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Synthetic studies on an oligosaccharide of a glycolipid from the spermatozoa of bivalves IX *. Syntheses of lipids I, II, and IV

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Abstract

Glycosphingolipids isolated from the spermatozoa of the freshwater bivalve, *Hyriopsis schlegelii*, have a unique structure containing one or two mannosyl residues and novel linkages, including an internal fucopyranosyl residue, as well as terminal xylosyl and 4-*O*-methyl-D-glucopyranosyluronic acid groups. The octasaccharide of lipid IV was synthesized as follows. Condensation of methyl (2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl)-(1 → 3)-[methyl(2,3-di-*O*-acetyl-4-*O*-methyl-β-D-glucopyranosyluronate)-(1 → 4)]-2-*O*-benzyl-1-thio-α,β-L-fucopyranoside (**18**) with (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**14**), in the presence of dimethyl (methylthio) sulfonium triflate (DMTST), gave the corresponding octasaccharide (**19**). Removal of the protecting groups gave 2-acetamido-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl-(1 → 3)-[4-*O*-methyl-β-D-glucopyranosyl uronic acid-(1 → 4)]-α-L-fucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-mannopyranosyl-(1 → 4)-1,6-anhydro-β-D-glucopyranose (**22**). The other two oligosaccharides that constitute the partial structure of lipid IV, called lipid I and II, were also synthesized.

1. Introduction

In the course of a systematic study on the spermatozoa glycosphingolipids of the freshwater bivalve, *Hyriopsis schlegerii*, T. Hori et al. [2–4] have isolated and

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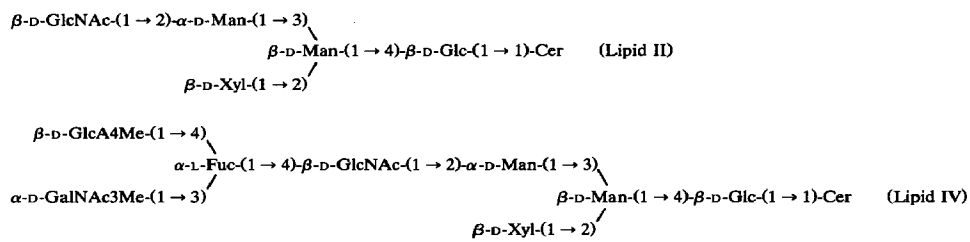
characterized seven glycolipids. These neutral glycolipids differ from mammalian glycolipids in having mannosyl residues. Furthermore, they also isolated a novel acidic glycolipid (lipid IV) containing 4-*O*-methyl glucuronic acid, which is concerned with the function in invertebrate animal species that does not have gangliosides [5]. It is speculated that this glycolipid plays a role in spermatocyte membranes as do gangliosides in mammalian membranes [6,7]. In our previous papers [8–10], we have reported the synthesis of the nonreducing end trisaccharide corresponding to the partial structure derived from lipid IV, namely 2-(trimethylsilyl) ethyl (2-azido-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl)-(1 → 3)-[methyl (2,3-di-*O*-acetyl-4-*O*-methyl-β-D-glucopyranosyluronate)-(1 → 4)]-2-*O*-benzyl-β-L-fucopyranoside [8], the reducing-end pentasaccharide derivative, (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose [9], and the precursor of lipid IV, β-D-Man-(1 → 4)-D-Glc. α-D-Man-(1 → 3)-β-D-Man-(1 → 4)-D-Glc and α-D-Man-(1 → 3)-[β-D-Xyl-(1 → 2)]-β-D-Man-(1 → 4)-D-Glc, respectively [10].

The oligosaccharides of lipid I, II, and IV were the target for the synthetic studies described here as part of our investigation into the synthesis of oligosaccharides of biological interest. Synthesis of the reducing end pentasaccharide has already been reported [9]. Herein we choose a systematic, integrated approach for the synthesis of lipid I and II.

β-D-GlcNAc-(1 → 2)-α-D-Man-(1 → 3)-β-D-Man-(1 → 4)-β-D-Glc-(1 → 1)-Cer (Lipid I)

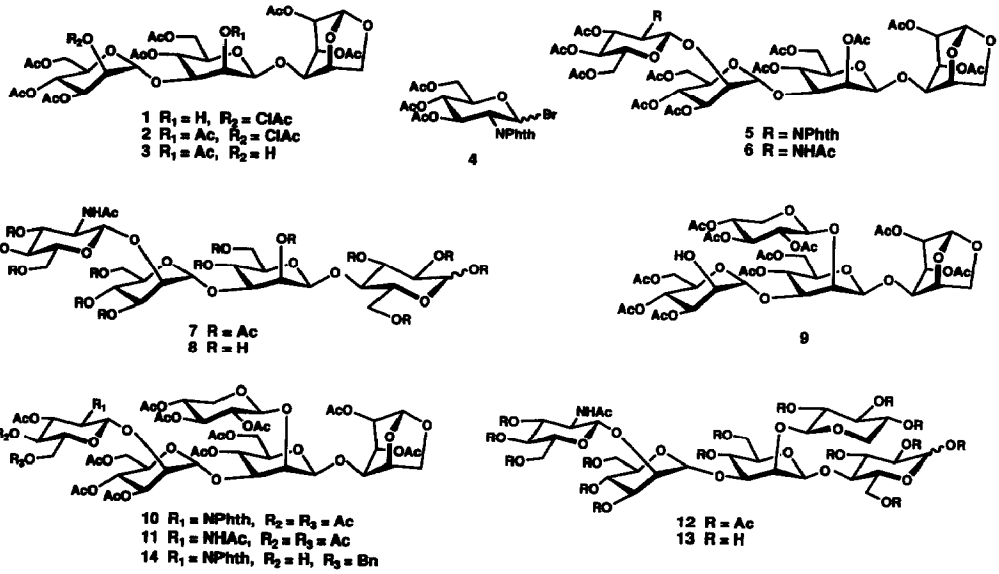


2. Results and discussion

In this work, oligosaccharides β-D-GlcNAc-(1 → 2)-α-D-Man-(1 → 3)-β-D-Man-(1 → 4)-Glc (**8**) and β-D-GlcNAc -(1 → 2)-α-D-Man-(1 → 3)[β-D-Xyl-(1 → 2)]-β-D-Man-(1 → 4)-Glc (**13**), corresponding to the carbohydrate parts of lipid I and II, respectively, were synthesized from **1**. A precursor of **8**, (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl) -(1 → 3)-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**5**), was synthesized as follows. Compound **3**, which was prepared by acetylation of **1** with acetic anhydride, followed by dechloroacetylation with thiourea, was condensed with **4** (ref. 11) in dichloromethane for 10 h at 20°C

