Bioorganic & Medicinal Chemistry 20 (2012) 2850–2859

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of truncated α-galactosylceramide derivatives focusing on cytokine induction profile

Tetsuya Toba [a], Kenji Murata [a], Junko Futamura [a], Kyoko Nakanishi [a], Bitoku Takahashi [a], Naohiro Takemoto [a], Minako Tomino [a], Takashi Nakatsuka [a], Seiichi Imajo [a], Megumi Goto [a], Takashi Yamamura [b], Sachiko Miyake [b], Hirokazu Annoura [a,*]

[a] Asubio Pharma Co., Ltd, 6-4-3, Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan
[b] National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan

ARTICLE INFO

Article history:
Received 13 February 2012
Revised 9 March 2012
Accepted 10 March 2012
Available online 20 March 2012

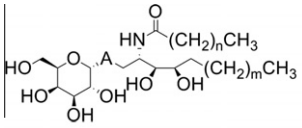
Keywords:
OCH
Th2 cytokine
Phytosphingosine-modified analogs
C-Glycoside

ABSTRACT

A series of truncated analogs of α-galactosylceramide with altered ceramide moiety was prepared, and evaluated for Th2-biased response in the context of IL-4/IFN-γ ratio. Phytosphingosine-modified analogs including cyclic, aromatic and ethereal compounds as well as the C-glycoside analog of OCH (**2**) with their cytokine inducing profile are disclosed.

1. Introduction

Natural killer T (NKT) cells are potent producers of immunoregulatory cytokines, and are restricted to glycolipid antigens presented by CD1d, a glycoprotein structurally and functionally related to non-classical major histocompatibility complex (MHC) class I.[1] Several natural glycolipids of bacterial[2] and mammalian[3] origin, and quite a few synthetic ligands of CD1d are identified and reported to date.[1b,4] Among them, synthetic α-galactosylceramide KRN7000 (**1**)[5] (Fig. 1) is the most extensively studied, for its strong activation of NKT cells as well as its effectiveness in in vivo animal disease models.[6] Compound **1** is known to induce various cytokines including proinflammatory Th1 cytokine interferon-γ (IFN-γ) and immunomodulatory Th2 cytokine interleukin-4 (IL-4), which oppose each other's response and may in part result in its marginal effect. Some studies are reported which aim to increase the selectivity of Th1 or Th2 cytokine induction. The majority are directed towards increased Th1 activity, and not few utilize the derivatives of the acyl chain and/or the sugar moiety which are relatively easy to prepare from a synthetic point of view. One of the most potent compounds reported to date is that with 8-(4-fluorophenyl)octanoyl chain as the acyl tail, which binds two orders of magnitude stronger with CD1d than **1**.[7] Another impressing finding was the conversion of **1** to its C-glycoside analog **3**, which leads to striking enhancement of activity in in vivo animal models of malaria and lung cancer.[8]



1 (**KRN7000**; A = O, m = 12, n = 24)
2 (**OCH**; A = O, m = 3, n = 22)
3 (A = CH_2, m = 12, n = 24)
4 (A = CH_2, m = 3, n = 22)

Figure 1. Structures of KRN7000 (**1**), OCH (**2**) and their C-glycoside analogs **3**, **4**.

An altered analog of **1** termed OCH (**2**) possessing a shorter phytosphingosine side chain[9] has been identified as NKT cell ligand which predominantly induces IL-4 over IFN-γ. Only compound **2** but not **1** is significantly effective in animal models of Th1-mediated autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) and collagen induced arthritis (CIA), which makes it an attractive lead for potential therapeutic application.[9,10]

Complete occupation of the binding groove of CD1d by **1** contributes to the sustained stimulation of NKT cells to induce robust immunological response, as indicated by several examples of X-ray crystallographic structures of compound **1**/CD1d complex.[11,12] Altered analogs such as **2** with short phytosphingosine chain is considered to result in short duration of stimulation and

* Corresponding author. Tel.: +81 78 306 5047; fax: +81 78 306 5971.
E-mail address: annoura.hirokazu.wk@asubio.co.jp (H. Annoura).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.03.025

cause differential polarization of NKT cells.[9d] Instability of the short-chain analogs to form binary and ternary complexes is shown by molecular dynamics simulation study[13] and more directly by using the surface plasmon resonance (SPR) technique.[14] It was also shown that truncation in the phytosphingosine and not the acyl chain will affect the NKT cell activation profile.[14]

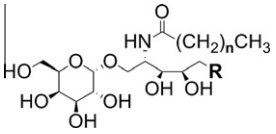
As part of our efforts to obtain more potent compounds for the enhancement of Th2 response, a series of analogs based on **2** with altered ceramide moiety was prepared and evaluated in vitro, some of which are the first to be reported. In this report, the structure–activity relationship in the context of IL-4/IFN-γ ratio is described. In the course of our study, the C-glycoside of **2** was prepared for the first time and its cytokine-inducing profile in vitro and in vivo are also described.

2. Results and discussion

2.1. Chemistry

The analogs were prepared by the versatile method developed by our group (Scheme 1).[15] The phytosphingosine side chain substituents R shown in Tables 1 and 2 were introduced to the known epoxide **5** by means of nucleophilic addition. In addition to the nucleophiles reported earlier utilizing alkyl or aryl lithium reagents or corresponding magnesium bromides,[16] alkoxides and phenoxide were also efficiently introduced. Liquid alcohols were reacted as a solvent, while dioxane was used as a solvent for solid hydroxyls such as phenol. Various nucleophiles, including short or long primary alkyl, secondary alkyl, aryl, alkoxy and aryloxy groups were successfully incorporated via this route. After regioselective mesylation of the more reactive axial hydroxyl group,[17] compound **6** was subjected to benzylidene cleavage and azidation, after which secondary hydroxyl groups were protected to provide isopropylidene acetal **7**. The order of de-benzylidene reaction and azidation could be reversed, but azidation first of the axial mesyloxy group of **6** needed higher temperature, longer time and gave lower yield presumably for its steric demand. On the other hand, azidation later to the deprotected **6** yielded small portions of regio- and stereoisomers as side products along with major product **7**, assumed to have formed via epoxide through nucleophilic addition of the vicinal hydroxyl groups. Generally, deprotection first of **6** gave higher yield in total. Glycosidation with tetra-*O*-benzyl-α-D-galactosyl fluoride in the presence of $BF_3{\cdot}OEt_2$, or with tetra-*O*-benzyl-α-D-galactosyl bromide or chloride in the presence of tetra-*n*-butylammonium bromide gave selectively the α-glycoside **9**. The selectivity over the β-isomer was improved in the latter protocol, to a ratio typically greater than 10:1.[15,18] The azido group in **9** was reduced to an amine and acylated with suitable carboxylic acids to give **10**. Finally, all the protective groups were removed to give the desired analogs.

Table 1
Dependency of cytokine induction on alkyl chain lengths[a]



Compound	R	*n*	IL-4[b] (%)	IFN-γ[b] (%)
11a	$-CH_2CH_3$	22	105	96
11b	$-(CH_2)_2CH_3$	21	97	148
11c	$-(CH_2)_2CH_3$	22	115	112
11d	$-(CH_2)_3CH_3$	18	6	9
11e	$-(CH_2)_3CH_3$	20	51	46
11f	$-(CH_2)_3CH_3$	21	103	93
2	$-(CH_2)_3CH_3$	22	100	100
11g	$-(CH_2)_3CH_3$	23	154	103
11h	$-(CH_2)_3CH_3$	24	129	504
11i	$-(CH_2)_3CH_3$	26	178	761
11j	$-(CH_2)_4CH_3$	21	97	113
1	$-(CH_2)_{12}CH_3$	24	128	569

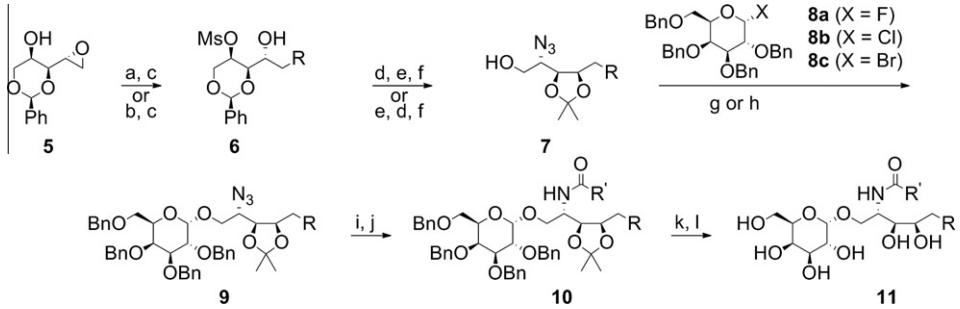
[a] At 100 ng/ml.
[b] Normalized to **2** at 100 ng/ml.

It is worthy of note that alkoxy derivatives (e.g., R = *n*-PrO) or aryl derivatives (e.g., R = Ph) with R at this position are not directly accessible via the Wittig reaction of stereo-fixed, sugar-based starting materials (e.g., D-lyxose).[5a,19]

C-Glycoside **4** was synthesized by short and efficient route as depicted in Scheme 2.[20] Known α-ethynylgalactose derivative **12**[21] and octanal derivative **13** synthesized from L-arabinose were coupled in a chelation-controlled manner to give a 1.6:1 mixture of **14a** and **14b**. Compounds **14a** and **14b** were easily separated by column chromatography over silica gel, and the stereochemistry of the newly formed diastereomeric center was determined for the major isomer **14a** applying modified Mosher's protocol[22] to have the *R*-configuration.[20] The acetylenic bond in **14a** was selectively and efficiently reduced by diimide reduction, after which the hydroxyl group was mesylated to give **15**. The synthesis of **4** was completed in a straightforward manner, after substitution by azido group, reduction, acylation and global deprotection.

