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Synthesis and Evaluation of Stimulatory Properties of Glycolipids for Natural Killer T Cells

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**SYNTHESIS AND EVALUATION OF STIMULATORY
PROPERTIES OF GLYCOLIPIDS FOR NATURAL KILLER T
CELLS**

by

Xiangtian Long

A dissertation submitted to the faculty of

Brigham Young University

In partial fulfillment of the requirements for degree of

Doctor of Philosophy

Department of Chemistry and Biochemistry

Brigham Young University

August 2009

BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a dissertation submitted by

Xiangtian Long

This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the dissertation of Xiangtian Long in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable fulfill university and department; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to be graduate committee is ready for submission to the university library.

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Abstract

**SYNTHESIS AND EVALUATION OF STIMULATORY
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Department of Chemistry and Biochemistry

Doctor of Philosophy

Natural killer T cells (NKT cells) are a subset of T cells. They regulate a wide range of diseases including infection, tumor growth, and autoimmune diseases, through recognizing glycolipid antigens in the context of CD1d. An understanding of the scope of glycolipid antigens would facilitate use of this cell type in controlling immune responses.

Till today, a lysosomal glycolipid, isoglobotrihexosylceramide (iGb3), is the only natural glycolipid that has been found to be recognized by both human and mouse NKT cells. To elucidate the molecular basis of this specific recognition, iGb3 variants were designed and prepared: i) replacement of the C26 acyl chain with shortened acyl chains; ii) replacement of the distal galactose with glucose and mannose; iii) replacement of the intermediate galactose with glucose; iv) replacement of the proximal glucose with galactose. Among these glycolipids, the iGb3 variants with shortened acyl chains are potent stimulators of NKT cells. The iGb3 variant with intermediate glucose also showed the ability to stimulate NKT cells,

but this finding needs to be verified. Our findings support the specific recognition of iGb3 by NKT cells.

The search for other natural glycolipid antigens focuses on glycolipids that are isolated from bacteria and parasites. Recently, glycosphingolipids (GSL-1, -3, and -4) isolated from the *sphingomonodaceae* family of bacteria were characterized. GSL-1 has been shown to be a potent stimulator of NKT cells. Moreover, it has been reported that GSL-4 is a stimulator as well. To verify the structures and stimulatory properties of GSLs, GSL-1 to -4 were prepared and tested for their abilities to stimulate NKT cells. The result that only GSL-1 can stimulate NKT cells suggests that synthesis of these higher order GSLs would be an immune evasion mechanism.

Neutral glycosphingolipids from sheep-derived *F. hepatica* liver flukes, a causative agent of fascioliasis, were isolated and characterized. Their structures are closely related to iGb3. Among these glycolipids, neo-iGb4s could be truncated to iGb3 in the lysosome and thus stimulate NKT cells. To test this hypothesis, these glycosphingolipids were prepared and tested. None of these synthetic glycolipids stimulates NKT cells, which suggests that the secretion of these glycolipids by *F. hepatica* could be the result of the parasite-immune-evasion mechanism.

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Table of Contents

Abstract	IV
Acknowledgements	VI
Chapter 1: Natural Killer T cells Recognize Glycolipids as Antigens	
1.1. T Lymphocytes	1
1.2. Natural Killer T Cells	2
1.2.1. CD1d Proteins Present Lipids to NKT Cells	3
1.2.2. KRN7000: the First Identified Glycolipid Antigen for CD1d Molecules	4
1.2.3. CD1d- α GalCer-TCR Complex	4
1.2.4. Activated NKT Cells Release Cytokines to Regulate Immune Responses	5
1.3. Identity of Natural Ligands for NKT Cells	7
1.3.1. Microbial Glycolipids	7
1.3.2. Other Glycolipids	8
1.3.3. Endogenous Glycolipids: iGb3	8
References	11
Chapter 2: Synthesis of Isoglobotrihexosylceramide Variants and Characterization as Stimulators of NKT Cells	
2.1. Introduction	15
2.1.1. Modifications of the Ceramide Moiety of iGb3	15
2.1.2. Modifications of the Sugar Head Moiety of iGb3	18
2.1.3. Evaluation of iGb3 Stimulant Property Considering Trace Amounts of the Alpha	

Anomer	21
2.2. Syntheses of iGb3 Variants	22
2.2.1. Synthesis of 2-1-2-3	23
2.2.2. Synthesis of 2-4-2-9	25
2.2.3. Synthesis of 2-10	28
2.2.4. Synthesis of 2-11	29
2.2.5. Synthesis of β LacCer and iGb3'	32
2.3. Results and Discussion	33
References	37
Experimental Procedures	40
Chapter 3: Synthesis and Evaluation of Stimulatory Properties of Glycosphingolipids from <i>Sphingomonadaceae</i> Family	
3.1. Introduction	84
3.2. Synthesis of GSLs	86
3.2.1. Synthesis of GSL-1	87
3.2.2. Synthesis of GSL-3	88
3.2.3. Synthesis of GSL-4	90
3.2.4. Synthesis of GSL-2	93
3.3. Results and Discussion	94
References	100
Experimental Procedures	102

Chapter 4: Synthesis and Evaluation of Stimulatory properties of Glycosphingolipids Isolated from *Fasciola hepatica*

4.1. Introduction	129
4.2. Identification of Glycolipids from <i>Fasciola hepatica</i>	132
4.3. Synthesis and Analysis	135
4.3.1. Synthesis of Neo-Gb3	135
4.3.2. Retrosynthesis of neo-iGb4	136
4.3.3. Synthesis of neo-iGb4A and neo-iGb4B	137
4.4. Results and Discussion	141
References	143
Experimental Procedures	146
APPENDIX. ¹ H NMR AND ¹³ C NMR SPECTRA OF SYNTHETIC TARGETS	170

Chapter 1: Natural Killer T cells Recognize Glycolipids as Antigen

1.1. T Lymphocytes

The immune system consists of the molecules, cells, tissues, and organs that mediate the protection against infectious diseases caused by pathogenic organisms such as bacteria and viruses.^{1,2} The cells of the immune system include two varieties of lymphocytes: B cells and T cells. B cells have unique cell surface proteins (B cell receptors, or BCRs) that bind soluble antigens in the blood or lymph and produce secreted forms of the BCR, called antibodies or immunoglobulins (Ig). T cells recognize antigens through their cell surface T cell receptors (TCRs). This recognition requires transmembrane proteins known as the *major histocompatibility complex* (MHC) proteins and antigen-presenting cells (APCs) such as dendritic cells and macrophages. The antigens are bound to MHC proteins to form complexes which are displayed on the surface of APCs. Once the TCRs are ligated by the MHC and antigen complexes, T cells are activated and produce a variety of signals to mediate the immune response. In this process, CD4 and CD8 proteins expressed on the surface of mature T cells usually function as important antigen co-receptors.

T lymphocytes are produced in the bone marrow and mature in the thymus. Several types of T lymphocytes serve specific immune functions. Helper T cells (Th cells) that express CD4 co-receptor on their surface help to increase the activity of other immune cells by producing signaling molecules called cytokines. Cytokines are

soluble hormone-like proteins and peptides that stimulate or inhibit the proliferation or function of immune cells. A subclass of Th cells, Th1, combat intracellular pathogens that are residing in phagocytic cells by producing a certain pattern of signaling cytokines (Th1 cytokines), such as interleukin-2 (IL-2) and interferon- γ (IFN- γ). In contrast, Th2 cells produce Th2 cytokines such as IL-4, IL-10 and IL-13 to increase antibody production that kills extracellular antigens.

The second major population of T cells is the CD8⁺ T cells, also known as cytotoxic T cells, CTLs, Tc cells. CD8⁺ T cells kill cells that are infected with pathogens, or are otherwise damaged or dysfunctional. These cells can recognize the complex of MHC class I and microbial peptides. The activated CD8⁺ T cells induce lyses of the target cells, or destroy the infected cells by introducing membrane perforations.

1.2. Natural Killer T Cells

In 1987, two groups of scientists reported that a mysterious subset of CD4⁻CD8⁻ (double-negative, DN) T cells over-express the TCR $\nu\beta 8$ gene segment.^{3, 4} These unconventional T cells express the natural killer cell-associated marker NK1.1,⁵ and are now known as invariant natural killer T cells (iNKT cells or NKT cells). Besides the DN NKT cells, there are other subsets of NKT cells such as CD4⁺ NKT cells and CD8⁺ NKT cells.⁶ NKT cells constitute only 0.2% of all peripheral blood T cells. A significant proportion of NKT cells express semi-invariant TCRs that are each composed of an invariant α chain and a limited set of β chains. These TCRs are

V α 24-J α 18/V β 11 in human or V α 14-J α 18/V β 8, V β 7, and V β 2 in mouse.⁶ In this dissertation, the abbreviation “NKT cells” will be used for this portion of cells.

1.2.1. CD1d Proteins Present Lipids to NKT Cells

CD1 proteins are MHC class I-like proteins that can present non-protein antigens to T cells to trigger antigen-specific activation of those T cells.⁷ These non-polymorphic CD1 proteins are expressed mainly on the surface of white blood cells, including B lymphocytes, macrophages and dendritic cells (DC). They consist of two chains: β 2 microglobulin (β 2m) and a heavy chain containing three extracellular domains (α 1- α 3). The antigen presented by the CD1 protein is accommodated within a predominantly hydrophobic groove between the α 1 and α 2 helices.

There are five members of CD1 family (CD1a-e) in humans, while mice and rats have only CD1d. MHC proteins present peptides to conventional T cells, while CD1d present lipids or glycolipids to NKT cells. The ligand-binding groove of CD1d consists of two large hydrophobic pockets, termed A' and F', which are capable of accommodating lipid chains up to 30 carbons.⁸

NKT cells are CD1d restricted T cells.⁹ It was reported that the generation of CD1d-deficient mice totally lacked NKT cells.¹⁰ NKT cells mature in the thymus and the maturation needs endogenous ligands presented by CD1d for positive selection of NKT cells.

1.2.2. KRN7000: the First Identified Glycolipid Antigen for CD1d Molecules

In 1994, Kirin Pharmaceutical in Japan identified several glycosphingolipid compounds from a marine sponge, *Agelas mauritianus*, collected in the Okinawan sea.¹¹ Among these compounds, an α -galactosylceramide showed strong anticancer activity in mice. Kirin Pharmaceutical later synthesized a slightly modified structure of the α -galactosylceramide, called KRN7000 (also commonly referred to α GalCer, Figure 1), as a candidate for clinical application. Two years later, KRN7000 was demonstrated by Kawano *et al.* as the first glycolipid antigen for CD1d molecules and stimulant for NKT cells.¹² KRN7000 consists of a hydrophilic carbohydrate moiety with an α -linkage to the hydrophobic ceramide portion and seems to be an exogenous ligand for NKT cells since glycosphingolipids with α -conformation do not exist in mammals. Due to the high affinity of interaction between CD1d-KRN7000 and mouse TCRs, KRN7000 has been used extensively in the studies of NKT cells.



Figure 1. Representative structure of glycolipid isolated from *Agelas mauritianus* and the structure of KRN7000.

1.2.3. CD1d- α GalCer-TCR Complex

The process of the TCR of NKT cell recognizing CD1d- α GalCer complex was proposed through a tri-molecular model.¹³⁻¹⁷ The orientation of the two hydrophobic chains of α GalCer in CD1d- α GalCer complex has been determined (Figure 2).¹⁴⁻¹⁶

The crystal structures of the CD1d- α GalCer complex show that the sphingosine chain always fits in the F' pocket and the long acyl chain fits in the large A' pocket. Loading of glycolipid antigens requires displacement of highly homologous endogenous ligands.¹⁵ The carbohydrate epitope of α GalCer is exposed at the CD1d surface and recognized by the TCR of NKT cells. The CDR loops of the TCR that contact with the CD1d- α GalCer complex minimally change conformation on ligation, which suggests a rigid “lock and key” interaction.^{17, 18}

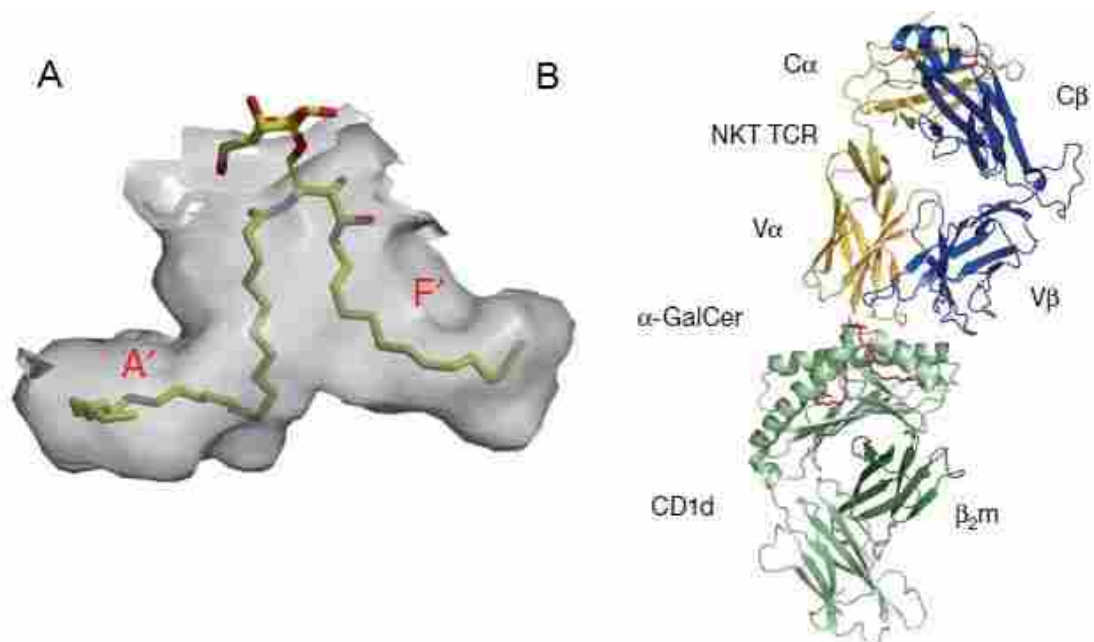


Figure 2. A) Mode of α GalCer-CD1d complex. The acyl chain fits into the A' pocket, while the sphingosine chain fits into the F' pocket. (Reproduced from ref. 15. Copyright 2005 Nature Immunology Publishing Group.) B) NKT TCR- α GalCer-human CD1d complex. (Reproduced from ref. 17. Copyright 2007 Nature Publishing Group.)

1.2.4. Activated NKT Cells Release Cytokines to Regulate Immune Responses

NKT cell-mediated regulation of immune responses has been reviewed.^{19, 20} DCs present the CD1d- α GalCer complex to NKT cells, inducing NKT cells to upregulate the expression of CD40L and cytokines. CD40L that cross-links to CD40 and

cytokines induce DCs to upregulate CD40, IL-12 and other signals, which in turn enhance NKT cell activation and cytokine production. This reciprocal activation of NKT cells and DCs augment immune responses, including the activation of NK cell cytotoxicity and upregulation of DC costimulatory properties and MHC class I- and II-mediated antigen presentation (Figure 3).

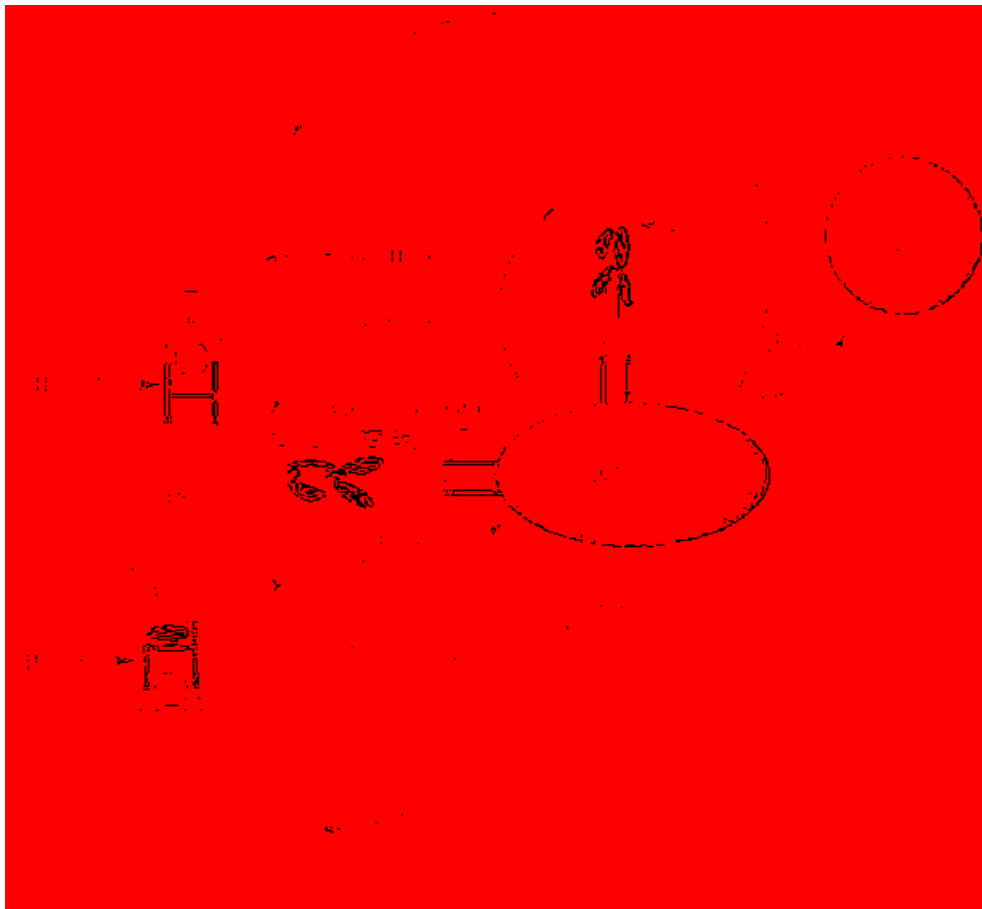


Figure 3. Cellular and molecular mechanisms of NKT cell-mediated immune responses.

NKT cells can rapidly produce large amounts of cytokines and chemokines. Release of IFN- γ and IL-2 cause an inflammatory response (Th1 response), while release of IL-4 and IL-13 result in an immunomodulatory response (Th2 response). Proinflammatory Th1 responses induced by NKT cells can regulate bacterial, parasitic and viral infections and control tumor growth. However, certain autoimmune diseases,

such as diabetes, lupus, atherosclerosis, and allergen-induced asthma, are Th1-mediated.^{19, 21} Th2 cytokines can attenuate proinflammatory Th1 responses and consequently ameliorate autoimmune diseases, which indicates NKT cells may play a protective role in inflammatory diseases. Th1 cytokines also can offset Th2 responses. For example, α GalCer-activated NKT cells inhibit Th2 responses by producing IFN- γ and subsequently suppress the antigen-specific IgE production.²² To fully understand the opposing responses of NKT cells, the mechanism of NKT cell activation *in vitro* and *in vivo* must be determined.

1.3. Identity of Natural Ligands for NKT Cells

For nearly a decade, α GalCer was the only known antigen presented by CD1d and recognized by NKT cells and other natural ligands remain poorly elucidated.

1.3.1. Microbial Glycolipids

α -Glucuronosylceramide (GSL-1) and α -galacturonosylceramide (GSL-1'), both structurally related to KRN7000, have been identified from the cell wall of *Sphingomonas*, a Gram-negative, LPS-negative member of the class of α -proteobacteria (Figure 4).^{23,24} These two glycosphingolipids have only one sugar, α -anomerically branched to the ceramide backbone, and can stimulate murine and human NKT cells in a CD1d-specific fashion.^{25, 26} It has been shown that both CD1d-deficient mice and NKT-deficient mice have a higher bacterial load than wild-type mice after exposure to *Sphingomonas*.^{25, 26} These data suggest that NKT

cells may provide protection from certain bacteria by recognizing glycolipid antigens.



Figure 4. Representative structures of natural ligands

1.3.2. Other Glycolipids

α -galactosyldiacylglycerols (BbGL-II) with two different fatty acid compositions have been isolated from Gram-negative LPS-positive *Borrelia burgdorferi*, a spirochete that causes Lyme disease (Figure 4).²⁷ These glycolipids resemble α -GalCer and appear to be recognized by NKT cells.²⁸ Other bacteria-derived glycolipids related to CD1d proteins and NKT cells include phosphatidylinositolmannoside PIM4 and ganglioside GD3 (Figure 4).²⁸⁻³¹ However, they are weak agonists and there is little support for their physiological importance of stimulating NKT cells.

1.3.3. Endogenous Glycolipids: iGb3

It is widely believed that the presentation of endogenous glycolipids requires

lysosomal trafficking of CD1d molecules and lipid-transfer proteins.¹⁹ NKT cells interact with CD1d and endogenous antigens to generate a TCR during development in the thymus. Mature NKT cells recognize the same endogenous CD1d-restricted antigens on the surface of APC in the periphery. This low-level auto-reactivity of NKT cells to CD1d molecules indicates the endogenous glycolipid antigen could be a lysosomal glycolipid. Zhou *et al.* found that mice deficient in β -hexosaminidase (Hexb), a lysosomal glycosphingolipid degrading enzyme, exhibited a 95% decrease in thymic NKT cell production.³² In addition, thymocytes from Hexb^{-/-} mice could not stimulate V α 14 NKT cell hybridomas. These results have led to the identification of isoglobotrihexosylceramide (iGb3) that can activate both mouse V α 14 and human V α 24 NKT cells (Figure 4). iGb3 consists of a trisaccharide head group linked to a ceramide in a β -anomeric conformation. In the lysosome, Hexb degrades the iGb3 precursor iGb4 to iGb3 by removing the terminal N-acetylgalactosamine (GalNAc) residue. Moreover, the *Griffonia simplicifolia* ioelectin B4 (IB4) that binds the terminal Gal α 1,3Gal of iGb3 blocked CD1d-mediated presentation of exogenous iGb3 and endogenous ligand, but not presentation of the unrelated ligand, α GalCer. These results suggest that iGb3 may be a key endogenous NKT cell antigen. Further observation that human IgG does not react with iGb3 suggests that iGb3 may not be considered foreign by human immune cells. iGb3 presented in the context of CD1d and the presence of IL-12 induce NKT cells to release IFN- γ during immune responses against Gram-negative, LPS-positive bacteria.²⁶

As iGb3 is the endogenous ligand for NKT cells, detection of iGb3 in both mouse

and human tissue would provide more information to understand the general biology of iGb3. Recently, it has been reported that iGb3 was not detected in all human tissues including spleen, thymus, and dendritic cells and the only tissue where iGb3 could be detected in mouse was the dorsal root ganglia.³³ This result suggests the human iGb3 synthase gene could be a pseudogene. However, the detection limit could be an important issue because of the low levels of iGb3 for positive selection of NKT cells. Another group of scientists reported that mice deficient in iGb3 synthase (iGb3S) have normal NKT cell number and function,³⁴ which suggests that iGb3 may not be the sole natural ligand for NKT cells. Christiansen *et al.* also reported that iGb3S is not expressed or active in humans.³⁵ Nevertheless, iGb3 and iGb4 had been identified in a CHO cell line transfected by iGb3S and in human thymus by MS⁵ ion trap mass spectrometry,³⁶ which contradicts the hypothesis that human do not express iGb3.³³⁻³⁵

Although iGb3 has been identified as a self ligand for NKT cells, little is known about the general biology of iGb3 and its function. Moreover, the fact that NKT cells recognize both iGb3 and α GalCer would lead to the search for self ligands other than iGb3.

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Chapter 2: Synthesis of Isoglobotrihexosylceramide Variants and Characterization as Stimulators of NKT Cells

2.1. Introduction

Unlike conventional T cells, NKT cells are stimulated by glycolipid antigens in the context of CD1d. Activated NKT cells produce large amounts of cytokines and chemokines to bridge the innate and acquired immune responses. It has been demonstrated that NKT cell-mediated regulation of immune responses influence a large number of disease states. Because activated NKT cells produce both Th1 and Th2 cytokines and these two types of cytokines regulate each other's activities, the cytokine release bias of NKT cells leading to Th1 and/or Th2 type responses may be key to exploiting NKT cell function for the treatment of various diseases. This requires studies of all processes that influence NKT cell stimulation. Of particular importance are the structural features of glycolipids and their influences on interactions between NKT cells and glycolipid-CD1d complex. The structure-activity relationship of iGb3 variants will be investigated to determine the relative influences of glycolipid structure on NKT cell stimulation.

2.1.1. Modifications of the Ceramide Moiety of iGb3

A key step of the activation of NKT cells involves loading of glycolipids into CD1d molecule. It has been demonstrated that the ceramide of iGb3 or α GalCer (Figure 1) can fit well into the two hydrophobic pockets of CD1d groove and the

sugar head group projects out of the binding groove.



Figure 1. Structures of iGb3 and α GalCer

iGb3 has a sphingosine chain in the ceramide moiety, while α GalCer has a phytosphingosine chain that bears an extra hydroxyl group at C4. It has been widely accepted that NKT cells have a high affinity for α GalCer plus CD1d. Interestingly, elimination of the hydroxyl groups at C3 and C4 on the ceramide group result in a loss of affinity.¹ The hydroxyl groups at C3 and C4 may facilitate orientation of the sugar head group by CD1d for recognition by the TCR.^{1,2} Further study showed that modified α GalCer without the hydroxyl group at C4 mirrors α GalCer in the cytokine responses. It was suggested that the hydroxyl group at C4 is much less important than that at C3 for orienting the glycolipid in CD1d.³ Moreover, Xia *et al.* reported that an iGb3 analogue that consists of a phytosphingosine chain instead of a sphingosine chain is more efficient for stimulation of NKT cells compared to iGb3 at very low concentrations.⁴

The bias of cytokine release profiles has been observed via modifications of the ceramide (Figure 2). OCH is an α GalCer analogue which has a substantially shorter sphingosine chain (Figure 2). OCH induces Th2 bias of NKT cells, possibly through alteration of glycolipid/CD1d complex stability.⁵ Further research demonstrated that

the chain-shortened glycolipids bias cytokine release toward an immunomodulatory response.⁶⁻⁸ Moreover, introduction of an aromatic group to the fatty acyl chain enables tuning of Th1/Th2 cytokine profile.⁸ Crystal structures of α GalCer-CD1d complex and α GalCer analogues-CD1d complexes reveal that the relatively short acyl chain of the glycolipid only partially fills the lipid-binding tunnel in CD1d and a “spacer lipid” fills in the bottom of the tunnel.^{2,9} Analogue PBS25 loads more efficiently into CD1d than α GalCer because loading α GalCer requires displacement of the spacer lipid. It was suggested that cell types presenting PBS25 are different from those presenting α GalCer.² A C-glycoside analogue of α GalCer, termed α -C-GalCer, stimulated very strong Th1 responses *in vivo* from NKT cells.¹⁰ α -C-GalCer exhibited a 1,000-fold more potent antimalaria activity and a 100-fold more potent antimetastatic activity than α GalCer. Because α -C-GalCer can not be truncated in lysosome due to the C-glycoside bond, it is hypothesized that the great stability of α -C-GalCer causes its great activity.¹⁰



Figure 2. Structures of α GalCer and its analogues

Based on these studies, we designed and synthesized three modified structures of

iGb3 with different acyl chains, including stearic, caprylic and nervonic acyl chains (Figure 3).



Figure 3. Structures of 2-1, 2-2, and 2-3

2.1.2. Modifications of the Sugar Head Moiety of iGb3

Taniguchi *et al.* reported that α GalCer is more active than α -glucosylceramide and both mannosylceramide and β -galactosylceramide are inactive.¹¹ It was confirmed by further study that TCR affinity for the CD1d- α -glucosylceramide complex was *ca.* 10-fold lower than CD1d- α GalCer complex and tetramers of α ManCer/CD1d and β GalCer/CD1d did not stain NKT cells.¹ In Taniguchi's report, α GalCer substituted with sugars at the C2, C3, or C6 position of the galactose (Figure 4) stimulated NKT cells nearly as well as α GalCer.¹¹ Later, two groups reported that the diglycoside ceramides with distal sugars at C2, C3, or C4 positions of the galactose must be truncated to the corresponding α GalCer to allow stimulation.^{12,13} However, 1-6 linked diglycolipid stimulates NKT cells without truncation to the α GalCer,¹³ which suggested that the glycolipid-CD1d-TCR interaction tolerates a small molecule at C6 on the galactose. This was confirmed by the observation that

small fluorophores and biotin appended at C6 position were tolerated in glycolipid-CD1d-TCR interaction.¹⁴



Figure 4. Structures of diglycolipids

The crystal structure of CD1d complexes of α GalCer and related glycolipids illustrated that two H-bonds were formed between CD1d and the hydroxyl groups at C2 and C3 positions of the sugar.² The additional sugars at C2 and C3 would interfere with the H-bonds. Recently, Borg *et al.* reported that the hydroxyl groups at C2, C3, and C4 positions of the galactose ring form H-bonds with the TCR α -chain.¹⁵ These observations are consistent with the fine specificity of the NKT TCR exhibits for α GalCer and its analogues.

In contrast to α GalCer, iGb3 contains a trisaccharide moiety linked to the ceramide in a β -anomeric conformation. Although the CD1d- α GalCer-TCR interactions were well understood, the CD1d-iGb3-TCR interactions remain poorly understood due to the low affinity of iGb3 binding to the TCR and/or CD1d.

NKT cells recognize iGb3, but not β LacCer and Gb3, which suggests that the orientation and the fine structure of the distal sugar moiety might play a central role

for TCR binding.¹³ Based on these observations, Chen *et al.* synthesized iGb3 analogues containing 2'''', 3'''', 4''' and 6''' deoxy terminal galactose.¹⁶ 2''' and 3''' hydroxyl groups play more important roles for TCR binding than 4''' and 6''' hydroxyl groups. It was suggested that modifications of 4''' and 6''' positions of iGb3 with other functional groups would be tolerated for NKT cell recognition. Elimination of the 4'''-OH of iGb3 with phytosphingosine chain elicited more TH1-biased responses than iGb3.¹⁷



Figure 5. Structures of β Lactose, Gb3 and iGb3

To determine the structural features of iGb3 responsible for stimulation of NKT cells, we prepared iGb3 variants **2-4-2-11** (Figure 6): i) replacement of the distal galactose with glucose and mannose (**2-4-2-9**); ii) replacement of the proximal glucose with galactose (**2-10**); replacement of the intermediate galactose with glucose (**2-11**).

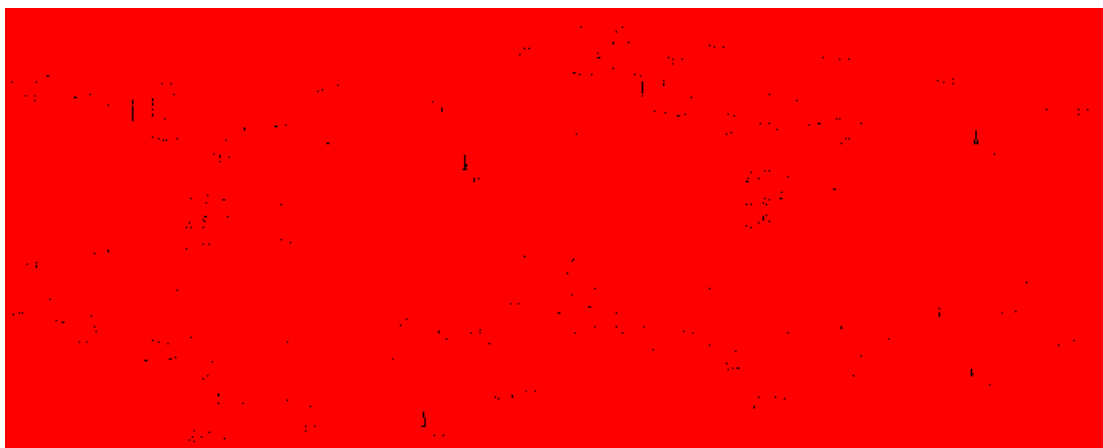


Figure 6. Structures of 2-4-2-11

2.1.3. Evaluation of iGb3 Stimulant Property Considering Trace Amounts of the Alpha Anomer

iGb3 is a relatively weak stimulator of NKT cells in comparison with α GalCer. Contamination of β -glycosylceramides with even trace amounts of alpha anomers can lead to the appearance of weak stimulation. Detection of trace amounts (i.e., < 0.1 %) of an alpha anomer contaminant is extremely difficult using traditional analytical methods. To avoid the contamination of trace amounts of alpha anomer, an iGb3 analogue, iGb3', and β LacCer have been synthesized from same batch of the precursor (Figure 7). If the β LacCer does not stimulate NKT cells while iGb3' does, it will be clear that iGb3' itself is a ligand for NKT cells.



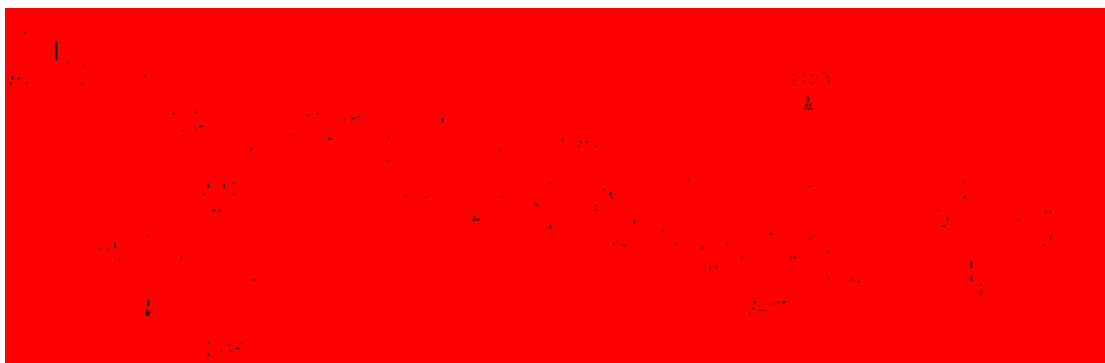
Figure 7. Structures of β LacCer and iGb3'

2.2. Syntheses of iGb3 Variants

The synthesis of iGb3 and Gb3 has been reported by several groups.¹⁸ In most of these reports, a 2-azidosphingosine instead of an amide was used for the acceptor to introduce the lipid portion of iGb3. For example, Xia *et al.* utilized the trisaccharide **2-12** and azide **2-13** as advanced intermediates in the glycosylation (Scheme 1).⁴ The reduction of coupling product **2-14** to amine followed by amide formation led to iGb3 in good overall yield.



Scheme 1. Xia's synthesis of iGb3



Scheme 2. Klike's synthesis of iGb3

In Klike's synthesis of iGb3, Ceramide **2-16** was directly used as the acceptor in the glycosylation step, which made the synthesis convergent (Scheme 2). However, the yield of the glycosylation was only 13%. It is most likely due to the amide which dramatically decreases the acceptor reactivity and leads to a low yield in glycosylation.

This low yield could be attributable to two effects as well: i) the potential of ceramide **2-16** to bind the glycosylation promoters; ii) the potential of ceramide **2-16** to form an orthoester (Scheme 3).¹⁹



Scheme 3. Formation of orthoester

To improve the yield, modifications of Klike's synthesis have been performed in our lab. For example, glycosylation of **2-18** with ceramide **2-19** gave **2-20** in 50% yield (Scheme 4). Although the reason is not clear, it was suggested that trisaccharide donor **2-18** with a benzoyl protecting group at C2 may play a role in decreasing the effects that were discussed above in the glycosylation. Moreover, AW 300 molecular sieves were used instead of normal molecular sieves.



Scheme 4. Synthesis of iGb3 in our lab

2.2.1. Synthesis of 2-1-2-3

Based on our experiences in the synthesis of iGb3, a convergent synthesis pathway was used to prepare **2-1-2-9**. The syntheses of **2-1-2-3** started with lactose **S1**

that was prepared according to a published method (Scheme 5).²⁰ Coupling of **2-21** and **S1** was achieved with the trichloroacetimidate method.²¹ The stereochemistry of newly generated glycosidic bond in **2-22** was alpha as determined by ¹H and 2D COSY NMR spectra. Hydrogenation of the benzyl groups followed by acylation gave trisaccharide **2-23**. The anomeric alcohol was liberated by treatment of TFA. Following coupling of this alcohol **2-24** and ceramide **C2** generated the desired β -glycosylation product **2-25** in a decent yield. Final deprotection of **2-25** with NaOMe provided **2-1**. Compound **2-2** and **2-3** were synthesized in a closely related process.

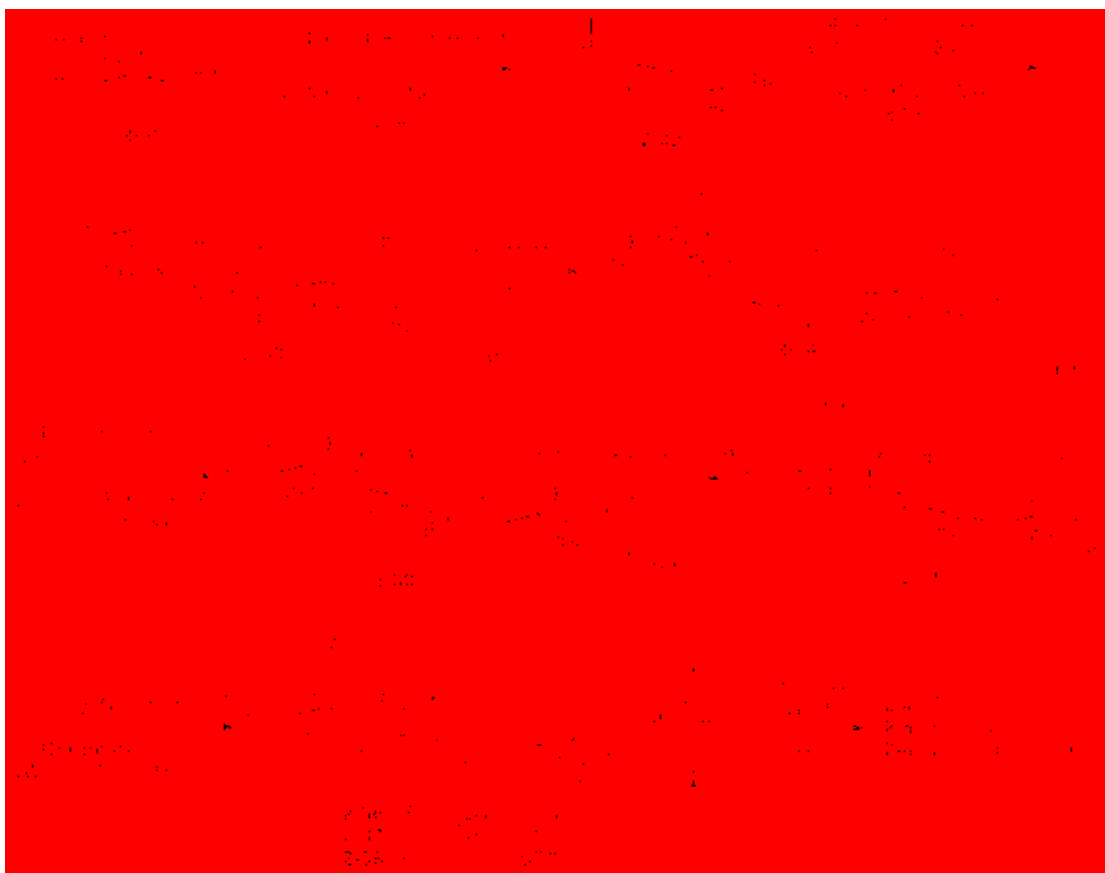
but not acceptable for biological evaluation. To achieve the synthetic goals, an appropriate thioglycoside **2-31** was used instead of **2-28**.



Scheme 6. Preparation toward compounds **2-4-2-6**

Selective protection of the hydroxyl groups at C4 and C6 of **2-31** with dimethoxypropane followed by benzylation of the remaining alcohols gave **2-32** (Scheme 7). Glycosylation of **2-32** with **S1** generated an inseparable mixture of the desired trisaccharide and its β -anomer. Treatment of this mixture with Ac_2O afforded mixture **2-33** which was inseparable as well. Fortunately, the desired α -anomer **2-34** was able to be separated from its β -anomer after removal of the acetonide. The benzyl groups were removed and all the resulting alcohols were protected with Ac_2O to generate compound **2-30**. As described in the synthesis of **2-1**, compound **2-4-2-6** were obtained in 4 steps.

For the preparation of **2-7-2-9**, an appropriate mannosyl trichloroacetimidate **2-39**²² was used to generate the α -glycosidic bond (Scheme 8). The attack of **S1** was only possible from the bottom face of the ring of **2-39** due to the participation of C2 benzoyl group that formed an acyloxonium cation intermediate.



Scheme 7. Synthesis of 2-4-2-6



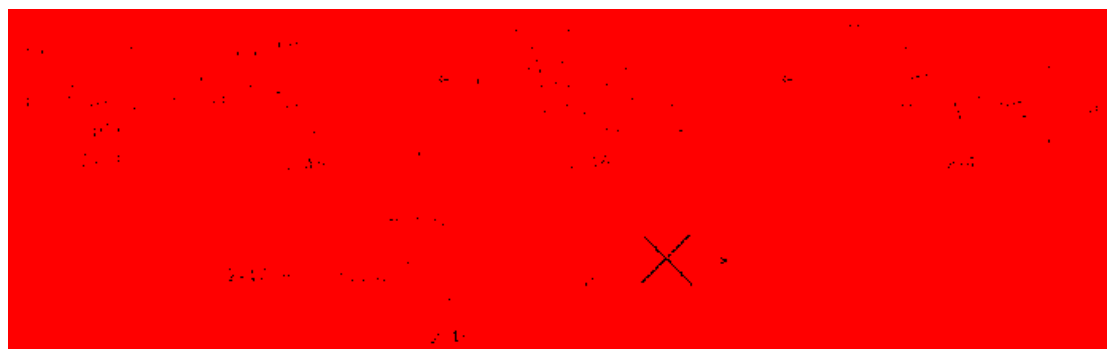
Scheme 8. Synthesis of 2-7-2-9

2.2.3. Synthesis of 2-10

The retrosynthetic strategy of **2-10** is illustrated in scheme 9. In this strategy, the trisaccharide donor **2-48** was constructed from three monosaccharide building blocks.



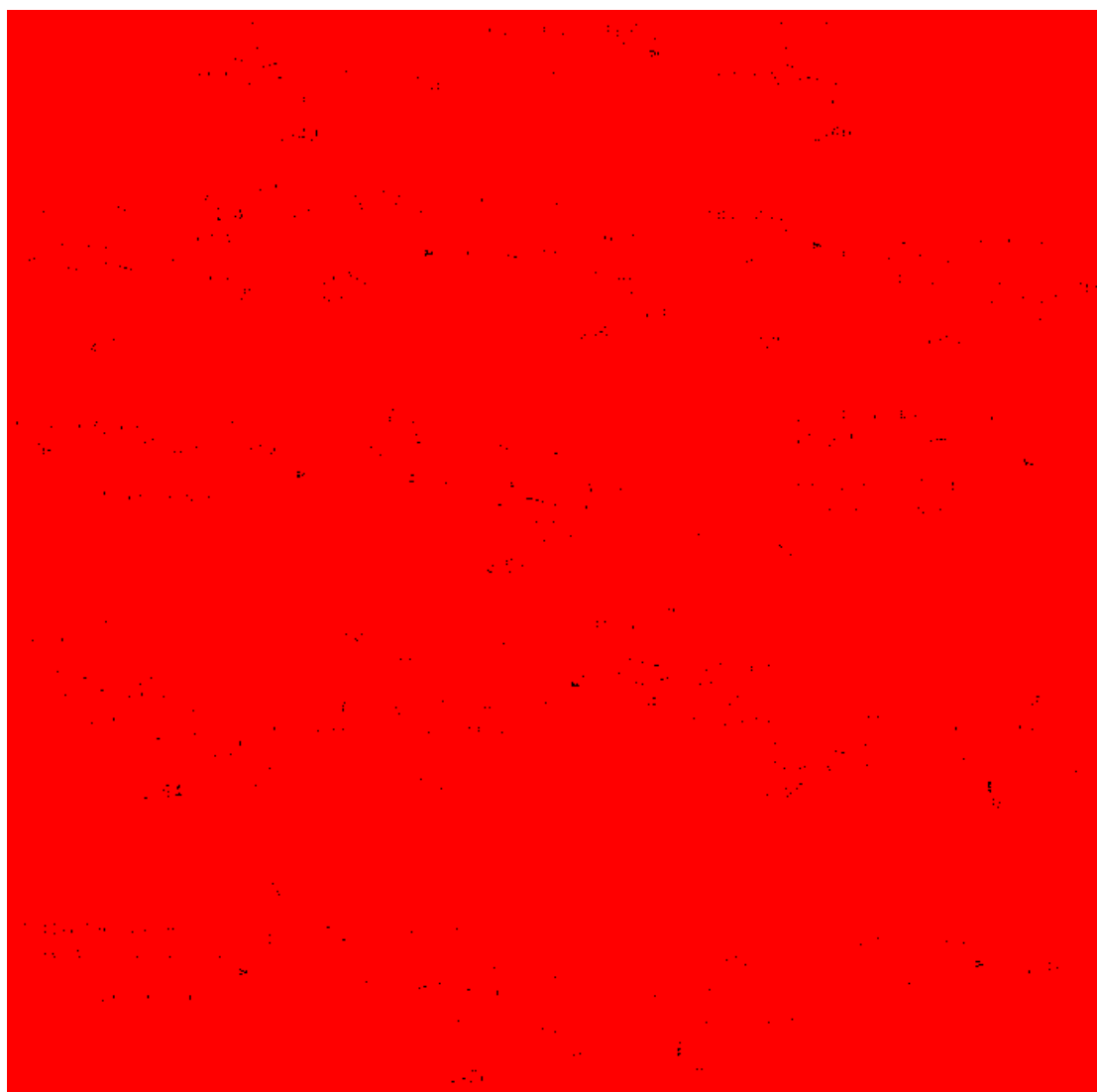
Scheme 9. Retrosynthesis of **2-10**



Scheme 10. Unsuccessful procedure to prepare **2-53**

Originally, building block **2-48** was prepared to install the proximal galactose (Scheme 10). Glycosylation of **2-21** with **2-45**²³ afforded disaccharide **2-46** in a decent yield. Transformation of **2-46** to **2-47** was achieved in 3 steps by using hydrogenation, acylation and cleavage of the silyl ether under acidic conditions. Unfortunately, coupling **2-47** and acceptor **2-48** gave us no trace of the desired product. It is probably due to two effects: First, the axial hydroxyl group at C4 in

galactose is much less accessible than an equatorial hydroxyl group. Second, three benzoyl groups decrease the nucleophilicity of the axial 4-OH in **2-48**. To achieve the synthetic goal, a more active acceptor **2-50** that bears two benzyl ethers at C2 and C6 was prepared.



Scheme 11. Synthesis of **2-10**

2.2.4. Synthesis of 2-11

Coupling **2-21** with **2-45**²³ followed by acylation of the remaining hydroxyl group afforded compound **2-51** with the desired α -glycosidic bond. Liberation of the

anomeric alcohol gave **2-52**. Selective protection of the equatorial 3-OH in **2-49**²³ with benzoyl chloride at 0 °C gave acceptor **2-50**. As we expected, coupling of acceptor **2-50** with **2-52** succeeded to generate **2-53** in a reasonable yield. With the trisaccharide **2-53** in hand, **2-10** was generated using a sequence similar to that used in the synthesis of **2-1**. Compound **2-55** was generated with some impurities that were difficult to separate at this stage, so the benzoyl groups were removed and the acyl groups were introduced to give **2-56** as a pure compound.

Three appropriately protected monosaccharides **2-21**, **2-57**²⁴ and **2-59**²³ were used to prepare **2-11**. Coupling of **2-21** with **2-57** in the presence of TMSOTf generated the desired disaccharide **2-58a** and its anomer **2-58b** in the ratio **2-58a** : **2-58b** = 1.15 : 1 (confirmed by ¹H NMR data) after careful purification. Probably, the promoter TMSOTf would facilitate the loss of the anomeric benzoyl group to form an oxonium ion. Reattaching this benzoyl group to oxonium ion would lead to an alpha and beta mixture. Separation of the anomeric mixture twice enabled the characterization of the alpha anomer. The anomeric mixture **2-58** was converted to hemiacetal using BnNH₂ in the presence of Et₂O. Glycosylation of the hemiacetal with **2-59** provided trisaccharide **2-60**. It was originally designed that direct hydrogenation of **2-60** would generate debenzylated compound. Actually, hydrogenation of **2-60** degraded the trisaccharide into disaccharide and monosaccharide due to the interactions between three congested monosaccharide moieties, which was further confirmed by unsuccessful perbenzoylation of compound **2-61**. Removal of the benzoyl groups of the intermediate saccharide would reduce the congestion of the trisaccharide building

block. Compound **2-61** was produced via treatment of **2-60** with NaOMe followed by hydrogenation over palladium. Perbenzoylation of **2-61** in the presence of pyridine and DMAP was not achieved even the reaction mixture was stirred at 50 °C for 10 h.



Scheme 11. Synthesis of **2-11**

The mass spectrum of the reaction mixture showed that the major product was the

compound with a free hydroxyl group probably at C2' and the minor product was **2-62a**. Ac₂O was added to the mixture led to **2-62a** and **2-62b**. To confirm the desired construction of three monosaccharide building blocks, 20 mg of mixture **2-62** was converted to **2-63** which was characterized with ¹H, ¹³C and 2D NMR. As described in the synthesis of **2-10**, **2-62** was converted to **2-11** in 6 steps.



Scheme 12. Syntheses of β LacCer and iGb3'

2.2.5. Synthesis of β LacCer and iGb3'

Syntheses of β LacCer and iGb3' was performed from **S1** (Scheme 12). Treatment of **S1** with levulinic acid in the presence of DCC gave disaccharide **2-66**. Liberation of the anomeric alcohol followed by glycosylation with ceramide **C5** generated **2-67**. Removal of the Levulinic groups afforded intermediate **2-68** which was used to

prepare both β LacCer and iGb3'.

2.3. Results and Discussion

The abilities of iGb3 variants to stimulate NKT cells were gauged by measuring IL-2 production. A mouse NKT cell hybridoma DN32.D3 was incubated with the indicated glycolipid in the presence of the bone marrow-derived dendritic cells (BMDCs) to observe the stimulation. Each experiment was performed a minimum of three times by collaborators at University of Chicago.

As expected, results indicated that cytokine release (IL-2) is dependent of lipid concentration. Among these variants, only **2-1** (PBS 73) and **2-2** (PBS74) stimulate NKT cells as potent as iGb3 (Figure 8). **2-2** caused larger amounts of IL-2 production than **2-1** and iGb3, which suggested shortened N-fatty acyl chains may increase the solubility of iGb3 and/or increase the iGb3 presentation. Since NKT cell development and function requires CD1d trafficking to lysosomal compartments,²⁵ stimulation of NKT cells by iGb3 is diminished in the presence of tail-deleted CD1d (CD1TD) that is unable to traffic into lysosomes. The CD1TD dendritic cells were used as well to test the abilities of **2-1** and **2-2** and they showed a defect in presenting **2-1** and **2-2**. The activity caused by **2-1** and **2-2** in CD1TD cell lines suggested that these two variants are partially independent of lysosome.

Compounds **2-5** and **2-8** did not stimulate NKT cells, suggesting that the terminal galactose is essential and unique for stimulation. These results confirmed that the CD1d-NKT cell recognition of glycolipids is specific for the molecular pattern of

iGb3. These results were confirmed by another collaborative group at the Scripps Research Institute (Figure 9, left).

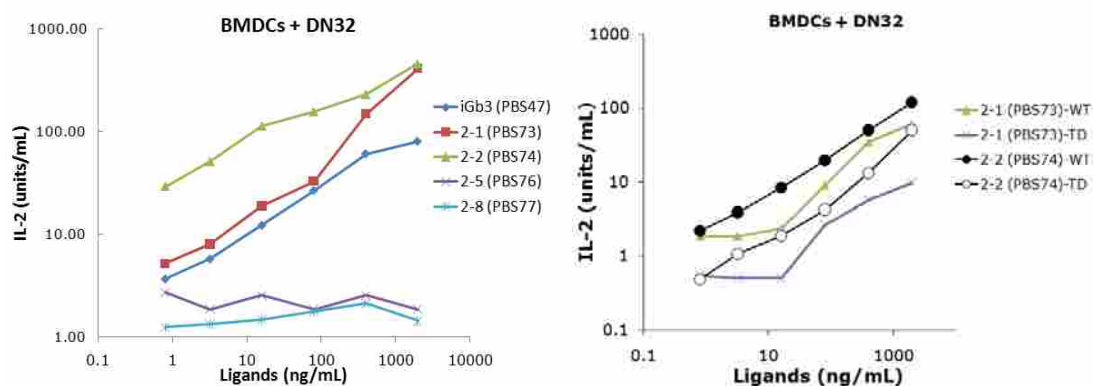


Figure 8. Dose response (IL-2 production) of NKT cells (mouse hybridoma DN32.D3) to glycolipids. A) glycolipids presented by BMDCs from CD1d mice; B) glycolipids presented by BMDCs from CD1TD mice.

To verify their cytokine release profiles, B6 mouse spleen cells were also exposed at the same concentration of iGb3 variants and IFN- γ production was measured instead of IL-2 production (Figure 9, right). The same tendency was found and PBS-74 (2-2) dissolved in MeOH was loaded more efficiently than in DMSO.

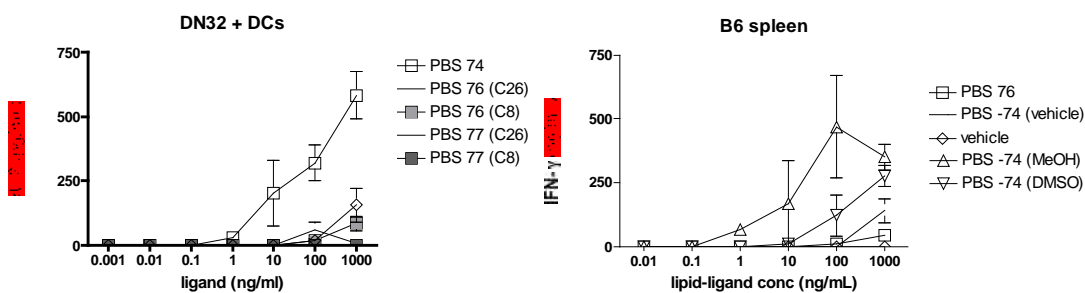


Figure 9. left, dose response (IL-2 production) of NKT cells (mouse hybridoma DN32.D3) to glycolipids; right, dose response (IFN- γ production) of NKT cells (B6 spleen cells).

Since PBS74 (2-2) is a better stimulator than iGb3, it was used to form the crystal structure with mouse CD1d (Figure 10). As expected, the A' pocket binds the acyl chain, while the sphingosine chain is inserted into the F' pocket. Because PBS74 (2-2) is a short-chain version with a C₈ fatty acid, deep inside the bottom of the A' pocket was sequestered a spacer lipid which stabilizes the A' pocket.

The crystal structure of the CD1d-PBS74 (2-2) complex revealed that only the proximal glucose was well ordered, while other two sugars showed a high degree of flexibility, as they do not interact extensively with CD1d.²⁶ This raises the question: how the same TCR recognized both α GalCer and the structurally different iGb3?

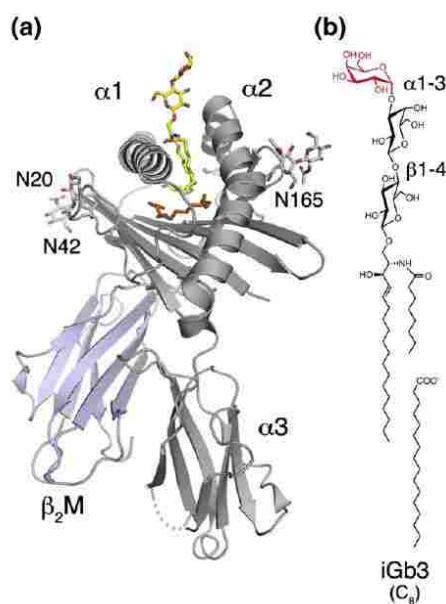


Figure 10. A representation of the mCD1d-PBS74 complex (a) and chemical structure of PBS74 (2-2) (b). A spacer lipid (C16, orange) present in the binding groove complements the short C8 alky chain of PBS74 (2-2). (Copyright 2008 Teyton's group)

Multiple $V\alpha 14$ recombinant TCRs were used to crystallize with PBS74-CD1d complex and low yields of crystallization were obtained due to the low affinity association of $V\alpha 14$ $\alpha\beta$ with carbohydrates. Thus, a cognate $V\alpha 14$ TCR was used to model CD1d-PBS74-TCR interactions for further studies. As discussed in Chapter 1, the crystal structure of human CD1d- α GalCer- $V\alpha 24$ TCR complex revealed a “parallel” TCR docking mode. Because PBS74 is very different in size and structure from α GalCer, this model caused serious steric clashes between the exposed iGb3 and several TCR residues. Thus, two alternative models were proposed. In the “cavity” model, a large and deep cavity of the cognate $V\alpha 14$ TCR can accommodate the distal

two sugars of PBS74. In the “squashed” model, three sugars of PBS74 are squashed between the CD1d and TCR surfaces without any steric clash, which conserves the CD1d-TCR specific interactions. Although the native TCR might be slightly different in size and shape, these models shed lights on the interaction of CD1d- α GalCer-V α 14 TCR complex. Further efforts will be focused on the crystallization of the CD1d- α GalCer-V α 14 TCR complex.

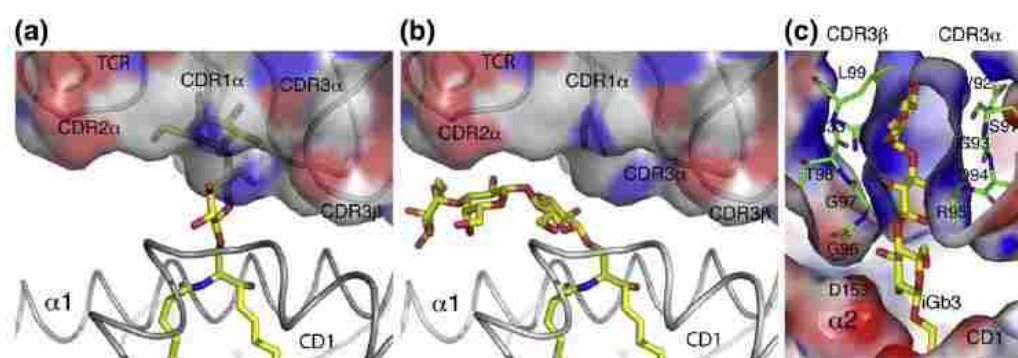


Figure 11. Models of CD1d-PBS74-V α 14 TCR binding site. a) A model similar to CD1d- α GalCer-V α 24 TCR complex demonstrated by Borg²⁷; b) the “squashed” model; c) the “cavity” model. (Copyright 2008 Teyton’s group)

It is accepted that the β LacCer with the same first two sugars as iGb3 is non-stimulatory and that iGb4 with one more sugar is also non-stimulatory, suggesting search for candidates of endogenous ligands requires focusing on trisaccharaide ceramides. The non-stimulatory PBS76, PBS77, and Gb3 established that the distal sugar of iGb3 is critical for TCR recognition. The stimulatory properties of compound **2-10** and **2-11** are under investigation. With all these studies, the intimate interaction between all of the sugars and the TCR will be elucidated in the near future.

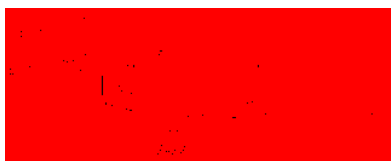
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Experimental Procedures:



Preparation of 2-22: Compound **2-21** (120 mg, 0.22 mmol) was dissolved in CCl_3CN (5 mL) followed by addition of K_2CO_3 (300 mg). The suspension was stirred for 3 h. The mixture was filtered through a celite pad. The filtrate was removed *in vacuo*. To the solution of the residue and **S1** (160 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (8 mL) was added 4 Å molecular sieves (400 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 18 h and filtered through a celite pad. The filter cake was washed with EtOAc/Hexanes (20 mL, 1:2). The combined filtrate was concentrated. The residue was purified by SiO_2 column chromatography (EtOAc/Hexanes 1:2) to afford **2-22** as a clear oil (120 mg, 43%). ^1H NMR (CDCl_3 , 500 MHz) δ 8.13-7.95 (m, 10 H), 7.67-7.03 (m, 35 H), 5.74 (t, $J = 9.5$ Hz, 1 H), 5.46-5.41 (m, 2 H), 4.82 (d, $J = 12.0$ Hz, 1 H), 4.77 (d, $J = 11.0$ Hz, 1 H), 4.70 (brs, 2 H), 4.67 (d, $J = 7.5$ Hz, 1 H), 4.64 (d, $J = 4.0$ Hz, 1 H), 4.62-4.57 (m, 3 H), 4.47 (dd, $J = 12.0, 5.0$ Hz, 1 H), 4.39 (d, $J = 11.0$ Hz, 1 H), 4.27 (dd, $J = 11.5, 4.5$ Hz, 1 H), 4.19 (t, $J = 9.5$ Hz, 1 H), 3.96-3.88 (m, 4 H), 3.85 (t, $J = 11.5$ Hz, 1 H), 3.82-3.79 (m, 2 H), 3.73-3.65 (m, 5 H), 3.54-3.48 (m, 2 H), 3.25 (t, $J = 8.5$ Hz, 1 H), 2.78 (dd, $J = 8.0, 5.0$ Hz, 1 H), 0.91-0.78 (m, 2 H), -0.18 (t, $J = 3.5$ Hz, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 166.27, 166.06, 165.82, 165.40, 165.04, 138.69, 138.59, 138.01, 137.62, 133.54, 133.36, 133.28, 133.20, 133.03, 130.13, 129.97, 129.75, 129.67, 129.42, 128.88, 128.74, 128.66, 128.59,

128.56, 128.44, 128.35, 128.27, 128.16, 127.70, 127.61, 100.82, 100.54, 96.69, 79.68, 78.65, 75.90, 75.14, 74.96, 74.78, 74.37, 73.15, 73.06, 72.85, 72.63, 72.20, 70.94, 69.76, 67.83, 67.69, 65.38, 63.25, 62.85, 17.99, -1.38; HRMS (ESI) calcd for $C_{86}H_{92}NO_{21}Si[M+NH_4]^+$: 1502.59256, found: 1502.59305.



