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Synthesis of a position isomer of ganglioside GD_3 having an α-Neu5Ac-(2→9)-α-Neu5Ac linkage*

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ABSTRACT

A position isomer of ganglioside GD_3 has been synthesized in which *N*-acetylneuraminic acid (Neu5Ac) is linked α-glycosidically at C-9 of the Neu5Ac residue of the ganglioside GM_3 structure. The coupling of 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside with methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→9)-(methyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate, prepared from the corresponding 2-(trimethylsilyl)ethyl glycoside by selective removal of the 2-(trimethylsilyl)ethyl group, 1-*O*-acetylation, and introduction of the methylthio group with trimethyl(methylthio)silane, using *N*-iodosuccinimide–trifluoromethanesulfonic acid as a glycosylation catalyst, gave a tetrasaccharide (**5**). Compound **5** was converted, *via* *O*-acetylation, selective removal of the 2-(trimethylsilyl)ethyl group, and subsequent imidate formation, into a protected α-Neu5Ac-(2→9)-α-Neu5Ac-(2→3′)-α-lactosyl trichloroacetimidate (**8**). Glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol with **8** afforded a ceramide precursor which was transformed, *via* selective reduction of the azido group, coupling with octadecanoic acid, *O*-deacylation, and hydrolysis of the methyl ester groups, into to the title ganglioside.

INTRODUCTION

Gangliosides are distinguished from other glycosphingolipids in that they contain sialic acid, usually α-linked to C-3 or C-6 of galactose, or C-8 of another sialic acid residue. Recently, as more and more biological functions[2–5] of gangliosides are being revealed, their synthesis is becoming increasingly stimulating and rewarding.

Our past efforts in this regard have resulted in the development of a facile procedure for the α-stereoselective coupling[6] of sialic acid using its protected methyl 2-thioglycoside as the glycosyl donor and suitably protected galactose and lactose acceptors, with dimethyl(methylthio)sulfonium triflate (DMTST) or *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH) as the promoter in acetonitrile solution.

This method has subsequently served us for the systematic synthesis of gangliosides[7] and their analogs[8], in order to elucidate the functions of gangliosides at the molecular level. Here we describe the synthesis of α-Neu5Ac-(2→9)-α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-β-D-Glc-(1→1)-ceramide, an isomer of ganglioside GD_3 (ref. 9) with respect to the position of linkage between the two sialic acid residues.

* Synthetic Studies on Sialoglycoconjugates, Part 28. For Part 27, see ref. 1.

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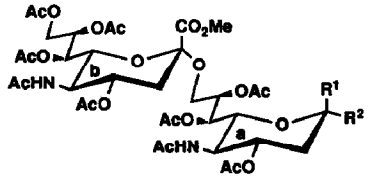
RESULTS AND DISCUSSION

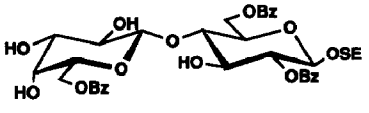
Methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→9)-(methyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**3**) was selected as the glycosyl donor, and 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside[6a,7b] (**4**) as the acceptor in the synthesis of tetrasaccharide **5**. Compound **5** could then, by coupling to the ceramide moiety, be transformed into the title ganglioside **13**.

Treatment of 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→9)-(methyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate[6b] (**1**) with trifluoroacetic acid in dichloromethane, followed by acetylation, gave an anomeric mixture (α:β = 1:3) of the 1-acetate **2** in quantitative yield. The conversion of **2** into the methyl thioglycoside **3** (93%; the α:β ratio was estimated as ~ 1:1 from the relative intensities of the methyl ester signals) was achieved by treatment[6c,10] with trimethyl(methylthio)silane and trimethylsilyl trifluoromethanesulfonate (TMS triflate) in dichloroethane.

