Bioorganic & Medicinal Chemistry 21 (2013) 3066–3079

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

RCAI-61 and related 6′-modified analogs of KRN7000: Their synthesis and bioactivity for mouse lymphocytes to produce interferon-γ in vivo ☆

Takuya Tashiro [a], Ryusuke Nakagawa [b,†], Tomokuni Shigeura [b], Hiroshi Watarai [b], Masaru Taniguchi [b], Kenji Mori [a,*]

[a] Glycosphingolipid Synthesis Group, Laboratory for Immune Regulation, Research Center for Allergy and Immunology, RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan
[b] Laboratory for Immune Regulation, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Suehiro-cho 1-7-22, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

ARTICLE INFO

Article history:
Received 5 February 2013
Revised 18 March 2013
Accepted 20 March 2013
Available online 30 March 2013

Keywords:
CD1d
Cytokine
α-Galactosyl ceramide
KRN7000
Natural killer T cell

ABSTRACT

We synthesized ten new analogs of 6′-modified KRN7000 (**A**): RCAI-58, 61, 64, 83, 85–87, 113, 119, and 125. They could be synthesized by α-selective galactosylation of ceramide **9** with the 6-modified D-galactopyranosyl fluorides (**8a**–**8f**) or L-arabinopyranosyl fluoride (**17**), or by etherification of the known alcohol **19**. Bioassay of the ten analogs demonstrated that RCAI-61 (**1**, 6′-O-methylated analog of **A**) was the most potent immunostimulant among them, and could induce the production of a large amount of IFN-γ even at a low concentration in mice in vivo.

1. Introduction

An anticancer drug candidate KRN7000 (**A**, α-GalCer, Fig. 1) was developed by researchers at Kirin Brewery Co. in 1995.[2] Although KRN7000 itself has not been successful in the clinic,[3] the study of its mechanism of action has led to the production of analogs and to methods of delivery which may lead to a useful drug in the clinic.[4] It has been known that **A** is a ligand which forms a complex with CD1d on the surface of antigen presenting cells, such as dendritic cells (DC), of the immune system.[5] CD1d is the major histocompatibility complex class I-like glycolipid presentation protein, and the structures of mouse and human CD1d–**A** complexes were solved by X-ray crystallographic analyses in 2005.[6]

Natural killer T (NKT) cells, one of the lineages of lymphocytes, recognize glycolipids presented by CD1d with their invariant (mouse: Vα14 Jα18, human: Vα24 Jα18) T cell receptor (TCR), and have an ability to secrete various types of cytokines in large quantities after activation.[7] The potent anticancer activity of KRN7000 (**A**) is produced by helper T type 1 (Th1) cytokines, such as interferon (IFN)-γ, secreted by activated NKT cells. The activation of the NKT cells requires their formation of a ternary complex with the CD1d liganded with **A**. However, CD1d–**A** complex induces NKT cells to produce not only Th1 cytokines but also Th2 cytokines such as interleukin (IL)-4 and IL-10. Th2 cytokines mediate immunosuppressive activities, and antagonize biological actions of Th1 cytokines.[8] Therefore, many research groups are making efforts to develop new analogs of **A**, which stimulate NKT cells to produce only (or highly-biased) Th1 cytokines (see reviews).[9]

In 2007 and 2009, X-ray crystallographic analyses of the ternary complexes of human and mouse CD1d–**A**–TCR were reported, respectively.[10] According to those reports, the 2′-, 3′- and 4′-hydroxy groups of the galactose part of **A** are involved in the hydrogen-bonding network with TCR or CD1d, while the 6′-hydroxy group makes no hydrogen-bonding with any residues in the ternary complexes. Therefore, to develop novel analogs of **A**, we thought that the 6′-hydroxy group of **A** can be modified. Indeed, Kirin's research group reported that their α-D-fucopyranosyl (=6-deoxy-α-D-galactopyranosyl) ceramide (**B**, AGL-571, Fig. 1) also showed strong lymphocytic proliferation stimulatory effect in mice in vitro.[11] In addition, in 2002, Savage and co-workers reported that introduction of large fluorophore groups, such as a dansyl group, to the

☆ Synthesis of sphingosine relatives, Part XXXVI. Part XXXV, see Ref. 1.

* Corresponding author. Tel.: +81 3 3816 6889; fax: +81 3 3813 1516.
E-mail address: kjk-mori@arion.ocn.ne.jp (K. Mor.
† Present address: Department of Immunology, Faculty of Medicine, University of Yamanashi, Yamanashi 409-3898, Japan.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.03.028

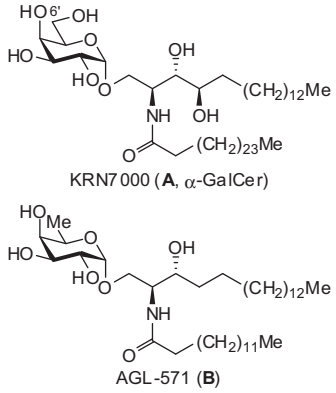


Figure 1. Structures of KRN7000 (**A**) and AGL-571 (**B**).

6′-position of **A** did not diminish the immunostimulatory activity.[12]

To date, many analogs of **A** having an α-D-galactopyranose of which the 6′-hydroxy(methyl) group is modified have been developed.[9] Some of them are shown in Figure 2. AGL-586 (**C**),[13] NU-α-GalCer (**D**)[14] and PBS-57 (**E**)[15] showed potent Th1-type immunostimulatory activities. On the other hand, a 6′-benzotriazolyl analog (**F**)[16] and αGalCerMPEG (**G**)[17] induced Th2-biased cytokine production. Synthesis of an α-D-galacturonyl analog of **A** (**H**, C14) has also been reported.[18,19] According to Calenbergh's observation, **H** induced only a small amount of Th2-biased cytokine production.[19] Interestingly, Wong, Yu and their co-workers reported that **H** showed significantly greater anticancer activity than **A**.[18]

Since the binding site of CD1d is highly flexible, a wide variety of analogs of **A** are acceptable. For example, in the crystal structure of CD1d–**D**–TCR complex resolved by Zajonc, Elewaut and co-workers, a small binding pocket was newly formed above the A′ hydrophobic pocket of CD1d by induced fit to accept the bulky naphthyl group of **D**.[20] This flexibility, at the same time, makes it difficult to find the definitive partial structures of **A** that make the analogs to be Th1-type immunostimulants or Th2-type ones. The substituents at 6′-position of **C** (Th1-type), **D** (Th1), **F** (Th2) and **G** (Th2) are bulky, whereas those of **E** (Th1) and **H** (Th2) are small. Therefore, the bulkiness of the appendant groups seems to have no relationship with Th1/Th2 profile of the analog.

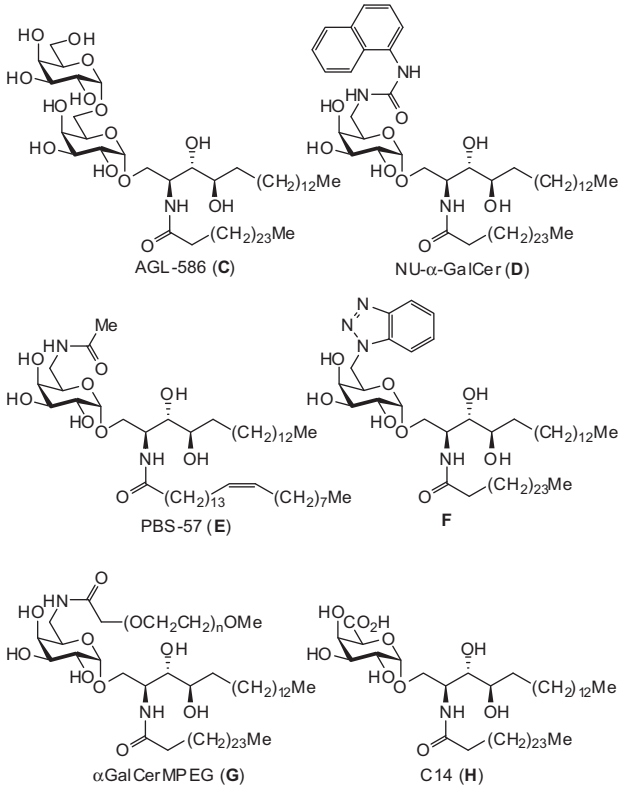


Figure 2. Structures of the analogs of **A** possessing an α-D-galactopyranose of which 6′-position is modified.

In 2007, we reported the synthesis of RCAI-56 (**I**, α-carba-GalCer, Fig. 3), a carbasugar analog of **A**,[21] and described its potent Th1-type immune responses.[22] It was derived by substituting the hydrophilic oxygen of the pyranose by a hydrophobic methylene. This pyranose oxygen has been shown not to participate as an H-bond acceptor within the ligand/protein complex. Similarly, the 6′-OH does not act as an H-bond donner or acceptor. Therefore, from these data, we anticipated that replacing the non-H-bond participating OH with a more hydrophobic ether would make **A** to be a potent Th1-type analog. Hence, to verify this assumption, we launched the synthesis of RCAI-61 (**1**) having an ether group at 6′-position.[23] If **1** induces potent Th1-type immune responses like RCAI-56 (**I**), RCAI-61 (**1**) would be a more promising anticancer drug candidate than **I**, because the synthesis of **1** must be simpler than that of **I**.

In 2008, we reported the synthesis of RCAI-61 (**1**) and its six relatives as a preliminary communication, and found them to be potent activators of mouse lymphocytes to produce a large amount of IFN-γ in mice in vivo.[23] The most potent IFN-γ inducer among those seven analogs was 6′-methoxy one, RCAI-61 (**1**). It should be noted that **1** could stimulate mouse lymphocytes strongly even when it was administered at low concentrations. On the other hand, as we expected, the water-solubility of **1** was lower than that of **A**. As a water-soluble analog, αGalCerMPEG (**G**) was developed by Guzmán and co-workers in 2007.[17] It has a long hydrophilic polyether chain, an (ω-methoxy)polyethylene glycol group, at its 6′-position, and reported to be an IL-4-biased cytokine production inducer.[17] We wondered whether the Th1/Th2 cytokine balances can be changed or not, if a short hydrophilic ether chain was introduced at the 6′-position. Accordingly, we started the synthesis of a 6′-*O*-methoxymethyl, a 6′-*O*-(2-methoxy)ethoxymethyl and a 6′-*O*-(2-hydroxy)ethoxy analogs of **A** (coded as RCAI-113, 119 and 125, respectively), and studied their immunostimulatory activity.

In this paper, we describe the synthesis of RCAI-58, 61, 64, 83, 85, 86 and 87 (**1**, **12**–**16** and **18**), in detail. The time course of the concentrations of IFN-γ, IL-4 and IL-12p70 in sera of mice after administration of these analogs, which were not mentioned in our preliminary communication, are also described in detail. Syn-

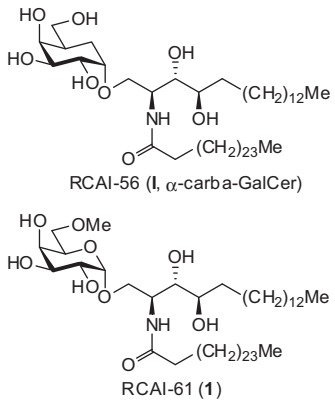


Figure 3. RCAI-56 (**I**, α-carba-GalCer) and RCAI-61 (**1**).

thesis and bioassay of three functionalized 6′-etherified analogs of **A**, RCAI-113 (**23**), 119 (**24**) and 125 (**25**), are also described. RCAI-113 (**23**) induces a larger amount of IFN-γ than KRN7000 (**1**), while the other analogs induce Th2-biased cytokine production.

2. Results and discussions

2.1. Synthesis of RCAI-61 and its related analogs derived by modification of 6′-position of KRN7000

As shown in Scheme 1, we synthesized six kinds of 6-modified methyl α-D-galactopyranosides **3a**–**3f** from the known alcohol **2**.[24] The 6-O-alkylated galactopyranosides **3a**–**3c** were prepared by Williamson's etherification in 87–98% yield. 6-Deoxy-6-methyl galactopyranoside **3d** was obtained by diimide reduction of alkene **4** (87%), which could be prepared according to Tatsuta et al.[25] 6-Deoxygalactopyranoside **3e** (= α-D-fucopyranoside) was synthesized from **2** by two-step deoxygenation as reported by Koto et al.[26] Fluorination of **2** under the XtalFluor-E/DBU conditions gave a cyclized by-product **6** predominantly in 72% yield together with the desired **3f** in only 4% yield.[27] Fortunately, treatment of **2** with (diethylamino)sulfur trifluoride (DAST) in the presence of Et_3N gave **3f** (36%, 71% based on consumed **2**) together with **6** (10%).[28] When this fluorination was performed without Et_3N, only **6** was obtained.[29]

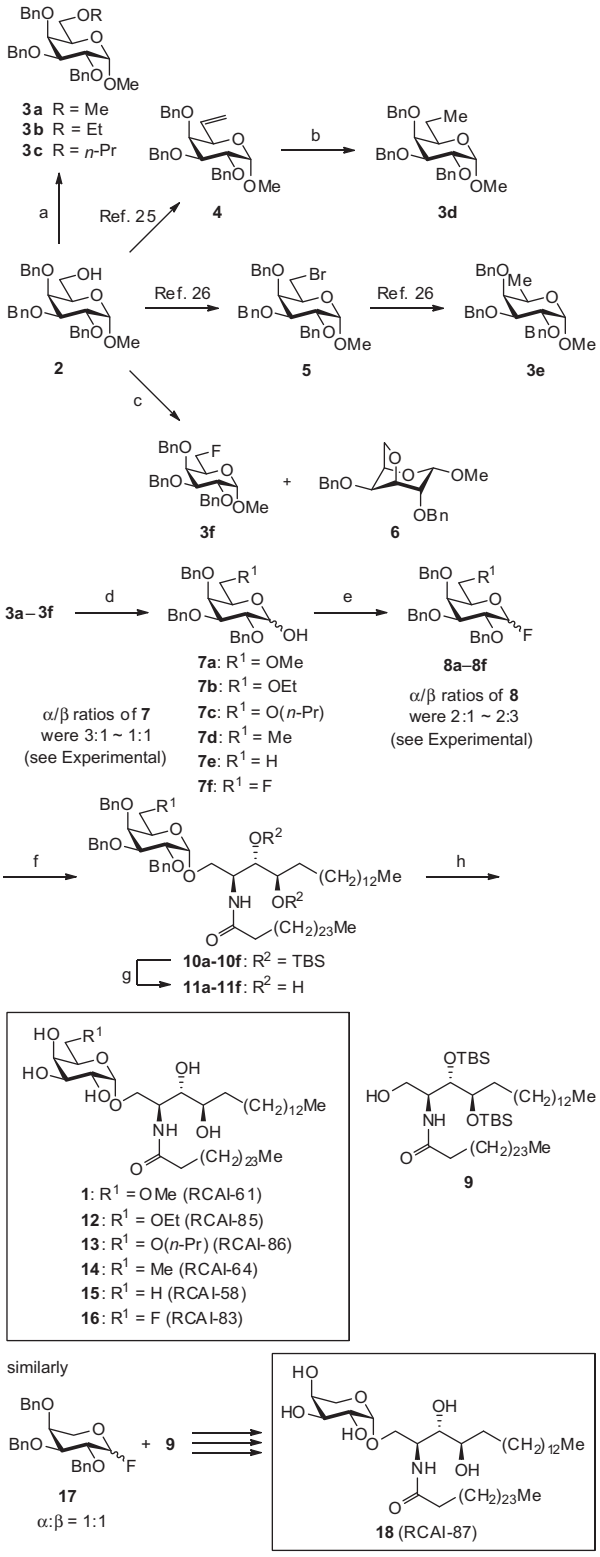
The obtained methyl α-D-galactopyranoside derivatives **3a**–**3f** were converted to the corresponding galactopyranoses **7a**–**7f** by acetolysis with acetic anhydride in the presence of a catalytic amount of sulfuric acid followed by O-deacetylation in 75–94% yield (two steps).[25] The galactopyranoses **7a**–**7f** were then treated with DAST to give fluorides **8a**–**8f** (84–96%) as anomeric mixtures. The fluorides **8a**–**8f** were coupled with the known alcohol **9**[30] under the Mukaiyama conditions[31] to give protected α-galactosyl ceramides **10a**–**10f**. Although **10a**–**10f** contained some unreacted fluorides, these inseparable impurities could be removed readily from **11a**–**11f** after deprotection of *tert*-butyldimethylsilyl (TBS) groups with tetrabutylammonium fluoride (TBAF). Finally, hydrogenolysis of all of the benzyl groups of **11a**–**11f** gave the desired analogs **1** and **12**–**16** in 61–91% yield as colorless powders. Similarly, β-L-arabinopyranosyl ceramide **18** was synthesized from the known fluoride **17** and ceramide **9** by glycosylation followed by deprotection.[32]

2.2. Results of bioassay of RCAI-58, 61, 64, 83 and 85–87

To investigate the ability of **1**, **12**–**16** and **18** to induce cytokine production by mouse lymphocytes in vivo, the concentrations of cytokines in sera of mice were monitored after intravenous administration of KRN7000 (**A**) or synthesized analogs (**1**, **12**–**16** or **18**) as phosphate buffered saline (PBS) solutions, which were prepared just before injection into C57BL/6 J mice.[33,34] The sera samples were collected at 3, 6, 12, 24, 36, 48, and 60 h after injection of glycolipid solutions.

