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Synthesis and biological activity of hydroxylated analogs of RCAI-80

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ABSTRACT

RCAI-80 is one of the ester analogs of KRN7000 (α-galactosylceramide). This compound released mainly T helper 2 (Th2) cytokines, such as IL-4 rather than T helper 1 (Th1) cytokines, such as IFNγ from the invariant natural killer T (iNKT) cells. In addition, it has been known that some of the hydroxylated derivatives of KRN7000 make the cytokine secretion bias to Th2 by decreasing the IFNγ production to almost zero. This time, the three compounds having these two characteristic properties, namely an ester group and also some extra hydroxy groups existing on the ester side chain and/or on the 2-acyloxy-3,4-dihydroxyoctadecyl main chain of RCAI-80, were synthesized to examine the biological activities. As a result, it was found that these compounds made the cytokine secretion skew to Th2. Therefore, their effectiveness for experimental autoimmune encephalomyelitis (EAE) was examined. It was recognized that one of them showed moderate suppression of EAE symptom.

1. Introduction[1]

Agelasphins are a series of glycosphingolipids isolated from the marine sponge (*Agelas mauritianus*), and they show potent effects on the immune system of mice.[2] KRN7000,[3] one of the α-galactosylceramides, was developed by Kirin Brewery Co. in the process of synthetic studies on the agelasphins. Invariant natural killer T (iNKT) cells[4] produce immunoregulatory cytokines, such as both IFNγ [T helper 1 (Th1) type] and IL-4 [T helper 2 (Th2) type] by the recognition of glycolipid antigens, such as KRN7000 bound by a major histocompatibility complex (MHC) class I-like protein, i.e., CD1d,[5] which is an antigen presenting protein for the T cell receptor of iNKT cells. The glycolipids stimulating the secretion of mainly Th1 type cytokines or of mainly Th2 type cytokines are expected to be promising anticancer drugs or immunosuppressive agents and autoimmune disease treatment medicine, respectively.

RCAI-80,[6] an ester analog of KRN7000, was found to induce more selectively IL-4 than IFNγ compared to KRN7000. Besides this, the compound OCH, reported by Miyamoto et al.,[7] has two truncated chains both on the main chain and on the amide side chain of the aglycon, and it is known that OCH induces strongly a Th2 bias. By analogy from the hydrophilicity of OCH relatively increasing compare to KRN7000, the more hydroxylated analogs of KRN7000 should become hydrophilic and may induce the secretion of Th2 cytokines from iNKT cells. Therefore, RCAI-147 and 160, hydroxylated analogs of KRN7000, had been synthesized and showed good suppression of experimental autoimmune encephalomyelitis (EAE)[7,8] symptoms as reported in the previous paper.[9] And these compounds were found to inhibit mostly the IFNγ production, and their IL-4 production ability still remained approximately more than 55% of KRN7000. Accordingly, it is anticipated that the hybrid compounds, having the mixed properties of the ester compound RCAI-80 and the hydroxylated compounds RCAI-147/-160, behave as inducing the secretion of Th2 cytokines from iNKT cells (Fig. 1). However, there is no specific evidence that hydrophobicity/hydrophilicity is key. It may be just binding/recognition (and not hydrophilicity per se) that effects biological activity.

For whatever reason, this time, three relevant compounds (**13**, **22**, and **38**) of RCAI-80's hydroxylated analogs were synthesized to evaluate their biological activities, because we were interested in the biological activities (EAE) of hydroxylated analogs of KRN7000.[9] (Schemes 1–6) (see Fig. 2).

At last, in the previous paper[6] for the synthesis of RCAI-80, we had to use the acidic condition (aqueous HF) to remove the

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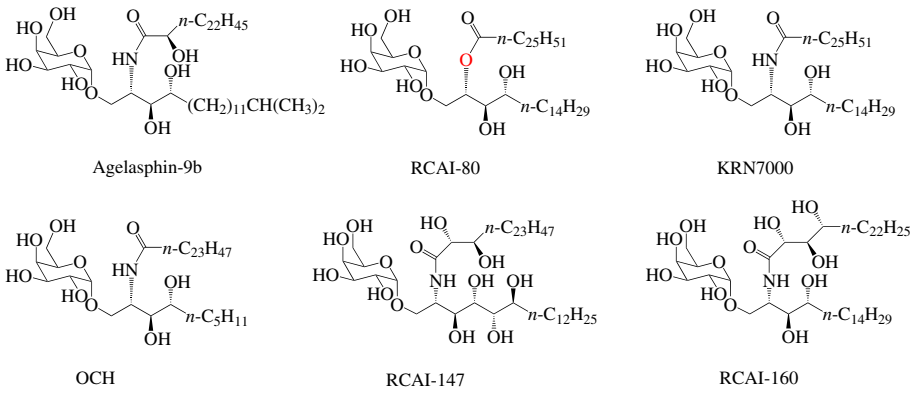
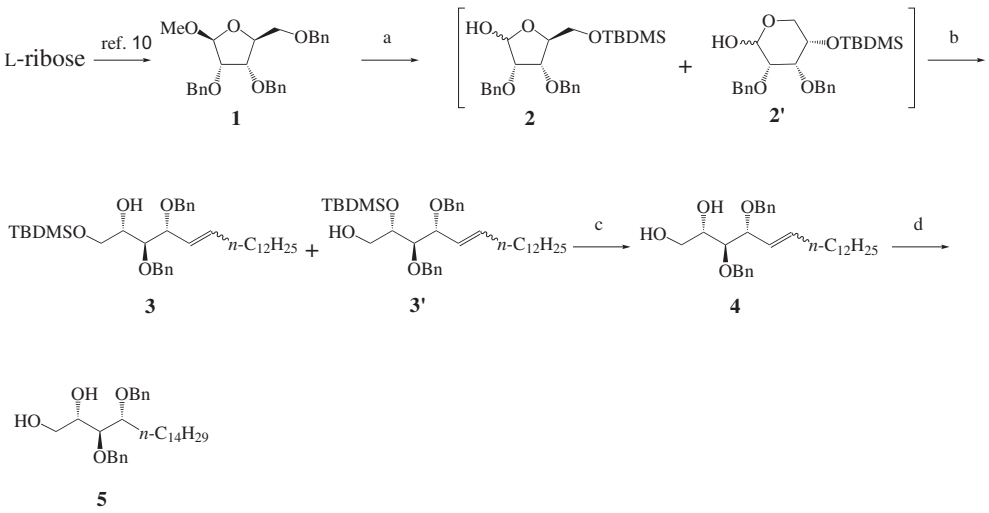


Fig. 1. Structures of agelasphin-9b, RCAI-80, KRN7000, OCH, RCAI-147, and RCAI-160.



Scheme 1. Reagents and conditions: (a) (1) Ac_2O, cat. H_2SO_4, 6–10 °C, 1 h; (2) MeOH, cat. KOH, rt, 2 h, two steps 53%; (3) TBDMS-Cl, imidazole, CH_2Cl_2, 15–20 °C, 2 h, 84% (three steps 45%); (b) *n*-$C_{13}H_{27}PPh_3Br$, *n*-BuLi, THF, −10 °C, 30 min, then **2** and **2′**, −10 °C, 10 min, and rt 16 h, **3** (54%), a mixture of **3** and **3′** (6%), and **3′** (4%); (c) TBAF, THF, rt, 30 min, 95%; (d) H_2, 10% Pd/C, hexane, rt, 25 min, 82%.

protecting groups. This condition caused to yield the acyl migrated compound from C2–O to C4–O positions as a side reaction. Therefore, an alternative route was attempted to prevent this migration and to improve the overall yield of RCAI-80 synthesis.

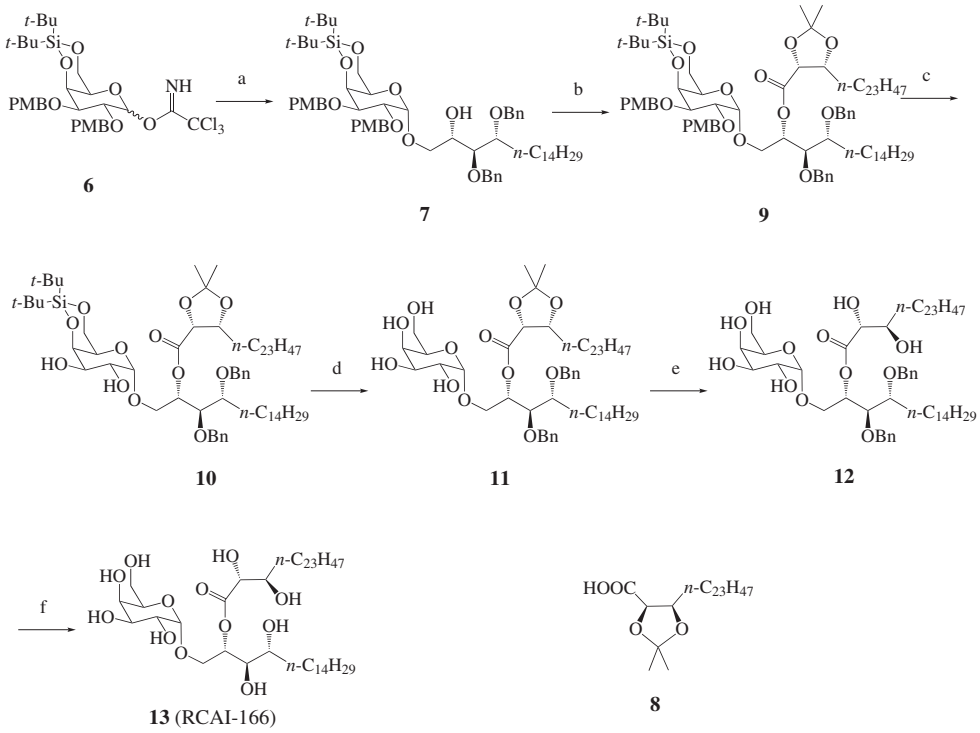
2. Results and discussion

2.1. Synthesis

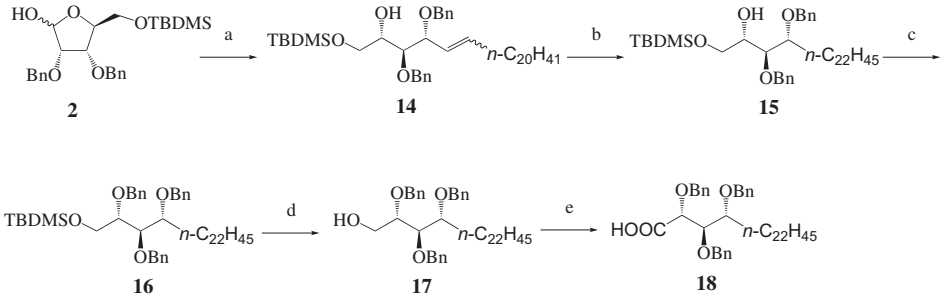
Firstly, compound **13** (RCAI-166), having two hydroxy groups on the C2′- and C3′-positions in the hexacosanoyl ester side chain, was synthesized via methyl 2,3,5-tri-*O*-benzyl-β-L-ribofuranoside (**1**) obtained easily from L-ribose.[10] Reaction of **1** with Ac_2O containing a catalytic amount of concd H_2SO_4 gave a diacetyl intermediate, which was further converted to a mixture of diols in MeOH containing a catalytic amount of KOH, and then a mixture of silyl ethers **2** and **2′** (84%) with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) using imidazole as a base. Wittig reaction of this mixture (**2** and **2′**) with the phosphorane derived from *n*-tridecyltriphenylphosphonium bromide and *n*-BuLi gave a mixture of **3** and **3′** (64%), which was converted to **4** (*E*,*Z*-mixture, 95%) by tetra-*n*-butylammonium fluoride (TBAF) treatment. The *E*,*Z*-double bonds of **4** were reduced to **5** (82%) by treatment with H_2 at room temperature for 30 min using Pd on carbon (Pd/C) as a catalyst (Scheme 1).

The diol **5** was treated with the imidate **6**[6] using silver trifluoromethanesulfonate (AgOTf) to yield α-anomer **7** (73%). Esterification of alcohol **7** with carboxylic acid **8**[9] using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) as a dehydrating agent in THF–CH_2Cl_2 (1:1) gave **9** (79%). Reaction of **9** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane–water (CH_2Cl_2–H_2O=10:1) gave **10**. Treatment of **10** with HF·pyridine in THF–pyridine gave **11**. The isopropylidene group of **11** was deprotected with aqueous HF in CH_2Cl_2–MeCN (1:1) to give **12**. Two benzyl groups of **12** were hydrogenolyzed using $Pd(OH)_2$ on carbon [$Pd(OH)_2$/C] in THF to give **13** (RCAI-166). The 2D COSY analysis of **13** showed that the (2′,3′-dihydroxy)hexacosanoyl group stayed on the C2–O position in the octadecyl chain. However, silica gel column chromatography of **13** by elution with $CHCl_3$–MeOH (9:1) yielded gradually a lower R_f product, which may be C4–O acyl migrated compound[6] (Scheme 2).

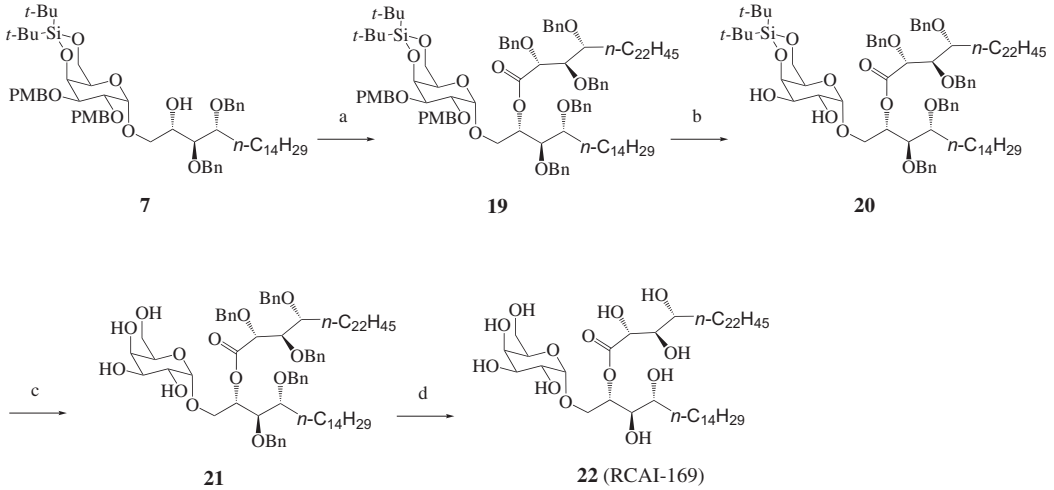
Secondly, compound **22**, having three hydroxy groups on the C2′-, C3′- and C4′-positions in the hexacosanoyl ester side chain, was synthesized via compound **7** and carboxylic acid **18**, which was obtained from compound **2**. Wittig reaction of a mixture of **2** and **2′** with the phosphorane derived from *n*-henicosyltriphenylphosphonium bromide (*n*-$C_{21}H_{43}PPh_3Br$) and *n*-BuLi gave **14** (*E*,*Z*-mixture). The double bond of **14** was reduced to **15** by treatment with H_2 at room temperature for 30 min using Pd/C as a catalyst. The alcohol of **15** was benzylated with benzyl bromide using NaH as a base to give **16**, which was converted to **17** by treatment with TBAF. The primary alcohol of **17** was oxidized to carboxylic acid **18** by using Jones reagent (Scheme 3).



