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# SYNTHESIS OF THE 6- AND 6′-PHOSPHATES OF 8-METHOXYCARBONYLOCTYL 2-*O*-α-D-MANNOPYRANOSYL-α-D-MANNOPYRANOSIDE

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ABSTRACT

Phosphorylated derivatives of 2-*O*-α-D-mannopyranosyl-α-D-mannopyranose bearing phosphoric monoesters at OH-6′ (**1**), OH-6 (**2**), and at both OH-6 and -6′ (**3**) were prepared from 8-methoxycarbonyloctyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4-di-*O*-benzyl-6-*O*-(4-methoxyphenyldiphenylmethyl)-α-D-mannopyranoside, from which each of the two primary hydroxyl groups was selectively deprotected for subsequent phosphorylation with diphenyl phosphorochloridate.

## INTRODUCTION

D-Mannose 6-phosphate (Man-6-*P*) is well known as the essential component of a recognition marker involved in the targeting of newly biosynthesized lysosomal enzymes to the lysosomes[1–3]. Cell surface receptors having a high affinity for Man-6-*P* have also been demonstrated on fibroblasts[4], Chinese hamster ovary cells[5,6], macrophages[7], and mouse lymphoma cells[8] where they mediate the internalization of phosphorylated glycoconjugates[9,10].

The Man-6-*P* recognition marker in these systems is normally present on "high-mannose", asparagine-linked oligosaccharides which bind to the receptors with affinities far greater than that of the simple Man-6-*P* monosaccharide (reviewed in ref. 10). These observations suggested that the receptors are able to recognize structures more complex than Man-6-*P* and which are present in the asparagine-linked carbohydrates. The elegant work of Varki and Kornfeld[11,12], and of Natowicz, Baenziger, and Sly[13] has shown that the phosphorylated oligosaccharides of lysosomal enzymes are highly heterogeneous in structure and may possess one, two, or even three Man-6-*P* residues, some of which are present in a phosphoric diester linkage to a 2-acetamido-2-deoxy-D-glucosyl group[11–13]. The unprocessed, high-mannose oligosaccharide[11] (**1**) (Scheme 1) is esterified at either terminal or subterminal units on the α-D-Man*p*-(1→2)-α-D-Man*p* terminal group.

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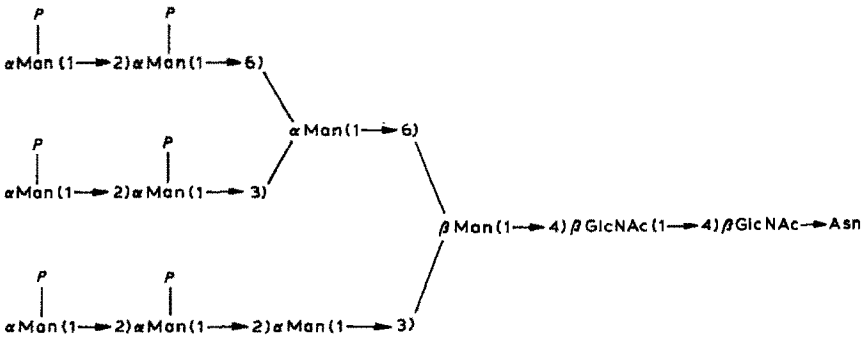



Scheme 1. The unprocessed, high-mannose, asparagine-linked oligosaccharide of lysosomal enzymes. The symbol *P* indicates residues where 6-phosphoric mono- or di-esters have been located[11–13]. The D configuration of the GlcNAc and Man units are assumed.

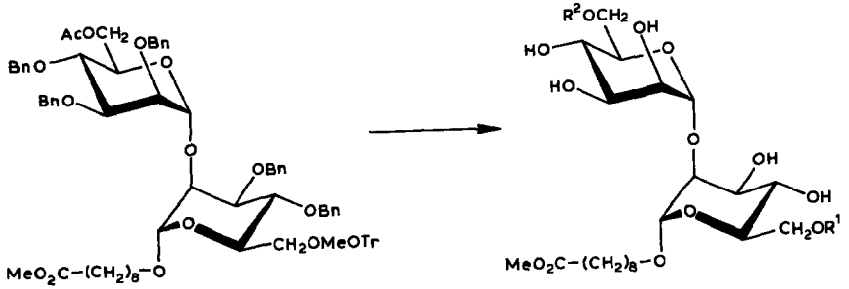
Lower-molecular-weight phosphorylated oligosaccharide components, where outer nonphosphorylated mannose residues have been enzymically cleaved, are also found in lysosomal enzymes[11–13].

In an effort to gain added insights into the structural requirements for high-affinity binding to the Man-6-*P* receptor, we have embarked on the synthesis of an extensive series of phosphorylated di- and tri-mannosides corresponding to partial structures of oligosaccharide **1**. We report herein the synthesis of the *O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside disaccharides **3–5** bearing phosphate groups on the terminal D-mannosyl group (**3**) and the subterminal D-mannosyl residue (**4**), as well as on both D-mannose units (**5**). These structures correspond to the three possible phosphorylated terminal groups of the unprocessed oligosaccharides. Compounds **3–5** were synthesized as their 8-methoxycarbonyloctyl glycosides[14,15] to allow for their eventual attachment to solid supports, protein carriers, and fluorescent or radioactive tags. These glycoconjugates should prove valuable both in examining the effect of polyvalency[16–20] on binding with the receptor and in studies on the endocytosis and intracellular transport[21–23] of the Man-6-*P* recognition marker.

## RESULTS AND DISCUSSION

Compounds **3–5** were prepared from a common *O*-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside disaccharide precursor **2** which was protected in a manner that allowed the selective liberation of either, or both, of the primary hydroxyl groups for subsequent phosphorylation. To achieve this protection pattern, the benzyl ether was used as the persistent blocking group for the secondary hydroxyl groups, and OH-6 was protected as the acid-labile 4-methoxyphenyldiphenylmethyl (mono-4-methoxytrityl, MeOTr) ether, and OH-6′ as the base-labile acetyl ester. This key disaccharide intermediate (**2**) could be assembled by a

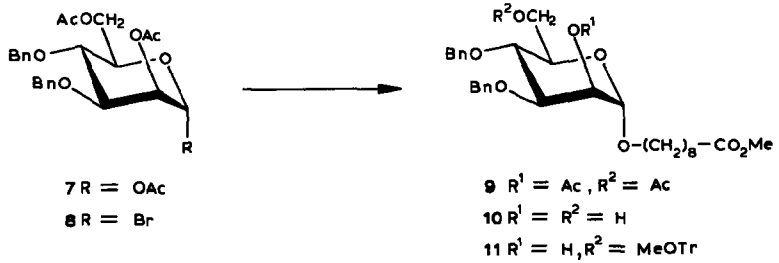
straightforward procedure making use of the ready availability of the protected D-mannose derivatives **8** and **13**.




