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SYNTHESIS OF FOUR STRUCTURAL ELEMENTS OF XYLOSE-CONTAINING CARBOHYDRATE CHAINS FROM *N*-GLYCOPROTEINS

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ABSTRACT

The synthesis of the oligosaccharides β-D-Xyl*p*-(1→2)-β-D-Man*p*-OMe (**12**), β-D-Xyl*p*-(1→2)-[α-D-Man*p*-(1→6)]-β-D-Man*p*-OMe (**17**), β-D-Xyl*p*-(1→2)-[α-D-Man*p*-(1→3)]-β-D-Man*p*-OMe (**21**), and β-D-Xyl*p*-(1→2)-[α-D-Man*p*-(1→3)][α-D-Man*p*-(1→6)]-β-D-Man*p*-OMe (**25**) is described. Methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-β-D-mannopyranoside (**6**) was prepared from the corresponding *gluco*-epimer (**4**) by oxidation, followed by stereoselective reduction. Condensation of **6** with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide in the presence of mercuric cyanide gave a 1:9 mixture of methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α- (**7a**) and -β-D-xylopyranosyl)-β-D-mannopyranoside (**7**), and then **7** was converted into the acetylated disaccharide-glycoside **11**. Regioselective mannosylation, with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide, at position 6 of deisopropylidenated **7** (**8**), using mercuric bromide as a promoter, afforded the trisaccharide-glycoside derivative **13**, which was transformed into the acetylated trisaccharide-glycoside **16**. The disaccharide derivative **10**, obtained from **8**, and the trisaccharide derivative **15**, obtained from **13**, were glycosylated at position 3 with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)trichloroacetimidate (**19**), using trimethylsilyl triflate as a promoter, giving rise to acetylated tri- (**20**) and tetra-saccharide (**24**) derivatives, respectively. *O*-Deacetylation of **11, 16, 20**, and **24** gave **12, 17, 21**, and **25**, respectively.

INTRODUCTION

Xylose-containing *N*-linked carbohydrate chains are integral parts of certain plant[1–11] (**1**) and animal[12–14] (**2**) glycoproteins. In all of the known structures, β-D-xylose is (1→2)-linked to β-D-mannose of the core element. If α-L-fucose is present

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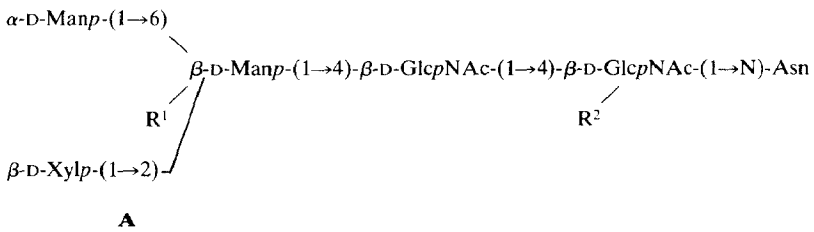
at the asparagine-linked 2-acetamido-2-deoxy-D-glucose, then there are differences in the sites of attachment. The α-D-mannose residues can be substituted with additional monosaccharides[10,12–14] or 3-*O*-methylated[13,14]. The biosynthesis of the carbohydrate chains of this type of *N*-glycoprotein is still a subject of discussion[7,10].



A

1 R^1 = H or α-D-Manp-(1→3), R^2 = H or α-L-Fucp-(1→3)
2 R^1 = α-D-Manp-(1→3), R^2 = H or α-L-Fucp-(1→6)

In seeking to determine the structure of xylose-containing *N*-linked carbohydrate chains, the synthesis of structural elements of this type of glycan was undertaken. In addition to their importance for conformational analysis, these elements are of value in biosynthesis and immunological studies. We now report the synthesis of the tetrasaccharide unit **A** [R^1 = α-D-Manp-(1→3)] of **1** and **2** as its methyl β-glycoside, and of partial structures.

RESULTS AND DISCUSSION

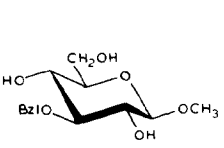
One of the difficulties in the synthesis of the carbohydrate chains of **1** and **2** is the formation of the β-D-Manp-(1→4)-β-D-GlcpNAc linkage[15]. Two routes are available which can provide acceptable yields. The first approach[16–18] is laborious, but reliable, and involves the synthesis of a β-D-glucopyranose derivative in which the participating protecting group at C-2 can be removed selectively. Oxidation of HO-2 to a ketone group followed by stereoselective reduction provides the corresponding β-D-mannopyranose derivative. The second procedure involves the coupling of a C-1-activated D-mannopyranosyl residue having a non-participating group at C-2 as glycosyl donor and a 2-amino-2-deoxy-D-glucose derivative in reversed chair conformation as acceptor, in the presence of insoluble silver or thallium salts on silicate or zeolite supporters[19–21].

