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Synthesis of some D-mannosyl-oligosaccharides containing the methyl and 4-nitrophenyl β-D-mannopyranoside units*

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ABSTRACT

Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (**2**), methyl 2,3-*O*-isopropylidene-β-D-mannopyranoside (**11**), and 4-nitrophenyl 2,3-*O*-isopropylidene-β-D-mannopyranoside (**12**) were each condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**1**) in the presence of mercuric cyanide, to give after deprotection, methyl 2- (**5**) and 6-*O*-α-D-mannopyranosyl-β-D-mannopyranoside (**15**), and 4-nitrophenyl 6-*O*-α-D-mannopyranosyl-β-D-mannopyranoside (**20**), respectively. A similar condensation of **11** with 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide (**21**) and 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide (**25**), followed by removal of protecting groups, afforded methyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-β-D-mannopyranoside (**24**) and methyl *O*-α-D-mannopyranosyl-(1→6)-*O*-α-D-mannopyranosyl-(1→6)-β-D-mannopyranoside (**28**), respectively. Bromide **25** was also condensed with **12** to give a trisaccharide derivative which was deprotected to furnish 4-nitrophenyl *O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-β-D-mannopyranoside (**31**). Phosphorylation of methyl 3,4,6-tri-*O*-benzyl-2-*O*-α-D-mannopyranosyl-β-D-mannopyranoside and **15** with diphenyl phosphorochloridate in pyridine gave the 6′-phosphates **6** and **16**, respectively. Hydrogenolysis of the benzyl and phenyl groups provided methyl 2-*O*-(disodium α-D-mannopyranosyl 6-phosphate)-β-D-mannopyranoside (**7**) and methyl 6-*O*-(disodium α-D-mannopyranosyl 6-phosphate)-β-D-mannopyranoside (**17**) after treatment with Amberlite IR-120 (Na^+) cation-exchange resin. The structures of compounds **5**, **7**, **15**, **17**, **20**, **24**, **28**, and **31** were established by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

Lysosomal enzymes carry a D-mannopyranosyl 6-phosphate recognition marker which is considered to be responsible for the binding of lysosomal enzymes to D-mannose 6-phosphate receptors and the translocation of these enzymes to lysosomes[2]. The biosynthesis of this recognition marker occurs by the sequential action of two enzymes[3–5], UDP-*N*-acetylglucosamine: lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (EC 2.7.8.17; "GlcNAc-*P*-transferase") and *N*-acetyl-α-D-glucosaminyl phosphodiesterase (EC 3.1.4.45), as shown in Scheme 1. The first enzyme

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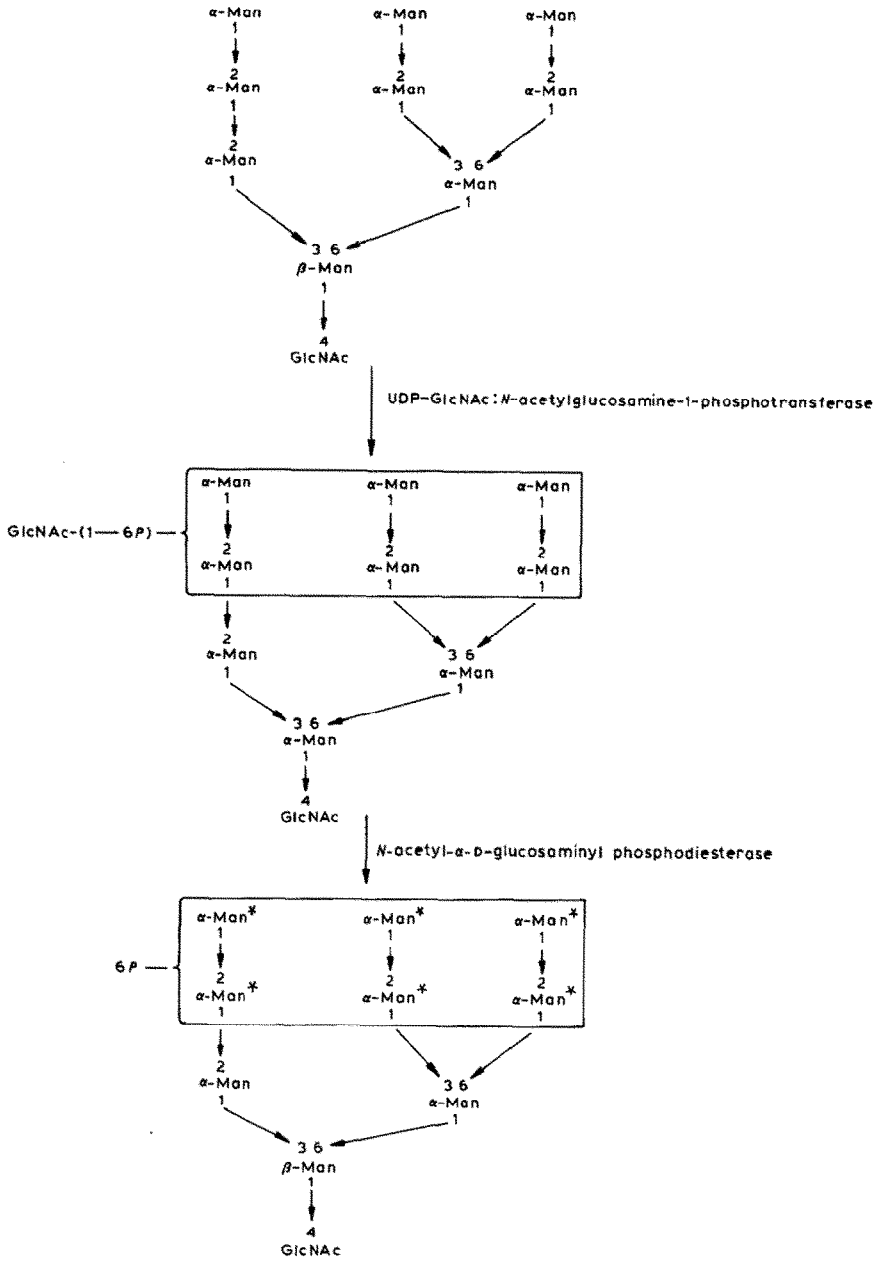


