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Total synthesis of α-galactosyl cerebroside

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Abstract

A highly convergent synthetic approach was developed to obtain α-galactosyl cerebroside *O*-(α-D-galactopyranosyl)-2-hexacosylamino-D-*ribo*-1,3,4-octadecantriol, which has previously been demonstrated to have immunostimulatory activity. Known 4,6-*O*-benzylidene galactose was the starting material for both the required α-galactosyl and the phytosphingosine building blocks *O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-galactopyranosyl) trichloroacetimidate (**4**) and 2-*O*-methanesulfonyl-D-*arabino*-1,2,3,4-octadecantetrol (**5**). The key step of the synthetic strategy is the highly regio- and stereoselective O-galactosylation of 1,3,4-*O*-unprotected phytosphingosine acceptor **5** using known **4** as donor. The total synthesis required only 11 synthetic steps starting from galactose.

Keywords: Glycosphingolipids; Phytosphingosine; Synthesis; Glycosylation; Trichloroacetimidates

1. Introduction

Monoglycosylated ceramides (cerebrosides) are the simplest class of glycosphingolipids; they are important surface molecules found in virtually all cells. Galactosyl ceramides and their metabolites have been shown to possess important functions in promoting the regulation of nerve cells [1], regulating protein kinase C activities [2], and modulating the function of hormone receptors [3].

Some α-galactosyl ceramides with phytosphingosine structures, isolated from the marine sponge *Agelas mauritianus*, were found to have strong in vivo antitumor activities against several murine tumor cells [4]. This kind of structure has hardly been detected in normal mammalian tissues [5], but mainly in cancer cells [6], foetus [7] and specific kidney and intestine cell lines [8]. Owing to several structural complexities of the natural product, Koezuka and co-workers [9] have synthesized several analogs for a structure–activity relationship study, taking the more prominent compound agelasphin-**9b** {(2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-16-methyl-2[*N*-(*R*)-2-hydroxytetracarbanoyl - amino] - 1,3,4 - heptadecanetriol} a lead structure, from which compound **1** arose as a potent candidate for clinical trial.

Compound **1** was also found to have immunostimulatory activity, since it is the ligand of a T-cell antigen receptor expressed by V_α 14 natural killer T-lymphocytes (NKT) [10]. NKT cells are known to play important roles in the regulation of the progress of several autoimmune diseases [11] and, when activated with compound **1** and interleukin **12**, they are able to suppress tumor metastases [12].

These findings have promoted an increasing demand for this compound for biological in-

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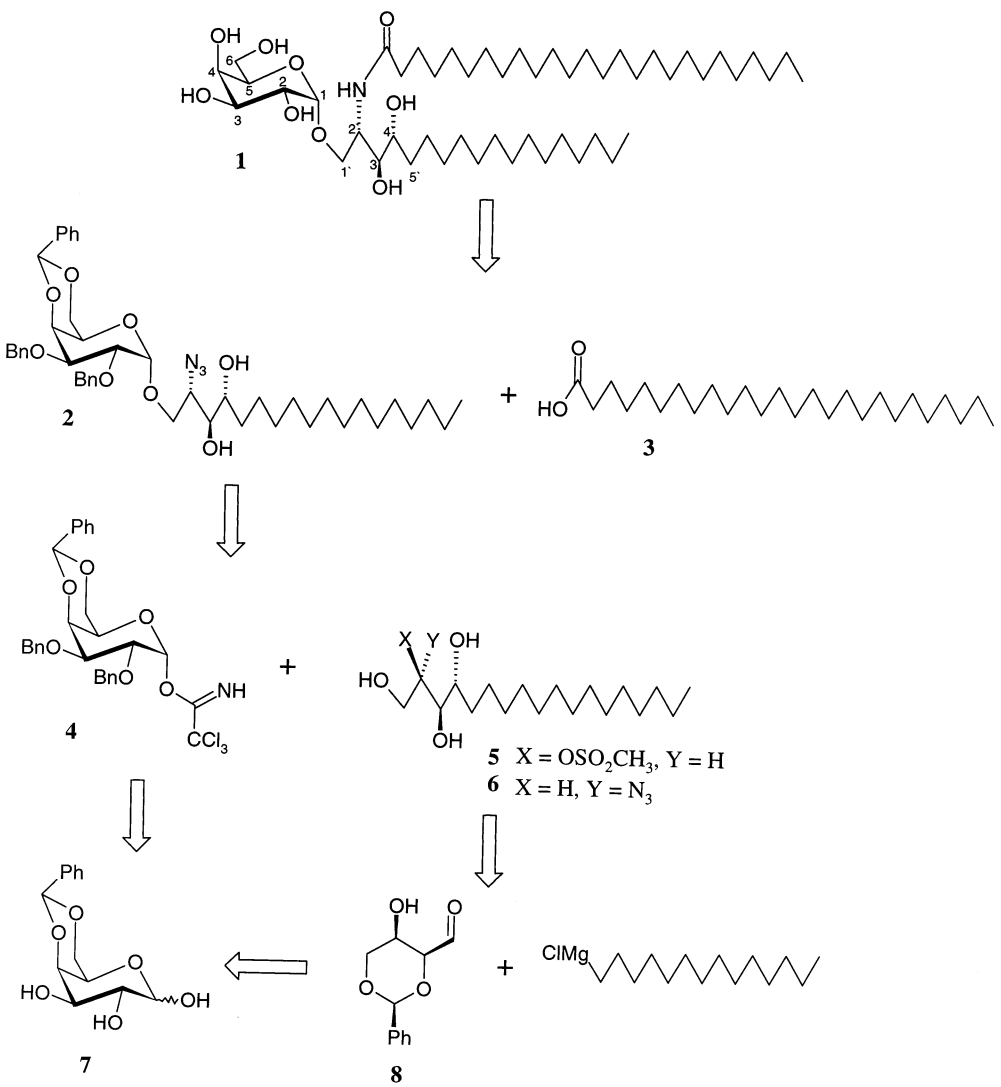
vestigations. Larger amounts of pure **1** are therefore highly desirable. We report here on a facile and convergent approach for the synthesis of cerebroside **1**, which can be easily scaled up.