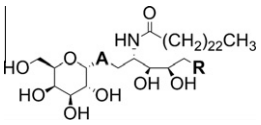
2.2. Biological evaluation

The analogs were evaluated in vitro for their ability to induce IL-4 and IFN-γ relative to **2**. IL-4 and IFN-γ secretion were assessed with spleen cells prepared from C57BL/6 mice, which were



Scheme 1. Synthesis of O-glycosides. Reagents and conditions: (a) RLi or RMgBr, CuI or CuOTf, THF, −40 °C, 52–98%; (b) alcohol or phenol, NaH, (dioxane), rt–80 °C, 83–88%; (c) MsCl, pyridine, −40 °C–rt, 34–93%; (d) H_2, $Pd(OH)_2$/C, EtOH, rt, or 6 N HCl, MeOH, rt, 68–100%; (e) NaN_3, DMF, 95–110 °C, 20–66%; (f) cat. *p*-TsOH, 2,2-dimethoxypropane, rt, 26–75%; (g) **8a**, $BF_3{\cdot}OEt_2$, MS 4 Å, $CHCl_3$, −50 °C, 13–73%; (h) **8b** or **8c**, *n*-Bu_4NBr, MS 4 Å, DMF–toluene, rt, 22–68%; (i) H_2, Lindlar catalyst, EtOH, rt; (j) R'CO_2H, EDCI·HCl, HOBt or HOAt, *i*-Pr_2NEt, DMF–CH_2Cl_2, 40 °C, 22–100% (two steps); (k) HCl–dioxane, MeOH–CH_2Cl_2, rt, or 80% AcOH, 80 °C; (l) H_2, $Pd(OH)_2$/C, MeOH–$CHCl_3$, rt–40 °C, 41–91% (two steps).

Table 2
Cytokine induction profile of the phytosphingosine-altered derivatives and the C-glycoside of OCH[a]



Compound	A	R	IL-4[b] (%)	IFN-γ[b] (%)
2	O	$-(CH_2)_3CH_3$	100	100
11k	O	–*c*-Pent	98	74
11l	O	–Ph	211	284
11m	O	$-CH_2Ph$	57	35
11n	O	–*p*-Tol	78	76
11o	O	$-OCH_3$	86	64
11p	O	$-O(CH_2)_2CH_3$	103	161
11q	O	$-O(CH_2)_{11}CH_3$	78	227
11r	O	–OPh	99	80
4	CH_2	$-(CH_2)_3CH_3$	1	0

[a] At 100 ng/ml.
[b] Normalized to **2** at 100 ng/ml.

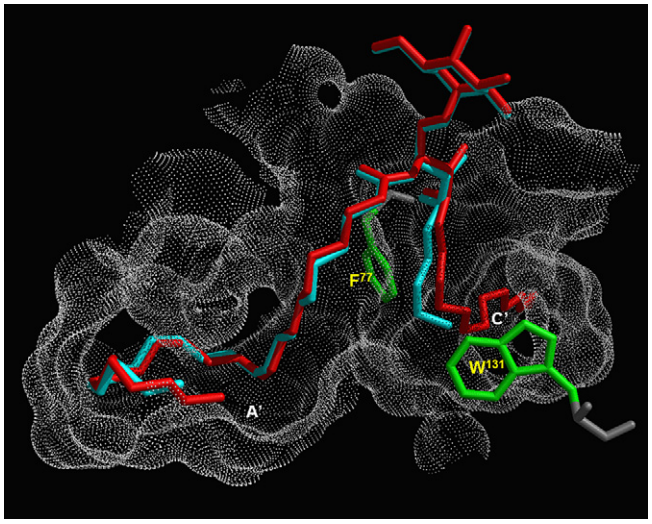


Figure 2. Side view of the optimized structure of **2** (blue)/hCD1d complex. X-ray structure of **1** (red) is superimposed.

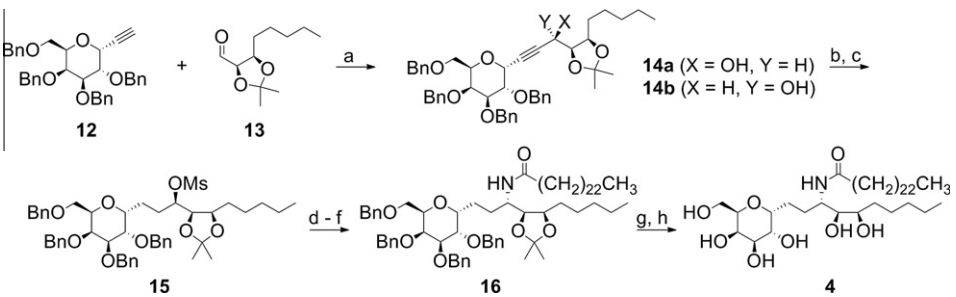
incubated with 100 ng/ml of glycolipids for 72 h. The cytokines in the culture supernatant were measured by ELISA.

Influence of the chain lengths was first examined (Table 1). When the phytosphingosine chain was fixed to that of **2** and acyl chain length altered (compounds **2** and **11d**–**11i**), the chain length proximal to **2** showed similar cytokine production. As the acyl chain became longer the cytokine release increased, and for chains longer than hexacosanoic acid there was a marked increase in IFN-γ production that dominated IL-4 (**11h**, **11i**), which was comparable to **1**. On the other hand, as the chain became shorter the induction of both cytokines decreased rather drastically, and icosanoyl derivative **11d** showed negligible efficacy.

When the acyl chain length was next fixed to that of **2** and sphingosine base altered, in our hands cytokine producing profile did not change for given derivatives (**11a**, **11c** and **2**; $n = 22$). Compounds **11b**, **11f** and **11j** which have tricosanoyl chain ($n = 21$) also showed similar profile. Taking above results together, we concluded that very close modification of **2** both in acyl and sphingosine chain are tolerated and shows similar profiles, and that further shortening of the acyl chain in aim for more Th2-biased response seems inappropriate.

Our interest was next focused on the phytosphingosine moiety, where it makes **2** a completely different switch of the NKT cell signal. Analogs bearing aliphatic ring (**11k**) or aromatic ring (**11l**–**11n**) were also prepared. To our knowledge, these are the first examples of non-linear hydrocarbon chain analogs of the phytosphingosine moiety that show similar cytokine inducing ratio to **2**.[23] Compound **11l** with an aromatic ring appears to show slight increase in both cytokines. This is suggestive of aromatic interaction(s) with residues such as Phe77 and Trp131 in CD1d (Fig. 2). Aromatic derivatives with one methylene unit longer (**11m**) or one additional methyl in the *para*-position (**11n**) showed reduced activity, which is indicative of the appropriate length and flexibility in reference to pocket depth, as well as for possible interaction with aromatic residues. We have next prepared analogs bearing ether linkage in the phytosphingosine chain. There is only one report for α-galactosylceramide derivatives with aliphatic ether chain, which assessed the release of IL-2, a Th1 cytokine.[24] Our compounds (**11o**–**11r**) including oxa- analog of **2** showed similar profiles to corresponding methylene derivatives, which is in line with one example of oxa- analog of **1** shown in the previous report.[24] The oxa- analogs were also shown for the first time to be comparable in the context of IL-4/IFN-γ ratio to the corresponding methylene analogs.

The binding groove of the CD1d consists of two hydrophobic channels A′ and C′ that accommodate two lipid chains of α-glycosyl ceramides. As can be seen from the X-ray structure of **1**/hCD1d complex,[12] the acyl chain occupies A′ pocket and the bent phytosphingosine chain enters the narrow C′ pocket beyond the length of **2** (Fig. 2). We assume the phytosphingosine-altered analogs in Tables 1 and 2 which in length do not reach the C′ pocket showed similar profiles to **2** owing to weak interaction with CD1d. There is a wide space before entering the C′ pocket which allows cycloalkyl and aromatic substituents, and as mentioned earlier the aromatic side chain might interact with aromatic residues such as Phe77 and Trp131. Molecular modeling of **2**/hCD1d complex based on



Scheme 2. Synthesis of C-glycoside **4**. Reagents and conditions: (a) *n*-BuLi, THF, −48 to −30 °C, 47% for **14a**, 30% for **14b** (BRSM); (b) $TsNHNH_2$, DME, NaOAc aq, reflux, 91%; (c) MsCl, pyridine, CH_2Cl_2, 0 °C–rt, 94%; (d) NaN_3, DMF, 90 °C; (e) H_2, Lindlar catalyst, EtOH, rt; (f) Lignoceric acid, EDCI·HCl, HOAt, Et_3N, DMF–CH_2Cl_2, rt, 48% (three steps); (g) 80% AcOH, 60 °C, 88%; (h) H_2, $Pd(OH)_2$/C, MeOH–CH_2Cl_2, rt, quant.

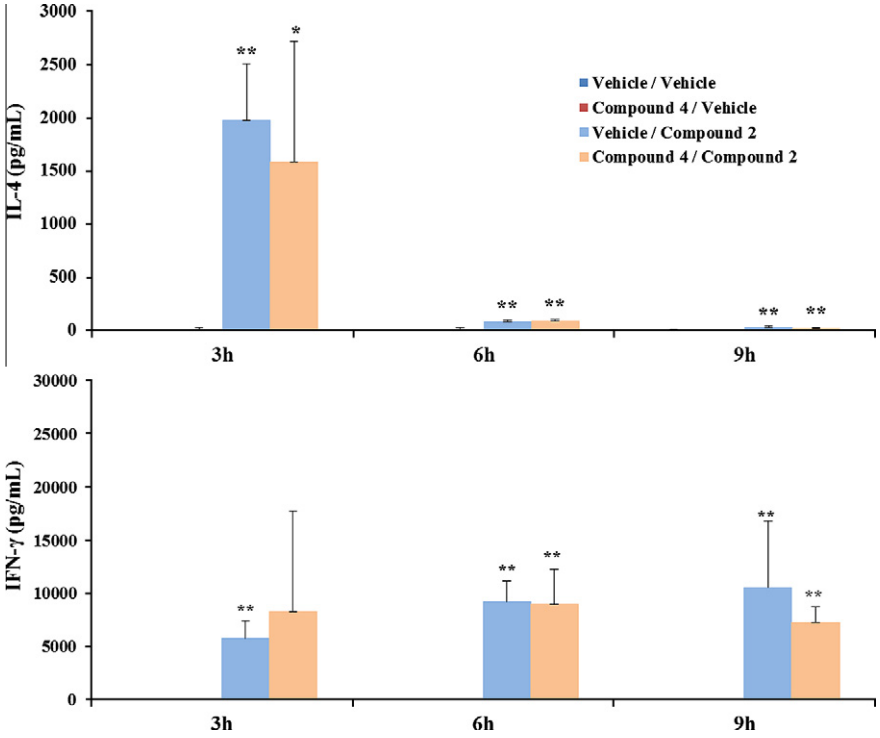


Figure 3. In vivo IL-4/IFN-γ production profile of **2** and **4** after iv administration to C57BL/6 mice. The data are expressed as mean ± SD (N = 4–5). $^{**}p$ <0.01, $^{*}p$ <0.05 compared with vehicle group (Student's t-test).

