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A synthetic approach to polysialogangliosides containing α-sialyl-(2 → 8)-sialic acid: total synthesis of ganglioside GD_3 †

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ABSTRACT

A stereocontrolled, facile total synthesis of ganglioside GD_3 is described as an example of a proposed systematic approach to the preparation of gangliosides containing an α-sialyl-(2 → 8)-sialic acid unit α-glycosidically linked to O-3 of a D-galactose residue in their oligosaccharide chains. Glycosylation of 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-, 3-*O*-benzoyl-, or 3-*O*-benzyl-β-D-galactopyranosides, or 2-(trimethylsilyl)ethyl 2,3,6,2′,6′-penta-*O*-benzyl-β-lactoside (**7**), 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-nonulopyranosyl-ono-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate (**3**), using *N*-iodosuccinimide–trifluoromethanesulfonic acid as a promoter, gave the corresponding α glycosides **8** (32%), **13** (33%), **14** (48%), and **17** (31%), respectively. The glycosyl donor **3** was prepared from *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2 → 8)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid by treatment with Amberlite IR-120 (H^+) in methanol, *O*-acetylation, and subsequent replacement of the anomeric acetoxy group with phenylthio. Compound **8** was converted into the methyl β-thioglycoside via *O*-benzoylation, replacement of the 2-(trimethylsilyl)ethyl group by acetyl, and introduction of the methylthio group by reaction with methylthiotrimethylsilane. Compound **17** was converted, via *O*-acetylation, selective removal of the 2-(trimethylsilyl)ethyl group, and reaction with trichloroacetonitrile, into the α-trichloroacetimidate, which was coupled with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol to give the β-glycoside. This glycoside was easily transformed, via selective reduction of the azido group, condensation with octadecanoic acid, *O*-deacylation, and hydrolysis of the methyl ester and lactone functions, into ganglioside GD_3.

INTRODUCTION

Recently, as more and more biological functions[2–10] of gangliosides are being revealed, their synthesis has become increasingly stimulating and rewarding. For

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some time our laboratory has had a program in this area, and our efforts resulted in the development of a facile procedure for the α-stereoselective coupling[11–13] of sialic acid using its protected methyl or phenyl 2-thioglycoside as the glycosyl donor and the suitably protected galactose acceptors, with dimethyl (methylthio)sulfonium triflate (DMTST) or *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (TfOH) (or trimethylsilyl trifluoromethanesulfonate) as the promoter in acetonitrile solution. This method has served us for the systematic synthesis of gangliosides[14] and their analogs[15], useful for elucidating the functions of these substances at the molecular level.

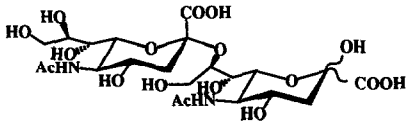
There are several gangliosides containing an α-sialyl-(2 → 8)-sialic acid unit in their molecules, such as GD_3, GT_{1a}, GQ_{1b}, and others, and these have many important biological roles[9]. As a continuation of our synthetic efforts, we describe here a facile preparation of ganglioside GD_3 by a method suitable for the systematic synthesis of polysialogangliosides containing an α-sialyl-(2 → 8)-sialic moiety. Ganglioside GD_3 is well known as a human melanoma associated antigen[16] and it is widely distributed in normal and pathological tissues. It was first synthesized by Ogawa et al.[17] by a multistep procedure.

RESULTS AND DISCUSSION

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 (**3**) was selected as the glycosyl donor, while 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-, 3-*O*-benzoyl-, and 3-*O*-benzyl-β-D-galactopyranoside[11,18] (**4–6**), and 2-(trimethylsilyl)ethyl 2,3,6,2′,6′-penta-*O*-benzyl-β-lactoside[14] (**7**) served as the acceptors in the syntheses of trisaccharides **8**, **13**, and **14**, and tetrasaccharide **17**. Compound **17** could then, by coupling to the ceramide moiety, be transformed into ganglioside GD_3. Compounds **8**, **13**, and **14**, along with **17**, could be used as building units for syntheses of polysialogangliosides.

Treatment of *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2 → 8)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (**1**), which was easily prepared by hydrolysis of colominic acid under mild, acidic conditions according to the literature[19], with Amberlite IR-120 (H^+) resin in methanol for 2 days at 40°C, followed by acetylation, gave an anomeric mixture ($\alpha:\beta$ ratio 1:10) of the ester-lactone **2** in 84% yield. The conversion of **2** into the phenyl thioglycoside **3** (89%; the $\alpha:\beta$ ratio was estimated as ~ 1:3 from the relative intensities of H-3*eq* signals) was achieved by treatment[20] with thiophenol and boron trifluoride etherate for 12 h at room temperature in dichloromethane.

Glycosylation of **4** with **3** thus obtained, in acetonitrile for 48 h at −35°C in the presence of NIS–TfOH, gave the expected α-glycoside **8** in 32% yield, together with the corresponding β-glycoside **12** (9%). Benzoylation of **8** with benzoic



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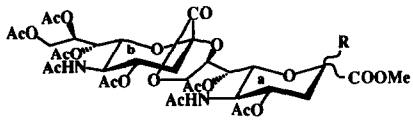
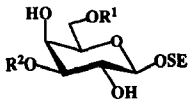
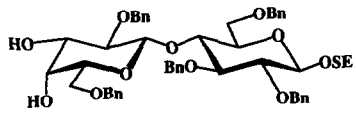


