Carbohydrate Research 370 (2013) 46–66

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

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

Synthesis and biological activity of hydroxylated analogues of KRN7000 (α-galactosylceramide)

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ARTICLE INFO

Article history:
Received 12 November 2012
Received in revised form 15 January 2013
Accepted 16 January 2013
Available online 4 February 2013

Keywords:
CD1d
iNKT cells
Glycosylation
EAE
Th1/Th2
Agelasphins

ABSTRACT

KRN7000 is one of the α-galactosylceramides, which has a 2-hexacosanoylamino-3,4-dihydroxyoctadecyl group. This compound, known as a ligand for the activation of CD1d mediated invariant natural killer T cells (iNKT cells) which release both T helper 1 (Th1) cytokines such as IFNγ and Th2 cytokines such as IL-4, has been anticipated as an antitumor drug, because of its strong secretion of IFNγ. This time, we focused on the hydroxylated analogues of KRN7000 which could be thought of as increasing hydrophilicity and showing bias to Th2 cytokine (IL-4) secretion. Therefore, they may become the drugs for autoimmune diseases for the following reasons: (i) compound OCH, one of the α-galactosylceramide analogues with a shorter sphingosine chain than KRN7000, increases hydrophilicity relative to KRN7000; and (ii) OCH is known to induce much more Th2 cytokines (IL-4) than Th1 cytokines from iNKT cells compared to KRN7000. Naturally, OCH has become one of the candidate drugs for autoimmune diseases. The more hydroxylated derivatives of KRN7000 are anticipated to induce Th2 bias. Therefore, eight analogues with 1–4 excess hydroxyl groups on the lipid chain of KRN7000 were synthesized to examine if they behave in the same way as OCH. As a result, three out of eight compounds biased largely to IL-4 secretion, and their effectiveness for experimental autoimmune encephalomyelitis (EAE) was examined. It was recognized that two compounds [†]RCAI-147/-160 showed good suppression of EAE symptoms.

1. Introduction

Agelasphins are a series of α-galactosylceramides isolated from a marine sponge (*Agelas mauritianus*), and they showed potent effects on the immune system of mice.[1] The compound known as KRN7000[2] (one of the α-galactosylceramides) was developed by Kirin Brewery Co. through synthetic studies on agelasphins. Invariant natural killer T cells (iNKT cells)[3] produce immunoregulatory cytokines such as IFNγ (Th1 type) and IL-4 or IL-10 (Th2 type) by the recognition of glycolipid antigens such as KRN7000 bound by a major histocompatibility complex (MHC) class I-like protein, CD1d.[4] These cytokines secreted by iNKT cells hold the promise of the therapeutic use for anticancer drugs or immunosuppressive agents and autoimmune disease treatment.

This time, we were interested in the compound OCH reported by Miyamoto et al.,[5] a truncated analogue of KRN7000 as shown in Figure 1, because it appears to induce a Th2 bias. In addition, also the ester analogue of KRN7000, namely RCAI-80,[6] biased largely the cytokine secretion from iNKT cells to Th2. However, in comparison to OCH, the induced Th2 bias of RCAI-80 was fairly lower than that of OCH.[5]

The compound OCH has been a potential candidate agent for the treatment of autoimmune diseases, immunosuppressant in association with organ transplantation, therapy of inflammatory conditions, etc. It will be considered that the lipophilicity of a truncated analogue of KRN7000 is decreasing. If this is true, reversely, this may mean that the hydrophilicity of OCH is increasing. It is known that KRN7000 forms the hydrogen bonding between the amide N–H hydrogen on the phytosphingosine and the oxygen on the hydroxyl group of the adjacent threonine-156 residue of mouse CD1d (mCD1d) (or threonine-154 residue of human CD1d). And also we can recognize from the X-ray crystallographic analysis[7] that crucial hydrogen bonding interactions were formed between mCD1d residue Arg79 and the 3′-OH of KRN7000 phytosphingosine, and residue Asp80 showed hydrogen bonds with both 3′- and 4′-OH of the phytosphingosine. X-ray crystallographic analysis reveals the presence of mCD1d residue Asp80 formed the same hydrogen bond with both 3′- and 4′-OH of compound OCH in the

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† RCAI- is a code number.

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http://dx.doi.org/10.1016/j.carres.2013.01.010

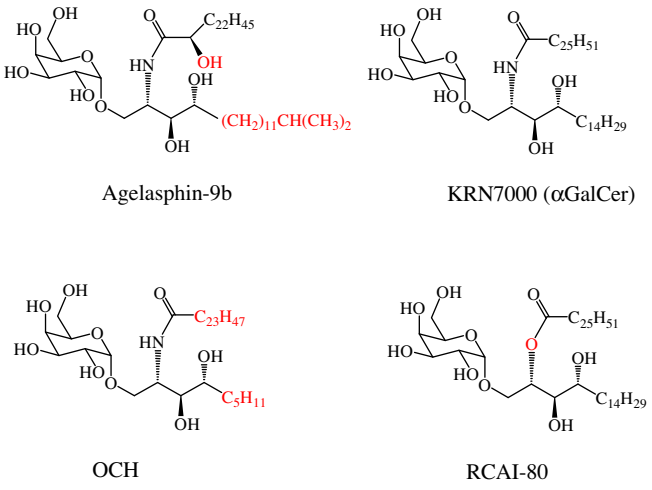


Figure 1. Structures of agelasphin-9b, KRN7000 (αGalCer), OCH, and RCAI-80.

ternary TCR/OCH/CD1d complex.[8] Introduction of an additional or multiple hydroxyl groups on the phytosphingosine main chain and/or amide side chain of KRN7000, may influence to simply increase the hydrophilicity of the compound without hydrogen bonding. Do these hydroxylated KRN7000 analogues bias toward Th2 cytokine secretion compared to KRN7000?

Therefore, eight hydroxylated KRN7000 analogues were synthesized to verify this hypothesis.

2. Results and discussion

2.1. Synthesis

Firstly, we synthesized compound **14**, which has another (5*S*)-hydroxyl group on the phytosphingosine main-chain carbon adjacent to the C_4-hydroxyl group of KRN7000. It should be possible to synthesize **14** via compound **10** obtained by coupling reaction of alcohol **7** and imidoyl derivative of **9** as shown in Schemes 1 and 2.

Compound **1**, which was converted into the primary alcohol **7** as a glycosyl accepter, was treated with acetic anhydride containing catalytic amount of conc. sulfuric acid to give 1,6-diacetate of **2**.[9] The diacetate was further converted into **2** in 95% yield by transesterification reaction in MeOH using KOH as a catalyst. Compound **2** was treated with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) using imidazole as a base to give TBDMS ether **3**. Wittig reaction of **3** with the phosphorane derived from *n*-dodecyltriphenylphosphonium bromide [$Ph_3P(Br)C_{12}H_{25}$] and *n*-BuLi gave **4** as a mixture of *E*- and *Z*-isomers. Reduction of the double bond in **4** under hydrogen using Pd on carbon (Pd/C) in hexane yielded alcohol **5**. Treatment of **5** with diethyl azodicarboxylate (DEAD) and triphenylphosphine (Ph_3P) in tetrahydrofuran (THF), and then with diphenylphosphoryl azide [$(PhO)_2P(O)N_3$] gave C_2-azide **6** with inversion of the configuration from *R* to *S* at C_2-OH position. Deprotection of TBDMS group from **6** with *n*-tetrabutylammonium fluoride (*n*-Bu_4NF) in THF afforded primary alcohol **7** as a glycosyl acceptor.

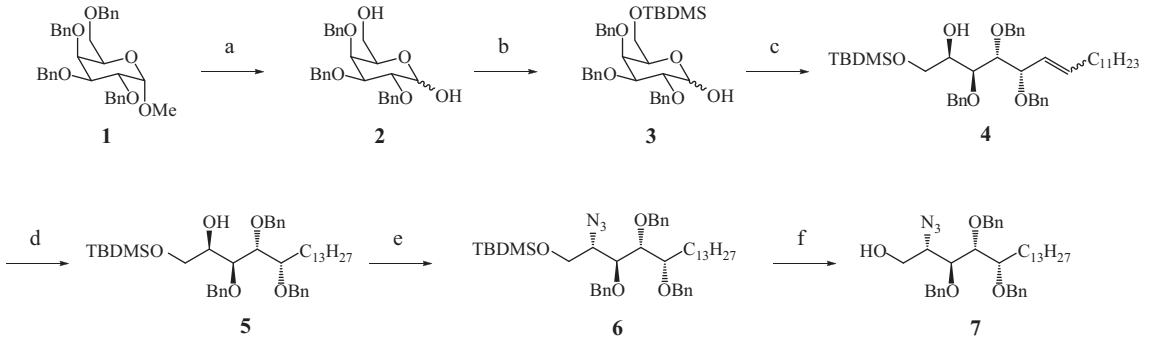
On the other hand, the reported compound **8**,[6] which was converted into imidate of compound **9** as a glycosyl donor, was benzylated with benzyl bromide (BnBr) and NaH as a base in *N*,*N*-dimethylformamide (DMF) to afford 2,3-di-*O*-benzylated compound of **8**, and then the anomeric *S*-tolyl group was deprotected with *N*-bromosuccinimide (NBS) in acetone to give **9**, which was converted into the imidate of **9** with trichloroacetonitrile (CCl_3CN) using cesium carbonate (Cs_2CO_3) as a base.

The glycosylation reaction of glycosyl acceptor **7** with this imidate of **9** was performed by use of silver trifluoromethanesulfonate (AgOTf) as a catalyst to give α-anomer **10** quantitatively. The azide of **10** was reduced to amine **11** by treatment with trimethylphosphine (Me_3P) in THF, then with aqueous 1 M NaOH. A condensation reaction of amine **11** with hexacosanoic acid (*n*-$C_{25}H_{51}COOH$) using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC) as a dehydrating agent in THF–CH_2Cl_2 (1:1) gave the amide **12**. Treatment of **12** with HF·pyridine in THF–pyridine gave **13** (92%). Five benzyl groups of **13** were hydrogenolyzed using $Pd(OH)_2$ on carbon [$Pd(OH)_2$/C] in THF to give **14** (RCAI-104) in 47% yield.

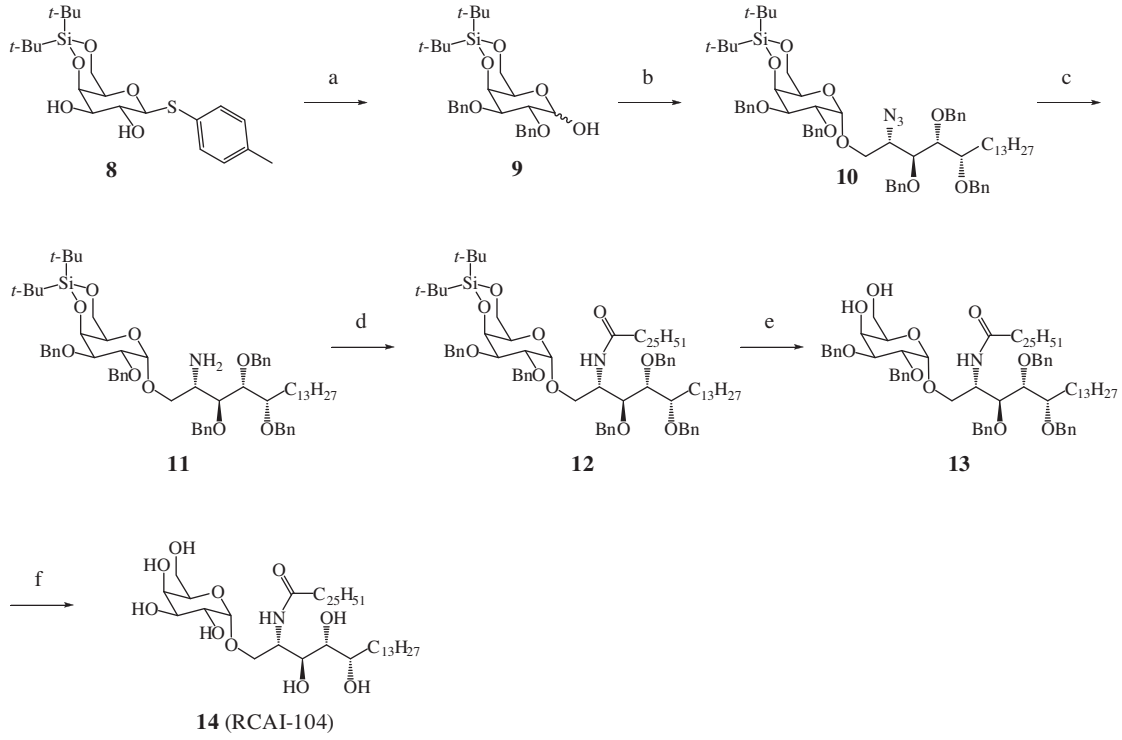
Secondly, we synthesized compound **30** (RCAI-120), which has another (6*S*)-hydroxyl group on the main-chain carbon adjacent to the C_5-hydroxyl group of compound **14** as shown in Schemes 2–4.

The known starting material **15**,[10] converted into the glycosyl acceptor **23** in eight steps, was changed to diol **16** (63% yield, unstable at room temperature) by osmium tetroxide (OsO_4) oxidation in acetone–H_2O (4:1) containing each 1.2 equiv of 4-methylmorpholine *N*-oxide and *p*-toluenesulfonic acid (TsOH). Treatment of **16** with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) using 2,6-lutidine as a base gave **17** (96%, stable at room temperature).

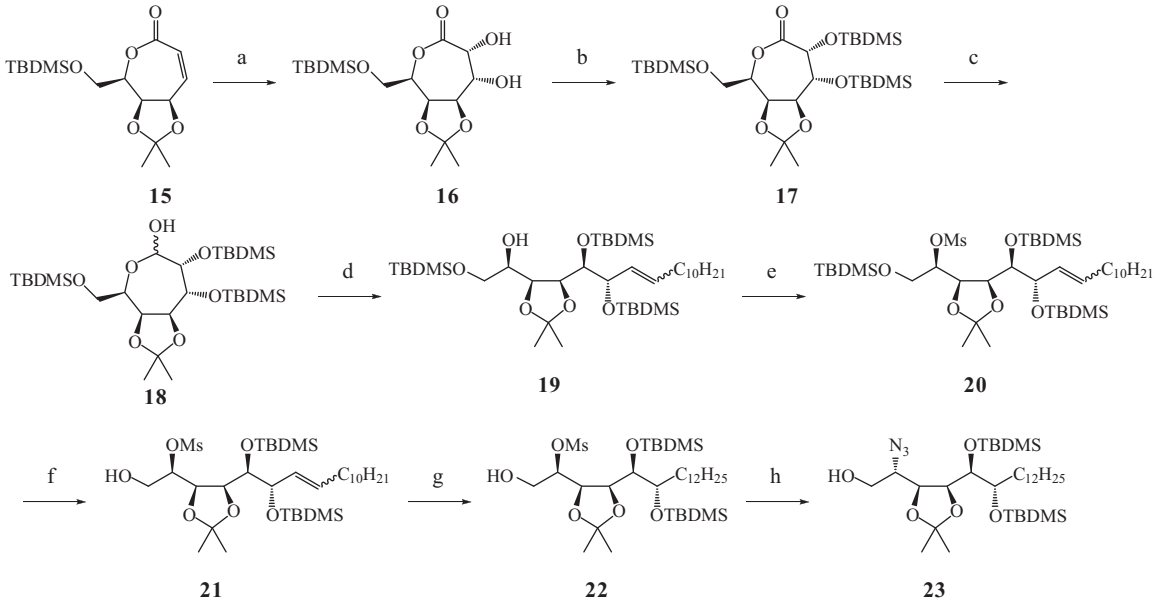
Stereochemistry of the *syn*-diol portion of **16** was determined by detailed NMR analysis of tri-TBDMS lactone derivative **17**. Complete ^1H and ^{13}C NMR assignments were carried out by analysis of 2D DQF-COSY, ^1H–^{13}C HSQC, and HMBC, and also ^1H–^{29}Si HMBC data of **17**. Relative stereochemistry of the oxygenated oxepan-2-one ring was confirmed by NOE correlations between an isopropylidene methyl at 1.48 ppm and silyloxyl methines (H-3 and H-4),



Scheme 1. Reagents and conditions: (a) (1) Ac_2O, concd H_2SO_4 (catalytic amount), rt, 1 h, (2) KOH (catalytic amount), MeOH, rt, 1 h, two steps 95%; (b) TBDMSCl, imidazole, CH_2Cl_2, rt, 1.5 h, 78%; (c) $Ph_3P(Br)C_{12}H_{25}$, *n*-BuLi (1.6 M in hexane), THF, −10 °C, then **3**, −10 °C → rt, 16 h, 45%; (d) H_2, Pd/C, hexane, rt, 50 min, 71%; (e) DEAD, Ph_3P, $(PhO)_2P(O)N_3$, THF, Ar, −10 → 0 °C, 30 min, then rt, 3 h, 78%; (f) *n*-Bu_4NF, THF, rt, 30 min, 93%.



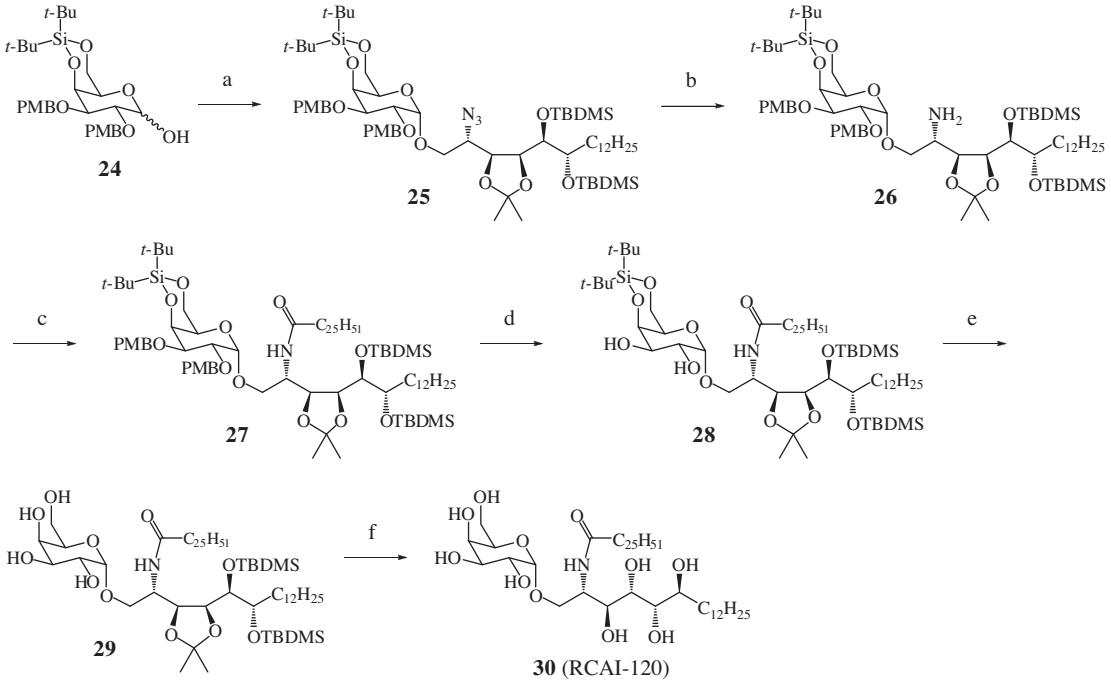
Scheme 2. Reagents and conditions: (a) (1) BnBr, NaH, DMF, Ar, 0 °C, 15 min, then rt, 15 min, quant, (2) NBS, acetone, −20 °C, 40 min, then quenched with satd aq $NaHCO_3$, 65%; (b) (1) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 16 h, (2) **7**, MS 4 Å, CH_2Cl_2, rt, 30 min, then 0 °C, AgOTf, 1 h, and rt, 16 h, quant; (c) Me_3P, THF, rt, 2 h, then aq 1 M NaOH, rt, 2 h, 77%; (d) *n*-$C_{25}H_{51}COOH$, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 1.5 h, 70%; (e) HF·pyridine, pyridine, THF, Ar, rt, 1 h, 92%; (f) H_2, $Pd(OH)_2$/C, THF, rt, 24 h, 47%.



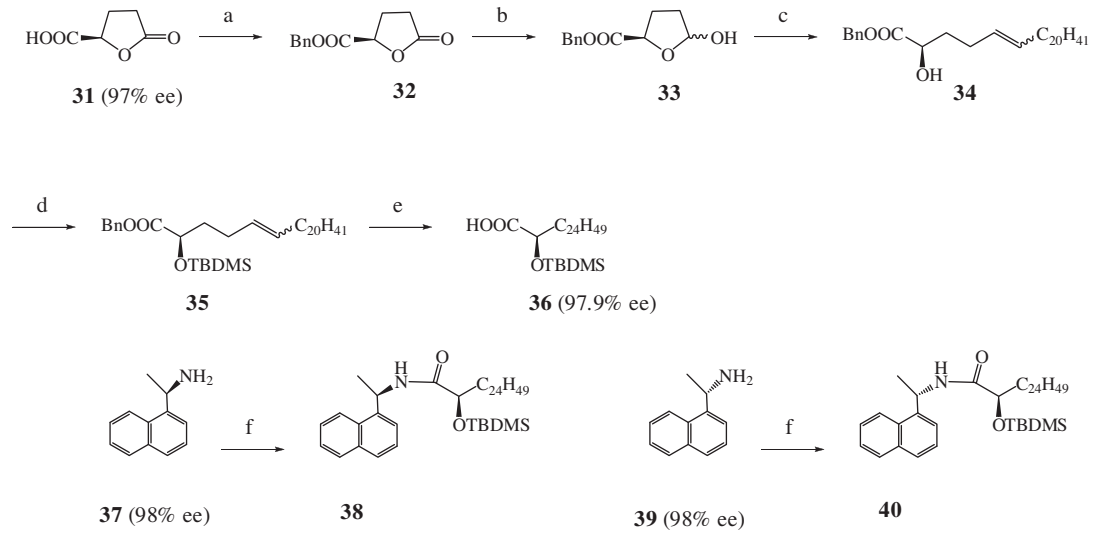
Scheme 3. Reagents and conditions: (a) OsO_4, 4-methylmorpholine *N*-oxide, *p*-TsOH, 0 °C, 15 min, then rt, 16 h, **16** (63%) and recovery **15** (11%); (b) *t*-$BuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2, rt, 16 h, 96%; (c) DIBAH, toluene, −78 °C, 45 min, 96%; (d) 4 equiv $Ph_3P(Br)C_{11}H_{23}$, THF, −30 °C, then $LiN(SiMe_3)_2$, −30 °C → −10 °C over 20 min, −10 °C, 15 min, then rt, 3 h, 54%; (e) $(MeSO_2)_2O$, pyridine, CH_2Cl_2, rt, 45 min, 77%; (f) HF·pyridine, pyridine, THF, 0 °C, 5 min, and rt, 5 h, 62%; (g) H_2, Pd/C, EtOAc, rt, 1 h, 100%; (h) NaN_3, DMF, 100 °C, 14 h, 76%.

and also between another methyl at 1.36 ppm and methines attached with the isopropylidene group (H-5 and H-6).