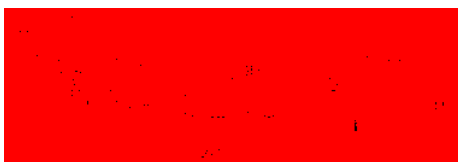
Preparation of 2-23: Compound **2-22** (120 mg, 0.081 mmol) was dissolved in THF (10 mL) and MeOH (10 mL) and transferred to a hydrogenation vessel. Palladium on carbon (10%, 100 mg) was added to the vessel, and the vessel was subjected to H_2 (300 psi) at room temperature for 12 h. The catalyst was then removed via filtration through a celite pad, and the filtrate was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (6 mL) and pyridine (4 mL) followed by introduction of a catalytic amount of DMAP (20 mg) and Ac_2O (50 μ L, 0.53 mmol). The solvent was removed *in vacuo* after 12 h and the residue was chromatographed (SiO_2 , EtOAc/hexane 1:1) to afford **2-23** as a white solid (70 mg, 64%). 1H NMR ($CDCl_3$, 500 MHz) δ 8.11-7.93 (m, 10 H), 7.62-7.34 (m, 15 H), 5.74 (t, $J = 9.5$ Hz, 1 H), 5.47-5.41 (m, 2 H), 5.24 (d, $J = 3.5$ Hz, 1 H), 5.15 (dd, $J = 11.0, 3.5$ Hz, 1 H), 5.11 (d, $J = 3.5$ Hz, 1 H), 4.97 (dd, $J = 11.5, 3.5$ Hz, 1 H), 4.81 (d, $J = 3.0$ Hz, 1 H), 4.68 (d, $J = 8.0$ Hz, 1 H), 4.65 (d, $J = 8.0$ Hz, 1 H), 4.56 (d, $J = 11.5$ Hz, 1 H), 4.48 (dd, $J = 12.0, 4.5$ Hz, 1 H), 4.17 (d, $J = 9.5$ Hz, 1 H), 3.93-3.88 (m, 3 H), 3.81 (dd, $J = 10.0, 3.5$ Hz, 1 H), 3.74-3.58 (m, 5 H), 3.53 (td, $J = 10.5, 6.5$ Hz, 1 H), 2.00 (s, 6 H), 1.97 (s, 3 H), 1.93 (s, 3 H), 1.87 (s, 3 H),

0.90-0.77 (m, 2 H), -0.13 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.38, 170.35, 170.16, 169.84, 169.58, 166.16, 165.88, 165.46, 165.42, 164.71, 133.95, 133.75, 133.63, 133.34, 130.21, 130.04, 129.86, 129.74, 129.59, 128.97, 128.93, 128.80, 128.53, 128.46, 101.00, 100.70, 93.77, 75.82, 73.78, 73.14, 73.07, 72.00, 71.14, 70.74, 67.89, 67.84, 67.10, 66.82, 66.62, 64.85, 62.84, 61.60, 61.17, 20.96, 20.84, 20.75, 20.72, 18.08, -1.31; HRMS (ESI) calcd for $\text{C}_{68}\text{H}_{78}\text{NO}_{26}\text{Si}[\text{M}+\text{NH}_4]^+$: 1352.45758, found: 1352.45688.



Preparation of 2-25: Compound **2-23** (76 mg, 0.057 mmol) was dissolved in TFA (4 mL) and CH_2Cl_2 (2 mL) and cooled to 0 °C. The solvent was removed *in vacuo* after 1 h, and the residue was purified by SiO_2 chromatography (EtOAc/hexanes 1:2) to afford **2-24** as a clear oil (58 mg, 83%). To a solution of **2-24** (30 mg, 0.024 mmol) in CCl_3CN (3 mL) was added K_2CO_3 (100 mg), the mixture was stirred for 3 h. K_2CO_3 was removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (4 mL) with **C2** (15 mg, 0.025 mmol) and AW 300 molecular sieves (100 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*. The residue was purified by SiO_2 column chromatography (EtOAc/hexanes 1:1) to afford **2-25** as a clear oil (24 mg, 54%). ^1H NMR (CDCl_3 , 500 MHz) δ 8.12-7.96 (m, 10 H),

7.64-7.37 (m, 15 H), 5.76 (t, $J = 9.5$ Hz, 1 H), 5.67 (dt, $J = 15.0, 7.5$ Hz, 1 H), 5.46 (t, $J = 10.0$ Hz, 2 H), 5.39 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.29 (t, $J = 7.5$ Hz, 1 H), 5.26 (d, $J = 3.5$ Hz, 1 H), 5.20-5.15 (m, 2 H), 5.12 (d, $J = 3.5$ Hz, 1 H), 4.97 (dd, $J = 11.0, 3.5$ Hz, 1 H), 4.81 (d, $J = 3.5$ Hz, 1 H), 4.67 (d, $J = 8.0$ Hz, 1 H), 4.61 (d, $J = 7.5$ Hz, 1 H), 4.55-4.48 (m, 2 H), 4.24-4.19 (m, 1 H), 4.17 (t, $J = 9.5$ Hz, 1 H), 3.98 (dd, $J = 10.0, 3.5$ Hz, 1 H), 3.93-3.90 (m, 2 H), 3.81-3.78 (m, 1 H), 3.75-3.68 (m, 3 H), 3.64-3.60 (m, 2 H), 3.44 (dd, $J = 10.0, 4.0$ Hz, 1 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.94-1.90 (m, 2 H), 1.87 (s, 3 H), 1.86 (s, 3 H), 1.70 (t, $J = 7.0$ Hz, 2 H), 1.34-1.04 (m, 52 H), 0.88 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.76, 170.34, 170.15, 169.85, 169.82, 169.56, 166.14, 165.85, 165.47, 165.31, 164.68, 137.31, 133.97, 133.75, 133.48, 130.01, 129.87, 129.76, 129.55, 129.28, 128.98, 128.90, 128.79, 128.53, 125.01, 101.16, 101.00, 93.77, 75.46, 73.73, 73.59, 73.22, 72.60, 72.24, 71.14, 70.72, 67.88, 67.08, 66.84, 66.61, 64.77, 62.62, 61.61, 61.11, 50.42, 36.62, 32.50, 32.16, 29.95, 29.79, 29.75, 29.61, 29.49, 29.40, 29.21, 25.68, 22.93, 21.15, 20.96, 20.83, 20.72, 14.37; HRFAB-MS (thioglycerol + Na^+ matrix) m/e : ($[\text{M} + \text{Na}]^+$) 1846.8867 (95.9%); calcd. 1846.8861.



Preparation of 2-1: Compound **2-25** (24 mg, 0.013 mmol) was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in THF). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was

purified by SiO₂ column chromatography (MeOH/CH₂Cl₂/H₂O 20:80:3) to afford **2-1** (11 mg, 80%) as a white solid. ¹H NMR (DMSO-d₆/D₂O, 500 MHz) δ 7.55 (d, *J* = 8.5 Hz, 1 H), 5.54-5.48 (m, 1 H), 5.31 (dd, *J* = 15.0, 7.5 Hz, 1 H), 4.82 (d, *J* = 3.5 Hz, 1 H), 4.26 (d, *J* = 7.5 Hz, 1 H), 4.15 (d, *J* = 7.5 Hz, 1 H), 3.98-3.96 (m, 2 H), 3.86-3.83 (m, 2 H), 3.76-3.71 (m, 3 H), 3.63-3.45 (m, 8 H), 3.41-3.39 (m, 3 H), 3.33-3.28 (m, 3 H), 3.02 (t, *J* = 8.0 Hz, 1 H), 2.00 (t, *J* = 7.5 Hz, 2 H), 1.93-1.88 (m, 2 H), 1.44-1.39 (m, 2 H), 1.26-1.08 (m, 50 H), 0.83 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (DMSO-d₆/D₂O, 125 MHz) δ 172.10, 131.61, 131.24, 103.50, 103.50, 96.26, 80.66, 78.66, 74.96, 74.85, 74.54, 73.14, 70.88, 70.71, 69.44, 69.15, 68.96, 68.79, 68.34, 64.40, 60.48, 60.27, 60.27, 53.03, 35.66, 31.74, 31.27, 29.05, 28.99, 28.78, 28.74, 28.68, 25.39, 22.06, 13.88; HRFAB-MS (thioglycerol + Na⁺ matrix) *m/e*: ([M+ Na]⁺) 1074.6924 (100%); calcd. 1074.6917.



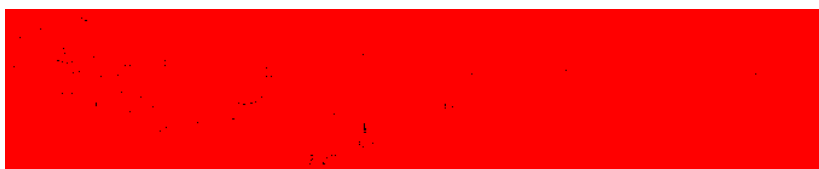
Preparation of 2-26: Compound **2-26** (24 mg, 61%) was obtained by coupling of **2-24** (28 mg, 0.023 mmol) with **C3** (15 mg, 0.032 mmol) according to the procedure used for the preparation of **2-25**. ¹H NMR (CDCl₃, 500 MHz) δ 8.12-7.96 (m, 10 H), 7.64-7.37 (m, 15 H), 5.77 (t, *J* = 9.5 Hz, 1 H), 5.67 (dt, *J* = 14.0, 7.0 Hz, 1 H), 5.49 (d, *J* = 8.5 Hz, 1 H), 5.47 (t, *J* = 10.0, 7.5 Hz, 1 H), 5.39 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.32 (s, 1 H), 5.30-5.26 (m, 2 H), 5.22-5.16 (m, 2 H), 5.13 (d, *J* = 4.0 Hz, 1 H), 4.99 (dd, *J* = 11.0, 3.5 Hz, 1 H), 4.82 (d, *J* = 3.5 Hz, 1 H), 4.68 (d, *J* = 7.5 Hz, 1 H), 4.62 (d, *J* =

7.5 Hz, 1 H), 4.56-4.52 (m, 1 H), 4.50 (dd, $J = 12.0, 4.0$ Hz, 1 H), 4.26-4.21 (m, 1 H), 4.19 (t, $J = 9.5$ Hz, 1 H), 3.99 (dd, $J = 10.0, 3.5$ Hz, 1 H), 3.94-3.92 (m, 2 H), 3.82-3.79 (m, 1 H), 3.76-3.68 (m, 3 H), 3.65-3.59 (m, 2 H), 3.46 (dd, $J = 10.0, 4.0$ Hz, 1 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.95 (s, 3 H), 1.94-1.92 (m, 2 H), 1.88 (s, 3 H), 1.88 (s, 3 H), 1.72 (t, $J = 7.5$ Hz, 2 H), 1.38-1.15 (m, 30 H), 1.08 (q, $J = 7.5$ Hz, 2 H), 0.91-0.86 (m, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.74, 170.38, 170.16, 169.84, 169.82, 169.57, 166.14, 165.85, 165.47, 165.31, 164.68, 137.31, 133.98, 133.76, 133.49, 130.01, 129.87, 129.53, 129.27, 128.90, 128.53, 125.01, 101.17, 101.00, 93.75, 75.46, 73.71, 73.60, 73.20, 72.60, 72.23, 71.13, 70.71, 67.88, 67.07, 66.83, 66.60, 64.76, 62.62, 61.62, 61.11, 50.41, 36.62, 32.50, 32.16, 31.94, 29.94, 29.74, 29.60, 29.49, 29.21, 25.66, 22.93, 21.15, 20.96, 20.83, 20.72, 14.34; HRFAB-MS (thioglycerol + Na^+ matrix) m/e : ($[\text{M} + \text{Na}]^+$) 1706.7299 (100.0%); calcd. 1706.7296.



Preparation of 2-2: Compound **2-2** (11 mg, 86%) was obtained by hydrolysis of compound **2-26** (24 mg, 0.014 mmol) as described in the preparation of **2-1**. ^1H NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$, 500 MHz) δ 7.60 (d, $J = 9.0$ Hz, 1 H), 5.55-5.49 (m, 1 H), 5.31 (dd, $J = 15.0, 7.0$ Hz, 1 H), 4.83 (d, $J = 3.5$ Hz, 1 H), 4.26 (d, $J = 8.0$ Hz, 1 H), 4.14 (d, $J = 8.0$ Hz, 1 H), 3.98-3.94 (m, 2 H), 3.87-3.84 (m, 2 H), 3.79-3.69 (m, 3 H), 3.63-3.45 (m, 8 H), 3.42-3.38 (m, 3 H), 3.34-.328 (m, 3 H), 3.03 (t, $J = 8.0$ Hz, 1 H), 2.01 (t, $J =$

7.0 Hz, 2 H), 1.94-1.86 (m, 2 H), 1.45-1.39 (m, 2 H), 1.27-1.09 (m, 32 H), 0.83 (t, $J = 7.0$ Hz, 3 H), 0.82 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (DMSO- d_6 /D $_2$ O, 125 MHz) δ 172.94, 132.53, 131.77, 104.10, 104.01, 96.68, 81.16, 78.97, 75.51, 75.37, 75.03, 73.68, 71.40, 71.28, 69.95, 69.71, 69.49, 69.30, 68.86, 64.90, 60.87, 60.87, 60.74, 53.44, 36.25, 32.36, 31.93, 29.66, 29.33, 29.27, 26.08, 22.79, 22.74, 14.61; HRFAB-MS (thioglycerol + Na $^+$ matrix) m/e : ($[\text{M} + \text{Na}]^+$) 934.5355 (100%); calcd. 934.5352.



Preparation of 2-27: Compound **2-27** (36 mg, 65%) was obtained by coupling of **2-24** (40 mg, 0.033 mmol) with **C4** (20 mg, 0.029 mmol) according to the procedure used for the preparation of **2-25**. ^1H NMR (CDCl $_3$, 500 MHz) δ 8.10-7.95 (m, 10 H), 7.64-7.36 (m, 15 H), 5.88 (t, $J = 9.5$ Hz, 1 H), 5.75 (t, $J = 10.0$ Hz, 1 H), 5.45 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.36-5.33 (m, 3 H), 5.25 (d, $J = 3.5$ Hz, 1 H), 5.15 (dd, $J = 11.0$ Hz, 3.5 Hz, 1 H), 5.11 (d, $J = 8.5$ Hz, 1 H), 5.06 (dd, $J = 8.5, 3.0$ Hz, 1 H), 4.96 (dd, $J = 11.0, 3.0$ Hz, 1 H), 4.86 (dt, $J = 10.5, 3.0$ Hz, 1 H), 4.80 (dd, $J = 3.5, 1.5$ Hz, 1 H), 4.65 (d, $J = 8.0$ Hz, 1 H), 4.57 (d, $J = 8.0$ Hz, 1 H), 4.53 (d, $J = 11.0$ Hz, 1 H), 4.47 (dd, $J = 12.0, 4.5$ Hz, 1 H), 4.28-4.24 (m, 1 H), 4.17 (t, $J = 9.5$ Hz, 1 H), 3.93-3.89 (m, 3 H), 3.79 (dq, $J = 10.0, 2.0$ Hz, 1 H), 3.74-3.67 (m, 3 H), 3.64-3.59 (m, 2 H), 3.42 (dd, $J = 9.5, 3.5$ Hz, 1 H), 2.03-1.99 (m, 2 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.95 (s, 3 H), 1.94 (s, 3 H), 1.86 (s, 3 H), 1.85 (s, 3 H), 1.78-1.73 (m, 2 H),

1.69-1.63 (m, 2 H), 1.60-1.54 (m, 2 H), 1.52-1.45 (m, 2 H), 1.38-1.13 (m, 54 H), 1.07-1.02 (m, 2 H), 0.88 (t, $J = 6.5$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.94, 171.06, 170.38, 170.36, 170.16, 169.84, 169.82, 169.58, 166.13, 165.87, 165.46, 165.31, 164.67, 133.97, 133.80, 133.73, 133.49, 130.14, 130.01, 129.86, 129.75, 129.49, 129.24, 128.93, 128.78, 128.53, 100.96, 100.94, 93.73, 75.34, 73.71, 73.25, 73.13, 72.51, 72.23, 72.13, 71.14, 70.71, 67.87, 67.69, 67.07, 66.82, 66.59, 64.75, 62.65, 61.63, 61.13, 47.53, 36.45, 32.50, 32.16, 29.94, 29.78, 29.60, 29.57, 29.39, 28.42, 27.46, 25.80, 25.55, 22.94, 21.22, 20.97, 20.84, 20.74, 14.38; HRMS (ESI) calcd for $\text{C}_{109}\text{H}_{151}\text{N}_2\text{O}_{31}[\text{M}+\text{Na}]^+$: 1984.02953, found: 1984.03339.



Preparation of 2-3: Compound **2-3** (19 mg, 88%) was obtained by hydrolysis of compound **2-27** (36 mg, 0.019 mmol) as described in the preparation of **2-1**. ^1H NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$, 500 MHz) δ 5.29 (t, $J = 5.5$ Hz, 1 H), 4.82 (d, $J = 4.0$ Hz, 1 H), 4.27 (d, $J = 7.5$ Hz, 1 H), 4.17 (d, $J = 8.0$ Hz, 1 H), 3.98 (t, $J = 7.0$ Hz, 1 H), 3.91-3.89 (m, 2 H), 3.84 (d, $J = 2.5$ Hz, 1 H), 3.74-3.72 (m, 2 H), 3.63-3.47 (m, 11 H), 3.42-3.29 (m, 6 H), 3.02 (t, $J = 8.0$ Hz, 1 H), 2.09-1.99 (m, 2 H), 1.95 (q, $J = 6.0$ Hz, 4 H), 1.49-1.34 (m, 4 H), 1.26-1.08 (m, 56 H), 0.83 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$, 125 MHz) δ 172.10, 129.67, 129.67, 103.64, 103.51, 96.30, 80.78, 78.61, 74.97, 74.85, 74.53, 73.23, 73.15, 70.93, 70.58, 69.76, 69.47, 68.96, 68.73, 68.38, 64.39, 60.37, 60.30, 60.11, 50.17, 35.66, 31.51, 31.49, 30.41, 29.50, 29.38, 29.34, 29.26,

29.08, 29.04, 28.95, 28.91, 28.78, 26.70, 25.70, 25.60, 22.29, 14.04; HRMS (ESI)

calcd for $C_{60}H_{113}NNaO_{19}[M+Na]^+$: 1174.77990, found: 1174.77625.



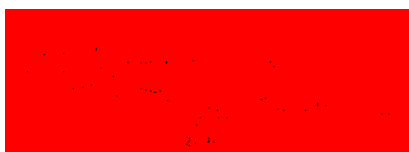
Preparation of 2-32: A solution of compound **2-31** (1.3 g, 4.8 mmol) in DMF (20 mL) was stirred with DMP (0.72 ml, 5.9 mmol) for 5 min followed by the addition of $TsOH \cdot H_2O$ (26 mg, 0.14 mmol). The mixture was stirred for 20 min and cooled to 0 °C. To the solution was added NaH (60% dispersion in mineral oil, 0.56 mg, 14 mmol) in portions and BnBr (1.4 mL, 12 mmol). After 1 h, MeOH (4 mL) was added and the mixture was concentrated and purified by SiO_2 chromatography (EtOAc/hexanes 1:4) to give **2-32** as a clear oil (1.9 g, 82%). 1H NMR ($CDCl_3$, 500 MHz) δ 7.51 (m, 2 H), 7.40-7.24 (m, 13 H), 4.87 (d, $J = 11.0$ Hz, 1 H), 4.83 (d, $J = 11.0$ Hz, 1 H), 4.79 (d, $J = 10.5$ Hz, 1 H), 4.73 (d, $J = 11.0$ Hz, 1 H), 4.70 (d, $J = 10.0$ Hz, 1 H), 3.95 (dd, $J = 10.0, 5.5$ Hz, 1 H), 3.78 (t, $J = 10.5$ Hz, 1 H), 3.72 (t, $J = 10.0$ Hz, 1 H), 3.66 (t, $J = 9.5$ Hz, 1 H), 3.45 (dd, $J = 10.0, 9.0$ Hz, 1 H), 3.28 (m, 1 H), 1.49 (s, 3 H), 1.41 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.90, 138.43, 133.62, 132.44, 129.24, 128.64, 128.60, 128.48, 128.28, 128.09, 127.99, 127.93, 99.54, 88.54, 83.70, 80.80, 76.14, 75.30, 74.58, 71.49, 62.50, 29.41, 19.48; HRFAB-MS (thioglycerol + Na^+ matrix) m/e: ($[M+Na]^+$) 515.1840 (100%); calcd. 515.1868.



Preparation of 2-34: A solution of **2-32** (125 mg, 0.28 mmol), BSP (79 mg, 0.38 mmol) and TTBP (200 mg, 0.80 mmol) in CH₂Cl₂ (7 mL) was stirred with 3 Å molecular sieves (350 mg) for 30 min. The mixture was cooled to -60 °C before Tf₂O (60 µL, 0.36 mmol) was added. The mixture was warmed to -40 °C in 20 min followed by the addition of **S1** (193 mg, 0.20 mmol) in CH₂Cl₂ (2 mL). The reaction mixture was allowed to warm to 0 °C and quenched by the addition of Et₃N (0.5 mL). The molecular sieves were then removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was purified via SiO₂ chromatography (EtOAc/hexane 1:2) to afford a clear oil. To this oil in pyridine (5 mL) was added Ac₂O (0.1 mL, 1.0 mmol) and DMAP (30 mg, 0.25 mmol). The mixture was stirred for 3 h before concentration. The residue was purified via SiO₂ chromatography (EtOAc/hexane 1:2) to afford **2-33** as a clear oil (170 mg, 0.12 mmol, mixture of α- and β-anomers, 61 %).

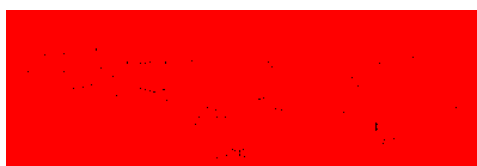
A mixture of **2-33** (170 mg, 0.12 mmol), AcOH (16 mL) and H₂O (4 mL) was stirred at 60 °C for 3 h. The solvent was removed *in vacuo*, and the remaining slurry was purified via SiO₂ chromatography (EtOAc/hexane 1:1) to afford **2-34** as a white solid (120 mg, α-anomer, 74 %). ¹H NMR (CDCl₃, 500 MHz) δ 8.05-7.94 (m, 10 H), 7.63-7.21 (m, 25 H), 5.74 (t, *J* = 9.5 Hz, 1 H), 5.45 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.21 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.38 (d, *J* = 3.5 Hz, 1 H), 4.99 (d, *J* = 3.0 Hz, 1 H), 4.84 (d, *J* = 10.0, 8.0 Hz, 1 H), 4.68 (d, *J* = 8.0 Hz, 1 H), 4.60-4.47 (m, 6 H), 4.13 (t, *J* = 9.5 Hz, 1

H), 3.94-3.89 (m, 1 H), 3.82-3.77 (m, 2 H), 3.70-3.66 (m, 1 H), 3.61 (d, $J = 8.5$ Hz, 1 H), 3.55-3.50 (m, 3 H), 3.45-3.40 (m, 2 H), 3.40 (t, $J = 9.0$ Hz, 1 H), 3.30 (dd, $J = 9.5$, 3.0 Hz, 1 H), 3.19 (t, $J = 9.5$ Hz, 1 H), 1.74 (s, 3 H), 0.91-0.78 (m, 2 H), -0.12 (t, $J = 3.5$ Hz, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.39, 166.45, 165.90, 165.57, 165.44, 164.83, 138.83, 138.41, 133.66, 133.32, 130.23, 130.06, 129.97, 129.90, 129.84, 129.77, 129.73, 129.68, 129.19, 128.90, 128.81, 128.51, 128.40, 128.20, 128.01, 127.96, 127.83, 101.31, 100.72, 93.79, 80.68, 79.56, 76.07, 75.32, 73.29, 73.12, 72.77, 72.02, 71.88, 71.46, 70.96, 70.30, 67.84, 64.89, 62.76, 62.46, 61.36, 29.95, 20.56, 18.09, -1.30; HRFAB-MS (thioglycerol + Na^+ matrix) m/e : ($[\text{M} + \text{Na}]^+$) 1369.4652 (100%); calcd. 1369.4664.



Preparation of 2-30: Compound **2-34** (120 mg, 0.089 mmol) was dissolved in THF (10 mL) and MeOH (10 mL) and transferred to a hydrogenation vessel. Palladium on carbon (10%, 100 mg) was added to the vessel, and the vessel was subjected to H_2 (300 psi) at room temperature for 12 h. The catalyst was then removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (6 mL) and pyridine (4 mL) followed by introduction of a catalytic amount of DMAP (20 mg) and Ac_2O (50 μL , 0.53 mmol). The solvent was removed *in vacuo* after 12 h and the residue was chromatographed (SiO_2 , EtOAc/hexane 1:2) to afford **2-30** as a clear oil (87 mg, 73%). ^1H NMR (CDCl_3 , 500

MHz) δ 8.11-7.93 (m, 10 H), 7.62-7.34 (m, 15 H), 5.74 (t, $J = 9.5$ Hz, 1 H), 5.49 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.43 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.23 (d, $J = 3.5$ Hz, 1 H), 5.09 (dd, $J = 10.5, 9.0$ Hz, 1 H), 5.08 (d, $J = 3.0$ Hz, 1 H), 4.90 (dd, $J = 10.0, 3.5$ Hz, 1 H), 4.81 (t, $J = 9.5$ Hz, 1 H), 4.68 (d, $J = 8.0$ Hz, 1 H), 4.62 (d, $J = 8.5$ Hz, 1 H), 4.51-4.50 (m, 2 H), 4.16 (t, $J = 9.5$ Hz, 1 H), 3.93-3.73 (m, 6 H), 3.65-3.50 (m, 4 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.88 (s, 3 H), 1.50 (s, 3 H), 0.87-0.80 (m, 2 H), -0.13 (t, $J = 3.5$ Hz, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.78, 170.09, 169.89, 169.76, 169.44, 166.18, 165.80, 165.47, 165.42, 164.37, 133.74, 133.66, 133.35, 130.20, 130.04, 129.99, 129.85, 129.79, 129.69, 129.53, 129.13, 128.91, 128.82, 128.53, 128.48, 101.06, 100.70, 93.00, 75.92, 73.43, 73.13, 73.04, 71.97, 71.07, 70.53, 69.73, 69.19, 67.91, 67.84, 67.66, 64.59, 62.76, 61.41, 60.97, 20.88, 20.83, 20.78, 20.42, 18.08, -1.31; HRFAB-MS (thioglycerol + Na^+ matrix) m/e : ($[\text{M} + \text{Na}]^+$) 1357.4133 (100%); calcd. 1357.4136.



Preparation of 2-36: Compound **2-30** (77 mg, 0.058 mmol) was dissolved in TFA (4 mL) and CH_2Cl_2 (2 mL) and cooled to 0 °C. The solvent was removed *in vacuo* after 1 h, and the residue was purified by SiO_2 chromatography (EtOAc/hexanes 1:2) to afford **2-35** as a clear oil (70 mg, 98%). To a solution of **2-35** (25 mg, 0.020 mmol) in CCl_3CN (3 mL) was added K_2CO_3 (100 mg), the mixture was stirred for 3 h. K_2CO_3 was removed via filtration through a celite pad, and the solvent was removed *in vacuo*.

The residue was dissolved in CH₂Cl₂ (4 mL) with **C1** (20 mg, 0.028 mmol) and AW 300 molecular sieves (100 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*, and purified by SiO₂ column chromatography (EtOAc/hexanes 1:2) to afford **2-36** as a clear oil (24 mg, 61%). ¹H NMR (CDCl₃, 500 MHz) δ 8.10-7.92 (m, 10 H), 7.60-7.38 (m, 15 H), 5.76 (t, *J* = 9.0 Hz, 1 H), 5.67 (dt, *J* = 15.0, 7.0 Hz, 1 H), 5.50-5.45 (m, 2 H), 5.43-5.37 (m, 1 H), 5.29 (t, *J* = 7.5 Hz, 1 H), 5.25-5.23 (m, 1 H), 5.18 (t, *J* = 7.5 Hz, 1 H), 5.10-5.07 (m, 2 H), 4.90 (dd, *J* = 10.5, 3.5 Hz, 1 H), 4.81 (t, *J* = 9.5 Hz, 1 H), 4.64 (d, *J* = 7.5 Hz, 1 H), 4.60 (d, *J* = 7.5 Hz, 1 H), 4.49 (brs, 2 H), 4.22-4.16 (m, 2 H), 3.98-3.85 (m, 3 H), 3.76-3.70 (m, 4 H), 3.58-3.55 (m, 2 H), 3.44 (dd, *J* = 10.0, 3.5 Hz, 1 H), 2.05 (s, 3 H), 2.01-1.87 (m, 2 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.88 (s, 3 H), 1.87 (s, 3 H), 1.70 (t, *J* = 7.5 Hz, 2 H), 1.50 (s, 3 H), 1.41-1.06 (m, 68 H), 0.88 (t, *J* = 6.5 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 172.75, 170.77, 170.08, 169.87, 169.74, 169.43, 166.16, 165.77, 165.47, 165.32, 164.35, 137.32, 134.89, 133.76, 133.50, 130.16, 130.01, 129.86, 129.82, 129.52, 129.27, 129.09, 128.89, 128.55, 127.79, 125.01, 101.17, 101.08, 93.00, 75.56, 73.59, 73.38, 73.19, 72.60, 72.23, 71.65, 71.07, 70.52, 69.72, 69.19, 67.91, 67.67, 64.52, 62.55, 61.41, 60.93, 50.40, 36.64, 32.50, 32.33, 32.17, 29.95, 29.80, 29.75, 29.61, 29.50, 29.43, 29.21, 29.00, 28.15, 26.09, 25.68, 22.94, 21.16, 20.83, 20.77, 20.41, 14.37; HRMS (ESI) calcd for C₁₀₉H₁₄₉NNaO₂₉[M+Na]⁺: 1959.01075, found: 1959.00919.



Preparation of 2-4: Compound **2-36** (24 mg, 0.012 mmol) was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in THF). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was purified by SiO₂ column chromatography (MeOH/CH₂Cl₂/H₂O 20:80:3) to afford **2-4** (12 mg, 85%) as a white solid. ¹H NMR (DMSO-d₆/D₂O, 500 MHz) δ 5.54-5.51 (m, 1 H), 5.34-5.30 (m, 1 H), 4.83 (d, *J* = 3.5 Hz, 1 H), 4.28 (d, *J* = 7.0 Hz, 1 H), 4.18 (d, *J* = 7.5 Hz, 1 H), 4.06-3.98 (m, 1 H), 3.87 (brs, 2 H), 3.76-3.30 (m, 16 H), 3.22 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.15 (t, *J* = 9.5 Hz, 1 H), 3.07 (t, *J* = 8.0 Hz, 1 H), 2.01-1.96 (m, 2 H), 1.89 (brs, 2 H), 1.44-1.40 (m, 2 H), 1.19 (brs, 66 H), 0.81-0.79 (m, 6 H); ¹³C NMR (Pyridine-d₅, 125 MHz) δ 173.62, 132.99, 132.62, 105.90, 105.90, 97.82, 82.55, 80.62, 77.02, 76.84, 75.69, 75.09, 74.67, 73.92, 73.06, 72.22, 70.96, 70.65, 66.33, 63.04, 62.33, 62.12, 55.27, 37.30, 33.10, 32.49, 30.39, 30.26, 30.16, 30.08, 29.97, 26.76, 23.30, 14.64; HRMS (ESI) calcd for C₆₂H₁₁₇NNaO₁₈[M+Na]⁺: 1186.81629, found: 1186.81543.



Preparation of 2-37: Coupling of compound **2-35** (20 mg, 0.016 mmol) with **C2** (15 mg, 0.021 mmol) afforded product **2-37** as a clear oil (20 mg, 64%). ¹H NMR (CDCl₃, 500 MHz) δ 8.12-7.96 (m, 10 H), 7.64-7.38 (m, 15 H), 5.77 (t, *J* = 9.5 Hz, 1 H), 5.68

(dt, $J = 14.5, 7.0$ Hz, 1 H), 5.52-5.48 (m, 2 H), 5.40 (dd, $J = 10.0, 7.5$ Hz, 1 H), 5.30 (t, $J = 8.0$ Hz, 1 H), 5.26 (d, $J = 3.5$ Hz, 1 H), 5.20 (t, $J = 7.5$ Hz, 1 H), 5.12-5.08 (m, 2 H), 4.92 (dd, $J = 10.5, 3.5$ Hz, 1 H), 4.83 (t, $J = 9.5$ Hz, 1 H), 4.66 (d, $J = 8.5$ Hz, 1 H), 4.61 (d, $J = 7.0$ Hz, 1 H), 4.52-4.50 (m, 2 H), 4.25-4.21 (m, 1 H), 4.17 (t, $J = 9.5$ Hz, 1 H), 3.99 (dd, $J = 10.0, 3.0$ Hz, 1 H), 3.94 (dd, $J = 10.0, 3.5$ Hz, 1 H), 3.88 (d, $J = 11.0$ Hz, 1 H), 3.81-3.78 (m, 3 H), 3.75-3.72 (m, 2 H), 3.61-3.55 (m, 3 H), 3.45 (dd, $J = 10.0, 4.0$ Hz, 1 H), 2.07 (s, 3 H), 2.02-1.90 (m, 2 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.90 (s, 3 H), 1.88 (s, 3 H), 1.71 (t, $J = 7.5$ Hz, 2 H), 1.51 (s, 3 H), 1.41-1.06 (m, 52 H), 0.89 (t, $J = 7.5$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.81, 170.79, 170.09, 169.88, 169.77, 169.45, 166.17, 165.78, 165.49, 165.34, 164.37, 137.34, 133.77, 133.52, 130.17, 130.01, 129.86, 129.52, 129.26, 129.08, 128.89, 128.56, 124.99, 101.08, 101.07, 93.00, 75.57, 73.58, 73.36, 73.19, 72.60, 72.22, 71.06, 70.52, 69.72, 69.19, 67.91, 67.66, 64.51, 62.55, 61.40, 60.91, 50.42, 36.62, 32.51, 32.17, 29.96, 29.80, 29.61, 29.50, 29.40, 29.21, 29.00, 25.68, 22.94, 21.17, 20.85, 20.42, 14.38; HRFAB-MS (thioglycerol + Na^+ matrix) m/e : ($[\text{M} + \text{Na}]^+$) 1846.8861 (100%); calcd. 1846.8861.



Preparation of 2-5: Treatment of compound **2-37** (20 mg, 0.010 mmol) with NaOMe afforded **2-5** as a white solid (10 mg, 95%). ^1H NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$, 500 MHz) δ 5.58-5.52 (m, 1 H), 5.37-5.32 (m, 1 H), 4.85 (d, $J = 4.0$ Hz, 1 H), 4.29 (d, $J = 8.0$ Hz,

1 H), 4.18 (d, $J = 8.0$ Hz, 1 H), 3.99 (dd, $J = 10.0, 4.5$ Hz, 1 H), 3.91-3.89 (m, 2 H), 3.79-3.74 (m, 4 H), 3.58-3.31 (m, 12 H), 3.22 (dd, $J = 9.5, 4.0$ Hz, 1 H), 3.17 (t, $J = 9.5$ Hz, 1 H), 3.07 (t, $J = 8.0$ Hz, 1 H), 2.04 (t, $J = 7.5$ Hz, 2 H), 1.95-1.90 (m, 2 H), 1.47-1.43 (m, 2 H), 1.28-1.19 (m, 50 H), 0.86 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR (DMSO- d_6 /D $_2$ O, 125 MHz) δ 172.55, 132.21, 132.13, 104.27, 104.18, 96.82, 81.25, 79.55, 75.51, 73.85, 72.93, 72.52, 71.36, 71.26, 70.41, 69.91, 69.57, 69.47, 65.17, 61.19, 61.05, 60.93, 60.83, 53.54, 36.32, 32.50, 32.04, 29.80, 29.50, 26.13, 22.82, 14.62; HRFAB-MS (thioglycerol + Na $^+$ matrix) m/e: ([M+ Na] $^+$) 1074.6913 (100%); calcd. 1074.6917.



Preparation of 2-38: Coupling of compound **2-35** (30 mg, 0.024 mmol) with **C3** (10 mg, 0.021 mmol) afforded product **2-38** as a clear oil (27 mg, 67%). ^1H NMR (CDCl $_3$, 500 MHz) δ 8.10-7.92 (m, 10 H), 7.61-7.35 (m, 15 H), 5.76 (t, $J = 9.0$ Hz, 1 H), 5.71-5.64 (m, 1 H), 5.51-5.46 (m, 2 H), 5.39 (dd, $J = 10.0, 7.5$ Hz, 1 H), 5.29 (t, $J = 7.5$ Hz, 1 H), 5.25 (brs, 1 H), 5.19 (t, $J = 7.5$ Hz, 1 H), 5.10-5.07 (m, 2 H), 4.90 (dd, $J = 10.5, 3.0$ Hz, 1 H), 4.82 (t, $J = 9.5$ Hz, 1 H), 4.64 (d, $J = 7.5$ Hz, 1 H), 4.60 (d, $J = 8.0$ Hz, 1 H), 4.50 (brs, 2 H), 4.22-4.20 (m, 1 H), 4.16 (t, $J = 9.0$ Hz, 1 H), 3.97 (dd, $J = 10.0, 3.0$ Hz, 1 H), 3.92 (dd, $J = 10.0, 3.5$ Hz, 1 H), 3.86 (d, $J = 11.0$ Hz, 1 H), 3.76-3.70 (m, 4 H), 3.58-3.55 (m, 2 H), 3.44 (dd, $J = 10.0, 3.5$ Hz, 1 H), 2.05 (s, 3 H), 2.02-1.92 (m, 2 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.88 (s, 3 H), 1.87 (s, 3 H), 1.70 (t, J

= 7.5 Hz, 2 H), 1.50 (s, 3 H), 1.35-1.06 (m, 32 H), 0.88 (t, $J = 6.5$ Hz, 6 H); ^{13}C NMR (CDCl₃, 125 MHz) δ 172.75, 170.78, 170.08, 169.88, 169.74, 169.43, 166.16, 165.77, 165.47, 165.32, 164.35, 137.31, 133.76, 133.50, 130.16, 130.01, 129.86, 129.81, 129.53, 129.27, 129.08, 128.89, 128.80, 128.55, 125.00, 101.18, 101.08, 93.00, 75.57, 73.60, 73.37, 73.20, 72.60, 72.22, 71.07, 70.52, 69.72, 69.19, 67.91, 67.83, 67.67, 64.51, 62.55, 61.40, 60.92, 50.42, 36.62, 32.50, 32.16, 31.94, 29.94, 29.74, 29.60, 29.49, 29.31, 29.21, 29.06, 28.14, 25.66, 22.93, 22.86, 21.16, 20.83, 20.77, 20.41, 14.37; HRMS (ESI) calcd for C₉₁H₁₁₃NNaO₂₉[M+Na]⁺: 1706.72905, found: 1706.76522.



Preparation of 2-6: Treatment of compound **2-38** (27 mg, 0.016 mmol) with NaOMe afforded **2-6** as a white solid (13 mg, 89%). ^1H NMR (DMSO-d₆/D₂O, 500 MHz) δ 5.55-5.50 (m, 1 H), 5.33 (dd, $J = 15.0, 7.0$ Hz, 1 H), 4.81 (d, $J = 3.5$ Hz, 1 H), 4.26 (d, $J = 7.5$ Hz, 1 H), 4.14 (d, $J = 8.0$ Hz, 1 H), 3.96 (dd, $J = 10.0, 4.5$ Hz, 1 H), 3.88-3.84 (m, 2 H), 3.76-3.71 (m, 3 H), 3.60-3.45 (m, 9 H), 3.40 (t, $J = 8.5$ Hz, 1 H), 3.34-3.29 (m, 3 H), 3.18 (dd, $J = 10.0, 4.5$ Hz, 1 H), 3.14 (t, $J = 9.5$ Hz, 1 H), 3.03 (t, $J = 8.0$ Hz, 1 H), 2.01 (t, $J = 7.5$ Hz, 2 H), 1.92 (t, $J = 7.0$ Hz, 2 H), 1.43 (t, $J = 7.5$ Hz, 2 H), 1.26-1.21 (m, 30 H), 0.84 (t, $J = 7.5$ Hz, 6 H); ^{13}C NMR (DMSO-d₆/D₂O, 125 MHz) δ 172.38, 131.91, 131.43, 103.76, 103.62, 96.26, 80.70, 78.94, 75.04, 74.94, 74.62, 73.28, 73.27, 72.41, 71.92, 70.85, 69.87, 69.32, 69.20, 64.58, 60.63, 60.39, 60.38,

53.12, 35.84, 31.94, 31.50, 29.25, 28.91, 28.84, 25.64, 22.35, 22.31, 14.18; HRMS (ESI) calcd for $C_{44}H_{81}NNaO_{18}[M+Na]^+$: 934.53459, found: 934.52682.



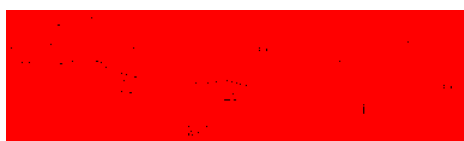
Preparation of 2-40: Compound **2-39** (450 mg, 0.61 mmol) and **S1** (450 mg, 0.47 mmol) were dissolved in CH_2Cl_2 (12 mL) followed by addition of 4 Å molecular sieves (600 mg). The mixture was stirred for 1 h then cooled to 0 °C before the addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*, and purified by SiO_2 column chromatography (EtOAc/hexanes 1:3) to afford a white solid. To this solid in pyridine (10 mL) was added Ac_2O (0.3 mL, 3.0 mmol) and DMAP (60 mg, 0.5 mmol). The mixture was stirred for 6 h before concentration. The residue was purified via SiO_2 chromatography (EtOAc/hexane 1:3) to afford **2-40** as a white solid (400 mg, 54%). 1H NMR ($CDCl_3$, 500 MHz) δ 8.18-8.17 (m, 2 H), 8.09-8.04 (m, 4 H), 7.98-7.89 (m, 8 H), 7.74-7.72 (m, 2 H), 7.58-7.18 (m, 29 H), 5.94 (t, $J = 10.0$ Hz, 1 H), 5.77 (t, $J = 9.5$ Hz, 1 H), 5.52-5.49 (m, 3 H), 5.43 (dd, $J = 10.0, 7.5$ Hz, 1H), 5.35 (d, $J = 3.5$ Hz, 1 H), 5.18 (s, 1 H), 4.70 (d, $J = 8.0$ Hz, 2 H), 4.56 (dd, $J = 11.5, 1.5$ Hz, 1 H), 4.48-4.44 (m, 2 H), 4.18 (t, $J = 9.5$ Hz, 1 H), 4.09 (dd, $J = 11.0, 2.0$ Hz, 1 H), 4.05 (d, $J = 10.0$ Hz, 1 H), 3.93-3.88 (m, 1 H), 3.84-3.80 (m, 2 H), 3.73-3.64 (m, 2 H), 3.56-3.50 (m, 2 H), 2.18 (s, 3 H), 0.93-0.79 (m, 2 H), -0.13 (t, $J = 3.5$ Hz, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.66, 166.19, 166.08, 165.79, 165.52, 165.45, 165.23,

164.86, 164.63, 133.85, 133.60, 133.49, 133.42, 133.14, 130.18, 130.05, 129.99, 129.90, 129.85, 129.60, 129.52, 129.41, 129.07, 128.97, 128.85, 128.75, 128.71, 128.62, 128.52, 128.44, 128.37, 101.14, 100.64, 95.38, 76.28, 74.38, 73.36, 73.14, 72.12, 71.08, 70.88, 69.96, 69.71, 69.51, 67.76, 66.10, 65.06, 62.84, 61.88, 61.12, 20.79, 18.10, -1.32; HRMS (ESI) calcd for C₈₈H₈₂NaO₂₆Si[M+Na]⁺: 1605.47558, found: 1605.48522.



Preparation of 2-42: Compound **2-40** (31 mg, 0.058 mmol) was dissolved in TFA (3 mL) and CH₂Cl₂ (2 mL) and cooled to 0 °C. The solvent was removed *in vacuo* after 2 h, and the residue was purified by SiO₂ chromatography (EtOAc/hexanes 1:2) to afford **2-41** as a white solid (25 mg, 85%). To a solution of **2-41** (25 mg, 0.020 mmol) in CCl₃CN (3 mL) was added K₂CO₃ (100 mg), the mixture was stirred for 3 h. K₂CO₃ was removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH₂Cl₂ (4 mL) with **C1** (20 mg, 0.028 mmol) and AW 300 molecular sieves (100 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*, and purified by SiO₂ column chromatography (EtOAc/hexanes 1:2) to afford **2-42** as a clear oil (24 mg, 63%). ¹H NMR (CDCl₃, 500 MHz) δ 8.18-7.90 (m, 14 H), 7.75-7.73 (m, 2 H), 7.58-7.20 (m, 29 H), 5.96 (t, *J* = 10.0 Hz, 1 H), 5.80 (t, *J* = 9.5 Hz,

1 H), 5.68 (dt, $J = 15.0, 6.5$ Hz, 1 H), 5.54-5.47 (m, 3 H), 5.39 (dd, $J = 9.5, 7.5$ Hz, 1 H), 5.37 (d, $J = 3.5$ Hz, 1 H), 5.29 (dd, $J = 15.0, 8.0$ Hz, 1 H), 5.21-5.18 (m, 2 H), 4.72 (d, $J = 8.0$ Hz, 1 H), 4.62 (d, $J = 8.0$ Hz, 1 H), 4.55 (d, $J = 11.0$ Hz, 1 H), 4.50-4.46 (m, 2 H), 4.24-4.21 (m, 1 H), 4.18 (t, $J = 9.5$ Hz, 1 H), 4.12 (t, $J = 7.0$ Hz, 1 H), 4.07-4.04 (m, 1 H), 3.98 (dd, $J = 9.5, 3.0$ Hz, 1 H), 3.83-3.73 (m, 4 H), 3.68 (dd, $J = 12.0, 7.0$ Hz, 1 H), 3.58 (t, $J = 7.0$ Hz, 1 H), 3.45 (dd, $J = 9.5, 3.0$ Hz, 1 H), 2.17 (s, 3 H), 1.94-1.85 (m, 4 H), 1.88 (s, 3 H), 1.71 (t, $J = 7.5$ Hz, 2 H), 1.58 (s, 3 H), 1.37-1.14 (m, 66 H), 0.88 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.77, 170.66, 169.91, 166.20, 165.80, 165.51, 165.38, 165.22, 164.85, 164.61, 137.30, 133.90, 133.64, 133.45, 130.00, 129.91, 129.62, 129.31, 129.00, 128.82, 128.63, 128.45, 128.38, 125.03, 101.17, 101.15, 95.37, 75.90, 74.30, 73.64, 73.28, 72.79, 72.31, 72.15, 71.05, 69.94, 69.65, 69.52, 67.82, 66.02, 64.96, 62.62, 61.80, 61.05, 50.42, 36.63, 32.51, 32.18, 29.96, 29.81, 29.62, 29.51, 29.22, 25.69, 22.94, 21.17, 20.80, 14.37; HRMS (ESI) calcd for $\text{C}_{129}\text{H}_{157}\text{NNaO}_{29}[\text{M}+\text{Na}]^+$: 2207.07390, found: 2207.07532.



Preparation of 2-7: Compound **2-42** (24 mg, 0.011 mmol) was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in THF). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was purified by SiO_2 column chromatography (MeOH/ CH_2Cl_2 / H_2O 20:80:3) to afford **2-7** (10 mg, 79%) as a white solid. ^1H NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$, 500 MHz) δ 7.45 (d, J

= 9.0 Hz, 1 H), 5.54 (dt, $J = 15.0, 7.0$ Hz, 1 H), 5.35 (dd, $J = 15.5, 6.5$ Hz, 1 H), 4.81 (s, 1 H), 4.27 (d, $J = 6.5$ Hz, 1 H), 4.16 (d, $J = 8.0$ Hz, 1 H), 3.97 (dd, $J = 10.0, 4.5$ Hz, 1 H), 3.90-3.87 (m, 2 H), 3.78-3.70 (m, 3 H), 3.63-3.03 (m, 16 H), 2.02 (t, $J = 7.0$ Hz, 2 H), 1.93 (brs, 2 H), 1.49-1.39 (m, 2 H), 1.24-1.10 (m, 66 H), 0.85 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR (DMSO- d_6 /D $_2$ O, 125 MHz) δ 172.51, 132.23, 132.06, 104.43, 104.16, 96.14, 81.25, 76.19, 75.96, 75.43, 75.09, 73.86, 73.76, 71.32, 71.20, 70.86, 69.88, 69.22, 67.42, 63.46, 61.72, 61.00, 60.81, 53.49, 36.27, 32.47, 32.01, 31.99, 29.81, 29.74, 29.44, 26.10, 22.80, 14.64; HRMS (ESI) calcd for $\text{C}_{62}\text{H}_{117}\text{NNaO}_{18}[\text{M}+\text{Na}]^+$: 1186.81629, found: 1186.81701.



Preparation of 2-41': Compound **2-39** (330 mg, 0.44 mmol) and **2-S1** (380 mg, 0.39 mmol) were dissolved in CH_2Cl_2 (12 mL) followed by addition of 4 Å molecular sieves (600 mg). The mixture was stirred for 1 h then cooled to 0 °C before the addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*, and purified by SiO_2 column chromatography (EtOAc/hexanes 1:3) to afford a white solid. To this solid in pyridine (10 mL) was added BzCl (0.12 mL, 1.0 mmol) and DMAP (60 mg, 0.5 mmol). The mixture was stirred for 6 h before concentration. The residue was purified via SiO_2 chromatography (EtOAc/hexane 1:3) to afford **2-40'** as a white solid (450 mg, 70%). HRMS (ESI) calcd for $\text{C}_{93}\text{H}_{88}\text{NO}_{26}\text{Si} [\text{M}+\text{NH}_4]^+$: 1662.53583,

found: 1662.53440.

Compound **2-40'** (450 mg, 0.27 mmol) was dissolved in TFA (6 mL) and CH₂Cl₂ (3 mL) and cooled to 0 °C. The solvent was removed *in vacuo* after 2 h, and the residue was purified by SiO₂ chromatography (EtOAc/hexanes 1:2) to afford **2-41'** as a white solid (230 mg, 55%). ¹H NMR (CDCl₃, 500 MHz) δ 8.20-7.95 (m, 16 H), 7.66-7.15 (m, 34 H), 6.19 (t, *J* = 10.0 Hz, 1 H), 5.92 (t, *J* = 10.0 Hz, 1 H), 5.65 (m, 2 H), 5.52 (m, 1 H), 5.45 (dd, *J* = 10.0, 3.0 Hz, 1 H), 5.37 (d, *J* = 2.5 Hz, 1 H), 5.29 (s, 1 H), 5.26 (dd, *J* = 10.0, 3.0 Hz, 1 H), 4.89 (d, *J* = 8.0 Hz, 1 H), 4.57-4.50 (m, 3 H), 4.39-4.37 (m, 1 H), 4.29-4.25 (m, 1 H), 4.24 (t, *J* = 9.5 Hz, 1 H), 4.13-4.05 (m, 2 H), 3.97 (dd, *J* = 11.0, 8.0 Hz, 1 H), 3.86-3.74 (m, 2 H), 3.51 (brs, 1 H); ¹³C NMR (CDCl₃, 125 MHz) δ 166.28, 166.23, 166.22, 166.09, 165.85, 165.50, 165.26, 164.88, 164.84, 164.50, 133.86, 133.81, 133.62, 133.42, 133.05, 130.57, 130.20, 130.09, 130.02, 129.92, 129.84, 129.65, 129.54, 129.41, 129.26, 128.99, 128.82, 128.79, 128.70, 128.67, 128.59, 128.41, 128.33, 101.43, 95.42, 90.53, 76.38, 74.32, 72.38, 71.36, 71.02, 70.33, 69.72, 69.71, 69.44, 68.77, 66.30, 65.54, 62.61, 61.91, 61.44; HRMS (ESI) calcd for C₈₈H₇₆NO₂₆[M+NH₄]⁺: 1562.46501, found: 1562.46409 .



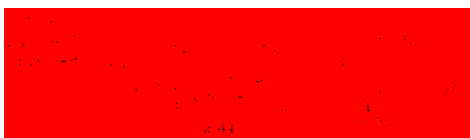
Preparation of 2-43: Coupling of compound **2-41'** (31 mg, 0.020 mmol) with **C2** (15 mg, 0.025 mmol) afforded product **2-43** as a clear oil (31 mg, 72%). ¹H NMR (CDCl₃, 500 MHz) δ 8.18-7.94 (m, 16 H), 7.64-7.14 (m, 34 H), 5.89 (t, *J* = 9.5 Hz, 1 H), 5.83

(t, $J = 10.0$ Hz, 1 H), 5.70-5.65 (m, 1 H), 5.62 (d, $J = 3.5$ Hz, 1 H), 5.60 (t, $J = 11.0$ Hz, 1 H), 5.51-5.48 (m, 2 H), 5.44-5.35 (m, 2 H), 5.35 (brs, 1 H), 5.29 (dd, $J = 15.0$, 7.5 Hz, 1 H), 5.19 (t, $J = 8.0$ Hz, 1 H), 4.77 (t, $J = 8.0$ Hz, 1 H), 4.64 (d, $J = 7.5$ Hz, 1 H), 4.55-4.53 (m, 3H), 4.24-4.17 (m, 2 H), 4.19 (t, $J = 9.5$ Hz, 1 H), 4.04 (d, $J = 10.0$ Hz, 1 H), 3.98 (dd, $J = 10.0$, 3.0 Hz, 1 H), 3.86-3.83 (m, 3 H), 3.74-3.67 (m, 2 H), 3.45 (dd, $J = 9.5$, 4.0 Hz, 1 H), 1.94-1.90 (m, 2 H), 1.88 (s, 3 H), 1.71 (t, $J = 7.5$ Hz, 2 H), 1.39-1.16 (m, 50 H), 1.08 (q, $J = 7.0$ Hz, 2 H), 0.88 (t, $J = 7.5$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.78, 169.91, 166.24, 166.11, 165.99, 165.79, 165.52, 165.40, 165.22, 164.84, 164.43, 137.30, 133.81, 133.64, 133.38, 133.02, 130.57, 130.02, 129.92, 129.83, 129.64, 129.51, 129.41, 129.32, 128.98, 128.83, 128.65, 128.39, 128.31, 125.04, 101.45, 101.17, 95.37, 76.30, 74.16, 73.64, 73.40, 72.81, 72.38, 71.39, 71.03, 69.68, 69.68, 69.44, 67.82, 66.30, 65.48, 62.66, 61.91, 61.33, 50.43, 36.65, 32.52, 32.18, 29.97, 29.81, 29.77, 29.61, 29.51, 29.41, 29.22, 25.70, 22.94, 21.17, 14.37; HRMS (ESI) calcd for $\text{C}_{126}\text{H}_{144}\text{NO}_{29}[\text{M}+\text{H}]^+$: 2134.98185, found: 2134.98364.



Preparation of 2-8: Treatment of compound **2-43** (30 mg, 0.014 mmol) with NaOMe afforded **2-8** as a white solid (13 mg, 85%). ^1H NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$, 500 MHz) δ 7.46 (d, $J = 9.0$ Hz, 1 H), 5.56-5.51 (m, 1 H), 5.35 (dd, $J = 15.0$, 7.5 Hz, 1 H), 4.81 (s, 1 H), 4.27 (d, $J = 6.5$ Hz, 1 H), 4.16 (d, $J = 8.0$ Hz, 1 H), 3.97 (dd, $J = 9.5$, 4.5 Hz, 1

H), 3.89-3.86 (m, 2 H), 3.80-3.70 (m, 3 H), 3.64-3.29 (m, 12 H), 3.04 (t, $J = 8.0$ Hz, 1 H), 2.02 (t, $J = 7.5$ Hz, 2 H), 1.93 (p, $J = 6.5$ Hz, 2 H), 1.47-1.43 (m, 2 H), 1.42-1.10 (m, 50 H), 0.85 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR (DMSO- d_6 /D $_2$ O, 125 MHz) δ 171.90, 131.62, 131.45, 103.82, 103.56, 95.53, 80.64, 75.58, 75.35, 74.82, 74.48, 73.25, 73.15, 70.60, 70.59, 70.25, 69.28, 68.62, 66.81, 62.86, 61.11, 60.39, 60.20, 52.88, 35.66, 31.86, 31.40, 31.38, 29.20, 29.13, 28.83, 25.49, 22.19, 14.03; HRMS (ESI) calcd for $\text{C}_{54}\text{H}_{101}\text{NNaO}_{18}[\text{M}+\text{Na}]^+$: 1074.69109, found: 1074.69074.

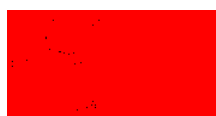


Preparation of 2-44: Coupling of compound **2-41'** (44 mg, 0.028 mmol) with **C3** (15 mg, 0.032 mmol) afforded product **2-44** as a white solid (35 mg, 63%). ^1H NMR (CDCl_3 , 500 MHz) δ 8.18-7.94 (m, 16 H), 7.64-7.14 (m, 34 H), 5.89 (t, $J = 10.0$ Hz, 1 H), 5.83 (t, $J = 9.5$ Hz, 1 H), 5.70-5.65 (m, 1 H), 5.62 (d, $J = 3.5$ Hz, 1 H), 5.60 (t, $J = 11.0$ Hz, 1 H), 5.50-5.489 (m, 2 H), 5.43-5.39 (m, 2 H), 5.35 (brs, 1 H), 5.28 (dd, $J = 15.0, 7.5$ Hz, 1 H), 5.19 (t, $J = 7.5$ Hz, 1 H), 4.77 (t, $J = 8.0$ Hz, 1 H), 4.64 (d, $J = 7.5$ Hz, 1 H), 4.55-4.53 (m, 3H), 4.24-4.17 (m, 2 H), 4.19 (t, $J = 9.5$ Hz, 1 H), 4.04 (dt, $J = 10.0, 3.5$ Hz, 1 H), 3.98 (dd, $J = 10.0, 3.0$ Hz, 1 H), 3.87-3.83 (m, 3 H), 3.73-3.67 (m, 2 H), 3.45 (dd, $J = 9.5, 4.0$ Hz, 1 H), 1.94-1.90 (m, 2 H), 1.88 (s, 3 H), 1.72 (t, $J = 7.5$ Hz, 2 H), 1.37-1.15 (m, 30 H), 1.08 (q, $J = 7.0$ Hz, 2 H), 0.88 (t, $J = 7.5$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.77, 169.91, 166.24, 165.99, 165.79, 165.51, 165.22, 164.84, 164.42, 137.30, 133.81, 133.64, 133.37, 130.57, 130.01, 129.92,

129.83, 129.64, 129.32, 128.98, 128.83, 128.65, 128.38, 128.31, 125.03, 101.44, 101.18, 95.36, 76.30, 74.16, 73.65, 73.40, 72.81, 72.37, 71.38, 71.03, 69.68, 69.68, 69.44, 67.83, 66.30, 65.48, 62.64, 61.90, 61.33, 50.45, 36.64, 32.51, 32.17, 31.96, 29.95, 29.76, 29.61, 29.50, 29.33, 29.23, 25.68, 22.88, 21.17, 14.37; HRMS (ESI) calcd for C₁₁₆H₁₂₄NO₂₉[M+H]⁺: 1994.82535, found: 1994.82149.



Preparation of 2-9: Treatment of compound **2-44** (35 mg, 0.018 mmol) with NaOMe afforded **9** as a white solid (12 mg, 75%). ¹H NMR (DMSO-d₆/D₂O, 500 MHz) δ 7.56 (d, *J* = 9.0 Hz, 1 H), 5.55-5.50 (m, 1 H), 5.34 (dd, *J* = 15.5, 7.5 Hz, 1 H), 4.79 (s, 1 H), 4.24 (d, *J* = 7.0 Hz, 1 H), 4.15 (d, *J* = 8.0 Hz, 1 H), 3.95 (dd, *J* = 9.5, 4.5 Hz, 1 H), 3.88-3.85 (m, 2 H), 3.77-3.72 (m, 3 H), 3.60-3.29 (m, 12 H), 3.03 (t, *J* = 8.0 Hz, 1 H), 2.01 (t, *J* = 7.0 Hz, 2 H), 1.92 (p, *J* = 7.0 Hz, 2 H), 1.43 (p, *J* = 7.0 Hz, 2 H), 1.27-1.08 (m, 30 H), 0.84 (t, *J* = 6.5 Hz, 6 H); ¹³C NMR (DMSO-d₆/D₂O, 125 MHz) δ 172.32, 131.86, 131.42, 103.88, 103.59, 95.62, 80.71, 75.66, 75.44, 74.92, 74.59, 73.34, 73.24, 70.81, 70.67, 70.35, 69.28, 68.74, 66.88, 62.98, 61.19, 60.48, 60.33, 53.09, 35.81, 31.91, 31.47, 31.44, 29.22, 28.88, 28.81, 25.61, 22.31, 22.28, 14.15; HRMS (ESI) calcd for C₄₄H₈₁NNaO₁₈[M+Na]⁺: 934.53459, found: 934.53537.



