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Syntheses of Derivatives of the Trisaccharide GlcNAcβ1→2Manα1→3Man

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The glycosphingolipids isolated from spermatozoa of a fresh-water bivalve, *Hyriopsis schlegelii*, have a unique structure containing one or two mannosyl residues. We synthesized the trisaccharide derivatives which constitute the partial structure of lipid I and/or II. Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide with methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside in the presence of mercuric cyanide gave methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside in 80% yield. Condensation of the disaccharide bromide with the appropriate C-3 hydroxyl free mannose derivatives afforded the corresponding GlcNAcβ1→2Manα1→3Man derivatives (**11** and **12**).

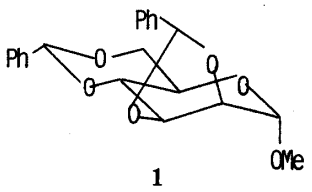
Keywords——glycosphingolipid; *Hyriopsis schlegelii*; lipid I; lipid II; condensation; trisaccharide

Hori *et al.*[1] isolated 7 mannose-containing glycolipids from spermatozoa of a fresh-water bivalve, *Hyriopsis schlegelii*, and characterized them as Manβ1→4Glc–Cer, Manα1→3Manβ1→4Glc–Cer, Manα1→3(Xylβ1→2)Manβ1→4Glc–Cer, GlcNAcβ1→2Manα1→3Manβ1→4Glc–Cer (lipid I), GlcNAcβ1→2Manα1→3(Xylβ1→2)Manβ1→4Glc–Cer (lipid II), Fuc3Meα1→2Xyl3Me β1→4(GalNAc3Meα1→3) Fucα1→4GlcNAcβ1→2Manα1→3 (Xylβ1→2) Manβ1→4Glc–Cer (lipid III), and Gal4Meβ1→3GalNAcβ1→3Fucα1→4GlcNAcβ1→2Manα1→3(Xylα1→2)2′-aminoethylphosphoryl(→6)Manβ1→4Glc–Cer. These glycolipids have a unique structure containing one or two mannosyl residues.

We chose trisaccharides corresponding to the partial structure of lipid I and/or II as targets for our synthetic studies on oligosaccharides of biological interest. In this work, fully protected trisaccharides **11** and **12** were synthesized by stepwise condensation of suitably protected monosaccharide units.

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**3**), which was prepared from methyl exo-2,3 : 4,6-di-*O*-benzylidene-α-D-mannopyranoside (**1**) according to the method of Lipták *et al.*,[2] was condensed with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**7**)[3] in dichloromethane for 3 h at 0 °C, in the presence of silver trifluoromethanesulfonate, 2,4,6-collidine, and molecular sieve (4 Å). Purification of the crude product by column chromatography afforded in 80% yield the disaccharide derivative, methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside (**8**), the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of which supported the presence of the β1→2 linkage (doublets at δ 5.44 with a spacing of 8 Hz). Acetolysis of **8** at 0 °C with the acetolysis reagent (1% H_2SO_4 in Ac_2O) described previously[4] afforded **9**. The corresponding α-bromide (**10**) was prepared from **9** by treatment with hydrogen bromide in acetic acid. Without purification, the bromide (**10**) was condensed in the presence of mercuric cyanide with compound **5**, which was prepared

from methyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**4**)[5] by hydrogenation using Pd/C in formic acid[6] or $Pd(OH)_2/C$ in cyclohexene.[7] This reaction gave the fully protected trisaccharide, methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**11**), in 49.4% yield. The ^{1}H-NMR spectrum showed a benzylidene proton signal at δ 5.60, 7 acetyl group signals at δ 1.84—2.15, and a methoxy signal at δ 3.39 ppm. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) data showed three anomeric carbon atoms at δ 95.8 (C-1″, J=153.8 Hz), 97.8 (C-1′, J=170.89 Hz) and 99.86 (C-1, J=170.89 Hz). The α configuration of the new inter-glycosidic bond was indicated by the $J_{C\text{-}1',H\text{-}1'}$ value of 170.89 Hz. The synthetic route is illustrated in Chart 1.



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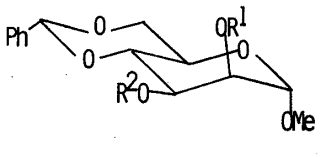
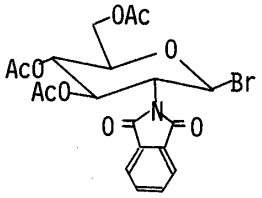


2 : R^1=R^2=H
3 : R^1=H, R^2=Bn
4 : R^1=Ac, R^2=Bn
5 : R^1=Ac, R^2=H
6 : R^1=All, R^2=H



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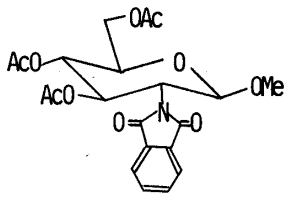

