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Synthesis of Natural and Unnatural Sulfatide Ligands for NKT Cell Activation and Olefin Cross-metathesis of α-Methylene-β-lactones as a Methodology for the Rapid Assembly of β-Lactones

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Kaddy Camara, Ph.D.
University of Connecticut, 2015

Sulfatides are innate glycosphingolipids shown to activate a subpopulation of type II NKT cells. Their activation has been reported to sometimes have antagonistic roles to those of type I NKT cells in some disease models. This has sparked a lot of interest in the synthesis of natural and unnatural sulfatides for an examination of their influence on NKT cell responses. The design, synthesis and evaluation of type II NKT cell activation of several sulfatide ligands are described in this thesis.

A two-step methodology has been developed for the rapid assembly of disubstituted β-lactones. The first step is olefin cross metathesis (CM) of α-methylene-β-lactones with various alkene cross partners to furnish α-alkylidine-β-lactones. These are subsequently diastereoselectively reduced. A diverse library of β-lactones, including (±)-nocardiolactone has been prepared. Combining this approach with competitive activity-based protein profiling (ABPP) identified lead β-lactone inhibitors for several serine hydrolases, including disease-associated enzymes and enzymes of uncharacterized function.

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Ac	acetyl
AcOH	acetic acid
APC	antigen presenting cell
Aq	aqueous
BF_3•OEt_2	boron trifluoro etherate
Boc	*t*-butyloxycarbonyl
Bu	butyl
Bu_2SnO	dibutyltin oxide
Bz	benzoyl
CD1d	cluster of differentiation
CH_2Cl_2/DCM	dichloromethane
Cl_3CCN	trichloroacetonitrile
d	day
DBU	1,8-diazabicyclo 5.4.0 undec-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
Me_3N•SO_3	trimethylamine sulfur trioxide complex
DIEA	diisopropylethylamine
DMAP	4-dimethylamino pyridine
DMF	*N*,*N*-dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide

EDC	1-[3-(dimethylamino)propyl]3-ethylcarbodiimide
ESI	electrospray ionization
Et	ethyl
EtOAc	ethyl acetate
equiv	equivalent
FAB	fast atom bombardment
α-GalCer	α-galactosylceramide
GSL	glycosphingolipid
h	hour
HRMS	high resolution mass spectroscopy
Hz	Hertz
IFN-γ	interferon-gamma
IL-4	interlukin-4
IR	infrared
4Å MS	molecular sieves
Me	methyl
MeOH	methanol
MHC	major histocompatability complex
min	minutes
NIS	*N*-iodo succinimide
NKT cells	natural killer T cell
Piv	pivaloyl

PNP	*p*-nitrophenyl
Pr	propyl
py	pyridine
rt	room temperature
SAR	structure activity relationship
TBAF	tetrabutyammonium fluoride
TBDMS/TBS	*t*-butyldimethylsilyl
TBDPS	*t*-butyldiphenylsilyl
TCR	T-cell receptor
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
Th	T helper
THF	tetrahydrofuran
TMS	trimethylsilyl
Tr	trityl
Ts	*p*-toluenesulfonyl

Chapter 1.

1.1. Introduction

1.1.1. Natural Killer (NK) T Cell

Natural killer (NK) T cells are among the body's first line of response in combating infectious diseases. They are a subset of T cell lymphocytes, a class of white blood cell, pre-armed with mRNA cytokines, which are produced quickly upon activation of their T cell receptors for diverse immune responses.[1-3] NKT cells are CD1d-restricted, which means they only recognize lipid antigens presented by CD1d proteins for their activation. NKT cells can be classified as either type I or type II, based on their T cell receptor (TCR), a heterodimer made up of a variable *N*-terminus (designated V) and a *C*-terminus.[4] NKT cells with a variable *N*-terminus (Vα14Jα18 in mice and Vα24Jα18 in humans) are called type I NKT cells (classical), or invariant (*i*NKT) cells. NKT cells which express more diverse TCR repertoires are termed type II NKT cells.[5]

Type I NKT cells are well characterized. Extensive research has been conducted on them using α-galactosylceramide (α-GalCer or KRN7000 (**1**), Figure 1) and its analogs as potent ligands for their stimulation.[6] Less, however, is known about type II NKT cells, due to a lack of potent ligands that activate them. Studies have shown that a specific type of glycolipid, known as sulfatides (see **2**, Figure 1), can activate one sub-population of type II NKT cells. This dissertation will focus on the synthesis of sulfatides as ligands for the activation of type II NKT cells.

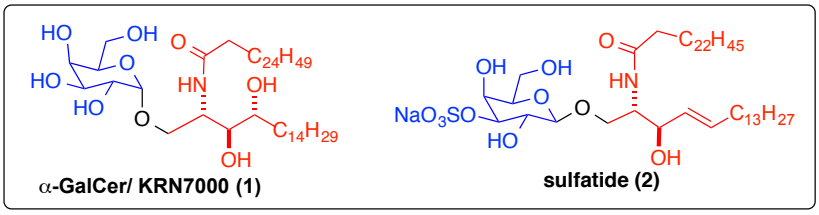


Figure 1. Examples of glycolipids

1.1.2. CD1 proteins

CD1 proteins are antigen-presenting proteins found on the surface of antigen presenting cells (APCs) such as dendritic cells, macrophages, and subsets of B cells. They are similar in action to class I major histocompatibility complex (MHC) proteins. Unlike MHC, CD1 proteins present lipids and glycolipids, both innate (e.g. phospholipids,[7] glycosphingolipids and sulfoglycolipids[8]) and foreign (e.g. mycobacterial),[9] to NKT cell receptors.[2] There are five known CD1 proteins, all of which are expressed in humans, but only CD1d is expressed in mice and rats. Group I proteins includes CD1a, CD1b, CD1c and CD1e, and group II is made up of CD1d.[2,10] Crystal structure studies showed CD1d (hCD1d, Figure 2) to be constituted of a *C*-terminal part and an *N*-terminus domain characterized by two helices (α1 and α2). They differ from MHC molecules by the presence of two hydrophobic pockets designated zone A and C (F in mCD1d) (Figure 2). CD1d is the antigen-presenting protein studied in this dissertation.

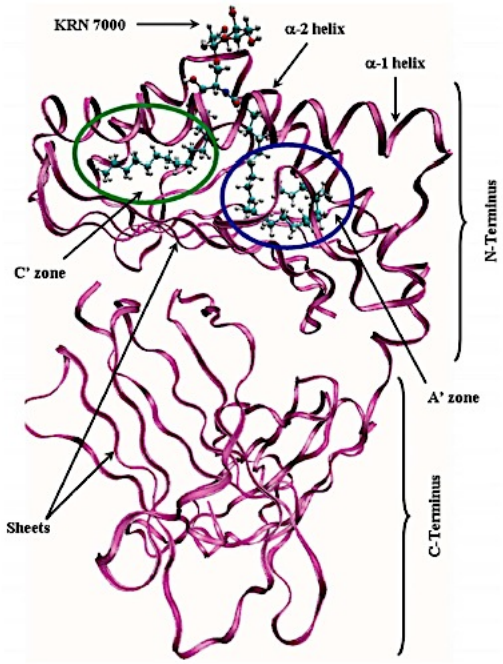


Figure 2. Crystal structure of hCD1d with KRN7000[2]

1.1.3. Glycosphingolipids

Glycosphingolipids (glycolipids) are amphiphilic molecules found on the cell membrane of eukaryotic cells. They can be either neutral (e.g., KRN7000) or acidic (e.g., sulfatides).

The structural skeleton of glycolipids is composed of a hydrophobic ceramide tail (lipid) and a hydrophilic sugar head (Figure 3a). The ceramide consists of a sphingoid base (phytosphingosine, sphingosine, or sphinganine (Figure 3b) and fatty acyl chain connected through *N*-acylation. The sugar is connected to the ceramide through a glycosidic bond that can be either alpha or beta linked (Figure 3c).

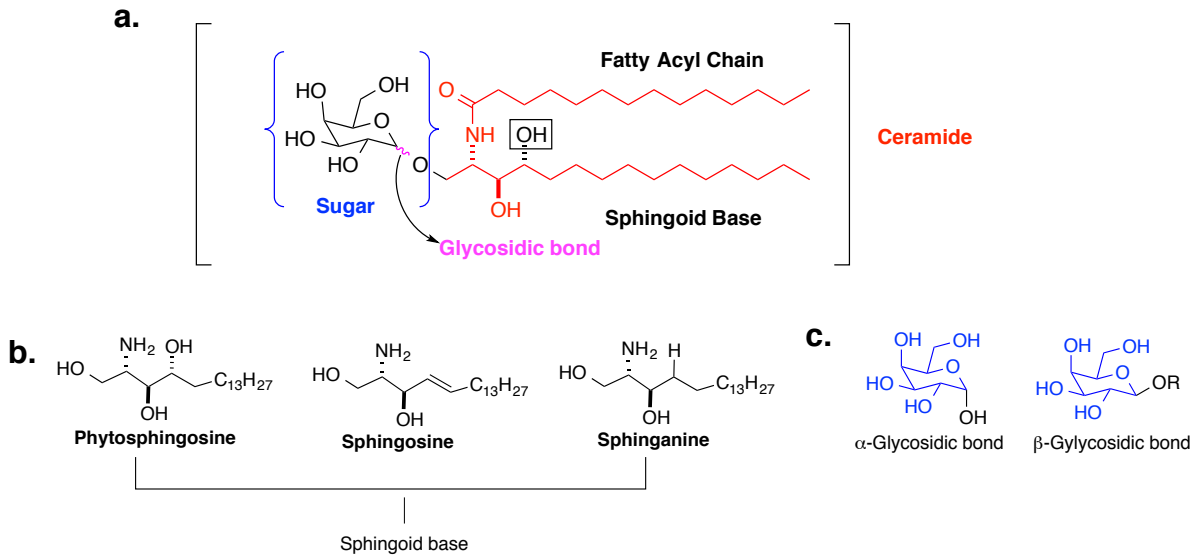


Figure 3. General composition of glycolipids: **a.** structural skeleton of glycolipids; **b.** types of sphingoid bases; **c.** glycosidic bonds

Glycolipids found in mammals have the β-configuration, until a recent study by Kain and co-workers reported the isolation of C24:1-α-galactosylceramide (**3**)[11] from mammals (Figure 4). Agelasphin-9b (**4**) (KRN7000 is a synthetic analog of Agelasphin-9b) was the first glycolipid isolated from a natural source with α-configuration connecting the sugar and ceramide.

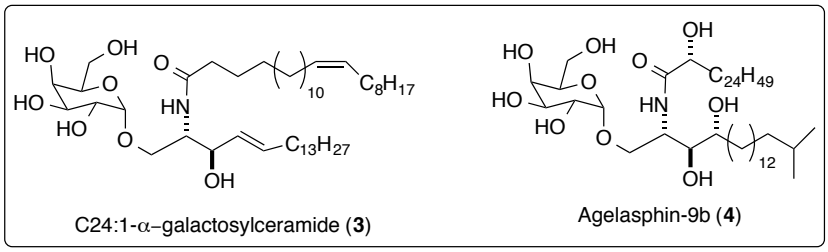


Figure 4. Examples of natural α-glycolipids

1.1.3.1. Glycolipids: Mode of activation of NKT cells

Two important complexes, binary and ternary, must be formed for glycolipids to activate NKT cells. The first complex, formed is the binary complex arising from the interaction of the glycolipids with CD1d. H-bonding interactions between amino acid residues in CD1d and the glycolipid ligands provide specificity and stability to the complex (Figure 5). The H-bond interaction between the C3-OH of the sphingoid base and amino acid D80 (Figure 5c and 5d) directs the fatty acyl chain into pocket A and the sphingoid base into pocket C (or F), as depicted in the binary complexes of KRN7000-hCD1d reported by Koch *et al.*[12] and of a sulfatide-mCD1d reported by Wilson *et al.*[13] (Figure 5a and 5b respectively).

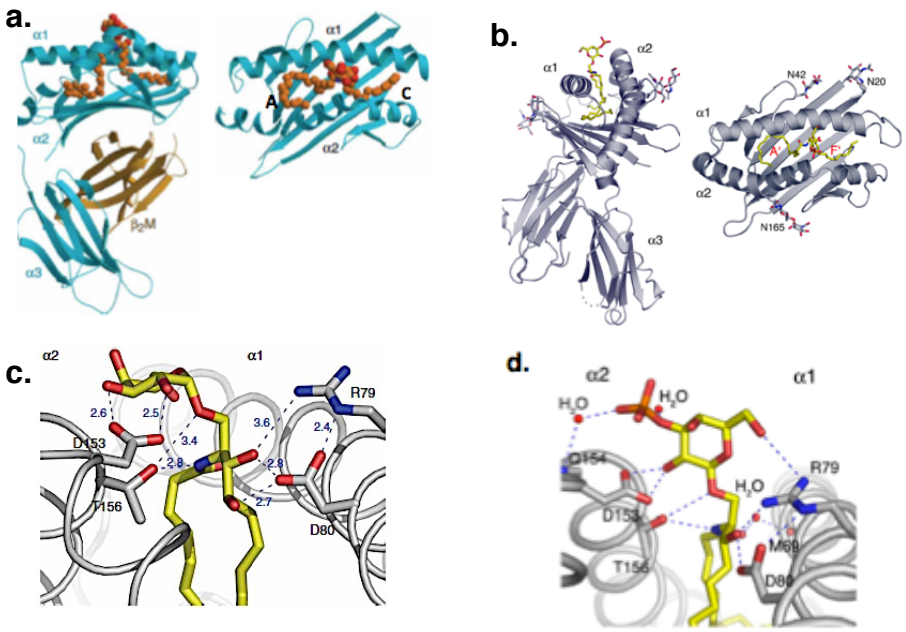


Figure 5. Binary complex of CD1d bound glycolipids: **a.** hCD1d-Bound KRN7000; **b.** mCD1d-bound sulfatide; **c.** H-bonding network of mCD1d amino acid residues and KRN7000; **d.** H-bonding network of mCD1d amino acid residues and sulfatide[12,13]

These pockets are further stabilized with β-helix sheets via van der Waals

interactions. Four of the same H-bonding interactions seen in the CD1d-KRN7000 binary complex are also observed in CD1d-sulfatide (galactose moiety C2-OH-D153, C1-O'-T156, sphingoid base moiety C3-OH-D80 and the amide NH-T156) (Figure 5c and 5d). In addition to these four interactions, sulfatide forms three extra H-bonds with CD1d (C6-OH-R79, sulfate group with Q154, and the carbonyl group of the amide with Met69). The sulfate group and carbonyl interactions are H_2O mediated (Figure 5d).

Upon formation of the binary complex, the sugar moieties of the glycolipids are pinned down between the α-1 and α-2 helices of CD1d, ready to interact with the T cell receptors on the surface of NKT cells. The interaction of the sugar moieties with the T cells results in the formation of the ternary complex. Successful formation of the ternary complex activates NKT cells to release different cytokines, communication devices for immune responses (Figure 6).[2,6]

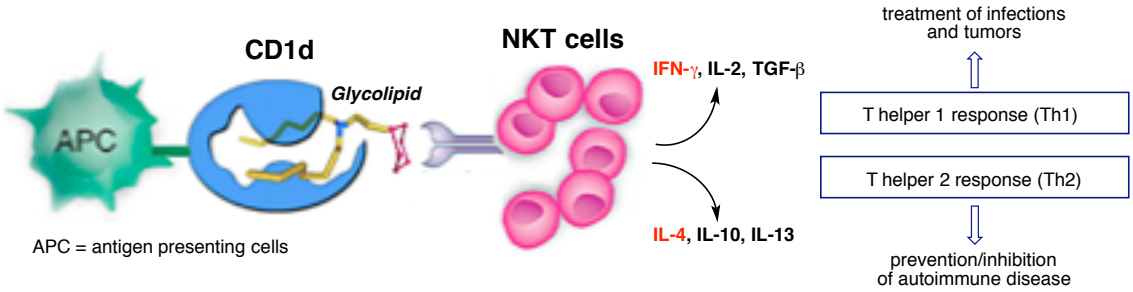


Figure 6. Activation of NKT cells for cytokine secretion[ref]

These cytokines, based on their biological activities, can be classed as having T helper 1 (Th1) and T helper 2 (Th2) types of responses. Th1 cytokines include IFN-γ, IL-2, IL-3 and TNF-β; they spawn pro-inflammatory activities, a defense

mechanism for the immune system to fight diseases, such as viral, bacterial, and parasitic infections. Th1 cytokines have also been shown to have antitumor activity.[6] Th2 cytokines include IL-4, IL-5, IL-10 and IL-13. They are responsible for autoimmuno-regulatory activities, which are relevant for the treatment of diabetes and other autoimmune conditions. The most studied glycolipids for NKT cell activation are KRN7000 for type I NKT cells and recently sulfatides for type II NKT cells.

1.1.4. Sulfatides

Sulfatides are natural glycolipids mostly found in the myelin sheaths in the central nervous system. Thudichum was the first person to isolate a lipid with a sulfate moiety from brain tissue and gave it the name sulfatide.[14] The sulfatides isolated from human cells are comprised of various molecular species that differ in their ceramide moieties expressly in the acyl chain including chain length, number and location of unsaturation, and presence/absence of a C-2 hydroxyl group (Figure 7).[13,15]

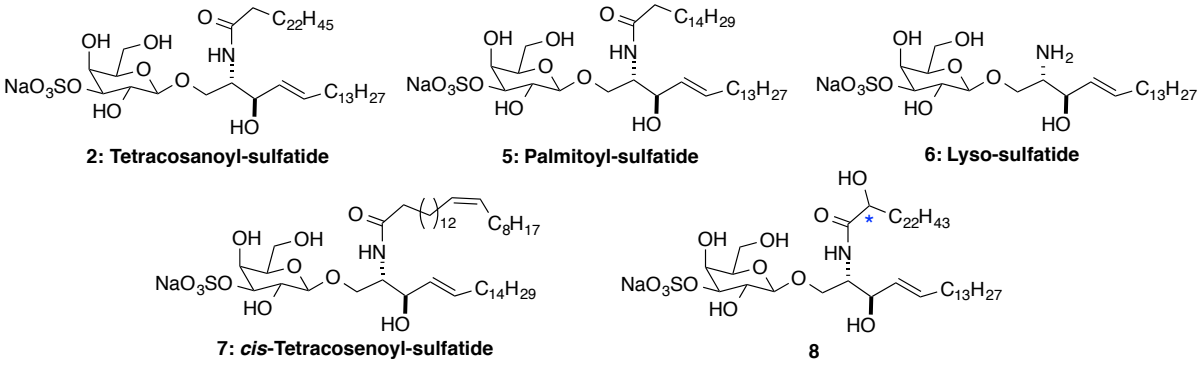


Figure 7. Selected examples of molecular species of natural sulfatides

Sulfatides can be presented by all classes of CD1 molecules to T cells to activate them for cytokine secretion.[10] The sulfate group at the C-3 position of the sugar differentiates sulfatides from other glycolipids structurally. They have a β-glycosidic configuration, in contrast to KRN7000, which has an α-glycosidic linkage. The β-glycosidic linkage in sulfatides causes their sugar moieties to elongate out farther from the α-helices of CD1d. There they interact in a diagonal docking mode with the TCR on the surface of some type II NKT cells, found above the A pocket of CD1d (Figure 8a and 8c).[16] The α-glycosidic bond in KRN7000 causes the sugar moiety to be buried more in the α-helices of CD1d, favoring it's presentation to type I NKT cells positioned above the F or C pocket of CD1d (Figure 8b) in a parallel docking mode (Figure 8d).

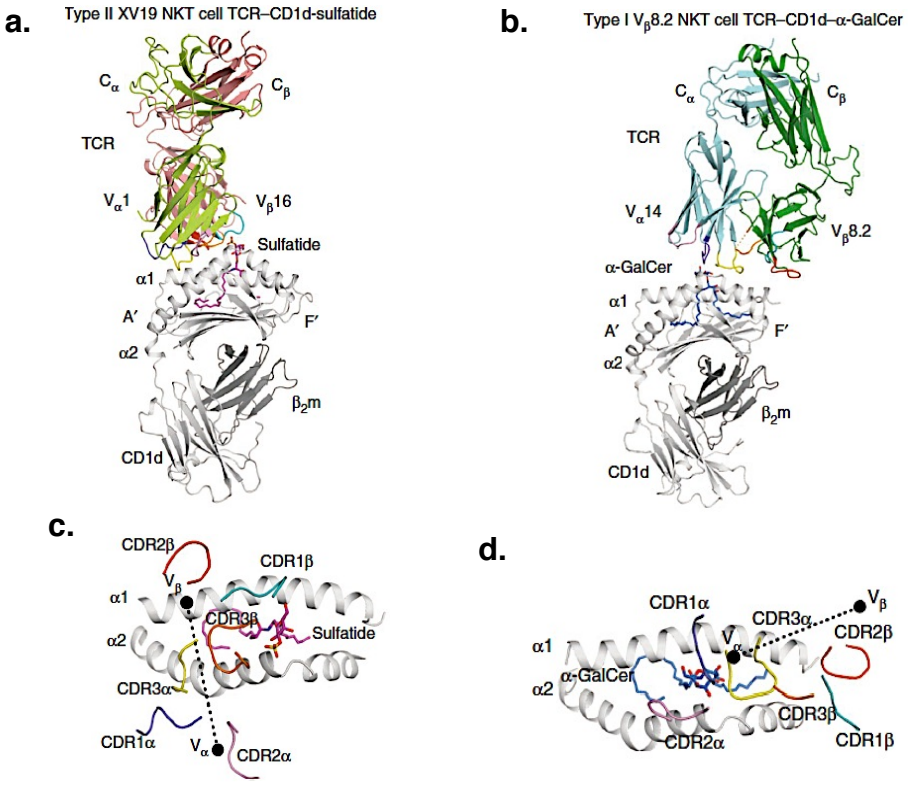


Figure 8. Overview of docking orientation; **a.** Type II NKT cell complex with the mCD1d-sulfatide; **b.** Type I NKT cell complex with mCD1d-KRN7000 (α-GalCer); **c.** Type II NKT cell- mCD1d-sulfatide complex viewed from the cleft of CD1d; **d.** Type I NKT cell-mCD1d-KRN7000 complex viewed from the cleft of CD1d[ref]

The recognition of sulfatides as activators of a subset of type II NKT cells has inspired crystal structure studies of CD1d-sulfatide-type II NKT complexes to eucidate how sulfatides interact with the NKT cells. Two independent research groups, one led by Rossjohn[16] and the other by Zajonc,[17] recently solved the crystal structure of sulfatide reactive-TCRs of type II NKT cells, using *cis*-tetracosenoyl sulfatide-CD1d-XV19 TCR complex and lyso-sulfatide (free amine, has no acyl chain)-CD1d-Hy19.3 TCR complex (9a and 9b), respectively. Neither study showed H-bonding between the sulfate moiety and any amino acid residues of the TCRs.

The only H-bonding interaction observed was between the C2-OH of the sugar and tryptophan residue W97β (unlike the KRN7000-type I NKT TCR interaction, which showed a compacted H-bonding interaction of the sugar moiety and TCRs).[Ref] The complexes were stabilized, in addition to the C2-OH-W97β H-bond, by van der Waal interactions of the TCRs amino acid residues and the ligands. The lack of an intricate H-bonding network between the sulfate moiety of sulfatides suggested that other β-glycolipids can activate type II NKT cells. It also validated β-GlCer stimulation of XV19 TCR, although this happens to a lesser extent than with sulfatides.[18]

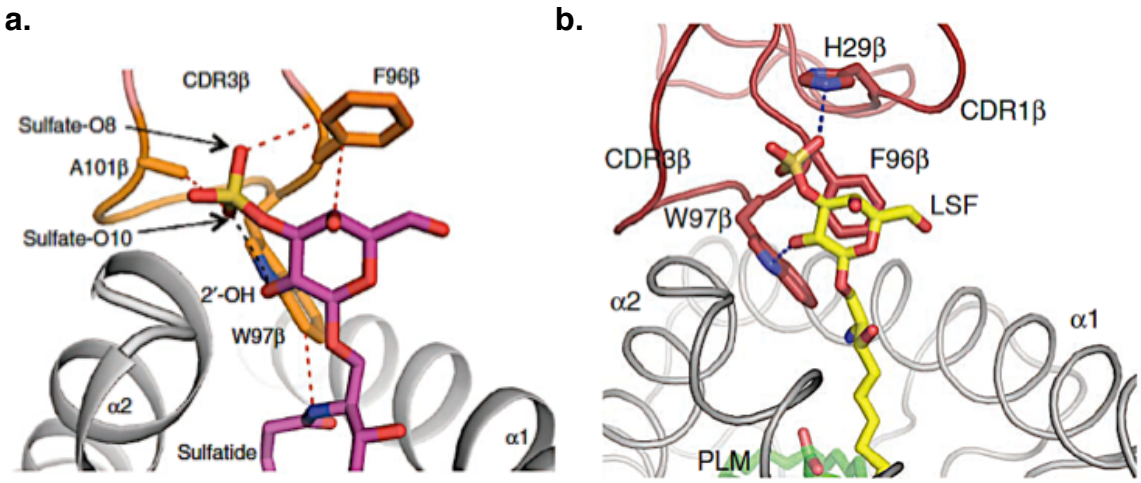


Figure 9. Type II NKT cells TCR interactions with sulfatides; **a.** XV19 TCR interaction with *cis*-tertracosenoyl sulfatide shown in magenta, van der Waal interaction shown in red dashes and H-bonds shown in black dash;[16] **b.** Hy19.3 TCR interaction with lyso-sulfatide (free amine, has no acyl chain attached) shown in yellow, H-bond and van der Waal interactions are shown in blue dashes[17]

1.1.4.1. Immunostimulatory activity of some natural sulfatide and type II NKT cells

The fact that NKT cells have been shown to play an important role in immunoregulatory activities has drawn lot of attention toward them as potential therapeutic agents. However in order to fully explore NKT cells for biological purposes it is critical for the less characterized type II NKT cell activation and cytokine secretion mechanism to be understood. Several recent studies have revealed type II NKT cells to have distinct and sometimes-complementary effects in comparison to type I NK T cells. For example, cytokines secreted by type II NKT cells have been demonstrated to enhance tumor growth, while type I NKT cells secrete cytokines cause tumor death.[6,19] Sulfatide activation of type II NKT cell subpopulations has provided a break-through for these studies, even though initial experiments conducted to identify active sulfatides were arduous. This is mainly due to their structural similarities as highlighted in Figure 7, which makes isolation of individual species challenging. Early analyses were carried out with a mixture of sulfatides. Modifications in the ceramide moiety can influence the interactions the ligands have with CD1d and T cell receptors and, consequently, also the type of responses induced.

The sulfatide-CD1d complex recognition by type II NKT cells was first reported by Jahng *et al.*[8] in their search for ligands that can activate type II NKT cells using Hy 19.3 (which is a subclone of XV19, a sulfatide-reactive type II NKT cell). Hy 19.3 was treated with different CD1d-glycolipid complexes, including sulfatide mixtures,

KRN7000 (α-GalCer), β-GalCer (β-anomer of KRN7000) and mono-GM1 (with a negatively charged sialic acid instead of sulfate), but only the sulfatide mixtures were able to stimulate the secretion of cytokines (IFN-γ and IL-4). In a later publication Kumar and co-workers demonstrated that the sulfatide active against Hy 19.3 was lyso-sulfatide **6** (see Figure 7 for structure).[20] Other individual sulfatides, **2**, **5** and **7**, which were part of the previously analyzed mixture, were inactive. Wilson and co-workers, when developing the first CD1d-sulfatide binary complex (CD1d-sulfatide **7**), tested **7** with other sulfatides (its saturated acyl chain version **2**, short chain **5** and lyso-sulfatide **6**) for cytokine proliferation, and **7** displayed the highest stimulation of ^{3}H-thymidine (a cell proliferation assay to test the potency of ligands in stimulating lymphocytes) secretion.[13]

Although many factors influence glycolipid stimulation of NKT cells, information thus far on sulfatide activation of type II NKT cells suggests the stability of the antigen-NKT cell ternary complex to be what differentiates individual sulfatide stimulatory results.[21] Highlighted below is information on the functional roles exhibited by type II NKT cells in immuno-regulation.

Multiple sclerosis (MS)

MS, also known as encephalomyelitis disseminate, is an inflammatory disease of the brain, and its animal model counterpart is called experimental autoimmune encephalomyelitis (EAE). NKT cells are targets for MS treatment because studies have shown MS infected regions to be enriched with glycolipid species. Early treatment of EAE mice with cytokines secreted from sulfatide-reactive type II NKT

cells prevented clinical disease symptoms by inactivating the IFN-γ and IL-4 production by pathogenic myelin glycoprotein reactive T cells that cause the inflammation.[8] The mechanism for prevention of EAE is proposed in a another report by Kumar and co-workers, one of the principal investigators in the previous studies, to be via the inactivation of dendritic cells and type I NKT cells after sulfatide administration.[20] This type II NKT cell mediated anergy induction of type I NKT cells is also argued by them to be important in the potential treatment of inflammatory liver disease.[22]

Tumor Immunity

The fact that type I NKT cells produce cytokines are capable of preventing tumor metastasis in mice in response to KRN7000 treatment has resulted in type I NKT cells been screened for tumor immunity. In contrast to type I NKT cells, sulfatide-reactive type II NKT cells released cytokines enhance tumor metastasis by suppressing immunosurveillance (the method employed by the immune system to recognize transformed cells in order to inhibit their growth into neoplastic tissues).[19] Ambrosino *et al.* evaluated sulfatides' stimulatory activity in tumor metastasis using three mice genotypes, Jα18KO mice (only express type II NKT cells not type I NKT cells), WT mice (contain both type I and type II NKT cells) and CD1dKO mice (do not express either NKT cells). The Jα18KO mice showed augmented tumor metastasis, depicted in the graph (Figure 10) by the number of nodules, while type I NKT cells prevented metastasis, indicated by the low nodules present in WT mice. The CD1dKO mice showed no response. The lower nodule levels in WT mice

suggests an antagonizing effect of type I and type II NKT cells in tumor immunity, invoking a new immunoregulatory axis that needs to be resolved to understand subsequent immune responses.

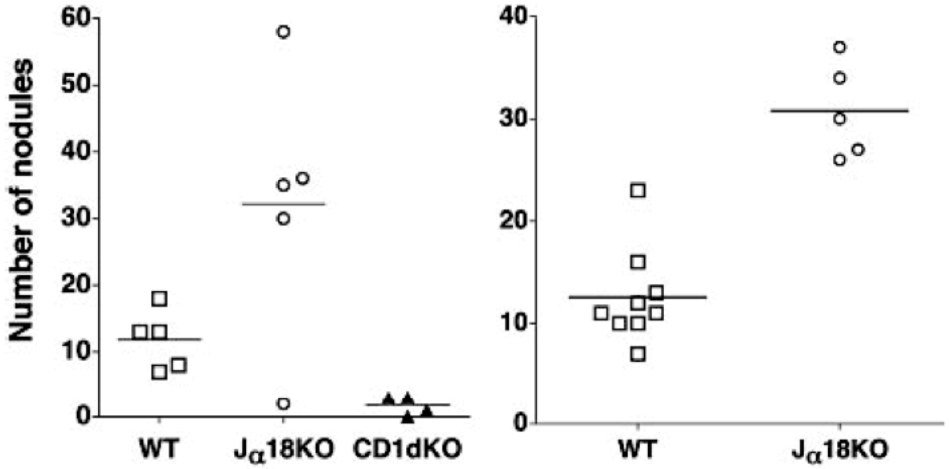


Figure 10. The CD1d-restricted type II NKT cells down-regulate tumor immunosurveillance and the absence of type I NKT cells correlates with a higher susceptibility of tumor growth[19]

Type I diabetes

Subramanian *et al.* have demonstrated that sulfatide **2** activation of type II NKT cells causes the secretion of IL-10 (a Th2 cytokine), which helps protect non-obese mice from type I diabetes.[21] A similar observation was made by Durate *et al*.[23]

Parasitic infection

The antagonistic role of type I and type II NKT cells is not only observed in tumor immunity but is also seen in the presence of parasitic infection. The evaluation of the role-played by NKT cells in *Trypanosoma cruzi* infection (the cause of Chagas disease, a chronic inflammation) revealed that type II NKT cells (Jα18KO mice expressed only type II NKT cells) enhance proinflammation in infected mice and

eventually caused their death (Figure 11). Conversely type I NKT cells performed a anti-inflammatory function, cross-regulating the type II NKT cells activities and increasing the survival rate of the mice (wild type).[24]

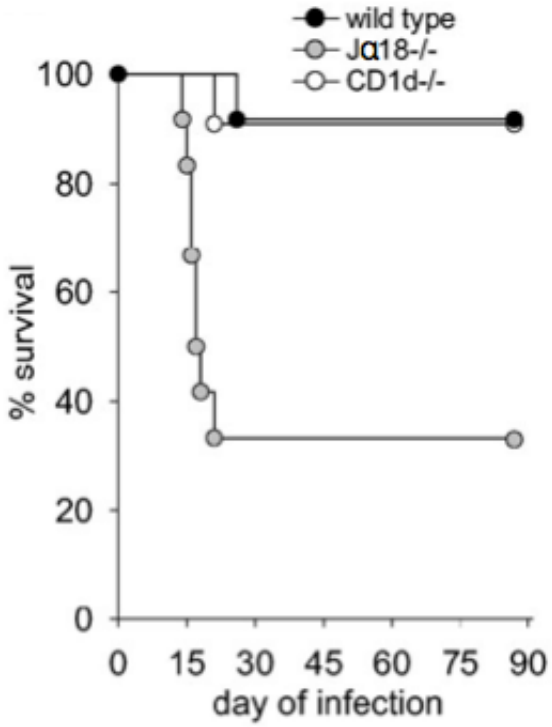


Figure 11. *T. cruzi* infection in mice, Jα18KO mice (only expressed type II NKT cells) enhances proinflammation, eventually causing mortality, while in wild type mice (which expressed both NKT cell types), the type I NKT cells present counter-balanced the type II NKT cells activity, indicated by the increase in mice survival rate [24]

Graft-versus-host disease (GVHD)

Bone marrow transplant (BMT), albeit an effective treatment for cancer, has the consequences of causing GVHD, a disease that frequently arises as a result of complications during the process. NKT cells have been shown to be effective in preventing it. Kim *et al.*[25] showed both IL-4 and INF-γ cytokines secreted by type II NKT cells to protect against GVHD. IL-4 guards against GVHD through the

deversion of the immune system toward a Th2-type response. Exley *et al.*[26] also reported a Th2-biased cytokine profile in GVHD treatment. The INF-γ derived mode of controlling GVHD is through the expression of Fas-L, inducing apoptosis of donor T cells. The type I NKT cells produced INF-γ also prevents GVHD but they engage the host T in contrast to the donor T cells by type II NKT cells.

Ulcerative colitis (UC)

This is an inflammatory bowel disease characterized as a Th2 response inflammation. Type II NKT cells from the lamina propria in ulcerative colitis patients expressed an increased level of IL-13, a Th2 cytokine, which in turn elevates the inflammation. Presence of type II NKT cells is a critical piece to be considered in the treatment of UC.[27]

Hepatitis

NKT cells are a target for hepatitis treatment, a disease condition characterized by the presence of inflammatory cells and tissues in the liver. Type I NKT cells been shown to mediate concanavalin A (a letin carbohydrate binding protein) induced experimental hepatitis. Kumar and co-workers have reported cytokines secreted by type II NKT cells upon sulfatide injection to prevent this type I induced liver damage.[22] The mode of this prevention is through the inactivation of pathogenic type I NKT cells.

1.1.5. Structure Activity Relationship (SAR) on Sulfatides

To date there have been no SAR studies done on sulfatides. Current research efforts are tfocused on identifying lead sulfatide targets from those isolated from

human cells. However, with the information now available from crystal structure studies on the sulfatide-TCR interaction of type II NKT cells, SAR studies should be easier.

1.1.6. Purpose of Study

The potential of exploring type II NKT cells for therapeutic purposes based on their vital role in immune responses inspired us to synthesize the natural and unnatural sufatides shown in Figures 12 and 13 for further studies. The targets in Figure 12 were selected for structure-activity studies on the effect of the number of unsaturations in the acyl chain on the ability of the lipids to stimulate type II NKT cells. Previous biological analyses by our collaborators at the NIH had shown that target **7**, which has a double bond in its acyl chain, stimulated higher secretion of both IFN-γ and IL-4 than sulfatide **2**, which has no double bond in its acyl chain. These data suggested that the degree of unsaturation in the acyl chain affects the ability of the lipids to stimulate type II cells. Sulfatide **9**, which has an extra double bond in its acyl chain, was also targeted to evaluate the effect of a higher degree of unsaturation. Phytosphingosine analogs **10–12** of compounds **2**, **7** and **9** were also targeted. These were of interest because the α-galactosylceramide family of agonists for *i*NKT cells are largely phytosphingosine-containing ceramides, whereas the natural sulfatides that had been found to stimulate type II NKT cells are sphingosine based. Therefore, it is of interest to determine the role of the sphingosine-phytosphingosine difference in the specificity of these glycolipids for different subsets of NKT cells.

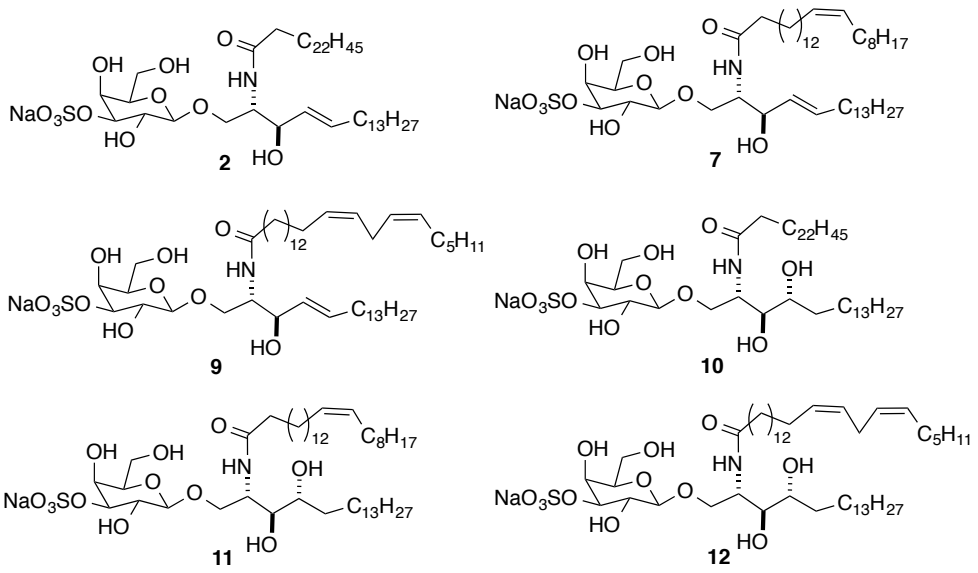


Figure 12. Initial sulfatide targets

Two novel sulfatides **13** and **14** (Figure 13) were also targeted to further probe the effect of the ceramide moiety on the nature of the immune response of sulfatide-reactive type II NKT cells. Their design was based on what is known about compounds that elicit a biased activation of type I NKT cells (*i*NKT cells). Ligands **13** and **14** have acyl chain and sphingoid base that are found in analogs **15** and **16** (respectively) of KRN7000 that have been shown to induce a Th1 type response in *i*NKT cells. For compound **15** the Th1 bias is attributed to π-π stacking with aromatic residues in the A' pocket of CD1d.[28] The ternary crystal structure of **16** shows that the sphingoid base has an increased buried surface area.[29] It is not clear how such interactions with CD1d are going to impact the response of type II NKT cells in comparison to *i*NKT cells, as structural information about CD1d/ceramide interactions have not been exploited with sulfatides. Sulfatides **13** and **14** represent

a starting point for this. This dissertation will focus on the synthesis and initial assays of **13**.

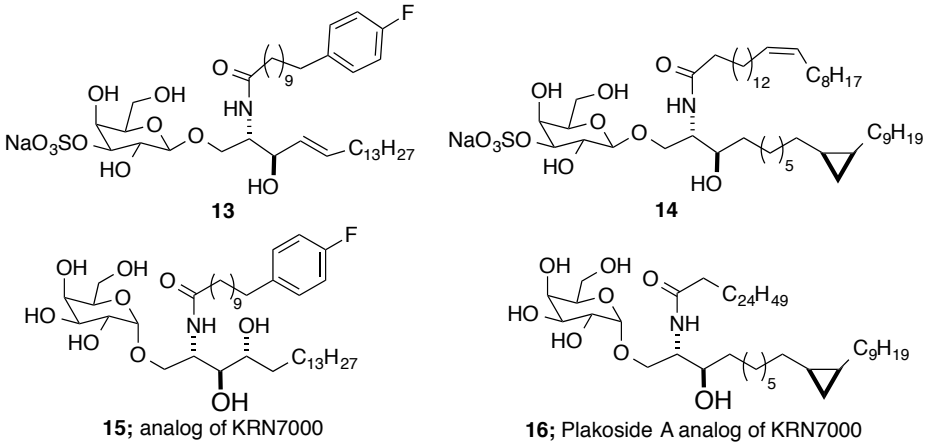


Figure 13. Unnatural sulfatides and KRN7000 analogs

1.1.7. Glycosylation approaches

Glycosphingolipids are normally synthesized by one of two approaches (A or B, Figure 14). In both pathways glycosylation is the key and most problematic step. In pathway A, the galactosyl donor (**i**) is coupled to a ceramide acceptor (**ii**). Pathway B uses a sphingoid base (**iv**) as the acceptor, and the product of this reaction (**v**) undergoes *N*-acylation to give the same product (**iii**) as pathway A.

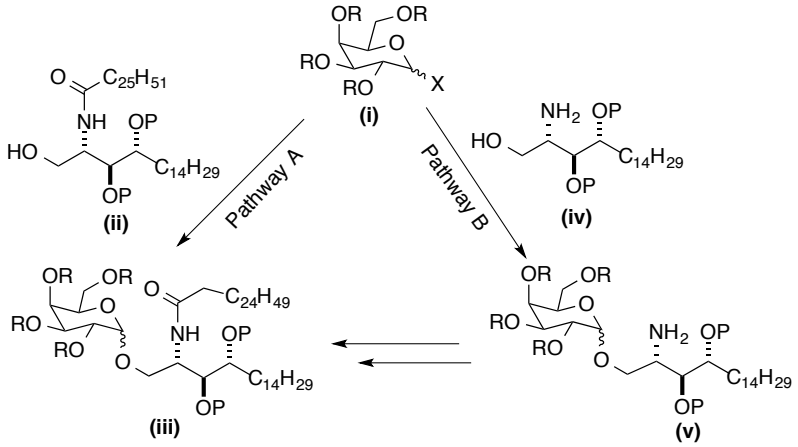


Figure 14. Glycosylation approaches

A key consideration when selecting protocols for glycosylation reactions is the α- and β-glycosidic bond selectivity. Although the thermodynamically stable product is the α-glycosidic linkage, it is not always the predominant product. Protecting groups,[ref] leaving groups[ref] and solvents[ref] have been manipulated to enhance α- or β-product preference. Solvent manipulations have been successful in favoring α-anomers and have been extensively used in the synthesis of α-glycosphingolipids (KRN7000 and analogs).

Just as solvents have been used to favor α-anomer formation, appropriate protecting groups have been used to give selectively β-anomers. This phenomenon is called anchimeric assistance, also referred to as neighboring group participation, and is a common strategy utilized in carbohydrate chemistry to give β-selectivity. The concept relies on having a protecting group (acetate, benzoyl or pivaloyl group) at the C-2 position of the sugar to stabilize the formed oxocarbeniun ion, as shown in Figure 15. The C-2 acetate-protecting group on the sugar interacts with the anomeric carbon, converting the open oxocarbenium ion **A** (formed after the leaving group departs) to a cyclic oxocarbenium ion **B**, blocking the α-face and leaving the β-face accessible for nucleophilic attack. This leads to the formation of β-glycosidic product **C**.

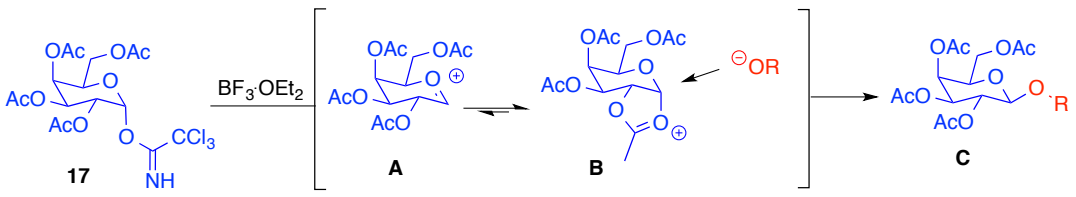
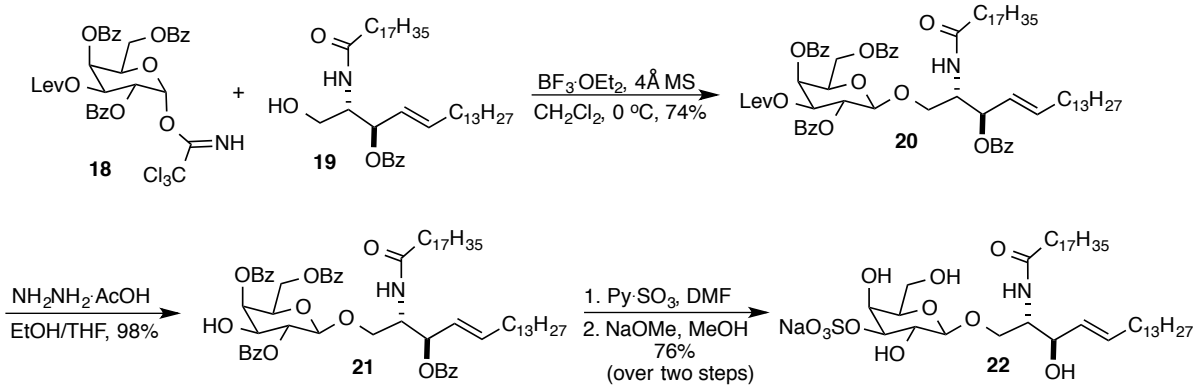


Figure 15. Anchimeric assistance

1.1.8. Approaches to previously synthesized sulfatides

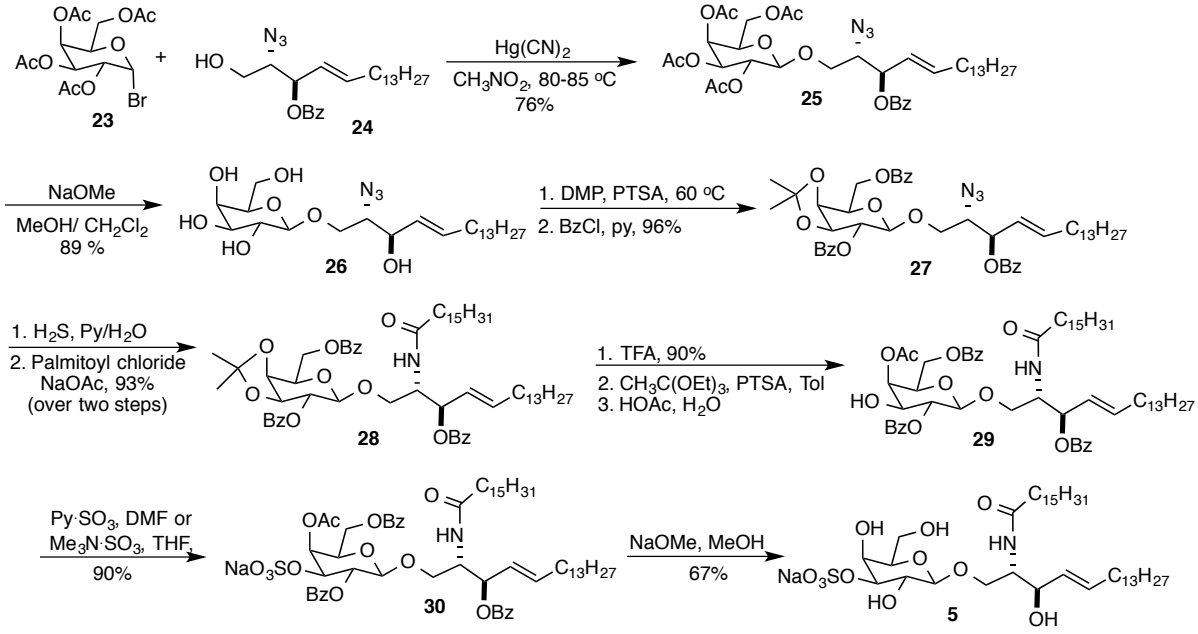
Sulfatides, like other glycosphingolipids, have been prepared using both glycosylation approaches highlighted in Figure 14. In addition to formation of the glycosidic bond, regioselective installation of the sulfate group at the sugar moiety (C3-OH) is a critical step in sulfatide synthesis. The sulfate moiety is acid sensitive and unstable to most reaction conditions, by default making its installation late stage and ideally the last step in the reaction sequence.

Early syntheses of sulfatides depended on protecting group manipulation strategies. In 1997 Tanahashi and co-workers[30] synthesized various sulfatides as selectin ligands/inhibitors using a pathway A glycosylation approach and protecting groups to systematically install the sulfate group (Scheme 1). Their synthesis of sulfatide **22** commences with coupling of trichloroacetimdate donor **18** (prepared from using at least three orthogonal protecting groups) with ceramide acceptor **19** to give **20**. Selective deprotection of the levulinoyl (Lev) group gave **21**. Sulfation of the C3-OH with sulfur trioxide pyridine (Py•SO_3) complex yielded a sulfatide intermediate that underwent deprotection of the benzoyl groups to give final sulfatide **22**.



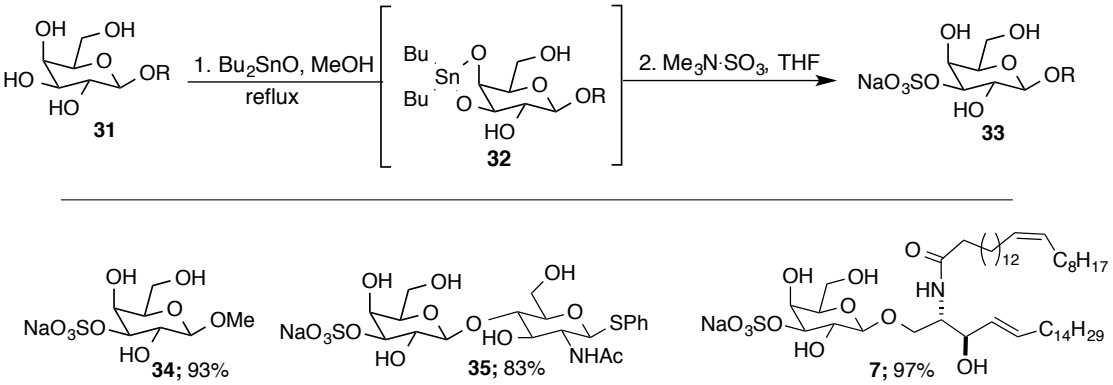
Scheme 1. Tanahashi's synthesis of sulfatide **22**

A similar protecting group sulfation strategy was used by Marinier *et al.*[31] to prepare sulfatide **5**, using a pathway B glycosylation strategy (Scheme 2). Helferich glycosylation conditions were used to couple bromo galactose donor **23** with sphingosine acceptor **24** to give glycolipid **25** with 9% α-product (separable). Global cleavage of the acetyl and benzoyl protecting groups gave azido-glycolipid **26**. Acetonide protection of the sugar moiety (C3-OH and C4-OH), followed by benzoyl protection of the rest of the hydroxyl groups, furnished glycolipid **27**. Reduction of the azide group, followed by acylation gave **28**. Cleavage of the acetonide group, followed by acetate protection of C4-OH via an orthoester gave the free C3-OH product **29**. This product underwent sulfation with either ammonium sulfur trioxide ($Me_3.N{\bullet}SO_3$) complex or sulfur trioxide pyridine ($Py{\bullet}SO_3$) complex to give **30**. Cleavage of the protecting groups in **30** gave final sulfatide product **5**.



Scheme 2. Marinier's synthesis of sulfatide **5**

To address the lengthy protecting group strategy and to achieve a more economical regioselective sulfation, Flitsch and co-workers developed a dibutylstannylene acetal approach.[32] The methodology relies on the formation of the dibutylstannylene acetal at the C3-OH and C4-OH of saccharides or glycolipids (Scheme 3). The reaction proceeds by refluxing saccharides or glycolipids like **31** with Bu_2SnO in MeOH to form the cyclic dibutylstannylene acetal **32**. This increases the nucleophilicity of the equatorial alcohol (C3-OH) to react selectively with the $Me_3N{\bullet}SO_3$ complex gave target-sulfated compounds **7**, **34** and **35**. This sulfation strategy is now the most widely applied synthetic route to prepare β-sulfatides[33-36] and is used in this dissertation.



Scheme 3. Flitsch's dibutylstannylene acetal synthesis of sulfatides

1.1.9. Summary

Although the effect of sulfatide-reactive type II NKT cells is not well defined with respect to their effect on immune responses, the summarized data presented here suggests they sometimes have different roles than type I NKT cells. These findings suggest a new immunoregulatory axis that needs to be analyzed to understand the

functional role-played by NKT cells and to develop drug candidates. The successful syntheses of sulfatide targets (**2** and **9**–**13**) and their analyses in the activation of type II NKT cells will contribute to the ongoing search towards defining their role.

1.2. Discussion

1.2. 1. Research objective

The goal of this study is to synthesize sulfatides **2**, and **9**–**13**. The synthesis of sulfatide **10** was embarked upon first, based on the priorities of our collaborators.

1.2. 2. Synthesis of sulfatide 10

Pathway A glycosylation route was explored in our initial strategy to sulfatide **10**, which would be obtained in three main reactions (sulfation, deprotection and glycosylation) from previously known compounds (Figure 16). Sulfation of advanced intermediate **36** would furnish target **10**. In turn, **36** could be obtained from **37** by cleavage of the ester protecting groups. Coupling of sugar donor **17** with ceramide acceptor **38** would give **37**. Sugar donor **17** could be obtained in two steps from commercially available β-pentaacetyl-D-galactose and ceramide **38** in four steps from commercially available phytosphingosine.

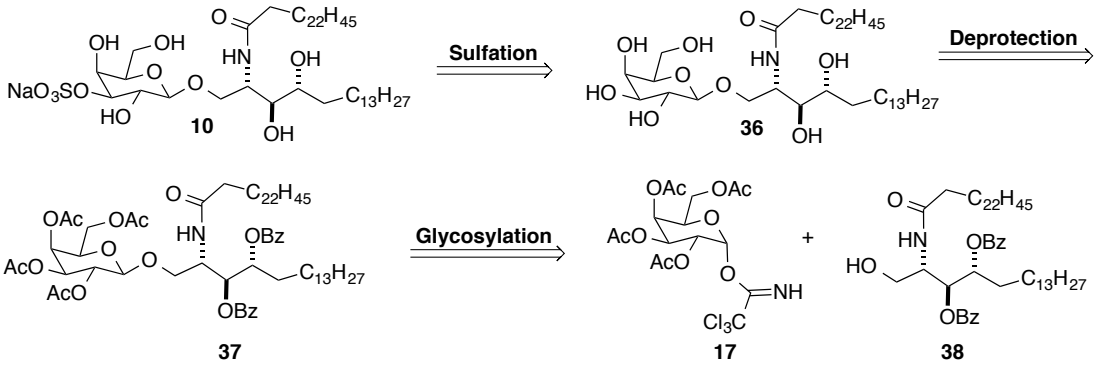
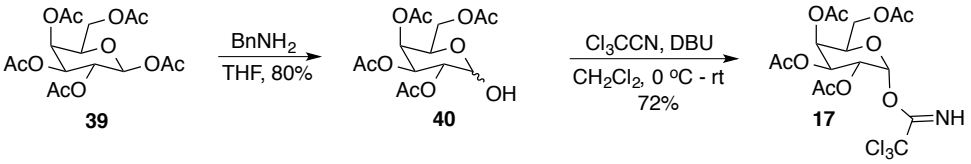


Figure 16. Retrosynthesis to sulfatide target **10**

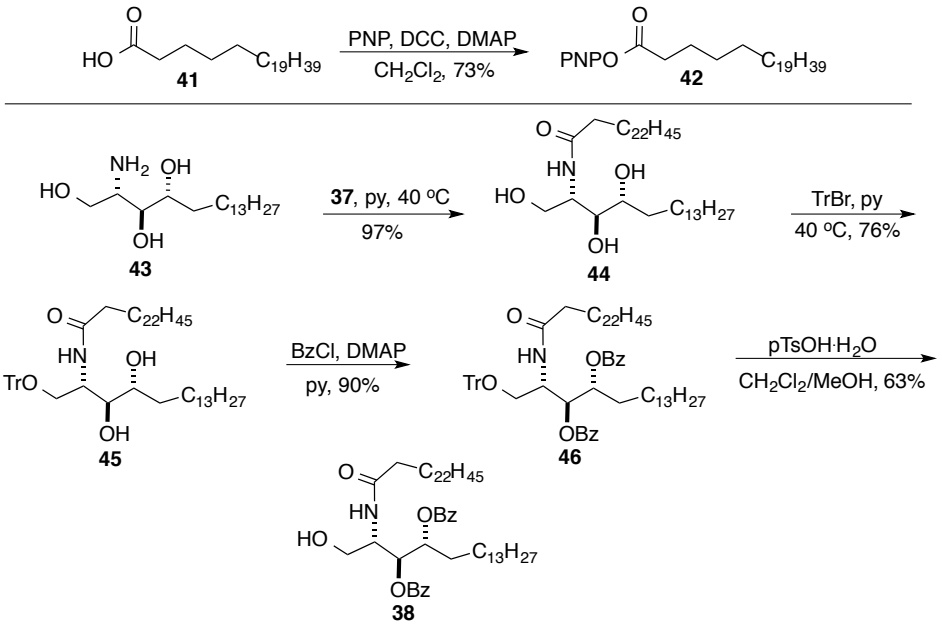
Tetraacetyl trichloroacetimidate sugar **17** was chosen as the sugar donor because of the successes reported in the literature in obtaining β-selectively and the ease of activating the trichloroacetimidate group under mildly acidic conditions.[37]

Chemoselective benzylamine cleavage of commercially available β-pentacetyl-D-galactose **39** gave lactol **40** (Scheme 4). This was converted to the desired trichloroacetimidate sugar donor **17** using trichloroacetonitrile in the presence of catalytic DBU[37]



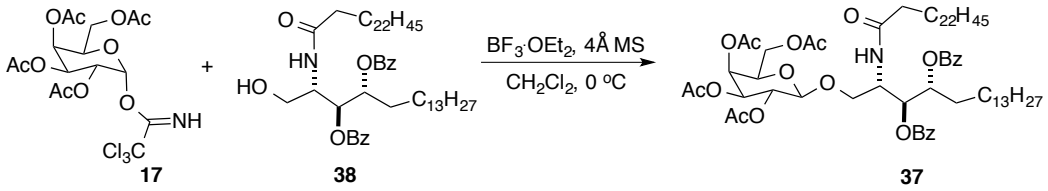
Scheme 4. Synthesis of trichloroacetimidate sugar donor **17**

The synthesis of ceramide acceptor **38** started with acylation of commercially available phytosphingosine (**43**) with the *p*-nitrophenol activated ester (**42**) of tetracosanoic acid **41** to give **44** (Scheme 5). Trityl protection of the primary alcohol gave diol **45**. Benzoyl protection of the secondary alcohols gave **46**. Cleavage of the trityl protecting group with pTsOH•H_2O gave ceramide acceptor **38**.



Scheme 5. Synthesis of ceramide acceptor **38**

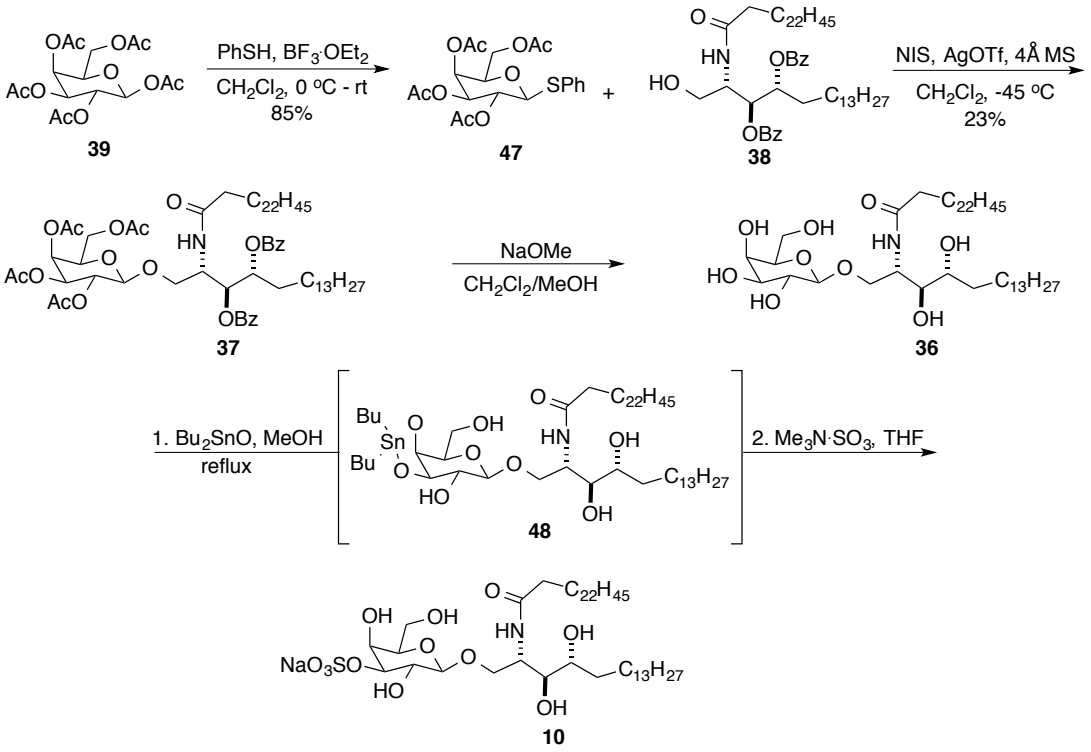
The sugar donor **17** and acceptor **38** were coupled in the presence of BF_3•OEt_2 as an activator to give glycolipid **37**, along with its α-anomer and other side products (Scheme 6). Purification of the desired product **37** from its α-anomer and an impurity assumed to be hydrolyzed sugar donor (on the basis of NMR analysis) was very difficult. Thus no clean product was isolated. Another sugar donor with a different leaving group was prepared.



