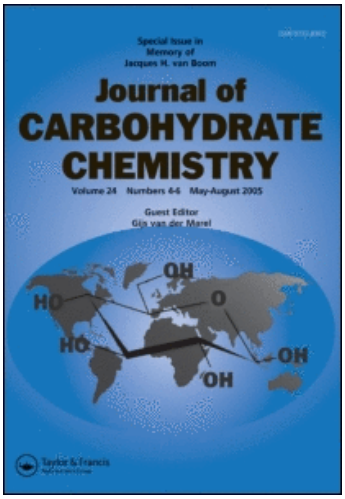

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 22: TOTAL SYNTHESIS OF TUMOR-ASSOCIATED GANGLIOSIDE, SIALYL LEWIS X[1]

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ABSTRACT

The first total synthesis of tumor-associated glycolipid antigen, sialyl Lewis X is described. Glycosylation of 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-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 (**1**) with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**4**) gave the α-glycoside (**5**), which was converted by reductive ring-opening of the benzylidene acetal into the glycosyl acceptor (**6**). Dimethyl(methylthio)sulfonium triflate-promoted coupling of **6** with methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*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 (**7**) afforded the desired hexasaccharide **8** in good yield. Compound **8** was converted into the α-trichloroacetimidate **11**, *via* reductive removal of the benzyl groups, *O*-acetylation, removal of the 2-(trimethylsilyl)ethyl group, and treatment with trichloroacetonitrile, which, on coupling with (2*S*, 3*R*, 4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**12**), gave the β-glycoside **13**. Finally, **13** was transformed, *via* selective reduction of the azide group, condensation with octadecanoic acid, *O*-deacylation, and hydrolysis of the methyl ester group, into the title compound **16**.

INTRODUCTION

Various important biological functions of sialoglycoconjugates in plasma membranes of animal cells such as glycolipids and glycoproteins have been reported by many groups.[2-8] Gangliosides are distinguished in that they contain sialic acid, usually α-linked to either C-3 or C-6 of galactose, or C-8 of another sialic acid residue of an oligosaccharide chain.

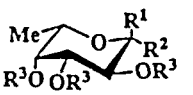
Recently, we have synthesized[9,10] sialyl-lactotetraosyl and -neolactotetraosyl ceramides (IV^{3}NeuAcLc$_4$Cer and IV^{3}NeuAcnLc$_4$Cer) by use of the methyl β-thioglycoside of sialyl-α(2→3)-galactose **7** as the glycosyl donor which is easily prepared according to our newly developed α-glycosylation of sialic acid.[11,12] As a part of our continuing efforts, on the synthesis and elucidation of the functions of sialoglycoconjugates, we describe here the first total synthesis of sialyl Lewis X, which has been isolated[13] from human kidney, and found[14] to be widespread as the tumor-associated ganglioside antigen.

RESULTS AND DISCUSSION

Methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside[10] (**7**) was selected as the glycosyl donor, and 2-(trimethylsilyl)ethyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-*O*-(2-acetamido-6-*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 (**6**) as the glycosyl acceptor in the synthesis of sialyl Lewis X.

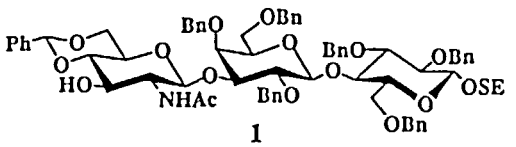
Methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[15] (**4**) was obtained in good yield from per-*O*-acetyl-L-fucopyranose (**2**) *via* replacement of the anomeric acetoxy group with the methylthio group by use of methylthiotrimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate, followed by de-*O*-acetylation and then *O*-benzylation.