The results were shown in Figure 4A, C and E. As can be seen, all of the synthesized analogs **1**, **12**–**16** and **18** induced mouse lymphocytes to produce a larger amount of IFN-γ than **A** did (Fig. 4A). They also induced almost the same level of IL-4 production as **A** did (Fig. 4C). Therefore, these 6′-modified analogs are regarded as potent Th1-biased cytokine inducers. As shown in Figure 4E, **1** and **12**–**16** induced increased IL-12p70 production in comparison to **A**. IL-12p70 produced by DC stimulates NKT cells to produce IFN-γ along with CD40/CD40 ligand interaction.[35] Therefore, the potent IFN-γ production seems to be related to the enhanced production of IL-12p70.

Among three 6′-O-alkylated analogs, RCAI-61 (**1**) induced a larger amount of IFN-γ than RCAI-85 (**12**) and 86 (**13**). The 6′-appendages of **12** and **13** are ethoxy and *n*-propoxy groups, respectively, and are bulkier than that of **1**, methoxy group. The increase in bulkiness of those hydrophobic appendages is relatively small, so



Scheme 1. Synthesis of RCAI-61 (**1**) and its relatives **12**–**16** and **18**. Reagents and conditions: (a) NaH, MeI or EtBr or *n*-PrBr, Bu_4NI (when the alkyl bromides were used), DMF, THF, rt (87–98%); (b) $H_2NNH_2{\cdot}H_2O$, 30% H_2O_2 aq, EtOH, rt (87%); (c) DAST, Et_3N, CH_2Cl_2, rt to reflux (36%, 71% based on consumed **2**); (d) (1) cat. concd H_2SO_4, Ac_2O, 0 °C; (2) NaOMe, MeOH, rt (75–94%, two steps); (e) DAST, CH_2Cl_2, rt (84–96%); (f) **9**, $SnCl_2$, $AgClO_4$, MS 4A, THF, −18 to 5 °C; (g) TBAF, THF, rt (27–87%, two steps); (h) H_2, 20% $Pd(OH)_2$-C, THF, rt (61–91%).

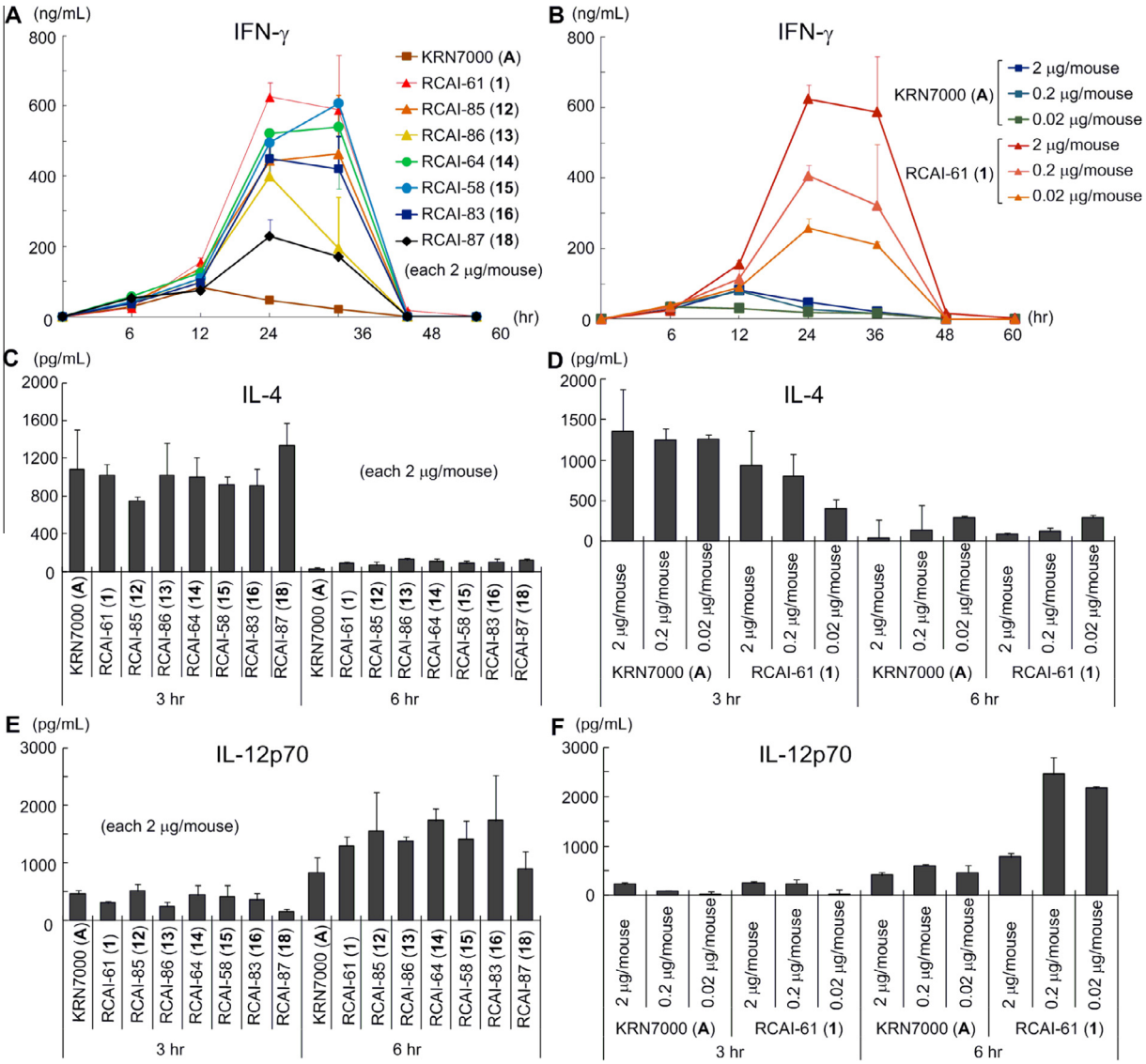


Figure 4. Cytokine level in serum after intravenous injection in mice of KRN7000 (**A**) or synthesized analogs (**1**, **12**–**16**, **18**). Serum concentrations of IFN-γ (A and B), IL-4 (C and D) and IL-12p70 (E and F) were measured by ELISA or CBA at the indicated time points. Data are means ± SD from three mice.[33]

both of those might be acceptable to the hydrophobic binding cleft of CD1d. However, they might somewhat disrupt the formation of a stable complex with CD1d or the binding of TCR to the binary complex of **12** or **13** with CD1d. The decrease of the binding affinity of TCR to those complexes might cause the reduced production of IFN-γ upon administration of **12** or **13**. Although the binding pocket of CD1d is flexible, the linear hydrophobic alkyl chain might not be able to generate an additional binding site on the roof of the hydrophobic pocket of CD1d as observed in the CD1d–**D**–TCR complex.[20] It should be added that the introduction of larger hydrophobic appendages decreased the water-solubility of the analogs (*M*log*P* of **A**, **1**, **12** and **13** are 4.16, 4.32, 4.47, and 4.63, respectively).[36]

The functional groups at 6′-position of RCAI-64 (**14**), RCAI-58 (**15**) and RCAI-83 (**16**) are smaller than that of **1**, and these analogs induced the production of a large amount of IFN-γ like **1**. Therefore, analogs possessing a sterically less bulky and hydrophobic 6′-functional group seems to induce a large amount of IFN-γ production. It could make a more rigid complex with CD1d than **A** which has a small but hydrophilic 6′-hydroxyl group. Presence of the 6′-carbon atom is, however, thought to be important for the analogs to be a potent IFN-γ inducer. The peak IFN-γ concentration induced by RCAI-87 (**18**), which has no 6′-methyl or methylene group, was lower than that of other 6′-functionalized analogs. As the result, due to its potent bioactivity and ready availability, we chose 6′-O-methylated galactose as the best sugar moiety to develop a potent anticancer glycosphingolipid.

We then investigated dose dependency of bioactivity of RCAI-61 (**1**) as shown in Figure 4B, D and F. Even at low concentrations, immunostimulatory activity of **1** was very powerful. The IFN-γ concentration at the peak time induced by administration of **1** at a dose of 0.02 μg/mouse was three times larger than that of **A** at a dose of 2 μg/mouse in mice in vivo. It is noteworthy that the IL-4 concentrations at 3 h induced by **A** were almost the same levels in every concentrations, however, those of **1** were decreased concentration-dependently. Therefore, administration of **1** in low concentration induced mouse lymphocytes to produce Th1 cytokines almost exclusively.

2.3. Synthesis and bioactivity of RCAI-113, 119 and 125

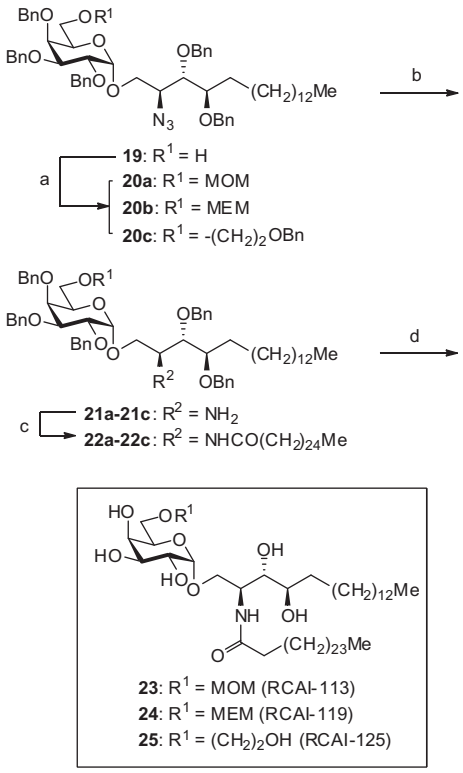
As described above, RCAI-61 (**1**) was derived by replacing the hydrophilic 6′-hydroxy group with the less hydrophilic methoxy group. Its water-solubility was therefore decreased. To improve

its solubility, we planned to introduce some hydrophilic functional groups at a proper position of **1**. In 2007, Guzmán and co-workers reported that introduction of a polyethyleneglycol (PEG) group into 6′-position of **A** improved the water-solubility of **A**, and did not diminish its immunostimulatory activities.[17] They developed αGalCerMPEG (**G**, Fig. 2), a 6′-[ω-(methoxy)polyethoxy]acetamide analog of **A**, as a water-soluble potent immunostimulatory agent. However, it induced Th2-type immune responses.[17] We expected that the introduction of a short hydrophilic ether unit instead of the long polyethylene group at the 6′-position would increase the water-solubility, and induce the Th1-type immunostimulatory activity like **1**. Based on this assumption, we synthesized three 6′-*O*-alkyl etherated analogs **23**–**25** as shown in Scheme 2.

The synthesis commenced with the known alcohol **19**.[19] The 6′-hydroxy group of **19** was converted to a methoxymethoxy (MOM-oxy), a (2-methoxy)ethoxymethoxy (MEM-oxy) or a (2-benzyloxy)ethoxy groups to yield **20a**, **20b** and **20c** in 83%, 78% and 61% yield, respectively. Azide groups of **20a**–**20c** were reduced by Staudinger reaction to give amines **21a**–**21c** (78–85%).[37] Amides **22a**–**22c** were obtained by acylation of amines **21a**–**21c** with hexacosanoyl chloride in 78–87% yield.[38] Deprotection of all of the benzyl groups furnished RCAI-113 (**23**), 119 (**24**) and 125 (**25**) in 44–72% yield. It should be added that the *M*log*P* values of **23**, **24** and **25** were calculated as 4.16, 3.74 and 3.75, respectively.[36] The value of **23** was identical with that of **A** (4.16). The water-solubility of these analogs are therefore no less than **A** based on the calculation.

The immunostimulatory activity of **23**–**25** were investigated by measuring the cytokine concentrations in sera of mice after treatment with these glycolipids. In the case of RCAI-61 (**1**) and its derivatives, their PBS/DMSO (99:1) solutions were prepared. On the other hand, to improve the solubility of the glycolipids, a surface-activating agent Tween® 20 was added to the PBS/DMSO (99:1) solutions of **23**–**25** and their positive standard **A** (see Section 4). The intravenous administration of these PBS solutions of **23**–**25** at a dose of 2 μg/mouse C57BL/6 J mice induced production of IL-4 and IL-12p70 at levels similar to those caused by **A**, while the highest concentrations of IFN-γ at the peak time were different as shown in Figure 5.[33,39] RCAI-113 (**23**) induced mouse lymphocytes to produce a larger amount of IFN-γ compared to **A**, while RCAI-119 (**24**) and RCAI-125 (**25**) were weaker inducers than **A**.

The chain length of the MOM group of **23** and 2-hydroxyethyl group of **25** are nearly identical to that of the *n*-propyl group of RCAI-86 (**13**). Comparison of Figures 4A and 5A ranked the immunostimulatory activity of these three analogs as **13** > **23** > **25**. This order is the same as that of the hydrophobicity of the functional groups at the 6′-position of these analogs since the polarity follows the order of hydroxyl, ether, and alkyl groups. Therefore, the hydrophobicity of the 6′-appendages might be one of the key factors enhancing the binding affinity of CD1d–glycolipid–TCR and



Scheme 2. Synthesis of the analogs of **A** (**23**–**25**) possessing short hydrophilic ether groups at the 6′-position. Reagents and conditions: (a) MOMCl or MEMCl, (*i*-Pr)$_2$NEt, CH_2Cl_2, rt (83% for **20a**, 78% for **20b**); NaH, BnO$(CH_2)_2$Br, Bu_4NI, DMF, THF, rt (61% for **20c**); (b) PMe_3, THF, rt; then 1 M NaOH aq, rt (78–85%); (c) *n*-$C_{25}H_{51}COCl$, Et_3N, CH_2Cl_2, rt (78–87%); (d) H_2, 20% $Pd(OH)_2$-C, THF, rt (44–72%).

