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SYNTHESIS OF PHOSPHORYLATED TRIMANNOSIDES CORRESPONDING TO END GROUPS OF THE HIGH-MANNOSE CHAINS OF LYSOSOMAL ENZYMES

OM P. SRIVASTAVA AND OLE HINDSGAUL*
Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2 (Canada)
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ABSTRACT

Glycosylation of suitably protected 8-methoxycarbonyloctyl α-D-mannopyranosides with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride provided α-D-Man*p*-(1→2)-α-D-Man, α-D-Man*p*-(1→3)-α-D-Man and α-D-Man*p*-(1→6)-α-D-Man derivatives from which the 2′-hydroxyl group was liberated by *O*-deacetylation. Addition of the terminal D-mannose 6-phosphate residues was achieved by reaction with the readily accessible 2,3,4-tri-*O*-acetyl-6-*O*-diphenoxyphosphoryl-α-D-mannopyranosyl bromide under standard glycosylation conditions. Conventional deprotection provided the terminal 6″-phosphate of α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man, α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-α-D-Man, and α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)-α-D-Man which are present as end groups on the high-mannose oligosaccharide chains of lysosomal enzymes.

INTRODUCTION

D-Mannose 6-phosphate (Man-6-*P*) residues on asparagine-linked oligosaccharide chains are known as subcellular sorting signals in the lysosomal targeting of acid hydrolases[1–3]. Man-6-*P* has also been shown to be a recognition marker for the binding and endocytosis of extracellular glycoproteins by high-affinity receptors on the surface of a variety of cells[4–10]. With the recent evidence[11,12] for the existence of at least two structurally distinct Man-6-*P* receptors, we have embarked on a synthetic program aimed at providing an extensive panel of *O*-phosphorylated oligosaccharides to study the detailed molecular specificity of these important recognition phenomena.

The Man-6-*P* recognition marker in these systems is found on asparagine-linked oligosaccharide chains whose structures have been studied by Varki and Kornfeld[13,14], and by Natowicz *et al.*[15]. Structure **1** shows the mannose residues of the "unprocessed" high-mannose oligosaccharide chains where 6-*O*-phosphorylation has been suggested to occur. The Man-6-*P* residues are always either terminal

*Author to whom correspondence should be addressed.

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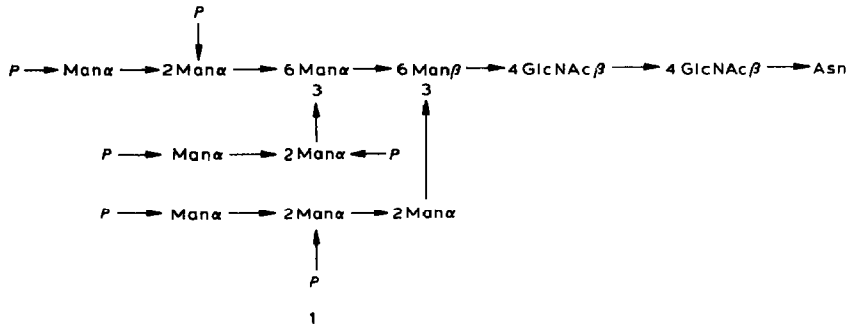

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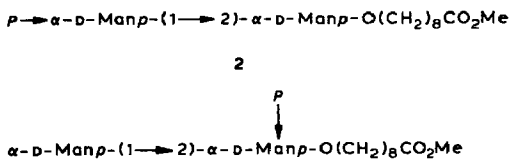
Manα = α-D-Manp
GlcNAcβ = β-D-GlcpNAc
Asn = L-Asn
P = PO_3H_2



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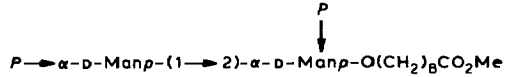

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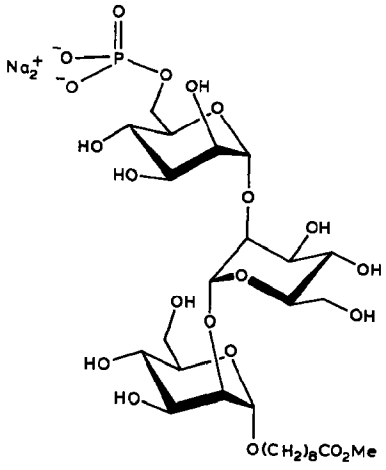
or subterminal and, in this unprocessed structure, are located on α-D-Manp-(1→2)-α-D-Man disaccharide terminii. Smaller phosphorylated oligosaccharides, where outer nonphosphorylated residues have been cleaved, have also been described[13–15].

We have already reported[16,17] the preparation of glycosides of the three possible terminal disaccharides present in the composite structure **1**, namely, **2, 3,** and **4**. We report herein the syntheses of the three phosphorylated trisaccharide glycosides **5, 6,** and **7** which are also partial structures of **1**. These trisaccharides were prepared as the 8-methoxycarbonyloctyl glycosides[18] to allow for their eventual covalent attachment to proteins and solid supports[19].

RESULTS AND DISCUSSION

Methods for the synthesis of oligomannosides are now well developed and have been reviewed by Ogawa *et al.*[20] who have elegantly synthesized a large number of structures related to the naturally occurring asparagine-linked manno-



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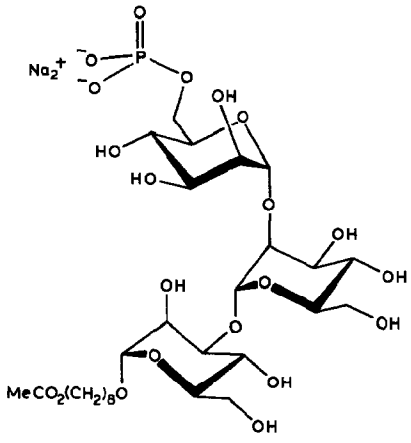

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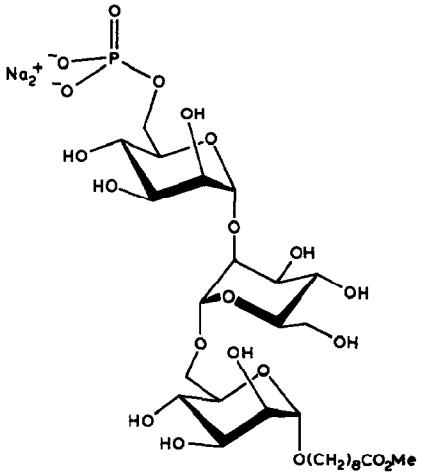

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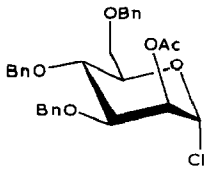
oligosaccharides[21]. For the synthesis of trisaccharides **5, 6,** and **7**, we have used very similar approaches starting with the preparation of selectively protected 8-methoxycarbonyloctyl α-D-mannopyranosides having free OH-2 (**12**), OH-3 (**18**), and OH-6 (**14**). Glycosylation of **12, 18,** and **14** with well known 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[22,23] (**8**), followed by *O*-deacetylation, provides access to the required disaccharides **20, 22,** and **24** having OH-2′ groups free. Addition of the terminal 6-*O*-phosphorylated α-D-mannopyranosyl units could then be accomplished by reaction with the prephosphorylated glycosyl bromide **10**, which is obtained on treating the readily accessible[24] β-acetate **9** with hydrogen bromide in acetic acid.