the crystal structure of **1**/hCD1d complex[12] was performed utilizing MAESTRO[25] program. Contrary to our expectation, the optimized structure of **2** in the complex had only a subtle, insignificant difference from **1** (Fig. 2). Some of the above derivatives were also calculated in silico, including aromatic derivative **11l** in expectation of aromatic interaction(s), but no significant difference was observed either (data not shown). No significant conformational change in the α1 and α2 helices of CD1d was observed in the minimization initiated from the X-ray structure. Molecular dynamics simulation might be more appropriate for the understanding of this exquisite signaling system.[13]

C-Glycoside derivative (**4**) of **2** was prepared and evaluated for its cytokine inducing profile. Conversion of **1** to its C-glycoside analog **3** is reported to lead to striking enhancement of activity in in vivo animal models of malaria and lung cancer.[8] It is the only example of the C-glycoside which is more potent than corresponding O-glycoside. C-Glycoside (**3**) is shown to somehow stimulate prolonged IL-12 secretion from dendritic cells, followed by prolonged IFN-γ stimulation from NK cells. Compound **4** did not show induction of either cytokines in vitro (Table 2), and in contrast to **3** did not elevate cytokine levels in vivo when administered intravenously to C57BL/6 mice (Fig. 3). In addition, **4** was co-administered intravenously with **2** to evaluate its antagonistic activity. Compound **4** did not antagonize the elevation of IL-4 or IFN-γ levels caused by **2** (Fig. 3). Although the anomeric oxygen does not participate in the hydrogen bond network in the ternary complex with CD1d and NKT T-cell receptor,[11] subtle difference from O to CH_2 was shown to have great influence on the signal transduction.

3. Conclusion

Several analogs related to **1** have been prepared to date, and many of them are equipotent to or even more potent than **1** in the aspect of IFN-γ secretion. In this study, a series of analogs based on **2** with altered ceramide moiety was prepared for its Th2-biased response, and evaluated in the context of IL-4/IFN-γ ratio. Compound **2** in terms of chain length was shown to be one of the optimal compounds for the desired profile. First examples of phytosphingosine-modified analogs were discovered with non-linear hydrocarbon chain or ether linkage that show similar cytokine inducing profile to **2**. Expected aromatic interaction in the sphingosine chain may be of use in the future derivatization. Unprecedented C-glycoside of **2** was prepared and evaluated, which was shown to have no cytokine production effect in vitro or in vivo. In the course of this study, versatile syntheses were developed which allowed preparation of unprecedented derivatives and new findings on Th2 biased immunomodulation. The method and the possibility of structure modification proven in this study should allow future access to the analogs improved in their pharmacological and physicochemical properties.

4. Experimental

4.1. Chemistry

Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on Brucker ARX-400 or Brucker Avance III (400 MHz) spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to the internal standard tetramethylsilane. High-resolution mass spectra (HRMS) and fast atom bombardment (FAB) mass spectra were recorded on JEOL JMS-700 mass spectrometer. Electro-spray ionization (ESI) mass spectra were recorded on Agilent G1956A MSD spectrometer system. Other chemical reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, Kanto Kagaku or Nacalai tesque and used without purification. Flash column chromatography was performed using Merck Silica Gel 60 (230–400 mesh) or Purif-Pack® SI 30um supplied by Shoko Scientific. The experimental procedure for alkyl chain derivative **2** is reported

previously.[15] Exemplified procedure for aryl derivative **11l**, alkoxy derivative **11p** and C-glycoside **4**, along with compound data for all compounds **11a**–**11r** are described.

4.1.1. (2*R*,3*R*,4*R*)-1,3-*O*-Benzylidene-2-*O*-methanesulfonyl-5-phenyl-1,2,3,4-pentanetetrol (6l)

To a suspension of CuI (4.28 g, 22.5 mmol) in THF (45 ml) was added 1.06 M PhLi in THF (85 ml, 90.1 mmol) dropwise at −40 °C and the mixture was stirred for 1 h. A solution of **5** (5.01 g, 22.6 mmol) in THF (15 ml) was added via cannula, and the reaction was slowly allowed to warm to rt over 6 h. The reaction was quenched with satd NH_4Cl aq, extracted with EtOAc and washed twice with half-satd NH_4Cl aq. The organic layer was filtered through Celite, dried over Na_2SO_4 and concentrated. The precipitation formed was filtered and purified by silica gel column chromatography (CH_2Cl_2/MeOH; 3%) to give a colorless solid (6.47 g, 96%). To the solution of this diol (6.40 g, 21.3 mmol) in pyridine (70 ml) was added methanesulfonyl chloride (1.65 ml, 21.3 mmol) at 0 °C, and the mixture was gradually warmed to rt. Pyridine was removed under reduced pressure after consumption of the starting diol, and the residue was diluted with EtOAc, washed twice with water and brine, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc; 50%) to yield **6l** as a colorless solid (2.75 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.55–7.52 (m, 2H), 7.44–7.20 (m, 8H), 5.59 (s, 1H), 4.91 (d, *J* = 1.3 Hz, 1H), 4.52 (dd, *J* = 1.5, 13.2 Hz, 1H), 4.12 (dd, *J* = 1.2, 13.3 Hz, 1H), 4.10–4.05 (m, 1H), 3.77 (dd, *J* = 1.2, 9.0 Hz, 1H), 3.18 (dd, *J* = 2.8, 13.9 Hz, 1H), 3.13 (s, 3H), 2.78 (dd, *J* = 7.7, 13.8 Hz, 1H), 2.61 (d, *J* = 5.2 Hz, 1H).

4.1.2. (2*S*,3*S*,4*R*)-2-Azido-3,4-*O*-isopropylidene-5-phenyl-1,3,4-pentanetriol (7l)

A mixture of **6l** (2.70 g, 7.14 mmol) and NaN_3 (5.57 g, 85.7 mmol) in DMF (35 ml) was stirred at 110 °C for 17 h. The reaction was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc; 40–66%) to yield the azide (892 mg, 38%). To this azide (860 mg, 2.65 mmol) in MeOH (14 ml) was added 6 N HCl (1.3 ml, 7.95 mmol) at 0 °C, and the mixture was stirred for 4 h. The reaction was neutralized with solid K_2CO_3, then filtered, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 40–66%) to yield the triol (434 mg, 69%). The triol (430 mg, 1.81 mmol) was dissolved in 2,2-dimethoxypropane (7 ml), catalytic amount of *p*-toluenesulfonic acid monohydrate (174 mg, 0.092 mmol) was added, and the mixture was stirred for 2 h. MeOH was added and the reaction was stirred for 1 h. The mixture was concentrated and directly purified by silica gel column chromatography (hexane/EtOAc; 17%) to yield **7l** as a colorless oil (350 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35–7.17 (m, 5H), 4.45 (ddd, *J* = 3.1, 5.6, 10.2 Hz, 1H), 4.12–4.00 (m, 2H), 3.98–3.88 (m, 1H), 3.65–3.55 (m, 1H), 3.01 (dd, *J* = 3.0, 14.1 Hz, 1H), 2.81 (dd, *J* = 10.4, 14.0 Hz, 1H), 2.07 (dd, *J* = 5.4, 6.8 Hz, 1H), 1.53 (s, 3H), 1.49 (s, 3H).

4.1.3. (2*S*,3*S*,4*R*)-2-Azido-3,4-*O*-isopropylidene-5-phenyl-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl)-1,3,4-pentanetriol (9l)

To a mixture of **7l** (175 mg, 0.633 mmol), **8a** (446 mg, 0.822 mmol) and molecular sieves 4 Å in $CHCl_3$ (14 ml) under Ar was added dropwise at −50 °C a solution of $BF_3{\cdot}OEt_2$ (80 μl, 0.631 mmol) in $CHCl_3$ (2.7 ml). After 1 h of stirring the reaction was quenched with satd $NaHCO_3$ aq, extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 12.5%) to give **9l** as a colorless oil (108 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40–7.15 (m, 25H), 4.96 (d, *J* = 3.7 Hz, 1H), 4.95 (d, *J* = 11.2 Hz, 1H), 4.84 (d, *J* = 12.2 Hz, 1H), 4.81 (d, *J* = 13.0 Hz, 1H), 4.72 (d, *J* = 11.8 Hz, 1H), 4.71 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 11.5 Hz, 1H), 4.48 (d, *J* = 11.9 Hz, 1H), 4.41 (d, *J* = 12.1 Hz, 1H), 4.42–4.33 (m, 1H), 4.20–3.90 (m, 6H), 3.77 (dd, *J* = 6.5, 10.8 Hz, 1H), 3.65–3.45 (m, 3H), 3.00 (dd, *J* = 2.8, 14.1 Hz, 1H), 2.78 (dd, *J* = 10.5, 14.0 Hz, 1H), 1.44 (s, 3H), 1.23 (s, 3H).

4.1.4. (2*S*,3*S*,4*R*)-3,4-*O*-Isopropylidene-5-phenyl-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl)-2-tetracosanoylamino-1,3,4-pentanetriol (10l)

A mixture of **9l** (98.2 mg, 0.123 mmol) and Lindlar catalyst (98 mg) in EtOH (5 ml) was stirred under H_2 atmosphere for 24 h. Additional Lindlar catalyst (96 mg) was added and the mixture was stirred for another 24 h. Insolubles were removed by filtration through membrane filter and the filtrate was concentrated to give an oil. The oil was diluted with CH_2Cl_2 (2 ml) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (26.8 mg, 0.140 mmol) was added. This mixture was added at 0 °C to the premixed suspension of Lignoceric acid (44.8 mg, 0.122 mmol), 1-hydroxybenzotrilazole (20.3 mg, 0.150 mmol) and Hunig's Base (49 μl, 0.281 mmol), in DMF (2.5 ml) and CH_2Cl_2 (5 ml), and the mixture was stirred at rt for 24 h. The reaction mixture was diluted with [Et_2O/EtOAc = 1:1] solution, quenched with satd $NaHCO_3$ aq, washed with 1 N HCl and brine, dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 25–33%) to give **10l** as a colorless solid (90.0 mg, 65% in two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40–7.08 (m, 25H), 6.32 (d, *J* = 8.4 Hz, 1H), 4.924 (d, *J* = 11.4 Hz, 1H), 4.919 (d, *J* = 3.9 Hz, 1H), 4.82 (d, *J* = 11.4 Hz, 1H), 4.81 (d, *J* = 11.7 Hz, 1H), 4.74 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 11.5 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.47 (d, *J* = 11.8 Hz, 1H), 4.37 (d, *J* = 11.8 Hz, 1H), 4.25–4.05 (m, 4H), 4.06 (dd, *J* = 3.3, 9.7 Hz, 1H), 3.98 (t, *J* = 6.1 Hz, 1H), 3.95–3.90 (m, 2H), 3.65 (d, *J* = 11.2 Hz, 1H), 3.55 (dd, *J* = 7.0, 9.5 Hz, 1H), 3.38 (dd, *J* = 5.6, 9.4 Hz, 1H), 2.75–2.70 (m, 2H), 2.18–1.93 (m, 2H), 1.60–1.50 (m, 2H), 1.47 (s, 3H), 1.28 (s, 3H), 1.35–1.20 (m, 40H), 0.87 (t, *J* = 6.5 Hz, 3H).