	R
2	OAc
3	SPh



	R^1	R^2
4	Bz	H
5	H	Bz
6	H	Bn



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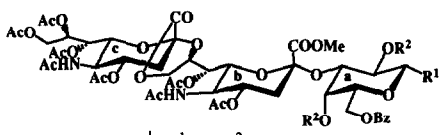
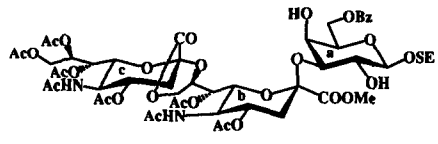
SE = 2-(trimethylsilyl)ethyl
Bz = benzoyl
Bn = benzyl

anhydride in pyridine in the presence of 4-dimethylaminopyridine gave the 2,4,6-tri-*O*-benzoate **9** in good yield. Characteristics signals of the Neu5Ac unit in the ^{1}H NMR spectrum of **9** were a one-proton doublet of doublets at δ 2.58 due to H-3b*eq* and a one-proton doublet of doublets at δ 5.01 ($J_{7,8}$ 9.2 Hz) due to H-7b, indicating the newly formed glycosidic linkage to be α. The observed chemical



	R^1	R^2
8	OSE	H
9	OSE	Bz
10	OAc	Bz
11	SMe	Bz



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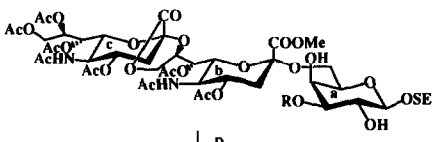
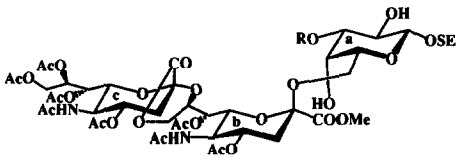


	R
13	Bz
14	Bn



	R
15	Bz
16	Bn

shifts for H-2a (δ 5.62, $J_{2,3}$ 8.1 Hz) and H-4a (δ 5.82, $J_{3,4}$ 3.3 Hz) clearly indicated the linkage position to be C-3 of the galactose residue. Other ^{1}H NMR data are given in the Experimental section and are consistent with the structure assigned.

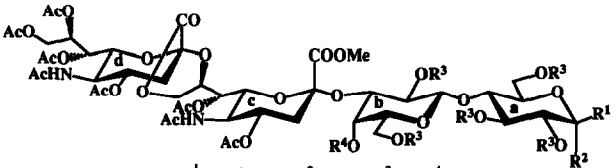
Treatment[21] of **9** with acetic anhydride and boron trifluoride etherate in toluene gave the β-1 acetate **10** in 82% yield. Conversion of **10** into the methyl β-thioglycoside **11** (76%) was achieved by treatment[21b,22] with methylthiotrimethylsilane and boron trifluoride etherate in dichloromethane for 4 h at room temperature.

In essentially the same way, glycosylation of **5**, **6**, or **7** with **3** gave the corresponding α-glycosides **13** (33%), **14** (48%), and **17** (31%) respectively, together with the β-glycosides **15** (10%) and **16** (16%). The moderate yields (31–48%) of the desired α anomers are quite appreciable, considering the bulkiness of the donor and the steric hindrance to glycosidation at C-3′ of the lactose unit in the synthesis of **17**.

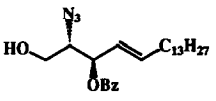
Hydrogenolytic removal of the benzyl groups in **17** over 10% Pd–C in 1:1 methanol–acetic acid for 2 days at 45°C, followed by acetylations of the free hydroxy groups with acetic anhydride–pyridine for 12 h at 45°C, afforded the fully acylated core oligosaccharide **18** in 90% yield. For the selective removal of the 2-(trimethylsilyl)ethyl group, **18** was treated[21] with trifluoroacetic acid in dichloromethane for 1.5 h at room temperature to give the 1-hydroxy compound **19** (90%) which, on further treatment[11,23] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene in dichloromethane for 2 h at 0°C, gave the trichloroacetimidate **20** in 86% yield. The ^{1}H NMR spectrum of **20** contained a one-proton doublet at δ 6.55 ($J_{1,2}$ 3.8 Hz, H-1) and a one proton singlet at δ 8.73 (C=N*H*), indicating the anomeric configuration of **20** to be α.

Glycosylation[11,24] of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[25] (**21**) with the donor **20** thus obtained was carried out in the presence of boron trifluoride etherate in dichloromethane for 6 h at −20°C, to give a 60% yield of the desired β-glycoside **22**. A significant signal in the ^{1}H NMR spectrum of **22** was a one-proton triplet at δ 4.95 ($J_{1,2} = J_{2,3} = 9.1$ Hz, H-2 of the lactose unit), showing the newly formed glycosidic linkage to be β.

Selective reduction[11,26] of the azido group in **22** with hydrogen sulfide in aqueous 83% pyridine for 3 days at 0°C, and subsequent condensation with octadecanoic acid, using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, furnished an 84% yield of acylated ganglioside GD_3 (**23**). *O*-Deacylation of **23** with sodium methoxide in methanol, with subsequent saponification of the methyl ester and lactone functions, yielded ganglioside GD_3 (**24**) quantitatively. The ^{1}H NMR data (400 MHz) for **24** in 1:1 $CDCl_3$–CD_3OD included δ 1.93, 1.94 (2 s, 6 H, 2 AcN), 2.52, 2.85 (2 m, 2 H, H-3c*eq*, H-3d*eq*), 4.20 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 4.35 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 5.35 (dd, 1 H, $J_{3,4}$ 7.7, $J_{4,5}$ 15.4 Hz, H-4 of ceramide), and 5.60 (dt, 1 H, $J_{5,6} = J_{5,6'} = 6.6$ Hz, H-5 of ceramide), and were good agreement with those of the natural sample reported by Yu et al.[27]



	R^1	R^2	R^3	R^4
17	OSE	H	Bn	H
18	OSE	H	Ac	Ac
19	OH, H		Ac	Ac
20	H	$OC(=NH)CCl_3$	Ac	Ac