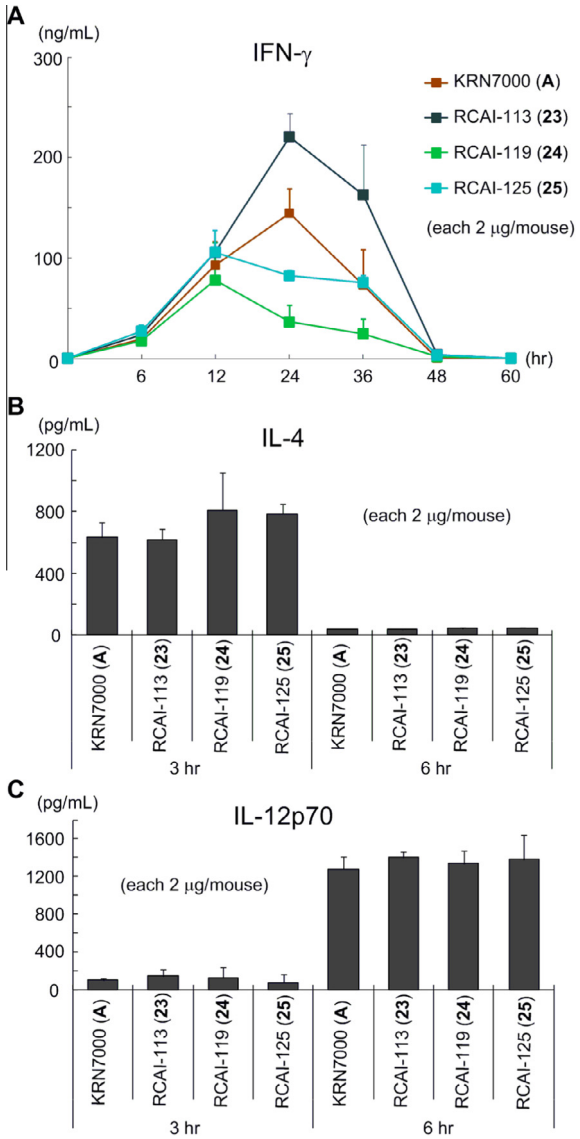


Figure 5. Mouse in vivo cytokine production after intravenous administration of KRN7000 (**A**) or synthesized analogs (**23**–**25**). Serum concentrations of IFN-γ (A), IL-4 (B) and IL-12p70 (C) were measured by ELISA or CBA at the indicated time points. Data are means ± SD from three mice.[33]

the strength of the stimulatory signals inside NKT cells. On the other hand, **24** has a MEM-oxy group, which is a longer ether chain than the MOM-oxy group of **23** at its 6′-position. Although **24** induced the same levels of IL-12p70 as shown in Figure 5C, the peak concentration of IFN-γ induced by it was approximately a half as much as that by **A**. Accordingly, **24** is regarded as Th2-type analog in contrast to Th1-type profiles of the other short 6′-etherified analogs, such as RCAI-61 (**1**) and 113 (**23**). Introduction of a linear (poly)ether chain with seven (the MEM-oxy group) or more atoms at the 6′-position seems to render the glycolipid to the Th2-type like αGalCerMPEG (**G**).

From these results, the analogs possessing a short hydrophobic appendage at 6′-position seem to induce potent Th1-type immune responses. On the other hand, to be a potent immunostimulant, the analog should have the 6′-carbon atom, which can keep the galactose in a chair-form rigidly. Calculation of the docking models would reveal the relationship between the hydrophobicity or the bulkiness at the 6′-position and the binding affinity of the ternary complexes. Molecular dynamics computer simulation of CD1d–**1**–TCR is in progress, and the results will be reported by our co-worker, Dr. T. Hirokawa (AIST, Japan).

Because it could be synthesized very concisely, we chose the 6′-O-methylated analog RCAI-61 (**1**) as the promising anticancer drug candidate. Unfortunately, the water-solubility of **1** is quite low.[40] To obtain the reproducible results of the powerful bioactivity of **1**, administration of DC pulsed with **1** would be an alternative method.[41] The detailed results of bioassay of DC-pulsed **1** will be reported in due course.

Very recently Bittman and coworkers reported that their introduction of a 6′-*O*-methyl group into a *C*-GalCer did not improve its immunostimulant activity.[42] Apparently, the *C*-GalCer framework of their compound caused the observed difference with RCAI-61.

3. Conclusion

We synthesized RCAI-58, 61, 64, 83, 85–87, 113, 119 and 125, the 6′-modified analogs of KRN7000. Bioassay of these analogs in mice in vivo demonstrated that RCAI-58, 61, 64, 83, 85–87 and 113 were the Th1-type cytokine inducers, while RCAI-119 and 125 were the Th2-type ones. Among those Th1-type analogs, RCAI-61 (**1**, 6′-*O*-methyl analog) was found to be the most potent IFN-γ inducer. It could stimulate mouse lymphocytes to produce a large amount of IFN-γ even at low concentrations.

4. Experimental

4.1. Chemistry

4.1.1. General

Refractive indices (n_D) were measured on an Atago 1T refractometer. Melting points were recorded using a Yanaco MP-S3 melting point measuring apparatus and are uncorrected. Optical rotation values were measured on a Jasco P-1010 polarimeter. IR spectra were measured on a Jasco FT/IR-460 plus spectrometer. ^{1}H NMR spectra (TMS at $\delta = 0.00$, or pyridine at $\delta = 7.55$ as the internal standards) and ^{13}C NMR spectra (pyridine at $\delta = 135.5$ as the internal standard) were recorded on a Varian VNMRS-500, Jeol A400 or a Jeol EX270 spectrometers. High resolution mass spectrometry (HRMS) was performed on a Jeol JMS-SX102A mass spectrometer [fast atom bombardment (FAB)-HRMS] or a Bruker BioAPEX II 70e FT-ICR mass spectrometer interfaced to an external Apollo-II™ electrospray ionization (ESI) source (ESI-HRMS). Column chromatography was performed by using Kanto Chemical silica gel 60 N irregular neutral (37572-79) or Fuji Silysia Chemical chromatorex® NH (DM2035).

4.1.2. 6-*O*-Alkylation of 2

4.1.2.1. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-galactopyranoside 3a. To a stirred solution of **2** (7.21 g, 15.5 mmol) in dry *N*,*N*-dimethylformamide (DMF) and THF (1:1, 100 mL), NaH (60% mineral oil suspension, 1.07 g, 26.8 mmol) was added at 0 °C. After stirring at 0 °C for 30 min, methyl iodide (2.90 mL, 46.6 mmol) was added to the mixture. The mixture was stirred at room temperature for 14 h. The reaction was then quenched with water, and the mixture was extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (150 g, hexane/EtOAc = 3:1) to give **3a** (6.49 g, 87%) as a colorless oil, n_D^{22} 1.5172; $[\alpha]_D^{26}$ +34.9 (*c* 1.10, $CHCl_3$); ν_{max} (film): 1600 (w), 1500 (m), 1100 (br s, C–O), 1050 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.42–7.26 (15H, m), 4.96 (1H, d, *J* = 12 Hz), 4.86 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.692 (1H, d, *J* = 12 Hz), 4.687 (1H, d, *J* = 3.2 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.04 (1H, dd, *J* = 9.6, 3.2 Hz), 3.94 (1H, dd, *J* = 10, 3.2 Hz), 3.91–3.89 (1H, m), 3.84 (1H, br t, *J* = 6.4 Hz), 3.44 (1H, dd, *J* = 10, 6.4 Hz), 3.37 (3H, s), 3.34 (1H, dd, *J* = 10, 6.4 Hz), 3.27 (3H, s) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{29}H_{34}O_6Na$ $[M+Na]^+$ 501.2253, found 501.2252.

4.1.2.2. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-ethyl-α-D-galactopyranoside 3b. In the same manner as described above, the 6-hydroxy group of **2** was alkylated with bromoethane in the presence of a catalytic amount of tetrabutylammonium iodide to give **3b** (98%) as a colorless oil, n_D^{23} 1.5170; $[\alpha]_D^{26}$ +25.3 (*c* 1.74, $CHCl_3$); ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1115 (br s, C–O), 1050 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.40–7.24 (15H, m), 4.95 (1H, d, *J* = 12 Hz), 4.85 (1H, d, *J* = 12 Hz), 4.83 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.685 (1H, d, *J* = 12 Hz), 4.682 (1H, d, *J* = 3.2 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.06–4.02 (1H, m), 3.96–3.92 (2H, m), 3.85 (1H, br t, *J* = 6.6 Hz), 3.49–3.42 (3H, m), 3.38–3.34 (1H, m), 3.37 (3H, s), 1.14 (3H, t, *J* = 7.2 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{30}H_{36}O_6Na$ $[M+Na]^+$ 515.2410, found: 515.2422.

4.1.2.3. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-propyl-α-D-galactopyranoside 3c. In the same manner as described above, the 6-hydroxy group of **2** was alkylated with 1-bromopropane in the presence of a catalytic amount of tetrabutylammonium iodide to give **3c** (94%) as a colorless oil, n_D^{27} 1.5177; $[\alpha]_D^{23}$ +24.7 (*c* 0.77, $CHCl_3$); ν_{max} (film): 1605 (w), 1495 (m), 1110 (br s, C–O), 1050 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.40–7.24 (15H, m), 4.96 (1H, d, *J* = 12 Hz), 4.85 (1H, d, *J* = 12 Hz), 4.83 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.685 (1H, d, *J* = 12 Hz), 4.680 (1H, d, *J* = 4.0 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.06–4.01 (1H, m), 3.96–3.92 (2H, m), 3.86 (1H, t, *J* = 6.4 Hz), 3.45 (2H, d, *J* = 6.4 Hz), 3.40–3.34 (1H, m), 3.37 (3H, s), 3.26 (1H, dt, *J* = 9.6, 7.2 Hz), 1.53 (1H, sext., *J* = 7.2 Hz), 0.89 (3H, t, *J* = 7.2 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{31}H_{38}O_6Na$ $[M+Na]^+$ 529.2566, found: 529.2552.

4.1.3. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-methyl-α-D-galactopyranoside 3d

The olefin **4** was prepared as reported by Tatsuta et al.[25] To a stirred solution of **4** (1.12 g, 2.43 mmol) and hydrazine monohydrate (10.0 mL, 20.6 mmol) in ethanol (50 mL), 30% aqueous H_2O_2 solution (4 mL) was added dropwise over 3 h. The mixture was stirred at room temperature overnight, and then cooled to 0 °C. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (20 mL). After stirring at room temperature for 30 min, the mixture was extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous $Na_2S_2O_3$ solution, a

saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 20:1) to give **3d** (981 mg, 87%) as a colorless oil, n_D^{22} 1.5163; $[\alpha]_D^{27}$ +33.5 (*c* 1.60, $CHCl_3$); ν_{max} (film): 1605 (w), 1500 (m), 1100 (br s, C–O), 1050 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.42–7.25 (15H, m), 4.99 (1H, d, *J* = 11 Hz), 4.89 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.65 (1H, d, *J* = 11 Hz), 4.66 (1H, d, *J* = 4.0 Hz), 4.05 (1H, dd, *J* = 10, 4.0 Hz), 3.92 (1H, dd, *J* = 10, 2.8 Hz), 3.72 (1H, d, *J* = 2.8 Hz), 3.50 (1H, dd, *J* = 8.4, 6.0 Hz), 3.35 (3H, s), 1.66 (1H, ddq, *J* = 14, 7.2, 6.0 Hz), 1.42–1.32 (1H, m), 0.81 (3H, t, *J* = 7.2 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{29}H_{34}O_5Na$ $[M+Na]^+$ 485.2304, found 485.2306.

4.1.4. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-galactopyranoside 3f

To a stirred solution of **2** (1.20 g, 2.58 mmol) and Et_3N (4.80 mL, 2.60 mmol) in dry CH_2Cl_2 (20 mL), (diethylamino)sulfur trifluoride (DAST, 681 μL, 5.15 mmol) was added at −40 °C. The mixture was stirred at room temperature for 2 h, and then refluxed for 1 h. The mixture was then cooled to −40 °C, and the reaction was quenched with methanol. After stirring at room temperature for 30 min, the mixture was concentrated in vacuo. The residue was poured into a saturated aqueous $NaHCO_3$ solution, and extracted with EtOAc. The organic phase was washed successively with a saturated aqueous $NaHCO_3$ solution, water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 20:3) to give **3f** (436 mg, 36%, 71% based on consumed **2**) as a colorless oil, n_D^{22} 1.5169; $[\alpha]_D^{22}$ +20.5 (*c* 1.01, $CHCl_3$); ν_{max} (film): 1605 (w), 1585 (w) 1495 (m), 1100 (br s, C–O), 1045 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.42–7.24 (15H, m), 4.97 (1H, d, *J* = 12 Hz), 4.89 (1H, d, *J* = 12 Hz), 4.85 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 4.0 Hz), 4.60 (1H, d, *J* = 12 Hz), 4.44 (1H, ddd, *J* = 48, 9.2, 6.0 Hz), 4.27 (1H, ddd, *J* = 46, 9.2, 5.2 Hz), 4.04 (1H, dd, *J* = 10, 4.0 Hz), 4.00–3.91 (2H, m), 3.89 (1H, br s), 3.37 (3H, s) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{31}O_5FNa$ $[M+Na]^+$ 489.2053, found 489.2064.

(1*R*,3*S*,4*R*,5*S*,8*S*)-4,8-Bis(benzyloxy)-3-methoxy-2,6-dioxabicyclo[3.2.1]octane (**6**, 117 mg, 10%, 19% consumed **2**) was eluted with hexane/EtOAc = 4:1 as a colorless oil, n_D^{26} 1.5179; $[\alpha]_D^{25}$ +26.7 (*c* 1.25, $CHCl_3$); ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1090 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.37–7.24 (10H, m), 4.86 (1H, d, *J* = 12 Hz), 4.76 (1H, d, *J* = 2.4 Hz), 4.58 (1H, d, *J* = 12 Hz), 4.52 (1H, d, *J* = 12 Hz), 4.51 (1H, d, *J* = 12 Hz), 4.40 (1H, br s), 4.36 (1H, br s), 4.27 (1H, d, *J* = 5.6 Hz), 4.05 (1H, d, *J* = 10 Hz), 4.00 (1H, dd, *J* = 10, 2.4 Hz), 3.72 (1H, dd, *J* = 5.6, 2.4 Hz), 3.53 (3H, s) ppm; HRMS (EI+) *m*/*z* calcd for $C_{21}H_{24}O_5$ $[M]^+$ 356.1624, found 356.1625.

Unreacted **2** (583 mg, 49%) was recovered by elution with hexane/EtOAc = 1:1.

4.1.5. Acetolysis and deacetylation

4.1.5.1. 2,3,4-Tri-*O*-benzyl-6-*O*-methyl-D-galactopyranose 7a. To a stirred solution of **3a** (733 mg, 1.53 mmol) in acetic anhydride (20 mL), a solution of concn H_2SO_4 (0.03 mL) in acetic anhydride (10 mL) was added at 0 °C. The mixture was stirred at 0 °C for 20 min. The reaction was then quenched with a saturated aqueous $NaHCO_3$ solution. After stirring at 0 °C for 10 min, the mixture was extracted with EtOAc. The organic phase was washed successively with a saturated aqueous $NaHCO_3$ solution, water and brine, dried with $MgSO_4$, and concentrated in vacuo to give 1-O-acetylated **7a** (766 mg, 99%) as a pale yellow oil.

To a stirred solution of the acetate (766 mg) in MeOH (20 mL), sodium methoxide (90 mg, 1.7 mmol) was added at room temperature. After stirring at room temperature for 30 min, the mixture was neutralized with Dowex® 50W-X8 ion exchange resin. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 3:1) to give **7a** (652 mg, 92% in two steps) as white powder. Mp 68.5–73.0 °C; $[\alpha]_D^{26}$ +23.5 (*c* 2.18, $CHCl_3$); ν_{max} (KBr): 3420 (br s, OH), 1605 (w), 1495 (m), 1100 (br s, C–O), 735 (s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$, characteristic data, α:β = ca. 5:4): 5.29 (0.56H, dd, *J* = 3.2, 2.4 Hz), 4.68 (0.44H, dd, *J* = 7.2, 6.4 Hz), 3.28 (3H, s) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{28}H_{33}O_6$ $[M+H]^+$ 465.2277, found 465.2273.

4.1.5.2. 2,3,4-Tri-*O*-benzyl-6-*O*-ethyl-D-galactopyranose 7b. In the same manner as described above, **3b** (559 mg, 1.13 mmol) was converted to **7b** (406 mg, 75% in two steps) as a colorless waxy solid. Mp 53.5–61.0 °C; $[\alpha]_D^{23}$ +21.5 (*c* 0.61, $CHCl_3$); ν_{max} (film): 3300 (br m, OH), 1495 (m), 1100 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$, characteristic data, α:β = ca. 1:1): 5.29 (0.5H, dd, *J* = 3.0, 2.5 Hz), 4.68 (0.5H, dd, *J* = 7.0, 6.5 Hz), 1.14 (3H, t, *J* = 7.0 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{29}H_{34}O_6Na$ $[M+Na]^+$ 501.2253, found 501.2251.