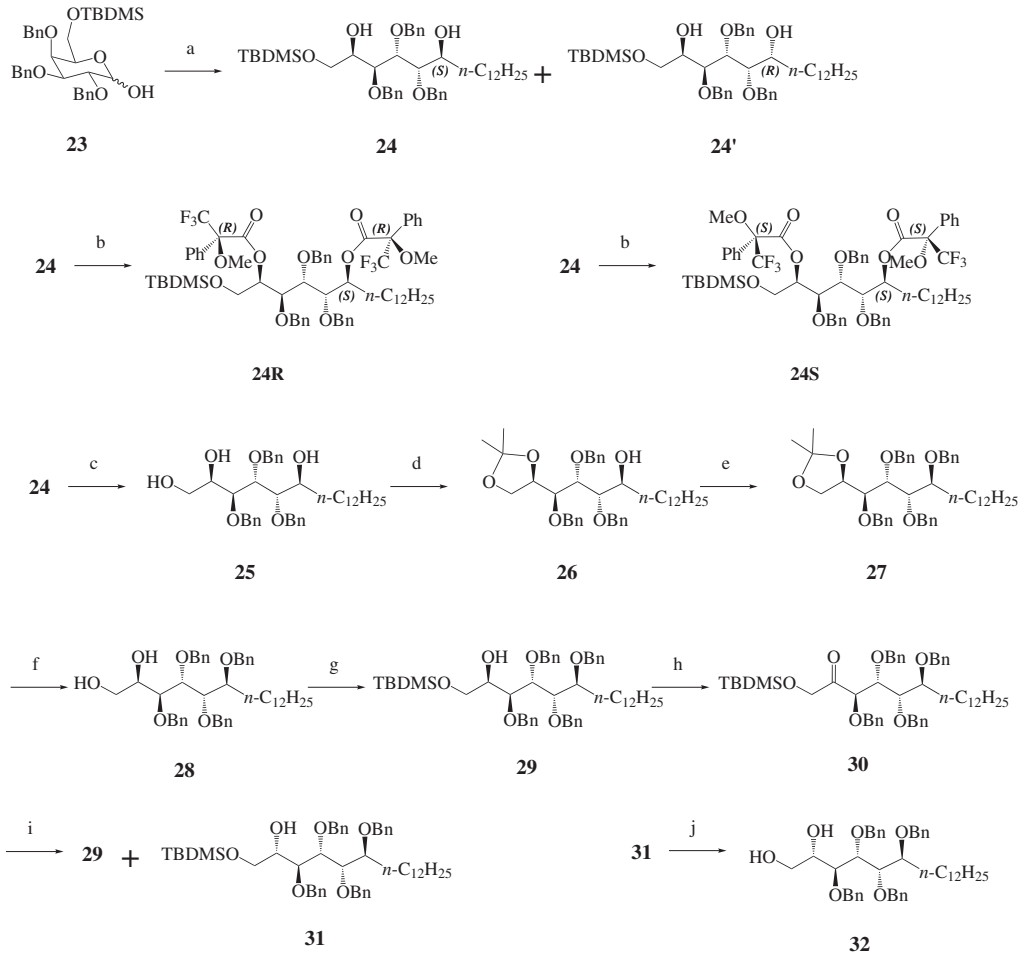
Scheme 2. Reagents and conditions: (a) **5**, MS 4 Å, AgOTf, CH_2Cl_2, 0 °C, 2 h, then rt 1 h, 73%; (b) **8**, DMAP, EDAC, THF–CH_2Cl_2 (1:1), rt, 5 h, 79%; (c) DDQ, CH_2Cl_2–H_2O (10:1), rt, 3 h, 57%; (d) HF–pyridine, pyridine, THF, rt, 1.5 h; (e) aq 46% HF, CH_2Cl_2–MeCN (1:1), rt, 1.5 h, two steps 72%; (f) H_2, $Pd(OH)_2$/C, THF, rt, 5 h, 80%.



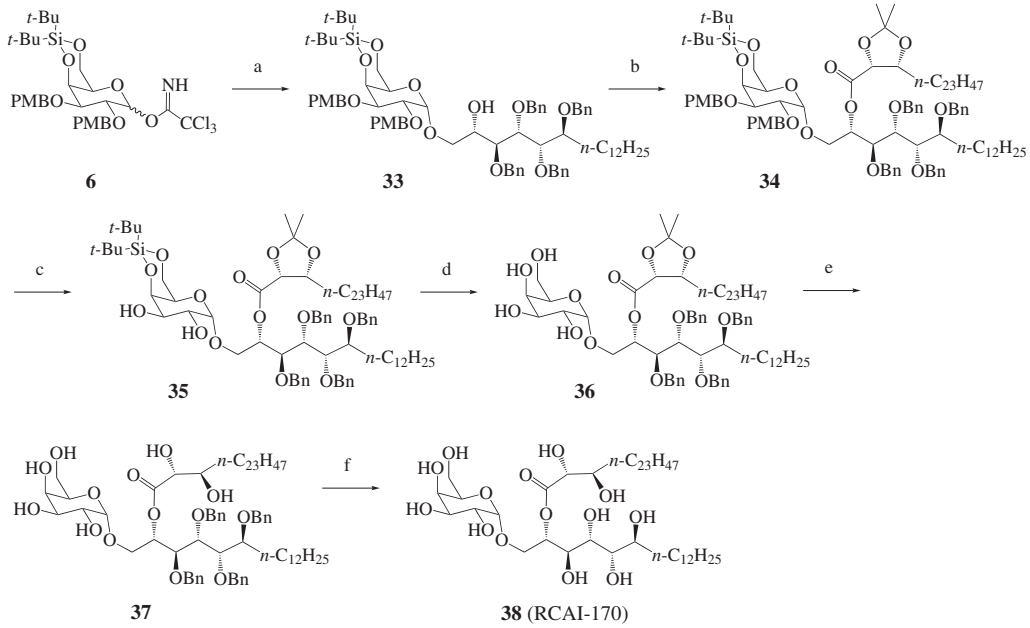
Scheme 3. Reagents and conditions: (a) n-$C_{21}H_{43}PPh_3Br$, n-BuLi, THF, −10 °C, 30 min, then **2**, THF, −10 °C, 20 min, then rt, 3 h, 53%; (b) H_2, 10% Pd/C, hexane, rt, 25 min, 90%; (c) BnBr, NaH, DMF, 5 °C, 16 h; (d) TBAF, THF, rt, 2 h, two steps 77%; (e) Jones reagent, acetone, 0 °C, 5 min, then rt, 30 min, 72%.



Scheme 4. Reagents and conditions: (a) **18**, DMAP, EDAC, THF–CH_2Cl_2 (1:1), rt, 3.5 days, 76%; (b) DDQ, CH_2Cl_2–H_2O (10:1), rt, 2.5 h; (c) HF–pyridine, pyridine, THF, rt, 2 h, two steps 26%; (d) H_2, $Pd(OH)_2$/C, THF, rt, 21 h, 78%.



Scheme 5. Reagents and conditions: (a) *n*-$C_{12}H_{25}$MgBr, Et_2O, reflux, **24** (44%) and **24′** (38%); (b) **24** (5 mg), (+)-MTPA chloride or (−)-MTPA chloride, pyridine, DMAP, THF, rt, 15 min, **24R** (5 mg) or **24S** (5 mg); (c) TBAF, THF, rt, 1 h, 71%; (d) 2,2-dimethoxypropane, *p*-TsOH·H_2O, rt, 1 h; (e) BnBr, NaH, DMF, rt, 1 h, two steps 93%; (f) aq 46% HF, CH_2Cl_2–MeCN (1:1), rt, 30 min, 99%; (g) TBDMS-Cl, imidazole, catalytic DMAP, rt, 16 h, 93%; (h) Dess–Martin periodinane, rt, 2 h, 91%; (i) $NaBH_4$, EtOH, rt, 30 min, **31** (34%) and **29** (52%); (j) TBAF, THF, rt, 1 h, 86%.



Scheme 6. Reagents and conditions: (a) **32**, AgOTf, MS 4 Å, CH_2Cl_2, rt, 1 h, 38%; (b) **8**, DMAP, EDAC, CH_2Cl_2–THF (1:1), 5 h, 63%; (c) DDQ, CH_2Cl_2–H_2O (10:1), rt, 2.5 h; (d) HF–pyridine, pyridine, THF, rt, 1.5 h, two steps 74%; (e) aq 46% HF, CH_2Cl_2–MeCN (1:1), rt, 1.5 h, 87%; (f) H_2, $Pd(OH)_2$/C, THF, rt, 16 h, 74%.

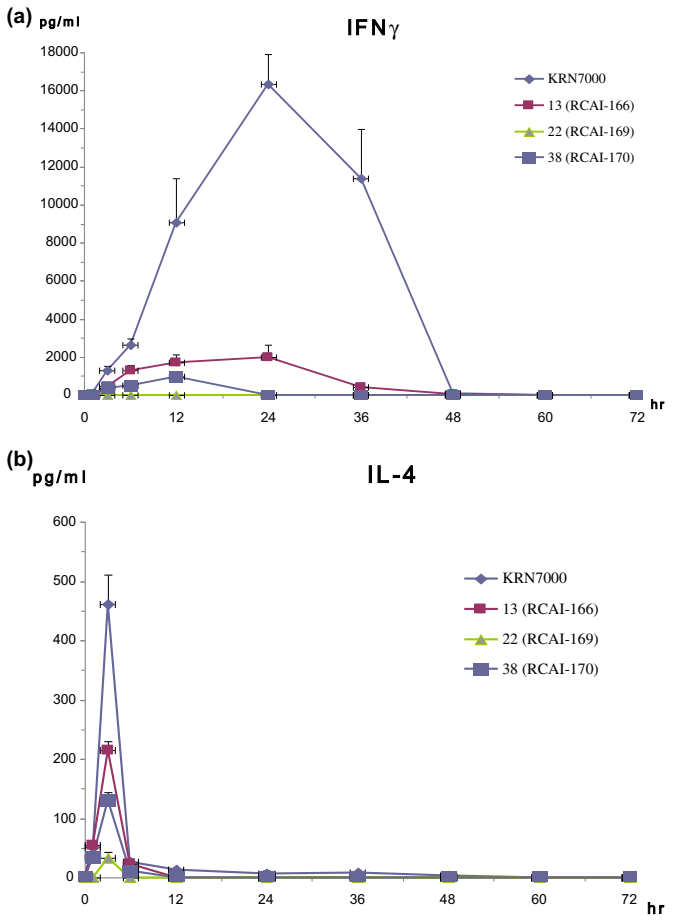


Fig. 2. The concentrations of IFNγ (panel a) and IL-4 (panel b) in sera of mice (2 μg/mouse, in vivo) upon administration of the RCAI-80 variants (ester analogs of KRN7000), namely three compounds **13**, **22** and **38** (the structures shown in Table 1).

Esterification of alcohol **7** with carboxylic acid **18** using EDAC as a dehydrating agent in THF–CH_2Cl_2 (1:1) afforded **19**. Reaction of **19** with DDQ in dichloromethane–water (CH_2Cl_2–H_2O=10:1) gave **20** as a mixture with 4-methoxybenzaldehyde. This mixture was treated with HF·pyridine in THF–pyridine to give **21** (two steps 26%). Five benzyl groups of **21** were hydrogenolyzed using $Pd(OH)_2$/C in THF to give **22** (RCAI-169) (Scheme 4).

Thirdly, compound **32**, constructing the aglycon part of **38**, was obtained in nine steps (4% yield) from 1-*O*-*tert*-butyldimethylsilyl-2,3,4-tri-*O*-benzyl-D-galactose (**23**).[9] Grignard reaction of **23** with dodecylmagnesium bromide gave a mixture of **24** and **24′**.

The absolute configuration of C6–OH of **24** was determined by Mosher's method using MTPA (α-methoxy-α-trifluoromethyl phenylacetyl) diesters for C2 and C6 hydroxy groups.[11] Complete NMR assignments of (*R*)-MTPA ester (**24R**) and (*S*)-MTPA ester (**24S**) were confirmed by 2D DQF-COSY, NOESY, HSQC, and HMBC experiments (see Experimental).

Compound **24** was desilylated by treatment with TBAF to yield triol **25**, in which 1- and 2-hydroxy groups were distinguished from C6-hydroxy group by construction of 1,2-*O*-isopropylidene using *p*-toluenesulfonic acid monohydrate ($TsOH·H_2O$) as a catalyst in 2,2-dimethoxypropane to afford **26**. The remaining C6–OH group was benzylated with BnBr and NaH as a base to yield **27**. The isopropylidene group of **27** was deprotected with aqueous HF to give 1,2-diol **28**. The primary alcohol of **28** was protected selectively with TBDMS-Cl using imidazole as a base to form **29**. Mitsunobu inversion at the C2–OH position of **29** was attempted. However, the target product could not be obtained. Therefore, another approach was tried to yield **32**. Dess–Martin oxidation of C2-secondary alcohol of **29** gave a ketone **30**, which was reduced to a mixture of **29** (52%) and **31** (34%) using sodium borohydride ($NaBH_4$). Treatment of **31** with TBAF afforded 1,2-diol **32**, i.e., a C2–OH epimer of **28** (Scheme 5).

Compound **38**, having two hydroxy groups on the C2′ and C3′-positions in the hexacosanoyl ester side chain, and four hydroxy groups on the C3, C4, C5, and C6-positions in the octadecyl aglycone main chain, was synthesized through alcohol **33** obtained by the coupling reaction with imidate **6** and diol **32**.

The glycosylation reaction of glycosyl acceptor **32** with glycosyl donor **6** was performed by use of AgOTf to construct α-anomer **33**. The C2–OH of **33** was esterified with carboxylic acid **8** in the presence of dehydrating agent, EDAC, to form **34**. Compound **34** was converted to **38** (RCAI-170) in four steps via intermediates **35**, **36**, and **37** by the same procedure to convert **9** to **13** through intermediates **10**, **11**, and **12**. It was obvious by 2D COSY analysis that the (2′,3′-dihydroxy)hexacosanoyl group of **38** was on the C2–O position on the octadecane chain (Scheme 6).

Finally, an alternative route other than going through compound **7** for the synthesis of RCAI-80 was attempted.

L-Ribose was converted to benzylidene compound **39** according to the reported method.[12] The primary alcohol of this compound was protected as a silyl ether with TBDMS-Cl and imidazole to yield a 10:2:1 mixture of **40β**, **40α**, and **41** (from ^{1}H NMR analysis). Anomeric configuration at C1 of **40β** and **40α** was determined by vicinal coupling constant values $^3J_{H1,H2}$ of 0 Hz and 4.1 Hz, respectively. Relative stereochemistry of benzylidene acetal was determined by NOESY data.

The Wittig reaction of this mixture (**40β**, **40α**, and **41**) with a phosphorane ($Ph_3P{=}CHC_{12}H_{25}$) obtained from *n*-tridecyltriphenylphosphonium bromide and *n*-BuLi afforded **42EZ** (a mixture of *E*- and *Z*-isomers) and **43EZ** (a mixture of *E*- and *Z*-isomers). These compounds **42EZ** and **43EZ** were separable chromatographically. The double bond of **42EZ** was reduced under H_2 atmosphere using Pd/C as a catalyst in hexane to furnish **44**, which was treated with TBAF to give diol **45** (Scheme 7).

The glycosylation reaction of glycosyl acceptor **45** with glycosyl donor **6** was performed by use of AgOTf as a catalyst to construct α-anomer **46**. The C2–OH of **46** was esterified with *n*-hexacosanoic acid in the presence of dehydrating agent, EDAC, to form **47**, which was further converted to **48** using DDQ in CH_2Cl_2–H_2O (10:1). Treatment of **48** with HF·pyridine in THF–pyridine yielded **49** (80%). Hydrogenolysis of **49** in THF under H_2 atmosphere using $Pd(OH)_2$/C as a catalyst gave **50** (RCAI-80) without occurring the acyl migration from C2–O to C4–O (Scheme 8). This synthetic route was improved in both total yield and steps as compared to the previously reported method.[6]

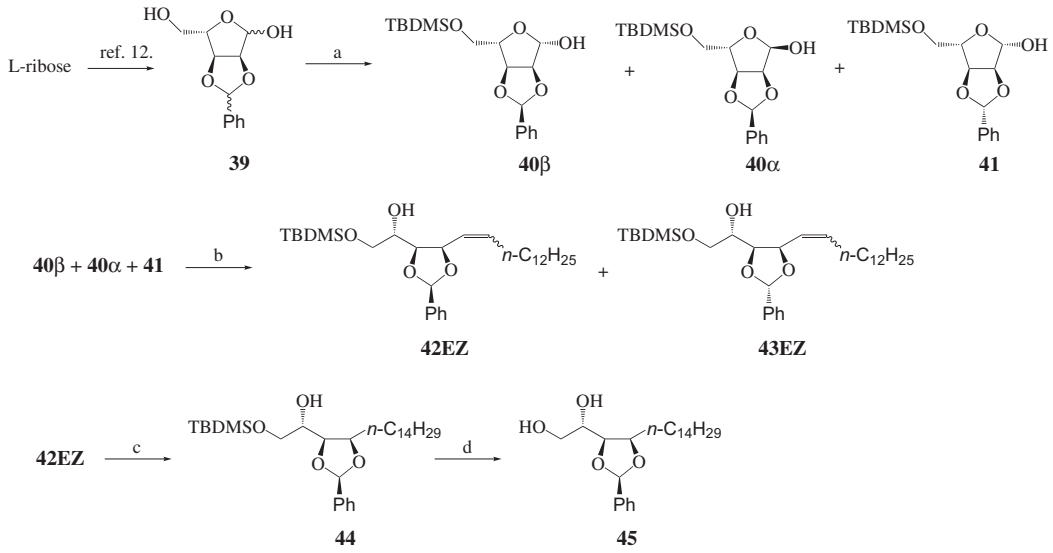
2.2. Biological activity

The cytokine-producing [IFNγ (Th1 response) and IL-4 (Th2 response)] activity from iNKT cells of mice in vivo of the three synthetic compounds **13** (RCAI-166), **22** (RCAI-169) and **38** (RCAI-170) was investigated using KRN7000 as a positive control as shown in Fig. 2 [IFNγ (panel a) and IL-4 (panel b)], and the structures of these compounds were listed in Table 1.

