

Electronic Supplementary Information

RCAI-133, the *N*-methylated analogue of KRN7000, activates mouse natural killer T cells to produce Th2-biased cytokines

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1. Experimental

1.1 Chemistry

1.1.1. General

Melting points are uncorrected values, and were measured on Yanako MP-S3 micro melting point apparatus. Refractive indices (n_D) were measured on an Atago 1T refractometer. Optical rotation values were measured on a Jasco P-1010 polarimeter. IR spectra were measured on a Jasco FT/IR-460 plus spectrometer. ^1H -NMR spectra (TMS at $\delta_H = 0.00$, CHCl_3 at $\delta_H = 7.26$ or pyridine at $\delta_H = 7.55$ as internal standards) and ^{13}C -NMR spectra (pyridine at $\delta_C = 135.5$ as internal standard) were recorded on a Varian VNMRS-500 spectrometer. HRMS were recorded on a Jeol JMS-SX102A or a Bruker BioAPEX II 70e FT-ICR. Column chromatography was performed by using Kanto Chemical silica gel 60N irregular neutral (37572-79) or Fuji Silysia Chemical chromatorex[®] DIOL (SMB100-75/200) or NH (DM2035). Thin layer chromatography was performed on Merck Silica gel 60 F₂₅₄.

1.1.2. (*R*)-2-(Benzyloxy)octadecan-1-ol (10)

To a stirred solution of octadecane-1,2-diol (Wako Pure Chemical Industries, Ltd., 248 mg, 0.866 mmol) in pyridine (10 mL), triphenylmethyl chloride (TrCl, 484 mg, 1.74 mmol) and 4-(*N,N*-dimethylamino)pyridine (DMAP, 0.05 g, catalytic amount) were added at 0 °C. After stirring for 16 h, the mixture was poured into water, and extracted with EtOAc. The separated organic phase was washed successively with water, a saturated aqueous CuSO_4 solution, water, a 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 (30 g, hexane/EtOAc = 20:1) to give (*R*)-1-(triphenylmethoxy)octadecan-2-ol (607 mg) with a small amount of triphenylmethanol as a white solid. This was used in the next step without further purification.

To a stirred solution of (*R*)-1-(triphenylmethoxy)octadecan-2-ol (607 mg, 1.15 mmol) in THF-*N,N*-dimethylformamide (DMF) (1:1, 10 mL), sodium hydride (ca. 60% in mineral oil, 93 mg, 2.3 mmol), benzyl bromide (164 μL , 1.38 mmol) and tetrabutylammonium iodide (0.02 g, catalytic amount) were added at 0 °C. After stirring at 80 °C for 6 h, the mixture was poured into water, and extracted with EtOAc. The separated organic phase was washed successively with water, a 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 (30 g, hexane/EtOAc = 20:1) to give (*R*)-2-benzyloxy-1-(triphenylmethoxy)octadecane (650 mg) with a small amount of impurities as a pale yellow oil. This was used in the next step without further purification.

To a stirred solution of (*R*)-2-benzyloxy-1-(triphenylmethoxy)octadecane (650 mg) in dioxane-MeOH (1:1, 16 mL), conc. H_2SO_4 (0.13 mL) was added at room temperature. After stirring at 50 °C for 2 h, the mixture was poured into a saturated aqueous NaHCO_3 solution at 0 °C, and extracted with EtOAc. The separated organic phase was washed successively with a saturated aqueous NaHCO_3 solution and brine, dried with K_2CO_3 , and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 10:1) to give **10** (185 mg, 57%, three steps) as a colorless solid. Mp 42.0–43.5 °C; $[\alpha]_D^{21} -14.9$ (*c* 1.00, CHCl_3); ν_{max} (KBr): 3440 (br s, OH), 1495 (w), 1070 (br s, C–O), 750 (br s), 720 (m), 695 (s) cm^{-1} ; δ_H (500 MHz, CDCl_3): 7.39–7.22 (5H, m), 4.63 (1H, d, $J = 12$ Hz), 4.54 (1H, d, $J = 12$ Hz), 3.72–3.67 (1H, m), 3.56–3.49 (2H, m), 1.91 (1H, dd, $J = 7.0, 4.5$ Hz), 1.67–1.60 (1H, m), 1.53–1.46 (1H, m), 1.38–1.21 (28H, m), 0.88 (3H, t, $J = 7.0$ Hz) ppm. The IR and ^1H -NMR data were in good accord with those reported by U. Massing *et al.*¹⁾; HRMS (ESI+): Calcd for $\text{C}_{25}\text{H}_{44}\text{O}_2\text{Na}$ $[\text{M}+\text{Na}]^+$ 399.3239; found 399.3241.

1.1.3. (*R*)-2-(Benzyloxy)octadecanoic acid (11)

To a stirred solution of **10** (397 mg, 1.05 mmol) in acetone (dried with CaCl_2 , 20 mL), a Jones CrO_3

solution (2.67 M, 1.77 mL, 4.73 mmol) was added at 0 °C. The mixture was stirred with gradually warm up to room temperature. After stirring for 6 h, the reaction was quenched with 2-propanol (360 µL), and the resulting mixture was stirred at room temperature for 30 min, and then concentrated *in vacuo*. The residue was added water, and extracted with EtOAc. The separated organic phase was washed successively with water and brine, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (DIOL, 30 g, hexane/EtOAc = 20:1) to give **11** (197 mg, 48%) as a colorless solid. Mp 52.5–55.0 °C; $[\alpha]_D^{24} +26.0$ (*c* 1.05, CHCl₃); ν_{\max} (KBr): 3040 (br s, COOH), 1700 (br s, CO), 1605 (w), 1495 (m), 1110 (br s, C–O), 945 (br m), 735 (br s), 700 (s) cm⁻¹; δ_H (500 MHz, CDCl₃): 7.89–7.31 (5H, m), 4.70 (1H, d, *J* = 12 Hz), 4.52 (1H, d, *J* = 12 Hz), 4.01 (1H, t, *J* = 6.0 Hz), 1.84–1.79 (2H, m), 1.46–1.39 (2H, m), 1.32–1.20 (27H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI⁻): Calcd for C₂₅H₄₁O₃ [M–H]⁻ 389.3056; found 389.3062.