Scheme 1. Proposed steps in the generation of phosphomannosyl recognition marker of lyosomal enzymes [3-5]. The asterisks indicate residues where 6-phosphate groups have been located; α-Man = α-D-Man*p*, β-Man = β-D-Man*p*, GlcNAc = D-GlcNAc.

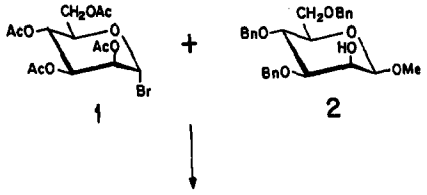
catalyzes the transfer of 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate from UDP-GlcNAc to OH-6 of D-mannose units of high-mannose-type oligosaccharides[4], and the second enzyme removes the masking α-D-GlcNAc residues to generate phosphorylated mannosyl oligosaccharides in the lysosomal enzymes[5]. In our study of lysosomal-enzyme targeting, we demonstrated recently that the disaccharide, methyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside, acts as a better acceptor among the various disaccharides tested[6] and that phosphorylation occurs at terminal D-mannopyranosyl groups[7]. Our interest was enhanced by our recent observation that GlcNAc-*P*-transferase activity is increased in ovarian microsomes from ovarian cancer patients and in several cultured cell-lines derived from tumors when compared with normal counterparts[8]. Another point of interest is that two of the oligosaccharides isolated from malignant thyroid have been reported[9] to contain the oligosaccharide, sequence α-D-Glc*p*NAc-(1→*P*-6)-α-D-Man*p*-(1→3 or 6)-β-D-Man*p*-(1→.

Thus, in a study of the substrate-specificity of GlcNAc-*P*-transferase, we describe herein the synthesis of three disaccharides and three trisaccharides, namely, α-D-Man*p*-(1→2)-β-D-Man*p*OMe (**5**), α-D-Man*p*-(1→6)-β-D-Man*p*OMe (**15**), α-D-Man*p*-(1→6)-β-D-Man*p*$OC_6H_4NO_2$(4) (**20**), α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)-β-D-Man*p*OMe (**24**), α-D-Man*p*-(1→6)-α-D-Man*p*-(1→6)-β-D-Man*p*OMe (**28**), and α-D-Man*p*-(1→6)-α-D-Man*p*-(1→6)-β-D-Man*p*$OC_6H_4NO_2$(4) (**31**). By use of such modified compounds, we intend to explore the effects of altered C-1 configuration and oligosaccharide chain elongation. These compounds will also be useful in specificity studies on antibody raised against a related, synthetic antigen currently under investigation. In addition, we also report the synthesis of two monophosphorylated derivatives of **5** and **15**, which we intend to employ as reference compounds for the identification of products formed when appropriate oligosaccharides are used as substrates for *N*-acetyl-α-D-glucosaminyl phosphodiesterase.

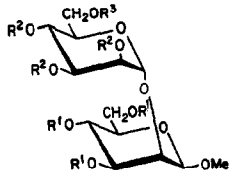
RESULTS AND DISCUSSION

Glycosylation of methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[10] (**2**) with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**1**) in the presence of powdered mercuric cyanide gave a crude mixture (containing **3**) which was not separated but directly *O*-deacetylated to give, after column-chromatographic purification, disaccharide derivative **4** in 62% yield. Hydrogenolysis of the benzyl groups of **4** then furnished amorphous **5** as a monohydrate in good (83%) yield. Compound **4** was treated with diphenyl chlorophosphate in pyridine at 0° to give, in 75% yield, the monophosphorylated derivative **6**, the ^{1}H-n.m.r. spectrum of which was in accord with the structure expected. Removal of benzyl and phenyl protecting groups of **6** by hydrogenolysis afforded disaccharide **7** that was isolated as its disodium salt in 68% yield. In its ^{13}C-n.m.r. spectrum, the noticeable downfield shift for the resonance of C-6′ (δ 65.16), which also showed the typical $^2J_{C,P}$ value of 4.6 Hz, was indicative of O-6′ as the site of phosphorylation.

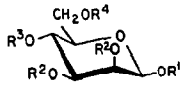
For the synthesis of disaccharide **15** and trisaccharides **24** and **28**, methyl 2,3-*O*-



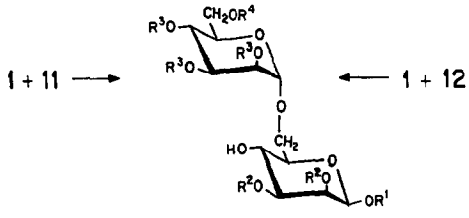
1 2



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



8 R^1 = Me;R^2=R^3=R^4=H
9 R^1 = Me;R^2=R^3=H,R^4=Bu^tPh_2Si
10 R^1 = Me;R^2,R^2=CMe_2;R^3=H;R^4=Bu^tPh_2Si
11 R^1 = Me;R^2,R^2=CMe_2;R^3=R^4=H
12 R^1 = C_6H_4-NO_2(4);R^2,R^2=CMe_2;R^3=R^4=H