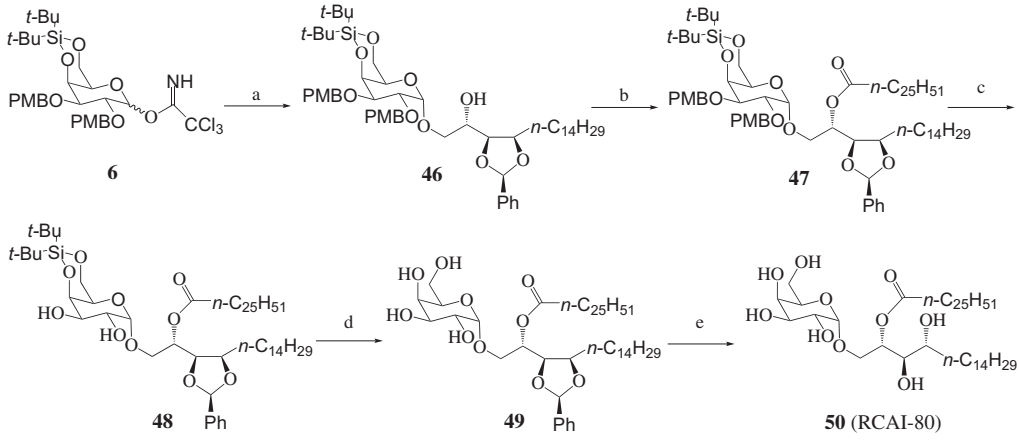
The results of biological activity are described below.

Both compounds **13** and **38** have consecutively (2*R*,3*R*)-dihydroxy groups in the immediate vicinity of ester carbonyl of RCAI-80. Furthermore, the compound **38** is attached on the extra (5*S*,6*S*)-dihydroxy groups on the aglycon main chain of RCAI-80. And the compound **22** has consecutively (2*R*,3*R*,4*R*)-trihydroxy groups in the immediate vicinity of ester carbonyl of RCAI-80.

The IFNγ production ability of compound **13** (RCAI-166) decreased to approximately 11.4% of KRN7000, and yet the IL-4

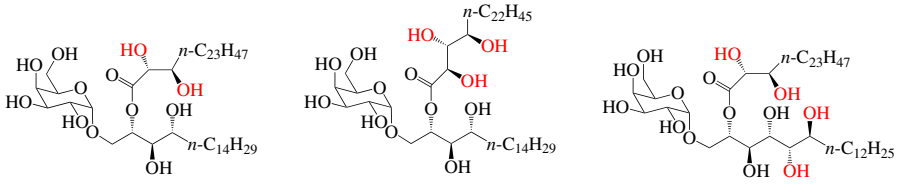


Scheme 7. Reagents and conditions: (a) TBDMS-Cl, imidazole, CH_2Cl_2, rt, 45 min, 74% (**40β**:**40α**:**41**=10:2:1); (b) *n*-$C_{13}H_{27}Ph_3PBr$, *n*-BuLi, THF, −10 °C, 71% (**42EZ**) and 6% (**43EZ**); (c) H_2, 10% Pd/C, hexane, rt, 30 min, 96%; (d) TBAF, THF, rt, 45 min, 68%.



Scheme 8. Reagents and conditions: (a) **45**, MS 4 Å, AgOTf, CH_2Cl_2, rt, 4 h, 87%; (b) *n*-$C_{25}H_{51}COOH$, DMAP, EDAC, THF−CH_2Cl_2 (1:1), rt, 4 days, 95%; (c) DDQ, CH_2Cl_2−H_2O (10:1), rt, 2.5 h, 85%; (d) HF−pyridine, pyridine, THF, rt, 2.5 h, 80%; (e) H_2, $Pd(OH)_2$/C, THF, rt, 4 h, 87%.

Table 1
Structures of newly synthesized three compounds. The extra hydroxy groups added to RCAI-80 are painted in red



production amount remained approximately more than 45.4% of KRN7000. Therefore, compound **13** biased to Th2 response (almost 4.0 times) as compared to that of KRN7000.

The IFNγ production ability of compound **22** (RCAI-169) was reduced to almost zero, and its IL-4 production amount remained merely 4.6% of that of KRN7000. However, compound **22** biased to Th2 response.

The IFNγ production ability of compound **38** (RCAI-170) decreased to approximately 2.5% of that of KRN7000, and its IL-4 production amount still remained approximately 24.8% of that of KRN7000. Therefore, compound **38** biased to Th2 response (about 9.9 times) as compared to that of KRN7000 (Fig. 2).

These compounds induced Th2 cytokine (IL-4) rather than Th1 cytokine (IFNγ) from iNKT cells compared to KRN7000. Therefore, these three compounds **13**, **22**, and **38** were tested for the suppression activity of the symptoms associated with experimental autoimmune encephalomyelitis (EAE),[7,8] an animal (mouse) model of multiple sclerosis, as shown in Fig. 3. In addition, the EAE test of RCAI-80 was shown in Fig. 4.

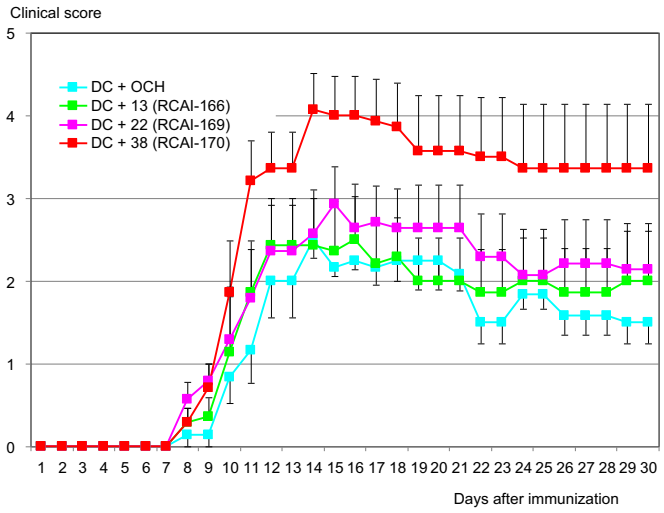


Fig. 3. Clinical score of compounds **13**, **22**, and **38** compared to OCH for EAE suppression.

IFNγ production to almost zero may be important for the preventive effects against EAE.

4. Experimental

4.1. General

IR spectra were measured with a Jasco FT/IR-460 plus spectrometer. ^{1}H NMR spectra (TMS as an internal standard) and ^{13}C NMR spectra were recorded with a Varian VNMRS-500 spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra and High Resolution MS were recorded with Jeol JMS-SX102A or Bruker BioAPEX II 70e FT-ICR mass spectrometers. Separation by HPLC was performed by use of Hitachi L7110 apparatus. Optical rotation values were measured with a Jasco P-1010 polarimeter. Column chromatography was carried out using silica gel 60N (70–230 mesh ASTM, using ca. 10–50 times the weight of substrate if there is no indication) under a slightly elevated pressure for elution. Preparative TLC was carried out on a PLC plate (Merck, Silica gel 60 F_{254}, 0.5 mm).

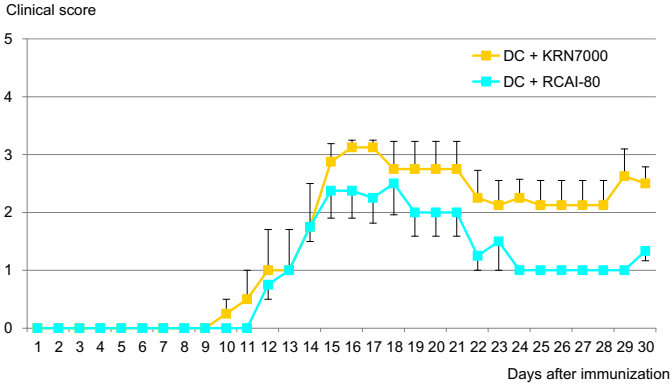


Fig. 4. Clinical score of RCAI-80 compared to KRN7000 for EAE suppression. RCAI-80 showed better clinical score than that of KRN7000.

Compound **13** (RCAI-166) showed moderate clinical score. However, its intensity is a little bit weaker than that of OCH. Nevertheless, the IL-4 production of compound **22** (RCAI-169) was merely 4.6% of KRN7000, it showed a weak depression tendency for EAE effectiveness. It may be due to no production of IFNγ. The EAE test of compound **38** (RCAI-170) was disappointing. It is not known exactly why.

3. Conclusion

The cytokine secretion ability of **13** (RCAI-166), **22** (RCAI-169), and **38** (RCAI-170) was biased to the Th2 response comparing to that of KRN7000, because they inhibited mostly the IFNγ production (approximately 88.6%, 100%, 97.5%, respectively), and their IL-4 production ability still remained approximately 45.4%, 4.6%, and 24.8% of that of KRN7000, respectively. However, compound **13** showed only moderate suppressive effects against EAE symptoms, and compound **22** showed the weak tendency for EAE effectiveness. Compound **38** did not show effectiveness against the EAE suppression. It may be difficult to deduce the structure–activity relationship of this series of hydroxylated RCAI-80 analogs, because of difficulty of finding out the rational and systematic regularity from the above mentioned results. However the balance of the hydroxy groups on the sphingosine main chain and the ester side chain may be important for EAE suppression. In addition, not only making the cytokine secretion bias to Th2 but also decreasing the

*4.1.1. 2,3-Di-O-benzyl-5-O-tert-butyldimethylsilyl-L-ribofuranose (**2**) and 2,3-Di-O-benzyl-4-O-tert-butyldimethylsilyl-L-ribopyranose (**2′**).* (i) A solution of 1 (2.17 g, 4.99 mmol) in Ac_2O (125 mL) containing concd H_2SO_4 (0.055 mL) was stirred for 1 h at 6–10 °C, and diluted with EtOAc (250 mL), which was washed with aq saturated $NaHCO_3$ (three times), and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give an oil. Toluene was added to this oil, and concentrated in vacuo to remove Ac_2O at 60 °C of bath temperature. This procedure was repeated three times to give a mixture of diacetates.

(ii) The above obtained mixture was dissolved in MeOH (350 mL) containing KOH (200 mg), stirred for 2 h at 15–20 °C to give alcohols from the diacetates, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1, then 1:2), gave a mixture (0.88 g, 53%) of 2,3-di-*O*-benzyl-L-ribofuranose and 2,3-di-*O*-benzyl-L-ribopyranose as an oil. ESI MS: *m*/*z* 353.14 $[M+Na]^+$. HRESI MS: calcd for $C_{19}H_{22}O_5Na$: 353.1365; observed 353.1370.

(iii) A solution of the above obtained mixture (0.88 g, 2.67 mmol), imidazole (545 mg, 8.01 mmol) and *tert*-butyldimethylsilyl chloride (643 mg, 4.27 mmol) in CH_2Cl_2 (70 mL) was stirred for 2 h at 15–20 °C, and diluted with CH_2Cl_2, which was washed with aq saturated $NaHCO_3$ (three times) and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1, then 9:2), gave an inseparable

mixture of **2** and **2′** [1.00 g, 84% (three steps 45%)] as an oil. R_f=0.383 (hexane–EtOAc=3:1). IR ν_{max} (KBr) 3509 (br), 2929, 2856, 1455, 1253 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ −0.02 to 0.05 (3H, m), 0.83–0.89 (9H, m), 3.33–4.40 (5H, m), 4.60–4.74 (4H, m), 5.26–5.29 (1H, m), 7.28–7.40 (10H, m). ESI MS: *m*/*z* 467.2 $[M+Na]^+$. HRESI MS: calcd for $C_{25}H_{36}O_5SiNa$: 467.2230; observed: 467.2219.

*4.1.2. (2S,3S,4R,5EZ)-1-tert-Butyldimethylsilyloxy-3,4-dibenzyloxy-5-octadecen-2-ol (**3**) and (2S,3R,4R,5EZ)-2-tert-Butyldimethylsilyloxy-3,4-di-benzyloxy-5-octadecen-1-ol (**3′**).* To a solution of *n*-tridecyltriphenylphosphonium bromide (1.17 g, 2.22 mmol) in dry THF (3 mL) was added a solution of *n*-BuLi (1.65 M in hexane, 1.80 mL, 2.97 mmol, after the lasting phosphorane red color) at −10 °C under argon with stirring. After stirring for 30 min at −10 °C, to this red-colored solution was added a solution of a mixture of 2 and 2′ (327 mg, 0.99 mmol) in THF (2 mL). This mixture was stirred for 10 min at −10 °C, and then overnight at room temperature, and diluted with EtOAc. The solution was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1) gave an *E*,*Z*-mixture of 3 (325 mg, 54%) as an oil, a mixture of 3 and 3′ (37 mg, 6%), and an *E*,*Z*-mixture of 3′ (26 mg, 4%) as an oil.

The *E*,*Z*-mixture of **3** (10 mg) was separated on a silica gel TLC plate by development with hexane–EtOAc (9:1) to give *Z*-isomer of **3** (4 mg) and *E*,*Z*-mixture (3 mg), and also the *E*,*Z*-mixture of **3′** (10 mg) was separated on a silica gel TLC plate by development with hexane–EtOAc (9:1) to give *Z*-isomer of **3′** (2 mg) and *E*,*Z*-mixture of **3′** (5 mg). Physical data of *Z*-isomer of **3**: R_f=0.634 (hexane–EtOAc=6:1). IR ν_{max} (KBr) 3567, 2925, 2854, 1517, 1457, 1255 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.88 (3H, t, *J*=6.8 Hz), 0.89 (9H, s), 1.25 (16H, br s), 1.31–1.37 (2H, m), 1.50–1.60 (2H, m), 2.05 (1H, m), 2.11 (1H, m), 3.57 (1H, m), 3.66–3.70 (2H, m), 3.76 (1H, dd, *J* 3.4, 10.0 Hz), 4.38, 4.65 (2H, AB-q, *J* 12.0 Hz), 4.59 (1H, m), 4.62, 4.87 (2H, AB-q, *J* 11.2 Hz), 5.55 (1H, t, *J* 11.0 Hz), 5.77 (1H, m), 7.27–7.34 (10H, m). ESI MS of *E*,*Z*-mixture of **3**: *m*/*z* 633.4 $[M+Na]^+$. HRESI MS: calcd for $C_{58}H_{62}O_4SiNa$: 633.4315; observed, 633.4309. Physical data of *Z*-isomer of **3′**: [R_f=0.488 (hexane–EtOAc=6:1)]; IR ν_{max} (KBr) 3566, 2925, 2854, 1456, 1252 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.05 (3H, s), 0.09 (3H, s), 0.88 (3H, t, *J*=7.1 Hz), 0.90 (9H, s), 1.26 (18H, m), 1.57 (2H, m), 1.92 (1H, m), 1.98 (1H, m), 3.60 (1H, m), 3.67–3.74 (2H, m), 3.99 (1H, m), 4.35 (1H, m), 4.36, 4.61 (2H, AB-q, *J* 11.9 Hz), 4.66, 4.73 (2H, AB-q, *J* 11.4 Hz), 5.43 (1H, t, *J* 11.0 Hz), 5.72 (1H, m), 7.30–7.33 (10H, m). ESI MS: *m*/*z* 633.4 $[M+Na]^+$. HRESI MS: calcd for $C_{58}H_{62}O_4SiNa$: 633.4315; observed, 633.4300.

*4.1.3. (2S,3S,4R,5EZ)-3,4-Dibenzyloxy-5-octadecene-1,2-diol (**4**).* To a solution of a mixture of **3** and **3′** (360 mg, 0.59 mmol) in dry THF (6 mL) was added tetra-*n*-butylammonium fluoride (1.0 M solution in THF, 3.0 mL). After stirring for 30 min at room temperature, the reaction mixture was diluted with EtOAc, which was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (6:1, then 2:1) gave **4** (279 mg, 95%) as an oily mixture of *E*- and *Z*-isomers. The *E*,*Z*-mixture of **4** (10 mg) was separated on a silica gel TLC plate by development with hexane–EtOAc (2:1) to give *Z*-isomer of **4** (4 mg) and *E*,*Z*-mixture of **4** (4 mg). Physical data of *Z*-isomer of **4**: R_f=0.338 (hexane–EtOAc=2:1). IR ν_{max} (KBr) 3411 (br), 2925, 2853, 1456 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J*=6.8 Hz), 1.25 (16H, s), 1.30–1.37 (2H, m), 1.57–1.62 (2H, m), 2.01 (1H, m), 2.08 (1H, m), 2.20 (1H, br, OH), 3.24 (1H, br, OH), 3.59 (1H, t, *J* 6.5 Hz), 3.72–3.76 (2H, m), 3.79 (1H, m), 4.35, 4.64 (2H, AB-q, *J* 11.6 Hz), 4.43 (1H, dd, *J* 6.3, 9.4 Hz), 4.58, 4.70 (2H, AB-q, *J* 11.0 Hz), 5.47 (1H, t, *J* 11.0 Hz), 5.81 (1H, m), 7.27–7.36 (10H, m). ESI MS: *m*/*z* 519.3 $[M+Na]^+$. HRESI MS: calcd for $C_{32}H_{48}O_4Na$: 519.3450; observed, 519.3441. Physical data of *E*-isomer of **4**: [R_f=0.263 (hexane–EtOAc=2:1)]; IR ν_{max} (KBr) 3415 (br), 2925, 2853, 1456, 1271 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J*=6.9 Hz), 1.26 (16H, br s), 1.38–1.43 (2H, m), 1.57–1.60 (2H, m), 2.10–2.14 (2H, m), 3.24 (1H, br, OH), 3.58 (1H, t, *J* 6.5 Hz), 3.70–3.74 (2H, m), 3.78 (1H, m), 4.00 (1H, dd, *J* 6.0, 8.4 Hz), 4.36, 4.64 (2H, AB-q, *J* 11.7 Hz), 4.59, 4.68 (2H, AB-q, *J* 10.9 Hz), 5.48 (1H, dd, *J* 8.4, 15.6 Hz), 5.80 (1H, td, *J* 6.7, 15.6 Hz), 7.28–7.36 (10H, m). ESI MS: *m*/*z* 519.3 $[M+Na]^+$. HRESI MS: calcd for $C_{32}H_{48}O_4Na$: 519.3450; observed, 519.3439.

