Synthetic Studies on Oligosaccharide of a Glycolipid from the Spermatozoa of Bivalves. VII.[1] The Synthesis of Di-, Tri-, Tetrasaccharides Related to Glycosphingolipid

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The three kinds of oligosaccharides that constitute the partial structure of lipid IV (1) were synthesized as follows. The disaccharide *O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose (13), the trisaccharide *O*-α-D-mannopyranosyl-(1→3)-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose (21), and the tetrasaccharide *O*-α-D-mannopyranosyl-(1→3)-*O*-[β-D-xylopyranosyl-(1→2)]-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose (25) were each synthesized by stepwise condensation of suitably protected monosaccharide units. A 1,6-anhydro-D-glucopyranose derivative was used as the glycosyl acceptor, and bromide derivatives of D-mannose and D-xylose as donors.

Keywords *Hyriopsis schlegelii*; fresh-water bivalve; glycosphingolipid; lipid IV; oligosaccharide; synthesis; condensation

In our previous paper, we reported the synthesis of the nonreducing end trisaccharide corresponding to the partial structure derived from lipid IV, 4-*O*-Me-β-D-Glc$_p$A-(1→4)-[3-*O*-Me-α-D-Gal$_p$NAc-(1→3)]-L-Fuc$_p$,[2] and the reducing end pentasaccharide, β-D-Glc$_p$NAc-(1→2)-α-D-Man$_p$-(1→3)-[β-D-Xyl$_p$-(1→2)]-β-D-Man$_p$-(1→4)-D-Glc$_p$.[1] The disaccharide (**13**), trisaccharide (**21**) and tetrasaccharide (**25**) reducing ends of lipid IV were the targets for the synthetic studies described here, as part of our investigations on the synthesis of oligosaccharides of biological interest. A synthetic plan for the target compounds **13**, **21** and **25** was designed as shown in Fig. 1.

The disaccharide derivative 2,3-di-*O*-acetyl-1,6-anhydro-4-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (**10**) was obtained by condensation of 2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**9**)[3] with 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-mannopyranosyl bromide (**8**), which was prepared from 2,2,2-trichloroethyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**3**)[1] in the presence of silver zeolite and molecular sieves. The disaccharide derivative **10** was acetolyzed (**11**), debenzylated (**12**), and then deacetylated to give the free disaccharide **13** ($\beta/\alpha = 1.5$).

An attempted debenzylation of **10** by a 10% Pd–C procedure gave quantitatively the disaccharide acceptor (**15**) required for synthesis of the trisaccharide **16**. The glycosylation of **15** with 3,4,6-tri-*O*-acetyl-2-chloroacetyl-α-D-mannopyranosyl bromide **14** in the presence of cupric bromide–tetrabutyl ammonium bromide–silver triflate according to Ogawa[4] afforded a 57.4% yield of a mixture of trisaccharide **16**, **17** and tetrasaccharide **18** in the ratio of 3.4 : 1.0 : 2.1. The positions of the new linkages were assigned on the basis of carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data. Comparing the ^{13}C-NMR data of **16** with those of **15** showed that the C-3 signal of the inner mannose residue was shifted downfield by 9.2 ppm. In the case of **17** the C-2 signal of the mannose residue was shifted downfield by 6.5 ppm. The C-2 and C-3 signals of the mannose residue of the tetrasaccharide **18** were shifted downfield by 6.4 and 5.0 ppm, respectively, suggesting that the new mannose residues were linked to C-3, C-2, and both of C-3, C-2, according to the glycosylation shift rule.[5] The configuration at C-1 of the non-reducing end mannose residue of compound **16** was assigned as α-D on the basis of the anomeric carbon signal at δ 98.7 with a $^1J_{C,H}$ value of 173 Hz. Deprotection of compound **16** gave quantitatively the free trisaccharide,

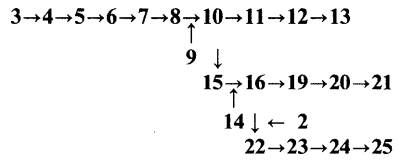
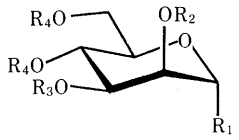