13 R^1 = Me;R^2,R^2=CMe_2;R^3=R^4=Ac
14 R^1 = Me;R^2=H;R^3=R^4=Ac
15 R^1 = Me;R^2=R^3=R^4=H
16 R^1 = Me;R^2=R^3=H;R^4=$PO(OPh)_2$
17 R^1 = Me;R^2=R^3=H;R^4=$PO(ONa)_2$
18 R^1 = C_6H_4-NO_2(4);R^2,R^2=CMe_2;R^3=R^4=Ac
19 R^1 = C_6H_4-NO_2(4);R^2,R^2=CMe_2;R_3=R_4=H
20 R^1 = C_6H_4-NO_2(4);R^2=R^3=R^4=H

isopropylidene-β-D-mannopyranoside (**11**) was required as a glycosyl acceptor, and was obtained in three steps from methyl β-D-mannopyranoside[10] (**8**). Thus, treatment of **8** with *tert*-butylchlorodiphenylsilane in *N,N*-dimethylformamide in the presence of imidazole afforded a good yield (~89%) of the 6-(*tert*-butyldiphenylsilyl) ether **9**. This was readily converted, in high yield, into its 2,3-*O*-isopropylidene derivative **10**. Removal of the *tert*-butyldiphenylsilyl group of **10** was accomplished by treatment with tetrabutylammonium fluoride in oxolane to give **11**. The ^{1}H-n.m.r. spectrum of chromatographically pure **11** was in accord with the structure expected (see Experimental section). Treatment of **11** with bromide **1**, in a manner analogous to that described for the reaction of **1** with **2** but at room temperature, gave a crude mixture (containing **13**) which was directly treated with aqueous trifluoroacetic acid in chloroform to afford, after column-chromatographic purification, disaccharide derivative **14**. *O*-Deacetylation of **14** provided, in 84% yield the disaccharide **15** as a hemihydrate.

Selective phosphorylation of **15**, in a manner analogous to that described for **4** (to give **6**), gave **16**; its identity as a monophosphorylated derivative of **15** was clearly evidenced by its ^{1}H-n.m.r. spectrum which contained a 10-proton multiplet at δ 7.40–7.06, attributable to the phosphoric ester phenyl protons. Hydrogenolytic cleavage of the phenyl groups, as described for **6** (to give **7**), afforded in 72% yield methyl 6-*O*-(disodium α-D-mannopyranosyl 6-phosphate)-β-D-mannopyranoside (**17**) as the monohydrate.

Condensation of diol[11] **12** with bromide **1**, followed by column-chromatography, gave, in 56% yield, 4-nitrophenyl 2,3-*O*-isopropylidene-6-*O*-α-D-mannopyranosyl-β-D-mannopyranoside (**18**). The ^{1}H-n.m.r. spectrum of **18** contained signals in support of its overall structure; two doublets at δ 8.15 and 7.01 (2 H each, J ~ 10 Hz) accounted for the aromatic ring protons, whereas four singlets (δ 2.05–1.91, 12 H) evidenced the presence of four acetyl-group methyl protons, and the isopropylidene methyl protons occurred as well-separated singlets at δ 1.57 and 1.42. *O*-Deacetylation of **18**, followed by cleavage of the acetal group of **19** with aqueous trifluoroacetic acid, furnished **20** in 87% yield.

Glycosylation of diol **11** with bromide[12] **21** in a manner analogous to that described for the reaction of **11** with **1** gave a crude product mixture (containing **22**) which was not separated but directly treated with aqueous acetic acid to afford, after column-chromatographic purification, trisaccharide derivative **23**. A similar reaction of diol **11** with bromide[12] **25** and removal of the isopropylidene group afforded, in 33% yield (based on **11**), amorphous **27**. The ^{1}H-n.m.r. spectrum of both **23** and **27** contained signals supporting the proposed structures (see Experimental section). Zemplén transesterification of **23** and **27** gave trisaccharides **24** and **28**, respectively, in excellent yields.

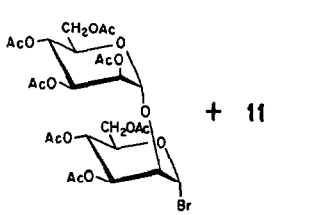
A similar coupling of bromide[12] **25** with **12**, followed by column-chromatographic purification, gave the partially protected trisaccharide derivative **29**, in 47% yield, the ^{1}H-n.m.r. spectrum of which was in good agreement with the structure assigned (see Experimental section). Sequential removal of the acetyl and acetal groups of **29**, in a manner analogous to that described for **18** and **19** (to give **19** and **20**, respectively),

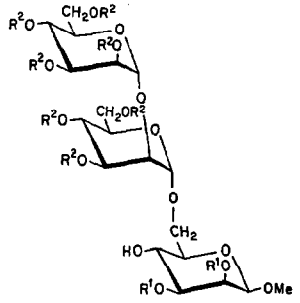
TABLE I

Proposed ^{13}C-n.m.r. chemical shifts[a] and coupling constants[b]

