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Note

Selective phosphorylation of the primary hydroxyl groups of methyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside*

RAKESH K. JAIN, SAEED A. ABBAS, AND KHUSHI L. MATTA*

Department of Gynecologic Oncology, Roswell Park Memorial Institute, New York State Department of Health, 666 Elm Street, Buffalo, New York 14263 (U.S.A.)

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In a recent communication in this series[2], we briefly described some attempts to synthesize D-*manno*-disaccharides having phosphoric ester groups at O-6 or O-6′, or at both. Such *manno*-oligosaccharides, which are known to occur as parts of lysosomal enzymes[3], were required in a study related to lysosomal-enzyme targeting. We now describe in more detail some of the synthetic targets we have hitherto accomplished.

A common intermediate, namely, methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside[4] (**1**) was utilized for the synthesis of all of the three disaccharides herein described. Cleavage of the benzylidene group of **1** in hot, 50% aqueous acetic acid gave known methyl 3-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside[4] (**2**). Compound **2** was treated with two molar equivalents of diphenyl phosphorochloridate in pyridine at 0° to give, in high (~93%) yield, the monophosphorylated derivative **5**, the ^{1}H- and ^{13}C-n.m.r. spectra (Table I) of which were in accord with the structure expected.

O-Deacetylation of **1** in methanolic sodium methoxide afforded methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside (**3**) as an amorphous solid whose ^{1}H- and ^{13}C-n.m.r. spectra were in support of the structure assigned. Hydrogenolytic cleavage of the benzylidene and benzyl groups of **3** furnished known methyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside[5] (**4**).

Selective phosphorylation of **3**, in a manner analogous to that described for **2** (to give **5**), gave **6** whose identity as a monophosphorylated derivative of **3** was clearly evidenced by its ^{1}H- and ^{13}C-n.m.r. spectral data.

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*To whom correspondence should be addressed.

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TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a]

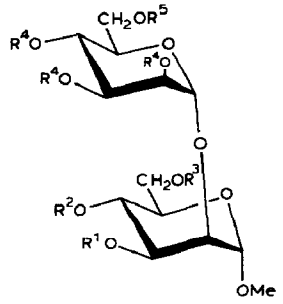
Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	OCH_3	CH_3CO	C_6H_5CH
[b]	101.89	71.68	71.03	67.88	73.63	62.08							55.84		
[c]	101.77	71.31	70.71	67.09	72.00 (7)	66.00 (5)							55.72		
3	100.69	78.38	78.17	78.57	68.73	70.79	101.35	71.38	72.72	68.64	72.90	63.85	54.78		101.57
4[d]	100.1	79.3	70.8	67.8	73.4	61.9	103.0	71.7	71.7	67.8	74.1	61.8	55.7		
5	99.16	78.92	78.23	66.07	71.22 (7)	68.23 (6)	99.56	68.93	68.93	66.26	69.29	62.51	54.80	20.74, 20.59	
6	100.69	78.18	76.93	78.82	66.97	70.29	101.39	71.68	72.77	63.75	71.33 (8)	68.57 (6.5)	54.71		101.89
7	101.83	79.36	70.20	65.84	71.18 (7)	68.94 (4)	102.32	70.27	70.27	67.16	71.18 (7)	68.94 (4)	54.51		
8	99.33	79.67	71.46	65.44	70.23 (7)	65.79 (5)	99.64	68.83	69.17	67.12	69.75	62.77	55.07	20.74, 18.22	
9	100.53	79.18	70.96	67.27	73.15 (7)	63.77 (4)	103.18	71.18	71.18	67.80	74.30	62.11	55.91		
10	100.30	79.57	70.92	67.38	73.60	61.91	103.31	71.20	71.20	67.90	73.20 (8)	65.21 (5)	55.99		
11	100.55	78.98	71.01	67.24	73.38 (8)	64.30 (5)	103.23	71.01	71.01	67.24	72.97 (7.5)	64.67 (4)	56.00		

[a]For solutions in D_2O with Me_4Si as the external standard, except for compounds **3**, **5**, **6–8**, the solvent was $CDCl_3$, with Me_4Si as the internal standard. Aromatic and carbonyl resonances are not shown. Where applicable, $^2J_{C,OP}$ and $^3J_{C,COP}$ in Hz are shown in parenthesis. [b]Methyl α-D-mannopyranoside[6]. [c]Methyl α-D-mannopyranoside 6-phosphate[6]. The chemical shifts of the last-mentioned two compounds are included for comparison. [d]Values taken from ref. 5.