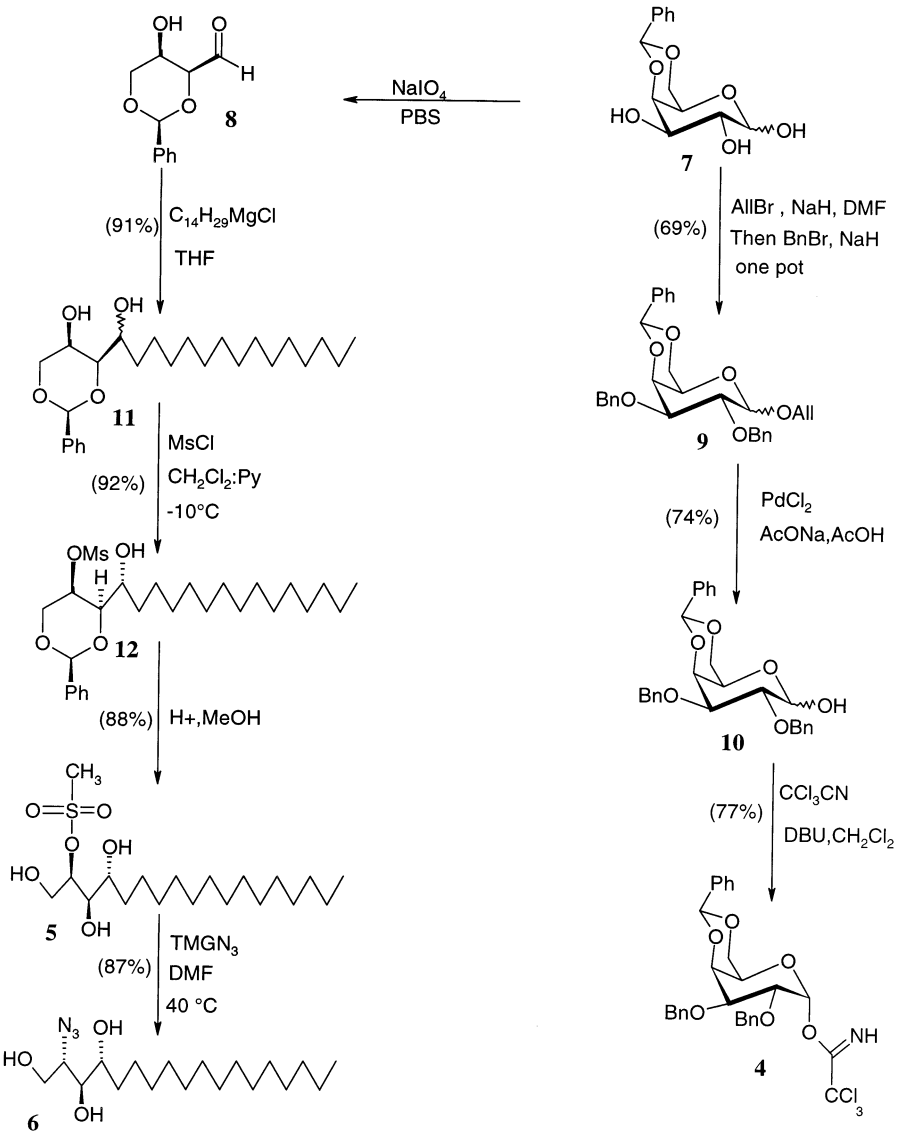
2. Results and discussion

The retrosynthetic analysis of compound **1** is depicted in Scheme 1. The first disconnection leads to fatty acid **3** and to the α-galactosylphytosphingosine synthon **2**, which in turn has to be obtained starting from a suitable protected galactosyl donor **4** and a phytosphingosine acceptor bearing three chiral centers in positions 2, 3 and 4. The key step of this sequence is the regioselective α-galactosylation of a 1,3,4-*O*-unprotected phytosphingosine derivative (**5** or **6**), thus minimizing the protection–deprotection steps, which would make the sequence shorter and therefore more efficient. Trichloroacetimidate **4** can be readily obtained from the known 4,6-*O*-benzylidene-D-galactose (**7**) [13], which via **8** should also provide **5** and **6**.

The galactosyl donor should be designed in order to obtain a high α:β ratio, thus easing the purification of the resulting α-glycoside. Generally, the 2,3,4,6-tetra-*O*-benzyl-galactosyl donor has been the choice for this case, but it often led to anomeric mixtures that sometimes were difficult to purify. In our approach we have placed a benzylidene group at C-4



