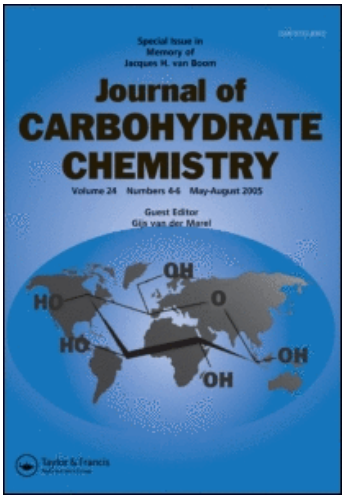

Journal of Carbohydrate Chemistry

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

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To cite this Article Hotta, Kenji , Komba, Shiro , Ishida, Hideharu , Kiso, Makoto and Hasegawa, Akira(1994) 'Synthetic Studies on Sialoglycoconjugates 61: Synthesis of α-Series Ganglioside GM1α', Journal of Carbohydrate Chemistry, 13: 5, 665 — 677
To link to this Article: DOI: 10.1080/07328309408011673
URL: http://dx.doi.org/10.1080/07328309408011673

J. CARBOHYDRATE CHEMISTRY, 13(5), 665-677 (1994)

SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 61: SYNTHESIS OF α-SERIES GANGLIOSIDE GM1α

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Received November 1, 1993 - Final Form February 8, 1994

ABSTRACT

A stereocontrolled synthesis of α-series ganglioside GM1α (III^{6}Neu5AcGgOse$_4$Cer) is described. Glycosylation of 2-(trimethylsilyl)ethyl *O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**1**) with the suitably protected galactosamine donor, methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**4**) gave the desired trisaccharide, which was transformed into the trisaccharide acceptor *via* removal of the phthaloyl and *O*-acetyl groups followed by *N*-acetylation. Glycosylation of this acceptor with methyl 3-*O*-benzyl-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**7**) gave the asialo GM1 saccharide derivative, which was transformed into the acceptor by removal of benzylidene group. Coupling of this gangliotetraose acceptor with phenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate by use of NIS-TfOH afforded the desired GM1α oligosaccharide derivative in high yield, which was transformed, *via* removal of the benzyl group followed by *O*-acetylation, selective removal of the 2-(trimethylsilyl)ethyl group and subsequent imidate formation, into the final glycosyl donor. Condensation of this imidate derivative with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**15**) gave the β-glycoside, which on channeling through selective reduction of azido group, coupling of the amino group with octadecanoic acid, *O*-deacylation and saponification of the methyl ester group, gave the title compound GM1α.

INTRODUCTION

Glycosphingolipids are covalent conjugates of hydrophilic oligosaccharide and hydrophobic ceramide moieties, and present in the plasma membranes of all mammalian

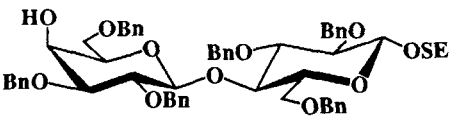
cells.[1,2] The biological function of these compounds has been the subject of much investigation and unveiled their important biological roles in cell growth, differentiation, adhesion, oncogenesis, receptor functions for viruses and bacterial toxins, and ligand activities for selectin family.[2] In order to investigate the function of sialoglycoconjugates at the molecular level, we have synthesized a series of gangliosides and their analogs,[3,4] by using our newly developed methods for ganglioside synthesis.[5-7]

In the studies on the isolation and characterization of mouse and adult bovine brain glycoconjugates, α-series gangliosides containing α-glycosidically linked sialic acid at C-6 of galactosamine residue in their molecules have been isolated as the components in tissues of the central nervous system, and focused on the biological roles in the cell-cell adhesion, metastasis of tumor cell and developmental regulation.[8-11] Additionally, two of α-series gangliosides, GT1aα and GQ1bα, have been recognized as the cholinergic neuron-specific antigen of bovine brain by Whittaker *et al.*[12] Previously, we reported[4] the synthesis of α-series ganglioside, GD1α. Here we describe the synthesis of α-series ganglioside GM1α, III[6] Neu5Ac α Gg_4 Cer, which is another member of α-series gangliosides.

