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Towards the Synthesis of alpha-Aminogalactosylceramides

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APPROVAL PAGE

Master of Science Thesis

Towards the Synthesis of α-Aminogalactosylceramides

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List of Abbreviations

Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
BAIB	[bis(acetoxy)iodo]benzene
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
BnBr	benzyl bromide
Boc	*tert*-butyloxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
br	broad
Bu_4NF	tetrabutylammonium fluoride
CBr_4	tetrabromomethane
CH_2Cl_2	methylene chloride
$CHCl_3$	chloroform
cm	centimeters
d	doublet
DCC	*N,N'*-dicyclohexylcarbodiimide
dd	doublet of doublets
ddd	doublet of doublet of doublets
dddd	doublet of doublet of doublet of doublets
DIAD	diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	dimethyl formamide
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
Et_2O	diethyl ether
EtOAc	ethyl acetate

EtOH	ethanol
Fmoc	fluorenylmethyloxycarbonyl
g	gram
GalCer	galactosylceramide
h	hour
H	hydrogen
H_2	dihydrogen
H_2NNH_2	hydrazine
H_2O	hydrogen oxide
HBr	hydrobromic acid
HCl	hydrochloric acid
HN(OMe)Me	methoxy(methyl)amine
HRMS	high resolution mass spectroscopy
Hz	hertz
IR	infrared
J	J-coupling
KBr	potassium bromide
$LiAl(O\textit{t}\text{-}Bu)_3H$	tri-*t*-butoxyaluminum hydride
M	molecular or molarity
m	multiplet
MeOH	methanol
$MeSO_3H$	methanesulfonic acid
MgCl	magnesium chloride
$MgSO_4$	magnesium sulfate
MHz	megahertz
min	minutes

mL	milliliter
mmol	millimole
N_2	dinitrogen
$Na_2S_2O_3$	sodium thiosulfate
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
NaOMe	sodium methoxide
NaOt-Bu	sodium tert-butyl alcohol
NH_4Cl	ammonium chloride
NHS	*N*-hydroxysuccinimide
NK T	natural killer T-cell
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NOD	non-obese diabetic mice
$OC(NH)CCl_3$	trichloroacetimidate
P_2S_5	phosphorus pentasulfide
$Pd_2(dba)_3$	tris(dibenzylideneacetone)dipalladium(0)
PhSH	thiophenol
PNP	*p*-nitrophenyl
PPh_3	triphenyl phosphine
ppm	parts per million
Pyr	pyridine
q	quartet
rt	room temperature
s	singlet

SEM	[2-(trimethylsilyl)ethoxy]methyl
t	triplet
TBAF	tetrabutylammonium fluoride
TBAHS	tetra-*n*-butylammonium hydrogensulfate
TBAI	tetrabutylammonium iodide
TBDMSOTf (TBSOTf)	tert-butyldimethylsilyl trifluoromethanesulfonate
TBDPSCl	tert-butyldiphenylsilyl chloride
t-BuOH	tert-butyl alcohol
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TFA	trifluoroacetic acid
THAI	tetrahexylammonium iodide
THF	tetrahydrofuran
TLC	thin layer chromatography
$TMSN_3$	trimethylsilyl azide
TOF	time of flight
TsOH	*p*-toluenesulfonic acid

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Introduction

I. Galactosylceramide Background

In 1993, Koezuka and coworkers were researching marine natural products with anti-tumor activities at the Kirin Brewery, which led to the discovery of agelasphin (e.g. agelasphin 9b). Research[1] on these natural products led to the creation of the KRN7000 (Figure 1).

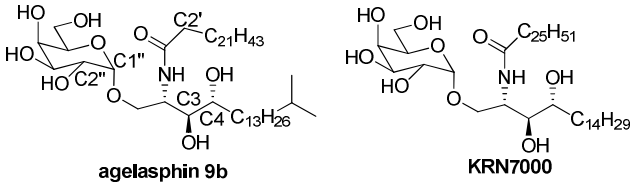


Figure 1: Structures of Agelasphin 9b and KRN7000

KRN7000 has been reported to stimulate NKT cells through its presentation by the CD1d protein. This stimulatory pathway may have potential for the treatment of tumors,[2-3] infectious diseases,[4-5] and autoimmune conditions[6-7]. Further studies involving alterations to this molecule are being investigated to observe how differences in glycolipid functionality alter immune responses. In the following paragraphs the structure and bioactivity of some of the glycolipids related to the goals of this thesis will be discussed.

A. Composition Of A Galactosylceramide

Generally, a galactosyl ceramide (a type of glycolipid) (Figure 2) is made up of a galactosyl moiety connected to a ceramide by what is known as a glycosidic bond. The ceramide consists of two hydrophobic lipid chains, one an amide acyl chain and the other a sphinganine, either of which can vary based on chain length, branching, or functional groups attached. In the next section the influence of some glycolipids on the immune system will be presented.

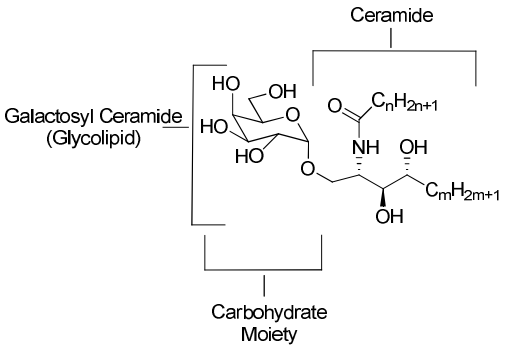


Figure 2: General Structure of Galactosylceramides

B. Immunomodulation

Glycolipids have been shown to increase immunoactivity by interacting with the immune systems lymphocytes.[8] Lymphocytes are white blood cells that serve various functions in the immune system. They are also related to adaptive immunity (immunity that develops as we age), as opposed to innate immunity (basic immunity that responds immediately but non-specifically to infections and pathogens).[9] The two major classes of lymphocytes are distinguished by cells which grow independently of the thymus (B cells) and that grow inside the thymus (T cells). Natural Killer T-lymphocytes (NKT cells) are a subset of lymphocytes that have T-cell receptors located on their surface.[9] The glycolipid's alkyl chains are utilized by binding to the CD1d protein of an antigen presenting cell through crevice-like tunnels. Once bound, the exposed sugar head of the glycolipid interacts with the T-cell receptor of the NKT cell. Interaction between the T-cell receptor and glycolipid causes the release of cytokines from the NKT cells.[10] There are two groups of cytokines, T helper 1 (Th1) and T helper 2 (Th2), categorized based on their response upon release. Cytokines like interferon-γ (IFN- γ) and interleukin-2 (IL-2) exhibit an inflammatory response (Th1) associated with controlling bacterial, parasitic and viral infections as well as tumors. Interleukin-4 (IL-4) and IL-10 are examples of two cytokines responsible for an immunomodulatory response (Th2), which has been known to ameliorate autoimmune diseases, such as multiple sclerosis, lupus, rheumatoid arthritis, and type I diabetes.[10] In the next section the research leading to the discovery of KRN7000 and its biological activity will be discussed.

II. Discovery Of KRN7000

In 1993, Koezuka and coworkers were investigating marine natural products for bioactivity and came across the agelasphins, in particular, agelasphin 9b (extracted from the sponge *Agelas Mauritianus*) (Figure 1). The interestingly rare feature about these agelasphins was their α-glycosidic bonds, as opposed to the β-glycosidic bonds found in more advanced organisms. Around this time it was also discovered that glycolipids cause lymphocyte cell proliferation. Further studies[11] of the bioactivity led to structural determination that indicated the C4 hydroxyl group as nonessential for bioactivity and the C3 hydroxyl group as necessary for T-cell stimulation. Alteration of the lipid chains led to the creation of the galactosylceramide KRN7000.

KRN7000 is now widely known for its interaction with NKT cells leading to the release of both Th1 and Th2 cytokines. It has been shown[10] that Th2 cytokines can antagonize the Th1 response. Ideally, controlling which cytokines are released would prove beneficial to those suffering from infectious or autoimmune diseases. Interest surrounding the medicinal properties of the glycolipid led researchers to investigate methods to manipulate the molecule resulting in the controlled release of Th1 and Th2 cytokines. In the next section previous research involving key KRN7000 derivatives that lead to a biased cytokine response will be discussed, including background information leading to the target structures of this thesis.

III. Research On KRN7000 Analogs

A. OCH – A Th2 Biasing Glycolipid

Among the analogs of KRN7000 is OCH (Figure 3), a compound which showed bias in the release of Th2 cytokines, IL-4 and IL-10. Further studies showed that OCH inhibited experimental autoimmune encephalitis[12] and collagen-induced arthritis[13]. Inspired by the ameliorative implications,

Miyake and coworkers researched the glycolipid's (Figure 3) effect on type 1 diabetes. Tests were conducted on non-obese diabetic mice (NOD), a model for type I diabetes in humans. KRN7000 was used as a control to test the activity of OCH.

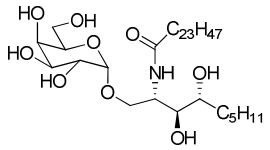


Figure 3: Structure of OCH

While observing the incidence of diabetes in 30 week old NOD mice that were given multiple doses of OCH at 5 weeks of age, the glycolipid showed comparable inhibition results (reduction from 75% to 27%) to KRN7000. In an experiment observing insulitis (inflammation of islets of the pancreas, which can lead to diabetes), the inflammations were categorized by the amount of inflamed areas observed (Figure 4).