4.1.5.3. 2,3,4-Tri-*O*-benzyl-6-*O*-propyl-D-galactopyranose 7c. In the same manner as described above, **3c** (594 mg, 1.17 mmol) was converted to **7c** (453 mg, 79% in two steps) as a colorless oil, n_D^{24} 1.5174; $[\alpha]_D^{24}$ +21.7 (*c* 1.11, $CHCl_3$); ν_{max} (film): 3420 (br s, OH), 1610 (w), 1585 (w), 1495 (m), 1110 (br s, C–O), 1065 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$, characteristic data, α:β = ca. 1:1): 5.29–5.27 (0.5H, m), 4.68 (0.5H, dd, *J* = 7.0, 6.5 Hz), 1.54 (2H, sext., *J* = 7.0 Hz), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{36}O_6Na$ $[M+Na]^+$ 515.2410, found 515.2431.

4.1.5.4. 2,3,4-Tri-*O*-benzyl-6-deoxy-6-methyl-D-galactopyranose 7d. In the same manner as described above, **3d** (913 mg, 1.97 mmol) was converted to **7d** (832 mg, 94% in two steps) as a white solid. Mp 81.0–86.5 °C; $[\alpha]_D^{26}$ +20.4 (*c* 2.17, $CHCl_3$); ν_{max} (KBr): 3400 (br s, OH), 1605 (w), 1495 (m), 1100 (br s, C–O), 1040 (br s, C–O), 735 (s), 690 (s) cm^{-1}; δ_H (400 MHz,$CDCl_3$, characteristic data, α:β = ca. 2:1): 5.27 (0.67H, br d, *J* = 3.2 Hz), 4.60 (0.33H, t, *J* = 6.8 Hz), 0.79 (1H, t, *J* = 7.2 Hz), 0.78 (2H, t, *J* = 7.6 Hz) ppm; HRMS (FAB+) calcd for $C_{28}H_{32}O_5Na$ $[M+Na]^+$ 471.2147, found 471.2146.

4.1.5.5. 2,3,4-Tri-*O*-benzyl-6-deoxy-6-fluoro-D-galactopyranose 7f. In the same manner as described above, **3f** (620 mg, 1.33 mmol) was converted to **7f** (521 mg, 87% in two steps) as colorless solid. Mp 74.0–79.0 °C; $[\alpha]_D^{22}$ +26.5 (*c* 1.05, $CHCl_3$); ν_{max} (KBr): 3420 (br s, OH), 1610 (w), 1585 (w), 1495 (m), 1215 (br m), 1100 (br s, C–O), 1030 (br s, C–O), 750 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$, characteristic data, α:β = ca. 3:1): 5.29 (0.75H, dd, *J* = 3.0, 2.5 Hz), 4.69 (0.25H, dd, *J* = 7.5, 6.5 Hz), 3.23 (0.25H, d, *J* = 6.5 Hz), 2.96 (0.75H, d, *J* = 2.5 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{29}O_5FNa$ $[M+Na]^+$ 475.1897, found 475.1891.

4.1.5.6. 2,3,4-Tri-*O*-benzyl-L-arabinopyranose 7g. Methyl 2,3,4-tri-*O*-benzyl-β-L-arabinopyranoside (**3g**) was prepared by the method of Szeja et al.,[43] n_D^{22} 1.5170; $[\alpha]_D^{22}$ +66.7 (*c* 1.05, $CHCl_3$), Lit.[43] $[\alpha]_D^{20}$ +83 (*c* 2, $CHCl_3$); ν_{max} (film): 1605 (w), 1585 (w), 1495 (s), 1140 (br s, C–O), 1100 (br s, C–O), 1055 (br s, C–O), 740 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.25 (15H, m, Ph x 3), 4.86 (1H, d, *J* = 12 Hz, PhCH_a), 4.76 (1H, d, *J* = 12 Hz, PhCH_a), 4.72 (2H, s, PhCH_2), 4.70 (1H, d, *J* = 12 Hz, PhCH_b), 4.68 (1H, d, *J* = 3.5 Hz, 1-H), 4.65 (1H, d, *J* = 12 Hz, PhCH_b), 4.01 (1H, dd, *J* = 10, 3.5 Hz, 2-H), 3.87 (1H, dd, *J* = 10, 3.5 Hz, 3-H), 3.75–3.74 (1H, m, 4-H), 3.65 (1H, dd, *J* = 13, 2.5 Hz, 5-H_a), 3.59 (1H, dd, *J* = 13,

1.0 Hz, 5-H_b), 3.37 (3H, s, OMe) ppm. This spectrum indicates the conformation of **3g** as $^4C_1$; HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{30}O_5Na$ $[M+Na]^+$ 457.1991, found 457.1978.

In the same manner as described above, the obtained **3g** (2.65 g, 6.10 mmol) was converted to **7g** (1.41 g, 55% in two steps) as colorless oil, n_D^{23} 1.5162; $[\alpha]_D^{23}$ −7.0 (*c* 1.04, $CHCl_3$). Pure β-anomer was reported as fine needles,[44] mp: 83–86 °C, $[\alpha]_D^{20}$ +51.1 (*c* 1.76, CH_2Cl_2); ν_{max} (film): 3440 (br s, OH), 1700 (w), 1600 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$, characteristic data, α:β = ca. 1:1): 5.17 (0.5H, dd, *J* = 5.5, 2.0 Hz), 4.88 (0.5H, dd, *J* = 10, 2.0 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{26}H_{28}O_5Na$ $[M+Na]^+$ 443.1834, found 443.1843.

4.1.6. Fluorination

4.1.6.1. 2,3,4-Tri-*O*-benzyl-6-*O*-methyl-D-galactopyranosyl fluoride 8a. To a stirred solution of **7a** (602 mg, 1.30 mmol) in dry CH_2Cl_2 (20 mL), DAST (2.50 mL, 18.9 mmol) was added at −40 °C. The mixture was stirred at 0 °C for 30 min. The reaction was then quenched with MeOH (1 mL) at −40 °C. The resulting mixture was diluted with EtOAc, and washed successively with a saturated aqueous $NaHCO_3$ solution and bride, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (60 g, hexane/EtOAc = 6:1) to give **8a** (554 mg, 91%) as a colorless oil. ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1110 (br s, C–O), 1055 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$, characteristic data, α:β = ca. 5:4): 5.58 (0.56H, dd, *J* = 54, 2.4 Hz), 5.16 (0.44H, dd, *J* = 53, 6.8 Hz), 3.27 (3H, s) ppm. This was immediately used in the next step without further purification.

4.1.6.2. 2,3,4-Tri-*O*-benzyl-6-*O*-ethyl-D-galactopyranosyl fluoride 8b. In the same manner as described above, **7b** (352 mg, 0.736 mmol) was converted to **8b** (332 mg, 94%) as a colorless oil. ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1115 (br s, C–O), 1055 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$, characteristic data, α:β = ca. 1:1): 5.59 (0.5H, dd, *J* = 54, 2.4 Hz), 5.18 (0.5H, dd, *J* = 53, 7.2 Hz), 1.51 (3H, t, *J* = 7.2 Hz) ppm. This was immediately used in the next step without further purification.

4.1.6.3. 2,3,4-Tri-*O*-benzyl-6-*O*-propyl-D-galactopyranosyl fluoride 8c. In the same manner as described above, **7c** (414 mg, 0.841 mmol) was converted to **8c** (368 mg, 88%) as a colorless oil. ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1110 (br s, C–O), 1060 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$, characteristic data, α:β = ca. 1:1): 5.59 (0.5H, dd, *J* = 54, 2.8 Hz), 5.18 (0.5H, dd, *J* = 53, 6.8 Hz), 1.54 (2H, sext., *J* = 7.0 Hz), 0.89 (3H, t, *J* = 7.0 Hz) ppm. This was immediately used in the next step without further purification.

4.1.6.4. 2,3,4-Tri-*O*-benzyl-6-deoxy-6-methyl-D-galactopyranosyl fluoride 8d. In the same manner as described above, **7d** (776 mg, 1.73 mmol) was converted to **8d** (745 mg, 96%) as a colorless oil. ν_{max} (film): 1605 (w), 1585 (w), 1500 (m), 1105 (br s, C–O), 740 (s), 700 (s) cm^{-1}; δ_H (270 MHz, $CDCl_3$, characteristic data, α:β = ca. 2:1): 5.58 (0.67H, dd, *J* = 54, 3.0 Hz), 5.14 (0.33H, dd, *J* = 53, 7.3 Hz), 0.82 (1H, t, *J* = 7.6 Hz), 0.80 (2H, t, *J* = 7.6 Hz) ppm. This was immediately used in the next step without further purification.

4.1.6.5. 2,3,4-Tri-*O*-benzyl-D-fucopyranosyl fluoride 8e. 2,3,4-Tri-*O*-benzyl-D-fucopyranose (**7e**) was prepared as reported by Koto et al.[26] In the same manner as described above, **7e** (460 mg, 1.06 mmol) was converted to **8e** (387 mg, 84%) as a colorless oil. ν_{max} (film): 1605 (w), 1585 (w), 1495 (s), 1100 (br s, C–O), 1060 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$, characteristic data, α:β = ca. 2:1): 5.56 (0.7H, dd, *J* = 54, 3.2 Hz), 5.13 (0.3H, dd, *J* = 54, 6.8 Hz), 1.23 (1H, d, *J* = 6.4 Hz), 1.15 (2H, d, *J* = 6.4 Hz) ppm. This was immediately used in the next step without further purification

4.1.6.6. 2,3,4-Tri-*O*-benzyl-6-deoxy-6-fluoro-D-galactopyranosyl fluoride 8f. In the same manner as described above, **7f** (469 mg, 1.04 mmol) was converted to **8f** (420 mg, 89%) as a colorless oil, ν_{max} (film): 1610 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1050 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$, characteristic data, α:β = ca. 2:3): 5.59 (0.4H, dd, *J* = 54, 2.4 Hz), 5.23 (0.6H, dd, *J* = 52, 6.0 Hz) ppm. This was immediately used in the next step without further purification.

4.1.6.7. 2,3,4-Tri-*O*-benzyl-L-arabinopyranosyl fluoride 8g. In the same manner as described above, **7g** (565 mg, 1.04 mmol) was converted to **8g** (461 mg, 81%) as a colorless oil, ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$, characteristic data, α:β = ca. 1:1): 5.61 (0.5H, dd, *J* = 54, 2.5 Hz), 5.31 (0.5H, dd, *J* = 51, 2.5 Hz) ppm. This was immediately used in the next step without further purification.[45]

4.1.7. Galactosylation and desilylation

4.1.7.1. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-galactopyranosyl)octadecane-1,3,4-triol 11a. To a stirred mixture of **9** (633 mg, 0.685 mmol) and dried MS 4A (powder, 10 g) in dry THF (20 mL), tin(II) chloride (1.00 g, 5.27 mmol) and silver(I) perchlorate (1.10 g, 5.31 mmol) were added under argon at room temperature. After stirring in the dark at room temperature for 1 h, the mixture was cooled to −18 °C. To this mixture, a solution of **8a** (α:β = ca. 5:4, 814 mg, 1.74 mmol) in dry THF (10 mL) was added dropwise at −18 °C. The mixture was gradually warmed up to room temperature with stirring over 2 h, and then diluted with Et_2O (50 mL), and filtered through a bed of Celite. The filtrate was washed successively with a saturated aqueous $NaHCO_3$ solution, water and brine, dried with K_2CO_3, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 10:1) to give **10a** (625 mg) as a colorless oil. We could not isolate the β-anomer of **10a**. The obtained **10a** contained a small portion of unreacted **8a**, and was used in the next step without further purification. The analytical sample was obtained by careful column chromatography on silica gel (hexane/EtOAc = 10:1), n_D^{24} = 1.4952; $[\alpha]_D^{22}$ +21.9 (*c* 0.76, $CHCl_3$); ν_{max} (film): 3360 (m, NH), 1680 (br s, CO), 1610 (w), 1520 (m), 1500 (m), 1250 (s, *t*-Bu, Si–Me), 1105 (br s, C–O), 1060 (br s, C–O), 835 (s), 780 (s), 735 (br m), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.38–7.26 (15H, m), 6.26 (1H, d, *J* = 8.0 Hz), 4.96 (1H, d, *J* = 12 Hz), 4.800 (1H, d, *J* = 4.0 Hz), 4.798 (2H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.10–3.98 (3H, m), 3.93–3.85 (3H, m), 3.79 (1H, br d, *J* = 7.6 Hz), 3.69 (1H, dd, *J* = 11, 2.8 Hz), 3.64–3.60 (1H, m), 3.44 (1H, dd, *J* = 9.2, 6.0 Hz), 3.29 (1H, dd, *J* = 9.2, 5.2 Hz), 3.26 (3H, s), 2.05–2.00 (2H, m), 1.59–1.18 (72H, m), 0.90 (9H, s), 0.88 (9H, s), 0.88 (6H, t, *J* = 6.8 Hz), 0.07 (3H, s), 0.03 (3H, s), 0.024 (3H, s), 0.016 (3H, s) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{84}H_{147}NO_9Si_2Na$ $[M+Na]^+$ 1393.0512, found 1393.0514.

To a stirred solution of **10a** (625 mg, mixture) in THF (30 mL), a solution of tetrabutylammonium fluoride (TBAF, 1.0 M in THF, 4.6 mL, 4.6 mmol) was added at room temperature. After stirring at room temperature overnight, the mixture was poured into water, and extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (30 g,

hexane/EtOAc = 3:1) to give **11a** (379 mg, 48% in two steps) as colorless powder. Mp 88.0–89.5 °C; $[\alpha]_D^{25}$ +40.1 (*c* 0.70, $CHCl_3$); ν_{max} (KBr): 3420 (br s, OH), 3320 (br m, NH), 1645 (s, CO), 1620 (s), 1540 (br s), 1500 (w), 1100 (br s, C–O), 1060 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.40–7.28 (15H, m), 6.40 (1H, d, *J* = 8.8 Hz), 4.94 (1H, d, *J* = 12 Hz), 4.90 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 4.0 Hz), 4.77 (2H, s), 4.68 (1H, d, *J* = 12 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.23–4.17 (1H, m), 4.05 (1H, dd, *J* = 10, 3.6 Hz), 3.94 (1H, br s), 3.90–3.84 (2H, m), 3.82 (1H, br t, *J* = 6.8 Hz), 3.79–3.77 (1H, m), 3.52–3.44 (2H, m), 3.41 (1H, dd, *J* = 9.6, 6.4 Hz), 3.34 (1H, dd, *J* = 9.6, 6.4 Hz), 3.26 (3H, s), 2.18–2.12 (3H, m), 1.64–1.53 (4H, m), 1.51–1.18 (69H, m), 0.88 (6H, t, *J* = 6.8 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{72}H_{120}NO_9$ $[M+H]^+$ 1142.8963, found 1142.8961.

4.1.7.2. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-ethyl-α-D-galactopyranosyl)octadecane-1,3,4-triol

11b. In the same manner as described above, **9** (304 mg, 0.329 mmol) was galactosylated with freshly prepared **8b** (324 mg, 0.675 mmol) to give **10b** (292 mg) with a small amount of unreacted **8b**, which was deprotected to give **11b** (186 mg, 49% in two steps) as a colorless solid. Mp 72.0–73.5 °C; $[\alpha]_D^{26}$ +39.1 (*c* 1.31, $CHCl_3$); ν_{max} (KBr): 3320 (br m, NH, OH), 1640 (s, CO), 1620 (m), 1545 (br s), 1495 (w), 1115 (br s, C–O), 1050 (br s, C–O), 730 (br m), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.39–7.25 (15H, m), 6.41 (1H, d, *J* = 8.8 Hz), 4.93 (1H, d, *J* = 12 Hz), 4.88 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 3.2 Hz), 4.77 (2H, s), 4.68 (1H, d, *J* = 12 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.23–4.18 (1H, m), 4.05 (1H, dd, *J* = 9.6, 4.0 Hz), 3.97 (1H, br d, *J* = 2.0 Hz), 3.90–3.78 (4H, m), 3.53–3.33 (6H, m), 2.18–2.10 (3H, m), 1.64–1.53 (4H, m), 1.51–1.18 (69H, m), 1.14 (3H, t, *J* = 6.8 Hz), 0.88 (6H, t, *J* = 6.8 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{73}H_{122}NO_9$ $[M+H]^+$ 1156.9120, found 1156.9116.