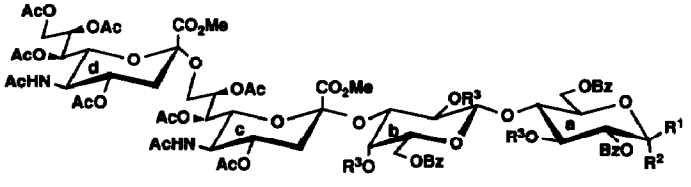
Glycosylation of **4** with **3** thus obtained, in acetonitrile for 4.5 h at −40° in the presence of NIS–TfOH, gave the expected α-glycoside **5** in 24% yield. Characteristic signals of the Neu5Ac units in the ^{1}H-n.m.r. spectrum of **5** were two one-proton doublets of doublets at δ 2.60 and 2.65 due to H-3c*eq* and H-3d*eq*, and two one-proton



1 R^1=CO_2Me, R^2=OSE
2 R^1, R^2=CO_2Me, OAc
3 R^1, R^2=CO_2Me, SMe



4

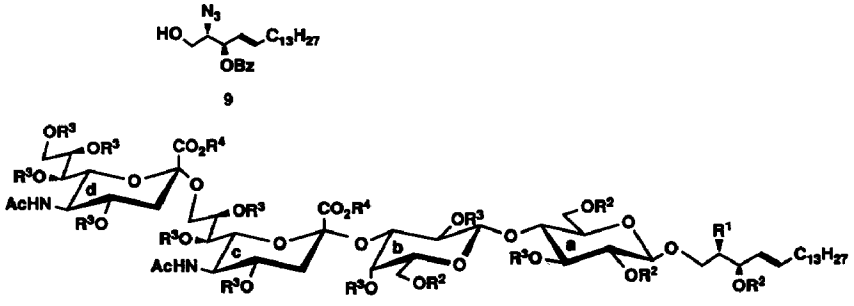


5 R^1=OSE, R^2=R^3=H
6 R^1=OSE, R^2=H, R^3=Ac
7 R^1,R^2=OH, H, R^3=Ac
8 R^1=H, R^2=OC(=NH)CCl_3, R^3=Ac

SE = $Me_3SiCH_2CH_2$-
Bz = benzoyl

multiplets at δ 4.82 and 4.97 due to H-4c and H-4d, respectively, indicating[11–13] the newly formed glycosidic linkage to be α.

The position of linkage of the α-Neu5Ac-(2→9)-Neu5Ac moiety to the acceptor was unambiguously proved by ^{1}H-n.m.r. data for the per-*O*-acetyl derivative **6**. The observed chemical shifts for H-3b (δ 4.55, $J_{2,3}$ 9.7, $J_{3,4}$ 3.3 Hz), H-2b (δ 5.04, $J_{1,2}$ 8.1 Hz), and H-4b (δ 4.93) clearly indicated the linkage position to be C-3 of the galactose residue. Other ^{1}H-n.m.r. data are given in the Experimental section and are consistent with the structure assigned. Selective removal of the 2-(trimethylsilyl)ethyl group in **6** by treatment[14] with trifluoroacetic acid in dichloromethane for 1 h at room temperature gave the 1-hydroxy compound **7**, quantitatively. Treatment[15] of **7** with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 2.5 h at 0° gave the α-trichloroacetimidate **8** in 82% yield after column chromatography. Characteristic signals in the ^{1}H-n.m.r. spectrum were at δ 6.64 (d, $J_{1,2}$ 3.8 Hz, H-1a) and 8.55 (C = N*H*), indicating the imidate to be α. Glycosylation[8a,15a] of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[8a,16] (**9**) with **8** in dichloromethane in the presence of boron trifluoride etherate for 3 h at 0° then afforded the desired β-glycoside **10** in 79% yield.



9

10 R^1=N_3, R^2=Bz, R^3=Ac, R^4=Me
11 R^1=NH_2, R^2=Bz, R^3=Ac, R^4=Me
12 R^1=NHCO$(CH_2)_{16}$Me, R^2=Bz, R^3=Ac, R^4=Me
13 R^1=NHCO$(CH_2)_{16}$Me, R^2=R^3=R^4=H

Selective reduction[7a,17] of the azido group in **10** with hydrogen sulfide in aqueous 83% pyridine for 48 h at 0–10° afforded the amine **11**, and this on condensation with octadecanoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane gave the acylated ganglioside **12** in 88% yield. *O*-Deacylation of **12** with sodium methoxide in methanol, and subsequent saponification of the methyl ester groups, yielded the desired ganglioside **13** in 87% yield after column chromatography. The ^{1}H-n.m.r. data for **13** in 49:1 $(CD_3)_2SO$–D_2O included δ 1.90 (2 s, 6 H, 2AcN), 4.32 (d, $J_{1,2}$ 7.7 Hz, H-1a), and 4.37 (d, $J_{1,2}$ 7.9 Hz, H-1b). The work described above shows that the use of the methyl 2-thioglycoside in the presence of NIS–TfOH may be effective for the synthesis of complex types of polysialoglycoconjugates.