The glycosylation of compound **1**[10] with **4**, in the presence of dimethyl(methylthio)-sulfonium triflate[11,16,17] (DMTST) as the glycosyl promoter and molecular sieves 4A (MS-4A) in benzene for 4 h at 6 °C gave the desired α-glycoside **5** in 86% yield; significant signals of the fucose unit in the ^{1}H NMR spectrum were a three-proton doublet at δ 0.84 ($J_{5,6}$ = 6.4 Hz, H-6) and a one-proton doublet at δ 5.07 ($J_{1,2}$ = 3.6 Hz, H-1), indicating the structure assigned. Reductive ring-opening of the benzylidene acetal in **5** with sodium cyanoborohydride-hydrogen chloride in dry ether, according to the method by Garegg *et al.*,[18] afforded compound **6** as a syrup in 75% yield. Glycosylation of **6** with **7** in dichloromethane for 20 h at room temperature in the presence of 3.0 equiv. of DMTST to the glycosyl donor and powdered MS-4A gave the hexasaccharide **8** in 41% yield, which had the expected stereochemistry. The ^{1}H NMR data were a three-proton doublet at δ 1.06 ($J_{5,6}$ = 6.6 Hz, H-6, fucose unit), two three-proton singlets at δ 1.45 and 1.50 (*N*-acetyl), four three-proton singlets at δ 1.78, 1.90, 1.93, and 2.13 (*O*-acetyl), a three-proton singlet at δ 3.77 (*O*-methyl), sixty five aromatic protons at δ 7.05-8.19 (13Ph), and a one-proton doublet of doublets at δ 5.43 ($J_{1,2}$ = 8.1, $J_{2,3}$ = 9.9 Hz, H-2, Gal unit), indicating the newly formed glycosidic linkage to be β.



2 R^1,R^2 = H, OAc , R^3 = Ac

3 R^1 = H , R^2 = SMe , R^3 = Ac



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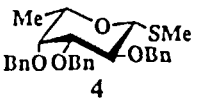
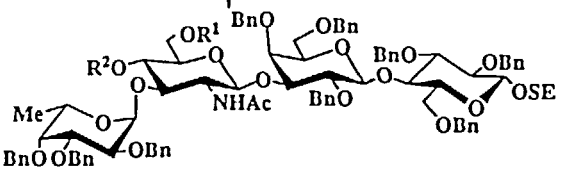


