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Synthetic studies on glycosphingolipids from the Protostomia phyla: syntheses of arthro-series glycosphingolipids

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

Glycosphingolipids isolated from larvae of the green-bottle fly, *Lucilia caesar*, have quite unique structures containing GlcNAcβ-(1→3)-Man and GalNAcβ-(1→4)-GlcNAcβ-(1→3)-Man. We have synthesized two glycosphingolipids, β-D-GlcNAc*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer and β-D-GalNAc*p*-(1→4)-β-D-GlcNAc*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer. A key reaction in the synthetic sequence is the application of the intramolecular aglycon delivery (IAD) approach for the synthesis of the β-mannopyranosidic linkages.

Keywords: Glycosphingolipids; Arthro-series; Chemical synthesis; *Lucilia caesar*

1. Introduction

The structure and the biological role of the oligosaccharide residues of glycosphingolipids in mammalian tissues have been extensively studied and are known to participate in various biological processes, i.e., immunological response, cell-growth regulation, a common metastatic inflammation, viral infection, and fertilization.[1] They are divided into separate series based on the nature of the core saccharide, such as Globo-, Isoglobo-, Lacto-, Neolacto-, and Ganglio-series.[2] Their core may be repeated or extended and finally terminated with blood group and other antigenic determinants, including sialic acid and sulfate groups.

Table 1
Glycosphingolipid series found in Protostomia phyla

Prefix of series	Structure
Arthro	β-D-Glc*p*NAc-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*
Mollu	α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*
Gara	α-D-Gal*p*-(1→6)-β-D-Gal*p*
Neogara	β-D-Gal*p*-(1→6)-β-D-Gal*p*
Schisto	β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*

On the other hand, the core saccharides of glycosphingolipids from invertebrate animal species differ greatly from those of mammals (Table 1). A unique series of glycolipids having mannose, internally located fucose, and *O*-methyl sugars have been found in various invertebrates.[3] These differences from common vertebrate glycolipids have prompted the study of glycolipid profiles of other unexplored invertebrates, especially in the Protostomia phyla. Furthermore, the biological function of these glycans has, for the most part, not been explored. We have been interested in the relationships between the structure and biological function of glycolipids from invertebrate animal species and have so far synthesized oligosaccharides from various P. phyla.[4] Sugita et al. found some novel glycolipids with β-D-GlcNAc*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer (**1**) and β-D-GalNAc*p*-(1→4)-β-D-GlcNAc*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer (**2**) in larvae of the green-bottle fly, *Lucilia caesar*,

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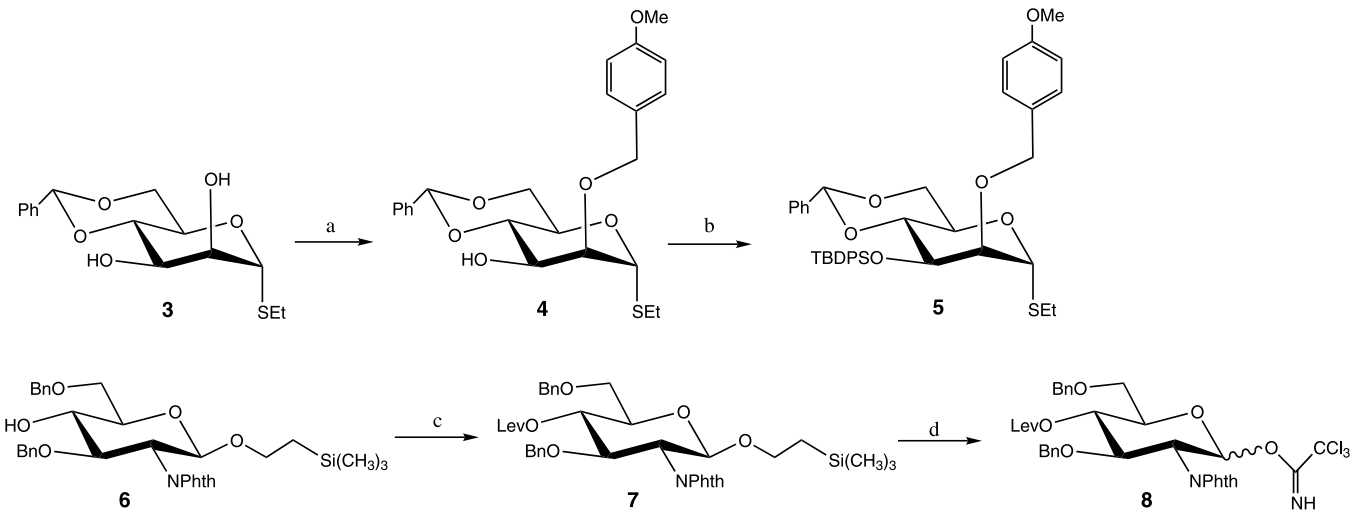


Scheme 1. (a) *p*-MBnCl, Bu_4NBr, CH_2Cl_2–NaOHaq; (b) TBDPSCl, imidazole, DMF; (c) LevOH, DCC, DMAP, CH_2Cl_2; (d) (1) CF_3COOH, CH_2Cl_2; (2) CCl_3CN, DBU, CH_2Cl_2.

which belongs to the class Insecta[5] (structures **1** + **2**). It is particularly interesting to note that these glycosphingolipids contain the unique structures GlcNAcβ(1→3)Man and GalNAcβ(1→4)GlcNAcβ(1→3)Man. The structure of other glycolipids with longer sugar chains has also been defined.[6] These compounds have also 1,2-*cis*-β-D-mannopyranosidic linkages, which are one of the most difficult linkages to be synthesized[7] and for which many carbohydrate chemists[8,9] have developed methods to make these linkages. Furthermore, application of these methods to the synthesis of asparagine (Asn)-linked oligosaccharides and the glycolipids from the fresh water bivalve *Hyriopsis schlegelii* is under current investigation.[9] Among those β-mannoglycoside approaches, Ito and co-workers reported[8g] a novel strategy for its stereoselective synthesis as a new entry into the so-called intramolecular aglycon delivery (IAD) approach. This strategy was subsequently applied to the completely stereocontrolled synthesis of the core structure of Asn-linked glycoprotein oligosaccharides.[9c,d] Herein we report on the application of this method to the total synthesis of the glycosphingolipid. The oligosaccharides of two glycolipids were the target of the synthetic studies described herein as part of our investigation into synthetic oligosaccharides of structural and biological interest.

β-D-GlcNAc*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer
1

β-D-GalNAc*p*-(1→4)-β-D-GlcNAc*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer
2