EXPERIMENTAL

General methods. — Optical rotations were determined with a Union PM-201 polarimeter at 25° and i.r. spectra were recorded with a Jasco IRA-100 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a JEOL JNM-GX 270 spectrometer. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Evaporative concentrations were conducted *in vacuo*.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)-methyl 5-acetamido-2,4,7,8-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-D-galacto-*2-nonulopyranosonate* (**2**). — To a solution of 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→9)-(methyl 5-acetamido-4,7,8-tri-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate[6b] (**1**, 1.21 g, 1.18 mmol) in dichloroethane (10 mL) was added trifluoroacetic acid (20 mL), and the mixture was stirred for 1 h at room temperature then concentrated. The residue was acetylated with acetic anhydride (5 mL)–pyridine (10 mL) overnight at room temperature. The product was purified by chromatography on a column of silica gel (60 g) with 30:1 CH_2Cl_2–MeOH to give **2** (1.14 g, quantitative) as an amorphous mass, $[\alpha]_D$ −24.6° (*c* 0.64, $CHCl_3$); *v* 3500 (NH), 1750 and 1230 (ester), and 1670 and 1550 cm^{-1} (amide); ^{1}H-n.m.r. ($CDCl_3$): δ 1.87, 1.92 (2 s, 6 H, 2 AcN), 2.02–2.15 (24 H, 8 AcO), 2.57 (m, 2 H, H-3a*eq*, 3b*eq*); the anomeric ratio (α:β) was estimated as ~ 1:3 from the ratio of intensity of the methyl ester signals.

Anal. Calc. for $C_{40}H_{76}N_2O_{25}$ (964.9): C, 49.79; H, 7.94; N, 2.90. Found: C, 49.71; H, 7.86; N, 2.79.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)-(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy-2-thio*-D-glycero-D-galacto-*2-nonulopyranosid)onate* (**3**). — To a cooled, stirred solution of **2** (710 mg, 0.74 mmol) in dichloroethane (10 mL) were added trimethyl(methylthio)silane (0.42 mL, 2.97 mmol), TMS triflate (0.21 mL, 1.09 mmol), and molecular sieves 4A (MS-4A, 300 mg), and the mixture was then heated, with stirring, for 4 h at 50°, the course of the reaction being monitored by t.l.c. Dichloromethane (20 mL) was added and the solution was washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel (50 g) with 30:1 CH_2Cl_2–MeOH, to give **3** (650 mg, 93%) as an amorphous mass, $[\alpha]_D$ −32.5° (*c* 0.74, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.88–2.17 (m, 30 H, 2 AcN, 7 AcO, MeS), 2.56 (m, 2 H, H-3a*eq*, H-3b*eq*), 3.79, 3.81, 3.82, and 3.84 (4 s, 6 H, 2 MeO); anomeric ratio (α:β) was estimated as ~1:1.

Anal. Calc. for $C_{39}H_{76}N_2O_{23}S$ (952.9): C, 49.15; H, 8.04; N, 2.94. Found: C, 49.11; H, 8.20; N, 2.98.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-2-*nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-