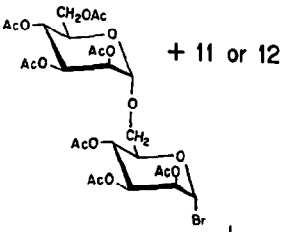
Residue or group	*Compd.*	*Solvent*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*
β-D-Man*p*OMe	**8**[c]	D_2O	102.40	71.57	74.30	68.19	77.55	62.36	58.07
β-D-Man*p*OMe	**5**	D_2O	102.34	78.09	73.91	68.43	77.44	62.15	58.22
α-D-Man*p*-(1→2)			102.70	71.52	71.77	67.94	74.95	62.55	
β-D-Man*p*OMe	**7**	D_2O	102.29	78.04	74.76	68.35	77.63	62.50	58.22
6-PO_4-α-D-Man*p*-(1→2)			102.87	71.42	71.51	67.38	72.87	65.16	
							(8.0)	(4.6)	
β-D-Man*p*OMe	**15**	D_2O	100.55	71.07	74.22	67.91	75.40	66.96	57.97
α-D-Man*p*-(1→6)			102.23	71.34	71.73	67.91	73.81	62.10	
β-D-Man*p*OMe	**17**	D_2O	101.09	71.67	74.51	68.00	75.60	67.18	58.27
6-PO_4-α-D-Man*p*-(1→6)			102.60	71.32	71.67	67.54	73.30	64.74	
							(7.7)	(5.0)	
β-D-Man*p*$OC_6H_4NO_2-4$	[d]	$(CD_3)_2SO$	97.68	70.22	73.41	66.88	77.95	61.11	
β-D-Man*p*$OC_6H_4NO_2-4$	**20**	$(CD_3)_2SO$	97.81	70.20	73.39	66.75	75.49	65.80	
α-D-Man*p*-(1→6)			99.55	70.50	71.15	67.11	73.72	61.24	
β-D-Man*p*OMe	**8**[c]	$(CD_3)_2SO$	101.43	70.38	73.66	67.21	77.49	61.43	55.91
β-D-Man*p*OMe	**24**	$(CD_3)_2SO$	101.44	70.26	73.57	67.28	75.25	66.24	55.80
α-D-Man*p*-(1→6)			98.11	77.57	70.71	67.28	73.79	61.44	
α-D-Man*p*-(1→2)			102.37	70.36	70.88	67.28	74.15	61.44	
β-D-Man*p*OMe	**28**	$(CD_3)_2SO$	101.43	70.41	71.26	67.19	75.45	65.81	55.87
α-D-Man*p*-(1→6)			99.46	70.41	71.02	67.19	73.72	66.77	
α-D-Man*p*-(1→6)			99.46	70.41	71.26	67.19	73.72	61.35	
β-D-Man*p*$OC_6H_4NO_2-4$	[d]	D_2O	99.95	73.06	75.44	69.35	79.35	63.64	
β-D-Man*p*$OC_6H_4NO_2-4$	**31**[e]	D_2O	99.49	72.94	73.59	69.26	77.45	68.30	
α-D-Man*p*-(1→6)			102.07	72.73	73.33	69.38	75.42	68.85	
α-D-Man*p*-(1→6)			102.20	72.73	73.51	69.52	75.56	63.68	

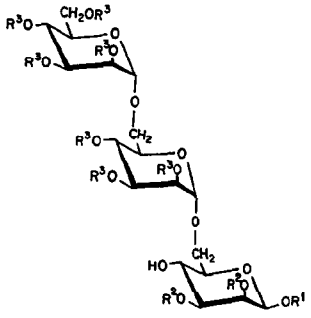
[a] The reference (Me_4Si) is internal for solutions in $(CD_3)_2SO$ and external for solutions in D_2O. Aromatic resonances are not shown. [b] Where applicable, $^2J_{C,COP}$ and $^3J_{C,COP}$ in Hz are shown in parentheses. [c] The chemical shifts for this compound[13] have been reported earlier for a solution in C_5D_5N. [d] The chemical shifts for this compound are included for comparison purposes. [e] With $(Me_3)_3Si(CD)_2CO_2Na$ (TSP) as an external standard.





22 $R^1,R^1 = CMe_2; R^2 = Ac$
23 $R^1 = H; R^2 = Ac$
24 $R^1 = R^2 = H$





26 $R^1 = Me; R^2,R^2 = CMe_2; R^3 = Ac$
27 $R^1 = Me; R^2 = H; R^3 = Ac$
28 $R^1 = Me; R^2 = R^3 = H$
29 $R^1 = C_6H_4\text{-}NO_2(4); R^2,R^2 = CMe_2; R^3 = Ac$
30 $R^1 = C_6H_4\text{-}NO_2(4); R^2,R^2 = CMe_2; R^3 = H$
31 $R^1 = C_6H_4\text{-}NO_2(4); R^2 = R^3 = H$

afforded trisaccharide **31** in fair yield. The ^{13}C-n.m.r. spectra of disaccharides **5**, **7**, **15**, **17**, and **20**; and trisaccharides **24**, **28**, and **31** were all in good agreement with the structures assigned (see Table I).

EXPERIMENTAL

General methods. — Optical rotations were measured at ~22° with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded at ~25°, ^1H-n.m.r. spectra with a Varian EM-390 instrument, and ^{13}C-n.m.r. spectra either with a Varian XL-100, or a Bruker WP-200, or a Bruker AM-400 instrument, operating at 90, 25.2, 50.3, and 100.6 MHz, respectively; the positions of the peak (δ) are expressed from the tetramethylsilane signal. T.l.c. was conducted on aluminum sheets, precoated with a 0.2-mm layer of Silica Gel 60F-25 (E. Merk, 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 (or both) and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). Generally ~25-mL fractions were collected and the flow rate was maintained at ~5 mL/min. Unless otherwise indicated, the following solvent systems (v/v) were used for chromatography: (*A*) 24:1 chloroform–methanol, (*B*) 13:6:1 chloroform–methanol–water, (*C*) 97:3 chloroform–methanol, (*D*) 4:1 chloroform–acetone, (*E*) 19:1 chloro-

form–methanol, (*F*) 17:3 chloroform–methanol, (*G*) 5:4:1 chloroform–methanol–water, and (*H*) 9:1 chloroform–methanol. Solutions in organic solvents were generally dried with anhydrous Na_2SO_4. Pyridine, benzene, and dichloromethane were dried over KOH, Na, and 4A molecular sieves, respectively. Nitromethane and acetonitrile were distilled from P_2O_5 immediately before use. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 07940.