Scheme 6. Glycosylation of sugar donor **17** and acceptor **38** to give **37**

Thiogalactosyl donor **47** was chosen as the alternative sugar donor, based on the successes we have had using thiophenol as a leaving group. It was obtained from BF_3•OEt_2-catalyzed thioglycosylation of β-pentaacetyl-D-galactose (**39**) with thiophenol. Glycosylation of sugar **47** with ceramide acceptor **38** gave desired product **37**, again along with its α-isomer. This time the separation was much easier, providing β-product **37**. Cleavage of the ester protecting groups of **37** gave free glycolipid **36**, which was subjected to Flitsch's protocol for selective sulfation of the C3-OH of the galactose moiety.[32] Glycolipid **36** was refluxed with Bu_2SnO in MeOH, furnishing cyclic dibutylstannylene acetal **48**. Treatment of **48** with Me_3N•SO_3 complex gave target sulfatide **10**. Although both ^{13}C NMR and Hi-Res mass spectrometry confirmed formation of sulfatide **10**, ^{1}H and ^{13}C NMR showed

Bu_2SnO related impurities that couldn't be removed after multiple purifications on silica gel, which resulted in ultimate decomposition of the sulfatide.

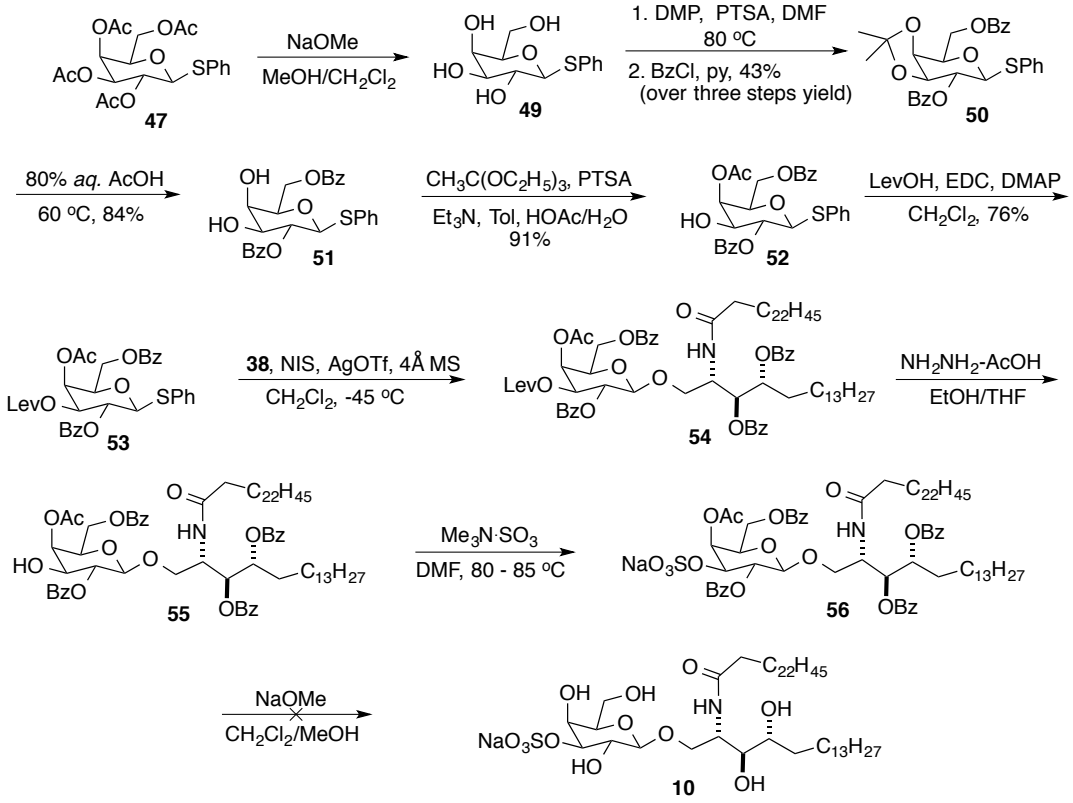


Scheme 7. Thioglycosidic sugar donor **47** route to prepare sulfatide **10**

To overcome the purification problem encountered following Flitsch's procedure for the synthesis of sulfatide **10**, we decided to explore a sulfation strategy in the presences of protecting groups to avoid using Bu_2SnO. Sugar donor **53**, a similar sugar donor to the one utilized by Tanahashi (see Scheme 1), was prepared.

Global cleavage of the acetate protecting groups of **47** gave **49** (Scheme 8), which was then subjected to two protection reactions. First acetonide protection then benzoyl protection of the remaining hydroxyl groups furnished galactose **50**. Chemoselective removal of the acetonide protecting group gave **51**. C4-OH acetate protection via *in situ* generation of the orthoester of **51** gave the free C3-OH **52**,

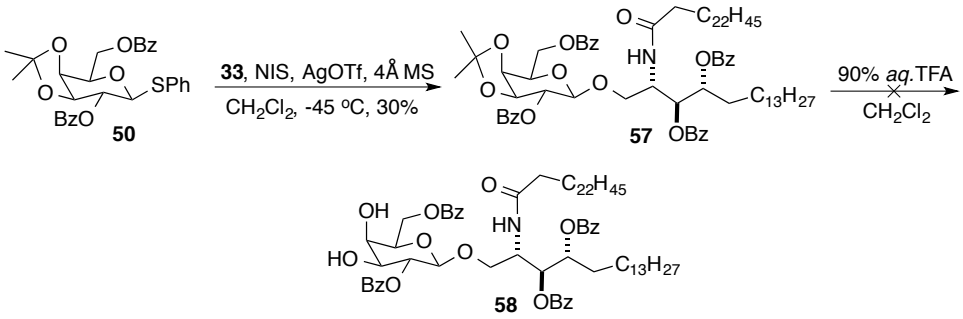
which was treated with levulinic acid to give Lev-protected galactose **53**. Glycosylation of sugar donor **43** with ceramide acceptor **38** gave impure product **54**. The rest of the reaction protocol was attempted with this to test the practically of the approach prior to purification studies. Hydrazine acetate catalyzed selective facilitatede of the levulinoyl group gave the free C3-OH glycolipid **55**, which underwent sulfation using $Me_3N{\bullet}SO_4$ in DMF to give impure **56**. Global deprotection of the ester groups gave no clean desired product **10**.



Scheme 8. Lev-protecting group approach to synthesize sulfatide **10**

Because of the unsuccessful formation of sulfatide **10** using Tanahashi's approach, Marinier's approach using sugar donor **50**, an intermediate available to us from the synthesis of donor **53** was tried (Scheme 9). Glycosylation of **50** with ceramide **38**

gave product **57**, but attempted cleavage of the acetonide protecting groups using 90% *aq*. TFA, cleaved the anomeric bond instead of the acetonide group.



Scheme 9. Acetonide sugar donor **50** approach to prepare sulfatide **10**

1.2.2.1. 1-Butanol/H_2O partition purification technique to remove the Bu_2SnO impurity

While exploring routes to prepare sulfatide **10**, we continued to search for purification techniques to remove the Bu_2SnO related impurity. Dr. Petr Illarionov from the University of Birmingham suggested partitioning of the sulfatide in a 1-butanol/H_2O mixture and then centrifiguing to isolate the sulfatide in the 1-butanol layer. Sulfatide **10** was prepared again following Scheme 7 and subjected to the new purification technique before column chromatography on silica gel. This resulted in removing >98% of the Bu_2SnO related impurity, providing sulfatide **10**, which was sent to our collaborators.

1.2.3. Retrosynthetic approaches to synthesize sulfatides (**2** and **9–13**) via pathway B glycosylation strategy

Pathway B glycosylation was chosen to prepare the rest of the sulfatide targets, as it provides amino-glycolipids **59** and **60**, functionalization of which through acylation would gave advanced intermediates (Figure 17). Sulfation of these intermediates would complete the synthesis of the sphingosine- (**2**, **9**, **13**) and phytosphingosine- (**10–12**) sulfatides.

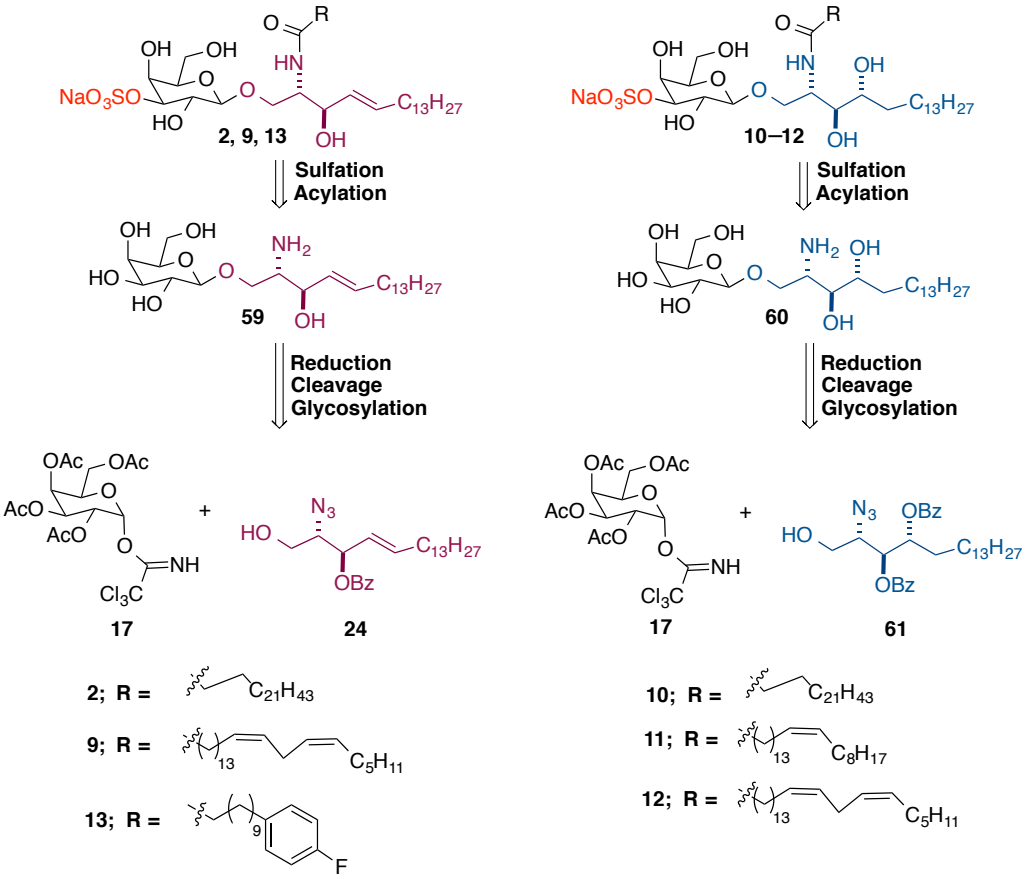
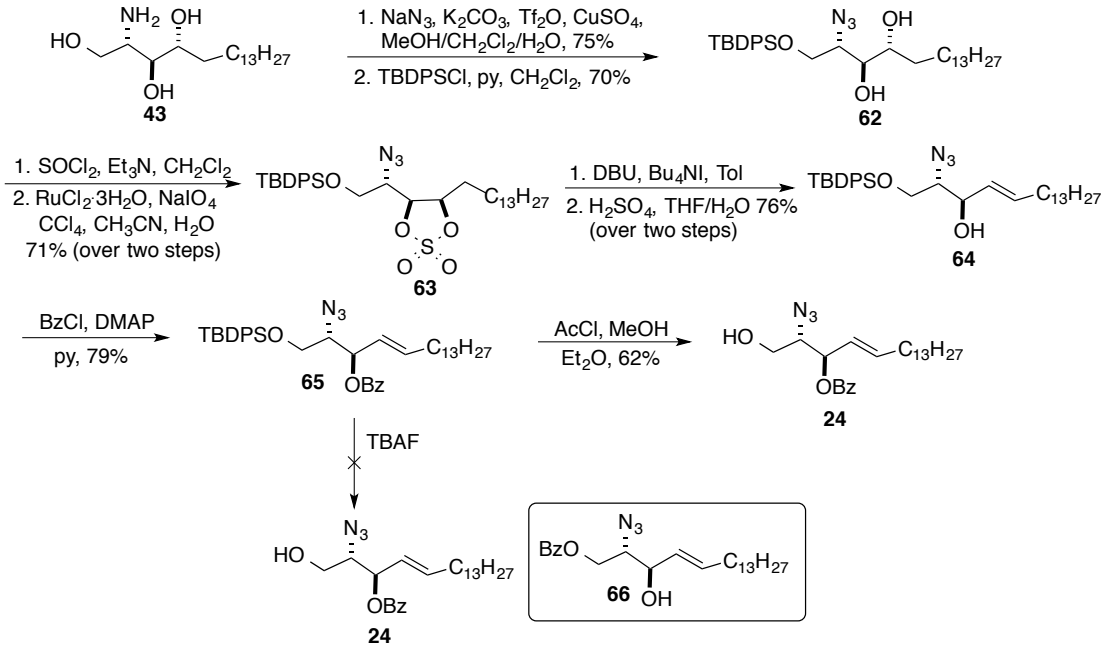


Figure 17. Retrosynthesis to sulfatide targets **2** and **9–13**

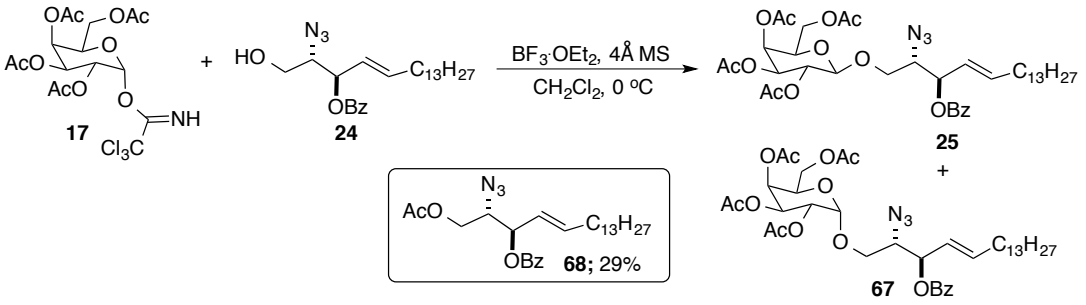
Amino-glycolipids **59** and **60** in turn can be obtained from cleavage of the ester protecting groups, followed by reduction of the azide in the glycosylation products of sugar donor **17** with acceptors **24** and **61** respectively.

To prepare the sphingosine-sulfatide targets (**2**, **9** and **13**) the sphingosine starting material required to give desired acceptor **24** was synthesized from D-*ribo*-phytosphingosine (**43**) instead of buying it from an expensive commercial source. The protocol of Wang *et al*. was followed for the azide protection of phytosphingosine **43** (Scheme 10). The reaction proceeded via a diazo transfer reaction of the amine with Tf_3N, generated *in situ* from NaN_3 and Tf_2O.[38] TBDPS protection of the primary alcohol gave diol **62**. The free diols in **62** were activated with thionyl chloride to give the cyclic sulfite, which was oxidized with catalytic $RuCl_2 \bullet 3H_2O$ to give cyclic sulfate **63**. Opening of **63** with Bu_4NI, followed by DBU promoted elimination gave a sulfated olefin, and subsequent aqueous H_2SO_4 hydrolysis gave sphingosine **64**. Benzoyl protection of the secondary alcohol gave **65**. Cleavage of the TBDPS group was first attempted with TBAF, but no desired product was isolated. Instead the benzoyl group migrated to the primary position, and **66** was obtained. 2 M HCl, generated *in situ* (AcCl in MeOH), following the procedure of Glaudemans *et al.*,[39] produced the desired sphingosine acceptor **24**.



Scheme 10. Synthesis of sphingosine acceptor **24**

The coupling of sugar donor **17** and acceptor **24**, catalyzed by BF_3•OEt_2, gave glycolipid **25** with its α-anomer **67** detected by NMR and acetylated-sphingosine **68** (Scheme 11). Separation of the desired product **25** from its α-anomer **67** was very difficult, although Marinier had reported separating the isomers (see Scheme 2).



Scheme 11. Glycosylation of sugar donor **17** and acceptor **24** to give **25**

The difficult separation of desired product **25** from α-anomer **67** and the competing formation of **68** prompted us to prepare another sugar donor with a different protecting group on the C-2 position that could provide anchimeric assistance, but be less prone to undergo trans-esterification. It was also important that the α-anomer be easily separable. Benzoyl- and pivaloyl-protected trichloroacetimidate sugar donors **69** and **70** were selected (Figure 18).

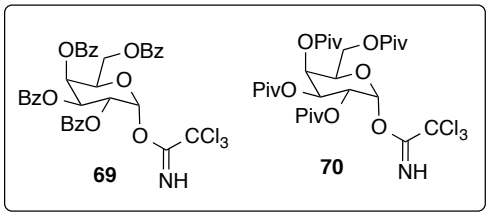
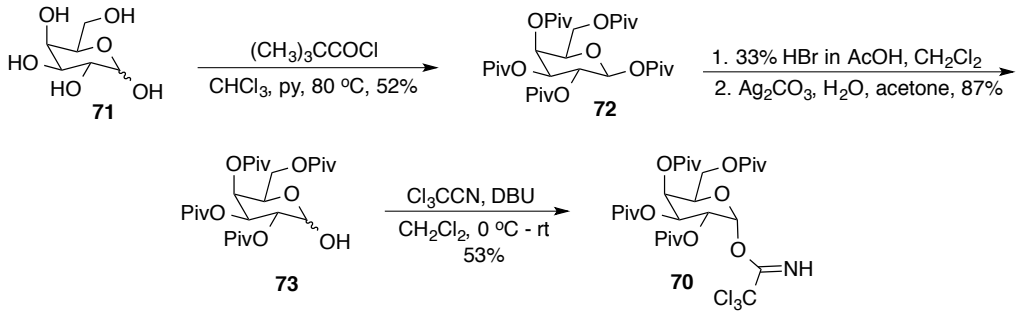


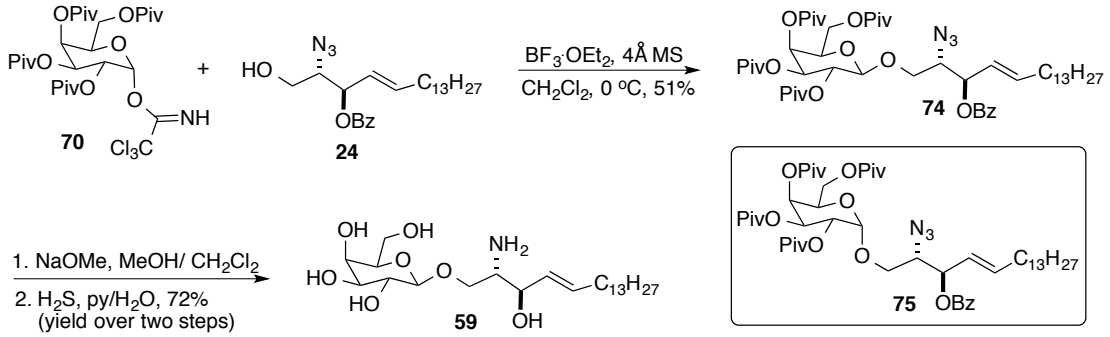
Figure 18. Alternative sugar donors **69** and **70**

Several methods were attempted to prepare benzoyl-protected trichloroacetimidate sugar donor **69**, but no clean product was isolated. Therefore the synthesis of sugar donor **70** was undertaken. Global pivalyol protection of D-galactose (**71**) gave **72** (Scheme 12).[40] Cleavage of the anomeric pivaloyl group using either hydrazine or $BnNH_2$ to give **73** was unsuccessful. A two- step hydrolysis employed by Menger and Mbadugha in the cleavage of anomeric benzoyl-protected sugar was utilized to give lactol **73**.[41] Pentapivaloyl galactose **72** was first treated with HBr (33% in AcOH) to give a bromo sugar, which was treated with Ag_2CO_3 in aqueous acetone to give lactol **73**. Activation of lactol **73** to trichloroacetimidate sugar donor **70** followed standard conditions.



Scheme 12. Synthesis of sugar donor **70**

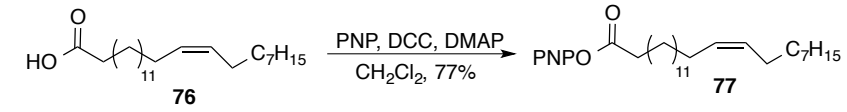
BF_3•OEt_2-catalyzed glycosylation of acceptor **23** with sugar donor **70** gave glycolipid **74** and its α-anomer **75** as a minor product (Scheme 13). Combi-flash column chromatography was used to separate **74** from **75**. NaOMe catalyzed cleavaged of the pivaloyl-protecting groups in **74**, followed by H_2S reduction[42] of the azido group, gave free amino-glycolipid **59**, the required intermediate for the preparation of sulfatides, **2**, **9**, and **13**.



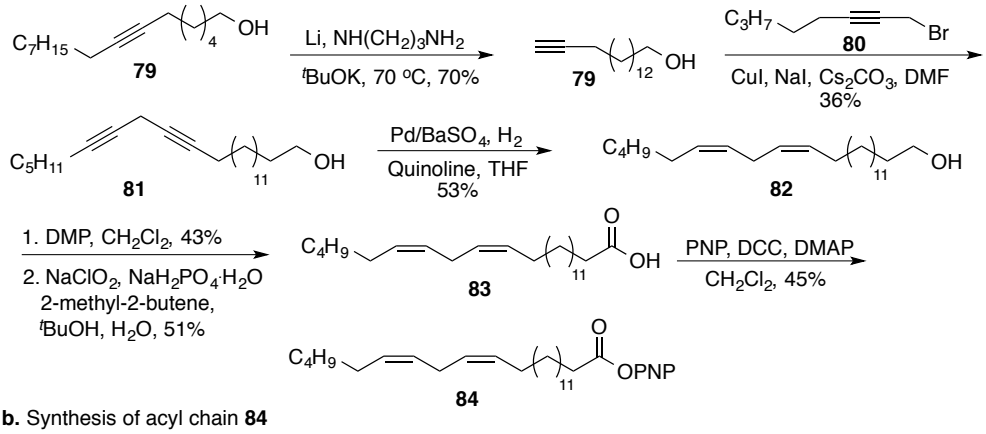
Scheme 13. Synthesis of amino-glycolipid **59**

Once amino-glycolipid **59** was obtained, the syntheses of the required activated esters (**77**, **84**, and **88**) were undertaken. The *p*-nitrophenol ester was chosen as the activating group for the acyl chain acids because of previous success with this

group. PNP ester **77** was easily prepared from its corresponding commercially available acid **76** (Scheme 14a).



a. Synthesis of acyl chain **77**

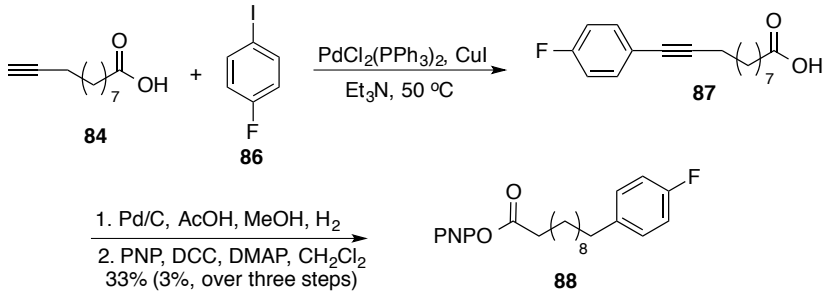


b. Synthesis of acyl chain **84**

Scheme 14. Synthesis of esters **77** and **84**

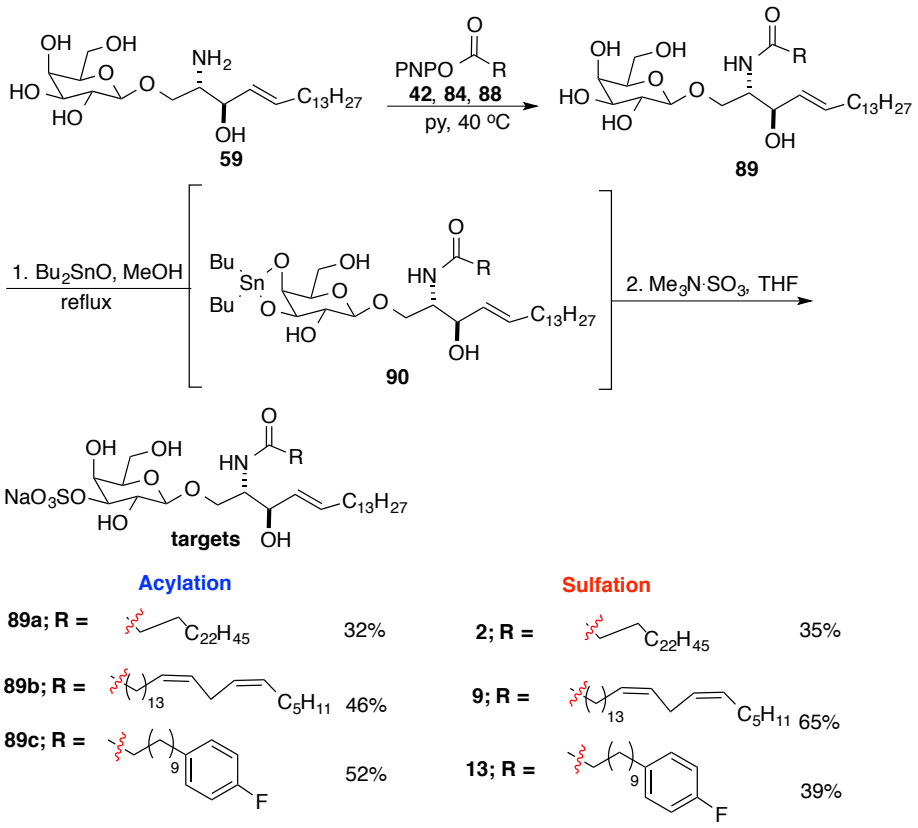
The required carboxylic acids for esters **84** and **88** were not commercially available and had to be prepared. Alkyne zipper reaction of **78** to produce terminal alkyne **79** (Scheme 14b) initiated the synthesis of ester **84**. CuI-catalyzed alkylation of **79** with propargyl bromide **80**, facilitated by NaI and $CsCO_3$ in DMF, gave skipped-diyne **81**. This was treated under Lindlar hydrogenation reaction conditions to give skipped-diene **82** with 17% of its inseparable *trans* isomer. Dess Martin perdiodinane (DMAP) oxidation of alcohol **82** to an aldehyde, and succeeding oxidation of the aldehyde with $NaClO_2$ using $NaH_2PO_4 \bullet H_2O$ as a buffer furnished acid **83**. PNP activation of **83** gave ester **84** (still with 17% *trans* isomer).

Activated ester **88** was prepared in 3 steps (Scheme 15). Sonogashira cross coupling of acid **85** with *p*-fluoro-iodo benzene **86** gave **87** with inseparable impurities. Hydrogenation of alkyne **87** to its corresponding alkane and subsequent PNP activation of the acid gave clean ester **88**.



Scheme 15. Synthesis of acyl chain **88**

Amino-glycolipid **59** was acylated with the appropriate *p*-nitrophenol activated esters (**42**, **84** or **88**) in pyridine to give the advanced intermediates (**89a–89c**), ready for sulfation (Scheme 16). Selective installation of the sulfate group at the C3-OH of the glycolipid's sugar moieties proceeded via Flitsch's Bu_2SnO approach with $Me_3N{\bullet}SO_3$ providing sulfatides **2**, **9**, and **13**. The amount of Bu_2SnO used was cut to 1.2 equiv. instead of the 2 equiv. described in the original procedure. This made the separation from the tin impurity more facile.



Scheme 16. Completion of sulfatide targets **2**, **9** and **13**

The partitioning of the sulfatides in a mixture of 1-butanol/H_2O was necessary before column chromatography as described in the early preparation of target **10** to remove the Bu_2SnO related impurity. Although this approach works, it was material depleting and the procedure had to be repeated multiple times even after column chromatography for some lipids (Figure 19a, shows the ^{1}H-NMR spectra of a sulfatide aliphatic region after column chromatography and Figure 19b is the ^{1}H-NMR taken after repetition of the partitioning of the sample used to take the NMR in 19a).

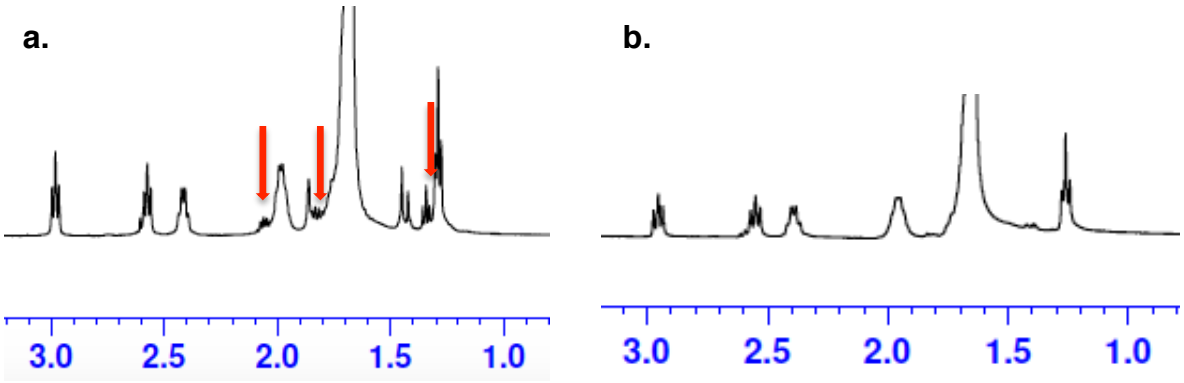
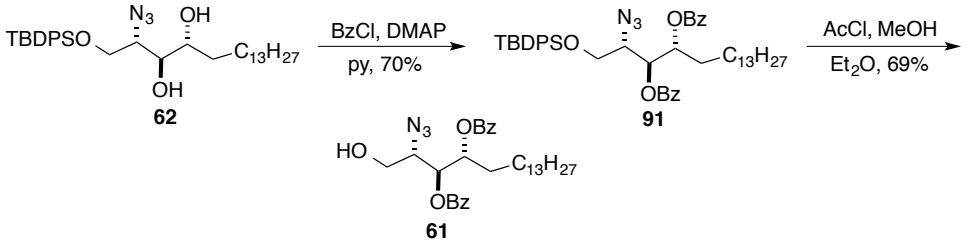


Figure 19. ^{1}H-NMR spectra of a sulfatide; **a.** After 1st partition in 1-Butanol/H_2O and column; **b.** After further partitioning of sample used to take NMR in (a)

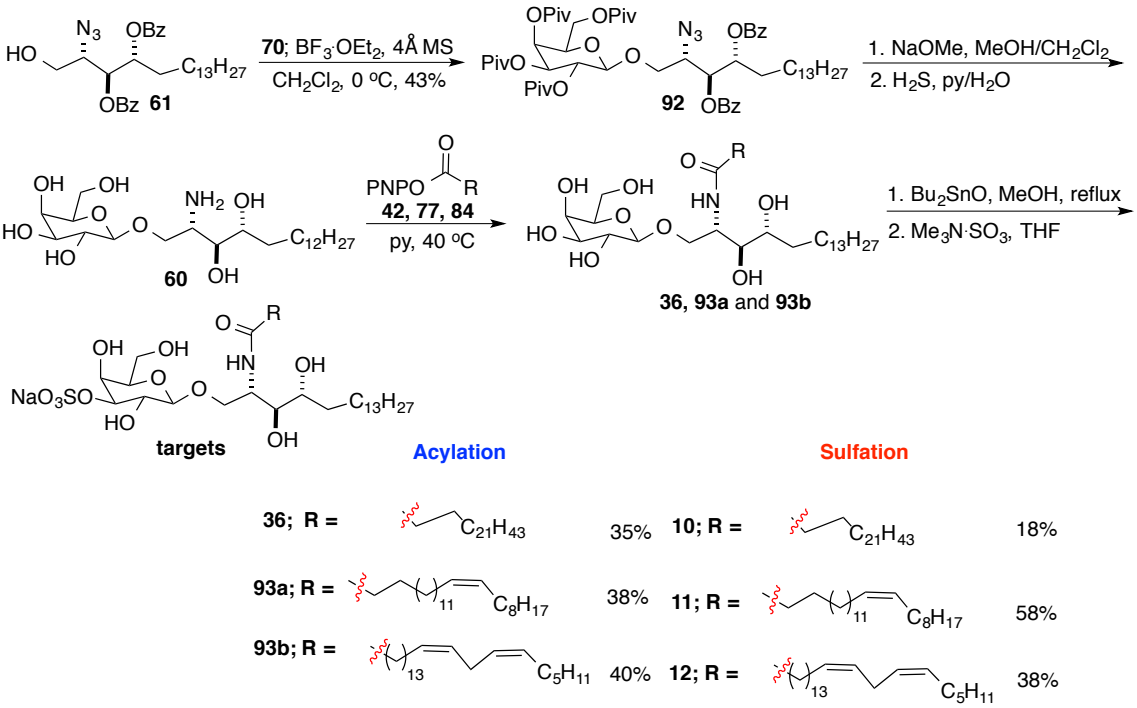
The phytosphingosine acceptor **61** (Scheme 17) was prepared to begin the synthesis of sulfatide targets **10**–**12**. Benzoyl protection of the secondary alcohols of **62** gave **91**. Desilylation of the primary TBDPS protecting-group with AcCl in MeOH gave acceptor **61**.



Scheme 17. Synthesis of phytosphingosine acceptor **61**

Similar reaction sequences as described in Schemes 13 and 16 were followed to prepare targets **10**–**12** (Scheme 18). Glycosylation of **61** with sugar donor **70** gave glycolipid **92** and its α-anomer as a minor product, which was separated using normal column chromatography. NaOMe catalyzed deprotection of the ester groups; followed by H_2S reduction of the azido group gave free amino-glycolipid **60**. This was carried forward without purification, because attempted purification gave less

than 20% yield (unclean). Acylation of **60** with the activated esters gave intermediates **36** **93a** and **93b**. These were subjected to the typical sulfation conditions to produce sulfatides **10**–**12**. Target **10** was synthesized again to obtain a purer product.



Scheme 18. Synthesis of sulfatides **10**–**12**

1.2.4. Tetrachlorophthalimide as an amino-protecting group: Improve glycosylation reaction yield

Switching from acetate- to pivaloyl-protecting groups on the sugar moiety produced a much cleaner reaction, resulting in easier purification of **74** and **92**. However, the search for reaction conditions that would give if not absolute β-selectivity but greater isolation yield by suppressing α-anomer formation was continued. Since the ester protecting groups on the sugar donors were chosen to give β-selectivity as a result

of participating in anchimeric assistance, factors that could lead to the α-anomer formation under these coupling conditions were examined and hypothesized as being as the result of two features: 1. the rate the cyclic oxocarbenium ion like **B** is formed before nucleophilic attack (Figure 15); 2. the nucleophilicity of the sphingosine acceptor. This latter theory is well studied; hence it was selected for analysis. Previous studies on glycosylation reactions have shown the presence of H-bond interaction between the C1-O and N-H of the amide withdraws electron density from the primary hydroxyl group causing it to be less nucleophilic (see sphingosine **94**, Figure 20).[43] To address this issue, acceptors with protecting groups on the C-2 amine, like azide (azido-sphingosine acceptor **24**), have been utilized. These donate electron density, by acting as H-bond acceptors to the primary hydroxyl group, consequently increasing its nucleophilicity (Figure 20). In response to this Panza and co-workers recently published the use of tetrachlorophthalimide (TCP) (with a greater electron donating effect on the C1-OH) as an effective protecting group for sphingoid bases in glycosylation reactions (sphingosine **95**, Figure 20).[44]

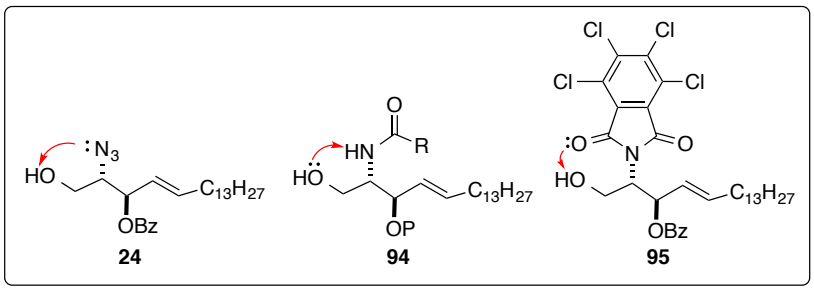
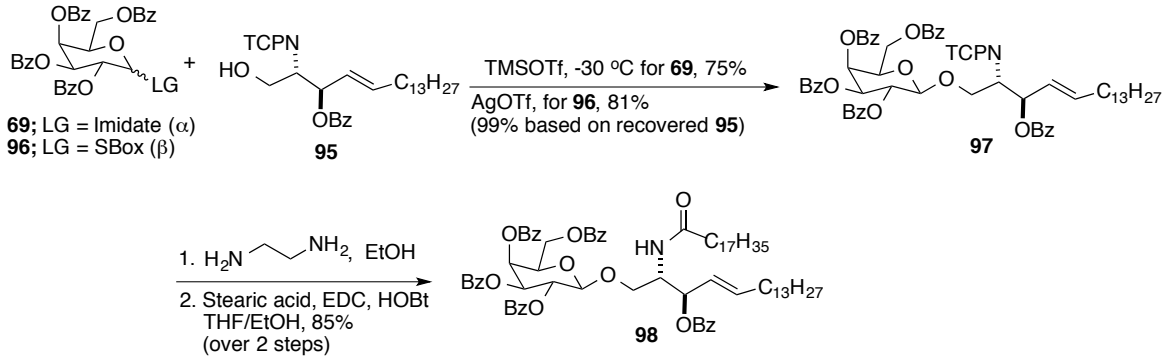


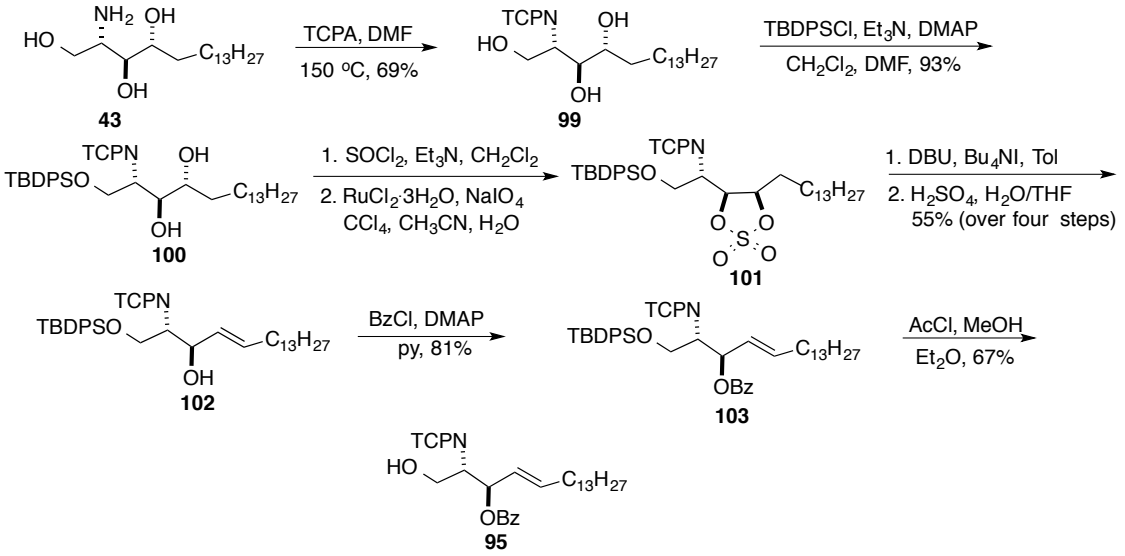
Figure 20. *N*-Protecting-group effect on the nuclephilicity of sphingoid bases

The TCP-protecting group was attractive in many ways; Panza and co-workers use of **95** in their glycosylations gave them **97** (100% isolated) regardless of the sugar donor (**69** or **96**) used. It was also easily removed with ethylene diamine to give the free amino glycolipid, which was acylated in a one-pot reaction to yield **98** (Scheme 19). As a result of these successes with acceptor **95**, we decided to prepare it to couple with our optimized sugar donor **70**.



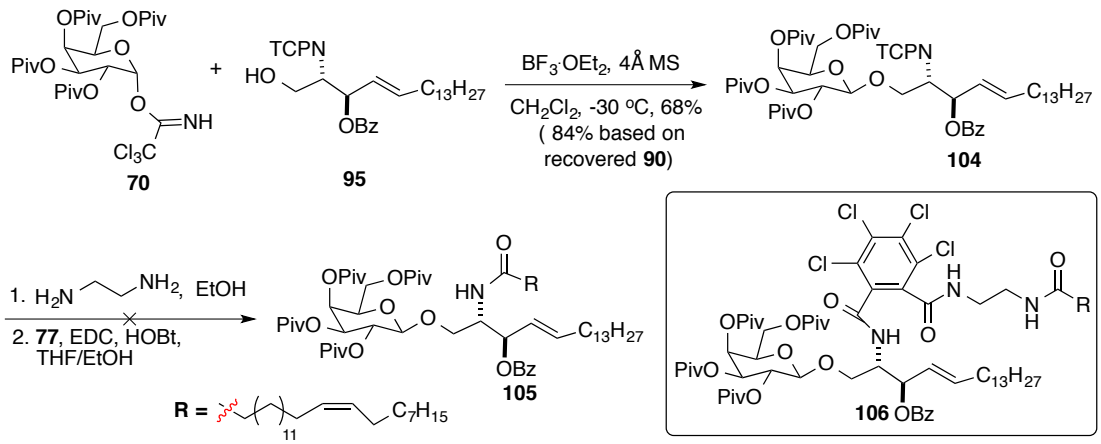
Scheme 19. Panza's application of **95** as an efficient acceptor in glycosylation

Treatment of phytosphingosine (**43**) with tetrachlorophthalic anhydride in DMF gave **99**. This was converted to *N*-TCP-sphingosine acceptor **95** (Scheme 20), following the same reaction sequences described previously for the preparation of sphingosine **24** from phytosphingosine **43** (Scheme 10).



Scheme 20. Synthesis of sphingosine **95**

Coupling of sugar donor **70** with acceptor **95** catalyzed by BF_3•OEt_2 gave β-product **104** (68%, 84% based on recovered **95**) and it's α-isomer (~5%) (Scheme 21). The reaction was not 100% β-selective as was observed by Panza and co-workers (Scheme 19). However, the separation of the two anomers was easier, and the glycosylation yield improved. Unfortunately cleavage of the *N*-TCP group was unsuccessful using ethylene diamine (at room temp as reported by Panza or by heating it to 60 °C). The isolated product was acylated and only partially *N*-TCP cleaved product and was proposed to be **106** based on NMR data.



Scheme 21. Attempted cleavage of *N*-TCP protecting group to give **105**

Other cleavage protocols known to remove the *N*-TCP group, such as hydrazine, $NaBH_4$ in THF/MeOH and benzylamine, were used but to no avail; all resulted in messy reactions. Cleavage of the ester groups before removing the *N*-TCP group also failed. ^{13}C NMR spectra's showed TCP related peaks after multiple chromatographic purifications. Glycosylation using the sugar donor **69** or **96** (sugar donors were not 100% clean, used by Panza were tried, but both gave inseparable products. In summary, switching the *N*-sphingosine protecting group from an *N*-azide to *N*-TCP improved the glycosylation yield and purification of the desired β-product, but its ineffective removal makes its utility limited.

The total synthesis of sulfatide sphingosine **2**, **9**, **13** and phytosphingosine targets **10**–**12** were completed in 13 and 9 steps respectively from commercially available phytosphingosine and D-galactose. Compounds were sent for evaluation to assess their ability to stimulate type II NKT cells by our collaborators at the NIH.

1.2.5. Results: activity of prepared sulfatides (**2**, **7**, **9**–**13**) in stimulating type II NKT cells

The first experiment conducted with the sulfatides was to evaluate if NKT cells in general would recognize their CD1d tetramers. Therefore, the sulfatide-CD1d prepared tetramers were treated with liver lymphocytes. As shown in Figure 21 and 22 (gated cells) all prepared sulfatides were reactive towards NKT cells, and novel sulfatide **13** had the lowest interaction (Figure 22, indicated by the small number of gated cells in comparison to those in 21).

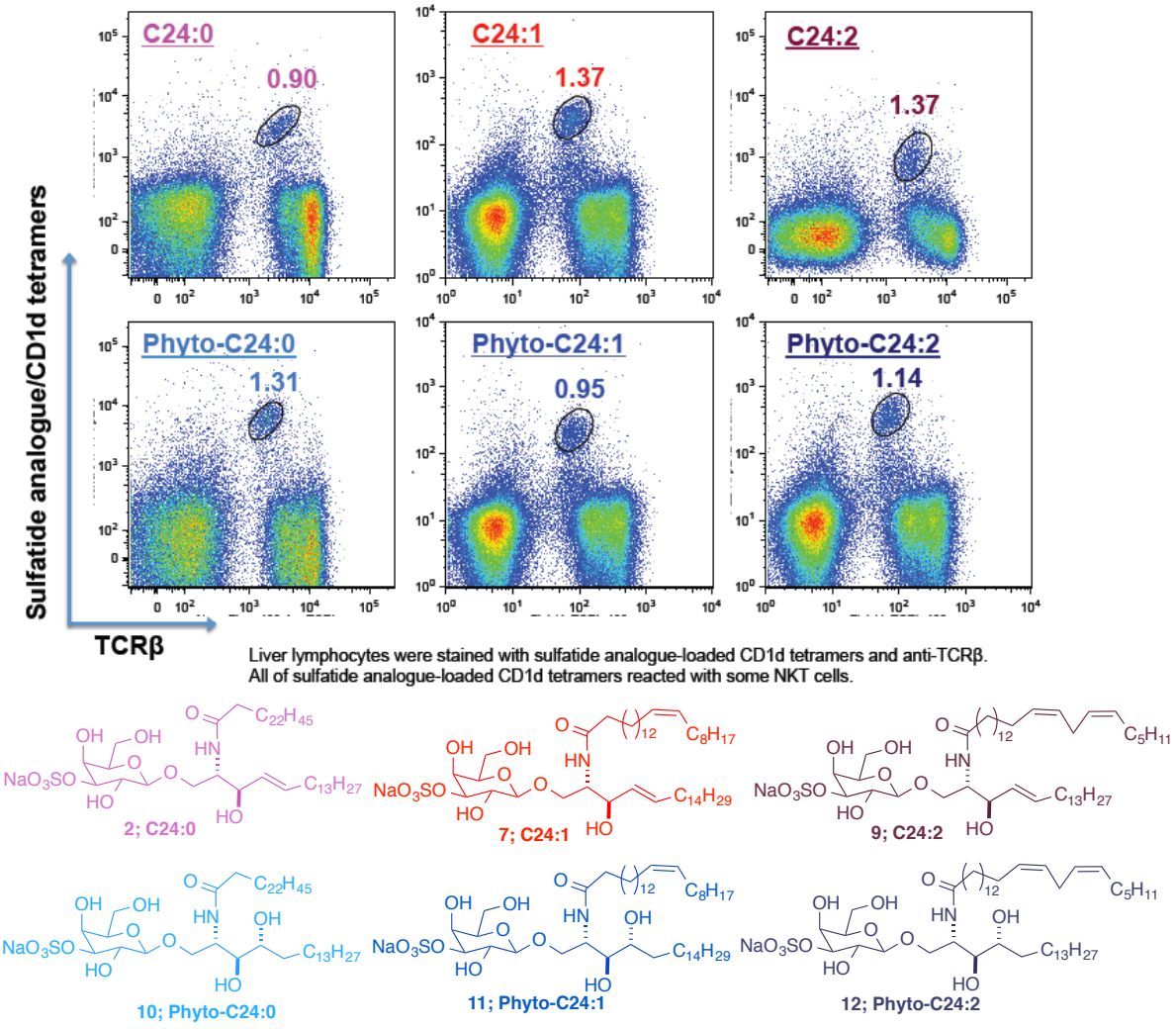


Figure 21. Sulfatides (**2**, **9**–**12**) reactive against liver lymphocytes

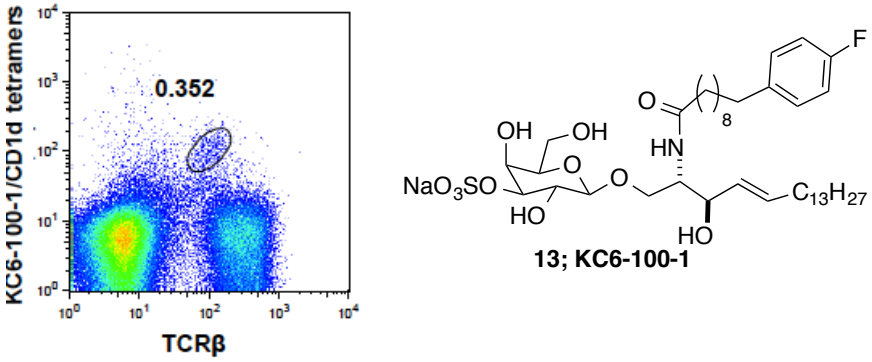


Figure 22. Sulfatide **13** reactive against liver lymphocytes

Once it was confirmed that the prepared ligands could interact with NKT cells, they were next screened simultaneously with PBS57 (**107**), an analog of KRN7000, against T cells (sulfatides stains shown on the y-axis and PBS57 stains shown on the x-axis, Figure 23). The purpose of this analysis was to check if the sulfatides would interact with the same NKT cells as PBS57 (in this case type I NKT cells). The data obtained showed sulfatides to be recognized by a distinct population of T cells (probably type II NKT cells) different from type I NKT cells, as no double positive stains were observed (depicted by gated cells, Figure 23).

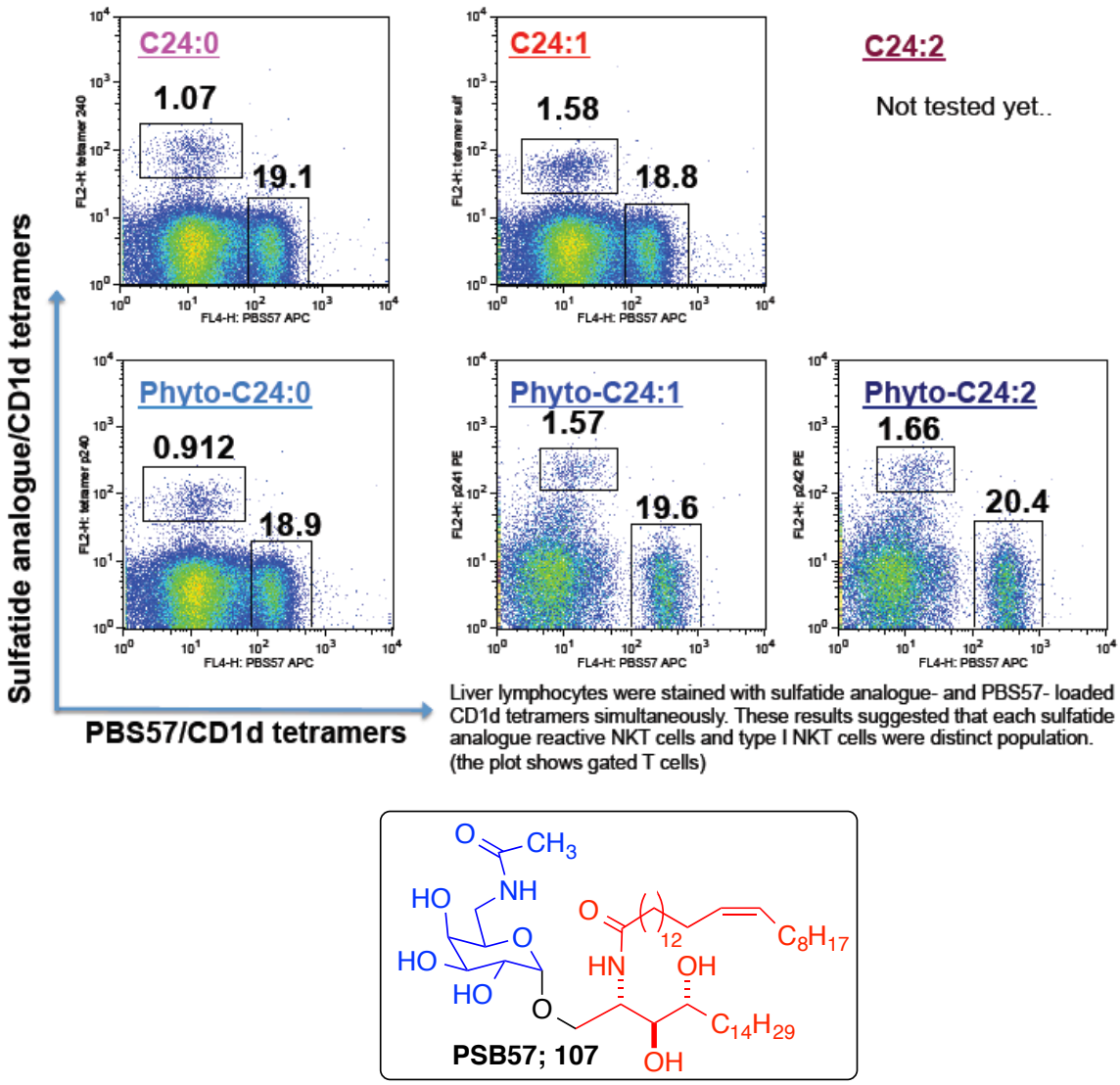


Figure 23. Cross-staining of sulfatides and PBS57 on NKT cells; sulfatide stains shown on the y-axis and PBS57 stains shown on the x-axis

Because some type II NKT cell hybridomas have been shown to recognize specific sulfatides, the sulfatide ligands were screened to determine if they would interact with the same subpopulation of type II NKT cells. The gated T cells (Figure 24) from concurrent staining of compounds (a) phyto-C24:0 (**10**) and C24:0 (**2**), (b) C24:0 (**2**)

and C24:1 (**7**) and (c) C24:0 (**2**) and C24:2 (**9**) suggested the ligands to have reactivity largely towards identical NKT cells.

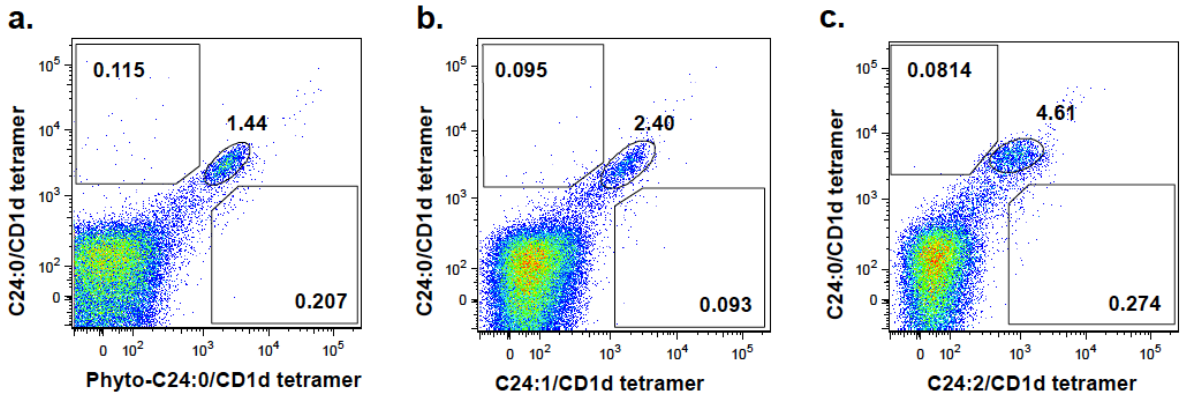


Figure 24. Sulfatides **2**, **7** and **11** targets largely identical subpopulation of NKT cells; **a.** phyto-C24:1 (**11**) and C24:0 (**2**) loaded CD1d tetramers; **b.** C24:1 (**7**) and C24:0 (**2**) loaded CD1d tetramers; **c.** C24:2 (**9**) and C24:0 (**2**) loaded CD1d tetramers

The ability of the prepared sulfatides to stimulate NKT cells to secrete cytokines was also examined, and preliminary in vitro studies revealed them to stimulate the secretion of both Th1 and Th2 cytokines (illustrated by IFN-γ and IL-4 production responsively, Figure 25). The phytosphingosine analogs exhibited the highest activity with phyto-C24:0 (**10**) being the most potent ligand and phyto-C24:2 (**10**) displayed the lowest (comparing phyto-sulfatides). In contrast to the phytosphingosine-sulfatides, the sphingosine-sulfatides displaying an opposite cytokine production pattern; the number of unsaturations present in their acyl chains correlates with their ability to elicite cytokine production, manifested clearly in the amount of IL-4 produced. Target C24:2 (**9**) stimulated a higher secretion of IL-4 than C24:1 (**7**), which showed stronger cytokine production than C24:0 (**2**).

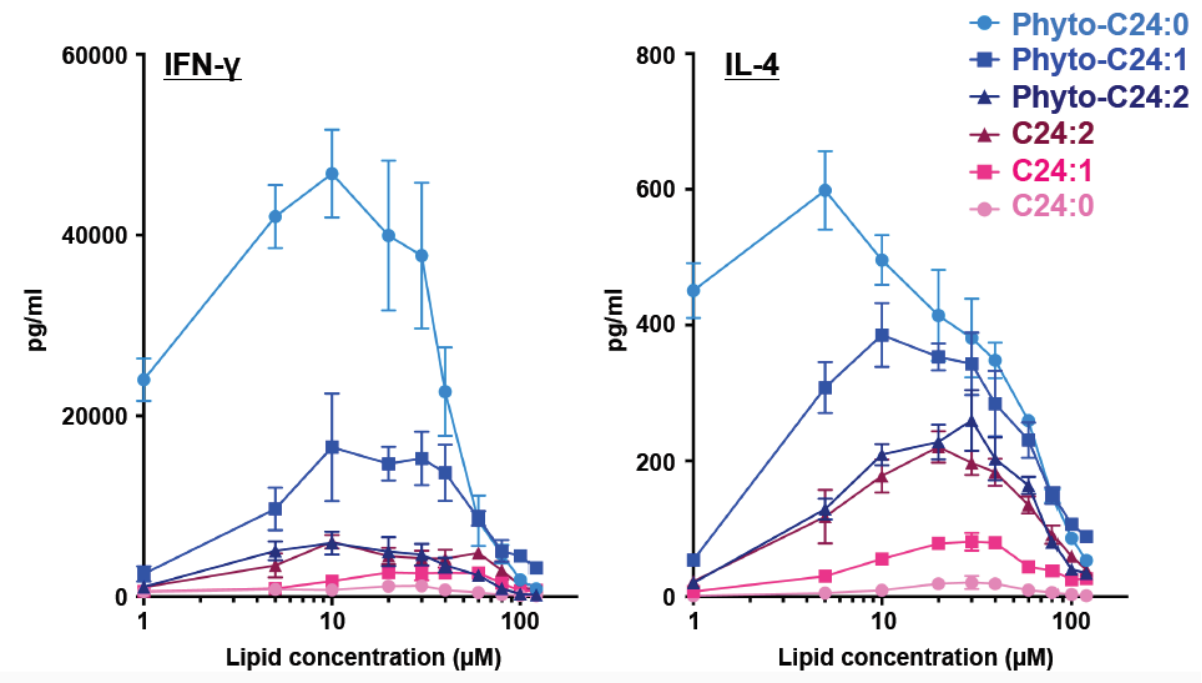


Figure 25. Cytokine secretion ability of prepared sulfatides

In conclusion, all the prepared natural and unnatural sulfatides activate NKT cells to release cytokines. The phytosphingosine analogs emerging as the strongest ligands revealed new data that both type I and type II NKT cells are potently stimulated by phytosphingosine sphingoid bases. The distinct NKT cells the compounds targeted in comparison to PBS57 implied these NKT cells to be type II NKT cells, as PBS57 interacts specifically with type I NKT cells. The sulfatide-reactive T cells need to be isolated to confirm this hypothesis, which is part of the future studies to be conducted with the ligands. All of the ligands have been prepared again to verify their stimulatory pattern. In vivo tumor immunity experiments will be conducted once all observed data are reproduced.

