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Note

Synthesis of 6′-*O*-phosphorylated *O*-α-D-mannopyranosyl-(1→3)- and -(1→6)-α-D-mannopyranosides

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D-Mannose 6-phosphate is known as the essential component of a recognition marker involved in the intracellular targeting of newly biosynthesized lysosomal enzymes to the lysosomes[1,2]. The recognition marker is carried on the "high-mannose" asparagine-linked oligosaccharide chains of these enzymes which may contain as many as nine mannose residues, more than one of which may be 6-*O*-phosphorylated in any given structure. The largest of these structures (**1**) is shown where the D-mannose residues on which 6-phosphoric mono- or di-esters have been located are indicated by an asterisk[3–5]. Targeting of enzymes to the lysosomes is dependent on the recognition and binding of the phosphorylated oligosaccharide chains by specific receptors.

In order to determine the structural features required for specific recognition by these receptors, we became involved in a synthetic effort to produce fourteen phosphorylated di- to penta-mannosides corresponding to partial structures of **1**, six of which we have already reported[6,7]. We describe herein the preparation of the 6′-*O*-phosphorylated *O*-α-D-mannopyranosyl-(1→3)- and -(1→6)-α-D-mannopyranoside disaccharides **2** and **3**. These disaccharides were synthesized as their 8-methoxycarbonyloctyl glycosides to allow their eventual attachment to proteins and solid supports.

The synthesis of **2** and **3** included the preparation of the selectively protected 8-methoxycarbonyloctyl α-D-mannopyranosides **4** and **5** having the required OH-3 and -6, respectively, free for glycosylation. The preparation of **4** (by benzylidenation of 8-methoxycarbonyloctyl α-D-mannopyranoside followed by selective 2-*O*-benzylation) and the synthesis of **5** (from 8-methoxycarbonyloctyl 3,4-di-*O*-benzyl-α-D-mannopyranoside by 2-*O*-benzylation of its 6-*O*-*tert*-butyldimethylsilyl derivative) have already been reported[7].

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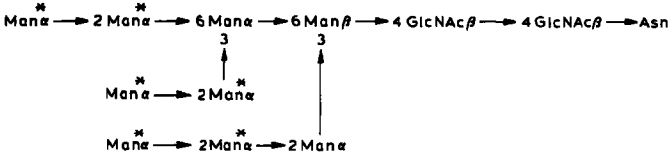
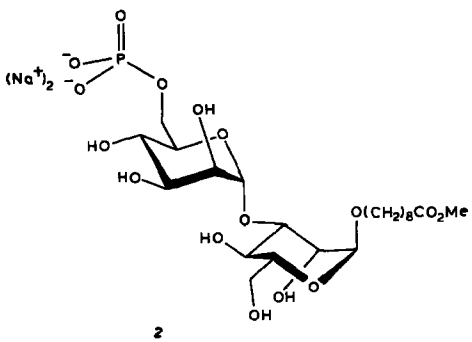
Glycosylation of **4** (Ref. 7) with 2,3,4-tri-*O*-acetyl-6-*O*-diphenoxyphosporyl-α-D-mannopyranosyl bromide (**7**, prepared[7] by reaction of its readily available β-acetate[8] **6** with hydrogen bromide in glacial acetic acid) in the presence of silver trifluoromethanesulfonate and *N,N,N',N'*-tetramethylurea in 1,2-dichloroethane provided the protected α-(1→3)-linked disaccharide phosphate **8** in 77% yield. That both glycosidic linkages in **8** had the α-D configuration was evident[9,10] from the ^{1}H-coupled ^{13}C-n.m.r. spectrum which showed the signals for the two anomeric carbon atoms at δ 99.02 ($^1J_{C,H}$ 170 Hz) and 98.77 ($^1J_{C,H}$ 170 Hz), along with the signal for the benzylidene acetal carbon atom at δ101.32. Reaction of **5** with **7** under the same conditions provided the phosphorylated α-(1→6)-linked disaccharide **9** (78%), which showed the corresponding signals for the anomeric carbon atoms at δ 97.84 ($^1J_{C,H}$ 167 Hz) and 97.61 ($^1J_{C,H}$ 172 Hz).

Deprotection of both **8** and **9** involved sequential hydrogenolysis of the benzyl ethers in the presence of 5% palladium-on-charcoal, removal of the phosphate phenyl protecting groups by hydrogenolysis in the presence of Adam's catalyst (PtO_2) and *O*-deacetylation with sodium methoxide in methanol to provide, after Bio-Gel P-2 chromatography and ion exchange, the target 6'-disodium phosphates **2** (80% from **8**) and **3** (78% from **9**). The structures of the 6'-*O*-phosphorylated disaccharides **2** and **3** are supported by their ^{1}H-, ^{31}P-, and ^{13}C-n.m.r. spectra which include the expected ^{13}C–^{31}P coupling constants, $^3J_{C_{5'},P}$ 7 and $^2J_{6',P}$ ~5 Hz.