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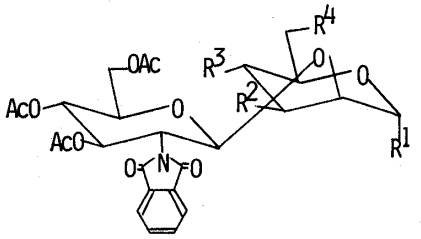
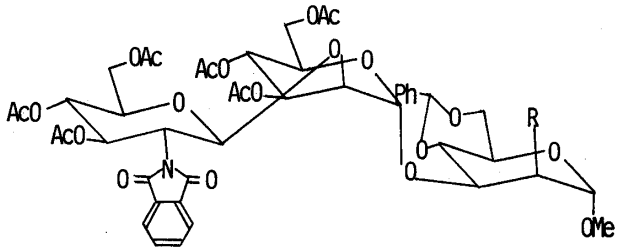


3 + **7** → **8**
5 + **10** → **11**
6 + **10** → **12**

8 : R^1=OMe, R^2=OBn, R^3=R^4=O-benzylidene
9 : R^1=R^2=R^3=R^4=OAc
10 : R^1=Br, R^2=R^3=R^4=OAc



11 : R=OAc
12 : R=OAll

Chart 1

The trisaccharide **12** was also synthesized *via* another route. Methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**6**) was obtained by the procedure of Winnik.[8] The

bromide **10** was treated with compound **6** in the presence of mercuric cyanide to give methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**12**). The ^{13}C-NMR data showed three anomeric carbon atoms at δ 95.6 (C-1″, J=158.70 Hz), 97.8 (C-1′, J=170.90 Hz) and 100.7 (C-1, J=170.90 Hz). The α configuration of the new inter-glycosidic bond was indicated by the $J_{C\text{-}1',H\text{-}1'}$ value of 170.90 Hz.[9] The ^{13}C-shifts of the trisaccharides and related compounds are listed in Table I.

TABLE I. ^{13}C Chemical Shifts in $CDCl_3$ (δ)

Carbon atom	Compounds								
	4	**5**	**6**	**3**	**13**	**8**	**9**	**11**	**12**
C-1	99.8	99.5	102.0					99.9	100.7
C-2	72.1	72.1	72.8					72.6	72.0
C-3	73.8	67.7	68.8					71.1	70.6
C-4	78.3	78.1	79.4					79.0	79.0
C-5	63.7	63.3	63.3					63.4	63.9
C-6	68.7	68.7	68.5					68.6	68.6
Ph-CH	101.6	102.1	99.6					102.5	102.6
Ph-CH_2	69.7								
–OA11			117.8, 134.2, 78.2						118.1, 134.1, 78.5
–OMe	55.0	55.1	54.9					55.2	54.9
C-1′				101.3		96.7	90.7	97.8	97.8
C-2′				69.7		73.8	73.2	72.9	72.7
C-3′				75.6		75.2	68.9	68.9	68.9
C-4′				78.7		78.1	65.0	65.5	66.2
C-5′				63.3		63.7	70.5	68.9	68.2
C-6′				68.8		68.5	61.9	62.6	63.9
Ph-CH				101.5		101.5			
Ph-CH_2				72.8		71.5			
–OMe				54.8		54.9			
C-1″					98.1	99.0	97.1	95.8	95.6
C-2″					56.7	54.1	54.4	54.1	54.1
C-3″					72.1	72.2	72.0	71.3	71.3
C-4″					69.4	69.2	69.6	69.5	69.4
C-5″					71.0	70.5	70.5	70.4	70.4
C-6″					62.2	62.2	61.9	61.2	61.2
–OMe					54.7				

Experimental

General Methods——Melting points were determined with a Yanagimoto microapparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JNM MH-100 spectrometer, and the ^{13}C-NMR spectra were obtained at 25.0 MHz in the pulsed Fourier-transform mode on JEOL FX-100 instruments. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Thin-layer chromatography (TLC) was conducted on precoated silica-gel plates (Merck GF-254), and the detection of compounds was achieved by quenching of ultraviolet (UV). fluorescence and with 10% sulfuric acid solution. Column chromatography was carried out using silica-gel (Merck Kieselgel 60).