2. Results and discussion

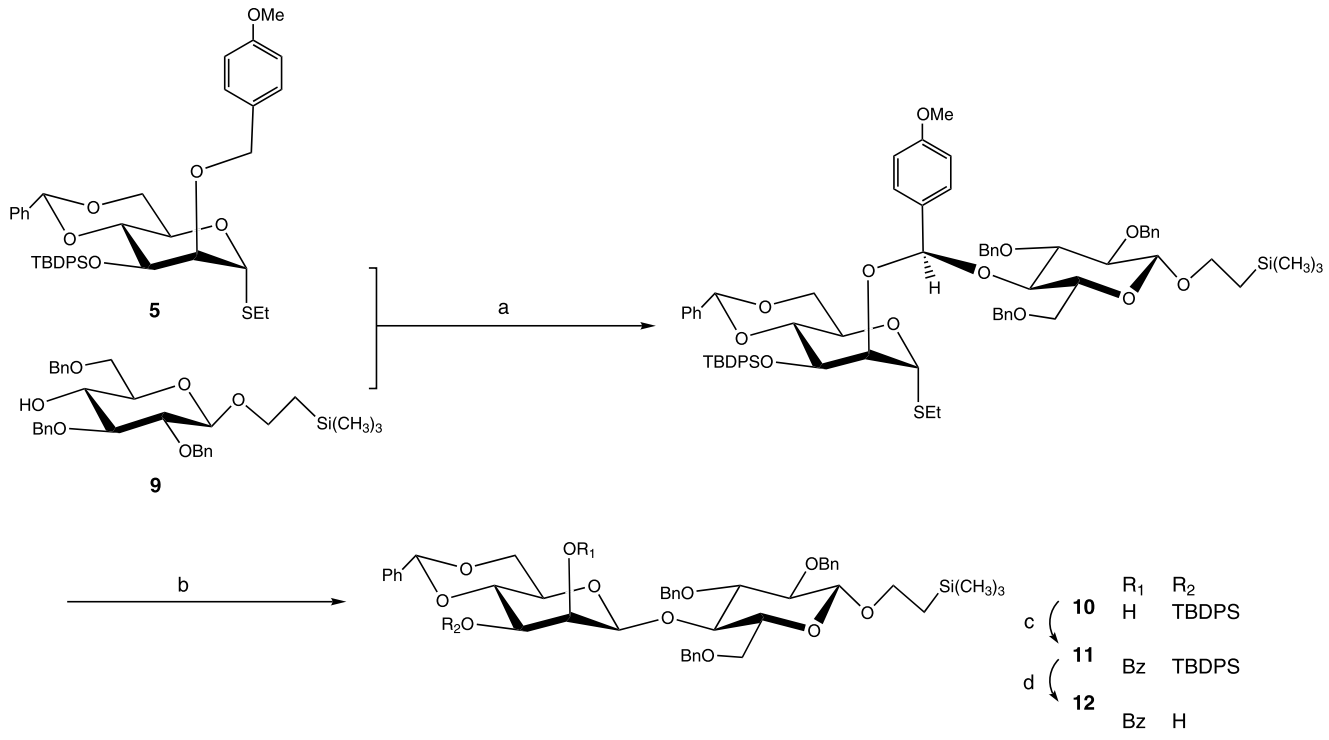
Syntheses of monosaccharide derivatives.—Syntheses of the mannopyranosyl and 2-acetamido-2-deoxy-D-glucopyranosyl building blocks **5** and **8** were carried out as depicted in Scheme 1. Compound **5** was prepared from known ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**3**)[10] by the following two-step procedure. Regioselective *p*-methoxybenzylation at the C-2 position of the starting material with *p*-methoxybenzyl chloride and tetrabutylammonium bromide, followed by silylation with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl),[9c] gave compound **5**. Imidate donor **8** was obtained from 2-(trimethylsilyl)ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6**),[11] which was prepared from the known phthalimido compound.[12] 3-*O*-Levulinoylation[13] of **6** with levulinic acid, dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)pyridine (DMAP) afforded compound **7**. For selective removal of the 2-(trimethylsilyl)ethyl (SE) group, **7** was treated[11] with trifluoroacetic acid in dichloromethane for 1 h at 0 °C to give the 1-hydroxy compound, which, on further treatment[14] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane for 2 h at 0 °C, gave the corresponding trichloroacetimidate **8**.

Syntheses of the target oligosaccharide.—β-Mannosylation was conducted in a standard manner. Thus, treatment of gluco-derived acceptor **9**[11] with **5** and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in dichloromethane in the presence of 4 Å molecular sieves (4 Å MS) afforded the acetal. IAD was effected by methyl trifluoromethanesulfonate (MeOTf) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in dichloromethane to afford a 37% yield of disaccharide, β-D-mannopyranosyl-(1→4)-β-D-glucopyranoside derivative **10**. The β-D configuration of the newly formed glycosidic bond was indicated by the $J_{C1,H1}$ value of 159.3 Hz in the ^{13}C NMR spectrum[15] (Table 2). Benzoylation of **10** and subsequent removal of the 3′-O-TBDPS group from **11** by Bu_4NF gave the disac-

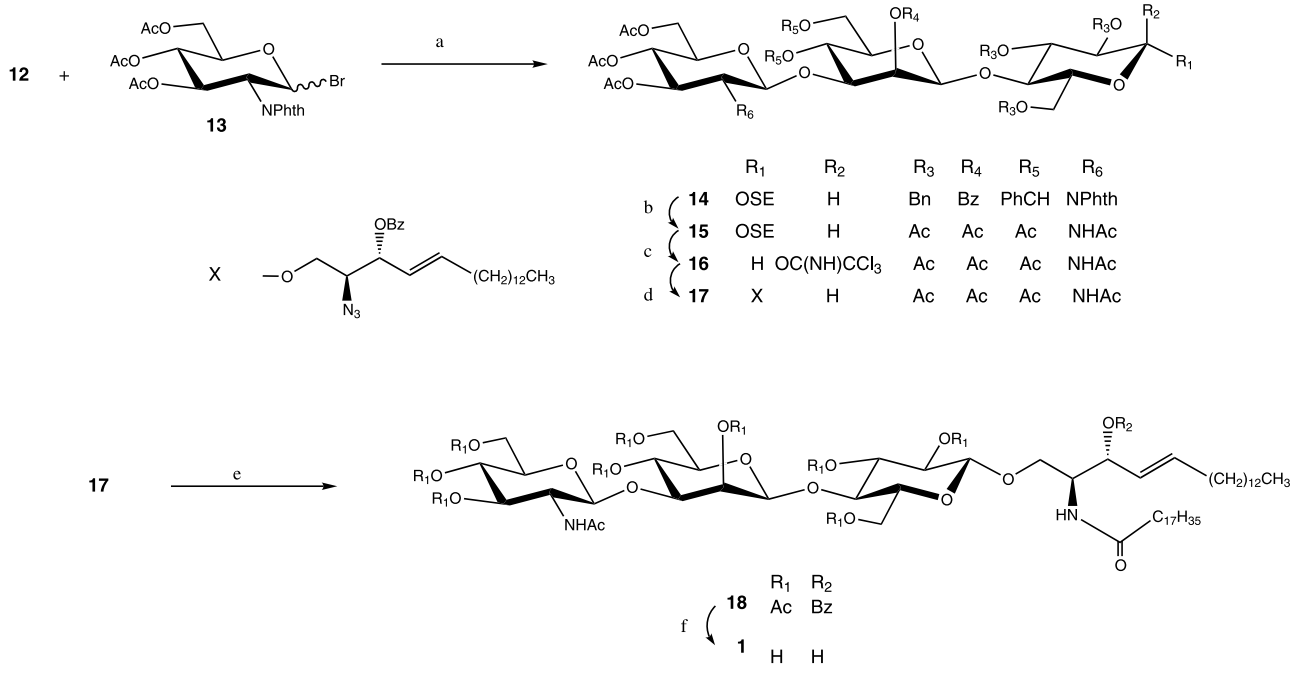
charide acceptor **12** (Scheme 2). Glycosylation of **12** with Lemieux's (phthalimido) donor **13**[12] in the presence of silver triflate (AgOTf) and 4 Å MS in dichloromethane for 24 h at rt gave the desired trisaccharide **14** (72%), as evidenced by ^{1}H NMR spectroscopy (H-1″, 5.57 ppm, J 8.6 Hz). After removal of the benzylidene and benzyl group with 10% Pd–C, the phthalimido and *O*-benzoyl groups were removed by refluxing with hydrazine hydrate in ethanol, giving free amino and hydroxyl groups, which were subsequently N- and O-acetylated with acetic anhydride and pyridine giving **15**. Selective removal of the SE group with trifluoroacetic acid in dichloromethane and treatment with trichloroacetonitrile in the presence of DBU gave the corresponding α-trichloroacetimidate **16**. Glycosylation[16] of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[17] with the glycosyl donor **16**, which was carried out in the presence of TMSOTf and AW300 MS for 8 h at 0 °C, afforded the desired β-glycoside **17** (48%). Selective reduction[18] of the azido group in **17** with triphenylphosphine in 20:1 benzene–water gave the amine, which on condensation with stearic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide, WSC) in dichloromethane, gave the fully protected derivative **18** (72%). Finally, removal of acyl groups in **18** under basic conditions and column chromatography on Sephadex LH-20 furnished target gly-

