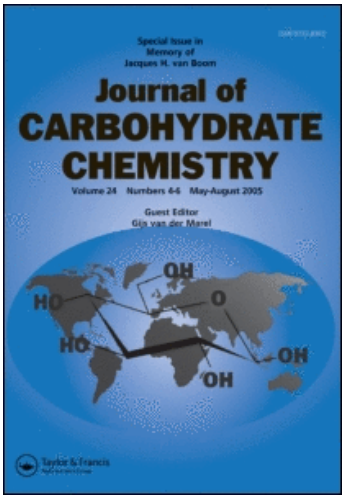

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 27: SYNTHESIS OF SIALYL-α(2→6)-LEWIS X

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ABSTRACT

Synthesis of a positional isomer of sialyl Lewis X with regard to the substitution of the terminal galactose residue of the pentasaccharide by N-acetylneuraminic acid is described. Dimethyl(methylthio)sulfonium triflate-promoted coupling of 2-(trimethylsilyl)ethyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-O-(2-acetamido-6-O-benzyl-2-deoxy-ß-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-ß-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-ß-D-glucopyranoside (1) with methyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,4-di-O-benzoyl-3-O-benzyl-1-thio-ß-D-galactopyranoside (2) gave the desired hexasaccharide 3. Compound 3 was converted into the α-trichloroacetimidate 6, via reductive removal of the benzyl groups, O-acetylation, removal of the 2-(trimethylsilyl)ethyl group, and treatment with trichloroacetonitrile, which, on coupling with (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (7), gave the ß-glycoside 8. Finally, 8 was transformed, via selective reduction of the azide group, coupling with octadecanoic acid, O-deacylation, and hydrolysis of the methyl ester group, into the title ganglioside 11 in good yield.

INTRODUCTION

Sialyl Lewis X was first isolated[1] from human kidney and found[2] to be widespread as the tumor-associated ganglioside antigen. Very recently, it has been demonstrated[3-5] that endothelial leukocyte adhesion molecule-1 (ELAM-1) recognizes sialyl Lewis X determinant, Neu5Acα(2→3)Galß(1→4)[Fuc α(1→3)]GlcNAc-, which is found as the terminal carbohydrate structure in both glycolipids and glycoproteins. Binding of myeloid cells to soluble ELAM-1 is inhibited by a monoclonal antibody recognizing sialyl Lewis X or proteins bearing sialyl Lewis X, indicating the carbohydrates may be

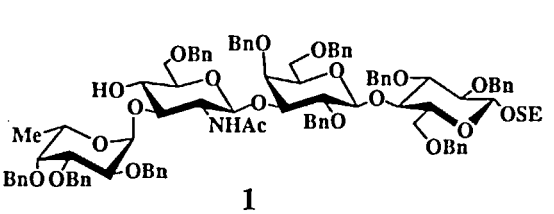
capable of blocking white cell from attacking ELAM-1 receptors, thereby reducing inflamation. In view of these facts, it is of interest to elucidate the structural requirements for expressing such functions.

Previously, we have synthesized sialyl Lewis X[6] and the analogs[7,8] by use of the methyl ß-thioglycosides of sialyl α(2→3)- and sialyl α(2→6)-galactoses as the glycosyl donors which are easily prepared according to our newly developed α-glycosylation of sialic acid.[7-12] We describe here the synthesis of sialyl α(2→6)-Lewis X, a positional isomer of sialyl Lewis X with regard to the substitution of the terminal galactose moiety of the oligosaccharide by Neu5Ac.

