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RCAI-17, 22, 24–26, 29, 31, 34–36, 38–40, and 88, the analogs of KRN7000 with a sulfonamide linkage: Their synthesis and bioactivity for mouse natural killer T cells to produce Th2-biased cytokines ☆

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ABSTRACT

RCAI-17, 22, 24–26, 29, 31, 34–36, 38–40, and 88, the analogs of KRN7000 (**1**) with a sulfonamide linkage instead of an amide bond, were synthesized to examine their bioactivity for mouse natural killer (NK) T cells. RCAI-17, 22, 24–26, 29, 31, 34–36, and 88 are the aromatic sulfonamide analogs, while RCAI-39 and 40 are the aliphatic ones. RCAI-38 is a *C*-galactoside analog of RCAI-26, which is the *p*-toluenesulfonamide analog of KRN7000. According to their bioassay, these sulfonamide analogs were shown to be the stimulants of mouse NKT cells to induce the production of Th2-biased cytokines in vitro, while RCAI-38 did not induce any cytokine production.

1. Introduction

In 1995 KRN7000 (**1**, α-GalCer, Fig. 1) was developed as an anticancer drug candidate by researchers at Kirin Brewery Co.[2] It is a derivative of agelasphins (main component is agelasphin-9b), which are anticancer glycosphingolipids isolated in 1993 from the extract of an Okinawan marine sponge, *Agelas mauritianus*.[3,4] It is shown that KRN7000 or a related α-galactosylceramide can be 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] Natural killer (NK) T cells are activated by recognition of this CD1d–glycosphingolipid complex with its invariant Vα14 (mouse) or Vα24 (human) T cell receptor (TCR). The X-ray analyses revealed the crystal structures of mouse and human CD1d-**1**,[6,7] and human CD1d-**1**-TCR[8] complexes.

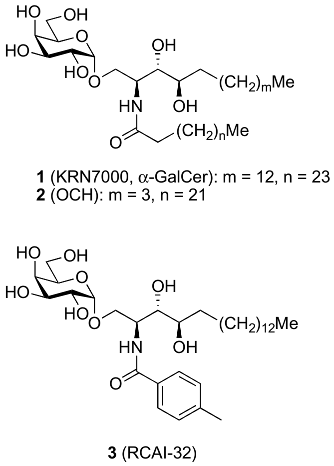

Figure 1. Structures of KRN7000 (**1**, α-GalCer), OCH (**2**), and RCAI-32 (**3**).

Activated NKT cells can release both helper T(Th) 1 and Th2 types of cytokines. Th1 type cytokines (IFN-γ, etc.) can induce protective immune response against pathogen infections or mediate tumor rejection, whereas Th2 type cytokines (IL-4, IL-13, etc.) mediate regulatory immune functions to ameliorate autoimmune

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☆ Synthesis of sphingosine relatives, Part 31. For Part 30, see Ref. 1.

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diseases or transplantation tolerance. These Th1 and Th2 type cytokines can antagonize each other's biological functions.[9] The effectiveness of KRN7000 for clinical use is limited because it induces both Th1 and Th2 cytokines at the same time.[10] To solve this problem, many groups are trying to develop a novel ligand, which induces either Th1 or Th2 type cytokines.[11]

In 2001, Miyamoto et al. found that OCH (**2**), an analog of KRN7000 with the truncated sphingosine and alkyl chains, induced predominant production of IL-4 by mouse NKT cells.[12] They hypothesized the reason for this phenomenon as follows:[13] NKT cells release IL-4 quickly after activation, while IFN-γ is produced at the later stage. OCH has the shorter alkyl chains, which bind in grooves in interior of CD1d by hydrophobic interaction. Less tight binding of OCH than that of KRN7000 to CD1d may make the CD1d–ligand complex unstable. As the result, NKT cells were stimulated only for a short period, and release IL-4 predominantly. Annoura and co-workers reported the synthesis of OCH,[14] its *C*-galactoside analog with no bioactivity in vitro,[15] and related compounds.[16] Additionally, Savage and co-workers also noticed that the analogs with shortened alkyl chains tend to induce IL-4 production.[17]

The hydrophobic pocket of CD1d protein is so narrow and deep that the bulky and short aromatic ring would not fit this pocket. The aromatic ring analog, therefore, could also stimulate NKT cells only for a short period like OCH. Based on this hypothesis, we synthesized RCAI-32 (**3**, Fig. 1), an benzamide analog. We expected that RCAI-32 also makes an unstable complex with CD1d, and it induces IL-4-biased cytokine production. However, RCAI-32 induced no cytokine production (Fig. 2). We reasoned that the stability of the complex might be too low to induce cytokine production. In the field of drug discovery, conversion of an amide bond to a sulfonamide linkage, a biologically equivalent functional group, is one of the major processes. In general, polarity of a sulfonamide bond is higher than that of an amide bond, due to the presence of two sulfur–oxygen linkages whereas the amide possesses only one carbon–oxygen linkage. We therefore anticipated that the benzenesulfonamide analogs might be able to bind CD1d for enough period to stimulate NKT cells. Based on this concept, we started the synthesis of the aromatic and aliphatic sulfonamide analogs. Most of the synthesized sulfonamide analogs induced Th2-biased cytokine production in vitro in mice. Among them, IL-4/IFN-γ ratio of the produced cytokines induced with RCAI-34 (**13h**), 35 (**13i**), and 36 (**13j**) were especially very high. It should be noted that RCAI-38 (**14**), the *C*-galactoside of RCAI-26 (**13a**, the *p*-toluenesulfonamide analog of KRN7000), did not induce any cytokine production by mouse NKT cells in vitro (Fig. 2). The detailed synthesis and the results of bioassay of these analogs are described below[18].

2. Results and discussion

2.1. Synthesis of the sulfonamide analogs

Scheme 1 summarizes the synthesis of the sulfonamide analogs RCAI-17, 22, 24–26, 29, 31, 34–36, 39, 40, and 88. The synthesis was commenced with commercially available phytosphingosine (**4**). Three hydroxy groups were protected as *tert*-butyldimethylsilyl (TBS) ether to give **5** in 81% yield. After protection of the amino group as 9-fluorenylmethoxycarbonyl (Fmoc) group (94%), the terminal TBS group was selectively removed with aqueous 10%

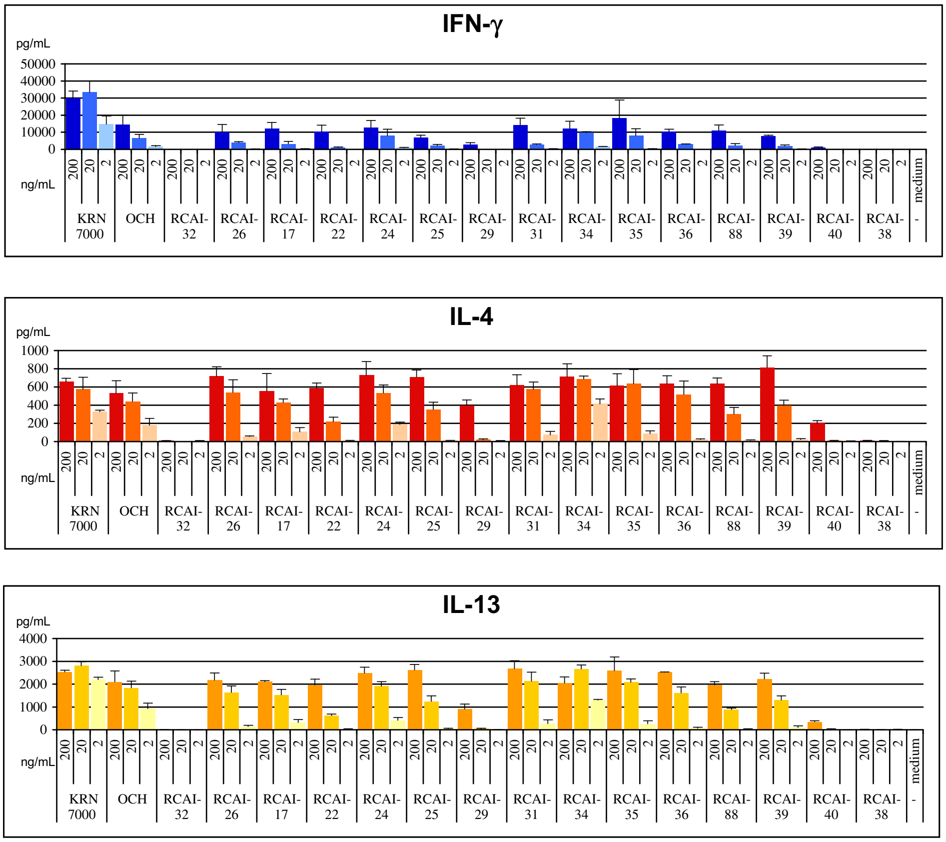
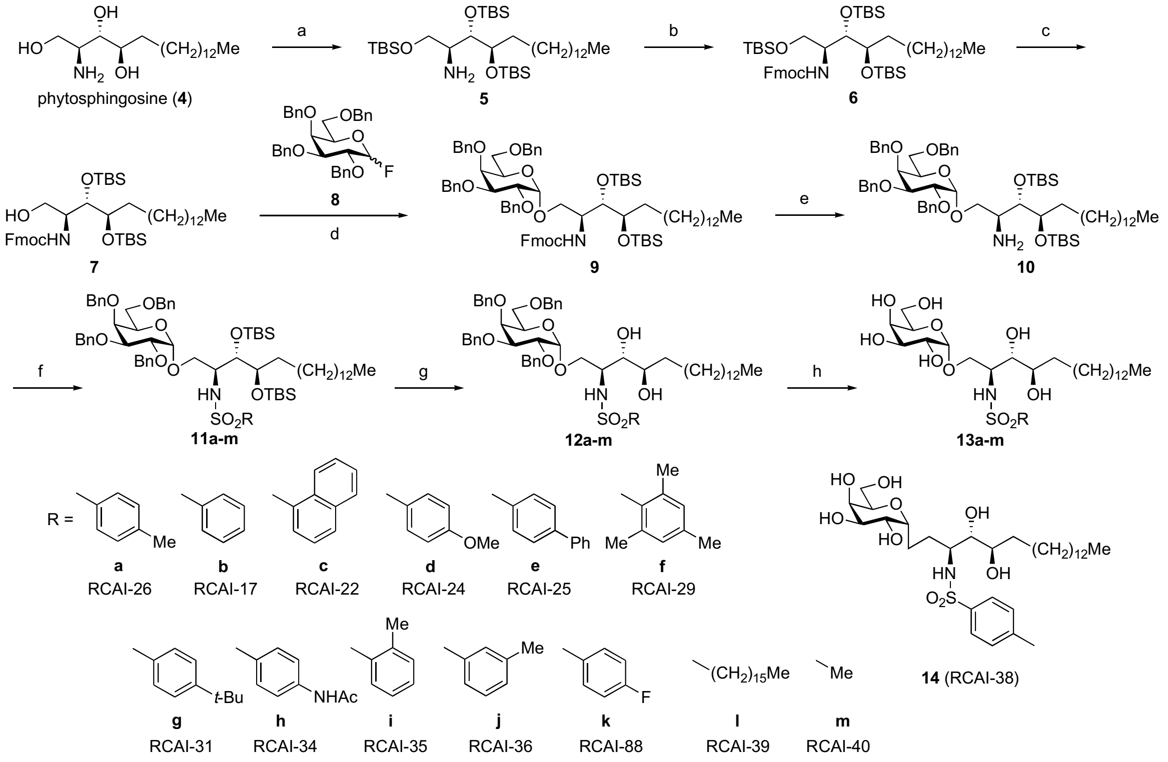
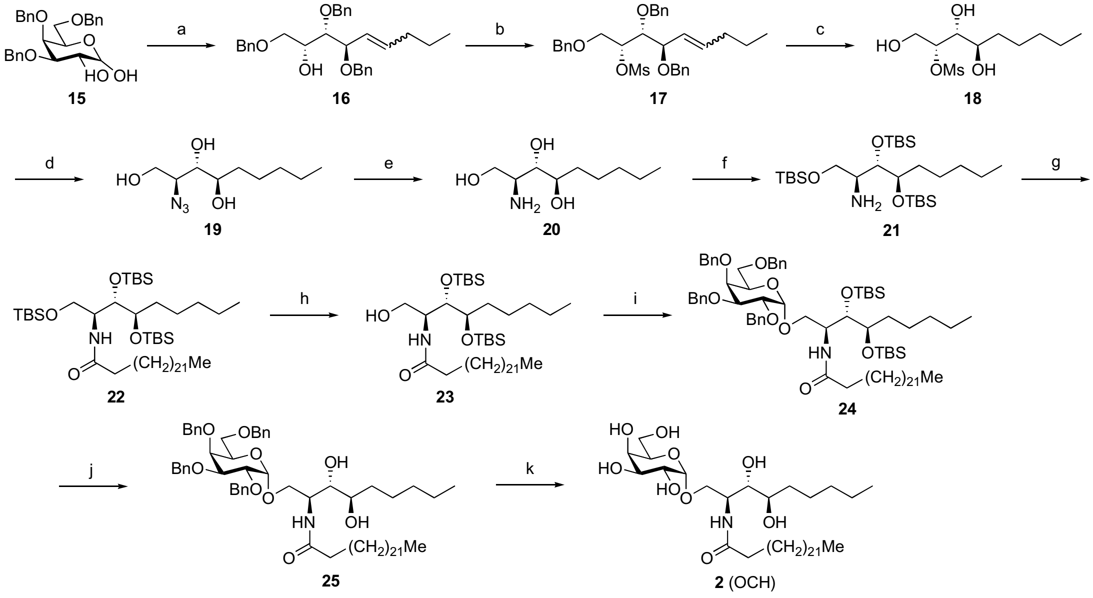