Table 2
^{13}C NMR data (δ) for selected compounds

Carbon atom	Compound						
	10	**11**	**12**	**14**	**19**	**20**	**22**
C-1 (Glc)	102.9	102.9	103.0	102.4	105.7	102.9	102.8
(*J*C,H)	(159.3)	(159.4)	(157.2)	(159.3)	(159.3)	(161.4)	
2	82.3	82.3	82.3	82.2	82.4	82.2	82.3
3	84.1	84.6	84.6	84.6	84.6	84.5	84.6
4	77.8	78.0	78.2	78.0	77.9	78.4	77.9
5	74.3	74.5	74.8	74.6	74.5	74.6	74.5
6	68.4	69.1	69.6	68.1	68.4	68.9	68.6
C-1 (Man)	100.7	100.1	100.4	99.9	100.6	99.9	99.8
(*J*C,H)	(151.0)	(155.2)	(157.2)	(157.2)	(159.7)	(161.4)	
2	71.4	71.6	71.6	69.6	69.1	69.3	68.9
3	72.7	72.2	70.2	77.2	75.4	75.0	75.4
4	78.4	79.0	79.1	75.6	76.6	75.6	75.2
5	66.6	66.8	67.7	69.5	68.6	68.5	67.8
6	68.6	68.5	68.6	69.4	69.0	69.3	68.1
C-1 (GlcNAc)				97.1	96.2	96.9	96.7
(*J*C,H)				(161.4)	(165.5)	(161.4)	
2				54.5	55.3	55.1	55.1
3				70.8	73.0	73.5	73.9
4				69.0	73.7	71.3	74.7
5				71.7	73.6	73.2	74.1
6				69.0	70.2	71.3	70.3
C-1 (GalNAc)							95.7
2							52.0
3							72.6
4							73.5
5							70.5
6							67.4
CH_2CH_2TMS	67.5	67.3	67.7	67.3	67.3	67.3	67.3
	18.4	18.5	18.5	18.3	18.3	18.4	18.3
	−1.4	−1.4	−1.4	−1.3	−1.3	−1.2	−1.3
PhCH=	101.7	101.8	102.4	101.3	101.2	101.5	101.8
$PhCH_2$–	749	747	74.3	74.4	74.4	74.4	74.4
	75.7	74.8	74.8	74.8	74.8	74.8	75.2
	77.2	75.5	75.6	77.2	74.9	75.0	76.2
					75.6	75.6	76.4
					76.7	77.2	77.2



Scheme 2. (a) DDQ, CH_2Cl_2; (b) DTBMP, MeOTf, CH_2Cl_2; (c) BzCl, Pyr; (d) TBAF, AcOH, THF.



Scheme 3. (a) AgOTf, CH_2Cl_2; (b) (1) Pd–C, AcOH, MeOH, (2) $NH_2NH_2{\cdot}H_2O$, EtOH, (3) Ac_2O, Pyr; (c) (1) CF_3COOH, CH_2Cl_2; (2) CCl_3CN, DBU, CH_2Cl_2; (d) (2*S*,3*R*,4*E*)-2-azido-3-benzoyl-4-octadecene-1,3-diol, TMSOTf, CH_2Cl_2; (e) (1) triphenylphosphine, benzene–water, (2) $C_{17}H_{35}COOH$, WSC, CH_2Cl_2; (f) NaOMe, 1,4-dioxane–MeOH.

colipid **1** (Scheme 3). The structure and purity of **1** was demonstrated by the ^1H NMR and MALDI-TOFMS data. The synthesis of tetrasaccharide **2** began with the TMSOTf-promoted coupling of **8** with **12**, which afforded trisaccharide **19** (72%), as evidenced by ^1H NMR spectroscopy (H-1″ 5.34 ppm, *J* 8.4 Hz). Selective removal of the Lev group[13] in **19** with hydrazine acetate gave the partially protected compound **20**. Glycosylation[19] of **20** with imidate **21**[20] in the presence of TMSOTf and 4 Å MS in dichloromethane for 24 h

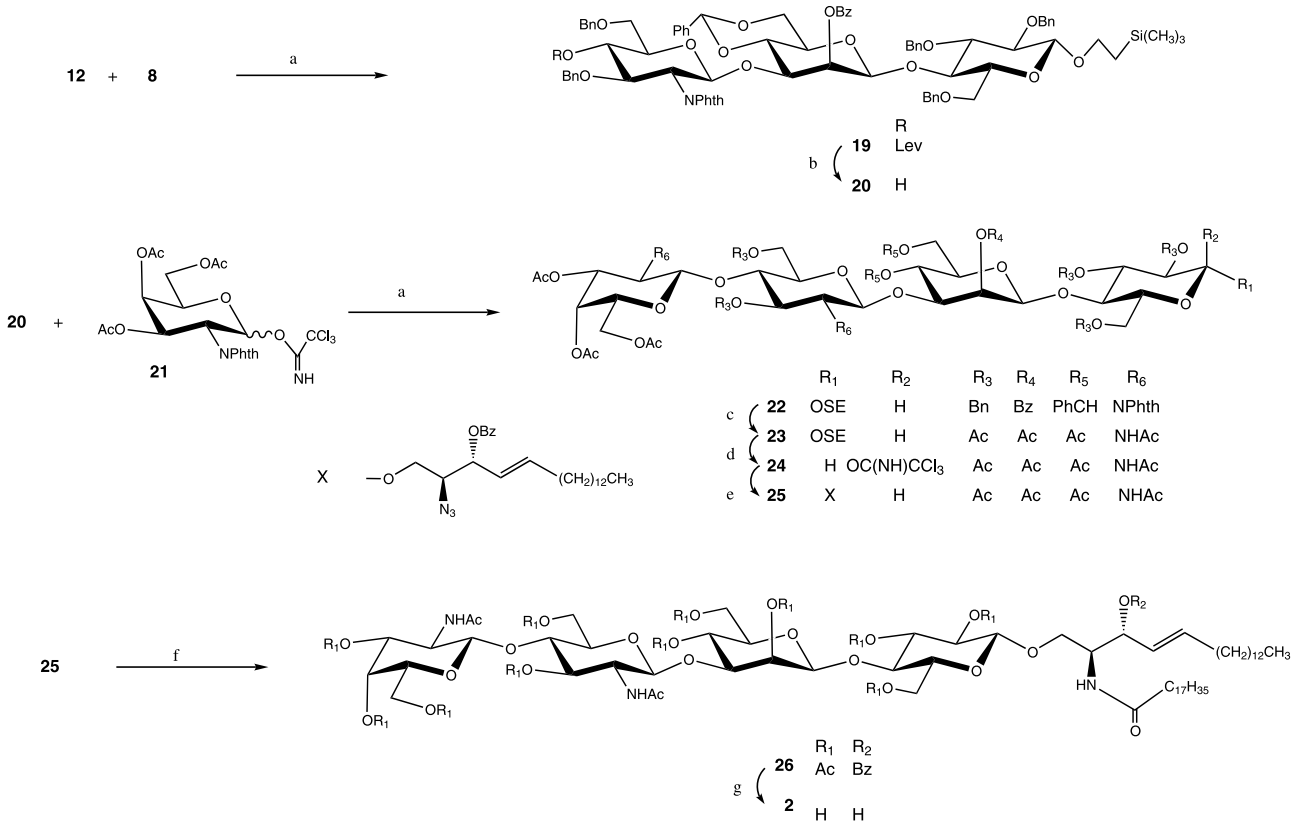
at rt gave the desired tetrasaccharide **22** (83%), as evidenced by ^{1}H NMR spectroscopy (H-1 of GalNAc, 5.43 ppm, J 8.5 Hz). Removal of the benzylidene and benzyl groups with 10% Pd–C and then cleavage of the phthalimido and the *O*-benzoyl groups with hydrazine hydrate in ethanol afforded the fully deprotected product, which was subsequently N- and O-acetylated with acetic anhydride and pyridine to give **23**. Selective removal of the SE group, and treatment with trichloroacetonitrile in the presence of DBU gave the corresponding α-trichloroacetimidate **24**. Glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol with the glycosyl donor **24**, which was carried out in the presence of TMSOTf and AW300 MS for 6 h at 0 °C, afforded the desired β-glycoside **25** (40%). Selective reduction of the azido group in **25** with triphenylphosphine in 20:1 benzene–water gave the amine, which on condensation with stearic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane, gave the fully protected derivative **26** (67%). Finally, removal of the acyl groups in **26** under the basic conditions and column chromatography on Sephadex LH-20 furnished target glycolipid **2** (Scheme 4). The structure and purity of **2** was demonstrated by the MALDI-TOFMS data.

In summary, the synthesis of oligosaccharides containing the GlcNAcβ(1→3)Man and GalNAcβ(1→4)GlcNAcβ(1→3)Man structural motifs, has been carried out. These structures may represent a new type of glycolipid core of glycosphingolipids from the larvae of the green-bottle fly, *L. caesar*.

3. Experimental

General methods.—Optical rotations were measured with a Jasco digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a JMN A500 FT NMR spectrometer with Me_4Si as the internal standard for solutions in $CDCl_3$. MALDI-TOFMS was recorded on a Perseptive Voyager RP mass spectrometer. High-resolution mass spectra were recorded on a JEOL JMS-700 under FAB conditions. TLC was performed on Silica Gel 60 F254 (E. Merck) with detection by quenching of UV fluorescence and by charring with 10% H_2SO_4. Column chromatography was carried out on Silica Gel 60 (E. Merck).

Ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**3**),[10] 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-



Scheme 4. (a) TMSOTf, CH_2Cl_2; (b) NH_2NH_2·AcOH, MeOH–THF; (c) (1) Pd–C, AcOH, MeOH, (2) NH_2NH_2·H_2O, EtOH, (3) Ac_2O, Pyr; (d) (1) CF_3COOH, CH_2Cl_2; (2) CCl_3CN, DBU, CH_2Cl_2; (e) (2*S*,3*R*,4*E*)-2-azido-3-benzoyl-4-octadecene-1,3-diol, TMSOTf, CH_2Cl_2; (f) (1) triphenylphosphine, benzene–water, (2) $C_{17}H_{35}COOH$, WSC, CH_2Cl_2; (g) NaOMe, 1,4-dioxane–MeOH.

D-glucopyranoside (**9**),[11] 2-(trimethylsilyl)ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6**),[11] 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**13**),[12] 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate (**21**)[20] and (2*S*,3*R*,4*E*)-2-azido-3-*O*-(benzoyl)-4-octadecene-1,3-diol[17] were prepared as reported in the literature.

*Ethyl 4,6-*O*-benzylidene-2-*O*-p-methoxybenzyl-1-thio-α-D-mannopyranoside* (**4**).—Ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**3**: 500 mg, 1.60 mmol), Bu_4NBr (153 mg, 0.50 mmol) and *p*-methoxybenzyl chloride (320 μL, 0.80 mmol) were dissolved in CH_2Cl_2 (10 mL). Aq NaOH (1.0 mL of a 5% solution) was added, and the mixture was stirred under reflux for 30 h. The reaction mixture was cooled, and the organic layer was separated, washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 5:1 hexane–AcOEt as eluent to give **4** (370 mg, 54%). $[\alpha]_D$ +12.9° (*c* 3.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.49–6.89 (m, 9 H, 2 *Ph*), 5.53 (s, 1 H, PhC*H*), 5.34 (s, 1 H, *H*-1), 4.67,4.56 (each d, 2 H, J_{gem} 11.6 Hz, PhCH_2), 4.21–4.18 (m, 2 H, *H*-3, *H*-6a), 4.01 (q, 1 H, *H*-5), 3.91 (t, 1 H, $J_{4,5}$ 9.2 Hz, *H*-4), 3.89 (d, 1 H, $J_{2,3}$ 9.7 Hz, *H*-2), 3.81 (t, 1 H, $J_{6a,6b}$ 11.0 Hz, *H*-6b), 3.78 (s, 3 H, PhOCH_3), 2.60 (q, 2 H, SCH_2CH$_3$), 2.47 (s, 1 H, O*H*), 1.25 (t, 3 H, SCH$_2$CH_3). MALDI-TOFMS: Calcd for $C_{23}H_{28}O_6S$: *m*/*z* 432. Found: *m*/*z* 455 $[M+Na]^+$.

*Ethyl 4,6-*O*-benzylidene-3-*O*-tert-butyldiphenylsilyl-2-O-p-methoxybenzyl-1-thio-α-D-mannopyranoside* (**5**).—To a solution of **4** (660 mg, 1.50 mmol) in DMF (5.0 mL) were added imidazole (210 mg, 3.0 mmol) and *tert*-butylchlorodiphenylsilane (0.80 mL, 3.0 mmol). The reaction mixture was stirred for 18 h at 50 °C, then extracted with AcOEt, washed with 5% HCl, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 8:1 hexane–AcOEt as eluent to give **5** (934 mg, 91%). $[\alpha]_D$ +14.2° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.67–6.83 (19H, m, 4 *Ph*), 5.41 (s, 1 H, PhC*H*), 5.01 (s, 1 H, *H*-1), 4.58, 4.40 (each d, 2 H, J_{gem} 11.8 Hz, PhCH_2), 4.29 (dd, 1 H, *H*-3), 4.14 (t, 1 H, $J_{4,5}$ 9.8 Hz, *H*-4), 4.11 (dd, 1 H, *H*-6a), 4.02 (q, 1 H, *H*-5), 3.81–3.77 (m, 4 H, *H*-6b, PhOCH_3), 3.40 (d, 1 H, *H*-2), 2.47 (q, 2 H, SCH_2CH$_3$), 1.13 (t, 3 H, SCH$_2$CH_3), 1.05 (s, 9 H, Si(CH_3)$_3$). MALDI-TOFMS: Calcd for $C_{39}H_{46}O_6SSi$: *m*/*z* 670. Found: *m*/*z* 693 $[M+Na]^+$. HR FABMS: Calcd for $C_{39}H_{47}O_6SSi$ $[M+H]^+$: 671.2862, Found 671.2849.

*2-(Trimethylsilyl)ethyl 3,6-di-*O*-benzyl-2-deoxy-4-O-levulinoyl-2-phthalimido-β-D-glucopyranoside* (**7**).—To a solution of **6** (630 mg, 1.10 mmol) in CH_2Cl_2 (5.0 mL) was added levulinic acid (181 μL, 1.90 mmol), DCC (407 mg, 2.0 mmol) and DMAP (36.0 mg, 0.30 mmol). The reaction mixture was stirred for 2 h at rt, then extracted with $CHCl_3$, washed with 5% HCl, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 2:1 hexane–AcOEt as eluent to give **7** (600 mg, 83%). ^{1}H NMR ($CDCl_3$): δ 7.68–6.87 (m, 14 H, 3 Ph), 5.15 (t, 1 H, $J_{4,5}$ 8.5 Hz, *H*-4), 5.13, 4.64, 4.60, 4.54 (each d, 4 H, J_{gem} 10.8 Hz, 2 PhCH_2), 4.41 (t, 1 H, $J_{2,3}$ 10.4 Hz, *H*-2), 4.30 (d, 1 H, $J_{1,2}$ 8.3 Hz, *H*-1), 4.28 (t, 1 H, $J_{3,4}$ 8.5 Hz, *H*-3), 3.92 (q, 1 H, OCH_2CH$_2$Si), 3.76 (q, 1 H, *H*-5), 3.65-3.63 (m, 2 H, *H*-6a, OCH_2CH$_2$Si), 3.46 (dd, 1 H, $J_{5,6a}$ 16.7 Hz, $J_{6a,6b}$ 9.8 Hz, *H*-6b), 2.19 (s, 3 H, CH_3), 1.57 (s, 4 H, CH_2CH_2), 0.83 (t, 2 H, OCH$_2$CH_2Si), −0.08 (s, 9 H, Si(CH_3)$_3$). MALDI-TOFMS: Calcd for $C_{38}H_{45}NO_9Si$: *m*/*z* 687. Found: *m*/*z* 710 $[M+Na]^+$. HR FABMS: Calcd for $C_{38}H_{46}NO_9Si$ $[M+H]^+$: 688.2941, Found 688.2923.

*3,6-Di-*O*-benzyl-2-deoxy-4-O-levulinoyl-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate* (**8**).—To a solution of **7** (292 mg, 0.43 mmol) in CH_2Cl_2 (5.0 mL), cooled to 0 °C was added CF_3COOH (5.0 mL), and the mixture was stirred for 1 h at rt and concentrated. AcOEt and toluene (1:2) were added and evaporated to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (2.0 mL) cooled at 0 °C were added CCl_3CN (850 μL, 8.60 mmol) and DBU (64.0 μL, 0.43 mmol). The mixture was stirred for 30 min at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography of the residue on silica gel (1:1 benzene–acetone) gave **8** (218 mg, 70%). ^{1}H NMR ($CDCl_3$): δ 8.62 (s, 1 H, N*H*), 6.42 (d, 1 H, $J_{1,2}$ 8.6 Hz, *H*-1β), 6.36 (d, 1 H, $J_{1,2}$ 3.7 Hz, *H*-1α).