Reaction of **4** with four molar equivalents of diphenyl phosphorochloridate in pyridine produced the disaccharide derivative **7**. That **7** was a diphosphorylated derivative of **4** could readily be shown by its ^{1}H-n.m.r. spectrum, which contained a 20-proton multiplet at δ 7.10–7.35, attributable to the phosphoric ester phenyl protons. In the ^{13}C-n.m.r. spectrum of **7**, the noticeable down-field shifts for the resonances of C-6 and C-6′, which also showed typical $^2J_{C,P}$ values of ~4 Hz each, were indicative of O-6 and O-6′ being the sites of phosphorylation.

Hydrogenolytic cleavage of the benzyl and then the phenyl groups of **5** in the presence of palladium-on-carbon and platinum oxide, respectively, gave an acetylated disaccharide derivative that was isolated as its disodium salt **8**. *O*-Deacetylation of **8** in methanolic sodium methoxide gave, after purification in a column of Bio-Gel P-2 and stirring in water with Amberlite IR-120 (Na^+) cation-exchange resin, disodium (methyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside) 6-phosphate (**9**).

On hydrogenolysis, exactly as described for **5** (to give **8**), **6** afforded, in 80% yield, methyl 2-*O*-(disodium α-D-mannopyranosyl 6-phosphate)-α-D-mannopyranoside (**10**), as the monohydrate. On similar treatment, **7** furnished disodium [methyl 2-*O*-(disodium α-D-mannopyranosyl 6-phosphate)-α-D-mannopyranoside] 6-phosphate (**11**), as the hemihydrate.



1 R^1 = Bn; R^2, R^3 = PhCH; R^4 = R^5 = Ac
2 R^1 = Bn; R^2 = R^3 = H; R^4 = R^5 = Ac
3 R^1 = Bn; R^2, R^3 = PhCH; R^4 = R^5 = H
4 R^1 = R^2 = R^3 = R^4 = R^5 = H
5 R^1 = Bn; R^2 = H; R^3 = $PO(OPh)_2$; R^4 = R^5 = Ac
6 R^1 = Bn; R^2, R^3 = PhCH; R^4 = H; R^5 = $PO(OPh)_2$
7 R^1 = R^2 = R^4 = H; R^3 = R^5 = $PO(OPh)_2$
8 R^1 = R^2 = H; R^3 = $PO(ONa)_2$; R^4 = R^5 = Ac
9 R^1 = R^2 = R^4 = R^5 = H; R^3 = $PO(ONa)_2$
10 R^1 = R^2 = R^3 = R^4 = H; R^5 = $PO(ONa)_2$
11 R^1 = R^2 = R^4 = H; R^3 = R^5 = $PO(ONa)_2$

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at ~25° with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on plates coated with a 0.2-mm layer of Silica gel 60F-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light, or by spraying the plates with 5% H_2SO_4 in ethanol and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh), and solvent *A* was 9:1 (v/v) chloroform–methanol. N.m.r. spectra were recorded at 25° with a Varian XL-100 instrument, ^{1}H-n.m.r. spectra at 100 MHz, and ^{13}C-n.m.r. spectra at 25.2 MHz in the F.t. mode; the positions of the peaks (δ) are expressed from the Me_4Si signal. Organic solutions were generally dried with anhydrous Na_2SO_4. Pyridine was distilled and stored over KOH. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A., and Galbraith Laboratories, Inc., Knoxville, Tennessee, U.S.A.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-α-D-mannopyranosyl-α-D-mannopyranoside (**3**). — This compound was obtained by *O*-deacetylation of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside[4] (**1**) in methanolic sodium methoxide, followed by column-chromatographic purification on silica gel (solvent *A*), amorphous, $[\alpha]_D^{25}$ +25° (*c* 2.1, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 3.23 (s, 3 H, OMe), 5.53 (s, 1 H, PhCH), and 7.10–7.58 (m, 10 H, arom.).