Scheme 1.



Scheme 2.

and C-6, since there are examples in the literature that show that in the galactose series the cis-decalin ring system with the equatorial phenyl group hinders the attack from the β-face [14]. Non-participating benzyl groups were chosen to protect positions 2 and 3 of the galactosyl trichloracetimidate **4**. The phytosphingosine triol acceptors (**5** and **6**) were readily accessible from known 4,6-*O*-benzylidene-D-galactose (**7**) [13].

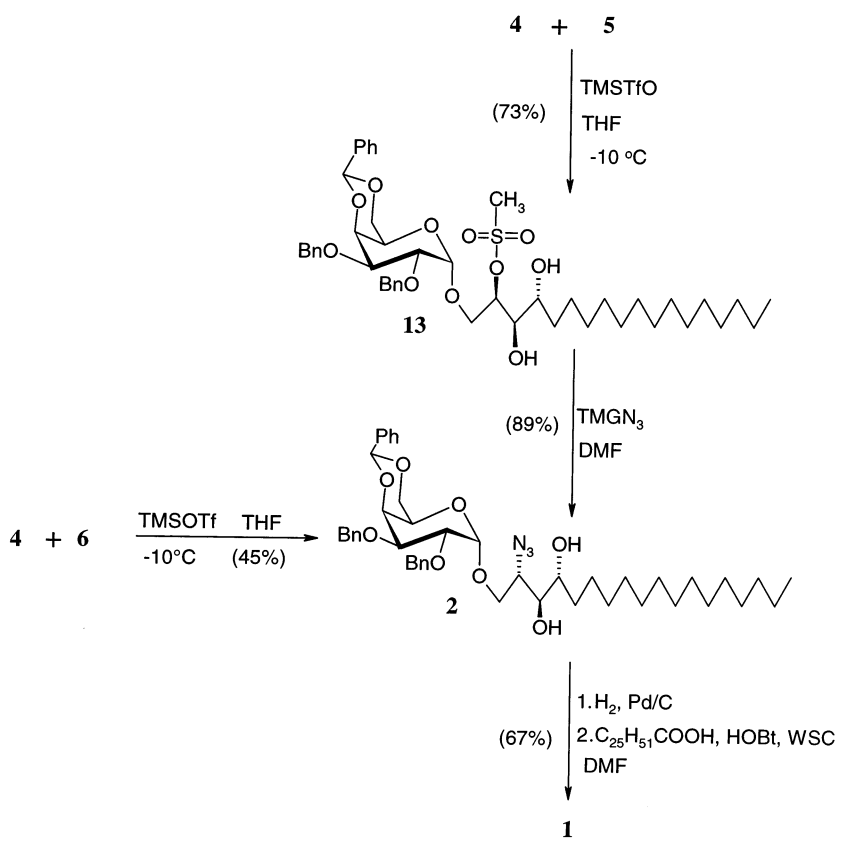
4,6-*O*-Benzylidene-D-galactose (**7**) was 1-*O*-allylated and 2,3-di-*O*-benzylated using a one-pot procedure to afford an α/β mixture of allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-galactopyranoside (**9**) in 69% yield (Scheme 2). The NMR data of both compounds were identical with those previously reported [15]. Compound **9** was deallylated using $PdCl_2$ without affecting the benzylidene group in 74% yield after purification by flash chromatography. The analytical data of compound **9** were in accordance with those already published [15,16].