RESULTS AND DISCUSSION

For the synthesis of desired α-series ganglioside GM1α, we have selected methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**4**) as a key glycosyl donor, suitable for the preparation of the gangliotetraose derivative and its transformation to the gangliotetraose acceptor for the construction of the core structure of α-series gangliosides. The appropriately protected galactosamine donor **4** was obtained in good yield from methyl 2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside[13] (**2**) by 4,6-*O*-benzylidenation and 3-*O*-acetylation. The glycosylation of 2-(trimethylsilyl)ethyl *O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[4] (**1**) with **4** in dichloromethane for 2 h at 0 °C in the presence of *N*-iodosuccinimide (NIS) - trifluoromethanesulfonic acid (TfOH) and powdered molecular sieves 4Å (MS-4Å) afforded the desired β-glycoside **5** in 65% yield. Significant signals of the galactosamine unit in the ^{1}H NMR spectrum of **5** were a one-proton doublet at δ 5.40 ($J_{1,2}$ = 8.3 Hz, H-1), a three-proton singlet at 1.97 (AcO) and a one-proton singlet at 5.64 (PhC*H*) indicating the structure assigned. *O*-Deacetylation and conversion of the phthalimide to the acetamido by heating with hydrazine hydrate in aqueous 95% ethanol followed by *N*-acetylation with acetic anhydride of **5** afforded gangliotriose acceptor **6.**

The condensation of **6** with methyl 3-*O*-benzyl-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside[3i] (**7**) in a similar condition as described for the glycosylation of **1** with



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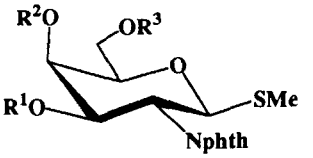
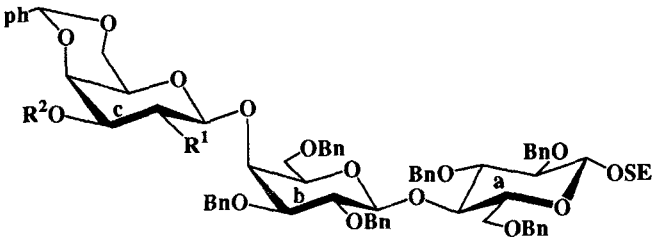
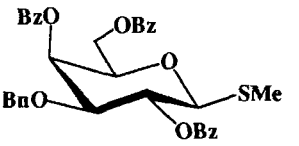


	R^1	R^2	R^3
2	H	H	H
3	H	benzylidene	
4	Ac	benzylidene	



	R^1	R^2
5	Nphth	Ac
6	NHAc	H



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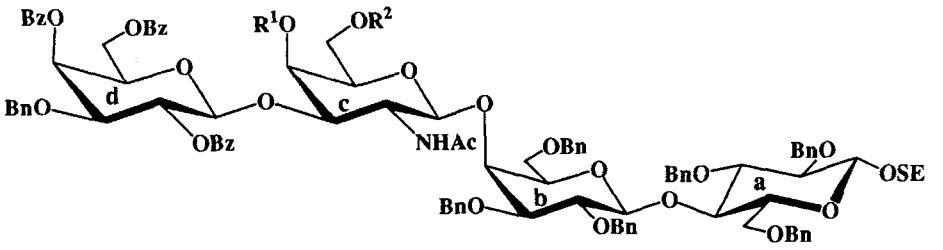
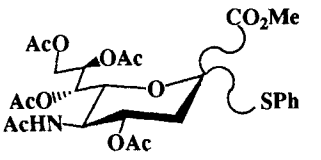