To observe levels of insulitis pancreatic sections were removed and examined. Data showed lower grade 3 levels and higher grade 0 levels in mice dosed with OCH compared to mice dosed with KRN7000. Although OCH was not as potent in the stimulation of NKT cells (as KRN7000), the glycolipid showed more inhibitory effect of insulitis in the microscopic appearance of pancreatic samples. Overall, OCH has provided evidence indicating its inhibition of insulitis and diabetes.[14]

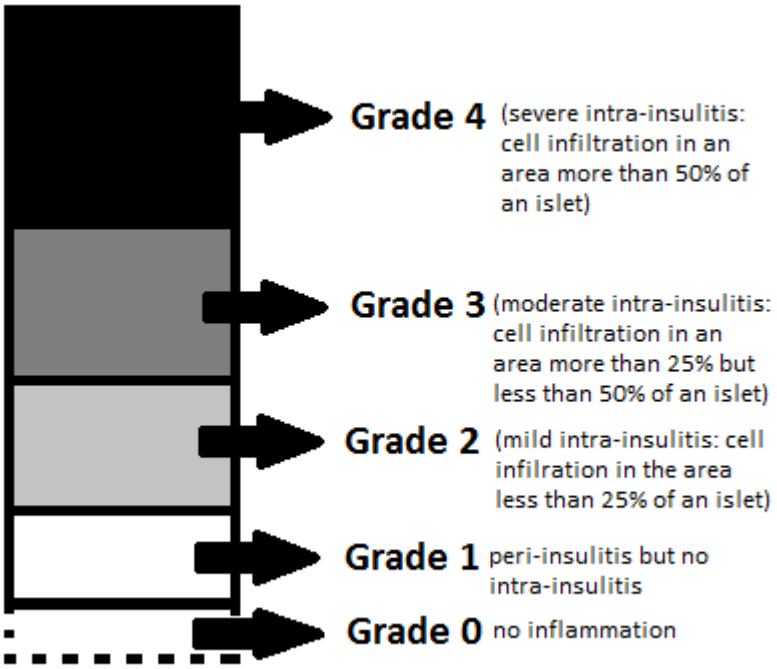


Figure 4: Levels of insulitis inflammation

OCH test results have been debated[14] between researchers trying to determine plausible reasons for its superiority over KRN7000 in models for diabetes. Variables, such as defects in NKT cells,[14] might have led to an increase of NKT cells in transgenic mice, which would give off more Th2 cytokines, contaminating the accuracy of the results for cytokine release. The results for the spontaneous development of diabetes could have been shifted in favor of the OCH glycolipid if there were any bacterial infections in the mice, because a combination of mycobacterium extract with KRN7000 would cause the NKT cells to predominately produce Th1 cytokines,[15] nullifying the Th2 effect. Although these are not definitively the reasons behind the results, the fact remains that OCH has shown a biased release of Th2 cytokines, IL-4 and IL-10, and has assisted validation of the pursuit of altering the glycolipid structure to provide controlled responses. Another area of interest concerning the alteration of the glycolipid structure and controlled cytokine release is the glycosidic bond between sugar and ceramide.

Discussion below will focus more on research concerning the glycosidic bond between sugar and ceramide.

B. Anomeric Replacement: α-*C*-GalCer

It is known that KRN7000 releases high levels of both Th1 and Th2 cytokines (i.e. IFN-γ and IL-4, respectively, are generally monitored). Moreover, the unbiased release antagonizes the health benefits related to both types of cytokines. Research[16] on encephalomyelitis in mice has shown that a synthetic analogue possessing a truncated sphingosine chain resulted in stimulating of the release of only IL-4, which better protected mice from encephalomyelitis. This alteration to the glycoside led researchers[16-17] to synthesize a KRN7000 derivative with a hydrophobic methylene (CH2) as the glycosidic link between the sugar and ceramide instead of the hydrophilic oxygen (O) (Figure 5). The substitution was meant to deter α-galatosidase catabolism in vivo[18-19] and allow observation of any difference in NKT cell stimulation.

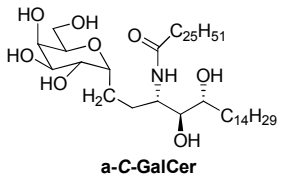


Figure 5: Franck and Tsuji's methylene galactosylceramide[17]

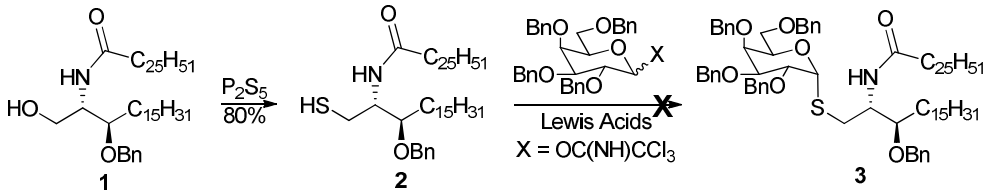
In multiple comparisons between KRN7000 and α-*C*-GalCer, test results consistently provided data[17,20-21] which led to the conclusion that α-*C*-GalCer is a better candidate for the treatment of infectious and autoimmune diseases than KRN7000. Comparison tests[17] regarding malaria have shown that α-*C*-GalCer's antimalaria effect lasted 3 days longer than KRN7000. In regards to tumor studies[20] α-*C*-GalCer has been shown to exhibit similar T cell stimulation to KRN7000 when using a dosage 1000 times less than KRN7000. A binding stability experiment[20] showed that α-*C*-GalCer required less time to bind to NKT cells and stimulated the cells more significantly than KRN7000. In more recent studies,[21] α-*C*-

GalCer was used as an adjuvant to a live attenuated influenza virus vaccine and was shown to increase the immunogenicity and enhance protection provided by the vaccine in wild type mice. The next section will discuss previous research conducted in the Howell group focusing particularly on the glycosidic bond.

C. α-*S*-GalCer

The emerging understanding and medicinal potential of the α-glycosylceramides had attracted the attention of the Howell group.[10] Previous research in our group related to glycolipids was inspired by the KRN7000 derivative α-*C*-GalCer. The comparison of the CH2 glycolipid to KRN7000 has previously been discussed and has led to the conclusion that alterations to the glycosidic bond can have biased and beneficial effects. Following that approach an investigation[22] was conducted exploring the link between the sugar and ceramide. One previous glycolipid target in the group was α-*S*-GalCer **3**.

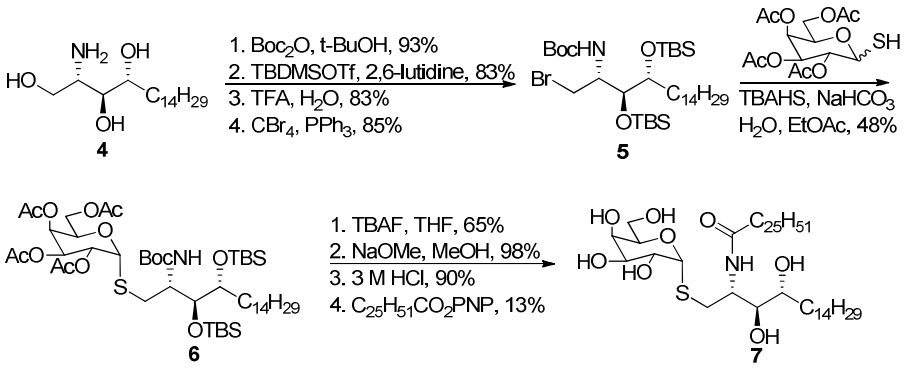
This approach took advantage of treating a nucleophilic ceramide with an electrophilic sugar donor. Ceramide **1** (Scheme 1) was treated with phosphorus pentasulfide forming **2**. Glycosylation was attempted using a trichloroacetimidate activated by a Lewis acid but unfortunately no desired product **3** was isolated. Thiol glycosylation following the above method had been previously reported successfully[23], but researchers used simple alkyl acceptor groups in comparison to the ceramide.



Scheme 1: Glycosylation of a protected α-*S*-GalCer

Another approach was attempted, targeting α-*S*-GalCer (Scheme 2). Beginning with phytosphingosine **4**, the molecule was Boc-protected, fully silyl protected, selectively deprotected, and then brominated following Yamamoto's procedure forming the electrophilic alkyl chain, **5**. Following precedent literature[22] α-*S*-GalCer **7** was synthesized as an inseparable anomeric mixture. The thiol sugar was then

alkylated using Schimdt's reported approach, resulting in the protected glycolipid **6**. The thiol glycoside was fully deprotected and acylated to form the targeted α-*S*-GalCer **7**.



Scheme 2: Synthesis of α-*S*-GalCer

The successfully synthesized α-*S*-GalCer was tested for biological activity,[22] and although it shared a similar structure to KRN7000, it did not show any biological activity. One speculation was that oxidation of the thiol glycoside could have occurred, interfering with interaction between the *S*-GalCer and NKT cell. Although the *S*-GalCer may not have shown beneficial effects in regards to NKT cell stimulation, it provided information regarding substitution of oxygen with a closely related atom. Continuation of the glycosidic link investigation by the Howell group led to the goals of this thesis.

IV. Target Of Research (α-*N*-GalCer)

The goals of this thesis were to synthesize α-*N*-GalCer **8** and α-*N*-formyl-*N*-GalCer **9** (Figure 6). These targets were chosen because we were inspired by α-*C*-GalCer's bioactivity and wanted to synthesize glycosylceramides that carried similarities but had not been explored previously. The next two sections will discuss literature methods used to approach *N*-glycosides.

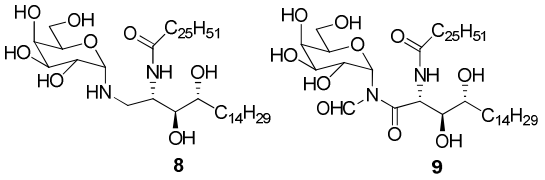
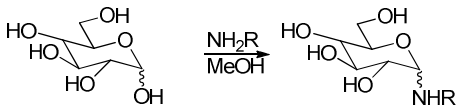


Figure 6: Target α-*N*-GalCer molecules

A. Approach To α-*N*-GalCer's

As with S-GalCer's there are two main approaches to synthesizing *N*-glycosides. The first couples an electrophilic sugar donor with an amine. The next method utilizes a nucleophilic sugar coupled with an electrophile.

Some researchers have reported creating alkylglycosylamines by coupling simple aminoalkyl groups with unprotected sugar donors (Scheme 3).[24-25] In relation to the sugar, mannose commonly results in alpha glycosylations, while glucose and galactose yield alpha and beta glycosylated products (beta predominating).[26] There have been no reports of the synthesis of *N*-glycosylceramides. Marisa Blauvelt, in the Howell group did try to glycosylate an aminoceramide using an unprotected sugar donor, but the approach was unsuccessful.



Scheme 3: Formation of an alkylglycosylamine using mannose.

An alternative approach would be to use a halide donor sugar. Our group has experience coupling galactosyl iodides or bromides with ceramides or related sphingoid bases. Consequently we will examine the reaction of these glycosyl halides with aminoceramides. Glycosylations reported by Jacquelyn Gervay-Hague[26] treated an alpha sugar donor with a sphinganine (acceptor) resulting in an alpha

glycosylated product. To synthesize the first target (**8**) a similar approach to Jacquelyn Gervay-Hague's will be attempted (Figure 7).

