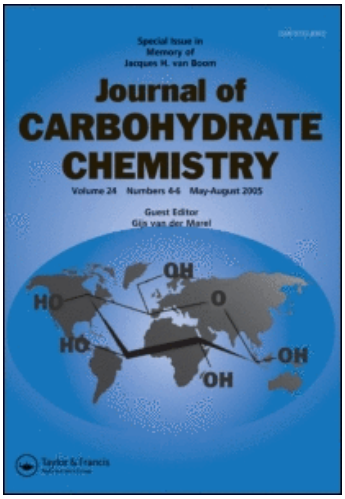

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 75: A TOTAL SYNTHESIS OF β-SERIES GANGLIOSIDE GQ1β

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ABSTRACT

A first total synthesis of a β-series ganglioside GQ1β ($IV^3Neu5Ac\alpha_2$, $III^6Neu5Ac\alpha_2$-Gg4Cer) is described. Regio- and stereoselective dimeric sialylation of the hydroxyl group at C-6 of the GalNAc residue in 2-(trimethylsilyl)ethyl *O*-(2-acetamido-2-deoxy-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3**) with methyl [phenyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate (**4**) using *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (TfOH) as a promoter gave the desired pentasaccharide **5** containing α-glycosidically-linked dimeric sialic acids. This was transformed into the acceptor **6** by removal of the levulinyl group. Condensation of methyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonate]-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**7**) with **6**, using dimethyl(methylthio)sulfonium triflate (DMTST) as a promoter, gave the desired octasaccharide derivative **8** in high yield. Compound **8** was converted into α-trichloroacetimidate **11**, *via* reductive removal of the benzyl groups, *O*-acetylation, removal of the 2-(trimethylsilyl)ethyl group, and treatment with trichloroacetonitrile, which, on coupling with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**12**), gave the β-glycoside **13**. Finally, **13** was transformed, *via* selective reduction of the azido group, coupling with octadecanoic acid, *O*-deacylation, and hydrolysis of the methyl ester group, into the title ganglioside **15** in good yield.

INTRODUCTION

The biosynthetic pathway for ganglio-series glycosphingolipids has been elucidated[1] and glycosphingolipids representing the products (i.e., GT1a, GT1b, GQ1b) of this pathway have been detected in the central nervous system. These ganglio-series gangliosides have been isolated and structurally characterized, and found to be important compounds for biological processes. Recently, α- and β-series gangliosides, which contain α-glycosidically-linked sialic acid at HO-6 of the *N*-acetylgalactosamine residue in the oligosaccharide chain, have been isolated[2-4] as the components in tissues of the central nervous system of mouse and adult bovine brains. In particular, β-series ganglioside GQ1β, which has Neu5Acα(2→8)Neu5Ac residues at HO-6 of the *N*-acetylgalactosamine residue and HO-3 of the galactose residue in the gangliotetraose, has been found in the frog brain.[5] In the previous papers,[6,7] we have reported the first synthesis of α-series gangliosides, GM1α, GD1α, and GQ1bα. As a part of our continuing efforts[8] on the synthesis and elucidation of the functions of sialoglycoconjugates, we describe here the first total synthesis of β-series ganglioside GQ1β which is one of the most complex structures among gangliosides.

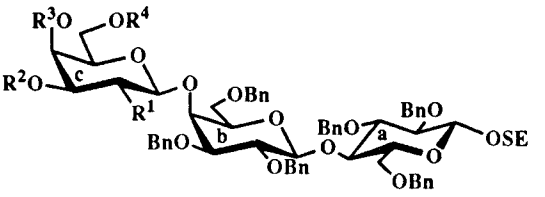
RESULTS AND DISCUSSION

For the synthesis of a β-series ganglioside GQ1β, we have selected a well designed trisaccharide derivative **3** as a key glycosyl acceptor, suitable for the preparation of the pentasaccharide derivative **5** and its transformation to the acceptor **6** for construction of the core structure of β-series gangliosides. This approach was taken cosidering the application for the synthesis of other α- and β-series gangliosides containing α-glycosidically linked sialic acid at OH-6 of galactosamine residues.