Preparation of 2-50: Compound **2-49** (1.0 g, 2.2 mmol) was dissolved in CH₂Cl₂ (5

mL) and Pyridine (10 mL). The mixture was cooled to 0 °C followed by addition of BzCl (0.30 mL, 2.6 mmol) dropwise. After 1 h, the mixture was quenched with MeOH (1 mL) and concentrated. The residue was chromatographed (SiO₂, EtOAc/hexane 1:3) to afford **2-50** as a clear oil (0.91 g, 73%). ¹H NMR (CDCl₃, 500 MHz) δ 7.94 (dd, *J* = 8.5, 1.0 Hz, 2 H), 7.48 (tt, *J* = 7.5, 1.0 Hz, 1 H), 7.33 (t, *J* = 8.0 Hz, 2 H), 7.27-7.09 (m, 10 H), 5.08 (dd, *J* = 10.0, 3.0 Hz, 1 H), 4.82 (d, *J* = 11.5 Hz, 1 H), 4.67 (d, *J* = 11.5 Hz, 1 H), 4.53-4.47 (m, 3 H), 4.18 (d, *J* = 3.0 Hz, 1 H), 4.07-4.01 (m, 1 H), 3.84 (dd, *J* = 10.0, 7.5 Hz, 1 H), 3.74-3.65 (m, 3 H), 3.64-3.58 (m, 1 H), 1.03 (t, *J* = 8.5 Hz, 2 H), 0.00 (t, *J* = 3.5 Hz, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 165.91, 138.28, 137.77, 133.21, 129.93, 129.82, 128.51, 128.42, 128.25, 128.11, 127.85, 127.76, 127.57, 103.63, 76.35, 75.56, 74.59, 73.70, 72.92, 69.54, 68.24, 67.66, 18.58, -1.29; HRMS (ESI) calcd for C₃₂H₄₄NO₇Si[M+NH₄]⁺: 582.28816, found: 582.28748.



Preparation of 2-51: Compound **2-21** (167 mg, 0.31 mmol) was dissolved in CCl₃CN (5 mL) followed by addition of K₂CO₃ (300 mg). The suspension was stirred for 3 h. The mixture was filtered through a celite pad. The filtrate was removed *in vacuo*. To the solution of the residue and **2-45** (252 mg, 0.39 mmol) were dissolved in CH₂Cl₂ (4 mL) followed by addition of molecular sieves 4 Å (200 mg). The mixture was stirred for 1 h then cooled to 0 °C before the addition of a catalytic

amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*. To the residue in pyridine (5 mL) was added Ac₂O (0.2 mL, 2.0 mmol) and DMAP (60 mg, 0.50 mmol). The mixture was stirred for 3 h before concentration. The residue was purified via SiO₂ chromatography (EtOAc/hexane 1:4) to afford **2-51** as a clear oil (204 mg, 63%). ¹H NMR (CDCl₃, 500 MHz) δ 8.16-8.10 (m, 4 H), 7.66-7.20 (m, 26 H), 5.73 (d, *J* = 3.0 Hz, 1 H), 5.63 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.31 (d, *J* = 3.5 Hz, 1 H), 4.83 (d, *J* = 11.5 Hz, 1 H), 4.75-4.71 (m, 3 H), 4.63-4.60 (m, 3 H), 4.48 (d, *J* = 12.0 Hz, 1 H), 4.43 (d, *J* = 11.5 Hz, 1 H), 4.41-4.37 (m, 2 H), 4.21 (dd, *J* = 10.0, 3.5 Hz, 1 H), 4.07 (dt, *J* = 15.5, 5.5 Hz, 1 H), 4.03-3.97 (m, 3 H), 3.67-3.60 (m, 2 H), 3.54 (dd, *J* = 10.0, 7.0 Hz, 1 H), 3.28 (d, *J* = 3.0 Hz, 1 H), 3.25 (dd, *J* = 9.5, 5.0 Hz, 1 H), 1.97 (s, 3 H), 1.05-0.91 (m, 2 H), 0.01 (t, *J* = 3.5 Hz, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.74, 166.34, 165.03, 138.99, 138.92, 138.67, 138.60, 133.55, 133.34, 130.09, 130.03, 129.97, 129.86, 128.76, 128.62, 128.55, 128.46, 128.42, 128.35, 128.14, 127.92, 127.87, 127.81, 127.67, 127.63, 127.56, 101.25, 93.64, 79.10, 75.91, 75.11, 74.69, 73.62, 73.52, 73.48, 71.88, 71.23, 70.97, 70.10, 69.91, 67.78, 65.10, 62.38, 20.80, 18.15, -1.25; HRMS (ESI) calcd for C₆₁H₇₂NO₁₄Si[M+NH₄]⁺: 1070.47166, found: 1070.47107.



Preparation of 2-53: Compound **2-51** (150 mg, 0.14 mmol) was dissolved in CH₂Cl₂

(2 mL) and cooled to 0 °C followed by addition of TFA (3 mL). The solvent was removed *in vacuo* after 1 h, and the residue was purified by SiO₂ chromatography (EtOAc/hexanes 1:2) to afford **2-52** as a clear oil (129 mg, 95%). To a solution of **2-52** (129 mg, 0.14 mmol) in CCl₃CN (5 mL) was added K₂CO₃ (200 mg), the mixture was stirred for 3 h. K₂CO₃ was removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH₂Cl₂ (6 mL) with **50** (64 mg, 0.11 mmol) and 4 Å molecular sieves (300 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*, and purified by SiO₂ column chromatography (EtOAc/hexanes 1:3) to afford **2-53** as a clear oil (95 mg, 56%). ¹H NMR (CDCl₃, 500 MHz) δ 8.13 (d, *J* = 7.0 Hz, 2 H), 8.03 (d, *J* = 7.5 Hz, 2 H), 7.98 (d, *J* = 7.5 Hz, 2 H), 7.61 (t, *J* = 7.5 Hz, 1 H), 7.57 (t, *J* = 7.5 Hz, 1 H), 7.50-6.90 (m, 37 H), 5.64 (dd, *J* = 10.5, 8.0 Hz, 1 H), 5.58 (d, *J* = 3.0 Hz, 1 H), 5.17-5.15 (m, 2 H), 4.82 (d, *J* = 12.0 Hz, 1 H), 4.75-4.72 (m, 2 H), 4.70 (d, *J* = 12.0 Hz, 1 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 4.62 (d, *J* = 11.5 Hz, 1 H), 4.56 (d, *J* = 11.5 Hz, 1 H), 4.51 (d, *J* = 12.0 Hz, 1 H), 4.49 (d, *J* = 12.0 Hz, 1 H), 4.47 (d, *J* = 7.0 Hz, 1 H), 4.41 (d, *J* = 11.5 Hz, 1 H), 4.36 (d, *J* = 11.0 Hz, 1 H), 4.32-4.29 (m, 3 H), 4.24 (dd, *J* = 11.0, 8.0 Hz, 1 H), 4.16-4.10 (m, 1 H), 4.00-3.95 (m, 3 H), 3.84-3.77 (m, 4 H), 3.72 (dd, *J* = 9.0, 3.0 Hz, 1 H), 3.67-3.64 (m, 2 H), 3.56 (s, 1 H), 3.52 (dd, *J* = 10.0, 8.0 Hz, 1 H), 3.44-3.40 (m, 2 H), 1.94 (s, 3 H), 1.11-1.02 (m, 2 H), 0.09-0.06 (m, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.48, 166.11, 166.05, 165.16, 138.75, 138.67, 138.63, 138.39, 138.15, 133.51, 133.46, 130.08, 129.99,

129.92, 129.73, 129.60, 128.72, 128.65, 128.58, 128.51, 128.48, 128.35, 128.23, 128.12, 127.86, 127.70, 127.68, 127.62, 127.45, 127.39, 103.22, 101.22, 94.74, 78.73, 77.93, 75.56, 75.47, 75.04, 74.88, 73.86, 73.77, 73.64, 73.54, 73.24, 72.69, 71.62, 71.03, 70.05, 69.96, 68.83, 67.68, 65.37, 62.17, 29.88, 20.70, 18.61, -1.13; HRMS (ESI) calcd for $C_{88}H_{98}NO_{20}Si[M+NH_4]^+$: 1516.64460, found: 1516.67725.



Preparation of 2-54: Compound **2-53** (50 mg, 0.033 mmol) was dissolved in THF (10 mL) and MeOH (10 mL) and transferred to a hydrogenation vessel. Palladium on carbon (10%, 100 mg) was added to the vessel, and the vessel was subjected to H_2 (350 psi) at room temperature for 12 h. The catalyst was then removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in MeOH (3 mL) and THF (1 mL). NaOMe (0.1 mL, 1 M in THF) was introduced, and the solution was stirred for 12 h. The solvent was removed *in vacuo*. The residue in pyridine (5 mL) and CH_2Cl_2 (2.5 mL) was cooled to 0 °C followed by addition of BzCl (0.3 mL). The solution was stirred for 12 h. The solvent was removed *in vacuo* and the residue was purified via SiO_2 chromatography (EtOAc/hexanes 1:3) to give compound **2-54** as a clear oil (23 mg, 42%). 1H NMR ($CDCl_3$, 500 MHz) δ 8.34-7.00 (m, 50 H), 5.84 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.77-5.75 (m, 2 H), 5.71 (d, $J = 3.0$ Hz, 1 H), 5.65 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.60 (dd, $J = 10.0, 3.0$ Hz, 1 H), 5.51 (d, $J = 3.5$ Hz, 1 H), 5.44 (dd, $J = 10.5, 3.0$ Hz, 1 H), 5.02 (d, $J =$

7.5 Hz, 1 H), 4.84 (dd, $J = 11.5, 6.0$ Hz, 1 H), 4.75 (d, $J = 8.0$ Hz, 1 H), 4.71 (d, $J = 2.5$ Hz, 1 H), 4.67 (dd, $J = 11.5, 7.5$ Hz, 1 H), 4.41 (dd, $J = 11.0, 6.5$ Hz, 1 H), 4.35 (t, $J = 7.0$ Hz, 1 H), 4.25 (dd, $J = 11.5, 6.5$ Hz, 1 H), 4.18-4.14 (m, 2 H), 4.13 (dd, $J = 12.0, 7.0$ Hz, 1 H), 4.07 (dd, $J = 10.5, 7.0$ Hz, 1 H), 3.96 (dt, $J = 15.5, 5.5$ Hz, 1 H), 3.63 (t, $J = 6.5$ Hz, 1 H), 3.59 (dt, $J = 10.0, 6.5$ Hz, 1 H), 0.92-0.80 (m, 2 H), -0.12 (t, $J = 3.5$ Hz, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 166.34, 166.29, 166.04, 165.88, 165.79, 165.53, 165.42, 164.93, 164.76, 133.99, 133.63, 133.41, 133.28, 133.16, 133.04, 130.42, 130.25, 130.12, 130.00, 129.96, 129.88, 129.79, 129.46, 129.38, 129.31, 129.23, 128.83, 128.75, 128.70, 128.66, 128.61, 128.55, 128.48, 128.38, 128.26, 100.76, 100.75, 92.98, 74.56, 73.28, 72.29, 71.98, 71.52, 71.02, 69.76, 68.59, 68.19, 67.78, 66.99, 66.95, 65.12, 63.82, 61.86, 61.77, 29.92, 18.05, -1.34; HRMS (ESI) calcd for $\text{C}_{93}\text{H}_{88}\text{NO}_{26}\text{Si}[\text{M}+\text{NH}_4]^+$: 1662.53583, found: 1662.56130.



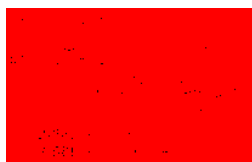
Preparation of 2-56: Compound **2-54** (23 mg, 0.014 mmol) was dissolved in TFA (2 mL) and CH_2Cl_2 (1 mL) and cooled to 0 °C. The solvent was removed *in vacuo* after 2 h, and the residue was purified by SiO_2 chromatography (EtOAc/hexanes 1:2) to afford a clear oil (19 mg, 88%). To a solution of the oil (19 mg, 0.012 mmol) in CCl_3CN (3 mL) was added K_2CO_3 (100 mg), the mixture was stirred for 3 h. K_2CO_3 was removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (4 mL) with **C3** (10 mg, 0.013 mmol) and AW

300 molecular sieves (100 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 16 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*, and purified by SiO₂ column chromatography (EtOAc/hexanes 1:2) to afford impure **2-55** as a clear oil (15 mg, 55%). The oil (15 mg, 0.0068 mmol) was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in THF). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was dissolved in pyridine (3 mL) followed by addition of Ac₂O (0.1 mL) and DMAP (10 mg). The solvent was removed *in vacuo* after 12 h and the residue was chromatographed (SiO₂, EtOAc/hexane 1:1) to afford **2-56** as a white solid (8.8 mg, 78%). ¹H NMR (CDCl₃, 500 MHz) δ 6.21 (d, *J* = 9.0 Hz, 1 H), 5.46 (d, *J* = 2.0 Hz, 1 H), 5.34 (d, *J* = 3.0 Hz, 1 H), 5.27-5.16 (m, 3 H), 5.15 (dd, *J* = 10.5, 3.0 Hz, 1 H), 5.04 (dd, *J* = 10.5, 8.0 Hz, 1 H), 5.00 (dd, *J* = 7.5, 3.5 Hz, 1 H), 4.96-4.94 (m, 1 H), 4.84 (dd, *J* = 10.5, 3.0 Hz, 1 H), 4.42 (d, *J* = 8.0 Hz, 1 H), 4.38-4.33 (m, 3 H), 4.30 (t, *J* = 7.0 Hz, 1 H), 4.15 (dd, *J* = 12.0, 7.0 Hz, 1 H), 4.13-4.04 (5 H), 3.89-3.85 (m, 2 H), 3.76 (t, *J* = 6.5 Hz, 1 H), 3.71-3.68 (m, 2 H), 2.19 (s, 3 H), 2.18 (s, 3 H), 2.14 (s, 3 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.08 (s, 6 H), 2.06 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.96 (s, 3 H), 1.68-1.56 (m, 4 H), 1.30-1.12 (m, 66 H), 0.88 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 172.92, 171.13, 171.01, 170.85, 170.81, 170.53, 170.40, 170.38, 170.28, 170.15, 170.05, 169.61, 169.32, 102.20, 100.20, 93.90, 74.28, 73.48, 73.41, 73.31, 73.22, 72.28, 70.96, 69.76, 68.53, 67.71, 67.40, 66.84, 66.64, 65.50, 65.14, 63.68, 61.74, 61.04, 53.68, 48.05, 37.01, 32.18, 29.95,

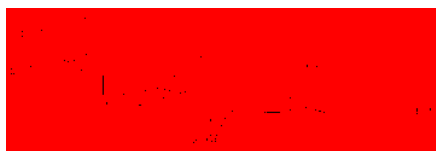
29.86, 29.61, 29.03, 25.87, 25.73, 22.94, 21.32, 21.13, 21.00, 20.94, 20.88, 20.70
14.38; HRMS (ESI) calcd for $C_{84}H_{139}NNaO_{31}[M+Na]^+$: 1680.92233, found:
1680.92592.



Preparation of 2-10: Compound **2-56** (8.8 mg, 0.0053 mmol) was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in THF). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was purified by SiO₂ column chromatography (MeOH/CH₂Cl₂/H₂O 20:80:3) to afford **2-10** (5.2 mg, 86%) as a white solid. ¹H NMR (DMSO-d₆/D₂O, 500 MHz) δ 7.72 (d, *J* = 9.5 Hz, 1 H), 4.82 (d, *J* = 3.5 Hz, 1 H), 4.31 (d, *J* = 7.0 Hz, 1 H), 4.06 (d, *J* = 7.0 Hz, 1 H), 3.98 (t, *J* = 6.5 Hz, 1 H), 3.89 (brs, 2 H), 3.82 (brs, 2 H), 3.73 (d, *J* = 3.0 Hz, 1 H), 3.65-3.33 (m, 14 H), 3.29 (d, *J* = 8.5 Hz, 1 H), 3.14-3.12 (m, 2 H), 2.09-2.02 (m, 2 H), 1.50-1.13 (m, 68 H), 0.83 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (DMSO-d₆/D₂O, 125 MHz) δ 171.98, 105.46, 104.12, 96.10, 78.73, 78.60, 74.74, 74.06, 73.35, 73.00, 71.48, 70.80, 70.58, 69.99, 69.57, 68.67, 68.42, 64.29, 60.33, 60.00, 59.84, 58.09, 50.17, 35.56, 31.35, 31.33, 30.66, 29.33, 29.28, 29.16, 29.10, 29.02, 28.79, 28.73, 25.55, 25.45, 22.15, 13.99; HRMS (ESI) calcd for $C_{60}H_{115}NNaO_{19}[M+Na]^+$: 1176.79555, found: 1176.79430.



Preparation of 2-58: As described in the preparation of **2-51**, coupling of compound **2-21** (300 mg, 0.56 mmol) with **57** (280 mg, 0.47 mmol) afforded a mixture of **2-58a** and **2-58b** as a clear oil (331 mg, 58a/58b = 1.15:1, 63%). The mixture was carefully chromatographed (SiO₂, EtOAc/hexane 1:4) again to afford **2-58b** (65 mg) and a mixture of **2-58a** and **2-58b** (250 mg). **2-58b** was characterized. ¹H NMR (CDCl₃, 500 MHz) δ 8.13-8.02 (m, 6 H), 7.81 (d, *J* = 7.5 Hz, 2 H), 7.66-7.09 (m, 32 H), 6.71 (d, *J* = 4.0 Hz, 1 H), 5.83 (t, *J* = 10.0 Hz, 1 H), 5.64 (dd, *J* = 10.0, 3.5 Hz, 1 H), 5.00 (d, *J* = 3.0 Hz, 1 H), 4.72 (d, *J* = 11.0 Hz, 1 H), 4.68 (d, *J* = 9.0 Hz, 1 H), 4.61 (dd, *J* = 12.0, 3.5 Hz, 1 H), 4.55 (dt, *J* = 9.0, 4.5 Hz, 1 H), 4.50-4.45 (m, 2 H), 4.43 (dd, *J* = 8.0, 4.5 Hz, 1 H), 4.35 (d, *J* = 11.0 Hz, 1 H), 4.31 (d, *J* = 12.0 Hz, 1 H), 4.31 (d, *J* = 12.0 Hz, 1 H), 4.19 (d, *J* = 12.5 Hz, 1 H), 4.15 (d, *J* = 12.5 Hz, 1 H), 4.08 (t, *J* = 7.0 Hz, 1 H), 3.78-3.72 (m, 2 H), 3.65 (brs, 1 H), 3.26-3.19 (m, 2 H); ¹³C NMR (CDCl₃, 125 MHz) δ 166.45, 165.42, 165.13, 164.62, 139.00, 138.80, 138.73, 138.20, 134.05, 133.60, 133.44, 133.31, 130.18, 130.12, 130.04, 129.97, 129.78, 129.66, 129.53, 129.26, 128.95, 128.75, 128.58, 128.55, 128.49, 128.44, 128.35, 128.32, 128.19, 128.04, 127.86, 127.80, 127.70, 127.62, 127.54, 99.21, 90.55, 78.77, 76.16, 75.26, 75.25, 75.06, 73.55, 73.39, 73.09, 71.67, 70.66, 70.64, 70.07, 68.29, 62.88; HRMS (ESI) calcd for C₆₈H₆₆NO₁₅[M+NH₄]⁺: 1136.44270, found: 1136.46410.



Preparation of 2-60: The disacchride **2-58** (200 mg, 0.18 mmol) was dissolved in Et₂O (20 mL) and cooled to 0 °C. BnNH₂ (2 mL) was added. The mixture was stirred for 18 h and treated with HCl (1 M, 20 mL). The aqueous layer was washed with EtOAc (20 mL x 3). The combined organic layers were washed with brine (10 mL), dried with Na₂SO₄, and concentrated. The residue was purified by SiO₂ column chromatography (EtOAc/Hexane 1:3) to afford a white solid (154 mg, 85%). As described in the preparation of **2-51**, coupling of this solid (154 mg, 0.15 mmol) with **2-59** (66 mg, 0.12 mmol) afforded **2-60** as a clear oil (123 mg, 66%). ¹H NMR (CDCl₃, 500 MHz) δ 7.98 (t, *J* = 8.5 Hz, 4 H), 7.82 (d, *J* = 7.0 Hz, 2 H), 7.50-7.03 (m, 44 H), 5.56 (t, *J* = 9.5 Hz, 1 H), 5.38 (t, *J* = 8.5 Hz, 1 H), 5.04 (d, *J* = 11.0 Hz, 1 H), 4.83 (d, *J* = 11.0 Hz, 1 H), 4.80 (d, *J* = 3.5 Hz, 1 H), 4.77 (d, *J* = 8.5 Hz, 1 H), 4.75 (d, *J* = 12.0 Hz, 1 H), 4.71 (d, *J* = 8.5 Hz, 1 H), 4.68 (d, *J* = 11.0 Hz, 1 H), 4.66 (d, *J* = 11.5 Hz, 1 H), 4.57-4.51 (m, 2 H), 4.42 (dd, *J* = 12.0, 3.5 Hz, 1 H), 4.33 (d, *J* = 11.0 Hz, 1 H), 4.32 (d, *J* = 12.5 Hz, 1 H), 4.30 (d, *J* = 10.0 Hz, 1 H), 4.27 (d, *J* = 12.5 Hz, 1 H), 4.25 (d, *J* = 12.0 Hz, 1 H), 4.24 (d, *J* = 7.5 Hz, 1 H), 4.20 (dd, *J* = 12.0, 5.0 Hz, 1 H), 4.00 (t, *J* = 9.5 Hz, 1 H), 3.98-3.88 (m, 4 H), 3.80 (dd, *J* = 10.0, 2.5 Hz, 1 H), 3.73-3.69 (m, 2 H), 3.68 (dd, *J* = 9.5, 3.0 Hz, 1 H), 3.60 (dd, *J* = 11.0, 3.5 Hz, 1 H), 3.55 (t, *J* = 9.0 Hz, 1 H), 3.48 (t, *J* = 10.0 Hz, 1 H), 3.47 (t, *J* = 10.5 Hz, 1 H), 3.32 (dd, *J* = 9.5, 7.5 Hz, 1 H), 3.22 (t, *J* = 8.5 Hz, 1 H), 3.17 (dd, *J* = 8.5, 5.0 Hz, 1 H), 3.12-3.09 (m, 1 H), 1.00-0.96 (m, 2 H), -0.01 (t, *J* = 3.5 Hz, 9 H); ¹³C NMR (CDCl₃,

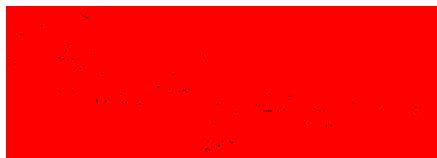
125 MHz) δ 166.41, 165.08, 164.84, 139.36, 139.16, 138.92, 138.90, 138.87, 138.61, 138.47, 133.38, 133.16, 130.06, 130.00, 129.75, 128.81, 128.67, 128.56, 128.50, 128.44, 128.38, 128.34, 128.30, 128.23, 127.96, 127.82, 127.70, 127.56, 127.36, 103.26, 100.60, 100.13, 82.84, 81.96, 79.54, 78.89, 75.60, 75.47, 75.37, 75.17, 75.04, 74.48, 73.82, 73.71, 73.47, 73.36, 72.85, 72.05, 71.26, 70.02, 68.06, 67.74, 67.62, 63.63, 18.68, -1.17; HRMS (ESI) calcd for $C_{93}H_{102}NO_{19}Si[M+NH_4]^+$: 1564.68098, found: 1564.68662.



Preparation of 2-62: To a stirring mixture of Compound **2-60** (100 mg, 0.065 mmol) and MeOH (3 mL) was added NaOMe (0.2 mL, 1 M in MeOH). After 18 h, the mixture was concentrated and the residue was purified by SiO₂ column chromatography (EtOAc/Hexane 1:1) to afford a clear oil. As described in the preparation of **30**, hydrogenation of this oil afforded **2-61** as a white solid (35 mg, 89%). HRMS (ESI) calcd for $C_{23}H_{45}O_{16}Si[M+H]^+$: 605.24714, found: 605.24563.

To a stirring solution of **2-61** (35 mg, 0.058 mmol) in pyridine (4 mL) was added BzCl (0.3 mL) and DMAP (10 mg). The mixture was stirred for 14 h at room temperature and 10 h to 50 °C. To the mixture was added Ac₂O (0.1 mL). After 1 h, the mixture was concentrated and the residue was purified by SiO₂ column chromatography (EtOAc/Hexane 1:2) to afford a mixture of **2-62a** and **2-62b** as a white solid (76 mg). **2-62a:** HRMS (ESI) calcd for $C_{93}H_{88}NO_{26}Si[M+NH_4]^+$:

1662.53583, found: 1662.53349. **2-62b**: HRMS (ESI) calcd for $C_{88}H_{86}NO_{26}Si[M+NH_4]^+$: 1600.52073, found: 1600.51977.



Preparation of 2-63: To a stirring mixture of **2-62** (20 mg) in MeOH (2 mL) was added NaOMe (0.1 mL, 1 M in MeOH). After 5 h, the mixture was concentrated and dissolved in Pyridine (2 mL) followed by addition of Ac₂O (0.1 mL). The mixture was concentrated after 12 h. The residue was purified by SiO₂ column chromatography (EtOAc/Hexane 1:1) to afford **2-63** as a white solid (10 mg). ¹H NMR (CDCl₃, 500 MHz) δ 5.33 (d, *J* = 3.5 Hz, 1 H), 5.19 (d, *J* = 3.5 Hz, 1 H), 5.13 (dd, *J* = 11.5, 3.5 Hz, 1 H), 5.10 (t, *J* = 9.5 Hz, 1 H), 5.06 (t, *J* = 9.5 Hz, 1 H), 4.97 (dd, *J* = 11.0, 3.5 Hz, 1 H), 4.92 (dd, *J* = 9.5, 8.5 Hz, 1 H), 4.81 (dd, *J* = 9.5, 8.0 Hz, 1 H), 4.40 (d, *J* = 8.0 Hz, 1 H), 4.37 (dd, *J* = 12.5, 2.0 Hz, 1 H), 4.28 (d, *J* = 8.0 Hz, 1 H), 4.22 (dd, *J* = 12.5, 4.5 Hz, 1 H), 4.14 (d, *J* = 7.0 Hz, 1 H), 4.09-4.03 (m, 2 H), 3.95-3.91 (m, 2 H), 3.89-3.84 (m, 1 H), 3.78 (t, *J* = 9.5 Hz, 1 H), 3.65 (t, *J* = 9.5 Hz, 1 H), 3.52-3.45 (m, 3 H), 2.05 (s, 6 H), 2.02 (s, 3 H), 2.01 (s, 6 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 1.94 (s, 3 H), 1.90 (s, 3 H), 0.90-0.78 (m, 2 H), -0.07 (t, *J* = 3.5 Hz, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.08, 170.86, 170.65, 170.56, 170.28, 170.08, 169.88, 169.75, 169.55, 168.89, 100.99, 100.20, 96.65, 77.40, 76.44, 72.87, 72.85, 72.38, 72.21, 71.85, 69.58, 68.48, 67.85, 67.78, 67.27, 66.83, 62.27, 61.84, 60.87, 29.94, 21.08, 21.00, 20.96, 20.88, 20.84, 18.08, -1.19; HRMS (ESI) calcd for

C₄₃H₆₈NO₂₆Si[M+NH₄]⁺: 1042.37933, found: 1042.37702.



Preparation of 2-65: As described in the preparation of **2-56**, coupling of **2-62** (56 mg) with **C5** (18 mg, 0.024 mmol) gave compound **2-64** as a clear oil (28 mg, ~51-53%) which was finally transformed to **2-65** (17 mg, ~81-83%). ¹H NMR (CDCl₃, 500 MHz) δ 6.24 (d, *J* = 9.5 Hz, 1 H), 5.40 (d, *J* = 2.5 Hz, 1 H), 5.26 (d, *J* = 3.5 Hz, 1 H), 5.20 (dd, *J* = 11.0, 3.0 Hz, 1 H), 5.17-5.09 (m, 3 H), 5.04 (dd, *J* = 11.0, 3.5 Hz, 1 H), 4.99 (t, *J* = 8.5 Hz, 1 H), 4.88 (dt, *J* = 10.0, 3.0 Hz, 1 H), 4.83 (dd, *J* = 9.5, 8.0 Hz, 1 H), 4.48 (dd, *J* = 12.0, 2.0 Hz, 1 H), 4.39 (d, *J* = 8.0 Hz, 1 H), 4.34 (d, *J* = 8.0 Hz, 1 H), 4.32-4.28 (m, 2 H), 4.20 (t, *J* = 7.0 Hz, 1 H), 4.15-4.10 (m, 2 H), 4.06-3.98 (m, 3 H), 3.84 (t, *J* = 9.5 Hz, 1 H), 3.83 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.71 (t, *J* = 9.5 Hz, 1 H), 3.57-3.51 (m, 2 H), 2.13 (s, 3 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 6 H), 1.96 (s, 3 H), 1.68-1.56 (m, 4 H), 1.38-1.12 (m, 66 H), 0.88 (t, *J* = 7.0 Hz, 3 H), 0.87 (t, *J* = 7.0 Hz, 3 H); ¹³C NMR (CDCl₃, 125 MHz) δ 173.04, 171.22, 171.06, 170.83, 170.57, 170.54, 170.26, 170.11, 169.99, 169.93, 169.72, 169.51, 168.84, 100.98, 100.46, 96.64, 75.99, 73.37, 72.95, 72.29, 72.28, 71.90, 71.85, 69.52, 68.48, 67.83, 67.39, 67.25, 66.80, 61.90, 61.80, 60.84, 47.87, 36.88, 32.15, 31.80, 29.93, 29.59, 28.12, 25.86, 25.77, 22.92, 21.25, 21.06, 20.96, 20.81, 20.75, 14.35; HRMS (ESI) calcd for C₈₄H₁₄₃N₂O₃₁ [M+NH₄]⁺: 1675.96693, found: 1675.96616.



Preparation of 2-11: As described in the preparation of **2-10**, hydrolysis of compound **2-65** (17 mg, 0.010 mmol) afforded **2-11** (10 mg, 85%) as a white solid. ^1H NMR (DMSO- d_6 /D $_2$ O, 500 MHz) δ 5.00 (d, J = 3.5 Hz, 1 H), 4.35 (d, J = 7.5 Hz, 1 H), 4.17 (d, J = 8.0 Hz, 1 H), 3.98-3.95 (m, 1 H), 3.93 (t, J = 7.0 Hz, 1 H), 3.88 (dd, J = 10.5, 6.0 Hz, 1 H), 3.75-3.67 (m, 3 H), 3.63-3.30 (m, 14 H), 3.16 (s, 1 H), 3.14 (t, J = 8.0, 1 H), 3.05-3.00 (m, 1 H), 2.12-2.00 (m, 2 H), 1.52-1.40 (m, 4 H), 1.34-1.13 (m, 62 H), 1.13 (t, J = 7.5 Hz, 2 H), 0.85 (t, J = 7.0 Hz, 6 H); ^{13}C NMR (DMSO- d_6 /D $_2$ O, 125 MHz) δ 171.97, 103.33, 102.85, 100.18, 85.38, 80.41, 76.10, 74.84, 74.50, 73.48, 73.04, 71.77, 71.35, 70.53, 69.50, 69.32, 69.30, 68.89, 68.71, 60.56, 60.37, 60.21, 50.12, 35.48, 31.18, 30.94, 29.14, 28.99, 28.86, 28.59, 28.57, 25.37, 25.30, 21.98, 13.80; HRMS (ESI) calcd for $\text{C}_{60}\text{H}_{115}\text{NNaO}_{19}[\text{M}+\text{Na}]^+$: 1176.79555, found: 1176.79452.



Preparation of 2-66: Compound **S1** (1.40 g, 1.4 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to 0 °C followed by addition of levulinic acid (0.46 mL, 4.5 mmol) and DMAP (0.68 g, 5.6 mmol). The mixture was stirred for 10 min and DCC (0.93 g, 4.5 mmol) was added. The solvent was removed *in vacuo*, and the residue was

purified by SiO₂ chromatography (EtOAc/hexanes 1:2) to give **2-66** (1.60 g, 94%). ¹H NMR (CDCl₃, 500 MHz) δ 8.04-9.03 (m, 10 H), 7.60-7.34 (m, 15 H), 5.74 (t, *J* = 9.5 Hz, 1 H), 5.40 (t, *J* = 9.5 Hz, 1 H), 5.38 (t, *J* = 10.5 Hz, 1 H), 5.32 (d, *J* = 3.5 Hz, 1 H), 5.01 (dd, *J* = 10.5, 3.5 Hz, 1 H), 4.72 (d, *J* = 8.0 Hz, 1H), 4.68 (d, *J* = 8.0 Hz, 1 H), 4.58 (d, *J* = 10.0 Hz, 1 H), 4.41 (dd, *J* = 12.0, 5.0 Hz, 1 H), 4.19 (t, *J* = 9.0 Hz, 1 H), 3.90 (ddd, *J* = 10.5, 10.5, 6.0 Hz, 1 H), 3.81-3.80 (m, 1 H), 3.78-3.74 (m, 1 H), 3.69-3.65 (m, 1 H), 3.51 (ddd, *J* = 10.5, 10.5, 6.5 Hz, 1 H), 2.72-2.68 (m, 2 H), 2.60-2.45 (m, 4 H), 2.40-2.30 (m, 2 H), 2.18 (s, 3 H), 2.04 (s, 3 H), 0.88-0.78 (m, 2 H), -0.13 (s, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.81, 171.68, 165.88, 165.65, 165.42, 165.28, 164.90, 133.54, 133.50, 133.38, 133.35, 133.21, 129.96, 129.91, 129.83, 129.75, 129.66, 129.59, 129.51, 128.86, 128.72, 128.64, 128.59, 128.48, 128.41, 100.92, 100.48, 76.11, 73.21, 72.94, 72.04, 71.17, 71.08, 69.58, 67.64, 66.92, 62.57, 60.88, 37.80, 37.76, 34.06, 29.84, 29.51, 27.93, 27.84, 25.74, 25.09, 17.94, 7.23, 7.08, -1.43; HRMS (ESI) calcd for C₆₂H₆₆NaO₂₀Si[M+Na]⁺: 1181.38089, found: 1181.38209.



Preparation of 2-67: Compound **2-66** (1.60 g, 1.38 mmol) in CH₂Cl₂ (6 mL) was cooled to 0 °C followed by addition of TFA (10 mL). The mixture was stirred for 2 h and the solvent was removed *in vacuo*. The residue was purified by SiO₂ chromatography (EtOAc/hexanes 1:1) to give clear oil (1.20 g, 1.13 mmol). To a

solution of the oil (0.60 g, 0.56 mmol) in CCl_3CN (10 mL) was added K_2O_3 (400 mg), the mixture was stirred for 1 h. K_2O_3 was removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (12 mL) with **C5** (0.35 g, 0.46 mmol) and AW 300 molecular sieves (600 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 18 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*, and purified by SiO_2 column chromatography (EtOAc/hexanes 1:1) to afford **2-67** as a white solid (0.66 g, 79%). ^1H NMR (CDCl_3 , 500 MHz) δ 8.04-7.93 (m, 10 H), 7.61-7.33 (m, 15 H), 5.90 (d, $J = 9.5$ Hz, 1 H), 5.74 (t, $J = 9.5$ Hz, 1 H), 5.36 (dd, $J = 10.5, 8.0$ Hz, 1 H), 5.32-5.29 (m, 2 H), 5.03 (dd, $J = 9.0, 2.5$ Hz, 1 H), 5.02 (dd, $J = 11.0, 3.5$ Hz, 1 H), 4.86 (dt, $J = 10.0, 3.0$ Hz, 1 H), 4.71 (d, $J = 8.5$ Hz, 1 H), 4.57 (d, $J = 7.5$ Hz, 1 H), 4.55 (d, $J = 13$ Hz, 1 H), 4.38 (dd, $J = 12.5, 4.5$ Hz, 1 H), 4.26 (dt, $J = 9.0, 2.5$ Hz, 1 H), 4.17 (t, $J = 10.0$ Hz, 1 H), 3.91 (dd, $J = 9.5, 2.5$ Hz, 1 H), 3.78 (dd, $J = 9.5, 2.5$ Hz, 1 H), 3.73 (d, $J = 7.0$ Hz, 2 H), 3.67 (t, $J = 7.0$ Hz, 1 H), 3.41 (dd, $J = 10.0, 2.5$ Hz, 1 H), 2.67-2.64 (m, 2 H), 2.59-2.53 (m, 3 H), 2.47 (t, $J = 7.5$ Hz, 1 H), 2.35 (dt, $J = 9.0, 6.5$ Hz, 2 H), 2.17 (s, 3 H), 1.96 (s, 3 H), 1.94 (s, 3 H), 1.86 (s, 3 H), 1.78-1.63 (m, 2 H), 1.57-1.47 (m, 2 H), 1.38-1.16 (m, 64 H), 1.05 (dt, $J = 7.5, 7.5$ Hz, 2 H), 0.88 (t, $J = 7.0$ Hz, 3 H), 0.87 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 206.18, 206.02, 172.89, 171.89, 171.74, 171.00, 169.79, 165.92, 165.71, 165.38, 165.33, 164.97, 133.60, 133.53, 130.00, 129.91, 129.82, 129.74, 129.52, 129.50, 129.20, 128.88, 128.77, 128.74, 128.70, 128.61, 100.99, 100.78, 75.76, 73.19, 73.08, 72.69, 72.32, 72.06, 71.17, 69.60,

67.61, 66.91, 62.46, 60.90, 37.85, 37.81, 36.38, 32.10, 29.89, 29.77, 29.72, 29.54, 29.49, 29.31, 28.36, 27.98, 27.87, 25.72, 25.50, 22.87, 21.15, 20.67, 14.31; HRMS (ESI) calcd for C₁₀₃H₁₄₁NNaO₂₅[M+Na]⁺: 1814.96849, found: 1814.93025.



Preparation of 2-68: Compound **2-67** (0.66 g, 0.37 mmol) was dissolved in THF (20 mL) and MeOH (2 mL) followed by addition of a mixture of N₂H₄ (0.83 mL, 1M in THF) and AcOH (1.1 mL, 19 mmol). The reaction mixture was stirred for 2 h and the solvent was removed *in vacuo*. The residue was dissolved in CH₂Cl₂ (50 mL) and washed with saturated NaHCO₃ (20 mL x 2) and brine (20 mL). The organic phase was dried with Na₂SO₄ and concentrated *in vacuo*. The residue was purified by SiO₂ column chromatography (EtOAc/hexanes 1:1) to afford **2-68** as a white solid (0.66 g, 79%). ¹H NMR (CDCl₃, 500 MHz) δ 8.05 (d, *J* = 7.0 Hz, 2 H), 8.02 (d, *J* = 7.0 Hz, 2 H), 7.93 (t, *J* = 8.5 Hz, 6 H), 7.58 (t, *J* = 7.5 Hz, 1 H), 7.54 (t, *J* = 7.5 Hz, 1 H), 7.50 (t, *J* = 8.0 Hz, 1 H), 7.46 (t, *J* = 7.5 Hz, 2 H), 7.40-7.35 (m, 6 H), 7.30 (t, *J* = 7.5 Hz, 2 H), 7.25 (t, *J* = 7.5 Hz, 2 H), 5.91 (d, *J* = 9.0 Hz, 1 H), 5.61 (t, *J* = 9.0 Hz, 1 H), 5.40 (t, *J* = 8.5 Hz, 1 H), 5.31 (t, *J* = 9.0 Hz, 1 H), 5.04 (dd, *J* = 8.5, 3.0 Hz, 1 H), 4.87 (dt, *J* = 9.5, 3.0 Hz, 1 H), 4.58 (d, *J* = 7.5 Hz, 1 H), 4.52-4.45 (m, 3 H), 4.25 (t, *J* = 9.0 Hz, 1 H), 4.08 (t, *J* = 9.5 Hz, 1 H), 4.03 (dd, *J* = 11.0, 6.0 Hz, 1 H), 3.89 (brs, 2 H), 3.72 (d, *J* = 9.0 Hz, 2 H), 3.59 (dd, *J* = 11.0, 6.5 Hz, 1 H), 3.52 (t, *J* = 6.5 Hz, 1 H), 3.39 (d, *J* = 6.5 Hz, 1 H), 1.94 (s, 3 H), 1.84 (s, 3 H), 1.78-1.64 (m, 2 H), 1.58-1.48 (m, 2 H),

1.40-1.11 (m, 64 H), 1.05 (dt, $J = 7.5, 7.5$ Hz, 2 H), 0.88 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.90, 170.91, 169.74, 166.15, 166.06, 166.03, 165.33, 133.56, 133.46, 133.36, 130.00, 129.93, 129.82, 129.75, 129.65, 129.54, 129.35, 129.12, 128.71, 128.64, 128.56, 128.45, 101.18, 100.57, 76.07, 73.52, 73.10, 72.98, 72.72, 72.61, 72.05, 68.87, 67.44, 62.74, 62.33, 47.44, 36.30, 32.04, 29.82, 29.70, 29.65, 29.48, 29.42, 29.24, 28.32, 25.65, 25.43, 22.81, 21.05, 20.57, 14.25; HRMS (ESI) calcd for $\text{C}_{93}\text{H}_{129}\text{NNaO}_{21}[\text{M}+\text{Na}]^+$: 1618.89493, found: 1618.87950.



Preparation of 2-69: As described in the preparation of **2-51**, glycosylation of **2-68** (30 mg, 0.018 mmol) with **2-21** (12 mg, 0.022 mmol) afforded **2-69** as a clear oil (34 mg, 85%). ^1H NMR (CDCl_3 , 500 MHz) δ 8.10 (d, $J = 6.5$ Hz, 2 H), 8.05 (d, $J = 7.0$ Hz, 2 H), 8.00 (d, $J = 7.5$ Hz, 2 H), 7.97 (d, $J = 7.0$ Hz, 2 H), 7.93 (d, $J = 6.5$ Hz, 2 H), 7.67-7.10 (m, 33 H), 7.01 (d, $J = 7.0$ Hz, 2 H), 5.86 (d, $J = 9.0$ Hz, 1 H), 5.74 (t, $J = 9.5$ Hz, 1 H), 5.41 (t, $J = 8.5$ Hz, 1 H), 5.31 (t, $J = 9.0$ Hz, 1 H), 5.03 (dd, $J = 8.5, 3.0$ Hz, 1 H), 4.87 (dt, $J = 10.0, 2.5$ Hz, 1 H), 4.80 (d, $J = 12.0$ Hz, 1 H), 4.50 (d, $J = 11.5$ Hz, 1 H), 4.68 (s, 2 H), 4.63-4.53 (m, 6 H), 4.44 (dd, $J = 9.0, 3.0$ Hz, 1 H), 4.37 (d, $J = 11.0$ Hz, 1 H), 4.26-4.23 (m, 2 H), 4.15 (t, $J = 9.5$ Hz, 1 H), 3.92-3.71 (m, 11 H), 3.51 (t, $J = 6.0$ Hz, 1 H), 3.39-3.36 (m, 1 H), 3.23 (t, $J = 9.0$ Hz, 1 H), 2.76 (dd, $J = 7.0, 6.0$ Hz, 1 H), 1.95 (s, 3 H), 1.84 (s, 3 H), 1.80-1.65 (m, 2 H), 1.59-1.43 (m, 2 H), 1.39-1.15 (m, 64 H), 1.05 (dt, $J = 7.5, 7.5$ Hz, 2 H), 0.89 (t, $J = 7.0$ Hz, 3 H), 0.88 (t, $J = 7.0$ Hz, 3 H).

= 7.0 Hz, 3 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.90, 170.97, 169.79, 166.30, 166.05, 165.67, 165.44, 165.04, 138.73, 138.62, 138.04, 137.64, 133.62, 133.51, 133.29, 133.19, 130.14, 130.02, 129.99, 129.78, 129.70, 129.60, 129.44, 129.28, 128.90, 128.77, 128.72, 128.58, 128.38, 128.29, 128.18, 127.72, 127.64, 127.50, 100.87, 100.87, 96.76, 79.72, 78.69, 75.57, 75.18, 75.00, 74.84, 74.40, 73.74, 73.32, 73.20, 73.10, 72.89, 72.72, 72.48, 72.40, 72.26, 70.95, 69.82, 67.87, 67.60, 65.40, 63.32, 62.72, 47.49, 36.42, 32.13, 29.92, 29.80, 29.74, 29.58, 29.53, 29.34, 28.52, 25.76, 25.52, 22.91, 21.16, 20.67, 14.34; HRMS (ESI) calcd for $\text{C}_{127}\text{H}_{163}\text{NNaO}_{26}[\text{M}+\text{Na}]^+$: 2136.18106, found: 2136.18591.



Preparation of iGb3': Compound **2-69** (34 mg, 0.016 mmol) was dissolved in THF (5 mL) and MeOH (5 mL) and transferred to a hydrogenation vessel. Palladium on carbon (10%, 50 mg) was added to the vessel, and the vessel was subjected to H_2 (300 psi) at room temperature for 18 h. The catalyst was then removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in THF). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was purified by SiO_2 column chromatography (MeOH/ CH_2Cl_2 / H_2O 20:80:3) to afford **iGb3'** (14 mg, 75%) as a white solid. ^1H NMR ($\text{DMSO}-d_6/\text{D}_2\text{O}$, 500 MHz) δ 4.82 (d, $J = 3.5$ Hz, 1 H), 4.27 (d, $J = 7.5$ Hz, 1 H), 4.17 (d, $J = 8.0$ Hz, 1

H), 3.97 (t, $J = 7.0$ Hz, 1 H), 3.92-3.87 (m, 2 H), 3.84 (d, $J = 3.0$ Hz, 1 H), 3.74-3.72 (m, 2 H), 3.63-3.28 (m, 16 H), 3.01 (t, $J = 8.0$ Hz, 1 H), 2.07-2.01 (m, 2 H), 1.52-1.34 (m, 4 H), 1.26-1.11 (m, 64 H), 0.84 (t, $J = 7.0$ Hz, 6 H); ^{13}C NMR (DMSO- d_6 , 125 MHz) δ 171.98, 103.54, 103.45, 96.29, 80.68, 78.63, 74.92, 74.80, 74.61, 73.48, 73.26, 70.94, 70.57, 69.79, 69.52, 69.51, 68.93, 68.65, 68.43, 64.34, 60.26, 60.25, 60.04, 50.20, 35.56, 31.32, 30.78, 29.15, 29.01, 28.78, 28.73, 25.54, 25.46, 22.13, 13.99; HRMS (ESI) calcd for $\text{C}_{60}\text{H}_{115}\text{NNaO}_{19}[\text{M}+\text{Na}]^+$: 1176.79555, found: 1176.79823.

Chapter 3: Synthesis and Evaluation of Stimulatory Properties of Glycosphingolipids from *Sphingomonadaceae* Family

3.1. Introduction

Innate immunity prevents a wide array of infections from bacterial, fungal, viral, and protozoan microorganisms by recognition of microbial molecular patterns, structural components or motifs that indicate the presence of microbes. For example, the lipopolysaccharide (LPS) that comprises the outer leaflet of the outer membrane of Gram-negative bacteria is not produced by human cells and its presence triggers an innate immune response characterized by cytokine production and immune system activation. However, some species of Gram-negative bacteria do not produce LPS, which leads to the question: does the innate immune system survey for the presence of molecular patterns from these bacteria? Studying the outer membrane components of these Gram-negative, LPS-negative bacteria would be key to exploring the mechanism of innate immunity against these bacteria.

Among these Gram-negative, LPS-negative bacteria, the *Sphingomonadaceae* family produces glycosphingolipids in the cell wall. Kawahara *et al.* reported two novel glycosphingolipids isolated from *Sphingomonas paucimobilis*.¹ The glycosyl portion of the glycosphingolipids consists of a tetrasaccharide or monosaccharide and the hydrophobic residue is the ceramide. Further research designates these two molecular as GSL-1 and GSL-4A.² These glycosphingolipids localize at the cell

heat-killed *Sphingomonas* spp. and GSL-1 from *Sphingomonas* spp. are potent stimulators of NKT cells.⁴⁻⁶ In addition, it has been reported that GSL-4A isolated from bacteria is able to stimulate NKT cells.⁶

As described in Chapter 2, the variants of α -GalCer such as diglycosylceramides stimulate NKT cells only if they are truncated by glycosidases to give α -GalCer. Glycolipids are usually transported to lysosomes in APCs, and exposed to a collection of glycosidases. Therefore, it would be expected that GSL-3 and GSL-4A would have to be truncated to GSL-1 to stimulate NKT cells. To evaluate the stimulatory properties of GSLs, we developed efficient synthetic procedures to prepare GSL-1, -2, -3 and -4 (Figure 2). GSL-2 was prepared to determine the extent to which the carbohydrates of the GSLs are recognized by NKT cells.



Figure 2. Structures of synthetic GSLs.

3.2. Synthesis of GSLs

To prepare the GSLs, we initially designed a convergent synthesis which included

the installment of other saccharides on GSL-1. Unfortunately, glucuronic acid is a poor glycosyl donor and acceptor, and its reactivity influenced the synthesis of all of the GSLs.

3.2.1. Synthesis of GSL-1

The synthesis of GSL-1 started from sphingosine and thioglycoside **3-1**⁷ (Scheme 1). Selective protection of the primary alcohol of **3-1** with bulky trityl ether gave compound **3-2**. Installation of PMB ether to the remaining alcohol followed by removal of the trityl ether generated **3-3**. The primary alcohol in compound **3-3** was oxidized to the glucuronic acid and converted to the methyl ester, affording **3-4**. We found the PMB group at C4 complicated later steps in the synthesis. Therefore, the PMB ether was replaced by a TBS ether at this stage. We also tried installment of a TBS ether in the very beginning, but the TBS ether did not completely withstand the oxidation conditions. Glycosylation of **3-5** with azide **3-6** generated **3-7** as a mixture of anomers in 67% yield. The poor glycosyl donor property of **3-5** made it necessary to use azide **3-6**, which is a better acceptor than the preformed ceramide. The anomers were separable only after the azide was reduced to the amine using H₂S and coupled with **3-8** (giving **3-9**). Removal of TBS ether afforded compound **3-10** which was used as an intermediate in the syntheses of GSL-2, -3 and -4. Deprotection of **3-10** generated GSL-1 directly.

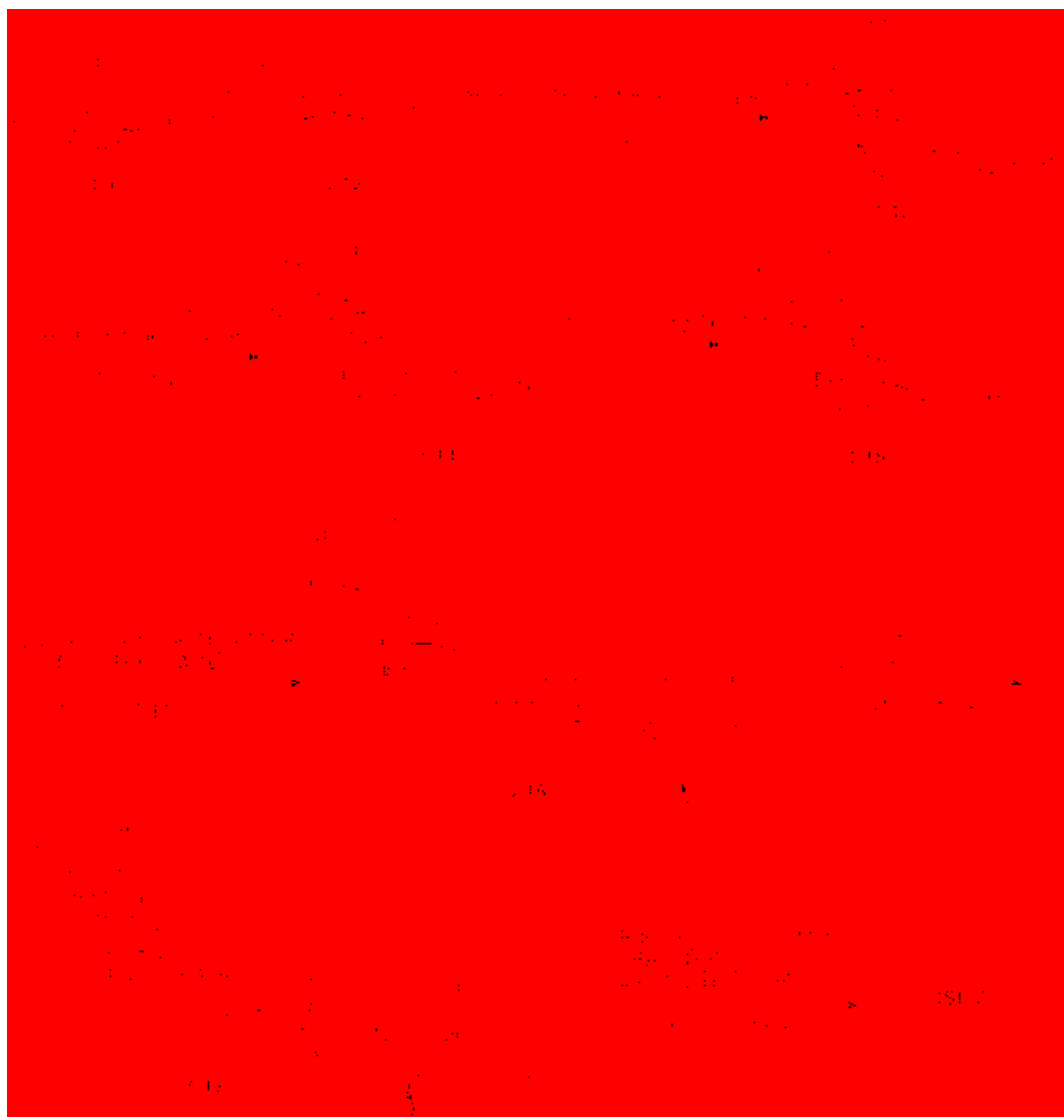


Scheme 1. Synthesis of GSL-1

3.2.2. Synthesis of GSL-3

Synthesis of GSL-3 started with glycosylation of galactoside **3-11**⁸ with azide **3-12**⁹, giving **3-13** (Scheme 2). The newly formed glycosidic bond was generated exclusively in α conformation due to the armed protecting PMB group. Because of the instability of the PMB ether under many conditions, such as acidic environment, the PMB ether was converted to acetate to give **3-14**. The acetate on C2 of galactose was designed for further reaction with mannose to generate GSI-4. Treatment of **3-14** with HF afforded donor **3-15** which was ready to couple with acceptor **3-10**. The

coupling reaction succeeded using Ph₂SO/Tf₂O mediated glycosylation in relatively good yield with good anomeric selectivity.¹⁰ Deprotection of **3-16** was the necessary. Reduction of the azide and deprotection of the resulting material proved problematic: yields were low and it was difficult to recover the glycolipid from the hydrogenation catalyst. Therefore, it was necessary to reduce the azide selectively and to protect the resulting amine as a *t*-butyloxy carbamate (Boc) (yielding **3-17**) to avoid material losses late in the synthesis. Removal of the Boc group, occurred without incident, and final cleavage of the esters gave GSL-3.



Scheme 2. Synthesis of GSL-3

3.2.3. Synthesis of GSL-4

Synthesis of GSL-4 was more difficult than expected. Originally, precursor **3-17** was selected as an acceptor to make GSL-4. Selective deprotection of acetate in **3-17** with NaOMe gave **3-18**. Glycosylation of **3-18** with mannoside **3-19** or **3-20** using different methods failed possibly due to the low nucleophilicity of the hindered alcohol in **3-18**. The mannoside needed to be installed earlier in the synthesis of GSL-4.



Scheme 3. First strategy to make GSL-4



Scheme 4. The 2nd strategy to make GSL-4



Scheme 6. Preparation of donor **3-29**



Scheme 7. Preparation of GSL-4 form **3-29**

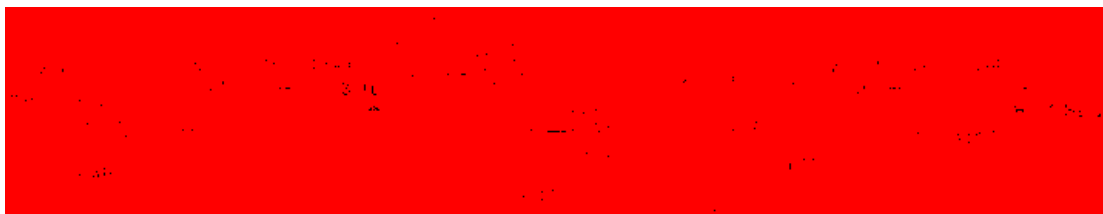
Glycosylation of **3-10** with azide **3-30** afforded **3-31** in good yields. To avoid the

loss of materials in later steps, we reduced the azide and protected the result amine, giving **3-32**. The TBDPS ether of **3-32** was cleaved under mild conditions to give acceptor **3-33**. With both donor **3-29** and acceptor **3-33** in hand, the glycosylation was succeeded with a satisfying yield and anomeric selectivity (only a trace of the β -anomer was detected). To facilitate removal of the β -anomer, the benzyl groups of **3-34** was removed and the result alcohol was peracylated to give **3-35**, which was amenable to purification via chromatography. Full deprotection of **3-35** produced GSL-4 in a good yield.

To verify the structure of synthetic GSL-4, we isolated GSL-4A from *Sphingomonas paucimobilis*. Although the structure of GSL-4A has been investigated spectroscopically, the comparison of synthetic GSL-4 to isolated material provided additional evidence in favor of this proposed structure. As shown in Figure 1, GSL-4A contains three different ceramides including a longer-chained (C21) sphinganine with a double bond. Resonances from this double bond appeared as minor resonances in the proton NMR spectrum of isolated GSL-4A. Neglecting these minor double bond resonances, the proton NMR spectra of isolated GSL-4A and synthetic GSL-4 are indistinguishable (see experiment figure). The chemical shifts of the protons (usually from 5.5 to 4.8 ppm) on anomeric carbons are characteristic of the configuration and identity of the sugar. Our spectra showed the resonances and chemical shifts of these protons were identical. The carbon spectra of both were indistinguishable as well.

3.2.4. Synthesis of GSL-2

We prepared GSL-2 to determine the extent to which the carbohydrates of the GSLs are recognized by NKT cells. As shown in preparation of GSL-4, GSL-2 was prepared in a similar fashion.



Scheme 7. Preparation of GSL-2

3.3. Results and Discussion

GSLs were evaluated for their stimulatory abilities to NKT cells by using a mouse hybridoma, a human NKT cell line, CD1d-restricted B6 spleen cells and NKT cells isolated from human plasma.^{5,11} APCs were present as well, thus the glycolipids were exposed to the glycosidases present in the lysosomes of these cells (Figure 4). Each of the experiments was run in triplicate.

The stimulatory properties of the GSLs were compared to those of PBS57¹² (Figure 3), a surrogate for KRN7000, and iGb3¹³. PBS57 cause significant stimulation by using



both mouse NKT cell hybridoma (DN32.D3) and human NKT cells, while iGb3 cause modest stimulation when dendritic cells were used as APCs (Figure 4 and 5).

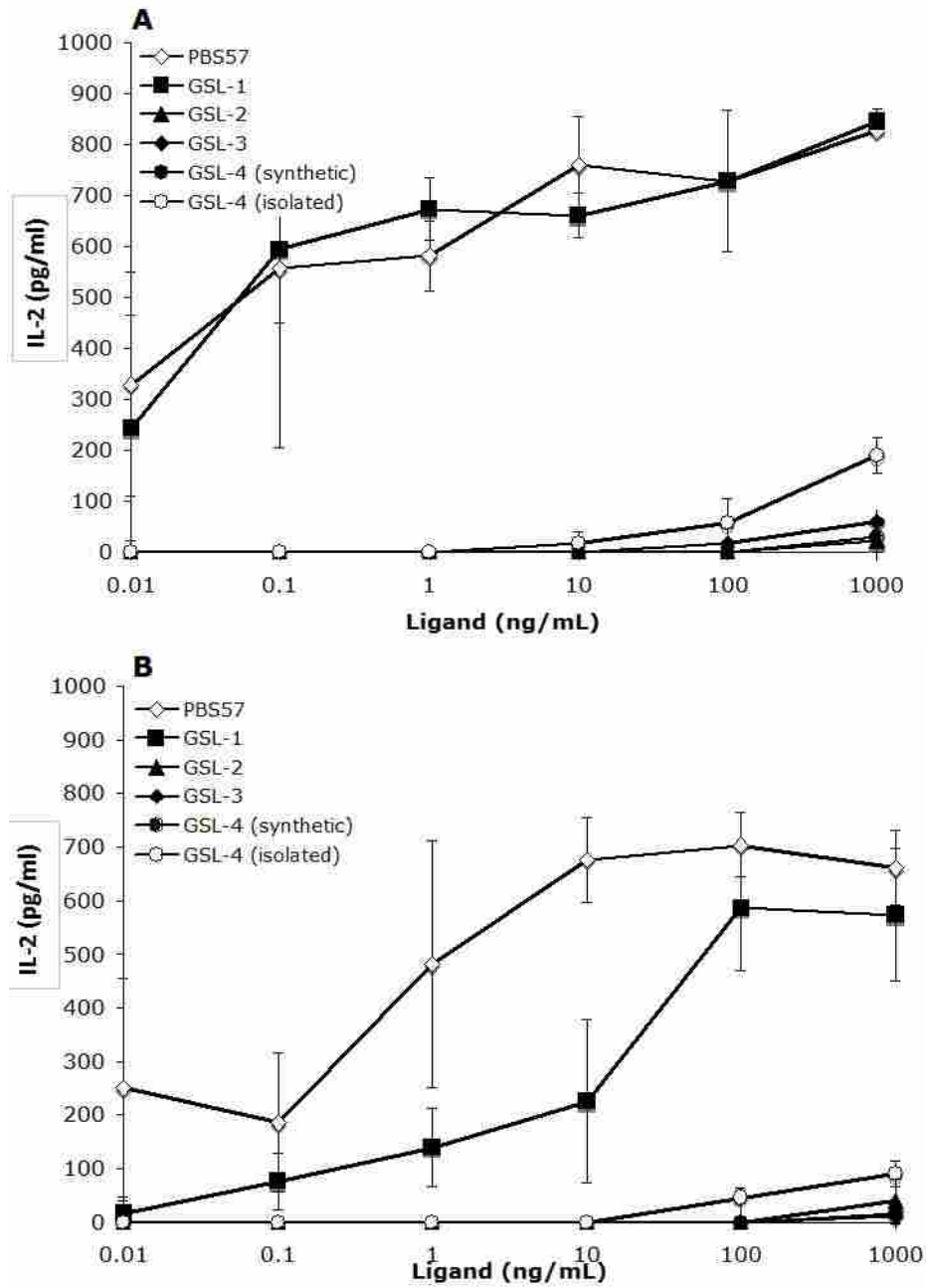


Figure 4. Stimulation of DN32.D3 cells (mouse NKT cell hybridomas) with the indicated glycolipids. **A:** Dendritic cells as antigen present cells. **B:** Macrophages as antigen presenting cells.