1.1.4. (2*RS*,2'*R*)-2-(2-Methyloctadecyloxy)tetrahydropyran (**14**)

To a stirred mixture of magnesium (853 mg, 35.1 mmol) and a piece of iodine (one piece, catalytic amount) in dry THF (1 mL), a solution of 1-bromopentadecane (8.50 g, 29.2 mmol) in dry THF (50 mL) was added dropwise at room temperature. After completion of the addition, the mixture was stirred at room temperature for 1 h. The mixture was then slowly added to a solution of the known tosylate **13**²⁾ (6.39 g, 19.5 mmol) in dry THF (30 mL) at –50 °C. To the resulting mixture, a solution of Li₂CuCl₄ (Schlosser catalyst, 0.1 M in THF, 3.0 mL, 0.3 mmol) was added at –40 °C. The mixture was gradually warmed up to room temperature with stirring over 15 h. The mixture was poured into a saturated aqueous NH₄Cl solution, and extracted with Et₂O. The separated organic phase was washed successively with a saturated aqueous NH₄Cl solution, water, a saturated aqueous NaHCO₃ solution and brine, dried with K₂CO₃, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (150 g, hexane/EtOAc = 15:1) to give **14** (4.16 g, 58%) as a colorless oil, n_D^{25} 1.4550; $[\alpha]_D^{25} -1.25$ (*c* 1.12, CHCl₃); ν_{\max} (film): 1120 (br s, C–O), 1035 (br s, C–O), 975 (m) cm⁻¹; δ_H (500 MHz, CDCl₃, Diastereomer ratio = ca. 1:1): 4.58–4.55 (1H, m), 3.89–3.84 (1H, m), 3.61 (0.5H, dd, *J* = 9.5, 6.5 Hz), 3.52–3.48 (1H, m), 3.49 (0.5H, dd, *J* = 9.5, 6.0 Hz), 3.24 (0.5H, dd, *J* = 9.5, 6.0 Hz), 3.13 (0.5H, dd, *J* = 9.5, 7.0 Hz), 1.87–1.79 (1H, m), 1.75–1.67 (2H, m), 1.62–1.48 (4H, m), 1.46–1.21 (29H, m), 1.15–1.06 (1H, m), 0.93 (1.5H, d, *J* = 7.0 Hz), 0.91 (1.5H, d, *J* = 7.0 Hz), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (EI⁺): Calcd for C₂₄H₄₈O₂ [M]⁺ 368.3654; found 368.3650.

1.1.5. (R)-2-Methyloctadecan-1-ol (**15**)

To a stirred solution of **14** (3.26 g, 8.84 mmol) in MeOH-CH₂Cl₂ (1:1, 100 mL), *p*-toluenesulfonic acid monohydrate (TsOH·H₂O, 104 mg, 0.547 mmol) was added at room temperature. After stirring at room temperature for 14 h, the mixture was added an aqueous 1.0 M NaOH solution (2.2 mL, 2.2 mmol), and concentrated *in vacuo*. The residue was added water, and extracted with EtOAc. The separated organic phase was washed successively with water, a saturated aqueous NaHCO₃ solution and brine, dried with K₂CO₃, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (70 g, hexane/EtOAc = 8:1) to give **15** (2.23 g, 89%) as a colorless solid. Mp 41.5–42.5 °C, Ref. 3: MP 44–45 °C; $[\alpha]_D^{25} +6.68$ (*c* 1.16, CHCl₃), $[\alpha]_D^{26} +5.17$ (*c* 1.16, benzene), Ref. 3: $[\alpha]_D^{22} +5.5$ (*c* 5.0, benzene); ν_{\max} (KBr): 3300 (br s, OH), 1045 (br s, C–O) cm⁻¹; δ_H (500 MHz, CDCl₃): 3.51 (1H, ddd, *J* = 11, 6.0, 5.5 Hz), 3.42 (1H, ddd, *J* = 11, 6.0, 5.0 Hz), 1.64–1.56 (1H, m), 1.42–1.21 (30H, m), 1.15–1.06 (1H, m), 0.91 (3H, d, *J* = 7.0 Hz), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (EI⁺): Calcd for C₁₉H₃₈ [M–H₂O]⁺ 266.2974; found 266.2979.

1.1.6. (R)-2-Methyloctadecanoic acid (**16**)

In the same manner as described above for the conversion of **10** to **11**, **15** (496 mg, 1.74 mmol) was

converted to **16** (428 mg, 82%) as a colorless solid. This was used in the next step without further purification. An analytical sample was obtained by recrystallization from MeOH. Mp 51.0–52.5 °C; Ref. 3: MP 53.5–54 °C; $[\alpha]_{\text{D}}^{23}$ –9.41 (*c* 1.19, CHCl₃), Ref. 3: $[\alpha]_{\text{D}}^{25}$ –9.7 (*c* 9.0, CHCl₃); ν_{max} (KBr): 3080 (br s, COOH), 1715 (br s, CO), 1200 (br s), 900 (br m) cm^{–1}; δ_{H} (500 MHz, CDCl₃): 10.1 (1H, br s), 2.46 (1H, sext, *J* = 7.0 Hz), 1.72–1.64 (1H, m), 1.47–1.39 (1H, m), 1.38–1.20 (28H, m), 1.18 (3H, d, *J* = 7.0 Hz), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI[–]): Calcd for C₁₉H₃₇O₂ [M–H][–] 297.2794; found 297.2793.

1.1.7. 2,2-Dimethyloctadecanoic acid (**19**)

To a stirred solution of **18** (1.01 g, 11.5 mmol) in dry THF (10 mL), sodium hydride (ca. 60% in mineral oil, 481 mg, 12.0 mmol) was slowly added at room temperature. After stirring for 30 min under reflux, the reaction mixture was cooled to 0 °C. To the mixture, **17** (4.00 mL, 12.7 mmol) was added at 0 °C. The mixture was gradually warmed up to room temperature with stirring for 18 h. The mixture was then poured into water, and extracted with Et₂O. The separated organic phase was acidified with a 3.0 M hydrochloric acid, and the aqueous phase was extracted with Et₂O. The combined organic phase was washed successively with water and brine, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (70 g, hexane/EtOAc = 20:1) to give **19** (2.93 g, 81%) as a colorless solid. This was used in the next step without further purification. An analytical sample was obtained by recrystallization from acetone. Mp 55.0–56.5 °C; Ref. 4: Mp 57.1–58.0 °C; ν_{max} (KBr): 3000 (br s, COOH), 1710 (br s, CO), 1200 (m), 940 (br s), 730 (s) cm^{–1}; δ_{H} (500 MHz, CDCl₃): 10.6 (1H, br s), 1.53 (2H, br t, *J* = 7.0 Hz), 1.32–1.23 (28H, m), 1.19 (6H, s), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI[–]): Calcd for C₂₀H₃₉O₂ [M–H][–] 311.2950; found 311.2959.

1.1.8. 2,2-Dimethyloctadecanoyl chloride (**20**)

To a stirred solution of **19** (59 mg, 0.19 mmol) in dry benzene (2 mL), oxalyl chloride (162 μL, 1.89 mmol) was added at 60 °C. After stirring at 60 °C for 90 min, the mixture was cooled to room temperature, and concentrated *in vacuo* to give crude **20** (64 mg, quant.) as a colorless oil. This was immediately used in the next step without further purification. ν_{max} (film): 1815 (shoulder), 1790 (s, CO), 900 (br s), 810 (br m) cm^{–1}.