4.1.5. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-5-phenyl-2-tetracosanoylamino-1,3,4-pentanetriol (11l)

To a solution of **10l** (90.0 mg, 0.0801 mmol) in CH_2Cl_2 (5 ml) and MeOH (1 ml) was added 4 M HCl in dioxane (100 μl, 0.4 mmol) at 0 °C and the mixture was stirred at rt for 3 h. Silica gel was added to the reaction mixture, then volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc; 25–33%) to give a colorless solid (68 mg, 78%). A mixture of this solid (67 mg, 0.062 mmol) and Pearlman's catalyst (26.8 mg) in $CHCl_3$ (1 ml) and MeOH (3 ml) was stirred under H_2 atmosphere for 1.5 h. Insolubles were removed by filtration through membrane filter and the filtrate was concentrated to give compound **11l** as a colorless solid (43.6 mg, 98%). ^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.53 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 6.8 Hz, 2H), 7.32–7.27 (m, 2H), 7.20–7.17 (m, 1H), 6.83 (d, *J* = 4.6 Hz, 1H), 6.58–6.44 (m, 3H), 6.33 (d, *J* = 6.7 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 5.51 (d, *J* = 3.9 Hz, 1H), 5.27 (qd, *J* = 4.7, 8.9 Hz, 1H), 4.69–4.59 (m, 2H), 4.58–4.31 (m, 8H), 3.70 (dd, *J* = 1.8, 13.5 Hz, 1H), 3.14 (dd, *J* = 9.3, 13.7 Hz, 1H), 2.49–2.38 (m, 2H), 1.81 (quin, *J* = 7.5 Hz, 2H), 1.39–1.18 (m, 40H), 0.87 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.4, 130.5, 128.5, 101.8, 76.6, 74.0, 73.1, 71.6, 71.0, 70.4, 69.3, 62.7, 51.7, 40.7, 36.8, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.6, 26.4, 23.0, 14.3; HRMS (FAB) Calcd for $C_{41}H_{73}NNaO_9^+$: 746.5178; Found: 746.5157.

4.1.6. (2*R*,3*R*,4*R*)-1,3-*O*-Benzylidene-2-*O*-methanesulfonyl-6-oxa-1,2,3,4-nonanetetrol (6p)

NaH (1.82 g, 45.4 mmol) was added to 1-propanol (60 ml) at 0 °C and stirred for 5 min. To the solution was added **5** (2.00 g, 9.01 mmol), and the mixture was stirred at rt for 20 h. To the reac-

tion mixture was added water (200 mL), and the product was extracted with EtOAc (200 mL × 1, 50 ml × 2). The combined organic layer was dried over Na_2SO_4, filtered, concentrated and purified over silica gel column chromatography (hexane/EtOAc; 50–67%) to yield the ether as a colorless solid (2.12 g, 83%). To the solution of above ether (451 mg, 1.60 mmol) in pyridine (15 ml) was added methanesulfonyl chloride (118 μl, 1.51 mmol) at −40 °C, and the mixture was gradually warmed to rt. After 36 h of stirring pyridine was removed azeotropically with heptane. The residue was directly purified by column chromatography (hexane/EtOAc; 40–66%) to yield **6p** as a colorless solid (357.0 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52–7.44 (m, 2H), 7.41–7.35 (m, 3H), 5.58 (s, 1H), 4.88 (dd, *J* = 1.5, 3.0 Hz, 1H), 4.59 (dd, *J* = 1.6, 13.2 Hz, 1H), 4.16 (dd, *J* = 1.3, 13.2 Hz, 1H), 4.04 (dd, *J* = 1.5, 9.2 Hz, 1H), 4.00–3.93 (m, 1H), 3.67 (dd, *J* = 2.9, 9.8 Hz, 1H), 3.63 (dd, *J* = 4.4, 9.8 Hz, 1H), 3.47 (ddd, *J* = 6.7, 9.5, 13.8 Hz, 2H), 3.17 (s, 3H), 2.76 (d, *J* = 6.4 Hz, 1H), 1.61 (sxt, *J* = 7.1 Hz, 2H), 0.93 (t, *J* = 7.5 Hz, 3H); MS (ESI) 361.1 $(M+H)^+$.

4.1.7. (2*S*,3*S*,4*R*)-2-Azido-3,4-*O*-isopropylidene-6-oxa-1,3,4-nonanetriol (7p)

A mixture of **6p** (325 mg, 0.901 mmol) and Pearlman's catalyst (61.3 mg, 0.437 mmol) in EtOH (10 ml) was stirred under H_2 atmosphere at rt for 90 min. Insolubles were removed by filtration through membrane filter and the filtrate was concentrated to give a colorless oil which contained EtOH (281.3 mg, calculated from ^{1}H NMR to contain 242 mg of the triol, 99%). EtOAc was added and removed under reduced pressure repeatedly for three times to remove EtOH. The residue was dissolved in DMF (5 ml), NaN_3 (236 mg, 3.63 mmol) was added and the mixture was stirred under Ar at 95 °C for 3 h. To the reaction mixture was added half-satd $NaHCO_3$ (100 mL), and the product was extracted with EtOAc (100 mL × 1, 50 ml × 8). The combined organic layer was dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography ([hexane/EtOAc = 1:1]/MeOH; 2–5%) to give the azido-triol as a colorless oil (119.0 mg, 60%). The residue was dissolved in 2,2-dimethoxypropane (2 ml), catalytic amount of *p*-toluenesulfonic acid monohydrate (5 mg, 0.026 mmol) was added at 0 °C, and the mixture was stirred for 21 h during which ice in the cooling bath gradually melted. MeOH was added and the reaction was stirred for 2 h. To the mixture was added half-satd $NaHCO_3$ aq (75 mL), and the product was extracted with EtOAc (75 mL × 1, 40 ml × 2). The combined organic layer was dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 20–50%, then to [hexane/EtOAc = 1:1]/MeOH; 5%) to yield **7p** as a colorless oil (36.9 mg, 26%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 5.10 (br s, 1H), 4.23 (q, *J* = 5.8 Hz, 1H), 3.93 (dd, *J* = 5.9, 9.0 Hz, 1H), 3.80 (dd, *J* = 1.5, 11.0 Hz, 1H), 3.62 (dd, *J* = 5.0, 10.5 Hz, 1H), 3.60–3.49 (m, 2H), 3.46 (dd, *J* = 5.8, 10.5 Hz, 1H), 3.39 (t, *J* = 6.7 Hz, 2H), 1.53 (sxt, *J* = 7.1 Hz, 2H), 1.34 (s, 3H), 1.25 (s, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 107.8, 75.6, 74.5, 72.3, 68.7, 62.2, 61.6, 27.4, 25.2, 22.2, 10.4; MS (ESI) 232.2 $(M-N_2+H)^+$.

4.1.8. (2*S*,3*S*,4*R*)-2-Azido-3,4-*O*-isopropylidene-6-oxa-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl)-1,3,4-nonanetriol (9p)

To a solution of **7p** (36.9 mg, 0.142 mmol) in toluene (3 ml) under Ar were added molecular sieves 4 Å (151.3 mg), a solution of tetra-*O*-benzyl-galactosyl chloride **8b** (162 mg, 0.29 mmol) in toluene (7 ml), tetra-*n*-butylammonium bromide (140.9 mg, 0.437 mmol) and Hunig's Base (50 μl, 0.286 mmol) at rt. The mixture was stirred at rt for 45 min, at 60 °C for 45 h, and at 80 °C for 15 h. MeOH was added at 50 °C and stirred for 6 h. The reaction mixture was passed through Celite pad to remove insolubles, and to the filtrate was added half-satd $NaHCO_3$ aq (100 ml). The product was extracted with EtOAc (100 ml × 1, 50 ml × 1), and the combined organic layer was dried over Na_2SO_4, filtered, concentrated and subjected to silica gel column chromatography (hexane/EtOAc; 11% to 14%) to give **9p** as a colorless oil (98.0 mg) as a mixture with tetra-*O*-benzyl-1-methoxygalactose. Tetra-*O*-benzyl-1-methoxygalactose was removed in the next step. MS (FAB) 804 $(M+Na)^+$.