The lactone carbonyl of **17** was reduced to hemiacetal **18** with diisobutylaluminum hydride (DIBAH) in toluene at −78 °C for 45 min in 96% yield. Wittig reaction of **18** with the phosphorane derived from *n*-undecyltriphenylphosphonium bromide [$Ph_3P(Br)C_{11}H_{23}$] and lithium bis(trimethylsilyl)amide gave **19** as a mixture of *E*- and *Z*-isomers. Mesylation of **19** with methanesulfonic anhydride in dichloromethane (CH_2Cl_2) using pyridine as a base gave **20**. Selective desilylation of primary silyl ether of **20** by



Scheme 4. Reagents and conditions: (a) (1) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 16 h, (2) **23**, MS 4 Å, CH_2Cl_2, rt, 30 min, then 0 °C, AgOTf, 1 h, and rt, 1.5 h, 78%; (b) Me_3P, THF, rt, 2 h, then aq 1 M NaOH, rt, 2 h, 77%; (c) *n*-$C_{25}H_{51}COOH$, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 3 h, 86%; (d) DDQ, CH_2Cl_2–H_2O (10:1), rt, 2 h, 85%; (e) HF·pyridine, pyridine, THF, rt, 1 h, 85%; (f) aq 46% HF, CH_2Cl_2–MeCN (1:1), H_2O, rt, 16 h, 92%.



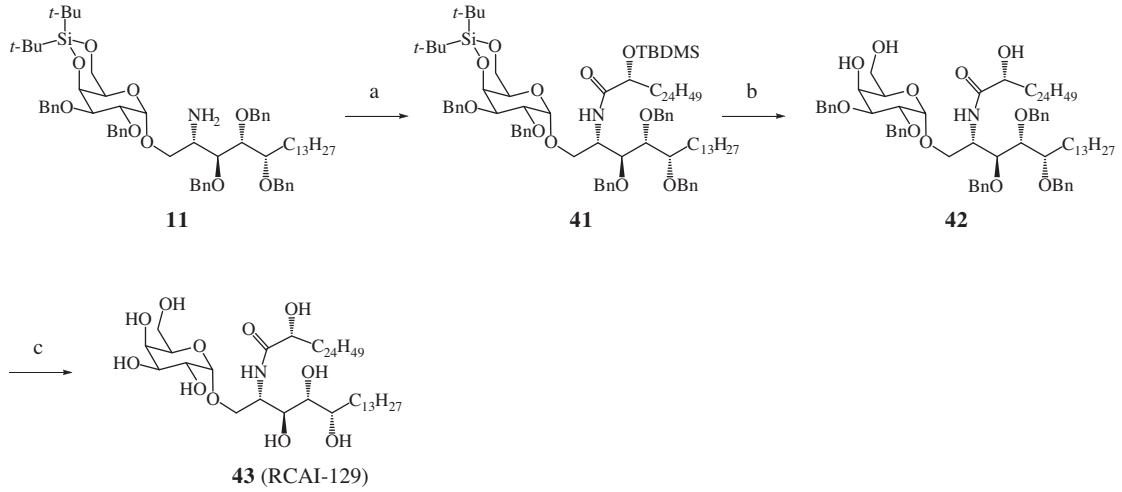
Scheme 5. Reagents and conditions: (a) BnOH, EDAC, CH_2Cl_2, rt, 1.5 h, 70%; (b) BH_3, 2-methyl-2-butene, THF, 0 °C, 75 min, then **32**, and rt, 15 h, 51%; (c) $Ph_3P(Br)C_{21}H_{43}$, $LiN(SiMe_3)_2$, THF, 0 °C, 1 h, and rt, 2 h, 61%; (d) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 1.5 h, 79%; (e) H_2, $Pd(OH)_2$/C, THF, quant; (f) **36**, EDAC, CH_2Cl_2, rt, 2 h, 70%.

HF·pyridine gave **21**. The double bond of **21** was reduced as described for the formation of **5** from **4** to give **22** (quantitatively). Treatment of **22** with sodium azide in DMF at 100 °C for 14 h gave azide **23** accompanied by inversion of the configuration.

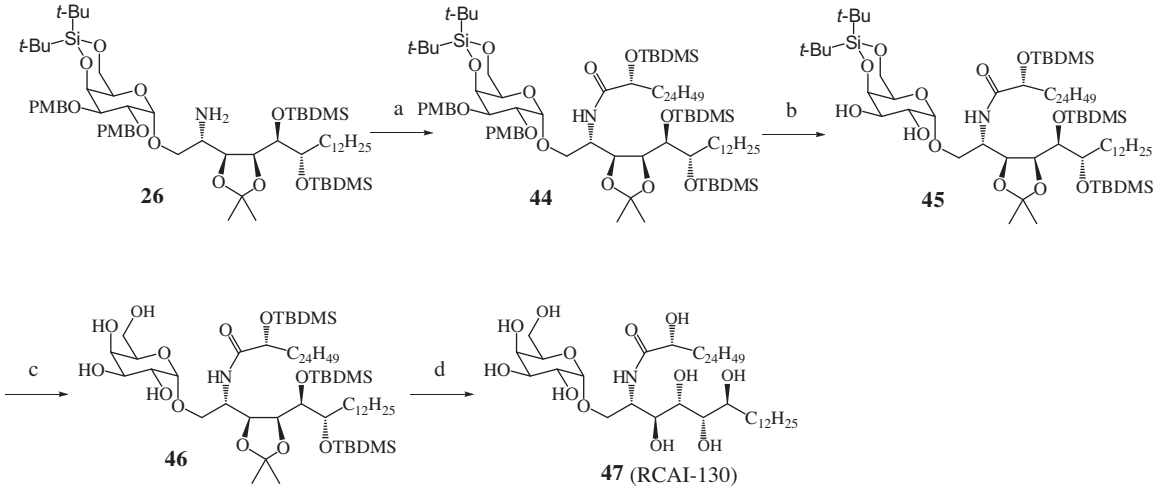
The α-imidoyl compound of **24**,[6] prepared by the same procedure from **9**, was treated with alcohol **23** according to the procedure from **9** to **10** to give **25**. Compound **25** was converted into **27** via amine **26** according to the same procedure from **10** to **12**. Treatment of **27** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane–water (CH_2Cl_2–H_2O) (10:1) gave **28**. The silylene group of **28** was deprotected to give **29** with HF·pyridine according to the procedure from **12** to **13**. In the last step, two TBDMS and acetonide were removed with aqueous HF in CH_2Cl_2–MeCN (1:1) to give **30** (RCAI-120).

Thirdly, we synthesized compound **43** (RCAI-129), which has another (2*R*)-hydroxyl group on the amide side-chain of compound **14** as shown in Schemes 5 and 6.

(*R*)-2-(*tert*-Butyldimethylsilyloxy)hexacosanoic acid (**36**) was synthesized from the commercially available (*R*)-5-oxo-2-tetrahydrofurancarboxylic acid (**31**, 97% ee) in five steps (12%). The carboxylic acid of **31** was esterified with benzyl alcohol using EDAC as a dehydrating agent to give benzyl ester **32**. The lactone carbonyl of **32** was reduced to a hemiacetal **33** using borane tetrahydrofuran complex (1.0 M in THF)/2-methyl-2-butene (2 M in THF).[11] Wittig



Scheme 6. Reagents and conditions: (a) **36**, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 3 h, 54%; (b) (1) aq 46% HF, CH_2Cl_2–MeCN (1:1), rt, 3 h, (2) HF·pyridine, pyridine, THF, rt, 1.5 h, two steps 68%; (c) H_2, $Pd(OH)_2$/C, THF, 48 h, 52%.



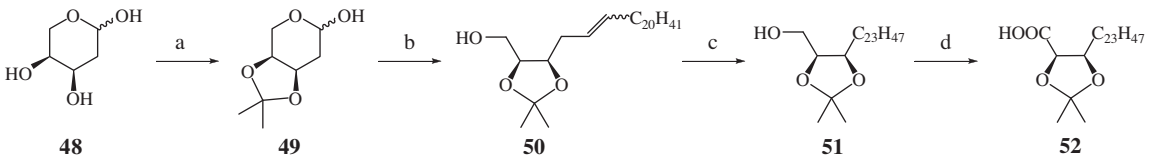
Scheme 7. Reagents and conditions: (a) **36**, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 16 h, quant; (b) DDQ, CH_2Cl_2–H_2O (10:1), rt, 3 h, 60%; (c) HF·pyridine, pyridine, THF, rt, 1 h, 68%; (d) aq 46% HF, CH_2Cl_2–MeCN (3:2), H_2O, rt, 16 h, 65%.

reaction of **33** with the phosphorane derived from *n*-henicosyltriphenylphosphonium bromide [n-$C_{21}H_{43}P(Br)Ph_3$] and $LiN(SiMe_3)_2$ gave **34** as a mixture of *E*- and *Z*-isomers. Silylation of this mixture with TBDMSOTf and 2,6-lutidine afforded **35**, which was reduced to **36** quantitatively using $Pd(OH)_2$/C as a catalyst.

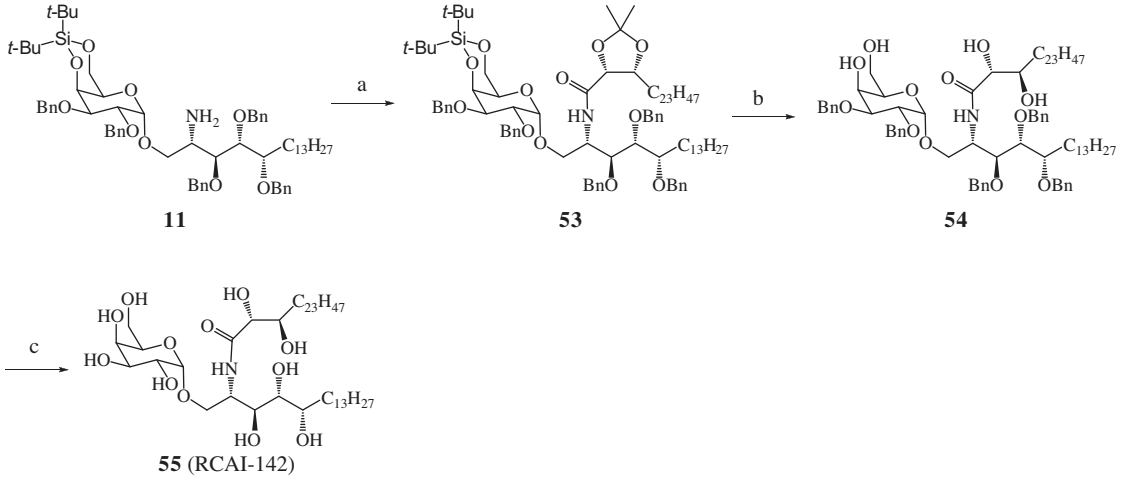
This carboxylic acid **36** was converted into amides **38** and **40** to determine the enantiomeric excess of **36**. Reaction of **36** with both (*R*)- and (*S*)-1-(1-naphthyl)ethylamines (98% ee) using EDAC as a condensing agent gave amides **38** [(*R*,*R*)-isomer] and **40** [(*S*,*R*)-isomer], respectively. The diastereomeric excess of crude sample of **40** was 97.9% as determined by HPLC analysis, and the enantiomeric excess of starting **31** was 97%. Therefore, no epimerization occurred during the conversion from **31** to **36**.

Condensation of amine **11** and carboxylic acid **36** using EDAC as a dehydrating agent gave amide **41**. The TBDMS and silylene groups of **41** were deprotected successively by aqueous HF and then HF·pyridine to give **42**. Hydrogenolysis of five benzyl groups of **42** using $Pd(OH)_2$/C as a catalyst gave **43** (RCAI-129) in 53% yield.

Fourthly, amine **26** and carboxylic acid **36** were converted into **47** (RCAI-130) in four steps with an overall yield of 21% via compounds **44**, **45**, and **46** according to almost the same procedure from **26** to **30** via **27**, **28**, and **29** as shown in Scheme 7.



Scheme 8. Reagents and conditions: (a) 2,2-dimethoxypropane (1.5 equiv), Amberlyst 15, DMF, rt, 18 h, 50%; (b) $Ph_3P(Br)C_{21}H_{43}$, $LiN(SiMe_3)_2$, THF, 0 °C, 1 h, and rt, 2 h, 50%; (c) H_2, Pd/C, hexane, rt, 2 h, 98%; (d) $RuCl_3 \cdot nH_2O$, $NaIO_4$, CCl_4–MeCN–H_2O (2:2:3), rt, 3 h, 80%.



Scheme 9. Reagents and conditions: (a) **52**, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 16 h, 68%; (b) (1) aq 46% HF, CH_2Cl_2–MeCN (1:1), rt, 3 h, (2) HF·pyridine, pyridine, THF, rt, 1.5 h, two steps 69%; (c) H_2, $Pd(OH)_2$/C, THF, 16 h, 52%.

Fifthly, the compound **55** (RCAI-142), which has two consecutive (2*R*,3*R*)-hydroxy groups adjacent to the carbonyl group on the amide side-chain of **43**, was synthesized as shown in Scheme 8.

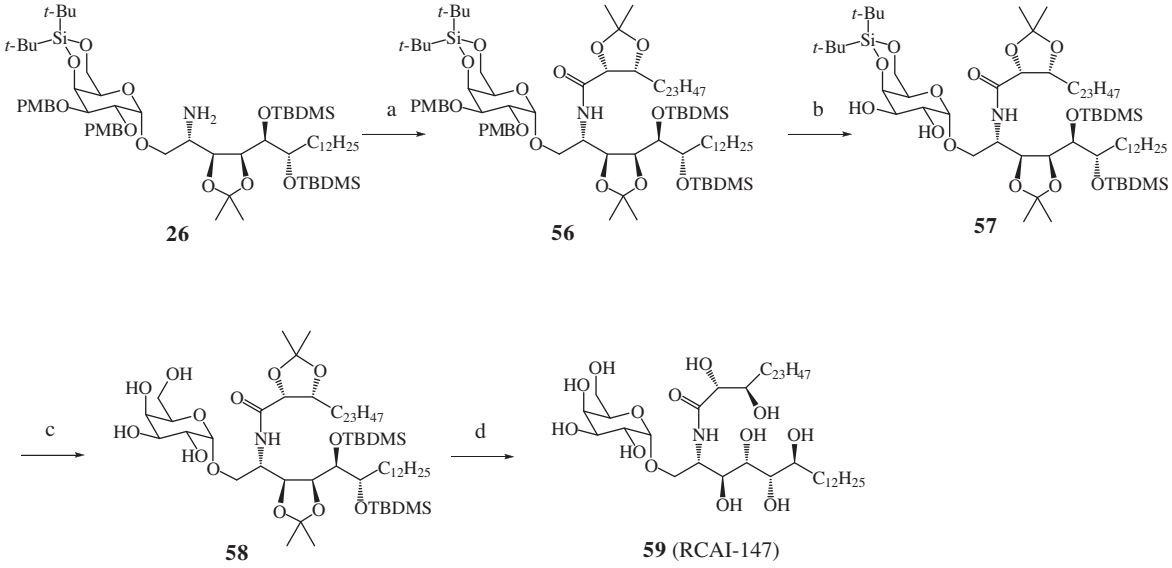
(2*R*,3*R*)-2,3-*O*-Isopropylidenehexacosanoic acid (**52**) was prepared from 2-deoxy-L-ribose (**48**). Compound **48** was treated with 2,2-dimethoxypropane in DMF using strongly acidic ion exchange resin (Amberlyst 15) as a catalyst to afford **49**. Wittig reaction of **49** with the phosphorane derived from *n*-$C_{21}H_{43}P(Br)Ph_3$ and $LiN(SiMe_3)_2$ gave **50** as a mixture of *E*- and *Z*-isomers, which was hydrogenated to **51** using $Pd(OH)_2$/C as a catalyst. The primary alcohol of **51** was oxidized to carboxylic acid **52** using ruthenium(III) chloride hydrate ($RuCl_3 \cdot nH_2O$) and sodium periodate ($NaIO_4$) in carbon tetrachloride–acetonitrile–water (CCl_4–MeCN–H_2O) (2:2:3).[12]

Amine **11** and carboxylic acid **52** were converted into **55** (RCAI-142) in three steps with 24% yield via **53** and **54** according to almost the same procedure from **11** to **43** via **41** and **42** as shown in Scheme 9.

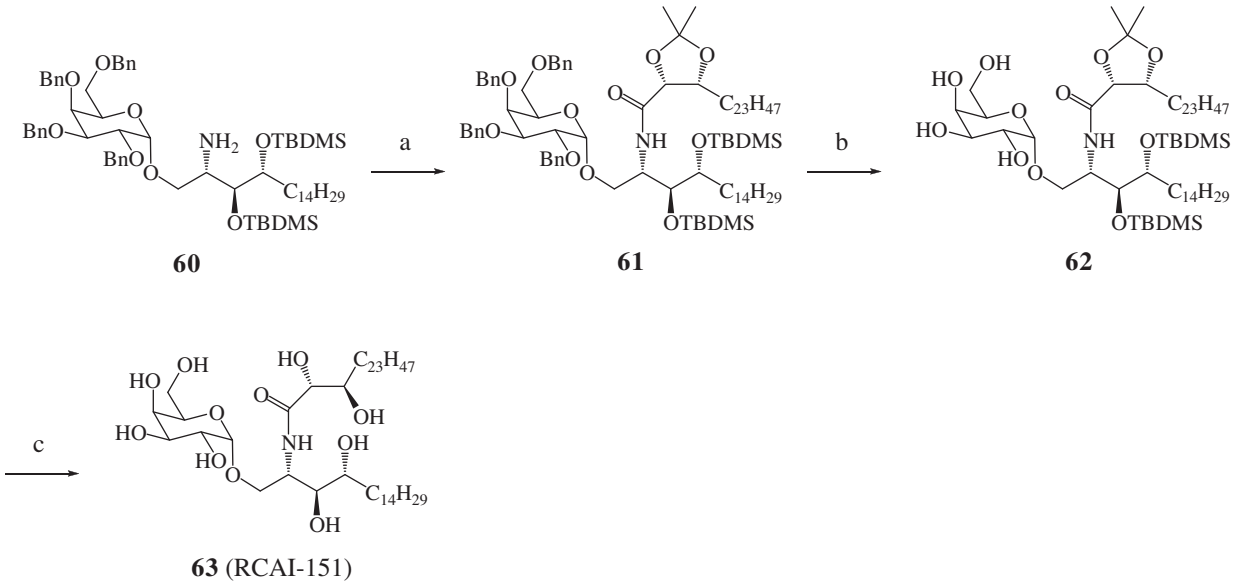
Sixthly, the compound **59** (RCAI-147), which has two consecutive (2*R*,3*R*)-hydroxyl groups on the amide side-chain and four successive (3*S*,4*S*,5*S*,6*S*)-hydroxyl groups on the main-chain carbon adjacent to C_2-amino group, was synthesized in four steps with an overall yield of 32% via **56**, **57**, and **58** from amine **26** and carboxylic acid **52** according to almost the same procedure from **26** to **47** via **44**, **45**, and **46** as shown in Scheme 10.

Seventh, compound **63** (RCAI-151), which has two (2*R*,3*R*)-hydroxyl groups on the amide side-chain of KRN7000, was synthesized. Reaction of amine **60**[13] and carboxylic acid **52** with EDAC as a dehydrating agent gave amide **61**. Hydrogenolysis of four benzyl groups of **61** in THF using $Pd(OH)_2$/C as a catalyst gave **62**, and the isopropylidene and two TBDMS groups of **62** were removed by treatment of aqueous HF to give **63** (RCAI-151) in 63% yield as shown in Scheme 11.

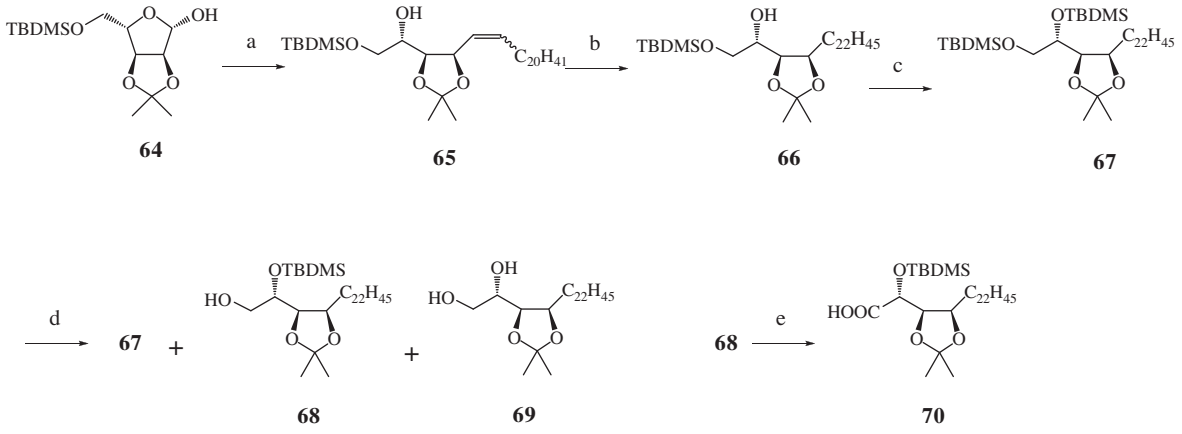
Finally, compound **73** (RCAI-160), which has three (2*R*,3*R*,4*R*)-hydroxyl groups on the amide side-chain of KRN7000, was synthesized as shown in Schemes 12 and 13.



