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The Synthesis and Evaluation of Chemical Adjuvants for Modulating Immunity

by

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Abstract

The immune system is extremely complex, consisting of the innate and the acquired immune systems, which work together to generate a response. The ability to influence these systems and result in a more desirable immune response would be extremely beneficial for treating a range of diseases, as well as for preventing them with vaccination strategies.

The acquired immune system is specific to particular antigens and is only activated after exposure to a pathogen. Invariant natural killer T (iNKT) cells are an important part of the acquired immune response. When activated they release a range of pro-inflammatory (T_H1) and regulatory (T_H2) cytokines, resulting in stimulation of the wider immune system. iNKT cells are activated by the recognition of glycolipids presented by the protein CD1d. A non-glycosidic analogue of the prototypical CD1d agonist, α-GalCer, is threitol ceramide (ThrCer), which shows promise as a therapeutic agent. ThrCer should retain four of the hydrogen bonds seen in the crystal structure of the CD1d-α-GalCer-iNKT cell receptor ternary complex. In order to ascertain the relative importance of these hydrogen bonds a series of deoxy ThrCer analogues, which systematically removed the hydroxyl groups in the sugar head group, were synthesised and then tested for iNKT cell activation. From this study we determined that all three hydroxyl groups of ThrCer are necessary for effective iNKT cell activation.

Postulating that the lower biological activity of ThrCer compared to α-GalCer was due to the conformational flexibility of the acyclic threitol head group, we next synthesised analogues which constrained the threitol head group into a six-, seven- and eight-membered carbocyclic ring. These analogues were then tested for iNKT cell activation to determine their therapeutic potential, and results indicated that constraining the threitol head group into a six- or seven-

membered carbocyclic ring restores activity to ThrCer, to the level produced by α-GalCer. Routes to conformationally less flexible double bond-containing carbocyclic analogues have also been explored.

In contrast to acquired immunity, innate immunity is non-specific and can act immediately to promote inflammation and recruit phagocytes to a site of infection. The phagocytes can then engulf any pathogens to disable them. Uptake of these pathogens is usually through pattern recognition receptors, which recognise specific pathogen-associated molecular patterns. Macrophage-inducible C-type lectin (Mincle) is one such receptor which recognises mycobacterial trehalose-6,6'-dimycolate (TDM). The synthetic analogue trehalose-6,6'-dibehenate, which has replaced the two mycolic acid chains of TDM with C_{22} acyl chains, has been shown to induce biological activity in the same manner as TDM, and has the potential to be used as a synthetic adjuvant. To investigate the effect of the acyl chain on the level of biological activity we synthesised TDM analogues with different length acyl chains, which were then tested for Mincle stimulation. Results indicate that acyl chain length can modulate Mincle stimulation, although the optimal chain length has not yet been determined.

Declaration

The work recorded in this thesis was carried out in the School of Biosciences at the University of Birmingham, U.K. during the period of November 2008 to November 2012. The work in this thesis is original except where acknowledged by reference.

No portion of this work is being, or has been submitted for a degree, diploma or any other qualification at any other university.

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Contents

Abstract **ii**

Declaration **iv**

Acknowledgements **v**

Table of contents **vi**

List of abbreviations **x**

Table of Contents

Chapter 1.

1. Introduction

1.1 The Immune System 2

1.2 Innate Immunity 6

1.3 C-type Lectin Receptors 9

1.4 Macrophage-inducible C-type Lectin 11

1.4.1 Antifungal Activity of Mincle 13

1.4.2 Necrotic Cell Recognition of Mincle 14

1.4.3 Anti-Mycobacterial Activity of Mincle 15

1.5 Trehalose-6,6'-dimycolate (TDM) 17

1.5.1 Adjuvanticity of TDM 17

1.5.2 Structural Requirements for Binding and Recognition 17

1.6 CD1 22

1.7 Natural Killer T Cells 28

1.8 α-Galactosyl Ceramide 33

1.9 The Crystal Structures of the α-GalCer-CD1d Complex and the α-GalCer-CD1d-TCR Ternary Complex 37

1.10 Analogues of α-GalCer 46

1.10.1 Analogues with Modifications to the C_{26} Acyl Chain 46

1.10.2 Analogues with Modifications to the Sphingosine Chain 49

1.10.3 Analogues with Modifications to the Amide Bond 51

1.10.4 Analogues with Modifications to the Glycosidic Bond 53

1.10.5 Analogues with Modifications to the Sugar Head Group 54

1.10.6 Non-glycosidic Analogues 57

1.11 Aims and objectives 60

Chapter 2.

2. Synthesis of Deoxy and Truncated ThrCer Analogues 64

2.1 Threitol Ceramide and our Target Compounds 64

2.2 Synthesis of Threitol Ceramide 66

2.2.1 Synthesis of the Nucleophile 68

2.2.2 Synthesis of the Threitol Electrophile and Etherification 69

2.3 Retrosynthetic Analysis of our Deoxy and Truncated Analogues of ThrCer 74

2.4 Synthesis of the 2-Deoxy and 3-Deoxy ThrCer Analogues 76

2.5 Synthesis of the Truncated ThrCer Analogue 80

2.6 Synthesis of the 2,3-Dideoxy ThrCer Analogue 83

2.7 Biological Analysis 86

2.8 Conclusions 88

Chapter 3.

3. Synthesis and Biological Evaluation of Conformationally Less Flexible ThrCer Analogues 90

3.1 Target Carbocyclic ThrCer Analogues 90

3.2 Reported Carbocyclic Analogues 92

3.3 The Configuration and Conformation of Our Target Analogues 95

3.4 Retrosynthetic Analysis 100

3.5 Synthesis of the Cycloheptyl ThrCer Analogue 101

3.5.1 Synthesis of the Aldehyde 102

3.5.2 Ring-Closing Metathesis 108

3.5.3 Etherification 109

3.6 Synthesis of the Cyclohexanyl ThrCer Analogue 118

3.7 Synthesis of the Cyclooctanyl ThrCer Analogue 123

3.8 Biological Analysis 126

3.9 Conclusions and Future Work 132

3.10 Synthesis of Double Bond-Containing Constrained Ring ThrCer Analogues 134

3.10.1 Synthesis of the Cyclooctenyl ThrCer Analogue 140

3.10.2 Towards the Synthesis of the Cyclohexenyl and Cycloheptenyl ThrCer Analogues 143

3.10.3 Conclusions and Future Work 153

Chapter 4.

4. Synthesis and Biological Evaluation of TDM/TMM analogues 156

4.1 Target Compounds and Retrosynthetic Analysis 156

4.1.1 Synthesis of Partially Deprotected Trehalose 158

4.1.2 Esterification of the Deprotected Trehalose 160

4.2 Synthesis of Unsaturated TDM/TMM Analogues 163

4.3 Synthesis of GMM Analogues 165

4.4 Biological Analysis 167

4.5 Conclusions and Further Work 171

Chapter 5.

5. Experimental 173

5.1 Instrumentation 173

5.2 Chemicals and Reagents 174

5.3 Reactions 174

Chapter 6.

6. References 322

List of Abbreviations

°C	Degrees centigrade
%	Percent
Å	Angstrom
AICD	Activation-induced cell death
α-GalCer	α-galactosyl ceramide
APC	Antigen Presenting Cell
Ar	Aromatic, aryl
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
β_2m	β_2-microglobulin
Bn	Benzyl (CH_2Ph)
BSA	Buried surface area
Bu	Butyl
CAN	Cerium (IV) ammonium nitrate
CD1	Cluster of Differentiation 1
CTLD	C-Type lectin-like domain
CLR	C-Type lectin receptor
CTL	Cytotoxic T cell
DC	Dendritic Cells
DCC	*N,N'*-Dicyclohexylcarbodiimide
DDM	Didehydroxymycobactin
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIBALH	Diisobutylaluminium hydride

DMAP	4-Dimethylaminopyridine
DMB	2,4-Dimethoxybenzyl
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethylsulfoxide
DN	Double negative
dNKT	Diverse natural killer T
DP	Double positive
DTBP	2,6-Di-*tert*-butylpyridine
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
Et	Ethyl
g	Grammes
GFP	Green Fluorescent Protein
Glu	Glutamic acid
Gly	Glycine
GlyCer	Glycerol ceramide
GMM	Glucose monomycolate
h	Hour
hCD1d	Human CD1d
HMBC	Heteronuclear multiple bond correlation
HMDS	Hexamethyldisilazane
HRMS	High resolution mass spectrometry
IFN	Interferon
Ig	Immunoglobulin
iGb3	Isoglobotrihexosylceramide
IL	Interleukin

Ile	Isoleucine
iNKT	Invariant natural killer T
IR	Infrared
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
K_d	Dissociation constant
Lys	Lysine
M	Molar
m	Milli
Me	Methyl
Met	Methionine
MHC	Major Histocompatability Complex
MHz	Mega-Hertz
min	Minute
MIP	Macrophage inflammatory protein
Mincle	Macrophage-inducible C-type lectin
ml	Millilitres
m.p	Melting point
MTP	Microsomal triglyceride protein
n	Nano
NFAT	Nuclear factor of activated T-cells
NK	Natural Killer
NKT	Natural Killer T
NMR	Nuclear magnetic resonance
NOD	Non-obese diabetic
PAMP	Pathogen-associated molecular pattern

Ph	Phenyl
Phe	Phenylalanine
PIM	Phosphatidylinositol mannoside
PMB	*para*-Methoxybenzyl
Pro	Proline
PRR	Pattern recognition receptor
rt	Room temperature
s	Second
SAR	Structure activity relationship
SAP	Spliceosone-associated protein
Ser	Serine
SH2	Src homology 2
TB	Tuberculosis
TBAF	Tetrabutylammonium fluoride
TBDMS	*Tert*-butyldimethylsilyl
TBDPS	*Tert*-butyldiphenylsilyl
TCR	T cell receptor
TDB	Trehalose dibehenate
TDM	Trehalose-6,6'-dimycolate
Tf	Trifluoromethanesulfonyl
T_H	T helper
THF	Tetrahydrofuran
THP	Tetrahydropyran
Thr	Threonine
ThrCer	Threitol ceramide
TFA	Trifluoroacetic acid

TLC	Thin layer chromatography
TMM	Trehalose monomycolate
TMS	Trimethylsilyl
TNF	Tumour necrosis factor
Trp	Tryptophan
Tyr	Tyrosine
wt	Wild type
µ	Micro

Chapter 1

Introduction

1. Introduction

1.1 The Immune System

Immunity is the ability to defend against biological infection. Immunology was already being explored back in circa 400BC, when Thucydides discovered that humans had protection against the recurrence of a disease – memory, and that this protection is only for one particular disease – specificity. This basic knowledge of immunology was only developed further in the 18th century, when Edward Jenner (1749-1823) observed that patients he had infected with cow pox, were immune against small pox. This process, where antigenic material is administered in order to develop immunity to a particular disease, was called vaccination. Louis Pasteur (1822-1895) subsequently established the germ theory of disease, which postulated that microorganisms are responsible for fermentation and disease, and that attenuated pathogenic organisms can be used as vaccines. In this same time frame, Emil Behring (1854-1917) and Shibasaburo Kitasato (1852-1931) discovered antiserum (now called antibodies) that could be raised against a toxin to provide protection even if no prior exposure to the toxin had occurred – immunity could be transferred.

There are two different types of immunity which work together to form the immune system: innate and acquired.

Innate immunity does not require any prior exposure to the pathogen to be able to defend against it; it is non-specific, independent of antigen-specific immune cells (B and T cells) and can act immediately. The innate immune system includes physical barriers, like the skin, and hydrolytic enzymes; however its main function is to promote inflammation and hence recruit

phagocytes and natural killer (NK) cells to the site of infection.[1] These phagocytic cells engulf pathogens and digest them with enzymes.

Acquired immunity is pathogen-specific and only occurs after an initial exposure to the pathogen. Lymphocytes are only activated when bound to antigens, and hence only develop to fight against the specific pathogen from which the antigen is derived. There are two main types of lymphocyte: B cells and T cells. B cells express antibodies (immunoglobulins) on their cell surface and hence activate upon encountering an antigen, proliferating and secreting more antibodies which bind specifically to the antigen. These immunoglobulins (Ig) have a variety of responses, for example IgM can agglutinate the antigen-presenting pathogen, hence easing phagocytosis. This phagocytosis can then activate T cells. T cells only become active when presented with antigen by specialised antigen-presenting cells (APC), like dendritic cells (DC), which express the Major Histocompatibility Complex (MHC) proteins. Once activated, T cells either secrete cytokines (CD4+ T helper cells) or become cytotoxic (CD8+ T cells), inducing apoptosis in cells which express specific peptides. CD4+ T helper (T_H) cells can become polarised to secrete certain cytokines, becoming T_H1 or T_H2 effector cells (Figure 1.1). This is dependent on the cytokine environment: in the presence of interferon-γ (IFN-γ) and interleukin-12 (IL-12) CD4+ cells differentiate into T_H1 effector cells, whilst with interleukin-4 (IL-4), they become T_H2 cells.[1] A T_H1 response protects against pathogen infections and tumour formation; it is a pro-inflammatory response. In contrast, a T_H2 response controls the regulatory immune functions; it is the suppressive response.[2]

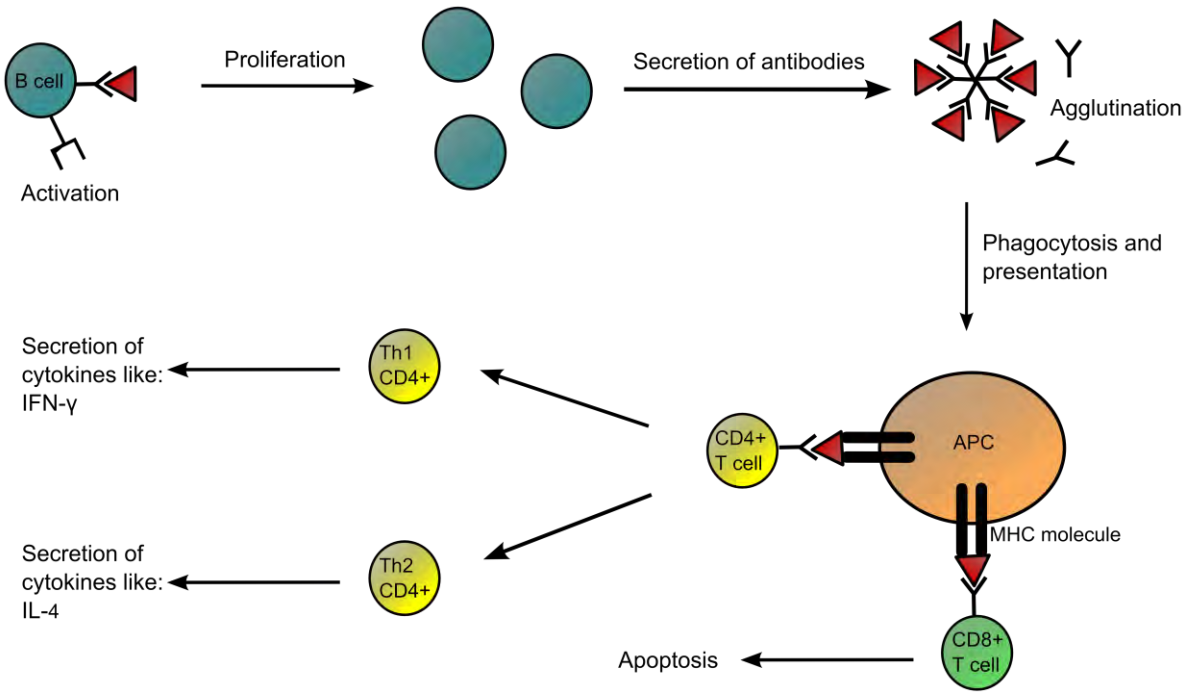


Figure 1.1. Diagram showing some of the functions of B and T cells. When B cells are activated after recognising an antigen they proliferate and secrete antibodies. Some of these antibodies cause agglutination of the antigens, which eases phagocytosis by APCs. The APCs then process and present these antigens to T cells. CD4+ T cells can become either T_H1 or T_H2 T-helper cells, depending on the cytokine environment. T_H1 T-helper cells then secrete pro-inflammatory cytokines like IFN-γ, whereas T_H2 T-helper cells secrete suppressive cytokines like IL-4. CD8+ T cells induce apoptosis in cells which express specific peptides.

The MHC molecule presents processed antigen, in the form of an oligopeptide. The antigen is first proteolytically digested inside the infected cell, leading to peptide fragments. The MHC molecule binds these fragments and carries them to the cell surface. Once on the surface they are presented to T cells, which bind to the peptide and parts of the MHC molecule through their T cell receptors (TCR). If foreign peptide and self MHC is recognised then the T cell initiates lysis of the cell. There are two subgroups of MHC molecule: class I and class II. MHC

class I molecules are present in all nucleated cells, and present intracellular antigen fragments to CD8+ T cells, resulting in cytotoxic T cells and cell lysis – the endogenous pathway of antigen presentation. MHC class II molecules are located on professional APC and present exogenous antigen fragments to CD4+ T helper cells. Cross-presentation, when exogenous antigen fragments are presented to CD8+ T cells, can also occur.[3]

1.2 Innate Immunity

The innate immune system is the first line of defence against any foreign body. It is active against any pathogen and provides immediate host defence. The initial barriers to infection are physical, chemical and microbiological, namely the skin, mucosal membranes and hydrolytic enzymes. However it is after these barriers that the innate response really begins. The innate response is highly conserved, and is found even in very simple animals, indicating the importance of this response in survival.

The main function of the innate response is to recruit and activate neutrophils and other phagocytosing cells to the site of infection. The recruited neutrophils phagocytose pathogens, forming a phagosome. This fuses with a cytoplasmic lysosome, forming the phagolysosome, in which the pathogen can be killed in one of two ways: the oxygen-dependent response, where oxygen is reduced by NADPH oxidase, forming toxic oxygen metabolites such as hydrogen peroxide, or the oxygen-independent response, which uses toxic cationic proteins and enzymes contained within the lysosome (Figure 1.2). This phagocytosis is 100-fold more effective if the pathogen is first opsonised (coated) with an antibody or complement protein, indeed some organisms cannot be phagocytosed without opsonisation.[4] This is one example of the interlinking between the innate and acquired immune systems – without the antibodies secreted by B cells of the acquired immune system, which are specific to the organism, the innate immune system is much less effective at eradicating pathogens.

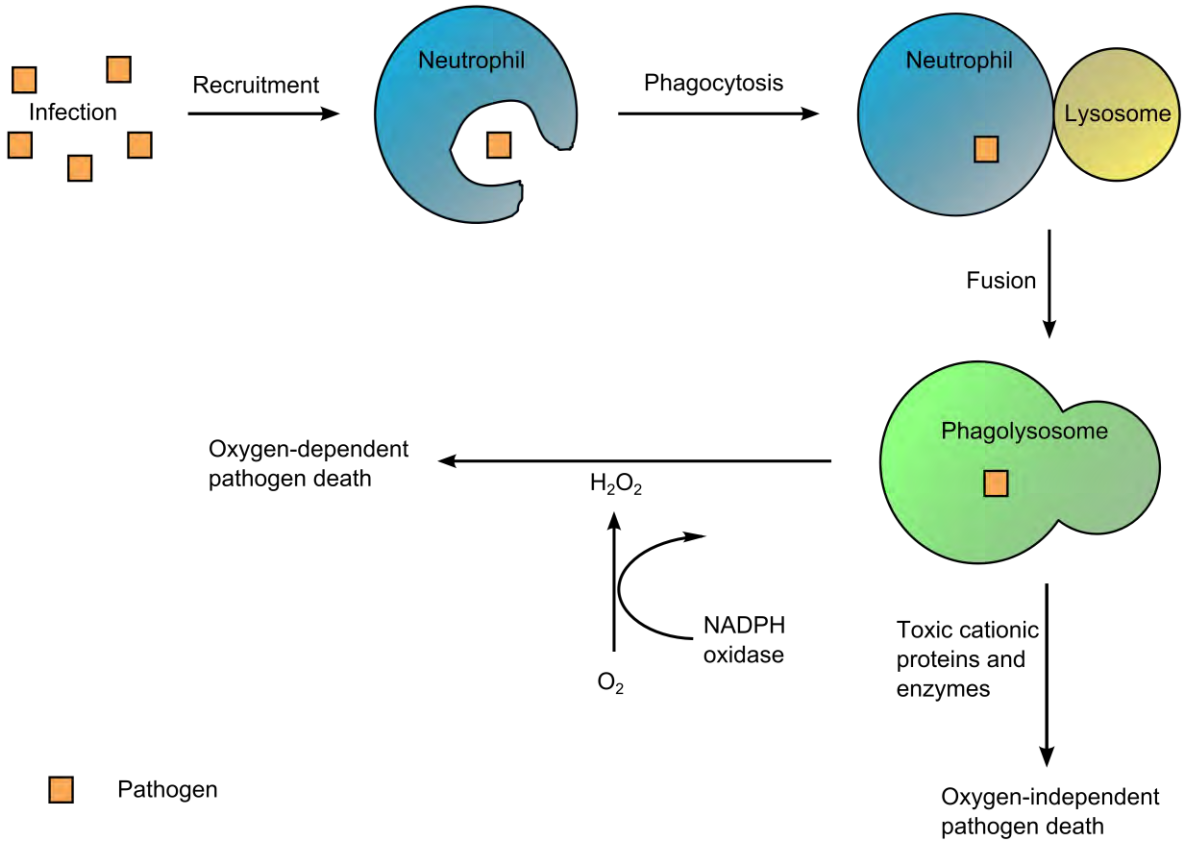


Figure 1.2. Diagram showing the main function of the innate immune system. Neutrophils and other phagocytosing cells are recruited to the site of an infection, where they engulf pathogens before fusing with a lysosome to form a phagolysosome. The pathogen is then killed, either via an oxygen- dependent method, where oxygen is reduced by NADPH oxidase to form toxic metabolites like hydrogen peroxide, or via an oxygen-independent method, using toxic cationic proteins and enzymes which were contained in the lysosome.

The innate immune response is not initiated by antigens, however it is still able to discriminate between self and foreign molecules. This is because phagocytes express a number of conserved pattern recognition receptors (PRRs), which recognise specific molecular structures found in pathogens, called pathogen-associated molecular patterns (PAMPs). Examples of PAMPs include lipoteichoic acid, lipopolysaccharide and mannans, which are found in the cell walls of Gram positive, Gram negative and yeast organisms, respectively (Figure 1.3). PAMPs

are not found in the host and so if a PAMP is recognised, the foreign body is deemed to be a pathogen and undergoes phagocytosis. There are three groups of PRRs: those that induce endocytosis and hence enhance antigen presentation, those that initiate nuclear factor κβ transduction and cell activation, and those which are secreted to act as opsonins.[4] PRRs include Toll-like receptors, Nod-like receptors and RIG-I-like receptors. C-Type lectin receptors are another type of PRR.

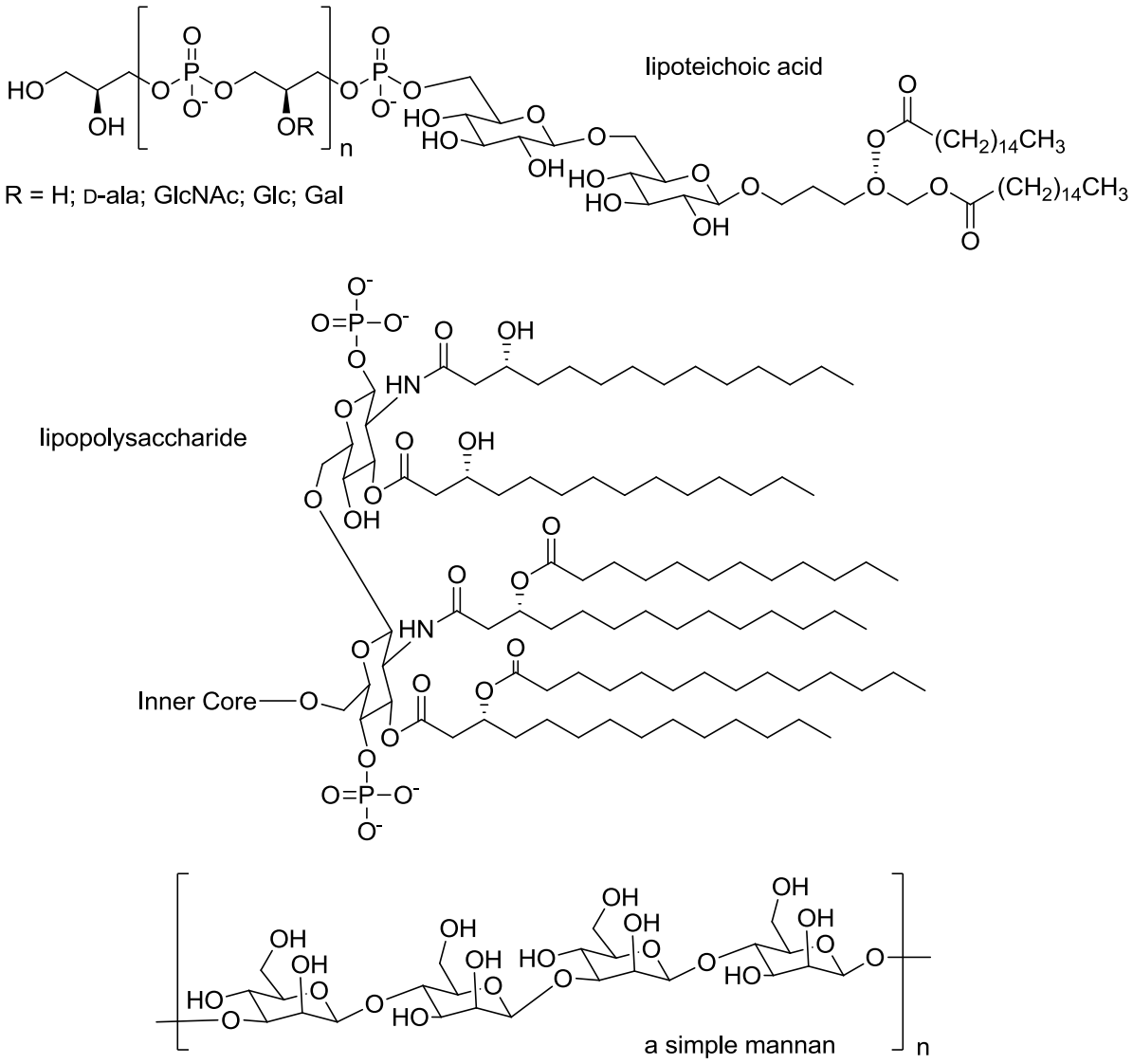


Figure 1.3. Some examples of PAMPS.

1.3 C-Type Lectin Receptors

C-Type lectin receptors (CLRs) are a family of proteins that were originally defined by their ability to recognise carbohydrate structures and so must contain at least one C-type lectin-like domain (CTLD, the domain which binds to the carbohydrate ligand).[5] This structure is a characteristic double-loop, which is formed by two disulfide bridges between conserved cysteine residues at the base of the loops.[5,6] The second long loop region, is structurally flexible, and is involved in Ca^{2+}-dependent carbohydrate binding. Classical C-type lectin receptor long loop domains generally contain conserved residues and motifs to form Ca^{2+} binding sites and typically bind carbohydrate ligands. There is another non-classical type of CLR, sometimes called lectin-like receptors, which generally do not contain these conserved motifs and hence are more likely to bind to non-carbohydrates (Figure 1.4).[6-8]

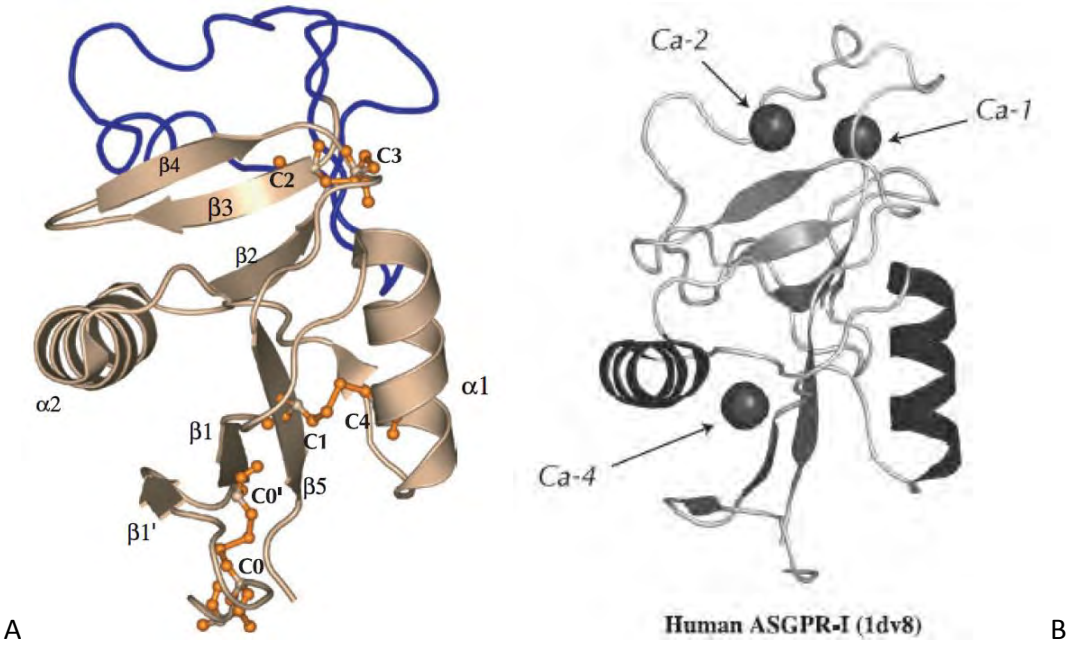


Figure 1.4. A - Cartoon representation of a typical CTLD structure. The long loop is shown in blue. Cysteine bridges are shown as orange sticks. B - Showing the Ca^{2+} binding sites. Figure adapted from ref.[5] RightsLink® licence number 2992010416664.

Ligand recognition causes a variety of cellular responses, depending on the particular CLR stimulated. These can either inhibit or induce cellular activation. Generally inhibitory CLRs contain an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domain, which upon activation, leads to recruitment of Src homology 2 (SH2) domain-containing phosphatases which dephosphorylate the tyrosines of activation kinases, leading to down-modulation of cellular activation. Activation CLRs, on the other hand, contain an immunoreceptor tyrosine-based activation motif (ITAM) or can associate with signalling adaptor molecules such as FcRγ chain, DAP10 or DAP12. This results in activation of SH2-containing protein tyrosine kinases, such as Syk, which leads to the production of cytokines and chemokines and the induction of phagocytosis. However there are some exceptions in which ITIMs have induced activation and ITAMs have induced cellular inhibition.[9,10] Generally though an ITIM/ITAM pair on cells maintains the balance between activation and inhibition, without which excessive inflammation, autoreactivity and disease can occur.[11-13]

The CLR family is divided into 17 groups based on the CTLD structure. Within Group II is the dectin-2 family of CLRs. Proteins within this family all have a similar structure, consisting of a short cytoplasmic tail, a type II transmembrane domain, where the N-terminus encodes the intracellular region of the protein and the C-terminus encodes the extracellular region, an extracellular stalk region of varying length and a single extracellular Ca^{2+} carbohydrate binding CTLD, making them a classical CLR. Members of this family tend to lack any signalling motifs in their cytoplasmic domain, but instead associate with ITAM signalling adaptor molecules through the presence of a positively charged residue in the transmembrane region.[8] They are also predominately expressed on cells of myeloid lineage, including dendritic cells and macrophages.[14] Mincle (Macrophage-inducible C-type lectin) is one receptor in the dectin-2 family.

1.4 Macrophage-inducible C-type Lectin

Mincle (also called Clec4e or Clecsf9) was originally identified as a protein whose expression was induced by lipopolysaccharide,[14] and also as a transcriptional target of nuclear factor NF-IL6 in peritoneal macrophages, with gene expression being induced by several proinflammatory cytokines, such as IFN-γ, tumour necrosis factor (TNF)-α and IL-6.[14] Like the other members of the dectin-2 family it is predominately expressed on cells of myeloid lineage, however this receptor is also found on B cells and microglia in the brain.[15,16] Normally the expression of Mincle is very low, however it is highly upregulated upon exposure to stimuli, such as inflammatory cytokines and TLR ligands. Mincle does not contain any signalling motifs in its cytoplasmic domain, so selectively associates with an ITAM-containing FcRγ chain, over other adaptors like DAP12, via a positively charged arginine at position 42 in the transmembrane region.[17] Signalling is dependent on the FcRγ chain; Mincle-induced production of inflammatory cytokines is abrogated in FcRγ-deficient macrophages.[17] MyD88, a crucial adaptor for TLR signalling was not necessary. After Mincle stimulation there is a signalling cascade where the ITAM tyrosine residues are phosphorylated by Src-family kinases, resulting in the recruitment and activation of Syk. Syk then activates a signalling cascade through CARD9, inducing the production of inflammatory cytokines like TNF-α, macrophage inflammatory protein (MIP)-2 and IL-6. CARD9 is essential for Syk inflammatory responses;[18,19] CARD9-deficient macrophages impair Mincle-induced MIP-2 production to a similar level as FcRγ-deficient macrophages.[17] These results indicate that Mincle activates macrophages through the FcRγ-Syk-CARD9 pathway (Figure 1.5).

Mincle has been shown to have a variety of ligands and is the receptor for both endogenous and exogenous ligands. It is involved in antifungal activity, necrotic cell recognition and antimycobacterial activity.

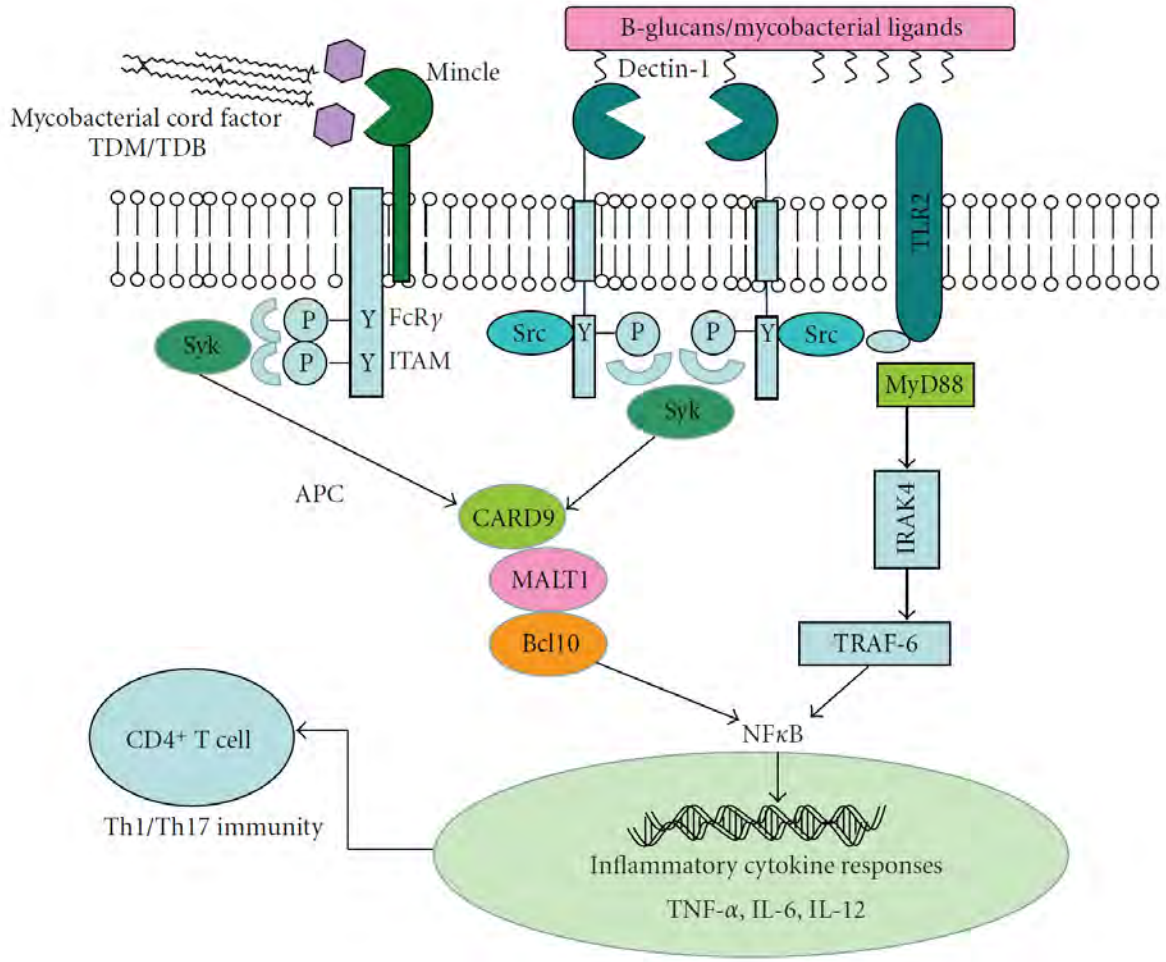


Figure 1.5. Diagram showing Mincle-mediated signalling. After Mincle-mediated recognition of a ligand, the associating ITAM-containing FcRγ chain is phosphorylated by Src-family kinases, activating the Syk-CARD9 pathway to produce inflammatory cytokines like TNF-α. Adapted from ref.[20]

1.4.1 Antifungal Activity of Mincle

Some C-type lectin receptors directly recognise specific fungi.[21] Dectin-2, for example, recognises *Candida albicans, Microsporum audouinii* and *Trichophyton rubrum*[22,23] and this recognition induces the production of inflammatory cytokines and chemokines, as well as mediating fungal uptake and killing.

Mincle had been reported by Wells *et al.* to also recognise *C. albicans* and induce the production of TNF-α by macrophages.[24] The group also reported that mice lacking Mincle were more susceptible to systemic candidiasis; they had higher fungal burdens than wild-type mice when infected with *C. albicans*, indicating that Mincle has an important role in clearing such infections.

However, Yamasaki *et al.* communicated that in their study, Mincle did not recognise *C. albicans*, but did recognise the fungal species *Malassezia*.[25] They did use different strains of *C. albicans* and noted that this might be the reason for the differing results; Mincle might be able to distinguish the structural differences in strains of *C. albicans. Malassezia* is commonly found on human skin, but can cause skin diseases and fatal sepsis, including intravascular catheter-associated sepsis.[26-27] Recognition of *Malassezia* caused the production of cytokines such as TNF-α, MIP-2, KC and IL-10.[25] Mincle-deficient mice produced far fewer cytokines and neutrophil infiltration against *Malassezia* injection, indicating that Mincle also plays a key role in the immune response to *Malassezia* fungi. The ligand causing this recognition is as of yet unknown; however for recognition to occur the Mincle CTLD must contain the mannose binding EPN-motif, and requires the presence of Ca^{2+},[25] indicating the ligand is a carbohydrate. α-Mannose has been shown to act as a ligand to Mincle,[25] and so it is possible that Mincle recognises α-mannosyl residues on the fungal surface.

1.4.2 Necrotic Cell Recognition of Mincle

Mincle can also sense necrosis and mediates inflammatory responses to the presence of necrotic cells.[28] Using the NFAT-GFP reporter cell system it was found that when the cells were cultured for prolonged periods alone, without exchange of the medium, the number of GFP^+ cells increased, indicating that activation was occurring. This increase was paralleled by an increase in the number of dead cells. GFP expression was also increased with supernatants from necrotic cells and lysates generated from normal cells. These results suggest that a component is released, or generated, during cell death and signals through Mincle.[17] Mutation of the mannose binding EPN-motif to a QPD galactose binding motif did not alter the signalling through Mincle,[29] indicating that Mincle recognises the ligand independently from any carbohydrate region. This suggests that the ligand could possibly be a non-carbohydrate; non-carbohydrates, like protein, lipids and inorganic ligands, have been shown before to bind to CLRs.[5] To test this hypothesis, a soluble Mincle protein was constructed by fusing the extracellular domain of Mincle to the carboxyl terminus of the human IgG Fc domain, creating an Ig-Mincle. This Ig-Mincle bound to annexin V-positive, propidium iodide-positive dead thermocytes, indicating that dead cells express a molecule that binds to Mincle. This binding occurred in the absence of Ca^{2+}, indicating that the ligand is not a carbohydrate, as the presence of Ca^{2+} is essential for carbohydrate recognition.[6] Therefore proteins from lysates of dead cells were screened with Ig-Mincle in the absence of Ca^{2+} to determine whether the ligand might be a protein. A protein of 130 kilodaltons was found to bind specifically to Ig-Mincle and was shown to be spliceosome-associated protein 130 (SAP130, also called Sf3b3), which is a component of the U2 snRNP complex.[30] In the U2 snRNP complex SAP130 interacts with SAP49, SAP145 and SAP155 to form the spliceosome complex. These other snRNP proteins also precipitated together with Ig-Mincle, but far less so than SAP130. Proteins which

are similar to SAP130, such as DDB1 (a protein that binds to DNA damaged by ultraviolet radiation), or proteins which are known to be secreted from dead cells such as HMGB1, were shown to not bind to Mincle. Therefore SAP130 selectively binds to Mincle. SAP130 is normally located in the nucleus in live cells,[30] consequently the movement of SAP130 to the outside milieu must be indicative of deregulated cell death. SAP130 is an endogenous ligand, indicating that Mincle can recognise both self- and non-self ligands. Once SAP130 is recognised, Mincle promotes neutrophil recruitment to the site of the necrosis and increases cytokine production of macrophages.

Excessive cell death has been shown to induce transient infiltration of inflammatory cells even in the absence of infection.[17] This is mainly due to Mincle, as Mincle mRNA is rapidly upregulated to increase the expression of Mincle on macrophages. These macrophages can then produce MIP-2, which recruits neutrophils. Neutrophil infiltration is believed to cause the acute inflammation that accompanies tissue damage; however, early, small-scale, neutrophil-mediated tissue destruction can in some cases promote tissue repair.[31] Therefore Mincle might accelerate diseases characterised by massive cell death, such as hepatitis or insulitis, by excessively activating macrophages, or it might instead promote repair and aid clearance of apoptotic cells through beneficial small-scale recruitment of neutrophils. However in rheumatoid arthritis Mincle has been reported to be greatly upregulated.[32] Therefore a Mincle-blocking compound could be beneficial in controlling these inflammatory diseases.

1.4.3 Anti-Mycobacterial Activity of Mincle

Tuberculosis (TB) is a major worldwide disease caused by *Mycobacterium tuberculosis*. It has infected one third of the world's population[33] and kills more than 1.5 million people each year.[34] *M. bovis* Bacille Calmette-Guérin (BCG), commonly used as a TB vaccination strategy, is

also a widely used antitumour adjuvant therapy for bladder cancer.[35] Injection of BCG causes a strong local immune response, which bathes tumours in cytokines and activated immune cells, resulting in regression of transitional cell carcinomas. Also, complete Freund's adjuvant (CFA) is an emulsion of mycobacterial cell wall in paraffin oil. It has been used experimentally for decades to optimise memory T- and B-cell responses in mice. One of the main immunostimulatory components in CFA is a cell-wall glycolipid called trehalose-6,6'-dimycolate (TDM).

1.5 Trehalose-6,6'-dimycolate (TDM)

In search of the factor which caused a characteristic bacterial growth pattern called cording, Hubert Bloch isolated a glycolipid from the tubercle bacillus.[36] This glycolipid was determined to be TDM, and although it is still called cord factor, it is now not thought to be involved in cording. TDM is the most abundant glycolipid in the cell wall of mycobacteria, and is a major component in making the cell wall hydrophobic, which is crucial for mycobacterial survival in the host. However TDM effectively stimulates the innate immune system of mammals, and so has been extensively studied for this adjuvant effect.[33] TDM is a potent stimulator of IL-1, TNF-α, nitric oxide and granuloma formation. It also enhances B-cell antibody production.

1.5.1 Adjuvanticity of TDM

When TDM is administered *in vivo* it induces granuloma formation, a large production of inflammatory cytokines, such as TNF-α and MIP-2, and activates macrophages to produce nitric oxide, which can kill mycobacterial cells directly. This effect is completely abrogated in Mincle-deficient mice and also in FcRγ-deficient cells.[36,37] TDM also appears to induce adaptive immunity; it can activate T_H1/T_H17 cellular immunity when administered with a subunit vaccine.[38] This is achieved by activating the Syk-CARD9-Bcl10-Malt1 pathway in antigen-presenting cells (APCs).

1.5.2 Structural Requirements for Binding and Recognition

TDM consists of a trehalose unit with two very long-chain α-branched, β-hydroxy fatty acids, called mycolic acids, linked through ester bonds on the 6 and 6'-positions (Figure 1.6). Mycolic acids are only found in mycobacteria and related actinobacteria, therefore molecules containing these compounds are easily recognisable as foreign lipids.

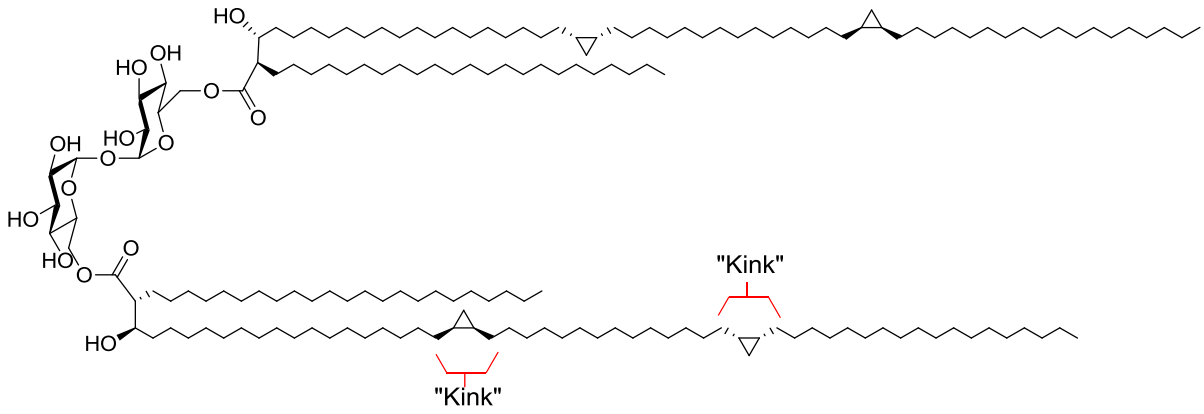


Figure 1.6. Structure of one type of TDM, with the kink highlighted.

Until recently, the receptor for TDM was unknown. In 2009 Ishikawa *et al.*[36] demonstrated that Mincle recognised *M. tuberculosis*. This recognition was dependent on the presence of the mannose binding EPN-motif in the CTLD; mutation into the galactose binding QPD-motif prevented any activation.[36] Because of this it was initially assumed that Mincle was recognising terminal α-1,2-mannose residues of mycobacterial molecules such as phosphatidylinositol mannosides (PIMs); however studies with PimE-deficient mycobacteria, which should not contain terminal α-1,2-mannose residues, still showed similar activity to wild-type cells. Therefore even though the mannose binding EPN-motif is necessary, Mincle does not seem to recognise mycobacterial α-1,2-mannose-containing glycolipids. However, this is not unheard of; proteins with mannose binding motifs can also recognise structurally related sugars like glucose.[34] To determine what was causing Mincle recognition Ishikawa *et al.* extracted lipids from the cell wall of *M. smegmatis* using various organic solvents. From this they discovered that the ligand was TDM, and that trehalose monomycolate (TMM) also stimulated Mincle,

albeit at a much lower level.[36,39] Purified mycolic acid chains by themselves did not activate Mincle, and soluble trehalose by itself also had no activity, indicating that both the sugar and the lipid are necessary for recognition. This suggests that the ester linkage of a fatty acid to trehalose might be important in Mincle recognition.

A synthetic TDM analogue, trehalose dibehenate (TDB, Figure 1.7) was also shown to activate Mincle strongly, in the same manner as TDM.[36,38] This is despite not having a mycolic acid chain, which had been previously reported to be necessary.[40] The mycolic acid chain had been hypothesised to contribute to optimal presentation of the polar head to Mincle, via the "kink".[36]

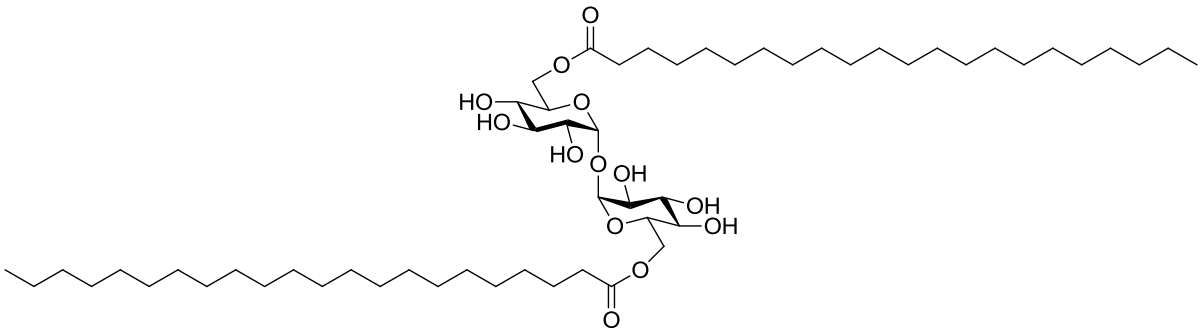


Figure 1.7. Structure of TDB

TDB may find application as a synthetic adjuvant,[37] being much simpler to synthesise while still retaining good activity.

It was recently reported that TDM is converted to glucose monomycolate (GMM) in the host cell environment, possibly to escape the host immune system and Mincle recognition.[41]

Trehalase, which should hydrolyse the glycosidic linkage in the trehalose unit to form two glucose units, was added to TDM and indeed Mincle activity was impaired.[36] This could be because one disaccharide head of TDM binds to two Mincle receptors, which can then cross-link.[34,36]

This conversion of TDM to GMM allows mycobacteria to evade the Mincle-mediated innate immune response. However the human immune system does not just consist of the innate immune response; there is often interlinking of the innate and the acquired immune systems. In this case the acquired immune system comes in to block this escape by mycobacteria; the GMM formed from hydrolysis of TDM is an antigen which can be presented to T cells to provoke an immune response (Figure 1.8).

Normally in the acquired immune system the antigen is a peptide fragment, which binds to the MHC for presentation to the T cell. GMM is not a peptide, it is a glycolipid and hence would not bind to the MHC molecule and so would not be presented by this route. Although the MHC is the usual route for antigen presentation there are other antigen-presenting molecules; the CD1 family are one such class of antigen-presenting molecules which present glycolipids. GMM can be bound by CD1b, which presents this glycolipid to T cells for an acquired immune response.

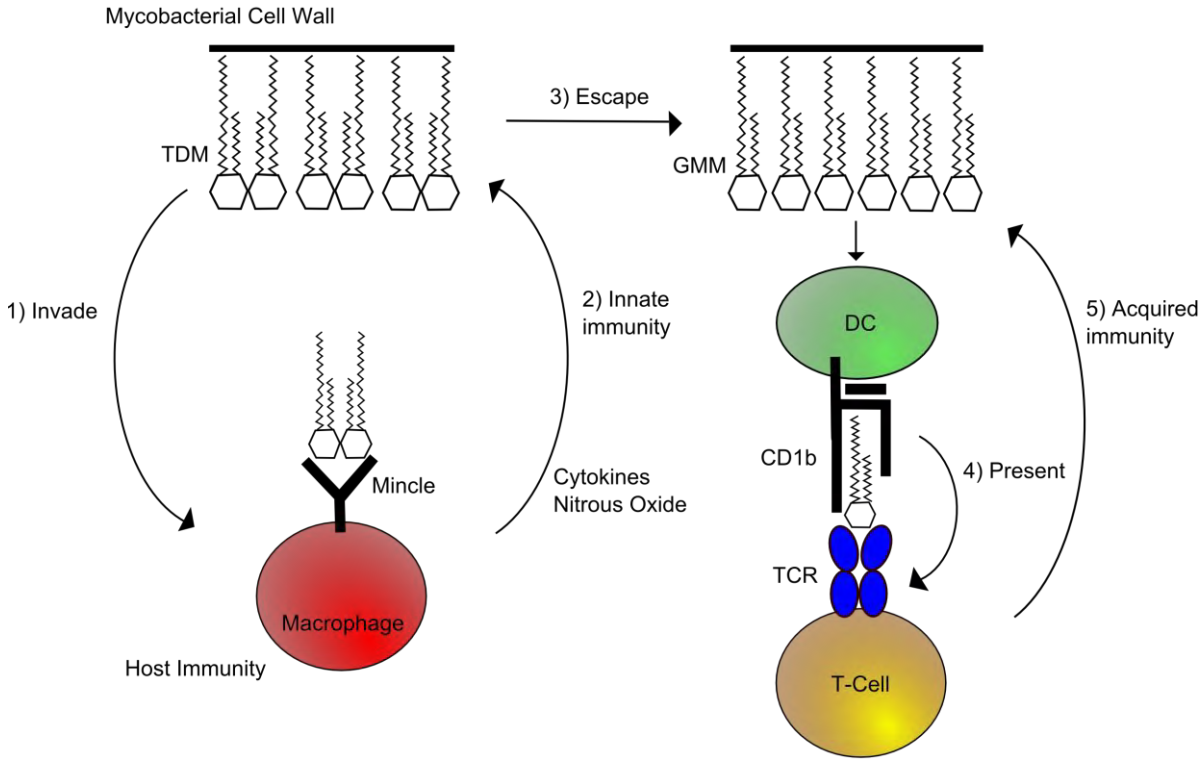


Figure 1.8. Schematic illustrating the attempted avoidance of the immune system by mycobacteria. TDM is one of the most abundant glycolipids present in the mycobacterial cell wall, however after invasion of the host the TDM is recognised by Mincle on macrophages, which then produce cytokines and nitrous oxide to activate the innate immune system (2). Mycobacteria try to avoid activation of the innate immune system by converting the TDM into GMM (3). However GMM can then be processed by DCs and presented by CD1b to T cells (4), which activates the acquired immune system (5).

1.6 CD1

The Cluster of Differentiation 1 (CD1) family of proteins are a class of antigen-presenting molecules, which present lipid, rather than peptide, antigens.[1,42] There are five different CD1 proteins, CD1a-CD1e, which can be separated into three groups based on their nucleotide and amino acid sequence homology; Group 1 consists of CD1a, CD1b and CD1c, Group 2 contains CD1d and Group 3 contains CD1e.[42-44] All five proteins are expressed in humans, however only CD1d is found in mice. There is very limited allelic variation of CD1 genes, unlike the polymorphism seen in MHC class I and class II genes. This could be because the lipid tails of antigens are structurally constrained and hence there is less variation, which means that the CD1 pockets also do not need to change significantly.[42]

Structurally, CD1 molecules are similar to MHC class I molecules, where a heavy α chain folds into three domains (α1-3) and is non-covalently associated with β_2-microglobulin (β_2m).[1,42] The α1 and α2 domains sit on a β-pleated sheet and fold to form a groove, deeper and larger in volume in CD1 molecules than in MHC class I, but also narrower. This groove provides the antigen binding site, although access to the groove is only through a narrow opening. MHC molecules characteristically have many small pockets in the wall of the groove to accommodate peptide side-chains; however in CD1 molecules these pockets have fused together to form between two and four big pockets, named A', C', F' and T'. These pockets are lined with mostly non-polar, and hence hydrophobic, amino acids.[1,42]

This structure allows CD1 molecules to bind antigens which have an amphipathic character, where there is a hydrophilic head group attached to a hydrophobic fatty acid or alkyl tail. The hydrophobic tail sits in the groove where it is stabilised by hydrophobic interactions, exposing the hydrophilic head on the surface of the molecule, for subsequent recognition by T cell

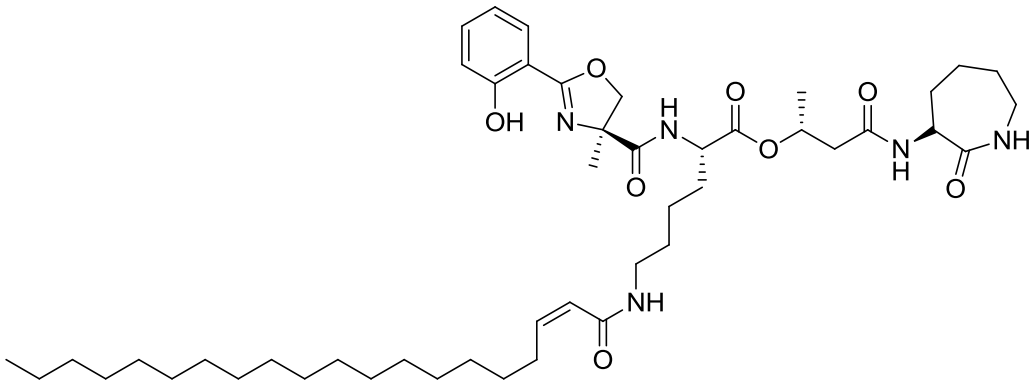
receptors (TCR).[1] The head group is stabilised by hydrogen bonds to the CD1 molecule, and these contribute to the correct positioning of the antigen for TCR recognition.

Although CD1 molecules have almost invariant binding grooves, they can still bind a wide variety of different glycolipids. The different CD1 molecules have unique binding groove architectures, which accounts for some of this variability; however some CD1 molecules can also present different classes of glycolipids themselves. CD1b, for example, can bind and present glycolipids containing diacylglycerol, sphingolipid or mycolate moieties.[45,46] CD1a has two pockets, A' and F'. The A' pocket is common to all of the CD1 molecules and is almost completely buried inside the CD1 molecule.

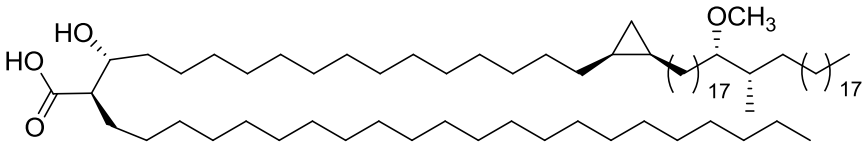
In CD1a the A' pocket is closed at one end, whereas for the other CD1 molecules the A' pocket circles back round to join the F' pocket. The F' pocket is long and extended, though shallower in CD1a than in the other CD1 molecules. It is able to accommodate both alkyl chains and peptides, allowing CD1a to bind and present molecules such as didehydroxymycobactin (DDM, Figure 1.9), a mycobacterial lipopeptide which contains one alkyl chain and a peptide moiety.[47] The lipid chain of DDM enters the A' pocket. It has been shown that DDM antigens with a C_{20} alkyl chain, which will fully occupy the A' pocket, are more potent agonists of T cells than DDM antigens with a C_{16} or C_{18} chain, indicating that CD1a selects lipids based on chain length.[47]

CD1b can bind and present glycolipids with very long lipid tails, like GMM and mycolic acids (Figure 1.9), because CD1b has two extra pockets, the C' and T' pockets. The C' pocket connects the F' pocket to the surface of the molecule and appears to provide an escape hatch for lipids which are longer than the pocket to protrude out of. The T' pocket connects the A' and F' pockets, creating a very long tunnel called the A'T'F' superchannel, which can accommodate the long lipid chains characteristic of mycolic acids.[48] CD1c can bind and present

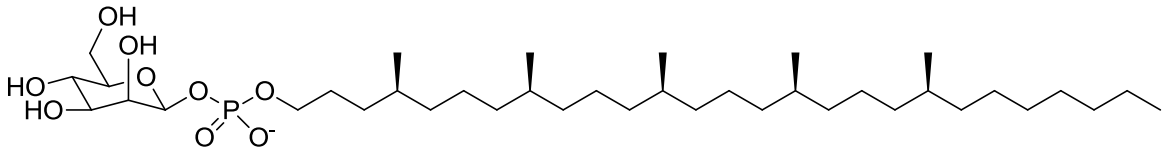
polyketides like mannosyl-1β-phosphomycoketide (Figure 1.9), which contain branched lipid tails.[49] Some lipids can bind to multiple CD1 proteins – sulfatide, a sulfate ester of β-D-galactosyl ceramide (Figure 1.9), can bind to CD1a, CD1b, CD1c and CD1d.[50,51]



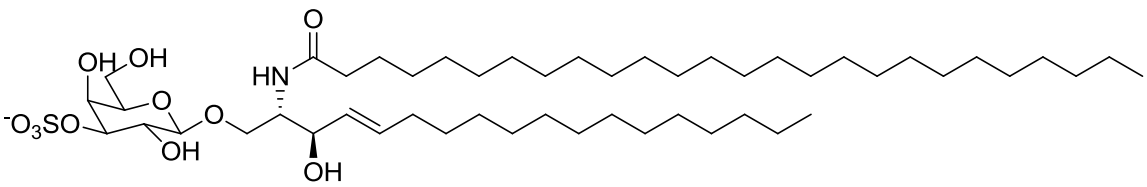
dideoxymycobactin (DDM)



free mycolic acid



mannosyl phosphomycoketide



sulfatide

Figure 1.9. Showing the structural diversity of lipid antigens presented by CD1 molecules.

The Group 3 CD1 molecule, CD1e, is the only CD1 molecule which is not involved in antigen presentation. It is not expressed on the cell surface but is instead located in late endosomes and lysosomes, as a soluble protein. It appears to be involved in intracellular lipid transport,[52] and is involved in antigen processing; CD1e facilitates the processing of PIM_6 into PIM_2 by an α-mannosidase.[53]

The structure of CD1d is similar to that of CD1a, containing both an F' and A' pocket, which in CD1d branches off the F' pocket, circles around and then rejoins the F' pocket.[54]

CD1d is assembled in the endoplasmic reticulum (ER), where it is loaded with a self-lipid, before binding to chaperones and to β_2m, which assist in their trafficking through the secretory pathway out of the ER, through the Golgi apparatus and out onto the plasma membrane. CD1d is then internalised via a clathrin-coated pit into the early or sorting endosomes, with the help of the AP2 protein. CD1d can also associate with MHC II molecules and the invariant chain in the ER, which allows them to go straight from the Golgi apparatus to endosomal compartments without having to go to the plasma membrane.[55,56] After internalisation into early endosomes, mouse CD1d can traffic to late endosomal and lysosomal compartments with the help of the AP3 protein, before being re-exported back out to the plasma membrane. However, human CD1d cannot interact with the AP3 protein and so can only enter early endosomes before returning to the plasma membrane (Figure 1.10).[57]

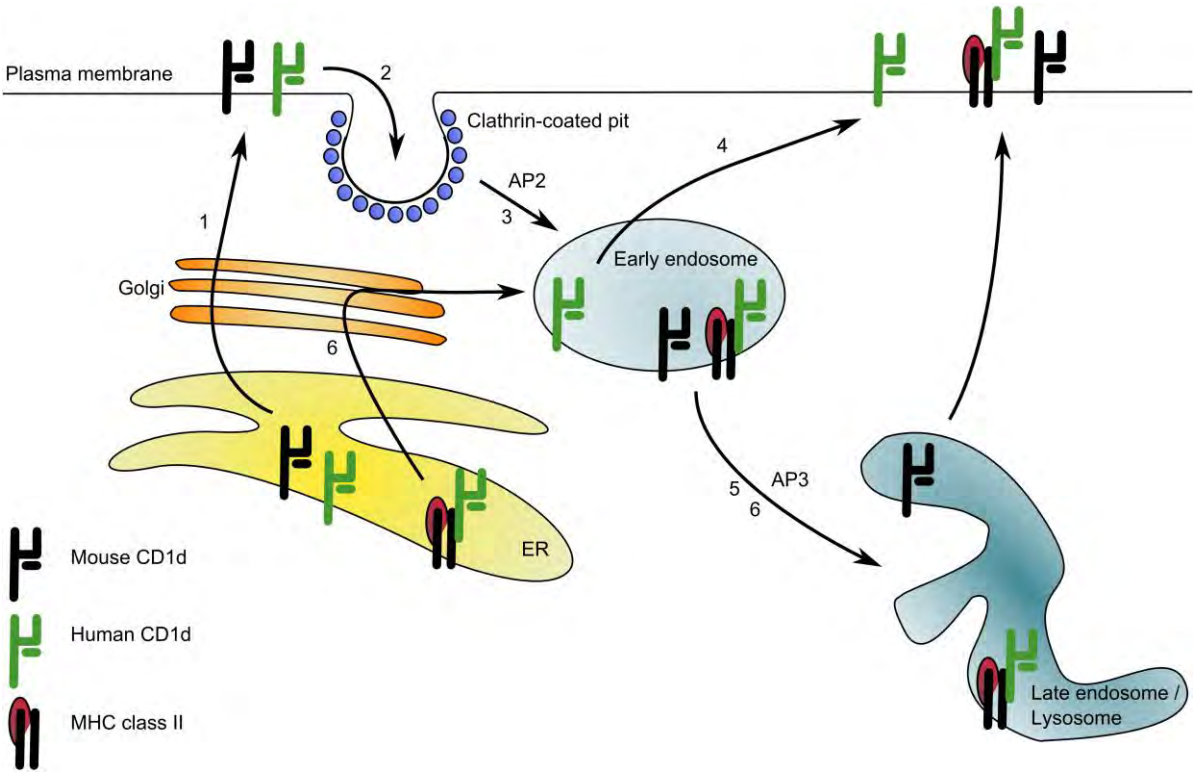


Figure 1.10. Intracellular trafficking of CD1d molecules. Both mouse and human CD1d molecules are assembled in the ER before being exported to the plasma membrane via the Golgi (1). These are then internalised via a clathrin-coated pit (2) and with the help of AP2, into an early endosome (3). Mouse CD1d can then move into the late endosome with the help of AP3 (4), however human CD1d cannot bind to AP3, and so is re-exported out to the plasma membrane from the early endosome (5). Human CD1d molecules can also associate with MHC class II molecules in the ER, which allows them to enter the late endosome or lysosome, before re-exportation to the plasma membrane (6).

This trafficking into endosomes is important in allowing CD1d to encounter and bind any lipid antigens for subsequent presentation to T cells, though some lipids can be directly loaded into CD1d molecules at the cell surface with no need for internalisation.[58] On the cell surface most CD1d molecules are associated with plasma membrane detergent-resistant membrane microdomains, also called rafts. These are domains which are enriched in cholesterol and

lipids, and this localisation can be important for efficient activation of T cells, especially at low antigen concentrations. The loading of lipids into CD1d is facilitated by several lipid transfer proteins. In the ER the self-lipids are loaded into CD1d with the assistance of the microsomal triglyceride transfer protein (MTP). Removal of this protein reduces surface expression of CD1d.[59] Saposin B can bind to lipids in bilayer membranes in the endosome to form soluble protein-lipid complexes before transporting the lipid to CD1d molecules for loading.[60]

The Group 2 CD1 molecule, CD1d is different to the Group 1 CD1 molecules in that it can present lipids to natural killer T (NKT) cells, which are specifically CD1d-restricted.[61]

1.7 Natural Killer T cells

NKT cells were first described in 1987[62-64] as T cells which contain an αβ TCR. They share some natural killer (NK) cell characteristics, most notably the expression of the NK1.1 marker (CD161 in humans), previously thought to be limited to NK cells, and are a potent source of immunoregulatory cytokines, including IL-4, IFN-γ and TNF. This last feature is what allows NKT cells to be important regulators of the immune response.

More recent work has determined that not all NKT cells express the NK1.1 marker, and that there are in fact many different types of NKT cells, which appear to have distinct functions. There are three main classes of NKT cells: Type I NKT cells (also called invariant NKT cells), Type II NKT cells (or diverse NKT cells) and NKT-like cells.[65] NKT-like cells are T cells which express the NK1.1 marker ($NK1.1^+$) but which are CD1d-independent. They are instead restricted by conventional MHC molecules, and as such are not really a type of NKT cell, despite having the NK1.1 marker. Type I and Type II NKT cells are both restricted by CD1d.

Type II NKT cells, or diverse NKT (dNKT) cells, contain a diverse TCR, and appear to be $NK1.1^+$ or $NK1.1^-$. These can be further separated into $CD4^+$ and double negative (DN; $CD4^-CD8^-$) subsets.

Type I NKT cells, or invariant NKT (iNKT) cells are the classical NKT cell. They express a semi-invariant αβ TCR. In mice the α chain is composed of Vα14-Jα18 and is predominantly paired with a β chain that uses Vβ8.2, Vβ7 or Vβ2. Human iNKT cells express homologous chains composed of Vα24-Jα18 and Vβ11 (homologous to Vβ8.2).[42,66,67] iNKT cells can be either $NK1.1^+$ or $NK1.1^-$, and $CD4^+$ or DN (or $CD8^+$ in humans, Table 1.1). These different subsets appear to have distinct functional phenotypes. In mice the proportions of the different subsets are

different depending on the tissue type and the $NK1.1^+CD4^+$ subset appears to produce more IL-4 than the $NK1.1^+CD4^-$ subset.[68] The $NK1.1^-$ subset in the thymus also produces more IL-4 and less IFN-γ than the $NK1.1^+$ subset.[69,70] In humans the $CD161^+CD4^+$ subset produces higher levels of IL-4, IL-2, IL-13 and granulocyte-macrophage colony-stimulating factor than the $CD161^+CD4^-$ subset, which produces mainly Th1 cytokines.[71]

	Type I NKT cells	Type II NKT cells	NKT-like cells
CD1d-dependent	Yes	Yes	No
α-GalCer reactive	Yes	Some	No
TCR α-chain	Vα14-Jα18 (mice) Vα24-Jα18 (humans)	Diverse	Diverse
TCR β-chain	Vβ8.2, Vβ7 or Vβ2 (mice) Vβ11 (humans)	Diverse	Diverse
NK1.1	+ (resting mature) − (immature or post-activation)	+ / −	+
Subsets	$CD4^+$ and DN (mice) $CD4^+$, $CD8^+$and DN (humans)	$CD4^+$ and DN (mice)	$CD4^+$, $CD8^+$and DN

Table 1.1. Showing the contrasting features of the different populations of NKT cells. Adapted from ref.[65] RightsLink® licence number 2992011383701.

iNKT cells develop in the thymus, like conventional T cells, but they branch off from conventional T cell development at the double positive (DP; $CD4^+CD8^+$) thymocyte stage.[72] The TCR is formed from random recombination of the V, J, and D genes, and positive selection occurs through the ability to recognise glycolipid antigens presented by CD1d on DP

thymocytes. The endogenous glycolipid which is used for positive selection is still unknown; isoglobotrihexosyl ceramide (iGb3), a lysosomal glycolipid, has been suggested as the self ligand,[73] however this view has been challenged.[74] It is possible that there is redundancy for this important function, and that iGb3 is just one of a selection of self glycolipid antigens that are used for positive selection. iNKT cells also appear to undergo negative selection, when the TCR has too high an affinity to the glycolipid and hence is deleted. However it is still not known when this negative selection occurs. The CD1d-restricted positive selection leads to immature $NK1.1^{-}CD4^{+}CD8^{+}$ NKT cells, which rapidly downregulate expression of the CD8 receptor, leaving $CD4^{+}$ cells. These then undergo at least three stages of phenotypical changes based on the expression of cell-surface molecules, specifically CD24, CD44 and DX5. The expression of CD4 can also be downregulated, giving $CD4^{-}$ cells, however it is uncertain when this occurs as both $NK1.1^{+}$ and $NK1.1^{-}CD4^{-}$ NKT cells have been observed.[69] After these changes most of the immature $NK1.1^{-}$ NKT cells leave the thymus and mature to $NK1.1^{+}$ cells in the periphery. However some $NK1.1^{-}$ cells mature in the thymus itself, becoming long-term intrathymic residents. The role of these mature iNKT cells in the thymus is still unclear. The maturation process is also restricted by CD1d, and requires the presence of IL-15 (Figure 1.11).[75,76]

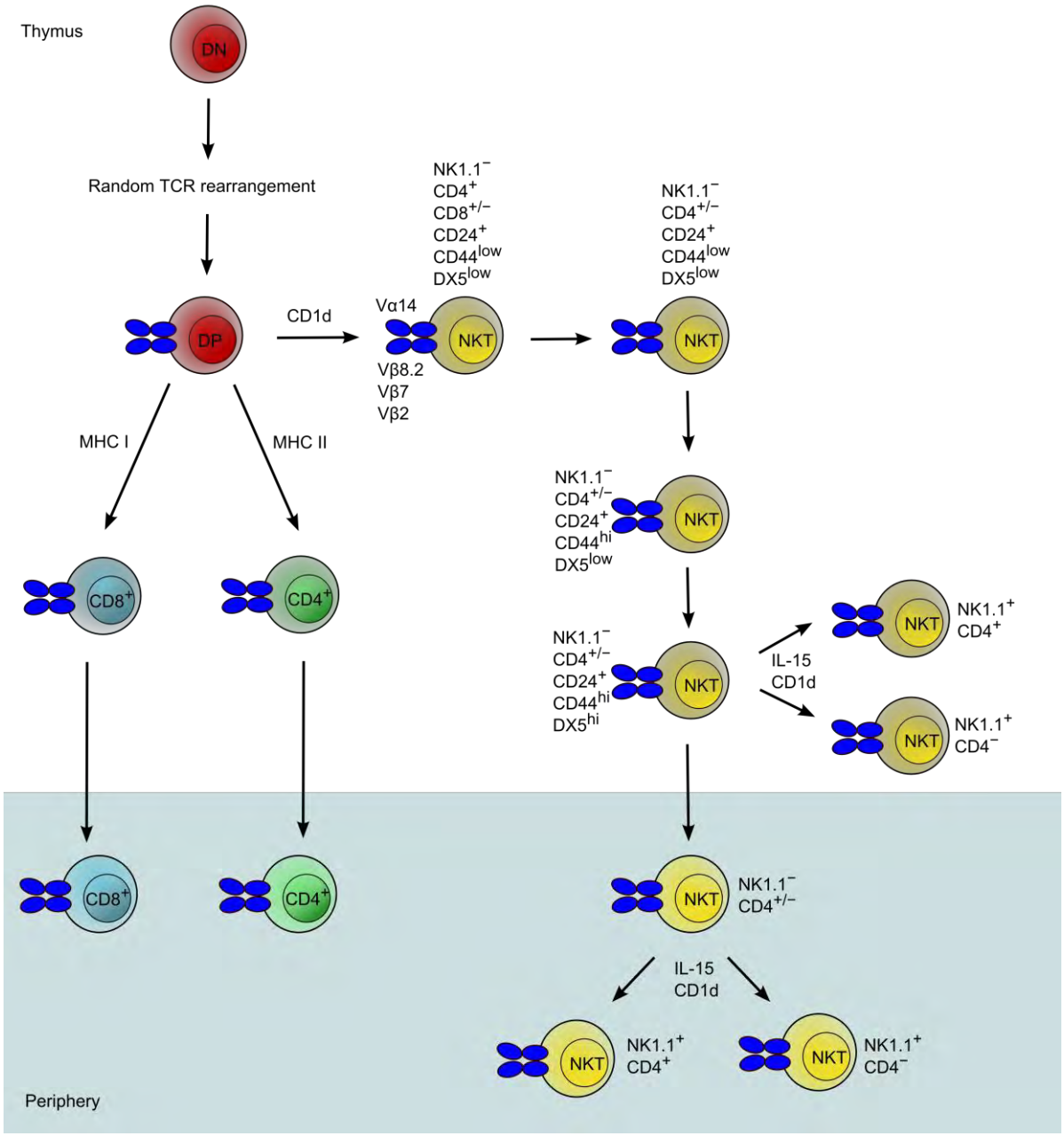


Figure 1.11. The development of iNKT cells. The TCR is randomly rearranged on DP thymocytes before positive selection with CD1d to select for cells containing the invariant TCR. These NKT cells then undergo a series of phenotypical changes based on the expression of surface markers like CD24, CD44 and DX5. Most of these cells are then exported to the periphery before maturation in the presence of CD1d and IL-15. Some of these cells however mature in the thymus instead, becoming long-term intrathymic residents.

iNKT cells can produce large amounts of cytokines upon activation, including the T_H1 cytokine IFN-γ and the T_H2 cytokine IL-4. This is possible because iNKT cells constitutively express IFN-γ and IL-4 mRNA. The ability of iNKT cells to secrete both T_H1 and T_H2 cytokines at the same time allows a role in immune regulation, being able to both suppress and inflame the immune system as required. iNKT cells also have T_H17 character; they secrete IL-17, which leads to inflammation and neutrophil activation. iNKT cell activation results in the activation of many types of cell, including B cells, T cells and NK cells. This results in waves of cytokine secretion, as these cells are activated: IL-4 secretion, produced mainly by iNKT cells, peaks at around 2 hours, IL-12, produced mainly by DC, peaks at around 6 hours and IFN-γ, produced mainly by NK cells, peaks at around 24 hours.[66] Activation of iNKT cells also results in rapid down-regulation of their surface TCR, as seen in conventional T cells,[77] which may protect against over-stimulation and activation-induced cell death (AICD). Then dramatic proliferation and recovery of the TCR allows the production of large amounts of cytokines.[78] The ability to secrete T_H1, T_H2 and T_H17 cytokines is how iNKT cells control tissue destruction, antitumour responses and inflammation. They are also implicated in the immune response against bacterial and parasitic infections, by their secretion of CD4+ T helper cell-activating cytokines. However misregulation of iNKT cells results in an imbalance of the T_H1 and T_H2 responses, causing allergy and autoimmunity.[79]

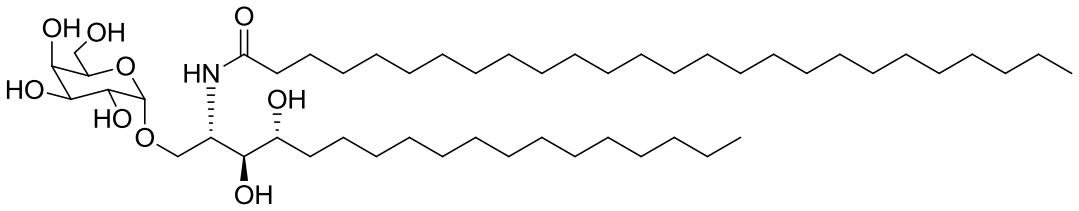
iNKT cells can recognise a wide variety of different glycolipids, even though they have an invariant α chain. This is most likely due to the diversity of their TCRβ; although the β chain is heavily biased towards Vβ8.2, Vβ7 or Vβ2, the Jβ genes and CDR3β regions are diverse.[80] This flexibility allows for recognition of many different antigens presented by CD1d, however the prototypical antigen, which provokes the strongest iNKT cell response seen so far, is a glycolipid called α-galactosyl ceramide (α-GalCer).

1.8 α-Galactosyl Ceramide

α-Galactosyl ceramide (α-GalCer) is a glycolipid which binds strongly to CD1d, having a dissociation constant (K_d) of 1.29 ± 0.08 μM.[81] This CD1d-αGalCer complex is recognised by iNKT cells, and serves to activate them.

α-GalCer (also referred to as KRN7000) is a synthetic glycolipid which was derived from agelasphins, compounds isolated from the marine sponge *Agelas mauritianus*. These natural products were found to prevent tumour metastasis.[82] They are unusual in that they contain an α-galactosyl linkage, rather than the more common β-glucosyl linkage found in ceramide lipids seen in mammals. This antitumour response was due to the fact that they had potent iNKT cell-stimulating properties, resulting in their proliferation and cytokine secretion. This resulted in activation of a variety of other cells, including B cells, NK cells and DC, initiating an immune response.[66]

The structure of α-GalCer consists of two hydrophobic hydrocarbon chains attached to a hydrophilic galactose sugar head, hence fitting in to the antigen binding groove of CD1d with the 26-carbon acyl hydrocarbon chain in the A' pocket, the 18-carbon phytosphingosine hydrocarbon chain in the F' pocket and the sugar head exposed for TCR recognition. The galactose head group is α-linked to a ceramide base, which consists of phytosphingosine that has been *N*-acylated with hexacosanoic acid (Figure 1.12).



α-GalCer (KRN7000)

Figure 1.12. The structure of α-GalCer

The ability of α-GalCer to potently stimulate iNKT cells allows the possibility of using this glycolipid for disease therapy and as an adjuvant. An adjuvant is a substance which can modulate the response of the immune system. This is particularly beneficial for vaccines; an adjuvant can be added together with the vaccine, allowing the use of lower doses or less immunogenic molecules. Therefore α-GalCer could be administered in addition to a vaccine, where it will stimulate iNKT cells to immediately secrete cytokines. These cytokines will activate the cells of the immune system, like B cells, T cells and DC. Maturation of DC requires direct contact with iNKT cells through CD40/CD40L signalling and results in an increase in cross-presentation of the exogenous protein antigens present in a vaccine by MHC class I molecules to CD8+ T cells. This increases cytotoxic T cell (CTL) activity and hence provides a greater immune response to the vaccine, aiding recognition and memory of the antigen – immunity.[3] The adjuvant ability of α-GalCer has been shown for both inactivated and replicating recombinant vaccines. An analogue of α-GalCer, α-*C*-GalCer, where the linking glycosidic oxygen is replaced with a CH_2 group (Figure 1.13), has been shown to act as an adjuvant for a live attenuated influenza vaccine in mice. α-*C*-GalCer also stimulates iNKT cells, increasing the immune response to the vaccine and allowing a reduced amount of virus to be used.[83]

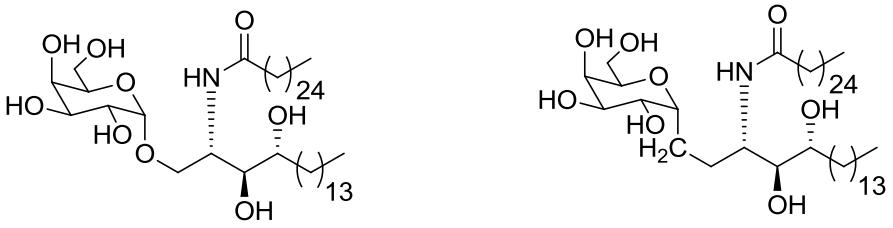


Figure 1.13. Showing the structural difference between α-GalCer and α-*C*-GalCer.

α-GalCer has also been shown to have therapeutic effects against certain autoimmune diseases including type I diabetes, experimental allergic encephalomyelitis, arthritis and systemic lupus erythematosus.[66] This could be because in autoimmune diseases there appears to be either an iNKT cell deficiency or dysfunction.[1] Type I diabetes is caused by host destruction of pancreatic β cells. It can be modelled in non-obese diabetic (NOD) mice, where it was found that they have fewer iNKT cells than normal, and that the cells present are unable to secrete IL-4 immediately after activation.[1,66] Chronic treatment with α-GalCer before the onset of insulitis has been shown to prevent diabetes from developing.[66,84] This is thought to be due to a bias towards a T_H2 response, and the emergence of tolerogenic DC, with a reduced ability to produce IL-12.

The deviation to a T_H2 response is known to protect against T_H1 dominated autoimmunity[85] and is thought to be due to a number of mechanisms. Secretion of IL-4 after administration of α-GalCer is very rapid; however in chronic α-GalCer treatment, IL-4 is continuously secreted at a significant level; IL-4 is known to promote T_H2 responses. This chronic secretion of IL-4 could also cause apoptosis of self-reacting T_H1 cells.[28] IL-13 and IL-10 are also known to

promote T_H2 responses and suppress T_H1 responses, respectively, and are also produced by α-GalCer-activated iNKT cells.[86] α-GalCer also leads to the emergence of tolerogenic DC.

However treatment with α-GalCer has also exacerbated autoimmune diseases;[66,67] this is usually attributed to deviation to a T_H1 response.

iNKT cells are important for tumour immunity. There have been reports of iNKT cell deficiency or dysfunction in certain types of human cancer.[67] α-GalCer can promote iNKT cell-mediated rejection of tumour cells by increasing production of IFN-γ; IFN-γ has been shown to have anti-angiogenic properties.[87] Activated iNKT cells produce large amounts of cytokines which result in the recruitment of DC and macrophages, which in turn secrete IL-12. This cytokine activates NK cells and T cells to produce IFN-γ, which activates CD8+ T cells and hence enhances cytotoxic T cell (CTL) activity, therefore promoting apoptosis of tumour cells.[1,67]

However there are a number of obstacles with using α-GalCer as a therapeutic agent:

1. α-GalCer over-stimulates iNKT cells. This results in cytokine storm and DC lysis. Over-stimulation also causes iNKT cell anergy and unresponsiveness to further stimulations.

2. α-GalCer possesses glycosidic and amide bonds, which can potentially be hydrolysed *in vivo* by glycosidases and amidases, respectively.

3. α-GalCer is a complex structure, making selective synthesis, especially of the α-glycosidic linkage, time-consuming.

4. α-GalCer causes iNKT cells to produce both T_H1 and T_H2 cytokines together, with a preference for neither. There is no bias of the immune response to either T_H1 or T_H2.

1.9 The Crystal Structures of the α-GalCer-CD1d Complex and the CD1d-α-GalCer-TCR Ternary Complex

The crystal structures of the CD1d-α-GalCer complex and the CD1d-α-GalCer-iNKT cell TCR ternary complex have been determined[61,88] and show the binding interactions between these molecules.

The α-GalCer-CD1d complex with human CD1d (hCD1d) has been determined (Figure 1.14) and indicates the various stabilising H-bonds between the glycolipid and CD1d.[88]

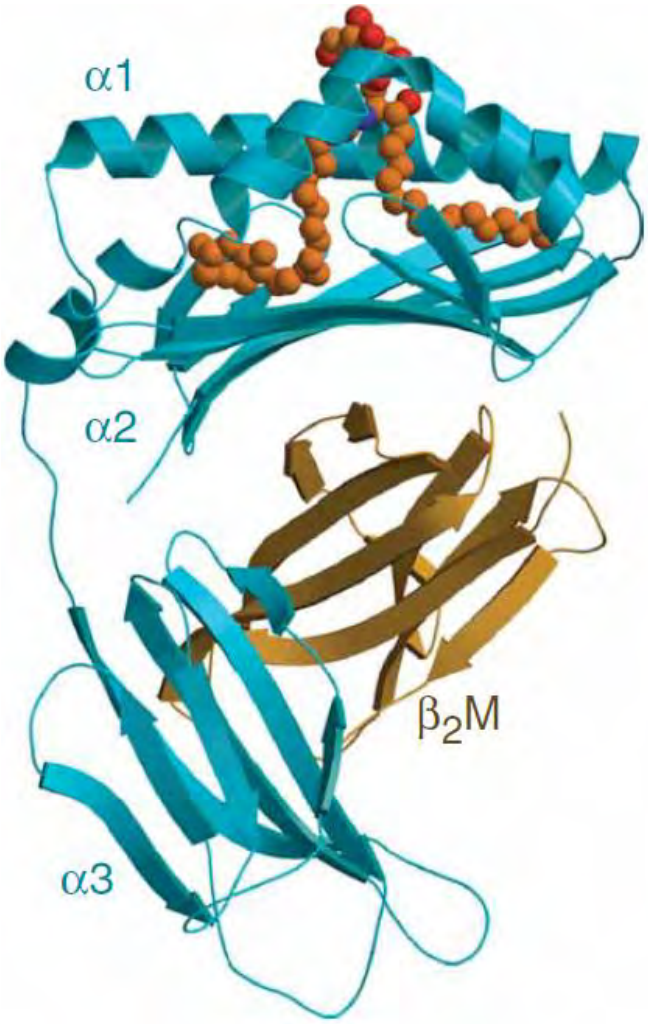


Figure 1.14. Ribbon representation of the CD1d-α-GalCer complex. Figure adapted from ref.[88] RightsLink® licence number 2992040354193.

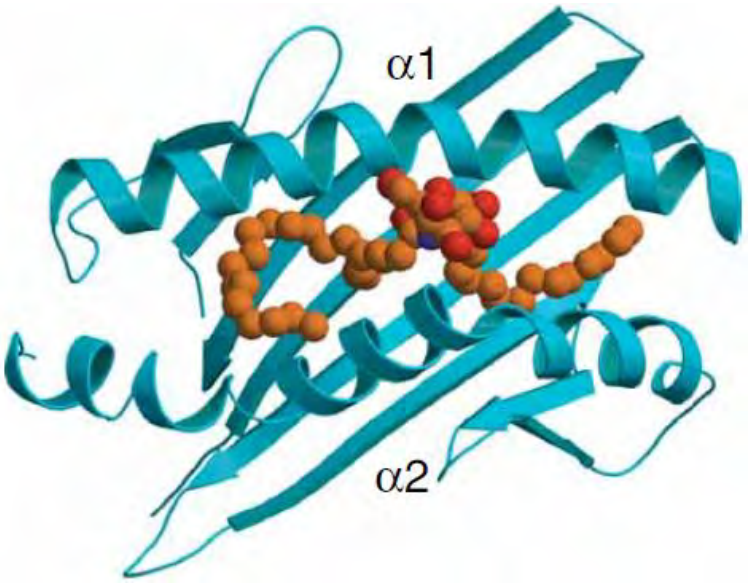


Figure 1.15. Ribbon representation of the CD1d-α-GalCer complex showing the binding groove from above. The anticlockwise curve around the A' pole is clearly seen. Figure adapted from ref.[88] RightsLink® licence number 2992040354193.

CD1d is a dimeric protein consisting of a heavy chain containing three domains, α1, α2 and α3, which are non-covalently associated with β_2m. The α1 and α2 domains make up the antigen binding groove, which consists of two anti-parallel α-helices sitting on top of a β-pleated sheet. The groove separates out into two channels, the A' and F' pockets, which are lined with hydrophobic amino acids. The acyl chain of α-GalCer occupies the A' pocket, adopting the curve of the pocket around the A' pole in an anticlockwise fashion (Figure 1.15).[88] The phytosphingosine chain occupies the straighter and less voluminous F' pocket. Both chains terminate at the end of their respective pockets, fully occupying the pocket and so are indicative of the maximum chain length which can be tolerated – 26 carbons in the A' pocket and 18 carbons in the F' pocket. As α-GalCer has this maximum number of atoms the number of hydrophobic interactions between the glycolipid antigen and CD1d is maximised; this explains why α-GalCer has such a high affinity (K_d = 1.29 ± 0.08 μM)[81] for CD1d. Longer lipid

chains would not fit correctly into the pockets and so would cause the head group to protrude out of CD1d more than it should, disrupting stabilising interactions at the branch point of the two alkyl chains and the galactose head group and hence also disrupting recognition by the iNKT cell TCR. Shorter lipid chains would not fully occupy the pockets, minimising the hydrophobic interactions and causing an increased rate of dissociation of the glycolipid from the CD1d molecule. The glycolipid is anchored into CD1d via the hydrophobic interactions between the lipid chains and the hydrophobic amino acids lining the binding pockets. This leaves the sugar head group exposed on the surface of CD1d for recognition by the iNKT cell TCR. Analysis of the crystal structure of the α-GalCer-CD1d complex[88] reveals several hydrogen bonds at the branch point, which not only stabilise the glycolipid-CD1d complex further, but also orient the head group into the correct position for iNKT-cell recognition.

The 2'-OH of the galactose head group forms a hydrogen bond to Asp151, on the α2-helix of the CD1d protein. The glycosidic linkage 1'-O is hydrogen bonded to Thr154, which is also on the α2-helix of the CD1d protein. The 3-OH on the sphingosine chain forms a hydrogen bond with Asp80. This crystal structure was of the human CD1d molecule, however the mouse CD1d is very similar and the residues are conserved for all three of these hydrogen bonds. So in mice the 2'-OH is hydrogen bonded to Asp153, the 1'-O to Thr156 and the 3-OH to Asp80. These hydrogen bonds help to orientate the sugar head group so that it is parallel to the plane of the α-helices, which is necessary for recognition by the iNKT cell TCR. A more recent, higher resolution, crystal structure of the α-GalCer-CD1d complex[89] revealed that the NH of the amide bond also forms a hydrogen bond to Thr154. The carbonyl group of the amide bond does not form a direct hydrogen bond with CD1d, however it does hydrogen bond with a water molecule, which in turn forms a hydrogen bond with the backbone carbonyl of Ile69, in the α1-helix of human CD1d (Table 1.2, Figure 1.16).[90]

Position on α-GalCer	Human CD1d	Mouse CD1d
1'-O	Thr154	Thr156
2'-OH	Asp151	Asp153
3-OH	Asp80	Asp80
NH	Thr154	Thr156
C=O	Ile69*	Met69*

Table 1.2. Showing the hydrogen bonds between specific positions on α-GalCer and amino acids on CD1d. These amino acid residues have been conserved between species, indicating the importance of these hydrogen bonds in orientating and positioning the glycolipid correctly for recognition. *This hydrogen bond involves a bridging H_2O molecule.

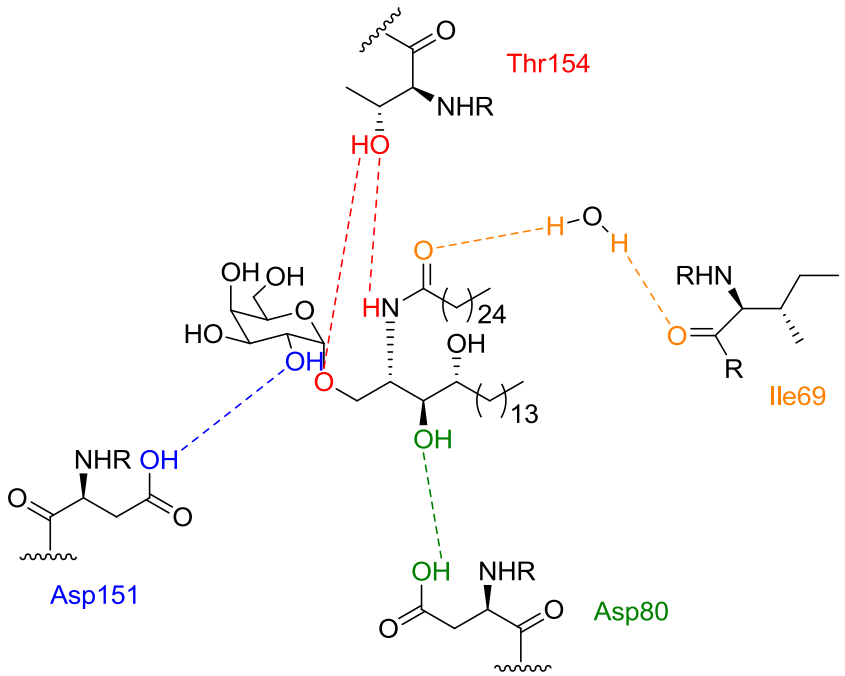


Figure 1.16. Schematic showing the H-bonds between α-GalCer and hCD1d

The crystal structure of human CD1d without any ligand bound has also been determined.[88] The structure is more like that of the MHC class I molecule, in that it has a wider binding groove than CD1d with α-GalCer bound. This indicates that the empty CD1d adopts a more "open" conformation which could allow easier access to the binding groove for lipid loading, before "closing" the binding groove after binding to restrict further lipid exchange.

The co-crystal structure of the CD1d-α-GalCer complex with the human iNKT cell TCR has also been determined (Figure 1.17).[61] From this we can tell that the iNKT cell TCR docks almost parallel to the antigen binding groove, directly over the F' pocket and at one extreme end of CD1d. The human iNKT cell TCR has an invariant Vα24-Jα18 α-chain combined with a Vβ11-containing β-chain, with the α-chain contributing many more contacts with the CD1d-α-GalCer complex than the β-chain (approximately 82 compared to 32).[61] There is a very small iNKT cell TCR-CD1d-α-GalCer interface, with the total buried surface area (BSA) being only around 910 $Å^2$, again with the α-chain contributing more of the BSA than the β-chain (65.5% compared to 34.5%).

