Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 950–964
Bioorganic & Medicinal Chemistry

RCAI-8, 9, 18, 19, and 49–52, conformationally restricted analogues of KRN7000 with an azetidine or a pyrrolidine ring: Their synthesis and bioactivity for mouse natural killer T cells to produce cytokines☆

Ken-ichi Fuhshuku,[a,†] Naomi Hongo,[b] Takuya Tashiro,[a] Yui Masuda,[a,‡] Ryusuke Nakagawa,[b] Ken-ichiro Seino,[b,¶] Masaru Taniguchi[b] and Kenji Mori[a,*]

[a]*Glycosphingolipid Synthesis Group, Laboratory for Immune Regulation, Research Center for Allergy and Immunology, RIKEN, Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan*
[b]*Laboratory for Immune Regulation, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Suehiro-cho 1-7-22, Tsurumi-ku, Yokohama-shi, Kanagawa 280-0045, Japan*

Received 22 August 2007; revised 29 September 2007; accepted 4 October 2007
Available online 10 October 2007

Abstract—Conformationally restricted analogues of KRN7000, an α-D-galactosyl ceramide, were synthesized to examine their bioactivity for mouse natural killer (NK) T cells to produce cytokines. RCAI-8, 9, 51, and 52 are the analogues with a pyrrolidine ring, and RCAI-18, 19, 49, and 50 are those with an azetidine ring. RCAI-18 was shown to be a potent inducer of cytokine production by mouse NKT cells, while RCAI-51 was a moderately active inducer.

1. Introduction

In 1995 an anticancer drug candidate KRN7000 (α-GalCer, **1**, Fig. 1) was developed by researchers at Kirin Brewery Co.[2] It was obtained through the modification of the structures of agelasphins (see Fig. 1 for the structure of agelasphin 9b), which had been isolated in 1993 as anticancer glycosphingolipids from the extract of an Okinawan marine sponge, *Agelas mauritianus*.[3,4] These glycosphingolipids exhibit anticancer activity in vivo in mice and humans.

It has been shown that KRN7000 (**1**) is a ligand to make a complex with CD1d protein, a glycolipid presentation protein on the surface of the antigen-presenting cells of the immune system.[5] The structure of CD1d-**1** complex was recently solved by X-ray crystallographic analysis.[6,7] Lipid alkyl chains of **1** are bound in grooves in the interior of the CD1d protein, and the galactose head group of **1** is presented to the invariant Vα14 antigen receptors of natural killer (NK) T cells. Very recent X-ray crystallographic analysis revealed the structure of a human NKT T cell receptor (TCR) in complex with CD1d bound to KRN7000.[8] The structure clearly showed a lock-and-key type interaction of the alkyl chains of **1** with CD1d-binding cleft as well as the critical role of the terminal galactose moiety in TCR binding.

After activation by recognition of CD1d-**1** complex, NKT cells release both helper T(Th)1 and Th2 types of cytokines at the same time in large quantities.[9] Th1 type cytokines such as interferon (IFN)-γ mediate protective immune functions like tumor rejection, whereas Th2 type cytokines such as interleukin (IL)-4 mediate regulatory immune functions to ameliorate autoimmune diseases. Th1 and Th2 type cytokines can antagonize

Keywords: Azetidines; α-Galactosyl ceramides; Glycosphingolipids; KRN7000; NKT cell; Pyrrolidines.
☆ Synthesis of sphingosine relatives, Part 30. For Part 29, see: Ref. 1.
* Corresponding author. Fax: +81 48 467 9381; e-mail: kjk-mori@arion.ocn.ne.jp
† Present address: Department of Biotechnology, Toyama Prefectural University, Toyama 939-0398, Japan.
‡ Present address: Technical Research Institute, T. Hasegawa Co., Kawasaki 211-0022, Japan.
¶ Present address: Institute of Medical Science, St. Marianna University School of Medicine, Kanagawa 216-8511, Japan.

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.10.008

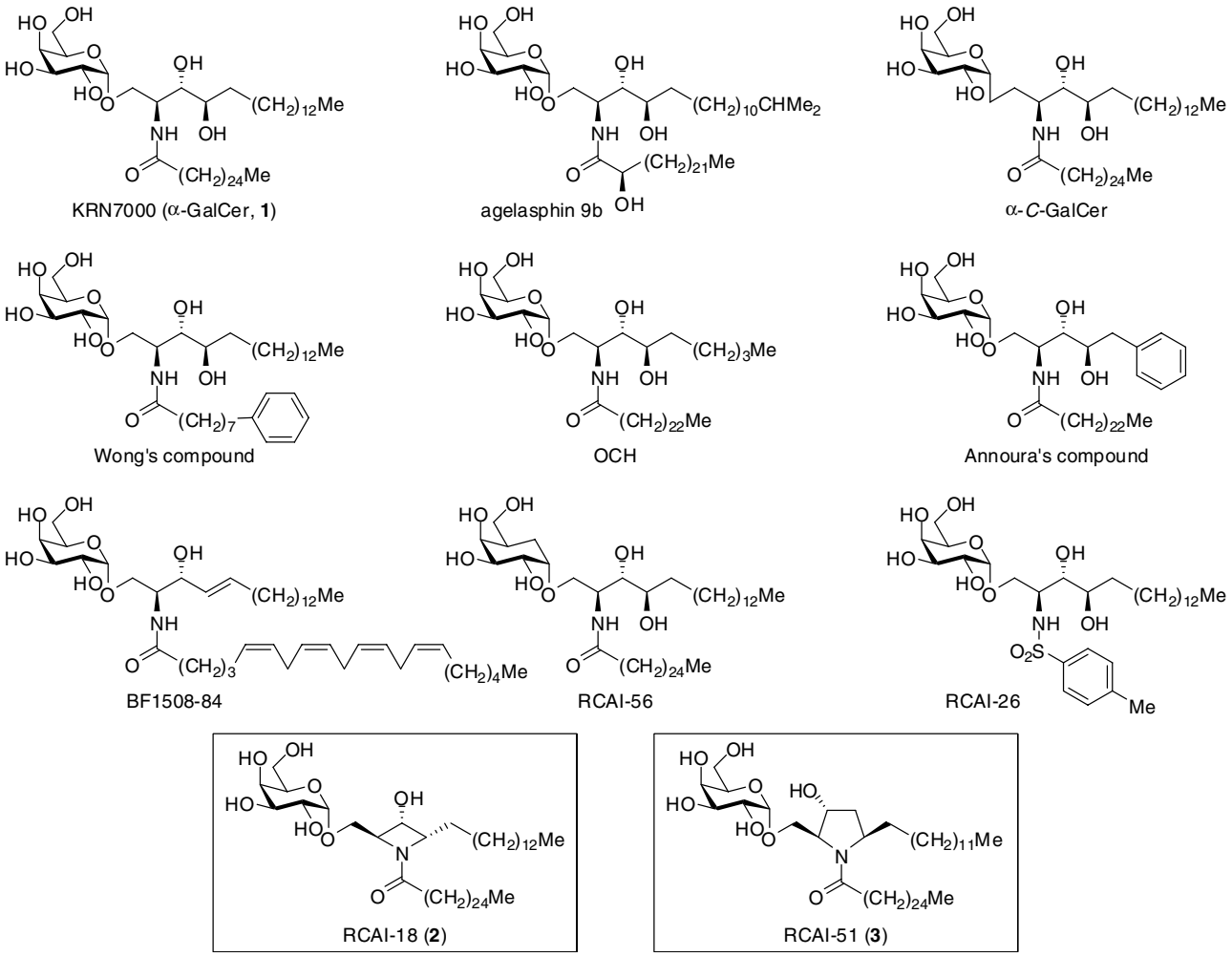


Figure 1. Structures of glycosphingolipids for NKT cell activation.

each other's biological actions.[10] Because of this antagonism, use of **1** for clinical therapy has not been successful yet. To circumvent this problem, many research groups are trying to develop new analogues of **1**, which induce NKT cells to produce either Th1 or Th2 type cytokines (see reviews[11–13]).

In 2003, Franck, Tsuji, and their co-workers reported that their synthetic α-D-*C*-galactosyl ceramide (α-*C*-GalCer, Fig. 1) caused an enhanced Th1 type response in vivo.[14] It is proposed that α-*C*-GalCer is stable against α-galactosidase in vivo, and therefore CD1d-α-*C*-GalCer complex can stimulate NKT cells for a longer period than CD1d-**1** can do, causing Th1-biased response.[12] Three different syntheses of α-*C*-GalCer have been reported to date.[15–17] A truncated 1-methylene-linked α-*C*-GalCer analogue was also synthesized by Bittman and co-workers, and confirmed to be bioactive.[18] In 2006, Wong and his co-workers found that the introduction of an aromatic group at the end of the fatty acyl chain (see Fig. 1) enhances IFN-γ production and enables the tuning of Th1/Th2 cytokine profile, perhaps through alteration of the stability of the glycosphingolipid-CD1d complex.[19]

In 2001, Miyamoto et al. found that OCH (Fig. 1), an analogue of KRN7000 with a truncated sphingosine alkyl chain, caused NKT cells to produce IL-4 predominantly.[20] Annoura and his co-workers then reported the synthetic procedures of OCH,[21] its *C*-galactosyl analogue with no bioactivity in vitro,[22] and related compounds.[23] In his 2007 paper Annoura demonstrated that his compound (Fig. 1) induced the production of both IFN-γ and IL-4.[23] This means that even a compound with a shortened sphingosine alkyl chain may induce the production of IFN-γ in addition to IL-4. Savage and co-workers also noticed that the analogues with shortened alkyl chains tend to induce IL-4 production.[24] There is a report that BF1508-84 (Fig. 1), an analogue containing arachidonic acid, shows Th2 bias like OCH.[25]

Since the inauguration of RIKEN (Institute of Physical and Chemical Research) Research Center for Allergy and Immunology (**RCAI**) in 2003, we started our project to synthesize glycosphingolipids, which induce NKT cells to produce preferentially either Th1 or Th2 type of cytokines. We already reported, in preliminary forms, the synthesis of RCAI-56 (Fig. 1), a carbocyclic ana-

logue with IFN-γ biased activity,[26] and RCAI-26, an analogue with a sulfonamide linkage instead of a carboxamide linkage with IL-4 biased activity.[27]

Another facet of our interest was to clarify the effect of conformational restriction in the ceramide part as caused by azetidine or pyrrolidine ring formation. In this paper, we report the synthesis of eight new analogues of KRN7000, which possess the four, or five-membered ring in the ceramide part. Among them, RCAI-18 (**2**) and RCAI-51 (**3**) showed remarkable and moderate bioactivity, respectively.

2. Results and discussion

2.1. Synthetic plan

A decade ago we reported the syntheses of bioactive marine alkaloids with an azetidine ring such as penaresidins A and B,[28,29] and penazetidine A (Fig. 2).[30] In these studies, the azetidine ring was generated by ring closure of phytosphingosine derivatives, which were synthesized from Garner's aldehyde by a conventional procedure. Accordingly, a synthetic plan as shown in Scheme 1 was devised.

Chain-elongation of Garner's aldehyde **A** with 1-pentadecyne **B** is followed by reduction of the triple bond and deprotection to give **C**.[31] After protection of two hydroxy groups as *tert*-butyldimethylsilyl (TBS) ether and tosylation of the amino group, the product is oxidized with *m*-chloroperbenzoic acid to furnish **D** and **D′**, which are separable by SiO_2 chromatography.[32] Conversion of the β-epoxide **D** to azetidine **F** is possible via mesylate **E** by the known method.[29,30] Further modification of **F** gives α-galactoside RCAI-18 (**2**), etc.

As to the pyrrolidine ring formation, mesylate **E′** is required, which is derivable from α-epoxide **D′** (vide infra). Ring closure of **E′** gives **F′**, which eventually furnishes α-galactoside RCAI-8 (**4**), etc. Eight new analogues of KRN7000 are available by adopting this synthetic plan.

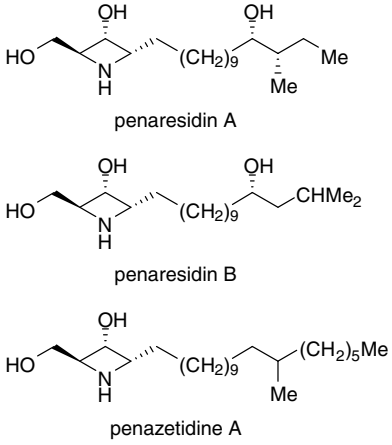
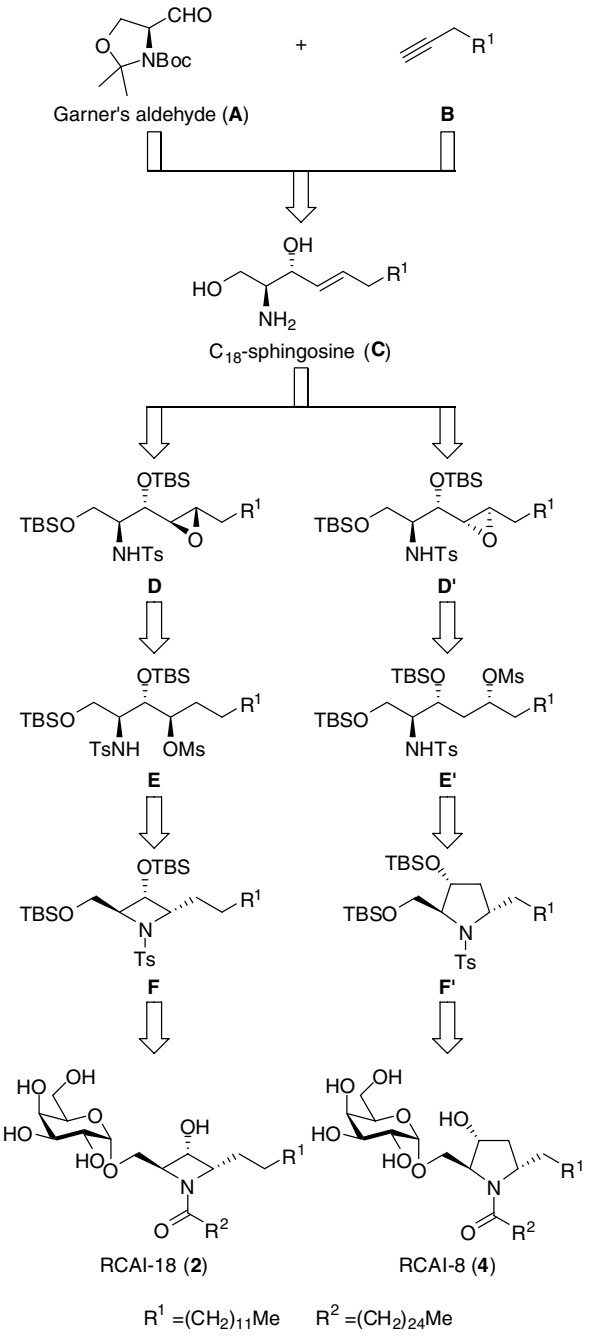


Figure 2. Structures of marine azetidine alkaloids.

