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RCAI-37, 56, 59, 60, 92, 101, and 102, cyclitol and carbasugar analogs of KRN7000: Their synthesis and bioactivity for mouse lymphocytes to produce Th1-biased cytokines ☆

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ABSTRACT

Cyclitol [RCAI-37 (**1**), 59 (**5**), 92 (**7**), and 102 (**2**)] and carbasugar analogs [RCAI-56 (**3**), 60 (**4**), and 101 (**6**)] of KRN7000 were synthesized through coupling reactions of the corresponding cyclitol or carbasugar derivatives with a cyclic sulfamidate (**9**) as the key step. Bioassay showed RCAI-56 (**3**, carbagalactose analog of KRN7000), 59 (**5**, 1-deoxy-*neo*-inositol analog), and 92 (**7**, 1-*O*-methylated **5**) to be remarkably potent stimulants of mouse lymphocytes to produce Th1-biased cytokines, such as interferon-γ, in vivo. RCAI-60 (**4**, carbafucose analog) and RCAI-101 (**6**, 6-*O*-methylated **3**) showed strong bioactivity, on the other hands, RCAI-37 (**1**, *myo*-inositol analog) and 102 (**2**, *neo*-inositol analog) induced little cytokine production.

1. Introduction

In 1993, agelasphins (the major component is agelasphin-9b, **A**, in Fig. 1)[2] were isolated as anticancer glycosphingolipids from the extract of an Okinawan marine sponge, *Agelas mauritianus*.[3,4] Through structure–activity relationship studies, the researchers at Kirin Brewery Co. developed an anticancer drug candidate KRN7000 (α-GalCer, **B**) in 1995.[5] Both **A** and **B** are structurally characteristic α-galactosylceramides and act as the immunostimulant agents to induce antitumor activity in vivo in mice and humans. In 1997, it was found that these sphingolipids bind to a CD1d protein, an MHC class I like glycolipid presentation protein on the surface of the antigen presenting cells of the immune system.[6] In 2005, X-ray crystallographic analyses revealed the structures of the complexes of mouse and human CD1d–**B**.[7,8] According those reports, the two lipid alkyl chains of **B** are bound in the interior of the CD1d protein, and the galactose head group of **B** is presented to the receptor. This CD1d–glycosphingolipid complex is recognized by the invariant (mouse: Vα14, human: Vα24) T cell receptor (TCR) of natural killer T (NKT) cells and activates them to release helper T(Th)1 and/or Th2 cytokines.[9] Th1 cytokines such as interferon(IFN)-γ mediate protective immune functions like tumor rejection, whereas Th2 cytokines such as interleukin(IL)-4 mediate regulatory immune functions to ameliorate autoimmune diseases. If there is a novel glycolipid, which can induce NKT cells to produce cytokines with directed Th1/Th2 balance, it will be a promising drug candidate against many types of diseases.

Unfortunately, KRN7000 (**B**) induces both Th1 and Th2 cytokines in large quantities at the same time by a single injection.[10] Since these Th1/Th2 cytokines can antagonize each other's biological functions,[11] use of **B** for clinical therapy is limited. To circumvent this problem, many groups are trying to develop new analogs of **B**, which induce NKT cells to produce only Th1 (or Th2) type cytokines (see reviews[12]). The structure **B** contains three modifiable parts as follows: (i) the galactose–ceramide linkage, (ii) the lipid chains, and (iii) the galactose part. As the representative Th1-biased analog belonging to category (i), Franck, Tsuji and their co-workers reported in 2003 that their synthetic α-*C*-galactosylceramide (α-*C*-GalCer, **C**) caused an enhanced Th1 type response in mice in vivo (m, vivo).[13] The derivative **C** was developed by

☆ Synthesis of sphingosine relatives, Part 32. For Part 31, see Ref. 1.

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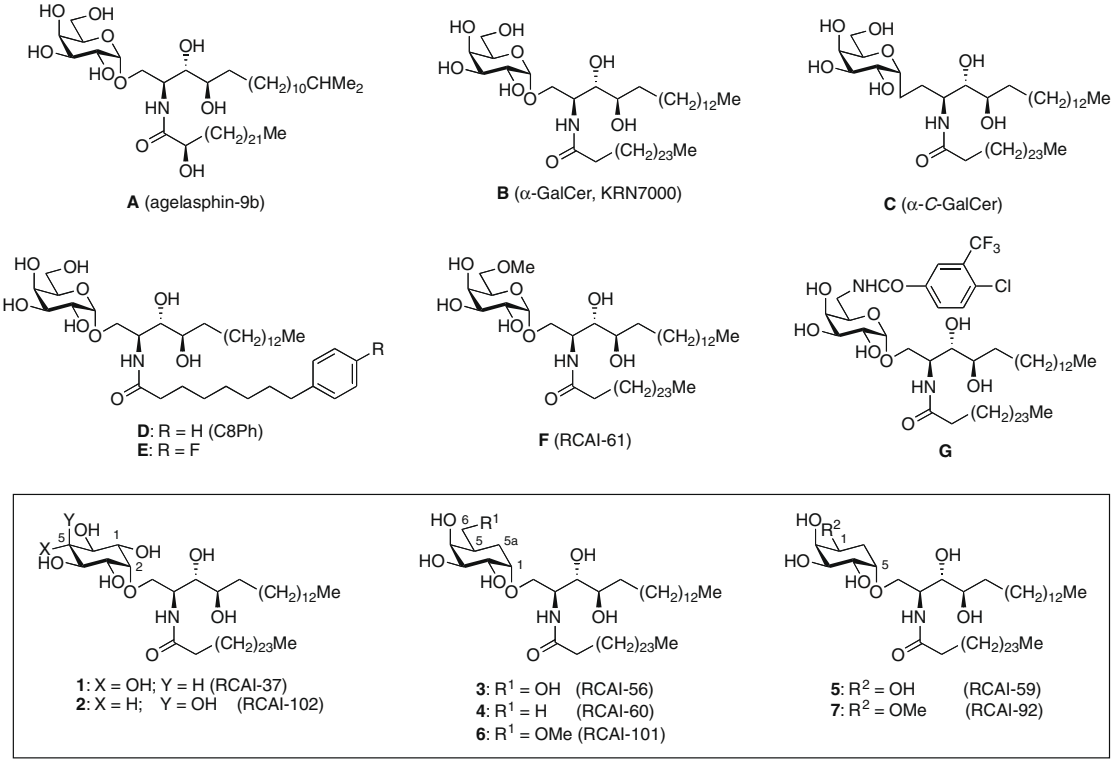


Figure 1. Structures of glycosphingolipids for NKT cell activation.

replacing the glycosidic O atom at the galactose–ceramide linkage to a CH_2 group, and five different syntheses of **C** have already been reported.[14] Why the analog **C** induces NKT cells to release Th1-biased cytokines? The analog **C** has the stable ether bond instead of an acetal linkage and is resistant against α-galactosidase in vivo. Therefore, CD1d–**C** complex can stimulate NKT cells for a longer period than CD1d–**B** can do. It is proposed that this long term stimulation causes Th1-biased response, although the detailed mechanism remains unclear. A novel analog **D**, which induces Th1 response in human in vitro (h, vitro), was developed by Wong and his co-workers in 2006.[15] This analog is included in category (ii).[15] According to their docking model of human (h) CD1d–**D**, the phenyl group of **D** is placed at the bottom of the hydrophobic pocket of hCD1d, and seems to possess further stability by π–π stacking interactions between the aromatic group of **D** and that of Tyr73 or Trp40 of hCD1d. They reported that the binding affinity of the ligand to hCD1d correlates with the amount of IFN-γ produced by NKT cells.[16] Through their further investigation by using microarray experiment, they developed 4-fluorophenyl-octanoyl analog **E** (two times stronger binding affinity with hCD1d and three times higher IFN-γ/IL-4 ratio than **B**) as more selective IFN-γ production inducer (h, vitro).[16]

The X-ray crystallographic analysis of Vα24TCR–**B**-hCD1d complex was reported by Rossjohn and his co-workers in 2007.[17] It was revealed that Vα24TCR of invariant NKT cell was interacting with the 2′-, 3′-, and 4′-hydroxy groups of the galactose part of **B**, while 6′-hydroxy group was not involved in this hydrogen bonding network with any residues of hCD1d or Vα24TCR.[17] Based on that report, RCAI-61 (**F**) and **G** were developed by modification at the 6′-hydroxy group as the Th1 analogs of category (iii) in 2008. In comparison with **B** (m, vivo), RCAI-61 (**F**) induces a significant amount of IFN-γ and comparable levels of IL-4,[18] and the analog **G** induces comparable levels of IFN-γ and only marginal amount of IL-4 (m, vivo).[19]

Incidentally, the O atom of the pyranose ring makes no hydrogen bonding with CD1d or TCR, hence this position could also be modified. Additionally, according to the X-ray crystallographic analysis of CD1d–**B** complex, the glycosidic O atom makes a hydrogen bonding with the α2 helix of CD1d (mouse: Thr156; human: Thr154) and seems to play an important role for making the stable complex with CD1d.[7,8] Based on these results, we began our attempt to synthesize a carbocyclic analogs of **B** with the linking O atom and hence the stable ether structure similar to **C**. Those carbocyclic analogs might make a more stable complex with CD1d and induce NKT cells to release larger amount of Th1-biased cytokines than **C**.

Based on this supposition, we synthesized the ceramidyl *myo*- and *neo*-inositol (**1**, **2**) as the cyclitol analogs of **B,** however, those were inactive (mice, vivo). We considered the additional hydroxy group of cyclitol part of **1** or **2** might obstruct the analog's binding with CD1d. We then planned to synthesize the carbasugar analogs, which have the 5a-methylene (Fig. 1) group. In 2007, we reported the synthesis of RCAI-56 (**3**), a carba-α-D-galactose analog of **B**, as a preliminary communication, and found it to be a potent activator of lymphocytes to produce a large amount of IFN-γ (m, vivo).[20] As described below, in addition to the carbagalactose analog (**3**), its 6-*O*-methylated analog (RCAI-101, **6**), a carbafucose analog (RCAI-60, **4**), a 1-deoxy-*neo*-inostol analog (RCAI-59, **5**), and its 1-*O*-methylated analog (RCAI-92, **7**) also induced mouse lymphocytes to produce Th1 cytokines (m, vivo). Herein, we report in detail our synthesis of **1**–**7**, the new cyclitol and carbasugar analogs of **B**, and their bioactivity.

2. Results and discussion

2.1. Synthesis of RCAI-37 and 102, the inositol analogs

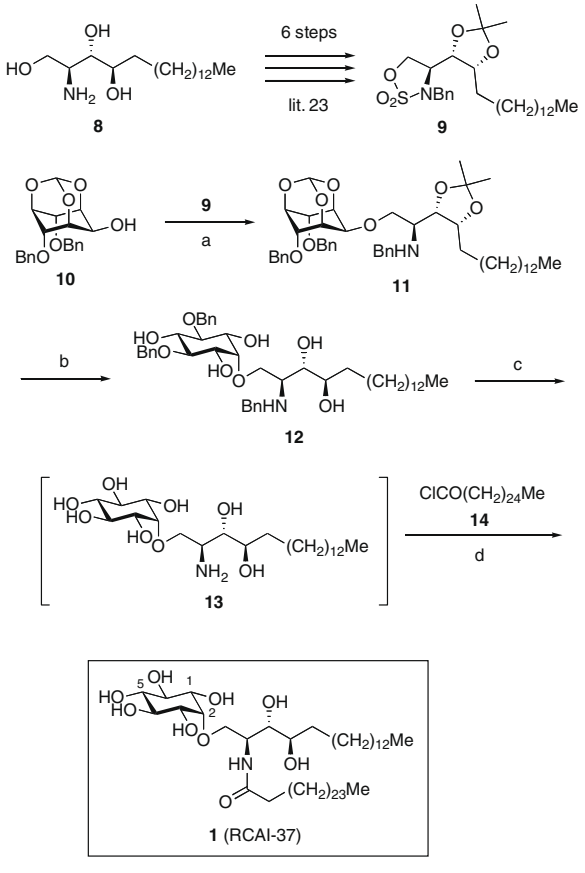
The binding pocket of CD1d protein is so flexible that it can recognize many types of glycolipids, and therefore identification of the 'true' natural ligand has been difficult. In 1998, Joyce et al. identified a candidate of the natural ligand of mouse CD1d as gly-

cosylphosphatidylinositol (GPI).[21] Based on their finding, we assumed that inositol analogs of **B** might also show NKT cell stimulatory activity. It has been known that both α-galactosyl- and α-glucosylceramide show strong bioactivity among the monoglycosylceramides,[6] and therefore we attempted to synthesize both *myo*- and *neo*-inositol analogs (**1** and **2**).

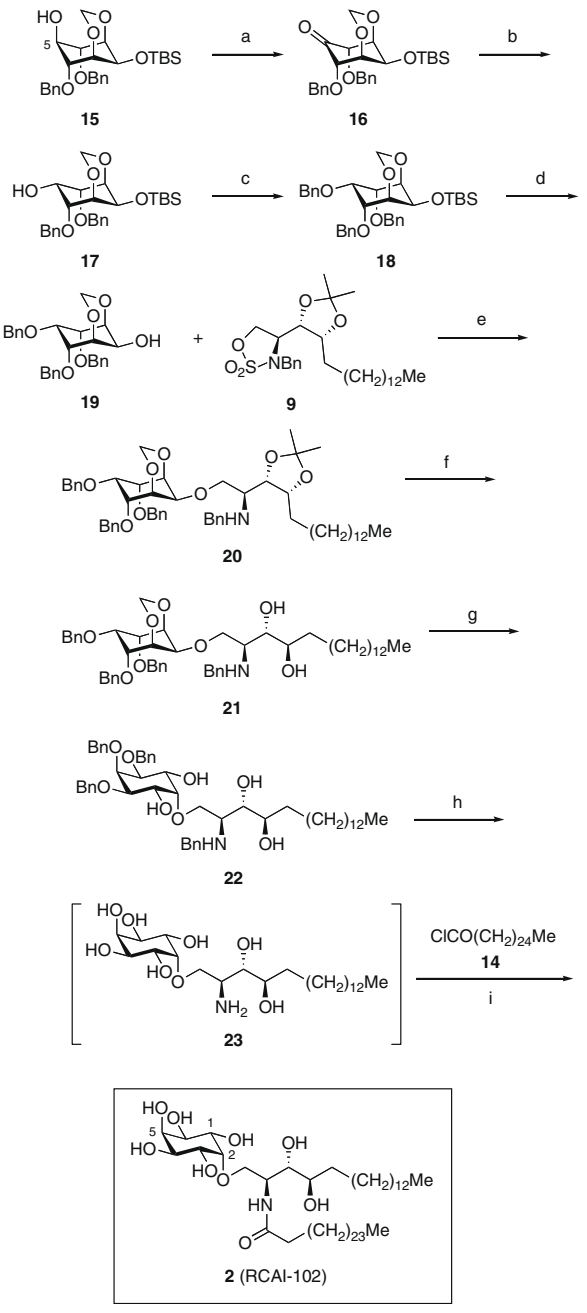
Synthesis of RCAI-37 (**1**), the *myo*-inositol analog, was summarized in Scheme 1. A known *myo*-inositol derivative **10** was chosen as a suitable starting material for the synthesis of **1**.[22] The alcohol **10** was coupled with cyclic sulfamidate **9**, which was prepared from commercially available phytosphingosine (**8**) by Bittman's protocol,[23] to give amine **11** in 86% yield via acid hydrolysis of the sodium sulfamidate intermediate. The acetonide and the orthoformate protective groups of **11** were simultaneously removed under acidic conditions to give amino alcohol **12** (79%). Hydrogenolysis of all of the benzyl groups of **12** was followed by acylation of the resulting **13** with freshly prepared cerotyl chloride (**14**)[14b,24] to afford RCAI-37 (**1**) as a colorless solid, mp 195–199 °C, in 39% yield (two steps). The overall yield of **1** was 26% based on the inositol derivative **10** through four steps.