As it is our goal to synthesize the carbohydrate chains of **1** and **2** *via* the *gluco*-route, for the synthesis of **A** as its methyl β-glycoside (**25**) and the derived oligosaccharides **12, 17**, and **21**, a methyl β-D-glucopyranoside derivative was preferred to a methyl β-D-mannopyranoside derivative such as methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside[22]. Moreover, the introduction of a β-D-xylopyranosyl unit generally proceeds with lower stereoselectivity; thus, this unit should be inserted at an early stage of the synthesis.

Methyl 3-*O*-benzyl-β-D-glucopyranoside[23] (**3**) was treated with 2,2-di-

methoxypropane in the presence of *p*-toluenesulfonic acid to give methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-β-D-glucopyranoside (**4**). Oxidation of **4** with methyl sulfoxide–acetic anhydride gave the ulose **5** (88%), which was reduced stereoselectively with $NaBH_4$ in 1:1 dichloromethane–methanol, yielding methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-β-D-mannopyranoside (**6**). Only traces of **4** could be detected by t.l.c.

Glycosylation of **6** with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide using the Helferich procedure resulted in a good yield of disaccharide derivative with an αβ-ratio of 1:9. The desired (1→2)-β-linked disaccharide **7** was isolated in a yield of 68% after column chromatography. Acid hydrolysis of the isopropylidene group in **7** gave crystalline **8**, which was acetylated (→**9**), debenzylated by catalytic hydrogenolysis over Pd/C (→crystalline **10**), and acetylated to give crystalline **11**. Saponification of **11** gave methyl 2-*O*-β-D-xylopyranosyl-β-D-mannopyranoside (**12**).



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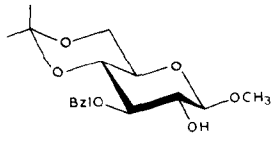

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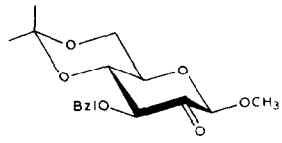

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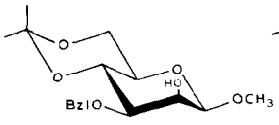

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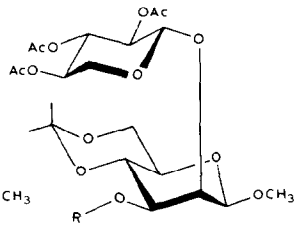
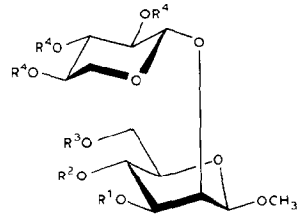


7 R = Bzl
22 R = H



8 R^1 = Bzl, R^2 = R^3 = H, R^4 = Ac
9 R^1 = Bzl, R^2 = R^3 = R^4 = Ac
10 R^1 = H, R^2 = R^3 = R^4 = Ac
11 R^1 = R^2 = R^3 = R^4 = Ac
12 R^1 = R^2 = R^3 = R^4 = H

Since HO-4 has the lowest reactivity among the hydroxyl groups of hexopyranosides, it was anticipated that methyl 3-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**8**) could be mannosylated regioselectivily at position 6. Treatment of **8** with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide, in the presence of $HgBr_2$ as a promoter, gave the trisaccharide derivative **13** (68%). However, the use of $Hg(CN)_2$ as a promoter led to the formation of orthoester instead of **13**. Acetylation of **13** (→**14**), followed by debenzylation using catalytic hydrogenolysis (→**15**) and acetylation, gave the crystalline acetylated tri-