Figure 2. Cytokine production of NKT cells stimulated with KRN7000, OCH or synthetic analogs. Spleen cells (4×10^5) from B6 mice were cultured with 2, 20, 200 ng/mL of KRN7000, OCH or synthetic analogs for 48 h. The concentration of IFN-γ, IL-4, and IL-13 in the culture supernatants were measured by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



Scheme 1. Synthesis of sulfonamide analogs **13a–m**. Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, room temperature, 24 h (81%); (b) Fmoc-NHS, THF, MeCN, room temperature, 16 h (94%); (c) 10% CF_3CO_2H aq, THF, −20 to −5 °C, 4 h (80%); (d) **8**, $SnCl_2$, $AgClO_4$, powdered MS 4A, THF, −15 to 5 °C, 1 h (61%); (e) piperidine, DMF, room temperature, 2 h (98%); (f) RSO_2Cl, pyridine, DMAP, $CHCl_3$, room temperature or reflux, 15 h (68% to quant.); (g) TBAF, THF, room temperature or 60 °C, 2 h (84% to quant.); (h) H_2, $Pd(OH)_2$–C, EtOH, $CHCl_3$, room temperature, 16 h (82 to 97%).

CF_3CO_2H solution in THF to give alcohol **7** (80%). Glycosidation of **7** with 2,3,4,6-tetrabenzyl-1-fluoro-D-galactopyranoside (**8**) under Mukaiyama conditions[19] afforded **9** in 61% yield. The undesired β-isomer of **9** could be removed by silica gel column chromatography. The Fmoc group of **9** was then removed with piperidine in DMF to give amine **10** (98%), which was treated with aryl- or alkylsulfonyl chloride to give **11a–m** in 68% to quantitative yield. For installation of sterically hindered sulfonyl groups, for example 2,4,6-trimethylbenzenesulfonyl group, the reaction had to be conducted under reflux in $CHCl_3$. Finally, deprotection of TBS groups



Scheme 2. Synthesis of OCH (**2** or RCAI-43 in our code name). Reagents and conditions: (a) i—$NaIO_4$, EtOH, H_2O, room temperature, 18 h; ii— n-BuLi, $Ph_3P^+(CH_2)_3MeBr^-$, THF, room temperature, 19 h (61%, two steps); (b) MsCl, pyridine, room temperature, 18 h (87%); (c) H_2, 10% Pd–C, EtOH, room temperature, 17 h; (d) NaN_3, DMF, 95 °C, 9 h (54%, two steps); (e) H_2, 10% Pd–C, EtOH, $CHCl_3$, room temperature, 12 h (quant.); (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, room temperature, 17 h (43%, two steps); (g) lignoceric acid, EDC·HCl, i-Pr_2NEt, DMAP, CH_2Cl_2, room temperature, 15 h (75%); (h) 10% CF_3CO_2H aq, THF, −15 to 0 °C, 5 h (77%); (i) **8**, $SnCl_2$, $AgClO_4$, powdered MS 4A, THF, −18 to 0 °C, 2 h; (j) TBAF, THF, room temperature, 20 h (41%, two steps); (k) H_2, 10% $Pd(OH)_2$–C, EtOH, $CHCl_3$, room temperature, 14 h (50%).

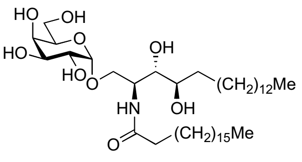
with tetra-*n*-butylammonium fluoride (TBAF) (84% ~ quant.) followed by hydrogenolysis of all of the benzyl groups furnished sulfonamide analogs **13a**–**m** in 82–97% yield.

OCH (**2**) was necessary as a reference sample, and it was synthesized as shown in Scheme 2. The known diol **15**[2] was subjected to the oxidative cleavage with sodium periodate, and the resulting aldehyde was elongated by Wittig reaction to give **16** in 61% yield (two steps). The alcohol **16** was converted to the corresponding mesylate **17** (87%). Hydrogenolysis of the three benzyl groups and reduction of the double bond were taken place at the same time to give **18**. Introduction of an azide group to **18** by S_N2 reaction (54% yield, two steps) followed by reduction of the resulting **19** afforded truncated phytosphingosine **20**. Subsequently, **20** was converted to OCH (**2**) as follows: (i) protection of the three hydroxy groups as the TBS ether (43%, two steps); (ii) acylation of the amino group with lignoceric acid (75%); (iii) deprotection of the terminal TBS group (77%); (iv) α-preferential galactosylation; (v) deprotection of TBS groups with TBAF (41%, two steps); and (vi) hydrogenolysis of benzyl groups (50%). All of the spectral data of synthesized OCH (**2**) were in good accordance with those reported by Annoura and co-workers.[14]

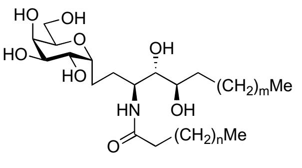
2.2. Results of bioassay

To assess the NKT cell stimulatory effects of RCAI-17, 22, 24–26, 29, 31, 32, 34–36, 38-40, and 88, we measured the level of Th1 and Th2 cytokines in supernatants from spleen cells cultured with KRN7000, OCH or synthetic analogs.[20] Spleen cells were prepared from C57BL/6 mice and were cultured for 48 h with various glycolipids. The levels of IFN-γ, IL-4, and IL-13 in the supernatants were measured by cytometric bead array (CBA) system (BD Biosciences).

Figure 2 shows the results of bioassay. KRN7000 and OCH were chosen as the standard samples in the production of cytokines. As can be seen from the figure, glycolipids with sulfonamide preferentially induce NKT cells to produce Th2 cytokines rather than Th1 cytokines. RCAI-22, 24–26, 34–36, and 39 stimulated NKT cells to produce higher amount of IL-4 and IL-13 than those of KRN7000 and OCH stimulation, whereas none of these analogs induced the production of a higher amount of IFN-γ than that could be induced with KRN7000 even with the high dose (200 ng/mL). Notably, RCAI-39 has an activity to produce Th2 cytokines in a dose-dependent fashion, indicating that it might be beneficial in its clinical application to enable the precise control of Th2 cytokine production. It must be added that RCAI-53 (**26**, Fig. 3), another analog of KRN7000 with a shorter octadecanamide ($NHCOC_{17}H_{35}$) side-chain, induced the production of much more IFN-γ and much less IL-4 than RCAI-39 did (unpublished observation).



26 (RCAI-53)



27: (α-*C*-GalCer): m = 12, n = 23
28: m = 3, n = 21

Figure 3. Structures of RCAI-53 (**26**), α-*C*-GalCer (**27**) and *C*-galactoside analog (**28**) of OCH.

In contrast to the high dose of stimulation, RCAI-34 was one of the remarkable Th2 inducer even in the low dose concentration (2 ng/mL) due to the higher level of IL-4 production than that of both KRN7000 and OCH. This is beneficial in its clinical application to enable the strong modification of Th1/Th2 balance to Th2.

Using a mixture of various glycolipids with sulfonamide matching with the symptoms might be useful for prevention and improvement of Th2-mediated autoimmune diseases and also for maintenance of transplantation tolerance.

In 2003, it was reported that the *C*-galactoside analog of KRN7000, α-*C*-GalCer (**27**) induces prolonged production of the Th1 cytokines, especially IFN-γ in mice.[21] In general, *C*-glycosides are resistant to enzymatic degradation by glycosidases and therefore it may exhibit sustained activity. Annoura and co-workers reported synthesis and its bioassay of the *C*-galactoside analog of OCH (**28**) in 2005, and it induced in mice neither IL-4 nor IFN-γ production in vitro, but increased serum level of IL-4 in vivo.[15] Based on this report, we synthesized RCAI-38 (**14**), the *C*-galactoside analog of RCAI-26, and assayed it. RCAI-38, however, induced no cytokine production in our assay system both in vitro and in vivo. In the case of the sulfonamide analogs, the hydrogen bonding between the galactosidic oxygen atom and α2 helix of CD1d (mouse, Thr156; human, Thr154) plays a critical role in exhibiting their bioactivities. It should be added that *C*-galactoside analogs are generally weak ligands in our bioassay system, presumably due to the lack of the galactosidic oxygen atom which can interact with CD1d through a hydrogen bonding.

3. Conclusion

We synthesized the aryl- and alkylsulfonamide analogs of KRN7000. It was found that most of these analogs induced NKT cells to produce Th2-biased cytokines (IL-4, IL-13, etc.) in mice in vitro. RCAI-34 (**13h**, *p*-acetamidobenzenesulfonamide analog), shows the highest value of IL-4/IFN-γ ratio among the synthesized analogs. The aliphatic sulfonamide analogs also showed bioactivity. Further studies to clarify the structure–activity relationship are in progress by means of the computational docking models.

It should be noted that the sulfonamide analogs did not induce any cytokine production in mice in vivo by iv direct injection. However, RCAI-17 induced only IL-4 production in mice in vivo by modification of drug delivery system such as the use of liposome technique.[22] It is known that formulation of the glycolipid in liposome enhances the uptake of the glycolipid by antigen-presenting cells in vivo.[22] Indeed, RCAI-17 in liposome induced Th2-biased cytokine production by NKT cells in vivo. We are now investigating the reason for these phenomena, and will report the results in due course.

4. Experimental

4.1. General

Melting point (Mp) data are uncorrected. Refractive indices (n_D) were measured on an Atago 1T refractometer. Optical rotations were measured on a Jasco P-1010 polarimeter. IR spectra were recorded on a Jasco FT/IR-460 plus spectrometer. ^1H NMR spectra (400 and 500 MHz, TMS at $\delta = 0.00$ or $CHCl_3$ at $\delta = 7.26$ as internal standard) and ^{13}C NMR spectra (100 and 126 MHz, $CHCl_3$ at $\delta = 77.0$ as internal standard) were recorded on a Jeol JNM-A400 or a Varian INOVA-AS500 spectrometers. HRMS were recorded on a JMS-SX102A. Column chromatography was performed on Merck Kieselgel 60 Art 1.07734.

4.2. (2*S*,3*S*,4*R*)-2-Amino-1,3,4-tris(*tert*-butyldimethylsilyloxy)octadecane (5)

To a stirred solution of phytosphingosine (**4**, 6.81 g, 21.4 mmol) in 2,6-lutidine (250 mL) and CH_2Cl_2 (250 mL), TBSOTf (29.5 mL, 128 mmol) was added at 0 °C. After stirring at room temperature for 24 h, the reaction was quenched with MeOH (10 mL). The resulting mixture was concentrated in vacuo, and the remaining 2,6-lutidine was removed azeotropically with toluene. The residue was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (300 g, hexane/EtOAc = 30:1) to give **5** (11.4 g, 81%) as a colorless oil, n_D^{17} = 1.4604; $[\alpha]_D^{17}$ −2.69 (*c* 1.05, $CHCl_3$); ν_{max} (film): 3400 (w, NH), 3320 (w, NH), 1255 (s, *t*Bu, Si–CH_3), 1090 (br s, C–O), 835 (s), 775 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 3.82 (1H, dd, *J* = 9.5, 3.5 Hz, 1-H_b), 3.83–3.79 (1H, m, 4-H), 3.52 (1H, dd, *J* = 7.5, 1.5 Hz, 3-H), 3.44 (1H, dd, *J* = 9.5, 7.5 Hz, 1-H_a), 2.88 (1H, dt, *J* = 7.5, 3.5 Hz, 2-H), 1.60–1.34 (4H, m, 5-H_2, NH_2), 1.34–1.23 (24H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 6-H_2), 0.906 (9H, s, *t*Bu), 0.898 (9H, s, *t*Bu), 0.897 (9H, s, *t*Bu), 0.88 (3H, t, *J* = 7.0 Hz, 18-H_3), 0.09 (6H, s, 2× $SiCH_3$), 0.062 (6H, s, 2× $SiCH_3$), 0.055 (3H, s, $SiCH_3$), 0.051 (3H, s, $SiCH_3$) ppm; δ_C (126 MHz, $CDCl_3$): 77.9, 76.4, 65.6, 55.4, 34.3, 31.9, 29.8, 29.70, 29.68, 29.67, 29.65, 29.63, 29.60, 29.4, 26.2, 26.1, 26.0, 25.9, 22.7, 18.25, 18.22, 18.20, 14.1, −3.6, −3.9, −4.7, −4.9, −5.28, −5.32 ppm; Anal. Calcd for $C_{36}H_{81}NO_3Si_3$ (660.29) C, 65.48; H, 12.36; N, 2.12; found: C, 65.79; H, 12.05; N, 2.04.