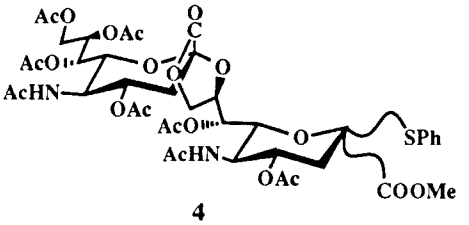
The appropriately protected trisaccharide acceptor **3** was obtained in good yield from 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[6a] (**1**) by 3-*O*-levulinylation and removal of the benzylidene group. The glycosylation of the trisaccharide acceptor **3** with methyl

[phenyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate[9] (**4**) by use of *N*-iodosuccinimide (NIS) - trifluoromethanesulfonic acid (TfOH) in the presence of powdered molecular sieves 3Å (MS-3Å) in acetonitrile for 10 h at -30 °C gave the expected pentasaccharide α-glycoside **5** in 48 % yield. The observed chemical shifts and coupling constants of the sialyl α(2→8) sialic acid residue were a one-proton doublet of doublets at δ 2.41 (J_{gem} = 13.7 Hz, $J_{3eq,4}$ = 5.4 Hz, H-3e*eq*), a one-proton doublet of doublets at δ 2.64 (J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.9 Hz, H-3d*eq*), a three-proton singlet at δ 3.82 (MeO), a one-proton multiplet at δ 5.05 (H-4d), a one-proton multiplet at δ 5.37 (H-4e), a one-proton doublet at δ 5.41 ($J_{7,8}$ = 8.6 Hz, H-7d), and a one-proton multiplet at δ 5.44 (H-8e), indicating the newly formed glycosidic linkage to be α.[9] The regiochemistry was deduced from the ^{1}H NMR spectrum of the acetylated compound of **5**; the observed chemical shift of the *N*-acetylgalactosamine residue for H-4 (δ 5.17), indicating the glycosylated position in **5** to be HO-6 of the GalNAc residue.

By removal[10] of the levulinyl group, the pentasaccharide acceptor **6** was formed from **5** in 57 % yield. Dimethyl(methylthio)sulfonium triflate[11] (DMTST)-promoted glycosylation of **6** with methyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside[12] (**7**) in dichloromethane for 2 days at 0 °C gave the desired octasaccharide **8** in 42 % yield. The regiochemistry of compound **8** was deduced from the ^{1}H NMR spectrum of the acetylated compound **9**. The observed chemical shift of GalNAc unit for H-4 (δ 5.77) indicated the position of glycosylation in **8** to be HO-3. Catalytic hydrogenolysis (10% Pd-C) of the benzyl groups of **8** in ethanol-acetic acid for 2 days at 40 °C and subsequent *O*-acetylation gave the per-*O*-acyl derivative **9** in 67 % yield. Treatment[13] of **9** with trifluoroacetic acid in dichloromethane for 1 h at 0 °C gave the 1-hydroxy compound **10**. When treated with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 1 h at 0 °C, **10** gave the α-trichloroacetimidate **11** in 92 % yield. The ^{1}H NMR data for the Glc unit in **11** [δ 6.49 ($J_{1,2}$ = 3.7 Hz, H-1a), 8.69 (C=NH)] indicated the imidate to be α.



	R^1	R^2	R^3	R^4
1	NHAc	H	benzylidene	
2	NHAc	Lev	benzylidene	
3	NHAc	Lev	H	H



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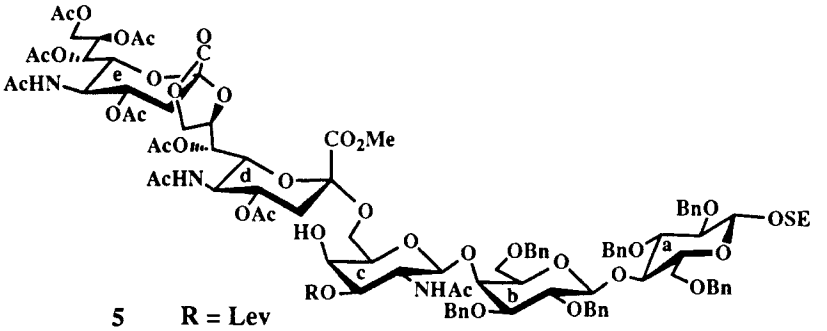
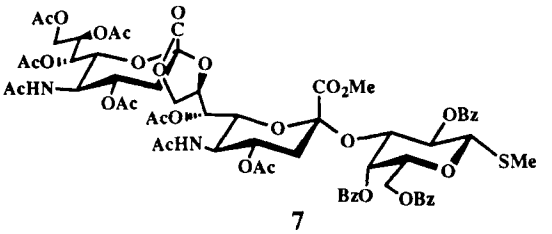