Anal. Calc. for $C_{27}H_{34}O_{11}$: C, 60.67; H, 6.37. Found: C, 60.89; H, 6.42.

Methyl 3-O-benzyl-6-O-(diphenoxy)phosphoryl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**5**). — To a cold (0°), stirred solution of methyl 3-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside[4] (**2**; 1.8 g, 2.9 mmol) in dry pyridine (40 mL) was added diphenyl phosphorochloridate (1.25 mL, 6 mmol), and the stirring was continued for 1 h at 0°. The mixture was allowed to gradually warm to room temperature, and the stirring was continued for an additional 1 h. Water (10 mL) was then added, the mixture evaporated under reduced pressure, and the residue dissolved in chloroform. The solution was successively washed with water, 5% HCl, saturated $NaHCO_3$, and water, dried, and evaporated, and the residue applied to a column of silica gel. On elution with 2% acetone in chloroform, evaporation of the fractions corresponding (t.l.c., 9:1 chloroform–acetone) to **5** afforded an amorphous compound (2.3 g, 93%), $[\alpha]_D^{25}$ +31° (*c* 1.9, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 1.86–2.16 (cluster of s, 12 H, OAc), 3.30 (s, 3 H, OMe), and 7.40–7.36 (m, 15 H, arom.).

Anal. Calc. for $C_{40}H_{49}O_{18}P \cdot H_2O$: C, 55.42; H, 5.88; P, 3.57. Found: C, 55.35; H, 5.70; P, 3.58.

Disodium [methyl 2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside] 6-phosphate (**8**). — A solution of **5** (2.6 g) in 95% ethanol (75 mL) was shaken under H_2 at ~345 kPa for 16 h at room temperature in the presence of

10% Pd–C (0.8 g). The suspension was filtered through a bed of Celite, the solids were thoroughly washed with ethanol, and the filtrate and washings were combined and evaporated. The residue was dissolved in 95% ethanol (60 mL), treated with PtO_2 (1.5 g), and shaken under H_2 at 345 kPa for an additional 3 h. The catalyst was filteredd off, the ethanol removed under diminished pressure, and the residue dissolved in 4:1 ethanol–water (50 mL) and stirred with Amberlite IR-120 (Na^+) cation-exchange resin for 16 h at 4°. The resin was removed by filtration, the solvent evaporated, and the residue dissolved in chloroform. Addition of hexane caused the precipitation of **8** (1.5 g, 77%), amorphous, $[\alpha]_D^{25}$ (*c* 1.2, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 1.90–2.23 (cluster of s, 12 H, OAc), and 3.39 (s, 3 H, OMe).

Anal. Calc. for $C_{21}H_{31}Na_2O_{17}P$: C, 39.87; H, 4.90; P, 4.90. Found: C, 39.67; H, 4.93; P, 4.87.

*Disodium (methyl 2-O-α-*D*-mannopyranosyl-α-*D*-mannopyranoside) 6-phosphate* (**9**). — Compound **8** (0.5 g) was stirred in 0.1M methanolic sodium methoxide (25 mL) for 4 h at room temperature. The solution was de-ionized with Amberlite IR-120 (H^+) cation-exchange resin, the resin removed by filtration, and the residue purified in a column of Bio-Gel P-2 by use of water as the eluent. The fractions corresponding (t.l.c. 4:1, 2-propanol–water) to **9** were combined and lyophilized, and the residue so obtained was dissolved in water and stirred for 16 h at 4° with Amberlite IR-120 (Na^+) cation-exchange resin. The resin was removed by filtration and the aqueous solution lyophilized to furnish amorphous **9** (0.3 g, 79%), $[\alpha]_D^{25}$ +32° (*c* 1.4, water).