in the presense of AgOTf–2,6-lutidine and 4A molecular sieves (MS-4A). Purification of the crude product by column chromatography afforded the tetrasaccharide derivative **5** in 71% yield. The ^{1}H NMR spectrum showed four signals for anomeric hydrogen atoms at δ 5.43 (br s, Glc), 5.27 (d, J 8.4 Hz, GlcNAc), 4.88 (s, β-Man), 4.61 (d, J 1.5 Hz, α-Man). The β-D configuration of the newly formed glycosidic bond was indicated by the $J_{C,H}$ value of 163.6 Hz in the ^{13}C NMR spectrum and the coupling constant of 8.4 Hz for the GlcNAc H-1 in the ^{1}H NMR spectrum. The phthalimido and the *O*-acetyl groups in **5** were removed by refluxing **5** with hydrazine hydrate in ethanol, giving free amino and hydroxy groups, which were subsequently *N*- and *O*-acetylated with acetic anhydride and pyridine, giving **6** in 84% yield [12]. Acetolysis of the 1,6-anhydro sugar in **6** by treatment with 1:15 trifluoroacetic acid–acetic anhydride [13], and finally treatment with methanolic sodium methoxide, gave **8** in 78% yield. On the other hand, (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**10**), a precursor of **13**, obtained by condensation of **4** with tetrasaccharide derivative **9** (ref 10), was prepared from **1** in the presence of silver triflate and molecular sieves. The ^{1}H NMR spectrum showed five signals for anomeric hydrogen atoms at δ 5.40 (br s, Glc), 5.30 (d, J 8.6 Hz, GlcNAc), 5.04 (d, J 4.7 Hz, Xyl), 4.71 (s, β-Man), 4.60 (d, J 1.5 Hz, α-Man). The β-D configuration of the newly formed glycosidic bond was indicated by the $J_{C,H}$ value of 162.9 Hz in the ^{13}C NMR spectrum and the coupling constant of 8.6 Hz for the GlcNAc H-1 in the ^{1}H NMR spectrum. Compound **10** was converted to **13** in three steps, in a manner analogous to that described for the deprotection of **5**. Nonreducing end trisaccharide derivative methyl (2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl)-(1 → 3)-[methyl(2,3-di-*O*-acetyl-4-*O*-methyl-β-D-glucopyranosyluronate)-(1 → 4)]-2-*O*-benzyl-1-thio-α,β-L-fucopyranoside (**18**), corresponding to the partical structure derived from lipid IV, was synthesized from **15** (ref 8) in 84% overall yield as described below. Compound **15**, after tretment with nickel chloride–sodium borohydride, followed by *N*-acetylation with acetic anhydride, provided the acetamido derivative **16** (refs 14 and 15) in 69% yield. Treatment of **16** with trifluoroacetic acid in dichloromethane, followed by acetylation, gave **17** in 95% yield [16]. Compound **17** was subsequently converted into the thioglycoside **18** by the use of methylthiotrimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) [17].

Glycosylation of nonreducing end trisaccharide derivative **18** and reducing end pentasaccharide (**14**) (ref 9), in the presence of dimethyl(methylthio) sulfonium triflate (DMTST) [17,18] as the glycosyl promoter and MS-4A in dichloromethane gave the desired α-fucosylated octasaccharide (**19**) in 29% yield. The ^{13}C NMR spectrum showed eight anomeric carbon atom signals at δ 101.0 (J 161.2 Hz, GlcA), 100.3 (J 169.2 Hz, GalNAc), 99.3 (J 175.3 Hz, α-Man), 99.2 (J 151.0 Hz, β-Man), 99.2 (J 187.3 Hz, Glc), 99.1 (J 171.2 Hz, Xyl), 99.0 (J 170.2 Hz, Fuc), and 96.4 (J 168.1 Hz, GlcNAc). The ^{1}H NMR spectrum of **19** showed a one-proton doublet at δ 4.71 (d, J 3.7 Hz, H-1) and a three-proton doublet at δ 1.10 (J 6.7 Hz,

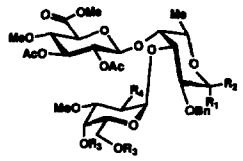


H-6) of an α-L-Fucose unit indicating the newly formed glycosidic linkage to be α-L. The deblocking of **19** was carried out by Paulsen's method [19,20]. The *O*-acetyl groups were cleaved, and the methyl ester was hydrolyzed in one step with aqueous N NaOH, which led to partial opening of the phthalimido group (**20**). On subsequent treatment with hydrazine, the phthalimido group was completely removed, and the NH_2 group was *N*-acetylated with acetic anhydride in methanol to give **21**. Finally, removal of the benzyl group with 10% Pd–C afforded the target compound **22** in 77% yield.

The ^1H and ^{13}C NMR specta of **22** showed eight signals due to the anomeric hydrogens and carbons, respectively; ^1H NMR: δ 5.43 (br s, Glc), 5.15 (br s, α-Man), 5.06 (*J* 4.2 Hz, GalNAc), 4.95 (*J* 3.7 Hz, Fuc), 4.92 (s, β-Man), 4.61 (*J* 7.3 Hz, Xyl), 4.51 (*J* 7.9 Hz, GlcNAc), and 4.47 (*J* 8.0 Hz, GlcA); ^{13}C NMR: δ 107.5 (Xyl), 106.5 (GlcA), 104.4 (Glc), 102.5 (GlcNAc), 102.4 (Fuc), 102.3 (GalNAc), 102.0 (α-Man), and 101.4 (β-Man). Compound **22** revealed an $[M + H]^+$ ion peak at *m/z* 1375 in the fast-atom-bombardment mass spectrum (FABMS).