5 R = Lev
6 R = H

SE = 2-(trimethylsilyl)ethyl
Bn = benzyl
Bz = benzoyl
Lev = levulinyl



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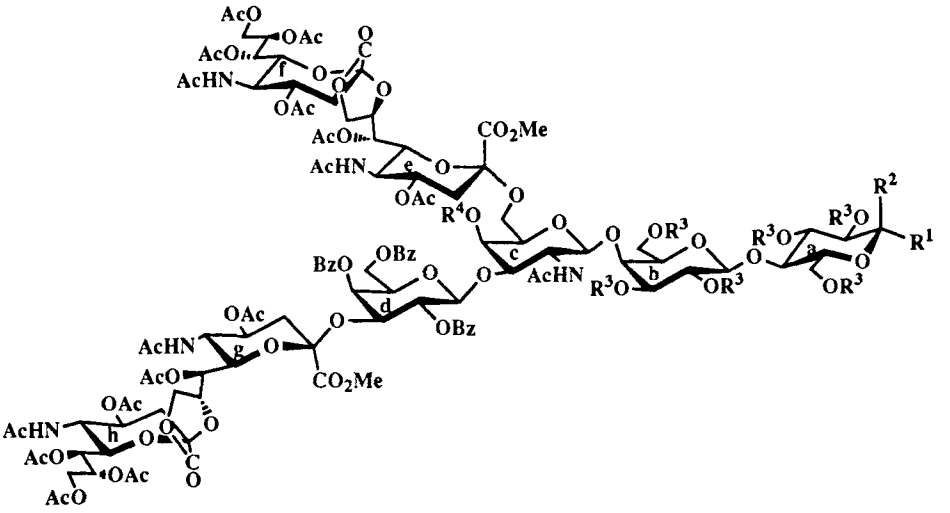
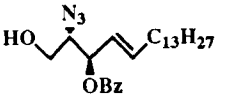
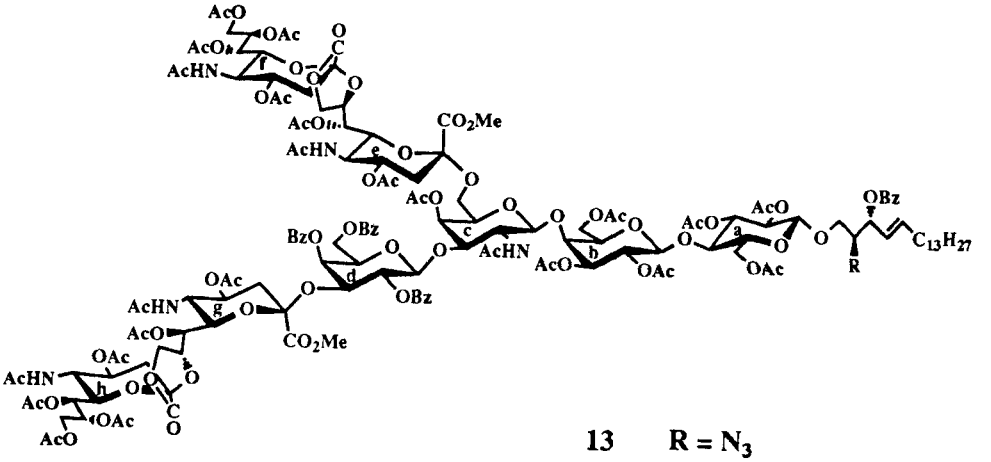


	R^1	R^2	R^3	R^4
8	OSE	H	Bn	H
9	OSE	H	Ac	Ac
10	OH , H		Ac	Ac
11	H	$OC(=NH)CCl_3$	Ac	Ac

