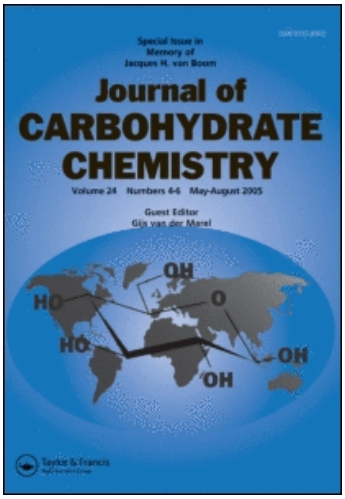

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 95: TOTAL SYNTHESIS OF SULFATED GLUCURONYL LACTOSAMINYL PARAGLOBOSIDES

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ABSTRACT

3-*O*-Sulfo glucuronyl neolactohexanosyl ceramide derivatives (heptasaccharides) have been synthesized. Condensation of 2-(trimethylsilyl)ethyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**2**) with 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**1**) gave the desired β-glycoside **3**, which was converted into 2-(trimethylsilyl)ethyl *O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**) *via* removal of the *O*-acetyl and *N*-phthaloyl groups, followed by *N*-acetylation. Glycosylation of **4** with *O*-(methyl 4-*O*-acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**5**) using trimethylsilyl trifluoromethanesulfonate gave the target tetrasaccharide **6**, which was transformed *via* removal of the benzyl group, *O*-benzoylation, removal of the 2-(trimethylsilyl)ethyl group and imidate formation into the tetrasaccharide donor **9**. Glycosylation of 2-(trimethylsilyl)ethyl *O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**10**) with the imidate donor **9** using trimethylsilyl trifluoromethanesulfonate gave the desired heptasaccharide **11**, which was transformed into the heptasaccharide imidate donor **14**. Glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**15**) with **14** gave β-glycoside **16**, which was transformed into the four target compounds, *via* reduction of the azido group, coupling with octadecanoic acid or tetracosanoic acid, selective removal of the levulinoyl group, *O*-sulfation, hydrolysis of the methyl ester group and *O*-deacylation.

INTRODUCTION

Patients with neuropathy associated with paraproteinemia carry monoclonal immunoglobulin M antibodies reacting[1] with myelin-associated glycoprotein (MAG) and sulfated glucuronyl glycolipids, indicating that the monoclonal antibodies may be responsible for these neuropathies. These glycolipids are characterized[2] as 3-*O*-sulfo glucuronyl paragloboside and 3-*O*-sulfo glucuronyl lactosaminyl paraglobosides. These glycolipids are also known to react with mouse IgM monoclonal antibody, HNK-1 (Leu-7) which is recognized[3] by a subset of human lymphocytes including natural killer cells. Recently, it has been reported[4] that the HNK-1 reactive glycolipids react with L- and P-selectin, but not with E-selectin. In view of these facts, it is of interest to synthesize these glycolipids to elucidate the structural requirements for recognition by selectin and HNK-1 antibody.

Previously, we have synthesized[5] the 3-*O*-sulfo glucuronyl paraglobosides and the corresponding glucuronyl paraglobosides. We describe here a facile total synthesis of the heptasaccharide 3-*O*-sulfo glucuronyl lactosaminyl paraglobosides (**21** and **26**) and the corresponding glucuronyl lactosaminyl paraglobosides (**20** and **25**), in which fatty acyl groups at the ceramide moiety consist of octadecanoyl and tetracosanoyl groups;

GlcAβ(1→3){Galβ(1→4)GlcNAcβ(1→3)}$_2$Galβ(1→4)Glcβ-(1→1)Ceramide (C-18, **20**)

GlcAβ(1→3){Galβ(1→4)GlcNAcβ(1→3)}$_2$Galβ(1→4)Glcβ-(1→1)Ceramide (C-24, **25**)

3-*O*-sulfo GlcAβ(1→3){Galβ(1→4)GlcNAcβ(1→3)}$_2$Galβ(1→4)Glcβ-(1→1)Ceramide (C-18, **21**)

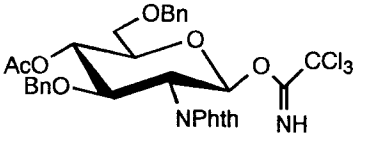
3-*O*-sulfo GlcAβ(1→3){Galβ(1→4)GlcNAcβ(1→3)}$_2$Galβ(1→4)Glcβ-(1→1)Ceramide (C-24, **26**)

RESULTS AND DISCUSSION

O-(Methyl 4-*O*-acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→ 3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**9**) was selected as the glycosyl donor for the basic

heptasaccharide unit (**11**) synthesis. The planned synthesis of **9** was *via* coupling of suitably protected disaccharide, **5** and **4**, removal of the benzyl group, *O*-benzoylation, removal of the 2-(trimethylsilyl)ethyl group and imidate formation. The trisaccharide **10** was chosen as a suitably protected glycosyl acceptor in the planned syntheses of **11**. Compound **9** has a levulinoyl group at C-3 of the GlcA residue and provides selectively of one free hydroxy group at the desired position for sulfation in the final step. Compound **10** has a free hydroxyl group at the C-4 position of the GlcN residue for coupling with **9** to give the heptasaccharide **11**. By further processing, according to our previous procedure,[5] it was anticipated that the intermediate **11** could be transformed into the end products by introduction of the ceramide moiety and/or sulfate.

