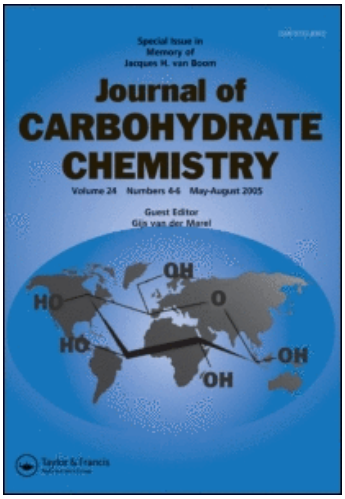


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthetic Studies on Sialoglycoconjugates 59: Total Synthesis of Tumor-Associated Ganglioside, Sialyl Le

Akihiko Kameyama[a]; Hideharu Ishida[a]; Makoto Kiso[a]; Akira Hasegawa[a]
[a] Department of Applied Bioorganic Chemistry, Gifu University, Gifu, Japan

To cite this Article Kameyama, Akihiko , Ishida, Hideharu , Kiso, Makoto and Hasegawa, Akira(1994) 'Synthetic Studies on Sialoglycoconjugates 59: Total Synthesis of Tumor-Associated Ganglioside, Sialyl Le', Journal of Carbohydrate Chemistry, 13: 5, 641 — 654
To link to this Article: DOI: 10.1080/07328309408011671
URL: http://dx.doi.org/10.1080/07328309408011671

J. CARBOHYDRATE CHEMISTRY, 13(5), 641-654 (1994)

SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 59: TOTAL SYNTHESIS OF TUMOR-ASSOCIATED GANGLIOSIDE, SIALYL Le^{a}[1]

Akihiko Kameyama, Hideharu Ishida, Makoto Kiso
and Akira Hasegawa*

Department of Applied Bioorganic Chemistry, Gifu University,
Gifu 501-11, Japan

Received September 30, 1993 - Final Form February 8, 1994

ABSTRACT

The first total synthesis of tumor-associated glycolipid antigen, sialyl Le^{a}, is described. Methylsulfenyl bromide-silver triflate-promoted coupling of 2-(trimethylsilyl)ethyl *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 (**2**) 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 (**3**) afforded the pentasaccharide **4a** and **5a** in good yields. Glycosylation of **4a** with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**6**) by use of *N*-iodosuccinimide (NIS) - trifluoromethanesulfonic acid (TfOH) as a promoter, gave the desired hexasaccharide **7**. Compound **7** was converted into the α-trichloroacetimidate **10**, *via* reductive removal of 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 (**11**), gave the β-glycoside **12**. Finally, **12** 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 **15** in good yield.

INTRODUCTION

Many carbohydrate antigens defined by monoclonal antibodies have been introduced as tumor-associated antigens.[2] Recently, the sialyl Le^{x} antigen,[3] one of these tumor-associated antigens, has been shown[4] to be one of the possible ligands for selectins, a family of lectin-type cell adhesion molecules (LEC-CAMs). The more recent reports[5]

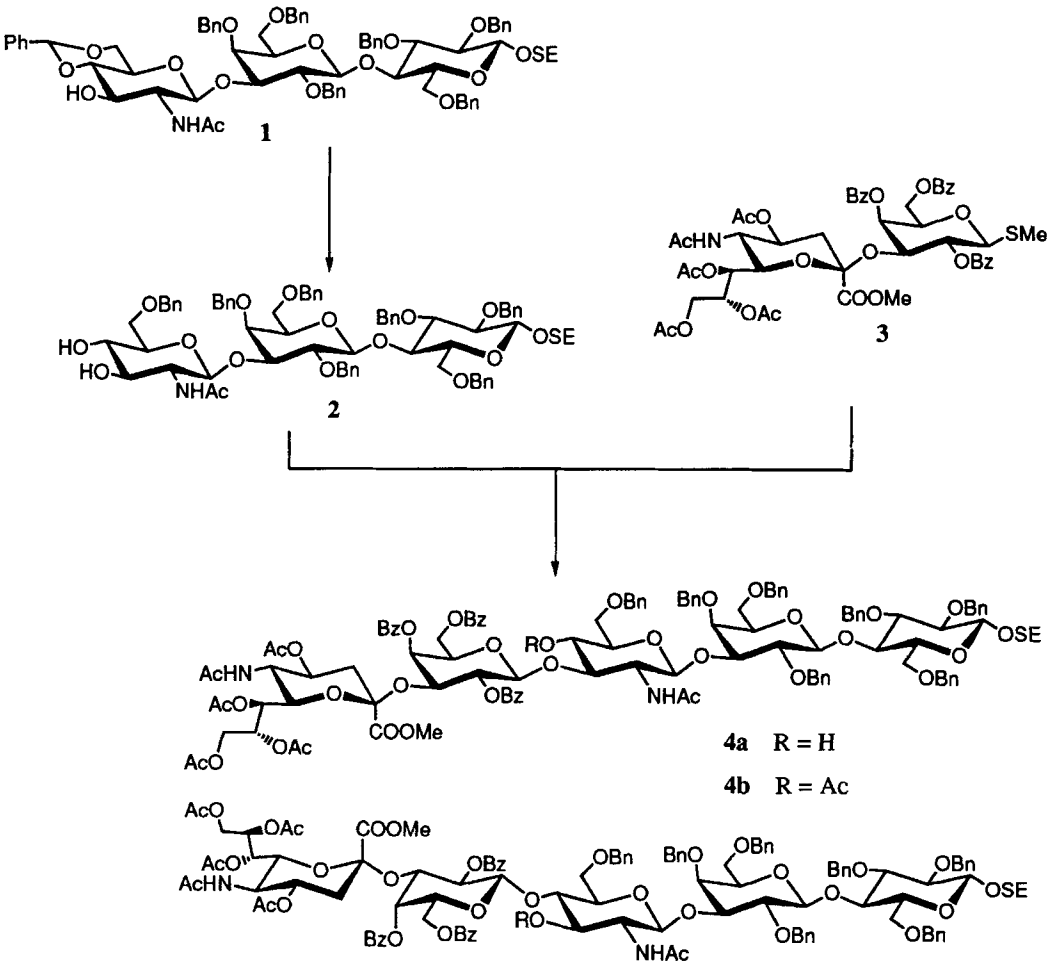
revealed that the sialyl Le^a antigen,[6] which has a chemical structure closely related to that of sialyl Le^x, was also recognized by LEC-CAMs. These findings suggest that expression of the sialyl Le^a and sialyl Le^x on cancer cells is involved in the process of hematogeneous metastasis of cancer cells. In a previous paper[7] we reported the total synthesis of sialyl Le^x ganglioside. 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 Le^a ganglioside, which has been isolated[8] from human adenocarcinoma cell line SW1116, and found[9] to be widespread as the tumor-associated glycolipid antigen of digestive organs.