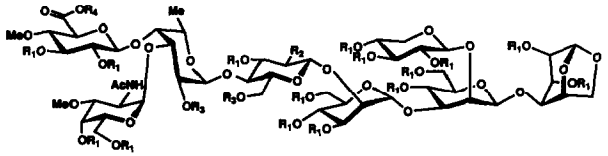
3. Experimental

General methods.—Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-140 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded with Jeol JNM-EX270, GSX400, and A500 FT-NMR 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. FABMS was recorded



15 $R_1 = O(CH_2)_2SiMe_3$, $R_2 = H$, $R_3 = H$, $R_4 = N_3$
16 $R_1 = O(CH_2)_2SiMe_3$, $R_2 = H$, $R_3 = H$, $R_4 = NHAc$
17 $R_1,R_2 = OAc,H$, $R_3 = Ac$, $R_4 = NHAc$
18 $R_1,R_2 = SMe,H$, $R_3 = Ac$, $R_4 = NHAc$

14 + 18 ⟶



19 $R_1 = Ac$, $R_2 = NPhth$, $R_3 = Bn$, $R_4 = Me$
20 $R_1 = R_4 = H$, $R_2 = NH\text{-}CO$-(C$_6$H$_3$)-COOH, $R_3 = Bn$
21 $R_1 = R_4 = H$, $R_2 = NHAc$, $R_3 = Bn$
22 $R_1 = R_3 = R_4 = H$, $R_2 = NHAc$

on a Jeol JMS-HX 110 mass spectrometer. TLC was conducted on precoated Silica Gel 60-F_{254} plates (E. Merck), with detection by quenching of UV fluorescence and by heating after spraying with aq 10% H_2SO_4. Column chromatography was carried out with Silica Gel-60 (E. Merck).

Materials and methods.—(3,4,6-Tri-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranosyl)-(1 → 3)-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**1**) (ref 10), (3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**9**) (ref 10), (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**14**) (ref 9), 2-(trimethylsilyl)ethyl (2-azido-2-deoxy-3-*O*-methyl-α-D-galactopyranosyl)-(1 → 3)-[methyl (2,3-di-*O*-acetyl-4-*O*-methyl-β-D-glucopyranosyl-uronate)-(1 → 4)]-2-*O*-benzyl-β-L-fucopyranoside (**15**) (ref 8) were obtained by the procedures described in our previous papers. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl bromide (**4**) (ref 11) was prepared as previously described.

*(3,4,6-Tri-*O*-acetyl-2-*O*-chloroacetyl-α-D-mannopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-D-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose* (**2**).—Compound **1** (72.0 mg, 0.085 nmol) was acetylated with 2:3 acetic anhydride–pyridine (3 mL) for 3 h at 20°C. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed sequentially with 3% HCl, aq

$NaHCO_3$, and water, dried (Na_2SO_4), filtered, and concentrated. The product was purified by silica gel column chromatography using 10:1 benzene–acetone as eluent, to give **2** (73.7 mg, 96.5%); $[\alpha]_D^{23}$ −33.3° (*c* 0.4, $CHCl_3$), TLC (3:1 benzene–acetone) R_f 0.43; ^1H NMR ($CDCl_3$): δ 5.48 (dd, 1 H, $J_{2',3'}$ 2.6 Hz, H-2'), 5.44 (br s, 1 H, H-1), 5.32 (t, 1 H, $J_{3',4'} = J_{4',5'} = 10.1$ Hz, H-4'), 5.23 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ 10.1 Hz, H-4''), 5.21 (dd, 1 H, $J_{2'',3''}$ 3.3 Hz, H-3''), 5.10 (br t, 1 H, $J_{2,3}$ 1.7 Hz, H-2), 5.07 (dd, 1 H, H-2''), 5.01 (d, 1 H, $J_{1'',2''}$ 1.8 Hz, H-1''), 4.95 (d, 1 H, $J_{1',3'}$ 0.9 Hz, H-1'), 4.61 (br d, 1 H, $J_{5,6b}$ 5.1 Hz, H-5), 4.56 (br s, 1 H, H-3), 4.28–4.08 (m, 7 H, H-5'', H-6'a, H-6'b, H-6''a, H-6''b and $ClCH_2CO$–), 3.95 (d, 1 H, $J_{6a,6b}$ 7.7 Hz, H-6a), 3.91 (dd, 1 H, H-3'), 3.79 (dd, 1 H, H-6b), 3.65 (ddd, 1 H, H-5'), 3.58 (br s, 1 H, H-4), 2.25, 2.13, 2.12, 2.11, 2.10, 2.06, 2.05, and 2.00 (each s, 24 H, 8 × OAc). Anal. Calcd for $C_{36}H_{47}ClO_{24}$: C, 48.09; H, 5.27. Found: C, 47.87; H, 5.05.

*(3,4,6-Tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-*D*-glucopyranose* (**3**).—Thiourea (70 mg) was added to a solution of **2** (71.0 mg, 0.079 nmol) in 6:1 pyridine–EtOH (4 mL). The mixture was stirred for 1.5 h at 80°C, $CHCl_3$ and water were added, and the organic layer was separated, washed with 3% HCl, aq $NaHCO_3$, and water, then dried (Na_2SO_4). Evaporation of the solvent gave a syrup, which was chromatographed on silica gel. Elution with 40:1 $CHCl_3$–EtOH provided **3** (50.0 mg, 77.0%); $[\alpha]_D^{21}$ −13.5° (*c* 1.0, $CHCl_3$); TLC (8:1 benzene–EtOH) R_f 0.30; ^1H NMR ($CDCl_3$): δ 5.44 (br s, 1 H, H-1), 4.97 (d, 1 H, $J_{1'',2''}$ 1.6 Hz, H-1''), 4.96 (d, 1 H, $J_{1',2'}$ 0.9 Hz, H-1'), 2.49 (br s, 1 H, OH), 2.24, 2.12, 2.11, 2.09, 2.08, 2.062, 2.060, and 2.04 (each s, 24 H, 8 × OAc) Anal. Calcd for $C_{34}H_{46}O_{23}$: C, 49.64; H, 5.64. Found: C, 49.16; H, 5.37.