Materials——Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**3**) was obtained by the procedure of Lipták *et al.*[2] Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**5**) was prepared from methyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**4**).[6,7] 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide was prepared according to the method of Lemieux *et al.*[3] Methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**6**) was obtained by the procedure of Winnik.[8]

Methyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside (8)——A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**7**) (1.5 g) in dichloromethane (7 ml) was added to a mixture of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-manno-

pyranoside (**3**) (1 g), silver trifluoromethanesulfonate (1.5 g), 2,4,6-collidine (370 mg) and molecular sieve (4 Å) (2 g) in the same solvent (10 ml). After being stirred for 3 h at 0 °C, the mixture was filtered and the filtrate was extracted with chloroform. The chloroform extracts were washed with water, dried and concentrated to a syrup, which was chromatographed on a column of silica-gel with 5 : 1 : 1 (v/v/v) hexane–ethyl acetate–acetone as an eluent. The disaccharide fraction was collected and evaporated to dryness to give pure **8** (1.7 g, 80%), $[\alpha]_D^{22}$ +22.7 °C (c = 0.08, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.90 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.06 (s, 3H, OAc), 3.17 (s, 3H, OMe), 4.66 (s, 3H, Ph-C$\underline{H}_2$, H-1), 5.44 (d, 1H, J = 8 Hz, H-1′), 7.28 (m, 10H, arom. H), 7.76 (m, 4H, phthalimido).

1,3,4,6-Tetra-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranose (9)——Compound **8** (1.5 g) was dissolved in acetic anhydride (5 ml) and acetolyzed with 1% sulfuric acid in acetic anhydride (15 ml) for 12 h at room temperature. After the usual processing, the mixture was chromatographed on silica-gel with the same solvent as that used for TLC. (4 : 1, v/v, benzene–acetone) to give **9**, $[\alpha]_D^{22}$ +22.0 ° (c = 0.08, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.86, 1.99, 2.00, 2.02, 2.07, 2.09 (s, each 3H, OAc), 5.47 (d, 1H, J = 10 Hz, H-1′), 5.77 (d, J = 3 Hz, H-1), 7.76 (m, 4H, phthalimido). *Anal.* Calcd for $C_{34}H_{39}NO_{19}$: C, 52.29; H, 4.98; N, 1.76. Found: C, 52.33; H, 5.13; N, 1.83.

3,4,6-Tri-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl Bromide (10)——A 25% solution of hydrogen bromide in acetic acid (6 ml) was added to a solution of **9** (0.5 g) in chloroform (6 ml) under cooling and the whole was stirred for 12 h at 0 °C. The reaction solution was poured into ice-water and extracted with chloroform. The chloroform extracts were washed with water, dried and concentrated *in vacuo* to yield a crystalline mass (0.4 g, 82.5%). TLC (4 : 1, v/v, benzene–acetone): Rf 0.52.

Methyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (11)——A solution of **10** (400 mg) in nitromethane (5 ml) was added to a mixture of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**5**) (210 mg), mercuric cyanide (260 mg) and molecular sieve 4 Å (200 mg) in the same solvent (5 ml). After being stirred for 24 h at 40 °C, the resulting solution was filtered and the filtrate was extracted with chloroform. The chloroform layer was washed with water and evaporated to a syrup, which was chromatographed on silica-gel using 4 : 1 (v/v) benzene–acetone as an eluent. The eluate containing the trisaccharide fraction was evaporated to dryness to give pure **11** (329.5 mg, 49.4%), $[\alpha]_D^{23}$ +12.9 ° (c = 0.43, $CHCl_3$). High mass 1029.3043 (Calcd for $C_{48}H_{55}NO_{24}$, 1029. 9548). ^{1}H-NMR ($CDCl_3$) δ: 1.84, 1.89, 1.96, 1.97 (s, each 3H, OAc), 2.03 (s, 6H, 2 × OAc), 2.15 (s, 3H, OAc), 3.39 (s, 3H, OMe), 4.61 (d, 1H, J = 2 Hz, H-1′), 4.88 (s, 1H, H-1), 5.48 (d, 1H, J = 11 Hz, H-1″), 5.60 (s, 1H, Ph-C$\underline{H}$), 7.12—7.94 (m, 9H, arom. H).

Methyl *O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (12)——A solution of **10** (52.5 mg) in nitromethane (4 ml) was added to a mixture of methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside[8] (200 mg), mercuric cyanide (200 mg), and molecular sieve 4 Å (200 mg) in the same solvent (4 ml). After being stirred for 24 h at 40 °C, the mixture was filtered and the filtrate was extracted with chloroform. The chloroform extracts were washed with water, dried and concentrated to a syrup, which was chromatographed on a column of silica-gel with 4 : 1 (v/v) benzene–acetone as an eluent. The trisaccharide fraction was collected and evaporated to dryness to give pure **12** (333 mg, 52.2%), $[\alpha]_D^{23}$ −4.6 ° (c = 0.02, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.89, 1.94, 1.99, 2.02 (s, each 3H, OAc), 2.08 (s, 6H, 2 × OAc), 3.34 (s, 3H, OMe), 4.70 (s, 1H, H-1′), 4.96 (d, J = 3 Hz, H-1), 5.44 (d, 1H, J = 8 Hz, H-1″), 5.60 (s, 1H, Ph-C$\underline{H}$), 7.38—8.00 (m, 9H, arom. H).

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