Reaction of the diol[16,17] **11** with *tert*-butyldimethylsilyl chloride provided the



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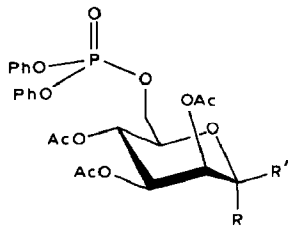
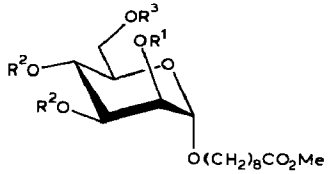


9 R = H, R′ = OAc

10 R = Br, R′ = H



11 $R^1 = R^3 = H$, $R^2 = Bn$

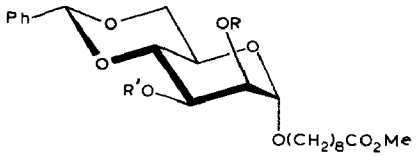
12 $R^1 = H$, $R^2 = Bn$, $R^3 = Bu^tMe_2Si$

13 $R^1 = R^2 = Bn$, $R^3 = Bu^tMe_2Si$

14 $R^1 = R^2 = Bn$, $R^3 = H$

15 $R^1 = R^2 = R^3 = Ac$

16 $R^1 = R^2 = R^3 = H$



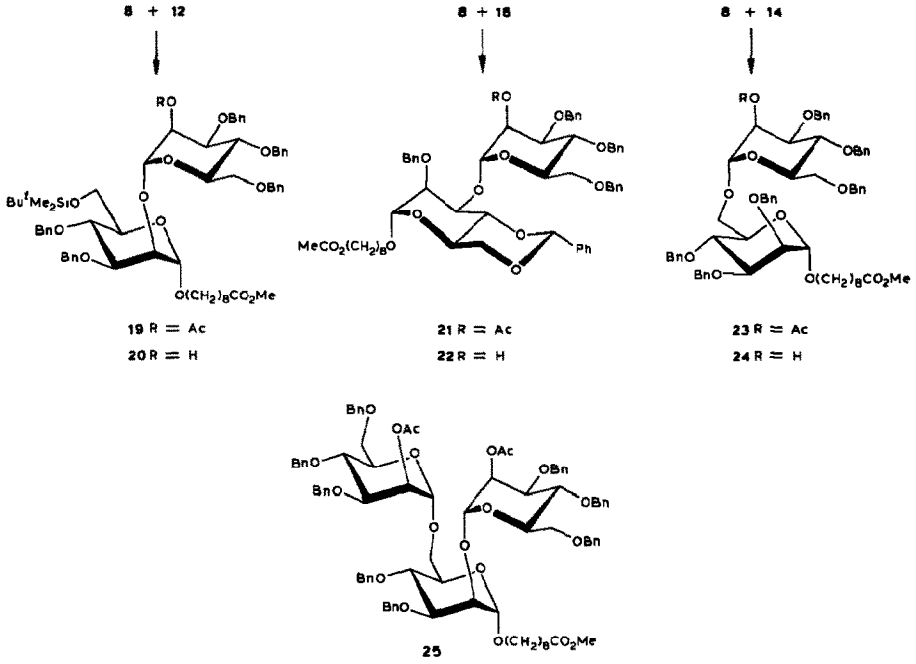
17 R = R′ = H

18 R = Bn, R′ = H

6-*O*-silyl derivative **12** (80%) having the required OH-2 free for further glycosylation. Benzylation of **12** with benzyl bromide and silver oxide in *N,N*-dimethylformamide provided **13** (69%) from which OH-6 was cleaved with tetrabutylammonium fluoride to provide **14** (87%).

Treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide with 8-methoxycarbonyloctanol gave the α-glycoside **15** (60%) which was *O*-deacetylated to provide **16**. Benzylidenation of **16**, according to the recently described procedure of Albert *et al.*[25], by use of dimethoxytoluene in *N,N*-dimethylformamide containing ethereal tetrafluoroboric acid provided the 4,6-*O*-benzylidene derivative **17** (74%). The structural assignment of **17** was verified by conversion of a small sample into the 2,3-di-*O*-acetyl derivative (acetic anhydride–pyridine) and observing the characteristic signals for H-2 (δ 5.345, $J_{1,2}$ 1.5, $J_{2,3}$ 3.5 Hz) and H-3 (δ 5.423, $J_{3,4}$ 10.0 Hz) in the ^{1}H-n.m.r. spectrum shifted downfield. Selective 2-*O*-benzylation of **17**, according to Borén *et al.*[26], provided the alcohol **18** (41%) with OH-3 free for chain extension.

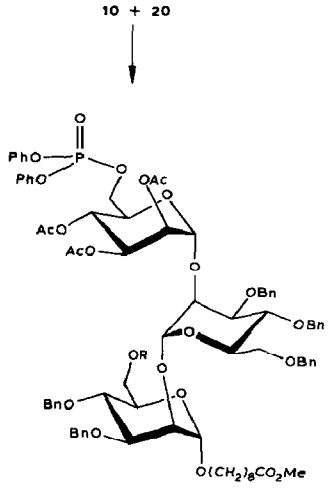
Reaction of **12** with the mannopyranosyl chloride **8**, in the presence of silver trifluoromethanesulfonate and *N,N,N′,N′*-tetramethylurea[27,28], gave the desired protected α-(1→2)-linked disaccharide **19** in 67% yield. Ogawa and Sasajima[29] have previously described the lability of the *tert*-butyldimethylsilyl group under standard



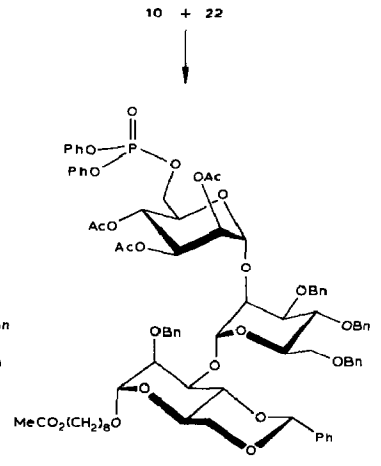
glycosylation conditions and some loss of this protecting group was anticipated in the glycosylation. Indeed, the α-(1→2)-α-(1→6)-linked trisaccharide **25** was isolated from the reaction mixture in 12% yield. The loss in the yield of **19** due to this side-reaction was still deemed acceptable, however, since an ample supply of the precursor diol[16,17] **11** was already available in our laboratory. Deprotection of OH-2 in **19** by transesterification with sodium methoxide in methanol provided the disaccharide alcohol **20** (86%).