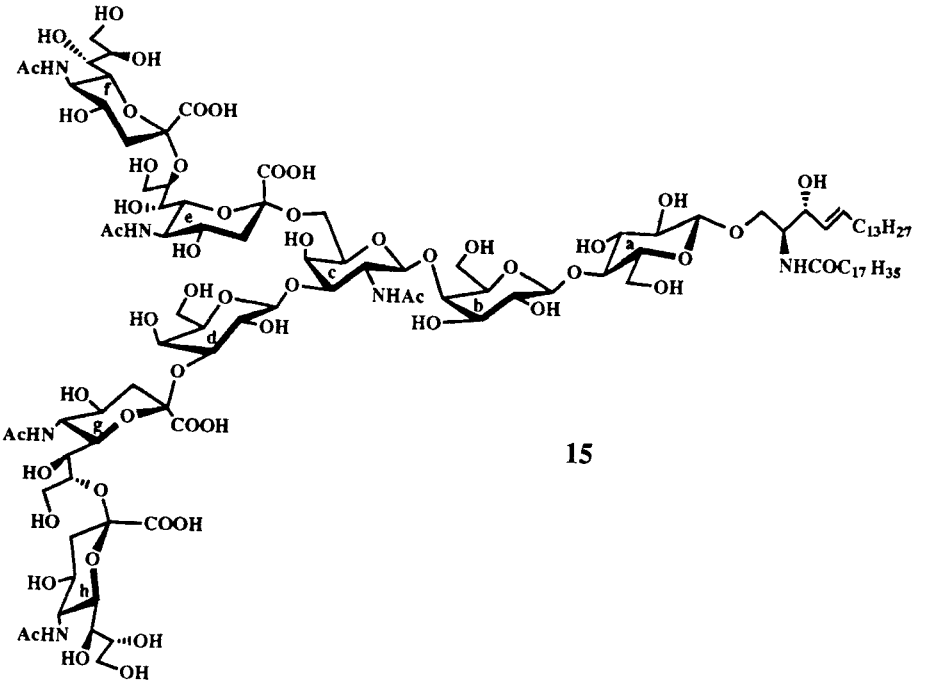


12



13 R = N_3

14 R = $NHCOC_{17}H_{35}$



15

The final glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol [14](**12**) with **11** in dichloromethane in the presence of boron trifluoride etherate[15] for 3 h at 0 °C afforded the desired β-glycoside **13** in 59 % yield. Selective reduction[14a,16] of azido group in **13** with hydrogen sulfide in aqueous 83% pyridine for 3 days at 0 °C gave the amine which on condensation with octadecanoic acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) in dichloromethane, gave the acylated GQ1β ganglioside **14** in 36% yield after chromatography.

Finally, *O*-deacylation of **14** with sodium methoxide in methanol, and subsequent saponification of the methyl ester group, yielded the desired β-series ganglioside GQ1β **15** in 86 % yield after chromatography on a column of Sephadex LH-20. The ^{1}H NMR data of the product thus obtained are consistent with the structure assigned.

EXPERIMENTAL

General methods. Optical rotations were determined with a Union PM-201 polarimeter at 25 °C and IR spectra were recorded with a Jasco IRA-100 spectrophotometer. ^{1}H NMR spectra were recorded at 270 MHz with a Jeol JNM-GX 270 and at 500 MHz with Varian VXR-500S spectrometers. Preparative chromatography was performed on silica gel (Fuji Silysia Co., 300 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl *O*-(2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2). To a solution of 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[6a] (**1**; 540 mg, 0.42 mmol) in pyridine (10 mL) was added levulinic anhydride (181 mg, 0.84 mmol). The mixture was stirred for 2 h at room temperature, and MeOH (2 mL) was then added. The solution was concentrated to a syrup which was extracted with CH_2Cl_2. The extract was successively washed with 2 M HCl acid and H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 EtOAc-hexane) of the residue on silica gel (20 g) gave **2** (476 mg, 82 %) as an amorphous mass: $[\alpha]_D$ +34.3° (*c* 2.1, $CHCl_3$); ^{1}H NMR

($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$), 1.80 (s, 3H, AcN), 2.18 (s, 3H, $CH_3COCH_2CH_2$), 5.59 (s, 1H, PhCH), and 7.22 - 7.58 (m, 35H, 7Ph).

Anal. Calcd for $C_{79}H_{93}NO_{18}Si$ (1372.7): C, 69.12; H, 6.83; N, 1.02. Found: C, 68.84; H, 6.64; N, 1.00.