*Methyl 3,4,6-tri-*O*-benzyl-2-O-(α-*D*-mannopyranosyl)-β-*D*-mannopyranoside* (**4**). — A mixture of methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside[10] (**2**; 5.5 g, 11.8 mmol) and powdered $Hg(CN)_2$ (3 g, 11.9 mmol) in 1:1 benzene–nitromethane (250 mL) was boiled until ~100 mL of the solvent had distilled off. The temperature was then adjusted to 50–55°, a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**1**) (5 g, 12.2 mmol) in benzene (20 mL) was added, and stirring was continued for 3 h. More portions of $Hg(CN)_2$ (1 g, 4 mmol) and bromide **1** (2.5 g, 6.1 mmol in 10 mL of benzene) were added and stirring was continued for an additional 16 h at the same temperature. The mixture was then cooled, diluted with benzene, and filtered. The filtrate was successively washed with saturated aqueous $NaHCO_3$, M KI solution, and water, and evaporated to a syrup.

The crude product mixture (~10 g; containing **3**) was thoroughly dried *in vacuo*, dissolved in 0.05M sodium methoxide in methanol (200 mL), and stirred for 3 h at room temperature. The base was neutralized by the dropwise addition of glacial acetic acid. The solution was concentrated to dryness, and the residue applied to a column of silica gel. Elution with solvent *A* and concentration of fractions corresponding to product gave **4** (4.5 g, 62%), amorphous, $[\alpha]_D^{22}$ +5° (*c* 0.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 3.42 (s, 3 H, OMe) and 7.03–7.40 (m, 15 H, arom.).

Anal. Calc. for $C_{33}H_{42}O_{11}$: C, 64.47; H, 6.90. Found: C, 64.29; H, 6.87.

*Methyl 2-*O*-α-*D*-mannopyranosyl-β-*D*-mannopyranoside* (**5**). — A mixture of **4** (2.5 g) and 10% Pd–C (2.5 g) in 4:1 ethanol–glacial acetic acid (75 mL) was shaken under H_2 at ~345 kPa for 2 days at room temperature. The suspension was filtered through a bed of Celite, the solid thoroughly washed with methanol, and the filtrate and washings were combined and concentrated under diminished pressure. The residue so obtained was purified by chromatography in a column of silica gel using solvent *B* as the eluent to afford **5** (1.2 g, 83%), amorphous, $[\alpha]_D^{22}$ +8° (*c* 1.2, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{13}H_{24}O_{11}\cdot H_2O$: C, 41.70; H, 7.01. Found: C, 41.84; H, 6.95.

*Methyl 3,4,6-tri-*O*-benzyl-2-*O*-[α-*D*-mannopyranosyl 6-(diphenyl phosphate)]-β-*D*-mannopyranoside* (**6**). — To a cold (0°, bath), stirred solution of **4** (1 g, 1.6 mmol) in pyridine (30 mL) was added diphenyl chlorophosphate (0.9 mL, 4.3 mmol), and the stirring was continued for 2 h at 0°. Water (10 mL) was then added, the mixture concentrated 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 concentrated, and the residue applied to a column of silica gel. On elution with solvent *C*, concentration of the fractions corresponding to the product furnished **6** (1 g, 75%), syrup, $[\alpha]_D^{22}$ +34° (*c* 1.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.0 (m, 25 H, arom.) and 3.43 (s, 3 H, OMe).

Anal. Calc. for $C_{46}H_{51}O_{14}P \cdot H_2O$: C, 63.00; H, 6.10. Found: C, 62.81; H, 6.15.

Methyl 2-O-(disodium α-D-mannopyranosyl 6-phosphate)-β-D-mannopyranoside (**7**). — A solution of **6** (0.9 g) in 95% ethanol (75 mL) was shaken under H_2 at ~345 kPa for 20 h at room temperature in the presence of 10% Pd–C (1 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 concentrated. The residue was dissolved in 9:1 ethanol–water (50 mL) and shaken under H_2 at ~345 kPa for an additional 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 (40 mL) 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 **7** (0.35 g, 68%), amorphous, $[\alpha]_D^{22}$ +42° (*c* 1.1, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{13}H_{23}Na_2O_{14}P \cdot H_2O$: C, 30.24; H, 5.28. Found: C, 30.51; H, 4.98.

Methyl 6-O-tert-butyldiphenylsilyl-β-D-mannopyranoside (**9**). — To a cold (0°, bath) stirred solution of methyl β-D-mannopyranoside[10] (**8**; 4 g, 20.6 mmol) and imidazole (4.0 g, 59.9 mmol) in dry *N,N*-dimethylformamide (40 mL) was added *tert*-butylchlorodiphenylsilane (6.8 mL, 26.1 mmol), and stirring was continued for 1.5 h at ~0°. The mixture was then poured into ice–water and extracted with chloroform. The chloroform solution was successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated. The concetrate was applied to a column of silica gel. Elution with solvent *C* and concentration of the fractions corresponding to the product afforded **9** (7.9 g, 88%), syrup, $[\alpha]_D^{22}$ −39° (*c* 1.4, chloroform); ^1H-n.m.r. ($CDCl_3$): δ 7.73–7.27 (m, 10 H, arom.), 3.50 (s, 3 H, OMe), and 1.07 (s, 9 H, CMe_3).