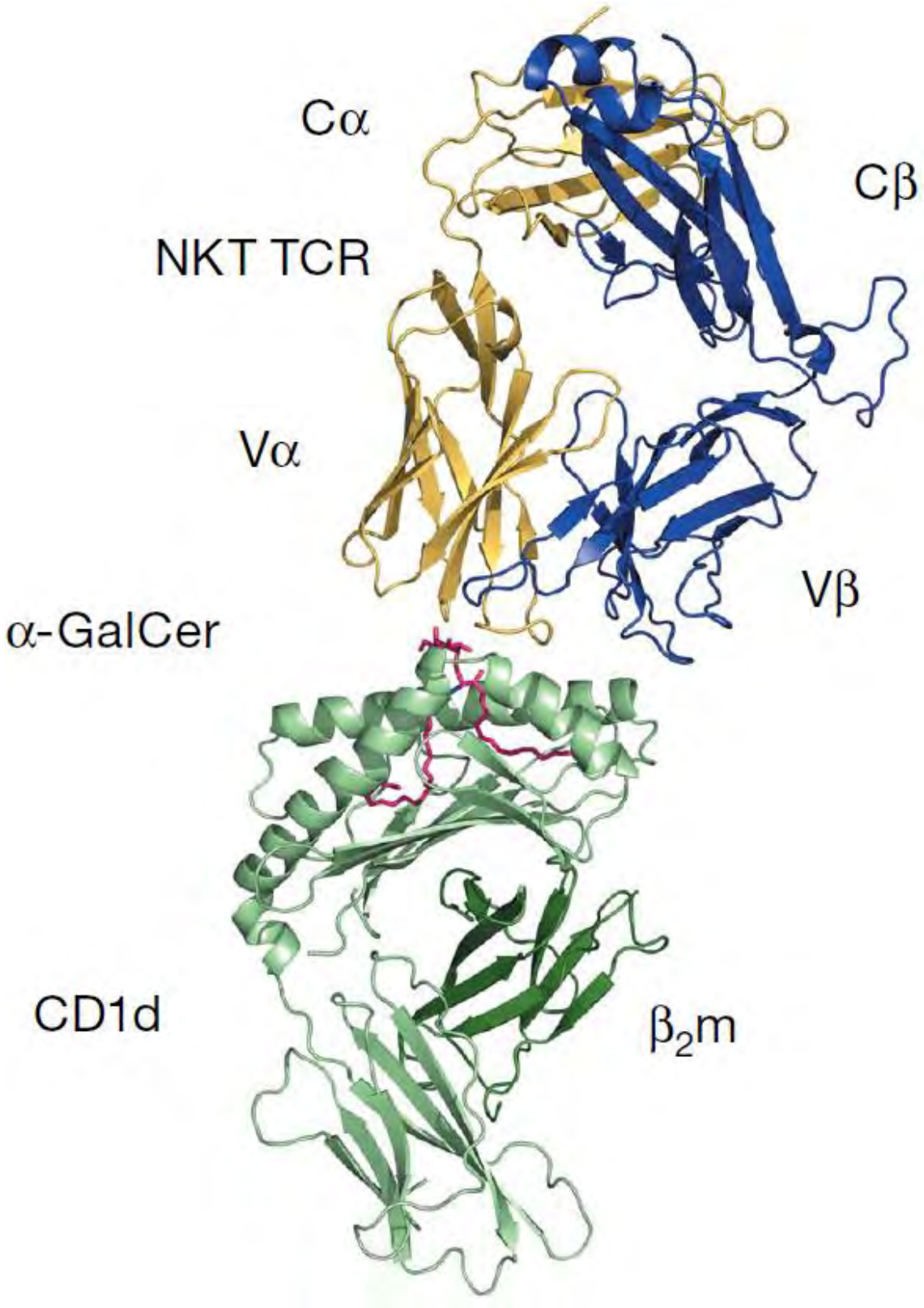


Figure 1.17. Ribbon representation of the human CD1d-α-GalCer-human iNKT cell TCR ternary complex. Figure adapted from ref.[61] RightsLink® licence number 2992040088535.

Of the β-chain the main contact is from the CDR2β loop to the α1-helix of CD1d. Tyr48β and Tyr50β form three hydrogen bonds with Glu83; Tyr48β also forms a hydrogen bond to Lys86, which forms a salt bridge with Glu56β. Arg89 forms van der Waals interactions with Asn53β, located at the tip of the CDR2β loop.

For the α-chain, contacts are with the CDR1α and CDR3α loops. The CDR3α interacts with the α1 and α2-helices, as well as with α-GalCer, whereas the CDR1α loop interacts only with α-GalCer. The galactose ring is positioned underneath the CDR1α loop and next to the CDR3α loop. Numerous hydrogen bonds stabilise this TCR-α-GalCer interaction:[61] the 3-hydroxyl of the sphingosine chain hydrogen bonds to the side-chain of Arg95α. The galactose 2' and 4'-hydroxyl groups hydrogen bond with the main chain of Gly96α and Phe29α, respectively, and the 3'-hydroxyl forms a hydrogen bond to the side-chain hydroxyl residue of Ser30α (Table 1.3, Figure 1.18).

Position on α-GalCer	Human iNKT cell TCR
2'-OH	Gly96α
3'-OH	Ser30α
4'-OH	Phe29α
3-OH	Arg 95α

Table 1.3. Showing the hydrogen bonds between specific positions on α-GalCer and amino acids on the iNKT cell TCR.

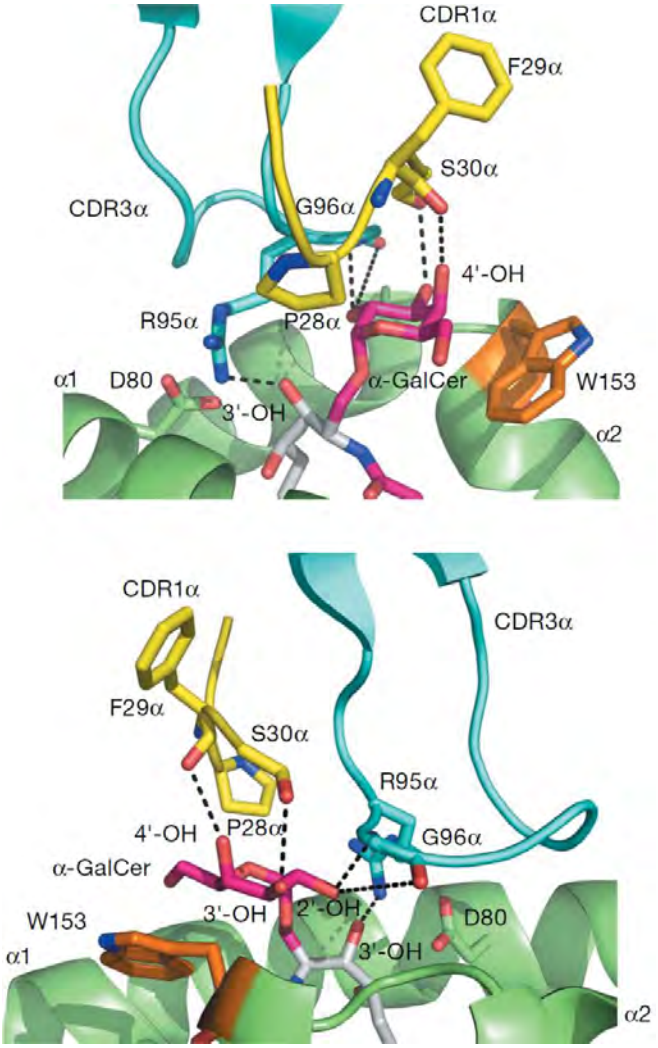


Figure 1.18. Diagram showing the hydrogen bonds between α-GalCer and the human iNKT cell TCR. Figure adapted from ref.[61] RightsLink® licence number 2992040088535.

These hydrogen bonds appear to be important for recognition by the TCR; glycolipids which cannot form these hydrogen bonds have less or no biological activity. α-Mannosyl ceramide does not activate iNKT cells,[91] which is probably due to the loss of two hydrogen bonds, as in mannose the 2'- and 4'-hydroxyls are in the opposite orientation compared to galactose, and so would not be in the correct position to hydrogen bond.

The α-linkage is also important for optimal activity; β-linked glycolipids have weaker activity than their α-linked counterparts, for instance β-GalCer is much less potent an agonist than α-GalCer.[92] This is probably due to the altered orientation of the head group; β-linked glycolipids are predicted to adopt a more perpendicular orientation which would disrupt contacts with the iNKT cell TCR CDR1α loop.[61]

1.10 Analogues of α-GalCer

There has been particular interest in α-GalCer due to its significant immunomodulating properties; however there are a number of problems which limit its therapeutic potential: α-GalCer over-stimulates iNKT cells resulting in cytokine storm, DC lysis and iNKT cell anergy. α-GalCer also possesses glycosidic and amide bonds, which can potentially be hydrolysed *in vivo* by glycosidases and amidases, respectively. The structure of α-GalCer is complex, making selective synthesis, especially of the α-glycosidic linkage, time-consuming. Also α-GalCer causes iNKT cells to produce both T_H1 and T_H2 cytokines together, with a preference for neither, so there is no bias of the immune response to either T_H1 or T_H2.

Analogues of α-GalCer with structural modifications have been synthesised to overcome some of these problems. These have also allowed us to learn more about how the structural features of the glycolipid can affect iNKT cell activation, whether there are any structure activity relationships (SAR) and also whether we can design analogues to contain certain structural features to give a certain immune response.

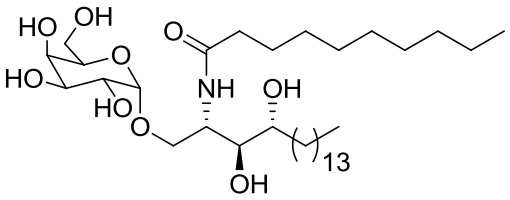
1.10.1 Analogues with Modifications to the C_{26} Acyl Chain

There have been numerous analogues with modified acyl chains, with the length of the chain and the degree of unsaturation being the main focus. Analogues with shorter acyl chains appear to skew the response towards T_H2 cytokines, and weaken the activation of iNKT cells, as seen in the analogue α-GalCer C10:0 (Figure 1.19).[93] One reason for this biasing effect could be due to the formation of a less stable glycolipid-CD1d complex. There will be fewer hydrophobic interactions between the shorter lipid chain and the antigen binding groove, and so dissociation will be faster. This will have less of an affect on IL-4 release, which is induced after

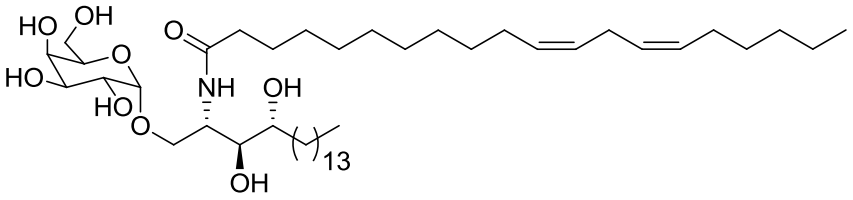
only 2 h, but might have more impact on the amount of IFN-γ released, as this requires a longer stimulation time by the glycolipid-CD1d complex. Most of the IFN-γ is secreted by natural killer (NK) cells, which are transactivated by iNKT cells. This also takes time, further supporting the notion that sustained iNKT cell stimulation is required for IFN-γ release.

The analogue α-GalCer C20:2 (Figure 1.19), which has an unsaturated acyl chain containing two *cis* double bonds at carbons 11 and 14, also skews the response towards T_H2, with a diminished IFN-γ production.[58] This analogue does not require trafficking to endosomal compartments for loading onto CD1d; it can load directly on to CD1d molecules which are non-raft-associated on the cell surface.[94] This rapid loading at the cell surface could be one reason why this analogue skews the response towards IL-4 release; the glycolipid can be immediately recognised and initiate activation of iNKT cells as soon as the glycolipid-CD1d complex is formed. Also the CD1d molecules are non-raft-associated, suggesting that the site of antigen loading might be important in determining the cytokine profile; in the raft are many other molecules which can interact with the iNKT cell as it binds to the glycolipid-CD1d complex, which might affect its response. It has been shown that localisation of MHC class II molecules into rafts can affect the polarisation of cytokine production by CD4+ T cells.[95]

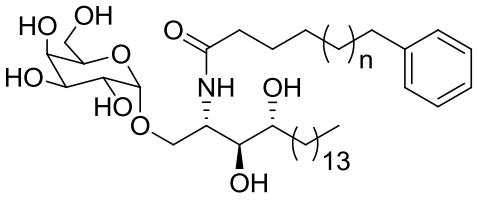
Other analogues have acyl chains with aromatic groups at the terminus (Figure 1.19).[96] These analogues skewed the response towards T_H1, resulting in more IFN-γ secretion. Fujio *et al.* postulated that this was due to increased stability of the glycolipid-CD1d complex due to extra aromatic interactions between the terminal phenyl group and Tyr73 or Trp40 in the A' pocket.



α-GalCer C10:0



α-GalCer C20:2



n = 0,3,6

aromatic analogues

Figure 1.19. α-GalCer analogues with modifications to the acyl chain.

1.10.2 Analogues with Modifications to the Sphingosine Chain

Truncation of the sphingosine chain has also been explored; OCH (Figure 1.20) is an analogue with a nine-carbon phytosphingosine chain, rather than the normal 18-carbon chain, and has also been shown to bias the response towards T_H2.[97] The acyl chain has also been truncated by two carbons, containing only 24 carbons. OCH is a weaker agonist than α-GalCer but it is T_H2 biasing. The reason for this has again been suggested to be because the OCH-CD1d complex is less stable, resulting in faster dissociation and hence a T_H2 cytokine-biased response. However it has also been noted that OCH is unable to transactivate NK cells, which are the source of most of the IFN-γ.

The functional groups of the phytosphingosine chain have also been modified to test for SAR. Analogues with both the 3-OH and the 4-OH removed did not exhibit any biological activity, whereas if only the 4-OH was removed, the analogue could initiate a strong biological response in mice, similar to that of α-GalCer (Figure 1.20).[98] The results for human iNKT cells have been more controversial, with some saying that the 4-deoxy analogue cannot activate human iNKT cells,[98] and some saying that it can.[99] There have also been analogues which have replaced the 4-OH with a *gem*-difluoro group, with retention of human iNKT cell activity, supporting the notion that the 4-OH is not required for recognition and activity. These results make sense when one looks at the crystal structure of the α-GalCer-CD1d complex; the 3-OH forms a hydrogen bond to Asp80 in both mouse and human CD1d, and also forms a hydrogen bond to Arg95α in the CDR3α loop of the TCR, whereas the 4-OH of α-GalCer does not make any hydrogen bonds with CD1d or the TCR, though in other glycolipids it has been shown to also hydrogen bond to Asp80.[100]

Analogues with an aromatic group attached to the end of a truncated phytosphingosine chain (Figure 1.20) have been shown to skew the immune response towards T_H1. This is a similar response to previous analogues where an aromatic group was attached to the end of the acyl chain, and again is postulated to be because of increased stability of the glycolipid-CD1d complex.[101]

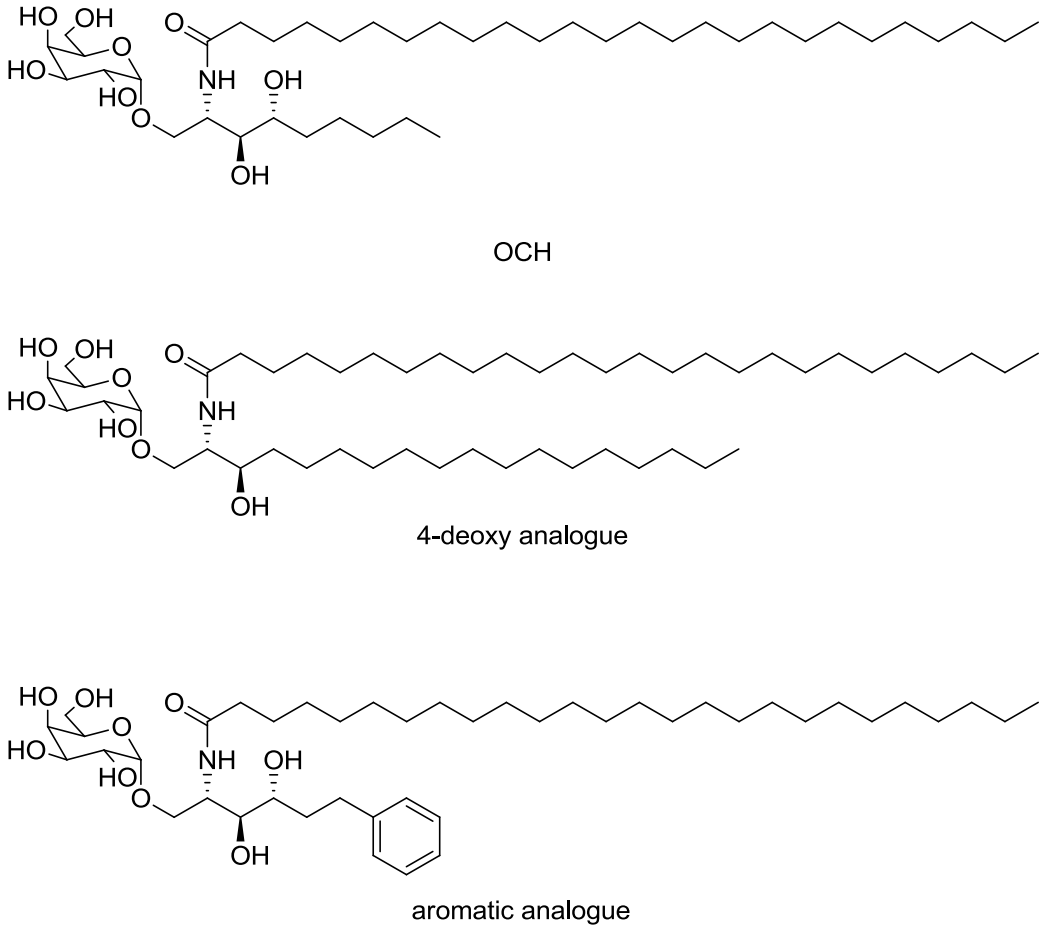


Figure 1.20. α-GalCer analogues with modifications to the phytosphingosine chain.

1.10.3 Analogues with Modifications to the Amide Bond

The NH of the amide bond of α-GalCer forms a hydrogen bond with the CD1d molecule; analogues have been synthesised to determine the role and importance of this hydrogen bond. An analogue with a *gem*-difluoro group at the α-position to the amide bond, which should increase the NH acidity and hence increase the strength of the hydrogen bond, was actually less potent than α-GalCer, indicating that this hydrogen bond does not contribute to the stability of the glycolipid-CD1d complex but rather is involved in orientation of the sugar head group (Figure 1.21).[102]

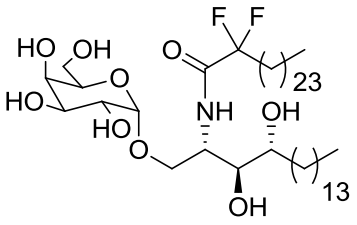
Analogues which replaced the amide bond with aliphatic and aromatic sulfonamides (Figure 1.21) resulted in a reduced but skewed biological response towards T_H2, however the reason for this is not clear. Possibly the bulky aromatic group destabilises the glycolipid-CD1d complex but this does not explain the result for the aliphatic sulfonamide analogues.[2]

The amide bond has also been replaced by a triazole group, an amide isostere which is hydrolytically more stable (Figure 1.21).[103] This group retains the ability to be a hydrogen bond acceptor, but cannot be a hydrogen bond donor. It also appears to mimic the atom arrangement of the amide group. These analogues also resulted in a T_H2 response, when the acyl chain was long; short and medium acyl chain lengths did not stimulate iNKT cells *in vivo*.

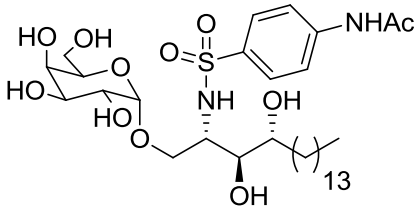
Preserving the hydrogen bond appears to be important in retaining the ability to stimulate iNKT cells. Analogues where the amide bond were replaced with an ether bond or an ester group have been synthesised (Figure 1.21). The ether analogue, which lacks both the NH and the carbonyl oxygen could not stimulate iNKT cells; the ester group analogue could, but only with

significantly reduced activity, highlighting the importance of the NH group and the hydrogen bond it forms with Thr154 on human CD1d.

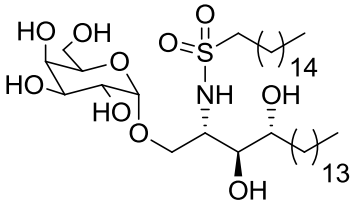
Recently there have been analogues which have replaced the amide bond with a thioamide or a carbamate (Figure 1.21). These retain the NH group but should be more hydrolytically stable *in vivo*. Both of these analogues cause a T_H1 bias in the immune response, however the reasons for this have not been investigated yet.



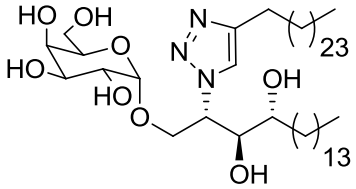
gem-difluoro analogue



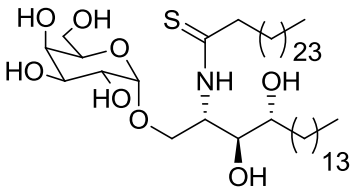
aromatic sulfonamide analogue



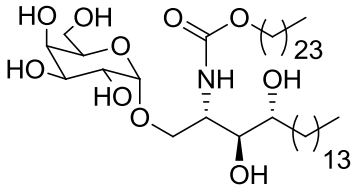
aliphatic sulfonamide analogue



triazole analogue



thioamide analogue



carbamate analogue

Figure 1.21. α-GalCer analogues with amide bond modifications.

1.10.4 Analogues with Modifications to the Glycosidic Bond

Since the *O*-glycosidic bond is hydrolytically unstable *in vivo*, replacement of the oxygen atom with a methylene would make the compound much more stable. α-*C*-GalCer (Figure 1.22)[104] exhibits a T_H1-biased immune response, and has more potent anti-malarial activity and anti-metastatic activity than does α-GalCer.[105] The replacement of the oxygen atom with a non-polar carbon atom removes the hydrogen bond to Thr154 on CD1d. This appears to affect the strength of binding of the glycolipid to CD1d; it has much weaker affinity to CD1d than α-GalCer and even OCH.[106] This weaker binding might alter the position of the glycolipid in the antigen binding groove, causing conformational alterations on the surface, and hence affecting iNKT cell TCR recognition. The higher levels of IFN-γ produced could also be due to the increased metabolic stability of the *C*-glycosidic linkage, resulting in longer stimulation times. However α-*C*-GalCer is only active in mice; it is unable to stimulate human iNKT cells significantly.[107]

(*E*)-Alkene-linked *C*-glycosides (Figure 1.22) are also potent iNKT cell agonists and also skew the response towards T_H1, giving greater levels of IL-12. It was suggested that the (*E*)-alkene linker may fix the orientation of the polar head group into one which is easily recognised by the iNKT cell TCR.[107] These analogues, in contrast to α-*C*-GalCer, can stimulate human iNKT cells, indicating that it is not the loss of the glycosidic hydrogen bond which caused α-*C*-GalCer to not stimulate human iNKT cells, but is probably due to the angle of the linking unit which alters the position of the galactose head group.

The glycosidic oxygen has also been replaced with a sulfur atom, giving a thioglycoside which is less susceptible to enzymatic hydrolysis than α-GalCer (Figure 1.22). Initial data suggested that

it was not active in mice *in vivo*;[108] however recent studies suggest that it is active towards human iNKT cells *in vitro*,[109] with a similar level of activity to that of α-GalCer.

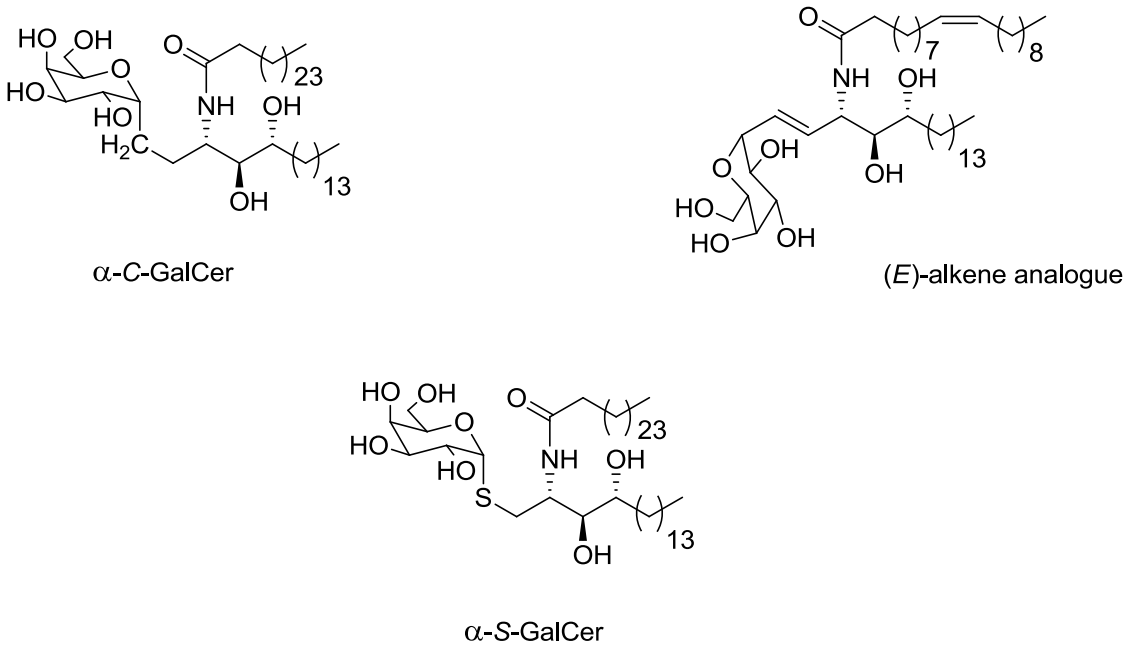


Figure 1.22. α-GalCer analogues with modifications to the glycosidic bond.

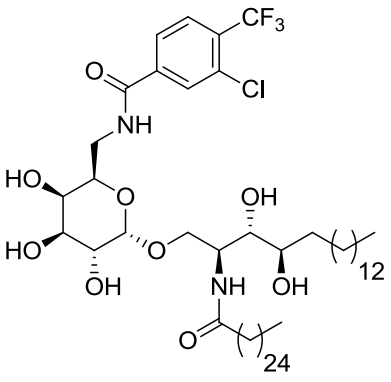
1.10.5 Analogues with modifications to the sugar head group

Modification to the sugar head group needs to be done with care, due to the number of hydrogen bonds it contributes towards stabilising the CD1d-glycolipid-TCR complex, and the fact that the orientation of the head group is vital in iNKT cell TCR recognition. The 6-position however does not appear to be involved in any hydrogen bonding, and is located in a large open pocket, and so modification at this position should be well tolerated. A disaccharide

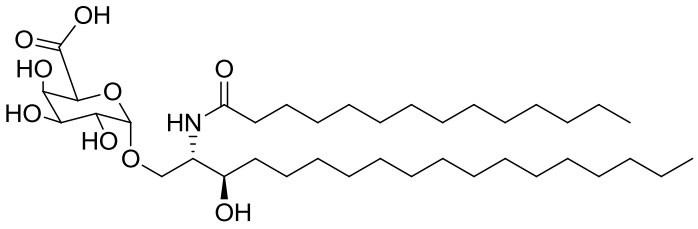
analogue with a galactose linked to the 6-position of α-GalCer has been shown to be an effective antigen, with no need for processing to remove the additional sugar unit,[110] demonstrating the versatility of the 6-position.

Modification of the 6-position does not appear to affect TCR recognition, and has been used extensively for attaching labels. However it has been shown that modification can also alter the cytokine profile; recent analogues have tried to introduce extra interactions between this position and the CD1d molecule, by the addition of aromatic groups (Figure 1.23).[111] The human CD1d molecule has a Trp153 residue in close proximity to the 6-position, which could allow additional π-π interactions with aromatic groups. These analogues cause the secretion of similar levels of IFN-γ, but very little IL-4 compared to α-GalCer, resulting in a T_H1 response, which could be explained by enhanced binding due to π-π interactions.

The flexibility of the 6-position is further corroborated by the fact that analogues with α-linked glucuronic acid or galacturonic residues, where the 6-OH has been oxidised into a carboxylic acid (Figure 1.23), stimulate iNKT cells in a similar fashion to α-GalCer.[112,113]



aromatic analogue



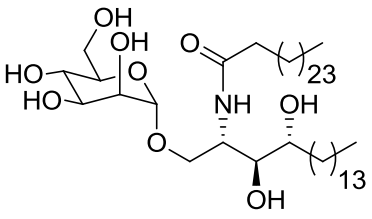
α-galacturonosyl ceramide

Figure 1.23. Structure of α-GalCer analogues with modification to the 6-position

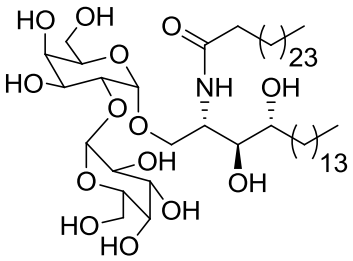
The 2, 3 and 4-alcohol residues in α-GalCer are involved in hydrogen bonding with the iNKT cell TCR, with the 2-OH also being involved with hydrogen bonding to the CD1d molecule. The equatorial orientation of the 2-position is vital for the antigenicity of the glycolipid; α-mannosyl ceramide, which has an axial 2-OH (Figure 1.24), does not stimulate iNKT cells. Not only does this axial orientation remove the hydrogen bond, but it could also clash with the TCR, as it will point out perpendicularly to the binding surface. An α-GalCer analogue which has been 2-*O*-methylated, and therefore is unable to function as a hydrogen bond donor at that position, exhibited significantly reduced activity, suggesting that this 2-hydroxyl also needs to be free for optimal activity. An analogue which does have modification on the 2-position and is still moderately active is the Gal-α-(1→2)GalCer analogue (Figure 1.24), where there is another galactose group linked to α-GalCer through the 2-position. However it has been shown that this extra galactose is actually removed during processing of the glycolipid to give antigenic α-GalCer, which is presented to iNKT cells.[110]

The 3- and 4-positions are more amenable to modifications, possibly as they only contribute one hydrogen bond each, whereas the 2-position has two. The 3-*O*-sulfate analogue of α-GalCer (Figure 1.24) can efficiently stimulate iNKT cells, with activity comparable to that of α-GalCer.[114] Glycosylation at the 3-position caused a dramatic reduction of activity, although this sugar again is cleaved before presentation to iNKT cells.[110] These analogues indicate that the 3-position is less sensitive to modifications than the 2-position. α-Glucosyl ceramide, which has an equatorial 4-hydroxyl rather than the axial one seen in α-GalCer (Figure 1.24), has slightly reduced activity, demonstrating that changing the configuration of the 4-position can be tolerated.

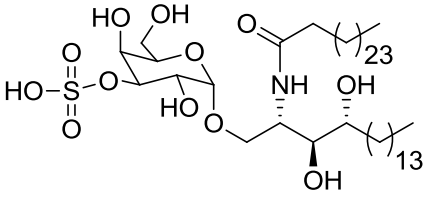
All these analogues demonstrate that these different hydrogen bonds do not make equal contributions to the efficacy of α-GalCer as a CD1d agonist.



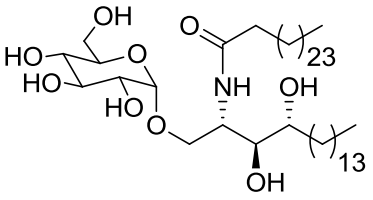
α-mannosyl ceramide



Gal-α-(1-2)GalCer



3-*O* sulfate α-GalCer



α-glucosyl ceramide

Figure 1.24. α-GalCer analogues with modifications to the 2-, 3- and 4-positions of the sugar residue.

1.10.6 Non-glycosidic Analogues

Analogues which have removed the labile glycosidic bond should be more stable *in vivo* and so might produce a more biased biological response. Tashiro *et al.* synthesised a carbocyclic analogue, where the ring oxygen was removed and so the glycosidic bond was replaced with an ether linkage (Figure 1.25). This analogue did indeed show a T_H1 bias, with an increase in the amount of IFN-γ released and a reduction in the amount of IL-4 produced, compared to α-

GalCer.[115] The group reasoned that the enhanced *in vivo* stability could allow for longer stimulation times, and hence more IFN-γ production. Also the CDR1 loop of the TCRα chain has a Pro28 in the proximity of the ring oxygen; with α-GalCer this causes repulsion between the polar oxygen and the non-polar Pro28. However this carbocyclic analogue has replaced the ring oxygen with a methylene group, which in contrast to α-GalCer will allow additional hydrophobic interactions. This extra binding will increase the stability of the glycolipid-CD1d-TCR complex, and could be another reason as to why the response is T_H1-biased.[116]

Threitol ceramide (ThrCer) is a truncated non-glycosidic analogue of α-GalCer, where the 5- and 6-positions of the galactose head group have been excised, leaving an acyclic sugar, threitol, which retains the absolute and relative stereochemistry of D-galactose (Figure 1.25). The glycosidic bond has again been replaced by a metabolically more stable ether linkage. However even with the removal of these functional groups ThrCer is still able to activate iNKT cells, albeit with reduced activity compared to α-GalCer. Furthermore, it does not cause the lysis of dendritic cells, unlike α-GalCer, but is still able to mature DC and cause proliferation of antigen-specific T and B cells, which greatly enhances its therapeutic potential.[117] Activation-induced anergy is also reduced after stimulation with ThrCer, compared to α-GalCer.[118] The formation of four hydrogen bonds with the 2-, 3- and 4-OH of ThrCer appears to be enough to stabilise the glycolipid-CD1d complex and allow recognition by the iNKT cell.

Looking at previous analogues we know that these hydrogen bonds are not equal in importance. Glycerol ceramide (GlyCer), where the threitol head group is further truncated to a glycerol unit (Figure 1.25), has been shown to activate human iNKT cells, but not murine iNKT cells.[118] Murine iNKT cells appear to require the hydrogen bond from the 4-OH, whereas human iNKT cell activation can occur with only three hydrogen bonds.

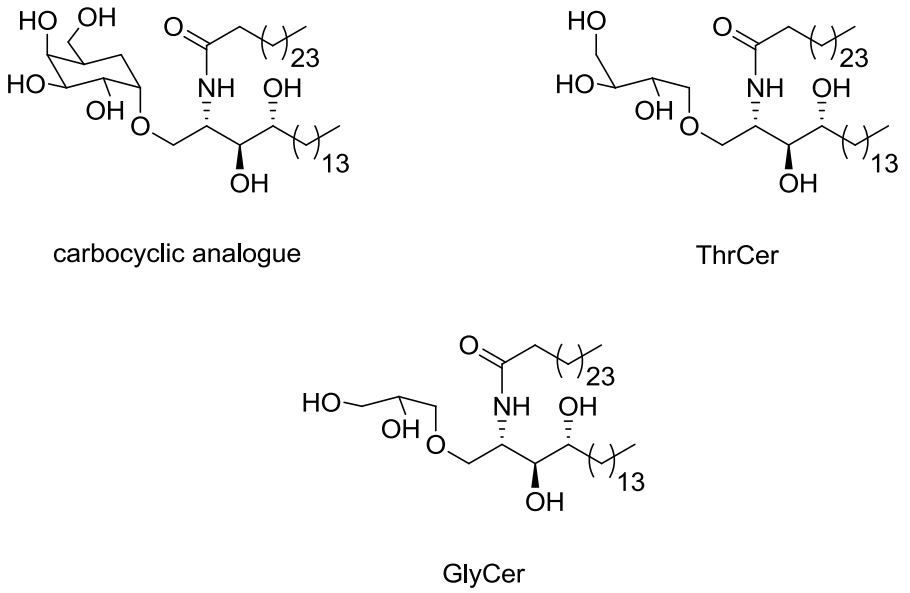


Figure 1.25. Non-glycosidic analogues of α-GalCer.

1.11 Aims and Objectives

The non-glycosidic analogues of α-GalCer are interesting CD1d agonists, being metabolically more stable yet still activating iNKT cells to produce an immune response. ThrCer also reduces the problem of DC lysis and anergy, which is seen in α-GalCer stimulation. Assuming that ThrCer adopts the same conformation as α-GalCer for recognition, it should form four hydrogen bonds with CD1d / iNKT cell TCR, however the relative importance of these hydrogen bonds is unknown; we know that the hydrogen bond from the 4-OH is not essential for activation, at least in human systems, as evidenced by the activity of GlyCer, which does not contain the 4-position. To determine the relative importance of the four hydrogen bonds, we proposed to synthesise ThrCer analogues which lack the ability to form all four hydrogen bonds; we will systematically remove the hydroxyl groups of ThrCer. These analogues will then be tested for iNKT cell activation, allowing us to determine which positions are necessary for biological activity (Figure 1.26).

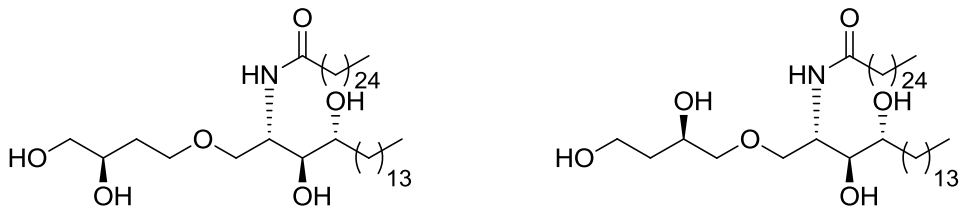


Figure 1.26. Examples of the deoxy target compounds

ThrCer has much weaker biological activity than α-GalCer. One reason for this weaker activity could be due to the conformational flexibility of the linear threitol head unit, allowing it to adopt many more conformations than the galactose head group in α-GalCer, some of which might not be recognised by the iNKT cell TCR. Therefore we proposed to synthesise analogues which constrain the head unit into a carbocyclic ring (Figure 1.27). This will reduce the conformational flexibility of the head unit, as carbocycles tend to have fewer and more defined conformations. It will also bring us closer to the structure of α-GalCer, which has a ring sugar as its head unit. These analogues will then be tested for iNKT cell activation, to see whether reducing the conformational flexibility restores biological activity to this type of CD1d agonist.

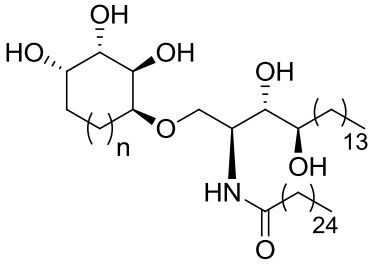


Figure 1.27. Structure of cyclic ThrCer analogues

The second part of this project will be to investigate Mincle activation with synthetic ligands. TDB was shown to activate Mincle, even though the lipid chains differ significantly from the natural TDM. We proposed to synthesise analogues of TDB with different length chains, to determine which length gives optimal activity (Figure 1.28).

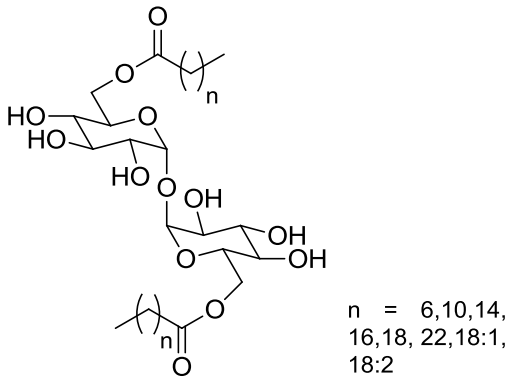


Figure 1.28. Structure of the TDB analogues

Chapter 2

Synthesis of Deoxy and Truncated ThrCer Analogues

2. Synthesis of Deoxy and Truncated ThrCer Analogues

2.1 Threitol Ceramide and our Target Compounds

Threitol ceramide is an attractive analogue of α-GalCer, addressing some of the problems associated with α-GalCer, like metabolic instability, DC lysis and iNKT cell anergy. The ThrCer-hCD1d complex has a weaker affinity for the human TCR than does the α-GalCer-hCD1d complex, having a K_d of 5.78 μM compared to 1.3 μM for α-GalCer.[118] This weaker affinity is due to a slower on rate of the TCR to the ThrCer-hCD1d complex and a faster off rate. However ThrCer still activates iNKT cells, inducing DC maturation and the secretion of cytokines. Although the overall activity is weaker than α-GalCer, ThrCer could potentially be a better therapeutic; ThrCer stimulation results in less DC lysis (50% DC survival compared to 10% for α-GalCer)[118] and a faster recovery from iNKT cell activation-induced anergy. However, like α-GalCer, it does not induce a T_H1 / T_H2 bias in the cytokine production.

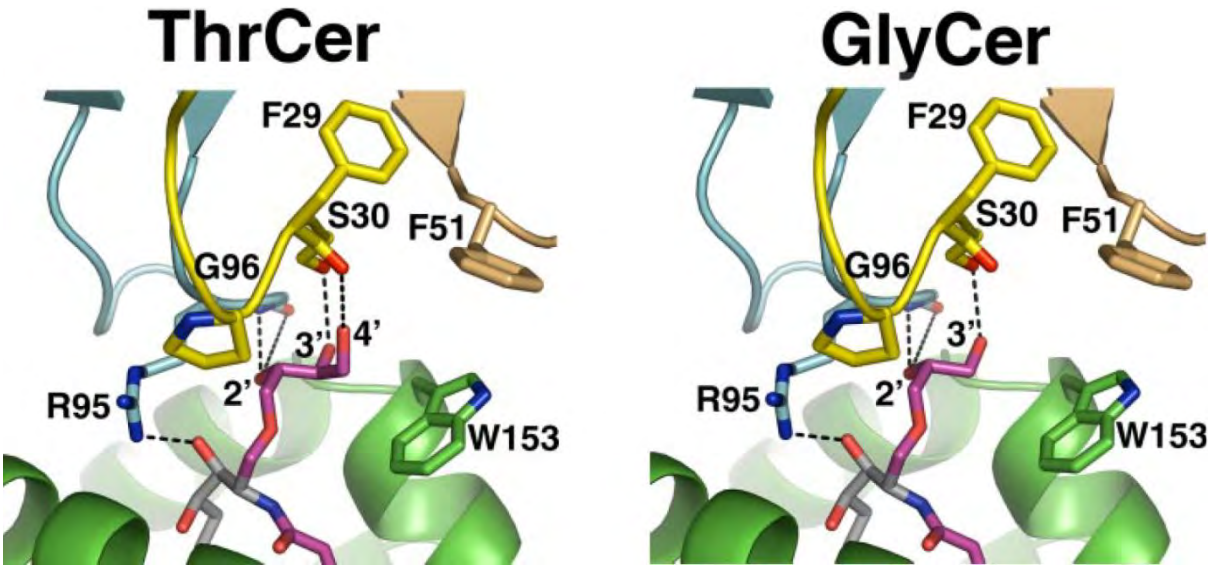


Figure 2.1. Diagram showing molecular modelling of the CD1d-glycolipid-TCR ternary complex, highlighting possible hydrogen bonds of ThrCer and GlyCer. Adapted from ref.[118]

The success of ThrCer shows that the whole galactose head group is not necessary for iNKT cell recognition and activation, however it is thought that even the four hydrogen bonds that should be retained with ThrCer might not all be necessary for orientation of the head group for TCR recognition. For example, the activity shown by GlyCer, a ThrCer analogue which has removed the fourth carbon of the head group and hence the hydrogen bond formed by the 4-OH, indicates that not all of the hydrogen bonds to the human iNKT cell are necessary, or, at least indicates that they are not of equal significance (Figure 2.1).[118]

With our target compounds, we proposed to systematically remove the hydroxyls of the sugar portion of ThrCer and then test these for biological activity to determine which hydroxyls / hydrogen bonds are important for iNKT cell activation (Figure 2.2).

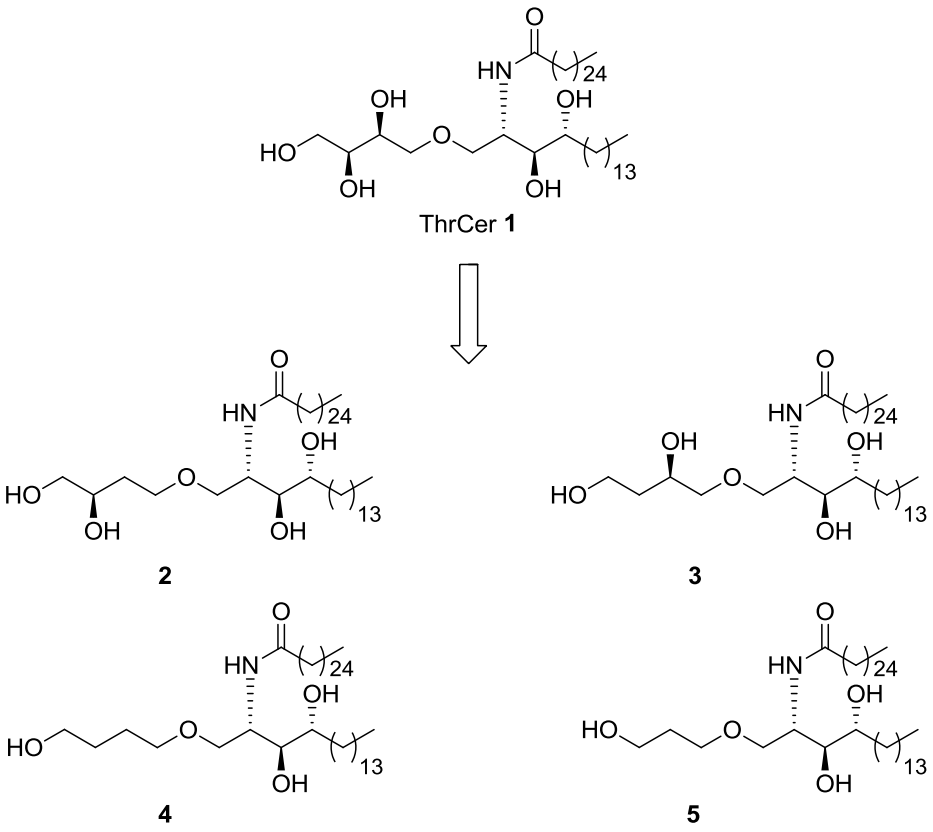


Figure 2.2. The structures of ThrCer and the analogues to be synthesised.

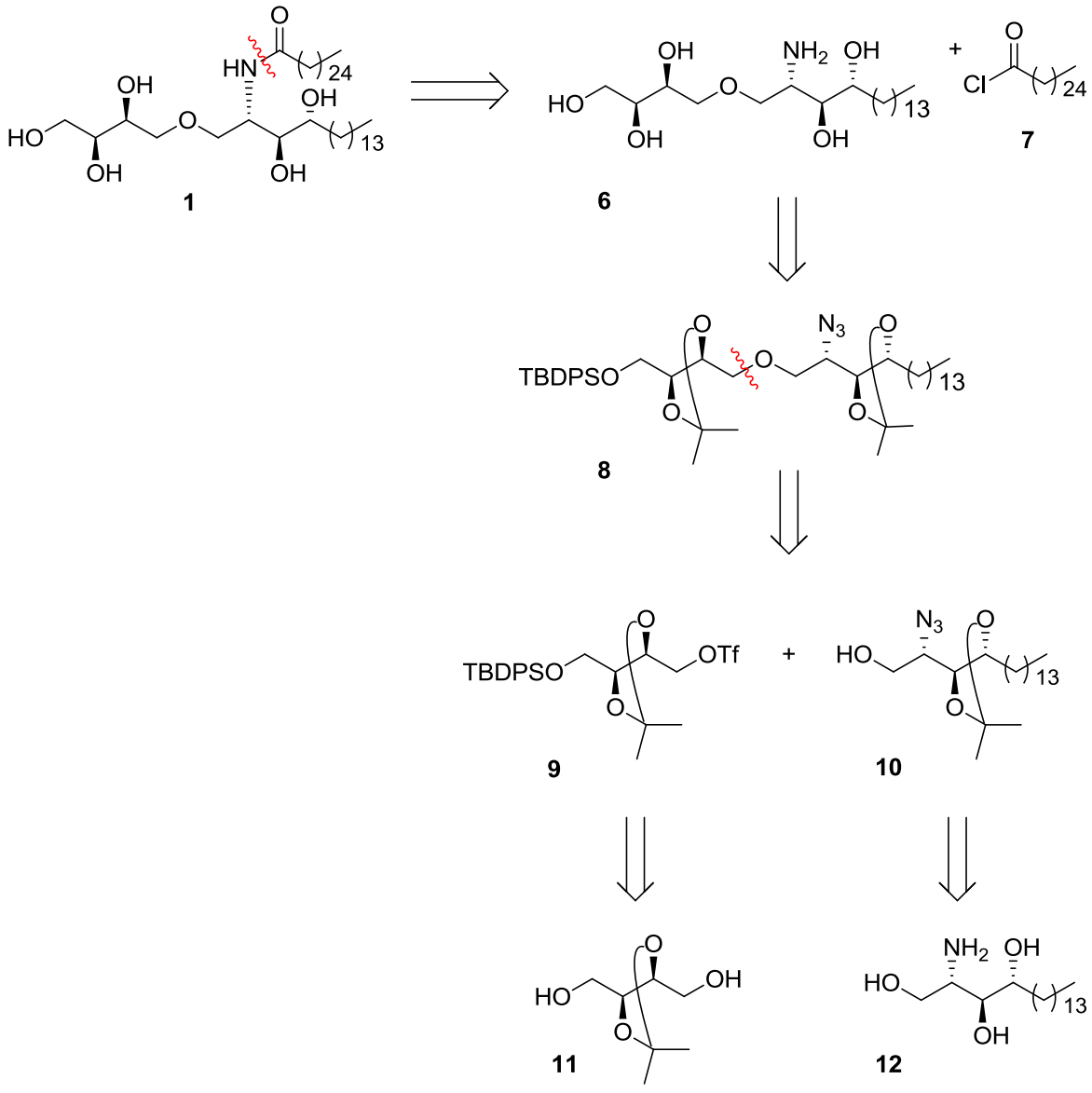
2.2 Synthesis of Threitol Ceramide

Threitol ceramide is our lead compound; the analogues we want to synthesise are based on its structure. Therefore it would be useful to synthesise ThrCer as it will be needed as a control against which our analogues will be compared in the biological assays. Also, since the synthetic pathway of ThrCer was similar to that we had planned for our deoxy and truncated analogues, synthesising ThrCer would allow us to see how these types of compounds react and also identify any possible problems.

There are two published synthetic routes of ThrCer, one by Reddy *et al.*[117] and the other by our research group.[119] However these two routes are slightly different from the one we chose to use.

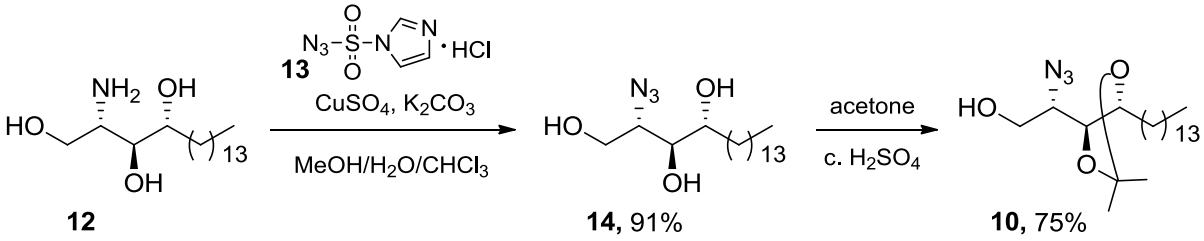
Our retrosynthetic analysis for synthesising ThrCer is shown in Scheme 2.1. The most important step is the coupling of the threitol electrophile **9** to the sphingosine nucleophile **10** via a Williamson etherification. This provides the azide **8**, which is similar to that employed in Reddy's strategy, except that the primary alcohol of the threitol unit was protected as a benzyl ether rather than the TBDPS group which we proposed to use. The TBDPS group was also used in our research group's previous synthesis, however the internal diol of the threitol unit was protected as a benzylidene acetal rather than an isopropylidene which we proposed to use. We preferred to use an isopropylidene as the benzylidene group would add an additional stereocentre which would make analysis of our intermediates more complex. In the forward synthesis, removal of the protecting groups and reduction of the azide provides the free amine, which is acylated with hexacosanoyl chloride to afford ThrCer **1**. We identified commercially available (+)-2,3-*O*-isopropylidene-L-threitol and phytosphingosine as the starting materials,

which should be easily converted into the threitol electrophile **9** and sphingosine nucleophile **10**, respectively.



Scheme 2.1. Retrosynthetic analysis of ThrCer **1**.

2.2.1 Synthesis of the Nucleophile



Scheme 2.2. Synthesis of azido alcohol **10**.

Azides are commonly used as an amine protecting group on phytosphingosine substrates. Previously in our group trifluoromethanesulfonyl azide (TfN_3) was used as the diazo donor, however there are a number of disadvantages to using this reagent: neat TfN_3 can be explosive and has a poor shelf-life, requiring preparation immediately before use.[120,121] In contrast, imidazole-1-sulfonyl azide hydrochloride[122] provides a much better alternative, being shelf-stable (as its crystalline hydrochloride salt) and less explosive. Another protecting group commonly used for amines is the carbamate, such as Boc or Cbz,[123] however carbamate protecting groups are easily deprotonated by strong bases, like NaH, which we will be using to form our alkoxide in the etherification step. Whilst the alkoxide we will form should be a better nucleophile than the carbamate anion, to prevent any interference from possible competing *N*-alkylation and elimination reactions, it was decided to protect the amine with an azide group. Also the azide can be deprotected selectively in the presence of the isopropylidene group we will be protecting the internal diol with. Commercially available phytosphingosine **12** was converted to azide **14** in a Cu(II)-catalysed diazo-transfer reaction,[122] with imidazole-1-sulfonyl azide hydrochloride **13** as the diazo donor. The internal 1,2-diol embedded in azide **14** was next selectively protected as an isopropylidene acetal using acetone

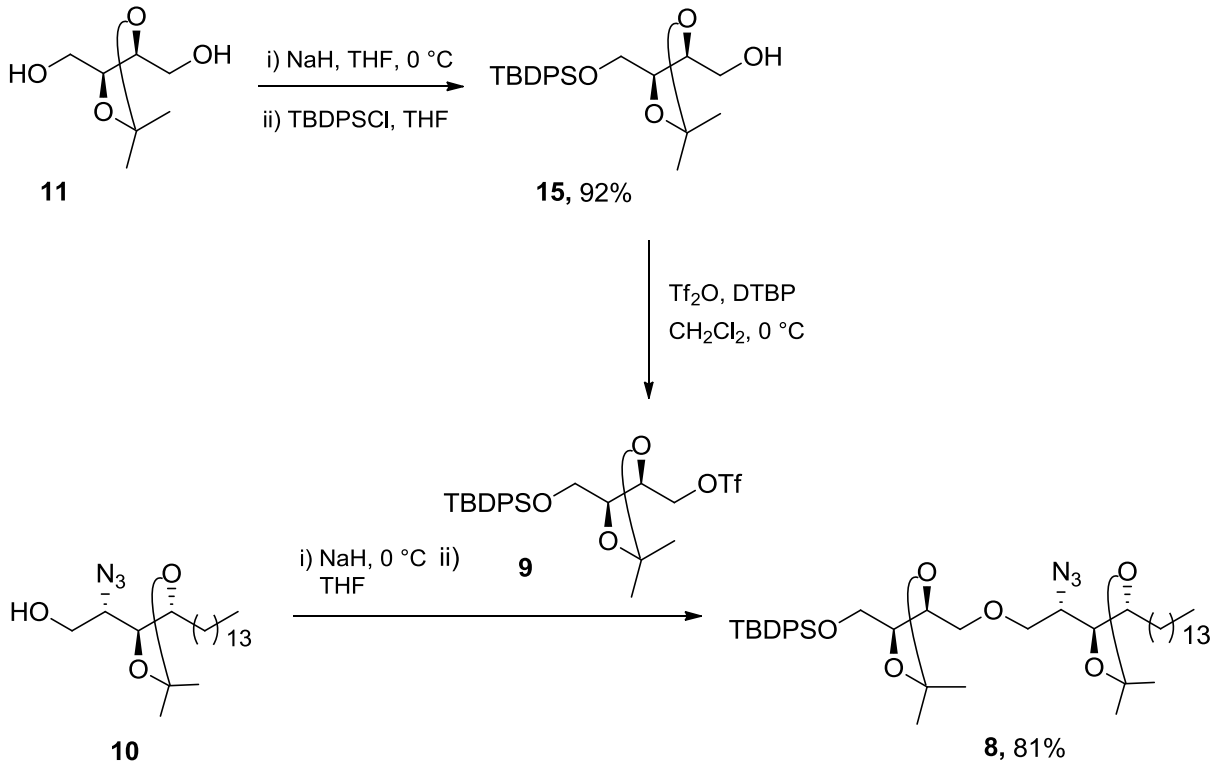
in the presence of a catalytic amount of concentrated H_2SO_4, to provide our target nucleophile **10** (Scheme 2.2). The 1,2-diol selectivity in this acetal protection step can be rationalised on thermodynamic grounds. Acetal formation is a reversible process, therefore the reaction is often selective for the thermodynamic product. As we are using acetone the thermodynamic product will be the 1,2-dioxolane, a five-membered ring in which the 1,2-diol is protected. If the 1,3-diol was protected, forming a 1,3-dioxane, a six-membered ring, there would be significant destabilising 1,3-diaxial interactions present. In contrast, had we chosen to employ benzylidene protection, the major thermodynamic product would be the 1,3-dioxane. This is because the phenyl acetal substituent can be placed equatorially in a six-membered ring, thereby minimising 1,3-diaxial interactions. Therefore benzylidene (and other aldehydes) are selective for 1,3-diols. As the isopropylidene acetal is formed on the internal 1,2-diol, the target protected nucleophile **10** can be produced in just two steps, without needing to worry about the primary hydroxyl interfering in these protection steps, leaving it free, with no other manipulation needed, to react further.

2.2.2 Synthesis of the Threitol Electrophile and Etherification

The threitol electrophile **9** was quickly and efficiently synthesised from commercially available (+)-2,3-*O*-isopropylidene-L-threitol **11**, which was first monoprotected with a bulky TBDPS silyl ether group to give alcohol **15**. We chose the TBDPS group as it is resistant to basic and nucleophilic conditions, which it will be subjected to in the subsequent etherification step. Less hindered silyl ethers are more susceptible to nucleophilic attack, and hence would be less stable in the presence of an alkoxide. The remaining alcohol was then converted into a triflate, a commonly employed leaving group (Scheme 2.3). Triflates are very good alkyl electrophiles, due to their three strongly electron-withdrawing fluoro substituents. The pK_a of triflic acid is

around −14; this extremely low pK_a indicates that the conjugate base does not need a counter ion but can exist separately in the solvent, which is reflected in its excellent leaving group ability. One disadvantage of triflates however is that their high reactivity can cause them to decompose rapidly before they react, usually leading to an elimination of the triflate, producing an alkene.

Diol **11** was treated with 1.2 equivalents of NaH, before the addition of 1.2 equivalents of TBDPSCl to provide, almost solely, the monoprotected alcohol **15**. Preparation of the triflate **9** involved reaction of the alcohol **15** with triflic anhydride in the presence of 2,6-di-*tert*-butylpyridine (DTBP), a hindered, non-nucleophilic base which was added as an acid scavenger, as Tf_2O is easily hydrolysed into TfOH, which could cause acetal or silyl ether hydrolysis. As a result of its instability, triflate **9** was reacted immediately, without purification, with the alkoxide of acceptor **10** in a Williamson etherification to afford ether **8** in 81% yield (Scheme 2.3).[119]

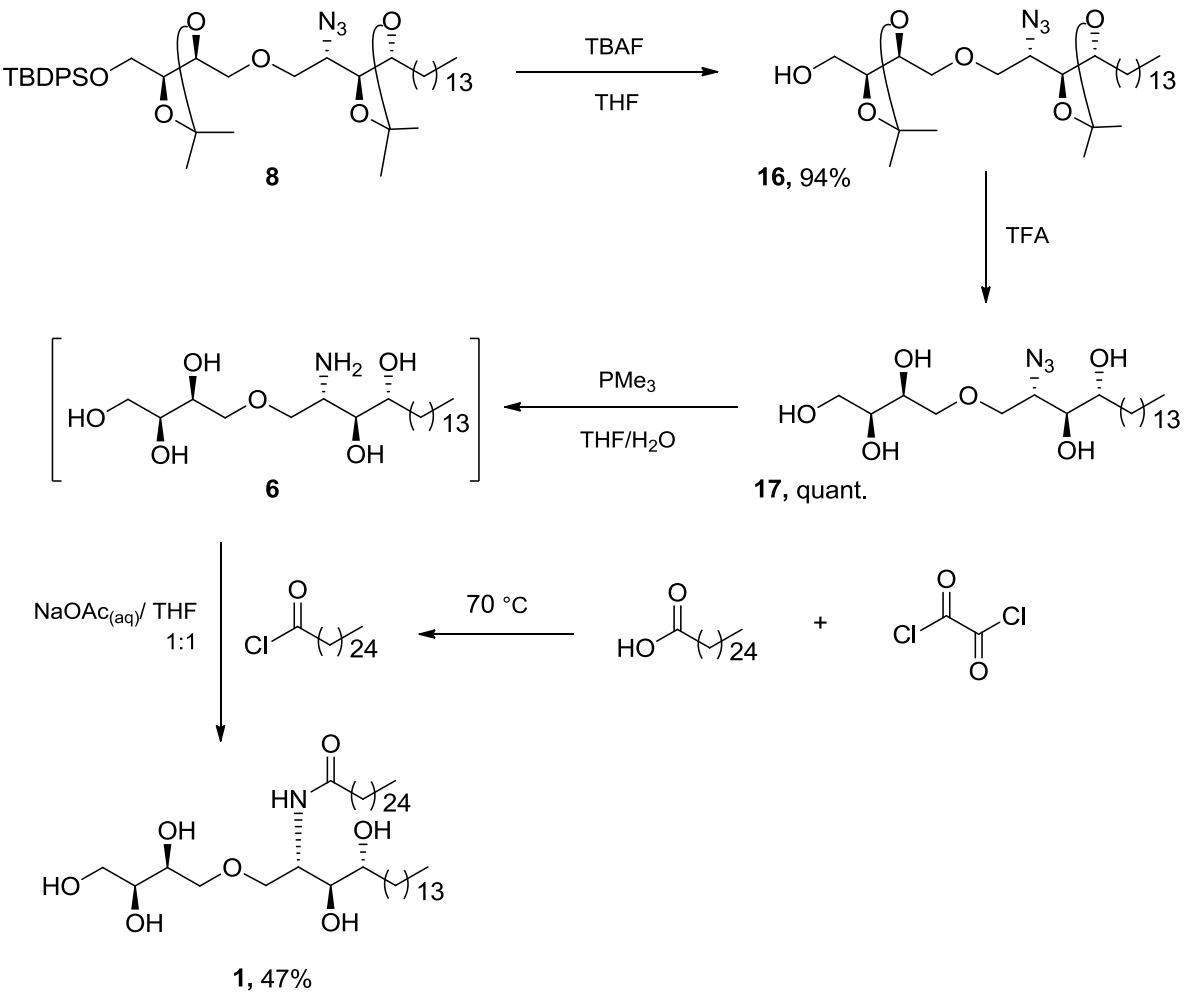


Scheme 2.3. Synthesis of threitol ether **8**.

The ether **8** was then deprotected, first by using tetra-*n*-butylammonium fluoride (TBAF), to selectively remove the TBDPS group, and then with neat trifluoroacetic acid (TFA), to hydrolyse the two acetals. Acetals are easily removed under even mildly acidic conditions, however we decided to use TFA, a relatively strong acid, for our deprotection. This is because TFA is volatile, and can be removed under reduced pressure. This negates the need for an aqueous work-up, which other commonly used acidic conditions would need. Since our product from the acetal deprotection would be highly polar, having five free hydroxyl groups, using TFA would remove the possibility of losing material in an aqueous work-up. The azide group in tetraol **17** was then reduced to the amine **6**, via a Staudinger reaction with trimethylphosphine.

The Staudinger reaction is a commonly used method for reducing azides to amines.[124,125] The azide is reacted with a phosphine, usually triphenylphosphine, to form an imino phosphorane, with concomitant release of nitrogen gas. The imino phosphorane is then broken down with water, releasing the amine and producing a phosphine oxide by-product. This phosphine oxide by-product can be tricky to separate from the product, especially when using PPh_3. We therefore chose to use PMe_3, as the phosphine oxide by-product formed is a solid with a high vapour pressure, and hence can be removed from the product mixture under reduced pressure.[126] This leaves an essentially pure product, which can be used in the next step without purification.

These three deprotection steps resulted in fully deprotected amine **6**, which only needs to be acylated to give our target threitol ceramide **1** (Scheme 2.4). Hexacosanoyl chloride was freshly prepared from hexacosanoic acid and oxalyl chloride in the absence of solvent and was added without further purification to amine **6,** to provide ThrCer **1**. Comparison with literature NMR data[119] confirmed the identity of the product.



Scheme 2.4. Synthesis of ThrCer **1**.

There were no major problems with the synthesis of ThrCer, therefore we were now ready to move on to the deoxy and truncated analogues. These analogues only differ from ThrCer in the electrophile part, which should allow us to employ many of the steps we had used in the synthesis of ThrCer.

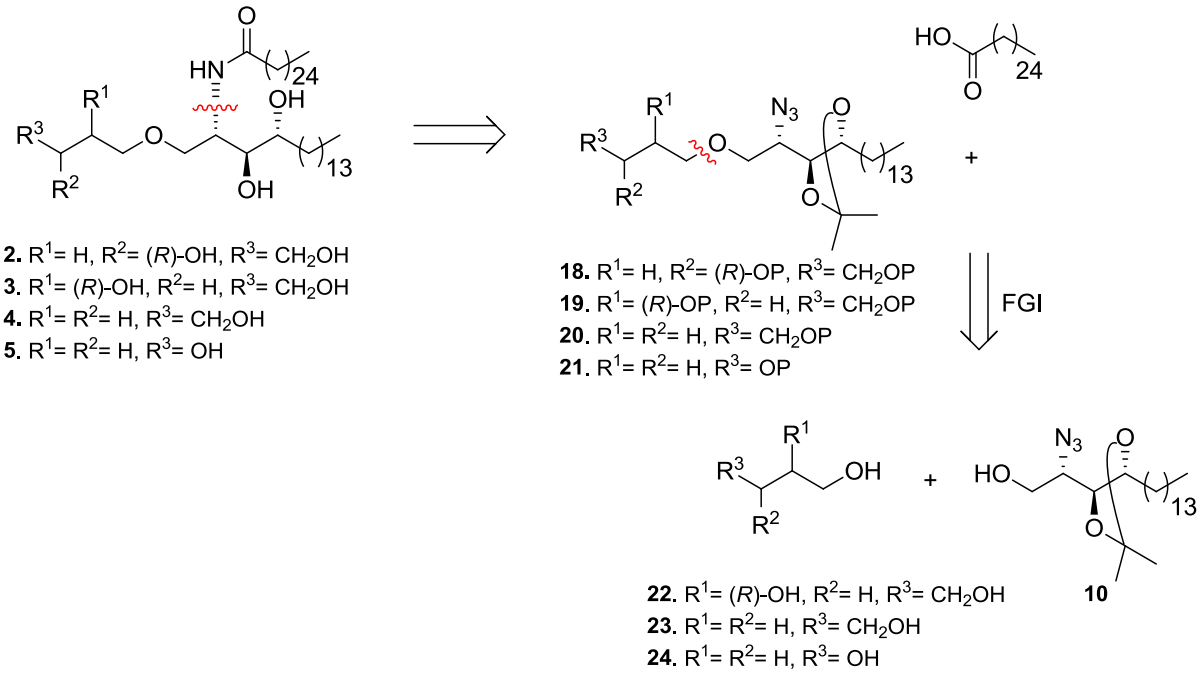
2.3 Retrosynthetic Analysis of our Deoxy and Truncated Analogues of ThrCer

The target truncated and deoxy analogues of ThrCer (Figure 2.2) will allow us to identify which hydroxyls / hydrogen bond interactions are important and ascertain how much further we might be able to simplify the structure of our CD1d agonists before losing activity.

We want these analogues to retain, where relevant, the absolute and relative configuration of ThrCer (and hence the configuration of α-GalCer) as it is known that this arrangement of the hydroxyl groups is recognised by the iNKT cell TCR, and will allow us to compare our analogues with these compounds. In this way we can be certain that any change in biological activity should be solely due to the removal of certain hydroxyl groups, which might either directly affect recognition by the iNKT cell TCR, due to the loss of hydrogen bonds, or result in a change in physical properties, like solubility, which would also have an impact on activity.

Our retrosynthetic pathway of the target analogues is shown in Scheme 2.5.

The azido intermediates **18**, **19**, **20** and **21,** accessed via a Williamson etherification, would be reduced to the corresponding amines and then reacted with freshly made hexacosanoyl chloride to give the corresponding ThrCer analogues. The azido alcohol **10** is synthesised as before (Scheme 2.2), but the electrophiles will be different for each analogue, and will have to be synthesised separately.



2. R^1= H, R^2= (*R*)-OH, R^3= CH_2OH
3. R^1= (*R*)-OH, R^2= H, R^3= CH_2OH
4. R^1= R^2= H, R^3= CH_2OH
5. R^1= R^2= H, R^3= OH

18. R^1= H, R^2= (*R*)-OP, R^3= CH_2OP
19. R^1= (*R*)-OP, R^2= H, R^3= CH_2OP
20. R^1= R^2= H, R^3= CH_2OP
21. R^1= R^2= H, R^3= OP

22. R^1= (*R*)-OH, R^2= H, R^3= CH_2OH
23. R^1= R^2= H, R^3= CH_2OH
24. R^1= R^2= H, R^3= OH

Scheme 2.5. Retrosynthetic analysis of the deoxy and truncated ThrCer analogues **2**, **3**, **4** and **5**.

2.4 Synthesis of the 2-Deoxy and 3-Deoxy ThrCer Analogues

The 2-deoxy and 3-deoxy ThrCer analogues **2** and **3**, respectively, are structurally more complex than the 2,3-dideoxy analogue **4** and the truncated analogue **5**, due to the presence of an additional stereogenic centre. For our analogues the loss of one neighbouring hydroxyl group alters the priority of the surrounding atoms, which means that to retain the same absolute configuration as ThrCer, the configuration of the stereogenic centres of the secondary hydroxyls need to be (*R*), rather than (*S*) (Figure 2.3).

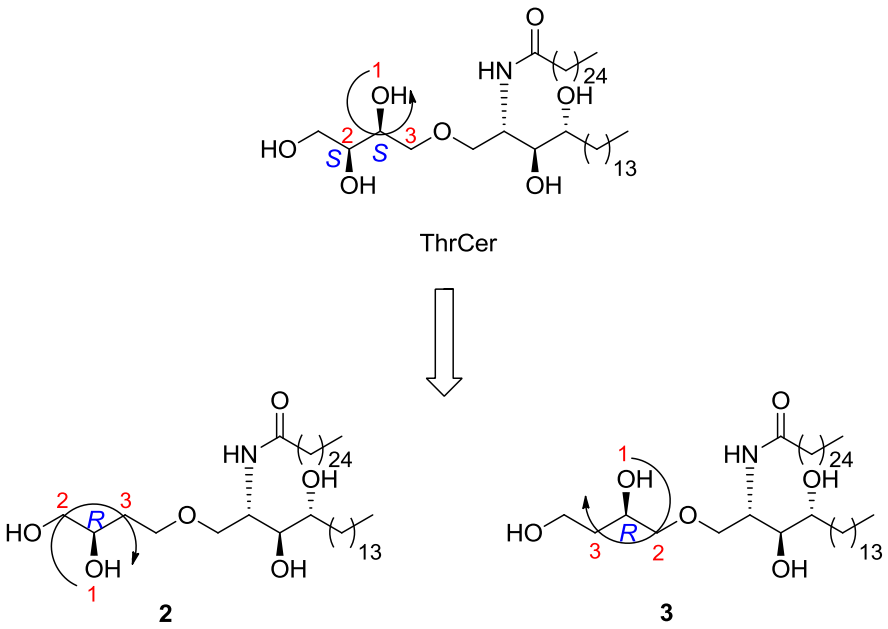
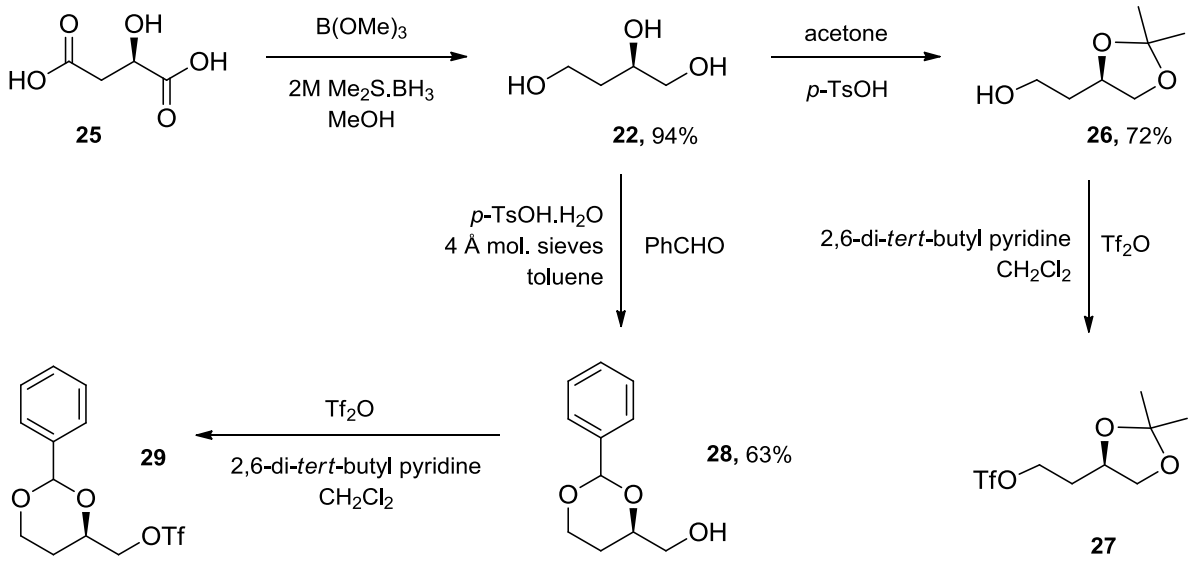


Figure 2.3. Illustrating the absolute stereochemistry of analogues **2** and **3.**

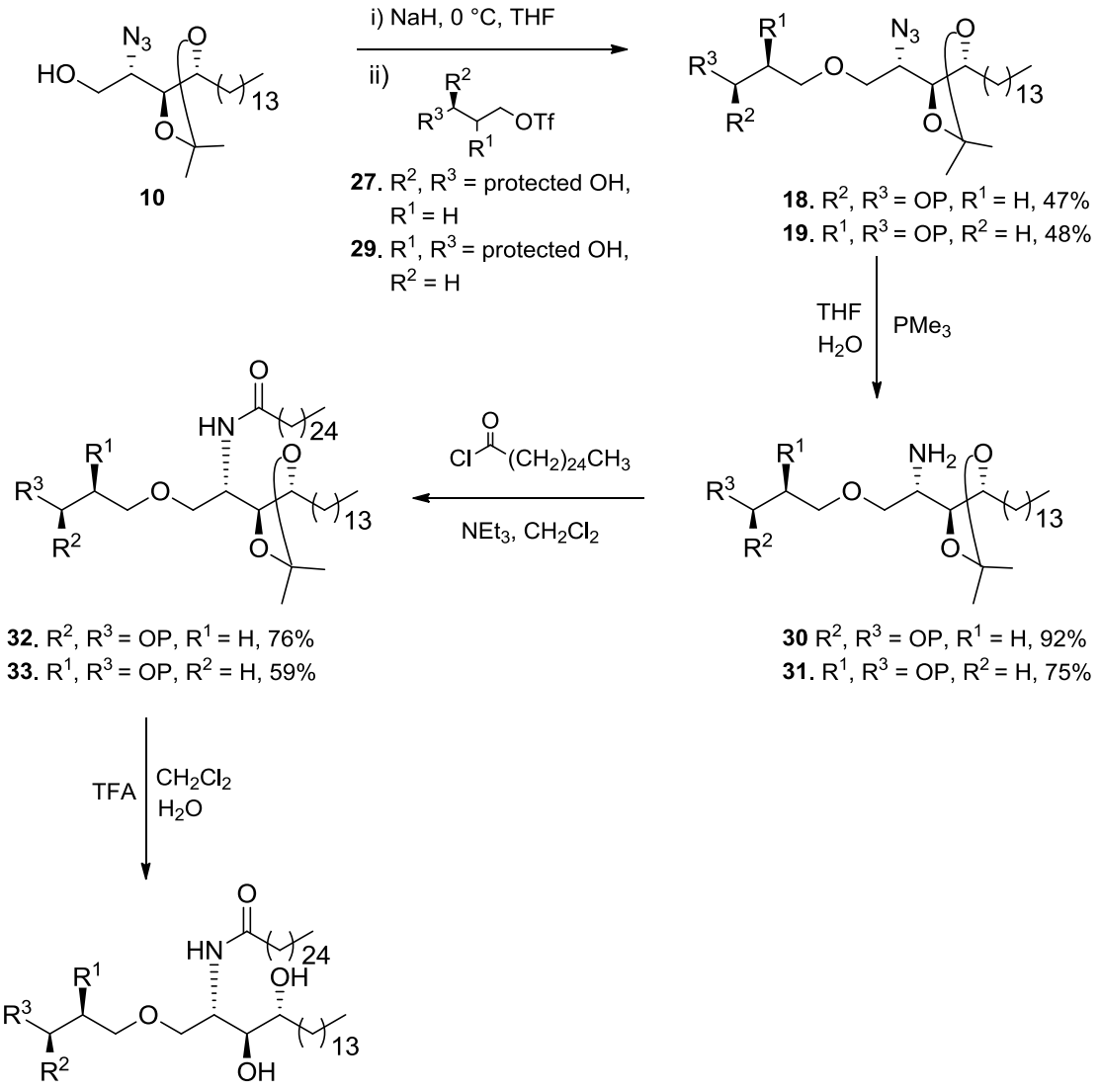
Analogues **2** and **3** are derived from the same starting material – (*R*)-1,2,4-butanetriol **22**, which was synthesised from (*R*)-malic acid **25** using trimethylborate and borane-dimethyl sulfide complex.[127] To make the two different analogues, the etherification reaction needed to occur using both of the primary alcohols. We therefore needed two different protecting group

strategies, one which protected the 1,2-diol but left the 4-hydroxyl group free and another which protected the 1,3-diol and left the other primary hydroxyl group free. This was achieved by using the isopropylidene and benzylidene protecting groups. As described above (Page 69), ketone-derived acetals afford selective protection for 1,2-diols over 1,3-diols, whereas aldehyde-derived acetals are selective for 1,3-diols. (*R*)-1,2,4-Butanetriol **22** was therefore reacted in the presence of a sub-stoichiometric amount of tosic acid with either acetone to install the isopropylidene, or with benzaldehyde to install the benzylidene. The reaction to establish the isopropylidene proceeded without event, providing acetal **26**. The reaction with benzaldehyde was performed over 4 Å molecular sieves to remove the water formed during the reaction, in order to drive the reaction forwards.[128] We confirmed that the 1,3-diol was protected with the benzylidene in 1,3-dioxane **28** by ^{13}C NMR spectroscopy, which showed a CH_2 resonance at 27.0 ppm, which was further upfield than in (*R*)-1,2,4-butanetriol and acetal **26** (CH_2 at 37.1 ppm and 35.7 ppm respectively), highlighting the difference in this area caused by the benzylidene. Also the resonances at 101.3 ppm, 126.6 ppm, 128.2 ppm, 128.7 ppm and 140.2 ppm confirmed that the benzylidene group was present and that the acetal was a six-membered acetal, rather than the five-membered acetal seen in acetal **26** (C resonance at 108.9 ppm). In both cases, the remaining primary hydroxyl was then made into the corresponding triflate using Tf_2O in the presence of 2,6-di-*tert*-butyl pyridine, giving the required electrophiles **27** and **29** for analogue **13** and analogue **16** respectively (Scheme 2.6).



Scheme 2.6. Synthesis of the triflates **27** and **29**.

Triflates **27** and **29** proved to be very reactive and prone to decomposition. They were therefore reacted immediately with the acceptor, azide **10**, to make the linking ether bond. The azide group was then reduced with PMe_3 in a Staudinger reaction[124-126] to give the amines **30** and **31**, which were separately coupled with hexacosanoyl chloride in the presence of NEt_3 to give the protected analogues **32** and **33**. We decided to incorporate the acyl chain before deprotection for this set of analogues, rather than deprotect first as we had for the synthesis of ThrCer. Although deprotection first had proven to be a successful synthetic route we decided to try this pathway to avoid working with very polar molecules, which can be tricky to purify. All remaining protecting groups on protected analogues **32** and **33** were acetals which were globally deprotected using TFA to give the analogues **2** and **3** (Scheme 2.7).



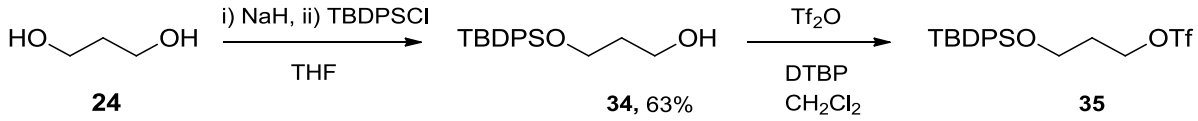
Scheme 2.7. Synthesis of the 2-deoxy and the 3-deoxy ThrCer analogues **2** and **3.**

2.5 Synthesis of the Truncated ThrCer Analogue

The 2,3-dideoxy analogue **4** and the truncated analogue **5**, have 1,4-butanediol and 1,3-propanediol, respectively, as starting materials. The planned scheme for synthesising these analogues involved mono-protecting the diol, making the triflate and then reacting the triflate with the nucleophile, azide **10**. However these simpler analogues proved surprisingly more difficult to synthesise than had been expected.

We first investigated THP etherification as a method for mono-protection of the diols. Whilst mono-protection and triflate synthesis proceeded without incident, the R_f of the mono-protected diols and the nucleophile azide **10** were very similar to the etherification product, which rendered analysis of this reaction by TLC difficult; it was difficult to determine whether or not the reaction was complete, or indeed had occurred at all. Purifying the reaction mixture via column chromatography would be unlikely to separate the compounds and the next step would be to reduce the azide to the amine, a step which would also affect one of the starting materials. Therefore it was decided to try a different protecting group.

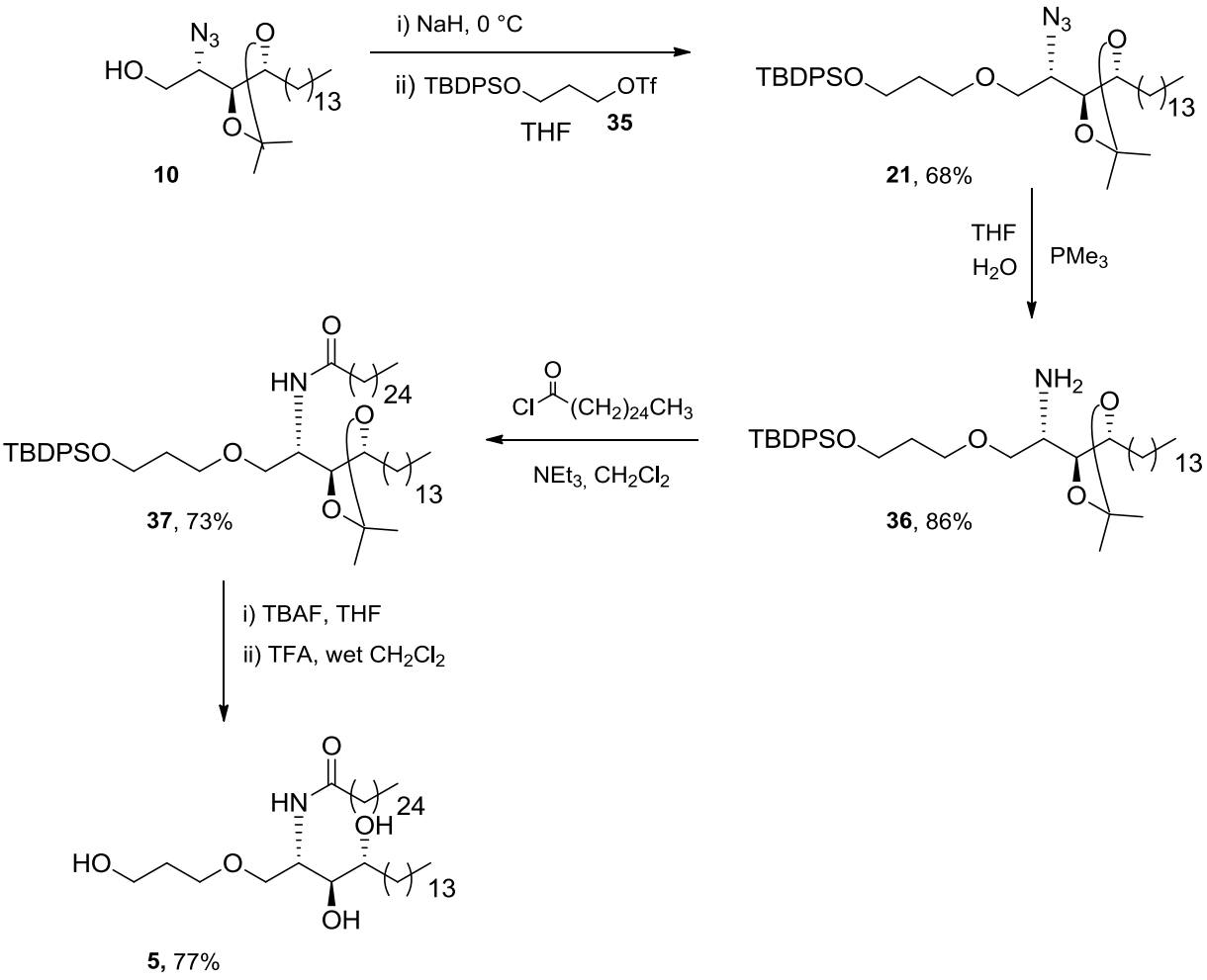
The next protecting group attempted was the *tert*-butyldiphenylsilyl (TBDPS) group. This protecting group afforded mono-protected diols which were significantly less polar than azide **10**, which facilitated TLC analysis of the Williamson etherification. Whilst this protection group allowed the synthesis of the truncated analogue **5**, we were unable to prepare the 2,3-dideoxy ThrCer analogue **4.**



24 **34**, 63% **35**

Scheme 2.8. Synthesis of the triflate **35.**

Thus 1,3-propanediol **24** was treated with NaH, and then reacted with 1.1 equiv of TBDPSCl, giving the mono-protected diol **34**, which was converted into the triflate **35** under our standard conditions (Scheme 2.8). Reaction of triflate **35** with the sodium alkoxide of azide **10**, generated the ether **21**. The azide functionality in ether **21** was then reduced and the resulting amine **36** coupled with hexacosanoyl chloride as before, to afford the protected analogue **37**. TBAF deprotection of the silyl ether followed by acetal hydrolysis, as before with TFA, generated our next target, truncated analogue **5** (Scheme 2.9).



Scheme 2.9. Synthesis of truncated analogue **5.**

2.6 Synthesis of the 2,3-Dideoxy ThrCer Analogue

In the case of 1,4-butanediol, the TBDPS protecting group proved unsuitable for making the corresponding triflate. This was obvious from TLC analysis of the triflation reaction; usually this is a very clean process however with the 1,4-butanediol there were several spots on the TLC plate. The R_f of one of the spots identified the silanol TBDPSOH as one of the products, indicating that the silyl protecting group was unstable under these conditions. One possible decomposition pathway is that the silyl ether reacts intramolecularly with the triflate to form tetrahydrofuran and the silanol on aqueous work-up (Figure 2.4).

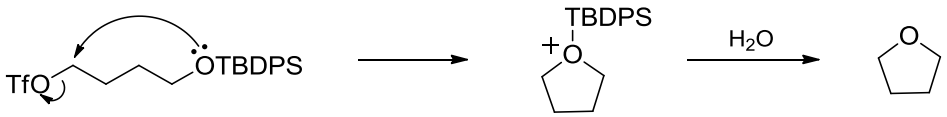
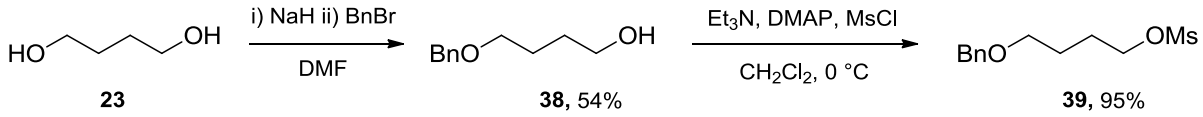


Figure 2.4. Showing the possible intramolecular reaction to form a tetrahydrofuran.

Since triflate is a very reactive leaving group we decided to try a less reactive mesylate, and a benzyl protecting group which would be less liable to acidic hydrolysis. The combination of both these changes did allow the reaction to proceed, although the yield was not particularly good and the etherification reaction required heating.

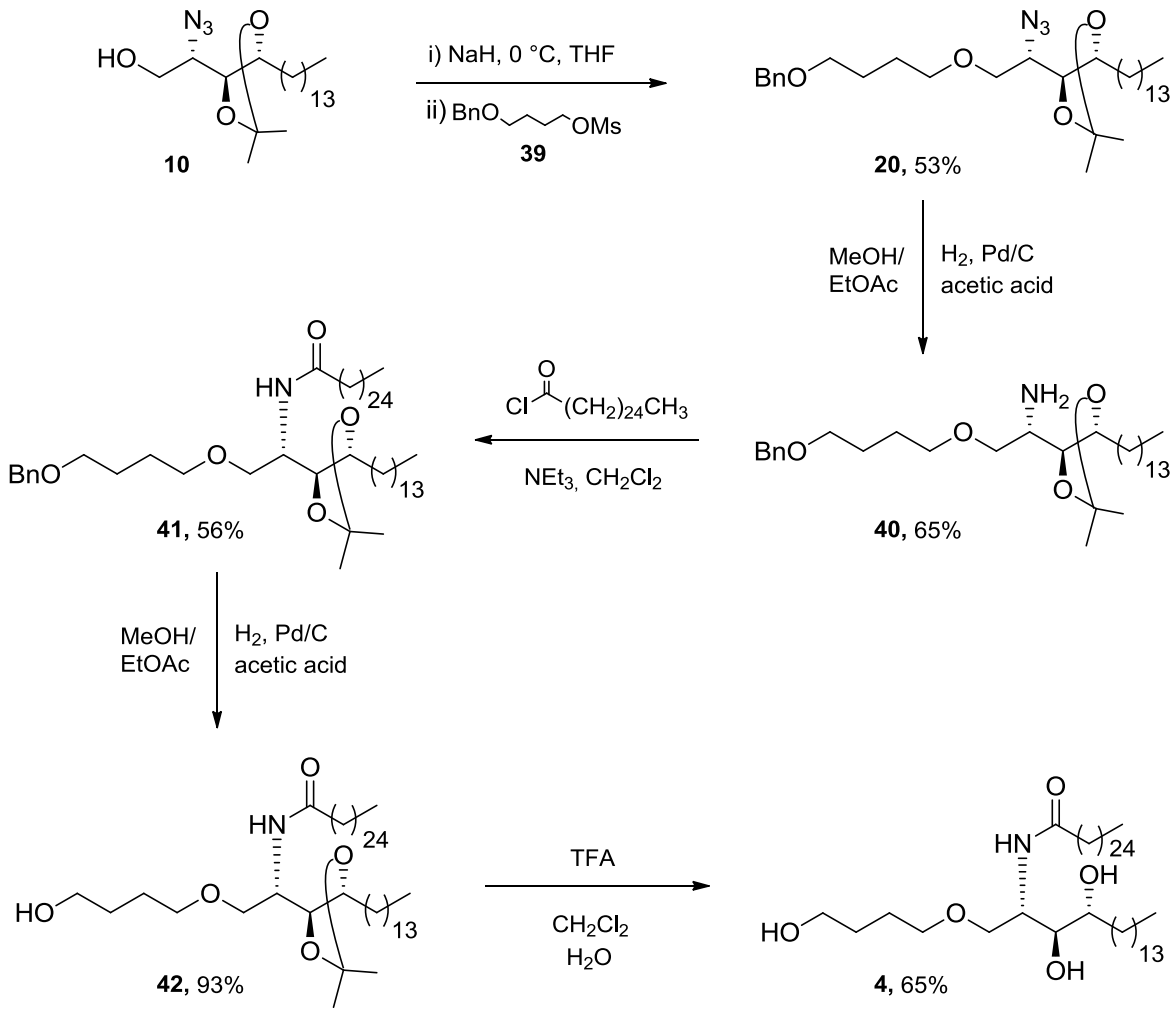


23 **38**, 54% **39**, 95%

Scheme 2.10. Synthesis of mesylate **39.**

The diol **23** was treated with NaH, and then treated with 0.7 equiv of benzyl bromide to give the mono-protected alcohol **38**,[129] which was then reacted with mesyl chloride in the presence of Et_3N and a sub-stoichiometric amount of DMAP to give the mesylate **39** (Scheme 2.10). Mesylate **39** was then coupled to the nucleophile, azide **10**. The mesylate proved to be much less reactive than the corresponding triflate which meant etherification required heating to 65 °C to reach completion. Hydrogenolysis with Pd/C catalyst was then performed in an attempt to reduce both the azide group to the amine and the benzyl group; however after 16 h, only the azide was reduced, the benzyl group was still present. This was likely due to the amine poisoning the Pd catalyst, even though acetic acid was added to the reaction mixture in an attempt to protonate the amine and avoid this problem. The amine **40** was then reacted with hexacosanoyl chloride to afford the protected analogue **41**. This protected analogue was then deprotected with another hydrogenolysis, which removed the benzyl group without event, followed by acetal deprotection with TFA to provide our final product **4** (Scheme 2.11).

There is plenty of scope to optimise this reaction scheme, however we obtained sufficient material to allow for preliminary biological testing.



Scheme 2.11. Synthesis of 2,3-dideoxy ThrCer analogue **4.**

2.7 Biological Analysis

Analogues **2**, **3**, **4** and **5** were submitted for biological testing, which was carried out by Dr John-Paul Jukes and Dr Hemza Ghadbane, members of Prof. Vincenzo Cerundolo's group at the Weatherall Institute of Molecular Medicine in Oxford, UK.

Initially they were tested for binding affinity of the CD1d/glycolipid complex to the TCR with a Biacore analysis using human Vα24/Vβ11 NKT cells (Figure 2.5). Our analogues, together with α-GalCer as a control, were first refolded by oxidative refolding chromatography with bacterially-expressed hCD1d and β_2m molecules before being immobilised on a BIAcore chip. Increasing concentrations of soluble human iNKT cell TCR were then passed over the chip for 5 seconds until the specific binding reached its plateau. Binding was then measured by surface plasmon resonance.

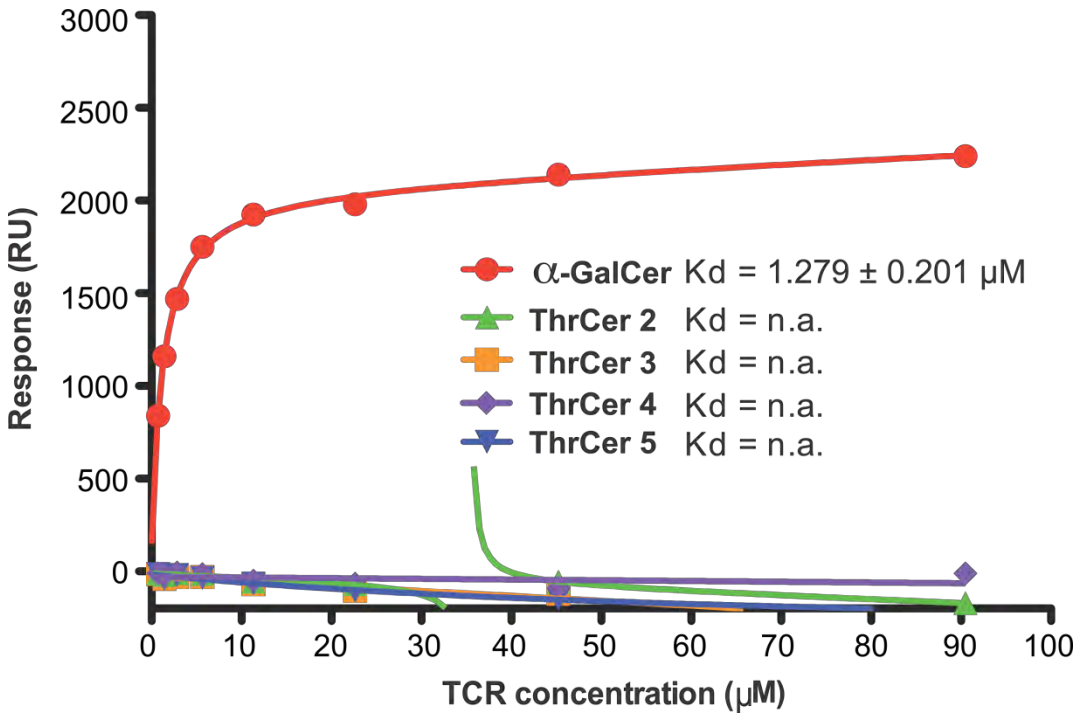


Figure 2.5. Binding affinities of the iNKT TCR for hCD1d molecules loaded with ThrCer analogues **2**, **3**, **4** and **5**. K_d values (μM) were calculated from equilibrium binding.

Unfortunately all of our analogues did not give any response, indicating that our analogues do not appear to bind to the TCR.

In parallel, the analogues were also tested *in vitro* for IL-2 release (Figure 2.6). To this end, dendritic cells (DC2.4) were pulsed overnight with the lipid, then the excess lipid was washed away. The DCs were then cultured for 24 hours with DN32, a mouse iNKT cell hybridoma, before the culture medium was tested for IL-2 by ELISA. IL-2 release was measured as a predictor of IFN-γ release, as IL-2 causes the proliferation of iNKT cells, which are the major secretor of IFN-γ. Again our analogues did not release detectable levels of IL-2, and hence do not seem to activate iNKT cells.