1.2.6. Synthesis of new natural sulfatides 8-(*S*), 111 and 112

At about the same time that we completed the synthesis of the sulfatide targets (**2**, **9**–**13**) we were approached by Prof. Lucia Mori's of the Singapore Immunology Network (SigN). Their lab is also interested in the evaluation of natural (bovine brain) sulfatide extracts for the activation of T cells. The Mori lab uses mass spectrometry for sulfatide structural analysis and identification of potential active ligands from mixtures of natural sulfatides bought from commercial providers. After multiple HPLC and mass spectrometric analyses, targets **8-(*S*)**, **108**–**112** (Figure 26), were selected as potential potent compounds against T cells present in the sulfatide mixtures. Thus a collaboration was initiated with our lab to complete their synthesis so they could confirm their activities and do further studies with them on the CD1b-mediated activation of T cells.

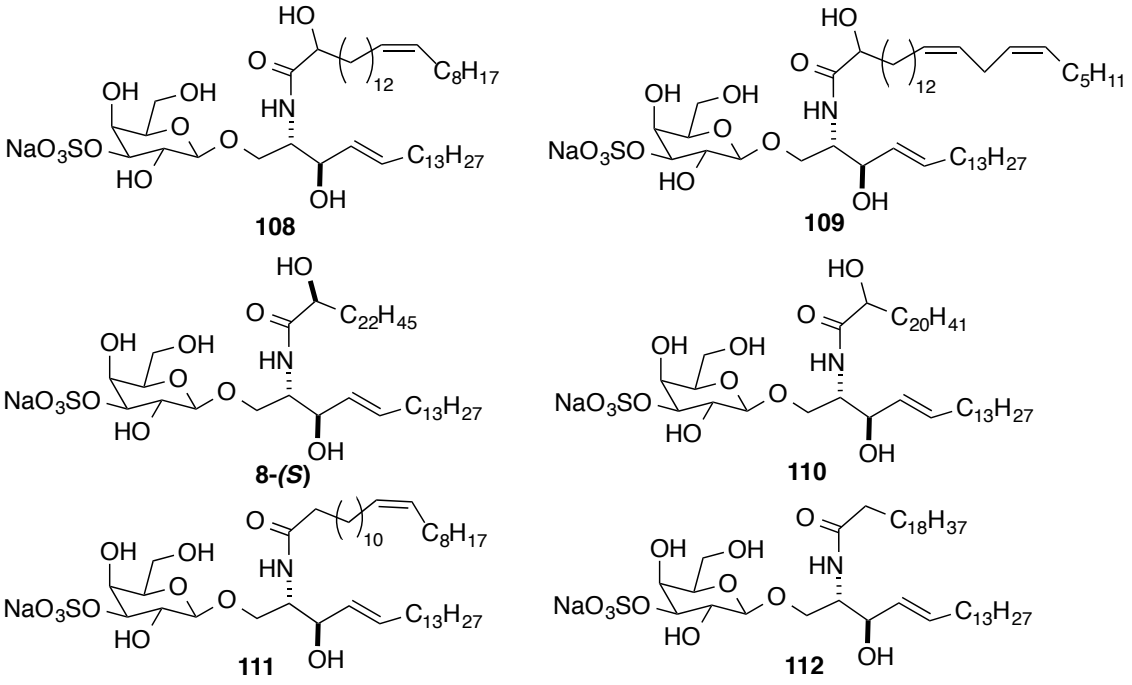
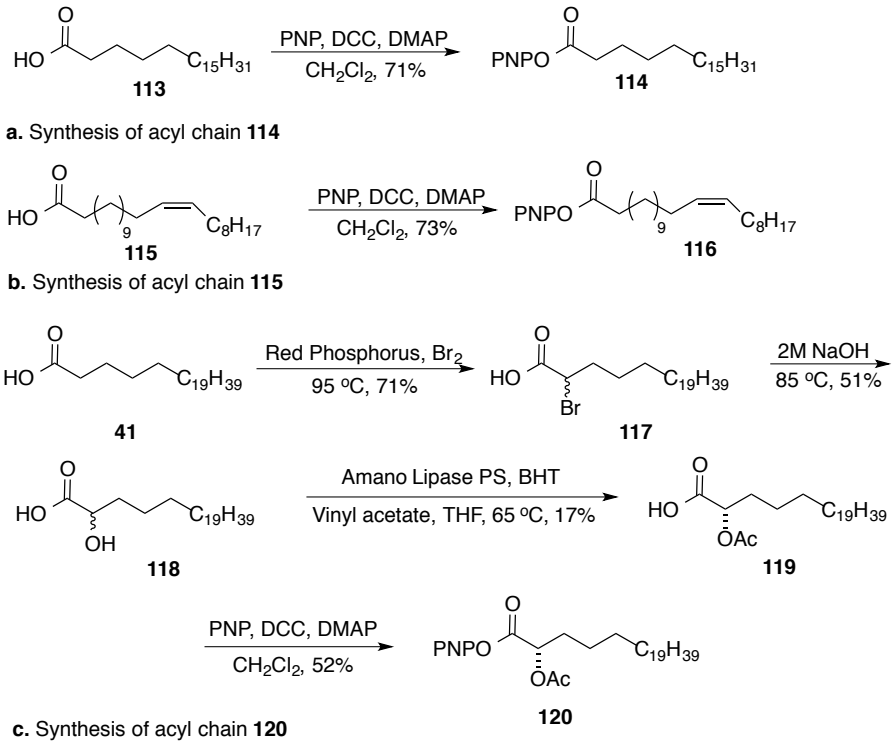


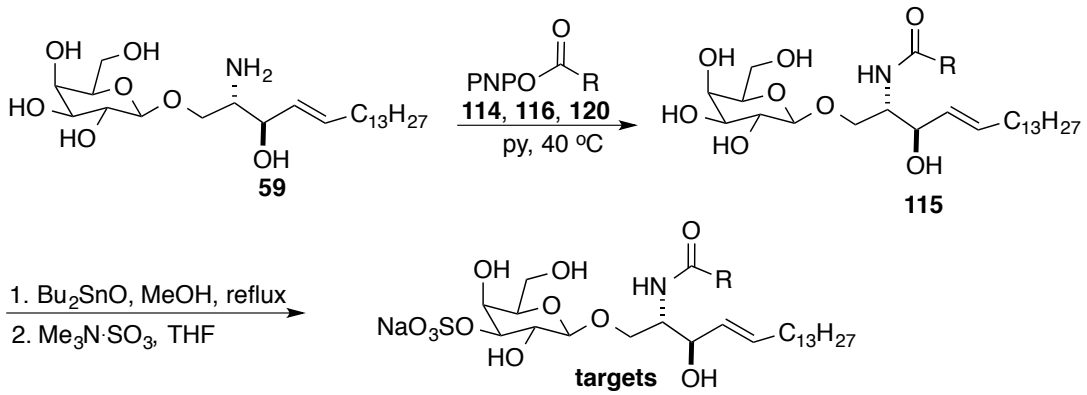
Figure 26. Synthesis of new natural sulfatide targets **8-*S***, **108**–**112**

The new targets (**8-*S***, **111** and **112**) can also be accessed from advanced amino-glycolipid **59** by doing acylation and sulfation as the previous targets. The order the compounds are presented in Figure 2.7 is a representation of the priority order, but because of the complicated reactions required to prepare some of the acyl chain, the wish list was narrowed first to synthesize targets **8-*S***, **111** and **112**. The preparation of the appropriate acyl chains launched their synthesis (Scheme 22). The commercially available fatty acids archidic (elcosanoic) acid (**113**) and erucic acid (**115**) were activated with *p*-nitrophenol to give their corresponding PNP-esters **114** and **116** respectively. The acid precursor **119** to acyl chain **120** is not commercially available and was prepared in three steps from tetracosanoic acid (**41**) following Mori's *et al.* protocol.[45] Red phosphorus catalyzed bromination of **41** in the presence of bromine gave (*rac*)-α-bromo tetracosanoic acid **117**. Hydrolysis of **117** with 2M NaOH gave (*rac*)-α-hydroxy tetracosanoic acid **118**. Amano-lipase PS facilitated kinetic resolution using vinyl acetate in the presence of BHT gave enantiopure acetate-protected (*S*)-α-hydroxy tetracosanoic acid **119**. *p*-Nitrophenol activation of acid **119** gave PNP-ester **120**.

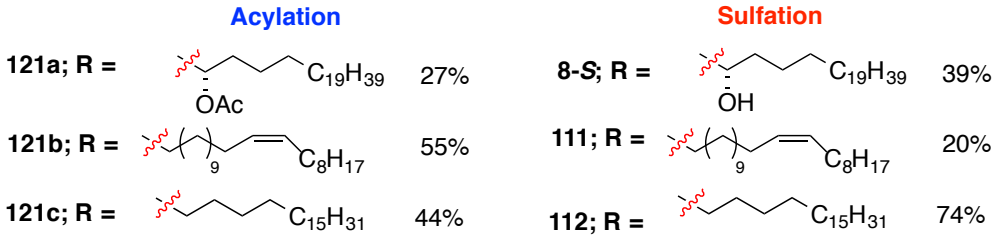


Scheme 22. Synthesis of acyl chains **114**, **116** and **120**

The acylation of amino-glycolipid **59** with PNP-activated esters **114**, **116** and **120** gave glycolipids **121a**–**121c**. NaOMe cleavage of the acetate group in **121a** was done before sulfation. The lipids were then subjected to the developed sulfation procedure to provide sulfatides **8-(*S*)**, **111** and **112**. The compounds were sent to the Mori lab for evaluation of their biological functions in the activation of T cells.



*Note: **115a** was first subjected to NaOMe, CH_2Cl_2/MeOH to cleave the actate group before sulfation*



Scheme 23. Completion of the synthesis of sulfatides **8-*S***, **111** and **112**

1.2.7. Activity of sulfatides 8-(*S*), 111 and 112

Unfortunately, none of the ligands were able to stimulate the T cells to secrete cytokines, even though their molecular weights correspond to previously analyze active sulfatide mixtures. Nonetheless they served as a tool, narrowing down the probable compounds to synthesize and test. Thorough HPLC and mass spectrometry were further conducted on mixtures of sulfatides bought from Fluka and Matreya. Upon further charaterization of HPLC fractions, the most potent fraction had a compound with molecular weight of m/z 860.6026 $-Na^+$. One possible structure for m/z 860.6026 is **111**, already synthesized and tested as inactive. Another possible structure to match the m/z 860.6026 is sulfatide **122** which would have a C-16 chain length (d:16-sphingosine base) and an acyl chain of

tetracosenoic acid (C24:1) instead of the C-18 chain length (d:18-sphingosine) and erucic acid acyl chain moiety (C22:1) as in **111** (Figure 27).

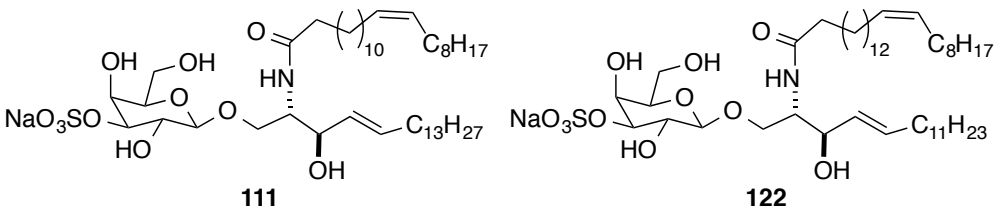


Figure 27. Possible structures for m/z 860.6026 $-Na^+$

The active HPLC fraction from Fluka showed both d:16- and d:18-sphingosine base sulfatides with m/z 860.6026 (Figure 28a and 28c), while the fraction from Matreya which had almost pure d:18 sulfatide was inactive (Figure 28b and 28d). The observed inactivity of **111** (see Figure 28c and d) coupled with the experimental data of the Fluka and Matreya sulfatide mixtures lead to the assumption that **122** might be the active lipid, and its synthesis was initiated.

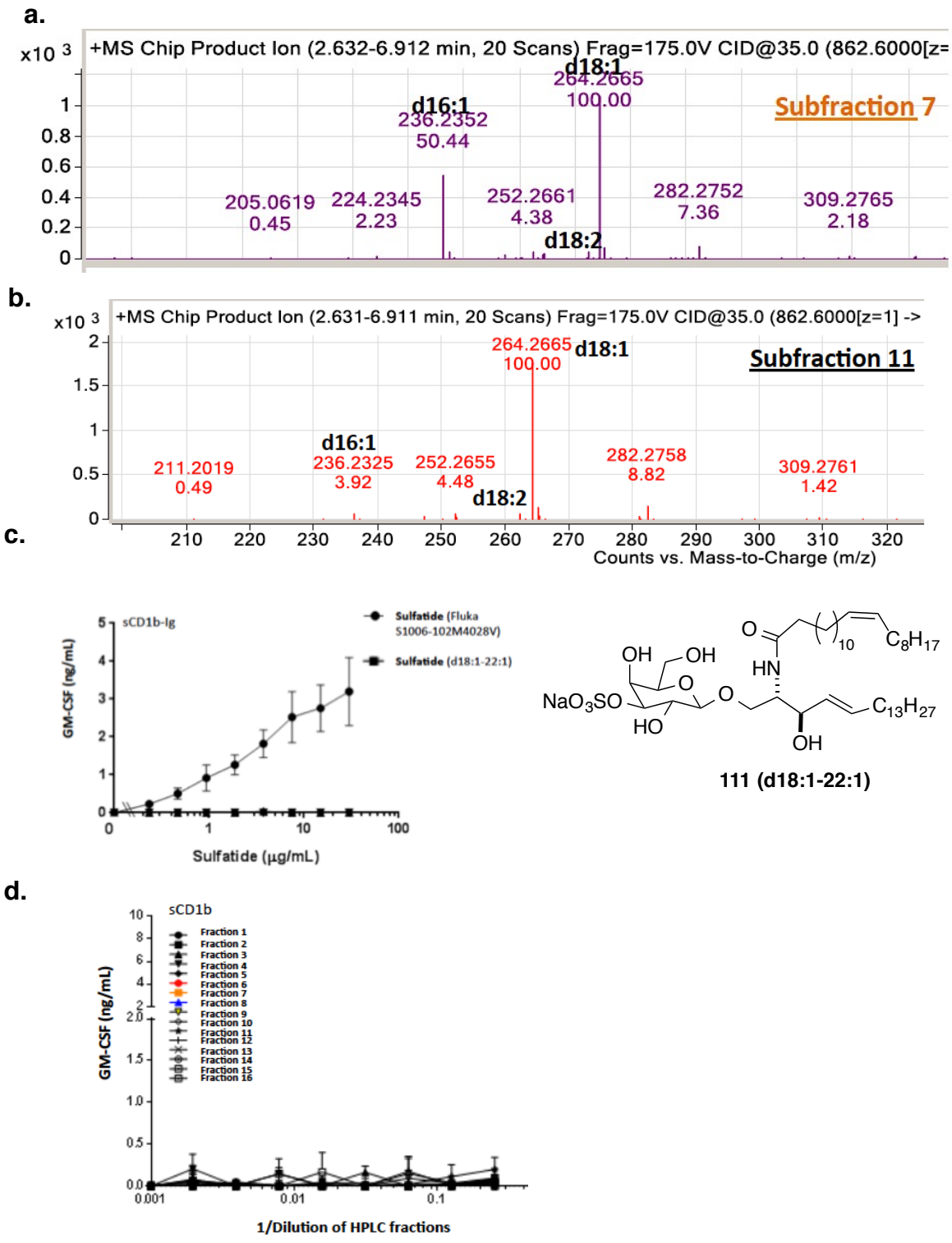


Figure 28. Structural deduction of m/z 860.6026 $-Na^+$; **a.** MS of sulfatide mixtures from Fluka; **b.** Sulfatides mixture from Matreya; **c.** stimulatory activity of sulfatide **105** and sulfatide fraction from Fluka; **d.** inactive sulfatide fractions from Matreya

1.2.8. Synthesis of sulfatide 122

To prepare sulfatide **122**, the synthesis of the d:16-sphingosine was required as it was expensive to buy from a commercial provider (10mg for $175.00 or 100mg for $1,400.00). The Garner aldehyde (**125**) was chosen as the chiral pool source for its enantiopure synthesis highlighted in the retrosynthetic scheme (Figure 29).

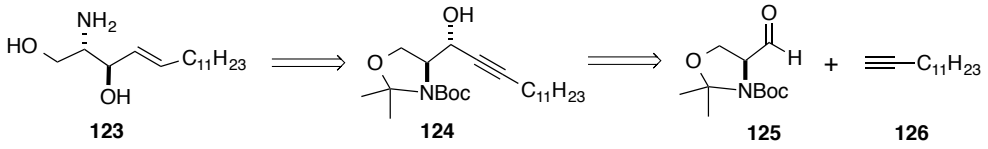
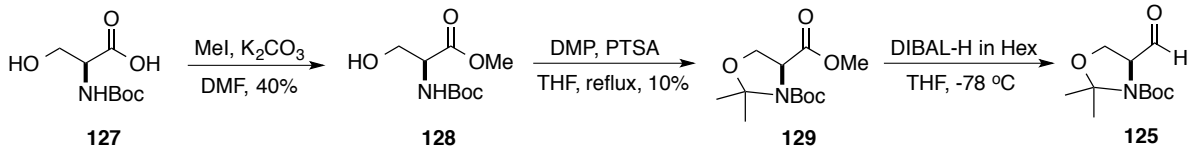


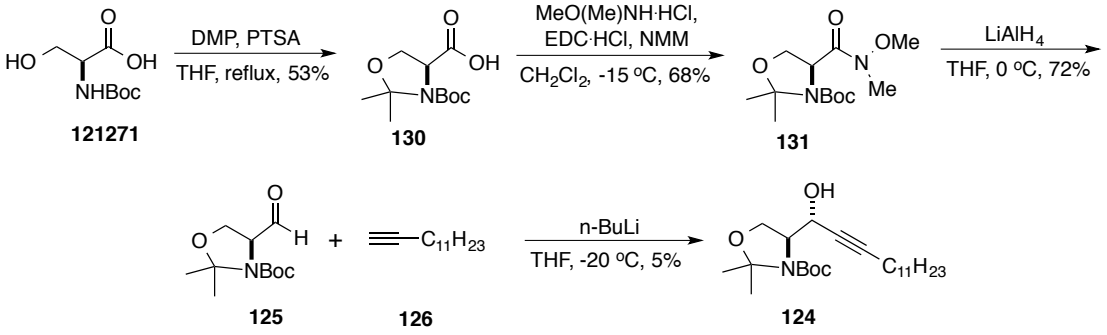
Figure 29. Retrosynthesis of d:16-sphingosine **125** using Garner aldehyde

The commercial cost of Garner aldehyde (**125**) was also very expensive (1g for $209.00); therefore, it was prepared from Boc-L-serine (**127**), which is much cheaper. Following the traditional Garner aldehyde synthesis from Boc-L-serine (Scheme 24), the carboxylic acid group was converted to a methyl ester using MeI and K_2CO_3 as the base, giving **128**. The primary alcohol and nitrogen of the carbamate were protected with DMP to give oxazolidine **129**. This was subjected under DIBAL-H reduction conditions to give Garner aldehyde (**125**) and its over reduced alcohol product. The low yielding steps and the inefficient DIBAL-H reduction caused us to pursue an alternative route.



Scheme 24. Synthesis of Garner aldehyde (**125**) from Boc-L-serine

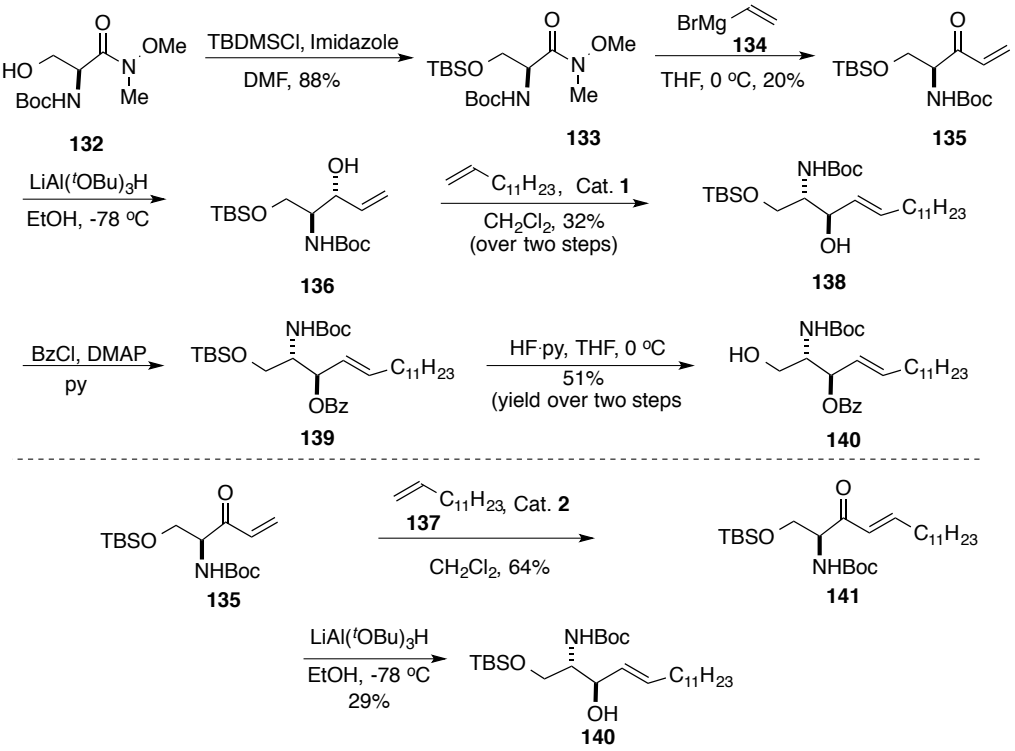
A Weinreb amide route was appealing; because of the high yield reported by Siciiano *et al.* in their reduction of various substituted Weinreb amides to aldehydes.[46] The protection of the alcohol and nitrogen of Boc-L-serine to give oxazolidine **130** commenced the strategy (Scheme 25). Activation of **130** with a Weinreb amide salt gave **131**. $LiAlH_4$ reduction of **131** granted Garner aldehyde **125**. Nucleophilic addition of deprotonated **126** to **125** provided **124** in only 5% yield.



Scheme 25. Synthesis of the Garner aldehyde via a Weinreb amide route

The low reactivity of Garner aldehydes under our reaction conditions calls for a new plan to prepare sphingosine **123**. The successful synthesis of d:18-sphingosine via olefin cross metathesis (CM) reported by Katsumura and co-workers[47] inspired us to investigate CM methodology (Scheme 26). The new path began with TBS protection of Weinreb amide **132** to give **133**. Nucleophilic addition of vinyl magnesium bromide (**134**) to **133** generated enone **135** in 20% yield. The low yield was as a result of the silyl group being cleaved during the acidic work-up. Since enough product was obtained, however, **135** was subjected to Hoffman's chemoselective and diastereoselective reduction[48] of the enone carbonyl using $LiAl(^tOBu)_3H$ in EtOH to furnish allylic alcohol **136** impure, no clean product was otained. Olefin CM

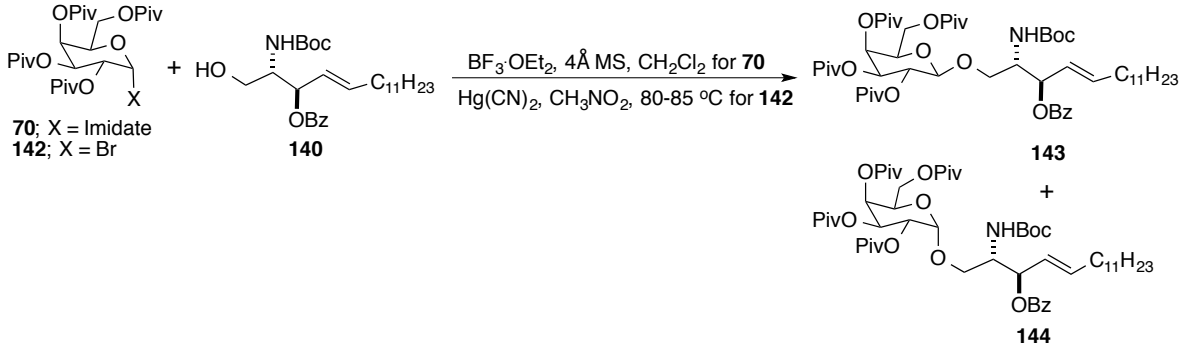
of allylic alcohol with 1-tridecene (**137**) gave sphingosine **138**. The secondary hydroxyl group in **138** was protected with a benzoyl group followed by silyl group deprotection to give desired sphingosine **140**. The purification of **138** from **136** was very tedious for a couple of reasons; 1. The amount of 1-tridecene for the CM reaction was 4 equiv. leading to a lot of its homo-coupled product; 2. the reaction didn't go to completion, and the product and starting material had close retention times. To overcome these issues, CM was done on enone **135** to give **141**, which was reduced to give **138**. Again, loss of the TBS group during acidic work-up accounts for the low yield in the conversion of **141** to **138**.



Scheme 26. Synthesis of d:16-sphingosine using CM

Glycosylation of acceptor **140** was first tried with our optimized sugar donor **70** (Scheme 27). This resulted in the formation of both the desired β-product **143**, its

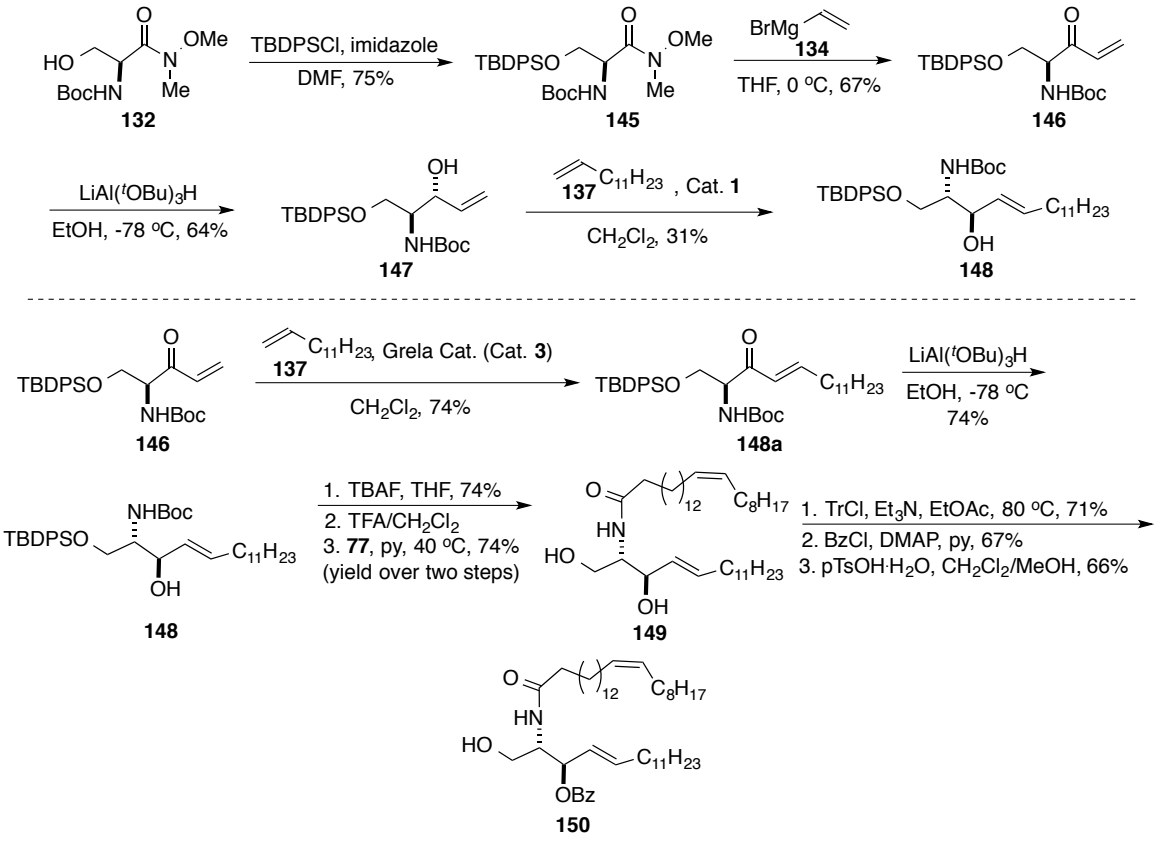
inseparable α-anomer **144** and other byproducts. The bromo sugar donor **142**, an intermediate in the synthesis of **70** was tried next. Acceptor **140** and bromo sugar **142** were reacted following a classical Helferich glycosylation procedure (Scheme 27). Although coupling occured, the outcome was the same as with the acetimidate sugar donor **70**; so the use of a ceramide acceptor was suggested.



Scheme 27. Attempted glycosylations

At this point we had run out of sphingosine intermediates to prepare the precusors to the ceramide and we had to start again from Weinreb amide **132**. The TBDPS analog **148** of **138** was prepared to avoid the the issues faced with TBS group cleavage (Scheme 28). As was seen in synthesis of **138**, CM before diastereoselective reduction of enone precursor **146** provided **148** more efficiently. TBAF was used for the removal of the silyl group in **148**. The treatment of the *N*-Boc-sphingosine with a TFA/CH_2Cl_2 mixture removed the Boc group and the free amino d:16-sphingosine was directly acylated with PNP-activated ester **77** in the presence of pyridine to give ceramide **149**. This product underwent protecting group manipulation reactions to give the desired acceptor **150**. First trityl protection of the

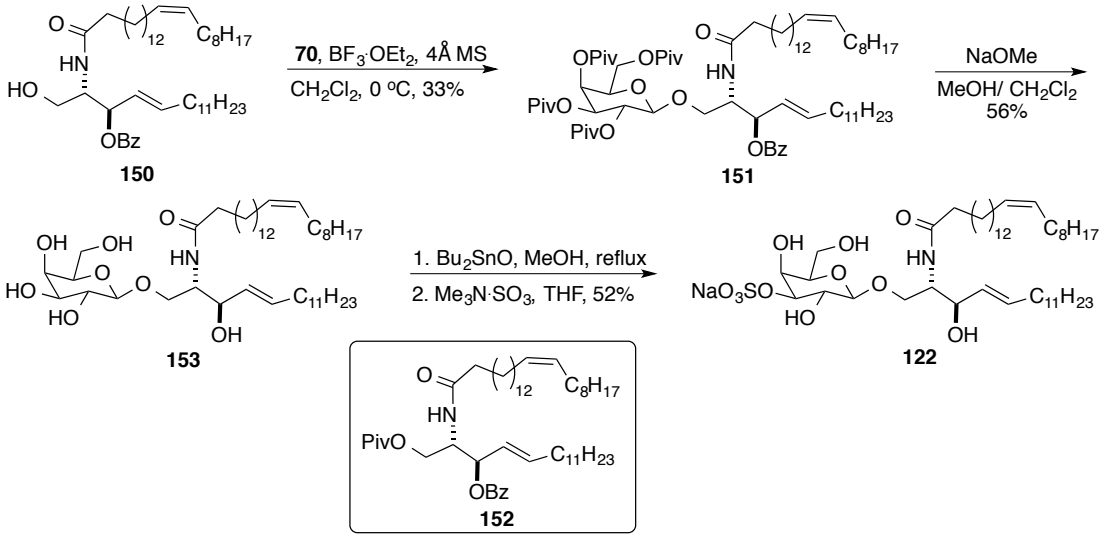
primary hydroxyl group, subsequent benzoyl protection of the secondary hydroxyl group and finally selective removal of the trityl group using pTsOH•H_2O.



Scheme 28. Synthesis of ceramide acceptor **150**

Gratifyingly the coupling of ceramide acceptor **150** with sugar donor **70** promoted by BF_3•OEt_2 furnished glycolipid **151** (Scheme 29). No α-anomer was isolated supporting hypothesis (1) stated in section 1.2.4. It is likely the α-anomers in the glycosylation reactions of Scheme 13 and 18 were formed because the cyclic oxocarbenium ion intermediate required wasn't completely formed before the azido-base acceptors **24** and **61** attacked, which are stronger nucleophiles. In contrast, **150**'s low reactivity favored the successful formation of the cyclic oxocarbenium ion before it attacks, thus resulting in exclusive formation of β-product **151**. The only

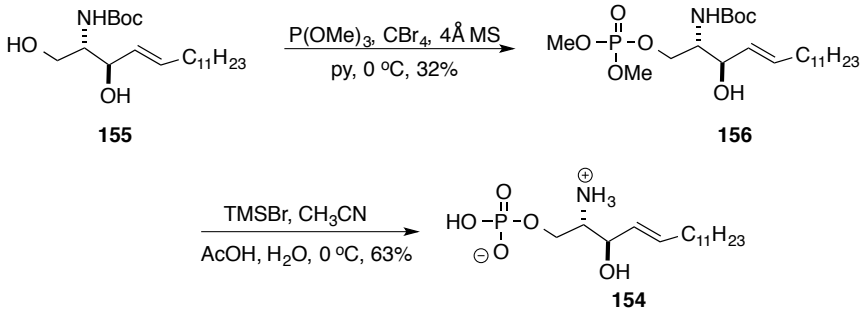
side product isolated was the trans-esterification product **152** (~9%). A NaOMe cleavage procedure was employed to remove the ester protecting groups to yield **153**. Sulfation of **153**, finally gave **122**. Sulfatide **122** and the free amino d:16 sphingosine **123** are currently being tested for their stimulation of T cells using CD1b as the antigent presenting protein.



Scheme 29. Completion of **122** syntheses

It happens that it is not only the Mori lab that is interested in d:16-sphingosine lipids. Dr. Torta and co-workers at Singapore Lipidomics Incubator (SLING) requested **154** a phosphate analog of **123**. Their lab is involved in studying long chain base phosphates (LCBP) and they believe **154** might be involved in important biological functions. To prepare **154**, the N-Boc protected sphingosine **155** (an intermediate after TBAF cleavage of **148**) was reacted with trimethoxy phosphate to give methyl phosphate **156**. The hydrolysis of **156** using TMSBr gave zitterionic amino-

phosphoric acid **154** (Scheme 30). Phosphate **154** was sent, along with **123**, as a control for analyses.



Scheme 30. Synthesis of d:16-sphingosine phosphate **154**

In summary we have completed the synthesis of four additional sulfatides (**8-*S***, **111**, **112** and **122**) and d:16-sphingosine phosphate **154**. Targets **8-*S***, **111** and **112** were prepared in 13 steps from commercially available phytosphingosine and D-galactose and target **122** was prepared in 14 steps from D-galactose and Boc-L-serine. Also, a more efficient CM methodology of enones have been developed for the preparation of d:16-sphingosine.

1.3. EXPERIMENTAL: Sulfatides

1.3.1. General Experimental

Tetrahydrofuran (THF) was dried using a solvent dispensing system (SDS) with a column of neutral alumina. Pyridine, toluene, dimethylformamide (DMF), methylene chloride (CH_2Cl_2), deuterated chloroform ($CDCl_3$), methanol (MeOH), deuterated methanol and ethanol (EtOH) were dried over 4Å molecular sieves (MS).

N-tert-BOC-*L*-serine and hexacosanoic acid were purchased from Novabiochem and TCI, respectively. The other reagents were purchased from Acros, Alfa Aesar or Aldrich and used without further purification.

All reactions were conducted under an atmosphere of N_2 in glassware that had been dried overnight in an oven at 120 ^{o}C. Where appropriate, control of the reaction temperature was achieved with a solid CO_2/acetone bath, an ice bath or a heated oil bath.

^{1}H NMR spectra were recorded at 500 MHz, 400 MHz and/or 300 MHz and calibrated to the residual $CHCl_3$ peak in $CDCl_3$ at 7.26 ppm, to the TMS peak at 0.0, or to the residual MeOH peak in MeOD at 3.34 ppm. ^{13}C NMR spectra were recorded at 125 MHz, 100 MHz, and/or 75 MHz and calibrated to the residual $CHCl_3$ peak in $CDCl_3$ at 77.23, or to the residual MeOH peak in MeOD at 49.5 ppm. The following abbreviations are used for peak multiplicities: app (apparent), s (singlet); br s (broadened singlet); d (doublet); dd (doublet of doublet); ddd (doublet of doublet of doublets); dddd (doublet of doublet of doublet of doublets); dt (doublet of triplets);

tt (triplet of triplets) t (triplet); q (quartet); quin (quintet); m (multiplet). Coupling constants, *J*, are reported in Hertz (Hz).

IR spectra were recorded on a Brucker FT-IR spectrometer. High-resolution mass spectra (HiRMS) were obtained on an AccuTOF instrument equipped with a DART ionization source.

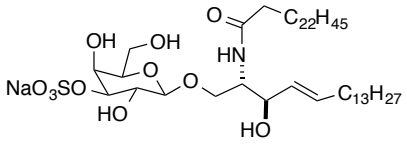
Melting points were observed in open Prex capillary tubes and are uncorrected. Specific rotations $[\alpha]_D$ were obtained on a JASCO polarimeter using the sodium D-line as a source, and the concentration (c) is expressed in g per 100 mL.

Flash chromatography was performed on Silica Gel, 40 micron, 32-63 flash silica from Sorbent. Thin layer chromatography was performed on silica gel (Silicycle Silica Gel 60 F_{254} glass plates). Compounds were visualized by UV, 5% phosphomolybdic acid in ethanol, 0.5% potassium permanganate in water or a solution of ethanol/H_2SO_4/AcOH/*p*-anisaldehyde (135:5:1.5:3.7). Ceric molybdate in a solution of H_2O/ammonium molybdate/ceric ammonium molybdate/ H_2SO_4 (235 mL: 12 g: 0.5 g: 15mL) was used for sulfatides.

Compounds **95**[44] and **99–103**[44] were prepared according to literature procedures.

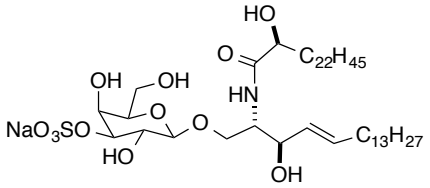
General sulfation procedure

Glycolipids (1 equiv) and Bu_2SnO (1.2 equiv) were refluxed in MeOH (0.016 M) for 2 h. The solvent was evaporated under reduced pressure. The resulting dibutylstannylene complex was treated with $Me_3N{\bullet}SO_3$ (2 equiv) in THF (2 mL).[32,34] The mixture was stirred at rt from between 2 to 6 h. TLC was used to monitor the reaction. The solvent was evaporated, and the residue dissolved in a 1:1 mixture of CH_3Cl_3/MeOH (4 mL). Dowex (Na^+ resin) was added. The mixture was then stirred for 10 min, followed by filteration and concentration. The crude product was partitioned in a mixture of 1-butanol/H_2O (1:1, v/v) and centrifuged. The supernatants (1-butanol, containing the sulfatides) was collected and concentrated. Purification by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 10 to 15%) gave the sulfatides.

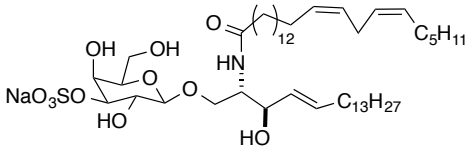


(*2S,3R,4E*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-tetracosanoylamino)octadec-4-en-3-ol (2). Sulfatide **2** was isolated as a white solid (4.2 mg, 35%): mp 204.0–205.0 °C; $[\alpha]^{25}_D$ 6.98 (*c* 0.38, $CHCl_3$/MeOH, 3:2); IR (neat) 3400 (br), 2917, 2850, 1646, 1466, 1258, 1066, 801 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.67 (dt, *J* = 15.3, 6.7 Hz, 1H), 5.42 (dd, *J* = 15.3, 6.6 Hz, 1H), 3.81–3.74 (m, 4H), 3.58–3.56 (m, 2H), 2.15 (t, *J* = 7.9 Hz, 2H), 2.00–1.98 (m, 2H), 1.66–1.45 (m, 2H), 1.41–1.25 (m, 62H), 0.87 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, $CHCl_3/CD_3OD$, 3:2) δ 175.7, 135.4, 130.3, 104.3, 81.4, 75.8, 72.7, 70.5, 69.7,

68.2, 62.2, 54.2, 37.4, 33.4, 32.9, 31.5, 30.7, 30.6, 30.6, 30.5, 30.4, 30.3, 30.3, 26.9, 23.5, 14.8; HRMS (TOF) *m/z* calcd for $C_{48}H_{92}NO_{11}S$ [M –Na]$^+$ 890.6397, found 890.6377.

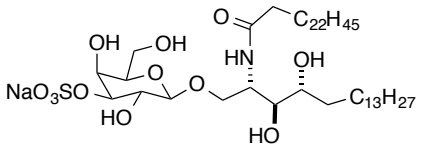


(*2S,3R,4E*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2(2*S*-hydroxyl-*N*-tetracosanoylamino)octadec-4-en-3-ol (8-*S*). Sulfatide **8-*S*** was isolated as a white solid (2.4 mg, 39%): ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.72 (dt, *J* = 15.2, 6.9 Hz, 1H), 5.45 (dd, *J* = 15.4, 7.2 Hz, 1H), 4.00–3.96 (m, 2H), 3.83 (dd, *J* = 11.8, 6.9 Hz, 1H), 3.72–3.70 (m, 1H), 3.69 (dd, *J* = 10.5, 3.4 Hz, 1H), 3.56 (dd, *J* = 5.5, 5.5 Hz, 1H), 2.05–1.98 (m, 2H), 1.81–1.74 (m, 1H), 1.65–1.61 (m, 1H), 1.56–1.49 (m, 1H), 1.46–1.26 (m, 61H), 0.88 (t, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, $CHCl_3/CD_3OD$, 3:2) δ 176.7, 135.3, 129.7, 104.4, 81.4, 75.8, 72.9, 72.5, 70.4, 69.3, 68.5, 62.5, 54.5, 35.5, 33.3, 32.8, 30.6, 30.6, 30.5, 30.4, 30.3, 30.3, 30.2, 26.3, 23.6, 14.8; HRMS (TOF) *m/z* calcd for $C_{48}H_{92}NO_{12}S$ [M –Na]$^+$ 906.6346, found 906.6381.

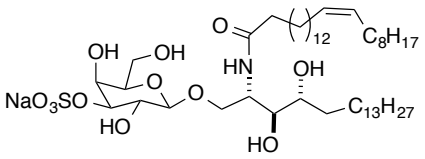


(2*S*,3*R*,4*E*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-15*Z*,18*Z*-tetracosadienoylamino)octadec-4-en-3-ol (9). Sulfatide **9** was ioslated as an off white solid (14.9 mg, 65%): mp 182.0–183.0 °C; $[\alpha]^{25}$D 8.28 (*c* 0.72, $CHCl_3$/MeOH,

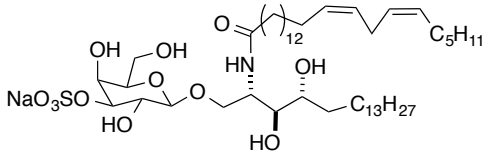
3:2); IR (neat) 3370 (br), 2918, 2850, 1644, 1467, 1258, 1066 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.70 (dt, *J* = 15.3, 6.6 Hz, 1H), 5.44 (dd, *J* = 15.4, 7.4 Hz, 1H), 5.40–5.29 (m, 4H), 4.34 (d, *J* = 7.7 Hz, 1H), 3.64 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.57 (dd, *J* = 5.7, 5.7 Hz, 1H), 2.77 (dd, *J* = 6.3, 6.3 Hz, 4H), 2.17 (t, *J* = 7.6 Hz, 2H), 2.08–2.00 (m, 7H), 1.65–1.51 (m, 2H), 1.40–1.27 (m, 48H), 0.91–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CHCl_3/CD_3OD$, 3:2) δ 175.8, 135.2, 131.1, 130.4, 128.9, 104.5, 81.4, 75.8, 72.9, 70.6, 69.9, 68.6, 62.4, 54.5, 37.4, 33.2, 32.8, 32.4, 30.6, 30.5, 30.5, 30.4, 30.4, 30.3, 30.2, 28.1, 28.1, 26.9, 26.6, 23.5, 23.4, 14.7; HRMS (TOF) calcd for $C_{48}H_{92}NO_{11}$ $[M-Na]^+$ *m/z* 886.6078, found 886.6058.



(*2S,3S,4R*)-1-(3-*O*-sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-tetracosanoylamino)octadecane-3,4-diol (10). Sulfatide **10** was isolated as a white solid (3.2 mg, 18%): mp 184.0.0–185.0 °C; $[\alpha]^{25}_D$ 10.43 (*c* 0.49, $CHCl_3$/MeOH, 3:2); IR (neat) 3429 (br), 2917, 2850, 1632, 1467, 1224, 1070, 801 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.35–5.32 (m, 1H), 3.81 (dd, *J* = 11.8, 7.2 Hz, 1H), 3.76–3.71 (m, 2H), 3.66–3.64 (m, 1H), 3.59–3.56 (m, 1H), 2.15 (t, *J* = 7.5 Hz, 2H), 2.04–2.00 (m, 1H), 1.60–1.51 (m, 5H), 1.41–1.26 (m, 62H), 0.87 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 175.8, 104.2, 81.2, 75.8, 74.8, 73.2, 70.5, 70.1, 68.3, 62.3, 51.2, 37.3, 32.8, 32.6, 30.6, 30.6, 30.4, 30.3, 30.2, 28.0, 26.9, 26.8, 23.5, 14.8; HRMS (TOF) *m/z* calcd for $C_{48}H_{94}NO_{12}S$ $[M-Na]^+$ 908.6502, found 908.6465.

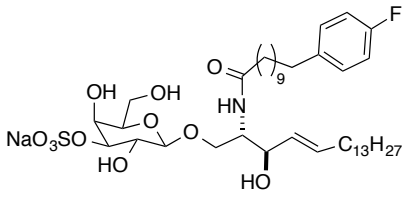


(*2S,3S,4R*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-15*Z*-tetracosenoylamino)octadecane-3,4-diol (11). Sulfatide **11** was isolated as a white solid (5.3 mg, 58%): mp 211.4–212.4 °C; $[\alpha]^{25}_{D}$ 8.33 (*c* 0.50, $CHCl_3$/MeOH, 3:2); IR (neat) 3367 (br), 2917, 2850, 1643, 1466, 1224, 1066, 812 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.37–5.30 (m, 2H), 4.34 (d, *J* = 7.7 Hz, 1H), 3.81 (dd, *J* = 4.9, 3.1 Hz, 1H), 3.76–3.56 (m, 2H), 3.70–3.64 (m, 2H), 3.59–3.57 (m, 2H), 2.20 (t, *J* = 7.6 Hz, 2H), 2.04–2.00 (m, 4H), 1.64–1.50 (m, 4H), 1.44–1.26 (m, 56H), 0.87 (t, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 175.8, 130.8, 104.2, 81.3, 75.8, 74.9, 73.2, 70.5, 70.1, 68.3, 62.3, 51.2, 37.4, 37.3, 32.9, 32.8, 30.7, 30.7, 30.6, 30.6, 30.5, 30.4, 30.4, 30.3, 30.2, 30.2, 28.1, 27.0, 26.9, 23.6, 14.8; HRMS (TOF) *m/z* calcd for $C_{48}H_{90}NO_{12}S$ $[M-Na]^+$ 906.6340, found 906.6339.



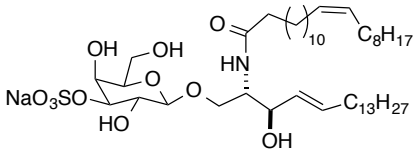
(2*S*,3*S*,4*R*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-15*Z*,18*Z*-tetracosadienoylamino)octadecan-3,4-diol (12). Sulfatide **12** was isolated as an off white solid (8.0 mg, 48%): mp 172.0–173.0 °C; $[\alpha]^{25}_{D}$ 11.15 (*c* 0.49, $CHCl_3$/MeOH, 3:2); IR (neat) 3400 (br), 2917, 2850, 1637, 1467, 1226, 1061 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.41–5.30 (m, 4H), 3.81–3.78 (m, 1H), 3.75–3.72 (m, 2H), 3.69–3.64 (m, 2H), 3.60–3.57 (m, 2H), 2.77 (t, *J* = 6.6 Hz, 1H),

2.20 (t, *J* = 7.3 Hz, 2H), 2.07–2.02 (m, 4H), 1.59–1.52 (m, 4H), 1.38–1.26 (m, 49H), 0.88 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.8, 131.1, 131.0, 128.9, 128.9, 104.2, 81.3, 75.8, 74.8, 73.3, 70.5, 70.1, 68.3, 62.3, 54.4, 37.3, 37.3, 32.8, 32.6, 32.4, 30.6, 30.5, 30.4, 30.3, 30.2, 28.1, 28.1, 26.9, 26.8, 26.5, 23.5, 23.4, 14.8; HRMS (TOF) calcd for $C_{48}H_{92}NO_{12}S^-$ [M –Na]$^+$ *m/z* 904.6189, found 904.6210.

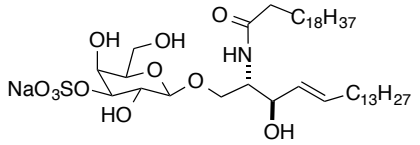


(2*S*,3*R*,4*E*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-11-*p*-fluorobenzylundecanoylamino)octadec-4-en-3-ol (13). Sulfatide **13** was isolated as a white solid (6.2 mg, 39%): mp 213.0–214.0 °C; $[\alpha]^{25}_D$ 1.68 (*c* 0.62, CH_2Cl_2/MeOH, 3:2); IR (neat) 3387 (br), 2919, 2850, 1634, 1511, 1466, 1225, 1065, 820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 7.12 (dd, *J* = 8.3, 5.7 Hz, 2H), 6.93 (t, *J* = 8.8 Hz, 2H), 5.68 (dt, *J* = 15.2, 6.8 Hz, 1H), 5.43 (dd, *J* = 15.2, 7.3 Hz ,1H), 4.33 (d, *J* = 7.7 Hz, 1H), 4.27–4.21 (m, 4H), 3.97–3.94 (m, 1H), 3.83–3.72 (m, 3H), 3.59–3.54 (m, 2H), 2.56 (t, *J* = 7.6 Hz, 2H), 2.16 (t, *J* = 7.6 Hz, 2H), 2.00 (m), 1.59–1.55 (m, 4H), 1.36–1.25 (m, 36H), 0.87 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 174.9, 162.1 (d_{C-F}, *J* = 240.7 Hz), 139.5, 135.3, 130.7, 130.6, 130.4, 128.6, 115.7 (d_{C-F}, *J* = 20.9 Hz), 115.6, 81.4, 75.8, 72.7, 70.6, 69.7, 68.4, 62.3, 54.3, 37.8, 36.0, 33.4, 32.9, 32.6, 30.6, 30.5, 30.5, 30.4, 30.4, 30.3.

30.2, 30.1, 28.1, 26.9, 23.6, 20.4, 14.8; HRMS (ESI) calcd for $C_{41}H_{69}FNO_{11}S^-$ [M + Na]$^+$ *m/z* 802.4581, found 802.4552.

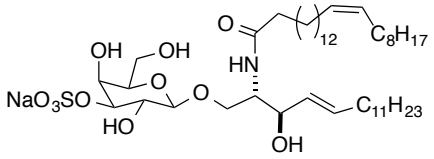


(*2S,3R,4E*)-2-(*N*-13*Z*-dodecenoylamino)-1-(3-*O*-sodiumsulfonyl-β-D-galactopyranosyloxy)octadec-4-en-3-ol (111). Sulfatide **111** was isolated as an off white solid (5.1 mg, 20%): ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.73–5.67 (m, 1H), 5.44 (dd, *J* = 14.8, 7.3 Hz, 1H), 5.34 (br s, 2H), 5.00 (br s, 1H), 3.63–3.58 (m, 4H), 2.17 (m, 2H), 2.02 (br s, 6H), 1.57 (br s, 2H), 1.27 (br s, 50H), 0.89–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 175.8, 135.3, 130.8, 130.4, 104.4, 83.5, 75.8, 72.8, 70.6, 69.8, 68.4, 62.3, 54.5, 37.4, 37.4, 33.3, 32.8, 30.7, 30.6, 30.5, 30.4, 30.4, 30.3, 30.2, 30.2, 28.1, 26.9, 23.5, 14.7; HRMS (TOF) *m/z* calcd for $C_{46}H_{86}NO_{11}S$ [M – Na]$^+$ 860.5922, found 860.5923.

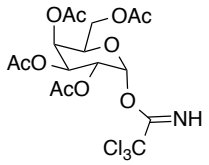


(*2S,3R,4E*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-eicosanoylamino)octadec-4-en-3-ol (112). Sulfatide **112** was isolated as an off white solid (6.3 mg, 74%): mp 189.2–191.4 °C; IR (neat) 2919, 2851, 1739, 1366, 1217 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 7.70 (s, 1H), 5.70–5.67 (m, 1H), 5.45–5.37 (m, 1H), 4.00–3.96 (m, 2H), 3.76–3.75 (m, 3H), 3.59–3.56 (m, 2H), 2.18–2.16 (m, 2H), 2.09–1.95 (m, 2H), 1.71–1.52 (m, 2H), 1.26 (br s, 55H),

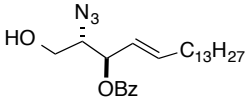
0.88–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 135.4, 130.3, 104.3, 81.3, 75.7, 72.7, 70.5, 69.7, 68.3, 62.2, 55.3, 37.4, 33.3, 32.8, 30.6, 30.3, 30.2, 26.9, 23.5, 14.8; HRMS (TOF) *m/z* calcd for $C_{44}H_{84}NO_{11}S$ $[M + H]^+$ 834.5765, found 834.5771.



(*2S,3R,4E*)-1-(3-*O*-Sodiumsulfonyl-β-D-galactopyranosyloxy)-2-(*N*-15*Z*-tetracosenoylamino)hexadec-4-en-3-ol (122). Sufatide **122** was isolated as a white solid (5.2 mg, 52%): mp 183.1–184.6 °C; $[\alpha]^{25}_D$ 2.99 (*c* 0.52 $CHCl_3$/MeOH, 3:2); IR (neat) 3351 (br), 2918, 2850, 1643, 1466, 1227, 1068 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.69 (dt, *J* = 15.3, 6.7 Hz, 1H), 5.43 (dd, *J* = 15.2, 7.6 Hz, 1H), 5.36–5.30 (m, 2H), 4.32 (d, *J* = 7.7 Hz, 1H), 4.26–4.25 (m, 2H), 4.21 (dd, *J* = 10.2, 4.0 Hz, 1H), 4.12–4.09 (m, 3H), 3.96–3.95 (m, 2H), 3.82 (dd, *J* = 11.6, 6.6 Hz, 1H), 3.78–3.73 (m, 2H), 3.60–3.55 (m, 2H), 2.16 (t, *J* = 7.6 Hz, 2H), 2.03–2.01 (m, 6H), 1.60–1.54 (m, 2H), 1.42–1.26 (m, 50H), 0.88 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.7, 135.3, 130.8, 130.3, 104.4, 81.3, 75.7, 72.8, 70.5, 69.8, 68.4, 62.4, 54.3, 37.4, 33.3, 32.8, 32.9, 30.6, 30.6, 30.5, 30.5, 30.5, 30.4, 30.4, 30.3, 30.3, 30.2, 30.2, 28.1, 26.9, 23.6, 14.8; HRMS (TOF) *m/z* calcd for $C_{46}H_{88}NO_{12}S^-$ $[M – Na]^+$ 860.5927, found 860.5901.

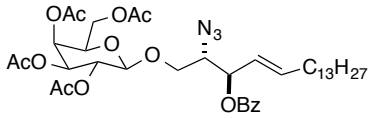


(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranoside)-1-trichloroacetimidate (17). DBU (43.0 μL, 0.29 mmol) was added drop-wise to a stirred solution of (2,3,4,6)-tetra-*O*-acetyl-α/β-D-galactopyranoside) (**40**) (0.20 g, 0.57 mmol) in CH_2Cl_2 (1 mL) at 0 °C. After 5 min, Cl_3CCN (0.12 mL, 1.15 mmol) was added drop-wise, the reaction was slowly warmed to rt and stirred overnight. The next day saturated aqueous NH_4Cl (2 mL) was added, and the product was extracted using CH_2Cl_2 (3 X 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 85:15) to give **17** with its β-anomer (0.15 g, 53%) as a yellowish oil.[37] Peak assignments for **17**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s 1H), 6.60 (d, *J* = 3.4 Hz, 1H), 5.56 (dd, *J* = 3.0, 1.0 Hz, 1H), 5.41 (app d, *J* = 3.1 Hz, 1H), 5.38 (app dd, *J* = 3.5 Hz, 1H), 4.44 (app dd, *J* = 6.5, 6.5 1H), 4.12 (dd, *J* = 11.3, 6.6, Hz, 1H), 4.08 (dd, *J* = 11.3, 6.6, Hz, 1H), 2.17 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.3, 170.3, 170.2, 161.2, 93.8, 69.2, 67.7, 67.6, 67.1, 61.5, 20.9, 20.8, 20.8, 20.8.



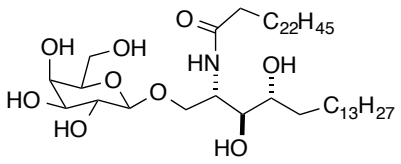
(*2S,3R,4E*)-2-Azido-3-(*O*-benzoyl)octadec-4-en-1-ol (24). AcCl (0.75 mL, 10.5 mmol) was added drop-wise to MeOH (19 mL) at 0 °C. (*2S,3R,4E*)-2-azido-(3-benzoyloxy-1-*tert*-butyldiphenylsilyloxy)octadec-4-ene (**65**) (0.37 g, 0.57 mmol) in

Et_2O (19 mL) was added to the *in situ* generated HCl solution.[39] The reaction solution was stirred for 2 d. Saturated aqueous $NaHCO_3$ was added. The two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 X 25 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 90:10) to give **24** (0.15 g, 62%) as slightly yellow oil.:[49] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07–8.05 (m, 2H), 7.60–7.56 (m, 1H), 7.45 (t, *J* = 7.8 Hz, 2H), 6.01–5.91 (m, 1H), 5.64–5.58 (m, 2H), 3.82–3.73 (m, 2H), 3.63 (ddd, *J* = 11.6, 6.9, 4.6 Hz, 1H), 2.11–2.03 (m, 3H), 1.42–1.35 (m, 2H), 1.32–1.25 (m, 20H), 0.88 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 139.0, 133.5, 130.0, 130.0, 128.7, 123.5, 74.8, 66.4, 62.2, 32.6, 32.1, 29.9, 29.8, 29.6, 29.6, 29.3, 28.9, 22.9, 14.3.

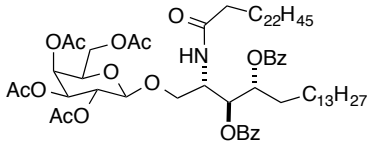


(*2S,3R,4E*)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside)-4-octadec-4-ene (25). (2,3,4,6-Tetra-*O*-acetyl-α/β-D-galactopyranoside)-1-trichloroacetimidate (**17**) (0.12 mg, 0.25 mmol) and (*2S,3R,4E*)-2-azido-3-(benzoyloxy)octadec-4-en-1-ol (**24**) (0.11 g, 0.26 mmol) were dissolved in dry CH_2Cl_2 (7 mL), and the solution was stirred in the presence of 4Å MS (100 mg) at rt for 30 min. BF_3•OEt_2 in dry CH_2Cl_2 (18 μL in 0.5 mL) was then added drop-wise at 0 °C. The reaction was slowly warmed to rt and stirred for 4 h. The reaction mixture was diluted with petroleum ether (5 mL) and then filtered. The filtrate was treated with saturated aqueous $NaHCO_3$ (13 mL). The

organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 X 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc, 80:20) gave **25** (8.1 mg, β-anomer) as a slightly yellowish oil:[34] ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.7 Hz, 2H), 7.55 (t, *J* = 7.3 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 2H), 5.92 (dt, *J* = 14.8, 6.8 Hz, 1H), 5.62–5.52 (m, 2H), 5.37–5.36 (m, 1H), 5.22 (dd, *J* = 10.2, 8.0 Hz, 1H), 5.01 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.13–4.07 (m, 2H), 3.92–3.88 (m, 3H), 3.60–3.56 (m, 1H), 2.13 (s, 4H), 2.09 (s, 4H), 2.00 (s, 4H), 1.97 (s, 4H), 1.38–1.34 (m, 2H), 1.45 (br s, 18H), 0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.4, 170.3, 169.5, 165.3, 139.2, 133.4, 130.1, 129.9, 128.6, 122.9, 101.2, 74.9, 71.0, 68.7, 68.1, 67.1, 63.7, 61.4, 32.5, 32.1, 29.8, 29.7, 29.6, 29.5, 29.3, 28.9, 22.8, 20.9, 20.8, 20.8, 20.7, 14.3; *trans*-esterification sphingosine product **68**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–8.05 (m, 2H), 7.60–7.56 (m, 1H), 7.46 (t, *J* = 8.0 Hz, 2H), 5.96 (dt, *J* = 14.4, 6.8 Hz, 1H), 5.61–5.55 (m, 2H), 4.23 (dd, *J* = 11.6, 4.5 Hz, 1H), 4.13 (dd, *J* = 11.5, 7.9 Hz, 1H), 3.98 (ddd, *J* = 8.5, 4.4, 4.4 Hz, 1H), 2.11 (s, 3H), 2.10–2.07 (m, 2H), 1.41–1.35 (quin, 2H), 1.32–1.24 (m, 20H), 0.88 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 165.4, 139.3, 133.5, 130.0, 128.7, 122.9, 74.8, 63.4, 63.2, 32.6, 32.1, 29.9, 29.8, 29.6, 29.6, 29.3, 28.9, 22.9, 20.9, 14.3.