*4.1.4. (2S,3S,4R)-3,4-(Dibenzyloxy)octadecane-1,2-diol (**5**).* A solution of **4** (516 mg, 1.04 mmol) in hexane (80 mL) was stirred for 25 min at 16–18 °C under hydrogen using 10% Pd/C (200 mg) as a catalyst, and filtered. The filtrate was concentrated in vacuo to give crude **5**, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:1, then 3:2) gave **5** (425 mg, 82%) as an oil. IR ν_{max} (KBr) 3853 (br), 2921, 2853, 1518, 1263 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* 6.8 Hz), 1.26 (20H, br s), 1.33–1.44 (4H, m), 1.61–1.74 (2H, m), 1.90 (2H, br, OH), 3.61 (1H, dd, *J* 4.6, 6.3 Hz), 3.73 (1H, m), 3.75–3.77 (2H, m), 3.82 (1H, m), 4.61 (2H, s), 4.62, 4.69 (2H, AB-q, *J* 11.3 Hz), 7.30–7.37 (10H, m). ESI MS: *m*/*z* 521.4 $[M+Na]^+$. HRESI MS: calcd for $C_{32}H_{50}O_4Na$: 521.3607; observed, 521.3606.

*4.1.5. (2S,3S,4R)-3,4-Dibenzyloxy-2-hydroxyoctadecyl 2,3-di-O-(4-methoxybenzyl)-4,6-O-(di-tert- butyl)silylene-α-D-galactopyranoside (**7**).* To a solution of imidate 6 [obtained from 2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranose (311 mg, 0.555 mmol) and CCl_3CN (801 mg, 5.55 mmol) using Cs_2CO_3 (266 mg) as a base in CH_2Cl_2 (6 mL) at room temperature over night] and diol 5 (166 mg, 0.333 mmol) in dry CH_2Cl_2 (15 mL) was added 4 Å MS (dry powder, 1.0 g). After stirring for 30 min at room temperature, the mixture was cooled at 0 °C, and to this mixture was added AgOTf (110 mg, 0.428 mmol). After stirring for 2 h at 0 °C and for 1 h at room temperature, the mixture was filtered, and the filter cake was washed with CH_2Cl_2. The combined filtrate was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (6:1, then 4:1) gave 7 (253 mg, 73%) as an oil. IR ν_{max} (KBr) 3442, 2925, 2854, 1511, 1249 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, *J* 6.8 Hz), 0.99 (9H, s), 1.05 (9H, s), 1.25–1.32 (24H, m), 1.53–1.70 (2H, m), 3.45 (1H, dd, *J* 8.6, 10.6 Hz), 3.60 (1H, s), 3.64 (1H, dd, *J* 3.1, 6.6 Hz), 3.71 (1H, td, *J* 3.4, 8.1 Hz), 3.75–3.81 (7H, m, containing two 3H singlets at 3.76 and 3.81 ppm), 3.88 (1H, m), 3.96 (1H, dd, *J* 2.7, 10.5 Hz), 3.98 (1H, dd, *J* 2.7, 10.5 Hz), 4.46 (1H, d, *J* 3.0 Hz), 4.52 (1H, d, *J* 11.5 Hz, benzylic H), 4.59–4.68 (5H, m, benzylic 5H), 4.71 (1H, d, *J* 3.6 Hz, anomeric H), 4.74 (1H, d, *J* 11.5 Hz, benzylic H), 4.79 (1H, d, *J* 11.2 Hz, benzylic H), 6.80 (2H, d, *J* 8.8 Hz), 6.88 (2H, d, *J* 8.8 Hz), 7.24–7.35 (14H, m). ESI MS: *m*/*z* 1063.6 $[M+Na]^+$. HRESI MS: calcd for $C_{62}H_{92}O_{11}SiNa$, 1063.6307; observed, 1063.6307.

*4.1.6. (2S,3S,4R)-3,4-Dibenzyloxy-2-[(2′R,3′R)-2′,3′-(isopropylidenedioxy)hexacosanoyloxy]octadecyl 2,3-di-O-(4-methoxybenzyl)-4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**9**).* To a solution of 7 (242 mg, 0.232 mmol) and DMAP (452 mg, 3.70 mmol) in dry THF–CH_2Cl_2 (1:1, 24 mL) were added (2*R*,3*R*)-2,3-(isopropylidenedioxy)hexacosanoic acid 8 (289 mg, 0.617 mmol) and EDAC (590 mg, 3.08 mmol). The solution was stirred for 5 h at room temperature, concentrated in vacuo, and diluted with CH_2Cl_2. The solution was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1, then 9:1)

gave 9 (275 mg, 79%) as an oil. IR ν_{max} (KBr) 2925, 2854, 1734, 1612, 1512, 1464, 1248 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 0.98 (9H, s), 1.04 (9H, s), 1.20–1.38 (62H, m, containing 3H singlet at 1.34 ppm), 1.42–1.68 (4H, m, containing 3H singlet at 1.53 ppm), 3.55 (1H, m), 3.60 (1H, s), 3.53–4.01 (11H, m, containing two 3H singlets at 3.74 and 3.77 ppm), 4.26 (1H, m), 4.40–4.46 (3H, m), 4.54–4.74 (9H, m, benzylic 8H and anomeric H), 5.36 (1H, m), 6.75 (2H, d, *J* 8.7 Hz), 6.84 (2H, d, *J* 8.7 Hz), 7.21 (2H, d, *J* 8.8 Hz), 7.27–7.33 (12H, m). ESI MS: *m*/*z* 1514.03 $[M+Na]^+$. HRESI MS: calcd for $C_{91}H_{146}O_{14}SiNa$: 1514.0380; observed: 1514.0377.

*4.1.7. (2S,3S,4R)-3,4-Dibenzyloxy-2-[(2′R,3′R)-2′,3′-(isopropylidenedioxy)hexacosanoyloxy]octadecyl 4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**10**).* To a solution of 9 (270 mg, 0.181 mmol) in CH_2Cl_2–H_2O (10:1, 24 mL) was added DDQ (270 mg, 1.19 mmol). The mixture was stirred for 3 h at room temperature, and diluted with CH_2Cl_2. The solution was washed twice with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1, 6:1, then 4:1) gave 10 (128 mg, 57%) as an oil. IR ν_{max} (KBr) 3470, 2925, 2854, 1734, 1466 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 1.01 (9H, s), 1.04 (9H, s), 1.25 (62H, br s), 1.37 (3H, s), 1.42–1.66 (7H, m, containing 3H singlet at 1.59 ppm), 3.54–3.60 (2H, m), 3.63 (1H, s), 3.72–3.77 (3H, m), 3.98 (1H, dd, *J* 1.6, 12.7 Hz), 4.05 (1H, dd, *J* 3.0, 11.3 Hz), 4.08 (1H, dd, *J* 2.2, 12.7 Hz), 4.29–4.36 (2H, m), 4.53 (1H, d, *J* 6.6 Hz, C2′-H), 4.54, 4.58 (2H, AB-q, *J* 11.5 Hz), 4.65, 4.70 (2H, AB-q, *J* 11.3 Hz), 4.86 (1H, d, *J* 3.9 Hz, anomeric H), 5.42 (1H, m, C2–H), 7.29–7.34 (10H, m). ESI MS: *m*/*z* 1273.92 $[M+Na]^+$. HRESI MS: calcd for $C_{75}H_{130}O_{12}SiNa$: 1273.9229; observed: 1273.9233.

*4.1.8. (2S,3S,4R)-3,4-Dibenzyloxy-2-[(2′R,3′R)-2′,3′-(isopropylidenedioxy)hexacosanoyloxy]octadecyl α-D-galactopyranoside (**11**).* To a solution of 10 (78 mg, 0.062 mmol) and in dry THF (6 mL) containing dry pyridine (50 mg) was added HF·pyridine (HF: ~70%; pyridine: ~30%, 25 mg) at room temperature. After stirring for 1.5 h at room temperature, the reaction mixture was diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue (69 mg), which was employed for the next reaction without purification. A part of this residue (3 mg) was purified on a preparative TLC plate. Development with $CHCl_3$–MeOH (7:1) gave 11 (2 mg) as a gum. IR ν_{max} (KBr) 3472 (br), 2924, 2853, 1732, 1466, 1456 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 1.20–1.30 (64H, m), 1.38 (3H, s), 1.43–1.72 (9H, m, containing 3H singlet at 1.60 ppm), 3.59 (1H, td, *J* 4.4, 7.4 Hz), 3.64–3.82 (7H, m), 4.00 (1H, d, *J* 2.3 Hz), 4.10 (1H, dd, *J* 2.3, 10.8 Hz), 4.32 (1H, m), 4.56 (1H, m), 4.56, 4.59 (2H, AB-q, *J* 11.5 Hz), 4.65, 4.71 (2H, AB-q, *J* 11.4 Hz), 4.87 (1H, d, *J* 3.7 Hz, anomeric H), 5.46 (1H, m, C2–H), 7.28–7.36 (10H, m). ESI MS: *m*/*z* 1133.82 $[M+Na]^+$. HRESI MS: calcd for $C_{67}H_{114}O_{12}Na$: 1133.8208; observed: 1133.8218.

*4.1.9. (2S,3S,4R)-3,4-Dibenzyloxy-2-[(2′R,3′R)-2′,3′-(dihydroxy)hexacosanoyloxy]octadecyl α-D-galactopyranoside (**12**).* To a solution of crude 11 (63 mg, 0.057 mmol) in CH_2Cl_2–MeCN (1:1, 30 mL) was added aq 46% HF solution (2.7 mL). The mixture was stirred for 1.5 h at room temperature, and diluted with $CHCl_3$. The solution was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH (96:4, then 94:6) gave 12 (44 mg, two steps 72%) as a powder. IR ν_{max} (KBr) 3412 (br), 2923, 2852, 1733, 1516, 1457 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 1.21–1.32 (64H, m), 1.44–1.70 (6H, m), 3.59–3.80 (7H, m), 4.01 (1H, s), 4.08–4.13 (2H, m), 4.56 (2H, s), 4.62, 4.68 (2H, AB-q, *J* 11.5 Hz), 4.84 (1H, d, *J* 3.2 Hz, anomeric H), 5.66 (1H, m, HC2–O), 7.28–7.36 (10H, m). ESI MS: *m*/*z* 1093.79 $[M+Na]^+$. HRESI MS: calcd for $C_{64}H_{110}O_{12}Na$: 1093.7895; observed: 1093.7908.

*4.1.10. (2S,3S,4R)-3,4-Dihydroxy-2-[(2′R,3′R)-2′,3′-(dihydroxy)hexacosanoyloxy]octadecyl α-D-galactopyranoside (**13**) (RCAI-166).* A solution of **12** (48 mg, 1.04 mmol) in THF (40 mL) was stirred for 5 h at room temperature under hydrogen using 20% $Pd(OH)_2$/C (wet, water ca. 50%, 52 mg) as a catalyst, and the reaction mixture was filtered. The catalyst was washed with THF, and combined THF solution was concentrated in vacuo to give a white powder, which was washed with EtOAc (3 mL). The powder was dried with a pump to give **13** (32 mg, 80%) as a powder. It was obvious by 2D COSY analysis that the (2′,3′-dihydroxy)hexacosanoyl group of **13** was on the C2–O position on the octadecane chain. However, silica gel column chromatography of **13** by elution with $CHCl_3$–MeOH (9:1) yields gradually C4–O acyl migrated compound. Physical data of **13**: IR ν_{max} (KBr) 3431 (br), 2919, 2850, 1732, 1468 cm^{-1}. $[\alpha]_D^{19}$ +42.2 (*c* 0.83, pyridine). ^1H NMR (500 MHz, pyridine-d_5) δ 0.88 (3H, t, *J* 6.8 Hz), 1.21–1.44 (62H, m), 1.58–1.68 (2H, m), 1.80–1.94 (3H, m), 2.02 (1H, m), 2.15 (1H, m), 2.23 (1H, m), 4.28 (1H, m), 3.73 (1H, m), 3.37–4.56 (8H, m), 4.64 (1H, dd, *J* 3.4, 9.8 Hz), 4.69 (1H, d, *J* 6.2 Hz), 5.51 (1H, d, *J* 3.6 Hz, anomeric H), 6.38 (1H, m). ^{13}C NMR (125 MHz, pyridine-d_5) δ 14.34, 22.99, 26.43, 29.66, 29.67, 29.97, 29.98, 30.05, 30.06, 30.08, 30.10, 30.15, 30.20, 30.38, 32.18, 34.11, 34.55, 62.68, 67.04, 70.49, 71.04, 71.57, 72.40, 72.83, 73.64, 74.00, 75.40, 76.85, 101.08, 174.22. ESI MS: *m*/*z* 913.70 $[M+Na]^+$. HRESI MS: calcd for $C_{50}H_{98}O_{12}Na$: 913.6956; observed, 913.6960.

*4.1.11. (2S,3S,4R,5EZ)-1-tert-Butyldimethylsilyloxy-3,4-dibenzyloxy-5-hexacosen-2-ol (**14**).* To a solution of *n*-henicosyltriphenylphosphonium bromide (5.13 g, 8.04 mmol, 3 equiv) in dry THF (24 mL) was added *n*-BuLi (1.65 M in hexane, 4.87 mL, 8.04 mmol, after appearance of red color) at −10 °C under argon with stirring. After stirring for 30 min at −10 °C, a solution of **2** (884 mg, 2.68 mmol) in dry THF (11 mL) was added to the above obtained phosphorane solution at −10 °C with stirring. After stirring for 20 min at −10 °C, the mixture was stirred for 3 h at room temperature, and diluted with $CHCl_3$, then washed with aq 3% H_2O_2, water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (29:1, then 19:1) gave **14** (1.03 g, 53%) as an oily *E*,*Z*-mixture. IR ν_{max} (KBr) 3630, 2925, 2853, 1457 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.86–0.89 (12H, m, containing 9H singlet at 0.89 ppm), 1.22–1.40 (34H, m, containing 30H singlet at 1.25 ppm), 1.50–1.55 (2H, m), 2.00–2.11 (2H, m), 2.65 (1H, br s, OH), 3.57–3.78 (4H, m), 4.35–4.40 (1H, m), 4.57–4.66 (3H, m), 4.84–4.88 (1H, m), 5.53–5.59 (1H, m), 5.75–5.78 (1H, m), 7.26–7.34 (10H, m). ESI MS: *m*/*z* 745.6 $[M+Na]^+$. HRESI MS: calcd for $C_{46}H_{78}O_4SiNa$: 745.5567; observed, 745.5554.

*4.1.12. (2S,3S,4R)-1-tert-Butyldimethylsilyloxy-3,4-(dibenzyloxy)hexacosan-2-ol (**15**).* A solution of **14** (660 mg, 0.913 mmol) in hexane (75 mL) was stirred for 25 min at room temperature under hydrogen using 10% Pd on carbon (260 mg), and filtered. The filtrate was concentrated in vacuo to give **15**, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1) gave **15** (596 mg, 90%) as an oil. IR ν_{max} (KBr) 3570, 2925, 2853, 1517, 1464 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.88 (3H, t, *J* 6.9 Hz), 0.90 (9H, s), 1.20–1.30 (40H, m), 1.50–1.75 (2H, m), 2.64 (1H, d, *J* 4.2 Hz, OH), 3.62–3.68 (3H, m), 3.76 (1H, m), 3.80 (1H, dd, *J* 2.4, 9.7 Hz), 4.55, 4.81 (2H, AB-q, *J* 11.6 Hz), 4.60, 4.65 (2H, AB-q, *J* 11.5 Hz), 7.26–7.37 (10H, m). ESI MS: *m*/*z* 747.6 $[M+Na]^+$. HRESI MS: calcd for $C_{46}H_{80}O_4SiNa$: 747.5724; observed, 747.5737.

*4.1.13. (2S,3S,4R)-1-tert-Butyldimethylsilyloxy-2,3,4-(tribenzyloxy)hexacosane (**16**).* To a solution of **15** (645 mg, 0.889 mmol) in dry

DMF (7 mL) were added NaH (60% oil dispersion, 175 mg, 4.32 mmol) and then BnBr (500 mg, 2.92 mmol) at 0 °C. The mixture was stirred for 16 h at 5 °C, and diluted with EtOAc. The whole was washed with ice water, and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (29:1, then 19:1) gave benzyl bromide contaminated **16** (780 mg) as an oil. This mixture was employed for the next reaction without further purification. IR ν_{max} (KBr) 2925, 2853, 1455 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.88 (3H, t, *J* 6.8 Hz), 0.91 (9H, s), 1.26 (40H, s), 1.42 (1H, m), 1.61 (1H, m), 3.60–3.70 (2H, m), 3.78–3.84 (2H, m), 3.97 (1H, m), 4.46–4.78 (6H, m), 7.26–7.37 (15H, m). ESI MS: *m*/*z* 837.6 $[M+Na]^+$. HRESI MS: calcd for $C_{53}H_{86}O_4SiNa$: 837.6193; observed, 837.6191.