4.1.9. (2*S*,3*S*,4*R*)-3,4-*O*-Isopropylidene-6-oxa-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl)-2-tetracosanoylamino-1,3,4-nonanetriol (10p)

The crude **9p** obtained in 4.1.8 was divided into two portions. One portion was dissolved in EtOH (3 ml) and stirred with Lindlar catalyst (20.8 mg) under H_2 atmosphere for 22 h. Insolubles were removed by filtration through membrane filter and the filtrate was concentrated to give an oil. The oil was diluted with CH_2Cl_2 (1 ml) and DMF (1 ml), and to the solution was added premixed suspension of Lignoceric acid (10.5 mg, 0.028 mmol), 3*H*-[1,2,3]-triazolo[4,5-*b*]pyridin-3-ol (4.5 mg, 0.033 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (7.4 mg, 0.039 mmol) in DMF (1 ml) and CH_2Cl_2 (1 ml), then Hunig's Base (12 μl, 0.069 mmol), and the mixture was stirred at 35 °C for 17 h. To the reaction mixture was added half-satd $NaHCO_3$ aq (100 mL), and the product was extracted with [hexane/EtOAc = 1:1] solution (100 mL × 1, 50 ml × 2). The combined organic layer was dried over Na_2SO_4, filtered, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 25%) to give **10p** as colorless oil (17.8 mg). The same procedure was applied to the other portion of the crude **9p**, and the products from both portions were combined to yield 35.1 mg (22% from **7p**) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.42–7.19 (m, 20H), 6.36 (d, *J* = 9.4 Hz, 1H), 4.93 (d, *J* = 11.5 Hz, 1H), 4.89 (d, *J* = 3.8 Hz, 1H), 4.81 (d, *J* = 11.4 Hz, 1H), 4.80 (d, *J* = 11.4 Hz, 1H), 4.74 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 11.5 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.48 (d, *J* = 11.8 Hz, 1H), 4.38 (d, *J* = 11.9 Hz, 1H), 4.23–4.02 (m, 5H), 3.98–3.88 (m, 3H), 3.61 (dd, *J* = 2.6, 11.4 Hz, 1H), 3.54 (dd, *J* = 6.8, 9.3 Hz, 1H), 3.45–3.34 (m, 4H), 3.30 (td, *J* = 7.0, 9.4 Hz, 1H), 2.10–1.94 (m, 2H), 1.61–1.52 (m, 4H), 1.44 (s, 3H), 1.33 (s, 3H), 1.32–1.19 (m, 40H), 0.88 (t, *J* = 7.2 Hz, 3H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.3, 128.5, 128.4, 128.4, 128.3, 128.3, 128.0, 127.9, 127.7, 127.6, 127.5, 108.7, 99.6, 78.9, 74.7, 74.6, 73.5, 73.4, 73.0, 36.8, 31.9, 29.7, 29.7, 29.6, 29.4, 29.4, 25.8, 25.6, 22.7, 14.1, 10.4; MS (FAB) 1128 $(M+Na-1)^+$.

4.1.10. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-6-oxa-2-tetracosanoylamino-1,3,4-nonanetriol (11p)

To a solution of **10p** (16.4 mg, 0.015 mmol) in CH_2Cl_2 (4 ml) and MeOH (0.8 ml) was added 4 M HCl in dioxane (80 μl, 0.320 mmol) and the mixture was stirred at rt for 2 h. Et_3N (90 μl, 0.646 mmol) was added, then volatiles were removed under reduced pressure to give solid, which was purified by silica gel column chromatography (hexane/EtOAc; 33% to 44%) to give colorless solid (13.8 mg, 87%). A mixture of above solid (12.5 mg, 0.012 mmol) and Pearlman's catalyst (7.5 mg) in CH_2Cl_2 (1 ml) and MeOH (3 ml) was stirred under H_2 atmosphere for 3.5 h. Insolubles were removed by filtration through membrane filter and the filtrate was concentrated to give compound **11p** as a colorless solid (8.8 mg, quant.) ^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.45 (d, *J* = 8.7 Hz, 1H), 6.86 (d, *J* = 6.7 Hz, 1H), 6.53 (d, *J* = 6.1 Hz, 1H), 6.50–6.41 (m, 2H), 6.34 (d, *J* = 6.4 Hz, 1H), 6.27 (d, *J* = 4.1 Hz, 1H), 5.54 (d, *J* = 3.8 Hz, 1H), 5.30–5.21 (m, 1H), 4.69–4.59 (m, 2H), 4.57–4.53 (m, 1H), 4.53–4.34 (m, 7H), 4.12 (dd, *J* = 2.7, 9.9 Hz, 1H), 3.99 (dd, *J* = 6.0, 9.9 Hz, 1H), 3.45 (tq, *J* = 6.7, 9.1 Hz, 2H), 2.42 (dt, *J* = 1.8, 7.5 Hz, 2H), 1.79 (quin, *J* = 7.5 Hz, 2H), 1.54 (sxt, *J* = 7.1 Hz, 2H), 1.39–1.15 (m, 40H), 0.87 (t, *J* = 6.8 Hz, 3H), 0.82 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.4, 101.6, 74.2, 74.1, 73.3, 73.1, 72.1, 71.7, 71.0, 70.4, 68.7, 62.7, 51.6, 36.8, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6,

26.4, 23.3, 23.0, 14.3, 10.8; HRMS (FAB) Calcd for $C_{38}H_{75}NNaO_{10}^+$: 728.5283; Found: 728.5311.

4.1.11. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-tetracosanoylamino-1,3,4-heptanetriol (11a)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.40 (d, J = 8.5 Hz, 1H), 6.91 (d, J = 6.1 Hz, 1H), 6.59 (d, J = 6.1 Hz, 1H), 6.48 (t, J = 5.6 Hz, 1H), 6.38 (d, J = 6.1 Hz, 1H), 6.26 (d, J = 3.9 Hz, 1H), 6.03 (d, J = 5.9 Hz, 1H), 5.57 (d, J = 3.8 Hz, 1H), 5.30–5.21 (m, 1H), 4.70–4.61 (m, 2H), 4.58–4.53 (m, 1H), 4.53–4.47 (m, 1H), 4.47–4.34 (m, 4H), 4.33–4.23 (m, 2H), 2.43 (t, J = 7.5 Hz, 2H), 2.27–2.14 (m, 1H), 1.94–1.75 (m, 4H), 1.74–1.57 (m, 1H), 1.41–1.14 (m, 40H), 0.96 (t, J = 7.3 Hz, 3H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.2, 101.6, 76.9, 73.1, 72.2, 71.7, 71.0, 70.3, 68.7, 62.7, 51.4, 36.8, 36.6, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 23.0, 19.6, 14.6, 14.3; HRMS (FAB) Calcd for $C_{37}H_{73}NNaO_9^+$: 698.5178; Found: 698.5151.

4.1.12. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-tricosanoylamino-1,3,4-octanetriol (11b)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.42 (d, J = 8.7 Hz, 1H), 5.57 (d, J = 3.8 Hz, 1H), 5.31–5.21 (m, 1H), 4.70–4.62 (m, 2H), 4.58–4.54 (m, 1H), 4.54–4.48 (m, 1H), 4.46–4.35 (m, 4H), 4.32–4.24 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.32–2.17 (m, 1H), 1.90–1.74 (m, 4H), 1.70–1.53 (m, 1H), 1.46–1.16 (m, 40H), 0.91–0.80 (m, 6H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.3, 101.6, 76.8, 73.1, 72.5, 71.6, 71.1, 70.3, 68.7, 62.7, 51.4, 36.8, 34.1, 32.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 28.6, 26.4, 23.3, 23.0, 14.4, 14.3; HRMS (FAB) Calcd for $C_{37}H_{73}NNaO_9^+$: 698.5178; Found: 698.5161.

4.1.13. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-tetracosanoylamino-1,3,4-octanetriol (11c)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.41 (d, J = 8.7 Hz, 1H), 6.96–6.86 (m, 1H), 6.65–6.53 (m, 1H), 6.53–6.43 (m, 1H), 6.37 (d, J = 6.1 Hz, 1H), 6.31–6.20 (m, 1H), 6.03 (d, J = 5.1 Hz, 1H), 5.57 (d, J = 3.9 Hz, 1H), 5.31–5.21 (m, 1H), 4.71–4.62 (m, 2H), 4.55 (br s, 1H), 4.53–4.48 (m, 1H), 4.47–4.35 (m, 4H), 4.32–4.22 (m, 2H), 2.43 (t, J = 7.2 Hz, 2H), 2.32–2.18 (m, 1H), 1.91–1.72 (m, 4H), 1.67–1.53 (m, 1H), 1.47–1.15 (m, 42H), 0.87 (t, J = 6.8 Hz, 3 H), 0.85 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.2, 101.6, 76.9, 73.1, 72.5, 71.7, 71.1, 70.3, 68.7, 62.7, 51.4, 36.8, 34.1, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 28.6, 26.4, 23.3, 23.0, 14.4, 14.3; HRMS (FAB) Calcd for $C_{38}H_{75}NNaO_9^+$: 712.5334; Found: 712.5316.

4.1.14. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-icosanoylamino-1,3,4-nonanetriol (11d)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.42 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 6.4 Hz, 1H), 6.57 (d, J = 4.8 Hz, 1H), 6.49 (t, J = 5.5 Hz, 1H), 6.39 (d, J = 6.1 Hz, 1H), 6.26 (d, J = 3.6 Hz, 1H), 6.03 (d, J = 5.8 Hz, 1H), 5.57 (d, J = 3.9 Hz, 1H), 5.30–5.21 (m, 1H), 4.71–4.61 (m, 2H), 4.55 (br s, 1H), 4.53–4.48 (m, 1H), 4.47–4.35 (m, 4H), 4.34–4.23 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.30–2.17 (m, 1H), 1.93–1.74 (m, 4H), 1.70–1.56 (m, 1H), 1.41–1.15 (m, 36H), 0.87 (t, J = 6.8 Hz, 3H), 0.81 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.3, 101.6, 76.8, 73.1, 72.5, 71.7, 71.0, 70.4, 68.7, 62.7, 51.5, 36.8, 34.4, 32.5, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 26.1, 23.0, 23.0, 14.3, 14.3; HRMS (FAB) Calcd for $C_{35}H_{69}NNaO_{9+}$: 670.4865; Found: 670.4880.

4.1.15. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-docosanoylamino-1,3,4-nonanetriol (11e)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.42 (d, J = 8.7 Hz, 1H), 6.90 (br s, 1H), 6.57 (d, J = 4.4 Hz, 1H), 6.49 (t, J = 5.3 Hz, 1H), 6.39 (d, J = 5.9 Hz, 1H), 6.26 (d, J = 3.9 Hz, 1H), 6.03 (d, J = 5.5 Hz, 1H), 5.57 (d, J = 3.8 Hz, 1H), 5.31–5.20 (m, 1H), 4.71–4.61 (m, 2H), 4.55 (br s, 1H), 4.53–4.48 (m, 1H), 4.47–4.36 (m, 4H), 4.33–4.24 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.30–2.18 (m, 1H), 1.93–1.75 (m, 4H), 1.70–1.54 (m, 1H), 1.45–1.11 (m, 40H), 0.87 (t, J = 6.8 Hz, 3H), 0.81 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.3, 101.6, 76.8, 73.1, 72.5, 71.7, 71.1, 70.4, 68.7, 62.7, 51.5, 36.8, 34.4, 32.5, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 26.1, 23.0, 23.0, 14.3, 14.3; HRMS (FAB) Calcd for $C_{37}H_{73}NNaO_9^+$: 698.5178; Found: 698.5145.