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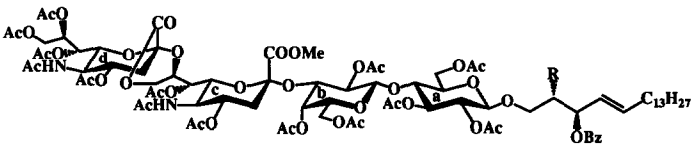
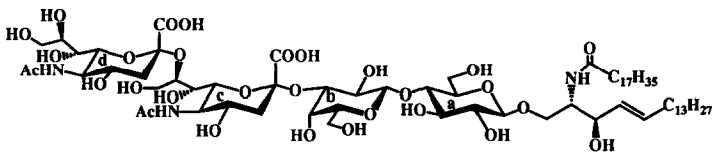


	R
22	N_3
23	$NHCOC_{17}H_{35}$



24 Ganglioside GD3

In summary, a facile, stereocontrolled total synthesis of ganglioside GD_3 was accomplished. The work showed that the use of the phenyl 2-thioglycoside derivative **3** of α-sialyl-(2 → 8)-sialic acid in the presence of NIS–TfOH in acetonitrile solution is effective for obtaining the α-glycosides, and the α-glycosides described herein could be used as the intermediates in the synthesis of polysialogangliosides.

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. ^1H NMR spectra were recorded at 270 and 400 MHz with JEOL JNM-GX spectrometers and at 500 MHz with a Varian VXR-500S spectrometer. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted in vacuo.

*Methyl 5-acetamido-2,4,7-tri-*O*-acetyl-3,5-dideoxy-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylono-1',9-lactone)-*D*-glycero-*D*-galacto-2-nonulopyranosonate* (**2**).—To a suspension of *O*-(5-acetamido-3,5-dideoxy-*D*-*glycero*-α-*D*-*galacto*-2-nonulpyransylonic acid)-(2 → 8)-5-acetamido-3,5-dideoxy-*D*-*glycero*-*D*-*galacto*-2-nonulopyranosonic acid[19] (**1**, 5.0 g, 8.3 mmol) in dry MeOH (300 mL) was added Amberlite IR-120 (H^+) resin (20 g) and the mixture was stirred for 2 days at 40°C; the progress of the reaction was monitored by TLC. The resin was filtered off, and washed with MeOH. The filtrate and washings were combined and concentrated. To a suspension of the residue in Ac_2O (15 mL) was added dropwise pyridine (15 mL) at 0°C, and the mixture was stirred for 24 h at 40°C. After the addition of MeOH (10 mL) at 0°C the mixture was concentrated, and the residue extracted with CH_2Cl_2. The extract was washed with 2 M HCl and M Na_2CO_3, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 CH_2Cl_2–MeOH) of the residue on silica gel (300 g) gave **2** (6.2 g, 84%) as an amorphous mass; ν 3300 (NH), 1740 and 1220 (ester), 1660 and 1530 cm^{-1} (amide); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.90 and 1.96 (2 s, 6 H, 2 AcN), 2.00, 2.04, 2.06, 2.12, 2.13, 2.17 and 2.18 (7 s, 21 H, 7 AcO), 2.49 (dd, 1 H, $J_{3ax,3eq}$ 13.0, $J_{3eq,4}$ 5.1 Hz, H-3b*eq*), 2.53 (dd, 1 H, $J_{3ax,3eq}$ 13.2, $J_{eq,4}$ 5.1 Hz, H-3a*eq*), 3.81 (s, 3 H, MeO), 5.18 (d, 1 H, $J_{7,8}$ 8.4 Hz, H-7a), 5.24 (m, 1 H, H-4b), and 5.34 (d, 1 H, $J_{7,8}$ 6.8 Hz, H-7b); the anomeric ratio (α:β) was estimated as ~1:10 from the ratio of intensity of the methyl ester signals. Anal. Calcd for $C_{37}H_{50}N_2O_{23}$ (890.8): C, 49.80; H, 5.66; N, 3.14. Found: C, 49.75; H, 5.83; N, 2.96.

*Methyl [phenyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylano-1',9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-*D*-glycero-*D*-galacto-2-nonulopyranosid]onate* (**3**).—To a solution of **2** (1.0 g, 1.1 mmol) in dry CH_2Cl_2 (10 mL) were added thiophenol (0.5 mL, 4.4 mmol) and boron trifluoride etherate (2 mL, 8.8 mmol), and the mixture was stirred for 12 h at room temperature; the progress of the reaction was monitored by TLC. Dichloromethane (50 mL) was added, and the solution was washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated to a syrup which was chromatographed on a column of silica gel with 50:1 CH_2Cl_2–MeOH to give **3** (940 mg, 89%) as an amorphous mass; ν 3300 (NH), 1740 and 1220 (ester), 1660 and 1530 (amide), and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.89, 1.95 (2 s, 6 H, 2 AcN), 2.01, 2.03, 2.05, 2.06, 2.07 and 2.16 (6 s, 18 H, 6 AcO), 2.41 (dd, 1 H, $J_{3ax,3eq}$ 13.4, $J_{3eq,4}$ 5.5 Hz, H-3b*eq*), 2.70 (dd, 1 H, $J_{3ax,3eq}$ 14.1, $J_{3eq,4}$ 5.1 Hz, H-3a*eq*), 3.63 (s, 3 H, MeO), 5.08 (m, 1 H, H-4a), 5.33 (dd, 1 H, $J_{6,7}$ 1.8, $J_{7,8}$ 9.2 Hz, H-7a), and 7.29–7.57 (m, 5 H, Ph); the anomeric ratio (α:β) was estimated as ~1:3 from the ratio of intensity of the methyl ester signals. Anal. Calcd for $C_{41}H_{52}N_2O_{21}S$ (940.9): C, 52.34; H, 5.57; N, 2.98. Found: C, 52.31; H, 5.27; N, 2.90.