1.1.9. (2*S*,3*S*,4*R*,2'*R*)-2-[2-(Benzyloxy)octadecanamido]-3,4-di-*O*-benzyl-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)octadecane-1,3,4-triol (**23a**)

To a stirred suspension of **22** (178 mg, 0.174 mmol), **11** (76 mg, 0.19 mmol) and DMAP (66 mg, 0.54 mmol) in dry CH₂Cl₂ (5 mL), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 74 mg, 0.39 mmol) was added at room temperature. After stirring at room temperature for 11 h, the mixture was poured into water, and extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous NaHCO₃ solution and brine, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 17:3) to give **23a** (199 mg, 82%) as a colorless oil, n_{D}^{23} 1.5175; $[\alpha]_{\text{D}}^{24}$ +32.0 (*c* 1.01, CHCl₃); ν_{max} (film): 3420 (w, NH), 1680 (s, CO), 1605 (w), 1580 (w), 1510 (m), 1500 (m), 1100 (br s, C–O), 1060 (br s, C–O), 740 (s), 700 (s) cm^{–1}; δ_{H} (500 MHz, CDCl₃): 7.32–7.20 (32H, m), 7.16–7.14 (3H, m), 7.01 (1H, d, *J* = 10 Hz), 4.87 (1H, d, *J* = 4.0 Hz), 4.85 (1H, d, *J* = 12 Hz), 4.78 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.59 (1H, d, *J* = 12 Hz), 4.57 (1H, d, *J* = 12 Hz), 4.559 (1H, d, *J* = 12 Hz), 4.555 (1H, d, *J* = 12 Hz), 4.50 (2H, d, *J* = 12 Hz), 4.49 (1H, d, *J* = 12 Hz), 4.48 (1H, d, *J* = 12 Hz), 4.37 (1H, d, *J* = 12 Hz), 4.34 (1H, d, *J* = 12 Hz), 4.33 (1H, d, *J* = 12 Hz), 4.32–4.27 (1H, m), 4.00 (1H, dd, *J* = 11, 3.5 Hz), 3.90 (1H, d, *J* = 7.5, 1.5 Hz), 3.86–3.80 (3H, m), 3.78 (1H, dd, *J* = 7.5, 3.5 Hz), 3.76–3.72 (2H, m), 3.48–3.44 (3H, m), 1.72–1.58 (4H, m), 1.52–1.42 (1H, m), 1.42–1.17 (51H, m), 0.88 (6H, t, *J* = 7.0 Hz) ppm; HRMS (ESI⁺): Calcd for

C₉₁H₁₂₅NO₁₀Na [M+Na]⁺ 1414.9201; found 1414.9215.

1.1.10. (2*S*,3*S*,4*R*,2'*R*)-3,4-Di-*O*-benzyl-2-(2-methyloctadecanamido)-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (23b)

In the same manner as described above, **22** (192 mg, 0.188 mmol) was condensed with **16** (56 mg, 0.19 mmol) to give **23b** (205 mg, 84%) as a colorless solid. Mp 38.5–40.0 °C; [α]_D²⁴ +15.9 (*c* 1.15, CHCl₃); ν_{\max} (KBr): 3320 (s, NH), 1640 (s, CO), 1605 (w), 1540 (br s), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 725 (br s), 695 (s) cm⁻¹; δ_{H} (500 MHz, CDCl₃): 7.37–7.20 (30H, m), 6.17 (1H, d, *J* = 8.5 Hz), 4.91 (1H, d, *J* = 12 Hz), 4.85 (1H, d, *J* = 3.5 Hz), 4.79 (3H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.64 (1H, d, *J* = 12 Hz), 4.59 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.52 (1H, d, *J* = 12 Hz), 4.47 (1H, d, *J* = 12 Hz), 4.41 (1H, d, *J* = 12 Hz), 4.37 (1H, d, *J* = 12 Hz), 4.18–4.13 (1H, m), 4.04 (1H, dd, *J* = 9.5, 3.0 Hz), 4.02 (1H, d, *J* = 11, 4.5 Hz), 3.94–3.88 (4H, m), 3.71 (1H, dd, *J* = 11, 4.0 Hz), 3.50–3.47 (1H, m), 3.48 (1H, dd, *J* = 9.0, 6.0 Hz), 3.42 (1H, dd, *J* = 9.0, 6.5 Hz), 1.96 (1H, sext., *J* = 7.0 Hz), 1.72–1.63 (1H, m), 1.63–1.50 (4H, m), 1.50–1.42 (1H, m), 1.32–1.17 (50H, m), 0.99 (3H, d, *J* = 7.0 Hz), 0.88 (6H, t, *J* = 7.0 Hz) ppm; HRMS (ESI⁺): Calcd for C₈₅H₁₂₁NO₉Na [M+Na]⁺ 1322.8939; found 1322.8934.

1.1.11. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-2-(2,2-dimethyloctadecanamido)-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (23c)

To a stirred solution of **22** (210 mg, 0.201 mmol) and triethylamine (83 μ L, 0.60 mmol) in dry CH₂Cl₂ (10 mL), a solution of freshly prepared **20** (81 mg, 0.25 mmol) in dry CH₂Cl₂ (2 mL) was added at 0 °C. After stirring at room temperature for 9 h, the mixture was poured into water, and extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous NaHCO₃ solution and brine, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 9:1) to give **23c** (245 mg, 93%) as a colorless oil, n_{D}^{23} 1.5173; [α]_D²² +18.9 (*c* 1.09, CHCl₃); ν_{\max} (film): 3460 (w, NH), 1670 (br s, CO), 1605 (w), 1585 (w), 1520 (shoulder), 1495 (s), 1100 (br s, C–O), 1055 (br s, C–O), 740 (br s), 695 (s) cm⁻¹; δ_{H} (500 MHz, CDCl₃): 7.37–7.20 (30H, m), 6.21 (1H, d, *J* = 9.0 Hz), 4.91 (1H, d, *J* = 12 Hz), 4.86 (1H, d, *J* = 4.0 Hz), 4.79 (2H, d, *J* = 12 Hz), 4.78 (1H, d, *J* = 12 Hz), 4.73 (1H, d, *J* = 12 Hz), 4.65 (1H, d, *J* = 12 Hz), 4.58 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.52 (1H, d, *J* = 12 Hz), 4.46 (1H, d, *J* = 12 Hz), 4.40 (1H, d, *J* = 12 Hz), 4.35 (1H, d, *J* = 12 Hz), 4.24–4.18 (1H, m), 4.04 (1H, dd, *J* = 10, 3.0 Hz), 3.96 (1H, br s), 3.94 (1H, br t, *J* = 5.0 Hz), 3.91–3.87 (2H, m), 3.89 (1H, dd, *J* = 7.5, 2.5 Hz), 3.74 (1H, dd, *J* = 12, 4.0 Hz), 3.52–3.44 (3H, m), 1.72–1.52 (5H, m), 1.52–1.16 (51H, m), 1.04 (3H, s), 1.02 (3H, s), 0.88 (6H, t, *J* = 7.0 Hz) ppm; HRMS (ESI⁺): Calcd for C₈₆H₁₂₃NO₉Na [M+Na]⁺ 1336.9096; found 1336.9103.