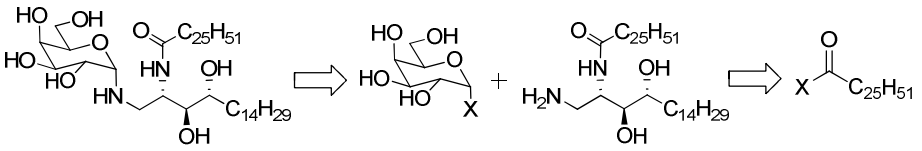
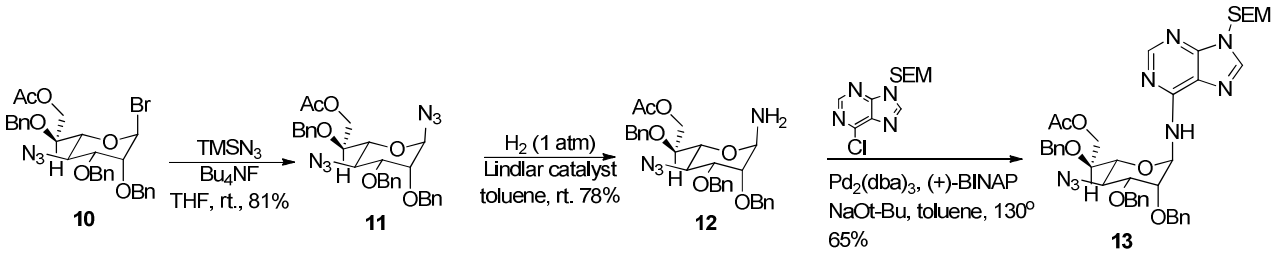


Figure 7: Approach to α-*N*-GalCer

Regarding the second approach to forming α-*N*-GalCer's (mentioned above) there have been reports[25] of successfully synthesizing α-*N*-GalCer's using nucleophilic sugar donors (Scheme 4). For example, the synthesis of spicamycin[27] involves a nucleophilic sugar donor reacting with an electrophilic chloropurine. The sugar **10** underwent azide formation using trimethylsilyl azide. Azide **11** was reduced using hydrogen with Lindlar's catalyst, forming **12**. The heterocylic alkylation via amide coupling formed **13**.



Scheme 4: Formation of alkylglycosylamine using galactose.

B. Approach To α-*N*-formyl-*N*-GalCer

A proposed method to the synthesis of α-*N*-formyl glycolipids was found in recent chemistry[28] performed by Danishefsky's group. Their investigation of complex chemistry was inspired by the Passerini reaction[29] (Figure 8). The reaction (also referred to as an acyl transfer) called for a carboxylic acid and an isonitrile resulting in an amide product.

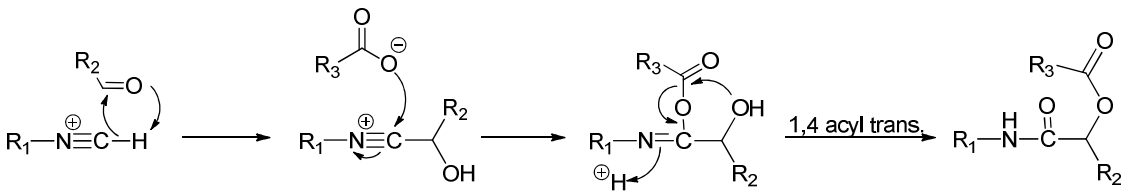


Figure 8: Passerini Reaction.

Danishefsky proposed a mechanism between the isonitrile and carboxylic acid (Figure 9), as illustrated in a reported reaction using a fully protected isonitrile and aspartic acid (Scheme 5). This reactions outcome has been shown to be influenced anomerically by the isonitrile used for the acyl transfer; an alpha isonitrile would result in an alpha formylated product and a beta isonitrile would result in a beta formylated product.

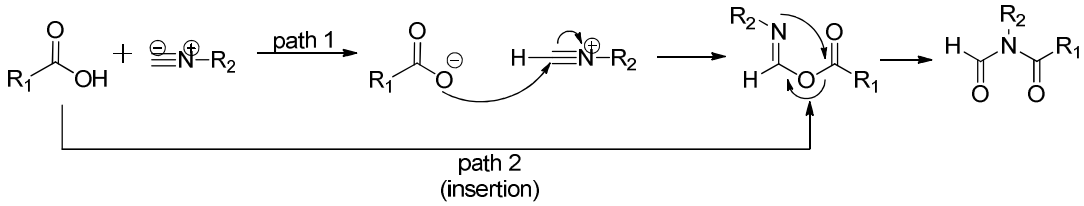
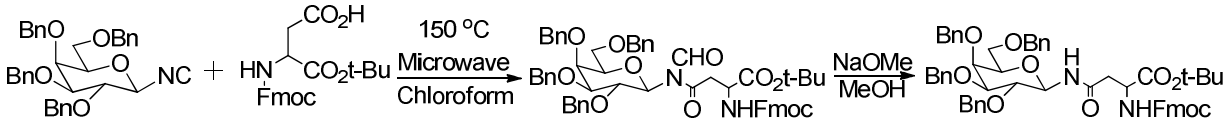


Figure 9: Danishefsky's proposed acyl transformation.



Scheme 5: Acyl transfer using tetrabenzylated isonitrile and aspartic acid.

Our initial approach to α-*N*-formyl-*N*-GalCer **9** will take advantage of Danishefsky's method (discussed above) to form *N*-formyl glycolipids. An alpha nucleophilic sugar donor will be treated with an electrophilic ceramide acceptor (Figure 10) to synthesize our desired formyl product.

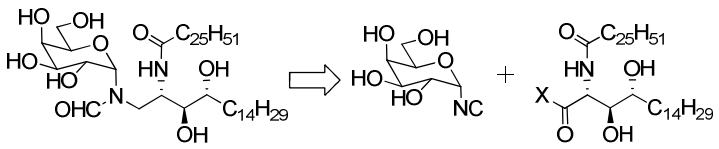


Figure 10: Approach to α-*N*-formyl-*N*-GalCer

V. Summary

Past research has shown that KRN7000 anomeric replacements (e.g. α-*C*-GalCer) have produced interesting results. Further investigations on these replacements would lead to a better understanding of the glycolipid structure and potentially biased T cell stimulation. The Howell group's research has reported that it is possible to synthesize α-*S*-galactosylceramides. The investigations in this thesis have been focused on anomeric replacement with nitrogen.

Results and Discussion

I. Research Objective

The goals of these studies were:

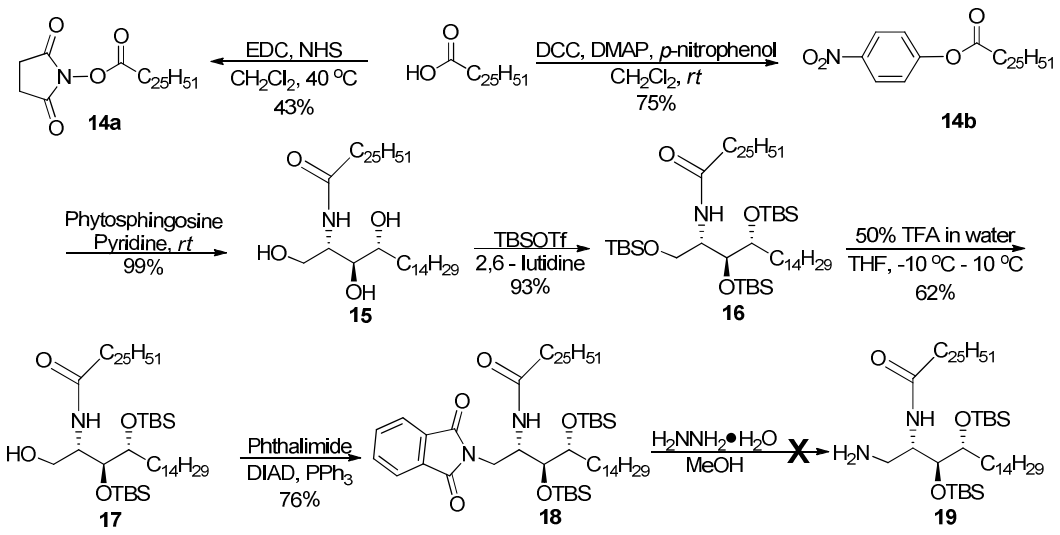
1. To synthesize an α-*N*-galactosylceramide analog of KRN7000
2. To synthesize an α-*N*-formyl-*N*-galactosylceramide analog of KRN7000
3. To compare NKT cell stimulatory abilities of α-*N*-galactosylceramide and α-*N*-formyl-*N*-galactosylceramide to their parent compound KRN7000 in collaboration with the lab of Professor Brian Wilson of the University of Florida

II. Synthesis Of α-*N*-galactosylceramide

A simple and straightforward method was required for the synthesis of the complex structure of α-*N*-galactosylceramide. Since the Howell group had success with coupling galactosyl halides with ceramides or related sphingoid bases this method was examined first. As mentioned before (Figure 7, pg.

10), the glycolipid will be broken down into two groups an aminoceramide and a halosugar. The ceramide will be synthesized by coupling a fatty acid chain with phytosphingosine.

Following Sanghee's reported[30] α-GalCer approach (Scheme 6), the synthesis of α-*N*-GalCer began by coupling commercially available cerotic acid with *N*-hydroxysuccinimide, providing ester **14a** in 43% yield. Due to the esters less than moderate yields an alternate ester formation was examined.[31] Cerotic acid was treated with *p*-nitrophenol to afford ester **14b** in 60% yield. Ester **14b** could be isolated using column chromatography, but it was also found that purification of **14b** could be accomplished by recrystallization using ethyl acetate giving 75% yield. The ester was then coupled to phytosphingosine, forming ceramide **15**, isolated in 99% yield. Previously this had been purified via column chromatography, but it was discovered that **15** could also be purified by recrystallization using ethyl acetate. Ceramide **15** was then fully silyl protected, providing **16** in 93% yield. Selective deprotection gave the free primary alcohol **17** in 62% yield. The original procedure[30] called for 10% trifluoroacetic acid (TFA), but the reaction did not reach completion until the concentration of TFA was increased to 50%. An amination similar to the Gabriel synthesis[32] was then performed on alcohol **17**. The selectively deprotected ceramide was treated with phthalimide under Mitsunobu conditions, forming **18** in 76% yield. Selective deprotection of **18** using hydrazine was attempted. Although the starting material was consumed, based on TLC, we were unable to isolate clean aminoceramide **19**. As proof of aminoceramide formation, it was acetylated. Although pure acetylated product was not isolated, ^{13}C NMR data from the acetylation showed two carbonyl peaks, which led us to believe that aminoceramide **19** was successfully synthesized. The glycosylation of **19** was attempted but no desired product was isolated. Consequently, the approach was revised.



