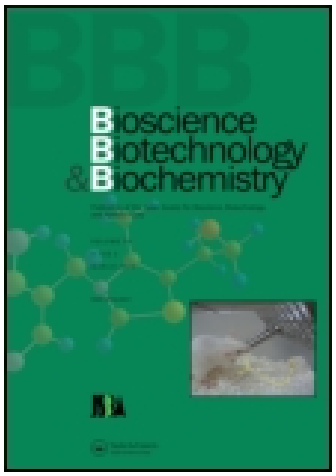


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# Practical Total Synthesis of (2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-N-hexacosanoyl-2-amino-1,3,4-octadecanetriol, the Antitumorial and Immunostimulatory α-Galactosylcer-amide, KRN7000

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# Practical Total Synthesis of (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-*N*-hexacosanoyl-2-amino-1,3,4-octadecanetriol, the Antitumorial and Immunostimulatory α-Galactosylceramide, KRN7000

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**A practical total synthesis of (2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-*N*-hexacosanoyl-2-amino-1,3,4-octadecanetriol (KRN7000), an antitumorial and immunostimulatory glycosphingolipid derived from agelasphins, was achieved in 14 steps starting from D-lyxose in a 16% overall yield.**

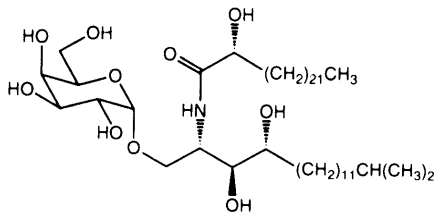
**Key words:** KRN7000; agelasphins; α-galactosylceramide; practical total synthesis; D-lyxose



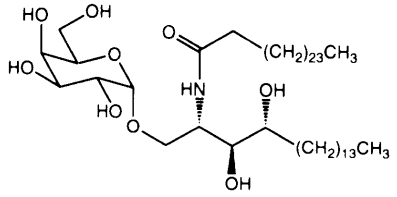
Agelasphins, having the characteristic α-galactosylceramide structure, have been isolated from an extract of a marine sponge (*Agelas mauritianus*) and shown to have antitumorial activity against mice intraperitoneally inoculated with melanoma B16 cells.[1,2] Agelaspin-9b (AGL-9b), (2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-*N*-[(*R*)-2-hydroxytetracosanoyl]-2-amino-16-methyl-1,3,4-heptadecanetriol, the main compound of agelasphins, has shown marked antitumorial activity against several murine tumors *in vivo* (0.1 mg/kg) and its acute toxicity was weak ($LD_{50}$ > 10 mg/kg).[1] These results imply that AGL-9b could be a useful agent for cancer treatment. The available synthetic route to this compound reported by this laboratory in 1993 was tedious and complicated, even on a small scale.[3] Therefore, a variety of AGL-9b analogues were synthesized, and on the basis of their structure-antitumorial activity relationships by several assay systems,[4] KRN7000 was selected as the most promising candidate for clinical development. On a small scale, KRN7000 has been synthesized by starting from D-galactose in 18 steps,[4] and in this synthesis, the phytosphingosine moiety was constructed according to the method reported by Ogawa and his co-workers.[5] So far, D-galactose has been used in several chiron approaches for phytosphingosines,[5–7] since C-3 to C-5 in D-galactose contains the requisite functionality and configuration corresponding to C-2 to C-4 of the phytosphingosine structure, noting that the introduction of the C-2 amino group proceeds with an inversion of configuration *via* an SN2-type process.

In order to supply a sufficiently substantial amount of KRN7000 for preclinical trials, it was necessary to develop a shorter and simpler process that would be suitable for a large scale. Therefore, we planned to construct the phytosphingosine moiety of KRN7000 by using another chiron, and D-lyxose was chosen as the starting material. D-Lyxose is a pentose in which C-2 to C-4 has the same configurations as C-3 to C-5 of D-galactose, and we expected that the C-1 acetal in furanose derivative **2**, which is accessible from D-lyxose with simple two-step protective reactions, could be directly used for alkylation under Wittig-type homologation.

As shown in the Scheme, D-lyxose was treated with a catalytic amount of sulfuric acid in an excess of acetone[8] and then tritylated (TrCl/pyridine, $CH_2Cl_2$) to give suitably protected lyxofuranose **2** as crystals in an 87% yield. Easily prepared and stable D-lyxofuranose derivative **2** was coupled with an ylid that had been generated from the corresponding phosphonium bromide which, in turn, was bromotridecane and triphenylphosphine, to afford a mixture of geometrically isomeric alcohols **4** (*E*/*Z* = 3/7, determined by 500 MHz $^1$H-NMR), which is a suitably functionalized $C_{18}$-alkyl chain with (2*R*,3*S*,4*R*) configuration. Mesylation (MsCl, pyridine/$CH_2Cl_2$) of **4** and subsequent deprotection (HCl/MeOH–$CH_2Cl_2$) provided unsaturated triol **6** as crystals. The olefinic portion of **6** was catalytically hydrogenated ($H_2$, 5% palladium on barium sulfate/THF) to **7** in a 64% overall yield from **2**. Saturated mesylate **7** was converted to azide **8** ($NaN_3$/DMF), and subsequent