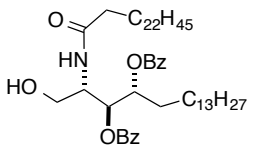


(2*S*,3*S*,4*R*)-1-(β-D-galactopyranosyloxy)-2-(*N*-tetracosanoylamino)octadecane-3,4-diol (36). *p*-Nitrophenyltetracosanoate (**42**) (0.03 g, 0.07 mmol) was added to a solution of (2*S*,3*S*,4*R*)-2-amino-1-(β-galactopyranosyloxy)octadecane-3,4-diol (**60**) (30.0 mg, 0.06 mmol) in pyridine (1 mL). The mixture was stirred in a preheated oil bath at 40 ^{o}C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **36** as a white solid (15.7 mg, 35%): mp 198.7–199.8 ^{o}C; $[\alpha]^{25}_{D}$ 10.20 (*c* 0.49, $CHCl_3$/MeOH, 3:2); IR (neat) 3304, 2915, 2849, 1625, 1468, 1077, 718 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$/MeOD, 3:2) δ 4.25–4.12 (m, 1H), 3.87–3,86 (m, 1H), 3.82 (dd, *J* = 11.6, 6.7 Hz, 2H), 3.75–3.69 (m, 2H), 3.61–3.47 (m, 5H), 2.20 (t, *J* = 7.5 Hz, 2H), 1.68–1.52 (m, 4H), 1.44–1.27 (m, 64H), 0.88 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD, 3:2) δ 175.6, 104.8, 76.3, 75.4, 74.5, 73.3, 72.4, 70.2, 70.1, 51.5, 37.4, 33.2, 32.8, 31.3, 30.7, 30.6, 30.6, 30.5, 30.3, 30.3, 30.2, 26.8, 26.8, 23.5, 14.7; HRMS (TOF) *m/z* calcd for $C_{48}H_{96}NO_9$ $[M + H]^+$ 830.7080, found 830.7052.

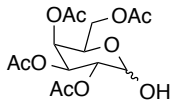


(2*S*,3*S*,4*R*)-3,4-Dibenzoyloxy-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside)-2-(*N*-tetracosanoylamino)octadecane (37). (2,3,4,6)-*tetra*-*O*-acetyl-1-thiophenyl-β-D-galactopyranose (**47**) (0.24 mg, 0.59 mmol) and (2*S*,3*S*,4*R*)-3,4-dibenzoyloxy-2-

(*N*-tetracosanoylamino)octadecane-1-ol (**38**) (0.28 g, 0.30 mmol) were azeotroped in toluene (3 x 10 mL). The mixture was then dissolved in dry CH_2Cl_2 (27 mL) under N_2, followed by the addition of activated 4Å MS (0.20 g). The mixture was cooled to −40 ^oC and (NIS) (0.08 g, 0.34 mmol) and AgOTf (0.03 g, 0.10 mmol) were then added. The mixture was stirred at −20 ^oC for 5 h. The generated magenta mixture was warmed to rt. Et_3N (0.5 mL) was added. The solution was filtered through a pad of celite, the celite was further washed with CH_2Cl_2 (20 mL) and the filtrate was concentrated. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc 80:20) afforded **37** (83 mg, 23%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 8.02–7.98 (m, 4H), 7.60–7.52 (m, 2H), 7.47–7.39 (m, 4H), 6.17 (d, *J* = 9.1 Hz, 1H), 5.56 (dd, *J* = 8.0, 3.4 Hz, 1H), 5.35–5.32 (m, 1H), 5.30–5.28 (m, 1H), 5.07 (dd, *J* = 10.4, 7.9 Hz, 1H), 4.93 (dd, *J* = 10.4, 3.4 Hz, 1H), 4.60 (dddd, *J* = 9.2, 9.2, 3.6, 3.6 Hz, 1H), 4.40 (app d, *J* = 7.8 Hz, 1H), 3.97–3.89 (m, 2H), 3.82–3.72 (m, 3H), 2.23 (t, *J* = 7.5 Hz, 2H), 2.22–2.17 (m, 1H), 2.10 (m, 3H), 2.03–1.99 (m, 2H), 2.03–2.00 (m, 1H), 1.97 (m, 3H), 1.96 (m, 3H), 1.94 (m, 3H), 1.90–1.82 (m, 2H), 1.72–1.60 (m, 2H), 1.24–1.20 (m, 60H), 0.88–0.85 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 170.4, 170.2, 169.7, 166.5, 165.2, 133.4, 133.2, 130.2, 130.0, 128.7, 128.6, 100.9, 74.0, 73.1, 70.9, 70.9, 68.9, 67.1, 67.0, 61.1, 48.4, 36.9, 32.1, 29.9, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 25.8, 25.8, 22.9, 20.9, 20.8, 20.7, 14.3.



(2*S*,3*S*,4*R*)-3,4-Dibenzoyloxy-2-(*N*-tetracosanoylamino)octadecan-1-ol (38). *p*TsOH•H_2O (0.77 g, 4.1 mmol) was added to solution of (2*S*,3*R*,4*S*)-3,4-dibenzoyloxy-2-(*N*-tetracosanoylamino)-1-triphenylmethoxyoctadecane (**46**) (2.42 g, 2.03 mmol) in a mixture of MeOH/CH_2Cl_2 (15:32 mL).[50] After 6 h TLC showed complete consumption of starting material. The reaction was quenched with saturated aqueous $NaHCO_3$ (25 mL), and the product was extracted with CH_2Cl_2 (3 X 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to give **38** (2.42 g, 63%) as a colorless solid: mp 45.6–46.5 °C; $[\alpha]^{25}_D$ 1.43 (*c* 0.85, CH_2Cl_2); IR (neat) 3350 (br), 2920, 2851, 1720, 1451, 1271, 1112, 710 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.2 Hz, 2H), 7.97 (d, *J* = 7.2 Hz, 2H), 7.59–7.52 (m, 2H), 7.46–7.39 (m, 4H), 6.08 (d, *J* = 8.2 Hz, 1H), 5.22 (ddd, *J* = 8.6, 4.4, 4.4 Hz, 1H), 4.69 (ddd, *J* = 7.2, 7.2, 7.2 Hz, 1H), 4.39–4.43 (m, 2H), 3.99–3.95 (m, 1H), 3.52 (d, *J* = 5.3 Hz, 1H), 2.28–2.16 (m, 2H), 1.96–1.82 (m, 2H), 1.61 (quin *J* = 7.2 Hz, 2H), 1.41–1.12 (m, 64H), 0.87 (t, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 167.1, 167.0, 133.5, 130.0, 129.9, 129.7, 128.7, 128.6, 75.8, 73.2, 64.2, 51.4, 37.0, 32.1, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 25.9, 25.7, 22.9, 14.3; HRMS (ESI) calcd for $C_{56}H_{94}NO_6$ $[M + H]^+$ *m/z* 876.7076, found 876.7044.

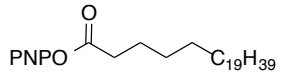


(2,3,4,6)-Tetra-*O*-acetyl-1-hydroxyl-α/β-D-galactopyranose (40). $BnNH_2$ (0.98 mL, 3.1 mmol) was added to a solution of (1,2,3,4,6)-penta-*O*-acetyl-β-D-galactopyranoside (**39**) (1.00 g, 2.56 mmol) in dry THF (15 mL) at rt. The solution was allowed to stir overnight. The next day the reaction was cooled to 0 ^oC and then neutralized to pH 7 with 1N HCl. The product was extracted with CH_2Cl_2 (3 X 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 70:30) to give **40** as a colorless oil (0.59 g, 64%, 4:1): ^1H NMR (400 MHz, $CDCl_3$) δ 5.47 (dd, *J* = 3.5, 3.5 Hz, 1H), 5.43 (app d, *J* = 2.3 Hz, 1H), 5.38 (dd, *J* = 10.8, 3.3 Hz, 1H) 5.10 (dd, *J* = 10.8, 3.2 Hz, 0.8H), 5.05–5.03 (m, 0.2H), 4.68 (dd, *J* = 7.9, 7.9, Hz, 0.2H), 4.44 (dd, *J* = 6.5, 6.5, Hz, 0.8H), 4.13 (dd, *J* = 12.9, 8.6, Hz, 0.8H), 4.09–4.02 (m, 1.2H), 3.94 (app d, *J* = 6.8 Hz, 0.2H), 3.91 (app d, *J* = 3.3 Hz, 0.8H), 2.13 (s, 0.6H), 2.11 (s, 2.4H), 2.06 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.5, 170.3, 90.7, 68.5, 68.4, 67.4, 66.2, 61.9, 20.9, 20.8, 20.8, 20.8.

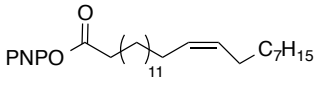
***p*-Nitrophenyl ester preparation**

p-Nitrophenol (1.1 equiv) and DMAP 0.2 equiv.) were added to a flask charged with carboxyl acid (1.0 equiv.) in dry CH_2Cl_2 (0.014 M) and stirred for 15 min. DCC (1.04 equiv) in dry CH_2Cl_2 (0.12 M) was then added slowly. The solution was allowed to stir at rt overnight. The reaction was filtered through a pad of celite and the celite was washed with more CH_2Cl_2. The combined filtrates were then concentrated.

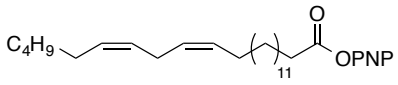
Purification via flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) yielded PNP-activated esters.



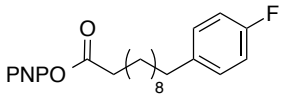
***p*-Nitrophenyltetracosanoate (42).** Compound **42** was isolated as a white solid (0.29 g, 73%): mp 81.9–82.2 °C; IR (neat) 2916, 2849, 1752, 1535, 1347, 1203, 1136, 1107, 927, 868, 717 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.29–8.25 (m, 2H), 7.29–7.26 (m, 2H), 2.59 (t, *J* = 7.4 Hz, 2H), 1.76 (quin, *J* = 7.3 Hz 2H), 1.45–1.26 (m, 40H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 155.8, 145.4, 125.4, 122.6, 34.6, 32.2, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 25.0, 22.9, 14.3; HRMS (ESI) calcd for $C_{30}H_{52}NO_4$ $[M + H]^+$ *m/z* 490.3891, found 490.3921.



***p*-Nitrophenyl 15*Z*-tetracosenoate (77).** Compound **77** was isolated as a colorless solid (0.50 g, 73%): mp 35.5–36.0 °C; IR (neat) 2916, 2850, 1753, 1593, 1536, 1490, 1471, 1350, 1203, 1138, 926, 868, 717 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.7 Hz, 2H), 7.19 (d, *J* = 8.6 Hz, 2H), 5.27 (dt, *J* = 15.3, 11.6 Hz, 2H), 2.51 (t, *J* = 7.3 Hz, 2H), 1.96–1.91 (m, 4H), 1.68 (quin, *J* = 7.0 Hz, 2H), 1.34–1.19 (m, 32H), 0.80 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 155.7, 145.4, 130.1, 130.0, 125.3, 122.6, 34.5, 32.1, 30.0, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 27.4, 24.9, 22.9, 14.3; HRMS (ESI) calcd for $C_{30}H_{50}NO_4$ $[M + H]^+$ *m/z* 488.3734, found 488.3755.

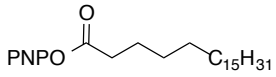


***p*-Nitrophenyl 15*Z*,18*Z*-tetracosedienoate (84).** Compound **84** was afforded as a colorless solid/oil (29.0 mg, 73%): IR (neat) 2922, 2852, 1768, 1593, 1524, 1490, 1464, 1345, 1208, 1098, 863 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.0 Hz, 2H), 7.19 (d, *J* = 8.9 Hz, 2H), 5.33–5.21 (m, 4H), 2.71–2.64 (m, 2H), 2.52 (t, *J* = 7.4 Hz, 2H), 1.97 (dt, *J* = 6.2, 6.2 Hz, 5H), 1.68 (quin, *J* = 7.2 Hz, 2H), 1.36–1.20 (m, 26H), 0.81 (t, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 155.7, 145.4, 130.3, 128.1,125.3, 122.6, 34.5, 31.7, 29.8, 29.8, 29.6, 29.5, 29.4, 29.2, 27.6, 27.4, 25.8, 24.9, 22.8, 14.2; HRMS (ESI) calcd for $C_{30}H_{48}NO_4$ $[M + H]^+$ *m/z* 486.3578, found 486.3570.

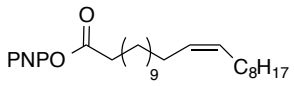


***p*-Nitrophenyl 11-(*p*-fluorophenyl)undecanoate (88).** Pd/C (0.16 g, 10% wt) and AcOH (0.3 mL) were added to a solution of 11-(*p*-fluorophenyl)-10-undecanoic acid (**87**) (0.23 g, 0.82 mmol) in MeOH (5.5 mL). The reaction was purged with H_2 for 10 min and then allowed to stir under H_2 until TLC showed complete consumption of starting material. The suspension was filtered through a pad of celite, and the celite was washed with $CHCl_3$/MeOH (1:1, 6 mL). The filtrate was concentrated under reduced pressure. The crude product was purified via flash column chromatography on silica gel (petroleum ether/EtOAc, 90:10) to give *p*-nitrophenyl-11-(*p*-fluorophenyl)undecanoic acid (0.17 g) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.12 (dd, *J* = 8.3, 5.6 Hz, 2H), 6.95 (t, *J* = 8.7 Hz, 2H), 2.57 (t, *J* = 7.5 Hz, 2H), 2.35

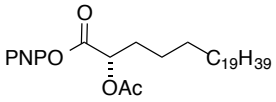
(t, *J* = 7.4 Hz, 2H), 1.68–1.53 (sept, *J* = 7.4 Hz, 4H), 1.35–1.28 (m, 12H); The 11-(*p*-fluorophenyl)undecanoic acid was then activated to its corresponding PNP ester following standard conditions to give clean ester **88** as a colorless solid/oil (72 mg, 33%): IR (neat) 2923, 2851, 1758, 1592, 1527, 1508, 1488, 1343, 1200, 1141, 1103, 925, 818, 716 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.0 Hz, 2H), 7.27 (d, *J* = 4.1 Hz, 2H), 7.11 (dd, *J* = 8.1, 5.6 Hz, 2H), 6.95 (t, *J* = 8.7 Hz, 2H), 2.61–2.55 (m, 4H), 1.75 (quin, *J* = 7.2 Hz, 2H), 1.58 (quin, *J* = 8.0 Hz, 2H), 1.44–1.30 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 161.3 (d_{C-F}, *J* = 241.4 Hz), 155.7, 145.5, 138.6, 129.9, 129.8, 125.4, 122.6, 115.13 (d_{C-F}, *J* = 20.8 Hz), 35.3, 34.5, 31.8, 29.7, 29.6, 29.6, 29.4, 29.4, 29.2; HRMS (ESI) calcd for $C_{23}H_{29}FNO_4$ $[M + H]^+$ *m/z* 402.2075, found 402.2109.



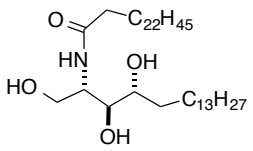
***p*-Nitrophenylelcosanoate (114).** Compound **114** was isolated as a white solid (0.18 g, 71%): mp 73.1–73.4 °C; IR (neat) 2916, 2849, 1752, 1535, 1472, 1347, 1203, 1136, 1104, 927, 856, 718 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (dt, *J* = 10.1, 3.1 Hz, 2H), 7.27 (dt, *J* = 10.2, 3.1 Hz, 2H), 2.59 (t, *J* = 7.4 Hz, 2H), 1.76 (quin, *J* = 7.4 Hz 2H), 1.45–1.26 (m, 32H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 155.8, 145.5, 125.4, 122.6, 34.6, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 25.0, 22.9, 14.3; HRMS (ESI) calcd for $C_{26}H_{44}NO_4$ $[M + H]^+$ *m/z* 434.3265, found 434.3296.



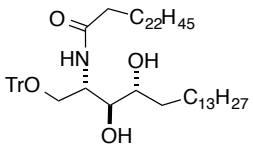
***p*-Nitrophenylerucicoate (116).** Compound **116** was afforded as a colorless solid/oil (0.497 g, 73%): IR (neat) 2918, 2850, 1753, 1592, 1533, 1489, 1345, 1203, 1136, 927, 854, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 9.0 Hz, 2H), 7.20 (d, *J* = 9.1 Hz, 2H), 5.31–5.24 (m, 2H), 2.52 (t, *J* = 7.5 Hz, 2H), 1.97–1.92 (m, 4H), 1.68 (quin, *J* = 7.3 Hz 2H), 1.36–1.26 (m, 28H), 0.81 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 155.8, 145.5, 130.1, 130.2, 125.4, 122.6, 34.6, 32.1, 30.0, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 25.0, 22.9, 14.3; HRMS (ESI) calcd for $C_{28}H_{46}NO_4$ $[M + H]^+$ *m/z* 460.3421, found 460.3438.



***p*-Nitrophenyl (2*S*)-*O*-acetyl tetracosanoate (120).** Compound **120** was afforded as a white solid (35 mg, 56%): mp 63.9–62.9 °C; $[\alpha]^{25}_D$ –14.23 (*c* 0.86, CH_2Cl_2); IR (neat) 2914, 2849, 1768, 1734, 1518, 1472, 1347, 1251, 1202, 1159, 922, 871, 714 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.29–8.26 (m, 2H), 7.29 (dt, *J* = 5.5, 3.5 Hz, 2H), 5.13 (dd, *J* = 6.5, 6.5 Hz, 1H), 2.18 (s, 3H), 2.02–1.97 (m, 2H), 1.52 (quin, *J* = 7.1 Hz, 2H), 1.41–1.26 (m, 38H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 168.6, 155.1, 145.8, 125.5, 122.5, 72.6, 32.1, 31.1, 29.9, 29.8, 29.8, 29.7, 29.6, 29.3, 25.3, 22.9, 20.7, 14.3; HRMS (ESI) calcd for $C_{32}H_{54}NO_6$ $[M + H]^+$ *m/z* 548.3946, found 548.3936.

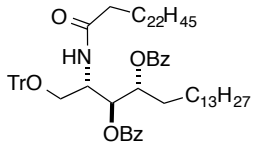


(2*S*,3*R*,4*S*)-2-(*N*-Tetracosanoylamino)octadecan-1,3,4-triol (44). *p*-Nitrophenyl tetracosanoate (**42**) (2.62 g, 5.34 mmol) was added to a solution of *ribo*-phytosphingosine (**43**) (1.41 g, 4.45 mmol) in dry pyridine (56 mL). The mixture was stirred in a preheated oil bath at 50 °C overnight. The solvent was evaporated, and the solid residue was purified via flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to yield **44** as a white solid (2.88 g, 97%): mp 119.0–119.5 °C; $[\alpha]^{25}_D$ 2.43 (*c* 0.16, $CHCl_3$/MeOH, 3:2); IR (neat) 2916, 2849, 1750, 1490, 718 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 4.09 (dd, *J* = 9.2, 4.9 Hz, 1H), 3.78 (dd, *J* = 11.3, 4.4 Hz, 1H), 3.71 (dd, *J* = 11.3, 5.0 Hz, 1H), 3.56–3.54 (m, 2H), 2.22 (t *J* = 7.4 Hz, 2H), 1.64–1.58 (m, 4H), 1.54–1.52 (m, 2H), 1.48–1.27 (m, 62H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.7, 76.6, 73.5, 62.3, 53.1, 37.4, 33.8, 32.8, 30.7, 30.6, 30.4, 30.3, 30.2, 26.8, 23.5, 14.7; HRMS (ESI) calcd for $C_{42}H_{86}NO_4$ $[M + H]^+$ *m/z* 668.6551, found 668.6562.



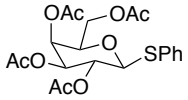
(2*S*,3*S*,4*R*)-2-(*N*-tetracosanoylamino)-1-triphenylmethoxyoctadecan-3,4-diol (45). Trityl bromide (2.08 g, 6.45 mmol) was added to a mixture of (2*S*,3*S*,4*R*)-2-(*N*-teracosanoylamino)octadecan-1,3,4-triol (**44**) (2.88 g, 4.30 mmol) in dry pyridine (18 mL).[50] The mixture was stirred in preheated oil bath at 50 °C overnight. The solvent was evaporated under reduced pressure, and the crude product was purified by

flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) to provide **45** (2.96 g, 76%) as a clear, crystalline solid: mp 41.9–42.3 °C; $[\alpha]^{25}_{D}$ 12.0 (*c* 1.35, CH_2Cl_2); IR (neat) 2917, 2850, 1656, 1467, 1069, 906, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41–7.39 (m, 6H), 7.32–7.29 (m, 6H), 7.26–7.23 (m, 3H), 6.03 (d, *J* = 8.3 Hz, 1H), 4.25 (dddd, *J* = 8.3, 4.2, 4.2, 4.2 Hz, 1H), 3.59–3.54 (m, 1H), 3.50 (dd, *J* = 9.8, 3.6 Hz, 1H), 3.42–3.77 (m, 1H), 3.34 (dd, *J* = 9.8, 4.6 Hz, 1H), 3.11 (d, *J* = 8.4 Hz, 1H), 2.24 (d, *J* = 7.7 Hz, 1H), 2.14 (t *J* = 7.4 Hz, 2H), 1.69–1.67 (m, 1H), 1.60 (quin *J* = 7.2 Hz, 2H), 1.47–1.37 (m, 3H), 1.33–1.26 (m, 62H), 0.88 (t, *J* = 6.6, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 143.4, 128.7, 127.6, 128.1, 87.9, 75.8, 73.4, 63.2, 50.7, 37.1, 33.5, 32.1, 29.9, 29.9, 29.7, 29.6, 29.6, 26.0, 26.0, 22.9, 14.3; HRMS (ESI) calcd for $C_{61}H_{99}NNaO_4$ $[M + Na]^+$ *m/z* 932.7466, found 932.7453.



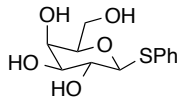
(2*S*,3*S*,4*R*)-2-(*N*-Tetracosanoylamino)-3,4-dibenzoyloxy-1-triphenylmethoxy-octadecane (46). BzCl (2.26 mL, 19.5 mmol) was added to a solution of (2*S*,3*S*,4*R*)-2-(*N*-tetracosanoylamino)-1-triphenylmethoxyoctadecan-3,4-diol (**45**) (2.96 g, 3.25 mmol) and DMAP (66 mg, 0.54 mmol) in pyridine (27 mL).[50] The reaction mixture was allowed to stir overnight. Ice-cold H_2O (50 mL) was added. The solution was extracted with CH_2Cl_2 (3 X 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) gave **46** as a colorless oil (2.42 g, 63%): $[\alpha]^{25}_{D}$ 10.6 (*c* 1.04, CH_2Cl_2); IR (neat) 2921,

2852, 1723, 1449, 1277, 1049, 703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.2 Hz, 2H), 7.88 (d, *J* = 7.2 Hz, 2H), 7.60–7.53 (m, 2H), 7.40 (dt, *J* = 7.4, 7.4 Hz, 2H), 7.30–7.28 (m, 8H), 7.15–7.08 (m, 9H), 5.94 (d, *J* = 9.3 Hz, 1H), 5.78 (dd, *J* = 9.0, 2.5 Hz, 1H), 5.34 (ddd, *J* = 10.0, 2.9, 2.9 Hz, 1H), 4.57 (dddd, *J* = 9.0, 9.0, 3.4, 3.4 Hz, 1H), 3.33 (dd, *J* = 9.8, 3.6 Hz, 1H), 3.28 (dd, *J* = 9.7, 3.2 Hz, 1H), 2.23–2.11 (m, 2H), 1.94–1.77 (m, 2H), 1.62 (q *J* = 6.9 Hz, 2H), 1.41–1.12 (m, 64H), 0.89–0.85 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 166.6, 165.3, 143.7, 133.2, 133.1, 130.6, 130.2, 130.0, 130.0 128.9, 128.6, 128.6, 128.0, 127.2, 87.2, 74.4, 73.4, 62.1, 49.0, 37.2, 32.2, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 25.9, 22.9, 14.3; HRMS (ESI) calcd for $C_{75}H_{107}NNaO_6$ $[M + Na]^+$ *m/z*, 1140.7991 found 1140.7941.

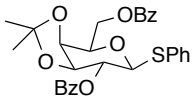


(2,3,4,6)-tetra-*O*-acetyl-1-β-D-thiophenylglactopyranoside (47). $BF_3•Et_2O$ (2.4 mL, 17.9 mmol) was added drop-wise to a solution of thiophenol (1.58 mL, 15.37 mmol) and penta-*O*-acetyl-D-galactose (**39**) (2.0 g, 5.1 mmol) in CH_2Cl_2 (20 mL) at 0 ^oC. The reaction solution was slowly allowed to warm to rt and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (28 mL) and neutralized with saturated aqueous $NaHCO_3$ (80 mL) at 0 ^oC. The layers were separated, and the aqueous layer was further extracted with CH_2Cl_2 (3 X 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 80:20) afforded **47** (1.75 g, 85%) as slightly yellow vicious oil:[51] ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 3.6, 2.4 Hz, 2H), 7.30

(t, *J* = 3.5 Hz, 3H), 5.40 (d, *J* = 3.1 Hz, 1H), 5.23 (dd, *J* = 9.9, 9.9 Hz, 1H), 5.04 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.71 (app dd, *J* = 10.0 Hz, 1H), 4.18 (dd, *J* = 11.4, 7.0 Hz, 1H), 4.10 (dd, *J* = 11.4, 6.2 Hz 1H), 3.93 (app ddd, *J* = 6.6, 6.6 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.3, 170.2, 169.6, 132.7, 132.6, 129.0, 128.3, 86.7, 74.6, 72.2, 67.4, 67.4, 61.8, 67.4, 67.4, 29.8, 21.0, 20.8, 20.8, 20.7.

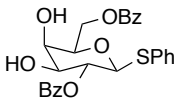


Phenyl-1-thio-β-D-galactopyranoside (49). (2,3,4,6)-tetra-*O*-acetyl-1-thiophenyl-β-D-galactopyranoside (**47**) (1.15 g, 2.83 mmol) was dissolved in a mixture of MeOH/CH_2Cl_2 (11:11 mL). NaOMe (3.3 mL, 17 mmol, 0.5 M in MeOH) was added drop-wise to pH = 9. The mixture was stirred for 2 h at rt. The solution was then neutralized to pH = 7 using Dowex 50W x 2-100 (H^+). The resin was filtered off, and the filtrate was concentrated to afford **49** as a yellowish solid (0.82 g).[51] The crude product was used in the next step without purification.



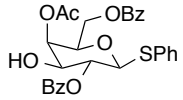
2,6-Dibenzoyloxy-3,4-*O*-isopropylidene-1-thiophenyl-β-D-galactopyranoside (50). 2,2-Dimethoxypropane (5.7 mL) and *p*TsOH•H_2O (0.03 g) were added to a solution of phenyl-1-thio-β-D-galactopyranoside (**49**) (1.27 g, 5.37 mmol) in DMF (5.7 mL).[52] The solution was stirred in a preheated oil bath at 80 °C for 1.5 h. The reaction was neutralized to pH 7 with saturated$NaHCO_3$ and filtered. The filtrate was concentrated and the product was dissolved in pyridine (2.4 mL) and treated with

BzCl (1.5 g, 1.2 mL, 10.3 mmol). The solution was allowed to stir overnight. The next day, MeOH (5 mL) was added and the reaction mixture was concentrated. The syrup was dissolved in CH_2Cl_2 (10 mL) and washed with 2 M HCl (10 mL) and then saturated aqueous $NaHCO_3$ (10 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to yield **50** as a white solid (1.08 g, 43%): mp 145.3–145.5 °C; $[\alpha]^{25}_D$ 32.9 (*c* 1.33, CH_2Cl_2); IR (neat) 2886, 1711, 1451, 1368, 1262, 1068, 1025, 874, 745, 705, 589, 551 cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.1 Hz, 4H), 7.63–7.56 (m, 2H), 7.49–7.43 (m, 6H), 7.17 (t, *J* = 7.4 Hz, 1H), 7.07 (t, *J* = 7.7 Hz, 2H), 5.35 (dd, *J* = 10.0, 7.0 Hz, 1H), 4.81 (d, *J* = 10.0 Hz, 1H), 4.74 (dd, *J* = 11.9, 3.6 Hz, 1H) 4.63 (dd, *J* = 11.9, 8.3 Hz, 1H), 4.42 (dd, *J* = 5.6, 5.6, Hz, 1H), 4.35 (dd, *J* = 5.4, 4.1 Hz, 1H), 4.24 (ddd, *J* = 5.6, 3.4, 3.4 Hz, 1H), 1.62 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 165.6, 133.9, 133.4, 132, 130.1, 130.1, 130.0, 129.9, 129.0, 128.7, 128.6, 127.8, 111.3, 86.3, 74.7, 73.9, 72.1, 64.3, 29.9, 27.9, 26.6; HRMS (ESI) calcd for $C_{29}H_{29}O_7S$ $[M + H]^+$ *m/z* 521.1629, found 521.1654.



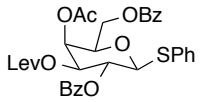
2,6-Dibenzoyloxy-1-thiophenyl-β-D-galactopyranoside-3,4-diol (51). 80% aqueous AcOH (9.3 mL) was added to a flask charged with 2,6-dibenzoyloxy-3,4-*O*-isopropylidene-1-thiophenyl-β-D-galactopyranoside (**50**) (1.08 g, 2.07 mmol).[52] The mixture was stirred in a preheated oil bath at 60 °C for 4 h. The reaction was allowed to cool to rt and then concentrated to give a white solid. The product was

purified using flash column chromatography on silica gel (Petroleum ether/EtOAc, 60:40) to give **51** (0.83 g, 84%) as a white solid: mp 160.4–161.9 °C; $[\alpha]^{25}_{D}$ 12.14 (*c* 1.49, CH_2Cl_2); IR (neat) 3523, 2158, 1976, 1701, 1601, 1450, 1264, 1069, 690, 597 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.5 Hz, 4H), 7.56 (tt, *J* = 7.4, 7.4 Hz, 2H), 7.45–7.38 (m, 6H), 7.18 (t, *J* = 7.4 Hz, 1H), 7.09 (t, *J* = 6.2 Hz, 2H), 5.37 (dd, *J* = 9.2, 9.2 Hz, 1H), 4.83 (d, *J* = 10.0 Hz, 1H), 4.66 (ddd, *J* = 12.1, 6.7, 5.3 Hz, 2H) 4.15 (s, 1H), 3.92 (dd, *J* = 6.2, 6.2 Hz, 3H), 3.70 (d, *J* = 4.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 166.7, 133.5, 133.4, 132.0, 130.2, 129.9, 129.8, 129.7, 128.9, 128.6, 127.8, 86.3, 76.6, 73.6, 72.0, 69.3, 64; HRMS (ESI) calcd for $C_{26}H_{25}O_7S$ $[M + H]^+$ *m/z* 481.1316, found 481.1345.



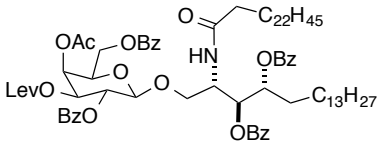
4-*O*-Acetyl-2,6-*O*-dibenzoyl-1-thiophenyl-β-D-galactopyranoside-3-ol (52). PTSA (5.0 mg, 0.26 mmol) was added to a solution of phenyl-2,6-dibenzoyloxy-1-thio-β-D-galactopyranoside-3,4-diol (**51**) (0.10 g, 0.21 mmol) and triethyl orthoacetate (5 mL, 27 mmol) in toluene (5 mL).[31] After 1 h, TLC showed complete consumption of starting material. Et_3N (0.2 mL was added, followed by the addition of H_2O (4 mL). The product was extracted with EtOAc (3 X 10 mL). The combined organic layers were washed with 1 M aqueous $NaHCO_3$ (3 X 10 mL), H_2O (2 X 10 mL) and brine (10 mL). The EtOAc was dried ($MgSO_4$), filtered and concentrated. The crude product was purified via flash column chromatography on silica gel to provide **52** and its inseparable C3-OAc regioisomer, ~10% (0.10 g) as a white fluffy solid: Peak assignment for **52**: ^1H NMR (400 MHz, $CDCl_3$) δ 8.08–8.00 (m, 4H),

7.62–7.58 (m, 2H), 7.49–7.44 (m, 6H), 7.23 (tt, *J* = 6.3, 0.4 Hz, 1H), 7.14 (t, *J* = 7.7 Hz, 2H), 5.52 (d, *J* = 3.1 Hz, 1H), 5.29 (dd, *J* = 9.7, 9.7 Hz, 1H), 4.90 (app d, *J* = 10.0 Hz, 1H) 4.50 (dd, *J* = 11.6, 7.6 Hz, 1H), 4.43 (dd, *J* = 11.5, 5.2 Hz, 1H), 4.14–4.02 (m, 2H), 2.78 (d, *J* = 6.0 Hz, 1H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 166.9, 166.3, 133.8, 133.5, 133.0, 132.6, 130.2, 130.0, 129.1, 128.7, 128.7, 128.2, 86.7, 75.4, 72.9, 71.9, 70.3, 63.0, 21.0.



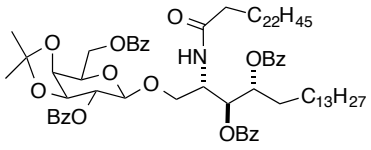
4-*O*-Acetyl-2,6-dibenzoyloxy-3-*O*-levulinoyl-1-thiophenyl-β-D-galactopyranoside (53). Levulinic acid (0.03 mL, 0.3 mmol), EDC•HCl (0.05 g, 0.3 mmol) and DMAP (6 mg, 0.05 mmol) were added to a solution of 4-*O*-acetyl-2,6-dibenzoyloxy-1-thiophenyl-β-D-galactopyranoside-3-ol (**52**) (0.1 g, 0.2 mmol) in dry CH_2Cl_2 (2 mL).[36] The reaction was wrapped in aluminum foil, and the solution was stirred until TLC showed complete consumption of starting material. The reaction was diluted with CH_2Cl_2 (10 mL) and then washed with H_2O (10 mL) and saturated aqueous $NaHCO_3$ (10 mL) successively. The organic phase was dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 80:20) to give **53** and its inseparable C-4 regiosiomer as a colorless oil (0.1 g): peak assignment for **53**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04–8.00 (m, 4H), 7.59 (t, *J* = 7.2 Hz, 2H), 7.48–7.43 (m, 6H), 7.23 (tt, *J* = 6.6, 1.4 Hz, 1H), 7.15 (t, *J* = 14.8 Hz, 2H), 5.61–5.52 (m, 2H), 5.28 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.52 (dd, *J* = 14.6, 3.2 Hz, 1H), 4.41–4.37 (m, 1H), 4.17 (app ddd, *J* = 6.3, 6.3 Hz, 2H), 2.64–2.51 (m, 2H), 2.49–2.34 (m, 2H), 2.19 (s, 3H),

2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.8, 171.9, 170.3, 166.1, 165.5, 133.6, 133.5, 132.9, 132.5, 130.1, 129.9, 129.0, 128.7, 128.2, 87.2, 74.9, 72.4, 68.0, 67.8, 62.6, 37.8, 29.6, 28.0, 20.9; HRMS (ESI) calcd for $C_{33}H_{33}O_{10}S$ $[M + H]^+$ *m/z* 621.1789, found 621.1761.



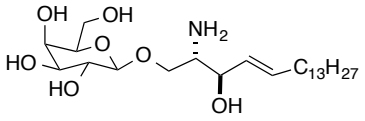
(2*S*,3*S*,4*R*)-3,4-Dibenzoyloxy-1-(4-*O*-acetyl-2,6-dibenzoyloxy-3-*O*-levulinoyl-β-D-galactopyranoside)-2-(*N*-tetracosanoylamino)octadecane (54). (2*S*,3*S*,4*R*)-3,4-dibenzoyloxy-2-(*N*-tetracosanoylamino)octadecan-1-ol (**38**) (0.46 g, 0.48 mmol) and 4-*O*-acetyl-2,6-dibenzoyloxy-3-*O*-levulinoyl-1-thiophenyl-β-D-galactopyranoside (**53**) (0.20 g, 0.32 mmol) were azeotroped in toluene (3 x 10 mL). The mixture was then dissolved in dry CH_2Cl_2 (9 mL) under N_2, followed by the addition of activated 4Å MS (0.20 g). The mixture was cooled to −40 °C, and then NIS (0.10 g, 0.40 mmol) and AgOTf (30 mg, 0.11 mmol) were added. The mixture was stirred at −20 °C for 5 h. The generated magenta mixture was warmed to rt. Et_3N (0.5 mL) was added. The mixture was filtered through a pad of celite, the celite was washed with CH_2Cl_2 (20 mL) and the filtrate was concentrated. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc 80:20) afforded **54** with a trace of an inseparable unknown product (0.38 g) as a slightly yellowish oil. Peak assignment for **54**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02–7.98 (m, 5H), 7.96 (d, *J* = 7.8 Hz, 2H), 7.91 (d, *J* = 7.9 Hz, 2H), 7.58–7.51 (m, 4H), 7.46–7.38 (m, 7H), 6.10 (d, *J* = 9.1 Hz, 1H), 5.58 (dd, *J* = 8.3, 3.3 Hz, 1H), 5.46–5.45 (m, 1H), 5.39–5.33 (m, 2H),

5.19 (dd, *J* = 10.3, 3.3 Hz, 1H), 4.63–4.56 (m, 2H), 4.10–4.06 (m, 3H), 3.96 (dd, *J* = 6.4, 6.4 Hz, 1H), 3.66 (dd, *J* = 9.9, 3.4 Hz, 1H), 2.64–2.54 (m, 2H), 2.47–2.41 (m, 1H), 2.40–2.33 (m, 1H), 2.17–2.13 (m, 4H), 2.05–2.00 (m, 4H), 1.96–1.90 (m, 1H), 1.87–1.76 (m, 3H), 1.51–1.40 (m, 2H), 1.38–1.19 (m, 60H), 0.88–0.85 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.9, 173.1, 171.9, 170.3, 166.3, 165.9, 165.5, 165.1, 133.6, 133.4, 133.3, 133.1, 130.3, 130.1, 130.0, 130.0, 129.9, 129.8, 129.5, 129.4, 128.7, 128.6, 128.6, 128.5, 100.8, 73.9, 72.9, 71.0, 70.9, 69.8, 67.3, 67.1, 61.5, 71.0, 70.9, 67.8, 67.3, 67.1, 48.0, 37.9, 36.6, 32.1, 29.9, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.1, 27.9, 25.7, 25.6, 22.9, 20.9, 14.3.



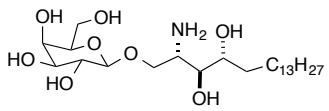
(2*S*,3*S*,4*R*)-3,4-Dibenzoyloxy-1-(2,6-dibenzoyloxy-3,4-*O*-isopropylidene-β-D-galactopyranoside)-2-(*N*-tetracosanoylamino)octadecane (57). (2*S*,3*S*,4*R*)-3,4-dibenzoyloxy-2-(*N*-tetracosanoylamino)octadecan-1-ol (**38**) (0.42 g, 0.44 mmol) and 2,6-dibenzoyloxy-3,4-*O*-isoproylidene-1-thiophenyl-β-D-galactopyranoside (**50**) (0.19 g, 0.36 mmol) were azeotroped in toluene (3 x 10 mL). The mixture was then dissolved in dry CH_2Cl_2 (10 mL) under N_2, followed by the addition of activated 4Å MS (0.20 g). The mixture was cooled to –40 °C; then (NIS) (0.10 g, 0.45 mmol) and AgOTf (0.03 g, 0.13 mmol) were added. The reaction mixture was stirred at –20 °C for 5 h. The magenta mixture was to warm to rt, followed by the addition of Et_3N (0.5 mL). The solution was filtered through a pad of celite, the celite was washed with CH_2Cl_2 (15 mL) and the filtrate was concentrated. Purification by flash column

chromatography on silica gel (petroleum ether/EtOAc 95:5) afforded **57** (0.15 g, 30%) as a slightly yellowish oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (dd, *J* = 8.1, 0.9 Hz, 2H), 8.01 (dd, *J* = 8.1, 1.0 Hz, 2H), 7.96 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.63–7.58 (m, 1H), 7.57–7.52 (m, 2H), 7.49–7.43 (m, 6H), 7.39 (t, *J* = 7.9 Hz, 2H), 7.28–7.23 (m, 1H), 7.13 (t, *J* = 7.8 Hz, 2H), 6.15 (d, *J* = 9.6 Hz, 1H), 5.71 (dd, *J* = 10.2, 2.2 Hz, 1H), 5.31–5.28 (m, 2H), 4.66 (dd, *J* = 5.1, 2.6 Hz, 1H), 4.53 (dd, *J* = 7.9, 2.5 Hz, 1H), 4.49 (ddd, *J* = 8.1, 2.6, 2.6 Hz, 1H), 4.41 (dd, *J* = 12.1, 3.1 Hz, 1H), 4.26 (dd, *J* = 12.0, 8.2 Hz, 1H), 4.11 (dd, *J* = 7.7, 1.3 Hz, 1H), 3.74 (ddd, *J* = 8.3, 2.7, 2.7 Hz, 1H), 3.57 (dd, *J* = 9.8, 2.2 Hz, 1H), 3.10 (dd, *J* = 9.8, 2.7 Hz, 1H), 2.29–2.17 (m, 2H), 1.95–1.82 (m, 3H), 1.38 (s, 3H), 1.35–1.20 (m, 68H), 0.89–0.86 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 166.6, 166.3, 165.2, 134.9, 133.6, 133.2, 133.1, 130.3, 130.2, 129.9, 129.9, 129.8, 128.9, 128.5, 128.5, 126.2, 120.3, 110.1, 97.2, 74.2, 71.9, 71.4, 71.2, 70.4, 67.6, 64.3, 62.6, 32.1, 29.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 28.1, 26.1, 25.9, 24.6, 22.9, 14.4.



(*2S,3R,4E*)-2-Amino1-(β-galactopyranosyloxy)octadec-4-en-3-ol (59). NaOMe (0.5 M, 4 mL, 2.10 mmol) was added to a solution of (2*S,*3*R,*4*E*)-2-azido-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-pivaloyl-β-galactopyranoside)octadec-4-ene (**74**) (0.32 g, 0.35 mmol) in a mixture of CH_2Cl_2/MeOH (2.8:2.8 mL). The reaction was stirred at rt for 1h. The reaction was neutralized with Dowex (H^+ resin). The mixture was filtered through a pad of celite, and the celite was washed with mixture of

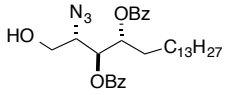
$CHCl_3$/MeOH (1:1, 10 mL). The filtrate was concentrated and purified using flash column chromatography on silica gel (CH_2Cl_2/MeOH, 85:15) to give (2*S*,3*R*,4*E*)-2-azido-1-(galactopyranosyloxy)octadec-4-en-3-ol as a white solid (0.12 g, 69%):[42] ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$, 3:2) δ 6.21 (dt, *J* = 11.7, 6.6 Hz, 1H), 5.94 (dd, *J* = 15.5, 7.3 Hz, 1H), 4.64–4.4 (m, 3H), 4.31 (s, 1H), 4.26–4.16 (m, 3H), 4.00 (dd, *J* = 9.2, 9.2 Hz, 1H), 3.96–3.92 (m, 3H), 2.49 (dt, *J* = 6.8, 6.8 Hz, 2H), 1.84–1.80 (m, 22H) 1.30 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDC1_3/CD_3OD$ = 3:2) δ 1361, 129.0, 104.4, 76.0, 74.4, 72.9, 72.8, 72.1, 72.0, 69.9, 69.7, 69.6, 66.5, 62.3, 62.2, 33.3, 32.8, 30.5, 30.5, 30.5, 30.4, 30.3, 30.2, 30.1, 29.9, 23.5, 14.7; (*2S,3R,4E*) -2-azido-1-(galactopyranosyloxy)octadec-4-en3-ol (90 mg, 0.20 mmol) in a mixture of pyridine/H_2O (1:1, 6 mL) was saturated with H_2S for 15 min. The reaction was stirred for 48 h. The solvent was evaporated, and the crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 80:20) to give **59** (0.71 g, 80%) as yellowish brown crystals:[42] $[\alpha]^{25}_D$ –3.65 (*c* 1.0, ($CHC1_3$/MeOH, 3:2); IR 3356, 2920, 2851, 1466, 1053 cm^{-1}; ^{1}H NMR (400 MHz, $CDC1_3/CD_3OD$, 3:2) δ 6.12 (dt, *J* = 14.7, 6.7 Hz, 1H), 5.78 (dd, *J* = 15.3, 7.3 Hz, 1H), 4.59 (d, *J* = 7.4 Hz, 1H), 4.43 (dd, *J* = 6.6, 6.6 Hz, 1H), 4.29 (dd, *J* = 10.4, 7.2 Hz, 2H), 4.24 (app d, *J* = 2.1 Hz, 1H), 4.19 (dd, *J* = 11.8, 7.0 Hz, 1H), 4.12–4.07 (m, 2H), 3.72–3.70 (m, 1H), 2.41 (dt, *J* = 6.6, 6.6 Hz, 2H), 1.80–1.61(m, 22H), 0.84 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 135.9, 129.2, 104.0, 75.7, 74.0, 73.2, 71.8, 69.7, 62.3, 55.6, 33.0, 32.5, 30.3, 30.2, 30.1, 30.0, 29.8, 23.3, 14.6; HRMS (TOP) m/z calcd for $C_{24}H_{48}NO_7$ (M^+ + H) 462.3425, found 462.3405.



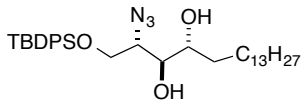
(2*S*,3*S*,4*R*)-2-Amino-1-(β-galactopyranosyloxy)octadecan-3,4-diol (60). NaOMe (3.9 mL, 1.97 mmol) was added to a solution of (2*S*,3*S*,4*R*)-2-azido-3,4-dibenzoyloxy-1-(tetra-*O*-pivaloyl-β-galactopyranoside)octadecane (**92**) (296 mg, 0.28 mmol) in a mixture of CH_2Cl_2/MeOH (3.4/3.4 mL).[42] The solution was stirred at rt for 1.5 h. The reaction was acidified to pH 2 with dowex (H^+ resin). The mixture was filtered through a pad of celite, and the celite was washed with 1:1 mixture of $CHCl_3$ and MeOH (15 mL). The filtrate was concentrated and triturated with petroleum ether/EtOAc (85:15) to give (2*S*,3*S*,4*R*)-2-azido-1-(β-galactopyranosyloxy)octadecan-3,4-diol (134 mg, 94%) as a white solid: $[\alpha]^{25}_D$ 18.88 (*c* 6.643, $CHCl_3$/MeOH, 3:2); IR (neat) 3355 (br), 2915, 2849, 2096, 1255, 1071, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 4.28 (d, *J* = 7.2 Hz, 1H), 4.13 (dd, *J* = 10.6, 5.0 Hz, 1H), 3.96 (d, *J* = 10.3 Hz, 1H), 3.97 (s, 1H), 3.82 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.70–3.63 (m, 4H), 3.58–3.49 (m, 3H), 1.67–1.56 (m, 2H), 1.42–1.25 (m, 24H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDC1_3/CD_3OD$, 3:2) δ 104.3, 76.1, 74.9, 74.4, 72.7, 72.1, 69.9, 69.4, 63.1, 62.3, 33.2, 32.8, 30.6, 30.5, 30.5, 30.2, 26.6, 23.5, 14.7; HRMS (ESI) calcd for $C_{24}H_{48}N_3O_8$ $[M + H]^+$ *m/z* 506.3436, found 506.3511.

The product was carried forward to reduction. (2*S*,3*S*,4*R*)-2-azido--1-(β-galactopyranosyloxy)octadecan-3,4-diol (13 mg, 0.27 mmol) in a mixture of pyridine/H_2O (1:1, 7.6 mL) was saturated with H_2S. The reaction was stirred for 48

h.[42] The solvent was evaporated to give **60** (136 mg, crude) as a yellowish brown powder, which was carried forward without purification.

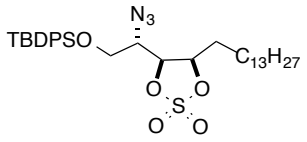


(2*S*,3*S*,4*R*)-2-Azido-(3,4-dibenzoyloxy)octadecan-1-ol (61). AcCl (9.10 mL) was added drop-wise to MeOH (230 mL). (2*S*,3*S*,4*R*)-2-azido-(3,4-dibenzoyloxy-1-*tert*-butyldiphenylsilyloxy)octadecane (**91**) (5.43 g, 6.99 mmol) in Et_2O (230 mL) was the added drop-wise.[39] The solution was stirred for 2 d. Saturated aqueous $NaHCO_3$ was added to neutralize the reaction to pH 7. The two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 X 100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (petroleum ether/ EtOAc 90:10) to give **61** (2.14 g, 69%) as a colorless solid:[53] mp 54.0–55.0 ^oC; $[\alpha]^{25}_D$ 17.3 (*c* 1.05, $CHCl_3$); IR (neat) 2918, 2107, 1712, 1246, 1109, 1026, 706, 685 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.3 Hz, 2H), 8.01 (d, *J* = 7.3 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.40 (t, *J* = 7.8 Hz, 2H), 5.61–5.56 (m, 2H), 4.03–4.00 (m, 1H), 3.88–3.79 (m, 2H), 3.11 (s, 1H), 2.02–1.87 (m, 2H), 1.57–1.38 (m, 2H), 1.40–1.26 (m, 22H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.7, 133.7, 133.3, 129.9, 129.8, 129.3, 128.7, 128.5, 73.4, 73.0, 63.3, 62.2, 32.0, 29.7, 29.7, 29.7, 29.5, 29.5, 29.4, 25.5, 22.8, 14.2; HRMS (ESI) calcd for $C_{32}H_{46}N_3O_5$ $[M + H]^+$ *m/z* 552.3432, found 552.3433.



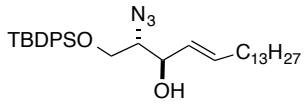
(*2S,3S,4R*)-2-Azido-1-(*tert*-butyldiphenylsilyloxy)octadecan-3,4-diol (62). NaN_3 (20.5 g, 31.5 mmol) was dissolved in a mixture of H_2O (50 mL) and CH_2Cl_2 (85 mL) and cooled to 5 ^oC. Tf_2O (10.5 mL, 63.0 mmol) was slowly added via syringe over 15 min. The resulting mixture was vigorously stirred in a ice bath for 2 h. The *in situ* generated Tf_2N_3 in the CH_2Cl_2 was separated and the aqueous phase was then extracted with CH_2Cl_2 (2 X 24 mL). The combined organic layers were washed with saturated aqueous Na_2CO_3 (100 mL). This solution was added to a suspension of *ribo*-phytosphingosine (**43**) (10.0 g 31.5 mmol), K_2CO_3 (6.53 g, 47.3 mmol) and Cu_2SO_4 (79.0 mg, 0.32 mmol) in a mixture of H_2O (100 mL) and MeOH (200 mL) at 0 ^oC. The solution was allowed to stir at rt overnight. The next day, the solvents were evaporated. H_2O (300 mL) was added to the solid residue and resulting mixture was allowed to stir for 2 h. The product was extracted with EtOAc (3 X 200 mL). The combined organic layers were filtered through celite, and the filtrate was concentrated to yield (2*S*,3*S*,4*R*)-2-azidooctadecan-1,3,4-triol (14.6 g, crude) as a bluish solid.[54] This product was carried forward to the next step without purification. TBDPSCl (13 mL, 51 mmol) was added drop-wise to 2*S*,3*S*,4*R*)-2-azidooctadecan-1,3,4-triol (14.6 g, 42.6 mmol) and DMAP (0.26 g, 2.1 mmol) mixture in dry pyridine (47 mL) and CH_2Cl_2 (200 mL). The mixture was stirred for 48 h. The reaction was diluted with EtOAc (200 ml) and washed with brine (200 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 90:10) to

provide **62** as slightly yellow oil (17.0 g, 70%):[53] $[\alpha]^{25}_{D}$ 16.9 (*c* 1.13, $CDCl_3$); IR (neat) 2923, 2853, 2097, 1428, 1111, 823, 738, 700, 503 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.2 Hz, 4H), 7.48–7.39 (m, 6H), 4.04 (dd, *J* = 11.0, 4.2 Hz, 1H), 3.92 (dd, *J* = 10.9, 5.7 Hz, 1H), 3.69 (s, 2H), 3.37 (ddd, *J* = 5.4, 5.4, 5.4 Hz, 1H), 2.49 (d, *J* = 3.1 Hz, 1H), 1.97 (s, 1H), 1.60–1.39 (m, 4H), 1.35–1.26 (m, 20H), 1.09 (s, 9H), 0.88 (t, *J* = 7.0, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.9, 135.8, 132.8, 132.7, 130.3, 128.1, 74.4, 72.6, 64.4, 63.6, 32.1, 32.1, 29.9, 29.9, 29.8, 29.8, 29.6, 27.0, 25.9, 22.9, 19.3, 14.3; HRMS (ESI) calcd for $C_{34}H_{56}N_3O_3Si$ $[M + H]^+$ *m/z* 582.4085, found 582.4119.



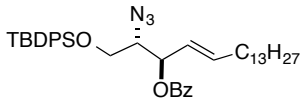
(*2S,3S,4R*)-2-Azido-3,4-dioxathiolan)-1-(*tert*-butyldiphenylsilyloxy)octadecane (63). Et_3N (5.2 mL, 55 mmol) and $SOCl_2$ (2.64 mL, 36.4 mmol) were successively added to a solution of (*2S,3S,4R*)-2-azido-1-(*tert*-butyldiphenylsilyloxy)octadecan-3,4-diol (**62**) (7.52 g, 18.2 mmol) in CH_2Cl_2 (70 mL) at 0 °C. The solution was stirred for 30 min. The reaction mixture was poured into brine solution (150 mL). The resulting mixture was separated and the aqueous layer was extracted with EtOAc (150 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The cyclic sulfite residue was dried under high vacuum for 2 h. The resulting oil was dissolved in $CCl_4/CHCl_3/H_2O$ (1:1:1, 78 mL). $RuCl_3•3H_2O$ (0.132 mg, 0.645 mmol) and $NaIO_4$ (8.06 g, 37.5 mmol) were added to the mixture, which warmed slightly. The mixture was stirred vigorously for

2 h. Sat. $NaHSO_4$ (100 mL) and saturated aqueous $NaSO_3$ (65 mL) were added to the mixture. The mixture was separated and the aqueous phase was extracted with EtOAc (100 mL). The combined organic layers were dried over ($MgSO_4$), filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to give **63** (7.01 g, 72%) as colorless oil:[55] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70–7.67 (m, 4H), 7.49–7.4 (m, 6H), 4.99 (ddd, *J* = 11.2, 5.1, 2.9 Hz, 1H), 4.94–4.91 (m, 1H), 4.05 (dd, *J* = 11.3, 2.3 Hz, 1H), 3.90 (dd, *J* = 11.3, 5.2 Hz, 1H), 3.69 (ddd, *J* = 7.6, 5.1, 2.3 Hz, 1H), 1.99–1.89 (m, 1H), 1.79–1.72 (m, 1H), 1.67–1.57 (m, 2H), 1.47–1.28 (m, 22H), 1.10 (s, 9H), 0.89 (t, *J* = 7.0 Hz, 3H); ^{13}H NMR (100 MHz, $CDC1_3$) δ 135.8, 135.8, 132.4, 132.2, 130.7, 130.4, 128.2, 86.7, 80.1, 63.8, 59.4, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.2, 28.3, 26.9, 25.4, 22.9, 19.4, 14.3.



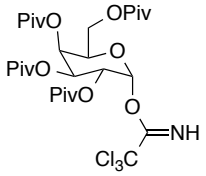
(2*S*,3*R*,4*E*)-2-Azido-1-(*tert*-butyldiphenylsilyloxy)octadecen-3-ol (64). TBAI (6.03 g, 16.3 mmol) was added to a solution of (*2S,3S,4R*)-2-azido-3,4-dioxathiolan)-1-(*tert*-butyldiphenylsilyloxy)octadecane (**63**) (7.01 g, 10.9 mmol) in toluene (220 mL). DBN (1.0 mL, 12 mmol) was added drop-wise to the solution. The reaction mixture was refluxed for 2 h. The solution was allowed to cool to rt. A mixture of $H_2O/H_2SO_{4(conc.)}$/THF (2.5 mL, 1.9 mL, 28 mL) was added to the solution and the reaction was stirred for 3 h. Saturated aqueous $NaHCO_3$ (76 mL) was added to neutralized the reaction solution to pH 7, and the resulting mixture was left to stir overnight. The solution was extracted with EtOAc (230 mL). The organic layer

was dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to give **64** (4.41 g, 71%) as a yellow oil:[55] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.67 (m, 4H), 7.47–7.38 (m, 6H), 5.74 (dt, *J* = 14.9, 6.6 Hz, 1H), 5.47–5.42 (m, 7.2 Hz, 1H), 4.2 (br s, 1H), 3.84–3.77 (m, 2H), 3.51 (ddd, *J* = 5.0, 5.0, 5.0 Hz, ,1H), 2.11 (d, *J* = 3.7 Hz, 1H), 2.02 (dt, *J* = 6.8, 6.8 Hz, 1H), 1.36–1.27 (m, 22H), 1.08 (s, 9H), 0.89 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDC1_3$) δ 135.8, 135.6, 133.0, 130.1, 128.0, 73.0, 67.1, 64.5, 42.8, 32.5, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.2, 26.9.

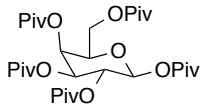


(2*S*,3*R*,4*E*)-2-Azido-(3-benzoyloxy-1-*tert*-butyldiphenylsilyloxy)octadec-4-ene (65). BzCl (2.3 mL, 20 mmol) was added to a solution of **(**2*S*,3*R*,*4E*)-2-azido-1-(*tert*-butyldiphenylsilyloxy)octadecen-3-ol (**64**) (2.70 g, 4.91 mmol) and DMAP (90 mg, 0.74 mmol) in pyridine (43 mL). The reaction mixture was stirred overnight. Ice-cold H_2O (50 mL) was added, and the solution was extracted with CH_2Cl_2 (3 X 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Flash column chromatography purification of the crude product on silica gel (petroleum ether/EtOAc, 95:5) gave **65** (2.51 g, 79%) as a slightly yellow oil: ^{1}H NMR (400 MHz, $CDC1_3$) δ 8.01 (dd, *J* = 8.0, 0.9 Hz, 2H), 7.69–7.64 (m, 4H), 7.57 (dt, *J* = 6.9, 1.6 Hz, 1H), 7.46–7.37 (m, 6H), 7.35–7.31 (m, 2H), 5.89 (dt, *J* = 15.1, 6.7 Hz, 1H), 5.67 (dd, *J* = 7.9, 4.8 Hz, 1H), 5.51 (dd, *J* = 15.4, 8.0 Hz, 1H), 3.85–3.81 (m, 1H), 3.78–3.74 (m, 2H), 2.02 (dt, *J* = 7.0, 7.0 Hz, 2H), 1.33–1.24 (m, 22H), 1.08 (s, 9H),

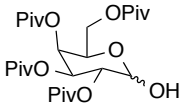
0.89 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDC1_3$) δ 165.4, 138.7, 135.8, 133.3, 133.1, 132.9, 130.3, 130.1, 130.0, 130.0, 128.6, 128.0, 128.0, 123.4, 74.5, 66.0, 63.6, 32.5, 32.1, 29.9, 29.8, 29.6, 29.6, 29.3, 28.9, 26.9, 22.9, 19.3, 14.4.



(2,3,4,6-Tetra-*O*-pivaloyl-α-D-galactopyranoside)-1-trichloroacetimidate (70). DBU (0.86 mL, 5.74 mmol) was added drop-wise to a stirred solution of *(*2,3,4,6)-tetra-*O*-pivaloyl-α/β-D-galactopyranoside-1-ol (**71**) (2.00 g, 5.74 mmol) in CH_2Cl_2 (10 mL). After 5 min, Cl_3CCN (1.2 mL, 11 mmol) was added drop-wise. The reaction was stirred at 0 °C for 2 h. Saturated aqueous NH_4Cl (50 mL) and the mixture was extracted with CH_2Cl_2 (3 X 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to give **70** (1.87 g, 53%) as a slightly yellowish viscious oil:[42] ^{1}H NMR (400 MHz, $CDC1_3$) δ 8.65 (br s, 1H), 6.60 (d, *J* = 3.5 Hz, 1H), 5.56–5.54 (m, 1H), 5.52–5.11 (m, 1H), 5.40 (dd, *J* = 10.6, 3.6 Hz, 1H), 4.47 (app ddd, *J* = 6.5, 6.5 Hz, 1H), 4.08 (dd, *J* = 11.4, 7.4 Hz, 1H), 4.03 (dd, *J* = 11.3, 6.0 Hz, 1H),1.25 (s , 9H), 1.13 (s , 9H), 1.12-1.11 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178, 177.6, 177.4, 177.0, 160.9, 93.7, 91.1, 70.0, 67.9, 67.6, 67.1, 61.8, 39.3, 39.1, 39.0, 38.9, 27.4, 27.3, 27.3, 27.2, 27.2, 27.2.