Manα = α-D-Manp
Manβ = β-D-Manp
GlcNAcβ = β-D-GlcpNAc
* = 6-$(PO_4)^{2-}$

1



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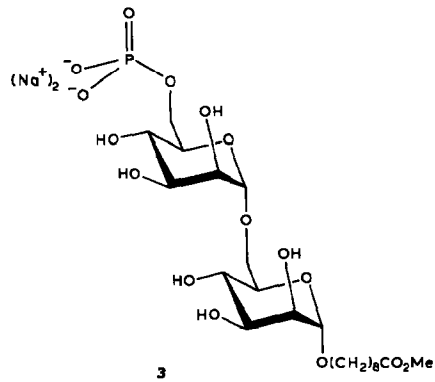

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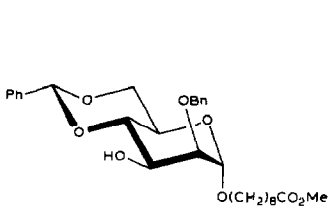

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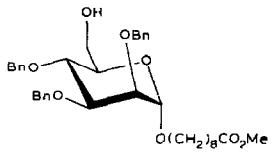

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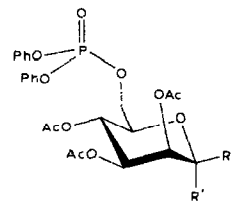
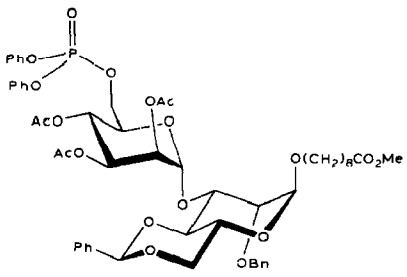


6 R = OAc, R' = H
7 R = H, R' = Br



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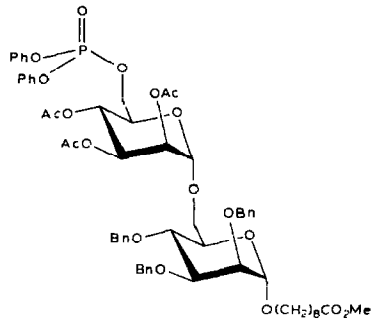

9

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 241 polarimeter at ambient temperatures (22 ±2°). T.l.c. was performed on precoated plates of silica gel (60-F_{254}, E. Merck, Darmstadt) with detection by quenching of fluorescence, or by charring, or both, after spraying with 5% H_2SO_4 in ethanol. Column chromatography was performed on Silica gel Merck 60 (30–63 μm); solvent *A* is 2:3 ethyl acetate–hexane, solvent *B* is 4:1 2-propanol–water. ^{1}H-N.m.r. spectra were recorded at 360 MHz (Bruker WM-360) with either tetramethylsilane (Me_4Si, δ 0 in $CDCl_3$) or acetone (δ 2.225 in D_2O) as internal standards at ambient temperature. ^{13}C-N.m.r. spectra were recorded at 100 MHz (Bruker WH-400) with either internal Me_4Si (δ 0 in $CDCl_3$) or external 1,4-dioxane (δ 67.4 in D_2O) as reference standards. ^{31}P-N.m.r. spectra were recorded at 145 MHz (Bruker WM-360) with external 85% H_3PO_4 in D_2O as reference (δ 0). Unless otherwise noted, all reactions were carried out at ambient temperature and, in the processing of reaction mixtures, solutions in organic solvents were washed with equal volumes of aqueous solutions. The microanalyses were carried out by the Analytical Services Laboratory of this department.

8-Methoxycarbonyloctyl 2-O-benzyl-4,6-O-benzylidene-3-O-(2,3,4-tri-O-acetyl-6-O-diphenoxyphosporyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**8**). — A mixture of **4** (221 mg, 0.42 mmol) and silver trifluoromethanesulfonate (645 mg, 2.51 mmol) was dried *in vacuo* in the presence of P_2O_5 for 5 h at 20° and