Glycosylation of **1**[5] with **2**[6] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the glycosyl promoter and MS-4Å in dichloromethane afforded the desired β-glycosidic disaccharide **3** in 94% yield (based on **1**); significant signals of **3** in the ^{1}H NMR spectrum were two one-proton doublets at δ 4.33 (d, $J_{1,2}$ = 7.9 Hz, H-1 for Gal) and 5.48 (d, $J_{1,2}$ = 8.2 Hz, H-1 for GlcN), and twenty-nine aromatic protons at δ 7.17-7.51 (5Ph and Phthaloyl-H). *O*-Deacetylation of **3** with sodium methoxide followed by heating with hydrazine monohydrate in aq 95% ethanol, and subsequent *N*-acetylation gave **4** in 85% yield. Glycosylation of **4** with the disaccharide imidate **5**[5] in the presence of 0.15 equiv of TMSOTf and MS-4Å for 5 h at room temperature afforded the tetrasaccharide **6** in 92% yield (based on **5**). Catalytic hydrogenolysis (10% Pd-C) of the benzyl groups in **6** in ethyl acetate-methanol for 24 h at room temperature and subsequent *O*-benzoylation gave the per-*O*-benzoyl derivative **7** in quantitative yield. Selective removal[7] of the 2-(trimethylsilyl)ethyl group in **7** with trifluoroacetic acid in dichloromethane for 3 h at room temperature gave **8** (94%). When treated[8] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 3 h at 0 °C, compound **8** gave the tetrasaccharide donor **9** as the α-anomer in 92% yield. Glycosylation of **10**[5] with the tetrasaccharide imidate **9** in the presence of 0.2 equiv of TMSOTf and MS-4Å overnight at room temperature afforded the heptasaccharide **11** in 95% yield (based on



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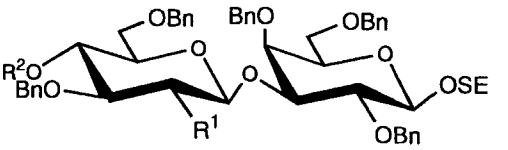
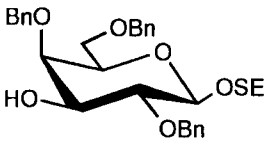


	R^1	R^2
3	NPhth	Ac
4	NHAc	H



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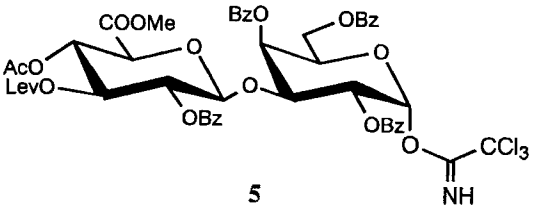

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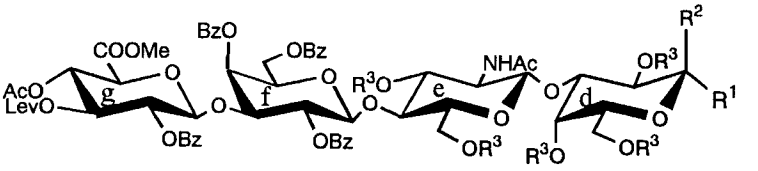
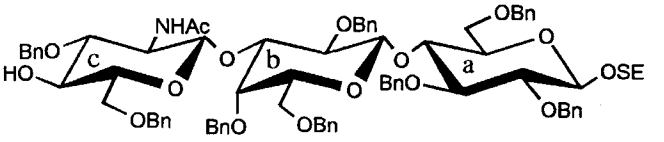
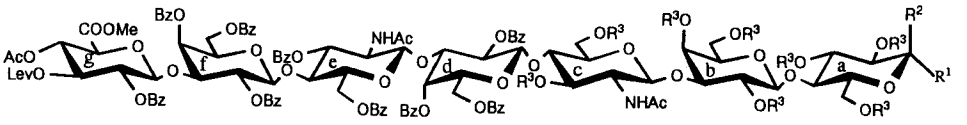


	R^1	R^2	R^3
6	OSE	H	Bn
7	OSE	H	Bz
8	OH ,H		Bz
9	H	$OC(=NH)CCl_3$	Bz

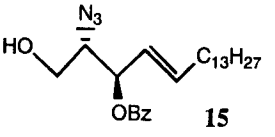


10

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



	R^1	R^2	R^3
11	OSE	H	Bn
12	OSE	H	Ac
13	OH ,H		Ac
14	H	$OC(=NH)CCl_3$	Ac



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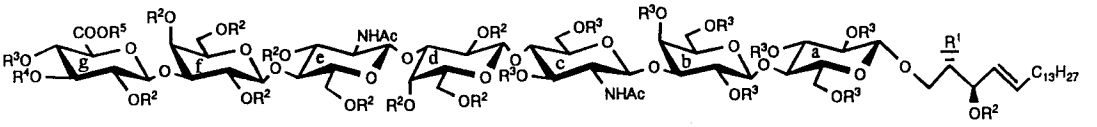