Scheme 6: Attempted synthesis of aminoceramide

The Howell group has found that working with sphinganines (aminodiols) rather than phytosphingosines (aminotriols), is sometimes more straightforward. We decided to proceed with the synthesis using a protected sphinganine (Figure 11). Prior to the ceramide formation the aminodiol will be coupled with the halosugar donor. The sphingoid base will be formed using a simple and selective route.[33]

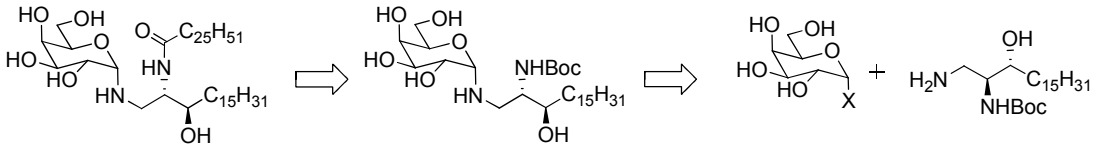
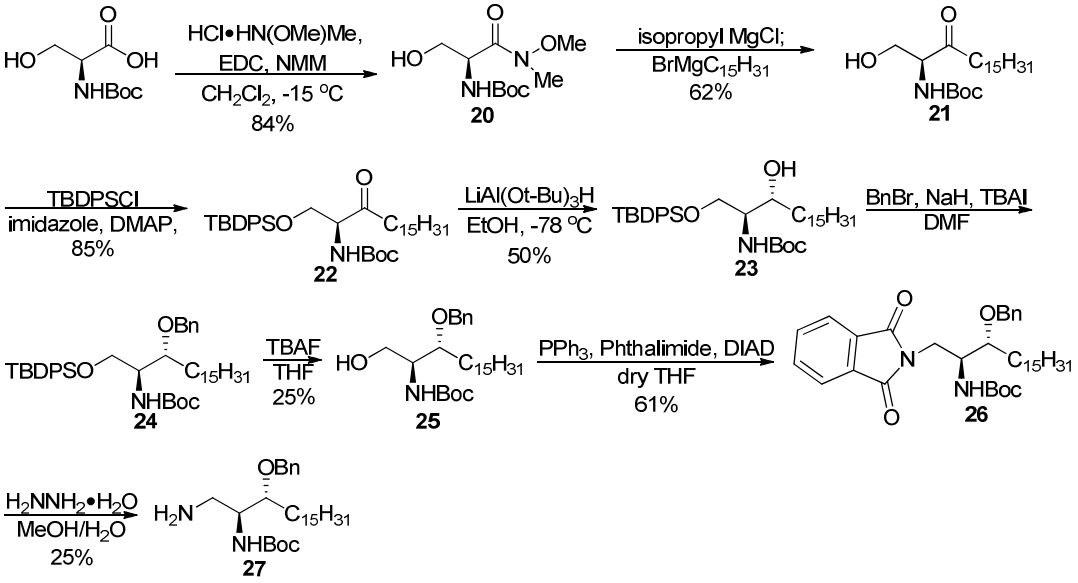


Figure 11: Approach to revised α-*N*-GalCer

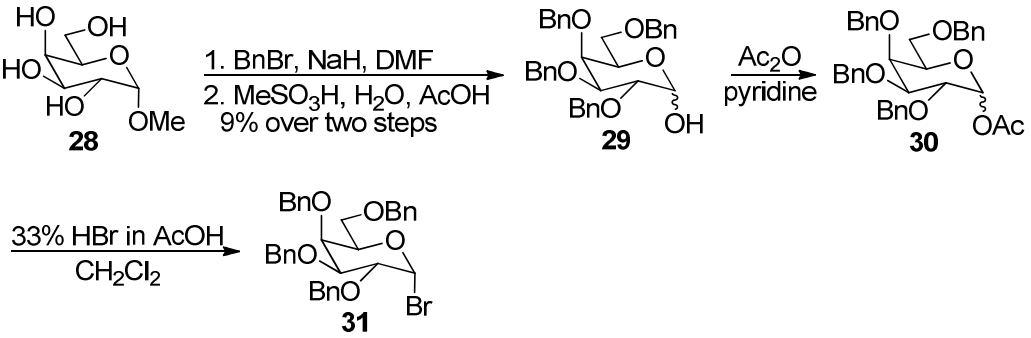
Following a procedure developed in the group[33] (Scheme 7) the synthesis of the selectively protected sphingoid base began by treating Boc-protected serine with methoxy(methyl)amine hydrochloride, forming Weinreb amide **20**. This was treated with two equivalents of a sacrificial base to deprotonate the primary alcohol of the carbonate. Then, pentadecylmagnesium bromide was added, forming ketone **21** in 62% yield over two steps. The alcohol was silyl protected, providing **22** in 85% yield. The protected sphinganine was then reduced, giving the secondary alcohol **23** in 50% yield. Benzylation gave the fully protected sphingoid base **24** in 54% yield. Selective deprotection provided **25** in 25% yield. The low yield in the deprotection was due to loss of material during work-up. The primary

amine was formed following the same procedure described in the attempted synthesis of the aminoceramide **19**. Reaction with phthalimide gave **26** in 61% yield. A deprotection was attempted using sodium borohydride with acetic acid in isopropyl alcohol,[34] but no desired product was isolated. An alternate deprotection with hydrazine produced **27** in 25% yield. During purification of the phthalimide deprotection via TLC plate a second spot was observed close to the desired product. This had also been observed with the attempted aminoceramide purification. The ^{1}H and ^{13}C NMRs showed only the desired aminodiol in the isolated product.



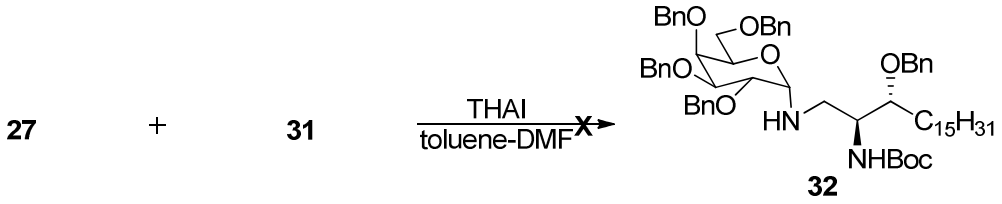
Scheme 7: Synthesis of sphingoid base

The synthesis of the glycosyl donor began with commercially available methyl-α-D-galactopyranoside(**28**) (Scheme 8). Following the approach of Jacquelyn Gervay-Hague's reported halosugar synthesis,[35] global benzyl protection of methoxygalactoside, followed by anomeric hydrolysis gave **29** in 9% yield over two steps. Acetalyation of the lactol formed **30**. Purification of **30** did not afford a sufficient yield so the product was obtained by a labmate. Treatment of the acetylated sugar with hydrobromic acid provided bromosugar **31**. Due to its sensitivity to silica gel, the sugar was directly used in the glycosylation without purification.



Scheme 8: Synthesis of bromosugar

Glycosylation of the protected aminosphingoid base **27** with bromosugar **31** was performed following a method reported by Jacquelyn Gervay-Hague (Scheme 9).[26] A compound that displayed similar spectral characteristics to the desired product was observed but, unfortunately, could not be isolated.



Scheme 9: Attempted synthesis of revised α-*N*-GalCer

III. Synthesis Of α-*N*-formyl-*N*-galactosylceramide

Our synthesis of α-*N*-formyl-*N*-galactosylceramide (**9**) will require treatment of an electrophilic sugar with a nucleophilic ceramide (Figure 12). Based on the study reported by Danishefsky[28] (Scheme 5, pg. 12) we will treat an isonitrile with a carboxylic acid derived from the corresponding ceramide. The beta isonitrile will be used to test the methodology of the acyl transfer reaction. Once the methodology proves successful, the alpha isonitrile will be used to create the desired *N*-formyl glycolipid. The isonitrile will be synthesized using Danishefsky's methods, and the acid will be synthesized by oxidation of the ceramide's primary alcohol.

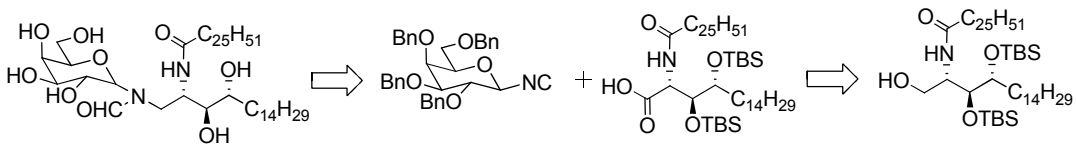
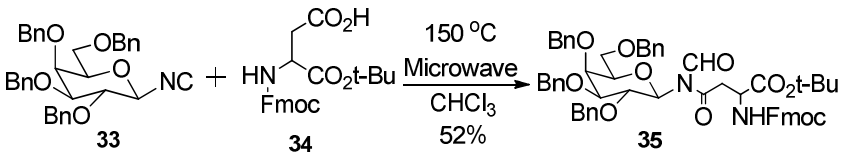


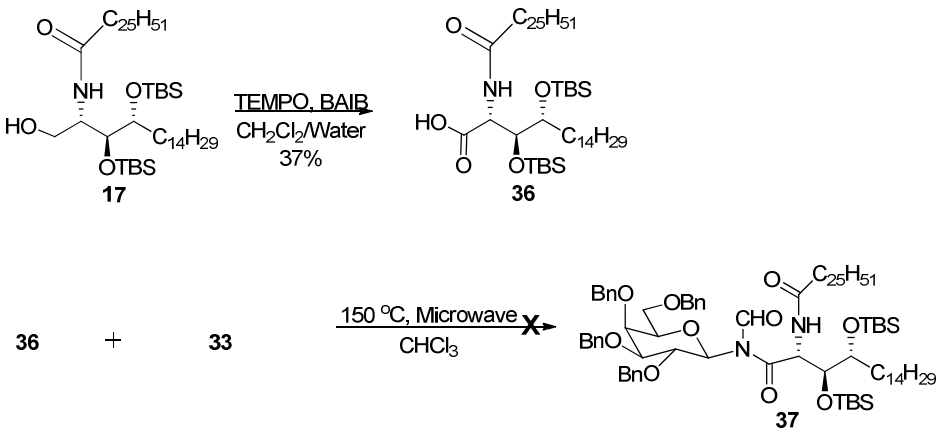
Figure 12: Approach to α-*N*-formyl-*N*-GalCer

A model study was conducted prior to the α-*N*-formyl-*N*-GalCer synthesis, to test Danishefsky's reported acyl transfer (Scheme 5, pg. 12).[28] Commercially available aspartic acid **34** was treated with isonitrile **33**, prepared by labmate Dr. Stewart Richardson, giving **35** in 52% yield (Scheme 10). The results of this study gave us enough cause to pursue the acyl transfer with ceramide.