Condensation of **18** with **8** gave the protected α-D-(1→3)-linked disaccharide **21** (81%) which was *O*-deacetylated to produce the alcohol **22** (85%). Similarly, reaction of **14** with **8** gave the α-D-(1→6)-linked disaccharide **23** (79%) from which the acetyl group was removed to provide the OH-2′ derivative **24** (85%).

Reaction of the disaccharides **20, 22,** and **24** having OH-2′ free with the 6-*O*-phosphorylated glycosyl bromide **10**, in the presence of silver trifluoromethanesulfonate and *N,N,N′,N′*-tetramethylurea in 1,2-dichloroethane, was sluggish and required a large excess of **10** to produce an acceptable yield. Reaction of **20** with **10** (7.5 equiv.) for 2 days at room temperature produced the protected α-D-(1→2)-α-D-(1→2)-linked trisaccharide **26** in 50% yield. That all three glycosidic linkages in **26** had the α-D-configuration was evident from its ^{1}H-coupled ^{13}C-n.m.r. spectrum which showed the presence of three resolved anomeric carbon atoms at δ 100.87 ($^1J_{C,H}$ 171 Hz), 99.23 ($^1J_{C,H}$ 173 Hz), and 98.86 ($^1J_{C,H}$ 170 Hz), in accord with the empirical rules formulated by Bock and Pedersen[30] for the dependence of the one-bond C–H coupling constants on the anomeric configuration of glycopyranosides



26 R = Bu^tMe_2Si
27 R = H



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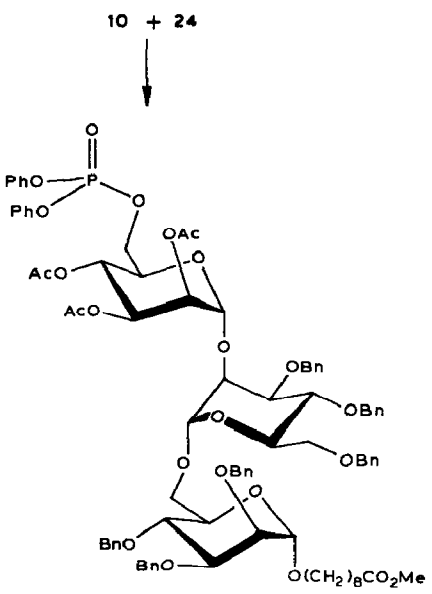

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and supported by a large number of observations[31] on synthetic oligomannosides. Although some loss of the *tert*-butyldimethylsilyl protecting group undoubtedly occurred under these reaction conditions, no tetrasaccharidic product was isolated. Combinations of mercuric cyanide and mercuric bromide were also evaluated as promoters in these glycosylation reactions with **10** but, in acetonitrile containing 4A molecular sieves, unacceptable yields (<10%) of trisaccharides were produced.

Glycosylation of **22** with **10** under the reaction conditions described gave the protected α-D-(1→2)-α-D-(1→3)-linked trisaccharide **28** in 56% yield. The ^{13}C-n.m.r. spectrum of **28** showed the expected one-bond C–H coupling constants for the three anomeric carbon atoms at δ 99.67 ($^1J_{C,H}$ 173 Hz), 99.16 ($^1J_{C,H}$ 167 Hz), and 98.47 ($^1J_{C,H}$ 172 Hz) along with the benzylidene acetal carbon atom at δ 101.86. Similarly, reaction of **24** with **10** gave the protected α-D-(1→2)-α-D-(1→6)-linked trisaccharide **29** in 65% yield. Compound **10** showed signals for the anomeric carbon atoms at δ 99.37 ($^1J_{C,H}$ 170 Hz), 99.19 ($^1J_{C,H}$ 170 Hz), and 97.95 ($^1J_{C,H}$ 168 Hz). The signals for C-6″ in the ^{13}C-n.m.r. spectra of the trisaccharides **26, 28,** and **29** all showed $^3J_{C,P} \approx 5$ Hz and the ^{31}P-n.m.r. spectra showed the presence of a single signal in the region expected for phosphoric triesters at δ −12.5.

Deprotection of **26** involved removal of the *tert*-butyldimethylsilyl group with aqueous acetic acid at room temperature for 24 h to give **27** (86%). Acidic conditions were selected for this deprotection step in order to avoid the potentially troublesome acetyl-transesterification side-reactions that may accompany desilyla-

TABLE I

SELECTED CHEMICAL SHIFTS (δ) AND COUPLING CONSTANTS (Hz)[a] FOR TRISACCHARIDES **5, 6,** AND **7**[b]

	Compound		
Nucleus[c]	**5** *(1→2,1→2)*	**6** *(1→2,1→3)*	**7** *(1→2,1→6)*
H-1 ($J_{1,2}$)	4.985 (1.4)	4.832 (1.6)	4.850 (1.6)
H-1′ ($J_{1',2'}$)	5.325 (1.6)	5.369 (1.6)	5.129 (1.4)
H-1″ ($J_{1'',2''}$)	5.042 (1.6)	5.068 (1.7)	5.027 (1.8)
H-2 ($J_{2,3}$)	4.006 (3.2)	4.029 (3.2)	3.929 (3.2)
H-2′ ($J_{2',3'}$)	4.125 (3.2)	4.120 (3.2)	3.984 (3.0)
H-2″ ($J_{2'',3''}$)	4.060 (3.0)	4.068 (2.8)	4.067 (3.2)
C-1 ($^1J_{C,H}$)	98.54 (170)	99.71 (171)	99.02 (172)
C-1′ ($^1J_{C,H}$)	100.66 (170)	100.45 (171)	100.64 (170)
C-1″ ($^1J_{C,H}$)	102.40 (170)	102.33 (170)	103.16 (169)
C-5″ ($^3J_{C,P}$)	72.77 (7)	72.73 (7)	73.20 (7)
C-6″ ($^2J_{C,P}$)	63.16 (4)	63.16 (3–4)	64.50 (3–4)
P	4.23	3.51	4.12

[a]In parentheses. [b]For solutions in deuterium oxide; experimental conditions and reference standards are described in the Experimental section. [c]Assignments are tentative. ^1H-Connectivities were established by homonuclear-decoupling.

tion with tetrabutylammonium fluoride[32,33]. Hydrogenolysis of the benzyl ethers of **27** in the presence of 5% palladium-on-carbon, followed by hydrogenolysis of the phosphate-protecting phenyl groups in the presence of Adam's catalyst (PtO_2), and *O*-deacetylation with sodium methoxide in methanol provided, after ion exchange on Dowex 50-X8 (Na^+), the disodium salt of the α-D-(1→2)-α-D-(1→2)-linked trisaccharide **5** (73% from **27**).

Deprotection of **28** and **29** in a similar fashion using hydrogenation over palladium-on-carbon, followed by Adam's catalyst, and finally *O*-deacetylation gave the target trisaccharides **6** (75%) and **7** (72%). Some key ^1H, ^{13}C-, and ^{31}P-n.m.r. data for **6**, **7**, and **8** are presented in Table I.