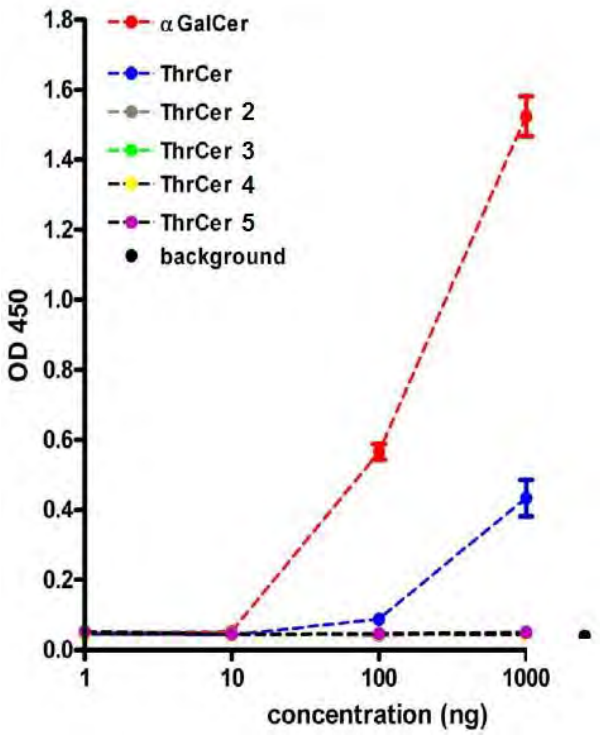


Figure 2.6. Graph showing IL-2 release by iNKT cell hybridoma after culture with mature DCs. The results shown are representative of three independent experiments.

2.8 Conclusions

Analyses of these compounds indicate that for all four structurally simplified ThrCer analogues, IL-2 release is diminished relative to ThrCer (Figure 2.6). This suggests that removal of any of the hydroxyl groups of ThrCer is not tolerated. This was surprising as the removal of one hydroxyl group was not predicted to have had such a dramatic change. However it was noted that our compounds were difficult to solubilise, which could result in poor delivery of the glycolipid to CD1d and as a result the CD1d/glycolipid complex itself might not be formed efficiently which would therefore provide an alternative explanation for the dramatic reduction in activity: thus our deoxy ThrCer analogues might still be able to bind to the iNKT cell TCR, but their solubility properties might be preventing access. With these disappointing results and mindful of the potential problems associated with more hydrophobic ThrCer analogues we hypothesised that future analogues would need to preserve the three hydroxyl groups of ThrCer as a scaffold in order to ensure the physical properties of the products are compatible with the TCR of iNKT cells and the conditions inside the cell.

Chapter 3

Synthesis and Biological Evaluation of Conformationally Less Flexible ThrCer Analogues

3. Synthesis and Biological Evaluation of Conformationally Less Flexible ThrCer Analogues

3.1 Target Carbocyclic ThrCer Analogues

The next aim of this project was to synthesise analogues of ThrCer which were less conformationally flexible. Although ThrCer is biologically active it displays lower activity than α-GalCer; it is thought that the flexibility of ThrCer might be a factor in this. In α-GalCer, the sugar head group is locked into a conformation which is primed for recognition by the iNKT cell TCR; in ThrCer the truncated sugar head group is acyclic and as such can adopt many different conformations, many of which will not be correct for recognition. Constraining the ThrCer head group into a ring should reduce conformational flexibility. A more rigid system will decrease the entropy of the molecule and so reduce the fall in entropy that will occur during binding to CD1d. This should be reflected in a higher binding affinity and hopefully improved biological activity. Therefore analogues in which the triol unit of ThrCer is constrained into a carbocyclic ring, and hence are structurally closer to α-GalCer, would be interesting to test (Figure 3.1).

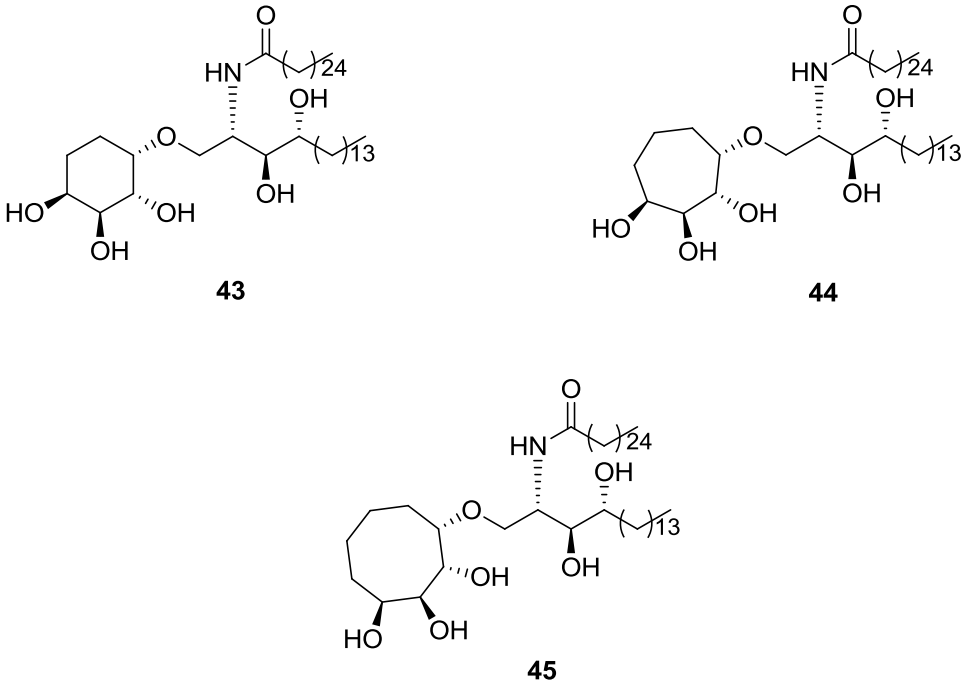
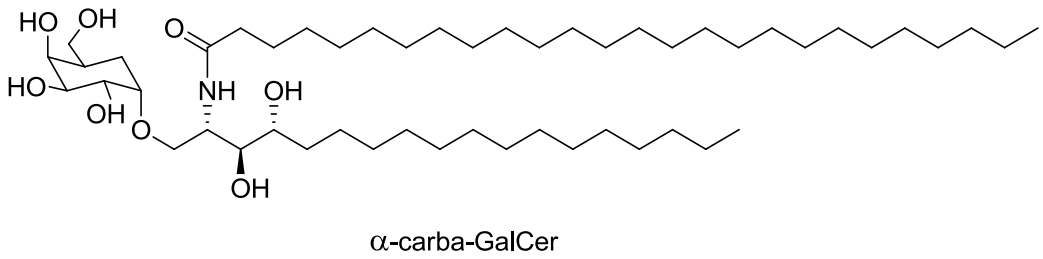


Figure 3.1. The target carbocyclic ThrCer analogues **43**, **44** and **45.**

3.2 Reported Carbocyclic Analogues

There have been a few examples of carbocyclic α-GalCer analogues in the literature. Tashiro *et al.* synthesised the carbocyclic analogue, α-carba-GalCer, in which the ring oxygen from α-GalCer was replaced with a methylene group, hence substituting the glycosidic bond with an ether linkage (Figure 3.2).[116] α-Carba-GalCer produced a T_H1-biased response, which the group proposed could be due to the greater *in vivo* stability of the ether linkage, as compared to the glycosidic linkage of α-GalCer, as the α-carba-GalCer analogue will be available for longer, allowing for a more stable CD1d-glycolipid-TCR complex and a longer stimulation time.



α-carba-GalCer

Figure 3.2. Structure of the α-GalCer analogue α-carba-GalCer.

Another group of interesting carbocyclic CD1d agonists are the aminocyclitol analogues, prepared by Harrak *et al*.[130,131] These analogues have again replaced the ring oxygen of α-GalCer with a methylene group, but have also replaced the glycosidic bond of α-GalCer with an amino linkage, which, like an ether bond, is metabolically more stable. HS44 and HS161 were the two analogues which showed the most biological activity. HS44 has a head group which is

more closely related to glucose, having an equatorial 4-OH; HS161 retains the absolute and relative configuration of D-galactose (Figure 3.3).

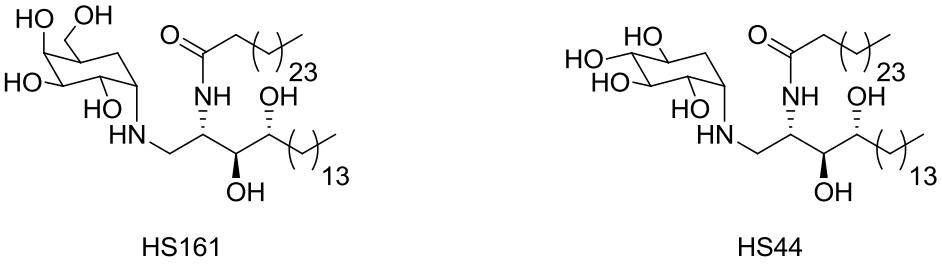


Figure 3.3. The structures of the α-GalCer analogues HS161 and HS44.

Both of these analogues are weaker agonists than α-GalCer, but result in a T_H1-biased response *in vivo*, producing large amounts of IFN-γ and little IL-4. The HS44 analogue has a much weaker binding affinity (K_d = 155 nM)[130] to the mouse TCR than does α-GalCer (K_d = 11.2 nM),[132] due to a much faster dissociation rate. This fast dissociation rate makes sense when one looks at the crystal structure of the mCD1d-HS44-TCR complex.[130] HS44 binds to CD1d in a similar fashion to α-GalCer, with the sphingosine chain occupying the F' pocket and the acyl chain in the A' pocket. The head group is orientated parallel to the groove, like in α-GalCer, allowing for recognition by the TCR. The head group of α-GalCer forms four hydrogen bonds with the TCR, however HS44 only forms three direct hydrogen bonds. This is due to the D-glucose configuration of the head group; the equatorial 4-OH is not in the correct position to form a hydrogen bond with Asn30α of the TCR CDR1α loop. Instead the 4-OH hydrogen bonds to a bridging water molecule, which in turn forms a hydrogen bond to Asn30α (Figure 3.4). This reduced interaction could be the cause of the faster dissociation rate of HS44 from the TCR. The HS44 analogue has been tested for anti-metastatic ability and is highly effective,

being almost as effective as α-GalCer.[130] This confirms the ability of HS44 to act in a T_H1-biasing manner. The group believe that the T_H1 bias is again due to the greater *in vivo* stability of their analogues, allowing for a more sustained stimulation of iNKT cells.

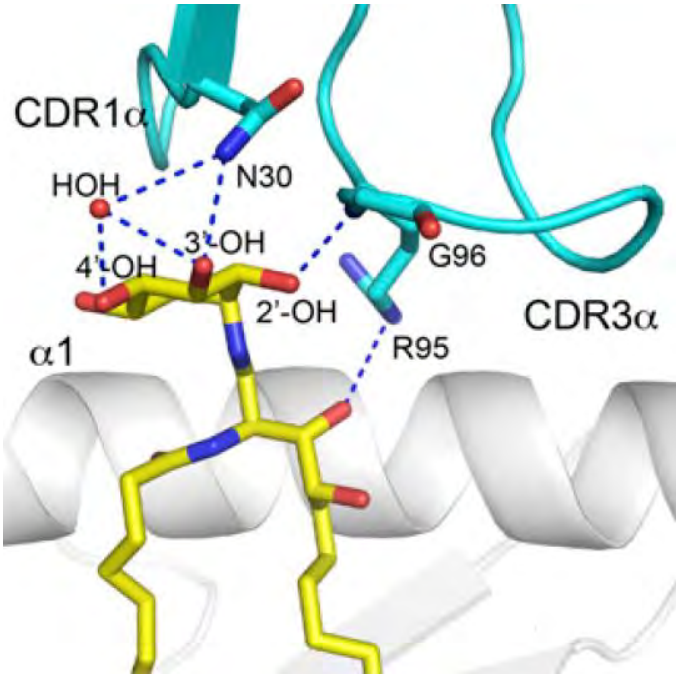


Figure 3.4. Crystal structure highlighting the hydrogen bonds of HS44 with the iNKT cell TCR. Figure adapted from ref.[130]

3.3 The Configuration and Conformation of Our Target Analogues

The analogues we targeted are similar to these carbocyclic compounds in that we are replacing the glycosidic bond with a metabolically more stable ether linkage. However our lead compound is ThrCer, not α-GalCer, and we are trying to restore activity to ThrCer by constraining the sugar head group into a carbocyclic ring, hence reducing the conformational flexibility. This means that our head group contains only the three OH groups of ThrCer, which we have previously shown to be essential for recognition (see Chapter 2); the ring oxygen and the hydroxymethyl substituent of α-GalCer have both been removed. We were keen to see whether reducing the conformational flexibility would restore biological activity, and also whether the removal of the glycosidic bond would allow our analogues to cause a T_H1 bias, as was observed for the α-carba-GalCer and HS44 analogues. However it is worth noting that ThrCer does not show any cytokine bias.

Our three target cyclic ThrCer analogues constrain the threitol head unit into a cyclohexyl, cycloheptyl and cyclooctyl ring (Figure 3.1). At the outset, we foresaw a potential conformational problem with the cyclohexyl ThrCer analogue **43**. α-GalCer adopts a $^4C_1$ chair conformation, which is recognised by CD1d and the iNKT cell TCR. The $^4C_1$ chair conformation is the lowest energy conformation for two reasons: the α-glycosidic linkage benefits from additional anomeric stabilisation in this conformation, and 1,3-diaxial interactions are minimised in this conformation (Figure 3.5). Therefore "ring-flipping" to the higher energy $^1C_4$ chair conformation is unfavourable.

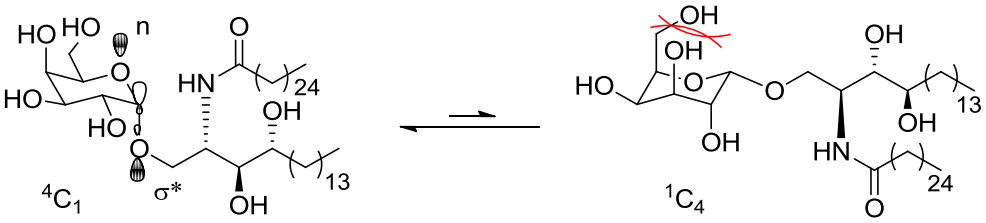


Figure 3.5. Showing the possible chair conformations of α-GalCer.

Our cyclohexyl ThrCer analogue **43**, being a six-membered ring, will also likely adopt low-energy chair conformations (Figure 3.6). However, as it is not a sugar there is no anomeric stabilisation of the pseudo $^4C_1$ chair conformation. Also, the absence of the hydroxymethyl substituent at position 5 in our cyclohexyl ThrCer analogue **43** will remove some of the 1,3-diaxial interactions of the pseudo $^1C_4$ chair conformation. These two structural modifications are likely to result in the two chair conformations being much more similar in energy. In fact, it is likely that the pseudo $^1C_4$ chair conformation will be lower in energy than the pseudo $^4C_1$ chair conformation, due to the equatorial positioning of the bulky ceramide group, which should minimise 1,3-diaxial interactions. Therefore we predicted the lowest energy conformation of cyclohexyl ThrCer analogue **43** would likely be the pseudo $^1C_4$ chair conformation, which may not be recognised by the iNKT cell TCR.

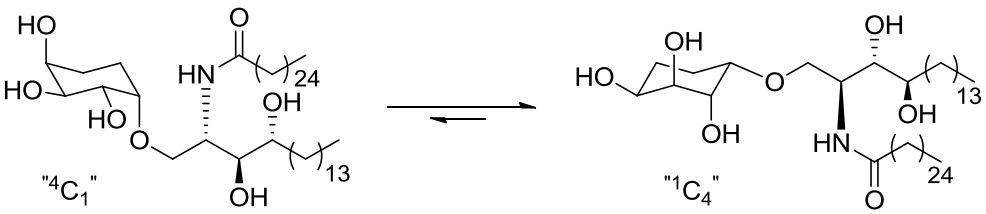


Figure 3.6. Showing the chair conformations of target cyclohexyl ThrCer analogue **43**

However, as the two conformations are likely to be similar in energy, the barrier of interconversion between the two could also be low, resulting in rapid flipping between the two conformational isomers. The lowest energy conformation would therefore be unimportant, as the correct conformation needed for binding and recognition would effectively be selected from the mixture of conformers by CD1d and the TCR.

Due to the possibility of the cyclohexyl ThrCer analogue **43** adopting the undesired conformation we decided to also synthesise the cycloheptyl and the cyclooctyl ThrCer analogues **44** and **45** (Figure 3.1). The cycloheptyl and cyclooctyl ring systems should not be as rigid as the cyclohexyl analogue, having more conformations which interconvert easily, and so when presented with CD1d and the TCR, will hopefully conform to the shape necessary for binding.

We want the analogues to retain the same absolute and relative configuration of D-galactose and L-threitol. The structure of D-galactose has all the OH groups equatorial, except at the C(4) position. This equates to all the relevant OH groups in our target compounds having the (*S*) configuration. At the C(1)/anomeric position, if we want an "α-configuration" the OH group also has to be in the (*S*) configuration (Figure 3.7).

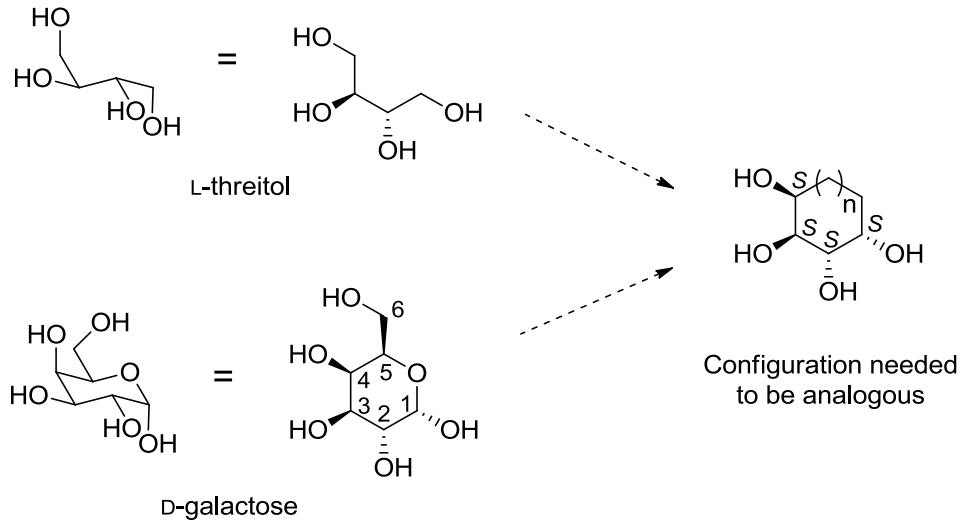


Figure 3.7. Highlighting the D-galactose and L-threitol configuration needed in our carbocyclic analogues.

However it is at this position that we are going to be reacting. Previously when forming the ether bonds in ThrCer and the deoxy and truncated ThrCer analogues (see Chapter 2) we have had the leaving group on the threitol head unit and the sphingosine has acted as the nucleophile. However this is an S_N2 type reaction, which leads to inversion of stereochemistry at the electrophilic centre undergoing substitution. Therefore if we want to create the ether bonds in our target compounds in the same way, we would need the C(1) position to be in the (*R*) configuration before reacting, in order to give the (*S*) configuration in the end product (Figure 3.8).

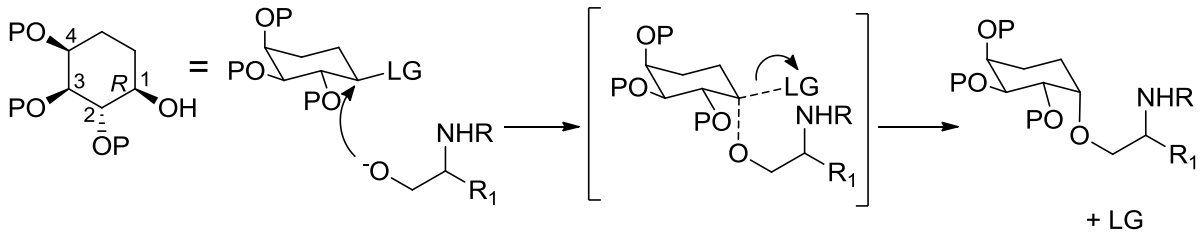
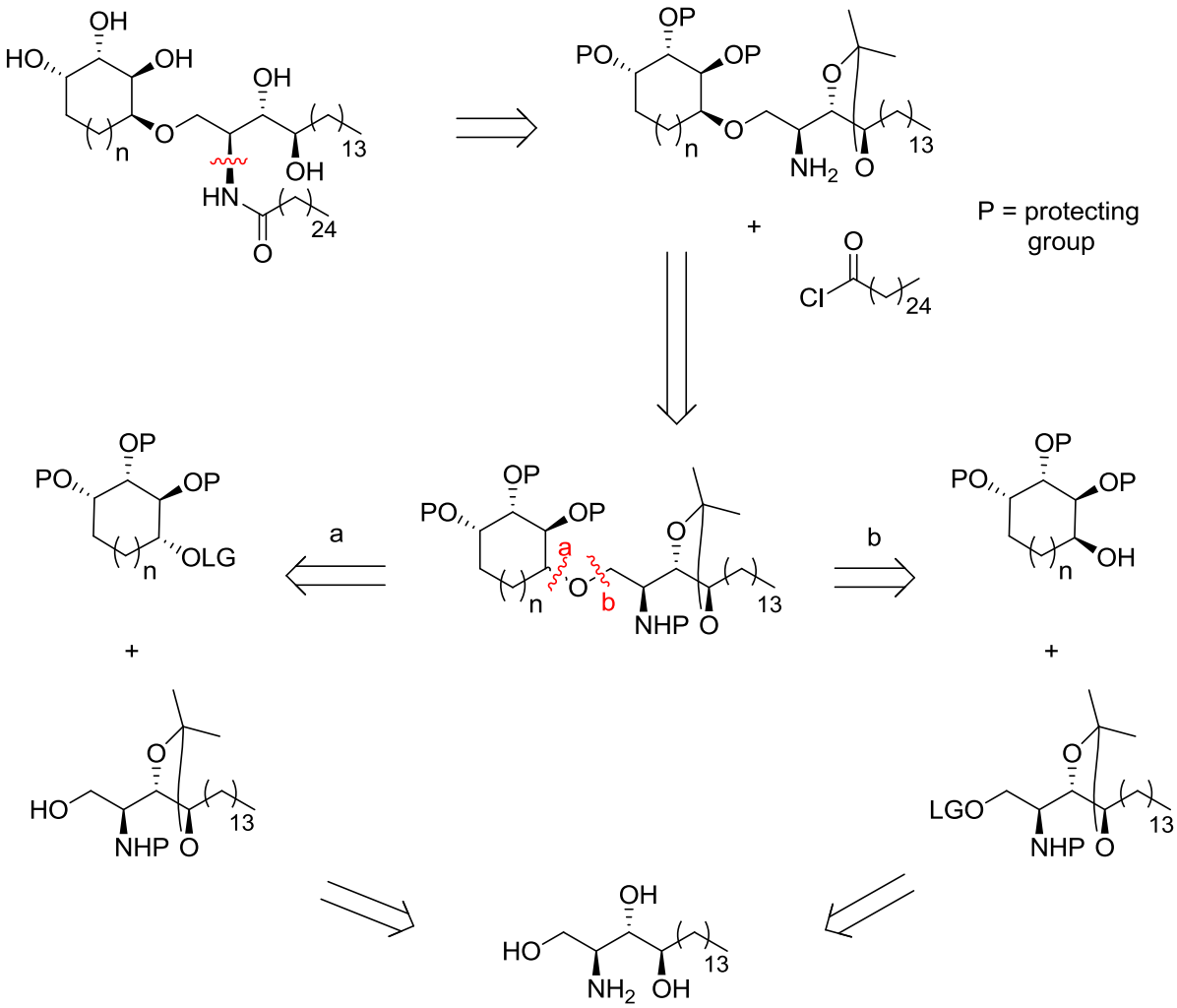


Figure 3.8. Inversion of stereochemistry.

Conversely if we had the leaving group on the sphingosine and the nucleophile on the threitol head unit then the C(1) position would need to be in the (*S*) configuration before ether bond formation.

3.4 Retrosynthetic Analysis

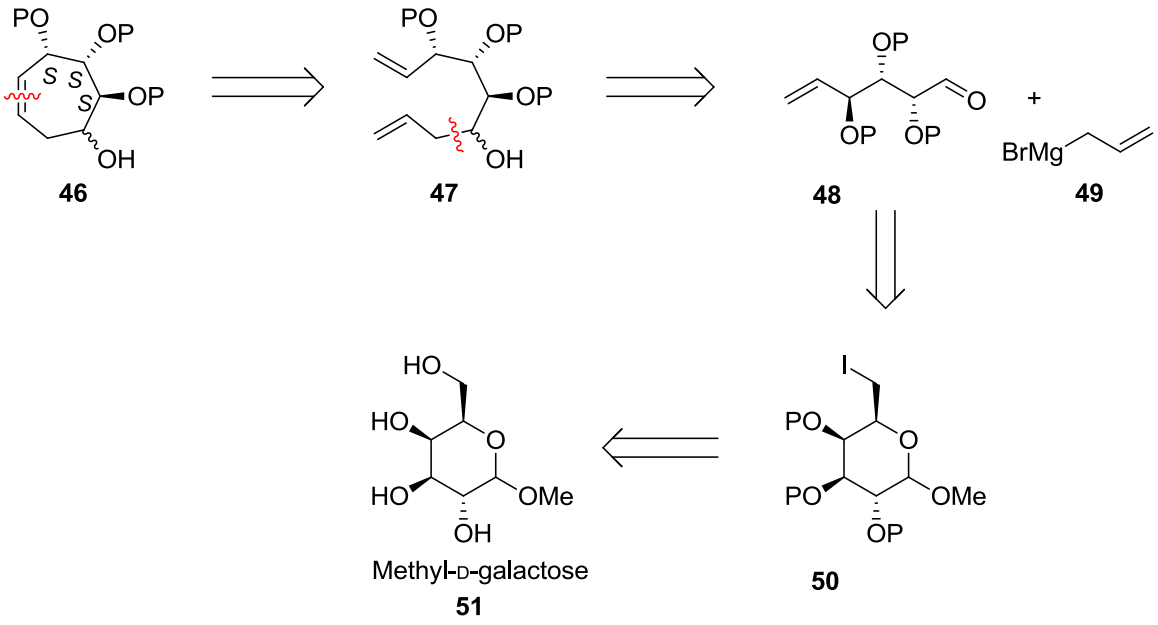
A retrosynthetic pathway for synthesising the analogues is shown in Scheme 3.1. We planned for all three ring analogues to employ the same method of etherification with the same phytosphingosine unit, with only the head unit changing to differently sized rings, which would need to be synthesised separately.



Scheme 3.1. Retrosynthetic analysis of cyclic ThrCer analogues.

3.5 Synthesis of the Cycloheptyl ThrCer Analogue

Ring-closing metathesis is a powerful method for the synthesis of differently sized rings.[133-135] Using sub-stoichiometric amounts of catalyst a linear diene can be efficiently cyclised. We decided to use this method to synthesise our rings. The retrosynthetic scheme for the synthesis of the cycloheptanol ring is shown below (Scheme 3.2).



Scheme 3.2. Retrosynthetic analysis of the cycloheptanol **46.**

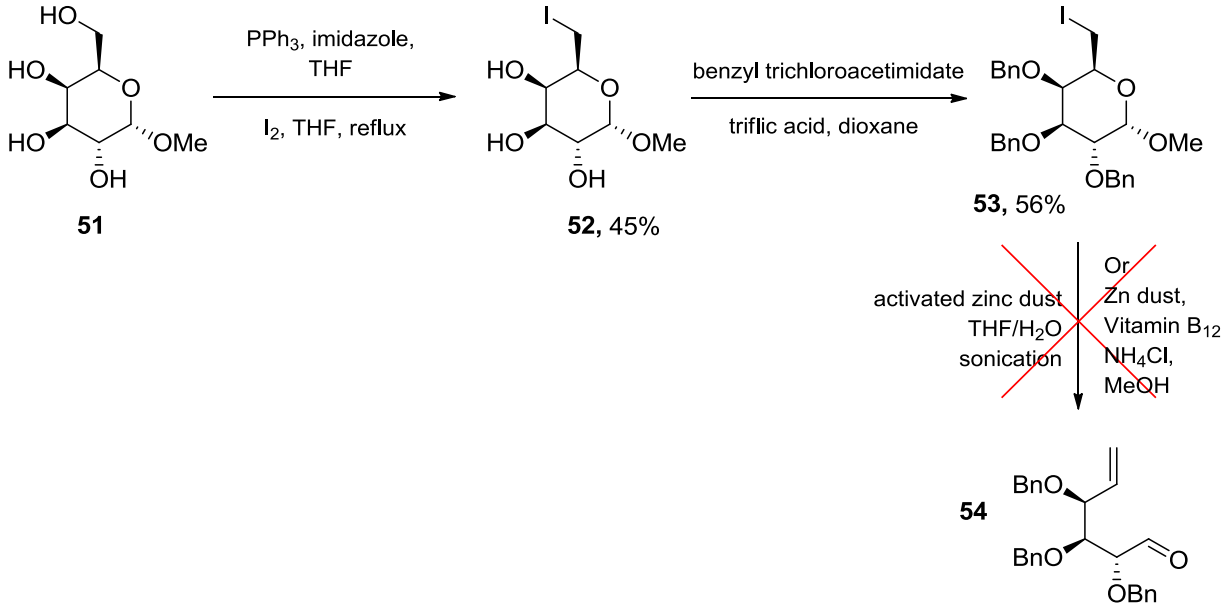
The 1,8-diene **47** will be synthesised via reaction of the Grignard allyl magnesium bromide **49** with the protected aldehyde **48**. These types of organometallic nucleophilic additions to α-alkoxy ketones can be highly stereoselective, as they can undergo chelation control, with the metal chelating with the two oxygen atoms, blocking that face, and so guiding the nucleophile

to attack from the opposite face.[136,137] However with compounds which have additional hydroxyl groups next to the α-alkoxy group, the stereoselectivity is often reduced, providing a mixture of diastereoisomers.[138,139] The reaction of our aldehyde **48** with allyl magnesium bromide to form 1,8-diene **47** is therefore likely to create diastereoisomers at the C(1) position, which will hopefully be separable. The aldehyde will be formed by a zinc-mediated fragmentation[140] of methyl-iodo-galactose. By using D-galactose as the starting material we now have an efficient synthesis, as the stereochemistry for three of the stereogenic centres is already defined as needed.

3.5.1 Synthesis of the Aldehyde

Aldehyde **54** was synthesised via a zinc-mediated fragmentation of methyl-6-deoxy-6-iodo-galactose **53**. We found in the literature a short synthetic route to the aldehyde by Skaanderup *et al.*,[141] and decided to follow their procedures (Scheme 3.3). The more reactive 6-position of methyl galactose was first converted to the iodide using triphenylphosphine and iodine, in the presence of imidazole, before protection of the remaining secondary hydroxyls with benzyl trichloroacetimidate and triflic acid. These acidic conditions ensure the survival of the iodide; basic conditions could deprotonate the unprotected hydroxyls, opening up the possibility for competing intermolecular nucleophilic substitution with the iodide. ^{13}C NMR spectroscopy confirmed the formation of the iodide on the 6-position, with the characteristically low CH_2 resonance at 3.6 ppm. The protected iodide was then subjected to a zinc-mediated fragmentation in THF/H_2O.[141,142] The addition of water was supposed to ensure that the surface of the zinc was kept free from adsorbants, and hence active for reaction, however in our hands this zinc-mediated fragmentation did not work, with only the starting material being recovered.

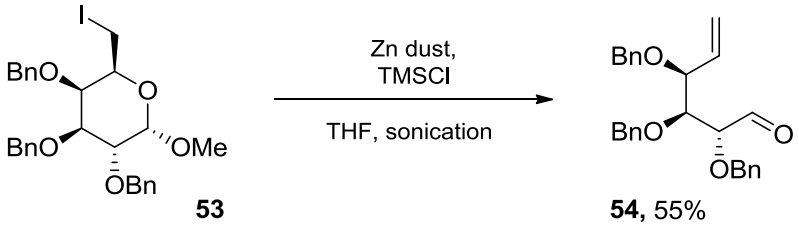
There are however many different conditions in the literature for zinc-mediated fragmentations, therefore we tried a few to see which was best.



Scheme 3.3. Failed synthetic route to aldehyde **54.**

Kleban *et al.* reported that zinc-mediated fragmentation of iodide **53** occurred in the presence of vitamin B_{12}, which was employed as a catalyst.[143] Under the reaction conditions, the Co(III) in vitamin B_{12} is reduced to Co(I), a strong nucleophile which forms intermediate Co(III) species with alkyl halides. These then undergo reductive fragmentation to give the aldehyde **54**.[143,144] In our hands however, this reaction only returned starting material (Scheme 3.3).

We tried another method with freshly activated zinc dust and trimethylsilyl chloride (TMSCl) in THF.[145] The reaction mixture was sonicated for 5.5 h (Scheme 3.4).



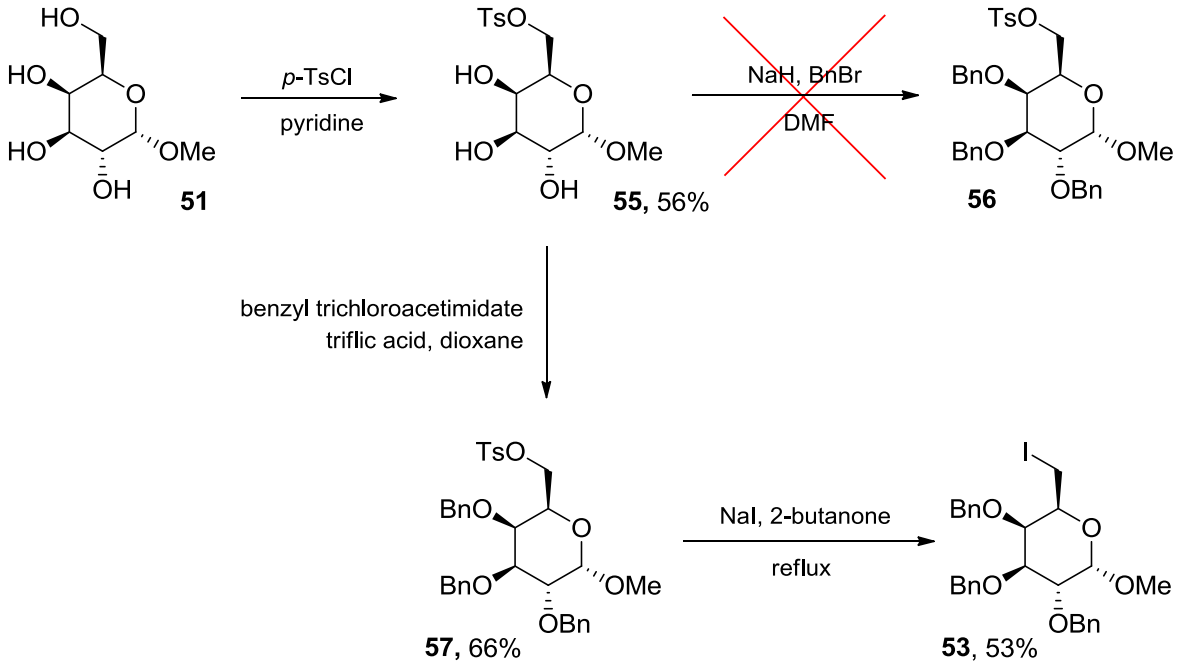
Scheme 3.4. Synthesis of aldehyde **54.**

Thankfully these conditions did give us the aldehyde **54**, although the yield was only moderate; however it furnished enough material to carry on the synthesis. TLC analysis of this reaction was quite tricky; the aldehyde has an R_f of 0.44 (16% EtOAc in hexanes), which is only very slightly above the iodide (R_f = 0.40 (16% EtOAc in hexanes)), therefore it was hard to tell when the reaction had gone to completion. Also the aldehyde did not stain in the conventional α-naphthol sugar stain. We therefore decided to use vanillin, a general purpose staining agent which tends to produce brightly coloured spots, and in our case it was now much easier to distinguish the two compounds; the iodide stained in a blackish brown colour, whereas the aldehyde was more of a reddish brown colour. Also the aldehyde tended to develop faster than the iodide upon heating, and at this lower temperature the aldehyde spot appeared orange.

We now had a suitable procedure for synthesising the aldehyde **54**, however the synthesis of the iodide **53** was also causing problems (Scheme 3.3). Purification of the unprotected iodide required a reverse phase silica column, which we did not have in our lab. We obtained some

reverse phase silica and purified the iodide using that, however in our hands this proved to be difficult. Therefore we decided to find a different route to the iodide.

The first route we attempted involved direct tosylation on the 6-position of unprotected galactose; this primary alcohol is usually more reactive than the other secondary alcohols, so regioselective reactions are possible.[146,147] Regioselective tosylation was almost entirely selective for the desired primary alcohol.[146,147] Initially for the subsequent benzylation we used sodium hydride and benzyl bromide in DMF, however this caused substitution of the tosyl group. The strongly basic nature of sodium hydride was probably the reason for this unwanted reaction, creating an alkoxide with one of the free hydroxyls, which resulted in intramolecular nucleophilic substitution and the formation of a bi-cycle, as evidenced by HRMS. Therefore we decided to use the acidic conditions of benzyl trichloroacetimidate and triflic acid in dioxane, which afforded the benzyl-protected tosylate **57** in 66% yield. We then tried to convert tosylate **57** to the iodide using sodium iodide in 2-butanone (Scheme 3.4).[148,149]

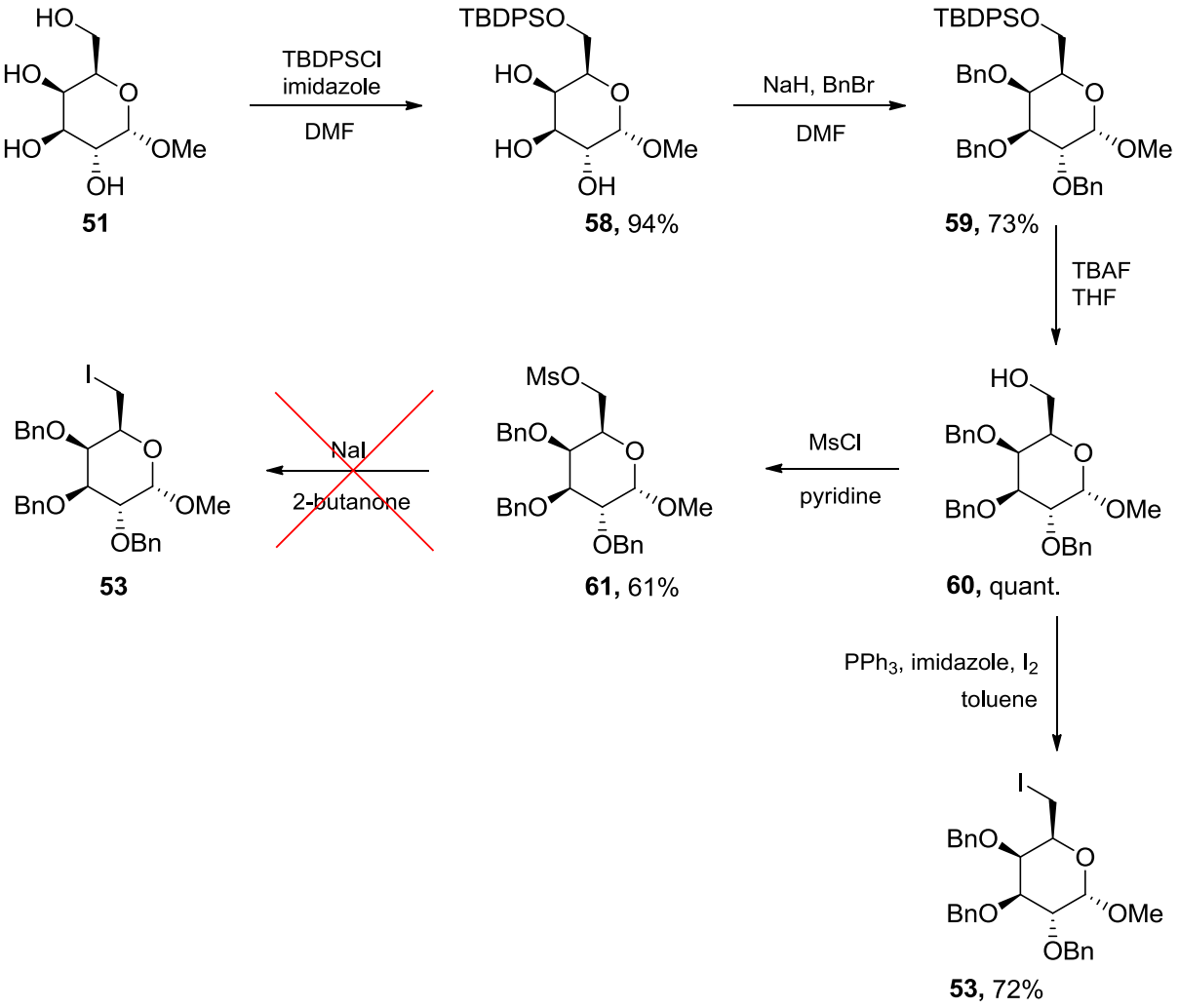


Scheme 3.4. Synthesis of iodide **53** via tosylation of the primary position.

However the Finkelstein reaction was very slow. We had elected to use 2-butanone as the solvent rather than the usual acetone due to its higher boiling point, however even with this higher temperature reflux the reaction was too slow, so we decided to investigate the more reactive mesylate. Although we could have installed the mesylate group directly on the 6-position of the unprotected galactose, as we had the tosylate, we decided against this route. The selective tosylation did not occur easily; the reaction took a long time and never seemed to go to completion, even with the addition of DMAP as a catalyst. Although the reaction was almost completely regioselective it was decided to go for a quicker and more reliable synthetic route, involving 6-position protection of galactose with a bulky silyl group, global protection of the remaining hydroxyls with an orthogonal protecting group before 6-position deprotection (Scheme 3.5). This provided protected galactose **60** with a free 6-position, which could then be

converted to mesylate **61,** before installation of the iodide via a Finklestein reaction as before (Scheme 3.5). However even with the more reactive mesylate group on the 6-position the Finkelstein reaction was very slow.

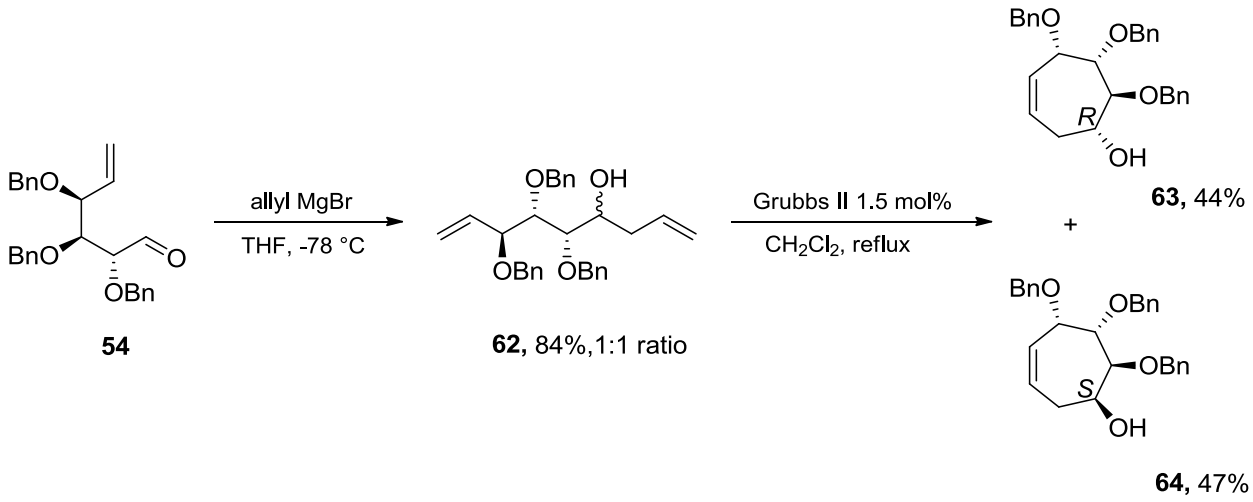
Reviewing the literature again we found a method to install the iodide directly onto the primary alcohol of **60**, by heating in toluene under reflux in the presence of PPh_3, imidazole and I_2,[150] which worked well (Scheme 3.5).



Scheme 3.5. Synthesis of iodide **53.**

3.5.2 Ring-Closing Metathesis

With a better synthetic route to the aldehyde **54** our attention turned to the ring-closing metathesis diene precursor.



54

62, 84%,1:1 ratio

63, 44%

64, 47%

Scheme 3.6. Synthesis of cycloheptenols **63** and **64.**

Reaction of commercially available allyl magnesium bromide with aldehyde **54** gave the required diene as a 1:1 mixture of diastereoisomers **62**, as predicted, which we did not separate at this stage (R_f = 0.47 (16% EtOAc in hexanes)). The protecting group on the α-alkoxy can govern the stereoselectivity, for example benzyl groups allow chelation control.[136] Therefore our lack of stereoselectivity is likely a consequence of the additional alkoxy groups also participating in chelation, creating a mixture of chelation products and hence providing a mixture of diastereoisomers.[139] The mixture of diastereoisomers **62** was subjected to ring-closing metathesis,[151] after which the two diastereoisomers **63** and **64** were separable by

column chromatography. We used Grubbs 2nd-generation Ru metathesis catalyst for the ring-closing metathesis and the reaction was complete within 2 hours. Grubbs 1st-generation Ru metathesis catalyst was also sufficiently active to cyclise this diene, only it required a longer reaction time, and higher catalyst loading (Scheme 3.6). To determine which of the products corresponded to which diastereoisomer we performed a hydrogenolysis on the two products. The (*R*)-stereoisomer **66** is non-symmetrical, whereas the (*S*)-stereoisomer **65** is C_2-symmetrical, therefore ^{13}C NMR spectroscopy allowed us to easily assign the products, as the C_2-symmetrical stereoisomer **65** had only four resonances compared with seven observed for its diastereoisomer **66**. From this analysis we were able to determine that the more polar product was **64**, the C_2-symmetrical stereoisomer and that the less polar product was **63**, the non-symmetrical stereoisomer (Figure 3.9).

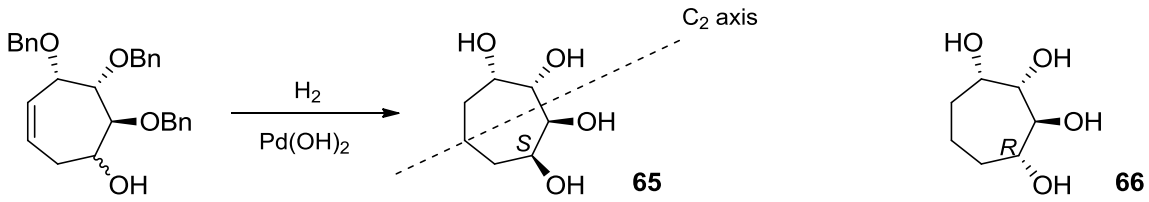
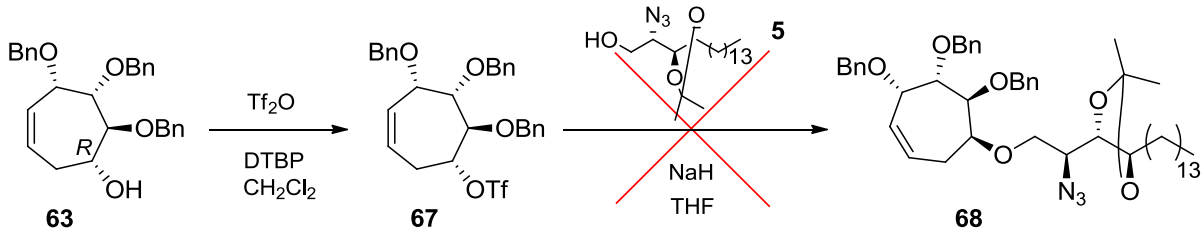


Figure 3.9. Showing the symmetry of the cycloheptan-tetraols **65** and **66**.

3.5.3 Etherification

Previously, when forming the ether bond of ThrCer, we had introduced a leaving group on the sugar and the phytosphingosine had acted as the nucleophile. To synthesise the correct

stereochemistry in this case we therefore needed to use the non-symmetrical stereoisomer, convert the free hydroxyl into a leaving group, and then react it with the alkoxide of a suitable phytosphingosine acceptor **10**. The leaving group previously used to synthesise the deoxy and truncated ThrCer analogues was a triflate, therefore we tried a triflate group with our cycloheptenol **63**.



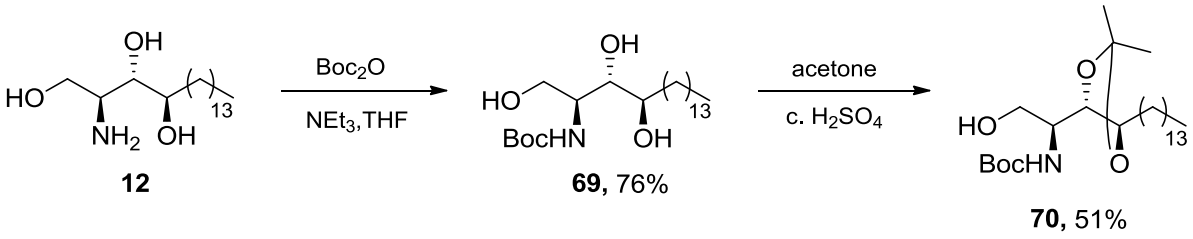
Scheme 3.7. Attempted Williamson etherification to form the cycloheptenyl ether **68.**

Formation of the triflate **67** proceeded without event, as evidenced by TLC analysis, however the etherification reaction did not work (Scheme 3.7). The triflate underwent preferential elimination, resulting in a substituted cycloheptadiene, as confirmed by mass spectrometry. Whilst we could have investigated some less reactive leaving groups, we decided instead to make the ether bond using the C_2-symmetrical stereoisomer **64**, and so we needed to install a leaving group on the phytosphingosine.

The first leaving group we trialled was again the triflate. TLC analysis indicated that formation of the triflate was successful, however the subsequent etherification did not work, providing no isolable products. Therefore we went to the less reactive mesylate, which was also

unsuccessful. For both these reactions we propose that the azide could be interfering in the reaction, perhaps by some kind of intramolecular elimination to form a five-membered ring. We therefore replaced the azide with a Boc group; however in this case, triflate formation did not occur cleanly as the Boc group was removed under the reaction conditions.

After another literature search a method by Tashiro *et al.*[116] looked promising; they had used a sulfamidate on the sphingosine to act as the leaving group. They had used an *N*-benzyl protected sulfamidate, however since hydrogenolysis of benzylamines can be difficult, we first decided to try protecting the nitrogen as a Boc carbamate (Scheme 3.8).

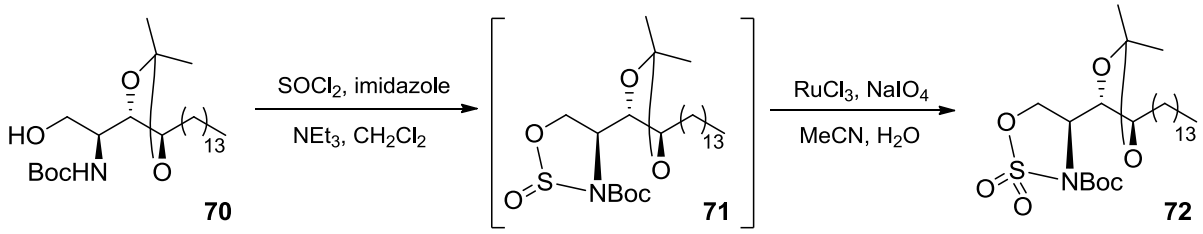


Scheme 3.8. Synthesis of Boc amino-protected phytosphingosine **70.**

Phytosphingosine **12** was reacted with Boc_2O in the presence of NEt_3 to give Boc-protected amine **69,** which was dissolved in acetone before the addition of a catalytic amount of c. H_2SO_4 to provide protected amine **70**. This was a fast route to a Boc-protected acceptor **70**, although for the last step we only isolated a 51% yield due to formation of the di-acetal product (with an N-O acetal). However, the ease of this route made up for that loss. As this is only a starting

material we could afford to perform large-scale reactions to provide enough material, and the sphingosine is easily recoverable.

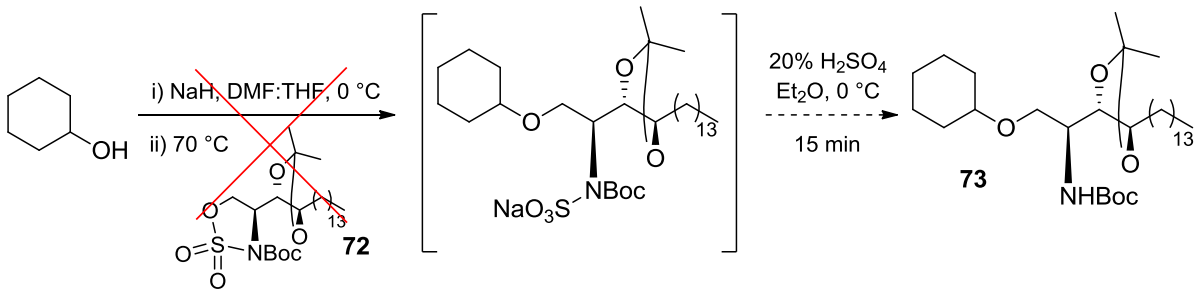
The sulfamidate was formed by reacting alcohol **70** with thionyl chloride, and then oxidising the resulting sulfamidite **71** with ruthenium(III) chloride and sodium periodate (Scheme 3.9).[116] Reaction with sulfuryl chloride to make the sulfamidate directly is generally not done, as flexible amino alcohols often form aziridines rather than sulfamidates.[152]



Scheme 3.9. Synthesis of Boc sulfamidate **72.**

The formation of the sulfamidate **72** proceeded without any problems. The sulfamidite was formed as an inconsequential mixture of diastereoisomers, which upon oxidation converged into one product. A test reaction with cyclohexanol, using the same conditions as we would employ for our ring compounds, was then performed (Scheme 3.10). The sodium alkoxide of cyclohexanol was heated to 70 °C in the presence of Boc sulfamidate **72**. This should provide the sodium salt of ether **73**, which would be hydrolysed using 20% H_2SO_4 to afford ether **73**. However this reaction did not work. The Boc group was removed under the reaction

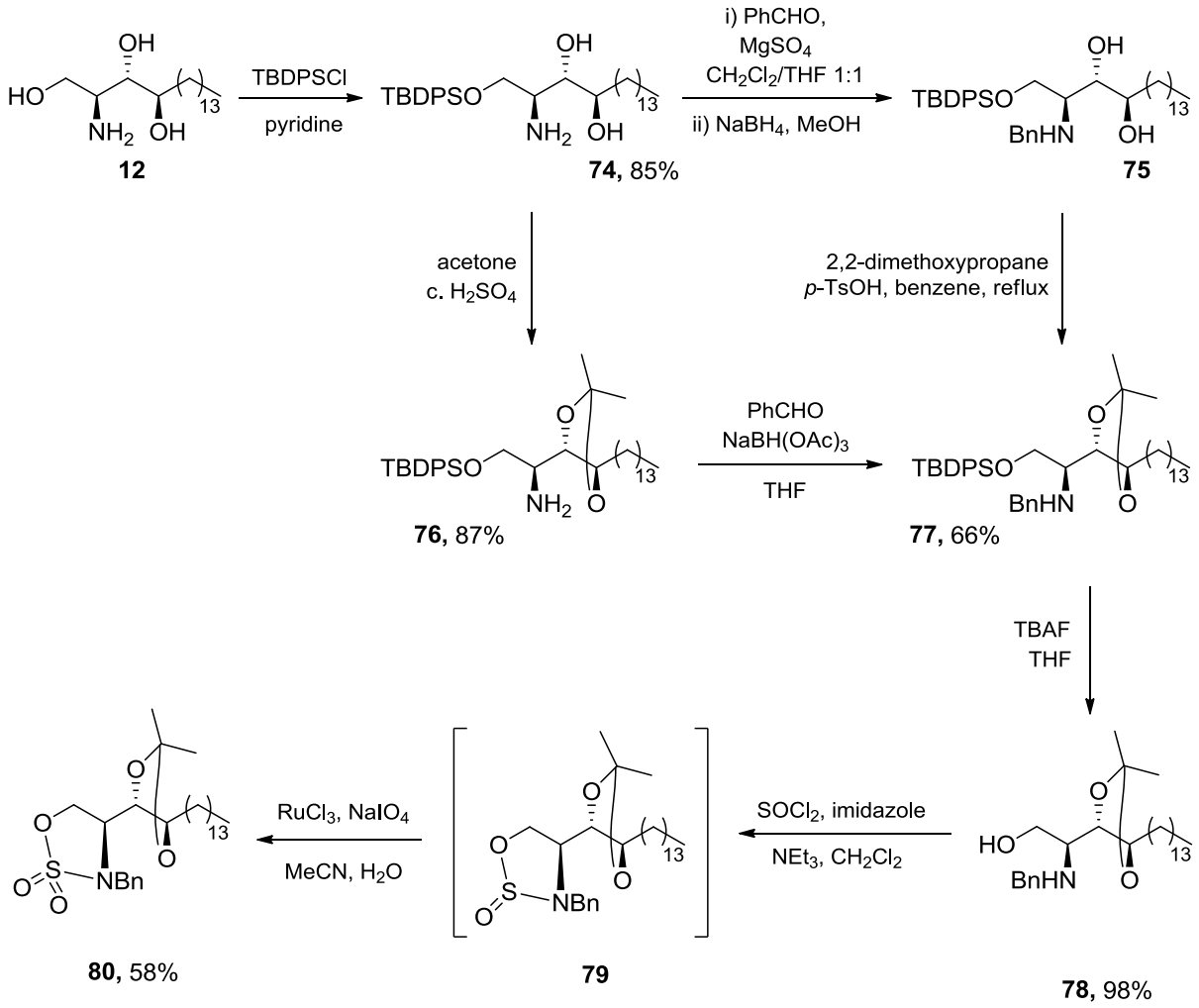
conditions, so all that could be isolated was the sphingosine with a free amine. Therefore we decided to protect the amine with a benzyl group, as Tashiro *et al*. had previously reported.[116]



Scheme 3.10. Failed test reaction with the Boc sulfamidate **72.**

We first tried a two-step reductive amination of phytosphingosine **12** with benzaldehyde and sodium borohydride. However we encountered problems in the second step, namely reducing the imine. It was thought that the primary alcohol was interfering with this reaction, so we protected the primary alcohol with a TBDPS group, giving diol **74** before reattempting the reductive amination, which was now successful; however we also devised an alternative route, in which we also protected the internal diol with an acetal, before attempting the reductive amination (Scheme 3.11). We found that by having all the other groups protected, the reductive amination proceeded far more efficiently. Also rather than using sodium borohydride as the reducing agent, which requires a two-step process, we used sodium triacetoxyborohydride instead.[153] Sodium borohydride requires a two-step process as it will reduce the aldehyde before it can react with the imine. Sodium triacetoxyborohydride is more selective for the iminium species,[153] and so can be added together with the aldehyde and the

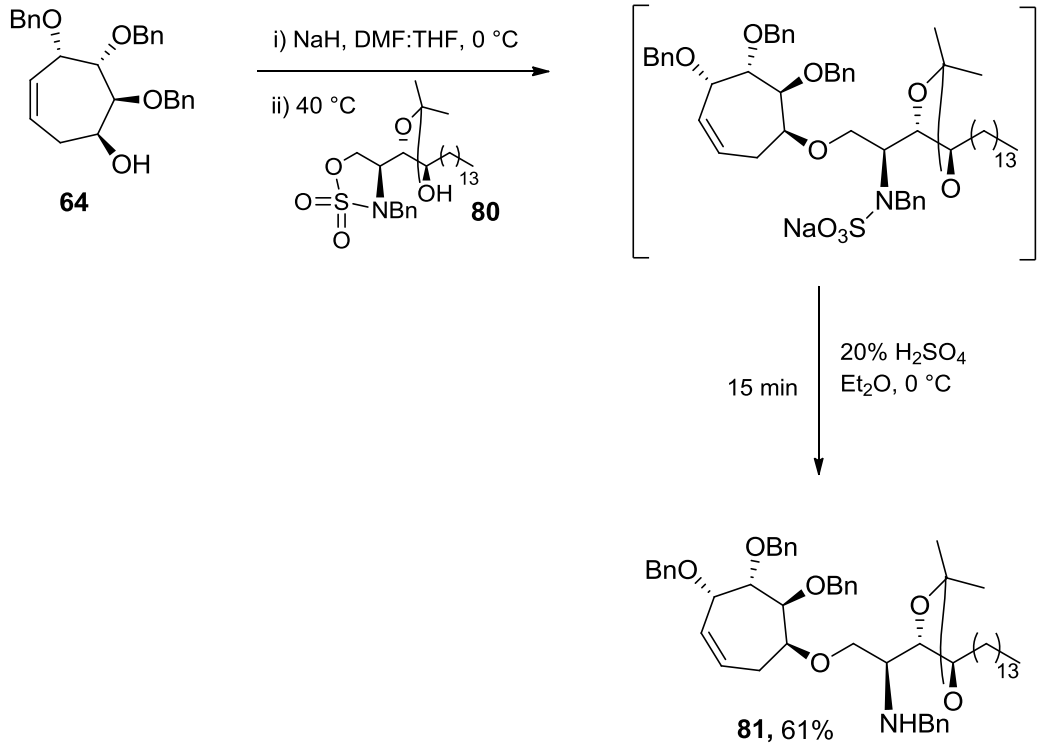
amine for a one-pot reaction. The TBDPS group of sphingosine **77** was then removed with TBAF, before synthesis of the sulfamidate as before (Scheme 3.11). Formation of the sulfamidate was evidenced by HRMS, and the ^{13}C NMR spectrum, which showed a downfield shift of the *C*H_2O resonance from 60.6 ppm in alcohol **78** to 68.1 ppm in sulfamidate **80**.



Scheme 3.11. Synthesis of benzyl-protected sulfamidate **80.**

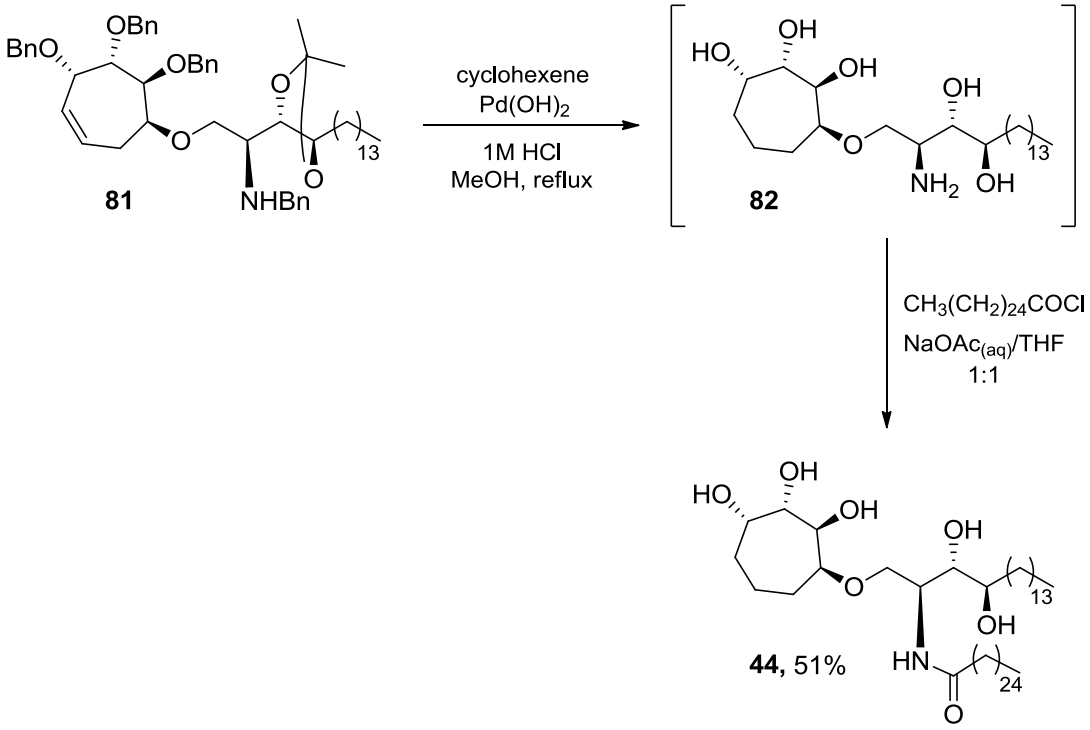
The Bn sulfamidate was then subjected to the same etherification test reaction with cyclohexanol (Scheme 3.10), and thankfully we were now able to isolate the desired ether product.

The Bn sulfamidate **80** was therefore heated with the sodium alkoxide of cycloheptenol **64** at only 40 °C compared to the 70 °C we had used for the cyclohexanol test reaction. This lower temperature was sufficient to effect full consumption of the Bn sulfamidate **80** starting material. This reaction formed the sodium salt of ether **81**, which was hydrolysed using 20% H_2SO_4 to furnish fully protected cycloheptenyl ether **81** (Scheme 3.12). The time duration (15 min) of the acid hydrolysis was important in determining the level of protection in the cycloheptenyl ether **81**, as the internal diol in the sphingosine unit is protected with an acetal, which is also acid labile. A duration of 15 min did not affect the acetal in this product, a longer time period would likely result in removal of the internal acetal group, to provide a partially deprotected ether product, which could be useful, depending on how the deprotection of cycloheptenyl ether **81** proceeds.



Scheme 3.12. Synthesis of the cycloheptenyl ether **81.**

The benzyl groups and the double bond in cycloheptenyl ether **81** were then removed by a transfer hydrogenolysis using cyclohexene and $Pd(OH)_2$,[116] before being acylated with freshly made C_{26} acid chloride. This hydrogen transfer reaction was not particularly clean, which was probably due to the number of benzyl groups being removed at once, and also the deprotection of the acetal from the internal diol, from the small amount of acid added. The acid was included in the reaction conditions to protonate the deprotected amine, and hence stop it from adsorbing to, and poisoning, the Pd catalyst. Gratifyingly, the transfer hydrogenolysis afforded the fully deprotected amine **82** as the major product. This amine **82** was then acylated with the C_{26} acid chloride to provide cycloheptyl ThrCer analogue **44** (Scheme 3.13).



Scheme 3.13. Synthesis of the cycloheptyl ThrCer analogue **44.**

3.6 Synthesis of the Cyclohexyl ThrCer Analogue

Now that we had synthesised the cycloheptyl ThrCer analogue **44,** the cyclohexyl ThrCer analogue **43** should be simpler. The synthetic route for forming the ether bond would be the same, the only significant difference in the total synthesis being formation of the cyclohexenol ring itself.

From the synthesis of the cycloheptyl ThrCer analogue **44,** we knew that we wanted the C_2-symmetrical stereoisomer. If we make the cyclohexenol ring via ring-closing metathesis it will contain a double bond, therefore the likely structure of the cyclohexenol etherification precursor is shown below (Figure 3.10).

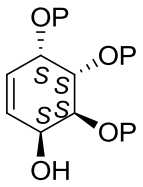
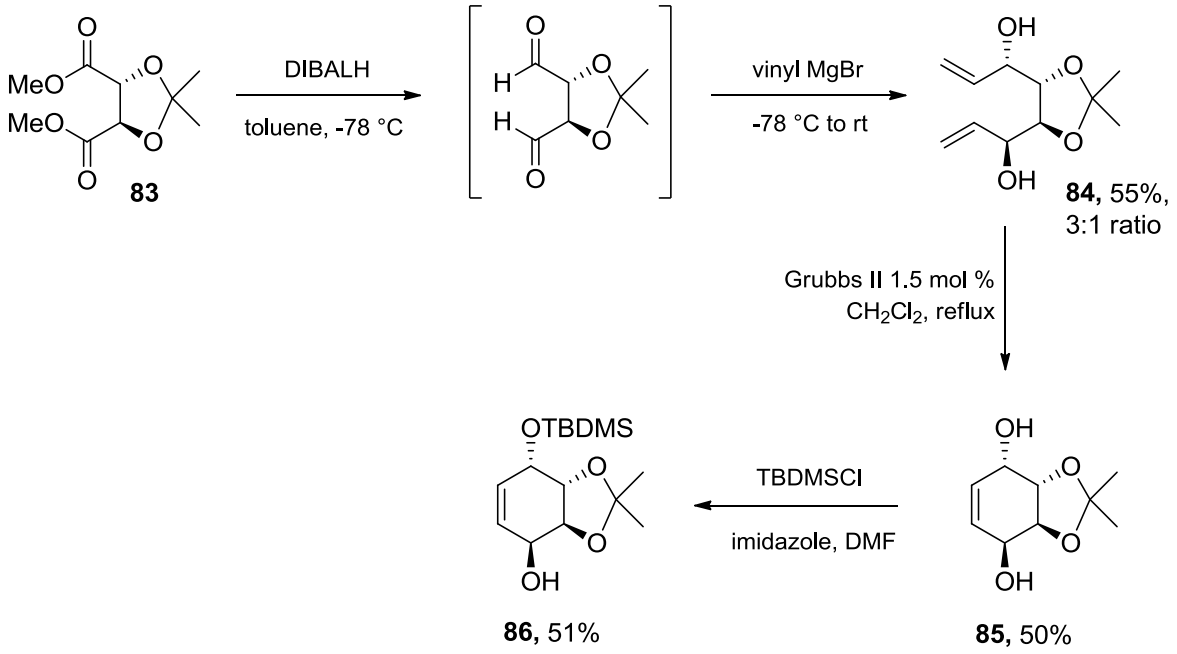


Figure 3.10. Structure of the cyclohexenol ring etherification precursor.

This structure is the same as that of a product called (+)-conduritol E. There are a group of six isomeric compounds called conduritols, which have this same structure but the hydroxyl groups have different relative stereochemistries. Conduritol E has the hydroxyl groups in the same stereochemistry as we need. There have been a few syntheses of conduritol E,[154-156] many of which employ a dialkyl (*2R,3R*)-2,3-*O*-isopropylidenetartrate as the starting material.

This di-ester is reduced to the di-aldehyde *in situ*, before undergoing a double Grignard reaction with vinyl magnesium bromide. The resulting diene can then be subjected to ring-closing metathesis, giving conduritol E with the 1 and 4-OH groups free. This would then need to be mono-protected before reaction with the sulfamidate (Scheme 3.14).



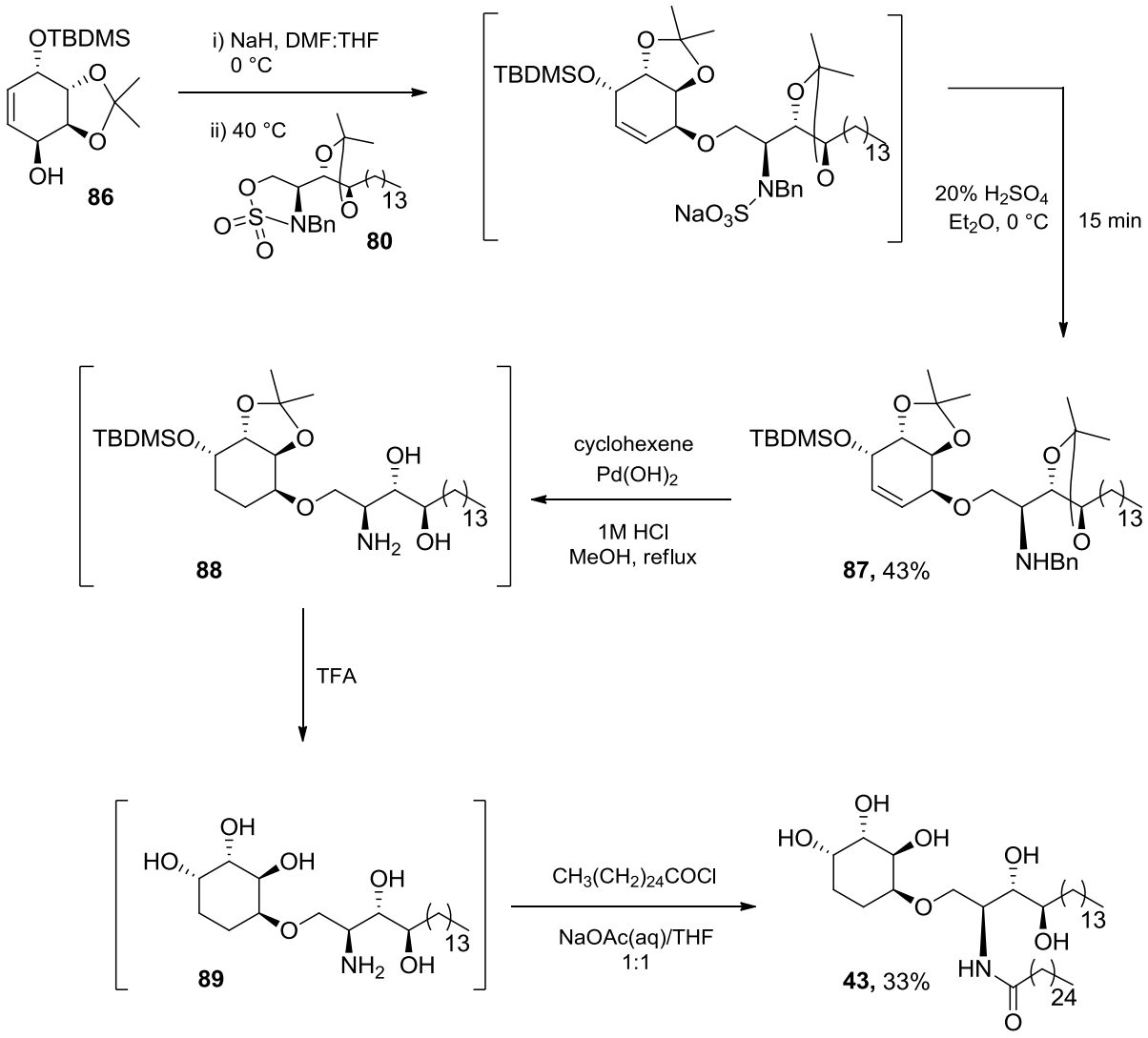
Scheme 3.14. Synthesis of the cyclohexenol **86.**

This is potentially a fast route to the cyclohexenol **86** with the desired absolute and relative configuration. Dimethyl (*2R,3R*)-2,3-*O*-isopropylidenetartrate **83** was reacted with two equivalents of DIBALH at −78 °C to provide the di-aldehyde, which was trapped *in situ* with vinyl magnesium bromide to supply the 1,7-diene **84**. Only two equivalents of DIBALH and low

temperature were used to ensure each ester was reduced to the aldehyde and not further reduced to the alcohol. The double Grignard reaction should produce four diastereoisomers, however the major isomer formed was the target compound **85**, in a 3: 1 ratio (with the one accounting for the remaining three diastereoisomers). This is probably due to chelation control, as the two α-alkoxy stereocentres are the same stereochemistry we want for the forming hydroxyl groups. The magnesium chelates with the α-alkoxy and the carbonyl oxygen, orientating them onto the same face and blocking that face, so alkylation can only occur on the opposite face. The diastereoisomers were inseparable at this stage, therefore the mixture was subjected to ring-closing metathesis (Scheme 3.14). Lee and Chang have reported problems with performing ring-closing metatheses on this unprotected compound.[155] The group hypothesised that the molecule could not ring close due to the strain caused by the *trans* isopropylidene group. However it could also have been because they had only used Grubbs 1st-generation Ru metathesis catalyst, which Ackermann *et al.* had reported to provide no product yield when metathesising compounds with free hydroxyls.[154] Indeed Ackermann *et al.* reported that this unprotected 1,7-diene **84** could undergo ring closing metathesis using Grubbs 2nd-generation Ru metathesis catalyst.[154] Therefore we decided to try the metathesis with Grubbs 2nd-generation Ru metathesis catalyst on the unprotected compound first, and this worked very well. The reaction only required two hours of heating under reflux; the greater reactivity of the 2nd-generation catalyst could therefore be necessary for cyclising this compound. The ring-closing metathesis provided two separate spots by TLC analysis; the more non-polar spot was the desired C_2-symmetrical stereoisomer **85**, as determined by comparison to literature NMR data.[154] The more polar spot appeared to be a mixture of the remaining diastereoisomers. The desired C_2-symmetrical cyclohexendiol **85** now had to be monoprotected. Initially we tried to monoprotect with TBDPS, however this bulky silyl group would not react, probably due to

steric hindrance. Therefore we tried monoprotecting with TBDMS, a smaller silyl protecting group, which now did react as intended to give alcohol **86**. Cyclohexendiol **85** was reacted with 1.2 equivalents of TBDMSCl, which provided a mixture of the mono-protected, di-protected and unreacted products, with the mono-protected cyclohexenol **86** being the major product in a 5:1:2 ratio. These products were also easily separable by column chromatography, allowing recovery of the unreacted product and the di-protected product, which could be deprotected with TBAF to afford the starting diol (Scheme 3.14).

The synthesis of the ether bond now followed the same route as for the cycloheptyl ether **44**, the only difference was in the deprotection step, as the two different rings contain different protecting groups. The cycloheptenol **54** only contained benzyl protecting groups, therefore when deprotecting the NBn all the other protecting groups were also removed. The sphingosine contained one acetal protecting group, which was also removed when deprotecting the NBn, due to the addition of a small amount of acid. The cyclohexenol **86** however contained a TBDMS group and an additional acetal. Since these groups were not removed with the small amount of acid added to the transfer hydrogenolysis, an additional deprotection step was needed. Due to the acid-labile nature of both these groups, global deprotection was afforded by treatment with TFA, before acylation as before to give the target compound **43** (Scheme 3.15).

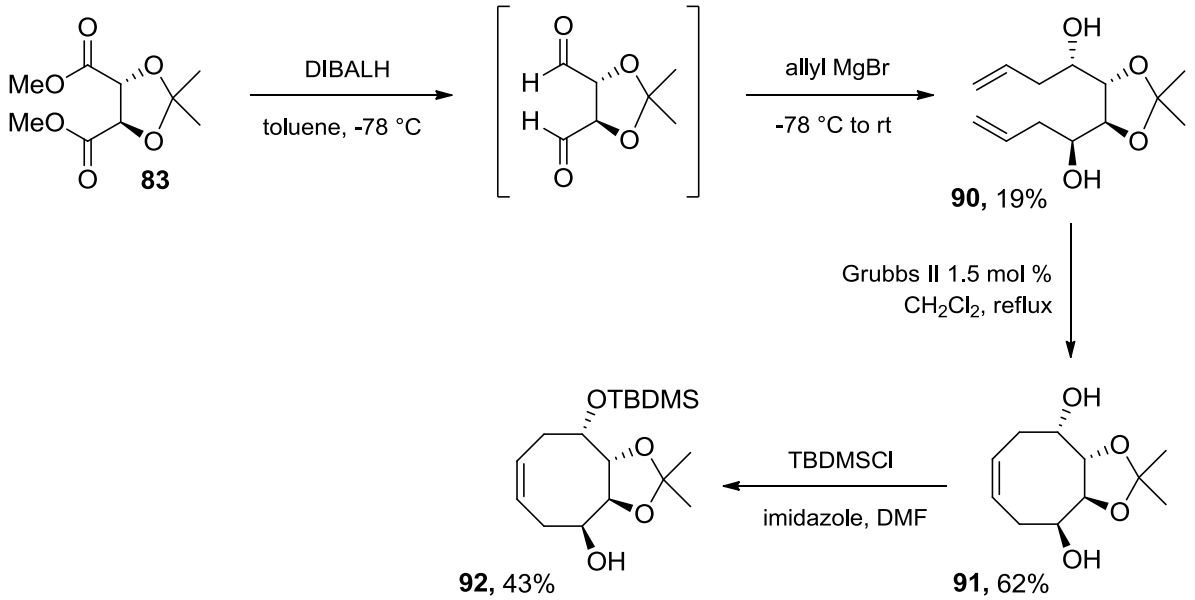


Scheme 3.15. Synthesis of the cyclohexyl ThrCer analogue **43.**

3.7 Synthesis of the Cyclooctyl ThrCer Analogue

We also wanted to synthesise the cyclooctyl ThrCer analogue **45**, due to the increased flexibility of the ring, and also to investigate whether additional carbon atoms in the ring might interfere with the TCR binding. We were unsure as to whether there might be too many carbons in a cyclooctane for it to be able to adopt a conformation that the TCR can recognise, but the additional flexibility could allow it to mould itself to the shape of the TCR and hence give very good binding. In fact the extra carbons in the ring could potentially provide additional hydrophobic interactions with the TCR, as there is a non-polar proline residue in the area that the extra lipid portion of the ring would come into contact with.

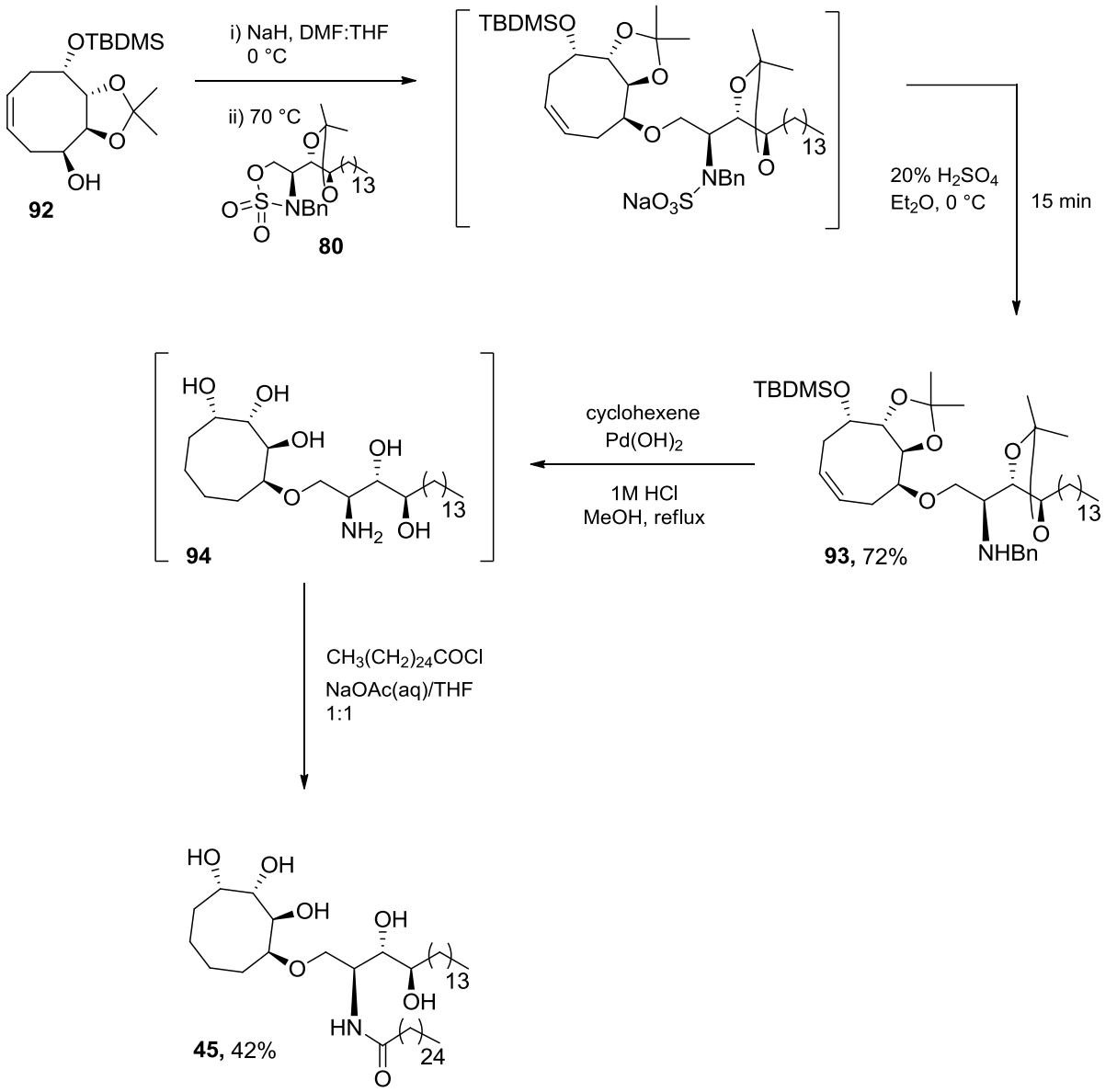
Synthesising cyclooctenol **92** proceeded in a similar fashion to that used for cyclohexenol **86**, only allyl magnesium bromide was used instead of vinyl magnesium bromide in the double Grignard reaction (Scheme 3.16).



Scheme 3.16. Synthesis of cyclooctenol **92.**

In this case of bis-allylation, the desired major diastereoisomer **90** was separable from the other diastereoisomer diols. The ring-closing metathesis on 1,9-diene **90** proceeded without any problems to form the cyclooctendiol **91**, as did mono silyl-etherification to give cyclooctenol **92**.

The synthesis of the ether bond now followed that of the cyclohexyl ThrCer analogue **43**. Formation of the ether bond using the cyclic sulfamidate proceeded without any problems to give ether **93**, which was then subjected to transfer hydrogenolysis as before. In the case of the cyclohexenyl ether **87,** the transfer hydrogenolysis had removed the NBn group and one acetal. When the same reaction was performed on the cyclooctenyl ether **93**, all of the protecting groups were fortuitously removed. This could be because a cyclooctane is less sterically hindered, and hence the protecting groups might be more accessible to the acid in the reaction. Transannular effects are also likely to be important. Fully deprotected amine **94** from the transfer hydrogenolysis was then acylated with freshly prepared hexacosanoyl chloride to give target compound **45** (Scheme 3.17).

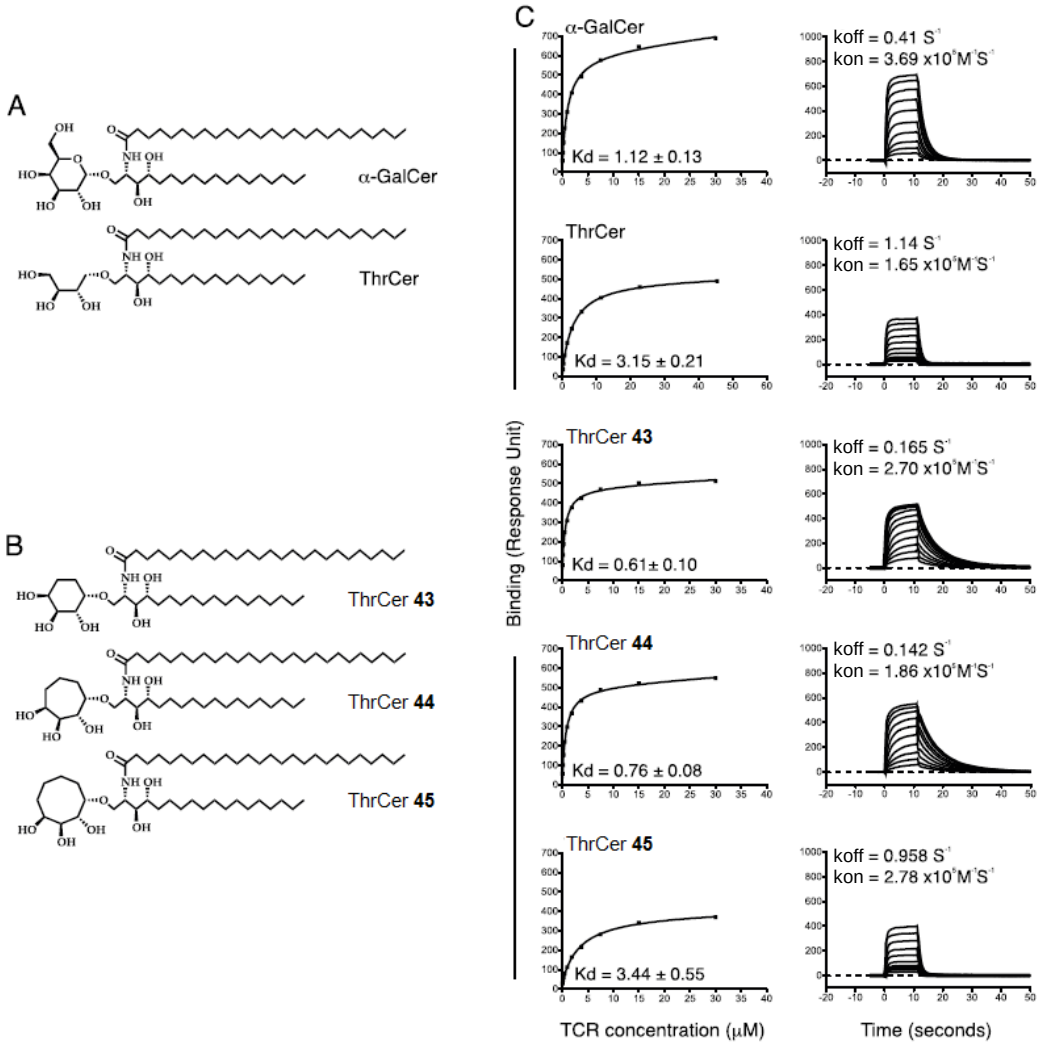


Scheme 3.17. Synthesis of cyclooctyl ThrCer analogue **45.**

3.8 Biological Analysis

Analogues **43**, **44** and **45** were submitted for biological testing, which was carried out by Dr John-Paul Jukes and Dr Hemza Ghadbane, members of Prof. Vincenzo Cerundolo's group at the Weatherall Institute of Molecular Medicine in Oxford, UK.

Initial testing was for binding affinity of the glycolipid/hCD1d complex to the human iNKT cell TCR using BIAcore analysis. Our analogues, together with α-GalCer and ThrCer as controls, were first refolded by oxidative refolding chromatography with bacterially-expressed hCD1d and β_2m molecules before being immobilised on a BIAcore chip. Increasing concentrations of soluble human iNKT cell TCR were then passed over the chip for 5 seconds until the specific binding reached its plateau (Figure 3.11). We can see that the cyclohexyl ThrCer ring analogue **43** does bind to the iNKT cell TCR, indicating that it likely adopts the desired chair conformation to be recognised by the TCR. The cyclohexyl ThrCer analogue **43** and the cycloheptyl ThrCer analogue **44** have a much more sustained binding to the iNKT cell TCR than does ThrCer, as was hoped for by constraining the threitol head unit into a ring and reducing its conformational flexibility. Moreover they also bind to the iNKT cell TCR for longer than α-GalCer. This might be because of the removal of the ring oxygen; crystal structures show that the iNKT cell TCR contains a proline in the area which would be close to the ring oxygen, so causing repulsion between the glycolipid and the TCR, which could destabilise the complex. Our ring analogues have removed the ring oxygen, and therefore removed this repulsion and at the same time introduced extra hydrophobic interactions, resulting in a more stable ternary complex. The cyclooctyl ThrCer analogue **45** exhibited similar binding affinity behaviour to ThrCer and α-GalCer. This could be because the extra carbons in the eight-membered ring cause steric issues in the binding site (Figure 3.11).



Lipid on CD1d	K_d (μM)	k_{off} (s^{-1})	k_{on} (M^{-1} s^{-1})
α-GalCer	1.12 ± 0.13	0.41	3.69×10^5
ThrCer	3.15 ± 0.21	1.14	1.65×10^5
Cyclohexyl ThrCer analogue **43**	0.61 ± 0.10	0.165	2.70×10^5
Cycloheptyl ThrCer analogue **44**	0.76 ± 0.08	0.142	1.86×10^5
Cyclooctyl ThrCer analogue **45**	3.44 ± 0.55	0.958	2.78×10^5

Figure 3.11. Binding affinities of the iNKT TCR for hCD1d molecules loaded with cyclic ThrCer analogues **43**, **44** and **45**. The structures of α-GalCer and ThrCer (A), and ThrCer **43**, **44** and **45** (B) are shown. Equilibrium binding and kinetic measurements of a

soluble human iNKT cell TCR were assessed for hCD1d molecules refolded with α-GalCer, ThrCer and cyclic ThrCer analogues **43**, **44** and **45** (C). K_d values (μM) were calculated from equilibrium binding.

The analogues were then tested for activation of murine iNKT cells, *in vitro* and *in vivo*. For the *in vitro* testing splenocytes from naive mice were incubated for 40 hours with various concentrations of our analogues, before determining the amount of IFN-γ released into the supernatant by ELISA (Figure 3.12, A). We can see from the graph that the amount of IFN-γ released by murine iNKT cells stimulated by the cyclohexyl and cycloheptyl ThrCer analogues **43** and **44** is comparable to that released by α-GalCer-stimulated iNKT cells and much higher than that caused by the parent ThrCer. The cyclooctyl ThrCer analogue **45** is very similar to that of ThrCer; incorporating the threitol unit into a cyclooctane does not appear to restore biological activity. The analogues were then tested *in vivo*, and the results corroborate those seen *in vitro* (Figure 3.12, B).

Our analogues were injected intravenously into mice before being tail-bled at 2 h, and the blood serum measured for IL-4 release by ELISA. After 18 h, the mice were sacrificed and the blood serum measured for the amount of IFN-γ released by ELISA. The cyclohexyl and cycloheptyl ThrCer analogues **43** and **44** both cause the secretion of significantly more IL-4 and IFN-γ than ThrCer, and are again similar to the amount released by α-GalCer-stimulated iNKT cells. Both analogues **43** and **44** also elict a mixed T_H1 and T_H2 cytokine profile, with significant levels of both IL-4 and IFN-γ released. There is no skewing towards T_H1 for our ether linked analogues, unlike the ether linked α-carba-GalCer and the amino linked HS161 and HS44 (Chapter 3.2). The cyclooctyl ThrCer analogue **45** proved inactive *in vivo* in mice, with no cytokine release observed.

The ability of our analogues to cause DC maturation was also tested *in vivo* in mice (Figure 3.12, C). This is measured by the level of up-regulation of the co-stimulatory molecule CD86. When the mice were sacrificed at 18 h the spleen was removed and the splenocytes stained with anti-CD11c and CD86. The level of expression of CD86 was then measured using flow cytometry. From the graph we can see that the cyclohexyl and cycloheptyl ThrCer analogues **43** and **44** cause a similar level of DC maturation to α-GalCer, which is higher than that of ThrCer. Again the cyclooctyl ThrCer analogue **45** is inactive *in vivo*, with no CD86 up-regulation seen.

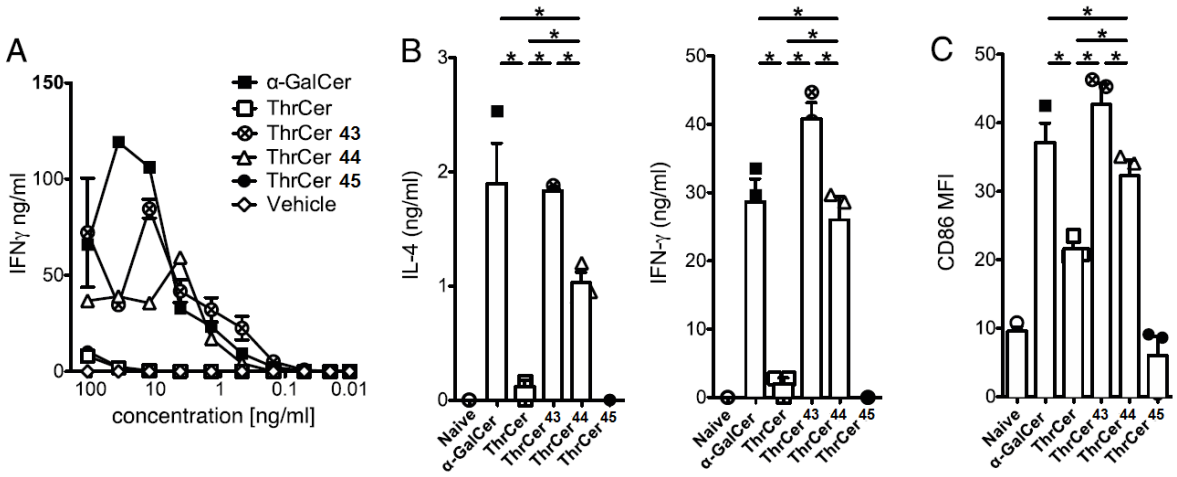


Figure 3.12. Cyclic six-, seven- and eight-membered ThrCer analogues **43**, **44** and **45** activate murine iNKT cells. Splenocytes from naïve mice were incubated with various concentrations of lipids for 40 h and IFN-γ in the supernatant detected by ELISA (A). Mice were immunised with lipids i.v. and IL-4 and IFN-γ detected in blood sera at either 2 h or 18 h, respectively, by ELISA (B). At 18 h, immunised mice were sacrificed and splenocytes stained with anti-CD11c and CD86 to determine the extent of maturation by the expression of CD86, on gated DCs ($CD11c^+$), using flow cytometry (C). *Median Fluorescent Intensity = MFI. Error bars are mean ± SEM (A - quadruplicate wells; C-D n=3/group). *p < 0.05.*

Mouse iNKT cells have a slightly different homology to human iNKT cells; sometimes compounds can be active in mice but not activate human iNKT cells. Therefore our analogues were also tested *in vitro* with human iNKT cells. To this end, our analogues were added to hCD1d C1R cells and incubated overnight. After washing, human iNKT cells were added and the mixture incubated for 40 h, after which the amount of IFN-γ released by the iNKT cells into the supernatant was measured by ELISA. These results mirror those seen by murine iNKT cells. The cyclohexyl and cycloheptyl ThrCer analogues **43** and **44** showed significant activation of iNKT cells and release of IFN-γ, much greater than that of ThrCer and comparable to α-GalCer. The cyclooctyl ThrCer analogue **45** caused the release of similar amounts of IFN-γ to that of ThrCer (Figure 3.13, A). DC maturation was also measured, by co-culturing DC with human iNKT cells and our analogues for 40 h, before determining the level of CD86 upregulation. Again the cyclohexyl and cycloheptyl ThrCer analogues **43** and **44** caused similar levels of maturation as α-GalCer, which is higher than that seen with ThrCer. The cyclooctyl ThrCer analogue **45** was on a par with ThrCer (Figure 3.13, B).