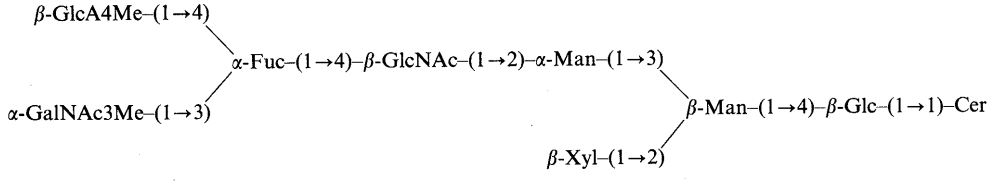
Fig. 1



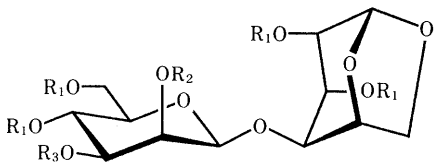
	R1	R2	R3	R4
3	OCH_2CCl_3	H	H	-PhCH-
4	OCH_2CCl_3	Bn	Bn	-PhCH-
5	OCH_2CCl_3	Bn	Bn	H
6	OH	Bn	Bn	H
7	OAc	Bn	Bn	Ac
8	Br	Bn	Bn	Ac
14	Br	CA	Ac	Ac

Bn: $-CH_2Ph$, CA: $-COCH_2Cl$

Chart 1



1 (lipid IV)



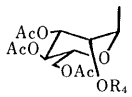
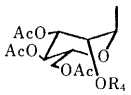
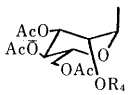
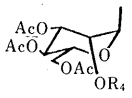
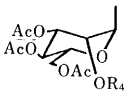
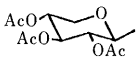
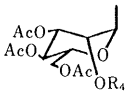
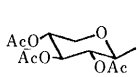
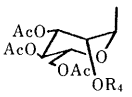
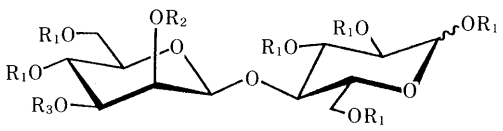
	R1	R2	R3	R4
10	Ac	Bn	Bn	
15	Ac	H	H	
16	Ac	H		CA
17	Ac		H	CA
18	Ac			CA
19	Ac	H		H
22	Ac			CA
23	Ac			H

Chart 2



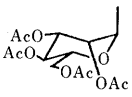
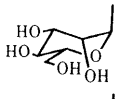
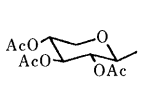
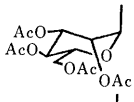
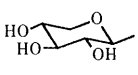
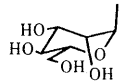
	R1	R2	R3
11	Ac	Bn	Bn
12	Ac	H	H
13	H	H	H
20	Ac	Ac	
21	H	H	
24	Ac		
25	H		

Chart 3

α-D-Man-(1→3)-β-D-Man-(1→4)-D-Glc (**21**).

To achieve a synthesis of the tetrasaccharide **22**, the trisaccharide **16** was condensed with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (**2**) in the presence of mercuric cyanide and molecular sieves. The ^{13}C-NMR spectrum of **22** revealed a signal at δ 99.5 with $^{1}J_{C,H}$ value of 174 Hz that was assignable to an anomeric carbon atom having the β-D-configuration; this could be rationalized as indicating that the β-xylopyranosyl residue had assumed the $^{1}C_4$ conformation. Compound **22** was transformed into the corresponding deblocked compound **25**. The β-D-configuration for the newly introduced anomeric proton of compound **25** was assigned according to ^{1}H-NMR data, which showed a doublet at δ 4.54 with a $J_{1,2}$ value of 7.3 Hz. Ogawa *et al.* have already reported the synthesis of the glycotriosyl- and glycotetraosyl ceramide[4] which were isolated from spermatozoa of *Hyriopsis schlegelii*. We report here syntheses of the di-, tri-, and tetrasaccharide in the course of the planned synthesis of the penta- and/or octasaccharide.

Experimental

Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-4 digital polarimeter. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR spectra were recorded with JEOL EX-270 and JEOL GSX-400 MHz spectrometers; tetramethylsilane was the internal standard for solutions in $CDCl_3$ and CD_3OD, and sodium 4,4-dimethyl-4-silapentane-1-sulfonate for solution in D_2O. Thin-layer chromatography (TLC) was conducted on precoated silica gel plates (Merck GF-254), and the compounds were detected by ultraviolet (UV) fluorescence and by heating after spraying with 10% H_2SO_4 solution. Column chromatography was carried on silica gel (Merck Kieselgel 60).