**2**

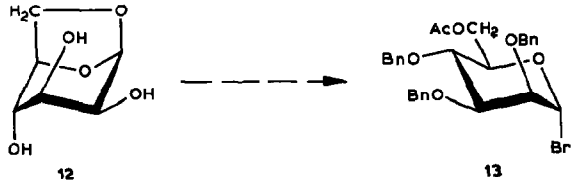
**3** $R^1 = H, R^2 = P$
**4** $R^1 = P, R^2 = H$
**5** $R^1 = R^2 = P$
**6** $R^1 = R^2 = H$

Reaction of the triacetate **7**, prepared according to Ponpipom[24], with hydrogen bromide in dichloromethane at room temperature provided the α-bromide **8**. Reaction of **8** with 8-methoxycarbonyloctanol[14] in the presence of mercuric bromide and mercuric cyanide in acetonitrile produced the α-D-linked glycoside **9** (81%). Deacetylation of **9** gave the diol **10** which could be selectively protected at the primary hydroxyl group by reaction with chloro(4-methoxyphenyl)diphenylmethane in pyridine to provide the alcohol **11** (79%) where the signal for unreacted OH-2 appeared as a doublet (δ 2.479, $J_{OH,H-2}$ 2.5 Hz) in the $^1$H-n.m.r. spectrum recorded for a solution in ($^2$H)chloroform. The elaboration of **11** into **15** required its glycosylation by bromide **13** which was prepared from D-mannose following literature procedures, specifically conversion of D-mannose into 1,6-anhydro-D-mannose (**12**) by the convenient procedure of Georges and Fraser-Reid[25], benzylation[26], and acetolysis of the anhydro ring, followed by treatment with hydrogen bromide in dichloromethane according to Paulsen and Lockhoff[27].



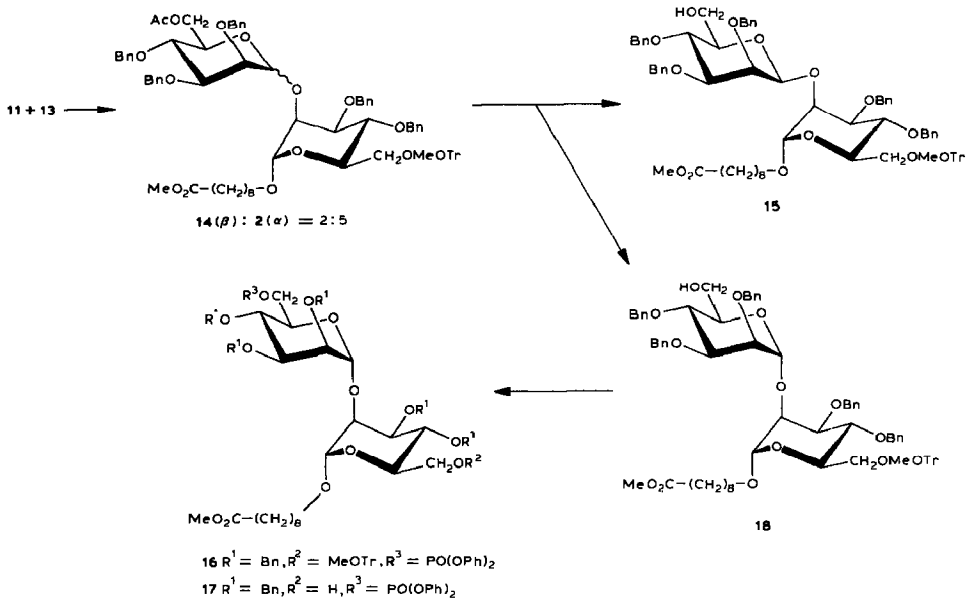

**7** R = OAc
**8** R = Br

**9** $R^1 = Ac, R^2 = Ac$
**10** $R^1 = R^2 = H$
**11** $R^1 = H, R^2 = MeOTr$




**12** **13**

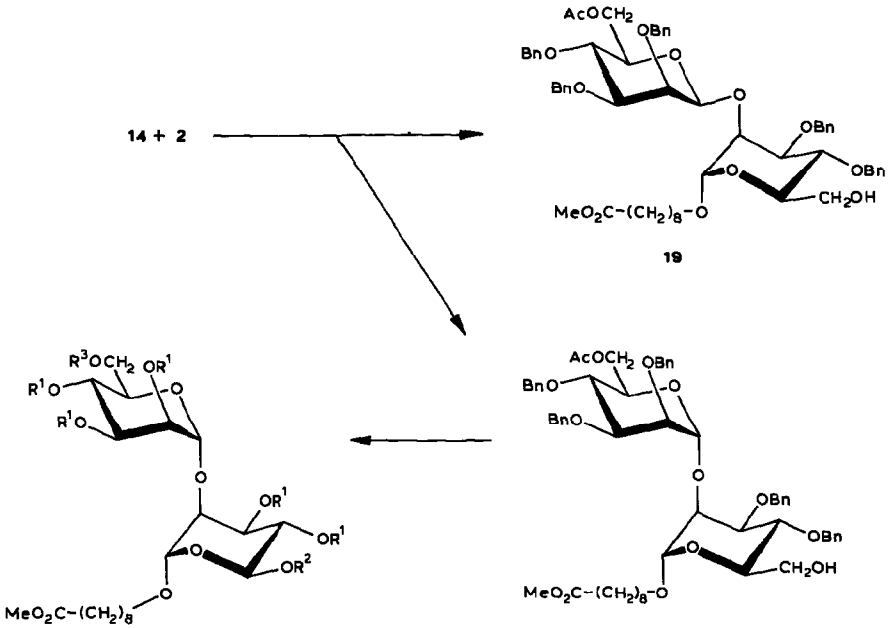
Coupling of **11** with **13** in the presence of silver trifluoromethanesulfonate, 2,4,6-trimethylpyridine, and molecular sieve 4A in dichloromethane produced a mixture of the $\beta$ and $\alpha$ disaccharides **14** and **2** in 70–80% yield. These disaccharide anomers could not be separated chromatographically at this stage, but their ratio could be determined from the $^1$H-n.m.r. spectrum of the crude reaction product and was found to vary with the temperature of the reaction. At −40°, **14** and **2** were produced in a near 1:1 ratio, and at 0° the ratio of **14** ($\beta$) to **2** ($\alpha$) was 2:5. Higher reaction temperatures were not examined. The formation of a significant proportion of $\beta$-linked disaccharide was not unexpected and has been observed by others[27–30] when 2-*O*-benzylated mannopyranosyl halides were used in the presence of various promoters. Since the anomeric products could be readily separated after removal of either the acetyl or MeOTr protecting groups, the formation of **14** was not considered a major problem and further reaction conditions were, therefore, not explored.