1.1.12. (2*S*,3*S*,4*R*,2'*R*)-1-*O*-(α -D-Galactopyranosyl)-2-(2-hydroxyoctadecanamido)octadecane-1,3,4-triol (4, RCAI-154)

A mixture of **23a** (149 mg, 0.107 mmol) and Pd(OH)₂-C (20%, Aldrich, wet, 40 mg) in THF (5 mL) was stirred under hydrogen atmosphere (balloon) at room temperature for 15 h. The mixture was then diluted with CHCl₃-MeOH (5:1, 20 mL), and stirred for 30 min. The resulting mixture was filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (6 g, CHCl₃/MeOH = 50:7) to give **4** (42 mg, 52 %) as colorless powder. Mp 218–220 °C; [α]_D²⁵ +49.7 (*c* 0.31, pyridine); ν_{\max} (KBr): 3350 (br s, OH, NH), 1645 (br s, CO), 1620 (br s, CO), 1535 (br m), 1075 (br s, C–O) cm⁻¹; δ_{H} (500 MHz, pyridine-*d*₅): 8.51 (1H, d, *J* = 9.5 Hz), 6.73 (1H, br s), 6.65–6.22 (5H, m), 6.12 (1H, br s), 5.60 (1H, d, *J* = 4.0 Hz), 5.31–5.26 (1H, m), 4.67–4.62 (2H, m), 4.59 (1H, dd, *J* = 7.0, 3.0 Hz), 4.53 (1H, br d, *J* = 2.5 Hz), 4.50–4.47 (1H, m), 4.47 (1H, dd, *J* = 11, 4.0 Hz), 4.42–4.30 (5H, m), 4.29–4.25 (1H, m), 2.32–2.24 (1H, m), 2.23–2.15 (1H, m), 2.03–1.94 (1H, m), 1.94–1.83 (2H, m), 1.79–1.61 (4H, m), 1.46–1.16 (47H, m), 0.84 (6H, t, *J* = 7.0 Hz) ppm;

δ_{C} (126 MHz, pyridine- d_5): 175.0, 101.3, 76.6, 73.1, 72.4, 72.3, 71.6, 71.0, 70.2, 68.2, 62.7, 50.5, 35.6, 34.5, 32.1, 30.4, 30.2, 30.04, 30.03, 30.00, 29.99, 29.98, 29.9, 29.6, 26.5, 25.8, 22.9, 14.3 ppm; HRMS (ESI⁺): Calcd for $\text{C}_{42}\text{H}_{83}\text{NO}_{10}\text{Na}$ $[\text{M}+\text{Na}]^+$ 784.5915; found 784.5912.

1.1.13. (2S,3S,4R,2'R)-1-O-(α -D-Galactopyranosyl)-2-(2-methyloctadecanamido)octadecane-1,3,4-triol (5, RCAI-156)

In the same manner as described above, **23b** (157 mg, 0.121 mmol) was converted to **5** (86 mg, 94%) as a colorless solid. Mp 143–145 °C; $[\alpha]_{\text{D}}^{21} +46.4$ (c 0.30, pyridine); ν_{max} (KBr): 3280 (br s, OH, NH), 1640 (br s, CO), 1540 (br s), 1145 (br m, C–O), 1075 (br s, C–O) cm^{-1} ; δ_{H} (500 MHz, pyridine- d_5): 8.35 (1H, d, $J = 8.5$ Hz), 6.93 (1H, br s), 6.70–6.24 (4H, m), 6.06 (1H, br s), 5.57 (1H, d, $J = 3.5$ Hz), 5.22–5.18 (1H, m), 4.65 (1H, dd, $J = 11, 5.0$ Hz), 4.63 (1H, d, $J = 9.5, 3.5$ Hz), 4.55 (1H, br d, $J = 2.5$ Hz), 4.50 (1H, dd, $J = 6.0, 5.5$ Hz), 4.46–4.39 (3H, m), 4.37 (1H, dd, $J = 11, 6.0$ Hz), 4.33–4.27 (2H, m), 2.62–2.55 (1H, m), 2.29–2.22 (1H, m), 1.94–1.84 (3H, m), 1.69–1.61 (1H, m), 1.49–1.17 (54H, m), 0.84 (6H, t, $J = 7.0$ Hz) ppm; δ_{C} (126 MHz, pyridine- d_5): 176.7, 101.7, 76.7, 73.0, 72.5, 71.7, 71.0, 70.3, 68.8, 62.7, 51.5, 41.4, 34.9, 34.2, 32.1, 30.3, 30.12, 30.11, 30.04, 30.01, 29.99, 29.98, 29.91, 29.89, 29.6, 28.0, 26.5, 22.9, 18.5, 14.3 ppm; HRMS (ESI⁺): Calcd for $\text{C}_{43}\text{H}_{85}\text{NO}_9\text{Na}$ $[\text{M}+\text{Na}]^+$ 782.6122; found 782.6113.

1.1.14. (2S,3S,4R)-2-(2,2-Dimethyloctadecanamido)-1-O-(α -D-galactopyranosyl)octadecane-1,3,4-triol (6, RCAI-158)