*4.1.14. (2S,3R,4R)-2,3,4-(Tribenzyloxy)hexacosanol (**17**).* To a solution of **16** (780 mg) in dry THF (30 mL) was added tetra-*n*-butylammonium fluoride (1.0 M solution in THF, 3.5 mL). After stirring for 2 h at room temperature, the reaction mixture was diluted with EtOAc, which was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (11:1, then 4:1) gave **17** (485 mg, two steps 77%) as an oil. IR ν_{max} (KBr) 3370 (br), 2919, 2850, 1496, 1469, 1454 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* 7.0 Hz), 1.20–1.35 (38H, s), 1.35–1.65 (4H, m), 2.46 (1H, dd, *J* 1.9, 5.4 Hz, OH), 3.59 (1H, m), 3.63 (1H, m), 3.77–3.87 (3H, m), 4.51, 4.62 (2H, AB-q, *J* 11.5 Hz), 4.53, 4.64 (2H, AB-q, *J* 11.6 Hz), 4.71, 4.77 (2H, AB-q, *J* 11.3 Hz), 7.26–7.35 (15H, m). ESI MS: *m*/*z* 723.5 $[M+Na]^+$. HRESI MS: calcd for $C_{47}H_{72}O_4Na$: 723.5328; observed, 723.5314.

*4.1.15. (2R,3R,4R)-2,3,4-(Tribenzyloxy)hexacosanoic acid (**18**).* To a solution of **17** (632 mg, 0.901 mmol) in acetone (30 mL) was added Jones reagent (0.65 mL) at 0 °C. After stirring for 5 min at 0 °C, the mixture was stirred for 30 min at room temperature, and diluted with $CHCl_3$, which was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (4:1, then 2:1) gave **18** (464 mg, 72%) as an oil. IR ν_{max} (KBr) 3032 (br), 2917, 2850, 1754, 1720 (shoulder), 1497, 1471, 1455 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* 6.9 Hz), 1.15–1.40 (40H, m), 1.50–1.58 (2H, m), 3.73 (1H, dd, *J* 5.5, 11.4 Hz), 3.89 (1H, dd, *J* 3.9, 4.6 Hz), 4.92 (1H, d, *J* 1.6 Hz), 4.55, 4.58 (2H, AB-q, *J* 11.7 Hz), 4.59, 4.75 (2H, AB-q, *J* 11.5 Hz), 4.61, 4.79 (2H, AB-q, *J* 12.0 Hz), 7.26–7.34 (15H, m). ESI MS: *m*/*z* 737.5 $[M+Na]^+$. HRESI MS: calcd for $C_{32}H_{50}O_4Na$: 737.5121; observed, 737.5121.

*4.1.16. (2S,3R,4R)-2-[(2′R,3′R,4′R)-2′,3′,4′-(Tribenzyloxy)hexacosanoyloxy]-3,4-(dibenzyloxy)octadecyl 2,3-di-O-(4-methoxybenzyl)-4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**19**).* To a solution of 7 (393 mg, 0.377 mmol) and DMAP (919 mg, 7.52 mmol) in dry THF–CH_2Cl_2 (1:1, 40 mL) were added carboxylic acid 18 (665 mg, 0.930 mmol) and EDAC (1.44 g, 7.51 mmol). The mixture was stirred for 3.5 days at room temperature, and diluted with $CHCl_3$, which was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1, then 9:1) gave 19 (498 mg, 76%) as an oil. IR ν_{max} (KBr) 2924, 2853, 1749, 1615, 1515, 1457, 1249 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 0.98 (9H, s), 1.01 (9H, s), 1.25 (64H, br s), 1.53–1.58 (4H, m), 3.46–3.92 (17H, m, containing two 3H singlets at 3.69 and 3.75 ppm), 4.30–4.75 (17H, m), 5.47 (1H, dd, *J* 5.1, 9.7 Hz), 6.70 (2H, d, *J* 8.8 Hz), 6.81 (2H, d, *J* 8.6 Hz), 7.15–7.32 (29H, m). ESI MS: *m*/*z* 1760.1 $[M+Na]^+$, 1761.2, 1762.2. HRESI MS: calcd for $C_{109}H_{160}O_{15}SiNa$: 1760.1424; observed: 1760.1426.

*4.1.17. (2S,3R,4R)-2-[(2′R,3′R,4′R)-2′,3′,4′-(Tribenzyloxy)hexacosanoyloxy]-3,4-(dibenzyloxy)octadecyl 4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**20**).* To a solution of 19 (536 mg, 0.308 mmol) in CH_2Cl_2–H_2O (10:1, 44 mL) was added DDQ (536 mg, 2.36 mmol). The mixture was stirred for 2.5 h at room temperature, and diluted with CH_2Cl_2. The whole was washed twice with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1, 6:1, then 4:1) gave a mixture (206 mg) of 20 and inseparable 4-methoxybenzaldehyde as an oil. This mixture was employed for the next reaction without further purification. IR ν_{max} (KBr) 3530, 2925, 2854, 1750 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 1.00 (9H, s), 1.02 (9H, s), 1.26 (64H, br s), 1.45–1.65 (4H, m), 2.37 (1H, d, *J* 9.7 Hz, OH), 2.66 (1H, d, *J* 11.5 Hz, OH), 3.43–4.04 (11H, m, excluding methoxy protons of the contaminated inseparable 4-methoxybenzalehyde at 3.90 ppm), 4.24 (1H, d, *J* 3.4 Hz), 4.41–4.59 (8H, m), 4.69–4.76 (4H, m), 5.48 (1H, m), 7.21–7.36 (25H, m). ESI MS: *m*/*z* 1520.0 $[M+Na]^+$. HRESI MS: calcd for $C_{93}H_{144}O_{13}SiNa$: 1520.0274; observed: 1520.0243.

*4.1.18. (2S,3R,4R)-2-[(2′R,3′R,4′R)-2′,3′,4′-(Tribenzyloxy)hexacosanoyloxy]-3,4-(dibenzyloxy)octadecyl α-D-galactopyranoside (**21**).* To a solution of a mixture (206 mg) of 20 and 4-methoxybenzaldehyde in dry THF (12 mL) containing dry pyridine (110 mg) was added HF·pyridine (HF: ~70%; pyridine: ~30%, 55 mg) at room temperature. After stirring for 2 h at room temperature, the reaction mixture was diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1, then 2:3) gave 21 (109 mg, two steps 26%) as a viscous oil. IR ν_{max} (KBr) 3420, 2924, 2853, 1749 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 6.9 Hz), 1.20–1.40 (64H, m), 1.58–1.71 (4H, m), 1.95 (1H, d, *J* 2.9 Hz, OH), 2.16 (1H, br s, OH), 2.42 (1H, d, *J* 11.9 Hz, OH), 2.58 (1H, s, OH), 3.14 (1H, dt, *J* 9.7, 1.1 Hz), 3.48–3.58 (5H, m), 3.62 (1H, dd, *J* 3.7, 4.4 Hz), 3.68–3.73 (2H, m), 3.78 (1H, d, *J* 2.7 Hz), 3.87 (1H, dd, *J* 2.0, 8.6 Hz), 4.09 (1H, dd, *J* 2.2, 11.0 Hz), 4.42–4.82 (12H, m), 5.55 (1H, dt, *J* 9.3, 2.9 Hz), 7.20–7.36 (25H, m). ESI MS: *m*/*z* 1379.9 $[M+Na]^+$. HRESI MS: calcd for $C_{85}H_{128}O_{13}Na$: 1379.9253; observed: 1379.9287.

*4.1.19. (2S,3R,4R)-2-[(2′R,3′R,4′R)-2′,3′,4′-(Trihydroxy)hexacosanoyloxy]-3,4-(dihydroxy)octadecyl α-D-galactopyranoside (**22**) (RCAI-169).* A solution of 21 (50 mg, 3.68×10^{-2} mmol) in THF (40 mL) was stirred for 21 h at room temperature under hydrogen using 20% $Pd(OH)_2$ on carbon (wet, water ca. 50%, 60 mg) catalyst, and the reaction mixture was filtered. The catalyst was washed with THF, and combined THF solution was concentrated in vacuo to give a white powder, which was washed with Et_2O (5 mL×2). This powder was dried with a pump to give 22 (26 mg, 78%) as a powder. It was obvious by 2D COSY analysis that the (2′,3′,4′-trihydroxy) hexacosanoyl group of 22 was on the C2–O position on the octadecane chain. However, silica gel column chromatography of 22 by elution with $CHCl_3$–MeOH (9:1) yields gradually C4–O acyl migrated compound. IR ν_{max} (KBr) 3390, 2919, 2851, 1734, 1468 cm^{-1}. $[\alpha]_D^{21}$ +44.6 (*c* 0.92, pyridine). ^1H NMR (500 MHz, pyridine-d_5) δ 0.87 (6H, t, *J* 6.9 Hz), 1.20–1.42 (60H, m), 1.60–1.72 (2H, m), 1.82–2.02 (4H, m), 2.02–2.35 (2H, m), 4.27 (1H, m), 4.37–4.58 (8H, m), 4.60–4.66 (2H, m), 4.93 (1H, dd, *J* 3.4, 7.6 Hz), 5.19 (1H, d, *J* 4.5 Hz), 5.53 (1H, d, *J* 3.5 Hz, anomeric H), 6.40 (1H, m, HC2–OCO). ^{13}C NMR (125 MHz, pyridine-d_5) δ 14.33, 22.98, 26.41, 29.66, 29.97, 30.05, 30.08, 30.18, 30.34, 32.17, 34.21, 34.73, 62.72, 66.73, 70.53, 71.06,

71.63, 71.91, 72.38, 72.81, 74.93, 75.35, 77.99, 101.01, 174.04. ESI MS: *m*/*z* 503.3, 929.7 $[M+Na]^+$. HRESI MS: calcd for $C_{50}H_{98}O_{12}Na$: 929.6905; observed, 929.6892.

*4.1.20. (2R,3S,4R,5S,6S)-3,4,5-Tribenzyloxy-1-tert-butyldimethylsilyloxyoctadecane-2,6-diol (**24**) and (2R,3S,4R,5S,6R)-3,4,5-tribenzyloxy-1-tert-butyldimethylsilyloxyoctadecane-2,6-diol (**24′**).* A solution of 1-bromododecane (20.0 g, 80.25 mmol) in dry Et_2O (100 mL) was gradually added to Mg turnings (2.43 g, 0.10 atom) containing catalytic amount of I_2 in a flask equipped with a reflux condenser at room temperature. After dropping the ether solution of 1-bromododecane, and ceasing the reflux, the resulting Grignard solution was left to stand for 30 min. To this solution was added dropwise a solution of 23 (8.89 g, 15.77 mmol) in Et_2O (50 mL) at room temperature. After ceasing the vigorous reaction, the mixture was stirred for 1 h at room temperature, and quenched with satd aq NH_4Cl carefully. The mixture was diluted with $CHCl_3$ (1 L), and the whole was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1, then 4:1) gave 24′ (4.44 g, 38%) as an oil and 24 (5.09 g, 44%) as an oil. Physical data of 24: R_f=0.415 (hexane–EtOAc (4:1); IR ν_{max} (KBr) 3551, 2925, 2854, 1456, 1251, 1106, 1065 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 0.05 (6H, s), 0.88 (3H, t, *J* 7.3 Hz), 0.89 (9H, s), 1.26 (18H, m), 1.28 (2H, m), 1.47 (2H, m), 3.60 (1H, dd, *J* 4.6, 5.5 Hz), 3.63 (1H, dd, *J* 7.3, 10.1 Hz), 3.69 (1H, dd, *J* 5.5, 10.1 Hz), 3.84 (1H, ddd, *J* 4.6, 5.3, 7.3 Hz), 3.90 (1H, dd, *J* 1.4, 5.6 Hz), 3.96 (1H, ddd, *J* 1.4, 5.9, 7.3 Hz), 3.99 (1H, dd, *J* 5.1, 5.5 Hz), 4.57, 4.60 (2H, AB-q, *J* 11.5 Hz), 4.63, 4.68 (2H, AB-q, *J* 11.5 Hz), 4.75, 4.80 (2H, AB-q, *J* 11.0 Hz), 7.26–7.34 (15H, m). ^{13}C NMR (150 MHz, $CDCl_3$) −5.42, −5.36, 14.11, 18.19, 22.68, 25.73, 25.91 (×3), 29.36, 29.42, 29.59, 29.60, 29.63, 29.66, 29.69, 31.92, 33.16, 63.49, 71.10, 71.25, 72.91, 73.79, 74.35, 76.57, 79.88, 80.94, 127.70, 127.82 (×2), 128.06, 128.17 (×2), 128.43 (×6), 128.81 (×2), 128.87 (×2), 137.94, 138.06, 138.26. ESI MS: *m*/*z* 757.48 $[M+Na]^+$. HRESI MS: calcd for $C_{45}H_{70}O_6SiNa$: 757.4839; observed: 757.4841. Physical data of 24′: R_f=0.537 hexane–EtOAc (4:1); IR ν_{max} (KBr) 3558, 2926, 2855, 1457, 1253, 1108, 1067 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 0.03 (3H, s), 0.04 (3H, s), 0.87 (9H, s), 0.88 (3H, t, *J* 7.3 Hz), 1.20 (1H, m), 1.26 (16H, m), 1.28 (2H, m), 1.39 (1H, m), 1.40 (1H, m), 1.54 (1H, m), 3.53 (1H, dd, *J* 2.3, 7.3 Hz), 3.60 (1H, ddd, *J* 2.3, 4.6, 7.8 Hz), 3.86 (1H, ddd, *J* 1.9, 5.9, 7.3 Hz), 3.90 (1H, dd, *J* 1.9, 3.2 Hz), 4.11 (1H, dd, *J* 3.2, 7.3 Hz), 4.59, 4.85 (2H, AB-q, *J* 11.0 Hz), 4.61, 4.79 (2H, AB-q, *J* 11.5 Hz), 4.71, 4.79 (2H, AB-q, *J* 11.0 Hz), 7.27–7.42 (15H, m). ^{13}C NMR (150 MHz, $CDCl_3$) −5.41 (×2), 14.11, 18.17, 22.68, 25.90 (×3), 25.96, 29.36, 29.59 (×2), 29.65 (×2), 29.68 (×2), 31.92, 34.74, 63.10, 71.05, 71.96, 73.28, 75.01, 75.04, 76.66, 81.25, 81.50, 127.74, 127.84 (×2), 127.95 (×2), 127.98 (×2), 128.12 (×2), 128.39 (×2), 128.41 (×4), 138.09 (×2), 138.15. ESI MS: *m*/*z* 757.48 $[M+Na]^+$. HRESI MS: calcd for $C_{45}H_{70}O_6SiNa$: 757.4839; observed: 757.4826.

*4.1.21. Mosher's ester of **24** (**24R**).* (*S*)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride (10 mg) was added to a solution of **24** (5 mg) and DMAP (5 mg) in THF (0.2 mL) containing pyridine (30 mg). After stirring for 15 min at room temperature, the reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a TLC plate. Development with hexane–EtOAc (9:1) gave **24R** (5 mg, 63%) as an oil. IR ν_{max} (KBr) 2927, 2855, 1746, 1497, 1454, 1256, 1170, 1121, 1019, 838 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ −0.023 (3H, s), −0.015 (3H, s), 0.84 (9H, s), 0.89 (3H, t *J* 7.1 Hz), 1.07 (1H, m, H-8a), 1.27 (1H, m, H-8b), 1.12–1.29 (18H, m), 1.75(2H, m, H-7), 3.51 (3H, s), 3.53 (3H, s), 3.70 (1H, dd, *J* 10.6, 6.4 Hz, H-1a), 3.70 (1H, dd, *J* 7.4, 4.1 Hz, H4), 3.79 (1H, dd, *J* 4.1, 1.8 Hz, H-5), 3.80 (1H, dd, *J* 10.6, 5.9 Hz, H-1b), 4.03 (1H, dd, *J* 7.4, 2.7 Hz, H-3), 4.04 (1H, d, *J* 11.9 Hz, H-5-O–Bn), 4.32 (1H, d, *J* 11.9 Hz, H-5-O–Bn), 4.51 (2H, s, H-4-O–Bn), 4.59 (1H, d, *J* 11.9 Hz, H-3-O–Bn), 4.63 (1H, d, *J* 11.9 Hz, H-3-O–Bn), 5.35 (1H, ddd, *J* 6.5, 6.5, 1.8 Hz, H-6), 5.47 (1H, ddd, *J* 6.4, 5.9, 2.7 Hz, H-2), 7.10–7.61 (25H, m). ^{13}C NMR (150 MHz, $CDCl_3$, ref. 77.00 ppm) −5.65, −5.58, 14.11, 18.12, 22.68, 25.77 (×3), 26.15, 28.92, 29.35, 29.45, 29.50, 29.52, 29.60, 29.64, 29.67, 31.92, 55.31, 55.64, 61.14, 73.35, 73.66, 74.10, 75.74, 76.07, 79.15, 79.52, 80.79, 84.30, 84.80, 126.89 (×2), 127.06 (×2), 127.21, 127.30, 127.39, 127.56, 127.79, 127.83, 128.32, 128.44, 129.47, 129.58, 131.74, 132.25, 137.82, 138.18, 138.27, 166.36, 166.42. ESI MS: *m*/*z* 1189.6 $[M+Na]^+$. HRESI MS: calcd for $C_{65}H_{84}O_{10}F_6SiNa$: 1189.5636; observed: 1189.5632.