RESULTS AND DISCUSSION

For the synthesis of the desired sialyl Le^a ganglioside, pentasaccharide derivative **4a**, having a sialyl α(2→3) galactose unit already linked and providing free hydroxyl group at C-4 of the GlcNAc residue for further glycosylation with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[10](**6**) as the donor, was selected as the glycosyl acceptor. The glycosyl acceptor (**4a**) was obtained by the coupling of the diol (**2**) of trisaccharide 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[11] (**3**).

Reductive ring-opening of the benzylidene acetal in a known trisaccharide derivative[11]**1** with sodium cyanoborohydride-hydrogen chloride in dry ether, according to the method by Garegg et al.,[12] afforded compound **2** in 61% yield. Methyl sulfenyl bromide (MSB) - silver trifluoromethanesulfonate promoted glycosylation[13] of **2** with **3** in dichloromethane for 16 h at -15 °C gave the pentasaccharide **4a** (45%) and it's positional isomer **5a** (36%) after chromatography. The regio-chemistry of compounds **4a** and **5a** was deduced from the 300 MHz ^1H NMR spectra of acetylated compounds **4b** and **5b**. The observed chemical shifts of GlcNAc unit, which were assigned from the cross-peaks in COSY spectrum, for H-3 (δ 4.60 for **4b**; δ 4.92 for **5b**) and for H-4 (δ 4.55 for **4b**; δ 4.00 for **5b**) indicate the position of glycosylation to be C-3 and C-4, respectively.