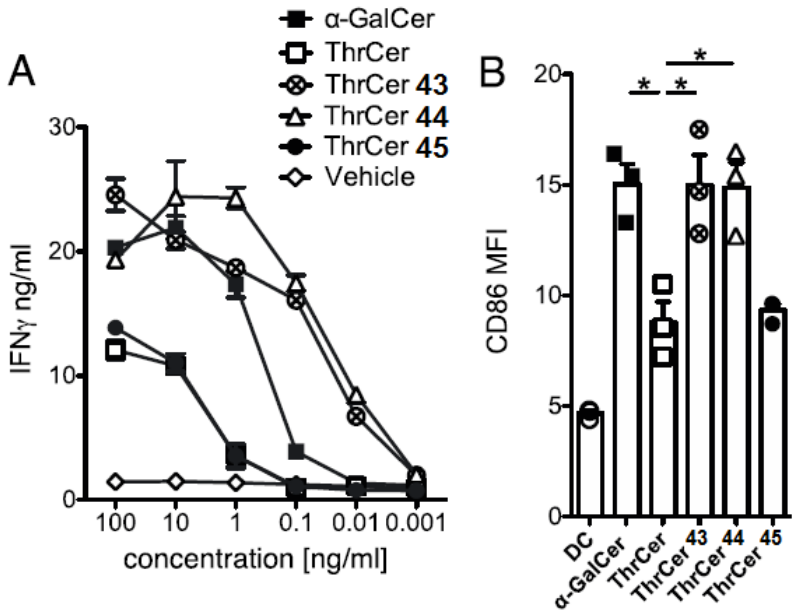


Figure 3.13. Cyclic six-, seven- and eight-membered ThrCer analogues **43**, **44** and **45** activate human iNKT cells. iNKT cell agonists were added to hCD1d C1R cells overnight at various concentration, washed and human iNKT cells added. At 40 h, IFN-γ in supernatant was determined by ELISA (A). Human DC maturation was assessed following coculture with human iNKT cells and l μg lipids after 40 h, as determined by CD86 upregulation on DCs (B) *Median Fluorescent Intensity = MFI. Error bars are mean ± SEM (quadruplicate wells). *p < 0.05.*

3.9 Conclusions and Future Work

The cyclohexyl and cycloheptyl ThrCer analogues **43** and **44** display significant iNKT cell-activating properties, both *in vitro* and *in vivo*, in both murine and human iNKT cells. These two analogues cause the secretion of large amounts of IL-4 and IFN-γ, and cause the maturation of DC, as evidenced by the up-regulation of CD86. Using a cyclohexane or cycloheptane to constrain the threitol head group appears to have restored biological activity back to the levels seen in α-GalCer. However, like α-GalCer, the cytokine profile is mixed, with significant amounts of both T_H1 and T_H2 cytokines released. The cyclooctyl ThrCer analogue showed no activity *in vivo* and activity similar to that of ThrCer *in vitro*, suggesting that a cyclooctyl ring is not as well tolerated.

The fact that the cyclohexyl ThrCer analogue **43** is recognised by the iNKT cell TCR is interesting, as we were unsure at the outset as to whether the conformation of this analogue would allow recognition. From these results we know that the ground state chair conformation of the cyclohexyl ThrCer analogue **43** must position the ceramide unit in an axial orientation, or that the energy of interconversion between the two chair conformations is very low. It would be interesting to do some computational calculations in the future to try to determine which of these scenarios is correct.

This synthetic route has not been optimised, and due to the promising biological results it would be useful to simplify the scheme for the future. One way we could do this is by changing the amine protecting group on the sulfamidate. The current synthetic route used a benzyl group, which required a lot of protection / deprotection steps before resulting in the sulfamidate. Carbamate protecting groups would reduce the number of steps required to

synthesise the sulfamidate. We already tried the Boc group, which did not survive the etherification conditions, however there are other carbamate groups which could be trialled, for example the Z group, which can be removed by hydrogenolysis.

The lack of cytokine bias displayed by these compounds is another aspect that we would like to investigate. Compounds which produce a cytokine bias would be more useful in treating specific diseases. It is known that certain structural modifications can alter the cytokine profile towards either T_H1 or T_H2, therefore we propose to synthesise analogues which replace the C_{26} acyl chain with a C20:2 chain, which causes a T_H2 bias (Page 47), and also synthesise analogues which replace the amide linkage with a thioamide, which results in a T_H1 bias (Page 52).

3.10 Synthesis of Double Bond-Containing Constrained Ring ThrCer Analogues

The constrained ring ThrCer analogues appear to be very attractive CD1d agonists, retaining the ability to activate the immune system at a comparable level to α-GalCer while reducing some of the problems associated with α-GalCer. Reducing the conformational flexibility of the sugar head group in ThrCer has restored activity back to levels seen in α-GalCer, therefore it would be interesting to investigate other cyclic analogues which might have different low-energy conformations. With what little time we had left, we decided to investigate the synthesis of conformationally less flexible analogues (Figure 3.13). This reduction in conformational flexibility could be achieved by retaining the double bond in the ring, which, in the previous synthesis of the cyclic ThrCer analogues, had been created by ring-closing metathesis, but was later removed by hydrogenation, whilst deprotecting the benzyl-protected amine. Therefore a different protection / deprotection strategy would be needed for the amine in order to retain the target double bond, were we to follow a similar synthetic strategy to these new targets.

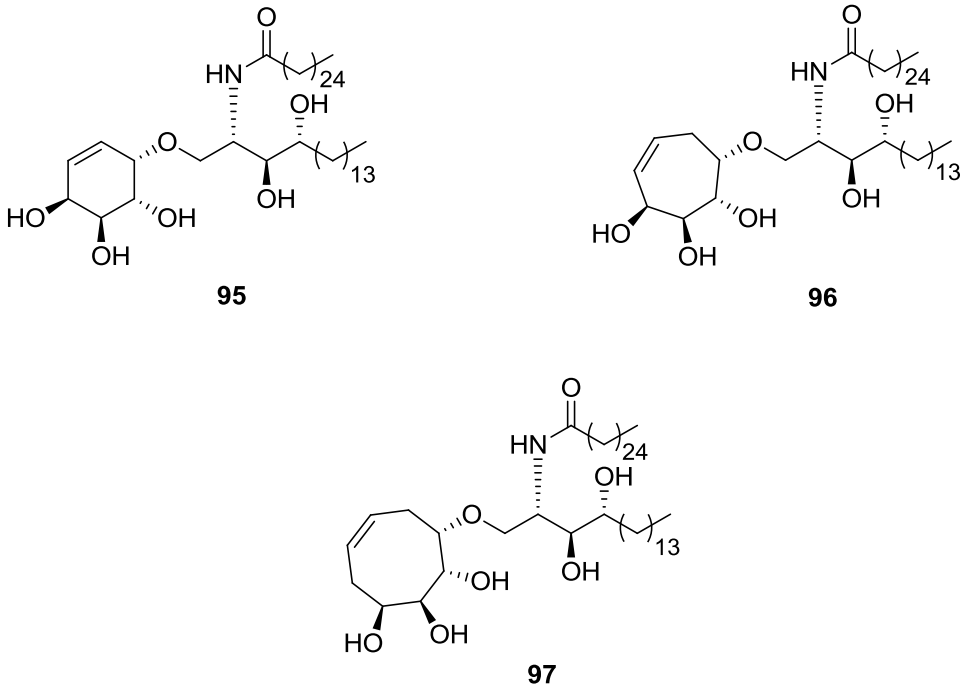
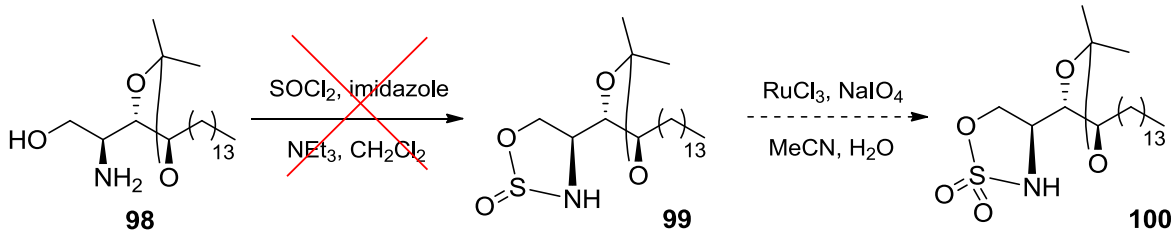


Figure 3.13. The target cyclohexenyl, cycloheptenyl and cyclooctenyl ThrCer analogues **95**, **96** and **97.**

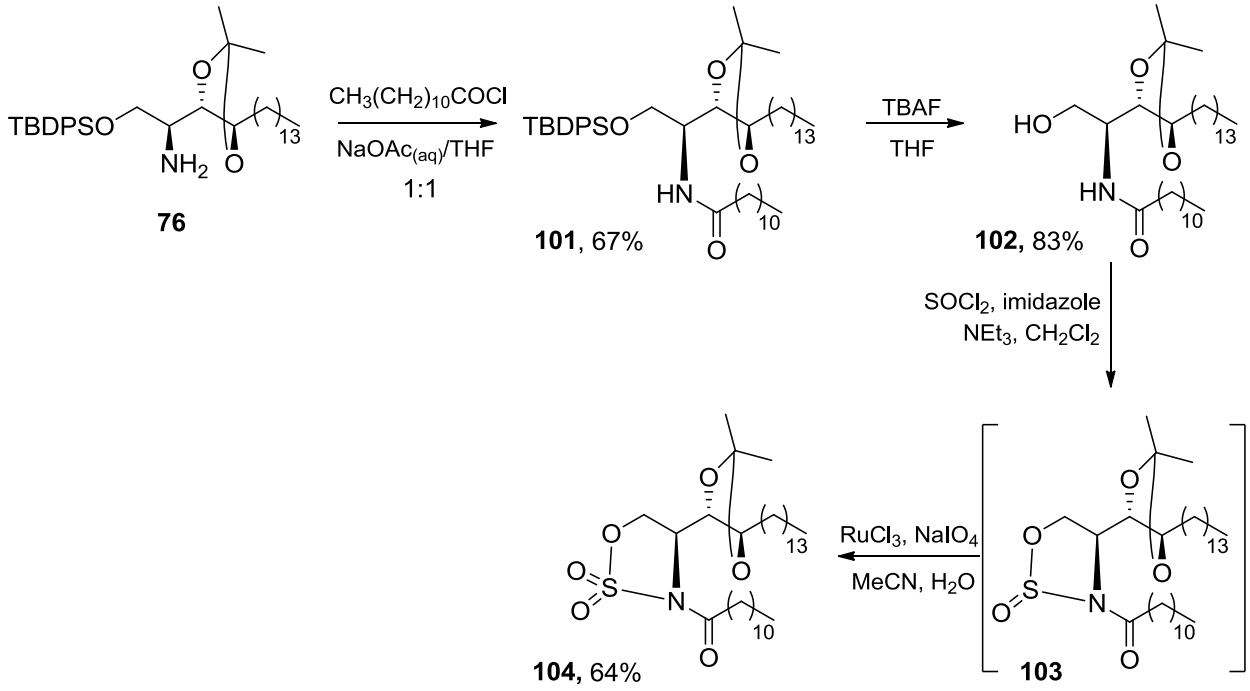
Previously we had tried a Boc protecting group for the amine, which could have been removed by acid hydrolysis, however this did not withstand the conditions used to form the ether bond.

One method to circumvent the problem of deprotecting the amine would be to not protect it in the first place. We therefore decided to install the sulfamidate on the free amine (Scheme 3.18). Although direct installation of the sulfamidate on a free amine had been reported,[157,158] in our hands this was not possible. No reaction occurred between the amine and the thionyl chloride. We found this strange, due to the nucleophilicity of the free amine, however there were reports in the literature which had also encountered this problem.[159,160]



Scheme 3.18. Attempted synthesis of an unprotected sulfamidate.

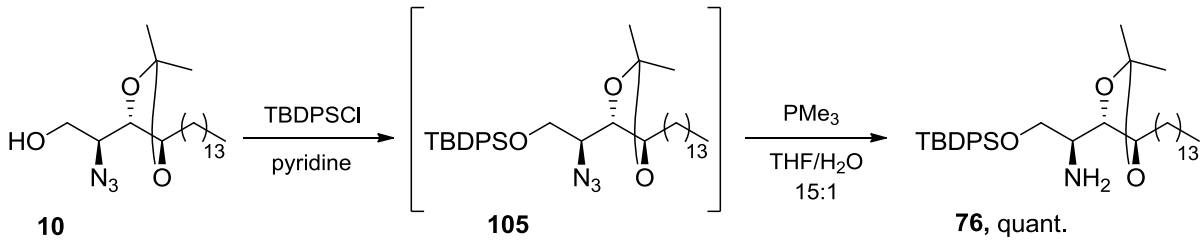
We then decided to attempt an amide. If we installed the C_{26} acyl chain *before* making the ether bond, the synthesis would be much easier. There would be no need to employ a nitrogen protecting group and all that would be left to do after forming the ether bond would be removal of the alcohol protecting groups. To investigate whether an amide would work we chose to test the synthetic route with an inexpensive C_{12} acyl chain (Scheme 3.19). Amine **76** was first acylated with the C_{12} acid chloride, before deprotection of the silyl ether and sulfamidate formation, all of which proceeded uneventfully. However the subsequent etherification with cyclohexanol did not work. A product was formed, however it does not appear to be the ether product – we are still unsure as to what happened and further studies would be worthwhile.



Scheme 3.19. Synthesis of the C_{12} amide sulfamidate **104.**

Due to these unsuccessful trials we elected to return to using a protected amine, and decided to investigate the *para*-methoxybenzyl (PMB) protecting group. Structurally this protecting group is similar to the benzyl group, however it is more labile. This protecting group can also be used to protect alcohols, where it can be removed via oxidative, reductive and acidic conditions. PMB ethers are not commonly used to protect amines, however we envisage that the PMB-protected amine would also be deprotected using these conditions, giving us much more flexibility in attempting to deprotect the amine selectively. Synthesis of the PMB-protected sulfamidate **109** was very similar to the route used to synthesise the Bn-protected sulfamidate **80**. However the amine **76** was synthesised from azide **10,** used previously to synthesise the deoxy and truncated analogues. Protection of the primary hydroxyl of azide **10**

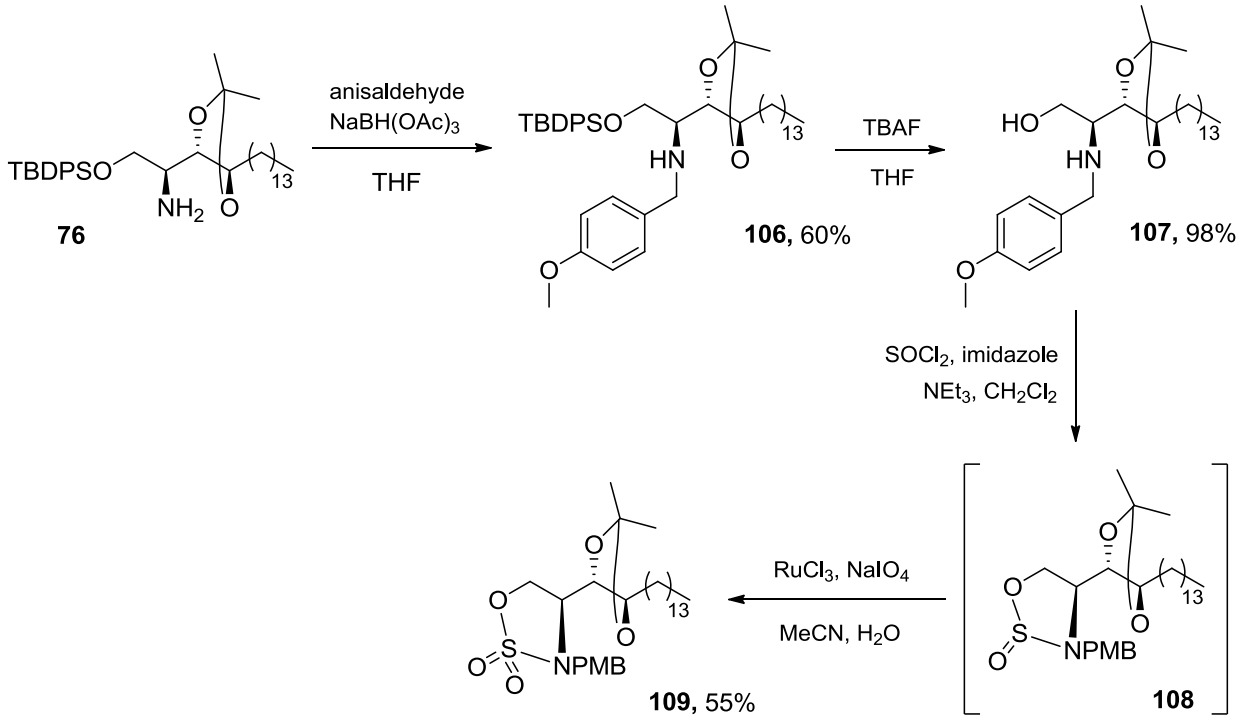
as its TBDPS ether, followed by Staudinger reduction of the azide with PMe_3 provided amine **76** (Scheme 3.20). This route proved to be much more reliable than the previously used synthetic scheme (Scheme 3.11).



10 **105** **76**, quant.

Scheme 3.20. Synthesis of amine **76.**

Amine **76** was then protected via a one-pot reductive amination with anisaldehyde and $NaBH(OAc)_3$, before the primary silyl ether was removed with TBAF and the sulfamidate formed as before (Scheme 3.21). Formation of the sulfamidate was evidenced by HRMS, and the ^{13}C NMR spectrum, which showed a downfield shift of the CH_2O resonance from 61.1 ppm in alcohol **107** to 68.1 ppm in sulfamidate **109**.

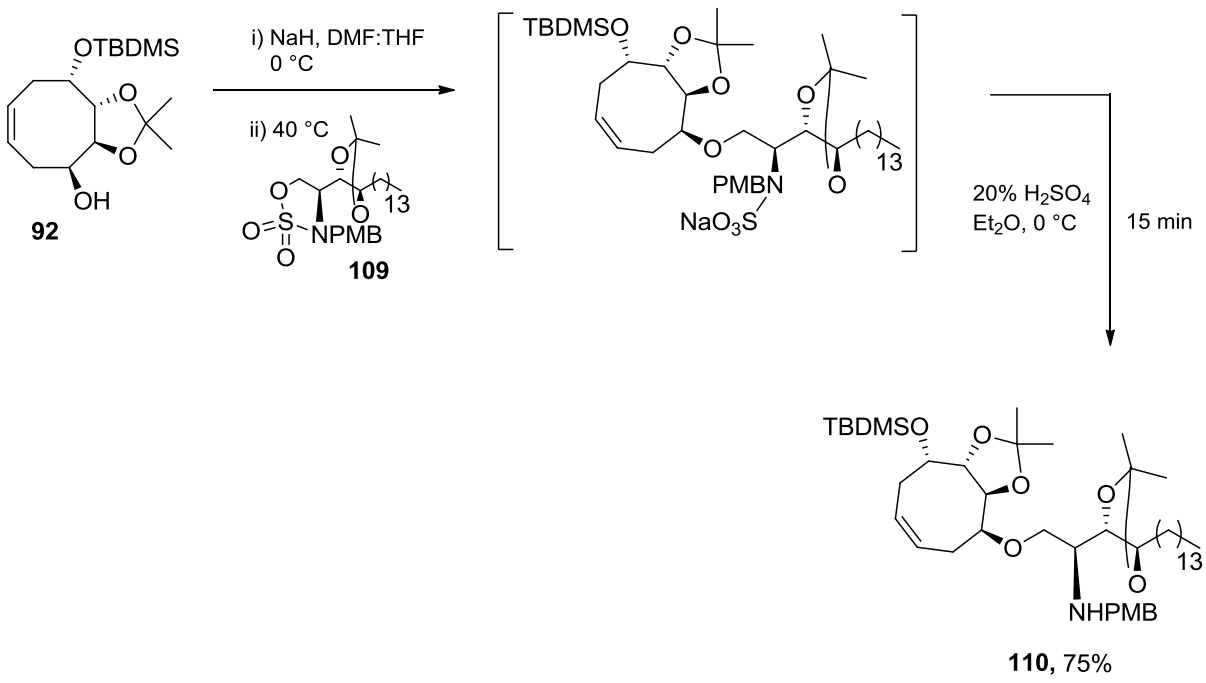


Scheme 3.21. Synthesis of PMB sulfamidate **109.**

3.10.1 Synthesis of the Cyclooctenyl ThrCer Analogue

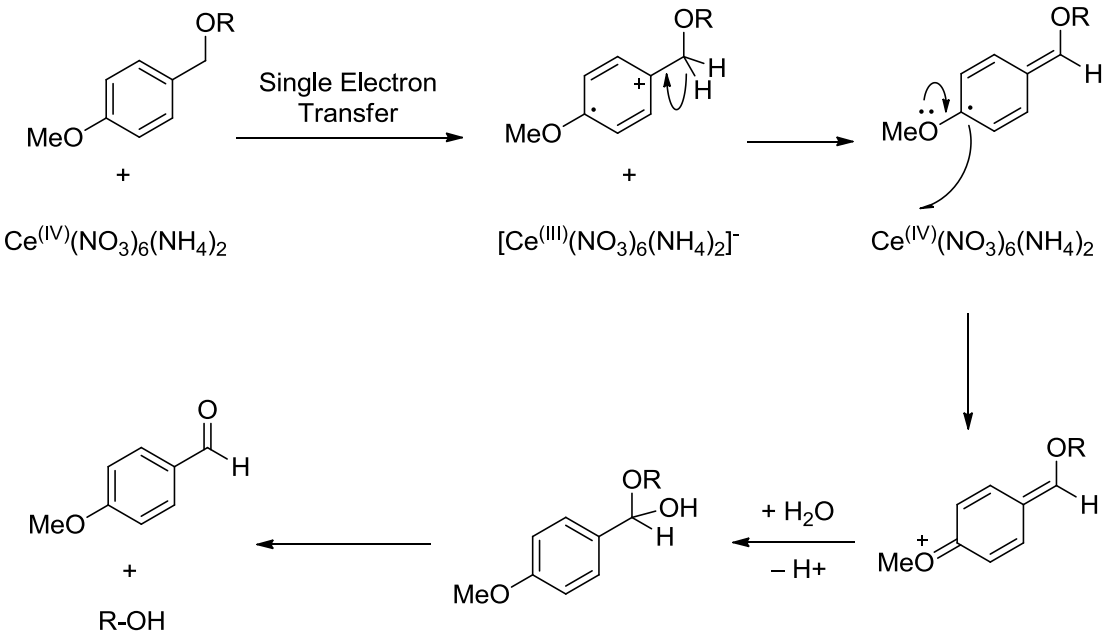
The synthetic route to the double bond ring analogues would be analogous to that used for the ring compounds synthesised previously, only replacing the Bn sulfamidate **80** with the PMB sulfamidate **109**.

The cyclooctenyl ThrCer analogue **97** was synthesised first (Scheme 3.22). Thus treatment of cyclooctenol **92** with NaH, followed by reaction of the resulting alkoxide with the PMB-protected sulfamidate **109** provided fully protected ether **110** after acidic work-up.



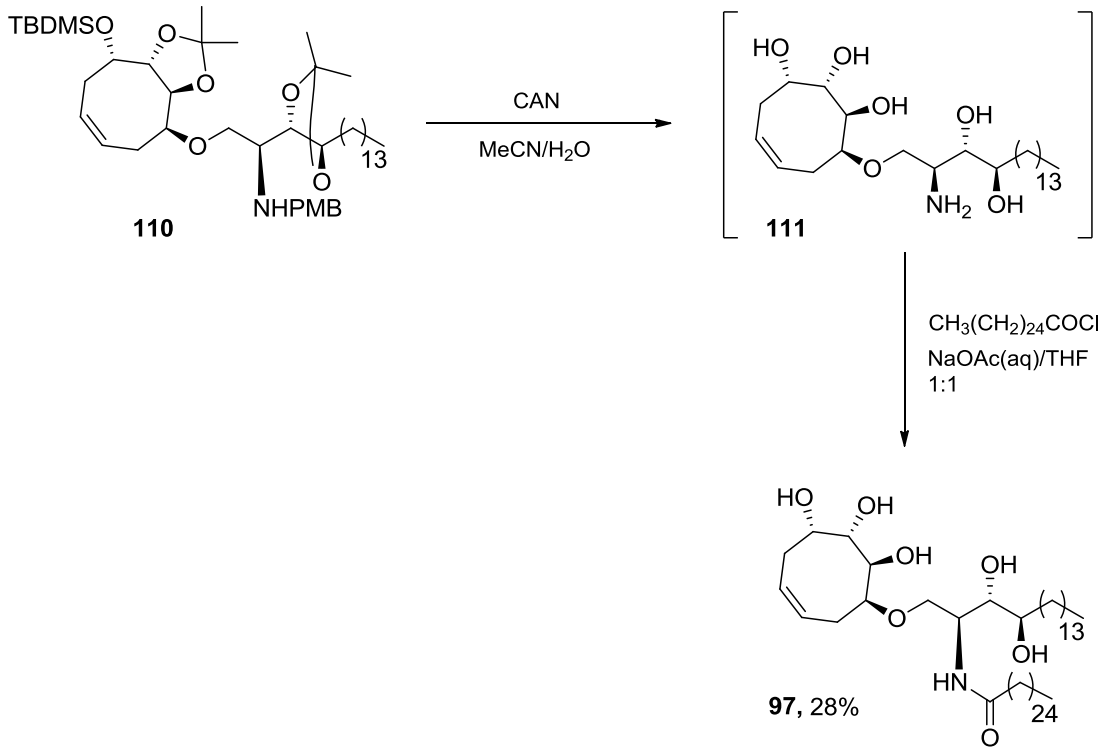
Scheme 3.22. Synthesis of cyclooctenyl ether **110.**

We attempted removal of the PMB group with TFA first, as these acidic conditions should also effect global deprotection of the acetal, and the silyl ether, leaving the fully deprotected amine which would then just need to be acylated to form the target compound. However the PMB group proved to be resistant to TFA hydrolysis. Therefore we attempted an oxidative deprotection of the PMB group, using the oxidant Cerium (IV) Ammonium Nitrate (CAN). This method of oxidative cleavage involves the transfer of a single electron to two different CAN molecules, forming an oxonium ion which is trapped by water, releasing the PMB group as the aldehyde (Scheme 3.23).



Scheme 3.23. Mechanism of CAN-mediated PMB-deprotection.

CAN successfully deprotected the N-PMB group, and due to the acidic reaction conditions, the acetals and silyl ether were also hydrolysed, affording the fully deprotected product **111**. This polar compound was not purified, but immediately acylated with the C_{26} acid chloride, forming our target compound **97** (Scheme 3.24). Resonances at 128.0 ppm and 128.8 ppm in the ^{13}C NMR spectrum confirmed the presence of the double bond.



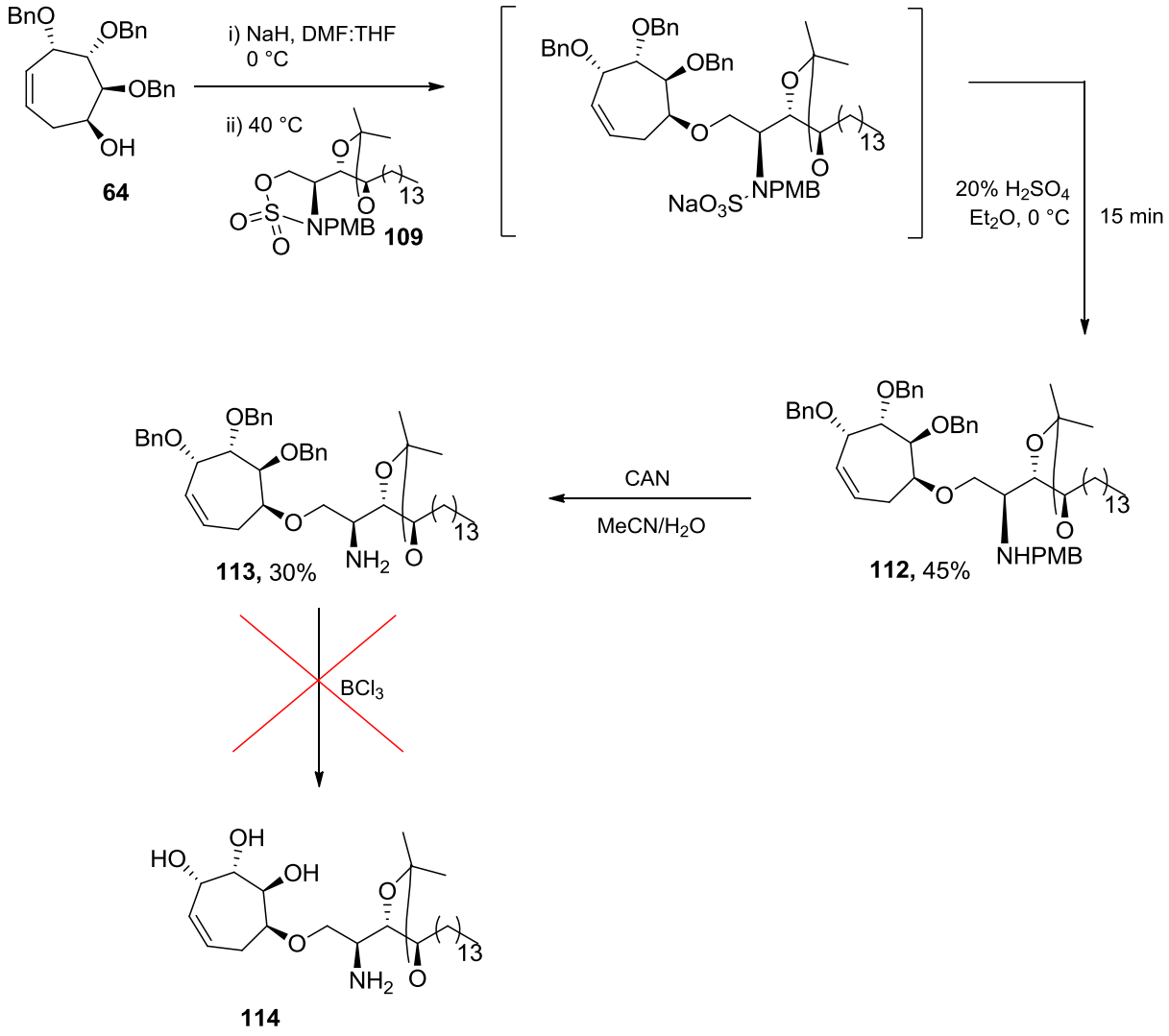
Scheme 3.24. Synthesis of the cyclooctenyl ThrCer analogue **97.**

3.10.2 Towards the Synthesis of the Cyclohexenyl and Cycloheptenyl ThrCer Analogues

Following the successful synthesis of the cyclooctenyl ThrCer analogue **97** our attention turned to the cyclohexenyl and cycloheptenyl ThrCer analogues **95** and **96** using the PMB sulfamidate **109** as before.

From the synthesis of the cycloheptyl ThrCer analogue **44,** we already had access to the benzyl-protected cycloheptenol **64**, and therefore decided to react this benzyl-protected cycloheptenol **64** with the PMB sulfamidate **109.** Previously the benzyl groups had been removed via a hydrogenolysis, which now would not be possible as we intended to retain the double bond. It was envisioned that the benzyl groups could be removed instead by reacting with BCl_3, which has been employed before for benzyl ether deprotection and does not affect isolated double bonds (Scheme 3.25).[161,162]

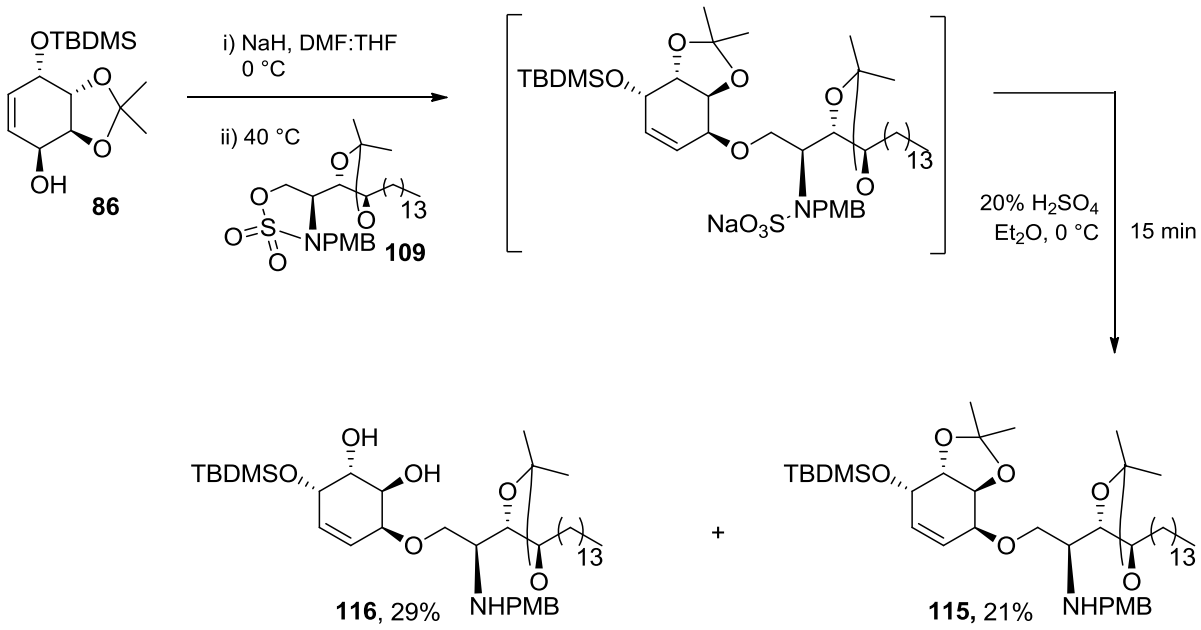
Reaction of the sodium alkoxide of cycloheptenol **64** with the PMB sulfamidate **109** provided ether **112**, and subsequent treatment with CAN effected PMB removal. Unfortunately attempted deprotection of the benzyl ethers with BCl_3 at −78 °C led to extensive decomposition. BCl_3 is a strong Lewis acid and so decomposition was always a potential problem. Performing the reaction at −78 °C, and using only 3.3 equivalents of BCl_3 (1.1 for each benzyl group) failed to improve matters. Therefore we decided to replace the benzyl protecting groups on the cycloheptenol with PMB ethers, to mirror the protecting group strategy on the sphingosine component. Global deprotection should then be possible with CAN to provide the fully deprotected amine. This route was performed in parallel with the synthesis of the cyclohexenyl ThrCer analogue **95**.



Scheme 3.25. Attempted synthesis of the cycloheptenyl ThrCer analogue **96.**

We envisaged the cyclohexenyl ThrCer analogue **95** would be synthesised by reacting cyclohexenol **86** with the PMB sulfamidate **109**, before deprotection with CAN and acylation. However we encountered a number of unexpected obstacles, which prevented us from accessing the target compound via this methodology. Synthesis of the ether **115** was successful (Scheme 3.26), however we were unable to separate the product from the excess

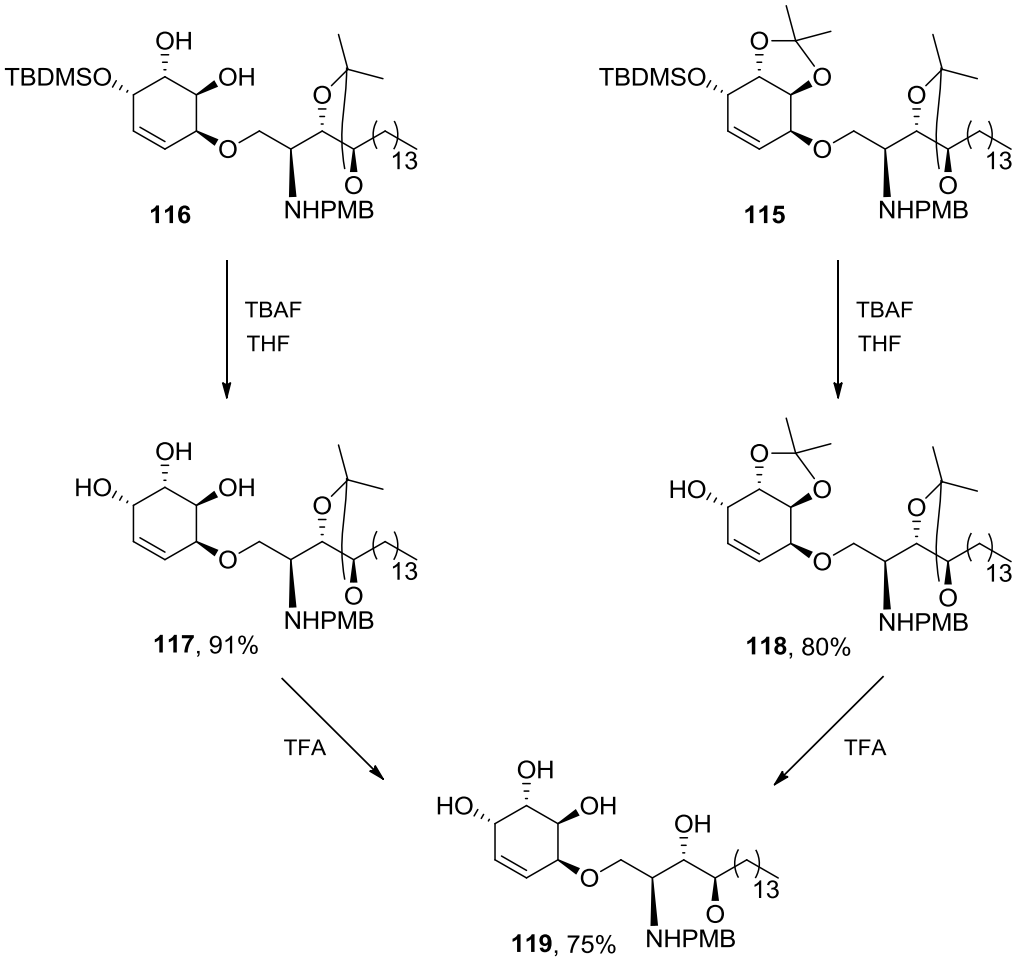
sulfamidate **109**, and therefore had to use the mixture in the next step. We also obtained partially deprotected ether **116,** even though the acidic work up was the same as for the cyclooctenyl target compound **97**, the acetal group in the cyclohexenyl ring was removed. This is presumably due to conformational effects and ring strain, making the cyclohexenyl acetal more susceptible to acid hydrolysis.



Scheme 3.26. Synthesis of cyclohexenyl ethers **115** and **116.**

The impure, fully protected ether **115** was then reacted with TBAF to provide alcohol **118**, which was now separable from the sulfamidate impurity. Ether **118** was then treated with TFA to hydrolyse both acetals and provide the protected amine **119**. In parallel, the partially

deprotected ether **116** was also reacted with TBAF, and then treated with TFA to provide protected amine **119** (Scheme 3.27).

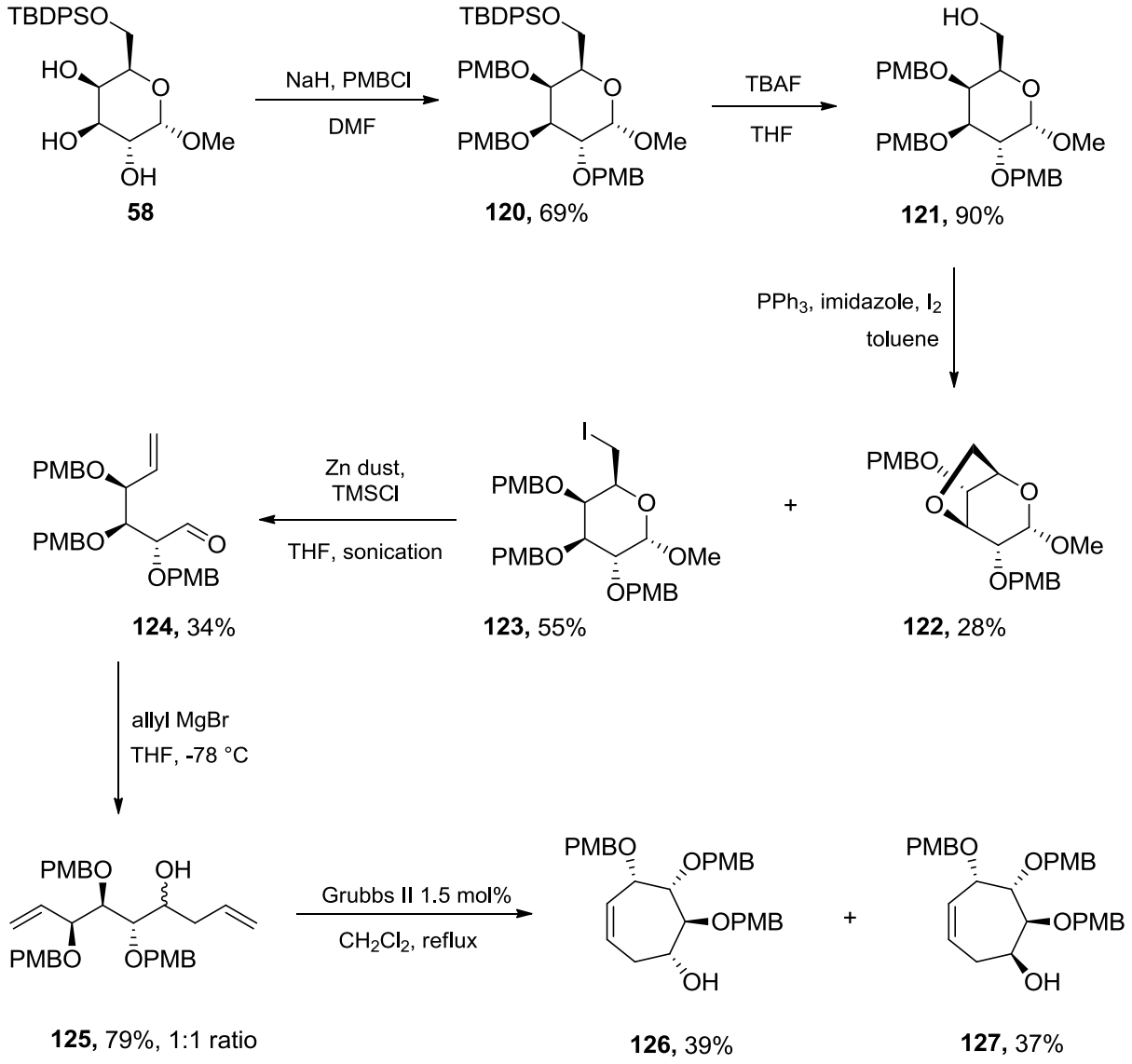


Scheme 3.27. Synthesis of the PMB amine-protected cyclohexenyl ether **119.**

Amine **119** then only needed to be deprotected and acylated to obtain the target compound. However deprotection with CAN was unsuccessful, and caused decomposition of the starting

material. Another oxidant, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), was also unsuccessful. Acid hydrolysis with TFA was unable to remove the PMB group, leaving the compound untouched. We had tried both oxidative and acidic conditions to remove this PMB group, reductive conditions would also remove the double bond and so were deemed unviable. To date, we have been unable to remove this lone PMB group.

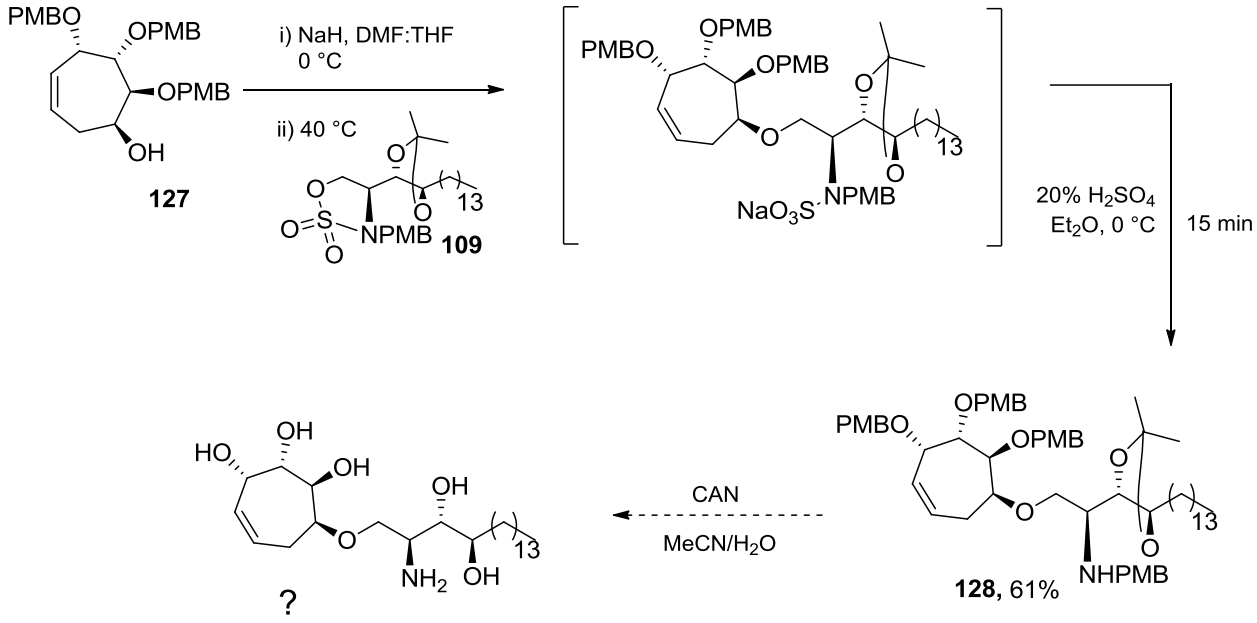
While this synthetic route to make the cyclohexenyl ThrCer analogue **95** with the PMB sulfamidate **109** was underway, we had also started to synthesise the cycloheptenyl ThrCer compound **96** using the same synthetic route. First we had to synthesise the cycloheptenol **127** with PMB protecting groups (Scheme 3.28).



Scheme 3.28. Synthesis of the PMB-protected cycloheptenol **127.**

This synthetic route was successful in providing the PMB-protected cycloheptenols **126** and **127**. The sodium alkoxide of triol **58** was reacted with PMBCl to give fully protected galactose **120**, before primary silyl ether deprotection with TBAF. The alcohol **121** was then heated

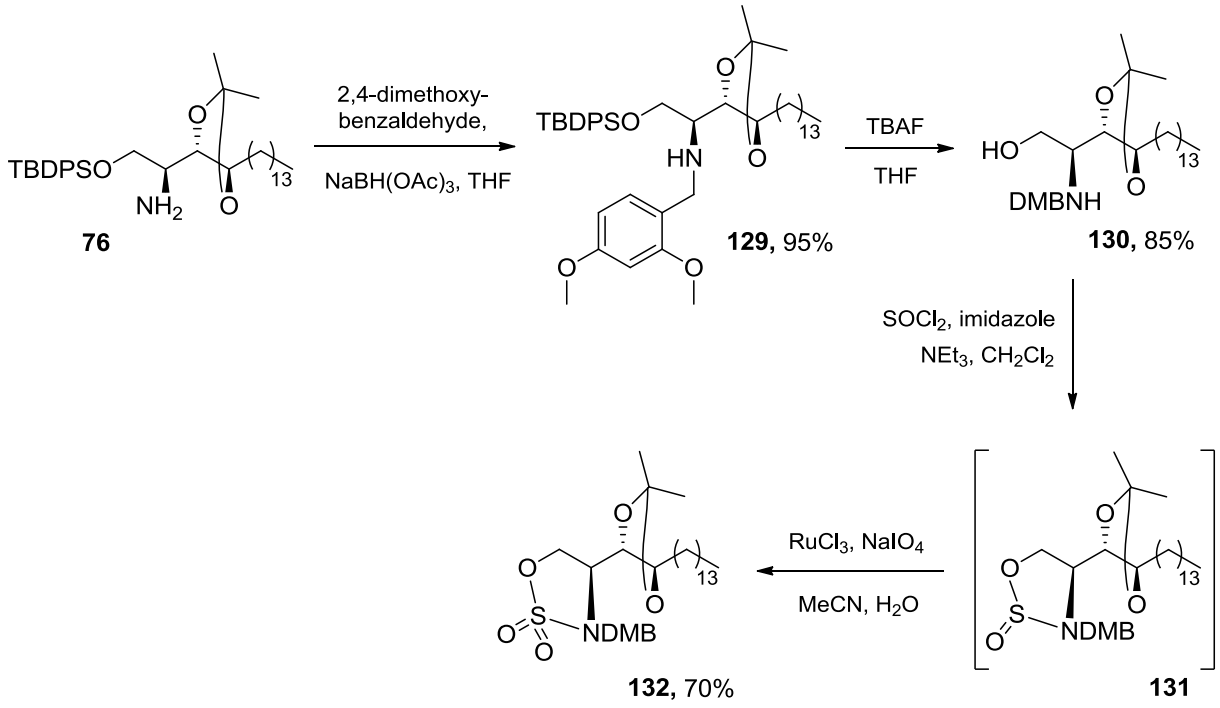
under reflux with I_2 and PPh_3, as before, to install the iodide. Although this method did result in formation of the iodide **123**, a by-product was also formed. This bi-cycle **122** is likely due to the greater acid sensitivity of the PMB ethers compared to Bn ethers, as the reaction conditions result in the formation of HI, a strong acid. This appears to have hydrolysed the PMB ether at the 3-position, as evidenced by HMBC experiments, which then reacts in an intramolecular fashion with the newly installed iodide, to provide the bridged bi-cycle **122**. Formation of the bi-cycle **122** could also be due to the greater nucleophilicity of the PMB ether oxygen, which could react in an intramolecular fashion with the iodide first, before hydrolysis of the PMB ether. To reduce the formation of this bi-cycle **122** we shortened the reaction time to 15 min, which allowed us to retrieve the target iodide **123** in a 2:1 ratio. Iodide **123** was then subjected to zinc-mediated fragmentation, Grignard reaction with allyl magnesium bromide on the resulting aldehyde, and ring-closing metathesis using Grubbs 2nd-generation catalyst to provide us with the target cycloheptenol **127** (Scheme 3.28)**.** This was then treated with NaH before reacting with the PMB sulfamidate **109** to form the fully protected ether **128** (Scheme 3.29). We hoped the next step would involve global deprotection with CAN, however due to the problems we had encountered with deprotecting the PMB amine in the cyclohexenyl ether **119** we have not attempted this step yet. We were unsure whether we would have the same problem with this substrate, and with the addition of three more PMB ethers we did not want to try this step before we had worked out a method with the cyclohexenyl ether **119**, which is a less labour-intensive compound to make. That said, the PMB amine in benzyl-protected cycloheptenyl ether **112** was removed by CAN successfully, so the only difference in this case would be the three extra PMB ethers, which should be relatively easy to remove.



Scheme 3.29. Synthesis of PMB-protected cycloheptenyl ether **128.**

Having unsuccessfully tried various different methods for removing the lone PMB group from partially deprotected cyclohexenyl ether **119**, we decided to change the amine protecting group.

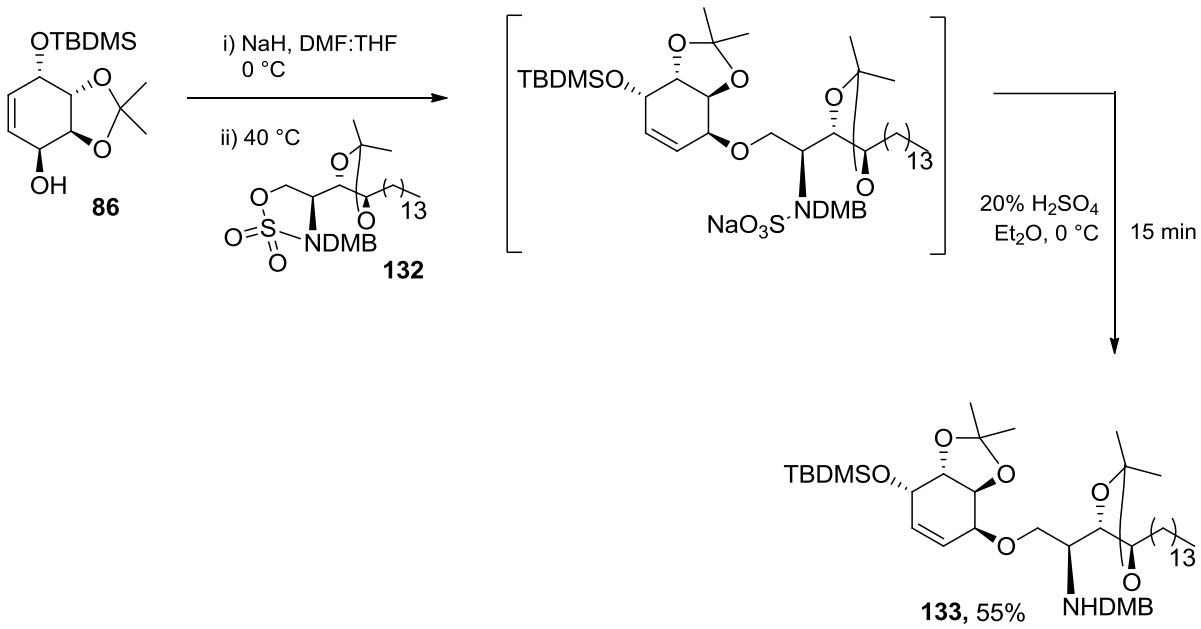
2,4-Dimethoxybenzyl (DMB) groups are similar to PMB groups, having the same structure but with an additional methoxy group on the 2-position of the benzyl ring. This DMB group can therefore also be removed via reductive, oxidative and acidic conditions. However the extra methoxy group should make the DMB group more labile, at least to oxidative and acidic conditions.



Scheme 3.30. Synthesis of DMB sulfamidate **132**.

A one-pot reductive amination of amine **76** with 2,4-dimethoxybenzaldehyde and $NaBH(OAc)_3$ provided protected amine **129.** Desilylation with TBAF followed by sulfamidate formation via the standard two-step protocol provided our target electrophile coupling partner **132** (Scheme 3.30).

The DMB sulfamidate **132** was then reacted with the sodium alkoxide of cyclohexenol **86** to afford the corresponding ether **133** (Scheme 3.31). The DMB protecting group survived these etherification conditions, including the acidic work-up conditions of the final step.



Scheme 3.31. Synthesis of the cyclohexenyl DMB ether **133.**

Synthesis of the cyclohexenyl DMB ether **133** is as far as we have progressed along this synthetic route. The cyclohexenyl DMB ether **133** now needs to be investigated for ease of DMB deprotection, using both acidic and oxidative conditions.

3.10.3 Conclusions and Future Work

Synthesis of the cyclohexenyl, cycloheptenyl and cyclooctenyl ThrCer analogues **95**, **96** and **97** has been more troublesome than we initially imagined. The synthesis of the ThrCer ring analogues **43**, **44** and **45** presented us with an intermediate which already contained the double bond within the ring; however debenzylation removed this double bond. We envisaged that changing the amine (and alcohol) protecting groups to ones which did not require hydrogenolysis would allow us to reach the double bond ThrCer ring analogues **95**, **96** and **97**. The protecting group we chose was the PMB group, which can be deprotected under acidic and oxidative (as well as reductive) conditions. Indeed this replacement allowed us to obtain the cyclooctenyl ThrCer analogue **97**, with CAN effecting deprotection of the PMB amine. However in the synthesis of cyclohexenyl ThrCer analogue **95**, CAN did not effect removal of the NPMB group, which was also untouched by DDQ and TFA. Therefore the DMB group, an even more labile protecting group, was chosen to protect the amine, and work along this synthetic route is ongoing. So far, we have synthesised cyclohexenyl DMB ether **133**, proving that the DMB group is suitable for sulfamidate synthesis and etherification. However the key deprotection step has not yet been attempted and needs to be investigated in the future.

Synthesis of the cycloheptenyl ThrCer analogue **96** is also ongoing. So far, we have synthesised the fully PMB-protected ether **128**, which now needs to be deprotected and acylated to provide cycloheptenyl ThrCer analogue **96**.

Other amine protecting groups should also be explored, for example carbamates, which can be removed without affecting the double bond. Boc is not suitable (see Page 112), however there are other carbamate protecting groups which could be investigated, such as Troc, Alloc and Z.

Alternative methods for deprotection of the Bn / PMB group should also be considered. Dissolving metal reduction can remove benzyl and PMB groups without touching double bonds, and should also be investigated.

Chapter 4

Synthesis and Biological Evaluation of TDM/TMM Analogues

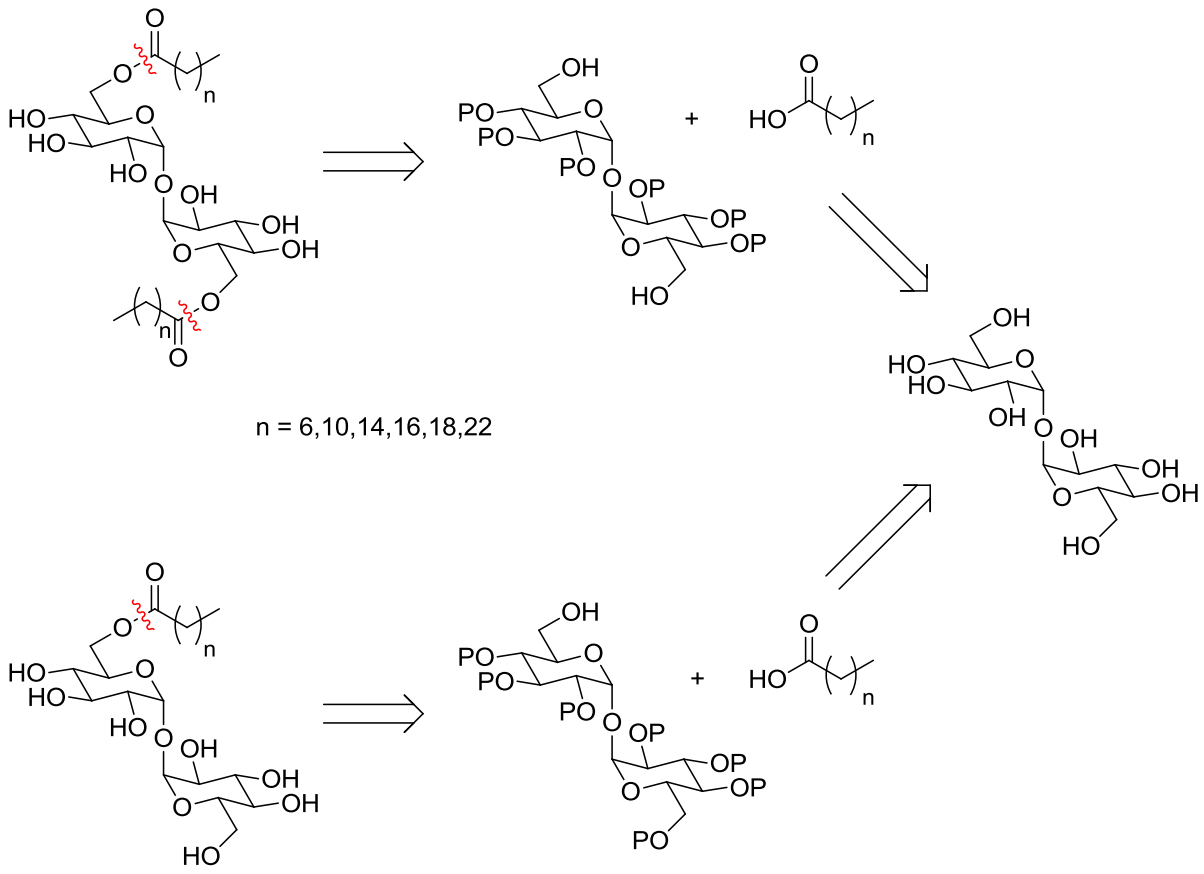
4. Synthesis and Biological Evaluation of TDM/TMM analogues

4.1 Target Compounds and Retrosynthetic Analysis

The adjuvanticity of TDM/TDB molecules is of great interest. There has not been much work on new adjuvants, and the development of a safe and effective adjuvant is now crucial for the use of many new vaccines, which are based on subunits and hence are not very immunogenic by themselves. Subunit vaccines are now becoming more popular due to their relative safety, however these vaccines only become effective at providing immunity when administered with an adjuvant. Previously, vaccines often used heat-treated pathogens (heat should kill the pathogen), or attenuated pathogens (passaged through a medium to make them less pathogenic) to invoke an immune response. However these are not ideal. Dead pathogens can often not provoke a high enough immune response for immunity to occur. Attenuated pathogens are still live, and so there is still a risk of becoming infected with the disease. Also the pathogen could revert back to a more deadly form and infect the host.

TDB has been shown to be effective as an adjuvant for a subunit vaccine against tuberculosis,[38] and therefore is a good structure to start basing different analogues on. TDB has two C_{22} fatty acid chains attached to the 6- and 6'-positions of trehalose. The stucture differs from TDM only in the lipid chain; TDM has a long branched mycolic acid. As this chain has already been altered it would be interesting to see whether different chains would also be recognised by Mincle and how any differences might affect biological activity. The first aim of this project is to synthesise a range of TDM analogues with different-length fatty acid chains. These will then be tested for Mincle activation. We also want to determine whether both fatty acid chains are necessary for activation, therefore the corresponding range of TMM analogues will also be

synthesised. Our first targets were TDM/TMM analogues with C_8, C_{12}, C_{16}, C_{18}, C_{20} and C_{24} fatty acid chain lengths. The retrosynthesis of the TDM/TMM analogues is shown in Scheme 4.1.



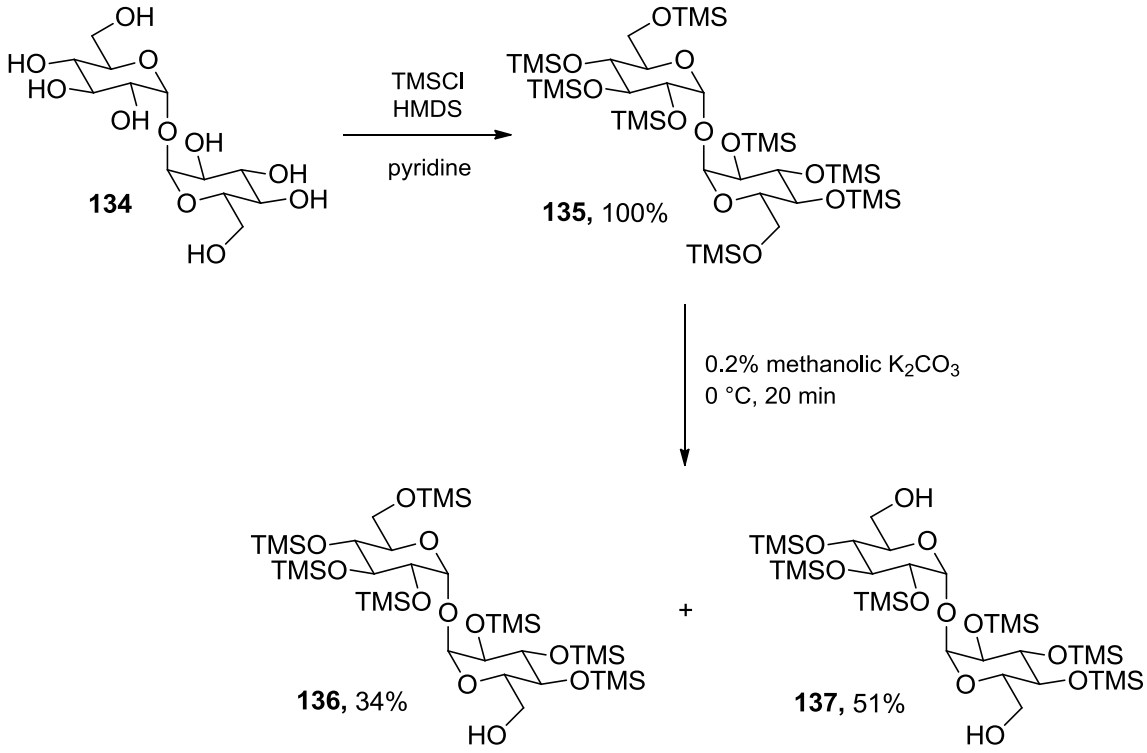
Scheme 4.1. Retrosynthetic analysis of the target TDM and TMM analogues.

Our retrosynthetic analysis identified commercially available α,α-trehalose as the starting material for this synthesis. This would be fully protected before selective deprotection of the 6 and/or 6' positions would release the primary alcohol residue for subsequent acylation. Global deprotection would then provide the target products.

4.1.1 Synthesis of Partially Deprotected Trehalose

The alcohol functionality in commercially available α,α-trehalose was globally protected with trimethylsilyl (TMS) groups. TMS groups have recently been used in many carbohydrate syntheses, and are relatively stable under a variety of conditions. They have been employed in Gervay-Hague glycosyl iodide-mediated one-pot stereoselective glycosylation procedures,[163,164] and by Beau who employed cat. $Cu(OTf)_2$ and $FeCl_3.6H_2O$ for sugar protection.[165,166] We chose to use TMS groups because of their ease of introduction and removal, their stability and to improve the solubility of the trehalose intermediates in organic solvents. Also primary TMS ethers can be selectively hydrolysed in the presence of secondary TMS ethers.

Trehalose **134** was reacted with hexamethyldisilazane (HMDS) and trimethylsilyl chloride, as the activating agent, with pyridine as the solvent and acid scavenger.[167,168] This afforded pure per-TMS trehalose **135**, without any need for purification. Extracting with hexanes removed most of the pyridine from the product, the remainder was removed under high vacuum. Per-TMS trehalose **135** was then selectively deprotected with 0.2% methanolic K_2CO_3 at 0 °C.[167] This afforded both the mono 6-deprotected trehalose **136** and the di 6, 6'-deprotected trehalose **137** in one reaction, which were easily separable (Scheme 4.2). We achieved a ratio of 1.0: 1.4 mono-deprotected: di-deprotected products after 20 minutes of reaction. To get both under one set of reaction conditions was very useful in simplifying the synthesis.



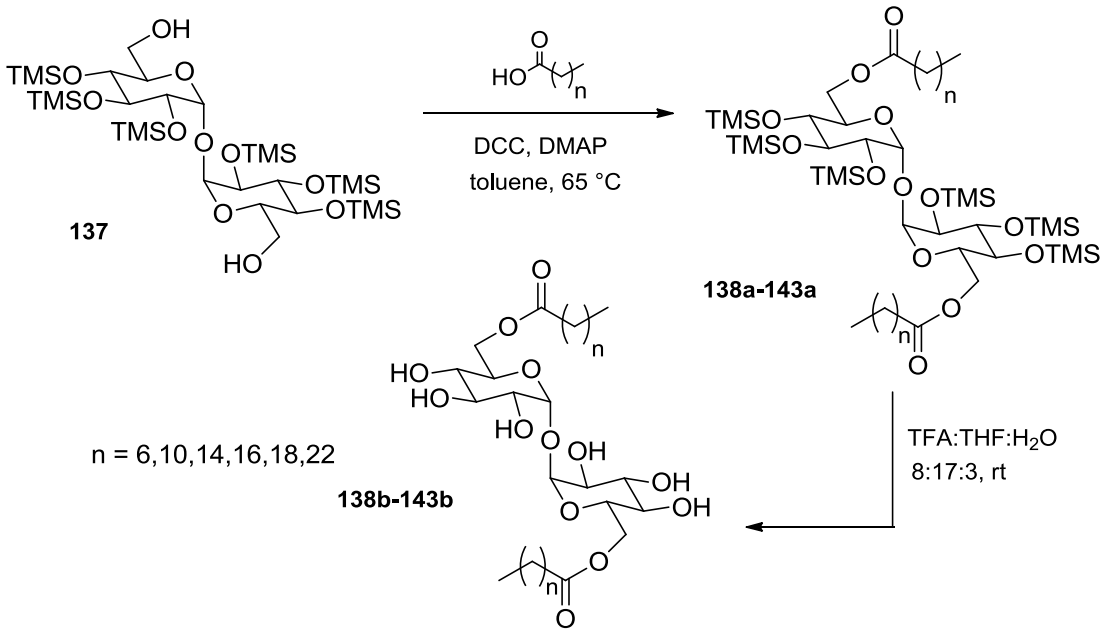
Scheme 4.2. Synthesis of the 6- and 6,6'-deprotected trehalose **136** and **137.**

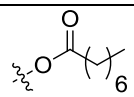
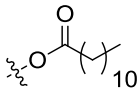
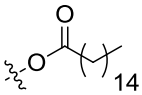
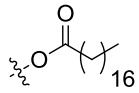
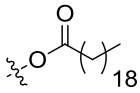
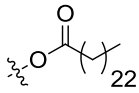
4.1.2 Esterification of the Deprotected Trehalose

Esterification of the two free hydroxyls in **137** was done using modified Steglich esterification conditions,[169] in which 1,3-dicyclohexylcarbodiimide (DCC) is used as the coupling reagent and 4-dimethylaminopyridine (DMAP) as the catalyst (Scheme 4.3). For efficient coupling, the reaction must be anhydrous, therefore the starting di-deprotected trehalose **137**, carboxylic acid, DCC and DMAP were all dried under high vacuum for at least one hour prior to the reaction, and freshly activated 4 Å molecular sieves were added to the reaction mixture to ensure any adventitious water was removed. Toluene was then added and the reaction was heated to 65 °C, to ensure good conversion. 3.0 Equivalents of the carboxylic acid and DCC were used to ensure both hydroxyls were acylated. Global deprotection of the TMS groups was achieved using an 8: 17: 3 TFA: THF: H_2O solution, giving TDM analogues **138b**-**143b** in good yield. This solvent system was able to dissolve the non-polar protected trehalose starting materials but was still polar enough to keep the partially deprotected product in solution to allow full deprotection. TFA was chosen as the deprotection acid as it is volatile and so is easily removed from the reaction mixture under reduced pressure (Scheme 4.3).

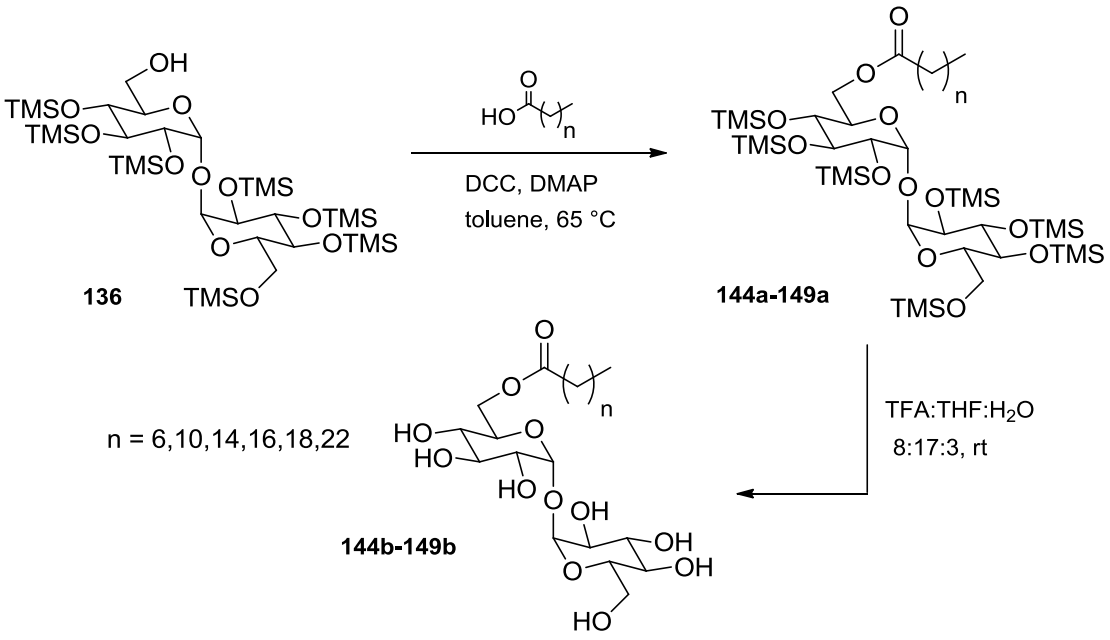
The mono-acylated analogues were synthesised from mono-alcohol **136** in the same way as the di-acylated analogues, except only 1.5 equivalents of the carboxylic acid and DCC coupling reagent were used. TMM analogues **144b**-**149b** were isolated in good yields (Scheme 4.4).

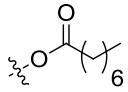
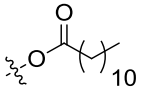
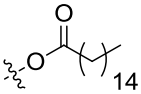
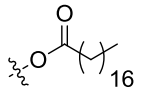
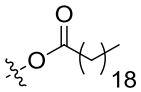
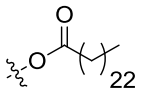
Esters are known to migrate, however HMBC analysis confirmed that the esters on our analogues remained on the 6- / 6'-position and did not migrate.



Compound	Chain length	Yield (over both steps)
138		69%
139		63%
140		70%
141		64%
142		75%
143		62%

Scheme 4.3. Synthesis of the different chain-length di-ester trehalose analogues **138b-143b.**



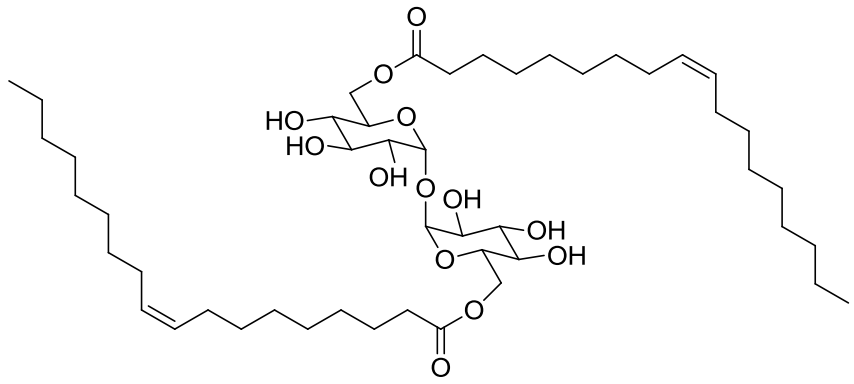
Compound	Chain length	Yield (over both steps)
144		75%
145		78%
146		72%
147		72%
148		66%
149		69%

Scheme 4.4. Synthesis of the different chain-length mono-ester trehalose analogues **144b-149b**.

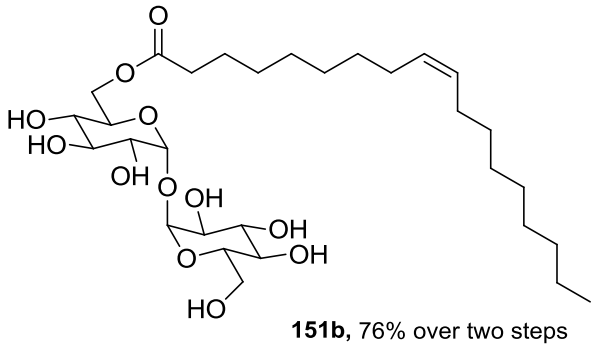
4.2 Synthesis of Unsaturated TDM/TMM Analogues

The mycolic acid chain of TDM has been hypothesised to contribute to optimal presentation of the polar head to Mincle, via the "kink".[36] Therefore we decided to synthesise TDB analogues with an unsaturated fatty acid chain, to see whether the additional "kink" affects activity (Figure 1.6). We chose to synthesise linoleic acid (C18 chain with 2 *cis* double bonds) and oleic acid (C18 chain with 1 *cis* double bond) derivatives, as a *cis* double bond is needed to mimic the "kink" effect of the cyclopropyl groups embedded in the mycolic acid side-chain (Figure 4.1).

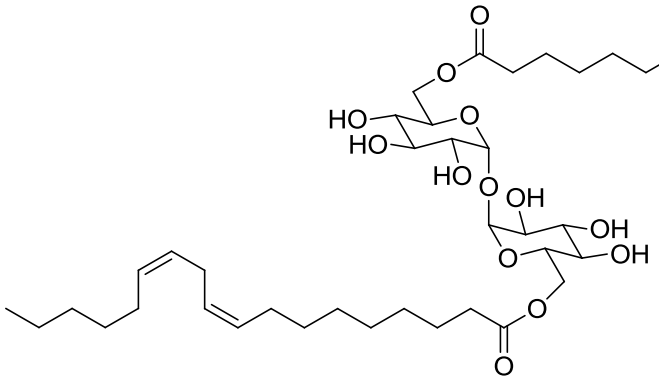
The synthesis of these analogues was analogous to that used to prepare the straight-chain derivatives (Scheme 4.3 and 4.4). The inclusion of (*Z*)-olefins in the chain did not affect any of the reactions.



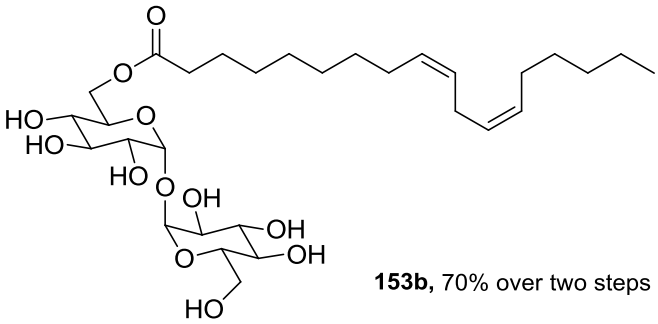
150b, 71% over two steps



151b, 76% over two steps



152b, 67% over two steps



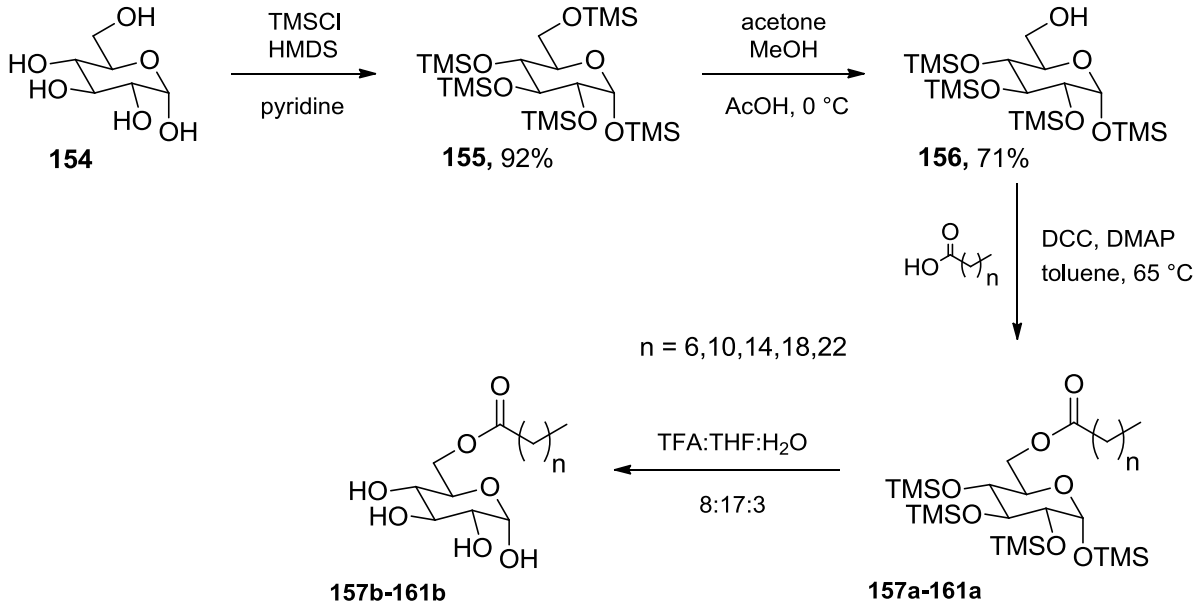
153b, 70% over two steps

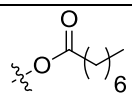
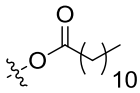
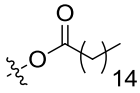
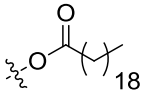
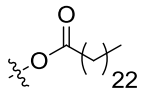
Figure 4.1. Target unsaturated chain analogues.

4.3 Synthesis of GMM Analogues

Although it has been previously shown that GMM does not activate Mincle[36] we wanted to verify this and see to what extent Mincle activity is decreased. Therefore we synthesised a range of GMM analogues, also with C_8, C_{12}, C_{16}, C_{20} and C_{24} fatty acid chain lengths.

The synthesis of the GMM analogues is analogous to that of the TMM analogues, with only a few differences: the starting sugar is glucose rather than trehalose, and the method of 6-position deprotection is different. Glucose was fully protected with TMS groups, again using TMSCl, HMDS and pyridine. The 6-position however was deprotected using different conditions to the trehalose compounds, using acetic acid in a methanol/acetone solution at 0 °C. These conditions had been used previously in our group for 6-TMS deprotection of glucose, and were shown to be effective.[123] The 6-deprotected glucose **154** was then acylated with the appropriate carboxylic acid, using DCC and DMAP, before affecting TMS-deprotection with an 8: 17: 3 TFA: THF: H_2O solution as before (Scheme 4.5).



Compound	Chain length	Yield (over both steps)
157		63%
158		71%
159		67%
160		58%
161		66%

Scheme 4.5. Synthesis of the different chain-length GMM analogues **157b-161b**.

4.4 Biological Analysis

These compounds were then tested for activity and Mincle activation in Chris O'Callaghan's lab in Oxford, using an NFAT7-luciferase reporter assay. In this assay Jurkat cells are stably transfected with a luciferase construct whose transcription is driven by the NFAT signalling pathway. These cells are then transfected with a lentivirus (made in 293T cells), which express either Mincle and FcRγ, or an empty construct with FcRγ. FcRγ is an adaptor protein that binds the intracellular portion of Mincle, and when activated, stimulates the NFAT pathway. An ELISA plate is coated with the compound to be tested overnight, before the two sets of cells are transferred to the ELISA plate. The cells are exposed to the compound for six hours before being lysed. Luciferase reagent is then added and any luminescence is measured. If the compound activates Mincle, it will activate the NFAT pathway via FcRγ, and drive luciferase expression. Cell lysates that contain more luciferase will generate more luminescence, therefore we can determine the level of Mincle activation by measuring the level of luminescence. The lentivirus also encodes GFP, so by measuring GFP fluorescence in each sample, we can obtain an estimate of how well the cells have been transfected, and normalise the luminescence results to the GFP fluorescence. For each assay each compound was tested in triplicate on both Mincle and empty cell lines.

Initial testing shows that the chain length does affect the ability of the compound to act as a ligand to Mincle. Amongst the TDM analogues **138b-143b**, the TDM analogue **140b** with C_{16} acyl chains produced the strongest signal in the assay. The general trend was for the measured luminescence to increase as the chain length increased, peaking at the TDM analogue with C_{16} chains **140b**, before decreasing as the chain length increased (Figure 4.2).

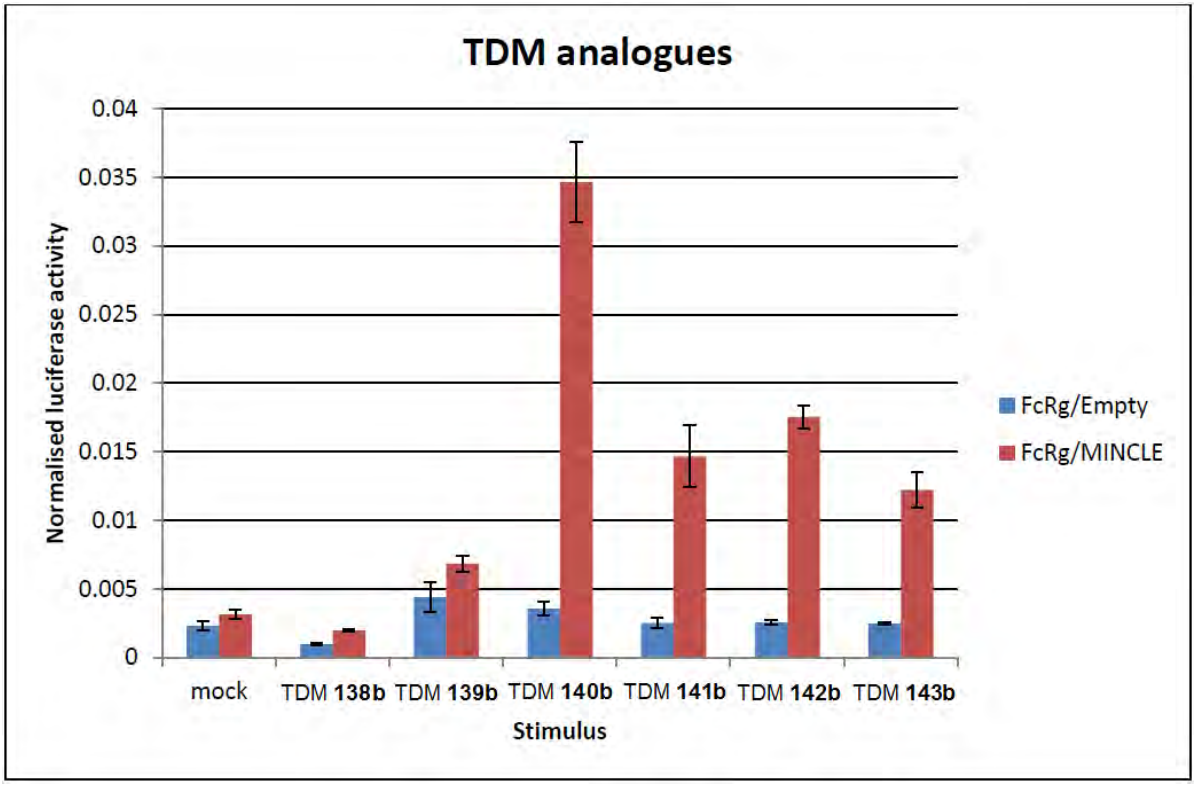


Figure 4.2. Graph showing the relative amounts of luminescence of the TDM analogues.

Amongst the TMM analogues **144b-149b**, the strongest stimulation was observed with analogue **149b**, containing a C_{24} acyl chain. However, the C_{16} TMM **146b** seemed to lyse the cells during the assay and the C_{18} TMM compound **147b** also produced a poor signal in the assay, therefore it is not conclusive that the C_{24} TMM **149b** activates Mincle the most. The general trend was again for the luminescence to increase as the chain length increased but these experiments need further investigation (Figure 4.3).

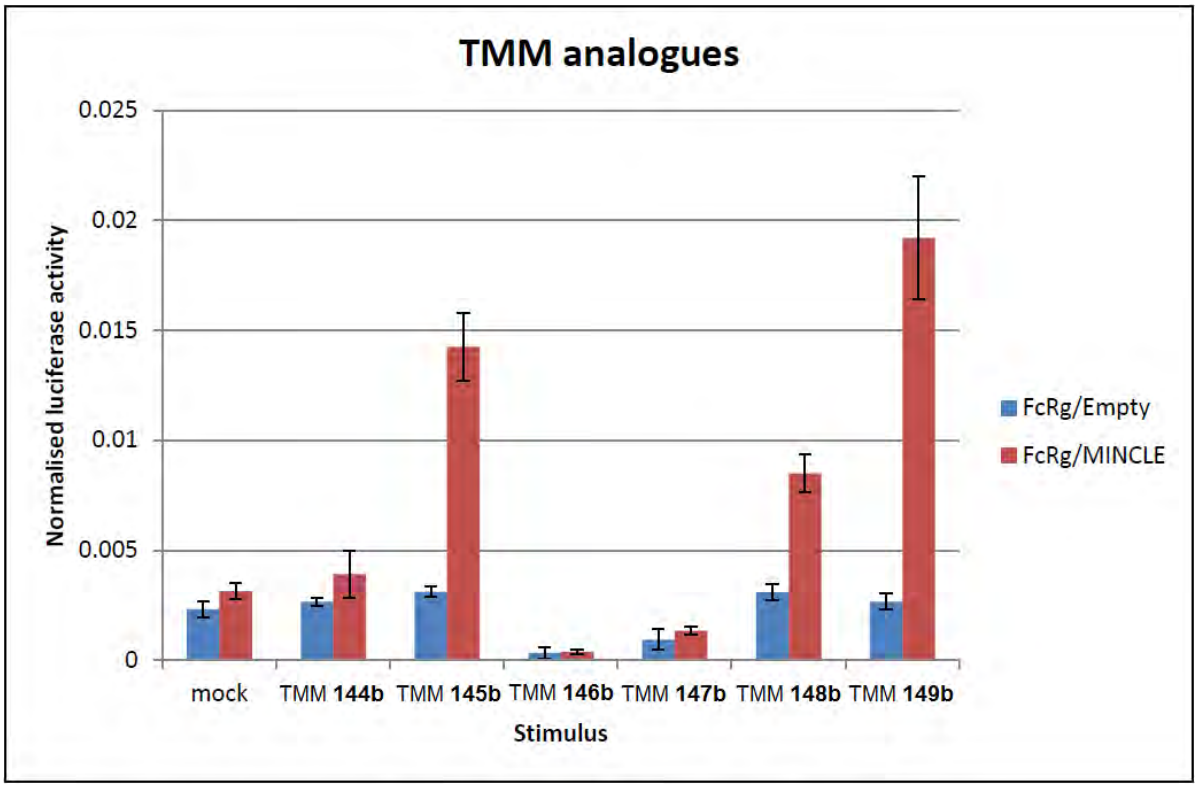


Figure 4.3. Graph showing the relative amounts of luminescence of the TMM analogues.

The TDM analogues appear to be more active than the TMM analogues, with medium chain lengths appearing to stimulate Mincle the most, with C_{16} being the best for TDM analogues. The results for the TMM analogues are currently inconclusive and need repeating. These compounds were tested with both human and mouse Mincle, and activated both with similar levels of expression.

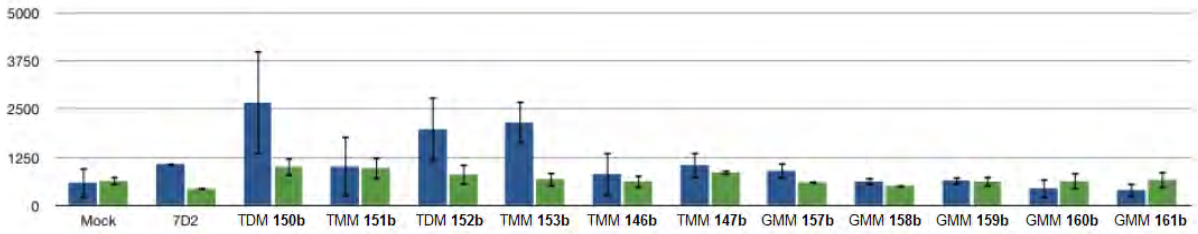


Figure 4.4. Graph showing the relative amounts of luminescence of the GMM and unsaturated analogues. NFAT assay 2: luminescence corrected for GFP fluorescence +/- standard deviation (7D2: anti-mincle antibody positive control), Blue: Mincle transfectants; green: empty vector transfectants.

The GMM analogues **157b-161b** were also tested alongside the unsaturated chain TDM/TMMs **150b-153b** (Figure 4.4). The oleic TDM analogue **150** and both the linoleic TDM and TMM analogues **152b** and **153b** displayed significant activity, indicating that the double bonds and the "kink" might affect binding to Mincle. However they have not yet been tested against the other trehalose analogues and so direct comparisons cannot be made.

None of the GMM analogues had any significant activity, which was in line with previous results that had shown that GMM inhibited Mincle activation.[36]

4.5 Conclusions and Further Work

Initial testing shows that the chain length does affect the ability of the compound to act as a ligand to Mincle. The TDM analogues appear to be more active than the TMM analogues, whilst the GMM analogues did not show any activity. Medium chain lengths appear to be more active, with C_{16} being the best for TDM analogues; the results for the TMM analogues are not complete, and hence are inconclusive. The unsaturated analogues also significantly activate Mincle.

In the future we need to test the unsaturated analogues against the saturated analogues, to determine whether the kink is having a positive effect on activity or not. The best analogues should then be tested for *in vivo* activation of Mincle.

Chapter 5

Experimental

5. Experimental

5.1 Instrumentation

Infra red spectra were recorded neat as thin films on a Perkin Elmer FT-IR PARAGON 1000 spectrometer. The intensity of each band is described as strong (s), medium (m) or weak (w) with the prefix br if the peak is broad. ^{1}H-NMR spectra were recorded at ambient temperature (unless stated otherwise) on a Bruker AC-300 (300 MHz), Bruker AVIII300 (300 MHz), Bruker AMX 400 (400 MHz), Bruker AVIII400 (400 MHz) or Bruker DRX 500 (500 MHz) spectrometer, and are reported as follows: chemical shift δppm (number of protons, multiplicity, coupling constant *J* (Hz), assignment). Connectivities were deduced from COSY90, HSQC and HMBC experiments. Multiplicities of ^{1}H-NMR resonances are reported as follows: s - singlet, d - doublet, t - triplet, p - pentet, m - multiplet, v - very, br - broad signal and stack. The term 'stack' is used to describe a region where resonances from non-equivalent nuclei are coincident. Multiplet is used to describe a region where a resonance arises from a single/equivalent nuclei but where coupling constants cannot be readily assigned. ^{13}C-NMR spectra were recorded at ambient temperature (unless stated otherwise) on a Bruker AV 300 (75 MHz), Bruker AMX 400 (100 MHz), Bruker AVIII400 (100 MHz) or Bruker DRX 500 (125 MHz) spectrometer, and are reported as follows: chemical shift δppm (multiplicity, assignment). EI (electron impact) mass spectra were recorded on a VG Prospec mass spectrometer and TOF-ES+ (time of flight electrospray) mass spectra were recorded on a Micromass LCT spectrometer, and are reported as (*m/z* (%)). High resolution mass spectra (HRMS) were recorded on a Micromass LCT spectrometer, using a lock mass incorporated into the mobile phase. Optical rotations were measured in $CHCl_3$ using an Optical Activity PolAAr2001 automatic polarimeter.

5.2 Chemicals and Reagents

All reagents were obtained from commercial sources and used without further purification unless stated otherwise. All solutions are aqueous and saturated unless stated otherwise.

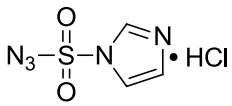
5.3 Reactions

All reactions were conducted in oven-dried (140 °C) or flame-dried glassware under an Ar atmosphere at ambient temperature with magnetic stirring unless stated otherwise. Volumes of 1 mL or less were measured and dispensed with Hamilton gastight syringes. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica aluminium sheets (60A F_{254}, Merck) and visualised by UV detection (at 254 nm) and with phosphomolybdic acid (lipid stain) and α-naphthol with H_2SO_4 (sugar stain). Column chromatography was of the flash type and performed on Fluka 60 (40-60 µm mesh) silica gel and on pre-packed column cartridges (Mega Bond Elut Si 5 g – 20 mL and 2 g – 12 mL, by Varian). Evaporation of volatiles and concentration of solutions under reduced pressure were performed at 50-700 mbar. Residual solvent was removed under high vacuum (<1 mbar).

General procedure for activation of molecular sieves

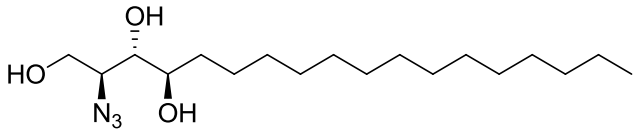
Molecular sieves were weighed into a round bottom flask before it was heated with a heat gun under high vacuum for 2 h. The sieves were then allowed to cool down before immediate transfer to the reaction vessel.