4.1.7.3. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-propyl-α-D-galactopyranosyl)octadecane-1,3,4-triol

11c. In the same manner as described above, **9** (333 mg, 0.360 mmol) was galactosylated with freshly prepared **8c** (363 mg, 0.733 mmol) to give **10c** (340 mg) with a small amount of unreacted **8c**, which was deprotected to give **11c** (199 mg, 47% in two steps) as a colorless solid. Mp 79.0–80.5 °C; $[\alpha]_D^{20}$ +38.4 (*c* 1.06, $CHCl_3$); ν_{max} (KBr): 3320 (br m, NH, OH), 1640 (s, CO), 1620 (m), 1545 (br s), 1495 (w), 1115 (br s, C–O), 1045 (br s, C–O), 730 (br m), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.26 (15H, m), 6.42 (1H, d, *J* = 8.5 Hz), 4.93 (1H, d, *J* = 12 Hz), 4.87 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 3.5 Hz), 4.78 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.68 (1H, d, *J* = 12 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.24–4.19 (1H, m), 4.05 (1H, dd, *J* = 10, 3.5 Hz), 3.96 (1H, br d, *J* = 1.5 Hz), 3.90–3.87 (2H, m), 3.87 (1H, dd, *J* = 10, 3.0 Hz), 3.83 (1H, br t, *J* = 7.0 Hz), 3.80 (1H, d, *J* = 8.5 Hz), 3.53–3.46 (2H, m), 3.44 (2H, br d, *J* = 6.0 Hz), 3.36 (1H, dt, *J* = 9.5, 6.5 Hz), 3.27 (1H, dt, *J* = 9.5, 6.5 Hz), 2.20 (1H, br s), 2.17 (1H, dt, *J* = 15, 7.5 Hz), 2.13 (1H, dt, *J* = 15, 7.5 Hz), 1.69 (1H, br s), 1.64–1.56 (3H, m), 1.53 (2H, sext., *J* = 7.5), 1.50–1.42 (1H, m), 1.40–1.19 (68H, m), 0.89 (3H, t, *J* = 7.5 Hz), 0.88 (6H, t, *J* = 7.5 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{74}H_{123}NO_9Na$ $[M+Na]^+$ 1192.9096, found 1192.9064.

4.1.7.4. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-methyl-α-D-galactopyranosyl)octadecane-1,3,4-triol

11d. In the same manner as described above, **9** (434 mg, 0.469 mmol) was galactosylated with freshly prepared **8d** (703 mg, 0.156 mmol) to give **10d** (533 mg) with a small amount of unreacted **8d**, which was deprotected to give **11d** (294 mg, 56% in two steps) as a colorless solid. Mp 93.0–94.5 °C; $[\alpha]_D^{26}$ +48.9 (*c* 1.18, $CHCl_3$); ν_{max} (KBr): 3420 (br s, OH), 3300 (br m, NH), 1620 (br s, CO), 1540 (br s), 1495 (w), 1100 (br s, C–O), 1050 (br s, C–O), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.40–7.25 (15H, m), 6.30 (1H, d, *J* = 9.0 Hz), 4.97 (1H, d, *J* = 12 Hz), 4.87 (1H, d, *J* = 12 Hz), 4.86 (1H, d, *J* = 4.0 Hz), 4.81 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.27 (1H, ddd, *J* = 8.5, 3.5, 3.5 Hz), 4.06 (1H, dd, *J* = 10, 3.5 Hz), 3.89 (1H, dd, *J* = 10, 3.5 Hz), 3.84 (1H, dd, *J* = 10, 2.5 Hz), 3.78 (1H, dd, *J* = 10, 2.5 Hz), 3.74 (1H, br s), 3.67 (1H, d, *J* = 10 Hz), 3.53–3.47 (1H, m), 3.47–3.42 (2H, m), 2.22 (1H, br s), 2.17 (1H, dt, *J* = 14, 7.0 Hz), 2.14 (1H, dt, *J* = 14, 7.0 Hz), 1.75–1.60 (4H, m), 1.59 (2H, quint., *J* = 7.0 Hz), 1.52–1.42 (1H, m), 1.42–1.20 (66H, m), 0.88 (6H, t, *J* = 7.0 Hz), 0.79 (3H, t, *J* = 7.0 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{72}H_{120}NO_8$ $[M+H]^+$ 1126.9014, found 1126.9017.

4.1.7.5. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(2,3,4-tri-*O*-benzyl-α-D-fucopyranosyl)octadecane-1,3,4-triol 11e.

In the same manner as described above, **9** (412 mg, 0.446 mmol) was fucosylated with freshly prepared **8e** (387 mg, 0.887 mmol) to give **10e** (435 mg, 73%) with a small amount of unreacted **8e**, which was deprotected to give **11e** (315 mg, 87%) as a colorless waxy solid. Mp 81.5–83.5 °C; $[\alpha]_D^{28}$ +47.7 (*c* 1.16, $CHCl_3$); ν_{max} (KBr): 3360 (br m, OH), 3320 (m, NH), 1640 (br s, CO), 1540 (s), 1500 (w), 1105 (br s, C–O), 1050 (br s, C–O), 730 (br s), 695 (s) cm^{-1}; δ_H (400 MHz,$CDCl_3$): 7.38–7.24 (15H, m), 6.27 (1H, d, *J* = 8.4 Hz), 4.94 (1H, d, *J* = 12 Hz), 4.86 (1H, d, *J* = 12 Hz), 4.82 (1H, d, *J* = 3.2 Hz), 4.78 (1H, d, *J* = 12 Hz),4.74 (1H, d, *J* = 12 Hz), 4.67 (1H, d, *J* = 12 Hz), 4.63 (1H, d, *J* = 12 Hz),4.26–4.21 (1H, m), 4.03 (1H, dd, *J* = 9.6, 3.2 Hz), 3.88 (1H, dd, *J* = 10, 3.2 Hz), 3.83 (1H, dd, *J* = 10, 3.2 Hz), 3.80–3.73 (2H, m), 3.64 (1H, d, *J* = 7.2 Hz), 3.63 (1H, s), 3.51–3.39 (2H, m), 2.18 (1H, d, *J* = 5.6 Hz), 2.13 (2H, t, *J* = 7.2 Hz), 1.67 (1H, s), 1.61–1.53 (2H, m), 1.48–1.18 (70H, m), 1.10 (3H, d, *J* = 6.4 Hz), 0.86 (3H, t, *J* = 6.8 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{71}H_{118}NO_8$ $[M+H]^+$: 1112.8857; found, 1112.8857.

4.1.7.6. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-galactopyranosyl)octadecane-1,3,4-triol

11f. In the same manner as described above, **9** (310 mg, 0.335 mmol) was galactosylated with freshly prepared **8f** (381 mg, 0.838 mmol) to give **10f** (363 mg) with a small amount of unreacted **8f**, which was deprotected to give **11f** (104 mg, 27% in two steps) as a colorless solid. Mp 76.0–78.0 °C; $[\alpha]_D^{25}$ +44.2 (*c* 1.01, $CHCl_3$); ν_{max} (KBr): 3400 (w, OH), 3320 (br s, OH, NH), 1635 (br s, CO), 1545 (br s), 1495 (w), 1100 (br s, C–O), 1045 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.40–7.27 (15H, m), 6.26 (1H, d, *J* = 8.5 Hz), 4.95 (1H, d, *J* = 12 Hz), 4.89 (1H, d, *J* = 12 Hz), 4.88 (1H, d, *J* = 4.0 Hz), 4.81 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.60 (1H, d, *J* = 12 Hz), 4.42 (1H, ddd, *J* = 48, 8.5, 6.5 Hz), 4.33 (1H, ddd, *J* = 47, 9.5, 6.0 Hz), 4.26–4.22 (1H, m), 4.06 (1H, dd, *J* = 10, 4.0 Hz), 3.94–3.88 (3H, m), 3.88 (1H, dd, *J* = 10, 2.5 Hz), 3.84 (1H, dd, *J* = 11, 3.5 Hz), 3.52–3.48 (1H, m), 3.48–3.44 (1H, m), 2.15 (2H, t, *J* = 7.5 Hz), 1.70–1.53 (7H, m), 1.50–1.42 (1H, m), 1.40–1.10 (66H, m), 0.88 (6H, t, *J* = 7.0 Hz) ppm; HRMS (FAB+) *m*/*z* calcd for $C_{71}H_{117}NO_8F$ $[M+H]^+$ 1130.8763, found 1130.8765.

4.1.7.7. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(2,3,4-tri-*O*-benzyl-β-L-arabinopyranosyl)octadecane-1,3,4-triol 11g.

In the same manner as described above, **9** (518 mg, 0.560 mmol) was glycosylated with freshly prepared **8g** (470 mg, 1.11 mmol) to give **10g** (499 mg) with a small amount of unreacted **8g**, which was deprotected to give **11g** (318 mg, 52% in two steps) as a colorless solid. Mp 79.0–81.0 °C; $[\alpha]_D^{23}$ +61.1 (*c* 1.01, $CHCl_3$); ν_{max} (KBr): 3400 (s, OH), 3320 (m, NH), 1645 (br s, CO), 1540 (br s), 1495 (w), 1140 (m), 1060 (br s, C–O), 730 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.27 (15H, m), 6.27 (1H, d, *J* = 8.5 Hz), 4.92 (1H, d, *J* = 12 Hz), 4.88 (1H, d, *J* = 4.0 Hz, 1′-H), 4.72 (1H, d,

J = 12 Hz), 4.68 (1H, d, J = 12 Hz), 4.67 (1H, d, J = 12 Hz), 4.65 (2H, s), 4.24–4.20 (1H, m, 2-H), 4.02 (1H, dd, J = 9.5, 4.0 Hz, 2′-H), 3.92 (1H, dd, J = 10, 3.0 Hz, 1-H_a), 3.83–3.79 (2H, m, 1-H_b, 3′-H), 3.78 (1H, br s), 3.71 (1H, dd, J = 12, 2.5 Hz, 5′-H_a), 3.61 (1H, d, J = 9.5 Hz, OH), 3.53 (1H, br d, J = 12 Hz, 5-H_b), 3.50–3.40 (2H, m, 3-, 4-H), 2.13 (2H, t, J = 7.5 Hz, 2″-H_2), 2.12 (1H, d, J = 5.5 Hz), 1.62–1.52 (4H, m), 1.47–1.18 (70H, m), 0.88 (6H, t, J = 7.0 Hz) ppm. This spectrum indicates the conformation of **11g** as $^4C_1$; HRMS (FAB+) m/z calcd for $C_{70}H_{115}NO_8Na$ $[M+Na]^+$ 1120.8520, found 1120.8531.

4.1.8. 6′-*O*-Alkylation

4.1.8.1. (2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(methoxymethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 20a. The alcohol **19** was prepared according to Calenbergh.[19] To a stirred solution of **19** (279 mg, 0.292 mmol) in CH_2Cl_2 (5 mL), $(i\text{-Pr})_2NEt$ (254 μL, 1.46 mmol) and chloromethyl methyl ether (66 μL, 0.877 mmol) were added at 0 °C. After stirring at room temperature for 15 h, the mixture was poured into water, and extracted with EtOAc. The organic phase was washed successively with water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 9:1) to give **20a** (241 mg, 83%) as a colorless oil, n_D^{23} 1.5170; $[\alpha]_D^{24}$ +33.5 (c 1.01, $CHCl_3$); ν_{max} (film): 2100 (s, N_3), 1610 (w), 1585 (w), 1495 (m), 1105 (br s, C–O), 1045 (br s, C–O), 735 (br s), 700 (br s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.18 (25H, m), 4.98 (1H, d, J = 12 Hz), 4.90 (1H, d, J = 4.0 Hz), 4.85 (1H, d, J = 12 Hz), 4.80 (1H, d, J = 12 Hz), 4.75 (1H, d, J = 12 Hz), 4.68 (1H, d, J = 12 Hz), 4.67 (1H, d, J = 12 Hz), 4.60 (2H, d, J = 12 Hz), 4.59 (1H, d, J = 12 Hz), 4.50 (1H, d, J = 6.5 Hz), 4.49 (1H, d, J = 12 Hz), 4.46 (1H, d, J = 6.5 Hz), 4.07 (1H, dd, J = 10, 3.5 Hz), 4.01 (1H, dd, J = 10, 2.5 Hz), 4.00 (1H, dd, J = 10, 3.0 Hz), 3.930 (1H, br t, J = 6.5 Hz), 3.925 (1H, br s), 3.76–3.69 (3H, m), 3.62 (1H, dt, J = 8.0, 3.5 Hz), 3.58 (1H, dd, J = 10, 6.5 Hz), 3.51 (1H, dd, J = 10, 6.5 Hz), 3.26 (3H, s), 1.70–1.63 (1H, m), 1.56–1.49 (2H, m), 1.45–1.37 (1H, m), 1.33–1.21 (22H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{61}H_{81}N_3O_9Na$ $[M+Na]^+$ 1022.5871, found 1022.5875.

4.1.8.2. (2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2-methoxyethoxymethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 20b. In the same manner as described above, **19** (264 mg, 0.276 mmol) was converted to **20b** (225 mg, 78%) as a colorless oil, n_D^{25} 1.5172; $[\alpha]_D^{23}$ +32.6 (c 1.00, $CHCl_3$); ν_{max} (film): 2100 (s, N_3), 1610 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1050 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.20 (25H, m), 4.97 (1H, d, J = 12 Hz), 4.90 (1H, d, J = 3.5 Hz), 4.84 (1H, d, J = 12 Hz), 4.79 (1H, d, J = 12 Hz), 4.75 (1H, d, J = 12 Hz), 4.68 (1H, d, J = 12 Hz), 4.67 (1H, d, J = 12 Hz), 4.600 (1H, d, J = 12 Hz), 4.596 (1H, d, J = 7.0 Hz), 4.596 (1H, d, J = 12 Hz), 4.59 (1H, d, J = 12 Hz), 4.56 (1H, d, J = 7.0 Hz), 4.79 (1H, d, J = 12 Hz), 4.07 (1H, dd, J = 10, 3.5 Hz), 4.003 (1H, dd, J = 11, 1.5 Hz), 3.999 (1H, dd, J = 11, 2.5 Hz), 3.93 (1H, br t, J = 6.0 Hz), 3.91 (1H, br s), 3.75 (1H, dd, J = 5.0, 1.5 Hz), 3.72 (1H, dd, J = 10, 3.5 Hz), 3.71–3.68 (1H, m), 3.64–3.60 (2H, m), 3.60–3.56 (2H, m), 3.53 (1H, dd, J = 10, 6.0 Hz), 3.47–3.44 (2H, m), 3.34 (3H, s), 1.70–1.63 (1H, m), 1.56–1.48 (2H, m), 1.45–1.36 (1H, m), 1.32–1.21 (22H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{63}H_{85}N_3O_{10}Na$ $[M+Na]^+$ 1066.6133, found 1066.6118.

4.1.8.3. (2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2-benzyloxyethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 20c. In the similar manner as described above for the conversion of **2** to **3b**, **19** (243 mg, 0.254 mmol) was converted to **20c** (170 mg, 61%) as a colorless oil, n_D^{24} 1.5174; $[\alpha]_D^{24}$ +28.6 (c 1.03, $CHCl_3$); ν_{max} (film): 2090 (s, N_3), 1610 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.18 (30H, m), 4.93 (1H, d, J = 12 Hz), 4.89 (1H, d, J = 3.5 Hz), 4.81 (1H, d, J = 12 Hz), 4.79 (1H, d, J = 12 Hz), 4.72 (1H, d, J = 12 Hz), 4.67 (1H, d, J = 12 Hz), 4.65 (1H, d, J = 12 Hz), 4.604 (1H, d, J = 12 Hz), 4.596 (1H, d, J = 12 Hz), 4.58 (1H, d, J = 12 Hz), 4.50 (2H, s), 4.48 (1H, d, J = 12 Hz), 4.05 (1H, dd, J = 10, 3.5 Hz), 3.99 (1H, dd, J = 10, 2.5 Hz), 3.98 (1H, dd, J = 10, 3.0 Hz), 3.95 (1H, br d, J = 2.0 Hz), 3.94 (1H, br t, J = 6.5 Hz), 3.74 (1H, dd, J = 6.5, 4.5 Hz), 3.71–3.64 (2H, m), 3.61 (1H, dt, J = 8.0, 3.5 Hz), 3.59–3.55 (1H, m), 3.53–3.45 (5H, m), 1.70–1.62 (1H, m), 1.56–1.49 (2H, m), 1.44–1.36 (1H, m), 1.33–1.20 (22H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{68}H_{87}N_3O_9Na$ $[M+Na]^+$ 1112.6340, found 1112.6337.