Reaction of the reducing sugar **10** with trichloroacetonitrile and DBU in dry dichloromethane afforded donor **4** in 77% yield. The NMR data of this trichloroacetimidate were identical with those published by Koeman et al. for the α anomer [17]. This compound is relatively unstable although it can be stored under argon for at least 3 weeks at −18 °C.

Acceptor **5** was obtained in 88% yield through a controlled acid hydrolysis of acetal **12**, which in turn was obtained from 4,6-*O*-benzylidene-D-galactose (**7**), via **8** and **11**, as previously described [18]. The multiplet at 4.72 ppm in the ^{1}H NMR spectrum corresponding to H-2 indicated the substitution of this position by the methanesulfonyl (mesyl) group, this was also confirmed by the presence of a singlet at 3.14 ppm owing to the methyl group of the mesylate. On the other hand, acceptor **6** was prepared by azide substitution of the mesylate group of **5**, using tetramethylguanidinium azide as soluble azide ion donor. The multiplet of proton 2 in the ^{1}H NMR spectrum was strongly shifted upfield (4.72 → 3.45 ppm) and the signal of carbon 2 in the ^{13}C NMR was also shifted to 59.2 ppm, evidencing the replacement of the mesylate group by the azide group.

Regioselective galactosylation of compound **5** was performed with 0.75 equivalents of trichloracetimidate **4** in THF at −10 °C, using trimethylsilyl trifluoromethanesulfonate catalysis to afford, after flash chromatography, the α-glycoside **13** in 73% yield based on trichloroacetimidate **4**, and 83% based on the consumed triol **5** (Scheme 3). Neither the β anomer nor any other regioisomer was detected in the reaction. The ^{1}H NMR spectrum shows a doublet at 5.12 ppm with a coupling constant of 3.54 Hz that corresponds to the anomeric proton, it evidences α-stereochemistry of the glycosidic bond. In the ^{13}C NMR spectrum the signal of C-1 of the phytosphingosine unit appears at 68.3 ppm (C-1′ in **5**: 59.9 ppm), which clearly indicated O-alkylation.

When the same reaction was performed with acceptor **6**, the yield of pure compound **2** was only 45% based on **4** and 61% based on reacted **6**; obviously, the regioselectivity of the galactosylation reaction is lower, thus resulting in a complex purification problem. The presence of the more electron withdrawing mesyl group seems to increase the reactivity difference between primary and secondary hydroxyl groups.



Scheme 3.

Table 1
Coupling constant values of H-2′ in Hz

	$J_{1'a-2'}$	$J_{1'b-2'}$	$J_{2'-3'}$	$J_{3'-4'}$
13	5.6	4.4	<1	8.3
2	2.8	3.4	6.0	12.1

The replacement of the mesylate group by azide in compound **13** was performed by treatment with five equivalents of tetramethylguanidinium azide in DMF at 40 °C for 2 days. Compound **2** was obtained in 89% yield with complete inversion of configuration. The ^{13}C and ^{1}H NMR signals for position 2 of the phytosphingosine moiety were considerably shifted (82.3 → 59.6 and 5.05 → 3.41 ppm), thus confirming the replacement of the mesylate group by the azido group. All the mesyl related signals disappeared. The dramatic change of the coupling constant pattern of proton 2′ (Table 1) confirms the inversion of configuration at carbon 2′.