The *neo*-inositol analog, RCAI-102 (**2**), was synthesized as shown in Scheme 2. Synthesis of **2** was commenced with the known alcohol **15**, which could be prepared from the silyl ether of **10**.[25] In order to invert the configuration at C-5 of **15**, we adopted the oxidation-reduction procedure. The alcohol **15** was oxidized with Dess–Martin periodinane[26] to furnish ketone **16** in 62% yield. Stereoselective reduction with lithium tri-*sec*-butylborohydride[27] cleanly gave the desired alcohol **17** (99%). When this reduction was performed with sodium borohydride (2 mol equiv, 0 °C, 30 min), an inseparable diastereomeric mixture was obtained (**17**:**15** = 4:1, determined by 500 MHz ^{1}H NMR). Benzylation of the free hydroxy group of **17** yielded **18** (96%). Removal of the *tert*-butyldimethylsilyl (TBS) group of **18** with tetra(*n*-butyl)ammonium fluoride (TBAF) furnished the desired *neo*-inositol derivative **19** in 97% yield.

The obtained alcohol **19** was coupled with **9** to give **20** (96%). Successive removal of the acetonide and methylidene protective groups of amine **20** afforded tetrol **22** via diol **21** (80%, two steps). Deprotection of all of the benzyl groups of **22** by hydrogenolysis followed by acylation of the resulting **23** with cerotyl chloride



Scheme 1. Synthesis of RCAI-37 (**1**). Reagents and conditions: (a) NaH, DMF/THF (1:1), 70 °C; 20% H_2SO_4 aq, Et_2O, 0 °C (86%); (b) *p*-TsOH·H_2O, MeOH/CH_2Cl_2 (2:1), reflux (79%); (c) 10% Pd–C, cyclohexene, 1 M HCl aq, MeOH, reflux; (d) Et_3N, $CHCl_3$/MeOH/THF (5:1:1), rt (39%, two steps).



Scheme 2. Synthesis of RCAI-102 (**2**). Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2, rt (62%); (b) LiB(*sec*-Bu)$_3$H, THF, −78 °C; 34% H_2O_2 aq, 3 M NaOH aq, rt (99%); (c) NaH, BnBr, (*n*-Bu)$_4$NI, DMF/THF (1:1), rt (96%); (d) TBAF, THF, rt (97%); (e) NaH, DMF/THF (1:1), 70 °C; 20% H_2SO_4 aq, Et_2O, 0 °C (96%); (f) *p*-TsOH·H_2O, MeOH/CH_2Cl_2 (2:1), reflux (92%); (g) concd HCl, MeOH, reflux (87%); (h) 10% Pd–C, cyclohexene, 1 M HCl aq, MeOH, reflux; (i) Et_3N, $CHCl_3$/MeOH/THF (5:1:1), rt (12%, two steps).

(**14**) furnished RCAI-102 (**2**) as a colorless solid, mp 179–183 °C, in 12% yield (two steps).

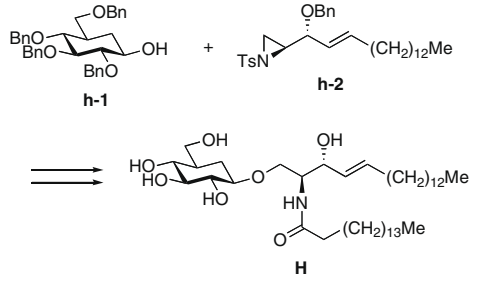
2.2. Synthesis of RCAI-56 and 60, the carbasugar analogs

In 1995, Tsunoda and Ogawa reported the synthesis of carba-β-D-glucosylceramide (**H**, Scheme 3) as an immunomodulator.[28] Their glycolipid **H** was synthesized by coupling of a carbaglucose derivative (**h-1**) with a 1,2-aziridine (**h-2**) as the key step. It must be added that an independent attempt of Chung and his co-workers to provide the carba-α-D-galactosylceramide was reported in 2006.[29]

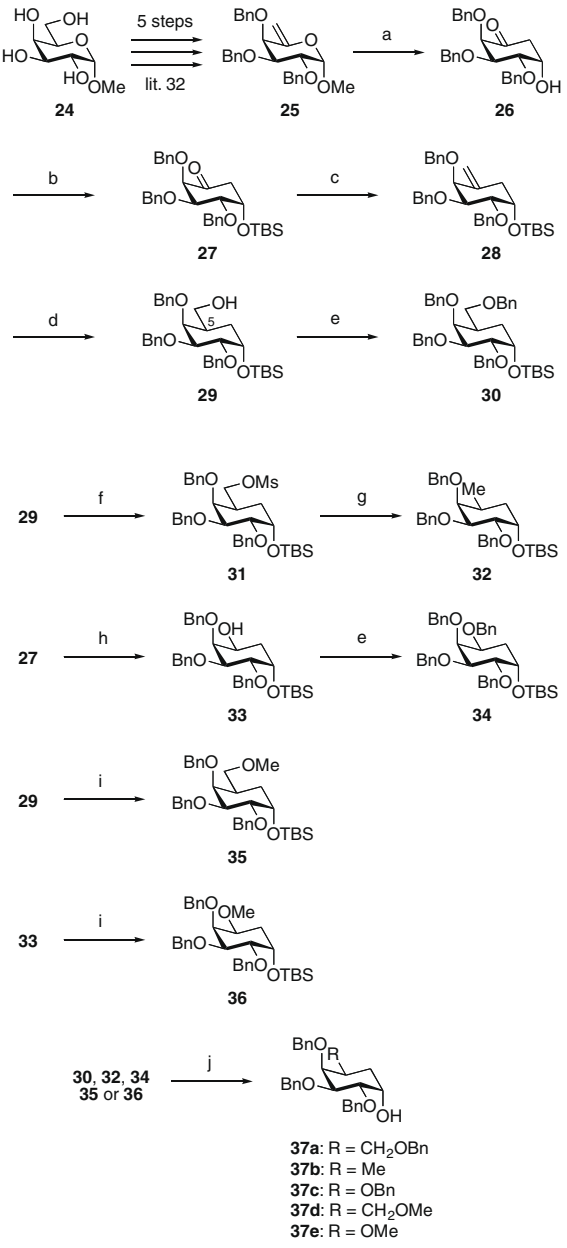
Our synthesis of carba-α-D-galactose and carba-α-D-fucose derivatives (**37a, 37b**) were shown in Scheme 4. We adopted a method similar to the one developed for the synthesis of a carba-α-D-glucose derivative by Pohl and his co-workers.[30] Ikegami's modification of the Ferrier rearrangement (**25**→**26**)[31] was employed as the key reaction. The known building block **25** was prepared from the commercially available methyl α-D-galactopyranoside (**24**) through five steps in 49% yield.[32] The cyclohexane framework of ketone **26** was constructed by Pd(II)-catalyzed Ferrier rearrangement of **25** (α:β = 10:1, determined by 400 MHz ^{1}H NMR).[31] Resulting two epimers could be separated by SiO_2 column chromatography. The free hydroxy group of the desired α-isomer (**26**) was protected as TBS ether by treatment with *tert*-butyldimethylsilyl triflate (TBSOTf) at −20 °C to give TBS ether **27** (89%, two steps). Compound **27** was then converted to alkene **28** by methylenation with Tebbe reagent (88%). It should be noted that compounds **27** and **28** take $_4C^1$ conformation as judged by the coupling constants of their ^{1}H NMR spectra (see Section 4). Alkene **28** was subjected to hydroboration–oxidation by treatment with borane–THF and NaOH aq/H_2O_2 to furnish alcohol **29** with the desired β-configuration at C-5 (84%). Because of the steric hindrance of β-benzyloxy group at C-4, borane could approach only from the α-face to give the β-isomer selectively.[33] Benzylation of the free hydroxy group of **29** yielded **30** (91%). Removal of the TBS group of **30** with TBAF furnished the desired tetrabenzylated carba-α-D-galactose derivative **37a** in 77% yield.[34]

Carba-α-D-fucose derivative was prepared by deoxygenation of **29**. The alcohol **29** was converted to the corresponding mesylate **31**. Subsequent reduction of **31** with lithium triethylborohydride yielded **32** in 84% yield (two steps). The TBS protective group of **32** was removed with TBAF to provide **37b**, the tribenzylated carba-α-D-fucose derivative, in 98% yield.

Completion of the synthesis of RCAI-56 (**3**) is illustrated in Scheme 5. In the same manner as described in Scheme 1, four-step-conversion of carba-α-D-galactose derivative (**37a**) to RCAI-56 (**3**) was achieved as follows: (i) [**37a**→**38a**] coupling with cyclic sulfamidate **9** followed by acid hydrolysis (76%, two steps); (ii) [**38a**→**39a**] removal of acetonide protective group (97%); (iii) [**39a**→**40a**] hydrogenolysis of all of the benzyl groups; and (iv) [**40a**→**3**] acylation with cerotyl chloride **14** (60%, two steps). RCAI-56 (**3**) was obtained as a colorless solid, mp 147–149 °C. Similarly, carba-α-D-fucose derivative **37b** was converted to RCAI-60 (**4**) as a colorless solid, mp 126–127 °C, in four steps (38%). The overall yields of **3** and **4** were 20% (10 steps) and 21% (11 steps), respectively, from the known vinyl ether **25**.



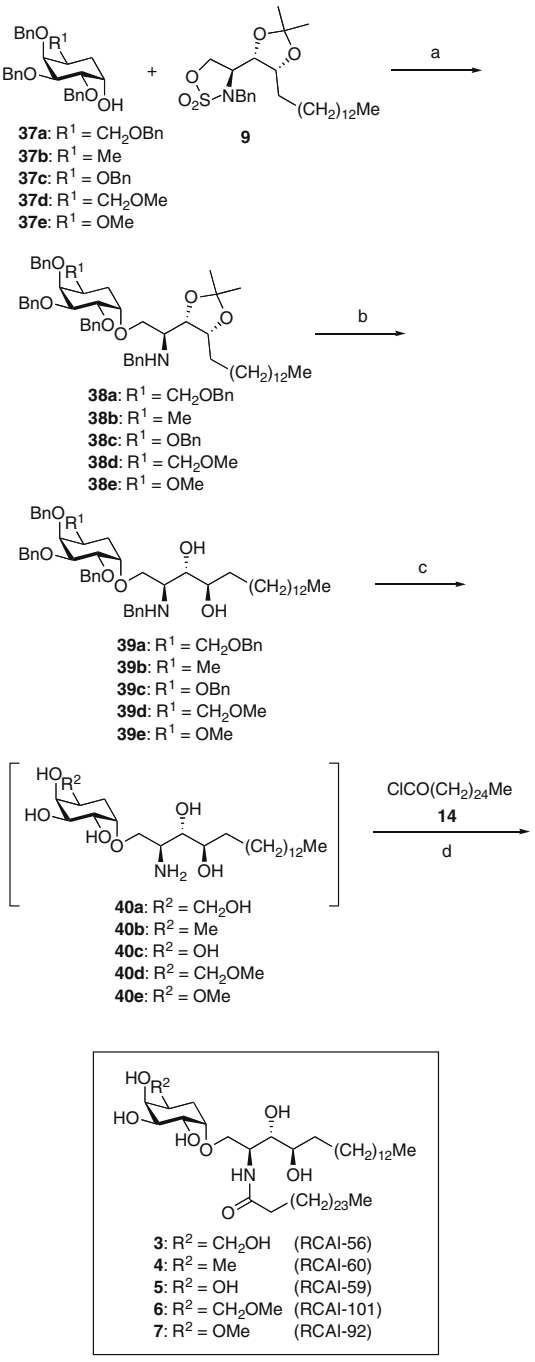
Scheme 3. Tsunoda and Ogawa's synthesis of carba-β-D-glucosylceramide (**H**).



Scheme 4. Syntheses of carbasugar derivatives (**37a**, **37b**, and **37e**) and cyclitol derivatives (**37c**, **37d**). Reagents and conditions: (a) 5 mol % $PdCl_2$, 1,4-dioxane/H_2O (2:1), 60 °C (b) TBSOTf, CH_2Cl_2/2,6-lutidine (6:1), −20 °C (89%, two steps); (c) Tebbe reagent, pyridine, THF, −40 °C to rt, (88%); (d) BH_3·THF, THF, rt; then 30% H_2O_2 aq, 3 M NaOH aq, 0 °C (84%); (e) NaH, BnBr, $(n\text{-Bu})_4NI$, DMF/THF (1:1), rt (91% for **30**; quant. for **34**); (f) MsCl, pyridine, rt (90%); (g) $LiBEt_3H$, THF, rt; 30% H_2O_2 aq, 3 M NaOH aq, 0 °C (84%); (h) $NaBH_4$, EtOH, 0 °C to rt (89%); (i) NaH, MeI, DMF/THF (1:1), rt (98% for **35**, quant. for **36**); (j) TBAF, THF, rt [77% for **37a**; 98% for **37b**; 76% (two steps) for **37c**; 97% for **37d**; 96% for **37e**].

2.3. Synthesis of RCAI-59, 92, and 101, the modified cyclitol and carbagalactose analogs

As described previously, 6′-hydroxy group of galactose part of **B** is not interacting with CD1d or TCR, and is modifiable. We synthesized three other analogs derived by modification at this position.



Scheme 5. Synthesis of RCAI-56 (**3**), 60 (**4**), 59 (**5**), 92 (**6**), and 101 (**7**). Reagents and conditions: (a) NaH, DMF/THF (1:1), 70 °C; 20% H_2SO_4 aq, Et_2O, 0 °C (76% for **38a**; 91% for **38b**; 54% for **38c**; 74% for **38d**; 87% for **38e**); (b) *p*-TsOH·H_2O, MeOH/CH_2Cl_2 (2:1), reflux (97% for **39a**; 74% for **39b**; 88% for **39c**; 86% for **39d**; 85% for **39e**); (c) 10% Pd–C, cyclohexene, 1 M HCl aq, MeOH, reflux; (d) Et_3N, $CHCl_3$/MeOH/THF (5:1:1), rt (60% for **3**; 56% for **4**; 51% for **5**; 69% for **6**; 79% for **7**, two steps).

As summarized in Scheme 4, a 1-deoxy-*neo*-inositol derivative (**37c**) was synthesized from ketone **27**. The ketone **27** was reduced with sodium borohydride to give alcohol **33** as a single product (89%). Benzylation of the hydroxy group of **33** yielded **34**, which was treated with TBAF to give **37c** in 76% yield (two steps). 6-*O*-Methylated (**37d**) and 1-*O*-methylated (**37e**) derivatives were prepared from alcohols **29** and **33** by methylation of the hydroxy group followed by deprotection of the TBS protective group to give **37d** and **37e** in 95% and 96% yields (two steps), respectively.

The three derivatives (**37c–e**) were converted to RCAI-59 (**5**), RCAI-101 (**6**), and RCAI-92 (**7**) in the same manner as shown in Scheme 5. Each of these analogs was obtained as a colorless solid, and the overall yields of **5** (mp 177–181 °C), **6** (mp 116–118 °C), and **7** (mp 144–146 °C) were 15% (nine steps), 27% (10 steps), and 44% (nine steps), respectively, from the known vinyl ether **25**.

2.4. Results of bioassay

Figure 2 shows the results of bioassay.[35] To investigate the ability of RCAI-37, 56, 59, 60, 92, 101, and 102 to induce cytokine production by mouse lymphocytes in vivo, the concentrations of cytokines in sera of mice were monitored after their intravenous injection of KRN7000 (**B**) or synthesized analogs (**1**–**7**) into C57BL/6 mice. The sera samples were collected at 3, 6, 12, 24, 36, 48, and 60 h, and the measurement of cytokine concentrations were performed by ELISA system (BD Biosciences) for IFN-γ (A and B, in Fig. 2) and cytometric bead array (CBA) system (BD Bioscience) for IL-4 (C and D) and IL-12p70 (E and F).