2-(Trimethylsilyl)ethyl *O*-(2-Acetamido-2-deoxy-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3). To a solution of **2** (417 mg, 0.30 mmol) in MeOH (10 mL) was added *p*-toluenesulfonic acid monohydrate (20 mg). The mixture was stirred for 1 h at room temperature, then neutralized with Amberlite IRA-410 (OH^-) resin and concentrated. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel (20 g) gave **3** (390 mg, quantitative) as an amorphous mass: $[\alpha]_D$ +15.4° (*c* 3.1 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.03 (m, 2H, $Me_3SiCH_2CH_2$), 1.60 (s, 3H, AcN), 2.14 (s, 3H, $CH_3COCH_2CH_2$), 5.57 (s, 1H, $J_{1,2}$ = 7.7 Hz, H-1c), and 7.22-7.42 (m, 30H, 6Ph).

Anal. Calcd for $C_{72}H_{89}NO_{18}Si$ (1284.6): C, 67.32; H, 6.98; N, 1.09. Found: C, 67.17; H, 6.78; N, 0.95.

2-(Trimethylsilyl)ethyl *O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)-*O*-(2-acetamido-2-deoxy-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5). To a solution of **3** (571 mg, 0.30 mmol) and methyl [phenyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate[9] (**4**; 390 mg, 0.60 mmol) in MeCN (8 mL) were added 3Å molecular sieves (1.5 g). The mixture was stirred for 5 h at room temperature, then cooled to -30 °C. To the stirred mixture were added NIS (273 mg, 1.2 mmol) and TfOH (13 μL, 0.12 mmol), and stirring was continued for 10 h at -30 °C. The solids were removed by filtration and washed with CH_2Cl_2. The combined filtrate and washings were successively washed with M Na_2CO_3 and M $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated. Column chromatography (10:1 toluene-MeOH) of the

residue on silica gel (20 g) gave **5** (310 mg, 48 %) as an amorphous mass: $[\alpha]_D$ -1.2° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.87-2.24 (10s, 30H, 3AcN, 6AcO, and $CH_3COCH_2CH_2$), 2.41 (dd, 1H, J_{gem} = 13.7 Hz, $J_{3eq,4}$ = 5.4 Hz, H-3e*eq*), 2.64 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.9 Hz, H-3d*eq*), 3.82 (s, 3H, MeO), 5.05 (m, 1H, H-4d), 5.37 (m, 1H, H-4e), 5.41 (d, 1H, $J_{7,8}$ = 8.6 Hz, H-7d), 5.44 (m, 1H, H-8e), and 7.25-7.40 (m, 30H, 6Ph).

Anal. Calcd for $C_{97}H_{135}N_3O_{39}Si$ (1995.2): C, 58.39; H, 6.82; N, 2.11. Found: C, 58.25; H, 6.54; N, 2.03.

2-(Trimethylsilyl)ethyl *O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6). To a solution of **5** (310 mg, 0.15 mmol) in EtOH (3 mL) was added hydrazine acetate[10] (270 mg). The mixture was stirred for 0.5 h at room temperature and then concentrated. Column chromatography (30:1 CH_2Cl_2-MeOH) of the residue on silica gel (20 g) gave **6** (170 mg, 57 %) as an amorphous mass: $[\alpha]_D$ +8.9° (*c* 2.0 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.01 (m, 2H, $Me_3SiCH_2CH_2$), 1.87 - 2.25 (9s, 27H, 6AcO and 3AcN), 2.47 (m, 1H, H-3d*eq*), 2.63 (dd, 1H, J_{gem} = 12.7 Hz, $J_{3eq,4}$ = 5.1 Hz, H-3e*eq*), 3.79 (s, 3H, MeO), and 7.26 - 7.38 (m, 30H, 6Ph).

Anal. Calcd for $C_{92}H_{129}N_3O_{37}Si$ (1897.1): C, 58.25; H, 6.85; N, 2.21. Found: C, 58.08; H, 6.81; N, 2.14.