4

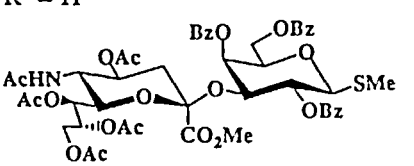
Bn = benzyl

SE = $Me_3SiCH_2CH_2$



5 R^1,R^2 = benzylidene

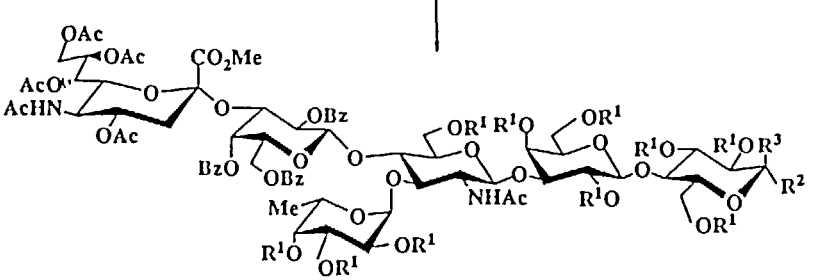
6 R^1 = Bn , R^2 = H



6

7

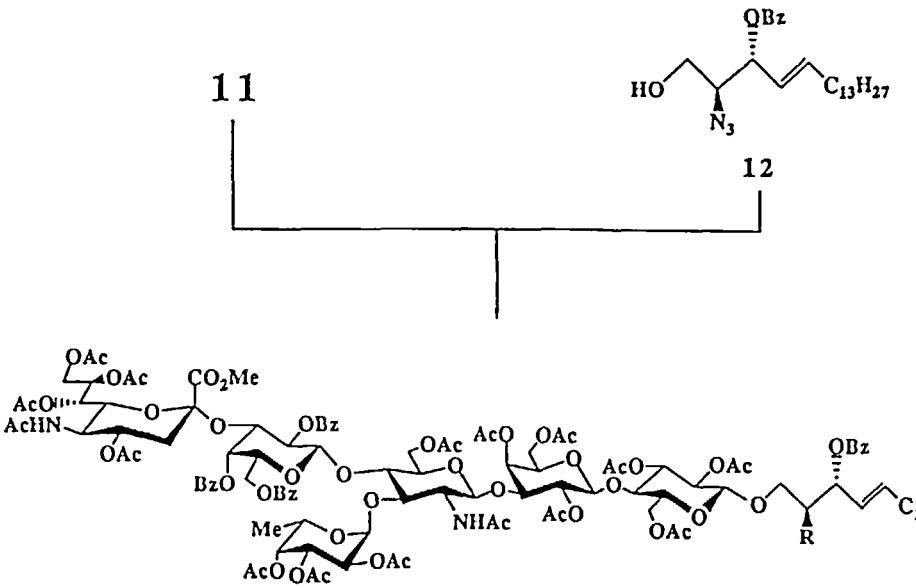
Bz = benzoyl



8 R^1 = Bn , R^2 = OSE , R^3 = H

9 R^1 = Ac , R^2 = OSE , R^3 = H

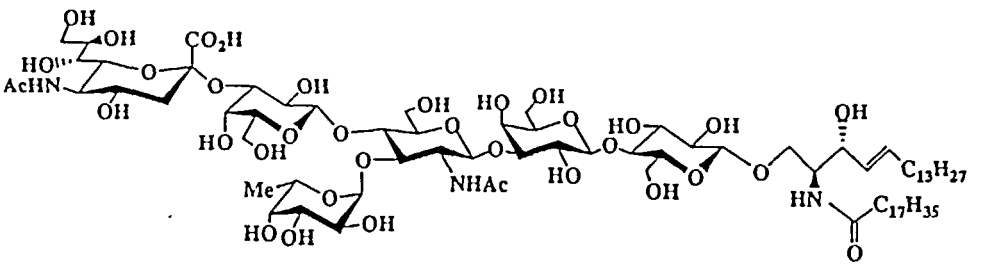
10 R^1 = Ac , R^2,R^3 = H, OH



13 R = N_3

14 R = NH_2

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



16

Catalytic hydrogenolysis (10% Pd-C) of the benzyl groups of **8** in ethanol-acetic acid (3:1) for 4 days at 45 °C , and subsequent *O*-acetylation gave the per-*O*-acetyl compound **9** in 81% yield after column chromatography. Treatment[19] of **9** with trifluoroacetic acid in dichloromethane for 1 h at room temperature gave the 1-hydroxy compound **10** in 94% yield. When this selective removal of the 2-(trimethylsilyl)ethyl group in **9** was performed by use of boron trifluoride etherate, the reaction was complete after 24 h at 0 °C. When treated with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) for 3 h at 0 °C, **10** gave the α-trichloroacetimidate **11** in 91% yield. Significant signals in the ^{1}H NMR spectrum of **11** were a one-proton doublet at δ 6.47 ($J_{1,2}$ = 3.9 Hz, H-1) and a one-proton singlet at δ 8.65 (C=NH), indicating the α-trichloroacetimidate formation.

The final glycosylation of (2*S*, 3*R*, 4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[20] (**12**) with **11** thus obtained, in dichloromethane in the presence of boron trifluoride etherate[20a,21] for 3 h at 0 °C afforded only the expected β-glycoside **13** in 56% yield. The observed chemical shifts and coupling constants due to the newly coupled 2-azido-sphingosin analog were a one-proton doublet at δ 4.49 ($J_{1,2}$ = 7.7 Hz, H-1, Glc unit) and a one-proton doublet of triplets at δ 5.91 ($J_{4,5}$ = 13.9, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5, sphingosin unit). Selective reduction[20a,22] of the azide group in **13** with hydrogen sulfide in aqueous pyridine for 2 days at 0 °C gave the amine **14**, which, on condensation with octadecanoic acid, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) in dichloromethane, gave the acylated sialyl Lewis X **15** in 81% yield, after chromatography. Finally, *O*-deacylation of **15** with sodium methoxide in methanol, with subsequent saponification of the methyl ester group, yielded the desired sialyl Lewis X in almost quantitative yield after chromatography on a column of Sephadex LH-20.

The work described above shows that the use of thioglycosides in the presence of DMTST is effective for the synthesis of complex types of sialoglycoconjugates. The 2-(trimethylsilyl)ethyl group is useful for protecting the anomeric hydroxyl group because of the easy and selective deprotection with trifluoroacetic acid in dichloromethane and its stability towards many reagents used in carbohydrate synthesis.

EXPERIMENTAL

General Procedures. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. 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 Co., 200 mesh) with the solvent systems specified. Concentration were conducted *in vacuo*.