RESULTS AND DISCUSSION

The glycosylation of the suitably protected tetrasaccharide[6] 1 with methyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,4-di-O-benzoyl-3-O-benzyl-1-thio-ß-D-galactopyranoside[8] (2), in the presence of dimethyl(methylthio)-sulfonium triflate[13] (DMTST) as the glycosyl promoter and molecular sieves 4A (MS-4A) in dichloromethane for 24 h at 0 °C, gave the desired ß-glycoside in 38% yield. The ^{1}H NMR data were a three-proton doublet at δ 1.10 ($J_{5,6}$ = 6.4 Hz, H-6, fucose unit), two three-proton singlets at δ 1.60 and 1.85 (N-acetyl), four three-proton singlets at δ 1.93, 2.00, 2.01, and 2.07 (O-acetyl), a three-proton singlet at δ 3.74 (O-methyl), sixty five aromatic protons at δ 7.08-8.12 (Ph), and a one-proton doublet of doublets at δ 5.40 ($J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.6 Hz, H-2, Gal unit), indicating the newly formed glycosidic linkage to be ß. Catalytic hydrogenolysis (10% Pd-C) in ethanol-acetic acid (4:1) for 30 h at 45 °C of the benzyl groups in 3, and subsequent O-acetylation gave the per-O-acetyl derivative 4 in 80% yield after column chromatography. Treatment[14] of 4 with trifluoroacetic acid in dichloromethane for 1 h at room temperature afforded the 1-hydroxy compound 5 in 94% yield, which, on treatment[15] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane for 4 h at 0 °C, afforded the α-trichloroacetimidate 6 in 87% yield. Significant signals in the ^{1}H NMR spectrum of 6 were a one-proton doublet at δ 6.47 ($J_{1,2}$ = 3.7 Hz, H-1) and a one-proton singlet at δ 8.66 (C=NH), indicating the α-trichloroacetimidate formation.

The final glycosylation[15a,16] of (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol[16,17] (7) with 6 thus obtained, in dichloromethane in the presence of boron trifluoride etherate for 8 h at 0 °C, gave the expected ß-glycoside 8 in 37% yield. Selective reduction[16,18] of the azide



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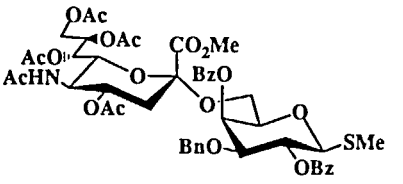

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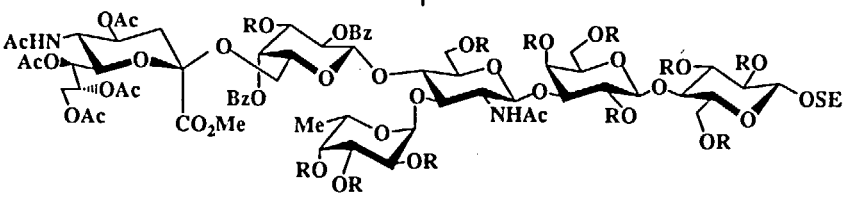
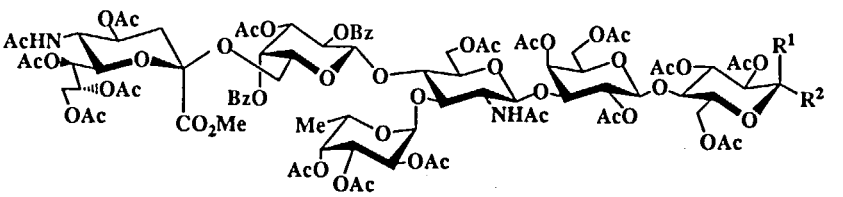


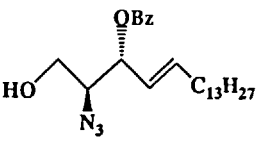
3 R = Bn

4 R = Ac



5 R^1 , R^2 = H, OH

6 R^1 = OC(=NH)CCl_3, R^2 = H



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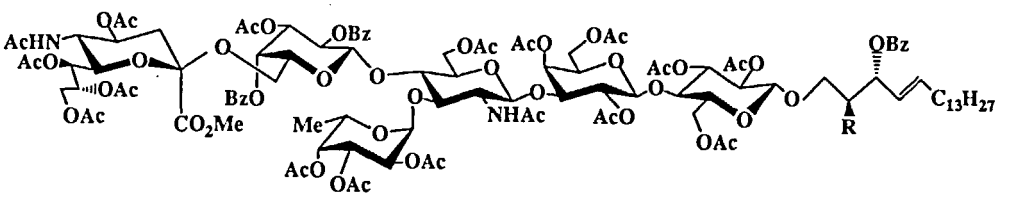
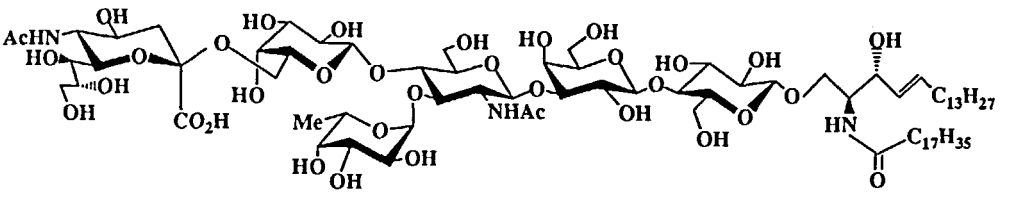