The bioassay data of the carbasugar analogs, RCAI-56 (**3**), 60 (**4**), and 101 (**6**), are depicted in A, C, and E, and those of the inositol analogs, RCAI-37 (**1**), 59 (**5**), 92 (**7**), and 102 (**2**), in B, D, and F. KRN7000 (**B**) was chosen as the standard sample for the production of cytokines. As can be seen from A, C, and E in Figure 2, RCAI-56 (**3**) resulted in the quick and increased IL-12p70 production at 3 and 6 h after injection, and brought about remarkable increase in the production of IFN-γ (at 24 and 36 h), while it induced almost a half level of IL-4 production in comparison to **B**. Accordingly, RCAI-56 (**3**) is regarded as one of the most potent Th1-biased cytokine inducer.[36]

The carbafucose analog RCAI-60 (**4**) and 6′-*O*-methylated carbagalactose derivative RCAI-101 (**6**) also showed strong activity, and they induced a half amount of IFN-γ in comparison to **3**. As we reported in the synthesis of RCAI-61 (**F**, in Fig. 1),[18] methylation of the 6′-hydroxy group of **B** enhanced ability of IFN-γ production, however, 6′-*O*-methylation of **3** to give RCAI-101 (**6**) resulted in the decrease of bioactivity. Furthermore, a separately synthesized α-D-fucopyranose (not carbasugar) analog of **B** induces as much amount of IFN-γ production as **3** can do,[18,37] while **4** could only induce the same level of IFN-γ as **6**. The carbasugar analogs have the hydrophobic CH_2 group instead of the hydrophilic pyranose O atom, therefore, their solubilities are lower than that of **B**. We considered that methylation of a hydroxy group of a carbasugar caused further enhancement of hydrophobicity and consequently decreased their bioactivities.

From B, D, and F in Figure 2, it became clear that RCAI-37 (**1**) and 102 (**2**), inositol analogs of **B**, were almost inactive, whereas deoxy-inositol analog, RCAI-59 (**5**), and its 1-*O*-methylated derivative, RCAI-92 (**7**), showed potent activity to induce a large amount of IFN-γ production.[38] The analogs **5** and **7** caused IL-4 production at the almost same level as **B** could do, therefore these analogs are also presumed as inducers of Th1-biased cytokine production.

We also studied the ability of IFN-γ production and proliferation activity of **3** and **5** against human peripheral blood mononuclear cells (unpublished observation). According to these experiments, both **3** and **5** induced similar levels of IFN-γ production, and showed more potent proliferation activity in comparison to **B**. For **3** or **5**, further improvement of synthetic efficiency would be necessary to regard them as the drug candidates for Th1-cytokine inducers.

The binding conformations of **B** and **3** to mouse (m) CD1d were calculated and found to be not so different. The averages of ligand binding XP Score (Gride score)[39] of the ligand–mCD1d complexes were calculated as −16.66 for **B** and −14.74 for **3**.

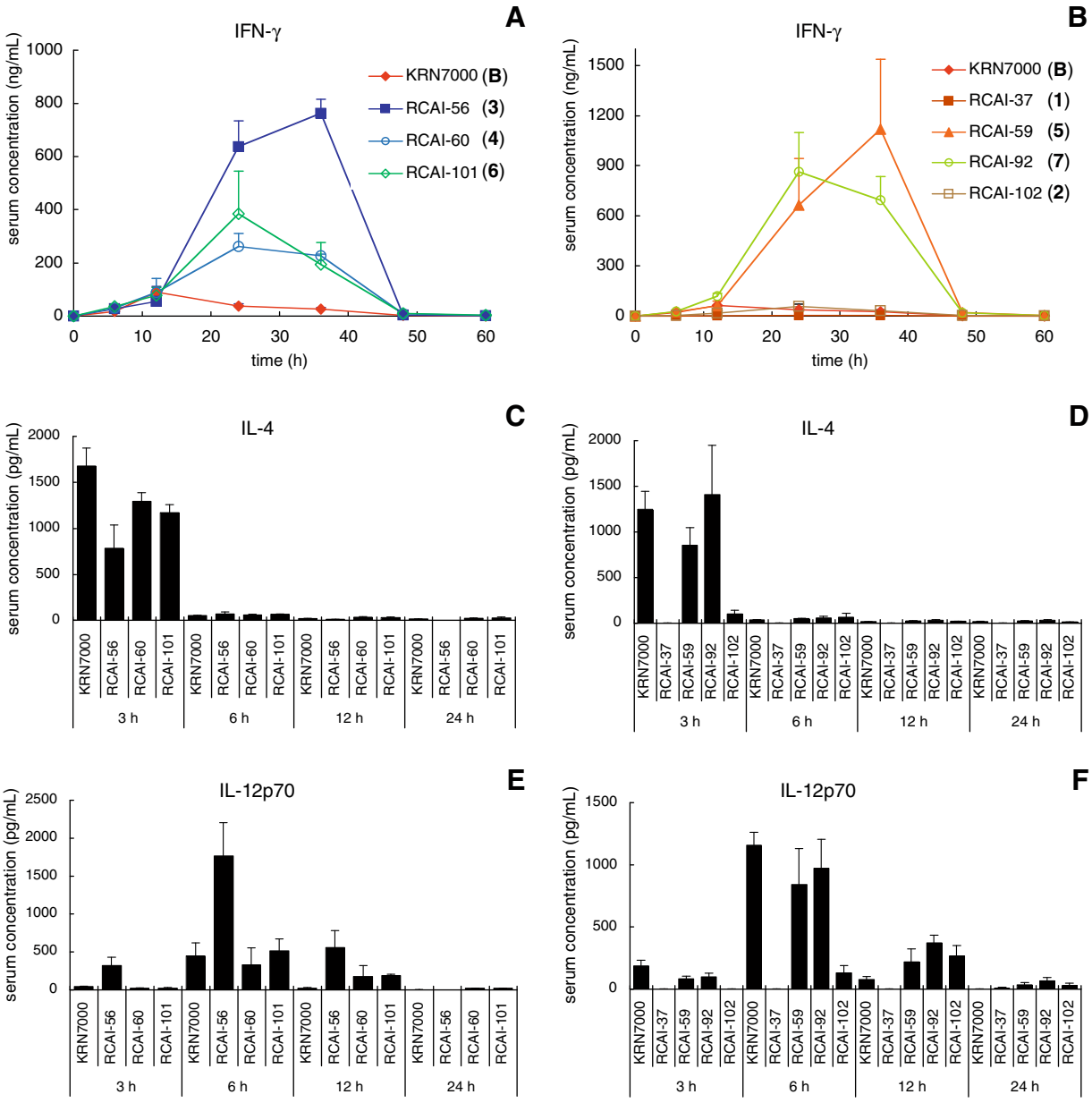


Figure 2. In vivo cytokine production after intravenous injection of synthesized analogs.[35] Serum concentrations of IFN-γ (A, B), IL-4 (C, D), or IL-12p70 (E, F) were measured by ELISA or CBA at the indicated time points. Data are means ± SD from 3 mice and repeated three times with similar results.

Although the lowest docking score of ligand–mCD1d of **3** (−22.76) was much lower than that of **B** (−16.66), and it was found that **3** could make a more stable complex with CD1d than **B**. The docking conformations of the complexes of ligand–mCD1d are now under investigating, and will be reported separately in due course by Hirokawa.

3. Conclusion

We synthesized RCAI-37, 56, 59, 60, 92, 101, and 102, the carba-sugar and cyclitol analogs of KRN7000. Among them, RCAI-56 (**3**, the carba-α-D-galactose analog), RCAI-59 (**5**, the 1-deoxy-*neo*-inositol analog), and RCAI-92 (1-*O*-methylated **5**) were found to be the remarkably potent inducers of Th1-biased cytokine production in mice in vivo. Further studies are in progress to clarify the structural requirements for a glycosphingolipid ligand in controlling the ratio of Th1/Th2 responses.

4. Experimental

4.1. Chemistry

4.1.1. General

Refractive indices (n_D) were measured on an Atago 1T refractometer. 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 $\delta_H = 0.00$, $CHCl_3$ at $\delta_H = 7.26$ or pyridine at $\delta_H = 7.55$ as internal standards) and ^{13}C NMR spectra ($CDCl_3$ at $\delta_C = 77.0$ or pyridine at $\delta_C = 123.5$ as internal standards) were recorded on a Jeol JNM-A400 or a Varian VNMRS-500 spectrometers. HRMS were recorded on a Jeol JMS-SX102A or a

Bruker BioAPEX II 70e FT-ICR. Column chromatography was carried out on Merck Kieselgel 60 Art 1.07734. Thin layer chromatography was performed on Merck Silica gel 60 F_{254}.

4.1.2. Coupling reaction

4.1.2.1. (2′*S*,3′*S*,4′*R*)-4,6-Di-*O*-benzyl-2-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxy-3′,4′-*O*-isopropylideneoctadecyl)-myo-inositol 1,3,5-orthoformte (11). To a stirred solution of **10** (308 mg, 0.832 mmol) in DMF (15 mL) and THF (8 mL), NaH (60% mineral oil suspension, 106 mg, 2.65 mmol) was added at 0 °C. After stirring at 0 °C for 1 h, a solution of **9** (636 mg, 1.25 mmol) in THF (7 mL) was added at 0 °C. The resulting mixture was stirred at 70 °C overnight. To this mixture, NaH (60% mineral oil suspension, 34 mg, 0.84 mmol) and a solution of **9** (122 mg, 0.239 mmol) in THF (2 mL) were successively added at 70 °C, and the mixture was stirred overnight at that temperature (if the reaction had not proceeded completely, this procedure should be repeated until the alcohol was consumed completely). The reaction mixture was then cooled to room temperature, and concentrated in vacuo to give a crude suspension of sodium salt intermediate.

The residue was diluted with Et_2O (40 mL) and cooled to 0 °C. To this mixture, 20% aqueous H_2SO_4 solution (40 mL) was added at 0 °C. After stirring at 0 °C for 20 min, the mixture was neutralized with K_2CO_3 at 0 °C. After stirring at 0 °C for 40 min, Et_2O and water were added to this mixture. The mixture was then filtered to remove the precipitates. The aqueous phase of the filtrate was extracted with Et_2O, and then the combined organic phase was washed with water, 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 (20 g, hexane/EtOAc = 8:1) to give **11** (570 mg, 86%) as a colorless gummy oil, n_D^{25} 1.5173; $[\alpha]_D^{23}$ +30.6 (*c* 1.71, $CHCl_3$); ν_{max} (film): 3340 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1065 (s, C–O), 1100 (br s, C–O), 1005 (br s), 755 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.33–7.20 (15H, m), 5.48 (1H, d, *J* = 1.0 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.67 (1H, d, *J* = 12 Hz), 4.57 (2H, d, *J* = 12 Hz), 4.46 (1H, br s), 4.37 (1H, br t, *J* = 3.8 Hz), 4.35 (2H, br s), 4.31 (1H, br s), 4.16–4.13 (1H, m), 4.10 (1H, dd, *J* = 9.5, 6.0 Hz), 3.98 (1H, br s), 3.95 (1H, dd, *J* = 9.5, 2.5 Hz), 3.93 (1H, d, *J* = 13 Hz), 3.69 (1H, d, *J* = 13 Hz), 3.61 (1H, dd, *J* = 9.5, 4.0 Hz), 2.81 (1H, dt, *J* = 9.5, 4.0 Hz), 1.75 (1H, br s), 1.58–1.20 (26H, m), 1.37 (3H, s), 1.27 (3H, s), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 140.5, 137.6, 137.5, 128.5, 128.44, 128.38, 128.2, 127.92, 127.91, 127.72, 127.71, 126.9, 107.4, 103.2, 78.2, 76.5, 74.2, 74.0, 71.8, 71.7, 70.3, 70.2, 68.1, 68.0, 66.4, 56.1, 51.0, 31.9, 29.7, 29.64, 29.62, 29.59, 29.5, 29.3, 28.4, 26.1, 25.9, 22.7, 14.1 ppm; HR-ESIMS: calcd for $C_{49}H_{70}NO_8$ $[M+H]^+$ 800.5096; found 800.5094.

4.1.2.2. (2′*S*,3′*S*,4′*R*)-4,5,6-Tri-*O*-benzyl-2-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxy-3′,4′-*O*-isopropylideneoctadecyl)-1,3-*O*-methylidene-*neo*-inositol (20). In the same manner as described above, **19** was converted to **20** (96%, based on consumed starting material: 76%) as a colorless oil, n_D^{25} 1.5172; $[\alpha]_D^{23}$ +22.0 (*c* 1.28, $CHCl_3$); ν_{max} (film): 3320 (w, NH), 1605 (w), 1590 (w), 1495 (m), 1130 (br s, C–O), 1020 (br s, C–O), 735 (s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.20 (20H, m), 5.40 (1H, d, *J* = 4.5 Hz), 4.96 (1H, d, *J* = 12 Hz), 4.93 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.68 (1H, d, *J* = 12 Hz), 4.59 (2H, s), 4.53 (1H, d, *J* = 4.5 Hz), 4.39–4.37 (1H, m), 4.36 (1H, br s), 4.32 (1H, t, *J* = 4.0 Hz), 4.29–4.27 (1H, m), 4.15–4.08 (3H, m), 3.99 (1H, dd, *J* = 9.5, 5.5 Hz), 3.88 (1H, d, *J* = 13 Hz), 3.78 (1H, dd, *J* = 9.5, 2.5 Hz), 3.67 (1H, dd, *J* = 9.5, 2.5 Hz), 3.65 (1H, d, *J* = 13 Hz), 2.76 (1H, dt, *J* = 9.5, 2.5 Hz), 1.58–1.22 (26H, m), 1.38 (3H, s), 1.27 (3H, s), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 140.4, 139.0, 138.9, 138.6, 128.34, 128.31, 128.2, 127.68, 127.65, 127.5, 127.42, 127.41, 127.2, 127.0, 107.4, 85.4, 78.2, 76.6, 74.4, 73.6, 73.4, 71.9, 71.6, 70.8, 70.4, 65.6, 55.9, 51.0, 31.9, 29.70, 29.69, 29.65, 29.63, 29.60, 29.5, 29.3, 28.4, 26.0, 25.8, 22.7, 14.1 ppm; HR-ESIMS: calcd for $C_{56}H_{78}NO_8$ $[M+H]^+$ 892.5722; found 892.5716.

4.1.2.3. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-5a-carba-α-D-galactopyranosyl)-2-*N*-benzylamino-3,4-*O*-isopropylideneoctadecane-1,3,4-triol (38a). In the same manner as described above, **37a** was converted to **38a** (76%) as a colorless oil, n_D^{21} 1.5177; $[\alpha]_D^{22}$ +40.6 (*c* 1.73, $CHCl_3$); ν_{max} (film): 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1095 (br s, C–O), 735 (s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.35–7.20 (25H, m), 4.98 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.50 (1H, d, *J* = 12 Hz), 4.43 (2H, s), 4.12 (1H, br s), 4.08 (1H, dd, *J* = 10, 3.2 Hz), 4.05 (1H, dd, *J* = 8.8, 5.6 Hz), 3.93–3.82 (5H, m), 3.72–3.67 (1H, m), 3.68 (1H, d, *J* = 13 Hz), 3.51 (1H, t, *J* = 8.8 Hz), 3.32–3.28 (1H, m), 2.76 (1H, ddd, *J* = 8.4, 4.0, 3.6 Hz), 2.22–2.13 (1H, m), 1.74–1.23 (28H, m), 1.38 (3H, s), 1.27 (3H, s), 0.88 (3H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, $CDCl_3$): 140.8, 139.6, 139.3, 138.4, 128.4, 128.3, 128.22, 128.17, 128.11, 127.7, 127.63, 127.58, 127.4, 127.3, 127.2, 127.1, 126.8, 107.3, 81.3, 80.1, 78.3, 76.2, 75.7, 74.5, 73.15, 73.12, 72.4, 70.8, 68.2, 56.5, 51.2, 35.8, 31.9, 29.71, 29.66, 29.61, 29.5, 29.3, 28.3, 26.6, 26.2, 25.9, 22.7, 14.1 ppm; HR-FABMS: calcd for $C_{63}H_{86}NO_7$ $[M+H]^+$ 968.6404; found 968.6401.