Methyl 2,3,4-Tri-O-acetyl-1-thio-β-L-fucopyranoside (3). To a cooled solution of 1,2,3,4-tetra-*O*-acetyl-L-fucopyranose (**2**; 880 mg, 2.7 mmol) in dry dichloromethane (5 mL) were added, with stirring, (methylthio)trimethylsilane (637 mg, 5.3 mmol) and trimethylsilyl trifluoromethanesulfonate (TMS-triflate; 588 mg, 2.65 mmol), and the stirring was continued for 2 h at 0 °C; the course of the reaction was monitored by TLC. Dichloromethane (50 mL) was added to the mixture, and the solution was washed with M sodium carbonate and water, dried (Na_2SO_4), and concentrated to a syrup that was chromatographed on a column of silica gel (30 g) with 4:1 hexane-ethyl acetate, to give **3** (435 mg, 51%); mp 138-141 °C, *lit.*[15] mp 139-141 °C; $[\alpha]_D$ +1.6° (*c* 1.0, chloroform); ^{1}H

NMR ($CDCl_3$) δ 1.22 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6), 1.99, 2.07, 2.17, 2.20 (4s, 12H, 3AcO, MeS), 3.85 (m, 1H, H-5), 4.36 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 5.05 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 5.24 (t, 1H, H-2), and 5.28 (dd, 1H, $J_{4,5}$ = 1.1 Hz, H-4).

Anal. Calcd for $C_{13}H_{20}O_7S$ (332.3): C, 50.60; H, 6.07. Found: C, 50.59; H, 6.14.

Methyl 2,3,4-Tri-O-benzyl-1-thio-β-L-fucopyranoside (4). To a solution of **3** (1.3 g, 4.0 mmol) in methanol (8 mL) was added sodium methoxide (30 mg), and the mixture was stirred for 10 min at room temperature and treated with Amberlite IR-120 (H^+) resin to remove the base. The solution was concentrated, and the residue was dissolved in dry *N,N*-dimethylformamide (7 mL). To the stirred solution was added sodium hydride in oil suspension (723 mg; 60% of sodium hydride by weight), and the mixture was stirred for 30 min at 0 °C, and then benzyl bromide (2.15 mL, 18.1 mmol) was added. The stirring was continued for 2 h, and methanol (2 mL) was added. The mixture was concentrated to a syrup which was chromatographed on a column of silica gel (50 g) with 10:1 hexane-ethyl acetate to give **4** (1.57 g, 84%) as a syrup; $[\alpha]_D$ -0.8° (*c* 1.0, chloroform), *lit.*[15] $[\alpha]_D$-0.2° (chloroform); ^1H NMR ($CDCl_3$) δ 1.20 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 2.18 (s, 3H, MeS), 3.45 (m, 1H, H-5), 3.83 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.3 Hz, H-2), 4.28 (d, 1H, H-1), and 7.26-7.39 (m, 15H, 3Ph).

Anal. Calcd for $C_{28}H_{32}O_4S$ (464.6): C, 72.38; H, 6.94. Found: C, 72.20; H, 7.14.

2-(Trimethylsilyl)ethyl O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-O-(2-acetamido-4,6-O-benzylidene-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 (5). To a solution of 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-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] (**1**; 1.35 g, 1.06 mmol) and **4** (558 mg, 1.27 mmol) in dry benzene (20 mL), was added powdered molecular sieves 4A (MS-4A; 4 g), and the mixture was stirred for 4 h at room temperature. DMTST (980 mg, 3.8 mmol) and MS-4A (920 mg) were added to the stirred mixture at 6 °C, and the stirring was continued for 4 h at 6 °C; the course of the reaction was monitored by TLC. Methanol (3 mL) and triethylamine (1 mL) were added to the mixture, and stirred for 30 min. The precipitates were filtered off, and washed with dichloromethane. The filtrate and washings were combined, 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 (60 g) with 2:1 hexane-ethyl acetate to give **5** (1.53 g, 86%) as an amorphous mass; $[\alpha]_D$ -37.2° (*c* 0.9, chloroform); ^1H NMR ($CDCl_3$) δ 0.84 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6, fucose unit), 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.35 (s, 3H, AcN), 5.07 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1, fucose unit), 5.51 (s, 1H, PhC*H*), and 7.08-7.47 (m, 50H, 10Ph).