	R^1	R^2
8	benzylidene	
9	H	H

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



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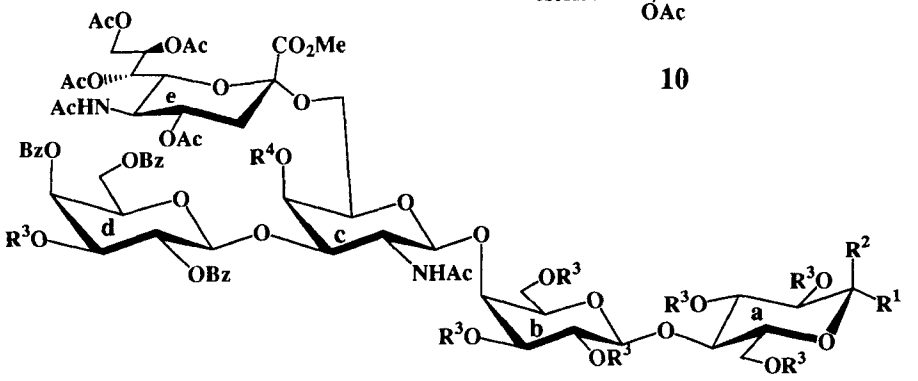
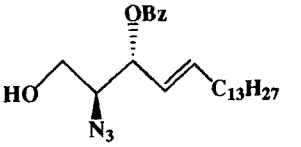


	R^1	R^2	R^3	R^4
11	OSE	H	Bn	H
12	OSE	H	Ac	Ac
13	H , OH		Ac	Ac
14	H	$OC(=NH)CCl_3$	Ac	Ac



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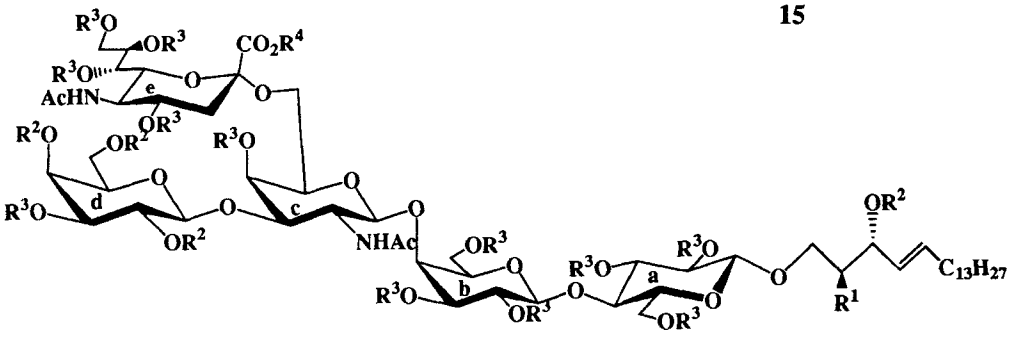

	R^1	R^2	R^3	R^4
16	N_3	Bz	Ac	Me
17	$NHCO(CH_2)_{16}CH_3$	Bz	Ac	Me
18	$NHCO(CH_2)_{16}CH_3$	H	H	H

4 gave an asialo-GM1 oligosaccharide derivative **8** in 91% yield. The observed chemical shifts and coupling constants of the newly introduced galactose residue for H-2 (δ 5.54, $J_{1,2}$ = 10.3 Hz, $J_{2,3}$ = 7.9 Hz) indicated the newly formed glycosidic linkage to be β. By removal of the benzylidene group, the gangliotetraose acceptor **9** was formed from **8** in a quantitative yield. The glycosylation of **9** with phenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate[7] (**10**) by use of NIS-TfOH in the presence of powdered MS-4Å in acetonitrile solution for 5 h at -30°C gave the expected GM1α oligosaccharide derivative **11** in 36% yield; significant signals of **11** in ^{1}H NMR spectrum were a one-proton multiplet at δ 2.50 (H-3e-*eq*), one three-proton singlets at δ 3.74 (*O*-methyl), a one-proton multiplet at δ 4.80 (H-4e), a one-proton multiplet at δ 5.30 (H-8e), and fifty aromatic protons (10Ph) at δ 7.03-8.31, indicating the structure assigned. Catalytic hydrogenolysis (10% Pd-C) of the benzyl groups in **11** in ethanol-acetic acid for 48 h at 45 °C and subsequent *O*-acetylation gave the per-*O*-acyl compound **12** in 91% yield. Treatment[14] of **12** with trifluoroacetic acid in dichloromethane for 30 min at 0 °C gave the 1-hydroxy compound **13**. When treated with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) for 3h at 0 °C, **13** gave the α-trichloroacetimidate **14** in 91% yield. The ^{1}H NMR data for Glc unit in **14** [δ 6.44 ($J_{1,2}$ = 3.8 Hz, H-1a), 8.61 (C=NH)] indicated the imidate to be α.