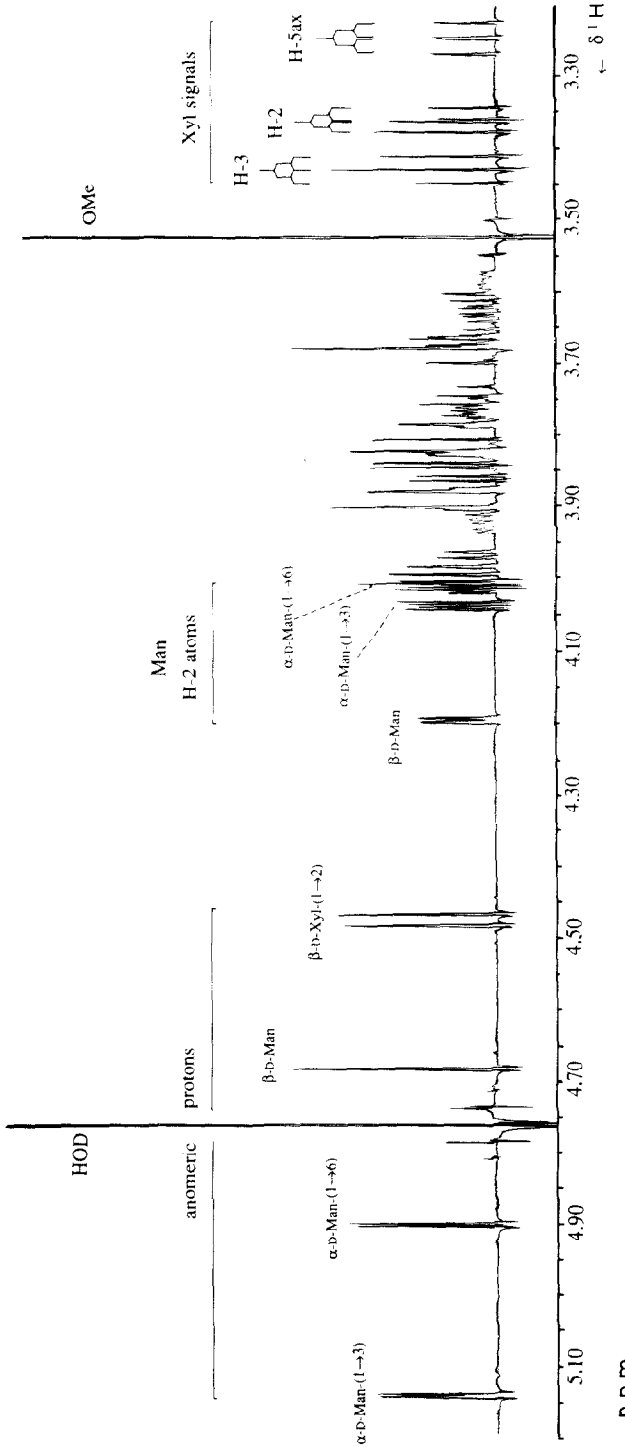
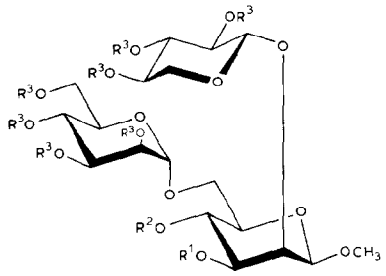
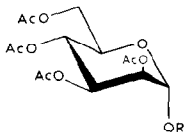


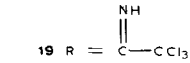
Fig. 1. 500-MHz ^{1}H-n.m.r. spectrum of β-D-Xyl*p*-(1→2)-[α-D-Man*p*-(1→3)][α-D-Man*p*-(1→6)]-β-D-Man*p*-OMe (**25**).



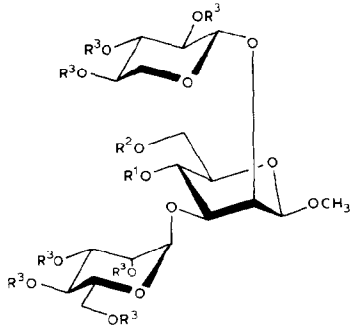
13 R^1 = Bzl, R^2 = H, R^3 = Ac
14 R^1 = Bzl, R^2 = R^3 = Ac
15 R^1 = H, R^2 = R^3 = Ac
16 R^1 = R^2 = R^3 = Ac
17 R^1 = R^2 = R^3 = H



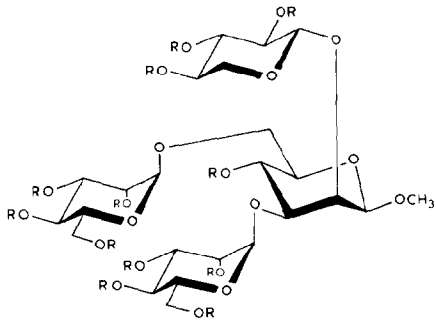
18 R = H



19 R = C(=NH)—CCl3



20 R^1 = R^2 = R^3 = Ac
21 R^1 = R^2 = R^3 = H
23 R^1, R^2 = $C(CH_3)_2$, R^3 = Ac



24 R = Ac
25 R = H

saccharide derivative **16**. Zemplèn deacetylation of **16** gave methyl 6-*O*-α-D-mannopyranosyl-2-*O*-β-D-xylopyranosyl-β-D-mannopyranoside (**17**).

Compound **15**, with HO-3 of the β-D-mannopyranosyl unit unsubstituted, was chosen for preparation of the tetrasaccharide derivative. However, attempted glycosylation of **15** using 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide, with various catalysts [$Hg(CN)_2$, $Hg(CN)_2$–$HgBr_2$, and Ag triflate] and reaction conditions, resulted mainly in cleavage of the xylopyranosyl residue and the formation of complex mixtures. The low reactivity of HO-3 in **15** may be due to the presence of two glycosyl residues on the same side of the β-D-mannopyranosyl unit and by the presence of AcO-4 which can deactivate the neighbouring hydroxyl group. Attempted mannosylation of **10**, the intermediate which lacks the α-D-mannopyranosyl residue at position 6, also failed to give an acceptable yield and only low yields were obtained on reaction with penta-*O*-acetyl-α-D-mannopyranose catalyzed by trimethylsilyl triflate. However, the trichloroacetimidate method[24] with trimethylsilyl triflate as a catalyst was successful.