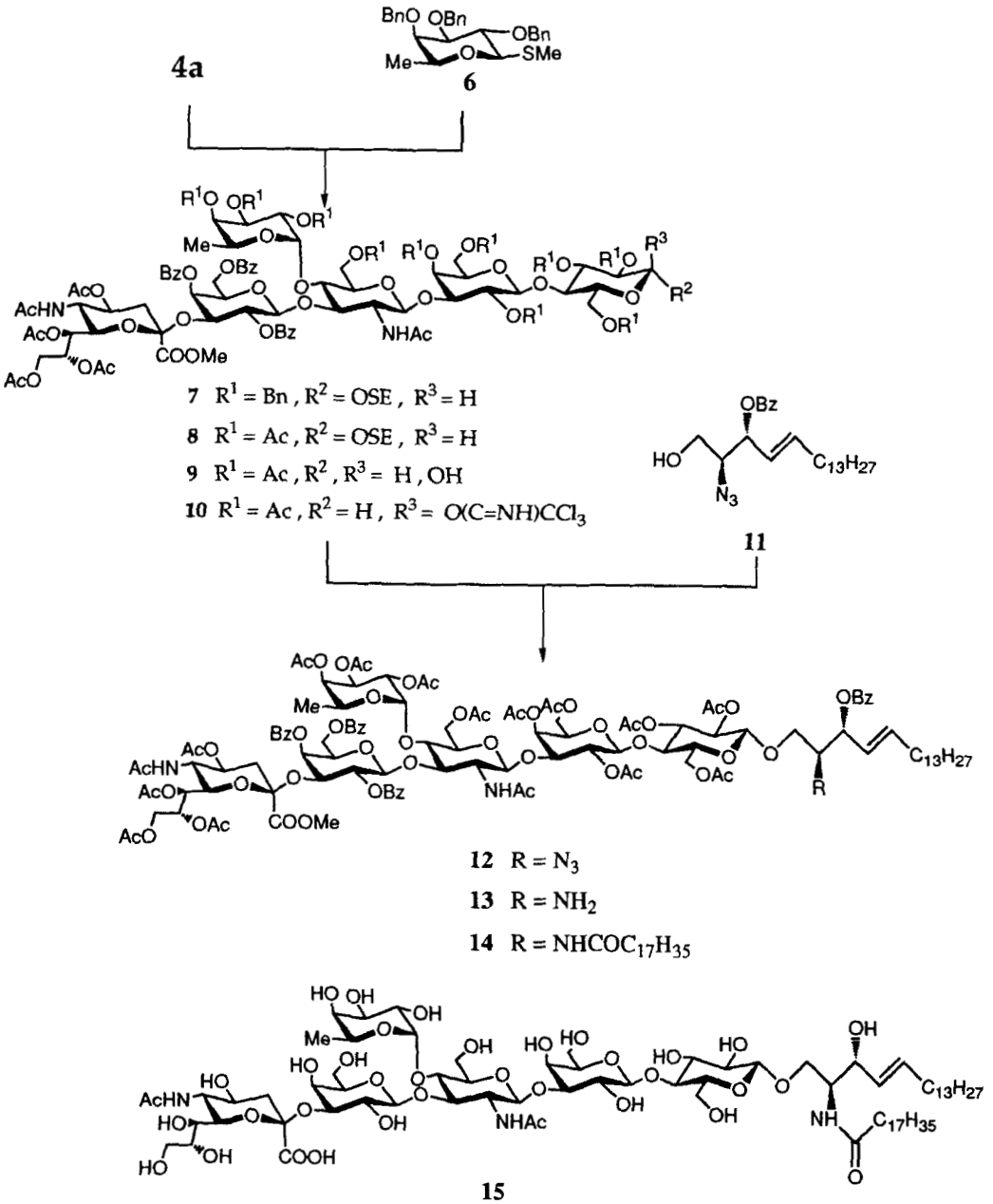
The glycosylation of **4a** with thio glycoside (**6**) was performed in toluene for 8 h at 0 °C in the presence of *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid[14] (TfOH) and molecular sieves 4 A, to give the desired hexasaccharide **7** in 60% yield; significant signals of the fucose unit in the ^1H NMR spectrum were a three-proton doublet at δ 1.39 ($J_{5,6}$ = 6.4 Hz, H-6) and a one proton doublet at δ 5.04 ($J_{1,2}$ = 2.7 Hz, H-1), indicating the structure assigned.



SE = 2-(trimethylsilyl)ethyl

Bn = benzyl

Bz = benzoyl



Catalytic hydrogenolysis (10% Pd-C) of the benzyl groups of **7** in ethanol-acetic acid (3 : 1) for 4 days at 45 °C, and subsequent *O*-acetylation, gave the per-*O*-acyl compound **8** in 82% yield. Selective removal of the 2-(trimethylsilyl)ethyl group from **8** was performed by treatment[15] with trifluoroacetic acid in dichloromethane for 2 h at room temperature, to give the 1-hydroxy compound **9** in 84% yield. Treatment[16] of **9** with trichloroacetonitrile in the presence of 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) for 3 h at 0 °C gave the α-trichloroacetimidate **10** in 98% yield. Significant signals in the ^{1}H NMR spectrum of **10** were a one-proton doublet at δ 6.48 ($J_{1,2}$ = 3.8 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[17] (**11**) with **10** thus obtained, in dichloromethane in the presence of boron trifluoride etherate[16a] for 4 h at 0 °C afforded only the expected β-glycoside **12** in 45% yield. The observed chemical shifts and coupling constants were a one-proton doublet at δ 4.50 ($J_{1,2}$ = 7.8 Hz, H-1 for the glucose unit) and a one- proton doublet of triplets at δ 5.91 ($J_{4,5}$ = 13.9 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5 for sphingosine unit).

Selective reduction[17a,18] of the azide group in **12**, which, on condensation with octadecanoic acid, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) in dichloromethane, gave the acylated sialyl Le^a ganglioside **14** in 67% yield, after chromatography. Finally, *O*-deacylation of **14** with sodium methoxide in methanol, with subsequent saponification of the methyl ester group, yielded the desired sialyl Le^a ganglioside in almost quantitative yield after chromatography on a column of Sephadex LH-20. The ^{1}H NMR data of the product thus obtained are consistent with the structure assigned.

In conclusion, the first total synthesis of sialyl Le^a ganglioside was achieved by employing the thioglycoside donors **3** and **6**, and suitably protected tri-and pentasaccharide derivatives (**2**, **4a**) as glycosyl acceptors. The introduction of a fucose unit into a pentasaccharide acceptor was accomplished in moderate yield using NIS-TfOH as a glycosyl promoter.