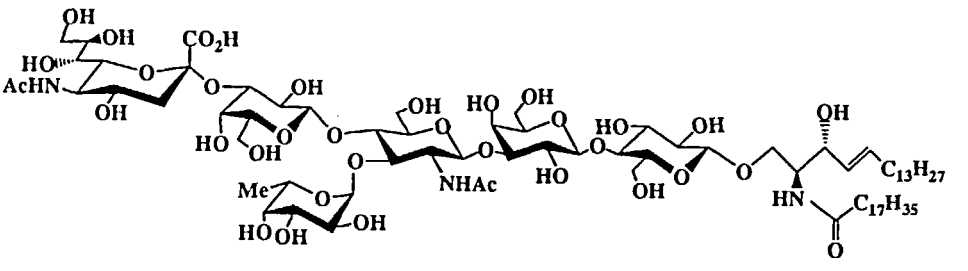
8 R = N_3

9 R = NH_2

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



11 [α(2-6)Sialyl Lewis X]



Sialyl Lewis X

group in 8 with hydrogen sulfide in aqueous pyridine for 2 days at 0 °C gave the amine 9 which, on condensation with octadecanoic acid, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) in dichloromethane, gave the acylated sialyl α(2→6)-Lewis X 10 in 85% yield, after column chromatography. Finally, O-deacylation with sodium methoxide in methanol and subsequent saponification of the methyl ester group in 10, yielded a positional isomer 11 of sialyl Lewis X in 90% yield. The ^{1}H NMR data were a one-proton doublet at δ 0.99 ($J_{5,6}$ = 6.4 Hz, H-6, fucose unit), two three-proton singlets at δ 1.80 and 1.87 (N-acetyl), a one-proton doublet of doublets at δ 2.66 (J_{gem} = 12.0 Hz, $J_{3e,4}$ = 3.6 Hz, H-3e, Neu5Ac unit), four one-proton doublets due to the ß-glycosidic linkage at δ 4.16, 4.24 (2), and 4.74 ($J_{1,2}$ = 7.5-7.7 Hz, H-1), and a one-proton doublet at δ 4.85 ($J_{1,2}$ = 3.5 Hz, H-1α, fucose unit). Other ^{1}H NMR data, given in the Experimental, are consistent with the structure 11.

EXPERIMENTAL

General Procedures. Specific rotations were determined with a Union PM-201 polarimeter at 25 °C, and IR spectra were recorded with a Jasco A-100 spectrophotometer. ^{1}H NMR spectra were recorded with a Jeol JNM-GX 270 spectrometer. 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-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-(2,4-di-O-benzoyl-3-O-benzyl-ß-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-benzyl-2-deoxy-ß-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-ß-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-ß-D-glucopyranoside (3). To a solution of 1[6] (512 mg, 0.31 mmol) and 2[8] (445 mg, 0.48 mmol) in dry dichloromethane (8 mL) was added MS-4A (1.0 g), and the mixture was stirred for 8 h at room temperature. DMTST (470 mg, 1.82 mmol) and MS-4A (450 mg) were added, and stirring was continued for 24 h at 0 °C; the progress of the reaction was monitored by TLC. Methanol (1 mL) and triethylamine (0.5 mL) were added to the mixture, and the precipitates were filtered and washed with dichloromethane. The combined filtrate and washings were washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (4:1 ethyl acetate-hexane) of the residue on silica gel (50 g) gave 3 (303 mg, 38%) as an amorphous mass; $[\alpha]_D$ -13.0° (c 0.83, chloroform); IR (KBr) 3400 (NH), 1750 and 1250 (ester), 1680 and 1530 (amide), 860 and 840 (Me_3Si), and 740 and 700 cm^{-1}