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)-6-O-*

benzoyl-β-D-galactopyranoside (**8**) *and the corresponding β-glycoside* (**12**).—To a solution of **3** (600 mg, 0.65 mmol) and 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside[11] (**4**, 750 mg, 2.0 mmol) in MeCN (2 mL) was added molecular sieves (2.5 g), and the mixture was stirred for 5 h at room temperature, then cooled to −35°C. To the cooled mixture were added, with stirring, *N*-iodosuccinimide (NIS, 300 mg, 1.3 mmol) and trifluoromethanesulfonic acid (TfOH, 23 μL, 0.13 mmol), and the stirring was continued for 2 days at −35°C; the progress of the reaction was monitored by TLC. The solids were filtered off and washed with CH_2Cl_2. The combined filtrate and washings were washed with M Na_2CO_3 and M $Na_2S_2O_3$, dried (Na_2SO_4), and concentrated. Column chromatography (40:1 CH_2Cl_2–MeOH) of the residue on silica gel (60 g) gave **8** (250 mg, 32%) and **12** (70 mg, 9%). Compound **8** had $[\alpha]_D$ −21.8° (*c* 1.1, $CHCl_3$); *ν* 3600–3100 (OH, NH), 1730 and 1220 (ester), 1650 and 1540 (amide), 860 and 840 (Me_3Si), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) at 400 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.91, 1.95 (2 s, 6 H, 2 AcN), 2.04–2.16 (6 s, 18 H, 6 AcO), 2.49 (dd, 1 H, $J_{3ax,3eq}$ 13.4, $J_{3eq,4}$ 5.5 Hz, H-3c*eq*), 2.73 (dd, 1 H, $J_{3ax,3eq}$ 13.4, $J_{3eq,4}$ 4.9 Hz, H-3b*eq*), 3.65 and 4.12 (2 m, 2 H, $Me_3SiCH_2CH_2$), 3.72 (dd, 1 H, $J_{1,2}$ 7.3, $J_{2,3}$ 9.2 Hz, H-2a), 3.83 (s, 3 H, MeO), 3.95 (t, 1 H, $J_{4,5} = J_{5,6} = 9.2$ Hz, H-5b), 3.95 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 3.1 Hz, H-3a), 4.04 (dd, 1 H, $J_{8,9}$ 4.9, $J_{9,9'}$ 12.8 Hz, H-9c), 4.20 (t, 1 H, $J_{4,5} = J_{5,6} = 10.4$ Hz, H-5c), 4.28 (dd, 1 H, $J_{8,9'}$ 2.4, $J_{9,9'}$ 12.8 Hz, H-9′c), 4.36 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1a), 4.38 (dd, 1 H, $J_{8,9}$ 3.7, $J_{9,9'}$ 12.2 Hz, H-9b), 4.52 (m, 1 H, H-9′b), 4.54 (dd, 1 H, $J_{5,6}$ 7.3, $J_{6,6'}$ 11.6 Hz, H-6a), 4.65 (dd, 1 H, $J_{5,6'}$ 4.9, $J_{6,6'}$ 11.6 Hz, H-6′a), 5.14 (d, 1 H, $J_{7,8}$ 8.5 Hz, H-7b), 5.09–5.17 (m, 2 H, H-4b and H-8c), 5.36 (dd, 1 H, $J_{6,7}$ 1.8, $J_{7,8}$ 9.1 Hz, H-7c), 5.43 (m, 1 H, H-4c), and 7.42–8.05 (m, 5 H, Ph). Anal. Calcd for $C_{53}H_{74}N_2O_{28}Si$ (1215.3): C, 52.38; H, 6.14; N, 2.31. Found: C, 52.13; H, 6.10; N, 2.60.

Compound **12** had $[\alpha]_D$ −15.1° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.92 and 1.95 (2 s, 6 H, 2 AcN), 1.97–2.17 (6 s, 18 H, 6 AcO), 2.48 (m, 2 H, H-3b*eq* and H-3c*eq*), 3.82 (s, 3 H, MeO), 5.40 (m, 2 H, H-4b and H-4c), and 7.29–8.14 (m, 5 H, Ph). Anal. Calcd for $C_{53}H_{74}N_2O_{28}Si$ (1215.3): C, 52.38; H, 6.14; N, 2.31. Found: C, 52.25; H, 6.30; N, 2.30.

*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)-2,4,6-tri-O-benzoyl-β-*D*-galactopyranoside* (**9**).—To a solution of **8** (200 mg, 0.165 mmol) in pyridine (1.0 mL) were added benzoic anhydride (150 mg, 0.66 mmol) and 4-dimethylaminopyridine (23 mg, 0.165 mmol), and the mixture was stirred for 10 h at room temperature. Methanol (1 mL) was added to the mixture and it was concentrated, then the residue was extracted with CH_2Cl_2. The extract was washed with 2 M HCl and water, dried (Na_2SO_4), and concentrated. Column chromatography (100:1 CH_2Cl_2–MeOH) of the residue on silica gel (30 g) gave **9** (180 mg, 77%) as an amorphous mass; $[\alpha]_D$ −5.6° (*c* 0.7, $CHCl_3$); *ν* 3300 (NH), 1730 and 1220 (ester), 1650 and 1540 (amide), 860 and 840 (Me_3Si), and 710 cm^{-1}