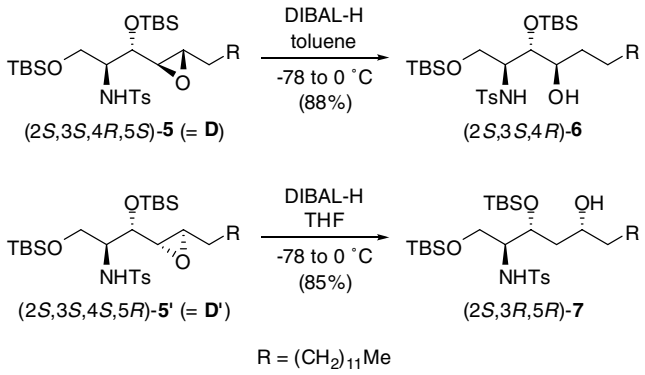


Scheme 1. Synthetic plan for RCAI-18 (**2**) and RCAI-8 (**4**).

2.2. Reductive cleavage of the epoxy ring of the stereoisomers of 4,5-epoxysphingosine derivatives

The important preliminary stage of the present work was to clarify the regiochemistry of the reductive opening of the stereoisomeric epoxides **5** and **5′** with diisobutylaluminum hydride (DIBAL-H) as shown in Scheme 2. Reduction of the β-epoxide **5** with DIBAL-H in toluene was known to give 4-hydroxy compound **6**.[32] When THF or CH_2Cl_2 was employed as the solvent instead of toluene, the reduction yielded complex mixtures.

Reduction of the α-epoxide **5′** was also examined by employing DIBAL-H in THF as the reducing agent.



Scheme 2. Reduction of epoxides **5** and **5′** with DIBAL-H.

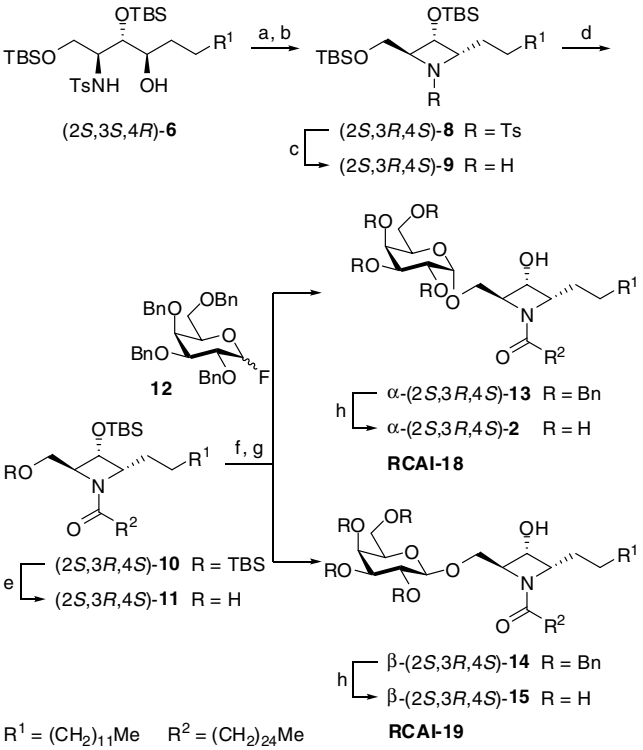
The reaction cleanly gave 5-hydroxy compound **7** in 85% yield, while in toluene or in CH_2Cl_2, a messy complex mixture was the product. In the ^1H NMR spectrum of **7** (270 MHz, $CDCl_3$), the proton at C-5 absorbed at $\delta = 4.07$ (ddd, $J = 5.3$, 5.6, 5.6 Hz). This NMR signal pattern was distinctly different from that of **6′**, and therefore structure **7** was assigned to this reduction product. It therefore is clear that we can employ **6** to prepare azetidine compounds, and **7** for the synthesis of pyrrolidine compounds.

2.3. Synthesis of RCAI-18 and RCAI-19, the (2*S*,3*R*,4*S*)-azetidine derivatives

Conversion of the known (2*S*,3*S*,4*R*)-**6** to RCAI-18 (**2**) and RCAI-19 (**15**) is shown in Scheme 3. The alcohol **6** was mesylated, and then the resulting mesylate was treated with sodium hydride to give (2*S*,3*R*,4*S*)-**8**. (cf.[29,30]) Six stereoisomers of **8** were synthesized by Kobayashi and co-workers very recently.[33] After removing the tosyl group of **8** with sodium naphthalenide, the resulting azetidine (2*S*,3*R*,4*S*)-**9** was converted to amide **10** by treatment with cerotic acid and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in the presence of *N*,*N*-diisopropylethylamine and *N*,*N*-4-dimethylaminopyridine (DMAP). The TBS protective group at the primary hydroxy group of **10** was selectively removed with aqueous trifluoroacetic acid to give **11**. Glycosylation of **11** with tetra-*O*-benzyl-D-galactopyranosyl fluoride under Mukaiyama's conditions[34] yielded both α-(2*S*,3*R*,4*S*)-**13** and β-(2*S*,3*R*,4*S*)-**14** in 43 and 49% yield, respectively, after desilylation with tetra(*n*-butyl)ammonium fluoride (TBAF). The reason was unclear for the predominant formation of β-galactoside **14**. Finally, hydrogenolysis of **13** and **14** furnished RCAI-18 (**2**) and RCAI-19 (**15**), respectively, as amorphous solids.

2.4. Synthesis of RCAI-8 and RCAI-9, the (2*S*,3*R*,5*S*)-pyrrolidine derivatives

Conversion of (2*S*,3*R*,5*R*)-**7** to RCAI-8 (**4**) and RCAI-9 (**22**) is shown in Scheme 4. The alcohol **7** was mesylated and cyclized to give pyrrolidine (2*S*,3*R*,5*S*)-**16**. Its detosylation to **17** was followed by acylation with cerotic acid to furnish amide (2*S*,3*R*,5*S*)-**18**. Partial desilylation of **18** furnished alcohol **19**, which was



Scheme 3. Synthesis of RCAI-18 (**2**) and RCAI-19 (**15**). Reagents and conditions: (a) MsCl, C_5H_5N, 4 °C; (b) NaH, THF, 80% (2 steps); (c) Na, naphthalene, DME, −78 °C, quant; (d) $Me(CH_2)_{24}CO_2H$, EDC, *i*-Pr_2NEt, DMAP, CH_2Cl_2. 88%; (e) CF_3CO_2H, H_2O, THF, 99%; (f) **12**, $SnCl_2$, $AgClO_4$, THF, MS 4A, −20 to 10 °C; (g) TBAF, THF, 43% for α-**13** (2 steps), 49% for β-**14** (2 steps); (h) H_2, $Pd(OH)_2$/C, EtOH, $CHCl_3$, 84% for **2**, 85% for **15**.

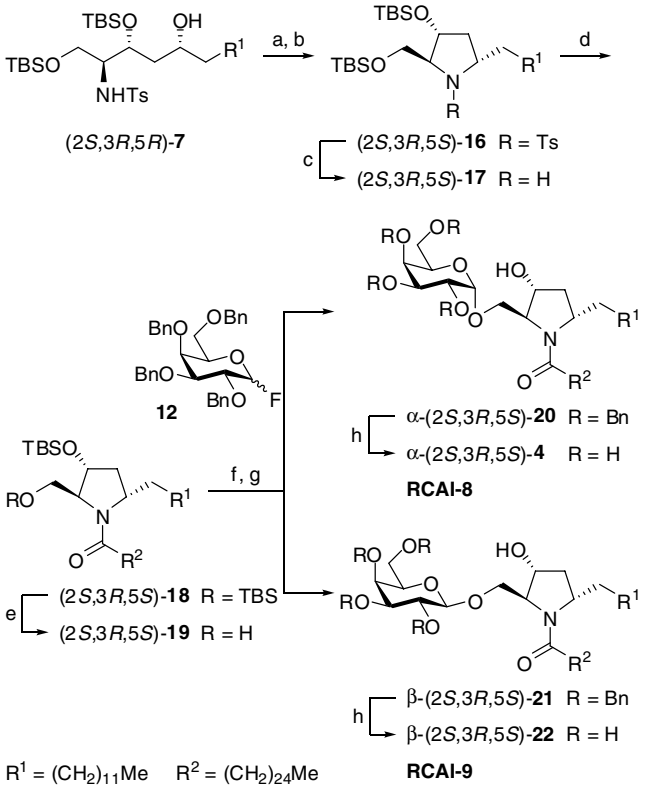
galactosylated to give α-galactoside **20** and β-galactoside **21**. Debenzylation of **20** afforded RCAI-8 (**4**), while that of **21** provided RCAI-9 (**22**).

2.5. Synthesis of RCAI-49 and RCAI-50, the (2*S*,3*R*,4*R*)-azetidine derivatives

Conversion of (2*S*,3*S*,4*R*)-**6** to RCAI-49 (**24**) and RCAI-50 (**25**) is shown in Scheme 5. So as to obtain azetidines RCAI-49 and 50 with two *cis*-alkyl groups attached to the four-membered ring, it was necessary to invert the 4*R*-configuration of alcohol **6** to give (4*S*)-alcohol **6′**. The successful route for this purpose was to oxidize **6** with DMSO-Ac_2O (Albright-Goldman reagent)[35] and then reduce the resulting ketone **23** with a bulky reducing agent, lithium triethylborohydride. Subsequently (2*S*,3*S*,4*S*)-alcohol **6′** afforded RCAI-49 (**24**) and RCAI-50 (**25**) in the same manner as described in Scheme 3.

2.6. Synthesis of RCAI-51 and RCAI-52, the (2*S*,3*R*,5*R*)-pyrrolidine derivatives

Conversion of (2*S*,3*R*,5*R*)-**7** to RCAI-51 (**3**) and RCAI-52 (**27**) is shown in Scheme 6. In the present case, oxidation of (2*S*,3*R*,5*R*)-**7** to ketone (2*S*,3*R*)-**26** was executed with tetra(*n*-propyl)ammonium perruthenate (TPAP) and *N*-methylmorpholine-*N*-oxide (NMO).[36] Subse-



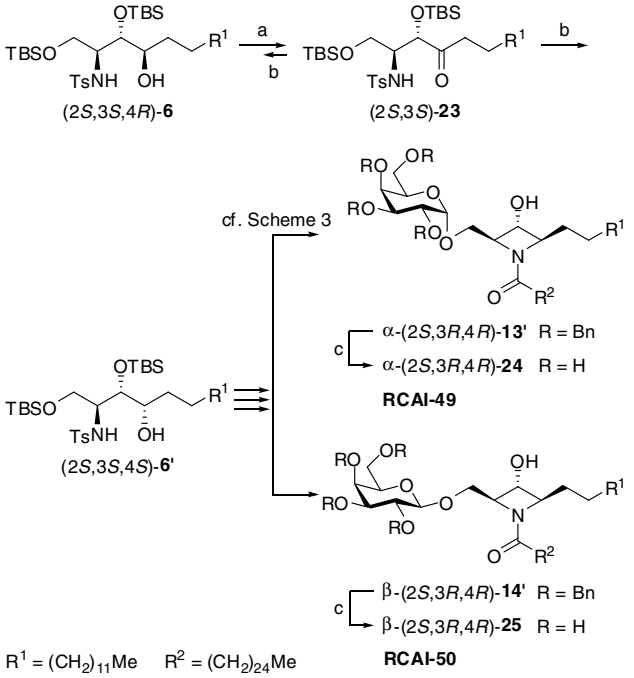
Scheme 4. Synthesis of RCAI-8 (**4**) and RCAI-9 (**22**). Reagents and conditions: (a) MsCl, C_5H_5N, 4 °C; (b) NaH, THF, 99% (2 steps); (c) Na, naphthalene, DME, −78 °C, 99%; (d) $Me(CH_2)_{24}CO_2H$, EDC, *i*-Pr_2NEt, DMAP, CH_2Cl_2, 84%; (e) CF_3CO_2H, H_2O, THF, 92%; (f) **12**, $SnCl_2$, $AgClO_4$, THF, MS 4A, −20 to 10 °C; (g) TBAF, THF, 49% for α-**20** (2 steps), 19% for β-**21** (2 steps); (h) H_2, $Pd(OH)_2$/C, EtOH, $CHCl_3$, 95% for **4**, 64% for **22**.

quent reduction of the ketone **26** with lithium triethylborohydride gave (2*S*,3*R*,5*S*)-**7**′. The alcohol **7**′ furnished RCAI-51 (**3**) and RCAI-52 (**27**) in the same manner as described in Scheme 4.

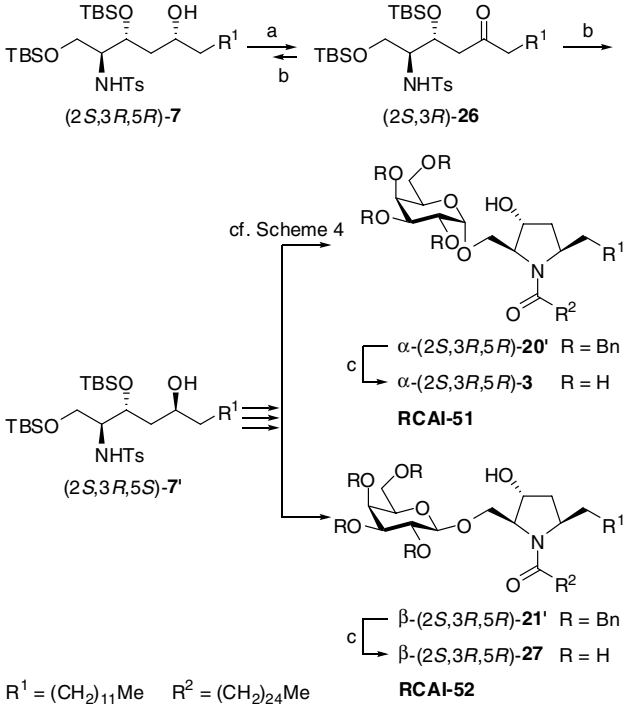
2.7. Results of bioassay

To assess the NKT cell stimulatory effect of RCAI-8, 9, 18, 19 and 49–52, we measured the level of various cytokines in supernatants from spleen cells cultured with KRN7000 or these synthetic analogues.[37,38] Spleen cells were prepared from B6 mice and were cultured for 48 h with various glycolipids. The level of IFN-γ, IL-2, IL-4, IL-10, IL-13 in the supernatants was measured by cytometric bead array (CBA) system (BD Biosciences).[38]

Figure 3 shows the results of bioassay. KRN7000 (α-GalCer, **1**) was chosen as the positive standard. As can be seen from the Figure, RCAI-18 and 51 showed notable activities. Other compounds were of marginal activity. The bioactivity of RCAI-18 was more dose-dependent than that of KRN7000, although both of them showed almost the same activity at the dosage of 200 ng/mL. The high dose-dependent activity of RCAI-18 might be beneficial in its clinical application to enable the precise control of cytokine production.