4.1.2.4. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4-Tri-*O*-benzyl-5a-carba-α-D-fucopyranosyl)-2-*N*-benzylamino-3,4-*O*-isopropylideneoctadecane-1,3,4-triol (38b). In the same manner as described above, **37b** was converted to **38b** (91%) as a colorless oil, n_D^{20} 1.5173; $[\alpha]_D^{24}$ +53.9 (*c* 1.43, $CHCl_3$); ν_{max} (film): 3340 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1090 (br s, C–O), 730 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.36–7.20 (20H, m), 5.00 (1H, br d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.70 (2H, s), 4.69 (1H, d, *J* = 12 Hz), 4.56 (1H, d, *J* = 12 Hz), 4.12–4.06 (1H, m), 4.05 (1H, dd, *J* = 8.5, 6.0 Hz), 3.91 (1H, dd, *J* = 10, 2.5 Hz), 3.89 (1H, d, *J* = 13 Hz), 3.86 (1H, dd, *J* = 10, 2.5 Hz), 3.85–3.80 (2H, m), 3.73 (1H, br s), 3.70 (1H, dd, *J* = 9.5, 4.5 Hz), 3.69 (1H, d, *J* = 13 Hz), 2.79–2.74 (1H, m), 1.96–1.88 (1H, m), 1.64–1.46 (6H, m), 1.44–1.20 (26H, m), 1.38 (3H, s), 0.96 (3H, d, *J* = 7.0 Hz), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 140.9, 139.7, 139.5, 139.4, 128.29, 128.27, 128.2, 128.13, 128.11, 127.7, 127.5, 127.3, 127.21, 127.18, 127.1, 126.9, 107.3, 81.5, 80.7, 79.9, 78.33, 78.27, 76.1, 74.7, 73.2, 72.4, 68.1, 56.6, 51.3, 31.9, 31.6, 30.1, 29.73, 29.70, 29.68, 29.6, 29.5, 29.4, 28.3, 26.2, 25.9, 22.7, 17.5, 14.1 ppm; HR-FABMS: calcd for $C_{56}H_{80}NO_6$ $[M+H]^+$: 862.5986; found 862.5986.

4.1.2.5. (2′*S*,3′*S*,4′*R*)-1D-(1,2,3/4,5)-1,2,3,4-Terta-*O*-benzyl-5-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxy-3′,4′-*O*-isopropylideneoctadecyl)-cyclohexanepentol (38c). In the same manner as described above, **37c** was converted to **38c** (54%) as a colorless oil, n_D^{23} 1.5172; $[\alpha]_D^{21}$ +29.0 (*c* 1.04, $CHCl_3$); ν_{max} (film): 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1095 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.41–7.21 (25H, m), 4.89 (1H, br d, *J* = 12 Hz), 4.82 (1H, br d, *J* = 12 Hz), 4.73 (2H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.50 (1H, br d, *J* = 12 Hz), 4.45 (1H, br d, *J* = 12 Hz), 4.13 (1H, br s), 4.11–4.05 (1H, m), 3.97 (1H, dd, *J* = 9.6, 6.0 Hz), 3.95 (1H, dd, *J* = 10, 3.2 Hz), 3.89 (1H, br s), 3.82–3.79 (1H, m), 3.81 (1H, d, *J* = 13 Hz), 3.78–3.73 (1H, m), 3.72 (1H, dd, *J* = 10, 4.4 Hz), 3.70–3.62 (1H, m), 3.62 (1H, d, *J* = 13 Hz), 2.72 (1H, ddd, *J* = 8.8, 2.8, 2.8 Hz), 2.10–2.02 (1H, m), 1.97 (1H, br t, *J* = 12 Hz), 1.76–1.67 (1H, m), 1.60–1.21 (26H, m), 1.38 (3H, s), 1.27 (3H, s), 0.88 (3H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, $CDCl_3$): 140.7, 139.4, 139.10, 139.07, 138.5, 128.4, 128.22, 128.19, 128.12, 128.06, 127.7, 127.5, 127.42, 127.35, 127.32, 127.25, 127.2, 126.9, 107.3, 79.7, 79.2, 78.2, 76.8, 76.2, 74.6, 73.9, 72.9, 72.6, 70.7, 68.2, 56.4, 51.1, 38.0, 31.9, 29.69, 29.66, 29.61, 29.5, 29.4, 29.3, 28.3,

26.2, 25.9, 22.7, 14.1 ppm; HR-FABMS: calcd for $C_{62}H_{84}NO_7$ $[M+H]^+$ 954.6248; found 954.6245.

4.1.2.6. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-methyl-5a-carba-α-D-galactopyranosyl)-2-*N*-benzylamino-3,4-*O*-isopropylideneoctadecane-1,3,4-triol (38d). In the same manner as described above, **37d** was converted to **38d** (74%) as a colorless oil, n_D^{26} 1.5172; $[\alpha]_D^{23}$ +56.4 (*c* 1.06, $CHCl_3$); ν_{max} (film): 3340 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1105 (br s, C–O), 730 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.36–7.20 (20H, m), 4.99 (1H, br d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 13 Hz), 4.71 (1H, d, *J* = 13 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.10 (1H, dd, *J* = 6.0, 3.0 Hz), 4.10–4.06 (1H, m), 4.05 (1H, dd, *J* = 9.0, 6.0 Hz), 3.93–3.84 (4H, m), 3.84 (1H, dd, *J* = 9.0, 2.5 Hz), 3.71 (1H, dd, *J* = 9.0, 4.5 Hz), 3.68 (1H, d, *J* = 13 Hz), 3.39 (1H, t, *J* = 9.0 Hz), 3.28 (3H, s), 3.22–3.17 (1H, m), 2.78–2.75 (1H, m), 2.12 (1H, br s), 1.70 (1H, br s), 1.67–1.20 (31H, m), 1.38 (3H, s), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 140.8, 139.6, 139.29, 139.27, 128.3, 128.22, 128.18, 128.113, 128.105, 127.7, 127.4, 127.3, 127.22, 127.15, 127.12, 126.8, 107.3, 81.2, 80.0, 78.2, 76.0, 75.8, 74.6, 73.2, 73.1, 72.4, 68.2, 58.6, 56.5, 51.2, 35.6, 31.9, 29.71, 29.70, 29.66, 29.65, 29.61, 29.5, 29.3, 28.3, 26.6, 26.2, 25.9, 22.7, 14.1 ppm; HR-ESIMS: calcd for $C_{57}H_{82}NO_7$ $[M+H]^+$ 892.6086; found 892.6083.

4.1.2.7. (2′*S*,3′*S*,4′*R*)-1D-(1,2,3/4,5)-2,3,4-Tri-*O*-benzyl-5-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxy-3′,4′-*O*-isopropylideneoctadecyl)-1-*O*-methylcyclohexanepentol (38e). In the same manner as described above, **37e** was converted to **38e** (87%) as a colorless oil, n_D^{22} 1.5178; $[\alpha]_D^{22}$ +45.9 (*c* 1.07, $CHCl_3$); ν_{max} (film): 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 730 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.41–7.20 (20H, m), 4.87 (1H, br d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.14–4.09 (2H, m), 4.04 (1H, dd, *J* = 9.0, 6.0 Hz), 3.94 (1H, dd, *J* = 10, 3.0 Hz), 3.90–3.86 (2H, m), 3.89 (1H, d, *J* = 13 Hz), 3.79–3.72 (2H, m), 3.70 (1H, d, *J* = 13 Hz), 3.44 (1H, br d, *J* = 9.5 Hz), 3.27 (3H, s), 2.79–2.75 (1H, m), 2.04 (1H, br d, *J* = 13 Hz), 1.89 (1H, br t, *J* = 13 Hz), 1.76–1.69 (1H, m), 1.61–1.46 (2H, m), 1.46–1.20 (26H, m), 1.39 (3H, s), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 140.8, 139.4, 139.2, 139.1, 128.3, 128.24, 128.18, 128.1, 127.7, 127.5, 127.4, 127.3, 127.24, 127.22, 126.9, 107.4, 79.7, 79.2, 78.2, 77.0, 75.6, 74.6, 73.9, 73.0, 72.7, 68.6, 56.7, 56.6, 51.3, 31.9, 29.74, 29.73, 29.70, 29.68, 29.63, 29.5, 29.4, 29.3, 28.3, 26.2, 25.9, 22.7, 14.1 ppm; HR-FABMS: calcd for $C_{56}H_{80}NO_7$ $[M+H]^+$: 878.5935; found 878.5938.

4.1.3. Deprotection of diisopropylidene, ortho ester, and methylidene protective groups

4.1.3.1. (2′*S*,3′*S*,4′*R*)-4,6-Di-*O*-benzyl-2-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxyoctadecyl)-*myo*-inositol (12). To a stirred solution of **11** (407 mg, 0.508 mmol) in MeOH and CH_2Cl_2 (2:1, 20 mL), *p*-TsOH·H_2O (196 mg, 1.03 mmol) was added at room temperature. After stirring and heating under reflux for 8 h, the mixture was cooled to room temperature, and then concentrated in vacuo. The residue was diluted with EtOAc, and neutralized with saturated aqueous $NaHCO_3$ solution. The separated organic phase was washed with 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 (10 g, $CHCl_3$/MeOH = 50:3) to give **12** (300 mg, 79%) as a colorless oil, n_D^{22} 1.5161; $[\alpha]_D^{25}$ +25.8 (*c* 1.49, $CHCl_3$); ν_{max} (film): 3400 (br s, OH), 3340 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1070 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.25 (15H, m), 4.87 (1H, d, *J* = 12 Hz), 4.85 (1H, d, *J* = 12 Hz), 4.76 (1H, d, *J* = 11 Hz), 4.74 (1H, d, *J* = 11 Hz), 4.13 (1H, dd, *J* = 10, 2.5 Hz), 3.90 (1H, dd, *J* = 10, 2.5 Hz), 3.88 (1H, d, *J* = 13 Hz), 3.78 (1H, br s), 3.73 (1H, d, *J* = 13 Hz), 3.66 (1H, dt, *J* = 8.0, 2.5 Hz), 3.56–3.46 (5H, m), 3.42 (1H, t, *J* = 8.0 Hz), 2.80 (1H, dt, *J* = 7.5, 2.5 Hz), 2.55 (1H, br s), 1.80–1.72 (1H, m), 1.59–1.50 (1H, m), 1.49–1.19 (28H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.0, 138.3, 138.2, 128.71, 128.65, 128.6, 128.4, 128.1, 128.04, 127.98, 127.95, 127.5, 82.0, 81.8, 80.4, 75.31, 75.25, 75.1, 74.9, 72.0, 71.9, 71.7, 70.5, 61.9, 51.1, 34.4, 31.9, 30.0, 29.8, 29.71, 29.69, 29.64, 29.3, 25.2, 22.7, 14.1 ppm; HR-FABMS: calcd for $C_{45}H_{68}NO_8$ $[M+H]^+$ 750.4945; found 750.4945.

4.1.3.2. (2′*S*,3′*S*,4′*R*)-4,5,6-Tri-*O*-benzyl-2-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxyoctadecyl)-1,3-*O*-methylidene-*neo*-inositol (21). In the same manner as described above, **20** was converted to **21** (92%) as colorless oil, n_D^{23} 1.5170; $[\alpha]_D^{23}$ +11.7 (*c* 1.39, $CHCl_3$); ν_{max} (film): 3400 (br s, OH), 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1130 (br s, C–O), 1020 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.23 (20H, m), 5.31 (1H, d, *J* = 5.0 Hz), 4.96 (2H, d, *J* = 12 Hz), 4.66 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.60 (2H, s), 4.55 (1H, d, *J* = 5.0 Hz), 4.36 (1H, br s), 4.31 (1H, t, *J* = 4.0 Hz), 4.31–4.28 (1H, m), 4.28–4.26 (1H, m), 4.12–4.08 (2H, m), 3.82 (1H, d, *J* = 13 Hz), 3.74 (1H, d, *J* = 13 Hz), 3.71 (2H, d, *J* = 3.5 Hz), 3.59 (1H, br dt, *J* = 7.5, 2.5 Hz), 3.36 (1H, t, *J* = 7.5 Hz), 2.84 (1H, dt, *J* = 7.5, 3.5 Hz), 2.36 (2H, br s), 1.67–1.60 (1H, m), 1.56–1.46 (1H, m), 1.40–1.21 (25H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.0, 138.9, 138.8, 138.5, 128.6, 128.4, 128.3, 127.72, 127.71, 127.55, 127.52, 127.51, 127.4, 127.2, 85.4, 74.8, 74.3, 73.7, 73.6, 71.7, 71.5, 71.4, 71.1, 71.0, 65.0, 61.0, 51.3, 33.8, 31.9, 29.8, 29.68, 29.65, 29.64, 29.3, 25.2, 22.7, 14.1 ppm; HR-ESIMS: calcd for $C_{53}H_{74}NO_8$ $[M+H]^+$ 852.5409; found 852.5406.

4.1.3.3. (2′*S*,3′*S*,4′*R*)-4,5,6-Tri-*O*-benzyl-2-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxyoctadecyl)-*neo*-inositol (22). To a stirred solution of **21** (184 mg, 0.216 mmol) in MeOH (15 mL), concd HCl (3 mL) was added at room temperature. After stirring under reflux for 2 h, the mixture was cooled to room temperature, and then concentrated in vacuo. The residue was diluted with EtOAc, and neutralized with saturated aqueous $NaHCO_3$ solution. The separated organic phase was washed with 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 (10 g, $CHCl_3$/MeOH = 25:1) to give **22** (158 mg, 87%) as a colorless oil, n_D^{23} 1.5171; $[\alpha]_D^{23}$ +26.1 (*c* 0.97, $CHCl_3$); ν_{max} (film): 3380 (br s, OH), 3300 (w, NH), 1605 (w), 1590 (w), 1495 (m), 1130 (br s, C–O), 1070 (br s, C–O), 760 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.24 (20H, m), 4.73 (2H, s), 4.61 (1H, d, *J* = 12 Hz), 4.60 (1H, d, *J* = 12 Hz), 4.47 (1H, d, *J* = 12 Hz), 4.45 (1H, d, *J* = 12 Hz), 4.16 (1H, br d, *J* = 8.5 Hz), 4.10–4.08 (2H, m), 4.08–4.06 (1H, m), 3.93–3.90 (2H, m), 3.88 (1H, d, *J* = 13 Hz), 3.69 (1H, d, *J* = 13 Hz), 3.65 (1H, br dt, *J* = 8.0, 2.5 Hz), 3.49–3.44 (2H, m), 3.30 (1H, t, *J* = 8.0 Hz), 2.77 (1H, br d, *J* = 8.0 Hz), 2.64 (2H, br s), 1.84–1.75 (1H, m), 1.60–1.50 (1H, m), 1.44–1.16 (27H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.2, 138.6, 137.6, 137.5, 128.64, 128.56, 128.4, 128.3, 128.1, 128.0, 127.81, 127.76, 127.69, 127.6, 127.4, 79.45, 79.38, 78.9, 75.6, 74.3, 72.4, 72.2, 71.5, 70.2, 70.0, 69.8, 62.5, 51.1, 34.6, 31.9, 30.0, 29.8, 29.72, 29.70, 29.69, 29.68, 29.64, 29.3, 25.3, 22.7, 14.1 ppm; HR-ESIMS: calcd for $C_{52}H_{74}NO_8$ $[M+H]^+$ 840.5409; found 840.5406.