Scheme 10. Reagents and conditions: (a) **52**, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 16 h, 90%; (b) DDQ, CH_2Cl_2–H_2O (10:1), rt, 2 h, 94%; (c) HF·pyridine, pyridine, THF, rt, 1 h, 78%; (d) aq 46% HF, CH_2Cl_2–MeCN (1:1), H_2O, rt, 16 h, 48%.



Scheme 11. Reagents and conditions: (a) **52**, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 16 h, 78%; (b) H_2, $Pd(OH)_2$/C, THF, rt, 16 h, 77%; (c) aq 46% HF, CH_2Cl_2–MeCN (1:1), H_2O, rt, 16 h, 63%.



Scheme 12. Reagents and conditions: (a) 3 equiv $Ph_3P(Br)C_{21}H_{43}$, 3 equiv *n*-BuLi, THF, −10 °C, 30 min, then **64**, 4 h, rt, 63%; (b) H_2, $Pd(OH)_2$/C, EtOAc, rt, 1 h, quant; (c) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, rt, 1 h, 90%; (d) HF·pyridine, pyridine, THF, rt, 16 h, **67** (recovery 32%), **68** (47%), and **69** (15%); (e) $RuCl_3 \cdot nH_2O$, $NaIO_4$, CCl_4–MeCN–H_2O (2:2:3), rt, 3 h, 77%.

Preparation of carboxylic acid **70** was performed from 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-L-ribofuranose **64**[6] as shown in Scheme 12. Wittig reaction of **64** with the phosphorane derived from $Ph_3P(Br)C_{21}H_{43}$ and *n*-BuLi gave **65** as a mixture of *E*- and *Z*-isomers. The double bond reduction of **65** under hydrogen using $Pd(OH)_2$/C as a catalyst in ethyl acetate (EtOAc) gave alcohol **66**. Treatment of **66** with TBDMSOTf using 2,6-lutidine as a base gave **67**. Selective cleavage of C_1-primary TBDMS ether of **67** was performed using HF·pyridine to give **68** (47%) accompanying recovered **67** (32%) and 1,2-diol **69** (15%). Oxidation of the primary alcohol of **68** using $RuCl_3 \cdot nH_2O$ and $NaIO_4$ in CCl_4–MeCN–H_2O (2:2:3)[12] afforded carboxylic acid **70**.

Reaction of amine **60**[13] and carboxylic acid **70** with EDAC as a dehydrating agent gave amide **71** (38%) as shown in Scheme 13. Hydrogenolysis of four benzyl groups of **71** in THF using $Pd(OH)_2$/C as a catalyst gave **72**, and the isopropylidene as well as three TBDMS groups of **72** were removed by treatment of aqueous HF to give **73** (RCAI-160) in 48% yield.

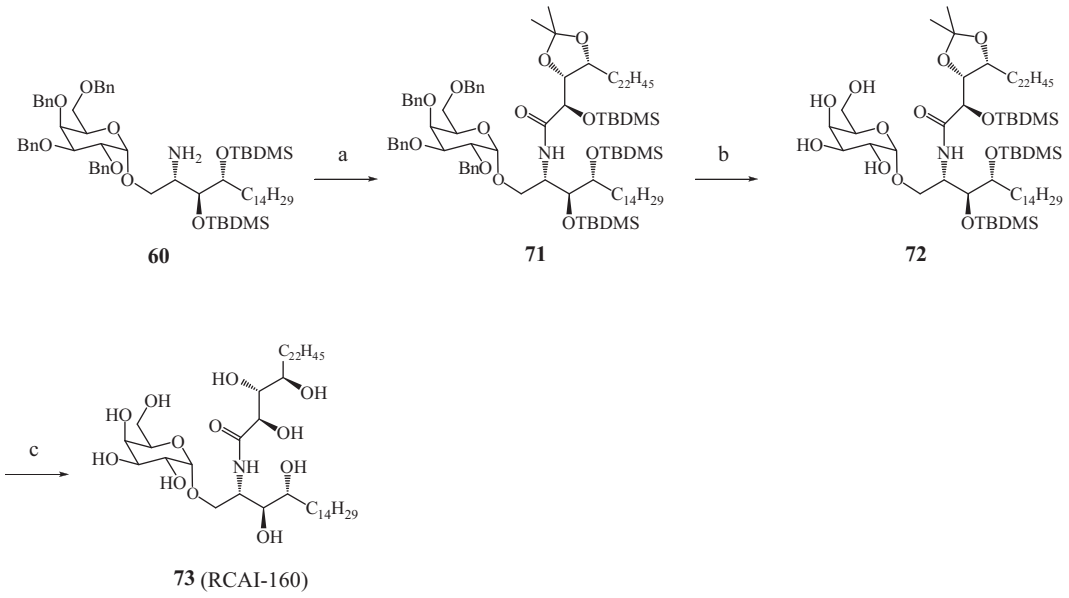
2.2. Biological activity

The cytokine-producing [IFNγ (Th1 response) and IL-4 (Th2 response)] activity from iNKT cells of mice in vivo of the eight synthetic compounds was investigated using KRN7000 as a positive control as shown both in Figure 2 [IFNγ (panel a) and IL-4 (panel b), compounds **14**, **30**, **43**, and **47**] and in Figure 3 [IFNγ (panel a) and IL-4 (panel b), compounds **55**, **59**, **63**, and **73**], and the structures of newly synthesized eight compounds are listed in Table 1.

Three compounds **59**, **63**, and **73** out of these eight compounds strongly induced Th2 cytokine (IL-4) rather than Th1 cytokine (IFNγ) from iNKT cells compared to KRN7000. Therefore, these three compounds **59**, **63**, and **73** were tested for the suppression activity of symptoms associated with experimental autoimmune encephalomyelitis (EAE)[14] as shown in Figure 4.

The results of biological activity are described below.

Both compounds **14** and **43** are holding another (5*S*)-hydroxyl group in the immediate vicinity of C4-hydroxyl group on the



Scheme 13. Reagents and conditions: (a) **70**, EDAC, DMAP, THF–CH_2Cl_2 (1:1), rt, 16 h, 38%; (b) H_2, $Pd(OH)_2$/C, THF, 16 h, 54%; (c) aq 46% HF, CH_2Cl_2–MeCN (3:2), H_2O, rt, 16 h, 48%.

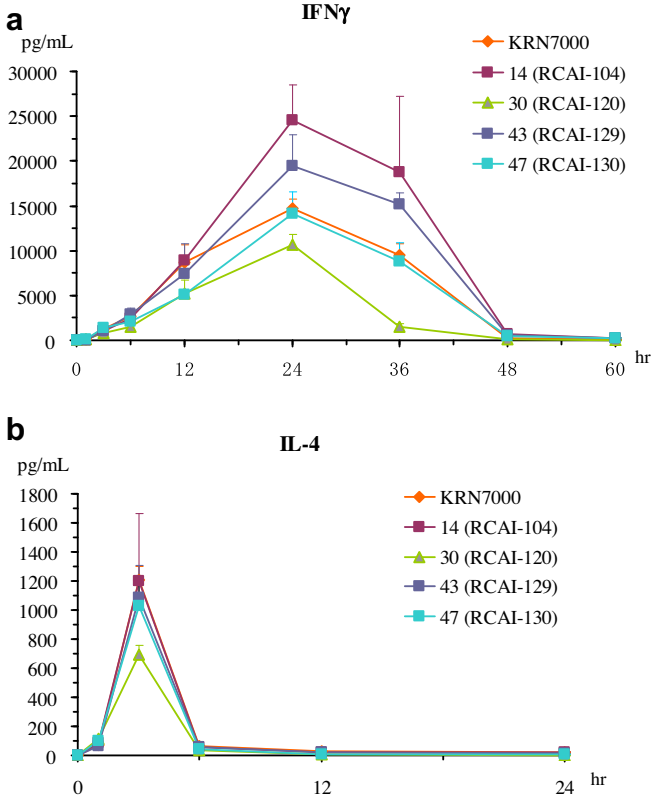


Figure 2. (Panels a and b) The concentrations of IFNγ (panel a) and IL-4 (panel b)[15] in sera of mice (2 μg/mouse) upon administration of the α-galactosylceramide variants, namely four compounds **14**, **30**, **43**, and **47** (the structures shown in Table 1).

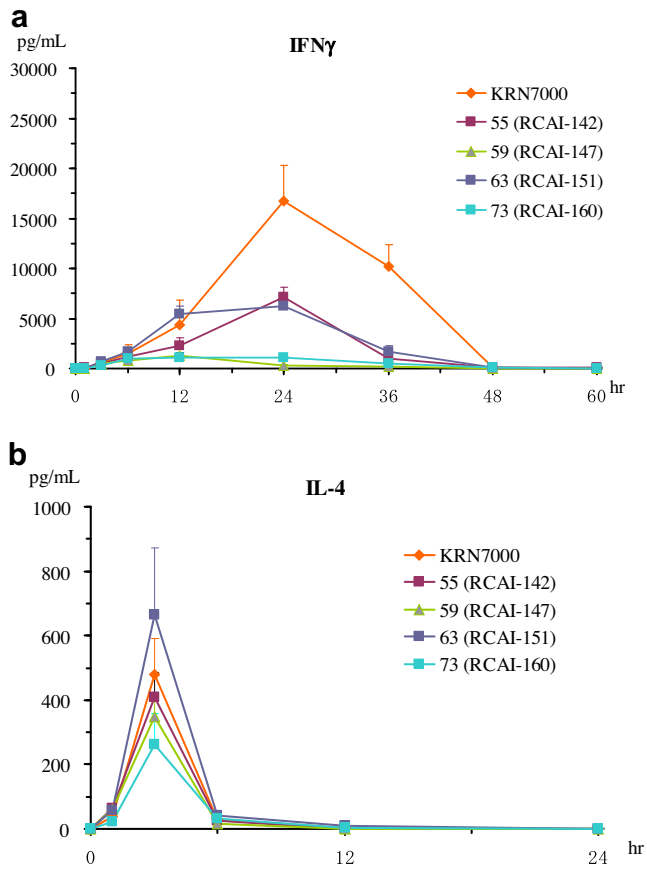
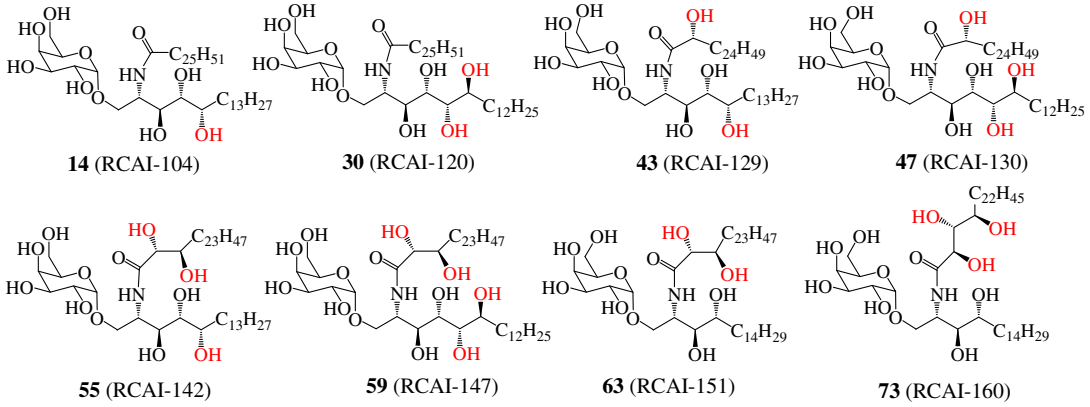


Figure 3. (Panels a and b) The concentrations of IFNγ (panel a) and IL-4 (panel b)[15] in sera of mice (2 μg/mouse) upon administration of the α-galactosylceramide variants, namely four compounds **55**, **59**, **63**, and **73** (the structures shown in Table 1).

main-chain of phytosphingosine. Also the compound **14** is retaining a hexacosanamide such as KRN7000 and **43** is having a (2*R*)-2-hydroxyhexacosanamide in the amide side-chain. The amount of IFNγ secretion mediated by compounds **14** and **43** was approximately 1.7 and 1.4 times of KRN7000, respectively. On the other hand, the IL-4 secretion of both compounds **14** and **43** was almost the same level with KRN7000. Therefore, it can be said that the cytokine secretion ability of these two compounds

Table 1
Structures of newly synthesized eight compounds



The extra hydroxy groups added to KRN7000 are painted in red.

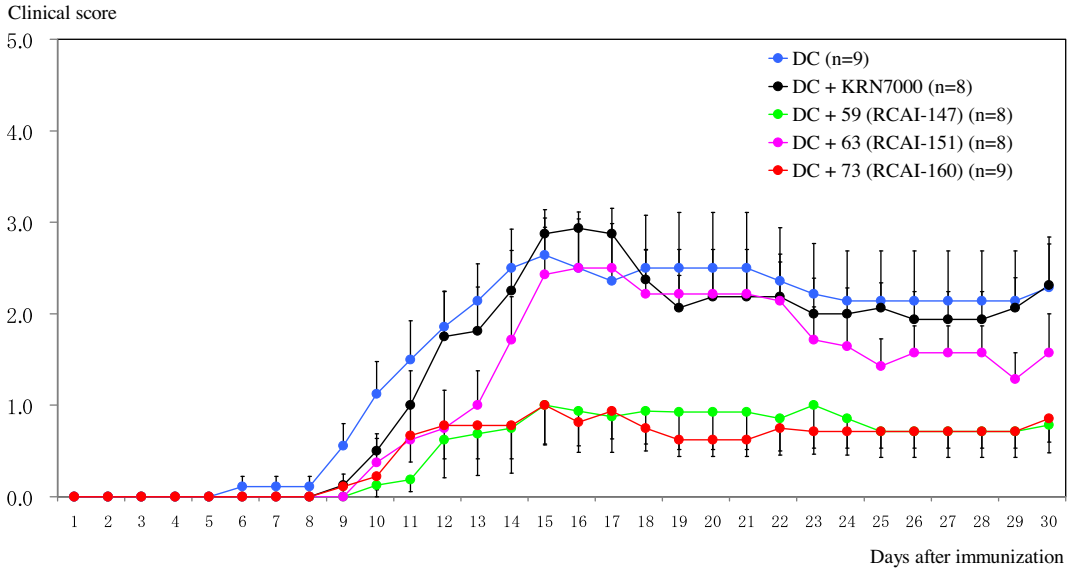


Figure 4. Preventive effects of the KRN7000 analogues against EAE.

is biased toward the Th1 response comparing to that of KRN7000. However, the compound **55**, which has a (2*R*,3*R*)-2,3-dihydroxyhexacosanamide in the amide side-chain, decreased both IFNγ and IL-4 productions to ~40% and ~85% of that of KRN7000, respectively, even though it has the same (5*S*)-hydroxyl group on the phytosphingosine main-chain as compounds **14** and **43**. Therefore, compound **55** showed a weak tendency to bias to Th2 response compared to that of KRN7000.

Compounds **30** and **47** have two successive (5*S*,6*S*)-5,6-dihydroxyl groups next to C_4-hydroxyl group on the phytosphingosine main-chain. The amide side-chain of **30** is a hexacosanamide, and that of **47** is a (2*R*)-2-hydroxyhexacosanamide. Each amount of both IFNγ and IL-4 secretions mediated by compound **47** was almost the same level as KRN7000, and compound **30** decreased both IFNγ and IL-4 productions to ~70% and ~56% of that of KRN7000, respectively. Thus, the compound **47** has almost the same profile to KRN7000 about both IFNγ and IL-4 secretions, and compound **30** shows a little bit bias to Th1 response, but it will be close to the same with that of KRN7000.

Compound **59** (RCAI-147) has two successive (5*S*,6*S*)-5,6-dihydroxy groups next to C_4-hydroxyl group on the phytosphingosine main-chain such as compounds **30** and **47**, and also (2*R*,3*R*)-2,3-dihydroxyhexacosanamide on the amide side-chain. The IFNγ production ability of compound **59** decreased to ~5% of KRN7000, and yet the IL-4 production amount remained more than 70% of KRN7000. Therefore, compound **59** biased to Th2 response (almost 14 times) compared to that of KRN7000.

Compound **63** (RCAI-151) has a phytosphingosine as the main-chain, and also has a (2*R*,3*R*)-2,3-(dihydroxy)hexacosanamide as the amide side-chain. The IFNγ production ability of compound **63** decreased to ~41% of KRN7000, and its IL-4 production amount increased to ~138% of that of KRN7000. Therefore, compound **63** biased to Th2 response (almost 3.4 times) compared to that of KRN7000.

Compound **73** (RCAI-160) has a phytosphingosine moiety as the main-chain, and has a (2*R*,3*R*,4*R*)-2,3,4-trihydroxyhexacosanamide as the amide side-chain. The IFNγ production ability of compound **73** decreased to ~7% of that of KRN7000, and its IL-4 production amount still remained ~55% of that of KRN7000. Therefore, compound **73** biased to Th2 response (almost 7.8 times) comparing to that of KRN7000.

The EAE test of compounds **59**, **63**, and **73** was performed as shown in Figure 4. Compounds **59** (RCAI-147) and **73** (RCAI-160) were effective for EAE test. This may be because the IFNγ production by **59** (RCAI-147) and **73** (RCAI-160) was close to zero, and the IL-4 production was still remaining in high level (approximately

~70% and ~55% of KRN7000, respectively). Although, compound **63** (RCAI-151) produced IL-4 more than KRN7000, it could not suppress the development of EAE. It may be attributable to masking of the therapeutic effect of IL-4 by the IFNγ derived simultaneously from iNKT cells.

3. Conclusions

The cytokine secretion ability of **14** and **43** was biased toward the Th1 response compared to that of KRN7000. Compounds **30**, **47**, and **55** were much the same with KRN7000. On the contrary, compounds **59**, **63**, and **73** behaved obviously as Th2 type antigens. Most of all, both compounds **59** and **73** were biased largely to Th2 response, and showed good suppressive effects against EAE symptoms. From these results, it can be said that the existence of 2–3 consecutive hydroxyl groups on the amide side chain and 2–4 consecutive hydroxyl groups on the octadecyl main chain should contribute to the secretion of Th2 cytokines from iNKT cells. And also these results might show that the balance of hydroxyl groups both on the amide side chain and on the octadecyl main chain should be essential for Th2 response. In addition, not only making the cytokine secretion bias to Th2 but also decreasing the INFγ 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 60 N (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).

4.1.1. 2,3,4-Tri-*O*-benzyl-D-galactopyranose (2)

(1) To a solution of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside **1** (20.0 g, 36.1 mmol) in Ac_2O (600 mL) was added conc H_2SO_4 (0.6 mL). The solution was stirred for 1 h at room temperature, and diluted with EtOAc (1.2 L). The solution was washed with aq satd $NaHCO_3$ (200 mL × 2) and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, to which toluene (100 mL) was added and evaporated under reduced pressure. This procedure was repeated once more, and dried in vacuo to give crude 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-galactopyranose (23.4 g).

(2) The above obtained diacetate was dissolved in MeOH (1.2 L) containing KOH (1.4 g). After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1, and then EtOAc) gave **2** (15.5 g, 95%) as a 1:1 anomeric mixture as a viscous oil. IR ν_{max}(KBr) 3410 (br), 1497, 1454 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.05 (1H, br s, OH), 3.36 (1H, br s, OH), 3.45–3.52 (1.5H, m), 3.57 (0.5H, dd, *J* 2.8, 9.4 Hz), 3.71–3.81 (2H, m), 3.90–3.92 (1H, m), 3.99 (0.5H, t, *J* 5.7 Hz), 4.05 (0.5H, m), 4.63–4.98 (6.5H, m), 5.31 (0.5H, d, *J* 3.4 Hz, anomeric H). ESI-MS: *m*/*z* 473.19 [M+Na]$^+$. HR ESI-MS: calcd for $C_{27}H_{30}O_6Na$: 473.1940; observed: 473.1926.

4.1.2. 2,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-D-galactopyranose (3)

To a solution of **2** (10.5 g, 23.3 mmol) in CH_2Cl_2 (250 mL) were added imidazole (1.67 g, 24.5 mmol, 1.05 equiv) and TBDMS-Cl (3.65 g, 24.2 mmol, 1.04 equiv). After stirring for 1.5 h at room temperature, the reaction mixture 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 **3** (10.3 g, 78%) as an oil and *tert*-butyldimethylsilyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (0.47 g, 3%). Physical data of **3**; IR ν_{max}(KBr): 3411 (br), 2952, 2928, 2883, 2856, 1496, 1470, 1454 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.03 (3H, s), 0.04 (3H, s), 0.88 (9H, s), 2.93 (0.5H, d, *J* 2.0 Hz, OH), 3.06 (0.5H, d, *J* 7.1 Hz, OH), 3.43–3.76 (3H, m), 3.88–4.07 (3H, m), 4.61–4.97 (6.5H, m), 5.26 (0.5H, dd, *J* 2.0, 3.4 Hz), 7.26–7.39 (15H, m). ESI-MS: *m*/*z* 587 [M+Na]$^+$. HR ESI-MS: calcd for $C_{33}H_{44}O_6SiNa$: 587.2799; observed: 587.2796. Physical data of *tert*-butyldimethylsilyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside; IR ν_{max}(KBr) 2954, 2929, 2857, 1496, 1471, 1254 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (6H, s), 0.12 (3H, s), 0.13 (3H, s), 0.87 (9H, s), 0.92 (9H, s), 3.34 (1H, t, *J* 6.4 Hz), 3.48 (3H, dd, *J* 3.0, 9.9 Hz), 3.58 (1H, dd, *J* 6.9, 10.1 Hz), 3.65 (1H, dd, *J* 5.1, 9.8 Hz), 3.75 (1H, dd, *J* 7.4, 9.9 Hz), 3.81 (1H, d, *J* 2.6 Hz), 4.58 (1H, d, *J* 7.3 Hz), 4.63, 4.95 (2H, AB-q, *J* 11.6 Hz), 4.69, 4.77 (2H, AB-q, *J* 12.0 Hz), 4.78, 4.93 (2H, AB-q, *J* 10.9 Hz), 7.26–7.37 (15H, m). ESI-MS: *m*/*z* 701 [M+Na]$^+$. HR ESI-MS: calcd for $C_{39}H_{58}O_6Si_2Na$: 701.3664; observed: 701.3664.