*(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-*D*-glucopyranose* (**5**).—A solution of the bromide **4** (100 mg, 0.21 mmol) in CH_2Cl_2 (2 mL) was added to a cooled (−15°C) solution of **3** (49.0 mg, 0.06 mmol), silver triflate (AgOTf) (80 mg), MS-4A (300 mg), and 2,6-lutidine (20 μL) in CH_2Cl_2 (1 mL). After stirring at −15°C for 3 h and at 20°C for 10 h, the mixture was diluted with $CHCl_3$. The solid was removed by filtration and washed with $CHCl_3$. The combined filtrates were washed with cold water, 3% HCl, aq $NaHCO_3$, and water, dried (Na_2SO_4) and concentrated to a syrup, which was purified by chromatography on a column of silica gel with 5:1 benzene–acetone as eluent. The solvent was removed under reduced pressure to give **5** (52.5 mg, 71.1%); $[\alpha]_D^{20}$ −41.1° (*c* 1.0, $CHCl_3$); TLC (8:1 benzene–EtOH) R_f 0.40; ^1H NMR ($CDCl_3$): δ 7.86–7.84 (m, 4 H, Phth), 5.43 (br s, 1 H, H-1 Glc), 5.27 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1 GlcNAc), 4.88 (s, 1 H, H-1 β-Man), 4.61 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 α-Man), 2.31, 2.19, 2.13, 2.102, 2.100, 2.07, 2.02, 2.01, 2.00, 1.95, and 1.86 (each s, 33 H, 11 × OAc); ^{13}C NMR δ 99.2, ($J_{C,H}$ 173.9 Hz, Glc), 98.6 (*J* 175.7 Hz, α-Man), 97.6 (*J* 153.7 Hz, β-Man), 97.1 (*J* 163.6 Hz, GlcNAc). Anal. Calcd for $C_{54}H_{25}NO_{32}$: C, 52.30; H, 5.28; N, 1.13. Found: C, 51.85; H, 5.19; N, 1.24.

*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-mannopyranosyl)-(1 →*

*4)-2,3-di-*O*-acetyl-1,6-anhydro-β-*D*-glucopyranose* (**6**).—Compound **5** (41.0 mg, 0.033 mmol) was boiled for 3 h under N_2 in a mixture of EtOH (6 mL) and hydrazine hydrate (1.5 mL). The solution was coevaporated with water, the residue was dissolved in 2:3 Ac_2O–pyridine (3 mL), and stirred overnight at 20°C. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed sequentially with 3% HCl, aq $NaHCO_3$, and water, then dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 4:1 benzene–acetone as eluent, to give **6** (32.0 mg, 84.0%); $[\alpha]_D^{23}$ −38.5° (*c* 0.6, $CHCl_3$), TLC (8:1 benzene–EtOH) R_f 0.31; ^{1}H NMR ($CDCl_3$): δ 5.81 (br d, 1 H, *J* 5.9 Hz, –NH), 5.44 (br s, 1 H, H-1 Glc), 5.16 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1 GlcNAc), 4.96 (s, 1 H, H-1 β-Man), 4.84 (d, 1 H, $J_{1,2}$ 2.2 Hz, H-1 α-Man), 2.12, 2.11, 2.09, 2.08, 2.071, 2.067, 2.04, 2.01, and 2.00 (each s, 27 H, 9 × OAc), 2.22 (s, 6 H, 2 × OAc), 1.94 (s, 3 H, NH*Ac*).

*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-mannopyranosyl)-(1 → 4)-1,2,3,6-tetra-*O*-acetyl-*D*-glucopyranose* (**7**).—Compound **6** (30.0 mg, 0.026 mmol) was treated with the acetolysis reagent, 15:1 Ac_2O–CF_3CO_2H (6.4 mL), for 30 h at 20°C. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 30:1 $CHCl_3$–EtOH as eluent, to give **7** (28.2 mg, 80.7%); TLC (8:1 benzene–EtOH) R_f 0.34; ^{1}H NMR ($CDCl_3$): δ 6.26 (d, 0.75 H, $J_{1,2}$ 3.8 Hz, H-1 α-Glc), 5.78 (br d, 1 H, *J* 6.6 Hz, –NH), 5.68 (d, 0.25 H, $J_{1,2}$ 8.3 Hz, H-1 β-Glc), 5.12 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1 GlcNAc), 4.84 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 α-Man), 4.62 (s, 1 H, H-1 β-Man), 2.21, 2.190, 2.186, 2.13, 2.11, 2.08, 2.06, 2.04, 2.014, 2.011, and 1.99 (each s, 33 H, 11 × OAc), 2.09 (s, 6 H, 2 × OAc), 1.94 (s, 3 H, NH*Ac*). Anal. Calcd for $C_{58}H_{71}NO_{35}$: C, 51.90; *H*, 5.33; N, 1.04. Found: C, 51.50; H, 5.58; N, 1.00.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-α-*D*-mannopyranosyl-(1 → 3)-β-*D*-mannopyranosyl-(1 → 4)-*D*-glucopyranose* (**8**).—Compound **7** (28.0 mg, 0.021 mmol) was treated with NaOMe (5 mg) in MeOH (3 mL) for 2 h at 25°C, and then water (1 mL) was added. After the usual work-up, **8** was obtained (14.5 mg, 98.2%); mp 159–161°C; $[\alpha]_D^{22}$ +5.0° (*c* 0.4, H_2O), TLC (3:3:1 $CHCl_3$–MeOH–H_2O) R_f 0.28; ^{1}H NMR (D_2O): δ 5.22 (d, 0.4 H, $J_{1,2}$ 3.7 Hz, H-1 α-Glc), 5.14 (br s, 1 H, H-1 α-Man), 4.76 (s, 1 H, H-1 β-Man), 4.65 (d, 0.6 H, $J_{1,2}$ 7.9 Hz, H-1 β-Glc), 4.58 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1 GlcNAc), 2.05 (s, 3 H, NH*Ac*). Anal. Calcd for $C_{26}H_{46}NO_{21} \cdot 3.5H_2O$: C, 40.52; H, 5.89; N, 1.82. Found: C, 40.16; H, 6.16; N, 1.56.