Compound **2** was submitted to hydrogenolysis to remove the benzyl and benzylidene groups and to reduce the azido group to the amine group. The resulting compound was isolated and without further purification treated with hexacosanoic acid and the reagent system *N*-hydroxybenzotriazole and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride in DMF at 40 °C. The target compound **1** was obtained in pure form after purification by RP column chromatography and then by silica gel adsorption chromatography in 69% yield. All the analytical data were in accord with those previously published [9].

In conclusion, the total synthesis of compound **1** was successfully achieved starting from 4,6-*O*-benzylidene-D-galactose (**7**), following a convergent approach, in 11 efficient steps which involved a highly regio- and stereoselective α-galactosylation of a 2-*O*-methanesulfonyl-D-*arabino*-1,2,3,4-octadecanetetrol acceptor. This facile sequence permits the scaling up of this synthesis in order to obtain bulk quantities of **1** for biological studies.

3. Experimental

General methods.—Solvents were purified in the usual way. Thin-layer chromatography (TLC) was performed on plastic plates Silica Gel 60 F_{254} and on HPTLC plates NH_2 F_{254} S (E. Merck, layer thickness 0.2 mm). The detection was achieved by treatment with a solution of ammonium molybdate (20 g) and cerium(IV) sulfate (0.4 g) in 400 mL of 10% H_2SO_4 or with 15% H_2SO_4, and heating at 150 °C. Flash chromatography was carried out on silica gel (Baker, 30–60 μm) and Lichroprep NH_2, particle size 40–63 μm (E. Merck). Reversed-phase (RP) chromatography: RP 18 (32–63 μm) ICN Biomedicals. Medium-pressure liquid chromatography (MPLC): LiChroprep Si 60 (E. Merck; size 15–25 μm), detection by differential refractometer. Optical rotations were determined at 25 °C with a Perkin–Elmer 241/MC polarimeter (1 dm cell). NMR spectra were recorded with Bruker AC 250 and 600 DRX instruments using tetramethylsilane as internal reference for $CDCl_3$ and CD_3OD, while for D_2O the water peak in 4.63 ppm was taken as the reference. The assignments of ^{1}H NMR spectra were based on chemical shift correlation (DQF COSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY). The assignments of ^{13}C NMR spectra were based on carbon–proton shift-correlation heteronuclear multiple quantum coherence (HMQC). MS spectra were recorded with MALDI-Kompakt (Kratos), EI and FAB with Finnigan MAT 312/AMD equipment. Microanalyses were performed in the Microanalyses Unit at the Fakultät für Chemie, Universität Konstanz.

*Allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*D*-galactopyranoside* (**9**).—Sodium chloride (0.96 g, 22 mmol) was slowly added to a cooled (ice-bath) solution of compound **7** [13] (5 g, 18.5 mmol) and allyl bromide (2.25 mL, 27.7 mmol) in dry DMF (70 mL). After the addition was completed, the mixture was stirred 30 min at room temperature (rt) and then diluted with benzyl bromide (8.4 mL, 74 mmol). The clear yellow solution was cooled again and more NaH (1.92 g, 55.5 mmol) was supplied in portions. The resulting suspension was fur-

ther stirred at rt for 1 h, when a clear solution was already formed. The reaction mixture was then poured into ice water (1 L) and the suspension was stirred until it reached rt. The water was decanted and the residue was dissolved in CH_2Cl_2 (50 mL) and successively washed with an aq 0.1 M HCl soln (20 mL), aq satd $NaHCO_3$ soln (15 mL), and water (15 mL), dried ($MgSO_4$) and concentrated. Flash chromatography of the residue (3:1 petroleum ether–EtOAc) and recrystallisation from EtOH provided compound **9** (α/β mixture) as a white solid (6.28 g, 69%). TLC (3:1 petroleum ether–EtOAc): R_f 0.31, 0.38. The NMR data of each anomer was identical, as reported in the literature [15].

2,3-Di-O-*benzyl-4,6*-O-*benzylidene*-D-*galactopyranose* (**10**).—Palladium chloride (3.34 g, 21 mmol) was added to a solution of compound **9** (1.5 g, 3.3 mmol) in 95% aq AcOH (67 mL) containing AcONa (2.1 g). The dark suspension was stirred until TLC evidenced complete conversion of the reactand into a slower moving spot. The reaction mixture was filtered through a bed of Celite, diluted with CH_2Cl_2 (100 mL), and washed with water (50 mL) satd $NaHCO_3$ soln (2 × 30 mL) and water (20 mL), dried ($MgSO_4$) and concentrated. The resulting dark yellow syrup was purified by flash chromatography (2:1 petroleum ether–EtOAc) to provide compound **10** (α/β mixture) as a solid (1 g, 74%). The NMR data of each anomer were identical with those reported in the literature [15,16].