Scheme 5. Synthesis of RCAI-49 (**24**) and RCAI-50 (**25**). Reagents and condition: (a) DMSO, Ac_2O; (b) $LiBEt_3H$, THF, −78 °C, 90% (2 steps, **6**′/**6** = 8:1). Subsequent steps were executed in the same manner as described in Scheme 3. α-(2*S*,3*R*,4*R*)-**13**′ could be separated from β-(2*S*,3*R*,4*R*)-**14**′ by SiO_2 chromatography.



Scheme 6. Synthesis of RCAI-51 (**3**) and RCAI-52 (**27**). Reagents and condition. (a) TPAP, NMO, CH_2Cl_2, MS 4A; (b) $LiBEt_3H$, THF, −78 °C, 37% for **7**′ and 62% recovery of **7** after SiO_2 chromatography. Subsequent steps were executed in the same manner as described in Scheme 4.

None of these new analogues, RCAI-8, 9, 18, 19, and 49–52, induced NKT cells to produce preferentially either Th1 or Th2 type of cytokines at higher concentrations,

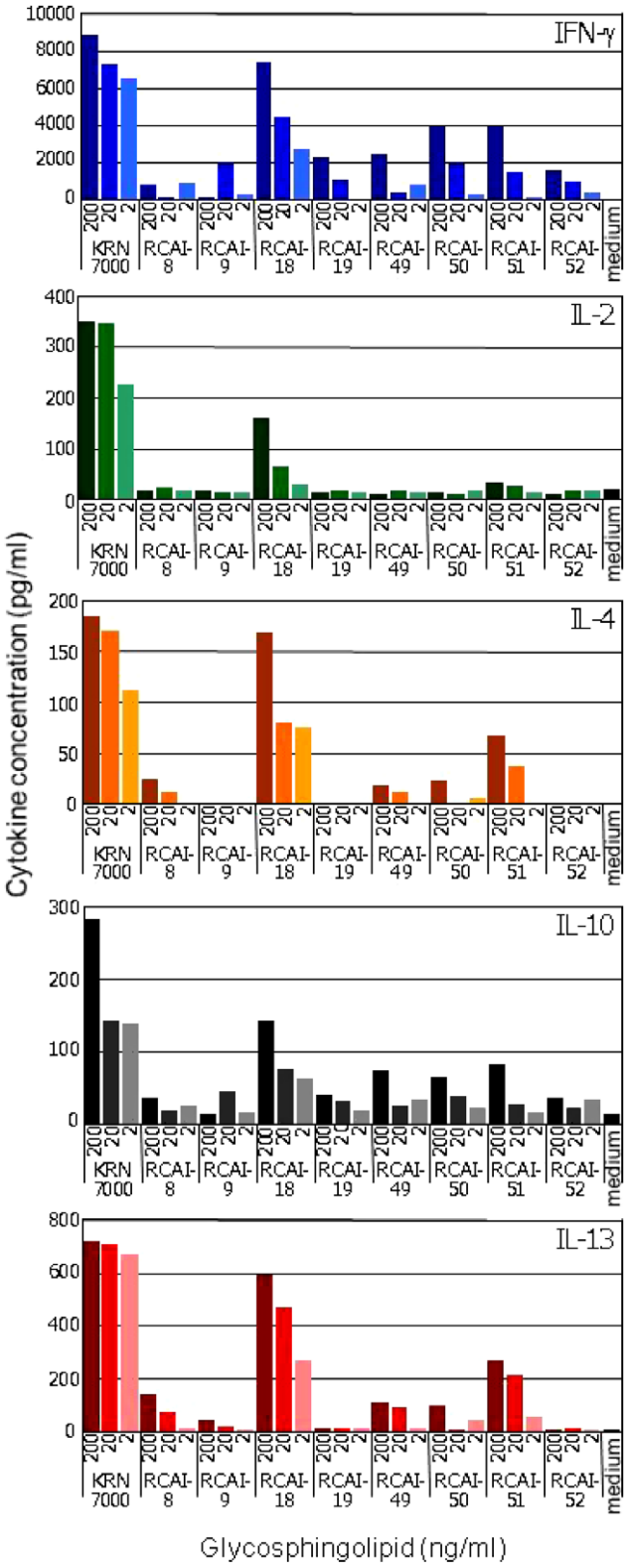


Figure 3. Cytokine production of NKT cells stimulated with KRN7000 or synthetic analogues. Spleen cells (2×10^5) from B6 mice were cultured with 2, 20, 200 ng/mL of KRN7000 or synthetic analogues for 48 h. The amount of IFN-γ, IL-2, IL-4, IL-10, IL-13 in the culture supernatants was measured by cytometric bead array (CBA). Data are shown as means of three wells. Medium indicates the amount of cytokines in the supernatant without glycolipid. Similar results were obtained from 2 independent experiments.

although RCAI-18 induced production of Th2-type cytokines more than that of Th1-type ones at lower dosage of 2–20 ng/mL. This slightly Th2-biased activity of RCAI-18 at low concentrations was probably due to the less stable nature of the complex between RCAI-18 and CD1d protein, and might be useful in ameliorating autoimmune diseases. Further studies to clarify the structure–activity relationship are in progress by means of the computational docking models.

3. Conclusion

Among eight synthetic analogues of KRN7000, RCAI-18 (**2**) with a *trans*-2,4-dialkylated azetidine ring showed bioactivity remarkable enough to induce the production of IFN-γ, IL-4, and IL-13. RCAI-51 (**3**) with a *cis*-2,4-dialkylated pyrrolidine ring showed decreased amount of cytokine induction compared with RCAI-18. RCAI-50 (**25**) induced IFN-γ production moderately, although it was a β-galactoside.

In conclusion, conformational restriction at the ceramide part of KRN7000 by four- or five-membered ring formation did not bring about highly encouraging result. Nevertheless, it became clear that even a conformationally restricted analogue RCAI-18 (**2**) possesses high cytokine inducing activity. Dose-dependent and slightly Th2-biased bioactivity of RCAI-18 might be of interest in future biological research.

4. Experimental

4.1. General

All melting points (mp) are uncorrected. Refractive indices (n_D) were measured on an Atago 1T refractometer. Optical rotation values were measured on Jasco DIP-1010 instruments. IR spectra were recorded on Jasco FT/IR-460 plus spectrometer. ^1H and ^{13}C NMR spectra were recorded on Jeol AL-270 (270 MHz) and AL-400 (400 MHz) ($CHCl_3$ at $\delta_H = 7.26$ and $\delta_C = 77.00$ as an internal standard). Mass spectra were recorded on Jeol MS-700. Column chromatography was carried out with silica gel 60 (spherical; 100–210 μm) purchased from Kanto Chemical Co., and thin-layer chromatography was carried out with Merck silica gel 60 F_{254} thin-layer plates (1.05715).

4.2. (2*S*,3*R*,4*S*)-3-*tert*-Butyldimethylsilyloxy-2-*tert*-butyldimethylsilyloxymethyl-4-tetradecyl-1-*p*-toluenesulfonylazetidine (2*S*,3*R*,4*S*)-8

To an ice-cooled solution of (2*S*,3*S*,4*R*)-**6** (460 mg, 0.657 mmol) in dry pyridine (5.0 mL), MsCl (0.20 mL, 2.58 mmol) was added in one portion. The reaction mixture was stirred for 18 h at 4 °C and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was successively washed with a saturated aqueous $CuSO_4$ solution, water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in dry THF (5.0 mL), cooled to 0 °C, and NaH

(60% in mineral oil, 79.0 mg, 1.98 mmol) was added. The reaction mixture was stirred for 40 h at room temperature and diluted with water and a saturated aqueous NH_4Cl solution. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (25 g). Elution with hexane–EtOAc (60:1 to 30:1) afforded (2*S*,3*R*,4*S*)-**8** (360 mg, 80%) as an oil, n_D^{25} 1.4815; $[\alpha]_D^{25}$ +44.0 (*c* 0.56, $CHCl_3$); IR (film) ν_{max} 2927, 2854, 1600, 1463, 1345, 1254, 1159, 1106, 838, 779, 674 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.02 (3H, s, SiMe), 0.03 (3H, s, SiMe), 0.04 (3H, s, SiMe), 0.05 (3H, s, SiMe), 0.85–0.91 (21H, m, *t*Bu × 2, 14′-Me), 1.26 (24H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 13′-H_2), 1.75–1.80 (2H, m, 1′-H_2), 2.41 (3H, s, Ar-Me), 3.80 (1H, dd, *J* = 3.3, 11.2 Hz, 2-C*H*H-OTBS), 3.86 (1H, dd, *J* = 4.6, 11.2 Hz, 2-CH*H*-OTBS), 3.97 (1H, ddd, *J* = 3.3, 3.3, 4.0 Hz, 2-H), 4.22 (1H, m, 4-H), 4.41 (1H, dd, *J* = 3.3, 6.6 Hz, 3-H), 7.26 (2H, d, *J* = 7.9 Hz, *m*Ar), 7.71 (2H, d, *J* = 7.9 Hz, *o*Ar); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.5, −5.3, −5.1, −4.4, 14.2, 17.9, 18.4, 21.6, 22.8, 25.7, 26.0, 27.0, 29.4, 29.66, 29.73, 29.8, 32.0, 61.5, 65.8, 69.1, 73.7, 127.1, 129.3, 138.4, 142.7; Anal. Calcd for $C_{37}H_{71}NO_4SSi_2$: C, 65.14; H, 10.49; N, 2.05. Found: C, 65.03; H, 10.69; N, 1.97.

4.3. (2*S*,3*R*,4*S*)-3-*tert*-Butyldimethylsilyloxy-2-*tert*-butyldimethylsilyloxymethyl-4-tetradecylazetidine (2*S*,3*R*,4*S*)-9

To a solution of (2*S*,3*R*,4*S*)-**8** (286 mg, 0.419 mmol) in dry DME (3.0 mL), a solution of sodium naphthalenide (5.0 mL) [Prepared as follows. To a solution of naphthalene (516 mg, 4.03 mmol) in dry DME (5.0 mL), sodium (77.4 mg, 3.37 mmol) was added under argon. The mixture was stirred for 3 h at room temperature.] was added at −78 °C under argon. The reaction mixture was stirred for 90 min and diluted with water. The mixture was extracted with $CHCl_3$. The combined organic extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (15 g). Elution with hexane–EtOAc (1:0 to 20:1) afforded (2*S*,3*R*,4*S*)-**9** (221 mg, quant) as an oil, n_D^{23} 1.4574; $[\alpha]_D^{23}$ − 16.2 (*c* 1.05, $CHCl_3$); IR (film) ν_{max} 2926, 2854, 1463, 1253, 1132, 837, 777, 669 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.057 (6H, s, SiMe × 2), 0.059 (6H, s, SiMe × 2), 0.84–0.91 (21H, m, *t*Bu × 2, 14′-Me), 1.24 (24H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 13′-H_2), 1.55–1.72 (2H, m, 1′-H_2), 2.37 (1H, br s, NH), 3.50–3.66 (4H, m, 2-CH_2-OTBS, 2, 4-H), 4.42 (1H, dd, *J* = 5.3, 7.3 Hz, 3-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.3, −4.9, −4.6, 14.2, 18.2, 18.4, 22.8, 25.9, 26.0, 29.4, 29.7, 29.9, 30.7, 32.0, 61.5, 63.4, 67.7, 68.0. This compound was immediately employed for the next step without further purification.

4.4. (2*S*,3*R*,4*S*)-3-*tert*-Butyldimethylsilyloxy-2-*tert*-butyldimethylsilyloxymethyl- 4-tetradecyl-1-hexacosanoylazetidine (2*S*,3*R*,4*S*)-10

To an ice-cooled solution of (2*S*,3*R*,4*S*)-**9** (194 mg, 0.367 mmol) and *i*-Pr_2NEt (510 μL, 2.92 mmol) in dry CH_2Cl_2 (7.3 mL), EDC (106 mg, 0.553 mmol), cerotic acid (220 mg, 0.554 mmol), and a catalytic amount of DMAP were added. The reaction mixture was stirred for 48 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (10 g). Elution with hexane–EtOAc (50:1 to 30:1) afforded (2*S*,3*R*,4*S*)-**10** (293 mg, 88%) as an oil, n_D^{23} 1.4615; $[\alpha]_D^{23}$ + 42.8 (*c* 1.05, $CHCl_3$); IR (film) ν_{max} 2925, 2853, 1652, 1463, 1423, 1254, 837, 778 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.02–0.07 (12H, m, SiMe × 4), 0.85–0.90 (24H, m, *t*Bu × 2, 14′-Me, 26″-Me), 1.25 (68H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 13′, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″, 18″, 19″, 20″, 21″, 22″, 23″, 24″, 25″-H_2), 1.59–2.07 (6H, m, 1′, 2″, 3″-H_2), 3.67 (0.75H, dd, *J* = 2.0, 10.9 Hz, 2-C*H*H-OTBS), 3.75 (0.25H, dd, *J* = 3.3, 11.2 Hz, 2-C*H*H-OTBS), 3.84 (0.25H, dd, *J* = 4.0, 11.2 Hz, 2-CH*H*-OTBS), 4.00–4.23 (2H, m, 2, 4-H), 4.29 (0.75H, dd, *J* = 3.0, 10.9 Hz, 2-CH*H*-OTBS), 4.35 (0.25H, dd, *J* = 3.3, 6.6 Hz, 3-H), 4.58 (0.75H, dd, *J* = 3.6, 6.9 Hz, 3-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.5, −5.4, −5.33, −5.27, −5.1, −5.0, −4.5, −4.4, 14.2, 18.0, 18.30, 18.34, 22.8, 25.17, 25.21, 25.7, 25.8, 25.9, 26.1, 26.5, 28.9, 29.4, 29.55, 29.58, 29.63, 29.8, 29.9, 30.0, 32.0, 33.2, 33.6, 59.1, 62.4, 64.6, 66.0, 66.1, 70.7, 71.9, 172.8, 173.0; Anal. Calcd for $C_{56}H_{115}NO_3Si_2$: C, 74.18; H, 12.78; N, 1.54. Found: C, 74.07; H, 13.01; N, 1.62.