Removal of the acetyl group of **2** and **14** with sodium methoxide in methanol produced **15** and **18** whose anomeric configurations could be assigned from the $^1$H-coupled $^{13}$C-n.m.r. spectrum. The $\beta$-linked disaccharide **15**, the minor component, showed $^1J_{C\text{-}1',H\text{-}1'}$ 154.5 and its $\alpha$ anomer **18** 168.5 Hz, in accord with the empirical rules formulated by Bock and Pedersen[31] for the dependence of the one-bond C–H coupling constants on the anomeric configuration. Phosphorylation of **18** with diphenyl phosphorochloridate and 4-dimethylaminopyridine in pyridine gave the phosphoric triester **16** (83%). The position of the phosphoryl group in **16** could be confirmed by the observation of coupling between $^{31}$P and C-6′ ($\delta$ 68.24,

$^{2}J_{C,P}$ 7.0 Hz), and C-5′ (δ 71.36, $^{3}J_{C,P}$ 6.5 Hz). The MeOTr group of **16** was removed with 4:2:1 acetic acid–dichloromethane–water at room temperature for 4 h to provide **17** in 87% yield after chromatography. The benzyl and phenyl protecting groups of **17** were then removed successively by hydrogenolysis in the presence of 10% palladium-on-carbon and Adam's catalyst (platinum dioxide). The target disaccharide **3** was obtained as the disodium salt in 85% yield from **17** after passage through Dowex 50 ion-exchange resin in the sodium form.

Removal of the MeOTr group from the anomeric mixture of **14** and **2** provided the α-linked disaccharide **23** and its β anomer **19** which were separated by silica gel chromatography. The α-linked disaccharide **23** showed the resonance for C-1′ at δ 99.34 ($^{1}J_{C\text{-}1',H\text{-}1'}$ 171 Hz) and the β-disaccharide the corresponding signal at δ 99.60 ($^{1}J_{C\text{-}1',H\text{-}1'}$ 155 Hz) in support[31] of the assigned structures. Reaction of **23** with diphenyl phosphorochloridate gave **20** where the coupling of $^{31}P$ with C-6 (δ 68.16, $^{2}J_{C,P}$ 4.5 Hz) and C-5 (δ 70.80, $^{3}J_{C,P} \approx 7$ Hz) could be observed in the $^{13}C$-n.m.r. spectrum, thus confirming the position of the phosphoryl group. Sequential removal of the benzyl, phenyl, and acetyl protecting groups of **20** then yielded, after ion exchange, the disodium salt of the 6-phosphate **4** (81% from **20**).



To prepare the diphosphate **5**, the alcohol **23** was deacetylated with sodium methoxide in methanol to provide the diol **21** (94%), the structure of which was substantiated by the observation of two hydroxylic protons, both doublets of doublets due to the coupling with H-6, in the $^1$H-n.m.r. spectrum recorded for a solution in ($^2$H)chloroform. Phosphorylation of **21** provided the diphosphate **22** (85%), the $^{13}$C-n.m.r. spectrum of which showed the expected doublets for the two C-5 atoms ($\delta$ 71.56, $^3J_{C,P}$ 7.5 and $\delta$ 70.72, $^3J_{C,P}$ 8.5 Hz). The signals for C-6 and -6′ overlapped at $\delta$ 68.19 obscuring the two-bond $^{13}$C–$^{31}$P coupling constants. Depro-

TABLE I

SELECTED CHEMICAL SHIFTS ($\delta$) AND COUPLING CONSTANTS (Hz)[a] FOR 2-*O*-($\alpha$-D-MANNOPYRANOSYL)-$\alpha$-D-MANNOPYRANOSE DERIVATIVES

| *Compound* | *$^{13}$C-N.m.r.*[b] | | | | | | |
|---|---|---|---|---|---|---|---|
| | *C-1* | *C-1′* | *C-2* | *C-5* ($^3J_{C,P}$) | *C-5′* ($^3J_{C,P}$) | *C-6* ($^2J_{C,P}$) | *C-6′* ($^2J_{C,P}$) |
| **3** | 99.10 | 103.10 | 79.51 | | 73.09 (7.5) | 61.71 | 64.55 (4.5) |
| **4** | 99.24 | 103.10 | 79.22 | 73.24 (7.0) | | 63.61 (4.5) | 61.97 |
| **5** | 99.44 | 103.09 | 78.84 | 73.26[c] (7.5) | 72.99[c] (7.0) | 64.20[d] (~4) | 64.25[d] (~4) |
| **6** | 98.95 | 103.19 | 79.58 | | | 61.95[e] | 61.85[e] |
| | *$^1$H-N.m.r.*[f] | | | | | | |
| | *H-1* ($J_{1,2}$) | *H-2* ($J_{2,3}$) | *H-6a* ($J_{5,6a}$) ($J_{6a,6b}$) ($J_{6a,P}$) | *H-1′* ($J_{1',2'}$) | *H-2′* ($J_{2',3'}$) | *H-6′a* ($J_{5',6'a}$) ($J_{6'a,6'b}$) ($J_{6'a,P}$) | |
| **3** | 5.021 (1.4) | 3.949 (3.2) | | 5.041 (1.6) | 4.057 (2.8) | 4.096 (3.5) (12.2) (6.5) | |
| **4** | 5.049 (1.5) | 3.954 (3.0) | 4.081 (3.4) (12.0) (7.0) | 5.026 (1.7) | 4.093 (3.4) | | |
| **5** | 4.982 (1.6) | 4.090 (3.2) | 4.129 (3.8)[g] (11.8) (6.6) | 5.069 (1.7) | 3.973 (3.0) | 4.09[g] | |
| **6** | 5.094 (1.6) | 3.946 (3.4) | | 5.023 (1.8) | 4.073 (3.3) | | |

[a]In parentheses. [b]At 90 MHz, for a solution in $D_2O$ at 308°K. [c,d,e]May be interchanged. [f]At 360 MHz, for a solution in $D_2O$ at 297°K. [g]Measured at 338°K; exact chemical shifts and coupling constants could not be determined at 297°K owing to signal overlap.

tection of **22** by hydrogenolysis finally provided, after ion exchange, the 6,6′-diphosphate **5** (84%) as the tetrasodium salt.

Table I presents selected $^{13}C$- and $^{1}H$-n.m.r. data for the new compounds **3–5** as well as for the parent disaccharide 8-methoxycarbonyloctyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside[32] (**6**). In each case, phosphorylation of the primary hydroxyl groups caused a downfield shift of the corresponding C-6 signals by ~2 p.p.m. and these signals showed the expected $^{2}J_{C,P}$ 4.5 Hz. In addition, the signals for the corresponding C-5 atoms could be assigned due to their coupling with $^{31}P$ ($^{3}J_{C,P}$ 7.0–7.5 Hz). The chemical shifts for the carbon atoms involved in the glycosidic linkages are also seen to remain remarkably constant throughout this series of compounds with C-1 at δ 99.20 ±0.25, C-1′ at 103.14 ±0.05, and C-2 at 79.21 ±0.37. Since it is widely held[33–35] that the chemical shifts of both the glycosidic and aglyconic carbon atoms involved in a glycosidic linkage should be sensitive to changes in conformation about that glycosidic linkage, the inference is that phosphorylation of **6** to produce **3–5** causes, at most, minor changes in the conformation of the disaccharide.