Imidazole-1-sulfonyl azide hydrochloride (13)



SO_2Cl_2 (16.1 mL, 200 mmol) was added dropwise over 5 min to an ice-cooled suspension of NaN_3 (13.0 g, 200 mmol) in dry MeCN (200 mL). The reaction mixture was stirred overnight at rt. Imidazole (25.9 g, 380 mmol) was then added portionwise to the ice-cooled mixture and the resulting slurry stirred for 3 h at rt. The mixture was diluted with EtOAc (400 mL), washed with H_2O (2 × 400 mL) and $NaHCO_3$ solution (2 × 400 mL), dried over Na_2SO_4 and filtered. A solution of HCl in EtOH [obtained by dropwise addition of AcCl (21.2 mL, 300 mmol) to ice-cooled dry EtOH (75 mL)] was added dropwise over 10 min to the filtrate with stirring. The mixture was chilled in an ice-bath, filtered and the filter cake washed with EtOAc (3 × 100 mL) to give azide **13** as a white solid.[122]

(2*R*,3*R*,4*S*)-2-azido-1,3,4-octadecanetriol (14)

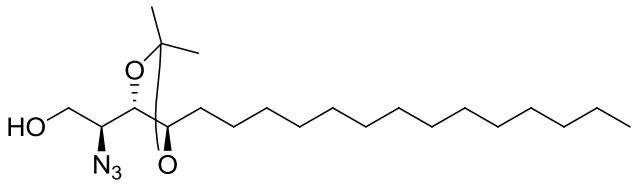


A solution of imidazole-1-sulfonyl azide hydrochloride **13**[122] in CH_2Cl_2 (47 mL) was added to a solution of phytosphingosine (5.00 g, 15.6 mmol), $CuSO_4$ (40 mg, 0.16 mmol) and K_2CO_3 (3.23 g, 23.4 mmol) in H_2O (47 mL) with vigorous stirring. MeOH (157 mL) was added dropwise over 10 min and the mixture was stirred vigorously at rt for at least 18 h. The solution was diluted with CH_2Cl_2 (400 mL), washed with $NaHCO_3$ solution (100 mL), H_2O (70 mL) and brine (70 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced

pressure. The crude product was dissolved in $CHCl_3$ (plus CH_2Cl_2 to facilitate solubility) and purified on a silica plug (90% EtOAc, 10% acetone) to give azide **14** as a white solid (4.88 g, 91%): R_f = 0.70 (100% EtOAc); mp 92 – 94 °C (lit.[170] mp 90 °C); $[\alpha]_D^{22}$ = 9.6 (c = 1.0, $CHCl_3$) (lit.[171] $[\alpha]_D^{25}$ = 10.0 (c = 1.0, $CHCl_3$,); ν_{max}(film)/cm^{-1} 3261 br w, 2916 s, 2847 s, 2096 s, 1590 w, 1461 m, 1248 m, 1099 w, 1058 s, 1008 m, 923 w, 857 m, 723 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.66 (3H, t, *J* = 6.5), 0.95-1.22 (24H, stack), 1.37-1.42 (2H, stack), 3.34-3.39 (3H, stack), 3.58 (1H, dd, *J* = 6.1, 5.7), 3.72 (1H, dd, *J* = 3.9, 3.7), exchangeable hydrogens not observed; ^{13}C NMR (75 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 14.4 (CH_3), 23.7 (CH_2), 26.7 (CH_2), 30.5 (CH_2), 30.7 (CH_2), 30.8 (CH_2), 33.1 (CH_2), 33.9 (CH_2), 62.5 (CH_2), 66.6 (CH), 72.9 (CH), 76.0 (CH), some overlap in the methylene resonances; *m/z* (TOF ES+) 366.2 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 366.2728 $C_{18}H_{37}N_3O_3Na$ $[M+Na]^+$ requires 366.2733.

Data were in agreement with those reported in the literature.[170,171]

(2*R*,3*R*,4*S*)-2-azido-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (10)

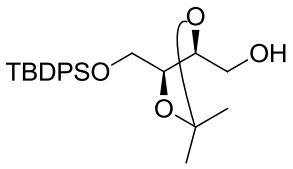


Concentrated H_2SO_4 (4 drops) was added to a solution of azide **14** (500 mg, 1.46 mmol) in dry acetone (10 mL) at 0 °C. After stirring for 2.5 h, the reaction mixture was quenched with $NaHCO_3$ solution (20 mL), and then concentrated under reduced pressure. The mixture was extracted with EtOAc (3 × 20mL) and the combined organic layers were washed with brine (10 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure.

The crude product was purified by column chromatography (10% EtOAc in hexane) to give acetonide **10** as a colourless oil (420 mg, 75%): R_f = 0.16 (10% EtOAc in hexanes); $[\alpha]_D^{20}$ = 26.2 (c = 1.0, $CHCl_3$) (lit.[119] $[\alpha]_D^{22}$ = 23.0 (c = 1.0, $CHCl_3$)); ν_{max}(film)/cm^{-1} 3432 br w, 2920 s, 2850 s, 2095 s, 1471 m, 1427 w, 1382 m, 1370 m, 1344 w, 1317 w, 1261 m, 1207 s, 1164 m, 1102 m, 1053 m, 1019 s, 882 m, 856 m, 826 w, 718 m; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.91 (3H, t, *J* = 6.7), 1.22-1.69 (32H, stack), 3.50 (1H, ddd, *J* = 9.6, 5.4, 4.5), 3.90 (1H, dd, *J* = 11.6, 5.5), 3.96-4.06 (2H, m), 4.17-4.26 (1H, m), O*H* resonance not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.9 (CH_3), 22.7 (CH_2), 25.5 (CH_3), 26.5 (CH_2), 28.0 (CH_3), [29.4, 29.5, 29.6, 29.7 (CH_2, significant resonance overlap)], 31.9 (CH_2), 61.1 (CH), 63.9 (CH_2), 76.7 (CH), 77.7 (CH), 108.6 (C); *m/z* (TOF ES+) 406.5 ($[M+Na]^+$, 100%).

Data were in agreement with those reported in the literature.[119]

(2*S*, 3*S*)-1-*O*-(*tert*-butyldiphenysilyl)-2,3-*O*-isopropylidene-1,2,3,4-butanetetraol (15)

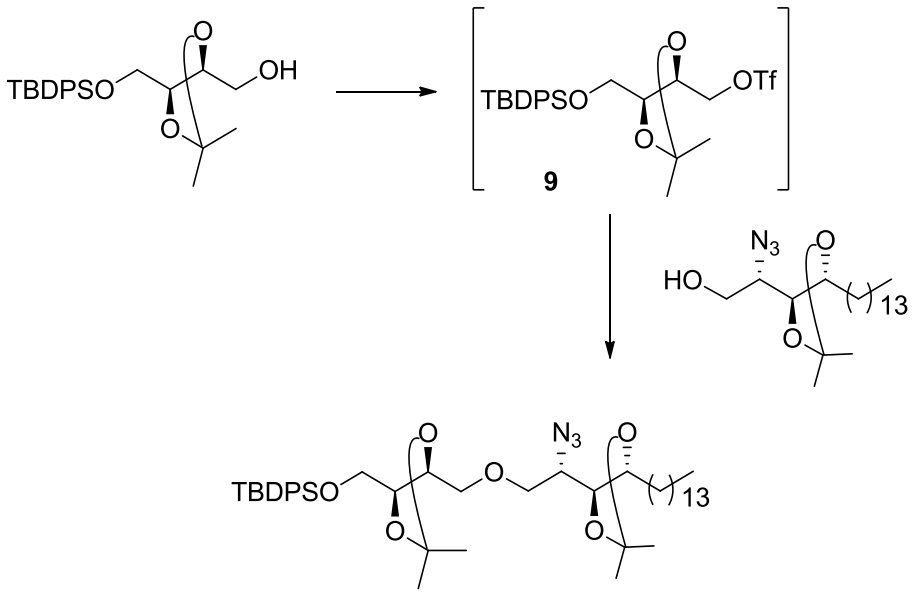


A solution of (+)-2,3-*O*-isopropylidene-L-threitol **11** (250 mg, 1.54 mmol) in THF (5 mL) was added dropwise over 10 min to a suspension of NaH (60% dispersion in mineral oil, 62 mg, 1.70 mmol) in THF (10 mL) at 0 °C. After 30 min, a solution of TBDPSCl (423 mg, 1.70 mmol) in THF (5 mL) was added dropwise over 20 min. After stirring at rt for 12 h, the reaction was quenched by the sequential addition of MeOH (5 mL) and $NaHCO_3$ solution (60 mL). The resulting layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 60 mL).

The combined organic phases were washed with brine (60 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (15% EtOAc in hexanes) to give silyl ether **15** as a colourless oil (0.56 g, 92%): R_f = 0.25 (15% EtOAc in hexanes); $[\alpha]_D^{20}$ = 3.5 (c = 1.0, $CHCl_3$), lit.[172] $[\alpha]_D^{22}$ = 8.5 (c = 0.2, $CHCl_3$); ν_{max}(film)/cm^{-1} 3465 br w, 3071 w, 3049 w, 2986 m, 2931 s, 2858 s, 1589 w, 1473 m, 1463 m, 1428 s, 1380 m, 1371 m, 1246 m, 1216 m, 1113 s, 1080 s, 823 m, 704 s; ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.13 (9H, s, C(C*H*$_3$)$_3$), 1.45 (3H, s, 1 × C(C*H*$_3$)$_2$), 1.48 (3H, s, 1 × C(C*H*$_3$)$_2$), 2.61 (1H, br s, O*H*), 3.67-3.93 (4H, stack), 3.99-4.08 (1H, m), 4.12-4.20 (1H, m), 7.39-7.52 (6H, stack, Ph), 7.70-7.78 (4H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 19.1 (C, *C*(CH$_3$)$_3$), [26.9, 27.0, 27.2 (CH$_3$, C(*C*H$_3$)$_3$, C(*C*H$_3$)$_2$)], 62.6 (CH$_2$, *C*H$_2$O), 64.2 (CH$_2$, *C*H$_2$O), 77.6 (CH, *C*HO), 79.6 (CH, *C*HO), 109.1 (C, *C*(CH$_3$)$_2$), 127.8 (CH, Ph), 129.9 (CH, Ph), 132.9 (C, *ipso* Ph), 135.6 (CH, Ph); *m/z* (TOF ES+) 423.3 ([M+Na]$^+$, 100%).

Data were in agreement with those reported in the literature.[172]

(2*R*,3*R*,4*S*,2'*S*,3'*S*)-2-azido-1-*O*-[4'-*O*-(*tert*-butyldiphenylsilyl)-2',3'-*O*-isopropylidene-2',3',4'-trihydroxybutyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (8)

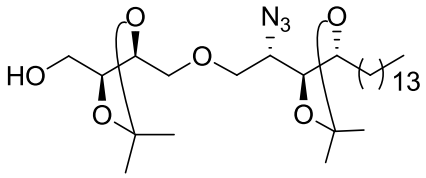


Tf_2O (122 µL, 0.73 mmol) was added dropwise over 10 min to a solution of silyl ether **15** (290 mg, 0.73 mmol) and 2,6-di-*tert*-butylpyridine (179 µL, 0.80 mmol) in CH_2Cl_2 (15 mL) at 0 °C. After 30 min, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and the resulting solution washed sequentially with cold H_2O (2 × 30 mL) and brine (10 mL), dried over Na_2SO_4, and filtered. Removal of the solvent under reduced pressure provided crude triflate **9** as a colourless oil, which was used immediately in the next etherification step: R_f = 0.70 (15% EtOAc in hexanes). A solution of alcohol **10** (253 mg, 0.66 mmol) in THF (10 mL) was treated with NaH (60% dispersion in mineral oil, 29 mg, 0.73 mmol) at 0 °C. After 1 h, a solution of triflate **9** (assuming 100% conversion, 0.73 mmol) in THF (5 mL) was added dropwise over 5 min. The reaction mixture was stirred at 0 °C for 1 h and then at rt for 12 h. The reaction was then quenched by the addition of MeOH (2 mL) followed by $NaHCO_3$ solution (10 mL). The resulting layers were separated and the phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate

concentrated under reduced pressure. The crude product was purified by column chromatography (5% EtOAc in hexanes) to give ether **8** as a colourless oil (0.41 g, 81%): R_f = 0.20 (5% EtOAc in hexanes); $[\alpha]_D^{20}$ = 10.0 (c = 1.0, $CHCl_3$), lit.[173] $[\alpha]_D^{21}$ = 10.0 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2925 s, 2855 s, 2098 s (N_3), 1589 w, 1463 m, 1428 s, 1380 m, 1371 s, 1218 s, 1112 s, 1082 s, 823 s, 701 s; ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.77 (3H, t, *J* = 6.7, CH_2CH_3), 0.98-1.03 (23H, stack, alkyl chain), 1.24 (9H, s, $C(CH_3)_3$), 1.33 (6H, s, $C(CH_3)_2$), 1.36 (6H, s, $C(CH_3)_2$), 1.49-1.63 (3H, stack), 3.50-3.68 (4H, stack), 3.71-3.38 (3H, stack), 3.80-3.89 (2H, stack), 3.97-4.08 (1H, m), 4.09-4.18 (1H, m), 7.39-7.52 (6H, stack, Ph), 7.70-7.78 (4H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2 (CH_3, CH_2CH_3), 19.3 (C, $C(CH_3)_3$), 22.8 (CH_2), 25.7 (CH_3, 1 × CH_3 from 1,2-*anti*-diol acetonide), 26.5 (CH_2), 26.9 (1 × CH_3 from 1,2-*syn*-diol acetonide), 27.0 (CH_3, $C(CH_3)_3$), 27.2 (CH_3, 1 × CH_3 from 1,2-*syn*-diol acetonide), 28.2 (CH_3, 1 × CH_3 from 1,2-*anti*-diol acetonide), [29.4, 29.61, 29.63, 29.72, 29.73 (CH_2, alkyl chain, resonance overlap)], 32.0 (CH_2), 60.0 (CH), 64.2 (CH_2), 72.4 (CH_2), 73.1 (CH_2), 75.8 (CH), 76.8 (CH), 77.9 (CH), 78.0 (CH), 108.3 (C, $C(CH_3)_2$), 109.5 (C, $C(CH_3)_2$), 127.7 (CH, Ph), 129.7 (CH, Ph), 133.2 (C, *ipso* Ph), 135.6 (CH, Ph); *m/z* (TOF ES+) 788.6 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 788.5001 ($[M+Na]^+$) $C_{44}H_{71}N_3O_6Si$ requires 788.5010.

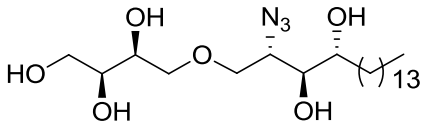
Data were in agreement with those reported in the literature.[173]

(2*R*,3*R*,4*S*,2'*S*,3'*S*)-2-azido-1-*O*-[2',3'-*O*-isopropylidene-2',3',4'-trihydroxybutyl]-3,4-*O*-isopropylidene-1,3,4-octadecantriol (16)



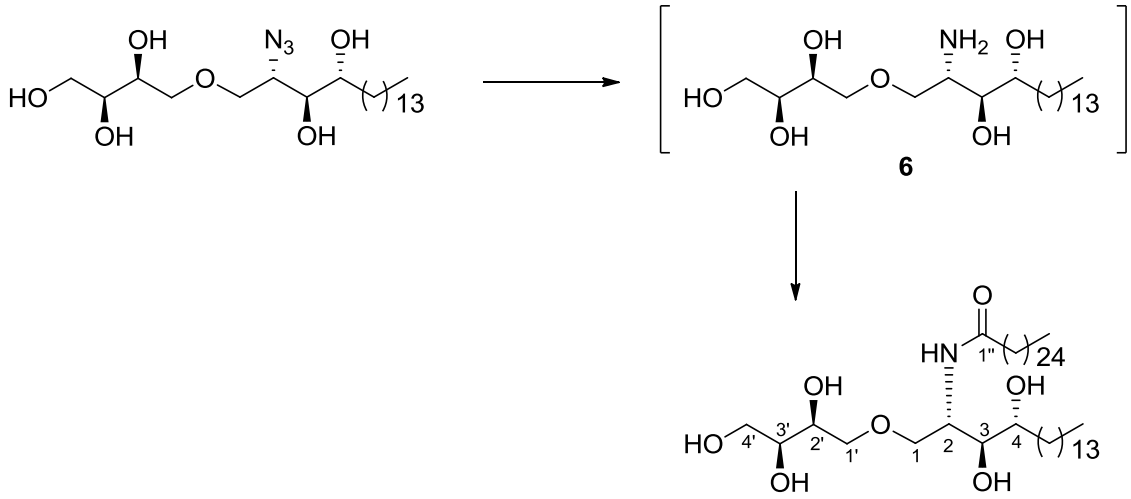
TBAF (1.0 M solution in THF, 0.65 mL, 0.65 mmol) was added to a solution of silyl ether **8** (250 mg, 0.33 mmol) in THF (5 mL) at rt. After 4 h, NH_4Cl solution (10 mL) was added. The resulting layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography to give alcohol **16** as a colourless oil (164 mg, 94%): R_f = 0.35 (30% EtOAc in hexanes); $[\alpha]_D^{20}$ = 22.0 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3487 br (O–H), 2925 s, 2098 s (N_3), 1589 w, 1458 m, 1370 m, 1250 s, 1219 s, 1168 m, 1058 s, 846 s, 707 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.81 (3H, t, *J* = 6.7, CH_2CH_3), 1.13-1.23 (24H, stack, alkyl chain), 1.24 (3H, s, 1 × $C(CH_3)_2$), 1.33 (3H, s, 1 × $C(CH_3)_2$), 1.35 (6H, s, 2 × $C(CH_3)_2$, resonance overlap), 1.43-1.56 (2H, stack), 2.33 (1H, br s, O*H*), 3.45-4.11 (11H, broad stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.6 (CH_3, 1 × $C(CH_3)_2$), 26.4 (CH_2), 26.9 (CH_3, 1 × $C(CH_3)_2$), 27.0 (CH_3, 1 × $C(CH_3)_3$), 28.1 (CH_3, 1 × $C(CH_3)_3$), [29.3, 29.4, 29.5, 29.6, 29.7 (CH_2, alkyl chain, resonance overlap)], 31.9 (CH_2), 59.9 (CH), 62.3 (CH_2), 71.9 (CH_2), 72.8 (CH_2), 75.6 (CH), 76.1 (CH), 77.7 (CH), 79.4 (CH), 108.3 (C, $C(CH_3)_2$), 109.4 (C, $C(CH_3)_2$); *m/z* (TOF ES+) 550.5 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 550.3823 ($[M+Na]^+$) $C_{28}H_{53}N_3O_6$ requires 550.3832.

(2*R*,3*R*,4*S*,2'*S*,3'*S*)-2-azido-1-*O*-[2',3',4'-trihydroxybutyl]-1,3,4-octadecantriol (17)



TFA (2.00 mL) was added over 1 min to azide **16** (400 mg, 0.76 mmol). After stirring for 1 h at rt the reaction mixture was concentrated under reduced pressure and the residual TFA was removed by co-evaporation with Et_2O (3 × 10 mL) to provide the crude pentaol **17** as a white solid (315 mg, quant), which was used in the next step without further purification: R_f = 0.23 (10% MeOH in $CHCl_3$); $[\alpha]_D$ = 29.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3306 br (O–H), 2916 s, 2848 s, 2095 s (N_3), 1465 m, 1380 m, 1271 s, 1166m, 1097 s, 1075 m, 981 w, 932 w, 881 w, 859 s, 723 s, 685 m; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.80 (3H, t, *J* = 6.6, CH_2CH_3), 1.13-1.35 (24H, stack, alkyl chain), 1.43-1.63 (2H, stack, CH_2), 3.49-3.68 (8H, stack), 3.69-3.84 (3H, stack), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 15.2 (CH_3, CH_2CH_3), 24.0 (CH_2), 27.1 (CH_2), [30.7, 31.0 (CH_2, alkyl chain, resonance overlap)], 33.2 (CH_2), 33.5 (CH_2), 63.5 (CH), 64.8 (CH_2), 71.6 (CH), 72.0 (CH_2), 73.1 (CH), 73.3 (CH), 73.9 (CH_2), 75.3 (CH); *m/z* (TOF ES+) 470.4 ($[M+Na]^+$, 100%).

(2*R*,3*R*,4*S*,2'*S*,3'*S*)-1-*O*-[2',3',4'-trihydroxybutyl]-2-hexacosanoylamino-1,3,4-octadecantriol [ThrCer] (1)

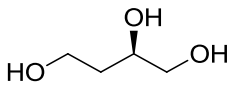


PMe_3 (1.0 M solution in THF, 0.21 mL, 0.21 mmol) was added dropwise over 5 min to a solution of pentaol **17** (80 mg, 0.18 mmol) in THF/H_2O (3 mL, 15:1). The reaction mixture was stirred at rt for 4 h and then concentrated under reduced pressure. The residual H_2O was removed by co-evaporation with toluene (3 × 10 mL) to provide amine **6** as a white solid, which was used directly in the next step without further purification. $(COCl)_2$ (2 mL) was added to hexacosanoic acid (85 mg, 0.21 mmol) and heated at 70 °C for 2 h, after which time the solution was cooled to rt, and the $(COCl)_2$ removed under a stream of dry argon. The residual volatiles were removed under reduced pressure. The resulting crude acyl chloride was dissolved in THF (0.5 mL) and added with vigorous stirring to a solution of amine **6** (assuming 100% conversion, 0.18 mmol) in THF/$NaOAc_{(aq)}$ (8M) (1:1, 2 mL). Vigorous stirring was maintained for 2 h, after which time the reaction mixture was left to stand and the layers were separated. The aqueous layer was extracted with THF (3 × 2.0 mL) and the organic layers were combined and concentrated under reduced pressure. The crude product was purified by column chromatography (10% MeOH in $CHCl_3$) to give ThrCer **1** as a white solid (68 mg, 47% over 2 steps): R_f = 0.30 (8% MeOH

in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; mp 107–109 °C; ν_{max}(film)/cm^{-1} 3308 br (O–H), 2915 s, 2849 s, 2098 w, 1634 m (C=O), 1540 m, 1471 m, 1108 m, 1070 m, 1026 m, 718 m; ^{1}H NMR (400 MHz, $CDCl_3$: CD_3OD, 2:1, 40 °C) δ ppm 0.85 (6H, t, *J* = 6.6, 2 × CH_2CH_3), 1.15-1.33 (70H, stack, alkyl chain), 1.52-1.68 (2H, stack, CH_2), 2.17 (2H, app t, *J* = 7.7), 3.46-3.58 (4H, stack, C(1')H_2, C(4)*H*, C(3)*H*), 3.58-3.65 (4H, stack, C(1)H_aH_b, C(2')*H* or C(3')*H*, C(4')H_2), 3.69-3.79 (2H, stack, C(1)H_aH_b, C(3')*H* or C(2')*H*), 4.13-4.18 (1H, m, C(2)*H*), N*H* and O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2 (CH_3, CH_2CH_3), 23.1 (CH_2), 26.4 (CH_2), [29.6, 29.8, 29.9, 30.1 (CH_2, alkyl chains, resonance overlap)], 32.4 (CH_2), 33.0 (CH_2), 36.9 (CH_2), 50.8 (CH, C(2)), 63.9 (CH_2, C(4')), 70.8 (CH, C(2') or C(3')), 71.0 (CH_2, C(1')), 72.5 (CH, C(4)), 73.0 (CH, C(3') or C(2')), 73.4 (CH_2, C(1)), 175.1 (C, C(1'')); *m/z* (TOF ES+) 823.9 ($[M+Na]^+$, 100%).

Data were in agreement with those reported in the literature.[119]

(2*R*)-1,2,4-butanetriol (22)

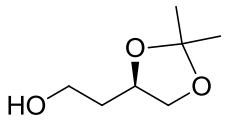


$(MeO)_3B$ (12.5 mL, 0.112 mol) was added to a solution of $BH_3{\cdot}SMe_3$ complex (2 M solution in THF, 60.0 mL, 0.12 mol) at 0 ˚C. (*R*)-Malic acid (5.0 g, 0.037 mol) in dry THF (25 mL) was added to the solution dropwise over 5 min. The resulting mixture was stirred for 5 min at 0 ˚C, then for 24 h at rt. MeOH (30 mL) was added very slowly (over 1-2 h) at 0 ˚C before the solution was concentrated under reduced pressure. Three further co-evaporations with MeOH (25 mL added portionwise) under reduced pressure afforded the crude product which was purified by column chromatography (12% MeOH in CH_2Cl_2) to provide triol **22** as a colourless oil (3.7 g,

94%): R_f = 0.21 (10% MeOH in $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.53-1.66 (1H, m), 1.67-1.79 (1H, m), 3.42-3.53 (2H, m), 3.67-3.80 (3H, stack), exchangeable hydrogens not observed; ^{13}C NMR (100 MHz, CD_3OD) δ ppm 37.1 (CH_2), 60.0 (CH_2), 67.5 (CH_2), 70.8 (CH); *m/z* (EI) 107 (M^+), 75, 57, 45.

Data were in agreement with those reported in the literature.[127]

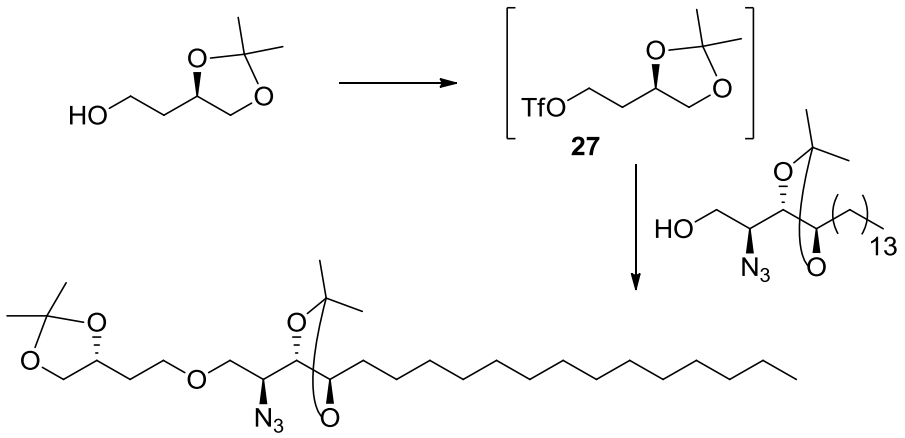
(2*R*)-1,2-*O*-isopropylidene-1,2,4-butanetriol (26)



Alcohol **22** (1.14 g, 10.7 mmol) and *p*TsOH (0.40 g, 2.14 mmol) were dissolved in dry acetone (35 mL) and stirred overnight at rt. The reaction was quenched by addition of solid $NaHCO_3$ (5 g), and stirred for 30 min. The solution was then filtered and concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL), washed sequentially with $NaHCO_3$ solution (10 mL) and brine (10 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (50% EtOAc in hexane) to provide acetonide **26** as a colourless oil (1.13 g, 72%): R_f = 0.74 (10% MeOH in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.35 (3H, s), 1.42 (3H, s), 1.76-1.85 (2H, stack), 2.19 (1H, t, *J* = 5.4), 3.59 (1H, dd, *J* = 8.0, 7.4), 3.75-3.86 (2H, stack), 4.08 (1H, dd, *J* = 8.1, 6.0), 4.27 (1H, dt, *J* = 12.6, 6.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 25.6 (CH_3), 26.8 (CH_3), 35.7 (CH_2), 60.1 (CH_2), 69.4 (CH_2), 74.7 (CH), 108.9 (C); *m/z* (EI) 131 $[M - Me]^+$, 101, 71, 59, 43.

Data were in agreement with those reported in the literature.[174]

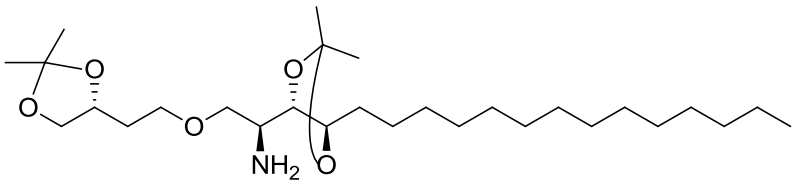
(2*R*,3*R*,4*S*,3'*S*)-2-azido-1-*O*-[3',4'-*O*-isopropylidene-butyl]-3,4-isopropylidene-1,3,4-octadecanetriol (18)



Tf_2O (331 µL, 1.97 mmol) was added dropwise over 10 min to a solution of alcohol **26** (225 mg, 1.54 mmol) and 2,6-di-*tert*-butyl pyridine (487 µL, 2.17 mmol) in dry CH_2Cl_2 (20 mL) at 0 ˚C. The reaction mixture was stirred for 15 min, then diluted with CH_2Cl_2 (20 mL), washed with cold H_2O (2 × 20 mL) and then brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was used immediately in the next step: R_f = 0.60 (25% EtOAc in hexanes). Azide **10** (150 mg, 0.40 mmol) in dry THF (2.0 mL) was treated with NaH (60% by wt in mineral oil, 18 mg, 0.44 mmol) at 0 ˚C. After stirring for 1 h, a solution of triflate **27** (0.40 mmol) in dry THF (2.0 mL) was added dropwise over 5 min at 0 ˚C. The mixture was stirred at 0 ˚C for 1 h, then at rt overnight. The reaction was quenched by addition of NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (hexane-7% EtOAc in hexane, gradient) to provide azide **18** as a colourless oil (95 mg, 47%): R_f = 0.60 (15% EtOAc in hexanes); $[\alpha]_D^{22}$ = 26.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 2098 s (N_3), 1458 m, 1378 m, 1369 m, 1246 m, 1219 m, 1161 w, 1101 m, 1059 s, 861 m, 721 w; ^{1}H NMR

(300 MHz, $CDCl_3$) δ ppm 0.81 (3H, t, *J* = 6.7), 1.10-1.59 (38H, stack), 1.73-1.89 (2H, stack), 3.39-3.61 (5H, stack), 3.75-3.85 (2H, stack), 3.98-4.10 (2H, stack), 4.10-4.22 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.9 (CH_3), 22.7 (CH_2), 25.6 (CH_3), 25.7 (CH_3), 26.4 (CH_2), 26.9 (CH_3), 28.1 (CH_3), [29.3, 29.4, 29.6 (CH_2, significant resonance overlap)], 31.9 (CH_2), 33.9 (CH_2), 59.8 (CH), 68.2 (CH_2), 69.6 (CH_2), 71.9 (CH_2), 73.6 (CH), 75.6 (CH), 77.8 (CH), 108.3 (C), 108.8 (C); *m/z* (TOF ES+) 534.4 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 534.3881 ($[M+Na]^+$) $C_{28}H_{53}N_3O_5Na$ requires 534.3883.

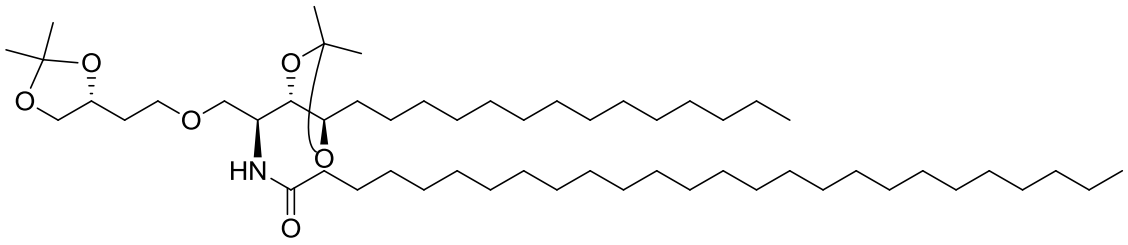
(2*R*,3*R*,4*S*,3'*S*)-2-amino-1-*O*-[3',4'-*O*-isopropylidene-butyl]-3,4-isopropylidene-1,3,4-octadecanetriol (30)



PMe_3 (1.0 M solution in THF, 150 µL, 0.15 mmol) was added dropwise over 5 min to a solution of azide **18** (70 mg, 0.14 mmol) in THF (1.4 mL). The reaction mixture was stirred for 4 h at rt, then H_2O (1 mL) was added and the mixture left to stir overnight. The mixture was then concentrated under reduced pressure and residual H_2O removed by co-evaporation with toluene (3 × 2 mL). The crude product was purified by column chromatography (30% EtOAc in hexane) to provide amine **30** as a white solid (61 mg, 92%): R_f = 0.06 (30% EtOAc in hexanes); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 1468 w, 1369 w, 1218 w, 1062 w, 858 w; ^1H NMR (300 MHz, C_6D_6) δ ppm 0.92 (3H, t, *J* = 6.6), 1.58-1.16 (36H, stack), 1.58-1.87 (4H, stack), 2.93-3.01 (1H, m), 3.31-3.48 (4H, stack), 3.54 (1H, dd, *J* = 9.0, 2.9), 3.87 (1H, dd, *J* = 5.8, 2.2), 3.90 (1H, d, *J* = 5.9), 4.09

(1H, ddd, *J* = 13.0, 7.1, 6.0), 4.17-4.25 (1H, m), N*H*$_2$ not observed; ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.2 (CH_3), 23.0 (CH_2), 25.9 (CH_3), 26.1 (CH_3), 26.6 (CH_2), 27.2 (CH_3), 28.7 (CH_3), [29.7, 30.0 (CH_2, significant resonance overlap)], 32.2 (CH_2), 34.2 (CH_2), 50.9 (CH), 68.1 (CH_2), 69.8 (CH_2), 74.0 (CH), 74.7 (CH_2), 78.2 (CH), 79.0 (CH), 107.7 (C), 108.5 (C); *m/z* (TOF ES+) 486.3 ($[M]^+$, 100%); HRMS *m/z* (TOF ES+) 508.3963 ($[M+Na]^+$) $C_{28}H_{55}NO_5Na$ requires 508.3978.

(2*R*,3*R*,4*S*,3'*S*)-2-hexacosanoylamino-1-*O*-[3',4'-*O*-isopropylidene-butyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (32)

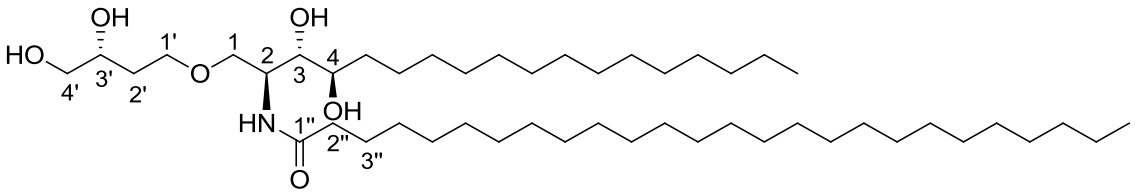


$(COCl)_2$ (2 mL) was added to hexacosanoic acid (26 mg, 0.0615 mmol) in a flame-dried tube, which was tightly closed, parafilmed and heated at 70 ˚C for 2 h. The volatiles were evaporated under a flow of argon and any residual solvent evaporated under high vacuum for at least 1 h. The resulting acid chloride was used immediately without further purification.

A solution of freshly prepared acid chloride (26 mg, 0.0615 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise over 5 min to a solution of amine **30** (20 mg, 0.041 mmol) and NEt_3 (12 µL, 0.082 mmol) in CH_2Cl_2 (0.27 mL) at 0 ˚C. The reaction mixture was stirred overnight, then diluted with CH_2Cl_2 (20 mL), washed sequentially with $NaHCO_3$ solution (20mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexane)

to provide amide **32** as a white solid (27 mg, 76%): R_f = 0.46 (30% EtOAc in hexanes); mp = 77–78 °C; $[\alpha]_D^{22}$ = −1.8 (c = 0.6, CH_3Cl); ν_{max}(film)/cm^{-1} 3323 w (N–H), 2917 s, 2850 s, 1734 w, 1643 m (C=O), 1532 w, 1472 w, 1370 w, 1244 w, 1060 w, 870 w, 719 w; ^{1}H NMR (300 MHz, C_6D_6) δ ppm 0.82-1.04 (6H, stack), 1.20-2.02 (88H, stack), 3.27-3.40 (2H, stack), 3.40-3.53 (2H, stack), 3.70 (1H, dd, *J* = 9.4, 3.5), 3.93 (1H, dd, *J* =7.9, 6.0), 4.02-4.17 (3H, stack), 4.47-4.58 (1H, m), 5.23 (1H, d, *J* = 9.6); ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.2 (CH_3), 23.0 (CH_2), 26.0 (2 × CH_3), 26.7 (CH_2), 27.1 (CH_3), 28.3 (CH_3), [29.6, 30.1 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 34.1 (CH_2), 36.7 (CH_2), 48.3 (CH), 68.2 (CH_2), 69.8 (CH_2), 71.0 (CH_2), 74.1 (CH), 76.6 (CH), 78.2 (CH), 107.9 (C), 108.6 (C), 171.3 (C); *m/z* (TOF ES+) 887.0 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 886.7834 ($[M+Na]^+$) $C_{54}H_{105}NO_6Na$ requires 886.7840.

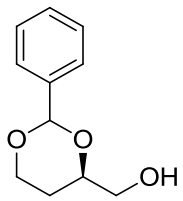
(2*R*,3*R*,4*S*,3'*S*)-1-*O*-[3',4'-dihydroxybutyl]-2-hexacosanoylamino-1,3,4-octadecanetriol (2)



TFA (0.23 mL, 0.3 mmol) was added to a solution of amide **32** (26 mg, 0.03 mmol), in CH_2Cl_2 (1 mL) and H_2O (10 µL). The solution was stirred at rt overnight. The reaction mixture was then poured into CH_2Cl_2 (10 mL) and quenched with $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with chloroform (3 × 10 mL) and the combined organic layers were washed with $NaHCO_3$ (30 mL) and then brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (5% MeOH in $CHCl_3$) to provide tetraol **2** as a white solid (15 mg, 63%): R_f =

0.44 (10% MeOH in $CHCl_3$); mp = 106–107 °C; $[\alpha]_D$ the insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3321 br w (O–H,), 2957 w, 2918 m, 2850 m, 2322 w, 1972 w, 1626 m (C=O), 1464 w, 719 w; ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD, 2:1, 40 °C) δ ppm 0.83 (6H, t, *J* = 6.8, 2 × CH_2CH_3), 1.07-1.38 (68H, stack, CH_2 resonances in alkyl chains), 1.44-1.53 (1H, m, C(3'')H_aH_b), 1.53-1.66 (4H, stack, C(2')H_aH_b, C(3'')H_aH_b, 1 × CH_2 in alkyl chain), 1.66-1.75 (1H, m, C(2')H_aH_b), 2.16 (2H, app t, *J* = 7.6, C(2'')H_2), 3.41 (1H, dd, *J* = 11.2, 6.6, C(4')H_aH_b), 3.44-3.52 (3H, stack, C(4)*H*, C(3)*H*, C(4')H_aH_b), 3.52-3.61 (3H, stack, C(1)H_aH_b, C(1')H_2), 3.66 (1H, dd, *J* =9.8, 4.6 Hz, C(1)H_aH_b), 3.69-3.75 (1H, m, C(3')*H*), 4.15 (1H, dd, *J* = 8.7, 4.3, C(2)*H*); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD, 2:1, 40 °C) δ ppm 14.1 (CH_3, 2 × CH_2CH_3), 22.9 (CH_2), 26.2 (CH_2, C(3'')), [29.7, 29.8, 29.9, 30.0 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 33.1 (CH_2), 33.2 (CH_2, C(2')), 36.8 (CH_2, C(2'')), 50.6 (CH, C(2)), 66.6 (CH_2, C(4')), 68.7 (CH_2 C(1')), 70.2 (CH, C(3')), 70.3 (CH_2, C(1)), 72.9 (CH, C(4)), 75.6 (CH, C(3)), 174.8 (C, C(1'')); *m/z* (TOF ES+) 806.9 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 806.7186 ($[M+Na]^+$) $C_{48}H_{97}NO_6Na$ requires 806.7214.

(2*R*)-2,4-*O*-benzylidene-1,2,4-butanetriol (28)

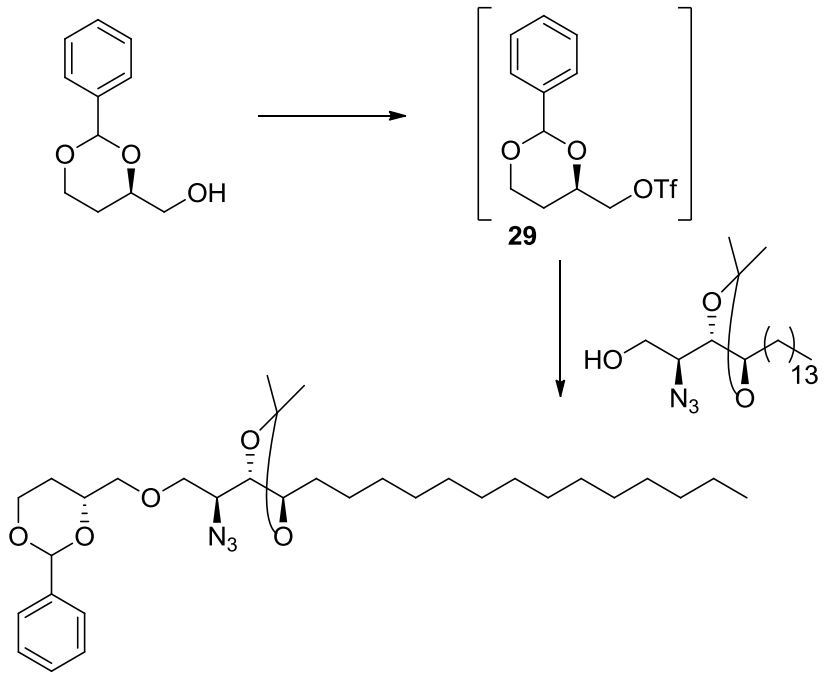


PhCHO (0.93 mL, 9.2 mmol) and freshly activated 4 Å molecular sieves were added to a solution of triol **22** (650 mg, 6.1 mmol) in dry toluene (65 mL) at 95 °C. After stirring for 30 min, *p*TsOH·H_2O (0.11 g, 0.6 mmol) was added and the mixture left to stir overnight. The reaction

was quenched with $NaHCO_3$ solution (30 mL), the molecular sieves were filtered off using Celite and the resulting solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed sequentially with $NaHCO_3$ solution (30 mL) and H_2O (30 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (50% EtOAc in hexane) to provide 1,3-dioxane **28** as a colourless oil (746 mg, 63 %): R_f = 0.74 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.85-2.07 (2H, stack), 3.64-3.75 (2H, stack), 3.94-4.09 (2H, stack), 4.32 (1H, ddd, *J* = 11.3, 5.1, 1.2), 5.56 (1H, s), 7.31-7.43 (3H, stack), 7.43-7.53 (2H, stack), O*H* not observed; ^{13}C NMR (100 MHz, C_6D_6) δ ppm 27.0 (CH_2), 65.6 (CH), 66.4 (CH), 77.7 (CH_2), 101.3 (CH), 126.6 (CH), 128.2 (CH), 128.7 (CH), 140.2 (C); *m/z* (EI) 194 (M^+), 163, 105, 91, 79, 71, 57, 51, 43.

Data were in agreement with those reported in the literature.[128]

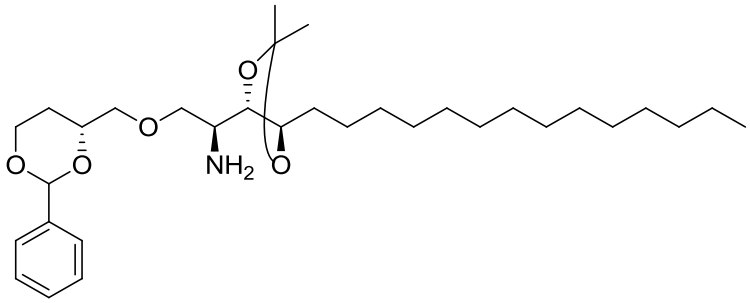
(2*R*,3*R*,4*S*,2'*S*)-2-azido-1-*O*-(2',4'-*O*-benzylidene-butyl)-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (19)



Tf_2O (106 µL, 0.63 mmol) was added dropwise over 10 min to a solution of alcohol **28** (122 mg, 0.63 mmol) and 2,6-di-*tert*-butyl pyridine (213 µL, 0.93 mmol) in dry CH_2Cl_2 (6.3 mL) at 0 ˚C. The reaction mixture was stirred for 15 min, then diluted with CH_2Cl_2 (20 mL), washed with cold H_2O (2 × 20 mL) and then brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was used immediately in the next step: R_f = 0.43 (25% EtOAc in hexanes). Azide **10** (200 mg, 0.52 mmol) in dry THF (2.6 mL) was treated with NaH (60% by wt in mineral oil, 23 mg, 0.57 mmol) at 0 ˚C. The solution was stirred for 1 h, then a solution of triflate **29** (0.63 mmol) in dry THF (2.6 mL) was added dropwise over 5 min at 0 ˚C. The mixture was stirred at 0 ˚C for 1 h, then at rt overnight. The reaction was quenched by addition of NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column

chromatography (0%-5% EtOAc in hexane, gradient) to provide ether **19** as a colourless oil (140 mg, 48%): R_f = 0.44 (5% EtOAc in hexanes); ν_{max}(film)/cm^{-1} 2917 s, 2849 s, 2140 m, 2099 s (N_3), 1451 w, 1369 w, 1312 w, 1254 w, 1218 w, 1109 m, 1031 w, 877 w, 750 w, 697 m; ^{1}H NMR (300 MHz, C_6D_6) δ ppm 0.92 (3H, t, *J* = 6.7), 1.11-1.46 (30H, stack), 1.47-1.76 (4H, stack), 3.35 (1H, dd, *J* = 10.5, 4.7), 3.43-3.55 (3H, stack), 3.67 (1H, dd, *J* = 10.2, 7.4), 3.75-4.00 (4H, stack), 4.01-4.11 (1H, m), 5.47 (1H, s), 7.18-7.26 (3H, stack), 7.66-7.74 (2H, stack); ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.9 (CH_3), 22.9 (CH_2), 25.6 (CH_3), 26.8 (CH_2), 28.0 (CH_2), 28.2 (CH_3), [29.7. 29.8, 30.0 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 60.1 (CH), 66.5 (CH_2), 73.1 (CH_2), 74.5 (CH_2), 75.8 (CH), 76.5 (CH), 77.9 (CH), 101.3 (CH), 108.2 (C), 126.6 (CH), [127.6, 127.9, 128.1 (CH, coincident with solvent)], 128.6 (CH), 139.5 (C); *m/z* (TOF ES+) 582.4 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 582.3887 ([M+Na]$^+$) $C_{32}H_{53}N_3O_5Na$ requires 528.3883.

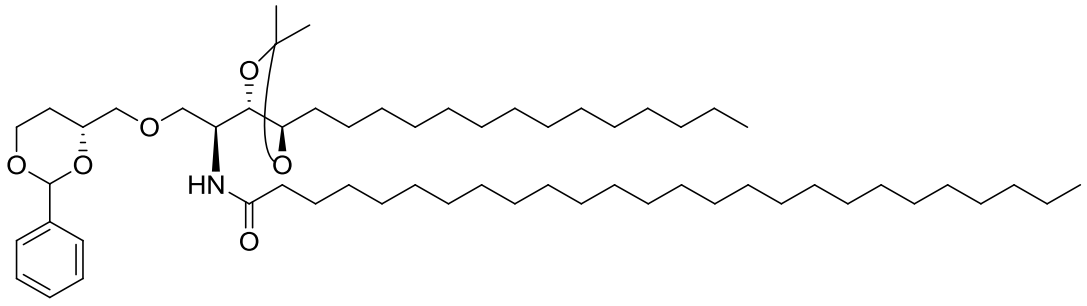
(2*R*,3*R*,4*S*,2'*S*)-2-amino-1-*O*-(2',4'-*O*-benzylidene-butyl)-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (31)



PMe_3 (1.0 M solution in THF, 280 µL, 0.28 mmol) was added dropwise over 5 min to a solution of the azide **19** (140 mg, 0.25 mmol) in THF (2.5 mL). The reaction mixture was stirred for 4 h at rt, then H_2O (1 mL) was added and the mixture left to stir overnight. The mixture was then concentrated under reduced pressure and the residual H_2O removed by co-evaporation with

toluene (3 × 2 mL). The crude product was purified by column chromatography (50% EtOAc in hexane) to provide amine **31** as a white solid (100 mg, 75%): R_f = 0.09 (50% EtOAc in hexanes); $[\alpha]_D$ the insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3401 w, 2923 s, 2853 s, 1729 w, 1459 w, 1367 w, 1243 w, 1216 w, 1109 m, 1063 w, 1033 w, 876 w, 698 w; ^{1}H NMR (300 MHz, C_6D_6) δ ppm 0.92 (3H, t, *J* = 6.6), 1.20-1.86 (34H, stack), 3.01 (1H, ddd, *J* = 9.5, 6.6, 2.9), 3.33 (1H, dd, *J* =10.4, 4.5), 3.43-3.43 (3H, stack), 3.71 (1H, dd, *J* = 9.2, 2.8), 3.74-3.83 (1H, m), 3.87-4.01 (2H, stack), 4.21 (1H, ddd, *J* = 9.9, 5.6, 3.0), 5.48 (1H, s), 7.19-7.27 (3H, stack), 7.64-7.74 (2H, stack), N*H*$_2$ not observed; ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.2 (CH_3), 23.0 (CH_2), 26.1 (CH_3), 26.6 (CH_2), 28.1 (CH_2), 28.7 (CH_3), [29.7, 30.0 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 50.8 (CH), 66.5 (CH_2), 74.3 (CH_2), 75.4 (CH_2), 76.3 (CH), 78.2 (CH), 79.0 (CH), 101.3 (CH), 107.7 (C), 126.6 (CH), [127.6, 127.9, 128.1 (CH, coincident with solvent)], 128.6 (CH), 139.5 (C); *m/z* (TOF ES+) 534.4 ([M]$^+$, 100%).

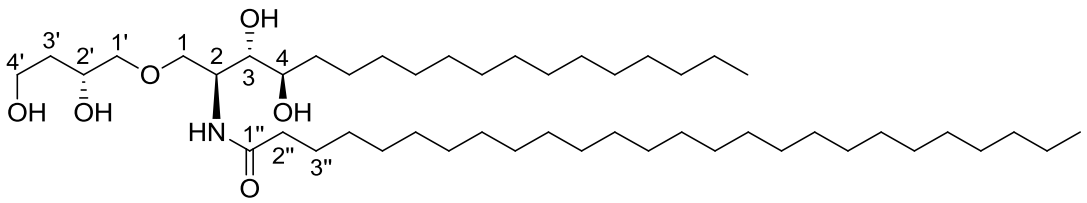
(2*R*,3*R*,4*S*,2'*S*)-1-*O*-(2',4'-*O*-benzylidene-butyl)-2-hexacosanoylamino-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (33)



$(COCl)_2$ (2 mL) was added to hexacosanoic acid (91 mg, 0.22 mmol) in a flame-dried tube, which was tightly closed, parafilmed and heated at 70 ˚C for 2 h. The volatiles were evaporated under a flow of argon and any residual solvent evaporated under high vacuum for

at least 1 h. The resulting acid chloride was used immediately without further purification. A solution of freshly prepared acid chloride (91 mg, 0.22 mmol) in CH_2Cl_2 (2.2 mL) was added dropwise over 5 min to a solution of amine **31** (80 mg, 0.15 mmol) and NEt_3 (42 µL, 0.3 mmol) in CH_2Cl_2 (1.0 mL) at 0 ˚C. The reaction mixture was stirred overnight, then diluted with CH_2Cl_2 (20 mL), washed sequentially with $NaHCO_3$ solution (20mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (15% EtOAc in hexane) to provide amide **33** as a white solid (80 mg, 59%): R_f = 0.21 (20% EtOAc in hexanes); mp = 89–91 °C; $[\alpha]_D^{22}$ = 1.8 (c = 0.9, CH_3Cl); ν_{max}(film)/cm^{-1} 3276 w (N–H), 2916 s, 2849 s, 1646 m (C=O), 1560 w, 1470 m, 1368 w, 1243 w, 1218 w, 1139 1, 1019 m, 870 w; ^{1}H NMR (300 MHz, C_6D_6) δ ppm 0.86-1.30 (6H, stack), 1.20-1.95 (82H, stack), 3.26-3.58 (4H, stack), 3.71-3.83 (1H, m), 3.90-4.00 (2H, stack), 4.08-4.17 (1H, m), 4.22-4.32 (1H, stack), 4.54 (1H, tt, *J* = 9.9, 2.9), 5.38 (1H, s), 5.62 (1H, d, *J* = 9.6), 7.19-7.28 (3H, stack), 7.62-7.70 (2H, stack); ^{13}C NMR (100 MHz, C_6D_6) δ ppm 15.6 (CH_3), 23.3 (CH_2), 25.9 (CH_2), 26.0 (CH_3), 26.8 (CH_2), 27.6 (CH_2), 28.9 (CH_3), [29.6, 30.1 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 36.0 (CH_2), 48.9 (CH), 66.4 (CH_2), 71.7 (CH_2), 76.4 (CH_2), 76.6 (CH), 76.7 (CH), 78.5 (CH), 107.8 (C), 101.3 (CH), 126.5 (CH), [127.6, 127.9, 128.1 (CH, coincident with solvent)], 128.9 (CH), 171.1 (C); *m/z* (TOF ES+) 934.7 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 934.7858 ($[M+Na]^+$) $C_{58}H_{105}NO_6Na$ requires 934.7840.

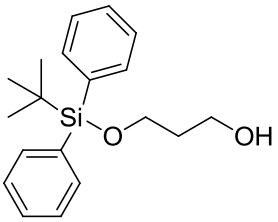
(2*R*,3*R*,4*S*,2'*S*)-1-*O*-(2',4'-dihydroxybutyl)-2-hexacosanoylamino-1,3,4-octadecanetriol (3)



TFA (0.3 mL, 0.4 mmol) was added to a solution of amide **33** (40 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) and H_2O (10 μL). The solution was stirred at rt overnight. The reaction mixture was then poured into CH_2Cl_2 (10 mL) and quenched with $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 10 mL) and the combined organic layers were washed sequentially with $NaHCO_3$ solution (30 mL) and brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography chromatography (0-5% MeOH in $CHCl_3$, gradient) to provide tetraol **3** as a white solid (20 mg, 65%): R_f = 0.51 (10% MeOH in $CHCl_3$); mp = 88–89 °C; $[\alpha]_D$ the insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3415 br w (O–H, N–H), 2920 m, 2850 w, 1756 m, 1705 m, 1589 w, 1494 w, 1460 w, 1365 w, 1297 s, 1258 w, 1200 m, 1145 m, 1115 s, 1010 w, 921 w; ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD, 2:1, 40 °C) δ ppm 0.84 (6H, t, *J* = 6.8, 2 × CH_2CH_3), 1.18-1.40 (68H, stack, CH_2 resonances in alkyl chains), 1.45-1.52 (1H, m, CH_aH_b in alkyl chain), 1.53-1.66 (5H, stack, C(3')H_2, C(3'')H_2, CH_aH_b in alkyl chain), 2.16 (2H, app t, *J* = 7.5, C(2'')H_2), 3.35 (1H, dd, *J* = 10.0, 7.0, C(1')H_aH_b), 3.43 (1H, dd, *J* = 10.0, 3.5, C(1')H_aH_b), 3.46-3.52 (2H, stack, C(3)*H*, C(4)*H*), 3.54 (1H, dd, *J* = 10.0, 4.0, C(1)H_aH_b), 3.69 (2H, app t, *J* = 5.5, C(4')H_2), 3.73 (1H, dd, *J* = 10.0, 4.5, C(1)H_aH_b), 3.88-3.92 (1H, m, C(2')*H*), 4.13-4.18 (1H, m, C(2)*H*), N*H* and O*H*s not observed; ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD, 2:1, 40 °C) δ ppm 14.2 (CH_3, 2 × CH_2CH_3), 23.0 (CH_2), 26.3 (CH_2, C(3'')), 29.7 (CH_2), 29.8 (CH_2), [29.9, 30.0, 30.1 (CH_2, v broad, significant resonance overlap)], 32.3 (CH_2), 33.1 (CH_2), 35.8 (CH_2, C(3')), 36.9

(CH_2, C(2'')), 50.7 (CH, C(2)), 59.8 (CH_2, C(4')), 68.8 (CH, C(2')), 70.8 (CH_2, C(1)), 73.0 (CH, C(4)), 75.5 (CH, C(3)), 76.2 (CH_2, C(1')), 175.0 (C, C(1'')); *m/z* (TOF ES+) 806.7 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 806.7208 ($[M+Na]^+$) $C_{48}H_{97}NO_6Na$ requires 806.7214.

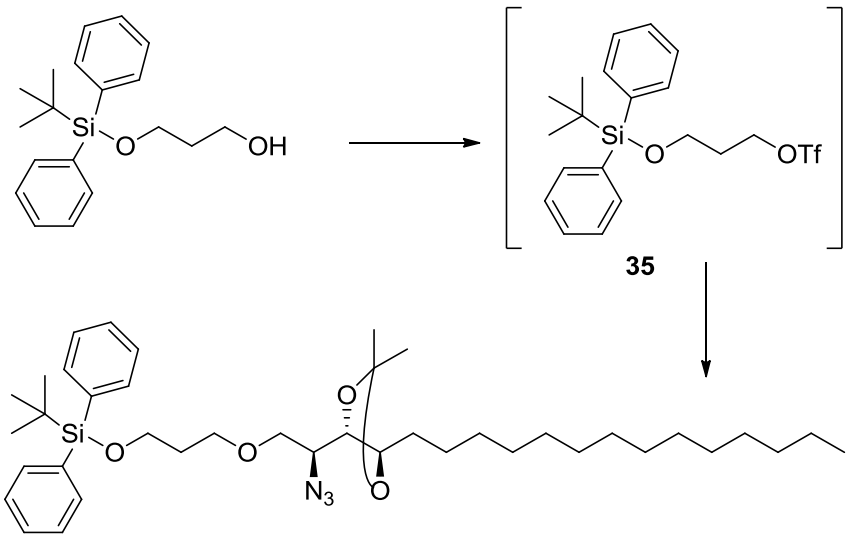
3-*O*-(*tert*-butyldiphenylsilyl)-propan-1-ol (34)



1,3-Propanediol **24** (0.5 g, 6.5 mmol) in dry THF (20 mL) was treated with NaH (60% by wt in mineral oil, 260 mg, 6.5 mmol). The solution was stirred for 30 min, then TBDPSCl (1.87 mL, 7.2 mmol) was added dropwise over 10 min. The reaction mixture was stirred for 16 h, then diluted with EtOAc (200 mL), washed sequentially with H_2O (3 × 100 mL) and brine (2 × 100 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (hexane-8% EtOAc in hexane, gradient) to provide silyl ether **34** as a colourless oil (1.3 g, 63%): R_f = 0.18 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.05-1.10 (9H, stack), 1.84 (2H, pentet, *J* = 5.7), 2.34 (1H, t, *J* = 5.5), 3.84-3.91 (4H, stack), 7.38-7.51 (10H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 18.7 (C), 26.9 (CH_3), 34.4 (CH_2), 61.7 (CH_2), 63.1 (CH_2), 127.8 (CH), 129.8 (CH), 133.7 (C), 135.6 (CH); *m/z* (TOF ES+) 337.1 ($[M+Na]^+$, 100%).

Data were in agreement with those reported in the literature.[175]

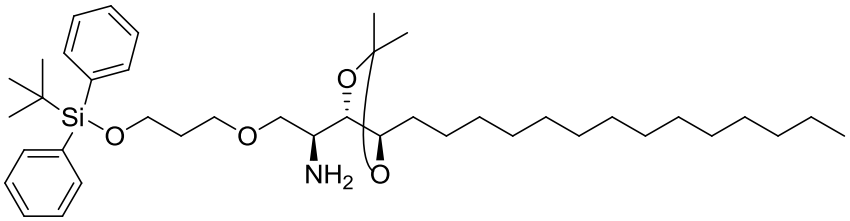
(2*R*,3*R*,4*S*)-2-azido-1-*O*-[3'-*O*-*tert*-butyldiphenylsilyl-propyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (21)



Tf_2O (131 μL, 0.78 mmol) was added dropwise over 10 min to a solution of alcohol **34** (245 mg, 0.78 mmol) and 2,6-di-*tert*-butyl pyridine (263 μL, 1.17 mmol) in dry CH_2Cl_2 (7.8 mL) at 0 ˚C. The reaction mixture was stirred for 15 min, then diluted with CH_2Cl_2 (20 mL), washed with cold H_2O (2 × 20 mL) and then brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was used immediately in the next step: R_f = 0.83 (15% EtOAc in hexanes). Azide **10** (250 mg, 0.65 mmol) in dry THF (3.5 mL) was treated with NaH (60% by wt in mineral oil, 29 mg, 0.72 mmol) at 0 ˚C. The solution was stirred for 1 h, then a solution of triflate **35** (0.78 mmol) in dry THF (3.0 mL) was added dropwise over 5 min at 0 ˚C. The mixture was stirred at 0 ˚C for 1 h, then at rt overnight. The reaction was quenched by addition of NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (0%-5% EtOAc in hexane, gradient) to provide ether **21** as a colourless oil (300 mg, 68%): R_f = 0.59 (5% EtOAc in hexanes); ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 2098 s (N_3), 1463 w,

1427 w, 1379 w, 1369 w, 1245 m, 1219 m, 1106 s, 823 m, 735 m, 701 s; ^{1}H NMR (300 MHz, C_6D_6) δ ppm 0.92 (3H, t, *J* = 6.0), 1.04-1.47 (38H, stack), 1.49-1.74 (3H, stack), 1.75-1.85 (2H, pentet, *J* =6.2), 3.60-3.42 (4H, stack), 3.76-3.90 (4H, stack), 4.02-4.11 (1H, m), 7.20-7.32 (6H stack), 7.76-7.83 (4H, stack); ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.8 (CH_3), 20.0 (C), 22.2 (CH_2) 25.6 (CH_3), 26.7 (CH_2), 27.0 (CH_3), 28.3 (CH_3), [29.6, 29.8, 29.9, 30.0 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 33.0 (CH_2), 60.1 (CH), 60.8 (CH_2), 68.0 (CH_2), 72.2 (CH_2), 75.8 (CH), 77.9 (CH), 108.3 (C), 127.9 (CH), 129.7 (CH), 133.8 (C), 135.8 (CH); *m/z* (TOF ES+) 702.3 ($[M+Na]^+$, 100%).

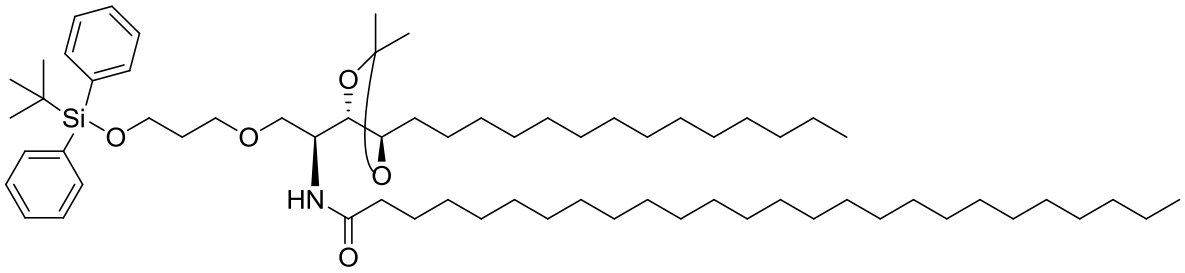
(2*R*,3*R*,4*S*)-2-amino-1-*O*-[3'-*O*-*tert*-butyldiphenylsilyl-propyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (36)



PMe_3 (1.0 M solution in THF, 530 µL, 0.53 mmol) was added dropwise over 5 min to a solution of the azide **21** (330 mg, 0.48 mmol) in THF (4.8 mL). The reaction mixture was stirred for 4 h at rt, then H_2O (1 mL) was added and the mixture left to stir overnight. The mixture was then concentrated under reduced pressure and residual H_2O removed by co-evaporation with toluene (3 × 2 mL). The crude product was purified by column chromatography (15% EtOAc in hexane) to provide amine **36** as a white solid (270 mg, 86%): R_f = 0.10 (15% EtOAc in hexanes); $[\alpha]_D$ the insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 1463 w, 1428 w, 1378 w, 1218 m, 1111 s, 823 m, 736 m, 701 s; ^{1}H

NMR (300 MHz, C_6D_6) δ ppm 0.80-0.98 (3H, m), 1.14-1.67 (40H, stack), 1.73-1.90 (3H, stack), 3.00 (1H, ddd, *J* = 9.7, 6.8, 2.8), 3.35 (1H, dd, *J* = 9.0, 6.8), 3.41-3.58 (2H, stack), 3.62 (1H, dd, *J* = 9.0, 2.8), 3.73-3.93 (3H, stack), 4.17-4.25 (1H, m), 7.19-7.34 (6H, stack), 7.71-7.82 (4H, stack), N*H*$_2$ not observed; ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.3 (CH_3), 19.3 (C), 23.0 (CH_2), 26.2 (CH_3), 26.6 (CH_2), 27.0 (CH_3), 28.7 (CH_3), [29.7, 30.11, 30.13 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 33.1 (CH_2), 50.9 (CH), 61.0 (CH_2), 67.7 (CH_2), 74.6 (CH_2), 78.3 (CH), 79.0 (CH), 107.7 (C), 127.9 (CH), 129.8 (CH), 134.2 (C), 135.9 (CH); *m/z* (TOF ES+) 676.4 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 676.4741 ([M+Na]$^+$) $C_{40}H_{67}NO_4NaSi$ requires 676.4737.

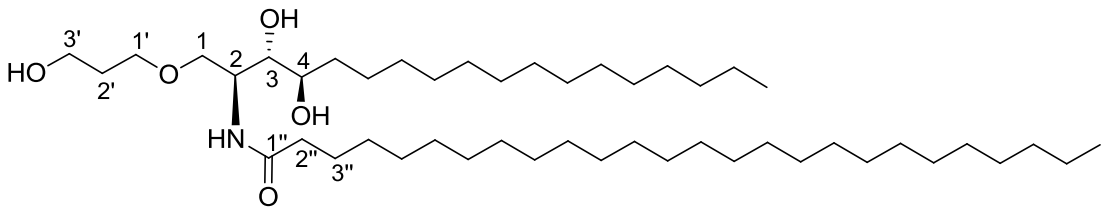
(2*R*,3*R*,4*S*)-1-*O*-[3'-*O*-tert-butyldiphenylsilyl-propyl]-2-hexacosanoylamino-3,4-*O*-isopropylidene-1,3,4-octadecantriol (37)



$(COCl)_2$ (2 mL) was added to hexacosanoic acid (195 mg, 0.47 mmol) in a flame-dried tube, which was tightly closed, parafilmed and heated at 70 ˚C for 2 h. The volatiles were evaporated under a stream of argon and any residual solvent evaporated under high vacuum for at least 1 h. The resulting acid chloride was used immediately without further purification. A solution of freshly prepared acid chloride (195 mg, 0.47 mmol) in CH_2Cl_2 (2.0 mL) was added dropwise over 5 min to a solution of amine **36** (204 mg, 0.31 mmol) and NEt_3 (86 µL, 0.62 mmol) in CH_2Cl_2 (2.1 mL) at 0 ˚C. The reaction mixture was stirred overnight, then diluted with

CH_2Cl_2 (20 mL), washed with $NaHCO_3$ solution (20mL) and then brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (12% EtOAc in hexane) to provide amide **37** as a white solid (236 mg, 73%): R_f = 0.49 (15% EtOAc in hexanes); mp = 77–79 °C; $[\alpha]_D^{20}$ = 14.4 (c = 1.0, CH_3Cl); ν_{max}(film)/cm^{-1} 3318 w (N–H), 2916 s, 2850 s, 1640 m (C=O), 1537 w, 1469 w, 1427 w, 1367 w, 1244 w, 1220 m, 1111 m, 823 w, 701 m; ^{1}H NMR (300 MHz, C_6D_6) δ ppm 0.91 (6H, t, *J* = 7.1), 1.21-1.60 (85H, stack), 1.66-1.87 (6H, stack), 3.45 (2H, app d, *J* = 6.2), 3.71 (1H, dd, *J* = 9.1, 3.2), 3.78 (2H, app t, *J* = 6.2), 3.89 (2H, app q, *J* = 7.1), 4.07-4.18 (2H, stack), 4.45-4.60 (1H, m), 7.19-7.32 (6H, stack), 7.72-7.82 (4H, stack); ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.3 (CH_3), 19.8 (C), 23.0 (CH_2), 25.9 (CH_2), 26.1 (CH_3), 27.0 (CH_3), 28.4 (CH_3), [29.6, 29.7, 29.8, 30.1 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 32.9 (CH_2), 36.7 (CH_2), 48.3 (CH), 61.1 (CH_2), 67.9 (CH_2), 70.5 (CH_2), 76.4 (CH), 78.2 (CH), 107.8 (C), 127.9 (CH), 129.8 (CH), 134.2 (C), 135.8 (CH), 171.2 (C); *m/z* (TOF ES+) 1054.6 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1054.8584 ([M+Na]$^+$) $C_{66}H_{117}NO_5NaSi$ requires 1054.8599.

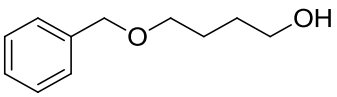
(2*R*,3*R*,4*S*)-2-hexacosanoylamino-1-*O*-[3'-hydroxypropyl]-1,3,4-octadecanetriol (5)



TBAF (1.0 M solution in THF, 0.23 mL, 0.23 mmol) was added to a solution of amide **37** (215 mg, 0.21 mmol) in THF (2 mL). The reaction mixture was stirred for 4 h, after which time NH_4Cl solution (10 mL) was added. The resulting layers were separated and the aqueous layer was

extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were washed with $NaHCO_3$ solution (10 mL) and then brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The acetal crude product was used in the next step without further purification: R_f = 0.12 (30% EtOAc in hexanes). TFA (0.6 mL) was added to a solution of acetal (assuming 100% conversion, 0.21 mmol) in CH_2Cl_2/H_2O (2 mL, 15:1). The reaction mixture was stirred at 32 °C for 24 h, before being diluted with CH_2Cl_2 (20 mL) and quenched with $NaHCO_3$ solution (10 mL). The resulting layers were separated and the aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were washed with $NaHCO_3$ solution (20 mL) and then brine (20 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (0-5% MeOH in $CHCl_3$, gradient) to give amide **5** as a white solid (58 mg, 77% over 2 steps): R_f = 0.68 (10% MeOH in $CHCl_3$); mp = 94–99 °C; $[\alpha]_D$ the insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3289 br m (O–H, N–H), 2917 m, 2850 w, 1642 m, 1528 w, 1468 w, 1078 w; ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD, 2:1, 40 °C) δ ppm 0.82 (6H, t, *J* = 6.9, 2 × CH_2CH_3), 1.13-1.36 (70H, stack, CH_2 resonances in alkyl chains), 1.52-1.59 (2H, m, C(3'')H_2), 1.73 (2H, m, C(2')H_2), 2.15 (2H, app t, *J* = 7.5, C(2'')H_2), 3.42-3.48 (2H, stack, C(3)*H*, C(4)*H*), 3.48-3.57 (3H, stack, C(1)H_aH_b, C(3')H_2), 3.61 (2H, app t, *J* = 5.7, C(1')H_2), 3.67 (1H, dd, *J* = 9.9, 4.1, C(1)H_aH_b), 4.15 (1H, dd, *J* = 7.9, 4.1, C(2)*H*), exchangeable hydrogens not observed; ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD, 2:1, 40 °C) δ ppm 14.1 (CH_3, 2 × CH_2CH_3, resonance overlap), 22.8 (CH_2), 26.0 (CH_2, C(3'')), [29.3, 29.4, 29.5, 29.5, 29.7, 29.8 (CH_2, v broad, significant resonance overlap)], 32.0 (CH_2), 32.1 (CH_2, C(2')), 36.7 (CH_2, C(2'')), 50.0 (CH, C(2)), 59.7 (CH_2, C(3')), 69.1 (CH_2, C(1')), 70.0 (CH_2, C(1)), 72.8 (CH, C(4)), 75.5 (CH, C(3)), 174.5 (C, C(1'')); *m/z* (TOF ES+) 776.5 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 776.7114 ($[M+Na]^+$) $C_{47}H_{95}NO_5Na$ requires 776.7108.

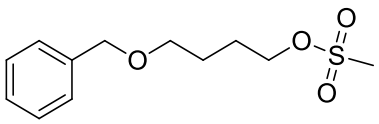
4-(benzyloxy)butan-1-ol (38)



1,4-Butanediol **23** (0.98 mL, 11 mmol) was added dropwise over 5 min to a suspension of NaH (60% by wt in mineral oil, 0.44 g, 11 mmol) in dry DMF (10 mL). The mixture was stirred for 1 h and then cooled to 0 °C. BnBr (0.92 mL, 7.7 mmol) was added dropwise over 5 min, and the mixture was stirred at rt for 15 h. The reaction was quenched with NH_4Cl solution (20 mL), then poured into H_2O (20 mL) and extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (20% EtOAc in hexane) to provide benzyl ether **38** as a colourless oil (1.07 g, 54%): R_f = 0.14 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.63-1.75 (4H, stack), 2.19 (1H, br s), 3.53 (2H, t, *J* = 5.8), 3.65 (2H, t, *J* = 5.8), 4.53 (2H, s), 7.27-7.39 (5H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 24.8 (CH_2), 28.1 (CH_2), 60.6 (CH_2), 68.7 (CH_2), 71.3 (CH_2), 125.96 (CH), 126.02 (CH), 126.7 (CH), 136.6 (C); *m/z* (TOF ES+) 203.1 ([M+Na]$^+$, 100%).

Data were in agreement with those reported in the literature.[129]

4-(benzyloxy)-1-*O*-methanesulfonyl-butan-1-ol (39)

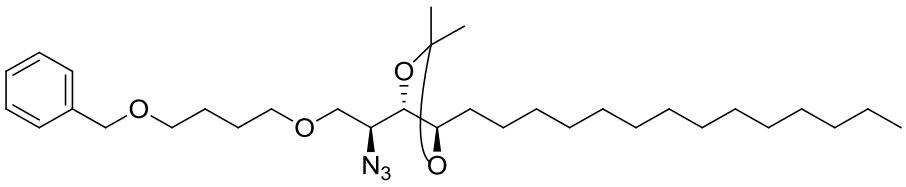


Et_3N (1.13 mL, 8.15 mmol), DMAP (0.07 g, 0.53 mmol) and MsCl (0.50 mL, 6.52 mmol) were added sequentially to a solution of alcohol **38** (0.979 g, 5.43 mmol) in CH_2Cl_2 (10.7 mL) at 0 °C.

The reaction mixture was stirred for 1 h and then poured into H_2O (10 mL) and extracted sequentially with CH_2Cl_2 (2 × 10 mL) and EtOAc (2 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (30% EtOAc in hexane) to provide mesylate **39** as a colourless oil (1.33 g, 95%): R_f = 0.28 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.67-1.80 (2H, m), 1.82-1.93 (2H, m), 2.98 (3H, s), 3.51, (2H, t, *J* = 6.0), 4.26 (2H, t, *J* = 6.4), 4.50 (2H, s), 7.27-7.39 (5H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 26.0 (CH_2), 26.5 (CH_2), 37.4 (CH_3), 69.6 (CH_2), 70.4 (CH_2), 73.2 (CH_2), 127.9 (2 × CH), 128.6 (CH), 137.9 (C).

Data were in agreement with those reported in the literature.[129]

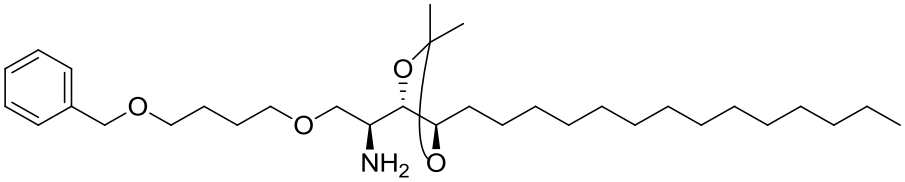
(2*R*,3*R*,4*S*)-2-azido-1-*O*-[4'-benzyloxy-butyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (20)



Azide **10** (260 mg, 0.68 mmol) in dry THF (1.0 mL) was treated with NaH (60% by wt in mineral oil, 32 mg, 0.81 mmol) at 0 ˚C. The solution was stirred for 1 h, then a solution of mesylate **39** (0.81 mmol) in dry THF (0.5 mL) was added dropwise over 5 min at 0 ˚C. The mixture was stirred at 0 ˚C for 1 h, then at rt overnight. The reaction was quenched by addition of NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (5% EtOAc in hexane) to provide ether **20** as a colourless oil (196 mg, 53%): R_f = 0.52 (5% EtOAc in hexanes); $[\alpha]_D^{21}$ = 15.3 (c = 0.6, $CHCl_3$);

ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 2137 w, 2098 m (N_3), 1454 w, 1368 w, 1245 w, 1219 w, 1102 m, 1069 w, 733 w, 697 w; ^{1}H NMR (300 MHz, C_6D_6) δ ppm 0.91 (3H, t, *J* = 6.7), 1.16-1.50 (28H, stack), 1.49-1.77 (8H, stack), 3.25-3.35 (4H, stack), 3.45-3.64 (2H, stack), 3.80 (1H, dd, *J* = 9.4, 1.9), 3.90 (1H, dd, *J* = 9.4, 5.6), 4.02-4.10 (1H, m), 4.32 (2H, s), 7.16-7.22 (3H, stack), 7.31 (2H, d, *J* = 7.4); ^{13}C NMR (100 MHz, C_6D_6) δ ppm 14.2 (CH_3), 23.0 (CH_2), 25.7 (CH_3), 26.6 (CH_2), 26.8 (CH_2), 28.3 (CH_3), [29.7, 29.8, 29.9, 30.0 (CH_2, v broad, significant resonance overlap)], 32.2 (CH_2), 60.1 (CH), 70.0 (CH_2), 71.3 (CH_2), 72.0 (CH_2), 72.7 (CH_2), 75.8 (CH), 77.9 (CH), 108.1 (CH), 127.4 (CH), 127.5 (CH), 128.4 (CH), 139.4 (C); *m/z* (TOF ES+) 568.5 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 568.4066 ([M+Na]$^+$) $C_{32}H_{55}N_3O_4Na$ requires 568.4090.

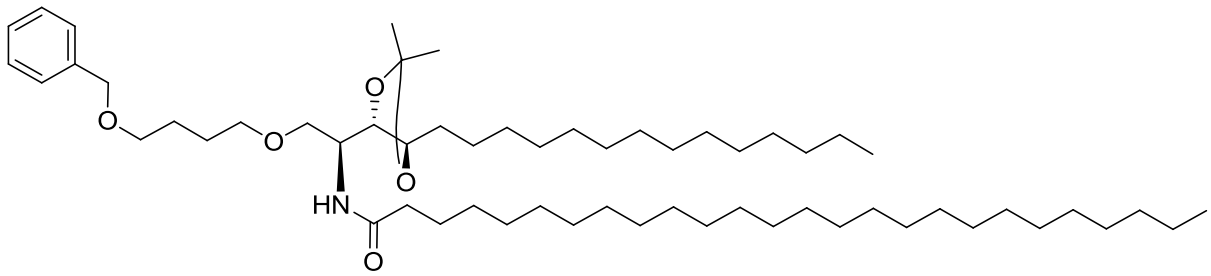
(2*R*,3*R*,4*S*)-2-amino-1-*O*-[4'-(benzyloxy)-butyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (40)



A spatula tip of Pd/C was added to a solution of amine **20** (130 mg, 0.24 mmol) in MeOH (10 mL), EtOAc (10 mL) and glacial acetic acid (2 drops). H_2 gas was bubbled through the solution overnight. The reaction mixture was then filtered over Celite, concentrated under reduced pressure and the crude product purified by column chromatography (50% EtOAc in hexane) to provide amine **40** as a white solid (80 mg, 65%): R_f = 0.29 (50% EtOAc in hexanes); mp = 105–111 °C; $[\alpha]_D^{21}$ = 13.8 (c = 1, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1727 w, 1455 w, 1367 w, 1245 m, 1217 m, 1097 s, 732 w, 697 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.91 (3H, t, *J* = 6.7), 1.21-1.38 (27H, stack), 1.42 (3H, s), 1.49-1.81 (6H, stack), 3.27-3.33 (1H, m), 3.45-3.61 (5H, stack),

3.68-3.76 (1H, m), 4.20-4.30 (1H, m), 4.33-3.40 (1H, m), 4.52 (2H, s), 7.25-7.40 (5H, stack), NH_2 not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3), 22.7 (CH_2), 25.9 (CH_3), 26.2 (CH_2), 26.5 (CH_2), 28.3 (CH_3), [29.4, 29.6, 29.7, 29.8 (CH_2, v broad, significant resonance overlap)], 31.9 (CH_2), 50.4 (CH), 70.1 (CH_2), 71.1 (CH_2), 72.9 (CH_2), 73.4 (CH_2), 77.9 (CH), 78.9 (CH), 107.9 (C), 127.5 (CH), 127.6 (CH), 128.4 (CH), 138.6 (C); *m/z* (TOF ES+) 520.3 ($[M]^+$, 100%).

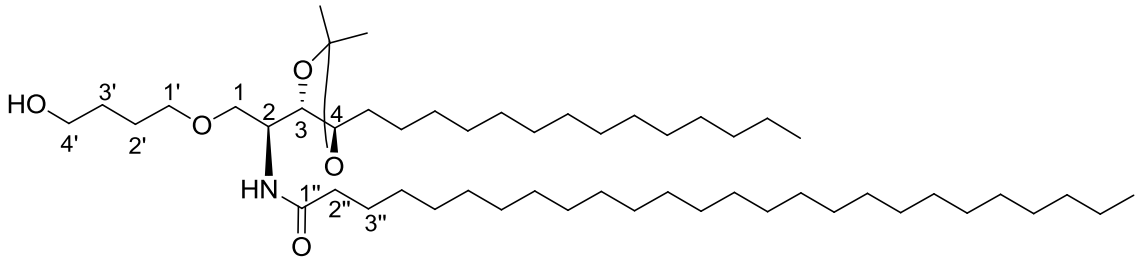
(2*R*,3*R*,4*S*)-1-*O*-[4'-(benzyloxy)-butyl]-2-hexacosanoylamino-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (41)



$(COCl)_2$ (2 mL) was added to hexacosanoic acid (75 mg, 0.18 mmol) in a flame-dried tube, which was tightly closed, parafilmed and heated at 70 ˚C for 2 h. The volatiles were evaporated under a flow of argon and any residual solvent evaporated under high vacuum for at least 1 h. The resulting acid chloride was used immediately without further purification. A solution of freshly prepared acid chloride (75 mg, 0.18 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise over 5 min to a solution of amine **40** (60 mg, 0.15 mmol) and NEt_3 (33 µL, 0.24 mmol) in CH_2Cl_2 (1.0 mL) at 0 ˚C. The reaction mixture was stirred overnight, then diluted with CH_2Cl_2 (20 mL), washed with $NaHCO_3$ solution (20mL) and then brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (15% EtOAc in hexane) to provide amide **41**

as a white solid (63 mg, 56%): R_f = 0.23 (15% EtOAc in hexanes); mp = 90–92 °C; $[\alpha]_D^{21}$ = 18.6 (c = 0.7, $CHCl_3$); ν_{max}(film)/cm^{-1} 3289 br m (N–H), 2917 m, 2850 w, 1642 m (C=O), 1528 w, 1468 w, 1078 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.81 (6H, t, *J* = 6.7, 2 × CH_2CH_3), 1.14-1.24 (70H, stack, CH_2 resonances in alkyl chains), 1.26 (3H, s, 1 × $C(CH_3)_2$), 1.35 (3H, s, 1 × $C(CH_3)_2$), 1.49-1.64 (6H, stack), 2.01-2.09 (2H, stack, O=CCH_2), 3.33-3.46 (5H, stack), 3.59 (1H, dd, *J* = 9.6, 3.2), 3.95-4.15 (3H, stack), 4.44 (2H, s, CH_2Ph), 5.58 (1H, d, *J* = 9.1, CHN*H*), 7.31-7.22 (5H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, 2 × CH_2CH_3, resonance overlap), 22.7 (CH_2), 26.4 (CH_2), 26.5 (CH_2), 28.0 (CH_3, $C(CH_3)_2$), [29.1, 29.3, 29.4, 29.4, 29.6, 29.7 (CH_2, v broad, significant resonance overlap)], 31.9 (CH_2), 37.0 (CH_2), 48.2 (CH), 70.1 (CH_2), 70.1 (CH_2), 71.1 (CH_2), 72.9 (CH_2), 76.2 (CH), 77.8 (CH), 107.9 (C), 127.5 (CH), 127.6 (CH), 128.4 (CH), 137.8 (C), 171.6 (C); *m/z* (TOF ES+) 920.9 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 920.8041 ($[M+Na]^+$) $C_{58}H_{101}NaNO_5$ requires 920.8047.

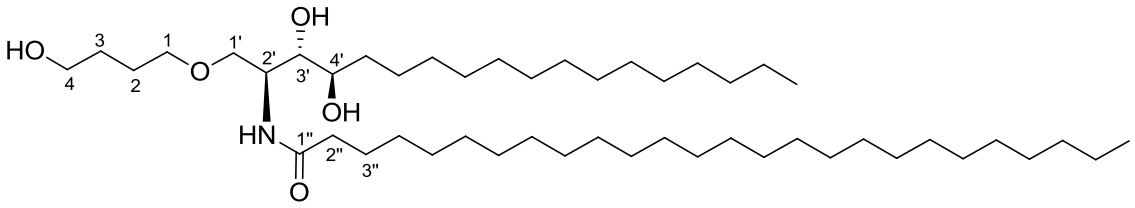
(2*R*,3*R*,4*S*)-1-*O*-[4'-hydroxybutyl]-2-hexacosanoylamino-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (42)



A spatula tip of Pd/C was added to a solution of benzyl ether **41** (63 mg, 0.07 mmol) in hexane (10 mL) and EtOAc (5 mL). H_2 gas was bubbled through the solution overnight. The reaction mixture was then filtered over Celite, concentrated under reduced pressure and the crude product purified by column chromatography (30% EtOAc in hexane) to provide alcohol **42** as a

white solid (53 mg, 93%): R_f = 0.13 (30% EtOAc in hexanes); mp = 95−98 °C, $[\alpha]_D$ = 14.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3289 br m (O–H, N–H), 2917 m, 2850 w, 1642 m (C=O), 1528 w, 1468 w, 1078 w; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.87 (6H, t, *J* = 6.9, 2 × CH_2CH_3), 1.21-1.30 (68H, stack, CH_2 resonances in alkyl chains), 1.32 (3H, s, 1 × $C(CH_3)_2$), 1.42 (3H, s, 1 × $C(CH_3)_2$), 1.56-1.63 (4H, stack), 1.63-1.70 (4H, stack, C(2')H_2, C(3')H_2), 2.10-2.21 (2H, m, C(2'')H_2), 3.46-3.52 (3H, stack, C(1)H_aH$_b$, C(1')H_2), 3.64 (2H, app t, *J* = 5.6, C(4')H_2), 3.71 (1H, dd, *J* = 9.7, 3.6, C(1)H$_a$$H_b$), 4.05 (2H, stack, C(3)*H*, C(4)*H*), 4.15-4.21 (1H, m, C(2)*H*), 5.73 (1H, d, *J* = 9.4, CHN*H*), O*H* not observed; ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, 2 × CH_2CH_3, resonance overlap), 22.7 (CH_2), 25.7 (CH_2), 26.51 (CH_2, C(2')), 26.52 (CH_2), 28.0 (CH_3, $C(CH_3)_2$), 28.7 (CH_2), [29.0, 29.1, 29.3, 29.4, 29.6, 29.7 (CH_2, v broad, significant resonance overlap)], 30.0 (CH_2, C(3')), 31.9 (CH_2), 37.0 (CH_2, C(2'')), 48.2 (CH, C(2)), 62.6 (CH_2, C(4')), 70.4 (CH_2, C(1)), 71.3 (CH_2, C(1')), 76.2 (CH, C(3)), 77.8 (CH, C(4)), 107.9 (C, $C(CH_3)_2$), 172.6 (C, C(1'')); *m/z* (TOF ES+) 830.9 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 830.7593 ([M+Na]$^+$) $C_{51}H_{101}NaNO_5$ requires 830.7577.

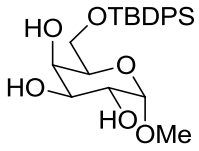
(2*R*,3*R*,4*S*)-1-*O*-[4'-hydroxybutyl]-2-hexacosanoylamino-1,3,4-octadecanetriol (4)



TFA (0.25 mL, 0.33 mmol) was added to a solution of acetal **42** (27 mg, 0.033 mmol) in CH_2Cl_2 (2 mL) and H_2O (20 µL). The solution was stirred at rt overnight. The reaction mixture was then poured into CH_2Cl_2 (10 mL) and quenched with $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 10 mL) and the combined organic layers were washed with

$NaHCO_3$ solution (30 mL) and then brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (0-5% MeOH in $CHCl_3$, gradient) to provide triol **4** as a white solid (16 mg, 65%): R_f = 0.31 (5% MeOH in $CHCl_3$); mp = 93–94 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3215 br w (O–H, N–H), 2920 m, 2849 w, 1776 m, 1707 m, 1528 w, 1494 w, 1258 w, 1200 m, 1015 w, 931 w; ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD, 2: 1, 40 °C) δ ppm 0.84 (6H, t, *J* = 6.9, 2 × CH_2CH_3), 1.05-1.38 (69H, stack, CH_2 resonances in alkyl chains), 1.41-1.69 (7H, stack, C(2)H_aH_b, C(3'')H_aH_b, 2 × CH_2 in alkyl chain), 2.15 (2H, app t, *J* = 7.6, C(2'')H_aH_b), 3.40-3.49 (4H, stack, C(3')*H*, C(4')*H*, C(4)H_aH_b), 3.49-3.60 (3H, stack, C(1')H_aH_b, C(1)H_aH_b), 3.66 (1H, dd, *J* = 9.8, 4.2, C(1')H_aH_b), 4.15 (1H, dd, *J* = 8.1, 4.0, C(2')*H*), N*H* and O*Hs* not observed; ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD, 2:1, 40 °C) δ ppm 14.1 (CH_3, 2 × CH_2CH_3, resonance overlap), 22.8 (CH_2), 25.9 (CH_2, C(3'')), 26.1 (CH_2), [29.2, 29.4, 29.5, 29.5, 29.7, 29.8 (CH_2, v broad, significant resonance overlap)], 32.0 (CH_2), 33.2 (CH_2, C(2)), 36.7 (CH_2, C(2'')), 50.1 (CH, C(2')), 61.9 (CH_2, C(1)), 69.8 (CH, C(1')), 71.4 (CH_2, C(4)), 72.8 (CH, C(4')), 75.6 (CH, C(3')), 175.0 (C, C(1'')); *m/z* (TOF ES+) 790.7 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 790.7271 ($[M+Na]^+$) $C_{48}H_{97}NO_5Na$ requires 790.7264.

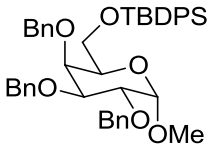
Methyl 6-*O*-*tert*-butyldiphenylsilyl-α-D-galactoside (58)



Imidazole (0.77 g, 11.30 mmol) and TBDPSCl (1.74 mL, 6.70 mmol) were added sequentially to a solution of methyl-α-D-galactopyranoside **51** (1.00 g, 5.15 mmol) in DMF (5 mL). After 24 h, the reaction mixture was diluted with Et_2O (30 mL), and then washed sequentially with H_2O (20 mL) and NH_4Cl solution (20 mL). The isolated organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (30% hexanes in EtOAc) to give silyl ether **58** as a colourless oil (2.10 g, 94%): R_f = 0.82 (30% MeOH in EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.94 (9H, s, C(C*H*$_3$)$_3$), 3.62-3.72 (2H, stack), 3.73-3.85 (3H, stack), 3.91 (1H, app s), 4.09 (3H, broad s), 4.65 (1H, d, *J* = 3.6), 7.20-7.31 (6H, stack), 7.54-7.63 (4H, stack), exchangeable hydrogens not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 19.2 (C), 26.9 (C*H*$_3$), 55.1 (CH_3), 63.5 (CH_2), 69.2 (CH), 69.8 (CH), 70.6 (CH), 70.9 (CH), 99.8 (CH), 127.8 (CH), 129.8 (CH), 133.3 (C), 133.4 (C), 135.7 (CH); *m/z* (TOF ES+) 455.3 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 455.1880 ([M+Na]$^+$) $C_{23}H_{32}NaO_6Si$ requires 455.1866.

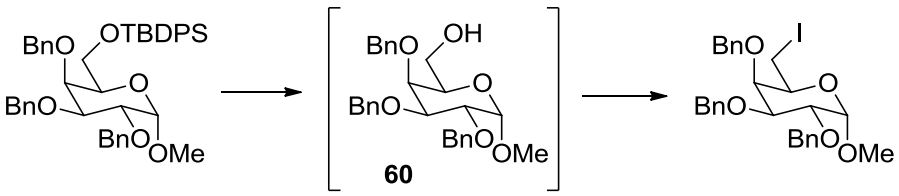
Data were in agreement with those reported in the literature.[176]

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactoside (59)



NaH (60% wt in mineral oil, 0.61 g, 15.5 mmol) was added to a solution of glycoside **58** (1.20 g, 2.78 mmol) in DMF (10 mL) at 0 °C. The reaction mixture was stirred for 20 min, then BnBr (1.33 mL, 11.1 mmol) was added at 0 °C. After warming to rt and stirring overnight, the reaction was quenched by the addition of MeOH over 5 min, and then diluted with EtOAc (30 mL). The separated organic layer was washed with H_2O (20 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give tribenzyl ether **59** as a colourless oil (1.43 g, 73%): R_f = 0.60 (15% EtOAc in hexanes); $[\alpha]_D^{21}$ = 26.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2930 w, 2893 w, 2857 w, 1588 w, 1496 w, 1471 w, 1454 w, 1427 w, 1390 w, 1349 w, 1193 w, 1150 m, 1131 m, 1092 s, 1046 s, 823 m, 800 m, 769 w, 735 s, 696 s, 612 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.05 (9H, s), 3.28 (3H, s), 3.67-3.75 (3H, stack), 3.88-3.99 (2H, stack), 4.03 (1H, dd, *J* = 10.0, 3.6), 4.58-5.01 (7H, stack), 7.19-7.50 (22H, stack), 7.58-7.68 (3H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 19.3 (C), 27.0 (CH_3), 55.1 (CH_3), 62.7 (CH_2), 70.8 (CH), 73.4 (CH_2), 73.7 (CH_2), 74.9 (CH_2), 75.3 (CH), 76.6 (CH), 79.2 (CH), 98.8 (CH), [127.6, 127.7, 127.8, 128.2, 128.3, 128.4, 128.5 (CH, resonance overlap], 129.8 (CH), 133.4 (C), 135.6 (CH), 135.7 (CH), 138.7 (C), 138.8 (C), 139.0 (C); *m/z* (TOF ES+) 725.6 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 725.3255 ([M+Na]$^+$) $C_{44}H_{50}NaO_6Si$ requires 725.3274.

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-iodo-α-D-galactoside (53)

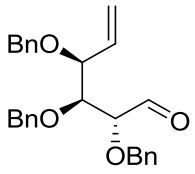


TBAF (1.0 M solution in THF, 1.27 mL, 1.27 mmol) was added to a solution of glycoside **59** (460 mg, 0.63 mmol) in THF (5 mL). The reaction mixture was stirred overnight before being quenched with H_2O (15 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to provide the crude alcohol product [R_f = 0.19 (40% EtOAc in hexanes)], which was used directly in the next step without further purification. A solution of glycoside **60** (206 mg, 0.44 mmol) and PPh_3 (139 mg, 0.53 mmol) in toluene (5 mL) was heated under reflux for 10 min. The reaction mixture was cooled to 80 °C, and then imidazole (89 mg, 1.32 mmol) and I_2 (142 mg, 0.57 mmol) were added. The mixture was heated under reflux for 20 min before being concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed sequentially with $Na_2S_2O_3$ solution (20 mL) and H_2O (20 mL). The organic layer was then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (8% EtOAc in hexanes) to give iodide **53** as a colourless oil (183 mg, 72%): R_f = 0.55 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ = 19.7 (c = 1.0, $CHCl_3$), lit.[150] $[\alpha]_D^{20}$ = 23.0 (c = 1.0, $CHCl_3$) ; ν_{max}(film)/cm^{-1} 3029 w, 2901 w, 1496 w, 1453 m, 1348 m, 1244 w, 1199 m, 1127 s, 1093 s, 1041 s, 909 w, 735 s, 696 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.99 (1H, dd, *J* = 10.0, 6.2), 3.15 (1H, dd, *J* = 10.0, 7.6), 3.35 (3H, s), 3.77 (1H, app t, *J* = 6.9), 3.85 (1H, dd, *J* = 10.2, 2.6), 3.91-3.99 (2H, stack), 4.54-5.01 (7H, stack), 7.15-7.41 (15H, stack); ^{13}C

NMR (100 MHz, $CDCl_3$) δ ppm 3.6 (CH_2), 55.8 (CH_3), 71.4 (CH), 73.7 (2 × CH_2), 75.1 (CH_2), 75.9 (CH), 76.1 (CH), 79.1 (CH), 98.9 (CH), [127.6, 127.7, 127.8, 127.9, 128.2, 128.4, 128.5 (CH, resonance overlap)], 138.3 (C), 138.4 (C), 138.7 (C); *m/z* (TOF ES+) 597.1 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 597.1119 ($[M+Na]^+$) $C_{28}H_{31}NaIO_5$ requires 597.1114.