*(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-acetyl-β-*D*-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-*D*-glucopyranose* (**10**).—A solution of the glycosyl bromide **4** (140.0 mg, 0.294 mmol) in CH_2Cl_2 (3 mL) was added to a cooled (−15°C) solution of **9** (102.0 mg, 0.098 mmol), silver triflate (AgOTf) (160 mg), MS-4A (600 mg), and 2,6-lutidine (50 μL) in CH_2Cl_2 (2 mL). After stirring at −15°C for 3 h and at 20°C for 10 h, the mixture was diluted with $CHCl_3$. The solid was removed by filtration and washed

with $CHCl_3$. The combined filtrates were washed with cold water, 3% HCl, aq $NaHCO_3$, and water, dried (Na_2SO_4) and concentrated to a syrup which was purified by chromatography on a column of silica gel with 4:1 benzene–acetone. Solvent removal under reduced pressure gave **10** (59.6 mg, 41.8%); $[\alpha]_D^{22}$ −66.1° (*c* 1.1, $CHCl_3$); TLC (8:1 benzene–EtOH) R_f 0.36; ^1H NMR ($CDCl_3$): δ 7.86–7.74 (m, 4 H, Phth), 5.40 (br s, 1 H, H-1 Glc), 5.30 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1 GlcNAc), 5.04 (d, 1 H, $J_{1,2}$ 4.7 Hz, H-1 Xyl), 4.71 (s, 1 H, H-1 β-Man), 4.60 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 α-Man), 2.24, 2.10, 2.09, 2.08, 2.07, 2.033, 2.029, 2.026, 2.00, 1.97, and 1.86 (each s, 33 H, 11 × OAc). 2.14 (s, 6 H, 2 × OAc); ^{13}C NMR δ 99.5 ($J_{C,H}$ 169.8 Hz, Xyl), 99.28 (*J* 169.1 Hz, α-Man), 99.25 (*J* 180.2 Hz, Glc), 99.2 (*J* 155.5 Hz, β-Man), 97.2 (*J* 162.7 Hz, GlcNAc). Anal. Calcd for $C_{63}H_{77}NO_{38}$; C, 51.96; H, 5.33; N, 0.96. Found: C, 51.74; H, 5.50; N, 0.86.

*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-(2,3,4,-tri-*O*-acetyl-β-*D*-xylopyranosyl)-(1 → 2)]-(4,6-di-O-acetyl-β-*D*-mannopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-1,6-anhydro-β-*D*-glucopyranose* (**11**).—Compound **10** (19.6 mg, 0.014 mmol) was boiled for 3 h under N_2 in a mixture of EtOH (4 mL) and hydrazine hydrate (1 mL). The solution was coevaporated with water, the residue was dissolved in 2:3 Ac_2O–pyridine (2 mL), and stirred overnight at 20°C. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed sequentially with 3% HCl, aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 3:1 benzene–acetone as eluent to give **11** (17.1 mg, 92.9%); $[\alpha]_D^{23}$ −60.4° (*c* 0.4, $CHCl_3$); TLC (8:1 benzene–EtOH) R_f 0.18; ^1H NMR ($CDCl_3$): δ 5.81 (br d, 1 H, *J* 7.1 Hz, –NH), 5.42 (br s, 1 H, H-1 Glc), 5.14 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1 GlcNAc), 5.12 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1 Xyl), 4.85 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1 α-Man), 4.80 (s, 1 H, H-1 β-Man), 2.17, 2.15, 2.11, 2.097, 2.095, 2.07, 2.06, 2.043, 2.041, 2.012, and 2.008 (each s, 33 H, 11 × OAc), 2.087 (s, 6 H, 2 × OAc), 1.95 (s, 3 H, NH*Ac*). Anal. Calcd for $C_{57}H_{79}NO_{37}$: C, 50.04; H, 5.67; N, 1.02. Found: C, 49.86; H, 5.73; N, 0.87.

*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-acetyl-β-*D*-mannopyranosyl)-(1 → 4)-1,2,3,6-tetra-*O*-acetyl-*D*-glucopyranose* (**12**).—Compound **11** (15.8 mg, 0.012 mmol) was treated with the acetolysis reagent, 15:1 Ac_2O–CF_3CO_2H (3.2 mL), for 30 h at 20°C. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 30:1 $CHCl_3$–EtOH as eluent to give **12** (13.0 mg, 76.9%); TLC (8:1 benzene–EtOH) R_f 0.30; ^1H NMR ($CDCl_3$): δ 6.26 (d, 0.7 H, $J_{1,2}$ 3.7 Hz, H-1 α-Glc), 5.72 (d, 0.3 H, $J_{1,2}$ 8.3 Hz, H-1 β-Glc), 5.12 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1 GlcNAc), 4.96 (d, 1 H, $J_{1,2}$ 6.2 Hz, H-1 Xyl), 4.87 (br s, 1 H, H-1 α-Man), 4.48 (s, 1 H, H-1 β-Man), 2.13, 2.12, 2.113, 2.105, 2.10, 2.09, 2.08, 2.061, 2.057, 2.052, 2.04, 2.032, 2.026, 2.01, and 2.00 (each s, 45 H, 15 × OAc), 1.95 (s, 3 H, NH*Ac*).