(Ph); ^{1}H NMR ($CDCl_3$) δ 0.99 (m, 2H, $Me_3SiCH_2CH_2O$), 1.11 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6, fucose unit), 1.60, 1.85 (2s, 6H, 2AcN), 1.93, 2.00, 2.01, 2.07 (4s, 12H, 4AcO), 2.53 (dd, 1H, J_{gem} = 12.9 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e, Neu5Ac unit), 3.74 (s, 3H, MeO), 5.13 (d, 1H, $J_{5,NH}$ = 9.2 Hz, NH), 5.40 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.6 Hz, H-2, Gal unit), 5.88 (broad d, 1H, H-4, Gal unit), and 7.08-8.14 (m, 65H, 13Ph).

Anal. Calcd for $C_{148}H_{168}N_2O_{39}Si$ (2627.0): C, 67.67; H, 6.45; N, 1.07. Found: C, 67.60; H, 6.51; N, 1.05.

2-(Trimethylsilyl)ethyl O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-(3-O-acetyl-2,4-di-O-benzoyl-ß-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-acetyl-2-deoxy-ß-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-ß-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-ß-D-glucopyranoside (4). A solution of 3 (304 mg, 0.116 mmol) in ethanol (40 mL) and acetic acid (10 mL) was hydrogenolysed in the presence of 10% Pd-C (300 mg) for 30 h at 45 °C, then filtered, and concentrated. The residue was acetylated with acetic anhydride (3 mL)-pyridine (5 mL) overnight at 45 °C. The product was purified by chromatography on a column of silica gel (40 g) with 50:1 dichloromethane-methanol, to give 4 (194 mg, 80%) as an amorphous mass; $[\alpha]_D$ -25.7° (c 1.1, chloroform); ^{1}H NMR ($CDCl_3$) δ 0.90 (m, 2H, $Me_3SiCH_2CH_2O$), 1.28 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6, fucose unit), 1.86, 1.87 (2s, 6H, 2AcN), 1.92-2.21 (15s, 45H, 15AcO), 2.44 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e, Neu5Ac unit), 3.71 (s, 3H, MeO), 4.27 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, Glc unit), 4.46 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, Gal residue of Lac unit), 4.54 (dd, 1H, J_{gem} = 12.4 Hz, $J_{8,9'}$ = 2.4 Hz, H-9', Neu5Ac unit), 4.80 (m, 1H, H-4, Neu5Ac unit), 4.86 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2, Gal residue of Lac unit), 5.04 (dd, 1H, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 3.8 Hz, H-3, fucose unit), 5.14 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3, Glc unit), 5.20 (broad d, 1H, H-4, Gal residue of Lac unit), 5.30 (dd, 1H, $J_{6,7}$ = 2.0 Hz, $J_{7,8}$ = 7.7 Hz, H-7, Neu5Ac unit), 5.37 (broad d, 1H, H-4, fucose unit), 5.93 (broad s, 1H, H-4, Gal unit), and 7.45-8.06 (m, 10H, 2Ph).

Anal. Calcd for $C_{93}H_{124}N_2O_{50}Si$(2098.1): C, 53.24; H, 5.96; N, 1.34. Found: C, 53.19; H, 6.12; N, 1.33.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-(3-O-acetyl-2,4-di-O-benzoyl-ß-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-acetyl-2-deoxy-ß-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-ß-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranose (5). To a solution of 4 (191 mg, 91 μmol) in dry dichloromethane (1 mL)

was added trifluoroacetic acid (2 mL), and the mixture was stirred for 1 h at room temperature and concentrated. Column chromatography of the residue on silica gel (20 g) with 20:1 dichloromethane-methanol afforded 5 (170 mg, 94%) as an amorphous mass; $[\alpha]_D$ -12.5° (c 0.93, chloroform); IR (KBr) 3400 (NH, OH), 1740 and 1220 (ester), 1680 and 1540 (amide), and 750 and 710 cm^{-1} (Ph).