O-*(2,3-Di*-O-*benzyl-4,6*-O-*benzylidene*-D-*galactopyranosyl) trichloroacetimidate* (**4**).—To a solution of compound **10** (1 g, 2.4 mmol) and CCl_3CN (2.4 mL, 24 mmol) in dry CH_2Cl_2 (20 mL) was added DBU (0.15 mL, 1.2 mmol) and the solution was stirred for 40 min at rt. The dark solution was then concentrated and purified by flash chromatography (2:1 petroleum ether–EtOAc containing 0.1% Et_3N) to afford compound **4** (1.04 g, 77%) as a white foam [17].

2-O-*Methanesulfonyl*-D-arabino-*1,2,3,4-octadecantetrol* (**5**).—To a solution of compound **12** [18] (1.7 g, 3.49 mmol) in methanol (50 mL) was added *p*-toluenesulfonic acid (40 mg), and the solution was stirred at rt until the starting material was completely transformed into a single slower moving spot (~3 h). The solution was then neutralized with Et_3N and concentrated to a thick syrup, which was purified by flash chromatography (15:1 CH_2Cl_2–MeOH) to afford **5** (1.22 g, 88%) as an amorphous solid. An analytical sample was recrystallized from methanol: mp 129 °C; TLC (3:1 petroleum ether–EtOAc): R_f 0.07 and (10:1 CH_2Cl_2–MeOH): R_f 0.36; $[\alpha]_D$ +18° (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, Me_2SO-d_6): δ 4.99 (s, 1 H, OH-1), 4.85 (d, 1 H, $J_{3,OH}$ 6.7 Hz, OH-3), 4.72 (t, 1 H, $J_{2,3} = J_{1,2}$ 5.7 Hz, H-2), 4.43 (d, 1 H, $J_{4,OH}$ 6 Hz, OH-4), 3.62 (m, 2 H, H-1), 3.37 (m, 1 H, H-3), 3.33 (m, 1 H, H-4), 3.14 (s, 3 H, SCH_3), 1.44 (m, 2 H, H-5), 1.25 (m, 24 H, CH_2), 0.84 (t, 3 H, *J* 6.7 Hz, CH_3). ^{13}C NMR (150.9 MHz, Me_2SO-d_6): δ 82.3 (C-2), 71.2 (C-3), 68.8 (C-4), 59.9 (C-1), 37.6 (SCH_3), 33.1 (C-5), 31.2–22.0 (CH_2), 13.8 (CH_3). MALDIMS (positive mode, DHB/THT-matrix): *m/z* 419.6 [MNa^+], 435.6 [MK^+]. Anal. Calcd for $C_{19}H_{40}O_6S$ (396.58): C, 57.54; H, 10.16; S, 8.08. Found: C, 57.47; H, 10.19.

2-O-*Azido*-D-ribo-*1,2,3,4-octadecantetrol* (**6**).—Compound **5** (1.3 g, 3.27 mmol) was stirred with tetramethylguanidinium azide (2.6 g, 16.39 mmol) in dry DMF (10 mL) at 50 °C until TLC of the reaction mixture (10:1 CH_2Cl_2–MeOH) showed complete conversion of the starting material into a faster moving spot. The solution was then concentrated and purified using silica gel flash column chromatography (30:1 CH_2Cl_2–MeOH) to afford **6** (1.22 g, 87%) as an amorphous solid. An analytical sample was recrystallized from MeOH: mp 90 °C; TLC (3:1 petroleum ether–EtOAc): R_f 0.09 and (10:1 CH_2Cl_2–MeOH 10:1): R_f 0.73; $[\alpha]_D$ +10° (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.89 (m, 2 H, H-1), 3.66 (m, 1 H, H-3′), 3.64 (m, 1 H, H-4′), 3.45 (m, 1 H, H-2′), 2.77 (d, 1 H, $J_{3,OH}$ 7.1 Hz, OH-3′), 2.17 (d, 1 H, $J_{4,OH}$ 4.5 Hz, OH-4′), 1.50 (m, 2 H, H-5′), 1.26 (m, 24 H, CH_2), 0.88 (t, 3 H, *J* 6.7 Hz, CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 74.3 (C-3′), 72.9 (C-4′), 61.6 (C-1′), 59.2 (C-2′), 33.4 (C-5′), 31.9–22.6 (CH_2), 14.1 (CH_3). MALDIMS (positive mode, DHB/THT-matrix): *m/z* 366.5 [MNa^+], 382.5 [MK^+]. Anal. Calcd for $C_{18}H_{37}O_3N_3$ (343.50): C, 62.93; H, 10.85; N, 12.23. Found: C, 62.77; H, 10.99; N, 12.15.