4.1.3. (2*R*,3*S*,4*R*,5*S*,6*EZ*)-3,4,5-Tris(benzyloxy)-1-[(*tert*-butyldimethylsilyl)oxy]octadec-6-en-2-ol (4)

To a solution of $Ph_3P(Br)C_{12}H_{25}$ (25.3 g, 49.5 mmol) in dry THF (300 mL) was added *n*-BuLi (1.59 M in hexane, 32 mL, 50.9 mmol) at −10 °C under an atmosphere of argon. After stirring for 30 min at −10 °C, a solution of **3** (9.31 g, 16.48 mmol) in THF (50 mL) was added dropwise at −10 °C. The mixture was stirred for 15 min at −10 °C at room temperature for 16 h, and then concentrated in vacuo to one-fifth of the volume, and quenched with MeOH (30 mL). The mixture was diluted with hexane, and the whole was washed with water, and aq 3.5% H_2O_2, and again 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 (10:1) gave **4** (5.36 g, 45%) as an oily mixture of *E*- and *Z*-isomers. IR ν_{max}(KBr) 3489, 2926, 2855, 1455, 1253 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.03 (6H, s), 0.86–0.89 (12H, m), 1.26 (16H, br s), 1.31–1.38 (2H, m), 2.00–2.08 (2H, m), 3.09 (1H, d, *J* 4.9 Hz, OH), 3.59–3.62 (2H, m), 3.78 (1H, m), 3.87 (1H, m), 3.90 (1H, m), 4.31, 4.63 (2H, AB-q, *J* 12.0 Hz), 4.44, 4.50 (2H, AB-q, *J* 11.4 Hz), 4.46 (1H, m), 4.77 (2H, s), 5.43 (1H, m), 5.64 (1H, m), 7.22–7.37 (15H, m). ESI-MS: *m*/*z* 739.5 [M+Na]$^+$. HR ESI-MS: calcd for $C_{45}H_{68}O_5SiNa$: 739.4728; observed: 739.4727.

4.1.4. (2*R*,3*S*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-1-[(*tert*-butyldimethylsilyl)oxy]octadecan-2-ol (5)

A solution of **4** (1.00 g, 1.39 mmol) in hexane (100 mL) was stirred for 50 min under an atmosphere of H_2 using 10% Pd/C (500 mg) at room temperature. The reaction mixture was filtered, and the filtrate was concentrated to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1) gave **5** (710 mg, 71%) as an oil. IR ν_{max}(KBr) 3495, 2927, 2854 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.07 (3H, s),

0.86–0.90 (12H, m), 1.26 (22H, br s), 1.52–1.58 (2H, m), 3.36 (1H, d, *J* 4.4 Hz, OH), 3.58–3.66 (3H, m), 3.82–3.86 (2H, m), 3.90 (1H, m), 4.56, 4.58 (2H, AB-q, *J* 11.7 Hz), 4.61 (2H, s), 4.73, 4.81 (2H, AB-q, *J* 11.2 Hz), 7.22–7.35 (15H, m). ESI-MS: *m*/*z* 741.49 $[M+Na]^+$. HR ESI-MS: calcd for $C_{45}H_{70}O_5SiNa$: 741.4885; observed: 741.4887.

4.1.5. (2*S*,3*S*,4*R*,5*S*)-2-Azido-1-(*tert*-butyldimethylsilyl)oxy-3,4,5-tris(benzyloxy)octadecane (6)

To a solution of **5** (72 mg, 0.10 mmol) in dry THF (2 mL) were added Ph_3P (118 mg, 0.45 mmol) and diethyl azodicarboxylate (2.2 M in toluene, 0.20 mL, 0.44 mmol) under an atmosphere of argon. After stirring for 10 min at room temperature, $(PhO)_2P(O)N_3$ (124 mg, 0.45 mmol) was added at 0 °C. The mixture was stirred vigorously for 30 min at 0 °C, then at room temperature for 3 h, and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1) gave **6** (58 mg, 78%) as a viscous oil. IR ν_{max}(KBr) 2926, 2854, 2097 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.05 (3H, s), 0.88 (3H, t, *J* 7.1 Hz), 0.90 (9H, s), 1.26 (20H, br s), 1.30–1.44 (2H, br), 1.52–1.61 (2H, br), 3.65–3.75 (3H, m), 3.79–3.87 (2H, m), 4.01 (1H, dd, *J* 2.4, 10.3 Hz), 4.53–4.58 (3H, m), 4.65 (1H, d, *J* 11.2 Hz), 4.71, 4.75 (2H, AB-q, *J* 11.4 Hz), 7.27–7.36 (15H, m). ESI-MS: *m*/*z* 766.49 $[M+Na]^+$. HR ESI-MS: calcd for $C_{45}H_{69}O_4SiNa$: 766.4950; observed: 766.4950.

4.1.6. (2*S*,3*S*,4*R*,5*S*)-2-Azido-3,4,5-tris(benzyloxy)octadecan-1-ol (7)

A solution of **6** (580 mg, 0.779 mmol) in THF (2 mL) containing *n*-Bu_4NF (1 M solution in THF, 3.3 mL) was stirred for 30 min at room temperature, and the solution was concentrated in vacuo to give a mixture, which was diluted with EtOAc. The solution was washed with H_2O 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 **7** (456 mg, 93%) as an oil. IR ν_{max}(KBr) 3721 (br), 2925, 2854, 2097, 1454 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.26 (20H, s), 1.39 (2H, br s), 1.53 (2H, m), 2.59 (1H, t, *J* 6.4 Hz, OH), 3.61 (1H, m), 3.75–3.84 (4H, m), 3.95 (1H, m), 4.56, 4.64 (2H, AB-q, *J* 11.3 Hz), 4.57, 4.61 (2H, AB-q, *J* 11.3 Hz), 4.75 (2H, s), 7.27–7.35 (15H, m). ESI-MS: *m*/*z* 624.40, 652.40 $[M+Na]^+$ (on addition of HCOONa). HR ESI-MS: calcd for $C_{39}H_{55}N_3O_4Na$: 652.4090; observed: 652.4083.

4.1.7. 2,3-Di-*O*-Benzyl-4,6-*O*-di-*tert*-butylsilylene-D-galactopyranose (9)

(1) To a solution of **8**[6] (2.93 g, 6.86 mmol) in DMF (8 mL) were added BnBr (4.69 g, 27.4 mmol) and NaH (60% oil dispersion, 1.65 g, 41.3 mmol) at 0 °C under an atmosphere of argon. After stirring for 15 min at 0 °C, the mixture was stirred for 15 min at room temperature, diluted with EtOAc, and quenched with ice water. The organic layer was washed with 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 a di-benzyl ether (4.17 g, quantitatively) as a viscous oil. ^1H NMR (500 MHz, $CDCl_3$): δ 1.08 (9H, s), 1.13 (9H, s), 2.32 (3H, s), 3.25 (1H, s), 3.46 (1H, dd, *J* 2.9, 9.0 Hz), 3.83 (1H, t, *J* 9.5 Hz), 4.16 (1H, dd, *J* 2.3, 12.4 Hz), 4.20 (1H, dd, *J* 1.7, 12.4 Hz), 4.49 (1H, d, *J* 2.4 Hz), 4.58, (1H, d, *J* 3.9 Hz), 4.69, 4.77 (2H, AB-q, *J* 12.0 Hz), 4.89, 4.91 (2H, AB-q, *J* 10.4 Hz), 7.07 (2H, d, *J* 8.0 Hz), 7.27–7.36 (6H, m), 7.40–7.45 (6H, m). ESI-MS: *m*/*z* 629.27 $[M+Na]^+$. HR ESI-MS: calcd for $C_{35}H_{46}O_5SSiNa$: 629.2727; observed: 629.2726.

(2) The above obtained compound (4.17 g, 6.86 mmol) was dissolved in acetone (140 mL), and the solution was cooled to −20 °C. To this solution was added *N*-bromosuccinimide (1.47 g, 8.26 mmol). After stirring for 40 min at −20 °C, the reaction mixture was quenched with aq satd $NaHCO_3$ (35 mL), concentrated in vacuo, and diluted with EtOAc, which was washed with aq 10% $Na_2S_2O_3$ 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 (2:1, then 3:2, finally 2:3) gave **9** (2.24 g, 65%) as a gum. IR ν_{max}(KBr) 3539, 3419 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.01 (4.5H, s), 1.06 (4.5H, s), 1.076 (4.5H, s), 1.084 (4.5H, s), 2.83–2.85 (1H, m, OH), 3.35 (0.5H, s), 3.45 (0.5H, dd, *J* 3.0, 7.9 Hz), 3.71 (0.5H, dd, *J* 7.8, 9.3 Hz), 3.77 (0.5H, dd, *J* 2.8, 9.7 Hz), 3.85 (0.5H, s), 3.98 (0.5H, dd, *J* 3.7, 10.0 Hz), 4.13–4.23 (2H, m), 4.47 (0.5H, d, *J* 2.9 Hz), 4.52 (0.5H, d, *J* 3.2 Hz), 4.64–4.78 (3H, m), 4.88–4.91 (1.5H, m), 5.20 (0.5H, d, *J* 3.9 Hz, on addition of D_2O), 7.27–7.43 (10H, m). ESI-MS: *m*/*z* 523.25 $[M+Na]^+$. HR ESI-MS: calcd for $C_{28}H_{40}O_6SiNa$: 523.2486; observed: 523.2486.

4.1.8. (2*S*,3*S*,4*R*,5*S*)-2-Azido-3,4,5-tris(benzyloxy)octadecyl 2,3-di-*O*-benzyl-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (10)

To a solution of **9** (462 mg, 0.923 mmol) in dry CH_2Cl_2 (10 mL) were added Cl_3CCN (1.33 g, 9.23 mmol) and Cs_2CO_3 (350 mg), and the mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with 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 crude imidate (590 mg). To the solution of this imidate (590 mg) and **7** (348 mg, 0.55 mmol) in CH_2Cl_2 (8 mL) was added molecular sieves (MS) 4 Å (890 mg). The mixture was stirred for 30 min at room temperature, and cooled to 0 °C. To this mixture was added AgOTf (83 mg, 0.323 mmol), and stirred for 1 h at 0 °C, and then 16 h at room temperature. The mixture was filtered, and the filter cake was washed with CH_2Cl_2. The combined filtrate was washed with aq satd $NaHCO_3$ 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 (9:1) gave **10** (615 mg, quantitative) as a gum. IR ν_{max}(KBr) 2927, 2856, 2099 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 6.8 Hz) 0.98 (9H, s), 1.04 (9H, s), 1.20–1.31 (20H, m), 1.31–1.43 (2H, m), 1.50–1.60 (2H, m), 3.51 (1H, br s), 3.62 (1H, m), 3.69–3.73 (2H, m), 3.79–3.83 (2H, m), 3.93–4.03 (5H, m), 4.42 (1H, d, *J* 3.0 Hz), 4.42–4.85 (12H, m containing 1H, s, at 4.58 ppm, and 1H, d, *J* 3.4 Hz at 4.79 ppm as an anomeric proton), 7.22–7.42 (25H, m). ESI-MS: *m*/*z* 1134.64 $[M+Na]^+$. HR ESI-MS: calcd for $C_{67}H_{93}N_3O_9SiNa$: 1134.6579; observed: 1134.6588.

4.1.9. (2*S*,3*S*,4*R*,5*S*)-2-Amino-3,4,5-tris(benzyloxy)octadecyl 2,3-di-*O*-benzyl-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (11)

To a solution of **10** (318 mg, 0.29 mmol) in dry THF (5.6 mL) was added Me_3P (1 M solution in THF, 1.5 mL). After stirring for 2 h at room temperature, aq NaOH (1 M solution in H_2O, 5.45 mL) was added to this solution. The mixture was stirred for 2 h at room temperature, diluted with water, and extracted with $CHCl_3$, which 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 (4:1, then 1:1) gave **11** (240 mg, 77%) as a gum. IR ν_{max}(KBr) 2926, 2856 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 6.8 Hz) 0.99 (9H, s), 1.04 (9H, s), 1.20–1.30 (22H, m), 1.50–1.70 (4H, br s, containing NH_2), 3.53 (1H, br s), 3.76 (1H, m), 3.81 (1H, dd, *J* 3.2, 10.1 Hz), 3.97–4.02 (2H, m), 4.08 (1H, m), 4.47 (1H, m), 4.52–4.75 (9H, m), 4.78 (1H, d, *J* 3.0 Hz), 4.83 (1H, d, *J* 12.0 Hz, benzylic H), 7.22–7.33 (23H, m), 7.42 (2H, d, *J* 7.0 Hz). ESI-MS: *m*/*z* 1086.67 $[M+H]^+$. HR ESI-MS: calcd for $C_{67}H_{96}NO_9Si$: 1086.6854; observed: 1086.6843.

4.1.10. (2*S*,3*S*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-(hexacosanoylamino)octadecyl 2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranoside (12)

To a solution of **11** (170 mg, 0.15 mmol) in THF–CH_2Cl_2 (1:1, 14 mL) were added DMAP (150 mg, 1.22 mmol), *n*-hexacosanoic acid (182 mg, 0.46 mmol), and EDAC (176 mg, 0.919 mmol). The mixture was stirred for 1.5 h at room temperature, and concentrated in vacuo to give a mixture, which was diluted with CH_2Cl_2. 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) gave **12** (161 mg, 70%) as a gum. IR ν_{max}(KBr) 2925, 2853, 1674 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 0.99 (9H, s), 1.02 (9H, s), 1.25 (6H, br s), 1.37–1.44 (2H, m), 1.53–1.66 (2H, m), 1.73–1.78 (2H, m), 3.50–3.55 (2H, m), 3.69 (1H, dd, *J* 4.0, 5.9 Hz), 3.75 (1H, dd, *J* 2.7, 10.1 Hz), 3.79 (1H, dd, *J* 7.6, 11.0 Hz), 3.87 (1H, t, *J* 3.8 Hz), 3.96 (1H, dd, *J* 3.7, 10.1 Hz), 4.01–4.08 (3H, m), 4.33 (1H, m), 4.46 (1H, d, *J* 2.3 Hz), 4.49–4.74 (9H, m), 4.79 (1H, d, *J* 3.6 Hz), 4.82 (1H, d, *J* 11.5 Hz), 6.12 (1H, d, *J* 8.4 Hz, NH), 7.22–7.34 (23H, m), 7.40 (2H, m). ESI-MS: *m*/*z* 1487.05 $[M+Na]^+$. HR ESI-MS: calcd for $C_{93}H_{145}NO_{10}SiNa$: 1487.0535; observed: 1487.0523.

4.1.11. (2*S*,3*S*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-(hexacosanoylamino)octadecyl 2,3-di-*O*-benzyl-α-D-galactopyranoside (13)

To a solution of **12** (55 mg, 0.04 mmol) and pyridine (64 mg, 0.79 mmol) in dry THF (4 mL) was added HF·pyridine (HF: ~70%; pyridine: ~30%, 30 mg, ca. 1.05 mmol) under an atmosphere of argon at room temperature. After stirring for 50 min, the reaction mixture was diluted with $CHCl_3$, and washed with aq satd $NaHCO_3$ 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 (2:1, then 1:1) gave **13** (46 mg, 92%) as a gum. IR ν_{max}(KBr) 3744–3535 (br), 2923, 2852, 1650 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.879 (3H, t, *J* 7.0 Hz), 0.882 (3H, t, *J* 6.9 Hz), 1.25 (66H, br s), 1.44 (2H, quintet, *J* 7.2 Hz), 1.52–1.70 (2H, m), 1.80–1.84 (2H, m), 2.50 (1H, s, OH), 3.60 (1H, m), 3.64–3.69 (2H, m), 3.72–3.78 (4H, m), 3.81 (1H, dd, *J* 3.7, 9.8 Hz), 3.86 (1H, dd, *J* 8.6, 11.6 Hz), 3.96 (1H, d, *J* 2.9 Hz), 4.13 (1H, dd, *J* 4.4, 11.5 Hz), 4.42 (1H, d, *J* 11.7 Hz), 4.45 (1H, m), 4.54–4.75 (9H, m), 4.84 (1H, d, *J* 3.7 Hz, anomeric H), 5.94 (1H, d, *J* 8.4 Hz, NH), 7.24–7.35 (25H, m). ESI-MS: *m*/*z* 1346.95 $[M+Na]^+$. HR ESI-MS: calcd for $C_{85}H_{129}NO_{10}Na$: 1346.9514; observed: 1346.9498.

4.1.12. (2*S*,3*S*,4*R*,5*S*)-2-Hexacosanoylamino-3,4,5-trihydroxyoctadecyl α-D-galactopyranoside (14) (RCAI-104)

To a solution of **13** (32 mg, 0.024 mmol) in THF (25 mL) was added 20% $Pd(OH)_2$/C (Degussa type, wet, ~50%, 32 mg). After stirring for 24 h under an atmosphere of H_2 at room temperature, the catalyst was removed by filtration, and washed with THF, and then $CHCl_3$–MeOH (5:1). The combined filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel (1 g) column. Elution with $CHCl_3$–MeOH (19:1, then 9:1) gave **14** (10 mg, 47%) as a white powder. $[\alpha]_D^{29}$ +39.9 [*c* 1.2, $CHCl_3$–CH_3OH (1:1)]. IR ν_{max}(KBr) 3372 (br), 2920, 2851, 1645, 1541, 1468 cm^{-1}. ^1H NMR [500 MHz, $CDCl_3$–CD_3OD (9:1)]: δ 0.88 (6H, t, *J* 7.0 Hz), 1.25 (64H, br s), 1.40–1.63 (4H, m), 2.20 (2H, t, *J* 7.6 Hz), 3.43 (1H, d, *J* 7.9 Hz), 3.66 (1H, dd, *J* 4.4, 10.8 Hz), 3.72 (1H, dd, *J* 3.3, 9.9 Hz), 3.74–3.84 (7H, m), 3.91 (1H, dd, *J* 5.4, 11.0 Hz), 3.96 (1H, d, *J* 3.1 Hz), 4.22 (1H, m), 4.93 (1H, d, *J* 3.9 Hz, anomeric H), 7.15 (1H, d, *J* 8.3 Hz). ^{13}C NMR (126 MHz, pyridine-d_5): δ 14.28, 22.93, 26.36, 26.88, 29.60, 29.61, 29.75, 29.79, 29.85, 29.91, 29.92, 30.00, 30.02, 30.04, 30.11, 30.23, 32.12, 35.01, 36.75, 51.74, 62.68, 68.22, 70.31, 71.00, 71.13, 71.64, 73.06, 73.78, 73.97, 101.31, 173.32. ESI-MS: *m*/*z* 896.72 $[M+Na]^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{10}Na$: 896.7161; observed: 896.7157.

4.1.13. (3*R*,4*R*,5*S*,6*S*,7*R*)-7-[(*tert*-Butyldimethylsilyloxy)methyl]-5,6-*O*-isopropylidene-3,4,5,6-tetrahydroxyoxepan-2-one (16)

To a solution of **15** (3.00 g, 9.13 mmol), 4-methylmorpholine *N*-oxide (1.28 g, 10.96 mmol), and *p*-TsOH·H_2O (2.07 g, 10.90 mmol) in acetone–H_2O (4:1, 150 mL) was added OsO_4 (1% solution in *t*-BuOH, 30 mL) at 0 °C. The solution was stirred for 15 min at 0 °C, and for 16 h at room temperature. The reaction mixture was concentrated in vacuo to half volume at 25 °C, and diluted with EtOAc (500 mL), which was washed with 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 **16** (2.10 g, 63%); mp 107–108 °C (as a floc from hexane–EtOAc (5:1)). This compound decomposes gradually at room temperature. IR ν_{max}(KBr) 3390 (br), 3000–2858, 1725, 1472 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.089 (3H, s), 0.092 (3H, s), 0.90 (9H, s), 1.38 (3H, s), 1.50 (3H, s), 2.72 (1H, d, *J* 3.9 Hz, OH), 3.51 (1H, s, OH), 3.81 (1H, dd, *J* 9.3, 9.6 Hz), 3.94 (1H, dd, *J* 5.6, 9.5 Hz), 4.24 (1H, m, changed to dd, *J* 1.5, 3.9 Hz, on addition of D_2O), 4.44 (1H, dd, *J* 4.3, 7.6 Hz), 4.52 (1H, br s, changed to doublet, *J* 1.5 Hz, on addition of D_2O), 4.70 (1H, d, *J* 7.6 Hz), 4.85 (1H, dd, *J* 5.6, 9.3 Hz). FABMS: *m*/*z* 363.2 $[M+H]^+$. HRFABMS: calcd for $C_{16}H_{31}O_7Si$: 363.1839; observed: 363.1853.

4.1.14. (3*R*,4*R*,5*S*,6*S*,7*R*)-7-[(*tert*-Butyldimethylsilyloxy)methyl]-3,4-bis(*tert*-butyldimethylsilyloxy)-5,6-*O*-isopropylidene-5,6-dihydroxyoxepan-2-one (17)

To a solution of **16** (580 mg, 1.60 mmol) in dry CH_2Cl_2 (30 mL) containing 2,6-lutidine (1.72 g, 16.0 mmol) was added TBDMSOTf (2.54 g, 9.60 mmol). After stirring for 16 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, and washed with H_2O 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) gave **17** (908 mg, 96%) as an amorphous. IR ν_{max}(KBr) 2990–2858, 1732, 1471, 1464 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 0.07 (6H, s), 0.10 (3H, s), 0.11(3H, s), 0.12 (3H, s), 0.14 (3H, s), 0.88 (9H,s), 0.89 (9H, s), 0.91 (9H, s), 1.36 (3H, s), 1.48 (3H, s), 3.81 (1H, dd, *J* 8.2, 9.6 Hz), 3.87 (1H, dd, *J* 6.1, 9.6 Hz), 3.89 (1H, dd, *J* 1.0, 6.4 Hz), 4.22 (1H, dd, *J* 6.4, 6.9 Hz), 4.50 (1H, d, *J* 1.0 Hz, C3-H), 4.50 (1H, d, *J* 6.9 Hz, C6-H), 5.13 (1H, dd, *J* 6.1, 8.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ −5.50, −5.43, −5.40, −5.08, −4.81, −4.51, 18.02, 18.11, 18.20, 25.31, 25.70, 25.78, 27.62, 62.51, 73.51, 74.51, 74.97, 77.64, 78.24, 108.59, 170.07. ESI-MS: *m*/*z* 613.34 $[M+Na]^+$. HR ESI-MS: calcd for $C_{28}H_{58}O_7Si_3Na$: 613.3383; observed: 613.3381.