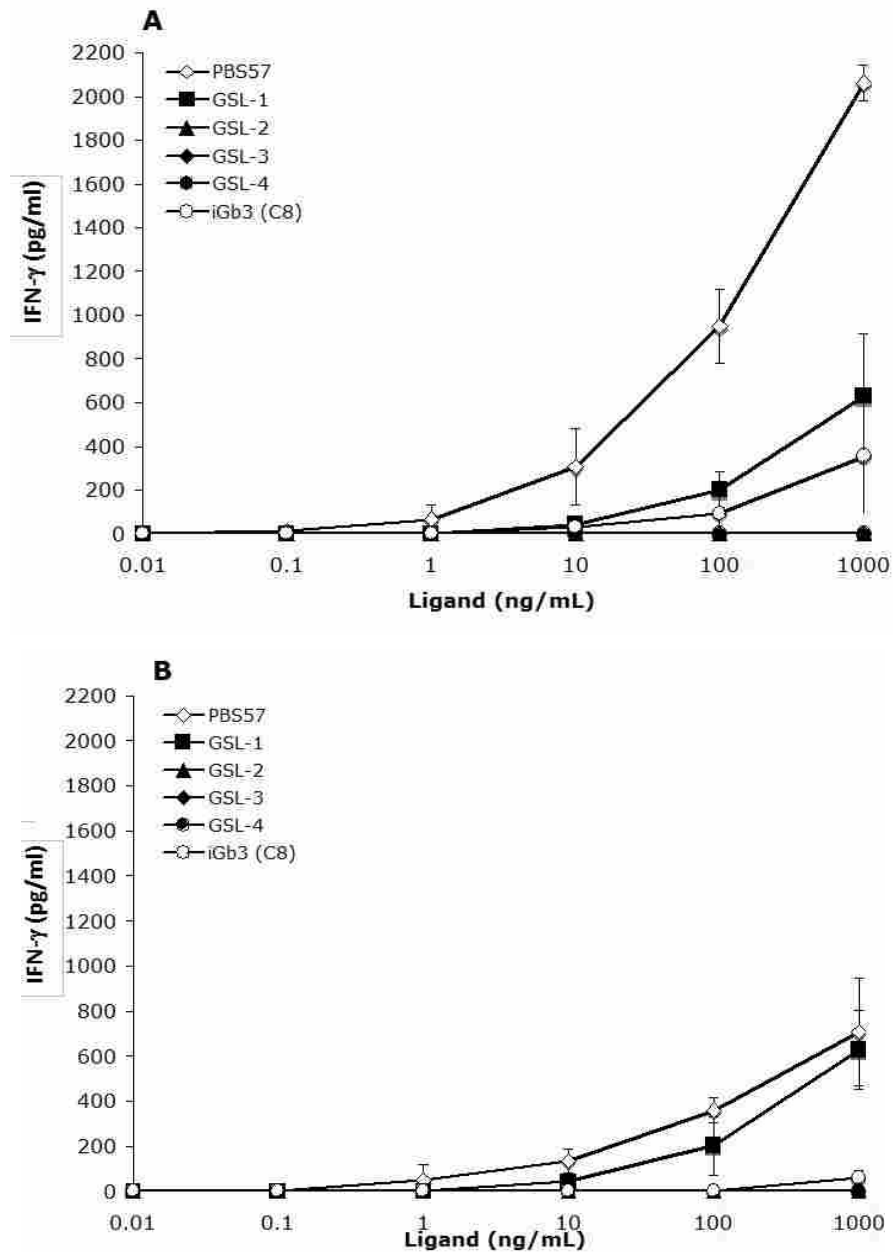


Figure 5. Stimulation of human NKT cells with the indicated glycolipids. **A:** Dendritic cells as antigen presenting cells. **B:** PBLs as antigen presenting cells.

In initial experiments with isolated GSL-4, low stimulation of cytokine production was observed at high concentrations of the GSL-4 (~ 100 ng/ml). Careful purification of this glycolipid yielded GSL-4 in pure form that stimulated only at higher concentrations. Because GSL-1 is difficult to separate completely from GSL-4, the stimulation could be the result of the contamination of GSL-1 in isolated GSL-4 which was confirmed by that synthetic GSL-4 does not stimulate the mouse NKT cell

hybridoma (Figure 4). Synthetic GSL-3 and GSL-2 are non-stimulatory as well. Macrophages and dendritic cells were used to verify that the result was not APC dependent. In all experiments with GSLs, only GSL-1 shows stimulatory ability with NKT cells using dendritic cells, and peripheral blood lymphocytes (PBLs) as APCs (Figure 5). With dendritic cells as APCs, GSL-1 and iGb3 showed modest stimulation; with PBLs as APCs, GSL-1 was nearly as potent as PBS57. Similar results were observed when splenocytes from B6 mice and NKT cells isolated from human plasma were used. GSL-3 and GSL-4 did not stimulate, but GSL-1 caused IFN- γ and IL-4 release.

If GSL-2, -3 and -4 were truncated to GSL-1 in lysosome of APCs, the generated GSL-1 would stimulate NKT cells to produce cytokines. There are many different types of lysosomal glycosidases in APCs, such as α -mannosidase¹⁴ and α -glucosidase A¹⁵. These glycosidases should truncate GSL-4 to GSL-3 and GSL-3 to GSL-2. However, GSL-2 is not truncated to GSL-1, which suggests that the glycosidase necessary to cleave glucosamine from GSL-2 might be absent or inactive in lysosomes. In higher organisms, amino sugars are generally acylated at nitrogen. Moreover, lysosomes contain an acyltransfer enzyme that acylates the amines of the terminal α -glucosamine in heparin¹⁶. For some reason, the acylation does not happen with the amine group in GSL-2 (and GSL-3 and -4), therefore GSL-2 cannot be truncated to GSL-1. It is possible that GSL-2 is not an effective substrate for acyltransfer enzymes.

To verify that glucosamine cannot be acylated and subsequently cleaved from

glycosylceramides in lysosomes, we prepared the GSL-KRN7000 hybrids: α -GlcNH₂-(1-4)- α -GalCer and α -GlcNAc-(1-4)- α -GalCer (Figure 6). Like GSL-2, α -GlcNH₂-(1-4)- α -GalCer contains glucosamine. The control compound, α -GlcNAc-(1-4)- α -GalCer, would be the product of acylation of α -GlcNH₂-(1-4)- α -GalCer. As discussed in Chap. 2, diglycosylceramides, such as α -Gal-(1-4)- α -GalCer and β -GalNAc-(1-4)- α -GalCer, can be truncated to α -GalCer and stimulate NKT cells. If these two glycosylceramides can be truncated, the resulting α -GalCer should give a strong stimulation of NKT cells. The experiments showed α -GlcNAc-(1-4)- α -GalCer is stimulatory, whereas α -GlcNH₂-(1-4)- α -GalCer is non-stimulatory. It is suggested that the lysosomal α -N-acetylglucosaminidase could cleave the N-acetylglucosamine from α -GlcNAc-(1-4)- α -GalCer to yield α -GalCer. The result that α -GlcNH₂-(1-4)- α -GalCer does not stimulate provided evidence of the inability of the APC to acylate and cleave glucosamine from GSL-2.



Figure 6. Structures of GSL-KRN7000 hybrids

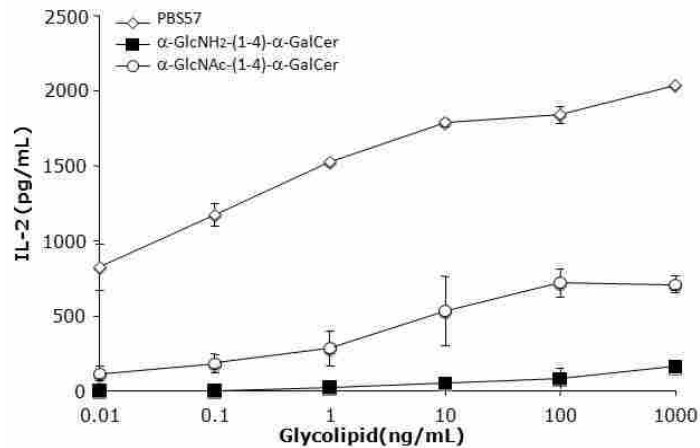


Figure 7. Stimulation of DN32.D3 cells with dendritic cells as antigen present cells.

The NKT cell system provides a mechanism for surveillance for bacterial glycolipids. Our observation that, the GSL-1 is a potent stimulator and other GSLs are not stimulator of NKT cells, suggests that synthesis of these higher-order GSLs might be a mechanism of immune evasion.

Recently, Kinjo *et al.* reported the synthesized GSL-4A was a much weaker antigen than GSL-1 using either unfixed APC that expressing a CD1d mutant for lysosomal trafficking or CD1d-coated plates without APC.¹⁷ For example, the lowest dose of GSL-4A without processing induced IL-2 release much less than 16-fold less GSL-1 (125 ng/well) in the CD1d-coated plate assay. By contrast, our result showed GSL-4 is non-stimulatory. To explain the weak activity of GSL-4A, they reasoned that the CD1d-coated plate assay is highly sensitive, and they used A20 B cell transfectants expressing very high amounts of CD1d. However, the weak stimulatory property of GSL-4A might be the result of trace amounts of contaminating GSL with a monosaccharide head group.

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Experimental Procedures:



Preparation of 3-2: To a solution of **3-1** (4.61 g, 10.2 mmol) in pyridine (30 mL) was added trityl chloride (3.41 g, 12.2 mmol) and DMAP (0.06 g, 0.51 mmol). The mixture was warmed to 60 °C and stirred overnight. After the starting material was completely consumed (by TLC), the solvent was removed on rotavapor. The residue obtained was further dissolved in CH₂Cl₂ (400 mL), washed with H₂O, saturated aqueous NaHCO₃ and brine. The organic layer was further dried over anhydrous Na₂SO₄, followed by filtration and evaporation. The crude mixture was then subject to column chromatography (1:3, EtOAc/hexane) to afford **3-2** as a whit solid (5.85g, 83%). ¹H NMR (CDCl₃, 500 MHz) δ 7.64 (m, 30 H), 4.91 (d, *J* = 10.5 Hz, 1 H), 4.87 (d, *J* = 11.5 Hz, 1 H), 4.75 (d, *J* = 11.5 Hz, 1 H), 4.73 (d, *J* = 10.5 Hz, 1 H), 4.67 (d, *J* = 9.0 Hz, 1 H), 3.66-3.22 (m, 1 H), 3.52-3.47 (m, 2 H), 3.45 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.38-3.32 (m, 2 H), 2.23 (d, *J* = 2.5 Hz, 1 H); ¹³C NMR (CDCl₃, 125 MHz) δ 144.03, 138.62, 138.26, 133.99, 132.25, 129.21, 128.95, 128.86, 128.72, 128.53, 128.27, 128.16, 127.74, 87.69, 86.49, 80.65, 78.55, 75.85, 75.61, 71.55, 63.97; HRMS (ESI) calcd for C₄₅H₄₂NaO₅S [M+Na]⁺: 717.26452, found: 717.26401.



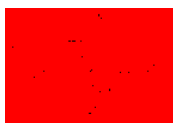
Preparation of 3-3: To a solution of **3-2** (5.85 g, 8.43 mmol) in DMF (30 mL), was added NaH (0.51 g, 12.6 mmol, 60% dispersion in mineral oil) and TBAI (0.05 g,

0.14 mmol) at 0 °C. The mixture was stirred for 30 min followed by addition of PMBCl (1.58 g, 10.1 mmol). The reaction mixture was then warmed to room temperature and stirred for another 4 h. After MeOH (1 mL) was added, the reaction mixture was filtered and concentrated. The residue was further dissolved in CH₂Cl₂, washed with brine. The organic phase was dried over NaHSO₄ and filtered through a celite pad. The filtrate was concentrated, and the residue was purified by flash column chromatography (1:3, EtOAc/hexane) to afford **3-3** as a white solid (3.75 g, 75%). ¹H NMR (CDCl₃, 500 MHz) δ 7.52-6.83 (m, 19 H), 4.91 (d, *J* = 10.5 Hz, 1 H), 4.90 (d, *J* = 10.5 Hz, 1 H), 4.88 (d, *J* = 10.5 Hz, 1 H), 4.76 (d, *J* = 10.5 Hz, 2 H), 4.70 (d, *J* = 10.0 Hz, 1 H), 4.58 (d, *J* = 11.0 Hz, 1 H), 3.85 (dd, *J* = 12.0, 2.5 Hz, 1 H), 3.81 (t, *J* = 10.0 Hz, 1 H), 3.77 (s, 3 H), 3.67 (dd, *J* = 12.0, 5.0 Hz, 1 H), 3.56 (t, *J* = 9.5 Hz, 1 H), 3.48 (t, *J* = 10.0 Hz, 1 H), 3.36 (m, 1 H), 1.99 (brs, 1 H); ¹³C NMR (CDCl₃, 125 MHz) δ 159.71, 138.64, 138.16, 133.78, 132.09, 130.24, 130.06, 129.33, 128.76, 128.72, 128.50, 128.19, 128.03, 127.94, 114.21, 87.81, 86.85, 81.37, 79.62, 77.58, 76.08, 75.83, 75.07, 62.43, 55.54; HRMS (ESI) calcd for C₃₄H₃₆NaO₆S [M+Na]⁺: 595.21301, found: 595.21316.



Preparation of 3-4: A mixture of **3-3** (640 mg, 1.12 mmol), TEMPO (35 mg, 0.22 mmol) and BAIB (900 mg, 2.79 mmol) was dissolved in dichloromethane (10 mL) and water (5 mL). The reaction was stirred at room temperature for 8 h. After dilution with dichloromethane (50 mL), the reaction mixture was washed with H₂O (2 x 30

mL) and dried over Na₂SO₄. After filtration, the solvent was evaporated. The crude acid was then dissolved in dichloromethane (10 mL) and a solution of diazomethane in diethyl ether (freshly prepared) was added in small portions until acid was totally esterified (monitored by TLC). Acetic acid (2 ml) was added. The reaction mixture was concentrated, and the residue was purified by SiO₂ column chromatography (1:3, EtOAc/hexane) to afford **3-4** as white solid (420 mg, 63%). ¹H NMR (CDCl₃, 500 MHz) δ 7.59-6.86 (m, 19 H), 4.91 (d, *J* = 10.0 Hz, 1 H), 4.90 (s, 2 H), 4.75 (d, *J* = 12.0 Hz, 1 H), 4.73 (d, *J* = 9.5 Hz, 1 H), 4.71 (d, *J* = 10.0 Hz, 1 H), 4.57 (d, *J* = 10.0 Hz, 1 H), 3.94 (d, *J* = 9.5 Hz, 1 H), 3.86 (t, *J* = 9.5 Hz, 1 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 3.73 (t, *J* = 9.5 Hz, 1 H), 3.55 (t, *J* = 9.5 Hz, 1 H); ¹³C NMR (CDCl₃, 125 MHz) δ 168.99, 159.66, 138.47, 138.11, 133.46, 132.47, 130.11, 129.97, 129.32, 128.76, 128.72, 128.48, 128.21, 128.09, 128.05, 114.11, 88.59, 86.15, 80.55, 79.25, 78.28, 76.14, 75.79, 75.05, 55.54, 52.81; HRMS (ESI) calcd for C₃₅H₄₀NO₇S [M+NH₄]⁺: 618.25200, found: 618.25143.



Preparation of 3-5: To a solution of **3-4** (0.40 g, 0.73 mmol) and DDQ (0.214 g, 0.94 mmol) in CH₂Cl₂ (10 mL) was added H₂O (2 mL). The mixture was stirred at room temperature for 4 h, then diluted with dichloromethane (50 mL), washed with H₂O (3 x 30 mL) and dried over Na₂SO₄. After filtration and evaporation, the crude mixture was purified by column chromatography (EtOAc/hexane = 1/3) to afford the corresponding alcohol as a white solid (0.21 g, 72%). ¹H NMR (CDCl₃, 500 MHz) δ

7.58-7.28 (m, 15 H), 4.89 (d, $J = 11.0$ Hz, 1 H), 4.88 (s, 2 H), 4.76 (d, $J = 11.0$ Hz, 1 H), 4.70 (d, $J = 10.0$ Hz, 1 H), 3.91 (t, $J = 10.0$ Hz, 1 H), 3.85 (d, $J = 9.5$ Hz, 1 H), 3.84 (s, 3 H), 3.60 (t, $J = 9.5$ Hz, 1 H), 3.49 (t, $J = 9.5$ Hz, 1 H), 2.91 (bs, 1 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 169.82, 138.53, 138.08, 133.46, 132.50, 129.26, 128.80, 128.69, 128.48, 128.25, 128.18, 128.12, 88.68, 85.46, 79.98, 77.76, 75.91, 75.80, 72.09, 53.08; HRMS (ESI) calcd for $\text{C}_{27}\text{H}_{32}\text{NO}_6\text{S}$ $[\text{M}+\text{NH}_4]^+$: 498.1945, found: 498.1949. To a solution of the alcohol formed above (0.24 g, 0.49 mmol) in DMF (20 mL) was added imidazole (0.06 g, 0.90 mmol) and *tert*-butyldimethylsilyl chloride (0.21 g, 0.75 mmol) in sequence. The reaction was warmed to 100 and allowed to stir overnight. The solvent was removed, and the residue was dissolved in EtOAc (100 mL). The solution was washed with saturated aqueous NaHCO_3 and dried over Na_2SO_4 . After filtration and evaporation, the crude mixture was purified by column chromatography (1:3, EtOAc/hexane) to afford **3-5** as a colorless oil (0.23 g, 84%). ^1H NMR (CDCl_3 , 500 MHz) δ 7.58-7.26 (m, 15 H), 5.00 (d, $J = 12.0$ Hz, 1 H), 4.91 (d, $J = 10.0$ Hz, 1 H), 4.80 (d, $J = 12.0$ Hz, 1 H), 4.75 (d, $J = 9.0$ Hz, 1 H), 4.63 (d, $J = 10.0$ Hz, 1 H), 3.98 (t, $J = 9.0$ Hz, 1 H), 3.90 (d, $J = 9.0$ Hz, 1 H), 3.80 (s, 3 H), 3.58 (t, $J = 9.0$ Hz, 1 H), 3.54 (d, $J = 9.0$ Hz, 1 H), 0.86 (s, 9 H), 0.02 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 168.89, 138.77, 137.98, 133.82, 132.21, 129.29, 128.60, 128.47, 128.44, 128.10, 127.93, 127.48, 126.97, 88.63, 86.23, 81.00, 80.39, 75.49, 75.37, 72.25, 52.59, 25.97, 18.14, -3.75; HRMS (ESI) calcd for $\text{C}_{33}\text{H}_{44}\text{NO}_6\text{SSi}$ $[\text{M}+\text{NH}_4]^+$: 612.28096, found: 612.28134.

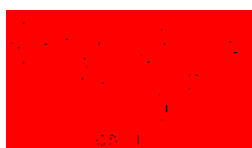


Preparation of 3-9: A mixture of donor **3-5** (0.17 g, 0.28 mmol), **3-6** (0.09 g, 0.23 mmol), DTBMP (0.14 g, 0.69 mmol) was dissolved in dichloromethane (5 mL). The solution was stirred with 4 Å MS (150 mg) at room temperature. After 30 min, freshly prepared promoter DMTST (0.15 g, 0.58 mmol) was added (methyl disulfide (307 mg, 3.21 mmol) and MeOTf (533 mg, 3.21 mmol) were mixed in a vial at 0 °C to form DMTST as uniform white solid). The reaction was stirred overnight under N₂. After the acceptor was completely consumed, Et₃N (1 mL) was added to quench the reaction. The mixture was filtered through silica gel pad. The filtrate was concentrated, and the residue was purified by flash column chromatography, affording **3-7** as a light yellow oil (150 mg). HRMS (ESI) calcd for C₅₂H₈₁N₄O₈Si [M+NH₄]⁺: 917.58182, found: 917.58649. Compound **3-7** (150 mg, 0.165 mmol) was dissolved in a mixture of dry pyridine (7.5 mL) and Et₃N (1.32 mL). To this solution was introduced H₂S over 15 min at 0 °C. After the solution became dark blue, H₂S stream was stopped; the reaction was allowed to continue at 0 °C for 12 h. Then H₂S was introduced into the solution over again at 0 °C for another 15 min. The solution was stirred for additional 12 h. Thin layer chromatography showed that the starting material was completely consumed; the solvent was subsequently removed in vacuo. The remaining crude product was purified using flash silica gel plug. The polar fractions were collected and concentrated. The resulting amine was dissolved in dry dichloromethane (10 mL). And to this solution was added a solution of **3-8** in

dichloromethane (3 mL) over 10 min at 0°C. The reaction mixture was stirred at room temperature for another 3 h. The resulting solution was diluted with dichloromethane (20 mL), and washed with H₂O (50 mL), concentrated in vacuo. The residue was subject to SiO₂ column chromatography (1:3, EtOAc/hexane) to afford the α -anomer **3-9** as a colorless oil (45 mg, 23%) with the β -anomer as a colorless oil (37.5 mg, 20%). α -anomer: ¹H NMR (CDCl₃, 500 MHz) δ 7.34-7.16 (m, 15 H), 6.39 (d, *J* = 10.0 Hz, 1 H), 5.62 (tt, *J* = 7.0, 15.5 Hz, 1 H), 5.37 (dd, *J* = 8.5, 15.5 Hz, 1 H), 5.19 (dd, *J* = 4.5, 8.5 Hz, 1 H), 5.03 (d, *J* = 11.5 Hz, 1 H), 4.83 (d, *J* = 3.5 Hz, 1 H), 4.70 (d, *J* = 11.5 Hz, 1 H), 4.55 (d, *J* = 12.0 Hz, 1 H), 4.54 (d, *J* = 11.5 Hz, 1 H), 4.49 (d, *J* = 12.0 Hz, 1 H), 4.31-4.28 (m, 1 H), 4.20 (d, *J* = 11.5 Hz, 1 H), 4.08 (d, *J* = 9.5 Hz, 1 H), 3.92 (t, *J* = 8.5 Hz, 1 H), 3.87 (t, *J* = 9.0 Hz, 1 H), 3.80 (d, *J* = 3.5 Hz, 2 H), 3.74 (s, 3 H), 3.69 (t, *J* = 9.0 Hz, 1 H), 3.59 (dd, *J* = 3.5, 9.0 Hz, 1 H), 2.07-2.01 (m, 2 H), 1.95-1.88 (m, 1 H), 1.86-1.78 (m, 1 H), 1.37-1.22 (m, 51 H), 0.88 (t, *J* = 7.0 Hz, 6 H), 0.83 (s, 9 H), -0.16 (s, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 176.74, 170.13, 170.01, 138.98, 138.66, 138.06, 128.62, 128.57, 128.41, 128.06, 127.96, 127.69, 127.55, 127.45, 127.21, 126.98, 98.07, 81.37, 80.17, 79.72, 75.24, 73.75, 72.98, 72.63, 72.49, 70.18, 67.09, 52.58, 51.69, 39.03, 32.49, 32.28, 32.17, 29.95, 29.92, 29.88, 29.74, 29.62, 29.43, 29.37, 27.51, 25.98, 25.17, 22.94, 18.17, 14.37, -3.71; HRMS (ESI) calcd for C₇₁H₁₁₄NO₁₁Si [M+H]⁺: 1184.81557, found: 1184.81604.



Preparation of 3-10: To a solution of **3-8** (14 mg, 0.012 mmol) in dichloromethane (0.5 mL) was added CH₃CN (5 mL) and aqueous HF (48%, 1 mL). The mixture was stirred at room temperature for 5 h, then diluted with dichloromethane (20 mL), washed with saturated NaHCO₃, and dried over Na₂SO₄. The resulting solution was concentrated, and the residue was purified by flash column chromatography (1:3, EtOAc/hexane, R_f = 0.35) to afford **3-10** as an oil (11 mg, 87%). ¹H NMR (CDCl₃, 500 MHz) δ 7.38-7.22 (m, 15 H), 6.35 (d, *J* = 9.5 Hz, 1 H), 5.61 (tt, *J* = 6.5, 15.5 Hz, 1 H), 5.36 (dd, *J* = 8.5, 15.5 Hz, 1 H), 5.07 (dd, *J* = 4.5, 7.8 Hz, 1 H), 4.91 (d, *J* = 3.0 Hz, 1 H), 4.84 (s, 2 H), 4.69 (d, *J* = 12.0 Hz, 1 H), 4.59 (d, *J* = 12.0 Hz, 1 H), 4.54 (d, *J* = 12.0 Hz, 1 H), 4.28-4.22 (m, 1 H), 4.23 (d, *J* = 12.0 Hz, 1 H), 4.21 (d, *J* = 7.5 Hz, 1 H), 3.94-3.81 (m, 5 H), 3.77 (s, 3 H), 3.53 (dd, *J* = 3.0, 8.5 Hz, 1 H), 3.23 (bs, 1 H), 2.04 (tt, overlapped, *J* = 6.5, 6.5 Hz, 2 H), 1.89-1.74 (m, 2 H), 1.44-1.20 (m, 38 H), 1.15 (s, 9 H), 0.88 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 177.42, 170.89, 169.96, 138.74, 138.59, 138.17, 138.03, 128.67, 128.59, 128.14, 128.03, 127.75, 127.65, 126.93, 98.34, 80.46, 79.71, 78.41, 75.35, 73.85, 73.66, 71.99, 71.63, 70.23, 67.62, 52.84, 51.99, 38.99, 32.52, 32.22, 32.17, 29.96, 29.93, 29.74, 29.61, 29.48, 29.39, 27.35, 25.14, 22.94, 14.39; HRMS (ESI) calcd for C₆₄H₉₆NO₁₂ [M+H]⁺: 1070.69270, found: 1070.72711.

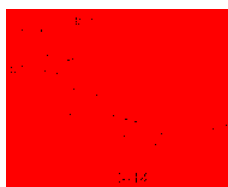


Preparation of GSL-1: To a solution of **3-10** in a mixture of MeOH (1.5 ml), THF (1.5 mL) and H₂O (0.2 mL) was added NaOMe (0.3 mL, 1 M in MeOH). The reaction mixture was stirred for 5 h, and then concentrated in vacuo. The residue was neutralized with acetic acid (0.1 ml) and using silica gel plug to afford a colorless oil (11 mg, 0.011 mmol). HRMS (ESI) calcd for C₅₉H₉₀NO₁₀ [M+H]⁺: 972.65592, found: 972.65979. The resulting compound (11 mg, 0.011 mmol) was dissolved in a mixture of THF (2 mL), MeOH (2 mL) and H₂O (0.2 mL). To this solution was added Pd (10 mg, 10 wt. % on activated carbon), and the reaction mixture was stirred under H₂ at 500 psi for 24 h, then filtered through a silica gel pad. The filtrate was concentrated, and the residue was purified by column chromatography (25:65:4, MeOH/dichloromethane/H₂O) to give **GSL-1** as a white solid (4.1 mg, 40 % for 2 steps). ¹H NMR (DMSO-d₆/acetic acid-d₄, 500 MHz) δ 7.55 (d, *J* = 9.0 Hz, 1 H), 4.71 (d, *J* = 4.0 Hz, 1 H), 8.30 (dd, *J* = 4.5, 8.0 Hz, 1 H), 3.78 (d, *J* = 9.5 Hz, 1 H), 3.74-3.71 (m, 1 H), 3.63 (dd, *J* = 4.0, 10.5 Hz, 1 H), 3.56 (dd, *J* = 4.0, 10.0 Hz, 1 H), 3.53-3.50 (m, 1 H), 3.41 (t, *J* = 9.5 Hz, 1 H), 3.29 (t, *J* = 9.5 Hz, 1 H), 3.21 (dd, *J* = 4.0, 10.0 Hz, 1 H), 1.61-1.55 (m, 2 H), 1.46-1.35 (m, 4 H), 1.30-1.15 (m, 44 H), 0.81 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (DMSO-d₆/acetic acid-d₄, 125 MHz) δ 174.38, 171.78, 100.28, 73.37, 71.42, 69.94, 67.82, 53.27, 34.96, 34.11, 31.95, 31.93, 29.85, 29.76, 29.57, 29.39, 29.36, 25.84, 25.23, 22.72, 14.39; HRMS (ESI) calcd for C₃₈H₇₄NO₁₀ [M+H]⁺: 704.53072, found: 704.52966.



Preparation of 3-13: A solution of sulfide donor **3-11** (1.0 g, 1.63 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (660 mg, 3.21 mmol) and **3-12** (660 mg, 1.32 mmol) in dry toluene (18 mL) was stirred with 4 Å MS (600 mg) for 1 h. Methyl disulfide (307 mg, 3.21 mmol) and MeOTf (533 mg, 3.21 mmol) were mixed in a vial at 0 °C to form DMTST as uniform white solid. The solid was added to the above stirred mixture at -10 °C. The reaction mixture was stirred at -5 °C for 4 h, then Et₃N (3 mL) was added. The solids were filtered off and the cake was washed with CH₂Cl₂ (3 x 20 mL). The filtrate was washed with saturated NaHCO₃, brine, and dried over Na₂SO₄. The dried organic layer was concentrated, and the residue was purified by SiO₂ column chromatography (1:6, EtOAc/hexanes) to yield **3-13** as a colorless oil (1.1 g, 1.05 mmol, 79%). ¹H NMR (CDCl₃, 500 MHz) δ 7.37-7.22 (m, 27 H), 6.82-6.81 (m, 2 H), 5.02 (d, *J* = 3.0 Hz, 1 H), 4.95 (d, *J* = 11.5 Hz, 1 H), 4.82 (d, *J* = 12.0 Hz, 1 H), 4.82 (d, *J* = 10.5 Hz, 1 H), 4.78 (d, *J* = 11.0 Hz, 1 H), 4.73 (d, *J* = 10.5 Hz, 1 H), 4.73 (d, *J* = 11.5 Hz, 1 H), 4.71 (d, *J* = 11.5 Hz, 1 H), 4.64 (d, *J* = 11.5 Hz, 1 H), 4.58 (d, *J* = 10.5 Hz, 1 H), 4.57 (d, *J* = 11.0 Hz, 1 H), 4.46 (d, *J* = 11.5 Hz, 1 H), 4.44 (d, *J* = 8.0 Hz, 1 H), 4.40 (d, *J* = 11.5 Hz, 1 H), 4.03 (dd, *J* = 3.5, 10.0 Hz, 1 H), 3.94 (t, *J* = 6.5 Hz, 1 H), 3.91 (d, *J* = 3.0 Hz, 1 H), 3.88 (dd, *J* = 3.0, 10.0 Hz, 1 H), 3.82 (dd, *J* = 4.5, 12.0 Hz, 1 H), 3.74 (s, 3 H), 3.70 (dd, *J* = 1.5, 12.0 Hz, 1 H), 3.65 (d, *J* = 9.5 Hz, 1 H), 3.54-3.48 (m, 2 H), 3.39 (ddd, *J* = 1.5, 4.5, 9.5 Hz, 1 H), 3.31 (t, *J* = 9.5 Hz, 1 H), 3.06 (dd, *J* = 8.0, 10.0 Hz, 1 H), 0.92 (s, 9 H), 0.13 (s, 3 H), 0.11 (s,

3 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 159.24, 139.12, 138.91, 138.50, 138.44, 138.32, 131.09, 129.24, 128.67, 128.64, 128.60, 128.56, 128.47, 128.26, 128.15, 128.05, 128.01, 127.95, 127.89, 127.83, 127.72, 127.67, 114.00, 98.54, 97.34, 83.00, 78.27, 77.77, 76.74, 75.55, 75.46, 75.31, 75.19, 75.11, 73.55, 73.25, 72.19, 69.58, 69.24, 68.81, 66.46, 55.44, 25.82, 18.16, -3.85, -5.11; HRMS (FAB) calcd for $\text{C}_{61}\text{H}_{73}\text{N}_3\text{NaO}_{11}\text{Si}$ $[\text{M}+\text{Na}]^+$: 1074.4912, found: 1074.4896.



Preparation of 3-14: To a solution of **3-13** (800 mg, 0.76 mmol) in a mixture of CH_2Cl_2 (20 mL) and H_2O (4 mL) was added 2, 3-dichloro-5, 6-dicyanoquinone (210 mg, 0.92 mmol). After being stirred at room temperature for 3 h, the mixture was filtered through a celite pad with the aid of CH_2Cl_2 (50 mL). The organic layer was washed with saturated NaHCO_3 , brine, and dried over Na_2SO_4 . The dried organic layer was concentrated. The residue was purified by SiO_2 column chromatography (1:4, EtOAc/hexanes) to afford the desired product as a colorless oil (580 mg, 0.62 mmol, 82%). A solution of this oil (580 mg, 0.62 mmol) in pyridine (10 mL) was stirred with 4-(dimethylamino)-pyridine (16 mg, 0.12 mmol), and acetic anhydride (0.11 mL, 1.2 mmol) was added. After being stirred at room temperature for 4 h, the reaction mixture was concentrated. The residue was purified by SiO_2 column chromatography (1:6, EtOAc/hexanes) to afford the desired product **3-14** as a colorless oil (570 mg, 0.59 mmol, 95%). ^1H NMR (CDCl_3 , 500 MHz) δ 7.38-7.19 (m,

25 H), 5.32 (dd, $J = 3.8, 10.3$ Hz, 1 H), 5.09 (d, $J = 3.5$ Hz, 1 H), 4.93 (d, $J = 11.0$ Hz, 1 H), 4.90 (d, $J = 10.5$ Hz, 1 H), 4.78 (d, $J = 11.5$ Hz, 1 H), 4.77 (d, $J = 11.0$ Hz, 1 H), 4.68 (d, $J = 12.0$ Hz, 1 H), 4.63 (d, $J = 12.0$ Hz, 1 H), 4.56 (d, $J = 11.0$ Hz, 1 H), 4.53 (d, $J = 10.5$ Hz, 1 H), 4.48 (d, $J = 8.0$ Hz, 1 H), 4.45 (d, $J = 12.0$ Hz, 1 H), 4.38 (d, $J = 12.0$ Hz, 1 H), 3.95-3.90 (m, 3 H), 3.60 (dd, $J = 1.5, 11.5$ Hz, 1 H), 3.57-3.50 (m, 3 H), 3.37 (t, $J = 9.5$ Hz, 1 H), 3.37 (dd, $J = 9.5, 1.5$ Hz, 1 H), 3.25 (dd, $J = 8.0, 9.5$ Hz, 1 H), 2.07 (s, 3 H), 0.92 (s, 9 H), 0.13 (s, 3 H), 0.12 (s, 3 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.39, 138.69, 138.50, 138.24, 138.06, 128.70, 128.66, 128.58, 128.56, 128.42, 128.35, 128.22, 128.14, 128.06, 127.89, 127.86, 127.76, 127.53, 97.59, 97.27, 83.05, 77.93, 76.59, 75.68, 75.23, 74.98, 74.87, 74.74, 73.54, 72.67, 71.22, 69.67, 69.13, 68.92, 66.96, 25.80, 21.30, 18.11, -3.93, -5.10; HRMS (ESI) calcd for $\text{C}_{55}\text{H}_{67}\text{N}_3\text{NaO}_{11}\text{Si}$ $[\text{M}+\text{Na}]^+$: 996.44371, found: 996.44710.



Preparation of 3-16: Compound **3-14** (246 mg, 0.25 mmol) was dissolved in acetonitrile (5 mL) followed by introduction of aqueous HF (1.5 mL). The reaction mixture was stirred for 18 h. Saturated NaHCO_3 was added to neutralize the acid. The mixture was extracted with CH_2Cl_2 . The extracts were washed with brine, and dried over Na_2SO_4 . The organic layer was concentrated, and the residue was purified by SiO_2 column chromatography (1:4, EtOAc/hexanes) to give the desired compound

3-15 as a colorless oil (140 mg, 0.19 mmol, 75%). A solution of **3-15** (120 mg, 0.14 mmol), diphenylsulfoxide (70 mg, 0.35 mmol) and tri-*tert*-butylpyrimidine (105 mg, 0.42 mmol) in CH₂Cl₂ (4 mL) was stirred over activated 3 Å MS (200 mg) for 30 min. The mixture was cooled to -60 °C before triflic acid anhydride (35 µL, 0.21 mmol) was added. The mixture was allowed to warm to -40 °C in 1 hour followed by addition of **3-10** (100 mg, 0.094 mmol) in CH₂Cl₂ (1 mL). The stirred reaction mixture was allowed to warm to 0 °C. Subsequently the reaction was quenched by addition of Net₃ (0.1 mL). SiO₂ column chromatography (1:4, EtOAc/hexanes) afforded the desired compound **3-16** (53 mg, 0.028 mmol, 68%) as a colorless oil and the acceptor **3-10** (56 mg, 0.053 mmol). ¹H NMR (CDCl₃, 500 MHz) δ 7.34-7.19 (m, 40 H), 6.32 (d, *J* = 9.0 Hz, 1 H), 5.59 (tt, *J* = 6.5, 15.5 Hz, 1 H), 5.46 (d, *J* = 3.5 Hz, 1 H), 5.36 (d, *J* = 8.5, 15.0 Hz, 1 H), 5.24 (dd, *J* = 4.0, 11.0 Hz, 1 H), 5.16 (d, *J* = 4.0 Hz, 1 H), 5.14 (d, *J* = 4.0 Hz, 1 H), 4.99 (d, *J* = 10.0 Hz, 1 H), 4.90 (d, *J* = 11.5 Hz, 1 H), 4.84-4.82 (m, 4 H), 4.75 (d, *J* = 10.5 Hz, 1 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 4.60 (d, *J* = 12.0 Hz, 1 H), 4.58-4.49 (m, 6 H), 4.41 (d, *J* = 11.5 Hz, 1 H), 4.33 (d, *J* = 11.5 Hz, 1 H), 4.28-4.23 (m, 1 H), 4.19 (d, *J* = 12.0 Hz, 1 H), 4.16 (d, *J* = 10.0 Hz, 1 H), 4.00 (t, *J* = 10.0 Hz, 1 H), 3.97 (d, *J* = 8.5 Hz, 1 H), 3.96-3.95 (m, 1 H), 3.92-3.76 (m, 8 H), 3.67 (s, 3 H), 3.64-3.43 (m, 6 H), 3.45-3.43 (m, 1 H), 3.13 (dd, *J* = 4.0, 10.0 Hz, 1 H), 2.06-2.02 (m, 2 H), 1.88-1.76 (m, 2 H), 1.25-1.17 (m, 51 H), 0.87 (tt, *J* = 2.5, 7.0 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 176.88, 170.46, 170.11, 169.84, 138.76, 138.60, 138.51, 138.35, 138.22, 138.08, 137.96, 128.71, 128.68, 128.61, 128.47, 128.40, 128.17, 128.03, 128.00, 127.92, 127.75, 127.70, 127.66, 126.86, 98.28, 98.01, 97.45,

81.43, 80.16, 79.85, 79.70, 78.28, 76.832, 76.41, 75.67, 75.52, 75.08, 74.97, 74.57, 73.72, 73.63, 73.31, 72.61, 71.65, 71.54, 70.67, 70.14, 69.79, 68.95, 67.33, 66.11, 63.78, 52.91, 52.03, 39.00, 32.52, 32.28, 32.18, 29.97, 29.90, 29.76, 29.68, 29.61, 29.47, 29.38, 27.44, 25.20, 22.95, 21.16, 15.53, 14.37; HRMS (ESI) calcd for $C_{114}H_{151}N_4O_{21}$ $[M+H]^+$: 1912.08653, found: 1912.08652.



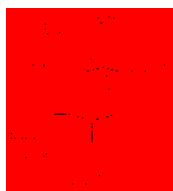
Preparation of 3-17: Compound **3-16** (53 mg, 0.028 mmol) was dissolved in dry pyridine (20 mL) and Et_3N (3.5 mL). To this solution was bubbled H_2S at 0 °C over 15 min. After the solution became dark blue, H_2S stream was stopped. The reaction mixture was stirred at 0 °C for 12 h. Then H_2S was bubbled into the solution over again for another 15 min. at 0 °C. The solution was stirred for additional 12 h, then concentrated. The residue was dissolved in CH_2Cl_2 (20 mL). To this solution was added Et_3N (5 mL) and di-*tert*-butyldicarbonate (121 mg, 0.56 mmol, 20 eq.) in sequence. After being stirred for 18 h, the reaction mixture was concentrated and the residue was purified by SiO_2 column chromatography (1:4, EtOAc/hexanes) to afford **3-17** as a colorless oil (47 mg, 0.024 mmol, 84%). 1H NMR ($CDCl_3$, 500 MHz) δ 7.39-7.22 (m, 40 H), 6.34 (d, $J = 9.0$ Hz, 1 H), 6.63 (tt, $J = 6.5, 16.0$ Hz, 1 H), 5.34 (dd, $J = 8.5, 15.5$ Hz, 1 H), 5.25 (dd, $J = 4, 10.5$ Hz, 1 H), 5.21 (d, $J = 3.0$ Hz, 1 H), 5.16-5.12 (m, 3 H), 4.92 (d, $J = 11.5$ Hz, 1 H), 4.85 (d, $J = 11.0$ Hz, 1 H), 4.84 (d, $J =$

3.5 Hz, 1 H), 4.76 (d, $J = 11.5$ Hz, 1 H), 4.72 (d, $J = 11.0$ Hz, 1 H), 4.69-4.64 (m, 3 H), 4.60-4.53 (m, 4 H), 4.41 (d, $J = 11.5$ Hz, 1 H), 4.33 (d, $J = 12.0$ Hz, 1 H), 4.30-4.26 (m, 1 H), 4.22 (d, $J = 11.5$ Hz, 1 H), 4.16 (d, $J = 8.5$ Hz, 1 H), 3.99 (t, $J = 8.5$ Hz, 1 H), 3.94-3.77 (m, 11 H), 3.72-3.69 (m, 1 H), 3.67 (s, 3 H), 3.58-3.47 (m, 8 H), 2.07-2.03 (m, 2 H), 2.01 (s, 3 H), 1.92-1.84 (m, 1 H), 1.84-1.78 (m, 1 H), 1.34-1.18 (m, 51 H), 0.89 (ddd, $J = 1.5, 7$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 177.02, 170.81, 170.02, 169.59, 155.49, 138.80, 138.52, 138.23, 137.97, 128.70, 128.59, 128.44, 128.11, 127.77, 127.63, 126.65, 99.23, 97.86, 81.43, 79.84, 79.72, 79.24, 77.87, 76.54, 75.49, 75.07, 74.96, 74.75, 73.81, 73.62, 73.35, 72.90, 71.91, 71.52, 70.14, 69.81, 68.98, 67.46, 66.69, 54.69, 52.89, 53.31, 38.99, 32.55, 32.16, 29.95, 29.74, 29.60, 29.47, 28.61, 27.38, 25.23, 22.93, 21.27, 14.36; HRMS (ESI) calcd for $\text{C}_{119}\text{H}_{161}\text{N}_2\text{O}_{23}$ $[\text{M}+\text{H}]^+$: 1986.14847, found: 1986.14541.



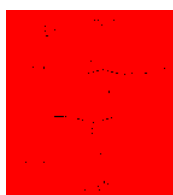
Preparation of GSL-3: A solution of **3-17** (54 mg, 0.027 mmol) in a mixture of MeOH (5 mL) and THF (5 mL) was stirred with palladium hydroxide (100 mg, 20 wt. % Pd on activated carbon) under H_2 at 400 psi for 18 h. The mixture was filtered and the filtrate was concentrated. The residue was purified by SiO_2 column chromatography (MeOH/ CH_2Cl_2 1:9) to afford a colorless syrup. A solution of this syrup in CH_2Cl_2 (1.5 mL) was stirred in the presence of TFA (3 mL) for 8 h. The

reaction mixture was co-evaporated with toluene (20 mL). The residue was dissolved in a mixture of THF (1 mL), MeOH (1 mL) and H₂O (4 drops). The mixture was stirred with NaOMe (0.1 mL, 1 M in MeOH) for 24 h, then concentrated. SiO₂ column chromatographic purification of the residue (CH₂Cl₂/MeOH/H₂O 65:25:4) afforded **GSL-3** as a white solid (17 mg, 0.017 mmol, 61%). ¹H NMR (DMSO-d₆/acetic acid-d₄, 500 MHz) δ 7.62 (d, *J* = 9.5 Hz, 1 H), 5.38 (d, *J* = 3.5 Hz, 1 H), 4.76 (d, *J* = 3.5 Hz, 1 H), 4.68 (d, *J* = 3.0 Hz, 1 H), 3.95 (d, *J* = 10.0 Hz, 1 H), 3.82 (dd, *J* = 4.5, 7.5 Hz, 1 H), 3.79-3.69 (m, 6 H), 3.65-3.55 (m, 5 H), 3.54-3.41 (m, 5 H), 3.28 (dd, *J* = 3.5, 9.5 Hz, 1 H), 3.22 (t, *J* = 9.5 Hz, 1 H), 2.93 (dd, *J* = 3.5, 10.5 Hz, 1 H), 1.64-1.58 (m, 2 H), 1.45-1.41 (m, 4 H), 1.34-1.13 (m, 44 H), 0.85 (t, *J* = 6.5 Hz, 6 H); ¹³C NMR (DMSO-d₆/acetic acid-d₄, 125 MHz) δ 173.71, 170.88, 99.44, 98.89, 95.31, 78.36, 72.02, 71.46, 70.94, 70.80, 70.32, 69.90, 69.43, 69.33, 68.76, 68.50, 67.65, 65.94, 60.54, 54.10, 53.11, 34.46, 33.56, 31.37, 29.29, 29.19, 29.09, 28.82, 25.17, 24.80, 22.16, 13.20; HRMS (ESI) calcd for C₅₀H₉₃N₂O₁₉ [M+H]⁺: 1027.65236, found: 1027.65236.



Preparation of 3-27: A mixture of donor **3-25** (220 mg, 0.37 mmol) and K₂CO₃ (300 mg, 2.17 mmol) in CCl₃CN (6 mL, 60 mmol) was stirred at room temperature for 4 h. The mixture was concentrated and the residue was filtered through a SiO₂ gel pad with the aid of a mixture of EtOAc and hexane (100 mL, 1:2, EtOAc/hexane). The

filtrate was concentrated, and the resulting trichloroacetimidate, acceptor **3-26** (190 mg, 0.35 mmol), and powdered 4 Å MS (300 mg) in CH₂Cl₂ (8 mL) were stirred for 1 h. TMSOTf (0.01 mL) was added to the solution at 0 °C. The reaction mixture was stirred for 18 h and filtered. The filtrate was concentrated, and the residue was purified by SiO₂ column chromatography (1:4, EtOAc/hexanes) to afford **3-27** as colorless oil (370 mg, 0.33 mmol, 94%). ¹H NMR (CDCl₃, 500 MHz) δ 8.11-8.06 (m, 4 H), 7.84-7.80 (m, 4 H), 7.60-7.48 (m, 5 H), 7.43-7.16 (m, 27 H), 6.10 (t, *J* = 10.0 Hz, 1 H), 5.92 (dd, *J* = 3.0, 10.0 Hz, 1 H), 5.86 (dd, *J* = 2.0, 3.0 Hz, 1 H), 5.60 (d, *J* = 2.0 Hz, 1 H), 4.96 (d, *J* = 11.0 Hz, 1 H), 4.93 (d, *J* = 11.0 Hz, 1 H), 4.69 (d, *J* = 10.0 Hz, 2 H), 4.64 (d, *J* = 11.5 Hz, 1 H), 4.60 (d, *J* = 11.5 Hz, 1 H), 4.52 (d, *J* = 12.0 Hz, 1 H), 4.47 (d, *J* = 12.0 Hz, 1 H), 4.31 (t, *J* = 9.0 Hz, 1 H), 4.19 (d, *J* = 2.5 Hz, 1 H), 4.17 (dd, *J* = 2.0, 12.0 Hz, 1 H), 3.71 (s, 3 H), 3.71-3.68 (m, 2 H); ¹³C NMR (CDCl₃, 125 MHz) δ 166.33, 165.90, 165.41, 165.32, 138.68, 137.97, 137.37, 133.99, 133.51, 133.46, 133.36, 133.03, 131.91, 130.40, 130.08, 129.97, 129.94, 129.75, 129.41, 129.39, 129.21, 128.89, 128.75, 128.71, 128.60, 128.55, 128.50, 128.40, 128.24, 128.14, 128.01, 127.92, 127.87, 127.73, 98.62, 89.02, 83.15, 77.42, 74.92, 74.81, 73.89, 72.54, 72.04, 70.44, 69.10, 68.75, 66.74, 62.24; HRMS (ESI) calcd for C₆₇H₆₄NO₁₄S [M+NH₄]⁺: 1138.40420, found: 1138.40433.



Preparation of 3-28: To a solution of **3-27** (370 mg, 0.33 mmol) in MeOH (10 mL) was added NaOMe (0.2 mL, 1 M in MeOH,) at room temperature. The mixture was stirred for 2 h, then concentrated. A solution of the residue in pyridine (10 mL) was treated with 4-(dimethylamino)-pyridine (32 mg, 0.24 mmol) and acetic anhydride (0.22 mL, 2.4 mmol). After being stirred for 4 h at room temperature, the reaction mixture was concentrated. The residue was purified by SiO₂ column chromatography (EtOAc/hexanes 1:2) to afford the desired product tetraacetate as a colorless oil (270 mg, 0.31 mmol, 94%). ¹H NMR (CDCl₃, 500 MHz) δ 7.53-7.52 (m, 2 H), 7.35-7.27 (m, 16 H), 7.21-71.8 (m, 2 H), 5.39 (dd, *J* = 2.0, 3.0 Hz, 1 H), 5.34 (s, 1 H), 5.30 (dd, *J* = 3.5, 10.0 Hz, 1 H), 5.22 (t, *J* = 10.0 Hz, 1 H), 4.92 (d, *J* = 11.5 Hz, 1 H), 4.80 (d, *J* = 11.0 Hz, 1 H), 4.61 (d, *J* = 12.0 Hz, 1 H), 4.57 (d, *J* = 10.0 Hz, 1 H), 4.50 (d, *J* = 11.0 Hz, 2 H), 4.44 (d, *J* = 12.0 Hz, 1 H), 4.30 (tt, *J* = 3.5, 8.5 Hz, 1 H), 4.17 (t, *J* = 9.5 Hz, 1 H), 4.09 (d, *J* = 2.5 Hz, 1 H), 3.72-3.64 (m, 4 H), 3.56 (dd, *J* = 2.5, 9.5 Hz, 2 H), 2.15 (s, 3 H), 1.99 (s, 3 H), 1.96 (s, 3 H), 1.92 (s, 3 H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.84, 170.22, 170.00, 169.65, 138.63, 137.89, 137.31, 133.82, 131.73, 129.13, 128.76, 128.64, 128.47, 128.29, 128.14, 128.07, 127.77, 127.71, 127.66, 98.34, 88.73, 82.96, 77.33, 74.81, 74.16, 73.79, 72.71, 72.14, 59.50, 69.40, 68.57, 65.81, 61.72, 21.11, 20.923, 20.84; HRMS (ESI) calcd for C₄₇H₅₂NaO₁₄S [M+Na]⁺: 895.29700, found: 895.29754.



Preparation of 3-31: A mixture of donor **3-30** (45.40 mg, 0.073 mmol), diphenylsulfoxide (28.37 mmg, 0.1403 mmol) and tri-tert-butylpyrimidine (48.77 mg, 0.1964 mmol) was dissolved in dry dichloromethane (2.5 mL) and dried over 3 Å MS with stirring for 30 min. After the reaction mixture was cooled to -60 °C, triflic acid anhydride (12.89 µL, 0.0785 mmol) was added. The reaction mixture was allowed to warm to -40 °C. A solution of acceptor **3-10** (60 mg, 0.056 mmol) in dichloromethane (1 mL) was subsequently added. The reaction mixture was warmed to 0C, followed by addition of Et₃N (0.2 mL). The solids were filtered off and the filtrate was concentrated. The residue was purified by SiO₂ column chromatography (1:3, EtOAc/Hexane, R_f = 0.7) to afford **3-31** as a colorless oil (48 mg, 64%). ¹H NMR (CDCl₃, 500 MHz) δ 7.73-7.19 (m, 35 H), 6.33 (d, *J* = 9.5 Hz, 1 H), 5.59 (tt, *J* = 6.5, 15.5 Hz, 1 H), 5.54 (d, *J* = 4.0 Hz, 1 H), 5.36 (dd, *J* = 8.5, 15.5 Hz, 1 H), 5.14 (dd, *J* = 4.0, 8.5 Hz, 1 H), 5.03 (d, *J* = 10.5 Hz, 1 H), 4.92-4.81 (m, 4 H), 4.74 (d, *J* = 10.5 Hz, 1 H), 4.61 (d, *J* = 12.0 Hz, 1 H), 4.53 (d, *J* = 12.0 Hz, 1 H), 4.52 (d, *J* = 11.5 Hz, 1 H), 4.27-4.22 (m, 1 H), 4.19 (d, *J* = 5.0 Hz, 1 H), 4.18 (d, *J* = 3.5 Hz, 1 H), 4.06-3.86 (m, 6 H), 3.81-3.74 (m, 3 H), 5.90 (dd, *J* = 3.5, 8.5 Hz, 1 H), 3.51-3.45 (m, 1 H), 3.50 (s, 3 H), 3.39-3.35 (m, 1 H), 3.28 (dd, *J* = 4.0, 10.0 Hz, 1 H), 2.10-2.01 (m, 2 H), 1.88-1.77 (m, 2 H), 1.33-1.19 (m, 51 H), 1.14 (s, 9 H), 0.88 (t, *J* = 7.0 Hz, 3 H), 0.87 (t, *J* = 7.5 Hz, 3 H); ¹³C NMR (CDCl₃, 125 MHz) δ 176.90, 170.15, 169.85, 138.63, 138.15, 138.03, 137.96, 136.17, 136.10, 135.91, 135.84, 133.85, 133.25, 129.88, 128.75, 128.69, 128.62, 128.43, 128.33, 128.14, 128.05, 127.95, 127.82, 127.77, 127.66, 127.53, 127.45, 126.83, 98.18, 98.00, 81.55, 80.27, 79.92, 79.69, 78.03, 75.88, 75.34,

75.11, 74.75, 73.73, 73.25, 72.46, 70.72, 70.14, 67.36, 63.68, 62.07, 52.79, 52.00, 39.02, 32.53, 32.29, 29.99, 29.94, 29.91, 29.78, 29.68, 29.62, 29.47, 29.38, 27.45, 27.38, 27.14, 25.20, 22.97, 19.63, 14.39; HRMS (ESI) calcd for C₁₀₁H₁₃₉N₄O₁₅Si [M+H]⁺: 1676.0006, found: 1675.9618.



Preparation of 3-32: Compound **3-31** (200 mg, 0.12 mmol) was dissolved in a mixture of dry pyridine (20 mL) and Et₃N (3.52 mL). To this solution was introduced H₂S over 15 min. at 0 °C, after the solution became dark blue. H₂S stream was stopped. The reaction mixture was stirred at 0°C for 12 h. Then H₂S was introduced into the solution over again for another 15 min. at 0°C. The solution was kept stirring for additional 12 h. The thin layer chromatography showed that the starting material was completely consumed. The solvent was subsequently removed and the residue was further dissolved in dichloromethane (20 mL), followed by addition of Et₃N (5 mL) and di-*tert*-butyl dicarbonate (521.50 mg, 2.39 mmol). The solution was stirred at room temperature overnight. After the free amine was totally consumed (by TLC), the solution was concentrated. The residue was subject to SiO₂ column chromatography (1:3, EtOAc/hexane) to afford the desired carbamate as a colorless oil (170 mg, 82%). ¹H NMR (CDCl₃, 500 MHz) δ 7.73 (d, *J* = 7.0 Hz, 2 H), 7.68 (d, *J* = 7.0 Hz, 2 H), 7.43-7.27 (m, 29 H), 7.21-7.19 (m, 2 H), 6.41 (d, *J* = 9.0 Hz, 1 H), 5.67 (tt, *J* = 7.0, 15.5 Hz, 1 H), 5.41 (dd, *J* = 8.5, 15.5 Hz, 1 H), 5.33 (d, *J* = 5.0 Hz, 1 H), 5.32 (d,

$J = 3.5$ Hz, 1 H), 5.17 (dd, $J = 4.5, 8.5$ Hz, 1 H), 4.92 (d, $J = 10.0$ Hz, 1 H), 4.90 (d, $J = 3.5$ Hz, 1 H), 4.89 (d, $J = 10.0$ Hz, 1 H), 4.79 (d, $J = 11.5$ Hz, 1 H), 4.75 (dd, $J = 5.0, 10.5$ Hz, 1 H), 4.68 (d, $J = 11.5$ Hz, 1 H), 4.63 (d, $J = 12.0$ Hz, 1 H), 4.59 (d, $J = 12.0$ Hz, 1 H), 4.58 (d, $J = 12.5$ Hz, 1 H), 4.35-4.29 (m, 1 H), 4.25 (d, $J = 12.5$ Hz, 1 H), 4.21 (d, $J = 9.0$ Hz, 1 H), 4.10-3.81 (m, 10 H), 3.68 (t, $J = 9.5$ Hz, 1 H), 3.62 (dd, $J = 3.5, 9.0$ Hz, 1 H), 3.53-3.47 (m, 1 H), 3.52 (s, 3 H), 2.10-2.06 (m, 2 H), 1.95-1.81 (m, 2 H), 1.40 (s, 9 H), 1.35-1.22 (m, 51 H), 1.09 (s, 9 H), 0.92 (t, $J = 7.5$ Hz, 6 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 177.05, 170.08, 169.61, 155.74, 138.87, 138.55, 138.00, 137.96, 136.22, 135.89, 133.94, 133.28, 129.84, 128.78, 128.73, 128.57, 128.49, 128.21, 128.13, 128.06, 128.02, 127.94, 127.84, 127.74, 127.65, 126.71, 99.24, 97.98, 81.54, 79.86, 79.80, 78.95, 77.82, 75.57, 75.06, 74.88, 74.52, 73.84, 73.33, 73.05, 71.73, 70.18, 67.59, 62.33, 54.79, 52.76, 52.28, 39.02, 32.56, 32.20, 29.98, 29.94, 29.90, 29.86, 29.76, 29.58, 29.48, 29.37, 28.67, 27.42, 27.14, 25.24, 22.97, 19.61, 14.42; HRMS (ESI) calcd for $\text{C}_{106}\text{H}_{149}\text{N}_2\text{O}_{17}\text{Si}$ $[\text{M}+\text{H}]^+$: 1750.06200, found: 1750.05749.



Preparation of 3-33: The carbamate **3-32** (170 mg, 0.097 mmol) was dissolved in anhydrous THF (10 mL). To this solution was added hydrogenfluoride-pyridine complex (con. 20%, 4.5 mL). The solution was stirred at room temperature for 16 h, then diluted with EtOAc (100 mL), and washed with saturated NaHCO_3 (3 x 100

mL). The organic layer was dried over Na₂SO₄ and concentrated. The residue was purified by SiO₂ column chromatography (1:3, EtOAc/hexane) to afford **3-33** as an oil (130 mg, 89%). ¹H NMR (CDCl₃, 500 MHz) δ 7.35-7.25 (m, 25 H), 6.41 (d, *J* = 9.0 Hz, 1 H), 5.68 (tt, *J* = 6.0, 15.5 Hz, 1 H), 5.41 (d, *J* = 8.5, 15.5 Hz, 1 H), 5.34 (d, *J* = 3.0 Hz, 1 H), 5.17 (dd, *J* = 4.5, 8.5 Hz, 1 H), 5.14 (d, *J* = 11.0 Hz, 1 H), 4.89 (d, *J* = 11.0 Hz, 1 H), 4.87 (d, *J* = 3.0 Hz, 1 H), 4.84 (d, *J* = 11.5 Hz, 1 H), 4.77 (d, *J* = 10.5 Hz, 1 H), 4.69 (d, *J* = 10.5 Hz, 1 H), 4.67 (d, *J* = 11.5 Hz, 1 H), 4.62 (d, *J* = 12.0 Hz, 1 H), 4.59 (d, *J* = 12.0 Hz, 1 H), 4.56 (d, *J* = 12.0 Hz, 1 H), 4.35-4.29 (m, 1 H), 4.26 (d, *J* = 12.0 Hz, 1 H), 4.22 (d, *J* = 9.0 Hz, 1 H), 4.06-3.76 (m, 7 H), 3.77 (s, 3 H), 3.70-3.49 (m, 5 H), 2.11-2.06 (m, 2 H), 1.95-1.84 (m, 2 H), 1.48-1.23 (m, 60H), 0.91 (t, *J* = 7.5 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 177.07, 170.10, 169.86, 155.57, 138.65, 138.38, 137.96, 137.85, 128.78, 128.74, 128.67, 128.62, 128.49, 128.24, 128.15, 128.04, 17.97, 127.80, 127.67, 126.67, 99.08, 98.07, 81.17, 80.22, 79.88, 79.45, 77.98, 75.40, 75.10, 73.85, 73.37, 72.66, 71.37, 70.18, 67.49, 61.88, 54.51, 53.04, 52.36, 39.02, 32.58, 32.24, 32.18, 29.97, 29.94, 29.89, 29.76, 29.70, 29.62, 29.51, 29.37, 28.63, 27.41, 25.26, 22.96, 14.40; HRMS (ESI) calcd for C₉₀H₁₃₁N₂O₁₇ [M+H]⁺:1511.94423, found: 1511.93851.