4.1.16. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-tricosanoylamino-1,3,4-nonanetriol (11f)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.42 (d, J = 8.5 Hz, 1H), 5.58 (d, J = 3.9 Hz, 1H), 5.30–5.22 (m, 1H), 4.71–4.62 (m, 2H), 4.58–4.54 (m, 1H), 4.54–4.49 (m, 1H), 4.47–4.36 (m, 4H), 4.33–4.25 (m, 2H), 2.44 (t, J = 7.3 Hz, 2H), 2.31–2.18 (m, 1H), 1.94–1.76 (m, 4H), 1.69–1.56 (m, 1H), 1.41–1.18 (m, 42H), 0.87 (t, J = 6.9 Hz, 3H), 0.81 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 101.6, 76.8, 73.1, 72.5, 71.7, 71.0, 70.3, 62.7, 51.5, 36.8, 34.4, 32.5, 32.1, 30.0, 29.9, 29.8, 29.6, 26.4, 26.1, 23.0, 23.0, 14.3, 14.3; HRMS (FAB) Calcd for $C_{38}H_{75}NNaO_9^+$: 712.5334; Found: 712.5302.

4.1.17. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-pentacosanoylamino-1,3,4-nonanetriol (11g)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.43 (d, J = 8.7 Hz, 1H), 6.92 (d, J = 4.0 Hz, 1H), 6.58 (d, J = 3.6 Hz, 1H), 6.50 (t, J = 5.3 Hz, 1H), 6.40 (d, J = 6.0 Hz, 1H), 6.27 (d, J = 3.4 Hz, 1H), 6.03 (d, J = 5.6 Hz, 1H), 5.58 (d, J = 3.8 Hz, 1H), 5.32–5.20 (m, 1H), 4.71–4.61 (m, 2H), 4.55 (br s, 1H), 4.54–4.48 (m, 1H), 4.47–4.35 (m, 4H), 4.29 (br s, 2H), 2.44 (t, J = 7.3 Hz, 2H), 2.30–2.18 (m, 1H), 1.94–1.75 (m, 4H), 1.70–1.56 (m, 1H), 1.38–1.20 (m, 46H), 0.87 (t, J = 6.8 Hz, 3H), 0.81 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.3, 101.6, 76.8, 73.1, 72.5, 71.7, 71.0, 70.4, 68.7, 62.7, 51.5, 36.8, 34.4, 32.5, 32.1, 30.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 26.1, 23.0, 23.0, 14.3, 14.3; HRMS (FAB) Calcd for $C_{40}H_{79}NNaO_9^+$: 740.5647; Found: 740.5618.

4.1.18. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-hexacosanoylamino-1,3,4-nonanetriol (11h)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.43 (d, J = 8.7 Hz, 1H), 6.92 (br s, 1H), 6.58 (br s, 1H), 6.49 (br s, 1H), 6.39 (d, J = 5.9 Hz, 1H), 6.27 (br s, 1H), 6.03 (d, J = 4.5 Hz, 1H), 5.58 (d, J = 3.9 Hz, 1H), 5.30–5.22 (m, 1H), 4.71–4.62 (m, 2H), 4.55 (br s, 1H), 4.54–4.48 (m, 1H), 4.48–4.34 (m, 4H), 4.33–4.24 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.31–2.18 (m, 1H), 1.94–1.76 (m, 4H), 1.70–1.55 (m, 1H), 1.42–1.16 (m, 48H), 0.87 (t, J = 6.9 Hz, 3H), 0.81 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.3, 101.6, 76.8, 73.1, 72.5, 71.7, 71.0, 70.4, 68.7, 62.7, 51.5, 36.8, 34.4, 32.5, 32.1, 30.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 26.1, 23.0, 23.0, 14.3; HRMS (FAB) Calcd for $C_{41}H_{81}NNaO_9^+$: 754.5804; Found: 754.5757.

4.1.19. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-octacosanoylamino-1,3,4-nonanetriol (11i)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.42 (d, J = 8.7 Hz, 1H), 6.90 (d, J = 4.1 Hz, 1H), 6.57 (d, J = 4.9 Hz, 1H), 6.49 (t, J = 5.3 Hz, 1H), 6.39 (d, J = 6.1 Hz, 1H), 6.26 (d, J = 3.5 Hz, 1H), 6.02 (d, J = 5.6 Hz, 1H), 5.57 (d, J = 3.9 Hz, 1H), 5.30–5.20 (m, 1H), 4.71–4.61 (m, 2H), 4.57–4.48 (m, 2H), 4.47–4.36 (m, 4H), 4.33–4.25 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.30–2.19 (m, 1H), 1.93–1.77 (m, 4H), 1.69–1.56 (m, 1H), 1.38–1.22 (m, 52H), 0.87 (t, J = 6.8 Hz, 3H), 0.81 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.3, 101.6, 76.8, 73.1, 72.5, 71.7, 71.0, 70.4, 68.7, 62.7, 51.5, 36.8, 34.4, 32.5, 32.1, 30.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 26.1, 23.0, 23.0, 14.3, 14.3; HRMS (FAB) Calcd for $C_{43}H_{85}NNaO_9^+$: 782.6117; Found: 782.6116.

4.1.20. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-2-tricosanoylamino-1,3,4-decanetriol (11j)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.43 (d, J = 8.7 Hz, 1H), 6.92 (d, J = 6.3 Hz, 1H), 6.58 (d, J = 6.0 Hz, 1H), 6.49 (t, J = 5.6 Hz, 1H), 6.40 (d, J = 6.1 Hz, 1H), 6.27 (d, J = 4.0 Hz, 1H), 6.04 (d, J = 5.9 Hz, 1H), 5.58 (d, J = 3.9 Hz, 1H), 5.31–5.21 (m, 1H), 4.71–4.61 (m, 2H), 4.58–4.54 (m, 1H), 4.54–4.49 (m, 1H), 4.47–4.35 (m, 4H), 4.34–4.25 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.31–2.19 (m, 1H), 1.94–1.75 (m, 4H), 1.70–1.57 (m, 1H), 1.44–1.16 (m, 44H), 0.87 (t, J = 6.8 Hz, 3H), 0.80 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.3, 101.6, 76.8, 73.1, 72.5, 71.7, 71.1, 70.4, 68.7, 62.7, 51.5, 36.8, 34.4, 32.2, 32.1, 30.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 23.0, 22.9, 14.3, 14.3; HRMS (FAB) Calcd for $C_{39}H_{77}NNaO_9^+$: 726.5491; Found: 726.5509.

4.1.21. (2*S*,3*S*,4*R*)-5-Cyclopentyl-1-*O*-(α-D-galactosyl)-2-tetracosanoylamino-1,3,4-pentanetriol (11k)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.41 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 4.9 Hz, 1H), 6.60 (d, J = 4.3 Hz, 1H), 6.49 (t, J = 5.5 Hz, 1H), 6.37 (d, J = 6.4 Hz, 1H), 6.26 (d, J = 3.9 Hz, 1H), 5.97 (d, J = 6.5 Hz, 1H), 5.57 (d, J = 3.9 Hz, 1H), 5.29–5.19 (m, 1H), 4.71–4.61 (m, 2H), 4.56 (br s, 1H), 4.54–4.49 (m, 1H), 4.47–4.25 (m, 6H), 2.51–2.35 (m, 3H), 2.20–2.11 (m, 1H), 2.01–1.88 (m, 2H), 1.88–1.76 (m, 3H), 1.61–1.49 (m, 2H), 1.49–1.16 (m, 44H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.2, 101.6, 77.2, 73.1, 71.7, 71.1, 70.4, 68.7, 62.7, 51.4, 37.3, 36.8, 34.1, 32.4, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 25.5, 25.4, 23.0, 14.3; HRMS (FAB) Calcd for $C_{40}H_{77}NNaO_9^+$: 738.5491; Found: 738.5444.

4.1.22. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-6-phenyl-2-tetracosa noylamino-1,3,4-hexanetriol (11m)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.35 (d, J = 8.7 Hz, 1H), 7.38–7.27 (m, 4H), 7.20–7.17 (m, 1H), 7.05 (br s, 1H), 6.59 (br s, 1H), 6.52–6.41 (m, 2H), 6.29 (br s, 1H), 6.22 (d, J = 5.9 Hz, 1H), 5.57 (d, J = 3.8 Hz, 1H), 5.31–5.22 (m, 1H), 4.69–4.59 (m, 2H), 4.56 (br s, 1H), 4.48–4.25 (m, 7H), 3.21 (ddd, J = 4.5, 9.8, 13.9 Hz, 1H), 3.00 (ddd, J = 6.8, 9.7, 13.5 Hz, 1H), 2.66–2.55 (m, 1H), 2.47–2.33 (m, 2H), 2.23–2.10 (m, 1H), 1.80 (quin, J = 7.6 Hz, 2H), 1.40–1.14 (m, 40H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.2, 143.6, 129.1, 128.7, 125.9, 101.5, 76.8, 73.0, 71.7, 71.6, 71.0, 70.3, 68.4, 62.7, 51.3, 36.8, 36.5, 32.7, 32.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 23.0, 14.3; HRMS (FAB) Calcd for $C_{42}H_{75}NNaO_9^+$: 760.5334; Found: 760.5322.

4.1.23. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-5-(*p*-tolyl)-2-tetracosano ylamino-1,3,4-pentanetriol (11n)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.51 (d, J = 8.5 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 7.7 Hz, 2H), 6.92–6.12 (m, 6H), 5.52 (d, J = 4.0 Hz, 1H), 5.28 (qd, J = 4.7, 8.9 Hz, 1H), 4.71–4.58 (m, 2H), 4.58–4.47 (m, 3H), 4.47–4.30 (m, 5H), 3.67 (dd, J = 1.8, 13.5 Hz, 1H), 3.12 (dd, J = 9.2, 13.8 Hz, 1H), 2.43 (dt, J = 3.3, 7.5 Hz, 2H), 2.21 (s, 3H), 1.81 (quin, J = 7.6 Hz, 2H), 1.43–1.10 (m, 40H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.4, 138.2, 130.3, 129.1, 101.8, 76.5, 74.1, 73.1, 71.6, 71.0, 70.4, 69.2, 62.7, 51.7, 36.8, 32.1, 30.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 23.0, 21.0, 14.3; HRMS (FAB) Calcd for $C_{42}H_{75}NNaO_9^+$: 760.5334; Found: 760.5358.