The final glycosylation[15b,16] of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[15] (**15**) with **14** in dichloromethane in the presence of boron trifluoride etherate for 4 h at 0 °C afforded the desired β-glycoside **16** in 63% yield. Selective reduction[17] of azido group in **16** with hydrogen sulfide in aqueous 83% pyridine for 3 days at 0 °C gave the amine, and this on condensation with octadecanoic acid, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) in dichloromethane gave the acylated GM1α ganglioside **17** in 37% yield, after chromatography.

Finally, *O*-deacylation of **17** with sodium methoxide in methanol, and subsequent saponification of the methyl ester group, yielded the desired α-series ganglioside GM1α **18**, β-D-Gal-(1→3)-[α-Neu5Ac-(2→6)-]β-D-GalNAc-(1→4)-β-D-Gal-(1→4)-β-D-Glc-(1→1)-Cer, in a quantitative yield after chromatography on a column of Sephadex LH-20.

EXPERIMENTAL

General Procedures. Optical rotations were determined with a Union PM-201 polarimeter at 25 °C and IR spectra were recorded with a Jasco A-100 spectro-

photometer. ^{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*.

Methyl 4,6-*O*-Benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (3). To a solution of methyl 2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside[13] (2; 11.7 g, 34 mmol) in *N,N*-dimethylformamide (DMF, 120 mL) was added Drierite (10 g) and the mixture was stirred for 2 h at room temperature. Benzaldehyde dimethyl acetal (10 mL, 68 mmol) and *p*-toluenesulfonic acid monohydrate (50 mg) were added, and the mixture was stirred for 48 h at room temperature, then neutralized with Amberlite IR-410 (OH^-) resin and concentrated. Column chromatography (1:1 AcOEt-hexane) of the residue on silica gel (200 g) afforded **7** (11.6 g, 79 %) as an amorphous mass: $[\alpha]_D$ +48.2° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H, MeS), 3.62 (t, 1H, Jgem = $J_{5,6}$ = 8.8 Hz, H-6), 3.73 (dt, 1H, $J_{4,5}$ = 8.8 Hz, $J_{5,6'}$ = 4.2 Hz, H-5), 3.82 (t, 1H, H-6'), 4.42 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-4), 4.70 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 10.3 Hz, H-2), 5.31 (d, 1H, H-1), 5.58 (s, 1H, PhC*H*), and 7.25-8.04 (m, 9H, 2Ph).

Anal. Calcd for $C_{22}H_{21}NO_6S$ (427.5): C, 61.81; H, 4.95; N, 3.28. Found: C, 61.61; H, 4.67; N, 3.30.

Methyl 3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (4). A solution of **3** (3.51 g, 8.2 mmol) in acetic anhydride (12 mL) and pyridine (25 mL) was stirred for 2 h at room temperature, and methanol (10 mL) was added. The solution was concentrated to a syrup which was extracted with dichloromethane. The extract was successively washed with 2 M hydrochloric acid and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 AcOEt-hexane) of the residue on silica gel (100 g) gave **4** (3.16 g, 82%) as an amorphous mass: $[\alpha]_D$ +62.9° (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.80, 2.10 (2s, 6H, AcO and MeS), 4.08 (dd, 1H, $J_{5,6}$ = 1.8 Hz, J_{gem} = 12.5 Hz, H-6), 4.40 (dd, 1H, $J_{5,6'}$ = 1.8 Hz, J_{gem} = 12.5 Hz, H-6'), 4.54 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 4.91 (dd, 1H, $J_{1,2}$ = 10.3 Hz, $J_{2,3}$ = 11.0 Hz, H-2), 5.33 (d, 1H, H-1), 5.57 (s, 1H, PhC*H*), 6.08 (dd, 1H, H-3), and 7.30-7.90 (m, 9H, 2Ph).