4.1.9. Reduction of azide

4.1.9.1. (2*S*,3*S*,4*R*)-2-Amino-3,4-di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(methoxymethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 21a. To a stirred solution of **20a** (208 mg, 0.208 mmol) in dry THF (10 mL), a solution of PMe_3 (1.0 M in THF, 2.1 mL, 2.1 mmol) was added at 0 °C. The mixture was stirred at room temperature for 16 h. The mixture was then added an aqueous solution of NaOH (1.0 M, 4.2 mL, 4.2 mmol) at room temperature. After stirring at room temperature for 6 h, the mixture was poured into water, and extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with K_2CO_3, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (NH silica, 30 g, hexane/EtOAc = 3:1) to give **21a** (173 mg, 85%) as a colorless oil, n_D^{24} 1.5172; $[\alpha]_D^{23}$ +37.7 (c 1.00, $CHCl_3$); ν_{max} (film): 3380 (w, NH), 1605 (w), 1585 (w), 1500 (m), 1100 (br s, C–O), 1045 (br s, C–O), 740 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.40–7.21 (25H, m), 4.97 (1H, d, J = 12 Hz), 4.89 (1H, d, J = 3.5 Hz), 4.83 (1H, d, J = 12 Hz), 4.79 (1H, d, J = 12 Hz), 4.76 (1H, d, J = 12 Hz), 4.72 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.63 (1H, d, J = 12 Hz), 4.60 (1H, d, J = 12 Hz), 4.54 (1H, d, J = 12 Hz), 4.51 (1H, d, J = 12 Hz), 4.49 (1H, d, J = 6.5 Hz), 4.45 (1H, d, J = 6.5 Hz), 4.06 (1H, dd, J = 9.5, 3.5 Hz), 4.00 (1H, dd, J = 10, 2.5 Hz), 3.95 (1H, dd, J = 10, 2.5 Hz), 3.94 (1H, br s), 3.91 (1H, br t, J = 6.5 Hz), 3.70 (1H, dt, J = 8.0, 3.5 Hz), 3.58 (1H, dd, J = 9.5, 6.5 Hz), 3.57–3.52 (1H, m), 3.52 (1H, dd, J = 9.5, 6.5 Hz), 3.39 (1H, br t, J = 9.0 Hz), 3.26 (3H, s), 3.21–3.16 (1H, m), 1.73–1.64 (1H, m), 1.64–1.52 (2H, m), 1.52–1.42 (1H, m), 1.35–1.22 (22H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{61}H_{84}NO_9$ $[M+H]^+$ 974.6146, found 974.6157.

4.1.9.2. (2*S*,3*S*,4*R*)-2-Amino-3,4-di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2-methoxyethoxymethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 21b. In the same manner as described above, **20b** (161 mg, 0.154 mmol) was reduced to **21b** (134 mg, 85%) as a colorless oil, n_D^{24} 1.5171; $[\alpha]_D^{24}$ +38.6 (c 1.04, $CHCl_3$); ν_{max} (film): 3380 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1045 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.20 (25H, m), 4.97 (1H, d, J = 12 Hz), 4.89 (1H, d, J = 3.5 Hz), 4.83 (1H, d, J = 12 Hz), 4.78 (1H, d, J = 12 Hz), 4.76 (1H, d, J = 12 Hz), 4.71 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.63 (1H, d, J = 12 Hz), 4.60 (1H, d, J = 12 Hz), 4.59 (1H, d, J = 12 Hz), 4.55 (1H, d, J = 6.5 Hz), 4.54 (1H, d, J = 12 Hz), 4.51 (1H, d, J = 12 Hz), 4.06 (1H, dd, J = 9.5, 3.5 Hz), 3.99 (1H, dd, J = 10, 3.0 Hz), 3.96–3.93 (2H, m), 3.91 (1H, br t, J = 6.5 Hz), 3.70 (1H, dt, J = 8.0, 3.5 Hz), 3.60 (1H, dd, J = 10, 6.5 Hz), 3.61–3.52 (4H, m), 3.46–3.43 (2H, m), 3.39 (1H, br t, J = 9.5 Hz), 3.34 (3H, s), 3.20–3.16 (1H, m), 1.73–1.64 (1H, m), 1.64–1.52 (4H, m), 1.52–1.43 (1H, m), 1.34–1.20 (22H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{63}H_{87}NO_{10}Na$ $[M+Na]^+$ 1040.6228, found 1040.6223.

4.1.9.3. (2*S*,3*S*,4*R*)-2-Amino-3,4-di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2-benzyloxyethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 21c. In the same manner as described above, **20c** (140 mg, 0.128 mmol) was reduced to **21c** (106 mg, 78%) as a colorless oil, n_D^{25} 1.5170; $[\alpha]_D^{25}$ +32.7 (*c* 1.05, $CHCl_3$); ν_{max} (film): 3380 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.20 (30H, m), 4.92 (1H, d, *J* = 12 Hz), 4.88 (1H, d, *J* = 4.0 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.78 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.65 (1H, d, *J* = 12 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.54 (1H, d, *J* = 12 Hz), 4.500 (3H, s), 4.501 (1H, d, *J* = 12 Hz), 4.04 (1H, dd, *J* = 10, 3.0 Hz), 3.99–3.93 (3H, m), 3.93 (1H, dd, *J* = 7.0, 3.0 Hz), 3.70 (1H, dt, *J* = 8.0, 3.5 Hz), 3.60–3.50 (5H, m), 3.49–3.44 (2H, m), 3.38 (1H, br t, *J* = 8.5 Hz), 3.19–3.14 (1H, m), 1.72–1.64 (1H, m), 1.64–1.52 (4H, m), 1.51–1.43 (1H, m), 1.34–1.21 (22H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{68}H_{89}NO_9Na$ $[M+Na]^+$ 1086.6435, found 1086.6432.

4.1.10. Acylation

4.1.10.1. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-2-hexacosanamido-1-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-methoxymethyl-α-D-galactopyranosyl)octadecane-1,3,4-triol 22a. To a stirred solution of **21a** (140 mg, 0.144 mmol) in dry CH_2Cl_2 (8 mL), Et_3N (100 μL, 0.721 mmol) and a solution of freshly prepared hexacosanoyl chloride (Ref. 38, 65 mg, 0.164 mmol) in dry dichloromethane (2 mL) were added at 0 °C. After stirring at room temperature for 2 h, the mixture was poured into water, and extracted with ethyl acetate. The organic phase was washed successively with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (30 g, hexane/ethyl acetate = 9:1) to give **22a** (170 mg, 87%) as a colorless solid. Mp 72.0–73.0 °C; $[\alpha]_D^{23}$ +19.5 (*c* 1.04, $CHCl_3$); ν_{max} (KBr): 3320 (s, NH), 1650 (br s, CO), 1605 (w), 1590 (w), 1535 (s), 1500 (m), 1120 (br s, C–O), 1050 (br s, C–O), 730 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.22 (25H, m), 6.08 (1H, d, *J* = 9.0 Hz), 4.97 (1H, d, *J* = 12 Hz), 4.86 (1H, d, *J* = 4.0 Hz), 4.82 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.65 (1H, d, *J* = 12 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.59 (1H, d, *J* = 12 Hz), 4.53 (1H, d, *J* = 6.0 Hz), 4.52 (1H, d, *J* = 12 Hz), 4.48 (1H, d, *J* = 6.0 Hz), 4.44 (1H, d, *J* = 12 Hz), 4.20–4.15 (1H, m), 4.06 (1H, dd, *J* = 10, 3.5 Hz), 3.98 (1H, dd, *J* = 11, 6.0 Hz), 3.92–3.88 (3H, m), 3.84 (1H, dd, *J* = 7.0, 2.5 Hz), 3.76 (1H, dd, *J* = 11, 4.0 Hz), 3.60 (1H, dd, *J* = 10, 7.0 Hz), 3.51–3.48 (1H, m), 3.49 (1H, dd, *J* = 10, 6.0 Hz), 1.97 (1H, dt, *J* = 15, 7.5 Hz), 1.91 (1H, dt, *J* = 15, 7.5 Hz), 1.70–1.62 (1H, m), 1.62–1.53 (2H, m), 1.50 (2H, quint., *J* = 7.5 Hz), 1.49–1.40 (1H, m), 1.35–1.17 (66H, m), 0.88 (6H, t, *J* = 7.0 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{87}H_{133}NO_{10}Na$ $[M+Na]^+$ 1374.9827, found 1374.9799.

4.1.10.2. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-2-hexacosanamido-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2-methoxyethoxymethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 22b. In the same manner as described above, **21b** (140 mg, 0.137 mmol) was acylated with hexacosanoyl chloride to give **22b** (150 mg, 78%) as a colorless solid. Mp 68.0–69.0 °C; $[\alpha]_D^{26}$ +19.8 (*c* 1.03, $CHCl_3$); ν_{max} (KBr): 3320 (s, NH), 1650 (s, CO), 1610 (w), 1585 (w), 1540 (s), 1500 (m), 1105 (br s, C–O), 1060 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.21 (25H, m), 6.08 (1H, d, *J* = 8.5 Hz), 4.97 (1H, d, *J* = 12 Hz), 4.85 (1H, d, *J* = 4.0 Hz), 4.82 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.65 (1H, d, *J* = 12 Hz), 4.63 (1H, d, *J* = 7.0 Hz), 4.60 (1H, d, *J* = 12 Hz), 4.58 (1H, d, *J* = 7.0 Hz), 4.59 (1H, d, *J* = 12 Hz), 4.52 (1H, d, *J* = 12 Hz), 4.44 (1H, d, *J* = 12 Hz), 4.20–4.15 (1H, m), 4.06 (1H, dd, *J* = 10, 4.0 Hz), 3.97 (1H, dd, *J* = 11, 5.5 Hz), 3.92–3.88 (3H, m), 3.83 (1H, dd, *J* = 7.0, 2.5 Hz), 3.76 (1H, dd, *J* = 11, 4.0 Hz), 3.63 (1H, dd, *J* = 10, 7.0 Hz), 3.57–3.55 (2H, m), 3.52 (1H, dd, *J* = 10, 5.5 Hz), 3.49 (1H, br dt, 8.0, 2.5 Hz), 3.45–3.43 (2H, m), 1.97 (1H, dt, *J* = 15, 7.5 Hz), 1.91 (1H, dt, *J* = 15, 7.5 Hz), 1.70–1.55 (3H, m), 1.50 (2H, quint., *J* = 7.5 Hz), 1.48–1.42 (1H, m), 1.34–1.18 (66H, m), 0.88 (6H, t, *J* = 7.0 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{89}H_{137}NO_{11}Na$ $[M+Na]^+$ 1419.0089, found 1419.0062.

4.1.10.3. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-2-hexacosanamido-1-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2-benzyloxyethyl)-α-D-galactopyranosyl]octadecane-1,3,4-triol 22c. In the same manner as described above, **21c** (106 mg, 0.0996 mmol) was acylated with hexacosanoyl chloride to give **22c** (116 mg, 81%) as a colorless solid. Mp 55.0–56.5 °C; $[\alpha]_D^{28}$ +22.2 (*c* 1.03, $CHCl_3$); ν_{max} (KBr): 3320 (s, NH), 1645 (s, CO), 1540 (br m), 1495 (m), 1140 (br s, C–O), 1050 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.21 (30H, m), 6.12 (1H, d, *J* = 9.0 Hz), 4.92 (1H, d, *J* = 12 Hz), 4.85 (1H, d, *J* = 4.0 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.58 (1H, d, *J* = 12 Hz), 4.50 (1H, d, *J* = 12 Hz), 4.48 (2H, s), 4.43 (1H, d, *J* = 12 Hz), 4.18–4.13 (1H, m), 4.04 (1H, dd, *J* = 10, 3.5 Hz), 3.97–3.93 (2H, m), 3.92–3.89 (1H, m), 3.89 (1H, dd, *J* = 7.0, 3.0 Hz), 3.86 (1H, dd, *J* = 7.0, 2.5 Hz), 3.74 (1H, dd, *J* = 11, 4.0 Hz), 3.61–3.57 (1H, m), 3.53–3.47 (6H, m), 1.98 (1H, dt, *J* = 15, 7.5 Hz), 1.91 (1H, dt, *J* = 15, 7.5 Hz), 1.70–1.63 (1H, m), 1.63–1.54 (2H, m), 1.49 (2H, quint., *J* = 7.5 Hz), 1.48–1.41 (1H, m), 1.32–1.17 (66H, m), 0.88 (6H, t, *J* = 7.0 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{94}H_{139}NO_{10}Na$ $[M+Na]^+$ 1465.0297, found 1465.0288.

4.1.11. Hydrogenolysis

4.1.11.1. (2*S*,3*S*,4*R*)-1-*O*-(6-*O*-Methyl-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol 1 (RCAI-61). To a solution of **11a** (1.83 g, 1.60 mmol) in THF (180 mL), $Pd(OH)_2$-C (20%, wet, 182 mg) was added. After stirring under hydrogen atmosphere (balloon) at room temperature for 24 h, the mixture was diluted with $CHCl_3$/MeOH (5:1, 180 mL), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (40 g, $CHCl_3$/MeOH = 50:3) to give **1** (RCAI-61, 1.27 g, 91%) as white powder. An analytical sample was obtained by recrystallization from EtOH. Mp 114.0–116.0 °C; $[\alpha]_D^{28}$ +46.7 (*c* 0.31, pyridine); ν_{max} (KBr): 3440 (br s, OH), 3280 (w, NH), 1640 (br s, CO), 1540 (br m), 1080 (br s, C–O), 720 (w) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.36 (1H, d, *J* = 8.5 Hz), 6.60–5.60 (5H, m), 5.51 (1H, d, *J* = 4.0 Hz), 5.26–5.21 (1H, m), 4.63 (1H, dd, *J* = 11, 5.5 Hz), 4.59 (1H, dd, *J* = 9.0, 3.5 Hz), 4.45 (1H, br t, *J* = 6.5 Hz), 4.38–4.34 (3H, m), 4.32–4.27 (2H, m), 3.97 (1H, dd, *J* = 10, 5.5 Hz), 3.93 (1H, dd, *J* = 10, 6.5 Hz), 3.33 (3H, s), 2.43 (1H, dt, *J* = 15, 7.5 Hz), 2.40 (1H, dt, *J* = 15, 7.5 Hz), 2.30–2.23 (1H, m), 1.95–1.83 (2H, m), 1.80 (2H, br quint., *J* = 7.5 Hz), 1.71–1.62 (1H, m), 1.47–1.18 (66H, m), 0.85 (6H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.1, 101.4, 76.4, 73.0, 72.4, 71.3, 70.7, 70.6, 70.1, 68.7, 58.8, 51.3, 36.7, 34.1, 32.1, 32.0, 30.3, 30.1, 30.0, 29.93, 29.90, 29.86, 29.84, 29.80, 29.75, 29.7, 29.6, 29.5, 26.4, 26.3, 22.9, 14.2 ppm; HRMS (FAB+) *m*/*z* calcd for $C_{51}H_{102}NO_9$ $[M+H]^+$ 872.7555, found 872.7553.