Preparation of 3-35: A solution of **3-28** (270 mg, 0.31 mmol) in acetone (10 mL) and H₂O (1 mL) was stirred with N-bromosuccinimide (83 mg, 0.46 mmol) at -15 °C for 1 h. The reaction mixture was quenched with saturated NaHCO₃ (10 mL), followed by extraction with CH₂Cl₂ (2 x 15 mL). The combined organic extracts were washed with saturated NaHCO₃ followed by brine, then dried over Na₂SO₄. The dried organic layer was concentrated. SiO₂ column chromatography (EtOAc/hexanes 1:2) afforded the desired alcohol **3-29** as a colorless oil (186 mg, 0.24 mmol, 77%). A solution of **3-29** (75 mg, 0.096 mmol), diphenylsulfoxide (35 mg, 0.18 mmol) and tri-*tert*-butylpyrimidine (50 mg, 0.20 mmol) in CH₂Cl₂ (4 mL) was stirred over activated 3 Å MS (150 mg) for 30 min. The mixture was cooled to -60 °C before triflic acid anhydride (18 µL, 0.10 mmol) was added. The mixture was allowed to warm to -40 °C during 1 h followed by addition of **3-31** (110 mg, 0.074 mmol) in CH₂Cl₂ (1 mL). The stirred mixture was allowed to warm to 0° C. Subsequently, the reaction was quenched by addition of Et₃N (0.1 mL). SiO₂ column chromatography (1:6, EtOAc/hexanes) afforded the desired tetrasaccharide **3-34** as a colorless oil (138 mg, 0.061 mmol, 82%). HRMS (ESI) calcd for C₁₃₁H₁₈₀N₃O₃₁ [M+NH₄]⁺: 2291.25953, found: 2291.26474. A solution of **3-34** (60 mg, 0.026 mmol) in MeOH (5 mL) and THF (5 mL) was stirred with palladium (100 mg, 10 wt. % pd on activated carbon) under H₂ at 600 psi for 72 h. The mixture was filtered and the filtrate was concentrated. A solution of the residue in pyridine (10 mL) was treated with 4-(dimethylamino)-pyridine (30 mg, 0.22 mmol) and acetic anhydride (0.20 mL, 2.2 mmol). After stirring at room temperature for 18 h, the reaction mixture was

concentrated. The residue was purified by SiO₂ column chromatography (1:7, MeOH/CH₂Cl₂) to afford the desired product **3-35** as a colorless oil (27 mg, 0.014 mmol, 55%). ¹H NMR (CDCl₃, 500 MHz) δ 6.48 (d, *J* = 9.0 Hz, 1 H), 5.50 (d, *J* = 3.5 Hz, 1 H), 5.45 (t, *J* = 9.5 Hz, 1 H), 5.41 (dd, *J* = 3.5, 11.0 Hz, 1 H), 5.34-5.21 (m, 3 H), 5.16-5.09 (m, 3 H), 5.05 (d, *J* = 3.0 Hz, 1 H), 5.02 (t, *J* = 10.0 Hz, 1 H), 4.98-4.90 (m, 4 H), 4.55 (d, *J* = 9.5 Hz, 1 H), 4.32-4.25 (m, 4 H), 4.19 (t, *J* = 9.5 Hz, 1 H), 4.14-4.06 (m, 5 H), 3.97 (ddd, *J* = 3.5, 10.5 Hz, 1 H), 3.81 (s, 3 H), 3.76 (dd, *J* = 5.0, 10.5 Hz, 1 H), 3.71-3.61 (m, 3 H), 3.52 (dd, *J* = 3.5, 11.0 Hz, 1 H), 2.15, 2.13, 2.12, 2.08, 2.07, 2.04, 1.98, 1.96 (s, 3 H each), 2.05, 2.01 (s, 6 H each), 1.94-1.88 (m, 1 H), 1.87-1.80 (m, 1 H), 1.70 (s, 2 H), 1.61 (bs, 2 H), 1.42-1.25 (m, 62 H), 0.88 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 177.54, 170.88, 170.63, 170.41, 170.29, 170.14, 170.01, 169.77, 169.65, 169.60, 169.26, 168.85, 155.09, 99.12, 97.04, 96.74, 95.65, 79.93, 74.72, 73.91, 73.16, 71.68, 71.28, 71.18, 70.73, 70.31, 69.84, 69.49, 69.23, 69.05, 68.97, 68.71, 68.52, 67.68, 66.62, 66.38, 66.07, 62.54, 61.84, 53.15, 52.96, 50.44, 39.13, 32.16, 32.08, 31.36, 29.96, 29.91, 29.85, 29.79, 29.65, 29.62, 29.39, 28.44, 27.35, 25.46, 25.20, 22.94, 21.21, 21.16, 20.99, 20.91, 20.88, 14.37; HRMS (ESI) calcd for C₉₁H₁₄₆N₂NaO₃₉ [M+Na]⁺: 1913.93949, found: 1913.93569.



Preparation of GSL-4: A solution of **3-35** (27 mg, 0.014 mmol) in a mixture of THF (1 mL), MeOH (1 mL) and H₂O (4 drops) was stirred in the presence of NaOMe (0.1 mL, 1 M in MeOH) for 60 h. To the solution was added acetic acid (0.1 mL). The mixture was concentrated. The residue in CH₂Cl₂ (1.5 mL) was stirred in the presence of TFA (3 mL) for 8 h. The reaction mixture was co-evaporated with toluene (20 mL). SiO₂ column chromatographic purification of the residue (65:25:4, CH₂Cl₂/MeOH/H₂O) afforded **GSL-4** as a white solid (11 mg, 0.0093 mmol, 66%). ¹H NMR (DMSO-d₆/acetic acid-d₄, 500 MHz) δ 7.59 (d, *J* = 8.5 Hz, 1 H), 5.40 (d, *J* = 3.5 Hz, 1 H), 4.95 (d, *J* = 3.0 Hz, 1 H), 4.86 (s, 1 H), 4.79 (d, *J* = 3.5 Hz, 1 H), 3.94 (d, *J* = 9.5 Hz, 1 H), 3.84-3.81 (m, 2 H), 3.77-3.70 (m, 5 H), 3.67-3.44 (m, 18 H), 3.39 (t, *J* = 9.5 Hz, 1 H), 3.32 (dd, *J* = 3.5, 9.5 Hz, 1 H), 3.02 (dd, *J* = 4.0, 10.5 Hz, 1 H), 1.64-1.58 (m, 1 H), 1.45-1.41 (m, 3 H), 1.30-1.21 (m, 44 H), 0.83 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (DMSO-d₆/acetic acid-d₄, 125 MHz) δ 174.02, 170.74, 99.74, 96.49, 96.37, 96.02, 78.17, 73.33, 72.77, 72.37, 71.25, 71.02, 70.78, 70.69, 69.74, 69.06, 68.96, 68.01, 67.85, 67.04, 64.53, 61.18, 60.74, 54.20, 53.30, 34.67, 33.75, 31.60, 31.61, 29.42, 29.32, 29.26, 29.04, 29.02, 25.41, 25.00, 22.39, 14.14; HRMS (ESI) calcd for C₅₆H₁₀₅N₂O₂₄ [M+H]⁺: 1189.70518, found: 1189.70462.



Preparation of 3-37: A solution of donor **3-36** (20.5 mg, 0.046 mmol), diphenylsulfoxide (19.50 mg, 0.096 mmol) and 2,4,6- tri-*tert*-butylpyrimidine (30.61

mg, 0.123 mmol) in dichloromethane (3 mL) was stirred over 70 mg of activated 3 Å MS for 30 min. The mixture was cooled to -60°C before triflic acid anhydride (9.07 μL, 0.054 mmol) was added. The mixture was warmed to -40°C over 45 min, followed by addition of acceptor **3-10** (45 mg, 0.0385 mmol) in dichloromethane (1 mL). The reaction mixture was warmed to 0°C, and quenched with Et₃N (0.4 mL). The solids were filtered off and the filtrate was concentrated. Purification by SiO₂ column chromatography (1:3, EtOAc/hexane) afforded **3-37** as a colorless oil (40 mg, 70%). ¹H NMR (CDCl₃, 500 MHz) δ 7.36-7.22 (m, 25 H), 6.33 (d, *J* = 9.0 Hz, 1 H), 5.61 (tt, *J* = 6.5, 15.5 Hz, 1 H), 5.60 (d, *J* = 4.0 Hz, 1 H), 5.38 (dd, *J* = 9.0, 15.5 Hz, 1 H), 5.15 (dd, *J* = 4.0, 8.0 Hz, 1 H), 5.03 (d, *J* = 10.0 Hz, 1 H), 4.86 (d, *J* = 10.5 Hz, 1 H), 4.85 (s, 2 H), 4.83 (d, *J* = 10.5 Hz, 1 H), 4.81 (d, *J* = 10.0 Hz, 1 H), 4.61 (d, *J* = 11.5 Hz, 1 H), 4.56 (d, *J* = 11.5 Hz, 1 H), 4.55 (d, *J* = 3.5 Hz, 1 H), 4.52 (d, *J* = 3.5 Hz, 1 H), 4.30-4.21 (m, 1 H), 4.22 (dd, *J* = 1.5, 3.5 Hz, 1 H), 4.22 (bs, 2 H), 4.19 (d, *J* = 3.0 Hz, 1 H), 4.05-4.01 (m, 2 H), 3.91 (t, *J* = 9.0 Hz, 1 H), 3.89 (t, *J* = 9.0 Hz, 1 H), 3.84-3.80 (m, 2 H), 3.74 (s, 3 H), 3.58 (dd, *J* = 3.5, 10.0 Hz, 1 H), 3.55 (t, *J* = 3.5 Hz, 1 H), 3.50 (dd, *J* = 9.0, 10.0 Hz, 1 H), 3.27 (dd, *J* = 4.0, 10.5 Hz, 1 H), 2.07-2.02 (m, 2 H), 1.90-1.78 (m, 2 H), 1.39-1.16 (m, 51 H), 0.88 (t, *J* = 7.0 Hz, 3 H), 0.87 (t, *J* = 7.5 Hz, 3 H); ¹³C NMR (CDCl₃, 125 MHz) δ 176.89, 170.93, 170.10, 169.87, 138.56, 138.51, 138.11, 137.86, 137.80, 128.76, 128.72, 128.65, 128.61, 128.22, 128.04, 127.86, 127.76, 127.69, 127.64, 127.79, 98.18, 97.98, 81.42, 80.43, 79.86, 79.68, 77.65, 75.76, 75.46, 75.24, 75.13, 73.71, 73.25, 70.42, 70.13, 69.77, 67.32, 63.44, 62.52, 52.97, 51.99, 32.52, 32.27, 32.16, 29.96, 29.88, 29.75, 29.68, 29.60, 29.48,

29.36, 27.41, 25.19, 22.95, 21.09, 14.38; HRMS (ESI) calcd for C₈₇H₁₂₃N₄O₁₆ [M+H]⁺: 1479.89286, found: 1479.89420.



Preparation of GSL-2: Compound **3-37** (40 mg, 0.027 mmol) was dissolved in a mixture of MeOH (3 mL), THF (4 mL) and H₂O (0.2 mL). To this solution was added NaOMe (0.4 mL, 1 M in MeOH). The mixture was stirred at room temperature for 8 h. The solvent was removed, and the residue was purified by silica gel plug (1:9, MeOH/dichloromethane). A colorless oil (15 mg) was obtained and dissolved in a mixture of MeOH (3 mL), THF (4.5 mL) and H₂O (0.4 mL). To this solution was added Pd (15 mg, 10 wt. % on activated carbon) as catalyst. The suspension was stirred under H₂ at 500 psi for 48 h. The mixture was filtered through a silica gel pad. The filtration was concentrated, and the residue was purified by SiO₂ column chromatography (25:65:4, MeOH/dichloromethane/H₂O) to afford **GSL-2** (4.7 mg, 22% for 2 steps). ¹H NMR (TFA-d₄/DMSO-d₆/D₂O, 500 MHz) δ 5.36 (d, *J* = 3.5 Hz, 1 H), 4.77 (d, *J* = 3.0 Hz, 1 H), 3.96 (d, *J* = 8.0 Hz, 1 H), 3.82 (d, *J* = 4.5, 7.0 Hz, 1 H), 3.73-3.66 (m, 3 H), 3.62 (t, *J* = 9.5 Hz, 1 H), 3.60-3.43 (m, 5 H), 3.37-3.27 (m, 3 H), 2.96 (dd, *J* = 3.5, 11.0 Hz, 1 H), 1.62-1.54 (m, 2 H), 1.44-1.10 (m, 48 H), 0.81 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (TFA-d₄/DMSO-d₆/D₂O, 125 MHz) δ 174.53, 1780.88, 99.95, 96.71, 78.35, 73.49, 72.93, 71.53, 71.33, 70.98, 69.88, 69.29, 68.10, 59.74, 55.32, 54.67, 53.72, 49.09, 35.02, 34.03, 31.92, 29.70, 29.34, 29.29, 25.59, 25.18,

22.71, 14.48; HRMS (ESI) calcd for $C_{44}H_{85}N_2O_{14}$ $[M+H]^+$ 865.59953, found 865.60145.

Chapter 4: Synthesis and Evaluation of Stimulatory properties of Glycosphingolipids Isolated from *Fasciola hepatica*

4.1. Introduction

NKT cells have been shown to either suppress or enhance the acquired immune response in a Th1 or a Th2 direction¹, thus regulating a number of conditions, including autoimmune diseases, inflammation, resistance to tumors, and antimicrobial host responses. Although activation of NKT cells during some parasite infections has been reported²⁻⁵, much less is known about the role of human NKT cells in parasite infections.

To elucidate this issue, several published studies were conducted in anti-CD1d mAb-treated and CD1d gene-disrupted (CD1d^{-/-}) mice, which manipulations abolished most of the NKT cells, and J α 281-deficient (J α 281^{-/-}) mice, which lacked V α 14J α 281 NKT cells. Other studies examined the effect of α -GalCer on the clinical course of infectious diseases. Ishikawa *et al.* reported that NKT cells were the main effector cells for an early stage of protective immunity against infection with *Leishmania major*.⁶ Furthermore, other types of innate cells such as $\gamma\delta$ T and NK cells appeared not to participate in providing protection. It was suggested that NKT cells play a key role in defining the resistance and susceptibility of mice against *L. major*, probably by producing IFN- γ and IL-4 respectively. More recently, it was reported that NKT cells lowered *L. major* burden, and exerted bystander effects on *Leishmania*

antigen-specific T cell responses in the spleen.⁷ In visceral *Leishmania donovani* infection, the parasite burden was significantly higher in the liver and spleen of CD1d^{-/-} mice compared to wild-type mice.⁸

Following intraperitoneal infection with *Toxoplasma cruzi*, mice without NKT cells died from immunopathology, as opposed to rampant parasite infection, indicating that in this system NKT cells have a beneficial, anti-inflammatory role.⁹ Ronet *et al.* also found this protective effect.¹⁰ After infection with *Toxoplasma gondii*, NKT cell-deficient mice (J α 281^{-/-}) are more resistant than C57BL/6 mice to the development of lethal ileitis. J α 281^{-/-} mice failed to overexpress IFN- γ in the intestine early after infection. Treatment with α -GalCer blocked this detrimental effect of NKT cells, which prevents death in C57BL/6, but not in NKT cell deficient mice. It was suggested the mediator role of NKT cells in the parasite infection by shifting the cytokine profile toward a Th1 or Th2 response. Mallevaey *et al.* reported that NKT cells exhibited an activated phenotype during murine *S. mansoni* infection *in vivo* and *schistosome* egg-sensitized DCs activate NKT cells to secrete IFN- γ and IL-4 *in vitro*.¹¹

These examples proved that NKT cells play a role during parasite infections. The administration of cytokine profiles during the infections would be helpful to elucidate this issue.

In murine malaria infections, it is observed that an initial Th1 response switches to Th2 after 7 to 10 days of infection.¹² Another report suggested that this process was regulated by CD1d-restricted NKT cells.¹³

NKT cells promote disease progress in BALB/c mice infected with *L. major*, via

the secretion of IL-4, and cause the deviation of the immune response from protective Th1 cytokines to disease-promoting Th2 cytokines.¹⁴ Gumy *et al.* reported that in the murine model of infection with *L. major* cytokines played an important role in Th cell differentiation, which induced the immune responses to prevent and/or control infections with pathogens.¹⁵

In *S. mansoni* infections, it was reported that CD1d^{-/-} mice had a reduced Th2 response after egg laying and developed a less marked fibrotic pathology compared with wild-type mice.¹⁶ Mallevaey *et al.* reported that transfer to schistosome egg-sensitized DCs promoted a strong Th2 response *in vivo*.¹¹

Bruner *et al.* reported that wild-type mice were protected from superinfection of *Schistosoma mansoni*, but resistance is abrogated in the absence of IL-4.¹⁷ This conclusion, together with Th2 cytokines promote disease progress in BALB/c mice infected with *L. major*¹⁴, suggests a crucial role of Th2 cytokines in parasite infections.

Studies with different parasite infections illustrate the perplexing dual nature of NKT cell function and a shifting immune response from Th1 to Th2 during parasite infections. One of the possible reasons for deleterious effects of NKT cells is the parasite-immune-evasion mechanism by which surface presentation of host-derived antigens and self-made glycolipids mimicking host antigens may contribute to parasite camouflage or induce autoimmune processes.¹⁸

Another possible reason for the dual roles of NKT cells is concomitant immunity (CI). CI is characterized by the ability of a host to mount an effective defense against

larva stages while being unable to clear a persistent burden of adult parasites.^{19,20}

Adult parasites constantly excrete and secrete antigens into the blood, thereby stimulating the immune system against larva. In addition, the immune system recognizes the antigens from larva itself. These two distinct sources of antigen strengthen immunity against larva challenge. This mechanism creates a barrier against continual infection and restricts burden size within the host.

The two mechanisms described above indicate that parasites are capable of affecting the immune responses by secreting and excreting glycolipids. These glycolipids may be originated from parasites themselves or made by parasites to mimic host antigens. Since NKT cells activated by different glycolipid antigens can release cytokines leading to Th1 and/or Th2 type responses, it could be very useful to identify target glycolipid antigens from parasites and understand their regulation after parasite infections. Moreover, there are many glycolipids that are not identified from human bodies due to low detection limits and low abundances of many glycolipids. The uptake of host glycolipids by parasites might cause accumulation of rare host glycolipids in parasites, and therefore identification of glycolipids from parasites may provide a means of identifying NKT cell self-antigens.

4.2. Identification of Glycolipids from *Fasciola hepatica*

F. hepatica is a causative agent of fascioliasis, which affects sheep, cattle, various other animal species, and humans. Recently, Two types of neutral glycosphingolipids from sheep-derived *Fasciola hepatica* liver flukes were isolated and characterized.²¹

The first type of glycolipids represents mammalian-type species comprising globo- and isoglobotrihexosylceramides, as well as the Forssman antigen. One of the isoglobotrihexosylceramides is structurally characterized as Gal α 1-3Gal β 1-4Glc β 1-1Cer (iGb3') (Figure 2). iGb3' has the same sugar head group with iGb3, which indicates that iGb3' could directly stimulate NKT cells. As we know, parasite antigens that unequivocally activate the TCR of NKT cells have not been reported. Therefore, identification of iGb3' from *F. hepatica* was a first breakthrough in the research of glycolipid antigens from parasites that directly stimulate NKT cells. The Forssman antigen (GalNAc α 1-3GalNAc β 1-3/4Gal α 1-3/4Gal β 1-4Glc β 1-1Cer) from the parasite and sheep host led to identical MALDI-TOF MS profiles, indicating that it might be acquired from the host. This could be an evidence of uptake of the blood glycolipid antigens by the parasite. Interestingly, β -hexosaminidases can degrade Forssman Ag-1 to iGb3'.

The second type of antigenic glycolipids exhibit terminal Gal β 1-6Gal-units (named as Neo-iGbs, Figure 2) and was recognized as cross-reacting target structures of antibodies from various *F. hepatica* and cestode infection sera.²² This type of *F. hepatica* glycosphingolipid motif, structurally related to iGb3, is not shared with schistosomes.²³ The role for these glycolipids in host-parasite interactions remains to be defined. Synthesis of these glycolipids will confirm the structures and identify their abilities to stimulate NKT cells.



Figure 1. Representative of structure of glycolipids isolated from *F. hepatica*.



Figure 2. Structures of iGb3' and neo-iGb's

4.3. Synthesis and Analysis

Syntheses of iGb3' has been reported in Chapter 1. As I know, the syntheses of Neo-Gb3, Neo-iGb4 have not been reported. Neo-Gb3, Neo-iGb4A and Neo-iGb4B with a C24 acyl chain in the ceramide moiety were selected as the synthetic targets.

4.3.1. Synthesis of Neo-Gb3



Scheme 1. Synthesis of neo-Gb3

The glycosylation method used in this chapter is the trichloroacetimidate method developed by Schmidt *et al.*²⁴ Synthesis of Neo-Gb3 is given in Scheme 1. The coupling of galactoside **4-1** with diol **4-2**²⁵ occurred at the less hindered primary

alcohol, giving trisaccharide **4-3** and its β anomer with the ratio of 4.8 to 1. Hydrogenation of **4-3** followed by acylation in the pyridine afforded fully protected trisaccharide **4-4**, which was transformed to donor **4-5** by using TFA. Glycosylation of **4-5** with ceramide **C1** followed by deprotection gave the desired product, Neo-Gb3.

4.3.2. Retrosynthesis of neo-iGb4



Scheme 2. Retrosynthesis of neo-iGb4

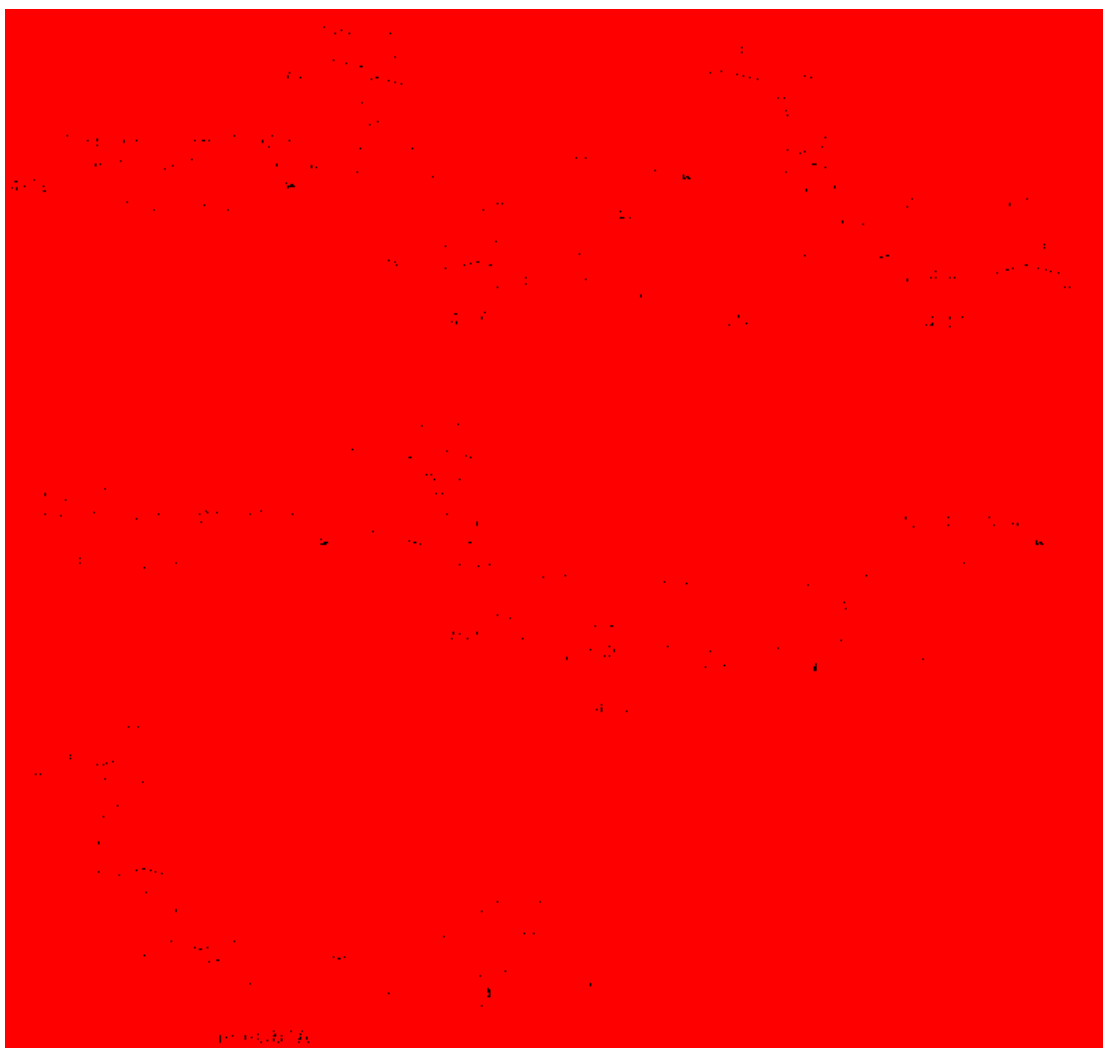
neo-iGb4A and neo-iGb4B contain the same lactosylceramide moiety with iGb3. To make the synthesis convergent, we chose distal disaccharide **4-7**, lactose **S1** and ceramide **C1** as the building blocks (Scheme 2). Initially, in the synthesis of Neo-iGb4A the assembly of three building blocks began with **4-7** and **S1**. The resulted tetrasaccharide then was coupled with **C1**, giving the fully protected neo-iGb4A. To avoid the contamination of trace amount of neo-iGb4 anomer with α glycosidic bond

connected to ceramide, we made β -Laccer first and tested its stimulatory property of NKT cells. The non-stimulatory β -Laccer was then glycosylated with **4-7** to afford neo-iGb4.

4.3.3. Synthesis of neo-iGb4A

Synthesis of neo-iGb4A started with an appropriate protected galactose **4-8**²⁶(Scheme 3). The glycosylation of **4-1** with **4-8** gave the desired disaccharide **4-9** and its β anomer ($\alpha:\beta = 1.3:1$) in decent yield. The remaining alcohol of **4-9** was protected as acetate before **4-9** was transformed to donor **4-10**. The coupling of **4-10** with lactose **S1** afforded a mixture of two products that are difficult to separate at this stage. Fortunately, the mixture was effectively separated after acylation to give **4-11** and **4-12** (**4-11/4-12** = 1.4/1). The formation of **4-12** was due to the high activity of the fully benzylated donor which enabled the nucleophilic attack of the axial hydroxyl group of the acceptor. Removal of all benzyl ether of **4-11** and the resulting alcohols was acylated to afford **4-13**. With **4-13** in hands, neo-iGb4A was obtained in a closely related process used in the preparation of neo-iGb3.

Interestingly, compound **4-12** contains the same internal trisaccharide moiety with Gb3, which triggered us to synthesize neo-Gb4A from **4-12** with the same process used in the synthesis of neo-iGb4A (Scheme 4). Since Gb3, the degraded product of neo-Gb4A, does not stimulate NKT cells *in vivo* and *in vitro*, neo-Gb4A is not an antigen for NKT cells. neo-Gb4A would be used as reference in the evaluation of the stimulant properties of neo-iGb4's.



Scheme 4. Synthesis of neo-Gb4A

We also prepared neo-iGb4A in a different manner (Scheme 5). An appropriate protected galactose **4-19**²⁷ was used in the glycosylation instead of **4-8**, giving **4-20**. The liberation of the C1 hydroxyl group of **4-20** and then coupling to LacCer **S2** gave an inseparable mixture of anomers. Treatment of the anomers with Ac₂O in pyridine enabled the separation, giving **4-22** and **4-23** with a ratio of 1.1 to 1. neo-iGb4A and neo-Gb4A were generated after fully deprotection.



Scheme 5. Synthesis of neo-iGb4A and neo-Gb4A from **S2**

neo-iGb4B was obtained in a closely related process used in the preparation of neo-iGb4A (Scheme 6). Noteworthy, the glycosylation of **4-26** with **S2** gave predominately α anomer **27** which was able to be separated from its diastereomer, while **22** and **23** were obtained with a ratio of 1.1 to 1 in the glycosylation of **21** with **S2**. These two distinct results demonstrated the influences of the substitution groups at C6 position of donor, which was consistent with the result that the coupling of the glucuronic acid with ceramide gave GSL-1 in poor yield (Chapter 3).



Scheme 6. Synthesis of neo-iGb4B

4.4. Results and Discussion

neo-iGb's were characterized and tested for their abilities to stimulate V α 14NKT cells. Stimulation of V α 14 NKT cell hybridomas on CD1d-coated plates and intracellular cytokine staining of tetramer-positive cells were performed as described in published protocols.²⁸ Supernatant levels of IL-2, IL-4 and IFN- γ were measured by the enzyme-linked immunosorbent assay (ELISA).

As we discussed before, if neo-iGb4A is truncated by enzyme to generate the antigen iGb3, it will stimulate NKT cells to produce cytokines. This truncation usually happens in the lysosome, which requires the lipid transfer proteins to deliver

the glycolipids. This trafficking was observed by the truncation of α GalCer with an addition sugar (Chapter 1). Our observation showed that none of neo-iGb's stimulates NKT cells. One explanation is that neo-iGb4s can not enter the lysosome for lacking the lipid transfer proteins, thus can not be truncated by the enzymes. The synthesis and secretion of these neo-iGb's might be a mechanism of immune evasion.

More experiments using different cell lines and APCs are still ongoing.

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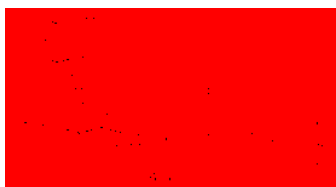
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Experimental Procedures:



Preparation of 4-3: Compound **4-1** (160 mg, 0.23 mmol) and **4-2** (180 mg, 0.19 mmol) in anhydrous CH₂Cl₂ (8 mL) was added 4 Å molecular sieves (400 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 18 h and filtered through a celite pad. The filter cake was washed with EtOAc/Hexanes (60 mL, 1:2). The combined filtrate was concentrated. The residue was purified by SiO₂ column chromatography (EtOAc/Hexanes 1:2) to afford **4-3** as a clear oil (120 mg, 42%) and its β anomer (25 mg, 8.8%). ¹H NMR (CDCl₃, 500 MHz) δ 8.01-7.94 (m, 10 H), 7.59-7.10 (m, 35 H), 5.74 (t, *J* = 9.5 Hz, 1 H), 5.76 (dd, *J* = 10.0, 3.0 Hz, 1 H), 5.74 (dd, *J* = 8.0, 3.0, 1 H), 5.38 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.16 (dd, *J* = 10.0, 3.0 Hz, 1 H), 4.96 (d, *J* = 12.0 Hz, 1 H), 4.77 (d, *J* = 11.0 Hz, 1 H), 4.75 (d, *J* = 7.5 Hz, 1 H), 4.71 (d, *J* = 2.0 Hz, 1 H), 4.69 (d, *J* = 6.0 Hz, 1 H), 4.65 (d, *J* = 9.5 Hz, 1 H), 4.62 (d, *J* = 12.5 Hz, 2 H), 4.59 (d, *J* = 11.5 Hz, 2 H), 4.55 (d, *J* = 12.0 Hz, 1 H), 4.49 (d, *J* = 11.5 Hz, 1 H), 4.42 (dd, *J* = 12.0, 5.0 Hz, 1 H), 4.20-4.11 (m, 2 H), 3.97 (d, *J* = 3.0 Hz, 1 H), 3.95 (dd, *J* = 10.5, 4.0 Hz, 1 H), 3.91 (td, *J* = 10.5, 5.5 Hz, 1 H), 3.82-3.79 (m, 2 H), 3.77 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.62-3.52 (m, 3 H), 3.39 (dd, *J* = 7.5, 4.0 Hz, 1 H), 3.28 (dd, *J* = 10.5, 4.5 Hz, 1 H), 2.95 (dd, *J* = 10.5, 8.5 Hz, 1 H), 2.83 (d, *J* = 3.0 Hz, 1 H), 0.91-0.78 (m, 2 H), -0.11 (t, *J* = 3.5 Hz, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 165.90, 165.87, 165.42, 165.29, 165.21, 138.76, 138.28, 138.05, 133.42, 133.21, 130.05, 129.98,

129.91, 129.81, 129.65, 129.36, 129.06, 128.65, 128.57, 128.48, 128.42, 128.11, 128.05, 127.99, 127.79, 127.76, 127.58, 101.84, 100.28, 99.13, 78.91, 76.29, 74.98, 74.85, 74.01, 73.89, 73.70, 73.25, 73.08, 73.00, 72.62, 72.18, 70.22, 69.76, 68.89, 67.56, 66.79, 66.21, 62.70, 60.57, 17.95, -1.41; HRMS (ESI) calcd for $C_{86}H_{92}NO_{21}Si[M+NH_4]^+$: 1502.59256, found: 1502.59260.



Preparation of 4-4: Compound **4-3** (120 mg, 0.081 mmol) was dissolved in THF (10 mL) and MeOH (10 mL) and transferred to a hydrogenation vessel. Palladium on carbon (10%, 100 mg) was added to the vessel, and the vessel was subjected to H_2 (300 psi) at room temperature for 12 h. The catalyst was then removed via filtration through a celite pad, and the filtrate was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (6 mL) and pyridine (4 mL) followed by introduction of a catalytic amount of DMAP (20 mg) and Ac_2O (50 μ L, 0.53 mmol). The solvent was removed *in vacuo* after 12 h and the residue was chromatographed (SiO_2 , EtOAc/hexane 1:1) to afford **4-4** as a white solid (82 mg, 76%). 1H NMR ($CDCl_3$, 500 MHz) δ 8.00-7.78 (m, 10 H), 7.62-7.25 (m, 15 H), 5.72 (t, $J = 9.5$ Hz, 1 H), 5.59 (dd, $J = 10.5, 7.5$ Hz, 1 H), 5.43 (dd, $J = 10.5, 3.5$ Hz, 2 H), 5.35 (dd, $J = 9.5, 8.0$ Hz, 1 H), 5.20 (dd, $J = 10.5, 3.5$ Hz, 1 H), 5.12 (dd, $J = 10.5, 3.5$ Hz, 1 H), 5.04 (dd, $J = 11.0, 3.5$ Hz, 1 H), 4.81 (d, $J = 8.0$ Hz, 1 H), 4.71 (d, $J = 8.0$ Hz, 1 H), 4.57 (dd, $J = 11.5, 2.0$ Hz, 1 H), 4.50 (d, $J = 3.5$ Hz, 1 H), 4.41 (dd, $J = 12.0, 4.5$ Hz, 1 H), 4.21-3.99 (m, 5 H), 3.89 (td, $J = 10.5,$

5.5 Hz, 1 H), 3.83 (ddd, $J = 10.0, 5.0, 2.0$, 1 H), 3.56-3.50 (m, 2 H), 3.19 (dd, $J = 10.0, 4.0$ Hz, 1 H), 2.13 (s, 3 H), 2.12 (s, 3 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 1.94 (s, 3 H), 0.91-0.75 (m, 2 H), -0.14 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.89, 170.66, 170.30, 170.07, 169.50, 165.93, 165.47, 165.34, 165.23, 165.12, 133.55, 133.49, 133.45, 133.40, 133.24, 130.14, 129.92, 129.85, 129.81, 129.66, 128.99, 128.82, 128.59, 128.53, 128.42, 101.40, 100.38, 97.21, 76.58, 73.80, 72.89, 72.06, 72.04, 71.20, 69.94, 67.96, 67.58, 67.54, 67.14, 66.64, 66.52, 64.51, 62.59, 61.73, 20.87, 20.72, 20.69, 20.44, 17.96, -1.42; HRMS (ESI) calcd for $\text{C}_{68}\text{H}_{78}\text{NO}_{26}\text{Si}[\text{M}+\text{NH}_4]^+$: 1352.45758, found: 1352.45707.



Preparation of 4-6: Compound **4-4** (82 mg, 0.061 mmol) was dissolved in TFA (4 mL) and CH_2Cl_2 (2 mL) and cooled to 0 °C. The solvent was removed *in vacuo* after 1 h, and the residue was purified by SiO_2 chromatography (EtOAc/hexanes 1:2) to afford **4-5** as a white solid (68 mg, 90%). To a solution of **4-5** (68 mg, 0.055 mmol) in CCl_3CN (4 mL) was added K_2CO_3 (100 mg), the mixture was stirred for 3 h. K_2CO_3 was removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (5 mL) with **C1** (34 mg, 0.045 mmol) and AW 300 molecular sieves (200 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*.

The residue was purified by SiO₂ column chromatography (EtOAc/hexanes 1:1) to afford **4-6** as a clear oil (48 mg, 54%). ¹H NMR (CDCl₃, 500 MHz) δ 8.02-7.95 (m, 8 H), 7.79-7.78 (m, 2 H), 7.65-7.25 (m, 15 H), 5.87 (d, *J* = 9.5 Hz, 1 H), 5.73 (t, *J* = 9.5 Hz, 1 H), 5.59 (dd, *J* = 10.5, 8.0 Hz, 1 H), 5.45 (d, *J* = 3.0 Hz, 1 H), 5.41 (d, *J* = 3.5 Hz, 1 H), 5.30 (dd, *J* = 10.0, 7.5 Hz, 1 H), 5.19 (dd, *J* = 10.5, 3.5 Hz, 1 H), 5.12 (dd, *J* = 11.0, 3.5 Hz, 1 H), 5.07-5.03 (m, 2 H), 4.85 (dt, *J* = 9.5, 3.0 Hz, 1 H), 4.79 (d, *J* = 8.0 Hz, 1 H), 4.61 (d, *J* = 8.0 Hz, 1 H), 4.54 (dd, *J* = 12.5, 4.0 Hz, 1 H), 4.49 (d, *J* = 3.5 Hz, 1 H), 4.41 (dd, *J* = 12.5, 4.5 Hz, 1 H), 4.26 (tt, *J* = 9.0, 3.0 Hz, 1 H), 4.20 (dd, *J* = 11.0, 6.5 Hz, 1 H), 4.15 (t, *J* = 9.5 Hz, 1 H), 4.10 (dd, *J* = 11.0, 6.5 Hz, 1 H), 4.01 (t, *J* = 6.5 Hz, 1 H), 3.91 (dd, *J* = 10.0, 2.5 Hz, 1 H), 3.82 (ddd, *J* = 10.0, 4.5, 2.0 Hz, 1 H), 3.52 (dd, *J* = 10.0, 4.5 Hz, 1 H), 3.43 (dd, *J* = 9.5, 3.0 Hz, 1 H), 3.23 (dd, *J* = 10.0, 4.0 Hz, 1 H), 2.46 (t, *J* = 10.0, 1 H), 2.14 (s, 3 H), 2.13 (s, 3 H), 1.99 (s, 6 H), 1.97 (s, 3 H), 1.93 (s, 3 H), 1.99-1.88 (m, 2 H), 1.85 (s, 3 H), 1.57-1.48 (m, 2 H), 1.37-1.00 (m, 66 H), 0.88 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 172.91, 171.06, 171.00, 170.75, 170.39, 170.20, 169.82, 169.55, 165.98, 165.53, 165.40, 165.16, 165.15, 133.70, 133.63, 133.57, 130.02, 129.95, 129.88, 129.72, 129.58, 129.21, 129.00, 128.77, 128.73, 128.64, 128.60, 101.43, 100.61, 97.29, 76.20, 73.23, 72.98, 72.25, 72.09, 71.92, 71.25, 69.90, 67.97, 67.56, 67.15, 66.70, 66.49, 64.57, 62.55, 61.75, 47.52, 36.40, 32.12, 29.91, 29.80, 29.74, 29.57, 29.51, 29.34, 28.26, 25.75, 25.51, 22.89, 21.19, 20.98, 20.82, 20.77, 20.70, 20.50, 14.33; HRMS (ESI) calcd for C₁₀₉H₁₄₉NaO₃₁[M+Na]⁺: 1991.00058, found: 1990.99986.



Preparation of neo-Gb3: Compound **4-6** (48 mg, 0.024 mmol) was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in MeOH). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was purified by SiO₂ column chromatography (MeOH/CH₂Cl₂/H₂O 20:80:3) to afford **neo-Gb3** (21 mg, 75%) as a white solid. ¹H NMR (DMSO-d₆/D₂O, 500 MHz) δ 7.62 (d, *J* = 8.5 Hz, 1 H), 5.07 (brs, 1 H), 5.02 (brs, 1 H), 4.74-4.71 (m, 2 H), 4.56-4.39 (m, 4 H), 4.28 (brs, 1 H), 4.24 (t, *J* = 3.5 Hz, 1 H), 4.18 (d, *J* = 7.5 Hz, 1 H), 4.14 (brs, 1 H), 3.96 (brs, 1 H), 3.90 (t, *J* = 4.5 Hz, 1 H), 3.73-3.26 (m, 11 H), 3.06 (t, *J* = 6.5 Hz, 1 H), 2.10-2.03 (m, 2 H), 1.53-1.42 (m, 4 H), 1.30-1.10 (m, 64 H), 0.85 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (DMSO-d₆/D₂O, 125 MHz) δ 171.85, 103.60, 103.33, 98.84, 80.12, 74.68, 74.38, 73.40, 73.11, 73.05, 72.93, 71.23, 70.59, 70.32, 69.72, 69.48, 68.84, 68.53, 68.24, 66.25, 60.63, 60.21, 50.13, 35.50, 31.20, 30.73, 29.18, 29.02, 28.89, 28.64, 28.60, 25.42, 25.33, 22.06, 13.82; HRMS (ESI) calcd for C₆₀H₁₁₅NaO₁₉[M+Na]⁺: 1176.79555, found: 1176.79419.



Preparation of 4-9: Compound **4-1** (365 mg, 0.53 mmol) and **4-8** (280 mg, 0.61 mmol) in anhydrous CH₂Cl₂ (10 mL) was added 4 Å molecular sieves (500 mg). The

mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 18 h and filtered through a celite pad. The filter cake was washed with EtOAc/Hexanes (80 mL, 1:2). The combined filtrate was concentrated. The residue was purified by SiO₂ column chromatography (EtOAc/Hexanes 1:3) to afford **4-9** as a clear oil (220 mg, 42%) and its β anomer (175 mg, 33%). **4-9**: ¹H NMR (CDCl₃, 500 MHz) δ 7.27-7.09 (m, 30 H), 4.86 (d, *J* = 3.5 Hz, 1 H), 4.82 (d, *J* = 4.0 Hz, 1 H), 4.79 (d, *J* = 3.0 Hz, 1 H), 4.72 (d, *J* = 11.5 Hz, 1 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 4.62-4.60 (m, 3 H), 4.52 (s, 2 H), 4.44 (d, *J* = 11.5 Hz, 1 H), 4.35 (d, *J* = 12.0 Hz, 1 H), 4.26 (d, *J* = 11.5 Hz, 1 H), 4.21 (d, *J* = 7.5 Hz, 1 H), 3.94-3.91 (m, 2 H), 3.89-3.84 (m, 2 H), 3.82 (t, *J* = 6.5 Hz, 1 H), 3.78 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.75 (dd, *J* = 10.5, 5.5 Hz, 1 H), 3.69 (dd, *J* = 10.5, 6.0 Hz, 1 H), 3.53 (t, *J* = 8.5 Hz, 1 H), 3.46-3.40 (m, 4 H), 3.34 (dd, *J* = 9.5, 3.5 Hz, 1 H), 2.63 (brs, 1 H), 0.89 (t, *J* = 8.5 Hz, 2 H), 0.12 (s, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 138.96, 138.90, 138.76, 138.68, 138.24, 138.06, 128.55, 128.50, 128.43, 128.37, 128.26, 128.04, 128.00, 127.92, 127.87, 127.76, 127.71, 127.61, 127.51, 103.34, 98.61, 80.67, 79.27, 79.15, 76.44, 75.33, 75.02, 74.88, 73.61, 73.24, 73.20, 72.60, 72.34, 69.60, 69.50, 67.37, 67.33, 67.02, 18.58, -1.23; HRMS (ESI) calcd for C₅₉H₇₄NO₁₁Si[M+NH₄]⁺: 1000.50257, found: 1000.50201. **4-9β**: ¹H NMR (CDCl₃, 500 MHz) δ 7.44-7.31 (m, 30 H), 5.00-4.95 (m, 3 H), 4.82-4.73 (m, 6 H), 4.66 (d, *J* = 12.0 Hz, 1 H), 4.53 (d, *J* = 7.5 Hz, 1 H), 4.51 (d, *J* = 10.0 Hz, 1 H), 4.42 (d, *J* = 12.5 Hz, 1 H), 4.39 (d, *J* = 8.0 Hz, 1 H), 4.11 (dd, *J* = 11.0, 5.5 Hz, 1 H), 4.06-4.01 (m, 3 H), 3.94 (d, *J* = 2.0 Hz, 1 H), 3.86 (dd, *J* = 10.0, 7.5 Hz, 1 H), 3.70 (dd, *J* = 9.5, 7.5 Hz, 1 H), 3.66-3.50 (m, 7 H),

2.65 (brs, 1 H), 1.02 (t, $J = 8.5$ Hz, 2 H), -0.18 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 138.91, 138.90, 138.73, 138.63, 138.14, 137.90, 128.57, 128.49, 128.41, 128.33, 128.23, 128.10, 127.94, 127.70, 127.65, 104.35, 103.32, 82.34, 80.59, 79.53, 79.20, 75.28, 75.14, 74.68, 73.71, 73.64, 73.54, 73.37, 73.22, 72.45, 68.73, 68.64, 67.48, 66.81, 18.55, -1.31; HRMS (ESI) calcd for $\text{C}_{59}\text{H}_{74}\text{NO}_{11}\text{Si}[\text{M}+\text{NH}_4]^+$: 1000.50257, found: 1000.50187.



Preparation of 4-11 and 4-12: Compound **4-9** (220 mg, 0.22 mmol) was dissolved in pyridine (8 mL) followed by introduction of a catalytic amount of DMAP (30 mg) and Ac_2O (50 μL , 0.53 mmol). The solvent was removed *in vacuo* after 12 h and the residue was chromatographed (SiO_2 , EtOAc/hexane 1:8) to afford a clear oil, which was dissolved in TFA (4 mL) and CH_2Cl_2 (2 mL) and cooled to 0 $^\circ\text{C}$. The solvent was removed *in vacuo* after 1 h, and the residue was purified by SiO_2 chromatography (EtOAc/hexanes 1:4) to afford **4-10** as a clear oil (180 mg, 88%). To a solution of **4-10** (180 mg, 0.19 mmol) in CCl_3CN (6 mL) was added K_2CO_3 (200 mg), the mixture was stirred for 3 h. K_2CO_3 was removed via filtration through a celite pad, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (6 mL) with **S1** (150 mg, 0.16 mmol) and 4 \AA molecular sieves (300 mg). The mixture was stirred for 1 h and cooled to 0 $^\circ\text{C}$ before addition of a catalytic

amount TMSOTf drop wise. The mixture was stirred for 12 h then filtered with a celite pad. The filtrate was concentrated *in vacuo*. The residue was purified by SiO₂ column chromatography (EtOAc/hexanes 1:2) to afford a mixture (210 mg, 70%). To a solution of the mixture in pyridine (5 mL) was added Ac₂O (50 μL, 0.53 mmol) and DMAP (30 mg). The reaction mixture was stirred for 10 h and concentrated. The residue was chromatographed (SiO₂, EtOAc/hexane 1:3) to afford **4-11** (107 mg, 50%) and **4-12** (79 mg, 37%) as white solids. **4-11**: ¹H NMR (CDCl₃, 500 MHz) δ 8.04-7.84 (m, 10 H), 7.61-7.08 (m, 45 H), 5.72 (t, *J* = 9.5 Hz, 1 H), 5.42 (dd, *J* = 10.0, 7.5 Hz, 1 H), 5.39 (dd, *J* = 9.5, 8.5 Hz, 1 H), 5.31 (d, *J* = 3.0 Hz, 1 H), 5.13 (brs, 1 H), 4.98 (d, *J* = 3.0 Hz, 1 H), 4.95 (d, *J* = 11.5 Hz, 1 H), 4.81 (d, *J* = 11.5 Hz, 1 H), 4.78 (d, *J* = 3.5 Hz, 1 H), 4.75 (d, *J* = 11.5 Hz, 2 H), 4.69 (d, *J* = 11.5 Hz, 1 H), 4.65 (d, *J* = 8.0 Hz, 1 H), 4.61 (d, *J* = 12.0 Hz, 1 H), 4.57 (d, *J* = 8.0 Hz, 1 H), 4.55 (d, *J* = 11.5 Hz, 1 H), 4.48 (d, *J* = 12.0 Hz, 1 H), 4.47-4.40 (m, 3 H), 4.37 (d, *J* = 11.5 Hz, 1 H), 4.36 (d, *J* = 11.0 Hz, 1 H), 4.13 (d, *J* = 10.5 Hz, 1 H), 4.04 (dd, *J* = 10.0, 3.5 Hz, 1 H), 4.03 (d, *J* = 3.5 Hz, 1 H), 4.00 (d, *J* = 9.5 Hz, 1 H), 3.97 (d, *J* = 5.0 Hz, 1 H), 3.94-3.92 (m, 2 H), 3.88 (td, *J* = 10.0, 5.5 Hz, 1 H), 3.84 (dd, *J* = 10.0, 3.5 Hz, 1 H), 3.77 (ddd, *J* = 10.0, 5.5, 2.0 Hz, 1 H), 3.64-3.58 (m, 3 H), 3.54-3.35 (m, 7 H), 1.93 (s, 3 H), 1.70 (s, 3 H), 0.94-0.76 (m, 2 H), -0.14 (s, 9 H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.24, 170.24, 165.95, 165.78, 165.45, 165.35, 164.70, 139.08, 138.94, 138.92, 138.71, 138.19, 138.10, 133.57, 133.52, 133.30, 133.22, 133.16, 130.13, 129.94, 129.85, 129.79, 129.74, 129.63, 128.97, 128.82, 128.61, 128.57, 128.49, 128.41, 128.37, 128.32, 128.26, 128.12, 128.08, 128.00, 127.93, 127.69, 127.60, 127.58, 127.54,

127.49, 101.08, 100.50, 98.48, 95.37, 79.22, 76.84, 76.19, 76.15, 75.23, 74.93, 74.79, 73.65, 73.52, 73.21, 73.00, 71.93, 71.79, 71.35, 71.25, 70.01, 69.40, 68.05, 67.68, 67.64, 66.60, 65.28, 62.85, 61.58, 21.03, 20.54, 17.99, -1.39; HRMS (ESI) calcd for $C_{110}H_{118}NO_{28}Si[M+NH_4]^+$: 1928.76096, found: 1928.75782. **4-12**: 1H NMR ($CDCl_3$, 500 MHz) δ 8.03-7.90 (m, 10 H), 7.59-7.02 (m, 45 H), 5.75 (d, $J = 3.0$ Hz, 1 H), 5.70 (t, $J = 9.0$ Hz, 1 H), 5.50 (dd, $J = 10.5, 8.0$ Hz, 1 H), 5.33 (dd, $J = 9.5, 7.5$ Hz, 1 H), 4.95 (d, $J = 11.5$ Hz, 1 H), 4.94-4.85 (m, 4 H), 4.78 (d, $J = 11.5$ Hz, 1 H), 4.74 (d, $J = 8.0$ Hz, 1 H), 4.71 (d, $J = 11.5$ Hz, 1 H), 4.69 (d, $J = 10.5$ Hz, 1 H), 4.63 (d, $J = 3.5$ Hz, 1 H), 4.61-4.58 (m, 3 H), 4.57 (d, $J = 3.0$ Hz, 1 H), 4.53 (d, $J = 12.0$ Hz, 1 H), 4.47 (dd, $J = 11.0, 6.5$ Hz, 1 H), 4.40 (dd, $J = 12.5, 5.0$ Hz, 1 H), 4.36 (d, $J = 10.5$ Hz, 1 H), 4.36-4.33 (m, 1 H), 4.29 (d, $J = 12.0$ Hz, 1 H), 4.23-4.05 (m, 6 H), 4.03 (d, $J = 2.0$ Hz, 1 H), 3.98 (dd, $J = 11.5, 6.0$ Hz, 1 H), 3.87-3.82 (m, 2 H), 3.73 (ddd, $J = 9.5, 4.5, 2.0$ Hz, 1 H), 3.70 (dd, $J = 9.5, 4.0$ Hz, 1 H), 3.64 (dd, $J = 10.5, 4.0$ Hz, 1 H), 3.54 (t, $J = 6.0$ Hz, 1 H), 3.49-3.35 (m, 4 H), 2.03 (s, 3 H), 1.67 (s, 3 H), 0.88-0.74 (m, 2 H), -0.15 (s, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.98, 170.14, 165.94, 165.72, 165.39, 165.36, 164.81, 139.45, 139.13, 139.01, 138.63, 138.40, 138.25, 133.39, 133.25, 133.22, 133.15, 130.01, 129.97, 129.80, 129.76, 129.72, 129.16, 128.75, 128.68, 128.54, 128.51, 128.43, 128.40, 128.33, 128.20, 127.89, 127.76, 127.70, 127.65, 127.51, 127.40, 101.43, 101.30, 100.37, 98.39, 78.95, 76.90, 76.68, 76.27, 75.48, 74.96, 74.75, 73.70, 73.47, 73.40, 73.35, 73.26, 72.99, 72.82, 72.42, 71.95, 69.95, 69.75, 69.47, 68.55, 67.85, 67.58, 65.79, 62.76, 62.31, 21.13, 20.80, 18.01,

-1.37; HRMS (ESI) calcd for $C_{110}H_{118}NO_{28}Si[M+NH_4]^+$: 1928.76096, found: 1928.76964.



Preparation of 4-13: Compound **4-11** (107 mg, 0.056 mmol) was dissolved in THF (10 mL) and MeOH (10 mL) and transferred to a hydrogenation vessel. Palladium on carbon (10%, 100 mg) was added to the vessel, and the vessel was subjected to H_2 (300 psi) at room temperature for 12 h. The catalyst was then removed via filtration through a celite pad, and the filtrate was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (6 mL) and pyridine (4 mL) followed by introduction of a catalytic amount of DMAP (30 mg) and Ac_2O (100 μ L, 1.06 mmol). The solvent was removed *in vacuo* after 12 h and the residue was chromatographed (SiO_2 , EtOAc/hexane 1:1) to afford **4-13** as a white solid (63 mg, 69%). 1H NMR ($CDCl_3$, 500 MHz) δ 8.18-7.27 (m, 25 H), 5.74 (t, $J = 9.5$ Hz, 1 H), 5.49 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.47-5.45 (m, 2 H), 5.40 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.32 (dd, $J = 10.5, 3.5$ Hz, 1 H), 5.23 (d, $J = 3.5$ Hz, 1 H), 5.22 (dd, $J = 11.0, 3.5$ Hz, 1 H), 5.11 (dd, $J = 11.0, 3.5$ Hz, 1 H), 4.98 (d, $J = 8.0$ Hz, 1 H), 4.93 (d, $J = 3.5$ Hz, 1 H), 4.89 (dd, $J = 11.5, 3.0$ Hz, 1 H), 4.65 (d, $J = 8.0$ Hz, 1 H), 4.63 (d, $J = 2.5$ Hz, 1 H), 4.53 (dd, $J = 11.5, 7.5$ Hz, 1 H), 4.41 (dd, $J = 12.0, 2.0$ Hz, 1 H), 4.33-4.39 (m, 1 H), 4.18-4.13 (m, 2 H), 3.95-3.65 (m, 8 H), 3.52-3.44 (m, 2 H), 3.11 (dd, $J = 10.5, 3.5$ Hz, 1 H), 2.17 (s, 3 H), 2.02 (s, 3 H), 2.01

(s, 9 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.84 (s, 3 H), 0.89-0.75 (m, 2 H), -0.18 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.03, 170.92, 170.82, 170.38, 170.21, 170.03, 169.90, 169.39, 165.90, 165.87, 165.52, 165.38, 164.73, 136.20, 133.68, 133.31, 133.20, 130.22, 129.97, 129.58, 129.02, 128.71, 128.60, 128.52, 128.45, 128.40, 123.95, 100.65, 100.45, 95.87, 92.72, 76.57, 73.70, 73.56, 73.14, 72.08, 70.90, 70.89, 68.60, 68.50, 68.18, 67.77, 67.68, 67.57, 67.54, 67.10, 66.85, 66.52, 64.91, 63.33, 62.91, 61.51, 20.94, 20.85, 20.73, 17.97, -1.43; HRMS (ESI) calcd for $\text{C}_{80}\text{H}_{94}\text{NO}_{34}\text{Si}[\text{M}+\text{NH}_4]^+$: 1640.54210, found: 1640.53928.



Preparation of 4-15: As described in the preparation of **4-6**, compound **4-13** (63 mg, 0.039 mmol) was treated with TFA (3 mL) in CH_2Cl_2 (2 mL), giving donor **4-14** (48 mg, 81%). HRMS (ESI) calcd for $\text{C}_{75}\text{H}_{82}\text{NO}_{34}[\text{M}+\text{NH}_4]^+$: 1540.47128, found: 1540.46220. The coupling of **4-14** (48 mg, 0.032 mmol) with **C1** (28 mg, 0.037 mmol) afforded product **4-15** as a clear oil (33 mg, 46%). ^1H NMR (CDCl_3 , 500 MHz) δ 8.14 (d, $J = 7.0$ Hz, 2 H), 8.04 (d, $J = 7.5$ Hz, 2 H), 8.03 (d, $J = 6.5$ Hz, 2 H), 7.95 (d, $J = 7.5$ Hz, 2 H), 7.80 (d, $J = 7.5$ Hz, 2 H), 7.58 (t, $J = 7.5$ Hz, 1 H), 7.54-7.26 (m, 14 H), 5.79 (d, $J = 8.5$ Hz, 1 H), 5.76 (t, $J = 9.5$ Hz, 1 H), 5.49-5.46 (m, 3 H), 5.33-5.30 (m, 2 H), 5.23-5.20 (m, 2 H), 5.11 (dd, $J = 11.5, 3.5$ Hz, 1 H), 5.00 (dd, $J = 8.5, 3.0$ Hz, 1 H), 4.98 (d, $J = 8.5$ Hz, 1 H), 4.92 (d, $J = 3.5$ Hz, 1 H), 4.89 (dd, $J =$

11.5, 3.0 Hz, 1 H), 4.85 (dt, $J = 10.0, 3.0$ Hz, 1 H), 4.64 (d, $J = 3.0$ Hz, 1 H), 4.56 (d, $J = 8.5$ Hz, 1 H), 4.52 (dd, $J = 12.0, 7.0$ Hz, 1 H), 4.39 (d, $J = 11.0$ Hz, 1 H), 4.31 (dd, $J = 7.0, 5.0$ Hz, 1 H), 4.24-4.20 (m, 1 H), 4.17-4.13 (m, 2 H), 3.94-3.82 (m, 6 H), 3.69 (dd, $J = 11.0, 7.0$ Hz, 1 H), 3.60 (dd, $J = 11.0, 7.0$ Hz, 1 H), 3.50 (dd, $J = 10.0, 8.0$ Hz, 1 H), 3.36-3.34 (m, 1 H), 3.12 (dd, $J = 10.5, 3.5$ Hz, 1 H), 2.18 (s, 3 H), 2.02 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.98 (s, 3 H), 1.94 (s, 3 H), 1.84 (s, 3 H), 1.83 (s, 3 H), 1.75-1.61 (m, 2 H), 1.59-1.40 (m, 2 H), 1.39-1.01 (m, 66 H), 0.88 (t, $J = 7.0$ Hz, 3 H), 0.87 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.86, 171.04, 170.93, 170.93, 170.82, 170.38, 170.25, 170.05, 169.80, 169.69, 169.41, 165.92, 165.90, 165.39, 165.39, 164.72, 133.68, 133.63, 133.40, 133.39, 133.32, 130.18, 129.98, 129.75, 129.58, 129.32, 128.96, 128.72, 128.64, 128.48, 100.78, 100.62, 95.90, 92.80, 76.03, 73.74, 73.22, 72.52, 72.27, 70.92, 68.59, 68.50, 67.81, 67.67, 67.48, 67.09, 66.86, 66.53, 64.87, 63.28, 62.92, 61.48, 47.49, 36.41, 32.14, 29.92, 29.76, 29.58, 29.55, 29.36, 28.53, 25.76, 25.52, 22.91, 21.16, 20.95, 20.86, 20.75, 20.66, 14.35; HRMS (ESI) calcd for $\text{C}_{121}\text{H}_{166}\text{NO}_{39}[\text{M}+\text{H}]^+$: 2257.10370, found: 2257.08412.



Preparation of neo-iGb4A: Compound **4-15** (33 mg, 0.015 mmol) was dissolved in MeOH (4 mL) followed by addition of NaOMe (0.5 mL, 1 M in MeOH). The mixture

was stirred for 24 h. The solvent was removed *in vacuo* and the residue was purified by SiO₂ column chromatography (MeOH/CH₂Cl₂/H₂O 20:80:3) to afford **neo-iGb4A** (13 mg, 68%) as a white solid. ¹H NMR (DMSO-d₆/D₂O, 500 MHz) δ 4.85 (d, *J* = 3.5 Hz, 1 H), 4.72 (d, *J* = 2.5 Hz, 1 H), 4.30 (d, *J* = 8.5 Hz, 1 H), 4.23 (t, *J* = 6.5 Hz, 1 H), 4.17 (d, *J* = 7.5 Hz, 1 H), 3.94-3.86 (m, 3 H), 3.74-3.72 (m, 2 H), 3.69 (d, *J* = 2.5 Hz, 1 H), 3.64-3.26 (m, 21 H), 3.02 (t, *J* = 8.0 Hz, 1 H), 2.09-1.98 (m, 2 H), 1.50-1.00 (m, 68 H), 0.82 (t, *J* = 7.0 Hz, 6 H); ¹³C NMR (DMSO-d₆/D₂O, 125 MHz) δ 172.07, 103.52, 103.50, 98.69, 95.88, 80.70, 77.95, 74.94, 74.82, 74.54, 73.21, 73.09, 71.24, 70.59, 69.75, 69.75, 69.41, 69.04, 68.93, 68.80, 68.51, 68.20, 66.34, 64.18, 60.61, 60.61, 60.29, 60.29, 50.16, 35.63, 31.51, 31.48, 30.35, 29.51, 29.36, 29.20, 28.96, 28.90, 25.70, 25.58, 22.26, 22.27, 13.98; HRMS (ESI) calcd for C₆₆H₁₂₆NO₂₄[M+H]⁺: 1316.86643, found: 1316.86501.