4.3. (2*S*,3*S*,4*R*)-1,3,4-Tris(*tert*-butyldimethylsilyloxy)-2-(9-fluorenylmethyloxycarbamido)octadecane (6)

To a stirred solution of **5** (1.24 g, 1.88 mmol) in MeCN (15 mL) and THF (15 mL), 9-fluorenylmethyl succinimidyl carbonate (779 mg, 2.31 mmol) was added at 0 °C. After stirring at room temperature for 16 h, the reaction mixture was diluted with water. The mixture was extracted with ether, and the combined extract was successively washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (25 g, hexane/EtOAc = 30:1) to give **6** (1.56 g, 94%) as a pale yellow oil, n_D^{20} = 1.4968; $[\alpha]_D^{20}$ +7.08 (*c* 0.52, $CHCl_3$); ν_{max} (film): 3445 (w, NH), 1735 (br s, C=O), 1500 (m), 1250 (s, *t*Bu, Si–CH_3), 1070 (br m, C–O), 1060 (br m, C–O), 835 (s), 780 (s), 740 (m) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.76 (2H, br d, *J* = 7.5 Hz, 2× Fmoc-aromat.H), 7.59 (2H, br d, *J* = 7.5 Hz, 2× Fmoc-aromat.H), 7.39 (2H, br t, *J* = 7.5 Hz, 2× Fmoc-aromat.H), 7.29 (2H, br tt, *J* = 7.5, 1.0 Hz, 2× Fmoc-aromat.H), 5.18 (1H, d, *J* = 8.0 Hz, NH), 4.35 (2H, d, *J* = 7.0 Hz, Fmoc-1-H_2), 4.22 (1H, br t, *J* = 7.0 Hz, Fmoc-9′-H), 3.86–3.81 (2H, m, 3-H, 1-H_a), 3.77–3.69 (3H, m, 4-, 2-H, 1-H_a), 1.57–1.46 (2H, m, 5-H_2), 1.43–1.18 (24H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 6-H_2), 0.91 (9H, s, *t*Bu), 0.901 (9H, s, *t*Bu), 0.897 (9H, s, *t*Bu), 0.88 (3H, t, *J* = 7.0 Hz, 18-H_3), 0.12 (3H, s, $SiCH_3$), 0.07 (3H, s, $SiCH_3$), 0.06 (9H, s, 3× $SiCH_3$), 0.03 (3H, s, $SiCH_3$) ppm; δ_C (126 MHz, $CDCl_3$): 156.0, 144.1, 143.0, 141.3, 127.6, 127.0, 125.1, 119.9, 75.4, 75.2, 66.7, 61.5, 54.7, 47.2, 32.6, 31.9, 29.9, 29.70, 29.68, 29.66, 29.63, 29.61, 29.4, 26.1, 26.1, 25.8, 22.7, 18.33, 18.18, 18.16, 14.1, −3.7, −4.0, −4.7, −5.2, −5.3, −5.6 ppm; Anal. Calcd for $C_{51}H_{91}NO_5Si_3$ (882.53) C, 69.41; H, 10.39; N, 1.59; found: C, 69.19; H, 10.13; N, 1.56.

4.4. (2*S*,3*S*,4*R*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-2-(9-fluorenylmethyloxycarbamido)octadecan-1-ol (7)

To a stirred solution of **6** (710 mg, 0.805 mmol) in THF (30 mL), 10% aqueous trifluoroacetic acid (v/v, 6 mL) was added dropwise at −20 °C. The reaction temperature was gradually raised up to −5 °C with stirring over 4 h. The reaction was then quenched with 15% aqueous NaOH solution, and then the mixture was extracted with ether. The combined organic extract was successively washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 10:1) to give **7** (492 mg, 80%) as a colorless oil, n_D^{20} = 1.5117; $[\alpha]_D^{20}$ −5.75 (*c* 0.59, $CHCl_3$); ν_{max} (film): 3440 (m, NH), 3340 (s, OH), 1710 (br s, C=O), 1510 (s), 1255 (s, *t*Bu, Si–CH_3), 1050 (br s, C–O), 940 (m), 835 (s), 780 (s), 760 (m), 740 (m) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.76 (2H, br d, *J* = 7.5 Hz, 2× Fmoc-aromat.H), 7.60 (1H, br d, *J* = 7.5 Hz, Fmoc-aromat.H), 7.59 (1H, br d, *J* = 7.5 Hz, Fmoc-aromat.H), 7.40 (1H, br t, *J* = 7.5 Hz, Fmoc-aromat.H), 7.39 (1H, br t, *J* = 7.5 Hz, Fmoc-aromat.H), 7.30 (2H, br tt, *J* = 7.5, 1.0 Hz, 2× Fmoc-aromat.H), 5.47 (1H, d, *J* = 8.5 Hz, NH), 4.44 (1H, dd, *J* = 10.5, 7.0 Hz, Fmoc-1-H_a), 4.38 (1H, dd, *J* = 10.5, 7.0 Hz, Fmoc-1-H_b), 4.21 (1H, br t, *J* = 7.0 Hz, Fmoc-9′-H), 4.17 (1H, dt, *J* = 11.5, 3.5 Hz, 1-H_a), 3.92 (1H, t, *J* = 3.5 Hz, 3-H), 3.83 (1H, dddd, *J* = 8.5, 4.0, 3.5, 3.5 Hz, 2-H), 3.77 (1H, dt, *J* = 6.0, 3.5 Hz, 4-H), 3.66 (1H, ddd, *J* = 11.5, 9.0, 4.0 Hz, 1-H_b), 2.96 (1H, dd, *J* = 9.0, 3.5 Hz, OH), 1.59–1.48 (2H, m, 5-H_2), 1.38–1.17 (24H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 6-H_2), 0.912 (9H, s, *t*Bu), 0.905 (9H, s, *t*Bu), 0.88 (3H, t, *J* = 7.0 Hz, 18-H_3), 0.10 (3H, s, $SiCH_3$), 0.09 (3H, s, $SiCH_3$), 0.08 (3H, s, $SiCH_3$), 0.04 (3H, s, $SiCH_3$) ppm; δ_C (126 MHz, $CDCl_3$): 155.9, 144.0, 143.8, 141.33, 141.30, 127.7, 127.02, 126.97, 125.1, 125.0, 120.0, 77.3, 75.9, 66.7, 63.3, 52.4, 47.2, 34.3, 31.9, 29.8, 29.68, 29.67, 29.66, 29.65, 29.61, 29.60, 29.5, 29.4, 26.0, 25.9, 25.6, 22.7, 18.2, 18.1, 14.1, −3.8, −4.2, −4.5, −5.1 ppm; Anal. Calcd for $C_{45}H_{77}NO_5Si_2$ (768.27) C, 70.35; H, 10.10; N, 1.82; found: C, 70.39; H, 10.01; N, 1.78.

4.5. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-bis(*tert*-butyldimethylsilyloxy)-2-(9-fluorenylmethyloxycarbamido)octadecane (9)

To a stirred solution of **7** (931 mg, 1.21 mmol) in dry THF (40 mL), tin(II) chloride (684 mg, 3.61 mmol), silver(I) perchlorate (750 mg, 3.61 mmol) and powdered MS 4A (5.08 g) were added under argon. After stirring at room temperature for 1 h, the mixture was cooled to −15 °C. To this mixture, a solution of **8** (1.52 g, 2.72 mmol) in dry THF (10 mL) was added dropwise at −15 °C. The reaction temperature was gradually raised up to 5 °C with stirring over 1 h. The reaction mixture was then diluted with ether, and the resulting mixture was filtered through a bed of Celite. The filtrate was successively washed with water and brine, dried with K_2CO_3, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (50 g, hexane/EtOAc = 20:1) to give **9** (742 mg, 61%) as a colorless oil, n_D^{21} = 1.5170; $[\alpha]_D^{21}$ +13.9 (*c* 0.61, $CHCl_3$); ν_{max} (film): 3340 (m, NH), 1730 (s, C=O), 1605 (w, aromat.), 1585 (w, aromat.), 1510 (w), 1500 (m, aromat.), 1250 (br s, *t*Bu, Si–CH_3), 1100 (br s, C–O), 1055 (br s, C–O), 835 (s), 780 (m) 740 (s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.71 (2H, d, *J* = 7.5 Hz, 2× Fmoc-aromat.H), 7.57 (1H, d, *J* = 7.5 Hz, Fmoc-aromat.H), 7.51 (1H, d, *J* = 7.5 Hz, Fmoc-aromat.H), 7.36–7.19 (24H, m, 20× Bn-aromat.H, 4× Fmoc-aromat.H), 5.51 (1H, d, *J* = 6.5 Hz, NH), 4.90 (1H, d, *J* = 11.5 Hz, Bn-H), 4.81 (1H, d, *J* = 4.0 Hz, 1′-H), 4.79 (1H, d, *J* = 12.0 Hz, Bn-H), 4.74 (1H, d, *J* = 12.0 Hz, Bn-H), 4.70 (1H, d, *J* = 12.0 Hz, Bn-H), 4.65 (1H, d, *J* = 12.0 Hz, Bn-H), 4.54 (1H, d, *J* = 11.5 Hz, Bn-H), 4.42 (1H, d, *J* = 12.0 Hz, Bn-H), 4.36 (1H, dd, *J* = 10.5, 7.0 Hz, Fmoc-1-H_a), 4.33 (1H, d, *J* = 12.0 Hz, Bn-H), 4.31 (1H, dd, *J* = 10.5, 7.0 Hz, Fmoc-1-H_b), 4.15 (1H, t, *J* = 7.0 Hz, Fmoc-9′-H), 4.02 (1H, dd, *J* = 10.0, 3.5 Hz, 2′-H), 3.99–3.83 (7H, m, 1-H_2, 2-, 3-, 3′-, 4′-, 5′-H), 3.69 (1H, br t, *J* = 6.0 Hz, 4-H), 3.48 (1H, dd, *J* = 9.0, 5.5 Hz, 6′-H$_a$), 3.45 (1H, dd, J = 9.0, 6.5 Hz, 6′-H$_b$), 1.51–1.46 (2H, m, 5-H$_2$), 1.38–1.16 (24H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 6-H$_2$), 0.90 (9H, s, *t*Bu), 0.88 (9H, s, *t*Bu), 0.88 (3H, t, J = 7.5 Hz, 18-H$_3$), 0.07 (3H, s, SiCH$_3$), 0.05 (3H, s, SiCH$_3$), 0.04 (3H, s, SiCH$_3$), 0.02 (3H, s, SiCH$_3$) ppm; δ_C (126 MHz, CDCl$_3$): 156.1, 144.1, 143.9, 141.3, 141.2, 138.8, 138.7, 138.6, 137.8, 128.29, 128.27, 128.16, 127.8, 127.8, 127.6, 127.52, 127.48, 127.4, 127.3, 126.9, 125.1, 125.0, 119.9, 100.1, 79.1, 76.3, 75.48, 75.42, 74.9, 74.7, 73.4, 73.1, 73.0, 69.7, 69.4, 68.8, 66.4, 53.6, 47.2, 33.3, 31.9, 29.9, 29.69, 29.65, 29.63, 29.36, 26.13, 26.05, 25.9, 22.7, 18.3, 18.2, 14.1, −3.72, −3.98, −4.64, −4.98 ppm; Anal. Calcd for $C_{79}H_{111}NO_{10}Si_2$ (1290.90) C, 73.50; H, 8.67; N, 1.09; found: C, 73.55; H, 8.59; N, 1.05.