	R^1	R^2	R^3	R^4	R^5
16	N_3	Bz	Ac	Lev	Me
17	$NHCOC_{17}H_{35}$	Bz	Ac	Lev	Me
18	$NHCOC_{17}H_{35}$	Bz	Ac	H	Me
19	$NHCOC_{17}H_{35}$	Bz	Ac	SO_3Na	Me
20	$NHCOC_{17}H_{35}$	H	H	H	Na
21	$NHCOC_{17}H_{35}$	H	H	SO_3Na	Na
22	$NHCOC_{23}H_{47}$	Bz	Ac	Lev	Me
23	$NHCOC_{23}H_{47}$	Bz	Ac	H	Me
24	$NHCOC_{23}H_{47}$	Bz	Ac	SO_3Na	Me
25	$NHCOC_{23}H_{47}$	H	H	H	Na
26	$NHCOC_{23}H_{47}$	H	H	SO_3Na	Na

13). Catalytic hydrogenolysis (10% Pd-C) of the benzyl groups in **11** in ethyl acetate-methanol for 24 h at room temperature and subsequent *O*-acetylation gave the per-*O*-acyl derivative **12** in quantitative yield. Compound **12** was transformed, in essentially the same way as described for **9**, into the heptasaccharide donor **14**. The ^{1}H NMR data for Glc unit in **14** [δ 6.46 (d, $J_{1,2}$ = 3.8 Hz, H-1), 8.64 (C=NH)] indicated the trichloroacetimidate to be α.

The final glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[9,10] (**15**) with **14** thus obtained, in dichloromethane in the presence of boron trifluoride etherate for 7 h at 0 °C afforded the expected β-glycoside **16** in 61% yield. Selective reduction[11,12] of the azido group in **16** with hydrogen sulfide in aq pyridine for 2.5 days at 10 °C gave the amine, which, on condensation with octadecanoic acid and tetracosanoic acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) in dichloromethane, afforded **17** (65%) and **22** (65%), respectively. Selective removal of the levulinoyl group in **17** and **22** with hydrazine-monoacetate gave **18** and **23** in good yields. Treatment of **18** and **23** in *N,N*-dimethylformamide (DMF) with excess of sulfur trioxide trimethylamine complex for 24 h at 45 °C afforded the sulfated **19** (92%) and **24** (92%), respectively. Finally, saponification of the methyl ester group in **18**, **19**, **23** and **24** with lithium hydroxide monohydrate in tetrahydrofuran and water, followed by *O*-deacylation with sodium methoxide in methanol-tetrahydrofuran at 10 °C, yielded the desired glycolipids **20**, **21**, **25** and **26**. The four target glycosphingolipids thus obtained, were purified by column chromatography on Sephadex LH-20, and the structures were confirmed by FAB-MS spectroscopy.

EXPERIMENTAL

General Procedures. Specific 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 with a JEOL JNM-GX 270 spectrometer. FAB-MS spectra were determined with a JEOL JMS-SX 102A mass

spectrometer/JMA-DA 7000 data system. Each sample was mixed with triethanolamine matrix on a target. The ion accelerating voltage was 8.0 KV, and the primary beam for the bombardment was 6.0 KeV of xenon. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted in vacuo.

2-(Trimethylsilyl)ethyl *O*-(4-*O*-Acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (3). To a solution of 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**1**; 4.5 g, 6.7 mmol) in CH_2Cl_2 (20 mL) were added 2-(trimethylsilyl)ethyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**2**; 7.8 g, 14.2 mmol) and powdered molecular sieves 4Å (MS-4Å; 4.0 g), and the mixture was stirred for 5 h at room temperature (mixture A). A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf; 0.4 g, 1.8 mmol) in CH_2Cl_2 (2 mL) was stirred with MS-4Å (0.7 g) for 1 h at room temperature and then it was added to the mixture A at -20 °C. After stirring for 5 h at -20 °C, the reaction mixture was neutralized with triethylamine and filtered, the residue was washed with CH_2Cl_2 and the combined filtrate and washings concentrated. Column chromatography (1:3 ethyl acetate-hexane) of the residue on silica gel (300 g) gave **3** (6.7 g, 94%) as crystals. Recrystallization from EtOH afforded needles: mp 89.0-91.0 °C; $[\alpha]_D$ +9.3° (*c* 0.6, $CHCl_3$); IR (KBr) 1750 and 1230 (ester), 1720 (imide), 860 and 840 (TMS), and 740, 720 and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.81 (m, 2H, $Me_3SiCH_2CH_2O$), 1.94 (s, 3H, AcO), 3.94 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4 for Gal), 4.33 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1 for Gal), 5.16 (dd, 1H, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.9 Hz, H-4 for GlcN), 5.48 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1 for Gal), and 7.17-7.51 (m, 29H, 5Ph, Phthaloyl-H).

Anal. Calcd for $C_{62}H_{69}NO_{13}Si$ (1064.3): C, 69.97; H, 6.53; N, 1.32. Found: C, 69.78; H, 6.48; N, 1.26.