Anal. Calcd for $C_{101}H_{115}NO_{20}Si$ (1691.1): C, 71.73; H, 6.85; N, 0.83. Found: C, 71.58; H, 6.91; N, 0.85.

2-(Trimethylsilyl)ethyl O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-O-(2-acetamido-6-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 (6). To a solution of **5** (610 mg, 0.36 mmol) in dry tetrahydrofuran (10 mL) was added MS-4A (2 g), and the mixture was stirred for 1 h at room temperature. Sodium cyanoborohydride (340 mg, 5.41 mmol) was gradually added under nitrogen atmosphere. After the reagent had dissolved, hydrogen chloride in ether was added at room temperature until the evolution of gas ceased. TLC indicated that the reaction was complete after 5 min. The mixture was diluted with dichloromethane (50 mL) and water (10 mL), filtered, washed with 2M hydrochloric acid and water, dried (Na_2SO_4), and concentrated. Column chromatography (1:1 hexane-ethyl acetate) of the residue on silica gel (40 g) gave **6** (460 mg, 75%) as an amorphous mass; $[\alpha]_D$ -19.5° (*c* 1.1, chloroform); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.12 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6, fucose unit), 1.31 (s, 3H, AcN), and 7.09-7.38 (m, 50H, 10Ph).

Anal. Calcd for $C_{101}H_{117}NO_{20}Si$ (1693.1): C, 71.64; H, 6.97; N. 0.83. Found: C, 71.63; H, 7.21; N, 0.85.

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→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-O-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (8). To a solution of **6** (473 mg, 0.28 mmol) and methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*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[10] (**7**; 417 mg, 0.42 mmol) in dry dichloromethane (8 mL) was added MS-4A (1 g), and the mixture was stirred for 4 h at room temperature. DMTST (325 mg, 1.26 mmol) and MS-4A (220 mg) were added to the stirred mixture, and the stirring was continued for 20 h at room temperature; 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 (30 g) gave **8** (300 mg, 41%) as an amorphous mass; $[\alpha]_D$ -14.5° (*c* 0.79, chloroform); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.06 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6, fucose unit), 1.45, 1.50 (2s, 6H, 2AcN), 1.78, 1.90, 1.93, 2.13 (4s, 12H, 4AcO), 2.42 (dd, 1H, J_{gem} = 12.4 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e, Neu5Ac unit), 3.77

(s, 3H, MeO), 5.21 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 12.1 Hz, H-7, Neu5Ac unit), 5.32 (broad d, 1H, $J_{3,4}$ = $J_{4,5}$ = 3.1 Hz, H-4, Gal unit), 5.43 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 9.9 Hz, H-2, Gal unit), 5.67 (m, 1H, H-8, Neu5Ac unit), and 7.05-8.19 (m, 65H, 13Ph).

Anal. Calcd for $C_{148}H_{166}N_2O_{40}Si$ (2641.0): C, 40.02; H, 6.34; N, 1.06. Found: C, 40.11; H, 6.39; N, 1.00.

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→3)-O-(2,-4,6-tri-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-β-D-glucopyranoside (9). A solution of **8** (458 mg, 0.17 mmol) in ethanol (60 mL) and acetic acid (22 mL) was hydrogenolysed in the presence of 10% Pd-C (400 mg) for 4 days at 45 °C, then filtered, and concentrated. The residue was acetylated with acetic anhydride (3 mL)-pyridine (5 mL) for 16 h at room temperature. The product was purified by chromatography on a column of silica gel (40 g) with 6:1 ethyl acetate-hexane, to give **9** (305 mg, 81%) as an amorphous mass; $[\alpha]_D$ -20.2° (*c* 0.74, chloroform); ^1H NMR ($CDCl_3$) δ 0.93 (m, 2H, $Me_3SiCH_2CH_2O$), 1.20 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6, fucose unit), 1.56, 1.77 (2s, 6H, 2AcN), 1.82-2.11 (14s, 42H, 14AcO), 2.41 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e, Neu5Ac unit), 3.43 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.6 Hz, H-3, Gal unit), 3.81 (s, 3H, MeO), 4.44 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1, Glc unit), 5.06 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1, fucose unit), 5.15 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3, Glc unit), 5.27 (dd, 1H, $J_{6,7}$ = 2.9 Hz, $J_{7,8}$ = 10.3 Hz, H-7, Neu5Ac unit), 5.66 (m 1H, H-8, Neu5Ac unit), and 7.42-8.17 (m, 15H, 3Ph).