4.1.11.2. (2*S*,3*S*,4*R*)-1-*O*-(6-*O*-Ethyl-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol 12 (RCAI-85). In the same manner as described above, **11b** (155 mg, 0.134 mmol) was converted to **12** (RCAI-85, 88 mg, 74%) as colorless powder. Mp 104.0–106.5 °C; $[\alpha]_D^{25}$ +46.8 (*c* 0.24, pyridine); ν_{max} (KBr): 3400 (br s, OH), 3280 (br m, NH), 1640 (br s, CO), 1545 (m), 1080 (br m, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.43 (1H, d, *J* = 9.0 Hz), 7.02 (1H, br s), 6.69 (1H, br s), 6.43 (1H, br s), 6.38 (1H, br s), 6.07 (1H, br s), 5.51 (1H, d, *J* = 4.0 Hz), 5.26–5.20 (1H, m), 4.63 (1H, dd, *J* = 10, 4.5 Hz), 4.60 (1H, dd, *J* = 9.0, 3.5 Hz), 4.45

(1H, t, J = 6.0 Hz), 4.40–4.33 (3H, m), 4.33–4.27 (2H, m), 4.04 (1H, dd, J = 10, 6.0 Hz), 3.96 (1H, dd, J = 10, 6.5 Hz), 3.54–3.47 (2H, m), 2.43 (1H, dt, J = 14, 7.0 Hz), 2.40 (1H, dt, J = 14, 7.0 Hz), 2.30–2.23 (1H, m), 1.95–1.84 (2H, m), 1.80 (2H, quint., J = 7.0 Hz), 1.71–1.62 (1H, m), 1.48–1.14 (66H, m), 1.14 (3H, dt, J = 1.0, 7.0 Hz), 0.84 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.1, 101.5, 76.6, 72.4, 71.4, 70.84, 70.79, 70.7, 70.1, 68.8, 66.7, 51.3, 36.8, 34.2, 32.2, 32.11, 30.3, 30.1, 30.03, 30.02, 30.00, 29.98, 29.97, 29.93, 29.91, 29.87, 29.81, 29.75, 29.62, 29.60, 26.5, 26.4, 22.9, 15.5, 14.3 ppm; HRMS (FAB+) m/z calcd for $C_{52}H_{104}NO_9$ $[M+H]^+$ 886.7711, found 886.7714.

4.1.11.3. (2*S*,3*S*,4*R*)-2-(Hexacosanamido)-1-*O*-(6-*O*-propyl-α-D-galactopyranosyl)octadecane-1,3,4-triol 13 (RCAI-86). In the same manner as described above, **11c** (169 mg, 0.144 mmol) was converted to **13** (RCAI-86, 80 mg, 61%) as colorless powder. Mp 104.0–106.5 °C; $[\alpha]_D^{23}$ +43.0 (c 0.30, pyridine); ν_{max} (KBr): 3420 (br s, OH), 3280 (br m, NH), 1645 (br s, CO), 1545 (br m), 1080 (br s, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.44 (1H, d, J = 8.5 Hz), 7.04 (1H, br s), 6.70 (1H, br s), 6.42 (1H, d, J = 6.0 Hz), 6.35 (1H, br s), 6.07 (1H, br s), 5.52 (1H, d, J = 4.0 Hz), 5.27–5.22 (1H, m), 4.65 (1H, dd, J = 11, 5.5 Hz), 4.61 (1H, br d, J = 9.5 Hz), 4.46 (1H, t, J = 6.5 Hz), 4.41–4.34 (2H, m), 4.36 (1H, dd, J = 11, 5.0 Hz), 4.34–4.28 (2H, m), 4.06 (1H, dd, J = 9.5, 6.0 Hz), 3.97 (1H, dd, J = 9.5, 6.5 Hz), 3.47–3.39 (2H, m), 2.44 (2H, dt, J = 15, 7.0 Hz), 2.41 (1H, dt, J = 15, 7.0 Hz), 2.31–2.24 (1H, m), 1.95–1.85 (2H, m), 1.81 (2H, quint., J = 7.5 Hz), 1.72–1.63 (1H, m), 1.56 (2H, sext., J = 7.0 Hz), 1.47–1.17 (66H, m), 0.87 (3H, br t, J = 7.0 Hz), 0.85 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.0, 101.5, 76.6, 73.0, 72.4, 71.4, 70.9, 70.8, 70.7, 70.1, 68.7, 51.2, 36.7, 34.3, 32.1, 30.3, 30.1, 30.02, 30.01, 29.99, 29.97, 29.92, 29.90, 29.86, 29.8, 29.7, 29.61, 29.59, 26.5, 26.4, 23.4, 22.9, 14.3, 10.8 ppm; HRMS (FAB+) m/z calcd for $C_{53}H_{106}NO_9$ $[M+H]^+$ 900.7868, found 900.7866.

4.1.11.4. (2*S*,3*S*,4*R*)-1-*O*-(6-Deoxy-6-methyl-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol 14 (RCAI-64). In the same manner as described above, **11d** (247 mg, 0.288 mmol) was converted to **14** (RCAI-64, 151 mg, 61%) as colorless powder. Mp 130.0–133.5 °C; $[\alpha]_D^{26}$ +55.7 (c 0.36, pyridine); ν_{max} (KBr): 3440 (br m, OH), 3270 (br m, NH), 1645 (br s, CO), 1540 (m), 1090 (br m, C–O), 1035 (br m, C–O) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 8.49 (1H, d, J = 8.8 Hz), 6.98 (1H, br s), 6.59 (1H, br s), 6.44 (1H, br d, J = 7.2 Hz), 6.13 (2H, br s), 5.48 (1H, d, J = 3.6 Hz), 5.33–5.26 (1H, m), 4.64 (1H, dd, J = 10, 5.6 Hz), 4.60–4.54 (1H, m), 4.38–4.27 (3H, m), 4.27 (1H, dd, J = 10, 4.8 Hz), 4.17 (1H, br s), 3.99 (1H, t, J = 6.8 Hz), 2.44 (2H, t, J = 6.8 Hz), 2.35–2.25 (1H, m), 2.15–2.03 (1H, m), 1.98–1.77 (5H, m), 1.74–1.61 (1H, m), 1.47–1.16 (66H, m), 1.05 (3H, t, J = 7.2 Hz), 0.84 (6H, t, J = 7.2 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 173.0, 101.2, 76.8, 72.9, 72.5, 71.7, 70.2, 68.1, 51.1, 36.8, 34.5, 32.1, 30.4, 30.1, 30.02, 29.99, 29.93, 29.86, 29.8, 29.7, 29.6, 26.5, 26.4, 24.4, 22.9, 14.3, 10.8 ppm; HRMS (FAB+) m/z calcd for $C_{51}H_{102}NO_9$ $[M+H]^+$ 856.7605, found 856.7606.

4.1.11.5. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Fucopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol 15 (RCAI-58). In the same manner as described above, **11e** (52 mg, 0.47 mmol) was converted to **15** (RCAI-58, 34 mg, 85%) as colorless powder. Mp 127.0–129.0 °C; $[\alpha]_D^{27}$ +47.4 (c 0.31, pyridine); ν_{max} (KBr): 3360 (br s, OH), 3300 (m, NH), 1645 (br s, CO), 1545 (m), 1085 (br m, C–O), 1050 (br m, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.41 (1H, d, J = 8.5 Hz), 6.38 (1H, br s), 5.44 (1H, d, J = 3.5 Hz), 5.27–5.22 (1H, m), 4.97 (4H, br s), 4.61 (1H, dd, J = 10, 5.0 Hz), 4.53 (1H, dd, J = 10, 4.0 Hz), 4.36 (1H, dd, J = 10, 3.5 Hz), 4.32–4.26 (3H, m), 4.28 (1H, dd, J = 10, 5.0 Hz), 4.06 (1H, br d, J = 2.0 Hz), 2.42 (2H, br t, J = 7.5 Hz), 2.31–2.23 (1H, m), 1.94–1.84 (2H, m), 1.80 (2H, quint., J = 7.5 Hz), 1.72–1.62 (1H, m), 1.48 (3H, d, J = 6.0 Hz), 1.46–1.15 (66H, m), 0.84 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.1, 101.4, 76.7, 73.2, 72.6, 71.6, 69.9, 68.5, 67.5, 51.3, 36.8, 32.11, 32.10, 30.4, 30.1, 30.02, 30.01, 29.98, 29.97, 29.96, 29.91, 29.90, 29.86, 29.8, 29.7, 29.60, 29.59, 26.5, 26.4, 22.9, 17.2, 14.3 ppm; HRMS (FAB+) m/z calcd for $C_{50}H_{100}NO_8$ $[M+H]^+$ 842.7449, found 842.7448.

4.1.11.6. (2*S*,3*S*,4*R*)-1-*O*-(6-Deoxy-6-fluoro-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol 16 (RCAI-83). In the same manner as described above, **11f** (81 mg, 0.072 mmol) was converted to **16** (RCAI-83, 49 mg, 79%) as colorless powder. Mp 123.5–127.0 °C; $[\alpha]_D^{22}$ +50.7 (c 0.24, pyridine); ν_{max} (KBr): 3440 (br s, OH), 3310 (br s, NH), 1635 (br s, CO), 1545 (br m), 1150 (br m), 1075 (br s, C–O), 1030 (br s, C–O) 795 (m), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.45 (1H, d, J = 9.0 Hz), 7.15 (1H, br s), 6.91 (1H, br s), 6.65 (1H, br s), 6.47 (1H, br s), 6.11 (1H, br s), 5.55 (1H, d, J = 4.0 Hz), 5.31–5.26 (1H, m), 5.00 (1H, ddd, J = 49, 9.5, 7.5 Hz), 4.95 (1H, ddd, J = 47, 9.5, 4.5 Hz), 4.67 (1H, dd, J = 11, 5.5 Hz), 4.60 (1H, dd, J = 10, 4.0 Hz), 4.50 (1H, br dt, J = 14, 6.5 Hz), 4.39 (1H, dd, J = 10, 3.5 Hz), 4.36–4.29 (4H, m), 2.43 (1H, dt, J = 15, 7.5 Hz), 2.41 (1H, dt, J = 15, 7.5 Hz), 2.32–2.25 (1H, m), 1.96–1.84 (2H, m), 1.80 (2H, quint., J = 7.5 Hz), 1.72–1.62 (1H, m), 1.47–1.16 (66H, m), 0.85 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.1, 101.4, 84.2 (d, J = 167 Hz), 76.7, 72.5, 71.0, 70.7 (d, J = 21 Hz), 70.3 (d, J = 6.9 Hz), 69.9, 68.6, 51.1, 36.8, 34.4, 32.1, 30.4, 30.1, 30.03, 30.02, 29.99, 29.98, 29.97, 29.92, 29.91, 29.85, 29.8, 29.7, 29.61, 29.60, 26.5, 26.4, 22.9, 14.3 ppm; HRMS (FAB+) m/z calcd for $C_{50}H_{99}NO_8F$ $[M+H]^+$ 860.7355, found 860.7357.

4.1.11.7. (2*S*,3*S*,4*R*)-1-*O*-(β-L-rabinopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol 18 (RCAI-87). In the same manner as described above, **11g** (205 mg, 0.187 mmol) was converted to **18** (RCAI-87, 49 mg, 32%) as colorless powder. Mp 151.0–153.0 °C; $[\alpha]_D^{24}$ +75.1 (c 0.20, pyridine); ν_{max} (KBr): 3480 (w, OH), 3240 (br s, OH), 3100 (w, NH), 1615 (s, CO), 1570 (br m), 1140 (br s, C–O), 1100 (m, C–O), 1080 (br s, C–O) 1020 (s, C–O), 1000 (s, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5, 45 °C): 8.14 (1H, d, J = 8.5 Hz, NH), 6.61 (1H, br s, OH), 6.30 (1H, br s, OH), 6.14 (1H, br d, J = 5.0 Hz, OH), 5.92 (1H, br s, OH), 5.82 (1H, br s, OH), 5.46 (1H, d, J = 3.5 Hz, 1′-H), 5.20–5.15 (1H, m, 2-H), 4.59 (1H, dd, J = 11, 5.5 Hz, 1-H_a), 4.53 (1H, dd, J = 9.5, 3.5 Hz, 2′-H), 4.36 (1H, dd, J = 9.5, 3.0 Hz, 3′-H), 4.29–4.22 (3H, m, 3-, 4-, 4′-H), 4.25 (1H, dd, J = 11, 4.5 Hz, 1-H_b), 4.15 (1H, br d, J = 12 Hz, 5′-H_a), 3.99 (1H, dd, J = 12, 2.5 Hz, 5′-H_b), 2.41 (1H, dt, J = 15, 7.5 Hz, 2″-H_a), 2.38 (1H, dt, J = 15, 7.5 Hz, 2″-H_b), 2.28–2.21 (1H, m, 5-H_a), 1.93–1.84 (2H, m, 5-H_b, 6-H_a), 1.80 (2H, quint., J = 7.5 Hz, 3″-H_2), 1.72–1.63 (1H, m, 6-H_b), 1.48–1.20 (66H, m), 0.87 (6H, t, J = 7.0 Hz, 18-, 26″-H_3) ppm; δ_C (126 MHz, pyridine-d_5): 173.0, 101.7, 76.7, 72.5, 70.8, 70.3, 70.2, 68.4, 64.4, 51.1, 36.8, 34.4, 32.1, 30.3, 30.1, 30.02, 30.01, 29.98, 29.97, 29.91, 29.90, 29.85, 29.8, 29.7, 29.61, 29.59, 26.5, 26.4, 22.9, 14.3 ppm. This spectrum indicates the conformation of **18** as $^4C_1$; HRMS (FAB+) m/z calcd for $C_{49}H_{98}NO_8$ $[M+H]^+$ 828.7292, found 828.7294.

4.1.11.8. (2*S*,3*S*,4*R*)-1-*O*-[6-*O*-(methoxylmethyl)-α-D-galactopyranosyl]-2-(hexacosanamido)octadecane-1,3,4-triol 23 (RCAI-113). In the same manner as described above, **22a** (175 mg, 0.129 mmol) was converted to **23** (RCAI-113, 84 mg, 72%) as colorless powder. Mp 109.5–111.0 °C; $[\alpha]_D^{21}$ +43.1 (c 0.30, pyridine); ν_{max} (KBr): 3440 (br s, OH), 3280 (s, NH), 1640 (br s, CO), 1545 (br m), 1140 (br s, C–O), 1075 (br s, C–O), 1050 (br s, C–O), 720 (br s) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.40 (1H, d, J = 8.5 Hz), 6.40 (1H, br s), 5.52 (1H, d, J = 4.0 Hz), 5.27–5.22 (1H, m), 5.20 (4H, br

s), 4.78 (2H, s), 4.65 (1H, dd, J = 11, 5.0 Hz), 4.60 (1H, dd, J = 9.5, 4.0 Hz), 4.48 (1H, dd, J = 7.0, 6.5 Hz), 4.41–4.36 (2H, m), 4.34 (1H, dd, J = 11, 4.5 Hz), 4.32–4.28 (2H, m), 4.20–4.17 (2H, m), 3.36 (3H, s), 2.44 (1H, dt, J = 14, 7.0 Hz), 2.40 (1H, dt, J = 14, 7.0 Hz), 2.30–2.24 (1H, m), 1.95–1.84 (2H, m), 1.84–1.77 (2H, m), 1.71–1.62 (1H, m), 1.46–1.16 (66H, m), 0.85 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.0, 101.5, 96.9, 76.7, 72.5, 71.4, 71.0, 70.7, 70.1, 68.7, 68.1, 55.1, 51.3, 36.8, 34.3, 32.1, 30.4, 30.1, 30.03, 30.02, 29.99, 29.97, 29.92, 29.91, 29.86, 29.80, 29.75, 29.6, 26.5, 26.4, 22.9, 14.3 ppm; HRMS (ESI+) m/z calcd for $C_{52}H_{103}NO_{10}Na$ $[M+Na]^+$ 924.7480, found 924.7483.

4.1.11.9. (2*S*,3*S*,4*R*)-1-*O*-[6-*O*-(2-methoxyethoxymethyl)-α-D-galactopyranosyl]-2-(hexacosanamido)octadecane-1,3,4-triol 24 (RCAI-119). In the same manner as described above, **22b** (150 mg, 0.107 mmol) was converted to **24** (RCAI-119, 56 mg, 55%) as colorless powder. Mp 86.0–88.0 °C; $[\alpha]_D^{21}$ +42.3 (c 0.31, pyridine); ν_{max} (KBr): 3440 (br m, OH), 3280 (br s, NH), 1640 (br s, CO), 1540 (br m), 1040 (br s, C–O), 1075 (br s, C–O), 1050 (br s, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.39 (1H, d, J = 9.0 Hz), 6.41 (2H, br s), 6.04 (1H, br s), 5.52 (1H, d, J = 3.5 Hz), 5.28–5.23 (1H, m), 4.95 (2H, br s), 4.89 (2H, s), 4.65 (1H, dd, J = 11, 4.5 Hz), 4.61 (1H, dd, J = 9.0, 3.5 Hz), 4.49 (1H, dd, J = 6.5, 6.0 Hz), 4.42–4.37 (1H, m), 4.34 (1H, dd, J = 11, 4.5 Hz), 4.34–4.28 (1H, m), 4.24 (1H, dd, J = 10, 6.0 Hz), 4.20 (1H, dd, J = 10, 7.0 Hz), 3.84–3.77 (2H, m), 3.54 (2H, dd, J = 5.0, 4.5 Hz), 3.26 (3H, s), 2.44 (1H, dt, J = 14, 7.0 Hz), 2.40 (1H, dt, J = 14, 7.0 Hz), 2.31–2.24 (1H, m), 1.96–1.85 (2H, m), 1.85–1.76 (2H, m), 1.72–1.62 (1H, m), 1.46–1.16 (66H, m), 0.85 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.1, 101.5, 96.0, 76.7, 72.5, 72.2, 71.4, 71.0, 70.8, 70.1, 68.8, 68.1, 67.2, 58.6, 51.3, 36.8, 34.4, 32.1, 30.4, 30.2, 30.05, 30.01, 30.00, 29.98, 29.93, 29.92, 29.88, 29.82, 29.77, 29.62, 29.61, 26.5, 26.4, 22.9, 14.3 ppm; HRMS (ESI+) m/z calcd for $C_{54}H_{107}NO_{11}Na$ $[M+Na]^+$ 968.7742, found 968.7747.