1-O-*(2,3-Di*-O-*benzyl-4,6*-O-*benzylidene*-α-D-*galactopyranosyl)*-*2*-O-*methanesulfonyl*-D-arabino-*1,2,3,4-octadecantetrol* (**13**).—To a solution of **5** (0.95 g, 2.4 mmol) and **4** (0.9 g, 1.6 mmol) in dry THF (20 mL) was added trimethylsilyl trifluoromethanosulfonate (30 μL, 0.16 mmol) at −10 °C, and the solution was stirred for 30 min. The solution was then neutralized with Et_3N and concentrated to dryness. Flash chromatography of the residue (3:1–1:1 petroleum ether–EtOAc) provided recovered **5** (0.4 g, 41% of the starting amount) and α-glycoside **13**, which was recrystallized from EtOAc–hexane (0.96 g, 73% based on **4** and 83% based on reacted **5**) as colorless needles: mp 137.5 °C; TLC (1:1 petroleum ether–EtOAc): R_f 0.28; $[\alpha]_D$ +62° (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.51–7.25 (m, 15 H, Ph–H), 5.49 (s, 1 H, CH), 5.05 (m, 1 H, H-2′), 5.00 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.77 (AB, *J* 11.4, CH_2Ph-2), 4.74 (AB, *J* 12.8, CH_2Ph-3), 4.23 (d, 1 H, $J_{3,4}$ 3.4 Hz, $J_{4,5}<1$ Hz, H-4), 4.22 (m, 1 H, H-6a), 4.09 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 4.03 (dd, 1 H, $J_{1'a,1'b}$ 11.7 Hz, H-1′a), 4.01 (m, 1 H, H-6b), 3.86 (m, 1 H, H-3), 3.77 (dd, 1 H, H-1′b), 3.54 (m, 1 H, H-4′), 3.49 (m, 1 H, H-3′), 2.96 (s, 3 H, SCH_3), 2.66 (d, 1 H, $J_{4,OH}$ 5.6 Hz, OH-4′), 2.62 (d, 1 H, $J_{3,OH}$ 7.6 Hz, OH-3′), 1.52 (m, 2 H, H-5′), 1.27 (m, 24 H, CH_2), 0.88 (t, 3 H, *J* 6.7 Hz, CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 138.3, 138.0, 137.6 (*ipso*), 128.9–126.2 (Ph), 101.1 (CH), 99.3 (C-1), 79.1 (C-2′), 75.5 (C-3), 75.2 (C-2), 74.0 (C-4, C-3′), 73.8 (CH_2Ph-2), 71.4 (CH_2Ph-3), 70.5 (C-4′), 69.2 (C-6), 68.2 (C-1′), 63.1 (C-5), 38.4 (SCH_3), 33.0 (C-5′), 31.9–22.6 (CH_2), 14.1 (CH_3). MALDIMS (positive mode, DHB/THT-matrix): *m*/*z* 849.7 [MNa^+]. Anal. Calcd for $C_{46}H_{66}O_{11}S$ (827.02): C, 66.80; H, 8.04; S, 3.87. Found: C, 66.69; H, 8.12.