Anal. Calcd for $C_{98}H_{126}N_2O_{50}Si$ (2160.2): C, 54.49; H, 5.41; N, 1.30. Found: C, 54.38; H, 5.54; N, 1.38.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→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-D-glucopyranose (10). To a solution of **9** (305 mg, 0.14 mmol) in dry dichloromethane (1 mL) was added one drop of trifluoroacetic acid, and the mixture was stirred for 1 h at room temperature. Ethyl acetate (1 mL) was added to the mixture, and concentrated to a syrup that was purified by chromatography on a column of silica gel (30 g) with 6:1 ethyl acetate-hexane, to give **10** (274 mg, 94%) as an amorphous mass; $[\alpha]_D$ -8.4° (*c* 0.95, chloroform); IR (KBr) 3400 (NH, OH), 1750 and 1230 (ester), 1690 and 1540 (amide), and 720 cm^{-1} (Ph).

Anal. Calcd for $C_{93}H_{114}N_2O_{50}$ (2059.9): C, 54.22; H, 5.58; N, 1.36. Found: C, 54.09; H, 5.66; N, 1.35.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→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 (11). A solution of **10** (146 mg, 0.07 mmol) and trichloroacetonitrile (0.3 mL) in dichloromethane (1 mL) was cooled to -5 °C, and to the solution was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 11 mg). The mixture was stirred for 3 h at 0 °C, then concentrated. Column chromatography of the residue on silica gel (20 g) with 30:1 dichloromethane-methanol afforded **11** (142 mg, 91%) as an amorphous mass; $[\alpha]_D$ +1.5° (*c* 1.4, chloroform); IR (KBr) 3350 (NH), 1740 and 1230 (ester), 1680 and 1540 (amide), and 750 and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 1.20 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6, fucose unit), 1.56, 1.77 (2s, 6H, 2AcN), 1.82-2.11 (14s, 42H, 14AcO), 2.40 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3e,4}$ = 4.8 Hz, H-3e, Neu5Ac unit), 3.45 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.4 Hz, H-3, Gal unit), 3.80 (s, 3H, MeO), 5.35 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 9.8 Hz, H-7, Neu5Ac unit), 5.66 (m, 1H, H-8, Neu5Ac unit), 6.47 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1 Glc unit), 7.45-8.17 (m, 15H, 3Ph), and 8.65 (s, 1H, C=NH).

Anal. Calcd for $C_{95}H_{114}N_3O_{50}Cl_3$ (2204.3): C, 51.76; H, 5.21; N, 1.91. Found: C, 51.70; H, 5.34; N, 1.95.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→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 (13). To a solution of **11** (142 mg, 64 μmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[20] (**12**; 56 mg, 130 μmol) in dry dichloromethane (2 mL) was added MS-4A (AW-300; 1.4 g), and the mixture was stirred for 30 min at room temperature, and cooled to 0 °C. To the cooled mixture was added boron trifluoride etherate (0.035 mL), and the mixture was stirred for 3 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 hydrogen carbonate and water; dried (Na_2SO_4), and concentrated. Chromatography (40:1 dichloromethane-methanol) of the residue on silica gel (20 g) gave **13** (88.5 mg, 56%) as an amorphous mass; $[\alpha]_D$ -23.0° (*c* 0.9, chloroform); IR (KBr) 3400 (NH), 2100 (azide), 1740 and 1220 (ester), 1690 and 1530 (amide), and 750 and 710 cm^{-1}

(Ph); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.24 (s, 22H, 11CH_2), 1.56, 1.77 (2s, 6H, 2AcN), 1.82-2.11 (14s, 42H, 14AcO), 2.41 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e, Neu5Ac unit), 3.43 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.6 Hz, H-3, Gal unit), 3.80 (s, 3H, MeO), 4.49 (d, 1H, $J_{1,2}$ = 7.7 Hz, Glc unit), 5.06 (d, 1H, $J_{1,2}$ = 2.6 Hz, H-1, fucose unit), 5.14 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3, Glc unit), 5.27 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 10.2 Hz, H-7, Neu5Ac unit), 5.65 (m, 1H, H-8, Neu5Ac unit), 5.91 (dt, 1H, $J_{4,5}$ = 13.9 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5, sphingosin unit), and 7.42-8.17 (m, 20H, 4Ph).