4.1.11.10. (2*S*,3*S*,4*R*)-1-*O*-[6-*O*-(2-hydroxyethyl)-α-D-galactopyranosyl]-2-(hexacosanamido)octadecane-1,3,4-triol 25 (RCAI-125). In the same manner as described above, **22c** (116 mg, 0.0804 mmol) was converted to **25** (RCAI-125, 32 mg, 44%) as colorless powder. Mp 115.0–117.0 °C; $[\alpha]_D^{23}$ +43.0 (c 0.32, pyridine); ν_{max} (KBr): 3440 (m, OH), 3350 (br s, NH), 1645 (br s, CO), 1145 (br m, C–O), 1075 (br s, C–O), 1040 (br s, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.46 (1H, d, J = 8.5 Hz), 6.42 (2H, br s), 5.50 (1H, d, J = 4.0 Hz), 5.25–5.20 (1H, m), 5.13 (4H, br s), 4.65 (1H, dd, J = 11, 5.0 Hz), 4.60 (1H, dd, J = 9.5, 3.5 Hz), 4.49 (1H, br t, J = 6.0 Hz), 4.40–4.27 (5H, m), 4.15 (1H, dd, J = 10, 5.5 Hz), 4.11 (1H, dd, J = 10, 6.5 Hz), 4.03–3.96 (2H, m), 3.83 (1H, dd, J = 10, 5.0 Hz), 3.79 (1H, dd, J = 10, 5.5 Hz), 2.45 (1H, dt, J = 15, 7.5 Hz), 2.41 (1H, dt, J = 15, 7.5 Hz), 2.29–2.22 (1H, m), 1.96–1.85 (2H, m), 1.85–1.75 (2H, m), 1.72–1.62 (1H, m), 1.46–1.17 (66H, m), 0.85 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.2, 101.5, 76.5, 73.9, 72.5, 71.5, 71.4, 70.83, 70.77, 70.2, 68.7, 61.7, 51.4, 36.8, 34.2, 32.12, 32.11, 30.4, 30.1, 30.04, 30.00, 29.99, 29.97, 29.93, 29.91, 29.87, 29.82, 29.76, 29.62, 29.60, 26.5, 26.4, 22.9, 14.3 ppm; HRMS (ESI+) m/z calcd for $C_{52}H_{103}NO_{10}Na$ $[M+Na]^+$ 924.7480, found 924.7490.

4.2. Pharmacology

4.2.1. Mice

C57BL/6J mice were purchased from Charles River Japan, Inc. or Clea Japan, Inc. Mice were kept under specific pathogen-free conditions and used at 8–16 wk of age.[33]

4.2.2. Preparation of glycolipid solutions

KRN7000 (**A**) or synthesized glycolipids (1.0 mg) were dissolved in dimethyl sulfoxide (1.0 mg/mL) at 80 °C.[34,39] After 30 min at 80 °C, the solutions of **A**, **1**, **12**–**16** and **18** were diluted to 200 μg/mL with Dulbecco's phosphate-buffered saline (PBS, Sigma–Aldrich). For bioassay in Section 2.3, Dulbecco's PBS (Invitrogen™) containing 0.5% Tween20 (polyethylene sorbitan monolaurate) was used for dilution of the solutions of **A**, **23**–**25** instead. The obtained solutions were diluted to 10 μg/mL with Dulbecco's PBS before injection into mice.

4.2.3. Administration of the glycolipid solutions and cytokine measurement

Each glycolipid solution (10 μg/mL, 200 μL) was administered intravenously (from the caudal vein) into mice. Peripheral blood was collected from the retro-orbital plexus of mice at 3, 6, 12, 24, 36, 48, and 60 h using heparin-coated capillary tubes (Hirschmann Laborgeräte GmbH & Co. KG), and plasma was prepared.

The cytokine concentrations in plasma were quantified by mouse IFN-γ ELISA kit (Thermo Fisher Scientific K.K.) for IFN-γ, and cytometric bead array (CBA) system (BD Bioscience) for IL-4 and IL-12p70 according to the manufacturer's protocol.

Acknowledgments

We thank Mrs. S. Suzuki (née Inoue, RIKEN RCAI) for her preliminary contribution. Our thanks are due to Drs. T. Nakamura and Y. Hongo (RIKEN) for HRMS analyses. We are grateful to Drs. M. Shiozaki (RIKEN RCAI) and K. Fuhshuku (Toyama Prefectural University) for their helpful comments. This work was partly supported by Mizutani Foundation for Glycoscience and Grant-in-Aid for Young Scientists (B) (No. 20790108) to T.T. from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

Supplementary data

Supplementary data (^1H and ^{13}C NMR spectra of RCAI-61, 85, 86, 64, 58, 83, 87, 113, 119 and 125) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.03.028.

References and notes

1. Tashiro, T.; Mori, K. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1715.
2. 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.
3. Schneiders, F. L.; Scheper, R. J.; von Blomberg, B. M. E.; Woltman, A. M.; Janssen, H. L. A.; van den Eertwegh, A. J. M.; Verheul, H. M. W.; de Gruijl, T. D.; van der Vliet, H. J. *Clin. Immunol.* **2011**, *140*, 130.
4. Motohashi, S.; Okamoto, Y.; Yoshino, I.; Nakayama, T. *Clin. Immunol.* **2011**, *140*, 167.
5. Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626.
6. (a) Zajonc, D. M.; Cantu, C., III; Mattner, J.; Zhou, D.; Savage, P. B.; Bendelac, A.; Wilson, I. A.; Teyton, L. *Nat. Immunol.* **2005**, *6*, 810; (b) 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.
7. (a) Taniguchi, M.; Harada, M.; Kojo, S.; Nakayama, T.; Wakao, H. *Annu. Rev. Immunol.* **2003**, *21*, 483; (b) Kronenberg, M. *Annu. Rev. Immunol.* **2005**, *26*, 877; (c) Bendelac, A.; Savage, P. B.; Teyton, L. *Annu. Rev. Immunol.* **2007**, *25*, 297.
8. Rissoan, M.-C.; Soumelis, V.; Kadowaki, N.; Grouard, G.; Briere, F.; Malefyt, R. d. W.; Liu, Y.-J. *Science* **1999**, *283*, 1183.
9. (a) Tashiro, T. *Biosci. Biotechnol. Biochem.* **2012**, *79*, 1055; (b) Franck, R. W. *C. R. Chimie.* **2012**, *15*, 46; (c) Banchet-Cadeddu, A.; Hénon, E.; Dauchez, M.; Renault, J.-H.; Monneaux, F.; Haudrechy, A. *Org. Biomol. Chem.* **2011**, *9*, 3080; (d) Mori, K.; Tashiro, T. *Heterocycles* **2011**, *83*, 951; (e) Murphy, N.; Zhu, X.; Schmidt, R. R. *Carbohydr. Chem.* **2010**, *36*, 64; (f) Wu, D.; Fujio, M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 1073; (g) Savage, P. B.; Teyton, L.; Bendelac, A. *Chem. Soc. Rev.* **2006**, *35*, 771; (h) Berkers, C. R.; Ovaa, H. *Trends Pharm. Sci.* **2005**, *26*, 252.
10. (a) 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; (b) Pellicci, D. G.; Patel, O.; Kjer-Nielsen, L.; Pang, S. S.; Sullivan,

L. C.; Kyparissoudis, K.; Brooks, A. G.; Reid, H. H.; Gras, S.; Lucet, I. S.; Koh, R.; Smyth, M. J.; Mallevaey, T.; Matsuda, J. L.; Gapin, L.; McCluskey, J.; Godfrey, D. I.; Rossjohn, J. *Immunity* **2009**, *31*, 47.
11. Motoki, K.; Morita, M.; Kobayashi, E.; Uchida, T.; Akimoto, K.; Fukushima, H.; Koezuka, Y. *Biol. Pharm. Bull.* **1995**, *18*, 1487.
12. Zhou, X.-T.; Forestier, C.; Goff, R. D.; Li, C.; Teyton, L.; Bendelac, A.; Savage, P. B. *Org. Lett.* **2002**, *4*, 1267.
13. Uchimura, A.; Shimizu, T.; Morita, M.; Ueno, H.; Motoki, K.; Fukushima, H.; Natori, T.; Koezuka, Y. *Bioorg. Med. Chem.* **1997**, *5*, 2245.
14. Trappeniers, M.; Van Beneden, K.; Decruy, T.; Hillaert, U.; Linclau, B.; Elewaut, D.; Van Calenbergh, S. *J. Am. Chem. Soc.* **2008**, *130*, 16468.
15. Liu, Y.; Goff, R. D.; Zhou, D.; Mattner, J.; Sullivan, B. A.; Khurana, A.; Cantu, C. I. I. I.; Ravkov, E. V.; Ibegbu, C. C.; Altman, J. D.; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Immunol. Methods* **2006**, *312*, 34.
16. Jervis, P. J.; Graham, L. M.; Foster, E. L.; Cox, L. R.; Porcelli, S. A.; Besra, G. S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4348.
17. Ebensen, T.; Link, C.; Riese, P.; Schulze, K.; Morr, M.; Guzmán, C. A. *J. Immunol.* **2007**, *179*, 2065.
18. Chang, Y. J.; Huang, J. R.; Tsai, Y. C.; Hung, J. T.; Wu, D.; Fujio, M.; Wong, C.-H.; Yu, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 10299.
19. Pauwels, N.; Aspeslagh, S.; Vanhoenacker, G.; Sandra, K.; Yu, E. D.; Zajonc, D. M.; Elewaut, D. E.; Linclau, B.; Van Calenbergh, S. *Org. Biomol. Chem.* **2011**, *9*, 8413.
20. Aspeslagh, S.; Li, Y.; Yu, E. D.; Pauwels, N.; Trappeniers, M.; Girardi, E.; Decruy, T.; Van Beneden, K.; Venken, K.; Drennan, M.; Leybaert, L.; Wang, J.; Franck, R. W.; Van Calenbergh, S. V.; Zajonc, D. M.; Elewaut, D. *EMBO J.* **2011**, *30*, 2294.
21. (a) Tashiro, T.; Nakagawa, R.; Hirokawa, T.; Inoue, S.; Watarai, H.; Taniguchi, M.; Mori, K. *Tetrahedron Lett.* **2007**, *48*, 3343; (b) Tashiro, T.; Nakagawa, R.; Hirokawa, T.; Inoue, S.; Watarai, H.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2009**, *17*, 6360.
22. Tashiro, T.; Sekine-Kondo, E.; Shigeura, T.; Nakagawa, R.; Inoue, S.; Omori-Miyake, M.; Chiba, T.; Hongo, N.; Fujii, S.; Shimizu, K.; Yoshiga, Y.; Sumida, T.; Mori, K.; Watarai, H.; Taniguchi, M. *Int. Immunol.* **2010**, *22*, 319.
23. Tashiro, T.; Nakagawa, R.; Inoue, S.; Shiozaki, M.; Watarai, H.; Taniguchi, M.; Mori, K. *Tetrahedron Lett.* **2008**, *49*, 6827.
24. Bernotas, R. C.; Pezzone, M. A.; Ganem, B. *Carbohydr. Res.* **1987**, *167*, 305.
25. Tatsuta, K.; Niwata, Y.; Umezawa, K.; Toshima, K.; Nakata, M. *Carbohydr. Res.* **1991**, *222*, 189.
26. Koto, S.; Kusunoki, A.; Hirooka, M. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 967.
27. L'Heureux, A.; Beaulieu, F.; Bennett, C.; Bill, D. R.; Clayton, S.; LaFlamme, F.; Mirmehrabi, M.; Tadayon, S.; Tovell, D.; Couturier, M. *J. Org. Chem.* **2010**, *75*, 3401.
28. Kovác, P.; Yeh, H. J. C.; Jung, G. L. *J. Carbohydr. Chem.* **1987**, *6*, 423.
29. Treatment of **2** with XtalFluor-E/$Et_3N{\cdot}3HF$ conditions reported by L'Heureux et al. (Ref. 27) gave **3f** in 7% yield (**6**: 87%).
30. Takikawa, H.; Muto, S.; Mori, K. *Tetrahedron* **1998**, *54*, 3141.
31. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, *10*, 431.
32. Fluoride **17** was prepared from methyl β-L-arabinopyranoside (Wako Pure Chemical Industries, Ltd) by benzylation, acetolysis followed by *O*-deacetylation, and treatment with DAST. The analytical data of each intermediates were shown in Section 4 as **3g**, **7g**, and **8g**, respectively.
33. All biological experiments were in accordance with protocols approved by RIKEN Animal Care and Use Committee.
34. KRN7000 (**A**) used in this assay (Fig. 4) was kindly provided by Kyowa Kirin Pharma (Takasaki, Japan), and its stock solution in DMSO (1.0 mg/mL) was employed (Section 2.2).
35. Kitamura, H.; Iwakabe, K.; Yahata, T.; Nishimura, S.; Ohta, A.; Ohmi, Y.; Sato, M.; Takeda, K.; Okumura, K.; Kaer, L. V.; Kawano, T.; Taniguchi, M.; Nishimura, T. *J. Immunol.* **1999**, *189*, 1121.
36. *M*log*P* (Moriguchi's estimation of log*P*: Moriguchi, I.; Hirono, S.; Liu, Q.; Nakagome, I.; Matsushita, Y. *Chem. Pharm. Bull.* **1992**, *40*, 127. values were calculated by MedChem Designer™ ver. 1.0 (Simulations Plus, Inc.).
37. (a) Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635; (b) Plettenburg, O.; Bodmer-Narkevitch, V.; Wong, C.-H. *J. Org. Chem.* **2002**, *67*, 4559.
38. (a) Wipf, P.; Pierce, J. G. *Org. Lett.* **2006**, *8*, 3375; (b) Heidelberg, T.; Martin, O. R. *J. Org. Chem.* **2004**, *69*, 2290.
39. KRN7000 (**A**) used in this assay (Fig. 5) was purchased from Funakoshi Corporation (Japan).
40. Due to enhancement of the hydrophobicity of the analog itself, methylation of the 6′-hydroxy group of some analogs depresses the immunostimulatory activity of the mother compounds Tashiro, T.; Shigeura, T.; Watarai, H.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2012**, *20*, 4540. See also Ref. 21b.
41. (a) Fujii, S.; Shimizu, K.; Kronenberg, M.; Steinman, R. M. *Nat. Immunol.* **2002**, *3*, 867; (b) Taniguchi, M.; Tashiro, T.; Dashtsoodol, N.; Hongo, N.; Watarai, H. *Int. Immunol.* **2009**, *22*, 1.
42. Liu, Z.; Courtney, A. N.; Metelitsa, L. S.; Bittman, R. *ChemBioChem* **2012**, *13*, 1733.
43. Szeja, W.; Fokt, I.; Grynkiewicz, G. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 224.
44. Tejima, S.; Fletcher, H. G., Jr. *J. Org. Chem.* **1963**, *28*, 2999.
45. Preparation of the pure β-anomer is reported Kumazawa, T.; Saito, T.; Matsuba, S.; Sato, S.; Onodera, J. *Carbohydr. Res.* **2000**, *329*, 855.