Agelasphin-9b




KRN7000

**Fig.** Structures of Agalasphin-9b and KRN7000.

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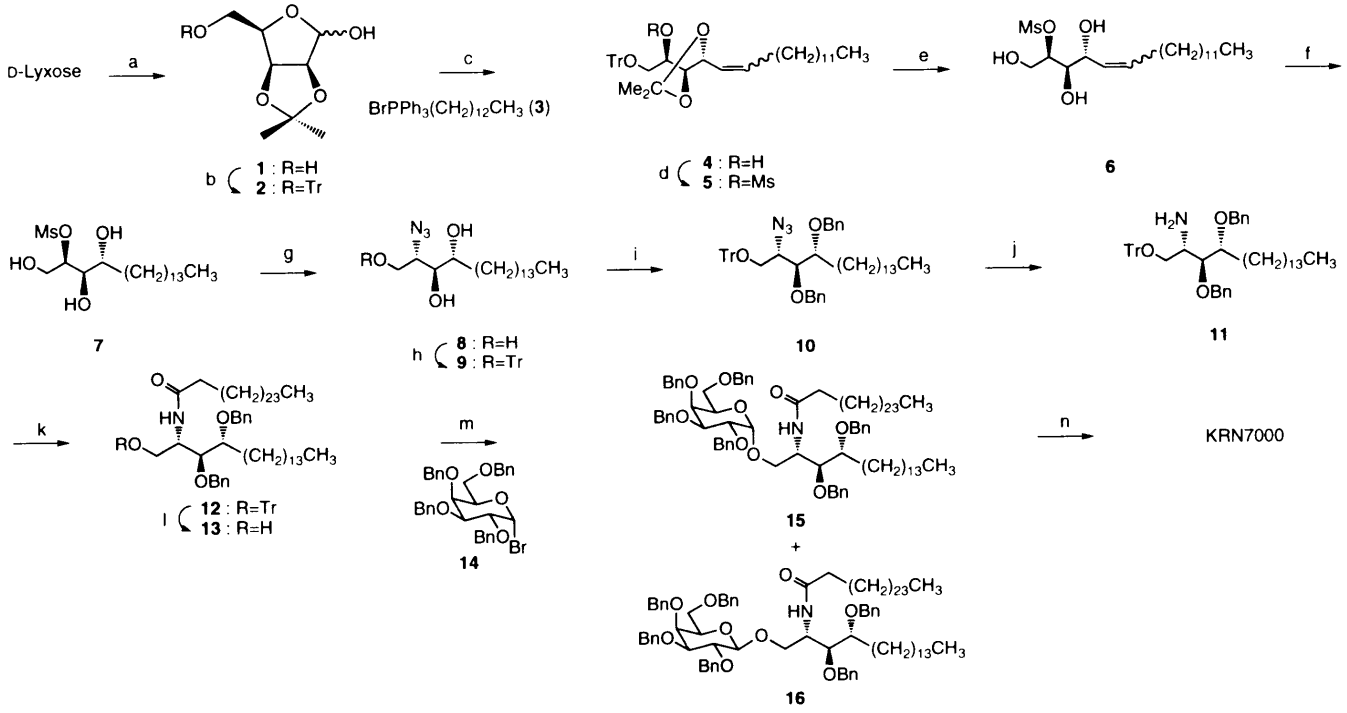
*Abbreviations*: Bn, benzyl; EtOAc, ethyl acetate; DMF, *N*,*N*-dimethylformamide; mesyl or Ms, methanesulfonyl; Pyr, pyridine; trityl or Tr, triphenylmethyl; THF, tetrahydrofuran; WSC, water-soluble carbodiimide = 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide.



**Scheme** (a) $H_2SO_4$/acetone, r.t., 18 h; (b) TrCl, Pyr/$CH_2Cl_2$, r.t., 4 h (a–b, 87%). (c) *n*-BuLi/THF, r.t., 18 h; (d) MsCl, Pyr/$CH_2Cl_2$, r.t., 24 h; (e) aq. HCl/$CH_2Cl_2$, MeOH, r.t., 5 h (c–e, 70%). (f) $H_2$, 5% Pd–$BaSO_4$/THF, r.t., 20 h (91%). (g) $NaN_3$/DMF, 95 °C, 4 h; (h) TrCl, Pyr/$CH_2Cl_2$, r.t., 16 h (g, h, 52%). (i) NaH, BnBr/DMF, 0 °C r.t., 18 h; (j) 10% Pd–C, $HCO_2NH_4$/1-propanol and MeOH, r.t., 16 h; (k) $CH_3(CH_2)_{24}CO_2H$, WSC·HCl/$CH_2Cl_2$, reflux, 2 h; (l) 10% HCl in MeOH/$CH_2Cl_2$ and MeOH, r.t., 2 h (i–l, 77%). (m) *n*-$Hex_4NBr$, MS4A/toluene and DMF, r.t., 72 h (74%). (n) 20% $Pd(OH)_2$–C, 4-methylcyclohexene/EtOH, reflux, 4 h (95%).

tritylation (TrCl, pyridine/$CH_2Cl_2$) of the primary hydroxyl group gave **9** (52% yield from **7** after silica-gel column purification). Two secondary hydroxyl groups in **9** were then benzylated to give **10** (NaH, benzyl bromide/DMF). Selective reduction of the azido group of **10** was achieved by catalytic hydrogenation over 10% palladium on charcoal, using ammonium formate as the hydrogen source,[9] to give amine **11**. Amine **11** was acylated with hexacosanoic acid in the presence of WSC·HCl in $CH_2Cl_2$ to afford amide **12**. De-*O*-tritylation of **12** under acidic conditions (10% HCl–MeOH/$CH_2Cl_2$) gave suitably protected ceramide **13** as a white powder in a 77% overall yield from **9**. Throughout these 12 steps, one chromatographic separation procedure was sufficient to obtain glycosyl aceptor **13** in a 22% overall yield from D-lyxose.