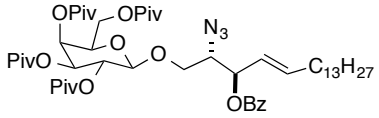


(1,2,3,4,6)-Penta-*O*-pivaloyl-β-D-galactopyranoside (72). A mixture of pyridine/CH_2Cl_2 (35/21 mL) was added to D-galactose (5.00 g, 27.8 mmol). Then pivaloyl chloride (27.0 mL, 222 mmol) was added drop-wise to the solution. The reaction was stirred at rt for 30 min. It was then placed in a preheated oil bath at 80 ^oC for 5 d. Then, the solvent was removed using high vacuum. The residue was transferred into a separatory funnel, and H_2O (100 mL) was added. The two layers were separated, and the organic layer was washed with H_2O (2 X 60 mL) and saturated aqueous $NaHCO_3$ (3 X 100 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The crude product was recrystallized from EtOH to give **72** (9.01 g, 69%) as a white solid:[40] ^1H NMR (400 MHz, $CDC1_3$) δ 5.71 (d, *J* = 8.3 Hz, 1H), 5.44–5.43 (m, 1H), 5.36 (dd, *J* = 10.2, 8.1 Hz, 1H), 5.17 (dd, *J* = 10.4, 7.4 Hz, 1H), 4.16 (dd, *J* = 10.3, 6.5 Hz, 1H), 4.09 (app ddd, *J* = 6.7, 6.7 Hz, 1H), 3.99 (dd, *J* = 10.3, 6.9 Hz, 1H), 1.28 (s, 9H), 1.19 (s, 9H), 1.16 (s, 9H), 1.12–1.12 (m, 18H); ^{13}C NMR (100 MHz, $CDC1_3$) δ 178.0, 177.4, 177.0, 176.8, 176.7, 92.4, 72.0, 71.3, 67.9, 66.9, 66.7, 60.9, 39.3, 39.0, 38.9, 27.4, 27.3, 27.2, 27.1.



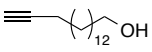
***(*2,3,4,6)-Tetra-*O*-pivaloyl-1-α/β-D-galactopyranoside-1-ol (73).** Hydrogen bromide (33% in AcOH, 9 mL) was added to a stirred solution of (1,2,3,4,6)-penta-*O*-pivaloyl-β-D-galactopyranoside (**72**) (9.00 g, 19.1 mmol) in CH_2Cl_2 (67 mL).[41] After

2 h, the reaction was quenched with sat. $NaHCO_3$ (100 mL); the two layers were separated. The organic layer was washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in acetone/H_2O solution (34 mL, 3 mL). To this solution, Ag_2CO_3 (3.65 g, 13.2 mmol) was added and mixture was stirred for 1.5 h. The mixture was filtered through a pad of celite, and the celite was washed with CH_2Cl_2 (30 mL). The crude product was purified by flash column chromatography on silica gel (Petroleum ether/ EtOAc, 90:10) to give **73** (5.85 g, 88%) as a colorless, viscious oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44–5.31 (m, 3H), 5.07–5.04 (m, 0.42H), 5.00 (dd, *J* = 10.7, 3.5 Hz, 1H), 4.68 (d, *J* = 7.1 Hz, 0.27H), 4.43–4.29 (m, 1.6H), 4.12–3.92 (m, 2.33H), 3.88–3.83 (m, 0.91H), 1.16–1.01 (m, 44H). ^{13}C NMR (100 MHz, $CDCl_3$) δ α-anomer: 178.2, 178.0, 177.4, 177.0, 68.7, 67.6, 67.5, 66.1, 61.1, 39.1, 38.8, 38.8, 38.7, 27.2, 27.1, 27.1; β-anomer: 178.1, 176.9, 71.0, 70.9, 70.7, 66.7, 61.0, 60.6, 38.9, the rest of the peaks are not disgusihable from the α-anomer.



(*2S,3R,4E*)-2-Azido-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-pivaloyl-β-galactopyranoside)octadec-4-ene (74). (2,3,4,6-Tetra-*O*-pivaloyl-α-D-galactopyranoside)-1-trichloroacetimidate (**70**) (0.38 mg, 0.58 mmol) and (*2S,3R,4E*)-2-azido-3-benzoyloxyoctadec-4-en-1-ol (**24**) (0.21 g, 0.48 mmol) were dissolved in dry CH_2Cl_2 (7 mL), and the solution was stirred in the presence of 4Å MS (300 mg) at rt for 30 min. BF_3•OEt_2 in dry CH_2Cl_2 (1.46 μL in 0.5 mL) was added

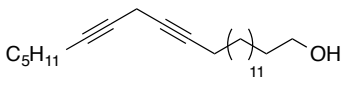
within 10 min at 0 °C. The reaction was slowly warmed to rt and stirred for 4 h. The reaction mixture was diluted with petroleum ether (15 mL) and then filtered. The filtrate was treated with saturated aqueous $NaHCO_3$ (5 mL). The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 X 5 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by combi flash column chromatography on silica gel (Acetone/hexane, 5:95) gave **74** (0.23 g, 51% β-product) as a colorless oil:[42] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.5 Hz, 2H), 7.54 (t, *J* = 7.4, Hz, 1H), 7.42 (t, *J* = 7.7, Hz 2H), 5.90 (dt, *J* = 14.3, 6.8 Hz, 1H), 5.59–5.50 (m, 2H), 5.38 (d, *J* = 1.3 Hz, 1H), 5.24 (dd, *J* = 10.5, 8.1 Hz, 1H), 5.08 (dd, *J* = 10.4, 3.1 Hz, 1H), 4.56 (d, *J* = 7.9 Hz, 1H), 4.11 (dd, *J* = 10.0, 5.7 Hz, 1H), 4.01–3.95 (m, 2H), 3.93–3.88 (m, 1H), 3.84 (dd, *J* = 10.2, 7.2 Hz, 1H), 3.64 (dd, *J* = 10.2, 4.8 Hz, 1H), 2.05 (dt, *J* = 7.0, 7.0 Hz, 2H), 1.40–1.32 (m, 3H), 1.30–1.22 (m, 28H), 1.16 (s, 9H), 1.14 (s, 9H), 1.10 (s, 9H), 0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 177.4, 177.0, 176.6, 165.1, 138.4, 133.3, 130.1, 129.9, 128.6, 123.0, 101.1, 74.8, 71.3, 71.1, 68.6, 68.0, 66.8, 64.0, 61.2, 39.2, 38.9, 38.9, 38.8, 32.5, 32.1, 29.8, 29.7, 29.5, 29.5, 29.3, 28.6, 27.3, 27.2, 22.8, 14.3.



15-Hexadecyn-1-ol (79)

1,3-Diaminopropane (13 mL) was added to a three neck round bottom flask charged with Li^+ wire (0.18 g, 25 mmol) under N_2.[56] The flask was fitted with a condenser. The mixture was stirred at rt for 30 min. A slight exothermic reaction resulted as the Li^+ dissolved, and a dark blue color was observed. The mixture was then stirred in a

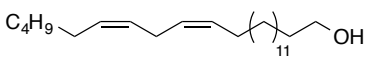
preheated oil bath at 70 °C until the blue color was discharged (approximately 1 h), affording a white suspension of lithium amide. This white color quickly changes to a magenta color. The reaction was cooled to rt, followed by addition of ^{t}BuOK (2.10 g, 16.8 mmol). The resulting dark yellow/reddish brown solution was stirred for 20 min at rt, and then 9-hexadecyn-1-ol (**78**) (1.00 g, 4.19 mmol) was added via syringe drop-wise. The reaction was stirred for 1 h and then poured into ice H_2O (42 mL). The product was extracted with petroleum ether (3 X 20 mL). Extraction of product was difficult as it was crystalizing in the process; therefore Et_2O (3 X 50 mL) was used to finish the extraction. The combined organic layers were washed with H_2O (42 ml), 10 % HCl (42 mL) and brine (42 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (Petroleum ether/EtOAc, 90:10) to give **79** (697 mg, 70%) as a white crystalline solid: mp 48.0–49.0 °C; IR (neat) 3286, 2916, 2848, 1462, 1071, 720, 684, 627 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.57 (t, *J* = 6.7 Hz, 2H), 2.13 (dt, *J* = 7.0, 2.6 Hz, 2H), 2.00 (s, 1H), 1.89 (t, *J* = 2.6 Hz, 1H), 1.50 (sept, *J* = 6.6 Hz, 4H), 1.38–1.23 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 84.9, 68.2, 63.0, 32.9, 29.8, 29.7, 29.6, 29.6, 29.2, 28.9, 28.6, 25.9, 22.8, 18.5; HRMS (ESI) calcd for $C_{16}H_{31}O$ $[M + H]^+$ *m/z* 239.2369, found 239.2374.



15,16-Tetracosadiyn-1-ol (81)

Cs_2CO_3 (4.22 g, 13.0 mmol), NaI (2.06 g, 13.7 mmol), and CuI (3.14 g, 16.4 mmol) were suspended in dry DMF (16 mL). After 5 min of stirring 15-hexadecyn-1-ol (**79**)

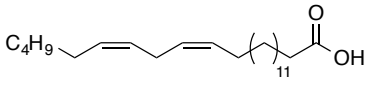
(3.00 g, 12.6 mmol) in dry DMF (5 mL) was added, drop-wise and the solution stirred for 10 min. Propargyl bromide **80** was added and the reaction mixture was stirred for 24 h (monitored by ^{1}H-NMR).[57] Saturated aqueous NH_4Cl (30 ml) was added, and the product was extracted with Et_2O (3 X 50 mL). The combined organic layers were washed with brine (3 X 15 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/Et_2O, 90:10), yielding **81** (1.60 g, 36%) as a yellowish solid. The product was light sensitive; therefore it was wrapped with aluminum foil and placed in the freezer immediately: mp 41.0–42.0 ^oC; IR (neat) 3286, 2917, 2849, 1462, 1071, 683, 628 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.61 (t, *J* = 6.6 Hz, 2H), 3.09 (dq, *J* = 2.4, 2.4 Hz, 2H), 2.12 (tt, *J* = 7.0, 2.2 Hz, 4H), 1.67 (br s, 1H), 1.54 (quin, *J* = 6.7 Hz, 2H), 1.46–1.42 (m, 4H), 1.36–1.24 (m, 24H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 80.7, 74.7, 63.2, 33.0, 31.3, 29.8, 29.8, 29.7, 29.6, 29.3, 29.1, 28.9, 28.6, 25.9, 22.4, 18.9, 18.9, 14.1, 9.9; HRMS (ESI) calcd for $C_{24}H_{43}O$ $[M + H]^+$ *m/z* 347.3308, found 347.3334.



15*Z*,18*Z*-Tetracosadien-1-ol (82)

Pd/$BaSO_4$ (30.0 mg, 10% wt) and quinoline (0.1 mL) were added to a solution of 15,16-tetracosadiyn-1-ol (**81**) (0.50 g, 1.4 mmol) in hexane.[57] The reaction was purged for 10 min with H_2 and then stirred under H_2 for 3 h. The reaction was monitored by ^{1}H-NMR. The reaction mixture was filtered through a pad of celite and the celite, was washed with hexane (20 mL). The filtrate was concentrated and

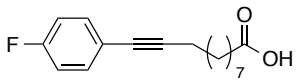
purified using flash column chromatography on silica gel (Petroleum ether/Et_2O, 95:5) to give **82** with 17% of its inseparable *E*-isomer (0.27 g, 53%) as a colorless oil: IR (neat) 2917, 2849, 1462, 1071, 683 cm^{-1}; Peak assignments for **82**: ^1H NMR (400 MHz, $CDCl_3$) δ 5.41–5.30 (m, 4H), 3.64 (t, *J* = 6.6 Hz, 2H), 2.77 (t, *J* = 6.5 Hz, 2H), 1.57 (quin, *J* = 7.3 Hz, 4H), 1.39–1.26 (m, 31H), 0.89 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.4, 128.2, 33.0, 31.8, 29.8, 29.8, 29.8, 29.7, 29.6, , 29.6, 27.5, 27.4, 26.0, 25.9, 14.3; HRMS (ESI) calcd for $C_{24}H_{47}O$ $[M + H]^+$ *m/z* 351.3621, found 351.3621.



15*Z*,18*Z*-Tetracosadienoic acid (83)

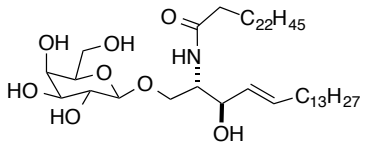
Des Martin periodinane (0.16 g, 0.38 mmol) was added to a solution of 15*Z*,18*Z*-tetracosedien-1-ol (**82**) (0.12 g, 0.35 mmol) in dry CH_2Cl_2 (1.3 mL) at 0 ^oC. The reaction was stirred at rt for 6h. TLC still showed incomplete consumption of the alcohol, and the reaction was placed in the fridge overnight. The next day, the reaction mixture was filtered through a pad of celite, the celite was washed with CH_2Cl_2 (10 mL). The combined filtrates were concentrated and purified by flash column chromatography on silica gel to provide 15*Z*,18*Z*-tetracosadienal as a colorless oil (53 mg, 43%, with *E*-isomer): IR (neat) 2920, 2850, 1700, 1650, 1510, 1100, 1050, 850 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.76 (s, 1H), 5.41–5.30 (m, 4H), 2.77 (t, *J* = 6.0 Hz, 2H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.04 (dt, *J* = 6.3, 6.4 Hz, 4H), 1.61 (quin, *J* = 6.7 Hz, 4H), 1.34–1.26 (m, 25H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.2, 130.4, 128.2, 44.1, 31.8, 29.9, 29.9, 29.8, 29.6, 29.6, , 29.6,

27.4, 27.5, 27.4, 25.9, 22.8, 22.3, 14.3; HRMS (ESI) calcd for $C_{24}H_{45}O$ $[M + H]^+$ *m/z* 349.3465, found 343.2579. $NaHPO_4$ (0.14 g, 1.0 mmol) was added to a mixture of 15*Z*,18*Z*-tetracosadienal (0.06 g, 0.18 mmol) and 2-methyl-2-butene (0.4 mL, 3.78 mmol) in ^{t}BuOH (7 mL) and H_2O (1.5 mL) at 0 ^{o}C. $NaClO_2$ (0.02 g, 0.22 mmol) was added in small portions and the mixture stirred for 6 h. One more equiv of $NaClO_2$ was added, and the reaction was left in the fridge overnight. The next day, TLC still showed remaining aldehyde; so another equiv of $NaClO_2$ was added, and the reaction mixture was stirred for 40 min at 0 ^{o}C. After this, TLC showed complete consumption of the aldehyde. Saturated aqueous Na_2SO_3 and pH7 phosphate buffer (1:1, 2 mL) was added to quench the reaction. The product was extracted with EtOAc (3 X 10 mL). The combined organic layers were washed with sat. NH_4Cl (5 mL), brine (5 mL), dried ($MgSO_4$), filtered and concentrated to give **83** (0.036 g, 51%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41–5.30 (m, 4H), 2.77 (t, *J* = 5.8 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.05 (dt, *J* = 7.0, 7.0 Hz, 4H), 1.63 (quin, *J* = 7.2 Hz, 4H), 1.40–1.26 (m, 25H), 0.91–0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 130.4, 128.2, 34.3, 32.8, 31.8, 29.9, 29.8, 29.7, 29.6, 29.5, 29.3, 27.5, 27.4, 25.9, 22.8, 14.3.



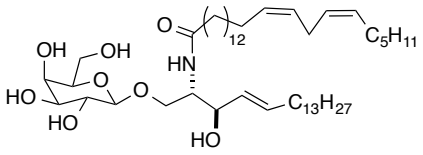
11-(*p*-Fluorophenyl)-10-undecynoic acid (87). $PdCl_2(PPh_3)_2$ (0.31 g, 0.44 mmol) was added to a solution of *p*-fluoroiodobenzene (**86**) (1.67 g, 7.52 mmol) and 10-undecynoic acid (**85**) (1.00 g, 5.49 mmol) in Et_3N (12 mL). The reaction mixture was stirred for 10 min, and then CuI (0.57 g, 3.0 mmol) was added. The resulting mixture

was stirred in a preheated oil bath at 50 °C overnight. The reaction mixture was cooled to rt, and then passed through a pad of celite to remove the generated ammonium salt. The celite was further washed with Et_2O (20 mL). The combined filtrates were concentrated and purified via flash column chromatography on silica gel (petroleum ether/EtOAc, 90:10) to give **87** (0.23 g) as a yellowish solid. ^{1}H-NMR showed extra, unknown peaks. Peak assignments for the product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (t, *J* = 5.7 Hz, 2H), 6.96 (t, *J* = 6.6 Hz, 2H), 2.39–2.33 (m, 4H), 1.64 (quin, *J* = 5.0 Hz, 2H), 1.58 (q, *J* = 5.8 Hz, 2H), 1.44 (quin, *J* = 4.5 Hz, 2H), 1.37–1.34 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.5, 162.2 ($d_{C\text{-}F}$, *J* = 246.2 Hz) 133.5, 133.5, 120.4, 115.6 ($d_{C\text{-}F}$, *J* = 21.8 Hz), 90.2, 79.8, 34.2, 29.3, 29.2, 29.1, 29.1, 28.9, 24.9, 19.5.



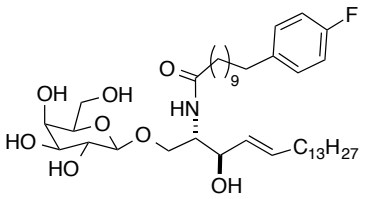
(2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(*N*-tetracosanoylamino)octadec4en-3-ol (89a). *p*-Nitrophenyltetracosaneoate (**42**) (0.04 g, 0.08 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2-amino-1-(β-galactopyranosyloxy)octadec-4-en-3-ol (**59**) (0.03 g, 0.7 mmol) in pyridine (1 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction solution was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) to give **89a** as a white solid (17.2 mg, 32%): mp 182.0–183.0 °C; $[\alpha]^{25}_D$ 1.10 (c 1.25, $CHCl_3$/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 3:2) δ 7.27 (d, *J* = 8.8 Hz, 1H), 5.69 (dt, *J* = 15.2, 7.2 Hz, 1H), 5.45 (dd, *J* = 15.0, 6.6 Hz, 1H), 4.21 (d, *J* = 6.8 Hz, 1H), 4.00 (br

s, 1H), 3.88 (br s, 1H), 3.81–3.72 (m, 2H), 3.61 (app dd, *J* = 9.8 Hz, 1H), 3.56–3.50 (m, 3H), 2.77 (t, *J* = 6.9 Hz, 2H), 2.02 (dt, *J* = 6.9, 6.9 Hz, 2H), 1.60–1.57 (m, 2H), 1.36–1.26 (m, 62H), 0.88 (t, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.5, 135.0, 130.2, 104.7, 76.0, 74.4, 73.0, 72.3, 70.0, 69.6, 62.5, 54.5, 50.1, 33.2, 32.7, 30.5, 30.5, 30.4, 30.3, 30.2, 30.2, 26.8, 23.5, 14,6; HRMS (ESI) calcd for $C_{48}H_{94}NO_8$ $[M + H]^+$ *m/z* 812.6974, found 812.6982.



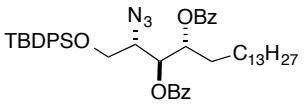
(2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(*N*-15*Z*,18*Z*-tetracosadienoylamino)octadec-4-en-3-ol (89b). *p*-Nitrophenyl 15*Z*,18*Z*-tetracosadieneoate (**84**) (28.0 mg, 0.06 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2-amino-1-(β-galactopyranosyloxy)octadec-4-en-3-ol **(59)** (25 mg, 0.60 mmol) in pyridine (1 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) to give **89b** (21 mg, 46%) as an off white solid: mp 129.0–130.0 °C; $[\alpha]^{25}_D$ – 0.68 (*c* 1.83, CH_2Cl_2/MeOH, 3:2); IR (neat) 3302, 2915, 1641, 1544, 1467, 1082, 718 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.70 (dt, *J* = 14.6, 6.6 Hz, 1H), 5.46 (dd, *J* = 15.3, 7.2 Hz, 1H), 5.41–5.29 (m, 4H), 4.21 (d, *J* = 7.4 Hz, 1H), 4.00 (ddd, *J* = 7.3, 3.7, 3.7 Hz, 1H), 3.82 (app dd, *J* = 2.6 Hz, 1H), 3.81 (dd, *J* = 11.5, 6.6 Hz, 1H), 3.75 (dd, *J* = 11.5, 5.0 Hz 1H), 3.62 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.57–3.47 (m, 3H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 2.07–1.99 (m, 6H), 1.59 (quin, *J* = 7.1 Hz, 2H), 1.40–

1.27 (m, 55H), 0.88 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.6, 135.0, 131.0, 130.3, 128.9, 104.8, 76.1, 74.5, 73.1, 72.4, 70.0, 69.7, 62.5, 54.5, 37.4, 33.2, 32.8, 32.4, 30.5, 30.5, 30.4, 30.3, 30.3, 30.2, 28.1, 28.1, 26.8, 26.5, 23.5, 23.4, 14.7; HRMS (ESI) calcd for $C_{48}H_{90}NO_8$ $[M + H]^+$ *m/z* 808.6661, found 808.6660.



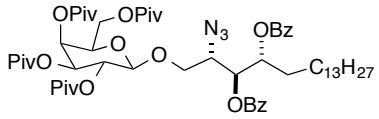
(2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(*N*-11-*p*-fluorobenzyl undecanoylamino)octadec-4-en-3-ol (89c). *p*-Nitrophenyl 11-*p*-fluorobenzyl undecanoate (**88**) (28.0 mg, 0.06 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2-amino-1-(β-galactopyranosyloxy)octadec-4-en-3-ol (**59**) (20.0 mg, 0.04 mmol) in pyridine (1 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **89c** (17 mg, 52%) as an off white crystalline solid: mp 145.0–146.0 °C; $[\alpha]^{25}_D$ 0.14 (*c* 1.36, CH_2Cl_2/MeOH, 3:2); IR (neat) 3385, 2916, 2849, 1636, 1511, 1468, 1224, 1068, 821.9, 719 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 7.13–7.09 (m, 2H), 6.93 (t, *J* = 8.8 Hz, 2H), 5.67 (dt, *J* = 14.7, 6.6 Hz, 1H), 5.44 (dd, *J* = 15.3, 7.4 Hz ,1H), 4.20 (d, *J* = 3.6 Hz, 1H), 4.17 (dd, *J* = 10.2, 4.5 Hz ,1H), 4.11 (dd, *J* = 7.4, 7.4 Hz, 1H), 3.98 (ddd, *J* = 7.3, 3.5, 3.5 Hz 1H), 3.87 (app dd, *J* = 2.6 Hz, 1H), 3.81 (dd, *J* = 11.6, 6.7 Hz, 1H), 3.73 (dd, *J* = 11.5, 5.0 Hz, 1H), 3.56 (dd, *J* = 10.2, 3.1 Hz, 1H), 3.54–3.47 (m, 3H), 2.56 (t, *J* = 7.6 Hz, 2H),

2.16 (t, J = 7.4 Hz, 2H), 2.01 (dt, J = 6.6, 6.6 Hz, 2H), 1.57 (quin, J = 6.8 Hz, 2H), 1.37–1.25 (m, 36H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.5, 162.0 (d_{C-F}, J = 240.0 Hz), 160.8, 139.4, 135.1, 130.5, 130.4, 130.1, 128.6, 115.6 (d_{C-F}, J = 21.8 Hz), 75.9, 74.3, 72.9, 72.2, 69.9, 69.5, 37.3, 35.9, 33.2, 32.8, 32.5, 30.5, 30.5, 30.4, 30.6, 30.3, 30.3, 30.2, 30.2, 30.1, 30.026.8, 23.5; HRMS (ESI) calcd for $C_{41}H_{71}FNO_8$ $[M + H]^+$ *m/z* 724.5158, found 724.5147.



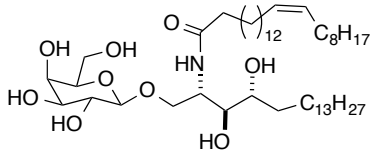
(2*S*,3*S*,4*R*)-2-Azido-(3,4-dibenzoyloxy-1-*tert*-butyldiphenylsilyloxy)octadecane (91). BzCl (6.10 mL, 52.8 mmol) was added to a solution of (*2S,3S,4R*)-2-azido-1-(*tert*-butyldiphenylsilyloxy)octadecane-3,4-diol (**62**) (5.00 g, 8.80 mmol) and DMAP (0.19 g, 1.6 mmol) in pyridine (78 mL). The reaction mixture was allowed to stir overnight. Ice-cold H_2O (120 mL) was added to the reaction to quench it. The solution was extracted with CH_2Cl_2 (3 X 100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Flash chromatography purification of the crude product on silica gel (petroleum ether/EtOAc, 95:5) gave **91** (5.43 g, 80%) as a colorless oil:[53] $[\alpha]^{25}_D$ 6.21 (*c* 1.28, CH_2Cl_2); IR (neat) 2923, 2853, 2099, 1724, 1451, 1260, 1104, 1068, 707, 503 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 7.2 Hz, 2H), 7.79 (d, J = 7.2 Hz, 2H), 7.56 (dd, J = 7.8, 1.2 Hz, 2H), 7.49 (d, J = 7.3 Hz, 3H), 7.46–7.42 (m, 1H), 7.33–7.22 (m, 8H), 7.14 (t, J = 7.3 Hz, 2H), 5.46–5.41 (m, 2H), 3.93–3.87 (m, 1H), 3.81–3.75 (m, 2H), 1.72 (dt, J = 9.1, 9.1 Hz, 2H), 1.35–1.14 (m, 24H), 0.96 (s, 9H), 0.79 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 165.2, 135.7, 135.7, 133.5, 133.2, 132.9, 132.6, 130.1, 130.0,

130.0, 129.9, 129.7, 128.7, 128.6, 128.0, 127.9, 73.3, 72.4, 64.3, 63.4, 32.1, 30.0, 29.8, 29.8, 29.7, 29.6, 29.6, 26.8, 25.6, 22.9, 19.2, 14.3; HRMS (ESI) calcd for $C_{48}H_{64}N_3O_5Si$ *m/z* 790.4610, found 790.4652.



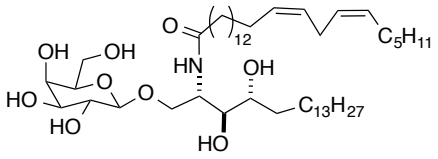
(2*S*,3*S*,4*R*)-2-Azido-3,4-dibenzoyloxy-1-(2,3,4,6-tetra-*O*-pivaloyl-β-galactopyranoside)octadecane (92). (2,3,4,6-Tetra-*O*-pivaloyl-α-D-galactopyranoside)-1-trichloroacetimidate (**70**) (0.60 mg, 0.99 mmol) and (2*S*,3*S*,4*R*)-2-azido-(3,4-dibenzoyloxy)octadecan-1-ol (**61**) (0.45 g, 0.82 mmol) were dissolved in dry CH_2Cl_2 (13 mL), and the solution was stirred in the presence of 4Å MS (600 mg) at rt for 10 min. BF_3•OEt_2 in dry CH_2Cl_2 (1.46 μL in 2 mL) was added within 10 min at –10 °C and the reaction was slowly warmed to rt and stirred for 1.5 h.[42] The reaction mixture was diluted with petroleum ether (50 mL) and then filtered. The filtrate was treated with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 X 15 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentration. Purification by flash column chromatography on silica gel (petroleum ether/ EtOAc 95:5) gave **92** (0.34 g, 39%, β-product) as a colorless oil: $[\alpha]^{25}_D$ –3.65 (*c* 1.00, CH_2Cl_2); IR (neat) 2926, 2103, 1728, 1480, 1261, 1140, 710 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.8 Hz, 4H), 7.57 (dt, *J* = 12.0, 7.4 Hz, 2H), 7.44 (t, *J* = 15.6, 7.8 Hz, 4H), 5.49– 5.44 (m, 2H), 5.37 (d, *J* = 3.1 Hz, 1H), 5.22 (dd, *J* = 10.5, 8.1 Hz, 1H), 5.05 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.56 (d, *J* = 7.9 Hz, 1H), 4.08–4.02 (m, 2H), 3.98–3.90

(m, 4H), 1.88–1.80 (m, 2H), 1.43–1.32 (m, 3H), 1.29–1.20 (m, 30H), 1.14 (s, 9H), 1.09 (s, 9H), 1.08 (s, 9H), 0.86 (t, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDC1_3$) δ 177.9, 177.4, 177.0, 176.5, 133.7, 133.4, 130.0, 129.9, 129.8, 129.5, 128.7, 128.6, 100.9, 73.0, 71.2, 71.1, 68.7, 68.6, 66.7, 61.4, 61.1, 38.9, 38.9, 38.8, 32.0, 30.3, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 25.4, 22.8, 14.2; HRMS (ESI) calcd for $C_{58}H_{88}N_3O_{14}$ $[M + H]^+$ *m/z* 1050.6261, found 1050.6300.

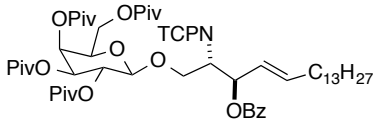


(2*S*,3*S*,4*R*)-1-(β-D-Galactopyranosyloxy)-2-(*N*-15*Z*-tetracosenoylamino)octadecan-3,4-diol (93a). *p*-Nitrophenyl 15*Z*-tetracosenoate (**77**) (33 mg, 0.07 mmol) was added to a solution of (2*S*,3*S*,4*R*)-2-amino-1-(β-galactopyranosyloxy)octadecan-3,4-diol (**60**) (30 mg, 0.06 mmol) in pyridine (1 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **93a** (19 mg, 38%) as a white solid: mp 169.0–171.0 °C; $[\alpha]^{25}_D$ 7.38 (*c* 0.82, $CHCl_3$/MeOH, 3:2); IR (neat) 3330, 2917, 2849, 1637, 1545, 1465, 1081, 1049, 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.38–5.30 (m, 2H), 4.24–4.20 (m, 2H), 4.16 (dd, J = 10.3, 4.6 Hz, 1H), 3.87 (app dd, J = 2.7 Hz, 1H), 3.82 (dd, J = 11.6, 6.8 Hz, 1H), 3.72 (ddd, J = 11.1, 11.1, 4.8 Hz, 2H), 3.61–3.47 (m, 5H), 2.20 (t, J = 7.6 Hz, 2H), 2.01 (dt, J = 6.8, 6.8 Hz, 2H), 2.04–2.00 (m, 2H), 1.68–1.57 (m, 3H), 1.53–1.51 (m, 1H), 1.47–1.27 (m, 56H), 0.88 (t, J = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.5, 130.7, 104.8, 76.2, 75.5,

74.5, 73.3, 72.4, 70.1, 70.1, 51.5, 37.4, 33.6, 32.7, 30.6, 30.6, 30.5, 30.4, 30.3, 30.3, 30.2, 30.1, 28.0, 26.8, 26.7, 23.5, 14.6; HRMS (TOF) *m/z* calcd for $C_{48}H_{94}NO_9$ $[M - H]^+$ 828.6923, found 828.6915.

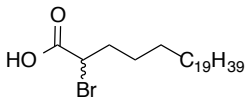


(2*S*,3*S*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(*N*-15*Z*,18*Z*-tetracosadienoylamino)octadecan-3.4-diol (93b). *p*-Nitrophenyl 15*Z*,18*Z*-tetracosadieneoate (**84**) (28 mg, 0.06 mmol) was added to a solution of (2*S*,3*S*,4*R*)-2-amino-1-(β-galactopyranosyloxy)octadecan-3,4-diol (**60**) (27.0 mg, 0.06 mmol) in pyridine (1 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **93b** (19 mg, 40%) as a white solid: mp 169.0–171.0 °C; $[\alpha]^{25}_D$ 8.81 (c 1.86, $CHCl_3$/MeOH, 3:2); IR (neat) 3302, 2918, 2850, 1637, 1467, 1082, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.41–5.29(m, 4H), 4.23–4.22 (m, 2H), 4.14–4.12 (m, 1H), 3.87 (app dd, *J* = 2.0 Hz, 1H), 3.82 (dd, *J* = 11.6, 6.8 Hz, 1H), 3.72 (ddd, *J* = 13.4, 13.4, 4.7 Hz, 2H), 3.61–3.47 (m, 5H), 2.77 (t, *J* = 6.1 Hz, 2H), 2.20 (t, *J* = 7.4 Hz, 2H), 2.05 (dt, *J* = 6.4, 6.4 Hz, 2H), 2.04–2.00 (m, 2H), 1.68–1.51 (m, 5H), 1.44–1.27 (m, 49H), 0.88 (t, *J* = 4.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ $CDCl_3/CD_3OD$, 3:2) δ 175.6, 131.0, 128.9, 76.3, 75.5, 74.5, 73.3, 72.4, 70.1, 70.1, 62.6, 51.5, 37.4, 33.2, 32.8, 32.4, 30.7, 30.6, 30.4, 30.3, 30.3, 30.2, 28.1, 28.1, 26.8, 26.8, 26.5, 23.5, 23.4, 14.7; HRMS (TOF) *m/z* calcd for $C_{48}H_{92}NO_9$ $[M - H]^+$ 826.6767, found 826.6777.



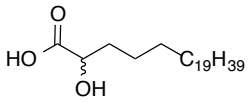
(*2S,3R,4E*)-3-Benzoyloxy-2-(*N*-tetrachlorophthalimido)-1-(2,3,4,6-tetra-*O*-pivaloyl-β-galactopyranoside)octadec-4-ene (104). (2,3,4,6-Tetra-*O*-pivaloyl-α-D-galactopyranoside)-1-trichloroacetimidate (**70**) (0.53 mg, 0.81 mmol) and (*2S,3R,4E*)-3-benzoyloxy-2-(*N*-tetrachlorophthalimido)octadec-4-en-1-ol (**95**) (0.69 g, 1.03 mmol) were dissolved in dry CH_2Cl_2 (14 mL), and the solution was stirred in the presence of 4Å MS (500 mg) at rt for 30 min. BF_3•OEt_2 in dry CH_2Cl_2 (1.46 μL in 0.5 mL) was added within 10 min at –30 °C. The reaction was stirred at this temperature for 2.5 h, diluted with CH_2Cl_2 (20 mL) and then filtered. The filtrate was extracted with H_2O (3 X 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) gave **104** (0.64 g, 68% β-product) as a fluffy, clear solid: mp 61.7–62.9 °C; $[\alpha]^{25}_D$ 8.84 (c 1.05, CH_2Cl_2); IR (neat) 2925, 1724, 1480, 1363, 1276, 1134, 739, 712 cm^{-1}; ^1H NMR (400 MHz, $CDC1_3$) δ 8.00 (d, *J* = 7.2 Hz, 2H), 7.54 (tt, *J* = 6.9, 1.2 Hz, 1H), 7.42 (t, *J* = 7.8, Hz, 2H), 5.93 (dd, *J* = 8.9, 8.9 Hz, 1H), 5.85 (dt, *J* = 14.8, 7.2 Hz, 1H), 5.34 (dd, *J* = 15.3, 8.5 Hz, 1H), 5.26 (s, 1H), 5.04–5.00 (m, 2H), 4.71 (ddd, *J* = 10.0, 10.0, 4.1 Hz, 1H), 4.53–4.47 (m, 2H), 3.91–3.81 (m, 3H), 3.73 (ddd, *J* = 12.8, 12.8, 7.2 Hz, 1H), 1.95–1.79 (m, 2H), 1.28–0.93 (m, 58H), 0.84 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDC1_3$) δ 177.6, 177.3, 176.7, 176.5, 133.4, 130.0, 129.9, 129.8, 128.6, 127.4, 124.3, 101.0, 72.7, 71.0, 70.9, 68.6, 66.5, 65.4, 60.5, 54.4, 39.0, 38.8, 38.7, 32.2, 32.0, 29.8, 29.7, 29.7, 29.5, 29.5, 29.5, 27.1,

27.1, 22.8, 14.2; HRMS (TOF) *m/z* calcd for $C_{59}H_{81}Cl_4NO_4$ $[M - Cl_4]^+$ 1029.5606, found 1048.4062.; **α-anomer**: ^{1}H NMR (400 MHz, $CDC1_3$) δ 8.04 (d, *J* = 7.2 Hz, 2H), 7.54 (t, *J* = 7.4 Hz, 1H), 7.42 (t, *J* = 7.8, Hz 2H), 5.99–5.84 (m, 2H), 5.38 (dd, *J* = 15.4, 8.5 Hz, 1H), 5.21 (ddd, *J* = 3.6, 3.6, 3.6 Hz, 1H), 4.94 (dd, *J* = 6.2, 1.8 Hz, 1H), 4.91 (s, 1H), 4.84 (ddd, *J* = 10.3, 10.3, 4.6 Hz, 1H), 4.75 (d, *J* = 1.8 Hz, 1H), 4.49 (dd, *J* = 10.6, 10.6 Hz, 1H), 4.31 (dd, *J* = 12.0, 3.3 Hz, 1H), 4.14–4.06 (m, 1H), 4.03 (dd, *J* = 12.1, 12.1 Hz, 1H), 3.92 (dd, *J* = 10.6, 4.6 Hz, 1H), 1.97–1.83 (m, 2H), 1.33–1.25 (m, 12H), 1.17–1.14 (m, 30H), 1.06–1.04 (m, 16H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDC1_3$) δ 177.9, 177.5, 177.3, 177.2, 130.0, 129.9, 128.7, 127.4, 124.3, 105.0, 81.8, 80.1, 75.6, 73.2, 69.0, 63.0, 62.8, 53.4, 39.0, 38.9, 38.8, 38.6, 32.3, 32.1, 29.9, 29.6, 29.6, 29.6, 29.1, 29.0, 27.3, 27.3, 27.1, 26.9, 22.9, 14.3.

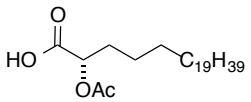


(±)-α-bromotetracosanoic acid (117). Br_2 (1.28 mL, 25.3 mmol) was added slowly over 10 min to a mixture of red phosphorus (0.25 g, 8.2 mmol) and tetracosanoic acid (**41**) (2.33 g, 6.32 mmol) at rt. The reaction mixture was then placed in a preheated oil bath at 95 °C and stirred for 6 h. ^{1}H NMR showed incomplete consumption of starting material. Br_2 (0.5 mL) was added, and the mixture was stirred for another 2 h, after which there was no starting material. H_2O (20 mL) was then added, and the reaction was stirred for 20 min at 95 °C. The reaction was cooled to rt and then extracted with EtOAc (3 X 75 mL). The combined

organic layers were concentrated, and the residue recrystallized from hexane to give **117** as a brownish solid (2.02 g, 71%):[45] ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.67 (br s, 1H), 4.24 (dd, *J* = 7.5, 7.5 Hz, 1H), 2.14–2.06 (m, 1H), 2.05–1.94 (m, 1H), 1.54–1.46 (m, 1H), 1.43–1.26 (m, 37H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 45.6, 34.9, 32.2, 29.9, 29.8, 29.7, 29.6, 29.5, 29.0, 27.8, 27.4, 22.9, 14.3.

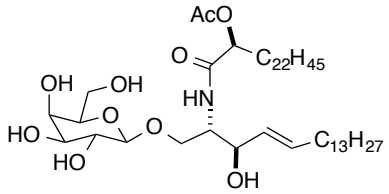


(±)-α-hydroxytetracosanoic acid (118). 2 M aq. NaOH (23 mL) was added to (±)-α-bromotetracosanoic acid (**117**) (2.0 g, 4.5 mmol), and the mixture was allowed to stir at 85 °C for 30 h. The reaction was cooled to rt and then acidified to pH 2 with aqueous 2 M HCl. The product was extracted with Et_2O (5 X 100 mL). The combined extracts were washed with H_2O (100 ML), dried (Na_2SO_4), filtered and concentrated. The crude product was recrystallized from acetone to give (±)-α-hydroxytetracosanoic acid as a white solid (0.88 g, 51%):[45] ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (dd, *J* = 6.0, 4.4 Hz, 1H), 1.83–1.74 (m, 1H), 1.71–1.62 (m, 1H), 1.45 (quin, *J* = 7.2 Hz, 2H), 1.35–1.28 (m, 38H), 0.89 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 71.6, 35.5, 33.1, 30.8, 30.8, 30.7, 30.6, 30.5, 26.2, 23.8, 14.8.



(*S*)-α-Acetoxytetracosanoic acid (119). Amano lipase PS (0.22 g) was suspended in a solution of (±)-α-hydroxytetracosanoic acid (**118**) (0.23 g, 0.60 mmol) in THF (2.3 mL). BHT (2.07 mg) in vinyl acetate (2.3 mL) was added, and the mixture was

stirred in a preheated oil bath at 65 °C for 20 h. The reaction mixture was filtered through a pad of celite, and the celite was further washed with THF (30 mL). The combined filtrates were concentrated, and the resdue purified by flash column chromatography on silica gel (petroleum ether/EtOAc, 80:20%) gave **120** (0.02 g, 17%, considering only the *S*-enantiomer reacted) as a white solid:[45] Lit $[\alpha]^{25}_{D}$ –8.9 (c 1.0, $CHCl_3$);[58] $[\alpha]^{25}_{D}$ –7.85 (c 1.0, $CHCl_3$), IR (neat) 2915, 2848, 1725, 1464, 1224, 1079, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.01 (dd, *J* = 6.7, 6.7 Hz, 1H), 2.14 (s, 3H), 1.89–1.83 (m, 2H), 1.46–1.39 (m, 3H), 1.34–1.26 (m, 37H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 170.9, 72.1, 32.2, 31.2, 29.9, 29.9, 29.8, 29.8, 29.6, 29.3, 25.3, 22.9, 20.8, 14.3.

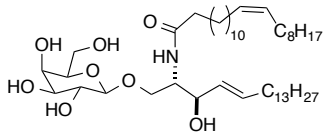


(2*S*,3*R*,4*E*)-2*S*-(*O*-Acetyl-*N*-tetracosanoylamino)-1-(β-D-galactopyranosyloxy) octadec-4-en-3-ol (121a). *p*-Nitrophenyl α-*O*-acetyl tetracosanoate (**120**) (26 mg, 0.05 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2-amino-1-(β-galactopyranosyloxy)octadec-4-en-3-ol (**59**) (19 mg, 0.04 mmol) in pyridine (0.8 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **121a** (14 mg, 38%) as a white solid: mp 155.6–156.7 °C; $[\alpha]^{25}_{D}$ –0.792 (*c* 1.42, $CHCl_3$/MeOH); IR (neat) 2917, 2850, 1724, 1651, 1469, 1242, 1074, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 7.70 (d, *J* = 9.2 Hz,

1H), 5.70 (dt, *J* = 14.9, 6.6 Hz, 1H), 5.43 (dd, *J* = 15.4, 7.5 Hz, 1H), 4.90 (dd, *J* = 6.1, 6.1 Hz, 1H), 4.28 (d, *J* = 7.2 Hz, 1H), 4.20 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.13 (dd, *J* = 7.8, 7.8 Hz, 1H), 3.96 (dddd, *J* = 8.6, 8.6, 5.1, 5.1 Hz, 1H), 3.87 (app dd, *J* = 2.7 Hz, 1H), 3.82 (dd, *J* = 11.5, 6.7 Hz, 1H), 3.73 (dd, *J* = 11.5, 5.0 Hz, 1H), 3.63 (dd, *J* = 10.4, 3.1 Hz, 1H), 3.58–3.49 (m, 3H), 2.13 (m, 3H), 2.01 (dt, *J* = 7.7, 7.7 Hz, 2H), 1.42–1.26 (m, 64H), 0.88 (t, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 172.1, 172.0, 135.4, 130.3, 104.7, 76.1, 75.3, 74.5, 72.5, 72.4, 70.0, 69.3, 62.4, 54.5, 54.4, 33.3, 33.0, 32.9, 30.6, 30.6, 30.5, 30.4, 30.4, 30.3, 30.1, 26.2, 23.6, 21.3, 14.8; HRMS (TOF) *m/z* calcd for $C_{50}H_{96}NO_{10}$ $[M + H]^+$ 870.7029, found 870.6997.

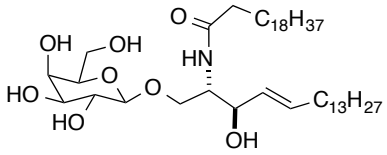
NaOMe (6.4 μL, 0.03 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2*S*-(*O*-acetyl-*N*-tetracosanoylamino)-1-(β-D-galactopyranosyloxy)octadec-4-en-3-ol (**121a**)**.** (14 mg, 0.02 mmol) in a mixture of CH_2Cl_2/MeOH (0.3:0.3 mL). The reaction was stirred at rt for 1 h, then neutralized with dowex (H^+ resin). The mixture was filtered through a pad of celite, and the celite was washed with a 1:1 mixture of $CHCl_3$ and MeOH (10 mL). The filtrate was concentrated and purified using flash column chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) to give (2*S*,3*R*,4*E*)-(β-D-galactopyranosyloxy)-2*S*-(hydroxy-*N*-tetracosanoylamino)octadec-4-en-3-ol (9.4 mg, 71%) as a white solid: mp 181.0–182.0 °C; $[\alpha]^{25}_D$ –3.03 (*c* 0.94, $CHCl_3$/MeOH); IR (neat) 3382, 2918, 2850, 1724, 1468, 1072, 720 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 7.57 (d, *J* = 8.3 Hz, 1H), 5.73 (dt, *J* = 14.5, 6.6 Hz, 1H), 5.46 (dd, *J* = 15.4, 6.9 Hz, 1H), 4.22–4.19 (m, 2H), 4.08 (dd, *J* = 10.1, 5.6 Hz, 1H), 3.86–3.85 (m, 1H), 3.82

(dd, *J* = 11.7, 6.8 Hz, 1H), 3.76–3.67 (m, 2H), 3.55–3.47 (m, 3H), 2.03 (dt, *J* = 6.5, 6.5 Hz, 2H), 1.83–1.74 (m, 1H), 1.59–1.50 (m, 1H), 1.36–1.26 (m, 66H), 0.88 (t, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 135.2, 129.6, 104.8, 76.1, 74.4, 72.9, 72.6, 70.0, 69.3, 62.5, 54.5, 35.5, 33.3, 32.8, 30.6, 30.5, 30.5, 30.4, 30.3, 30.1, 26.2, 23.6, 14.8; HRMS (TOF) *m/z* calcd for $C_{48}H_{94}NO_9$ $[M - H]^+$ 826.6778, found 826.6788.



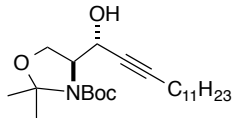
(2*S*,3*R*,4*E*)-1-(β-D-Galactopyranosyloxy)-2-(*N*-15*Z*-docosenoylamino)octadec-4-en-3-ol (121b). *p*-Nitrophenyl erucicoate (**116**) (29.5 mg, 0.06 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2-amino-1-(β-galactopyranosyloxy)octadec-4-en-3-ol (**59**) (24 mg, 0.05 mmol) in pyridine (0.9 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **121b** (23 mg, 55%) as an off white solid: mp 166.1–167.2 °C; $[\alpha]^{25}_D$ 0.877 (*c* 1.02, $CHCl_3$/MeOH, 3:2); IR (neat) 3278, 2917, 2850, 1645, 1551, 1468, 1069, 717 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 7.47 (d, *J* = 9.1 Hz, 1H), 5.69 (dt, *J* = 14.9, 6.6 Hz, 1H), 5.44 (dd, *J* = 15.4, 7.4 Hz, 1H), 5.37–5.30 (m, 2H), 4.21 (d, *J* = 7.4 Hz, 1H), 4.17 (dd, *J* = 10.2, 4.4 Hz, 1H), 4.10 (dd, *J* = 7.4, 7.4 Hz, 1H), 4.00 (dddd, *J* = 7.6, 3.6, 3.6, 3.6 Hz, 1H), 3.87 (app dd, *J* = 2.8 Hz, 1H), 3.81 (dd, *J* = 11.6, 6.7 Hz, 1H), 3.73 (dd, *J* = 11.6, 5.0 Hz, 1H), 3.60–3.47 (m, 4H), 2.17 (t, *J* = 7.4 Hz, 2H), 2.01 (dt, *J* = 6.9, 6.9 Hz, 6H), 1.58 (quin, *J* = 6.8 Hz, 2H), 1.38–1.26 (m, 50H), 0.88 (t, *J* = 6.3 Hz,

6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.5, 135.1, 133.3, 130.7, 130.7, 130.2, 104.7, 76.0, 74.3, 72.9, 72.3, 69.9, 69.6, 62.4, 54.3, 37.4, 33.3, 32.9, 32.8, 30.7, 30.6, 30.5, 30.5, 30.4, 30.3, 30.2, 30.2, 30.2, 28.1, 26.8, 23.5, 14.7; HRMS (TOF) *m/z* calcd for $C_{46}H_{88}NO_8$ $[M + H]^+$ 782.6504, found 782.6468.

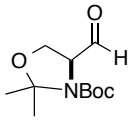


(2*S*,3*R*,4*E*)-1-(β-D-galactopyranosyloxy)2-(*N*-elcosanoylamino)octadec-4-en-3-ol (121c). *p*-Nitrophenyl elcosanoate (**114**) (12.0 mg, 0.03 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2-amino-1-(β-galactopyranosyloxy)octadec-4-en-3-ol **(59)** (10 mg, 0.03 mmol) in pyridine (0.4 mL). The mixture was stirred in a preheated oil bath at 40 °C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **121c** (7.5 mg, 44%) as a white solid: mp 166.1–167.7 °C; $[\alpha]^{25}_D$ 1.72 (*c* 0.86, $CHCl_3$/MeOH, 3:2); IR (neat) 3297, 2916, 2849, 1645, 1545, 1467, 1083, 718 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.67 (dt, *J* = 14.8, 6.6 Hz, 1H), 5.43 (dd, *J* = 15.4, 7.4 Hz, 1H), 4.19 (d, *J* = 7.3 Hz, 1H), 4.16 (dd, *J* = 10.2, 4.5 Hz, 1H), 4.09 (dd, *J* = 7.5, 7.5 Hz, 1H), 3.97 (dddd, *J* = 3.5, 3.5, 3.5, 3.5 Hz, 1H), 3.86 (app dd, *J* = 2.7 Hz, 1H), 3.80 (dd, *J* = 11.5, 6.7 Hz, 1H), 3.73 (dd, *J* = 11.5, 5.0 Hz, 1H), 3.58–3.46 (m, 4H), 2.15 (t, *J* = 7.4 Hz, 2H), 2.00 (dt, *J* = 6.8, 6.8 Hz, 2H), 1.57 (quin, *J* = 6.5 Hz, 2H), 1.38–1.24 (m, 54H), 0.86 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.6, 135.1, 130.2, 104.7, 76.0, 74.4, 72.9, 72.3, 69.9, 69.6, 62.4, 54.3, 37.4, 33.3,

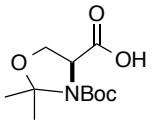
32.8, 30.6, 30.4, 30.4, 30.3, 30.2, 30.2, 28.1, 26.7, 23.5, 14.7; HRMS (TOF) *m/z* calcd for $C_{44}H_{86}NO_8$ $[M + H]^+$ 756.6348, found 756.6345.



2*S*-(*N*-*tert*-Butyl carboxylate dimethyl oxazolidine)hexadec-4-yn-3-ol (124). *n*-BuLi (2.5 M in Hex, 0.46 mL, 1.15 mmol) was added drop-wise to a solution of 1-tridecyne (**126**) (0.22 mL, 0.96 mmol) in THF (1 mL) at -20 ^oC. After 10 min Garner aldydehe (**125**) (0.200 g, 0.87 mmol) in THF (0.5 mL) was added drop-wise.[59] The solution was stirred at -20 ^oC for 7 h. Saturated aqueous NH_4Cl (5 mL) was added. The two layers were separated, and the aqueous phase was extracted with Et_2O (2 X 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to give **124** as a colorless oil (17 mg, 5% pure isolated product, 85.9 mg **124** with aldehyde): ^1H NMR (400 MHz, $CDCl_3$) δ 4.71–4.50 (m, 2H), 4.12–4.05 (m, 2H), 3.91 (br s, 1H), 2.19 (dt, *J* = 7.0, 1.5 Hz, 2H), 1.58–1.54 (m, 3H), 1.51–1.41 (m, 14H), 1.36–1.31 (m, 2H), 1.29–1.26 (m, 14H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 95.2, 86.9, 81.4, 78.1, 76.9, 65.3, 64.4, 63.1, 32.1, 29.8, 29.7, 29.5, 29.3, 29.1, 28.8, 28.6, 26.0, 25.6, 22.9, 19.0, 14.3.

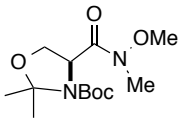


Garner's aldehyde (125). $LiAlH_4$ (0.24 g, 6.2 mmol) was added to (2*S*)-*tert*-butyl carboxylate dimethyl oxazolidine-*N*-methoxy-*N*-methyl propanamide (**131**) (0.90 g, 3.1 mmol) in THF (14 mL) at 0 ^oC.[46] After 10 min TLC showed complete consumption of **131**. $NaHCO_3$ (5% aq., 10 mL) was added drop-wise slowly. The two layers were separated and the aqueous layer was further extracted with EtOAc (3 X 20 mL). The combined EtOAc layers were dried ($MgSO_4$), filtered and concentrated. Purification via flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) gave Garner's aldehyde as a colorless oil (0.51 g, 72%): ^1H NMR (400 MHz, $CDCl_3$) δ 9.60 (br s, 0.32H), 9.54 (br s, 0.57H), 4.32 (br s, 0.4H), 4.19–4.18 (m, 0.58H), 4.11–4.02 (m, 2H), 1.64–1.42 (m, 15H).



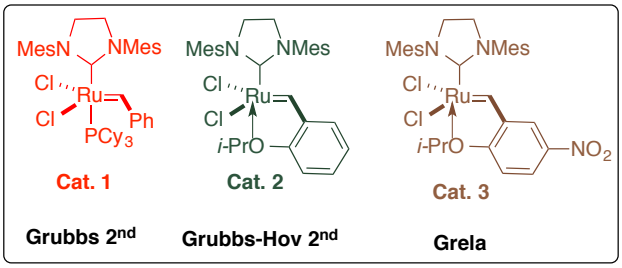
(2*S*)-*N*-*tert*-Butylcarboxylatedimethyloxazolidinepropanoic acid (130). 2,2-Dimethoxy propane (2.5 mL) was added to a solution of Boc-L-serine (**127**) (2.00 g, 9.75 mmol) and PTSA (53.0 mg) in THF (10 ml). The solution was stirred under reflux conditions for 24 h. The reaction was neutralized to pH 7 with solid Na_2CO_3. The mixture was filtered, and the filtrate was concentrated. Purification by flash column chromatography on silca gel (CH_2Cl_2/MeOH, 97:3) provided **125** as a brownish yellow oil (1.12 g, 47%): ^1H NMR (400 MHz, $CDCl_3$) δ 4.50 (app d, J = 6.2 Hz, 0.5H), 4.41 (dd, J = 6.7, 2.5 Hz, 0.5H), 4.31(app dd, J = 4.7 Hz, 0.5H), 4.19 (dd,

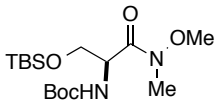
J = 9.2, 9.2 Hz, 0.5H), 4.14–4.10 (m, 1H), 1.67 (s, 1.5H), 1.61 (s, 1.5H), 1.53–1.51 (m, 8H), 1.44–1.42 (m, 4H).



(2*S*)-*N*-*tert*-Butylcarboxylatedimethyloxazolidine-*N*-methoxy-*N*-methyl propanamide (131). *N,O*-Dimethylhydrochloride (0.46 g, 4.7 mmol) was added to a solution of **(**2*S*)-*tert*-butylcarboxylatedimethyloxazolidinepropanoic acid (**130**) (1.12g, 4.55 mmol) in dry CH_2Cl_2 (18 mL) at −15 ^oC. NMM (0.51 mL, 4.66 mmol) was then added drop-wise, and the reaction was stirred for 5 min. EDC•HCl (0.90 g, 4.69 mmol) was added in five portions over 20 min.[60] After 1 h aqueous HCl (1 M, 5 mL) was added to quench the reaction. The two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (4 mL) and H_2O (4 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) to yield **131** (0.90 g, 68%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 4.79 (dd, *J* = 7.1, 2.8 Hz, 0.5H), 4.71 (dd, *J* = 7.3, 3.6 Hz, 0.5H), 4.20–4.15 (m, 1H), 3.97–3.90 (m, 1H), 3.71 (d, *J* = 9.0 Hz, 3H), 3.20 (s, 3H), 1.69–1.67 (m, 3H), 1.53 (d, *J* = 9.0 Hz, 3H), 1.48 (s, 4.5H), 1.40 (s, 4.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 170.8, 152.4, 151.5, 95.2, 94.6, 80.7, 80.2, 66.3, 66.1, 61.4, 61.4, 58.1, 57.9, 37.8, 32.7, 28.6, 28.6, 25.9, 25.7, 24.9, 24.8.

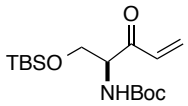
Catalysts used in olefin cross metathesis



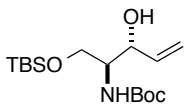


(*2S*)-*N*-Methoxy-*N*-methyl 3-(*tert*-butyldimethylsilyloxy)-*2S*-(*N*-tert-butyloxycarbonylamino)propanamide (133). Imidazole (2.64 g, 38.7 mmol) was added to solution of (2*S*)-*N*-methoxy-*N*-methyl-*2*-(*N*-*tert*-butyloxycarbonylamino)propanamide (**132**) (8.01 g, 32.3 mmol) in dry DMF (64 mL). TBDMSCl (5.10 g, 33.9 mmol) was then added, and the reaction was allowed to stir for 2 h. Saturated aqueous NH_4Cl (150 mL) was added, and the product was extracted with Et_2O (3 X 100 mL). The combined Et_2O extracts were washed with H_2O (3X 150 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to give **133** as a colorless oil (10.3 g, 88%):[47] $[\alpha]^{25}_D$ 21.57 (*c* 0.2, CH_2Cl_2); IR (neat) 2930, 1714, 1663, 1471, 1390, 1365, 1251, 1167, 1110, 990, 836, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.34 (d, *J* = 8.2 Hz, 1H), 4.75 (app ddd, *J* = 3.9, 3.9 Hz, 1H), 3.85 (dd, *J* = 10.0, 4.7 Hz, 1H), 3.78 (dd, *J* = 9.9, 5.2 Hz, 1H), 3.75 (s, 3H), 3.21 (s, 3H), 1.43 (s, 9H), 0.86 (s, 9H), 0.03 (br s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$)

δ 171.0, 155.6, 79.8, 63.7, 61.7, 52.7, 32.4, 28.6, 26.0, 18.5, 0.2, -0.5; HRMS (ESI) calcd for $C_{16}H_{35}NO_5Si$ $[M + H]^+$ *m/z* 363.2310, found 363.2292.

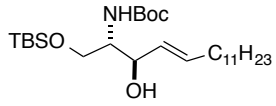


(2*S*)-1-(*tert*-Butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4-penten-3-one (135). Vinyl magnesium bromide (**134**) (1 M in THF, 113 mL, 113 mmol) was added drop-wise via an addition funnel to a solution of *N*-methoxy-*N*-methyl (2*S*)-3-(*tert*-butyldimethylsilyloxy)-2 -(*N*-*tert*-butyloxycarbonylamino)proanamide (**133**) (10.3 g, 28.3 mmol) in THF (86 mL) at 0 ^oC. The reaction was slowly warmed to rt and allowed to stirred for 1h. The reaction was cooled to 0 ^oC and then HCl (0.1 M aq., 30 mL) was added to quench the reaction. The product was extracted with Et_2O (3 X 75 mL). The combined Et_2O layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash column chromatography on silica gel (Petreoleum ether/EtOAc, 95:5) yielded **135** (1.90 g, 20%) as a colorless oil:[47] ^1H NMR (400 MHz, $CDCl_3$) δ 6.56 (dd, *J* = 17.4, 10.6 Hz, 1H), 6.43 (dd, *J* = 17.4, 0.9 Hz, 1H), 5.83 (app d, *J* = 10.6 Hz, 1H), 5.51 (d, *J* = 6.8 Hz, 1H), 4.59 (ddd, *J* = 7.5, 3.9, 3.9 Hz, 1H), 4.00 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.85 (dd, *J* = 10.3, 4.3 Hz, 1H), 1.44 (s, 9H), 0.83 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.1, 155.4, 133.4, 129.6, 80.0, 63, 7, 59.8, 28.6, 26.0, 18.4, -5.4, -5.4.



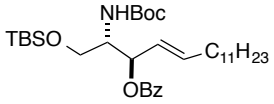
(2*S*,3*R*)-1-(*tert*-Butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4-

penten-3-ol (136). LiAl(*Ot*-Bu)$_3$H (3.22 g, 12.7 mmol) was added to a solution of (2*S*)-1-(*tert*-butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4-pentene-3-one (**135**) (1.19 g, 5.76 mmol) in dry EtOH (23 mL) at −78 °C under N_2. After being stirred for 5 h at −78 °C, the mixture was quenched slowly with aqueous 1 N HCl (19 mL). The mixture was then diluted with H_2O (50 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (3 X 75 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5 to 90:10) gave impure **136** (1.33 g) as colorless oil, which was carried forward to the next step without further purification.