2-(Trimethylsilyl)ethyl *O*-(2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (4). To a solution of **3** (6.5 g, 6.1 mmol) in MeOH (70 mL) was added NaOMe (100 mg). The solution was stirred for 2 h at room temperature, treated with Amberlite IR-120

(H^+) resin and then concentrated. A solution of the residue in aq 95% EtOH (70 mL) was heated with hydrazine monohydrate (3 mL) for 5 h under reflux. The precipitate was collected and washed with EtOH, and the combined filtrate and washings concentrated. The residue was treated with acetic anhydride (5 mL) in MeOH (70 mL) for 1 h at room temperature, and pyridine (10 mL) was added, the reaction mixture was concentrated and then extracted with CH_2Cl_2 (250 mL). The extract was successively washed with 2M HCl, water, and M Na_2CO_3, dried (Na_2SO_4) and concentrated. Column chromatography (1:1 ethyl acetate-hexane) of the residue on silica gel (300 g) afforded **4** (4.8 g, 85%) as crystals. Recrystallization from EtOH gave needles: mp 112.5-114.0 °C; $[\alpha]_D$ -15.8° (*c* 0.4, $CHCl_3$); IR (KBr) 3450-3300 (NH, OH) 1660 and 1530 (amide), 860 and 840 (TMS), and 740, and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.99 (m, 2H, $Me_3SiCH_2CH_2O$), 1.52 (s, 3H, AcN), and 7.20-7.31 (m, 25H, 5Ph).

Anal. Calcd for $C_{54}H_{67}NO_{11}Si$ (934.2): C, 69.43; H, 7.23; N, 1.50. Found: C, 69.29; H, 7.14; N, 1.49.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (6). To a solution of *O*-(methyl 4-*O*-acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**5**; 2.8g, 2.61 mmol) in CH_2Cl_2 (15 mL) were added **4** (4.2 g, 4.50 mmol) and MS-4Å (2.3 g), and the mixture was stirred for 5 h at room temperature (mixture A). A dried solution of TMSOTf (87 mg, 0.39 mmol) and MS-4Å (0.5 g) in CH_2Cl_2 (1.5 mL) was added to the mixture A at -10 °C and it was stirred overnight at room temperature. Conventional workup described for **3** gave **6** (4.4 g, 92%) as a syrup: $[\alpha]_D$ +3.4° (*c* 0.8, $CHCl_3$); IR (film) 3390 (NH), 1730 and 1270 (ester), 1680 and 1530 (amide), 860 and 840 (TMS), and 750 and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.97 (m, 2H, $Me_3SiCH_2CH_2O$), 1.62 (s, 3H, AcN), 1.97 and 2.02 (2s, 6H, AcO and

$CH_3COCH_2CH_2CO$), 2.32 (m, 4H, $CH_3COCH_2CH_2CO$), 3.70 (s, 3H, MeO), and 7.00-8.06 (m, 45H, 9Ph).

Anal. Calcd for $C_{102}H_{111}NO_{29}Si$ (1843.1): C, 66.47; H, 6.07; N, 0.76. Found: C, 66.21; H, 6.03; N, 0.75.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (7). A solution of **6** (4.5 g, 2.44 mmol) in MeOH (50 mL) and ethyl acetate (10 mL) was hydrogenolyzed in the presence of 10% Pd-C (2.0 g) for 24 h at room temperature, and then the reaction mixture was filtered and concentrated. The residue was treated with benzoic anhydride (3.3 g) in pyridine (50 mL) in the presence of 4-dimethylaminopyridine (2.0 g) for 2 h at 70 °C. Column chromatography (50:1 CH_2Cl_2-MeOH) of the crude product on silica gel (200 g) afforded **7** (3.7 g, 79%) as an amorphous mass: $[\alpha]_D$ +32.2° (*c* 0.9, $CHCl_3$); IR (film) 3370 (NH), 1740 and 1270 (ester), 1680 and 1540 (amide), and 710 and 690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.84 (m, 2H, $Me_3SiCH_2CH_2O$), 1.74 (s, 3H, AcN), 1.84 and 2.00 (2s, 6H, AcO and $CH_3COCH_2CH_2CO$), 2.33 (m, 4H, $CH_3COCH_2CH_2CO$), 3.71 (s, 3H, MeO), 4.48, 4.59, 4.71 and 4.87 (4d, 4H, $J_{1,2}$ = 7.3-8.2 Hz, H-1d-g), and 6.90-8.04 (m, 45H, 9Ph).

Anal. Calcd for $C_{102}H_{101}NO_{34}Si$ (1913.0): C, 64.04; H, 5.32; N, 0.73. Found: C, 63.75; H, 5.04; N, 0.50.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1 → 3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-D-galactopyranose (8).** To a solution of **7** (3.8 g, 1.99 mmol) in CH_2Cl_2 (30 mL) was added trifluoroacetic acid (9 mL) at 0 °C, and the solution was stirred for 3 h at room temperature and then concentrated. Column chromatography (30:1 CH_2Cl_2-MeOH) of the residue on silica gel (200 g) gave **8** (3.4

g, 94%) as a syrup: $[\alpha]_D$ +44.3° (*c* 0.8, $CHCl_3$); IR (film) 3380 (NH, OH), 1730 and 1270 (ester), and 710 and 690 cm^{-1} (Ph).