4.6. (2*S*,3*S*,4*R*)-2-Amino-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-bis(*tert*-butyldimethylsilyloxy)-octadecane (10)

To a stirred solution of **9** (788 mg, 0.626 mmol) in DMF (40 mL), piperidine (10 mL) was added at room temperature. After stirring for 2 h, the mixture was diluted with ethyl acetate. The organic phase was then washed with water and brine, dried with MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 15:4) to give **10** (655 mg, 98%) as a white waxy solid. n_D^{23} = 1.5125; $[\alpha]_D^{23}$ +25.5 (c 1.01, CHCl$_3$); ν_{max} (KBr): 3380 (w, NH), 3300 (w, NH), 1605 (w, aromat.), 1585 (w, aromat.), 1495 (m, aromat.), 1250 (s, *t*Bu, Si–CH$_3$), 1100 (br s, C–O), 1060 (br s, C–O), 835 (s), 780 (s) 735 (s), 695 (s) cm^{-1}; δ_H (500 MHz, CDCl$_3$): 7.39–7.23 (20H, m, 20× Bn-aromat.H), 4.94 (1H, d, J = 11.5 Hz, Bn-H), 4.87 (1H, d, J = 4.0 Hz, 1′-H), 4.80 (1H, d, J = 12.0 Hz, Bn-H), 4.78 (1H, d, J = 12.0 Hz, Bn-H), 4.73 (1H, d, J = 12.0 Hz, Bn-H), 4.67 (1H, d, J = 12.0 Hz, Bn-H), 4.56 (1H, d, J = 11.5 Hz, Bn-H), 4.43 (1H, d, J = 11.5 Hz, Bn-H), 4.38 (1H, d, J = 11.5 Hz, Bn-H), 4.05 (1H, dd, J = 10.0, 4.0 Hz, 2′-H), 4.02–4.00 (1H, m, 4′-H), 3.98 (1H, dd, J = 9.5, 3.0 Hz, 1-H$_a$), 3.98–3.94 (1H, br d, J = 3.0 Hz, 5′-H), 3.94 (1H, dd, J = 10.0, 3.0 Hz, 3′-H), 3.74 (1H, ddd, J = 7.0, 5.0, 2.5 Hz, 4-H), 3.58 (1H, t, J = 9.0 Hz, 6′-H$_a$), 3.49 (1H, dd, J = 6.5, 2.5 Hz, 3-H), 3.46 (1H, dd, J = 9.0, 5.5 Hz, 6′-H$_b$), 3.20 (1H, t, J = 9.5 Hz, 1-H$_b$), 3.09 (1H, ddd, J = 9.5, 6.5, 3.0 Hz, 2-H), 1.52–1.43 (2H, m, 5-H$_2$), 1.41–1.22 (24H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 6-H$_2$), 0.888 (9H, s, *t*Bu), 0.879 (3H, t, J = 7.0 Hz, 18-H$_3$), 0.875 (9H, s, *t*Bu), 0.073 (3H, s, SiCH$_3$), 0.070 (3H, s, SiCH$_3$), 0.06 (3H, s, SiCH$_3$), 0.05 (3H, s, SiCH$_3$) ppm; δ_C (126 MHz, CDCl$_3$): 138.79, 138.76, 138.6, 137.9, 128.4, 128.30, 128.28, 128.2, 128.1, 127.84, 127.75, 127.7, 127.51, 127.45, 127.4, 99.1, 79.0, 77.2, 76.7, 76.0, 74.79, 74.76, 73.5, 73.3, 72.8, 72.3, 69.2, 68.5, 53.6, 34.2, 31.9, 29.8, 29.69, 29.68, 29.65, 29.6, 29.4, 26.1, 25.9, 22.7, 18.23, 18.17, 14.1, −3.7, −3.9, −4.6, −4.7 ppm; Anal. Calcd for $C_{64}H_{101}NO_8Si_2$ (1068.66) C, 71.93; H, 9.53; N, 1.31; found: C, 72.03; H, 9.54; N, 1.28.

4.7. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-bis(*tert*-butyldimethylsilyloxy)-2-(4-toluenesulfonamido)octadecane (11a)

To a stirred solution of **10** (109 mg, 0.102 mmol) in CHCl$_3$ (5 mL) and pyridine (1 mL), *p*-toluenesulfonyl chloride (45 mg, 0.24 mmol), and a catalytic amount of DMAP (0.04 mg) were successively added at 0 °C. After stirring at room temperature for 15 h, the reaction was quenched with water. The resulting mixture was extracted with ether, and the combined organic extract was successively washed with water, saturated aqueous CuSO$_4$ solution, water, saturated aqueous NaHCO$_3$ solution and brine, dried with MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (10 g, hexane/EtOAc = 50:3) to give **11a** (94 mg, 75%) as a colorless oil, n_D^{25} = 1.5178; $[\alpha]_D^{25}$ −2.02 (c 0.88, CHCl$_3$); ν_{max} (film): 3270 (m, NH), 1600 (m, aromat.), 1495 (s, aromat.), 1340 (m, SO$_2$), 1255 (s, *t*Bu, Si–CH$_3$), 1165 (m, SO$_2$), 1090 (br s, C–O), 1055 (br s, C–O), 835 (s), 780 (s), 735 (m), 700 (s) cm^{-1}; δ_H (500 MHz, CDCl$_3$): 7.76 (2H, d, J = 8.0 Hz, 2× aromat.H), 7.43–7.24 (20H, m, 20× Bn-aromat.H), 7.11 (2H, d, J = 8.0 Hz, 2× aromat.H), 5.66 (1H, d, J = 4.0 Hz, NH), 4.91 (1H, d, J = 11.5 Hz, Bn-H), 4.88 (1H, d, J = 12.0 Hz, Bn-H), 4.80 (1H, d, J = 12.0 Hz, Bn-H), 4.76 (1H, d, J = 12.0 Hz, Bn-H), 4.67 (1H, d, J = 12.0 Hz, Bn-H), 4.55 (1H, d, J = 11.5 Hz, Bn-H), 4.49 (1H, d, J = 4.0 Hz, 1′-H), 4.43 (1H, d, J = 12.0 Hz, Bn-H), 4.40 (1H, d, J = 12.0 Hz, Bn-H), 4.10 (1H, dd, J = 4.0, 3.5 Hz, 3-H), 3.98 (1H, dd, J = 10.0, 4.0 Hz, 2′-H), 3.88 (1H, br d, J = 3.0 Hz, 4′-H), 3.72–3.68 (1H, m, 4-H), 3.70 (1H, dd, J = 10.0, 3.0 Hz, 3′-H), 3.67 (1H, dd, J = 11.0, 4.0 Hz, 1-H$_a$), 3.65 (1H, br t, J = 7.0 Hz, 5′-H), 3.58 (1H, dd, J = 11.0, 8.0 Hz, 1-H$_b$), 3.41 (1H, dd, J = 9.0, 7.0 Hz, 6′-H$_a$), 3.39 (1H, dd, J = 9.0, 6.0 Hz, 6′-H$_b$), 3.25 (1H, dddd, J = 8.0, 4.0, 4.0, 4.0 Hz, 2-H), 2.33 (3H, s, CH$_3$), 1.45–1.23 (26H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 6-, 5-H$_2$), 0.889 (9H, s, *t*Bu), 0.881 (3H, t, J = 7.0 Hz, 18-H$_3$), 0.80 (9H, s, *t*Bu), 0.18 (3H, s, SiCH$_3$), 0.13 (3H, s, SiCH$_3$), 0.02 (3H, s, SiCH$_3$), −0.04 (3H, s, SiCH$_3$) ppm; δ_C (126 MHz, CDCl$_3$): 142.9, 138.6, 138.5, 138.3, 137.7, 137.0, 129.5, 128.5, 128.4, 128.3, 128.22, 128.21, 128.14, 128.12, 127.77, 127.75, 127.62, 127.60, 127.2, 100.7, 79.3, 75.9, 75.2, 75.0, 74.7, 74.5, 73.7, 73.5, 72.7, 69.7, 68.7, 68.3, 55.2, 33.2, 31.9, 30.0, 29.70, 29.69, 29.65, 29.62, 29.4, 26.2, 25.9, 25.1, 22.7, 21.5, 18.4, 18.0, 14.1, −4.0, −4.17, −4.23, −4.6 ppm; Anal. Calcd for $C_{71}H_{107}NO_{10}SSi_2$ (1222.85) C, 69.74; H, 8.82; N, 1.15; found: C, 69.73; H, 8.66; N, 1.06.

4.8. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(4-toluenesulfonamido)octadecane-3,4-diol (12a)

To a stirred solution of **11a** (94 mg, 0.077 mmol) in THF (5 mL), a solution of TBAF (1.0 M in THF, 616 μL, 0.616 mmol) was added at room temperature. After stirring at 60 °C for 2 h, the reaction mixture was cooled to room temperature. The reaction was quenched with water, and the resulting mixture was extracted with EtOAc. The combined organic extract was successively washed with water and brine, dried with MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (10 g, hexane/EtOAc = 4:1) to give **12a** (69 mg, 90%) as a colorless oil, n_D^{25} = 1.5179; $[\alpha]_D^{25}$ +44.7 (c 1.18, CHCl$_3$); ν_{max} (film) = 3480 (s, OH), 3280 (m, NH), 1600 (m, aromat.), 1495 (s, aromat.), 1330 (br s, SO$_2$), 1165 (s, SO$_2$), 1095 (br s, C–O), 1055 (br s, C–O), 835 (s), 735 (br s) 695 (s), 665 (m) cm^{-1}; δ_H (500 MHz, CDCl$_3$): 7.74 (2H, d, J = 8.5 Hz, 2× aromat.H), 7.39–7.25 (20H, m, 20× Bn-aromat.H), 7.22 (2H, d, J = 8.5 Hz, 2× aromat.H), 5.56 (1H, d, J = 8.5 Hz, NH), 4.89 (1H, d, J = 11.0 Hz, Bn-H), 4.86 (1H, d, J = 11.5 Hz, Bn-H), 4.76 (1H, d, J = 12.0 Hz, Bn-H), 4.74 (1H, d, J = 12.0 Hz, Bn-H), 4.72 (1H, d, J = 3.5 Hz, 1′-H), 4.63 (1H, d, J = 11.5 Hz, Bn-H), 4.55 (1H, d, J = 11.0 Hz, Bn-H), 4.52 (1H, d, J = 12.0 Hz, Bn-H), 4.42 (1H, d, J = 12.0 Hz, Bn-H), 4.00 (1H, dd, J = 10.0, 4.0 Hz, 2′-H), 3.98 (1H, br d, J = 2.0 Hz, 4′-H), 3.86 (1H, br t, J = 6.5 Hz, 5′-H), 3.84 (1H, dd, J = 10.0, 2.0 Hz, 3′-H), 3.82 (1H, dd, J = 10.5, 3.5 Hz, 1-H$_a$), 3.69 (1H, dd, J = 10.5, 3.0 Hz, 1-H$_b$), 3.55 (1H, dddd, J = 8.5, 3.5, 3.0, 3.0 Hz, 2-H), 3.50 (1H, dd, J = 9.0, 7.0 Hz, 6′-H$_a$), 3.47 (1H, dd, J = 9.0, 6.0 Hz, 6′-H$_b$), 3.40–3.35 (1H, m, 4-H), 3.28 (1H, br d, J = 10.0 Hz, OH), 3.21–3.16 (1H, m, 3-H), 2.37 (3H, s, CH$_3$), 1.93 (1H, d, J = 5.0 Hz, OH), 1.45–1.03 (26H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 6-, 5-H$_2$), 0.88 (3H, t, J = 7.0 Hz, 18-H$_3$) ppm; δ_C (126 MHz, CDCl$_3$): 143.3, 138.4, 138.3, 137.9, 137.82, 137.76, 129.7, 128.5, 128.4, 128.2, 128.14, 128.09, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.0, 99.1, 79.5, 75.71, 75.66, 74.8, 74.32, 74.30, 73.5, 72.8, 72.6, 70.2, 69.7, 68.5, 53.0, 33.1, 31.9, 29.69, 29.68, 29.65, 29.63, 29.3, 25.5, 22.7, 21.5, 14.1 ppm; HR-FABMS: Calcd for $C_{59}H_{79}O_{10}NSNa$ [M+Na]$^+$ 1016.5322; found: 1016.5319.