(Ph); ^{1}H NMR ($CDCl_3$) at 270 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.95–2.17 (8 s, 24 H, 2 AcN and 6 AcO), 2.58 (dd, 1 H, $J_{3ax,3eq}$ 12.1, $J_{3eq,4}$ 5.6 Hz, H-3b*eq*), 3.33 (s, 3 H, MeO), 4.36 (dd, 1 H, $J_{8,9}$ 3.2, $J_{9,9'}$ 13.3 Hz, H-9b), 4.46 (dd, 1 H, $J_{5,6}$ 5.2, $J_{6,6'}$ 11.8 Hz, H-6a), 4.64 (dd, 1 H, $J_{8,9'}$ 6.9, $J_{9,9'}$ 13.3 Hz, H-9'b), 4.70 (dd, $J_{5,6'}$ 4.0, $J_{6,6'}$ 11.8 Hz, H-6'a), 4.75 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 5.01 (d, 1 H, $J_{7,8}$ 9.2 Hz, H-7b), 5.25 (m, 2 H, H-4b and H-8c), 5.42 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 8.6 Hz, H-7c), 5.62 (t, 1 H, $J_{1,2} = J_{2,3} = 8.1$ Hz, H-2a), 5.66 (m, 1 H, H-4c), 5.82 (d, 1 H, $J_{3,4}$ 4.0 Hz, H-4a), and 7.36–8.02 (m, 15 H, 3 Ph). Anal. Calcd for $C_{67}H_{82}N_2O_{30}Si$ (1423.5): C, 56.53; H, 5.81; N, 1.97. Found: C, 56.50; H, 5.61; N, 1.89.

*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)-1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-β-*D*-galactopyranose* (**10**).—To a solution of **9** (180 mg, 0.125 mmol) in toluene (2 mL) and Ac_2O (0.2 mL) was added boron trifluoride etherate (60 μL, 0.25 mmol), and the mixture was stirred for 24 h at room temperature. After dilution with CH_2Cl_2 (50 mL) the solution was washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (40:1 CH_2Cl_2–MeOH) of the residue on silica gel (30 g) gave **10** (142 mg, 82%) as an amorphous solid; $[\alpha]_D$ +7.7° (*c* 0.9, $CHCl_3$); ν 3300 (NH), 1730 and 1220 (ester), 1650 and 1540 (amide), and 710 cm^{-1} (Ph). ^{1}H NMR ($CDCl_3$) at 270 MHz: δ 1.94–2.18 (9 s, 27 H, 2 AcN and 7 AcO), 2.40 (dd, 1 H, $J_{3ax,3eq}$ 11.0, $J_{3eq,4}$ 4.1 Hz, H-3c*eq*), 2.67 (dd, 1 H, $J_{3ax,3eq}$ 11.8, $J_{3eq,4}$ 4.0 Hz, H-3b*eq*), 3.50 (s, 3 H, MeO), 5.26 (m, 2 H, H-4b and H-8c), 5.41 (d, 1 H, $J_{7,8}$ 8.4 Hz, H-7b), 5.53 (dd, $J_{6,7}$ 3.3, $J_{7,8}$ 10.6 Hz, H-7c), 5.62 (m, 1 H, H-4c), 5.73 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-4a), 5.80 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1a), and 7.23–8.19 (m, 15 H, 3 Ph). Anal. Calcd for $C_{64}H_{72}N_2O_{31}$ (1365.3): C, 56.30; H, 5.31; N, 2.05. Found: C, 56.16; H, 5.48; N, 1.87.

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* (**11**).—To a solution of **10** (140 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) were added methylthiotrimethylsilane (35 μL, 0.25 mmol) and boron trifluoride etherate (25 μL, 0.1 mmol) at 0°C, and the mixture was stirred for 4 h at room temperature. After dilution with CH_2Cl_2 (50 mL) the solution was 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 (25 g) with 40:1 CH_2Cl_2–MeOH to give **11** (106 mg, 76%) as an amorphous solid; $[\alpha]_D$ +10.7° (*c* 1.4, $CHCl_3$); ν 3400 (NH), 1750 and 1230 (ester), 1670 and 1540 (amide), and 710 cm^{-1} (Ph); ^{1}H NMR ($CDCl_3$) at 270 MHz: δ 1.86–2.10 (9 s, 27 H, 2 AcN, 6 AcO, and MeS), 2.33 (dd, 1 H, $J_{3ax,3eq}$ 12.2, $J_{3eq,4}$ 4.4 Hz, H-3c*eq*), 2.68 (dd, 1 H, $J_{3ex,3eq}$ 13.0, $J_{3eq,4}$ 4.8 Hz, H-3b*eq*), 3.43 (s, 3 H, MeO), 5.06 (d, 1 H, $J_{7,8}$ 10.0 Hz, H-7b), 5.36 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1a), 5.46 (dd, 1 H, $J_{6,7}$ 3.7, $J_{7,8}$ 9.6 Hz, H-7c), 5.56 (d, 1 H, $J_{3,4}$ 4.0 Hz, H-4a), and 7.28–8.11 (m, 15 H, 3 Ph). Anal. Calcd for $C_{63}H_{72}N_2O_{29}S$ (1353.3): C, 55.91; H, 5.36; N, 2.07. Found: C, 56.13; H, 5.10; N, 2.33.