Anal. Calcd for $C_{97}H_{89}NO_{34}$ (1812.8): C, 64.27; H, 4.95; N, 0.77. Found: C, 64.12; H, 4.86; N, 0.57.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl Trichloroacetimidate (9).** To a solution of **8** (3.5 g, 1.93 mmol) in CH_2Cl_2 (40 mL) and trichloroacetonitrile (5 mL) was added 1,8 diazabicyclo[5,4,0]undec-7-ene (DBU; 60 mg) at -10 °C, and the reaction mixture was stirred for 3 h at 0 °C. The solution was directly chromatographed on silica gel (200 g) with 2:1 ethyl acetate-hexane to give **9** (3.5 g, 92%): $[\alpha]_D$ +48.3° (*c* 0.9, $CHCl_3$); IR (film) 3390 (NH), 1730 and 1270 (ester), 1680 and 1590 (amide), and 710 and 690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 1.70 (s, 3H, AcN), 1.84 and 1.99 (2s, 6H, AcO and $CH_3COCH_2CH_2CO$), 2.28 (m, 4H, $CH_3COCH_2CH_2CO$), 3.70 (s, 3H, MeO), 4.51, 4.72 and 4.89 (3d, 3H, $J_{1,2}$ = 7.2-8.2 Hz, H-1e-g), 5.58 and 5.92 (2d, 2H, $J_{3,4}$ = 2.8-3.7 Hz, H-4d and 4f), 5.79 (dd, 1H, $J_{2,3}$ = 10.1 Hz, H-2d), 6.69 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1d), 6.90-8.01 (m, 45H, 9Ph), and 8.51 (s, 1H, C=NH).

Anal. Calcd for $C_{99}H_{89}Cl_3N_2O_{34}$ (1957.1): C, 60.76; H, 4.58; N, 1.43. Found: C, 60.71; H, 4.50; N, 1.14.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11). To a solution of **9** (2.6 g, 1.33 mmol) in CH_2Cl_2 (15 mL) were added 2-(trimethylsilyl)ethyl *O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyrano-

syl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**10**; 4.0 g, 2.93 mmol) and MS-4Å (2.0 g), and the reaction mixture was stirred for 5 h at room temperature (mixture A). A dried solution of TMSOTf (60 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) was added to the mixture A at room temperature, the mixture was stirred overnight at room temperature and the usual workup gave **11** (4.0 g, 95%) as an amorphous mass: $[\alpha]_D$ +13.3° (*c* 0.9, $CHCl_3$); IR (film) 3390 (NH), 1740 and 1270 (ester), 1680 and 1540 (amide), and 710 and 690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.73 and 1.76 (2s, 6H, 2AcN), 1.94 and 2.00 (2s, 6H, AcO and $CH_3COCH_2CH_2CO$), 2.26 (m, 4H, $CH_3COCH_2CH_2CO$), 3.63 (s, 3H, MeO), and 6.91-7.99 (m, 85H, 17Ph).

Anal. Calcd for $C_{178}H_{182}N_2O_{49}Si$ (3161.5): C, 67.63; H, 5.80; N, 0.89. Found: C, 67.47; H, 5.59; N, 0.59.

2-(Trimethylsilyl)ethyl *O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (12). A solution of **11** (3.9 g, 1.23 mmol) in MeOH (40 mL) and ethyl acetate (20 mL) was hydrogenolyzed in the presence of 10% Pd-C (2.0 g) for 24 h at room temperature, and the reaction mixture was then filtered and concentrated. The residue was acetylated with acetic anhydride (20 mL)-pyridine (40 mL) for 20 h at room temperature and concentrated. The product was purified by column chromatography on a column of silica gel (200 g) with ethyl acetate giving **12** (3.4 g, quantitative) as an amorphous mass: $[\alpha]_D$ +17.6° (*c* 0.9, $CHCl_3$); IR (film) 3380 (NH), 1740 and 1270 (ester), and 1680 and 1540 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 0.93 (m, 2H, $Me_3SiCH_2CH_2O$), 1.85 and 1.86 (2s, 6H, 2AcN), 1.97-2.08 (10s, 30H, 9AcO and $CH_3COCH_2CH_2CO$), 2.30 (m, 4H, $CH_3COCH_2CH_2CO$), 3.70 (s, 3H, MeO), 4.44, 4.49, 4.53, 4.59, 4.61, 4.71 and

4.81 (7d, 7H, $J_{1,2}$ = 7.3-8.1 Hz, H-1a-g), 5.55 and 5.72 (2d, 2H, $J_{3,4}$ = 3.3-3.7, H-4b, 4d, or 4f), and 6.90-8.00 (m, 45H, 9Ph).

Anal. Calcd for $C_{138}H_{150}N_2O_{57}Si$ (2776.8): C, 59.69; H, 5.45; N, 1.01. Found: C, 59.42; H, 5.40; N, 0.88.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranose (13).** To a solution of **12** (3.2 g, 1.15 mmol) in CH_2Cl_2 (30 mL) was added trifluoroacetic acid (7 mL) at 0 °C, and the reaction mixture was stirred for 3 h at room temperature and then concentrated. Column chromatography (ethyl acetate) of the residue on silica gel (150 g) afforded **13** (2.6 g, 85%) as a syrup: $[\alpha]_D$ +29.1° (*c* 1.9, $CHCl_3$); IR (film) 3380 (NH, OH), 1730 and 1270 (ester), 1680 and 1540 (amide), and 760, 710 and 690 cm^{-1} (Ph).