Scheme 10: Danishefsky's reported acyl transfer reaction

The approach towards **9** began with previously synthesized ceramide alcohol **17** (Scheme 6, pg. 14). Following a procedure presented by Danishefsky,[28] compound **17** was oxidized to carboxylic acid **36** in 37% yield (Scheme 11). Excess TEMPO and BAIB were necessary to complete the conversion to carboxylic acid. The acid was then treated with the protected isonitrile **33** to form product **37**. Unfortunately, the desired product from the acyl transfer reaction was not observed.



Scheme 11: Attempted synthesis of α-*N*-formyl-*N*-GalCer

Messy TLC and NMR data led to concerns about the stability of the ceramide under the high temperature reaction conditions. Byproducts observed via TLC showed potential signs of ceramide decomposition (e. g. deprotections). An alternate approach will be taken to address steric complications with the ceramide and take advantage of our previously synthesized sphingoid base **25**.

The reaction conditions for the acyl transfer reaction reported by Danishefsky appeared to be too harsh for the ceramide-derived carboxylic acid. It was decided that a simpler structure and different protecting groups may tolerate the reaction conditions better. The alternative route will use the protected isonitrile **33** and an oxidized form of the sphingoid base (creating the acid) for the acyl transfer (Figure 13). As stated previously, a beta isonitrile will be used to test the methodology of the acyl transfer with the sphingoid base.

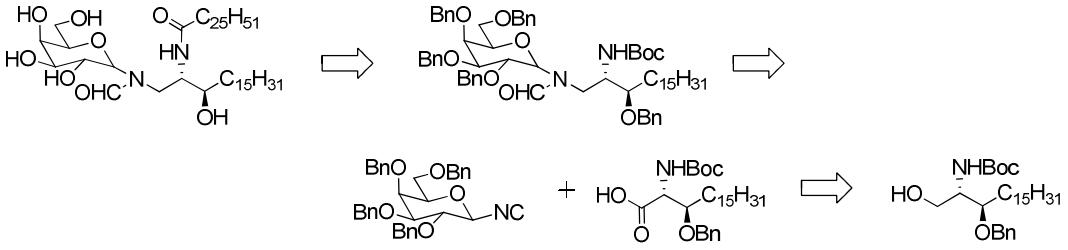
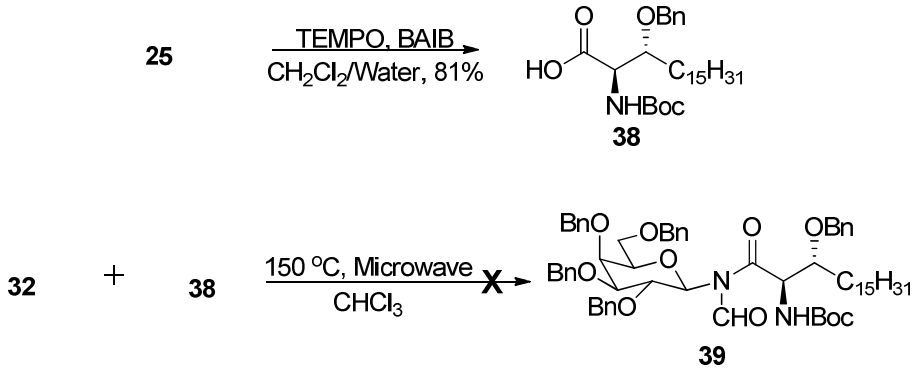


Figure 13: Revised approach to α-*N*-formyl-*N*-GalCer

Sphingoid base **25** (Scheme 7, pg. 16) was oxidized to carboxylic acid **37** in 81% yield (Scheme 12). Acid **38** was reacted with **32** to form the revised α-*N*-formyl-*N*-GalCer product **39**. Unfortunately no desired product was isolated, and NMR data showed evidence of byproducts. It was concluded that high temperatures caused the decomposition of the protected sphingoid base and ceramide which led to the unsuccessful acyl transfer with the protected isonitrile.



Scheme 12: Attempted synthesis of revised α-*N*-formyl-*N*-GalCer

Conclusion

The syntheses of complex amino glycosides have been shown to hold a few obstacles. The synthesis of the aminoceramide was not completed due to complications during purification. The acetylation of an isolated aminotriol did suggest that the aminoceramide was synthesized, but in the next reaction no glycosylated product was isolated. The alternative α-*N*-GalCer glycosylation using a sphingoid base, resulted in a compound with similar spectral characteristics to the desired product, but the glycosylated sphinganine was not isolated. The α-*N*-formyl-*N*-GalCer synthesis was also not successful. Based on ^{1}H NMR and TLC, it appeared that decomposition of the ceramide and sphinganine carboxylic acids occurred under the reaction conditions.

Future attempts to synthesize amino galactosylceramides will continue to focus on using aminosphingoid base derivative **35**. The α-*N*-formyl-*N*-GalCer approach will focus on identifying less extreme conditions. One approach would use a fully deprotected isonitrile **40** (Figure 14). The formation

of the *N*-formyl group is driven by hydrogen bonding of the free C2'' hydroxyl group on the sugar providing compound **41**,[36] followed by deprotection of the silyl groups to obtain target **9**.

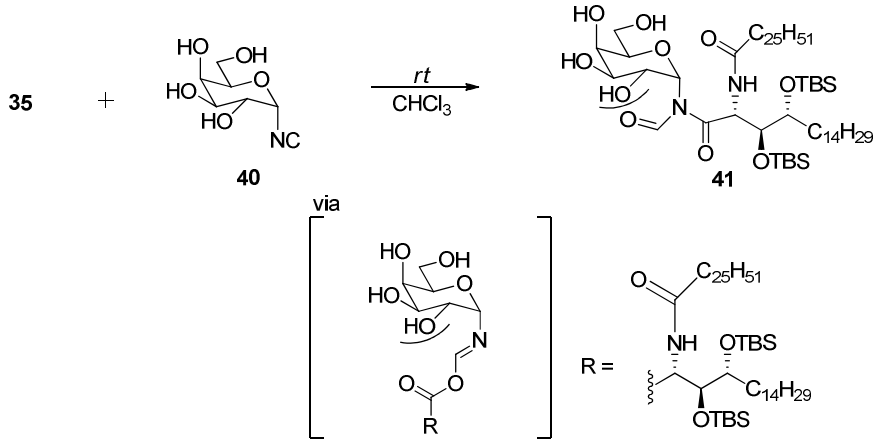
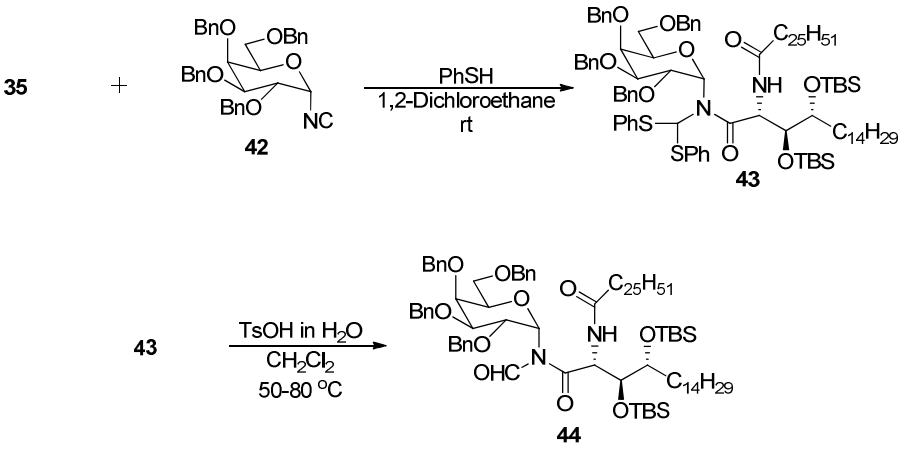


Figure 14: Formation of *N*-formyl-*N*-GalCer using unprotected isonitrile

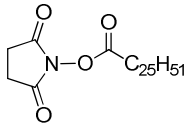
Another approach uses an acylated thiolphenol as opposed to a carboxylic acid in the acyl transfer reaction.[36] The product will be a di-thiophenol substituted carbon **43** (Scheme 13), but this can be hydrolyzed to the formyl group using *p*-toluenesulfonic acid in water to afford the α-*N*-formyl-*N*-galactosylceramide product **44**, followed by full deprotection to provide desired target **9**.



Scheme 13: Formation of *N*-formyl-*N*-GalCer using thiophenol

Experimental

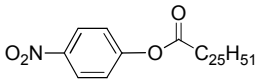
General. Melting points were determined in open Pyrex capillary tubes and are uncorrected. Infrared spectra were recorded on a Nicolet 750 FT-IR spectrometer. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker Avance Ultrashield 300-NMR spectrometer, at 400 MHz on a Bruker Avance DRX-400 NMR spectrometer, and at 500 MHz on a Bruker Avance 500-NMR spectrometer. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hertz. Abbreviations used are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, ddd = doublet of double of doublts, dt = doublet of triplets. ^{13}C NMR spectra were recorded on a Bruker Avance Ultrashield 300-NMR spectrometer at 75 MHz, on a Bruker Avance DRX-400 NMR spectrometer at 100 MHz, or on a Bruker Avance 500-NMR spectrometer at 125 MHz. High-resolution mass spectra were determined by the Mass Spectroscopy Facility in the Department of Chemistry at the University of Connecticut in Storrs, Connecticut and the Mass Spectroscopy Facility in the Department of Chemistry and Biochemistry at the University of Notre Dame in Notre Dame, Indiana. Column chromatography was performed with flash silica, 40 microns. Thin-layer chromatography was carried out on silica gel (Silica Gel 60 F_{254}) glass plates. Spots were visualized by UV and/or 10% molybdic acid in ethanol. Tetrahydrofuran was obtained from a distillation dispensing unit under neutral alumina (CH_2Cl_2, pyridine, and toluene were obtained in the Chemistry Department stock room located at University of Connecticut in Storrs, Connecticut and dried over 4Å MS).