Preparation of 4-16: As described in the preparation of **4-13**, **4-16** (50 mg, 75%) was obtained as a white solid from compound **4-12** (79 mg, 0.041 mmol). ¹H NMR (CDCl₃, 500 MHz) δ 8.04-7.90 (m, 9 H), 7.62-7.20 (m, 16 H), 5.75 (t, *J* = 9.5 Hz, 1 H), 5.69 (d, *J* = 3.5 Hz, 1 H), 5.66 (d, *J* = 3.0 Hz, 1 H), 5.60 (dd, *J* = 10.5, 3.5 Hz, 1 H), 5.49 (dd, *J* = 11.0, 8.0 Hz, 1 H), 5.41 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.36 (dd, *J* = 10.5, 3.5 Hz, 1 H), 5.16 (dd, *J* = 11.0, 3.5 Hz, 1 H), 5.14 (dd, *J* = 10.5, 3.5 Hz, 1 H),

4.94 (d, $J = 3.5$ Hz, 1 H), 4.93 (d, $J = 3.5$ Hz, 1 H), 4.81 (dd, $J = 11.0, 2.0$ Hz, 1 H), 4.73 (d, $J = 7.5$ Hz, 1 H), 4.67 (d, $J = 8.0$ Hz, 1 H), 4.58 (dd, $J = 10.0, 4.5$ Hz, 1 H), 4.52-4.45 (m, 2 H), 4.43 (t, $J = 7.0$ Hz, 1 H), 4.12 (t, $J = 9.0$ Hz, 1 H), 3.99 (d, $J = 2.0$ Hz, 1 H), 3.99 (dd, $J = 10.5, 7.0$ Hz, 1 H), 3.92-3.77 (m, 6 H), 3.60 (t, $J = 6.5$ Hz, 1 H), 3.52 (td, $J = 10.5, 6.5$ Hz, 1 H), 3.33 (t, $J = 9.5$ Hz, 1 H), 2.19 (s, 3 H), 2.15 (s, 3 H), 2.11 (s, 3 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 1.93 (s, 3 H), 1.86 (s, 3 H), 1.58 (s, 3 H), 0.90-0.75 (m, 2 H), -0.15 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.50, 170.83, 170.79, 170.55, 170.39, 170.01, 169.97, 169.59, 166.10, 165.72, 165.47, 165.25, 165.16, 133.75, 133.62, 133.35, 133.23, 132.92, 130.23, 130.12, 130.04, 129.89, 129.75, 129.50, 129.15, 128.92, 128.62, 128.49, 128.40, 101.64, 100.43, 99.75, 97.32, 77.67, 73.58, 73.23, 73.11, 72.53, 72.17, 69.53, 69.10, 68.50, 68.20, 68.17, 67.83, 67.71, 66.35, 64.96, 62.88, 62.04, 61.36, 21.25, 21.07, 20.94, 20.85, 20.78, 20.40, 18.03, -1.33; HRMS (ESI) calcd for $\text{C}_{80}\text{H}_{90}\text{NaO}_{34}\text{Si}[\text{M}+\text{Na}]^+$: 1645.49750, found: 1645.51812.



Preparation of 4-18: As described in the preparation of **4-15**, **4-18** (28 mg, 40%) was obtained as a white solid from compound **4-16** (50 mg, 0.031 mmol). ^1H NMR (CDCl_3 , 500 MHz) δ 7.98-7.83 (m, 10 H), 7.55-7.15 (m, 15 H), 5.78 (d, $J = 9.0$ Hz, 1 H), 5.70 (t, $J = 9.5$ Hz, 1 H), 5.61 (d, $J = 2.5$ Hz, 1 H), 5.57 (d, $J = 3.0$ Hz, 1 H), 5.51

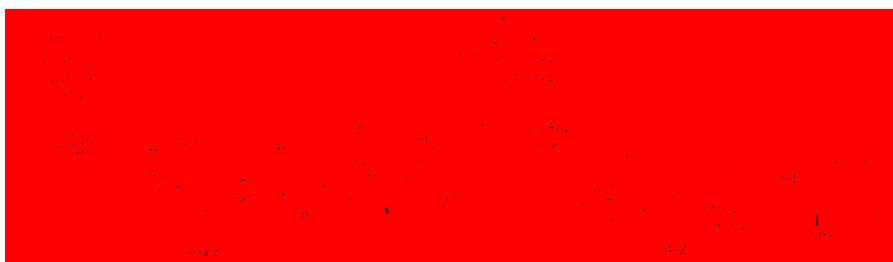
Preparation of neo-Gb4A: As described in the preparation of **neo-iGb4A**, **neo-Gb4A** (11 mg, 67%) was obtained as a white solid from compound **4-18** (28 mg, 0.012 mmol). ^1H NMR (DMSO- d_6 /D $_2$ O, 500 MHz) δ 4.77 (d, J = 3.5 Hz, 1 H), 4.67 (d, J = 3.5 Hz, 1 H), 4.26 (d, J = 7.0 Hz, 1 H), 4.21 (t, J = 6.5 Hz, 1 H), 4.17 (d, J = 8.5 Hz, 1 H), 3.94-3.88 (m, 2 H), 3.76-3.48 (m, 16 H), 3.44-3.26 (m, 9 H), 3.01 (t, J = 8.0 Hz, 1 H), 2.09-2.02 (m, 2 H), 1.50-1.10 (m, 68 H), 0.84 (t, J = 7.0 Hz, 6 H); ^{13}C NMR (DMSO- d_6 /D $_2$ O, 125 MHz) δ 172.04, 103.78, 103.45, 101.01, 99.03, 80.81, 78.75, 74.80, 74.80, 74.34, 73.33, 73.15, 72.93, 70.87, 70.87, 70.52, 69.88, 69.63, 68.97, 68.94, 68.77, 68.55, 68.46, 68.27, 66.43, 60.29, 60.29, 59.56, 50.14, 35.58, 31.39, 30.70, 29.19, 29.13, 29.05, 28.82, 28.76, 25.56, 25.48, 22.18, 14.01; HRMS (ESI) calcd for $\text{C}_{66}\text{H}_{126}\text{NO}_{24}[\text{M}+\text{H}]^+$: 1316.86643, found: 1316.86939.



Preparation of 4-20: Compound **4-1** (450 mg, 0.66 mmol) and **4-19** (300 mg, 0.54 mmol) in anhydrous CH_2Cl_2 (10 mL) was added 4 Å molecular sieves (500 mg). The mixture was stirred for 1 h and cooled to 0 °C before addition of a catalytic amount TMSOTf drop wise. The mixture was stirred for 18 h and filtered through a celite pad. The filter cake was washed with EtOAc/Hexanes (80 mL, 1:2). The combined filtrate was concentrated. The residue was chromatographed (SiO_2 , EtOAc/hexane 1:4) to afford **4-20** as a clear oil (300 mg, 51%) and its β anomer (104 mg, 18 %). ^1H NMR (CDCl_3 , 500 MHz) δ 7.36-7.14 (m, 35 H), 4.92-4.88 (m, 3 H), 4.79-4.67 (m, 6 H),

4.63 (d, $J = 12.0$ Hz, 1 H), 4.60 (d, $J = 11.5$ Hz, 1 H), 4.58 (d, $J = 11.5$ Hz, 1 H), 4.54 (d, $J = 12.0$ Hz, 1 H), 4.44 (d, $J = 11.5$ Hz, 1 H), 4.35 (d, $J = 11.5$ Hz, 1 H), 4.31 (d, $J = 7.5$ Hz, 1 H), 4.02-3.92 (m, 3 H), 3.90-3.88 (m, 2 H), 3.82 (d, $J = 3.5$ Hz, 1 H), 3.79-3.73 (m, 2 H), 3.58-3.46 (m, 6 H), 0.99 (t, $J = 8.5$ Hz, 2 H), -0.03 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 139.07, 138.93, 138.84, 138.80, 138.67, 138.00, 128.57, 128.51, 128.45, 128.38, 128.30, 128.10, 127.96, 127.89, 127.77, 127.71, 127.63, 127.51, 127.48, 103.49, 98.56, 82.30, 79.79, 79.22, 76.43, 75.28, 74.90, 74.90, 74.55, 74.05, 73.68, 73.65, 73.07, 72.99, 72.92, 69.51, 68.92, 67.39, 67.29, 18.55, -1.24; HRMS (ESI) calcd for $\text{C}_{66}\text{H}_{78}\text{NO}_{11}\text{Si}[\text{M}+\text{H}]^+$: 1073.52351, found: 1073.54234.

4-20 β : ^1H NMR (CDCl_3 , 500 MHz) δ 7.33-7.14 (m, 35 H), 4.89 (d, $J = 12.5$ Hz, 2 H), 4.87 (d, $J = 11.5$ Hz, 1 H), 4.79 (d, $J = 11.0$ Hz, 1 H), 4.72 (d, $J = 13.0$ Hz, 2 H), 4.69-4.66 (m, 3 H), 4.62 (d, $J = 13.0$ Hz, 2 H), 4.57 (d, $J = 11.5$ Hz, 1 H), 4.56 (d, $J = 12.0$ Hz, 1 H), 4.38-4.35 (m, 3 H), 4.29 (d, $J = 7.5$ Hz, 1 H), 3.92 (t, $J = 8.5$ Hz, 1 H), 3.87 (d, $J = 3.5$ Hz, 1 H), 3.83 (dd, $J = 10.5, 5.5$ Hz, 1 H), 3.79-3.71 (m, 4 H), 3.56-3.41 (m, 6 H), 0.93 (t, $J = 9.0$ Hz, 2 H), -0.12 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 139.07, 138.94, 138.85, 138.71, 138.71, 138.66, 137.99, 128.68, 128.61, 128.48, 128.41, 128.32, 128.29, 128.20, 128.06, 127.99, 127.67, 127.59, 127.10, 103.90, 103.62, 82.40, 82.32, 79.77, 79.61, 75.29, 75.12, 74.73, 74.40, 73.95, 73.66, 73.66, 73.54, 73.24, 73.13, 73.13, 68.60, 68.30, 67.53, 18.59, -1.30; HRMS (ESI) calcd for $\text{C}_{66}\text{H}_{78}\text{NO}_{11}\text{Si}[\text{M}+\text{H}]^+$: 1073.52351, found: 1073.49643.



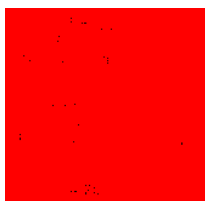
Preparation of 4-22 and 4-23: As described in the preparation of **4-11** and **4-12**, compound **4-20** (300 mg, 0.28 mmol) was treated with TFA (4 mL) and CH₂Cl₂ (2 mL), giving **4-21** (194 mg, 71%). The coupling of **4-21** (90 mg, 0.092 mmol) with **S2** (120 mg, 0.075 mmol) gave a mixture which was treated with Ac₂O (30 μL, 0.32 mmol) and DMAP (20 mg) in pyridine to afford **4-22** (55 mg, 28%) and **4-23** (42 mg, 22%) as white solids. **4-22:** ¹H NMR (CDCl₃, 500 MHz) δ 8.03-7.84 (m, 10 H), 7.63-7.07 (m, 50 H), 5.87 (d, *J* = 9.5 Hz, 1 H), 5.72 (t, *J* = 9.5 Hz, 1 H), 5.39 (dd, *J* = 9.5, 8.0 Hz, 1 H), 5.30 (d, *J* = 2.5 Hz, 1 H), 5.28 (dd, *J* = 10.0, 8.0, 1 H), 5.04 (dd, *J* = 8.5, 3.0 Hz, 1 H), 4.95 (d, *J* = 3.0 Hz, 1 H), 4.94 (d, *J* = 11.0 Hz, 1 H), 4.86 (dt, *J* = 10.0, 3.0 Hz, 1 H), 4.78 (d, *J* = 3.0 Hz, 1 H), 4.77 (d, *J* = 12.0 Hz, 1 H), 4.75 (s, 2 H), 4.71 (d, *J* = 10.5 Hz, 1 H), 4.61 (d, *J* = 12.0 Hz, 1 H), 4.59 (d, *J* = 11.5 Hz, 1 H), 4.55 (d, *J* = 11.0 Hz, 1 H), 4.55 (d, *J* = 8.5 Hz, 1 H), 4.54 (d, *J* = 8.0 Hz, 1 H), 4.50 (d, *J* = 12.5 Hz, 2 H), 4.48 (d, *J* = 9.5 Hz, 1 H), 4.43-4.39 (m, 3 H), 4.35 (d, *J* = 11.5 Hz, 1 H), 4.31 (d, *J* = 11.0 Hz, 1 H), 4.25 (tt, *J* = 9.0, 3.0 Hz, 1 H), 4.06 (d, *J* = 2.5 Hz, 1 H), 4.03 (dd, *J* = 10.0, 3.5 Hz, 1 H), 3.97 (t, *J* = 9.5 Hz, 1 H), 3.91-3.89 (m, 2 H), 3.87 (dd, *J* = 10.0, 3.5 Hz, 1 H), 3.85 (dd, *J* = 10.0, 3.5 Hz, 1 H), 3.82-3.79 (m, 2 H), 3.74 (ddd, *J* = 10.0, 5.5, 2.0 Hz, 1 H), 3.62-3.52 (m, 6 H), 3.48-3.38 (m, 4 H), 1.95 (s, 3 H), 1.82 (s, 3 H), 1.77-1.62 (m, 2 H), 1.71 (s, 3 H), 1.59-1.44 (m, 2 H), 1.39-1.01 (m, 66 H), 0.88 (t, *J* = 7.5 Hz, 3 H), 0.87 (t, *J* = 7.5 Hz, 3 H); ¹³C NMR (CDCl₃, 125 MHz) δ

172.89, 170.98, 170.19, 169.73, 165.96, 165.82, 165.42, 165.32, 164.76, 138.92, 138.80, 138.73, 138.19, 133.62, 133.49, 133.37, 133.35, 130.00, 129.92, 129.87, 129.82, 129.67, 129.59, 129.33, 129.26, 128.87, 128.71, 128.67, 128.58, 128.45, 128.42, 128.39, 128.31, 128.25, 128.16, 128.06, 128.01, 127.79, 127.70, 127.54, 127.44, 127.45, 101.10, 100.87, 98.86, 95.70, 79.68, 78.91, 76.49, 75.65, 75.08, 75.03, 74.63, 73.82, 73.70, 73.58, 73.42, 73.23, 72.89, 72.82, 72.48, 72.19, 71.56, 71.40, 69.79, 69.71, 69.30, 67.63, 66.39, 65.50, 62.79, 61.58, 47.52, 36.40, 34.16, 32.13, 29.91, 29.81, 29.75, 29.57, 29.53, 29.35, 28.48, 25.75, 25.52, 21.17, 20.66, 20.57, 14.34; HRMS (ESI) calcd for $C_{156}H_{197}N_2O_{32}[M+NH_4]^+$: 2610.38440, found: 2610.38982. **4-23**: 1H NMR ($CDCl_3$, 500 MHz) δ 8.02-7.91 (m, 10 H), 7.60-6.92 (m, 50 H), 5.86 (d, $J = 9.0$ Hz, 1 H), 5.71 (t, $J = 9.5$ Hz, 1 H), 5.53 (dd, $J = 10.5, 7.5$ Hz, 1 H), 5.25 (dd, $J = 9.0, 7.5$ Hz, 1 H), 5.02 (dd, $J = 8.5, 3.0$ Hz, 1 H), 4.96 (d, $J = 11.5$ Hz, 3 H), 4.88-4.82 (m, 4 H), 4.75 (d, $J = 10.5$ Hz, 1 H), 4.72 (d, $J = 7.5$ Hz, 1 H), 4.66-4.59 (m, 5 H), 4.56-4.52 (m, 3 H), 4.45-4.37 (m, 5 H), 4.26-4.21 (m, 3 H), 4.15-4.00 (m, 8 H), 3.89-3.87 (m, 3 H), 3.74 (dd, $J = 8.0, 4.0$ Hz, 1 H), 3.67 (ddd, $J = 10.0, 4.5, 2.0$ Hz, 1 H), 3.58 (t, $J = 6.5$ Hz, 1 H), 3.54 (t, $J = 9.5$ Hz, 1 H), 3.45 (dd, $J = 9.5, 6.5$ Hz, 1 H), 3.36 (dd, $J = 9.5, 3.0$ Hz, 1 H), 3.27 (dd, $J = 10.0, 6.5$ Hz, 1 H), 1.94 (s, 3 H), 1.82 (s, 3 H), 1.76-1.63 (m, 2 H), 1.61 (s, 3 H), 1.56-1.44 (m, 2 H), 1.39-1.02 (m, 66 H), 0.88 (t, $J = 7.5$ Hz, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.90, 171.23, 170.99, 169.80, 165.99, 165.77, 165.48, 165.38, 164.79, 139.31, 139.16, 139.00, 138.93, 138.48, 138.19, 133.65, 133.46, 133.44, 133.35, 128.99, 129.84, 129.78, 129.74, 129.63, 129.30, 129.23, 128.76, 128.72, 128.56, 128.52, 128.43,

128.40, 128.35, 128.28, 127.97, 127.78, 127.71, 127.64, 127.58, 127.54, 127.43, 101.72, 101.57, 100.71, 98.78, 79.83, 79.53, 76.62, 76.44, 76.03, 75.42, 75.12, 74.84, 74.76, 73.80, 73.54, 73.42, 73.32, 73.21, 72.84, 72.64, 72.21, 69.91, 69.82, 69.74, 69.62, 67.59, 65.31, 62.65, 62.26, 47.52, 36.41, 32.14, 29.92, 29.81, 29.75, 29.58, 29.53, 29.36, 28.50, 25.76, 25.53, 22.91, 21.16, 20.92, 20.67, 14.34; HRMS (ESI) calcd for C₁₅₆H₁₉₇N₂O₃₂[M+NH₄]⁺: 2610.38440, found: 2610.38198.

Preparation of neo-iGb4A: Compound **4-22** (55 mg, 0.021 mmol) was dissolved in THF (5 mL) and MeOH (5 mL) and transferred to a hydrogenation vessel. Palladium on carbon (10%, 50 mg) was added to the vessel, and the vessel was subjected to H₂ (300 psi) at room temperature for 36 h. The catalyst was then removed via filtration through a celite pad, and the filtrate was removed *in vacuo*. The residue was dissolved in MeOH (4 mL) and THF (2 mL) followed by addition of NaOMe (0.5 mL, 1 M in MeOH). The mixture was stirred for 24 h. The solvent was removed *in vacuo* and the residue was purified by SiO₂ column chromatography (MeOH/CH₂Cl₂/H₂O 20:80:3) to afford **neo-iGb4A** (15 mg, 54%) as a white solid. The NMR spectra are identical to those of the **neo-iGb4A** synthesized previously.

Preparation of neo-Gb4A: **neo-Gb4A** (13 mg, 62%) was obtained from **4-23** (42 mg, 0.016 mmol). The NMR spectra are identical to those of the **neo-Gb4A** synthesized previously.



Preparation of 4-25: As described in the preparation of **4-20**, the coupling of **4-24** (372 mg, 0.50 mmol) with **4-19** (228 mg, 0.41 mmol) gave the desired compound **4-25** (292 mg, 63%) as a clear oil. ^1H NMR (CDCl_3 , 500 MHz) δ 7.94-7.09 (m, 35 H), 5.89 (d, $J = 3.5$ Hz, 1 H), 5.68 (dd, $J = 10.5, 7.5$ Hz, 1 H), 5.50 (dd, $J = 10.0, 3.5$ Hz, 1 H), 4.83 (d, $J = 7.5$ Hz, 1 H), 4.78 (d, $J = 11.0$ Hz, 1 H), 4.77 (d, $J = 11.5$ Hz, 1 H), 4.62 (d, $J = 12.0$ Hz, 1 H), 4.58 (d, $J = 11.0$ Hz, 1 H), 4.52 (dd, $J = 11.5, 6.5$ Hz, 1 H), 4.40 (d, $J = 12.0$ Hz, 1 H), 4.34 (d, $J = 12.0$ Hz, 1 H), 4.30 (dd, $J = 11.0, 7.0$ Hz, 1 H), 4.23 (t, $J = 6.5$ Hz, 1 H), 4.17 (d, $J = 8.0$ Hz, 1 H), 3.94 (dd, $J = 10.0, 7.0$ Hz, 1 H), 3.82 (dt, $J = 9.0, 8.5$ Hz, 1 H), 3.74 (dd, $J = 10.5, 6.0$ Hz, 1 H), 3.70 (d, $J = 2.5$ Hz, 1 H), 3.62 (dd, $J = 10.0, 8.0$ Hz, 1 H), 3.40-3.35 (m, 2 H), 3.24 (dd, $J = 9.5, 3.0$ Hz, 1 H), 0.85 (t, $J = 8.5$ Hz, 1 H), -0.12 (s, 9 H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 166.18, 165.69, 165.66, 165.35, 138.85, 138.59, 138.52, 133.76, 133.59, 133.48, 130.08, 129.90, 129.86, 129.74, 129.40, 129.33, 129.08, 128.77, 128.68, 128.63, 128.46, 128.43, 128.35, 128.25, 127.73, 127.65, 127.63, 127.57, 103.48, 101.46, 82.08, 79.47, 75.28, 74.75, 73.41, 73.27, 72.77, 71.78, 71.39, 70.11, 68.21, 67.99, 67.52, 61.99, 18.49, -1.24; HRMS (ESI) calcd for $\text{C}_{66}\text{H}_{72}\text{NO}_{15}\text{Si}[\text{M}+\text{NH}_4]^+$: 1146.46657, found: 1146.46684.



Preparation of 4-27: As described in the preparation of **4-22**, **4-25** (292 mg, 0.26 mmol) was treated with TFA (4 mL) in CH₂Cl₂ (2 mL), giving **4-26** (207 mg, 77%) as a clear oil. **4-27** (63 mg, 50%) was obtained as clear oil by coupling **4-26** (60 mg, 0.058 mmol) with **C2** (77 mg, 0.048 mmol). ¹H NMR (CDCl₃, 500 MHz) δ 8.21-7.10 (m, 60 H), 5.85 (d, *J* = 3.5 Hz, 1 H), 5.82 (d, *J* = 9.5 Hz, 1 H), 5.73 (t, *J* = 9.5 Hz, 1 H), 5.57 (dd, *J* = 10.5, 8.0 Hz, 1 H), 5.42 (t, *J* = 8.5 Hz, 1 H), 5.29 (dd, *J* = 9.5, 8.0 Hz, 1 H), 5.18 (dd, *J* = 10.5, 3.5 Hz, 1 H), 5.01 (dd, *J* = 8.5, 3.0 Hz, 1 H), 4.86 (dt, *J* = 10.0, 2.5 Hz, 1 H), 4.69 (d, *J* = 11.5 Hz, 1 H), 4.64 (d, *J* = 11.0 Hz, 1 H), 4.56-4.47 (m, 6 H), 4.41 (dd, *J* = 12.0, 5.0 Hz, 1 H), 4.33 (dd, *J* = 11.0, 7.0 Hz, 1 H), 4.27-4.23 (m, 1 H), 4.21 (dd, *J* = 11.5, 5.0 Hz, 1 H), 4.13 (t, *J* = 10.0 Hz, 1 H), 3.91-3.77 (m, 6 H), 3.72-3.64 (m, 3 H), 3.60-3.55 (m, 4 H), 3.50 (brs, 1 H), 3.47-3.42 (m, 1 H), 3.42-3.35 (m, 2 H), 3.09 (dd, *J* = 11.0, 6.0 Hz, 1 H), 1.94 (s, 3 H), 1.82 (s, 3 H), 1.78-1.64 (m, 2 H), 1.58-1.47 (m, 2 H), 1.39-1.02 (m, 66 H), 0.88 (t, *J* = 6.5 Hz, 6 H); ¹³C NMR (CDCl₃, 125 MHz) δ 172.90, 170.95, 169.79, 166.33, 165.30, 165.95, 165.81, 165.73, 165.69, 165.42, 165.34, 164.90, 138.73, 138.40, 137.42, 133.92, 133.78, 133.64, 133.48, 133.20, 130.19, 130.03, 129.90, 129.78, 129.68, 129.57, 129.42, 129.30, 129.17, 128.94, 128.83, 128.68, 128.55, 128.45, 128.33, 127.67, 127.58, 127.41, 102.33, 100.86, 100.86, 96.58, 79.43, 78.66, 75.58, 75.15, 74.79, 74.63, 73.68, 73.35, 73.20, 72.66, 72.50, 72.45, 72.31, 71.80, 71.57, 71.09, 71.03, 69.57, 69.06, 67.95,

67.80, 67.62, 65.33, 63.37, 62.64, 62.09, 47.48, 36.43, 32.14, 29.92, 29.76, 29.58, 29.54, 29.36, 28.56, 25.77, 25.52, 22.90, 21.17, 20.68, 14.34; HRMS (ESI) calcd for $C_{154}H_{187}N_2O_{35}[M+NH_4]^+$: 2624.29144, found: 2624.29470.



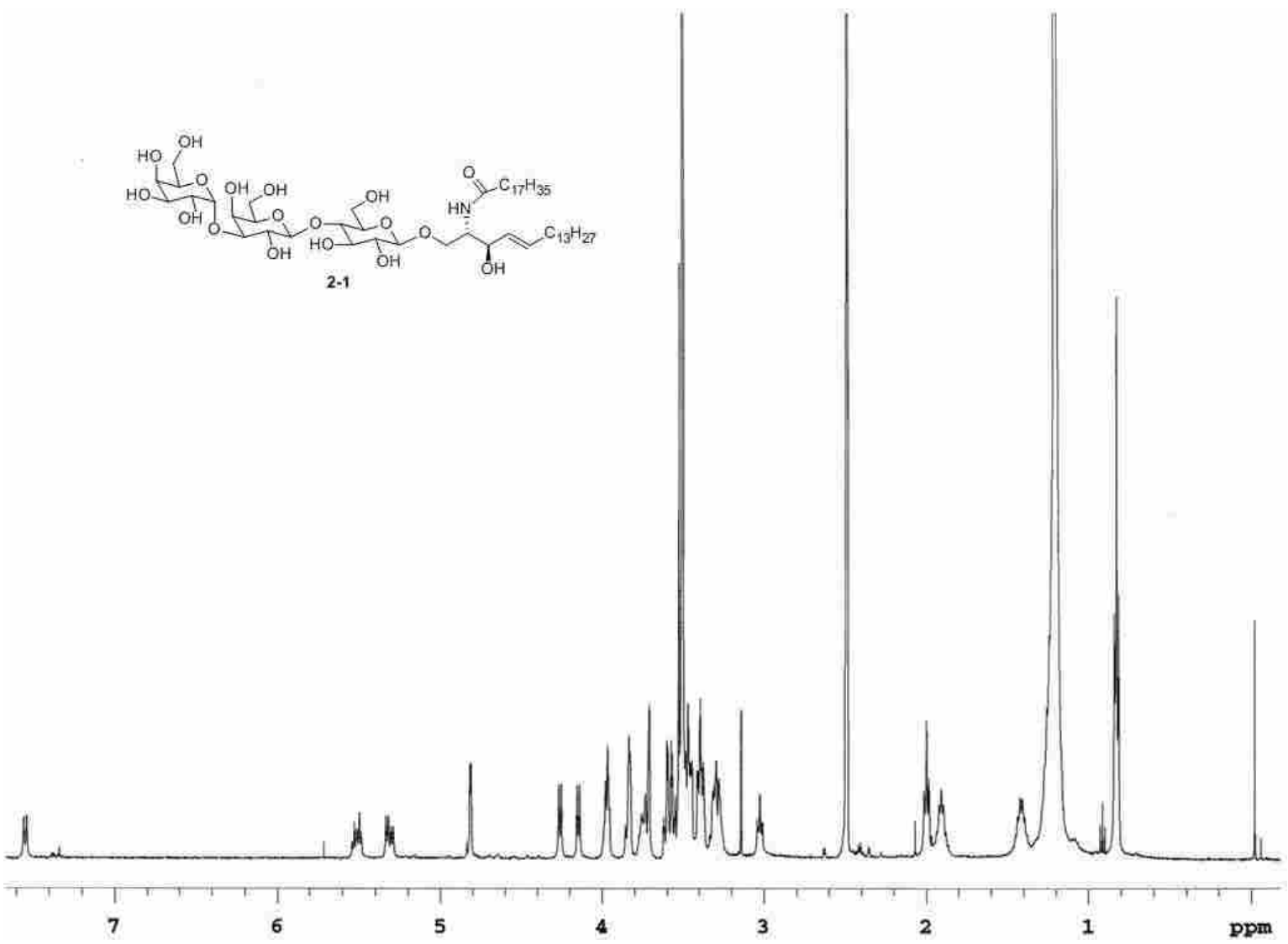
Preparation of 4-28: As described in the preparation of **4-13**, **4-28** (44 mg, 73%) was obtained as a white solid from compound **4-27** (63 mg, 0.024 mmol). 1H NMR ($CDCl_3$, 500 MHz) δ 8.30-8.24 (m, 4 H), 8.03-7.93 (m, 11 H), 7.68-6.88 (m, 30 H), 6.05 (d, $J = 3.5$ Hz, 1 H), 5.92-5.88 (m, 2 H), 5.81 (t, $J = 9.5$ Hz, 1 H), 5.73 (d, $J = 10.0, 3.0$ Hz, 1 H), 5.39 (dd, $J = 9.5, 7.5$ Hz, 1 H), 5.35 (dd, $J = 10.0, 8.0$ Hz, 1 H), 5.05 (d, $J = 3.0$ Hz, 1 H), 5.03 (t, $J = 3.0$ Hz, 1 H), 4.94 (d, $J = 8.5$ Hz, 1 H), 4.88-4.84 (m, 3 H), 4.76 (dd, $J = 11.0, 6.5$ Hz, 1 H), 4.73 (d, $J = 3.5$ Hz, 1 H), 4.68 (d, $J = 7.5$ Hz, 1 H), 4.61-4.56 (m, 3 H), 4.49-4.47 (m, 1 H), 4.42-4.35 (m, 3 H), 4.28 (tt, $J = 9.0, 2.0$ Hz, 1 H), 4.07 (d, $J = 9.0$ Hz, 1 H), 3.99-3.94 (m, 2 H), 3.79-3.74 (m, 3 H), 3.64 (t, $J = 7.0$ Hz, 1 H), 3.54 (dd, $J = 9.5, 2.5$ Hz, 1 H), 3.43 (dd, $J = 9.5, 3.0$ Hz, 1 H), 3.26 (t, $J = 9.5$ Hz, 1 H), 1.98 (s, 3 H), 1.94 (s, 3 H), 1.87 (s, 3 H), 1.81 (s, 3 H), 1.80 (s, 3 H), 1.76 (s, 3 H), 1.75-1.62 (m, 2 H), 1.56-1.46 (m, 2 H), 1.42-1.02 (m, 66 H), 0.88 (t, $J = 7.0$ Hz, 3 H), 0.87 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.91, 171.01, 170.22, 169.96, 169.78, 169.66, 169.31, 166.29, 166.02, 165.93, 165.77, 165.64, 165.49, 165.36, 165.21, 164.85, 134.04, 133.76, 133.64, 133.20, 133.12, 138.82, 130.32, 130.22, 130.05, 129.96, 129.86, 129.68, 129.47, 129.34, 129.03,

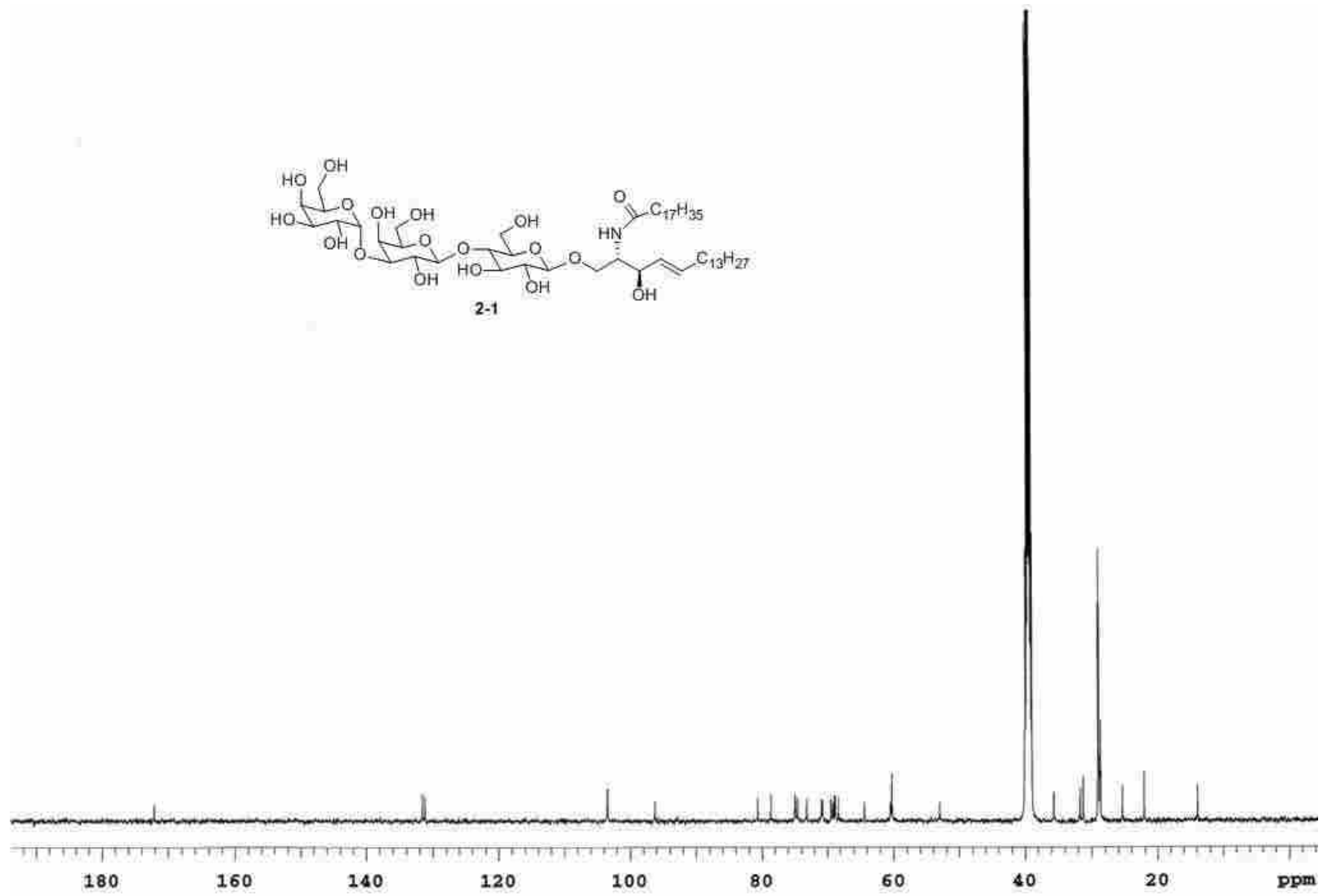
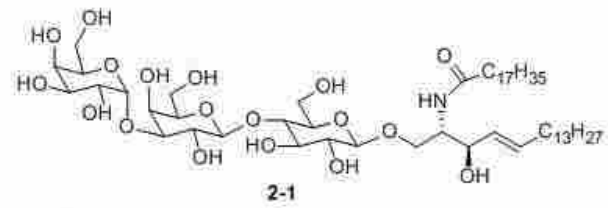
128.95, 128.88, 128.84, 128.80, 128.74, 128.54, 128.46, 128.38, 128.18, 102.52, 101.00, 100.34, 92.57, 74.47, 73.32, 73.24, 73.07, 72.38, 72.24, 71.91, 71.71, 71.56, 70.87, 70.53, 70.18, 69.49, 68.76, 68.49, 67.68, 67.28, 67.25, 66.61, 64.53, 62.42, 62.09, 61.16, 47.56, 36.39, 32.13, 29.91, 29.76, 29.57, 29.35, 28.56, 25.77, 25.51, 22.90, 21.16, 20.92, 20.79, 20.74, 20.62, 14.33; HRMS (ESI) calcd for $C_{141}H_{177}N_2O_{39}[M+NH_4]^+$: 2522.19285, found: 2522.20440.

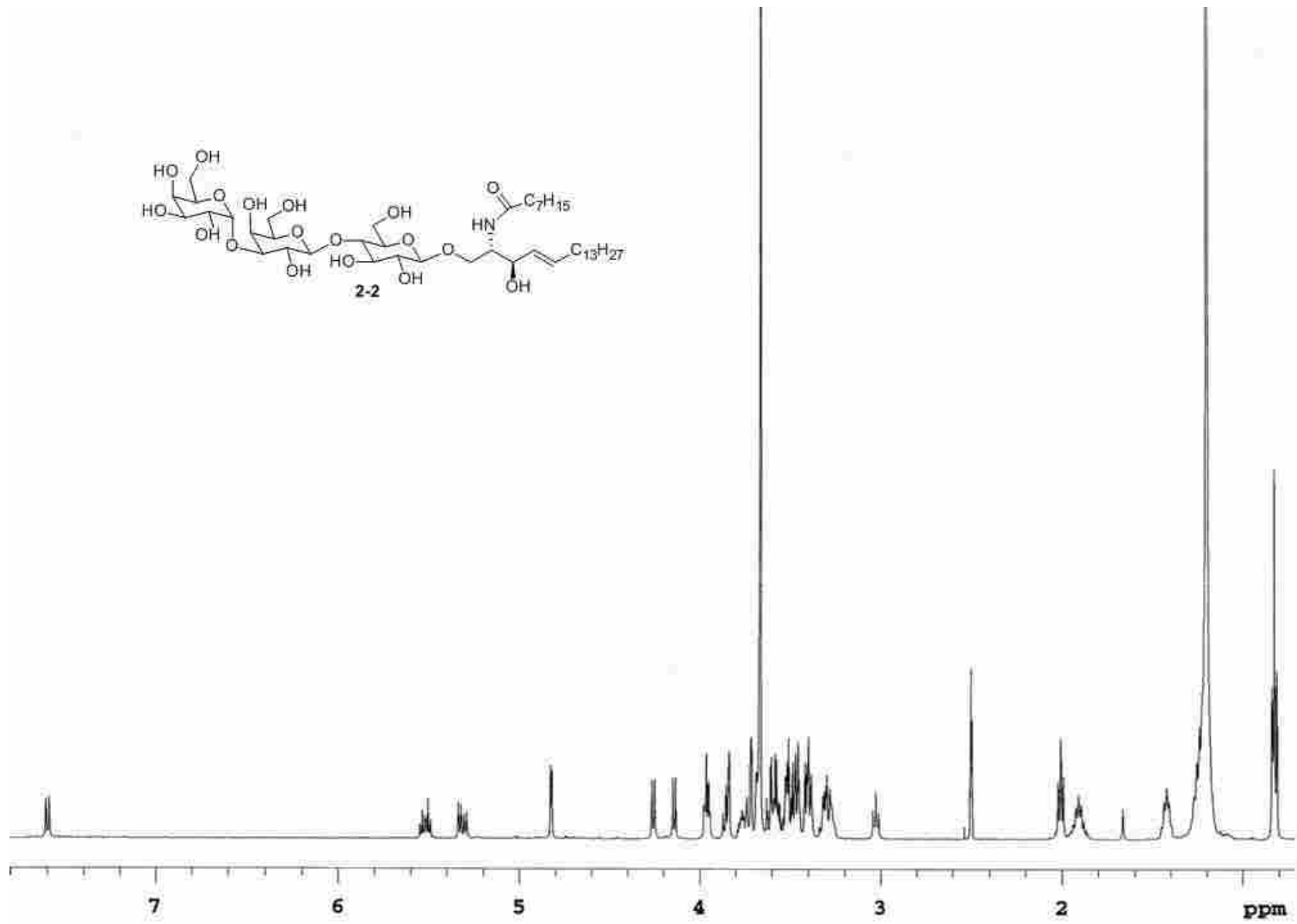
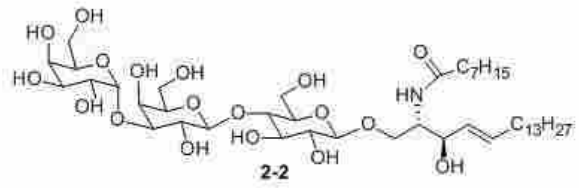


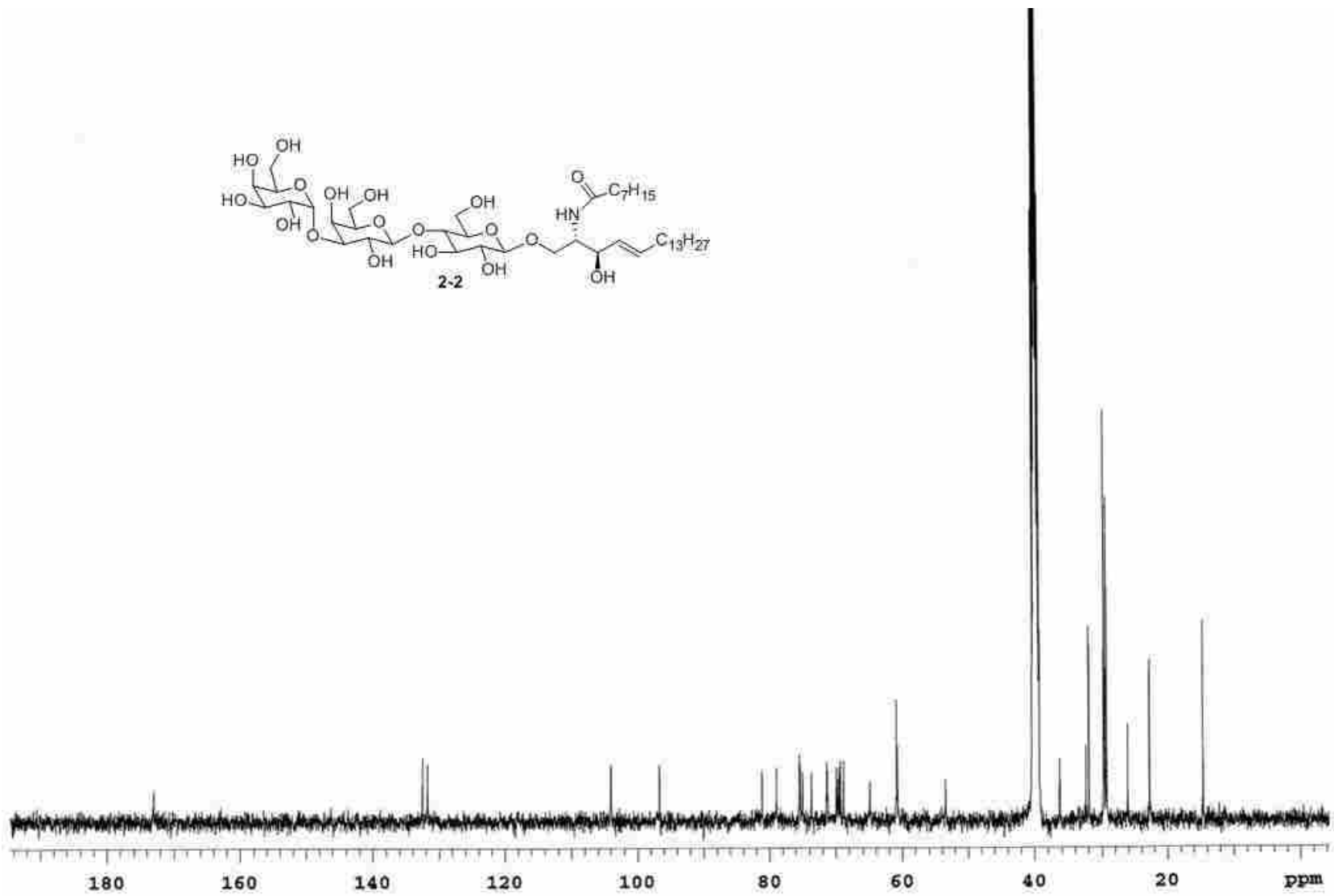
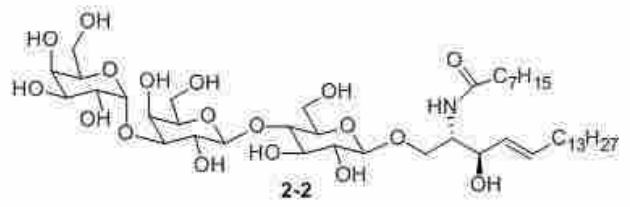
Preparation of neo-iGb4B: As described in the preparation of neo-iGb4A, neo-iGb4B (16 mg, 69%) was obtained as a white solid from compound **4-28** (44 mg, 0.018 mmol). 1H NMR (DMSO- d_6 /D $_2$ O, 500 MHz) δ 4.81 (d, J = 3.5 Hz, 1 H), 4.30 (d, J = 8.0 Hz, 1 H), 4.25 (t, J = 6.0 Hz, 1 H), 4.20 (s, J = 7.0 Hz, 1 H), 4.17 (d, J = 7.5 Hz, 1 H), 3.91-3.89 (m, 2 H), 3.84 (brs, 1 H), 3.74-3.72 (m, 3 H), 3.60-3.25 (m, 21 H), 3.02 (t, J = 7.5 Hz, 1 H), 2.07-2.02 (m, 2 H), 1.48-1.04 (m, 68 H), 0.84 (t, J = 7.5 Hz, 6 H); ^{13}C NMR (DMSO- d_6 /D $_2$ O, 125 MHz) δ 172.20, 103.58, 103.49, 103.40, 96.64, 80.79, 78.63, 75.20, 74.97, 74.84, 74.57, 73.27, 70.82, 70.58, 69.93, 69.70, 69.42, 69.03, 68.35, 68.20, 67.60, 64.67, 60.50, 60.33, 60.21, 55.04, 50.17, 35.68, 31.50, 30.60, 29.30, 29.14, 28.93, 28.87, 25.70, 25.60, 22.29, 14.13; HRMS (ESI) calcd for $C_{66}H_{126}NO_{24}[M+H]^+$: 1316.86643, found: 1316.87101.

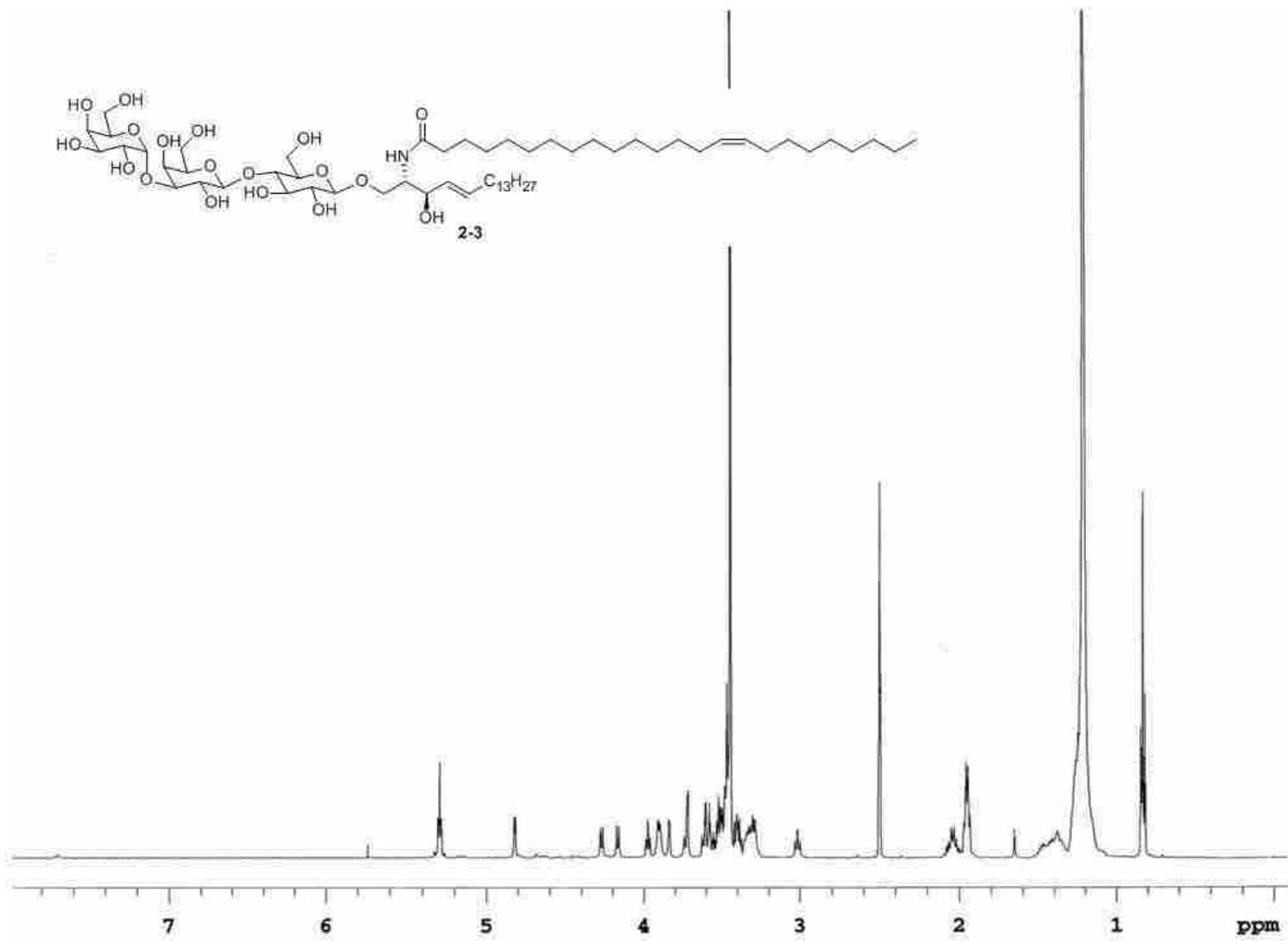
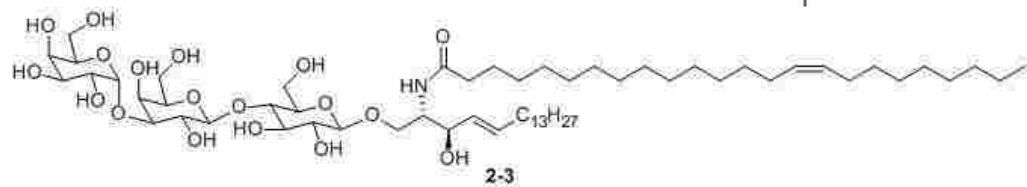
APPENDIX. ¹H NMR AND ¹³C NMR SPECTRA OF SYNTHETIC TARGETS:

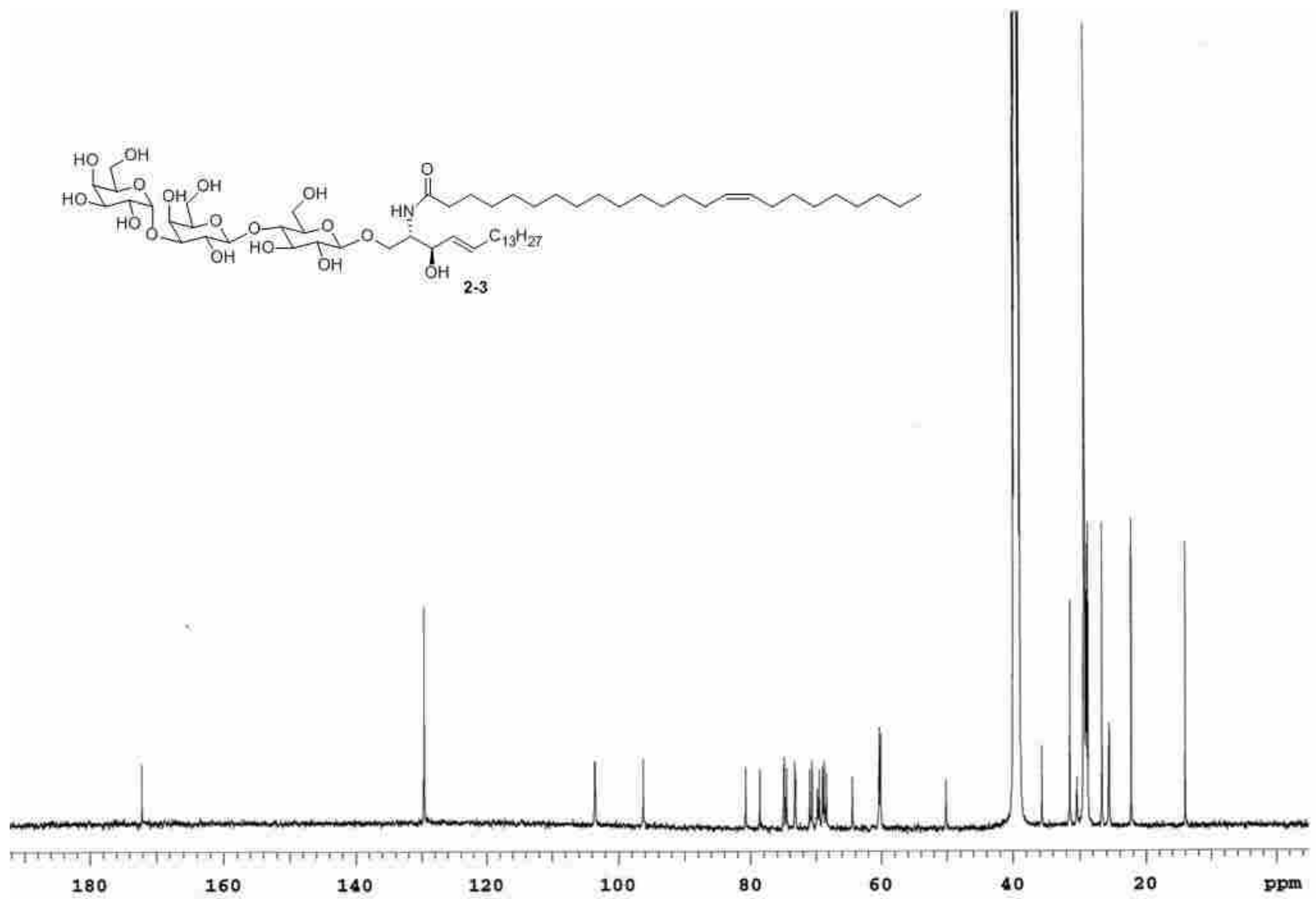


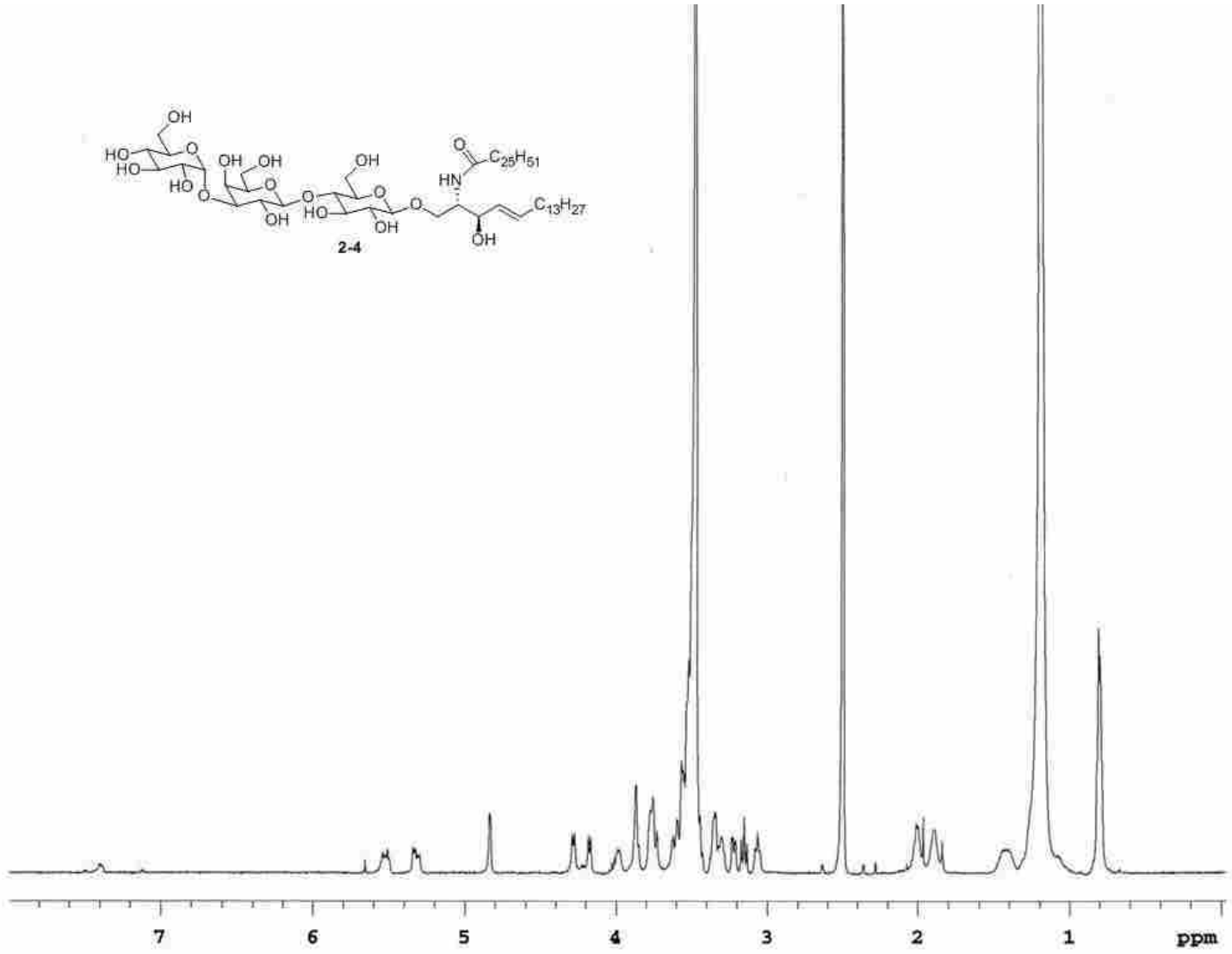
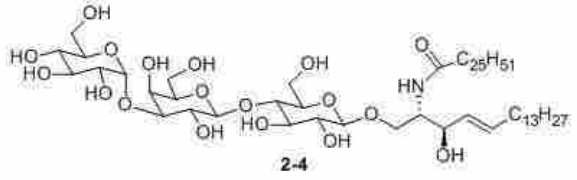