EXPERIMENTAL

General methods. — Melting points are uncorrected. 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). ^1H-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 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). The microanalyses were carried out by the Analytical Services Laboratory of this department. 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. 8-Methoxycarbonyloctanol was a generous gift from Chembiomed Ltd., Edmonton, Alberta, Canada.

2,3,4-Tri-O-acetyl-6-O-[(diphenyloxy)phosphoryl]-α-D-mannopyranosyl bromide (**10**). — Hydrogen bromide in glacial acetic acid (45%, w/v; 5 mL) containing 3% (v/v) acetic anhydride was added to a solution of 1,2,3,4-tetra-*O*-acetyl-6-*O*-[(diphenoxy)phosphoryl]-β-D-mannopyranose[24] (**9**; 1.20 g, 2.07 mmol) in dichloromethane (2 mL). After 4 h, the mixture was diluted with dichloromethane (50 mL) and washed 5 times with ice–water (50 mL) before drying (Na_2SO_4) and evaporation. T.l.c. examination of the residual syrup showed the presence of a single component (R_F 0.26 in 2:3 ethyl acetate–hexane) and this material was used in the glycosylation reactions without further purification; ^1H-n.m.r. ($CDCl_3$): δ 7.364–7.178 (m, 10 H, arom.), 6.237 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.717 (dd, 1 H, $J_{2,3}$ 2.3, $J_{3,4}$ 10.1 Hz, H-3), 5.425 (dd, 1 H, H-2), 5.374 (dd, 1 H, $J_{4,5}$ 10 Hz, H-4), 4.363 (dd, 2 H, $J_{5,6}$ 4.0, $J_{6,P}$ 7.5 Hz, H-6a,6b), and 4.265 (ddd, 1 H, $^4J_{5,P}$ 3 Hz, H-5).

8-Methoxycarbonyloctyl 3,4-di-O-benzyl-6-O-(tert-butyldimethylsilyl)-α-D-

mannopyranoside (**12**). — A solution of **11** (refs. 16, 17; 2.00 g, 3.77 mmol) and *tert*-butyldimethylsilyl chloride (681 mg, 4.52 mmol) in dry pyridine (15 mL) was stirred for 15 h. The mixture was poured into ice–water and, after 0.5 h, the solution was extracted with dichloromethane. The organic phase was taken to dryness and chromatography of the residue with 1:3 ethyl acetate–hexane as eluent provided **12** (1.95 g, 80%) as a syrup, $[\alpha]_D^{22}$ +31.9° (*c* 1.17 chloroform), R_F 0.50 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 4.825 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.660 (s, 3 H, CO_2CH_3), and 0.900 (s, 9 H, Me_3); ^{13}C-n.m.r. ($CDCl_3$): δ 138.65 and 138.19 (quat. arom.), 99.15 (C-1), 62.73 (C-6), 51.27 (OCH_3), 34.01 (CH_2CO_2), and 25.94 [$C(CH_3)_3$].

Anal. Calc. for $C_{36}H_{56}O_8Si$: C, 67.04; H, 8.75. Found: C, 66.86; H, 8.71.

*8-Methoxycarbonyloctyl 2,3,4-tri-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-*D*-mannopyranoside* (**13**). — Benzyl bromide (0.55 mL, 4.65 mmol) and Ag_2O (1.6 g) were added to a solution of **12** (1.0 g, 1.55 mmol) in dry *N,N*-dimethylformamide (7 mL) and the mixture was stirred for 48 h. Dichloromethane (50 mL) was added and the organic layer was washed with water (5 × 50 mL) before concentration to a syrup which was purified by chromatography with 1:4 ethyl acetate–hexane as eluent to provide **13** (790 mg, 69%) as a clear syrup, $[\alpha]_D^{22}$ +24.9° (*c* 0.85, chloroform), R_F 0.65 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 4.803 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.663 (s, 3 H, OCH_3), and 0.895 (s, 9 H, Me_3); ^{13}C-n.m.r. ($CDCl_3$): δ 138.92, 138.83 and 138.74 (quat. arom.), 97.73 (C-1), 62.97 (C-6), 51.33 (OCH_3), 34.10 (CH_2CO_2), and 25.98 [$C(CH_3)_3$].

Anal. Calc. for $C_{43}H_{62}O_8Si$: C, 70.26; H, 8.50. Found: C, 70.47; H, 8.55.

*8-Methoxycarbonyloctyl 2,3,4-tri-*O*-benzyl-α-*D*-mannopyranoside* (**14**). — Treatment of **13** (698 mg, 0.95 mmol) with tetrabutylammonium fluoride trihydrate (600 mg, 1.91 mmol) in oxolane (20 mL) for 5 h, followed by evaporation, gave crude **14** which was dissolved in dichloromethane (50 mL) and washed with water (3 × 20 mL) before concentration to a syrup. Chromatography with 1:3 ethyl acetate–hexane as eluent provided **14** (512 mg, 87%) as a clear syrup, $[\alpha]_D^{22}$ +32.9° (*c* 1.25, chloroform), R_F 0.28 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 4.781 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.659 (s, 3 H, OCH_3), and 1.999 (dd, 1 H, $J_{6,OH} = J_{6'OH}$ 7 Hz, OH); ^{13}C-n.m.r. ($CDCl_3$): δ 138.56, 138.47 and 138.42 (quat. arom.), 98.24 (C-1), 62.43 (C-6), 51.33 (CO_2CH_3), and 34.03 (CH_2CO_2).

*Anal.*Calc. for $C_{37}H_{48}O_8$: C, 71.59; H, 7.79. Found: C, 71.75; H, 7.59.

*8-Methoxycarbonyloctyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranoside* (**15**). — To a solution of 8-methoxycarbonyloctanol (964 mg, 5.12 mmol) in dry acetonitrile (15 mL) containing 4A molecular sieves were added sequentially $HgBr_2$ (2.77 g, 7.79 mmol) and $Hg(CN)_2$ (1.94 g, 7.79 mmol), followed by a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (7.7 mmol) in acetonitrile (15 mL). After being stirred for 15 h, the mixture was diluted with dichloromethane (50 mL), filtered, and evaporated to dryness. The residue was extracted three times with dichloromethane, and the extracts were combined and washed with saturated KCl (3 times), saturated $NaHCO_3$ (3 times), and twice with water, and solvent was

removed by evaporation. Chromatography using 1:2 ethyl acetate–hexane as eluent provided **15**, syrup (1.6 g, 60%), $[\alpha]_D^{22}$ +38.5° (*c* 1.22, chloroform): ^{1}H-N.m.r. ($CDCl_3$): δ 5.235 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, H-2), 4.803 (d, 1 H, H-1), 3.675 (s, 3 H, OCH_3), 2.163, 2.110, 2.053, and 2.002 (each s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 97.49 ($J_{C\text{-}1,H\text{-}1}$ 170.9 Hz), 51.17 (OCH_3), 33.92 (CH_2CO_2).