Hexacosanoic acid 2,5-Dioxo-pyrrolidin-1-yl ester (14a)

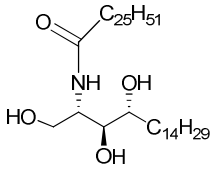
Hexacosanoic acid (0.50 g, 1.3 mmol) was dissolved in CH_2Cl_2 (15 mL) under N_2 at rt. EDC (0.27 g, 1.4 mmol) and *N*-hydroxysuccinimide (0.18 g, 1.6 mmol) were added to the solution. The reaction mixture

was heated to 40 °C for 6 h. The reaction was diluted with H_2O (10 mL), and the organic layer was extracted with Et_2O (30 mL). The organic layer was then washed with brine (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography on silica gel (PetEt/EtOAc 85:15) afforded **14a** as a white solid (0.28 g, 43%):[33] ^{1}H NMR (300 MHz, CDCl3) δ 2.83 (s, 4H), 2.59 (t, *J* = 7.5 Hz, 2H), 1.74 (m, 2H), 1.39 (m, 2H), 1.25 (m, 42H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 168.6, 31.9, 30.9, 29.7, 29.6, 29.5, 29.3, 29.1, 28.8, 25.6, 24.5, 22.7, 14.1



***p*-Nitrophenyl hexacosanoate (14b)**

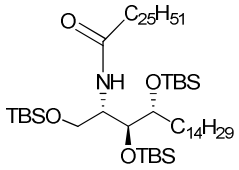
Hexacosanoic acid (1.0 g, 2.5 mmol) was dissolved in CH_2Cl_2 (260 mL) under N_2 at rt. *p*-Nitrophenol (0.39 g, 2.8 mmol), DMAP (0.060 g, 0.51 mmol), and DCC (0.55 g, 2.7 mmol) were added to the reaction flask, and the mixture was stirred for 8 h. The solution was filtered, and the filtrate was concentrated under reduced pressure. Purification by recrystallization (EtOAc) afforded **14b** as a yellow solid (0.98 g, 75%):[31] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 9.1 Hz, 2H), 7.30 (d, *J* = 9.3 Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H), 1.78 (m, 2H), 1.28 (m, 44H), 0.90 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 155.5, 145.2, 125.2, 122.4, 34.3, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.4, 29.2, 29.0, 24.7, 22.7, 14.1.



Hexacosanoic acid (2,3-Dihyroxy-1-hydroxymethylheptadecyl) amide (15)

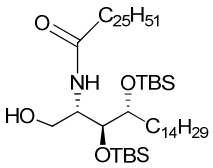
p-Nitrophenyl hexacosanoate (**14b**) (0.46 g, 0.89 mmol) was dissolved in pyridine (17 mL) under N_2 at rt. Phytosphingosine (0.23 g, 0.74 mmol) was then added, and the solution was allowed to stir for 65 h. The solution was then concentrated under reduced pressure. Purification by recrystallization (EtOAc) afforded **15** as a yellow solid (0.51 g, 99%):[30] ^{1}H NMR (500 MHz, pyr) δ 8.34 (d, *J* = 8.5 Hz, 1H), 5.10-5.05 (m,

1H), 4.49 (m, 1H), 4.45 (m, 1H), 4.33 (m, 1H), 4.23 (m, 1H), 2.46 (t, *J* = 7.3 Hz, 2H), 2.18-1.21 (m, 72H), 0.88 (t, *J* = 6.7 Hz, 6H).



(2*S*,3*S*,4*R*)-1,3,4-Tri-*t*-butyldimethylsilyloxy-2-hexacosanoylaminooctadecane (16)

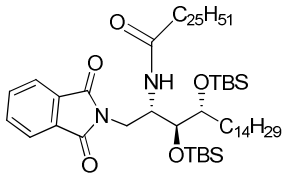
Hexacosanoic acid (2,3-dihyroxy-1-hydroxymethylheptadecyl) amide (**15**) (1.5 g, 2.2 mmol) was dissolved in CH_2Cl_2 (50 mL) at 0 °C. TBSOTf (4.4 g, 17 mmol) and 2,6-lutidine (3.5 g, 33 mmol) were added, and the solution was stirred at rt for 10 h. The reaction was quenched with MeOH (12 mL). The mixture was then diluted with Et_2O (100 mL) and washed with H_2O (55 mL), saturated aqueous $NaHCO_3$ (55 mL), and brine (55 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) afforded **16** as a colorless oil (2.13 g, 93%):[30] ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (d, *J* = 8.7 Hz, 1H) 3.94 (m, 1H), 3.87 (dd, *J* = 9.9, 4.2 Hz, 1H), 3.82 (dd, *J* = 7.2, 1.2 Hz, 1H), 3.67 (m, 1H), 3.63 (dd, *J* = 9.9, 4.5 Hz, 1H), 2.14 (t, *J* = 7.7 Hz, 2H), 1.64-1.46 (m, 6H), 1.25 (m, 66H), 0.93-0.86 (m, 33H), 0.13-0.04 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 75.4, 75.4, 61.4, 52.5, 37.2, 32.2, 31.9, 30.0, 29.7, 29.5, 29.4, 29.4, 29.4, 26.4, 26.1, 26.1, 25.9, 25.8, 22.7, 18.4, 18.2, 18.2, 14.1, -3.5, -3.8, -4.6, -5.2, -5.2, -5.6.



(2*S*,3*S*,4*R*)-3,4-Bis-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-1-octadecanol (17)

(2*S*,3*S*,4*R*)-1,3,4-Tri-*t*-butyldimethylsilyloxy-2-hexacosanoylaminooctadecane (**16**) (2.1 g, 2.1 mmol) was dissolved in dry THF (31 mL) and cooled to -10 °C. TFA (50% in H_2O, 0.84 mL, 11 mmol) was slowly added. The reaction was gradually warmed to 10 °C over 5 h. The solution was quenched with saturated

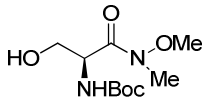
aqueous NaOH (22 mL) and diluted with Et_2O (64 mL). The two layers were separated, and the organic layer was washed with H_2O (31 mL), saturated aqueous $NaHCO_3$ (31 mL), and brine (31 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 80:20) afforded **17** as a colorless oil (1.18 g, 62%):[30] ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.24 (d, *J* = 7.8 Hz, 1H), 4.21 (m, 1H), 4.06 (m, 1H) 3.90 (m, 1H), 3.76 (m, 1H), 3.59 (m, 1H), 3.15 (m, 1H) 2.18 (t, *J* = 7.5 Hz, 2H), 1.48-1.62 (m, 6H), 1.25 (m, 66H), 0.93 (s, 9H), 0.91 (s, 9H), 0.85-0.94 (m, 6H), 0.11 (s, 6H), 0.08 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 77.5, 76.4, 63.6, 51.3, 37.0, 34.4, 31.9, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 26.0, 26.0, 25.8, 25.6, 22.7, 18.2, 16.9, 14.1, -3.8, -4.1, -4.5, -4.9.



(2S,3S,4R)-2-(*N*-Pentacosanoylamino)-3,4-di-*t*-butyldimethylsilanyloxy-1-octadecaphthalimide (18)

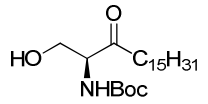
(2*S*,3*S*,4*R*)-3,4-Bis-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-1-octadecanol (**17**) (0.16 g, 0.18 mmol) was dissolved in dry THF (26 mL) under N_2 at rt. Triphenylphosphine (0.23 g, 0.88 mmol), DIAD (0.039 g, 0.19 mmol), and phthalimide (0.031 g, 0.21 mmol) were added, and the solution was stirred for 3.5 h. The mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) afforded **18** as a clear oil (0.14 g, 76%): $[\alpha]^{25}_D$ -5.19 (*c* 1.4, $CHCl_3$); IR (KBr) 2924, 2854, 1717, 1683 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (dd, *J* = 2.4, 5.4 Hz, 2H), 7.72 (dd, *J* = 2.4, 5.4 Hz, 2H) , 5.98 (d, J = 8.1 Hz, 1H), 4.42 (m, 2H), 3.81 (m, 3H), 2.12 (ddd, *J* = 7.6, 7.6, 7.6 Hz, 1H), 2.01 (ddd, *J* = 7.6, 7.6, 7.6 Hz, 1H), 1.70 (m, 4H), 1.60 (m, 4H), 1.41 (m, 4H), 1.28 (br, s, 60H), 1.02 (s, 9H), 0.95 (s, 9H), 0.90 (m, 6H), 0.13 (m, 12H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 169.1, 134.2, 132.2, 123.4, 51.1, 37.8, 37.1, 34.6, 32.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 29.5, 29.4, 26.3,

26.3, 26.1, 25.6, 22.9, 18.6, 18.4, 14.3, -3.3, -3.8, -4.3, -4.7; HRMS (TOF) calcd for $C_{64}H_{121}N_2O_5Si_2$ (M^+ + H) 1053.8814. Found 1053.8859.



[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]carbamic acid *tert*-butyl ester (20)

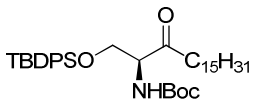
Boc-L-serine (6.00 g, 29 mmol) was dissolved in dry CH_2Cl_2 (115 mL) and the solution cooled to -15 °C under N_2. *N*,*O*-Dimethylhyroxylamine hydrochloride (3.0 g, 30 mmol) was added, followed by *N*-methylmorpholine (3.1 g, 30 mmol). After 5 min 1-(3-methylaminopropyl-3-ethylcarbodoiimide hydrochloride (5.8 g, 30 mmol) was added in five portions over 30 min. After being stirred for 1 h at -15 °C, the reaction was quenched with HCl (1 M, 17 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 34 mL). The organic extracts were combined, washed with saturated $NaHCO_3$ (17 mL) and H_2O (17 mL), dried ($MgSO_4$), and concentrated to provide **20** as a white solid (5.55 g, carried to the next step without purification):[33] ^{1}H NMR (400MHz, $CDCl_3$) δ 5.71 (br, s, 1H), 4.95 (br, s, 1H), 3.82-3.78 (m, 5H), 3.23 (s, 3H), 2.90 (br s, 1H), 1.43 (s, 9H).