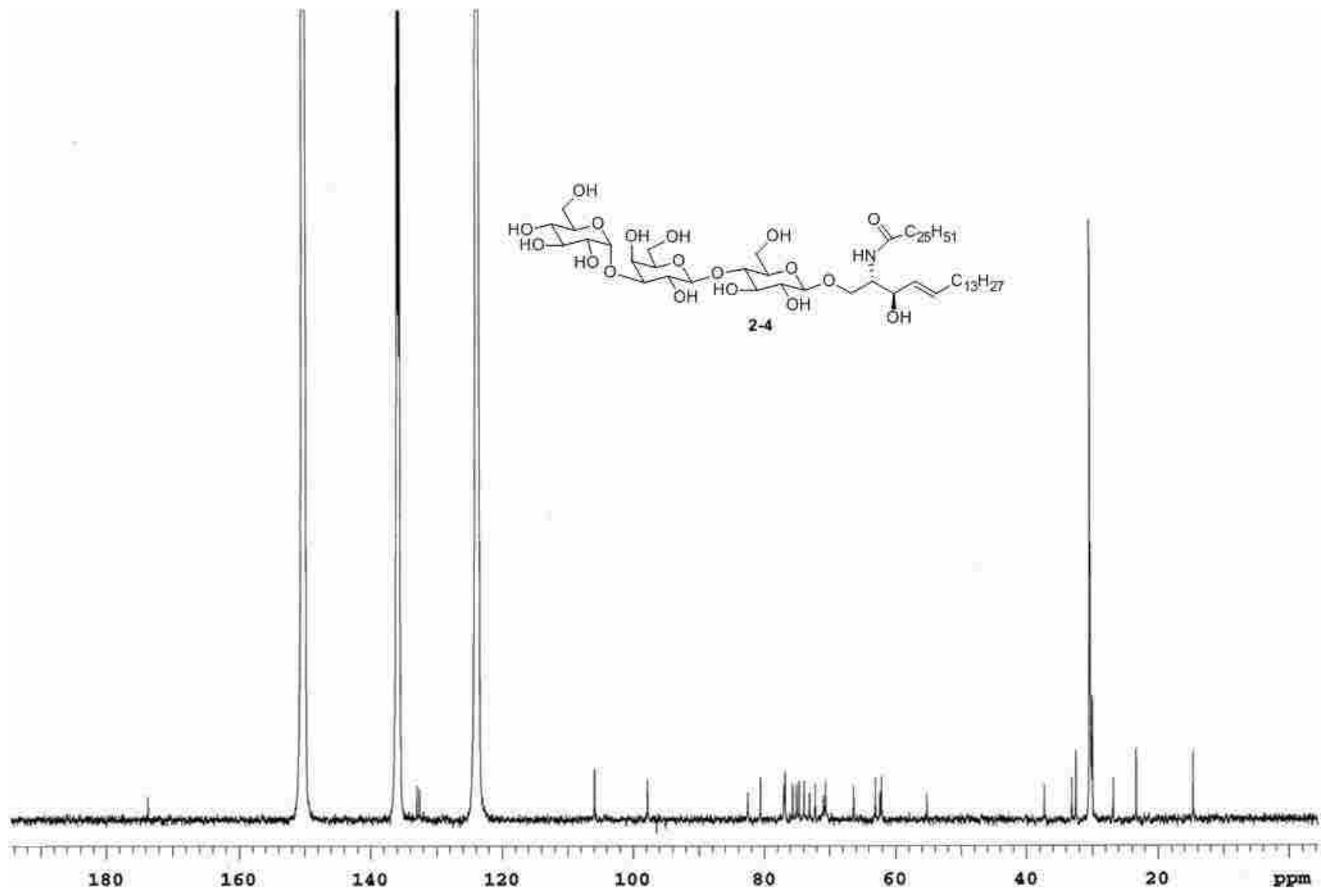


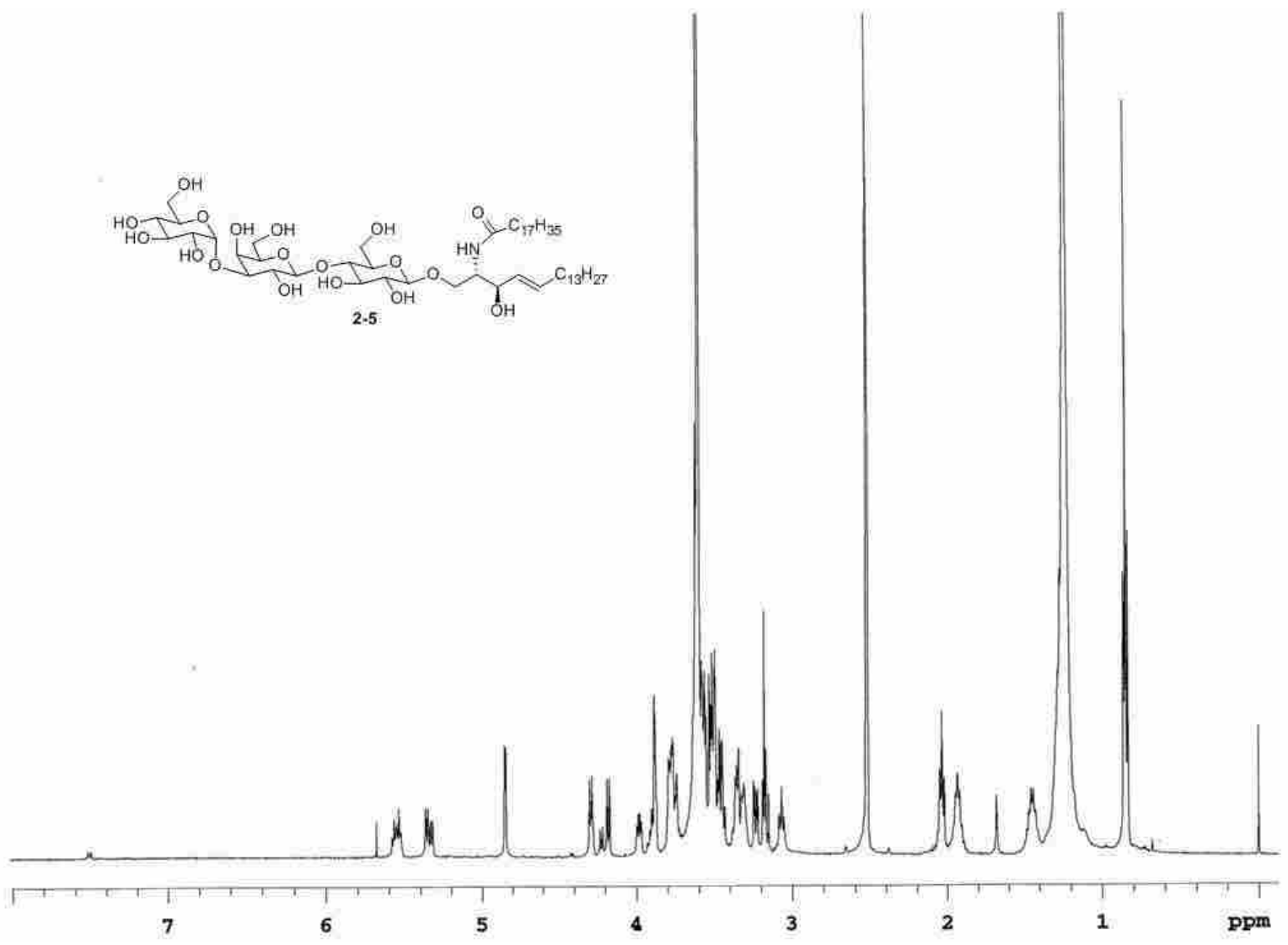
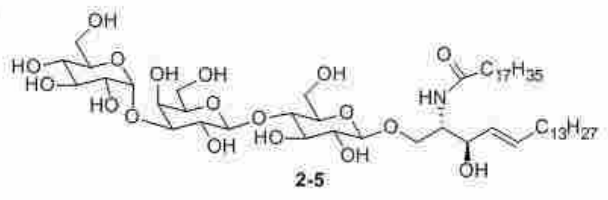


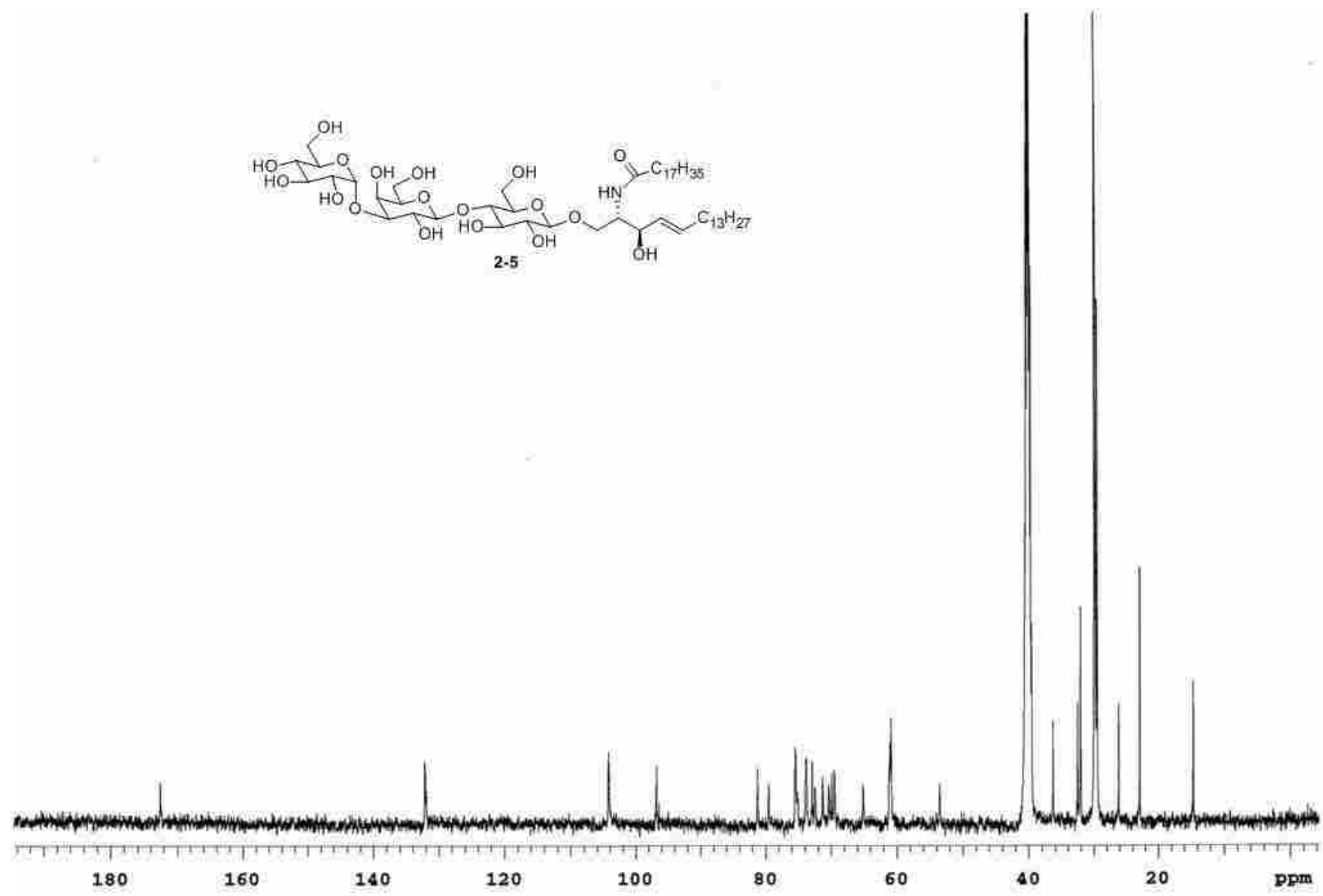
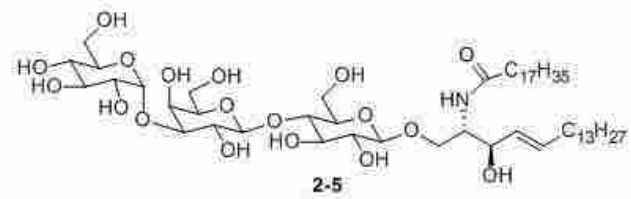


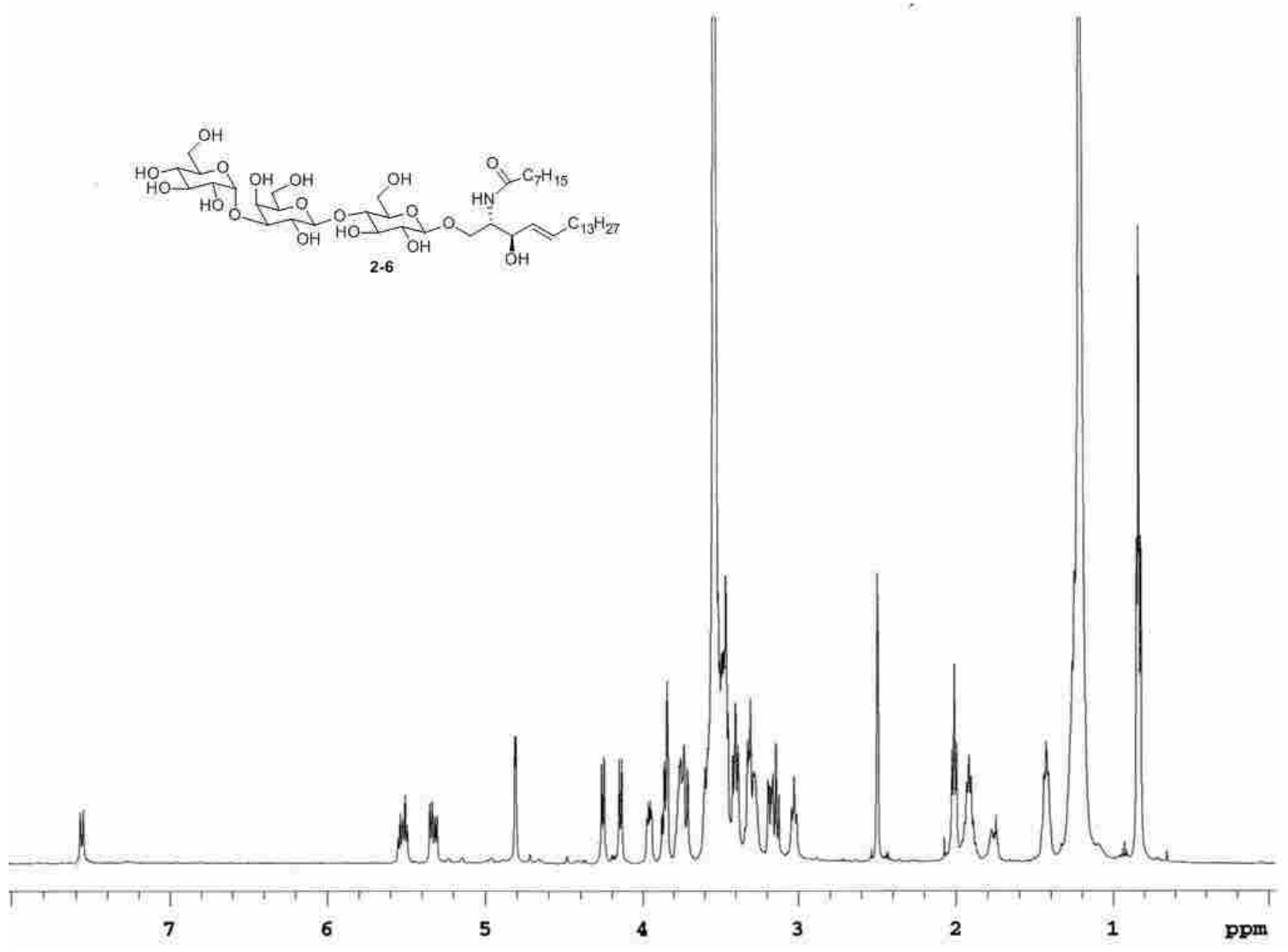
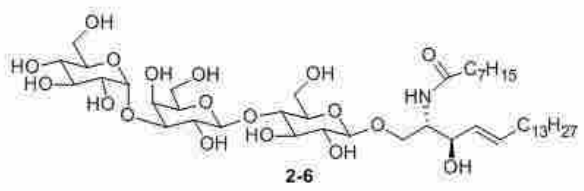


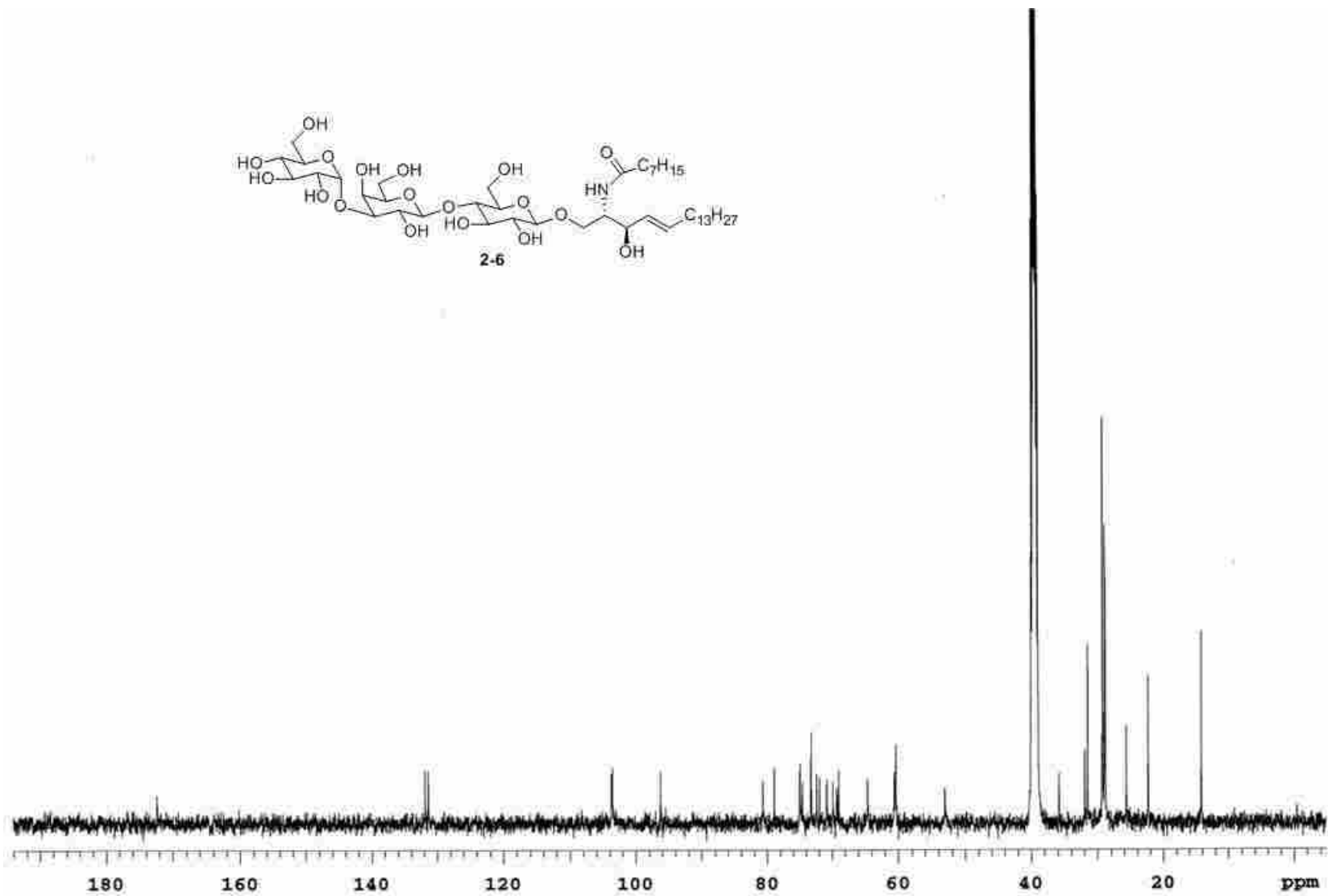
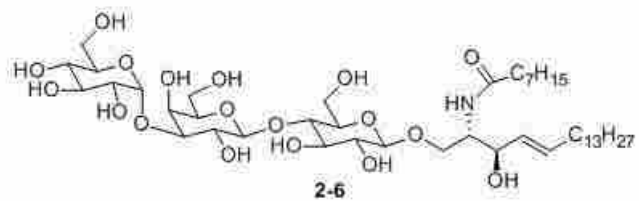


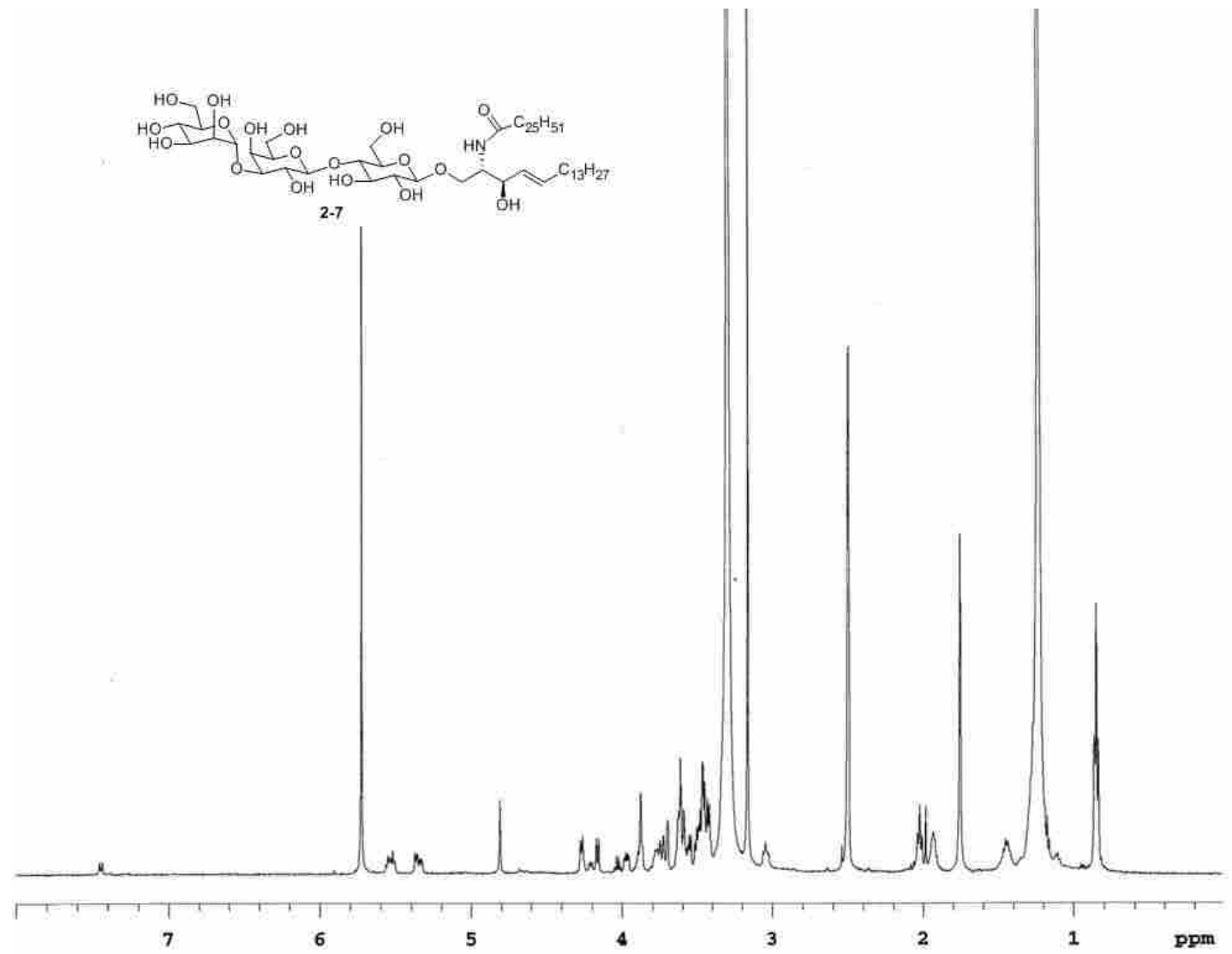
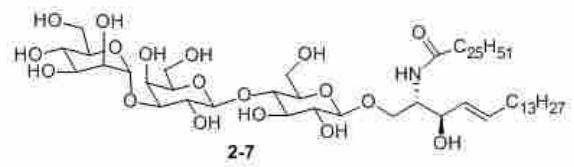


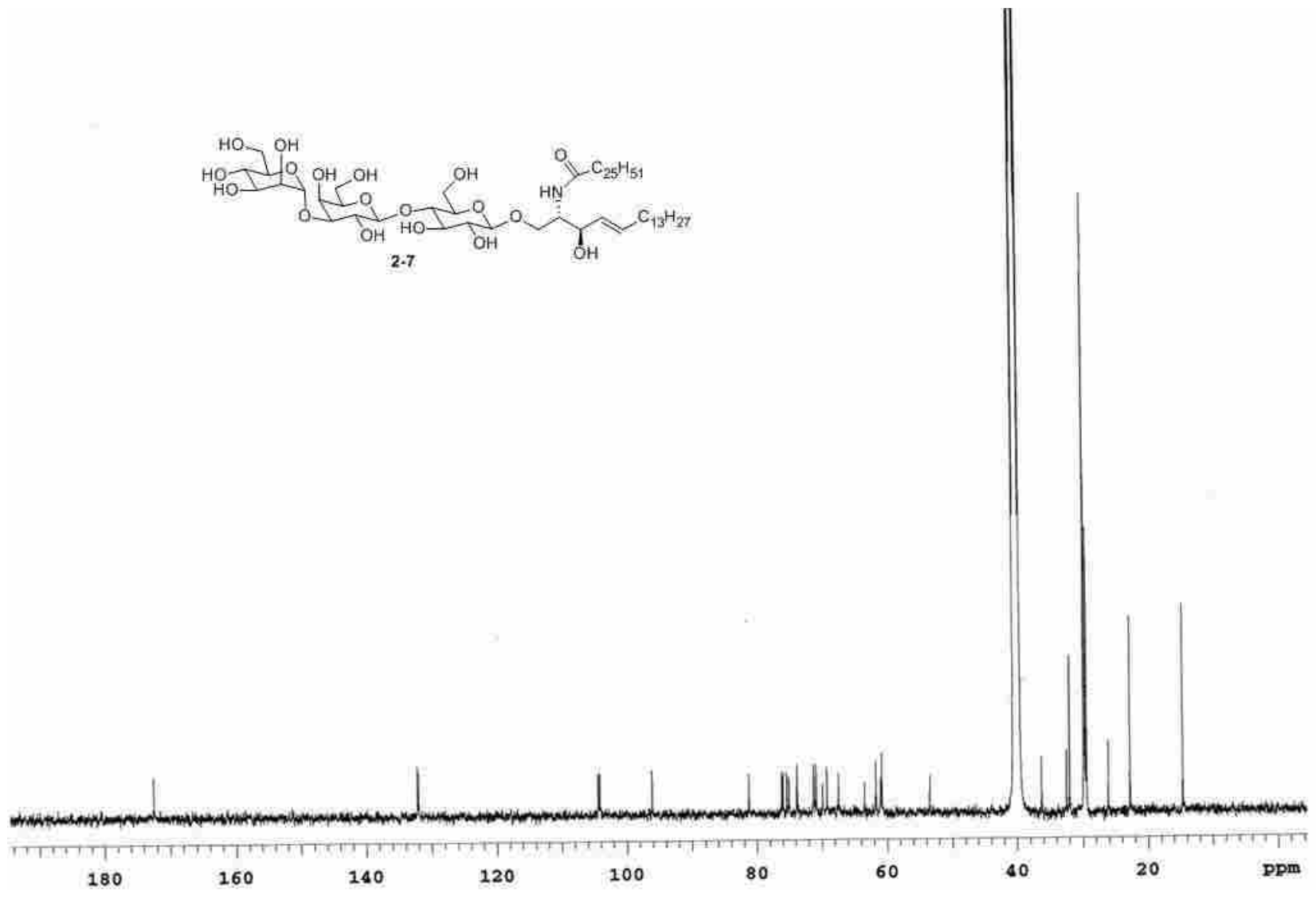
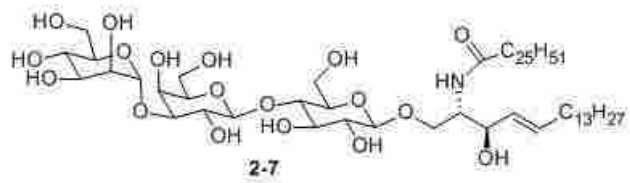


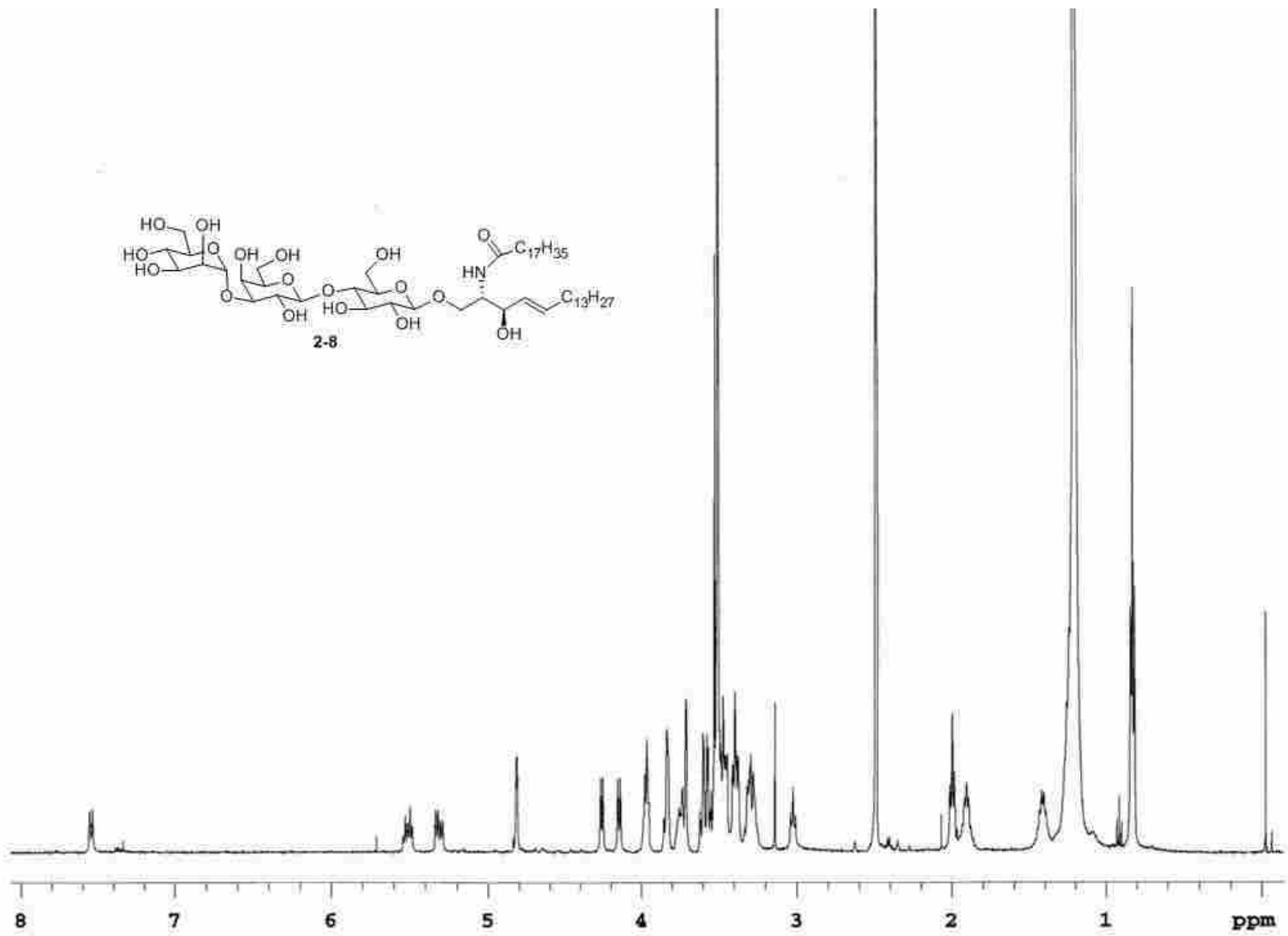
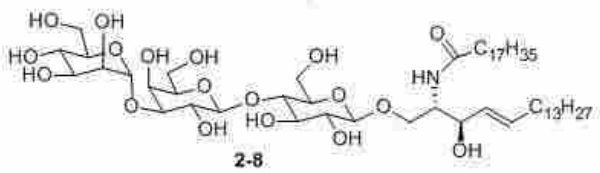


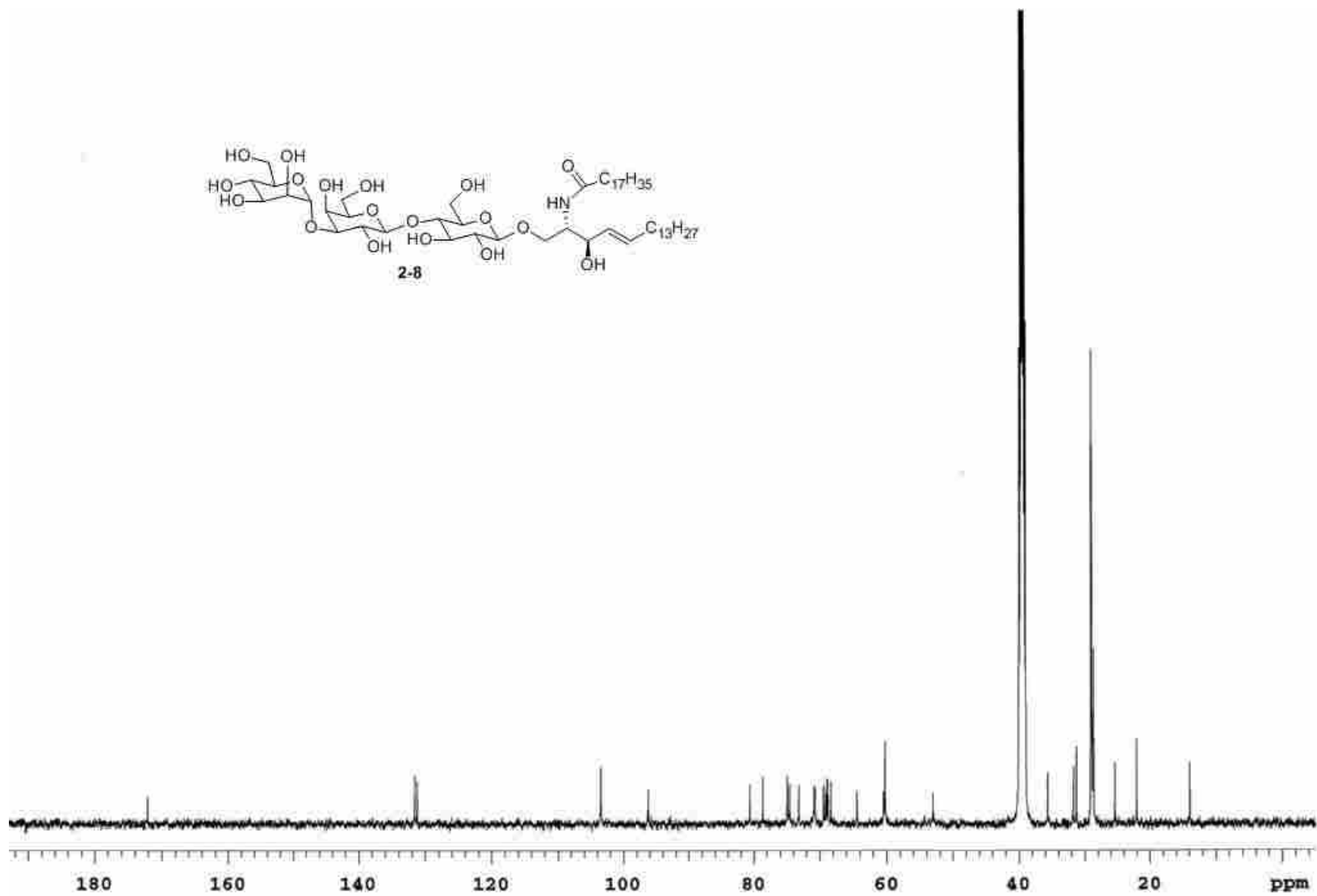
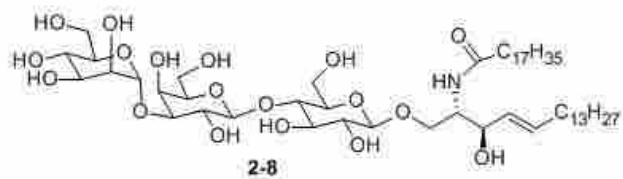


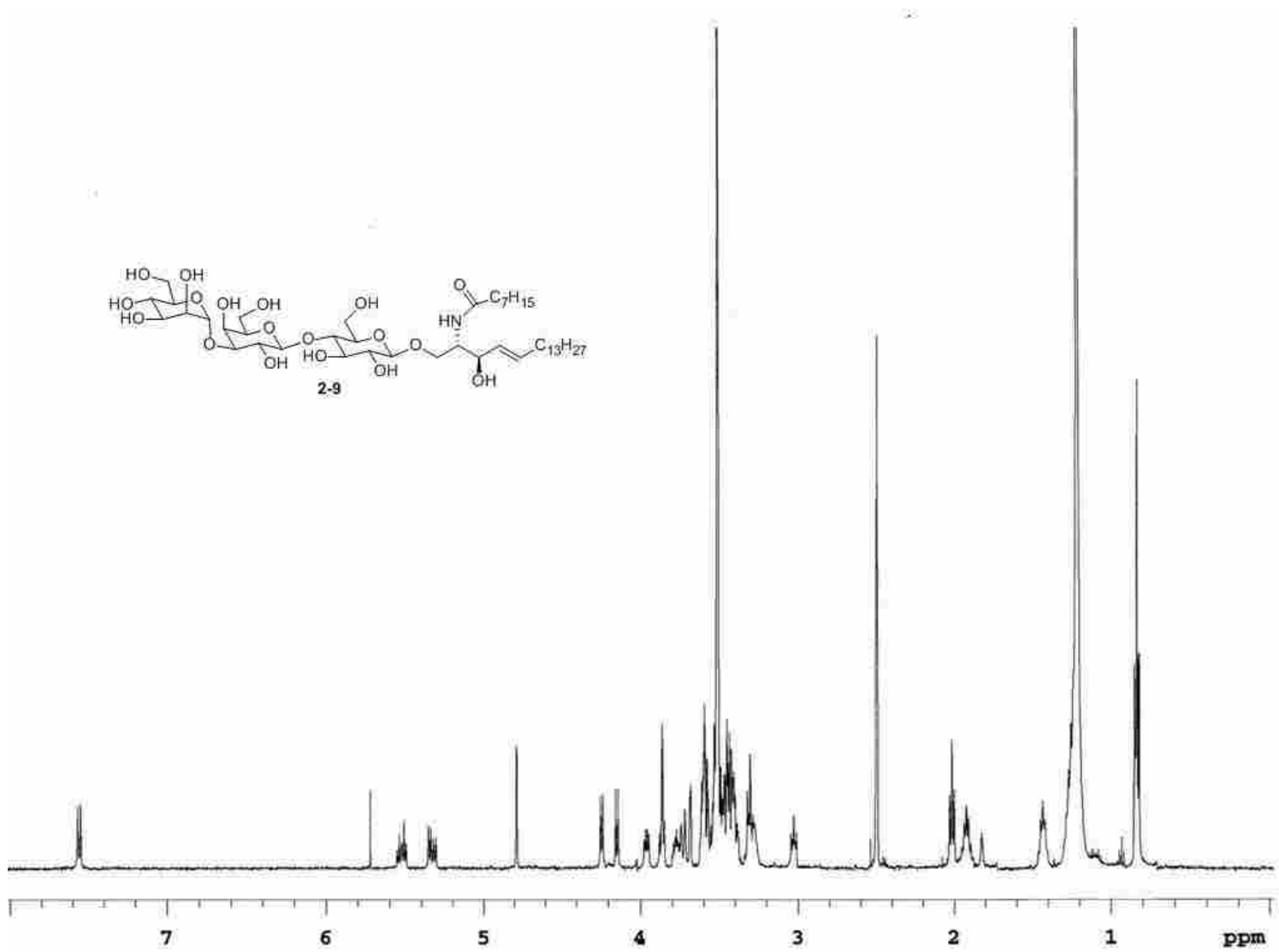
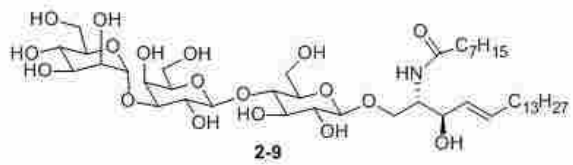


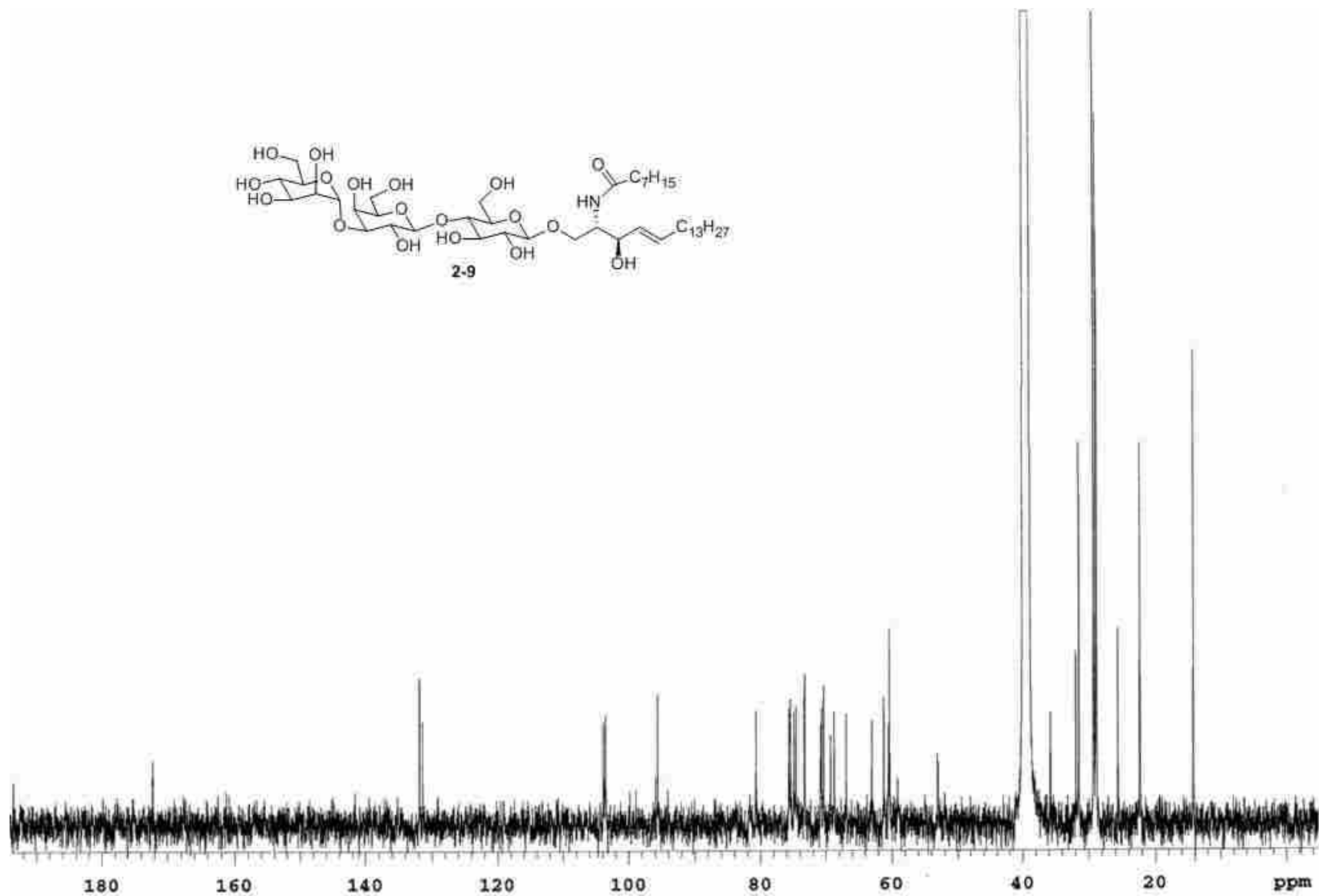
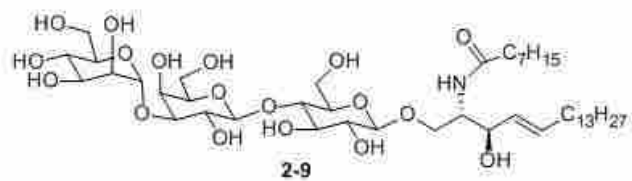


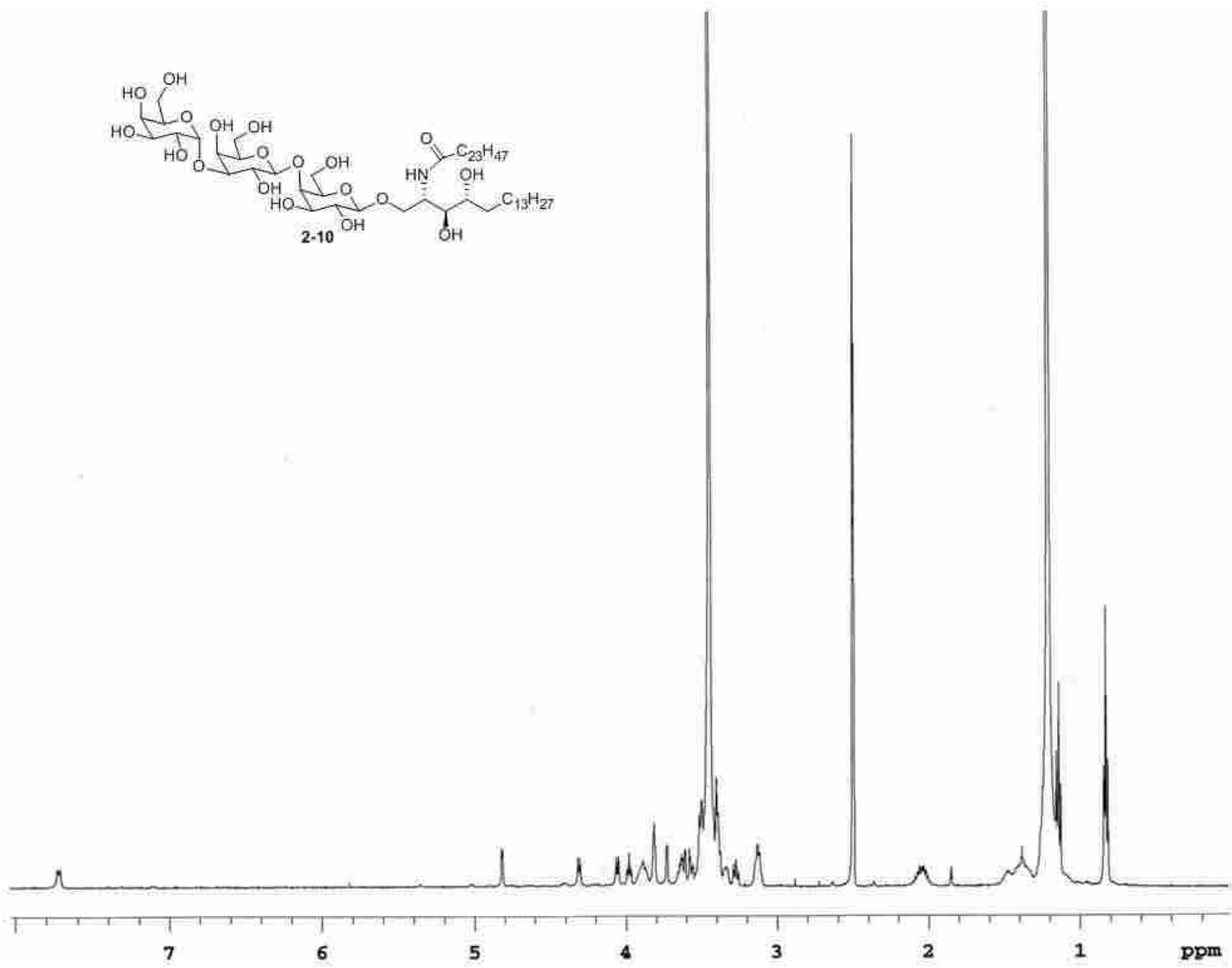
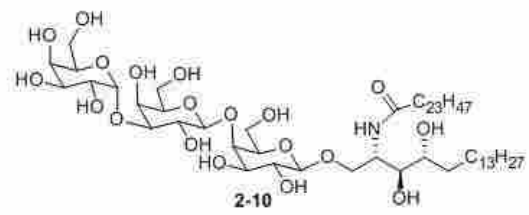


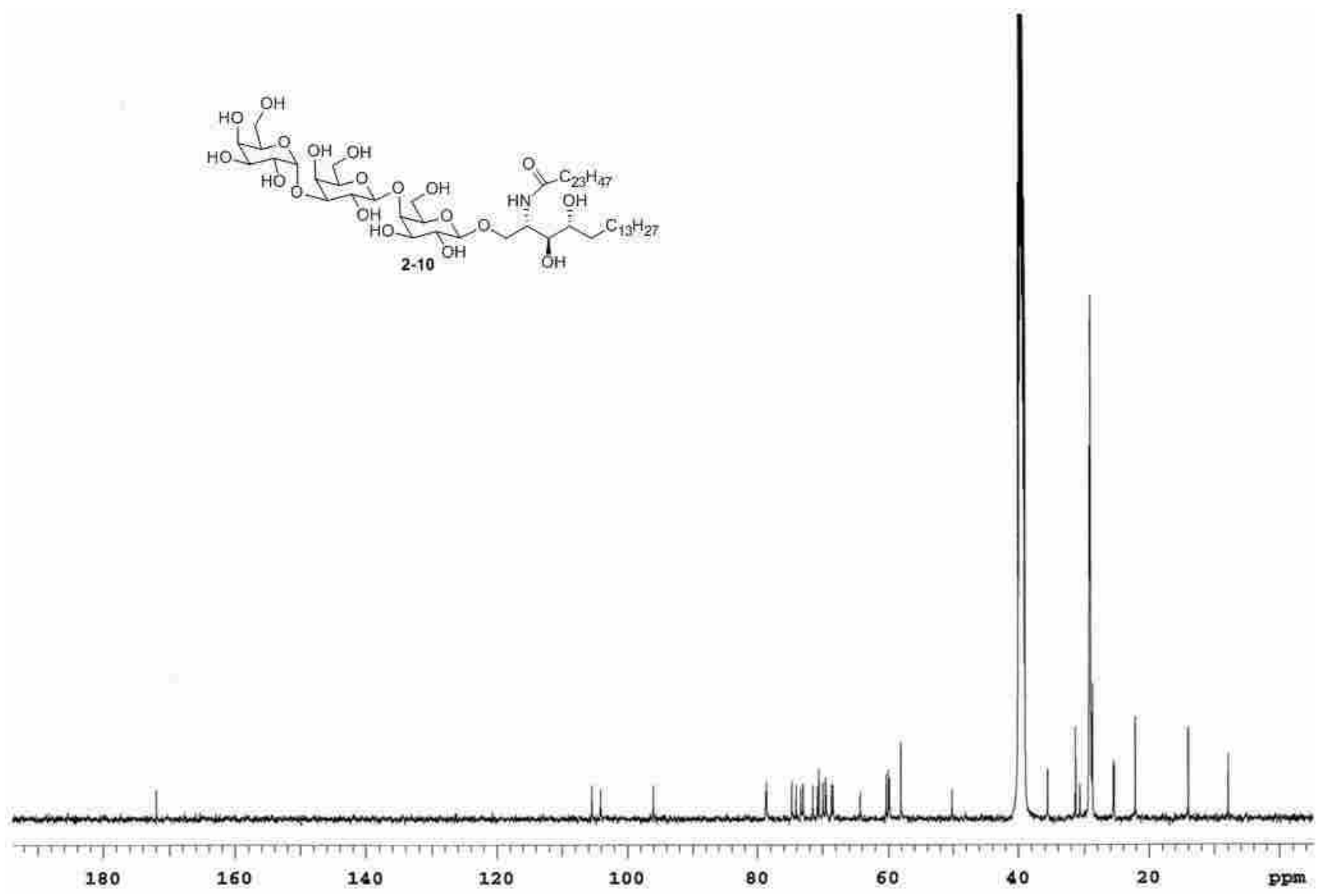
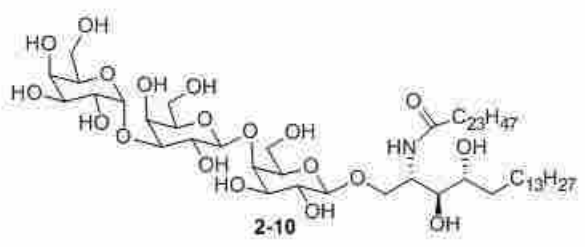


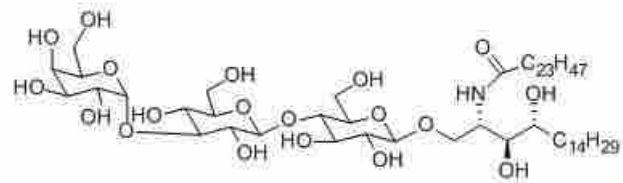




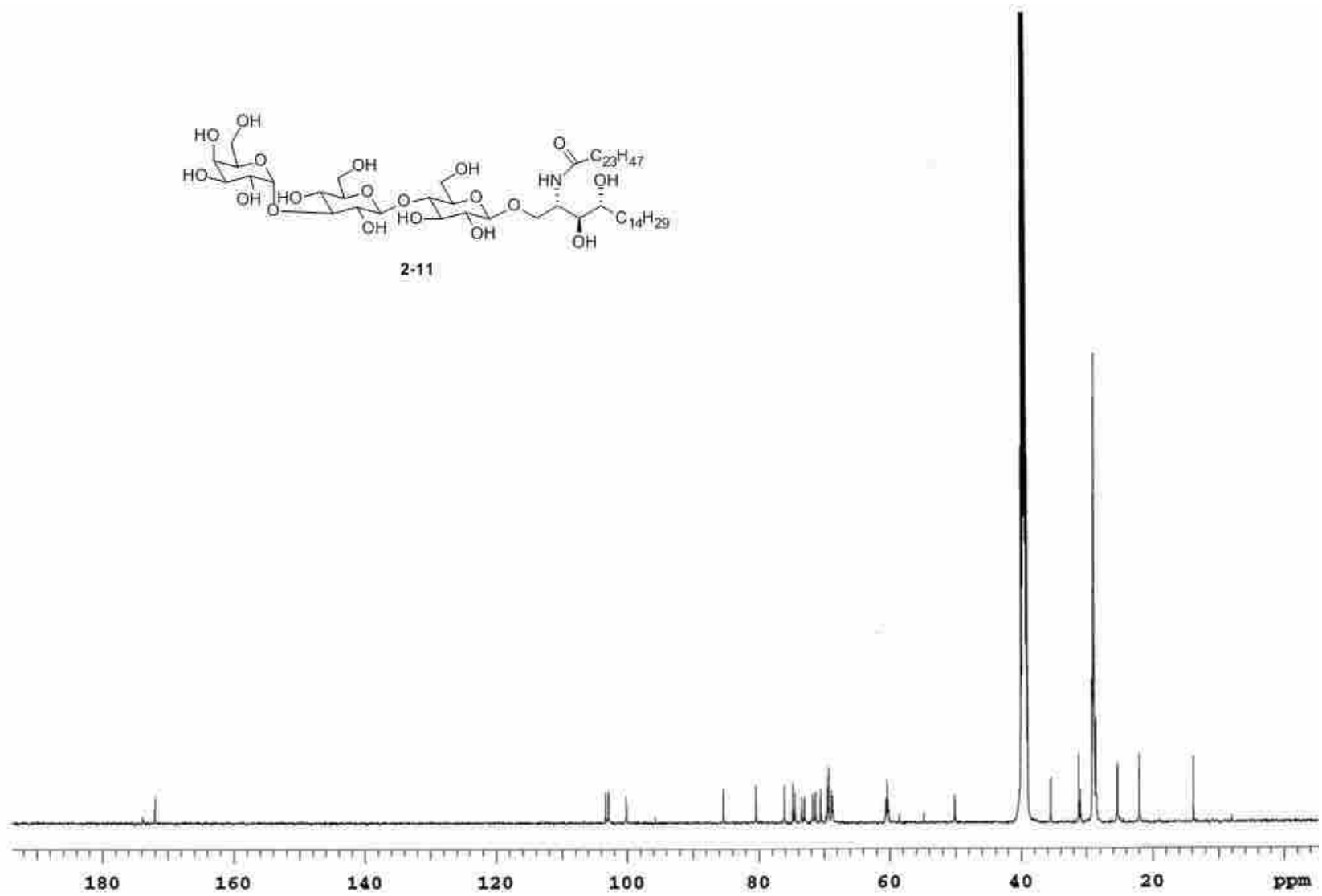


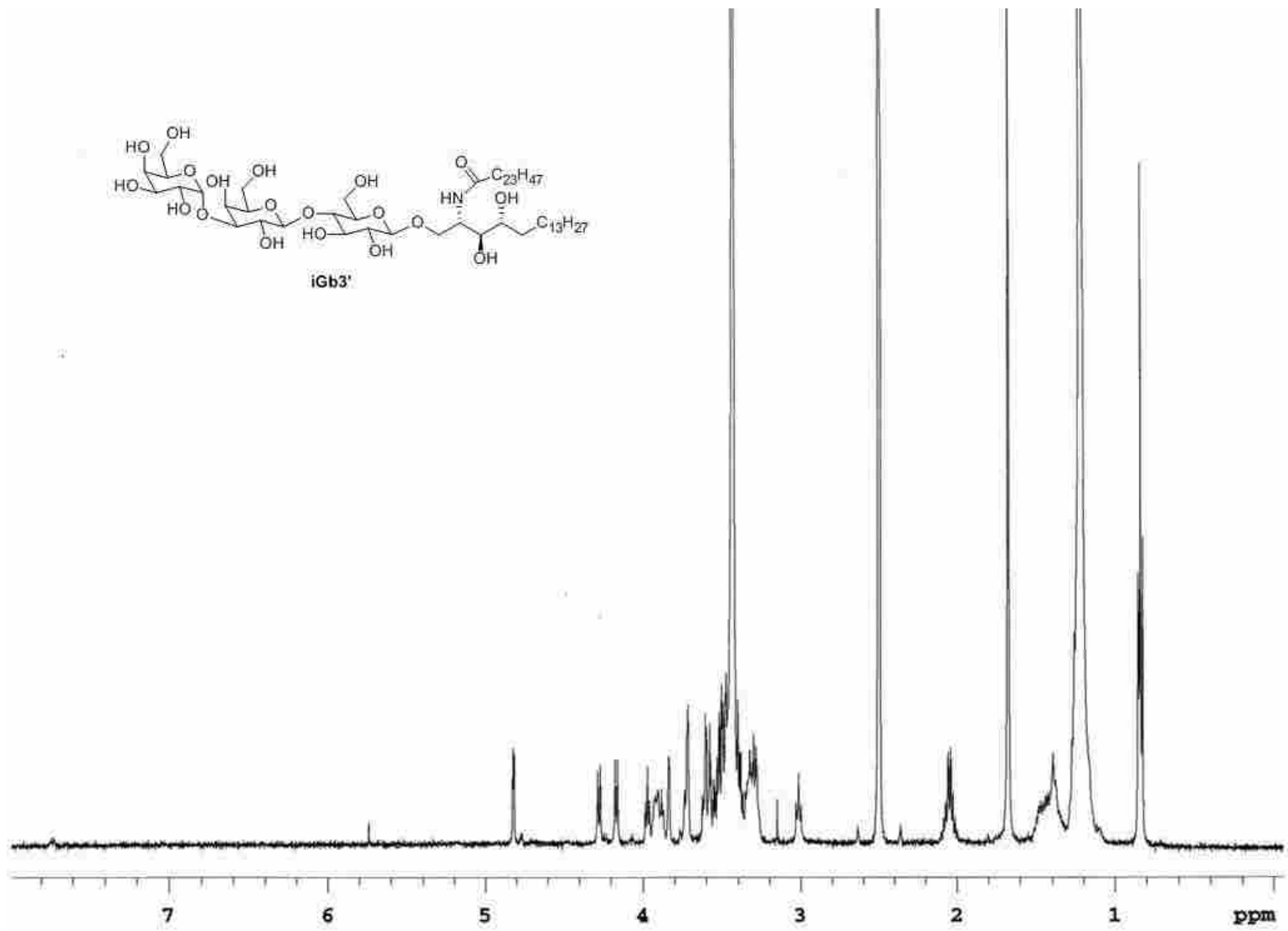
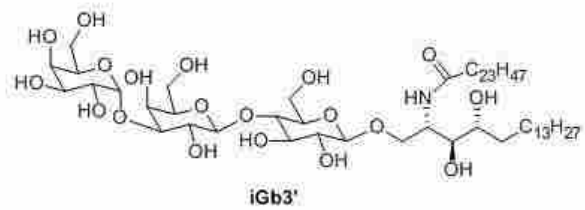


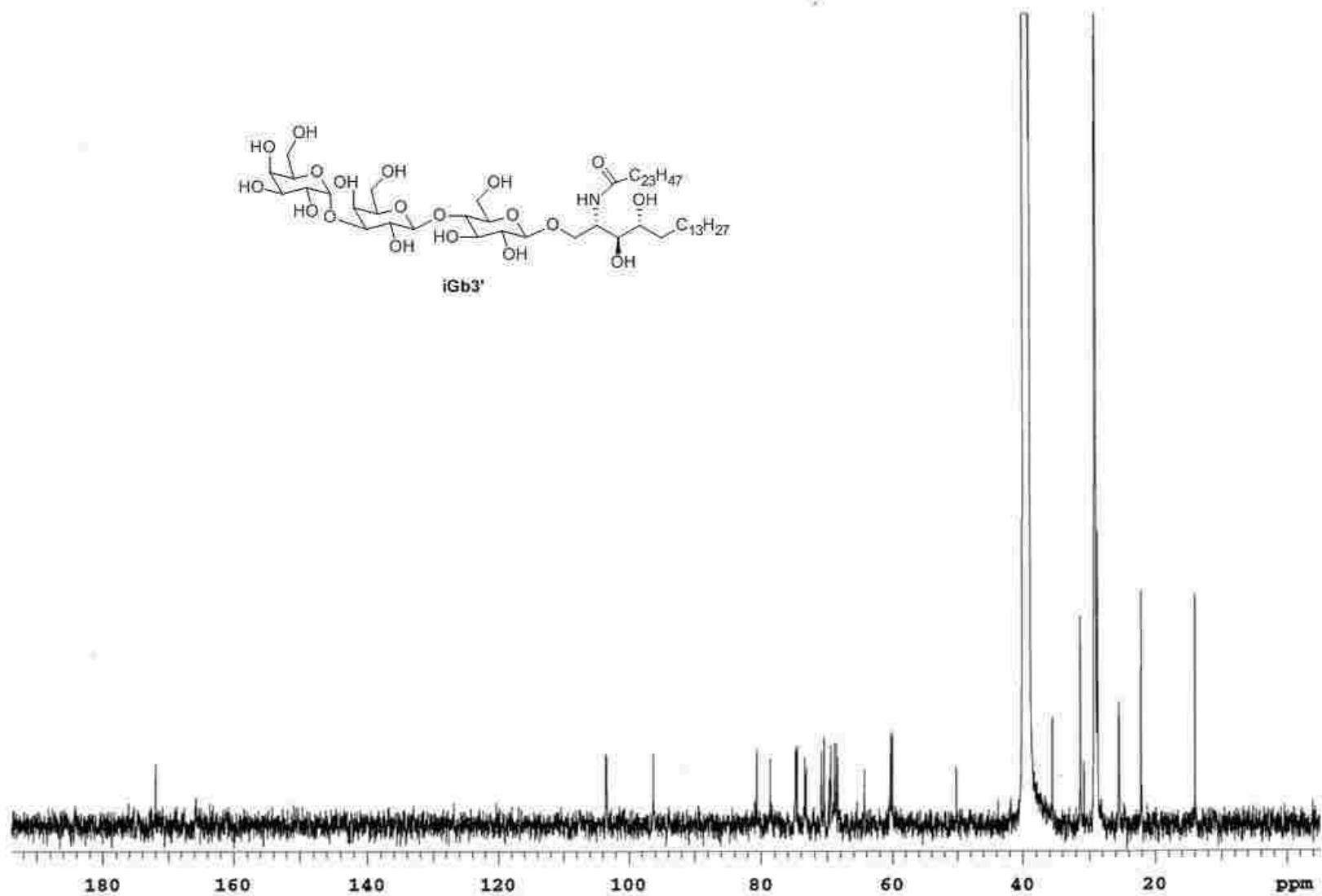
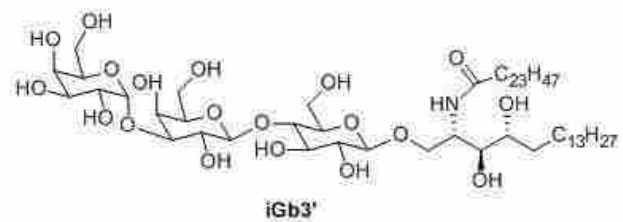