4.5. 3-*tert*-Butyldimethylsilyloxy-2-hydroxymethyl-4-tetradecyl-1-hexacosanoylazetidine

4.5.1. (2*S*,3*R*,4*S*)-Isomer 11. To an ice-cooled solution of (2*S*,3*R*,4*S*)-**10** (64.2 mg, 46.3 μmol) in dry THF (3.0 mL), TFA (10% in water, 1.0 mL) was added. The reaction mixture was stirred for 3 h at room temperature and neutralized with aqueous NaOH solution. The mixture was extracted with Et_2O. The combined organic extract was successively washed with a saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (5 g). Elution with hexane–EtOAc (50:1 to 4:1) afforded (2*S*,3*R*,4*S*)-**11** (55.7 mg, 99%) as an oil, IR (KBr) ν_{max} 3368, 2918, 2850, 1618, 1467, 1254, 836, 781, 721 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.04 (3H, s, SiMe), 0.06 (3H, s, SiMe), 0.85–0.90 (15H, m, *t*Bu, 14′-Me, 26″-Me), 1.25 (68H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 13′, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″, 18″, 19″, 20″, 21″, 22″, 23″, 24″, 25″-H_2), 1.62–2.12 (6H, m, 1′, 2″, 3″-H_2), 3.64 (1H, dd, *J* = 9.2, 11.5 Hz, 2-C*H*H-OTBS), 3.80 (1H, m, 2-CH*H*-OTBS), 4.17 (1H, dd, *J* = 5.6, 6.9 Hz, 3-H), 4.27 (2H, m, 2, 4-H), 4.69 (1H, br s, OH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.0, −4.5, 14.2, 17.9, 22.8, 25.1, 25.6, 25.8, 28.9, 29.4, 29.6, 29.67, 29.72, 29.8, 29.9, 32.0, 32.8, 64.4, 64.7, 66.5, 73.8, 174.0. This compound was immediately employed for the next step without further purification.

4.5.2. (2*S*,3*R*,4*R*)-Isomer 11′. A mixture of (2*S*,3*S*,4*R*)-**6** (201 mg, 0.287 mmol), DMSO (2.3 mL), and Ac_2O

(0.6 mL) was stirred for 20 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was filtered through a silica gel pad and concentrated in vacuo to give crude (2*S*,3*S*)-**23** (200 mg). This compound was immediately employed for the next step without further purification. To a solution of crude (2*S*,3*S*)-**23** (200 mg) in dry THF (4.5 mL), $LiBEt_3H$ (1.35 mL of a 1.06 M solution in THF, 1.43 mmol) was added at −78 °C. The reaction mixture was stirred for 4 h at −78 °C, quenched with saturated aqueous Na_2CO_3 solution and H_2O_2 (30% in water), and diluted with Et_2O. The mixture was extracted with Et_2O. The combined organic extract was successively washed with saturated aqueous $Na_2S_2O_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (10 g). Elution with hexane–EtOAc (30:1 to 20:1) afforded (2*S*,3*S*,4*S*)-**6′** (180 mg, 90% for 2 steps, 8:1 diastereomeric mixture). This compound was immediately employed for the next step without further purification. To an ice-cooled solution of (2*S*,3*S*,4*S*)-**6′** (159 mg, 0.227 mmol) in dry pyridine (2.5 mL), MsCl (0.14 mL, 1.80 mmol) was added in one portion. The reaction mixture was stirred for 40 h at 4 °C and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with a saturated aqueous $CuSO_4$ solution, water, a saturated aqueous $NaHCO_3$ solution, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in dry THF (2.5 mL), cooled to 0 °C, and NaH (60% in mineral oil, 27.8 mg, 0.695 mmol) was added. The reaction mixture was stirred for 44 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (8 g). Elution with hexane–EtOAc (50:1 to 20:1) afforded (2*S*,3*R*,4*R*)-**8′** (133 mg, 86%, 8:1 diastereomeric mixture). This compound was immediately employed for the next step without further purification. To a solution of (2*S*,3*R*,4*R*)-**8′** (133 mg, 0.195 mmol) in dry DME (3.0 mL), a solution of sodium naphthalenide (3.0 mL) [Prepared as follows. To a solution of naphthalene (868 mg, 6.77 mmol) in dry DME (10.0 mL), sodium (130 mg, 5.65 mmol) was added under argon. The mixture was stirred for 3 h at room temperature.] was added at −78 °C under argon. The reaction mixture was stirred for 90 min and diluted with water. The mixture was extracted with $CHCl_3$. The combined organic extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in dry CH_2Cl_2 (5.0 mL), cooled to 0 °C, and 2,6-lutidine (0.50 mL) and TBSOTf (0.25 mL) were added. The reaction mixture was stirred for 16 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was filtered through a silica gel pad and concentrated in vacuo. The residue was dissolved in dry CH_2Cl_2 (10.0 mL), and *i*-Pr_2NEt (275 μL, 1.58 mmol), EDC (58.0 mg, 0.303 mmol), cerotic acid (120 mg, 0.303 mmol), and a catalytic amount of DMAP were added. The reaction mixture was stirred for 96 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (7 g). Elution with hexane–EtOAc (30:1) afforded (2*S*,3*R*,4*R*)-**10′** (118 mg, 67% for 3 steps). To an ice-cooled solution of (2*S*,3*R*,4*R*)-**10′** (118 mg, 0.130 mmol) in dry THF (9.0 mL), TFA (10% in water, 3.0 mL) was added. The reaction mixture was then warmed gradually to room temperature with stirring in the course of 6 h and neutralized with aqueous NaOH solution. The mixture was extracted with Et_2O. The combined organic extract was successively washed with a saturated aqueous $NaHCO_3$ solution, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (12 g). Elution with hexane–EtOAc (20:1 to 3:1) afforded (2*S*,3*R*,4*R*)-**11′** (72.0 mg, 70%) as a solid, IR (KBr) ν_{max} 3390, 2918, 2850, 1626, 1468, 1254, 838, 778, 721 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.07 (6H, s, SiMe × 2), 0.85–0.90 (15H, m, *t*Bu, 14′-Me, 26″-Me), 1.25 (68H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 13′, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″, 18″, 19″, 20″, 21″, 22″, 23″, 24″, 25″-H_2), 1.62–2.21 (6H, m), 3.59 (1H, dd, J = 9.9, 10.9 Hz, 2-C*H*H-OTBS), 3.72 (1H, dd, J = 4.0, 4.3 Hz, 3-H), 3.79 (1H, ddd, J = 2.0, 8.9, 10.9 Hz, 2-CH*H*-OTBS), 4.02 (1H, ddd, J = 3.6, 4.0, 9.9 Hz, 4-H), 4.14 (1H, m, 2-H), 4.93 (1H, d, J = 8.9 Hz, OH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.0, −4.5, 14.2, 17.9, 22.8, 25.1, 25.6, 25.8, 28.9, 29.4, 29.6, 29.67, 29.72, 29.8, 29.9, 32.0, 32.8, 64.4, 64.7, 66.5, 73.8, 174.0. Its 4*S*-epimer could be removed at this stage. This compound was immediately employed for the next step without further purification.

4.6. 2-(2‴,3‴,4‴,6‴-Tetra-*O*-benzyl-α-D-galactopyranosyloxy)methyl-4-tetradecyl-1-hexacosanoylazetidin-3-ol

4.6.1. α-(2*S*,3*R*,4*S*)-Isomer 13 and β-(2*S*,3*R*,4*S*)-isomer 14. To a solution of (2*S*,3*R*,4*S*)-**11** (127 mg, 0.160 mmol) in dry THF (5.0 mL), $SnCl_2$ (91.8 mg, 0.485 mmol), $AgClO_4$ (99.8 mg, 0.481 mmol), and powdered MS 4A (300 mg) were added. The reaction mixture was stirred for 90 min at room temperature. A solution of benzylgalactosyl fluoride (**12**, 210 mg, 0.387 mmol) in dry THF (5.0 mL) was added at −20 °C. The reaction mixture was then warmed gradually to 10 °C with stirring in the course of 4 h and filtered through silica gel. The filtrate was successively washed with water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was separated into two fractions by silica gel column chromatography (20 g). Elution with hexane–EtOAc (1:0 to 6:1) afforded less polar residue (146 mg) and more polar residue (122 mg). The less polar residue (146 mg) was dissolved in dry THF (5.0 mL), and TBAF (1.0 M in THF, 0.75 mL, 0.75 mmol) was added at room temperature. The reaction mixture was stirred for 15 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic

extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (20 g). Elution with hexane–EtOAc (10:1 to 3:2) afforded α-(2*S*,3*R*,4*S*)-**13** (82.8 mg, 43%) as an oil, n_D^{23} 1.5138; $[\alpha]_D^{26}$ +47.0 (*c* 0.16, $CHCl_3$); IR (film) ν_{max} 3364, 2921, 2852, 1615, 1455, 1343, 1100, 1061, 733, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (68H, m), 1.56–2.08 (6H, m), 3.29 (0.33H, dd, *J* = 3.9, 9.9 Hz), 3.40 (0.67H, dd, *J* = 5.6, 9.7 Hz), 3.55 (1H, dd, *J* = 7.3, 9.4 Hz), 3.61 (0.33H, dd, *J* = 8.5, 10.6 Hz), 3.68 (0.67H, dd, *J* = 8.7, 10.4 Hz), 3.84–3.90 (2H, m), 3.96 (0.67H, m), 3.99–4.06 (1.67H, m), 4.11–4.22 (2H, m), 4.31–4.40 (2.67H, m), 4.47 (0.33H, d, *J* = 11.8 Hz), 4.48 (0.67H, d, *J* = 11.6 Hz), 4.54 (0.33H, d, *J* = 11.6 Hz), 4.55 (0.67H, d, *J* = 11.4 Hz), 4.64 (0.33H, d, *J* = 12.1 Hz), 4.66 (0.67H, d, *J* = 11.8 Hz), 4.73 (1H, d, *J* = 11.8 Hz), 4.77–4.94 (4H, m), 7.24–7.37 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.16, 25.20, 25.8, 26.15, 26.18, 28.8, 29.4, 29.5, 29.57, 29.60, 29.65, 29.69, 29.74, 29.8, 29.9, 32.0, 33.1, 33.3, 64.2, 65.7, 67.0, 67.2, 67.6, 68.8, 68.9, 69.7, 69.8, 70.3, 70.4, 70.7, 72.9, 73.3, 73.55, 73.57, 73.7, 73.9, 74.7, 75.1, 75.2, 76.5, 76.6, 77.2, 78.6, 78.7, 99.3, 99.9, 127.35, 127.39, 127.42, 127.5, 127.65, 127.72, 127.8, 127.89, 127.93, 128.10, 128.13, 128.2, 128.25, 128.28, 128.32, 128.33, 128.4, 137.0, 137.3, 138.1, 138.18, 138.24, 138.33, 138.4, 138.5, 172.8, 173.0; HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9171. Found 1200.9154. The more polar residue (122 mg) was dissolved in dry THF (5.0 mL), and TBAF (1.0 M in THF, 0.75 mL, 0.75 mmol) was added at room temperature. The reaction mixture was stirred for 15 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (20 g). Elution with hexane–EtOAc (10:1 to 3:2) afforded β-(2*S*,3*R*,4*S*)-**14** (93.4 mg, 49%) as an oil, n_D^{26} 1.5138; $[\alpha]_D^{26}$ +29.3 (*c* 0.16, $CHCl_3$); IR (film) ν_{max} 3347, 2923, 2852, 1614, 1455, 1362, 1102, 1076, 733, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (68H, m), 1.55–2.16 (6H, m), 3.46–3.61 (4H, m), 3.71 (0.33H, m), 3.81 (1H, dd, *J* = 9.4, 9.9 Hz), 3.84 (1H, br s), 4.06 (0.67H, dd, *J* = 5.3, 10.6 Hz), 4.09–4.30 (3H, m), 4.34–4.48 (3.33H, m), 4.57–4.62 (1.67H, m), 4.66–4.78 (3H, m), 4.86 (0.33H, d, *J* = 10.9 Hz), 4.86 (0.67H, d, *J* = 11.4 Hz), 4.91 (0.67H, d, *J* = 11.6 Hz), 4.94 (0.33H, d, *J* = 10.9 Hz), 7.25–7.37 (20 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.0, 25.1, 25.9, 26.26, 26.29, 28.8, 29.5, 29.55, 29.59, 29.65, 29.70, 29.8, 29.9, 32.0, 33.2, 33.3, 64.1, 65.1, 65.6, 66.2, 67.7, 68.0, 68.7, 68.8, 68.9, 69.0, 73.1, 73.2, 73.3, 73.35, 73.44, 73.5, 73.59, 73.63, 74.5, 74.9, 75.3, 77.2, 79.3, 79.4, 82.08, 82.13, 104.0, 104.2, 127.3, 127.4, 127.48, 127.51, 127.6, 127.7, 127.8, 127.9, 128.07, 128.12, 128.22, 128.24, 128.29, 128.33, 137.5, 137.6, 138.1, 138.2, 138.3, 138.6, 172.6, 173.1; HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9171. Found 1200.9174.