Anal. Calcd for $C_{88}H_{112}N_2O_{50}$ (1997.8): C, 52.91; H, 5.65; N, 1.40. Found: C, 52.78; H, 5.74; N, 1.43.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-(3-O-acetyl-2,4-di-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-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 (6). A solution of 5 (170 mg, 80 μmol) and trichloroacetonitorile (0.26 mL) in dichloromethane (2 mL) was cooled to -5 °C, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 15 mg) was added. The mixture was stirred for 4 h at 0 °C, and concentrated. Column chromatography of the residue on silica gel (20 g) with 30:1 dichloromethane-methanol gave 6 (158 mg, 87%) as an amorphous mass; $[\alpha]_D$ -3.2° (c 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 1.28 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6, fucose unit), 1.85, 1.87 (2s, 6H, 2AcN), 1.93-2.21 (15s, 45H, 15AcO), 2.44 (dd, 1H, J_{gem} = 12.4 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e, Neu5Ac unit), 3.70 (s, 3H, MeO), 5.03 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.8 Hz, H-3, fucose unit), 5.94 (broad s, 1H, H-4, Gal unit), 6.47 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1, Glc unit), 7.44-8.03 (m, 10H, 2Ph), and 8.66 (s, 1H, C=NH).

Anal. Calcd for $C_{90}H_{112}N_3O_{50}Cl_3$ (2142.2): C, 50.46; H, 5.27; N, 1.96. Found: C, 50.43; H, 5.33; N, 1.89.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-(3-O-acetyl-2,4-di-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-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)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (8). To a solution of 6 (158 mg, 74 μmol) and (2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol[16,17] (7; 63 mg, 130 μmol) in dry dichloromethane (2 mL) was added MS-4A (AW-300; 2.0 g), and the mixture was stirred for 30 min at room temperature, and cooled to 0 °C. Boron trifluoride etherate (0.004 mL) was added, and the mixture was stirred for 8 h at 0 °C and then filtered. The insoluble material was washed with dichloromethane, and the combined filtrate and washings were successively

washed with M sodium carbonate and water, dried (Na_2SO_4), and concentrated. Chromatography (25:1 dichloromethane-methanol) of the residue on silica gel (30 g) gave 8 (65 mg, 37%) as an amorphous mass; $[\alpha]_D$ -25.2° (c 1.3, chloroform); IR (KBr) 3350 (NH), 2920 and 2850 (methyl, methylene), 2100 (azide), 1740 and 1220 (ester), 1680 and 1530 (amide), and 740 and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J_{Me,CH_2} = 6.6 Hz, $MeCH_2$), 1.24 (s, 22H, $11CH_2$), 1.85, 1.87 (2s, 6H, 2AcN), 1.91-2.21 (15s, 45H, 15AcO), 2.43 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e, Neu5Ac unit), 4.49 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1, Gal residue of Lac unit), 4.87 (dd, 1H, $J_{2,3}$ = 10.7 Hz, H-2, Gal residue of Lac unit), 5.05 (dd, 1H, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 3.8 Hz, H-3, fucose unit), 5.14 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3, Glc unit), 5.20 (broad d, 1H, H-4, Gal residue of Lac unit), 5.35 (broad d, 1H, H-4, fucose unit), 5.89 (m, 1H, $J_{4,5}$ = 14.0 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5, sphingosin unit), 5.93 (broad s, 1H, H-4, Gal unit), and 7.42-8.05 (m, 15H, 3Ph).