2-11

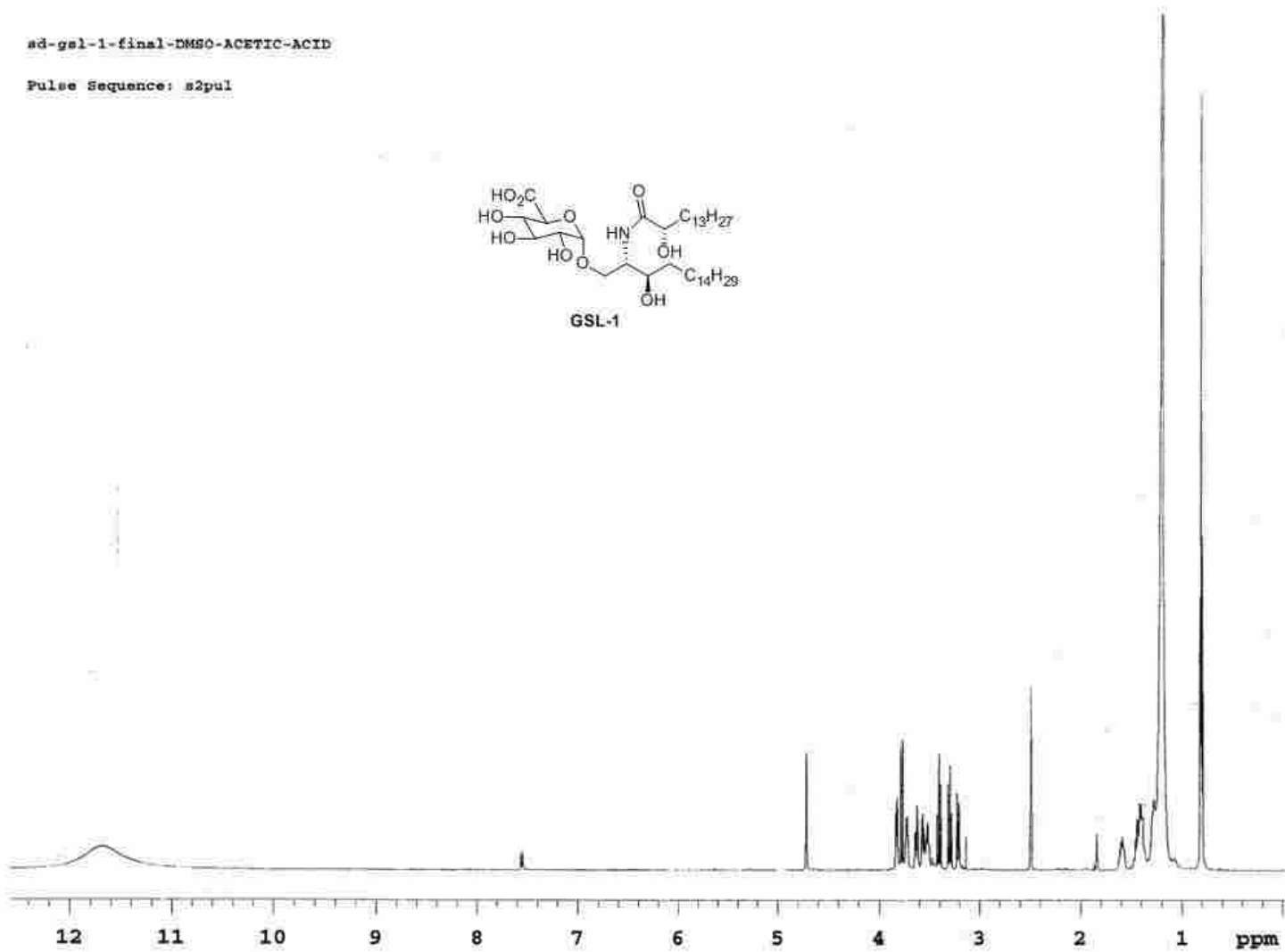
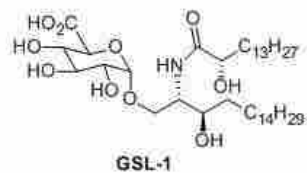






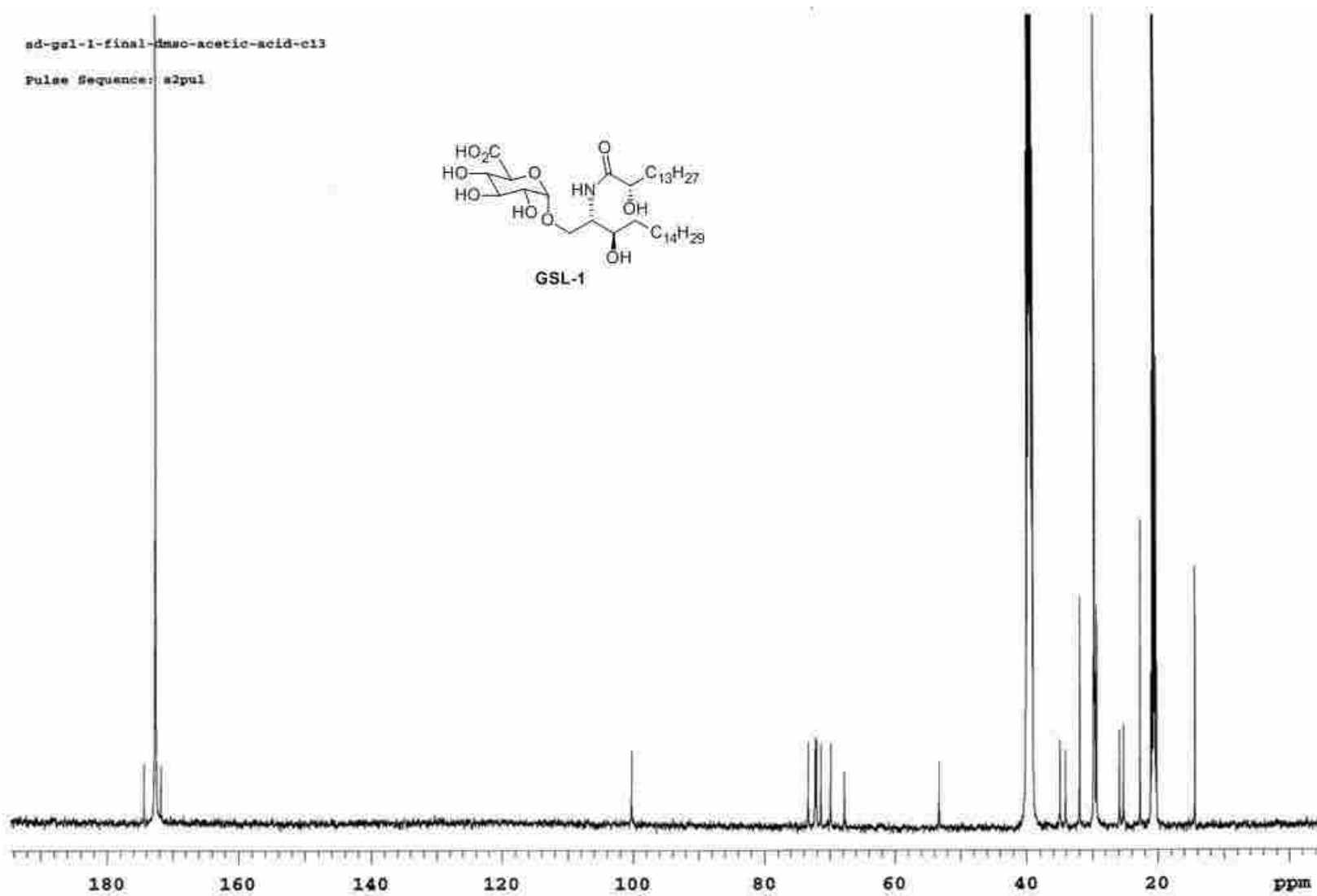
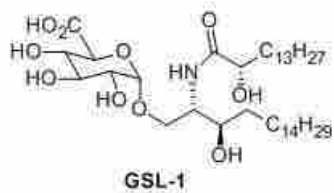
sd-gsl-1-final-DMSO-ACETIC-ACID

Pulse Sequence: s2pul



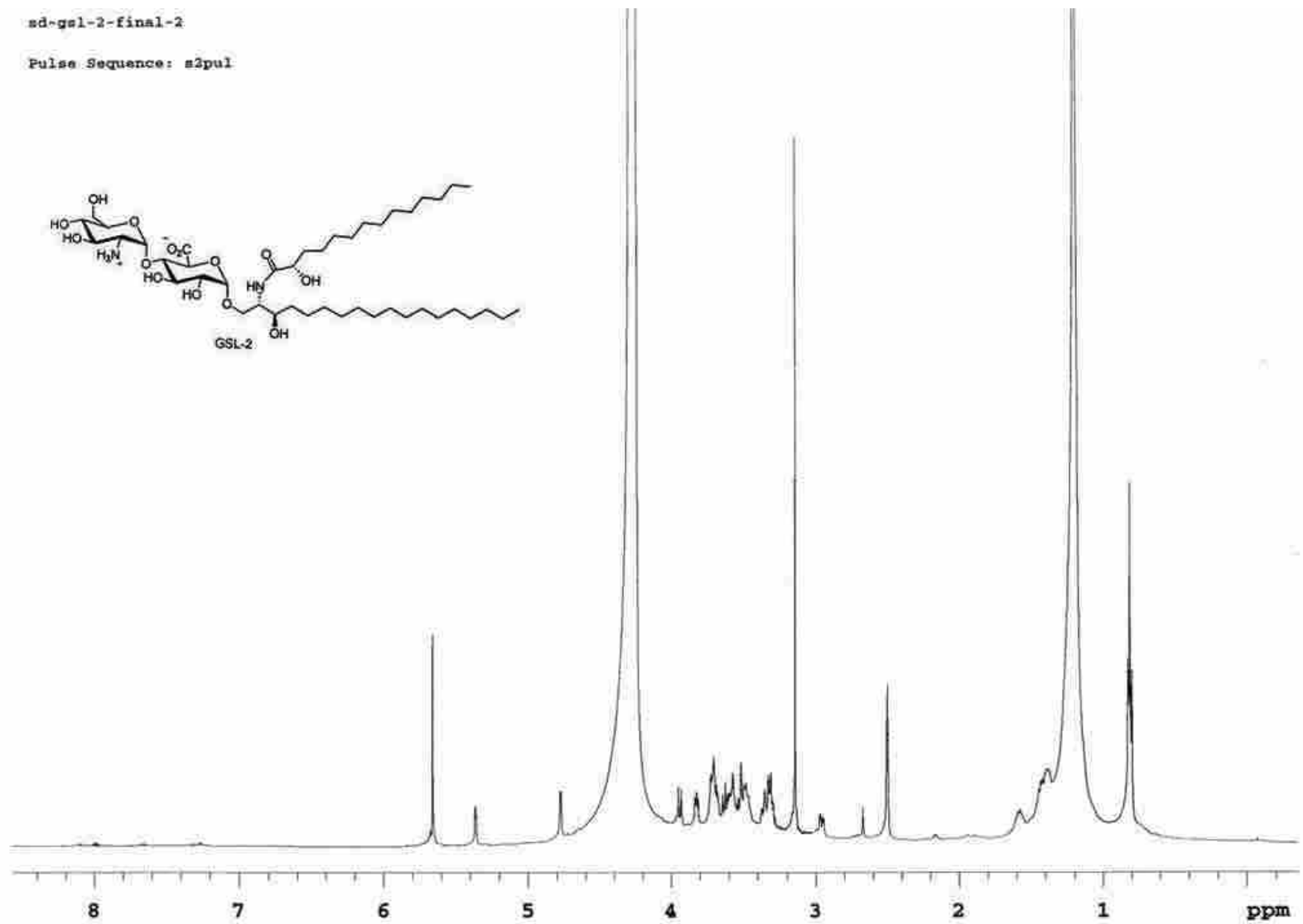
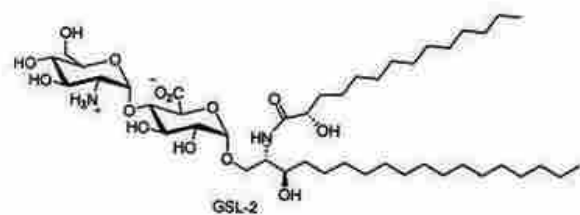
sd-gsl-1-final-dmsc-acetic-acid-c13

Pulse Sequence: a2pul



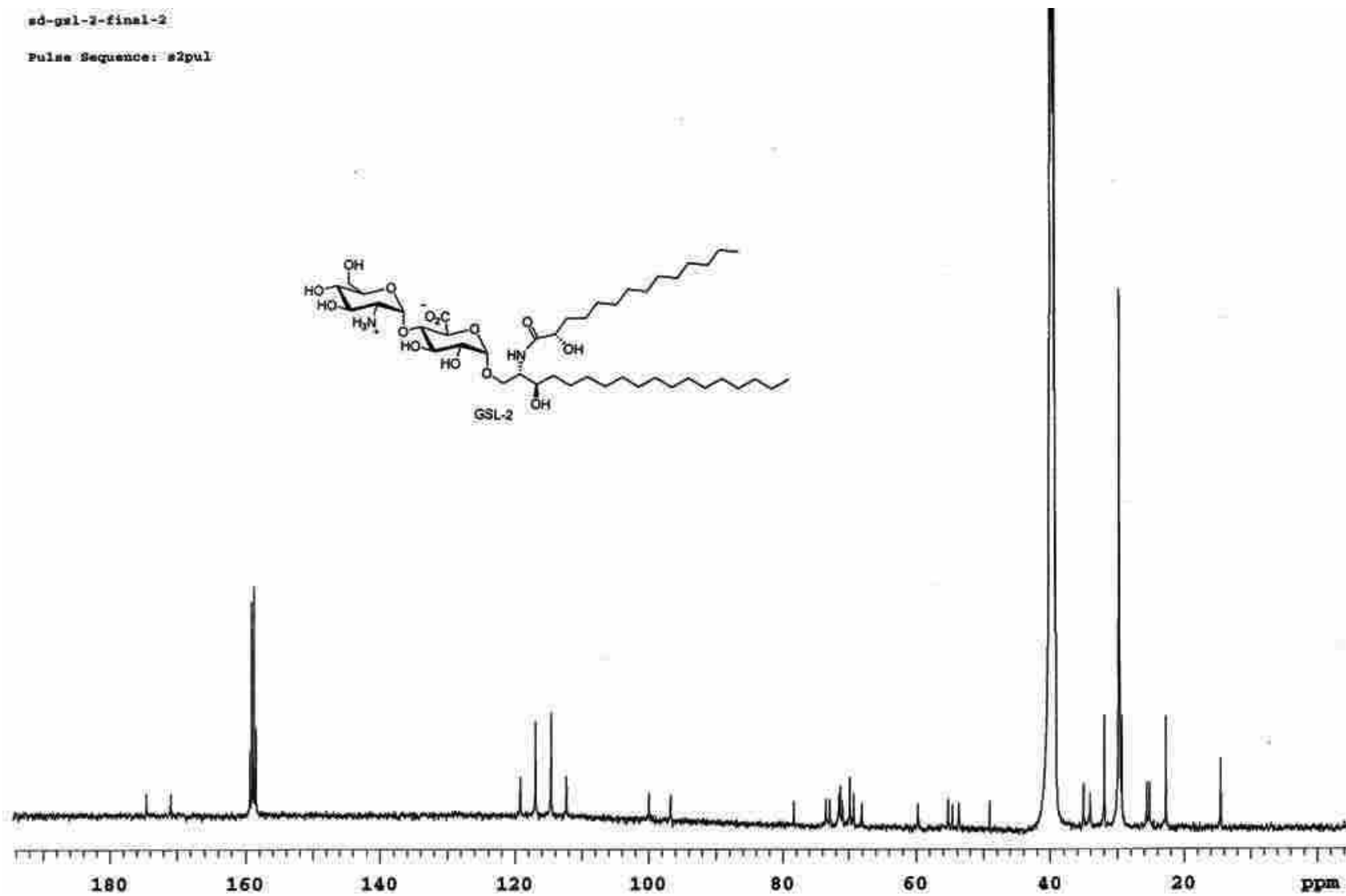
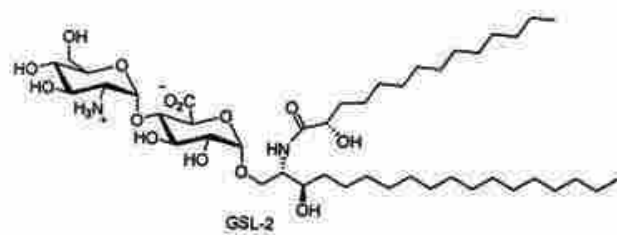
sd-gsl-2-final-2

Pulse Sequence: s2pul



sd-gsl-7-final-2

Pulse Sequence: s2pul

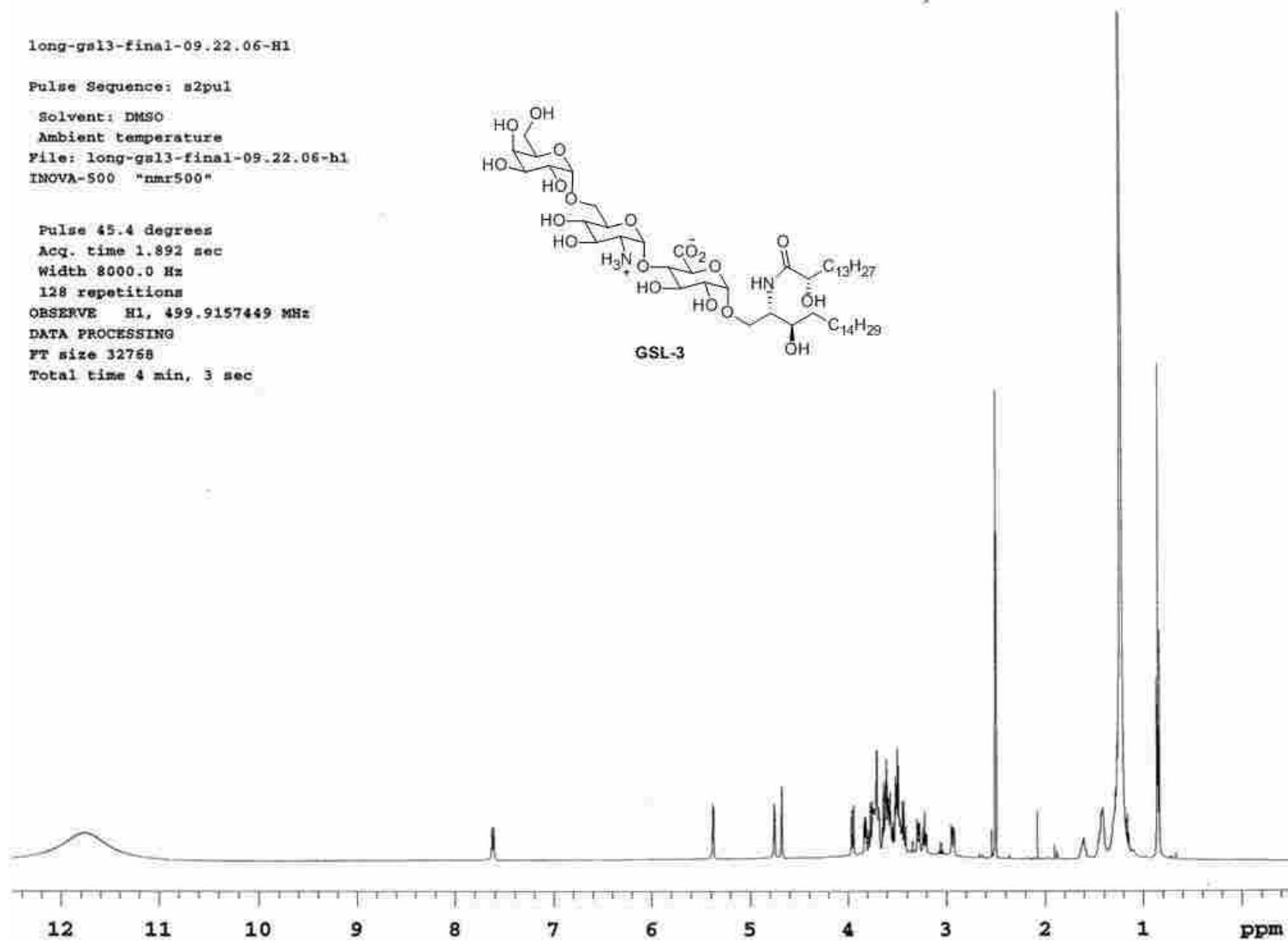
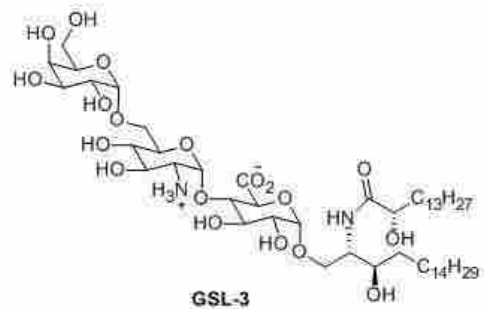


long-gsl3-final-09.22.06-H1

Pulse Sequence: s2pul

Solvent: DMSO
Ambient temperature
File: long-gsl3-final-09.22.06-h1
INOVA-500 "nmr500"

Pulse 45.4 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
128 repetitions
OBSERVE H1, 499.9157449 MHz
DATA PROCESSING
FT size 32758
Total time 4 min, 3 sec



C13 of MJH-282 on ID-probe, before HDEC. 03/11/2003.

Pulse Sequence: s3pul

Solvent: DMSO

Ambient temperature

User: 1-14-87

File: long-gal3-final-03.35.07-C13

INOVA-500 "mm500"

Pulse 69.2 degrees

Acq. time 1.300 sec

Width 25000.0 Hz

176608 repetitions

OBSERVE C13, 125.7039614 MHz

DECOUPLE H1, 499.9182481 MHz

Power 39 dB

continuously on

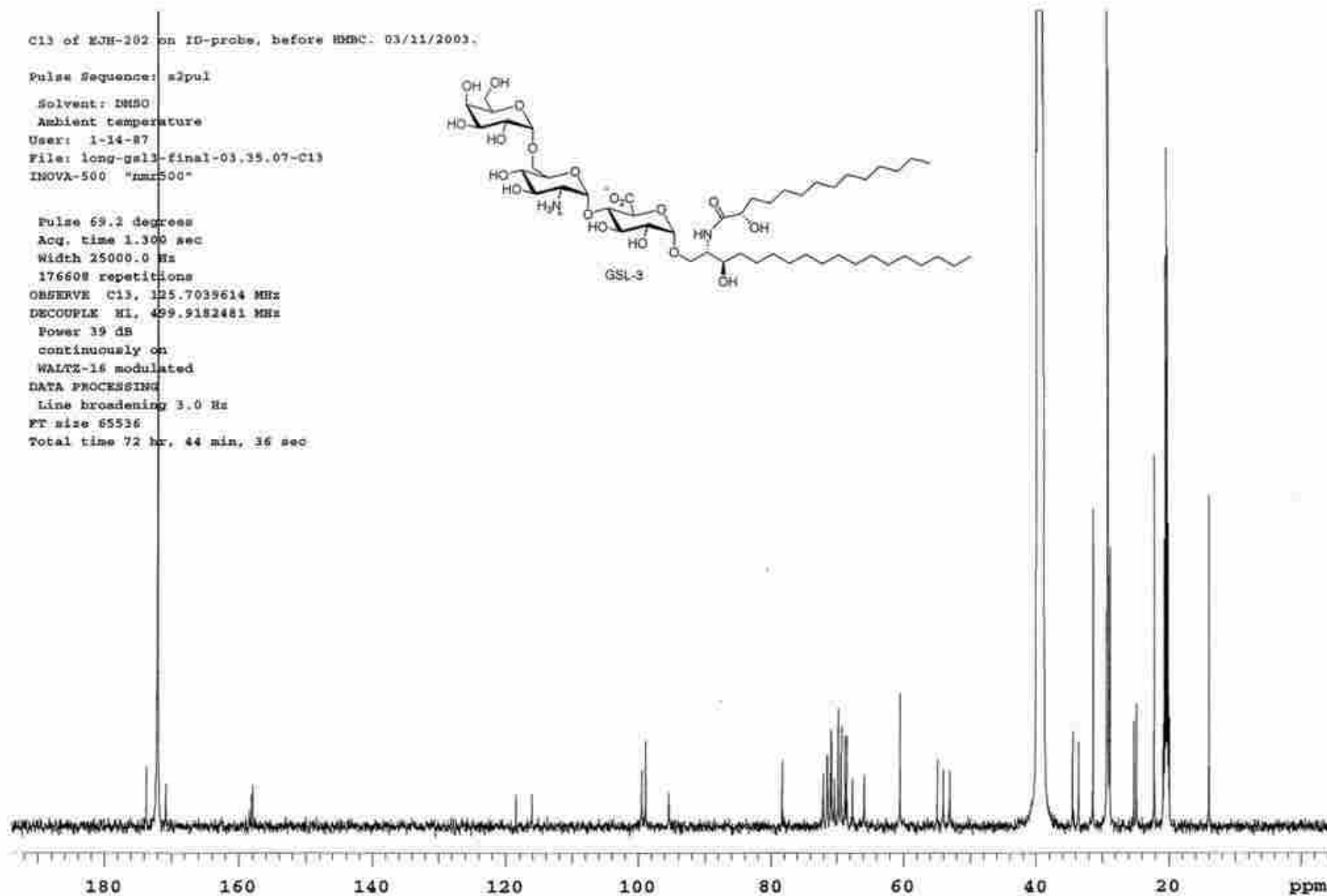
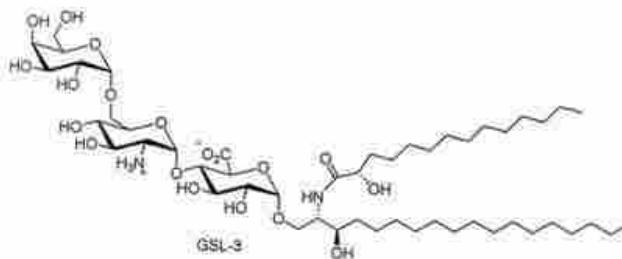
WALTZ-16 modulated

DATA PROCESSING

Line broadening 3.0 Hz

FT size 65536

Total time 72 hr, 44 min, 36 sec



long-gsl4-final-09.18.06-H1

Pulse Sequence: s2pul

Solvent: DMSO

Ambient temperature

File: long-gsl4-final-09.19.06-H1

INOVA-500 "nmr500"

Pulse 45.4 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

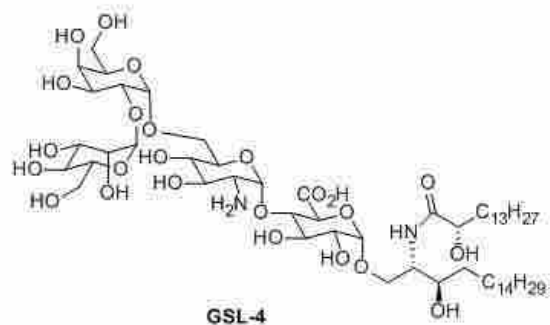
8 repetitions

OBSERVE H1, 499.9157449 MHz

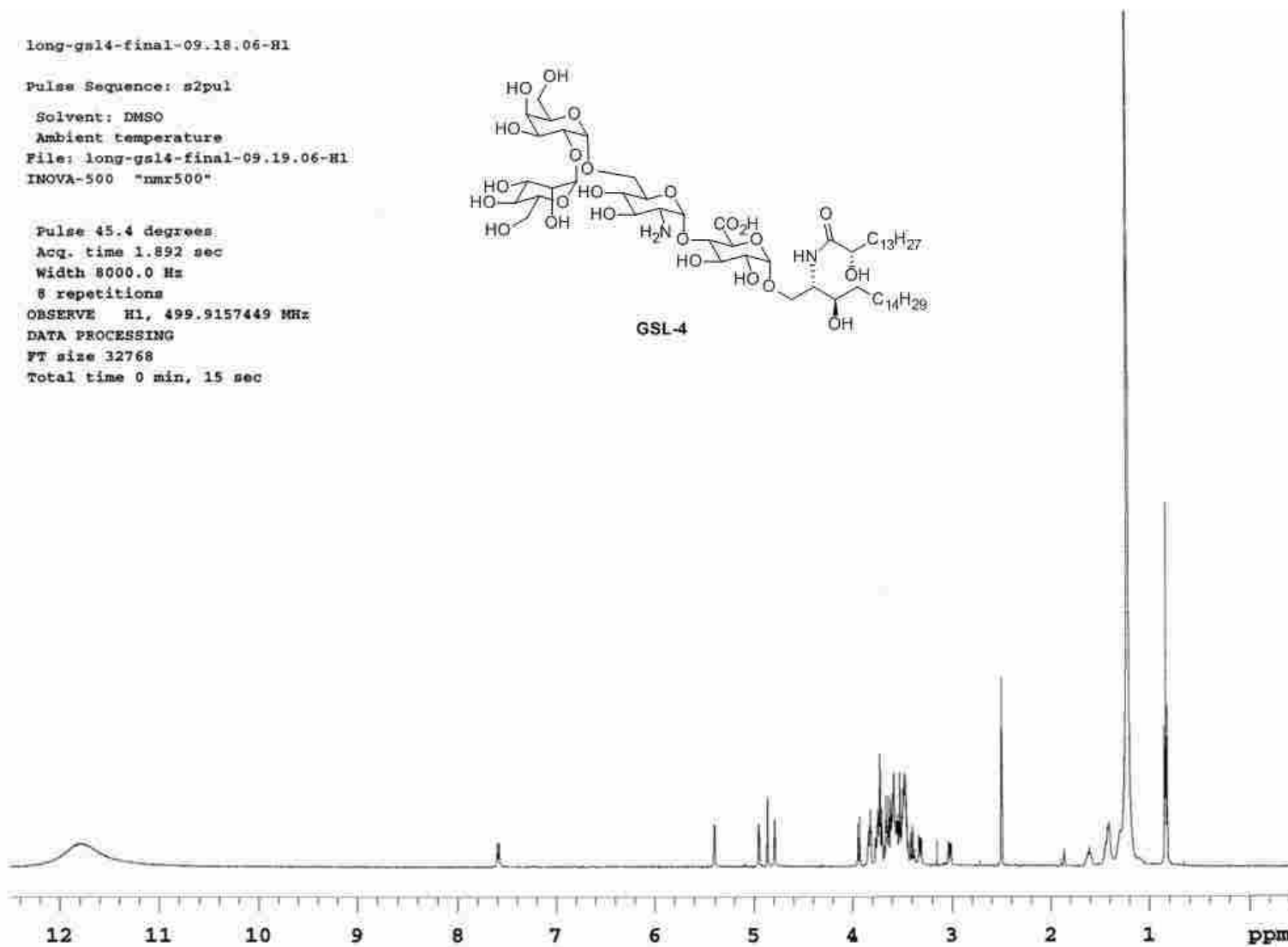
DATA PROCESSING

FT size 32768

Total time 0 min, 15 sec



GSL-4

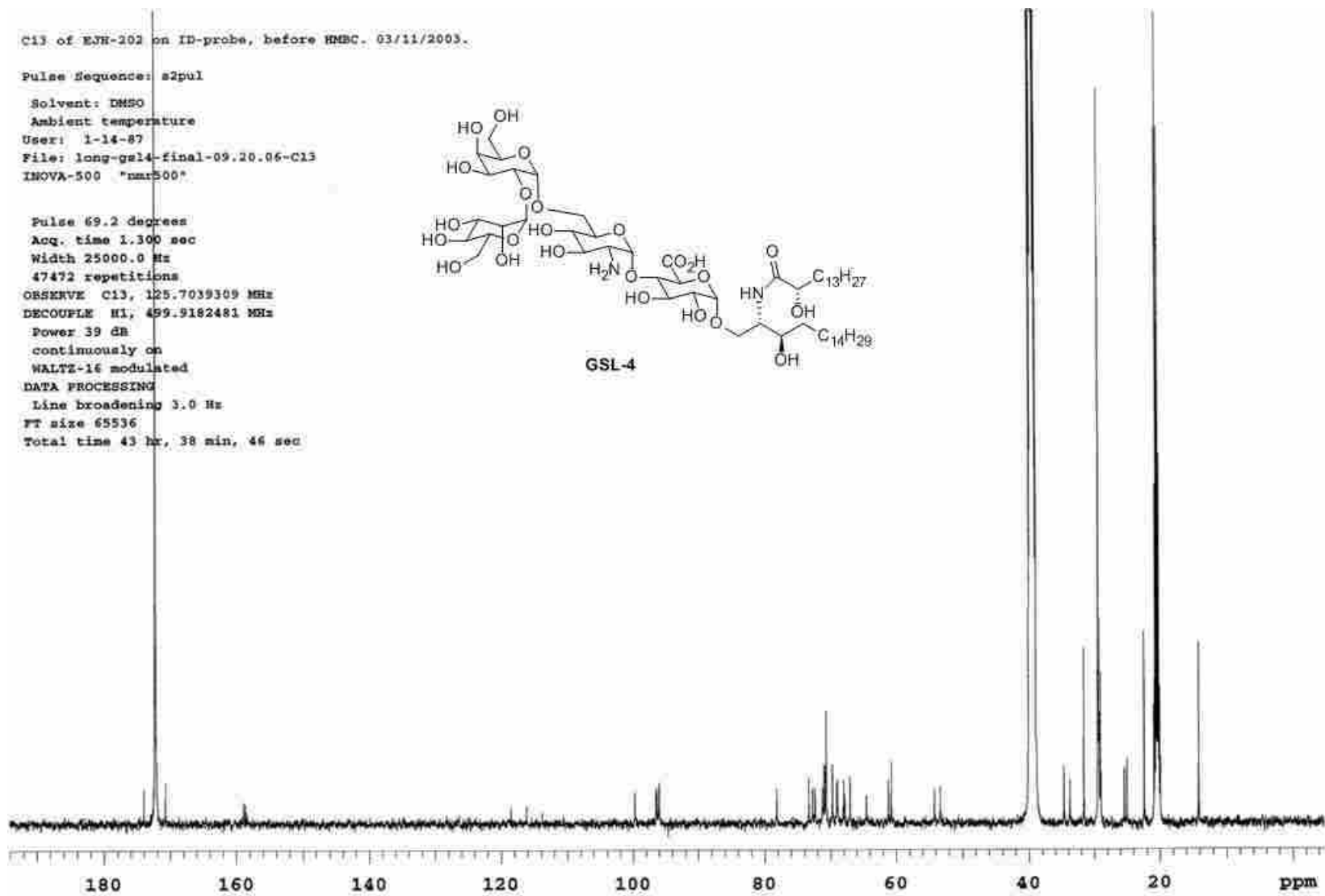
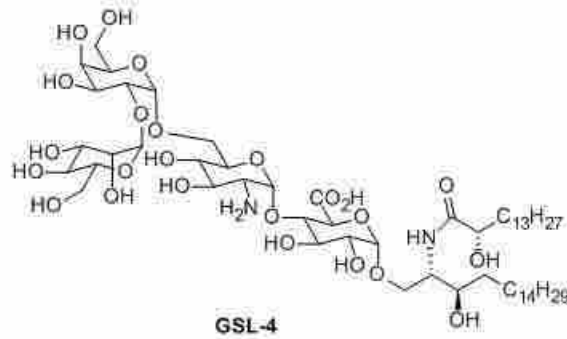


C13 of EJH-202 on ID-probe, before HMBC. 03/11/2003.

Pulse Sequence: s2pul

Solvent: DMSO
Ambient temperature
User: 1-14-87
File: long-gsl4-final-09.20.06-C13
INOVA-500 "nmr500"

Pulse 69.2 degrees
Acq. time 1.300 sec
Width 25000.0 Hz
47472 repetitions
OBSERVE C13, 125.7039309 MHz
DECOUPLE H1, 499.9182481 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 3.0 Hz
FT size 65536
Total time 43 hr, 38 min, 46 sec



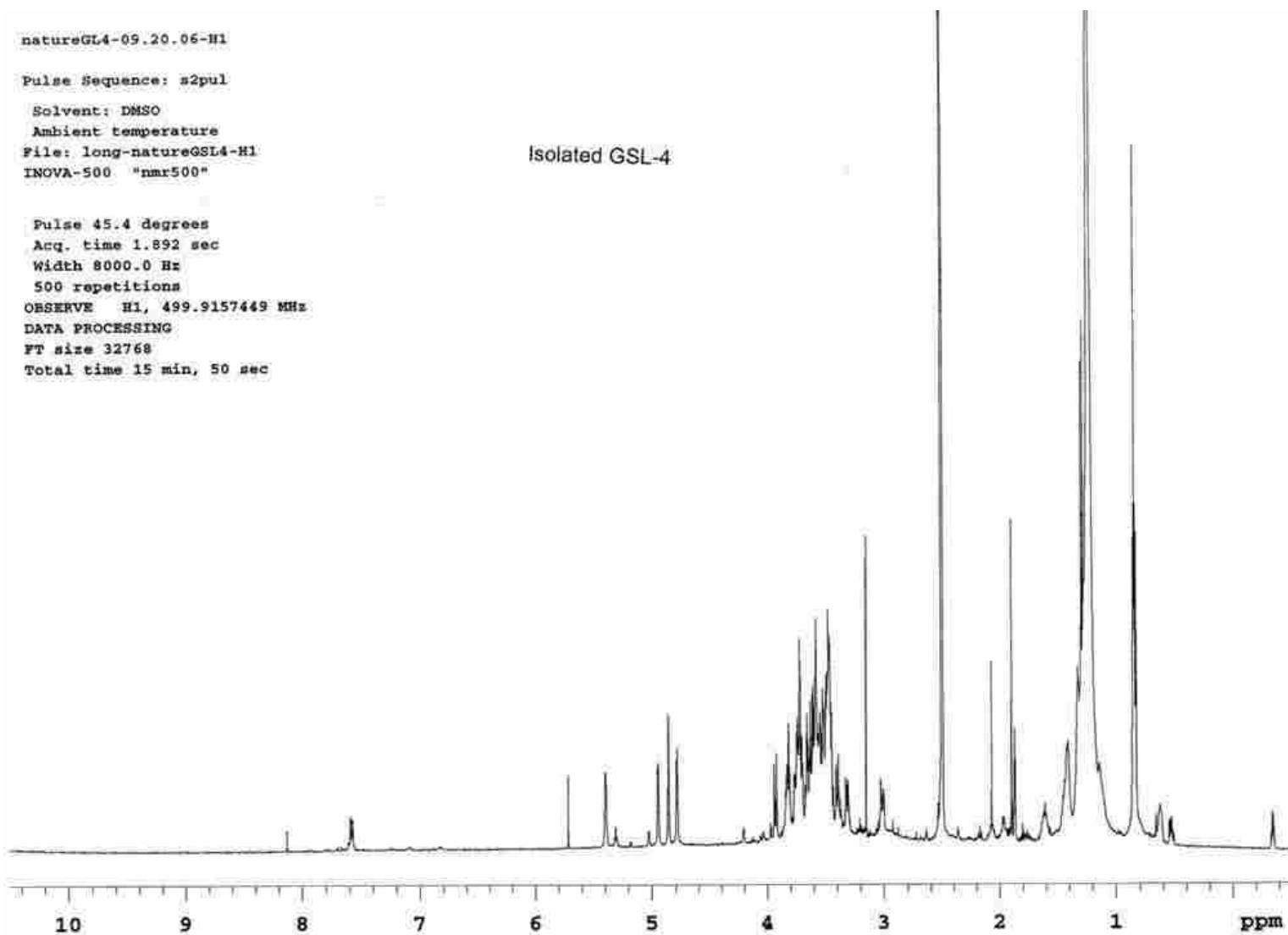
natureGL4-09.20.06-H1

Pulse Sequence: s2pul

Solvent: DMSO
Ambient temperature
File: long-natureGSL4-H1
INOVA-500 "nmr500"

Isolated GSL-4

Pulse 45.4 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
500 repetitions
OBSERVE H1, 499.9157449 MHz
DATA PROCESSING
FT size 32768
Total time 15 min, 50 sec



long-gsl4-final-09.18.06-H1

Pulse Sequence: s2pul

Solvent: DMSO

Ambient temperature

File: long-gsl4-final-09.19.06-H1

INOVA-500 "nmr500"

Pulse 45.4 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

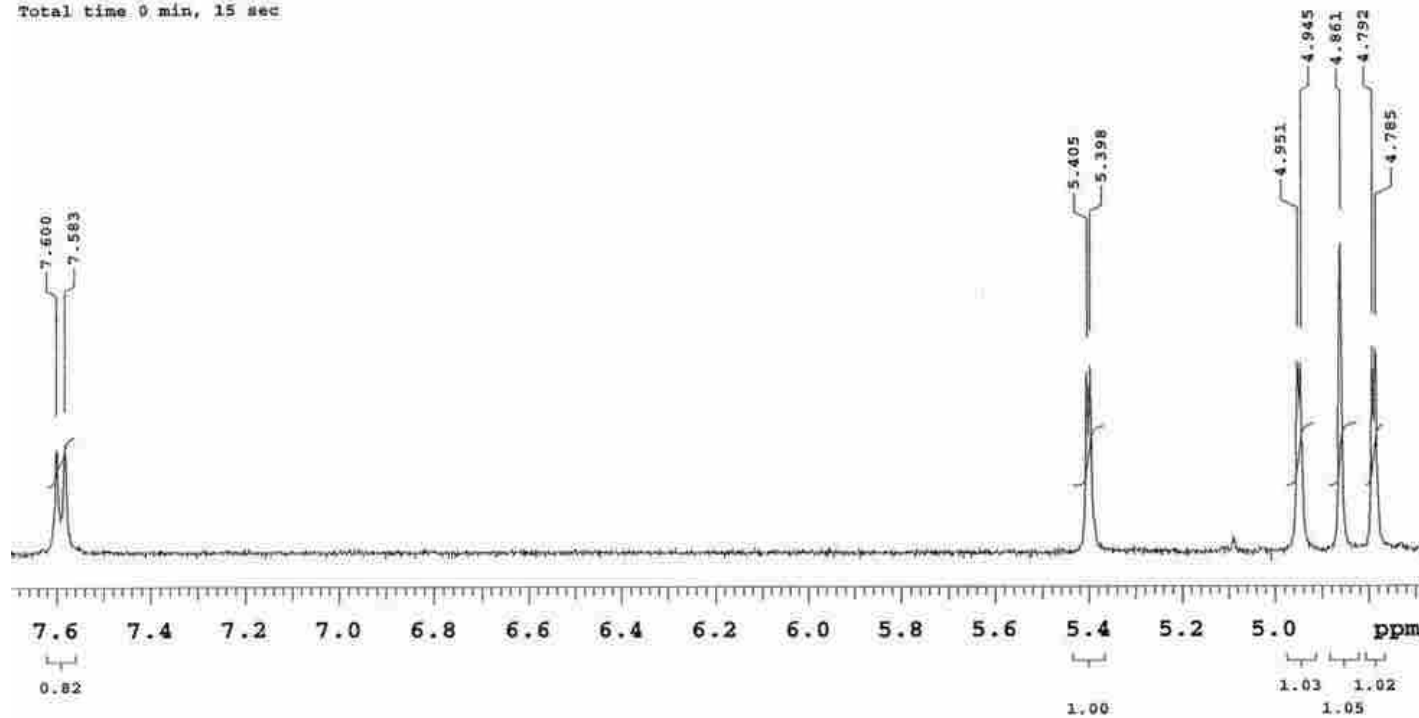
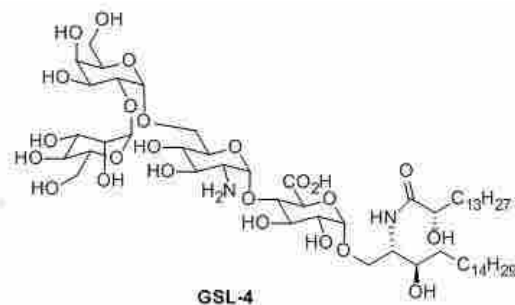
8 repetitions

OBSERVE H1, 499.9157449 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 15 sec



natureGL4-09.20.06-H1

Pulse Sequence: s2pul

Solvent: DMSO

Ambient temperature

File: long-natureGSL4-H1

INOVA-500 "nmr500"

Isolated GSL-4

Pulse 45.4 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

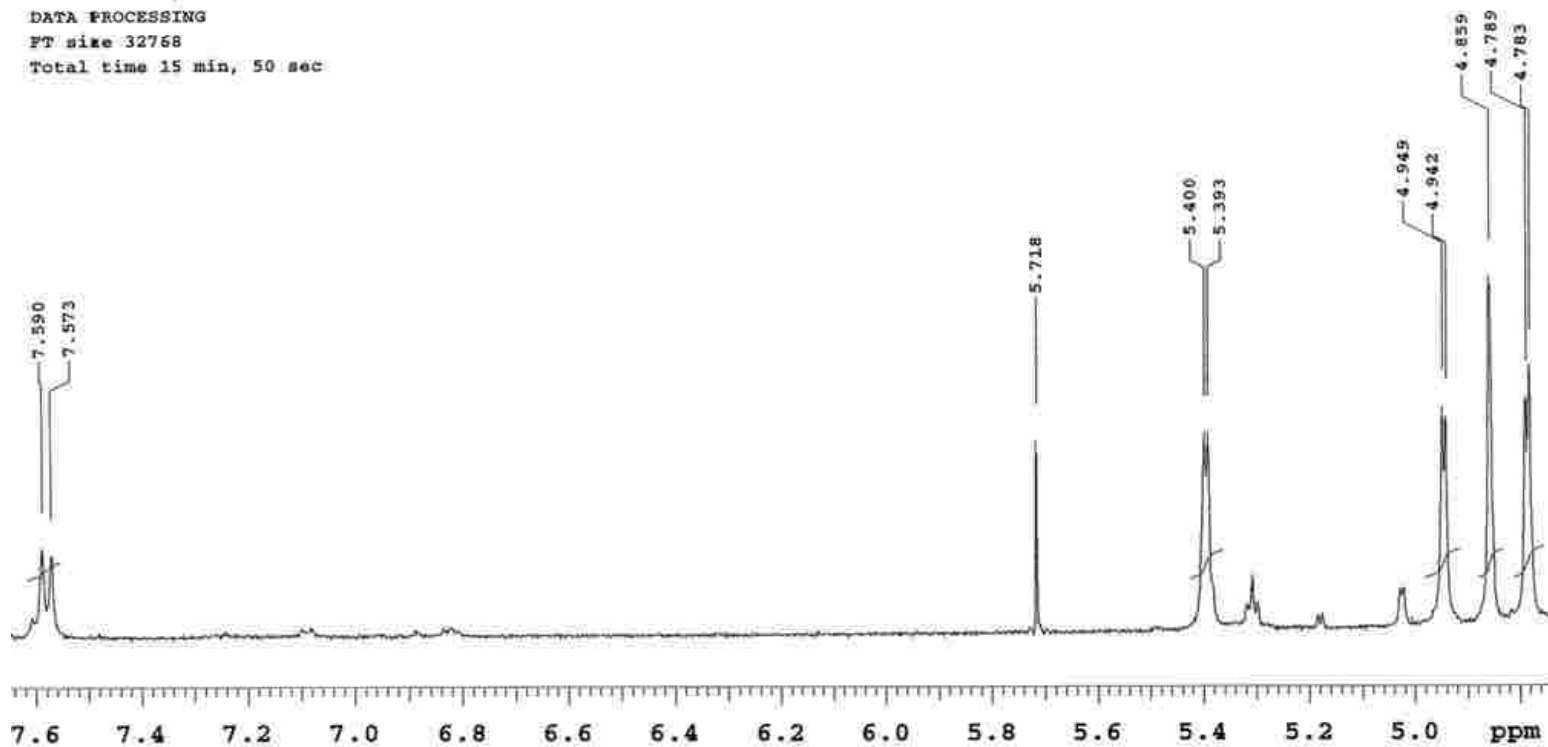
500 repetitions

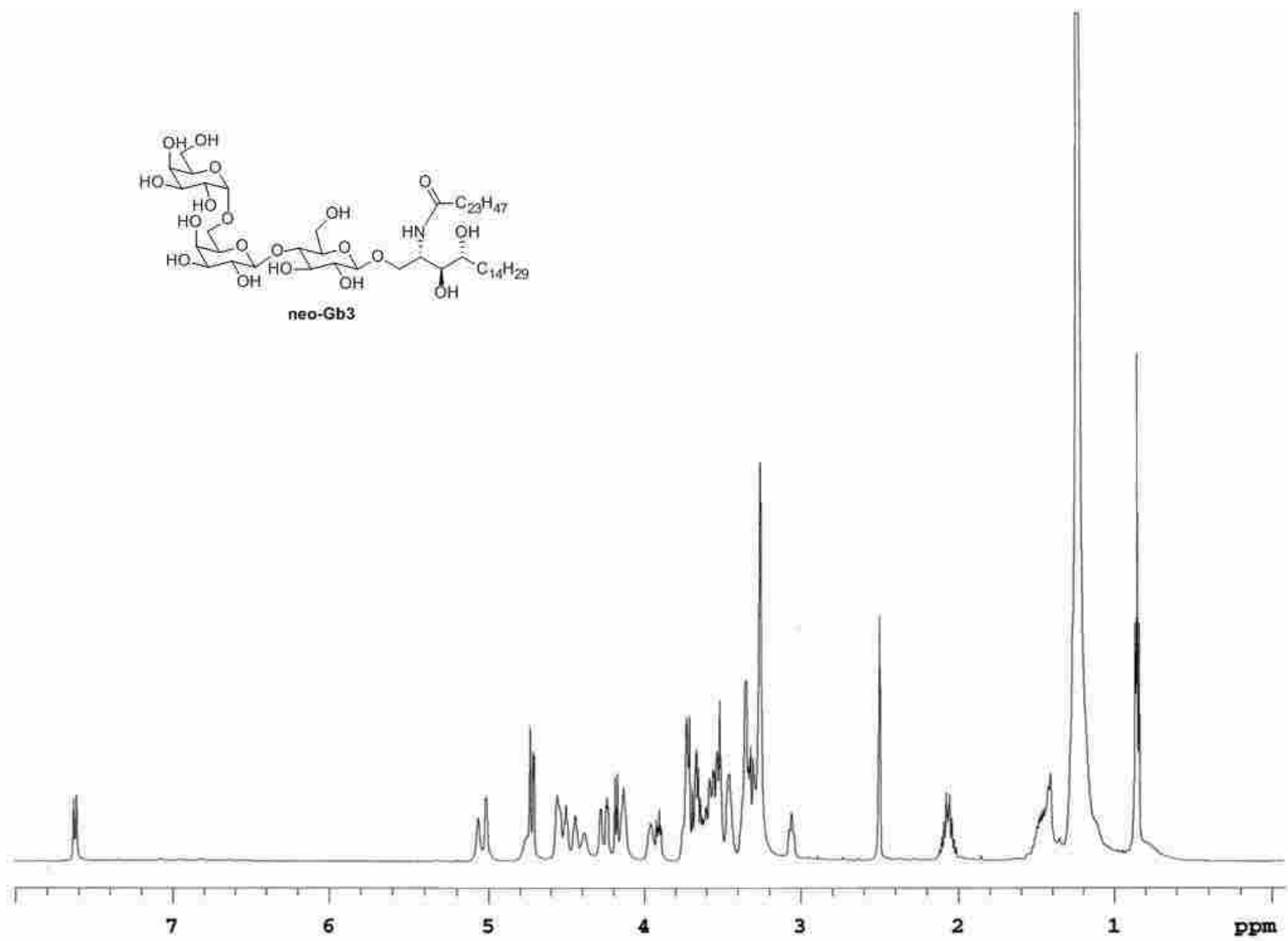
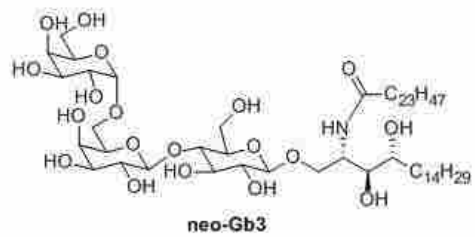
OBSERVE H1, 499.9157449 MHz

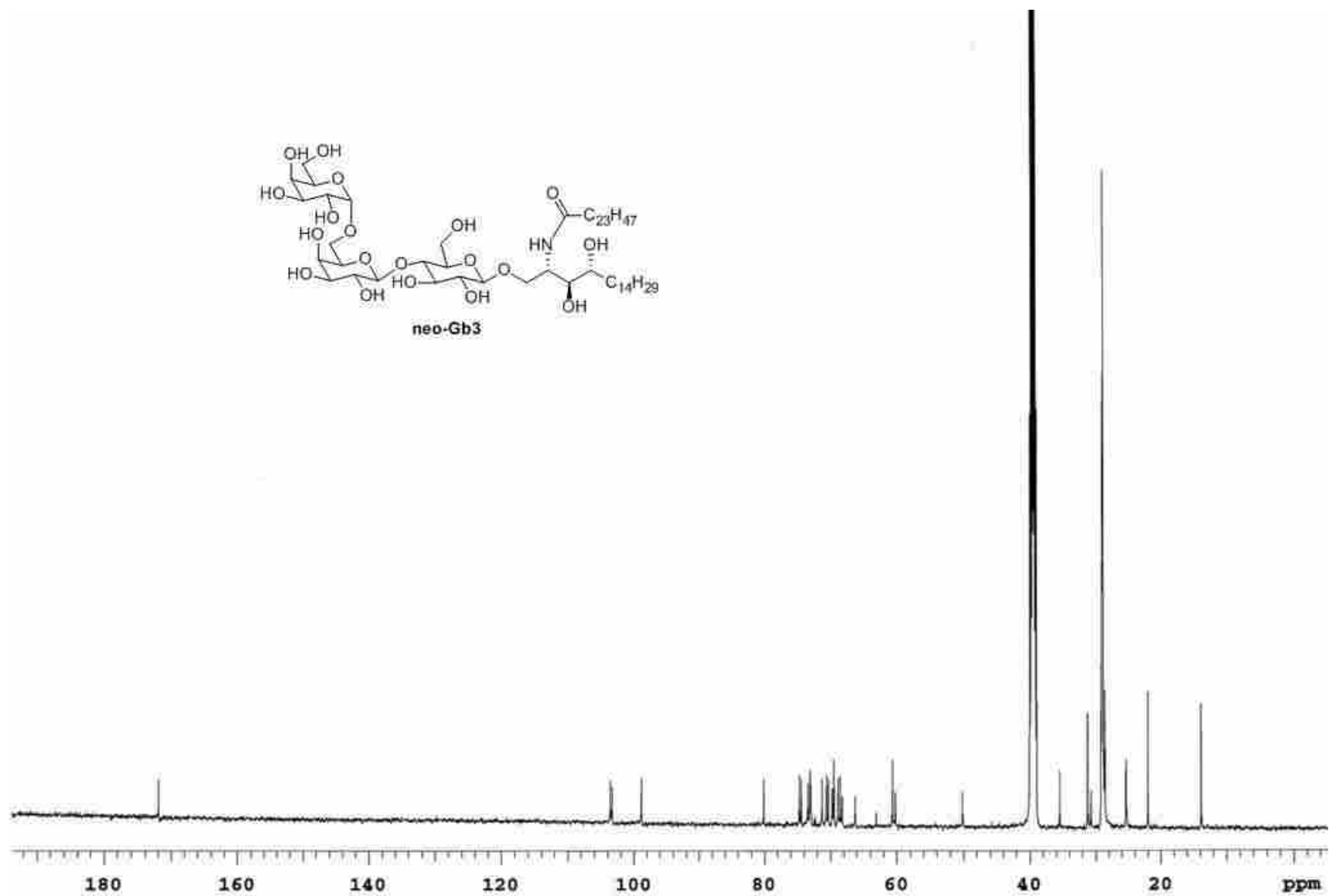
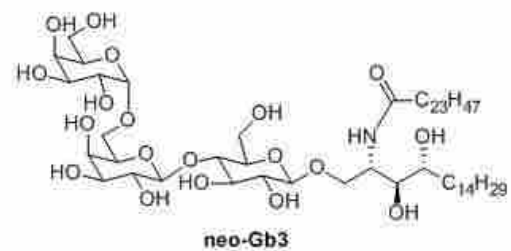
DATA PROCESSING

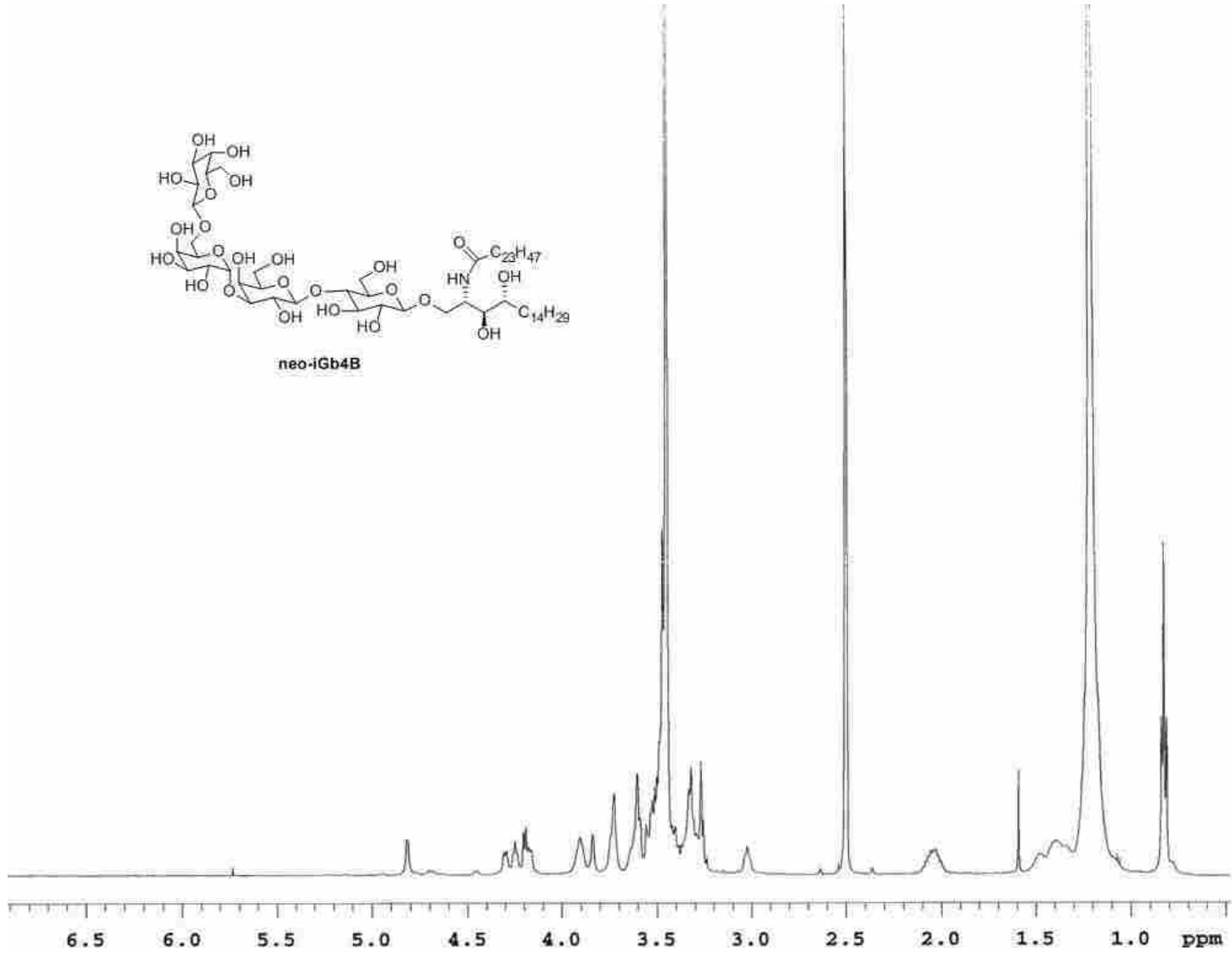
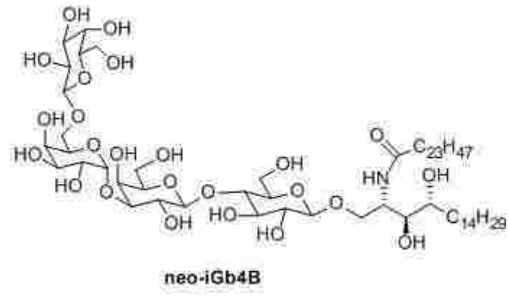
FT size 32768

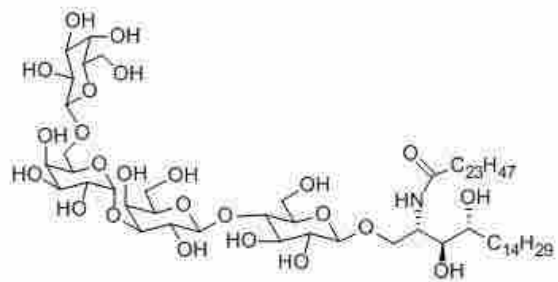
Total time 15 min, 50 sec











neo-iGb4B