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)-3-*O-*benzoyl-β-D-galactopyranoside* **(13)** *and the corresponding β-glycoside* **(15)**.—Coupling of **3** (300 mg, 0.32 mmol) with 2-(trimethylsilyl)ethyl 3-*O*-benzoyl-β-D-galactopyranoside[11,18] (**5**, 250 mg, 0.64 mmol) in the presence of NIS (150 mg) and TfOH (12 μL) in dry MeCN (1 mL) as described for **8**, gave **13** (128 mg, 33%) and **15** followed by chromatography of the amorphous residue, (39 mg, 10%). Compound **13** had $[\alpha]_D$ −18.6° (*c* 0.4, $CHCl_3$); ν 3600–3100 (OH, NH), 1730 and 1220 (ester), 1650 and 1540 (amide), 860 and 840 (Me_3Si), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) at 400 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.85, 1.88 (2 s, 6 H, 2 AcN), 1.98–2.16 (6 s, 18 H, 6 AcO), 2.41 (dd, 1 H, $J_{3ax,3eq}$ 13.4, $J_{3eq,4}$ 5.5 Hz, H-3c*eq*), 2.58 (dd, 1 H, $J_{3ax,3eq}$ 12.8, $J_{3eq,4}$ 4.9 Hz, H-3b*eq*), 3.60, 4.00 (2 m, 2 H, $Me_3SiCH_2CH_2$), 3.71 (dd, 1 H, $J_{5,6}$ 10.4, $J_{6,7}$ 1.8 Hz, H-6c), 3.80 (s, 3 H, MeO), 4.11 (dd, 1 H, $J_{5,6}$ 10.4, $J_{6,7}$ 1.2 Hz, H-6b), 4.13 (t, 1 H, $J_{4,5} = J_{5,6} = 10.4$ Hz, H-5c), 4.21 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 4.24 (dd, 1 H, $J_{8,9}$ 2.4, $J_{9,9'}$ 14.7 Hz, H-9c), 4.36 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1a), 4.48 (dd, 1 H, $J_{8,9}$ 4.3, $J_{9,9'}$ 12.2 Hz, H-9b), 5.02 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.1 Hz, H-3a), 5.04–5.14 (m, 2 H, H-4b and H-8c), 5.25 (dd, 1 H, $J_{6,7}$ 1.2, $J_{7,8}$ 5.5 Hz, H-7b), 5.31 (dd, 1 H, $J_{6,7}$ 1.8, $J_{7,8}$ 9.8 Hz, H-7c), 5.38 (m, 1 H, H-4c), and 7.39–8.08 (m, 5 H, Ph). Anal. Calcd for $C_{57}H_{74}N_2O_{28}Si$ (1215.3): C, 52.38; H, 6.14; N, 2.31. Found: C, 52.25; H, 6.42; N, 2.19.

Compound **15** had $[\alpha]_D$ −26.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.88, 1.92 (2 s, 6 H, 2 AcN), 2.00–2.20 (6 s, 18 H, 6 AcN), 2.45 (m, 2 H, H-3b*eq* and H-3c*eq*), 3.85 (s, 3 H, MeO), 5.10 (m, 1 H, H-8c), 5.38 (m, 2 H, H-4b and H-4c), and 7.29–8.18 (m, 5 H, Ph). Anal. Calcd for $C_{57}H_{74}N_2O_{28}Si$ (1215.3): C, 52.38; H, 6.14; N, 2.31. Found: C, 52.47; H, 6.01; N, 2.30.

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)-3-*O-*benzyl-β-D-galactopyranoside* **(14)** *and the corresponding β-glycoside* **(16)**.—Coupling of **3** (300 mg, 0.32 mmol) with 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside[18] (**6**, 310 mg, 0.84 mmol) in the presence of NIS (150 mg) and TfOH (12 μL) in dry MeCN (1 mL) as described for **8**, followed by chromatography of the amorphous residue, gave the α-glycoside **14** (183 mg, 48%) and the corresponding β-glycoside **16** (61 mg, 16%).

Compound **14** had $[\alpha]_D$ −23.5° (*c* 1.2, $CHCl_3$); ν 3600–3100 (OH, NH), 1730 and 1210 (ester), 1650 and 1540 (amide), 860 and 840 (Me_3Si), and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.88, 1.90 (2 s, 6 H, 2 AcN), 2.01–2.18 (6 s, 18 H, 6 AcO), 2.46 (dd, 1 H, $J_{3ax,3eq}$ 12.9, $J_{3eq,4}$ 5.7 Hz, H-3c*eq*), 2.62 (dd, 1 H, $J_{3ax,3eq}$ 12.6, $J_{3eq,4}$ 5.3 Hz, H-3b*eq*), 3.40 (dd, 1 H, $J_{2,3}$ 9.4, $J_{3,4}$ 3.3 Hz, H-3a), 3.81 (s, 3 H, MeO), 4.21 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1a), 5.02 (m, 1 H, H-4b), 5.14 (m, 1 H, H-8c), 5.43 (m, 1 H, H-4c), and 7.27–7.39 (m, 5 H, Ph). Anal.

Calcd for $C_{53}H_{76}N_2O_{27}Si$ (1201.3): C, 52.99; H, 6.38; N, 2.33. Found: C, 52.79; H, 6.10; N, 2.35.

Compound **16** had $[\alpha]_D$ −32.0° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.88, 1.92 (2 s, 6 H, 2 AcN), 2.01–2.20 (6 s, 18 H, 6 AcO), 2.43 (dd, 1 H, $J_{3ax,3eq}$ 14.4, $J_{3eq,4}$ 5.6 Hz, H-3c*eq*), 3.43 (dd, 1 H, $J_{2,3}$ 9.4, $J_{3,4}$ 3.7 Hz, H-3a), 3.85 (s, 3 H, MeO), 4.20 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1a), 5.09 (m, 1 H, H-8c), 5.44 (m, 1 H, H-4c), 5.53 (m, 1 H, H-4b), and 7.29–7.46 (m, 5 H, Ph). Anal. Calcd for $C_{53}H_{76}N_2O_{27}Si$ (1201.3): C, 52.99; H, 6.38; N, 2.33. Found: C, 52.71; H, 6.60; N, 2.29.