(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (21)

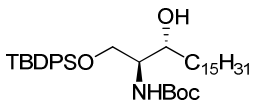
[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]carbamic acid *tert*-butyl ester (**20**) (15 g, 59 mmol) was dissolved in dry THF (117 mL) under N_2. The solution was cooled to -15 °C, and isopropylmagnesium chloride (60 mL, 2 M) was added dropwise, affording a clear solution. After 5 min, pentadecylmagnesium bromide (0.26 M in THF, 212 mL, 76 mmol) was added at -15 °C. The resulting solution was allowed to warm to rt overnight. The mixture was cooled to -15 °C, and HCl (1 M, 180 mL) was added, followed by EtOAc (135 mL). The two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x

30 mL). The organic extracts were combined, washed with H_2O (270 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 85:15) afforded **21** as a white solid (14 g, 62% over two steps):[33] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 (br s, 1H), 4.26 (m, 1H), 3.94 (m, 2H), 2.63 (br s, 1H), 2.55 (m, 2H), 1.57 (m, 2H), 1.45 (s, 9H), 1.27-1.22 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.1, 164.0, 80.3, 70.1, 68.9, 63.3, 61.6, 52.3, 49.8, 39.9, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 28.3, 23.5, 22.7, 14.1.



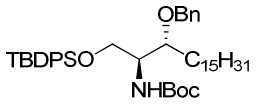
(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-one (22)

A catalytic amount of DMAP was added to a stirred solution under N_2 of (2*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (**21**) (7.4 g, 19 mmol) and imidazole (3.8 g, 56 mmol) in dry DMF (21 mL). After 20 min TBDPSCl (6.1 g, 22 mmol) was added dropwise, and the reaction mixture was stirred overnight. In the morning the reaction was diluted with saturated aqueous NH_4Cl (50 mL), and the aqueous layer was extracted with CH_2Cl_2 (37 mL). The organic layer was washed with H_2O (37 mL) and brine (37 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) afforded **22** as a clear oil (10 g, 85%):[33] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (m, 4H), 7.39 (m, 6H), 5.53 (d, *J* = 7.7 Hz, 1H), 4.33 (m, 1H), 4.04 (dd, *J* = 10.6, 3.1 Hz, 1H), 3.90 (dd, *J* = 10.9, 3.8 Hz, 1H), 2.51 (m, 2H), 1.58 (m, 2H), 1.44 (s, 9H), 1.26 (m, 24H), 1.03 (s, 9H), 0.89 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.4, 155.3, 135.5, 132.8, 129.9, 129.9, 127.8, 79.8, 64.2, 61.3, 60.3, 41.3, 40.0, 32.1, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 29.0, 28.3, 27.6, 26.7, 26.2, 23.3, 22.7, 22.6, 20.9, 20.4, 19.4, 19.2, 18.7, 14.3.



(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-ol (23)

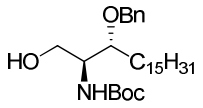
$LiAl(O\text{-}t\text{-}Bu)_3H$ (1.1 g, 4.3 mmol) was added to dry EtOH (7.6 mL) at -78 °C under N_2. (2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-one (**22**) (0.46 g, 0.72 mmol) in dry EtOH (7.6 mL) was added to the reaction flask dropwise. After stirring for 6 h at -78 °C the reaction mixture was diluted with CH_2Cl_2 (2.2 mL) and neutralized with 10% citric acid (22 mL). The solution was allowed to stir and reach rt over 1.5 h. The cloudy suspension was extracted with CH_2Cl_2 (3 x 7 mL). The organic extracts were combined and washed with H_2O (4 x 7 mL). The organic layer was then washed with brine (7 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) afforded **23** as a clear oil (0.23 g, 50%):[33] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (m, 4H), 7.41 (m, 6H), 5.30 (d, *J* = 8.1 Hz, 1H), 3.93 (m, 1H), 3.90 (m, 1H), 3.67 (m, 1H), 3.57 (br s, 1H), 2.88 (m, 2H), 1.44-1.06 (m, 45H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9, 135.8, 132.8, 132.7, 130.2, 130.1, 128.1, 128.0, 79.6, 74.0, 64.4, 54.7, 34.7, 32.1, 31.1, 29.8, 29.6, 28.6, 27.1, 26.1, 22.9, 19.4, 14.3.



(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxy-3-benzyloxyoctadecane (24)

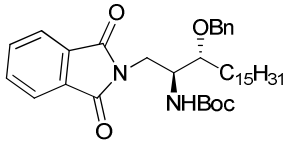
(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-ol (**23**) (0.14 g, 0.21 mmol) was dissolved in dry DMF (1 mL) under N_2 and cooled to 0 °C. Tetrabutylammonium iodide (0.12 g, 0.32 mmol) and sodium hydride (60% in mineral oil, 0.0070 g, 0.30 mmol) were then added. After 15 min benzylbromide (0.055 g, 0.32 mmol) was added dropwise via syringe. The solution was allowed to stir at 0 °C for an additional 15 min before it was removed from the cooling bath and allowed to stir at rt for 45 min. The reaction was then quenched with saturated aqueous NH_4Cl (2 mL), and the solution was extracted with EtOAc (4 x 3 mL). The combined organic extracts were washed with H_2O (3 mL) and brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 97:3) afforded **24** as a clear oil (0.085 g, 54%):[33] ^{1}H NMR (300 MHz, $CDCl_3$) δ

7.65 (m, 4H), 7.63-7.27 (m, 11H), 4.71 (d, *J* = 8.0 Hz, 1H), 4.54 (d, *J* = 11.3 Hz, 1H), 4.49 (d, *J* = 11.3 Hz, 1H), 3.86 (m, 2H), 3.72 (dd, *J* = 9.0, 4.1 Hz, 1H), 3.58 (m, 1H), 1.42 (s, 9H), 1.42 (s, 2H), 1.26 (br s, 26H), 1.05 (s, 9H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 138.8, 135.9, 133.5, 130.0, 128.5, 128.0, 127.7, 79.3, 79.1, 72.3, 63.1, 53.7, 32.2, 30.7, 30.1, 29.9, 29.9, 29.8, 29.6, 28.6, 27.1, 25.6, 22.9, 19.5, 14.4.



(2*S*,3*R*)-2-(*N-tert*-Butoxycarbonyl)amino-3-benzyloxyoctadecan-1-ol (25)

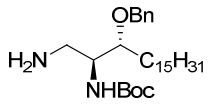
(2*S*,3*R*)-2-(*N-tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxy-3-benzyloxyoctadecane (**24**) (0.15 g, 0.20 mmol) was dissolved in THF (0.8 mL) under N_2, and the solution was cooled to 0 °C. TBAF (0.11 mL, 1.0 M in THF) was added, and the solution was stirred for 4 h at rt. The reaction mixture was concentrated, and the residue was dissolved in CH_2Cl_2 (2.4 mL). The solution was washed with brine (5 mL), dried ($MgSO_4$) and concentrated again. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 80:20) afforded **25** as a white solid (0.025 g, 25%):[33] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 5H), 5.27 (d, *J* = 7.4 Hz, 1H), 4.63 (d, J = 11.4 Hz, 1H), 4.48 (d, *J* = 11.4 Hz, 1H), 3.95 (m, 1H), 3.65 (m, 3H), 2.95 (s, 1H), 1.67 (m, 1H), 1.44 (s, 9H), 1.26 (br s, 27H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 138.2, 128.7, 128.1, 128.0, 82.0, 79.6, 73.0, 62.5, 53.5, 32.1, 31.5, 30.0, 29.9, 29.9, 29.8, 29.7, 29.6, 28.6, 25.8, 22.9, 14.3.



(2*S*,3*R*)-3-Benzyloxy-2-(*N-t*-butoxycarbonyl)amino-1-octadecapthalimide (26)

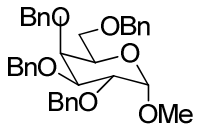
(2*S*,3*R*)-2-(*N-tert*-Butoxycarbonyl)amino-3-benzyloxyoctadecan-1-ol (**25**) (1.0 g, 2.0 mmol) was dissolved in dry THF (160 mL) at rt under N_2. Triphenylphosphine (2.7 g, 10 mmol), DIAD (0.45 g, 2.2

mmol), and phthalimide (0.36 g, 2.4 mmol) were added, and the solution was stirred for 3.5 h. The mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 90:10) afforded **26** as a white solid (0.77 g, 61%): mp 84-88 °C; $[\alpha]^{25}_{D}$ -27.3 (*c* 3.4, $CHCl_3$); IR (KBr) 2924, 2854, 1717 cm^{-1}: ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (br s, 2H), 7.61 (br s, 2H), 7.24 (m, 5H), 4.87 (d, J = 9.3 Hz, 1H), 4.61 (d, *J* = 11.7 Hz, 1H), 4.41 (d, *J* = 11.7 Hz, 1H), 4.06 (br s, 1H), 3.75 (m, 2H), 3.51 (br s, 1H), 1.69 (br s, 1H), 1.40 (br s, 3H), 1.20 (s, 23H), 1.11 (s, 9H), 0.81 (t, *J* = 6.3 Hz, 3H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 155.9, 138.6, 133.9, 132.4, 128.6, 127.2, 123.3, 80.7, 79.3, 77.6, 77.2, 76.8, 72.3, 51.8, 38.0, 32.1, 31.2, 30.0, 29.9, 29.9, 29.8, 29.7, 29.5, 28.2, 25.7, 23.1, 22.9, 14.3; HRMS (TOF) calcd for $C_{38}H_{57}N_2O_5$ (M^+ + H) 621.4267. Found 621.4289.