4.1.3.4. (2*S*,3*S*,4*R*)-2-*N*-Benzylamino-1-*O*-(2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-galactopyranosyl)octadecane-1,3,4-triol (39a). In the same manner as described above, **38a** was converted to **39a** (97%) as colorless needles, mp 44.5–47.0 °C; $[\alpha]_D^{23}$ +31.0 (*c* 1.40, $CHCl_3$); ν_{max} (KBr): 3450 (br s, OH), 3340 (w, NH), 1605 (w), 1585 (w), 1495 (s), 1095 (br s, C–O), 745 (br s), 695 (s) cm^{-1}; δ_H

(400 MHz, $CDCl_3$): 7.39–7.22 (25H, m), 4.95 (1H, d, J = 11 Hz), 4.84 (1H, d, J = 12 Hz), 4.75 (2H, s), 4.69 (1H, d, J = 12 Hz), 4.47 (1H, d, J = 11 Hz), 4.42 (2H, s), 4.09 (1H, br s), 3.94–3.89 (2H, m), 3.84 (1H, d, J = 13 Hz), 3.78–3.74 (2H, m), 3.72 (1H, d, J = 13 Hz), 3.68–3.61 (2H, m), 3.46 (1H, t, J = 8.8 Hz), 3.38 (1H, t, J = 8.0 Hz), 3.26 (1H, dd, J = 8.8, 5.6 Hz), 2.80 (1H, br d, J = 8.0 Hz), 2.12–2.02 (1H, m), 1.79–1.69 (1H, m), 1.57–1.20 (27H, m), 0.88 (3H, t, J = 7.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$): 139.4, 139.1, 138.7, 138.2, 137.9, 128.5, 128.4, 128.3, 128.24, 128.21, 128.1, 127.8, 127.7, 127.6, 127.43, 127.35, 127.30, 81.7, 79.5, 76.8, 75.7, 75.4, 74.6, 73.8, 73.2, 73.0, 71.2, 70.6, 67.1, 62.1, 51.0, 35.8, 34.7, 31.9, 30.0, 29.8, 29.74, 29.71, 29.6, 29.3, 27.2, 25.2, 22.7, 14.1 ppm; HR-FABMS: calcd for $C_{60}H_{82}NO_7$ $[M+H]^+$ 928.6091; found 928.6085.

4.1.3.5. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4-Tri-*O*-benzyl-5a-carba-α-D-fucopyranosyl)-2-*N*-benzylaminooctadecane-1,3,4-triol (39b). In the same manner as described above, **38b** was converted to **39b** (74%) as a colorless oil, n_D^{23} 1.5177; $[\alpha]_D^{23}$ +46.6 (c 2.42, $CHCl_3$); ν_{max} (film): 3450 (br s, OH), 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1090 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.21 (20H, m), 4.98 (1H, d, J = 11 Hz), 4.84 (1H, d, J = 12 Hz), 4.77 (1H, d, J = 12 Hz), 4.73 (1H, d, J = 12 Hz), 4.69 (1H, d, J = 12 Hz), 4.55 (1H, d, J = 11 Hz), 3.93–3.89 (2H, m), 3.84 (1H, d, J = 13 Hz), 3.76 (2H, dd, J = 9.5, 2.5 Hz), 3.73 (1H, d, J = 13 Hz), 3.70 (1H, br s), 3.66–3.62 (2H, m), 3.40 (1H, t, J = 8.0 Hz), 2.81 (1H, dt, J = 8.0, 2.5 Hz), 1.88–1.81 (1H, m), 1.80–1.73 (1H, m), 1.56–1.46 (3H, m), 1.42–1.20 (26H, m), 0.94 (3H, d, J = 7.0 Hz), 0.88 (3H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.4, 139.2, 138.9, 138.0, 128.5, 128.4, 128.3, 128.20, 128.19, 128.1, 127.8, 127.6, 127.42, 127.37, 127.28, 127.27, 82.0, 80.3, 79.4, 77.1, 75.4, 74.7, 73.7, 73.1, 71.5, 67.2, 62.0, 51.1, 34.6, 32.1, 31.9, 30.2, 30.0, 29.8, 29.72, 29.69, 29.68, 29.67, 29.6, 29.3, 25.3, 22.7, 17.5, 14.1 ppm; HR-FABMS: calcd for $C_{53}H_{76}NO_6$ $[M+H]^+$: 822.5673; found 822.5674.

4.1.3.6. (2′*S*,3′*S*,4′*R*)-1D-(1,2,3/4,5)-1,2,3,4-Terta-*O*-benzyl-5-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxyoctadecyl)cyclohexanepentol (39c). In the same manner as described above, **38c** was converted to **39c** (88%) as a white solid, mp 52.5–54.0 °C; $[\alpha]_D^{26}$ +25.8 (c 1.10, $CHCl_3$); ν_{max} (KBr): 3460 (s, OH), 3380 (m, NH), 1605 (w), 1585 (w), 1495 (m), 1095 (br s, C–O), 1070 (s, C–O), 1045 (s), 740 (br s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.40–7.24 (25H, m), 4.86 (1H, br d, J = 12 Hz), 4.85 (1H, d, J = 12 Hz), 4.80 (1H, d, J = 12 Hz), 4.70 (1H, d, J = 12 Hz), 4.68 (1H, d, J = 12 Hz), 4.64 (1H, d, J = 12 Hz), 4.47 (1H, d, J = 12 Hz), 4.42 (1H, d, J = 12 Hz), 4.11 (1H, br s), 3.95 (1H, dd, J = 10, 3.2 Hz), 3.91 (1H, br d, J = 7.6 Hz), 3.81 (1H, d, J = 12 Hz), 3.74 (1H, dd, J = 10, 2.4 Hz), 3.69 (1H, d, J = 12 Hz), 3.68–3.54 (4H, m), 3.31 (1H, t, J = 8.4 Hz), 2.78 (1H, br d, J =7.6 Hz), 1.98–1.92 (2H, m), 1.78–1.70 (1H, m), 1.57–1.48 (1H, m), 1.38–1.21 (27H, m), 0.88 (3H, t, J = 7.2 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.3, 138.9, 138.6, 138.3, 137.9, 128.6, 128.5, 128.42, 128.40, 128.33, 128.32, 128.1, 128.0, 127.71, 127.66, 127.6, 127.53, 127.49, 127.4, 127.3, 79.6, 79.2, 75.9, 75.8, 75.3, 74.6, 74.10, 74.06, 72.9, 71.3, 70.9, 67.5, 61.8, 51.0, 34.6, 31.9, 30.1, 30.0, 29.82, 29.77, 29.74, 29.73, 29.69, 29.4, 25.3, 22.7, 14.1 ppm; HR-FABMS: calcd for $C_{59}H_{80}NO_7$ $[M+H]^+$ 914.5935; found 914.5932.

4.1.3.7. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-methyl-5a-carba-α-D-galactopyranosyl)-2-*N*-benzylaminooctadecane-1,3,4-triol (39d). In the same manner as described above, **38d** was converted to **39d** (86%) as colorless oil, n_D^{24} 1.5172; $[\alpha]_D^{25}$ +46.1 (c 1.86, $CHCl_3$); ν_{max} (film): 3460 (br s, OH), 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1090 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.41–7.20 (20H, m), 4.97 (1H, d, J = 11 Hz), 4.85 (1H, d, J = 12 Hz), 4.76 (1H, d, J = 12 Hz), 4.74 (1H, d, J = 12 Hz), 4.70 (1H, d, J = 12 Hz), 4.54 (1H, d, J = 11 Hz), 4.07 (1H, br s), 3.95–3.91 (2H, m), 3.85 (1H, d, J = 13 Hz), 3.79–3.74 (2H, m), 3.73 (1H, d, J = 13 Hz), 3.70–3.67 (1H, m), 3.66–3.61 (1H, m), 3.39 (1H, t, J = 8.5 Hz), 3.35 (1H, t, J = 8.5 Hz), 3.27 (3H, s), 3.17 (1H, dd, J = 8.5, 6.0 Hz), 2.81 (1H, br d, J = 8.5 Hz), 2.07–2.00 (1H, m), 1.79–1.70 (1H, m), 1.57–1.22 (30H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.4, 139.1, 138.7, 138.0, 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.44, 127.36, 127.32, 127.27, 81.7, 79.5, 76.8, 75.6, 75.4, 74.6, 73.8, 73.0, 72.9, 71.3, 67.2, 62.0, 58.6, 51.0, 35.7, 34.6, 31.9, 30.0, 29.8, 29.72, 29.69, 29.68, 29.63, 29.3, 27.1, 25.2, 22.7, 14.1 ppm; HR-ESIMS: calcd for $C_{54}H_{78}NO_7$ $[M+H]^+$ 852.5773; found 852.5768.

4.1.3.8. (2′*S*,3′*S*,4′*R*)-1D-(1,2,3/4,5)-2,3,4-Tri-*O*-benzyl-5-*O*-(2′-*N*-benzylamino-3′,4′-dihydroxyoctadecyl)-1-*O*-methylcyclohexanepentol (39e). In the same manner as described above, **38e** was converted to **39e** (85%) as a colorless oil, n_D^{23} 1.5175; $[\alpha]_D^{23}$ +43.0 (c 1.35, $CHCl_3$); ν_{max} (film): 3460 (br s, OH), 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1095 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.21 (20H, m), 4.852 (1H, d, J = 12 Hz), 4.847 (1H, d, J = 12 Hz), 4.78 (1H, d, J = 12 Hz), 4.72 (1H, d, J = 12 Hz), 4.68 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.11 (1H, br s), 3.98–3.93 (2H, m), 3.85 (1H, d, J = 13 Hz), 3.77 (1H, dd, J = 9.5, 2.5 Hz), 3.73 (1H, d, J = 13 Hz), 3.69–3.61 (3H, m), 3.38–3.32 (2H, m), 3.26 (3H, s), 2.81 (1H, br d, J = 8.5 Hz), 1.96–1.84 (2H, m), 1.79–1.73 (1H, m), 1.57–1.48 (1H, m), 1.41–1.20 (27H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.2, 139.1, 138.5, 137.8, 128.6, 128.43, 128.38, 128.3, 128.2, 128.1, 127.9, 127.63, 127.56, 127.43, 127.37, 127.3, 79.6, 79.1, 76.5, 75.5, 75.4, 74.9, 74.01, 73.96, 72.9, 71.2, 67.4, 62.1, 56.5, 51.1, 34.6, 31.9, 30.0, 29.9, 29.8, 29.72, 29.70, 29.69, 29.6, 29.3, 25.3, 22.7, 14.1 ppm; HR-FABMS: calcd for $C_{53}H_{76}NO_7$ $[M+H]^+$: 838.5622; found 838.5620.

4.1.4. Debenzylation and acylation

4.1.4.1. RCAI-37: (2′*S*,3′*S*,4′*R*)-2-*O*-(2′-Hexacosanoylamino-3′,4′-dihydroxyoctadecyl)-*myo*-inositol (1). To a stirred solution of **12** (253 mg, 0.337 mmol) in MeOH (15 mL) and cyclohexene (3 mL), a 1 M aqueous HCl solution (337 μL, 0.337 mmol) and Pd–C (Kawaken Fine Chemicals Co. Ltd, 10%, dry, 72 mg) were added at room temperature. After stirring under reflux for 8 h, the mixture was cooled to room temperature. It was then diluted with $CHCl_3$/MeOH (5:1) and filtered through a bed of Celite. The filtrate was concentrated in vacuo to give crude **13** (ca. 190 mg) as a colorless solid.

To a stirred solution of crude **13** in $CHCl_3$/MeOH (5:1, 30 mL), Et_3N (140 μL, 1.01 mmol) and a solution of cerotyl chloride (**14**, freshly prepared, 155 mg, 0.373 mmol) in THF (5 mL) were successively added at 0 °C. The resulting mixture was stirred at room temperature overnight, and then concentrated in vacuo. The residual solid was washed with water and MeOH/H_2O (1:2) solution for removing the ammonium salt, and then dried in the air. The solid was purified by column chromatography on silica gel (15 g, $CHCl_3$/MeOH = 50:7) to give **1** (112 mg, 39%, two steps) as colorless powder, mp 195.0–199.0 °C; $[\alpha]_D^{22}$ +1.95 (c 0.30, pyridine); ν_{max} (KBr): 3360 (br s, OH, NH), 1640 (br s, CO), 1540 (br s), 1045 (br s, C–O), 720 (br m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.68 (1H, d, J = 8.0 Hz), 7.02 (5H, br s), 6.44 (1H, br s), 5.69 (1H, br s), 5.05–4.98 (1H, m), 4.95 (1H, dd, J = 11, 4.0 Hz), 4.70 (1H, dd, J = 11, 4.0 Hz), 4.51 (2H, t, J = 9.5 Hz), 4.46 (1H, br s), 4.39 (1H, t, J = 6.0 Hz), 4.26 (1H, br t, J = 6.0 Hz), 4.11–4.06 (2H, m), 3.96 (1H, t, J = 9.5 Hz), 2.37 (2H, t, J = 7.0 Hz), 2.26–2.18 (1H, m), 1.97–1.85 (2H, m), 1.83–1.71 (2H, m), 1.71–1.59 (1H, m), 1.46–1.18 (66H, m), 0.85 (3H, t, J = 7.0 Hz), 0.84 (3H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.1, 85.8, 77.0, 75.8, 75.3, 75.0, 74.9, 73.81, 73.80, 72.7, 52.6, 36.8, 33.4, 32.11, 32.10, 30.4, 30.2, 30.1, 30.03, 30.02, 30.01, 29.99, 29.98, 29.96, 29.93, 29.90, 29.8, 29.7,

29.62, 29.59, 26.7, 26.4, 22.93, 22.92, 14.3 ppm; HR-ESIMS: calcd for $C_{50}H_{100}NO_9$ $[M+H]^+$ 858.7393; found 858.7387.

4.1.4.2. RCAI-102: (2′*S*,3′*S*,4′*R*)-2-*O*-(2′-Hexacosanoylamino-3′,4′-dihydroxyoctadecyl)-*neo*-inositol (2). In the same manner as described above, **22** was converted to **2** (12%, two steps) as colorless powder, mp 178.5–183.0 °C; $[\alpha]_D^{25}$ +4.56 (*c* 0.14, pyridine); ν_{max} (KBr): 3350 (br s, OH), 3240 (w, NH), 1620 (s, CO), 1535 (m), 1145 (m, C–O), 1040 (s, C–O), 730 (br m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.71 (1H, d, *J* = 8.0 Hz), 5.30 (7H, br s), 5.10–5.04 (1H, m), 4.95–4.89 (1H, m), 4.72 (1H, dd, *J* = 10, 3.0 Hz), 4.65 (1H, br s), 4.59 (2H, br t, *J* = 7.0 Hz), 4.47–4.38 (4H, m), 4.32–4.28 (1H, m), 2.41 (2H, t, *J* = 7.0 Hz), 2.33–2.22 (1H, m), 1.98–1.88 (2H, m), 1.79 (2H, quint., *J* = 7.0 Hz), 1.74–1.64 (1H, m), 1.48–1.16 (66H, m), 0.849 (3H, t, *J* = 7.0 Hz), 0.845 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.2, 76.2, 72.8, 72.4, 72.3, 72.2, 71.7, 52.6, 36.9, 33.6, 32.1, 30.4, 30.2, 30.1, 30.04, 30.02, 29.99, 29.98, 29.93, 29.90, 29.8, 29.7, 29.62, 29.60, 26.7, 26.4, 22.9, 14.3 ppm; HR-ESIMS: calcd for $C_{50}H_{99}NO_9Na$ $[M+Na]^+$ 880.7212; found 880.7209.