EXPERIMENTAL

General Procedures. Specific rotations were determined with a Jasco DIP-370 digital polarimeter at 25 °C, and IR spectra were recorded with a Jasco IR-700 infrared spectrometer. ^{1}H NMR spectra were recorded at 300 MHz with General Electric QE-plus spectrometer. Preparative chromatography was performed on silica gel (Wako

Chemical Co., 300 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl *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 (2). To a solution of **1** (ref. 11) (315 mg, 0.25 mmol) in dry tetrahydrofuran (6 mL) was added powdered molecular sieves 4A (MS-4A; 400 mg), the mixture was stirred for 4 h at room temperature, and sodium cyanoborohydride (234 mg, 3.72 mmol) was gradually added. After the reagent had dissolved, hydrogen chloride in ether was added dropwise 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 (20 mL) and water (20 mL), filtered, washed with M sodium hydrogen carbonate and water, dried (Na_2SO_4), and concentrated. Column chromatography (30:1 dichloromethane-methanol) of the residue on silica gel (40 g) gave **2** (193 mg, 61%) as an amorphous mass; $[\alpha]_D$ -9.0° (*c* 0.81, chloroform); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.37 (s, 3H, AcN), 7.09-7.37 (m, 35H, 7Ph).

Anal. Calcd for $C_{74}H_{89}NO_{16}Si$ (1276.6): C, 69.62; H, 7.03; N, 1.10. Found: C, 69.35; H, 6.85; N, 1.04.

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→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 (4a), and **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-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 (5a).** To a solution of **2** (1.34 g, 1.05 mmol) and **3** (1.36 g, 1.36 mmol) in dry dichloromethane (36 mL) was added MS-4A (6.0 g), and the mixture was stirred for 8 h at room temperature. Silver trifluoromethanesulfonate (539 mg, 2.10 mmol) was added to the mixture, which was cooled to -15 °C. Methyl sulfenyl bromide (MSB) solution (2.2 mL, 2.20 mmol) was injected in two equal portions at an interval of 30 min, and the mixture was stirred for 4 h at -15 °C. Methanol (1 mL) and triethylamine (0.5 mL) were added to the mixture, and the precipitates were removed by filtration and washed with dichloromethane. The combined filtrate and washings were washed with

water, dried (Na_2SO_4), and concentrated. Column chromatography of silica gel (200 g) of the residue was performed using (a) 6:1 and (b) 4:1 dichloromethane-acetone as the eluants. Eluant (a) gave compound **4a** (1.06 g, 45%), and eluant (b) afforded **5a** (847 mg, 36%) as an amorphous mass, respectively.

4a; $[\alpha]_D$ +13.8° (*c* 1.23, chloroform); ^1H NMR ($CDCl_3$) δ 0.99 (m, 2H, $Me_3SiCH_2CH_2O$), 1.05, 1.75 (2s, 6H, 2AcN), 1.81, 1.90, 2.02, 2.18 (4s, 12H, 4AcO), 2.41 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3*eq*, Neu5Ac unit), 3.84 (s, 3H, MeO), 4.82 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, Gal unit), 5.28 (dd, 1H, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 9.5 Hz, H-7, Neu5Ac unit), 5.35 (broad d, 1H, $J_{3,4} = J_{4,5}$ = 2.9 Hz, H-4, Gal unit), 5.50 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2, Gal unit), 5.64 (m, 1H, H-8, Neu5Ac unit), 7.04-8.19 (m, 50H, 10Ph).

Anal. Calcd for $C_{121}H_{138}N_2O_{36}Si$ (2224.5): C, 65.33; H, 6.25; N, 1.26. Found: C, 65.26; H, 6.17; N, 1.25.

5a; $[\alpha]_D$ +26.2° (*c* 1.38, chloroform); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.45, 1.57 (2s, 6H, 2AcN), 1.77, 1.91, 2.03, 2.19 (4s, 12H, 4AcO), 2.45 (dd, 1H, J_{gem} = 12.7 Hz, $J_{3eq,4}$ = 4.7 Hz, H-3*eq*, Neu5Ac unit), 3.87 (s, 3H, MeO), 5.07 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1, Gal unit), 5.22 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 9.5 Hz, H-7, Neu5Ac unit), 5.33 (d, 1H, $J_{3,4} = J_{4,5}$ = 3.1 Hz, H-4, Gal unit), 5.54 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2, Gal unit), 5.66 (m, 1H, H-8, Neu5Ac unit), 7.04-8.29 (m, 50H, 10Ph).

Anal. Calcd for $C_{121}H_{138}N_2O_{36}Si$ (2224.5): C, 65.33; H, 6.25; N, 1.26. Found: C, 65.07; H, 6.21; N, 1.04.