With key intermediate **13** in hand, we next focussed our attention on exploring the optimum conditions for α-selective galactosidation on a large scale. Concerning the practical production of KRN7000, the use of dangerous reagents such as explosive $ClO_4$ salts[10–12] or harmful heavy metal reagents,[13] which have been widely employed in α-selective glycosidation, should be avoided. There is no precedent for the α-glycosidation of ceramides, except that in our reports[4,14] using $AgClO_4$ and $SnCl_2$.[10] Although several α-selective glycosidation methods considered safe for a large-scale use are known,[15–18] to the best of our knowledge, there is no report on α-glycosidation on several tens of grams scale. The examination of these methods on a small scale resulted in low to moderate yields (not optimized): a trichloroacetimidate donor with TMSOTf[15] (44%), and thioethyl donor with DMTST[16] and thiopyridyl donor with MeI[17] (38% and 40%, respectively). However, the halide ion catalyzation method,[18] using *n*-tetrahexylammonium bromide (*n*-$Hex_4NBr$) in a mixture of toluene and DMF (4:1) gave **15** in a *ca.* 70% yield. This solvent system was employed due to the solubility of **13**. In the case of $Et_4NBr$ or *n*-$Bu_4NBr$, the isolation yields of **15** were 40–50% under the same conditions. Thus, the treatment of **13** (60.0 g) with benzyl-protected galactosyl bromide **14** (2.0 eq.),[19,20] *n*-$Hex_4NBr$ (1.5 eq. for **14**), and 4A molecular sieve (MS4A) powder (60.0 g) in a mixture of toluene and DMF gave 70.9 g of desired α-galactosylceramide **15** in a 74% isolation yield after silica-gel column purification, along with a small amount of β-galactosylceramide **16** ($\alpha/\beta = 8.5$). The final deprotection of **15** turned out to be troublesome due to the poor solubility of KRN7000 or to partly deprotected intermediates being precipitated during the reaction under usual catalytic hydrogenolysis conditions ($H_2$, palladium catalyst/THF, at room temperature). Therefore, we investigated catalytic transfer hydrogenation by using other hydrogen sources.[21–23] As shown in Table, when **15** was treated with cyclohexene or 4-methylcyclohexene in the presence of palladium hydroxide on charcoal in refluxing EtOH, de-*O*-benzylation proceeded smoothly to give KRN7000 in high yields. Although cyclohexene forms carcinogenic benzene as a product, 4-methylcyclohexene forms less toxic toluene and is more reactive than cyclohexene. Thus, treatment of protected α-galactosylceramide **15** (60.0 g) with a catalytic amount of 20% palladium hydroxide on charcoal in the presence of 4-methylcyclohexene in EtOH under reflux conditions and subsequent purification by precipitation in aq. EtOH afforded 35.0 g (95% yield) of pure KRN7000. All of the physical data of KRN7000 were identical with those reported previously.[4]

In conclusion, an efficient synthetic method for producing

**Table** Catalytic Transfer Hydrogenation of **15**[a]

| Entry | Hydrogen source | Solvent | Catalyst | Time | Yield |
|---|---|---|---|---|---|
| 1 |  | EtOH | $Pd(OH)_2/C$ | 2 h | 91% |
| 2 |  | EtOH | $Pd(OH)_2/C$ | 7 h | 89% |
| 3 | $HCOONH_4$ | MeOH | Pd/C | 5 h | Trace |
| 4 | HCOOH | THF | Pd/C | 18 h | Complex mixture |
| 5 | $(CH_3)_2CHOH$ | $(CH_3)_2CHOH$ | Pd/C | 3 h | No reaction |

[a] All reactions were performed under reflux conditions with 10 wt% of catalyst for **15**.

KRN7000 by starting from D-lyxose was developed. Note that a phytosphingosine derivative could be synthesiszed stereoselectively under practical conditions, and that α-galactosidation of a ceramide was achieved in a satisfactory yield in several tens of grams scale. Our strategy would be useful for the syntheses of other phytosphingolipids and related compounds.

## Experimental

Column chromatography was performed on silica-gel (WAKO-gel C200, 0.075–0.150 mm particle size). TLC analyses were done on silica-gel plates (Merck, Art 5554). All melting point (mp) data were measured with Yanagimoto micromelting point apparatus and are uncorrected. Mass spectra were measured with a JEOL JMS SX-102 mass spectrometer, and optical rotation values with a JASCO DIP-140 digital polarimeter. Elemental analyses were recorded with a Perkin-Elmer 240C elemental analyzer. $^1$H-NMR spectra were obtained with a JEOL JNM-GX-500 FT NMR spectrometer, chemical shifts being expressed in $\delta$ units from tetramethylsilane (TMS) as an internal standard, and coupling constants ($J$) reported in hertz (Hz).

*2,3-O-Isopropylidene-*D*-lyxofuranose* (**1**). To a solution of D-lyxose (200 g, 1.33 mol) in acetone (300 ml, dried over $CaCl_2$) was added dropwise conc $H_2SO_4$ (0.5 ml), and the mixture was stirred for 18 h at room temperature. After IRA-400 resin ($OH^-$ type) had been added to the reaction mixture to neutralize it, the resulting mixture was filtered through Celite and washed with acetone. The filtrate was concentrated to give 240 g (95%) of crude **2** as a white solid, which was employed in the next step without further purification. An analytical sample was purified by silica-gel column chromatography, using hexane–acetone (9 : 1) as the eluent, to give pure **1**. mp 76–78°C [lit.,[6] 79–80°C]; FDMS $m/z$ 191 $(M+1)^+$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 5.45 (1H, d, $J$=1.8 Hz, H-1), 4.83 (1H, dd, $J$=3.7, 5.5 Hz, H-3), 4.64 (1H, d, $J$=6.1 Hz, H-2), 4.27–4.30 (1H, m, H-4), 3.90–3.99 (2H, m, H-5 and H-5b), 1.48 (3H, s, $OCH_3$), 1.32 (3H, s, $OCH_3$). *Anal.* Found: C, 50.67; H, 7.68%. Calcd. for $C_8H_{14}O_5$: C, 50.52; H, 7.42%.

