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TOTAL SYNTHESIS OF OCTASACCHARIDE RELATED TO GLYCOSPHINGOLIPID FROM THE SPERMATOZOA OF BIVALVES

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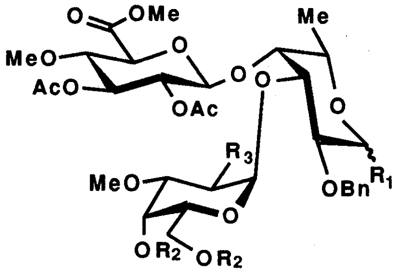
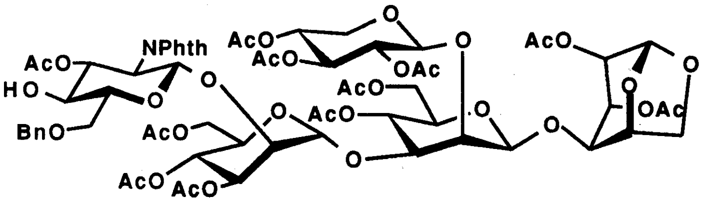
Octasaccharide corresponding to the partial structure derived from lipid IV has been prepared by coupling of the nonreducing end trisaccharide with reducing end pentasaccharide as an acceptor in the presence of dimethyl(methylthio)sulfonium triflate, followed by deprotection of a desirable protected octasaccharide.

KEYWORDS *Hyriopsis schlegelii*; fresh-water bivalve; glycosphingolipid; lipid IV; octasaccharide; synthesis

In the course of a systematic study on the spermatozoa glycosphingolipids classified as mollu (a name derived from mollusc) series[1)] of the fresh-water bivalve *Hyriopsis schlegelli*, T. Hori [2)]*et al*. have isolated a novel acidic glycolipid called lipid IV containing 4-*O*-methylglucuronic acid. It has a unique structure containing both α- and β-linked mannosyl residues, α-linked 3-*O*-methyl-*N*-acetylgalactosamine, and also novel linkages including an internal fucopyranosyl residue, as well as terminal xylosyl and 4-*O*-methyl-D-glucopyranosyluronic acid groups. In our previous papers, we reported the synthesis of the trisaccharide nonreducing end of lipid IV, 4-*O*-Me-β-D-Glc$_p$A-(1→4)-[3-*O*-Me-α-D-Gal$_p$NAc-(1→ 3)]-L-Fuc$_p$,[3)] and the pentasaccharide reducing end, β-D-Glc$_p$NAc-(1→2)-α-D-Man$_p$-(1→ 3)-[β-D-Xylp-(1→ 2)]-β-D-Manp-(1→ 4)-D-Glcp.[4)] The octasaccharide, the carbohydrate part of lipid IV, was the target for the synthetic studies described here as part of our investigations on the synthesis of oligosaccharides of biological interest. Various synthesized oligosaccharides have shown inhibition activity against the corresponding antiserum (data are not shown in this paper). In the presence of a hydroxyl, aminodeoxy group at C-2, eight main types of situations exist in glycosylations (1,2-*cis* α,β, 1,2-*trans*-α,β : OH, NHAc). In the construction of this octasaccharide, at least six types of situations exist, and this report is of one of the most complex types of oligosaccharide synthesis. Each of these approaches will be discussed in turn. Previously, we reported the synthesis of 2-(trimethylsilyl) ethyl 3-*O*-(2-azido-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl)-2-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-β-D-glucopyranosyluronate)-β-L-fucopyranoside (**1**).[3)] This trisaccharide azido derivative was converted into *N*-acetylated trisaccharide (**2**) by the procedure of R. R. Schmidt *et al.*[5)] Trisaccharide donor, designed as a thioglycoside (**4**), was derived from compound **2** in 84% overall yield as described below. Compound **2** was first converted into acetate compound **3**[6)] (CF_3COOH, Ac_2O), which was subsequently converted into thioglycoside **4** (Me_3SiSMe, TMSOTf).[7)] The glycosylation of compound **4** with pentasaccharide derivative **5**,[4,8)] in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)[9] as the glycosyl promoter in CH_2Cl_2, gave the octasaccharide **6**[10] in 29% yield; significant signals of the fucose unit in the ^1H-NMR spectrum were a three-proton doublet at δ 1.10 ($J_{5,6}$=6.7 Hz, H-6) and a one-proton doublet at δ 4.71 ($J_{1,2}$=3.7 Hz, H-1), indicating the structure assigned. For the deblocking of **6**, the *O*-acetyl groups are cleaved and the methyl ester is hydrolyzed in one step with aqueous 1N NaOH[11]; this leads to partial opening of the phthalimido group (**7**). After the phthalimido group was completely cleaved with hydrazine,[12] the resultant amino group was *N*-acetylated with acetic anhydride in methanol to give compound **8** in 78% from **6**. Removal of the benzyl group with 10% Pd-C in a hydrogen atmosphere afforded the target compound **9** ($[\alpha]_D$ -62.4 °) in 77% yield. The ^1H and ^{13}C-NMR data were in accordance with the proposed structure.[13]