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-α-*D*-mannopyranosyl-(1 → 3)-[β-*D*-xylopyranosyl-(1 → 2)]-β-*D*-mannopyranosyl-(1 → 4)-*D*-glucopyranose* (**13**).—

Compound **12** (13.0 mg, 0.009 mmol) was treated with NaOMe (3 mg) in MeOH (1.5 mL) for 2 h at 25°C, and then water (1 mL) was added. After the usual work-up, **13** was obtained (7.2 mg, 98.9%); mp 161–163°C; $[\alpha]_D^{22}$ −7.4° (*c* 0.2, H_2O); TLC (3:3:1 $CHCl_3$–MeOH–H_2O) R_f 0.21; ^1H NMR (D_2O): δ 5.23 (d, 0.38 H, $J_{1,2}$ 3.8 Hz, H-1 α-Glc), 5.16 (br s, 1 H, H-1 α-Man), 4.83 (s, 1 H, H-1 β-Man), 4.66 (d, 0.62 H, $J_{1,2}$ 8.1 Hz, H-1 β-Glc), 4.56 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1 GlcNAc), 4.52 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1 Xyl), 2.05 (s, 3 H, NH*Ac*). Anal. Calcd for $C_{31}H_{53}NO_{24} \cdot 4.5H_2O$: C, 41.15; H, 5.90; N, 1.55. Found: C, 40.83; H, 6.05; N, 1.31.

2-(Trimethylsilyl)ethyl(2-acetamido-2-deoxy-3-O-methyl-α-D-galactopyranosyl)-(1 → 3)-[methyl(2,3-di-O-acetyl-4-O-methyl-β-D-glucopyranosyluronate)-(1 → 4)]-2-O-benzyl-β-L-fucopyranoside (**16**).—To a solution of **15** (153.0 mg, 0.181 mmol), $NiCl_2 \cdot 6H_2O$ (4%), and boric acid (2%) in EtOH (25 mL) was added dropwise a filtered solution of $NaBH_4$ in EtOH until the black color persisted. After 3 h Ac_2O (20 mL) was added, and the mixture was stirred for 15 h, then poured into water, and extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4), concentrated, and then chromatographed on silica gel using 10:1 benzene–EtOH to give **16** (108.0 mg, 69.3%); $[\alpha]_D^{24}$ +3.4° (*c* 1.0, $CHCl_3$): TLC (5:1 benzene–EtOH) R_f 0.42; ^1H NMR ($CDCl_3$): δ 7.29–7.18 (m, 5H, Ph), 6.30 (d, 1 H, *J* 9.9 Hz, NH), 5.15 (d, 1 H, $J_{1'',2''}$ 4.2 Hz, H-1′), 4.70 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1″), 4.27 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 3.80 (s, 3 H, COOMe), 3.46 (s, 3 H, OMe), 3.36 (s, 3 H, OMe), 2.07 (s, 6 H, 2 × OAc), 1.79 (s, 3H, NH*Ac*), Anal. Calcd for $C_{39}H_{61}NO_{18}Si$: C, 54.47; H, 7.03; N, 1.61. Found: C, 53.98; H, 6.82; N, 1.33.

(2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-methyl-α-D-galactopyranosyl)-(1 → 3)-[methyl(2,3-di-O-acetyl-4-O-methyl-β-D-glucopyranosyluronate)-(1 → 4)]-1-O-acetyl-2-O-benzyl-L-fucopyranose (**17**).—To a solution of **16** (108.0 mg, 0.126 nmol) in dry CH_2Cl_2 (0.6 mL) was added CF_3CO_2H (1.2 mL) at 0°C, and the mixture was stirred for 1 h at 25°C. 1:2 Ethyl acetate–toluene (9 mL) was added to the mixture and concentrated. The residue was dissolved in 3:2 pyridine–Ac_2O (10 mL) and stirred overnight at 40°C. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed sequentially with 3% HCl, aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography using 20:1 benzene–EtOH as eluent, to give **17** (106.0 mg, 95.0%); TLC (5:1 benzene–EtOH) R_f 0.50; ^1H NMR ($CDCl_3$): δ 6.20 (d, 0.7 H, $J_{1,2}$ 3.3 Hz, H-1α), 5.46 (d, 0.3 H, $J_{1,2}$ 7.7 Hz, H-1β), 5.13 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1′), 4.66 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″). Anal. Calcd for $C_{40}H_{55}NO_{21}$: C, 54.23; H, 6.26; N, 1.58. Found: C, 54.11; H, 6.04; N, 1.29.

Methyl(2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-methyl-α-D-galactopyranosyl)-(1 → 3)-[methyl(2,3-di-O-acetyl-4-O-methyl-β-D-glucopyranosyluronate)-(1 → 4)]-2-O-benzyl-1-thio-L-fucopyranoside (**18**).—To a cooled and stirred solution of **17** (104.0 mg, 0.117 mmol) in dry CH_2Cl_2 (1.5 mL) was added (methylthio) trimethylsilane (66 μL, 0.48 mmol), and trimethylsilyl trifluoromethanesulfonate (23 μL, 0.12 mmol), and the stirring was continued for 2 h at 0°C. Chloroform was added to the mixture, and the solution was washed with aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated to a syrup, which was chromatographed on a column of silica gel

with 3:1 benzene–acetone to give **18** (92.6 mg, 88.9%); TLC (5:1 benzene–EtOH) R_f 0.52; ^{1}H NMR ($CDCl_3$): δ7.32–7.22 (m, 5 H, Ph), 5.95 (d, 1 H, *J* 10.4 Hz, NH), 5.19 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1'), 5.04 (d, 0.2 H, $J_{1,2}$ 4.2 Hz, H-1α), 4.94 and 4.66 (each d, 2 H, J_{gem} 10.4 Hz, PhCH_2), 4.68 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1''), 4.20 (d, 0.8 H, $J_{1,2}$ 9.2 Hz, H-1β), 3.93 (s, 3 H, COOMe), 3.46 and 3.39 (each s, 6 H, 2 × OMe), 2.16, 2.15, 2.12, 2.10, and 2.06 (each s, 15 H, 4 × OAc, SMe), 1.72 (s, 3 H, NH*Ac*), 1.36 (d, 3 H, $J_{5,6}$ 6.7 Hz, H-6). Anal. Calcd for $C_{39}H_{59}NO_{11}S$: C, 53.60; H, 6.34; N, 1.60. Found: C, 53.21; H, 6.09; N, 1.49.