Anal. Calcd for $C_{24}H_{23}NO_7S$ (469.5): C, 61.40; H, 4.94; N, 2.98. Found: C, 61.12; H, 4.83; N, 2.77

2-(Trimethylsilyl)ethyl *O*-(3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5). To a solution of **1** (320 mg, 0.33 mmol) and **4** (260 mg, 0.56 mmol) in dry dichloromethane (4 mL) were added the powderd molecular sieves 4Å (MS-4Å, 1.5 g),

and the mixture was stirred for 5 h at room temperature then cooled to 0 °C. To the cooled mixture were added, with stirring, *N*-iodosuccinimide (NIS, 250 mg, 1.1 mmol) and trifluoromethanesulfonic acid (TfOH, 12 μL, 0.13 mmol), and the stirring was continued for 2 h at 0 °C. The precipitate was removed by filtration and washed thoroughly with dichloromethane. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3, M $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 AcOEt-hexane) of the residue on silica gel (20 g) gave **5** (300 mg, 65%) as an amorphous mass: $[\alpha]_D$ +32.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.01 (m, 2H, $Me_3SiCH_2CH_2$), 1.97 (s, 3H, AcO), 4.58 (d, 1H, $J_{3,4}$ = 3.8 Hz, H-4c), 5.40 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1c), 6.08 (dd, 1H, $J_{2,3}$ = 11.5 Hz, H-3c), 5.64 (s, 1H, PhC*H*), and 7.10-7.90 (m, 39H, 8Ph)

Anal. Calcd for $C_{82}H_{89}NO_{18}Si$ (1404.7): C, 70.12; H, 6.39; N, 1.00. Found: C, 70.10; H, 6.21; N, 0.73.

2-(Trimethylsilyl)ethyl *O*-(2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6). A solution of **5** (0.30 g, 0.21 mmol) in aqueous 95% ethanol (6 mL) was treated with hydrazine hydrate (0.2 mL) for 9 h under reflux. The precipitate was collected and washed with ethanol, and the combined filtrate and washings were concentrated. The residue was treated with acetic anhydride (1 mL) in methanol (10 mL) for 2 h at room temperature, pyridine (2 mL) was added, the mixture was concentrated, and a solution of the residue in dichloromethane was successively washed with 2 M HCl, water, and M Na_2CO_3, dried (Na_2SO_4) and concentrated. Column chromatography (2:3 AcOEt-hexane) of the residue on silica gel (10 g) afforded **6** (0.24 g, 89%) as an amorphous mass: $[\alpha]_D$ +22.6° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$), 1.59 (s, 3H, AcN), 5.57 (s, 1H, PhC*H*) and 7.10-7.40 (m, 35H, 7Ph)

Anal. Calcd for $C_{74}H_{87}NO_{16}Si$ (1274.6): C, 69.73; H, 6.88; N, 1.10. Found: C, 69.44; H, 6.59; N, 1.06.