Data were in agreement with those reported in the literature.[141,150]

(2*R*,3*S*,4*S*)-2,3,4-tri-benzyloxy-hex-5-en-1-al (54)



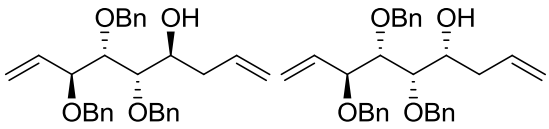
Zinc dust was activated by stirring in hydrochloric acid (1.0 M, 50 mL) at rt for 15 min, before being filtered and washed sequentially with H_2O (30 mL), acetone (30 mL) and Et_2O (30 mL). The resulting activated zinc was then dried under high vacuum with a heat-gun. The activated zinc (0.71 mg, 10.8 mmol) was added to a solution of glycoside **53** (620 mg, 1.08 mmol) and TMSCl (0.137 mL, 1.08 mmol) in THF (20 mL) and the reaction mixture sonicated at 40 °C. After 5 h, Et_2O (50 mL) and H_2O (50 mL) were added to the suspension, which was then filtered through Celite. The layers were separated and the aqueous layer was extracted with Et_2O (3 × 25 mL). The combined organic layers were washed sequentially with H_2O (2 × 15 mL) and brine (15 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (8% EtOAc in hexanes) to give aldehyde **54** as a colourless oil (247 mg, 55%): R_f = 0.44 (16% EtOAc in hexanes); $[\alpha]_D^{21}$ = 16.4 (c = 1.0, $CHCl_3$) ; ν_{max}(film)/cm^{-1} 3031 w, 2879 w, 1723 s, 1701 s, 1598 w, 1584 w, 1496 m, 1454 s,

1391 w, 1311 w, 1268 w, 1204 m, 1069 s, 1026 s, 933 m, 828 w, 736 s, 697 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.75-3.80 (1H, m), 3.95-4.11 (3H, stack), 4.34-4.56 (5H, stack), 5.28-5.38 (2H, stack, CH=CH_2), 5.72-5.86 (1H, m, C*H*=CH_2), 7.09-7.34 (15H, stack), 9.50 (1H, d, *J* = 1.5, C*H*O); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 70.1 (CH_2), 73.5 (CH_2), 74.4 (CH_2), 79.3 (CH), 81.2 (CH), 84.0 (CH), 120.6 (CH_2), [127.75, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6 (CH, resonance overlap)], 135.6 (CH), 137.2 (C), 137.6 (C), 137.9 (C), 202.7 (CH); *m/z* (TOF ES+) 439.2 ([M+Na]$^+$,100%); HRMS *m/z* (TOF ES+) 439.1868 ([M+Na]$^+$) $C_{27}H_{28}NaO_4$ requires 439.1885.

Data were in agreement with those reported in the literature.[145]

(4*S*,5*S*,6*S*,7*S*)-5,6,7-tri-benzyloxy-nona-1,8-dien-4-ol and

(4*R*,5*S*,6*S*,7*S*)-5,6,7-tri-benzyloxy-nona-1,8-dien-4-ol (62)



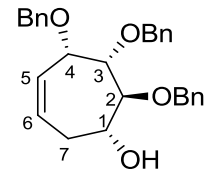
Allyl magnesium bromide (1.0 M in Et_2O, 1.44 mL, 1.44 mmol) was added dropwise over 5 min to a solution of aldehyde **54** (200 mg, 0.48 mmol) in THF (10 mL) at −78 °C. The reaction mixture was stirred at this temperature for 4 h before being quenched with NH_4Cl solution (30 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (0-4% EtOAc in hexanes, gradient) to give alcohol **62** as a mixture of two diasteroisomers (185 mg, 84%, ratio 1:1). Data for the mixture unless specified otherwise: R_f = 0.47 (16% EtOAc in hexanes); ν_{max}(film)/cm^{-1} 3452 br w, 3065 w, 3030

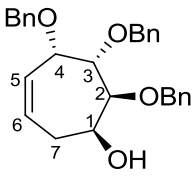
w, 2867 w, 1640 w, 1496 m, 1454 m, 1391 w, 1348 w, 1208 w, 1063 s, 1027 s, 996 m, 916 m, 867 w, 733 s, 697 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.42 (1H, br s, OH), 3.28-3.75 (6H, stack), 3.87-3.96 (1H, m), 4.06-4.23 (1H, m), 4.33-4.67 (4H, stack), 4.81-4.97 (2H, stack, CH=C*H*$_2$), 5.15-5.31 (2H, stack, CH=C*H*$_2$), 5.49-5.65 (1H, m, C*H*=CH_2), 5.77-5.92 (1H, m, C*H*=CH_2), 7.08-7.23 (15H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [38.1, 38.9 (CH_2, C(3))], [70.1, 70.2 (CH_2, CH_2Ph)], [70.4, 70.8 (CH, CHO)], [73.7, 74.1 (CH_2, CH_2Ph)], [74.4, 74.9 (CH_2, CH_2Ph)], [80.4, 80.6 (CH, CHO)], [80.7, 81.0 (CH, CHO)], [81.4, 81.8 (CH, CHO)], [117.4, 117.7 (CH_2, C(1) or C(9))], [119.6, 119.8 (CH_2, C(9) or C(1))], [127.0, 127.6, 127.70, 127.71, 127.9, 128.11, 128.14, 128.3, 128.4, 128.6 (CH, Ph)], [134.9, 135.1 (CH, C(2) or C(8))], [135.6, 135.8 (CH, C(8) or C(2))], [138.2, 138.3, 138.5, 138.6 (C, *ipso* Ph)]; *m/z* (TOF ES+) 481.2 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 481.2347 ($[M+Na]^+$) $C_{30}H_{34}NaO_4$ requires 481.2355.

(1*R*,2*S*,3*S*,4*S*)-2,3,4-tri-benzyloxy-cyclohept-2-en-1-ol (63) and

(1*S*,2*S*,3*S*,4*S*)-2,3,4-tri-*O*-benzyl-cyclohept-5-en-1-ol (64)

63





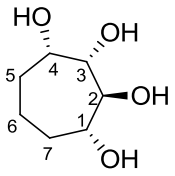
64

A solution of diene **62** (270 mg, 0.59 mmol) in CH_2Cl_2 (60 mL) was degassed by bubbling argon through the solvent while sonicating for 10 min. Grubbs 2nd generation Ru metathesis catalyst (8 mg, 0.009 mmol) was then added and the solution was heated under reflux. After 2 h, the solution was concentrated under reduced pressure and the crude product purified by column chromatography (20% EtOAc in hexanes) to give, in order of elution cycloheptenes **63** (113 mg,

44%) and **64** (120 mg, 47%) as colourless oils: Less polar diastereoisomer (**63**): R_f = 0.30 (16% EtOAc in hexanes; $[\alpha]_D^{20}$ = 66.8 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3498 br w, 3029 m, 2863 m, 1605 w, 1496 m, 1453 m, 1347 w, 1310 w, 1206 m, 1067 s, 1027 s, 910 w, 813 m, 733 s, 695 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.39 (2H, app t, *J* = 5.9, C(7)H_2), 3.30 (1H, br s, O*H*), 3.70-3.84 (3H, stack, C(1)H, C(2)H, C(3)H)), 4.34-4.70 (7H, stack, C(4)H, 3 × OCH_2Ph), 5.57-5.70 (1H, m, C(6)H), 5.77 (1H, dd, *J* = 11.8, 4.5, C(5)H), 7.12-7.28 (15H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 30.6 (CH_2, C(7)), 69.6 (CH, *CH*O), 71.3 (CH_2, *CH_2*Ph), 72.8 (CH_2, *CH_2*Ph), 73.0 (CH_2, *CH_2*Ph), 76.5 (CH, C(3)), 80.2 (CH, C*H*O), 81.3 (CH, *CH*O), [127.5, 127.6, 127.7, 127.81, 127.83, 127.9, 128.2, 128.4, 128.6 (CH, Ph, C(6), resonance overlap)], 131.6 (CH, C(5)), 138.2 (C, *ipso* Ph), 138.3 (2 × C, *ipso* Ph); *m/z* (TOF ES+) 453.3 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 453.2047 ($[M+Na]^+$) $C_{28}H_{30}NaO_4$ requires 453.2042.

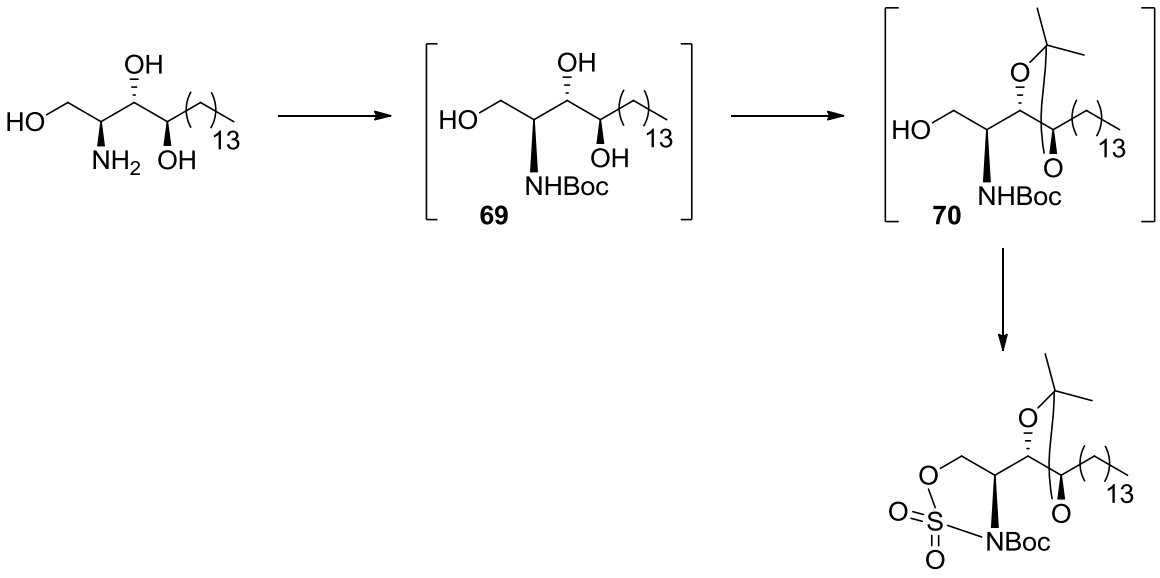
More polar diastereoisomer (**64**): R_f = 0.21 (16% EtOAc in hexanes); $[\alpha]_D^{20}$ = 71.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3416 w, 3031 w, 2869 w, 1717 m, 1602 w, 1584 w, 1496 w, 1452 m, 1315 m, 1268 m, 1207 m, 1177 w, 1089 s, 1069 s, 1025 s, 847 w, 818 w, 735 s, 712 s, 696 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.96-2.02 (1H, m, C(7)$H_a$$H_b$), 2.18 (1H, br s, O*H*), 2.50 (1H, app t, *J* = 12.1, C(7)H_a*H_b*), 3.73-3.89 (3H, stack, C(1)H, C(2)H, C(3)H)), 4.30-4.70 (7H, stack, C(4)H, 3 × OC*H_2*Ph), 5.68-5.75 (2H, stack, C(5)H, C(6)H), 7.08-7.30 (15H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 31.2 (CH_2, C(7)), 67.5 (CH, *CH*O), 71.2 (CH_2, *CH_2*Ph), 72.8 (CH_2, *CH_2*Ph), 73.6 (CH_2, *CH_2*Ph), 76.1 (CH, C*H*O), 78.8 (CH, C*H*O), 81.7 (CH, *CH*O), 126.8 (CH, C(5) or C(6)), [127.5, 127.6, 127.8, 127.9, 128.0, 128.42, 128.44, 128.6 (CH, Ph, resonance overlap)], 132.6 (CH, C(6) or C(5)), 138.2 (C, *ipso* Ph), 138.6 (C, *ipso* Ph), 138.7 (C, *ipso* Ph); *m/z* (TOF ES+) 453.3 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 453.2047 ($[M+Na]^+$) $C_{28}H_{30}NaO_4$ requires 453.2042.

(1*R*,2*S*,3*S*,4*S*)-cycloheptan-1,2,3,4-tetraol (66)



A spatula tip of Pd/C was added to a solution of alcohol **63/126** (40 mg, 0.08 mmol) in MeOH (15 mL). H_2 gas was bubbled through the solution overnight. The reaction mixture was then filtered over Celite, and concentrated under reduced pressure to provide the crude product **66** (mg, mmol) as a white solid. Selected data: R_f = 0.45 (20% MeOH in $CHCl_3$); ^{13}C NMR (100 MHz, CD_3OD) δ ppm 20.5 (CH_2, C(6)), [32.5, 33.2 (2 × CH_2, C(5), C(7))], 72.9 (CH, *C*HO), 75.7 (CH, *C*HO), 78.1 (CH, *C*HO), 78.3 (CH, *C*HO).

(3*R*,1'*S*,2'*S*)-3-*tert*-butoxycarbonyl-4-[1',2'-*O*-isopropylidene-dihydroxyhexadecyl]-1,2,3-oxathiazolidine-2,2-dioxide (72)



Et_3N (2.1 mL, 15.0 mmol) and Boc_2O (2.86 g, 13.1 mmol) were added to a stirring emulsion of phytosphingosine **12** (4.0 g, 12.5 mmol) in THF (100 mL). After stirring for 30 min, the solvent

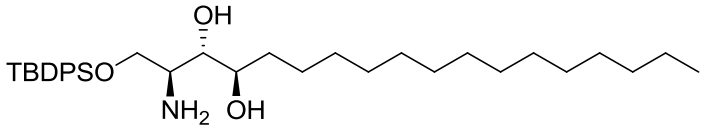
was removed under reduced pressure and the residue dissolved in EtOAc (100 mL) before cooling to 0 °C, upon which amine **69** precipitated out of solution as white crystals [R_f = 0.47 (10% MeOH in CH_2Cl_2)], which were used in the next step without further purification.

Concentrated H_2SO_4 (4 drops) was added to a solution of triol **69** (1.36 mg, 3.36 mmol) in dry acetone (10 mL) at 0 °C. After stirring for 2.5 h, the reaction mixture was quenched with $NaHCO_3$ solution (20 mL), and then concentrated under reduced pressure. The mixture was extracted with EtOAc (3 × 20 mL) and the combined organic layers were washed with brine (10 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (20% EtOAc in hexane) to give acetonide **70** as a colourless oil (768 mg, 50%): R_f = 0.50 (50% EtOAc in hexanes).

A solution of acetonide **70** (475 mg, 1.04 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 30 min to a solution of $SOCl_2$ (83 µL, 1.14 mmol), imidazole (283 mg, 4.16 mmol) and NEt_3 (319 µL, 2.29 mmol) in CH_2Cl_2 (15 mL) at −50 °C. The reaction mixture was warmed to 0 °C and stirred for 21 h, before adding H_2O (15 mL). The organic layer was isolated and washed with brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give the crude sulfamidite as a mixture of diastereoisomers [R_f = 0.92 (30% EtOAc in hexanes)], which was used immediately in the next step: $NaIO_4$ (244 mg, 1.14 mmol), $RuCl_3$ (11 mg, 0.052 mmol) and H_2O (5 mL) were added sequentially to a solution of the crude sulfamidite in MeCN (5 mL) at 0 °C. After 2.5 h, the reaction mixture was diluted with H_2O (50 mL) and Et_2O (50 mL). The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give

sulfamidate **72** as a colourless oil (334 mg, 62%): R_f = 0.35 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.82 (3H, t, *J* = 6.8, CH_2CH_3), 1.14-1.29 (26H, stack, CH_2 resonances in alkyl chains), 1.32 (3H, s, 1 × $C(CH_3)_2$), 1.40 (3H, s, 1 × $C(CH_3)_2$), 1.49 (9H, s, $C(CH_3)_3$), 4.16-4.28 (2H, stack), 4.39 (1H, dd, *J* = 6.9, 1.9), 4.51 (1H, dd, *J* = 9.0, 6.9), 4.67 (1H, dd, *J* = 9.0, 1.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (C, $SiC(CH_3)_3$), 24.7 (CH_3, 1 × $C(CH_3)_2$), 26.4 (CH_3, 1 × $C(CH_3)_2$), 26.8 (CH_2), 27.9 (CH_3, $SiC(CH_3)_3$), [29.4, 29.5, 29.6, 29.7, 29.8 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 57.4 (CH, *C*HN), 66.4 (CH_2, CH_2O), 75.5 (CH, *C*HO), 76.5 (CH, *C*HO), 85.8 (C), 108.7 (C, $C(CH_3)_2$), 149.0 (C, *C*=O); *m/z* (TOF ES+) 542.4 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 542.3132 ($[M+Na]^+$) $C_{26}H_{49}NaNO_7S$ requires 542.3127.

(2*R*,3*R*,4*S*)-2-amino-1-*O*-*tert*-butyldiphenylsilyl-1,3,4-octadecanetriol (74)

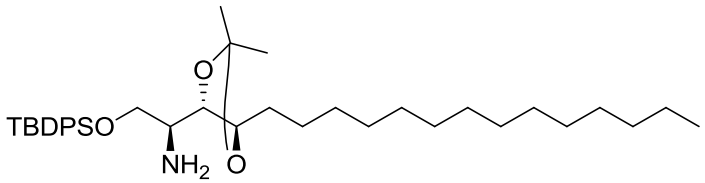


TBDPSCl (2.46 mL, 9.45 mmol) was added to a solution of phytosphingosine **12** (2.0 g, 6.3 mmol) in pyridine (20 mL). After stirring overnight, the reaction was quenched with MeOH (5 mL), and then the solvent was removed under reduced pressure. The residue was taken up in EtOAc (40 mL) and washed with water (2 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give the crude product, which was purified by column chromatography (50% EtOAc in hexanes, EtOAc, 0% – 7% MeOH in EtOAc, gradient) to provide silyl ether **74** as a colourless oil (2.8 g, 85%): R_f = 0.33 (5% MeOH in EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.77 (3H, t, *J* = 6.7, CH_2CH_3), 0.96 (9H, s, $C(CH_3)_3$), 1.13-1.21 (26H, stack, CH_2 resonances in alkyl chain), 2.56-2.62 (1H, m, C*H*NH),

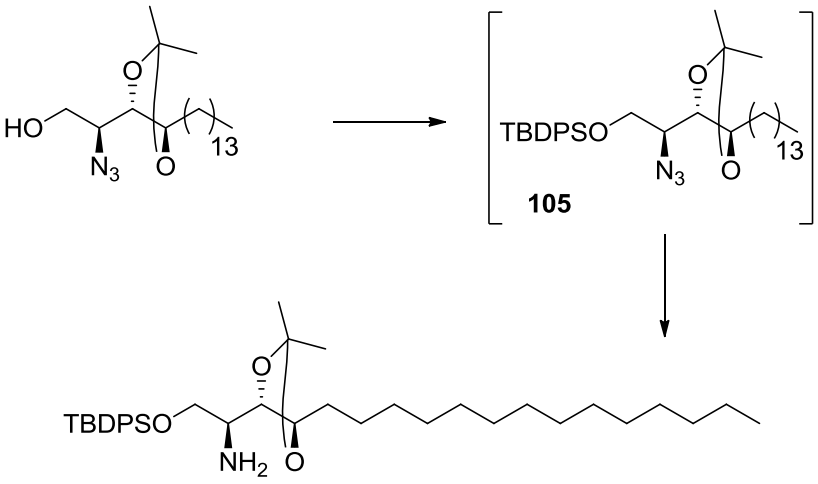
3.38-3.42 (1H, m), 3.60-3.66 (1H, m), 3.90-3.93 (2H, stack), 7.23-7.36 (6H, stack, Ph), 7.54-7.63 (4H, stack, Ph), exchangeable hydrogens not observed; *m/z* (TOF ES+) 556.4 ($[M+H]^+$, 100%); HRMS *m/z* (TOF ES+) 556.4188 ($[M+H]^+$) $C_{34}H_{58}NO_3Si$ requires 556.4186.

Data were in agreement with those reported in the literature.[177]

(2*R*,3*R*,4*S*)-2-amino-1-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (76)



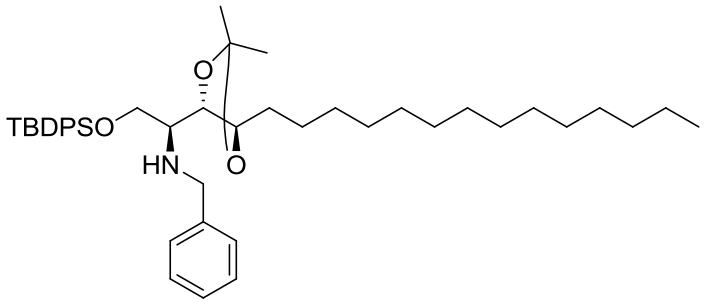
Concentrated H_2SO_4 (4 drops) was added to a solution of sphingosine **74** (450 mg, 0.81 mmol) in dry acetone (10 mL) at 0 °C. The reaction mixture was stirred for 5 h and then quenched with $NaHCO_3$ solution (20 mL), before being concentrated under reduced pressure. The mixture was then extracted with EtOAc (3 × 20 mL) and the combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (20% EtOAc in hexanes) to provide acetonide **76** as a colourless oil (420 mg, 87%): R_f = 0.44 (20% EtOAc in hexanes). Or:



TBDPSCl (1.32 mL, 5.09 mmol) was added to a solution of alcohol **10** (1.30 g, 3.39 mmol) in pyridine (20 mL). After stirring overnight, the reaction was quenched with MeOH (5 mL), and then the solvent was removed under reduced pressure. The residue was taken up in EtOAc (40 mL) and washed sequentially with H_2O (2 × 20 mL) and brine (20 mL). The separated organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give the crude silyl ether product, which was used directly in the next step: R_f = 0.59 (5% EtOAc in hexanes). PMe_3 (1.0 M solution in THF, 0.39 mL, 0.39 mmol) was added dropwise over 5 min to a solution of azide **105** (200 mg, 0.32 mmol) in a 15:1 THF: H_2O solution (5 mL). The reaction mixture was stirred for 4 h at rt before being concentrated under reduced pressure. The residual H_2O was removed by co-evaporation with toluene (3 × 2 mL). The crude product was then purified by column chromatography (20% EtOAc in hexanes) to give amine **76** as a colourless oil (0.19 g, 99%): R_f = 0.08 (10% EtOAc in hexanes); $[\alpha]_D^{20}$ = 41.0 (c = 0.8, $CHCl_3$); ν_{max}(film)/cm^{-1} 3675 w, 2923 s, 2854 s, 1589 w, 1463 m, 1427 m, 1377 m, 1367 m, 1244 m, 1217 m, 1168 w, 1111 s, 1066 s, 998 m, 822 m, 783 w, 739 m, 701 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.89 (3H, t, *J* = 6.6, CH_2CH_3), 1.09 (9H, s, $SiC(CH_3)_3$), 1.18–1.31 (26H, stack, CH_2 resonances in alkyl chain), 1.31 (3H, s, $C(CH_3)_3$), 1.35 (3H, s, $C(CH_3)_3$), 2.88–2.97 (1H, m, $CHNH_2$), 3.74–3.83 (2H, stack), 4.01 (1H, dd, *J* = 9.2, 5.5), 4.07–4.21 (1H, m), 7.32–7.44 (6H, stack, Ph),

7.64–7.75 (4H, stack, Ph), exchangeable hydrogens not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 19.4 (C, $SiC(CH_3)_3$), 22.7 (CH_2), 26.0 (CH_3, 1 × $C(CH_3)_2$), 26.2 (CH_2), 26.9 (CH_3, $SiC(CH_3)_3$), 28.4 (CH_3, 1 × $C(CH_3)_2$), [29.4, 29.7, 29.8 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 51.8 (CH, *CH*NH), 66.7 (CH_2, CH_2OSi), 78.0 (CH, CHO), 78.4 (CH, CHO), 107.8 (C, $C(CH_3)_2$), 127.6 (CH, Ph), 127.7 (CH, Ph), 129.70 (CH, Ph), 129.73 (CH, Ph), 133.4 (C, *ipso* Ph), 133.6 (C, *ipso* Ph), 135.6 (CH, Ph), 135.7 (CH, Ph); *m/z* (TOF ES+) 596.2 ($[M+H]^+$, 100%); HRMS *m/z* (TOF ES+) 596.4504 ($[M+H]^+$) $C_{37}H_{62}NO_3Si$ requires 596.4499.

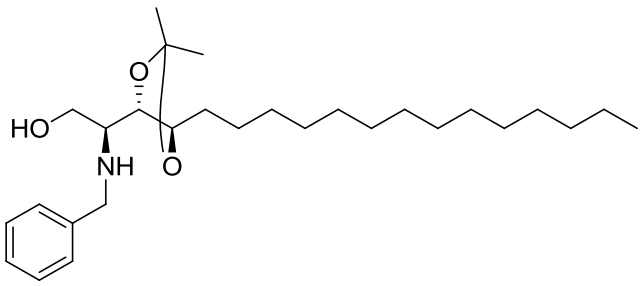
(2*R*,3*R*,4*S*)-2-benzylamino-1-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (77)



PhCHO (86 µL, 0.85 mmol) was added to a stirred suspension of amine **76** (420 mg, 0.71 mmol) and $NaBH(OAc)_3$ (377 mg, 1.78 mmol) in THF (5 mL). After stirring overnight, the reaction mixture was diluted with Et_2O (20 mL) and $NaHCO_3$ solution (20 mL). The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 20 mL). The organic layers were combined and washed with brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (0-2% EtOAc in hexanes, gradient) to give benzyl amine **77** as a colourless oil (320 mg, 66%): R_f = 0.64 (10% EtOAc in hexanes);$[\alpha]_D^{20}$ = 37.6 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1}

3070 w, 2923 s, 2853 s, 1589 w, 1455 m, 1427 m, 1377 m, 1366 m, 1245 m, 1216 m, 1173 w, 1111 s, 1081 s, 998 m, 938 w, 875 w, 822 m, 737 s, 699 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.77 (3H, t, *J* = 6.7, CH_2CH_3), 0.96 (9H, s, $C(CH_3)_3$), 1.13-1.21 (26H, stack, CH_2 resonances in alkyl chains), 1.28 (6H, s, $C(CH_3)_2$), 2.56-2.62 (1H, m, CHNH), 3.62 (1H, A of AB, $J_{A\text{-}B}$ = 12.5, CH_aH_bPh), 3.79 (1H, B of AB, $J_{B\text{-}A}$ = 12.5, CH_aH_bPh), 3.80-3.85 (2H, stack), 4.06-4.19 (3H, stack), 7.16-7.34 (11H, stack, Ph), 7.57-7.66 (4H, stack, Ph), NH not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2 (CH_3, CH_2CH_3), 19.5 (C, $SiC(CH_3)_3$), 22.8 (CH_2), 26.1 (CH_3, 1 × $C(CH_3)_2$), 26.2 (CH_3, $SiC(CH_3)_3$), 28.6 (CH_3, 1 × $C(CH_3)_2$), [29.5, 29.6, 29.80, 29.81 (CH_2, broad stack, significant resonance overlap)], 32.1 (CH_2), 51.2 (CH_2, CH_2NH), 57.3 (CH, CHNH), 60.3 (CH_2, CH_2O), 76.4 (CH, CHO), 78.4 (CH, CHO), 107.5 (C, $C(CH_3)_2$), 127.1 (CH, Ph), 127.7 (CH, Ph), 127.8 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 129.7 (CH, Ph), 129.8 (CH, Ph), 133.4 (C, *ipso* Ph), 133.8 (C, *ipso* Ph), 135.6 (CH, Ph), 135.8 (CH, Ph), 140.6 (C, *ipso* Ph); *m/z* (TOF ES+) 708.3 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 708.4786 ($[M+Na]^+$) $C_{44}H_{67}NNaO_3Si$ requires 708.4788.

(2*R*,3*R*,4*S*)-2-benzylamino-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (78)

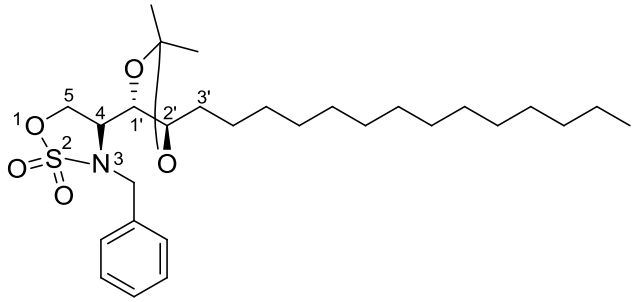


TBAF (1.0 M solution in THF, 1.27 mL, 1.27 mmol) was added to a solution of acetonide **77** (440 mg, 0.64 mmol) in THF (20 mL). The reaction mixture was stirred overnight before being quenched with H_2O (15 mL). The resulting layers were separated and the aqueous layer was

extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (25% EtOAc in hexanes) to provide alcohol **78** as a colourless oil (280 mg, 98%): R_f = 0.25 (25% EtOAc in hexanes); $[\alpha]_D^{20}$ = 27.8 (c = 0.9, $CHCl_3$), lit.[178] $[\alpha]_D^{25}$ = 30.1 (c = 6.3, $CHCl_3$); ν_{max}(film)/cm^{-1} 3411 br w, 2922 s, 2852 s, 1455 m, 1368 m, 1244 m, 1217 s, 1171 m, 1056 s, 873 m, 735 m, 699 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.88 (3H, t, *J* = 6.7, CH_2CH_3), 1.24-1.30 (26H, stack, CH_2 resonances in alkyl chains), 1.32 (3H, s, 1 × $C(CH_3)_2$), 1.40 (3H, s, 1 × $C(CH_3)_2$), 2.24 (1H, br s, O*H*), 2.67-2.75 (1H, m, C*H*NH), 3.65-3.90 (4H, stack), 4.01 (1H, dd, *J* = 8.3, 5.9), 4.09-4.19 (1H, m), 7.18-7.33 (5H, stack, Ph), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.3 (CH_3, 1 × $C(CH_3)_2$), 26.4 (CH_2). 28.0 (CH_3, 1 × $C(CH_3)_2$), [29.4, 29.62, 29.63, 29.7 (CH_2, broad stack, significant resonance overlap)], 32.0 (CH_2), 51.1 (CH_2, CH_2NH), 57.1 (CH, *CH*NH), 60.6 (CH_2, CH_2OH), 77.8 (CH, CHO), 78.0 (CH, CHO), 107.7 (C, $C(CH_3)_2$), 127.1 (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 140.1 (C, *ipso* Ph); *m/z* (TOF ES+) 448.3 ($[M+H]^+$, 100%); HRMS *m/z* (TOF ES+) 448.3783 ($[M+H]^+$) $C_{28}H_{50}NO_3$ requires 448.3791.

Data were in agreement with those reported in the literature.[178]

(3*R*,1'*S*,2'*S*)-3-benzyl-4-[1',2'-*O*-isopropylidene-dihydroxyhexadecyl]-1,2,3-oxathiazolidine-2,2-dioxide (80)

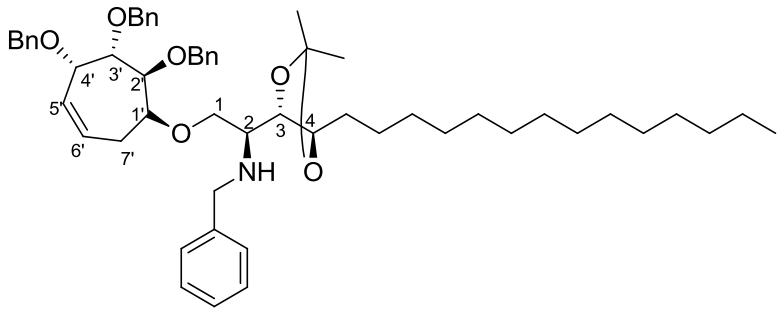


A solution of benzyl amine **78** (280 mg, 0.63 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 30 min to a solution of $SOCl_2$ (50 µL, 0.69 mmol), imidazole (172 mg, 2.52 mmol) and NEt_3 (194 µL, 1.39 mmol) in CH_2Cl_2 (6 mL) at −50 °C. The reaction mixture was warmed up to 0 °C and stirred for 21 h, before adding H_2O (10 mL). The organic layer was isolated and washed with brine (5 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give the crude sulfamidite as a mixture of diastereoisomers [R_f = 0.88 (30% EtOAc in hexanes)], which was used immediately in the next step. $NaIO_4$ (148 mg, 0.69 mmol), $RuCl_3$ (14 mg, 0.064 mmol) and H_2O (5 mL) were added sequentially to a solution of the crude sulfamidite in MeCN (5 mL) at 0 °C. After 2.5 h, the reaction mixture was diluted with H_2O (50 mL) and Et_2O (50 mL). The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed with H_2O (30 mL), brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give sulfamidate **80** as a colourless oil (183 mg, 58%): R_f = 0.40 (10% EtOAc in hexanes); $[\alpha]_D^{20}$ = 13.0 (c = 1.0, $CHCl_3$), lit.[178] $[\alpha]_D^{25}$ = 2.31 (c = 5.2, $CHCl_3$); ν_{max}(film)/cm^{-1} 2922 s, 2852 s, 1743 w, 1497 w, 1456 m, 1351 m, 1244 m, 1211 m, 1187 s, 1061 m, 1028 m, 977 m, 800 m, 732 m, 698 m; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.80 (3H, t, *J* = 6.7, CH_2CH_3), 1.12-1.20 (26H, stack,

CH_2 resonances in alkyl chain), 1.21 (3H, s, 1 × C(CH_3)$_2$), 1.32 (3H, s, 1 × C(CH_3)$_2$), 3.51-3.58 (1H, m), 3.91-4.00 (1H, m), 4.10 (1H, app t, J = 5.9), 4.23 (1H, dd, J = 8.7, 7.4), 4.36 (1H, A of AB, $J_{A\text{-}B}$ = 12.5, CH_aH$_b$Ph), 4.46 (1H, B of AB, $J_{B\text{-}A}$ = 12.5, CH$_a$$H_b$Ph), 4.53 (1H, dd, J = 8.7, 3.9), 7.22-7.37 (5H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, $CH_2$$CH_3$), 22.7 ($CH_2$), 25.1 ($CH_3$, 1 × C($CH_3$)$_2$), 26.5 ($CH_2$), 27.4 ($CH_3$, 1 × C($CH_3$)$_2$), [29.4, 29.53, 29.54, 29.7, 29.9 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 52.3 (CH_2, CH_2N), 58.8 (CH, *CH*N), 68.1 (CH_2, CH_2O), 75.7 (CH, CHO), 76.8 (CH, CHO), 108.2 (C, *C*(CH$_3$)$_2$), 128.5 (CH, Ph), 128.7 (CH, Ph), 128.9 (CH, Ph), 134.7 (C, *ipso* Ph); *m/z* (TOF ES+) 532.5 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 532.3079 ([M+Na]$^+$) $C_{28}H_{47}NaNO_5S$ requires 532.3073.

Data were in agreement with those reported in the literature.[178]

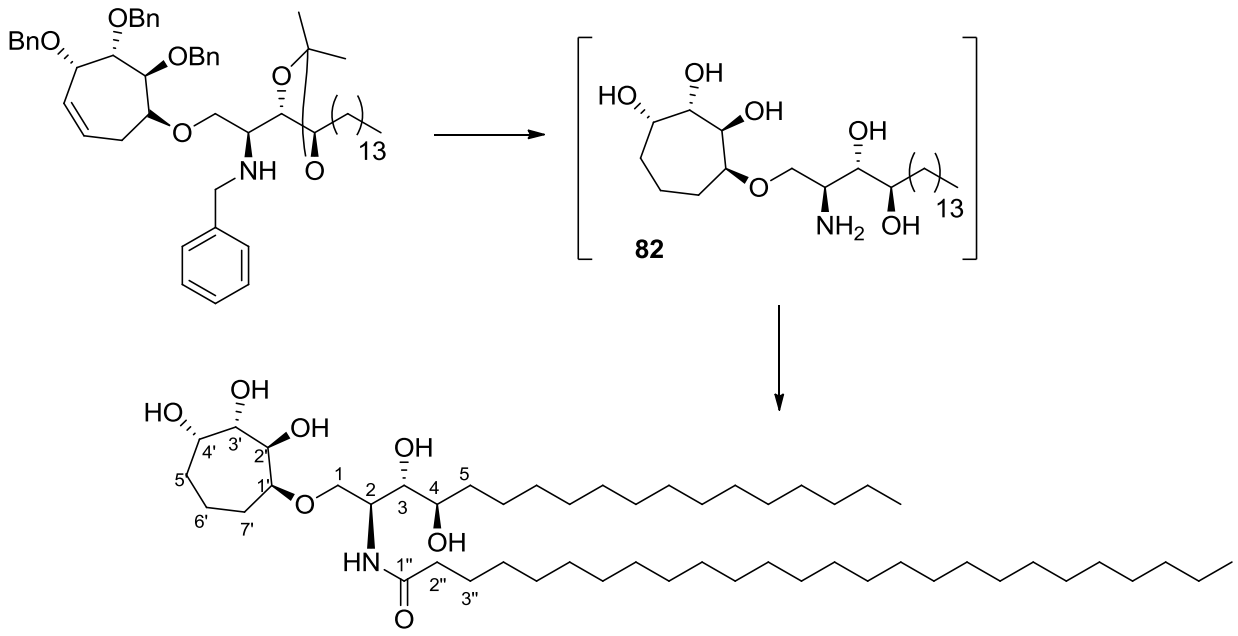
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-benzylamino-1-*O*-[2',3',4'-*O*-benzyl-trihydroxycyclohept-5'-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (81)



NaH (60% wt in mineral oil, 10 mg, 0.24 mmol) was added to a solution of alcohol **54** (300 mg, 0.70 mmol) in DMF (2 mL) and THF (1 mL) at 0 °C. After stirring for 1 h, a solution of sulfamidate **80** (426 mg, 0.84 mmol) in THF (1 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated and the residue was dissolved in Et_2O (10 mL). A

20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 20 min before being neutralised with K_2CO_3 (1 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) were added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give ether **81** as a colourless oil (367 mg, 61%): R_f = 0.68 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ = 47.6 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2922 s, 2853 s, 1743 w, 1496 w, 1454 m, 1377 m, 1367 m, 1241 m, 1216 m, 1172 w, 1127 s, 1090 s, 1067 s, 1027 s, 873 w, 833 m, 778 m, 733 s, 696 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.80 (3H, t, *J* = 6.8, CH_2CH_3), 1.14-1.24 (26H, stack, CH_2 resonances in alkyl chains), 1.31 (6H, s, $C(CH_3)_2$), 2.00-2.08 (1H, m, C(7')H_aH_b), 2.61-2.73 (2H, stack, CHNH, C(7')H_aH_b), 3.50-3.60 (3H, stack, NHCH_aH_b, C(1)H_aH_b, CHO), 3.67 (1H, dd, *J* = 9.3, 2.7, C(1)H_aH_b), 3.75-3.87 (3H, stack, NHCH_aH_b, 2 × CHO), 3.90 (1H, dd, *J* = 9.3, 5.7, CHO), 4.02-4.08 (1H, m, CHO), 4.31-4.57 (5H, stack), 4.66 (2H, stack), 5.68-5.72 (2H, stack, C(5')*H*, C(6')*H*), 7.11-7.26 (20H, stack, Ph), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2 (CH_3, CH_2CH_3), 22.8 (CH_2), 26.1 (CH_3, 1 × $C(CH_3)_2$), 26.2 (CH_2), 27.2 (CH_2). 28.5 (CH_3, 1 × $C(CH_3)_2$), [29.4, 29.6, 29.7, 29.8 (CH_2, broad stack, significant resonance overlap)], 32.0 (CH_2), 51.2 (CH_2, CH_2NH), 56.3 (CH, CHNH), 66.3 (CH_2, CH_2O), 71.2 (CH_2, CH_2Ph), 72.8 (CH_2, CH_2Ph), 73.8 (CH_2, CH_2Ph), 76.7 (CH, CHO) 76.9 (CH, CHO), 77.0 (CH, CHO), 78.4 (CH, CHO), 78.6 (CH, CHO), 79.3 (CH, CHO), 107.4 (C, $C(CH_3)_2$), 125.7 (CH, CH=CH), [127.1, 127.5, 127.6, 127.8, 128.3, 128.42, 128.43 (CH, resonance overlap, Ph)], 133.8 (CH, CH=CH), 138.7 (C, *ipso* Ph), 138.9 (C, *ipso* Ph), 139.0 (C, *ipso* Ph), 140.5 (C, *ipso* Ph); *m/z* (TOF ES+) 882.8 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 882.5670 ([M+Na]$^+$) $C_{56}H_{77}NaNO_6$ requires 882.5649.

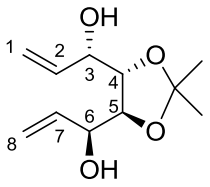
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-hexacosanoylamino-1-*O*-[2',3',4'-trihydroxycycloheptanyl]-1,3,4-octadecantriol (44)



A 1.0 M solution of hydrochloric acid (150 µL, 0.15 mmol) and Pd/C (10% wt, 32 mg, 0.03 mmol) were added to a solution of ether **81** (130 mg, 0.15 mmol) and cyclohexene (2 mL) in MeOH (10 mL) and heated under reflux. After stirring overnight the reaction mixture was cooled to rt and diluted with a 5:1 solution of $CHCl_3$: MeOH (30 mL), before being filtered thought a bed of Celite. The filtrate was concentrated under reduced pressure to provide the crude amino-pentaol **82,** R_f = 0.42 (10% MeOH in $CHCl_3$), which was used directly in the next step. $(COCl)_2$ (2 mL) was added to hexacosanoic acid (139 mg, 0.39 mmol) and heated at 70 °C for 2 h, after which time the solution was cooled to rt, and the $(COCl)_2$ removed under a stream of dry argon. The residual volatiles were removed under reduced pressure. The resulting crude acyl chloride was dissolved in THF (0.5 mL) and added with vigorous stirring to a solution of amine **82** (81 mg, 0.18 mmol) in THF/$NaOAc_{(aq)}$ (8 M) (1:1, 2 mL). Vigorous stirring was maintained for 2 h, after which time the reaction mixture was left to stand and the layers were

separated. The aqueous layer was extracted with THF (3 × 2.0 mL) and the organic layers were combined and concentrated under reduced pressure. The crude product was purified by column chromatography (10% MeOH in $CHCl_3$) to give amide **44** as a white solid (64 mg, 51% over two steps): R_f = 0.31 (10% MeOH in $CHCl_3$); mp = 106–112 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3310 br w, 2917 s, 2849 s, 1636 m, 1562 m, 1473 m, 1463 m, 1361 w, 1299 w, 1127 w, 1106 m, 1029 m, 1043 m, 969 w, 890 w, 851 w, 790 w, 729 m, 718 m, 642 w, 575 w; ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD, 2 : 1, 40 °C) δ ppm 0.84 (6H, t, *J* = 6.9, 2 × CH_2CH_3), 1.16-1.31 (70H, stack), 1.46-1.53 (1H, m, C(6')H_aH_b), 1.53-1.63 (4H, stack, C(6')H_aH_b, C(7')H_aH_b, C(3'')H_aH_b), 1.65-1.73 (2H, m, C(5')H_aH_b), 1.78-1.86 (1H, m, C(7')H_aH_b), 2.16 (2H, app t, *J* = 7.6, C(2'')H_aH_b), 3.48-3.52 (1H, m, C(3)*H*), 3.54 (1H, dd, *J* = 6.3, 2.3, C(4)*H*), 3.60 (1H, dd, *J* = 9.9, 3.8, C(1)H_aH_b), 3.68-3.71 (2H, stack, C(1')*H*, C(1)H_aH_b), 3.74 (1H, dd, *J* = 7.8, 2.9, C(3')*H*), 3.78 (1H, dd, *J* = 7.8, 2.2, C(2')*H*), 3.96-3.98 (1H, m, C(4')*H*), 4.09-4.13 (1H, stack coincident with solvent, C(2)*H*), exchangeable hydrogens not observed; ^{13}C NMR (500 MHz, $CDCl_3$: CD_3OD 2 : 1, 40 °C) δ ppm 14.2 (CH_3), 18.8 (CH_2, C(6')), 23.0 (CH_2), 26.2 (CH_2), 26.2 (CH_2, C(3'')), 28.2 (CH_2, C(7')), [29.7, 29.82, 29.84, 29.9, 30.0, 30.1 (CH_2, resonance overlap)], 30.9 (CH_2, C(5')), 32.3 (CH_2), 32.9 (CH_2, C(5)), 36.9 (CH_2, C(2'')), 50.6 (CH, C(2)), 69.0 (CH_2, C(1)), 71.0 (CH, C(4')), 72.8 (CH, C(4)), 73.3 (CH, C(2')), 74.0 (CH, C(3')), 75.4 (CH, C(3)), 80.7 (CH, C(1')), 174.6 (C, C(1'')); *m/z* (TOF ES+) 862.7 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 862.7515 ([M+Na]$^+$) $C_{51}H_{101}NNaO_7$ requires 862.7476.

(3*S*,4*S*,5*S*,6*S*)-4,5-*O*-isopropylidene-octa-1,7-dien-3,4,5,6-tetraol (84)

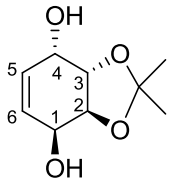


A solution of (2*R*,3*R*)-2,3-*O*-isopropylidene tartrate **83** (1.5 g, 6.9 mmol) in toluene (25 mL) was degassed by bubbling argon through the solvent while sonicating for 10 min. DIBALH (1.0 M in toluene, 14.4 mL, 14.4 mmol) was then added dropwise over 10 min to the solution at −78 °C. After 2.5 h at −78 °C, vinyl magnesium bromide (1.0 M in THF, 20.6 mL, 20.6 mmol) was added and the reaction mixture left to stir for 2 h at −78 °C, before being allowed to warm up to rt slowly. The reaction was carefully quenched with NH_4Cl solution (50 mL) and the resulting layers were separated. The aqueous layer was extracted with EtOAc (3 × 35 mL). The organic layers were combined and washed with H_2O (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (25% EtOAc in hexanes) to give diene **84** as the major product in a mixture of diastereoisomers (ratio, 3:1) as a colourless oil (810 mg, 55%): Data for the mixture unless specified otherwise. R_f = 0.21 (25% EtOAc in hexanes); ν_{max}(film)/cm^{-1} 3357 w, 2987 w, 2887 w, 1644 w, 1455 m, 1427 m, 1371 s, 1239 s, 1214 m, 1131 m, 1074 s, 1048 s, 993 s, 925 s, 877 s, 811 m, 736 s, 698 s; Data for the major isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.42 (6H, s, C(C*H*$_3$)$_2$), 2.10 (2H, br s, O*H*), 3.88-3.95 (2H, stack, 2 × C*H*O), 4.17-4.23 (2H, stack, 2 × C*H*O), 5.27-5.47 (4H, stack, 2 × CH=C*H*$_2$), 5.95-6.07 (2H, stack, 2 × C*H*=CH$_2$); Selected data for minor isomer (relative stereochemistry not determined): ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.88-6.01 (2H, stack, 2 × C*H*=CH$_2$); Data for the major isomer: ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 26.9 (CH_3, C(*C*H$_3$)$_2$), 73.6 (CH), 82.0 (CH), 109.5 (C, *C*(CH$_3$)$_2$), 117.1 (CH$_2$, CH=*C*H$_2$), 131.6 (CH,

CH=CH_2); *m/z* (TOF ES+) 237.2 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 237.1098 ([M+Na]$^+$) $C_{11}H_{18}NaO_4$ requires 237.1103.

Data were in agreement with those reported in the literature.[154,155]

(1*S*,2*S*,3*S*,4*S*)-2,3-*O*-isopropylidene-cyclohex-5-en-1,2,3,4-tetraol (85)

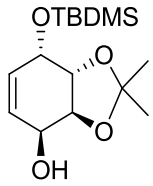


A solution of diene **84** (217 mg, 1.01 mmol) in CH_2Cl_2 (230 mL) was degassed by bubbling argon through the solvent while sonicating for 10 min. Grubbs 2nd generation Ru metathesis catalyst (12 mg, 0.015 mmol) was added and the solution was heated under reflux. After 2 h the solution was concentrated under reduced pressure and the crude product purified by column chromatography (5% MeOH in $CHCl_3$) to give diol **85** as a colourless oil (67 mg, 50%) as the major (2:1 ratio) less polar diastereoisomer[1]: R_f = 0.23 (5% MeOH in $CHCl_3$); $[\alpha]_D^{20}$ = 242.9 (c = 0.9, MeOH), lit.[179] $[\alpha]_D^{25}$ = 338.6 (c = 0.7, $CHCl_3$); ν_{max}(film)/cm^{-1} 3295 br m (OH), 2989 m, 2903 m, 2453 w, 1450 w, 1369 m, 1210 s, 1129 s, 1148 s, 1017 m, 931 m, 838 s, 795 m; ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.46 (6H, s, C(C*H*$_3$)$_2$), 3.93 (2H, s, 2 × C*H*O), 4.45 (2H, s, 2 × C*H*O), 5.97 (2H, s, C*H*=C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 27.3 (CH3, C(*C*H$_3$)$_2$), 65.9 (CH), 74.9 (CH), 111.0 (C, *C*(CH$_3$)$_2$), 131.6 (CH, *CH*=*CH*); *m/z* (TOF ES+) 209.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 209.0779 ([M+Na]$^+$) $C_9H_{14}NaO_4$ requires 209.0790.

Data were in agreement with those reported in the literature. [154,155,179]

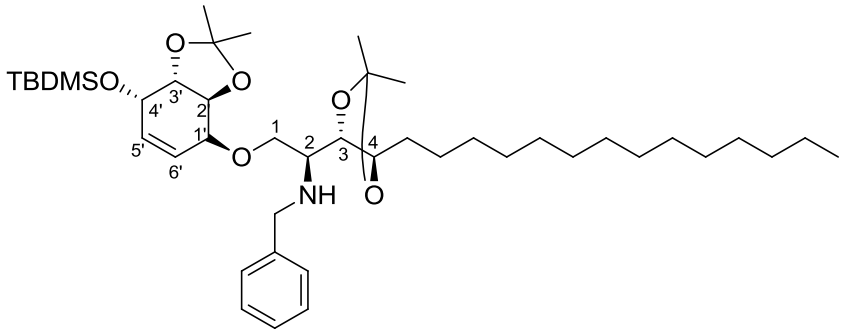
[1] The minor diastereoisomer was not isolated in a pure form.

(1*S*,2*S*,3*S*,4*S*)-4-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-cyclohex-5-en-1,2,3,4-tetraol (86)



Imidazole (275 mg, 4.04 mmol) and TBDMSCl (486 mg, 3.23 mmol) were added sequentially to a solution of diol **85** (500 mg, 2.69 mmol) in DMF (5 mL). After stirring overnight, the reaction mixture was diluted with Et_2O (30 mL), and washed sequentially with H_2O (15 mL) and NH_4Cl solution (15 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (20% EtOAc in hexanes) to give alcohol **86** as a colourless oil (412 mg, 51%): R_f = 0.33 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ = 252.8 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3352 m, 2987 m, 2887 m, 1644 w, 1455 m, 1214 m, 1166 w, 1107 s, 1074 s, 1048 s, 996 m, 919 w, 832 m, 812 m, 785 m, 709 m; ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.00 (3H, s, 1 × Si(C*H*$_3$)$_2$), 0.01 (3H, s, 1 × Si(C*H*$_3$)$_2$), 0.80 (9H, s, SiC(C*H*$_3$)$_3$), 1.362 (3H, s, 1 × C(C*H*$_3$)$_2$), 1.363 (3H, s, 1 × C(C*H*$_3$)$_2$), 3.79 (1H, dd, *J* = 10.0, 3.4, C*H*O), 3.93 (1H, dd, *J* = 10.0, 3.8, C*H*O), 4.36-4.39 (1H, m, CH=CHC*H*O), 4.40-4.43 (1H, m, CH=CHC*H*O), 5.79-5.81 (2H, stack, C*H*=C*H*), O*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−4.8, −4.6 (2 × CH_3, Si(*CH*$_3$)$_2$)], 18.2 (C, Si*C*(CH$_3$)$_3$), 25.7 (CH_3, SiC(C*H*$_3$)$_3$), 27.0 (2 × CH_3, C(*CH*$_3$)$_2$), [65.1, 65.8 (2 × CH, 2 × CH=CH*CH*O)], [73.2, 73.7 (2 × CH, 2 × *CH*O)], 110.2 (C, *C*(CH$_3$)$_2$), [128.4, 132.2 (CH, 2 × *CH*=CH)]; *m/z* (TOF ES+) 323.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 323.1647 ([M+Na]$^+$) $C_{15}H_{28}NaO_4Si$ requires 323.1655.

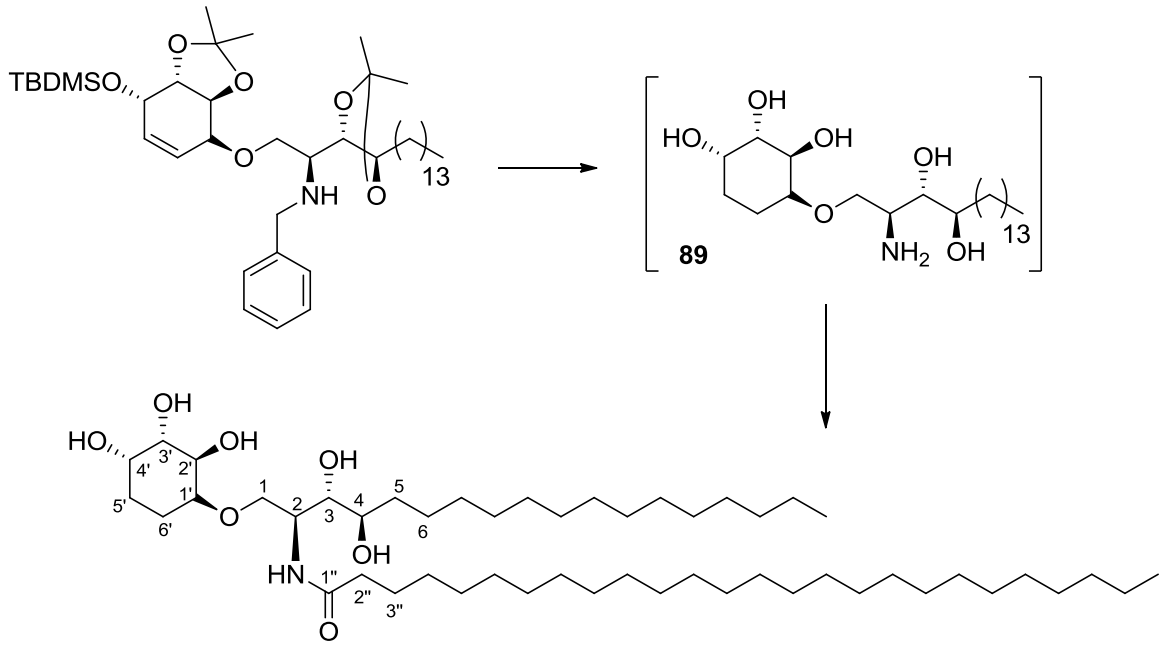
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-benzylamino-1-*O*-[4'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-2',3',4'-trihydroxycyclohex-5-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (87)



NaH (60% wt in mineral oil, 78 mg, 1.95 mmol) was added to a solution of alcohol **86** (195 mg, 0.65 mmol) in DMF (2 mL) and THF (1 mL) at 0 °C. After stirring for 1 h, a solution of sulfamidate **80** (397 mg, 0.78 mmol) in THF (1 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated and the residue was dissolved in Et_2O (10 mL). A 20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 20 min before being neutralised with K_2CO_3 (1 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) was added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give ether **87** as a colourless oil (204 mg, 43%): R_f = 0.56 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ = 122.8 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2924 s, 2853 s, 1651 w, 1456 w, 1377 m, 1368 m, 1218 m, 1172 m, 1147 m, 1128 s, 1092 s, 1064 s, 1026 m, 967 m, 923 w, 832 s, 801 m, 778 m, 738 m, 697 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (3H, 1 × Si(CH_3)$_2$), 0.01 (3H, 1 × Si(CH_3)$_2$), 0.78 (3H, t, *J* = 7.0, CH_2CH_3), 0.79 (9H, s, Si(CH_3)$_3$), 1.14-1.18

(26H, stack, CH_2 resonances in alkyl chains), 1.20 (3H, s, C(CH_3)$_2$), 1.29 (3H, s, C(CH_3)$_2$), 1.32 (6H, app s, C(CH_3)$_3$), 2.66-2.72 (1H, m, C(2)*H*), 3.62 (1H, A of AB, $J_{A\text{-}B}$ = 12.8, CH_2Ph), 3.70 (1H, dd, *J* = 9.8, 2.9, C(1)H_aH$_b$), 3.81 (1H, B of AB, $J_{B\text{-}A}$ = 12.8, CH_2Ph), 3.85 (1H, dd, *J* = 10.0, 3.6, C(2')*H* or C(3')*H*), 3.90-4.06 (4H, stack, C(3')*H* or C(2')*H*, C(3)*H*, C(1)H$_a$$H_b$, C(4)*H*), 4.08 (1H, app t, *J* = 4.0, C(1')*H* or C(4')*H*), 4.36 (1H, app t, *J* = 4.0, C(4')*H* or C(1')*H*), 5.74 (2H, stack, C(5')*H*, C(6')*H*), 7.09-7.26 (5H, stack, Ph), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−4.8, −4.6 (2 × CH_3, Si(*CH*$_3$)$_2$)], 14.1 (CH_3, CH_2*C*H$_3$), 18.3 (C, Si*C*(C*H*$_3$)$_3$), 22.7 (CH_2), 25.8 (CH_3, 1 × C(*CH*$_3$)$_2$), 25.9 (CH_3, 1 × C(*CH*$_3$)$_2$), 26.2 (CH_2), 26.9 (CH_3, 1 × C(*CH*$_3$)$_2$), 27.0 (CH_3, 1 × C(*CH*$_3$)$_2$, 28.3 (CH_3, SiC(*C*H$_3$)$_3$), [29.4, 29.5, 29.70, 29.73 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 51.3 (CH_2, NH*C*H$_2$), 56.7 (CH, C(2)), 66.0 (CH, C(1') or C(4')), 69.9 (CH_2, C(1)), 73.7 (CH, C(4') or C(1')), 74.0 (2 × CH, C(2') and C(3')), 76.9 (CH, C(3)), 78.3 (CH, C(4)), 107.4 (C, *C*(CH$_3$)$_2$), 109.9 (C, *C*(CH$_3$)$_2$), 126.9 (CH, C*H*=CH), 127.8 (CH, Ph), 128.0 (CH, Ph), 128.3 (CH, Ph), 131.6 (CH, C*H*=CH), 140.7 (C, *ipso* Ph)); *m/z* (TOF ES+) 752.6 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 752.5270 ([M+Na]$^+$) $C_{43}H_{75}NaNO_6Si$ requires 752.5261.

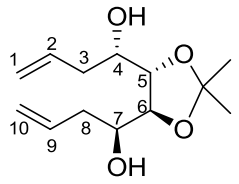
2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-hexacosanoylamino-1-*O*-[2',3',4'-trihydroxycyclohexyl]-1,3,4-octadecantriol (43)



A 1.0 M solution of hydrochloric acid (71 µL, 0.071 mmol) and Pd/C (10% wt, 15 mg, 0.014 mmol) were added to a solution of ether **87** (50 mg, 0.071 mmol) and cyclohexene (1 mL) in MeOH (5 mL). After heating at reflux overnight, the reaction mixture was cooled to rt and diluted with a 5:1 solution of $CHCl_3$: MeOH (30 mL), before being filtered thought a bed of Celite. The filtrate was concentrated under reduced pressure to provide the crude amine **88** [R_f = 0.35 (10% MeOH in $CHCl_3$)], which was used directly in the next step. Neat TFA (2 mL) was added to ether **88** (assuming 100% conversion, 0.071 mmol) for 15 min before removal of the TFA under reduced pressure. This procedure was repeated if necessary until all starting material was consumed, providing the crude amine **89** R_f = 0.33 (30% MeOH in $CHCl_3$), which was used directly in the next reaction. $(COCl)_2$ (2 mL) was added to hexacosanoic acid (21 mg, 0.054 mmol) and the resulting solution was heated at 70 °C for 2 h, after which time the solution was cooled to rt, and the residual $(COCl)_2$ removed under a stream of dry argon. The

residual volatiles were removed under reduced pressure. The resulting crude acyl chloride was dissolved in THF (0.5 mL) and added with vigorous stirring to a solution of amine **89** (20 mg, 0.045 mmol) in THF/$NaOAc_{(aq)}$ (8 M) (1:1, 2 mL). Vigorous stirring was maintained for 2 h, after which time the reaction mixture was left to stand and the layers were separated. The aqueous layer was extracted with THF (3 × 2.0 mL) and the organic layers were combined and concentrated under reduced pressure. The crude product was purified by column chromatography (10% MeOH in $CHCl_3$) to give amide **43** as a white solid (20 mg, 34% over three steps): R_f = 0.24 (10% MeOH in $CHCl_3$); mp = 105–110 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3284 w, 2916 s, 2849 s, 1638 m, 1543 w, 1468 m, 1230 w, 1070 m, 1007 w, 851 w, 719 m; ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD, 2 : 1, 40 °C) δ ppm 0.84 (6H, t, *J* = 7.0, 2 × CH_2CH_3), 1.12-1.32 (68H, stack), 1.45-1.67 (8H, stack, C(5')H_aH_b, C(6')H_aH_b, C(5)H_aH_b, C(6)H_aH_b, C(3'')H_aH_b), 2.16 (2H, app t, *J* = 7.6, C(2'')H_aH_b), 3.49-3.54 (2H, stack, C(3)*H*, C(4)*H*), 3.56 (1H, dd, *J* = 9.9, 3.5, C(1)H_aH_b), 3.63-3.68 (2H, stack, C(1')*H*, C(3')*H*), 3.70 (1H, dd, *J* = 9.9, 4.5, C(1)H_aH_b), 3.75 (1H, dd, *J* = 8.3, 2.5, C(2')*H*), 3.92-3.97 (1H, m, C(4')*H*), 4.12 (1H, app dt, *J* = 4.5, 4.2, C(2)*H*), exchangeable hydrogens not observed; ^{13}C NMR (500 MHz, $CDCl_3$: CD_3OD 2: 1, 40 °C) δ ppm 14.3 (CH_3), 22.5 (CH_2, C(5')), 23.1 (CH_2), 25.6 (CH_2, C(6')), 26.3 (CH_2, C(5) or C(6)), 26.4 (CH_2, C(3'')), [29.7, 29.82, 29.84, 30.0, 30.1, 30.2 (CH_2, resonance overlap)], 32.3 (CH_2), 32.9 (CH_2, C(6) or C(5)), 36.9 (CH_2, C(2'')), 50.6 (CH, C(2)), 68.7 (CH_2, C(1)), 69.5 (CH, C(4')), 71.4 (CH, C(2')), 72.7 (CH, C(3')), 72.7 (CH, C(4)), 75.3 (CH, C(3)), 78.9 (CH, C(1')), 174.7 (C, C(1'')); *m/z* (TOF ES+) 848.7 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 848.7311 ($[M+Na]^+$) $C_{50}H_{99}NNaO_7$ requires 848.7319.

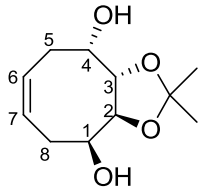
(4*S*,5*S*,6*S*,7*S*)-5,6-*O*-isopropylidene-deca-1,9-dien-4,5,6,7-tetraol (90)



A solution of (2*R*,3*R*)-2,3-*O*-isopropylidene tartrate **83** (1.88 g, 8.6 mmol) in toluene (25 mL) was degassed by bubbling argon through the solution while sonicating for 10 min. DIBALH (1.0 M in toluene, 18.1 mL, 18.1 mmol) was then added dropwise over 10 min to the solution at −78 °C.. After 2.5 h at −78 °C, allyl magnesium bromide (1.0 M in THF, 25.9 mL, 25.9 mmol) was added and the reaction mixture left to stir for 2 h at −78 °C, before being allowed to warm up to rt overnight. The reaction was carefully quenched with NH_4Cl solution (50 mL) and the resulting layers were separated. The aqueous layer was extracted with EtOAc (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (25% EtOAc in hexanes) to give diene **90** as a single diastereoisomer as a colourless oil (400 mg, 19%): R_f = 0.41 (25% EtOAc in hexanes); $[\alpha]_D^{21}$ = 1.75 (c = 0.8, $CHCl_3$); ν_{max}(film)/cm^{-1} 3310 br w, 2917 s, 2849 s, 1637 m, 1563 m, 1473 m, 1462 m, 1371 w, 1235 w, 1106 w, 1044 m, 969 w, 922 w, 875 w, 730 m, 718 m, 643 w,577 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.23 (6H, s, C(CH_3)$_2$), 2.04-2.14 (2H, m, C(3)H_aH$_b$, C(8)H_aH$_b$), 2.40-2.48 (2H, m, C(3)H$_a$$H_b$, C(8)H$_a$$H_b$), 3.46-3.56 (4H, stack, C(4)*H*, C(5)*H*, C(6)*H*, C(7)*H*), 4.98-5.07 (4H, stack, C(1)H_2, C(10)H_2), 5.69-5.81 (2H, m, C(2)*H*, C(9)*H*), O*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 26.9 (CH_3, C(*C*H_3)$_2$), 38.6 (CH_2, C(3), C(8)), [72.0, 82.5 (CH, C(4), C(5),C(6), C(7))], 108.9 (C, *C*(CH_3)$_2$), 118.2 (CH_2, C(1), C(10)), 134.2 (CH, C(2), C(9)); *m/z*

*(*TOF ES+) 265.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 265.1410 ([M+Na]$^+$) $C_{13}H_{22}NaO_4$ requires 265.1416.

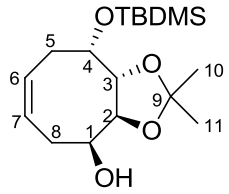
(1*S*,2*S*,3*S*,4*S*)-2,3-*O*-isopropylidene-cycloocta-6-en-1,2,3,4-tetraol (91)



A solution of diene **90** (400 mg, 1.65 mmol) in CH_2Cl_2 (750 mL) was degassed by bubbling argon through the solution while sonicating for 10 min. Grubbs 2nd-generation Ru metathesis catalyst (21 mg, 0.025 mmol) was added and the solution was heated under reflux. After 2 h, the solution was concentrated under reduced pressure and the crude product purified by column chromatography (40% EtOAc in hexanes) to give diol **91** as a colourless oil (220 mg, 62%): R_f = 0.34 (40% EtOAc in hexanes); $[\alpha]_D^{20}$ = 171.5 (c = 0.8, $CHCl_3$); ν_{max}(film)/cm^{-1} 3390 br m, 2917 s, 2850 s, 1638 w, 1544 m, 1466 m, 1376 m, 1328 m, 1307 m, 1255 m, 1216 m, 1166 w, 1107 s, 1062 s, 1049 s, 996 m, 919 w, 886 m, 858 m, 832 m, 812 m, 785 m, 709 m; ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.36 (6H, s, C(C*H*$_3$)), 2.25-2.46 (4H, stack, C(5)*H*$_2$, C(8)*H*$_2$), 2.72 (2H, s, O*H*), 4.05-4.13 (2H, m, C(1)*H*, C(4)*H*), 4.17 (2H, s, C(2)*H*, C(3)*H*), 5.71-5.74 (2H, m, C(6)*H*, C(7)*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 27.2 (CH_3, C(*C*H$_3$)$_2$), 29.1 (CH_2, C(5), C(8)), 67.4 (CH, *C*HO), 77.1 (CH, *C*HO), 108.1 (C, *C*(CH$_3$)$_2$), 128.0 (CH, C(6), C(7)); *m/z* (TOF ES+) 237.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 237.1112 ([M+Na]$^+$) $C_{11}H_{18}NaO_4$ requires 237.1103.

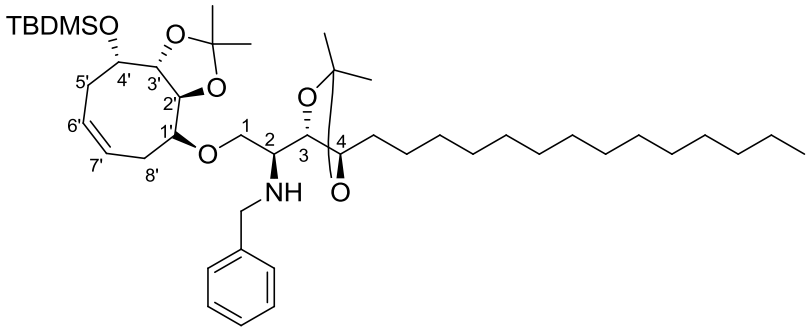
Data were in agreement with those reported in the literature.[180]

(1*S*,2*S*,3*S*,4*S*)-4-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-cyclooct-6-en-1,2,3,4-tetraol (92)



Imidazole (140 mg, 2.1 mmol) and TBDMSCl (187 mg, 1.2 mmol) were added sequentially to a solution of diol **91** (220 mg, 1.0 mmol) in DMF (5 mL). After stirring overnight, the reaction mixture was diluted with Et_2O (30 mL), washed sequentially with H_2O (15 mL) and NH_4Cl solution (15 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (20% EtOAc in hexanes) to give alcohol **92** as a colourless oil (162 mg, 43%): R_f = 0.50 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ = 163.0 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3498 br w, 2934 m, 2884 m, 2857 m, 1461 m, 1378 m, 1367 m, 1250 s, 1216 m, 1168 m, 1111 s, 1063 s, 1002 s, 942 m, 873 m, 826 s, 774 s, 745 m, 692 m, 665 m; ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.00 (3H, 1 × Si(C*H*$_3$)$_2$), 0.03 (3H, 1 × Si(C*H*$_3$)$_2$), 0.83 (9H, s, C(C*H*$_3$)$_3$), 1.31 (3H, s, C(10)*H*$_3$ or C(11)*H*$_3$), 1.32 (3H, C(11)*H*$_3$ or C(10)*H*$_3$), 2.17-2.31 (3H, stack), 2.34-2.42 (1H, m), 2.56 (1H, s, O*H*), 3.95-4.01 (1H, m, C*H*O), 4.07 (1H, dd, *J* = 8.4, 2.2, C*H*O), 4.15 (1H, td, *J* = 7.2, 2.5, C*H*O), 4.23 (1H, dd, *J* = 8.4, 3.4, C*H*O), 5.58-5.63 (2H, stack, C*H*=C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−4.9, −4.3 (2 × CH_3, Si(*C*H$_3$)$_2$)], 18.2 (C, *C*(CH$_3$)$_3$), 26.0 (CH$_3$, C(*C*H$_3$)$_3$), [27.2, 27.5 (2 × CH_3, C(10), C(11))], [30.8, 29.6 (2 × CH_2, C(5), C(8))], [66.8, 69.2 (2 × CH, C(1), C(4))], [76.5, 78.3 (2 × CH, C(2), C(3))], 108.4 (C, C(9)), [126.6, 129.1 (2 × CH, C(6), C(7))]; *m/z* (TOF ES+) 351.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 351.1958 ([M+Na]$^+$) $C_{17}H_{32}NaO_4Si$ requires 351.1968.

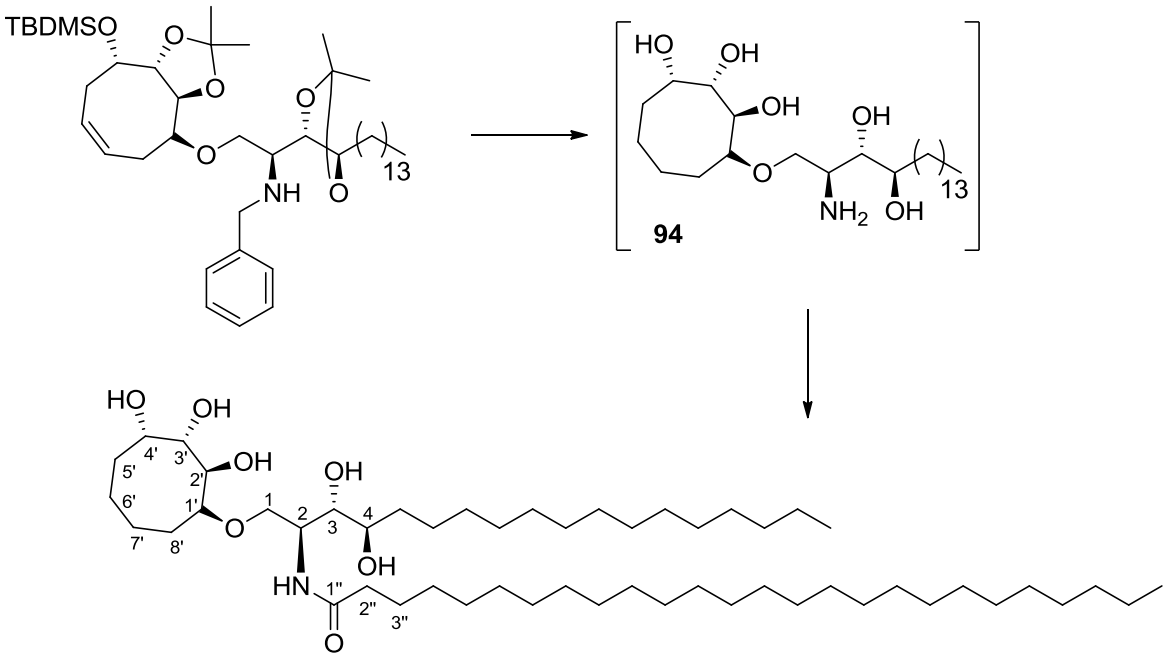
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-benzylamino-1-*O*-[4'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-2',3',4'-trihydroxycyclooct-6'-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (93)



NaH (60% wt in mineral oil, 83 mg, 2.07 mmol) was added to a solution of alcohol **92** (225 mg, 0.64 mmol) in DMF (2 mL) and THF (1 mL) at 0 °C. After stirring for 1 h, a solution of sulfamidate **80** (419 mg, 0.82 mmol) in THF (1 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated and the residue was dissolved in Et_2O (10 mL). A 20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 20 min before being neutralised with K_2CO_3 (1 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) were added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give ether **93** as a colourless oil (349 mg, 72%): R_f = 0.77 (20% EtOAc in hexanes); $[\alpha]_D^{21}$ = 119.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3600, 2925 s, 2854 s, 1612 w, 1512 m, 1461 m, 1366 m, 1247 s, 1217 m, 1170 m, 1115 m, 1005 m, 874 m, 834 s, 776 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (3H, 1 × Si(CH_3)$_2$), 0.02 (3H, 1 × Si(CH_3)$_2$), 0.76-0.86 (12H, stack, CH_2(CH_3), C(CH_3)$_3$), 1.15-1.21 (26H, stack, CH_2 resonances in alkyl chain), 1.22 (3H,

s, C(C*H*$_3$)$_2$), 1.23 (3H, s, C(C*H*$_3$)$_2$), 1.29 (3H, s, C(C*H*$_3$)$_2$), 1.30 (3H, s, C(C*H*$_3$)$_2$), 2.14-2.34 (4H, m, C(5')*H,* C(8')*H*), 2.65-2.70 (1H, m, C(2)*H*), 3.62 (1H, A of AB, $J_{A\text{-}B}$ = 12.7, C*H*$_a$H$_b$Ph), 3.75-3.80 (3H, stack, C(1)*H*$_a$*H*$_b$, C(1')*H* or C(4')*H*), 3.81 (1H, B of AB, $J_{B\text{-}A}$ = 12.7, CH$_a$*H*$_b$Ph), 3.98-4.10 (3H, stack, C(4')*H* or C(1')*H*, C(3)*H*, C(4)*H*), 4.18-4.27 (2H, stack, C(2')*H*, C(3')*H*), 5.48-5.64 (2H, stack, C(6')*H*, C(7')*H*), 7.13-7.27 (5H, stack, Ph), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−4.9, −4.2 (2 × CH_3, Si(*C*H$_3$)$_2$)], 14.1 (CH_3, CH_2*C*H_3), 18.2 (C, SiC(CH$_3$)$_3$), 22.7 (CH_2), 26.01 (CH_3, 1 × C(*C*H$_3$)$_2$), 26.02 (CH_3, SiC(*C*H$_3$)$_3$), 26.1 (CH_2), 27.2 (CH_3, 1 × C(*C*H$_3$)$_2$), 27.5 (CH_3, 1 × C(*C*H$_3$)$_2$), 28.4 (CH_3, 1 × C(*C*H$_3$)$_2$), 28.5 (CH_2, C(5') or C(8')), [29.4, 29.5, 29.7 (CH_2, broad stack, alkyl chain resonances)], 31.9 (CH_2), 32.0 (CH_2, C(8') or C(5')), 51.3 (CH_2, NH*C*H_2), 56.8 (CH, C(2)), 68.4 (CH_2, C(1)), 68.5 (CH, C(4)), 76.7 (CH, C(3)), 77.2 (CH, C(1') or C(4')), [77.7, 77.9 (CH, C(2'), C(3'))], 78.3 (CH, C(4') or C(1')), 107.4 (C, *C*(CH$_3$)$_2$), 108.7 (C, *C*(CH$_3$)$_2$), 126.9 (CH, Ph), 127.0 (CH, Ph), 127.5 (CH, C*H*=CH), 128.3 (CH, C*H*=CH), 128.4 (CH, Ph), 140.7 (C, *ipso* Ph); *m/z* (TOF ES+) 758.7 ([M+H]$^+$, 100%); HRMS *m/z* (TOF ES+) 755.5762 ([M+H]$^+$) $C_{45}H_{80}NO_6Si$ requires 758.5755.

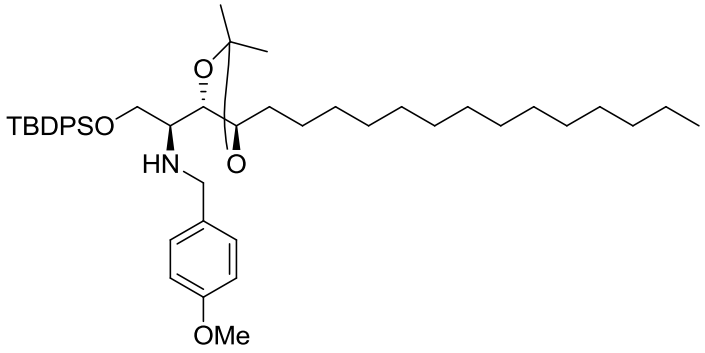
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-hexacosanoylamino-1-*O*-[2',3',4'-trihydroxycycloheptyl]-1,3,4-octadecanetriol (45)



A 1.0 M solution of hydrochloric acid (53 µL, 0.053 mmol) and Pd/C (10% wt, 11 mg, 0.011 mmol) were added to a solution of ether **93** (40 mg, 0.053 mmol) and cyclohexene (1 mL) in MeOH (5 mL). After stirring overnight at reflux, the reaction mixture was cooled to rt and diluted with a 5:1 solution of $CHCl_3$: MeOH (30 mL), before being filtered through a bed of Celite. The filtrate was concentrated under reduced pressure to provide the crude amine **94 [**R_f = 0.40 (10% MeOH in $CHCl_3$)], which was used directly in the next step. $(COCl)_2$ (2 mL) was added to hexacosanoic acid (25 mg, 0.064 mmol) and heated at 70 °C for 2 h, after which time the solution was cooled to rt, and the $(COCl)_2$ removed under a stream of dry argon. The residual volatiles were removed under reduced pressure. The resulting crude acyl chloride was dissolved in THF (0.5 mL) and added, with vigorous stirring, to a solution of amine **94** (0.053 mmol assuming complete conversion in previous step) in THF/$NaOAc_{(aq)}$ (8 M) (1:1, 2 mL). Vigorous stirring was maintained for 2 h, after which time the reaction mixture was left to

stand and the layers were separated. The aqueous layer was extracted with THF (3 × 2.0 mL) and the organic layers were combined and concentrated under reduced pressure. The crude product was purified by column chromatography (10% MeOH in $CHCl_3$) to give amide **45** as a white solid (19 mg, 42% over two steps): R_f = 0.40 (10% MeOH in $CHCl_3$); mp = 106–112 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3353 br w, 2917 s, 2850 s, 1722 w, 1626 m, 1542 w, 1464 m, 1267 w, 1075 s, 980 w, 936 w, 890 w, 769 w, 718 m; ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD, 2 : 1, 40 °C) δ ppm 0.84 (6H, t, *J* = 6.8, 2 × CH_2CH_3), 1.19-1.30 (66H, stack), 1.33-1.38 (1H, m), 1.38-1.45 (2H, stack), 1.45-1.53 (1H, m), 1.53-1.69 (8H, stack, C(5')H_aH_b, C(8')H_aH_b, C(3'')H_aH_b), 1.69-1.79 (2H, stack, C(5')H_aH_b, C(8')H_aH_b), 2.16 (2H, app t, *J* = 7.5, C(2'')H_aH_b), 3.49-3.55 (2H, stack, C(3)*H*, C(4)*H*), 3.62-3.71 (3H, stack, C(1')*H*, C(1)H_aH_b), 3.79 (1H, dd, *J* = 8.0, 2.5, C(3')*H*), 3.84 (1H, dd, *J* = 8.0, 2.2, C(2')*H*), 3.91-3.97 (1H, m, C(4')*H*), 4.09-4.16 (1H, resonance coincident with solvent, C(2)*H*), exchangeable hydrogens not observed; ^{13}C NMR (500 MHz, $CDCl_3$: CD_3OD, 2 : 1, 40 °C) δ ppm 14.2 (CH_3), 22.9 (CH_2), 23.0 (CH_2), 23.1 (CH_2), 26.3 (2 × CH_2), 28.1 (CH_2, C(8')), [29.7, 29.8, 29.9, 30.11, 30.12, 30.2 (CH_2, resonance overlap)], 30.4 (CH_2, C(5)), 32.3 (CH_2), 32.9 (CH_2), 36.9 (CH_2, C(2'')), 50.6 (CH, C(2)), 69.1 (CH_2, C(1)), 71.3 (CH, C(2')), 71.6 (CH, C(4')), 72.6 (CH, C(3')), 72.9 (CH, C(4)), 75.4 (CH, C(3)), 80.9 (CH, C(1')), 174.7 (C, C(1'')); *m/z* (TOF ES+) 876.6 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 876.7629 ($[M+Na]^+$) $C_{52}H_{103}NNaO_7$ requires 876.7632.

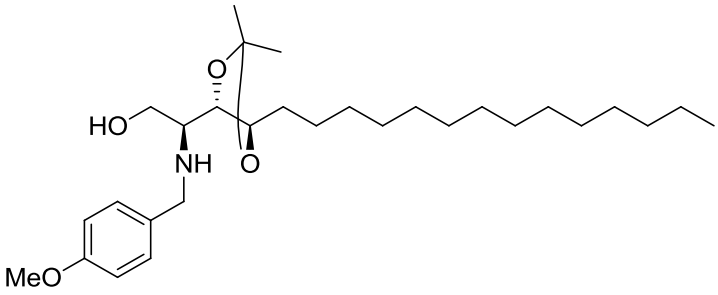
(2*R*,3*R*,4*S*)-2-*p*-methoxybenzylamino-1-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (106)



Anisaldehyde (427 µL, 3.49 mmol) was added to a stirred suspension of amine **76** (1.73 g, 2.91 mmol) and $NaBH(OAc)_3$ (1.85 g, 8.73 mmol) in THF (15 mL). After stirring overnight, the reaction mixture was diluted with Et_2O (20 mL) and $NaHCO_3$ solution (20 mL). The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 20 mL). The organic layers were combined and washed with brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (5% EtOAc in hexanes) to give *p*-methoxybenzyl amine **106** as a colourless oil (1.25 g, 60%): R_f = 0.64 (10% EtOAc in hexanes); $[\alpha]_D^{20}$ = 46.8 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2924 s, 2853 s, 1612 w, 1589 w, 1512 s, 1463 m, 1428 m, 1377 m, 1366 m, 1301 w, 1246 s, 1217 m, 1172 m, 1111 s, 1082 s, 1040 s, 998 w, 938 w, 878 w, 822 m, 740 m, 702 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.80 (3H, t, *J* = 6.9, CH_2CH_3), 0.98 (9H, s, $C(CH_3)_3$), 1.17-1.21 (26H, stack, CH_2 resonances in alkyl chains), 1.28 (3H, s, 1 × $C(CH_3)_2$), 1.30 (3H, s, 1 × $C(CH_3)_2$), 2.56-2.62 (1H, m, C*H*NH), 3.44 (1H, A of AB, $J_{A\text{-}B}$ = 12.4, CH_aH_bAr), 3.70 (1H, B of AB, $J_{B\text{-}A}$ = 12.4, CH_aH_bAr), 3.71 (3H, s, OCH_3), 3.81-3.84 (2H, stack, CH_2O), 4.06-4.17 (2H, stack), 6.76 (2H, d, *J* = 8.6, Ar), 7.11 (2H, d, *J* = 8.6, Ar), 7.26-7.38 (6H, stack, Ph), 7.59-7.66 (4H, stack, Ph), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2 (CH_3, CH_2CH_3), 19.4 (C, $SiC(CH_3)_3$), 22.7 (CH_2), 26.0 (CH_3, 1 ×

C(CH_3)$_2$), 26.1 (CH_2), 26.9 (CH_3, SiC(CH_3)$_3$), 28.5 (CH_3, 1 × C(CH_3)$_2$), [29.4, 29.6, 29.8 (CH_2, broad stack, significant resonance overlap)], 32.0 (CH_2), 50.4 (CH_2, CH_2NH), 55.2 (CH_3, OCH_3), 57.0 (CH, *C*HNH), 60.2 (CH_2, CH_2O), 76.3 (CH, *C*HO), 78.4 (CH, *C*HO), 107.4 (C, *C*(CH_3)$_2$), 113.7 (CH, Ar), 127.6 (CH, Ph), 127.7 (CH, Ph), 129.61 (CH, Ar), 129.62 (CH, Ph), 129.7 (CH, Ph), 132.7 (C, *ipso* Ph), 133.4 (C, *ipso* Ph), 133.7 (C, *ipso* Ph), 135.6 (CH, Ph), 135.7 (CH, Ph), 158.7 (C, *C*OCH$_3$); *m/z* (TOF ES+) 716.5 ([M+H]$^+$, 100%); HRMS *m/z* (TOF ES+) 716.5079 ([M+H]$^+$) $C_{45}H_{70}NO_4Si$ requires 716.5074.

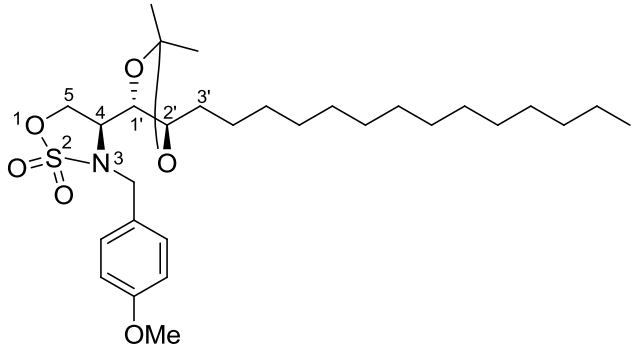
(2*R*,3*R*,4*S*)-2-*p*-methoxybenzylamino-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (107)



TBAF (1.0 M solution in THF, 2.66 mL, 2.66 mmol) was added to a solution of silyl ether **106** (950 mg, 1.33 mmol) in THF (20 mL). The reaction mixture was stirred overnight before being quenched with H_2O (15 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (25% EtOAc in hexanes) to provide alcohol **107** as a colourless oil (623 mg, 98%): R_f = 0.24 (25% EtOAc in hexanes); $[\alpha]_D^{20}$ = 40.0 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3386 br w, 2922 s, 2852 s, 1612 m, 1512 s, 1464 m, 1368 m, 1301 w, 1246 s, 1172 m, 1040 m, 873 m, 822 m; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.90 (3H, t, *J* = 6.9,

CH_2CH_3), 1.26-1.32 (26H, stack, CH_2 resonances in alkyl chains), 1.35 (3H, s, 1 × C(CH_3)$_2$), 1.43 (3H, s, 1 × C(CH_3)$_2$), 2.24 (1H, br s, O*H*), 2.73-2.78 (1H, m, C*H*NH), 3.66-3.85 (4H, stack), 3.81 (3H, s, OCH_3), 4.05 (1H, dd, *J* = 8.2, 5.9), 4.14-4.20 (1H, m), 6.87 (2H, d, *J* = 8.6, Ar), 7.24 (2H, d, *J* = 8.6, Ar), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.7 (CH_3, 1 × C(CH_3)$_2$), 26.4 (CH_2). 28.0 (CH_3, 1 × C(CH_3)$_2$), [29.4, 29.71, 29.72 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 50.4 (CH_2, CH_2NH), 55.2 (CH_3, OCH_3), 56.9 (CH, *CH*NH), 61.1 (CH_2, CH_2OH), 78.0 (CH, CHO), 78.1 (CH, CHO), 107.8 (C, *C*(CH_3)$_2$), 113.8 (CH, Ar), 129.5 (CH, Ar), 132.2 (C, *ipso* Ar), 158.8 (C, *C*OCH_3); *m/z* (TOF ES+) 478.7 ([M+H]$^+$, 100%); HRMS *m/z* (TOF ES+) 500.3731 ([M+Na]$^+$) $C_{29}H_{51}NNaO_4$ requires 500.3716.

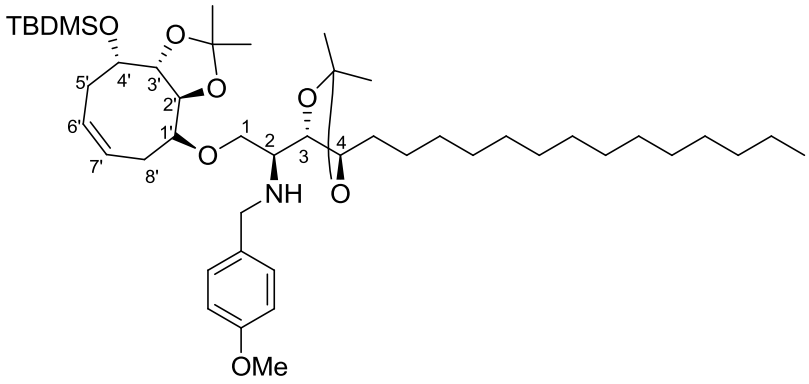
(3*R*,1'*S*,2'*S*)-3-*p*-methoxybenzyl-4-[1',2'-*O*-isopropylidene-dihydroxyhexadecyl]-1,2,3-oxathiazolidine-2,2-dioxide (109)



A solution of amine **107** (1.40 g, 2.94 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 30 min to a solution of $SOCl_2$ (235 µL, 3.23 mmol), imidazole (800 mg, 11.76 mmol) and NEt_3 (902 µL, 6.47 mmol) in CH_2Cl_2 (15 mL) at −50 °C. The reaction mixture was warmed to 0 °C and stirred for 21 h, before adding H_2O (15 mL). The organic layer was isolated and washed with brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give the crude sulfamidite as a mixture of diastereoisomers [R_f = 0.92 (30% EtOAc in hexanes)], which was used immediately in the next step: $NaIO_4$ (691 mg, 3.23 mmol), $RuCl_3$ (60 mg, 0.29

mmol) and H_2O (5 mL) were added sequentially to a solution of the crude sulfamidite in MeCN (5 mL) at 0 °C. After 2.5 h, the reaction mixture was diluted with H_2O (50 mL) and Et_2O (50 mL). The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give sulfamidate **109** as a colourless oil (873 mg, 55%): R_f = 0.33 (10% EtOAc in hexanes); $[\alpha]_D^{20}$ = 11.6 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1612 w, 1514 m, 1465 w, 1370 w, 1305 w, 1251 s, 1186 s, 1034 m, 834 m, 735 s, 703 m; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.83 (3H, t, *J* = 6.8, CH_2CH_3), 1.16-1.23 (26H, stack, CH_2 resonances in alkyl chains), 1.24 (3H, s, 1 × C(CH_3)$_2$), 1.34 (3H, s, 1 × C(CH_3)$_2$), 3.56-3.62 (1H, m, CHN), 3.71 (3H, s, OCH_3), 3.97-4.03 (1H, m), 4.16 (1H, app t, *J* = 6.0), 4.22 (1H, app t, *J* = 8.0), 4.28 (2H, br s, CH_2N), 4.52 (1H, dd, *J* = 8.7, 3.6), 6.82 (2H, d, *J* = 8.6, Ar), 7.28 (2H, d, *J* = 8.6, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.1 (CH_3, 1 × C(CH_3)$_2$), 26.5 (CH_2), 27.4 (CH_3, 1 × C(CH_3)$_2$), [29.4, 29.5, 29.7, 29.9 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 51.9 (CH_2, CH_2N), 55.2 (CH_3, OCH_3), 58.2 (CH, CHN), 68.1 (CH_2, CH_2O), 75.8 (CH, CHO), 76.8 (CH, CHO), 108.2 (C, C(CH_3)$_2$), 114.2 (CH, Ar), 126.3 (C, *ipso* Ar), 130.3 (CH, Ph), 159.8 (C, $COCH_3$); *m/z* (TOF ES+) 562.7 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 562.3184 ($[M+Na]^+$) $C_{29}H_{49}NaNO_6S$ requires 562.3178.

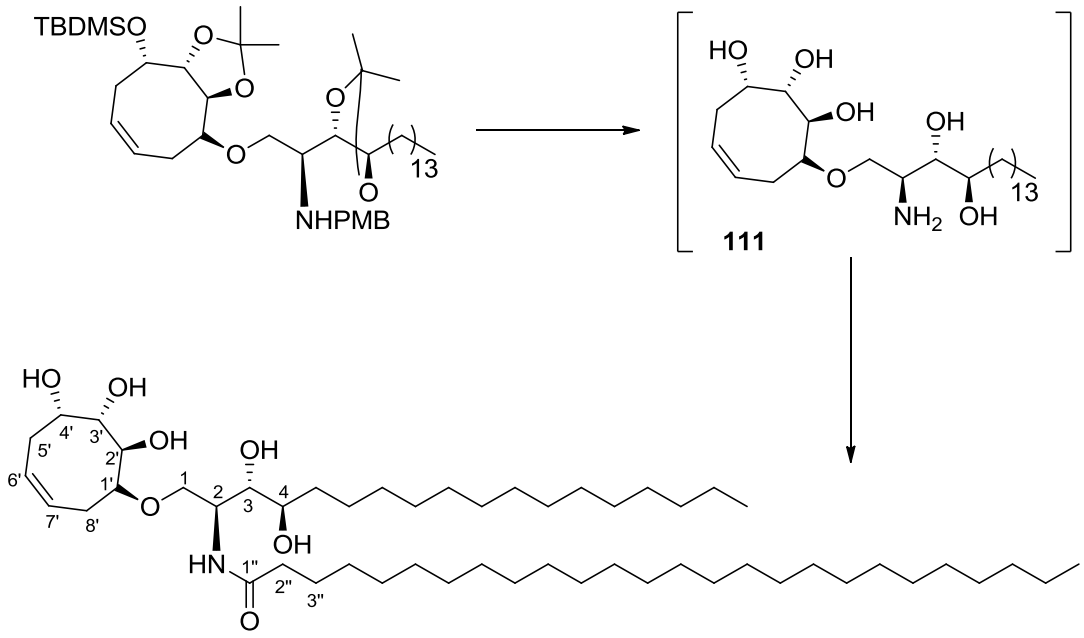
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[4'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-2',3',4'-trihydroxycyclooct-6'-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (110)



NaH (60% wt in mineral oil, 80 mg, 2.01 mmol) was added to a solution of alcohol **92** (220 mg, 0.67 mmol) in DMF (2 mL) and THF (1 mL) at 0 °C. After stirring for 1 h, a solution of sulfamidate **109** (433 mg, 0.80 mmol) in THF (1 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated and the residue was dissolved in Et_2O (10 mL). A 20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 20 min before being neutralised with K_2CO_3 (1.0 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) were added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give ether **110** as a colourless oil (396 mg, 75%): R_f = 0.62 (20% EtOAc in hexanes); ν_{max}(film)/cm^{-1} 3600 w, 2925 s, 2854 s, 1611 w, 1513 m, 1461 m, 1366 m, 1248 s, 1217 m, 1170 m, 1115 m, 1067 s, 1005 m, 874 w, 835 m, 776 m; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (3H, 1 × Si(CH_3)$_2$), 0.01 (3H, 1 × Si(CH_3)$_2$), 0.77-0.84 (12H, stack, CH_2CH_3, C(CH_3)$_3$), 1.15-1.20 (26H, stack, CH_2 resonances in alkyl chain), 1.21 (3H, s, 1 × C(CH_3)$_2$),

1.29 (3H, s, 1 × C(C*H*$_3$)$_2$), 1.30 (6H, app s, C(C*H*$_3$)$_2$), 2.14-2.33 (4H, m, C(5')*H,* C(8')*H*), 2.62-2.68 (1H, m, C(2)*H*), 3.56 (1H, A of AB, $J_{A\text{-}B}$ = 12.5, C*H*$_a$H$_b$Ar), 3.70 (3H, s, OC*H*$_3$), 3.72-3.79 (4H, stack, C(1)*H*$_a$*H*$_b$, C(1')*H* or C(4')*H*, B of AB, CH$_a$*H*$_b$Ar), 3.97-4.09 (3H, stack, C(4')*H* or C(1')*H*, C(3)*H*, C(4)*H*), 4.20 (1H, dd, *J* = 8.0, 2.5, C(2')*H* or C(3')*H*), 4.26 (1H, dd, *J* = 8.0, 2.8, C(3')*H* or C(2')*H*), 5.48-5.61 (2H, stack, C(6')*H*, C(7')*H*), 6.76 (2H, d, *J* = 8.6, Ar), 7.14 (2H, d, *J* = 8.6, Ar), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−4.9, −4.2 (2 × CH_3, Si(*C*H$_3$)$_2$)], 14.1 (CH_3, CH_2*C*H$_3$), 18.2 (C, Si*C*(CH$_3$)$_3$), 22.7 (CH_2), 26.0 (CH_3, 1 × C(*C*H$_3$)$_2$, SiC(*C*H$_3$)$_3$, resonance overlap), 26.1 (CH_2), 27.2 (CH_3, 1 × C(*C*H$_3$)$_2$), 27.6 (CH_3, 1 × C(*C*H$_3$)$_2$), 28.4 (CH_3, 1 × C(*C*H$_3$)$_2$), 28.5 (CH_2, C(5') or C(8')), [29.4, 29.5, 29.7 (CH_2, broad stack, alkyl chain resonances)], 31.91 (CH_2), 31.93 (CH_2, C(8') or C(5')), 50.6 (CH_2, NH*C*H$_2$), 55.2 (CH_3, O*C*H$_3$), 56.6 (CH, C(2)), 68.3 (CH_2, C(1)), 68.5 (CH, C(1') or C(4')), 76.7 (CH, C(3) or C(4)), 77.2 (CH, C(4') or C(1')), 77.7 (CH, C(2') or C(3')), 77.9, (CH, C(3') or C(2')), 78.3 (CH, C(4) or C(3)), 107.4 (C, *C*(CH$_3$)$_2$), 108.7 (C, *C*(CH$_3$)$_2$), 113.7 (CH, Ar), 127.5 (CH, C*H*=CH), 128.4 (CH, C*H*=CH), 129.5 (CH, Ar), 132.7 (C, *ipso* Ar), 158.7 (C, *C*OCH$_3$); *m/z* (TOF ES+) 788.6 ([M+H]$^+$, 100%); HRMS *m/z* (TOF ES+) 788.5853 ([M+H]$^+$) $C_{46}H_{82}NO_7Si$ requires 788.5861.