*2,3-O-Isopropylidene-5-O-trityl-*D*-lyxofuranose* (**2**). To a solution of crude **1** (239 g, *ca.* 1.26 mol) in $CH_2Cl_2$ (168 ml) and pyridine (10 ml) was added chlorotriphenylmethane (39.0 g), and the mixture was stirred for 4 h at 32°C. After the addition of EtOH (8 ml), the reaction mixture was stirred for 2 h more at room temperature. The resulting mixture was successively washed with a saturated aq. ammonium chloride solution, saturated aq. sodium hydrogen carbonate solution and brine, and then concentrated. The residue was dissolved in EtOAc then stored at 0°C to give 501 g of crystalline **2** (87% from D-lyxose). mp 174–176°C; FDMS $m/z$ 432 $M^+$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.21–7.49 (15H, m, aromatic-H), 5.38 (1H, d, $J$=2.4 Hz, H-1), 4.75 (1H, dd, $J$=3.7, 6.1 Hz, H-3), 4.59 (1H, d, $J$=6.1 Hz, H-2), 4.31–4.35 (1H, m, H-4), 3.43 (1H, dd, $J$=4.9, 9.8 Hz, H-5a), 3.39 (1H, dd, $J$=6.7, 9.8 Hz, H-5b), 1.29 (3H, s, $OCH_3$), 1.28 (3H, s, $OCH_3$). *Anal.* Found: C, 75.20; H, 6.75%. Calcd. for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53%.

*(2R,3S,4R)-3,4-O-Isopropylidene-1-O-trityl-5-octadecene-1,2,3,4-tetrol* (**4**). To a suspension of tridecanetriphenylphospnoium bromide **3** (962 g, 1.16 mol, prepared by heating 1-bromotridecane and triphenylphosphine for 4.5 h at 140°C) in THF (1500 ml) was added a hexane solution of *n*-butyllithium (462 ml of a 2.5 M solution, 366 mmol) at 0°C under an argon atmosphere while stirring. After the addition was complete, stirring was continued for an additional 15 min. To this mixture was added dropwise a solution of **2** (250 g, 579 mmol) in THF (450 ml). After the addition, the resulting mixture was allowed to warm to room temperature and stirred for 18 h. After the mixture had been concentrated, to the residue was added a mixture of hexane–MeOH–water (10 : 7 : 3, 1000 ml),[5] and the resulting mixture was separated. The aqueous layer was extracted with hexane (500 ml), and then the organic layers were combined, dried over $MgSO_4$ and concentrated to give 339 g (98%) of crude **4** as an oil, which was employed in the next step without further purification. An analytical sample was purified by silica-gel column chromatography, using hexane–EtOAc (9 : 1) as the eluent, to give pure **4** ($E/Z$=3/7) as a colorless oil. FDMS $m/z$ 598 $M^+$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.21–7.45 (15H, m, aromatic-H), 5.48–5.59 (2H, m, H-5$E$, H-5$Z$, H-6$E$, and H-6$Z$), 4.91 (0.7H, t, $J$=7.3 Hz, H-4$Z$), 4.44 (0.3H, t, $J$=7.3 Hz, H-4$E$), 4.26 (0.3H, dd, $J$=4.3, 7.3 Hz, H-3$E$), 4.21 (0.7H, dd, $J$=4.3, 6.7 Hz, H-5a$Z$), 3.75 (0.7H, m, H-2$Z$), 3.69 (0.3H, m, H-2$E$), 3.24 (0.3H, dd, $J$=4.9, 9.8 Hz, H-1a$E$), 3.17 (0.7H, dd, $J$=4.9, 9.8 Hz, H-1a$Z$), 3.09–3.14 [1H, (3.11, dd, $J$=4.9, 9.2 Hz, H-1b$Z$), H-1b$E$ overlapped], 1.75–2.03 (2H, m, H-7a$Z$, H-7a$E$, H-7b$E$, and H-7b$Z$), 1.49 (3H, s, $OCH_3$), 1.39 and 1.38 (3H, each s, 2 × $OCH_3$), 1.21–1.34 (20H, m, $CH_2$), 0.88 (3H, t, $J$=6.7 Hz, terminal $CH_3$). *Anal.* Found: C, 80.47; H, 9.41%. Calcd. for $C_{40}H_{54}O_4$: C, 80.23; H, 9.09%.