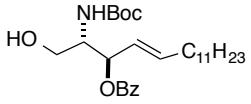
(2*S*,3*R*,4*E*)-1-(*tert*-Butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonyl amino) hexadecen-3-ol (138). Pathway 1: Cat.**1** (34 mg, 0.04 mmol) was added to a solution of (2*S*,3*R*)-1-(*tert*-butyldimethylsilyloxy)-2*S*-(*N*-*tert*-butyloxycarbonylamino)-4-penten-3-ol (**136**) (1.33 g, 4.01 mmol) and 1-tridecene (2.9 g, 3.8 mL, 16 mmol) in dry CH_2Cl_2 (60 mL) at rt.[47] The reaction was allowed to stir overnight. The solvent was evaporated, and the crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to provide **138** (0.22 g, 44%) as a slightly brownish oil.; Pathway 2: LiAl(*Ot*-Bu)$_3$H (4.00 g, 15.7 mmol) was added to (2*S,*4*E*)-1-(*tert*-butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)hexadecen-3-one (**141**) (3.45 g, 7.12 mmol) in dry EtOH (107 mL) at −78 °C under N_2.[61] After being stirred for 5 h, aqueous HCl (1 M, 30 mL)

was slowly added. The mixture was then diluted with H_2O (50 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (3 X 75 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5 to 90:10) gave **138** as a colorless oil (0.77 g, 29%): $[\alpha]^{25}_D$ 8.80 (*c* 0.8, CH_2Cl_2); IR (neat) 2924, 2854, 1698, 1497, 1365, 1365, 1252, 1170, 1096, 965, 835, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.75 (dt, *J* = 14.7, 6.7 Hz, 1H), 5.51 (dd, *J* = 15.5, 6.0 Hz, 1H), 5.22 (d, *J* = 7.4 Hz, 1H), 4.19 (app ddd, *J* = 5.8, 5.8, 5.8 Hz, 1H), 3.94 (dd, *J* = 10.3, 2.9 Hz, 1H), 3.75 (app d, *J* = 8.8 Hz, 1H), 3.57 (d, *J* = 4.3 Hz, 1H), 3.30 (app d, *J* = 7.8 Hz, 1H), 2.05 (dt, *J* = 7.0, 7.0 Hz, 1H), 1.45 (s, 9H), 1.37 (quin, *J* = 6.6 Hz, 2H), 1.32–1.26 (m, 16H), 0.89–0.87 (m, 12H), 0.07 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 133.3, 129.7, 79.6, 74.9, 63.7, 54.7, 32.5, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.4, 28.6, 26.0, 22.9, 18.4, 14.3, -5.4, -5.4; HRMS (ESI) calcd for $C_{27}H_{56}NO_4Si$ $[M + H]^+$ *m/z* 486.3973, found 486.3952.



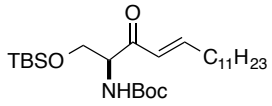
(2*S*,3*R*-4*E*)-3-benzoyloxy-1-(*tert*-Butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-ene (139). BzCl (0.52 g, 0.43 mL, 3.7 mmol) was added to a solution of (2*S*,3*R*,4*E*)-1-(*tert*-butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-en-3-ol (**138**) (0.45 g, 0.93 mmol) and DMAP (11 mg, 0.09 mmol) in pyridine (10 mL). After 6 h more BzCl (0.43 mL) was added, and the reaction mixture was stirred overnight. Ice-cold H_2O (10 mL) was added to the

reaction. The solution was extracted with CH_2Cl_2 (3 X 15 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Flash chromatography purification of the crude product on silica (petroleum ether/EtOAc, 95:5) gave **139** (0.21 g, 38%) as a colorless oil: $[\alpha]^{25}_D$ 14.28 (*c* 1.53, CH_2Cl_2); IR (neat) 2925, 2854, 1718, 1495, 1365, 1258, 1169, 1107, 1026, 965, 835, 776, 710 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.4 Hz, 2H), 7.55 (tt, *J* = 7.2, 1.4 Hz, 1H), 7.23 (t, *J* = 7.8 Hz, 2H), 5.92–5.83 (m, 1H), 5.56–5.49 (m, 2H), 4.81 (d, *J* = 9.4 Hz, 1H), 4.04–4.01 (m, 1H), 3.79 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.67 (dd, *J* = 10.0, 4.2 Hz, 1H), 2.01 (dd, *J* = 7.0, 7.0 Hz, 2H), 1.44 (s, 9H), 1.38–1.34 (m, 2H), 1.31–1.24 (m, 16H), 0.88–0.86 (m, 12H), 0.02 (s, sH), 0.00 (s, sH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 155.6, 137.3, 133.1, 130.8, 129.9, 128.5, 125.0, 79.6, 74.6, 62.1, 54.2, 32.6, 32.1, 29.9, 29.8, 29.8, 29.7, 29.6, 29.4, 29.1, 28.6, 26.1, 22.9, 18.4, 14.3, -5.3, -5.4; HRMS (ESI) calcd for $C_{34}H_{60}N_5Si$ $[M + H]^+$ *m/z* 590.4235, found 590.4231.



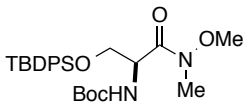
(*2S,3R,4E*)-3-(Benzoyloxy)-2-*(N-tert*-butyloxycarbonylamino)hexadec-4-en-1-ol (140). HF•py (0.1 mL, 1.2 mmol) was added drop-wise to a solution of (2*S*,3*R*-4*E*)-3-benzoyloxy-1-(*tert*-butyldimethylsilyloxy)-2-(*N-tert*-butyloxycarbonylamino) hexadec-4-ene (**139**) (0.19 g, 0.30 mmol). HF•py (0.2 mL) was added in two portions in a 3 h interval. Saturated $NaHCO_3$ (5 mL) was added. The product was extracted with CH_2Cl_2 (3 X 15 mL). The combined organic layers were dried

($MgSO_4$), filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc/, 90:10) to give **140** as a colorless oil (105 mg, 73%): $[\alpha]^{25}_D$ 13.56 (*c* 3.59, CH_2Cl_2); IR (neat) 2920, 2851, 1715, 1670, 1528, 1367, 1316, 1250, 1162, 1108, 1069, 1026, 972, 714 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.9 Hz, 2H), 7.56 (t, *J* = 7.7 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 2H), 5.88 (dt, *J* = 6.7, 6.7 Hz, 1H), 5.59 (dd, *J* = 15.0, 7.6 Hz, 1H), 5.52 (dd, *J* = 7.2, 7.2 Hz, 1H), 5.08 (d, *J* = 8.2 Hz, 1H), 3.94 (br s, 1H), 3.72 (s, 2H), 2.68 (br s, 1H), 2.04 (dt, *J* = 7.1, 7.1 Hz, 2H), 1.43 (s, 9H), 1.37 (quin, *J* = 6.1 Hz, 2H), 1.30–1.23 (m, 16H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 156.0, 137.5, 133.5, 130.1, 130.0, 128.7, 124.9, 80.0, 75.1, 62.1, 54.8, 32.5, 32.1, 29.8, 29.8, 29.8, 29.6, 29.5, 29.4, 29.0, 28.5, 22.9, 14.3; HRMS (ESI) calcd for $C_{28}H_{46}NO_5$ $[M + H]^+$ *m/z* 476.3371, found 476.3385.



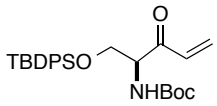
(2*S,* 4*E*)-1-(*tert*-Butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino) hexadec-4-en-3-ol (141). Cat. **2** (0.05 g, 0.08 mmol) was added to a solution of (2*S*)-1-(*tert*-butyldimethylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4-penten-3-one (**135**) (3.69 g, 11.2 mmol) and 1-tridecene (3 mL, 16 mmol) in dry CH_2Cl_2 (170 mL) at rt. The reaction was placed in a preheated oil bath at 40 °C and stirred for 2 h. The solvent was evaporated, and the crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to provide **141** (3.45 g, 64%) as a light brownish oil: $[\alpha]^{25}_D$ 30.92 (*c* 1.0, CH_2Cl_2); IR

(neat) 2926, 2854, 1695, 1488, 1251, 1168, 1113, 834, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.94 (dt, *J* = 15.6, 7.0 Hz, 1H), 6.26 (app d, *J* = 15.7 Hz, 1H), 5.51 (d, *J* = 7.4 Hz, 1H), 4.52 (ddd, *J* = 8.0, 4.4, 4.4 Hz, 1H), 3.95 (dd, *J* = 10.2, 3.2 Hz, 1H), 3.81 (dd, *J* = 10.1, 4.5 Hz, 1H), 2.20 (dt, *J* = 6.9, 1.2 Hz, 2H), 1.48–1.42 (m, 12H), 1.30–1.24 (m, 15H), 0.86 (t, *J* = 6.6 Hz, 3H), 0.82 (s, 9H), –0.01 (s, 3H), –0.02 (s, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.6, 155.5, 149.4, 127.1, 80.0, 63.9, 59.7, 32.8, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 28.5, 28.2, 25.9, 22.9, 18.4, 14.3, -5.4, -5.4; HRMS (ESI) calcd for $C_{27}H_{54}NO_4Si$ $[M+H]^+$ *m/z* 484.3817, found 484.3838.



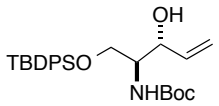
***(2S)-N*-Methoxy-*N*-methyl 3-(*tert*-butyldiphenylsilyloxy)-(*2S*)-(*N*-*tert*-butyloxycarbonylamino)propanamide (145).** Imidazole (3.18 g, 46.7 mmol) was added to solution of (2*S*)-*N*-methoxy-*N*-methyl *2*-(*N*-*tert*-butyloxycarbonylamino)propanamide (**132**) (3.86 g, 15.6 mmol) in dry DMF (10 mL). TBDPSCl (6 mL, 23 mmol) was then added and the reaction was stirred overnight.[47] H_2O (200 mL) was added to quench the reaction and the product was extracted out with Et_2O (3 X 100 mL). The combined Et_2O layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (Petroleum ether/EtOAc, 95:5) to give **145** (5.68 g, 75%) as a colorless oil: $[\alpha]^{25}_D$ 11.00 (*c* 1.09, CH_2Cl_2); IR (neat) 2932, 1713, 1664, 1427, 1365, 1249, 1166, 1108, 733, 701, 503 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (tt, *J* = 5.9, 1.5 Hz, 4H), 7.44–7.35 (m, 6H), 5.41 (d, *J* = 8.7 Hz, 1H), 4.84 (app dd, *J* = 4.2, 4.2 Hz,

1H), 3.87 (app d, *J* = 4.8 Hz, 2H), 3.66 (m 3H), 3.19 (s, 3H), 1.44 (s, 9H), 1.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 155.6, 135.8, 133.4, 129.9, 127.9, 79.7, 64.3, 61.6, 52.7, 32.4, 28.6, 26.9, 19.4; HRMS (ESI) calcd for $C_{26}H_{39}N_2O_5Si$ $[M+H]^+$ *m/z* 487.2623, found 487.2632.

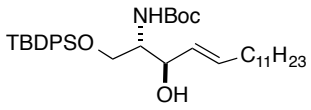


(2*S*)-1-(*tert*-Butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4-penten-3-one (146). Vinyl magnesium bromide (**134**) (1 M in THF, 95 mL, 95 mmol) was added drop-wise via an addition funnel to a solution of (2*S*)-*N*-methoxy-*N*-methyl 3-(*tert*-butyldiphenylsilyloxy)-*2*-(*N*-*tert*-butyloxycarbonylamino)propanamide (**145**) (11.5 g, 23.7 mmol) in THF (70 mL) at 0 °C.[47] The reaction was slowly allowed to warm to rt and allowed to stirred for 1.5 h. The reaction was cooled to 0 °C, and then HCl (aqueous 10%, 50 mL) was added. The product was extracted with EtOAc (3 X 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) yielded **146** (7.22 g, 67%) as a colorless oil: $[\alpha]^{25}_D$ 56.61 (*c* 1.09, CH_2Cl_2); IR (neat) 2931, 1699, 1489, 1428, 1364, 1243, 1168, 1108, 867, 733, 823, 738, 701 614, 503 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62–7.58 (m, 4H), 7.45–7.35 (m, 6H), 6.52 (dd, *J* = 17.4, 10.6 Hz, 1H), 6.34 (app d, *J* = 17.3 Hz, 1H), 5.82 (app d, *J* = 10.6 Hz, 1H), 5.59 (d, *J* = 7.4 Hz, 1H), 4.68 (ddd, *J* = 7.8, 4.0, 4.0 Hz, 1H), 4.01 (dd, *J* = 10.4, 3.4 Hz, 1H), 3.94 (dd, *J* = 10.4, 3.8 Hz, 1H), 1.46 (s, 9H), 1.01 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.8, 155.5, 135.8, 135.7, 133.3,

133.0, 132.9, 130.1, 129.7, 128.0, 79.9, 64.4, 59.6, 28.6, 27.0, 19.4; HRMS (ESI) calcd for $C_{26}H_{36}NO_4Si$ $[M + H]^+$ *m/z* 454.2408, found 454.2424.

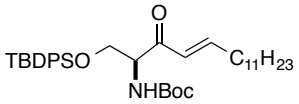


(2*S,3R*)-1-(*tert*-Butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4penten-3-ol (147). LiAl(*Ot*-Bu)$_3$H (4.17 g, 16.4 mmol) was added to a solution of (2*S*)-1-(*tert*-butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4-penten-3-one (**146**). (3.38 g, 7.45 mmol) in dry EtOH (15 mL) at −78 °C under N_2.[47] After being stirred for 6 h, the mixture was quenched slowly with aqueous HCl (1M, 25 mL) was added. The mixture was then diluted with H_2O (50 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (3 X 75 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5 to 90:10) gave **147** (2.15 g, 63%) as a colorless oil: $[\alpha]^{25}_D$ 20.88 (*c* 1.66, CH_2Cl_2); IR (neat) 3500 (br) 2960, 2930, 2888, 2857, 1695, 1499, 1427, 1365, 1167, 1110, 822, 740, 701, 611, 503 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.65–7.62 (m, 4H), 7.47–7.37 (m, 6H), 5.91 (ddd, *J* = 17.1, 10.6, 5.0 Hz, 1H), 5.41 (dt, *J* = 16.9, 1.6 Hz, 1H), 5.25 (dt, *J* = 10.6, 1.8 Hz, 2H), 4.34–4.29 (m, 1H), 3.91(dd, *J* = 10.5, 3.6 Hz, 1H), 3.76 (dd, *J* = 10.0, 3.3 Hz, 1H), 3.71 (br s, 1H), 3.29 (app dd, *J* = 7.6 Hz, 1H), 1.45 (s, 9H), 1.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 137.9, 135.8, 132.7, 130.2, 128.1, 116.3, 79.8, 74.8, 64.3, 55.0, 29.1, 28.6, 27.1, 19.4; HRMS (ESI) calcd for $C_{26}H_{38}NOSi$ $[M + H]^+$ *m/z* 456.6775, found 456.2582.



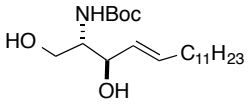
(2*S*,3*R*,4*E*)-1-(*tert*-Butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino) hexadec-4-en-3-ol (148). Pathway 1: Cat.**1** (19 mg, 0.02 mmol) was added to a solution of (2*S,3R*)-1-(*tert*-butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)-4-penten-3-ol (**147**) (1.00 g, 2.19 mmol) and 1-tridecene (**137**) (2 mL, 8.8 mmol) in dry CH_2Cl_2 (33 mL) at rt. The reaction was stirred overnight. The solvent was evaporated and the crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to provide **148** (0.42 g, 31%) as a light brownish oil; Pathway 2: LiAl(*Ot*-Bu)$_3$H (5.03 g, 19.8 mmol) was added to (2*S,*4*E*)-1-(*tert*-butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-ene-3-one (**148a**) (5.48 g, 8.99 mmol) in dry EtOH (36 mL) at −78 °C.[61] After being stirred for 1 h, the mixture was quenched slowly with aqueous HCl (1M, 20 mL) was added slowly. The mixture was then diluted with H_2O (50 mL). The two layers were separated and the aqueous layer was extracted with EtOAc (3 X 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5 to 90:10) gave **148** (4.08 g, 74%) as a colorless oil: $[\alpha]^{25}_D$ 13.05 (*c* 1.64, CH_2Cl_2); IR (neat) 2924, 2854, 1698, 1498, 1428, 1365, 1169, 1111, 966, 822, 740, 701, 613, 504 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (t, *J* = 12.2 Hz, 4H), 7.46–7.36 (m, 6H), 5.78 (dt, *J* = 14.4, 6.5 Hz, 1H), 5.48 (dd, *J* = 15.4, 6.0 Hz, 1H), 5.19 (d, *J* = 7.0 Hz, 1H), 4.24 (app ddd, *J* = 6.5, 6.5, 6.5 Hz, 1H), 3.91 (dd, *J* = 10.5, 3.6 Hz, 1H), 3.76 (dd, *J* = 11.1, 3.1 Hz, 1H), 3.66 (br s, 1H), 3.15 (app d, *J* = 6.3 Hz, 1H), 2.04 (dt, *J* = 6.7, 6.7

Hz, 2H), 1.45 (s, 9H), 1.37–1.32 (m, 3H), 1.31–1.26 (m, 15H), 1.07 (s, 9H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 135.8, 133.6, 132.8, 132.8, 130.2, 129.4, 128.1, 79.7, 74.5, 64.3, 55.3, 32.6, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.6, 27.1, 22.9, 19.4, 14.3; HRMS (ESI) calcd for $C_{37}H_{60}NO_4Si$ $[M + H]^+$ *m/z* 610.4286, found 610.4309.



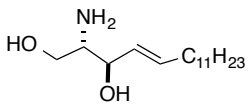
(2*S,* 4*E*)-1-(*tert*-Butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-en-3-one (148a). Cat. **3** (30 mg, 0.04 mmol) was added to a solution of (2*S*)-1-(*O*-tert-butyldiphenylsilyl)-2-(*N*-tert-butyloxycarbonylamino)-4-pentene-3-one (**146**). (2.02 g, 0.441 mmol) and 1-tridecene (2.2 mL, 8.8 mmol) in dry CH_2Cl_2 (65 mL). The reaction was allowed to stir in a preheated oil bath at 40 °C for 4h. The solvent was evaporated and the crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 95:5) to give **148a** (2.38 g, 88%) as a slightly brownish oil: $[\alpha]^{25}_D$ 41.8 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62–7.58 (m, 4H), 7.44–7.34 (m, 6H), 6.96 (dt, *J* = 14.1, 6.8 Hz, 1H), 6.23 (app d, *J* = 15.7 Hz, 1H), 5.62 (d, *J* = 7.7 Hz, 1H), 4.63 (ddd, *J* = 7.9, 4.0, 4.0 Hz, 1H), 3.97 (dd, *J* = 10.4, 3.6 Hz, 1H), 3.92 (dd, *J* = 14.4, 4.0 Hz, 1H), 2.20 (dt, *J* = 7.0, 7.0 Hz, 2H), 1.45 (s, 9H), 1.31–1.26 (m, 19H), 1.01 (s, 9H), 0.88 (t, *J* = 6.7 Hz, 3H), 0.82 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 155.5, 149.7, 135.8, 135.7, 133.1, 133.1, 130.0, 127.9, 127.9, 127.0, 79.8, 64.6, 59.6, 32.9, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 28.6, 28.2, 26.9, 22.9,

19.5, 14.4; HRMS (ESI) calcd for $C_{37}H_{58}NO_4Si$ $[M + H]^+$ *m/z* 608.4130, found 608.4124.

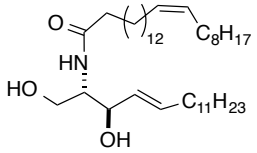


(2*S*,3*R*,4*E*)-2-(*N*-*tert*-Butyloxycarbonylamino)hexadec-4-en-1,3-diol (155).

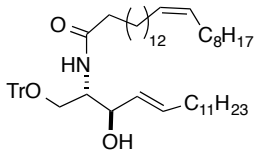
TBAF (1.0 M in THF, 6.6 mmol, 6.6 mL) was added drop-wise to a solution of (2*S*,3*R*,4*E*)-1-(*tert*-butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-en-3-ol (1**48**) (1.82 g, 2.98 mmol) in THF (60 mL) at 0 °C. The cooling bath was removed, and the mixture was stirred for 2 h at rt. Saturated aqueous NH_4Cl (30 mL) was added. The product was extracted with Et_2O (3 X 100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 60:40) provided **155** (0.82 g, 74%) as a white solid: mp 54.1–55.4 °C; $[\alpha]^{25}_D$ –2.57 (*c* 1, CH_2Cl_2); IR (neat) 3342, 2916, 2849, 1687, 1531, 1464, 1365, 1244, 1172, 1062, 1004, 906, 649 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.75 (dt, *J* = 14.6, 6.6 Hz, 1H), 5.50 (dd, *J* = 15.4, 6.3 Hz, 1H), 5.34 (d, *J* = 8.0 Hz, 1H), 4.27 (app ddd, *J* = 5.0, 5.0 Hz, 1H), 3.90 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.67 (app d, *J* = 11.0 Hz, 1H), 3.57 (br s, 1H), 3.10 (br s, 2H), 2.03 (dt *J* = 6.5, 6.5 Hz, 2H), 1.43 (s, 9H), 1.35 (t, *J* = 14.9 Hz, 2H), 1.24 (s, 16H), 0.86 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 134.2, 129.1, 80.0, 74.8, 65.3, 55.7, 32.5, 32.1, 29.8, 29.8, 29.7, 29.5, 29.4, 29.3, 28.6, 22.9, 14.3; HRMS (ESI) calcd for $C_{21}H_{42}NO_4$ $[M + H]^+$ *m/z* 372.3108, found 372.3133.



(2*S*,3*R*,4*E*)-2-(Amino)hexadec-4-en-1,3-diol (123). TFA (4 mL) (2*S*,3*R*,4*E*)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-en-1,3-diol (**155**) (0.77 g, 2.08 mmol) in CH_2Cl_2 (4 mL) at 0 ^oC. The reaction was stirred at 0 ^oC for 3.5 h, after which TLC still showed starting material. TFA (2 mL) was added, and the solution was stirred for an extra 30 min. 1 M NaOH was then added to pH 10. The product was extracted with EtOAc (3 X 75 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was carried forward to acylation, but some of it was saved and purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 70:30) to give **123** as a yellowish, viscious oil: $[\alpha]^{25}_D$ –2.22 (*c* 0.64, MeOH); IR (neat) 2920, 2851, 1465, 1138, 1045, 970, 719 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 5.84 (dt, *J* = 14.6, 6.8 Hz, 1H), 5.51 (dd, *J* = 15.4, 7.0 Hz, 1H), 4.19 (dd, *J* = 5.8, 5.8 Hz, 1H), 3.77 (dd, *J* = 11.2, 3.9 Hz, 1H), 3.63 (dd, *J* = 11.2, 7.8 Hz, 1H), 3.05 (ddd, *J* = 7.9, 4.8, 4.8 Hz, 1H), 2.13 (dt *J* = 6.8, 6.8 Hz, 2H), 1.45 (quin, *J* = 7.6 Hz, 2H), 1.37–1.32 (m, 16H), 0.93 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 136.6, 129.9, 73.1, 61.9, 58.8, 33.9, 33.6, 31.3, 31.2, 31.1, 31.0, 30.8, 30.8, 24.2, 14.9; HRMS (ESI) calcd for $C_{16}H_{34}NO_2$ $[M + H]^+$ *m/z* 272.2584, found 272.2617.

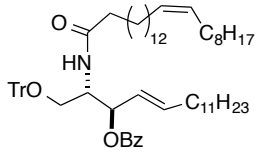


(2*S*,3*R*,4*E*)-2-(*N*-15*Z*-Tetracosenoylamino)hexadec-4-en-1,3-diol (149). *p*-Nitrophenyl 15*Z*-tetracosenoate (**77**) (1.26 g, 2.50 mmol) was added to a solution of (2*S*,3*R*,4*E*)-2-(amino)hexadec-4-en-1,3-diol **123** (0.77 g, 2.1 mmol) in pyridine (36 mL). The mixture was stirred in a preheated oil bath at 40 ^{o}C overnight. The reaction was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **149** (0.61 g, 75%) as a white solid: mp 72.1–73.4 ^{o}C; $[\alpha]^{25}_{D}$ –2.51 (*c* 0.84, $CDCl_3$/MeOH, 3:2); IR (neat) 2916, 2849, 1645, 1465, 720 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (d, *J* = 7.3 Hz, 1H), 5.78 (dt, *J* = 14.5, 6.3 Hz, 1H), 5.53 (dd, *J* = 15.4, 6.4 Hz, 1H), 5.38–5.31 (m, 2H), 4.31 (br s, 1H), 3.97–3.88 (m, 2H), 3.70 (app d, *J* = 11.1 Hz, 1H), 2.70 (br s, 2H), 2.23 (t *J* = 7.4 Hz, 2H), 2.08–1.99 (m, 6H), 1.67–1.60 (m, 3H), 1.40–1.26 (m, 49H), 0.88 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 134.5, 130.1, 129.0, 74.9, 62.8, 54.7, 37.1, 32.5, 32.1, 30.0, 29.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 26.0, 22.9, 14.3; HRMS (ESI) calcd for $C_{40}H_{78}NO_3$ $[M + H]^+$ *m/z* 620.5976, found 620.5962.



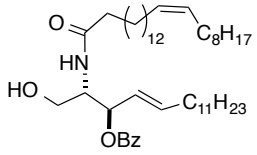
2*S*,3*R*,4*E*)-2-(*N*-15*Z*-Tetracosenoylamino)-1-triphenylmethoxyhexadec-4-en-3-ol. TrCl (0.13 g, 0.45 mmol) was added to a mixture of (2*S*,3*R*,4*E*)-2-(*N*-15*Z*-tetracosenoylamino)hexadec-4-en-1,3-diol (**149**) (0.24 g, 0.38 mmol), Et_3N (0.6 mL,

0.45 mmol) in dry EtOAc (1.6 mL).[62] The mixture was stirred in a preheated oil bath at 80 °C overnight. The solvent was evaporated under reduced pressure, and the crude product was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) to provide (2*S*,3*R*,4*E*)-2-(*N*-15*Z*-tetracosenoylamino)-1-triphenylmethoxy-hexadec-4-en-3-ol (0.21 g, 66%) as a yellowish oil: $[\alpha]^{25}_{D}$ −1.54 (*c* 0.85, CH_2Cl_2); IR (neat) 2921, 2851, 1642, 1448, 1074, 702, 632 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 7.2 Hz, 5H), 7.31 (t, *J* = 7.8 Hz, 5H), 7.27–7.23 (m, 5H), 6.05 (d, *J* = 7.9 Hz, 1H), 5.64 (dt, *J* = 14.5, 6.4, 1H), 5.38–5.31 (m, 1H), 5.26 (dd, *J* = 15.4, 6.2 Hz, 1H), 4.20–4.16 (m, 1H), 4.06 (dddd, *J* = 7.7, 3.8, 3.8, 3.8 Hz, 1H), 3.39 (dd, *J* = 9.7, 3.7 Hz, 1H), 3.35 (dd, *J* = 8.2, 0.0 Hz, 1H), 3.30 (dd, *J* = 9.8, 4.0 Hz, 1H), 2.20 (t, *J* = 7.5 Hz, 2H), 2.01 (dt, *J* = 6.3, 6.3 Hz, 4H), 2.00–1.89 (m, 2H), 1.64 (quin, *J* = 6.8 Hz, 2H), 1.34–1.26 (m, 50H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 143.5, 134.8, 133.7, 130.1, 128.9, 128.7, 128.2, 127.5, 87.6, 74.6, 63.3, 53.6, 37.1, 32.4, 32.1, 30.0, 29.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 27.4, 26.1, 24.7, 22.9, 19.8, 14.3; HRMS (ESI) calcd for $C_{59}H_{92}NO_3$ $[M + H]^+$ *m/z* 862.7072, found 862.7116.



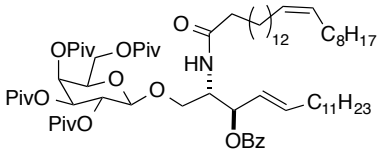
(2*S*,3*S*,4*E*)-3-Benzoyloxy-2-(*N*-15*Z*-tetracosanoylamino)-1-triphenylmethoxyhexadec-4-ene. BzCl (2.73 g, 2.26 mL, 19.5 mmol) was added to a solution of 2*S*,3*R*,4*E*)-2-(*N*-tetracosanoylamino)-1-triphenylmethoxyhexadec-4-en-3-ol (2.96 g, 3.25 mmol) and DMAP (66 mg, 0.54 mmol) in pyridine (27 mL).[50]

The reaction mixture was allowed to stir overnight. Ice-cold H_2O (50 mL) was added. The resulting mixture was extracted with CH_2Cl_2 (3 X 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Flash chromatography purification of the crude product on silica gel (petroleum ether/EtOAc, 95:5) gave (2*S*,3*S*,4*E*)-3-benzoyloxy-2-(tetracosanoylamino)-1-triphenylmethoxyhexadec-4-ene (2.42 g, 63%) as colorless oil: $[\alpha]^{25}_D$ 5.56 (*c* 1.4, CH_2Cl_2); IR (neat) 2922, 2852, 1720, 1449, 1265, 1108, 707 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 7.2 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 1H), 7.34–7.30 (m, 8H), 7.27–7.23 (m, 9H), 5.80 (dt, *J* = 14.8, 6.7 Hz, 1H), 5.64–5.58 (m, 2H), 5.38 (dd, *J* = 15.4, 7.5 Hz, 1H), 5.29–5.24 (m, 2H), 4.45–4.39 (m, 1H), 3.37 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.12 (dd, *J* = 9.6, 4.2 Hz, 1H), 2.03 (t, *J* = 7.5 Hz, 2H), 1.96–1.89 (m, 2H), 1.49 (quin, *J* = 8.6 Hz, 2H), 1.34–1.26 (m, 54H), 0.80 (t, *J* = 6.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 165.5, 143.7, 137.4, 133.1, 130.5, 130.1, 129.9, 128.8, 128.5, 128.1, 127.3, 125.3, 87.0, 74.6, 61.9, 51.3, 37.2, 32.5, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.529.1, 27.4, 26.0, 22.9, 14.3; HRMS (ESI) calcd for $C_{66}H_{96}NO_4$ $[M + H]^+$ *m/z* 966.7334, found 966.7241.



(2*S*,3*R*,4*E*)-3-Benzoyloxy-2-(*N*-15*Z*-tetracosenoylamino)hexadec-4-en-1-ol (150). pTsOH•H_2O (56 mg, 0.30 mmol) was added to a solution of (2*S*,3*R*,4*E*)-3-benzoyloxy-2-(*N*-tetracosanoylamino)-1-triphenylmethoxyhexadec-4-ene (0.26 g, 0.27 mmol) in a mixture of MeOH/CH_2Cl_2 (3.7:3.7 mL).[50] After 5 h, the reaction was

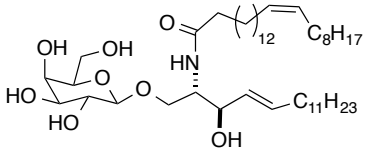
quenched with sat. $NaHCO_3$ (10 mL) and the product was extracted with CH_2Cl_2 (3 X 15 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to give **150** (99 mg, 56%) as a creamy white solid: $[\alpha]^{25}_D$ 12.6 (*c* 1.4, CH_2Cl_2); IR (neat) 2917, 2849, 1721, 1645, 1547, 1466, 1265, 1111, 963, 711 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.7 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.38 (t, *J* = 7.7 Hz, 2H), 6.06 (d, *J* = 8.6 Hz, 1H), 5.85 (dt, *J* = 14.5, 6.7 Hz, 1H), 5.61 (dd, *J* = 15.2, 7.7 Hz, 1H), 5.53 (dd, *J* = 7.5, 7.5 Hz, 1H), 5.35 (dt, *J* = 4.8, 0.0 Hz, 2H), 4.27 (dddd, *J* = 10.7, 10.7, 2.9, 2.9 Hz, 1H), 3.75–3.68 (m, 2H), 2.19 (dt, *J* = 14.3, 7.0 Hz, 2H), 2.07–1.97 (m, 6H), 1.61 (quin, *J* = 7.3 Hz, 2H), 1.33–1.24 (m, 51H), 0.86 (t, *J* = 6.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 166.8, 137.8, 133.7, 130.1, 130.0, 129.9, 128.8, 125.0, 74.9, 62.1, 53.7, 37.1, 32.5, 32.1, 30.0, 30.0, 29.9, 29.9, 29.9, 29.9,29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 27.4, 27.4, 26.0, 22.9, 14.3; HRMS (ESI) calcd for $C_{47}H_{82}NO_4$ $[M + H]^+$ *m/z* 724.6238, found 724.6189.



(2*S*,3*R*,4*E*)-3-Benzoyloxy-1-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-2-(*N*-15*Z*-tetracosenoylamino)hexadec-4-ene (151). (2,3,4,6-Tetra-*O*-pivaloyl-α-D-galactopyranosyl)-1-trichloroacetimidate (**70**) (0.19 g, 0.26 mmol) and (2*S*,3*R*,4*E*)-3-benzoyloxy-2-(*N*-15*Z*-tetracosenoylamino)hexadec-4-en-1-ol (**150**) (0.11 g, 0.17 mmol) were dissolved in dry CH_2Cl_2 (3.7 mL), and the solution was stirred in the presence of 4Å MS (200 mg) at rt for 10 min.[42] BF_3•OEt_2 in dry CH_2Cl_2 (0.54 μL in 2

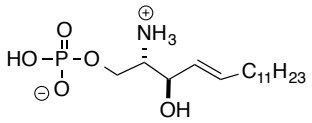
mL) was added within 10 min at –10 °C and the reaction was stirred at this temperature for 4 h. The reaction mixture was diluted with petroleum ether (5 mL) and then filtered. The filtrate was treated with saturated $NaHCO_3$ (50 mL). The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 X 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentration. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc, 90:10) gave **151** (69 mg, 33%, β) as a colorless oil: $[\alpha]^{25}_D$ 0.591 (*c* 1.04, CH_2Cl_2); IR (neat) 2921, 2852, 1740, 1461, 1275, 1141, 1073, 711 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 7.2 Hz, 2H), 7.54 (tt, *J* = 6.7, 1.3, Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 2H), 5.88 (dt, *J* = 14.5, 6.8 Hz, 1H), 5.73 (d, *J* = 9.1 Hz, 1H), 5.54 (dd, *J* = 14.6, 7.3 Hz, 1H), 5.47 (app t, *J* = 7.5 Hz, 1H), 5.38–5.29 (m, 3H), 5.18 (dd, *J* = 7.7, 2.7 Hz, 1H), 5.07 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.49–4.44 (m, 2H), 4.07 (dd, *J* = 9.8, 3.6 Hz, 1H), 3.89–3.79 (m, 3H), 3.64 (dd, *J* = 9.8, 3.6 Hz, 1H), 2.12 (dt, *J* = 14.0, 7.4 Hz, 2H), 2.05–1.99 (m, 6H), 1.61–1.51 (m, 3H), 1.32–1.24 (m, 57H), 1.19–1.16 (m, 1H), 1.14–1.11 (m, 27H), 0.87 (t, *J* = 5.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDC1_3$) δ 177.8, 177.4, 177.0, 172.8, 165.2, 137.6, 133.1, 130.5, 130.1, 129.8, 128.6, 125.1, 100.9, 74.2, 71.2, 71.0, 69.2, 67.3, 66.7, 60.9, 51.0, 39.3, 39.0, 39.0, 38.8, 37.1, 32.6, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 29.2, 27.4, 27.4, 27.3, 27.2, 25.9, 22.9, 14.3; HRMS (ESI) calcd for $C_{47}H_{124}NO_{13}$ $[M + H]^+$ *m/z* 1222.9067, found 1222.9059.; trans-esterification sphingosine product **152**: ^1H NMR (400 MHz, $CDC1_3$) δ 8.03–8.01 (m, 2H), 7.57 (tt, *J* = 6.7, 1.6, Hz, 1H), 7.47–7.43 (m, 2H), 5.88 (dt, *J* = 14.4, 6.8 Hz, 1H), 5.76 (d, *J* =

9.2 Hz, 1H), 5.56 (dd, *J* = 12.4, 7.2 Hz, 1H), 5.50 (dddd, *J* = 7.3, 7.3, 0.9, 0.9 Hz, 1H), 5.39–5.31 (m, 2H), 4.66–4.60 (m, 1H), 4.34 (dd, *J* = 11.6, 7.1 Hz, 1H), 4.18 (dd, *J* = 11.6, 4.3 Hz, 1H), 2.15 (t, *J* = 7.1 Hz, 2H), 2.07–2.00 (m, 6H), 1.59 (quin, *J* = 8.0 Hz, 2H), 1.37–1.24 (m, 50H), 1.20 (s, 9H), 0.88 (t, *J* = 6.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDC1_3$) δ 178.8, 172.9, 165.6, 137.5, 133.4, 130.1, 129.9, 128.7, 124.5, 74.9, 62.7, 51.2, 39.1, 37.1, 32.6, 31.1, 30.0, 29.9, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, , 29.5, 29.4, 29.1, 27.4, 27.4, 25.9, 22.9, 14.3.



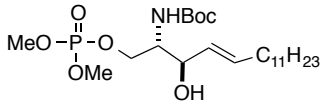
(2*S*,3*R*,4*E*)--1-(β-D-Galactopyranosyloxy)-2-(*N*-15*Z*-tetracosenoylamino)hexadec-4-en-3-ol (153). NaOMe (0.35 mL, 0.18 mmol) was added to a solution of (2*S*,3*R*,4*E*)-3-benzoyloxy-1-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranoside)-2-(*N*-15*Z*-tetracosenoylamino)hexadec-4-ene (**151**) (36 mg, 0.03 mmol) in a mixture of CH_2Cl_2/MeOH (0.24:0.24 mL). The reaction was stirred at rt for 1 h and then neutralized to pH 2 with dowex (H^+ resin). The mixture was filtered through a pad of celite, and the celite was washed with a 1:1 mixture of $CHCl_3$ and MeOH (10 mL). The filtrate was concentrated and purified using flash column chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give **153** (17 mg, 76%) as a white solid: mp 158.0–159.1 ^{o}C; $[\alpha]^{25}_{D}$ 0.267 (*c* 1.01, $CHCl_3$/MeOH, 3:2); IR (neat) 3298, 2916, 2849, 1644, 1545, 1467, 1083, 718 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 3:2) δ 5.69 (dt, *J* = 14.9, 6.7 Hz, 1H), 5.45 (dd, *J* = 15.4, 7.4 Hz, 1H), 5.37–5.30 (m, 2H), 4.21 (d, *J* = 7.4 Hz, 1H), 4.17 (dd, *J* = 10.4, 4.6 Hz, 1H), 4.10

(dd, *J* = 7.5, 7.5 Hz, 1H), 4.00 (dddd, *J* = 7.9, 7.9, 4.4, 4.4 Hz, 1H), 3.87 (app d, *J* = 2.6 Hz, 1H), 3.81 (dd, *J* = 11.5, 6.7 Hz, 1H), 3.73 (dd, *J* = 11.5, 5.0 Hz, 1H), 3.60–3.48 (m, 4H), 2.17 (t, *J* = 7.4 Hz, 2H), 2.01 (dt, *J* = 6.3, 6.3 Hz, 6H), 1.58 (quin, *J* = 6.8 Hz, 2H), 1.38–1.26 (m, 50H), 0.88 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 3:2) δ 175.6, 135.1, 130.8, 130.2, 104.7, 76.1, 74.4, 72.9, 72.3, 70.0, 69.7, 62.4, 54.4, 37.4, 33.3, 32.9, 32.8, 30.6, 30.6, 30.5, 30.4, 30.4, 30.3, 30.2, 28.1, 26.9, 23.6, 14.8; HRMS (TOF) *m/z* calcd for $C_{46}H_{88}NO_8$ $[M + H]^+$ 782.6504, found 782.6502.



(2*S*,3*R*,4*E*)-2-(*ammonium*)hexadecen-3-ol-1-phosphate (154). TMSBr (0.09 mL, 0.70 mmol) was added drop-wise to a solution of (2*S*,3*R*,4*E*)-2-(amino)hexadecen-3-ol-1-methyl phosphate (**156**) (0.04 g, 0.08 mmol) in CH_2Cl_2 (0.43 mL) at 0 °C. The reaction was stirred at rt for 30 min. AcOH (0.5 mL) was added, followed by the addition of ice water until precipitates were formed. The mixture was centrifuged and the H_2O layer was removed. The solid residue was further washed with H_2O (3 X 10 mL) and centrifugation was repeated each time to remove the H_2O. The solid residue was washed with a mixture of acetone/H_2O (1:1 v/v, 3 X 5 mL). The sample was then left on the high vacuum to remove excess solvents to provide **154** (19 mg, 63%) as a white solid: mp 154.9–156.4 °C; $[\alpha]^{25}_D$ 4.15 (*c* 0.17, MeOH); IR (neat) 3435, 2918, 2849, 1543, 1463, 1243, 1028, 923, 510 cm^{-1}; ^{1}H NMR (400 MHz, MeOD) δ 5.90 (dt, *J* = 14.7, 6.8 Hz, 1H), 5.52 (dd, *J* = 15.4, 7.0 Hz, 1H), 4.30 (dd, *J*

= 5.9, 5.9 Hz, 1H), 4.12 (ddd, *J* = 11.6, 7.3, 3.4 Hz, 1H), 4.04–3.96 (m, 1H), 3.39–3.33 (m, 1H), 2.13 (dt *J* = 7.0, 7.0 Hz, 2H), 1.46 (quin, *J* = 7.2 Hz, 2H), 1.39–1.32 (m, 17H), 0.93 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, MeOD) δ 135.7, 127.0, 63.6, 61.8 (d, $J_{C\text{-}P}$ = 4.8 Hz), 56.4 (d, $J_{C\text{-}P}$ = 6.4 Hz), 32.2, 31.9, 29.6, 29.5, 29.6, 29.5, 29.3, 29.2, 29.0, 22.5, 13.2, –1.2; ^{31}p NMR (400 MHz, MeOD) δ 1.31; HRMS (ESI) calcd for $C_{16}H_{35}NO_5P$ $[M + H]^+$ *m/z* 352.2247, found 352.2286.



(2*S*,3*R*,4*E*)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-en-3-ol-1-methyl phosphate (156). $P(OMe)_3$ (0.04 mL, 0.034 mmol) was added drop-wise to a solution of (2*S*,3*R*,4*E*)-1-(*tert*-butyldiphenylsilyloxy)-2-(*N*-*tert*-butyloxycarbonylamino)hexadec-4-en-3-ol (**148**) (100 mg, 0.27 mmol) and CBr_4 (0.123 g, 0.371 mmol) in pyridine (0.13 mL) at 0 °C. The reaction was stirred at the same temperature for 4.5 h, after which the precipitate formed was filtered off and rinsed with EtOAc (15 mL). The filtrate was washed with brine (10mL). The two layers were separated and the organic layer was dried ($MgSO_4$), filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (petroleum ether/EtOAc, 60:40) to yield **156** (41 mg, 32%) as a colorless oil: $[\alpha]^{25}_D$ 5.56 (*c* 0.17, CH_2Cl_2); IR (neat) 2923, 2853, 1714, 1457, 1365, 1249, 1171, 1036, 825 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.76 (dt, *J* = 14.9, 6.7 Hz, 1H), 5.48 (dd, *J* = 15.4, 6.7 Hz, 1H), 5.06 (d, *J* = 7.0 Hz, 1H), 4.31 (dddd, *J* = 6.0, 6.0, 6.0, 6.0 Hz, 1H), 4.13–4.08 (m, 2H), 3.77 (d, *J* = 10.7 Hz, 6H), 3.04 (br s,

1H), 2.01 (dt J = 6.8, 6.8 Hz, 2H), 1.42 (s, 9H), 1.37–1.33 (m, 2H), 1.30–1.24 (m, 17H), 0.86 (t, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.9, 128.7, 79.9, 72.6, 66.9 (d, $J_{C\text{-}P}$ = 2.8 Hz), 55.1, 54.7 (d, $J_{C\text{-}P}$ = 5.5 Hz), 32.5, 32.1, 29.9, 29.8, 29.8, 29.7, 29.5, 29.4, 29.3, 28.5, 22.9, 14.3; ^{31}p NMR (400 MHz, $CDCl_3$) δ 1.85; HRMS (ESI) calcd for $C_{23}H_{47}NO_7P$ $[M + H]^+$ *m/z* 480.3085, found 480.3092.

Chapter 2.

2.1. Introduction

2.1.1. Oxetanes as important scaffolds in natural product synthesis and methodological development

A major area of research in the Howell group focuses on exploring the reactivity of oxetanes, particularly 2-methyleneoxetanes **158** and α-methylene-β-lactones **161** respectively in designing new synthetic routes for the preparation of biologically interesting natural products and their analogs (Figure 30). In addition, the unusual exocyclic unsaturation present in **158** and **161** is taken advantage of to develop novel transformations. For example 2-Methyleneoxetanes such as **158** have been used by Liang *et al.* in a three-component reaction to synthesize a *psico*-oxetanocin **164** and its analogs,[63] and Keshipeddy *et al.*[64] employed dispirohexanes (see **163**) derived from **158** in their efforts to prepare laureatin (**165**). The focus of this dissertation is the olefin cross metathesis (CM) of α-methylene-β-lactones to access α-alkylidene-β-lactones **162** and their subsequent 1,4-reduction.

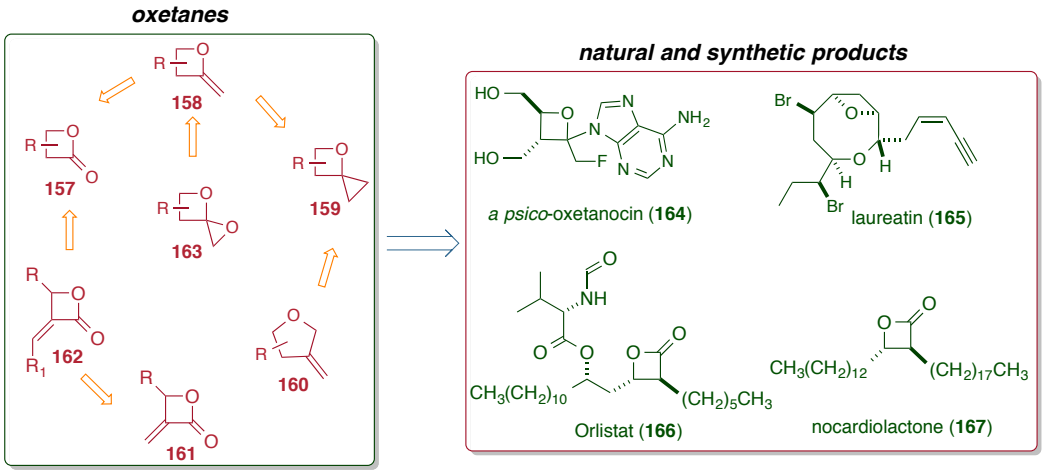
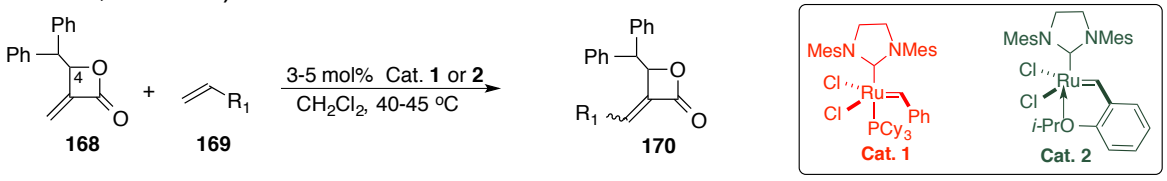


Figure 30. Utility of oxetanes in the Howell lab

2.1.2. Olefin cross metathesis of α-methylene-β-lactones

In 2006, Howell and Raju demonstrated that α-methylene-β-lactone **168**[65] can undergo olefin cross metathesis (CM) with a variety of type I[65] alkene cross partners **169** to give tri-substituted α-alkylidine-β-lactones **170** (Table 1). The reactions proceeded in excellent yields with high *Z*-selectivity using either Grubb's 2nd or Grubbs-Hoveyda 2nd generation catalysts (Cat. **1** and **2** respectively). The high *Z*-selectivity of the reactions was attributed to steric effects from the $CHPh_2$ group at the C-4 position of lactone **168**. This steric argument was confirmed by a drop in the selectivity when the $CHPh_2$ group was substituted with the $(CH_2)_2Ph$ (CM of **171** to give **172**, Table 1).



Entry	R_1	Catalyst	Yield (%)	*Z:E*
1	$(CH_2)_2OAc$	1	84	>20:1
2	$(CH_2)_3OAc$	1	90	>20:1
3	$(CH_2)_4OAc$	1	85	>14.1
4	$(CH_2)_8OAc$	1	84	>20:1
5	Ph	1	55[b]	>20:1
6	$(CH_2)_2CH_3$	1	94	11:1
7	$(CH_2)_2Br$	1	93	>20:1
8	CH_2Cl	2	80	12:1
9	$(CH_2)_2OTBDMS$	1	88	9:1
10	CH_2OR[a]	1 or 2	NR	

[a]Reaction did not work with allylic alcohols, whether protected or not (R = Bn, Ac, TBDMS, or TBDPS); [b]isolated *Z*-isomer yield

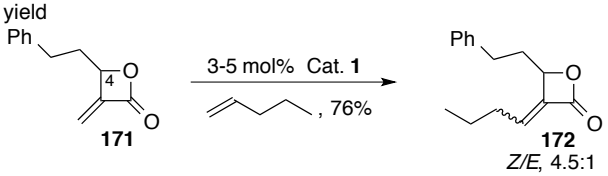
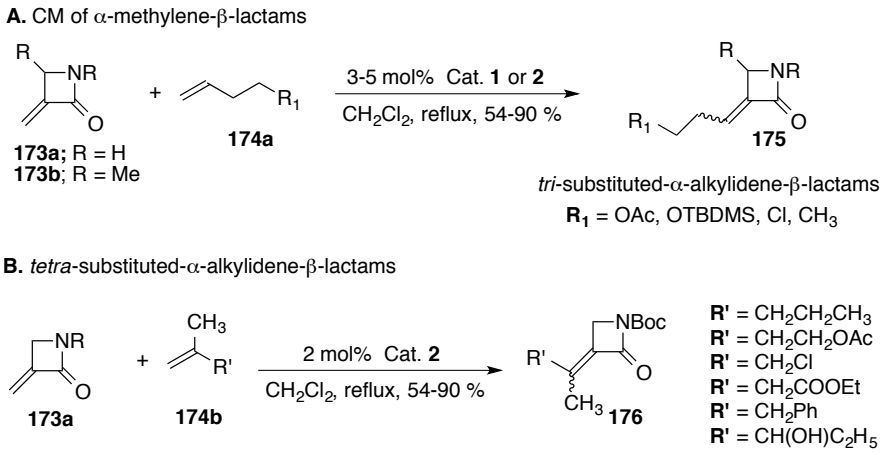


Table 1. CM of α-methylene-β-lactones[65]

The CM methodology was extended in 2009 by Liang *et al.* to α-methylene-β-lactams **173**, furnishing tri- and tetra-substituted α-alkylidine-β-lactams **175** and **176** respectively (Scheme 31).[66] The tetra-substituted α-alkylidine-β-lactams exemplified the first application of CM to give tetra-substituted alkene products.



Scheme 31. CM of α-methylene-β-lactams

The versatility of the CM reactions prompted application studies, and substituted β-lactones attracted our attention. β-lactones are a common moiety present in natural products, and many of these have been shown to have important biological activities.

2.1.3. β-Lactones as inhibitors of fatty acid synthase (FAS)

β-Lactones have been shown to be potent, irreversible inhibitors of serine hydrolase enzymes. For example, tetrahydrolipstatin (THL, orlistat **166**), an anti-obesity drug, is an irreversible inhibitor of the thioesterase domain of fatty acid synthase (FAS).[67] FAS is the largest multi-component enzyme in eukaryotes, with six enzymatic domains (acyl carrier protein (ACP), *β*-ketoacyl synthase (KS), malonyl/acetyl transferase (MT/AT, MAT), dehydratase (DH), *β*-enoyl reductase (ER), and

thioesterase (TE)) (Figure 31) that work together to synthesize palmitic acid (**177**).[68,69] FAS became a target for cancer therapy when it was found to be up-regulated in tumor cells found in breast tissues and prostate glands.[70-72] Cell proliferation was observed with the secretion of palmitic acid, and the inhibition of its formation causes tumor cell apoptosis.

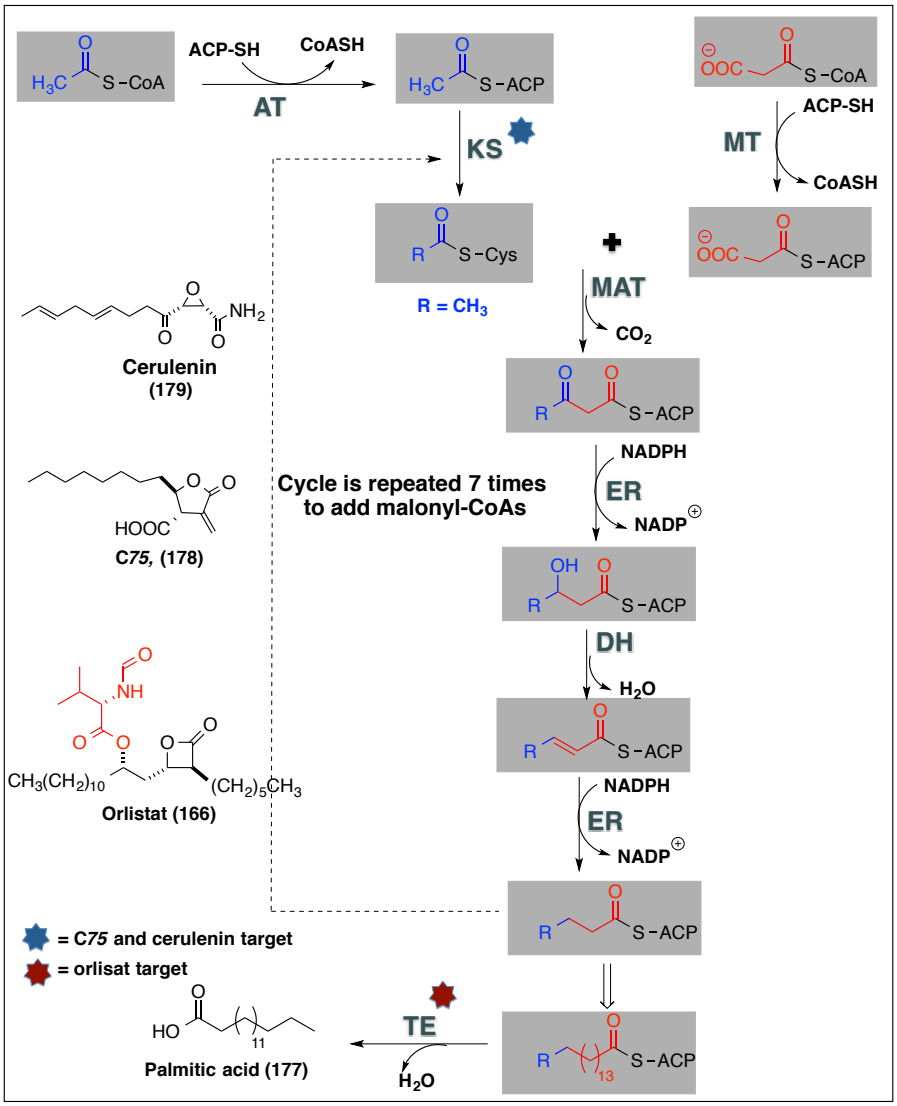


Figure 31. Schematic diagram of the FAS synthesis of palmitic acid (**177**)

Since this discovery, many research endeavors have been aimed at discovering compounds that can selectively inhibit FAS. Cerulenin (**179**) and C*75* (**178**) inhibit FAS by binding to the β-ketoacyl synthase (KS) domain (Figure 31). Unfortunately, their therapeutic exploration was halted because their inhibitory mechanism was non-selective. They also bind to other important biological proteins, causing excessive weight loss.[73] Orlistat (**166**), in contrast, selectively targets the thioesterase (TE) domain (Figure 32). This pocket has a serine residue (Ser 2308) that interacts with a palmitic acid precursor, causing hydrolytic cleavage of its thioester bond, thus producing palmitic acid (**177**). Orlistat competes and obstructs this action via nucleophilic addition of the enzyme to its carbonyl group leading to the formation of a stable acyl-enzyme complex (Figure 32 a, b and c).[74]

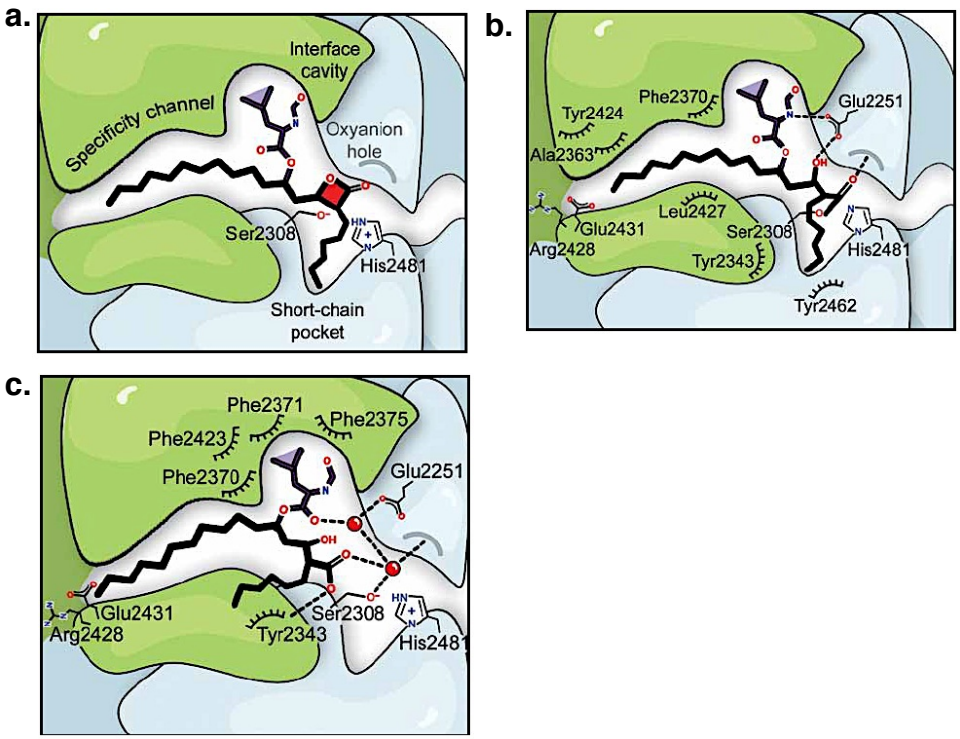
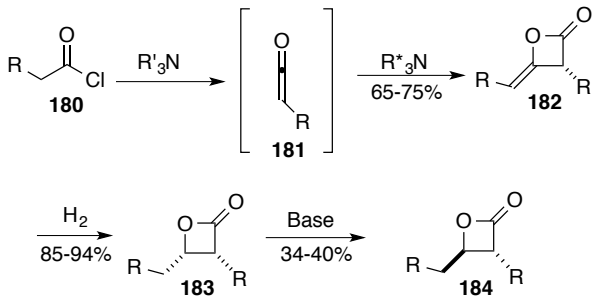


Figure 32. Orlistat mediated inhibition of FAS: **a.** Proposed interactions of orlistat in FAS; **b.** Orlistat acyl-enzyme intermediate; **c.** Hydrolyzed orlistat[74]

2.1.4. Development of synthetic routes to prepare β-lactones as FAS inhibitors

The fact that orlistat is an irreversible inhibitor of FAS has generated a lot of interest in exploring β-lactones as cancer therapeutic agents and in the development of synthetic routes for their easy availability for biological analyses. A major pioneer in this area has been the Romo research group. They have made remarkable contributions by developing asymmetric reaction sequences that provide β-lactone libraries. Their first route was a ketene dimerization pathway to give alkylidene β-lactones **182** that underwent hydrogenation to give *cis* β-lactones **183**, which upon epimerization under basic conditions gave their corresponding *trans* β-lactones **184** (Scheme 32).[75] This methodology has two major drawbacks; the epimerization step is low yielding and is only applicable for synthesizing substrates with identical substituents (R groups). Regardless, a focused library of β-lactones, including the ketene dimers and *cis*- and *trans*-β-lactones, was assembled (Table 2) and the compounds were evaluated as FAS inhibitors. In general, the *trans*-β-lactones displayed the highest inhibitory activity, but it was *cis* -**183d** that showed the highest potency, ~10 fold lower than orlistat (**166**).



Scheme 32. Ketene dimerization strategy

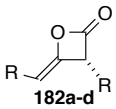
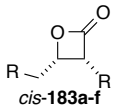
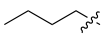
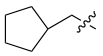
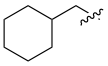
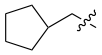
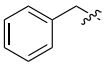
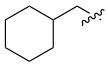
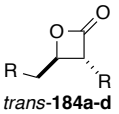
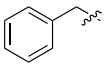
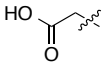
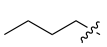
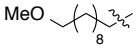
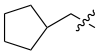
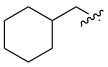
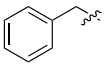
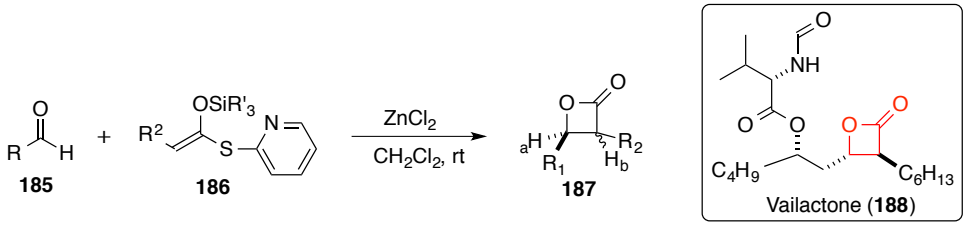
 **182a-d**				 *cis*-**183a-f**			
Entry	Compd.	R	K_i (μM)	Entry	Compd.	R	K_i (μM)
1	**182a**		17± 3	9	orlistat (**166**)		0.28± 0.06
2	**182b**		13 ± 1	10	**183a**		23± 4
3	**182c**		>100	11	**183b**		14± 8
4	**182d**		5.0± 2.1	12	**183c**		7.7 ±0.4
 *trans*-**184a-d**				13	**183d**		2.5± 0.5
Entry	Compd.	R	K_i (μM)	14	**183e**		>100
5	**184a**		4.0± 1.9	15	**183f**		35± 5
6	**184b**		6.7 ± 1.0				
7	**184c**		6.3 ± 0.7				
8	**184d**		3.3 ± 0.2				

Table 2. Inhibitory activity of orlistat inspired β-lactones[75]

The material depleting epimerization step in the ketene dimerization strategy was avoided by developing a second route; a tandem Mukaiyama Aldol lactonization (TMAL) to give *trans* β-lactones with high enantiomeric purities (Scheme 33).[76] This

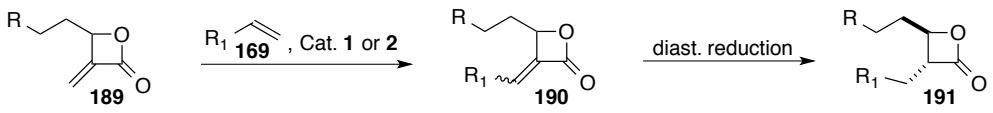
approach is also more suitable for diversification studies. It was used to synthesized orlistat (**166**), some derivatives, and other *trans* β-lactones, such as valollactone (**188**), which has a higher inhibitory activity than orlistat due to its better solubility.