2,3-Di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**9**),[3] 2,2,2-trichloroethyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**3**),[1] and 3,4,6-tri-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranosyl bromide (**14**)[3] were obtained by the procedures described in our previous papers.

2,2,2-Trichloroethyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (4) Sodium hydride (600 mg) was added portionwise to a stirred mixture of compound **3** (2.0 g, 5.0 mmol), benzyl bromide (7.4 ml), and *n*-Bu_4NBr (300 mg) in *N*,*N*-dimethylformamide (DMF) (60 ml) at −10 °C. The mixture was stirred for 2 h at −10 °C, and methanol was added dropwise to destroy the excess NaH. The mixture was diluted with $CHCl_3$, washed with water, dried (Na_2SO_4), and evaporated *in vacuo*. The residue was chromatographed on silica gel using hexane–ethyl acetate (10 : 1) to give 4 (2.8 g, 96.5%), $[\alpha]_D^{24}$ +64.3° (*c* = 2.48, chloroform); TLC (hexane–ethyl acetate, 5 : 1) *Rf* 0.44. ^{1}H-NMR ($CDCl_3$) δ: 7.60—7.24 (15H, m, Ph), 5.64 (1H, s, benzylidene methine), 5.00 (1H, br s, H-1). *Anal.* Calcd for $C_{29}H_{29}Cl_3O_6$: C, 60.07; H, 5.04. Found: C, 60.14; H, 5.02.

2,2,2-Trichloroethyl 2,3-Di-*O*-benzyl-α-D-mannopyranoside (5) A solution of compound **4** (2.8 g, 4.03 mmol) in 80% aqueous AcOH was stirred for 3 h at 40 °C, and evaporated *in vacuo*. The residual syrup was chromatograhed on silica gel with chloroform–methanol (80 : 1). The fractions having *Rf* 0.45 (chloroform–methanol, 15 : 1) were pooled and concentrated (2.32 g, 97.7%), $[\alpha]_D^{24}$ +42.0° (*c* = 0.69, chloroform). ^{1}H-NMR ($CDCl_3$) δ: 7.42—7.00 (10H, m, Ph), 5.06 (1H, br s, H-1), 2.85 (2H, br s, OH). *Anal.* Calcd for $C_{22}H_{25}Cl_3O_6$: C, 53.73; H, 5.12. Found: C, 53.75; H, 5.09.

2,3-Di-*O*-benzyl-α-D-mannopyranose (6) A solution of compound **5** (2.1 g, 4.27 mmol) in a mixture of acetic acid (7 ml) and acetic anhydride (0.35 ml) was added with stirring to a Zn–Cu reagent which was prepared by addition of zinc dust (12 g) to acetate buffer (AcONa (22.8 g)/AcOH (22.8 ml)–H_2O (32.5 ml)) containing $CuSO_4$ (1.2 g) solution (4.8 ml). The solution was stirred at room temperature for 10 h. The suspension was filtered and the filtrate was extracted with chloroform. The extract was washed with water and concentrated to give a syrup, which was chromatographed on silica gel using chloroform–methanol (20 : 1) to give **6** (1.2 g, 79.2%), $[\alpha]_D^{24}$ −20.1° (*c* = 2.79, methanol–water, 4 : 1); TLC (chloroform–methanol, 10 : 1) *Rf* 0.28. ^{1}H-NMR (CD_3OD) δ: 7.40—7.23 (10H, m, Ph), 5.18 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1). *Anal.* Calcd for $C_{22}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.54; H, 6.67.

1,4,6-Tri-*O*-acetyl-2,3-di-*O*-benzyl-α-D-mannopyranose (7) Acetic anhydride (20 ml) was added portionwise to a solution of compound **6** (1 g, 2.77 mmol) in pyridine (30 ml) at 0 °C. The mixture was stirred for 2 h at room temperature, then poured into ice-water, and extracted with

chloroform, the organic layer was washed with water, aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated to give **7** (1.32 g, quant.), $[\alpha]_D^{24}$ +9.2° (c=1.47, chloroform). ^{1}H-NMR ($CDCl_3$) δ: 7.44—7.08 (10H, m, Ph), 6.17 (1H, d, $J_{1,2}$=2.0 Hz, H-1), 5.46 (1H, t, $J_{3,4}$=$J_{4,5}$=10.0 Hz, H-4), 2.07, 2.05, 2.03 (9H, each s, 3×OAc).