dissolved in 1,2-dichloroethane (2 mL) under Ar. To this stirred mixture was added *N,N,N',N'*-tetramethylurea (150 μL, 1.25 mmol) and bromide[7] **7** (0.84 mmol) in 1,2-dichloroethane (1.0 mL). After both 5 h and 20 h, further additions of *N,N,N',N'*-tetramethylurea (150 μL) and bromide **7** (0.84 mmol) were made. The mixture was then stirred for an additional 36 h and diluted with 1,2-dichloroethane (10 mL). 2,4,6-Trimethylpyridine (330 μL), followed by silver trifluoromethanesulfonate (215 mg, 0.84 mmol) were then added to remove any unreacted **7** and, after 0.5 h, excess Ag was precipitated by addition of tetraethylammonium bromide (175 mg). Solids were removed by filtration and washed with dichloromethane (50 mL). The filtrate was then washed twice with saturated aqueous $NaHCO_3$ and twice with water before evaporation to a yellow oil. Purification by chromatography provided **8** as a clear syrup (336 mg, 77%), $[\alpha]_D^{22}$ +28.8° (*c* 0.99, chloroform); R_F 0.20 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.618 (s, 1 H, $C_6H_5CHO_2$), 5.462–5.425 (m, 2 H, H-2',3'), 5.255 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.845 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.655 (s, 3 H, OCH_3), 3.595 and 3.350 (each m, 1 H, $OCHHCH_2$), 2.034, 1.996, and 1.989 (each s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 137.99 and 137.48 (quat. arom.), 101.32 ($C_6H_5CHO_2$), 99.02 ($^1J_{C,H}$ 170 Hz), 98.77 ($^1J_{C,H}$ 170 Hz), 69.67 (d, $^3J_{C,P}$ 8 Hz, C-5'), 67.36 (d, $^2J_{C,P}$ 5 Hz, C-6'), 64.10 (C-6), 51.35 (OCH_3), and 34.11 (CH_2CO_2); ^{31}P-n.m.r. ($CDCl_3$): δ −12.61.

Anal. Calc. for $C_{54}H_{65}O_{19}P$: C, 61.82; H, 6.25. Found: C, 61.85; H, 6.46.

*8-Methoxycarbonyloctyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-diphenoxyphosphoryl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**9**). — Reaction of the alcohol[7] **5** (175 mg, 0.28 mmol) with the bromide **7** (6 equiv.) added in three equal portions, in the presence of silver trifluoromethanesulfonate and *N,N,N',N'*-tetramethylurea, exactly as described for the preparation of **8**, gave, after chromatographic purification using 2:3 ethyl acetate–hexane, **9** as a clear syrup (250 mg, 78%), $[\alpha]_D^{22}$ +64° (*c* 0.32, chloroform); R_F 0.23 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.385–5.255 (m, 3 H, H-2',3',4'), 4.940 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.773 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.655 (s, 3 H, OCH_3), 3.323 (m, 1 H, $OCHHCH_2$), 2.028, 1.970, and 1.934 (each s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 138.63 and 138.57 (2 C) (quat. arom.), 97.84 ($^1J_{C,H}$ 167 Hz) and 97.61 ($^1J_{C,H}$ 172 Hz, C-1,1'), 67.42 (d, $^2J_{C,P}$ 5 Hz, C-6'), 51.33 (OCH_3), and 34.08 (CH_2COO); ^{31}P-n.m.r. ($CDCl_3$): δ −12.75.

Anal. Calc. for $C_{61}H_{73}O_{19}P$: C, 64.20; H, 6.45. Found: C, 64.10; H, 6.33.

*8-Methoxycarbonyloctyl 3-*O*-(disodium α-*D*-mannopyranosyl 6-phosphate)-α-*D*-mannopyranoside* (**2**). — The benzyl and benzylidene groups of **8** (231 mg, 0.22 mmol) were hydrogenolyzed in the presence of 5% Pd–C (115 mg) in 95% ethanol (5 mL) at 0.1 MPa pressure of H_2 for 15 h, by which time t.l.c. showed a major component (~90%) with R_F 0.90 (60:35:6 chloroform–methanol–water) and a more polar product (R_F 0.65 in the same solvent), presumably the monophenyl ester. The catalyst was removed by filtration and washed with 95% ethanol (20 mL), and the filtrate was evaporated. The residue was dissolved in 95% ethanol (3 mL) and stirred with Adam's catalyst (PtO_2, 46 mg) for 3 h under H_2 (0.1 MPa) by