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→3)-*O*-(2-acetamido-4-*O*-acetyl-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 (4b). To a solution of **4a** (50 mg, 22.4 μmol) in pyridine (2 mL) was added acetic anhydride (1 mL), and the mixture was stirred for 16 h at room temperature. After completion of the reaction, methanol (2 mL) was added, and the mixture was stirred for 20 min at room temperature, concentrated, and extracted with dichloromethane. The extract was washed with 2 M hydrochloric acid, M sodium carbonate, and water, dried (Na_2SO_4), and concentrated to give **4b** (50 mg, quantitative) as an amorphous mass ; $[\alpha]_D$ +2.4° (*c* 0.78, chloroform); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.43, 1.52 (2s, 6H, 2AcN), 1.77, 1.88, 1.89, 1.99, 2.12 (5s, 15H, 5AcO), 2.43 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3*eq*, Neu5Ac unit),

2.85 (broad quartet, 1H, H-2, GlcNAc unit), 3.79 (s, 3H, MeO), 4.55 (1H, H-4, GlcNAc unit), 4.60 (1H, H-3, GlcNAc unit), 5.21 (dd, 1H, $J_{6,7}$ = 2.8 Hz, $J_{7,8}$ = 9.9 Hz, H-7, Neu5Ac unit), 5.58 (m, 1H, H-8, Neu5Ac unit), 7.07-8.11 (m, 50H, 10Ph).

Anal. Calcd for $C_{123}H_{140}N_2O_{36}Si$ (2250.5): C, 65.64; H, 6.27; N, 1.24. Found: C, 65.57; H, 6.07; N, 1.17.

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-acetamido-3-*O*-acetyl-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 (5b). Acetylation of **5a** (50 mg, 22.4 mmol), as described for **4b**, gave amorphous **5b** (50 mg, quantitative) ; $[\alpha]_D$ +12.7° (*c* 1.00, chloroform); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.44, 1.47 (2s, 6H, 2AcN), 1.77, 1.91, 2.01, 2.11 (4s, 15H, 5AcO), 2.47 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3*eq*, Neu5Ac unit), 3.79 (s, 3H, MeO), 4.00 (1H, H-4, GlcNAc unit), 4.92 (1H, H-3, GlcNAc unit), 5.07 (d, 1H, $J_{1,2}$ = 7.9Hz, H-1, Gal unit), 5.19 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 9.8 Hz, H-7, Neu5Ac unit), 5.34 (d, 1H, $J_{3,4} = J_{4,5}$ = 3.0 Hz, H-4, Gal unit), 5.38 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2, Gal unit), 5.69 (m, 1H, H-8, Neu5Ac unit), 7.05-8.27 (m, 50H, 10Ph).

Anal. Calcd for $C_{123}H_{140}N_2O_{36}Si$ (2250.5): C, 65.64; H, 6.27; N, 1.24. Found: C, 65.43; H, 6.13; N, 1.22.

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→3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-*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 (7). To a solution of **4a** (1.06 g, 0.48 mmol) and methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[10] (**6**; 376 mg, 0.72 mmol) in dry toluene (15 mL) was added MS-4A (4.0 g), the mixture was stirred for 8 h at room temperature. *N*-iodosuccinimide (NIS; 161 mg, 0.72 mmol) was added, and cooled to -15 °C. Trifluoromethanesulfonic acid (TfOH; 6.3 mL) was added at -15 °C to the mixture, and this was stirred for 24 h at -5° C. The precipitate was removed by filtration and washed with dichloromethane. The filtrate and washings were combined, and the solution was successively washed with M $Na_2S_2O_3$ and water, dried (Na_2SO_4), and concentrated to a syrup which was chromatographed on a column of silica gel (130 g) with 20:10: 0.3 ethyl acetate-hexane-methanol to give amorphous **7**

(755 mg, 60%): $[\alpha]_D$ -29.5° (*c* 0.88, chloroform); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2O$), 1.39 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6, Fuc unit), 1.60, 1.62 (2s, 6H, 2AcN), 1.78, 1.88, 1.95, 2.13 (4s, 12H, 4AcO), 2.39 (dd, 1H, J_{gem} = 12.7 Hz, $J_{3eq,4}$ = 4.7 Hz, H-3*eq*, Neu5Ac unit), 3.65 (s, 3H, MeO), 4.97 (d, 1H, $J_{1,2}$ = 8.5Hz, H-1, Gal unit), 5.04 (d, 1H, $J_{1,2}$ = 2.7 Hz, H-1, Fuc unit), 5.25 (dd, 1H, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 9.5 Hz, H-7, Neu5Ac unit), 5.28 (d, 1H, $J_{3,4}$ = $J_{4,5}$ = 3.6 Hz, H-4, Gal unit), 5.47 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2, Gal unit), 5.59 (m, 1H, H-8, Neu5Ac unit), 7.02-8.16 (m, 65H, 13Ph).

Anal. Calcd for $C_{148}H_{166}N_2O_{40}Si$ (2641.0): C, 67.31; H, 6.34; N, 1.06. Found: C, 67.01; H, 6.19; N, 0.96.