2-(Trimethylsilyl)ethyl *O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-*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** (500 mg, 0.39 mmol) and methyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside[3i] (**7**, 410 mg, 0.66 mmol) in dry dichloromethane (4 mL) was added MS-4Å (1.8 g), and the mixture was stirred for 5 h at room temperature then cooled to 0 °C. To the cooled mixture were added, with stirring, NIS (260 mg, 1.2 mmol) and TfOH (14 μL, 0.16

mmol), and the stirring was continued for 1 h at 0 °C. The precipitate was removed by filtration, and washed thoroughly with dichloromethane. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3, M $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 AcOEt-hexane) of the residue on silica gel (20 g) gave **8** (610 mg, 91%) as an amorphous mass: $[\alpha]_D$ +49.6° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$), 1.74 (s, 3H, AcN), 5.46 (s, 1H, PhC*H*), 5.54 (dd, 1H, $J_{1,2}$ = 10.3 Hz, $J_{2,3}$ = 7.9 Hz, H-2c), and 7.00-8.20 (m, 55H, 11Ph)

Anal. Calcd for $C_{108}H_{115}NO_{17}Si$ (1727.2): C, 75.10; H, 6.71; N, 0.81. Found: C, 74.96; H, 6.60; N, 0.75.

2-(Trimethylsilyl)ethyl *O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9). A solution of **8** (170 mg, 0.1 mmol) in aqueous 80% acetic acid (5 mL) was heated for 24 h at 60 °C and concentrated. Column chromatography (1:1 AcOEt-hexane) of the residue on silica gel (10 g) gave **9** (160 mg, quantitative) as an amorphous mass: $[\alpha]_D$ +48.6° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.00 (m, 2H, $Me_3SiCH_2CH_2$), 2.01 (s, 3H, AcN), 5.51 (dd, $J_{1,2}$ = 9.9 Hz, $J_{2,3}$ = 8.1 Hz, H-2d), 5.86 (d, $J_{3,4}$ = 2.9 Hz, H-3d), and 7.00-8.20 (m, 50H, 10Ph).

Anal. Calcd for $C_{101}H_{111}NO_{17}Si$ (1639.1): C, 74.01; H, 6.83; N, 0.85. Found: C, 74.01; H, 6.63; N, 0.63.

2-(Trimethylsilyl)ethyl *O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[*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-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2, 3, 6-tri-*O*-benzyl-β-D-glucopyranoside (11). To a solution of **9** (500 mg, 0.3 mmol) and phenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-1-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[7] (**10**, 500 mg, 0.90 mmol) in dry acetonitrile (10 mL) was added MS-3Å (1.5 g), and the mixture was stirred for 5 h at room temperature then cooled to 0 °C. To the cooled mixture were added, with stirring, NIS (385 mg, 1.8 mmol) and TfOH (15 μL, 0.18 mmol), and the stirring was continued for 1 h at 0 °C. The precipitate was removed by filtration, and washed thoroughly with dichloromethane. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3, M $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:1 AcOEt-hexane) of the residue on silica gel (30 g) gave **11** (240 mg, 36%) as an amorphous mass: $[\alpha]_D$

+26.8° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 1.80-2.20 (6s, 18H, 4AcO and 2AcN), 2.50 (m, 1H, H-3e*eq*), 3.74 (s, 3H, MeO), 4.80 (m, 1H, H-4e), 5.30 (m, 1H, H-8e), 5.50 (dd, 1H, $J_{1,2}$ = 9.89 Hz, $J_{2,3}$ = 8.79 Hz, H-2d), 5.91 (d, 1H, $J_{3,4}$ = 3.30 Hz, H-4d), and 7.00-8.20 (m, 50H, 10Ph)

Anal. Calcd for $C_{121}H_{138}N_2O_{29}Si$ (2112.5): C, 68.80; H, 6.58; N, 1.33. Found: C, 68.59; H, 6.43; N, 1.22.