The reaction of the glycosyl donor **19**, prepared from 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose (**18**) by treatment with trichloroacetonitrile in the presence of NaH, with **10** was extremely fast, even at −30°; after 10 min, only the trisaccharide derivative **20** could be detected by t.l.c. and it was isolated in a yield of 88%. Deacetylation of **20** afforded methyl 3-*O*-α-D-mannopyranosyl-2-*O*-β-D-xylopyranosyl-β-D-mannopyranoside (**21**). Similarly, the coupling of **19** with **22**, obtained by catalytic hydrogenolysis of **7**, afforded methyl 4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**23**, 92%). Hydrolysis of the isopropylidene group in **23** and then acetylation gave **20**. The latter experiment showed that the isopropylidene acetal is a suitable protecting group in the presence of a strong Lewis acid such as trimethylsilyl triflate, if low temperatures, anhydrous conditions, and short reaction times can be used.

Mannosylation of aglycon **15** with **19**, under the conditions mentioned above, yielded the acetylated tetrasaccharide-glycoside **22** (94%), which was saponified to give methyl 3,6-di-*O*-α-D-mannopyranosyl-2-*O*-β-D-xylopyranosyl-β-D-mannopyranoside (**25**), the 500-MHz ^{1}H-n.m.r. spectrum of which is shown in Fig. 1.

EXPERIMENTAL

General methods. — Melting points (uncorrected) were determined on a Kofler apparatus. Optical rotations were measured at 20° with a Perkin–Elmer 241 polarimeter. Reactions were monitored by t.l.c. on Kieselgel 60 F_{254} (Merck) with detection by u.v. light and/or by charring with aqueous 50% sulfuric acid. Column chromatography was performed on Kieselgel 60 (Merck, 70–230 mesh). All solvents were distilled from appropriate drying agents. Concentrations were performed under reduced pressure at 40°. 500-MHz ^{1}H-n.m.r. spectra were recorded on a Bruker AM-500 spectrometer (Department of NMR Spectroscopy, Utrecht University) for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal 4,4-dimethyl-4-silapentane-1-sulfonate; indirectly to internal acetone, δ 2.225).

Methyl 3-O-benzyl-4,6-O-isopropylidene-β-D-glucopyranoside (**4**). — *p*-Toluenesulfonic acid (150 mg) was added to a stirred suspension of methyl 3-*O*-benzyl-β-D-glucopyranoside[23] (**3**; 10.0 g, 35.2 mmol) in 2,2-dimethoxypropane (37.8 mL, 308 mmol) at room temperature. The reaction was stopped after 20 min by adding sodium hydrogencarbonate. The mixture was diluted with dichloromethane (250 mL), and the organic layer was washed with water, dried, and concentrated. Column chromatography (3:1 toluene–acetone) of the syrupy residue gave **4** (10.7 g, 94%), $[\alpha]_D$ −16° (*c* 0.9, chloroform), R_F 0.51. ^{1}H-N.m.r. data ($CDCl_3$): δ 7.40–7.25 (m, 5 H, Ph), 4.90 and 4.75 (2 d, each 1 H, ^2J −11.7 Hz, PhCH_2O), 4.27 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.93 (dd, 1 H, H-6*e*), 3.79 (dd, 1 H, H-6*a*), 3.71 (dd, 1 H, H-4), 3.55 (s, 3 H, OMe), 3.55–3.45 (m, 2 H, H-2,3), 3.27 (m, 1 H, H-5), 2.41 (d, 1 H, HO-2), 1.48 and 1.43 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{17}H_{24}O_6$: C, 63.0; H, 7.5. Found: C, 62.9; H, 7.5.

Methyl 3-O-benzyl-4,6-O-isopropylidene-β-D-mannopyranoside (**6**). — A solution of **4** (6.5 g, 20 mmol) in 1:2 acetic anhydride–methyl sulfoxide (120 mL) was kept at room temperature for 18 h and then concentrated. Column chromatography (3:1 toluene–acetone) of the residue afforded **5** (5.7 g, 88%), $[\alpha]_D$ +59° (*c* 0.3, chloroform), ν_{max}^{KBr} 1740 cm^{-1} (C=O), R_F 0.42. To a vigorously stirred solution of **5** (6.0 g, 18.6 mmol) in 1:1 dichloromethane–methanol (100 mL) at 0° was added sodium borohydride (2.8 g, 73.7 mmol) in portions during 30 min. After stirring for 3 h at room temperature, the mixture was diluted with dichloromethane, washed with water, dried, and concentrated. T.l.c. (3:1 toluene–acetone) of the residue showed complete disappearance of **5** (R_F 0.42), traces of **4** (R_F 0.51), and a major product **6** (R_F 0.34). Column chromatography of the residue gave **6** (5.0 g, 83%) as a syrup, $[\alpha]_D$ −28° (*c* 0.8, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.38–7.28 (m, 5 H, Ph), 4.83 and 4.73 (2 d, each 1 H, ^2J −12.3 Hz, PhCH_2O), 4.36 ("s", 1 H, H-1), 4.14 (dd, 1 H, H-4), 4.06 ("d", 1 H, H-2), 3.92 (dd, 1 H, H-6*e*), 3.88 (dd, 1 H, H-6*a*), 3.54 (s, 3 H, OMe), 3.48 (dd, 1 H, H-3), 3.16 (m, 1 H, H-5), 2.49 (s, 1 H, HO-2), 1.52 and 1.44 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{17}H_{24}O_6$: C, 63.0; H, 7.5. Found: C, 63.1; H, 7.6.