4.1.24. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-6-oxa-2-tetracosanoy lamino-1,3,4-heptanetriol (11o)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.47 (d, J = 8.7 Hz, 1H), 5.54 (d, J = 3.9 Hz, 1H), 5.30–5.22 (m, 1H), 4.69–4.61 (m, 2H), 4.57–4.47 (m, 3H), 4.46–4.33 (m, 5H), 4.07 (dd, J = 2.7, 9.9 Hz, 1H), 3.94 (dd, J = 6.1, 9.8 Hz, 1H), 3.35 (s, 3H), 2.42 (dt, J = 1.5, 7.5 Hz, 2H), 1.79 (quin, J = 7.5 Hz, 2H), 1.38–1.14 (m, 40H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.4, 101.6, 76.0, 74.0, 73.1, 72.0, 71.6, 71.0, 70.3, 68.5, 62.7, 59.0, 51.5, 36.8, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 23.0, 14.3; HRMS (FAB) Calcd for $C_{36}H_{71}NNaO_{10}^+$: 700.4970; Found: 700.4920.

4.1.25. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-6-oxa-2-tetracosanoy lamino-1,3,4-octadecanetriol (11q)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.47 (d, J = 8.7 Hz, 1H), 6.86 (br s, 1H), 6.62–6.42 (m, 3H), 6.36 (d, J = 6.3 Hz, 1H), 6.28 (br s, 1H), 5.54 (d, J = 3.8 Hz, 1H), 5.31–5.22 (m, 1H), 4.71–4.60 (m, 2H), 4.58–4.33 (m, 8H), 4.17 (dd, J = 2.6, 9.9 Hz, 1H), 4.04 (dd, J = 6.1, 9.9 Hz, 1H), 3.62–3.49 (m, 2H), 2.43 (dt, J = 1.6, 7.5 Hz, 2H), 1.80 (quin, J = 7.6 Hz, 2H), 1.64–1.55 (m, 2H), 1.40–1.16 (m, 58H), 0.90–0.85 (m, 6H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.4, 101.6, 74.3, 74.1, 73.1, 72.1, 71.9, 71.7, 71.0, 70.4, 68.7, 62.7, 51.6, 36.8, 32.2, 30.3, 30.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.6, 26.4, 23.0, 14.3; HRMS (FAB) Calcd for $C_{47}H_{93}NNaO_{10}^+$: 854.6692; Found: 854.6697.

4.1.26. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactosyl)-5-phenoxy-2-tetracosa noylamino-1,3,4-pentanetriol (11r)

^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.56 (d, J = 8.7 Hz, 1H), 7.29–7.23 (m, 2H), 7.09–7.03 (m, 2H), 6.96–6.91 (m, 1H), 6.70 (br s, 1H), 5.56 (d, J = 3.8 Hz, 1H), 5.37–5.30 (m, 1H), 4.77–4.58 (m, 5H), 4.58–4.49 (m, 3H), 4.47–4.35 (m, 4H), 2.44 (dt, J = 2.1, 7.5 Hz, 2H), 1.80 (quin, J = 7.6 Hz, 2H), 1.38–1.17 (m, 40H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.5, 160.1, 129.8, 120.8, 115.2, 101.6, 73.7, 73.1, 71.7, 71.6, 71.4, 71.0, 70.3, 68.6, 62.7, 51.5, 36.8, 32.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 26.4, 23.0, 14.3; HRMS (FAB) Calcd for $C_{41}H_{73}NNaO_{10}^+$: 762.5127; Found: 762.5139.

4.1.27. (3*R*,4*S*,5*R*)-4,5-*O*-Isopropylidene-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl)-1-decyne-3,4,5-triol (14)

To a solution of **12** (92.7 mg, 0.17 mmol) in THF (2 ml) was added dropwise a solution of 1.57 M *n*-BuLi in hexane (120 μl, 0.19 mmol) at −45 °C, and the reaction temperature was raised to 0 °C. After 30 min of stirring the mixture was cooled to −48 °C and a solution of **13** (117 mg, 0.584 mmol) in THF (1.5 ml) was added. After 90 min of stirring the mixture was allowed to gradually warm to −30 °C. The mixture was quenched with 0.1 M phosphonate buffer (2 ml, pH 7.4) at −30 °C and allowed to warm to rt. Satd NaCl aq (5 ml) and water (40 ml) was added, and the product was extracted with EtOAc (40 ml × 1, 30 ml × 2). Combined organic layer was dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 9–25%) to yield **14** as a pale yellow oil (43.7 mg, 35% (47% based on recovered starting material)), along with its epimer (27.9 mg, 22% (30% br sm)) and recovered **12** (24.5 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38–7.21 (m, 20H), 4.91 (d, J = 11.4 Hz, 1H), 4.86 (dd, J = 1.6, 5.7 Hz, 1H), 4.81 (d, J = 11.8 Hz, 1H), 4.74 (d, J = 11.8 Hz, 1H), 4.73 (d, J = 11.8 Hz, 1H), 4.67 (d, J = 11.8 Hz, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.48 (d, J = 12.2 Hz, 1H), 4.40 (d, J = 11.8 Hz, 1H), 4.38–4.33 (m, 1H), 4.15–4.04 (m, 4H), 3.96 (d, J = 1.6 Hz, 1H), 3.82 (dd, J = 2.8, 10.1 Hz, 1H), 3.55–3.49 (m, 2H), 2.65 (d, J = 3.7 Hz, 1H), 1.47 (s, 3H), 1.69–1.41 (m, 3H), 1.37 (s, 3H), 1.30–1.19 (m, 5H), 0.82 (t, J = 6.9 Hz, 3H); MS (FAB) 749 $(M+H)^+$.

4.1.28. (3*R*,4*R*,5*R*)-4,5-*O*-Isopropylidene-3-*O*-methanesulfonyl-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl)-3,4,5-decanetriol (15)

To a warmed solution of **14** (15.6 mg, 0.021 mmol) and *p*-toluenesulfonylhydrazine (38.8 mg, 0.208 mmol) in dimethoxyethane was added 1 N NaOAc aq solution in 10 portions over 5 h. The mixture was stirred at 85 °C for 4.5 h after the final addition. After cooling to rt, the reaction was diluted with water (10 ml) and extracted with CH_2Cl_2 (30 ml × 1, 20 ml × 1, 10 ml × 1). Combined organic layers was dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 25%) to yield saturated alcohol as a colorless oil (14.2 mg, 91%). The alcohol was

dissolved in CH_2Cl_2 (1 ml) and pyridine (0.5 ml), and to the solution was added methanesulfonyl chloride (four drops) at 0 °C. After overnight stirring at rt the reaction was diluted with EtOAc (30 ml) and washed with satd NH_4Cl aq (20 ml) and water (10 ml). The organic layer was dried over Na_2SO_4, concentrated and purified over silica gel column chromatography (hexane/EtOAc; 25%) to yield compound **15** as a colorless oil (14.7 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35–7.22 (m, 20H), 4.78–4.70 (m, 2H), 4.68 (s, 2H), 4.60 (s, 2H), 4.55 (d, J = 11.8 Hz, 1H), 4.49 (d, J = 11.4 Hz, 1H), 4.43 (d, J = 11.8 Hz, 1H), 4.11–4.04 (m, 2H), 3.99–3.92 (m, 2H), 3.88 (br s, 2H), 3.77–3.70 (m, 2H), 3.56 (dd, J = 4.7, 10.3 Hz, 1H), 3.08 (s, 3H), 1.97–1.46 (m, 5H), 1.44 (s, 3H), 1.33 (s, 3H), 1.32–1.22 (m, 7H), 0.88 (t, J = 6.9 Hz, 3H); MS (FAB) 831 $(M+H)^+$.

4.1.29. (3*S*,4*S*,5*R*)-4,5-*O*-Isopropylidene-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl)-3-tetracosanoylamino-4,5-decanediol (16)

To a solution of **15** (14.7 mg, 0.0177 mmol) in DMF (1 ml) was added NaN_3 (18.0 mg, 0.277 mmol) at 0 °C, and the mixture was stirred at 90 °C for 17 h. After cooling to rt the mixture was diluted with EtOAc (50 ml), washed with water (30 ml × 3), dried over Na_2SO_4, concentrated and passed through silica gel column to give the crude azide. The crude azide was stirred overnight with Lindlar catalyst (14.6 mg) in EtOH (2 ml) under H_2 atmosphere. Insolubles were removed by passing through membrane filter, and the filtrate was concentrated to give amine as a pale yellow oil. A mixture of this amine, Lignoceric acid (11.7 mg, 0.0317 mmol), 1-hydroxy-7-azabenzotriazole (5.8 mg, 0.0426 mmol), Et_3N (3 drops), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (9.0 mg, 0.0469 mmol) in DMF (1 ml) and CH_2Cl_2 (1 ml) was stirred overnight at rt. The reaction was diluted with EtOAc (40 ml) and washed with satd $NaHCO_3$ aq (30 ml) and water (30 ml). The organic layer was dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (hexane/EtOAc; 17% to 20%) to yield **16** as a colorless solid (9.3 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35–7.22 (m, 20H), 5.68 (d, J = 8.9 Hz, 1H), 4.74 (d, J = 11.4 Hz, 1H), 4.68 (d, J = 12.2 Hz, 1H), 4.64 (d, J = 11.8 Hz, 1H), 4.63 (d, J = 11.8 Hz, 1H), 4.57–4.48 (m, 3H), 4.45 (d, J = 11.8 Hz, 1H), 4.06–3.99 (m, 2H), 3.99–3.92 (m, 4H), 3.87–3.74 (m, 2H), 3.68 (dd, J = 2.4, 7.7 Hz, 1H), 3.54 (dd, J = 4.3, 10.3 Hz, 1H), 2.10–1.99 (m, 2H), 1.40 (s, 3H), 1.30 (s, 3H), 1.83–1.13 (m, 54H), 0.88 (t, J = 6.5 Hz, 3H), 0.86 (t, J = 6.5 Hz, 3H); MS (FAB) 1103 $(M+H)^+$.