*2-(Trimethylsilyl)ethyl 4,6-*O*-benzylidene-3-*O*-tert-butyldiphenylsilyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside* (**10**).—To a stirred mixture of DDQ (68.1 mg, 0.30 mmol) and 4A MS in dry CH_2Cl_2 (2.0 mL) were added 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**9**, 105 mg, 0.20 mmol) and **5** (174 mg, 0.26 mmol) as a solution in dry CH_2Cl_2 (5.0 mL) at 0 °C. The mixture was stirred for 10 min at 0 °C and for 1 h at rt. The resulting mixture was quenched with a solution of ascorbic acid (0.7%) and NaOH (0.9%) in water (3.0 mL), diluted with AcOEt and filtered off. The filtrate was successively washed with water, aq $NaHCO_3$, dried (Na_2SO_4), and concentrated to give mixed acetal. To a solution of this acetal, 2,6-di-*tert*-butyl-4-methyl-pyridine (DTBMP: 225 mg, 1.10 mmol) and 4A MS in dry CH_2Cl_2 (5.0 mL) were stirred for 2 h at rt, then cooled to 0 °C. Methyl trifuruoromethanesufonate (MeOTf, 113 μL, 5.0 mmol) was added to the mixture, which was stirred for 10 min at 0 °C and for 24 h at rt, then neutralized with Et_3N. The mixture was filtered off and washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 10:1 hexane–AcOEt as eluent to give **10** (73.7 mg, 37%). $[\alpha]_D$ −4.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.66–7.17 (m, 30 H, 6 *Ph*), 5.35 (s, 1 H,

PhC*H*), 4.95, 4.93, 4.81, 4.76, 4.70, 4.45 (each d, 6 H, J_{gem} 10.9 Hz, 3 PhCH_2), 4.37 (d, 1 H, $J_{1,2}$ 7.3 Hz, *H*-1), 4.22 (t, 1 H, $J_{4,5}$ 4.9 Hz, *H*-4′), 4.19 (s, 1 H, *H*-1′), 4.10 (dd, 1 H, *H*-6a), 3.99 (t, 1 H, $J_{5,6b}$ 9.2 Hz, *H*-6b), 3.93 (q, 1 H, OCH_2CH$_2$Si), 3.82 (dd, 1 H, $J_{5,6b}$ 9.2 Hz, $J_{6a,6b}$ 3.1 Hz, *H*-6a′), 3.78–3.76 (m, 2 H, *H*-2′, *H*-3′), 3.63 (t, 1 H, $J_{3,4}$ 8.5 Hz, *H*-3), 3.56 (q, 1 H, OCH_2CH$_2$Si), 3.51–3.48 (m, 2 H, *H*-5, *H*-6b′), 3.40–3.35 (m, 2 H, *H*-2, *H*-4), 3.10 (t, 1 H, *H*-5′), 1.62 (s, 1 H, O*H*), 1.01 (s, 9 H, 3 CH_3), 0.98 (t, 2 H, OCH$_2$CH_2Si), −0.08 (s, 9 H, Si(CH_3)$_3$). MALDI-TOFMS: Calcd for $C_{61}H_{74}O_{11}Si_2$: *m*/*z* 1038. Found: *m*/*z* 1061 $[M+Na]^+$. HR FABMS: Calcd for $C_{61}H_{75}O_{11}Si_2$ $[M+H]^+$: 1039.4847, Found 1039.4828.

*2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-tert-butyldiphenylsilyl-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**11**).—To a solution of **10** (550 mg, 0.53 mmol) in pyridine (10 mL) were added benzoyl chloride (5.0 mL). The reaction mixture was stirred for 5 h at rt, then extracted with $CHCl_3$, washed with 5% HCl, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 10:1 hexane–AcOEt as eluent to give **11** (540 mg, 89%). $[\alpha]_D$ −9.3° (*c* 3.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.17–6.97 (m, 35 H, 7 *Ph*), 5.55 (d, 1 H, H-2′), 5.18 (s, 1 H, PhC*H*), 4.90, 4.86, 4.76, 4.71, 4.64, 4.37 (each d, 6 H, J_{gem} 11.0 Hz, 3 PhCH_2), 4.47 (s, 1 H, *H*-1′), 4.27 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1), 4.22 (dd, 1 H, $J_{5,6a}$ 10.4 Hz, $J_{6a,6b}$ 4.9 Hz, *H*-6a′), 4.03 (dd, 1 H, $J_{2,3}$ 3.7 Hz, $J_{3,4}$ 9.8 Hz, *H*-3′), 3.98 (dd, 1 H, *H*-6a), 3.97 (t, 1 H, $J_{4,5}$ 9.2 Hz, *H*-4′), 3.94 (q, 1 H, OCH_2CH$_2$Si), 3.79 (t, 1 H, $J_{5,6b}$ 10.4 Hz, *H*-6b′), 3.59–3.50 (m, 3 H, H-3, *H*-6b, OCH_2CH$_2$Si), 3.35 (q, 1 H, *H*-5), 3.32 (t, 1 H, $J_{4,5}$ 9.8 Hz, *H*-4), 3.26 (d, 1 H, *H*-2), 3.20 (q, 1 H, *H*-5′), 0.92 (t, 2 H, OCH$_2$CH_2Si), 0.82 (s, 9 H, 3 CH$_3$), −0.08 (s, 9 H, Si(CH_3)$_3$). MALDI-TOFMS: Calcd for $C_{68}H_{78}O_{12}Si_2$: *m*/*z* 1142. Found: *m*/*z* 1165 $[M+Na]^+$.

*2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**12**).—To a solution of **11** (270 mg, 0.24 mmol) in THF (3.0 mL) were added *t*-Bu$_4$NF (TBAF, 1.0 M solution in THF 650 μL). The reaction mixture was stirred for 1 h at rt, then extracted with $CHCl_3$, washed with 5% HCl, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 1:1 hexane–AcOEt as eluent to give **12** (140 mg, 80%). $[\alpha]_D$ −20.5° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.07–7.16 (m, 25 H, 5 *Ph*), 5.67 (d, 1 H, *H*-2′), 5.57 (s, 1 H, PhC*H*), 4.89, 4.85, 4.79, 4.74, 4.70, 4.65 (each d, 6 H, J_{gem} 11.0 Hz, 3 PhCH_2), 4.76 (s, 1 H, *H*-1′), 4.35 (dd, 1 H, $J_{5,6a}$ 10.4 Hz, $J_{6a,6b}$ 4.9 Hz, *H*-6a′), 4.33 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1), 4.23 (t, 1 H, *H*-6a), 4.09 (dd, 1 H, $J_{5,6a}$ 9.7 Hz, $J_{6a,6b}$ 4.8 Hz, *H*-6b), 4.01–3.85 (m, 5 H, *H*-3′, OCH_2CH$_2$Si, *H*-4, *H*-4′, *H*-6b′), 3.61 (q, 1 H, OCH_2CH$_2$Si), 3.57 (t, 1 H, $J_{3,4}$ 9.2 Hz, *H*-3), 3.44–3.36 (m, 2 H, *H*-5, *H*-5′), 3.27 (t, 1 H, $J_{2,3}$ 9.2 Hz, *H*-2), 0.92 (t, 2 H, OCH$_2$CH_2Si), −0.08 (s, 9 H, Si(CH_3)$_3$). MALDI-TOFMS: Calcd for $C_{52}H_{60}O_{12}Si$: *m*/*z* 904. Found: *m*/*z* 927 $[M+Na]^+$.

*2-(Trimethylsilyl)ethyl 3,4,6,-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**14**).—A solution of **12** (140 mg, 0.15 mmol), 3,4,6-tri-O-acetyl-2-phthalimido-β-D-glucopyranosyl bromide (**13**: 169 mg, 0.31 mmol) and 4 Å MS in dry CH_2Cl_2 (2.0 mL) was stirred for 2 h at rt, then cooled to −25 °C. Silver triflate (AgOTf: 159 mg, 0.20 mmol) was added to the mixture, which was stirred for 1 h at −25 °C and for 24 h at rt. The insoluble materials were filtered off, and the filtrate was washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 1:1 hexane–AcOEt as eluent to give **14** (147 mg, 72%). $[\alpha]_D$ −15.3° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.08–7.14 (m, 29 H, 6 *Ph*), 5.64 (t, 1 H, $J_{2'',3''=3'',4''}$ 9.8 Hz, *H*-3″), 5.59 (s, 1 H, PhC*H*), 5.57 (d, 1 H, $J_{1,2}$ 8.6 Hz, *H*-1″), 5.46 (d, 1 H, $J_{2',3'}$ 3.0 Hz, *H*-2′), 4.86, 4.84, 4.73, 4.68, 4.62, 4.42 (each d, 6 H, J_{gem} 11.2 Hz, 3 PhCH_2), 4.53 (s, 1 H, *H*-1′), 4.35 (dd, 1 H, $J_{5,6a}$ 10.4 Hz, $J_{6a,6b}$ 4.9 Hz, *H*-6a′), 4.23 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1), 4.30–3.89 (m, 9 H, *H*-3′, *H*-4′, *H*-6a, *H*-6b, *H*-6b′ *H*-2″, *H*-6a″, *H*-6b″, OCH_2CH$_2$Si,), 3.83 (q, 1 H, OCH_2CH$_2$Si), 3.77 (q, 1H, *H*-5), 3.57 (t, 1 H, $J_{3,4}$ 9.2 Hz, *H*-3), 3.45–3.25 (m, 3 H, *H*-4, *H*-5′, *H*-5″), 3.22 (t, 1 H, $J_{2,3}$ 8.5 Hz, *H*-2), 2.10, 2.08, 2.06 (each S, 9H, 3 Ac), 0.95 (t, 2 H, OCH_2CH_2Si), −0.08 (s, 9 H, Si(CH_3)$_3$), 4.23 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1). MALDI-TOFMS: Calcd for $C_{72}H_{79}NO_{21}Si$: *m*/*z* 1321. Found: *m*/*z* 1344 $[M+Na]^+$. HR FABMS: Calcd for $C_{72}H_{80}NO_{21}Si$ $[M+H]^+$:1322.4991, Found 1322.4978.