*(2R,3S,4R)-3,4-O-Isopropylidene-2-O-methanesulfonyl-1-O-trityl-5-octadecene-1,2,3,4-tetrol* (**5**). To a solution of crude **4** (338 g, *ca.* 565 mmol) in $CH_2Cl_2$ (1500 ml) and pyridine (500 ml) was added dropwise methanesulfonyl chloride (49 ml, 633 mmol), and the mixture was stirred for 24 h at 31°C. After the addition of EtOH (40 ml), the reaction mixture was stirred for 1 h more at room temperature. The mixture was then concentrated, the residue was diluted with a mixture of hexane–MeOH–water (10 : 7 : 3, 1000 ml), and the resulting mixture separated. The aqueous layer was extracted with hexane (200 ml × 3), and the combined organic layers were dried over $MgSO_4$ and concentrated to give 363 g (95%) of crude **5** as an oil, which was employed in the next step without further purification. An analytical sample was purified by silica-gel column chromatography, using hexane–EtOAc (9 : 1) as the eluent, to give pure **5** ($E/Z$=3/7) as a colorless oil. FDMS $m/z$ 676 $M^+$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.21–7.47 (15H, m, aromatic-H), 5.41 (0.7H, ddd, $J$=5.5, 9.2, 11.0 Hz, H-6$Z$), 5.32 (0.7H, br. t, $J$=11.0 Hz, H-5$Z$), 5.22 (0.3H, br. dd, $J$=9.2, 15.0 Hz, H-5$E$), 5.02 (0.3H, dt, $J$=7.3 Hz, $J_d$=7.3 Hz, $J_d$=15.0 Hz, H-6$E$), 4.8 (0.7H, ddd, $J$=3.1, 5.5, 7.9 Hz, H-2$Z$), 4.73 (0.7H, dd, $J$=5.5, 9.8 Hz, H-4$Z$), 4.64–4.67 (0.3H, m, H-2$E$), 4.61 (0.3H, dd, $J$=5.5, 9.2 Hz, H-3$E$), 4.48 (0.7H, dd, $J$=5.5, 7.9 Hz, H-3$Z$), 4.22 (0.3H, dd, $J$=5.5, 9.2 Hz, H-4$E$), 3.55 (0.3H, dd, $J$=2.4, 11.6 Hz, H-1a$E$), 3.45 (0.7H, dd, $J$=3.2, 11.0 Hz, H-1a$Z$), 3.06–3.12 [4H, (3.12, s, $SCH_3E$), (3.11, s, $SCH_3Z$), (3.09, dd, $J$=3.1, 11.0 Hz, H-1b$Z$), H-1b$E$ overlapped], 1.66–1.82 (2H, m, H-7a$Z$, H-7b$Z$, H-7a$E$, and H-7b$E$), 1.47, and 1.46 (3H, each s, $OCH_3$), 1.39 (3H, s, $OCH_3$), 1.13–1.35 (20H, m, $-CH_2-$), 0.88 (3H, t, $J$=6.8 Hz, terminal $CH_3$). *Anal.* Found: C, 72.96; H, 8.63%. Calcd. for $C_{41}H_{56}SO_6$: C, 72.75; H, 8.34%.

*(2R,3S,4R)-2-O-Methanesulfonyl-5-octadecene-1,2,3,4-tetrol* (**6**). To a solution of crude **5** (362 g, *ca.* 536 mmol) in $CH_2Cl_2$ (1500 ml) and MeOH (350 ml) was added dropwise hydrochloric acid (200 ml), and the mixture was stirred for 5 h at room temperature. The reaction mixture was neutralized with powdered sodium hydrogen carbonate and then filtered. The filtrate was concentrated to a syrup, which was diluted with EtOAc and washed with brine. The aqueous layer was extracted with EtOAc and all the organic layers were combined, dried over $MgSO_4$ and concentrated to give an oil, which was crystalized from hexane to give 161 g of **6** (70% from **2**, $E/Z$=3/7). mp 66–67°C; FDMS $m/z$ 377 $(M-H_2O)^+$; $^1$H-NMR

(500 MHz, $CDCl_3+D_2O$) $\delta$: 5.86 (0.3H, dt, $J_t=7.3$ Hz, $J_d=14.7$ Hz, H-6*E*), 5.77 (0.7H, dt, $J_t=7.3$, $J_d=10.4$ Hz, H-5*Z*), 5.55 (0.3H, br. dd, $J=7.3$, 14.7 Hz, H-5*E*), 5.49 (0.7H, br. t, $J=9.8$ Hz, H-5*Z*), 4.91–4.97 (1H, m, H-2*Z* and H-2*E*), 4.51 (0.7H, br. t, $J=9.8$ Hz, H-4*Z*), 4.11 (0.3H, br. t, $J=7.3$ Hz, H-4*E*), 3.94–4.03 (2H, m, H-1a*Z* and H-1a*E*), 3.67–3.73 [1H, (3.70, dd, $J=3.1$, 6.7 Hz, H-3*Z*), (3.69, dd, $J=3.1$, 7.3 Hz, H-3*E*)], 3.20 and 3.19 (3H, each s, $SCH_3Z$ and $SCH_3E$), 2.05–2.22 (2H, m, H-7a*Z*, H-7b*Z*, H-7a*E*, and H-7b*E*), 1.22–1.43 (20H, m, $-CH_2-$), 0.88 (3H, t, $J=6.7$ Hz, terminal $CH_3$). *Anal.* Found: C, 58.01; H, 10.05%. Calcd. for $C_{19}H_{38}SO_6$: C, 57.84; H, 9.41%.

*(2R,3R,4R)-2-O-Methanesulfonyl-1,2,3,4-octadecanetetrol* (**7**). To a solution of **6** (160 g, 405 mmol) in THF (780 ml) was added 5% palladium on $BaSO_4$ (16 g). After the reaction vessel had been purged with hydrogen, the mixture was stirred at room temperature for 20 h, filtered through Celite, and the filter cake was washed with a mixture of $CHCl_3$–MeOH (1:1). The combined filtrate and washings were concentrated, and the residue was crystallized from EtOAc to give 146 g (91%) of **7**. $[\alpha]_D^{23}$ +12° (*c* 1, $CHCl_3$/MeOH = 1:1), [lit.,[7] +10° (*c* 1, $CHCl_3$/MeOH = 1:1)]; mp 124–126 °C [lit.,[7] 126 °C]; FDMS *m/z* 397 $(M+1)^+$; $^1$H-NMR (500 MHz, $CDCl_3$/$CD_3OD$ = 1:1) $\delta$: 4.93–4.96 (1H, m, H-2), 3.91 (1H, dd, $J=6.7$, 12.2 Hz, H-1a), 3.85 (1H, dd, $J=4.9$, 12.2 Hz, H-1b), 3.54–3.60 (1H, m, H-4), 3.50 (1H, dd, $J=1.8$, 8.5 Hz, H-3), 3.19 (3H, s, $SCH_3$), 1.75–1.83 (1H, m, H-5a), 1.53–1.62 (1H, m, H-5b), 1.21–1.45 (24H, m, $CH_2$–), 0.89 (3H, t, $J=6.7$ Hz, terminal $CH_3$). *Anal.* Found: C, 57.72; H, 10.52%. Calcd. for $C_{19}H_{40}SO_6$: C, 57.54; H, 10.17%.