4.9. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(4-toluenesulfonamido)octadecane-3,4-diol (13a, RCAI-26)

To a stirred solution of **12a** (59 mg, 0.059 mmol) in EtOH (4 mL) and $CHCl_3$ (1 mL), $Pd(OH)_2$–C (20%, wet type, 48 mg) was added under argon at room temperature. Under hydrogen atmosphere (balloon), the mixture was stirred at room temperature for 16 h. The suspension was then filtered through a bed of Celite, and the filter cake was washed with $CHCl_3$/MeOH (5:1). The combined filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (6 g, $CHCl_3$/MeOH = 50:7) to give **13a** (RCAI-26, 36 mg, 97%) as a colorless solid, $[\alpha]_D^{26}$ +60.4 (c 0.72, pyridine); ν_{max} (KBr): 3360 (br s, OH, NH), 1650 (br w), 1600 (w, aromat.), 1490 (w), 1330 (br m, SO_2), 1160 (s, SO_2), 1070 (br s, C–O), 1040 (m, C–O), 815 (m), 665 (s) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 9.40 (1H, d, J = 8.5 Hz, NH), 8.15 (2H, d, J = 8.5 Hz, 2× aromat.H), 7.17 (2H, d, J = 8.5 Hz, 2× aromat.H), 6.78–6.62 (6H, br s, 6× OH), 5.38 (1H, d, J = 3.5 Hz, 1′-H), 4.74–4.67 (1H, m, 2-H), 4.67 (1H, dd, J = 10.5, 6.0 Hz, 1-H_a), 4.62 (1H, dd, J = 9.5, 3.5 Hz, 2′-H), 4.51 (1H, d, J = 3.5 Hz, 4′-H), 4.33 (1H, dd, J = 9.5, 3.5 Hz, 3′-H), 4.33–4.26 (3H, m, 3-H, 6′-H_2), 4.26–4.20 (2H, m, 4-, 5′-H), 4.20 (1H, dd, J = 10.5, 4.0 Hz, 1-H_b), 2.23–2.16 (1H, m, 5-H_a), 2.14 (3H, s, CH_3), 1.84–1.68 (2H, m, 5-H_b, 6-H_a), 1.59–1.51 (1H, m, 6-H_b), 1.39–1.16 (22H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-H_2), 0.84 (3H, t, J = 7.5 Hz, 18-H_3) ppm; δ_C (126 MHz, pyridine-d_5): 142.7, 140.5, 129.9, 127.4, 101.2, 77.3, 72.8, 72.3, 71.5, 70.9, 70.1, 67.8, 62.4, 55.3, 34.9, 32.1, 30.3, 30.1, 29.98, 29.96, 29.9, 29.6, 26.2, 22.9, 21.1, 14.3 ppm; HR-FABMS: Calcd for $C_{31}H_{56}O_{10}NS$ [M+H]$^+$ 634.3625; found: 634.3621.

4.10. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(benzenesulfonamido)octadecane-3,4-diol (13b, RCAI-17)

In the same manner as described above for the conversion of **10** to **13a**, **13b** (RCAI-17) was synthesized from **10** as white powder. $[\alpha]_D^{22}$ +55.8 (c 0.58, pyridine); ν_{max} (KBr): 3400 (br s, OH, NH), 1330 (br m, SO_2), 1160 (m, SO_2), 1070 (br s, C–O) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 9.58 (1H, d, J = 8.4 Hz), 8.28–8.25 (2H, m), 7.42–7.38 (3H, m), 6.58–5.67 (6H, m), 5.36 (1H, d, J = 3.6 Hz), 4.74–4.65 (2H, m), 4.62 (1H, dd, J = 10, 3.6 Hz), 4.52 (1H, d, J = 3.2 Hz), 4.35–4.28 (4H, m), 4.24–4.22 (2H, m), 4.18 (1H, dd, J = 10, 3.6 Hz), 2.22–2.17 (1H, m), 1.87–1.67 (2H, m), 1.62–1.50 (1H, m), 1.43–1.18 (22H, m), 0.84 (3H, t, J = 7.2 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 143.4, 132.2, 129.3, 127.4, 101.3, 77.4, 72.8, 72.3, 71.5, 70.9, 70.1, 67.8, 62.5, 55.3, 34.9, 32.1, 30.3, 30.1, 30.0, 29.9, 29.6, 26.2, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{30}H_{53}O_{10}NSNa$ [M+Na]$^+$ 642.3288; found: 642.3289.

4.11. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(1-naphthalenesulfonamido)octadecane-3,4-diol (13c, RCAI-22)

In the same manner as described above for the conversion of **10** to **13a**, **13c** (RCAI-22) was synthesized from **10** as white powder. $[\alpha]_D^{25}$ +58.2 (c 0.43, pyridine); ν_{max} (KBr): 3400 (br s, OH, NH), 1595 (w, aromat.), 1510 (m, aromat.), 1320 (br s, SO_2), 1160 (m, SO_2), 1070 (br s, C–O), 1030 (br s, C–O), 800 (s), 770 (s) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 9.79 (1H, d, J = 8.8 Hz), 9.32 (1H, dd, J = 6.4, 3.6 Hz), 8.70 (1H, d, J = 8.0 Hz), 7.99 (1H, d, J = 8.0 Hz), 7.92 (1H, dd, J = 6.4, 3.6 Hz), 7.50–7.45 (3H, m), 6.59 (1H, br s), 6.40–5.02 (5H, m), 5.30 (1H, d, J = 3.6 Hz), 4.72–4.64 (1H, m), 4.60 (1H, dd, J = 11, 5.6 Hz), 4.56 (1H, dd, J = 9.6, 4.0 Hz), 4.44 (1H, d, J = 2.4 Hz), 4.39–4.10 (6H, m), 4.15 (1H, dd, J = 14, 5.6 Hz), 2.12–1.96 (1H, m), 1.75–1.56 (2H, m), 1.43–1.14 (23H, m), 0.84 (3H, t, J = 6.8 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 138.1, 134.8, 133.9, 129.34, 129.31, 129.0, 127.9, 127.0, 125.9, 124.8, 101.2, 77.3, 72.6, 72.2, 71.4, 70.9, 70.1, 68.0, 62.5, 55.4, 34.5, 32.1, 30.3, 30.04, 29.97, 29.9, 29.6, 26.1, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{34}H_{56}O_{10}NS$ [M+H]$^+$ 670.3625; found: 670.3630.

4.12. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(4-methoxybenzenesulfonamido)octadecane-3,4-diol (13d, RCAI-24)

In the same manner as described above for the conversion of **10** to **13a**, **13d** (RCAI-24) was synthesized from **10** as white powder. $[\alpha]_D^{23}$ +59.7 (c 0.55, pyridine); ν_{max} (KBr): 3380 (br s, OH, NH), 1600 (m, aromat.), 1500 (m, aromat.), 1325 (m, SO_2), 1155 (s, SO_2), 1080 (br s, C–O), 1030 (br s, C–O), 835 (m) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 9.31 (1H, d, J = 8.0 Hz), 8.22 (2H, d, J = 8.8 Hz), 7.07 (1H, br s), 6.99 (2H, d, J = 8.8 Hz), 6.68 (1H, br d, J = 8.8 Hz), 6.50–6.26 (3H, m), 6.16 (1H, br s), 5.40 (1H, d, J = 3.2 Hz), 4.73–4.66 (2H, m), 4.61 (1H, dd, J = 9.6, 3.2 Hz), 4.50 (1H, d, J = 2.8 Hz), 4.37–4.18 (7H, m), 3.61 (3H, s), 2.27–2.17 (1H, m), 1.86–1.69 (2H, m), 1.62–1.49 (1H, m), 1.43–1.15 (22H, m), 0.84 (3H, t, J = 7.2 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 162.7, 135.1, 129.6, 114.6, 101.3, 77.4, 72.9, 72.3, 71.5, 70.8, 70.2, 67.8, 62.4, 55.4, 55.3, 34.9, 32.1, 30.3, 30.1, 30.0, 29.9, 29.6, 26.3, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{31}H_{55}O_{11}NSNa$ [M+H]$^+$ 672.3394; found: 672.3396.

4.13. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(4-biphenylsulfonamido)octadecane-3,4-diol (13e, RCAI-25)

In the same manner as described above for the conversion of **10** to **13a**, **13e** (RCAI-25) was synthesized from **10** as white powder. $[\alpha]_D^{24}$ +56.0 (c 0.51, pyridine); ν_{max} (KBr): 3440 (br s, OH, NH), 1600 (w, aromat.), 1160 (s, SO_2), 1100 (s, C–O), 1030 (br s, C–O) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 9.63 (1H, d, J = 8.4 Hz), 8.37 (2H, d, J = 8.8 Hz), 7.73 (2H, d, J = 8.8 Hz), 7.64 (2H, dt-like, J = 6.8, 1.6 Hz), 7.43–7.32 (3H, m), 7.10 (1H, br s), 6.74 (1H, d, J = 6.0 Hz), 6.54–6.17 (4H, m), 5.43 (1H, d, J = 3.6 Hz), 4.83–4.76 (1H, m), 4.74 (1H, dd, J = 11, 6.0 Hz), 4.63 (1H, dd, J = 9.6, 4.0 Hz), 4.48 (1H, d, J = 3.6 Hz), 4.36–4.21 (6H, m), 4.26 (1H, dd, J = 11, 4.8 Hz), 2.27–2.18 (1H, m), 1.87–1.71 (2H, m), 1.62–1.50 (1H, m), 1.40–1.13 (22H, m), 0.85 (3H, t, J = 7.2 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 144.7, 142.2, 129.3, 128.6, 128.1, 127.9, 127.7, 101.3, 77.4, 72.9, 72.3, 71.5, 70.9, 70.2, 67.9, 62.5, 55.5, 34.9, 32.1, 30.3, 30.1, 30.0, 29.9, 29.6, 26.2, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{36}H_{58}O_{10}NS$ [M+H]$^+$ 696.3781; found: 696.3781.

4.14. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(2,4,6-trimethylbenzenesulfonamido)octadecane-3,4-diol (13f, RCAI-29)

In the same manner as described above for the conversion of **10** to **13a**, **13f** (RCAI-29) was synthesized from **10** as white powder. $[\alpha]_D^{23}$ +66.5 (c 0.53, pyridine); ν_{max} (KBr): 3440 (br s, OH, NH), 1600 (m, aromat.), 1330 (m, SO_2), 1160 (s, SO_2), 1040 (br s, C–O) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 9.19 (1H, d, J = 9.2 Hz), 6.90 (2H, s), 6.54 (1H, br s), 6.39–5.76 (5H, m), 5.37 (1H, d, J = 3.6 Hz), 4.62–4.47 (2H, m), 4.59 (1H, dd, J = 10, 4.4 Hz), 4.50 (1H, d, J = 2.8 Hz), 4.34–4.25 (4H, m), 4.23 (1H, dd, J = 10, 4.4 Hz), 4.22–4.16 (2H, m), 2.93 (6H, s), 2.18–2.09 (1H, m), 2.14 (3H, s), 1.83–1.64 (2H, m), 1.56–1.43 (1H, m), 1.38–1.15 (22H, m), 0.84 (3H, t, J = 6.8 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 141.7, 139.3, 136.7, 132.3, 101.5, 77.3, 72.8, 72.2, 71.5, 70.9, 70.2, 68.3, 62.5, 54.6, 34.6, 32.1, 30.3, 30.1, 30.0, 29.9, 29.6, 26.2, 23.3, 22.9, 20.7, 14.3 ppm; HR-FABMS: Calcd for $C_{33}H_{60}O_{10}NS$ [M+H]$^+$ 662.3938; found: 662.3936.

4.15. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(4-*tert*-butylbenzenesulfonamido)octadecane-3,4-diol (13g, RCAI-31)

In the same manner as described above for the conversion of **10** to **13a**, **13g** (RCAI-31) was synthesized from **10** as white powder. $[\alpha]_D^{23}$ +49.6 (*c* 0.29, pyridine); ν_{max} (KBr): 3420 (br s, OH, NH), 1600 (w, aromat.), 1320 (m, SO_2), 1160 (s, SO_2), 1080 (br s, C–O), 1010 (br s, C–O) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 9.48 (1H, d, *J* = 8.4 Hz), 8.25 (2H, d, *J* = 8.4 Hz), 7.47 (2H, d, *J* = 8.4 Hz), 7.08 (1H, br s), 6.72 (1H, d, *J* = 7.6 Hz), 6.64 (1H, br s), 6.44 (1H, br s), 6.34 (1H, br s), 6.15 (1H, d, *J* = 6.8 Hz), 5.33 (1H, d, *J* = 4.0 Hz), 4.81–4.73 (1H, m), 4.71 (1H, dd, *J* = 10, 6.4 Hz), 4.60 (1H, dd, *J* = 10, 3.6 Hz), 4.51 (1H, br s), 4.38–4.28 (4H, m), 4.28–4.20 (2H, m), 4.19 (1H, dd, *J* = 10, 4.4 Hz), 2.26–2.17 (1H, m), 1.86–1.67 (2H, m), 1.63–1.52 (1H, m), 1.44–1.15 (22H, m), 1.19 (9H, s), 0.85 (3H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 155.7, 140.5, 127.3, 126.4, 101.3, 77.4, 72.9, 72.2, 71.5, 70.8, 70.2, 67.9, 62.4, 55.3, 35.0, 32.1, 31.0, 30.4, 30.1, 30.0, 29.9, 29.6, 26.2, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{34}H_{62}O_{10}NS$ $[M+H]^+$ 676.4094; found: 676.4094.