Anal. Calc. for $C_{24}H_{38}O_{12}$: C, 55.59; H, 7.39. Found: C, 55.50; H, 7.41.

*8-Methoxycarbonyloctyl 4,6-O-benzylidene-α-*D*-mannopyranoside* (**17**). - Treatment of **15** (1.40 g, 2.70 mmol) with methanolic sodium methoxide for 7 h, followed by neutralization with IRC-50 (H^+) and subsequent removal of the resin provided a solution of 8-methoxycarbonyloctyl α-D-mannopyranoside (**16**, R_F 0.59 in 4:1 ethyl acetate–hexane) which was not further characterized. Evaporation gave a white solid which was dried overnight *in vacuo* over P_2O_5. Compound **16** (699 mg, 2.00 mmol) was dissolved in *N,N*-dimethylformamide (15 mL), and benzaldehyde dimethylacetal (0.33 mL, 2.2 mmol) and tetrafluoroboric acid (2.2 mmol of a 54% solution in diethyl ether) were added. After 15 h, triethylamine (0.35 mL) was added and the solvent was evaporated. The residue was purified by chromatography using 1:1 ethyl acetate–hexane as eluent to provide **17** (650 mg, 74%), clear syrup, R_F 0.22 (1:1 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.537 (s, 1 H, $C_6H_5CHO_2$), 4.794 (br, 1 H, H-1), 3.646 (s, 3 H, OCH_3), and 3.50 (br, O*H*); ^{13}C-n.m.r. ($CDCl_3$): δ 137.36 (quat. arom.), 102.05 and 100.31 (C_6H_5CH and C-1), 68.77 (C-6), 51.19 (OCH_3), and 33.93 (CH_2CO_2).

Anal. Calc. for $C_{23}H_{34}O_8$: C, 62.99; H, 7.82. Found: C, 62.91; H, 7.90.

*8-Methoxycarbonyloctyl 4,6-O-benzylidene-2-O-benzyl-α-*D*-mannopyranoside* (**18**). — Compound **17** (597 mg, 1.36 mmol) was stirred with benzyl bromide (0.23 mL, 1.92 mmol) and Ag_2O (0.47 g) in *N,N*-dimethylformamide (5 mL) for 48 h. After dilution with chloroform (50 mL), washing with water (5 × 50 mL), and evaporation, the residue was purified by chromatography using 1:3 ethyl acetate–hexane as eluent to give **18** (298 mg, 41%), clear syrup, $[\alpha]_D^{22}$ +13.3° (*c* 1.27, chloroform), R_F 0.47 (2:3 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.581 (s, 1 H, $C_6H_5CHO_2$), 4.828 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.105 (br. 1 H, collapses to dd, $J_{2,3}$ 4.0, $J_{3,4}$ 9.5 Hz on irradiation of O*H*, H-3), 3.667 (s, 3 H, OCH_3), and 2.378 (br., 1 H, OH); ^{13}C-n.m.r. ($CDCl_3$): δ 137.79 and 137.45 (quat. arom.), 101.99 ($C_6H_5CHO_2$), 98.41 (C-1), 68.86 (C-6), 51.30 (OCH_3), and 34.01 (CH_2CO_2).

Anal. Calc. for $C_{30}H_{40}O_8$: C, 68.16; H, 7.63. Found: C, 68.27; H, 7.46.

*8-Methoxycarbonyloctyl 2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-*D*-mannopyranosyl)-3,4-di-O-benzyl-6-O-*tert*-butyldimethylsilyl-α-*D*-mannopyranoside* (**19**) *and 8-methoxycarbonyloctyl 2,6-di-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-*D*-mannopyranosyl)-3,4-di-O-benzyl-α-*D*-mannopyranoside* (**25**). — A solution of **8** (0.9 mmol) in 1,2-dichloroethane (3 mL) was added dropwise to a stirred mixture of **12** (302 mg, 0.47 mmol), silver trifluoromethanesulfonate (0.48 g, 1.87 mmol), and *N,N,N'N'*-tetramethylurea (0.17 mL, 1.41 mmol) in the same solvent (3 mL). After 1 h at 20°, more **8** (0.9 mmol) and *N,N,N',N'*-tetramethylurea (0.17 mL) were added and stirring was continued for an additional hour. The mixture was diluted with 1,2-

dichloroethane (10 mL), and 2,4,6-trimethylpyridine (0.20 mL) followed by silver trifluoromethanesulfonate (0.24 g) were added to eliminate excess **8**. After 0.5 h, tetraethylammonium bromide (0.20 g) was added to precipitate excess Ag, and solids were removed by filtration. The filtrate was washed twice with a saturated $NaHCO_3$ solution and twice with water, before evaporation and purification by chromatography using 1:3 ethyl acetate–hexane as eluent. The disaccharide **19** was obtained as a clear syrup (350 mg, 67%), $[\alpha]_D^{22}$ +19.8° (*c* 0.99, chloroform), R_F 0.64 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.551 (dd, 1 H, $J_{1',2'}$ 1.8, $J_{2',3'}$ 3.2 Hz, H-2'), 5.085 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.816 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.654 (s, 3 H, OCH_3), 3.203 (m, 1 H, OC*H*HCH_2), 2.117 (s, 3 H, $COCH_3$), and 0.874 [s, 9 H, $C(CH_3)_3$]; ^{13}C-n.m.r. ($CDCl_3$): δ 99.80 ($J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1), 98.72 ($J_{C\text{-}1',H\text{-}1'}$ 168 Hz, C-1'), 62.70 (C-6), 51.32 (OCH_3), 34.11 (CH_2CO_2), and 24.97 [$C(CH_3)_3$].

Anal. Calc. for $C_{65}H_{86}O_{14}Si$: C, 69.74; H, 7.74. Found: C, 69.74; H, 7.86.

Evaporation of the later fractions provided the trisaccharide **25** (80 mg, 12%), $[\alpha]_D^{22}$ +37.9° (*c* 0.19, chloroform), R_F 0.47 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.570 (dd, 1 H, $J_{1',2'}$ 1.8, $J_{2',3'}$ 3.2 Hz, H-2'), 5.410 (dd, 1 H, $J_{1'',2''}$ 1.7, $J_{2'',3''}$ 3.0 Hz, H-2''), 5.094 (d, 1 H, H-1'), 4.928 (d, 1 H, H-1''), 4.798 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.645 (s, 3 H, OCH_3), 2.109 and 2.103 (both s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 99.62, 98.48 and 97.46 (C-1,1',1''), 51.30 (OCH_3), and 34.00 (CH_2CO_2).

Anal. Calc. for $C_{88}H_{102}O_{20}$: C, 71.43; H, 6.95. Found: C, 70.97; H, 7.08.