4.6.2. α-(2*S*,3*R*,4*R*)-Isomer 13′ and β-(2*S*,3*R*,4*R*)-isomer 14′. In the same manner, (2*S*,3*R*,4*R*)-**11′** (91.8 mg, 0.116 mmol) yielded 37.2 mg (27%) of α-(2*S*,3*R*,4*R*)-**13′** as an oil, n_D^{26} 1.5080; $[\alpha]_D^{26}$ +25.1 (*c* 0.22, $CHCl_3$); IR (film) ν_{max} 3365, 2923, 2852, 1624, 1455, 1366, 1100, 1060, 734, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (68H, m), 1.59–2.07 (6H, m), 3.27 (1H, br s), 3.35–4.23 (10H), 4.37 (1H, d, *J* = 12.1 Hz), 4.49 (1H, d, *J* = 12.3 Hz), 4.53 (1H, d, *J* = 11.6 Hz), 4.64 (1H, d, *J* = 11.8 Hz), 4.72 (1H, d, *J* = 11.6 Hz), 4.80 (1H, d, *J* = 3.6 Hz), 4.84 (2H, d, *J* = 11.4 Hz), 4.92 (1H, d, *J* = 11.4 Hz), 7.21–7.39 (20H, m); HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9170. Found 1200.9138, and 55.9 mg (40%) of β-(2*S*,3*R*,4*R*)-**14′** as an oil, n_D^{26} 1.5138; $[\alpha]_D^{26}$ +8.5 (*c* 0.20, $CHCl_3$); IR (film) ν_{max} 3376, 2924, 2852, 1626, 1455, 1362, 1100, 733, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (68H, m), 1.57–2.03 (6H, m), 3.39–4.29 (11H), 4.36–4.40 (2H, m), 4.49 (1H, d, *J* = 11.8 Hz), 4.60 (1H, d, *J* = 11.5 Hz), 4.68–4.76 (3H, m), 4.88 (1H, d, *J* = 11.1 Hz), 4.93 (1H, d, *J* = 11.6 Hz), 7.28–7.33 (20H, m); HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9171. Found 1200.9131.

4.7. 2-α-D-Galactopyranosyloxymethyl-4-tetradecyl-1-hexacosanoylazetidin-3-ol

4.7.1. α-(2*S*,3*R*,4*S*)-Isomer 2. To a solution of α-(2*S*,3*R*,4*S*)-**13** (35.6 mg, 29.6 μmol) in EtOH (3.0 mL) and $CHCl_3$ (1.0 mL), a catalytic amount of $Pd(OH)_2$ (20% on carbon) was added. The reaction mixture was vigorously stirred for 18 h at room temperature under H_2 and filtered through a Celite pad. The filter cake was washed with $CHCl_3$-MeOH, and the combined filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (3 g). Elution with $CHCl_3$-MeOH (20:1 to 8:1) afforded α-(2*S*,3*R*,4*S*)-**2** (21.0 mg, 84%) as an amorphous solid, $[\alpha]_D^{28}$ +73.1 (*c* 0.20, pyridine); IR (KBr) ν_{max} 3375, 2919, 2850, 1614, 1468, 1152, 1076, 721 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 0.84–0.86 (6H, m) 1.23–1.41 (67H, m), 1.64–1.68 (1H, m), 1.83–1.89 (2.6H, m), 2.24–2.61 (3.4H, m), 4.01 (0.4H, dd, *J* = 3.4, 10.9 Hz), 4.13 (0.6H, dd, *J* = 2.9, 10.4 Hz), 4.36–4.71 (8.40H, m), 4.80 (0.60H, dd, *J* = 4.6, 10.4 Hz), 5.05 (0.4H, m), 5.20 (0.6H, m), 5.39 (0.6H, *J* = 3.4 Hz), 5.43 (0.4H, *J* = 3.6 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 14.3, 22.9, 25.59, 25.62, 26.2, 26.6, 27.2, 29.5, 29.6, 29.7, 29.9, 30.0, 30.2, 30.4, 32.1, 33.4, 33.7, 62.88, 62.91, 65.3, 65.7, 66.4, 66.59, 66.61, 68.9, 69.6, 70.3, 70.55, 70.59, 71.1, 71.2, 71.5, 71.8, 72.7, 73.4, 101.2, 101.6, 173.2; HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7292. Found 840.7268.

4.7.2. β-(2*S*,3*R*,4*S*)-Isomer 15. In the same manner, β-(2*S*,3*R*,4*S*)-**14** (26.0 mg, 21.7 μmol) yielded 15.4 mg (85%) of β-(2*S*,3*R*,4*S*)-**15** as an amorphous solid, $[\alpha]_D^{28}$ +24.5 (*c* 0.15, pyridine); IR (KBr) ν_{max} 3410, 2919, 2850, 1601, 1469, 1084, 721 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 0.84–0.86 (6H, m), 1.23–1.43 (67H, m), 1.63–1.67 (1H, m), 1.75–1.93 (2.6H, m), 2.26–2.59 (3.4H, m), 4.01 (0.6H, dd, *J* = 5.8, 6.1 Hz), 4.06–4.20 (2H, m), 4.40–4.61 (5.8H, m), 4.68–4.73

(1.6H, m), 4.87 (1H, d, J = 7.7 Hz), 4.94 (0.4H, m), 5.10 (0.6H, m); ^{13}C NMR (100 MHz, pyridine-d_5) δ 14.3, 22.9, 25.5, 25.6, 26.2, 26.7, 27.2, 29.5, 29.6, 29.8, 29.95, 30.01; 30.2, 30.4, 32.1, 33.4, 33.7, 62.4, 65.1, 65.4, 66.1, 66.5, 67.4, 69.2, 69.9, 70.2, 70.3, 70.7, 72.2, 72.7, 75.35, 75.42, 77.0, 77.2, 105.5, 105.7, 173.1, 173.6; HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7292. Found 840.7264.

4.7.3. α-(2*S*,3*R*,4*R*)-Isomer 24. In the same manner, α-(2*S*,3*R*,4*R*)-**13′** (32.0 mg, 26.6 μmol) yielded 20.4 mg (91%) of α-(2*S*,3*R*,4*R*)-**24** as an amorphous solid, $[\alpha]_D^{26}$ + 52.3 (*c* 0.11, pyridine); IR (KBr) ν_{max} 3377, 2919, 2850, 1625, 1468, 1149, 1075, 721 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5, 70 °C) δ 0.88–0.91 (6H, m), 1.31–1.66 (68H, m), 1.82 (2H, dddd, J = 7.3, 7.3, 7.5, 7.5 Hz), 1.94 (1H, m), 2.16 (1H, m), 2.30–2.37 (2H, m), 4.10 (1H, dd, J = 4.4, 10.6 Hz), 4.25–4.47 (7H, m), 4.53 (2H, m), 4.59 (1H, m), 5.35 (1H, d, J = 3.9 Hz); HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7292. Found 840.7261.

4.7.4. β-(2*S*,3*R*,4*R*)-Isomer 25. In the same manner, β-(2*S*,3*R*,4*R*)-**14′** (52.2 mg, 43.5 μmol) yielded 33.2 mg (91%) of β-(2*S*,3*R*,4*R*)-**25** as an amorphous solid, $[\alpha]_D^{26}$ − 6.7 (*c* 0.21, pyridine); IR (KBr) ν_{max} 3365, 2919, 2850, 1622, 1468, 1074, 721 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5, 70 °C) δ 0.88–0.91 (6H, m), 1.31–1.60 (68H, m), 1.78 (2H, dddd, J = 7.3, 7.5, 7.5, 7.5 Hz), 1.88 (1H, m), 2.14 (1H, m), 2.25–2.39 (2H, m), 3.96 (1H, dd, J = 5.8, 5.8 Hz), 4.04 (1H, dd, J = 3.4, 9.2 Hz), 4.20–4.37 (6H, m), 4.43 (1H, d, J = 3.2 Hz), 4.46 (1H, m), 4.51 (1H, dd, J = 4.8, 10.9 Hz), 4.82 (1H, d, J = 7.7 Hz); HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7293. Found 840.7305.

4.8. 1,3-bis-*tert*-Butyldimethylsilyloxy-2-(*p*-toluenesulfonylamino)octadecan-5-ol

4.8.1. (2*S*,3*R*,5*R*)-Isomer 7. To a solution of (2*S*,3*S*,4*S*,5*R*)-**5′** (540 mg, 0.773 mmol) in dry THF (10.0 mL), DIBAL-H (4.2 mL of a 0.95 M solution in hexane, 3.99 mmol) was added at −78 °C. The reaction mixture was then warmed gradually to 0 °C with stirring in the course of 3 h, quenched with saturated aqueous sodium potassium tartrate solution, and diluted with Et_2O. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (25 g). Elution with hexane–EtOAc (30:1 to 15:1) afforded (2*S*,3*R*,5*R*)-**7** (462 mg, 85%) as an oil, n_D^{25} 1.4830; $[\alpha]_D^{25}$ − 4.1 (*c* 0.52, $CHCl_3$); IR (film) ν_{max} 3502, 3291, 2926, 2855, 1599, 1464, 1330, 1254, 1162, 1093, 837, 778, 668 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.07 (3H, s, SiMe), −0.03 (3H, s, SiMe), 0.01 (3H, s, SiMe), 0.05 (3H, s, SiMe), 0.83 (9H, s, *t*Bu), 0.85 (9H, s, *t*Bu), 0.88 (3H, t, J = 6.3 Hz, 18-Me), 1.26–1.41 (24H, m, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-H$_2$), 1.69–1.73 (2H, m, 4-H$_2$), 2.17 (1H, br s, OH), 2.41 (3H, s, Ar-Me), 3.32 (1H, dd, J = 6.3, 10.2 Hz, 1-H), 3.49 (1H, m, 2-H), 3.70 (1H, dd, J = 4.3, 10.2 Hz, 1-H), 3.79 (1H, m, 3-H), 4.07 (1H, ddd, J = 5.3, 5.6, 5.6 Hz, 5-H), 5.06 (1H, br s, NH), 7.28 (2H, d, J = 7.9 Hz, *m*Ar), 7.74 (2H, d, J = 7.9 Hz, *o*Ar); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.6, −5.3, −4.5, −4.3, 14.2, 18.1, 18.2, 21.6, 22.8, 25.7, 25.9, 29.4, 29.70, 29.73, 32.0, 38.2, 39.3, 58.3, 61.0, 68.6, 70.1, 127.1, 129.6, 137.5, 143.3; Anal. Calcd for $C_{37}H_{73}NO_5SSi_2$: C, 63.47; H, 10.51; N, 2.00. Found: C, 63.33; H, 10.72; N, 1.96.

4.8.2. (2*S*,3*R*,5*S*)-Isomer 7′. A mixture of (2*S*,3*R*,5*R*)-**7** (350 mg, 0.500 mmol), dry CH_2Cl_2 (10.0 mL), TPAP (35.6 mg, 0.101 mmol), NMO (238 mg, 2.03 mmol), and MS 4A (1.01 g) was stirred for 3 h at room temperature and concentrated in vacuo. The residue was filtered through a silica gel pad and concentrated in vacuo to give crude (2*S*,3*R*)-**26** (285 mg). This compound was immediately employed for the next step without further purification. To a solution of crude (2*S*,3*R*)-**26** (285 mg) in dry THF (8.0 mL), $LiBEt_3H$ (2.0 mL of a 1.06 M solution in THF, 2.12 mmol) was added at −78 °C. The reaction mixture was stirred for 4 h at −78 °C, quenched with saturated aqueous Na_2CO_3 solution and H_2O_2 (30% in water), and diluted with Et_2O. The mixture was extracted with Et_2O. The combined organic extract was successively washed with saturated aqueous $Na_2S_2O_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (30 g). Elution with hexane–EtOAc (20:1 to 10:1) afforded (2*S*,3*R*,5*S*)-**7′** (105 mg, 37%) as an oil, n_D^{26} 1.4842; $[\alpha]_D^{26}$ + 5.5 (*c* 0.62, $CHCl_3$); IR (film) ν_{max} 3522, 3312, 2926, 2855, 1599, 1470, 1335, 1254, 1163, 1091, 836, 778, 667 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.05 (3H, s, SiMe), −0.00 (3H, s, SiMe), 0.03 (3H, s, SiMe), 0.09 (3H, s, SiMe), 0.846 (9H, s, *t*Bu), 0.852 (9H, s, *t*Bu), 0.88 (3H, t, J = 6.6 Hz, 18-Me), 1.26 (24H, m, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-H$_2$), 1.51–1.68 (2H, m, 4-H$_2$), 2.42 (3H, s, Ar-Me), 3.36 (1H, m, 2-H), 3.49 (1H, dd, J = 5.3, 10.2 Hz, 1-H), 3.74 (1H, m, 3-H), 3.76 (1H, dd, J = 3.6, 10.2 Hz, 1-H), 4.05 (1H, ddd, J = 4.9, 5.3, 5.6 Hz, 5-H), 4.87 (1H, d, J = 6.9 Hz, NH), 7.29 (2H, d, J = 7.9 Hz, *m*Ar), 7.75 (2H, d, J = 8.2 Hz, *o*Ar); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.5, −5.2, −4.6, −4.2, 14.2, 18.1, 18.3, 21.6, 22.8, 25.5, 25.9, 26.0, 29.4, 29.7, 29.76, 29.81, 32.0, 38.4, 39.7, 58.2, 60.8, 68.5, 70.3, 127.1, 129.6, 137.5, 143.3; Anal. Calcd for $C_{37}H_{73}NO_5SSi_2$: C, 63.47; H, 10.51; N, 2.00. Found: C, 63.31; H, 10.69; N, 2.03, and (2*S*,3*R*,5*R*)-**7** (176 mg, 62%).

4.9. 3-*tert*-Butyldimethylsilyloxy-2-*tert*-butyldimethylsilyloxymethyl-5-tridecyl-1-*p*-toluenesulfonylpyrrolidine

4.9.1. (2*S*,3*R*,5*S*)-Isomer 16. To an ice-cooled solution of (2*S*,3*R*,5*R*)-**7** (336 mg, 0.480 mmol) in dry pyridine (5.0 mL), MsCl (0.30 mL, 3.88 mmol) was added in one portion. The reaction mixture was stirred for 40 h at 4 °C and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with a saturated aqueous $CuSO_4$ solution, water, a saturated aqueous $NaHCO_3$ solution, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in dry THF (9.5 mL), cooled to 0 °C, and NaH (60% in mineral oil, 57.5 mg,

1.44 mmol) was added. The reaction mixture was stirred for 40 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (8.5 g). Elution with hexane–EtOAc (30:1) afforded (2*S*,3*R*,5*S*)-**16** (323 mg, 99%) as an oil, n_D^{25} 1.4820; $[\alpha]_D^{25}$ +19.5 (*c* 0.55, $CHCl_3$); IR (film) ν_{max} 2927, 2855, 1601, 1464, 1343, 1254, 1159, 1100, 836, 777, 666 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.03 (6H, s, SiMe × 2), 0.06 (3H, s, SiMe), 0.07 (3H, s, SiMe), 0.79 (9H, s, *t*Bu), 0.88 (3H, t, *J* = 6.6 Hz, 13′-Me), 0.89 (9H, s, *t*Bu), 1.26 (22H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′-H_2), 1.49–1.61 (1H, m, 1′-H), 1.76 (1H, d, *J* = 13.5 Hz, 4-H), 1.88–1.94 (1H, m, 1′-H), 2.15 (1H, ddd, *J* = 4.3, 8.9, 13.2 Hz, 4-H), 2.39 (3H, s, Ar-Me), 3.37 (1H, dd, *J* = 9.2, 10.2 Hz, 2-C*H*H-OTBS), 3.74 (1H, dd, *J* = 3.6, 9.2 Hz, 2-H), 3.91 (1H, m, 5-H), 4.04 (1H, dd, *J* = 3.6, 10.2 Hz, 2-CH*H*-OTBS), 4.40 (1H, d, *J* = 4.0 Hz, 3-H), 7.22 (2H, d, *J* = 7.9 Hz, *m*Ar), 7.78 (2H, d, *J* = 7.9 Hz, *o*Ar); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.3, −5.2, −4.9, −4.8, 14.2, 17.8, 18.3, 21.5, 22.8, 25.6, 26.0, 26.8, 29.4, 29.58, 29.62, 29.7, 29.8, 32.0, 33.7, 35.8, 61.7, 64.0, 71.2, 74.1, 126.6, 129.1, 140.3, 142.2; Anal. Calcd for $C_{37}H_{71}NO_4SSi_2$: C, 65.14; H, 10.49; N, 2.05. Found: C, 65.07; H, 10.71; N, 2.04.