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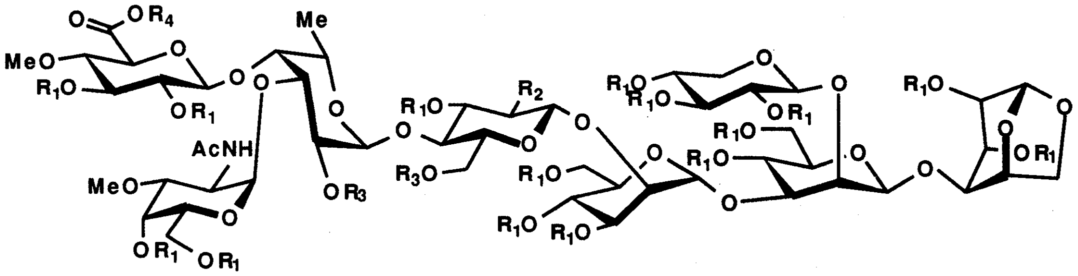
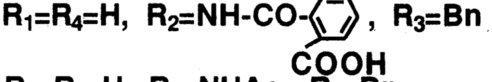
1 $R_1=O(CH_2)_2SiMe_3$, $R_2=H$, $R_3=N_3$
2 $R_1=O(CH_2)_2SiMe_3$, $R_2=H$, $R_3=NHAc$
3 $R_1=OAc$, $R_2=Ac$, $R_3=NHAc$
4 $R_1=SMe$, $R_2=Ac$, $R_3=NHAc$



6 $R_1=Ac$, $R_2=NPhth$, $R_3=Bn$, $R_4=Me$

7

$R_1=R_4=H$, $R_2=NH\text{-}CO\text{-}C_6H_4\text{-}COOH$, $R_3=Bn$

8 $R_1=R_4=H$, $R_2=NHAc$, $R_3=Bn$
9 $R_1=R_3=R_4=H$, $R_2=NHAc$

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REFERENCES AND NOTES

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2) T. Hori, M. Sugita, S. Ando, M. Kuwahara, K. Kumachi, O. Itasaka, *J. Biol. Chem.*, **256**, 10979 (1981).

3) O. Kanie, T. Takeda, Y. Ogihara, *J. Carbohydr. Chem.*, **9**, 159 (1990).

4) O. Kanie, T. Takeda, N. Hada, Y. Ogihara, *J. Carbohydr. Chem.*, **10**, 561 (1991).

5) W. Kinzy, R. R. Schmidt, *Carbohydr. Res.*, **193**, 33 (1989). To a solution of **1** (153 mg), $NiCl_2 \cdot 6H_2O$ and $B(OH)_3$ in EtOH (25 ml) was added a solution of $NaBH_4$ (100 mg) in EtOH and the mixture was stirred for 1 h at room temperature. After complete conversion of the starting material, amine compound was acetylated with acetic anhydride (20 ml) to give **2** in 69% yield.

6) To a solution of **2** (108 mg) in CH_2Cl_2 (0.6 ml), cooled to 0°C, was added TFA (1.2 ml). The mixture was stirred for 1 h at 20°C . After evaporation Ac_2O-pyridine (4 ml-6 ml) were added to the residue (yield; 95%). **3:** ^1H-NMR ($CDCl_3$):δ 6.20 (0.7H, d, J=3.3 Hz, Fuc H-1α), 5.46 (0.3 H, d, J=7.7, Fuc H-1β), 5.13 (1H, d, J=3.8, GalNAc H-1), 4.66 (1H, d, J=7.9, GlcA H-1).

7) To a stirred mixture of **3** (104 mg) and Me_3SiSMe (66 μl) in CH_2Cl_2 (1.5 ml) was added dropwise TMSOTf (23 μl) at 0° C for 2 h (yield; 89%). **4:** ^1H-NMR ($CDCl_3$) :δ 5.19 (1H, d, J=4.0 Hz, GalNAc H-1), 5.04 (0.2 H, d, J=4.2, Fuc H-1α), 4.68 (1H, d, J=7.9, GlcA H-1), 4.20 (0.8 H, d, J=9.2, Fuc H-1β).

8) **5:** $[\alpha]_D$ -76.2° (c=0.3, $CHCl_3$), ^1H-NMR ($CDCl_3$) :δ 5.39 (1H, br s, Glc H-1), 5.30 (1H, d, J=8.3, GlcNAc H-1), 5.04 (1H, d, J=5.0, Xyl H-1), 4.71 (1H, s, βMan H-1), 4.61 (1H, d, J=1.6, αMan H-1).

9) A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.*, **10**, 549 (1991). To a solution of **5** (55 mg) and **4** (51 mg) in CH_2Cl_2 (0.8 ml) was added powdered Molecular sieves 4A (MS-4A), and the mixture was stirred for 4 h at room temperature. DMTST (41mg) and MS-4A (41 mg) were added to the stirred mixture at 0° C, and the stirring was continued for 6 h at 20 °C.

10) **6:** ^{13}C-NMR ($CDCl_3$) :δ 101.1 ($J_{C,H}$ 161.2 Hz, GlcA C-1), 100.3 (J 169.2, GalNAc), 99.0 (J 170.2, Fuc), 96.4 (J 168.1, GlcNAc), 99.1 (J 171.2, Xyl), 99.3 (J 175.3, αMan), 99.2 (J 151.0, βMan), 99.2 (J 187.3, Glc).

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13) **9:** ^1H-NMR (D_2O) :δ 4.47 (1H, d, J =8.0 Hz, GlcA H-1), 5.06 (1H, d, J =4.2, GalNAc H-1), 4.95 (1H, d, J= 3.7, Fuc H-1), 4.51 (1H, d, J =7.9, GlcNAc H-1), 4.61 (1H, d, J= 7.3, Xyl H-1), 5.15 (1H, br s, αMan H-1), 4.93 (1H, br s, βMan H-1), 5.43 (1H, br s, Glc H-1); ^{13}C-NMR (D_2O) :δ 106.5 (GlcA C-1), 102.3 (GalNAc), 102.4 (Fuc), 102.5 (GlcNAc), 107.5 (Xyl), 102.0 (αMan), 101.4 (βMan), 104.4 (Glc).

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