*8-Methoxycarbonyloctyl 3,4-di-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-2-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**20**). — Deacetylation of **19** (299 mg, 0.27 mmol), as described for the preparation of **16**, gave **20** which was purified by chromatography using 1:3 ethyl acetate–hexane as eluent. Pure **20** was obtained as a syrup (250 mg, 86%), $[\alpha]_D^{22}$ +30.4° (*c* 0.53 chloroform), R_F 0.43 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.168 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.530 (d, 1 H, $J_{1,2}$ <2 Hz, H-1), 3.665 (s, 3 H, OCH_3), 2.452 (br., 1 H, OH), and 0.888 [s, 9 H, $C(CH_3)_3$]; ^{13}C-n.m.r. ($CDCl_3$): δ 101.27 (C-1'), 98.81 (C-1), 62.57 (C-6), 51.29 (OCH_3), 34.06 (CH_2CO_2), and 26.00 [$C(CH_3)_3$].

Anal. Calc. for $C_{63}H_{84}O_{13}Si$: C, 70.23; H, 7.86. Found: C, 70.25; H, 7.58.

*8-Methoxycarbonyloctyl 3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**21**). — A solution of **8** (1.18 mmol), in 1,2-dichloroethane (3 mL) was added dropwise to a stirred mixture of **18** (312 mg, 0.59 mmol), silver trifluoromethanesulfonate (303 mg, 1.18 mmol) and *N,N,N',N'*-tetramethylurea (212 mg, 1.77 mmol) in the same solvent (2 mL), and stirring was maintained for 2 h. After dilution with 1,2-dichloroethane (10 mL), the mixture was processed as described for the preparation of **19**. Chromatography using 1:3 ethyl acetate–hexane as eluent provided **21** as a syrup (480 mg, 81%), $[\alpha]_D^{22}$ +45° (*c* 0.27, chloroform), R_F 0.58 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.643 (s, 1 H, $C_6H_5CHO_2$), 5.620 (dd, 1 H, $J_{1',2'}$ 2.0, $J_{2',3'}$ 3.2 Hz, H-2'), 5.333 (d, 1 H, H-1'), 4.763 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.657 (s,

OCH_3), and 2.097 (s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 101.31 ($C_6H_5CHO_2$), 99.26 ($^3J_{C,H}$ 168 Hz) and 99.04 ($^3J_{C,H}$ 172 Hz) (C-1,1'), 64.17 (C-6), 51.34 (OCH_3), and 34.10 (CH_2CO_2).

Anal. Calc. for $C_{59}H_{70}O_{14}$: C, 70.64; H, 7.03. Found: C, 70.32; H, 6.88.

8-Methoxycarbonyloctyl 2-O-benzyl-4,6-O-benzylidene-3-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**22**). — Deacetylation of **21** (350 mg, 0.35 mmol) was accomplished as described for the preparation of **16**. Purification by chromatography using 1:3 ethyl acetate–hexane as eluent provided **22** as a clear syrup (285 mg, 85%), $[\alpha]_D^{22}$ +46° (*c* 0.19, chloroform), R_F 0.40 (1:2 ethyl acetate–hexane); ^{1}H-N.m.r. ($CDCl_3$): δ 5.592 (s, 1 H, $C_6H_5CHO_2$), 5.260 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.610 (d, 1 H, $J_{1,2}$ <2 Hz, H-1), and 3.658 (s, 3 H, OCH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 101.60, 100.69 and 99.16 ($C_6H_5CHO_2$, C-1,1'), 64.14 (C-6), 51.33 (OCH_3), and 34.10 (CH_2CO_2).

Anal. Calc. for $C_{57}H_{68}O_{13}$: C, 71.23; H, 7.13. Found: C, 70.74; H, 7.14.

8-Methoxycarbonyloctyl 6-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (**23**). — A solution of **8** (0.97 mmol) in 1,2-dichloroethane (2 mL) was added dropwise to a stirred mixture of **14** (301 mg, 0.49 mmol), silver trifluoromethanesulfonate (249 mg, 0.97 mmol), and *N,N,N',N'*-tetramethylurea (1.45 mmol) in the same solvent (3 mL). After 1 h, the mixture was processed as described for the preparation of **19**. Chromatography using 1:3 ethyl acetate–hexane as eluent provided **23** as a clear syrup (420 mg, 79%), $[\alpha]_D^{22}$ +33.4° (*c* 1.08, chloroform), R_F 0.47 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.495 (dd, 1 H, $J_{1',2'}$ 2.0, $J_{2',3'}$ 3.2 Hz, H-2'), 4.975 (d, 1 H, H-1'), 4.823 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 3.660 (s, 3 H, OCH_3), and 2.143 (s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 98.11 ($^3J_{C,H}$ 173 Hz) and 97.76 ($^3J_{C,H}$ 168 Hz) (C-1,1'), 51.31 (OCH_3), and 34.08 (CH_2CO_2).

Anal. Calc. for $C_{66}H_{78}O_{14}$: C, 72.37; H, 7.18. Found: C, 72.07; H, 7.21.

8-Methoxycarbonyloctyl 2,3,4-tri-O-benzyl-6-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**24**). — Deacetylation of **23** (350 mg, 0.32 mmol) was accomplished as described for the preparation of **16**. Chromatography using 1:2 ethyl acetate–hexane as eluent provided pure **24** as a clear syrup (285 mg, 85%), $[\alpha]_D^{22}$ +46.7° (*c* 0.67, chloroform), R_F 0.17 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.088 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.803 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 3.660 (s, 3 H, OCH_3), and 2.353 (d, 1 H, OH); ^{13}C-n.m.r. ($CDCl_3$): δ 99.79 (C-1), 97.84 (C-1'), 51.33 (OCH_3), and 34.10 (CH_2CO_2).

Anal. Calc. for $C_{64}H_{76}O_{13}$: C, 72.98; H, 7.27. Found: C, 73.07; H, 7.32.

8-Methoxycarbonyloctyl 2-O-[2,3,4-tri-O-acetyl-6-O-(diphenoxy)phosphoryl-α-D-mannopyranosyl]-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-3,4-di-O-benzyl-6-O-tert-butyldimethylsilyl-α-D-mannopyranoside (**26**). — Bromide **10** (0.40 mmol) in 1,2-dichloroethane (1 mL) was added to a stirred mixture of **20** (170 mg, 0.16 mmol), *N,N,N',N'*-tetramethylurea (71 μL, 0.59 mmol), and silver trifluoromethanesulfonate (304 mg, 1.19 mmol) in 3 mL of the same solvent. After both 5 h and a further 15 h, more tetramethylurea (71 μL) and **10** (0.40

mmol) were added. After an additional 36 h, the mixture was diluted with 1,2-dichloroethane (10 mL) and 2,4,6-trimethylpyridine, followed by excess silver trifluoromethanesulfonate in order to remove any unreacted **10**, as described for the preparation of **19**. After a further 0.5 h, excess Ag was precipitated with tetraethylammonium bromide. Processing, as described for **19**, followed by chromatography using 1:2 ethyl acetate–hexane gave **26** as a clear syrup (125 mg, 50%), $[\alpha]_D^{22}$ +34.8° (*c* 0.81, chloroform), R_F 0.28 (3:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.440–5.405 (m, 2 H, H-2″,3″), 5.328 (dd, $J_{3'',4''} = J_{4'',5''}$ 10 Hz), 5.215 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.873 (H-1″, $J_{1'',2''}$ <2 Hz), 4.862 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.660 (s, 3 H, OCH_3), 2.028, 2.003, 1.935 (each s, 3 H, $COCH_3$), and 0.885 [s, 9 H, $C(CH_3)_3$]; ^{13}C-n.m.r. ($CDCl_3$); δ 100.87 ($^1J_{C,H}$ 171 Hz), 99.23 ($^1J_{C,H}$ 173 Hz) and 98.86 ($^1J_{C,H}$ 170 Hz) (C-1,1′,1″), 67.16 (C-6″, broadened by $^3J_{P,H}$), 62.73 (C-6), 51.35 (OCH_3), 34.11 (CH_2CO_2), and 26.10 [$C(CH_3)_3$]; ^{31}P-n.m.r. ($CDCl_3$): δ −12.52.