*2-(Trimethylsilyl)ethyl 2-acetamido-3,4,6,-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-O-acetyl-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**15**).—A solution of **14** (147 mg, 0.11 mmol) in MeOH (3.0 mL) and AcOH (10 μL) was hydrogenolyzed in the presence of 10% Pd–C (140 mg) for 24 h at rt, then filtered and concentrated. The residue was dissolved in EtOH (5.0 mL), and hydrazine monohydrate (5.0 mL) was added. The reaction mixture was refluxed for 3 h, then concentrated. The residue was acetylated with Ac_2O (3.0 mL) in pyridine (4.0 mL). The mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with 5% HCl, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 2:1 hexane–AcOEt as eluent to give **15** (93.0 mg, 83%). $[\alpha]_D$ −88.1° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.63 (d, 1 H, N*H*) 5.39 (brs. 1 H, *H*-2′), 5.18–5.12 (m, 2 H, *H*-3, *H*-3″), 5.06 (t, 1 H, *H*-4′), 4.89–4.84 (m, 2 H, *H*-2, *H*-4″), 4.69 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1″), 4.51 (s, 1 H,

H-1′), 4.46 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1), 2.14–2.10 (m, 30H, 10 Ac). MALDI-TOFMS: Calcd for $C_{43}H_{65}NO_{25}Si$: *m*/*z* 1024. Found: *m*/*z* 1047 $[M + Na]^+$.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate* (**16**).—To a solution of **15** (93.0 mg, 0.09 mmol) in CH_2Cl_2 (2.0 mL), cooled to 0 °C was added CF_3COOH (2.0 mL), and the mixture was stirred for 30 min at rt and concentrated. AcOEt and toluene (1:2) were added and evaporated to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (2.0 mL) cooled at 0 °C were added CCl_3CN (325 μL, 3.20 mmol) and DBU (11.2 μL, 0.10 mmol). The mixture was stirred for 30 min at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography of the residue on silica gel (1:1 hexane–AcOEt) gave **16** (90.0 mg, 93%). ^{1}H NMR ($CDCl_3$): δ 8.58 (s, 1 H, N*H*), 6.55 (d, 1H, $J_{1,2}$ 3.6 Hz, *H*-1), 4.71 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1″), 4.49 (s, 1 H, *H*-1′). The product was used directly in the following step.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-*D*-mannopyranosyl-(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* (**17**).—To a solution of **16** (64.6 mg, 0.06 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (52.0 mg, 0.12 mmol) in dry CH_2Cl_2 (0.40 mL) was added AW300 MS (350 mg), and the mixture was stirred for 12 h at rt, then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 3.3 μL, 0.016 mmol) was added, and the mixture was stirred for 8 h at 0 °C. The solids were filtered off and washed with CH_2Cl_2. The combined filtrate and wash were washed with $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 3:2 benzene–acetone as eluent to give **17** (39.5 mg, 48%). $[\alpha]_D$ −28.1° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.94–5.90 (m, 1 H, *H*-5 of sphingosine), 5.68 (dd, 1 H, *H*-4 of sphingosine), 4.75 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1″), 4.65 (s, 1 H, *H*-1′), 4.23 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{63}H_{90}N_4O_{27}$: *m*/*z* 1335. Found: *m*/*z* 1358 $[M + Na]^+$. HR FABMS: Calcd for $C_{63}H_{91}N_4O_{27}$ $[M + H]^+$: 1335.5869, Found 1335.5832.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecamido-4-octadecene-1,3-diol* (**18**).—To an emulsion of **17** (30.0 mg, 0.02 mmol) in benzene (0.60 mL) and water (30.0 μL) was added triphenylphosphine (17.6 mg, 0.06 mmol), and the mixture was stirred for 24 h with the progress of the reaction being monitored by TLC. The mixture was concentrated, and the residue was stirred with stearic acid (19.0 mg, 0.06 mmol) and 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 12.8 mg, 0.06 mmol) in dry CH_2Cl_2 (1.0 mL) for 24 h at rt The mixture was diluted with CH_2Cl_2 (10 mL), washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 2:1 benzene–acetone as eluent to give **18** (24.0 mg, 72%). $[\alpha]_D$ −14.9° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.88–5.85 (m, 1 H, *H*-5 of sphingosine), 5.55 (dd, 1 H, *H*-4 of sphingosine), 4.74 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1″), 4.60 (s, 1 H, *H*-1′), 4.33 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1). MALDI-TOFMS: Calcd for $C_{81}H_{126}N_2O_{28}$: *m*/*z* 1575. Found: *m*/*z* 1576 $[M + H]^+$.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→3)-β-*D*-mannopyranosyl-(1→4)-β-*D*-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecamido-4-octadecene-1,3-diol* (**1**).—To a solution of **18** (24.0 mg, 0.02 mmol) in MeOH (1.0 mL) and 1,4-dioxane (1.0 mL) was added NaOMe (15.0 mg) at 40 °C, and the mixture was stirred for 20 h, then neutralized with Amberlite IR-120 $[H^+]$. The mixture was filtered off and concentrated. The product was purified by column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 to give **1** (12.5 mg, 82%). $[\alpha]_D$ −11.4° (*c* 0.5, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (1:1 $CDCl_3$–CD_3OD): δ 5.63–5.60 (m, 1 H, *H*-5 of sphingosine), 5.35 (dd, 1 H, *H*-4 of sphingosine), 4.53 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1″), 4.48 (s, 1 H, *H*-1′), 4.04 (d, 1 H, $J_{1,2}$ 7.6 Hz, *H*-1), 3.96 (t, 1 H, H-3 of sphingosine). HRFABMS: Calcd for $C_{56}H_{104}N_2NaO_{18}$ $[M + Na]^+$: 1115.7182, Found 1115.7191.

*2-(Trimethylsilyl)ethyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-phthalimido-β-*D*-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**19**).—To a solution of **12** (95.0 mg, 0.09 mmol), **8** (102 mg, 0.14 mmol) and 4 Å MS in dry CH_2Cl_2 (2.0 mL) were stirred for 2 h at rt, then cooled to −25 °C. TMSOTf (3.1 μL, 0.02 mmol) was added to the mixture, which was stirred for 1 h at −25 °C and for 24 h at rt. The mixture was filtered, and the filtrate was washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 50:1 benzene–acetone as eluent to give **19** (94.0 mg, 72%). $[\alpha]_D$ −9.1° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.51–6.84 (m, 39 H, 8 *Ph*), 5.60 (s, 1 H, PhC*H*), 5.43 (brs. 1 H, *H*-2′), 5.34 (d, 1 H, $J_{1,2}$ 8.4 Hz, *H*-1″), 5.10 (t, 1 H, $J_{3'',4''=4'',5''}$ 9.7 Hz, *H*-4″), 4.87, 4.80, 4.72, 4.67, 4.60, 4.50, 4.45, 4.43, 4.40, 4.32 (each d, 10 H, 5 benzyl methylene), 4.41 (s, 1 H, *H*-1′), 4.34 (t, 1 H, *H*-3″), 4.25 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1). 4.23 (t, 1 H, *H*-6a), 4.20 (t, 1 H, *H*-2″), 4.02 (dd, 1 H, *H*-3′), 3.94–3.82 (m, 4 H, *H*-6b, 4′, 6b, OCH_2CH_2), 3.75 (q, 1 H, *H*-5″), 3.61 (dd, 1H, *H*-6a″), 3.54–3.46 (m, 4 H, *H*-3′, 6b′, 6b″, OCH_2CH_2), 3.30–3.29 (m, 2 H, *H*-4, 5), 3.25–3.21 (m, 2 H, *H*-2, 5′), 2.09 (s, 3 H, CH_3), 1.25 (s, 4 H, CH_2CH_2), 0.94 (t, 2 H, OCH_2CH_2Si), −0.03 (s, 9 H, $Si(CH_3)_3$). MALDI-

TOFMS: Calcd for $C_{85}H_{91}NO_{20}Si$: m/z 1474. Found: m/z 1497$[M + Na]^+$.