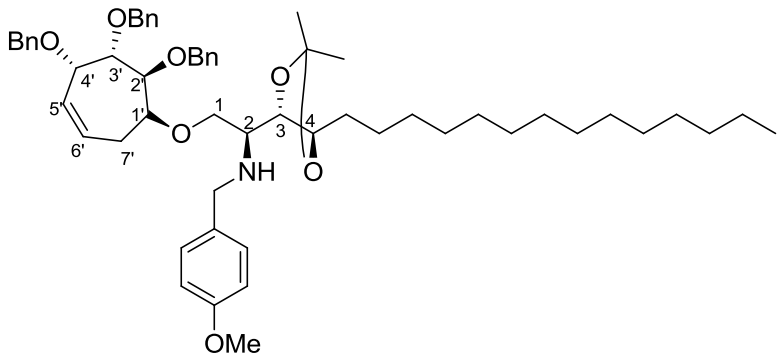
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-hexacosanoylamino-1-*O*-[2',3',4'-trihydroxycyclohept-6'-enyl]-1,3,4-octadecanetriol (97)



CAN (1.08 g, 1.97 mmol) was added to a solution of ether **110** (310 mg, 0.39 mmol) in a 2:1 mixture of MeCN/H_2O (6 mL). After stirring for 3 h, another portion of CAN (1.08 g, 1.97 mmol) was added. After 2 h, a solution of hydrochloric acid (0.5 M, 10 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL). The aqueous layer was then basified with 2 M NaOH solution until a purple precipitate formed, and then extracted with EtOAc (3 × 20 mL). The two organic layers were analysed by TLC, and if both contained the product, they were combined before being washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to provide the crude amine **111**, which was used directly in the next step: $(COCl)_2$ (2 mL) was added to hexacosanoic acid (38 mg, 0.10 mmol). The resulting mixture was heated at 70 °C for 2 h, after which time the solution was cooled to rt, and the $(COCl)_2$ removed under a stream of dry argon. The residual volatiles were removed under reduced pressure. The resulting crude acid chloride was dissolved in THF (0.5 mL) and

added, with vigorous stirring, to a solution of amine **97** (38 mg, 0.08 mmol) in THF/$NaOAc_{(aq)}$ (8 M) (1:1, 2 mL). Vigorous stirring was maintained for 2 h, after which time the reaction mixture was left to stand and the layers were separated. The aqueous layer was extracted with THF (3 × 2.0 mL) and the organic layers were combined and concentrated under reduced pressure. The crude product was purified by column chromatography (10% MeOH in $CHCl_3$) to give amide **xx** as a white solid (19 mg, 28%): R_f = 0.65 (10% MeOH in $CHCl_3$); mp = 123–131 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3320 br w, 2918 s, 2850 s, 1722 w, 1631 w, 1546 w, 1467 m, 1267 w, 1070 s, 779 w, 717 s; ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD, 2 : 1, 40 °C) δ ppm 0.83 (6H, t, *J* = 6.7, 2 × CH_2CH_3), 1.11-1.32 (68H, stack), 1.42-1.67 (4H, stack), 2.13-2.20 (2H, app t, *J* = 7.0, C(2'')H_aH_b), 2.29-2.39 (4H, stack, C(5')H_aH_b, C(8')H_aH_b), 3.44-3.55 (2H, stack, C(3)*H*, C(4)*H*), 3.66-3.71 (1H, m, C(1)H_aH_b), 3.80-3.83 (2H, stack, C(1)H_aH_b, C(1')*H*), 3.85-3.93 (2H, stack, C(2')*H*, C(3')*H*), 3.98-4.04 (1H, m, C(4')*H*), 4.10-4.17 (1H, m, C(2)*H*), 5.51-5.68 (2H, stack, C(6')*H*, C(7')*H*), exchangeable resonances not observed; ^{13}C NMR (500 MHz, $CDCl_3$: CD_3OD, 2 : 1, 40 °C) δ ppm 14.2 (CH_3), 23.0 (CH_2), 26.2 (CH_2), 26.3 (CH_2), 27.8 (CH_2, C(8')), [29.71, 29.73, 29.8, 29.9, 30.0, 30.1 (CH_2, resonance overlap)], 30.4 (CH_2, C(5)), 32.3 (CH_2), 33.0 (CH_2), 36.9 (CH_2, C(2'')), 50.3 (CH, C(2)), 68.8 (CH, C(2')), 68.9 (CH, C(3')), 69.4 (CH_2, C(1)), 71.1 (CH, C(4')), 72.7 (CH, C(3) or C(4)), 75.4 (CH, C(4) or C(3)), 80.9 (CH, C(1')), 128.0 (CH, C(7')), 128.8 (CH, C(6')), 174.6 (C, *C*=O); *m/z* (TOF ES+) 874.9 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 874.7474 ($[M+Na]^+$) $C_{52}H_{101}NNaO_7$ requires 874.7476.

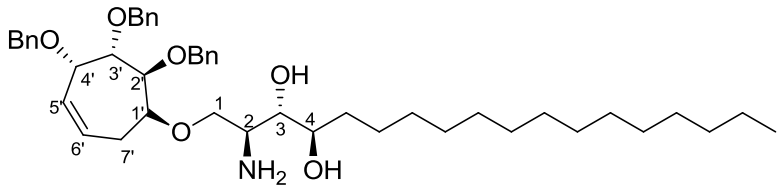
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[2',3',4'-*O*-benzyl-trihydroxycyclohept-5'-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (112)



NaH (60% wt in mineral oil, 126 mg, 3.15 mmol) was added to a solution of alcohol **64** (450 mg, 1.05 mmol) in DMF (2 mL) and THF (1 mL) at 0 °C. After stirring for 1 h, a solution of sulfamidate **109** (677 mg, 1.26 mmol) in THF (1 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in Et_2O (10 mL). A 20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 20 min before being neutralised with K_2CO_3 (1 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) were added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give ether **112** as a colourless oil (420 mg, 45%): R_f = 0.56 (20% EtOAc in hexanes); ν_{max}(film)/cm^{-1} 2922 s, 2853 s, 1743 w, 1496 w, 1454 m, 1377 m, 1367 m, 1241 m, 1216 m, 1172 w, 1127 s, 1090 s, 1067 s, 1027 s, 873 w, 833 m, 778 m, 733 s, 696 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.79 (3H, t, *J* = 6.8, CH_2CH_3), 1.14-1.22 (26H, stack, CH_2 resonances in alkyl chains), 1.23 (3H, s, 1 × $C(CH_3)_2$), 1.30 (3H, s, 1 × $C(CH_3)_2$), 1.99-2.08 (1H, m, C(7')H_aH_b), 2.60-2.70 (2H, stack, C(2)*H*, C(7')H_aH_b), 3.39-3.59 (3H,

stack, NHCH_aH$_b$, C(1)H_aH$_b$, C*H*O), 3.64-3.69 (4H, stack, OCH_3, C(1)H$_a$$H_b$), 3.70-3.79 (2H, stack, NHCH$_a$$H_b$, C(3')*H*), 3.82-3.96 (2H, stack, C(2')*H*, C*H*O), 4.00-4.08 (1H, m, C*H*O), 4.30-4.52 (4H, stack, CH_2Ph, 2 × CH_aH$_b$Ph), 4.55 (1H, s, C(4)*H*), 4.62-4.69 (2H, stack, 2 × CH$_a$$H_b$Ph), 5.67-5.72 (2H, stack, C(5')*H*, C(6')*H*), 6.73 (2H, d, *J* = 8.6), 7.09-7.26 (17H, stack, Ph), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2 (CH_3, CH_2CH_3), 22.8 (CH_2), 26.1 (CH_3, 1 × C(CH_3)$_2$), 26.3 (CH_2), 27.2 (CH_2, C(7')), 28.5 (CH_3, 1 × C(CH_3)$_2$), [29.4, 29.6, 29.8 (CH_2, broad stack, significant resonance overlap)], 32.0 (CH_2), 50.6 (CH_2, CH_2NH), 55.2 (CH_3, OCH_3), 56.1 (CH, C(2)), 66.3 (CH_2, C(1)), 71.2 (CH_2, CH_2Ph), 72.8 (CH_2, CH_2Ph), 73.8 (CH_2, CH_2Ph), 76.7 (CH, C(4)) 76.9 (CH, *C*HO), 77.0 (CH, *C*HO), 78.4 (CH, *C*HO), 78.6 (CH, *C*HO), 79.3 (CH, *C*HO), 107.4 (C, *C*(CH_3)$_2$), 113.8 (CH, Ar), 125.7 (CH, C*H*=CH), [127.5, 127.6, 127.8, 128.3 (CH, resonance overlap, Ph)], 132.9 (CH, Ar), 132.7 (C, *ipso* Ar), 133.8 (CH, C*H*=CH), 138.7 (C, *ipso* Ph), 138.9 (C, *ipso* Ph), 139.1 (C, *ipso* Ph), 158.8 (C, *C*OCH_3); *m/z* (TOF ES+) 890.7 ([M+H]$^+$, 100%); HRMS *m/z* (TOF ES+) 890.5921 ([M+H]$^+$) $C_{57}H_{80}NO_7$ requires 890.5935.

(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-amino-1-*O*-[2',3',4'-trihydroxycycloheptanyl]-1,3,4-octadecantriol (113)

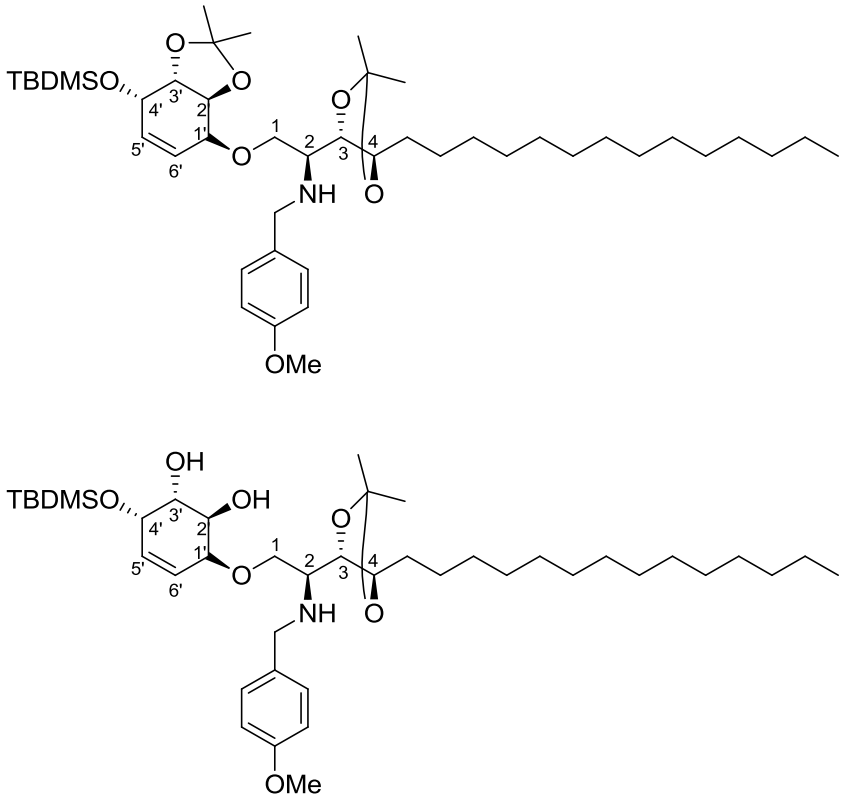


CAN (986 mg, 1.80 mmol) was added to a solution of ether **112** (320 mg, 0.36 mmol) in a 2:1 solution of MeCN/H_2O (6 mL). After stirring for 3 h, hydrochloric acid (0.5 M, 10 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL). The aqueous layer was then

basified with 2 M NaOH solution until a purple precipitate formed, and then extracted with EtOAc (3 × 20 mL). The two organic layers were analysed by TLC, and if both contained the product, they were combined before being washed with brine, dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% MeOH in chloroform) to give ether **113** as a colourless oil (80 mg, 30%): R_f = 0.22 (10% MeOH in chloroform); ν_{max}(film)/cm^{-1} 3600 w, 2922 s, 2853 s, 1743 w, 1496 w, 1454 m, 1377 m, 1367 m, 1241 m, 1216 m, 1172 w, 1127 s, 1090 s, 1067 s, 1027 s, 873 w, 833 m, 778 m, 733 s, 696 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.79 (3H, t, *J* = 6.9, CH_2CH_3), 1.11-1.20 (24H, stack, CH_2 resonances in alkyl chains), 1.33-1.43 (1H, m, CH_aH$_b$ from alkyl chain), 1.54-1.64 (1H, m, CH$_a$$H_b$ from alkyl chain), 2.04-2.14 (1H, m, C(7')H_aH$_b$), 2.45-2.56 (1H, m, C(7')H$_a$$H_b$), 3.32-3.41 (1H, m, C(3)*H*), 3.47-3.62 (4H, stack, C(1)H_aH$_b$, C(2)*H*, C(4)*H*, C(1')*H*), 3.69-3.80 (2H, stack, C(1)H$_a$$H_b$, C(3')*H*), 3.85 (1H, dd, *J* = 6.3, 1.4, C(2')*H*), 4.27 (1H, A of AB, $J_{A\text{-}B}$ = 12.2, CH_aH$_b$Ph), 4.39-4.44 (1H, m, C(4')*H*), 4.45-4.53 (4H, stack, CH_2Ph, 2 × CH$_a$$H_b$Ph), 4.62 (1H, A of AB, $J_{A\text{-}B}$ = 12.1, CH_aH$_b$Ph), 5.60-5.70 (2H, stack, C(5')*H*, C(6')*H*), 7.09-7.26 (15H, stack, Ph), NH_2 not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.8 (CH_2), 26.7 (CH_2, C(7')), [29.4, 29.7, 29.8 (CH_2, broad stack, significant resonance overlap)], 32.0 (CH_2), 34.1 (CH_2), 53.8 (CH, C(2)), 66.0 (CH_2, C(1)), 71.1 (CH_2, CH_2Ph), 72.5 (CH, C(4)), 72.9 (CH_2, CH_2Ph), 73.2 (CH, C(3)), 73.5 (CH_2, CH_2Ph), 76.1 (CH, C(4')) 77.4 (CH, C(1')), 78.6 (CH, C(3')), 79.4 (CH, C(2')), 126.4 (CH, C*H*=CH), [127.5, 127.6, 127.8, 127.9, 128.0, 128.3, 128.4 (CH, resonance overlap, Ph)], 132.8 (CH, C*H*=CH), 138.3 (C, *ipso* Ph), 138.5 (CH,*ipso* Ph), 138.6 (C, *ipso* Ph); *m/z* (TOF ES+) 730.7 ([M+H]$^+$, 100%); HRMS *m/z* (TOF ES+) 730.5067 ([M+H]$^+$) $C_{46}H_{68}NO_6$ requires 730.5047.

(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[4'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-2',3',4'-trihydroxycyclohex-5-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (115) and

(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[4'-*O*-*tert*-butyldimethylsilyl -2',3',4'-trihydroxycyclohex-5-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (116)

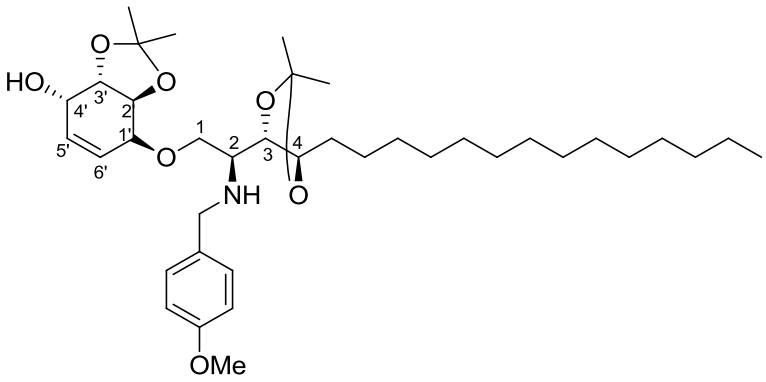


NaH (60% wt in mineral oil, 128 mg, 3.21 mmol) was added to a solution of alcohol **86** (320 mg, 1.07 mmol) in DMF (5 mL) and THF (2 mL) at 0 °C. After stirring for 1 h, a solution of sulfamidate **109** (632 mg, 1.17 mmol) in THF (3 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated and the residue was dissolved in Et_2O (10 mL). A 20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 20

min before being neutralised with K_2CO_3 (1.0 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) were added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give an inseparable mixture of ether **115** and sulfamidate **109** as a colourless oil, which was used directly in the next step (160 mg, 21%) [R_f = 0.59 (20% EtOAc in hexanes)] and ether **116** as a light yellow oil (220 mg, 29%): R_f = 0.12 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ = 77.4 (c = 0.8, $CHCl_3$); ν_{max}(film)/cm^{-1} 3457 br w, 2924 s, 2852 s, 1612 w, 1586 w, 1512 s, 1463 m, 1369 m, 1301 m, 1246 s, 1218 m, 1172 m, 1124 m, 1076 s, 1033 s, 939 m, 874 s, 835 s, 777 s, 722 w, 661 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm −0.001 (6H, app s, Si(CH_3)$_2$), 0.75 (3H, t, *J* = 6.8, CH_2CH_3), 0.79 (9H, s, SiC(CH_3)$_3$), 1.10-1.16 (26H, stack, CH_2 resonances in alkyl chains), 1.20 (3H, s, 1 × C(CH_3)$_2$), 1.28 (3H, s, 1 × C(CH_3)$_2$), 2.59-2.65 (1H, m, C(2)*H*), 3.50 (1H, A of AB, $J_{A\text{-}B}$ = 12.6, CH_2Ar), 3.57-3.65 (2H, stack, C(1)), 3.66 (3H, s, OCH_3), 3.71 (1H, B of AB, $J_{B\text{-}A}$ = 12.6, CH_2Ar), 3.88-3.96 (3H, stack, C(2')*H*, C(4')*H*, C(3)*H*), 4.01-4.07 (1H, m, C(4)*H*), 4.20-4.25 (1H, m, C(3')*H*), 4.31-4.35 (1H, m, C(1')*H*), 5.44-5.55 (2H, stack, C(5')*H*, C(6')*H*), 6.72 (2H, d, *J* = 8.6, Ar), 7.10 (2H, d, *J* = 8.6, Ar), exchangeable protons not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm −4.61 (CH_3, 1 × Si(*C*H_3)$_2$), −4.63 (CH_3, 1 × Si(*C*H_3)$_2$), 14.0 (CH_3, CH_2*C*H_3), 18.0 (C, Si*C*(CH$_3$)$_3$), 22.6 (CH_2), 25.4 (CH_3, 1 × C(*C*H_3)$_2$), 25.7 (CH_3, SiC(*C*H_3)$_3$), 26.7 (CH_2), 27.8 (CH_3, 1 × C(*C*H_3)$_2$), [29.3, 29.7 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 50.3 (CH_2, NH*C*H_2), 55.2 (CH_3, O*C*H_3), 55.8 (CH, C(2)), 65.0 (CH_2, C(1)), 67.1 (CH, C(1')), 67.5 (CH, C(3')), 70.7 (CH, C(2') or C(4')), 73.5 (CH, C(4') or C(2')), 77.0 (CH, C(3)), 78.1 (CH, C(4)), 107.5 (C, *C*(CH$_3$)$_2$), 113.7 (CH, Ar), 127.2 (CH, C(5) or C(6)), 129.3 (CH, C(6) or C(5)), 129.5 (CH, Ar), 132.0

(C, *ipso* Ar)), 158.7 (C, *C*OCH_3); *m/z* (TOF ES+) 742.1 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 742.5064 ($[M+Na]^+$) $C_{41}H_{73}NaNO_7Si$ requires 742.5054.

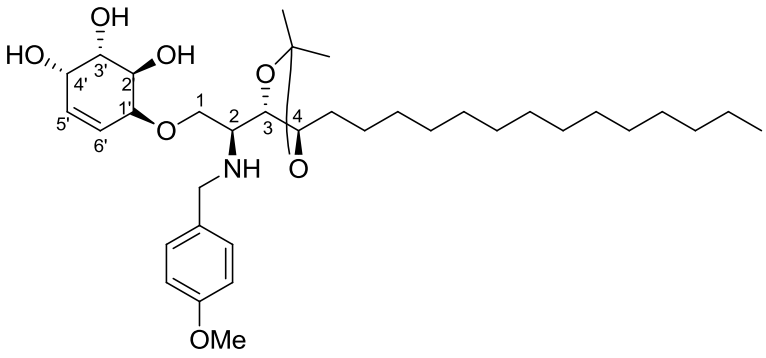
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[2',3'-*O*-isopropylidene-2',3',4'-trihydroxycyclohex-5-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (118)



TBAF (1.0 M solution in THF, 1.32 mL, 1.32 mmol) was added to a solution of silyl ether **115** (500 mg, 0.66 mmol) in THF (20 mL). The reaction mixture was stirred overnight before being quenched with H_2O (15 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (50% EtOAc in hexanes) to provide alcohol **118** as a colourless oil (341 mg, 80%): R_f = 0.40 (50% EtOAc in hexanes); $[\alpha]_D^{20}$ = 142.0 (c = 0.6, $CHCl_3$); ν_{max}(film)/cm^{-1} 3487 br w, 2984 w, 2920 s, 2851 s, 1611 w, 1585 w, 1512 m, 1464 m, 1370 m, 1333 w, 1301 w, 1244 s, 1218 s, 1170 m, 1143 m, 1127 s, 1090 s, 1058 s, 1034 s, 1017 m, 966 m, 876 w, 835 m, 798 m, 760 w, 729 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.81 (3H, t, *J* = 6.8, CH_2CH_3), 1.17-1.21 (26H, stack, CH_2 resonances in alkyl chains), 1.23 (3H, s, 1 × $C(CH_3)_2$), 1.31 (3H, s, 1 × $C(CH_3)_2$), 1.38 (6H, app s, $C(CH_3)_2$), 2.46 (1H, br s, O*H*), 2.66-2.72 (1H,

m, C(2)*H*), 3.59 (1H, A of AB, $J_{A\text{-}B}$ = 12.6, CH_2Ar), 3.71 (3H, s, OCH_3), 3.72-3.78 (2H, stack, C(1)H_aH$_b$, B of AB, CH_2Ar), 3.90-3.96 (3H, stack, C(2')*H*, C(3')*H*, C(3)*H*), 3.96-4.08 (2H, stack, C(1)H$_a$$H_b$, C(4)*H*), 4.15 (1H, dd, *J* = 4.6, 2.7, C(1')*H* or C(4')*H*), 4.44 (1H, dd, *J* = 4.3, 3.0, C(4')*H* or C(1')*H*), 5.86-5.96 (2H, stack, C(5')*H*, C(6')*H*), 6.76 (2H, d, *J* = 8.6, Ar), 7.14 (2H, d, *J* = 8.6, Ar), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.9 (CH_3, 1 × C(CH_3)$_2$), 26.2 (CH_2), 26.8 (CH_3, 1 × C(CH_3)$_2$), 26.9 (CH_3, 1 × C(CH_3)$_2$), 28.4 (CH_3, 1 × C(CH_3)$_2$), [29.4, 29.5, 29.7 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 50.6 (CH_2, NHCH_2), 55.2 (CH_3, OCH_3), 56.4 (CH, C(2)), 64.9 (CH, C(1') or C(4')), 69.7 (CH_2, C(1)), 73.2 (CH, C(4') or C(1')), 73.7 (CH, C(2') or C(3')), 74.2 (CH, C(3') or C(2')), 76.9 (CH, C(3)), 78.2 (CH, C(4)), 107.4 (C, $C(CH_3)_2$), 110.3 (C, $C(CH_3)_2$), 113.7 (CH, Ar), 129.4 (CH, Ar), 126.8 (CH, C(5) or C(6)), 129.9 (CH, C(6) or C(5)), 132.7 (C, *ipso* Ar)), 158.7 (C, $COCH_3$); *m/z* (TOF ES+) 668.7 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 668.4517 ([M+Na]$^+$) $C_{38}H_{63}NaNO_7$ requires 668.4502.

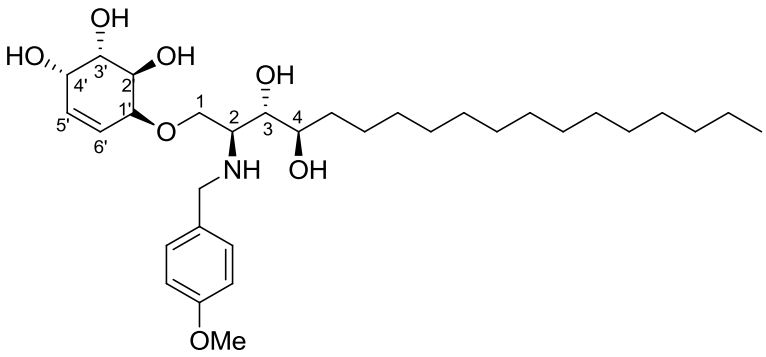
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[2',3',4'-trihydroxycyclohex-5-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (117)



TBAF (1.0 M solution in THF, 0.46 mL, 0.46 mmol) was added to a solution of silyl ether **116** (166 mg, 0.23 mmol) in THF (15 mL). The reaction mixture was stirred overnight before being

quenched with H_2O (15 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (0-5% MeOH in $CHCl_3$, gradient) to provide triol **117** as a colourless oil (130 mg, 93%): R_f = 0.30 (10% MeOH in $CHCl_3$); $[\alpha]_D^{20}$ = 100.2 (c = 0.8, $CHCl_3$); ν_{max}(film)/cm^{-1} 3395 br w, 2922 s, 2852 s, 1611 w, 1585 w, 1512 s, 1464 m, 1369 m, 1301 m, 1245 s, 1218 s, 1172 m, 1091 s, 1037 s, 927 w, 849 w, 820 m, 721 w, 606 w, 575 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.80 (3H, t, *J* = 6.9, CH_2CH_3), 1.14-1.24 (24H, stack, CH_2 resonances in alkyl chains), 1.26 (3H, s, 1 × C(CH_3)$_2$), 1.33 (3H, s, 1 × C(CH_3)$_2$), 1.37-1.45 (2H, stack), 2.66-2.72 (1H, m, C(2)*H*), 3.58 (1H, A of AB, $J_{A\text{-}B}$ = 12.6, CH_2Ar), 3.65 (1H, dd, *J* = 9.5, 3.7, C(1)H_aH$_b$), 3.71 (3H, s, OCH_3), 3.75 (1H, B of AB, $J_{B\text{-}A}$ = 12.6, CH_2Ar), 3.79 (1H, dd, *J* = 3.5, 1.8, C(1)H$_a$$H_b$), 3.92 (1H, dd, *J* = 7.6, 4.0, C(2')*H*), 3.95-4.02 (2H, stack, C(4')*H*, C(3)*H*), 4.04-4.12 (2H, stack, C(3')*H* , C(4)*H*), 4.22-4.25 (1H, m, C(1')*H*), 4.27 (1H, br s, N*H*), 5.70-5.72 (2H, stack, C(5')*H*, C(6')*H*), 6.78 (2H, d, *J* = 8.6, Ar), 7.15 (2H, d, *J* = 8.6, Ar), O*Hs* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 15.2 (CH_3, CH_2CH_3), 24.0 (CH_2), 26.6 (CH_3, 1 × C(CH_3)$_2$), 27.8 (CH_2), 29.0 (CH_3, 1 × C(CH_3)$_2$), [30.71, 30.72, 30.9, 31.02 31.04, 31.2 (CH_2, broad stack, significant resonance overlap)], 33.3 (CH_2), 51.6 (CH_2, NHCH_2), 56.4 (CH_3, OCH_3), 57.2 (CH, C(2)), 67.4 (CH, C(2')), 68.0 (CH_2, C(1)), 70.0 (CH, C(4')), 71.5 (CH, C(3)), 75.8 (CH, C(3')), 78.2 (CH, C(4)), 79.4 (CH, C(1')), 109.0 (C, $C(CH_3)_2$), 115.2 (CH, Ar), 128.8 (CH, C(5) or C(6)), 131.1 (CH, Ar), 131.3 (CH, C(6) or C(5)), 133.0 (C, *ipso* Ar)), 160.2 (C, $COCH_3$); *m/z* (TOF ES+) 628.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 628.4181 ([M+Na]$^+$) $C_{35}H_{59}NaNO_7$ requires 628.4189.

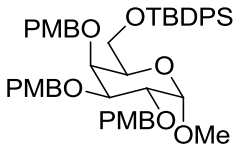
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[2',3',4'-trihydroxycyclohex-5-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (119)



TFA (1 mL) was added separately to ether **117** (60 mg, 0.09 mmol) and ether **118** (130 mg, 0.21 mmol). The reaction mixtures were stirred for 5 min before being concentrated under reduced pressure. The reaction mixtures were then analysed by to check for consumption of the starting material. If starting material was present the process was repeated until all starting material was consumed. Both reaction mixtures converged to the same crude product, which was combined before being purified by column chromatography (20% MeOH in $CHCl_3$) to provide pentaol **119** as a colourless oil (130 mg, 75%): R_f = 0.30 (20% MeOH in $CHCl_3$); $[\alpha]_D^{20}$ = 46.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3342 br m, 2923 s, 2853 s, 1671 s, 613 m, 1517 m, 1464 m, 1306 w, 1255 m, 1201 s, 1182 s, 1139 s, 1097 s, 1026 m, 921 w, 836 m, 801 m, 722 m, 614 w, 576 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.87 (3H, t, *J* = 6.8, CH_2CH_3), 1.22-1.35 (24H, stack, CH_2 resonances in alkyl chains), 1.45-1.56 (1H, m), 1.69-1.79 (1H, m), 3.42-3.50 (1H, m, C(4)*H*), 3.51-3.57 (1H, m, C(2)*H*), 3.70 (1H, dd, *J* = 8.5, 3.9, C(3)*H*), 3.80 (3H, s, OCH_3), 3.84-3.95 (2H, stack, C(1)H_aH_b, C(2')*H* or C(3')*H*), 4.03-4.11 (3H, stack, C(1)H_aH_b, C(3')*H* or C(2')*H*, C(1')*H* or C(4')*H*), 4.17 (1H, A of AB, $J_{A\text{-}B}$ = 13.2, CH_2Ar), 4.23 (1H, B of AB, $J_{B\text{-}A}$ = 13.2, CH_2Ar), 4.26-4.29 (1H, m, C(4')*H* or C(1')*H*), 4.83 (1H, br s, N*H*), 5.85-5.88 (2H, stack, C(5')*H*, C(6')*H*), 6.93 (2H, d, *J* = 8.7, Ar), 7.39 (2H, d, *J* = 8.7, Ar), O*Hs* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 15.2 (CH_3,

CH_2CH_3), 24.0 (CH_2), 26.6 (CH_2), [30.7, 31.0 (CH_2, broad stack, significant resonance overlap)], 33.2 (CH_2), 35.4 (CH_2), 50.2 (CH_2, $NHCH_2$), 56.5 (CH_3, OCH_3), 60.9 (CH, C(2)), 66.7 (CH_2, C(1)), 67.7 (CH, C(1') or C(4')), 70.0 (CH, C(2') or C(3')), 70.7 (CH, C(3') or C(2')), 71.4 (CH, C(3)), 73.8 (CH, C(4)), 77.4 (CH, C(4') or C(1')), 115.9 (CH, Ar), 123.9 (C, *ipso* Ar), 128.3 (CH, C(5) or C(6)), 132.3 (CH, C(6) or C(5)), 132.8 (CH, Ar), 162.0 (C, $COCH_3$); *m/z* (TOF ES+) 566.1 ($[M+H]^+$, 100%); HRMS *m/z* (TOF ES+) 566.4044 ($[M+H]^+$) $C_{32}H_{56}NO_7$ requires 566.4057.

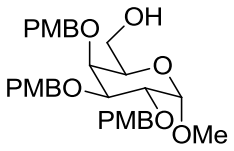
Methyl 2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactoside (120)



NaH (60% wt in mineral oil, 0.61 g, 15.5 mmol) was added to a solution of glycoside **58** (1.20 g, 2.78 mmol) in DMF (10 mL) at 0 °C. The reaction mixture was stirred for 20 min, then PMBCl (1.51 mL, 11.1 mmol) was added at 0 °C. After warming to rt and stirring overnight, the reaction was quenched by the addition of MeOH over 5 min, and then diluted with EtOAc (30 mL). The separated organic layer was washed with H_2O (20 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (15% EtOAc in hexanes) to give tri-*p*-methoxybenzyl ether **120** as a colourless oil (1.52 g, 69%): R_f = 0.16 (15% EtOAc in hexanes); $[\alpha]_D^{20}$ = 28.6 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2932 w, 2856 w, 2835 w, 1612 m, 1586 w, 1512 s, 1463 w, 1442 w, 1427 w, 1390 w, 1349 w, 1301 m, 1245 s, 1172 m, 1149 m, 1089 s, 1032 s, 820 s, 740 m, 701 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.94 (9H, s), 3.15 (3H, s), 3.55-3.58 (3H, stack), 3.62 (3H, s), 3.64 (3H, s), 3.67 (3H, s), 3.72-3.81 (2H, stack), 3.86 (1H, dd, *J* = 9.7, 3.6), 4.39-4.82 (7H, stack), 6.64 (2H,

d, *J* = 8.6), 6.72 (2H, d, *J* = 8.6), 6.79 (2H, d, *J* = 8.6), 7.05 (2H, d, *J* = 8.6), 7.18 (2H, d, *J* = 8.6), 7.20-7.34 (8H, stack), 7.48-7.54 (4H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 19.2 (C), 29.6 (CH_3), [55.1 , 55.2 (4 × CH_3, resonance overlap), 62.9 (CH_2), 70.8 (CH), 73.0 (CH_2), 73.2 (CH_2), 74.4 (CH_2), 74.8 (CH), 76.1 (CH), 79.0 (CH), 98.8 (CH), 113.6 (CH), 113.82 (CH), 113.83 (CH), 127.8 (CH), 129.2 (CH), 129.81 (CH), 129.82 (CH), 130.8 (C), 131.0 (C), 131.2 (C), 133.5 (C), 135.62 (CH), 135.63 (CH), 159.21 (C), 159.24 (C), 159.3 (C), some resonance overlap in the aromatic resonances; *m/z* (TOF ES+) 815.4 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 815.3595 ([M+Na]$^+$) $C_{47}H_{56}NaO_9Si$ requires 815.3591.

Methyl 2,3,4-tri-*O*-*p*-methoxybenzyl-α-D-galactoside (121)

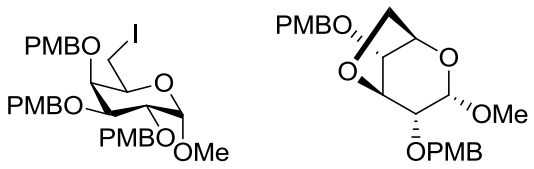


TBAF (1.0 M solution in THF, 8.08 mL, 8.08 mmol) was added to a solution of glycoside **120** (3.20 g, 4.04 mmol) in THF (20 mL). The reaction mixture was stirred overnight before being quenched with H_2O (15 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The organic layers were combined and washed with brine (25 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (50% EtOAc in hexanes) to give alcohol **121** as a colourless oil (2.02 g, 90%): R_f = 0.10 (40% EtOAc in hexanes); $[\alpha]_D^{21}$ = 7.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3344 br w, 2902 w, 2836 w, 1612 w, 1513 m, 1438 m, 1302 w, 1349 w, 1249 s, 1176 m, 1120 m, 1050 s, 822 w, 749 w, 723 s; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.25 (1H, br s), 3.27 (3H, s), 3.34-3.44 (1H, m), 3.57-3.66 (2H, stack), 3.67-3.74 (9H, stack), 3.75-3.78

(1H, m), 3.83 (1H, dd, *J* = 10.1, 2.7), 3.94 (1H, dd, *J* = 10.1, 3.5), 4.47-4.85 (7H, stack), 6.74-6.87 (6H, stack), 7.13-7.30 (6H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [55.2 , 55.3 (4 × CH_3, resonance overlap)], 62.3 (CH_2), 70.5 (CH), 73.1 (2 × CH_2, resonance overlap), 74.1 (CH_2), 74.9 (CH), 76.0 (CH), 78.8 (CH), 98.9 (CH), 113.81 (2 × CH, resonance overlap), 113.82 (CH), 129.2 (CH), 129.7 (CH), 130.1 (CH), 130.5 (C), 130.7 (C), 131.0 (C), 159.2 (C), 159.3 (C), 159.4 (C); *m/z* (TOF ES+) 577.3 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 577.2419 ([M+Na]$^+$) $C_{31}H_{38}NaO_9$ requires 577.2414.

Methyl 2,3,4-tri-*O*-*p*-methoxybenzyl-6-deoxy-6-iodo-α-D-galactoside (123) and

Methyl 4,6-anhydro-2,3-di-*O*-*p*-methoxybenzyl-α-D-galactoside (122)

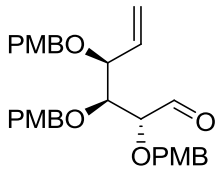


A solution of glycoside **121** (3.35 g, 6.05 mmol) and PPh_3 (1.90 g, 7.26 mmol) in toluene (5 mL) was heated under reflux for 10 min. The reaction mixture was cooled to 80 °C, and then imidazole (1.23 g, 18.2 mmol) and I_2 (2.00 g, 7.86 mmol) were added. The mixture was heated under reflux for 15 min before being concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed sequentially with $Na_2S_2O_3$ solution (20 mL) and H_2O (20 mL). The organic layer was then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (30% EtOAc in hexanes) to give iodide **123** [R_f = 0.62 (40% EtOAc in hexanes)] as a colourless oil (2.21 g, 55%) and bi-cycle **122** [R_f = 0.37 (40% EtOAc in hexanes)] as a colourless oil (1.11 g, 28%); Data for

iodide **123**: $[\alpha]_D^{20}$ = 9.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2998 w, 2904 w, 2835 w, 1611 m, 1585 w, 1511 s, 1462 m, 1441 w, 1421 w, 1348 w, 1301 m, 1244 s, 1199 m, 1172 m, 1089 s, 1031 s, 899 w, 817 s, 781 w, 753 w, 705 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.90 (1H, dd, *J* = 10.1, 5.9), 3.09 (1H, dd, *J* = 10.1, 7.9), 3.29 (3H, s), 3.646 (3H, s), 3.65 (3H, s), 3.67 (3H, s), 3.68-3.71 (1H, m), 3.77 (1H, dd, *J* = 10.1, 2.7), 3.82-3.88 (2H, stack), 4.42-4.86 (7H, stack), 6.70-6.81 (6H, stack), 7.09-7.24 (6H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 3.9 (CH_2), [55.3 , 55.7 (4 × CH_3, resonance overlap)], 71.3 (CH), 73.2 (2 × CH_2, resonance overlap), 74.6 (CH_2), 75.5 (CH), 75.6 (CH), 78.8 (CH), 99.0 (CH), 113.8 (2 × CH, resonance overlap), 113.9 (CH), 129.2 (CH), 129.8 (CH), 130.1 (CH), 130.5 (C), 130.6 (C), 130.9 (C), 159.2 (C), 159.41 (C), 159.42 (C); *m/z* (TOF ES+) 687.5 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 687.1399 ($[M+Na]^+$) $C_{31}H_{37}NaIO_8$ requires 687.1431.

Data for bi-cycle **122**: $[\alpha]_D^{20}$ = 37.6 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2954 w, 2891 w, 2836 w, 1714 w, 1611 m, 1585 w, 1511 s, 1463 m, 1338 w, 1301 m, 1244 s, 1173 m, 1079 s, 1031 s, 965 m, 926 m, 897 m, 878 w, 820 s, 765 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.52 (3H, s), 3.68-3.72 (1H, m), 3.75-3.79 (6H, stack), 3.94-4.05 (2H, stack), 4.22 (1H, m), 4.29-4.53 (5H, stack), 4.73-4.82 (2H, stack), 6.82-6.89 (4H, stack), 7.18-7.27 (4H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [55.2 , 57.6 (3 × CH_3, resonance overlap)], 69.5 (CH_2), 70.9 (CH_2), 73.4 (CH_2), 75.6 (CH), 76.4 (CH), 77.6 (CH), 78.3 (CH), 99.0 (CH), 113.7 (CH), 113.9 (CH), 129.4 (CH), 129.5 (CH), 130.0 (C), 130.5 (C), 159.3 (C), 159.4 (C); *m/z* (TOF ES+) 439.4 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 439.1751 ($[M+Na]^+$) $C_{23}H_{28}NaO_7$ requires 439.1733.

(2*R*,3*S*,4*S*)-2,3,4-tri-*p*-methoxybenzyloxy-hex-5-en-1-al (124)

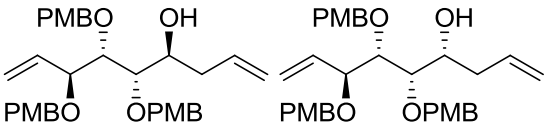


Zinc dust was activated by stirring in hydrochloric acid (1.0 M, 50 mL) at rt for 15 min, before being filtered and washed sequentially with H_2O (30 mL), acetone (30 mL) and Et_2O (30 mL). The resulting activated zinc was then dried under high vacuum with a heat-gun. The activated zinc (2.67 g, 40.8 mmol) was added to a solution of glycoside **123** (2.72 g, 4.08 mmol) and TMSCl (518 µL, 4.08 mmol) in THF (20 mL) and the reaction mixture sonicated at 40 °C. After 5 h, Et_2O (50 mL) and H_2O (50 mL) were added to the suspension, which was then filtered through Celite. The layers were separated and the aqueous layer was extracted with Et_2O (3 × 25 mL). The combined organic layers were washed sequentially with H_2O (2 × 15 mL) and brine (15 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (15% EtOAc in hexanes) to give aldehyde **124** as a colourless oil (710 mg, 34%): R_f = 0.27 (16% EtOAc in hexanes); $[\alpha]_D^{20}$ = 27.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2931 w, 1721 m, 1597 m, 1513 m, 1458 m, 1418 m, 1352 s, 1297 m, 1269 s, 1248 m, 1204 m, 1163 s, 1087 m, 1035 m, 982 m, 830 w, 812 m, 765 m, 733 m, 659 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.60 (3H, s), 3.62 (3H, s), 3.62 (3H, s), 3.71 (1H, dd, *J* = 7.8, 3.7), 3.90-4.02 (3H, stack), 4.25-4.47 (5H, stack), 5.26-5.35 (2H, stack, CH=C*H*$_2$), 5.70-5.81 (1H, m, C*H*=CH$_2$), 6.67-6.75 (6H, stack), 7.01-7.12 (6H, stack), 9.44 (1H, d, *J* = 1.6, C*H*O); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 55.2 (3 × CH_3, resonance overlap), 69.8 (CH_2), 73.0 (CH_2), 74.0 (CH_2), 79.0 (CH), 80.9 (CH), 83.7 (CH), 113.7 (CH), 113.8 (CH), 113.96 (CH), 120.1 (CH_2), [129.2, 129.4, 129.6, 129.82, 129.83 129.9, 130.0, 130.1 (CH and C, resonance overlap)], 136.0 (CH), 159.3 (C),

159.4 (C), 159.6 (C), 202.8 (CH); *m/z* (TOF ES+) 529.3 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 529.2211 ([M+Na]$^+$) $C_{30}H_{34}NaO_7$ requires 529.2202.

(4*S*,5*S*,6*S*,7*S*)-5,6,7-tri-*p*-methoxybenzyloxy-nona-1,8-dien-4-ol and

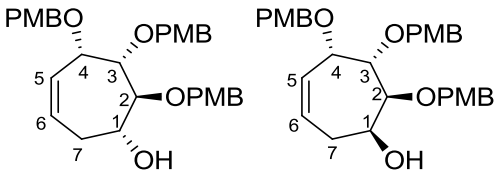
(4*R*,5*S*,6*S*,7*S*)-5,6,7-tri-*p*-methoxybenzyloxy-nona-1,8-dien-4-ol (125)



Allyl magnesium bromide (1.0 M in Et_2O, 4.18 mL, 4.18 mmol) was added dropwise over 5 min to a solution of aldehyde **124** (710 mg, 1.39 mmol) in THF (15 mL) at −78 °C. The reaction mixture was stirred at this temperature for 4 h before being quenched with NH_4Cl solution (30 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (20% EtOAc in hexanes) to give alcohol **125** as a mixture of two diasteroisomers (600 mg, 79%, ratio 1:1). Data for the mixture unless specified otherwise: R_f = 0.41 (25% EtOAc in hexanes; ν_{max}(film)/cm^{-1} 3352 w, 2932 w, 2856 w, 2835 w, 1612 m, 1586 w, 1512 s, 1463 w, 1442 w, 1427 w, 1390 w, 1349 w, 1301 m, 1245 s, 1172 m, 1149 m, 1089 s, 1032 s, 820 s, 740 m, 701 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.02-2.32 (2H, stack), 2.58-2.68 (1H, m), 3.35-3.48 (1H, m), 3.57-3.67 (9H, stack), 3.69-3.79 (1H, stack), 3.90-4.01 (1H, stack), 4.13 (1H, m), 4.31-4.41 (2H, stack), 4.41-4.49 (2H, stack), 4.52-4.62 (2H, stack), 4.87-5.00 (2H, stack, CH=CH_2), 5.20-5.34 (2H, stack, CH=CH_2), 5.58-5.79 (1H, m, C*H*=CH_2), 5.82-5.94 (1H, m, C*H*=CH_2), 6.67-6.76 (6H, stack, Ar), 7.02-7.16 (6H, stack, Ar); ^{13}C NMR (100 MHz,

$CDCl_3$) δ ppm [38.2, 38.9 (CH_2, C(3))], 55.2 (CH_3, resonance overlap), [69.7, 69.9 (CH_2, CH_2Ph)], [70.6, 70.9 (CH, CHO)], [73.2, 73.7 (CH_2, *C*H_2Ar)], [74.0, 74.5 (CH_2, *C*H_2Ar)], [80.1, 80.2 (CH, CHO)], [80.5, 80.7 (CH, CHO)], [81.1, 81.5 (CH, CHO)], 113.8 (CH, Ar, resonance overlap), [117.2, 117.5 (CH_2, C(1) or C(9))], [119.3, 119.5 (CH_2, C(9) or C(1))], [128.5, 129.42, 129.44, 129.6, 129.7, 129.8, 130.0 (CH, Ar)], [130.4, 130.7, 130.8, 130.9 (C, *ipso* Ar)], [135.3, 135.4 (CH, C(2) or C(8))], [136.0, 136.2 (CH, C(8) or C(2))], [159.2, 159.3 (C), resonance overlap]; *m/z* (TOF ES+) 571.4 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 571.2658 ($[M+Na]^+$) $C_{33}H_{40}NaO_7$ requires 571.2672.

(1*R*,2*S*,3*S*,4*S*)-2,3,4-tri-*p*-methoxybenzyloxy-cyclohept-2-en-1-ol (126) and (1*S*,2*S*,3*S*,4*S*)-2,3,4-tri-*p*-methoxybenzyloxy-cyclohept-5-en-1-ol (127)

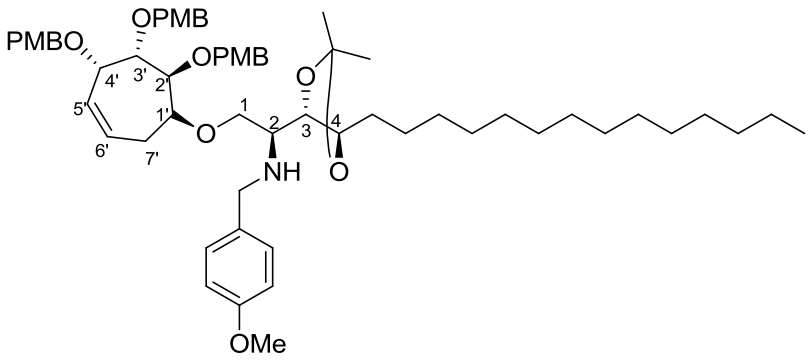


A solution of diene **125** (600 mg, 1.09 mmol) in CH_2Cl_2 (130 mL) was degassed by bubbling argon through the solvent while sonicating for 10 min. Grubbs 2nd-generation Ru metathesis catalyst (14 mg, 0.016 mmol) was then added and the solution was heated under reflux. After 2 h, the solution was concentrated under reduced pressure and the crude product purified by column chromatography (25% EtOAc in hexanes) to give, in order of elution cycloheptenes **126** (220 mg, 39%) and **127** (210 mg, 37%) as colourless oils: Less polar diastereoisomer (**126**): R_f = 0.21 (25% EtOAc in hexanes); $[\alpha]_D^{20}$ = 59.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3431 br w, 3028 w, 2868 w, 1702 w, 1496 w, 1453 m, 1311 w, 1206 m, 1064 s, 814 m, 732 s, 695 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.26-2.32 (2H, m, C(7)H_2), 3.37 (1H, br s, O*H*), 3.56-3.69 (12H, stack, 3 ×

OC*H*$_3$, C(1)H, C(2)H, C(3)H)), 4.22-4.50 (7H, stack, C(4)H, 3 × OC*H*$_2$Ar), 5.53-5.61 (1H, m, C(6)H), 5.66 (1H, dd, *J* = 11.3, 4.2, C(5)H), 6.65-6.72 (6H, stack, Ph), 6.97-7.11 (6H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 30.6 (CH_2, C(7)), 55.3 (3 × CH_3, O*C*H$_3$, resonance overlap), 69.6 (CH, *C*HO), 70.9 (CH_2, *C*H$_2$Ar), 72.4 (CH_2, *C*H$_2$Ar), 72.5 (CH_2, *C*H$_2$Ar), 76.1 (CH, C(4)H), 79.8 (CH, *C*HO), 80.9 (CH, *C*HO), 113.81 (CH, Ar), 113.82 (CH, Ar), 113.9 (CH, Ar), 127.3 (CH, C(5) or C(6)), [128.6, 129.2, 129.4 (CH, Ar, resonance overlap)], 130.41 (C, 3 × *ipso* C), 131.8 (CH, C(6) or C(5)), 159.21 (C, Ar), 159.24 (C, Ar), 159.4 (C, Ar); *m/z* (TOF ES+) 543.0 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 543.2348 ([M+Na]$^+$) $C_{31}H_{36}NaO_7$ requires 543.2359.

More polar diastereoisomer (**127**): R_f = 0.39 (25% EtOAc in hexanes); $[\alpha]_D^{20}$ = 49.8 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3418 br w, 3031 w, 2930 w, 2869 w, 1717 m, 1602 w, 1496 w, 1452 m, 1315 w, 1268 s, 1207 m, 1069 s, 1025 s, 847 w, 818 w, 735 s, 696 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.94-2.04 (1H, m, C(7)*H*$_a$H$_b$), 2.26 (1H, br s, O*H*), 2.48 (1H, app t, *J* = 12.0, C(7)H$_a$*H*$_b$), 3.65-3.73 (11H, stack, 3 × OC*H*$_3$, C(2)H, C(3)H)), 3.74-3.83 (1H, m, C(1)H), 4.20-4.61 (7H, stack, C(4)H, 3 × C*H*$_2$Ar), 5.62-5.70 (2H, stack, C(5)H, C(6)H), 6.69-6.80 (6H, stack, Ar), 7.01 (2H, d, *J* = 8.6, Ar), 7.11-7.17 (4H, stack, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 31.2 (CH_2, C(7)), 55.3 (3 × CH_3, O*C*H$_3$, resonance overlap), 67.4 (CH, *C*(1)H), 70.8 (CH_2, *C*H$_2$Ar), 72.3 (CH_2, *C*H$_2$Ar), 73.1 (CH_2, *C*H$_2$Ar), 75.7 (CH, C(4)H), 78.3 (CH, C(3)H), 81.3 (CH, *C*(2)H), 113.7 (CH, Ar), 113.8 (CH, Ar), 113.9 (CH, Ar), 126.5 (CH, C(5) or C(6)), [129.1, 129.3, 129.4, 129.5, 129.7 (CH, Ph, resonance overlap)], 130.4 (C, *ipso* Ar), 130.7 (C, *ipso* Ar), 130.8 (C, *ipso* Ar), 132.9 (CH, C(6) or C(5)), 159.1 (C, Ar), 159.2 (C, Ar), 159.4 (C, Ar); *m/z* (TOF ES+) 543.0 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 543.2348 ([M+Na]$^+$) $C_{31}H_{36}NaO_7$ requires 543.2359.

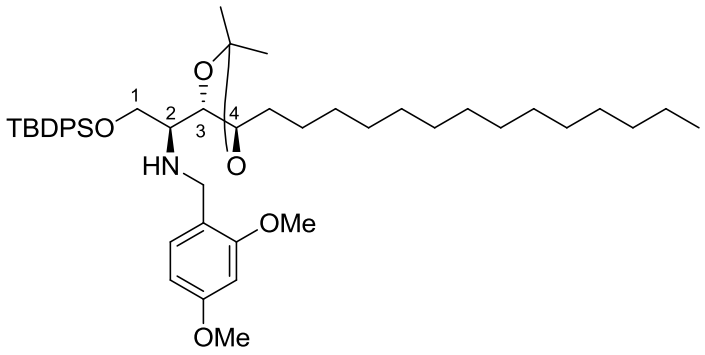
(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-*p*-methoxybenzylamino-1-*O*-[2',3',4'-*O*-benzyl-trihydroxycyclohept-5'-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (128)



NaH (60% wt in mineral oil, 48 mg, 1.20 mmol) was added to a solution of alcohol **127** (210 mg, 0.40 mmol) in DMF (2 mL) and THF (1 mL) at 0 °C. After stirring for 1 h, a solution of sulfamidate **109** (238 mg, 0.44 mmol) in THF (1 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated and the residue was dissolved in Et_2O (10 mL). A 20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 20 min before being neutralised with K_2CO_3 (1 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) were added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give ether **128** as a colourless oil (367 mg, 61%): R_f = 0.68 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ = 61.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1891 w, 1690 w, 1456 m, 1418 m, 1354 m, 1219 m, 1165 s, 1054 s, 881 m, 811 m, 736 m, 661 m; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.81 (3H, t, *J* = 6.8, CH_2CH_3), 1.16-1.25 (29H, stack, CH_2 resonances in alkyl chains, 1 × $C(CH_3)_2$), 1.32 (3H, s, 1 × $C(CH_3)_2$), 2.00-2.08 (1H, m, C(7')H_aH_b), 2.60-2.70 (2H, stack, C(2)*H*, C(7')H_aH_b), 3.48-3.58 (3H, stack, NHCH_aH_b, C(1)H_aH_b, C(1')*H*), 3.65-

6.77 (15H, stack, 4 × OC*H*$_3$, NHCH$_a$*H*$_b$, C(1)H$_a$*H*$_b$, C(3')*H*), 3.81 (1H, app d, *J* = 5.7, C(2')*H*), 3.90 (1H, dd, *J* = 9.4, 5.6, C(3)*H*), 4.02-4.08 (1H, m, C(4)*H*), 4.23-4.59 (7H, stack, 3 × OC*H*$_2$Ar, C(4')*H*), 5.65-5.73 (2H, stack, C(5')*H*, C(6')*H*), 6.68-6.81 (8H, stack, Ar), 7.02-7.19 (8H, stack, Ar), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2 (CH_3, CH_2CH_3), 22.7 (CH_2), 26.1 (CH_3, 1 × C(*C*H$_3$)$_2$), 26.2 (CH_2, C(7')), 27.2 (CH_2). 28.5 (CH_3, 1 × C(*C*H$_3$)$_2$), [29.4, 29.5, 29.8 (CH_2, broad stack, significant resonance overlap)], 32.0 (CH_2), 50.5 (CH_2, *C*H$_2$NH), 55.2 (4 × CH_3, O*C*H$_3$, resonance overlap), 56.1 (CH, C(2)), 66.2 (CH_2, C(1)), 70.7 (CH_2, O*C*H$_2$Ar), 72.3 (CH_2, O*C*H$_2$Ar), 73.3 (CH_2, O*C*H$_2$Ar), 76.4 (CH, C(4')) 76.7 (CH, C(3)), 77.0 (CH, C(1')), 78.0 (CH, C(2')), 78.4 (CH, C(4)), 78.6 (CH, C(3')), 107.4 (C, *C*(CH$_3$)$_2$), 113.71 (2 × CH, Ar), 113.73 (2 × CH, Ar), 125.5 (CH, C(5') or C(6')), [129.1, 129.2, 129.4, 129.5 (CH, resonance overlap, Ar)], 130.7 (C, *ipso* Ar), 130.9 (C, *ipso* Ar), 131.2 (C, *ipso* Ar), 132.7 (C, *ipso* Ar), 134.0 (CH, C*H*=CH), 158.7 (C, Ar), 159.7 (3 × C, Ar, resonance overlap); *m/z* (TOF ES+) 1002.8 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1002.6074 ([M+Na]$^+$) $C_{60}H_{85}NaNO_{10}$ requires 1002.6071.

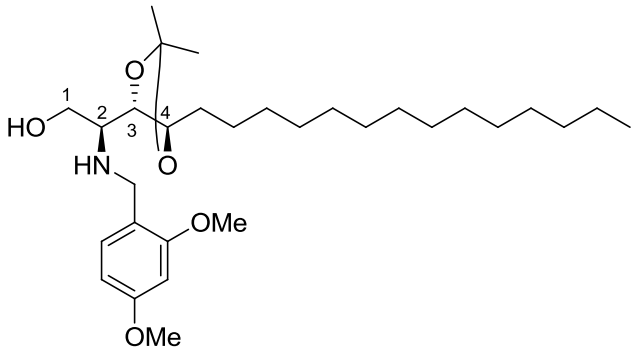
(2*R*,3*R*,4*S*)-2-(2',4'-dimethoxybenzylamino)-1-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (129)



2,4-Dimethoxybenzaldehyde (559 mg, 3.36 mmol) was added to a stirred suspension of amine **76** (1.82 g, 3.06 mmol) and $NaBH(OAc)_3$ (908 mg, 4.28 mmol) in THF (15 mL). After stirring overnight, the reaction mixture was diluted with Et_2O (20 mL) and $NaHCO_3$ solution (20 mL). The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 20 mL). The organic layers were combined and washed with brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to give 2,4-methoxybenzyl amine **129** as a colourless oil (2.16 g, 95%): R_f = 0.30 (10% EtOAc in hexanes); $[\alpha]_D^{22}$ = 23.2 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1613 m, 1589 m, 1507 m, 1463 s, 1427 m, 1377 m, 1366 m, 1286 m, 1246 s, 1208 s, 1156 s, 1112 s, 1076 s, 1039 s, 998 w, 936 w, 876 w, 822 m, 781 w, 739 m, 701 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.92 (3H, t, *J* = 6.8, CH_2CH_3), 1.10 (9H, s, $C(CH_3)_3$), 1.28-1.35 (26H, stack, CH_2 resonances in alkyl chains), 1.40 (3H, s, 1 × $(CH_3)_2$), 1.41 (3H, s, 1 × $C(CH_3)_2$), 2.02 (1H, br s, NH), 2.68-2.75 (1H, m, C(2)H), 3.65 (1H, A of AB, $J_{A\text{-}B}$ = 12.5, CH_aH_bAr), 3.74 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.90-3.99 (3H, stack, C(1)H_2, B of AB, CH_aH_bAr), 4.19-4.26 (1H, m, C(4)H), 4.29 (1H, dd, *J* = 9.4, 5.8, C(3)H), 6.45-6.47 (2H, stack, Ar), 7.16 (1H, d, *J* = 8.6, Ar), 7.36-7.48 (6H, stack, Ph), 7.71-7.81 (4H, stack, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2

(CH_3, CH_2CH_3), 19.4 (C, $SiC(CH_3)_3$), 22.7 (CH_2), 26.0 (CH_3, 1 × $C(CH_3)_2$), 26.2 (CH_2), 26.8 (CH_3, $SiC(CH_3)_3$), 28.5 (CH_3, 1 × $C(CH_3)_2$), [29.4, 29.7, 29.8 (CH_2, broad stack, significant resonance overlap)], 32.0 (CH_2), 46.0 (CH_2, CH_2NH), 55.2 (CH_3, OCH_3), 55.3 (CH_3, OCH_3), 57.5 (CH, C(2)), 60.8 (CH_2, C(1)), 76.3 (CH, C(3)), 78.4 (CH, C(4)), 98.6 (CH, Ar), 103.9 (CH, Ar), 107.3 (C, $C(CH_3)_2$), 121.5 (C, *ipso* Ar), 127.6 (CH, Ph), 127.7 (CH, Ph), 129.6 (CH, Ph), 129.7 (CH, Ph), 130.6 (CH, Ar), 133.5 (C, *ipso* Ph), 133.9 (C, *ipso* Ph), 135.6 (CH, Ph), 135.8 (CH, Ph), 158.6 (C, $COCH_3$), 160.1 (C, $COCH_3$); *m/z* (TOF ES+) 746.6 ($[M+H]^+$, 100%); HRMS *m/z* (TOF ES+) 768.5019 ($[M+Na]^+$) $C_{46}H_{71}NNaO_5Si$ requires 768.4999.

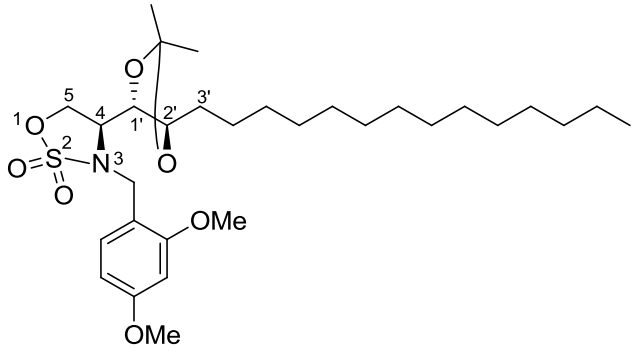
(2*R*,3*R*,4*S*)-2-(2',4'-di-methoxybenzylamino)-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (130)



TBAF (1.0 M solution in THF, 4.34 mL, 4.34 mmol) was added to a solution of silyl ether **129** (2.16 g, 2.90 mmol) in THF (20 mL). The reaction mixture was stirred overnight before being quenched with H_2O (15 mL). The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with brine (15 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (50% EtOAc in hexanes) to provide alcohol **130** as a colourless oil (1.25 g, 85%): R_f = 0.33 (50% EtOAc in hexanes); $[\alpha]_D^{22}$ = 32.8 (c =

1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 3413 br w, 2922 s, 2852 s, 1613 m, 1588 m, 1507 m, 1463 m, 1419 w, 1377 m, 1368 m, 1288 m, 1244 m, 1208 s, 1156 s, 1040 s, 921 w, 873 m, 833 m, 786 w, 721 w, 634 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.89 (3H, t, *J* = 6.8, CH_2CH_3), 1.25-1.30 (26H, stack, CH_2 resonances in alkyl chains), 1.34 (3H, s, 1 × C(CH_3)$_2$), 1.42 (3H, s, 1 × C(CH_3)$_2$), 2.16 (1H, br s, O*H*), 2.73-2.78 (1H, m, C(2)*H*), 3.65 (1H, A of AB, $J_{A\text{-}B}$ = 12.6, CH_aH_bAr), 3.76 (2H, app d, *J* = 4.1, C(1)H_2), 3.80 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.83-8.85 (1H, m, B of AB, CH_aH_bAr), 4.04 (1H, dd, *J* = 8.5, 5.8, C(3)*H*), 4.08-4.05 (1H, m, C(4)*H*), 6.42-6.47 (2H, stack, Ar), 7.12 (1H, d, *J* = 8.1), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.8 (CH_3, 1 × C(CH_3)$_2$), 26.3 (CH_2). 28.1 (CH_3, 1 × C(CH_3)$_2$), [29.4, 29.5, 29.7 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 45.8 (CH_2, CH_2NH), 55.31 (CH_3, OCH_3), 55.32 (CH_3, OCH_3), 56.7 (CH, C(2)), 61.0 (CH_2, C(1)), 77.8 (CH, C(4)), 78.0 (CH, C(3)), 98.7 (CH, Ar), 103.9 (CH, Ar), 107.7 (C, C(CH_3)$_2$), 120.6 (C, *ipso* Ar), 130.6 (CH, Ar), 158.5 (C, $COCH_3$), 160.3 (C, $COCH_3$); *m/z* (TOF ES+) 508.6 ($[M+H]^+$, 100%); HRMS *m/z* (TOF ES+) 508.4001 ($[M+H]^+$) $C_{30}H_{54}NO5$ requires 508.4002.

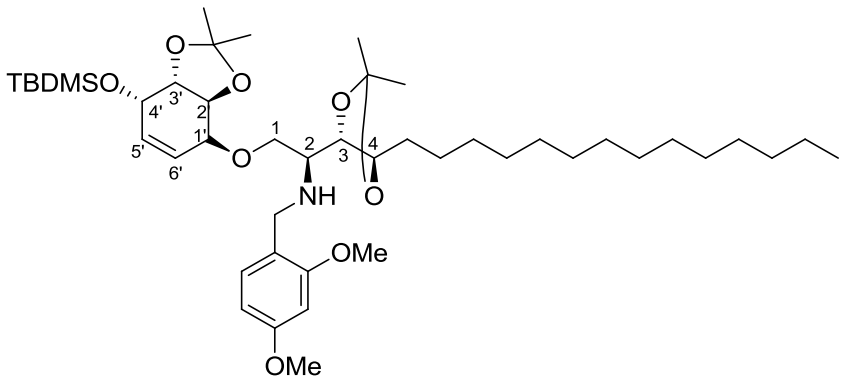
(3*R*,1'*S*,2'*S*)-3-(2'',4''-dimethoxybenzyl)-4-[1',2'-*O*-isopropylidene-dihydroxyhexadecyl]-1,2,3-oxathiazolidine-2,2-dioxide (132)



A solution of amine **130** (800 mg, 1.57 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 30 min to a solution of $SOCl_2$ (126 µL, 1.73 mmol), imidazole (428 mg, 6.28 mmol) and NEt_3 (656 µL, 4.71 mmol) in CH_2Cl_2 (15 mL) at −50 °C. The reaction mixture was warmed to 0 °C and stirred for 21 h, before adding H_2O (15 mL). The organic layer was isolated and washed with brine (10 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give the crude sulfamidite as a mixture of diastereoisomers [R_f = 0.81 (50% EtOAc in hexanes)], which was used immediately in the next step. $NaIO_4$ (370 mg, 1.73 mmol), $RuCl_3$ (16 mg, 0.08 mmol) and H_2O (5 mL) were added sequentially to a solution of the crude sulfamidite in MeCN (5 mL) at 0 °C. After 2.5 h, the reaction mixture was diluted with H_2O (50 mL) and Et_2O (50 mL). The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was filtered through a pad of silica, eluting with Et_2O, before purifying by column chromatography (10% EtOAc in hexanes) to give sulfamidate **132** as a colourless oil (627 mg, 70%): R_f = 0.30 (10% EtOAc in hexanes); $[\alpha]_D^{22}$ = 6.0 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1613 s, 1588 m, 1508 s, 1463 m, 1368 m, 1293 m, 1267 w, 1247 w, 1209 s, 1186 s, 1158

m, 1132 m, 1035 m, 834 m, 800 m, 721 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.88 (3H, t, *J* = 6.8, CH_2CH_3), 1.24-1.29 (26H, stack, CH_2 resonances in alkyl chains), 1.31 (3H, s, 1 × $C(CH_3)_2$), 1.37 (3H, s, 1 × $C(CH_3)_2$), 3.76 (1H, app td, *J* = 7.2, 3.4, C(4)*H*), 3.80 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.08-4.14 (2H, stack, C(5)H_aH_b, C(2')*H*), 4.27-4.34 (2H, stack, CH_aH_bAr, C(1')*H*), 4.47-4.55 (2H, stack, CH_aH_bAr, C(5)H_aH_b), 6.44-6.51 (2H, stack, Ar), 7.45 (1H, d, *J* = 8.4, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 25.2 (CH_3, 1 × $C(CH_3)_2$), 26.7 (CH_2), 27.6 (CH_3, 1 × $C(CH_3)_2$), [29.4, 29.5, 29.6, 29.7 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 46.3 (CH_2, CH_2N), 55.2 (CH_3, OCH_3), 55.3 (CH_3, OCH_3), 57.8 (CH, *C*(4)), 68.5 (CH_2, *C*(5)), 76.2 (CH, C(1')), 77.3 (CH, C(2')), 98.3 (CH, Ar), 104.8 (CH, Ar), 107.9 (C, $C(CH_3)_2$), 114.9 (C, *ipso* Ar), 132.5 (CH, Ar), 158.6 (C, $COCH_3$), 161.3 (C, $COCH_3$); *m/z* (TOF ES+) 592.6 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 592.3285 ($[M+Na]^+$) $C_{30}H_{51}NaNO_7S$ requires 592.3284.

(2*R*,3*R*,4*S*,1'*S*,2'*S*,3'*S*,4'*S*)-2-(2'',4''-dimethoxybenzylamino)-1-*O*-[4'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-2',3',4'-trihydroxycyclohex-5-enyl]-3,4-*O*-isopropylidene-1,3,4-octadecanetriol (133)

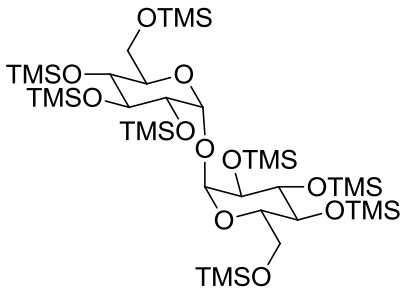


NaH (60% wt in mineral oil, 80 mg, 2.01 mmol) was added to a solution of alcohol **86** (200 mg, 0.67 mmol) in DMF (5 mL) and THF (2 mL) at 0 °C. After stirring for 1 h, a solution of

sulfamidate **132** (417 mg, 0.73 mmol) in THF (3 mL) was added at 0 °C. After stirring overnight at 40 °C, the reaction mixture was concentrated and the residue was dissolved in Et_2O (10 mL). 20% aq. H_2SO_4 solution (10 mL) was added at 0 °C and the reaction mixture was stirred for 15 min before being neutralised with K_2CO_3 (1.0 g). After 40 min, Et_2O (20 mL) and H_2O (20 mL) were added. The resulting layers were separated and the aqueous layer was extracted with Et_2O (3 × 35 mL). The organic layers were combined and washed sequentially with H_2O (30 mL), $NaHCO_3$ solution (20 mL) and brine (20 mL), and then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (20% EtOAc in hexanes) to give ether **133** as a colourless oil (291 mg, 55%): R_f = 0.25 (20% EtOAc in hexanes); $[\alpha]_D^{22}$ = 147.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 1614 w, 1589 w, 1507 m, 1463 m, 1419 w, 1377 m, 1368 m, 1287 m, 1247 m, 1208 s, 1148 m, 1129 s, 1063 s, 1040 s, 968 m, 922 m, 832 s, 801 m, 778 s, 704 w, 665 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm −0.005 (3H, s), −0.000 (3H, s), 0.76-0.80 (12H, stack, CH_2CH_3, $SiC(CH_3)_3$), 1.13-1.18 (26H, stack, CH_2 resonances in alkyl chains), 1.19 (3H, s, 1 × $C(CH_3)_2$), 1.28 (3H, s, 1 × $C(CH_3)_2$), 1.31 (3H, s, 1 × $C(CH_3)_2$), 1.34 (3H, s, 1 × $C(CH_3)_2$), 2.63-2.69 (1H, m, C(2)*H*), 3.53-3.62 (2H, stack, CH_aH_bAr, $C(1)H_aH_b$), 3.68 (6H, s, 2 × OCH_3, resonance overlap), 3.75 (1H, B of AB, $J_{B\text{-}A}$ = 12.9, CH_aH_bAr), 3.86 (1H, dd, *J* = 10.0, 3.5, C(2')*H* or C(3')*H*), 3.91-4.05 (5H, stack, $C(1)H_aH_b$, C(3')*H* or C(2')*H*, C(1')*H* or C(4')*H*, C(3)*H,* C(4)*H*), 4.34 (1H, app t, *J* = 3.9, C(4')*H* or C(1')*H*), 5.69-5.92 (2H, stack, C(5')*H*, C(6')*H*), 6.29-6.34 (2H, stack, Ar), 7.04 (1H, d, *J* = 7.9, Ar), N*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−4.8, −4.5 (2 × CH_3, $Si(CH_3)_2$)], 14.1 (CH_3, CH_2CH_3), 18.3 (C, $SiC(CH_3)_3$), 22.7 (CH_2), 25.8 (CH_3, 1 × $C(CH_3)_2$), 25.9 (CH_3, $SiC(CH_3)_3$), 26.2 (CH_2), 26.9 (CH_3, 2 × $C(CH_3)_2$, resonance overlap), 28.3 (CH_3, 1 × $C(CH_3)_2$), [29.4, 29.5, 29.7 (CH_2, broad stack, significant resonance overlap)], 31.9 (CH_2), 45.9 (CH_2, $NHCH_2$), 55.2 (CH_3, OCH_3), 55.3 (CH_3, OCH_3), 56.7 (CH, C(2)), 66.0 (CH, CHO), 70.2 (CH_2, C(1)), 73.7 (CH, CHO), 74.0 (CH, CHO), 74.2

(CH, CHO), 76.8 (CH, CHO), 78.2 (CH, CHO), 98.5 (CH, Ar), 103.8 (CH, Ar), 107.3 (C, *C*$(CH_3)_2$), 109.9 (C, *C*$(CH_3)_2$), 121.3 (C, *ipso* Ar), 127.6 (CH, C(5) or C(6)), 130.5 (CH, Ar), 131.4 (CH, C(6) or C(5)), 158.5 (C, *C*OCH_3), 160.1 (C, *C*OCH_3); *m/z* (TOF ES+) 791.0 ($[M+H]^+$, 100%); HRMS *m/z* (TOF ES+) 812.5475 ($[M+Na]^+$) $C_{45}H_{79}NaNO_8Si$ requires 812.5473.

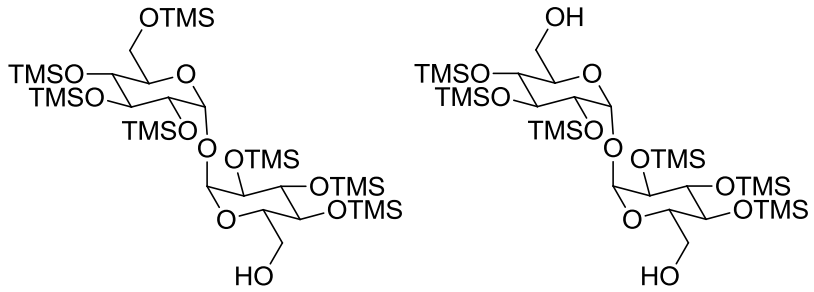
2,3,4,6,2',3',4',6'-octakis-*O*-trimethylsilyl-α,α-trehalose (135)



Pyridine (300 mL), HMDS (50 mL, 0.24 mol) and TMSCl (25 mL, 0.198 mol) were added sequentially to dry trehalose (5.0 g, 13.2 mmol). The reaction mixture was stirred overnight at rt before being poured into cold H_2O (300 mL) and extracted with hexane (3 × 150 mL). The organic layers were combined, washed with H_2O (3 × 300 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The material was isolated as a white solid, providing trehalose **135** (12.14 g, 100%): R_f = 0.62 (2.5% EtOAc in hexanes); mp = 79–83 °C; $[\alpha]_D^{21}$ = 104.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.05-0.18 (72H, stack), 3.21-3.33 (4H, stack), 3.51-3.56 (4H, stack), 3.65 (2H, ddd, *J* = 9.4, 3.9, 2.5), 3.74 (2H, t, *J* = 8.9), 4.77 (2H, d, *J* = 3.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm −0.9 (CH_3), [−1.4, −0.9, −0.1, −0.0 (CH_3, TMS, resonance overlap)], 61.1 (CH_2), 70.7 (CH), 71.8 (CH), 72.1 (CH), 72.5 (CH), 93.3 (CH); *m/z* (TOF ES+) 941.5 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 941.4222 ($[M+Na]^+$) $C_{36}H_{86}NaO_{11}Si_8$ requires 941.4250. Data were in agreement with those reported in the literature.[166]

2,3,4,6,2',3',4'-heptakis-*O*-trimethylsilyl-α,α-trehalose (136) and

2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (137)



K_2CO_3 (0.1 g) was added to a vigorously stirring solution of trehalose **135** (2.0 g, 2.17 mmol) in MeOH (50 mL) at 0 ˚C. After stirring for 30 min, glacial AcOH (2 mL) was added and the reaction mixture was diluted with $CHCl_3$ (50 mL) and H_2O (30 mL). The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (3 solvent systems – Et_2O in petroleum ether 1:12 (eluting unreacted starting material), 1:3 (eluting mono-alcohol **136**), 3:1 (eluting diol **137**)) to obtain mono-alcohol **136** and diol **137**, both as white solids (621 mg, 34% and 855 mg, 51% respectively). Data for alcohol **136**: R_f = 0.52 (4:1 petroleum ether: Et_2O); mp = 77−79 °C, lit.[181] mp = 76−78 °C; $[\alpha]_D^{21}$ = 100.2 (c = 1.0, $CHCl_3$), lit.[167] $[\alpha]_D^{20}$ = 114.5 (c = 2.3, Et_2O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.08-0.04 (63H, stack), 3.22-3.37 (4H, stack), 3.50-3.81 (8H, stack), 4.77 (2H, dd, *J* =14.7, 3.1), O*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.0, 0.1 (CH_3, TMS, resonance overlap)], 60.7 (CH_2), 61.1 (CH_2), 70.5 (CH), 70.7 (CH), 71.8 (2 × CH, resonance overlap), 72.4 (2 × CH, resonance overlap), 72.5 (2 × CH, resonance overlap), 93.3 (CH), 93.4 (CH); *m/z* (TOF ES+) 869.5 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 869.3827 ($[M+Na]^+$)$C_{33}H_{78}NaO_{11}Si_7$ requires 869.3837.

Data for diol **137**: R_f = 0.06 (4:1 petroleum ether: Et_2O), R_f = 0.48 (1:1 petroleum ether: Et_2O); mp = 110−114 °C, lit.[182] mp = 114−115 °C; $[\alpha]_D^{21}$ = 110.2 (c = 1.0, $CHCl_3$), lit.[167] $[\alpha]_D^{20}$ = 102.0 (c = 2.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.16-0.19 (54H, stack), 2.16 (2H, br s), 3.23-3.36 (4H, stack), 3.47-3.59 (4H, stack), 3.66-3.79 (4H, stack), 4.75 (2H, d, *J* = 3.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.7, 0.0, 0.1 (CH_3, TMS], 60.6 (CH_2), 70.5 (CH), 71.9 (CH), 72.3 (CH), 72.5 (CH), 93.8 (CH); *m/z* (TOF ES+) 797.5 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 797.3432 ([M+Na]$^+$) $C_{30}H_{70}NaO_{11}Si_6$ requires 797.3433.

Data were in agreement with those reported in the literature.[167,181,182]

General procedure for mono DCC coupling

Alcohol (1.0 equiv), fatty acid (1.2 equiv), DCC (1.2 equiv) and DMAP (catalytic amount) were dried under high vacuum before freshly activated 4 Å molecular sieves and toluene (5 mL) were added. The reaction mixture was stirred overnight at 65 ˚C before being diluted with toluene (15 mL) and filtered through Celite. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography (0-5% EtOAc in hexanes, gradient).

General procedure for di-DCC coupling

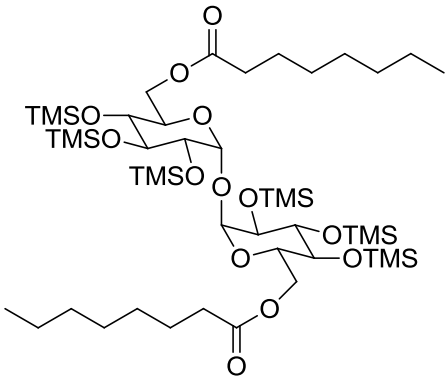
Alcohol (1.0 equiv), fatty acid (3.0 equiv), DCC (3.0 equiv) and DMAP (catalytic amount) were dried under high vacuum before freshly activated 4 Å molecular sieves and toluene (5 mL) were added. The reaction mixture was stirred overnight at 65 ˚C before being diluted with toluene

(15 mL) and filtered through Celite. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography (0-5% EtOAc in hexanes, gradient).

General procedure for removal of TMS groups

A TFA: THF: H_2O (8 : 17 : 3) solution (4 mL) was added to the TMS protected product (0.5 mmol) and stirred for 1.5 h. The reaction mixture was then concentrated under reduced pressure. The crude product was purified by column chromatography.

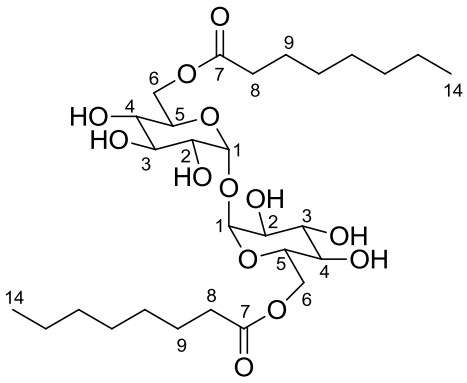
6,6'-di-*O*-octanoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (138a)



Di-ester **138a** was prepared from diol **137** (170 mg, 0.20 mmol), octanoic acid (94 µL, 0.60 mmol), DCC (122 mg, 0.60 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide di-ester **138a** as a colourless oil (146 mg, 71%): R_f = 0.36 (5% EtOAc in hexanes); $[\alpha]_D^{25}$ = 86.4 (c = 1.8, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1743 m (C=O), 1459 w, 1387 w, 1249 s, 1162 m, 1110 m, 1072 s, 1043 m, 1010 m, 965 m, 897 s, 872 s, 842 s, 748 s, 683 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ

ppm −0.02-0.05 (54H, stack), 0.75 (6H, t, *J* =6.7), 1.08-1.25 (16H, stack), 1.44-1.56 (4H, stack), 2.17-2.27 (4H, stack), 3.27-3.40 (4H, stack), 3.77 (2H, app t, *J* = 9.0), 3.80-4.00 (4H, stack), 4.15 (2H, dd, *J* = 11.6, 1.9), 4.79 (2H, d, *J* = 3.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.2, −0.0, (CH_3, TMS)], 13.0 (CH_3), 21.5 (CH_2), 23.7 (CH_2), 27.9 (CH_2), 28.0 (CH_2), 30.6 (CH_2), 33.1 (CH_2), 62.2 (CH_2), 69.7 (CH), 70.9 (CH), 71.6 (CH), 72.4 (CH), 93.3 (CH), 172.7 (C); *m/z* (TOF ES+) 1049.7 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1049.5503 ([M+Na]$^+$) $C_{48}H_{97}NaO_{13}Si_6$ requires 1049.5545.

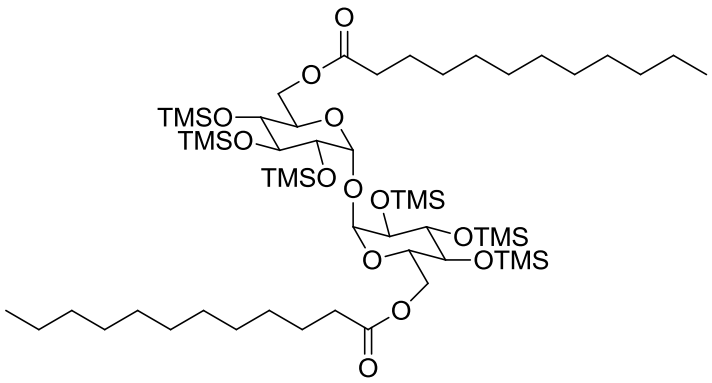
6,6'-di-*O*-octanoyl-α,α-trehalose (138b)



Hexaol **138b** was prepared from trehalose **138a** (220 mg, 0.21 mmol) and a TFA: THF: H_2O (8 : 17 : 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide hexaol **138b** as a colourless gel (82 mg, 66%): R_f = 0.33 (15% MeOH in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O–H), 2928 m, 2857 w, 1723 m (C=O), 1674 s, 1454 w, 1378 w, 1266 w, 1150 m, 1105 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD, 2:1)) δ ppm 0.89 (6H, t, *J* = 6.9, C(14)H_3), 1.24-1.37 (16H, stack), 1.57-1.69 (4H, m, C(9)H_aH_b), 2.35 (4H, t, *J* =

7.6, C(8)H_aH_b), 3.33-3.41 (2H, m, C(4)*H*), 3.54 (2H, dd, *J* = 9.5, 3.7, C(2)*H*), 3.83 (2H, app t, *J* = 9.5, C(3)*H*), 4.00 (2H, ddd, *J* = 10.1, 4.9, 2.2, C(5)*H*), 4.28 (2H, A of ABX, $J_{A\text{-}B}$ = 12.0, $J_{A\text{-}X}$ = 4.9, C(6)H_aH_b), 4.35 (2H, B of ABX, $J_{B\text{-}A}$ = 12.0, $J_{B\text{-}X}$ = 2.2, C(6)H_aH_b), 5.11 (2H, d, *J* = 3.7, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm 15.1 (CH_3, C(14)), 23.9 (CH_2), 26.2 (CH_2, C(9)), 30.2 (CH_2), 30.4 (CH_2), 33.0 (CH_2), 35.5 (CH_2, C(8)), 64.6 (CH_2, C(6)), 71.4 (CH, C(5)), 71.7 (CH, C(4)), 73.0 (CH, C(2)), 74.6 (CH, C(3)), 95.0 (CH, C(1)), 176.0 (C, C(7)); *m/z* (TOF ES+) 617.3 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 617.3158 ([M+Na]$^+$) $C_{28}H_{50}NaO_{13}$ requires 617.3149.

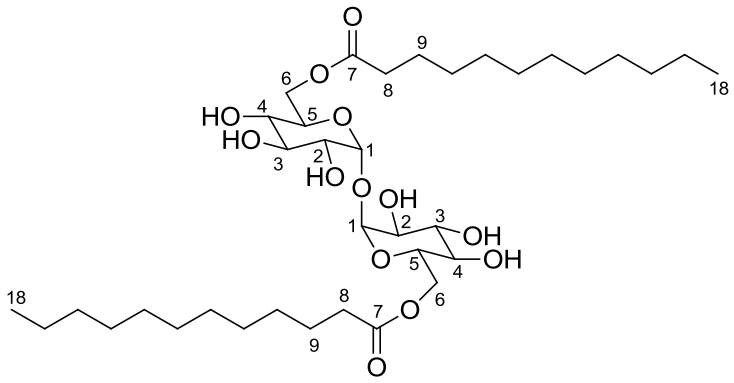
6,6'-di-*O*-dodecanoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (139a)



Di-ester **139a** was prepared from diol **137** (200 mg, 0.26 mmol), dodecanoic acid (155 mg, 0.78 mmol), DCC (161 mg, 0.78 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide di-ester **139a** as a colourless oil (186 mg, 62%): R_f = 0.35 (5% EtOAc in hexanes); $[\alpha]_D^{21}$ = 69.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1743 m (C=O), 1458 w, 1385 w, 1249 s, 1162 m, 1110 m, 1073 s, 1043 m, 1010 m, 965 m, 897 s, 872 s, 843 s, 748 s, 683 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ

ppm −0.03-0.03 (54H, stack), 0.75 (6H, t, *J* = 6.7), 1.03-1.24 (32H, stack), 1.44-1.61 (4H, stack), 2.16-2.23 (4H, stack), 3.26-3.42 (4H, stack), 3.77 (2H, app t, *J* = 9.0), 3.83-4.00 (4H, stack), 4.15 (2H, dd, *J* = 11.6, 1.9), 4.79 (2H, d, *J* = 3.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.2, −0.0 (CH_3, TMS)], 13.0 (CH_3), 21.6 (CH_2), 23.7 (CH_2), [28.1, 28.2, 28.3, 28.4, 28.6 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 62.2 (CH_2), 69.7 (CH), 70.9 (CH), 71.6 (CH), 72.4 (CH), 93.3 (CH), 172.6 (C); *m/z* (TOF ES+) 1162.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1161.6783 ([M+Na]$^+$) $C_{54}H_{114}NaO_{13}Si_6$ requires 1161.6773.

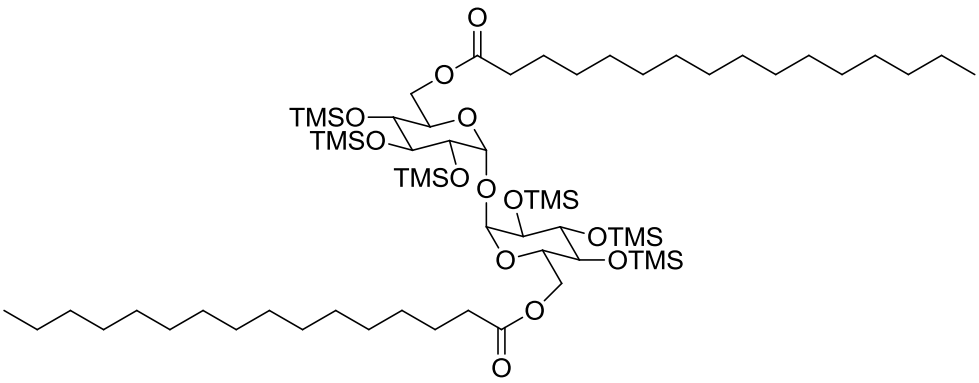
6,6'-di-*O*-dodecanoyl-α,α-trehalose (139b)



Hexaol **139b** was prepared from trehalose **139a** (186 mg, 0.16 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide hexaol **139b** as a white solid (72 mg, 64%): R_f = 0.36 (15% MeOH in $CHCl_3$); mp = 148-154 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O–H), 2928 m, 2857 w, 1723 m (C=O), 1674 s, 1454 w, 1378 w, 1266 w, 1150 m, 1105 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD 2:1)) δ ppm 0.89 (6H, t, *J* = 6.9, C(18)H_3), 1.18-1.42 (32H, stack), 1.63 (4H, m, C(9)H_aH_b), 2.35

(4H, t, *J* = 7.6, C(8)H_aH_b), 3.31-3.40 (2H, m, C(4)*H*), 3.53 (2H, dd, *J* = 9.5, 3.7, C(2)*H*), 3.80 (2H, app t, *J* = 9.5, C(3)*H*), 3.99 (2H, ddd, *J* = 10.1, 4.9, 2.2, C(5)*H*), 4.27 (2H, A of ABX, $J_{A\text{-}B}$ = 12.0, $J_{A\text{-}X}$ = 4.9, C(6)H_aH_b), 4.34 (2H, B of ABX, $J_{B\text{-}A}$ = 12.0, $J_{B\text{-}X}$ = 2.2, C(6)H_aH_b), 5.11 (2H, d, *J* = 3.7, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD 2:1) δ ppm 15.1 (CH_3, C(18)), 24.0 (CH_2), 26.2 (CH_2, C(9)), [30.5, 30.6, 30.7, 30.8, 31.0 (CH_2, resonance overlap)], 33.2 (CH_2), 35.5 (CH_2, C(8)), 64.6 (CH_2, C(6)), 71.4 (CH, C(5)), 71.8 (CH, C(4)), 73.1 (CH, C(2)), 74.7 (CH, C(3)), 95.0 (CH, C(1)), 175.9 (C, C(7)); *m/z* (TOF ES+) 729.3 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 729.4406 ([M+Na]$^+$) $C_{36}H_{66}NaO_{13}$ requires 729.4401.

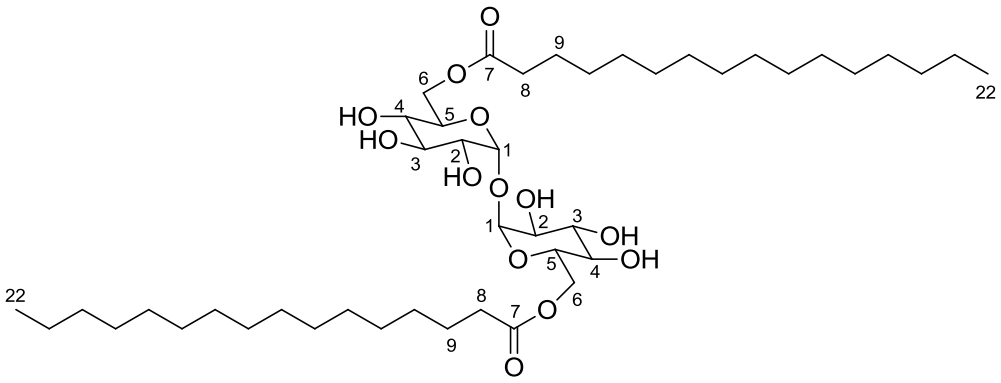
6,6'-di-*O*-hexadecanoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (140a)



Di-ester **140a** was prepared from diol **137** (180 mg, 0.23 mmol), hexadecanoic acid (179 mg, 0.70 mmol), DCC (144 mg, 0.70 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide di-ester **140a** as a colourless oil (193 mg, 67%): R_f = 0.43 (5% EtOAc in hexanes); $[\alpha]_D^{22}$ = 71.1 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1742 m (C=O), 1459 w, 1387 w, 1249 s, 1162 m, 1111 m, 1073 s, 1043 m, 1010 m, 964 m, 897 s, 874 s, 842 s, 748 s, 683 w; ^{1}H NMR

(300 MHz, $CDCl_3$) δ ppm −0.05-0.07 (54H, stack), 0.75 (6H, t, *J* = 7.0), 0.84-1.44 (48H, stack), 1.44-1.58 (4H, stack), 2.16-2.26 (4H, stack), 3.27-3.40 (4H, stack), 3.77 (2H, app t, *J* = 9.0), 3.83-4.00 (4H, stack), 4.15 (2H, dd, *J* = 11.9, 2.3), 4.79 (2H, d, *J* = 3.4); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.2, 0.0 (CH_3, TMS)], 13.1 (CH_3), 21.6 (CH_2), 23.8 (CH_2), [28.1, 28.32, 28.33, 28.4, 28.62, 28.64 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 62.2 (CH_2), 69.7 (CH), 70.9 (CH), 71.6 (CH), 72.4 (CH), 93.3 (CH), 172.6 (C); *m/z* (TOF ES+) 1274.2 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1273.7981 ($[M+Na]^+$) $C_{62}H_{130}NaO_{13}Si_6$ requires 1273.8025.

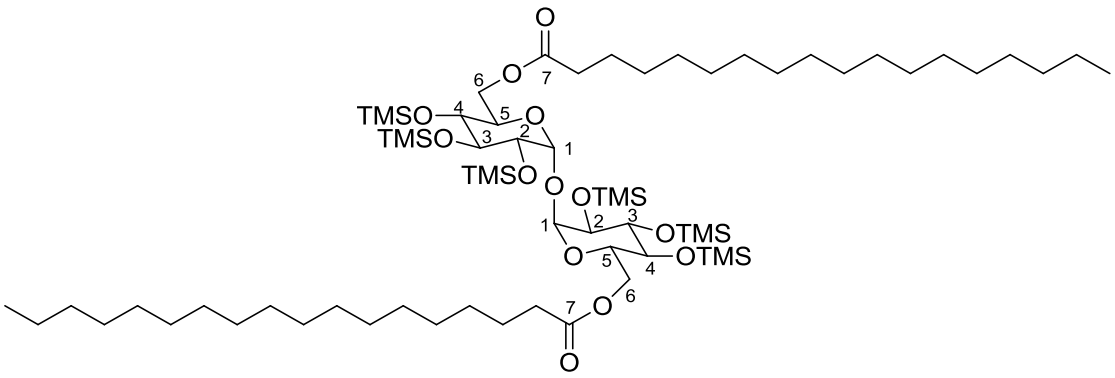
6,6'-di-*O*-hexadecanoyl-α,α-trehalose (140b)



Hexaol **140b** was prepared from trehalose **140a** (150 mg, 0.12 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide product **140b** as a white solid (72 mg, 73%): R_f = 0.32 (15% MeOH in $CHCl_3$); mp = 151-158 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3344 br s (O–H), 2916sm, 2850 m, 1722 w (C=O), 1644 w, 1467 w, 1150 w, 1104 m, 1016 m, 991 m; ^1H NMR (300 MHz, $CDCl_3$: CD_3OD 2:1)) δ ppm 0.89 (6H, t, *J* = 7.1, C(22)H_3), 0.94-1.58 (48H, stack), 1.63 (4H, m, C(9)H_aH_b), 2.35 (4H, t, *J* = 7.6, C(8)H_aH_b),

3.32-3.43 (2H, m, C(4)*H*), 3.53 (2H, dd, *J* = 9.8, 4.0, C(2)*H*), 3.87 (2H, app t, *J* = 9.8, C(3)*H*), 4.01 (2H, ddd, *J* = 10.5, 5.5, 2.3, C(5)*H*), 4.28 (2H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 5.5, C(6)H_aH_b), 4.35 (2H, B of ABX, $J_{B\text{-}A}$ = 12.4, $J_{B\text{-}X}$ = 2.3, C(6)H_aH_b), 5.11 (2H, d, *J* = 4.0, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD 2:1) δ ppm 15.2 (CH_3, C(22)), 24.0 (CH_2), 26.2 (CH_2, C(9)), [30.5, 30.6, 30.7, 30.8, 31.0 (CH_2, resonance overlap)], 33.2 (CH_2), 35.4 (CH_2, C(8)), 64.6 (CH_2, C(6)), 71.4 (CH, C(5)), 71.7 (CH, C(4)), 73.0 (CH, C(2)), 74.5 (CH, C(3)), 95.0 (CH, C(1)), 176.0 (C, C(7)); *m/z* (TOF ES+) 841.5 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 841.5679 ([M+Na]$^+$) $C_{44}H_{82}NaO_{13}$ requires 841.5653.