*4.1.22. Mosher's ester of **24** (**24S**).* The same treatment of **24** (5 mg) with (*R*)-(−)-MTPA chloride (10 mg) afforded **24S** (5 mg, 63%) as an oil. IR ν_{max} (KBr) 2928, 2855, 1747, 1497, 1454, 1258, 1170, 1122, 1021, 838 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 0.008 (3H, s), 0.017 (3H, s), 0.86 (9H, s), 0.89 (3H, t, *J* 7.1 Hz), 0.98 (1H, m, H-8a), 1.11(1H, m, H-8b), 1.09–1.29 (18H, m), 1.63 (1H, m, H-7a), 1.65 (1H, m, H-7b), 3.41 (3H, s), 3.54 (3H, s), 3.62 (1H, dd, *J* 7.8, 4.1 Hz, H-4), 3.75 (1H, dd, *J* 10.5, 6.4 Hz, H-1a), 3.76 (1H, dd, *J* 4.1, 2.7 Hz), 3.88 (1H, dd, *J* 10.5, 6.9 Hz, H-1b), 3.95 (1H, dd, *J* 7.8, 3.2 Hz, H-3), 4.27 (1H, d, *J* 11.0 Hz, 5-O–Bn), 4.34 (1H, d, *J* 11.0 Hz, H-3-O–Bn), 4.47 (1H, d, *J* 11.9 Hz, H-3-O–Bn), 4.53 (2H, d, *J* 11.0 Hz, H-3-O–Bn and H-4-O–Bn), 4.59 (1H, d, *J* 11.9 Hz, H-3-O–Bn), 5.40 (1H, ddd, *J* 9.7, 2.7, 2.7 Hz, H-6), 5.43 (1H, ddd, *J* 6.9, 6.4, 3.2 Hz, H-2), 7.16–7.60 (25H, m). ^{13}C NMR (150 MHz, $CDCl_3$, ref. 77.00 ppm) −5.52, −5.54, 14.11, 18.12, 22.68, 25.58, 25.76 (×3), 29.23, 29.33, 29.36, 29.43, 29.49, 29.62, 29.65, 29.67, 31.92, 55.40, 55.48, 61.05, 73.23, 73.59, 74.01, 76.07, 76.35, 77.89, 78.26, 80.24, 84.40, 84.50, 126.95, 127.31, 127.39, 127.43, 127.46, 127.50, 127.76, 128.16, 128.22, 128.32, 128.34, 129.49, 129.52, 131.74, 132.12 (×2), 137.89, 138.15 (×2), 166.15, 166.50. ESI MS: *m*/*z* 1189.6 $[M+Na]^+$. HRESI MS: calcd for $C_{65}H_{84}O_{10}F_6SiNa$: 1189.5636; observed: 1189.5621.

*4.1.23. (2R,3S,4R,5S,6S)-3,4,5-(Tribenzyloxy)octadecane-1,2,6-triol (**25**).* To a solution of **24** (5.23 g, 7.11 mmol) in THF (140 mL) was added TBAF (1.0 M in THF, 28 mL). After stirring for 1 h at room temperature, the reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:1, then 1:1) gave **25** (3.11 g, 71%) as an oil. IR ν_{max} (KBr) 3465, 2925, 2854, 1457, 1065 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 6.8 Hz), 1.22–1.31 (18H, m), 1.45–1.55 (4H, m), 1.99 (1H, m, OH), 2.47 (1H, d, *J* 5.6 Hz, OH), 3.13 (1H, d, *J* 5.9 Hz, OH), 3.55–3.61 (2H, m), 3.68–3.73 (2H, m), 3.84 (1H, m), 3.96–4.02 (2H, m), 4.52, 4.60 (2H, AB-q, *J* 11.6 Hz), 4.62, 4.66 (2H, AB-q, *J* 11.5 Hz), 4.77 (2H, s), 7.26–7.35 (15H, m). ESI MS: *m*/*z* 643.4 $[M+Na]^+$. HRESI MS: calcd for $C_{39}H_{56}O_6Na$: 643.3975; observed: 643.3953.

*4.1.24. (2R,3S,4R,5S,6S)-3,4,5-Tribenzyloxy-1,2-(isopropylidenedioxy)octadecan-6-ol (**26**).* A solution of **25** (3.20 g, 5.15 mmol) in 2,2-dimethoxypropane (60 mL) containing *p*-toluenesulfonic acid monohydrate (60 mg) was stirred for 1 h at room temperature, and diluted with EtOAc, which was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a crude **26** (3.46 g, quant.), which was employed for the next reaction without purification. A part of the crude **26** (10 mg) was purified on a preparative TLC plate. Development with hexane–EtOAc (4:1) gave **26** (8 mg) as an oil. IR ν_{max} (KBr) 3630, 2925, 2854, 1457, 1067 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.26 (18H, br s), 1.36 (3H, s), 1.41–1.50 (5H, m, containing 3H singlet at 1.44 ppm), 1.52–1.60 (2H, m), 2.81 (1H, d, *J* 5.1 Hz, OH), 3.47 (1H, dd, *J* 2.9, 6.1 Hz), 3.68–3.73 (2H, m), 3.77 (1H, dd, *J* 3.0, 6.4 Hz), 3.90 (1H, m), 3.94 (1H, dd, *J* 6.4, 8.3 Hz), 4.40 (1H, dd, *J* 6.5, 13.6 Hz), 4.48, 4.77 (2H, AB-q, *J* 11.5 Hz), 4.55, 4.57 (2H, AB-q, *J*

11.6 Hz), 4.59, 4.68 (2H, AB-q, *J* 11.3 Hz), 7.27–7.33 (15H, m). ESI MS: *m*/*z* 683.4 $[M+Na]^+$. HRESI MS: calcd for $C_{42}H_{60}O_6Na$: 683.4288; observed: 683.4289.

*4.1.25. (2R,3S,4R,5S,6S)-3,4,5,6-Tetrabenzyloxy-1,2-(isopropylidenedioxy)octadecane (**27**).* To a solution of crude **26** (3.40 g, 5.14 mmol) in dry DMF (30 mL) were added NaH (60% oil dispersion, 370 mg, 15.4 mmol) and BnBr (1.76 g, 10.3 mmol) at 0 °C, and the mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with EtOAc, which was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1, then 9:1) gave **27** (3.61 g, 93%, two steps) as an oil. IR ν_{max} (KBr) 2925, 2854, 1517, 1496, 1455, 1067 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 6.9 Hz), 1.21–1.30 (18H, m), 1.34 (3H, s), 1.40–1.46 (5H, m, containing 3H singlet at 4.21 ppm), 1.62–1.71 (2H, m), 3.62–3.66 (2H, m), 3.71–3.75 (2H, m), 3.86 (1H, t, *J* 3.9 Hz), 3.92 (1H, dd, *J* 6.5, 8.5 Hz), 4.29 (1H, dd, *J* 6.4, 14.0 Hz), 4.47 (2H, s), 4.48, 4.67 (2H, AB-q, *J* 11.5 Hz), 4.60, 4.73 (2H, AB-q, *J* 11.5 Hz), 4.60, 4.75 (2H, AB-q, *J* 11.6 Hz), 7.25–7.32 (20H, m). ESI MS: *m*/*z* 773.5 $[M+Na]^+$. HRESI MS: calcd for $C_{49}H_{66}O_6Na$: 773.4757; observed: 773.4745.

*4.1.26. (2R,3S,4R,5S,6S)-3,4,5,6-(Tetrabenzyloxy)octadecane-1,2-diol (**28**).* To a solution of crude **27** (3.60 g, 4.79 mmol) in CH_2Cl_2–MeCN (1:1, 200 mL) was added aq 46% HF (3.0 mL) at room temperature. The mixture was stirred for 30 min at room temperature, diluted with CH_2Cl_2, which was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give **28** (3.36 g, 99%) as an oil. IR ν_{max} (KBr) 3540, 2925, 2854, 1515, 1456 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.26 (18H, br s), 1.46–1.48 (2H, m), 1.72–1.78 (2H, m), 1.93 (1H, m, OH), 3.47–3.76 (4H, m, containing 1H doublet *J* 4.2 Hz at 3.48 ppm, OH), 3.66 (1H, m), 3.85 (1H, dd, *J* 3.5, 6.2 Hz), 3.88 (1H, m), 3.94 (1H, dd, *J* 3.8, 6.5 Hz), 4.43, 4.54 (2H, AB-q, *J* 11.7 Hz), 4.50, 4.52 (2H, AB-q, *J* 11.8 Hz), 4.71, 4.79 (2H, AB-q, *J* 11.5 Hz), 4.73, 4.77 (2H, AB-q, *J* 11.4 Hz), 7.25–7.33 (20H, m). ESI MS: *m*/*z* 733.4 $[M+Na]^+$. HRESI MS: calcd for $C_{46}H_{62}O_6Na$: 733.4444; observed: 733.4438.

*4.1.27. (2R,3S,4R,5S,6S)-3,4,5,6-Tetrabenzyloxy-1-(tert-butyldimethylsilyloxy)octadecan-2-ol (**29**).* To a solution of **28** (3.36 g, 4.70 mmol) in dry CH_2Cl_2 (200 mL) was added TBDMS-Cl (1.42 g, 9.40 mmol), imidazole (960 mg, 14.1 mmol) and a catalytic amount of DMAP (10 mg). The mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, which was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1, then 3:2) gave **29** (3.63 g, 93%) as an oil. IR ν_{max} (KBr) 3648, 2928, 2855, 1454 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.04 (6H, s), 0.86–0.90 (12H, m), 1.26 (18H, br s), 1.40–1.52 (2H, m), 1.73–1.77 (2H, m), 3.51–3.55 (2H, m), 3.63–3.70 (2H, m), 3.76 (1H, d, *J* 3.8 Hz, OH), 3.89–3.96 (3H, m), 4.46, 4.55 (2H, AB-q, *J* 11.5 Hz), 4.52 (2H, s), 4.68, 4.82 (2H, AB-q, *J* 11.4 Hz), 4.74, 4.77 (2H, AB-q, *J* 11.0 Hz), 7.25–7.34 (20H, m). ESI MS: *m*/*z* 847.5 $[M+Na]^+$. HRESI MS: calcd for $C_{52}H_{76}O_6SiNa$: 847.5309; observed: 847.5297.

*4.1.28. (3R,4R,5S,6S)-3,4,5,6-Tetrabenzyloxy-1-(tert-butyldimethylsilyloxy)octadecan-2-one (**30**).* A solution of **29** (3.62 g, 4.39 mmol) in dry CH_2Cl_2 (80 mL) containing Dess–Martin periodinane (3.80 g, 8.96 mmol) was stirred for 2 h at room temperature, and diluted with CH_2Cl_2, which was washed with aq 10% $Na_2S_2O_3$ and satd aq $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1) gave **30** (3.30 g, 91%) as an oil. IR ν_{max} (KBr) 2926, 2854, 1734, 1518, 1497, 1455 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.03 (3H, s), 0.04 (3H, s), 0.88–0.93 (12H, m), 1.18–1.45 (21H, m), 1.74 (1H, m), 3.58 (1H, td, *J* 2.9, 8.8 Hz), 3.92 (1H, dd, *J* 3.9, 7.1 Hz), 3.97 (1H, dd, *J* 3.1, 7.1 Hz), 4.12 (1H, d, *J* 3.9 Hz), 4.40–4.76 (10H, m), 7.26–7.36 (20H, m). ESI MS: *m*/*z* 731.4, 845.5 $[M+Na]^+$. HRESI MS: calcd for $C_{52}H_{74}O_6SiNa$: 845.5152; observed: 845.5135.

*4.1.29. (2S,3S,4R,5S,6S)-3,4,5,6-Tetrabenzyloxy-1-(tert-butyldimethylsilyloxy)octadecan-2-ol (**31**).* To a solution of **30** (3.30 g, 3.29 mmol) in dry EtOH (80 mL) was added $NaBH_4$ (powder, 220 mg, 5.82 mmol). The mixture was stirred for 30 min at room temperature. The reaction mixture was quenched with a few drops of AcOH, and neutralized with satd aq $NaHCO_3$, and concentrated in vacuo to give a residue, which was dissolved in EtOAc. The solution was washed with water, and dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture of **29** and **31** (3.33 g), which was separated on a silica gel column in three parts. Silica gel (111 g) was used for separation of the mixture (1.11 g). Elution with hexane–EtOAc (19:1) gave **31** (total amount of three times: 1.12 g, 34%) as an oil [R_f=0.402 (hexane–EtOAc=9/1)] and **29** (1.71 g, 52%) as an oil [R_f=0.348 (hexane–EtOAc=9/1)]. Physical data of **31**: IR ν_{max} (KBr) 3550, 2925, 2854, 1455 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.05 (3H, s), 0.06 (3H, s), 0.88 (3H, t, *J* 6.8 Hz), 0.90 (9H, s), 1.20–1.31 (18H, m), 1.42–1.52 (3H, m), 1.80 (1H, m), 2.67 (1H, d, *J* 4.4 Hz, OH), 3.58 (1H, m), 3.62 (1H, dd, *J* 2.2, 7.5 Hz), 3.67 (1H, dd, *J* 6.3, 10.2 Hz), 3.84 (1H, dd, *J* 3.1, 10.2 Hz), 3.93–3.97 (2H, m), 4.11 (1H, dd, *J* 3.1, 7.0 Hz), 4.42, 4.56 (2H, AB-q, *J* 11.7 Hz), 4.51, 4.62 (2H, AB-q, *J* 11.8 Hz), 4.75, 4.79 (2H, AB-q, *J* 11.0 Hz), 4.76 (2H, s), 7.23–7.35 (20H, m). ESI MS: *m*/*z* 847.5 $[M+Na]^+$, 848.5. HRESI MS: calcd for $C_{52}H_{76}O_6SiNa$: 847.5300; observed: 847.5287.

*4.1.30. (2S,3S,4R,5S,6S)-3,4,5,6-Tetrabenzyloxyoctadecane-1,2-diol (**32**).* To a solution of **31** (1.14 g, 7.11 mmol) in THF (50 mL) was added TBAF (1.0 M in THF, 10 mL). After stirring for 1 h at room temperature, the reaction mixture was diluted with EtOAc (350 mL), washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:1, then 1:1) gave **32** (845 mg, 86%) as an oil. IR ν_{max} (KBr) 3450, 2925, 2853, 1456 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 6.9 Hz), 1.20–1.30 (19H, m), 1.44 (1H, m), 1.61 (1H, m), 1.75 (1H, m), 2.15 (1H, t, *J* 6.4 Hz, OH), 3.19 (1H, d, *J* 4.7 Hz, OH), 3.55 (1H, quintuplet, *J* 3.9 Hz), 3.64 (1H, dd, *J* 2.7, 7.4 Hz), 3.67 (1H, m), 3.76–3.82 (2H, m), 3.84 (1H, m), 3.94 (1H, dd, *J* 2.7, 5.4 Hz), 4.45, 4.48 (2H, AB-q, J 11.5 Hz), 4.49, 4.56 (2H, AB-q, J 11.5 Hz), 4.69–4.75 (4H, m), 7.23–7.33 (20H, m). ESI MS: *m*/*z* 733.4 $[M+Na]^+$. HRESI MS: calcd for $C_{46}H_{62}O_6Na$: 733.4444; observed: 733.4438.