4.1.15. (3*R*,4*R*,5*S*,6*S*,7*R*)-7-[(*tert*-Butyldimethylsilyloxy)methyl]-3,4-bis(*tert*-butyldimethylsilyloxy)-5,6-*O*-(isopropylidene)oxepan-2,5,6-triol (18)

To a solution of **17** (5.97 g, 10.1 mmol) in toluene (180 mL) was added DIBAH (1.0 M in toluene, 10.8 mL, 10.8 mmol) under an atmosphere of argon at −78 °C. The solution was stirred for 45 min, and the reaction mixture was quenched with MeOH (15 mL). After stirring for 30 min at −78 °C, the reaction mixture was allowed to warm up to room temperature, and diluted with EtOAc, and the solution was washed with H_2O. The whole was filtered on Celite. Water, separated from the organic layer, was re-extracted with EtOAc. The combined organic layers were washed with 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 14:1) gave **18** (5.77 g, 96%) as an oily 2:1 anomeric mixture. IR ν_{max}(KBr) 3440, 2954, 2930, 2887, 2858, 1748 (w), 1472, 1464, 1389, 1379,

1362, 1255 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.05–0.06 (6H, m), 0.10–0.14 (12H, m), 0.87–0.93 (27H, m), 1.33 (2/3H, s), 1.34 (1/3H, s), 1.56 (3H, s), 2.63 (2/3H, d, *J* 4.2 Hz, OH), 3.68–3.74 (3H, m), 3.92–3.97 (1H, m), 4.22 (2/3H, m), 4.26–4.37 (2H, m), 4.55 (1/3H, m), 4.88 (2/3H, dd, *J* 4.4, 6.8 Hz, changed to doublet, *J* 6.8 Hz, on addition of D_2O), 5.06 (1/3H, m), 5.53 (1/3H, m, OH). ESI-MS: *m*/*z* 615.35 $[M+Na]^+$. HR ESI-MS: calcd for $C_{28}H_{60}O_7Si_3Na$: 615.3539; observed: 615.3540.

4.1.16. (2*R*,3*S*,4*S*,5*R*,6*S*,7*EZ*)-1,5,6-Tris(*tert*-butyldimethylsilyloxy)-3,4-*O*-(isopropylidene)octadec-7-en-2,3,4-triol (19)

To a solution of $Ph_3P(Br)C_{11}H_{23}$ (9.46 g, 19.0 mmol) in dry THF (47 mL) was added a solution of **18** (2.82 g, 4.76 mmol) in dry THF (23 mL) under an atmosphere of argon at −30 °C. To this solution was added $LiN(TMS)_2$ (1 M in THF, 28 mL) with stirring. After stirring for 5 min at −30 °C, the temperature was gradually elevated from −30 °C to −10 °C over 20 min. After stirring for 15 min at −10 °C, the solution was stirred for 15 min at −10 °C, and 3 h at room temperature. The reaction mixture was quenched with MeOH (4 mL) at 0 °C, and 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 mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (40:1) gave **19** (1.88 g, 54%) as an oily mixture of *E*- and *Z*-isomers, and elution with hexane–EtOAc (30:1) gave recovered **18** (380 mg, 14%). IR ν_{max}(KBr) 3484, 2927, 2856 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.03 (3H, s), 0.05 (3H, s), 0.07 (6H, s), 0.08 (3H, s), 0.09 (3H, s), 0.87–0.91 (30H, m, containing 9H singlet and 18H singlet at 0.87 and 0.90 ppm, respectively), 1.26–1.40 (19H, m, containing 3H singlet at 1.32 ppm), 1.48 (3H, s), 1.98 (1H, m), 2.06 (1H, m), 2.23 (1H, d, *J* 6.1 Hz, OH), 3.58 (1H, d, *J* 1.7 Hz), 3.59 (1H, s), 3.71 (1H, q, *J* 6.1 Hz), 3.99 (1H, dd, *J* 6.5, 9.7 Hz), 4.14 (1H, d, *J* 6.4 Hz), 4.23 (1H, dd, *J* 2.7, 9.8 Hz), 4.27 (1H, dd, *J* 2.6, 8.4 Hz), 5.44–5.53 (2H, m). ESI-MS: *m*/*z* 753.5 $[M+Na]^+$. HR ESI-MS: calcd for $C_{39}H_{82}O_6Si_3Na$: 753.5311; observed: 753.5314.

4.1.17. (2*R*,3*R*,4*S*,5*R*,6*S*,7*EZ*)-1,5,6-Tris(*tert*-butyldimethylsilyloxy)-3,4-*O*-isopropylidene-2-(methanesulfonyloxy)octadec-7-ene-3,4-diol (20)

To a solution of **19** (840 mg, 1.15 mmol) in dry CH_2Cl_2 (40 mL) and pyridine (4 mL) was added methanesulfonic anhydride (840 mg, 4.82 mmol). The mixture was stirred for 45 min at room temperature, and diluted with CH_2Cl_2, which was washed with aq satd $NaHCO_3$ (twice) 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 (30:1) gave **20** (719 mg, 77%) as an oily mixture of *E*- and *Z*-isomers. IR ν_{max}(KBr) 2929, 2857, 1471, 1345, 1254, 1178 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.088 (6H, s), 0.093 (3H, s), 0.10 (3H, s), 0.11 (3H, s), 0.88 (9H, s), 0.89 (3H, m), 0.90 (9H, s), 0.91 (9H, s), 1.25–1.40 (19H, m, containing 3H singlet at 1.30 ppm), 1.45 (3H, s), 1.99 (1H, m), 3.08 (3H, s), 3.84 (1H, dd, *J* 7.5, 10.0 Hz), 3.97–4.02 (2H, m), 4.21 (1H, dd, *J* 2.2, 9.7 Hz), 4.25 (1H, d, *J* 6.1 Hz), 4.31 (1H, dd, *J* 2.2, 8.5 Hz), 4.77 (1H, t, *J* 6.5 Hz), 5.44–5.53 (2H, m). ESI-MS: *m*/*z* 831.5 $[M+Na]^+$. HR ESI-MS: calcd for $C_{40}H_{84}O_8Si_3SNa$: 831.5087; observed: 831.5088.

4.1.18. (2*R*,3*R*,4*S*,5*R*,6*S*,7*EZ*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-3,4-*O*-isopropylidene-2-(methanesulfonyloxy)octadec-7-en-1,3,4-triol (21)

To a solution of **20** (128 mg, 0.16 mmol) and pyridine (1.0 mL) in dry THF (1.72 mL) was added HF·pyridine (HF: ~70%; pyridine: ~30%, 0.34 mL) under an atmosphere of argon at 0 °C. After stirring for 5 min, the mixture was stirred for 5 h at room temperature, the reaction mixture was quenched with aq satd $NaHCO_3$, and diluted with EtOAc, which was 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 (9:1) gave **21** (68 mg, 62%) as an oily 12:1 mixture of *E*- and *Z*-isomers. Partly this mixture was separated on a silica gel TLC plate by development with hexane–EtOAc (4:1). Physical data of the major isomer (larger R_f value than that of minor isomer). IR ν_{max}(KBr) 3540, 2927, 2856, 1466, 1342, 1253 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.05 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.12 (3H, s), 0.88 (18H, s, and 3H, t, *J* 7.1 Hz), 1.25–1.40 (19H, m, containing 3H singlet at 1.33 ppm), 1.49 (3H, s), 2.02 (1H, m), 2.10 (1H, m), 2.87 (1H, dd, dd, *J* 4.7, 7.9 Hz), 3.16 (3H, s), 3.94–3.99 (2H, m), 4.08 (1H, dd *J* 6.1, 9.6 Hz), 4.17 (1H, dd *J* 4.8, 9.5 Hz), 4.25 (1H, dd *J* 1.3, 6.3 Hz), 4.32 (1H, dd *J* 4.6, 9.0 Hz), 5.00 (1H, t *J* 4.4 Hz), 5.40 (1H, m), 5.48 (1H, m). ESI-MS: *m*/*z* 717.4 $[M+Na]^+$. HR ESI-MS: calcd for $C_{34}H_{70}O_8Si_2SNa$: 717.4222; observed: 717.4224.

4.1.19. (2*R*,3*R*,4*S*,5*R*,6*S*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-3,4-*O*-isopropylidene-2-(methanesulfonyloxy)octadecane-1,3,4-triol (22)

A solution of **21** (68 mg, 0.10 mmol) in EtOAc (7 mL) was stirred for 16 h at room temperature under hydrogen using 10% Pd/C (50 mg), and filtered. The filtrate was concentrated in vacuo to give **22** (68 mg, quantitatively) as a viscous oil, which was employed for the next reaction without purification. IR ν_{max}(KBr) 3526, 2928, 2856, 1517 (w), 1471, 1465, 1361, 1255, 1219, 1175 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.10 (6H, s), 0.12 (6H, s), 0.87–0.90 (21H, m, containing two 9H singlets at 0.88 and 0.90 ppm), 1.26 (18H, br s), 1.33 (3H, s), 1.38–1.49 (2H, m), 1.51 (3H, s), 1.73–1.77 (2H, m), 3.03 (1H, dd, *J* 3.0, 9.4 Hz, OH), 3.16 (3H, s), 3.65 (1H, m), 3.94 (1H, m), 4.00–4.07 (2H, m), 4.22 (1H, m), 4.30 (1H, d, *J* 6.4 Hz), 4.90 (1H, m). ESI-MS: *m*/*z* 719.4 $[M+Na]^+$. HR ESI-MS: calcd for $C_{34}H_{72}O_8Si_2SNa$: 719.4379; observed: 719.4380.

4.1.20. (2*S*,3*S*,4*S*,5*R*,6*S*)-2-Azido-5,6-bis(*tert*-butyldimethylsilyloxy)-3,4-*O*-(isopropylidene)octadecane-1,3,4-triol (23)

A solution of **22** (20 mg, 0.03 mmol) and NaN_3 (20 mg, 0.31 mmol) in dry DMF (2 mL) was stirred for 14 h at 100 °C, and concentrated in vacuo. The residual 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 mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1) gave **23** (14 mg, 76%) as a viscous oil. IR ν_{max}(KBr) 3470, 2927, 2856, 2100, 1518 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.09 (3H, s), 0.11 (6H, s), 0.14 (3H, s), 0.87–0.92 (21H, m, containing two 9H singlets at 0.90 and 0.92 ppm), 1.26 (18H, br s), 1.32 (3H, s), 1.41 (2H, m), 1.47 (3H, s), 1.52–1.65 (2H, m), 2.03 (1H, t, *J* 6.1 Hz, OH), 3.66 (3H, m), 3.71 (1H, quintet, *J* 3.4 Hz), 3.92 (1H, m), 3.98–4.01 (2H, m), 4.08–4.12 (2H, m). ESI-MS: *m*/*z* 666.5 $[M+Na]^+$. HR ESI-MS: calcd for $C_{33}H_{69}N_3O_5Si_2Na$: 666.4668; observed: 666.4667.

4.1.21. (2*S*,3*S*,4*S*,5*R*,6*S*)-2-Azido-5,6-bis(*tert*-butyldimethylsilyloxy)-3,4-*O*-isopropylidene-3,4-dihydroxyoctadecyl 2,3-bis-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (25)

To a solution of **24** (225 mg, 0.40 mmol) in dry CH_2Cl_2 (4 mL) were added Cl_3CCN (580 mg, 4.02 mmol) and Cs_2CO_3 (200 mg, 0.61 mmol), and the mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with 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 crude imidate (280 mg). This compound was unstable for silica gel column chromatography. Therefore, this imidate was used for the next reaction without purification. Physical data of this crude imidate:

IR ν_{max}(KBr) 3343 (w), 2935, 2859, 1728, 1672, 1514 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.00–1.07 (18H, m), 3.79–4.19 (12H, m, containing two methoxy 6H), 4.54–4.71 (5H, m, containing benzylic 4H), 6.39 (1H, d, *J* 3.6 Hz, anomeric H. Therefore, the major compound of this imidate is α-anomer.), 6.83–6.87 (4H, m), 7.24–7.33 (4H, m), 8.53 (1H, s, C=NH). ESI-MS: *m*/*z* 726.18 $[M+Na]^+$. HR ESI-MS: calcd for $C_{32}H_{44}NO_8SiCl_3Na$: 726.1794; observed: 726.1791.

To the solution of this imidate and **23** (127 mg, 0.20 mmol) in CH_2Cl_2 (6 mL) was added MS 4 Å (640 mg). The mixture was stirred for 30 min at room temperature, and cooled to 0 °C. To this mixture was added AgOTf (60 mg, 0.23 mmol), and stirred for 1 h at 0 °C, and then 1.5 h at room temperature. The mixture was filtered, and the filter cake was washed with CH_2Cl_2. The combined filtrate was washed with aq satd $NaHCO_3$ 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 **25** (184 mg, 78%) as a gum. IR ν_{max}(KBr) 2928, 2857, 2099, 1513 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.08 (6H, s), 0.09 (3H, s), 0.13 (3H, s), 0.86–0.89 (12H, m, containing 9H singlet at 0.88 ppm), 0.91 (9H, s), 0.99 (9H, s), 1.05 (9H, s), 1.25 (18H, br s), 1.29 (3H, s), 1.38–1.44 (5H, m, containing 3H singlet at 1.43 ppm), 1.54–1.59 (2H, m), 3.61 (1H, s), 3.65 (1H, m), 3.68–3.70 (2H, m), 3.78–3.81 (7H, m, containing 6H singlet at 3.80 ppm), 3.94–3.98 (2H, m), 4.04–4.06 (2H, m), 4.09–4.13 (2H, m), 4.18 (1H, dd, *J* 2.0, 12.5 Hz), 4.43 (1H, d, *J* 3.7 Hz, galactose C_4-H), 4.61, 4.74 (2H, AB-q, *J* 11.6 Hz), 4.63, 4.66 (2H, AB-q, *J* 11.7 Hz), 4.75 (1H, d, *J* 3.4 Hz, anomeric H), 6.83–6.86 (4H, m), 7.30–7.33 (4H, m). ESI-MS: *m*/*z* 1208.7 $[M+Na]^+$. HR ESI-MS: calcd for $C_{63}H_{111}N_3O_{12}Si_3Na$: 1208.7368; observed: 1208.7362.

4.1.22. (2*S*,3*S*,4*S*,5*R*,6*S*)-2-Amino-5,6-bis(*tert*-butyldimethylsilyloxy)-3,4-*O*-isopropylidene-3,4-dihydroxyoctadecyl 2,3-bis-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (26)

To a solution of **25** (180 mg, 0.15 mmol) in dry THF (3 mL) was added Me_3P (1 M solution in THF, 0.80 mL). After stirring for 2 h at room temperature, NaOH (1 M solution in H_2O, 2.9 mL) was added to this solution. The mixture was stirred for 2 h at room temperature, diluted with water, and extracted with $CHCl_3$, which 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 2:1) gave **26** (136 mg, 77%) as a gum. IR ν_{max}(KBr) 2928, 2856, 1613, 1513, 1472, 1464, 1250 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.03 (3H, s), 0.056 (3H, s), 0.061 (3H, s), 0.10 (3H, s), 0.86–0.89 (21H, m, containing two 9H singlets at 0.88 and 0.89 ppm), 1.00 (9H, s), 1.06 (9H, s), 1.25–1.29 (21H, br s, containing 3H singlet at 1.29 ppm), 1.39–1.42 (5H, m, containing 3H singlet at 1.42 ppm), 1.54–1.57 (4H, m, containing NH_2), 3.08 (1H, m), 3.28 (1H, dd, *J* 7.8, 10.0 Hz), 3.59 (1H, s), 3.67 (1H, m), 3.77 (1H, dd, *J* 2.8, 9.9 Hz), 3.79 (3H, s), 3.80 (3H, s), 3.89 (1H, m), 3.94–3.98 (3H, m), 4.03 (1H, m), 4.11 (1H, dd, *J* 1.6, 12.4 Hz), 4.18 (1H, dd, *J* 2.0, 12.4 Hz), 4.48 (1H, d, *J* 2.5 Hz, galactose C_4-H), 4.59, 4.74 (2H, AB-q, *J* 11.4 Hz), 4.63, 4.66 (2H, AB-q, *J* 11.4 Hz), 4.76 (1H, d, *J* 3.4 Hz, anomeric H), 6.83–6.87 (4H, m), 7.26–7.34 (4H, m). ESI-MS: *m*/*z* 1160.8 $[M+H]^+$. HR ESI-MS: calcd for $C_{63}H_{114}NO_{12}Si_3$: 1160.7643; observed: 1160.7644.

4.1.23. (2*S*,3*S*,4*S*,5*R*,6*S*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-2-hexacosanoylamino-3,4-*O*-isopropylidene-3,4-dihydroxyoctadecyl 2,3-bis-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (27)

Compound **26** (135 mg, 0.12 mmol) was treated as described for the formation of **12** from **11** to give **27** (154 mg, 86%) as a gum. IR ν_{max}(KBr) 3349 (w), 2925, 2854, 1678, 1613, 1514, 1465 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.01 (3H, s), 0.08 (3H, s), 0.10 (3H, s), 0.11 (3H, s), 0.86–0.89 (24H, m, containing two 9H singlets at 0.88 and 0.89 ppm), 0.99 (9H, s), 1.05 (9H, s), 1.22–1.36 (64H, m, containing 3H singlet at 1.35 ppm), 1.45 (3H, s), 1.53–1.58 (4H, m), 2.00 (1H, m), 2.08 (1H, m), 3.55 (1H, s), 3.59 (1H, dd, *J* 4.4, 10.5 Hz), 3.66 (1H, m), 3.72 (1H, dd, *J* 2.7, 10.0 Hz), 3.77–3.81 (7H, m, containing two 3H singlets at 3.79 and 3.81 ppm), 3.88–4.00 (4H, m), 4.08–4.18 (2H, m), 4.32 (1H, dd, *J* 5.4, 7.8 Hz), 4.47 (1H, d, *J* 2.4 Hz, galactose C_4-H), 4.58–4.71 (4H, benzylic 4H), 4.83 (1H, *J* 3.7 Hz, anomeric H), 6.24 (1H, d, *J* 6.1 Hz, NH), 6.81 (2H, d, *J* 8.7 Hz), 6.87 (2H, d, *J* 8.7 Hz), 7.28 (2H, d, *J* 8.6 Hz), 7.33 (2H, d, *J* 8.6 Hz). ESI-MS: *m*/*z* 1561.13 $[M+Na]^+$. HR ESI-MS: calcd for $C_{89}H_{163}NO_{13}Si_3Na$: 1561.1324; observed: 1561.1324.

4.1.24. (2*S*,3*S*,4*S*,5*R*,6*S*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-2-hexacosanoylamino-3,4-*O*-isopropylidene-3,4-dihydroxyoctadecyl 4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (28)

To a solution of **27** (154 mg, 0.10 mmol) in CH_2Cl_2–H_2O (10:1, 22 mL) was added DDQ (154 mg, 0.68 mmol), and the solution was stirred for 2 h at room temperature, then diluted with CH_2Cl_2. The solution was washed with satd aq $NaHCO_3$ (two 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 (6:1, then 3:1) gave **28** (111 mg, 85%) as a gum. IR ν_{max}(KBr): 3429, 3326, 2925, 2855, 1656, 1467 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.06 (3H, s), 0.09 (3H, s), 0.15 (3H, s), 0.16 (3H, s), 0.875 (9H, s), 0.882 (6H, t, *J* 6.8 Hz), 0.94 (9H, s), 1.02 (9H, s), 1.05 (9H, s), 1.25 (64H, br s), 1.32 (3H, s), 1.44 (3H, s), 1.53–1.66 (2H, m), 2.06–2.20 (2H, m), 2.52 (1H, d, *J* 10.0 Hz, OH), 2.58 (1H, d, *J* 10.3 Hz, OH), 3.60–3.65 (2H, m), 3.69 (1H, s), 3.77 (1H, m), 3.81 (1H, dd, *J* 6.3, 9.4 Hz), 4.03 (1H, dd, *J* 5.1, 9.3 Hz), 4.07 (1H, m), 4.14–4.16 (2H, m), 4.20–4.25 (2H, m), 4.39 (1H, dd, *J* 3.4 Hz, galactose C_4-H), 4.87 (1H, d, *J* 3.7 Hz, anomeric H), 6.17 (1H, d, *J* 6.3 Hz, NH). ESI-MS: *m*/*z* 1321.02 $[M+Na]^+$. HR ESI-MS: calcd for $C_{73}H_{147}NO_{11}Si_3Na$: 1321.0174; observed: 1321.0177.

4.1.25. (2*S*,3*S*,4*S*,5*R*,6*S*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-2-hexacosanoylamino-3,4-*O*-isopropylidene-3,4-dihydroxyoctadecyl α-D-galactopyranoside (29)

To a solution of **28** (61 mg, 0.05 mmol) and pyridine (40 mg, 0.77 mmol) in dry THF (5 mL) was added HF·pyridine (HF: ~70%; pyridine: ~30%, 30 mg, ca. 1.05 mmol) under an atmosphere of argon at room temperature. After stirring for 1 h, the reaction mixture was diluted with $CHCl_3$, and washed with aq satd $NaHCO_3$ 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 (1:4) gave **29** (46 mg, 85%) as a gum. IR ν_{max}(KBr): 3411 (br), 2925, 2854, 1655 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.07 (3H, s), 0.10 (3H, s), 0.15 (6H, s), 0.88 (9H, s, and 3H, t, *J* 6.6 Hz), 0.94 (9H, s), 1.25 (62H, br s), 1.32 (3H, s), 1.34–1.38 (2H, m), 1.44 (3H, s), 1.56–1.65 (4H, m), 2.15 (1H, t, *J* 7.0 Hz), 2.41 (1H, br s, OH), 2.65 (1H, br s, OH), 2.75 (1H, br s, OH), 2.87 (1H, s, OH), 3.47 (1H, m), 3.63 (1H, m), 3.71–3.79 (3H, m), 3.93 (1H, m), 4.04 (1H, m), 4.08 (1H, s), 4.12 (2H, s), 4.27 (1H, d, *J* 10.6 Hz), 4.88 (1H, d, *J* 3.4 Hz, anomeric H), 6.20 (1H, d, *J* 6.2 Hz, NH). ESI-MS: *m*/*z* 1180.91 $[M+Na]^+$. HR ESI-MS: calcd for $C_{65}H_{131}NO_{11}SiNa$: 1180.9153; observed: 1180.9149.