Anal. Calcd for $C_{118}H_{151}N_5O_{52}$ (2471.5): C, 57.34; H, 6.16; N, 2.83. Found: C, 57.29; H, 6.24; N, 2.81.

O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→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)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (15). Hydrogen sulfide was bubbled through a stirred solution of **13** (116 mg, 47 μmol) in aqueous 83% pyridine (12 mL) for 2 days at 0 °C. The reaction was monitored by TLC. The mixture was concentrated, and the residue **14** was stirred with octadecanoic acid (27 mg, 92 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 27 mg, 95 μmol) in dry dichloromethane (2 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 dichloromethane-methanol) of the residue on silica gel (25 g) gave **15** (103 mg, 81%) as an amorphous mass; $[\alpha]_D$ -11.8° (*c* 0.78, chloroform); IR (KBr) 3400 (NH), 2950 and 2900 (methyl, methylene), 1760 and 1240 (ester), 1690 and 1550 (amide), and 760 and 720 cm^{-1} (Ph); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, 2*Me*CH_2), 1.25 (s, 52H, 26CH_2), 1.56, 1.77 (2s, 6H, 2AcN), 1.82-2.11 (14s, 42H, 14AcO), 2.40 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e, Neu5Ac unit), 3.40 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.5 Hz, H-3, Gal unit), 3.80 (s, 3H, MeO), 4.23 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, Gal unit), 4.42 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1, Glc unit), 5.06 (d, 1H, $J_{1,2}$ = 2.9 Hz, H-1, fucose unit), 5.12 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3, Gal unit), 5.65 (m, 1H, H-8, Neu5Ac unit), 5.86 (dt, 1H, $J_{4,5}$ = 13.3 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.3 Hz, H-5, sphingosin unit), and 7.40-8.17 (m, 20H, 4Ph).

Anal. Calcd for $C_{136}H_{187}N_3O_{53}$ (2712.0): C, 60.67; H, 6.95; N, 1.55. Found: C, 60.51; H, 7.14; N, 1.53.

Sialyl-Lewis X (16). To a solution of **15** (103 mg, 34 μmol) in methanol (5 mL) was added sodium methoxide (30 mg), the mixture was stirred for 24 h at 40 °C, and 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 1:1 water-methanol, and the combined filtrate and washings were concentrated to a syrup that was chromatographed on a column of Sephadex LH-20 (50 g) with 5:4:0.7 chloroform-methanol-water, to give **16** (65 mg, quantitative) as an amorphous mass; $[\alpha]_D$ -17.5° (*c* 0.6, 5:4:0.7 chloroform-methanol-water); ^1H NMR [49:1 $(CD_3)_2SO$-D_2O] δ 0.85 (t, 6H, 2*Me*CH_2), 1.01 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6, fucose unit), 1.23 (s, 52H, 26CH_2), 1.82, 1.89 (2s, 6H, 2AcN), 2.03 (t, 2H, CO*CH*$_2$$CH_2$), 2.76 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3e,4}$ = 4.7 Hz, H-3e, Neu5Ac unit), 4.17 (d, 1H, $J_{1,2}$ = 7.3 Hz, Glc unit), 4.71 (d, 1H, $J_{1,2}$ = 7.3 Hz, GlcNAc unit), 4.87 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1, fucose unit), 5.36 (dd, 1H, $J_{3,4}$ = 7.0 Hz, $J_{4,5}$ = 15.2 Hz, H-4, sphingosin unit), and 5.54 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 7.1 Hz, H-5, sphingosin unit).

Anal. Calcd for $C_{79}H_{141}N_3O_{35}$ (1693.0): C, 56.04; H, 8.40; N, 2.48. Found: C, 56.14; H, 8.65; N, 2.40.

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