The complex $^{1}H$-n.m.r. spectra of **3–5** were not completely assigned, but some relevant data are included in Table I. Phosphorylation of the primary OH groups of **6** caused, in each case, a characteristic downfield shift of near 0.2 p.p.m. in the signal for one of the adjacent H-6 (labelled H-6a). This signal, which is normally found in the region δ 3.75–3.90 in α-D-mannopyranosides[32], where it is obscured by other resonances, was shifted to near δ 4.1 in a region where the only other signal present was that for H-2′. The couplings of H-6a in **3–5** with H-5 (3.4–3.8 Hz) and H-6b (11.8–12.2 Hz) were therefore readily measurable and proved very similar in each case. The measurement of $^{31}P$-decoupled $^{1}H$-n.m.r. spectra allowed the assignment of the two-bond $^{31}P$–$^{1}H$ coupling constants which were all in the range 6.5–7.0 Hz.

It was tempting* to assign the lowest-field anomeric proton in each of the disaccharides in Table I to the reducing end D-mannose unit as was done[32] for **6**. If this assignment were made, however, our decoupling experiments would require that phosphorylation of the 6′-OH group of **6** (to form **3**) causes a deshielding of 0.111 p.p.m. for H-2 and a concomitant shielding of 0.124 p.p.m. for H-2′. Such chemical-shift changes would suggest that a change in conformation about a glycosidic linkage accompany the phosphorylation, which is not consistent with the $^{13}C$-n.m.r. data discussed above. Definitive assignments for H-1 and -1′ of **3–5** were, therefore, sought from nuclear Overhauser enhancement (n.O.e.) experiments[34,37].

For compounds **3–6**, the signal for one of the aglyconic protons (dt, $^{2}J$ 9.5, $^{3}J$ 6.3 Hz) of the 8-methoxycarbonyloctyl group was clearly visible as the highest

*The deshielding of H-1 of *O*-α-D-mannopyranosyl-(1→2)-α-D-mannose disaccharides was proposed[36] to arise from the proximity of O-5′ (of the nonreducing D-mannosyl group) to H-1 in the preferred solution conformation.

field proton in the "envelope" region of the spectrum and always occurred in the range $\delta$ 3.52–3.57. Saturation of this signal provided enhancements of 3–8% in the intensity of the signal for only one of the anomeric protons in each of these compounds, thus allowing, with decoupling experiments, the unambiguous assignment of H-1, -1′, -2, and -2′ presented in Table I. The effect of phosphorylation of **6** to produce **3**, **4**, or **5** may, then, be seen to cause only trivial changes in the chemical shifts for H-2 and -2′, in accord with our proposal for an unchanged conformation. Only modest changes in the chemical shifts for H-1 and -1′ can be seen to accompany the phosphorylation of **6**, most noticeably the upfield shifting of the signal for H-1 observed on phosphorylation of 6′-OH. Although this may indeed reflect some conformational adjustment, changes of such small magnitude are difficult to interpret[38]. It might equally well be caused by the proximity of the 6′-phosphate group to H-1 in the unperturbed conformation.

## EXPERIMENTAL

*General methods.* — Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at ambient temperatures (22 ±2°). 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. T.l.c. was performed on precoated plates of Silica gel 60-$F_{254}$ (E. Merck, Darmstadt, West Germany) 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 (40–63 $\mu$m). $^1$H-N.m.r. spectra were recorded at 360 MHz (Bruker WM-360) with either tetramethylsilane ($Me_4Si$, $\delta$ 0 in $CDCl_3$) or acetone ($\delta$ 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 $Me_4Si$ ($\delta$ 0 in $CDCl_3$) or external 1,4-dioxane ($\delta$ 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 ($\delta$ 0). $^{31}$P-Decoupled $^1$H-n.m.r. spectra were recorded with a Bruker WH-400 spectrometer equipped with a B-SV3-BX heteronuclear decoupler. The microanalyses were carried out by the Analytical Services Laboratory of this department. 8-Methoxycarbonyloctanol and compound **6** were generous gifts from Chembiomed Ltd., Edmonton, Alberta, Canada.

*8-Methoxycarbonyloctyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-*D*-mannopyranoside* (**9**). — To a solution of 8-methoxycarbonyloctanol (337 mg, 1.79 mmol) in dry acetonitrile (10 mL) containing 4A molecular sieves were added, sequentially, $HgBr_2$ (645 mg, 1.79 mmol) and $Hg(CN)_2$ (452 mg, 1.79 mmol), followed by a solution of 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl bromide (**8**) [$^1$H-n.m.r. ($CDCl_3$), $\delta$ 6.000, 1 H, $J_{1,2}$ 2.0 Hz, H-1; $R_F$ 0.42 in 1:2 ethyl acetate–hexane] in acetonitrile (5 mL) which had been freshly prepared[27] from 1,2,6-tri-*O*-acetyl-2,3-di-*O*-benzyl-α-D-mannopyranose[24] (**4**, 1.0 g, 2.06 mmol). After being stirred

for 15 h, the mixture was diluted with dichloromethane (50 mL), filtered, and the filtrate evaporated to dryness. The residue was extracted three times with dichloromethane, and the extracts were combined and washed with saturated KCl (3 ×), saturated $NaHCO_3$ (3 ×), and twice with water, and the solvent was removed by evaporation. Chromatography with 1:3 ethyl acetate–hexane as eluent provided **9** as a syrup (890 mg, 81%), $[\alpha]_D^{22}$ +22.5° (*c* 0.95, chloroform); $R_F$ 0.33 (1:2 ethyl acetate–hexane), $^1$H-n.m.r. ($CDCl_3$): δ 8.2–8.4 (m, 5 H, arom.), 5.363 (dd, 1 H, $J_{1,2}$ 2.0, $J_{2,3}$ 3.5 Hz, H-2), 4.903 (d, 1 H, $J_{gem}$ 10.5 Hz, PhC*H*), 4.787 (d, 1 H, H-1), 4.720 (d, 1 H, $J_{gem}$ 11.0 Hz, PhC*H*), 4.555 (d, 1 H, $J_{gem}$ 10.5 Hz, PhC*H*), 4.545 (d, 1 H, $J_{gem}$ 11.0 Hz, PhC*H*), 4.383–4.295 (m, 2 H, H-6a,6b), 4.000 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.848 (ddd, 1 H, $J_{4,5}$ 10.0, $J_{5,6a}$ 2.5, $J_{5,6b}$ 5.0 Hz, H-5), 3.732 (dd, 1 H, H-4), 3.663 (s, 3 H, $OCH_3$), 3.633 (m, 1 H, OC*H*$CH_2$–), 3.393 (m, 1 H, OC*H*$CH_2$–), 2.300 (t, 2 H, *J* 7.5 Hz, C$H_2CO_2$Me), 2.150 and 2.063 (both s, 3 H, $COCH_3$), 1.7–1.5 (m, 4 H, –C$H_2$–), 1.3 (m, 8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): δ 174.10 (*C*$O_2$Me), 170.61 and 170.21 (*C*$OCH_3$), 138.02 and 137.81 (quat. arom.), 128.34, 128.03, 127.78 and 127.72 (arom.), 97.69 (C-1), 78.18 (C-3), 75.16 (PhCH), 74.15 (C-4), 71.72 (PhCH), 69.63 (C-2), 68.68 (C-5), 68.04 (O$CH_2CH_2$), 63.41 (C-6), 51.31 (O$CH_3$), 33.99 (*C*$H_2CO_2$Me), 29.26, 29.09, 29.06, 29.00, 25.97 and 24.85 (aliph.), 20.96 and 20.74 ($COCH_3$).