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→3)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-*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 (8). A solution of **7** (755 mg, 0.29 mmol) in ethanol (80 mL) and acetic acid (20 mL) was hydrogenolysed in the presence of 10% Pd-C (700 mg) for 4 days at 45 °C, the catalyst removed by filtration and the solution concentrated. The residue was acetylated with acetic anhydride (6 mL)-pyridine (10 mL) for 16 h at 45 °C. The product was purified by chromatography on a column of silica gel (80 g) with 35:1 dichloromethane-methanol, to give **8** (505 mg, 82%) as an amorphous mass; $[\alpha]_D$ -25.0° (*c* 1.08, chloroform); ^1H NMR ($CDCl_3$) δ 0.90 (m, 2H, $Me_3SiCH_2CH_2O$), 1.24 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6, Fuc unit), 1.58-2.12 (14s, 48H, 2AcN, 14AcO), 2.39 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.5 Hz, H-3*eq*, Neu5Ac unit), 2.72 (broad quartet, 1H, H-2, GlcNAc unit), 3.77 (s, 3H, MeO), 4.24 (dd, 1H, J_{gem} = 12.4 Hz, $J_{8,9}$ = 2.6 Hz, H-9, Neu5Ac unit), 4.30 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1, Gal residue of lactose unit), 4.46 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, Glc unit), 5.25 (dd, 1H, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 9.8 Hz, H-7, Neu5Ac unit), 5.33 (d, 1H, $J_{3,4}$ = $J_{4,5}$ = 3.7 Hz, H-4, Gal unit), 5.37 (dd, 1H, $J_{1,2}$ = 8.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2, Gal unit), 5.61 (m, 1H, H-8, Neu5Ac unit), 5.71 (d, 1H, $J_{2,NH}$ = 7.1 Hz, NH, GlcNAc unit), 7.39-8.21 (m, 15H, 3Ph).

Anal. Calcd for $C_{98}H_{126}N_2O_{50}Si$ (2160.2): C, 54.49; H, 5.88; N, 1.30. Found: C, 54.29; H, 5.64; N, 1.24.

***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→3)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyran-**

osyl)-(1→4)]-*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 (9). To a solution of **8** (316 mg, 0.15 mmol) in dry dichloromethane (1 mL) was added trifluoroacetic acid (2 mL), and the resulting solution was stirred for 2 h at room temperature. Ethyl acetate (6 mL) was added to the reaction mixture, which was then concentrated to a syrup that was purified by chromatography on a column of silica gel (30 g) with 20:1 dichloromethane-methanol, to give **9** (254 mg, 84%) as an amorphous mass; $[\alpha]_D$ -11.4° (*c* 1.14, chloroform); IR (KBr) 3380 (NH, OH), 1745 and 1225 (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, 54.05; H, 5.51; 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→3)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-*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 (10).** A solution of **9** (254 mg, 0.12 mmol) and trichloroacetonitrile (0.37 mL) in dichloromethane (2 mL) was cooled to -5 °C, and to the solution was added 1,8-diazabicyclo[5.4.0.]undec-7-ene (DBU; 19 mg). The mixture was stirred for 4 h at 0 °C, then concentrated. Column chromatography of the residue on silica gel (30 g) with 30:1 dichloromethane-methanol afforded **10** (265 mg, 98%) as an amorphous mass; $[\alpha]_D$ +0.92° (*c* 1.06, chloroform); ^1H NMR ($CDCl_3$) δ 1.23 (d, 3H, $J_{5,6}$ = 6.7 Hz, H-6, Fuc unit), 1.58-2.12 (16s, 48H, 2AcN, 14AcO), 2.40 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3*eq*, Neu5Ac unit), 2.69 (broad quartet, 1H, H-2, GlcNAc unit), 3.77 (s, 3H, MeO), 4.23 (dd, 1H, J_{gem} = 12.4 Hz, $J_{8,9}$ = 2.6 Hz, H-9, Neu5Ac unit), 4.34 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, Gal residue of lactose unit), 5.25 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 10.0 Hz, H-7, Neu5Ac unit), 5.33 (d, 1H, $J_{3,4}$ = $J_{4,5}$ = 3.8 Hz, H-4, Gal unit), 5.37 (dd, 1H, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 9.7 Hz, H-2, Gal unit), 5.61 (m, 1H, H-8, Neu5Ac unit), 5.71 (d, 1H, $J_{2,NH}$ = 7.1 Hz, NH, GlcNAc unit), 6.48 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1, Glc unit), 7.15-8.21 (m, 15H, 3Ph), 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, 51.65; H, 5.01; N, 1.67.