*(2S,3S,4R)-2-Azido-1,3,4-octadecanetriol* (**8**). To a solution of **7** (145 g, 365 mmol) in DMF (1000 ml) was added sodium azide (47 g, 730 mmol). After stirring at 95 °C for 4 h, the mixture was concentrated, and the residue was diluted with EtOAc (450 ml) and washed with water (300 ml). The aqueous layer was extracted with EtOAc (100 ml × 3). All the organic layers were combined and washed with brine (300 ml), dried over $MgSO_4$ and then concentrated to give 122 g (97%) of crude **8**, which was employed in the next step without further purification. An analytical sample was purified by silica-gel column chromatography, using hexane–EtOAc (9:1) as the eluent, to give pure **8** as a solid. $[\alpha]_D^{23}$ +16.5° (*c* 0.5, $CHCl_3$/MeOH, 1:1), [lit.,[7] +17 (*c* 0.25, $CHCl_3$/MeOH = 1:1)]; mp 92–93 °C, [lit.,[7] 92–93 °C]; FDMS *m/z* 344 $(M+1)^+$; $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$: 3.91 (1H, dd, $J=3.7$, 11.6 Hz, H-1a), 3.75 (1H, dd, $J=7.9$, 11.6 Hz, H-1b), 3.49–3.61 (3H, m, H-2, H-3, and H-4), 1.50–1.71 (2H, m, H-5a, and H-5b), 1.22–1.46 (24H, m, $CH_2$–), 0.90 (3H, t, $J=6.7$ Hz, terminal $CH_3$). *Anal.* Found: C, 76.09; H, 9.08; N, 7.16%. Calcd. for $C_{18}H_{37}N_3O_3$: C, 75.86; H, 8.78; N, 7.17%.

*(2S,3S,4R)-2-Azido-1-O-trityl-3,4-octadecanetriol* (**9**). To a solution of crude **8** (121 g, *ca.* 352 mmol) in $CH_2Cl_2$ (750 ml) and pyridine (250 ml) was added chlorotriphenylmethane (124 g, 445 mmol), and the mixture was stirred for 16 h at room temperature. After the addition of EtOH (30 ml), the reaction mixture was stirred for 0.5 h more at room temperature. The resulting mixture was successively washed with a saturated aq. sodium hydrogen carbonate solution (300 ml × 2), saturated aq. ammonium chloride solution (300 ml × 2) and brine (300 ml), and then concentrated. The residue was purified by silica-gel column chromatography (900 g), using hexane–EtOAc (10:1) as the eluent, to give 34.4 g (52% from **7**) of pure **9** as a colorless oil. $[\alpha]_D^{24}$ +11.9° (*c* 0.9, $CHCl_3$); FDMS *m/z* 585 $M^+$; $^1$H-NMR (500 MHz, $CDCl_3+D_2O$) $\delta$: 7.24–7.61 (15H, m, aromatic-H), 3.62–3.66 (2H, m, H-1a, and H-3), 3.51–3.57 (2H, m, H-2, and H-4), 3.42 (1H, dd, $J=6.0$, 10.4 Hz, H-1b), 1.23–1.56 (26H, m, $CH_2$–), 0.88 (3H, t, $J=6.7$ Hz, terminal $CH_3$). *Anal.* Found: C, 76.09; H, 9.08; N, 7.16%. Calcd. for $C_{37}H_{51}N_3O_3$: C, 75.86; H, 8.78; N, 7.17%.

*(2S,3S,4R)-3,4-Di-O-benzyl-1-O-trityl-2-azido-3,4-octadecanetriol* (**10**). To a solution of **9** (33.5 g, 57.3 mmol) in DMF (300 ml) was added 60% NaH (5.5 g, *ca.* 138 mmol as NaH), and the mixture was stirred for 40 min at room temperature. After the mixture had been cooled to 0 °C, to it was added dropwise benzylchloride (15 ml, 120 mmol). After this addition, the resulting mixture was allowed to warm to room temperature and stirred for 18 h. The resulting mixture was quenched with ice-cooled water (*ca.* 100 ml) and then extracted with EtOAc (150 ml × 3). The extracts were washed with brine (100 ml × 3), dried over $MgSO_4$ and then concentrated to give 42.2 g (96%) of crude **10**, which was employed in the next step without further purification. An analytical sample was purified by silica-gel column chromatography, using hexane–EtOAc (100:1) as the eluent, to give pure **10** as a colorless oil. $[\alpha]_D^{24}$ +9.8° (*c* 1.0, $CHCl_3$); FDMS *m/z* 738 $(M-N_2)^+$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.07–7.48 (25H, m, aromatic-H), 4.57 (1H, d, $J=11.6$ Hz, $-CH$Ph), 4.44 (1H, d, $J=11.6$ Hz, $-CH$Ph), 4.41 (2H, s, $-CH_2$Ph), 3.73–3.79 (1H, m, H-3), 3.46–3.56 (2H, m, H-1a, and H-4), 3.37 (1H, dd, $J=8.6$, 10.4 Hz, H-1b), 1.20–1.64 (26H, m, $-CH_2-$), 0.88 (3H, t, $J=6.7$ Hz, terminal $CH_3$). *Anal.* Found: C, 80.20; H, 8.58; N, 5.47%. Calcd. for $C_{51}H_{63}N_3O_3$: C, 79.96; H, 8.29; N, 5.49%.