In the same manner as described above, **23c** (213 mg, 0.162 mmol) was converted to **6** (91 mg, 73%) as a colorless solid. Mp 132–134 °C; $[\alpha]_{\text{D}}^{23} +56.3$ (c 0.31, pyridine); ν_{max} (KBr): 3380 (br s, OH, NH), 1640 (br s, CO), 1535 (br m), 1155 (m), 1070 (br s, C–O) cm^{-1} ; δ_{H} (500 MHz, pyridine- d_5): 7.46 (1H, d, $J = 8.5$ Hz), 6.46 (1H, br s), 5.57 (1H, d, $J = 4.0$ Hz), 5.31 (5H, br s), 5.15–5.10 (1H, m), 4.65 (1H, dd, $J = 10, 4.0$ Hz), 4.62–4.58 (2H, m), 4.51–4.47 (2H, m), 4.43–4.37 (3H, m), 4.28–4.25 (2H, m), 2.28–2.20 (1H, m), 1.94–1.82 (2H, m), 1.69–1.59 (3H, m), 1.45–1.17 (53H, m), 1.33 (3H, s), 0.84 (6H, t, $J = 7.0$ Hz) ppm; δ_{C} (126 MHz, pyridine- d_5): 177.5, 101.5, 76.5, 73.1, 72.4, 71.7, 71.0, 70.2, 68.5, 62.6, 51.5, 42.4, 41.7, 34.2, 32.1, 30.7, 30.4, 30.14, 30.08, 30.02, 30.01, 30.00, 29.98, 29.97, 29.93, 29.92, 29.6, 26.5, 25.8, 25.7, 25.3, 22.9, 14.3 ppm; HRMS (ESI⁺): Calcd for $\text{C}_{44}\text{H}_{87}\text{NO}_9\text{Na}$ $[\text{M}+\text{Na}]^+$ 796.6279; found 796.6264.

1.1.15. (2S,3S,4R)-3,4-Di-O-benzyl-2-(2,4-dinitrobenzenesulfonamido)-1-O-(2,3,4,6-tetra-O-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (24)

To a stirred solution of **22** (1.01 g, 0.990 mmol) in pyridine (20 mL), 2,4-dinitrobenzenesulfonyl chloride (DNsCl, 534 mg, 2.00 mmol) was added at room temperature. After stirring at room temperature for 15 h, the mixture was poured into water, and extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous CuSO_4 solution, water, a 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 (30 g, hexane/EtOAc = 17:3) to give **24** (848 mg, 68%) as a pale yellow oil, $n_{\text{D}}^{21} 1.5170$ $[\alpha]_{\text{D}}^{21} +38.0$ (c 1.04, CHCl_3); ν_{max} (film): 3360 (w, NH), 1605 (m), 1550 (s, NO_2), 1540 (s, NO_2), 1495 (m), 1350 (br s, SO_2 , NO_2), 1170 (m, SO_2), 1100 (br s, C–O), 1060 (br s, C–O), 750 (br s), 700 (s) cm^{-1} ; δ_{H} (500 MHz, CDCl_3): 8.34 (1H, d, $J = 2.0$ Hz), 8.04 (1H, d, $J = 8.5$ Hz), 7.93 (1H, dd, $J = 8.5, 2.0$ Hz), 7.41–7.14 (30H, m), 6.31 (1H, d, $J = 8.5$ Hz), 4.89 (1H, d, $J = 12$ Hz), 4.80 (1H, d, $J = 4.0$ Hz), 4.79 (1H, d, $J = 12$ Hz), 4.78 (1H, d, $J = 12$ Hz), 4.73 (1H, d, $J = 12$ Hz), 4.62 (1H, d, $J = 12$ Hz), 4.56 (1H, d, $J = 12$ Hz), 4.51 (1H, d, $J = 12$ Hz), 4.50 (1H, d, $J = 12$ Hz), 4.46 (1H, d, $J = 12$ Hz), 4.39 (1H, d, $J = 12$ Hz), 4.38 (1H, d, $J = 12$ Hz), 4.28 (1H, d, $J = 12$ Hz), 4.01 (1H, dd, $J = 10, 3.5$ Hz), 3.89–3.82 (3H, m), 3.79 (1H, dd, $J = 6.0, 4.0$ Hz), 3.70 (1H, dd, $J = 11, 5.0$ Hz), 3.64 (1H, dd, $J = 11, 5.0$ Hz), 3.64–3.60 (2H, m), 3.36 (2H, br d, $J = 6.0$ Hz), 1.61–1.54 (2H, m), 1.32–1.18 (24H, m), 0.88 (3H, t, $J = 7.0$ Hz) ppm; HRMS

(ESI⁺): Calcd for C₇₂H₈₇N₃O₁₄SNa [M+Na]⁺ 1272.5806; found 1272.5781.

1.1.16. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-2-(*N*-methyl-2,4-dinitrobenzenesulfonamido)-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (25**)**

To a stirred solution of **24** (792 mg, 0.633 mmol) in DMF (20 mL), K₂CO₃ (1.75 g, 12.7 mmol) and methyl iodide (394 μ L, 6.33 mmol) were added. After stirring at room temperature for 17 h, the mixture was poured into water, and extracted with EtOAc. The organic phase was washed successively with water, a saturated aqueous NaHCO₃ solution and brine, dried with K₂CO₃, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 5:1) to give **25** (542 mg, 68%) as a yellow oil, n_D^{20} 1.5160; $[\alpha]_D^{20}$ +4.68 (*c* 1.04, CHCl₃); ν_{\max} (film): 1605 (m), 1590 (w), 1555 (s, NO₂), 1540 (s, NO₂), 1495 (m), 1350 (br s, SO₂, NO₂), 1160 (m, SO₂), 1100 (br s, C–O), 1050 (br s, C–O), 750 (br s), 735 (br s), 700 (s) cm⁻¹; δ_H (500 MHz, CDCl₃): 8.36 (1H, d, *J* = 8.5 Hz), 8.23 (1H, d, *J* = 2.5 Hz), 8.05 (1H, dd, *J* = 8.5, 2.5 Hz), 7.44–7.19 (30H, m), 4.95 (1H, d, *J* = 3.5 Hz), 4.89 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 11 Hz), 4.72 (1H, d, *J* = 11 Hz), 4.68 (2H, s), 4.58 (1H, d, *J* = 11 Hz), 4.563 (1H, d, *J* = 11 Hz), 4.561 (1H, d, *J* = 11 Hz), 4.52 (1H, d, *J* = 11 Hz), 4.49 (1H, d, *J* = 12 Hz), 4.46 (1H, dt, *J* = 11, 3.5 Hz), 4.36 (1H, d, *J* = 12 Hz), 4.34 (1H, d, *J* = 11, 3.5 Hz), 4.27 (1H, d, *J* = 12 Hz), 4.08 (1H, dd, *J* = 10, 3.5 Hz), 3.96 (1H, dd, *J* = 4.5, 3.5 Hz), 3.92 (1H, br d, *J* = 2.5 Hz), 3.74 (1H, t, *J* = 11 Hz), 3.69 (1H, dd, *J* = 10, 2.5 Hz), 3.66 (1H, br t, *J* = 7.0 Hz), 3.59–3.55 (1H, m), 3.42 (1H, dd, *J* = 9.0, 7.0 Hz), 3.28 (1H, dd, *J* = 9.0, 6.0 Hz), 3.00 (3H, s), 1.61–1.53 (3H, m), 1.45–1.37 (1H, m), 1.34–1.18 (22H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI⁺): Calcd for C₇₃H₈₉N₃O₁₄SNa [M+Na]⁺ 1286.5963; found 1286.5969.