4.9.2. (2*S*,3*R*,5*R*)-Isomer 16′. In the same manner, (2*S*,3*R*,5*S*)-**7′** (531 mg, 0.758 mmol) yielded 516 mg (100%) of (2*S*,3*R*,5*R*)-**16′** as an oil, n_D^{26} 1.4838; $[\alpha]_D^{26}$ −14.5 (*c* 0.52, $CHCl_3$); IR (film) ν_{max} 2926, 2855, 1600, 1469, 1351, 1254, 1163, 1095, 837, 778, 663 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ −0.15 (3H, s, SiMe), −0.13 (3H, s, SiMe), 0.09 (3H, s, SiMe), 0.10 (3H, s, SiMe), 0.67 (9H, s, *t*Bu), 0.88 (3H, t, *J* = 6.9 Hz, 13′-Me), 0.90 (9H, s, *t*Bu), 1.27 (22H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′-H_2), 1.46–1.73 (3H, m, 4-H, 1′-H_2), 2.16 (1H, m, 4-H), 2.39 (3H, s, Ar-Me), 3.36 (1H, dd, *J* = 9.2, 10.2 Hz, 2-C*H*H-OTBS), 3.47–3.58 (2H, m, 2, 5-H), 3.87 (1H, dd, *J* = 3.6, 10.2 Hz, 2-CH*H*-OTBS), 4.19 (1H, m, 3-H), 7.22 (2H, d, *J* = 7.9 Hz, *m*Ar), 7.78 (2H, d, *J* = 7.9 Hz, *o*Ar); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.3, −5.2, −4.84, −4.81, 14.2, 18.0, 18.4, 21.6, 22.8, 25.8, 26.1, 29.4, 29.71, 29.73, 29.8, 32.0, 36.1, 39.7, 60.0, 65.0, 71.75, 71.82, 127.8, 129.3, 134.3, 142.9; Anal. Calcd for $C_{37}H_{71}NO_4SSi_2$: C, 65.14; H, 10.49; N, 2.05. Found: C, 65.07; H, 10.68; N, 2.08.

4.10. 3-*tert*-Butyldimethylsilyloxy-2-*tert*-butyldimethylsilyloxymethyl-5-tridecylpyrrolidine

4.10.1. (2*S*,3*R*,5*S*)-Isomer 17. To a solution of (2*S*,3*R*,5*S*)-**16** (495 mg, 0.726 mmol) in dry DME (10.0 mL), a solution of sodium naphthalenide (6.0 mL) [Prepared as follows. To a solution of naphthalene (1.86 mg, 14.5 mmol) in dry DME (12.0 mL), sodium (267 mg, 11.6 mmol) was added under argon. The mixture was stirred for 4 h at room temperature.] was added at −78 °C under argon. The reaction mixture was stirred for 90 min and diluted with water. The mixture was extracted with $CHCl_3$. The combined organic extract was dried over Mg_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (25 g). Elution with $CHCl_3$-MeOH (1:0 to 20:1) afforded (2*S*,3*R*,5*S*)-**17** (379 mg, 99%) as an oil, n_D^{23} 1.4560; $[\alpha]_D^{23}$ −19.8 (*c* 1.03, $CHCl_3$); IR (film) ν_{max} 2926, 2855, 1464, 1254, 1114, 837, 776, 669 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.04 (12H, s, SiMe × 4), 0.87–0.88 (21H, m, *t*Bu × 2, 13′-Me), 1.25–1.50 (25H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′-H_2, 4-H), 2.07–2.17 (2H, m, 4-H, NH), 2.95 (1H, ddd, *J* = 4.3, 4.6, 5.6 Hz, 2-H), 3.08 (1H, dddd, *J* = 6.6, 6.9, 6.9, 7.3 Hz, 5-H), 3.59 (2H, d, *J* = 4.3 Hz, 2-CH_2-OTBS), 4.09 (1H, ddd, *J* = 5.6, 6.3, 6.6 Hz, 3-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.33, −5.28, −4.7, −4.4, 14.2, 18.1, 18.4, 22.8, 25.9, 26.0, 27.2, 29.4, 29.7, 29.8, 32.0, 38.0, 41.4, 56.4, 62.4, 66.8, 73.5. This compound was immediately employed for the next step without further purification.

4.10.2. (2*S*,3*R*,5*R*)-Isomer 17′. In the same manner, (2*S*,3*R*,5*R*)-**16′** (203 mg, 0.298 mmol) yielded 151 mg (96%) of (2*S*,3*R*,5*R*)-**17′** as an oil, n_D^{26} 1.4573; $[\alpha]_D^{26}$ −20.5 (*c* 0.58, $CHCl_3$); IR (film) ν_{max} 2925, 2855, 1464, 1254, 1093, 836, 776, 667 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.037 (6H, s, SiMe × 2), 0.043 (6 H, s, SiMe × 2), 0.85–0.89 (21H, m*t*Bu × 2, 13′-Me), 1.24–1.48 (25H, m, 4-H, 1′, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′-H_2), 1.75 (1H, ddd, *J* = 3.0, 6.6, 12.9 Hz, 4-H), 1.90 (1H, br s, NH), 2.93 (1H, ddd, *J* = 4.3, 4.3, 4.3 Hz, 2-H), 3.24 (1H, m, 3-H), 3.59 (1H, dd, *J* = 4.6, 10.9 Hz, 2-C*H*H-OTBS), 3.63 (1H, dd, *J* = 4.6, 10.9 Hz, 2-CH*H*-OTBS), 4.09 (1H, dddd, *J* = 3.3, 3.3, 3.6, 4.0 Hz, 5-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.34, −5.31, −4.6, −4.5, 14.2, 18.1, 18.4, 22.8, 25.9, 26.0, 27.2, 29.4, 29.68, 29.74, 29.9, 32.0, 36.8, 42.3, 57.3, 63.3, 68.7, 73.9. This compound was immediately employed for the next step without further purification.

4.11. 3-*tert*-Butyldimethylsilyloxy-2-*tert*-butyldimethylsilyloxymethyl-5-tridecyl-1-hexacosanoylpyrrolidine

4.11.1. (2*S*,3*R*,5*S*)-Isomer 18. To an ice-cooled solution of (2*S*,3*R*,5*S*)-**17** (222 mg, 0.420 mmol) and *i*-Pr_2NEt (585 μL, 3.36 mmol) in dry CH_2Cl_2 (8.4 mL), EDC (121 mg, 0.631 mmol), cerotic acid (250 mg, 0.630 mmol), and a catalytic amount of DMAP were added. The reaction mixture was stirred for 48 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (10 g). Elution with hexane–EtOAc (50:1 to 30:1) afforded (2*S*,3*R*,5*S*)-**18** (322 mg, 84%), mp 34.0–35.0 °C (colorless needles from hexane–EtOAc); $[\alpha]_D^{23}$ +10.6 (*c* 1.13, $CHCl_3$); IR (KBr) ν_{max} 2918, 2850, 1631, 1470, 1410, 1256, 837, 774 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.02–0.09 (12H, m, SiMe × 4), 0.83–0.89 (24H, m, *t*Bu × 2, 13′-Me, 26″-Me), 1.25 (66H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″, 18″, 19″, 20″, 21″, 22″, 23″, 24″, 25″-H_2), 1.56–2.37 (8H, m, 4, 1′, 2″, 3″-H_2), 3.18 (0.33H, dd, *J* = 9.9, 10.2 Hz, 2-C*H*H-OTBS), 3.53 (0.67H, dd,

J = 6.6, 9.9 Hz, 2-C*H*H-OTBS), 3.56 (0.33H, m, 2-CH*H*-OTBS), 3.69–3.76 (1H, m, 2, 5-H), 3.83 (0.67H, dd, J = 3.0, 9.9 Hz, 2-CH*H*-OTBS), 3.94 (0.33H, m, 5-H), 4.02 (0.67H, dd, J = 3.0, 6.3 Hz, 2-H), 4.33 (0.67H, d, J = 4.3 Hz, 3-H), 4.38 (0.33H, d, J = 4.3 Hz, 3-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.4, −5.34, −5.31, −5.25, −4.9, −4.74, −4.68, −4.6, 14.2, 17.9, 18.3, 22.8, 25.77, 25.79, 25.9, 26.0, 26.1, 26.89, 26.93, 29.40, 29.44, 29.5, 29.56, 29.63, 29.7, 29.8, 32.0, 32.5, 34.5, 35.1, 35.2, 35.8, 36.7, 58.5, 59.6, 60.8, 64.0, 68.7, 69.2, 74.2, 74.5, 172.1, 172.4; Anal. Calcd for $C_{56}H_{115}NO_3Si_2$: C, 74.18; H, 12.78; N, 1.54. Found: C, 73.92; H, 13.03; N, 1.53.

4.11.2. (2*S*,3*R*,5*R*)-Isomer 18′. In the same manner, (2*S*,3*R*,5*R*)-**17′** (141 mg, 0.267 mmol) yielded 188 mg (78%) of (2*S*,3*R*,5*R*)-**18′**, mp 43.0–44.0 °C (colorless needles from hexane–EtOAc); $[\alpha]_D^{26}$ −2.8 (*c* 0.56, $CHCl_3$); IR (KBr) ν_{max} 2920, 2850, 1633, 1472, 1413, 1253, 836, 773 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.03–0.07 (12H, m, SiMe × 4), 0.85–0.91 (24H, m, *t*Bu × 2, 13′-Me, 26″-Me), 1.25 (66H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″, 18″, 19″, 20″, 21″, 22″, 23″, 24″, 25″-H_2), 1.63–2.36 (8H, m, 4, 1′, 2″, 3″-H_2),, 3.32 (0.65H, dd, J = 9.6, 10.2 Hz, 2-C*H*H-OTBS), 3.55 (0.65H, dd, J = 5.3, 10.6 Hz, 2-CH*H*-OTBS), 3.63–3.71 (1.35H, m, 2-CH_2-OTBS, 2-H), 3.83 (0.35H, m, 5-H), 3.96 (0.35H, m, 2-H), 4.10 (0.65H, m, 5-H), 4.30 (0.65H, d, J = 3.6 Hz, 3-H), 4.43 (0.35H, ddd, J = 3.6, 4.6, 4.6 Hz, 3-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.31, −5.25, −4.7, −4.59, −4.58, 14.2, 18.0, 18.3, 18.4, 22.8, 25.6, 25.7, 25.8, 25.85, 25.94, 26.1, 26.2, 26.3, 29.5, 29.6, 29.7, 29.8, 32.0, 33.8, 34.9, 36.0, 37.1, 38.7, 39.5, 40.3, 56.9, 57.3, 61.2, 63.8, 67.8, 69.5, 71.1, 72.5, 172.8, 173.0; Anal. Calcd for $C_{56}H_{115}NO_3Si_2$: C, 74.18; H, 12.78; N, 1.54. Found: C, 73.83; H, 12.89; N, 1.55.

4.12. 3-*tert*-Butyldimethylsilyloxy-2-hydroxymethyl-5-tridecyl-1-hexacosanoylpyrrolidine

4.12.1. (2*S*,3*R*,5*S*)-Isomer 19. To an ice-cooled solution of (2*S*,3*R*,5*S*)-**18** (86.2 mg, 95.1 μmol) in dry THF (9.0 mL), TFA (10% in water, 3.0 mL) was added. The reaction mixture was stirred for 5 h at room temperature and neutralized with aqueous NaOH solution. The mixture was extracted with Et_2O. The combined organic extract was successively washed with a saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (7 g). Elution with hexane–EtOAc (10:1 to 3:1) afforded (2*S*,3*R*,5*S*)-**19** (69.0 mg, 92%) as a solid, IR (KBr) ν_{max} 3390, 2919, 2851, 1614, 1469, 1256, 837, 776, 721 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.06 (3H, s, SiMe), 0.08 (3H, s, SiMe), 0.85–0.89 (15H, m, *t*Bu, 13′-Me, 26″-Me), 1.25 (66H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″, 18″, 19″, 20″, 21″, 22″, 23″, 24″, 25″-H_2), 1.62–2.09 (6H, m, 4, 1′, 3″-H_2), 2.31 (2H, t, J = 7.6 Hz, 2″-H_2), 3.36–4.15 (5H, m, 2-CH_2–OH, 2, 3, 5-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.8, −4.7, 14.2, 17.9, 22.8, 25.7, 25.9, 26.7, 29.4, 29.5, 29.6, 29.66, 29.73, 29.8, 32.0, 34.9, 35.5, 36.9, 59.9, 65.1, 70.4, 74.4, 174.4. This compound was immediately employed for the next step without further purification.