2-(Trimethylsilyl)ethyl *O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-{*O*-[methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)}-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-

(1→4)-*O*-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8). To a solution of **6** (50 mg, 0.024 mmol) and methyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside[12] (**7**, 65 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) were added 4Å molecular sieves (100 mg). The mixture was stirred for 5 h at room temperature then cooled to 0 °C. To the mixture was added, with stirring, dimethyl(methylthio)sulfonium triflate[11] (DMTST; 25 mg, 0.1 mmol), and stirring was continued for 1 day at 0 °C. The precipitates were removed by filtration, 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 H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel (50 g) gave **8** (33 mg, 42%) as an amorphous mass: $[\alpha]_D$ +8.4° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$), 1.81-2.18 (17s, 51H, 5AcN and 12AcO), 2.39 - 2.64 (m, 4H, H-3e*eq*, H-3f*eq*, H-3g*eq*, and H-3h*eq*), 3.50 and 3.82 (2s, 6H, 2MeO), and 7.18 - 8.20 (m, 45H, 9 Ph).

Anal. Calcd for $C_{164}H_{197}N_5O_{66}Si$ (3322.4): C, 59.29; H, 5.98; N, 2.11. Found: C, 58.99; H, 5.72; N, 1.84.

2-(Trimethylsilyl)ethyl *O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-{*O*-[methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)}-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (9). A solution of **8** (290 mg, 87 μmol) in EtOH (20 mL) and AcOH (4 mL) was hydrogenated in the presence of 10% Pd-C (500 mg) for 2 days at 40 °C, the catalyst removed by filtration and the solution concentrated. The residue was acetylated with Ac_2O (5 mL) and pyridine (10 mL) for 16 h at room temperature. The product was

purified by chromatography (20:1 CH_2Cl_2-MeOH) on a column of silica gel (20 g) to give **9** (180 mg, 67 %) as an amorphous mass: $[\alpha]_D$ -17.1° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (m, 2H, $Me_3SiCH_2CH_2$), 1.89 - 2.19 (m, 72H, 19AcO and 5AcN), 2.32 - 2.51 (m, 4H, H-3e*eq*, H-3f*eq*, H-3g*eq*, and H-3h*eq*), 3.24 and 3.72 (2s, 6H, 2MeO), 5.77 (d, 1H, $J_{3,4}$ = 3.8 Hz, H-4c), and 7.36 - 8.19 (m, 15H, 3Ph).

Anal. Calcd for $C_{136}H_{175}N_5O_{73}Si$ (3075.94): C, 53.11; H, 5.73; N, 2.28. Found: C, 53.03; H, 5.60; N, 1.99.

***O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-{*O*-[methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)}-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-2,3,6-tri-*O*-acetyl-D-glucopyranose (10).** To a solution of **9** (180 mg, 59 μmol) in CH_2Cl_2 (3 mL) was added CF_3CO_2H[13] (0.5 mL) at 0 °C, and the mixture was stirred for 0.5 h at 0 °C and concentrated. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel (10 g) gave **10** (150 mg, 86 %) as an amorphous mass: IR (KBr) 3600-3300 (OH, NH), 1740 and 1230 (ester), 1670 and 1550 (amide), and 760 and 720 cm^{-1} (Ph).

Anal. Calcd for $C_{131}H_{163}N_5O_{73}$ (2975.71): C, 52.88; H, 5.52; N, 2.35. Found: C, 52.72; H, 5.35; N, 2.17.

***O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-{*O*-[methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)}-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-**

galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (11). To a solution of **10** (150 mg, 50 μmol) in CH_2Cl_2 (2 mL) and trichloroacetonitrile (0.4 mL) at -5 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 10 mg), and the mixture was stirred for 1 h at 0 °C, then concentrated. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel (10 g) gave **11** (145 mg, 92 %) as an amorphous mass: $[\alpha]_D$ -3.44° (*c* 2.9 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.87 - 2.19 (m, 72H, 19AcO and 5AcN), 2.36 - 2.46 (m, 4H, H-3e*eq*, H-3f*eq*, H-3g*eq*, and H-3h*eq*), 3.24 and 3.70 (2s, 6H, 2MeO), 6.49 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1a), 7.31-8.19 (m, 15H, 3Ph), 8.69 (s, 1H, C=N*H*).

Anal. Calcd for $C_{133}H_{163}N_6O_{73}Cl_3$ (3120.1): C, 51.20; H, 5.27; N, 2.69. Found: C, 51.03; H, 4.99; N, 2.56.