Methyl 3-O-benzyl-4,6-O-isopropylidene-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**7**) *and methyl 3-O-benzyl-4,6-O-isopropylidene-2-O-(2,3,4-tri-O-acetyl-α-D-xylopyranosyl)-β-D-mannopyranoside* (**7a**). — A mixture of **6** (4.6 g, 14.2 mmol) and $Hg(CN)_2$ (5.4 g, 21.3 mmol) in 1:1 toluene–nitromethane (200 mL) was concentrated until 100 mL had been distilled off. The solution was cooled to room temperature, molecular sieves (4 Å, 10 g) were added, and the mixture was stirred for 30 min under argon. Then, 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (6.7 g, 19.8 mmol) was added in portions. After 2 h, t.l.c. (85:10:5 dichloromethane–ethyl acetate–acetone) revealed **7a** and **7** (R_F 0.60 and 0.56, respectively) in the ratio 1:9, but no **6**. The mixture was diluted with dichloromethane (100 mL), filtered, and concentrated, and a solution of the residue in dichloromethane (250 mL) was filtered, washed with aqueous 5% potassium iodide (3 × 50 mL) and water (3 × 50 mL), dried, and concentrated. Column chromatography (85:10:5 dichloromethane–ethyl acetate–acetone) of the residue gave **7a** (320 mg, 3.9%), m.p. 125° (from ethanol), $[\alpha]_D$ +55° (*c* 0.1, chloroform), then amorphous **7** (5.6 g, 68%), $[\alpha]_D$ −90° (*c* 0.3, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): **7**, δ 7.38–7.28 (m, 5 H, Ph), 5.17 (dd, 1 H, H-3′), 5.04 (dd, 1 H, H-2′), 4.93 (d, 1 H, $J_{1,2}$ 5.8 Hz, H-1′), 4.93 (m, 1 H, H-4′), 4.73 (s, 2 H, PhCH_2O), 4.28 (dd, 1 H, H-5′*e*), 4.24 ("s", 1 H, H-1), 4.10 ("d", 1 H, H-2), 4.06 (dd, 1 H, H-4), 3.86 (dd, 1 H, H-6*e*), 3.81 (dd, 1 H, H-6*a*), 3.43 (s, 3 H, OMe), 3.39 (dd, 1 H, H-3), 3.35 (dd, 1 H, H-5′*a*), 3.09 (m, 1 H, H-5), 2.04 (2×) and 2.03 (3 s, each 3 H, 3 OAc), 1.52 and 1.42 (2 s, each 3 H, CMe_2); **7a**, δ 7.40–7.30 (m, 5 H, Ph), 5.60 (dd, 1 H, H-3′), 5.33 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1′), 4.93 (m, 1 H, H-4′), 4.80 (dd, 1 H, H-2′), 4.65 and 4.57 (2 d, each 1 H, ^2J −12.5 Hz, PhCH_2O), 4.25 ("s", 1 H, H-1), 4.17 (dd, 1 H, H-4), 4.15 (dd, 1 H, H-5′*e*), 4.05–3.85 (m, 2 H, H-6*e*/*a*), 3.82 ("d", 1 H, H-2), 3.64 (dd, 1 H, H-5′*a*), 3.48 (s, 3 H, OMe), 3.37 (dd, 1 H, H-3), 3.12 (m, 1

H, H-5), 2.04, 2.03 and 1.96 (3 s, each 3 H, 3 OAc), 1.58 and 1.49 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{28}H_{38}O_{13}$: C, 57.7; H, 6.6. Found: **7**, C, 57.6; H, 6.6.

Methyl 3-O-benzyl-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**8**). — A solution of **7** (5.0 g, 8.6 mmol) in 1:1 acetic acid–water (60 mL) was kept at 80° for 30 min, then concentrated. Toluene (3 × 10 mL) was evaporated from the residue, which was crystallized from ethanol to give **8** (4.3 g, 92%), m.p. 124°, $[\alpha]_D$ −114° (*c* 0.5, chloroform).

Anal. Calc. for $C_{25}H_{34}O_{13}$: C, 55.4; H, 6.3. Found: C, 55.3; H, 6.3.

Methyl 4,6-di-O-acetyl-3-O-benzyl-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**9**). — Compound **8** (900 mg, 1.66 mmol) was treated overnight with 1:1 acetic anhydride–pyridine (20 mL). The mixture was concentrated using toluene (4 × 5 mL) and the residue was crystallized from ethanol to afford **9** (968 mg, 93%), m.p. 126°, $[\alpha]_D$ −153° (*c* 0.2, chloroform).

Anal. Calc. for $C_{29}H_{38}O_{15}$: C, 55.6; H, 6.1. Found: C, 55.7; H, 6.1.