4.1.4.3. RCAI-56: (2*S*,3*S*,4*R*)-1-*O*-(5a-Carba-α-D-galactopyranosyl)-2-hexacosanoylaminooctadecane-1,3,4-triol (3). In the same manner as described above, **39a** was converted to **3** (60%, two steps) as colorless powder, mp 147.0–149.0 °C; $[\alpha]_D^{23}$ +27.8 (*c* 0.32, pyridine); ν_{max} (KBr): 3360 (br s, OH), 3280 (m, NH), 1640 (br s, CO), 1545 (br m), 1075 (br m, C–O), 720 (m) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 8.43 (1H, d, *J* = 8.4 Hz), 6.84 (1H, br s), 6.37 (1H, d, *J* = 6.4 Hz,), 6.30 (1H, br s), 6.07 (1H, d, *J* = 5.2 Hz), 6.00 (1H, br s), 5.97 (1H, t, *J* = 5.4 Hz), 5.21–5.18 (1H, m), 4.69 (1H, br s), 4.50 (1H, dd, *J* = 10, 4.0 Hz), 4.47–4.43 (1H, m), 4.34–4.18 (5H, m), 4.26 (1H, dd, *J* = 10, 5.2 Hz), 4.00 (1H, ddd-like, *J* = 9.6, 5.4, 4.8 Hz), 2.51–2.42 (1H, m), 2.44 (2H, t, *J* = 7.6 Hz), 2.33–2.24 (1H, m), 2.14–2.06 (1H, m), 2.00 (1H, br t, *J* = 13 Hz), 1.98–1.84 (2H, m), 1.82 (2H, quint.-like, *J* = 7.6 Hz), 1.76–1.67 (1H, m), 1.50–1.17 (66H, m), 0.85 (6H, t, *J* = 7.2 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 173.1, 80.2, 76.6, 73.6, 72.8, 72.6, 71.5, 70.6, 64.2, 51.5, 38.6, 36.8, 34.2, 32.1, 30.4, 30.2, 30.04, 30.01, 29.94, 29.89, 29.83, 29.7, 29.6, 26.6, 26.4, 22.9, 14.3 ppm; HR-FABMS: calcd for $C_{51}H_{102}NO_8$ $[M+H]^+$ 856.7605; found 856.7603.

4.1.4.4. RCAI-60: (2*S*,3*S*,4*R*)-1-*O*-(5a-Carba-α-D-fucopyranosyl)-2-hexacosanoylaminooctadecane-1,3,4-triol (4). In the same manner as described above, **39b** was converted to **4** (56%, two steps) as colorless powder, mp 125.5–127.0 °C; $[\alpha]_D^{23}$ +36.9 (*c* 0.24, pyridine); ν_{max} (KBr): 3440 (br s, OH), 3280 (s, NH), 1645 (s, CO), 1545 (br m), 1100 (br m, C–O), 1060 (br m, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.38 (1H, d, *J* = 8.5 Hz), 6.72 (1H, br s), 6.28 (1H, br s), 6.13 (1H, br s), 6.00 (1H, br s), 5.70 (1H, br s), 5.18–5.14 (1H, m), 4.44 (1H, dd, *J* = 10, 4.0 Hz), 4.34 (1H, dd, *J* = 10, 3.0 Hz), 4.33–4.27 (2H, m), 4.23 (1H, dd, *J* = 10, 4.0 Hz), 4.19 (dd, *J* = 10, 3.0 Hz), 4.12–4.08 (2H, m), 2.45 (2H, t, *J* = 8.0 Hz), 2.31–2.24 (1H, m), 2.16–2.08 (1H, m), 1.97–1.86 (2H, m), 1.86–1.74 (4H, m), 1.74–1.65 (1H, m), 1.48–1.18 (66H, m), 1.13 (3H, d, *J* = 7.0 Hz), 0.85 (6H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.0, 80.2, 76.6, 74.5, 73.6, 72.7, 72.3, 70.7, 51.5, 36.8, 34.2, 32.13, 32.12, 31.3, 30.4, 30.21, 30.16, 30.05, 30.03, 30.02, 30.01, 30.00, 29.98, 29.97, 29.94, 29.92, 29.89, 29.83, 29.7, 29.62, 29.61, 26.6, 26.5, 22.9, 18.1, 14.3 ppm; HR-FABMS: calcd for $C_{51}H_{102}NO_7$ $[M+H]^+$ 840.7656; found 840.7659.

4.1.4.5. RCAI-59: (2′*S*,3′*S*,4′*R*)-1-D-(1,2,3/4,5)-5-*O*-(2′-Hexacosanoylamino-3′,4′-dihydroxyoctadecyl)cyclohexanepentol (5). In the same manner as described above, **39c** was converted to **5** (51%, two steps) as colorless powder, mp 177.0–181.0 °C; $[\alpha]_D^{26}$ +21.2 (*c* 0.30, pyridine); ν_{max} (KBr): 3420 (br s, OH), 3300 (br s, NH), 1645 (br s, CO), 1540 (br m), 1095 (br s, C–O), 1080 (br s, C–O), 720 (m) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 8.45 (1H, d, *J* = 8.4 Hz), 6.88 (1H, br s), 6.53 (1H, br s), 6.36 (1H, d, *J* = 6.4 Hz), 6.23 (1H, br s), 6.08 (1H, d, *J* = 4.8 Hz), 6.01 (1H, br s), 5.18 (1H, sext.-like, *J* = 4.4 Hz), 4.60 (1H, br s), 4.57–4.42 (2H, m), 4.48 (1H, dd, *J* = 10, 4.4 Hz), 4.37–4.23 (4H, m), 4.27 (1H, dd, *J* = 10, 4.4 Hz), 2.44 (2H, t, *J* = 7.6 Hz), 2.41–2.22 (3H, m), 1.97–1.86 (2H, m), 1.82 (2H, quint.-like, *J* = 7.6 Hz), 1.75–1.63 (1H, m), 1.48–1.17 (66H, m), 0.85 (6H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 173.1, 78.6, 76.5, 74.1, 72.8, 72.7, 72.1, 70.6, 68.1, 51.5, 36.8, 34.2, 32.5, 32.1, 30.4, 30.2, 30.04, 30.01, 29.94, 29.88, 29.83, 29.7, 29.6, 26.6, 26.4, 22.9, 14.3 ppm; HR-FABMS: calcd for $C_{50}H_{100}NO_8$ $[M+H]^+$ 842.7449; found 842.7449.

4.1.4.6. RCAI-101: (2*S*,3*S*,4*R*)-2-Hexacosanoylamino-1-*O*-(6-*O*-methyl-5a-carba-α-D-galactopyranosyl)octadecane-1,3,4-triol (6). In the same manner as described above, **39d** was converted to **6** (69%, two steps) as colorless powder, mp 115.5–118.0 °C; $[\alpha]_D^{26}$ +24.4 (*c* 0.32, pyridine); ν_{max} (KBr): 3400 (br s, OH), 3280 (m, NH), 1640 (br s, CO), 1545 (br m), 1100 (br s, C–O), 1075 (br s, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.37 (1H, d, *J* = 8.5 Hz), 6.80 (1H, br s), 6.32–6.16 (2H, m), 6.00 (1H, br s), 5.86 (1H, br s), 5.17–5.12 (1H, m), 4.47–4.43 (2H, m), 4.38 (1H, dd, *J* = 9.5, 3.0 Hz), 4.32–4.26 (2H, m), 4.21 (1H, dd, *J* = 9.5, 4.5 Hz), 4.21–4.14 (2H, m), 3.71 (1H, t, *J* = 8.5 Hz), 3.39 (1H, dd, *J* = 8.5, 6.5 Hz), 3.25 (3H, s), 2.46–2.39 (1H, m), 2.42 (2H, t, *J* = 7.0 Hz), 2.28–2.21 (1H, m), 1.97–1.85 (3H, m), 1.85–1.78 (3H, m), 1.72–1.62 (1H, m), 1.48–1.18 (66H, m), 0.85 (6H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.0, 79.9, 76.5, 74.5, 73.4, 72.6, 70.6, 58.5, 51.4, 36.8, 36.0, 34.1, 32.1, 30.4, 30.1, 30.02, 29.99, 29.96, 29.92, 29.91, 29.86, 29.8, 29.7, 29.61, 29.60, 26.6, 26.5, 26.4, 22.9, 14.3 ppm; HR-ESIMS: calcd for $C_{52}H_{104}NO_8$ $[M+H]^+$ 870.7756; found 870.7754.

4.1.4.7. RCAI-92: (2′*S*,3′*S*,4′*R*)-1-D-(1,2,3/4,5)-5-*O*-(2′-Hexacosanoylamino-3′,4′-dihydroxyoctadecyl)-1-*O*-methylcyclohexanepentol (7). In the same manner as described above, **39e** was converted to **7** (79%, two steps) as colorless powder, mp 143.5–145.5 °C; $[\alpha]_D^{23}$ +19.0 (*c* 0.26, pyridine); ν_{max} (KBr): 3440 (br s, OH), 3280 (br s, NH), 1640 (s, CO), 1540 (br m), 1065 (br s, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.42 (1H, d, *J* = 8.5 Hz), 6.91 (1H, br s), 6.34 (1H, br d, *J* = 7.0 Hz), 6.24 (1H, br s), 6.04 (2H, br s), 5.17 (1H, sext.-like, *J* = 4.0 Hz), 4.64 (1H, br s), 4.49 (1H, dd, *J* = 10, 4.5 Hz), 4.46 (1H, dd, *J* = 10, 3.0 Hz), 4.34–4.26 (3H, m), 4.27 (1H, dd, *J* = 10, 5.0 Hz), 4.16 (br d, *J* = 3.0 Hz), 3.78–3.74 (1H, m), 3.32 (3H, m), 2.46 (2H, dt, *J* = 7.5, 3.5 Hz), 2.32–2.18 (3H, m), 1.97–1.86 (2H, m), 1.83 (2H, quint. *J* = 7.5 Hz), 1.74–1.65 (1H, m), 1.47–1.18 (66H, m), 0.85 (6H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 173.0, 78.7, 77.2, 76.5, 72.6, 72.3, 72.2, 70.9, 70.2, 55.9, 51.5, 36.8, 34.2, 32.12, 32.11, 30.4, 30.2, 30.02, 30.00, 29.99, 29.97, 29.93, 29.91, 29.87, 29.83, 29.7, 29.62, 29.60, 29.1, 26.6, 26.4, 22.9, 14.3 ppm; HR-FABMS: calcd for $C_{51}H_{102}NO_8$ $[M+H]^+$ 856.7605; found 856.7602.

4.1.5. (2,6/3,4,5)-2,6-Di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3,5-*O*-methylenepentahydroxycyclohexanone (16)

To a stirred solution of **15** (175 mg, 0.360 mmol) in CH_2Cl_2 (6 mL), Dess–Martin periodinane (316 mg, 0.75 mmol) was added at 0 °C. After stirring at room temperature for 30 min, the reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (5 mL) and saturated aqueous $NaHCO_3$ solution (5 mL). The resulting mixture was diluted with Et_2O, and the separated aqueous phase was extracted with Et_2O. The combined organic phase was successively washed with water, 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 (10 g, hexane/EtOAc = 25:1) to give **16** (108 mg, 62%) as a colorless oil, n_D^{24} 1.5162; ν_{max} (film): 1725 (s, C=O), 1610 (w), 1585 (w), 1500 (m), 1255 (m, *t*-Bu, SiMe), 1150 (s, C–O), 1115 (br s, C–O), 1030 (s, C–

O), 870 (s), 740 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.31–7.23 (10H, m), 5.53 (1H, d, J = 4.5 Hz), 5.05 (1H, br t, J = 1.5 Hz), 4.64 (2H, d, J = 12 Hz), 4.57 (1H, d, J = 4.5 Hz), 4.50 (2H, d, J = 12 Hz), 4.27 (2H, dt, J = 2.5, 1.5 Hz), 3.86 (2H, br d, J = 4.0 Hz), 0.93 (9H, s) 0.15 (6H, s) ppm; δ_C (126 MHz, $CDCl_3$): 203.0, 137.1, 128.3, 127.8, 127.6, 105.3, 85.3, 81.9, 75.2, 72.3, 64.1, 25.7, 18.0, −4.8 ppm; HR-ESIMS: calcd for $C_{27}H_{36}O_6SiNa$ $[M+Na]^+$ 507.2173; found 507.2171.

4.1.6. 4,6-Di-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-1,3-*O*-methylene-*neo*-inositol (17)

To a stirred solution of **16** (327 mg, 0.675 mmol) in dry THF (10 mL), a solution of lithium tri-*sec*-butylborohydride (1.0 M in THF, 1.35 mL, 1.35 mmol) was added at −78 °C. After stirring at −78 °C for 30 min, the reaction was quenched with 34% aqueous H_2O_2 solution (1 mL) and 3 M aqueous NaOH solution (1 mL) at that temperature. The resulting mixture was stirred at room temperature for 1 h. The mixture was then diluted with Et_2O, and the separated organic phase was successively washed with water, 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 = 10:1) to give **17** (325 mg, 99%) as a colorless oil, n_D^{22} 1.5176; ν_{max} (film): 3560 (m, OH), 1610 (w), 1585 (w), 1495 (m), 1255 (m, *t*-Bu, SiMe), 1150 (s, C–O), 1105 (br s, C–O), 1015 (s, C–O), 870 (s), 840 (s), 735 (s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.32–7.26 (10H, m), 5.56 (1H, d, J = 4.5 Hz), 4.66 (4H, s), 4.600 (1H, d, J = 4.5 Hz), 4.598 (1H, d, J = 1.5 Hz), 4.40 (1H, dt, J = 12, 4.5 Hz), 4.14 (2H, br d, J = 3.0 Hz), 3.90 (2H, br t, J = 4.5 Hz), 2.81 (1H, d, J = 12 Hz), 0.88 (9H, s) 0.05 (6H, s) ppm; δ_C (126 MHz, $CDCl_3$): 138.2, 128.4, 127.7, 127.4, 85.3, 79.9, 73.9, 73.1, 64.3, 63.7, 25.7, 17.9, −4.8 ppm; HR-ESIMS: calcd for $C_{27}H_{38}O_6SiNa$ $[M+Na]^+$ 509.2330; found 509.2327.

4.1.7. Benzylation

4.1.7.1. 4,5,6-Tri-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-1,3-*O*-methylene-*neo*-inositol (18). To a stirred solution of **17** (285 mg, 0.586 mmol) in THF (5 mL) and DMF (5 mL), NaH (60% mineral oil suspension, 51 mg, 1.3 mmol) was added at 0 °C. After stirring at 0 °C for 30 min, BnBr (105 μL, 0.883 mmol) and $(n\text{-Bu})_4NI$ (0.04 g, catalytic amount) were added to this mixture at 0 °C. After stirring at room temperature for 50 min, the reaction was quenched with water. The mixture was diluted with EtOAc, the separated aqueous phase was extracted with EtOAc. The combined organic phase was successively washed with water, 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 **18** (325 mg, 96%) as a colorless oil, n_D^{23} 1.5174; ν_{max} (film): 1610 (w), 1585 (w), 1495 (m), 1255 (m, *t*-Bu, SiMe), 1110 (br s, C–O), 1015 (br s, C–O), 870 (s), 735 (s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.35–7.23 (15H, m), 5.59 (1H, d, J = 4.0 Hz), 4.91 (2H, d, J = 13 Hz), 4.79 (1H, t, J = 1.5 Hz), 4.67 (2H, d, J = 13 Hz), 4.571 (2H, s), 4.570 (1H, d, J = 4.0 Hz), 4.29 (1H, t, J = 4.0 Hz), 4.14 (2H, br d, J = 4.0 Hz), 4.06 (2H, br t, J = 4.0 Hz), 0.89 (9H, s) 0.08 (6H, s) ppm; δ_C (126 MHz, $CDCl_3$): 139.0, 138.7, 128.3, 128.1, 127.5, 127.4, 127.3, 127.2, 85.4 (d, $\Delta\delta$ = 0.02), 77.2, 74.4, 74.3, 73.3 (t, $\Delta\delta$ = 0.03), 71.6 (t, $\Delta\delta$ = 0.01), 63.9 (d, $\Delta\delta$ = 0.04), 25.8, 17.9, −4.7 ppm; HR-ESIMS: calcd for $C_{34}H_{44}O_6SiNa$ $[M+Na]^+$ 599.2799; found 599.2799.