Anal. Calc. for $C_{13}H_{23}Na_2O_{14}P \cdot H_2O$: C, 31.32; H, 5.02. Found: C, 31.56; H, 5.21.

*Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-[6-O-(diphenoxy)phosphoryl-α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**6**). — Compound **3** (1.2 g, 2.2 mmol) in pyridine (25 mL) was allowed to react with diphenyl phosphorochloridate (0.96 mL, 4.6 mmol) as described for **2** (to give **5**). After processing, as the above-described, the product was purified in a column of silica gel by use of 10% methanol in chloroform as the eluent to give **6** (1.6 g, 93%), amorphous, $[\alpha]_D^{25}$ +22° (*c* 1.4, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 3.20 (s, 3 H, OMe), 5.50 (s, 1 H, PhCH), and 7.10–7.50 (m, 20 H, aromatic).

Anal. Calc. for $C_{39}H_{43}O_{14}P \cdot 0.5\ H_2O$: C, 60.38; H, 5.67; P, 4.00. Found: C, 60.14; H, 5.68; P, 4.08.

*Methyl 2-O-(disodium α-*D*-mannopyranosyl 6-phosphate)-α-*D*-mannopyranoside* (**10**). — Compound **6** (1.0 g) in 95% aqueous ethanol was shaken under H_2 at 345 kPa for 16 h at room temperature in the presence of 10% Pd–C. After removal of the catalyst by filtration, the solvent was evaporated to dryness. The residue was dissolved in 8:1 ethanol–water (45 mL) and shaken under H_2 at 345 kPa for 3 h at room temperature in the presence of PtO_2 (0.7 g). The catalyst was filtered off, the solvent evaporated under reduced pressure, and the residue dissolved in water and passed through a Bio-Gel P-2 column with water as the eluent. The fractions corresponding (t.l.c., 4:1 2-propanol–water) to **10** were combined

and lyophilized, and the residue so obtained was dissolved in water and stirred with Amberlite IR-120 (Na^+) cation-exchange resin for 16 h at 4°. The resin was removed by filtration and the aqueous solution lyophilized to give amorphous **10** (0.5 g, 80%), $[\alpha]_D^{25}$ +35.1° (*c* 1.1, water).

Anal. Calc. for $C_{13}H_{23}Na_2O_{14}P$: C, 31.32; H, 5.02. Found: C, 31.41; H, 5.12.

Methyl 6-O-(diphenoxy)phosphoryl-2-O-[6-O-(diphenoxy)phosphoryl-α-D-mannopyranosyl]-α-D-mannopyranoside (**7**). — Disaccharide **4** (2 g, 5.4 mmol) was allowed to react with diphenyl phosphorochloridate (4.5 mL, 21.4 mmol) exactly as described for **3** (to give **6**). After processing as just described for the preparation of **5**, column-chromatographic purification using solvent *A* gave amorphous **7** (2.7 g, 58.6%), $[\alpha]_D^{25}$ +33.3° (*c* 2.5, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 3.16 (s, 3 H, OMe) and 7.10–7.35 (m, 20 H, arom.).

Anal. Calc. for $C_{37}H_{42}O_{17}P_2 \cdot 0.5\ H_2O$: C, 53.55; H, 5.18; P, 7.47. Found: C, 53.60; H, 5.42; P, 7.18.

Disodium [methyl 2-O-(disodium α-D-mannopyranosyl 6-phosphate)-α-D-mannopyranoside] 6-phosphate (**11**). — Compound **7** (0.9 g) was hydrogenated in the presence of PtO_2 (1.1 g) as just described. After purification by passage through a Bio-Gel P-2 column with water as the eluent, **11** (0.55 g, 82.9%) was obtained as its tetrasodium salt by stirring overnight with Amberlite IR-120 (Na^+) cation-exchange resin, $[\alpha]_D^{25}$ +24° (*c* 1.6, water).

Anal. Calc. for $C_{13}H_{22}Na_4O_{17}P_2 \cdot H_2O$: C, 25.08; H, 3.85. Found: C, 25.32; H, 3.61.

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