Anal. Calcd for $C_{133}H_{138}N_2O_{57}$ (2676.5): C, 59.68; H, 5.20; N, 1.05. Found: C, 59.59; H, 5.03; N, 0.82.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (14).** To a solution of **13** (3.0 g, 1.12 mmol) in CH_2Cl_2 (60 mL) and trichloroacetonitrile (5 mL) was added DBU (34 mg) at 0 °C, and the reaction mixture was stirred for 5 h at 0 °C. The solution was directly chromatographed on silica gel (100 g) with 6:1 ethyl acetate-hexane to give **14** (2.8 g, 90%) as an amorphous mass: $[\alpha]_D$ +32.7° (*c* 0.4, $CHCl_3$); IR (film) 3480 (NH), 1730 and 1270 (ester), and 1680 and 1540 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 1.69 and 1.73

(2s, 6H, 2AcN), 1.86-2.09 (10s, 30H, 9AcO and $CH_3COCH_2CH_2CO$), 2.34 (m, 4H, $CH_3COCH_2CH_2CO$), 3.70 (s, 3H, MeO), 4.48, 4.55, 4.59, 4.62, 4.71 and 4.82 (6d, 6H, $J_{1,2}$ = 7.3-8.1 Hz, H-1b-g), 5.73 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4b, 4d or 4f), 6.46 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1a), 6.93-8.00 (m, 45H, 9Ph), and 8.64 (s, 1H, C=NH).

Anal. Calcd for $C_{135}H_{138}Cl_3N_3O_{57}$ (2820.9): C, 57.48; H, 4.93; N, 1.49. Found: C, 57.29; H, 4.86; N, 1.29.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(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 (16).** To a solution of **14** (1.0 g, 0.35 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**15**; 0.30 g, 0.70 mmol) in CH_2Cl_2 (7 mL) were added powdered molecular sieves 4Å (AW-300, 1.2 g), the mixture was stirred for 5 h at room temperature and then cooled to 0 °C. Boron trifluoride etherate (0.17 g) was added, and the mixture was stirred for 7 h at 0 °C and filtered. The insoluble material was washed with CH_2Cl_2, and the combined filtrate and washings were washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (30:1 CH_2Cl_2-MeOH) of the residue on silica gel (100 g) gave amorphous **16** (0.67 g, 61%): $[\alpha]_D$ +9.0° (*c* 0.6, $CHCl_3$); IR (film) 3380 (NH), 2930 and 2860 (CH_3, CH_2), 2110 (azide), and 1730 and 1270 cm^{-1} (ester); ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, J_{Me,CH_2} = 6.6 Hz, $MeCH_2$), 1.23 (s, 22H, 11CH_2), 1.85 and 1.86 (2s, 6H, 2AcN), 1.94-2.08 (10s, 30H, 9AcO and $CH_3COCH_2CH_2CO$), 2.30 (m, 4H, $CH_3COCH_2CH_2CO$), 3.62 (s, 3H, MeO), 4.43, 4.47, 4.52, 4.58, 4.61, 4.71 and 4.81 (7d, 7H, $J_{1,2}$ = 7.1-8.1 Hz, H-1a-g), 5.91 (dt, 1H, H-5 of sphingosine), and 6.90-8.05 (m, 50H, 10Ph).

Anal. Calcd for $C_{158}H_{175}N_5O_{59}$ (3088.1): C, 61.45; H, 5.71; N, 2.27. Found: C, 61.42; H, 5.60; N, 2.05.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(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 (17).** Hydrogen sulfide was bubbled through a stirred solution of **16** (700 mg, 0.21 mmol) in aq 80% pyridine (50 mL) for 70 h at 10 °C. The reaction mixture was concentrated, and the residue was stirred with octadecanoic acid (210 mg, 0.74 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 192 mg, 1.00 mmol) in CH_2Cl_2 overnight at room temperature. Dichloromethane (50 mL) was added, and the mixture was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (45:1 CH_2Cl_2-MeOH) of the residue on silica gel (100 g) gave amorphous **17** (490 mg, 65%): $[\alpha]_D$ +22.5° (*c* 0.7, $CHCl_3$); IR (film) 3380 (NH), 2930 and 2860 (CH_3, CH_2), 1730 and 1270 (ester), 1680 and 1540 (amide), and 760, 710 and 690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.25 (s, 22H, 11CH_2), 1.84 (s, 6H, 2AcN), 1.92-2.09 (10s, 30H, 9AcO and *CH*$_3$COCH$_2$CH$_2$CO), 2.29 (m, 4H, $CH_3COCH_2CH_2CO$), 3.70 (s, 3H, MeO), 5.84 (dt, 1H, H-5 of sphingosine), and 6.90-8.00 (m, 50H, 10Ph).