4.12.2. (2*S*,3*R*,5*R*)-Isomer 19′. In the same manner, (2*S*,3*R*,5*R*)-**18′** (241 mg, 0.266 mmol) yielded 149 mg (71%) of (2*S*,3*R*,5*R*)-**19′** as a solid, IR (KBr) ν_{max} 3388, 2919, 2850, 1615, 1468, 1254, 836, 777, 721 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.06 (3H, s, SiMe), 0.07 (3H, s, SiMe), 0.86–0.90 (15H, m, *t*Bu, 13′-Me, 26″-Me), 1.25 (66H, m, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′, 12′, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″, 18″, 19″, 20″, 21″, 22″, 23″, 24″, 25″-H_2), 1.41–2.40 (8H, m, 4, 1′, 2″, 3′-H_2), 3.44–4.11 (5H, m, 2-CH_2-OH, 2, 3, 5-H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.7, −4.5, 14.2, 18.0, 22.8, 25.5, 25.8, 26.4, 29.4, 29.5, 29.55, 29.59, 29.63, 29.9, 32.0, 33.7, 36.8, 38.7, 58.1, 66.6, 69.2, 72.2, 174.9. This compound was immediately employed for the next step without further purification.

4.13. 2-(2‴,3‴,4‴,6‴-Tetra-*O*-benzyl-α-D-galactopyranosyloxy)methyl-5-tridecyl-1-hexacosanoylpyrrolidin-3-ol

4.13.1. α-(2*S*,3*R*,5*S*)-Isomer 20 and β-(2*S*,3*R*,5*S*)-isomer 21. To a solution of (2*S*,3*R*,5*S*)-**19** (114 mg, 0.144 mmol) in dry THF (5.0 mL), $SnCl_2$ (82.4 mg, 0.436 mmol), $AgClO_4$ (90.1 mg, 0.435 mmol), and powdered MS 4A (300 mg) were added. The reaction mixture was stirred for 90 min at room temperature. A solution of benzylgalactosyl fluoride (**12**, 189 mg, 0.348 mmol) in dry THF (5.0 mL) was added at −20 °C. The reaction mixture was then warmed gradually to 10 °C with stirring in the course of 4 h and filtered through silica gel. The filtrate was successively washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was separated into two fractions by silica gel column chromatography (2 g). Elution with hexane–EtOAc (10:1 to 6:1) afforded less polar residue (142 mg) and more polar residue (93 mg). The less polar residue was dissolved in dry THF (4.0 mL), and TBAF (1.0 M in THF, 0.50 mL, 0.50 mmol) was added at room temperature. The reaction mixture was stirred for 45 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (10 g). Elution with hexane–EtOAc (10:1 to 3:1) afforded α-(2*S*,3*R*,5*S*)-**20** (84.4 mg, 49%) as an oil, n_D^{26} 1.5145; $[\alpha]_D^{26}$ +32.3 (*c* 0.25, $CHCl_3$); IR (film) ν_{max} 3399, 2924, 2852, 1615, 1454, 1360, 1101, 1060, 734, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (66H, m), 1.59–1.63 (3H, m), 1.69 (0.33H, d, J = 14.5 Hz), 1.78 (0.67H, d, J = 13.5 Hz), 1.88 (0.67H, m), 2.05–2.33 (3.33H, m), 3.30 (0.67H, ddd, J = 2.2, 7.7, 9.4 Hz), 3.36 (0.67H, ddd, J = 8.7, 9.9 Hz), 3.41–3.55 (2H, m), 3.62 (0.67H, dd, J = 8.9, 9.2 Hz), 3.81–3.94 (4.33H, m), 4.03 (0.67H, dd, J = 3.6, 10.1 Hz), 4.05 (0.33H, dd, J = 3.9, 9.4 Hz), 4.12 (0.67H, m), 4.32–4.39 (2H, m), 4.46 (1H, d, J = 11.6 Hz), 4.56 (0.67H, d, J = 11.4 Hz), 4.57 (0.33H, d, J = 11.6 Hz),

4.62 (0.33H, d, J = 12.1 Hz), 4.66 (0.67H, d, J = 11.8 Hz), 4.73 (1H, d, J = 12.1 Hz), 4.73 (0.33H, d, J = 11.6 Hz), 4.76 (0.67H, d, J = 12.3 Hz), 4.80 (0.67H, d, J = 12.3 Hz), 4.82 (0.33H, d, J = 11.6 Hz), 4.84 (0.33H, d, J = 3.9 Hz), 4.86 (0.67H, d, J = 3.1 Hz), 4.91 (0.67H, d, J = 11.8 Hz), 4.94 (0.33H, d, J = 11.4 Hz), 7.23–7.40 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22,8, 25.8, 25.9, 26.9, 27.0, 29.5, 29.58, 29.63, 29.67, 29.70, 29.75, 29.79, 32.0, 33.3, 34.4, 34.9, 35.3, 35.7, 36.3, 58.5, 58.9, 66.4, 66.7, 67.4, 69.3, 69.7, 69.9, 70.4, 72.8, 73.2, 73.3, 73.5, 73.6, 73.7, 73.9, 74.6, 74.7, 74.9, 75.2, 76.5, 77.2, 78.6, 78.7, 98.25, 98.34, 127.37, 127.40, 127.49, 127.51, 127.6, 127.7, 127.81, 127.84, 127.9, 128.10, 128.12, 128.17, 128.24, 128.28, 128.31, 128.34, 137.3, 137.6, 138.1, 138.2, 138.3, 138.4, 138.5, 172.1, 172.5; HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9170. Found 1200.9131. The more polar residue was dissolved in dry THF (4.0 mL), and TBAF (1.0 M in THF, 0.50 mL, 0.50 mmol) was added at room temperature. The reaction mixture was stirred for 45 h at room temperature and diluted with water. The mixture was extracted with Et_2O. The combined organic extract was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (10 g). Elution with hexane–EtOAc (10:1 to 3:1) afforded β-(2*S*,3*R*,5*S*)-**21** (32.0 mg, 19%), mp 64.0–65.0 °C; $[\alpha]_D^{26}$ + 7.3 (*c* 0.16, $CHCl_3$); IR (film) ν_{max} 3420, 2923, 2852, 1615, 1455, 1362, 1100, 1076, 733, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (66H, m), 1.47–1.63 (3H, m), 1.80 (0.33H, d, J = 14.3 Hz), 1.88 (0.67H, d, J = 13.8 Hz), 1.95 (0.67H, m), 2.04–2.33 (3.33H, m), 3.20 (0.33H, dd, J = 10.6, 10.6 Hz), 3.29 (0.67H, dd, J = 4.8, 9.7 Hz), 3.41–3.84 (7.33H, m), 3.92–3.99 (0.67H, m), 4.23–4.26 (1.33H, m), 4.28 (0.33H, d, J = 7.7 Hz), 4.36 (0.67H, d, J = 11.8 Hz), 4.38 (0.33H, d, J = 11.8 Hz), 4.41 (0.67H, d, J = 7.7 Hz), 4.46 (0.33H, d, J = 11.8 Hz), 4.47 (0.67H, d, J = 11.8 Hz), 4.54 (0.67H, d, J = 5.0 Hz), 4.59 (0.67H, d, J = 11.6 Hz), 4.60 (0.67H, d, J = 10.4 Hz), 4.68 (0.67H, d, J = 11.8 Hz), 4.74 (0.67H, d, J = 11.6 Hz), 4.75 (0.33H, d, J = 5.6 Hz), 4.76 (0.67H, d, J = 11.4 Hz), 4.83 (1H, d, J = 10.4 Hz), 4.91 (0.67H, d, J = 11.8 Hz), 4.94 (0.33H, d, J = 11.4 Hz), 7.27–7.34 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.8, 25.9, 27.0, 27.1, 29.45, 29.54, 29.6, 29.7, 29.75, 29.79, 32.0, 33.3, 34.0, 34.9, 35.2, 35.4, 36.1, 58.6, 59.7, 67.0, 68.4, 68.9, 69.2, 70.5, 72.7, 73.0, 73.16, 73.23, 73.3, 73.55, 73.63, 74.1, 74.4, 74.6, 74.9, 75.4, 77.2, 79.4, 79.5, 82.1, 104.3, 104.6, 127.2, 127.4, 127.48, 127.50, 127.54, 127.58, 127.61, 127.7, 127.8, 127.9, 128.0, 128.10, 128.11, 128.2, 128.25, 128.33, 128.4, 137.2, 137.3, 138.1, 138.18, 138.22, 138.4, 138.8, 172.1, 172.6; HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9170. Found 1200.9177.

4.13.2. α-(2*S*,3*R*,5*R*)-Isomer 20′ and β-(2*S*,3*R*,5*R*)-isomer 21′. In the same manner, (2*S*,3*R*,5*R*)-**19′** (78.2 mg, 98.7 μmol) yielded 49.9 mg (42%) of α-(2*S*,3*R*,5*R*)-**20′** as an oil, n_D^{26} 1.5148; $[\alpha]_D^{26}$ + 32.3 (*c* 0.32, $CHCl_3$); IR (film) ν_{max} 3396, 2924, 2852, 1620, 1455, 1344, 1099, 1060, 734, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (66H, m), 1.53–1.64 (3.33H, m), 1.81–1.98 (2H, m), 2.08 (0.67H, m), 2.15–2.32 (2H, m), 3.34 (0.67H, dd, J = 10.1, 10.4 Hz), 3.38–3.60 (2.67H, m), 3.83–3.97 (4.67H, m), 4.02 (0.67H, dd, J = 3.6, 10.1 Hz), 4.06 (0.33H, dd, J = 3.6, 9.7 Hz), 4.18 (0.33H, m), 4.29 (0.67H, dd, J = 4.8, 10.9 Hz), 4.39 (1H, d, J = 11.6 Hz), 4.44 (2H, d, J = 11.6 Hz), 4.55 (0.67H, d, J = 11.4 Hz), 4.57 (0.33H, d, J = 11.4 Hz), 4.65 (0.33H, d, J = 11.8 Hz), 4.71–4.77 (2.67H, m), 4.80 (0.67H, d, J = 11.1 Hz), 4.83 (0.33H, J = 10.9 Hz), 4.84 (0.33H, d, J = 3.4 Hz), 4.88 (1H, m), 4.94 (0.67H, d, J = 13.0 Hz), 7.24–7.38 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.4, 26.4, 26.7, 29.45, 29.52, 29.6, 29.65, 29.68, 29.74, 29.8, 32.0, 33.7, 34.5, 35.7, 36.6, 36.7, 37.2, 56.7, 57.6, 63.3, 65.6, 68.7, 69.4, 69.56, 69.58, 69.63, 70.2, 72.9, 73.2, 73.3, 73.5, 73.7, 73.82, 73.84, 74.7, 74.9, 75.6, 76.2, 77.2, 79.1, 79.3, 98.0, 98.2, 127.25, 127.30, 127.39, 127.44, 127.5, 127.6, 127.77, 127.79, 128.0, 128.1, 128.16, 128.23, 128.27, 128.33, 137.5, 137.6, 137.8, 138.1, 138.2, 138.38, 138.41, 138.5, 172.5, 172.6; HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9170. Found 1200.9193, and 19.3 mg (16%) of β-(2*S*,3*R*,5*R*)-**21′** as an oil, n_D^{26} 1.5152; $[\alpha]_D^{26}$ + 5.0 (*c* 0.18, $CHCl_3$); IR (film) ν_{max} 3410, 2924, 2852, 1625, 1455, 1362, 1101, 1076, 733, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87–0.90 (6H, m), 1.26 (66H, m), 1.62–1.80 (4H, m), 2.00–2.32 (4H, m), 3.29 (0.4H, ddd, J = 4.6, 10.5, 11.0 Hz), 3.39 (0.4H, dd, J = 4.6, 7.7 Hz), 3.43–3.66 (4.6H, m), 3.77–3.91 (3.6H, m), 4.16 (1H, m), 4.25 (0.6H, dd, J = 4.8, 10.6 Hz), 4.32 (0.4H, d, J = 7.7 Hz), 4.38 (0.6H, d, J = 12.1 Hz), 4.38 (0.4H, d, J = 11.8 Hz), 4.44 (1.6H, m), 4.48 (0.6H, d, J = 7.5 Hz), 4.53–4.78 (3.4H, m), 4.58 (0.6H, d, J = 11.8 Hz), 4.61 (0.4H, d, J = 13.2 Hz), 4.85 (0.4H, d, J = 11.4 Hz), 4.92 (0.6H, d, J = 11.6 Hz), 4.94 (0.4H, d, J = 11.6 Hz), 7.24–7.34 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 25.4, 26.2, 26.4, 29.0, 29.5, 29.6, 29.7, 29.75, 29.79, 32.0, 33.8, 34.6, 36.1, 37.2, 37.3, 37.5, 56.8, 57.6, 65.3, 66.7, 68.7, 68.8, 70.8, 71.4, 71.7, 72.7, 73.1, 73.15, 73.19, 73.3, 73.46, 73.54, 73.6, 74.5, 74.6, 75.1, 75.4, 77.2, 79.4, 79.5, 82.1, 82.2, 104.5, 104.6, 127.3, 127.5, 127.6, 127.65, 127.71, 127.8, 127.87, 127.91, 128.11, 128.13, 128.25, 128.29, 128.33, 128,4, 137.29, 137.34, 138.07, 138.14, 138.2, 138.4, 138.6, 172.66, 172.70; HRMS (FAB) for $C_{78}H_{122}NO_8^+$ $[M+H]^+$ Calcd 1200.9170. Found 1200.9144.

4.14. 2-α-D-Galactopyranosyloxymethyl-5-tridecyl-1-hexacosanoylpyrrolidin-3-ol

4.14.1. α-(2*S*,3*R*,5*S*)-Isomer 4. To a solution of α-(2*S*,3*R*,5*S*)-**20** (39.8 mg, 33.1 μmol) in EtOH (3.0 mL) and $CHCl_3$ (1.0 mL), a catalytic amount of $Pd(OH)_2$ (20% on carbon) was added. The reaction mixture was vigorously stirred for 15 h at room temperature under H_2 and filtered through a Celite pad. The filter cake was washed with $CHCl_3$-MeOH, and the combined filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (3 g). Elution with $CHCl_3$-MeOH (12:1 to 8:1) afforded α-(2*S*,3*R*,5*S*)-**4** (26.5 mg, 95%) as an amorphous solid, $[\alpha]_D^{26}$ + 66.8 (*c*

0.21, pyridine); IR (KBr) ν_{max} 3397, 2919, 2850, 1615, 1468, 1150, 1074, 720 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 0.83–0.87 (6H, m), 1.22–1.44 (66H, m), 1.82–1.94 (2.5H, m), 2.10 (0.5H, d, J = 13.6 Hz), 2.15 (0.5H, m), 2.20 (0.5H, d, J = 13.3 Hz), 2.44–2.70 (4H, m), 3.74 (0.5H, dd, J = 3.1, 10.2 Hz), 3.90–3.97 (1H, m), 4.08 (0.5H, dd, J = 8.7, 8.7 Hz), 4.24 (0.5H, dd, J = 2.9, 9.4 Hz), 4.30–4.46 (4.5H, m), 4.56 (1.5H, m), 4.67 (0.5H, dd, J = 3.4, 9.9 Hz), 4.72 (0.5H, dd, J = 2.4, 9.4 Hz), 4.95–5.00 (1.5H, m), 5.40 (0.5H, d, J = 3.6 Hz), 5.42 (0.5H, d, J = 3.6 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 14.3, 22.9, 26.29, 26.32, 27.3, 27.5, 29.61, 29.64, 29.8, 29.9, 29.99, 30.01, 30.1, 32.12, 32.14, 33.8, 35.2, 35.3, 36.6, 36.8, 59.3, 59.6, 62.8, 62.9, 66.7, 67.6, 67.7, 68.8, 70.2, 70.4, 70.97, 70.99, 71.7, 73.1, 73.4, 73.5, 74.6, 100.6, 100.9, 172.2, 172.4; HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7292. Found 840.7258.