Methyl 4,6-di-O-acetyl-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**10**). — A solution of **9** (950 mg, 1.52 mmol) in ethanol (10 mL), ethyl acetate (10 mL), and acetic acid (0.5 mL) was hydrogenolysed in the presence of 10% Pd/C (200 mg). When only one compound could be detected by t.l.c. (85:15 dichloromethane–acetone), which did not show any u.v. absorption, the catalyst was removed, and the filtrate concentrated using toluene. Recrystallization of the residue from ethanol gave **10** (725 mg, 89%), m.p. 180°, $[\alpha]_D$ −96° (*c* 0.5, chloroform), R_F 0.32 (85:15 dichloromethane–acetone).

Anal. Calc. for $C_{22}H_{32}O_{15}$: C, 49.3; H, 6.0. Found: C, 49.3; H, 5.9.

Methyl 3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**11**). — Compound **10** (200 mg, 0.37 mmol) was treated with 1:1 acetic anhydride–pyridine (8 mL) to give **11** (207 mg, 96%), m.p. 156° (from ethanol), $[\alpha]_D$ −124° (*c* 0.3, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.73 (d, 1 H, $J_{1,2}$ 6.3 Hz, Xyl H-1), 4.41 ("s", 1 H, β-Man H-1), 3.48 (s, 3 H, OMe), 2.12, 2.08, 2.06, 2.05 (2×) and 2.04 (6 s, each 3 H, 6 OAc).

Anal. Calc. for $C_{24}H_{34}O_{16}$: C, 49.8; H, 5.9. Found: C, 49.8; H, 6.0.

Methyl 2-O-β-D-xylopyranosyl-β-D-mannopyranoside (**12**). — Compound **11** (150 mg, 0.26 mmol) was deacetylated conventionally with methanolic M sodium methoxide (10 mL). After 24 h, the solution was neutralized with Dowex 50W (H^+) resin, filtered, and concentrated to give **12** (79 mg, 93%), $[\alpha]_D$ −74° (*c* 0.1, water), R_F 0.54 (2:1:1 1-butanol–methanol–water). ^{1}H-N.m.r. data (D_2O): δ 4.645 ("s", 1 H, β-Man H-1), 4.489 (d, 1 H, $J_{1,2}$ 7.7 Hz, Xyl H-1), 4.163 ("d", 1 H, β-Man H-2), 3.535 (s, 3 H, OMe), 3.429 (dd, 1 H, Xyl H-3), 3.348 (dd, 1 H, Xyl H-2), 3.268 (dd, 1 H, Xyl H-5*a*).

Methyl 3-O-benzyl-6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**13**). — A solution of **8** (2.0 g, 3.69 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (2.8 g, 6.81 mmol) in dry dichloromethane (40 mL) was stirred with molecular sieves (4

Å, 6 g) under argon for 30 min, followed by the addition of $HgBr_2$ (1.33 g, 3.69 mmol). After 1 h, the mixture was filtered through a bed of Celite, washed with aqueous 5% potassium iodide and water, dried, and concentrated. Column chromatography (83:17 dichloromethane–acetone) of the residue afforded **13** (2.2 g, 68%) as a glass, $[\alpha]_D$ −55° (*c* 1.0, chloroform), R_F 0.68. ^{1}H-N.m.r. data ($CDCl_3$): δ 7.41–7.29 (m, 5 H, Ph), 4.87 [d, 1 H, $J_{1,2}$ ~1 Hz, α-Man-(1→6) H-1], 4.85 (d, 1 H, $J_{1,2}$ 6.5 Hz, Xyl H-1), 4.29 ("s", 1 H, β-Man H-1), 4.81 and 4.43 (2 d, each 1 H, ^2J −11.5 Hz, PhCH_2O), 3.48 (s, 3 H, OMe), 2.19, 2.10, 2.08, 2.06 (2×), 2.04, and 1.98 (7 s, each 3 H, 7 OAc).

*Methyl 4-*O*-acetyl-3-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-2-*O*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-β-*D*-mannopyranoside* (**14**). — Compound **13** (2.0 g, 2.29 mmol) was treated with 1:1 acetic anhydride–pyridine (40 mL), as described for **9**, to give **14** (2.1 g, 98%), $[\alpha]_D$ −62° (*c* 0.4, chloroform).

Anal. Calc. for $C_{41}H_{54}O_{23}$: C, 53.8; H, 5.9. Found: C, 53.7; H, 5.9.

*Methyl 3,4-di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-2-*O*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-β-*D*-mannopyranoside* (**16**). — Compound **14** (1.8 g, 1.97 mmol) was hydrogenolysed in ethanol (20 mL), ethyl acetate (20 mL), and acetic acid (1 mL) in the presence of 10% Pd/C (350 mg), as described for **10**, to give **15** (1.58 g, 97%) as a glass, $[\alpha]_D$ −38° (*c* 0.5, chloroform), R_F 0.4 (8:2 dichloromethane–acetone). Compound **15** (412 mg, 0.5 mmol) was acetylated conventionally with 1:1 acetic anhydride–pyridine (10 mL) to give **16** (420 mg, 97%), m.p. 194° (from ethanol), $[\alpha]_D$ −44° (*c* 0.3, chloroform), R_F 0.81 (8:2 dichloromethane–acetone). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.81 [d, 1 H, $J_{1,2}$ 1.7 Hz, α-Man-(1→6) H-1], 4.72 (d, 1 H, $J_{1,2}$ 5.7 Hz, Xyl H-1), 4.43 ("s", 1 H, β-Man H-1), 3.50 (s, 3 H, OMe), 2.15, 2.12, 2.08, 2.06, 2.05 (2×), 2.04, 2.01, and 1.98 (9 s, each 3 H, 9 OAc).