4.1.7.2. *tert*-Butyldimethylsilyl 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-galactopyranoside (30). In the same manner as described above, **29** was converted into **30** (91%) as a colorless oil, n_D^{20} 1.5172; $[\alpha]_D^{25}$ +31.8 (*c* 1.24, $CHCl_3$); ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1255 (m, *t*-Bu, SiMe), 1095 (br s, C–O), 835 (s), 735 (br s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.38–7.20 (20 H, m), 4.99 (1H, br d, J = 11 Hz), 4.79 (1H, d, J = 12 Hz), 4.73 (1H, d, J = 12 Hz), 4.72 (1H, d, J = 12 Hz), 4.68 (1H, d, J = 12 Hz), 4.50 (1H, d, J = 11 Hz), 4.44 (2H, s), 4.18 (1H, br s), 4.12 (1H, br s), 3.87 (1H, dd, J = 10, 1.6 Hz), 3.80 (1H, dd, J = 10, 2.0 Hz), 3.52 (1H, t, J = 9.2 Hz), 3.30 (1H, dd, J = 9.2, 6.0 Hz), 2.35–2.26 (1H, m), 1.58–1.49 (1H, m), 1.44–1.36 (1H, m), 0.86 (9H, s), 0.03 (3H, s), 0.02 (3H, s) ppm; δ_C (100 MHz, $CDCl_3$): 139.8, 139.4, 139.3, 138.4, 128.4, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.4, 127.1, 127.0, 80.8, 79.9, 76.3, 74.5, 73.0, 72.8, 72.6, 70.9, 68.7, 35.3, 30.4, 25.8, 18.2, −4.5, −5.0 ppm; HR-FABMS: calcd for $C_{41}H_{52}O_5SiNa$ $[M+Na]^+$ 675.3482; found 675.3479.

4.1.7.3. 1D-(1,2,3/4,5)-1,2,3,4-Tetra-*O*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)cyclohexanepentol (34). In the same manner as described above, **33** was converted into **34** (quant.) as a colorless oil (contaminated with unreacted BnBr). This was immediately used in the next step without further purification. An analytical sample (pure **34**) was obtained as a colorless solid by further column chromatography on silica gel, mp 61.0–63.5 °C; $[\alpha]_D^{23}$ +14.0 (*c* 0.70, $CHCl_3$); ν_{max} (KBr): 1605 (w), 1585 (w), 1495 (m), 1250 (m, *t*-Bu, SiMe), 1100 (br s, C–O), 1070 (br s, C–O), 835 (br s), 745 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.42–7.23 (20 H, m), 4.90 (1H, d, J = 12 Hz), 4.85 (1H, d, J = 12 Hz), 4.74 (1H, d, J = 12 Hz), 4.73 (1H, d, J = 12 Hz), 4.67 (1H, d, J = 12 Hz), 4.64 (1H, d, J = 12 Hz), 4.50 (1H, d, J = 13 Hz), 4.44 (1H, d, J = 13 Hz), 4.17 (1H, br s), 4.11 (1H, br s), 3.83 (1H, dd, J = 9.5, 2.5 Hz), 3.77–3.73 (1H, m), 3.74 (1H, dd, J = 9.5, 2.5 Hz), 2.00 (1H, ddd, J = 13, 12, 2.5 Hz), 1.85 (1H, ddt, J = 13, 4.5, 1.5 Hz), 0.79 (9H, s), −0.01 (3H, s), −0.02 (3H, s) ppm; δ_C (126 MHz, $CDCl_3$): 139.6, 139.15, 139.10, 138.6, 128.3, 128.2, 128.03, 128.02, 127.7, 127.54, 127.52, 127.43, 127.40, 127.2, 127.10, 127.08, 79.6, 78.6, 76.3, 74.4, 73.9, 72.8, 72.7, 70.6, 67.6, 33.0, 25.7, 18.0, −4.6, −5.2 ppm; HR-EIMS: calcd for $C_{40}H_{50}O_5Si$ $[M]^+$: 638.3428; found 638.3425.

4.1.8. Desilylation

4.1.8.1. 4,5,6-Tri-*O*-benzyl-1,3-*O*-methylene-*neo*-inositol (19). To a stirred solution of **18** (297 mg, 0.515 mmol) in THF (6 mL), a solution of TBAF (1.0 M in THF, 1.6 mL, 1.6 mmol) was added at room temperature. After stirring at room temperature for 1 h, the reaction was quenched with water. The resulting mixture was diluted with EtOAc, and the separated aqueous phase was extracted with EtOAc. The combined organic phase was successively washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 5:1) to give **19** (232 mg, 97%) as a sticky colorless oil, n_D^{24} 1.5170; ν_{max} (film): 3460 (br s, OH), 1605 (w), 1585 (w), 1495 (m), 1130 (br s, C–O), 1015 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.35–7.23 (15H, m), 5.51 (1H, d, J = 5.0 Hz), 4.94 (2H, d, J = 12 Hz), 4.74 (1H, br d, J = 5.0 Hz), 4.66 (2H, d, J = 12 Hz), 4.61 (1H, d, J = 5.0 Hz), 4.59 (2H, s), 4.32 (1H, t, J = 4.0 Hz), 4.22 (2H, br d, J = 4.0 Hz), 4.10 (2H, br t, J = 4.0 Hz), 2.09 (1H, br d, J = 5.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 138.8, 138.5, 128.3, 128.2, 127.7, 127.49, 127.46, 127.2, 85.4, 77.2, 74.5, 73.8, 73.5, 71.7, 63.3 ppm; HR-ESIMS: calcd for $C_{28}H_{30}O_6Na$ $[M+Na]^+$ 485.1935; found 485.1931.

4.1.8.2. 2,3,4,6-Tetra-*O*-benzyl-5a-carba-α-D-galactopyranose (37a). In the same manner as described above, **30** was converted into **37a** (77%) as a colorless oil, n_D^{24} 1.5176; $[\alpha]_D^{25}$ +21.3 (*c* 1.09, $CHCl_3$); ν_{max} (film): 3460 (br s, OH), 1605 (w), 1585 (w), 1495 (s), 1095 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.40–7.21 (20H, m), 4.97 (1H, d, J = 11 Hz), 4.781 (1H, d, J = 12 Hz), 4.775 (1H, d, J = 12 Hz), 4.74 (1H, d, J = 12 Hz), 4.69 (1H, d, J = 12 Hz), 4.52 (1H, d, J = 11 Hz), 4.44 (2H, s) 4.19–4.13 (2H, m), 3.91 (1H, dd, J = 9.2, 2.8 Hz), 3.77 (1H, dd, J = 9.2, 2.0 Hz), 3.49 (1H, t, J = 9.2 Hz), 3.30 (1H, dd, J = 9.2, 5.2 Hz), 2.53 (1H, s), 2.33–2.23 (1H, m), 1.62–1.51 (2H, m) ppm; δ_C (100 MHz, $CDCl_3$):

139.5, 139.0, 138.6, 138.4, 128.4, 128.3, 128.1, 127.79, 127.75, 127.66, 127.61, 127.58, 127.4, 127.33, 127.25, 81.1, 80.1, 75.6, 74.5, 73.0, 72.9, 72.8, 70.6, 67.3, 35.1, 27.6 ppm; HR-FABMS: calcd for $C_{35}H_{39}O_5$ $[M+H]^+$ 539.2797; found 539.2797.

4.1.8.3. 2,3,4-Tri-*O*-benzyl-5a-carba-α-D-fucopyranose (37b). In the same manner as described above, **32** was converted into **37b** (98%) as a colorless oil, n_D^{25} 1.5170; $[\alpha]_D^{24}$ +46.2 (*c* 1.46, $CHCl_3$); ν_{max} (film): 3460 (br s, OH), 1605 (w), 1585 (w), 1495 (s), 1070 (br s, C–O), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.40–7.23 (15H, m), 5.00 (1H, d, *J* = 12 Hz), 4.78 (2H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.58 (1H, d, *J* = 12 Hz), 4.12 (1H, br d, *J* = 3.0 Hz), 3.90 (1H, ddd, *J* = 9.5, 3.5, 2.0 Hz), 3.79–3.74 (2H, m), 2.53 (1H, br s), 2.06–1.97 (1H, m), 1.66 (1H, br t, *J* = 13 Hz), 1.64–1.57 (1H, m), 0.97 (3H, d, *J* = 7.0 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.5, 139.1, 138.6, 128.4, 128.3, 128.1, 127.8, 127.6, 127.4, 127.3, 127.2, 81.3, 80.3, 80.0, 74.6, 72.9, 72.8, 67.6, 32.5, 29.5, 17.5 ppm; HR-FABMS: calcd for $C_{28}H_{32}O_4Na$ $[M+Na]^+$ 455.2198; found 455.2196.

4.1.8.4. 1D-(1,2,3/4,5)-1,2,3,4-Terta-*O*-benzylcyclohexanepentol (37c). In the same manner as described above, **34** was converted to **37c** (76%, two steps) as a colorless solid, mp 69.0–71.0 °C; $[\alpha]_D^{23}$ +21.8 (*c* 1.27, $CHCl_3$); ν_{max} (KBr): 3550 (s, OH), 1605 (w), 1585 (w), 1500 (m), 1095 (br s, C–O), 1060 (s, C–O), 1045 (s, C–O), 735 (s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.42–7.22 (20H, m), 4.87 (1H, d, *J* = 13 Hz), 4.84 (1H, d, *J* = 13 Hz), 4.80 (1H, d, *J* = 12 Hz), 4,69 (1H, d, *J* = 12 Hz), 4.67 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.53 (1H, d, *J* = 12 Hz), 4.49 (1H, d, *J* = 12 Hz), 4.14–4.17 (2H, m), 3.94 (1H, dd, *J* = 9.2, 3.2 Hz), 3.79 (1H, ddd, *J* = 11, 4.4, 2.0 Hz), 3.68 (1H, dd, *J* = 9.2, 2.4 Hz), 2.51 (1H, br s), 2.16–2.02 (2H, m) ppm; δ_C (100 MHz, $CDCl_3$): 139.3, 138.8, 138.6, 138.4, 128.4, 128.33, 128.29, 128.1, 127.76, 127.75, 127.72, 127.47, 127.46, 127.43, 127.3, 127.2, 79.7, 78.9, 75.9, 74.8, 73.8, 73.1, 72.5, 71.0, 66.3, 30.2 ppm; HR-EIMS: calcd for $C_{34}H_{36}O_5$ $[M]^+$ 524.2563; found 524.2563.

4.1.8.5. 2,3,4-Tri-*O*-benzyl-6-*O*-methyl-5a-carba-α-D-galactopyranose (37d). In the same manner as described above, **35** was converted into **37d** (97%) as a colorless oil, n_D^{24} 1.5177; $[\alpha]_D^{24}$ +39.0 (*c* 1.34, $CHCl_3$); ν_{max} (film): 3440 (br s, OH), 1605 (w), 1585 (w), 1495 (m), 1090 (br s, C–O), 1070 (br s, C–O), 735 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.24 (15H, m), 4.98 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.57 (1H, d, *J* = 12 Hz), 4.16 (1H, q, *J* = 3.0 Hz), 4.13 (1H, br s), 3.91 (1H, dd, *J* = 9.5, 3.0 Hz), 3.76 (1H, dd, *J* = 9.5, 2.5 Hz), 3.39 (1H, t, *J* = 9.0 Hz), 3.28 (3H, s), 3.20 (1H, dd, *J* = 9.0, 5.0 Hz), 2.55 (1H, br s), 2.26–2.19 (1H, m), 1.63–1.55 (2H, m) ppm; δ_C (126 MHz, $CDCl_3$): 139.5, 139.0, 138.6, 128.35, 128.27, 128.1, 127.8, 127.6, 127.4, 127.33, 127.25, 81.0, 80.1, 75.5, 74.5, 73.0, 72.9, 72.7, 67.3, 58.6, 34.9, 27.5 ppm; HR-ESIMS: calcd for $C_{29}H_{34}O_5Na$ $[M+Na]^+$ 485.2298; found 485.2296.

4.1.8.6. 1D-(1,2,3/4,5)-2,3,4-Tri-*O*-benzyl-1-*O*-methylcyclohexanepentol (37e). In the same manner as described above, **36** was converted into **37e** (96%, two steps) as a colorless solid, mp 65.5–67.0 °C; $[\alpha]_D^{22}$ +31.5 (*c* 1.49, $CHCl_3$); ν_{max} (KBr): 3550 (s, OH), 1605 (w), 1585 (w), 1500 (s), 1100 (br s, C–O), 1060 (br s, C–O), 760 (s), 735 (s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.41–7.23 (15H, m), 4.85 (1H, d, *J* = 12 Hz), 4.81 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.67 (1H, d, *J* = 12 Hz), 4.66 (1H, d, *J* = 12 Hz), 4.14 (2H, br s), 3.92 (1H, dd, *J* = 9.5, 3.5 Hz), 3.68 (1H, dd, *J* = 9.5, 2.5 Hz), 3.57–3.52 (1H, m), 3.30 (3H, s), 2.53 (1H, br s), 2.07 (1H, br d, *J* = 13 Hz), 1.96 (1H, dt, *J* = 13, 2.5 Hz) ppm; δ_C (126 MHz, $CDCl_3$): 139.4, 138.9, 138.5, 128.4, 128.3, 128.1, 127.84, 127.78, 127.76, 127.53, 127.48, 127.3, 79.7, 79.0, 76.5, 75.3, 73.9, 73.2, 72.6, 66.3, 56.7, 30.0 ppm; HR-FABMS: calcd for $C_{28}H_{33}O_5$ $[M+H]^+$ 449.2328; found 449.2321.

4.1.9. 2D-(2,3/4,5)-2,3,4-Tri-*O*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)tetrahydroxycyclohexanone (27)

To a stirred solution of **26** (2.17 g, 5.02 mmol) in dry CH_2Cl_2 (60 mL) and 2,6-lutidine (10 mL), TBSOTf (1.50 mL, 6.53 mmol) was added at –20 °C. The mixture was stirred at –20 °C for 1 h, the reaction was then quenched with MeOH (2 mL). The resulting mixture was concentrated in vacuo, and the residue was diluted with EtOAc. The organic phase was successively washed with 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 (80 g, hexane/EtOAc = 15:1) to give **27** (2.44 g, 89%) as a colorless oil, n_D^{21} 1.5176; $[\alpha]_D^{25}$ +60.8 (*c* 0.92, $CHCl_3$); ν_{max} (film): 1730 (s, C=O), 1605 (w), 1585 (w), 1495 (m), 1255 (m, *t*-Bu, SiMe), 1110 (br s, C–O), 840 (br s), 780 (s), 740 (s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.35–7.18 (15H, m), 4.86 (1H, d, *J* = 12 Hz), 4.84 (1H, d, *J* = 13 Hz), 4.76 (1H, d, *J* = 13 Hz), 4.56 (1H, d, *J* = 12 Hz), 4.52 (1H, d, *J* = 13 Hz), 4.44 (1H, d, *J* = 12 Hz), 4.38 (1H, d, *J* = 4.0 Hz), 4.35 (1H, ddd, *J* = 11, 5.2, 2.4 Hz), 3.96 (1H, dd, *J* = 4.4, 4.0 Hz), 3.79–3.75 (1H, m), 2.80 (1H, dd, *J* = 13, 11 Hz), 2.56 (1H, dd, *J* = 13, 5.2 Hz), 0.88 (9H, s), 0.06 (6H, s) ppm; δ_C (100 MHz, $CDCl_3$): 205.0, 138.4, 138.1, 137.9, 128.35, 128.32, 128.29, 127.8, 127.7, 127.6, 127.5, 81.3, 78.6, 78.3, 73.9, 73.4, 72.5, 69.8, 45.3, 25.7, 18.0, –4.8, –4.9 ppm; HR-FABMS: calcd for $C_{33}H_{42}O_5SiNa$ $[M+Na]^+$ 569.2699; found 569.2719.