2-(Trimethylsilyl)ethyl *O*-(3-*O*-Acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-[*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-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (12). A solution of **11** (240 mg, 0.11 mmol) in ethanol (25 mL) and acetic acid (4 mL) was hydrogenated in the presence of 10% Pd-C (250 mg) for 48 h at 40 °C, the catalyst removed by filtration and the solution concentrated. The residue was acetylated with acetic anhydride (0.8 mL)-pyridine (1.6 mL) for 16 h at room temperature. The product was purified by chromatography on a column of silica gel (20 g) with 2:1 AcOEt-hexane to give **12** (190 mg, 91%) as an amorphous mass: $[\alpha]_D$ -1.33° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.93 (m, 2H, $Me_3SiCH_2CH_2$), 1.86, 1.87 (2s, 6H, 2AcN), 1.92-2.20 (12s, 36H, 12AcO), 2.51 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 5.0 Hz, H-3e*eq*), 3.70 (1s, 3H, MeO), 4.89 (m, 1H, H-4e), 5.28 (m, 1H, H-8e), 5.54 (dd, 1H, $J_{1,2}$ = 9.9 Hz, $J_{2,3}$ = 7.7 Hz, H-2d), 5.54 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-4d), and 7.42-8.20 (m, 15H, 3Ph).

Anal. Calcd for $C_{88}H_{112}N_2O_{38}Si$ (1833.9): C, 57.63; H, 6.16; N, 1.53. Found: C, 57.62; H, 6.10; N, 1.43.

***O*-(3-*O*-Acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-[*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-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranose (13).** To a solution of **12** (190 mg, 0.1 mmol) in dichloromethane (1 mL) was added trifluoroacetic acid (1 mL) at 0 °C, and the mixture was stirred for 30 min at 0 °C and concentrated. Column chromatography (30:1 CH_2Cl_2-MeOH) of the residue on silica gel (10 g) gave **13** (110 mg, 62%) as an amorphous mass: $[\alpha]_D$ +15.8° (*c* 1.1, $CHCl_3$); IR (KBr) δ 3600-3300 (OH,NH), 1740 and 1230 (ester), 1670 and 1550 (amide), and 760 and 720 cm^{-1} (ph).

Anal. Calcd for $C_{83}H_{112}N_2O_{38}$ (1833.9): C, 57.50; H, 5.81; N, 1.62. Found: C, 57.28; H, 5.73; N, 1.43

***O*-(3-*O*-Acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-[*O*-(methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 6)]-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (14).** To a solution of **13** (100 mg, 0.055 mmol) in dichloromethane (2 mL) and trichloroacetonitrile (0.24 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 10 mg) at -5 °C, and the mixture was stirred for 1 h at 0 °C, then concentrated. Column chromatography (30:1 dichloromethane-methanol) of the residue on silica gel (10 g) gave **14** (100 mg, 91%) as an amorphous mass: $[\alpha]_D$ +1.76° (*c* 0.45, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.55, 1.87 (2s, 6H, 2AcN), 1.98-2.20 (12s, 36H, 12AcO), 2.53 (dd, 1H, J_{gem} = 12.6 Hz, $J_{3eq,4}$ = 4.6 Hz, H-3e*eq*), 3.78 (1s, 3H, MeO), 5.78 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4d), 6.49 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1a), and 7.41-8.21 (m, 15H, 3Ph).

Anal. Calcd for $C_{85}H_{100}N_3O_{38}Cl_3$ (1878.1): C, 54.36; H, 5.37; N, 2.24. Found: C, 54.26; H, 5.37; N, 2.23.

***O*-(3-*O*-Acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-[*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-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2, 3, 6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (16).** To a solution of **14** (100 mg, 0.05 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[13] (**15**, 50 mg, 0.1 mmol) in dichloromethane (1 mL) were added MS-4Å (AW-300, 0.7 g) and the mixture was stirred for 5 h at room temperature, then cooled to 0 °C. Boron trifluoride etherate (30 μL) 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 washed with M $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 dichloromethane-methanol) of the residue on silica gel (10 g) gave **16** (70 mg, 63%) as an amorphous mass: $[\alpha]_D$ -0.74° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$) (aglycon) δ 0.88 (s, 3H, *Me*CH_2), 1.24 (s, 22H, 11CH_2), 5.89 (m, 1H, H-5), and (pentasaccharide) δ 1.86 (s, 6H, 2AcN), 1.98-2.09 (12s, 36H, 12AcO), 2.51 (m, 1H, H-3e*eq*), 3.00 (m, 1H, H-2c), 3.78 (1s, 3H, MeO), and 7.28-8.26 (m, 20H, 4Ph).