1.1.17. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-2-(methylamino)-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (26**)**

To a stirred solution of **25** (510 mg, 0.403 mmol) in CH₂Cl₂ (10 mL), triethylamine (560 μ L, 4.04 mmol) and thioglycolic acid (170 μ L, 2.45 mmol) were successively added at room temperature. After stirring at room temperature for 16 h, the mixture was poured into a saturated aqueous NaHCO₃ solution, and extracted with EtOAc. The organic phase was successively washed with a saturated aqueous NaHCO₃ solution, water, and brine, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (NH silica, 30 g, hexane/EtOAc = 9:1) to give **26** (415 mg, 99%) as a pale yellow oil, n_D^{24} 1.5175; $[\alpha]_D^{24}$ +33.3 (*c* 1.05, CHCl₃); ν_{\max} (film): 3340 (w, NH), 1605 (w), 1580 (w), 1500 (m), 1100 (br s, C–O), 1060 (br s, C–O), 740 (br s), 700 (s) cm⁻¹; δ_H (500 MHz, CDCl₃): 7.37–7.20 (30H, m), 4.92 (1H, br d, *J* = 12 Hz), 4.88 (1H, br s), 4.80 (1H, d, *J* = 12 Hz), 4.78 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.64 (2H, d, *J* = 12 Hz), 4.59 (1H, d, *J* = 12 Hz), 4.56 (1H, d, *J* = 12 Hz), 4.47 (1H, d, *J* = 12 Hz), 4.44 (1H, d, *J* = 12 Hz), 4.36 (1H, d, *J* = 12 Hz), 4.04 (1H, br dd, *J* = 10, 3.5 Hz), 3.99 (1H, br d, *J* = 11 Hz), 3.96–3.89 (3H, m), 3.74–3.70 (1H, m), 3.70 (1H, br s), 3.62 (1H, br s), 3.49 (1H, br s), 3.46 (1H, br s), 2.85 (1H, br s), 2.35 (3H, s), 1.68–1.51 (3H, m), 1.49–1.41 (1H, m), 1.34–1.19 (22H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI⁺): Calcd for C₆₇H₈₈NO₈ [M+H]⁺ 1034.6510; found 1034.6500.

1.1.18. (2*S*,3*S*,4*R*,2'*R*)-3,4-Di-*O*-benzyl-2-[*N*-methyl-2-(benzyloxy)octadecanamido]-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (28a**)**

In the same manner as described above for the conversion of **22** to **23a**, **26** (215 mg, 0.208 mmol) was converted to **28a** (241 mg, 82%) as a colorless oil, n_D^{27} 1.5172; $[\alpha]_D^{25}$ +28.9 (*c* 1.02, CHCl₃); ν_{\max} (film): 1640 (br s, CO), 1610 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 735 (br s), 700 (s) cm⁻¹; δ_H (500 MHz, CDCl₃, Rotamer ratio = ca. 6:1): 7.32–7.18 (35H, m), 4.88 (1H, d, *J* = 12 Hz), 4.83 (1H, d, *J* = 12 Hz), 4.80 (1H, d, *J* = 3.5 Hz), 4.70 (1H, d, *J* = 12 Hz), 4.66–4.59

(2H, m), 4.63 (2H, br s), 4.57 (1H, d, $J = 12$ Hz), 4.56 (1H, d, $J = 12$ Hz), 4.52 (1H, d, $J = 12$ Hz), 4.48 (1H, d, $J = 12$ Hz), 4.45 (1H, d, $J = 12$ Hz), 4.44 (1H, d, $J = 12$ Hz), 4.35 (1H, d, $J = 12$ Hz), 4.21 (1H, d, $J = 12$ Hz), 4.09–4.00 (3H, m), 3.99 (1H, dd, $J = 10, 3.5$ Hz), 3.93 (1H, br s), 3.90 (1H, br t, $J = 7.0$ Hz), 3.83 (1H, br d, $J = 10$ Hz), 3.78–3.72 (1H, m), 3.52 (1H, dd, $J = 10, 8.0$ Hz), 3.45 (1H, dd, $J = 10, 6.0$ Hz), 3.41 (1H, br d, $J = 10$ Hz), 2.96 (0.4H, s, the NMe protons of the minor rotamer), 2.94 (2.6H, s, the NMe protons of the major rotamer), 1.78–1.65 (2H, m), 1.65–1.49 (2H, m), 1.49–1.40 (1H, m), 1.32–1.14 (51H, m), 0.88 (6H, t, $J = 7.0$ Hz) ppm; HRMS (ESI+): Calcd for $C_{92}H_{127}NO_{10}Na$ $[M+Na]^+$ 1428.9358; found 1428.9363.

1.1.19. (2S,3S,4R,2'R)-3,4-Di-O-benzyl-2-(N-methyl-2-methyloctadecanamido)-1-O-(2,3,4,6-tetra-O-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (28b)

In the same manner as described above for the conversion of **22** to **23a**, **26** (192 mg, 0.186 mmol) was converted to **28b** (241 mg, 99%) as a colorless oil, n_D^{23} 1.5170; $[\alpha]_D^{23} +20.7$ (c 1.01, $CHCl_3$); ν_{max} (film): 1645 (br s, CO), 1610 (w), 1590 (w), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 740 (br s), 700 (s) cm^{-1} ; δ_H (500 MHz, $CDCl_3$, Rotamer ratio = ca. 7:3): The typical peaks of the major rotamer: 2.88 (2.1H, s, NMe), 2.43 (0.7H, br s), 0.96 (2.1H, d, $J = 7.0$ Hz) ppm. The typical peaks of the minor rotamer: 2.82 (0.9H, s, NMe), 2.64 (0.3H, sext., $J = 7.0$ Hz), 1.01 (0.9H, d, $J = 7.0$ Hz) ppm. The common peaks: 7.36–7.22 (30H, m), 0.88 (6H, t, $J = 7.0$ Hz) ppm; HRMS (ESI+): Calcd for $C_{86}H_{123}NO_9Na$ $[M+Na]^+$ 1336.9096; found 1336.9083.