Anal. Calcd for $C_{176}H_{211}N_3O_{60}$ (3328.6): C, 63.51; H, 6.39; N, 1.26. Found: C, 63.21; H, 6.20; N, 1.21.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(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 (18). A solution of **17** (270 mg, 81.1 μmol) and hydrazine-monoacetate (40 mg, 0.43 mmol) in EtOH (8 mL) was stirred for 1 h at room temperature. Dichloromethane (50 mL) was added, and the solution was washed with M $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (30:1 CH_2Cl_2-MeOH) of the residue on silica gel (50 g) afforded amorphous **18** (260 mg, quantitative): $[\alpha]_D$ +14.7° (*c* 0.7, $CHCl_3$); IR (film) 3380 (NH, OH), 2930 and 2860 (CH_3, CH_2), 1740 and 1270 (ester), 1680 and 1540 (amide), and 760, 720 and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.25 (s, 22H, 11CH_2), 1.84 (s, 6H, 2AcN), 1.98-2.08 (9s, 27H, 9AcO), 3.62 (s, 3H, MeO), 5.85 (dt, 1H, H-5 of sphingosine), and 6.93-8.02 (m, 50H, 10Ph).

Anal. Calcd for $C_{171}H_{205}N_3O_{58}$ (3230.5): C, 63.58; H, 6.40; N, 1.30. Found: C, 63.34; H, 6.38; N, 1.30.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(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 Sodium Salt (19).** A solution of **18** (230 mg, 71.2 μmol) and sulfur trioxide trimethylamine complex (158 mg, 1.14 mmol) in DMF (3 mL) was stirred for 24 h at 45 °C and then cooled to room temperature. Methanol (0.5 mL) and CH_2Cl_2 (0.5 mL) were added, and the solution was applied to a column of Sephadex LH-20 with 1:1 CH_2Cl_2-MeOH. Glycolipid-containing fractions were concentrated. Column chromatography (MeOH) of the residue on Dowex-50×2 (Na^+) resin gave amorphous **19** (218 mg, 92%): $[\alpha]_D$ +12.1° (*c* 0.4, $CHCl_3$); IR (film) 3390 (NH), 2930 and 2850 (CH_3, CH_2), 1750 and 1270 (ester), 1680 and 1540 (amide), and 750, 710 and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.25 (s, 22H, 11CH_2), 1.85 and 1.86 (2s, 6H, 2AcN), 1.92-2.08 (9s, 27H, 9AcO), 3.62 (s, 3H, MeO), and 6.93-8.02 (m, 50H, 10Ph).

***O*-β-D-Glucopyranosyluronate-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→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 Sodium Salt (20).** To a solution of **18** (120 mg, 37.1 μmol) in THF (5 mL) was added lithium hydroxide monohydrate (10 mg, 0.24 mmol) in water (1 mL), the reaction mixture was stirred for 3 h at 5 °C and then concentrated at 30 °C. Tetrahydrofuran (7 mL), MeOH (7 mL) and NaOMe (10 mg) were added to the reaction mixture which was then stirred overnight at 10 °C, and chromatographed on a column of Sephadex LH-20 in 7:3:1 $CHCl_3$-MeOH-H_2O to give **20** (55 mg, 82%): FAB-MS (negative ion mode); *m/z* 1794.72 $(M-Na)^-$, 1816.80 $(M-H)^-$; $C_{82}H_{144}N_3O_{39}$ requires 1794.9377 and $C_{82}H_{143}N_3O_{39}Na$ requires 1816.9196.

***O*-3-*O*-Sulfo-β-D-glucopyranosyluronate-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→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 Disodium Salt (21).** Deacylation and saponification of **19** (110 mg, 33.0 μmol), as described for **20**, yielded **21** (49.0 mg, 78%): FAB-MS (negative ion mode); *m/z* 1896.82 $(M-Na)^-$, 1918.69 $(M-H)^-$; $C_{82}H_{143}N_3O_{42}SNa$ requires 1896.8765 and $C_{82}H_{142}N_3O_{42}SNa_2$ requires 1918.8584.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-tetracosanamido-4-octadecene-1,3-diol (22).** Selective reduction of the azido group in **16** (700 mg, 0.23 mmol) and

subsequent coupling with tetracosanoic acid (270 mg, 0.73 mmol), as described for **17**, afforded amorphous **22** (503 mg, 65%): $[\alpha]_D$ +16.3° (*c* 1.1, $CHCl_3$); IR (film) 3380 (NH), 2930 and 2860 (CH_3, CH_2), 1740 and 1270 (ester), 1680 and 1540 (amide), and 760, 710 and 690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.25 (s, 22H, 11CH_2), 1.76 (s, 6H, 2AcN), 1.87-2.09 (10s, 30H, 9AcO and *CH*$_3$COCH$_2$CH$_2$CO), 2.29 (m, 4H, CH$_3$COC*H*$_2$C*H*$_2$CO), 3.62 (s, 3H, MeO), 5.84 (dt, 1H, H-5 of sphingosine), and 6.90-8.00 (m, 50H, 10Ph).

Anal. Calcd for $C_{182}H_{223}N_3O_{60}$ (3412.8): C, 64.05; H, 6.59; N, 1.23. Found: C, 63.77; H, 6.38; N, 1.14.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*, 3*R*, 4*E*)-2-tetracosanamido-4-octadecene-1,3-diol (23).** Selective removal of the levulinoyl group in **22** (290 mg, 85.0 μmol), as described for **18**, afforded amorphous **23** (281 mg, quantitative): $[\alpha]_D$ +9.2° (*c* 0.5, $CHCl_3$); IR (film) 3390 (NH, OH), 2930 and 2860 (CH_3, CH_2) 1750 and 1280 (ester), 1680 and 1540 (amide), and 760, 710 and 690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.26 (s, 22H, 11CH_2), 1.78 (s, 6H, 2AcN), 1.99-2.08 (9s, 27H, 9AcO), 3.62 (s, 3H, MeO), 5.83 (dt, 1H, H-5 of sphingosine), and 6.93-8.02 (m, 50H, 10Ph).