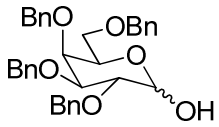
(2*S*,3*R*)-3-Benzyloxy-2-(*N*-*t*-butoxycarbonyl)amino-1-octadecamine (27)

(2*S*,3*R*)-3-Benzyloxy-2-(*N*-*t*-butoxycarbonyl)amino-1-octadecapthalimide (**26**) (1.5 g, 2.5 mmol) was dissolved in MeOH (15 mL) at rt under N_2. The solution was treated with hydrazine monohydrate (0.18 g, 3.7 mmol) and allowed to stir for 3 h at 65 °C. After 2 h a TLC (CH_2Cl_2/MeOH 98:2) showed starting material; so hydrazine (0.060 mL, 1.2 mmol) was added. A white precipitate resulted. After 1 h the mixture was passed through Celite and concentrated. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH 98:2) afforded **27** as a yellow oil (0.29 g, 25%): $[\alpha]^{25}_{D}$ -11.0 (*c* 1.7, $CHCl_3$); IR (KBr) 3447, 2924, 2854, 1717 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.21 (m, 5H), 5.01 (d, *J* = 7.8 Hz, 1H), 4.57 (d, *J* = 11.4 Hz, 1H), 4.43 (d, *J* = 11.4 Hz, 1H), 3.59 (m, 1H), 3.48 (m, 1H), 2.80 (br, s, 2H), 1.55 (m, 2H), 1.38 (s, 9H), 1.20 (s, 26H), 0.83 (t, *J* = 6.3 Hz, 3H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.2, 138.8, 128.7, 128.0, 127.9, 81.0, 79.4, 72.5, 54.5, 41.7, 32.1, 31.1, 30.1, 29.9, 29.9, 29.8, 29.7, 29.6, 28.6, 25.8, 22.9, 14.3; HRMS (TOF) calcd for $C_{30}H_{55}N_2O_3$ (M^+ + H) 491.4213. Found 491.4196.



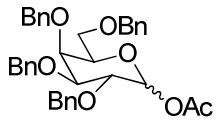
Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (28)

Sodium hydride (60% in mineral oil, 2.5 g, 103 mmol) in DMF (50 mL) was added to methyl-α-D-galactopyranoside (**28**) (2.0 g, 10 mmol) in two fractions. The flask was then placed in a cold water bath for 30 min. BnBr (11 g, 62 mmol) in DMF (10 mL) was then added to the reaction flask. The reaction was stirred at rt for 12 h under N_2 flow. The solution was neutralized with brine (25 mL), and the organic layer was extracted with Et_2O (50 mL x 3). The combined organic layers were washed with water (50 mL x 3) and brine (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The product was carried to the next step without further purification.[35]



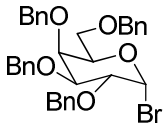
2,3,4,6-Tetra-*O*-benzyl-D-galactopyranose (29)

Methanesulfonic acid (2.1 g, 21 mmol) was dissolved in H_2O (10 mL). Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (**28**) (7.9 g, 14 mmol) was dissolved in acetic acid (60 mL). The methanic sulfonic acid solution was then added dropwise to the galactopyranoside solution. The resultant solution was heated to 80 °C for 6.5 h. The mixture was then cooled to rt and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 7:3) afforded **29** as a colorless oil (0.72 g, 9% over two steps):[35] ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40-7.18 (m, 20H), 5.97-4.36 (m, 8H), 5.27 (d, J = 3.6 Hz, 1H), 4.19-3.45 (m, 6H).



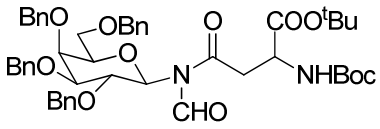
1-*O*-Acetyl, 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranose (30)

2,3,4,6-Tetra-*O*-benzyl-D-galactopyranose (**29**) (0.72 g, 1.3 mmol) was dissolved in pyridine (3 mL) and was cooled to 0 °C. Acetic anhydride (0.27 g, 2.6 mmol) was then added to the reaction flask dropwise. The reaction was stirred overnight. The mixture was diluted with EtOAc (3 mL) and washed with ice cold water (3 mL). The organic layer was then separated and washed with water (3 mL) and brine (3 mL), dried ($MgSO_4$), and concentrated under reduced pressure. 1-*O*-Acetyl, 2,3,4,6-tetra-O-benzyl-β-D-galactopyranose (**30**) was not purified.[35]



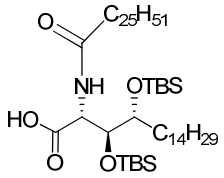
2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl bromide (31)

1-*O*-Acetyl, 2,3,4,6-tetra-O-benzyl-β-D-galactopyranose (**30**) (0.20 g, 0.34 mmol) was dissolved in CH_2Cl_2 (9 mL) and cooled to 0 °C. HBr (33% in AcOH) (0.17 mL) was then added to the reaction flask dropwise. The solution was allowed to stir for 1.5 h. The reaction mixture was then poured over ice cold saturated aqueous $NaHCO_3$ (10 mL) and stirred slowly. The organic and aqueous layers were separated. The aqueous layer was extracted with cold EtOAc (10 mL x 3). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The product (**31**) was used without further purification.[37]



N^{α}-(Fluoren-9-ylmethyloxycarbonyl)-N^{γ}-(2,3,4,6-tetra-*O*-benzyl-*β*-*D*-galactopyranosyl)-N^{γ}-formyl-*L*-asparagine-*t*-butyl-ester (35)

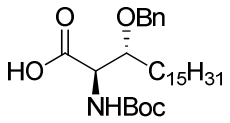
Aspartic acid (**34**) (0.027 g, 0.066 mmol) was added to 2,3,4,6-Tetra-*O*-benzyl-β -D-galactopyranosyl isonitrile (**33**) (0.025 g, 0.046 mmol) in anhydrous $CHCl_3$ under N_2. The reaction mixture was sealed and heated to 150 °C in a microwave for 45 minutes. Once the reaction was complete the solvent was remove via reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:1) afforded **35** as a yellow oil (0.023 g, 52%):[28] ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.3-8.5 (br, 1H), 7.68 (d, *J* = 7.5 Hz, 2H), 7.53-7.51 (m, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.26-7.11 (m, 22H), 5.61 (d, *J* = 9.0 Hz, 1H), 5.48 (d, *J* = 9.6 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 4.74 (d, *J* = 10.3 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 2H), 4.54-4.48 (m, 2H), 4.43-4.30 (m, 5H), 4.23 (t, *J* = 7.4 Hz, 1H), 4.15 (m, 1H), 3.94 (s, 1H), 3.60-3.59 (m, 2H), 3.49 (m, 1H), 3.42 (m, 1H), 3.32 (m, 1H), 3.20 (m, 1H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 164.5, 162.1, 156.0, 143.9, 143.8, 141.3, 141.3, 138.5, 137.9, 137.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.5, 127.1, 125.2, 125.1, 119.9, 82.4, 77.6, 75.9, 74.6, 73.6, 73.1, 72.6, 68.0, 67.1, 50.7, 47.1, 27.8



(2*S*,3*S*,4*R*)-2-(*N*-Hexacosanoylamino)-3,4-di-*tert*-butyldimethylsilanyloxy-1-octadecanoic acid (36)

(2*S*,3*S*,4*R*)-3,4-Bis-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-4-octadecanol (**17**) (0.10 g, 0.11 mmol) was dissolved in CH_2Cl_2 (4.4 mL)/H_2O (2.2 mL) at rt under N_2. TEMPO (0.0030 g, 0.022 mmol) followed by BAIB (0.087 g, 0.27 mmol), was added to the reaction flask. The mixture was stirred

vigorously until starting material was no longer visible via TLC (petroleum ether/EtOAc 7:3). The reaction was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $Na_2S_2O_3$ (11 mL), H_2O (11 mL), and brine (11 mL). The organic layer was combined, dried ($MgSO_4$) and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:2) afforded **36** as a white solid (0.038 g, 37%): mp 75-77 °C; $[\alpha]^{25}_D$ -9.6 (*c* 0.7, $CHCl_3$); IR (KBr) 3446, 2918, 2850, 1792, 1652 cm^{-1}: ^1H NMR (300 MHz, $CDCl_3$) δ 6.73 (d, *J* = 4.9 Hz, 1H), 4.69 (d, *J* = 5.1 Hz, 1H), 4.01 (s, 1H), 3.80 (d, *J* = 7.8 Hz, 1H), 2.20 (t, *J* = 7.8 Hz, 2H), 1.50 (m, 8H), 1.27 (s, 65H), 0.91 (s, 6H), 0.85 (s, 18H), 0.22 (s, 3H), 0.22 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 169.5, 80.3, 79.7, 55.4, 51.7, 36.9, 34.8, 32.1, 32.0, 29.9, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 26.5, 26.0, 25.9, 25.8, 25.8, 25.6, 22.7, 18.2, 18.0, 14.1, -3.9, -4.1, -4.5, -4.7; HRMS (TOF) calcd for $C_{56}H_{116}NO_5Si_2$ (M^+ + H) 938.84. Found 938.8387.



(2*S*,3*R*)-3-Benzyloxy-2-(*N*-*tert*-butoxycarbonyl)amino-1-octadecanoic acid (38)

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-3-benzyloxyoctadecan-1-ol (**25**) (0.10 g, 0.20 mmol) was dissolved in CH_2Cl_2 (4.4 mL)/H_2O (2.2 mL) at rt under N_2. TEMPO (0.0060 g, 0.041 mmol) followed by BAIB (0.16 g, 0.51 mmol), was added to the reaction flask. The mixture was stirred vigorously until starting material was no longer visible via TLC (petroleum ether/EtOAc 7:3). The reaction was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $Na_2S_2O_3$ (11 mL), H_2O (11 mL), and brine (11 mL). The organic layer was, dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH 99:1) afforded **38** as a light brown oil (0.083 g, 81%): $[\alpha]^{25}_D$ -0.3 (*c* 1.8, $CHCl_3$); IR (KBr) 3432, 2924, 2853, 1717, 1652 cm^{-1}: ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 (m, 5H), 5.15 (s, 1H), 4.62 (d, *J* = 11.1 Hz, 1H), 4.53 (d, *J* = 11.1 Hz, 1H), 3.71 (m, 1H), 1.60 (m, 2H), 1.40 (s, 9H), 1.22 (s, 26H), 0.84 (t, *J* = 6.9 Hz, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 155.9,

138.1, 128.6, 128.3, 128.0, 80.4, 79.6, 72.3, 32.1, 30.9, 29.9, 29.9, 29.8, 29.8, 29.6, 28.5, 25.9, 22.9, 14.3; HRMS (TOF) calcd for $C_{30}H_{52}NO_5$ (M^+ + H) 506.3845. Found 506.3887.

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