*(2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-α-*D*-galactopyranosyl)-(1 → 3)-[methyl-(2,3-di-*O*-acetyl-4-*O*-methyl-β-*D*-glucopyranosyluronate)-(1 → 4)]-(2-*O*-benzyl-α-*L*-fucopyranosyl)-(1 → 4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl-)(1 → 2)]-(4,6-di-*O*-acetyl-β-*D*-mannopyranosyl)-(1 → 4)-2,3-di-*O*-aceytl-1,6-anhydro-β-*D*-glucopyranose* (**19**).—A solution of **14** (55.0 mg, 37.6 μmol) and **18** (50.5 mg, 56.7 μmol) in dry CH_2Cl_2 (0.8 mL), containing powdered MS-4A (250 mg), was stirred for 1 h at 20°C. DMTST (41 mg, 159 μmol) and MS-4A (41 mg) were added to the mixture at 0°C, and the stirring was continued for 6 h at 20°C. The progress of the reaction was monitored by TLC. Methanol (0.2 mL) and Et_3N (0.1 mL) were added to the mixture, and the precipitates were filtered and washed with CH_2Cl_2. The combined filtrate and washings were washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (1:1 benzene–acetone) of the residue on silica gel gave **19** (24.5 mg 28.5%); $[\alpha]_D^{22}$ −116.9° (*c* 0.1, $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ7.74–7.72 (m, 4 H, Phth), 7.36–7.20 (m, 10 H, 2 × Ph), 5.82 (d, 1 H, *J* 9.8 Hz, NH), 5.39 (br s, 1 H, H-1 Glc), 5.28 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1 GlcNAc), 5.09 (d, 1 H, $J_{1,2}$ 6.7 Hz, H-1 Xyl), 5.02 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1 GalNAc), 4.71 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1 Fuc), 4.70 (s, 1 H, H-1 β-Man), 4.62 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 α-Man), 4.59 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1 GlcA), 3.91 (s, 3 H, COOMe), 3.45 and 3.37 (each s, 6 H, 2 × OMe), 2.184, 2.178, 2.129, 2.126, 2.099, 2.096, 2.08, 2.060, 2.055, 2.02, 1.98, 1.96, 1.94, 1.89, 1.77, and 1.73 (each s, 48 H, 16 × OAc, NH*Ac*), 1.10 (d, 3 H, $J_{5,6}$ 6.7 Hz, H-6 Fuc); ^{13}C NMR ($CDCl_3$): δ101.0 ($J_{C,H}$ 161.2 Hz, GlcA), 100.3 (*J* 169.2 Hz, GalNAc), 99.3 (*J* 175.3 Hz, α-Man), 99.2 (*J* 151.0 Hz, β-Man), 99.2 (*J* 187.3 Hz, Glc), 99.1 (*J* 171.2 Hz, Xyl), 99.0 (*J* 170.2 Hz, Fuc), 96.4 (*J* 168.1 Hz, GlcNAc). Anal. Calcd for $C_{104}H_{130}N_2O_{55}$: C, 54.59; H, 5.73; N, 1.22. Found: C, 54.35; H, 5.85; N, 1.08.

*(2-Acetamido-2-deoxy-3-*O*-methyl-α-*D*-galactopyranosyl)-(1 → 3)-[(4-*O*-methyl-β-*D*-glucopyranosyluronic acid)-(1 → 4)]-(2-*O*-benzyl-α-*L*-fucopyranosyl)-(1 → 4)-[2-(2-carboxy-benzamido)-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl]-(1 → 2)-(α-*D*-mannopyranosyl)-(1 → 3)-[(β-*D*-xylopyranosyl)-(1 → 2)]-(β-*D*-mannopyranosyl)-(1 → 4)-1,6-anhydro-β-*D*-glucopyranose* (**20**).—To a solution of **19** (21.2 mg, 9.27 μmol) in MeOH (1.5 mL) was added N NaOH (0.2 μL). The mixture was stirred for 26 h at 20°C, neutralized with Amberlite IR-120 (H^+) resin, and filtered. The resin was washed with MeOH, and the combined filtrate and washings were concentrated to give **20** (15.3 mg, 99.4%); $[\alpha]_D^{23}$ −33.4° (*c* 0.4, CH_3OH), TLC (3:3:1 $CHCl_3$–MeOH–H_2O) R_f 0.44; ^{1}H NMR (CD_3OD): δ 7.69–7.40 (m, 4 H, Phth), 7.34–7.25 (m, 10 H, 2 × Ph), 5.28 (br s, 1 H, H-1 Glc), 5.27 (br s, 1H, H-1 α-Man), 5.12 (d, 1

H, $J_{1,2}$ 4.3 Hz, H-1 GalNAc), 5.04 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1 Fuc), 4.86 (s, 1 H, H-1 β-Man), 4.64 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1 GlcNAc), 4.59 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1 Xyl), 4.38 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1 GlcA), 3.55 and 3.50 (each s, 6 H, 2 × OMe), 1.91 (s, 3 H, NH*Ac*), 1.31 (d, 3 H, $J_{5,6}$ 6.7 Hz, H-6 Fuc). ^{13}C NMR (CD_3OD): δ 106.0 (Xyl), 105.8 (GlcA), 103.8 (Glc), 102.0 (GlcNAc), 101.3 (GalNAc), 101.1 (α-Man), 100.7 (β-Man), 99.5 (Fuc).