O-(6-O-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-O-benzoyl-β-D-glucopyranoside (**5**). — To a solution of **3** (505 mg, 0.53 mmol) and 2-(trimethylsilyl)ethyl *O*-(6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside[6a,7b] (**4**, 505 mg, 0.265 mmol) in CH_3CN (3 mL) was added MS-3A (600 mg), and the mixture was stirred overnight at room temperature, then cooled to −40°. *N*-Iodosuccinimide (180 mg, 0.8 mmol) and trifluoromethanesulfonic acid (7.0 μL, 0.08 mmol) were added to the stirred mixture, and stirring was continued for 4.5 h at −40°. The solids were filtered off and washed thoroughly with CH_2Cl_2. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3, 2M $Na_2S_2O_3$, and water, dried (Na_2SO_4), and concentrated to a syrup. This was chromatographed on a column of silica gel (70 g) with 15:1 toluene–MeOH to give compound **5** (105 mg, 24%) as an amorphous mass, $[\alpha]_D$ −1.1° (*c* 0.74, $CHCl_3$); *v* 3700–3200 (OH, NH), 1750 and 1230 (ester), 1670 and 1550 (amide), 860 and 840 (Me_3Si), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.87 (m, 2 H, Me_3SiCH_2), 1.85, 1.92 (2 s, 6 H, 2 AcN), 2.60 (dd, 1 H, $J_{3ax,3eq}$ 13.0, $J_{3eq,4}$ 4.7 Hz, H-3c or 3d*eq*), 2.65 (dd, 1 H, $J_{3ax,3eq}$ 11.9, $J_{3eq,4}$ 4.9 Hz, H-3c or 3d*eq*), 3.62, 3.81 (2 s, 6 H, 2 MeO), 4.74 (d, 2 H, H-1a,1b), 4.82 (m, 1 H, H-4b), 4.97 (ddd, 1 H, $J_{3cax,4}$ 11.5, $J_{4,5}$ 10.3 Hz, H-4c), and 7.31–8.09 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{76}H_{98}N_2O_{37}Si$ (1659.7): C, 55.00; H, 5.95; N, 1.69. Found: C, 54.72; H, 6.13; N, 1.63.

A sample of **5** (100 mg, 0.06 mmol) was acetylated with acetic anhydride (1.5 mL)–pyridine (3 mL) overnight at room temperature. The product was purified by chromatography on a column of silica gel (30 g) with 30:1 CH_2Cl_2–MeOH to give **6** (98 mg, 91%) as an amorphous mass, $[\alpha]_D$ −7.9° (*c* 0.76, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 0.86 (m, 2 H, Me_3SiCH_2), 1.79, 1.86 (2 s, 6 H, 2 AcN), 1.87, 1.99, 2.00, 2.03, 2.05, 2.08, 2.10, 2.12, 2.17, 2.26 (10 s, 30 H, 10 AcO), 2.56 (m, 2 H, H-3c*eq*, 3b*eq*), 3.43 (dd, 1 H, H-9c), 3.53 (m, 1 H, $Me_3SiCH_2CH_2$), 3.64 (dd, 1 H, $J_{5,6}$ 12.0, $J_{6,7}$ 3.0 Hz, H-6c), 4.03 (dd, 1 H, $J_{8,9}$ 5.0, $J_{9,9'}$ 12.0 Hz, H-9d), 4.18 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4a), 4.30 (dd, 1 H, H-9'd), 4.55 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 3.3 Hz, H-3b), 4.64 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1a), 4.81 (m, 1 H, H-4d), 4.90 (m, 1 H, H-4c), 4.93 (d, 1 H, H-4b), 4.95 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1b), 5.03 (dd, 1 H, H-6d), 5.04 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2b), 5.15 (d, 1 H, N*H*), 5.20 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2a), 5.29 (m, 1 H, H-8d), 5.37 (dd, 1 H, H-7d), 5.40 (dd, 1 H, $J_{6,7}$ 3.0, $J_{7,8}$ 10.0 Hz, H-7c), 5.46 (t, 1 H, $J_{3,4}$ 9.5 Hz, H-3a), 5.68 (m, 1 H, H-8d), and 7.27–8.12 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{82}H_{104}N_2O_{40}Si$ (1785.8): C, 55.15; H, 5.87; N, 1.57. Found: C, 55.03; H, 5.90; N, 1.61.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→9)-O-(methyl 5-acetamido-4,7,8-tri-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4-di-O-acetyl-6-O-benzoyl-β-D-galactopyranosyl)-(1→4)-3-O-acetyl-2,6-di-O-benzoyl-D-glucopyranose (**7**). — To a solution of **6** (145 mg, 0.08 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (2 mL), and the mixture was stirred for 1 h at room temperature then concentrated. The residue was purified by chromatography on a column of silica gel (40 g) with 30:1 CH_2Cl_2–MeOH to give **7** (136 mg, quantitative) as an amorphous mass, $[\alpha]_D$ +5.1° (*c* 0.31, $CHCl_3$); *v* 3700–3200 (OH, NH), 1750 and 1230 (ester), 1670 and 1550 (amide), and 710 cm^{-1} (Ph).