*Anal.* Calc. for $C_{34}H_{46}O_{10}$: C, 66.43; H, 7.54. Found: C, 66.36; H, 7.54.

*8-Methoxycarbonyloctyl 3,4-di-*O*-benzyl-6-*O*-(4-methoxyphenyldiphenylmethyl)-α-*D*-mannopyranoside* (**11**). — Treatment of **9** (800 mg, 1.30 mmol) with sodium methoxide in methanol for 4 h at room temperature, followed by neutralization with IRC-150 ($H^+$) cation-exchange resin and subsequent removal of the resin, provided a solution of the diol **10** ($R_F$ 0.48, 4:1 ethyl acetate–hexane) which was not further characterized. Evaporation left a white solid which was dried *in vacuo* in the presence of $P_2O_5$ overnight. This material was dissolved in anhydrous pyridine (10 mL) and chloro-(4-methoxyphenyl)diphenylmethane (*P*-anisylchlorodiphenylmethane; 740 mg, 2.4 mmol) was added. After 24 h, the reaction was quenched by addition of methanol (4 mL) and, after an additional 0.5 h, the solution was taken to dryness. The residual syrup was dissolved in dichloromethane (40 mL) and washed sequentially with water, 5% HCl, water, saturated $NaHCO_3$, and finally twice with water before concentration to a syrup which was purified by chromatography with 1:3 ethyl acetate–hexane as eluent. Compound **11** (820 mg, 79%) was obtained as a clear syrup, $[\alpha]_D^{22}$ +26° (*c* 0.74, chloroform); $R_F$ 0.33 (1:2 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): δ 7.6–6.8 (24 H, arom.), 4.943 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 4.698 (AB, 2 H, $J_{gem}$ 11.0 Hz, PhC$H_2$), 4.668 (d, 1 H, $J_{gem}$ 10.5 Hz, PhC*H*), 4.274 (d, 1 H, $J_{gem}$ 10.5 Hz, PhC*H*), 4.065 (ddd, 1 H, $J_{2,3}$ 3.0, $J_{2,OH}$ 2.5 Hz, H-2), 3.867 (dd, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 3.833–3.762 (6 H, incl. H-4,5, –O$CH_2CH_2$–, and PhO$CH_3$ at δ 3.762), 3.649 (s, 3 H, $CH_2CO_2CH_3$), 3.469 (2 H, H-6a and OC*H*$CH_2$), 3.250 (dd, 1 H, *J* 10.0, $J_{5,6a}$ 5.0 Hz, H-6b), 2.479 (d, 1 H, $J_{OH,2}$ 2.5 Hz, O*H*), 2.275 (t, 2 H, *J* 7.5 Hz, C$H_2$C$O_2$Me), 1.60 (m, 4 H), and 1.30 (m, 8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): δ 174.00 (*C*$O_2$Me), 158.50, 144.73, 144.54, 138.04 and

135.75 (quat. arom.), 130.52, 128.62, 128.53, 128.50, 128.19, 128.12, 127.86, 127.83, 127.72, 127.60, 126.70, 113.05 (tert. arom.), 98.98 (C-1), 86.13 ($Ph_3C$), 80.48 (C-3), 75.11 (PhCH), 74.79 (C-4), 72.16 (PhCH), 71.20 (C-2), 68.71 (C-5), 67.49 ($OCH_2CH_2$), 62.95 (C-6), 55.14 ($PhOCH_3$), 51.39 ($CO_2CH_3$), 34.06 ($CH_2CO_2Me$), and 29.44, 29.27, 29.17, 29.07, 26.16, 24.92 (aliph.).

*Anal.* Calc. for $C_{50}H_{58}O_9$: C, 74.79; H, 7.28. Found: C, 75.06; H, 7.46.

*8-Methoxycarbonyloctyl 2-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-* (**14**) *and -α-*D*-mannopyranosyl)-3,4-di-O-benzyl-6-O-(4-methoxyphenyldiphenylmethyl)-α-*D*-mannopyranosides* (**2**). — A solution of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide (**13**), freshly prepared from 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranose[27] (650 mg, 1.22 mmol), was added dropwise, over 0.5 h, to a mixture of **11** (650 mg, 0.81 mmol), 2,4,6-trimethylpyridine (159 μL, 1.22 mmol), silver trifluoromethanesulfonate (312 mg, 1.22 mmol), and pulverized 4A molecular sieves (3 g) being stirred in dichloromethane (10 mL) at 0°. After 4 h, dichloromethane (30 mL) was added and the sieves were removed by filtration and washed with more dichloromethane (30 mL). 2,4,6-Trimethylpyridine (160 μL), followed by silver trifluoromethanesulfonate (312 mg) were then added to the filtrate to hydrolyze unreacted **13** and, after 0.5 h, tetraethylammonium bromide (255 mg) was added to precipitate excess silver. The solids were removed by filtration and the resulting solution was washed twice with saturated $NaHCO_3$ and twice with water, and taken to dryness. The residual syrup was purified by chromatography with 1:2 ethyl acetate–hexane as eluent to provide the fractions containing the disaccharide **14** and **2** ($R_F$ 0.38 in 1:2 ethyl acetate–hexane). They were pooled and evaporated to provide a syrup (782 mg, 76%) with a $^1$H-n.m.r. spectrum (for a solution in $CDCl_3$) showing it to consist of a mixture of the β-D-anomer **14** [δ 1.873 (s, $COCH_3$)] and the α-D-anomer **2** [δ 5.215 (d, $J_{1',2'}$ 1.8 Hz, H-1') and 2.070 (s, $COCH_3$)] in a ratio of ~2:5.

*8-Methoxycarbonyloctyl 3,4-di-O-benzyl-6-O-(4-methoxyphenyldiphenylmethyl)-2-O-[2,3,4-tri-O-benzyl-β-* (**15**) *and -α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**18**). — The mixture of **2** and **14** just described (328 mg) was dissolved in dry methanol (10 mL) containing sodium methoxide, kept for 4 h, and then made neutral with Amberlite IRC-50 ($H^+$) cation-exchange resin. After filtration, the solvent was evaporated and the residue chromatographed with 1:2 ethyl acetate–hexane as eluent. The early fractions ($R_F$ 0.29) were combined and evaporated to provide the β-D anomer **15** (80 mg) as a white powder, $[\alpha]_D^{22}$ −21.4° (*c* 0.93, chloroform); $^1$H-n.m.r. ($CDCl_3$): δ 7.5–6.7 (39 H, arom.), 5.085–4.843 [5 H including H-1, (δ 4.920, $J_{1,2}$ ~2 Hz) and 4 PhC*H* (d, $J_{gem}$ 10–12 Hz)], 4.703–4.430 [6 H including H-1' (δ 4.637, $J_{1',2'}$ <2 Hz) and 5 PhC*H* (d, $J_{gem}$ 10–12 Hz)], 4.253–4.218 (2 H, H-2' and PhC*H*), 4.008–3.728 [11 H, including Ph–$OCH_3$ (s 3.728, 3 H)], 3.638 (s, 3 H, $CO_2CH_3$), 3.548 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.5 Hz, H-3'), 3.497–3.443 [2 H, H-6a ($J_{5,H\text{-}6a}$ 2 Hz) and $OCHCH_2$], 3.343 (m, 1 H, $OCHCH_2$), 3.208 (dd, 1 H, $J_{6a,6b}$ 10.0, $J_{5,6b}$ 6.0 Hz, H-6b), 2.266 (t, 2 H, *J* 7.5 Hz, $CH_2CO_2Me$), 1.943 (dd, 1 H, $J_{OH,6'a}$ 6.0, $J_{OH,6'b}$ 7.5 Hz, O*H*), 1.62 (m, 4 H, $-CH_2-$), and 1.3 (8 H,