4.1.26. (2*S*,3*S*,4*S*,5*S*,6*S*)-2-Hexacosanoylamino-3,4,5,6-tetrahydroxyoctadecyl α-D-galactopyranoside (30) (RCAI-120)

To a solution of **29** (45 mg, 0.04 mmol) in CH_2Cl_2–CH_3CN (1:1, 20 mL) and H_2O (384 mg) was added aq 46% HF (294 mg, 7.71 mmol). The solution was stirred for 16 h at room temperature

to yield insoluble powder, and the whole was filtered. The filter cake was washed with aq satd $NaHCO_3$ and water, and a small amount of $CHCl_3$–CH_3CN (1:1), consecutively. The residual powder was dried under reduced pressure, and chromatographed on a silica gel (1 g) column. [It was loaded on the column with hot $CHCl_3$ or hot $CHCl_3$–MeOH (19:1).] Elution with $CHCl_3$–MeOH (9:1, then 7:1) gave **30** (32 mg, 92%) as a powder. $[\alpha]_D^{26}$ +35.1 (*c* 0.5, pyridine). IR ν_{max}(KBr): 3293, 2919, 2850, 1649 cm^{-1}. ^1H NMR [500 MHz, $CDCl_3$–CD_3OD (10:1)]: δ 0.88 (6H, t, *J* 6.8 Hz), 1.26 (62H, br s), 1.31–1.63 (4H, m), 1.67–1.74 (2H, m), 2.20 (1H, t, *J* 7.6 Hz), 3.58–3.64 (3H, m), 3.71–3.76 (2H, m), 3.77–3.83 (4H, m), 3.90–3.96 (3H, m), 4.26 (1H, m), 4.92 (1H, d, *J* 3.7 Hz, anomeric H). ^{13}C NMR (126 MHz, pyridine-d_5): δ 14.26, 22.92, 26.30, 29.59, 29.74, 29.78, 29.83, 29.90, 29.96, 29.99, 30.03, 30.12, 30.32, 32.10, 35.13, 36.76, 52.02, 62.64, 68.42, 70.35, 70.96, 71.44, 71.59, 72.91, 73.57, 73.77, 101.40, 173.50. ESI-MS: *m*/*z* 912.71 $[M+Na]^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{11}Na$: 912.7110; observed: 912.7116.

4.1.27. (2*R*)-Benzyl 5-oxo-2-tetrahydrofurancarboxylate (32)

To a solution of (*R*)-5-oxo-2-tetrahydrofurancarboxylic acid (**31**, 97% ee) (1.35 g, 10.4 mmol) and BnOH (2.25 g, 20.8 mmol) in CH_2Cl_2 (30 mL) was added EDAC (6.00 g, 31.2 mmol). After stirring for 1.5 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (100 mL), 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 (2:1, then 1:2) gave **32** (1.60 g, 70%) as an oil. IR ν_{max}(KBr): 1771, 1746, 1467 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 2.30 (1H, m), 2.47–2.65 (2H, m), 4.98 (1H, dd, *J* 4.3, 8.2 Hz), 5.22, 5.24 (2H, AB-q, *J* 12.1 Hz), 7.34–7.41 (5H, m). ESI-MS: *m*/*z* 220.07 $[M^{\cdot}]^+$. HR ESI-MS: calcd for $C_{12}H_{12}O_4$: 220.0736; observed: 220.0726.

4.1.28. (2*R*)-Benzyl 5-(hydroxyl)tetrahydrofuran-2-carboxylate (33)

A solution of disiamylborane was prepared by mixing BH_3 (1 M solution in THF, 14 mL) and 2-methyl-2-butene (2 M solution in THF, 14 mL) at 0 °C under Ar, and by stirring for 75 min at 0 °C. To this disiamylborane solution was introduced a solution of **32** (1.60 g, 7.26 mmol) in THF (2 mL). The combined solution was warmed to room temperature over a period of 2.5 h, and then stirred for another 15 h, and quenched with aq satd NH_4Cl, followed by dilution of EtOAc. The mixture was stirred for 10 min, and the organic phase was separated, washed with aq satd NH_4Cl 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 (3:2) gave **33** (0.83 g, 51%) as an oily 2:3 mixture of anomers. IR ν_{max}(KBr): 3650, 1740 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.97–2.05 (2H, m), 2.17–2.47 (2H, m), 2.92 (0.4H, s, OH), 3.55 (0.6H, s, OH), 4.63 (0.6H, dd, *J* 6.6, 8.3 Hz), 4.76 (0.4H, dd, *J* 4.1, 8.8 Hz), 5.17–5.23 (2H, m), 5.60 (0.6H, d, *J* 3.2 Hz), 5.74 (0.4H, d, *J* 3.9 Hz), 7.36–7.37 (5H, m). ESI-MS: *m*/*z* 204 $[M-H_2O]^+$, 222 $[M]^+$. HR ESI-MS: calcd for $C_{12}H_{14}O_4$: 222.0893; observed: 222.0901.

4.1.29. (2*R*,5*EZ*)-Benzyl 2-hydroxyhexacos-5-enoate (34)

To a suspension of $Ph_3P(Br)C_{21}H_{43}$ (1.59 g, 2.50 mmol) in dry THF (5 mL) was added $LiN(TMS)_2$ (1 M in THF, 4 mL) with stirring under an atmosphere of Ar. After stirring for 1 h at 0 °C, the mixture became a red-colored solution. To this solution was added a solution of **33** (222 mg, 1.00 mmol) in THF (3 mL). The solution was stirred for 1 h at 0 °C, and for 2 h at room temperature. The reaction mixture was quenched with aq satd NH_4Cl, and extracted with EtOAc–hexane (1:1), which was washed with 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 (16:1, then 9:1) gave **34** (307 mg, 61%) as an oily mixture of *E*- and *Z*-isomers. IR ν_{max}(KBr): 3519, 2917, 2850, 1735, 1468 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.21–1.31 (36H, m), 1.70 (1H, m), 1.85 (1H, m), 1.95 (2H, q like, *J* 6.6–7.4 Hz), 2.04–2.17 (2H, m), 2.69 (1H, d, *J* 5.9 Hz, OH), 4.23 (1H, m), 5.21 (2H, s), 5.33–5.46 (2H, m), 7.35–7.40 (5H, m). ESI-MS: *m*/*z* 523.41 $[M+Na]^+$. HR ESI-MS: calcd for $C_{33}H_{56}O_3$: 523.4127; observed: 523.4123.

4.1.30. (2*R*,5*EZ*)-Benzyl 2-(*tert*-butyldimethylsilyloxy)hexacos-5-enoate (35)

To a solution of **34** (305 mg, 0.607 mmol) in dry CH_2Cl_2 (10 mL) containing 2,6-lutidine (100 mg, 0.93 mmol) was added TBDMSOTf (190 mg, 0.72 mmol). After stirring for 1.5 h at room temperature, the reaction mixture was diluted with CH_2Cl_2. To this solution was added an aq 3% solution of H_2O_2 (5 mL), the mixture was stirred for 5 min at room temperature, and washed with H_2O 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 (39:1) gave **35** (297 mg, 79%) as an oily mixture of *E*- and *Z*-isomers. The R_f values of these isomers were different, but their ^1H NMR spectra were almost identical. IR ν_{max}(KBr): 2925, 2853, 1757, 1458 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.03 (3H, s), 0.04 (3H, s), 0.86–0.89 (12H, m), 1.25 (36H, m), 1.73–1.79 (2H, m), 1.93–2.00 (2H, m), 2.03–2.18 (2H, m), 4.24 (1H, dd, *J* 5.3, 6.8 Hz), 5.13, 5.17 (2H, AB-q, *J* 12.3 Hz), 5.29–5.42 (2H, m), 7.32–7.36 (5H, m). ESI-MS: *m*/*z* 637.50 $[M+Na]^+$. HR ESI-MS: calcd for $C_{39}H_{70}O_3SiNa$: 637.4992; observed: 637.4989.

4.1.31. (2*R*)-2-(*tert*-Butyldimethylsilyloxy)hexacosanoic acid (36)

The suspension of **35** (66 mg, 0.107 mmol) in THF (6.6 mL) containing 20% $Pd(OH)_2$ on carbon (66 mg) was stirred under an atmosphere of H_2 for 5–16 h. The resulting solution was filtered, and the catalyst was washed with THF. The combined THF solution was concentrated in vacuo to give **36** (57 mg, quantitatively) as a crude crystalline solid, which was employed for the next reaction without further purification. (This product was unstable for silica gel chromatography.) IR ν_{max}(KBr): 3300–3000 (br), 2919, 2850, 1727 (br) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.13 (3H, s), 0.14 (3H, s), 0.88 (3H, t, *J* 6.9 Hz), 0.94 (9H, s), 1.25 (44H, br s), 1.71–1.84 (2H, m), 4.30 (1H, t, *J* 5.0 Hz). FABMS (negative-ion): *m*/*z* 507.4, 525.5 $[M-H]^-$. HRFABMS: calcd for $C_{32}H_{65}O_3Si$: 525.4703; observed: 525.4679.

4.1.32. Authentic sample of *N*-[(*R*)-1-(1-naphthyl)ethyl]-(*R*)-2-(*tert*-butyldimethylsilyloxy)hexacosanamide (38) or *N*-[(*S*)-1-(1-naphthyl)ethyl]-(*R*)-2-(*tert*-butyldimethylsilyloxy)hexacosanamide (40)

To a solution of **36** (4.9 mg, 0.01 mmol) and (*R*)-1-(1-naphthyl)ethylamine (**37**, 3.2 mg, 0.02 mmol) or (*S*)-1-(1-naphthyl)ethylamine (**39**, 3.2 mg, 0.02 mmol) in CH_2Cl_2 (0.4 mL) was added EDAC (7.0 mg, 0.04 mmol). The mixture was stirred for 2 h at room temperature, and the whole was loaded on a preparative TLC plate. Development with hexane–EtOAc (6:1) gave **38** [(*R*,*R*)-isomer, 4 mg] or **40** [(*S*,*R*)-isomer, 4 mg]. Physical data of **38**: R_f = 0.446 (hexane:EtOAc = 6:1); ^1H NMR (500 MHz, $CDCl_3$): δ −0.37 (3H, s), −0.06 (3H, s), 0.68 (9H, s), 0.88 (3H, t, *J* 7.0 Hz), 1.25 (44H, br s), 1.68 (3H, d, *J* 6.8 Hz), 1.70–1.76 (2H, m), 4.11 (1H, dd, *J* 4.4, 5.7 Hz), 5.93 (1H, m), 6.84 (1H, d, *J* 8,8 Hz, NH), 7.44–7.53 (4H, m), 7.79 (1H, d, *J* 7.6 Hz), 7.85 (1H, d, *J* 7.8 Hz), 8.12 (1H, d, *J* 8.3 Hz). ESI-MS: *m*/*z* 702.56 $[M+Na]^+$. HR ESI-MS: calcd for $C_{44}H_{77}NO_2SiNa$, 702.5621; observed, 702.5635. Physical data of **40**: R_f= 0.429 (hexane:EtOAc = 6:1); ^1H NMR (500 MHz, $CDCl_3$): δ

0.08 (3H, s), 0.11 (3H, s), 0.88 (3H, t, *J* 7.0 Hz), 0.90 (9H, s), 1.12–1.32 (44H, m), 1.62–1.67 (5H, m, containing 3H, d, *J* 6.9 Hz at 1.65 ppm), 4.18 (1H, t, *J* 5.3 Hz), 5.93 (1H, m), 6.89 (1H, d, *J* 8.8 Hz, NH), 7.44–7.54 (4H, m), 7.79 (1H, m), 7.86 (1H, m), 8.10 (1H, d, *J* 8.5 Hz). ESI-MS: *m*/*z* 702.56 $[M+Na]^+$. HR ESI-MS: calcd for $C_{44}H_{77}NO_2SiNa$, 702.5621; observed, 702.5625.

HPLC conditions: [Column: PEGASIL silica 60-5, 4.6ø × 250 mm; Eluent: hexane–EtOAc (9:1); Flow rate: 1 mL/min; Detection: 254 nm; Column temperature: 10 °C.]

HPLC retention time of authentic **38** [(*R*,*R*)-isomer] was 5.41 min, and that of authentic **40** [(*S*,*R*)-isomer] was 6.56 min.

Preparation of HPLC analytical sample: To a solution of **36** (4.9 mg, 0.01 mmol) and (*S*)-1-(1-naphthyl)ethylamine (**39**, 3.2 mg, 0.02 mmol) in CH_2Cl_2 (0.4 mL) was added EDAC (7.0 mg, 0.04 mmol). The mixture was stirred for 2 h at room temperature, and evaporated in vacuo to give a residue. To this residue were added EtOAc (0.3 mL) and H_2O (0.3 mL). This mixture was stirred for 15 min at room temperature, and allowed to stand to separate into two layers. The upper EtOAc layer was concentrated in vacuo to obtain a HPLC analytical sample of **40**.

The HPLC sample of **40** showed that the area integrated at 5.41 min was 0.861, and the area integrated at 6.56 min was 82.463. The diastereomeric excess of compound **40** is 97.9% (81.602/83.324 = 0.97933). The enantiomeric excess of starting **31** was 97%, and that of **36** was 97.9%. Therefore, it is obvious that no racemization occurred during the conversion from **31** to **36**.

4.1.33. (2*S*,3*S*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-[(*R*)-2′-(*tert*-butyldimethylsilyloxy)hexacosanoylamino]octadecyl 2,3-di-*O*-benzyl-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (41)

To a solution of **11** (30 mg, 0.03 mmol) in THF–CH_2Cl_2 (1:1, 2 mL) were added DMAP (27 mg, 0.22 mmol), **36** (37 mg, 0.07 mmol), and EDAC (32 mg, 0.17 mmol). The mixture was stirred for 3 h at room temperature, and concentrated in vacuo to give a residue, which was diluted with CH_2Cl_2. The solution was washed with H_2O, 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 **41** (24 mg, 54%) as a gum. IR ν_{max}(KBr) 2925, 2854, 1675 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.01 (3H, s), 0.03 (3H, s), 0.87 (9H, s), 0.88 (6H, t, *J* 7.0 Hz) 0.96 (9H, s), 1.02 (9H, s), 1.19–1.32 (66H, m), 1.52–1.66 (4H, m), 3.52 (1H, s), 3.65 (1H, m), 3.70–3.78 (4H, m), 3.92 (1H, dd, *J* 3.5, 9.9 Hz), 3.96, 4.00 (2H, AB-q, *J* 12.4 Hz), 4.08 (1H, t, *J* 5.6 Hz), 4.16 (1H, dd, *J* 4.3, 11.4 Hz), 4.37 (1H, d, *J* 2.5 Hz), 4.45 (1H, d, *J* 11.3 Hz), 4.50 (1H, d, *J* 11.5 Hz), 4.57 (1H, d, *J* 11.5 Hz), 4.59–4.63 (4H, m, containing N-CH as a multiplet), 4.67 (1H, d, *J* 11.5 Hz), 4.68 (1H, d, *J* 11.5 Hz), 4.74–4.78 (3H, m, containing 1H, d, *J* 4.0 Hz at 4.76 ppm as an anomeric H), 6.95 (1H, d, *J* 9.0 Hz, NH), 7.19–7.40 (25H, m). ESI-MS: *m*/*z* 1617.14 $[M+Na]^+$. HR ESI-MS: calcd for $C_{99}H_{159}NO_{11}Si_2Na$: 1617.1349; observed: 1617.1344.

4.1.34. (2*S*,3*S*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-[(*R*)-2′-hydroxyhexacosanoylamino]octadecyl 2,3-di-*O*-benzyl-α-D-galactopyranoside (42)

(1) To a solution of **41** (40 mg, 0.03 mmol) in CH_2Cl_2–CH_3CN (1:1, 8 mL) was added aq 46% HF (0.6 mL, 0.03 mmol). The solution was stirred for 3 h at room temperature, and diluted with $CHCl_3$, which was washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture (34 mg).

(2) The above obtained mixture was dissolved in THF (3 mL) containing pyridine (40 mg). To this solution was added HF·pyridine (HF: ~70%; pyridine: ~30%, 33 mg, ca. 1.16 mmol) under an atmosphere of argon at room temperature. After stirring for 1.5 h, the reaction mixture was diluted with $CHCl_3$, and washed with aq satd $NaHCO_3$, 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 **42** (23 mg, two steps 68% yield) as a gum. IR ν_{max}(KBr): 3800–3400 (br), 2924, 2853, 1653 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 6.8 Hz), 1.22–1.36 (66H, m), 1.52–1.86 (4H, m), 2.49 (1H, s, OH), 2.54 (1H, br s, OH), 2.98 (1H, d, *J* 4.8 Hz, OH), 3.59–3.65 (2H, m), 3.67–3.72 (2H, m), 3.73–3.81 (4H, m), 3.82–3.87 (2H, m), 3.92 (1H, br s), 4.05 (1H, dd, *J* 3.6, 11.1 Hz), 4.48 (1H, m), 4.49–4.76 (12H, m), 6.86 (1H, d, *J* 9.5 Hz, NH), 7.24–7.37 (25H, m). ESI-MS: *m*/*z* 1362.94 $[M+Na]^+$. HR ESI-MS: calcd for $C_{85}H_{129}NO_{11}Na$: 1362.9463; observed: 1362.9497.

4.1.35. (2*S*,3*S*,4*R*,5*S*)-3,4,5-Tri(hydroxy)-2-[(*R*)-2′-hydroxyhexacosanoylamino]octadecyl α-D-galactopyranoside (43) (RCAI-129)

Compound **42** (23 mg, 0.02 mmol) was treated as described for the formation of **14** from **13** to give **43** (8 mg, 52%) as a powder. Column chromatography was performed by elution with $CHCl_3$–CH_3OH (9:1, then 7:1). $[\alpha]_D^{19}$ +63.2 (c 0.65, $CHCl_3$–CH_3OH (7:1)). IR ν_{max}(KBr) 3810–3291, 2919, 2850, 1646 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$–CD_3OD (9:1)): δ 0.88 (6H, t, *J* 6.9 Hz), 1.10–1.62 (69H, m), 1.77 (1H, m), 3.70–3.90 (10H, m), 3.92 (1H, d, *J* 2.9 Hz), 4.01(1H, dd, *J* 3.3, 8.7 Hz), 4.23 (1H, dd, *J* 4.3, 10.1 Hz), 4.88 (1H, d, *J* 3.9 Hz, anomeric H), 7.56 (1H, d, *J* 9.1 Hz, NH, gradually disappeared). ^{13}C NMR [126 MHz, $CDCl_3$–CD_3OD (9:1)]: δ 14.03, 22.63, 25.44, 25.89, 29.32, 29.43, 29.67, 31.87, 33.62, 34.40, 50.18, 61.87, 67.34, 68.87, 69.53, 70.04, 70.48, 70.86, 71.85, 71.96, 72.32, 99.19, 175.65. ESI-MS: *m*/*z* 912.73 $[M+Na]^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{11}Na$: 912.7166; observed: 912.7107.

4.1.36. (2*S*,3*S*,4*S*,5*R*,6*S*)-2-[(*R*)-2′-(*tert*-Butyldimethylsilyloxy)hexacosanoylamino]-5,6-bis(*tert*-butyldimethylsilyloxy)-3,4-dihydroxy-3,4-*O*-isopropylideneoctadecyl 2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (44)

To a solution of **26** (103 mg, 0.09 mmol) and **36** (172 mg, 0.36 mmol) in THF–CH_2Cl_2 (1:1, 8 mL) were added DMAP (96 mg, 0.79 mmol) and EDAC (115 mg, 0.60 mmol) at room temperature under Ar. After stirring for 16 h, the reaction mixture was diluted with CH_2Cl_2, washed with H_2O, 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 **44** (150 mg, 100%) as a gum. IR ν_{max}(KBr) 2925, 2854, 1685, 1678 (shoulder), 1612, 1516, 1466, 1250 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.04 (6H, s), 0.07 (3H, s), 0.09 (3H, s), 0.10 (3H, s), 0.13 (3H, s), 0.88 (6H, t, *J* 6.8 Hz), 0.88 (9H, s), 0.89 (9H, s), 0.91 (9H, s), 0.97 (9H, s), 1.05 (9H, s), 1.21–1.34 (67H, m), 1.44 (3H, s), 1.58–1.70 (4H, m), 3.64–3.72 (3H, m), 3.77–3.84 (8H, m, containing two 3H, singlets at 3.79 and 3.80 ppm), 3.92 (1H, m), 4.20 (1H, m), 4.09–4.21 (4H, m), 4.26 (1H, m), 4.51 (1H, s), 4.52, 4.72 (2H, AB-q, *J* 11.3 Hz), 4.59 4.63 (2H, AB-q, *J* 11.1 Hz), 4.83 (1H, d, *J* 3.7 Hz, anomeric H), 6.78–6.86 (5H, m, containing NH), 7.23–7.31 (4H, m). ESI-MS: *m*/*z* 1691.14 $[M+Na]^+$. HR ESI-MS: calcd for $C_{95}H_{177}NO_{14}Si_4Na$: 1691.2144; observed: 1691.2113.