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-*(2,6-di-*O-*benzyl-*β*-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O-*benzyl-*β*-D-glucopyranoside* (**17**).—Coupling of **3** (300 mg, 0.32 mmol) with 2-(trimethylsilyl)ethyl *O*-(2,6-di-*O*-benzyl-*β*-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside[14] (**7**, 570 mg, 0.64 mmol) in the presence of NIS (150 mg) and TfOH (12 μL) in dry MeCN (1 mL), as described for **8**, gave the tetrasaccharide **17** (170 mg, 31%) as an amorphous solid; $[\alpha]_D$ −12.3° (*c* 0.7, $CHCl_3$); ν 3600–3100 (OH, NH), 1730 and 1220 (ester), 1650 and 1540 (amide), 860 and 840 (Me_3Si), and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.86, 1.87 (2 s, 6 H, 2 AcN), 1.94–2.09 (6 s, 18 H, 6 AcO), 2.40 (m, 2 H, H-3b*eq* and H-3c*eq*), 3.80 (s, 3 H, MeO), 5.08 (m, 1 H, H-4b), 5.17 (m, 1 H, H-8c), 5.35 (m, 2 H, H-4c and H-7c), and 7.20–7.37 (m, 25 H, 5 Ph). Anal. Calcd for $C_{87}H_{110}N_2O_{32}Si$ (1723.9): C, 60.62; H, 6.43; N, 1.63. Found: C, 60.52; H, 6.59; N, 1.64.

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-*acetyl-*β*-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O-*acetyl-*β*-D-glucopyranoside* (**18**).—A solution of **17** (100 mg, 0.058 mmol) in MeOH (10 mL) and acetic acid (10 mL) was hydrogenolyzed in the presence of 10% Pd–C (100 mg) for 2 days at 45°C; the progress of the reaction was monitored by TLC. The solids were then filtered off and washed with MeOH. The combined filtrate and washings were concentrated, and the residue was acetylated with Ac_2O (1 mL) and pyridine (2 mL) for 12 h at 45°C. The product was purified by column chromatography (30:1 CH_2Cl_2–MeOH) on silica gel (20 g) to give **18** (80 mg, 90%) as an amorphous solid; $[\alpha]_D$ −5.0° (*c* 1.8, $CHCl_3$); ν 3300 (NH), 1730 and 1220 (ester), 1650 and 1540 (amide), and 860 and 840 (Me_3Si); ^1H NMR ($CDCl_3$) at 500 MHz: δ 0.90 (m, 2 H, $Me_3SiCH_2CH_2$), 1.52, 1.55 (2 s, 6 H, 2 AcN), 1.75 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4} =$ 12.5 Hz, H-3c*ax*), 2.02–2.18 (12 s, 36 H, 12 AcO), 2.52 (dd, 1 H, $J_{3ax,3eq}$ 12.5, $J_{3eq,4}$ 5.0 Hz, H-3c*eq*), 2.54 (dd, 1 H, $J_{3ax,3eq}$ 14.0, $J_{3eq,4}$ 5.5 Hz, H-3d*eq*), 3.56, 3.96 (2 m, 2 H, $Me_3SiCH_2CH_2$), 3.60 (m, 1 H, H-5a), 3.72 (dd, 1 H, $J_{5,6}$ 10.5, $J_{6,7}$ 2.5 Hz, H-6c), 3.82 (dd, 1 H, $J_{5,6}$ 10.5, $J_{6,7}$ 2.0 Hz, H-6d), 3.86 (s, 3 H, MeO), 4.22 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH} =$ 10.5 Hz, H-5c), 4.22 (dd, 1 H, $J_{5,6}$ 6.0, $J_{6,6'}$ 11.5 Hz, H-6a), 4.31

(dd, 1 H, $J_{8,9}$ 3.0, $J_{9,9'}$ 13.0 Hz, H-9d), 4.32 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.5 Hz, H-3b), 4.40 (dd, 1 H, $J_{8,9}$ 3.0, $J_{9,9'}$ 11.5 Hz, H-9c), 4.44 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1a), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1b), 4.55 (dd, 1 H, $J_{8,9'}$ 10.0, $J_{9,9'}$ 11.5 Hz, H-9'c), 4.86 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 9.5 Hz, H-2a), 4.95 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2b), 5.08 (m, 1 H, H-4c), 5.11 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4b), 5.15 (m, 1 H, H-8d), 5.19 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 8.5 Hz, H-7c), 5.19 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3a), 5.38 (dd, 1 H, $J_{6,7}$ 2.0, $J_{7,8}$ 9.0 Hz, H-7d), and 5.42 (m, 1 H, H-4d). Anal. Calcd for $C_{64}H_{92}N_2O_{32}Si$ (1525.5): C, 50.39; H, 6.08; N, 1.84. Found: C, 50.36; H, 5.78; N, 1.76.

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*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-*D*-glucopyranose* (**19**).—To a solution of **18** (75 mg, 0.05 mmol) in dry CH_2Cl_2 (0.6 mL) was added trifluoroacetic acid (1.3 mL), and the mixture was stirred for 1.5 h at room temperature then concentrated. Column chromatography (30:1 CH_2Cl_2–MeOH) of the residue on silica gel (20 g) gave **19** (63 mg, 90%) as an amorphous solid; ν 3600–3100 (OH, NH), 1730 and 1220 (ester), and 1650 and 1540 cm^{-1} (amide). Anal. Calcd for $C_{59}H_{80}N_2O_{38}$ (1425.3): C, 49.72; H, 5.66; N, 1.97. Found: C, 49.52; H, 5.58; 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*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate* (**20**).—To a solution of **19** (60 mg, 0.042 mmol) in dry CH_2Cl_2 (1 mL), cooled to 0°C, were added trichloroacetonitrile (0.2 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (10 μL, 0.05 mmol). The mixture was stirred for 2 h at 0°C, with the progress of the reaction monitored by TLC. The mixture was chromatographed on column of silica gel (20 g) with 25:1 CH_2Cl_2–MeOH to give **20** (54 mg, 86%) as an amorphous solid; $[\alpha]_D$ +27.5° (*c* 1.1, $CHCl_3$); ν 3300 (NH), 1730 and 1220 (ester), and 1650 and 1540 cm^{-1} (amide); ^1H NMR ($CDCl_3$) at 270 MHz: δ 1.96–2.25 (14 s, 42 H, 2 AcN and 12 AcO), 2.60 (m, 2 H, H-3c*eq* and H-3d*eq*), 3.93 (s, 3 H, MeO), 5.11 (dd, 1 H, $J_{1,2}$ 3.8, $J_{2,3}$ 9.9 Hz, H-2a), 5.20 (m, 1 H, H-4c), 5.48 (m, 1 H, H-4d), 6.55 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1a), and 8.73 (s, 1 H, C=NH). Anal. Calcd for $C_{61}H_{83}Cl_3N_3O_{38}$ (1572.4): C, 49.59; H, 5.32; N, 2.67. Found: C, 46.45; H, 5.49; N, 2.49.