***O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-{*O*-[methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)}-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (13).** To a solution of **11** (145 mg, 46 μmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[14,15] (**12**, 55 mg, 110 μmol) in CH_2Cl_2 (2 mL) was added 4Å molecular sieves (AW-300, 0.5 g). The mixture was stirred for 5 h at room temperature, then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (30 μL) was added, and the mixture was stirred for 3 h at 0 °C and then filtered. The insoluble materials were washed with CH_2Cl_2, and the combined filtrate and washings were washed with M $NaHCO_3$ and H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel (10 g) gave **13** (93 mg, 59 %) as an amorphous mass: $[\alpha]_D$ -14.3° (*c* 1.9 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.2

Hz, *Me*CH_2), 1.24 (s, 22H, 11CH_2), 1.84-2.17 (m, 72H, 19AcO and 5AcN), 2.32-2.46 (m, 4H, H-3e*eq*, H-3f*eq*, H-3g*eq*, and H-3h*eq*), 3.25 and 3.69 (2s, 6H, 3MeO), 5.90 (m, 1H, H-5 of sphingosine), 7.28-8.28 (m, 20H, 4Ph).

Anal. Calcd for $C_{156}H_{200}N_8O_{75}$ (3387.3): C, 55.32; H, 5.95; N, 3.31. Found: C, 55.29; H, 5.77; N, 3.29.

***O*-[Methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-{*O*-[methyl 5-Acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)}-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (14).** Hydrogen sulfide was bubbled[14a,16] through a stirred solution of **13** (93 mg, 27 μmol) in aqueous 83% pyridine (13 mL) for 3 days at 0 °C. The mixture was concentrated, and the residue was stirred with octadecanoic acid (25 mg, 95 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (25 mg, 138 μmol) in CH_2Cl_2 (5 mL) for 1 day at room temperature. Dichloromethane (20 mL) was added, and the mixture was washed with H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel (10 g) gave **14** (85 mg, 85 %) as an amorphous mass: $[\alpha]_D$ -12.9° (*c* 1.7 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, J_{Me,CH_2} = 7.0 Hz, 2*Me*CH_2), 1.26 (s, 52H, 26CH_2), 1.88 - 2.18 (24s, 72H, 19AcO and 5AcN), 2.32 - 2.46 (m, 4H, H-3e*eq*, H-3f*eq*, H-3g*eq*, and H-3h*eq*), 3.26 and 3.77 (2s, 6H, 2MeO), 5.84 (m, 1H, H-5 of sphingosine), 7.34-8.21 (m, 20H, 4Ph).

Anal. Calcd for $C_{174}H_{236}N_6O_{76}$ (3627.8): C, 57.61; H, 6.56; N, 2.32. Found: C, 57.40; H, 6.51; N, 2.15.

Ganglioside GQ1β (15). To a solution of **14** (85 mg, 23 μmol) in MeOH (5 mL) was added a catalytic amount of NaOMe, and the mixture was stirred for 72 h at

room temperature. Water (0.5 mL) was added, and the mixture was stirred for 10 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1:1 $CHCl_3$-MeOH, and the combined filtrate and washings were concentrated. Column chromatography (1:1 $CHCl_3$-MeOH) of the residue on Sephadex LH-20 (10 g) gave **15** (49 mg, 86 %) as an amorphous solid: $[\alpha]_D$ +24.9° (*c* 1.0, 1:1 $CHCl_3$-MeOH); ^1H NMR (1:1 DMSO-d_6-D_2O) δ 0.86 (t, 6H, J_{Me,CH_2} = 7.0 Hz, 2*Me*CH_2), 1.23 (s, 52H, 26*CH*$_2$), 1.85 - 1.89 (5s, 15H, 5AcN), 2.42 - 2.73 (m, 4H, H-3e*eq*, H-3f*eq*, H-3g*eq*, and H-3h*eq*), 4.22 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1a), 4.32 (m, 2H, H-1b and H-1d), 4.70 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1c), 5.35 (m, 1H, H-4 of sphingosine), and 5.55 (m, 1H, H-5 of sphingosine).

Anal. Calcd for $C_{106}H_{182}N_6O_{55}$ (2420.6): C, 52.60; H, 7.58; N, 3.47. Found: C, 52.34; H,7.30; N, 3.23.

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