*(2S,3S,4R)-3,4-Di-O-benzyl-N-hexacosanoyl-1-O-trityl-2-amino-3,4-octadecanetriol* (**12**). (i) To a solution of crude **10** (41.2 g, *ca.* 54 mmol) in *n*-PrOH (250 ml) and MeOH (30 ml) were added 5% palladium on charcoal (4.1 g) and ammonium formate (27.1 g, 4.3 mmol). The mixture was stirred at room temperature for 16 h, diluted with EtOAc (250 ml) and then filtered through Celite. The filtrate was concentrated, and the residue was diluted with EtOAc (150 ml), successively washed with a saturated aq. sodium hydrogen carbonate solution (100 ml × 2) and brine (150 ml), and then dried over $MgSO_4$. This mixture was concentrated to give 38.9 g (98%) of crude **11** [FDMS 740 $(M^+)$], which was employed in the following amidation step without further purification.

(ii) To a solution of crude **11** in $CH_2Cl_2$ (300 ml) were added hexacosanoic acid (22.4 g, 56.5 mmol) and WSC·HCl (12.6 g, 64.6 mmol), and the mixture was stirred under reflux for 2 h. After the mixture had been cooled to room temperature, it was concentrated. The residue was diluted with EtOAc (500 ml) and then filtered through Celite, and the residue was washed with EtOAc. The filtrate and washings were combined, and then successively washed with a 0.5 M aq. hydrochloric acid solution (250 ml), brine (250 ml), a saturated aq. sodium hydrogen carbonate solution (250 ml) and brine. After the organic solution had been dried over $MgSO_4$, it was concentrated to give 53.2 g (88%) of crude **12**, which was employed in the next step without further purification. An analytical sample was purified by silica-gel column chromatography, using hexane–EtOAc (100:1) as the eluent, to give pure **12** as a semi-solid. $[\alpha]_D^{24}$ +5.3° (*c* 0.4, $CHCl_3$); FDMS *m/z* 1118 $M^+$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.20–7.38 (25H, m, aromatic-H), 5.57 (1H, d, $J=9.1$ Hz, NH), 4.80 (1H, d, $J=11.6$ Hz, $-CH$Ph), 4.48–4.50 (3H, m, $-CH$Ph × 3), 4.24–4.32 (1H, m, H-2), 3.83 (1H, dd, $J=3.0$, 6.7 Hz, H-3), 3.43–3.51 (2H, m, H-1a, H-4), 3.29 (1H, dd, $J=4.3$, 9.8 Hz, H-1b), 1.92 (2H, t, $J=7.3$ Hz, H-2′, and H-2b′), 1.28–1.60 (72H, m, $-CH_2-$), 0.88 (6H, t, $J=6.7$ Hz, terminal $CH_3$). *Anal.* Found: C, 82.92; H, 10.72; N, 1.25%. Calcd. for $C_{77}H_{115}NO_4$: C, 82.67; H, 10.36; N, 1.25%.

*(2S,3S,4R)-3,4-Di-O-benzyl-N-hexacosanoyl-1-O-trityl-2-amino-1,3,4-octadecanetriol* (**13**). To a solution of crude **12** (52.2 g, *ca.* 47 mmol) in $CH_2Cl_2$ (180 ml) and MeOH (36 ml) was added dropwise a 10% HCl–MeOH solution (3.0 ml), and the mixture was stirred at room temperature for 2 h. After the mixture had been neutralized with powdered sodium hydrogen carbonate (18 g), it was filtered though Celite, and the residue was washed with $CH_2Cl_2$ (180 ml). The filtrate was washed with brine (100 ml × 2), dried over $MgSO_4$, and then concentrated. The residue was dissolved in hot acetone (500 ml) and stored at 0 °C to precipite 38.6 g of pure **13** (77% from **9**). $[\alpha]_D^{24}$ −29.7° (*c* 0.7, $CHCl_3$); mp 75–76.5 °C; FDMS *m/z* 876 $M^+$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.30–7.47 (10H, m, aromatic-H), 6.03 (1H, d, $J=7.9$ Hz, NH), 4.72 (1H, d, $J=11.6$ Hz, $-CH$Ph), 4.66 (1H, d, $J=11.6$ Hz, $-CH$Ph), 4.61 (1H, d, $J=11.6$ Hz, $-CH$Ph), 4.45 (1H, d, $J=11.6$ Hz, $-CH$Ph), 4.12–4.17 (1H, m, H-2), 4.00 (1H, dt, $J_t=4.3$, $J_d=7.3$ Hz, H-4), 3.67–3.72 (2H, m, H-1a, H-3), 3.61 (1H, ddd, $J=4.3$, 8.6, 11.6 Hz, H-1b), 1.94–2.05 (2H, m, H-2a′, and H-2b′), 1.15–1.69 (72H, m, $CH_2$–), 0.88 (6H, t, $J=6.1$ Hz, terminal $CH_3$). *Anal.* Found: C, 79.72; H, 12.02; N, 1.59%. Calcd. for $C_{58}H_{101}NO_4$: C, 79.49; H, 11.62; N, 1.60%.