Anal. Calcd for $C_{108}H_{137}N_5O_{40}$ (2145.3): C, 60.47; H, 6.44; N, 3.26. Found: C, 60.28; H, 6.24; N, 3.24.

***O*-(3-*O*-Acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-[*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-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-*O*-(2, 3, 6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2, 3, 6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (17). Hydrogen sulfide was bubbled through a stirred solution of **16** (70 mg, 0.038 mmol) in aqueous 83% pyridine (8 mL) for 3 days at 0 °C. The mixture was concentrated, and the residue was stirred with octadecanoic acid (20 mg, 0.076 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (21 mg, 0.11 mmol) in dry dichloromethane (4 mL) for 24 h at room temperature. Dichloromethane (50 mL) was added, and the mixture was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 CH_2Cl_2-MeOH) of the residue on silica gel (10 g) gave **17** (29 mg, 37%) as an amorphous mass: $[\alpha]_D$ +4.64° (*c* 0.56, $CHCl_3$);^{1}H NMR ($CDCl_3$) (aglycon) δ 0.89 (t, 6H, 2*Me*CH_2), 1.25 (s, 52H, 26CH_2), 5.89 (m, 1H, H-5), and (pentasaccharide) δ 1.85,1.86 (2s, 6H, 2AcN), 1.95-2.19 (12s, 36H, 12AcO), 2.51 (dd, 1H, J_{gem} = 12.3 Hz, $J_{3eq,4}$ = 5.3 Hz, H-3e*eq*), 3.03 (m, 1H, H-2c), 3.78 (1s, 3H, MeO), 5.78 (d, 1H, $J_{3,4}$ = 2.2 Hz, H-4d), and 7.26-8.21 (m, 20H, 4Ph).

Anal. Calcd for $C_{126}H_{173}N_3O_{41}$ (2385.8): C, 63.43; H, 7.31; N, 1.76. Found: C, 63.25; H, 7.12; N, 1.67

***O*-(β-D-Galactopyranosyl)-(1 → 3)-[*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)]-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (18).** To a solution of **17** (29 mg, 0.012 mmol) in methanol (1 mL) was added sodium methoxide (5 mg), and the mixture was stirred for 24 h at 40 °C, and water (0.2 mL) was added. The solution was stirred for 10 h at room temperature, then treated with Amberlite IR-120 (H^+) resin, and the resin removed by filtration. The resin was washed with methanol, and the combined filtrate and washings were concentrated. Column chromatography (7:40:50 H_2O-MeOH-$CHCl_3$) of the residue on Sephadex LH-20 (10 g) gave **18** (15 mg, quantitative) as an amorphous mass: $[\alpha]_D$ +5.42° (*c* 0.16, 7:40:50 H_2O-MeOH-$CHCl_3$); ^{1}H NMR [98:2 $(CD_3)_2SO$-D_2O] δ 0.99 (t, 6H, 2*Me*CH_2), 1.38 (s, 52H, 26CH_2), 1.87,1.89 (2s, 6H, 2AcN), 2.02 (t, 2H, CO*C*H_2CH_2), 2.75 (dd, 1H, J_{gem} = 12.8 Hz, $J_{3eq,4}$ = 5.1 Hz, H-3e*eq*), 4.24 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1a), 4.28 (d, 1H, $J_{1,2}$ = 8.9 Hz, H-1b), 4.39 (d, 1H, $J_{1,2}$ = 9.4 Hz, H-1d), 4.42 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1c), 5.55 (m, 1H, H-4 for sphingosine), and 5.98 (m, 1H, H-5 for sphingosine)

Anal. Calcd for $C_{73}H_{131}N_3O_{25}$ (1450.9): C, 60.43; H, 9.10; N, 2.90. Found: C, 60.39; H, 8.92; N, 2.83.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 05274102 and 05152053) for the Scientific Research on Priority Area and for the Cancer Research from the Ministry of Education, Science and Culture of Japan.

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