Scheme 33. Tandem Mukaiyama Aldol lactonization (TMAL) approach

2.1.5. Purpose of study

Extensive investigation of the promising therapeutic activities of β-lactones has been limited in scope, partly due to a lack of synthetic methodologies. Encouraged by the success in the preparation of α-alkylidine-β-lactones via CM, we proposed the hydrogenation or diastereoselective 1,4-reduction of α-alkylidine-β-lactones as a strategy for the rapid assembly of β-lactones (Scheme 34). This approach would serve as a simple, efficient and flexible methodology for the synthesis of a library of β-lactones in just two steps from α-methylene-β-lactones. The CM of appropriate α-methylene-β-lactones such as **189** with alkene cross partners **169** would facilitate access to a diverse class of unprecedented α-alkylidine-β-lactones like **190**.



Scheme 34. Proposed approach to β-lactones

In addition to these advantages, the diastereoselective 1,4-reduction of the α-alkylidine-β-lactones would represent the first application of such reductions with this class of exocylic enones. Hence, the successful implementation of this methodology would showcase CM as a valuable approach for disubstituted β-lactone synthesis, and would also demonstrate the synthetic utility of α-methylene-β-lactones.

2.2. Discussion and results

2.2.1. Research objective

The goal of this study is to synthesize a library of β-lactones. The synthesis of (±)-nocardiolactone (**167**), a *trans* β-lactone, was targeted to illustrate the practicality of the methodology. This was a starting point for the FAS inhibitor library development and was encouraged by the considerable inhibitory activities of compounds with simple alkyl side chains examined by Romo and colleagues in their SAR studies (Table 2).

2.2.2. Retrosynthesis to (±)-nocardiolactone (167)

Our plan to synthesize (±)-nocardiolactone (**167**) requires two reactions from α-methylene-β-lactone **194**, a CM with 1-nonadecene (**193**) to give α-alkylidene-β-lactone **192**, and diastereoselective 1,4-reduction (Figure 33). The α-methylene-β-lactone **194** can be constructed from tetradecanal (**195**) following pathway A or B. Both routes require Morita Baylis-Hillman (MBH) and lactonization as the key reactions.

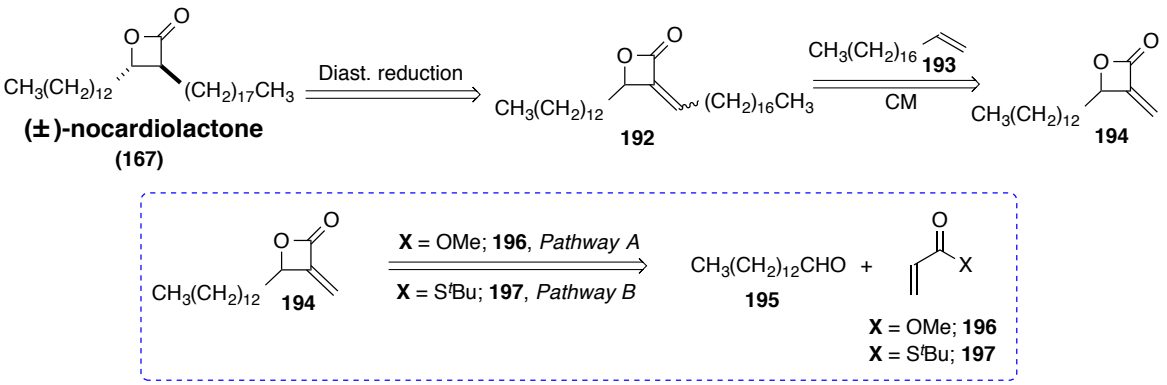
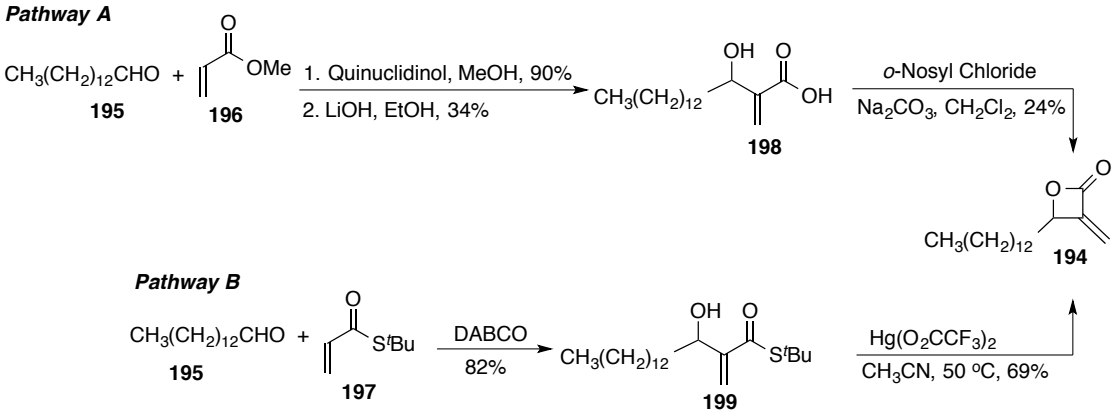


Figure 33. Retrosynthesis of (±)-nocardiolactone (**167**)

2.2.3. Synthesis of (±)-nocardiolactone (167)

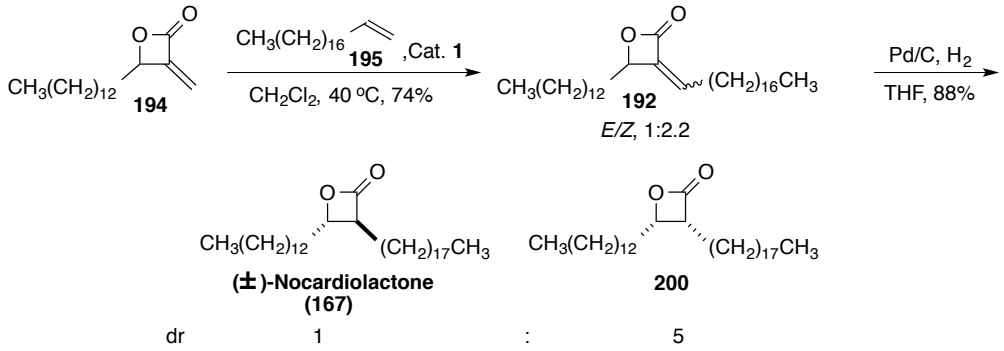
The synthesis of (±)-nocardiolactone (**167**) commenced with the preparation of α-methylene-β-lactone **194** (Scheme 35), initially via pathway A. Quinuclidinol-catalyzed MBH reaction of tetradecanal **195** and methyl acrylate **196** furnished a β-hydroxy ester, which was hydrolyzed with LiOH in EtOH to give acid **198**. *o*-Nosyl chloride mediated lactonization of **198** in the presence of Na_2CO_3 as a base produced **194**. While α-methylene-β-lactone **194** was readily prepared from this approach, pathway B provides it in a more efficient and direct sequence. DABCO catalyzed MBH reaction of tetradecanal **195** and *t*-butyl thioacrylate **197** gave MBH adduct **199** that was converted to **194** in a mercury-facilitated lactonization.



Scheme 35. Synthesis of α-methylene-β-lactone **194**

The prepared α-methylene-β-lactone **194** was then cross-coupled with 1-nonadecene (**193**) using Grubbs 2nd generation catalyst (Cat. **1**) to give (±)-nocardiolactone precursor **192** as a mixture of *Z/E*-isomers (2.2:1) (Scheme 36). Hydrogenation of **192** under standard conditions furnished (±)-nocardiolactone (**167**) and its *cis*-isomer **200**, favoring the *cis*-isomer as expected, in a 1:5 ratio. In

order to obtain (±)-nocardiolactone (**167**) predominantly, diastereoselective reduction conditions were screened next.



Scheme 36. CM to give (±)-nocardiolactone precursor **192** and its hydrogenation

2.2.3.1. Diastereoselective 1,4-reduction to give (±)-nocardiolactone (167)

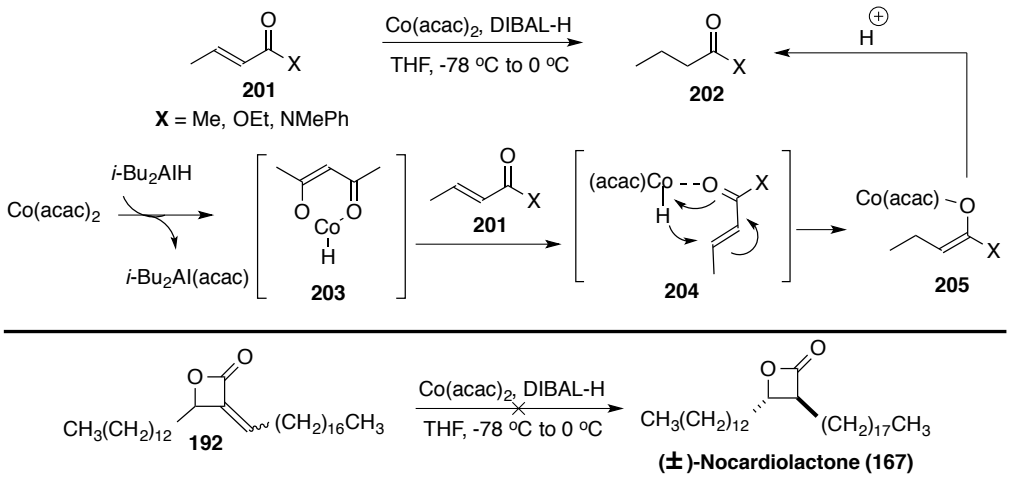
Since there is no literature precedent for the diastereoselective 1,4-reduction of exocyclic enones like **192**, known reduction conditions developed for acyclic enones and endo- and exo-cyclic enones of γ- and δ-lactones were investigated. As a starting point, main group metals shown to chemoselectively participate in 1,4-reduction were explored. Exocyclic enone **192** was subjected to reducing agents such as L- or K-selectride and DIBAL-H (Table 3). L-Selectride (entry 1) gave inconsistent yields upon repetition, and no reduction was observed with K-selectride or DIBAL-H. Mg^{077} catalyzed reduction was also attempted, and this gave opened reduced ester product (entry 4).

Entry	Reducing agent	Solvent	Temp (^oC)	Results
1	L-Selectride	THF	-78	2.5/1 (*trans/cis*) with sm[a]
2	K-Selectride	THF	-78 to rt	No reaction
3	DIBAL-H	THF	-78 to 0	No reaction
4	Mg^0	MeOH	rt	Reduced ring opened product

[a]Results were inconsistent on repetition, and material recovery was low, sm = starting material

Table 3. 1,4-Reduction attempted with main group metals

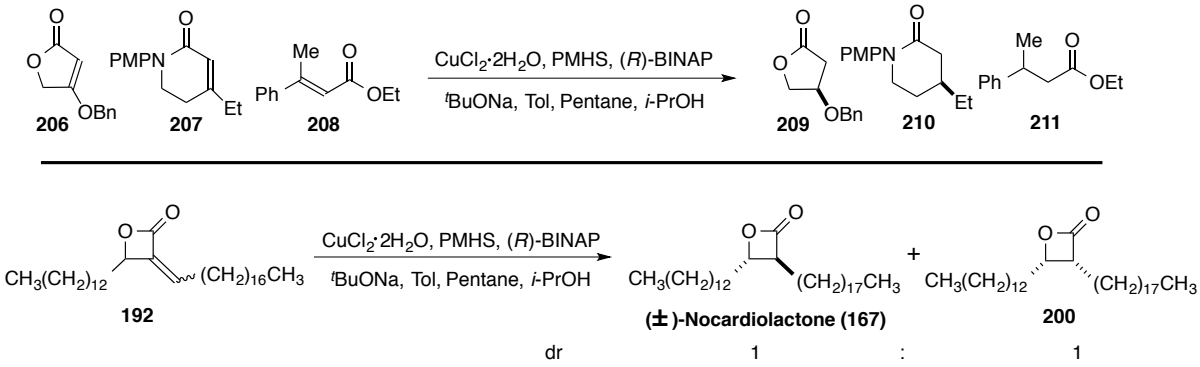
Consequently, a variety of 1,4-reduction conditions utilizing transition metal hydride complexes were explored. Cobalt-hydride complex (**203**), generated *in situ,* was reported by Ikeno *et al.*[78] as a successful reductant of various acyclic enones such **201** (Scheme 37) and was examined here. Unfortunately, when **192** was treated under the same conditions, no reduction occurred. Only the starting material was recovered, even though the observation of the deep red color of **203** confirmed the successful formation of the hydride-complex. Warming the reaction gradually to rt and letting it stir overnight did not have any effect.



Scheme 37. Cobalt-hydride (acetylacetonate) aluminum complex 1,4-reduction

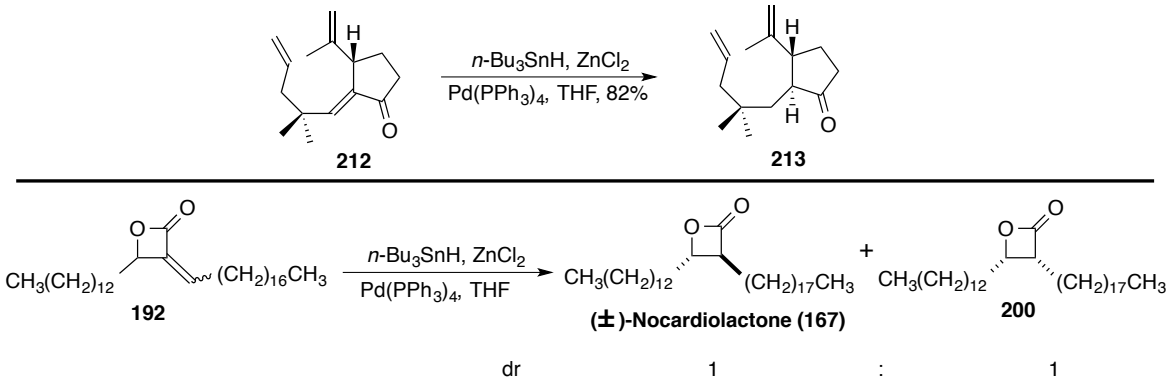
Standard Stryker $[(Ph_3P)Cu\text{-}H]_6$ reducing conditions were also tried. After 2h, ^{1}H-NMR analysis showed only the presence of starting material. Switching to the more reactive hot-Stryker reduction conditions utilized by Buchwald[79] in the diastereoselective reduction of enones of γ-lactone **206**, δ-lactam **207** and acylic enone **208** (Scheme 38) gratifyingly gave our first successful reduction. However, there was only 50% conversion and no diastereoselectivity. Purification wasn't

performed to isolate the products because the starting material (*E/Z* isomers) and reduced products (*cis/trans* isomers) were inseparable on TLC despite varying solvent systems.



Scheme 38. Hot Stryker 1,4-reduction

Next, Bu_3SnH catalyzed selective hydrogenation in the presence of $ZnCl_2$ and $Pd(PPh_3)_4$, used by Nakata and co-workers[80] for the reduction of exocyclic enone **212** to **213** in their synthesis of polymaxenolides, was tested (Scheme 39). This reduction was anticipated to proceed via a palladium-catalyzed hydrostannation, followed by kinetic protonation. Although the reaction worked, the outcome was similar to the hot-Stryker conditions, and the approach was not pursued further.



Scheme 39. Bu_3SnH catalyzed 1,4-reduction

After trying several more reduction conditions, $NaBH_4$ catalyzed conjugate reduction was studied. Iwasaki and co-workers have reported 1,4-reduction of α-alkylidene-γ-lactones **214** by $NaBH_4$ in the presence of transition metals (Co, Ni and Cu) (Figure 34).[81] The high diastereoselectivity of the reaction favoring *trans* isomer **216** attracted our attention. This was as a result of the stereoselective protonation of enolate **215**, rationalized by the authors on the basis of conformational 1,3-allylic strain.

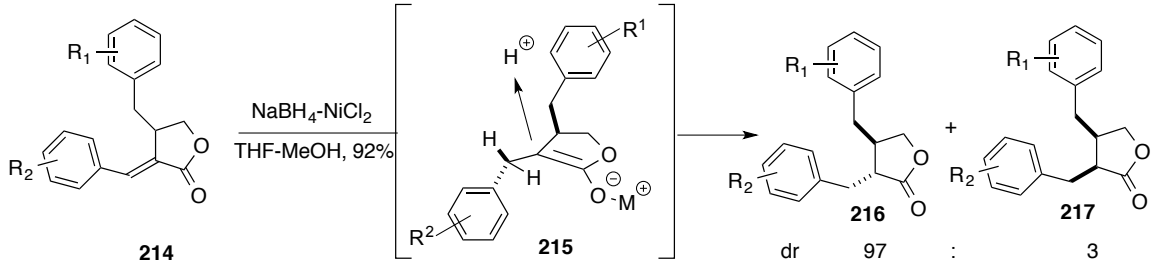


Figure 34. Iwasaki and co-workers procedure for reduction of exocylic enone **214**

Protonation of enolate **218** upon conjugate addition of hydride unto **192** would govern the selectivity of the reduction of α-alkylidene-β-lactones (Figure 35). Delivery of the proton from the top face of **218** would give (±)-nocardiolactone (**167**), while protonation from the bottom face would give the *cis*-siomer **200**. The latter would be projected to be predominant as a result of steric effects from the C4-substituent, hindering protonation from the top face. To achieve facially selective proton delivery to **218** to favor (±)-nocardiolactone (**167**), other factors that could govern the reaction outcome, such as catalyst (and ligands attached), proton source and temperature were systematically studied. The analysis began with a screening

of transition metals, and cobalt emerged as the best, rather than nickel as reported by Iwasaki. Hence further optimization studies were carried out with cobalt catalysts.

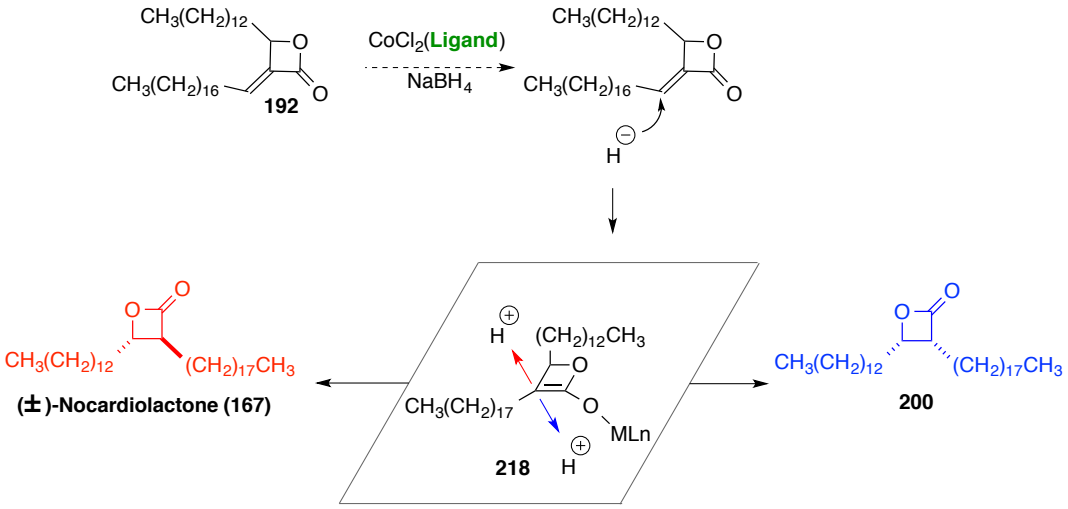
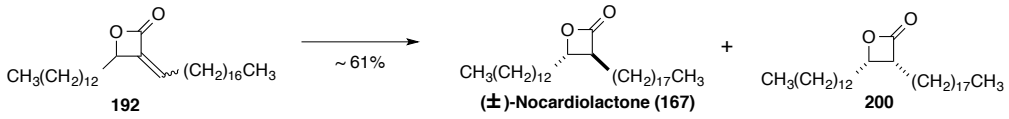


Figure 35. Proposed mechanism to obtained (±)-nocardiolactone (**167**)

The ligands on the catalyst were screened first, as depicted in Table 4 (entries 1, 2 and 5). The use of $Co(PPh_3)_2Cl_2$ (entry 5) gave the best diastereoselectivity. The temperature effect was investigated next. (±)-Nocardiolactone (**167**) being the thermodynamically more stable product, it was envisioned that perhaps elevating the reaction temperature would favor its formation. Unfortunately this hypothesis couldn't be successfully tested with MeOH (which acts both as solvent and proton source). The reactions done at elevated temperatures (0 ^{o}C or higher) in MeOH gave reduced opened lactone product (entry 3), as a result of MeOH being involved in nucleophilic addition to the lactone ring. Substituting MeOH with *i*PrOH or ^{t}BuOH, bulky and non-nucleophilic proton sources prevented the formation of the open ester product, providing (±)-nocardiolactone (**167**) and its *cis*-isomer in a 2:1 ratio

(entry 4 and 6). However, the reaction time (entry 4, reaction time not shown) was longer, and DMF (entry 6) was difficult to remove. The used of BINOL (entry 7), a chiral proton source caused a slight drop in the diastereoselectivity. The combined experimental results lead to entry 5 being selected as the optimized reduction conditions. Although the 2:1 diastereoselectivity ratio wasn't as high as desired, the first total synthesis of (±)-nocardiolactone (**167**) using CM and diastereoselective 1,4-reduction reactions was now completed.



Entry	Catalyst	Solvent	Temperature (°C)	Hydride	Results
1	$CoCl_2.6H_2O$	THF : MeOH (4 : 1)	-5	$NaBH_4$	2:1 *cis/trans*
2	$Co(acac)_2$	THF : MeOH (4 : 1)	-5	$NaBH_4$	2:3 *cis/ trans*
3	$Co(acac)_2$	THF : MeOH (4 : 1)	0	$NaBH_4$	1:4 *cis/trans* with opened ring
4	$Co(acac)_2$	THF : *i*-PrOH (4 : 1)	0	$NaBH_4$	1:2 *cis/trans*
5	$Co(Ph_3P)_2Cl_2$ (18 mol%)	THF : MeOH (4 : 1)	-5	$NaBH_4$	1:2 *cis/trans*
6	$Co(Ph_3P)_2Cl_2$ (18 mol%)	DMF : CH_2Cl_2: ^{t}BuOH (4 : 3 : 1)	0	$NaBH_4$	1:2 *cis/trans*
7	$Co(Ph_3P)_2Cl_2$ (18 mol%)	DMF : CH_2Cl_2: (R)-BINOL (4 : 3 : 1)	0	$NaBH_4$	1:1.3 *cis/trans*

Table 4. Screening of cobalt catalysts for optimum reduction conditions

2.2.3.2. Synthesis of C3-analogs of (±)-nocardiolactone (167)

The synthesis of (±)-nocardiolactone C3-analogs was undertaken after its successful preparation. In order to target a more comprehensive library, the x-ray structure of orlistat (**166**) in the thioesterase (TE) domain of FAS was examined.

The TE domain has four chambers (Figure 36a) in which it accommodates orlistat, namely the specificity channel (hosts the long chain of orlistat), the short-chain pocket (houses the short hexanoyl side chain), an interface cavity (fits the amide moiety) and an oxyanion hole (holds the carbonyl group). Because varied substituents at the C-3 position could reside in the short-chain pocket, it was targeted for the diversification studies. Substrate studies showing the optimum carbon chain for the specificity channel to be C-16 makes it less important for novel structure activity relationship (SAR) studies, reinforcing our choice of targeting the short chain pocket. When the short-chain pocket was viewed using PyMOL (a molecular visualization system), we noticed that the pocket has enough room to optimally fit a six-carbon chain, as in orlistat. Although the pocket was small, seemingly limiting the chain length of substituents at the C-3 position, the presence of certain amino acids residues couldn't be over looked, because specific interactions between these and the inhibitor might result in greater activity. Three amino acids residues (Thr2342, Tyr2343 and Tyr2462) were found to be in close proximity to the hexanoyl tail of orlistat, also seen in the stereo view reported as by Pemble *et al.*[74] (Figure 36b).

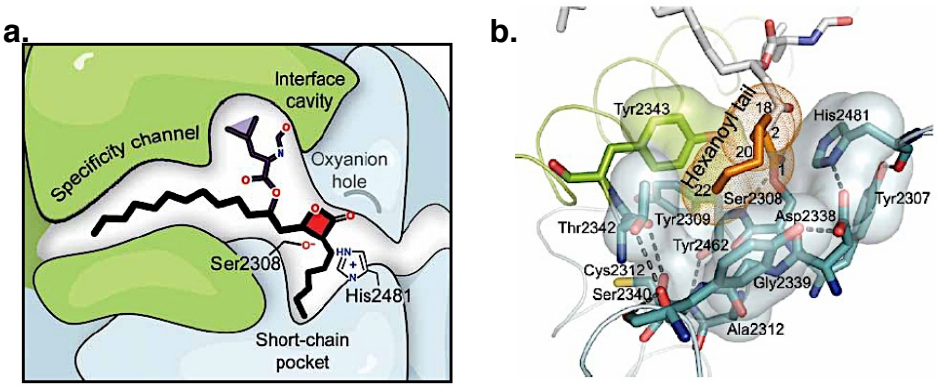
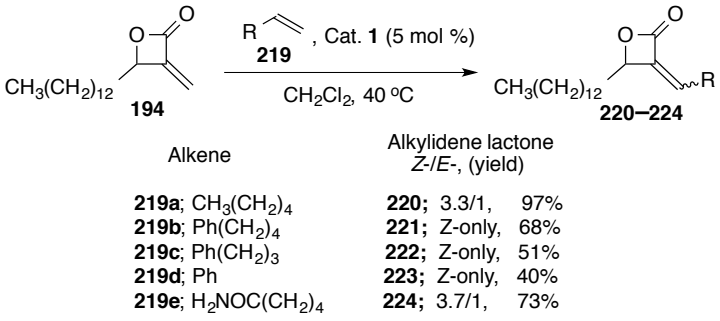


Figure 36. a. Chambers of the thioesterase domain (TE) of FAS; **b.** stereo view of the short-chain pocket of the TE[74]

These provided us with an insight for selecting substituents for our first diversification studies. Five additional alkene cross partners (**219a**–**219e**) were targeted including 1-heptene **219a** to give a C-3 hexanoyl analog of nocardiolactone to compare with orlistat, three compounds (**219b**–**219d**) that have terminal aromatic moieties with varying carbon chain lengths to tap into possible π-π stacking interactions with the tyrosine residues (Tyr2343 and Tyr2462) and one substituent with a terminal amide group (**219e**) for potential H-bonding interaction with amino acid residues (Thr2342, Tyr2343 and Tyr2462).

The CM reactions of **194** with the selected alkene cross partners (**219a**–**219e**) furnished seven novel α-alkylidene-β-lactones (*Z/E*-**220**, *Z*-**221**–*Z*-**223** and *Z/E*-**224**) in high *Z*-selectivity with the phenyl substituents gave the *Z*-isomers specifically (Scheme 40). The *Z/E* isomers of **220** were separable but the *Z/E* isomers of **224** were not fully separated, its *Z*-isomer was isolated pure after multiple column chromatography on silica gel. A 1:1 mixture of the *Z/E* isomers of **224** was included

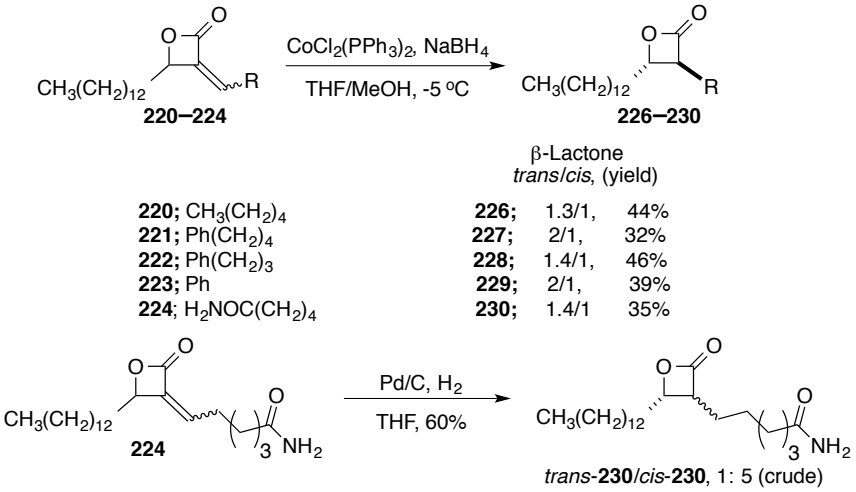
in the library to see if the presence of the *E*-isomer would show any noticeable activity in comparison to the pure *Z*-isomer.



Preparation of **219e**

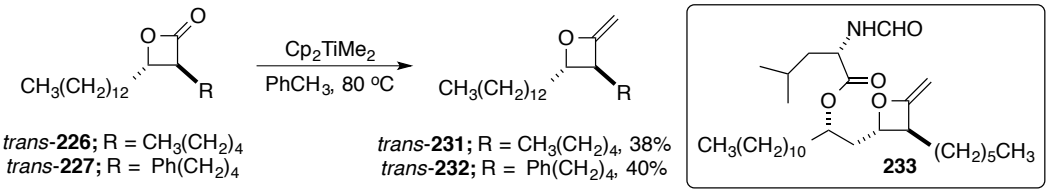
Scheme 40. Synthesis of α-alkylidine-β-lactones

The α-alkylidene-β-lactones (**220**–**224**) were subjected to the optimized reduction conditions to give their corresponding reduced β-lactone products (*trans/cis*-**226**–*trans/cis*-**230**) (Scheme 41), providing nine additional novel compounds. The *trans/cis* products (**226**–**229**) were separated but not the *trans/cis* isomers of **230**. This was evaluated as a mixture (*trans/cis*, 80:20) after partial purification. The *cis*-isomer (*cis*-**230**) was obtained with negligible *trans*-**230** from hydrogenation after column chromatography.



Scheme 41. Synthesis of reduced β-lactones

Additionally, *trans*-**226** and *trans*-**227** were methylenated to provide extra probes **231** and **232** respectively (Scheme 42). This was inspired by the earlier findings of Dollinger *et al*. of the comparable inhibitory activity of orlistat and its 2-methyleneoxetane analog **233** in a porcine pancreatic lipase (PPL) assay.[82]



Scheme 42. 2-Methyleneoxetane analogs of *trans* β-lactones

Thus, overall a library of 22 β-lactones was readily assembled for further evaluation from a single α-methylene-β-lactone **194** scaffold (Figure 37). This proves CM as a versatile approach for the rapid assembly of β-lactones.

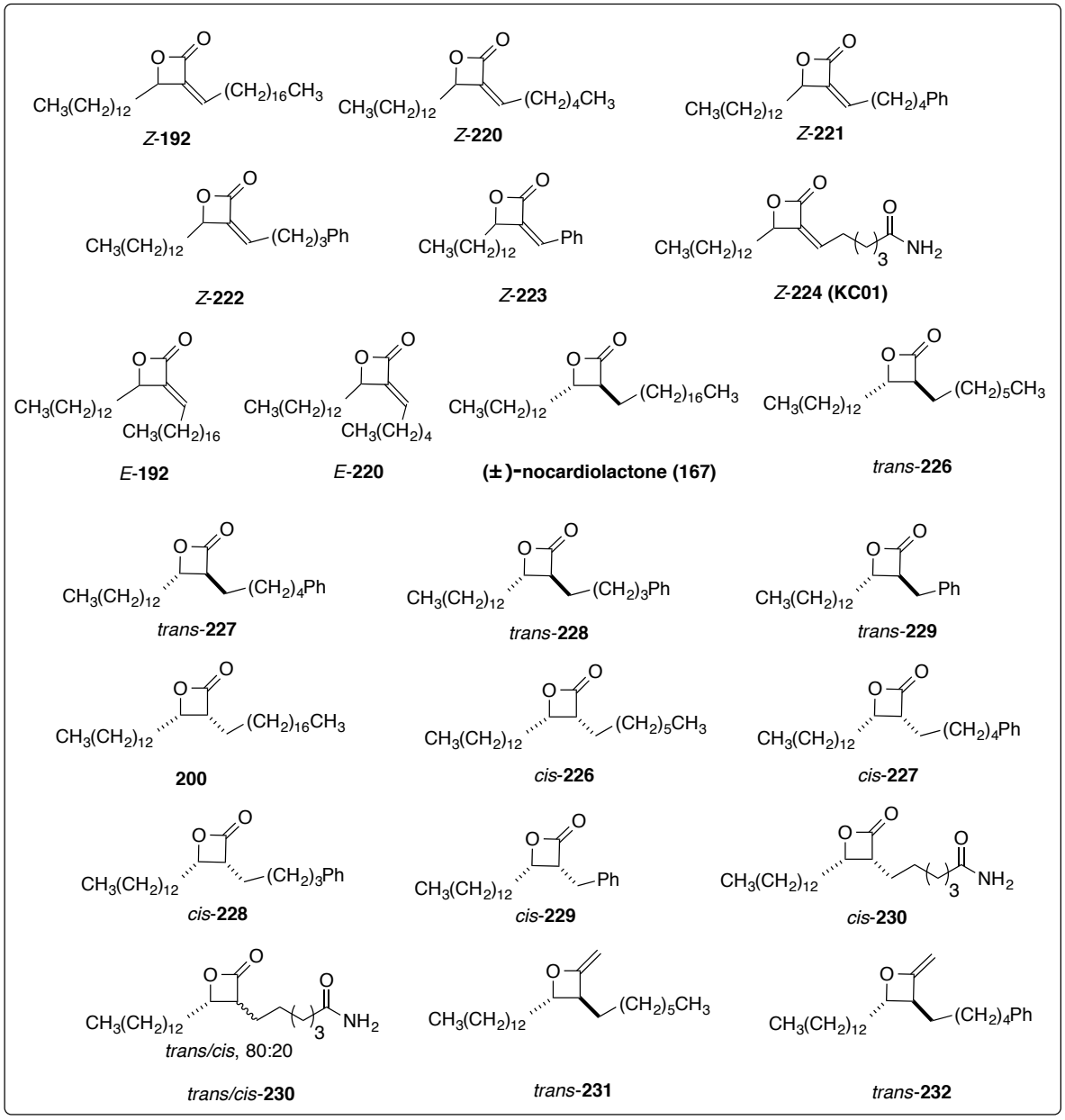


Figure 37. A library of 22 β-lactone chemical probes

2.2.4. Results:

2.2.4.1. Inhibitory activity of β-lactone library against serine hydrolases

The prepared β-lactone library was sent to the Cravatt lab at the Scripps Research Institute to be assayed as serine hydrolases inhibitors using competitive activity-based protein profiling (ABPP). Since β-lactones have been shown to inhibit several serine hydrolase enzymes (characterized by the presence of a serine moiety in the active site that acts as a nucleophile and interacts with electrophiles),[83] it was advantageous to screen our library against a broad spectrum of enzymes in a native biological sample, facilitating the targeting of different enzymes in a single screen. A powerful feature of ABPP is that it can be coupled to either LC/MS or gel readout to characterize numerous enzymes in parallel.[84] Figure 38,[85] shows a schematic workflow in ABPP. Known inhibitors of enzymes are added to a native biological sample as control vehicles to minimize unwanted interactions. The resulting mixture is then incubated with the chemical probes, which may bind selectively to the active site of enzymes. Gel or LC/MS reading allows detection and identification of enzymes in the biological samples. Faded spots in the gel or a decrease in bar sizes in LC/MS reflect enzymes targeted.

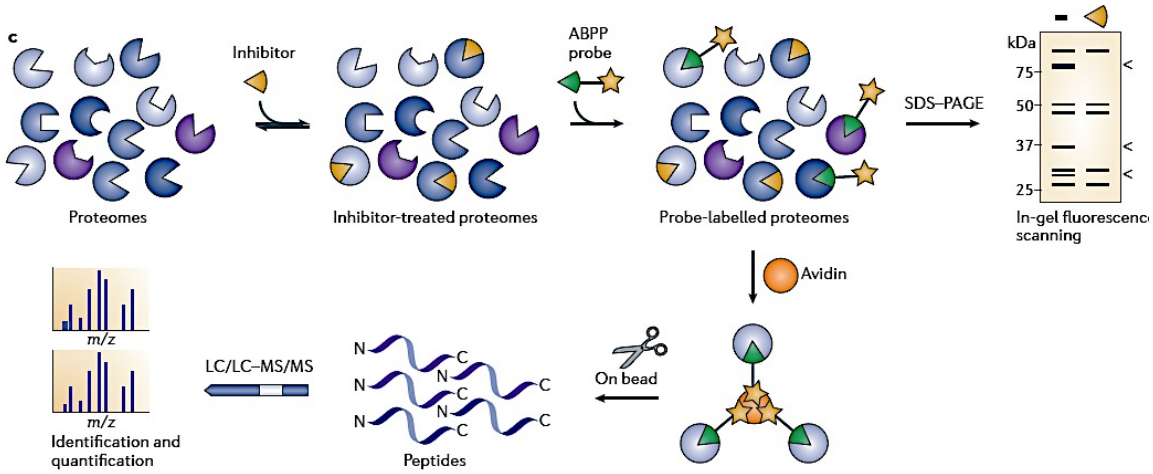


Figure 38. Schematic diagram of ABPP[85]

The inhibitory activity analysis of the prepared library probes using ABPP has worked in our favor, because when assayed with mouse membrane proteome none of the compounds except *trans*-**229** (KC6-10-3) showed comparable activity to orlistat (THL) against FAS (FASN) (Figure 39a). Instead, most of the β-lactones targeted other enzymes, and *trans*-**229** showed the broadest activity. Similar results were observed when β-lactones were profiled using the human membrane proteome (Figure 39b).[86] A stable isotope labeling of amino acids in cell culture (SILAC) study was conducted with *trans*-**229** to identify the inhibited enzymes (Figure 39c). Eight serine hydrolases were identified and divided into two groups based on their inhibition level. The first group included ABHD16A, PNPLA4, FAAH, ABHD2, CES2, and ABHD12 (all inhibited > 90%) and a second set was made up of FASN and ABHD3 (inhibited from 70-90%) at a final concentration of 25 μM. Among these enzymes, only FAAH and FASN, to our knowledge are well documented in

terms of biological functions and already have selective inhibitors suitable for cellular or *in vivo* pharmacology studies.

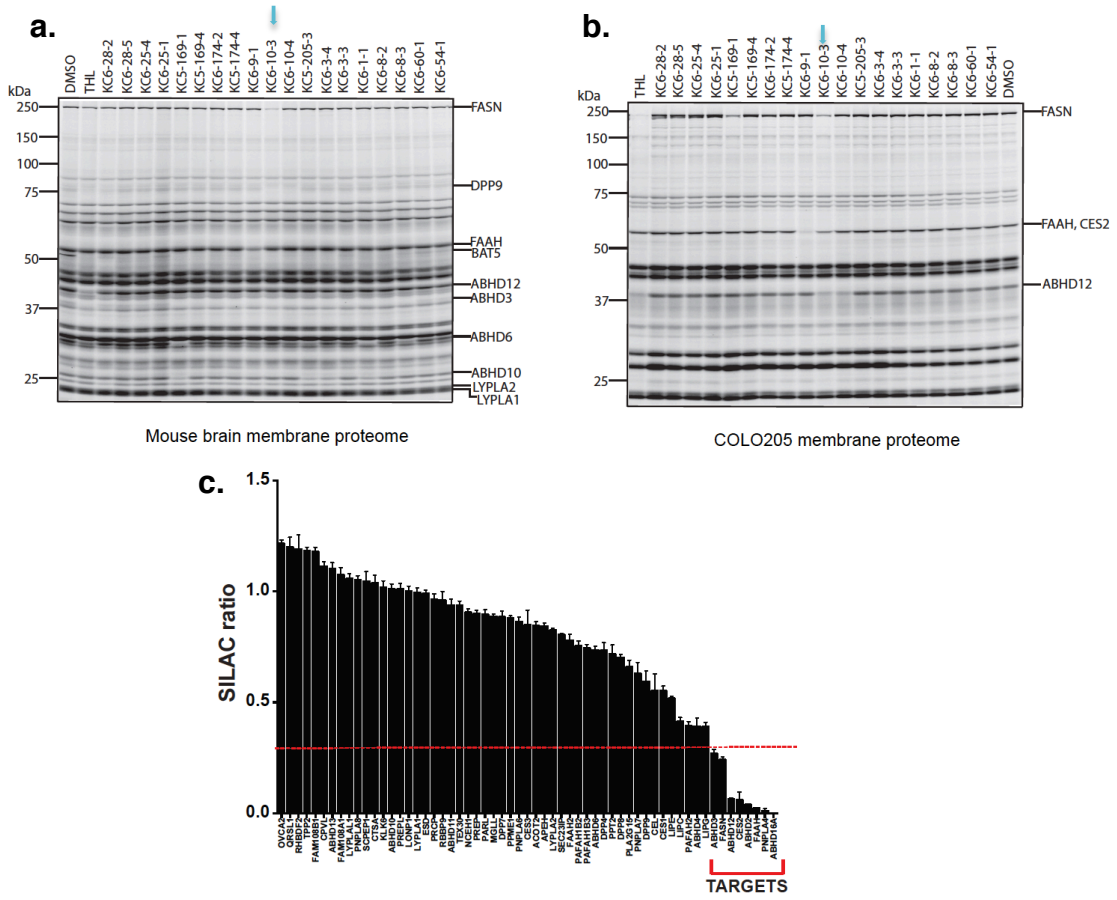


Figure 39. Identification of enzymes; **a.** screening of β-lactones against mouse brain membrane proteome; **b.** β-lactones against human membrane proteome; **c.** SILAC study with *trans*-**229** (KC6-10-3)[86]

ABHD16A (also called BAT5) was the most strongly inhibited enzyme and *Z*-**224** (KC01) was its most potent inhibitor (Figure 40).[87] ABHD16A is hypothesized to generate lysophosphatidylserine (lyso-PS), an inflammatory lipid in mammalian systems (specifically in the brain), and is a highly desirable target for immunological and neuroimmunological studies. *Z*-**224** (KC01) is the first reported potent inhibitor of ABHD16A, suggesting an α-alkylidene-β-lactone with a polar side chain could be

a useful starting point for developing selective inhibitors that target a diverse range of other poorly characterized serine hydrolases.

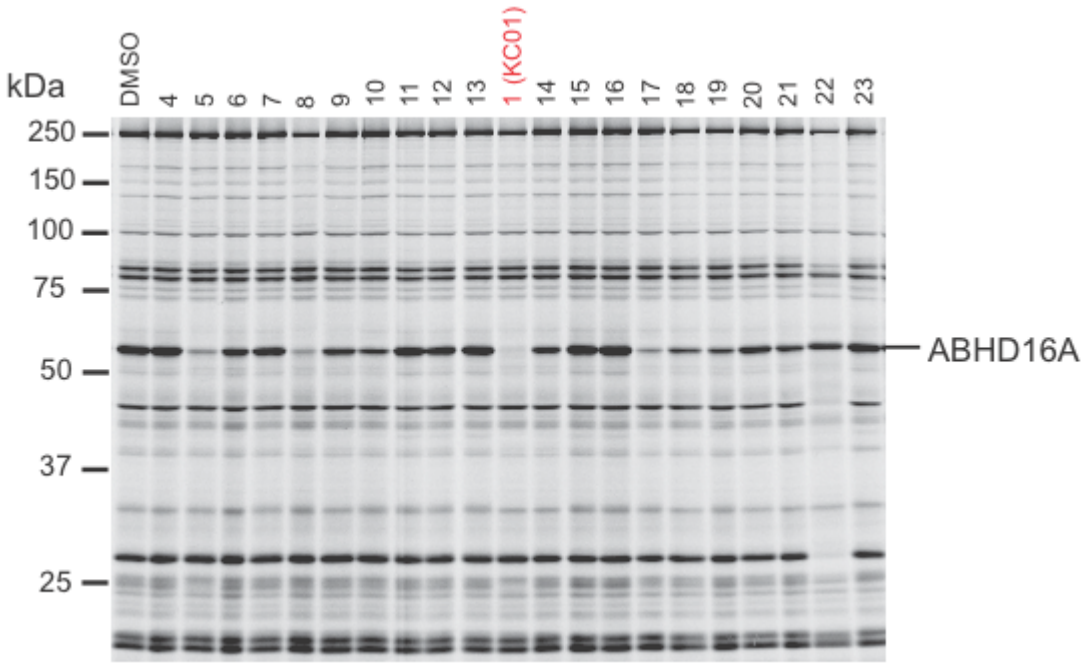


Figure 40. Screening of β-lactones against human ABHD16A by competitive ABPP[87]

To further develop *Z*-**224** (KC01) inhibitory activity, four structural analogs were generated (**234**–**237**) furnishing a second library (Figure 41). Their synthesis is shown in Scheme 43, following similar reaction sequences used in the preparation of the first library.

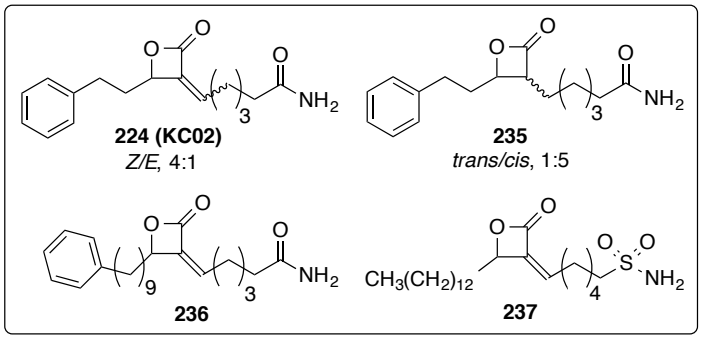
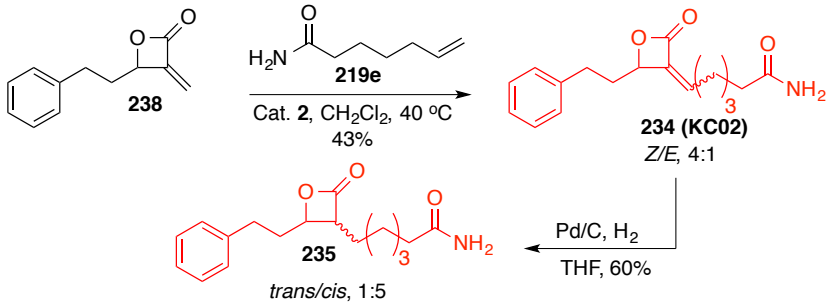


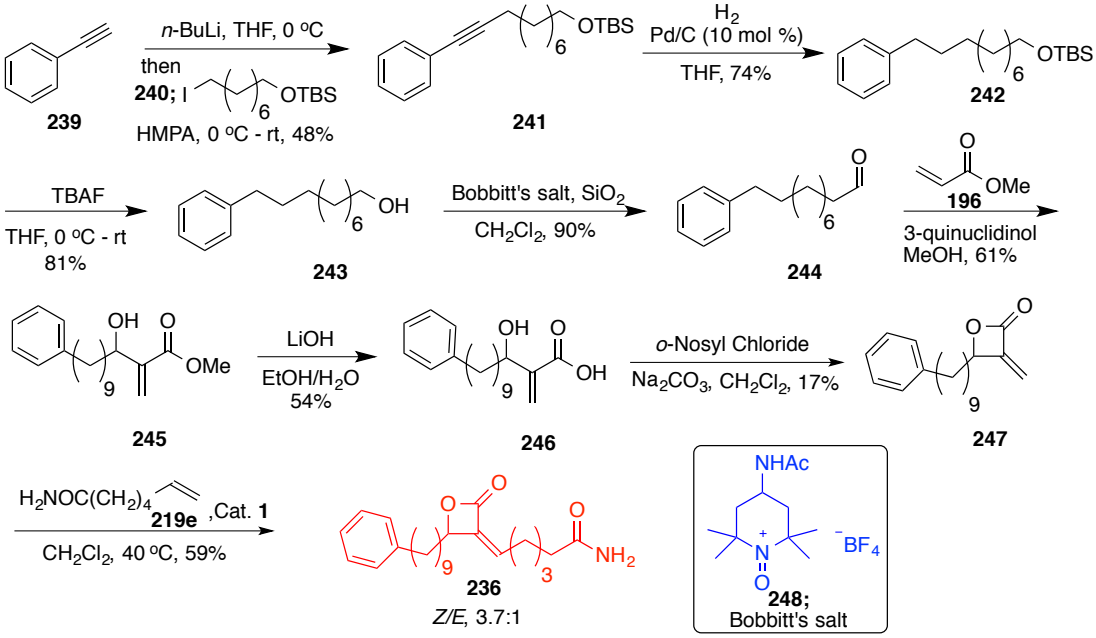
Figure 41. Chemical probe analogs of *Z*-**224** (KC01)

CM of α-methylene-β-lactone238 (obtained from Christain Malapit a graduate in our lab) with alkene cross partner **129e** yielded **234** (KCO2) as a mixture of *Z/E*-isomers (4:1 ratio) inseparable (Scheme 43). Therefore the isomers were evaluated as a mixture. Hydrogenation of **234** gave its corresponding reduced lactone **235** as a mixture of *trans/cis*-siomers (1:5 ratio) again inseparable and tested as a mixture. Because aldehyde **244**, required to prepare α-methylene-β-lactone **247** is not commercially available it was prepared starting with the alkylation of phenylacetylene with iodide **239** to give alkylated product **241**. This product was subjected under hydrogenation conditions to give its alkane analog **242**. Desilylation of the hydroxyl group using TBAF gave alcohol **243**, which was oxidized to aldehyde **244** in the presence of Bobbitt's salt (**248**) (courtesy of Dr. Bobbitt) and SiO_2. Aldehyde **244** was reacted with methyl acrylate under MBH conditions catalyzed by 3-quinuclidinol to afford MBH adduct **245**. LiOH facilitated hydrolysis of methyl ester **245** offered β-hydroxy acid **246**. Lactonization of **246** facilitated by *o*-nosyl chloride yielded α-methylene-β-lactone **247**, and its subsequent CM with **219e** gave α-alkylidene-β-lactone **245** as a mixture of *Z/E*-isomers. The *Z*-isomer **236** was obtained pure. The sulfonamide α-alkylidene-β-lactone **237** synthesis began with the preparation of cross partner **250** in three steps from 1-bromo-6-heptene **249**. The displacement of the bromide in **249** with Na_2SO_3 gives a sulfonic acid intermediate. Chlorination of the acid with phosphorus oxychloride provided a sulfonyl chloride product, aminolysis of which using NH_4OH finally gave sulfonamide **250**. The CM reaction of α-methylene-β-lactone **194** and **250** completed the

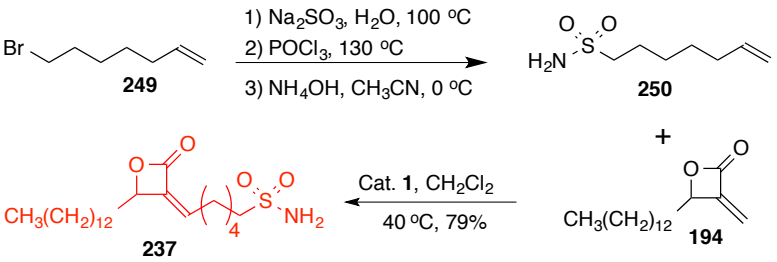
synthesis of **237** Both of its *Z/E*-isomers were formed and the pure *Z*-isomer was isolated using column chromatography.



Synthesis of β-lactone probes **234** and **235**



Synthesis of β-lactone probe **236**



Synthesis of β-lactone probe **237**

Scheme 43. Synthesis of analogs **234–237**

Assessment of the β-lactones (**234–237**) for the inhibition of ABDH16A led to the identification of an inactive control probe **234** (KC02, tested as a *Z/E*, 4:1 mixture), (Figure 42 a and b). A SILAC study confirmed that *Z*-**224** (KC01) is a potent, selective probe of ABHD16A (Figure 42c), indicated by the complete disappearance of the ABHD16A bar. Reduction in lyso-PSs in the human cancer cell line (COLO205 colon) was also observed as a result of *Z*-**224** (KC01) inhibiting ABHD16A, providing compelling pharmacological and genetic evidence that ABHD16A is a major enzyme responsible for generating lyso-PSs in mammalian cells (Figure 42d).

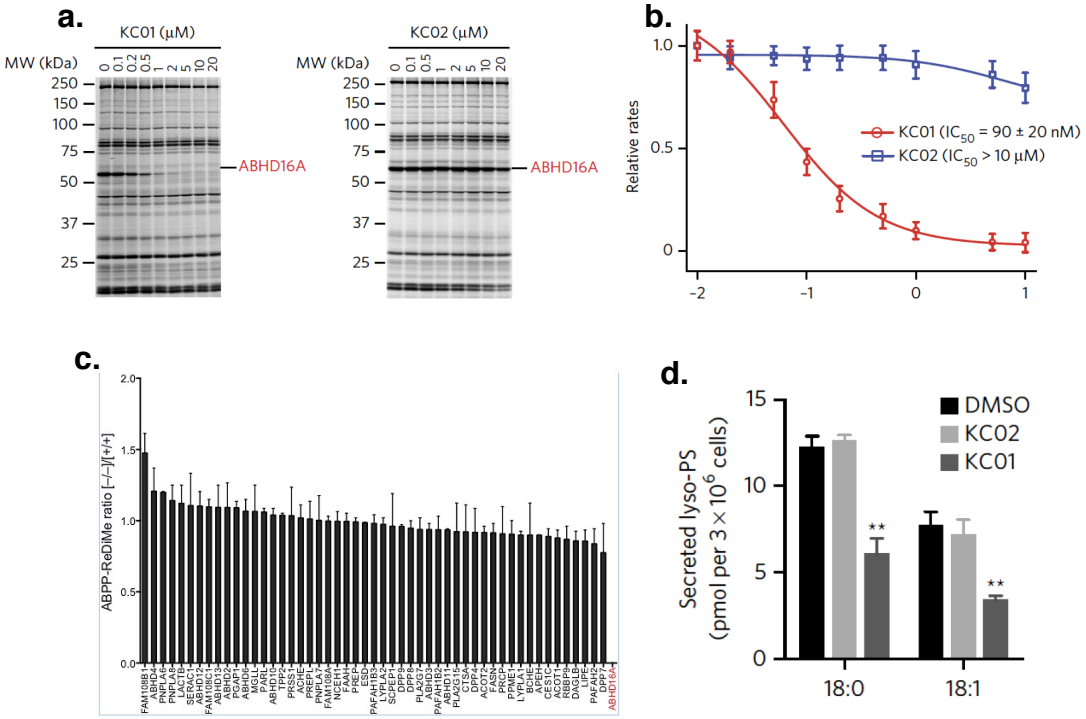


Figure 42. α-alkylidene-β-lactone *Z*-**224** (KC01) a selective inhibitor of ABHD16A; **a.** Gel spectrum of ABHD16A inhibitor *Z*-**224** (KC01) and its inactive control probe **234** (KC02); **b.** Concentration-dependent inhibition of the PS lipase activity of ABHD16A with *Z*-**224** (KC01) and **234** (KC02); **c.** Quantification of ABHD16A inhibiton with *Z*-**224** (KC01) using SILAC; **d.** Concentration of lyso-PS from COLO205 colon cancer cells treated *in situ* with inhibitors *Z*-**224** KC01 and **234** (KC02)[87]

2.2.5. Conclusion

In summary, a diverse set of 23 mostly new β-lactones including (±)-nocardiolactone (**167**) has been prepared from a single α-methylene-β-lactone scaffold **194**. The characterization of the library using competitive ABPP facilitated the discovery of previously uncharaterized serine hydrolase enzymes. Thus, ABPP amplifies the significance of a CM approach for discovering new chemical probes for enzyme inhibition in cell biology. This study is an initial tool for the identification/characterization of serine hydrolase enzymes, many of which are poorly understood in terms of the role they play in immunological functions, due to lack of suitable selective inhibitors for their characterization and in vivo analysis.

2.2.6. EXPERIMENTAL

2.2.6.1. General Experimental

Tetrahydrofuran (THF) was dried using a solvent dispensing system (SDS) with a column of neutral alumina. Pyridine, toluene, dimethylformamide (DMF), methylene chloride (CH_2Cl_2), deuterated chloroform ($CDCl_3$), methanol (MeOH), deuterated methanol (CD_3OD) and ethanol (EtOH) were dried over 4 Å molecular sieves.

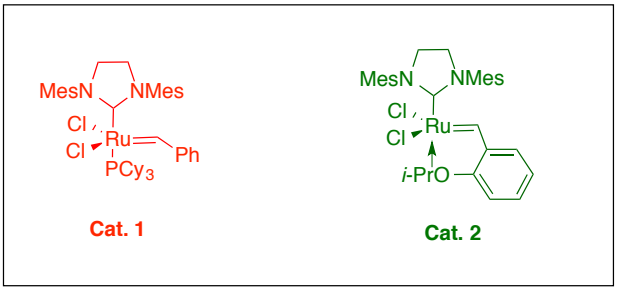
All reagents were purchased from Acros, Aldrich or Alfa Aesar and used without further purification. All reactions were conducted under an atmosphere of N_2 in glassware that had been dried overnight in an oven at 120 ^oC. Where appropriate, control of the reaction temperature was achieved with a solid CO_2/acetone bath, an ice bath or a heated oil bath.

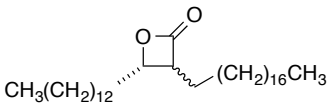
NMR Spectra were obtained on a Bruker Avance DRX-400 (400 MHz ^1H, 100 MHz ^{13}C), Bruker Avance (500 MHz ^1H, 125 MHz ^{13}C), or Bruker Avance (300 MHz ^1H, 75 MHz ^{13}C) spectrometer. ^1H and ^{13}C chemical shifts are reported in parts per million (ppm) and calibrated to the residual $CHCl_3$ peak at 7.26 and 77.23 respectively in $CDCl_3$. The following abbreviations are used for peak multiplicities: s (singlet); br s (broadened singlet); d (doublet); t (triplet); q (quartet); quin (quintet); m (multiplet); dd (doublet of doublet); ddd (doublet of doublet of doublet). Coupling constants, *J*, are reported in Hertz (Hz).

IR spectra were recorded on a Brucker FT-IR spectrometer. GC/MS spectra were obtained on a gas chromatograph equipped with a HP-1 methyl siloxane column and detected on a low-resolution 5970 series mass selective detector. High-

resolution mass spectra were obtained on a AccuTOF instrument equipped with a DART ionization source. Melting points were observed in open Pyrex capillary tubes and are uncorrected. Flash chromatography was performed on Silica Gel, 40 micron, 32-63 flash silica. Thin layer chromatography was performed on silica gel. Compounds were visualized by UV, 5% phosphomolybdic acid in ethanol, 0.5% potassium permanganate in water or a solution of ethanol/H_2SO_4/AcOH/*p*-anisaldehyde (135:5:1.5:3.7).

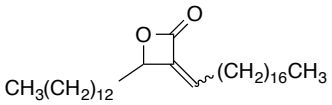
Catalyst used in olefin cross metathesis





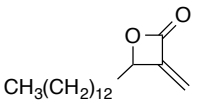
(cis/trans)*-3-Octadecyl-4-tridecyloxetan-2-one (*trans*-167/*cis*-200).** 3-Octylidene-4-tridecyloxetan-2-one (0.10 g, 0.20 mmol) (Z/E*-192**), was dissolved in a mixture of THF:MeOH (1.6:0.32 mL). This solution was cooled to −10 ˚C, followed by the addition of $CoCl_2(PPh)_3$ (0.02 g, 0.04 mmol) and then portion-wise addition of $NaBH_4$ (39.0 mg, 1.2 mmol) within 10 min.[81] The mixture was vigorously stirred for 2 h between −7 and −5 ˚C. The reaction mixture was filtered through a pad of celite, and the celite was then washed with $CHCl_3$ (10 mL). The filtrate was washed with 2M HCl (10 mL), dried ($MgSO_4$) and concentrated.