aliph.); $^{13}$C-n.m.r. ($CDCl_3$): δ 174.23 ($CO_2Me$), 158.5, 144.72, 144.52, 138.75, 138.62, 138.41, 138.20, 138.06, 135.78 (quat. arom.), 130.48–126.75 (14 lines, tert. arom.), 113.02 (–*C*H–OMe), 100.05 ($J_{C\text{-}1'\text{-}H\text{-}1'}$ 154.5 Hz, C-1′), 97.23 ($J_{C\text{-}1\text{-}H\text{-}1}$ 166.6 Hz, C-1), 86.08 ($Ph_3C$), 81.60 and 78.65, 76.09, 75.17, 74.92, 74.84, 74.56, 74.08, 74.00, 73.53, 71.82, 71.13, 70.81, 67.62 (–O$CH_2CH_2$), 63.48 and 62.38 (C-6,6′), 55.13 (PhO$CH_3$), 51.40 ($CO_2CH_3$), 34.05 ($CH_2CO_2Me$), and 29.54, 29.28, 29.19, 29.08, 26.22, 24.92 (aliph.).

*Anal.* Calc. for $C_{77}H_{86}O_{14}$: C, 74.85; H, 7.02. Found: C, 75.00; H, 7.11.

Evaporation of the later fractions ($R_F$ 0.24) provided the α-D-linked disaccharide **18** (190 mg) as a white powder, $[\alpha]_D^{22}$ +16.2° (*c* 0.62, chloroform); $^1$H-n.m.r. ($CDCl_3$): δ 7.6–6.7 (39 H, arom.), 5.248 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.903 (d, 1 H, $J_{gem}$ 11.0 Hz, PhC*H*), 4.867 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.697–4.487 (8 H, PhC*H*), 4.267 (d, 1 H, $J_{gem}$ 10.0 Hz, PhC*H*), 4.025 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.95–3.60 [14 H, including PhO$CH_3$ (δ 3.697, s, 3 H)], 3.648 (s, 3 H, $CO_2CH_3$), 3.42 (2 H, H-6a and O*CH*$CH_2$), 3.231 (dd, 1 H, $J_{5,6b}$ 5.0, $J_{6a,6b}$ 10.0 Hz, H-6b), 2.269 (t, 2 H, 7.5 Hz, $CH_2CO_2Me$), 1.977 (dd, 1 H, $J_{OH,6'a}$ 6.0, $J_{OH,6'b}$ 7.5 Hz, OH), 1.55 (m, 4 H, –$CH_2$–), and 1.30 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): δ 174.19 ($CO_2Me$), 158.57, 144.84, 144.76, 138.54, 138.44, 138.39, 138.25, 135.87 (quat. arom.), 130.53–126.72 (12 lines, tert. arom.), 113.11 (–*C*–OMe), 99.48 ($J_{C\text{-}1'\text{-}H\text{-}1'}$ 168.5 Hz, C-1), 98.68 ($J_{C\text{-}1\text{-}H\text{-}1}$ 169.5 Hz, C-1), 86.07 ($Ph_3C$), 80.43 and 79.71, 75.41, 75.22, 75.15 (2 C), 75.06, 74.41, 72.82, 72.72, 72.46, 72.24, 71.85, 67.50 (O$CH_2CH_2$), 63.29, 62.56, 55.09 (PhO$CH_3$), 51.35 ($CO_2CH_3$), 34.09 ($CH_2CO_2Me$), 29.48, 29.26, 29.18, 29.08, 26.16, and 24.94.

*Anal.* Calc. for $C_{77}H_{86}O_{14}$: C, 74.85; H, 7.02. Found: C, 74.68; H, 6.91.

*8-Methoxycarbonyloctyl 3,4-di-*O*-benzyl-6-*O*-(4-methoxyphenyldiphenylmethyl)-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-diphenoxyphosphoryl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**16**). — Diphenyl phosphorochloridate (30.5 μL, 0.15 mmol) and 4-dimethylaminopyridine (18 mg, 0.15 mmol) were added to a solution of **18** (125 mg, 0.1 mmol) in pyridine (2 mL) at 0°, and the mixture was allowed to warm to room temperature over 0.5 h. After 15 h, water (2 mL) was added and the mixture was taken to dryness after an additional 0.5 h. The residual syrup was dissolved in dichloromethane (20 mL) and the solution washed sequentially with water, 5% HCl, water, saturated $NaHCO_3$ and finally water before evaporation to a syrup which was purified by chromatography with 1:3 ethyl acetate–hexane. Pure **16** was obtained as a syrup (124 mg, 84%), $[\alpha]_D^{22}$ +16° (*c* 0.32, chloroform); $R_F$ 0.53 (1:2 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): δ 7.5–6.7 (~49 H, arom.), 5.118 (d, 1 H, $J_{gem}$ 11.5 Hz, PhC*H*), 4.975 (br, 1 H, $J_{1',2'}$ <2 Hz, H-1′), 4.875–4.218 [12 H, incl. H-1 (δ 4.675, $J_{1,2}$ <1.5 Hz), 9 PhC*H*, and H-6a′, 6b′], 4.048 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.953 (dd, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 3.90–3.45 [15 H, incl. PhO$CH_3$ (δ 3.730, s) and $CO_2CH_3$ (δ 3.635, s)], 3.195 (dd, 1 H, $J_{5,6b}$ 5.5, $J_{6a,6b}$ 10.0 Hz, H-6b), 2.270 (t, 2 H, *J* 7.5 Hz, $CH_2CO_2$), 1.60 (m, 4 H, –$CH_2$–), and 1.30 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): δ 174.17 ($CO_2Me$), 144.81, 144.75, 138.40 (2 C), 138.35, 138.25, 138.19, 135.85 (quat. arom.), 130.51–120.23 (19 lines, tert. arom.),

113.10 (–C–OMe), 99.23 (C-1′), 98.75 (C-1), 86.05 ($Ph_3C$), 80.38, 79.67, 75.37, 75.05 (2 C), 74.38, 72.54, 72.32, 72.07, 71.76, 71.36 (d, $J_{C,P}$ 6.5 Hz, C-5′), 68.24 (d, $J_{C,P}$ 7.0 Hz, C-6′), 67.60 ($OCH_2CH_2$), 63.31 (C-6), 55.08 (ar$OCH_3$), 51.32 ($CO_2CH_3$), 34.07 ($CH_2CO_2Me$), and 29.54, 29.28, 29.16, 29.08, 26.15, 24.93 (aliph.); $^{31}$P-n.m.r. ($CDCl_3$): $\delta$ −12.47.