Anal. Calcd for $C_{177}H_{217}N_3O_{58}$ (3314.7): C, 64.14; H, 6.60; N, 1.27. Found: C, 64.03; H, 6.36; N, 1.17.

***O*-(Methyl 4-*O*-Acetyl-2-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyluronate)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyrano-**

syl)-(1→1)-(2*S*,3*R*,4*E*)-3-2-tetracosanamido-4-octadecene-1,3-diol Sodium Salt (24). Sulfation of **23** (274 mg, 82.7 μmol) and workup, as described for **19**, yielded amorphous **24** (260 mg, 92%): $[\alpha]_D$ +12.3° (*c* 0.6, $CHCl_3$); IR (film) 3390 (NH), 2930 and 2860 (CH_3, CH_2), 1750 and 1270 (ester), 1680 and 1550 (amide), and 760, 720 and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.25 (s, 22H, 11CH_2), 1.85-1.86 (2s, 6H, 2AcN), 1.93-2.09 (9s, 27H, 9AcO), 3.62 (s, 3H, MeO), and 6.93-8.02 (m, 45H, 9Ph).

***O*-β-D-Glucopyranosyluronate-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-tetracosanamido-4-octadecene-1,3-diol Sodium Salt (25).** Deacylation and saponification of **23** (130 mg, 39.2 μmol), as described for **20**, yielded **25** (60.5 mg, 82%): FAB-MS (negative ion mode); *m/z* 1879.01 $(M-Na)^-$, 1901.10 $(M-H)^-$, $C_{88}H_{156}N_3O_{39}$ requires 1879.0316 and $C_{88}H_{155}N_3O_{39}Na$ requires 1901.0135.

***O*-3-*O*-Sulfo-β-D-glucopyranosyluronate-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-tetracosanamido-4-octadecene-1,3-diol Disodium Salt (26).** Deacylation and saponification of **24** (125 mg, 36.6 μmol), as described for **20**, yielded **26** (56.6 mg, 78%): FAB-MS (negative ion mode); *m/z* 1980.92 $(M-Na)^-$, 2003.85 $(M-H)^-$, $C_{88}H_{155}N_3O_{42}SNa$ requires 1980.9704 and $C_{88}H_{154}N_3O_{42}SNa_2$ requires 2002.9593.

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REFERENCES

1. a) E. Nobie-Orgazio, A.P. Hays, N. Latov, G. Perman, J. Glier, M.E. Shy and L. Freddo, *Neurology*, **34**, 1336 (1984); b) J. Kuruse, R. Mailhammer, H. Wernecke, A. Faissner, I. Sommer, C. Gorodis and M. Schachner, *Nature*, **311**, 153 (1984).
2. a) K.H. Chou, A.A. Ilyas, J.E. Evans, C. Costello, R.H.Quarles and F.B. Jungalwala, *Biochem. Biophys. Res. Commun.*, **261**, 11717 (1986); b) T. Ariga, T. Kohriyama, L. Freedo, N. Latov, M. Saito, K. Kon, S. Ando, M. Suzuki, M.E. Hemling, K.L. Rinehart, Jr., S. Kusunoki and R.K. Yu, *J. Biol. Chem.*, **262**, 848 (1987).
3. a) A.A. Ilyas, R.H. Quarles, T.D. McIntosh, M.J. Doberson, B.D. Trapp, M.C. Dalakas and R.O. Brady, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1225 (1984); b) A.A. Ilyas, R.H. Quarles and R.O.Brady, *Biochem. Biophys. Res. Commun.*, **122**, 1206 (1984); c) T. Abo, M.D. Coper and C.M. Balch, *J. Immunol.*, **129**, 1752 (1982).
4. D. Asa, T. Gant, Y. Oda and B. K. Brandley, *Glycobiology*, **2**, 395 (1992).
5. Y. Isogai, T. Kawase, H. Ishida, M. Kiso and A. Hasegawa, submitted *J. Carbohydr. Chem.*
6. A. Hasegawa, T. Ando, A, Kameyama and M. Kiso, *J. Carbohydr. Chem.*, **11**, 645 (1992).
7. K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmén, G. Noori and K. Stenvall, *J. Org. Chem.*, **53**, 5629 (1988).
8. a) R.R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, **19**, 731 (1980); b) R.R. Schmidt, J. Michel and M. Roos, *Liebigs Ann. Chem.*, 1343 (1984).
9. Y. Ito, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 285 (1989).
10. R.R. Schmidt and P. Zimmermann, *Angew. Chem. Int. Ed. Eng.*, **25**, 725 (1986).
11. T. Adachi, Y. Yamada, I. Inoue and M. Saneyoshi, *Synthesis*, 45 (1977).
12. T. Murase, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **188**, 71 (1989).