which time t.l.c. showed the presence of a single, non-u.v. absorbing component with R_F 0.50 (60:35:6 chloroform–methanol–water). The catalyst was removed by filtration and washed with 95% ethanol; the filtrate was evaporated, dried overnight (P_2O_5), and the residue redissolved in anhydrous methanol. The solution was then rendered basic by addition of sodium methoxide, kept at 0° for 15 h, and made neutral with IRC-50 (H^+) resin, following which the latter was removed and the solvent evaporated. The residue was passed through a column of Bio-Gel P-2 (200–400 mesh, 2 cm i.d. × 50 cm) with 10% ethanol as eluent. The carbohydrate-containing fractions were pooled, concentrated, and converted into the sodium salt by passage through Dowex 50-X8 (Na^+; 5 mL) resin. Lyophilization of the residue provided **2** as a white powder (112 mg, 80%), $[\alpha]_D^{22}$ +53° (*c* 0.30, water); R_F 0.45 (solvent *B*); ^{1}H-n.m.r. (D_2O): δ 5.074 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.832 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 4.139 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 4.054 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.011 (m, 2 H, $J_{5,6}$ 3.0, $^3J_{H,P}$ 6.8 Hz, H-6'a,6'b), 3.686 (s, 3 H, OCH_3), 3.540 (m, 1 H, OC*H*HCH$_2$), and 2.389 (t, 2 H, *J* 7.5 Hz, CH_2CO$_2$); ^{13}C-n.m.r. (D_2O): δ 171.60 (*C*O$_2$CH$_3$), 103.47 ($^1J_{C,H}$ 168 Hz, C-1'), 100.71 ($^1J_{C,H}$ 170 Hz, C-1), 80.55 (C-3), 73.66, 73.58 (d, $^3J_{C,P}$ 7 Hz, C-5'), 71.08, 71.01, 70.31, 68.81, 67.32, 66.68, 63.93 (br., $^2J_{C,P}$ <5 Hz, C-6'), 61.78 (C-6), 52.91 (OCH_3), 34.57 (*C*H$_2$CO$_2$), 29.34, 29.04 (2 C), 28.99 (2 C), 26.09, and 25.11; ^{31}P-n.m.r. (D_2O): δ 6.42.

Anal. Calc. for $C_{22}H_{39}Na_2O_{16}P \cdot 1.5\ H_2O$: C, 39.82; H, 6.38. Found: C, 39.87; H, 6.41.

*8-Methoxycarbonyloctyl 6-O-(disodium α-*D*-mannopyranosyl 6-phosphate)-α-*D*-mannopyranoside* (**3**). — Deprotection of **9** (120 mg, 0.11 mmol) was accomplished, as described for the preparation of **2**, with 5% Pd–C (60 mg) followed by Adam's catalyst (24 mg). After deacetylation and ion-exchange, the product was obtained as a white lyophilized powder (52.5 mg, 78%), $[\alpha]_D^{22}$ +35.3° (*c*, 0.32, water); R_F 0.38 (solvent *B*); ^{1}H-n.m.r. (D_2O): δ 4.890 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.842 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.061 (m, 2 H, H-6'a,6'b), 3.963 (dd, $J_{2',3'}$ 3.0 Hz, H-2'), 3.923 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.682 (s, OCH_3), 3.550 (m, 1 H, OC*H*HCH$_2$), and 2.386 (t, 2 H, *J* 7.5 Hz, CH_2COO); ^{13}C-n.m.r. (D_2O): δ 171.95 (*C*O$_2$CH$_3$), 100.84 ($^1J_{C,H}$ 170 Hz) and 100.60 ($^1J_{C,H}$ 169 Hz) (C-1,1'), 72.79 (d, $^3J_{C,P}$ 7.5 Hz, C-5'), 71.89, 71.84, 71.35, 71.06, 70.98, 68.91, 67.43, 67.07, 66.67, 64.36 (d, $^2J_{C,P}$ 5 Hz, C-6'), 53.01 (OCH_3), 34.67 (*C*H$_2$CO$_2$), 29.46, 29.23 (3 C), 26.30, and 25.27; ^{31}P-n.m.r. (D_2O): δ 5.00.

Anal. Calc. for $C_{22}H_{39}Na_2O_{16}P \cdot H_2O$: C, 40.37; H, 6.31. Found: C, 40.11; H, 6.21.

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REFERENCES

1 G. G. SAHAGIAN, *Biol. Cell*, 51 (1984) 207–214.
2 L. LANG, M. REITMAN, J. TANG, R. M. ROBERTS, AND S. KORNFELD, *J. Biol. Chem.*, 259 (1984) 14663–14671.
3 A. VARKI AND S. KORNFELD, *J. Biol. Chem.*, 255 (1980) 10847–10858.
4 A. VARKI AND S. KORNFELD, *J. Biol. Chem.*, 258 (1983) 2808–2818.
5 N. NATOWICZ, J. U. BAENZIGER, AND W. S. SLY, *J. Biol. Chem.*, 257 (1982) 4412–4420.
6 O. P. SRIVASTAVA AND O. HINDSGAUL, *Carbohydr. Res.*, 155 (1986) 57–82.
7 O. P. SRIVASTAVA AND O. HINDSGAUL, *Carbohydr. Res.*, 161 (1987) 195–210.
8 T. POSTERNAK AND J. P. ROSSELET, *Helv. Chim. Acta*, 36 (1953) 1614–1623.
9 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
10 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.