*Anal.* Calc. for $C_{89}H_{95}O_{17}P$: C, 72.83; H, 6.52. Found: C, 72.43; H, 6.52.

*8-Methoxycarbonyloctyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-diphenoxyphosphoryl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**17**). — Compound **16** (94 mg) was dissolved in dichloromethane (4 mL) and 80% aqueous acetic acid (10 mL) was added. After 4 h, the solvents were evaporated and the residue was chromatographed with 1:2 ethyl acetate–hexane as eluent to give **17** as a white foam (66.4 mg, 86.7%), $[\alpha]_D^{22}$ +17.5° (*c* 0.74, chloroform); $R_F$ 0.27 (1:2 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): 7.4–7.2 (35 H, arom.), 5.139 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.878 and 4.848 (each d, 1 H, $J_{gem}$ 11 Hz, PhC*H*), 4.741 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.661–4.419 (10 H, incl. 8 PhC*H*, H-6a′,6b′), 3.984 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 3.900–3.481 [13 H, incl. $OCH_3$ ($\delta$ 3.651, s)], 3.256 (m, 1 H, $OCHCH_2$), 2.279 (t, 2 H, *J* 7.5 Hz, $CH_2CO_2Me$), 1.944 (dd, 1 H, $J_{OH,6a}$ 5.2, $J_{OH,6b}$ 8.2 Hz, O*H*), 1.65–1.45 (m, 4 H, $-CH_2-$), and 1.25 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): $\delta$ 174.19 ($CO_2Me$), 150.72, 138.40, 138.37, 138.28 (2 C) and 138.17 (quat. arom.), 129.75–120.22 (14 lines, tert. arom.), 99.45 (C-1′), 99.00 (C-1), 80.04, 79.43, 75.19, 75.11, 74.97, 74.77 (2 C), 74.38, 72.49, 72.41, 72.09, 72.04, 71.51 (d, $^2J_{C,P}$ 7.5 Hz, C-5′), 68.26 (d, $^2J_{C,P}$ 4.5 Hz, C-6′), 67.94 ($OCH_2CH_2$), 62.27 (C-6), 51.36 ($OCH_3$), 34.11 ($CH_2CO_2Me$), and 29.45, 29.22, 29.13, 29.09, 26.07, 24.94 (aliph.); $^{31}$P-n.m.r. ($CDCl_3$): $\delta$ −12.53.

*Anal.* Calc. for $C_{69}H_{79}O_{16}P$: C, 69.33; H, 6.66. Found: C, 69.15; H, 6.79.

*8-Methoxycarbonyloctyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-* (**19**) *and -α-*D*-mannopyranosyl)-2,3-di-*O*-benzyl-α-*D*-mannopyranoside* (**23**). — The mixture of **2** and **14** described earlier (170 mg) was dissolved in dichloromethane (4 mL) and 80% aqueous acetic acid (16 mL) was added. After 20 h, the solvent was evaporated and the residue purified by chromatography with 1:2 ethyl acetate–hexane as eluent. The early fractions yielded the $\alpha$ anomer **23** (80 mg) as a syrup, $[\alpha]_D^{22}$ +19° (*c* 0.69, chloroform); $R_F$ 0.45 (1:2 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 7.4–7.2 (25 H, arom.), 5.123 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.921 (d, 1 H, $J_{gem}$ 10.5 Hz, PhC*H*), 4.883 (d, 1 H, $J_{gem}$ 11.0 Hz, PhC*H*), 4.762 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 4.703–4.453 (8 H, PhC*H*), 4.375 (dd, 1 H, $J_{5',6a'}$ 1.5, $J_{6a',6b'}$ 12.0 Hz, H-6a′), 4.258 (dd, 1 H, $J_{5',6b'}$ 5.0 Hz, H-6b′), 3.987 (1 H, dd, $J_{2,3}$ 3.0 Hz, H-2), 3.92–3.56 [13 H, incl. $OCH_3$ ($\delta$ 3.652, s)], 3.310 (m, 1 H, $OCHCH_2$), 2.295 (t, 2 H, *J* 7.5 Hz, $CH_2CO_2Me$), 2.056 (s, 3 H, $COCH_3$), 1.990 (br, 1 H, O*H*), 1.65–1.45 (4 H, $-CH_2-$), and 1.3 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): 174.17 ($CO_2Me$), 170.87 ($COCH_3$), 138.23 (2 C), 138.16 (2 C), 138.06 (quat. arom.), 128.40–127.49 (9 lines, tert. arom.), 99.34 ($^3J_{C,H}$ 171 Hz, C-1′), 98.84 ($^3J_{C,H}$ 170 Hz, C-1), 79.78 and 79.25, 75.16, 75.07, 74.88, 74.62, 74.55 (2 C), 72.41, 72.13, 71.97, 71.95, 70.42, 67.79 ($OCH_2CH_2$), 63.83, 62.04, 51.36 ($OCH_3$), 34.00 ($CH_2CO_2Me$), 29.38, 29.14, 29.07, 29.01, 26.02, 24.85 (aliph.), and 20.87 ($COCH_3$).

*Anal.* Calc. for $C_{59}H_{72}O_{14}$: C, 70.49; H, 7.22. Found: C, 70.25; H, 7.26.

The later fractions were evaporated to provide the β anomer **19** (40 mg) as a syrup, $[\alpha]_D^{22}$ −30.2° (*c* 0.28, chloroform); $R_F$ 0.34 (1:2 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): δ 7.6–7.1 (25 H, arom.), 5.106–4.800 [6 H, incl. H-1 (δ 4.845, d, $J_{1,2}$ 1.8 Hz) and 5 PhC*H*], 4.606–4.428 [7 H, incl. H-1′ (δ 4.583, $J_{1',2'} \leq 1.5$ Hz), H-6′a (δ 4.447), and 5 PhC*H*], 4.336 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.0 Hz, H-2), 4.215 (dd, 1 H, $J_{5,6b}$ 5.5, $J_{6,6b}$ 11.5 Hz, H-6b), 4.003 (d, 1 H, $J_{2',3'}$ 3.0 Hz, H-2′), 3.973 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.0 Hz, H-3), 3.918 (dd, 1 H, $J_{3',4'} \approx 9.5$ Hz, H-4′), 3.808–3.605 [10 H, incl. δ 3.778 (dd, $J_{3,4} \approx J_{4,5} \approx 9.5$ Hz, H-4) and δ 3.650 (s, OC*H*$_3$)], 3.538 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 9.5 Hz, H-3′), 3.500 (m, 1 H, H-5′), 3.373 (m, 1 H, OC*H*CH$_2$), 2.293 (t, 2 H, *J* 7.5 Hz, C*H*$_2$CO$_2$Me), 1.869 (s, 3 H, OC*H*$_3$), 1.74 (br, 1 H, O*H*), 1.6 (m, 4 H, –CH$_2$–), and 1.3 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): δ 174.15 (*C*O$_2$Me), 170.64 (*C*OCH$_3$), 138.87, 138.84, 138.37, 138.27, 138.09 (quat. arom.), 128.38–127.35 (9 lines, tert. arom.), 99.60 ($^3J_{C,H}$ 155 Hz, C-1′), 97.19 ($^3J_{C,H}$ 166 Hz, C-1), 81.91, 78.07, 75.15, 74.85, 74.59, 74.09, 73.97 (2 C), 73.78, 72.04, 71.85, 71.12, 70.14, 67.96 (OCH$_2$CH$_2$), 63.67 and 62.57, 51.35 (OCH$_3$), 34.06 (*C*H$_2$CO$_2$Me), 29.48, 29.20, 29.12, 29.07, 26.09, 24.91, and 20.64 (COC*H*$_3$).