Anal. Calc. for $C_{87}H_{109}O_{24}PSi$: C, 65.39; H, 6.87. Found: C, 65.39; H, 6.88.

8-Methoxycarbonyloctyl O-[*2,3,4-tri*-O-*acetyl*-*6*-O-*(diphenoxy)phosphoryl*-α-D-*mannopyranosyl*]-*(1→2)*-O-*(3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl)*-*(1→3)*-*2*-O-*benzyl*-*4,6*-O-*benzylidene*-α-D-*mannopyranoside* (**28**). — Disaccharide **22** (198 mg, 0.21 mmol) was condensed with bromide **10** (8 equiv.), added in three equal portions as described for the preparation of **26**. Chromatography using 2:3 ethyl acetate–hexane as eluent gave **28** as a syrup (170 mg, 56%), $[\alpha]_D^{22}$ +44.5° (*c* 0.31, chloroform), R_F 0.20 (2:3 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.523 (s, 1 H, $C_6H_5CHO_2$), 5.393–5.270 (4 H, H-1′,2″,3″,4″), 5.010 (d, 1 H, $J_{1'',2''}$ 1.6 Hz, H-1″), 4.743 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.367 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.0 Hz, H-3), 3.655 (s, 3 H, OCH_3), 1.993, 1.977, and 1.937 (all s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 101.86 ($C_6H_5CHO_2$), 99.67 ($^1J_{C,H}$ 173 Hz), 99.16 ($^1J_{C,H}$ 167 Hz) and 98.47 ($^1J_{C,H}$ 172 Hz) (C-1,1′,1″), 66.40 ($^3J_{C,P}$ 5 Hz, C-6″), 51.32 (OCH_3), and 34.06 (CH_2CO_2); ^{31}P-n.m.r. ($CDCl_3$): δ −12.59.

Anal. Calc. for $C_{81}H_{93}O_{24}P$: C, 65.66; H, 6.33. Found: C, 65.45; H, 6.30.

8-Methoxycarbonyloctyl O-[*2,3,4-tri*-O-*acetyl*-*6*-O-*(diphenoxy)phosphoryl*-α-D-*mannopyranosyl*]-*(1→2)*-O-*(3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl)*-*(1→6)*-*2,3,4-tri*-O-*benzyl*-α-D-*mannopyranoside* (**29**). — Disaccharide **24** (202 mg, 0.19 mmol) was condensed with bromide **10** (1.4 mmol), added in three portions, as described for the preparations of **26** and **28**. Processing of the mixture as just described, followed by chromatography using 2:3 ethyl acetate–hexane as eluent, gave **29** as a syrup (195 mg, 65%), $[\alpha]_D^{22}$ +43.2° (*c* 0.33, chloroform), R_F 0.24 (2:3 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.448–5.410 (m, 2 H, H-2″,3″), 5.348 (dd, 1 H, $J_{3'',4''} = J_{4'',5''}$ 10 Hz, H-4″), 5.080 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.910 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.748 (H-1, $J_{1,2}$ <2 Hz), 3.653 (s, 3 H, OCH_3), 2.020, 1.995 and 1.910 (each s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 99.37 ($^1J_{C,H}$ 170 Hz), 99.19 ($^1J_{C,H}$ 170 Hz) and 97.95 ($^1J_{C,H}$ 168 Hz) (C-1,1′,1″), 67.35 (C-6″, $^3J_{C,P}$ 5 Hz), 51.37 (OCH_3), and 34.11 (CH_2CO_2); ^{31}P-n.m.r. ($CDCl_3$): δ −12.46.

Anal. Calc. for $C_{88}H_{101}O_{24}P$; C, 67.17; H, 6.47. Found: C, 67.03; H, 6.55.

8-Methoxycarbonyloctyl O-[*2,3,4-tri*-O-*acetyl*-6-O-*(diphenoxy)phosphoryl*-α-D-*mannopyranosyl*]-*(1→2)*-O-*(3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl)*-*(1→2)*-*3,4-di*-O-*benzyl*-α-D-*mannopyranoside* (**27**). — Compound **26** (125 mg, 0.08 mmol) was dissolved in dichloromethane (1 mL) and 80% aqueous acetic acid (15 mL) was added. After 24 h, the solvent was evaporated and chromatography of the residue using 2:3 ethyl acetate–hexane as eluent provided **27** as a syrup (100 mg, 86%), $[\alpha]_D^{22}$ +41.3° (*c* 0.40, chloroform), R_F 0.18 (1:2 ethyl acetate–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 5.324 (dd, $J_{3'',4''} = J_{4'',5''}$ = 10 Hz, H-4''), 5.230 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.630 (H-1'', $J_{1'',2''}$ <2 Hz), 4.590 (d, 1 H, $J_{1,2}$ <2 Hz), 3.645 (s, 3 H, OCH_3), 2.075 (br, OH), 2.055, 2.008, and 1.932 (each s, 3 H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 100.30, 98.88 and 98.76 (C-1,1',1''), 67.07 (C-6'', $^3J_{C,P}$ 5 Hz), 62.15 (C-6), 51.27 (OCH_3), and 33.97 (CH_2CO_2).

Anal. Calc. for $C_{81}H_{95}O_{24}P$: C, 65.57; H, 6.46. Found: C, 65.42; H, 6.40.