***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→3)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyran-**

osyl)-(1→4)]-*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-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (12). To a solution of **10** (260 mg, 118 μmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[17] (**11**; 101 mg, 235 μmol) in dry dichloromethane (2 mL) was added MS-4A (AW-300; 2.0 g), and the reaction mixture was stirred for 30 min at room temperature, and cooled to 0 °C. To the cooled mixture was added boron trifluoride etherate (30 μL), and the mixture was stirred for 4 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 (30 g) gave **12** (132 mg, 45%) as an amorphous mass; $[\alpha]_D$ -22.7° (*c* 1.32, chloroform); ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, J_{Me,CH_2} = 6.6 Hz, *Me*CH_2), 1.24 (s, 22H, 11CH_2), 1.65 (t, 1H, J_{gem} = $J_{3ax,4}$ = 12.6 Hz, H-3*ax*, Neu5Ac unit), 1.58-2.10 (13s, 48H, 2AcN, 14AcO), 2.40 (dd, 1H, $J_{3eq,4}$ = 4.5 Hz, H-3*eq*, Neu5Ac unit), 2.71 (broad quartet, 1H, H-2, GlcNAc unit), 3.77 (s, 3H, MeO), 4.23 (dd, 1H, J_{gem} = 12.4 Hz, $J_{8,9}$ = 2.6 Hz, H-9, Neu5Ac unit), 4.29 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, Gal residue of lactose unit), 4.50 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1, Glc unit), 5.25 (dd, 1H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 9.9 Hz, H-7, Neu5Ac unit), 5.33 (d, 1H, $J_{3,4}$ = $J_{4,5}$ = 3.7 Hz, H-4, Gal unit), 5.37 (dd, 1H, $J_{1,2}$ = 8.6 Hz, $J_{2,3}$ = 10.6 Hz, H-2, Gal unit), 5.64 (m, 1H, H-8, Neu5Ac unit), 5.69 (d, 1H, $J_{2,NH}$ = 7.1 Hz, NH, GlcNAc unit), 5.91 (dt, 1H, $J_{4,5}$ = 13.9 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5, sphingosine unit), 7.28-8.23 (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, 57.33; H, 5.99; N, 2.78.

***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→3)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)]-*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-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (14).** Hydrogen sulfide was bubbled through a stirred solution of **12** (65 mg, 26 μmol) in aqueous 83% pyridine (12 mL) for 2 days at 0 °C. The reaction was monitored by TLC. The reaction mixture was concentrated, and the amine **13** was stirred with octadecanoic acid (15 mg, 53 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 15 mg, 78 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 dichloromethane-methanol) of the residue on silica gel (20 g) gave **14** (48 mg, 67%) as an amorphous mass; $[\alpha]_D$ -16.0° (*c* 0.96, chloroform); IR (KBr) 3380 (NH, OH), 2920 and 2850 (methyl, methylene), 1750 and 1230 (ester), 1680 and 1530 (amide), and 720 cm^{-1} (Ph). ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.25 (s, 52H, 26CH_2), 1.58-2.09 (16s, 48H, 2AcN, 14AcO), 2.39 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.1 Hz, H-3*eq*, Neu5Ac unit), 2.70 (broad quartet, 1H, H-2, GlcNAc unit), 3.77 (s, 3H, MeO), 4.23 (dd, 1H, J_{gem} = 12.4 Hz, $J_{8,9}$ = 2.6 Hz, H-9, Neu5Ac unit), 4.55 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3, GlcNAc unit), 5.25 (dd, 1H, $J_{6,7}$ = 2.6 Hz, $J_{7,8}$ = 9.9 Hz, H-7, Neu5Ac unit), 5.33 (d, 1H, $J_{3,4} = J_{4,5}$ = 3.6 Hz, H-4, Gal unit), 5.37 (dd, 1H, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 9.7 Hz, H-2, Gal unit), 5.45 (dd, 1H, $J_{3,4}$ = 7.4 Hz, $J_{4,5}$ = 14.9 Hz, H-4, ceramide unit), 5.53 (t, 1H, $J_{2,3} = J_{3,4}$ = 7.4 Hz, H-3, ceramide unit), 5.61 (m, 1H, H-8, Neu5Ac unit), 5.68 (d, 1H, $J_{2,NH}$ = 7.0 Hz, NH, GlcNAc unit), 5.74 (d, 1H, $J_{2,NH}$ = 9.1 Hz, NH, ceramide unit), 5.86 (dt, 1H, $J_{5,6} = J_{5,6'}$ = 7.2 Hz, H-5, ceramide unit), 7.39-8.20 (m, 20H, 4Ph).

Anal. Calcd for $C_{136}H_{187}N_3O_{53}$ (2712.0): C, 60.23; H, 6.95; N, 1.55. Found: C, 60.13; H, 6.76; N, 1.41.

Sialyl α(2→3)Le^a ganglioside (15). To a solution of **14** (48 mg, 19.5 μmol) in methanol (3 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 **15** (30 mg, quantitative) as an amorphous mass; $[\alpha]_D$ -18.8° (*c* 0.45, 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), 1.00 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6, Fuc unit), 1.24 (s, 52H, 26CH2), 1.83, 1.89 (2s, 6H, 2AcN), 2.03 (t, 2H, COC*H*$_2$CH$_2$), 2.77 (dd, 1H, J_{gem} = 11.8 Hz, $J_{3eq,4}$ = 4.3 Hz, H-3*eq*, Neu5Ac unit), 4.18 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1, Glc unit), 4.35 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1, Gal of lactose unit), 4.65 (d, 1H, $J_{1,2}$ = 7.1 Hz, H-1, Gal unit), 4.73 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1, GlcN unit), 4.79 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1, Fuc unit), 5.36 (dd, 1H, $J_{3,4}$ = 7.1 Hz, $J_{4,5}$ = 15.3 Hz, H-4, ceramide unit), 5.55 (dt, 1H, $J_{5,6} = J_{5,6'}$ = 7.5 Hz, H-5, ceramide unit).