4.1.10. *tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-6-deoxy-5a-carba-α-L-*arabino*-hex-5-enopyranoside (28)

To a stirred solution of **27** (645 mg, 1.18 mmol) in THF (15 mL) and pyridine (4 mL), a solution of Tebbe reagent (0.5 M in toluene, 4.7 mL, 2.4 mmol) was added at –40 °C. The mixture was stirred at room temperature for 1 h, then cooled to –40 °C. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (30 drops), the resulting mixture was then stirred at room temperature for 30 min. The mixture was diluted with Et_2O and filtered through a bed of Celite. The filtrate was concentrated in vacuo, and the residue was diluted with EtOAc. The organic phase was successively washed with water, saturated aqueous $CuSO_4$ solution, water, 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 (40 g, hexane/EtOAc = 20:1) to give **28** (566 mg, 88%) as a pale yellow oil, n_D^{16} 1.5169; $[\alpha]_D^{26}$ +22.1 (*c* 1.08, $CHCl_3$); ν_{max} (film): 1660 (m, C=C), 1605 (w), 1585 (w), 1495 (m), 1250 (s, *t*-Bu, SiMe), 1100 (br s, C–O), 890 (m), 840 (s), 780 (s), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.34–7.18 (15H, m), 4.95 (2H, d, *J* = 12 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.66 (1H, d, *J* = 12 Hz), 4.63 (1H, d, *J* = 12 Hz), 4.60 (1H, d, *J* = 13 Hz), 4.59 (1H, d, *J* = 12 Hz), 4.36 (1H, d, *J* = 13 Hz), 4.12 (1H, br s), 4.07 (1H, s), 3.82–3.78 (2H, m), 2.39 (1H, dd, *J* = 13, 2.4 Hz), 2.28–2.17 (1H, m), 0.81 (9H, s), 0.03 (3H, s), 0.01 (3H, s) ppm; δ_C (100 MHz, $CDCl_3$): 141.4, 139.2, 139.0, 138.6, 128.2, 128.12, 128.07, 127.7, 127.5, 127.31, 127.27, 127.2, 79.5, 78.9, 78.5, 73.2, 72.5, 69.8, 69.3, 37.2, 25.8, 18.1, –4.6, –4.9 ppm; HR-FABMS: calcd for $C_{34}H_{44}O_4SiNa$ $[M+Na]^+$ 567.2907; found 567.2907.

4.1.11. *tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-5a-carba-α-D-galactopyranoside (29)

To a stirred solution of **28** (1.85 g, 3.40 mmol) in THF (40 mL), a solution of BH_3·THF complex (1.03 M in THF, 16.5 mL, 17.0 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature for 3.5 h, then cooled to 0 °C. To this mixture, a 3 M aqueous NaOH solution (12 mL) and a 30% aqueous H_2O_2 solution (12 mL) were successively added to this mixture at 0 °C. The

resulting mixture was stirred at 0 °C for 3 h. The mixture was then diluted with Et_2O. The separated organic phase was successively washed with water, saturated aqueous $NaHCO_3$ solution, saturated aqueous $Na_2S_2O_3$ solution, water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (40 g, hexane/EtOAc = 10:1) to give **29** (1.60 g, 84%) as a colorless oil, n_D^{20} 1.5177; $[\alpha]_D^{26}$ +27.9 (*c* 1.18, $CHCl_3$); ν_{max} (film): 3450 (s, OH), 1605 (w), 1585 (w), 1495 (s), 1250 (s, *t*-Bu, SiMe), 1090 (br s, C–O), 835 (br s), 775 (s), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.38–7.24 (15H, m), 5.02 (1H, d, *J* = 12 Hz), 4.83 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.72 (2H, s), 4.64 (1H, d, *J* = 12 Hz), 4.25–4.22 (1H, m), 4.08 (1H, br s), 3.88 (1H, dd, *J* = 10, 2.4 Hz), 3.80 (1H, dd, *J* = 10, 2.4 Hz), 3.58–3.53 (2H, m), 2.10–2.02 (1H, m), 1.75 (1H, br s), 1.69 (1H, br t, *J* = 13 Hz), 1.46–1.38 (1H, m), 0.85 (9H, s), 0.04 (3H, s), 0.02 (3H, s) ppm; δ_C (100 MHz, $CDCl_3$): 139.2, 139.1, 139.0, 128.4, 128.3, 128.2, 128.1, 127.7, 127.6, 127.3, 127.1, 80.6, 79.9, 77.5, 74.0, 73.3, 72.6, 68.5, 64.6, 36.9, 30.1, 25.8, 18.1, –4.5, –4.9 ppm; HR-FABMS: calcd for $C_{34}H_{46}O_5SiNa$ $[M+Na]^+$ 585.3012; found 585.3011.

4.1.12. *tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-6-*O*-methanesulfonyl-5a-carba-α-D-galactopyranoside (31)

To a stirred solution of **29** (710 mg, 1.26 mmol) in pyridine (20 mL), methanesulfonyl chloride (295 μL, 3.81 mmol) was added at 0 °C. After stirring at room temperature for 18 h, the reaction was quenched with water. The resulting mixture was diluted with EtOAc, the separated organic phase was successively washed with water, saturated aqueous $CuSO_4$ solution, water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residual **31** (726 mg, 90%) was used in the next step without further purification. ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1360 (br s, SO_2), 1255 (br m, *t*-Bu, SiMe), 1090 (br s, C–O), 945 (br s), 835 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.24 (15H, m), 5.04 (1H, br s), 4.82 (1H, br d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.71 (2H, s), 4.55 (1H, d, *J* = 12 Hz), 4.25–4.18 (2H, m), 4.08–3.99 (1H, m), 4.01 (1H, br s), 3.88 (1H, dd, *J* = 9.5, 2.0 Hz), 3.80 (1H, dd, *J* = 9.5, 2.0 Hz), 2.90 (3H, s), 2.38 (1H, br s), 1.63–1.44 (2H, m), 0.86 (9H, s), 0.05 (3H, s), 0.03 (3H, s) ppm.

4.1.13. *tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-5a-carba-α-D-fucopyranoside (32)

To a stirred solution of crude **31** (726 mg, 1.13 mmol) in dry THF (15 mL), a solution of $LiBEt_3H$ (1.06 M in THF, 16.0 mL, 17.0 mmol) was added at 0 °C. After stirring at room temperature for 15 h, the mixture was poured into water at 0 °C. To the resulting mixture, 3 M aqueous NaOH solution (7 mL) and 30% aqueous H_2O_2 solution (7 mL) were added dropwise at 0 °C. After stirring at 0 °C for 1 h, the mixture was extracted with Et_2O. The combined organic phase was successively washed with water, 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 = 40:1) to give **32** (524 mg, 84%, two steps) as a colorless oil, n_D^{24} 1.5177; $[\alpha]_D^{22}$ +47.6 (*c* 1.38, $CHCl_3$); ν_{max} (film): ν_{max} 1605 (w), 1585 (w), 1495 (m), 1250 (s, *t*-Bu, SiMe), 1085 (br s, C–O), 835 (s), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.22 (15H, m), 5.00 (1H, br d, *J* = 12 Hz), 4.81 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.70 (2H, s), 4.57 (1H, d, *J* = 12 Hz), 4.16–4.14 (1H, m), 3.87 (1H, dd, *J* = 9.5, 2.5 Hz), 3.79 (1H, dd, *J* = 9.5, 2.5 Hz), 3.71 (1H, br s), 2.08–2.00 (1H, m), 1.59 (1H, dt, *J* = 13, 2.0 Hz), 1.38 (1H, br d, *J* = 13 Hz), 0.94 (3H, d, *J* = 7.0 Hz), 0.86 (9H, s), 0.03 (3H, s), 0.01 (3H, s) ppm; δ_C (126 MHz, $CDCl_3$): 139.8, 139.5, 139.4, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.1, 127.0, 81.0, 80.9, 79.9, 74.6, 72.9, 72.5, 69.0, 35.4, 29.7, 25.8, 18.2, 17.5, –4.5, –5.0 ppm; HR-FABMS: calcd for $C_{34}H_{46}O_4SiNa$ $[M+Na]^+$ 569.3063; found 569.3063.

4.1.14. 1D-(1,2,3/4,5)-2,3,4-Tri-*O*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)cyclohexanepentol (33)

To a stirred solution of **27** (553 mg, 1.01 mmol) in EtOH (20 mL), sodium borohydride (125 mg, 3.30 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 30 min and at room temperature for 30 min. The reaction was then quenched with water. The mixture was concentrated in vacuo, and the residue was diluted with EtOAc. The organic phase was successively washed with water, 1 M aqueous HCl solution, water, 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 = 40:3) to give **33** (495 mg, 89%) as a colorless oil, n_D^{26} 1.5174; $[\alpha]_D^{26}$ +35.8 (*c* 1.29, $CHCl_3$); ν_{max} (film): 3460 (br m, OH), 1610 (w), 1585 (w), 1495 (s), 1255 (m, *t*-Bu, SiMe), 1090 (br s, C–O), 1070 (br s, C–O), 830 (br s), 735 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.36–7.24 (15H, m), 4.85–4.57 (4H, m), 4.76 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.23 (1H, br s), 4.04 (1H, br s), 3.93–3.88 (2H, m), 3.78–3.72 (1H, m), 1.92–1.68 (2H, m), 1.62 (1H, s), 0.89 (9H, s), 0.07 (6H, s) ppm; δ_C (126 MHz, $CDCl_3$): 138.9, 138.8, 128.4, 128.3, 128.2, 127.8, 127.7, 127.6, 127.5, 127.3, 79.1, 73.6, 73.1, 67.1, 36.1, 25.8, 18.1, –4.7, –4.9 ppm; HR-FABMS: calcd for $C_{33}H_{44}O_5SiNa$ $[M+Na]^+$ 571.2856; found 571.2851.

4.1.15. Methylation

4.1.15.1. *tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-5a-carba-α-D-galactopyranoside (35). To a stirred solution of **29** (734 mg, 1.30 mmol) in THF (10 mL) and DMF (10 mL), NaH (60% mineral oil suspension, 258 mg, 6.45 mmol) was added at 0 °C. After stirring at 0 °C for 10 min, MeI (400 μL, 6.42 mmol) was added to this mixture at 0 °C. After stirring at room temperature overnight, the reaction was quenched with water. The mixture was diluted with EtOAc, the separated aqueous phase was extracted with EtOAc. The combined organic phase was successively washed with water, 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 (15 g, hexane/EtOAc = 20:1) to give **35** (736 mg, 98%) as a colorless oil, n_D^{22} 1.5174; $[\alpha]_D^{23}$ +44.0 (*c* 1.10, $CHCl_3$); ν_{max} (film): 1610 (w), 1585 (w), 1495 (m), 1250 (m, *t*-Bu, SiMe), 1090 (br s, C–O), 830 (br s), 735 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.37–7.23 (15H, m), 4.99 (1H, br d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.69 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.20–4.17 (1H, m), 4.08 (1H, br s), 3.87 (1H, dd, *J* = 9.5, 2.5 Hz), 3.80 (1H, dd, *J* = 9.5, 2.5 Hz), 3.38 (1H, t, *J* = 9.5 Hz), 3.28 (3H, s), 3.21–3.17 (1H, m), 2.28–2.20 (1H, m), 1.53 (1H, br t, *J* = 13 Hz), 1.38 (1H, br d, *J* = 13 Hz), 0.86 (9H, s), 0.03 (3H, s), 0.02 (3H, s) ppm; δ_C (126 MHz, $CDCl_3$): 139.8, 139.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.4, 127.2, 127.1, 127.0, 80.7, 80.0, 76.1, 74.5, 73.2, 72.8, 72.6, 68.7, 58.5, 35.2, 30.4, 25.8, 18.1, –4.5, –5.0 ppm; HR-ESIMS: calcd for $C_{35}H_{48}O_5SiNa$ $[M+Na]^+$ 599.3163; found 599.3162.

4.1.15.2. 1D-(1,2,3/4,5)-2,3,4-Tri-*O*-benzyl-5-*O*-(*tert*-butyldimethylsilyl)-1-*O*-methylcyclohexanepentol (36). In the same manner as described above, **33** was converted into **36** (quant.) as a colorless oil, n_D^{22} 1.5175; $[\alpha]_D^{22}$ +40.0 (*c* 0.64, $CHCl_3$); ν_{max} (film): 1605 (w), 1585 (w), 1495 (m), 1250 (m, *t*-Bu, SiMe), 1105 (br s, C–O), 830 (br s), 735 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.41–7.22 (15H, m), 4.88 (1H, d, *J* = 12 Hz), 4.80 (1H, d, *J* = 12 Hz), 4.75 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.68 (1H, d, *J* = 12 Hz), 4.67 (1H, d, *J* = 12 Hz), 4.20–4.17 (1H, m), 4.12 (1H, br s), 3.83 (1H, dd, *J* = 9.5, 2.5 Hz), 3.77 (1H, dd, *J* = 9.5, 2.5 Hz), 3.54 (1H, ddd, *J* = 12, 4.5, 2.0 Hz), 3.28 (3H, s), 1.91 (1H, dt, *J* = 13, 2.0 Hz), 1.84 (1H, ddt,

J = 13, 4.5, 1.5 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.03 (3H, s) ppm; δ_C (126 MHz, $CDCl_3$): 139.5, 139.13, 139.09, 128.2, 128.0, 127.7, 127.6, 127.5, 127.2, 127.1, 79.6, 78.7, 76.8, 75.8, 73.8, 72.8, 72.7, 67.5, 56.6, 32.6, 25.8, 18.1, −4.5, −5.1 ppm; HR-FABMS: calcd for $C_{34}H_{46}O_5SiNa$ $[M+Na]^+$ 585.3012; found 585.3024.

4.2. Pharmacology

4.2.1. General

4.2.1.1. Mice. C57BL/6. 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. All experiments were in accordance with protocols approved by RIKEN Animal Care and Use Committee.

4.2.1.2. Reagent. KRN7000 (α-GalCer) was kindly provided by Kyowa Kirin Pharma (Takasaki, Gunma, Japan).

4.2.2. Bioassay (mouse in vivo)

4.2.2.1. In vivo experiment. The stock solution (1.0 mg/mL in DMSO) of KRN7000 (**B**) and synthesized samples (**1-7**) was diluted to 10 μg/mL in Dulbecco's phosphate buffered saline (Sigma, Product No. D8537) just before injection into mice. Each glycolipid solution (10 μg/mL, 200 μL) was administered intravenously. Peripheral blood was collected from the retro-orbital plexus of mice at indicated time points, using heparin-coated capillary tubes (Funakoshi Pharmaceutical, Japan), and plasma was prepared.

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

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.025.

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