8-Methoxycarbonyloctyl O-*(*α-D-*mannopyranosyl disodium 6-phosphate)*-*(1→2)*-O-α-D-*mannopyranosyl*-*(1→2)*-α-D-*mannopyranoside* (**5**). — Compound **27** (102 mg, 0.069 mmol) was dissolved in 95% ethanol (3 mL) containing 5% Pd–C (50 mg) and stirred under H_2 (0.1 MPa) for 15 h, by which time t.l.c. showed a major product (~80%) with R_F 0.80 (60:35:6 chloroform–methanol–water) and a more polar product (~20%) with R_F 0.60 (same solvent), presumably the monophenyl phosphate. The catalyst was removed by filtration and washed with 95% ethanol (25 mL), and the filtrate evaporated and redissolved in 95% ethanol (3 mL). Hydrogenation (0.1 MPa) in the presence of Adam's catalyst (PtO_2, 20 mg) for 3 h provided a product with R_F 0.37 (60:35:6 chloroform–methanol–water) which was devoid of u.v. absorption in t.l.c. Removal of the catalyst by filtration, followed by evaporation and drying for 15 h (P_2O_5), gave a glass which was dissolved in dry methanol containing a trace of sodium methoxide. The solution was kept at 0° until all the material was converted into a single compound (R_F 0.30; 4:1 2-propanol–water). After neutralization with IRC-50 (H^+), removal of the resin, and evaporation, the residue was passed through a column of Bio-Gel P-2 (200–400 mesh) (50 × 2.5 cm) with 10% ethanol as eluent. The carbohydrate-containing fractions were pooled, concentrated, and passed through a column of Dowex 50-X8 (Na^+; 10 mL). The eluate was lyophilized to provide **5** (40.2 mg, 73%), white powder, $[\alpha]_D^{22}$ +50.6° (*c* 0.174, water); ^{1}H-n.m.r. (D_2O): δ 5.325 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1'), 5.042 (d, 1 H, $J_{1'',2''}$ 1.6 Hz, H-1''), 4.985 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.125 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.060 (dd, 1 H, $J_{2'',3''}$ 3.0 Hz, H-2''), 4.006 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.686 (s, OCH_3), 3.534 (m, 1 H, $OCHHCH_2$), and 2.387 (t, 2 H, CH_2CO_2); ^{13}C-n.m.r. (D_2O): δ 178.04 (CO_2CH_3), 102.40 ($^1J_{C,H}$ 170 ±3 Hz, C-1''), 100.66 ($^1J_{C,H}$ 170 ±3 Hz, C-1'), 98.54 ($^1J_{C,H}$ 170 Hz, C-1), 78.18, 77.62, 73.53, 72.99, 72.77 (d, $^3J_{C,P}$ 7 Hz, C-5''), 70.57, 70.21 (2 C), 70.16, 68.14, 67.10, 66.89, 66.48, 63.16 (d, $^2J_{C,P}$ 4 Hz, C-6''), 61.14 and 61.06 (C-6,6'), 52.22 (OCH_3), 33.87 (CH_2CO_2), 28.58, 28.38, 28.33 (2 C), 28.29, 25.39, and 24.43; ^{31}P-n.m.r. (D_2O): δ 4.23.

Anal. Calc. for $C_{28}H_{49}Na_2O_{21}P \cdot H_2O$: C, 41.17; H, 6.30. Found: C, 41.15; H, 6.32.

8-Methoxycarbonyloctyl O-*(α-*D*-mannopyranosyl disodium 6-phosphate)-(1→2)*-O-*α-*D*-mannopyranosyl-(1→3)*-*α*-*D*-mannopyranoside* (**6**). — Compound **28** (101 mg, 0.068 mmol) was deprotected, as described for the preparation of **5**, with 5% Pd–C (50 mg) for 40 h, followed by Adam's catalyst (20 mg) for 3 h. After deacetylation at 0°, Bio-Gel P-2 chromatography, and Dowex 50-X8 (Na^+) treatment and lyophilization, **6** was obtained as a white powder (41 mg, 75%), $[\alpha]_D^{22}$ +65.1° (*c* 0.166, water), R_F (4:1 2-isopropanol–water); ^{1}H-n.m.r. (D_2O): δ 5.369 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1'), 5.068 (d, 1 H, $J_{1'',2''}$ 1.7 Hz, H-1''), 4.832 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.120 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.068 (dd, 1 H, $J_{2'',3''}$ 2.8 Hz, H-2''), 4.029 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.687 (s, OCH_3), 3.537 (OC*H*HCH$_2$), and 2.388 (t, 2 H, *J* 7.4 Hz, CH_2CO_2); ^{13}C-n.m.r. (D_2O): δ 178.01 (*C*O_2CH_3), 102.33 ($^1J_{C,H}$ 170 Hz, C-1''), 100.45 ($^1J_{C,H}$ 171 Hz, C-1') and 99.71 ($^1J_{C,H}$ 171 Hz, C-1), 77.67, 76.81, 73.52, 73.03, 72.73 (d, $^3J_{C,P}$ 7 Hz, H-5''), 70.37, 70.17, 70.10, 70.01, 68.14, 67.02, 66.69, 66.24, 63.16 (br., $^2J_{C,P}$ ≈ 3–4 Hz, C-6''), 61.12 and 60.80 (C-6,6'), 52.22 (OCH_3), 33.87 (*C*H_2CO_2), 28.50, 28.36, 28.34, 28.39, 25.34, and 24.42; ^{31}P-n.m.r. (D_2O): δ 3.51.

Anal. Calc. for $C_{28}H_{49}Na_2O_{21}P \cdot 1.5\ H_2O$: C, 40.73; H, 6.35. Found: C, 40.61; H, 6.28.

8-Methoxycarbonyloctyl O-*(α-*D*-mannopyranosyl disodium 6-phosphate)-(1→2)*-O-*α-*D*-mannopyranosyl-(1→6)*-*α*-*D*-mannopyranoside* (**7**). — Compound **29** (120 mg, 0.08 mmol) was deprotected as described for the preparation of **5**. The disodium salt (R_F 0.30, 4:1 2-propanol–water) was isolated as white lyophilized powder (44 mg, 72%), $[\alpha]_D^{22}$ +47.1° (*c* 0.23, water); ^{1}H-n.m.r. (D_2O): δ 5.129 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1'), 5.027 (d, 1 H, $J_{1'',2''}$ 1.8 Hz, H-1''), 4.850 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.067 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-2''), 3.984 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.929 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.688 (s, OCH_3), 3.555 (m, 1 H, OC*H*HCH$_2$), and 2.392 (t, 2 H, *J* 7.5 Hz); ^{13}C-n.m.r. (D_2O): δ 178.55 (*C*O_2CH_3), 103.16 ($^1J_{C,H}$ 169 Hz, C-1''), 100.64 ($^1J_{C,H}$ 170 Hz, C-1'), 99.02 ($^1J_{C,H}$ 172 Hz, C-1), 79.65, 73.63, 73.20 (d, $^3J_{C,P}$ 7 Hz, C-5''), 72.20, 71.71, 71.15, 70.98 (2 C), 70.87, 68.84, 67.84, 67.64, 67.20, 67.13, 64.50 (br., $^2J_{C,P}$ ~3–4 Hz, C-6''), 61.80 (C-6'), 52.91 (OCH_3), 34.56 (*C*H_2CO_2), 29.27, 29.02 (2 C), 28.95, 26.12, and 25.13; ^{31}P-n.m.r. (D_2O): δ 4.12.

Anal. Calc. for $C_{28}H_{49}Na_2O_{21}P \cdot 2\ H_2O$: C, 40.29; H, 6.40. Found: C, 39.93; H, 6.26.

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