2-(Trimethylsilyl)ethyl 3,6-di-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-2*-O-*benzoyl-4,6*-O-*benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**20**).—To a solution of **19** (94.0 mg, 0.63 mmol) in MeOH (0.3 mL) and THF (3.0 mL) was added hydrazine acetate (24.0 mg, 2.6 mmol), and the mixture was stirred for 2 h at rt The mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was washed with $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 2:1 hexane–AcOEt as eluent to give **20** (80.0 mg, 91%). $[\alpha]_D$ −25.0° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.51–6.88 (m, 39 H, 8 *Ph*), 5.56 (s, 1 H, PhC*H*), 5.43 (brs. 1 H, *H*-2′), 5.34 (d, 1 H, $J_{1,2}$ 8.4 Hz, *H*-1″), 4.87, 4.84, 4.73, 4.69, 4.65, 4.63, (each d, 6 H, 3 benzyl methylene), 4.48–4.40 (m, 4 H, 2 benzyl methylene), 4.41 (s, 1 H, *H*-1′), 4.25 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1), 4.26–4.23 (m, 2 H, *H*-3″, 6a), 4.18–4.07 (m, 2 H, *H*-2″, 4″), 3.98 (dd, 1 H, *H*-3′), 3.93–3.82 (m, 4 H, *H*-6b, 4′, 6b, OCH_2CH$_2$), 3.75 (q, 1 H, *H*-5″), 3.61 (dd, 1H, *H*-6a″), 3.54-3.46 (m, 4 H, *H*-3, 6b′, 6b″, OCH_2CH$_2$), 3.30–3.29 (m, 3 H, *H*-4, 5, 5′), 3.21 (m, 1 H, *H*-2), 3.02 (s, 1 H, O*H*), 0.98 (t, 2 H, OCH$_2$CH_2Si), −0.03 (s, 9 H, Si(CH_3)$_3$). MALDI-TOFMS: Calcd for $C_{80}H_{85}NO_{18}Si$: m/z 1376. Found: m/z 1399 $[M + Na]^+$.

2-(Trimethylsilyl)ethyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β-D-glalactopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-2*-O-*benzoyl-4,6*-O-*benzylidene-β*-D-*mannopyranosyl-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**22**).—A solution of **20** (115 mg, 0.08 mmol), 3,4,6-tri-*O*-acetyl-2-phthalimido-2-deoxy-β-D-galactopyranosyl trichloroacetimidate (**21**: 73.0 mg, 0.12 mmol) and MS 4 Å in dry CH_2Cl_2 (2.0 mL) was stirred for 2 h at rt, then cooled to −25 °C. TMSOTf (3.0 μL, 0.24 mmol) was added to the mixture, which was then stirred for 1 h at −25 °C and for 24 h at rt. The insoluble materials were filtered off and the filtrate was washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 2:1 hexane–AcOEt as eluent to give **22** (124 mg, 83%). $[\alpha]_D$ −34.1° (*c* 2.5 $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.92–6.88 (m, 43 H, 9 *Ph*), 5.71 (dd, 1 H, $J_{2''',3'''}$ 11.5 Hz, $J_{3''',4'''}$ 3.7 Hz, *H*-3‴), 5.56 (s, 1 H, PhC*H*), 5.43 (d, 1 H, $J_{1''',\ 2'''}$ 8.5 Hz, *H*-1‴), 5.42 (br. s. 1 H, *H*-2′), 5.29 (d, 1 H, *H*-4‴), 5.20 (d, 1 H, $J_{1,2}$ 8.4 Hz, *H*-1″), 4.87, 4.83, 4.76, 4.70, 4.63, 4.47, 4.41, 4.40 (m, 8 H, 4 benzyl methylene), 4.46 (t, 1 H, *H*-2‴), 4.40 (s, 1 H, *H*-1′), 4.28 (dd, 1 H, *H*-6a′), 4.21 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1), 4.26–4.08 (m, 6H, *H*-6a, 2″, 4″, 6a‴, benzyl methylene), 3.98–3.80 (m, 6 H, *H*-4, 3′, 4′, 6b′, 6b″, OCH_2CH$_2$), 3.55–3.21 (m, 10 H, *H*-2, 3, 5, 6b, 5′, 3″, 5″, 6a″, 6b″, OCH_2CH$_2$) 2.36–2.00 (m, 9 H, 3 Ac), 0.70 (t, 2 H, OCH$_2$CH_2Si), −0.03 (s, 9 H, Si(CH_3)$_3$). MALDI-TOFMS: Calcd for $C_{100}H_{104}N_2O_{27}Si$: m/z 1793. Found: m/z 1816 $[M + Na]^+$. HRFABMS: Calcd for $C_{100}H_{105}N_2O_{27}Si$ $[M + H]^+$: 1793.6673, Found 1793.6652.

2-(Trimethylsilyl)ethyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β-D-glalactopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*mannopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**23**). A solution of **22** (76.0 mg, 0.04 mmol) in MeOH (3.0 mL) and AcOH (1.0 mL) was hydrogenolyzed in the presence of 10% Pd–C (90.0 mg) for 24 h at rt, then filtered and concentrated. The residue and hydrazine monohydrate (6.0 m L) were dissolved EtOH (8.0 mL). The reaction mixture was refluxed for 3 h, then concentrated. The residue was acetylated with Ac_2O (3.0 mL) in pyridine (4.0 mL). The mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with 5% HCl, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 2:1 benzene–acetone as eluent to give **23** (47.6 mg, 83%). $[\alpha]_D$ −22.7° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.54 (d, 1 H, $J_{1,2}$ 8.3 Hz, *H*-1 of GlcNAc), 4.46 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1 of GalNAc), 4.30 (s, 1 H, *H*-1 of Man), 4.24 (d, 1 H, $J_{1,2}$ 7.9 Hz, *H*-1 of Glc). MALDI-TOFMS: Calcd for $C_{55}H_{82}N_2O_{32}Si$: m/z 1311. Found: m/z 1334 $[M + Na]^+$.

2-Acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*glalactopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*mannopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl tichloroacetimidate* (**24**).—To a solution of **23** (47 mg, 0.04 mmol) in CH_2Cl_2 (1.0 mL), cooled to 0 °C was added CF_3COOH (1.0 mL), and the mixture was stirred for 1 h at rt and concentrated. Ethyl acetate and toluene (1:2) were added and evaporated to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (1.0 mL) cooled at 0 °C were added CCl_3CN (164 μL, 1.1 mmol) and DBU (5.50 μL, 0.04 mmol). The mixture was stirred for 30 min at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography of the residue on silica gel (1:1 benzene–acetone) gave **24** (47.0 mg, quantitative). $[\alpha]_D$ −3.3° (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.84 (s, 1 H, *N*H), 6.47 (d, 1 H, $J_{1,2}$ 3.7 Hz, *H*-1 of Glc) 4.71 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1 of GalNAc), 4.62 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1 of GlcNAc), 4.61 (s, 1 H, H-1 of Man).

2-Acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*galactopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*mannopyranosyl-(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* (**25**).—To a solution of **24** (25.0 mg, 18.7 μmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (32.0 mg, 38.0 μmol) in dry

CH_2Cl_2 (0.40 mL) was added AW300 MS (350 mg), and the mixture was stirred for 12 h at rt, then cooled to 0 °C. TMSOTf (1.30 μL, 1.0 μmol) was added, and the mixture was stirred for 6 h at 0 °C. The solids were filtrated off and washed with CH_2Cl_2. The combined filtrate and washings were washed with $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 1:1 benzene–acetone as eluent to give **25** (12.1 mg, 40%). $[\alpha]_D$ −28.4° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.96–5.93 (m, 1 H, *H*-5 of sphingosine), 5.61 (dd, 1 H, *H*-4 of sphingosine), 4.68 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1 of GlcNAc), 4.41 (s, 1 H, H-1 of Man), 4.40 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1 of GalNAc), 4.18 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1 of Glc). MALDI-TOFMS: Calcd for $C_{75}H_{107}N_5O_{34}$: *m*/*z* 1622. Found: *m*/*z* 1655 $[M + Na]^+$. HRFABMS: Calcd for $C_{75}H_{108}N_5O_{34}$ $[M + H]^+$: 1622.6874, Found 1622.6849.