*4.1.31. (2S,3S,4S,5S,6S)-2-Hydroxy-3,4,5,6-tetrabenzyloxyoctadecyl 2,3-di-O-(4-methoxybenzyl)-4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**33**).* To a solution of imidate 6 [obtained from 2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-D-galactopyranose (1.10 g, 1.97 mmol) and CCl_3CN (2.84 g, 5.55 mmol) using Cs_2CO_3 (943 mg) as a base in CH_2Cl_2 (20 mL) at room temperature over night] and diol 32 (838 mg, 1.18 mmol) in dry CH_2Cl_2 (60 mL) was added MS 4 Å (dry powder, 3.2 g). After stirring for 30 min at room temperature, AgOTf (400 mg, 1.56 mmol) was added to this mixture. After stirring for 1 h at room temperature, the reaction mixture was filtered, and the filter cake was washed with CH_2Cl_2. The combined filtrate was washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1, then 6:1) gave 33 (568 mg, 38%) as an oil. IR ν_{max} (KBr) 3465, 2926, 2855, 1612, 1514, 1249 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, *J* 7.0 Hz),

0.99 (9H, s), 1.05 (9H, s), 1.20–1.30 (19H, m), 1.46–1.49 (2H, m), 1.64 (1H, br s), 1.74 (1H, m), 3.48 (1H, dd, *J* 8.1, 10.5 Hz), 3.56 (1H, m), 3.62 (1H, s), 3.67 (1H, dd, *J* 2.9, 6.6 Hz), 3.70 (1H, s), 3.77–3.80 (4H, m, containing 3H singlet at 3.79 ppm), 3.87 (1H, dd, *J* 3.0, 6.0 Hz), 3.94–3.97 (2H, m), 4.02–4.03 (2H, m), 4.10–4.15 (2H, m), 4.41, 4.51 (2H, AB-q, *J* 11.6 Hz), 4.43 (1H, d, *J* 3.0 Hz), 4.56–4.79 (11H, m, containing anomeric H), 6.74 (2H, d, *J* 8.8 Hz), 6.86 (2H, d, *J* 8.8 Hz), 7.22–7.34 (24H, m). ESI MS: *m*/*z* 1275.7 $[M+Na]^+$. HRESI MS: calcd for $C_{76}H_{104}O_{13}SiNa$: 1275.7144; observed: 1275.7131.

*4.1.32. (2S,3S,4R,5S,6S)-2-[(2′R,3′R)-2′,3′-(Isopropylidenedioxy)hexacosanoyloxy]-3,4,5,6-(tetrabenzyloxy)octadecyl 2,3-di-O-(4-methoxybenzyl)-4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**34**).* To a solution of 33 (553 mg, 0.441 mmol) in dry CH_2Cl_2–THF (1:1, 50 mL) were added DMAP (1.23 g, 10.14 mmol), carboxylic acid 8 (620 mg, 1.32 mmol) and then EDAC (1.69 g, 8.82 mmol) at room temperature. After stirring for 5 h at room temperature, the reaction mixture was concentrated in vacuo to give a mixture, which was dissolved into CH_2Cl_2. The solution was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (14:1, then 9:1) gave 34 (472 mg, 63%) as an oil. IR ν_{max} (KBr) 2925, 2854, 1735, 1614, 1513, 1468, 1457 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.878 (3H, t, *J* 7.0 Hz), 0.882 (3H, t, *J* 6.8 Hz), 0.95 (9H, s), 1.01 (9H, s), 1.18–1.27 (62H, m), 1.42–1.48 (3H, m), 1.68 (1H, m), 3.43 (1H, s), 3.59 (1H, m), 3.67 (1H, dd, *J* 2.8, 10.1 Hz), 3.74 (1H, s), 3.78–3.90 (10H, m, containing 3H singlet at 3.78 ppm), 4.07 (1H, dd, *J* 4.2, 11.3 Hz), 4.24–4.32 (2H, m), 4.35–4.77 (12H, m), 5.62 (1H, m, HC2–OC=O), 6.75 (2H, d, *J* 8.8 Hz), 6.84 (2H, d, *J* 8.5 Hz), 7.19–7.31 (24H, m). ESI MS: *m*/*z* 1726.1 $[M+Na]^+$, 1727.1, 1728.1. HRESI MS: calcd for $C_{105}H_{158}O_{16}SiNa$: 1726.1217; observed: 1726.1215.

*4.1.33. (2S,3S,4R,5S,6S)-2-[(2′R,3′R)-2′,3′-(Isopropylidenedioxy)hexacosanoyloxy]-3,4,5,6-(tetrabenzyloxy)octadecyl 4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**35**).* To a solution of 34 (468 mg, 0.275 mmol) in CH_2Cl_2–H_2O (10:1, 36.3 mL) was added DDQ (470 mg, 2.07 mmol). The mixture was stirred for 2.5 h at room temperature, and diluted with CH_2Cl_2. The solution was washed twice with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (6:1, then 3:1) gave a 1:1 mixture (255 mg) of 35 and inseparable 4-methoxybenzaldehyde as an oil. IR ν_{max} (KBr) 3567, 2925, 2853, 1734 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 0.99 (9H, s), 1.02 (9H, s), 1.20–1.31 (62H, m), 1.38 (3H, s), 1.45–1.56 (3H, m), 1.59 (3H, s), 1.68 (1H, m), 2.26 (1H, d, *J* 10.0 Hz, OH), 2.44 (1H, d, *J* 10.2 Hz, OH), 3.43 (1H, s), 3.52 (1H, dt, *J* 3.4, 10.1 Hz), 3.58 (1H, m), 3.73 (1H, dd, *J* 3.6, 9.9 Hz), 3.77–3.86 (4H, m), 3.89 (1H, dd, *J* 1.7, 12.5 Hz), 3.94 (1H, dd, *J* 2.0, 12.5 Hz), 4.18 (1H, dd, *J* 2.9, 11.2 Hz), 4.21 (1H, d, *J* 3.4 Hz), 4.32 (1H, m), 4.40–4.75 (9H, m), 4.84 (1H, d, *J* 3.9 Hz, anomeric H), 5.60 (1H, dd, J 2.3, 8.7 Hz, HC2OC=O), 7.23–7.33 (20H, m). ESI MS: *m*/*z* 1486.0 $[M+Na]^+$. HRESI MS: calcd for $C_{89}H_{142}O_{14}SiNa$: 1486.0067; observed: 1486.0056.

*4.1.34. (2S,3S,4R,5S,6S)-2-[(2′R,3′R)-2′,3′-(Isopropylidenedioxy)hexacosanoyloxy]-3,4,5,6-(tetrabenzyloxy)octadecyl α-D-galactopyranoside (**36**).* To a solution of 35 (250 mg, 0.170 mmol) in dry THF (16 mL) containing dry pyridine (140 mg) was added HF·pyridine (HF: ~70%; pyridine: ~30%, 70 mg) at room temperature. After stirring for 1.5 h at room temperature, the reaction mixture was diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1) and then $CHCl_3$–MeOH (47:3, then 19:1) gave 36 (168 mg, 74%, two steps) as an oil. IR ν_{max} (KBr) 3446, 2925, 2853, 1733, 1456 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 1.20–1.32 (62H, m), 1.39 (3H, s), 1.45–1.57 (3H, m), 1.60 (3H, s), 1.69 (1H, m), 2.07 (1H, m, OH), 2.23 (1H, d, *J* 10.8 Hz, OH), 2.48 (1H, br s, OH), 2.72 (1H, br s, OH), 3.52–3.90 (11H, m), 4.24 (1H, dd, *J* 3.0, 11.3 Hz), 4.33 (1H, m), 4.41–4.77 (9H, m), 4.85 (1H, d, *J* 3.9 Hz, anomeric H), 5.61 (1H, td, *J* 1.2, 8.8 Hz, HC2OC=O), 7.25–7.34 (20H, m). ESI MS: *m*/*z* 1345.9 $[M+Na]^+$, 1346.9. HRESI MS: calcd for $C_{81}H_{126}O_{14}Na$: 1345.9045; observed: 1345.9019.

*4.1.35. (2S,3S,4R,5S,6S)-2-[(2′R,3′R)-2′,3′-(Dihydroxy)hexacosanoyloxy]-3,4,5,6-(tetrabenzyloxy)octadecyl α-D-galactopyranoside (**37**).* To a solution of crude 36 (165 mg, 0.125 mmol) in CH_2Cl_2–MeCN (1:1, 50 mL) was added aq 46% HF solution (5.4 mL). The mixture was stirred for 1.5 h at room temperature, and diluted with $CHCl_3$. The solution was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH (19:1) gave 37 (140 mg, 87%) as a gum. IR ν_{max} (KBr) 3425, 2924, 2853, 1741, 1456 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 1.20–1.33 (62H, m), 1.40–1.70 (4H, m), 2.14 (1H, br s, OH), 3.12 (1H, br s, OH), 3.42 (1H, m), 3.60 (1H, m), 3.56–3.96 (12H, m, containing four OH), 4.05 (1H, m), 4.21 (1H, d, *J* 8.6 Hz), 4.31 (1H, t, *J* 11.4 Hz), 4.41 (1H, d, *J* 11.5 Hz, benzylic H), 4.50 (2H, s, benzylic 2H), 4.60–4.73 (6H, m), 4.82 (1H, d, *J* 3.5 Hz, anomeric H), 5.80 (1H, d, *J* 9.5 Hz), 7.21–7.33 (20H, m). ESI MS: *m*/*z* 1305.9 $[M+Na]^+$, 1306.9. HRESI MS: calcd for $C_{78}H_{122}O_{14}Na$: 1305.8732; observed: 1305.8723.

*4.1.36. (2S,3R,4R,5S,6S)-2-[(2′R,3′R)-2′,3′-(Dihydroxy)hexacosanoyloxy]-3,4,5,6-(tetrahydroxy)octadecyl α-D-galactopyranoside (**38**) (RCAI-170).* A solution of 37 (68 mg, 0.053 mmol) in THF (40 mL) was stirred for 16 h at room temperature under hydrogen using 20% $Pd(OH)_2$ on carbon (wet, water ca. 50%, 74 mg) as a catalyst, and the reaction mixture was filtered. The catalyst was washed with THF, and combined THF solution was concentrated in vacuo to give a white powder, which was washed twice with Et_2O (5 mL) by sucking the supernatant Et_2O cleaning solution. The powder was dried with a pump to give 38 (36 mg, 74%) as a powder. It was obvious by 2D COSY analysis that the (2′,3′-dihydroxy)hexacosanoyl group of 38 was on the C2–O position on the octadecane chain. IR ν_{max} (KBr) 3400, 2920, 2851, 1734, 1468 cm^{-1}. $[\alpha]_D^{21}$ +40.1 (*c* 0.64, pyridine). ^1H NMR (500 MHz, pyridine-d_5) δ 0.87 (6H, t, *J* 7.0 Hz), 1.20–1.40 (58H, m), 1.55–1.68 (2H, m), 1.78–1.95 (3H, m), 2.00 (1H, m), 2.15 (1H, m), 2.26 (1H, m), 4.36 (1H, d, *J* 5.9 Hz), 4.38–4.45 (4H, m), 4.48–4.54 (4H, m), 4.61–4.65 (2H, m), 4.91 (1H, dd, *J* 3.9, 11.2 Hz), 4.96 (1H, d, *J* 8.8 Hz), 5.00 (1H, dd, *J* 3.3, 8.4 Hz), 5.48 (1H, d, *J* 3.7 Hz, anomeric H), 6.40 (1H, quintuplet, *J* 3.6 Hz, HC2–OC=O). ^{13}C NMR (125 MHz, pyridine-d_5) δ 14.33, 22.98, 26.39, 29.49, 29.65, 29.96, 30.02, 30.05, 30.07, 30.10, 30.15, 30.17, 30.19, 30.37, 32.16, 34.15, 35.13, 62.64, 66.81, 70.56, 71.00, 71.10, 71.57, 72.36, 72.53, 72.73, 73.89, 73.92, 75.64, 76.90, 101.02, 174.30. ESI MS: *m*/*z* 945.7 $[M+Na]^+$. HRESI MS: calcd for $C_{50}H_{98}O_{14}Na$: 945.6854; observed: 945.6839.

*4.1.37. (S)-2,3-O-Benzylidene-5-O-tert-butyldimethylsilyl-β-L-ribofuranose (**40β**), (S)-2,3-O-benzylidene-5-O-tert-butyldimethylsilyl-α-L-ribofuranose (**40α**) and (R)-2,3-O-benzylidene-5-O-tert-butyldimethylsilyl-β-L-ribofuranose (**41**).* To a solution of (*R*,*S*)-2,3-*O*-benzylidene-β-L-ribofuranose (39, 239 mg, 1.00 mmol), obtained from L-ribose according to the reported procedure,[12] in dry CH_2Cl_2 (10 mL) were added imidazole (82 mg, 1.20 mmol) and TBDMS-Cl (181 mg, 1.20 mmol). This solution was stirred for 45 min at room temperature, and diluted with CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column.

Elution with hexane–EtOAc (6:1, then 4:1) gave a 10:2:1 inseparable mixture of **40β**, **40α**, and **41** (261 mg, 74%) as an oil. IR ν_{max} (KBr): 3375, 2960, 2933, 2860, 1463, 1414, 1254 cm^{-1}. ESI MS: *m/z* 375.16 $[M+Na]^+$. HRESI MS: calcd for $C_{18}H_{28}O_5SiNa$: 375.1604; observed: 375.1592. ^1H NMR of 40β (600 MHz, $CDCl_3$): δ 0.16 (3H, s), 0.17 (3H, s), 0.95 (9H, s), 3.80 (1H, dd, *J* 11.0, 2.3 Hz), 3.83 (1H, dd, *J* 11.0, 1.8 Hz), 4.54 (1H, dd, *J* 2.3, 1.8 Hz), 4.61 (1H, d, *J* 6.4 Hz), 4.79 (1H, d, *J* 6.4 Hz), 4.87 (1H, d, *J* 12.0 Hz, OH), 5.45 (1H, d, *J* 12.0 Hz), 5.77 (1H, s), 7.39 (3H, m), 7.51 (2H, m). ^{13}C NMR (150 MHz, $CDCl_3$) −5.67, −5.64, 18.26, 25.78, 64.86, 82.60, 86.67, 88.25, 103.14, 105.79, 126.96, 128.44, 129.87, 135.84. ^1H NMR of **40α** (600 MHz, $CDCl_3$): δ 0.08 (6H, s), 0.91 (9H, s), 3.71 (1H, dd, *J* 11.0, 2.3 Hz), 3.80 (1H, dd, *J* 11.0, 1.8 Hz), 3.85 (1H, d, *J* 11.9 Hz, OH), 4.38 (1H, dd, *J* 2.3, 1.8 Hz), 4.67 (1H, dd, *J* 6.4, 4.1 Hz), 4.83 (1H, d, *J* 6.4 Hz), 5.56 (1H, dd, *J* 11.9, 4.1 Hz), 5.92 (1H, s), 7.40 (1H, m), 7.42 (2H, m), 7.54 (1H, m). ^{13}C NMR (150 MHz, $CDCl_3$) −5.67, −5.64, 18.26, 25.83, 65.56, 79.98, 80.73, 83.19, 98.21, 106.30, 126.83, 128.65, 130.08, 135.59. ^1H NMR of **41** ^1H NMR (600 MHz, $CDCl_3$): δ 3.83 (2H, m), 4.49 (1H, dd, *J* 2.3, 1.8 Hz), 4.64 (1H, d, *J* 5.5 Hz), 4.86 (1H, d, *J* 12.0 Hz, OH), 4.90 (1H, d, *J* 5.5 Hz), 5.42 (1H, d, *J* 12.0 Hz), 5.96 (1H, s), 7.39–7.54 (5H, m). ^{13}C NMR (150 MHz, $CDCl_3$) −5.67, −5.64, 18.26, 25.88, 64.91, 81.52, 86.53, 86.88, 103.01, 103.87, 126.81, 128.54, 129.64, 129.75.