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*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 1)-(2S,3R,4E)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol* (**22**).—To a solution of **20** (120 mg, 0.076 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[25] (**21**, 60 mg, 0.15 mmol) in dry CH_2Cl_2 (1 mL) was added 4A molecular sieves (2 g), and the mixture was stirred for 6 h at room temperature, then cooled to −20°C. Boron trifluoride etherate (20 μL) was added and the mixture was stirred for a further 6

h at −20°C; the course of the reaction was monitored by TLC. The solids were filtered off and washed with CH_2Cl_2, and the combined filtrate and washings were concentrated. Column chromatography (25:1 CH_2Cl_2–MeOH) of the residue on silica gel (20 g) gave **22** (84 mg, 60%) as an amorphous solid; $[\alpha]_D$ −1.6° (*c* 1.1, $CHCl_3$); ν 3300 (NH), 2100 (N_3), 1730 and 1220 (ester), 1650 and 1540 (amide), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) at 270 MHz: δ 0.88 (t, 3 H, J_{Me,CH_2} 6.2 Hz, CH_3CH_2), 1.24 (s, 22 H, 11 CH_2), 1.89–2.21 (14 s, 42 H, 2 AcN and 12 AcO), 2.60 (m, 2 H, H-3c*eq* and H-3d*eq*), 3.86 (s 3 H, MeO), 4.95 (t, 1 H, $J_{1,2} = J_{2,3} = 9.1$ Hz, H-2a), 5.03 (m, 1 H, H-4c), 5.51 (m, 1 H, H-4d), 5.92 (dt, 1 H, $J_{4,5}$ 14.5, $J_{5,6} = J_{5,6'} = 6.8$ Hz, H-5 of sphingosine), and 7.23–8.06 (m, 5 H, Ph). Anal. Calcd for $C_{84}H_{120}N_5O_{40}$ (1839.9): C, 54.83; H, 6.57; N, 3.80. Found: C, 54.71; H, 6.70; N, 3.69.

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*-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* (**23**).—Hydrogen sulfide was bubbled through a stirred solution of **22** (56 mg, 0.03 mmol) in aq 83% pyridine (7.2 mL) for 3 days at 0°C with the progress of the reaction monitored by TLC. The mixture was concentrated and the residue was dissolved in CH_2Cl_2 (1 mL). Octadecanoic acid (25 mg, 0.1 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (18 mg, 0.1 mmol) were added to the solution, and the mixture was stirred for 12 h at room temperature. After completion of the reaction, CH_2Cl_2 (30 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 (20 g) with 30:1 CH_2Cl_2–MeOH, to give **23** (53 mg, 84%) as an amorphous solid; $[\alpha]_D$ +10.0° (*c* 1.1, $CHCl_3$); ν 3300 (NH), 1730 and 1220 (ester), 1650 and 1540 (amide), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) at 270 MHz: δ 0.88 (t, 6 H, J_{Me,CH_2} 6.8 Hz, 2 CH_3CH_2), 1.25 (s, 52 H, 26 CH_2), 1.87–2.21 (14 s,42 H, 2 AcN and 12 AcO), 2.60 (m 2 H, H-3c*eq* and H-3d*eq*), 3.85 (s, 3 H, MeO), 5.03 (m, 1 H, H-4c), 5.85 (dt, 1 H, $J_{4,5}$ 14.3, $J_{5,6} = J_{5,6'} = 6.7$ Hz, H-5 of ceramide), and 7.27–8.02 (m, 5 H, Ph). Anal. Calcd for $C_{102}H_{156}N_3O_{41}$ (2080.4): C, 57.09; H, 6.00; N, 1.98. Found: C, 58.75; H, 7.72; N, 1.83.

Ganglioside GD$_3$ (**24**).—To a solution of **23** (53 mg, 0.025 mmol) in MeOH (2 mL) was added NaOMe (20 mg), and the mixture was stirred for 24 h at room temperature, with the course of the reaction monitored by TLC. Water (0.5 mL) was added and the mixture was again stirred for 24 h at room temperature, then neutralized with Amberite 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 (20 g) gave **24** (38 mg, quantitative) as an amorphous solid; $[\alpha]_D$ −2.6° (*c* 0.8, 1:1 $CHCl_3$–MeOH); ^1H NMR (1:1 $CDCl_3$–CD_3OD) at 400 MHz: δ

0.80 (t, 6 H, J_{Me,CH_2} 27.0 Hz, 2 CH_3CH_2), 1.18 (s, 52 H, 26 CH_2), 1.93, 1.94 (2 s, 6 H, 2 AcN), 2.08 (t, 2 H, $COCH_2CH_2$), 2.52 and 2.85 (2 m, 2 H, H-3c*eq* and H-3d*eq*), 4.20 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 4.35 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1b), 5.35 (dd, 1 H, $J_{3,4}$ 7.7, $J_{4,5}$ 15.4 Hz, H-4 of ceramide), 5.60 (dt, 1 H, $J_{4,5}$ 15.4, $J_{5,6} = J_{5,6'} = 6.6$ Hz, H-5 of ceramide). Anal. Calcd for $C_{70}H_{125}N_3O_{29}$ (1473.8): C, 57.08; H, 8.55; N, 2.85. Found: C, 57.17; H, 8.35; N, 2.95.

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