Anal. Calcd for $C_{113}H_{149}N_5O_{52}$ (2409.4): C, 56.33; H, 6.23; N, 2.91. Found: C, 56.30; H, 6.29; N, 2.86.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-(3-O-acetyl-2,4-di-O-benzoyl-ß-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-acetyl-2-deoxy-ß-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-ß-D-galactopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-ß-D-gluco-pyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (10). Hydrogen sulfide was bubbled through a stirred solution of 8 (65 mg, 27 μmol) in aqueous 83% pyridine (6 mL) for 2 days at 0 °C. The reaction was monitored by TLC. The mixture was concentrated, and the amine 9 was stirred with octadecanoic acid (16 mg, 56 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 16 mg, 83 μmol) in dry dichloromethane (1 mL) for 16 h at room temperature. Dichloromethane (30 mL) was added, and the mixture was washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (30:1 dichloro-methane-methanol) of the residue on silica gel (20 g) gave 10 (61 mg, 85%) as an amorphous mass; $[\alpha]_D$ -16.3° (c 1.2, chloroform); IR (KBr) 3350 (NH), 2920 and 2850 (methyl, methylene), 1740 and 1220 (ester), 1680 and 1530 (amide), and 750 and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, $2MeCH_2$), 1.25 (s, 50H, $25CH_2$), 1.60 (m, 2H, $COCH_2CH_2$), 2.43 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e, Neu5Ac unit), 3.72 (s, 3H, MeO), 4.20 (dd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 1.8 Hz, H-6, Neu5Ac unit), 4.23 (d, 1H, $J_{1,2}$ =7.9 Hz, H-1, Glc unit), 4.41 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1, Gal residue of Lac unit), 4.53 (dd, 1H, J_{gem} = 12.4 Hz, $J_{8,9'}$ = 2.4 Hz, H-9', Neu5Ac unit),

4.78 (m, 1H, H-4, Neu5Ac unit), 4.88 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2, Gal residue of Lac unit), 5.03 (dd, 1H, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 4.0 Hz, H-3, fucose unit), 5.17 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1, GlcNAc unit), 5.30 (dd, 1H, $J_{7,8}$ = 7.9 Hz, H-7, Neu5Ac unit), 5.36 (broad d, 1H, H-4, fucose unit), 5.86 (m, 1H, $J_{4,5}$ = 14.8 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.4 Hz, H-5, Cer unit), 5.94 (broad s, 1H, H-4, Gal unit), and 7.40-8.05 (m, 15H, 3Ph).

Anal. Calcd for $C_{131}H_{185}N_3O_{53}$ (2649.9): C, 59.38; H, 7.04; N, 1.59. Found: C, 59.35; H, 7.19; N, 1.60.

Sialyl α(2-6)-Lewis X (11). To a solution of 10 (61 mg, 23 μmol) in methanol (5 mL) was added sodium methoxide (20 mg), and the mixture was stirred for 48 h at 50 °C, and then water (0.5 mL) was added. The solution was stirred for 8 h at room temperature, neutralized with Amberlite IR-120 (H^+) resin, and filtered. The resin was washed with 5:4:0.7 chloroform-methanol-water, and the combined filtrate and washings were concentrated to a syrup that was chromatographed on a column of Sephadex LH-20 (40 g) with 5:4:0.7 chloroform-methanol-water, to give 11 (35 mg, 90%) as an amorphous mass; $[\alpha]_D$ -16.6° (c 0.7, 5:4:0.7 chloroform-methanol-water); ^{1}H NMR [49:1 $(CD_3)_2SO$-D_2O, at 60 °C] Cer unit δ 0.83 (t, 6H, 2MeCH$_2$), 1.22 (s, 50H, 25CH$_2$), 1.44 (m, 2H, COCH$_2$CH$_2$), 1.94 (m, 2H, CH=CHCH$_2$), 2.03 (t, 2H, COCH$_2$CH$_2$), 5.34 (dd, 1H, $J_{3,4}$ = 6.8 Hz, $J_{4,5}$ = 15.6 Hz, H-4), 5.54 (m, 1H, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5); hexasaccharide unit δ 0.99 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6, fucose unit), 1.80, 1.87 (2s, 6H, 2AcN), 2.66 (dd, 1H, J_{gem} = 12.0 Hz, $J_{3e,4}$ = 3.6 Hz, H-3e, Neu5Ac unit), 4.16 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1, Glc unit), 4.24 (2d, 2H, $J_{1,2}$ = 7.8 Hz, H-1, Gal unit), 4.55 (m, 1H, H-5, fucose unit), 4.74 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1, GlcNAc unit), and 4.85 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1, fucose unit).

Anal. Calcd for $C_{79}H_{141}N_3O_{35}$ (1693.0): C, 56.05; H, 8.39; N, 2.48. Found: C, 56.01; H, 8.65; N, 2.45.

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