*4.1.38. (2S,3S,4R,5EZ)-[(R)-3,4-Benzylidenedioxy]-1-tert-butyldimethylsilyloxy-5-octadecen-2-ol (**42EZ**) and (2S,3S,4R,5EZ)-[(S)-3,4-Benzylidenedioxy]-1-tert-butyldimethylsilyloxy-5-octadecen-2-ol (**43EZ**).* To a solution of *n*-tridecyltriphenylphosphonium bromide (1.58 g, 3.00 mmol) in dry THF (6 mL) was added a solution of *n*-BuLi (1.67 M in hexane, 2.4 mL, 4.01 mmol) at −10 °C under argon with stirring. After stirring for 30 min at −10 °C, a mixture of **40β**, **41** and **40α** (353 mg, 1.00 mmol) in THF (3 mL) was added at −10 °C to the resulting red-colored phosphorane solution. This mixture was stirred for 20 min at −10 °C, and then at room temperature for 16 h, and the whole was concentrated under reduced pressure to one-fifth of the volume, and quenched with water. The mixture was diluted with EtOAc, washed with water, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1, then 9:1) at 8–12 °C or containing 1% Et_3N (v/v) at room temperature gave a 5:6 mixture of **42Z** and **42E** (370 mg, 71%; or 359 mg, 69%; stored in a freezer at −80 °C) and a 1:2 mixture of **43Z** and **43E** (30 mg, 6%). The mixture of **42EZ** (15 mg) was separated using a preparative silica gel TLC plate. Development with hexane–EtOAc (9:1) at 5 °C gave *Z*-isomer (5 mg) and *E*-isomer (6 mg). Physical data of 42Z: R_f=0.488 (hexane–EtOAc=9:1); IR ν_{max} (KBr) 3569 (br), 2924, 2853, 1460, 1254 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 0.06 (3H, s), 0.07 (3H, s), 0.88 (3H, t, *J* 7.0 Hz), 0.90 (9H, s), 1.25 (18H, br s), 1.38–1.42 (2H, m), 2.12–2.22 (2H, m), 2.52 (1H, d, *J* 4.9 Hz, OH), 3.72 (1H, dd, *J* 5.5, 9.7 Hz), 3.80 (1H, m, C_2–H), 3.82 (1H, dd, *J* 3.2, 9.8 Hz), 4.11 (1H, dd, *J* 6.7, 8.4 Hz), 5.11 (1H, m), 5.63 (1H, m), 5.76 (1H, td, *J* 7.5, 11.0 Hz), 5.82 (1H, s), 7.36–7.38 (3H, m), 7.47–7.48 (2H, m). HRESI MS: calcd for $C_{31}H_{54}O_4SiNa$: 541.3689; observed: 541.3674. Physical data of **42E**: R_f=0.418 (hexane–EtOAc=9:1); IR ν_{max} (KBr) 3569 (br), 2925, 2854, 1458, 1254 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 0.070 (3H, s), 0.074 (3H, s), 0.88 (3H, t, *J* 6.8 Hz), 0.91 (9H, s), 1.25 (18H, br s), 1.38–1.41 (2H, m), 2.10 (2H, q, *J* 6.8 Hz), 2.52 (1H, d, *J* 4.9 Hz, OH), 3.72–3.84 (3H, m), 4.09 (1H, dd, *J* 6.8, 8.5 Hz), 4.74 (1H, t, *J* 7.1 Hz), 5.69 (1H, dd, *J* 7.5, 15.4 Hz), 5.81 (1H, s), 5.88 (1H, td, *J* 6.8, 15.4 Hz), 7.38–7.40 (3H, m), 7.47–7.49 (2H, m). ESI MS: *m/z* 541.37 $[M+Na]^+$. ESI MS: *m/z* 541.37 $[M+Na]^+$. HRESI MS: calcd for $C_{31}H_{54}O_4SiNa$: 541.3689; observed: 541.3676. The mixture of **43EZ** (30 mg) was separated using a preparative silica gel TLC plate. Development with hexane–EtOAc (6:1) at 5 °C gave *Z*-isomer (5 mg) and *E*-isomer (10 mg). Physical data of **43Z**: R_f=0.425 (hexane–EtOAc=6:1); IR ν_{max} (KBr) 3564 (br), 2925, 2854, 1462, 1251, 1094, 1068 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 0.08 (3H, s), 0.11 (3H, s), 0.88 (3H, t, *J* 7.1 Hz), 0.89 (9H, s), 1.26 (18H, br s), 1.38–1.40 (2H, m), 2.03 (1H, br s, OH), 2.09 (1H, m), 2.22 (1H, m), 3.71 (1H, m), 3.75 (1H, m), 3.95 (1H, m), 4.71 (1H, dd, *J* 6.1, 6.8 Hz), 5.05 (1H, dd, *J* 7.1, 9.1 Hz), 5.61 (1H, dd, *J* 9.2, 10.9 Hz), 5.73 (1H, m), 5.81 (1H, m), 7.36–7.38 (3H, m), 7.48–7.50 (2H, m). ESI MS: *m/z* 541.37 $[M+Na]^+$. HRESI MS: calcd for $C_{31}H_{54}O_4SiNa$: 541.3689; observed: 541.3674. Physical data of **43E**: R_f=0.388 (hexane–EtOAc=6:1); IR ν_{max} (KBr) 3628 (br), 2925, 2854, 1459, 1250, 1069 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 0.07 (3H, s), 0.11 (3H, s), 0.88 (3H, t, *J* 6.9 Hz), 0.90 (9H, s), 1.26 (18H, br s), 1.38–1.41 (2H, m), 2.06–2.10 (3H, m, containing OH), 3.75–3.80 (2H, m), 3.95 (1H, m), 4.24 (1H, dd, *J* 6.8, 7.1 Hz), 4.69 (1H, dd, *J* 7.1, 7.3 Hz), 5.60 (1H, dd, *J* 8.1, 15.4 Hz), 5.82 (1H, m), 7.36–7.39 (3H, m), 7.47–7.50 (2H, m). ESI MS: *m/z* 541.37 $[M+Na]^+$. HRESI MS: calcd for $C_{31}H_{54}O_4SiNa$: 541.3689; observed: 541.3680.

*4.1.39. (2S,3S,4R)-[(R)-3,4-Benzylidenedioxy]-1-(tert-butyldimethylsilyloxy)octadecan-2-ol (**44**).* A solution of **42EZ** (250 mg, 0.482 mmol) in hexane (50 mL) was stirred for 30 min under hydrogen using 10% Pd/C (200 mg) at room temperature. The reaction mixture was filtered, and the catalyst was washed with a small amount of EtOAc containing 1% Et_3N, and the combined filtrate was concentrated in vacuo to give **44** (240 mg, 96%, stored in a freezer at −80 °C) as an oil (at room temperature). IR ν_{max} (KBr) 3558, 2925, 2854, 1461 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.065 (3H, s), 0.069 (3H, s), 0.88 (3H, t, *J* 7.1 Hz), 0.90 (9H, s), 1.25 (20H, br s), 1.30–1.90 (6H, m), 2.64 (1H, d, *J* 5.1 Hz, OH), 3.69 (1H, dd, *J* 5.7, 9.7 Hz), 3.76 (1H, m), 3.82 (1H, dd, *J* 3.2, 9.7 Hz), 4.00 (1H, dd, *J* 6.5, 9.2 Hz), 4.26 (1H, m), 5.76 (1H, s), 7.34–7.39 (3H, m), 7.40–7.47 (2H, m). ESI MS: *m/z* 543.39 $[M+Na]^+$. HRESI MS: calcd for $C_{31}H_{56}O_4SiNa$: 543.3846; observed: 541.3843.

*4.1.40. (2S,3S,4R)-[(R)-3,4-Benzylidenedioxy]octadecane-1,2-diol (**45**).* To a solution of **44** (370 mg, 0.710 mmol) in dry THF (10 mL) was added TBAF (1 M in THF, 1.40 mL) at room temperature. After stirring for 45 min, the reaction mixture was concentrated in vacuo to give a residue, which was diluted with EtOAc. The solution was washed with H_2O and brine, dried over Na_2SO_4, and filtered, and the filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (6:1, then 3:2) at 8–12 °C or containing 1% Et_3N (v/v) at room temperature gave **45** (280 mg, 97% or 196 mg, 68%, respectively; stored in a freezer at −80 °C; unstable for the long-term storage at room temperature) as a solid. IR ν_{max} (KBr) 3349, 2919, 2851, 1465 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.25 (21H, br s), 1.36 (1H, m), 1.44 (1H, m), 1.63 (1H, m), 1.69 (1H, m), 1.85 (1H, m), 1.87 (1H, m, OH), 2.33 (1H, *J* 5.9 Hz, OH), 3.76 (1H, m), 3.80–3.86 (2H, m), 4.08 (1H, dd, *J* 6.6, 8.1 Hz), 4.27 (1H, ddd, *J* 3.4, 6.6, 10.0 Hz), 5.76 (1H, s), 7.37–7.39 (3H, m), 7.44–7.46 (2H, m). ESI MS: *m/z* 429.30 $[M+Na]^+$. HRESI MS: calcd for $C_{25}H_{42}O_4Na$: 429.2981; observed: 429.2974.

*4.1.41. (2S,3S,4R)-[(R)-3,4-Benzylidenedioxy]-2-hydroxyoctadecyl 2,3-di-O-(4-methoxybenzyl)-4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**46**).* To a solution of imidate 6 [obtained from 2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-D-galactopyranose (620 mg, 1.11 mmol) and CCl_3CN using Cs_2CO_3 as a base] and diol 45 (135 mg, 0.332 mmol) in dry CH_2Cl_2 (13 mL) was added.

MS 4 Å (dry powder, 1.3 g) under argon. After stirring for 30 min at room temperature, the mixture was cooled at 0 °C, and to this solution AgOTf (100 mg, 0.389 mmol) was added. After stirring for 30 min at 0 °C and for 4 h at room temperature, the solution was filtered, and the filter cake was washed with CH_2Cl_2. The combined filtrate was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give

a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (6:1, then 4:1) at 8–12 °C or containing 1% Et_3N (v/v) at room temperature gave **46** (275 mg, 87% or 284 mg, 90%, respectively; stored in a freezer at −80 °C) as a gum. IR ν_{max} (KBr): 3440, 2927, 2855, 1737, 1613, 1513, 1465, 1250 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.1 Hz), 0.98 (9H, s), 1.05 (9H, s), 1.26 (21H, br s), 1.40–1.84 (5H, m), 3.43 (1H, dd, *J* 8.0, 10.9 Hz), 3.61 (1H, s), 3.77–3.82 (8H, m), 3.87 (1H, m), 3.94–4.00 (2H, m), 4.05 (1H, dd, *J* 1.6, 12.5 Hz), 4.16 (1H, dd, *J* 2.0, 12.5 Hz), 4.20 (1H, m), 4.48 (1H, d, *J* 2.9 Hz), 4.60, 4.80 (2H, AB-q, *J* 11.3 Hz), 4.61, 4.67 (2H, AB-q, *J* 11.3 Hz), 4.75 (1H, d, *J* 3.6 Hz, anomeric H), 5.72 (1H, s), 6.84 (2H, d *J* 8.6 Hz), 6.89 (2H, d *J* 8.6 Hz), 7.33–7.43 (4H, m). ESI MS: *m*/*z* 971.6 $[M+Na]^+$. HRESI MS: calcd for $C_{55}H_{84}O_{11}SiNa$, 971.5681; observed, 971.5660.

*4.1.42. (2S,3S,4R)-[(R)-3,4-Benzylidenedioxy]-2-(hexacosanoyloxy) octadecyl 2,3-di-O-(4-methoxybenzyl)-4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**47**).* To a solution of 46 (54 mg, 5.69×10^{-2} mmol) in THF–CH_2Cl_2 (1:1, 8 mL) were added DMAP (160 mg, 1.31 mmol, 23 equiv), *n*-hexacosanoic acid (68 mg, 1.71×10^{-1} mmol, 3 equiv) and EDAC (218 mg, 1.14 mmol, 20 equiv). The mixture was stirred for 4 days at room temperature, and diluted with $CHCl_3$. The solution was washed with H_2O and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1, then 4:1) gave 47 (72 mg, 95%) as a viscous oil. IR ν_{max} (KBr) 2925, 2853, 1741, 1613, 1512, 1466 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 0.97 (9H, s), 1.04 (9H, s), 1.25 (66H, br s), 3.73–3.77 (4H, m, containing 3H, singlet at 3.76 ppm), 3.81 (3H, s), 3.84 (1H, dd, *J* 2.4, 11.5 Hz), 3.91 (1H, dd, *J* 3.5, 10.1 Hz), 4.02 (1H, dd, *J* 1.4, 12.4 Hz), 4.12–4.18 (2H, m), 4.39 (1H, dd, *J* 6.4, 8.1 Hz), 4.44 (1H, d, *J* 2.9 Hz), 4.57, 4.72 (2H, AB-q, *J* 11.5 Hz), 4.64 (2H, s), 4.69 (1H, d, *J* 3.4 Hz, anomeric H), 5.05 (1H, m), 5.74 (1H, s), 6.78 (2H, d, *J* 8.8 Hz), 6.87 (1H, d, *J* 8.8 Hz), 7.26–7.46 (9H, m). ESI MS: *m*/*z* 1349.9 $[M+Na]^+$. HRESI MS: calcd for $C_{81}H_{134}O_{12}SiNa$: 1349.9542; observed: 1349.9542.

*4.1.43. (2S,3S,4R)-[(R)-3,4-Benzylidenedioxy]-2-(hexacosanoyloxy) octadecyl 4,6-O-(di-tert-butyl)silylene-α-D-galactopyranoside (**48**).* To a solution of **47** (110 mg, 0.083 mmol) in CH_2Cl_2–H_2O (10:1, 11 mL) was added DDQ (110 mg, 0.48 mmol). The mixture was stirred for 2.5 h at room temperature, and diluted with CH_2Cl_2. The solution was washed twice with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1, then 3:1) gave **48** (77 mg, 85%) as a gum. IR ν_{max} (KBr) 3535 (br), 2922, 2851, 1733, 1470, 1168 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 6.8 Hz), 1.00 (9H, s), 1.04 (9H, s), 1.25 (66H, s), 1.27–1.42 (2H, m), 1.50–1.63 (4H, m), 2.27–2.32 (2H, m), 3.62–3.68 (3H, m), 3.75 (1H, m, changed to dd, *J* 4.0, 9.8 Hz on addition of D_2O), 3.96 (1H, dd, *J* 2.2, 11.3 Hz), 4.08 (1H, dd, *J* 1.5, 12.5 Hz), 4.17–4.23 (2H, m), 4.27 (1H, dd, *J* 6.1, 8.8 Hz), 4.39 (1H, d, *J* 3.2 Hz), 4.39 (1H, d, *J* 3.2 Hz), 4.84 (1H, d, *J* 3.9 Hz, anomeric H), 5.09 (1H, m), 7.39–7.45 (5H, m). ESI MS: *m*/*z* 1109.8 $[M+Na]^+$. HRESI MS: calcd for $C_{65}H_{118}O_{10}SiNa$: 1109.8392; observed: 1109.8394.

*4.1.44. (2S,3S,4R)-[(R)-3,4-Benzylidenedioxy]-2-(hexacosanoyloxy) octadecyl α-D-galactopyranoside (**49**).* To a solution of **48** (75 mg, 0.069 mmol) in dry THF (7 mL) containing dry pyridine (60 mg) was added HF·pyridine (HF: ~70%; pyridine: ~30%, 58 mg, ca. 2.03 mmol) under argon at room temperature. After stirring for 2.5 h, the reaction mixture was diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (2:3) and EtOAc gave **49** (52 mg, 80%) as a powder. IR ν_{max} (KBr) 3440, 2919, 2850, 1732, 1469 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 7.0 Hz), 1.25 (66H, br s), 1.38–1.62 (6H, m), 2.10 (4H, br, OH), 2.29–2.33 (2H, m), 3.64 (1H, dd, *J* 5.6, 11.5 Hz), 3.71 (1H, dd, *J* 3.3, 11.8 Hz), 3.75–3.79 (3H, m), 3.85 (1H, m), 4.00 (1H, dd, *J* 2.2, 11.3 Hz), 4.05 (1H, d, *J* 2.9 Hz), 4.21 (1H, m), 4.28 (1H, dd, *J* 6.2, 8.2 Hz), 4.85 (1H, d, *J* 3.7 Hz, anomeric H), 5.10 (1H, m), 5.77 (1H, s), 7.38–7.40 (3H, m), 7.43–7.45 (2H, m). ESI MS: *m*/*z* 969.7 $[M+Na]^+$. HRESI MS: calcd for $C_{57}H_{102}O_{10}Na$: 969.7371; observed: 969.7361.

*4.1.45. (2S,3S,4R)-3,4-Dihydroxy-2-(hexacosanoyloxy)octadecyl α-D-galactopyranoside (**50**) (RCAI-80).* To a solution of **49** (24 mg, 0.025 mmol) in THF (10 mL) was added $Pd(OH)_2$/C (20 wt. %, Degussa type, wet, ~50%, 30 mg). After stirring for 4 h under hydrogen at room temperature, the catalyst was filtered, and washed with THF, and then $CHCl_3$–MeOH (5:1). The combined filtrate was concentrated in vacuo to give a white powder, which was washed with EtOAc to give **50** (19 mg, 87%), or the powder was chromatographed on a silica gel (1 g) column. Elution with $CHCl_3$–MeOH (19:1, then 9:1) gave **50** (11 mg, 51%) as a powder. The ^1H NMR data of **50** were identical with those of previously reported data of RCAI-80.[6]

4.2. Methods for measurement of biological activity

4.2.1. Bioassay (mouse in vivo).[13] In vivo experiment. The stock solutions (1.0 mg/mL in DMSO) of αGalCer and synthesized samples were 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. Cytokine measurement. The cytokine concentrations in plasma were quantified by cytometric bead array (CBA) system (BD Biosciences) for mouse IFNγ and IL-4 according to the manufacturer's protocol.

4.2.3. Experimental autoimmune encephalomyelitis (EAE) induction by active immunization in C57BL/6 (B6) mice, and Clinical Score..[7,8] EAE is induced in B6 female mice by immunization with an emulsion of MOG35-55 peptide in complete Freund's adjuvant (CFA). MOG in CFA (Hooke Lab) solution (200 μL) was subcutaneously injected at two sites on lower back (one injection over each hip/base of tail) with 100 μL of emulsion at each site on day 1. 5 ng/mL pertussis toxin (200 μL in PBS) was intraperitoneally injected on day 1 and day 3. Mice were observed daily until day 30 for clinical score as 0–5 graduations with 0.5 for intermediate scores. 0: no clinical signs, 1: flaccid tail, 2: hind limb weakness or abnormal gait, 3: complete hind limb paralysis, 4: complete hind limb paralysis+forelimb weakness or paralysis, 5: moribund or deceased. In order to analyze the effect of glycolipid on EAE, each glycolipd-pulsed GM-CSF induced DC (GM-DC) (5×10^5 cells/mouse) were intravenously injected on day 5 and day 3. GM-DC were induced by bone marrow by culturing with GM-CSF (10 ng/mL) for 5 days, and then enriched by CD11c MACS beads (Miltenyi Biotec). Each glycolipid (100 ng/mL) was pulsed by culturing GM-DC (5×10^6/mL) for 24 h.

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