4,6-Di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-mannopyranosyl Bromide (8) Compound **7** (1.1 g, 2.26 mmol) was treated with saturated hydrogen bromide in dichloromethane (30 ml) for 3 h at 0 °C. The solution was extracted with chloroform, washed with water, dried, and concentrated to give **8** (1.0 g, 87.2%), TLC (hexane–ethyl acetate, 2:1) *Rf* 0.54.

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (10) A mixture of compound **9** (0.47 g, 1.91 mmol), compound **8** (1.23 g, 2.42 mmol), and molecular sieves 4A (1.8 g), in dichloromethane (26.5 ml) was stirred for 1 h at room temperature, then silver zeolite (2.8 g) was added at 0 °C. The mixture was stirred for 40 h at room temperature, and filtered through Celite. The filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel using benzene–acetone (10:1) to give **10** (0.92 g, 71.7%), $[\alpha]_D^{21}$ −101.9° (c=1.18, chloroform), TLC (benzene–acetone, 5:2) *Rf* 0.56. ^{1}H-NMR ($CDCl_3$) δ: 7.32—7.20 (10H, m, Ph), 5.47 (1H, br s, H-1), 5.34 (1H, t, $J_{3',4'}$=$J_{4',5'}$=9.7 Hz, H-4'), 5.23 (1H, br t, H-2), 4.94 (2H, each d, J_{gem}=12.3 Hz, benzyl methylene), 4.69 (1H, s, H-1'), 4.65 (1H, br d, $J_{5,6b}$=5.1 Hz, H-5), 4.58 (1H, br s, H-3), 4.45 (2H, each d, J_{gem}=12.3 Hz, benzylmethylene), 4.16 (1H, dd, $J_{5',6'b}$=3.1 Hz, H-6'b), 4.12 (1H, dd, $J_{5',6'a}$=5.1, $J_{6'a,6'b}$=12.1 Hz, H-6'a), 4.01 (1H, br d, $J_{6a,6b}$=7.0 Hz, H-6a), 3.94 (1H, d, $J_{2',3'}$=2.9 Hz, H-2'), 3.80 (1H, dd, H-6b), 3.65 (1H, br s, H-4), 3.55 (1H, ddd, H-5'), 3.45 (1H, dd, H-3'), 2.08, 2.06, 2.03, 2.02 (12H, each s, 4×OAc). *Anal.* Calcd for $C_{34}H_{40}O_{14}$: C, 60.71; H, 5.99. Found: C, 60.30; H, 5.95.

***O*-(4,6-Di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-1,2,3,-6-tetra-*O*-acetyl-D-glucopyranose (11)** Compound **10** (150 mg, 0.22 mmol) was treated with acetolysis reagent (Ac_2O–H_2SO_4, 45 ml–45 μl) for 15 min at 0 °C. The mixture was poured into ice-water, and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), concentrated *in vacuo*, and then chromatographed on silica gel using benzene–acetone (20:1) to give compound **11** (164 mg, 94.9%), TLC (hexane–ethyl acetate, 1:1) *Rf* 0.38. ^{1}H-NMR ($CDCl_3$) δ: 7.33—7.24 (10H, m, Ph), 6.22 (0.75H, d, $J_{1,2}$=3.7 Hz, H-1α), 5.70 (0.25H, d, $J_{1,2}$=8.3 Hz, H-1β), 5.50 (1H, t, $J_{2,3}$=$J_{3,4}$=10.1 Hz, H-3), 5.29 (1H, t, $J_{3',4'}$=$J_{4',5'}$= 9.5 Hz, H-4'), 5.02 (1H, dd, H-2), 4.35 (1H, s, H-1'), 2.21, 2.09, 2.07, 2.02, 2.01, 2.00 (18H, each s, 6×OAc). *Anal.* Calcd for $C_{38}H_{46}O_{17}$: C, 58.91; H, 5.98. Found: C, 58.57; H, 5.95.