*(2-Acetamido-2-deoxy-3-*O*-methyl-α-*D*-galactopyranosyl)-(1 → 3)-[(4-*O*-methyl-β-*D*-glucopyranosyluronic acid)-(1 → 4)]-(2-*O*-benzyl-α-*L*-fucopyranosyl)-(1 → 4)-(2-acetamido-6-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-(α-*D*-mannopyranosyl)-(1 → 3)-[(β-*D*-xylopyranosyl)-(1 → 2)]-(β-*D*-mannopyranosyl)-(1 → 4)-1,6-anhydro-β-*D*-glucopyranose* (**21**).—Hydrazine hydrate (0.2 mL) was added to a mixture of **20** (15.1 mg, 9.09 μmol) in 19:1 EtOH–H_2O (1.6 mL). The mixture was refluxed overnight and then concentrated. The residue was dissolved in MeOH (2 mL) and treated with Ac_2O (0.2 mL) at 20°C. After 20 h the mixture was concentrated and coevaporated with toluene. The residue was chromatographed on a column of Sephadex LH-20 with MeOH to give **21** (11.0 mg, 77.8%); $[\alpha]_D^{21}$ −42.3° (*c* 0.3 CH_3OH); TLC (3:3:1 $CHCl_3$–MeOH–H_2O) R_f 0.45; ^{1}H NMR (CD_3OD): δ 7.32–7.21 (m, 10 H, 2 × Ph), 5.27 (br s, 1 H, H-1 Glc), 5.13 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1 GalNAc), 5.11 (br s, 1 H, H-1 α-Man), 5.07 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1 Fuc), 4.83 (s, 1 H, H-1 β-Man), 4.60 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1 Xyl), 4.58 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1 GlcNAc), 4.38 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1 GlcA), 3.55 and 3.49 (each s, 6 H, 2 × OMe), 1.99 and 1.98 (each s, 6 H, 2 × NH*Ac*), 1.30 (d, 3 H, $J_{5,6}$ 6.7 Hz, H-6 Fuc).

*2-Acetamido-2-deoxy-3-*O*-methyl-α-*D*-galactopyranosyl-(1 → 3)-[4-*O*-methyl-β-*D*-glucopyranosyluronic acid-(1 → 4)]-α-*L*-fucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-*O*-α-*D*-mannopyranosyl-(1 → 3)-[β-*D*-xylopyranosyl-(1 → 2)]-β-*D*-mannopyranosyl-(1 → 4)-1,6-anhydro-β-*D*-glucopyranose* (**22**).—A mixture of **21** (10.0 mg, 6.43 μmol) and 10% Pd–C (20 mg) in 1:1 MeOH–H_2O (4 mL) was stirred for 2 days at 20°C under H_2 until the reaction was complete. The mixture was filtered, washed with water, and concentrated. Column chromatography (1:1 MeOH–H_2O) of the residue on Sephadex LH-20 gave **22** (6.8 mg, 76.9%); mp 240–243°C $[\alpha]_D^{21}$ −62.4° (*c* 0.2 H_2O); TLC (3:3:1 $CHCl_3$–MeOH–H_2O) R_f 0.15; ^{1}H NMR (D_2O): δ 5.43 (br s, 1 H, H-1 Glc), 5.15 (br s, 1 H, H-1 α-Man), 5.06 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1 GalNAc), 4.95 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1 Fuc), 4.93 (s, 1 H, H-1 β-Man), 4.61 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1 GlcNAc), 4.51 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1 Xyl), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1 GlcA), 3.47 and 3.45 (each s, 6 H, 2 × OMe), 2.02 and 2.00 (each s, 6 H, 2 × NH*Ac*), 1.24 (d, 3 H, $J_{5,6}$ 6.7 Hz, H-6 Fuc). ^{13}C NMR (D_2O): δ 107.5 (Xyl), 106.5 (GlcA), 104.4 (Glc), 102.5 (GlcNAc), 102.4 (Fuc), 102.3 (GalNAc), 102.0 (α-Man), 101.4 (β-Man). FABMS *m/z* 1375 $[M + H]^+$.

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References

[1] T. Takeda, N. Hada, and Y. Ogihara, *Chem. Pharm. Bull.*, 41 (1993) 2058–2060.
[2] T. Hori, M. Sugita, S. Ando, M. Kuwahara, K. Kumauchi, E. Sugie, and O. Itasaka, *J. Biol. Chem.*, 256 (1981) 10979–10985.
[3] T. Hori, M. Sugita, J. Kanbayashi, and O. Itasaka, *J. Biochem.*, 81 (1977) 107–114.
[4] T. Hori, H. Takeda, M. Sugita, and O. Itasaka, *J. Biochem.*, 82 (1977) 1281–1285.
[5] T. Hori, M. Sugita, S. Ando, K. Tsukada, K. Shiota, M. Tsuzuki, and O. Itasaka, *J. Biol. Chem.*, 258 (1983) 2239–2245.
[6] H. Beitinger, F. Schifferer, M. Sugita, S. Araki, M. Satake, D. Mobius, and H. Rahmann, *J. Biochem.*, 105 (1989) 664–669.
[7] M. Sugita, S. Itonori, F. Inagaki, and T. Hori, *J. Biol. Chem.*, 264 (1989) 15028–15033.
[8] O. Kanie, T. Takeda, and Y. Ogihara, *Carbohydr. Res.*, 190 (1989) 53–64.
[9] O. Kanie, T. Takeda, N. Hada, and Y. Ogihara, *J. Carbohydr. Chem.*, 10 (1991) 561–581.
[10] T. Takeda, N. Hada, and Y. Ogihara, *Chem. Pharm. Bull.*, 40 (1992) 1930–1933.
[11] R.U. Lemieux, T. Takeda, and B.Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90–115.
[12] S.H. Khan, S.A. Abbas, and K.L. Matta, *Carbohydr. Res.*, 205 (1990) 385–397.
[13] H. Paulsen, M. Heume, Z. Gyorgydeak, and R. Lebuhn, *Carbohydr. Res.*, 144 (1985) 57–70.
[14] W. Kinzy and R.R. Schmidt, *Carbohydr. Res.*, 193 (1989) 33–47.
[15] H. Paulsen and A. Bunsch, *Carbohydr. Res.*, 101 (1982) 21–30.
[16] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, and G. Magnusson, *J. Org. Chem.*, 53 (1988) 5629–5647.
[17] A. Kameyama, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 10 (1991) 549–560.
[18] P. Fugedi and P.J. Garegg, *Carbohydr. Res.*, 149 (1986) c9–c12.
[19] H. Paulsen and H. Tietz, *Carbohydr. Res.*, 144 (1985) 205–229.
[20] H. Paulsen and H. Tietz, *Angew. Chem. Int. Ed. Engl.*, 24 (1985) 128–129.