*(2S,3S,4R)-3,4-Di-O-benzyl-N-hexacosanoyl-1-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-2-amino-1,3,4-octadecanetriol* (**15**) *and (2S,3S,4R)-3,4-Di-O-benzyl-N-hexacosanoyl-1-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyransoyl)-2-amino-1,3,4-octadecanetriol* (**16**). To a mixture of **13** (60.0 g, 68.6 mmol), tetrahexylammonium bromide (89.4 g, 206 mmol) and 4A molecular sieve powder (60 g) in toluene (420 ml) and DMF (140 ml) was added dropwise freshly prepared tetra-*O*-benzyl galactopyranosyl bromide **14**[11] (*ca.* 137 mmol) in a toluene solution (250 ml), and the mixture was stirred at room temperature for 72 h. To the mixture was added MeOH (12 ml) to decompose remaining **14**, and the resulting mixture was stirred at room temperature for 2 h and then filtered though Celite. The filtrate was successively washed with a 4.5% aq. sodium hydrogen carbonate solution, brine and water, dried over $MgSO_4$, and then concentrated. The residue was diluted with acetonitrile (600 ml) and stirred for 2 h. The precipite formed was filtered, and the filter cake was dried under reduced

pressure at 50 C, before being purified by chromatography in a silica-gel column (1800 g), using hexane–EtOAc (8 : 1) as the eluent, to give pure **15** (70.9 g, 74%) and its β-anomer **16** (7.9 g, 8.2%). Physical data for **15**: $[\alpha]_D^{24}$ +18.8 (*c* 0.9, $CHCl_3$); mp 74–75 C; FDMS *m/z* 1399 $(M+1)^+$; $^1$H-NMR (500 MHz, $CDCl_3$) δ: 7.21–7.37 (30H, m, aromatic-H), 6.12 (1H, d, *J* = 9.0 Hz, NH), 4.91 (1H, d, *J* = 11.6 Hz, -C*H*Ph), 4.84 (1H, d, *J* = 3.7 Hz, H-1″), 4.72–4.80 (4H, m, C*H*Ph × 4), 4.35–4.65 (7H, m, -C*H*Ph × 7), 4.12–4.18 (1H, m, H-2), 3.99–4.05 (2H, m, H-2″ and H-1a), 3.84–3.93 (4H, m, H-4″, H-5″, H-3″, and H-3), 3.73 (1H, dd, *J* = 3.7, 11.0 Hz, H-1b), 3.47–3.51 (2H, m, H-6a″, and H-4), 3.42 (1H, dd, *J* = 6.1, 9.1 Hz, H-6″), 1.87–1.99 (2H, m, H-2a′, and H-2b′), 1.18–1.70 (72H, m, $-CH_2-$), 0.88 (6H, t, *J* = 7.4 Hz, terminal $CH_3$). *Anal.* Found: C, 79.22; H, 10.07%; N, 1.00. Calcd. for $C_{92}H_{135}NO_9$: C, 78.98; H, 9.73; N, 1.00%.

Physical data for **16**: $[\alpha]_D^{22}$ −0.4 (*c* 1.0, $CHCl_3$); mp 54–56 C; FDMS *m/z* 1399 $M^+$; $^1$H-NMR (500 MHz, $CDCl_3$) δ: 7.21–7.33 (30H, m, aromatic-H), 5.88 (1H, d, *J* = 8.6 Hz, NH), 4.94 (1H, d, *J* = 11.6 Hz, -C*H*Ph), 4.83 (1H, d, *J* = 11.6 Hz, -C*H*Ph), 4.76–4.80 [2H, (4.78, d, *J* = 11.6 Hz, -C*H*Ph), (4.77, d, *J* = 11.6 Hz, -C*H*Ph)], 4.72 (2H, s, $-CH_2$Ph), 4.59–4.62 [2H, (4.61, d, *J* = 11.6 Hz, -C*H*Ph), (4.60, d, *J* = 11.6 Hz, -C*H*Ph)], 4.55 (1H, d, *J* = 11.6 Hz, -C*H*Ph), 4.36–4.44 (3H, m, -C*H*Ph × 3), 4.30 (1H, d, *J* = 7.3 Hz, H-1″), 4.14–4.26 (2H, m, H-1a, and H-2), 3.94 (1H, d, *J* = 3.1 Hz, H-4″), 3.82 (1H, dd, *J* = 7.3, 9.2 Hz, H-2″), 3.79 (1H, dd, *J* = 2.4, 7.3 Hz, H-3), 3.66 (1H, dd, *J* = 3.7, 11.0 Hz, H-1b), 3.49–3.63 (4H, m, H-3″, H-5″, H-6a″, and H-6b″), 3.40–3.46 (1H, m, H-4), 1.10–1.88 (74H, m, $-CH_2-$), 0.88 (6H, t, *J* = 6.7 Hz, terminal $CH_3$). *Anal.* Found: C, 79.32; H, 9.97; N, 0.98%. Calcd. for $C_{92}H_{135}NO_9$: C, 78.98; H, 9.73; N, 1.00%.

*(2S,3S,4R)-1-O-(α-D-Galactopyranosyl)-N-hexacosanoyl-2-amino-1,3,4-octadecanetriol (KRN7000).* To a suspension of **15** (60.0 g, 42.9 mmol) in EtOH (960 ml) were added 20% palladium hydroxide on charcoal (6.0 g) as an EtOH suspension (120 ml) and 4-methylcyclohexane (120 ml, 93.5 mmol). After the mixture had been stirred at reflux for 4 h, it was successively filtered through paper (ADVANTEC, No. 5C) and a PALL filter (Japan PALL, SBF1JO12PH4). The filtrate was cooled to room temperature and the precipite formed was filtered and washed with EtOH (180 ml) to give a white cake of KRN7000. Crude KRN7000 was suspended in 92% aq. EtOH (400 ml) and stirred in a water bath at 85 C for 1.5 h. The mixture was allowed to cool to room temperature and stood for 12 h. The precipite formed was filtered and dried under reduced pressure to give pure KRN7000 (35.0 g, 95%) as a white solid.

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