4.1.30. (3*S*,4*S*,5*R*)-1-(α-D-Galactopyranosyl)-3-tetracosanoylamino-4,5-decanediol (4)

A mixture of **16** in 80% AcOH was stirred at 60 °C for 3 h, after which all of the volatiles were removed and the residue was purified by silica gel column chromatography (hexane/EtOAc; 33% to 50%) to yield diol as a colorless solid (7.9 mg, 88%). A mixture of this diol and Pearlman's catalyst (10.6 mg) in MeOH (2.5 ml) and CH_2Cl_2 (1 ml) was stirred under H_2 atmosphere for 4.5 h. Insolubles were removed by passing through membrane filter, and washed thoroughly with mixed solution of MeOH and CH_2Cl_2. The filtrate was concentrated to give compound **4** as a colorless solid (5.4 mg, quant.). ^{1}H NMR (400 MHz, Pyr-d_5) δ = 8.41 (d, J = 9.0 Hz, 1H), 6.81–5.71 (m, 6H), 5.17–5.07 (m, 1H), 4.72 (dd, J = 5.5, 8.9 Hz, 1H), 4.57–4.45 (m, 3H), 4.36 (dd, J = 4.6, 11.2 Hz, 1H), 4.29–4.11 (m, 4H), 2.78–2.65 (m, 1H), 2.65–2.53 (m, 1H), 2.53–2.38 (m, 2H), 2.38–2.13 (m, 3H), 1.97–1.76 (m, 4H), 1.74–1.57 (m, 1H), 1.49–1.09 (m, 44H), 0.87 (t, J = 6.8 Hz, 3H), 0.81 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, Pyr-d_5) δ = 173.4, 78.5, 77.0, 73.8, 72.7, 72.2, 70.6, 70.4, 62.8, 52.7, 37.0, 34.5, 32.5, 32.1, 30.1, 30.0, 29.9, 29.9, 29.8, 29.6, 26.6, 26.2, 23.1, 23.0, 14.3; HRMS (FAB) Calcd for $C_{40}H_{79}NNaO_8^+$: 724.5698; Found: 724.5693.

4.2. Biological evaluation

4.2.1. In vitro cytokine production

Splenocytes were prepared from the spleens of C57BL/6 mice (6–8 weeks old, female) and suspended in a RPMI1640 medium (purchased from Nacalai) containing 10% fetal bovine serum (purchased from GIBCO), 5×10^{-5} M 2-mercaptoethanol (purchased from GIBCO), 1 mM pyruvate (purchased from SIGMA), and 25 mM HEPES (purchased from SIGMA). The cells (5×10^5 cells/well) were stimulated with glycolipid derivatives at a concentration of 100 ng/ml for 72 h at 37 °C in a 96-well flat bottom plate (purchased from IWAKI), and the concentration of IL-4 and IFN-γ in the culture supernatant were measured by ELISA (BD Pharmingen EIA Kit). Compound **2** was always included in the assay as a control and the cytokine release were expressed as relative to that of **2** for the mean of at least three experiments.

4.2.2. In vivo cytokine production

Each glycolipid was dissolved in 0.5% DMSO in saline. To clarify the antagonist activity of **4**, the compound was injected intravenously into C57BL/6 mice (9 weeks old, female) though tail vein at 0.1 μg/mouse, 15 min before OCH injection (0.1 μg/mouse). 3, 6, 9 h after second injection, sera were collected, and the content of serum IL-4 and IFN-γ were measured by ELISA (BioLegend ELISA Set). The data were presented as mean ± SD (N = 4–5). Statistical analysis was performed by Student's t-test by using JMP9.0.2 (SAS Institute Inc., Cary, NC).

4.2.3. In silico optimization of 2/hCD1d complex

Both acyl and phytosphingosine chain of **1** bound to hCD1d in the crystal structure was truncated in silico to correspond to **2**, and the complex thus obtained was further optimized. Optimization of the complex was performed stepwise as follows, utilizing Macromodel Ver. 9.0[26] [force field OPLS2005/solv. Water] (convergence threshold .05 kJ/mol/Å): (i) main chain, Asp80, Asp151, Thr154 and ligand fixed, (ii) main chain, Asp80(Oδ1, Oδ2), Asp151(Oδ1, Oδ2), Thr154(Oγ) and oxygen atoms of the ligand fixed, (iii) main chain, distances among selected ligand atoms and Asp80, Asp151, Thr154 fixed, (iv) main chain fixed, (v) optimization of all the atoms.

Acknowledgements

Authors thank Dr. Y. Tanaka, Dr. K. Yamaoka-Kadoshima, Ms. F. Nakanishi-Izumi, and Ms. M. Nakajima-Komori for performing cytokine assays. Dr. T. Nishihara is acknowledged for support and encouragement throughout this study.

References and notes

1. For recent reviews: (a) Joyce, S.; Girardi, E.; Zajonc, D. M. *J. Immunol.* **2011**, *187*, 1081; (b) Venkataswamya, M. M.; Porcelli, S. A. *Semin. Immunol.* **2010**, *22*, 68; (c) Van Kaer, L. *Immunol. Res.* **2004**, *30*, 139; (d) Wu, L.; Gabriel, C. L.; Parekh, W.; Van Kaer, L. *Tissue Antigens* **2009**, *73*, 535; (e) Cohen, N. R.; Garq, S.; Brenner, M. B. *Adv. Immunol.* **2009**, *102*, 1.
2. (a) Wu, D.; Xing, G.-W.; Poles, M. A.; Horowitz, A.; Kinjo, Y.; Sullivan, B.; Bodmer-Narkevitch, V.; Plettenburg, O.; Kronenberg, M.; Tsuji, M.; Ho, D. D. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1351; (b) Zajonc, D. M.; Kronenberg, M. *Immunol. Rev.* **2009**, *230*, 188.
3. Zhou, D.; Mattner, J.; Cantu, C., III; Schrantz, N.; Yin, N.; Gao, Y.; Sagiv, Y.; Hudspeth, K.; Wu, Y.-P.; Yamashita, T.; Teneberg, S.; Wang, D.; Proia, R. L.; Levery, S. B.; Savage, P. B.; Teyton, L.; Bendelac, A. *Science* **2004**, *306*, 1786.
4. Wu, D.; Fujio, M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 1073.
5. (a) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176; (b) Morita, M.; Sawa, E.; Yamaji, K.; Sakai, T.; Natori, T.; Koezuka, Y.; Fukushima, H.; Akimoto, K. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 288; (c) Takikawa, H.; Muto, S.; Mori, K. *Tetrahedron* **1998**, *54*, 3141; (d) Graziani, A.; Passacantilli, P.; Piancatelli, G.; Tani, S. *Tetrahedron: Asymmetry* **2000**, *11*, 3921.
6. Yu, K. O. A.; Porcelli, S. A. *Immunol. Lett.* **2005**, *100*, 42. and references cited therein.

7. Liang, P.-H.; Imamura, M.; Li, X.; Wu, D.; Fujio, M.; Guy, R. T.; Wu, B.-C.; Tsuji, M.; Wong, C.-H. *J. Am. Chem. Soc.* **2008**, *130*, 12348.
8. (a) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818; (b) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631.
9. (a) Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531; (b) Yamamura, T.; Miyake, S. PCT Int. Appl. WO 2003/016326, 2003; *Chem. Abstr.* **2003**, *138*, 205292m.; (c) Yamamura, T.; Miyamoto, K.; Illes, Z.; Pal, E.; Araki, M.; Miyake, S. *Curr. Top. Med. Chem.* **2004**, *4*, 561; (d) Oki, S.; Chiba, A.; Yamamura, T.; Miyake, S. *J. Clin. Invest.* **2004**, *113*, 1631.
10. Chiba, A.; Oki, S.; Miyamoto, K.; Hashimoto, H.; Yamamura, T.; Miyake, S. *Arthritis Rheum.* **2004**, *50*, 305.
11. Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C. J.; Pellicci, D. G.; Koh, R.; Besra, G. S.; Bharadwaj, M.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *Nature* **2007**, *448*, 44.
12. Koch, M.; Stronge, V. S.; Shepherd, D.; Gadola, S. D.; Mathew, B.; Ritter, G.; Fersht, A. R.; Besra, G. S.; Schmidt, R. R.; Jones, E. Y.; Cerundolo, V. *Nat. Immunol.* **2005**, *6*, 819.
13. Henon, E.; Dauchez, M.; Haudrechy, A.; Banchet, A. *Tetrahedron* **2008**, *64*, 9480.
14. McCarthy, C.; Shepherd, D.; Fleire, S.; Stronge, V. S.; Koch, M.; Illarinov, P. A.; Bossi, G.; Salio, M.; Denkberg, G.; Reddington, F.; Tarlton, A.; Reddy, B. G.; van der Merwe, P. A.; Bersa, G. S.; Jones, W. Y.; Bartisa, F. D.; Cerundolo, V. *J. Exp. Med.* **2007**, *204*, 1131.
15. Murata, K.; Toba, T.; Nakanishi, K.; Takahashi, B.; Yamamura, T.; Miyake, S.; Annoura, H. *J. Org. Chem.* **2005**, *70*, 2398.
16. Toba, T.; Murata, K.; Nakanishi, K.; Takahashi, B.; Takemoto, N.; Akabane, M.; Nakatsuka, T.; Imajo, S.; Yamamura, T.; Miyake, S.; Annoura, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2781.
17. Schmidt, R. R.; Maier, T. *Carbohydr. Res.* **1988**, *174*, 169.
18. The anomeric diastereomers were separable by column chromatography over silica gel. The stereochemistry of the galactoside linkage was determined by ^{1}H NMR spectra. The anomeric proton of the α-isomer appears more downfield than β-isomer (typically ~0.5 ppm) and shows smaller coupling constant (typically less than 5 Hz). Example data of the anomeric proton (400 MHz, $CDCl_3$): For the α-isomer **9** (R = *n*-Bu): δ 4.94 (d, J = 3.5 Hz, 1H), and its corresponding β-isomer: δ 4.41 (d, J = 7.7 Hz, 1H).
19. Taniguchi, M.; Kawano, T.; Koezuka, Y. PCT Int. Appl. WO1998/044928A, 1998, 129, 310889.
20. Toba, T.; Murata, K.; Yamamura, T.; Miyake, S.; Annoura, H. *Tetrahedron Lett.* **2005**, *46*, 5043.
21. Dondoni, A.; Mariotti, G.; Marra, A. *J. Org. Chem.* **2002**, *67*, 4475.
22. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
23. Annoura, H.; Murata, K.; Yamamura, T. PCT Int. Appl. WO2004/072091, 2004; *Chem. Abstr.* **2004**, *141*, 225769n.
24. Michieletti, M.; Bracci, A.; Compostella, F.; De Libero, G.; Mori, L.; Fallarini, S.; Lombardi, G.; Pnaza, L. *J. Org. Chem.* **2008**, *73*, 9192.
25. *MAESTRO Ver. 7.5.112*; Schrödinger, LLC: New York.
26. Macromodel Ver. 9.0 (Schrödinger, LLC): Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskcamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.