4.16. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(4-acetamiobenzenesulfonamido)octadecane-3,4-diol (13h, RCAI-34)

In the same manner as described above for the conversion of **10** to **13a**, **13h** (RCAI-34) was synthesized from **10** as white powder. $[\alpha]_D^{25}$ +56.3 (*c* 0.59, pyridine); ν_{max} (KBr): 3320 (br s, OH, NH), 1670 (br s, C=O), 1595 (s, aromat.), 1535 (br s), 1320 (br s, SO_2), 1155 (s, SO_2), 1080 (br s, C–O), 1040 (br m, C–O) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 11.15 (1H, s), 9.35 (1H, d, *J* = 8.4 Hz), 8.22 (2H, d, *J* = 8.8 Hz), 8.07 (2H, d, *J* = 8.8 Hz), 6.38–5.23 (6H, m), 5.39 (1H, d, *J* = 4.0 Hz), 4.73–4.64 (2H, m), 4.61 (1H, dd, *J* = 10, 3.6 Hz), 4.52 (1H, d, *J* = 2.8 Hz), 4.36–4.17 (7H, m), 2.25–2.14 (1H, m), 2.12 (3H, s), 1.86–1.67 (2H, m), 1.61–1.49 (1H, m), 1.40–1.16 (22H, m), 0.84 (3H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 169.4, 143.8, 137.1, 128.6, 119.5, 101.1, 77.3, 72.7, 72.3, 71.4, 70.9, 70.1, 67.7, 62.4, 55.2, 34.8, 32.1, 30.3, 30.1, 30.0, 29.9, 29.6, 26.3, 24.3, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{32}H_{56}O_{11}N_2SNa$ $[M+Na]^+$ 699.3503; found: 699.3508.

4.17. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(2-toluenesulfonamido)octadecane-3,4-diol (13i, RCAI-35)

In the same manner as described above for the conversion of **10** to **13a**, **13i** (RCAI-35) was synthesized from **10** as white powder. $[\alpha]_D^{24}$ +68.2 (*c* 0.35, pyridine); ν_{max} (KBr): 3400 (br s, OH, NH), 3060 (w), 1650 (br w), 1595 (w, aromat.), 1325 (br s, SO_2), 1160 (s, SO_2), 1070 (br s, C–O), 1035 (br s, C–O), 735 (m), 710 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 9.55 (1H, br d, *J* = 8.5 Hz, NH), 8.40 (1H, dd, *J* = 8.0, 1.5 Hz, aromat.H), 7.32 (1H, dt, *J* = 7.5, 1.5 Hz, aromat.H), 7.26 (1H, dd, *J* = 7.5, 0.5 Hz, aromat.H), 7.23 (1H, ddd, *J* = 8.0, 7.5, 0.5 Hz, aromat.H), 7.01 (1H, br s, OH), 6.66 (1H, br s, OH), 6.60 (1H, br d, *J* = 6.5 Hz, OH), 6.47 (1H, br t, *J* = 5.5 Hz, OH), 6.33 (1H, d, *J* = 3.0 Hz, OH), 6.11 (1H, br d, *J* = 5.0 Hz, OH), 5.34 (1H, d, *J* = 4.0 Hz, 1′-H), 4.63 (1H, dd, *J* = 10.0, 5.5 Hz, 1-H_a), 4.63–4.57 (2H, m, 2-, 2′-H), 4.49 (1H, br s, 4′-H), 4.34–4.26 (4H, m, 3-, 3′-H, 6′-H_2), 4.21 (1H, dd, *J* = 10.0, 4.0 Hz, 1-H_b), 4.22–4.17 (2H, m, 4-, 5′-H), 2.89 (3H, s, CH_3), 2.20–2.13 (1H, m, 5-H_a), 1.83–1.75 (1H, m, 6-H_a), 1.75–1.67 (1H, m, 5-H_b), 1.56–1.47 (1H, m, 6-H_b), 1.37–1.16 (22H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-H_2), 0.84 (3H, t, *J* = 7.5 Hz, 18-H_3) ppm; δ_C (126 MHz, pyridine-d_5): 141.0, 137.6, 132.8, 132.4, 129.6, 126.4, 101.4, 77.5, 72.8, 72.3, 71.5, 70.8, 70.1, 68.1, 62.4, 55.2, 34.8, 32.1, 30.3, 30.04, 29.95, 29.9, 29.6, 26.3, 22.9, 20.4, 14.3 ppm; HR-FABMS: Calcd for $C_{31}H_{56}O_{10}NS$ $[M+H]^+$ 634.3625; found: 634.3629.

4.18. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(3-toluenesulfonamido)octadecane-3,4-diol (13j, RCAI-36)

In the same manner as described above for the conversion of **10** to **13a**, **13j** (RCAI-36) was synthesized from **10** as white powder. $[\alpha]_D^{24}$ +75.4 (*c* 0.35, pyridine); ν_{max} (KBr): 3400 (br s, OH, NH), 1600 (w, aromat.), 1330 (br s, SO_2), 1155 (br s, SO_2), 1080 (br s, C–O), 1035 (br s, C–O), 785 (m), 690 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 9.44 (1H, br d, *J* = 7.5 Hz, NH), 8.08 (1H, dd, *J* = 7.5, 0.5 Hz, aromat.H), 8.06 (1H, s, aromat.H), 7.30 (1H, t, *J* = 7.5, Hz, aromat.H), 7.19 (1H, dd, *J* = 7.5, 0.5 Hz, aromat.H), 7.03 (1H, br s, OH), 6.72 (1H, br d, *J* = 6.5 Hz, OH), 6.65 (1H, br s, OH), 6.42 (1H, br s, OH), 6.33 (1H, br d, OH), 6.15 (1H, br d, *J* = 4.0 Hz, OH), 5.35 (1H, d, *J* = 3.5 Hz, 1′-H), 4.75–4.71 (1H, m, 2-H), 4.69 (1H, dd, *J* = 10.5, 6.0 Hz, 1-H_a), 4.61–4.57 (1H, m, 2′-H), 4.50 (1H, br s, 4′-H), 4.35–4.27 (4H, m, 3-, 3′-H, 6′-H_2), 4.25–4.20 (2H, m, 4-, 5′-H), 4.17 (1H, dd, *J* = 10.5, 4.5 Hz, 1-H_b), 2.23–2.16 (1H, m, 5-H_a), 2.15 (3H, s, CH_3), 1.84–1.69 (2H, m, 5-H_b, 6-H_a), 1.59–1.51 (1H, m, 6-H_b), 1.38–1.13 (22H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-H_2), 0.84 (3H, t, *J* = 7.0 Hz, 18-H_3) ppm; δ_C (126 MHz, pyridine-d_5): 143.2, 139.4, 133.0, 129.2, 127.8, 124.5, 101.3, 77.3, 72.9, 72.2, 71.5, 70.8, 70.2, 67.8, 62.4, 55.3, 34.9, 32.1, 30.3, 30.1, 29.97, 29.96, 29.9, 29.6, 26.2, 22.9, 21.0, 14.3 ppm; HR-FABMS: Calcd for $C_{31}H_{56}O_{10}NS$ $[M+H]^+$ 634.3625; found: 634.3625.

4.19. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(4-fluorobenzenesulfonamido)octadecane-3,4-diol (13k, RCAI-88)

In the same manner as described above for the conversion of **10** to **13a**, **13k** (RCAI-88) was synthesized from **10** as white powder. $[\alpha]_D^{25}$ +61.3 (*c* 0.38, pyridine); ν_{max} (KBr): 3360 (br s, OH, NH), 1590 (m, aromat.), 1495 (m, aromat.), 1330 (br m, SO_2), 1080 (br s, C–O), 1045 (br m, C–O), 840 (s) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 9.65 (1H, d, *J* = 8.0 Hz), 8.32–8.26 (2H, m), 7.22–7.15 (2H, m), 6.73 (1H, br s), 6.60–5.80 (5H, m), 5.39 (1H, d, *J* = 3.6 Hz), 4.74–4.67 (2H, m), 4.62 (1H, dd, *J* = 10, 4.0 Hz), 4.49 (1H, d, *J* = 3.2 Hz), 4.35–4.27 (3H, m), 4.30 (1H, dd, *J* = 10, 3.2 Hz), 4.27–4.15 (3H, m), 2.26–2.17 (1H, m), 1.86–1.68 (2H, m), 1.62–1.49 (1H, m), 1.40–1.17 (22H, m), 0.84 (3H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 164.8 (d, *J* = 250 Hz), 139.7 (d, *J* = 3.3 Hz), 130.3 (d, *J* = 9.9 Hz), 116.4 (d, *J* = 23 Hz), 101.2, 77.4, 72.9, 72.3, 71.5, 70.8, 70.1, 67.7, 62.5, 55.5, 35.0, 32.1, 30.3, 30.1, 30.0, 29.9, 29.6, 26.2, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{30}H_{53}O_{10}NFS$ $[M+H]^+$ 638.3374; found: 638.3373.

4.20. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(hexadecanesulfonamido)octadecane-3,4-diol (13l, RCAI-39)

In the same manner as described above for the conversion of **10** to **13a**, **13l** (RCAI-39) was synthesized from **10** as white powder. $[\alpha]_D^{14}$ +55.0 (*c* 0.32, pyridine); ν_{max} (KBr): 3400 (br s, OH, NH), 1320 (br m, SO_2), 1150 (br s, SO_2), 1075 (br s, C–O), 1040 (br s, C–O), 720 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.89 (1H, d, *J* = 9.5 Hz, NH), 6.81 (1H, br s, OH), 6.44–6.03 (2H, br s, 2× OH), 5.52 (1H, d, *J* = 4.0 Hz, 1′-H), 5.36–4.75 (3H, br s, 3× OH), 4.88 (1H, dd, *J* = 10.5, 5.0 Hz, 1-H_a), 4.88–4.83 (1H, m, 2-H), 4.66 (1H, dd, *J* = 10.0, 4.0 Hz, 2′-H), 4.52 (1H, br d, *J* = 3.5 Hz, 4′-H), 4.49 (1H, br t, *J* = 6.0 Hz, 5′-H), 4.43 (1H, dd, *J* = 10.0, 3.5 Hz, 3′-H), 4.41 (1H, dd, *J* = 11.0, 6.0 Hz, 6′-H_a), 4.38–4.33 (1H, m, 3-H), 4.36 (1H, dd, *J* = 11.0, 5.5 Hz, 6′-H_b), 4.33–4.27 (2H, m, 1-H_b, 4-H), 3.51 (2H, ddd, *J* = 9.0, 6.5, 3.5 Hz, 1″-H_2), 2.36–2.28 (1H, m, 5-H_a), 2.10–2.01 (2H, m, 2″-H_2), 1.94–1.79 (2H, m, 5-H_b, 6-H_a), 1.69–1.61 (1H, m, 6-H_b), 1.45–1.14 (48H, m, 17-, 16-, 15-, 14-, 13-, 12-, 11-, 10-, 9-, 8-, 7-, 15″-, 14″-, 13″-, 12″-, 11″-, 10″-, 9″-, 8″-, 7″-, 6″-, 5″-, 4″-, 3″-H_2), 0.84 (6H, t, *J* = 7.0 Hz, 18-, 16″-H_3) ppm; δ_C (126 MHz, pyridine-d_5): 101.0, 78.3, 73.0, 72.4, 71.6, 71.0, 70.2, 68.3, 62.7, 55.9, 54.3, 35.1, 32.1, 30.0, 29.94, 29.92, 29.91, 29.89, 29.7, 29.6, 28.7, 26.4, 24.5, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{40}H_{82}O_{10}NS$ $[M+H]^+$ 768.5659; found: 768.5655.