***O*-(4,6-Di-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-D-glucopyranose (12)** A mixture of compound **11** (130 mg, 0.17 mmol) and 10% Pd–C (60 mg) in methanol (5 ml) was stirred at room temperature in a hydrogen atmosphere until the reaction was complete. The product was isolated in the usual manner to give **12** (98 mg, 97.9%), TLC (chloroform–methanol, 10:1) *Rf* 0.70. ^{1}H-NMR ($CDCl_3$) δ: 6.30 (0.9H, d, $J_{1,2}$=3.7 Hz, H-1α), 5.69 (0.1H, d, $J_{1,2}$=8.4 Hz, H-1β), 4.49 (1H, s, H-1'), 2.17, 2.13, 2.11, 2.10, 2.08, 2.03 (18H, each s, 6×OAc). *Anal.* Calcd for $C_{24}H_{34}O_{17}$: C, 48.49; H, 5.76. Found: C, 48.38; H, 5.94.

***O*-β-D-Mannopyranosyl-(1→4)-D-glucopyranose (13)** Compound **12** (65 mg, 0.11 mmol) was treated with sodium methoxide (5 mg) in methanol (2.5 ml) at room temperature for 2 h, and the base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. The solution was taken to dryness *in vacuo* to give **13** (37 mg, 95.7%), mp 128—130 °C, $[\alpha]_D^{23}$ +6.5° (c=0.75, H_2O), TLC ($CHCl_3$–MeOH–H_2O, 5:5:1) *Rf* 0.31. ^{1}H-NMR (D_2O) δ: 5.23 (0.4H, br d, $J_{1,2}$=3.9 Hz, H-1α), 4.74 (1H, d, $J_{1',2'}$=1.1 Hz, H-1'), 4.66 (0.6H, d, $J_{1,2}$=8.1 Hz, H-1β). *Anal.* Calcd for $C_{12}H_{22}O_{11}\cdot H_2O$: C, 40.00; H, 6.15. Found: C, 40.20; H, 6.45.

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-β-D-glucopyranose (15) A mixture of compound **10** (198 mg, 0.29 mmol) and 10% Pd–C (80 mg) in methanol (6 ml) was stirred at room temperature in a hydrogen atmosphere until the reaction was complete, and then filtered through Celite. The filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel using chloroform–methanol (20:1) to give **15** (140 mg, 98.0%), mp 159—160 °C, $[\alpha]_D^{22}$ −83.3° (c=2.24, chloroform), TLC (chloroform–methanol, 10:1) *Rf* 0.48. ^{1}H-NMR ($CDCl_3$) δ: 5.46 (1H, br s, H-1), 5.25 (1H, br t, J=1.7 Hz, H-2), 5.09 (1H, t, $J_{2',3'}$=$J_{3',4'}$=9.7 Hz, H-4'), 4.83 (1H, d, $J_{1',2'}$=1.1 Hz, H-1'), 4.07 (1H, dd, $J_{2',3'}$=3.3 Hz, H-2'), 3.71 (1H, br s, H-3'), 2.14, 2.13, 2.11, 2.07 (12H, each s, 4×OAc). ^{13}C-NMR ($CDCl_3$) δ: 99.3 (Man-1), 70.9 (Man-2), 72.3 (Man-3), 68.9 (Man-4), 72.3 (Man-5), 62.5 (Man-6), 99.2 (Glc-1), 75.8 (Glc-4). *Anal.* Calcd for $C_{20}H_{28}O_{14}$: C, 48.78; H, 5.73. Found; C, 48.56; H, 5.83.

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-{4,6-di-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl}-β-D-glucopyranose (16), 2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-{4,6-di-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl}-β-D-glucopyranose (17), 2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-{4,6-di-*O*-acetyl-2,3-di-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl}-β-D-glucopyranose (18) A solution of compound **15** (30 mg, 0.061 mmol) and compound **14** (46 mg, 0.103 mmol) in dichloromethane (1.5 ml) was added to a mixture of silver triflate (AgOTf, 47 mg, 0.185 mmol), $CuBr_2$ (40 mg, 0.18 mmol), n-Bu_4NBr (20 mg, 0.025 mmol), and molecular sieves 4A (400 mg). The mixture was stirred for 6 h at 10 °C under argon gas, then diluted with chloroform, and filtered. The filtrate was washed with aqueous $NaHCO_3$ and water, dried with Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel using benzene–acetone (4:1) to give **16** (15 mg, 29.9%), **17** (4.4 mg, 8.8%), and **18** (14 mg, 18.7%).