Anal. Calc. for $C_{77}H_{92}N_2O_{40}$ (1685.6): C, 54.86; H, 5.50; N, 1.66. Found: C, 54.83; H, 5.59; N, 1.64.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)*-3-O-*acetyl-2,6-di*-O-*benzoyl-α*-D-*glucopyranosyl trichloroacetimidate* (**8**). — To a solution of **7** (140 mg, 0.08 mmol) in CH_2Cl_2 (1.5 mL), cooled to 0°, were added Cl_3CCN (0.083 mL) and 1.8-diazabicyclo[5.4.0]undec-7-ene (DBU, 12.4 μL, 0.08 mmol). The mixture was stirred for 2.5 h at 0° and then concentrated. The residue was chromatographed on a column of silica gel (20 g) with 30:1 CH_2Cl_2–MeOH to give **8** (120 mg, 82%) as an amorphous mass, $[\alpha]_D$ +16.2° (*c* 1.1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.74, 1.87 (2 s, 6 H, 2 AcN), 1.87, 2.00, 2.02 (2), 2.04, 2.09, 2.11, 2.14 (2), 2.26 (8 s, 30 H, 10 AcO), 2.57 (m, 2 H, H-3c*eq*, 3d*eq*), 3.72, 3.82 (2 s, 6 H, 2 MeO), 4.56 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 3.3 Hz, H-3b), 4.80 (m, 1 H, H-4c), 4.91 (m, 1 H, H-4d), 5.01 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 5.80 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3a), 6.64 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1a), 7.32–8.12 (m, 15 H, 3 Ph), and 8.55 (s, 1 H, C=N*H*).

Anal. Calc. for $C_{79}H_{92}Cl_3N_3O_{40}$ (1830.0): C, 51.85; H, 5.07; N, 2.30. Found: C, 51.71; H, 5.04; N, 2.23.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-O-(2,4-di*-O-*acetyl-6*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-azido-3*-O-*benzoyl-4-octadecene-1,3-diol* (**10**). — To a solution of **8** (120 mg, 0.066 mmol) and (2*S*, 3*R*, 4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[8a,16] (**9**, 56 mg, 0.13 mmol) in dry CH_2Cl_2 (3 mL) was added MS-4A (AW-300, 1.0 g), the mixture was stirred for 45 min at room temperature, and then cooled to 0°. Boron trifluoride etherate (16 μL, 0.13 mmol) was added to the mixture, and this was stirred for 3 h at 0°, the progress of the reaction being monitored by t.l.c. The solids were filtered off and washed thoroughly with CH_2Cl_2. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated to a syrup that was chromatographed on a column of silica gel (30 g) with 30:1 CH_2Cl_2–MeOH to give **10** (108 mg, 79%) as an amorphous mass, $[\alpha]_D$ −12.5° (*c* 0.16, $CHCl_3$); *v* 3500 (NH), 2950 and 2850 (Me, CH_2), 2100 (N_3), 1750 and 1230 (ester), 1680 and 1550 (amide), and 710 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 3 H, *Me*CH_2), 1.18, 1.25 (2 s, 22 H, 11 CH_2), 1.79, 1.87 (2 s, 6 H, 2 AcN), 1.89, 2.00, 2.01, 2.02, 2.04, 2.08, 2.10, 2.12, 2.17, 2.26 (10 s, 30 H, 10 AcO), 2.56 (m, 2 H, H-3c*eq*, 3d*eq*), 3.73, 3.78 (2 s, 6 H, 2 MeO), 4.56 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.0 Hz, H-3b), 4.66 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1a), 4.79, 4.90 (2 m, 2 H, H-4c, 4d), 4.94 (br. d, 1 H, H-4b), 4.96 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 5.04 (dd, 1 H, H-2b), 5.62 (dt, 1 H, $J_{4,5}$ 15.0 Hz, $J_{5,6}$ = $J_{5,6'}$ = 6.5 Hz, H-5 of sphingosine), and 7.30–8.12 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{102}H_{129}N_5O_{42}$ (2097.2): C, 58.41; H, 6.20; N, 3.34. Found: C, 58.33; H, 6.34; N, 3.39.