4.21. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(methanesulfonamido)octadecane-3,4-diol (13m, RCAI-40)

In the same manner as described above for the conversion of **10** to **13a**, **13m** (RCAI-40) was synthesized from **10** as white powder. $[\alpha]_D^{15}$ +62.4 (*c* 0.31, pyridine); ν_{max} (KBr): 3400 (br s, OH), 3280 (w, NH), 1300 (br s, SO_2), 1140 (br s, SO_2), 1075 (br s, C–O), 1040 (br s, C–O), 770 (m) cm^{-1}; δ_H (400 MHz, pyridine-d_5): 8.94 (1H, d, *J* = 8.4 Hz), 6.86 (2H, br s), 6.50–5.80 (4H, m), 5.42 (1H, d, *J* = 4.0 Hz), 4.88–4.78 (2H, m), 4.62 (1H, dd, *J* = 9.6, 3.6 Hz), 4.47–4.28 (6H, m), 4.24–4.17 (2H, m), 3.34 (3H, s), 2.31–2,23 (1H, m), 1.90–1.72 (2H, m), 1.65–1.54 (1H, m), 1.41–1.10 (22H, m), 0.80 (3H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 101.0, 78.5, 73.0, 72.5, 71.5, 71.1, 70.2, 67.8, 62.8, 56.5, 42.2, 35.2, 32.1, 30.3, 30.1, 30.0, 29.9, 29.6, 26.4, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{25}H_{52}O_{10}NS$ $[M+H]^+$ 558.3312; found: 558.3316.

4.22. (2*R*,3*R*,4*R*)-1,3,4-Tribenzyloxy-5-nonen-2-ol (16)

To a stirred solution of **15** (15.2 g, 33.7 mmol) in EtOH (190 mL) and H_2O (45 mL), sodium periodate (14.7 g, 68.7 mmol) was added at room temp. After stirring at room temperature for 18 h, the mixture was diluted with Et_2O. The reaction was quenched with water, and the separated aqueous phase was extracted with Et_2O. The combined organic phase was washed with water and brine, dried with Na_2SO_4, and concentrated in vacuo to give the crude aldehyde (12.8 g).

To a stirred suspension of *n*-butyltriphenylphosphonium bromide (40.9 g, 102 mmol) in dry THF (200 mL), a solution of *n*-BuLi (1.59 M in hexane, 61.0 mL, 97.0 mmol) was added dropwise at 0 °C under argon. The resulting solution was stirred at 0 °C for 30 min. The solution of the crude aldehyde (12.8 g) in dry THF (75 mL) was added dropwise to this solution at 0 °C. After stirring at room temperature for 19 h, the mixture was concentrated in vacuo. The residue was dissolved in hexane/MeOH/H_2O (2:2:1, 500 mL) and the resulting mixture was stirred for 1 h. The separated aqueous phase was extracted with hexane. The combined organic phase was successively washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (100 g, hexane/EtOAc = 50:3) to give **16** (9.52 g, 61%, two steps) as a yellow oil, n_D^{21} = 1.5173; $[\alpha]_D^{21}$ −39.1 (*c* 1.31, $CHCl_3$); ν_{max} (film): 3480 (br m, OH), 1660 (w, C=C), 1605 (w, aromat.), 1585 (w, aromat.), 1500 (s, aromat.), 1090 (br s, C–O), 740 (s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.34–7.20 (15H, m), 5.73 (1H, dt, *J* = 10, 7.2 Hz), 5.47 (1H, t, *J* = 10 Hz), 4.68 (1H, d, *J* = 12 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.51 (1H, d, *J* = 12 Hz), 4.48 (1H, d, *J* = 12 Hz), 4.47 (1H, d, *J* = 12 Hz), 4.44 (1H, dd, *J* = 10, 5.2 Hz), 4.33 (1H, d, *J* = 12 Hz), 4.10–4.05 (1H, m), 3.56 (1H, dd, *J* = 5.2, 2.4 Hz), 3.51 (2H, d, *J* = 6.0 Hz), 3.04 (1H, br s), 2.00–1.83 (2H, m), 1.34 (2H, sext., *J* = 7.6 Hz), 0.86 (3H, t, *J* = 7.6 Hz) ppm; δ_C (100 MHz, $CDCl_3$): 138.1, 136.1, 128.34, 128.27, 128.2, 127.84, 127.79, 127.7, 127.6, 127.2, 79.7, 74.6, 73.8, 73.3, 71.0, 70.2, 69.9, 29.9, 22.7, 13.8 ppm; HR-FABMS: Calcd for $C_{30}H_{36}O_4Na$ $[M+Na]^+$ 483.2511; found: 483.2510.

4.23. (2*R*,3*R*,4*R*)-2-Methanesulfonyloxy-1,3,4-tribenzyloxy-5-nonene (17)

To a stirred solution of **16** (9.37 g, 20.3 mmol) in pyridine (100 mL), methanesulfonyl chloride (3.4 mL, 43.9 mmol) was added at 0 °C. After stirring at room temperature for 18 h, the reaction was quenched with water. The mixture was diluted with Et_2O, and the separated organic phase was successively washed with water, saturated aqueous $CuSO_4$ solution, water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (100 g, $CHCl_3$) to give **17** (9.46 g, 87%) as a pale yellow oil, n_D^{21} = 1.5171; $[\alpha]_D^{21}$ +4.00 (*c* 1.28, $CHCl_3$); ν_{max} (film): 1655 (w, C=C), 1605 (w, aromat.), 1585 (w, aromat.), 1500 (m, aromat.), 1175 (s, SO_2), 1085 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.38–7.25 (15H, m), 5.82 (1H, dt, *J* = 11, 7.6 Hz), 5.51 (1H, br t, *J* = 11 Hz), 5.07 (1H, dt, *J* = 7.6, 3.2 Hz), 4.77 (1H, d, *J* = 11 Hz), 4.53 (1H, d, *J* = 11 Hz), 4.52 (1H, d, *J* = 11 Hz), 4.50 (1H, d, *J* = 11 Hz), 4.42 (1H, d, *J* = 7.2 Hz), 4.41 (1H, d, *J* = 11 Hz), 4.40 (1H, d, *J* = 11 Hz), 3.78 (1H, dd, *J* = 6.4, 4.0 Hz), 3.67 (1H, dd, *J* = 11, 6.4 Hz), 3.49 (1H, dd, *J* = 11, 4.0 Hz), 2.95 (3H, s), 2.12–1.92 (2H, m), 1.46–1.33 (2H, m), 0.90 (3H, t, *J* = 7.6 Hz) ppm; δ_C (100 MHz, $CDCl_3$): 138.3, 137.2, 128.44, 128.37, 128.3, 128.1, 127.91, 127.86, 127.6, 126.5, 80.8, 79.2, 74.8, 73.3, 70.0, 69.4, 38.6, 29.9, 22.7, 13.8 ppm; HR-FABMS: Calcd for $C_{31}H_{38}O_6SNa$ $[M+Na]^+$ 561.2287; found: 561.2286.

4.24. (2*R*,3*R*,4*R*)-2-Methanesulfonyloxy-1,3,4-nonanetriol (18)

To a stirred solution of **17** (9.27 g, 17.2 mmol) in EtOH (200 mL), 10% Pd–C (Kawaken Co., 1.56 g) was added under argon. After stirring at room temperature for 17 h under hydrogen atmosphere (balloon), the mixture was filtered through a bed of Celite. The filter cake was washed with $CHCl_3$/MeOH (5:1), and the combined filtrate was concentrated in vacuo to give **18** (4.8 g) as a yellow oil. This was immediately used in the next step without further purification.

4.25. (2*S*,3*S*,4*R*)-2-Azido-1,3,4-nonanetriol (19)

A solution of **18** (4.8 g) and sodium azide (4.66 g, 71.7 mmol) in DMF (150 mL), was stirred at 95 °C for 9 h. The mixture was then cooled to room temperature, and the reaction was quenched with water. The mixture was diluted with EtOAc, and the separated aqueous phase was extracted with EtOAc. The combined organic phase was washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (80 g, $CHCl_3$/MeOH = 20:1) to give **19** (2.08 g, 54%, two steps) as a yellow solid. $[\alpha]_D^{22}$ +13.1 (*c* 0.78, MeOH); ν_{max} (film): 3320 (br s, OH), 2160 (s, N_3), 1010 (br m, C–O) cm^{-1}; δ_H (400 MHz, CD_3OD): 3.91 (1H, dd, *J* = 12, 3.2 Hz), 3.74 (1H, dd, *J* = 12, 7.6 Hz), 3.61–3.56 (1H, m), 3.54–3.49 (2H, m), 1.71–1.26 (8H, m), 0.91 (3H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, CD_3OD): 76.0, 72.9, 66.7, 62.5, 33.9, 33.1, 26.4, 23.7, 14.4 ppm; HR-FABMS: Calcd for $C_9H_{20}O_3N_3$ $[M+H]^+$ 218.1505; found: 218.1503. This was immediately used in the next step without further purification.

4.26. (2*S*,3*S*,4*R*)-2-Amino-1,3,4-nonanetriol (20)

To a stirred solution of **19** (1.98 g, 9.11 mmol) in EtOH (50 mL) and $CHCl_3$ (20 mL), 10% Pd–C (Kawaken Co., 266 mg) was added under argon. After stirring at room temperature for 12 h under hydrogen atmosphere (balloon), the mixture was filtered through a bed of Celite. The filter cake was washed with $CHCl_3$/MeOH (5:1), and the combined filtrate was concentrated in vacuo to give **20** (2.5 g) as a yellow solid. HR-FABMS: Calcd for $C_9H_{22}O_3N$ $[M+H]^+$ 192.1600; found: 192.1601. This was immediately used in the next step without further purification.

4.27. (2*S*,3*S*,4*R*)-2-Amino-1,3,4-tris(*tert*-butyldimethylsilyloxy)nonane (21)

To a solution of **20** (2.50 g) in 2,6-lutidine (20 mL) and CH_2Cl_2 (20 mL), TBSOTf (10.0 mL, 43.5 mmol) was added at 0 °C. After stirring at room temperature for 17 h, the reaction was quenched with MeOH. The mixture was concentrated in vacuo, and the remaining 2,6-lutidine was azeotropically removed with toluene. The residue was diluted with EtOAc, and neutralized with saturated aqueous $NaHCO_3$ solution. The separated organic phase was washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (80 g, hexane/EtOAc = 15:1) to give **21** (1.74 g, 43%, two steps) as a pale yellow oil, n_D^{21} = 1.4560; $[\alpha]_D^{21}$ −2.78 (*c* 1.29, $CHCl_3$); ν_{max} (film): 3400 (w, NH), 3320 (w, NH), 1255 (s, *t*Bu, Si–CH_3), 1100 (br s, C–O), 835 (br s), 780 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 3.83-3.79 (1H, m), 3.82 (1H, dd, *J* = 9.6, 3.2 Hz), 3.52 (1H, dd, *J* = 6.8, 1.6 Hz), 3.44 (1H, dd, *J* = 9.6, 6.8 Hz), 2.88 (1H, dt, *J* = 6.8, 3.2 Hz), 1.54–1.25 (10H, m), 0.91 (9H, s), 0.90 (18H, s), 0.89 (3H, t, *J* = 7.2 Hz), 0.09 (6H, s), 0.062 (6H, s), 0.055 (3H, s), 0.050 (3H, s) ppm; δ_C (100 MHz, $CDCl_3$): 77.9, 76.4, 65.5, 55.4, 34.3, 32.0, 26.1, 25.9, 22.6, 18.3, 18.23, 18.19, 14.0, −3.6, −3.9, −4.6, −4.9, −5.27, −5.32 ppm; HR-FABMS: Calcd for $C_{27}H_{64}O_3NSi_3$ $[M+H]^+$ 534.4194; found: 534.4192.

4.28. (2*S*,3*S*,4*R*)-1,3,4-Tris(*tert*-butyldimethylsilyloxy)-2-(tetracosanoylamino)nonane (22)

To a stirred solution of **21** (930 mg, 2.22 mmol), *i*-Pr_2NEt (2.0 mL, 12 mmol) and lignoceric acid (1.00 g, 2.71 mmol) in CH_2Cl_2 (20 mL), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (673 mg, 3.51 mmol) and a catalytic amount of DMAP (0.02 g) were added at 0 °C. After stirring at room temperature for 15 h, the reaction was quenched with water. The mixture was diluted with EtOAc, and the separated organic phase was washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 40:1) to give **22** (1.47 g, 75%) as a pale yellow oil, n_D^{21} = 1.4639; $[\alpha]_D^{21}$ +1.37 (*c* 1.04, $CHCl_3$); ν_{max} (film): 3440 (m, NH), 1690 (br s, C=O), 1255 (s, *t*Bu, Si–CH_3), 1080 (br s, C–O), 835 (br s), 780 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 5.82 (1H, d, *J* = 8.8 Hz), 3.98–3.91 (1H, m), 3.88 (1H, dd, *J* = 9.6, 4.0 Hz), 3.82 (1H, br d, *J* = 6.4 Hz), 3.69–3.65 (1H, m), 3.63 (1H, dd, *J* = 9.6, 4.4 Hz), 2.14 (2H, t, *J* = 7.6 Hz), 1.67–1.21 (50H, m), 0.908 (9H, s), 0.904 (9H, s), 0.895 (9H, s), 0.88 (6H, t, *J* = 7.2 Hz), 0.13 (3H, s), 0.057 (9H, s), 0.049 (3H, s), 0.037 (3H, s) ppm; δ_C (100 MHz, $CDCl_3$): 172.3, 75.4, 61.4, 52.5, 37.2, 32.2, 32.1, 31.9, 29.71, 29.66, 29.6, 29.5, 29.41, 29.37, 26.1, 26.0, 25.9, 25.8, 22.7, 22.6, 18.4, 18.2, 18.1, 14.13, 14.08, −3.5, −3.8, −4.6, −5.2, −5.6 ppm; HR-FABMS: Calcd for $C_{51}H_{110}O_4NSi_3$ $[M+H]^+$ 884.7743; found: 884.7745.