2-Azido-1-O-*(2,3-di*-O-*benzyl-4,6*-O-*benzylidene*-α-D-*galactopyranosyl)*-D-ribo-*1,3,4-octadecantriol* (**2**).—Method (a): to a solution of compound **13** (0.9 g, 1.08 mmol) in dry DMF (5 mL) was added tetramethylguanidinium azide (0.86 g, 5.44 mmol), and the solution was stirred for 48 h at rt. The mixture was then concentrated to dryness and the resulting syrup dissolved in CH_2Cl_2 (20 mL). The solution was successively washed with 0.03 M HCl (3 × 5 mL), satd $NaHCO_3$ (7 mL) soln, water (7 mL), then dried ($MgSO_4$), filtered and concentrated. Flash chromatography of the residue (3:1 petroleum ether–EtOAc) and recrystallization (EtOAc–pentane) provided **2** (0.64 g, 89%) as colorless crystals. Method (b): to a solution of compound **6** (0.51 g, 1.5 mmol) and compound **4** (0.42 g, 0.75 mmol) in dry THF (10 mL) was added trimethylsilyl trifluoromethanosulfonate (13 μL, 0.075 mmol) at −20 °C, and the solution was stirred for 30 min. The solution was then neutralized with Et_3N and concentrated to dryness. Flash chromatography of the residue (3:1–1:1 petroleum ether–EtOAc) provided recovered **6** (0.32 g, 62% of the starting material) and **2** (0.26 g, 49% based on trichloroacetimidate **4**, and 61% based on consumed acceptor **6**): mp 70.8 °C; TLC (1:1 petroleum ether–EtOAc): R_f 0.39. $[\alpha]_D$ +65° (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.51–7.25 (m, 15 H, Ph–H), 5.46 (s, 1 H, CH), 4.86 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.79 (AB, *J* 11.6, CH_2Ph-2), 4.74 (d, *J* 12.0, CH_2Ph-3), 4.23 (m, 1 H, $J_{6a,6b}$ 12.4, H-6a), 4.19 (d, 1 H, $J_{3,4}$ 3.4 Hz, $J_{4,5}<1$ Hz, H-4), 4.17 (dd, 1 H, $J_{1'a,1'b}$ 10.6 Hz, H-1′a), 4.08 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 4.02 (m, 1 H, $J_{6b,5}$ 1.2, H-6b), 3.97 (m, 1 H, H-3), 3.86 (dd, 1 H, H-1′b), 3.75 (m, 1 H, H-3′), 3.60 (m, 1 H, H-4′), 3.47 (d, 1 H, $J_{3,OH}$ 7.1 Hz, OH-3′), 3.41 (m, 1 H, H-2′), 2.17 (d, 1 H, $J_{4,OH}$ 4.5 Hz, OH-4′), 1.50 (m, 2 H, H-5′), 1.26 (m, 24 H, CH_2), 0.88 (t, 3 H, *J* 6.7 Hz, CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 138.3, 137.8, 137.7 (*ipso*), 128.9–126.2 (Ph), 101.0 (CH), 99.9 (C-1), 76.3 (C-3), 74.8 (C-2), 74.7 (C-3′), 74.1 (C-4), 73.8 (CH_2Ph-2), 71.4 (CH_2Ph-3), 72.8 (C-4′), 69.6 (C-1′), 69.4 (C-6), 63.3 (C-5), 59.6 (C-2′), 32.4 (C-5′), 31.9–22.6 (CH_2), 14.1 (CH_3). MALDIMS (positive mode, DHB/THT-matrix): *m*/*z* 796.9 [MNa^+]. Anal. Calcd for $C_{45}H_{63}N_3O_8$ (774.00): C, 69.83; H, 8.20; N, 5.42. Found: C, 69.67; H, 8.35; N, 5.38.

1-O-*(*α-D-*Galactopyranosyl)-2-hexacosylamino*-D-ribo-*1,3,4-octadecantriol* (**1**).—A mixture of compound **2** (123 mg, 0.15 mmol) and Pd–C 10% (0.1 g) in MeOH (10 mL) containing AcOH (0.1 mL) was stirred under a H_2 atmosphere at rt for 12 h. The mixture was filtered, concentrated and coevaporated with toluene. The resulting syrup was then

dissolved in dry DMF (3 mL), hexacosanoic acid (58 mg, 0.15 mmol), *N*-hydroxybenzotriazol (23 mg, 0.15 mmol) and 1-[3-(dimethylamino) - propyl] - 3 - ethylcarbodiimide hydrochloride (32 mg, 0.15 mmol) were successively added and the solution was stirred at 40 °C for 12 h. The mixture was injected onto a RP silica gel C-18 column and eluted with MeOH containing increasing amounts of CH_2Cl_2, the nearly pure white solid so obtained was rechromatographed on a short silica gel column (25:1 CH_2Cl_2–MeOH) to provide **1** (94 mg, 69%) as an amorphous solid. The NMR data were identical with those reported in the literature [9]. TLC (15:1 CH_2Cl_2–MeOH): R_f 0.49. Anal. Calcd for $C_{50}H_{99}NO_9$ (858.33): C, 69.96; H, 11.62; N, 1.63. Found: C, 69.88; H, 11.81; N, 1.54.

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