Compound **16**: $[\alpha]_D^{22}$ −36.3° (c=1.40, chloroform), TLC (benzene–acetone, 2:1) *Rf* 0.45. ^{1}H-NMR ($CDCl_3$) δ: 5.45 (1H, br s, H-1), 5.35 (1H, dd, $J_{2'',3''}$=2.9, $J_{3'',4''}$=9.3 Hz, H-3''), 5.34 (1H, t, $J_{4'',5''}$=9.3 Hz, H-4''), 5.27 (1H, t, $J_{3',4'}$=$J_{4',5'}$=10.1 Hz, H-4'), 5.24 (1H, br t, $J_{2,3}$=1.5 Hz, H-2), 5.19 (1H, dd, $J_{1'',2''}$=1.8 Hz, H-2''), 4.97 (1H, d, H-1''), 4.82 (1H, s, H-1'), 4.25—4.11 (7H, m, H-2', H-6'a, H-6'b, H-6''a, H-6''b, $ClCH_2CO$–), 3.70 (1H, br s, H-4), 3.67 (1H, dd, $J_{2',3'}$=3.1 Hz, H-3'), 2.13, 2.12, 2.00 (9H, each s, 3×OAc), 2.11, 2.06 (12H, each s, 4×OAc). ^{13}C-NMR ($CDCl_3$) δ: 98.9 (βMan-1, $J_{C,H}$ 158 Hz), 70.4 (βMan-2), 81.5 (βMan-3), 65.8 (βMan-4), 72.4 (βMan-5), 62.4 (βMan-6), 99.2 (Glc-1, $J_{C,H}$ 178 Hz), 76.0 (Glc-4), 98.7 (αMan-1, $J_{C,H}$ 173 Hz). *Anal.* Calcd for $C_{34}H_{45}ClO_{23}$: C, 47.64; H, 5.29. Found: C, 47.96; H, 5.35.

Compound **17**: $[\alpha]_D^{22}$ −36.0° (c=0.89, chloroform), TLC (benzene–acetone, 2:1) *Rf* 0.33. ^{1}H-NMR ($CDCl_3$) δ: 5.52 (1H, br s, H-1), 5.25 (1H, d, $J_{1'',2''}$=1.5 Hz, H-1''), 4.75 (1H, s, H-1'), 4.19 (2H, each d, J_{gem}=15.0 Hz, $ClCH_2CO$–), 2.16, 2.13, 2.112, 2.110, 2.09, 2.02, 2.00 (21H, each s, 7×OAc). ^{13}C-NMR ($CDCl_3$) δ: 100.9 (βMan-1), 77.4 (βMan-2), 73.3 (βMan-3), 70.1 (βMan-4), 72.4 (βMan-5), 62.1 (βMan-6), 99.1 (Glc-1), 77.8 (Glc-4), 98.3 (αMan-1).

Compound **18**: $[\alpha]_D^{20}$ −19.9° (c=0.98, chloroform), TLC (benzene–acetone, 2:1) *Rf* 0.51. ^{1}H-NMR ($CDCl_3$) δ: 5.43 (1H, br s, H-1 of reducing end Glc unit), 5.24 (1H, d, $J_{1,2}$=1.5 Hz, H-1 of 2-linked Man unit), 5.07 (1H, d, $J_{1,2}$=2.0 Hz, H-1 of 3-linked Man unit), 4.79 (1H, s, H-1 of inner Man unit), 4.24 and 4.14 (4H, each s, $ClCH_2CO$), 2.17, 2.12, 2.11, 2.100, 2.096, 2.09, 2.07, 2.02, 2.00, 1.99 (30H, each s, 10×OAc). ^{13}C-NMR ($CDCl_3$) δ: 100.7 (βMan-1), 77.31 (βMan-2), 77.27 (βMan-3), 67.9 (βMan-4), 72.7 (βMan-5), 62.2 (βMan-6), 99.2 (Glc-1), 77.9 (Glc-4), 98.3 (αMan-1), 97.8 (αMan-1).