1.1.20. (2S,3S,4R)-3,4-Di-O-benzyl-2-(N-methyl-2,2-dimethyloctadecanamido)-1-O-(2,3,4,6-tetra-O-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (28c)

In the same manner as described above for the conversion of **22** to **23c**, **26** (158 mg, 0.153 mmol) was converted to **28c** (179 mg, 88%) as a colorless oil, n_D^{22} 1.5174; $[\alpha]_D^{18} +16.1$ (c 1.01, $CHCl_3$); ν_{max} (film): 1640 (br s, CO), 1610 (w), 1590 (w), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 740 (s), 700 (s) cm^{-1} ; δ_H (500 MHz, $CDCl_3$): 7.36–7.20 (30H, m), 4.90 (1H, d, $J = 12$ Hz), 4.84 (1H, br d, $J = 12$ Hz), 4.82 (1H, d, $J = 3.5$ Hz), 4.75 (1H, d, $J = 12$ Hz), 4.73 (1H, d, $J = 12$ Hz), 4.69 (1H, d, $J = 12$ Hz), 4.61 (1H, d, $J = 12$ Hz), 4.54 (1H, d, $J = 12$ Hz), 4.48 (1H, d, $J = 12$ Hz), 4.44 (1H, d, $J = 12$ Hz), 4.42 (2H, br d, $J = 12$ Hz), 4.35 (1H, d, $J = 12$ Hz), 4.02–3.98 (2H, m), 4.00 (1H, dd, $J = 10, 4.0$ Hz), 3.97–3.94 (1H, m), 3.88–3.84 (3H, m), 3.55 (1H, br t, $J = 8.0$ Hz), 3.44 (1H, dd, $J = 9.0, 6.0$ Hz), 3.40 (2H, br d, $J = 10$ Hz), 3.00 (3H, br s), 1.76–1.67 (1H, m), 1.58–1.46 (5H, m), 1.32–1.17 (53H, m), 1.13 (3H, s), 0.88 (6H, t, $J = 7.0$ Hz) ppm; HRMS (ESI+): Calcd for $C_{87}H_{125}NO_9Na$ $[M+Na]^+$ 1350.9252; found 1350.9250.

1.1.21. (2S,3S,4R)-3,4-Di-O-benzyl-2-(N-methylhexacosanamido)-1-O-(2,3,4,6-tetra-O-benzyl- α -D-galactopyranosyl)octadecane-1,3,4-triol (28d)

To a stirred and warmed solution of cerotic acid (122 mg, 0.308 mmol) in dry benzene (5 mL), oxalyl chloride (264 μ L, 3.08 mmol) was added at 60 °C. After stirring at 60 °C for 1 h, the mixture was cooled to room temperature, and then concentrated *in vacuo* to give cerotyl chloride **27** (140 mg, quant.) as a colorless solid. This was used in acylation of **26** without further purification.

In the same manner as described above for the conversion of **22** to **23c**, **26** (290 mg, 0.280 mmol) was converted to **28d** (372 mg, 94%) as a colorless oil, n_D^{19} 1.5170; $[\alpha]_D^{20} +20.2$ (c 1.25, $CHCl_3$); ν_{max} (film): 1650 (br s, CO), 1610 (w), 1585 (w), 1495 (m), 1100 (br s, C–O), 1060 (br s, C–O), 750 (br s), 735 (br s), 695 (s) cm^{-1} ; δ_H (500 MHz, $CDCl_3$, Rotamer ratio = ca. 2:1): The typical peaks of the major rotamer: 3.36 (0.7H, dd, $J = 9.0, 8.0$ Hz), 3.48–3.43 (1.4H, m), 2.12–2.00 (1.4H, m) ppm. The typical peaks of the minor rotamer: 3.61 (0.3H, dd, $J = 10, 8.0$ Hz), 3.34 (0.6H, br d, $J = 9.0$ Hz), 2.35 (0.3H, dt, $J = 15, 7.5$ Hz), 2.25 (0.3H, dt, $J = 15, 7.5$ Hz) ppm. The common peaks: 7.36–7.20 (30H, m), 2.79 (3H, br s), 0.88 (6H, t, $J = 7.0$ Hz) ppm; HRMS (ESI+): Calcd for $C_{93}H_{137}NO_9Na$ $[M+Na]^+$ 1435.0191; found 1435.0193.

1.1.22. (2*S*,3*S*,4*R*,2'*R*)-1-*O*-(α -D-Galactopyranosyl)-2-(*N*-methyl-2-hydroxyoctadecanamido)octadecane-1,3,4-triol (7, RCAI-155)

In the same manner as described above for the conversion of **23a** to **4**, **28a** (236 mg, 0.168 mmol) was converted to **7** (114 mg, 88%) as a colorless solid. Mp 118–122 °C; $[\alpha]_{\text{D}}^{21} +46.0$ (*c* 0.30, pyridine); ν_{max} (KBr): 3300 (br s, OH), 1640 (br s, CO), 1610 (br s, CO), 1075 (br s, C–O) cm^{-1} ; δ_{H} (500 MHz, CDCl_3 , Rotamer ratio = ca. 1:1): The typical peaks: 5.42 (1H, d, $J = 3.5$ Hz, 1''-H), 5.30–5.26 (1H, m, 2-H), 3.45 (1.5H, s, NMe), 3.44 (1.5H, s, NMe), 0.85 (3H, t, $J = 7.0$ Hz), 0.84 (3H, t, $J = 7.0$ Hz) ppm; δ_{C} (126 MHz, pyridine- d_5): The typical peaks of the major rotamer: 176.4 (1'-C), 101.9 (1''-C) ppm. The typical peaks of the minor rotamer: 176.2 (1'-C), 101.5 (1''-C) ppm; HRMS (ESI+): Calcd for $\text{C}_{43}\text{H}_{85}\text{NO}_{10}\text{Na}$ $[\text{M}+\text{Na}]^+$ 798.6071; found 798.6074.

1.1.23. (2*S*,3*S*,4*R*,2'*R*)-1-*O*-(α -D-Galactopyranosyl)-2-(*N*-methyl-2-methyloctadecanamido)octadecane-1,3,4-triol (8, RCAI-157)

In the same manner as described above for the conversion of **23a** to **4**, **28b** (197 mg, 0.150 mmol) was converted to **8** (86 mg, 74%) as a colorless solid. Mp 131–133 °C; $[\alpha]_{\text{D}}^{21} +30.9$ (*c* 0.29, pyridine); ν_{max} (KBr): 3320 (br s, OH), 1610 (br s, CO), 1150 (m), 1080 (br s, C–O) cm^{-1} ; δ_{H} (500 MHz, pyridine- d_5 , Rotamer ratio = ca. 7:3): The typical peaks of the major rotamer: 5.40 (0.7H, d, $J = 4.0$ Hz, 1''-H), 3.34 (2.1H, s, NMe), 0.84 (4.2H, d, $J = 7.0$ Hz, 18-H₃, 18'-H₃) ppm. The typical peaks of the minor rotamer: 5.47 (0.3H, d, $J = 4.0$ Hz, 1''-H), 3.45 (0.9H, s, NMe), 0.85 (1.8H, t, $J = 7.0$ Hz, 18-H₃, 18'-H₃) ppm; δ_{C} (126 MHz, pyridine- d_5): The typical peaks of the major rotamer: 177.3 (1'-C), 101.8 (1''-C), 17.8 (2'-Me) ppm. The typical peaks of the minor rotamer: 177.9 (1'-C), 101.1 (1''-C), 18.8 (2'-Me) ppm; HRMS (ESI+): Calcd for $\text{C}_{44}\text{H}_{87}\text{NO}_9\text{Na}$ $[\text{M}+\text{Na}]^+$ 796.6279; found 796.6274.