Anal. Calc. for $C_{23}H_{32}O_6Si$: C, 63.85; H, 7.47. Found: C, 63.72; H, 7.61.

Methyl 6-O-tert-butyldiphenylsilyl-2,3-O-isopropylidene-β-D-mannopyranoside (**10**). — To a solution of **9** (7.5 g) in dry acetone (50 mL) were added 2,2-dimethoxypropane (50 mL) and 4-toluenesulfonic acid monohydrate (1.2 g). The mixture was stirred for 1 h at room temperature, made neutral by the addition of triethylamine, and concentrated. The residue was dissolved in chloroform, and the solution washed with water, dried, and concentrated. The concentrate was applied to a column of silica gel and eluted with chloroform to give **10** (7.5 g, 91%), a white amorphous solid, $[\alpha]_D^{22}$ −49° (*c* 2.9, chloroform); ^1H-n.m.r. ($CDCl_3$): δ 7.77–7.27 (m, 10 H, arom.), 3.53 (s, 3 H, OMe), 1.50 and 1.40 (s, 3 H each, CMe_2), and 1.07 (s, 9 H, CMe_3).

Anal. Calc. for $C_{22}H_{36}O_6Si$: C, 66.06; H, 7.69. Found: C, 65.98; H, 7.82.

Methyl 2,3-O-isopropylidene-β-D-mannopyranoside (**11**). — A solution of **10** (7.4 g) in anhydrous oxolane (140 mL) was treated with M tetrabutylammonium fluoride in oxolane (16.5 mL) and stirring was continued for 1 h at room temperature. The mixture was concentrated to dryness and the residue was purified in a column of silica gel with solvent *C* as the eluent to give **11** (3.4 g, 94%), syrup, $[\alpha]_D^{22}$ −64° (*c* 0.9, chloroform), R_F 0.15 (*D*); ^1H-n.m.r. ($CDCl_3$): δ 3.60 (s, 3 H, OMe), and 1.57 and 1.40 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.26; H, 7.76. Found: C, 50.99; H, 7.76.

Methyl 6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**14**). — A stirred mixture of diol **11** (2.9 g, 12.4 mmol) and $Hg(CN)_2$ (4.9 g, 19.4 mmol) in

1:1 benzene–nitromethane (250 mL) was boiled until ~100 mL of the solvent had been distilled. It was then cooled to room temperature, bromide **1** (6.8 g, 16.5 mmol) in benzene (25 mL) was added, and stirring continued for 16 h at room temperature. After processing in the usual manner, the crude product mixture (~8 g; containing **13**) was taken up in chloroform (120 mL) containing trifluoroacetic acid (9.5 mL) and water (0.5 mL), and stirred for 1 h at room temperature. It was then concentrated under diminished pressure, the last traces being removed by evaporation with several added portions of toluene, and the residue was applied to a column of silica gel. On elution with solvent *E*, concentration of the fractions corresponding to the product afforded **14** (4 g, 62%), a white amorphous solid, $[\alpha]_D^{22}$ +3° (*c* 1.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 3.20 (s, 3 H, OMe) and 2.17–1.98 (cluster of s, 12 H each, 4 OAc).

Anal. Calc. for $C_{21}H_{32}O_{15}$: C, 48.08; H, 6.16. Found: C, 48.09; H, 6.35.

Methyl 6-O-α-D-mannopyranosyl-β-D-mannopyranoside (**15**). — Compound **14** (1.4 g) in 20mM methanolic sodium methoxide (100 mL) was stirred for 3 h at room temperature. The base was neutralized by the dropwise addition of glacial acetic acid, the solution concentrated to dryness, and the residue applied to a column of silica gel. Elution with solvent *B* yielded **15** (0.8 g, 84%), a white amorphous solid, $[\alpha]_D^{22}$ +8° (*c* 0.4, methanol); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{13}H_{24}O_{11}\cdot 0.5H_2O$: C, 42.73; H, 6.91. Found: C, 42.69; H, 6.64.

Methyl 6-O-[α-D-mannopyranosyl-6-(diphenyl phosphate)]-β-D-mannopyranoside (**16**). — Compound **15** (0.5 g, 1.4 mmol) in pyridine was treated with diphenyl chlorophosphate (0.9 mL, 4.3 mmol) as described for **5** (to give **6**). After processing as described, the product was purified in a column of silica gel by use of solvent *F* as the eluent to give **16** (0.45 g, 55.5%), syrup, $[\alpha]_D^{22}$ −3° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.06 (m, 10 H, arom.) and 3.46 (s, 3 H, OMe).

Anal. Calc. for $C_{25}H_{23}O_{14}P\cdot H_2O$: C, 50.34; H, 4.23. Found: C, 50.28; H, 4.15.

Methyl 6-O-(disodium α-D-mannopyranosyl 6-phosphate)-β-D-mannopyranoside (**17**). — A solution of **16** (0.3 g) in 6:1 ethanol–water (35 mL) was shaken under H_2 at ~345 kPa for 3 h at room temperature in the presence of PtO_2 (0.3 g). The catalyst was filtered off, the solvent evaporated under reduced pressure, and the residue dissolved in water (20 mL) 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 **17** (0.18 g, 72%), amorphous, $[\alpha]_D^{22}$ −1° (*c* 1.0, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{13}H_{22}O_{14}P\cdot H_2O$: C, 31.32; H, 5.02. Found: C, 31.15; H, 4.96.