4.14.2. β-(2*S*,3*R*,5*S*)-Isomer 22. In the same manner, β-(2*S*,3*R*,5*S*)-**21** (30.6 mg, 25.5 μmol) yielded 13.6 mg (64%; not optimized) of β-(2*S*,3*R*,5*S*)-**22** as an amorphous solid, $[\alpha]_D^{26}$ +1.2 (*c* 0.15, pyridine); IR (KBr) ν_{max} 3398, 2919, 2850, 1609, 1468, 1080, 720 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 0.83–0.86 (6H, m), 1.22–1.40 (66H, m), 1.82–1.92 (2H, m), 2.08 (0.6H, d, J = 13.8 Hz), 2.16 (0.4H, d, J = 13.5 Hz), 2.07–2.18 (0.6H, m), 2.29 (0.4H, m), 2.42–2.62 (3.6H, m), 2.70 (0.4H, m), 3.60 (0.6H, dd, J = 10.1, 10.4 Hz), 3.84 (0.6H, dd, J = 8.9, 9.4 Hz), 3.97–4.19 (3.8H, m), 4.30–4.32 (1.2H, m), 4.41–4.50 (3.2H, m), 4.60–4.71 (1H, m), 4.69 (0.6H, dd, J = 3.1, 10.1 Hz), 4.84 (0.4H, d, J = 7.0 Hz), 4.86 (0.6H, J = 7.5 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 14.5, 23.2, 26.5, 26.6, 27.5, 27.7, 29.8, 29.85, 29.89, 30.0, 30.1, 30.15, 30.21, 32.3, 33.8, 34.9, 35.4, 35.6, 36.5, 37.0, 59.3, 59.8, 62.3, 62.8, 64.6, 68.30, 68.33, 68.8, 70.2, 70.5, 71.1, 72.6, 72.7, 73.1, 74.1, 75.55, 75.63, 77.2, 77.3, 106.2, 172.1, 172.4; HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7292. Found 840.7268.

4.14.3. α-(2*S*,3*R*,5*R*)-Isomer 3. In the same manner, α-(2*S*,3*R*,5*R*)-**20′** (21.4 mg, 17.8 μmol) yielded 13.5 mg (90%) of α-(2*S*,3*R*,5*R*)-**3** as an amorphous solid, $[\alpha]_D^{26}$ +42.2 (*c* 0.20, pyridine); IR (KBr) ν_{max} 3365, 2919, 2850, 1611, 1468, 1150, 1072, 720 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 0.83–0.86 (6H, m), 1.22–1.39 (67H, m), 1.78–1.87 (2.33H, m), 2.12–2.55 (4.67H, m), 3.86 (0.67H, dd, J = 4.6, 9.9 Hz), 4.08 (0.67H, dd, J = 9.4, 9.7 Hz), 4.17 (0.67H, m), 4.26 (0.33H, dd, J = 3.6, 9.9 Hz), 4.36–4.55 (4.67H, m), 4.61–4.69 (2H, m), 4.76 (0.67H, dd, J = 3.6, 9.9 Hz), 4.90 (0.33H, m), 4.97 (0.67H, d, J = 3.4 Hz), 5.04 (0.33H, m), 5.46 (0.33H, d, J = 3.4 Hz), 5.51 (0.67H, d, J = 3.4 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 14.3, 22.9, 25.9, 26.0, 26.6, 29.6, 29.8, 29.9, 30.0, 32.1, 34.0, 34.9, 36.4, 37.5, 38.6, 39.3, 57.3, 57.7, 62.7, 62.8, 66.1, 67.5, 68.1, 68.7, 70.3, 70.4, 71.1, 71.7, 71.9, 72.6, 73.1, 73.6, 100.6, 100.7, 172.9; HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7292. Found 840.7286.

4.14.4. β-(2*S*,3*R*,5*R*)-Isomer 27. In the same manner, β-(2*S*,3*R*,5*R*)-**21′** (18.6 mg, 15.5 μmol) yielded 12.3 mg (93%) of β-(2*S*,3*R*,5*R*)-**27** as an amorphous solid, $[\alpha]_D^{26}$ −15.3 (*c* 0.10, pyridine); IR (KBr) ν_{max} 3377, 2919, 2850, 1618, 1468, 1419, 1072, 721 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 0.83–0.87 (6H, m), 1.24–1.49 (67H, m), 1.77–2.00 (3H, m), 2.12 (0.33H, m), 2.36–2.60 (3.67H, m), 3.79 (0.67H, dd, J = 10.4, 10.4 Hz), 3.88 (0.33H, dd, J = 9.4, 9.7 Hz), 4.05 (0.33H, m), 4.14 (1H, dd, J = 6.1, 6.1 Hz), 4.19–4.21 (1H, m), 4.27–4.39 (1H, m), 4.46–4.63 (5H, m), 4.85–4.94 (2.33H, m), 5.08 (0.33H, m); ^{13}C NMR (100 MHz, pyridine-d_5) δ 14.3, 22.9, 25.95, 26.00, 26.4, 26.5, 29.6, 29.76, 29.81, 29.9, 30.0, 32.1, 34.0, 35.0, 36.4, 37.9, 38.4, 39.7, 57.3, 57.5, 62.2, 62.6, 67.4, 68.4, 70.1, 70.4, 70.6, 71.1, 71.5, 72.2, 72.5, 72.7, 75.5, 77.1, 77.3, 106.1, 106.2, 173.0, 173.1; HRMS (FAB) for $C_{50}H_{98}NO_8^+$ $[M+H]^+$ Calcd 840.7292. Found 840.7318.

4.15. Method of bioassay

4.15.1. Mice. C57BL/6 mice were purchased from Charles River Laboratories. Six to eight week-aged female mice were used for experiments. Experimental plans were approved by the Committee on Institutional Animal Care and Use at RIKEN.

4.15.2. Stimulation in vitro with glycolipid. Splenocytes from C57BL/6 mice were obtained by grinding the spleen between glass slides and removing red blood cells with red blood cell lysing buffer (Sigma–Aldrich). Approximately 4×10^5 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 mediumsupplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 150 μg/mL streptomycin, 150 U/mL penicillin, 10 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (Hepes), and 50 mM β-mercaptoethanol in humidified 5% CO_2 at 37 °C in 96-well U-bottom plates as reported by Hayakawa et al.[37] Cells were stimulated with 2 ng/mL, 20 ng/mL or 200 ng/mL of KRN7000 or other glycolipids. Forty-eight hoursafter the initiation of culture, cell-free supernatants were collected and stored at −70 °C for the measurement of cytokine levels.

4.15.3. Analysis of secreted cytokines by cytometric bead array. Cytometric bead array (CBA) was performed according to the manufacturer's protocols(BD Biosciences) for measurement of IL-2, IL-4, IL-10, IL-13, and IFN-γ levels,as described by Morgan et al.[38] In brief, IL-2, IL-4, IL-10, IL-13, and IFN-γ were detected simultaneously using the CBA Mouse Flex Set (BD Biosciences). Briefly, 50 μL of each sample was mixed with 50 μL of mixedcapture beads and 50 μL of the mouse soluble protein phycoerythrin (PE) detection reagent consisting of PE-conjugated anti-mouse IL-2, IL-4, IL-10, IL-13, and IFN-γ. The samples were incubated at room temperature for 1 h in the dark. After incubation with the PE detection reagent, the samples were washed once and resuspended in 300 μL of wash buffer before acquisition on the fluorescence activated cell sorter (FACS) Calibur (BD Biosciences). Data were analyzed using CBA software (BD Biosciences). Standard curves were generated for each cytokine using the mixed cytokine standard provided by the kit. The concentration

for each cytokine in cell supernatants was determined by interpolation from the corresponding standard curve. The range of detection was 0.8–5000 pg/mL for each cytokine measured by CBA.

Acknowledgments

We thank Dr. H. Nomi, Ms. A. Wakamatsu, Ms. N. Hirai, Ms. K. Utsunomiya, Ms. Y. Nagata, and Mr. H. Kamijuku for their technical support. Our thanks are due to Mr. K. Sakai and Dr. M. Iida (both at Seikagaku Kogyo Co.) for their helpful comments.

References and notes

1. Fuhshuku, K.; Mori, K. *Tetrahedron: Asymmetry* **2007**, *18*, 2104–2107.
2. Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176–2187.
3. Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592.
4. Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2776.
5. Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626–1629.
6. Zajonc, D. M.; Cantu, C., III; Mattner, J.; Zhou, D.; Savage, P. B.; Bendelac, A.; Wilson, I. A.; Teyton, L. *Nat. Immunol.* **2005**, *6*, 810–818.
7. Koch, M.; Stronge, V. S.; Shepherd, D.; Gadola, S. D.; Mathew, B.; Ritter, G.; Fersht, A. R.; Besra, G. S.; Schmidt, R. R.; Jones, E. Y.; Cerundolo, V. *Nat. Immunol.* **2005**, *6*, 819–826.
8. Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C. J.; Pellicci, D. G.; Koh, R.; Besra, G. S.; Bharadwaj, M.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *Nature* **2007**, *448*, 44–49.
9. Wu, D.; Xing, G.-W.; Poles, M. A.; Horowitz, A.; Kinjo, Y.; Sullivan, B.; Bodmer-Narkevitch, V.; Plettenburg, O.; Kronenberg, M.; Tsuji, M.; Ho, D. D.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1351–1356.
10. Rissoan, M.-C.; Soumelis, V.; Kadowaki, N.; Grouard, G.; Briere, F.; Malefyt, R. deW.; Liu, Y.-J. *Science* **1999**, *283*, 1183–1186.
11. Savage, P. B.; Teyton, L.; Bendelac, A. *Chem. Soc. Rev.* **2006**, *35*, 771–779.
12. Franck, R. W.; Tsuji, M. *Acc. Chem. Res.* **2006**, *39*, 692–701.
13. Berkers, C. R.; Ovaa, H. *Trends Pharmacol. Sci.* **2005**, *26*, 252–257.
14. Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631–1641.
15. Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem. Int. Ed.* **2004**, *43*, 3818–3822.
16. Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077–4080.
17. Wipf, P.; Pierce, J. G. *Org. Lett.* **2006**, *8*, 3375–3378.
18. Lu, X.; Song, L.; Metelitsa, L. S.; Bittman, R. *ChemBioChem.* **2006**, *7*, 1750–1756.
19. Fujio, M.; Wu, D.; Garcia-Navarro, R.; Ho, D. D.; Tsuji, M.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 9022–9023.
20. Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531–534.
21. Murata, K.; Toba, T.; Nakanishi, K.; Takahashi, B.; Yamamura, T.; Miyake, S.; Annoura, H. *J. Org. Chem.* **2005**, *70*, 2398–2401.
22. Toba, T.; Murata, K.; Yamamura, T.; Miyake, S.; Annoura, H. *Tetrahedron Lett.* **2005**, *46*, 5043–5047.
23. Toba, T.; Murata, K.; Nakanishi, K.; Takahashi, B.; Takemoto, N.; Akabane, M.; Nakatsuka, T.; Imajo, S.; Yamamura, T.; Miyake, S.; Annoura, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2781–2784.
24. Goff, R. D.; Gao, Y.; Mattner, J.; Zhou, D.; Yin, N.; Cantu, C., III; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Am. Chem. Soc.* **2004**, *126*, 13602–13603.
25. Yu, K. O. A.; Im, J. S.; Molano, A.; Dutronc, Y.; Illarionov, P. A.; Forestier, C.; Fujiwara, N.; Arias, I.; Miyake, S.; Yamamura, T.; Chang, Y.-T.; Besra, G. S.; Porcelli, S. A. *Proc. Natl. Acad. U.S.A.* **2005**, *102*, 3383–3388.
26. Tashiro, T.; Nakagawa, R.; Hirokawa, T.; Inoue, S.; Watarai, H.; Taniguchi, M.; Mori, K. *Tetrahedron Lett.* **2007**, *48*, 3343–3347.
27. Tashiro, T.; Fuhshuku, K.; Seino, K.; Watarai, H.; Taniguchi, M.; Mori, K. 48th Symposium on the Chemistry of Natural Products, Sendai, 2006, Symposium Papers, pp. 109–114.
28. Kobayashi, J.; Tsuda, M.; Cheng, J.; Ishibashi, M.; Takikawa, H.; Mori, K. *Tetrahedron Lett.* **1996**, *37*, 6775–6776.
29. Takikawa, H.; Maeda, T.; Seki, M.; Koshino, H.; Mori, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 97–111.
30. Yajima, A.; Takikawa, H.; Mori, K. *Liebigs Ann.* **1996**, 1083–1089.
31. Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988**, *53*, 4395–4398.
32. Takikawa, H.; Muto, S.; Mori, K. *Tetrahedron* **1998**, *54*, 3141–3150.
33. Ohshita, K.; Ishiyama, H.; Takahashi, Y.; Ito, J.; Mikami, Y.; Kobayashi, J. *Bioorg. Med. Chem.* **2007**, *15*, 4910–4916.
34. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431–432.
35. Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1967**, *89*, 2416–2423.
36. Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639–666.
37. Hayakawa, Y.; Takeda, K.; Yagita, H.; Van Kaer, L.; Saiki, I.; Okumura, K. *J. Immunol.* **2001**, *166*, 6012–6018.
38. Morgan, E.; Varro, R.; Sepulveda, H.; Ember, J. A.; Apgar, J.; Wilson, J.; Lowe, L.; Chen, R.; Shivraj, L.; Agadir, A.; Campos, R.; Ernst, D.; Gaur, A. *Clin. Immunol.* **2004**, *110*, 252–266.