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-{4,6-di-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl}-β-D-glucopyranose (19) Thiourea (20 mg) was added to a solution of compound **16** (23.0 mg, 0.027 mmol) in pyridine–ethanol (6:1, 1 ml). The mixture was stirred for 1.5 h at 80 °C, chloroform and water were added, and the organic layer was separated, washed with saturated sodium hydrogen carbonate solution and water, and then dried. Evaporation of the solvent gave a syrup, which was chromatographed on silica gel. Elution with chloroform–ethanol (30:1) provided compound **19** (15.2 mg, 72.0%); $[\alpha]_D^{20}$ −5.6° (c=0.31, chloroform), TLC (benzene–acetone, 3:1) *Rf* 0.26. ^{1}H-NMR ($CDCl_3$) δ: 5.45 (1H, br s, H-1), 5.31 (1H, t, $J_{3'',4''}$=$J_{4'',5''}$=9.7 Hz, H-4''), 5.30 (1H, t, $J_{3',4'}$=$J_{4',5'}$=9.7 Hz, H-4'), 5.25 (1H, br t, H-2), 5.24 (1H, dd, $J_{2'',3''}$=3.1 Hz, H-3''), 4.97 (1H, d, $J_{1'',2''}$=2.0 Hz, H-1''), 4.83 (1H, s, H-1'), 2.13, 2.111, 2.109, 2.10, 2.07, 2.05, 2.04 (21H, each s, 7×OAc).

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-D-glucopyranose (20)** Compound **19** (17.1 mg, 0.022 mmol) was treated with acetolysis reagent (Ac_2O–CF_3COOH, 15:1, 2 ml) for 24 h at 20 °C. The mixture was poured into ice-water and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), concentrated *in vacuo* and then chromatographed on silica gel using benzene–acetone (20:1) to give compound **20** (16.6 mg, 78.0%), mp 93—95 °C, TLC (benzene–acetone, 3:1) *Rf* 0.47. ^{1}H-NMR ($CDCl_3$) δ: 6.27 (0.75H, d, $J_{1,2}$=3.7 Hz, H-1α), 5.67 (0.25H, d, $J_{1,2}$=8.3 Hz, H-1β), 4.99 (1H, br s, H-1''), 4.58 (1H, s, H-1'), 2.23, 2.19, 2.14, 2.12, 2.100, 2.09, 2.05, 2.01, 1.99 (27H, each s, 9×OAc), 2.096 (6H, s, 2×OAc). *Anal.* Calcd for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.45; H, 5.44.

***O*-α-D-Mannopyranosyl-(1→3)-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose (21)** Compound **20** (7.0 mg, 0.0072 mmol) was treated with sodium methoxide (2 mg) in methanol (1 ml) at room temperature for 2 h. After the usual work-up, compound **21** was obtained (3.6 mg, 99.1%),

mp 157—159 °C, $[\alpha]_D^{23}$ −11.4° (c=0.16, water), TLC (ethyl acetate–2-propanol–water, 3:5:2) *Rf* 0.33. ^{1}H-NMR (D_2O) δ: 5.23 (0.32H, d, J=3.7 Hz, H-1α), 5.14 (1H, br s, H-1″), 4.76 (1H, s, H-1′), 4.66 (0.68H, d, J=7.9 Hz, H-1β). *Anal.* Calcd for $C_{18}H_{32}O_{16}\cdot 2.5H_2O$: C, 39.35; H, 5.87. Found: C, 39.15; H, 5.77.