4.1.37. (2*S*,3*S*,4*S*,5*R*,6*S*)-2-[(*R*)-2′-(*tert*-Butyldimethylsilyloxy)hexacosanoylamino]-5,6-bis(*tert*-butyldimethylsilyloxy)-3,4-dihydroxy-3,4-*O*-isopropylideneoctadecyl 4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (45)

Compound **44** (150 mg, 0.09 mmol) was treated as described for the formation of **28** from **27** to give **45** (77 mg, 60%) as a gum. IR ν_{max}(KBr) 3406, 2925, 2855, 1675, 1520, 1509, 1471 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.05 (3H, s), 0.06 (3H, s), 0.10 (6H, s),

0.101 (3H, s), 0.12 (3H, s), 0.87–0.92 (24H, m), 0.95 (9H, s), 1.02 (9H, s), 1.06 (9H, s), 1.05 (9H, s), 1.23–1.32 (63H, m, containing 3H, singlet at 3.31 ppm), 1.42 (3H, s), 1.50–1.70 (4H, m), 2.50 (1H, d, *J* 8.8 Hz, OH), 2.83 (1H, d, *J* 11.3 Hz, OH), 3.27 (1H, t, *J* 9.7 Hz), 3.59–3.74 (4H, m), 3.90 (1H, dd, *J* 5.6, 8.1 Hz), 4.04–4.26 (7H, m), 4.41 (1H, m), 4.77 (1H, d, *J* 3.5 Hz, anomeric H), 7.20 (1H, d, *J* 9.5 Hz, NH). ESI-MS: *m*/*z* 1451.02 $[M+Na]^+$. HR ESI-MS: calcd for $C_{79}H_{161}NO_{12}Si_4Na$: 1451.0994; observed: 1451.1016.

4.1.38. (2*S*,3*S*,4*S*,5*R*,6*S*)-2-[(*R*)-2′-(*tert*-Butyldimethylsilyloxy)hexacosanoylamino]-5,6-bis(*tert*-butyldimethylsilyloxy)-3,4-dihydroxy-3,4-*O*-isopropylideneoctadecyl α-D-galactopyranoside (46)

Compound **45** (75 mg, 0.05 mmol) was treated as described for the formation of **29** from **28** to give **46** (50 mg, 68%) as a gum. Column chromatography was performed by elution with hexane–EtOAc (1:1). IR ν_{max}(KBr) 3409 (br), 2925, 2854, 1670, 1523, 1515, 1465 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.08 (3H, s), 0.09 (6H, s), 0.10 (3H, s), 0.11 (3H, s), 0.12 (3H, s), 0.87–0.89 (24H, m), 0.95 (9H, s), 1.25 (64H, br s), 1.32 (3H, s), 1.41 (3H, s), 1.44–1.75 (4H, m), 2.58 (1H, m, OH), 2.65 (1H, s, OH), 2.80 (1H, s, OH), 3.09 (1H, d, *J* 12.0 Hz, OH), 3.25 (1H, t, *J* 10.3 Hz), 3.60 (1H, m), 3.70 (1H, m), 3.76–3.80 (2H, m), 3.89 (1H, m), 3.93–3.98 (2H, m), 4.04–4.13 (4H, m), 4.25–4.31 (2H, m), 4.83 (1H, d, *J* 3.6 Hz, anomeric H), 7.30 (1H, d, *J* 9.5 Hz, NH). ESI-MS: *m*/*z* 1310.94 $[M+Na]^+$. HR ESI-MS: calcd for $C_{71}H_{145}NO_{12}Si_3Na$: 1310.9972; observed: 1310.9994.

4.1.39. (2*S*,3*S*,4*S*,5*S*,6*S*)-2-[(*R*)-2′-(Hydroxy)hexacosanoylamino]-3,4,5,6-(tetrahydroxy)octadecyl α-D-galactopyranoside (47) (RCAI-130)

To a solution of **46** (20 mg, 0.02 mmol) in CH_2Cl_2–CH_3CN (3:2, 10 mL) and H_2O (152 mg) was added aq 46% HF (140 mg, 3.67 mmol). The solution was stirred for 16 h at room temperature to yield insoluble powder, and the whole was filtered. The filter cake was washed with aq satd $NaHCO_3$ and water, and a small amount of $CHCl_3$–CH_3CN (1:1), consecutively. The residual powder was dried under reduced pressure, and chromatographed on a silica gel (1 g) column. [It was loaded on the column with hot $CHCl_3$ or hot $CHCl_3$–MeOH (19:1).] Elution with $CHCl_3$–MeOH (9:1, then 5:1) gave **47** (9.2 mg, 65%) as a powder. $[\alpha]_D^{27}$ +50.6 [(*c* 0.7, $CHCl_3$–CH_3OH (5:1)). IR ν_{max}(KBr): 3396 (br), 2919, 2850, 1652, 1516, 1469 cm^{-1}. ^1H NMR [500 MHz, $CDCl_3$–CD_3OD (10:1)]: δ 0.88 (6H, t, *J* 7.0 Hz), 1.12–1.84 (68H, m), 3.64–3.70 (2H, m), 3.72–3.89 (8H, m), 3.90–3.98 (2H, m), 4.01 (1H, dd, *J* 3.6, 8.5 Hz), 4.25 (1H, m), 4.91 (1H, d, *J* 3.8 Hz, anomeric H). ^{13}C NMR (126 MHz, $CDCl_3$–CD_3OD (6:1)): δ 13.91, 22.55, 25.38, 25.73, 29.23, 29.24, 29.33, 29.45, 29.52, 29.54, 29.57, 29.58, 29.61, 29.68, 31.80, 33.66, 34.29, 49.60, 61.74, 66.97, 68.70, 69.25, 69.49, 69.91, 70.81, 71.86, 71.96, 72.00, 72.54, 99.27, 175.62. ESI-MS: *m*/*z* 928.58 $[M+Na]^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{12}Na$: 928.7056; observed: 928.7080.

4.1.40. 2-Deoxy-3,4-*O*-isopropylidene-L-ribopyranose (49)

To a solution of **48** (2-deoxy-L-ribose, 5.29 g, 39.44 mmol) in DMF (40 mL) containing 2,2-dimethoxypropane (6.16 g, 59.2 mmol) was added Amberlyst 15 ion-exchange resin (395 mg). The mixture was stirred for 18 h at room temperature, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2, then 2:3) gave **49** (3.41 g, 50%) as an oily 4:1 mixture of anomers. IR ν_{max}(KBr) 3534 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.35 (2.4H, s), 1.37 (0.6H, s), 1.51 (2.4H, s), 1.57 (0.6H, s), 1.79 (1H, m), 2.22–2.27 (1H, m), 2.69 (0.8H, d, *J* 3.9 Hz, OH), 3.70 (1H, dd, *J* 3.4, 12.2 Hz), 3.84 (0.2H, d, *J* 9.1 Hz, OH), 3.95 (1H, dd, *J* 3.4, 12.7 Hz), 4.16–4.20 (1H, m), 4.43 (0.2H, m), 4.49 (0.8H, m), 5.08 (0.2H, m, changed to triplet, *J* 3.6 Hz, on addition of D_2O), 5.26 (0.8H, m, changed to dd, *J* 4.3, 7.0 Hz, on addition of D_2O). EIMS: *m*/*z* 159.0 $[M-Me]^{\cdot+}$. HREIMS: calcd for $C_7H_{11}O_4$: 159.0657; observed: 159.0663.

4.1.41. (2*S*,3*R*,5*EZ*)-2,3-*O*-Isopropylidene-5-hexacosene-1,2,3-triol (50)

To a suspension of $Ph_3P(Br)C_{21}H_{43}$ (5.62 g, 8.81 mmol) in THF (19 mL) was added $LiN(TMS)_2$ (1 M in THF, 20.4 mmol), and the mixture was stirred for 1 h at 0 °C under Ar. To this resulting solution was added a solution of **49** (1.03 g, 5.91 mmol) in THF (11 mL) at 0 °C, and the mixture was stirred for 2 h at room temperature, quenched with aq satd NH_4Cl, diluted with $CHCl_3$, which was washed well with water, and aq 1% H_2O_2, and again with water, 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, finally 4:1) gave **50** (1.35 g, 50%) as an oily mixture of *E*- and *Z*-isomers. IR ν_{max}(KBr) 3536, 2918, 2850, 1468 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.25 (36H, m), 1.38 (3H, s), 1.49 (3H, s), 1.84 (1H, dd, *J* 5.4, 6.6 Hz), 2.04 (1H, m), 2.28 (1H, m), 2.38 (1H, m), 3.62–3.68 (2H, m), 4.15–4.23 (2H, m), 5.38 (1H, m), 5.52 (1H, m). ESI-MS: *m*/*z* 475.41 $[M+Na]^+$. HR ESI-MS: calcd for $C_{29}H_{56}O_3Na$: 475.4127; observed: 475.4127.

4.1.42. (2*S*,3*R*)-2,3-*O*-(Isopropylidene)hexacosane-1,2,3-triol (51)

The suspension of **50** (1.74 g, 3.80 mmol) in hexane (50 mL) containing 10% Pd/C (580 mg) was stirred under an atmosphere of H_2 for 2 h. The resulting solution was filtered, and the catalyst was washed with THF. The combined THF solution was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (19:1, then 4:1) gave **51** (1.71 g, 98%) as an oil. IR ν_{max}(KBr) 3458, 2924, 2848, 1468 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.23–1.61 (50H, m, containing two 3H singlets at 1.37 and 1.48 ppm), 1.82 (1H, dd, *J* 4.9, 7.3 Hz, OH), 3.59–3.63 (2H, m), 4.13–4.17 (2H, m). ESI-MS: *m*/*z* 477.4 $[M+Na]^+$. HR ESI-MS: calcd for $C_{29}H_{58}O_3Na$: 477.4284; observed: 477.4298.

4.1.43. (2*R*,3*R*)-2,3-Dihydroxy-2,3-*O*-(isopropylidene)hexacosanoic acid (52)

A solution of **51** (50 mg, 0.11 mmol), $NaIO_4$ (236 mg, 1.10 mmol), and $RuCl_3{\cdot}nH_2O$ (3 mg) in CCl_4–CH_3CN–H_2O (2:2:3, 7 mL) was stirred for 3 h at room temperature, and diluted with $CHCl_3$, washed with water, 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 1:2) gave **52** (41 mg, 80%) as a solid. IR ν_{max}(KBr) 3290–2560, 1728, 1469 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* 7.0 Hz), 1.23–1.71 (50H, m, containing two 3H singlets at 1.40 and 1.61 ppm), 4.38 (1H, m), 4.55 (1H, d, *J* 7.3 Hz). ESI-MS: *m*/*z* 467.4 $[M-H]^-$. HR ESI-MS: calcd for $C_{29}H_{55}O_4$: 467.4100; observed: 467.4107.

4.1.44. (2*S*,3*S*,4*R*,5*S*)-2-[(2′*R*,3′*R*)-2′,3′-Dihydroxy-2′,3′-*O*-(isopropylidene)hexacosanoylamino]-3,4,5-tris(benzyloxy)octadecyl 2,3-di-*O*-benzyl-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (53)

To a solution of **11** (150 mg, 0.14 mmol) and **52** (130 mg, 0.28 mmol) in dry THF–CH_2Cl_2 (1:1, 10 mL) were added DMAP (170 mg, 1.38 mmol) and EDAC (270 mg, 1.38 mmol) at room temperature under Ar. After stirring for 16 h, the reaction mixture was diluted with CH_2Cl_2, washed with H_2O, 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 6:1) gave **53** (145 mg, 68%) as a gum. IR ν_{max}(KBr) 2924, 2853, 1683 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 6.8 Hz), 0.97 (9H, s), 1.03 (9H, s), 1.18–1.30 (64H, m), 1.31 (3H, s), 1.36–1.38 (2H, m), 1.40 (3H, s), 1.56–1.66 (2H, m), 3.53 (1H, s), 3.65 (1H, m), 3.72 (1H, m), 3.74–3.78 (2H, m), 3.84 (1H, m), 3.92–4.09 (4H, m), 4.32 (1H, m), 4.37–4.41 (2H, m), 4.48–4.78 (11H, m), 7.00 (1H, d, *J* 8.5 Hz, NH), 7.21–7.41 (25H, m). ESI-MS: *m*/*z* 1537.08 [M+H]$^+$, 1559.07 [M+Na]$^+$. HR ESI-MS: calcd for $C_{96}H_{149}NO_{12}SiNa$: 1559.0747; observed: 1559.0747.

4.1.45. (2*S*,3*S*,4*R*,5*S*)-2-[(2′*R*,3′*R*)-2′,3′-(Dihydroxyl)hexacosanoylamino]-3,4,5-tris(benzyloxy)octadecyl 2,3-di-*O*-benzyl-α-D-galactopyranoside (54)

Compound **53** (129 mg, 0.08 mmol) was treated as described for the formation of **42** from **41** to give **54** (78 mg, two steps 69%) as a gum. Column chromatography was performed by elution with hexane–EtOAc (2:3, then 1:4). IR ν_{max}(KBr) 3645, 2924, 2853, 1655 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* 6.9 Hz), 1.21–1.31 (64H, m), 1.54–1.70 (4H, m), 2.23 (1H, m, OH), 2.49 (1H, s, OH), 3.38–3.48 (3H, m, containing one OH), 3.51–3.68 (6H, m), 3.70–3.73 (2H, m), 3.74–3.79 (2H, m, containing one OH), 3.82 (1H, t, *J* 3.8 Hz), 3.90 (1H, m, changed to doublet, *J* 3.2 Hz, on addition of D_2O), 4.16 (1H, dd, *J* 3.7, 10.2 Hz), 4.48–4.78 (11H, m), 6.84 (1H, d, *J* 9.0 Hz, NH), 7.24–7.37 (25H, m). ESI-MS: *m*/*z* 1378.86 [M+Na]$^+$. HR ESI-MS: calcd for $C_{85}H_{129}NO_{12}Na$: 1378.9412; observed: 1378.9444.

4.1.46. (2*S*,3*S*,4*R*,5*S*)-2-[(2′*R*,3′*R*)-2′,3′-Dihydroxyhexacosanoylamino]-3,4,5-trihydroxyoctadecyl α-D-galactopyranoside (55) (RCAI-142)

To a solution of **54** (29 mg, 0.02 mmol) in THF (9 mL) was added $Pd(OH)_2$/C (20 wt %, Degussa type, wet, ~50%, 55 mg). After stirring for 16 h under hydrogen at room temperature, the catalyst was removed by filtration, and washed with THF, and then $CHCl_3$–MeOH (5:1). The combined filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel (1 g) column. Elution with $CHCl_3$–CH_3OH (9:1, then 6:1) gave **55** (10 mg, 52%) as a powder. $[\alpha]_D^{22}$ +51.2 (*c* 0.71, pyridine). IR ν_{max}(KBr) 3383 (br), 2918, 2850, 1645 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5, one drop of 1% TMS in $CDCl_3$): δ 0.88 (6H, t, *J* 6.9 Hz), 1.21–1.35 (60H, m), 1.57–2.10 (8H, m), 4.32–4.81 (14H, m), 5.38 (1H, m), 5.62 (1H, br s), 5.65 (1H, d, *J* 4.0 Hz, anomeric H), 6.18 (1H, br s), 6.33 (1H, br s), 6.35 (1H, br s), 6.57 (1H, br s), 6.68 (1H, br s), 6.94 (1H, d, *J* 6.1 Hz), 7.77 (1H, br s), 8.68 (1H, d, *J* 9.0 Hz, NH). ^{13}C NMR (126 MHz, pyridine-d_5, one drop of 1% TMS in $CDCl_3$): δ 14.38, 23.04, 26.57, 27.01, 29.71, 29.72, 30.01, 30.03, 30.07, 30.08, 30.10, 30.12, 30.14, 30.22, 30.29, 30.32, 30.37, 32.22, 32.23, 32.70, 35.13, 50.81, 62.73, 70.32, 71.08, 71.24, 71.74, 73.21, 73.28, 73.57, 74.00, 76.18, 101.11, 174.00. ESI-MS: *m*/*z* 928.63 [M+Na]$^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{12}Na$: 928.7065; observed: 928.7069.

4.1.47. (2*S*,3*S*,4*S*,5*R*,6*S*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-2-[(2′*R*,3′*R*)-2′,3′-*O*-(isopropylidene)hexacosanoylamino]-3,4-*O*-(isopropylidene)octadecyl 2,3-bis-*O*-(4-methoxybenzyl)-4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (56)

Compound **26** (103 mg, 0.09 mmol), **52** (125 mg, 0.27 mmol), DMAP (130 mg, 1.06 mmol), and EDAC (205 mg, 1.06 mmol) in THF–CH_2Cl_2 (1:1, 10 mL) were treated as described for the formation of **12** from **11** to give **56** (129 mg, 90%) as a gum. Column chromatography was performed by elution with hexane–EtOAc (19:1, then 6:1). IR ν_{max}(KBr) 2925, 2855, 1684 cm^{-1}. ^{1}H NMR^{1}H NMR (500 MHz, $CDCl_3$): δ 0.05 (3H, s), 0.06 (3H, s), 0.09 (3H, s), 0.13 (3H, s), 0.87–0.89 (24H, m, containing two 9H, singlets at 0.88 and 0.89 ppm), 0.98 (9H, s), 1.05 (9H, s), 1.22–1.36 (71H, m, containing two 3H singlets at 1.28 and 1.31 ppm), 1.42–1.62 (7H, m, containing 3H singlet at 1.44 ppm), 3.51 (1H, m), 3.61 (1H, s), 3.72–3.78 (2H, m), 3.78–3.82 (7H, m), 3.86 (1H, m), 3.93 (1H, dd, *J* 3.4, 10.0 Hz), 4.06–4.11 (2H, m), 4.12–4.16 (2H, m), 4.20 (1H, m), 4.30 (1H, m), 4.38 (1H, d, *J* 7.3 Hz), 4.44 (1H, d, *J* 2.9 Hz), 4.57, 4.70 (2H, AB-q, *J* 11.4 Hz), 4.60, 4.63 (2H, AB-q, *J* 11.7 Hz), 4.67 (1H, d, *J* 3.9 Hz, anomeric H), 6.79–6.87 (5H, m, containing NH), 7.26–7.34 (4H, m). ESI-MS: *m*/*z* 1633.15 [M+Na]$^+$. HR ESI-MS: calcd for $C_{92}H_{167}NO_{15}Si_3Na$: 1633.1541; observed: 1633.1559.

4.1.48. (2*S*,3*S*,4*S*,5*R*,6*S*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-2-[(2′*R*,3′*R*)-2′,3′-*O*-(isopropylidene)hexacosanoylamino]-3,4-*O*-(isopropylidene)octadecyl 4,6-*O*-(di-*tert*-butyl)silylene-α-D-galactopyranoside (57)

Compound **56** (124 mg, 0.08 mmol) was treated as described for the formation of **28** from **27** to give **57** (99 mg, 94%) as a gum. Column chromatography was performed by elution with hexane–EtOAc (6:1, then 3:1). IR ν_{max}(KBr) 3405 (br), 2926, 2855, 1680, 1518, 1470 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.05 (3H, s), 0.07 (3H, s), 0.12 (3H, s), 0.13 (3H, s), 0.88 (6H, m), 0.89 (9H, s), 0.90 (9H, s), 1.01 (9H, s), 1.06 (9H, s), 1.25 (64H, br s), 1.31 (3H, s), 1.37 (3H, s), 1.57 (3H, s), 2.52 (1H, d, *J* 9.5 Hz, OH), 3.39 (1H, t, *J* 9.8 Hz), 3.59 (1H, m), 3.62–3.73 (4H, m), 3.87 (1H, m), 4.06–4.18 (4H, m), 4.22–4.26 (2H, m), 4.34 (1H, m), 4.41 (1H, m), 4.46 (1H, d, *J* 7.8 Hz), 4.86 (1H, d, *J* 3.9 Hz, anomeric H), 6.94 (1H, d, *J* 8.8 Hz, NH). ESI-MS: *m*/*z* 1393.05 [M+Na]$^+$. HR ESI-MS: calcd for $C_{76}H_{151}NO_{13}Si_3Na$: 1393.0385; observed: 1393.0405.

4.1.49. (2*S*,3*S*,4*S*,5*R*,6*S*)-5,6-Bis(*tert*-butyldimethylsilyloxy)-2-[(2′*R*,3′*R*)-2′,3′-*O*-(isopropylidene)hexacosanoylamino]-3,4-*O*-(isopropylidene)octadecyl α-D-galactopyranoside (58)

Compound **57** (96 mg, 0.07 mmol) was treated as described for the formation of **29** from **28** to give **58** (67 mg, 78%) as a gum. Column chromatography was performed by elution with hexane–EtOAc (2:3, then 1:3). IR ν_{max}(KBr) 3418 (br), 2925, 2854, 1676, 1516, 1461, 1381 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.08 (3H, s), 0.10 (3H, s), 0.12 (3H, s), 0.15 (3H, s), 0.88 (6H, t, *J* 7.0 Hz), 0.89 (9H, s), 0.91 (9H, s), 1.25 (60H, br s), 1.31 (3H, s), 1.38 (3H, s), 1.42–1.73 (14H, m, containing two 3H singlets at 1.43 and 1.58 ppm), 2.46 (1H, dd, *J* 4.2, 8.4 Hz, OH), 2.63 (1H, d, *J* 2.5 Hz, OH), 2.68 (1H, d, *J* 11.5 Hz, OH), 2.83 (1H, s, OH), 3.38 (1H, dd, *J* 9.3, 10.8 Hz), 3.56 (1H, m), 3.69–3.79 (3H, m), 3.85–3.98 (3H, m), 4.03–4.12 (3H, m), 4.18 (1H, m), 4.25 (1H, m), 4.34 (1H, m), 4.45 (1H, d, *J* 7.6 Hz), 4.88 (1H, d, *J* 3.7 Hz, anomeric H), 7.03 (1H, d, *J* 9.3 Hz, NH). ESI-MS: *m*/*z* 1252.93 [M+Na]$^+$. HR ESI-MS: calcd for $C_{68}H_{135}NO_{13}Si_2Na$: 1252.9370; observed: 1252.9390.