1.1.24. (2*S*,3*S*,4*R*)-1-*O*-(α -D-Galactopyranosyl)-2-(*N*-methyl-2,2-dimethyloctadecanamido)octadecane-1,3,4-triol (9, RCAI-159)

In the same manner as described above for the conversion of **23a** to **4**, **28c** (107 mg, 0.0805 mmol) was converted to **9** (33 mg, 52%) as a colorless solid. Mp 137–139 °C; $[\alpha]_{\text{D}}^{20} +49.7$ (*c* 0.30, pyridine); ν_{max} (KBr): 3450 (br s, OH), 1590 (br s, CO), 1080 (br s, C–O) cm^{-1} ; δ_{H} (500 MHz, pyridine- d_5): 6.85–5.80 (6H, m), 4.76 (1H, br d, $J = 8.5$ Hz), 4.67 (1H, dd, $J = 10, 4.0$ Hz), 4.62 (1H, d, $J = 2.5$ Hz), 4.48–4.37 (5H, m), 4.46 (1H, dd, $J = 10, 3.5$ Hz), 4.37–4.32 (1H, m), 4.13–4.07 (1H, m), 3.43 (3H, br s), 2.23–2.14 (1H, m), 1.97–1.85 (2H, m), 1.74–1.59 (3H, m), 1.32–1.16 (56H, m), 0.85 (6H, t, $J = 7.0$ Hz) ppm; δ_{C} (126 MHz, pyridine- d_5): 177.9, 101.6, 73.0, 72.7, 71.7, 71.0, 70.4, 62.6, 43.3, 41.1, 32.1, 30.7, 30.4, 30.2, 30.11, 30.05, 30.03, 30.01, 30.00, 29.96, 29.9, 29.6, 27.4, 26.6, 25.6, 22.9, 14.3 ppm; HRMS (ESI+): Calcd for $\text{C}_{45}\text{H}_{89}\text{NO}_9\text{Na}$ $[\text{M}+\text{Na}]^+$ 810.6435; found 810.6433.

1.1.25. (2*S*,3*S*,4*R*)-1-*O*-(α -D-Galactopyranosyl)-2-(*N*-methylhexacosanamido)octadecane-1,3,4-triol (3, RCAI-133)

In the same manner as described above for the conversion of **23a** to **4**, **28d** (233 mg, 0.165 mmol) was converted to **3** (46 mg, 32%) as a colorless solid. Mp 174–176 °C; $[\alpha]_{\text{D}}^{20} +41.6$ (*c* 0.31, pyridine); ν_{max} (KBr): 3440 (br s, OH), 3300 (s, OH), 1610 (br s, CO), 1270 (m), 1070 (s, C–O), 1055 (s, C–O), 720 (m) cm^{-1} ; δ_{H} (500 MHz, pyridine- d_5 , Rotamer ratio = ca. 2:1): The typical peaks of the major rotamer: 5.41 (0.7H, d, $J = 3.5$ Hz, 1''-H), 3.25 (2H, s, NMe), 2.35 (0.7H, dt, $J = 15, 7.5$ Hz, 2'-H_a), 2.32 (0.7H, dt, $J = 15, 7.5$ Hz, 2'-H_b), 0.84 (4H, d, $J = 7.0$ Hz, 18-H₃, 18'-H₃) ppm. The typical peaks of the minor rotamer: 5.44 (0.3H, d, $J = 3.5$ Hz, 1''-H), 3.39 (1H, s, NMe), 2.89 (0.3H, dt, $J = 15, 7.5$ Hz, 2'-H_a), 2.85 (0.3H, dt, $J = 15, 7.5$ Hz, 2'-H_b), 0.85 (2H, t, $J = 7.0$ Hz,

18-H₃, 18'-H₃) ppm; δ_C (126 MHz, pyridine-*d*₅): The typical peaks of the major rotamer: 173.8 (1'-C), 101.7 (1''-C) ppm. The typical peaks of the minor rotamer: 174.2 (1'-C), 101.3 (1''-C) ppm; HRMS (ESI⁺): Calcd for C₅₁H₁₀₂NO₉ [M+H]⁺ 872.7549; found 872.7547.

2. Pharmacology

2.1. Mice.

C57BL/6 mice were purchased from Charles River Japan, Inc. or Clea Japan, Inc. Mice were kept under specific pathogen-free conditions and used at 8 wk of age. All biological experiments were in accordance with protocols approved by RIKEN Animal Care and Use Committee.

2.2. Preparation of glycolipid solutions

KRN7000 (**A**) used in this study was purchased from Funakoshi Corporation (Japan). KRN7000 (**A**) or synthesized glycolipids (1.0 mg) were dissolved in dimethyl sulfoxide (1.0 mg/mL) at 80 °C. After 30 min at 80 °C, the solutions of **A** and **3–9** were diluted to 200 µg/mL with Dulbecco's phosphate-buffered saline (PBS, Sigma-Aldrich®) containing 0.5% Tween20 (polyethylene sorbitan monolaurate). The obtained solutions were diluted to 10 µg/mL with PBS (Invitrogen™) in just before injection into mice.

2.3. Administration of the glycolipid solutions and cytokine measurement

Each glycolipid solution (10 µg/mL, 200 µL) was administered intravenously (from the caudal vein) into mice. Peripheral blood was collected from the retro-orbital plexus of mice at 1, 3, 6, 12, 24, 36, 48, and 60 h using heparin-coated capillary tubes (Hirschmann Laborgeräte GmbH & Co. KG), and plasma was prepared.

The cytokine concentrations in plasma were quantified by mouse IFN- γ ELISA kit (Thermo Fisher Scientific K.K.) for IFN- γ , and cytometric bead array (CBA) system (BD Bioscience) for IL-2, 4, 5, 6, 10, 13, and 12p70. according to the manufacturer's protocol.

3. References

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4. ^1H - and ^{13}C -NMR spectra of synthesized glycolipids

4.1. RCAI-154	p.12, 13
4.2. RCAI-155	p.14, 15
4.3. RCAI-156	p.16, 17
4.4. RCAI-157	p.18, 19
4.5. RCAI-158	p.20, 21
4.6. RCAI-159	p.22, 23
4.7. RCAI-133	p.24, 25

