Anal. Calc. for $C_{36}H_{50}O_{24}$: C, 49.9; H, 5.8. Found: C, 49.8; H, 5.8.

*Methyl 6-*O*-α-*D*-mannopyranosyl-2-*O*-β-*D*-xylopyranosyl-β-*D*-mannopyranoside* (**17**). — Compound **16** (220 mg, 0.25 mmol) was deacetylated with methanolic M sodium methoxide (15 mL). Conventional work-up gave amorphous **17** (112 mg, 90%), $[\alpha]_D$ −28° (*c* 0.3, water), R_F 0.46 (2:1:1 1-butanol–methanol–water). ^{1}H-N.m.r. data (D_2O): δ 4.906 [d, 1 H, $J_{1,2}$ 1.8 Hz, α-Man-(1→6) H-1], 4.665 ("s", 1 H, β-Man H-1), 4.490 (d, 1 H, $J_{1,2}$ 7.8 Hz, Xyl H-1), 4.169 ("d", 1 H, β-Man H-2), 4.004 [dd, 1 H, α-Man-(1→6) H-2], 3.524 (s, 3 H, OMe), 3.438 (dd, 1 H, Xyl H-3), 3.366 (dd, 1 H, Xyl H-2), 3.268 (dd, 1 H, Xyl H-5*a*).

*O-(2,3,4,6-Tetra-*O*-acetyl-α-*D*-mannopyranosyl)trichloroacetimidate* (**19**). — To a stirred solution of 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose (3.9 g, 10 mmol) in *N,N*-dimethylformamide (10 mL) was added[25] hydrazine acetate (1.1 g, 12 mmol). The temperature was raised to 50°, and the solution became clear within 10 min, when t.l.c. [1:1 light petroleum (b.p. 40–70°)–ethyl acetate] showed that the reaction was complete. Then the mixture was cooled to room temperature, diluted with ethyl acetate, washed with aqueous 5% sodium chloride, dried ($MgSO_4$), and concentrated. Column chromatography [1:1 light petroleum (b.p. 40–70°)–ethyl acetate] of the residue gave syrupy **18** (2.9 g, 83%), $[\alpha]_D$ +19° (*c* 0.7,

chloroform), R_F 0.32. To a solution of **18** (1.04 g, 3 mmol) and trichloroacetonitrile (1.8 mL, 18 mmol) in dichloromethane (9 mL) was added sodium hydride (144 mg, 6 mmol) at 0°. The mixture was stirred at room temperature for 20 min and then passed through a short column of Kieselgel (1:1 light petroleum–ethyl acetate) to give **19** (790 mg, 54%), $[\alpha]_D$ +45° (*c* 0.4, chloroform), R_F 0.65. ^{1}H-N.m.r. data ($CDCl_3$): δ 8.79 (s, 1 H, NH), 6.28 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 2.20, 2.09, 2.07 and 2.01 (4 s, each 3 H, 4 OAc).

*Methyl 4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-2-*O*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-β-*D*-mannopyranoside* (**20**). — A solution of **10** (107 mg, 0.2 mmol) and **19** (118 mg, 0.24 mmol) in dry dichloromethane (4 mL) was stirred in the presence of molecular sieves (4 Å, 1 g) under argon for 30 min and then cooled to −30°, and a solution of trimethylsilyl triflate (44 μL, 0.24 mmol) in dry dichloromethane (2 mL) was added dropwise. The temperature was kept below −30° and, after 10 min, pyridine was added, the mixture was filtered through a bed of Celite, and co-concentrated with toluene. T.l.c. (85:15 dichloromethane–acetone) revealed no **10** (R_F 0.32) and **19** (R_F 0.71), but one product (R_F 0.45). Column chromatography (85:15 dichloromethane–acetone) of the crude product gave amorphous **20** (152 mg, 88%), $[\alpha]_D$ −56° (*c* 0.2, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.95 [d, 1 H, $J_{1,2}$ ~1 Hz, α-Man-(1→3) H-1], 4.92 (d, 1 H, $J_{1,2}$ 5.9 Hz, Xyl H-1), 4.35 ("s", 1 H, β-Man H-1), 3.48 (s, 3 H, OMe), 2.14, 2.12, 2.11 (2×), 2.08, 2.06, 2.05, 2.04, and 2.00 (9 s, each 3 H, 9 OAc).

Anal. Calc. for $C_{36}H_{50}O_{24}$: C, 49.9; H, 5.8. Found: C, 49.8; H, 5.8.