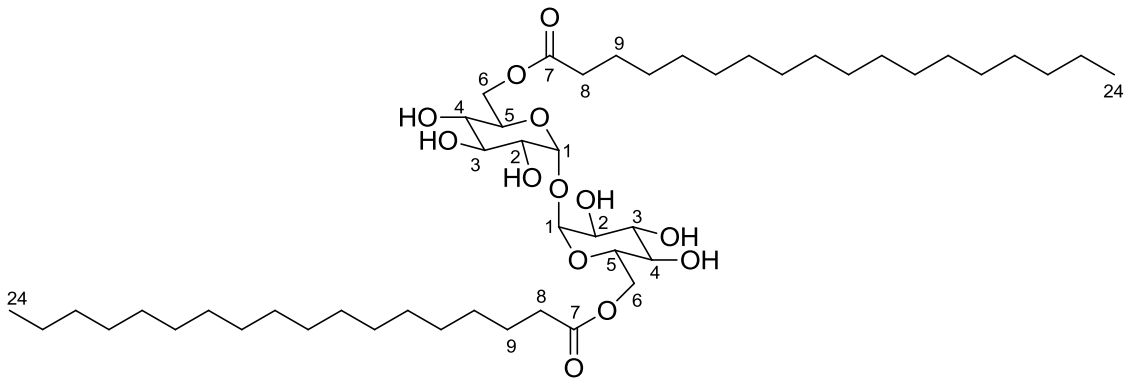
6,6'-di-*O*-octadecanoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (141a)



Di-ester **141a** was prepared from diol **137** (220 mg, 0.28 mmol), octadecanoic acid (239 mg, 0.84 mmol), DCC (173 mg, 0.84 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide di-ester **141a** as a colourless oil (231 mg, 61%): R_f = 0.37 (5% EtOAc in hexanes); $[\alpha]_D^{21}$ = 78.4 (c = 0.8, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1743 m (C=O), 1458 w, 1387 w, 1249 s, 1162 m, 1110 m, 1072 s, 1043 m, 1010 m, 966 m, 897 s, 872 s, 843 s, 748 s, 683 w; ^{1}H NMR

(300 MHz, $CDCl_3$) δ ppm −0.03-0.29 (54H, stack, Si(C*H*$_3$)$_3$), 0.86 (6H, t, *J* = 7.1, 2 × CH_2C*H*$_3$), 1.08-1.46 (56H, stack, alkyl chain C*H*$_2$), 1.55-1.76 (4H, stack, alkyl chain C*H*$_2$), 2.28-2.37 (4H, stack, C*H*$_2$ alkyl chain), 3.40-3.51 (4H, stack), 3.90 (2H, app t, *J* = 9.1), 3.95-4.12 (4H, stack), 4.28 (2H, dd, *J* = 11.9, 2.2), 4.91 (2H, d, *J* = 3.5, C(1)H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [0.1, 0.8, 1.0 (CH_3, TMS)], 14.1 (CH_3), 22.7 (CH_2), 24.8 (CH_2), [29.1, 29.3, 29.3, 29.4, 29.7 (CH_2, alkyl chain, resonance overlap)], 31.9 (CH_2), 34.1 (CH_2), 63.2 (CH_2), 70.7 (CH), 71.9 (CH), 72.7 (CH), 73.5 (CH), 94.3 (CH, C(1)H), 173.6 (C, C(7)); *m/z* (TOF ES+) 1330.6 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1329.8629 ([M]$^+$) $C_{66}H_{138}NaO_{13}Si_6$ requires 1329.8651.

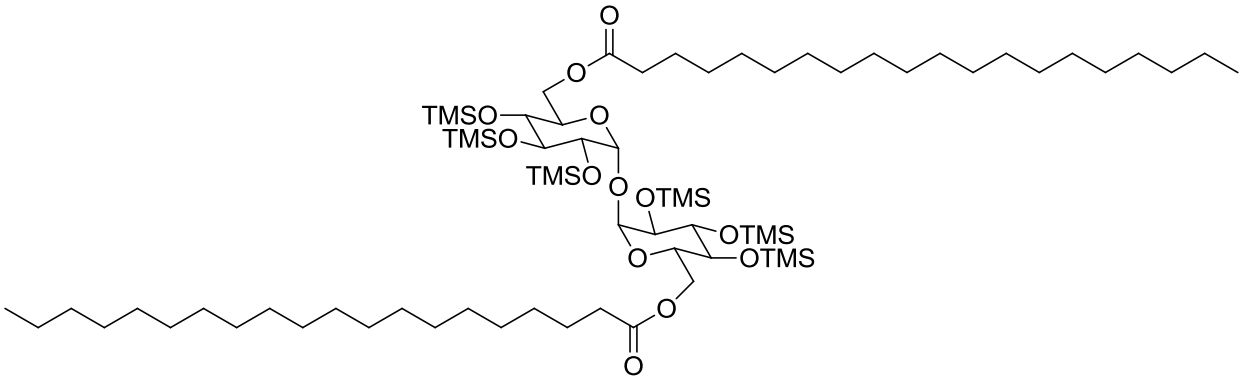
6,6'-di-*O*-octadecanoyl-α,α-trehalose (141b)



Hexaol **141b** was prepared from trehalose **141a** (136 mg, 0.10 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide product **141b** as a white solid (58 mg, 66%): R_f = 0.35 (15% MeOH in $CHCl_3$); mp = 158-164 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3339 br s (O–H), 2921 m, 2868 w, 1734 w (C=O), 1654 s, 1453 w, 1151 m, 1102 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD 2:1)) δ ppm 0.80

(6H, t, *J* = 7.0, C(24)H_3), 0.97-1.38 (56H, stack, CH_2 alkyl chain), 1.50-1.60 (4H, m, C(9)H_aH_b), 2.26 (4H, t, *J* = 8.2, C(8)H_aH_b), 3.30-3.42 (2H, m, C(4)*H*), 3.53 (2H, dd, *J* = 10.0, 4.0, C(2)*H*), 3.86 (2H, app t, *J* = 10.0, C(3)*H*), 4.01 (2H, ddd, *J* = 10.5, 4.8, 2.6, C(5)*H*), 4.35 (2H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 4.8, C(6)H_aH_b), 4.41 (2H, B of ABX, $J_{B\text{-}A}$ = 12.4, $J_{B\text{-}X}$ = 2.6, C(6)H_aH_b), 5.03 (2H, d, *J* = 4.0, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD 2:1) δ ppm 14.0 (CH_3, C(24)), 22.6 (CH_2), 24.8 (CH_2, C(9)), [29.1, 29.31, 29.32, 29.5, 29.6 (CH_2, alkyl chain resonance overlap)], 31.8 (CH_2), 34.1 (CH_2, C(8)), 63.1 (CH_2, C(6)), 70.0 (CH, C(5)), 70.1 (CH, C(4)), 71.5 (CH, C(2)), 72.8 (CH, C(3)), 93.4 (CH, C(1)), 174.5 (C, C(7)); *m/z* (TOF ES+) 897.8 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 897.6308 ([M+Na]$^+$) $C_{48}H_{90}NaO_{13}$ requires 897.6279.

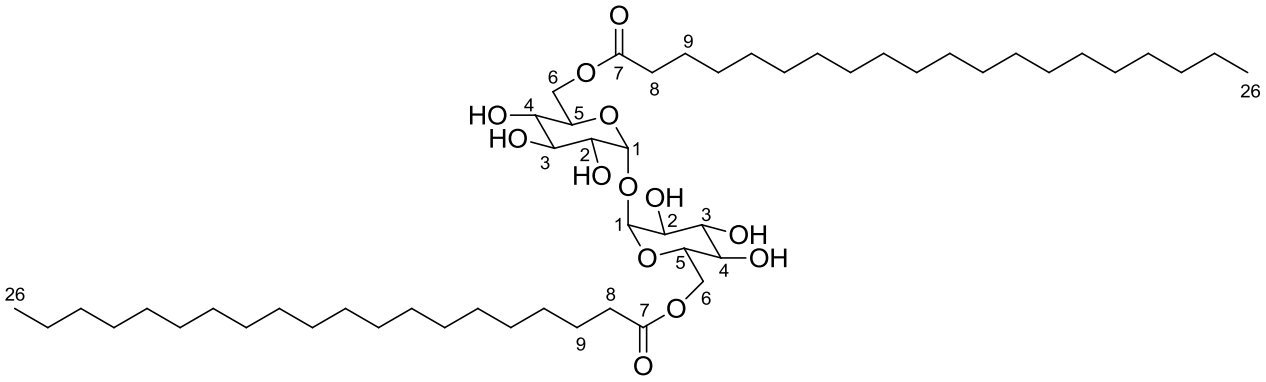
6,6'-di-*O*-eicosanoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (142a)



Di-ester **142a** was prepared from diol **137** (200 mg, 0.26 mmol), eicosanoic acid (243 mg, 0.77 mmol), DCC (159 mg, 0.77 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide di-ester **142a** as a colourless oil (300 mg, 85%): R_f = 0.38 (5% EtOAc in hexanes); $[\alpha]_D^{22}$ = 73.1 (c = 1.0,

$CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1743 m (C=O), 1459 w, 1388 w, 1249 s, 1162 m, 1072 s, 1044 m, 1010 m, 965 m, 897 s, 875 s, 842 s, 748 s, 683 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.20-0.23 (54H, stack), 0.75 (6H, t, *J* = 7.0), 0.80-1.45 (64H, stack), 1.45-1.64 (4H, stack), 2.16-2.27 (4H, stack), 3.26-3.43 (4H, stack), 3.78 (2H, app t, *J* = 9.1), 3.83-4.00 (4H, stack), 4.16 (2H, dd, *J* = 11.9, 2.1), 4.80 (2H, d, *J* = 3.5); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.2, −0.0 (CH_3, TMS)], 13.1 (CH_3), 21.6 (CH_2), 23.8 (CH_2), [28.1, 28.31, 28.32, 28.4, 28.7 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 62.2 (CH_2), 69.7 (CH), 70.9 (CH), 71.6 (CH), 72.4 (CH), 93.3 (CH), 172.6 (C); *m/z* (TOF ES+) 1386.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1385.9292 ([M+Na]$^+$) $C_{70}H_{146}NaO_{13}Si_6$ requires 1385.9277.

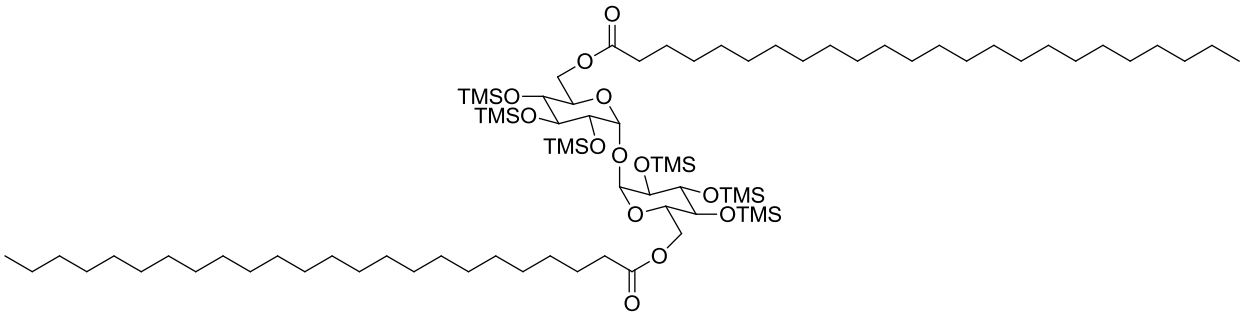
6,6'-di-*O*-eicosanoyl-α,α-trehalose (142b)



Hexaol **142b** was prepared from trehalose **142a** (300 mg, 0.22 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide hexaol **142b** as a white solid (127 mg, 62%): R_f = 0.37 (15% MeOH in $CHCl_3$); mp = 149-152 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate

optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O–H), 2929 m, 1722 m (C=O), 1674 s, 1454 w, 1377 w, 1266 w, 1150 m, 1015 s, 991 s, 941 w; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD 2:1)) δ ppm 0.66 (6H, t, *J* = 7.0, C(26)H_3), 0.70-1.35 (64H, stack), 1.35-1.47 (4H, m, C(9)H_aH_b), 2.13 (4H, t, *J* = 7.9, C(8)H_aH_b), 3.31-3.40 (2H, m, C(4)*H*), 3.53 (2H, dd, *J* = 9.7, 4.0, C(2)*H*), 3.55 (2H, app t, *J* = 9.7, C(3)*H*), 3.75 (2H, ddd, *J* = 10.5, 4.8, 2.6, C(5)*H*), 4.06 (2H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 5.3, C(6)H_aH_b), 4.11 (2H, B of ABX, $J_{B\text{-}A}$ = 12.4, $J_{B\text{-}X}$ = 2.3, C(6)H_aH_b), 4.88 (2H, d, *J* = 4.0, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD 2:1) δ ppm 14.1 (CH_3, C(26)), 22.7 (CH_2), 24.9 (CH_2, C(9)), [29.2, 29.42, 29.43, 29.6, 29.8 (CH_2, resonance overlap)], 32.0 (CH_2), 34.2 (CH_2, C(8)), 63.2 (CH_2, C(6)), 70.1 (CH, C(5)), 70.2 (CH, C(4)), 71.6 (CH, C(2)), 73.0 (CH, C(3)), 93.6 (CH, C(1)), 174.6 (C, C(7)); *m/z* (TOF ES+) 953.8 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 953.6949 ([M+Na]$^+$) $C_{52}H_{98}NaO_{13}$ requires 953.6905.

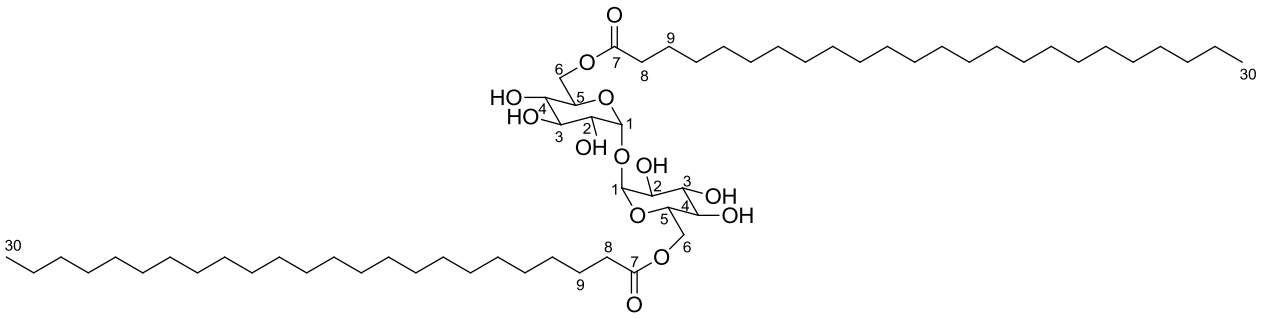
6,6'-di-*O*-tetracosanoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (143a)



Di-ester **143a** was prepared from diol **137** (180 mg, 0.23 mmol), tetracosanoic acid (258 mg, 0.70 mmol), DCC (144 mg, 0.70 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to

provide di-ester **143a** as a white solid (197 mg, 58%): R_f = 0.37 (5% EtOAc in hexanes); mp = 54-58 °C; $[\alpha]_D^{22}$ = 65.8 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 1742 m (C=O), 1457 w, 1379 w, 1250 s, 1162 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 898 s, 873 s, 843 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08-0.19 (54H, stack), 0.87 (6H, t, *J* = 7.0), 1.00-1.52 (80H, stack), 1.58-1.72 (4H, stack), 2.29-2.39 (4H, stack), 3.40-3.53 (4H, stack), 3.90 (2H, app t, *J* = 9.1), 3.96-4.11 (4H, stack), 4.28 (2H, dd, *J* = 11.9, 2.2), 4.92 (2H, d, *J* = 3.4); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.0, 0.2, 0.9 (CH_3, TMS)], 14.1 (CH_3), 22.7 (CH_2), 24.8 (CH_2), [29.2, 29.3, 29.4, 29.5, 29.7 (CH_2, resonance overlap)], 31.9 (CH_2), 34.1 (CH_2), 63.3 (CH_2), 70.7 (CH), 71.9 (CH), 72.7 (CH), 73.5 (CH), 94.3 (CH), 173.7 (C); *m/z* (TOF ES+) 1498.3 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1498.0564 ($[M+Na]^+$) $C_{78}H_{162}NaO_{13}Si_6$ requires 1498.0529.

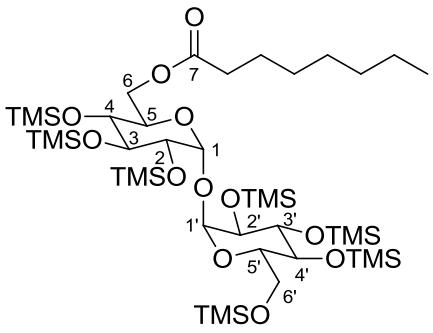
6,6'-di-*O*-tetracosanoyl-α,α-trehalose (143b)



Hexaol **143b** was prepared from trehalose **143a** (115 mg, 0.078 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide hexaol **143b** as a white solid (54 mg, 66%): R_f = 0.45 (15% MeOH in $CHCl_3$); mp = 118-125 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate

optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O–H), 2928 m, 2857 w, 1723 m (C=O), 1674 s, 1454 w, 1378 w, 1266 w, 1150 m, 1105 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD 2:1)) δ ppm 0.66 (6H, t, *J* = 7.0, C(30)H_3), 0.70-1.35 (80H, stack), 1.34-1.42 (4H, m, C(9)H_aH_b), 2.13 (4H, t, *J* = 7.9, C(8)H_aH_b), 3.33-3.41 (2H, m, C(4)*H*), 3.53 (2H, dd, *J* = 9.7, 4.0, C(2)*H*), 3.55 (2H, app t, *J* = 9.7, C(3)*H*), 3.75 (2H, ddd, *J* = 10.5, 4.8, 2.6, C(5)*H*), 4.06 (2H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 5.3, C(6)H_aH_b), 4.11 (2H, B of ABX, $J_{B\text{-}A}$ = 12.4, $J_{B\text{-}X}$ = 2.3, C(6)H_aH_b), 4.88 (2H, d, *J* = 4.0, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD 2:1) δ ppm 14.1 (CH_3, C(30)), 22.8 (CH_2), 25.0 (CH_2, C(9)), [29.3, 29.4, 29.5, 29.6, 29.8 (CH_2, resonance overlap)], 32.0 (CH_2), 34.3 (CH_2, C(8)), 63.3 (CH_2, C(6)), 70.2 (CH, C(5)), 70.4 (CH, C(4)), 71.8 (CH, C(2)), 73.4 (CH, C(3)), 93.7 (CH, C(1)), 174.7 (C, C(7)); *m/z* (TOF ES+) 1065.4 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1065.8177 ([M+Na]$^+$) $C_{60}H_{114}NaO_{13}$ requires 1065.8157.

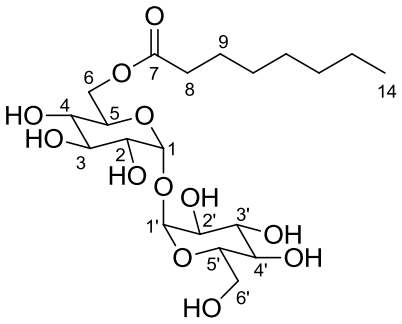
6-*O*-octanoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (144a)



Mono-ester **144a** was prepared from alcohol **136** (170 mg, 0.2 mmol), octanoic acid (47 µL, 0.3 mmol), DCC (61 mg, 0.3 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide mono-ester **144a** as a colourless oil (148 mg, 76%): R_f = 0.43 (5% EtOAc in hexanes); $[\alpha]_D^{22}$ = 74.2 (c = 1.0,

$CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2854 s, 1742 m (C=O), 1457 w, 1379 w, 1249 s, 1162 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 898 s, 873 s, 843 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.01-0.10 (63H, stack), 0.75 (3H, t, *J* = 6.0), 1.10-1.28 (10H, stack), 2.18-2.30 (2H, m), 3.24-3.40 (4H, stack), 3.54 (2H, app. d, *J* = 3.1), 3.64 (1H, dt, *J* = 9.4, 3.0), 3.77-3.83 (2H, stack), 3.85-4.13 (2H, stack), 4.17 (1H, dd, *J* = 11.6, 1.9), 4.77-4.83 (2H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.0, 0.3 (CH_3, TMS, resonance overlap)], 13.0 (CH_3), 21.5 (CH_2), 23.7 (CH_2), 27.9 (CH_2), 28.0 (CH_2), 30.6 (CH_2), 33.1 (CH_2), 60.8 (CH_2), 61.9 (CH_2), 69.5 (CH), 70.5 (CH), 70.9 (CH), 71.6 (CH), 71.8 (CH), 72.3 (CH), 72.4 (CH), 72.5 (CH), 93.6 (CH), 94.0 (CH), 171.6 (C); *m/z* (TOF ES+) 995.8 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 995.4871 ([M+Na]$^+$) $C_{41}H_{92}NaO_{12}Si_7$ requires 995.4866.

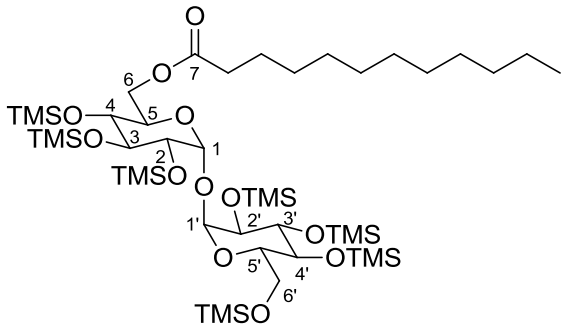
6-*O*-octanoyl-α,α-trehalose (144b)



Heptaol **144b** was prepared from trehalose **144a** (234 mg, 0.24 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide heptaol **144b** as a colourless oil (83 mg, 74%): $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3316 br s (O–H), 2928 m, 2858 w, 1741 w (C=O), 1674 s, 1437 w, 1357 w, 1202 s, 1144 s, 1106 s, 1078 s, 992 s,

942 m, 842 m, 804 m, 724 m; R_f = 0.40 (30% MeOH in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.89 (3H, t, *J* = 6.9, C(14)H_3), 1.19-1.43 (8H, stack), 1.58-1.69 (2H, m, C(9)H_aH_b), 2.35 (2H, t, *J* = 7.6, C(8)H_aH_b), 3.34-3.43 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.51-3.60 (2H, stack, C(2)*H,* C(2')*H*), 3.71 (1H, dd, *J* = 12.6, 6.0, C(6')H_aH_b), 3.79-3.95 (3H, stack, C(6')H_aH_b, C(3)*H*, C(3')*H*), 4.02 (1H, ddd, *J* = 10.1, 4.6, 2.1, C(5)*H*), 4.28 (1H, A of ABX, J_{A-B} = 12.1, J_{A-X} = 4.6, C(6)H_aH_b), 4.32 (1H, B of ABX, J_{A-B} = 12.1, J_{B-X} = 2.1, C(6)H_aH_b), 5.09-5.16 (2H, stack, C(1)*H*, C(1')*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 15.1 (CH_3, C(14)), 23.9 (CH_2), 26.2 (CH_2, C(9)), 30.2 (CH_2), 30.4 (CH_2), 33.0 (CH_2), 35.4 (CH_2, C(8)), 63.0 (CH_2, C(6')), 64.5 (CH_2, C(6)), 71.4 (CH, C(5)), 71.7 (CH, C(5')), 72.0 (CH, C(4)), 73.01 (CH, C(4')), 73.02 (CH, C(2)), 73.7 (CH, C(2')), 74.2 (CH, C(3)), 74.5 (CH, C(3')), 95.0 (CH, C(1)), 95.2 (CH, C(1')), 176.0 (C, C(7)); *m/z* (TOF ES+) 491.2 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 491.2103 ($[M+Na]^+$) $C_{20}H_{36}NaO_{12}$ requires 491.2104.

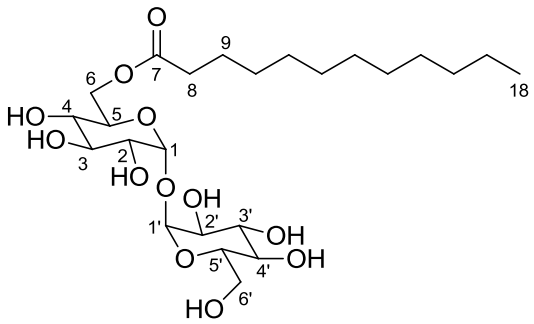
6-*O*-dodecanoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (145a)



Mono-ester **145a** was prepared from alcohol **136** (170 mg, 0.2 mmol), dodecanoic acid (60 mg, 0.3 mmol), DCC (61 mg, 0.3 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide mono-

ester **145a** as a colourless oil (161 mg, 78%): R_f = 0.42 (5% EtOAc in hexanes); $[\alpha]_D^{25}$ = 61.6 (c = 1.5, CH_3Cl); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1742 m (C=O), 1456 w, 1379 w, 1250 s, 1162 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 898 s, 872 s, 842 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.20-0.23 (63H, stack), 0.75 (3H, t, *J* = 6.7), 1.07-1.25 (16H, stack), 1.45-1.56 (2H, m), 2.18-2.26 (2H, m), 3.22-3.42 (4H, stack), 3.54 (2H, app. d, *J* = 3.0), 3.65 (1H, dt, *J* = 9.4, 3.0), 3.71-3.82 (2H, stack), 3.85-4.03 (2H, stack), 4.17 (1H, dd, *J* = 11.3, 1.4), 4.74-4.84 (2H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−8.9, −1.3, 0.0 (CH_3, TMS, resonance overlap)], 13.0 (CH_3), 21.7 (CH_2), 23.9 (CH_2), [28.1, 28.3, 28.4, 28.5, 28.6 (CH_2, resonance overlap)], 30.9 (CH_2), 33.0 (CH_2), 60.8 (CH_2), 61.9 (CH_2), 69.9 (CH), 70.4 (CH), 71.2 (CH), 72.0 (2 × CH), 72.5 (CH), 72.8 (2 × CH), 93.5 (CH), 94.0 (CH), 171.5 (C); *m/z* (TOF ES+) 1051.7 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1051.5539 ($[M+Na]^+$) $C_{45}H_{100}NaO_{12}Si_7$ requires 1051.5497.

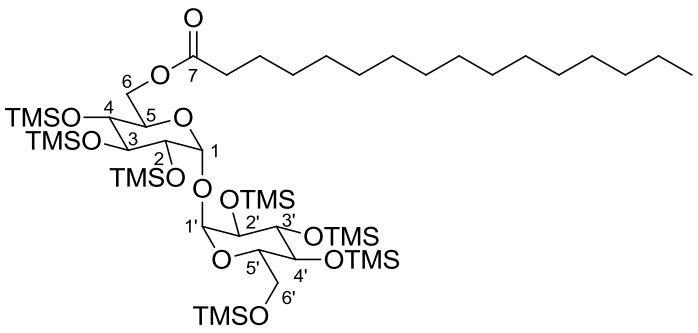
6-*O*-dodecanoyl-α,α-trehalose (145b)



Heptaol **145b** was prepared from trehalose **145a** (160 mg, 0.16 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide heptaol **145b** as a colourless gel (65 mg, 78%): R_f = 0.44 (30% MeOH in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical

rotation; ν_{max}(film)/cm^{-1} 3380 br s (O–H), 2924 s, 2858 m, 1718 m (C=O), 1675 m, 1050 s, 994 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.89 (3H, t, *J* = 6.8, C(18)H_3), 1.19-1.41 (16H, stack), 1.56-1.66 (2H, m, C(9)H_aH_b), 2.35 (2H, t, *J* = 7.6, C(8)H_aH_b), 3.32-3.45 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.53-3.60 (2H, stack, C(2)*H,* C(2')*H*), 3.72 (1H, dd, *J* = 12.6, 6.0, C(6')H_aH$_b$), 3.79-3.95 (3H, stack, C(6')H$_a$*H$_b$*, C(3)*H*, C(3')*H*), 4.02 (1H, ddd, *J* = 10.1, 4.6, 2.1, C(5)*H*), 4.28 (1H, A of ABX, $J_{A\text{-}B}$ = 12.1, $J_{A\text{-}X}$ = 4.6, C(6)H_aH_b), 4.36 (1H, B of ABX, $J_{A\text{-}B}$ = 12.1, $J_{B\text{-}X}$ = 2.1, C(6)H$_a$*H$_b$*), 5.13 (2H, app. dd, *J* = 12.4, 3.6, C(1)*H*, C(1')*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 15.2 (CH_3, C(18)), 24.0 (CH_2), 26.2 (CH_2, C(9)), [30.4, 30.5, 30.6, 30.8, 31.0 (CH_2, resonance overlap)], 33.2 (CH_2), 35.5 (CH_2, C(8)), 63.0 (CH_2, C(6')), 64.5 (CH_2, C(6)), 71.4 (CH, C(5)), 71.7 (CH, C(5')), 72.0 (CH, C(4)), 73.0 (CH, C(4')), 73.0 (CH, C(2)), 73.8 (CH, C(2')), 74.2 (CH, C(3)), 74.4 (CH, C(3')), 95.0 (CH, C(1)), 95.2 (CH, C(1')), 176.0 (C, C(7)); *m/z* (TOF ES+) 547.1 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 547.2727 ([M+Na]$^+$) $C_{24}H_{44}NaO_{12}$ requires 547.2730.

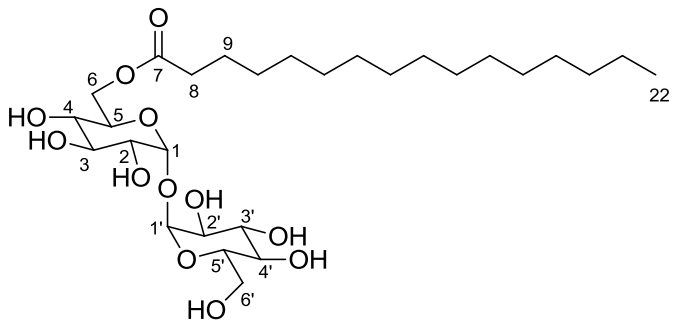
6-*O*-hexadecanoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (146a)



Mono-ester **146a** was prepared from alcohol **136** (170 mg, 0.2 mmol), hexadecanoic acid (62 mg, 0.24 mmol), DCC (50 mg, 0.24 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to

provide mono-ester **146a** as a colourless oil (150 mg, 69%): R_f = 0.42 (5% EtOAc in hexanes); $[\alpha]_D^{22}$ = 62.9 (c = 0.7, $CHCl_3$); ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 1742 m (C=O), 1457 w, 1381 w, 1250 s, 1162 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 896 s, 872 s, 842 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.20-0.23 (63H, stack), 0.75 (3H, t, *J* = 6.7), 1.07-1.25 (24H, stack), 1.42-1.55 (2H, m), 2.18-2.25 (2H, m), 3.22-3.42 (4H, stack), 3.54 (2H, app. d, *J* = 3.0), 3.65 (1H, dt, *J* = 9.4, 3.0), 3.71-3.82 (2H, stack), 3.85-4.03 (2H, stack), 4.17 (1H, dd, *J* = 11.3, 1.4), 4.74-4.84 (2H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−2.1, −1.4, −0.9, −0.2, −0.0, 0.3 (CH_3, TMS, resonance overlap)], 13.1 (CH_3), 21.7 (CH_2), 23.8 (CH_2), [28.1, 28.27, 28.3, 28.4, 28.6 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 60.9 (CH_2), 62.3 (CH_2), 69.5 (CH), 70.5 (CH), 70.9 (CH), 71.7 (CH), 71.8 (CH), 72.3 (CH), 72.4 (CH), 72.5 (CH), 93.1 (CH), 93.4 (CH), 172.6 (C); *m/z* (TOF ES+) 1107.8 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1107.6132 ($[M+Na]^+$) $C_{49}H_{108}NaO_{12}Si_7$ requires 1107.6123.

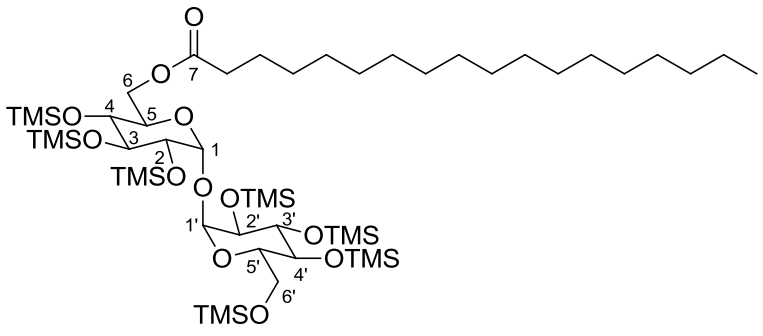
6-*O*-hexadecanoyl-α,α-trehalose (146b)



Heptaol **146b** was prepared from trehalose **146a** (90 mg, 0.083 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide heptaol **146b** as a colourless oil (36 mg, 75%): R_f = 0.44 (30%

MeOH in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3342 brs (O–H), 2918 s, 2850 s, 2476 w, 1736 m (C=O), 1032 s, 993 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.80 (3H, t, *J* = 7.1, C(22)H_3), 0.99-1.39 (24H, stack), 1.48-1.61 (2H, m, C(9)H_aH_b), 2.30 (2H, t, *J* = 7.9, C(8)H_aH_b), 3.16-3.36 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.37-3.54 (2H, stack, C(2)*H,* C(2')*H*), 3.62 (1H, dd, *J* = 12.6, 6.0, C(6')H_aH_b), 3.59-3.68 (3H, stack, C(6')H_aH_b, C(3)*H*, C(3')*H*), 3.94 (1H, ddd, *J* = 10.1, 4.8, 2.1, C(5)*H*), 4.18 (1H, A of ABX, $J_{A\text{-}B}$ = 12.2, $J_{A\text{-}X}$ = 4.8, C(6)H_aH_b), 4.36 (1H, B of ABX, $J_{A\text{-}B}$ = 12.2, $J_{B\text{-}X}$ = 2.1, C(6)H_aH_b), 5.00-5.10 (2H, stack, C(1)*H*, C(1')*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD 2:1) δ ppm 15.1 (CH_3, C(22)), 24.0 (CH_2), 26.2 (CH_2, C(9)), [30.4, 30.5, 30.6, 30.8, 31.0 (CH_2, resonance overlap)], 33.2 (CH_2), 35.4 (CH_2, C(8)), 63.0 (CH_2, C(6')), 64.5 (CH_2, C(6)), 71.4 (CH, C(5)), 71.8 (CH, C(5')), 72.0 (CH, C(4)), 73.0 (CH, C(4')), 73.1 (CH, C(2)), 73.8 (CH, C(2')), 74.4 (CH, C(3)), 74.6 (CH, C(3')), 95.1 (CH, C(1)), 95.2 (CH, C(1')), 176.0 (C, C(7)); *m/z* (TOF ES+) 603.3 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 603.3368 ($[M+Na]^+$) $C_{28}H_{52}NaO_{12}$ requires 603.3356.

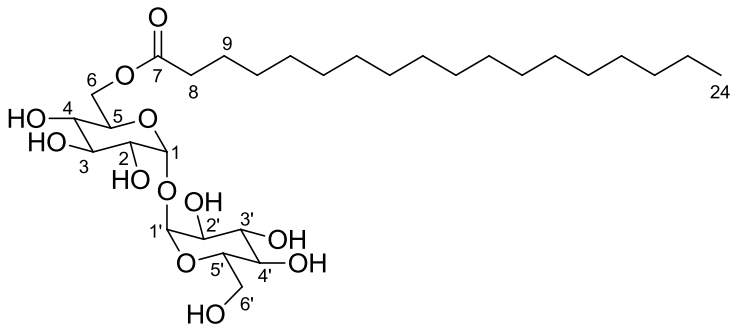
6-*O*-octadecanoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (147a)



Mono-ester **147a** was prepared from alcohol **136** (200 mg, 0.24 mmol), octadecanoic acid (108 mg, 0.38 mmol), DCC (78 mg, 0.38 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude

product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide mono-ester **147a** as a white solid (168 mg, 63%): R_f = 0.43 (5% EtOAc in hexanes); mp = 130-136 °C; $[\alpha]_D^{25}$ = 65.6 (c = 1.3, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2854 s, 1742 m (C=O), 1456 w, 1379 w, 1249 s, 1159 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 898 s, 873 s, 842 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.35-0.34 (63H, stack, Si(CH_3)$_3$), 0.74 (3H, t, *J* = 7.0, CH_2CH_3), 0.97-1.25 (28H, stack, CH_2 alkyl chain), 1.46-1.52 (2H, m), 2.15-2.26 (2H, m), 3.21-3.40 (4H, stack), 3.53 (2H, app. d, *J* = 3.5), 3.63 (1H, dt, *J* = 9.6, 3.1), 3.70-3.81 (2H, stack), 3.84-3.97 (2H, stack), 4.16 (1H, dd, *J* = 11.9, 2.1), 4.73-4.83 (2H, stack, C(1)*H*, C(1')*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm[−0.3, 0.1, 0.9, 1.1 (CH_3, TMS, resonance overlap)], 14.1 (CH_3, CH_2CH_3), 22.7 (CH_2), 24.8 (CH_2), [29.2, 29.3, 29.4, 29.7 (CH_2, alkyl chain resonance overlap)], 31.9 (CH_2), 34.2 (CH_2), 61.9 (CH_2), 63.3 (CH_2), 70.6 (CH), 71.6 (CH), 72.0 (CH), 72.7 (CH), 72.8 (CH), 73.4 (CH), 73.5 (CH), 73.6 (CH), 94.2 (CH), 94.5 (CH), 173.7 (C(7)); *m/z* (TOF ES+) 1135.3 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1135.6482 ([M+Na]$^+$) $C_{51}H_{112}NaO_{12}Si_7$ requires 1135.6436.

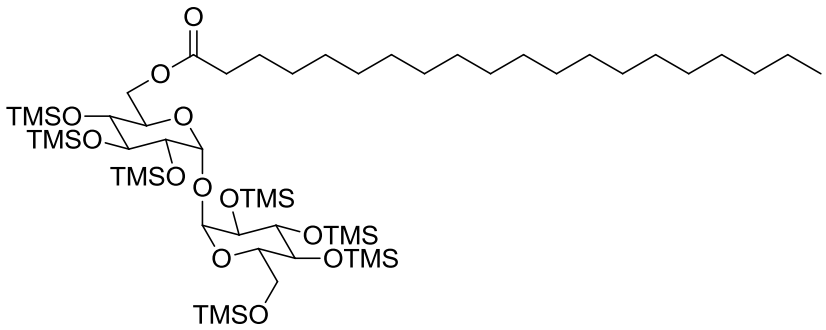
6-*O*-octadecanoyl-α,α-trehalose (147b)



Heptaol **147b** was prepared from trehalose **147a** (156 mg, 0.14 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in

hexane, gradient) to provide heptaol **147b** as a colourless oil (69 mg, 81%): R_f = 0.45 (30% MeOH in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3380 br s (O–H), 2935 s, 2858 m, 1732 w (C=O), 1050 s, 994 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.68 (3H, t, *J* = 7.1, C(24)H_3), 0.89-1.24 (28H, stack, CH_2 alkyl chain), 1.35-1.48 (2H, m, C(9)H_aH_b), 2.15 (2H, t, *J* = 8.0, C(8)H_aH_b), 3.14-3.23 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.32-3.41 (2H, stack, C(2)*H,* C(2')*H*), 3.51 (1H, dd, *J* = 13.1, 6.2, C(6')H_aH$_b$), 3.59-3.77 (3H, stack, C(6')H$_a$$H_b$, C(3)*H*, C(3')*H*), 3.83 (1H, ddd, *J* = 9.9, 4.8, 2.5, C(5)*H*), 4.08 (1H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 4.8, C(6)H_aH$_b$), 4.16 (1H, B of ABX, $J_{A\text{-}B}$ = 12.4, $J_{B\text{-}X}$ = 2.5, C(6)H$_a$$H_b$), 4.88-4.97 (2H, stack, C(1)*H*, C(1')*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 13.6 (CH_3, C(24)), 22.3 (CH_2), 24.5 (CH_2, C(9)), [28.8, 28.9, 29.1, 29.3 (CH_2, alkyl chain resonance overlap)], 31.5 (CH_2), 33.7 (CH_2, C(8)), 61.4 (CH_2, C(6')), 62.8 (CH_2, C(6)), 69.7 (CH, C(5)), 69.9 (CH, C(5')), 70.2 (CH, C(4)),71.2 (CH, C(4')), 71.3 (CH, C(2)), 72.1 (CH, C(2')), 72.4 (CH, C(3)), 72.7 (CH, C(3')), 93.4 (CH, C(1)), 93.5 (CH, C(1')), 174.2 (C, C(7)); *m/z* (TOF ES+) 631.4 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 631.3679 ($[M+Na]^+$) $C_{30}H_{56}NaO_{12}$ requires 631.3669.

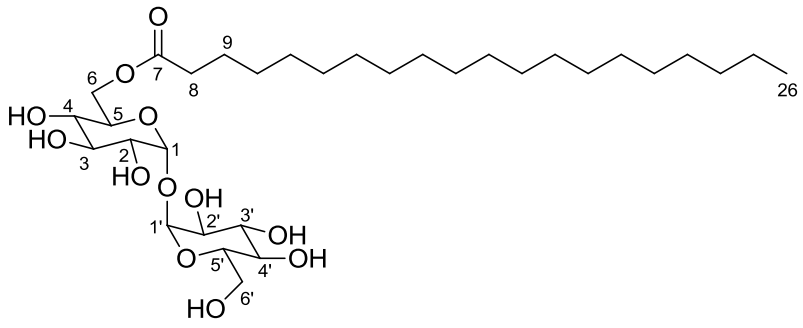
6-*O*-eicosanoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (148a)



Mono-ester **148a** was prepared from alcohol **136** (200 mg, 0.24 mmol), eicosanoic acid (111 mg, 0.35 mmol), DCC (72 mg, 0.35 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL)

according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide mono-ester **148a** as a colourless oil (175 mg, 64%): R_f = 0.40 (5% EtOAc in hexanes); $[\alpha]_D^{25}$ = 78.3 (c = 1.4, $CHCl_3$); ν_{max}(film)/cm^{-1} 2924 s, 2853 s, 1742 m (C=O), 1455 w, 1379 w, 1250 s, 1161 m, 1110 m, 1075 s, 1044 m, 1010 m, 965 m, 895 s, 872 s, 843 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.20-0.23 (63H, stack), 0.75 (3H, t, *J* = 6.7), 1.07-1.25 (32H, stack), 1.45-1.55 (2H, m), 2.17-2.25 (2H, m), 3.22-3.44 (4H, stack), 3.54 (2H, app. d, *J* = 3.0), 3.65 (1H, dt, *J* = 9.4, 3.0), 3.71-3.84 (2H, stack), 3.85-4.00 (2H, stack), 4.18 (1H, dd, *J* = 11.3, 1.4), 4.74-4.86 (2H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.2, 0.0 (CH_3, TMS, resonance overlap)], 13.1 (CH_3), 21.7 (CH_2), 23.8 (CH_2), [28.1, 28.32, 28.33, 28.4, 28.6 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 60.9 (CH_2), 62.3 (CH_2), 69.5 (CH), 70.5 (CH), 70.9 (CH), 71.7 (CH), 71.9 (CH), 72.3 (CH), 72.4 (CH), 72.5 (CH), 93.1 (CH), 93.4 (CH), 172.6 (C); *m/z* (TOF ES+) 1163.6 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1163.6792 ([M+Na]$^+$) $C_{53}H_{116}NaO_{12}Si_7$ requires 1163.6749.

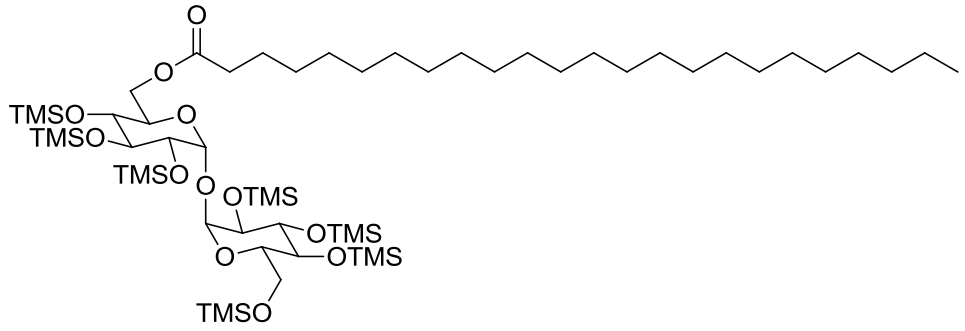
6-*O*-eicosanoyl-α,α-trehalose (148b)



Heptaol **148b** was prepared from trehalose **148a** (145 mg, 0.13 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up

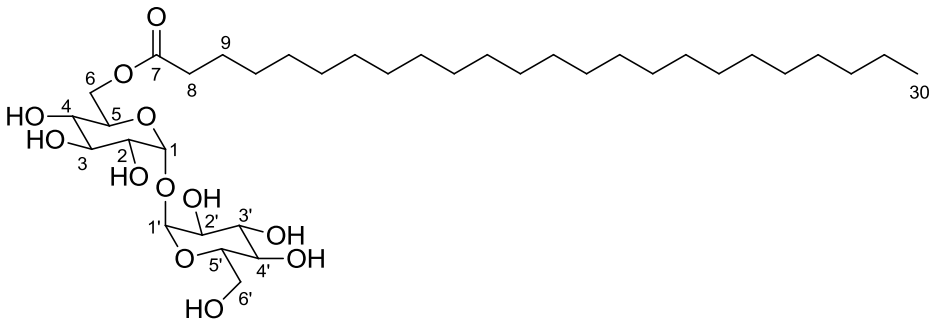
provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide heptaol **148b** as a white solid (55 mg, 67%): R_f = 0.45 (30% MeOH in $CHCl_3$); mp = 131-135 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3352 br w (O–H), 2917 s, 2850 m, 1721 w (C=O), 1467 w, 1024 s, 989 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.79 (3H, t, *J* = 7.0, C(26)H_3), 0.84-1.46 (32H, stack), 1.50-1.64 (2H, m, C(9)H_aH_b), 2.26 (2H, t, *J* = 7.9, C(8)H_aH_b), 3.19-3.34 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.38-3.51 (2H, stack, C(2)*H,* C(2')*H*), 3.61 (1H, dd, *J* = 12.0, 5.8, C(6')H_aH_b), 3.65-3.80 (3H, stack, C(6')H_aH_b, C(3)*H*, C(3')*H*), 3.91 (1H, ddd, *J* = 10.3, 4.7, 2.4, C(5)*H*), 4.19 (1H, A of ABX, $J_{A\text{-}B}$ = 12.0, $J_{A\text{-}X}$ = 4.7, C(6)H_aH_b), 4.26 (1H, B of ABX, $J_{A\text{-}B}$ = 12.0, $J_{B\text{-}X}$ = 2.4, C(6)H_aH_b), 4.99-5.01 (2H, stack, C(1)*H*, C(1')*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 15.1 (CH_3, C(26)), 24.0 (CH_2), 26.2 (CH_2, C(9)), [30.4, 30.62, 30.63, 30.8, 31.0 (CH_2, resonance overlap)], 33.2 (CH_2), 35.4 (CH_2, C(8)), 63.1 (CH_2, C(6')), 64.5 (CH_2, C(6)), 71.4 (CH, C(5)), 71.7 (CH, C(5')), 72.1 (CH, C(4)), 73.0 (CH, C(4')), 73.1 (CH, C(2)), 73.7 (CH, C(2')), 74.4 (CH, C(3)), 74.6 (CH, C(3')), 95.0 (CH, C(1)), 95.2 (CH, C(1')), 176.0 (C, C(7)); *m/z* (TOF ES+) 659.2 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 659.3991 ($[M+Na]^+$) $C_{32}H_{60}NaO_{12}$ requires 659.3982.

6-*O*-tetracosanoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (149a)



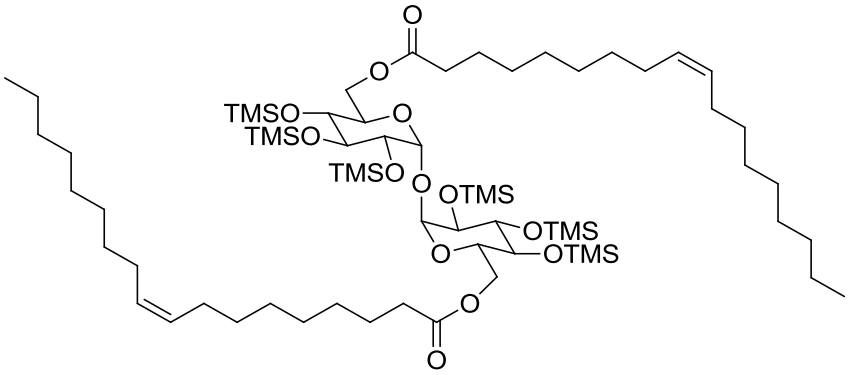
Mono-ester **149a** was prepared from alcohol **136** (200 mg, 0.24 mmol), tetracosanoic acid (131 mg, 0.35 mmol), DCC (72 mg, 0.35 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide mono-ester **149a** as a colourless oil (207 mg, 72%): R_f = 0.44 (5% EtOAc in hexanes); $[\alpha]_D^{25}$ = 76.4 (c = 1.0, CH_3Cl); ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 1742 m (C=O), 1457 w, 1375 w, 1252 s, 1162 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 896 s, 874 s, 842 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.20-0.23 (63H, stack), 0.76 (3H, t, *J* = 6.7), 1.06-1.23 (40H, stack), 146-1.53 (2H, m), 2.18-2.26 (2H, m), 3.23-3.41 (4H, stack), 3.54 (2H, app. d, *J* = 3.5), 3.64 (1H, dt, *J* = 9.4, 3.5), 3.71-3.85 (2H, stack), 3.85-4.00 (2H, stack), 4.17 (1H, dd, *J* = 11.3, 1.4), 4.75-4.84 (2H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.2, 0.0 (CH_3, TMS, resonance overlap)], 13.1 (CH_3), 21.7 (CH_2), 23.8 (CH_2), [28.1, 28.31, 28.32, 28.4, 28.7 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 60.9 (CH_2), 62.3 (CH_2), 69.6 (CH), 70.5 (CH), 70.9 (CH), 71.6 (CH), 71.8 (CH), 72.3 (CH), 72.4 (CH), 72.5 (CH), 93.1 (CH), 93.4 (CH), 172.7 (C); *m/z* (TOF ES+) 1220.0 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1219.7332 ($[M+Na]^+$) $C_{57}H_{124}NaO_{12}Si_7$ requires 1219.7375.

6-*O*-tetracosanoyl-α,α-trehalose (149b)



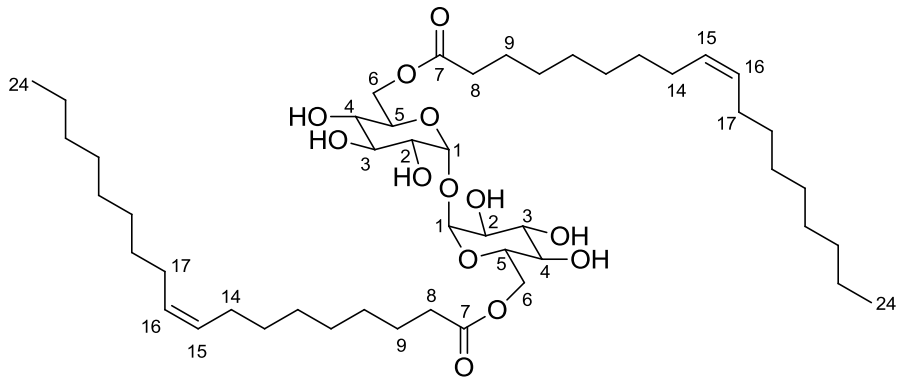
Heptaol **149b** was prepared from trehalose **149a** (360 mg, 0.30 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide heptaol **149b** as a white solid (137 mg, 66%): R_f = 0.45 (30% MeOH in $CHCl_3$); mp = 161-168 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3381 br s (O–H), 2925 s, 2858 m, 1717 m (C=O), 1676 m, 1050 s, 995 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.64 (3H, t, *J* = 6.9, C(30)H_3), 0.71-1.30 (40H, stack), 1.36-1.44 (2H, m, C(9)H_aH_b), 2.11 (2H, t, *J* = 7.9, C(8)H_aH_b), 3.05-3.21 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.24-3.40 (2H, stack, C(2)*H,* C(2')*H*), 3.61 (1H, dd, *J* = 12.8, 6.3, C(6')H_aH$_b$), 3.54-3.71 (3H, stack, C(6')H$_a$$H_b$, C(3)*H*, C(3')*H*), 3.91 (1H, ddd, *J* = 9.9, 4.5, 2.4, C(5)*H*), 4.04 (1H, A of ABX, $J_{A\text{-}B}$ = 12.2, $J_{A\text{-}X}$ = 4.5, C(6)H_aH$_b$), 4.12 (1H, B of ABX, $J_{A\text{-}B}$ = 12.2, $J_{B\text{-}X}$ = 2.4, C(6)H$_a$$H_b$), 4.99-5.01 (2H, stack, C(1)*H*, C(1')*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 14.2 (CH_3, C(30)), 23.0 (CH_2), 25.1 (CH_2, C(9)), [29.5, 29.6, 29.8, 29.9 (CH_2, resonance overlap)], 32.2 (CH_2), 34.4 (CH_2, C(8)), 62.0 (CH_2, C(6')), 63.4 (CH_2, C(6)), 70.4 (CH, C(5)), 70.6 (CH, C(5')), 70.9 (CH, C(4)),71.9 (CH, C(4')), 71.9 (CH, C(2)), 72.7 (CH, C(2')), 73.1 (CH, C(3)), 73.4 (CH, C(3')), 94.0 (CH, C(1)), 94.1 (CH, C(1')), 175.0 (C, C(7)); *m/z* (TOF ES+) 715.4 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 715.4606 ([M+Na]$^+$) $C_{36}H_{68}NaO_{12}$ requires 715.4608.

6,6'-di-*O*-oleoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (150a)



Di-ester **150a** was prepared from diol **137** (150 mg, 0.19 mmol*),* oleic acid (164 mg, 0.58 mmol), DCC (120 mg, 0.58 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide di-ester **150a** as a colourless oil (186 mg, 75%): R_f = 0.35 (5% EtOAc in hexanes); $[\alpha]_D^{22}$ = 59.6 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2854 s, 1742 m (C=O), 1459 w, 1379 w, 1249 s, 1162 m, 1110 m, 1072 s, 1044 m, 1010 m, 965 m, 897 s, 873 s, 843 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08-0.19 (54H, stack), 0.86 (6H, t, *J* = 7.1), 1.10-1.47 (40H, stack), 1.56-1.70 (4H, stack), 1.93-2.06 (8H, stack), 2.28-2.38 (4H, stack), 3.40-3.53 (4H, stack), 3.90 (2H, app t, *J* = 9.1), 3.95-4.11 (4H, stack), 4.27 (2H, dd, *J* = 11.9, 2.1), 4.91 (2H, d, *J* = 3.5), 5.17-5.25 (4H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.0, 0.7, 0.9 (CH_3, TMS)], 14.1 (CH_3), 22.6 (CH_2), 24.7 (CH_2), 27.1 (CH_2), 27.2 (CH_2), [29.1, 29.2, 29.3, 29.5, 29.71, 29.72 (CH_2, resonance overlap)], 31.9 (CH_2), 34.1 (CH_2), 63.3 (CH_2), 70.7 (CH), 71.9 (CH), 72.6 (CH), 73.4 (CH), 94.3 (CH), 129.7 (CH), 129.9 (CH), 173.7 (C); *m/z* (TOF ES+) 1326.0 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1325.8323 ($[M+Na]^+$) $C_{66}H_{134}NaO_{13}Si_6$ requires 1325.8338.

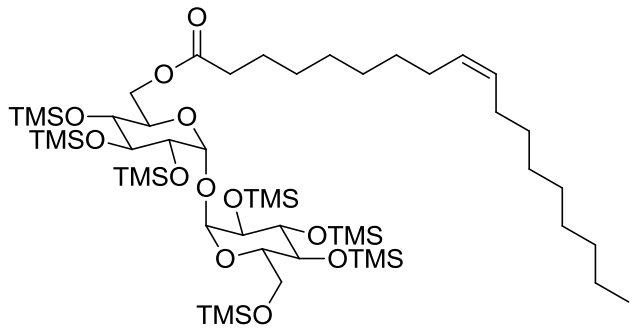
6,6'-di-*O*-oleoyl-α,α-trehalose (150b)



Hexaol **150b** was prepared from trehalose **150a** (180 mg, 0.14 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide hexaol **150b** as a white solid (80 mg, 66%): R_f = 0.40 (15% MeOH in $CHCl_3$); mp = 148-160 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O–H), 2928 m, 2857 w, 1723 m (C=O), 1674 s, 1454 w, 1378 w, 1266 w, 1150 m, 1105 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1)) δ ppm 0.63 (6H, t, *J* = 7.2, C(24)H_3), 0.80-1.25 (40H, stack), 1.33-1.45 (4H, m, C(9)H_aH_b), 1.70-1.85 (8H, stack, C(14)H_aH_b, C(17)H_aH_b) 2.10 (4H, t, *J* = 7.9, C(8)H_aH_b), 3.30-3.42 (2H, m, C(4)*H*), 3.45 (2H, dd, *J* = 9.8, 4.0, C(2)*H*), 3.60 (2H, app t, *J* = 9.8, C(3)*H*), 3.76 (2H, ddd, *J* = 10.6, 5.2, 2.4, C(5)*H*), 4.02 (2H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 5.2, C(6)H_aH_b), 4.10 (2H, B of ABX, $J_{B\text{-}A}$ = 12.4, $J_{B\text{-}X}$ = 2.4, C(6)H_aH_b), 4.85 (2H, d, *J* = 4.0, C(1)*H*), 5.06-5.12 (4H, stack, C(15)*H*, C(16)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 14.3 (CH_3, C(24)), 23.1 (CH_2), 25.3 (CH_2, C(9)), 27.6 (CH_2, C(14), C(17), resonance overlap), [29.61, 29.62, 29.7, 30.0, 30.2, 30.3, 30.4 (CH_2, resonance overlap)], 32.4 (CH_2), 34.6 (CH_2, C(8)), 63.7 (CH_2, C(6)), 70.5 (CH, C(5)), 70.8 (CH, C(4)), 72.1 (CH, C(2)), 73.6 (CH, C(3)), 94.2 (CH, C(1)), [130.1, 130.4 (CH, C(15), C(16)],

175.0 (C, C(7)); *m/z* (TOF ES+) 893.8 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 893.5974 ($[M+Na]^+$) $C_{48}H_{86}NaO_{13}$ requires 893.596.

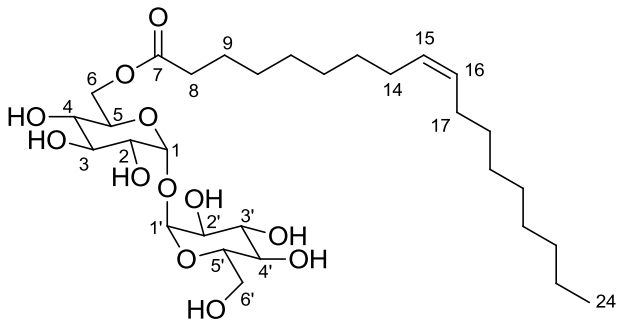
6-*O*-oleoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (151a)



Mono-ester **151a** was prepared from alcohol **136** (150 mg, 0.18 mmol), oleic acid (76 mg, 0.27 mmol), DCC (56 mg, 0.27 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide mono-ester **151a** as a colourless oil (148 mg, 74%): R_f = 0.39 (5% EtOAc in hexanes); $[\alpha]_D^{22}$ = 74.4 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2853 s, 1742 m (C=O), 1457 w, 1387 w, 1250 s, 1163 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 897 s, 873 s, 843 s, 757 s, 683 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.39-0.35 (63H, stack), 0.74 (3H, t, *J* = 6.7), 0.97-1.27 (20H, stack), 1.45-1.53 (2H, m), 1.80-1.96 (4H, stack), 2.16-2.23 (2H, m), 3.19-3.42 (4H, stack), 3.53 (2H, app. d, *J* = 3.3), 3.63 (1H, dt, *J* = 9.6, 3.3), 3.70-3.84 (2H, stack), 3.84-4.02 (2H, stack), 4.16 (1H, dd, *J* = 11.8, 2.1), 4.71-4.90 (2H, stack), 5.15-5.28 (2H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−1.3, −0.9, −0.2, 0.0, 0.3, (CH_3, TMS, resonance overlap)], 13.1 (CH_3), 21.7 (CH_2), 23.8 (CH_2), 26.1 (CH_2), 26.2 (CH_2), [28.1, 28.2, 28.5, 28.71, 28.72 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 60.9 (CH_2), 62.3 (CH_2), 69.6 (CH), 70.5 (CH), 71.0 (CH), 71.7 (CH), 71.8 (CH), 72.3 (CH), 72.4 (CH), 72.5

(CH), 93.1 (CH), 93.4 (CH), 128.7 (CH), 128.9 (CH), 172.7 (C); *m/z* (TOF ES+) 1133.8 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1133.6268 ($[M+Na]^+$) $C_{51}H_{110}NaO_{12}Si_7$ requires 1133.6280.

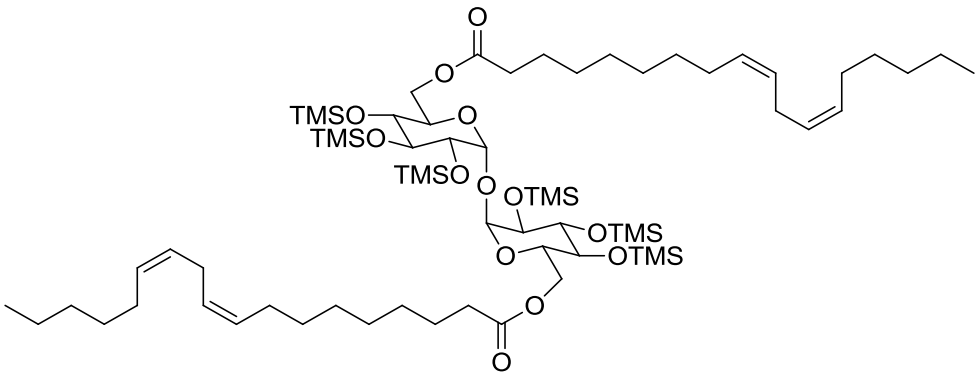
6-*O*-oleoyl-α,α-trehalose (151b)



Heptaol **151b** was prepared from trehalose **151a** (100 mg, 0.090 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide heptaol **151b** as a colourless oil (43 mg, 78%): R_f = 0.42 (30% MeOH in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3380 br s (O–H), 2925 s, 2858 m, 1718 m (C=O), 1676 m, 1050 s, 994 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.66 (3H, t, *J* = 7.2, C(24)H_3), 0.71-1.23 (20H, stack), 1.36-1.45 (2H, m, C(9)H_aH_b), 1.75-1.86 (4H, stack, C(14)H_aH_b, C(17)H_aH_b), 2.13 (2H, t, *J* = 7.9, C(8)H_aH_b), 3.09-3.21 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.28-3.38 (2H, stack, C(2)*H,* C(2')*H*), 3.48 (1H, dd, *J* = 13.0, 6.4, C(6')H_aH$_b$), 3.57-3.71 (3H, stack, C(6')H$_a$$H_b$, C(3)*H*, C(3')*H*), 3.80 (1H, ddd, *J* = 10.4, 4.8, 2.4, C(5)*H*), 4.06 (1H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 4.8, C(6)H_aH$_b$), 4.13 (1H, B of ABX, $J_{A\text{-}B}$ = 12.4, $J_{B\text{-}X}$ = 2.4, C(6)H$_a$$H_b$), 4.85-4.94 (2H, stack, C(1)*H*, C(1')*H*) 5.09-5.15 (2H, stack, C(15)*H*, C(16)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 14.2 (CH_3 ,C(24)), 23.0 (CH_2), 25.1 (CH_2, C(9)), 27.5 (CH_2, (C(14), C(17), resonance overlap), [29.4, 29.5,

29.6, 29.8, 30.0 (CH_2, resonance overlap)], 32.2 (CH_2), 34.4 (CH_2, C(8)), 62.1 (CH_2, C(6')), 63.5 (CH_2, C(6)), 70.4 (CH, C(5)), 70.6 (CH, C(5')), 71.0 (CH, C(4)),72.0 (CH, C(4')), 72.0 (CH, C(2)), 72.7 (CH, C(2')), 73.2 (CH, C(3)), 73.4 (CH, C(3')), 94.1 (CH, C(1)), 94.2 (CH, C(1')), [130.0, 130.2 (CH, C(15), C(16)], 175.0 (C, C(7)); *m/z* (TOF ES+) 629.5 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 629.3518 ($[M+Na]^+$) $C_{30}H_{54}NaO_{12}$ requires 629.3513.

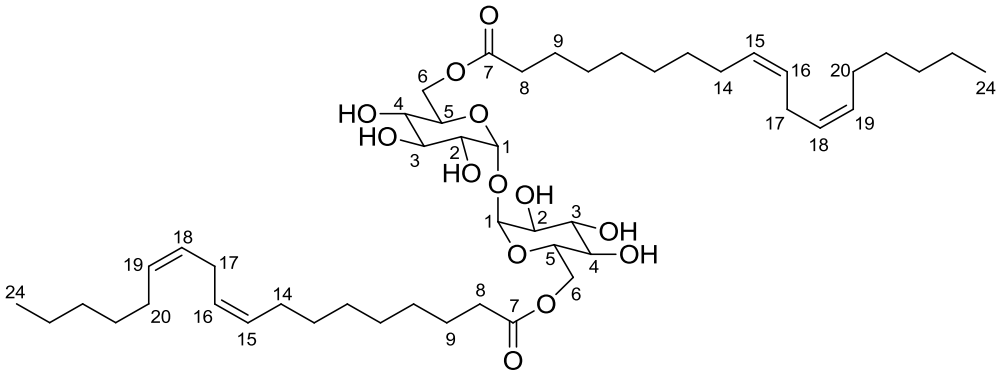
6,6'-di-*O*-linoleoyl-2,3,4,2',3',4'-hexakis-*O*-trimethylsilyl-α,α-trehalose (152a)



Di-ester **152a** was prepared from diol **137** (130 mg, 0.17 mmol), linoleic acid (141 mg, 0.50 mmol), DCC (103 mg, 0.50 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide di-ester **152a** as a colourless oil (150 mg, 68%): R_f = 0.37 (5% EtOAc in hexanes); $[\alpha]_D^{21}$ = 57.1 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2924 s, 2854 s, 1743 m (C=O), 1457 w, 1379 w, 1250 s, 1165 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 894 s, 875 s, 844 s, 757 s, 684 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.15-0.11 (54H, stack, Si(CH_3)$_3$), 0.74 (6H, t, *J* = 7.1, 2 × CH_2CH_3), 1.07-1.28 (28H, stack, CH_2, alkyl chain), 1.40-1.55 (4H, stack), 1.85-1.97 (8H, stack), 2.17-2.28 (4H, stack), 2.64 (4H, app t, *J* = 9.1), 3.28-3.40 (4H, stack), 3.80 (2H, app. t, *J* = 9.1), 3.83-3.98 (4H, stack), 4.18 (2H,

dd, *J* = 11.9, 2.1), 4.80 (2H, d, *J* = 3.5), 5.15-5.33 (8H, stack, 4 × C*H*=C*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.9, −0.3, 0.0 (CH_3, TMS)], 13.0 (CH_3), 21.5 (CH_2), 23.7 (CH_2), 24.6 (CH_2), 25.9 (CH_2), 26.1 (CH_2), [28.1, 28.2, 28.3, 28.6 (CH_2, resonance overlap)], 30.9 (CH_2), 33.1 (CH_2), 62.3 (CH_2), 79.7 (CH), 71.0 (CH), 71.6 (CH), 72.4 (CH), 93.3 (CH), 126.8 (CH), 127.0 (CH), 129.0 (CH), 129.1 (CH), 172.6 (C); *m/z* (TOF ES+) 1321.6 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 1321.8047 ([M+Na]$^+$) $C_{66}H_{130}NaO_{13}Si_6$ requires 1321.8025.

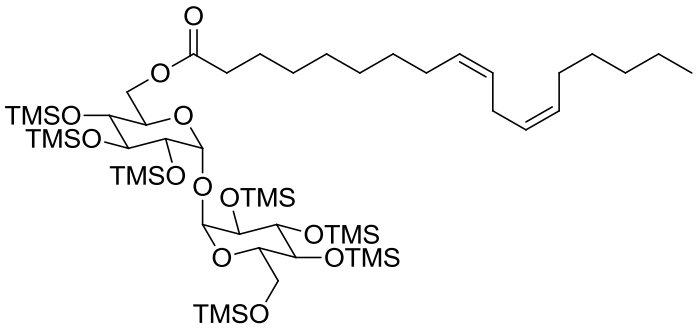
6,6'-di-*O*-linoleoyl-α,α-trehalose (152b)



Hexaol **152b** was prepared from trehalose **152a** (150 mg, 0.12 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide hexaol **152b** as a colourless oil (69 mg, 66%): R_f = 0.41 (15% MeOH in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O–H), 2928 m, 2857 w, 1723 m (C=O), 1674 s, 1454 w, 1378 w, 1266 w, 1150 m, 1105 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1)) δ ppm 0.75 (6H, t, *J* = 7.2, C(24)H_3), 1.11-1.26 (28H, stack, CH_2 alkyl chain), 1.43-1.52 (4H, m, C(9)H_aH_b), 1.86-1.95 (8H, stack, C(14)H_aH_b, C(20)H_aH_b), 2.21 (4H, t, *J* = 7.8, C(8)H_aH_b), 2.63 (4H, app t, *J* =

6.9, C(17)H_aH_b), 3.16-3.22 (2H, m, C(4)*H*), 3.39 (2H, dd, *J* = 9.9, 3.9, C(2)*H*), 3.67 (2H, app t, *J* = 9.5, C(3)*H*), 3.85 (2H, ddd, *J* = 10.6, 5.0, 2.5, C(5)*H*), 4.02 (2H, A of ABX, $J_{A\text{-}B}$ = 12.2, $J_{A\text{-}X}$ = 5.0, C(6)H_aH_b), 4.19 (2H, B of ABX, $J_{B\text{-}A}$ = 12.2, $J_{B\text{-}X}$ = 2.5, C(6)H_aH_b), 4.96 (2H, d, *J* = 4.1, C(1)*H*), 5.13-5.27 (8H, stack, C(15)*H*, C(16)*H,* C(18)*H*, C(19)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 14.2 (CH_3, C(24)), 22.9 (CH_2), 25.3 (CH_2 C(9)), 26.0 (CH_2, C(17)), 27.6 ((CH_2), C(14), C(20), resonance overlap), [29.5, 29.6, 29.7, 30.0 (CH_2, alkyl chain resonance overlap)], 31.9 (CH_2), 34.5 (CH_2, C(8)), 63.6 (CH_2, C(6)), 70.5 (CH, C(5)), 70.6 (CH, C(4)), 72.1 (CH, C(2)), 73.7 (CH, C(3)), 94.1 (CH, C(1)), [128.3, 128.4, 130.4, 130.5 (CH, C(15), C(16), C(18), C(19)], 175.0 (C, C(7)); *m/z* (TOF ES+) 889.8 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 889.5663 ([M+Na]$^+$) $C_{48}H_{82}NaO_{13}$ requires 889.5653.

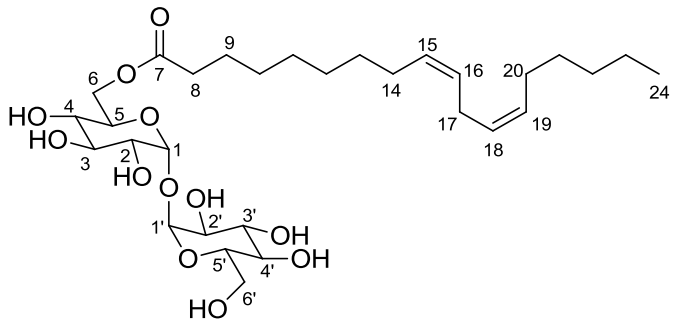
6-*O*-linoleoyl-2,3,4,2',3',4',6'-heptakis-*O*-trimethylsilyl-α,α-trehalose (153a)



Mono-ester **153a** was prepared from alcohol **136** (150 mg, 0.18 mmol), linoleic acid (75 mg, 0.27 mmol), DCC (56 mg, 0.27 mmol) DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide mono-ester **153a** as a colourless oil (155 mg, 78%): R_f = 0.39 (5% EtOAc in hexanes); $[\alpha]_D^{21}$ = 76.5 (c = 1.0, $CHCl_3$); ν_{max}(film)/cm^{-1} 2923 s, 2854 s, 1742 m (C=O), 1457 w, 1379 w,

1250 s, 1164 m, 1110 m, 1076 s, 1044 m, 1010 m, 965 m, 898 s, 876 s, 843 s, 756 s, 685 w; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.09-0.10 (63H, stack), 0.76 (3H, t, *J* = 7.2), 1.08-1.30 (14H, stack), 1.47-1.56 (2H, m), 1.86-1.99 (4H, stack), 2.14-2.25 (2H, m), 2.65 (2H, app t, *J* = 6.9), 3.24-3.40 (4H, stack), 3.53 (2H, app. d, *J* = 3.5), 3.64 (1H, dt, *J* = 9.8, 3.5), 3.72-3.83 (2H, stack), 3.86-3.99 (2H, stack), 4.17 (1H, dd, *J* = 12.0, 2.4), 4.74-4.86 (2H, stack), 5.15-5.31 (4H, stack); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm [−0.2, 0.1, 0.9, 1.0 (CH_3, TMS, resonance overlap)], 14.1 (CH_3), 22.6 (CH_2), 24.8 (CH_2), 25.6 (CH_2), 27.2 (CH_2), [29.1, 29.2, 29.6 (CH_2, resonance overlap)], 31.5 (CH_2), 34.1 (CH_2), 61.9 (CH_2), 63.4 (CH_2), 70.6 (CH), 71.6 (CH), 72.0 (CH), 72.6 (CH), 72.8 (CH), 73.4 (CH), 73.5 (CH), 73.6 (CH), 93.2 (CH), 93.5 (CH), 127.9 (CH), 128.0 (CH), 130.0 (CH), 130.2 (CH), 172.7 (C); *m/z* (TOF ES+) 1131.9 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 1131.6117 ($[M+Na]^+$) $C_{51}H_{108}NaO_{12}Si_7$ requires 1131.6123.

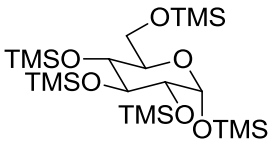
6-*O*-linoleoyl-α,α-trehalose (153b)



Heptaol **153b** was prepared from trehalose **153a** (220 mg, 0.20 mmol) and a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide heptaol **153b** as a colourless oil (74 mg, 61%): R_f = 0.40 (30% MeOH in $CHCl_3$); $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical

rotation; ν_{max}(film)/cm^{-1} 3380 br s (O–H), 2925 s, 2858 m, 1718 m (C=O), 1676 m, 1050 s, 994 s; ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 0.81 (3H, t, *J* = 7.2, C(24)H_3), 1.12-1.34 (14H, stack), 1.50-1.61 (2H, m, C(9)H_aH_b), 1.91-2.02 (4H, stack, C(14)H_aH_b, C(20)H_aH_b), 2.26 (2H, t, *J* = 7.9, C(8)H_aH_b), 2.68 (2H, t, *J* = 7.0, C(17)H_aH_b), 3.19-3.34 (3H, stack, C(5')*H*, C(4)*H*, C(4')*H*), 3.39-3.50 (2H, stack, C(2)*H,* C(2')*H*), 3.61 (1H, dd, *J* = 12.4, 6.0, C(6')H_aH$_b$), 3.69-3.80 (3H, stack, C(6')H$_a$$H_b$, C(3)*H*, C(3')*H*), 3.93 (1H, ddd, *J* = 10.5, 4.9, 2.4, C(5)*H*), 4.19 (1H, A of ABX, $J_{A\text{-}B}$ = 12.2, $J_{A\text{-}X}$ = 4.9, C(6)H_aH$_b$), 4.26 (1H, B of ABX, $J_{A\text{-}B}$ = 12.2, $J_{B\text{-}X}$ = 2.4, C(6)H$_a$$H_b$), 4.98-5.07 (2H, stack, C(1)*H*, C(1')*H*), 5.17-5.36 (4H, stack, C(15)*H*, C(16)*H*, C(18)*H*, C(19)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 2:1) δ ppm 13.4 (CH_3 , C(24)), 22.1 (CH_2), 24.4 (CH_2, C(9)), 25.2 (CH_2, C(17)), 27.5 (CH_2, C(14), C(20), resonance overlap), [28.7, 28.8, 28.9, 29.2 (CH_2, resonance overlap)], 31.9 (CH_2), 33.7 (CH_2, C(8)), 61.4 (CH_2, C(6')), 62.8 (CH_2, C(6)), 69.7 (CH, C(5)), 69.9 (CH, C(5')), 70.3 (CH, C(4)),71.3 (CH, C(4')), 71.4 (CH, C(2)), 72.0 (CH, C(2')), 72.6 (CH, C(3)), 72.9 (CH, C(3')), 93.4 (CH, C(1)), 93.5 (CH, C(1')), [127.5, 127.6, 129.6, 129.7 (CH, C(15), C(16), C(18), C(19)], 174.2 (C, C(7)); *m/z* (TOF ES+) 627.4 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 627.3369 ([M+Na]$^+$) $C_{30}H_{52}NaO_{12}$ requires 627.3356.

1,2,3,4,6-Pentakis-*O*-trimethylsilyl-α-D-glucose (155)

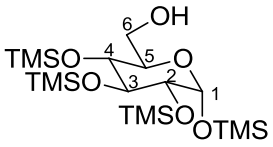


HMDS (100 mL, 0.48 mol) and TMSCl (50 mL, 0.39 mol) were added sequentially to a solution of D-glucose (10.0 g, 55.5 mmol) in pyridine (500 mL). The solution was stirred at 75 °C for 1 h under an Ar atmosphere before being allowed to cool to rt. The mixture was poured into ice-

water (500 mL) and extracted with hexane (3 × 300 mL). The combined organic extracts were washed with H_2O (3 × 300 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to afford glycoside **155** as a viscous, colourless oil (27.6 g, 92%): R_f = 0.25 (4% EtOAc in hexanes); $[\alpha]_D^{20}$ = 62.5 (c = 0.5, $CHCl_3$) (lit.[183] $[\alpha]_D^{22}$ = 65.4 (c = 3.3, $CHCl_3$)); R_f = 0.25 (4% EtOAc in hexanes); ν_{max}(film)/cm^{-1} 3608 w, 3582 w, 3074 w, 2957 s, 2935 s, 2876 s, 1780 w, 1734 w, 1458 m, 1415 w, 1380 m, 1362 w, 1342 w, 1251 s, 1175 w, 1131 m, 1070 s, 1005 s, 988 s, 945 m, 896 m, 880 s, 841 s, 811 m, 741 s, 726 s, 665 m, 627 m; ^{1}H NMR (300 MHz, $CDCl_3$)) δ ppm 0.10 (9H, s, Si(C*H*$_3$)$_3$), 0.12 (9H, s, Si(C*H*$_3$)$_3$), 0.14 (18H, s, 2 × Si(C*H*$_3$)$_3$), 0.17 (9H, s, Si(C*H*$_3$)$_3$), 3.26-3.42 (2H, stack), 3.57-3.80 (4H, stack), 4.99 (1H, d, *J* = 3,1); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm [−0.3, 0.2, 0.5, 1.0, 1.3 (*C*H$_3$, Si(*C*H$_3$)$_3$)], 62.3 (CH_2), 72.3 (CH), 72.5 (CH), 74.0 (CH), 74.2 (CH), 93.9 (CH); *m/z* (TOF ES+) 564.1 ($[M+Na]^+$, 100%).

Data were in agreement with those reported in the literature.[183]

6-hydroxy-1,2,3,4-tetrakis-*O*-trimethylsilyl-α-D-glucose (156)

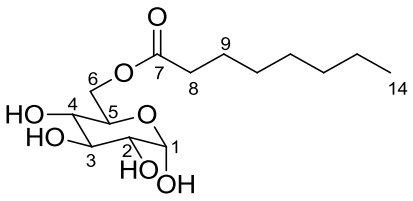


MeOH (10.4 mL) and glacial AcOH (0.38 mL, 6.64 mmol) were added to a solution of per-TMS protected glucose **155** (2g, 3.70 mmol) in acetone (7.6 mL) at 0 °C. After stirring for 6 h, the reaction mixture was quenched with solid $NaHCO_3$ before being concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL), washed sequentially with $NaHCO_3$ solution (10 mL) and brine (10 mL), then dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column

chromatography (10% EtOAc in hexane) to provide alcohol **156** as a colourless oil (1.23 g, 71%): R_f = 0.22 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD, 2:1)) δ ppm −0.31-0.29 (36H, stack, TMS), 3.19 (1H, dd, *J* = 9.2, 3.2), 3.30 (1H, dd, *J* = 9.2, 8.9), 3.50-3.70 (4H, stack), 4.86 (1H, d, *J* = 3.4, C(1)*H*), O*H* not observed; ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm [−0.7, −0.5, −0.0, 0.3 (CH_3, TMS], 61.0 (CH_2, C(6)), 71.0 (CH), 71.1 (CH), 72.7 (CH), 73.2 (CH), 93.1 (CH, C(1)); *m/z* (TOF ES+) 491.2 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 491.2107 ([M+Na]$^+$) $C_{18}H_{44}NaO_6Si_4$ requires 491.2113.

Data were in agreement with those reported in the literature.[184]

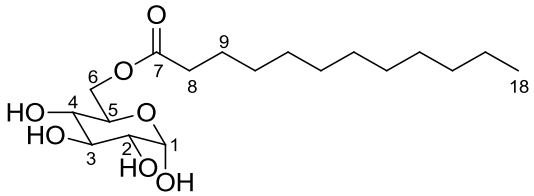
6-octanoyl-α-D-glucose (157b)



Tetraol **157b** was prepared from alcohol **156** (110mg, 0.24 mmol), octanoic acid (50 mg, 0.35 mmol), DCC (72 mg, 0.35 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was deprotected with a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. The crude product was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide tetraol **157b** as a white solid (46 mg, 63%): R_f = 0.30 (15% MeOH in $CHCl_3$); mp = 119-121 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O−H), 2928 m, 2857 w, 1723 m (C=O), 1674 s, 1454 w, 1378 w, 1266 w, 1150 m, 1105 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD, 2:1)) δ ppm 0.80

(3H, t, *J* = 7.3, C(14)H_3), 1.09-1.33 (8H, stack), 1.50-1.62 (2H, m, C(9)H_aH_b), 2.33 (2H, t, *J* = 7.9, C(8)H_aH_b), 3.32-1.40 (1H, m, C(4)*H*), 3.53 (1H, dd, *J* = 9.7, 4.0, C(2)*H*), 3.62 (1H, app t, *J* = 9.4, C(3)*H*), 3.90 (1H, ddd, *J* = 10.5, 5.4, 2.4, C(5)*H*), 4.06 (1H, A of ABX, $J_{A\text{-}B}$ = 12.4, $J_{A\text{-}X}$ = 5.3, C(6)H_aH_b), 4.11 (1H, B of ABX, $J_{B\text{-}A}$ = 12.4, $J_{B\text{-}X}$ = 2.3, C(6)H_aH_b), 5.07 (1H, d, *J* = 4.1, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm 14.1 (CH_3, C(14)), 24.9 (CH_2), 26.7 (CH_2, C(9)), 29.2 (CH_2), 29.4 (CH_2), 32.0 (CH_2), 34.2 (CH_2, C(8)), 63.2 (CH_2, C(6)), 70.1 (CH, C(5)), 70.2 (CH, C(4)), 71.6 (CH, C(2)), 73.0 (CH, C(3)), 93.6 (CH, C(1)), 175.6 (C, C(7)); *m/z* (TOF ES+) 329.2 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 329.1569 ([M+Na]$^+$) $C_{14}H_{26}NaO_7$ requires 329.1576.

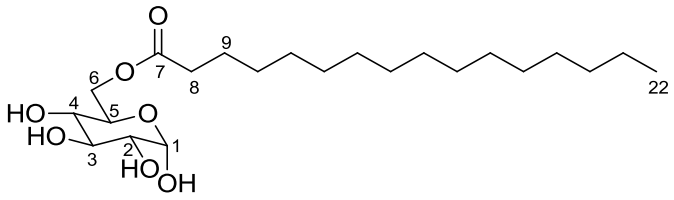
6-dodecanoyl-α-D-glucose (158b)



Tetraol **158b** was prepared from alcohol **156** (105mg, 0.22 mmol), dodecanoic acid (67 mg, 0.34 mmol), DCC (70 mg, 0.34 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was deprotected with a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. The crude product was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide tetraol **158b** as a white solid (57 mg, 71%): R_f = 0.30 (15% MeOH in $CHCl_3$); mp = 120-126 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3338 br s (O−H), 2857 w, 1723 m (C=O), 1675 s, 1454

w, 1151 m, 1105 m, 1016 s, 991 s; ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD, 2:1)) δ ppm 0.79 (3H, t, *J* = 7.2, C(18)$H_aH_bH_c$), 1.10-1.29 (16H, stack), 1.49-1.57 (2H, m, C(9)H_aH_b), 2.26 (2H, t, *J* = 7.8, C(8)H_aH_b), 3.18-3.28 (1H, m, C(4)*H*), 3.32 (1H, dd, *J* = 9.5, 3.9, C(2)*H*), 3.61 (1H, app. t, *J* = 9.5, C(3)*H*), 3.90 (1H, ddd, *J* = 10.5, 5.4, 2.5, C(5)*H*), 4.16 (1H, A of ABX, $J_{A\text{-}B}$ = 12.1, $J_{A\text{-}X}$ = 5.4, C(6)H_aH_b), 4.27 (1H, B of ABX, $J_{B\text{-}A}$ = 12.1, $J_{B\text{-}X}$ = 2.5, C(6)H_aH_b), 5.05 (1H, d, *J* = 3.9, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm 15.0 (CH_3, C(18)), 23.9 (CH_2), 26.2 (CH_2, C(9)), [30.4, 30.5, 30.6, 30.7, 30.8 (CH_2, resonance overlap)], 33.2 (CH_2), 35.3 (CH_2, C(8)), 65.0 (CH_2, C(6)), 70.7 (CH, C(5)), 71.8 (CH, C(4)), 73.7 (CH, C(2)), 74.9 (CH, C(3)), 93.9 (CH, C(1)), 175.6 (C, C(7)); *m/z* (TOF ES+) 385.3 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 385.296 ($[M+Na]^+$) $C_{18}H_{34}NaO_7$ requires 329.1576.

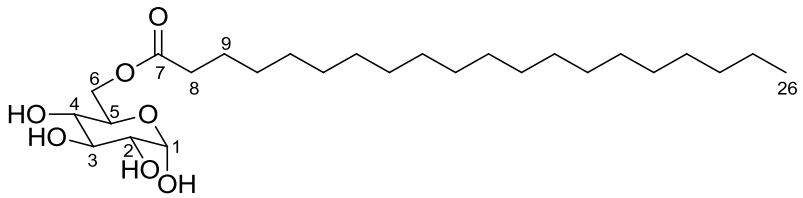
6-hexadecanoyl-α-D-glucose (159b)



Tetraol **159b** was prepared from alcohol **156** (110mg, 0.24 mmol), hexadecanoic acid (72 mg, 0.28 mmol), DCC (58 mg, 0.28 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was deprotected with a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. The crude product was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide tetraol **159b** as a white solid (67 mg, 67%): R_f = 0.33 (15% MeOH in $CHCl_3$); mp = 123-136 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an

accurate optical rotation; ν_{max}(film)/cm^{-1} 3337 br s (O−H), 2928 m, 2857 w, 1742 m (C=O), 1674 s, 1454 w, 1377 w, 1266 w, 1150 m, 1105 m, 1015 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD, 2:1)) δ ppm 0.80 (3H, t, *J* = 7.0, C(22)$H_aH_bH_c$), 1.14-1.24 (24H, stack), 1.50-1.59 (2H, m, C(9)H_aH_b), 2.25 (2H, t, *J* = 7.7, C(8)H_aH_b), 3.18-3.26 (1H, m, C(4)*H*), 3.31 (1H, dd, *J* = 9.5, 3.9, C(2)*H*), 3.60 (1H, app. t, *J* = 9.5, C(3)*H*), 3.90 (1H, ddd, *J* = 10.5, 5.4, 2.5, C(5)*H*), 4.15 (1H, A of ABX, $J_{A\text{-}B}$ = 12.0, $J_{A\text{-}X}$ = 5.4, C(6)H_aH_b), 4.27 (1H, B of ABX, $J_{B\text{-}A}$ = 12.0, $J_{B\text{-}X}$ = 2.5, C(6)H_aH_b), 5.04 (1H, d, *J* = 3.9, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm 14.9 (CH_3, C(22)), 23.9 (CH_2), 26.1 (CH_2, C(9)), [30.4, 30.5, 30.6, 30.7, 30.9 (CH_2, resonance overlap)], 33.2 (CH_2), 35.3 (CH_2, C(8)), 65.0 (CH_2, C(6)), 70.7 (CH, C(5)), 71.8 (CH, C(4)), 73.8 (CH, C(2)), 74.9 (CH, C(3)), 94.0 (CH, C(1)), 175.9 (C, C(7)); *m/z* (TOF ES+) 441.4 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 441.2822 ([M+Na]$^+$) $C_{22}H_{42}NaO_7$ requires 441.2828.

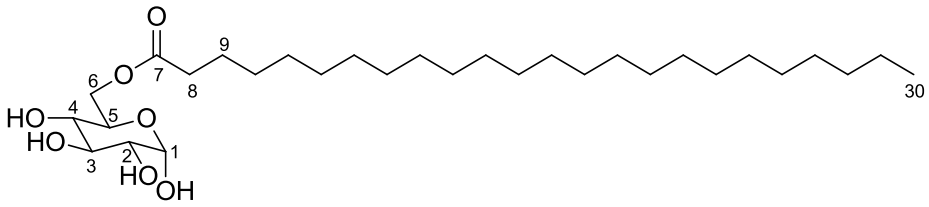
6-eicosanoyl-α-D-glucose (160b)



Tetraol **160b** was prepared from alcohol **156** (80mg, 0.17 mmol), eicosanoic acid (64 mg, 0.21 mmol), DCC (43 mg, 0.21 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was deprotected with a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. The crude product was purified by column chromatography (0-5% EtOAc in hexane, gradient) to provide tetraol **160b** as a white solid (47 mg, 58%): R_f = 0.35 (15% MeOH in $CHCl_3$);

mp = 125-136 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3332 br s (O−H), 2931 m, 2857 w, 1723 w (C=O), 1378 w, 1270 w, 1150 m, 1105 m, 1016 s, 991 s; ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD, 2:1)) δ ppm 0.79 (3H, t, *J* = 7.0, C(26)*H*$_3$), 0.96-1.38 (32H, stack), 1.50-1.62 (2H, m, C(9)H_aH_b), 2.26 (2H, t, *J* = 7.8, C(8)H_aH_b), 3.20-3.33 (1H, m, C(4)*H*), 3.31 (1H, dd, *J* = 9.4, 3.9, C(2)*H*), 3.61 (1H, app. t, *J* = 9.4, C(3)*H*), 3.90 (1H, ddd, *J* = 10.4, 5.2, 2.4, C(5)*H*), 4.16 (1H, A of ABX, $J_{A\text{-}B}$ = 12.0, $J_{A\text{-}X}$ = 5.2, C(6)H_aH_b), 4.27 (1H, B of ABX, $J_{B\text{-}A}$ = 12.0, $J_{B\text{-}X}$ = 2.4, C(6)H_aH_b), 5.05 (1H, d, *J* = 3.9, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm 14.9 (CH_3, C(26)), 23.9 (CH_2), 26.1 (CH_2, C(9)), [30.4, 30.5, 30.6, 30.7, 30.9 (CH_2, resonance overlap)], 33.2 (CH_2), 35.3 (CH_2, C(8)), 65.0 (CH_2, C(6)), 70.7 (CH, C(5)), 71.8 (CH, C(4)), 73.8 (CH, C(2)), 74.9 (CH, C(3)), 94.0 (CH, C(1)), 175.9 (C, C(7)); *m/z* (TOF ES+) 497.5 ([M+Na]$^+$, 100%); HRMS *m/z* (TOF ES+) 497.3466 ([M+Na]$^+$) $C_{26}H_{50}NaO_7$ requires 497.3454.

6-tetracosanoyl-α-D-glucose (161b)



Tetraol **161b** was prepared from alcohol **156** (100 mg, 0.21 mmol), tetracosanoic acid (95 mg, 0.26 mmol), DCC (53 mg, 0.26 mmol), DMAP and 4 Å molecular sieves in toluene (5 mL) according to the general procedure. After stirring overnight, work up provided the crude product, which was deprotected with a TFA: THF: H_2O (8: 17: 3) solution (4 mL) according to the general procedure. The crude product was purified by column chromatography (0-5%

EtOAc in hexane, gradient) to provide tetraol **161b** as a white solid (74 mg, 66%): R_f = 0.39 (15% MeOH in $CHCl_3$); mp = 128-144 °C; $[\alpha]_D$ insolubility at rt prevented the determination of an accurate optical rotation; ν_{max}(film)/cm^{-1} 3335 br s (O−H), 2930 m, 2857 w, 1723 m (C=O), 1674 s, 1454 w, 1388 w, 1276 w, 1150 m, 1105 m, 1016 s, 991 s, 940 w; ^{1}H NMR (300 MHz, $CDCl_3$:,CD_3OD 2:1)) δ ppm 0.79 (3H, t, *J* = 6.9, C(30)H_3), 1.01-1.36 (40H, stack), 1.49-1.59 (2H, m, C(9)H_aH_b), 2.26 (2H, t, *J* = 7.8, C(8)H_aH_b), 3.15-3.28 (1H, m, C(4)*H*), 3.31 (1H, dd, *J* = 9.5, 3.9, C(2)*H*), 3.61 (1H, app. t, *J* = 9.5, C(3)*H*), 3.90 (1H, ddd, *J* = 10.4, 5.2, 2.3, C(5)*H*), 4.16 (1H, A of ABX, $J_{A\text{-}B}$ = 12.0, $J_{A\text{-}X}$ = 5.2, C(6)H_aH_b), 4.27 (1H, B of ABX, $J_{B\text{-}A}$ = 12.0, $J_{B\text{-}X}$ = 2.3, C(6)H_aH_b), 5.05 (1H, d, *J* = 3.9, C(1)*H*), O*H*s not observed; ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm 15.0 (CH_3, C(30)), 23.8 (CH_2), 26.1 (CH_2, C(9)), [30.2, 30.3, 30.6, 30.7, 30.9 (CH_2, resonance overlap)], 32.9 (CH_2), 35.3 (CH_2, C(8)), 65.0 (CH_2, C(6)), 70.7 (CH, C(5)), 71.6 (CH, C(4)), 73.8 (CH, C(2)), 74.9 (CH, C(3)), 93.9 (CH, C(1)), 175.9 (C, C(7)); *m/z* (TOF ES+) 553.5 ($[M+Na]^+$, 100%); HRMS *m/z* (TOF ES+) 553.4077 ($[M+Na]^+$) $C_{30}H_{58}NaO_7$ requires 553.4080.

Chapter 6

References

6. References

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