Anal. Calcd for $C_{79}H_{141}N_3O_{35}$ (1693.0): C, 56.04; H, 8.40; N, 2.48. Found: C, 55.90; H, 8.22; N, 2.42.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 03255207 and 03660132) for the Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

REFERENCES AND NOTES

1. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *XVth International Carbohydrate Symposium, Yokohama*, Japan, August 12-17, 1990, Abstract A096.

2. S. Hakomori and R. Kannagi, *J. Natl. Cancer Inst.* **71**, 231 (1983).

3. K. Fukushima, M. Hirota, P. I. Terasaki, A. Wakisaka, H. Togashi, D. Chia, N. Suyama, Y. Fukushi, S. Nudelman and S. Hakomori, *Cancer Res.*, **201**, 31 (1990).

4. G. Walz, A. Aruffo, W. Kolanus, M. Bevilacqua and S. Seed, *Science*, **250**, 1132 (1990); M. L. Phillips, E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori and J.C. Paulson, *ibid.*, **250**, 1130 (1990); J. B. Lowe, L. M. Stoolman, R. P. Nair, R. D. Larsen, T. L. Berhend and R. M. Marks, *Cell*, **63**, 475 (1990).

5. E. L. Berg, M. K. Robinson, O. Mansson, E. C. Bucher and J. L. Magnani, *J. Biol. Chem.*, **266**, 14869 (1991); A. Takada, K. Ohmori, N. Takahashi, K. Tsuyuoka, A. Yago, K. Zenita, A. Hasegawa and R. Kannagi, *Biochem. Biophys. Res. Commun.*, **179**, 713 (1991); D. Tyrrell, P. James, N. Rao, C. Foxall, S. Abbas, F. Dasgupta, M. Nashed, A. Hasegawa, M. Kiso, D. Asa, J. Kidd and B. K. Brandley, *Proc. Natl. Acad. Sci. USA.*, **88**, 10372 (1991); K. Handa, E. D. Nudelman, M. R. Stroud, T. Shiozawa and S. Hakomori, *Biochem. Biophys. Res. Commun.*, **181**, 1223 (1991); M. -L. Majuri, P. Mattila and R. Renkonen, *Biochem. Biophys. Res. Commun.*, **182**, 1376 (1991); M. Larkin, T. J. Ahern, M. S. Stoll, M. Shaffer, D. Sako, J. O'Brien, C. -T. Yuen, A. M. Lawson, R. A. Childs, K. M. Barone, P. R. Langer-Safer, A. Hasegawa, M. Kiso, G. R. Larsen and T. Feizi, *J. Biol. Chem.*, **267**, 13661 (1992).

6. J. L. Magnani, B. Nilsson, M. Brockhaus, D. Zopf, Z. Steplewski, H. Koprowski and V. Ginsburg, *J. Biol. Chem.*, **257**, 14365 (1982).

7. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **10**, 549 (1991).

8. H. Koprowski, Z. Steplewski, K. Mitchell, M. Herlyn, D. Herly and P. Fuhrer, *Somat. Cell Genet.*, **5**, 957 (1979).

9. S. Itai, J. Nishikata, T. Yoneda, K. Ohmori, S. Tsunekawa, N. Hiraiwa, H. Yamabe, S. Arii, T. Tobe and R. Kannagi, *Cancer*, **67**, 1576 (1991) and references therein.

10. F. Yamazaki, S. Sato, T. Nukada, Y. Ito and T. Ogawa, *Carbohydr. Res.*, **201**, 31 (1990).

11. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **200**, 269 (1990).

12. P. J. Garegg, H. Hultberg and S. Wallin, *Carbohydr. Res.*, **108**, 97 (1982).

13. F. Dasgupta and P. J. Garegg, *Carbohydr. Res.*, **177**, c13 (1988).

14. G. H. Veeneman, S. H. van Leeuwen and J. H. van Boom, *Tetrahedron Lett.*, **31**, 1331 (1990); P. Konradsson, D. R. Mootoo, R. E. McDevitt and B. Fraser-Reid, *J. Chem. Soc., Chem. Commun.*, 270 (1990); A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida and M. Kiso, *J. Carbohydr. Chem.*, **10**, 493 (1991).

15. K. Jannson, S. Ahlfors, T. Frejd, J. Kirberg, G. Magnusson, J. Dahmen, G. Noori and K. Stenvall, *J. Org. Chem.*, **53**, 5629 (1988).

16. a) R. R. Schmidt and G. Grundler, *Synthesis*, 885 (1981); b) M. Numata, M. Sugimoto, K. Koike and T. Ogawa, *Carbohydr. Res.*, **163**, 209 (1987).

17. a) Y. Ito, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 285 (1989); b) R. R. Schmidt and P. Zimmermann, *Angew. Chem. Int. Ed. Engl.*, **25**, 725 (1986).

18. T. Adachi, Y. Yamada, I. Inoue and M. Saneyoshi, *Synthesis*, 45 (1977).