4-Nitrophenyl 2,3-O-isopropylidene-6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**18**). — To a solution of 4-nitrophenyl 2,3-*O*-isopropylidene-β-D-mannopyranoside[11] (**12**) (1.0 g, 2.9 mmol) in acetonitrile (5 mL) were added powdered $Hg(CN)_2$ (0.74 g, 2.9 mmol) and powdered 4A molecular sieves (1.5 g). The mixture was stirred at room temperature while a solution of bromide **1** (1.81 g, 4.4 mmol) in acetonitrile (9 mL) was added, and stirring was continued for 4 h at room temperature. The acetonitrile was evaporated under diminished pressure, the residue taken up in chloroform, and the solids were filtered off (a bed of Celite) and thoroughly washed with chloroform. The filtrate and washings were combined and successively

washed with water, aqueous M KI solution, aqueous $NaHCO_3$, and water, dried, and concentrated, and the concentrate applied to a column of silica gel. Elution with a stepwise solvent gradient consisting of 0–4% acetone in chloroform (2% steps) gave **18** (1.1 g, 56%), amorphous, $[\alpha]_D^{22}$ −47° (*c* 0.4, chloroform), R_F 0.43 (*D*); ^{1}H-n.m.r. ($CDCl_3$): δ 8.15 and 7.01 (d, 2 H each, $J \sim 10$ Hz, arom.), 5.55 (d, 1 H, $J \sim 1.5$ Hz, H-1), 2.05, 2.03, 1.99, and 1.91 (s, 3 H each, 4 OAc), and 1.57 and 1.42 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{29}H_{37}NO_{17}$: C, 51.86; H, 5.55; N, 2.09. Found: C, 51.73; H, 5.66; N, 1.88.

4-Nitrophenyl 2,3-O-isopropylidene-6-O-α-D-mannopyranosyl-β-D-mannopyranoside (**19**). — Compound **18** (0.22 g) in 30mM methanolic sodium methoxide (16 mL) was stirred overnight at room temperature. The base was neutralized with a few drops of glacial acetic acid and the solution was de-ionized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off (Celite bed) and thoroughly washed with methanol, and the filtrate and washings were combined and concentrated to give a material which was dissolved in a little methanol. Addition of ether–hexane caused the precipitation of **19** (0.16 g, 91%), amorphous, $[\alpha]_D^{22}$ −87° (*c* 0.7, methanol), R_F 0.75 (*G*); ^{1}H-n.m.r. ($CDCl_3$ plus a little CD_3OD): δ 8.20 and 7.12 (d, 2 H each, $J \sim 10$ Hz, arom.), 5.58 (d, 1 H, $J \sim 1.5$ Hz, H-1), 1.55 and 1.42 (s, 3 H, CMe_2).

Anal. Calc. for $C_{21}H_{29}NO_{13}$: C, 50.10; H, 5.81; N, 2.78. Found: C, 49.93; H, 5.58; N, 2.77.

4-Nitrophenyl 6-O-α-D-mannopyranosyl-β-D-mannopyranoside (**20**). — A mixture of **19** (0.15 g) in trifluoroacetic acid (0.25 mL), water (0.1 mL), and chloroform (10 mL) was stirred for 2 h at room temperature. It was then concentrated and several portions of toluene were added to and evaporated from the residue which was dissolved in a little methanol. Addition of ether caused the precipitation of **20** (0.12 g, 87%), amorphous, $[\alpha]_D^{22}$ −62° (*c* 1.1, water), R_F 0.46 (*G*); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{18}H_{25}NO_{13}$: C, 46.65; H, 5.44; N, 3.02. Found: C, 46.37; H, 5.73; N, 2.70.

Methyl O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1→6)-β-D-mannopyranoside (**23**). — A stirred mixture of diol **11** (0.5 g, 2.13 mmol) and $Hg(CN)_2$ (0.34 g, 2.88 mmol) in 1:1 benzene–nitromethane (150 mL) was boiled until ~50 mL of the solvent had distilled off. It was then cooled to room temperature and 3,4,6-tri-*O*-acetyl-2-O-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide[12] (**21**) (1.79 g, 2.56 mmol) was added, and the stirring continued for 17 h at room temperature. After customary processing, the crude product mixture (~2.2 g, containing **22**) was taken up in 50% aqueous acetic acid (50 mL) and heated for 1 h at ~50°. The acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several added portions of toluene, and the residue was applied to a column of silica gel. Elution with a solvent gradient consisting of 0–4% methanol in chloroform (2% steps) gave **23** (0.52 g, 30%, based on **11**), amorphous, $[\alpha]_D^{22}$ +10° (*c* 0.6, chloroform), R_F 0.22 (*H*); ^{1}H-n.m.r. ($CDCl_3$): δ 3.13 (s, 3 H, OMe) and 2.11–1.98 (cluster of s, 21 H, 7 OAc).

Anal. Calc. for $C_{33}H_{48}O_{23}$: C, 48.77; H, 5.95. Found: C, 48.63; H, 5.72.

Methyl O-α-D-*mannopyranosyl-(1→2)*-O-α-D-*mannopyranosyl-(1→6)*-β-D-*mannopyranoside* (**24**). — Compound **23** (0.5 g) was suspended in 20mM sodium methoxide in methanol (82 mL) and stirred overnight at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off and washed wth methanol, and the filtrate and washings were combined and concentrated to furnish **24** (0.29 g, 91%), $[\alpha]_D^{22}$ +33° (*c* 1.2, water), R_F 0.20 (*G*); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{19}H_{34}O_{16}\cdot 0.5H_2O$: C, 43.26; H, 6.68. Found: C, 43.41; H, 6.57.