*Methyl 3-*O*-α-*D*-mannopyranosyl-2-*O*-β-*D*-xylopyranosyl-β-*D*-mannopyranoside* (**21**). — Conventional deacetylation of **20** (100 mg, 0.12 mmol) in methanolic M sodium methoxide (5 mL) gave **21** (53 mg, 94%) as a glass, $[\alpha]_D$ −16° (*c* 0.2, water), R_F 0.47 (2:1:1 1-butanol–methanol–water). ^{1}H-N.m.r. data (D_2O): δ 5.144 [d, 1 H, $J_{1,2}$ 2.0 Hz, α-Man-(1→3) H-1], 4.661 ("s", 1 H, β-Man H-1), 4.484 (d, 1 H, $J_{1,2}$ 8.0 Hz, Xyl H-1), 4.182 ("d", 1 H, β-Man H-2), 4.039 [dd, 1 H, α-Man-(1→3) H-2], 3.537 (s, 3 H, OMe), 3.427 (dd, 1 H, Xyl H-3), 3.328 (dd, 1 H, Xyl H-2), 3.244 (dd, 1 H, Xyl H-5*a*).

*Methyl 4,6-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-β-*D*-mannopyranoside* (**22**). — Compound **7** (500 mg, 0.86 mmol) was hydrogenolysed in ethanol (10 mL) in the presence of 10% Pd/C (100 mg), as described for **10**, to yield **22** (388 mg, 92%), m.p. 214–216° (from ethanol), $[\alpha]_D$ −84° (*c* 0.3, chloroform), R_F 0.47 (87:13 dichloromethane–acetone).

Anal. Calc. for $C_{21}H_{32}O_{13}$: C, 51.2; H, 6.5. Found: C, 51.2; H, 6.5.

*Methyl 4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-2-*O*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-β-*D*-mannopyranoside* (**23**). — Compound **22** (148 mg, 0.3 mmol) was glycosylated with **19** (148 mg, 0.3 mmol) in the presence of trimethylsilyl triflate (55 μL, 0.3 mmol) in dry dichloromethane at −30° as described for **20**. The reaction was complete after 10 min, and, after work-up, t.l.c. revealed only one product. Column chromatography (85:15 dichloromethane–acetone) afforded **23** (227 mg, 92%), $[\alpha]_D$ −39° (*c* 0.4, chloroform), R_F

0.67. Compound **23** (60 mg) was hydrolysed with 1:1 acetic acid–water (10 mL) as described for **8**, and acetylated with 1:1 acetic anhydride–pyridine (10 mL) to give **20** (56 mg, 88%). For ^{1}H-n.m.r. data of **20**, see above.

Methyl 4-O-acetyl-3,6-di-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-mannopyranoside (**24**). — Compound **15** (264 mg, 0.32 mmol) was glycosylated with **19** (156 mg, 0.32 mmol) in the presence of trimethylsilyl triflate (58 μL, 0.32 mmol) in dry dichloromethane at −30° as described for **20**. The reaction was stopped after 10 min by the addition of pyridine. Only one product could be detected by t.l.c. Column chromatography (8:2 dichloromethane–acetone) afforded amorphous **24** (347 mg, 94%), $[\alpha]_D$ −24° (*c* 0.2, chloroform), R_F 0.47. ^{1}H-N.m.r. data ($CDCl_3$): δ 4.97 [d, 1 H, $J_{1,2}$ 1.2 Hz, α-Man-(1→3) H-1], 4.92 (d, 1 H, $J_{1,2}$ 5.1 Hz, Xyl H-1), 4.80 [d, 1 H, $J_{1,2}$ 1.5 Hz, α-Man-(1→6) H-1], 4.38 ("s", 1 H, β-Man H-1), 3.50 (s, 3 H, OMe), 2.15, 2.14, 2.13, 2.12, 2.11, 2.09, 2.06 (2×), 2.04, 2.02, 2.00, and 1.98 (12 s, each 3 H, 12 OAc).

Methyl 3,6-di-O-α-D-mannopyranosyl-2-O-β-D-xylopyranosyl-β-D-mannopyranoside (**25**). — Conventional deacetylation of **24** (200 mg, 0.17 mmol) yielded **25** (107 mg, 95%), $[\alpha]_D$ +5° (*c* 0.2, water), R_F 0.37 (2:1:1 1-butanol–methanol–water). ^{1}H-N.m.r. data (D_2O): δ 5.138 [d, 1 H, $J_{1,2}$ 1.8 Hz, α-Man-(1→3) H-1], 4.900 [d, 1 H, $J_{1,2}$ 1.8 Hz, α-Man-(1→6) H-1], 4.681 ("s", 1 H, β-Man H-1), 4.474 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), 4.197 ("d", 1 H, β-Man H-2), 4.035 [dd, 1 H, α-Man-(1→3) H-2], 4.010 [dd, 1 H, α-Man-(1→6) H-2], 3.523 (s, 3 H, OMe), 3.425 (dd, 1 H, Xyl H-3), 3.360 (dd, 1 H, Xyl H-2), 3.249 (dd, 1 H, Xyl H-5*a*).

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