2-Acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*galacto-pyranosyl-(1→4)-2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*mannopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranosyl-(1→1)-(2*S,*3*R,*4*E*)-3*-O-*benzoyl-2-octadecamido-4-octadecene-1,3-diol* (**26**).—To an emulsion of **25** (7.6 mg, 4.6 μmol) in benzene (0.6 mL) and water (30 μL) was added Ph_3P (3.5 mg, 13.8 μmol), and the mixture was stirred for 24 h with the progress of the reaction being monitored by TLC. The mixture was concentrated, and the residue was stirred with stearic acid (2.5 mg, 14 μmol) and WSC (3.50 mg, 14 μmol) in dry CH_2Cl_2 (0.6 mL) for 24 h at rt. The mixture was diluted with CH_2Cl_2 (10 mL), washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 2:1 benzene–acetone as eluent to give **26** (5.8 mg, 67%). $[\alpha]_D$ −27.3° (*c* 0.13, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.91–5.90 (m, 1 H, *H*-5 of sphingosine), 5.52 (dd, 1 H, *H*-4 of sphingosine), 4.68 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1 of GlcNAc), 4.41 (s, 1 H, *H*-1 of Man), 4.40 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1 of GalNAc), 4.20 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1 of Glc). MALDI-TOFMS: Calcd for $C_{93}H_{143}N_3O_{35}$: *m*/*z* 1862. Found: *m*/*z* 1885 $[M + Na]^+$.

2-Acetamido-2-deoxy-β-D-*galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β*-D-*glucopyranosyl-(1→3)-β*-D-*mannopyranosyl-(1→4)-β*-D-*glucopyranosyl-(1→1)-(2*S,*3*R,*4*E*)-*2-octadecamido-4-octadecene-1,3-diol* (**2**).—To a solution of **26** (5.8 mg, 3.10 μmol) in MeOH (1.0 mL) and 1,4-dioxane (1.0 mL) was added NaOMe (8.0 mg) at 40 °C and the mixture was stirred for 20 h, then neutralized with Amberlite IR 120[H^+]. The mixture was filtered off and concentrated. The product was purified by column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 giving **2** (3.0 mg, 74%). $[\alpha]_D$ −12.7° (*c* 0.5, MeOH); ^1H NMR (1:1 $CDCl_3$–CD_3OD): δ 5.90–5.89 (m, 1 H, *H*-5 of sphingosine), 4.68 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1 of GlcNAc), 4.42 (s, 1 H, *H*-1 of Man), 4.41 (d, 1 H, $J_{1,2}$ 8.5 Hz, *H*-1 of GalNAc), 4.18 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1 of Glc). MALDI-TOFMS: Calcd for $C_{64}H_{117}N_3O_{23}$: *m*/*z* 1296. Found: *m*/*z* 1319 $[M + Na]^+$. HR-FABMS: Calcd for $C_{64}H_{118}N_3O_{23}$ $[M + H]^+$: 1296.8155, Found 1296.8134.

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References

1. Varki, A. *Glycobiology* **1993**, *3*, 97–130.
2. Wiegandt, H. *Glycolipids*; Elsevier: New York, 1985.
3. (a) Hori, T.; Sugita, M.; Kanbayashi, J.; Itasaka, O. *J. Biochem.* **1977**, *81*, 107–114;
   (b) Hori, T.; Takeda, H.; Sugita, M.; Itasaka, O. *J. Biochem.* **1977**, *82*, 1281–1285;
   (c) Hori, T.; Sugita, M.; Ando, S.; Kuwahara, M.; Kumauchi, K.; Sugie, E.; Itasaka, O. *J. Biol. Chem.* **1981**, *256*, 10979–10985;
   (d) Dennis, R. D.; Geyer, R.; Egge, H.; Menges, H.; Stirm, S.; Wiegandt, H. *Eur. J. Biochem.* **1985**, *146*, 51–58.
4. (a) Takeda, T.; Hada, N.; Ogihara, Y. *Chem. Pharm. Bull.* **1992**, *40*, 1930–1933;
   (b) Takeda, T.; Hada, N.; Ogihara, Y. *Chem. Pharm. Bull.* **1993**, *41*, 2058–2060;
   (c) Hada, N.; Takeda, T.; Ogihara, Y. *Carbohydr. Res.* **1994**, *258*, 93–104;
   (d) Hada, N.; Hayashi, E.; Takeda, T. *Carbohydr. Res.* **1999**, *316*, 58–70;
   (e) Hada, N.; Matsuzaki, A.; Takeda, T. *Chem. Pharm. Bull.* **1999**, *47*, 1265–1268;
   (f) Hada, N.; Kuroda, M.; Takeda, T. *Chem. Pharm. Bull.* **2000**, *48*, 1160–1165;
   (g) Hada, N.; Ohtsuka, I.; Sugita, M.; Takeda, T. *Tetrahedoron Lett.* **2000**, *41*, 9065–9068.
5. Sugita, M.; Nishida, M.; Hori, T. *J. Biochem.* **1982**, *92*, 327–334.
6. Sugita, M.; Inagaki, F.; Naito, H.; Hori, T. *J. Biochem.* **1990**, *107*, 899–903.
7. Pozsgay, V. Stereoselective Synthesis of β-Mannoside. In *Carbohydrates in Chemistry and Biology*; Ernst, B.; Hart, G. W.; Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, 2000; Vol. 1, pp 319–344.
8. (a) Lichtenthaler, F. W.; Adams, T. S. *J. Org. Chem.* **1994**, *59*, 6728–6734;
   (b) Sato, K.; Seki, H.; Yoshitomo, A.; Nanaumi, H.; Takai, Y.; Ishido, Y. *J. Carbohydr. Chem.* **1998**, *17*, 703–727;
   (c) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435–436;
   (d) Hodosi, G.; Kovac, P. *J. Am. Chem. Soc.* **1998**, *119*, 2335–2336;

(e) Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447–1465;
(f) Stork, G.; La Clair, J. J. *J. Am. Chem. Soc.* **1996**, *118*, 247–248;
(g) Lergenmuller, M.; Nukada, T.; Kuramochi, K.; Dan, A.; Ogawa, T.; Ito, Y. *Eur. J. Org. Chem.* **1999**, 1367–1376;
(h) Ohtake, H.; Ichiba, N.; Ikegami, S. *J. Org. Chem.* **2000**, *65*, 8171–8179.
9. (a) Lichtenthaler, F. W.; Adams, T. S. *J. Org. Chem.* **1994**, *59*, 6735–6738;
(b) Crich, D.; Dai, Z. *Tetrahedron* **1999**, *55*, 1569–1580;
(c) Dan, A.; Ito, Y.; Ogawa, T. *J. Org. Chem.* **1995**, *60*, 4680–4681;
(d) Ohnishi, Y.; Ando, H.; Kawai, T.; Nakahara, Y.; Ito, Y. *Carbohydr. Res.* **2000**, *328*, 263–276.
10. Garegg, P. J.; Kvarnstrom, I.; Niklasson, A.; Niklasson, G.; Svensson, S. C. T. *J. Carbohydr. Chem.* **1993**, *12*, 933–953.
11. Jansson, K.; Ahlfors, S.; Kihlberg, T.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629–5647.
12. Lemieux, R. U.; Takeda, T.; Chung, B. Y. *ACS Symp. Ser.* **1976**, *39*, 90–115.
13. Kanie, O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1996**, *37*, 4551–4554.
14. Schmidt, R. R.; Grundler, G. *Synthesis* **1985**, 885–887.
15. Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.
16. Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235.
17. Kiso, M.; Nakamura, A.; Tomita, Y.; Hasegawa, A. *Carbohydr. Res.* **1986**, *158*, 101–111.
18. (a) Ohtsubo, N.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1998**, *306*, 517–530;
(b) Gege, C.; Oscarson, S.; Schmidt, R. R. *Tetrahedron Lett.* **2001**, *42*, 377–380.
19. Seifert, J.; Ogawa, T.; Ito, Y. *Tetrahedron Lett.* **1999**, *40*, 6803–6807.
20. van Seeventer, P. B.; Corsten, M. A.; Sanders, M. P.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1997**, *299*, 171–179.