Methyl O-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*acetyl*-α-D-*mannopyranosyl)-(1→6)*-β-D-*mannopyranoside* (**27**). — A stirred mixture of **11** (0.53 g, 2.26 mmol) and $Hg(CN)_2$ (0.77 g, 3.05 mmol) in 1:1 benzene–nitromethane (150 mL) was boiled until ~50 mL of solvent had distilled off and cooled to room temperature. 2,3,4-Tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide[12] (**25**; 1.89 g, 2.7 mmol) was added, and stirring was continued for 44 h at room temperature. T.l.c. (solvent *D*) revealed the presence of a product faster-migrating than **11** with a small proportion of **11** remaining. More portions of bromide **25** (0.1 g, 0.14 mmol) and $Hg(CN)_2$ (0.12 g, 0.48 mmol) were added, and stirring was continued for another 20 h at room temperature. After being processed in the usual manner, the product mixture (~1.66 g, containing **26**) was taken up in 50% aqueous acetic acid (50 mL) and heated for 3 h at ~55°. The acetic acid was evaporated as described above and the residue so obtained was purified in a column of silica gel by use of a stepwise solvent-gradient consisting of 0–2% methanol in chloroform (1% steps) to afford **27** (0.6 g, 33% based on **11**), amorphous, $[\alpha]_D^{22}$ +35° (*c* 0.8, chloroform), R_F 0.37 (*H*); ^{1}H-n.m.r. ($CDCl_3$): δ 3.53 (s, 3 H, OMe) and 2.15–1.96 (cluster of s, 21 H, 7 OAc).

Anal. Calc. for $C_{33}H_{48}O_{23}$: C, 48.77; H, 5.95. Found: C, 48.48; H, 5.75.

Methyl O-α-D-*mannopyranosyl-(1→6)*-O-α-D-*mannopyranosyl-(1→6)*-β-D-*mannopyranoside* (**28**). — Compound **27** (0.3 g) was *O*-deacetylated in 20mM methanolic sodium methoxide (41 mL), exactly as described for **23** (to give **24**) to afford **28** (0.18 g, 94%), amorphous, $[\alpha]_D^{22}$ +29° (*c* 0.6, water), R_F 0.21 (*G*); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{19}H_{34}O_{16}$: C, 44.01; H, 6.61. Found: C, 43.74; H, 6.55.

4-Nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*acetyl*-α-D-*mannopyranosyl)-(1→6) -2,3-* O-*isopropylidene*-β-D-*mannopyranoside* (**29**). — A stirred mixture of diol[11] **12** (0.75 g, 2.19 mmol) and $Hg(CN)_2$ (0.55 g, 2.18 mmol) in 1:1 benzene–nitromethane (70 mL) was boiled until 20 mL of the solvent had distilled off. After cooling to room temperature, disaccharide bromide[12] **25** (1.84 g, 2.63 mmol) was added and stirring was continued for 4 h at room temperature. T.l.c. (solvent *D*) revealed the disappearance of **25** and the presence of a major product faster migrating than **12**, some unchanged **12**, as well as some slower- and faster-migrating contaminants (presumably resulting from the decomposition of **25**). More portions of **25** (0.3 g, 0.43 mmol) and $Hg(CN)_2$ (0.13 g, 0.5 mmol) were added and the stirring was continued for 14 h at room temperature. After the usual processing, the crude product mixture was subjected to column chromatography on silica gel using chloroform and then 1%

methanol in chloroform as the eluent to afford **29** (1 g, 47%), amorphous, $[\alpha]_D^{22}$ −5° (*c* 0.3, chloroform), R_F 0.35 (*D*); ^{1}H-n.m.r. ($CDCl_3$): δ 8.13 and 7.10 (d, 2 H, each, $J \sim 10$ Hz, arom.), 5.56 (d, 1 H, $J \sim 2$ Hz, H-1), 2.13–2.0 (cluster of s, 21 H, 7 OAc), and 1.58 and 1.41 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{41}H_{53}NO_{25}$: C, 51.30; H, 5.57; N, 1.46. Found: C, 51.07; H, 5.63; N, 1.26.

4-Nitrophenyl O-α-D-*mannopyranosyl-(1→6)*-O-α-D-*mannopyranosyl-(1→6)-2,3*-O-*isopropylidene-β*-D-*mannopyranoside* (**30**). — Compound **29** (0.4 g) was *O*-deacetylated in 0.25M methanolic sodium methoxide (20 mL) exactly as described for **18** (to give **19**) to afford **30** (0.2 g, 72%), amorphous, $[\alpha]_D^{22}$ −35° (*c* 0.4, methanol), R_F 0.30 (*B*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.88 and 6.81 (d, 2 H each, $J \sim 10$ Hz), 5.33 (d, 1 H, $J \sim 1.5$ Hz, H-1), and 1.24 and 1.10 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{27}H_{39}NO_{18}$: C, 48.72; H, 5.91; N, 2.10. Found: C, 48.51; H, 6.10; N, 2.03.

4-Nitrophenyl O-α-D-*mannopyranosyl-(1→6)*-O-α-D-*mannopyranosyl-(1→6)*-β-D-*mannopyranoside* (**31**). — A mixture of **30** (0.15 g) in trifluoroacetic acid (0.8 mL), water (0.2 mL), and chloroform (5 mL) was stirred for 4 h at room temperature. After processing as described for **19** (to give **20**), the crude product was subjected to column chromatograhpy on silica gel. Elution with a stepwise solvent gradient consisting of 0–30% methanol in chloroform (10% steps) and concentration of fractions corresponding to the product afforded **31** (0.08 g, 57%), amorphous, $[\alpha]_D^{22}$ −17° (*c* 0.8, water), R_F 0.38 (*G*); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{24}H_{35}NO_{18}$: C, 46.08; H, 5.64; N, 2.24. Found: C, 46.31; H, 5.90; N, 1.92.

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