4.1.50. (2*S*,3*S*,4*S*,5*S*,6*S*)-2-[(2′*R*,3′*R*)-(2′,3′-Dihydroxy)hexacosanoylamino]-3,4,5,6-tetrahydroxyoctadecyl α-D-galactopyranoside (59) (RCAI-147)

Compound **58** (56 mg, 0.07 mmol) was treated as described for the formation of **30** from **29** to give **59** (20 mg, 48%) as a powder. Column chromatography was performed by elution with $CHCl_3$–MeOH (9.1, then 7:1, finally 5:1). $[\alpha]_D^{28}$ +49.6 (*c* 0.52, pyridine). IR ν_{max}(KBr) 3361 (br), 2920, 2851, 1646, 1542, 1468 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5, one drop of 1% TMS in $CDCl_3$): δ 0.87 (6H, t, *J* 7.0 Hz), 1.22–1.33 (60H, m), 1.53–1.62 (2H, m), 1.80–2.02 (5H, m), 2.26 (1H, m), 4.31–4.40 (4H, m), 4.43–4.48 (3H, m), 4.51–4.55 (2H, m), 4.63 (1H, dd, *J* 3.7, 9.8 Hz), 4.70 (1H, d, *J* 5.1 Hz), 4.73 (1H, dd, *J* 6.2, 10.8 Hz), 4.83 (1H, dd, *J* 3.1, 8.5 Hz), 4.99 (1H, m), 5.40 (1H, m), 5.57 (1H, d, *J* 3.7 Hz, anomeric H), 8.71 (1H, d, *J* 9.5 Hz, NH). ^{13}C NMR (126 MHz, pyridine-d_5): δ 14.26, 22.91, 26.42, 26.45, 29.58, 29.89, 29.95, 29.98, 30.02, 30.13, 30.16, 30.18, 30.32, 32.09, 32.69, 35.16, 50.89, 62.58, 67.89, 70.24, 70.91, 71.31, 71,59, 72.72, 72.73, 72.97, 73.49,

73.80, 76.02, 101.08, 173.98. ESI-MS: *m*/*z* 944.70 [M+Na]$^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{13}Na$: 944.7014; observed: 944.7007.

4.1.51. (2*S*,3*S*,4*R*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-2-[(2′*R*,3′*R*)-2′,3′-dihydroxy-2′,3′-*O*-(isopropylidene)hexacosanoylamino]octadecyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (61)

Compound **60** (160 mg, 0.15 mmol) and **52** (210 mg, 0.45 mmol) were treated as described for the formation of **12** from **11** to give **61** (177 mg, 78%) as a gum. IR ν_{max}(KBr) 2925, 2853, 1686 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.047 (3H, s), 0.054 (3H, s), 0.055 (3H, s), 0.11 (3H, s), 0.87–0.90 (24H, m), 1.20 (3H, s), 1.25 (62H, br s), 1.31–1.40 (4H, m), 1.44 (3H, s), 1.46–1.60 (2H, m), 1.65–1.70 (2H, m), 3.47 (1H, dd, *J* 5.7, 9.1 Hz), 3.54 (1H, t, *J* 8.4 Hz), 3.69–3.72 (2H, m), 3.81–3.85 (2H, m), 3.91–3.95 (2H, m), 3.99 (1H, d, *J* 1.7 Hz), 4.04 (1H, d, *J* 3.5, 10.1 Hz), 4.18 (1H, m), 4.27 (1H, m), 4.37, 4.48 (2H, AB-q, *J* 11.7 Hz), 4.40 (1H, d, *J* 7.6 Hz), 4.55, 4.91 (2H, AB-q, *J* 11.3 Hz), 4.70, 4.74 (2H, AB-q, *J* 11.7 Hz), 4.70, 4.81 (2H, AB-q, *J* 11.7 Hz), 4.87 (1H, d, *J* 3.5 Hz, anomeric H), 6.80 (1H, d, *J* 9.5 Hz, NH), 7.24–7.36 (20H, m). ESI-MS: *m*/*z* 1541.10 [M+Na]$^+$. HR ESI-MS: calcd for $C_{93}H_{155}NO_{11}Si_2Na$: 1541.1036; observed: 1541.0992.

4.1.52. (2*S*,3*S*,4*R*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-2-[(2′*R*,3′*R*)-2′,3′-dihydroxy-2′,3′-*O*-(isopropylidene)hexacosanoylamino]octadecyl α-D-galactopyranoside (62)

A solution of **61** (160 mg, 0.11 mmol) in THF (40 mL) containing 20% $Pd(OH)_2$/C (300 mg) was stirred under an atmosphere of H_2 for 16 h at room temperature, and filtered. The filtrate was concentrated to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1, then 1:4) gave **62** (94 mg, 77%) as a gum. IR ν_{max}(KBr) 3415, 2925, 2854, 1667, 1523, 1466 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.07 (3H, s), 0.08 (3H, s), 0.12 (3H, s), 0.14 (3H, s), 0.88 (6H, t, *J* 7.0 Hz), 0.91 (9H, s), 0.92 (9H, s), 1.25 (62H, br s), 1.37 (3H, s), 1.45–1.67 (11H, m, containing 3H singlet at 1.53 ppm), 2.25 (1H, d, *J* 10.8 Hz, OH), 2.46 (1H, dd, *J* 4.8, 7.7 Hz, OH), 2.61 (1H, d, *J* 3.7 Hz, OH), 2.84 (1H, s, OH), 3.50 (1H, dd, *J* 8.3, 11.0 Hz), 3.67–3.87 (6H, m), 3.92 (1H, m), 4.09 (1H, s), 4.15 (1H, dd, *J* 3.2, 10.8 Hz), 4.34–4.40 (2H, m), 4.52 (1H, d, *J* 7.4 Hz), 4.90 (1H, d, *J* 3.6 Hz, anomeric H), 6.71 (1H, d, *J* 9.8 Hz, NH). ESI-MS: *m*/*z* 1180.91 [M+Na]$^+$. HR ESI-MS: calcd for $C_{65}H_{131}NO_{11}Si_2Na$: 1180.9158; observed: 1180.9149.

4.1.53. (2*S*,3*S*,4*R*)-3,4-Dihydroxy-2-[(2′*R*,3′*R*)-2′,3′-(dihydroxy)hexacosanoylamino]octadecyl α-D-galactopyranoside (63) (RCAI-151)

Compound **62** (80 mg, 0.07 mmol) was treated as described for the formation of **30** from **29** to give **63** (40 mg, 63%) as a powder. Column chromatography was performed by elution with $CHCl_3$–MeOH (9:1, then 6:1). $[\alpha]_D^{29}$ +62.4 (c 1.04, pyridine). IR ν_{max}(KBr) 3364, 2918, 2850, 1646, 1636, 1541, 1469 cm^{-1}. ^{1}H NMR (500 MHz, pyridine-d_5, one drop of 1% TMS in $CDCl_3$): δ 0.88 (6H, t, *J* 6.8 Hz), 1.20–1.34 (60H, m), 1.34–1.49 (2H, m), 1.60–1.71 (2H, m), 1.82–1.96 (3H, m), 2.00–2.08 (2H, m), 2.31 (1H, m), 4.27 (1H, m), 4.32–4.43 (4H, m), 4.47–4.57 (4H, m), 4.64–4.70 (2H, m), 4.74 (1H, m), 5.33 (1H, m), 5.61 (1H, d, *J* 3.7 Hz, anomeric H), 6.11 (1H, d, *J* 6.6 Hz, OH), 6.32 (2H, br s, OH), 6.52 (1H, br s, OH), 6.65 (1H, br s, OH), 6.76 (1H, d, *J* 6.9 Hz, OH), 7.05 (1H, br s, OH), 7.78 (1H, s, OH), 8.65 (1H, d, *J* 9.5 Hz, NH). ^{13}C NMR (126 MHz, pyridine-d_5): δ 14.31, 22.96, 26.48, 26.55, 29.64, 29.65, 29.94, 29.97, 30.01, 30.03, 30.05, 30.09, 30.19, 30.22, 30.30, 30.47, 32.15, 32.16, 32.64, 34.62, 50.57, 62.67, 67.99, 70.24, 71.02, 71.65, 72.37, 73.13, 73.55, 76.21, 76.55, 101.20, 173.79. ESI-MS: *m*/*z* 912.72 [M+Na]$^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{11}Na$: 912.7116; observed: 912.7134.

4.1.54. (2*S*,3*S*,4*R*,5*EZ*)-1-[(*tert*-Butyldimethylsilyl)oxy]-3,4-*O*-isopropylidene-5-hexacosene-2,3,4-triol (65)

To a solution of $Ph_3P(Br)C_{21}H_{43}$ (638 mg, 1.00 mmol) in dry THF (3 mL) was added a solution of *n*-BuLi (1.57 M in hexane, 1.20 mL, 1.88 mmol) at −10 °C under an atmosphere of argon with stirring. After stirring for 30 min at −10 °C, to this red-colored solution was added a solution of **64** (152 mg, 0.50 mmol) in dry THF (2 mL). This mixture was stirred for 4 h at room temperature, quenched with MeOH at ice cooling temperature, concentrated in vacuo, then 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 (39:1, then 19:1) gave **65** (183 mg, 63%) as an oily mixture of *E*- and *Z*-isomers. IR ν_{max}(KBr): 3540, 2926, 2855, 1465 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.09 (6H, s), 0.88 (3H, t, *J* 6.8 Hz), 0.91 (9H, s), 1.25–1.44 (41H, m, containing 3H singlet at 1.34 ppm), 1.45 (3H, s), 2.02–2.20 (2H, m), 2.45 (1H, d, *J* 4.6 Hz, OH), 3.66–3.71 (2H, m), 3.81 (1H, m), 3.83–4.03 (1H, m), 4.64 (0.6H, m), 5.00 (0.4H, dd, *J* 7.1, 8.8 Hz), 5.51–5.85 (2H, m). ESMS: *m*/*z* 621.49 [M+Na]$^+$. HREIMS: calcd for $C_{35}H_{70}O_5SiNa$: 621.4890; observed, 621.4877.

4.1.55. (2*S*,3*S*,4*R*)-1-(*tert*-Butyldimethylsilyloxy)-3,4-*O*-(isopropylidene)hexacosane-2,3,4-triol (66)

A solution of a mixture of *E*- and *Z*-isomers (**65**, 3.45 g, 5.92 mmol) in EtOAc (120 mL) was stirred for 1 h at room temperature under hydrogen, using 20% $Pd(OH)_2$/C (1.3 g) as a catalyst. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to give **66** (3.45 g, 100%) as a wax, which was employed for the next reaction without further purification. IR ν_{max}(KBr) 3539 (br), 2923, 2853, 1518, 1464 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.09 (6H, s), 0.88 (3H, t, *J* 6.8 Hz), 0.91 (9H, s), 1.26 (38H, br s), 1.32 (3H, s), 1.40 (3H, s), 1.50–1.80 (4H, m), 2.57 (1H, d, *J* 3.9 Hz), 3.64–3.69 (2H, m), 3.82 (1H, m), 3.91 (1H, dd, *J* 5.7, 8.8 Hz), 4.17 (1H, m). ESMS: *m*/*z* 607.51 [M+Na]$^+$. HRESMS: calcd for $C_{35}H_{72}O_4SiNa$: 607.5098; observed, 607.5100.

4.1.56. (2*S*,3*S*,4*R*)-1,2-Bis(*tert*-butyldimethylsilyloxy)-3,4-*O*-(isopropylidene)hexacosane-3,4-diol (67)

To a solution of **66** (3.45 g, 5.90 mmol) in CH_2Cl_2 (150 mL) were added 2,6-lutidine (3.17 g, 29.6 mmol, 5 equiv) and TBDMSOTf (4.69 g, 17.8 mmol, 3 equiv). After stirring for 1 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (150 mL), washed 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 (39:1) gave **67** (3.71 g, 90%) as an oil. IR ν_{max}(KBr) 2925, 2855, 1464, 1254 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.09 (3H, s), 0.12 (3H, s), 0.87 (9H, s), 0.88 (3H, t, *J* 7.0 Hz), 0.91 (9H, s), 1.26 (40H, br s), 1.31 (3H, s), 1.40 (3H, s), 1.52–1.54 (2H, m), 3.72 (1H, m), 3.76–3.81 (2H, m), 4.03–4.10 (2H, m). ESMS: *m*/*z* 641.58, 659.58, 721.60 [M+Na]$^+$. HRESMS: calcd for $C_{41}H_{86}O_4Si_2Na$: 721.5962; observed, 721.5974.

4.1.57. (2*S*,3*R*,4*R*)-2-(*tert*-Butyldimethylsilyloxy)-3,4-*O*-(isopropylidene)hexacosane-1,3,4-triol (68) and (2*S*,3*S*,4*R*)-3,4-*O*-(isopropylidene)hexacosane-1,2,3,4-tetraol (69)

(1) To a solution of **67** (100 mg, 0.14 mmol) in pyridine (0.9 mL) and dry THF (1.5 mL) was added HF·pyridine (0.2 mL; HF: ∼70%, pyridine: ∼30%) at room temperature with stirring, and the mixture was stirred for 16 h at room temperature. The reaction mixture was quenched with aq satd $NaHCO_3$, diluted with EtOAc, and washed with brine. The organic solution was 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, 4:1, and 1:1) gave **67** (32 mg, 32%,

recovery), **68** (39 mg, 47%) as a gum, and **69** (10 mg, 15%) as a powder. Physical data of **68**: IR ν_{max}(KBr) 3496 (br), 2925, 3854 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.11 (3H, s), 0.13 (3H, s), 0.87–0.89 (12H, m), 1.25–1.34 (40H, m), 1.35 (3H, s), 1.43 (3H, s), 1.50–1.58 (2H, m), 2.27 (1H, dd, *J* 3.7, 9.1 Hz, OH), 3.68–3.77 (2H, m), 3.83 (1H, m), 4.07 (1H, dd, *J* 5.7, 8.2 Hz), 4.13 (1H, m). ESMS: *m*/*z* 607.51 $[M+Na]^+$. HRESMS: calcd. for $C_{35}H_{72}O_4SiNa$: 607.5098; observed, 607.5090. Physical data of **69**: IR ν_{max}(KBr) 3355, 2917, 2850, 1472 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* 7.0 Hz), 1.24–1.75 (48H, m, containing two 3H singlets at 1.34 and 1.42 ppm), 1.91 (1H, m, OH), 2.18 (1H, d, *J* 5.6 Hz, OH), 3.72–3.76 (2H, m), 3.82 (1H, m), 3.96 (1H, m), 4.20 (1H, m). ESMS: *m*/*z* 493.42 $[M+Na]^+$. HRESMS: calcd for $C_{29}H_{58}O_4Na$: 493.4233; observed, 493.4227.

4.1.58. (2*R*,3*R*,4*R*)-2-*tert*-Butyldimethylsilyloxy-3,4-dihydroxy-3,4-*O*-(isopropylidene)hexacosanoic acid (70)

A solution of **68** (66 mg, 0.11 mmol), $NaIO_4$ (242 mg, 1.13 mmol), and $RuCl_3{\cdot}nH_2O$ (3 mg) in CCl_4–CH_3CN–H_2O (2:2:3, 7 mL) was stirred for 3 h at room temperature, and diluted with $CHCl_3$, washed with water, 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 4:1) gave **70** (52 mg, 77%) as a solid. IR ν_{max}(KBr) 2924, 2853, 1725, 1465 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.11 (3H, s), 0.14 (3H, s), 0.88 (3H, t, *J* 6.8 Hz), 0.92 (9H, s), 1.25 (40H, br s), 1.34 (3H, s), 1.45 (3H, s), 1.54–1.66 (2H, m), 4.19–4.20 (2H, m), 4.30 (1H, d, *J* 6.1 Hz). ESMS: *m*/*z* 621.49 $[M+Na]^+$. HRESMS: calcd for $C_{35}H_{70}O_5SiNa$: 621.4890; observed: 621.4877.

4.1.59. (2*S*,3*S*,4*R*)-2-[(2′*R*,3′*R*,4′*R*)-2′-(*tert*-Butyldimethylsilyloxy)-3′,4′-dihydroxy-3′,4′-*O*-(isopropylidene)hexacosanoylamino]-3,4-bis(*tert*-butyldimethylsilyloxy)octadecyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (71)

To a solution of **60** (200 mg, 0.19 mmol), DMAP (320 mg, 2.62 mmol), and **70** (336 mg, 0.56 mmol) in dry THF–CH_2Cl_2 (1:1, 20 mL) was added EDAC (503 mg, 2.61 mmol). The mixture was stirred for 16 h at room temperature, and concentrated in vacuo to give a mixture, which was diluted with CH_2Cl_2. 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 (19:1, then 4:1) gave **71** (116 mg, 38%) as a gum. IR ν_{max}(KBr) 2925, 2854, 1685 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.05 (3H, s), 0.06 (3H, s), 0.068 (3H, s), 0.072 (3H, s), 0.10 (6H, s), 0.87–0.90 (33H, m), 1.25 (67H, br s), 1.40 (3H, s), 1.44–1.54 (4H, m), 3.48 (1H, m), 3.58 (1H, t, *J* 8.5 Hz), 3.71–3.77 (3H, m), 3.90 (1H, dd, *J* 2.8, 10.3 Hz), 3.98–4.07 (5H, m), 4.22–4.27 (2H, m), 4.33 (1H, m), 4.37, 4.88 (2H, AB-q, *J* 11.7 Hz), 4.49, 4.53 (2H, AB-q, *J* 11.5 Hz), 4.69, 4.74 (2H, AB-q, *J* 12.0 Hz), 4.69, 4.75 (2H, AB-q, *J* 12.0 Hz), 4.87 (1H, d, *J* 3.4 Hz, anomeric H), 6.62 (1H, d, *J* 8.3 Hz, NH), 7.24–7.35 (20H, m). ESMS: *m*/*z* 1649.20 $[M+H]^+$. HRESMS: calcd for $C_{99}H_{170}NO_{12}Si_3$: 1649.2031; observed: 1649.2056.

4.1.60. (2*S*,3*S*,4*R*)-2-[(2′*R*,3′*R*,4′*R*)-2′-(*tert*-Butyldimethylsilyloxy)-3′,4′-dihydroxy-3′,4′-*O*-(isopropylidene)hexacosanoylamino]-3,4-bis(*tert*-butyldimethylsilyloxy)octadecyl α-D-galactopyranoside (72)

Compound **71** (116 mg, 0.04 mmol) was hydrogenated as described for the formation of **62** from **61** using 20% $Pd(OH)_2$/C (200 mg) as a catalyst to give **72** (49 mg, 54%) as a gum after silica gel column chromatography by eluting with hexane–EtOAc (2:1, then 1:2). IR ν_{max}(KBr) 3419 (br), 2925, 2854, 1684, 1465 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.08 (3H, s), 0.09 (3H, s), 0.12–0.13 (12H, m), 0.88 (6H, t, *J* 7.1 Hz), 0.90 (9H, s), 0.92 (9H, s), 0.93 (9H, s), 1.25 (64H, br s), 1.31 (3H, s), 1.42 (3H, s), 1.50–1.62 (4H, m), 2.45 (1H, br s, OH), 2.61–2.80 (3H, m, OH), 3.42 (1H, t, *J* 10.5 Hz), 3.65 (1H, t, *J* 2.7 Hz), 3.68–3.75 (3H, m), 3.83–3.87 (2H, m), 3.92 (1H, m), 4.08 (1H, m), 4.12 (1H, m), 4.21 (2H, s), 4.30 (1H, m), 4.49 (1H, m), 4.88 (1H, d, *J* 3.4 Hz, anomeric H), 6.46 (1H, d, *J* 10.0 Hz, NH). ESMS: *m*/*z* 1289.01 $[M+H]^+$, 1290.02, 1291.02. HRESMS: calcd for $C_{71}H_{146}NO_{12}Si_3$: 1289.0153; observed: 1289.0162.

4.1.61. (2*S*,3*S*,4*R*)-2-[(2′*R*,3′*R*,4′*R*)-2′,3′,4′-Trihydroxyhexacosanoylamino]-3,4-dihydroxyoctadecyl α-D-galactopyranoside (73) (RCAI-160)

Compound **72** (46 mg, 0.04 mmol) was treated as described for the formation of **63** from **62** to give **73** (16 mg, 48%) as a powder. The silica gel column chromatography was performed by elution with $CHCl_3$–MeI_3–MeOH (9:1, then 6:1). $[\alpha]_D^{25}$ +56.2 (*c* 0.75, pyridine). IR ν_{max}(KBr) 3372 (br), 2920, 2851, 1638, 1541, 1469 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5, one drop of 1% TMS in $CDCl_3$): δ 0.88 (6H, t, *J* 6.9 Hz), 1.20–1.45 (60H, m), 1.58–1.69 (2H, m), 1.79 (1H, m), 1.85–1.97 (3H, m), 2.20–2.29 (2H, m), 4.26–4.35 (3H, m), 4.36–4.41 (2H, m), 4.43–4.52 (4H, m), 4.55 (1H, s), 4.66 (1H, dd, *J* 3.8, 9.9 Hz), 4.70 (1H, dd, *J* 5.8, 10.7 Hz), 5.05 (1H, s), 5.32 (1H, m), 5.60 (1H, d, *J* 3.7 Hz, anomeric H), 6.11 (1H, br s, OH), 6.16 (1H, br s, OH), 6.35 (1H, br s, OH), 6.56 (1H, br s, OH), 6.66 (1H, br s, OH), 6.67 (1H, br s, OH), 6.73 (1H, br s, OH), 7.05 (1H, br s, OH), 7.66 (1H, br s, OH), 8.70 (1H, d, *J* 9.0 Hz, NH). ^{13}C NMR (126 MHz, pyridine-d_5, one drop of 1% TMS in $CDCl_3$): δ 14.39, 23.05, 26.45, 26.58, 29.71, 29.73, 30.02, 30.04, 30.07, 30.09, 30.10, 30.11, 30.14, 30.26, 30.46, 30.51, 32.22, 32.23, 34.63, 34.81, 51.15, 62.74, 67.84, 70.34, 71.09, 71.70, 72.39, 73.06, 73.14, 74.37, 76.16, 77.77, 101.24, 174.29. ESMS: *m*/*z* 928.70 $[M+Na]^+$. HR ESI-MS: calcd for $C_{50}H_{99}NO_{12}Na$: 928.7065; observed: 928.7056.

4.2. Methods for measurement of biological activity

4.2.1. Bioassay (mouse in vivo)[16]

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) (BD Bioscience) for IL-4, and mouse IFNγ ELISA kit (Thermo Scientific Endogen, Rockford, IL, USA) for IFNγ 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[5a,14e]

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 2 sites on the 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 glycolipid-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.

Acknowledgments

We thank Drs. Takemichi Nakamura and Yayoi Hongo, The Institute of Physical and Chemical Research (RIKEN), for measurements of high-resolution mass spectra.

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