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-{4,6-di-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranosyl)-2-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-mannopyranosyl}-β-D-glucopyranose (22) Molecular sieves 4A (200 mg) and mercuric cyanide (150 mg) were added to a solution of compound **16** (70 mg, 0.082 mmol) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide **2** (138 mg, 0.407 mmol) in dichloromethane (1.5 ml), and the mixture was stirred for 10 h at 0 °C. The solid was removed by filtration. The filtrate was diluted with chloroform, and filtered throug Celite. The filtrate was washed with water, dried, and concentrated to give a syrup, which was chromatographed on silica gel using benzene–acetone (4:1) as the eluent to give compound **22** (67 mg, 73.5%), $[\alpha]_D^{20}$ −48.8° (c=1.26, chloroform), TLC (benzene–ethanol, 8:1) *Rf* 0.47. ^{1}H-NMR ($CDCl_3$) δ: 5.41 (1H, br s, H-1 of Glc-unit), 5.14 (1H, d, $J_{1,2}$=5.0 Hz, H-1 of Xyl unit), 5.01 (1H, d, $J_{1,2}$=1.7 Hz, H-1 of αMan unit), 4.79 (1H, s, H-1 of βMan unit), 4.20, 4.14 (2H, each d, J_{gem}=15.0 Hz, $ClCH_2CO$), 2.17, 2.13, 2.12, 2.102, 2.099, 2.09, 2.060, 2.056, 2.05, 2.01 (30H, each s, 10×OAc). ^{13}C-NMR ($CDCl_3$) δ: 99.3 (Glc-1, $J_{C,H}$ 180 Hz), 99.4 (βMan-1, $J_{C,H}$ 157 Hz), 101.5 (αMan-1, $J_{C,H}$ 171 Hz), 99.5 (Xyl-1, $J_{C,H}$ 174 Hz). *Anal.* Calcd for $C_{45}H_{59}ClO_{30}$: C, 48.45; H, 5.33. Found: C, 48.22; H, 5.01.

2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-{4,6-di-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-mannopyranosyl}-β-D-glucopyranose (23) Thiourea (60 mg) was added to a solution of compound **22** (62.0 mg, 0.056 mmol) in pyridine–ethanol (6:1, 3 ml). The same reaction conditions were used as those described for the preparation of compound **19**. The product was chromatographed on silica gel using chloroform–ethanol (30:1) to provide compound **23** (38.0 mg, 65.8%), $[\alpha]_D^{20}$ −48.7° (c=0.75, chloroform), TLC (benzene–ethanol, 8:1) *Rf* 0.25.

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)]-*O*-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-D-glucopyranose (24)** Compound **23** (12.0 mg, 0.012 mmol) was treated with acetolysis reagent (Ac_2O–CF_3COOH, 15:1, 3.2 ml) for 24 h at 0 °C. The same work-up was used as described for the preparation of compound **20**. The residue was chromatographed on silica gel using benzene–acetone (5:1) to give compound **24** (11.0 mg, 77.5%), TLC (benzene–acetone, 2:1) *Rf* 0.42. ^{1}H-NMR ($CDCl_3$) δ: 6.27 (0.4H, d, $J_{1,2}$=3.7 Hz, H-1 of Glcα), 5.72 (0.6H, d, $J_{1,2}$=8.2 Hz, H-1 of Glcβ), 5.02 (1H, br s, H-1 of αMan), 5.01 (1H, d, $J_{1,2}$=4.8 Hz, H-1 of Xyl), 4.46 (1H, s, H-1 of βMan), 2.15, 2.14, 2.13, 2.124, 2.120, 2.114, 2.105, 2.01, 2.00 (27H, each s, 9×OAc), 2.12 (6H, s, 2×OAc), 2.09 (6H, s, 2×OAc). *Anal.* Calcd for $C_{49}H_{66}O_{33}$: C, 49.75; H, 5.62. Found: C, 49.48; H, 5.67.

***O*-α-D-Mannopyranosyl-(1→3)-*O*-[β-D-xylopyranosyl-(1→2)]-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose (25)** Compound **24** (6.8 mg, 0.0057 mmol) was treated with sodium methoxide (2 mg) in methanol (1 ml) at room temperature for 2 h. After the usual work-up, compound **25** was obtained (3.4 mg, 92.9%), mp 135—138 °C, $[\alpha]_D^{20}$ −46.2° (c=0.08, water), TLC (ethyl acetate–2-propanol–water, 3:2:2) *Rf* 0.34. ^{1}H-NMR (D_2O) δ: 5.23 (0.4H, d, J=3.7 Hz, H-1α of Glc unit), 5.15 (1H, br s, H-1 of αMan unit), 4.83 (1H, s, H-1 of βMan unit), 4.66 (0.6H, d, J=7.9 Hz, H-1β of Glc unit), 4.54 (1H, d, J=7.3 Hz, H-1 of Xyl unit). *Anal.* Calcd for $C_{23}H_{40}O_{20}\cdot 6H_2O$: C, 37.10; H, 5.41. Found: C, 36.81; H, 5.63.

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