O-*(Methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galac-

to-*2-nonulopyranosylonate)-(2→9)*-O-*(methyl 5-acetamido-4,7,8-tri*-O-*acetyl-3,5-di-deoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)*-O-*(2,4-di*-O-*acetyl-6-*O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*gluco-pyranosyl)-(1→1)-(2*S,*3*R,*4*E)-*3*-O-*benzoyl-2-octadecanamido-4-octadecene-1,3-diol* (**12**). — Hydrogen sulfide was bubbled through a solution of **10** (105 mg, 0.05 mmol) in pyridine (6 mL) and water (1.2 mL) for 2 days while the solution was stirred at 0–10°, the course of the reaction being monitored by t.l.c. The mixture was concentrated to give the syrupy amine **11**, which was used without purification. In the next step a solution of **11** in dry CH_2Cl_2 (6 mL) was treated with octadecanoic acid (43 mg, 0.15 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 29 mg, 0.15 mmol), and the mixture was stirred for 3 h at room temperature. After completion of the reaction, CH_2Cl_2 (20 mL) was added to the mixture, and the solution was washed with water, dried (Na_2SO_4), and concentrated to a syrup that was chromatographed on a column of silica gel (30 g) with 30:1 CH_2Cl_2–MeOH to give **12** (105 mg, 88%) as an amorphous mass, $[\alpha]_D$ +5.0° (*c* 0.2, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 6 H, 2 *Me*CH_2), 1.21, 1.26 (2 s, 50 H, 25 CH_2), 1.78, 1.86 (2 s, 6 H, 2 AcN), 1.88, 2.00, 2.01 (2), 2.04, 2.08, 2.09, 2.11, 2.13, 2.25 (9 s, 30 H, 10 AcO), 2.56 (m, 2 H, H-3*ceq*, 3d*eq*), 3.72, 3.79 (2 s, 6 H, 2 MeO), 4.55 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 2.9 Hz, H-3b), 4.60 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1a), 4.90 (br. d, 1 H, H-4b), 5.71 (m, 1 H, H-5 of ceramide), and 7.28–8.11 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{120}H_{165}N_3O_{43}$ (2337.6): C, 61.65; H, 7.11; N, 1.80. Found: C, 61.41; H, 7.34; N, 1.83.

O-*(5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic-acid)(2→9)*-O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl-onic acid)-(2→3)*-O-*β*-D-*galactopyranosyl-(1→4)*-O-*β*-D-*glucopyranosyl)-(1→1)-(2*S,*3*R,*4*E)-*2-octadecanamido-4-octadecene-1,3-diol* (**13**). — To a solution of **12** (100 mg, 0.043 mmol) in MeOH (4 mL) was added NaOMe (20 mg), and the mixture was stirred for 7 h at 45°. Potassium hydroxide (0.2 M, 2 mL) was added to the mixture, and this was stirred overnight at room temperature, then treated with Amberlite IR-120(H^+) resin to remove the base. The resin was filtered off and washed thoroughly with 1:1 $CHCl_3$–MeOH. The filtrate and washings were combined, and concentrated to a syrup that was chromatographed on a column of Sephadex LH-20 (40 g) with 1:1 $CHCl_3$–MeOH to give **13** (55 mg, 87%) as an amorphous mass, $[\alpha]_D$ +8.5° (*c* 0.1, 1:1 $CHCl_3$–MeOH); ^{1}H-n.m.r. [49:1 $(CD_3)_2SO$–D_2O]: δ 0.83 (t, 6 H, 2 *Me*CH_2), 1.22 (s, 50 H, 25 CH_2), 1.90 (2 s, 6 H, 2 AcN), 2.03 (t, 2 H, *J* 6.4 Hz, COCH_2CH$_2$), 4.32 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1a), 4.37 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1b), 5.34 (dd, 1 H, $J_{3,4}$ 6.8, $J_{4,5}$ 15.0 Hz, H-4 of ceramide), and 5.55 (dt, 1 H, $J_{5,6}$ = $J_{5,6'}$ = 6.5 Hz, H-5 of ceramide).

Anal. Calc. for $C_{70}H_{125}N_3O_{29}$ (1472.8): C, 57.08; H, 8.56; N, 2.85. Found: C, 56.83; H, 8.74; N, 2.81.

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