4.29. (2*S*,3*S*,4*R*)-3,4-Bis(*tert*-butyldimethylsilyloxy)-2-(tetracosanoylamino)-1-nonanol (23)

To a stirred solution of **22** (1.40 g, 1.58 mmol) in THF (40 mL), a 10% aqueous CF_3CO_2H solution (v/v, 8 mL) was added dropwise at −15 °C. The mixture was gradually warmed up to 0 °C with stirring over 5 h. The reaction was then quenched with a 15% aqueous NaOH solution. The mixture was diluted with Et_2O, and the separated organic phase was washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 40:3) to give **23** (937 mg, 77%) as colorless needles. Mp = 35.5–37.0 °C; $[\alpha]_D^{22}$ −14.3 (*c* 0.45, $CHCl_3$); ν_{max} (film): 3360 (br m, OH), 3260 (m, NH), 1645 (br s, C=O), 1560 (br m), 1255 (s, *t*Bu, Si–CH_3), 1040 (m, C–O), 840 (s), 780 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 6.25 (1H, d, *J* = 7.6 Hz), 4.22 (1H, dt, *J* = 12, 2.8 Hz), 4.07 (1H, m), 3.91 (1H, t, *J* = 2.8 Hz), 3.77 (1H, dt, *J* = 6.4, 2.8 Hz), 3.59 (1H, ddd, *J* = 12, 9.2, 4.0 Hz), 3.16 (1H, dd, *J* = 9.2, 2.8 Hz), 2.18 (2H, t, *J* = 6.8 Hz), 1.67–1.20 (50H, m), 0.93 (9H, s), 0.91 (9H, s), 0.89 (3H, t, *J* = 6.8 Hz), 0.88 (3H, t, *J* = 6.8 Hz), 0.11 (6H, s), 0.09 (6H, s) ppm; δ_C (100 MHz, $CDCl_3$): 173.0, 77.8, 76.7, 64.0, 51.5, 37.3, 34.7, 32.3, 32.2, 30.02, 29.97, 29.9, 29.8, 29.7, 26.32, 26.27, 25.9, 25.8, 22.9, 18.4, 14.44, 14.36, −3.4, −4.2, −4.6 ppm; HR-FABMS: Calcd for $C_{45}H_{96}O_4NSi_2$ $[M+H]^+$ 770.6878; found: 770.6878.

4.30. (2*S*,3*S*,4*R*)-1-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranoxy)-3,4-bis(*tert*-butyldimethylsilyloxy)-2-(tetracosanoylamino)nonane (24)

To a stirred suspension of **23** (819 mg, 1.06 mmol) and powdered MS 4A (4.96 g) in dry THF (40 mL), tin(II) chloride (630 mg, 3.32 mmol) and silver(I) perchlorate (704 mg, 3.40 mmol) were added under argon. After stirring at room temperature for 1 h, the mixture was cooled to −18 °C. To this mixture, a solution of **8** (1.34 g, 2.40 mmol) in dry THF (10 mL) was added dropwise at −18 °C. The mixture was gradually warmed up to 0 °C with stirring over 2 h. The reaction mixture was then diluted with ether, and the resulting mixture was filtered through a bed of Celite. The filtrate was successively washed with water and brine, dried with K_2CO_3, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (50 g, hexane/EtOAc = 10:1) to give **24** (940 mg, contaminating unreacted **8**) as a colorless oil, n_D^{21} = 1.5084; $[\alpha]_D^{22}$ +14.2 (*c* 1.01, $CHCl_3$); ν_{max} (film): 3360 (m, NH), 1680 (br s, C=O), 1255 (s, *t*Bu, Si–CH_3), 1100 (br s, C–O), 1060 (br s, C–O), 840 (s), 780 (s), 735 (br s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.37–7.22 (20H, m), 6.01 (1H, d, *J* = 6.8 Hz), 4.91 (1H, d, *J* = 12 Hz), 4.83 (1H, d, *J* = 4.0 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.77 (1H, d, *J* = 12 Hz), 4.71 (1H, d, *J* = 12 Hz), 4.66 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.48 (1H, d, *J* = 12 Hz), 4.39 (1H, d, *J* = 12 Hz), 4.12–4.04 (1H, m), 4.03 (1H, dt, *J* = 9.6, 3.2 Hz), 3.97–3.84 (6H, m), 3.65 (1H, ddd, *J* = 6.8, 5.2, 2.0 Hz), 3.55–3.45 (2H, m), 1.98 (2H, t, *J* = 7.2 Hz), 1.57–1.18 (50H, m), 0.90 (9H, s), 0.88 (9H, s), 0.88 (3H, t, *J* = 7.2 Hz), 0.87 (3H, t, *J* = 7.2 Hz), 0.07 (3H, s), 0.05 (3H, s), 0.03 (3H, s), 0.02 (3H, s) ppm; δ_C (100 MHz, $CDCl_3$): 173.1, 138.7, 138.62, 138.57, 128.4, 128.34, 128.30, 128.2, 127.9, 127.8, 127.7, 127.6, 127.52, 127.48, 127.4, 100.3, 79.0, 76.5, 75.7, 74.9, 74.7, 73.5, 73.4, 72.9, 69.8, 69.6, 68.7, 51.7, 36.7, 33.1, 32.1, 31.9, 29.69, 29.65, 29.6, 29.5, 29.4, 26.14, 26.06, 25.7, 25.6, 22.7, 22.6, 18.3, 18.2, 14.1, −3.7, −3.9, −4.7 ppm; HR-FABMS: Calcd for $C_{79}H_{130}O_9NSi_2$ $[M+H]^+$ 1292.9284; found: 1292.9280.

4.31. (2*S*,3*S*,4*R*)-1-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranoxy)-2-(tetracosanoylamido)nonane-3,4-diol (25)

To a stirred solution of **24** (887 mg) in THF (10 mL), a solution of TBAF (1.0 M in THF, 7.0 mL, 7.0 mmol) was added at room temperature. After stirring at room temperature for 20 h, the reaction was quenched with water, and the resulting mixture was extracted with EtOAc. The combined organic extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 4:1) to give **25** (458 mg, 41%, two steps) as a white solid. $[\alpha]_D^{23}$ +36.2 (*c* 1.55, $CHCl_3$); ν_{max} (film): 3420 (br s, OH), 3340 (s, NH), 1640 (s, C=O), 1620 (w), 1540 (br s), 1500 (m, aromat.), 1110 (br s, C–O), 1045 (br s, C–O), 730 (br s), 695 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.38–7.25 (20H, m), 6.40 (1H, br d, *J* = 6.8 Hz), 4.91 (1H, d, *J* = 12 Hz), 4.88 (1H, d, *J* = 11 Hz), 4.84 (1H, d, *J* = 3.6 Hz), 4.78 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.56 (1H, d, *J* = 11 Hz), 4.47 (1H, d, *J* = 12 Hz), 4.38 (1H, d, *J* = 12 Hz), 4.23–4.18 (1H, m), 4.04 (1H, dd, *J* = 10, 4.0 Hz), 3.97 (1H, br d, *J* = 2.4 Hz), 3.92–3.81 (5H, m), 3.52–3.43 (4H, m), 2.19 (1H, br s), 2.11 (2H, t, *J* = 6.8 Hz), 1.77–1.65 (1H, m), 1.63–1.53 (4H, m), 1.52–1.41 (1H, m), 1.41–1.21 (44H, m), 0.88 (6H, t, *J* = 6.8 Hz) ppm; δ_C (100 MHz, $CDCl_3$): 173.1, 138.4, 138.3, 137.8, 137.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 99.2, 79.3, 76.2, 76.0, 74.7, 74.4, 74.2, 73.6, 73.2, 72.7, 70.0, 69.9, 68.9, 49.5, 36.7, 33.2, 32.1, 31.9, 31.8, 29.71, 29.68, 29.5, 29.38, 29.35, 29.3, 25.7, 25.5, 22.7, 22.6, 14.11, 14.05 ppm; HR-FABMS: Calcd for $C_{67}H_{102}O_9N$ [M + H]$^+$ 1064.7555; found: 1064.7551.

4.32. (2*S*,3*S*,4*R*)-1-(α-D-Galactopyranoxy)-2-(tetracosanoylamido)octadecane-3,4-diol (2, OCH or RCAI-43 in our code name)

To a stirred solution of **25** (366 mg, 0.344 mmol) in EtOH (16 mL) and $CHCl_3$ (4 mL), 20% $Pd(OH)_2$–C (Aldrich, wet type, 70 mg) was added under argon. The mixture was stirred at room temperature for 14 h under hydrogen atmosphere (balloon). The suspension was then filtered through a bed of Celite, and the filter cake was washed with $CHCl_3$/MeOH (5:1). The combined filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (15 g, $CHCl_3$/MeOH = 25:2) to give **2** (OCH or RCAI-43, 142 mg, 50%) as white powder. An analytical sample was recrystallized from EtOH/H_2O = 10:1. Mp = 140.5–142.0 °C [lit.[14] Mp = 142–145 °C (EtOH/H_2O = 10:1)]; $[\alpha]_D^{23}$ +54.4 (*c* 0.52, pyridine) {lit.[14] $[\alpha]_D^{30}$ +53.9 (*c* 0.5, pyridine)}; ν_{max} (film): 3360 (br s, OH), 3280 (s, NH), 1645 (s, C=O), 1615 (w), 1540 (br s), 1145 (br m, C–O), 1075 (br s, C–O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 8.46 (1H, d, *J* = 8.8 Hz), 6.80–5.70 (6H, m), 5.56 (1H, d, *J* = 4.0 Hz), 5.20–5.23 (1H, m), 4.68–4.62 (2H, m), 4.53 (1H, d, *J* = 2.8 Hz), 4.49 (1H, t, *J* = 5.6 Hz), 4.45–4.34 (4H, m), 4.30–4.24 (2H, m), 2.42 (2H, t, *J* = 7.2 Hz), 2.27–2.17 (1H, m), 1.91–1.77 (2H, m), 1.79 (2H, quint., *J* = 7.2 Hz), 1.66–1.53 (1H, m), 1.36–1.17 (44H, m), 0.85 (3H, t, *J* = 7.2 Hz), 0.78 (3H, t, *J* = 7.2 Hz) ppm; δ_C (100 MHz, pyridine-d_5): 173.2, 101.5, 76.8, 73.1, 72.4, 71.6, 71.0, 70.3, 68.6, 62.7, 51.4, 36.8, 34.3, 32.4, 32.1, 30.02, 29.99, 29.91, 29.86, 29.8, 29.7, 29.6, 26.4, 26.1, 23.0, 22.9, 14.3 ppm; HR-FABMS: Calcd for $C_{39}H_{78}O_9N$ [M+H]$^+$ 704.5677; found: 704.5673.

4.33. 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.

4.34. 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 medium supplemented 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 previously. Cells were stimulated with 2, 20, or 200 ng/mL of KRN7000 or other glycolipids.[1,20]

4.35. 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 IFN-γ, IL-4, and IL-13 levels, as described previously.[1,20] In brief, IFN-γ, IL-4, and IL-13 were detected simultaneously using the CBA Mouse Flex Set (BD Biosciences). Briefly, 50 μL of each sample was mixed with 50 μL of mixed capture beads and 50 μL of the mouse soluble protein phycoerythrin (PE) detection reagent consisting of PE-conjugated anti-mouse IFN-γ, IL-4, and IL-13. The samples were incubated at room temperature for 1 h in the dark. After incubation with the PE detection reagent, the samples were washed twice 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.

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