Purification by flash chromatography on silica gel (petroleum ether/ EtOAc, 98:2) gave a mixture of ***trans*-167/*cis*-200** (*trans*/*cis* 2/1), (0.06 g, 61%) as a white solid. The isomers were separated by careful chromatography using the same solvent system. ***cis*-200:** mp 63–64 ˚C; IR (neat) 2916, 2849, 1790, 1464, 1146, 1076 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (ddd, *J* = 9.9, 6.3, 4.1 Hz, 1H), 3.59 (ddd, *J* = 8.1, 6.9, 6.9 Hz, 1H), 1.82–1.72 (m, 2H), 1.69–1.55 (m, 2H), 1.52–1.48 (m, 2H), 1.39–1.26 (m, 52H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 76.0, 52.9, 32.1, 30.4, 29.9, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.6, 29.5, 27.8, 25.8, 24.2, 22.9,14.3; MS (EI) *m/z* 463 $(M - CO_2)^+$, 281, 207, 111, 97(100), 83, 57; HRMS (ESI) calcd for $C_{34}H_{67}O_2$ $[M + H]^+$ *m/z* 507.5136, found 507.5158. ***trans*-167; (±)-nocardiolactone**: mp 64–65 ˚C (Lit.[88] 66–68 °C); IR (neat) 2915, 2847, 1796, 1468, 1154, cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (ddd, *J* = 6.8, 6.8, 3.9 Hz, 1H), 3.17 (ddd, *J* = 10.3, 6.5, 4.0 Hz, 1H), 1.89–1.79 (m, 2H), 1.76–1.66 (m, 2H), 1.45–1.25 (m, 54H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 78.4, 56.4, 34.7, 32.1, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 28.1, 27.2, 25.2, 22.9, 14.3; MS (EI) *m/z* 462 $(M - CO_2)^+$, 281, 207, 111, 97 (100), 83, 57; HRMS (ESI) calcd for $C_{34}H_{67}O_2$ $[M + H]^+$ *m/z* 507.5136, found 507.5157.



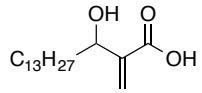
(*E/Z*)-3-Octadecylidene-4-tridecyloxetan-2-one (***Z/E*-192**)**.** Cat. **1** (40 mg, 0.047 mmol) was added to a solution of 3-methylene-4-tridecyloxetan-2-one (**194**) (0.25 g, 0.94 mmol) and 1-nonadecene (**195**) (0.38 g, 1.4 mmol) in dry CH_2Cl_2 (46 mL).

The mixture was stirred overnight at 40 ˚C.[65] The next day ^{1}H NMR showed complete consumption of **194**. The reaction was allowed to cool to rt followed by removal of CH_2Cl_2 under reduced pressure to yield a brownish residue. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) gave ***Z/E*-192***,* (*Z/E*, 2.2/1), (0.56 g, 75%) as a white solid. The isomers were separated by careful chromatography using the same solvent system. ***E*-192:** mp 49–50 ˚C; IR (neat) 2917, 2849, 1791, 1466, 1127 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.33 (dt, *J* = 7.9, 1.4 Hz, 1H), 4.99 (m, 1H), 2.10 (dt, *J* = 7.4, 7.4 Hz, 2H), 2.00–1.88 (m, 1H), 1.82–1.72 (m, 1H), 1.52–1.43 (m, 4H), 1.37–1.26 (m, 48H), 0.88 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 137.8, 134.2, 79.4, 33.5, 32.1, 29.9, 29.9, 29.9, 29.9, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.1, 28.6, 24.9, 22.9, 14.3; MS (EI) *m/z* 194,117, 91 (100), 77, 65, 51; HRMS (ESI) calcd for $C_{34}H_{65}O_2$ $[M + H]^+$ *m/z* 505.4957, found 505.5003. ***Z*-192:** mp 61.5–62.5 ˚C; IR (neat) 2914, 2848, 1795, 1471, 1117, 1070 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 (t, *J* = 7.5 Hz, 1H), 4.85 (t, *J* = 6.3 Hz, 1H), 2.48 (dt, *J* = 7.4, 7.4 Hz, 2H), 1.86–1.73 (m, 2H), 1.47–1.39 (m, 4H), 1.35–1.26 (m, 48H), 0.88 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 137.7, 136.5, 78.9, 34.0, 32.1, 29.9, 29.9, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.5, 29.3, 29.1, 29.1, 24.8, 22.9, 14.3; MS (EI) *m/z* 460 $(M - CO_2)^+$, 355, 341, 293, 281, 236, 207 (100), 194, 110, 95, 81, 67, 55; HRMS (ESI) calcd for $C_{34}H_{65}O_2$ $[M + H]^+$ *m/z* 505.4957, found 505.4957.



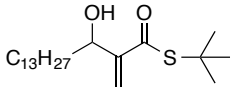
3-Methylene-4-tridecyloxetan-2-one (**194**)**. Pathway A:**[89] 3-Hydroxy-2-methyl-enehexadecanoic acid (**198**) (5.38 g, 18.9 mmol) was dissolved in dry CH_2Cl_2 (70 mL). Oven-dried Na_2CO_3 (20.1 g, 18.9 mmol) was added, and the resulting suspension was stirred for 30 min. *o*-Nosyl chloride (8.50 g, 37.7 mmol) was added, and the resulting mixture was stirred for 3 d. The reaction was diluted with CH_2Cl_2 (200 mL), and then 1 M HCl (200 mL) was added and separated. The aqueous layer was then extracted with CH_2Cl_2 (3 X 75 mL); the combined organic layers were dried (Na_2SO_4) and concentrated. Purification of the residue via flash chromatography on silica gel (petroleum ether/EtOAc 95:5) gave **194** (2.19 g, 43%) as a white solid: **Pathway B:**[90] $Hg(O_2CCF_3)_2$ (0.72 g, 1.68 mmol), was added to a solution of *S*-*tert*-butyl 3-hydroxy-2-methylenehexadecanethioate (**199**) (0.30 g, 0.84 mmol) in dry CH_3CN (50 mL). The reaction mixture was stirred in a preheated oil bath for 10 min at 50 ˚C. ^1H NMR was used to monitor the reaction. The reaction mixture was filtered, and the filtrate concentrated. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) gave **194** (0.15 g, 67%) as a white solid: mp: 34–35.5 ˚C; IR (neat) 2916, 2849, 1810, 1468, 1085, 962, 818 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.90 (dd, *J* = 2.0, 2.0 Hz, 1H), 5.41 (dd, *J* = 1.7 1.7 Hz, 1H), 4.98–4.94 (m, 1H), 1.92–1.79 (m, 2H), 1.54–1.40 (m, 2H), 1.37–1.26 (m, 20H), 0.88 (t, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 146.7, 115.0, 79.9, 33.5, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6,

29.6, 29.5, 24.8, 22.9, 14.3; MS (EI) *m/z* 266 (M^+), 108, 95, 83 (100), 67, 55; HRMS (ESI) calcd for $C_{17}H_{31}O_2$ $[M + H]^+$ *m/z* 267.2319, found 267.2335.

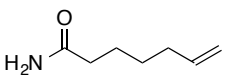


3-Hydroxy-2-methylenehexadecanoic acid (**198**)**.** Tetradecanal (**195**) (4.93 g, 23.2 mmol) and methyl acrylate (**196**) (4.20 mL, 46.8 mmol) were combined in an empty flask. 3- Hydroxyquinuclidine (0.74 g, 5.81 mmol) was added, followed by MeOH (0.70 mL). The resulting mixture was allowed to stir for 3 d.[89] MeOH and excess methyl acrylate were removed under reduced pressure. The resulting residue was diluted with a H_2O/sat. NH_4Cl solution (5:1, 120 mL), and the resulting mixture was extracted with CH_2Cl_2 (3 X 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Methyl 3-hydroxy-2-methylenehexadecanoate was obtained as a white solid (5.82 g, 84%) and used without further purification. Methyl 3-hydroxy-2-methylenehexadecanoate (5.82 g, 19.5 mmol) was dissolved in EtOH/H_2O (2:1, 45 mL). Lithium hydroxide monohydrate (0.45 g, 19.5 mmol) was added; the resulting solution was allowed to stir overnight. The reaction was quenched with 1 M HCl (200 mL), and the solution was extracted with CH_2Cl_2 (3 X 100 mL). The combined organic layers were washed with H_2O (100 mL) and dried ($MgSO_4$); CH_2Cl_2 was removed under reduced pressure.[89] Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc 85:15) gave **198** (5.38 g, 95%) as a white solid: mp: 72.4–73.5 ˚C; IR (KBr) 3853, 2918, 2850, 2594, 2360, 1697, 1637 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.38 (s, 1H), 5.91 (s,

1H), 4.43 (dd, *J* = 6.5, 6.5 Hz, 1H), 1.68–1.66 (m, 2H), 1.43–1.41 (m, 1H), 1.30–1.26 (m, 22H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 142.1, 127.6, 71.8, 36.4, 32.1, 29.9, 29.9, 29.9, 29.8, 29.8, 29.6, 29.6, 26.0, 22.9, 14.3; MS (EI) *m/z* 266 $(M - OH)^+$, 221, 192, 116, 101 (100), 83, 71, 57; HRMS (FAB) calcd for $C_{17}H_{32}NaO_3$ $[M + Na]^+$ *m/z* 307.2244, found 307.2258.

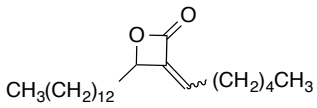


***S-tert*-Butyl 3-hydroxy-2-methylenehexadecanethioate** (**199**)**.** DABCO (0.040 g, 0.37 mmol) was added to a mixture of thio-tert-butyl acrylate (**197**)[91] (1.14 g, 7.87 mmol) and tetradecanal (**195**) (0.84 g, 3.7 mmol). The reaction mixture was allowed to stir for a week. It was diluted with CH_2Cl_2 (100 mL) and washed with 1 M aqueous HCl (50 mL), followed by sat. $NaHCO_3$ (50 mL). The organic layer was dried (Na_2SO_4) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) gave **199** (1.03 g, 84%) as slightly yellow oil: IR (neat) 2921, 2852, 1655, 1456, 1363, 967, 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (s, 1H), 5.71 (d, *J* = 1.0 Hz, 1H), 4.38 (dd, *J* = 6.7, 6.7 Hz, 1H), 2.40 (br. s, 1H), 1.65–1.56 (m, 2H), 1.49 (s, 9H), 1.45–1.41 (m, 1H), 1.33–1.25 (m, 21H), 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.8, 151.4, 121.7, 72.3, 48.4, 36.4, 32.1, 30.0, 29.9, 29.8, 29.7, 29.6, 29.6, 25.9, 22.9, 14.3; HRMS (ESI) calcd for $C_{21}H_{41}O_2S$ $[M + H]^+$ *m/z* 357.2822, found 357.2822.



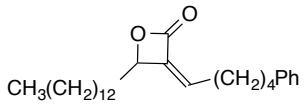
Hept-6-enamide (219e). A solution of 6-heptenoic acid (**225**) (0.50 g, 3.9 mmol),

$SOCl_2$ (0.34 mL, 4.7 mmol), and a drop of DMF in $CHCl_3$ (6 mL) was heated at reflux for 2 h. The reaction mixture was cooled to rt and then poured into a mixture of aqueous NH_4OH (28-30%, 6 mL) and ice (6.3 g) and stirred for 2 h. The layers were separated, and the organic layer was dried (Na_2SO_4) and concentrated. Purification by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) gave **219e** (0.42 g, 85%) as a white solid: mp: 84.8–84.9 ^oC; IR (neat) 3355, 3178, 2938, 1630, 1460, 1410, 1221 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.32 (br s, 1H), 5.84 (br s, 1H), 5.79–5.69 (m, 1H), 4.95 (d, *J* = 17.2 Hz, 1H), 4.88 (d, *J* = 10.2 Hz, 1H), 2.17 (t, *J* = 7.2 Hz, 2H), 2.02 (q, *J* = 6.4 Hz, 2H), 1.60 (quin, *J* = 7.5 Hz, 2H), 1.39 (quin, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 138.5, 114.8, 35.9, 33.5, 28.5, 25.1 MS (EI) *m/z* 127 (M^+), 72, 59 (100); HRMS (ESI) calcd for $C_7H_{14}NO$ $[M + H]^+$ *m/z* 128.1070, found 128.1057.



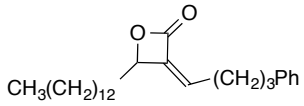
(E/Z)*-3-Hexylidene-4-tridecyloxetan-2-one** (Z/E*-220**)**.** Cat. **1** (0.02 g, 0.04 mmol) was added to a solution of 3-methylene-4-tridecyloxetan-2-one (**194**) (0.20 g, 0.75 mmol) and 1-heptene (**219a**) (0.15 g, 1.5 mmol) in dry CH_2Cl_2 (29 mL). The mixture was stirred overnight at 40 ˚C.[65] The next day ^1H NMR showed complete consumption of **194**. The reaction was cooled to rt followed removal of the CH_2Cl_2 under reduced pressure to yield a brownish residue. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) gave ***Z/E*-220** (*Z/E*, 3.3/1), (0.24 g, 97%) as a colorless oil. The isomers were separated by

careful chromatography using the same solvent system. ***E*-220:** IR (neat) 2922, 2852, 1813, 1464, 1117 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.33 (t, *J* = 7.6 Hz, 1H), 4.99 (m, 1H), 2.11 (dt, *J* = 7.1, 7.1 Hz, 2H), 1.96–1.88 (m, 1H), 1.81–1.72 (m, 1H), 1.50–1.43 (m, 4H), 1.31–1.26 (m, 24), 0.90 (m, 3H), 0.88 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 137.8, 134.1, 79.4, 33.5, 32.1, 31.6, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6 29.5, 29.0, 28.3, 24.9, 22.9, 22.6, 14.3, 14.1; MS (EI) *m/z* 336 (M^+), 178 (100); HRMS (ESI) calcd for $C_{22}H_{41}O_2$ $[M + H]^+$ *m/z* 337.3101, found 337.3124. ***Z*-220:** IR (neat) 2914, 2848, 1793, 1721, 1470, 1116, 1070, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.84 (dt, *J* = 7.9, 1.0 Hz, 1H), 4.84 (dd, *J* = 6.2, 6.2 Hz, 1H), 2.55–2.41 (m, 2H), 1.84–1.73 (m, 2H), 1.50–1.39 (m, 4H), 1.33–1.26 (m, 24H), 0.89 (m, 3H), 0.88 (t, *J* = 7.0 Hz 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 137.7, 136.4, 78.8, 33.9, 32.1, 31.4, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.0, 28.7, 24.8, 22.9, 22.6, 14.3, 14.1; MS (EI) *m/z* 336 (M^+), 275, 111, 81, 67, 55 (100); HRMS (ESI) calcd for $C_{22}H_{41}O_2$ $[M + H]^+$ *m/z* 337.3101, found 337.3123.



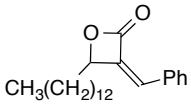
(Z)*-3-(5-Phenylpentylidene)-4-tridecyloxetan-2-one** (Z*-221**)**.** Catalyst **1** (0.03 g, 0.04 mmol) was added to a solution of 3-methylene-4-tridecyloxetan-2-one (**194**) (0.20 g, 0.75 mmol) and 6-phenyl-1-hexene (**219b**) (0.24 g, 1.50 mmol) in dry CH_2Cl_2 (29 mL). The mixture was stirred overnight at 40 ˚C.[65] The next day TLC showed incomplete consumption of **194**; so 1 equiv more of 6-phenyl-1-hexene was added, and the reaction was allowed to stir for 6 h at 40 ˚C. After the reaction

was allowed to cool to rt, the CH_2Cl_2 was removed under reduced pressure to yield a brown residue. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc 97:3) gave ***Z*-221** (0.20 g, 68%) as a wax: mp 34–35 ˚C; IR (neat) 2914, 2848, 1793, 1723, 1468, 1182.70, 1120, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.22–7.19 (m, 2H), 7.13–7.09 (m, 3H), 5.75 (t, *J* = 7.8 Hz, 1H), 4.77 (dd, *J* = 6.0, 6.0 Hz, 1H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.51– 2.38 (m, 2H), 1.77–1.69 (m, 2H), 1.60 (quin, *J* = 7.6 Hz, 2H), 1.47–1.33 (m, 4H), 1.28–1.19 (m, 20H), 0.81 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 142.4, 138.0, 136.0, 128.6, 128.5, 126.0, 78.9, 35.7, 33.9, 32.1, 30.9, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 28.9, 28.5, 24.8, 22.9, 14.3; MS (EI) *m/z* 354 $(M - CO_2)^+$, 104, 91(100); HRMS (ESI) calcd for $C_{27}H_{43}O_2$ $[M + H]^+$ *m/z* 399.3263, found 399.3258.



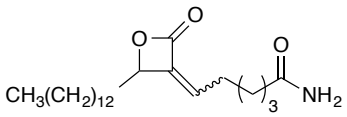
(Z)*-3-(4-Phenylbutylidene)-4-tridecyloxetan-2-one** (Z*-222**)**.** Cat.1 **1** (0.02 g, 0.04 mmol) was added to a solution of 3-methylene-4-tridecyloxetan-2-one (**194**) (0.20 g, 0.75 mmol) and 5-phenyl-1-pentene (**219c**) (0.44 g, 3.00 mmol) in dry CH_2Cl_2 (29 mL). The mixture was stirred overnight at 40 ˚C.[65] The next day TLC showed incomplete consumption of **194**; so 1 equiv more of 5-phenyl-1-pentene (**219c**) was added and the reaction was allowed to stir for 6 h at 40 ˚C. After the reaction was allowed to cool to rt, the CH_2Cl_2 was removed under reduced pressure to yield a brown residue. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc 97:3) gave ***Z*-222** (0.15 g, 51%) as a colorless oil: IR

(neat) 2921, 2852, 1805, 1454, 1067 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.31–7.27 (m, 2H), 7.21–7.18 (m, 3H), 5.84 (t, *J* = 7.6 Hz, 1H), 4.86 (dd, *J* = 6.0, 6.0 Hz, 1H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.56 (dt, *J* = 7.8, 7.8 Hz, 2H), 1.84–1.77 (m, 4H), 1.50–1.43 (m, 2H), 1.36–1.27 (m, 20H), 0.89 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 141.8, 138.1, 135.8, 128.6, 126.2, 78.9, 35.6, 33.9, 32.1, 30.8, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 28.8, 24.8, 22.9, 14.3; MS (EI) *m/z* 384 (M^+), 104 (100), 91; HRMS (ESI) calcd for $C_{26}H_{41}O_2$ $[M + H]^+$ *m/z* 385.3101, found 385.3114.



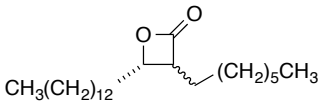
(Z)*-3-Benzylidene-4-tridecyloxetan-2-one** (Z*-223**)**.** Cat. **1** (0.01 g, 0.01 mmol) was added to a solution of 3-methylene-4-tridecyloxetan-2-one (**194**) (0.060 g, 0.21 mmol) and styrene (**219d**) (0.050 g, 0.43 mmol) in dry CH_2Cl_2 (8 mL) The mixture was stirred overnight at 40 ˚C.[65] The next day TLC showed incomplete consumption of **194**; so 1 equiv more of styrene was added, and the reaction was allowed to stir for 6 h at 40 °C. After the reaction mixture was allowed to cool to rt, the CH_2Cl_2 was removed under reduced pressure to yield a brown residue. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) gave ***Z*-223** (0.030 g, 40%) as a white solid: mp 61–62 ˚C; IR (neat) 2914, 2848, 1780, 1687, 1468, 1212, 1156, 1128, 1068 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 6.8 Hz, 2H), 7.43–7.41 (m, 3H), 6.53 (s, 1H), 4.96 (dd, *J* = 5.6, 5.6 Hz, 1H), 1.97–1.83 (m, 2H), 1.59–1.45 (m, 2H), 1.40–1.26 (m, 20H),

0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 136.0, 133.4, 133.2, 130.8, 130.4, 129.1, 78.0, 34.0, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 24.8, 22.9, 14.3; MS (EI) *m/z* 342 (M^+), 159, 130 (100); HRMS (ESI) calcd for $C_{23}H_{35}O_2$ [M + H]$^+$ *m/z* 343.2632, found 343.2655.



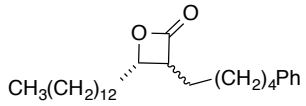
***(Z)*-6-(2-Oxo-4-tridecyloxetan-3-ylidene)hexanamide (*Z/E*-224; KC01).** Cat. **2** (0.03 g, 0.04 mmol) was added to a solution of 3-methylene-4-tridecyloxetan-2-one (**194**) (0.20 g, 0.75 mmol) and hept-6-enamide (**219e**) (0.19 g, 1.5 mmol) in dry CH_2Cl_2 (29 mL). The mixture was stirred at 40 °C for 48 h. After cooling to rt, the CH_2Cl_2 was removed under reduced pressure to yield a greenish brown residue. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) gave ***Z/E*-224** (*Z/E*, 3.7:1), (0.20 g, 73%) as an off white solid. The *Z*-isomer was separable from the *E*-isomer by careful column chromatography. The isomer ***E*-224** was not obtained as a single compound, but chemical shifts for a number of the protons and carbons were identifiable: ***E*-224**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (t, J = 7.5 Hz, 1H), 5.35 (br s, 2H) 5.00 (m, 1H), 2.28–2.23 (m, 2H), 2.16 (dt, J = 7.4, 7.4 Hz, 2H), the remaining proton signals cannot be readily distinguished from those of the *Z*-isomer; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 164.4, 138.3, 133.2, 79.4, 35.5, 33.5, 32.1, 29.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 28.9, 28.2, 28.1, 25.1, 25.0, 24.8, 24.6, 22.9, 14.3. ***Z*-224** (**KC01**): mp: 106–107 °C; IR (neat) 3384, 3196, 2915, 2850, 1792, 1656, 1468, 1422, 1180, 1124, 1074, 803 cm^{-1}; ^{1}H NMR (500 MHz,

$CDCl_3$) δ 5.86 (t, *J* = 8.0 Hz, 1H), 5.51 (br s, 1H), 5.27 (br s, 1H), 4.87 (dd, *J* = 6.5, 6.5 Hz, 1H), 2.59–2.47 (m, 2H), 2.26 (t, *J* = 7.5 Hz, 2H), 1.82–1.77 (m, 2H), 1.73–1.67 (m, 2H), 1.58-1.52 (m, 2H), 1.47–1.42 (m, 2H), 1.37–1.26 (m, 20H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 164.5, 136.2, 135.5, 79.0, 35.8, 33.9, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 28.2, 28.0, 24.8, 24.5, 22.8, 14.3; HRMS (ESI) calcd for $C_{22}H_{40}NO_3$ $[M + H]^+$ *m/z* 366.3003, found 366.3009.



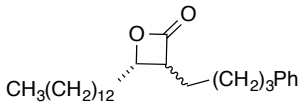
(cis/trans)*-3-Hexyl-4-tridecyloxetan-2-one** (trans/cis*-226**). *(E/Z)*-3-Hexylidene-4-tridecyloxetan-2-one (***Z/E*-220**) (0.03 g, 0.09 mmol), was dissolved in a mixture of THF:MeOH (0.70 mL: 0.14 mL). This solution was cooled to −10 ˚C, followed by the addition of $CoCl_2(PPh)_3$ (0.01 g, 0.01 mmol) and then portion-wise addition of $NaBH_4$ (9.9 mg, 27.0 mmol) within 10 min.[81] The mixture was vigorously stirred for 2 h between −7 to −5 ˚C. The reaction mixture was filtered through a pad of celite, and the celite was then washed with $CHCl_3$ (5 mL). The filtrate was washed with 2M HCl (5 mL), dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/ EtOAc 98:2) gave mixture of ***trans/cis*-226** (*trans*/*cis* 1.3/1), (14 mg, 44%). The isomers were separated by careful column chromatography using the same solvent system. ***cis*-226** (colorless oil): IR (neat) 2921, 2852, 1820, 1464, 1121 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.57–4.49 (m, 1H), 3.61–3.56 (m, 1H), 1.78–1.51 (m, 6H), 1.39–1.26 (m, 28H),

0.91–0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 76.0, 52.9, 32.1, 31.7, 30.4, 29.9, 29.9, 29.7, 29.6, 29.6, 29.5, 29.3, 27.8, 25.8, 24.2, 22.9, 22.8, 14.3, 14.2; MS (EI) *m/z* 294 $(M - CO_2)^+$, 207, 125, 111, 97 (100), 83, 69, 55; HRMS (ESI) calcd for $C_{22}H_{43}O_2$ $[M + H]^+$ *m/z* 339.3258, found 339.3295. ***trans*-226** (waxy solid): mp 29–30 ˚C; IR (neat) 2918, 2850, 1794, 1466, 1142, 1076 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.24–4.17 (m, 1H), 3.18–3.13 (m, 1H), 1.85–1.79 (m, 2H), 1.73–1.69 (m, 2H), 1.43–1.26 (m, 30H), 0.89–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 78.4, 56.4, 34.7, 32.1, 31.7, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.2, 28.1, 27.2, 25.2, 22.9, 22.7, 14.3, 14.2; MS (EI) *m/z* 294 $(M - CO_2)^+$, 281, 207, 125, 111, 97, 83 (100), 69, 55; HRMS (ESI) calcd for $C_{22}H_{43}O_2$ $[M + H]^+$ *m/z* 339.3258, found 339.3296.



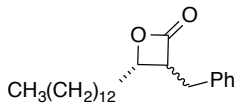
(cis/trans)*-3-(5-Phenylpentyl)-4-tridecyloxetan-2-one** (trans/cis*-227**)**.** *(Z)*-3-(5-Phenylpentyli-dene)-4-tridecyloxetan-2-one (***Z*-221**) (0.10 g, 0.25 mmol) was dissolved in a mixture of THF:MeOH (2.0:0.4 mL). This solution was cooled to –10 ˚C, followed by the addition of $CoCl_2(PPh)_3$ (0.03 g, 0.04 mmol) and then portion-wise addition of $NaBH_4$ (28.0 mg, 0.75 mmol), within 10 min.[81] The mixture was vigorously stirred for 4 h between –7 to –5 ˚C. The reaction mixture was filtered through a pad of celite, and the celite was washed with $CHCl_3$ (15 mL). The filtrate was washed with 2M HCl (10 mL), dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 99:1) gave a mixture of

***trans/cis*-227** (*trans*/cis, 2/1), (0.03 g, 32%) as a colorless oil. The isomers were separated by careful column chromatography using the same solvent system. ***cis*-227:** IR (neat) 2916, 2846, 1805, 1464, 1135, 1064 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H), 7.20–7.16 (m, 3H), 4.55–4.49 (m, 1H), 3.57 (ddd, *J* = 8.9, 6.8, 6.8 Hz, 1H), 2.61 (t, *J* = 7.4 Hz, 2H), 1.82–1.70 (m, 2H), 1.68–1.52 (m, 4H), 1.45–1.26 (m, 26H), 0.88 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 142.7, 128.6, 128.5, 125.9, 75.9, 52.8, 36.0, 32.1, 31.3, 30.4, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.2, 27.7, 25.8, 24.1, 22.9, 14.3; MS (GC) *m/z* 356 $(M - CO_2)^+$, 117, 104 (100), 91; HRMS (ESI) calcd for $C_{27}H_{45}O_2$ $[M + H]^+$ *m/z* 401.3414, found 401.3441. ***trans*-227:** IR (neat) 2921, 2852, 1819, 1454, 1114 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.28 (m, 2H), 7.20 (m, 3H), 4.23–4.19 (m, 1H), 3.19–3.14 (m, 1H), 2.64 (t, *J* = 7.1 Hz, 2H), 1.87–1.80 (m, 2H), 1.74–1.63 (m, 4H), 1.50–1.29 (m, 26H), 0.91 (br. t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 142.6, 128.6, 128.5, 125.9, 78.3, 56.3, 36.0, 34.6, 32.1, 31.3, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.1, 28.0, 27.1, 25.2, 22.9, 14.3; MS (EI) *m/z* 356 $(M - CO_2)^+$, 117, 104 (100), 91; HRMS (ESI) calcd for $C_{27}H_{45}O_2$ $[M + H]^+$ *m/z* 401.3414, found 401.3447.



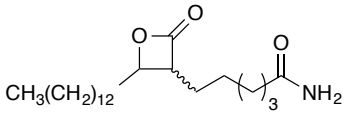
(cis/trans)*-3-(4-Phenylbutyl)-4-tridecyloxetan-2-one** (trans/cis*-228**)**.** *(Z)*-3-(4-Phenylbutylidene)-4-tridecyloxetan-2-one (***Z*-222**) (0.05 g, 0.13 mmol)) was dissolved in a mixture of THF:MeOH (1:0.2 mL). This solution was cooled to –10

˚C, followed by the addition of $CoCl_2(PPh)_3$ (15.0 mg, 0.02 mmol) and then portion-wise addition of $NaBH_4$ (14 mg, 0.39 mmol) within 10 min.[81] The mixture was vigorously stirred for 1.5 h between −7 to −5 ˚C. The reaction mixture was filtered through a pad of celite, and the celite was washed with $CHCl_3$ (10 mL). The filtrate was washed with 2M HCl (5 mL), dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) gave ***trans/cis*-228** (*trans*/cis, 1.4/1), (24 mg, 46%) as a colorless oil. The isomers were separated by careful column chromatography using the same solvent system. ***cis*-228**: IR (neat) 2919, 2850, 1818, 1462, 1052 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29−7.26 (m, 2H), 7.20−7.16 (m, 3H), 4.51 (ddd, *J* = 10.0, 6.2, 4.0 Hz, 1H), 3.58 (ddd, *J* = 8.6, 6.7, 7.7 Hz, 1H), 2.63 (t, *J* = 7.5 Hz, 2H), 1.85−1.77 (m, 1H), 1.75−1.62 (m, 6H), 1.51−1.26 (m, 21H), 0.88 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 142.3, 128.6, 126.0, 75.9, 52.8, 35.8, 32.1, 31.3, 30.4, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 27.4, 25.7, 24.0, 22.9, 14.3; MS (EI) *m/z* 342 $(M - CO_2)^+$, 117, 104 (100), 91; HRMS (ESI) calcd for $C_{26}H_{43}O_2$ $[M + H]^+$ *m/z* 387.3258, found 387.3261. *trans*-**228**: IR (neat) 2917, 2851, 1804, 1470, 1140 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30−7.26 (m, 2H), 7.20−7.15 (m, 3H), 4.19 (ddd, *J* = 6.5, 6.5, 3.9 Hz, 1H), 3.15 (m, 1H), 2.63 (t, *J* = 7.5 Hz, 2H), 1.90−1.81 (m, 2H), 1.79−1.62 (m, 4H), 1.51−1.26 (m, 24H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 142.3, 128.6, 126.1, 78.3, 56.3, 35.8, 34.6, 32.1, 31.3, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 28.0, 26.8, 25.2, 22.9, 14.3; MS (EI) *m/z* 342 $(M - CO_2)^+$, 117, 104 (100), 91; HRMS (ESI) calcd for $C_{26}H_{43}O_2$ $[M + H]^+$ *m/z* 387.3258, found 387.3259.



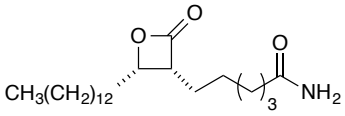
(cis/trans)*-3-Benzyl-4-tridecyloxetan-2-one** (trans/cis*-229**). *(Z)*-3-Benzylidene-4-tridecyloxetan-2-one (***Z*-223**) (0.05 g, 0.15 mmol) was dissolved in a mixture of THF:MeOH (1.1 mL:0.05 mL). This solution was cooled to −10 ˚C, followed by the addition of $CoCl_2(PPh)_3$ (0.02 g, 0.03 mmol) and then portion-wise addition of $NaBH_4$ (16.0 mg, 0.44 mmol) within 10 min.[81] The mixture was vigorously stirred for 2 h between −7 to −5 ˚C. The reaction mixture was filtered through a pad of celite, and the celite was then washed with $CHCl_3$ (5 mL). The filtrate was washed with 2M HCl (5 mL), dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 99:1) gave ***trans/cis*-229** (*trans*/*cis,* 2/1), (0.02 g, 39%) as a wax. The isomers were separated by careful column chromatography using the same solvent system. ***cis*-229** (white solid): mp 43–44 ˚C; IR (neat) 2917, 2849, 1797, 1466, 1136, 1067 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34–7.30 (m, 2H), 7.24–7.21 (m, 3H), 4.60 (ddd, *J* = 10.0, 6.4, 3.6 Hz, 1H), 4.01 (ddd, *J* = 9.0, 6.9, 6.9 Hz, 1H), 3.19 (dd, *J* = 15.1, 7.1 Hz, 1H), 2.98 (dd, *J* = 15.1, 9.0 Hz, 1H), 1.86–1.76 (m, 1H), 1.71–1.63 (m, 1H), 1.57–1.46 (m, 1H), 1.37–1.26 (m, 21H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 137.9, 129.0, 128.6, 127.0, 76.3, 53.5, 32.1, 30.6, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 25.8, 22.9, 14.3; MS (EI) *m/z* 300 $(M - CO_2)^+$, 117, 104 (100), 91; HRMS (ESI) calcd for $C_{23}H_{37}O_2$ $[M + H]^+$ *m/z* 345.2788, found 345.2817. ***trans*-229** (white solid): mp 40–41 ˚C; IR (neat) 2916, 2849, 1800, 1134, 1072 cm^{-1}; ^1H NMR

(400 MHz, $CDCl_3$) δ 7.34–7.30 (m, 2H), 7.27–7.25 (m, 2H), 7.20 (d, *J* = 7.0 Hz, 1H), 4.27 (ddd, *J* = 6.7, 6.7, 4.1 Hz, 1H), 3.45 (ddd, *J* = 9.5, 5.6, 4.2 Hz, 1H), 3.18 (dd, *J* = 14.3, 5.7 Hz, 1H), 3.00 (dd, *J* = 14.3, 9.4 Hz, 1H), 1.83–1.74 (m, 1H), 1.62–1.57 (m, 1H), 1.35–1.18 (m, 22H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 137.4, 129.1, 128.9, 127.3, 77.8, 57.6, 34.4, 34.0, 32.1, 29.9, 29.9, 29.8, 29.6, 29.6, 29.6, 29.3, 24.8, 22.9, 14.3; MS (EI) *m/z* 300 (M – $CO_2)^+$, 117, 104 (100), 91; HRMS (ESI) calcd for $C_{23}H_{37}O_2$ $[M + H]^+$ *m/z* 345.2788, found 345.2814.

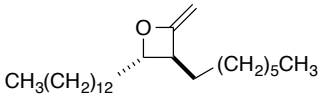


***trans/cis*-6-(2-Oxo-4-tridecyloxetan-3-yl)hexanamide (*trans/cis*-230).** *Z*-6-(2-Oxo-4-tridecyloxetan-3-ylidene)hexanamide (**Z/E-224**) (0.050 g, 0.14 mmol) was dissolved in mixture of THF:MeOH (0.9:0.2 mL). This solution was cooled to –10 ^oC, followed by the addition of $CoCl_2(PPh_3)_2$ (0.01 g, 0.04 mmol) and then portion-wise addition of $NaBH_4$ (13 mg, 0.41 mmol) within 10 min. The mixture was vigorously stirred for 4 h between –10 to –7 ^oC. The reaction mixture was filtered through a pad of celite, and the celite was then washed with $CHCl_3$ (10 mL). The filtrate was washed with 2M HCl (5 mL), dried ($MgSO_4$) and concentrated. Purification by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) gave an inseparable mixture of ***trans/cis*-230** (*trans/cis*, 1.4:1) (18 mg, 35%) as an off white solid. Peak assignments for *trans*-isomer: ^1H NMR (400 MHz, $CDCl_3$) δ 5.34 (br, 2H), 4.21 (ddd, *J* = 6.2, 6.2, 4.0 Hz, 1H), 3.16 (ddd, *J* = 7.8, 7.8, 4.0 Hz, 1H),),

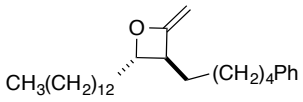
2.23 (t, *J* = 7.4 Hz, 2H), 1.87–1.81 (m, 2H), 1.78–1.65 (m, 4H), 1.55–1.26 (m, 26H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 171.8, 78.3, 56.3, 35.7, 34.6, 32.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.0, 27.9, 26.8, 25.2, 22.9, 14.3; HRMS (ESI) calcd for $C_{22}H_{42}NO_3$ $[M + H]^+$ *m/z* 368.3159, found 368.3186.



cis*-6-(2-Oxo-4-tridecyloxetan-3-yl)hexanamide (*cis*-230).** *Z*-6-(2-Oxo-4-tridecyloxetan-3-ylidene)hexanamide (Z/E*-224**) (50.0 mg, 0.14 mmol) was dissolved in THF (2 mL). Pd/C (10 mol%, 4 mg, 0.004 mmol) was added to the solution. The mixture was purged with H_2 gas for 5 min. It was then stirred under H_2 gas for 2 h. The mixture was filtered through a pad of celite, and the celite was washed with THF (5 mL). The filtrate was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) to give ***trans/cis*-230** (*trans/cis*, 1:9), (16 mg, 65%) as an off white solid. Careful chromatography under the same conditions provided the ***cis*-230** in >95% purity: mp 98–101 ˚C; IR (neat) 3393, 2918, 2849, 1795, 1649, 1417, 1132, 804 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40–5.35 (br, 2H), 4.54–4.50 (m, 1H), 3.61–3.55 (m, 1H), 2.23 (t, *J* = 6.1 Hz, 2H), 1.79–1.51 (m, 8H), 1.40–1.26 (m, 24H), 0.89–0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 172.5, 75.9, 52.7, 35.8, 32.1, 30.4, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.0, 27.4, 25.8, 25.2, 23.9, 22.9, 14.3; HRMS (ESI) calcd for $C_{22}H_{42}NO_3$ $[M + H]^+$ *m/z* 368.3159, found 368.3145.

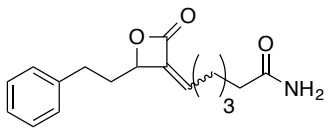


(*trans*)-3-Hexyl-2-methylene-4-tridecyloxetane (*trans*-231). *(trans)*-3-Hexyl-4-tridecyloxetan-2-one (***trans*-226**) (12 mg, 34 mmol) and Petasis solution (0.2 mL, 0.72 M) were stirred in a pre-heated oil bath at 80 ˚C for 6 h.[92] TLC showed remaining starting material so 0.2 mL more of the Petasis solution was added, and stirring was continued for 2 h. Petroleum ether (10 mL) was added to quench the reaction, and the mixture was allowed to stir overnight. The mixture was then filtered through a pad of celite until the filtrate was colorless, and the filtrate was concentrated to 1 mL solution. Purification by flash column chromatography on silica gel (petroleum ether/ EtOAc/ Et_3N, 96:3:1) gave *trans*-**231**(4.5 mg, 38%) as a slightly yellow oil: IR (neat) 2924, 2854, 1689, 784 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.39 (m, 1H), 4.06 (br s, 1H), 3.72 (br s, 1H), 2.96–2.91 (m, 1H), 1.85–1.78 (m, 1H), 1.71–1.62 (m, 3H), 1.29–1.26 (m, 30H), 0.89–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 86.3, 78.5, 47.5, 36.0, 32.3, 32.2, 31.9, 29.9 29.9, 29.7, 29.7, 29.6, 29.4, 27.0, 24.7, 22.9, 22.8, 14.3, 14.3; HRMS (ESI) calcd for $C_{23}H_{45}O$ $[M + H]^+$ *m/z* 337.3465, found 337.3469**.**



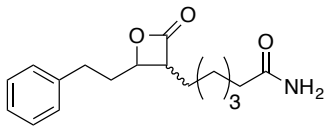
(*trans*)-2-Methylene-3-(5-phenylpentyl)-4-tridecyloxetane (*trans*-232). (*trans*)-3-(5-Phenylpentyl)-4-tridecyloxetan-2-one (***trans*-227**), (11 mg, 27 mmol) and Petasis solution (0.080 mL, 0.50 M) were stirred in a pre-heated oil bath at 80 ˚C for 5 h.[92] Petroleum ether (10 mL) was added to quench the reaction, and the mixture was allowed to stir overnight. The mixture was then filtered through a pad of celite, and the filtrate was

concentrated to 1 mL solution. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc/Et_3N 96:3:1) gave ***trans*-232** (3.2 mg, 40%) as a colorless oil: IR (neat) 2922, 2852, 1687, 1454 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H), 7.19–7.16 (m, 3H), 4.38 (m, 1H), 4.06 (m, 1H), 3.71 (m, 1H), 2.94–2.90 (m, 1H), 2.64 (t, *J* = 7.5 Hz, 2H), 1.84–1.76 (m, 1H), 1.70–1.62 (m, 5H), 1.36–1.26 (m, 26H), 0.91 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.8, 142.8, 128.6, 128.5, 125.9, 86.2, 78.5, 47.5, 36.1, 36.0, 32.2, 32.1, 31.5, 29.9, 29.9, 29.8, 29.7, 29.6, 29.4, 27.0, 24.7, 22.9, 14.3; MS (EI) *m/z* 398 (M^+), 104 (100), 91; HRMS (ESI) calcd for $C_{28}H_{47}O$ $[M + H]^+$ *m/z* 399.3621, found 399.3594.



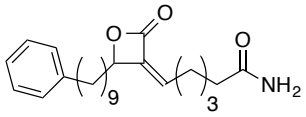
***(Z/E)*-6-(2-Oxo-4-(2-phenethyl)oxetan-3-ylidene)hexanamide (*Z/E*-234; KC02).** Cat. **2** (0.026 g, 0.041 mmol) was added to a solution of 3-methylene-4-(2-phenethyl)oxetan-2-one (**238**) (0.073 g, 0.41 mmol) and hept-6-enamide (**219e**) (0.053 g, 0.41 mmol) in dry CH_2Cl_2 (16 mL). The solution was stirred at 40 °C for 24 h.[65] After the reaction was allowed to cool to rt, the CH_2Cl_2 was removed under reduced pressure to yield a greenish brown residue. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH, 99:1) gave ***Z/E*-234** (**KC02**) (*Z/E*, 4:1) (0.050 g, 43%) as an off white solid: IR (neat) 3384, 3182, 2942, 1783, 1644, 1416, 1218, 1179, 1135, 1096, 1014 cm^{-1}; Peak assignment for ***Z*-234**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.28 (m, 2H), 7.23–7.18 (m, 3H), 5.74 (td, *J* = 8.0, 1.2 Hz, 1H), 5.35 (br s, 2H) 4.87 (dd, *J* = 6.1, 6.1 Hz, 1H), 2.89–2.70 (m, 2H), 2.56–2.42 (m, 2H),

2.27–2.21 (m, 2H), 2.15–2.08 (m, 2H), 1.70–1.62 (m, 2H), 1.54–1.47 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 164.3, 140.5, 137.9, 136.1, 128.8, 128.6, 126.6, 78.1, 35.6, 35.1, 31.1, 28.3, 28.1, 24.6; The ***E*-234**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.28 (m, 2H), 7.23–7.18 (m, 3H), 6.32 (td, *J* = 7.8, 1.6 Hz, 1H), 5.49 (br s, 2H) 4.99 (m, 1H), the remaining proton signals cannot be readily distinguished from those of the *Z*-isomer; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 164.2, 140.6, 138.0, 133.7, 128.8, 128.6, 126.6, 78.2, 35.4, 35.3, 31.1, 28.8, 28.1, 25.0; HRMS (ESI) calcd for $C_{17}H_{22}NO_3$ $[M + H]^+$ *m/z* 288.1594, found 288.1630.



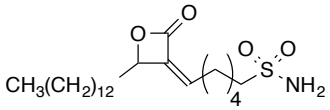
trans/cis*-6-(2-Oxo-4-(2-phenethyl)oxetan-3-yl)hexanamide (*trans/cis*-235).** (*Z/E*)-6-(2-Oxo-4-(2-phenethyl)oxetan-3-ylidene)hexanamide (Z/E*-234**) (0.031 g, 0.011 mmol) was dissolved in THF (1 mL). Pd/C (10 mol%, 1.2 mg, 0.0011 mmol) was added to the solution. The mixture was purged with H_2 gas for 5 min. It was then stirred under H_2 gas for 2 h 15 min. The mixture was filtered through a pad of celite, and the celite was washed with THF (5 mL). The filtrate was concentrated and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 98:1) ***trans/cis*-235** (*trans/cis*, 1:5,) (19 mg, 60%) as an inseparable mixture and off white solid. IR (neat) 3392, 2938, 1796, 1646, 1416, 1135 cm^{-1}; ***cis*-235**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.29 (m, 2H), 7.24–7.19 (m, 3H), 5.58–5.50 (br, 2H), 4.54 (ddd, *J* = 10.0, 6.4, 3.4 Hz, 1H), 3.59 (ddd, *J* = 9.2, 6.5, 6.5 Hz, 1H), 2.88 (ddd, *J* = 14.0, 9.4, 5.0 Hz, 1H), 2.69 (ddd, *J* = 13.9, 6,8, 6.8 Hz, 1H), 2.21 (t, *J* =

7.4 Hz, 2H), 2.10–2.01 (m, 1H), 1.98–1.78 (m, 1H), 1.83–1.90 (m, 1H), 1.67–1.50 (m, 4H), 1.43–1.32 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 172.2, 140.6, 128.8, 128.7, 126.6, 74.7, 52.6, 35.7, 32.4, 31.8, 28.9, 27.3, 25.1, 23.9; ***trans*-235**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54 (ddd, *J* = 8.6, 5.4, 4.1 Hz, 1H), 3.59 (ddd, *J* = 7.6, 7.6, 3.9 Hz, 1H), the remaining proton signals cannot be readily distinguished from those of the *cis*-isomer; ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 171.5, 140.3, 128.8, 128.6, 126.6, 77.4, 56.3, 36.2, 32.4, 31.5, 28.9, 27.7, 26.7, 25.1, 23.9; ^{1}H HRMS (ESI) calcd for $C_{17}H_{24}NO_3$ $[M + H]^+$ *m/z* 290.1751, found 290.1754.

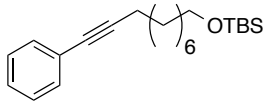


***(Z)*-6-(2-Oxo-4-(9-phenylnonyl)oxetan-3-ylidene)hexanamide (236).** Catalyst **1** (0.025 g, 0.30 mmol) was added to a solution of 3-methylene-4-(9-phenylnonyl)oxetan-2-one (**247**) (0.085 g, 0.30 mmol) and hept-6-enamide (**219e**) (0.038 g, 0.30 mmol) in dry CH_2Cl_2 (11 mL). The solution was stirred at 40 °C for 3 d.[65] After the reaction was allowed to cool to rt, the CH_2Cl_2 was removed under reduced pressure. Purification of the residue by flash chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) gave **236** (*Z/E*, 3.7:1), (0.068 g, 59%) as an off white solid. Further chromatography using Et_2O gave pure *Z*-isomer: mp: 93–94 °C; IR (neat) 3384, 3194, 2916, 2850, 1791, 1655, 1467, 1421, 1180, 1124, 1074 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.25 (m, 2H), 7.18–7.15 (m, 3H), 5.84 (t, *J* = 8.0 Hz, 1H), 5.50 (br s, 1H), 5.29 (br s, 1H), 4.86 (t, *J* = 6.2 Hz, 1H), 2.60 (t, *J* = 7.8 Hz, 2H), 2.56–2.45 (m, 2H), 2.26 (t, *J* = 7.3 Hz, 2H), 1.84–1.77 (m, 2H), 1.73–1.66 (m,

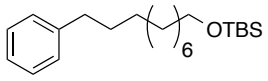
2H), 1.62–1.53 (m, 4H), 1.49–1.41 (m, 2H), 1.30–1.29 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 164.6, 143.1, 138.3, 135.6, 128.6, 128.4, 125.8, 79.1, 36.2, 35.1, 33.9, 31.7, 29.6, 29.6, 29.6, 29.5, 29.5, 28.3, 28.1, 24.8, 24.6 HRMS (ESI) calcd for $C_{24}H_{36}NO_3$ $[M + H]^+$ *m/z* 386.2690, found 386.2699.



***(Z)*-6-(2-Oxo-4-tridecyloxetan-3-ylidene)hexane-1-sulfonamide (237).** Cat. **2** (0.02 g, 0.03 mmol) was added to a solution of 3-methylene-4-tridecyloxetan-2-one (**194**) (0.16 g, 0.60 mmol) and hept-6-ene-1-sulfonamide (**250**) (0.15 g, 0.90 mmol) in dry CH_2Cl_2 (23 mL). The solution was stirred at 40 °C for 48 h.[65] After the reaction was allowed to cool to rt, the CH_2Cl_2 was removed under reduced pressure to yield a greenish brown residue. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) gave ***Z/E*-237** (*Z/E*, 4:1) (0.19 g, 79%) as an off white solid. The *Z*-isomer, isolated as a white solid, was separable from the *E*-isomer by careful chromatography using the same solvent system. ***Z*-237:** mp: 79–80 °C; IR (neat) 2914, 2849, 1791, 1469, 1331, 1143 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 (t, *J* = 7.6 Hz, 1H), 4.92 (s, 2H), 4.86 (dd, *J* = 6.2, 6.2 Hz, 1H), 3.11 (t, *J* = 7.3 Hz, 2H), 2.58–2.24 (m, 2H), 1.91–1.85 (m, 2H), 1.82–1.77 (m, 2H), 1.51 (m, 4H), 1.45–1.39 (m, 2H), 1.34–1.25 (m, 20H), 0.87 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 138.2, 135.6, 79.1, 55.1, 33.9, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.5, 28.3, 28.2, 27.4, 24.8, 23.6, 22.9, 14.3; HRMS (ESI) calcd for $C_{22}H_{42}NO_3$ $[M + H]^+$ *m/z* 416.2829, found 416.2842.

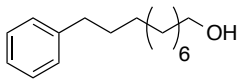


1-*(tert*-Butyldimethylsilyloxy)-10-phenyldec-9-yne (241). *n*-BuLi (2.5 M in THF, 12.5 mL, 31.2 mmol) was added to a solution of phenylacetylene (**239**) (3.43 mL, 31.2 mmol) in THF (10 mL) at 0 ^{o}C. After 10 min a solution of 1-*(tert*-butyldimethylsilyloxy)-8-iodooctane (**240**) (5.87 g, 15.6 mmol) and HMPA (10 mL) was added drop-wise. The resultant solution was stirred at 0 ^{o}C for 30 min, then allowed to slowly warm to rt overnight. Saturated aqueous NH_4Cl (60 mL) was added. The layers were separated, followed by extraction of the aqueous layer with Et_2O (3 X 30 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The crude residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 99:1) to yield **241** (2.56 g, 48%) as a colorless oil: IR (neat) 2927, 2855, 1462, 1253, 1093, 834, 774, 754, 691 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.38 (m, 2H), 7.30–7.23 (m, 3H), 3.61 (t, *J* = 6.6 Hz, 2H), 2.40 (t, *J* = 7.1 Hz, 2H), 1.64–1.57 (m, 2H), 1.53–1.44 (m, 4H) 1.38–1.33 (m, 6H), 0.90 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 131.8, 128.4, 127.6, 124.3, 90.6, 80.8, 63.5, 33.1, 29.6, 29.4, 29.1, 29.0, 26.2, 26.0, 19.6, 18.6, –5.0; HRMS (ESI) calcd for $C_{22}H_{37}OSi$ $[M + H]^+$ *m/z* 345.2608, found 345.2596.



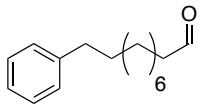
1-*(tert*-Butyldimethylsilyloxy)-10-phenyldecane (242). Pd/C (10 mol%, 0.27 g, 0.26 mmol) was added to a solution of 1-*(tert*-butyldimethylsilyloxy)-10-phenyldec-9-yne (**241**) (2.96 g, 8.59 mmol) in THF (34 mL). The mixture was purged with H_2

for 5 min and then stirred under H_2 for 7.5 h. The reaction mixture was filtered through a pad of celite, and the filtrate was concentrated to provide **242** (2.21g, 74%) as a colorless oil, which was clean based on ^1H and ^{13}C NMR and was used without further purification in the next reaction: IR (neat) 2925, 2853, 1462, 1252, 1096, 834, 774, 679 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.25 (m, 2H), 7.18–7.15 (m, 3H), 3.60 (t, *J* = 6.6 Hz, 2H), 2.60 (t, *J* = 7.6 Hz, 2H), 1.65–1.59 (m, 2H), 1.51 (quin, *J* = 6.8 Hz, 2H) 1.34–1.28 (m, 12H), 0.90 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.2, 128.6, 128.4, 125.8, 63.6, 36.2, 33.1, 31.7, 29.8, 29.7, 29.7, 29.7, 29.6, 26.2, 26.0, 18.6, –5.0; HRMS (ESI) calcd for $C_{22}H_{41}OSi$ $[M + H]^+$ *m/z* 349.2921, found 349.2919.

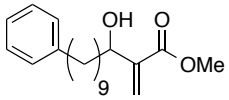


10-Phenyldecane-1-ol (243). TBAF (1 M in THF, 15.0 mL, 15.0 mmol) was added drop-wise to a stirred solution of 1-*(tert*-butyldimethylsilyloxy)-10-phenyldecane **242** (2.81 g, 7.5 mmol) in THF (140 mL) at 0 ^oC. The reaction was allowed to warm to rt and stir overnight. Saturated aqueous NH_4Cl (100 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (3 X 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The crude residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) to give (**243**) (1.41 g, 81%) as a colorless oil: IR (neat) 3431, 2919, 2848, 1494, 1453, 1348, 1060, 717, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.31–7.26 (m, 2H), 7.20–7.17 (m, 3H), 3.64 (t, *J* = 6.6 Hz, 2H), 2.60 (t, *J* = 7.6 Hz, 2H), 1,64–1.52 (m, 4H), 1.48 (br. s, 1H), 1.30–1.28 (m, 12H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 143.1, 128.6, 128.4, 125.7, 63.2, 36.2, 32.9, 31.8, 29.8, 29.7, 29.6, 29.5, 25.9; HRMS (ESI) calcd for $C_{16}H_{27}O$ $[M + H]^+$ *m/z* 235.2056, found 235.2065.

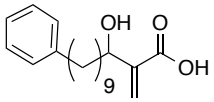


10-Phenyldecanal (244). 4-Acetamido-2,2,6,6-tetramethylpiperidine-1-oxoammonium tetrafluoroborate (**248**) (2.01 g, 6.68 mmol) and SiO_2 (2.01 g) were added to a solution of 10-phenyldecane-1-ol (**243**) (1.41 g, 6.07 mmol) in CH_2Cl_2 (48 mL). The reaction mixture was stirred at rt overnight. The reaction mixture was then filtered through a pad of SiO_2, and the filtrate was concentrated and used without further purification, as both ^1H and ^{13}C NMR showed clean **244** (1.26 g, 90%) as colorless crystals: mp: 41–42 ^oC; IR (neat) 2913, 2847, 1698, 1411, 1282, 1250, 1218, 942, 744, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.8 Hz, 1H), 7.29–7.25 (m, 2H), 7.18–7.15 (m, 3H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.41 (td, *J* = 7.3, 1.8 Hz, 2H), 1,66–1.57 (m, 4H), 1.30–1.29 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.1, 143.1, 128.6, 128.4, 125.8, 44.1, 36.2, 31.7, 29.6, 29.5, 29.5, 29.3, 22.3; HRMS (ESI) calcd for $C_{16}H_{23}O$ $[M - H]^+$ *m/z* 231.1754, found 231.1734.



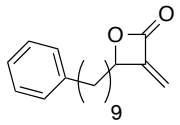
Methyl 3-hydroxy-2-methylene-12-phenyldodecanoate (245). 10-Phenyldecanal (**244**) (1.26 g, 5.45 mmol) and methyl acrylate (**196**) (0.98 mL, 10.9 mmol) were combined in an empty flask. 3-Hydroxyquinuclidine (0.173 g, 1.36 mmol) was added, followed by MeOH (0.17 mL).[89] The resulting mixture was

stirred for 2 d. MeOH and excess methyl acrylate were removed under reduced pressure. The resulting residue was diluted with a H_2O/saturated aqueous NH_4Cl solution (5:1, 30 mL), and the resulting mixture was extracted with CH_2Cl_2 (3 X 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) gave **245** (1.06 g, 61%) as a colorless oil: IR (neat) 3350 (br), 2924, 2853, 1717, 1438, 1194, 1153 cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.25 (m, 2H), 7.19–7.15 (m, 3H), 6.22 (m, 1H), 5.81 (m, 1H), 4.39 (m, 1H), 3.78 (s, 3H), 2.62–2.56 (m, 3H), 1.69–1.57 (m, 4H), 1.48–1.43 (m, 1H), 1.30–1.28 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 143.1, 142.7, 128.6, 128.4, 125.7, 125.1, 72.0, 52.0, 36.4, 36.2, 31.7, 29.7, 29.7, 29.7, 29.6, 29.5, 26.0; HRMS (ESI) calcd for $C_{20}H_{31}O_3$ $[M + H]^+$ *m/z* 319.2268, found 319.2281.



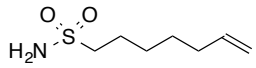
3-Hydroxy-2-methylene-12-phenyldodecanoic acid (246). Methyl 3-hydroxy-2-methylene-12-phenyldodecanoate (**245**) (1.06 g, 3.37 mmol) was dissolved in EtOH/H_2O (2:1, 8 mL). Lithium hydroxide (77 mg, 3.4 mmol) was added; the resulting solution was stirred overnight.[89] The reaction was quenched with 1 M HCl (30 mL), and the solution was extracted with CH_2Cl_2 (3 X 20 mL). The combined organic layers were washed with H_2O (30 mL) and dried ($MgSO_4$); CH_2Cl_2 was removed under reduced pressure. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc, 85:15) gave **246** (0.396 g,

54%) as a colorless oil: IR (neat) 2919, 2848, 1685, 1622, 1493, 1435, 1284, 1181, 1118, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.25 (m, 2H), 7.18–7.15 (m, 3H), 6.38 (s, 1H), 5.91 (s, 1H), 4.42 (dd, *J* = 6.3, 6.3 Hz, 1H), 2.60 (t, *J* = 7.6 Hz, 2H), 1.74–1.57 (m, 4H), 1.48–1.43 (m, 1H), 1.30–1.28 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 143.2, 142.0, 128.6, 128.4, 127.6, 125.8, 71.8, 36.4, 36.2, 31.7, 29.7, 29.7, 29.6, 29.5, 26.0; HRMS (ESI) calcd for $C_{19}H_{29}O_3$ $[M + H]^+$ *m/z* 305.2111, found 305.2142.



3-Methylene-4-(9-phenylnonyl)oxetan-2-one (247). 3-Hydroxy-2-methylene-12-phenyldodecanoic acid (**246**) (0.40 g, 1.8 mmol) was dissolved in dry CH_2Cl_2 (7 mL). Oven-dried Cs_2CO_3 (0.64 g, 1.8 mmol) was added, and the resulting suspension was stirred for 20 min. *o*-Nosyl chloride (800 mg, 3.6 mmol) was added, and the resulting mixture was stirred for 3 d. The reaction was diluted with CH_2Cl_2 (20 mL), and then 1 M HCl (20 mL) was added. The aqueous layer was separated and extracted with CH_2Cl_2 (3 X 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification of the residue by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) gave **247** (0.089 g, 17%) as a colorless oil: IR (neat) 2926, 2854, 1821, 1080 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.27–7.23 (m, 2H), 7.17–7.13 (m, 3H), 5.87 (dd, *J* = 1.8, 1.8 Hz, 1H), 5.38 (dd, *J* = 1.5, 1.5 Hz, 1H), 4.95–4.91 (m, 1H), 2.59 (t, *J* = 7.6 Hz, 2H), 1.88–1.78 (m, 2H), 1.60 (quin, *J* = 8.0 Hz, 2H), 1.50–1.40 (m, 2H), 1.38–1.29 (m,

10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 146.6, 143.0, 128.5, 128.4, 125.7, 115.0, 79.8, 36.1, 33.4, 31.6, 29.6, 29.5, 29.5, 29.4, 29.4, 24.7; HRMS (ESI) calcd for $C_{19}H_{27}O_2$ $[M + H]^+$ *m/z* 287.2006, found 287.1999.



Hept-6-ene-1-sulfonamide (250). A solution of 7-bromo-1-heptene (**249**) (0.50 g, 4.3 mmol), and sodium sulfite (0.65 g, 5.1 mmol) in H_2O (3.0 mL) was refluxed overnight.[93] After cooling to rt the aqueous solution was washed with Et_2O (2.2 mL) before being evaporated to dryness. The resulting white solid was dried under vacuum at 130 °C for 1 h and then $POCl_3$ (4.3 mL) was added, and the mixture was stirred for 4 h at 130 °C. The reaction mixture was evaporated, and the residue was taken up in CH_3CN (5 mL), and a solution of NH_4OH (28.0–30.0%, 10 mL) in CH_3CN (4 mL) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 1 h before being diluted with CH_2Cl_2 (30 mL) and washed with H_2O (20 mL). The organic layer was dried (Na_2SO_4) and concentrated. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc, 80:20) gave **250** (0.29 g, 42%) as a white solid: mp: 52–52.5 °C; IR (neat) 3338, 3253, 2927, 1469, 1294, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.78 (ddt, *J* = 17.0, 10.2, 6.4 Hz, 1H), 5.03–4.94 (m, 2H), 4.84 (br s, 2H), 3.13–3.09 (m, 2H), 2.06 (q, *J* = 6.3 Hz, 2H), 1.89–1.81 (m, 2H), 1.45–1.43 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 114.9, 55.3, 33.4, 28.4, 27.7, 23.8; HRMS (ESI) calcd for $C_7H_{16}NO_2S$ $[M + H]^+$ *m/z* 178.0896, found 178.0913.

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