Anal. Calc. for $C_{59}H_{72}O_{14}$: C, 70.49; H, 7.22. Found: C, 70.17; H, 7.18.

*8-Methoxycarbonyloctyl 2-O-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-3,4-di-O-benzyl-6-O-diphenoxyphosphoryl-α-D-mannopyranoside* (**20**). — Compound **23** (80 mg, 8 μmol) was phosphorylated as described for the preparation of **16** with 4-dimethylaminopyridine (15 mg, 0.12 mmol) and diphenyl phosphorochloridate (25 μL, 0.12 mmol). After washing and solvent evaporation, the residue was purified by chromatography with 1:2 ethyl acetate–hexane as eluent. Pure **20** (82 mg, 83%) was obtained as a syrup, $[\alpha]_D^{22}$ +14.9° (*c* 0.94, chloroform); $R_F$ 0.36 (1:2 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): δ 7.4–7.1 (35 H, arom.), 5.185 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.915 (d, 1 H, $J_{gem}$ 11.0 Hz, PhC*H*), 4.855 (d, 1 H, $J_{gem}$ 10.5 Hz, PhC*H*), 4.769 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.713–4.302 (12 H, incl. 8 PhC*H*, H-6a,6b,6′a,6′b), 4.045 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.92–3.64 [10 H, incl. OC*H*$_3$ (δ 3.658, s)], 3.563 and 3.288 (each m, 1 H, OC*H*CH$_2$), 2.288 (t, 2 H, *J* 7.5 Hz, C*H*$_2$CO$_2$Me), 2.050 (s, 3 H, COC*H*$_3$), 1.65–1.45 (m, 4 H, –CH$_2$–), and 1.3 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): δ 174.16 (*C*O$_2$Me), 170.71 (*C*OCH$_3$), 150.66, 147.90, 138.49, 138.39 (2 C), 138.33, 138.09 (quat. arom.), 129.76–120.16 (16 lines, tert. arom.), 99.52 (C-1′), 98.95 (C-1), 80.12, 79.72, 75.14, 75.08 (2 C), 74.81, 74.38, 74.28, 72.61, 72.30, 72.26, 70.80 (2 C incl. $^3J_{C,P} \approx 7$ Hz, C-5), 68.16 (d, $^2J_{C,P}$ 4.5 Hz, C-6), 67.94 (OCH$_2$CH$_2$), 63.82 (C-6′), 51.36 (OCH$_3$), 34.10 (*C*H$_2$CO$_2$Me), 29.47, 29.25, 29.16, 29.12, 26.12, 24.94 (aliph.), and 20.83 (*C*OCH$_3$); $^{31}$P-n.m.r. ($CDCl_3$): δ −12.44.

*Anal.* Calc. for $C_{71}H_{81}O_{17}P$: C, 68.92; H, 6.60. Found: C, 68.49; H, 6.55.

*8-Methoxycarbonyloctyl 3,4-di-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside* (**21**). — Compound **23** (90 mg) was *O*-deacetylated as described for the preparation of **10**. After removal of the resin by filtration, methanol was evaporated and the product was purified by chromatography with

1:2 ethyl acetate–hexane as eluent. Compound **21** (81 mg, 94%) was obtained as a white powder, $[\alpha]_D^{22}$ +19.6° (*c* 0.43, chloroform); $R_F$ 0.27 (1:2 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 7.4–7.2 (m, 25 H, arom.), 5.132 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.888 (d, 1 H, $J_{gem}$ 10.5 Hz, PhC*H*), 4.870 (d, 1 H, $J_{gem}$ 11.0 Hz, PhC*H*), 4.803 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.72–4.53 (8 H, PhC*H*), 3.983 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.920 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.89–3.55 [14 H incl. $OCH_3$ ($\delta$ 3.658, s)], 3.325 (m, 1 H, OC*H*$CH_2$), 2.298 (t, 2 H, *J* 7.5 Hz, $CH_2CO_2Me$), 2.210 (dd, *J* 6 Hz, OH), 2.185 (dd, 1 H, *J* 5 and 8 Hz, OH), 1.65–1.45 (4 H, $-CH_2-$), and 1.3 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): $\delta$ 174.21 ($CO_2Me$), 138.50, 138.46, 138.37 (3 C) (quat. arom.), 128.48–127.57 (11 lines, tert. arom.), 99.62 (C-1'), 98.85 (C-1), 79.97 and 79.32, 75.42, 75.35, 75.24, 74.98, 74.79, 74.63, 72.94, 72.57 (2 C), 72.33 (2 C), 67.92 ($OCH_2CH_2$), 62.74, 62.08, 51.38 ($OCH_3$), 34.11 ($CH_2CO_2Me$), and 29.42, 29.19, 29.14, 29.10, 26.06, 24.95 (aliph.).

*Anal.* Calc. for $C_{57}H_{70}O_{13}$: C, 71.08; H, 7.33. Found: C, 70.59; H, 7.30.

*8-Methoxycarbonyloctyl 3,4-di-*O*-benzyl-6-*O*-diphenoxyphosphoryl-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-diphenoxyphosphoryl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**22**). — The diol **21** (64 mg, 0.067 mmol) was phosphorylated as described for the preparation of **16** but with 4-dimethylaminopyridine (24 mg, 0.2 mmol) and diphenyl phosphorochloridate (41 μL, 0.2 mmol). After being processed, the diphosphate **22** was purified by chromatography with 1:2 ethyl acetate–hexane to provide a syrup (81 mg, 85.4%) $[\alpha]_D^{22}$ +12.5° (*c* 0.57, chloroform); $R_F$ 0.54 (2:3 ethyl acetate–hexane); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 7.4–7.0 (45 H, arom.), 5.180 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.830 (2 H, each d, $J_{gem}$ 10.5 Hz, PhC*H*), 4.738 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.655–4.338 (12 H, incl. 8 PhC*H*, H-6a,6b,6'a,6'b), 4.050 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.925–3.635 [10 H, incl. $OCH_3$ (s, 3.653)], 3.505 and 3.260 (each m, 1 H, OC*H*$CH_2$), 2.282 (t, 2 H, *J* 7.5 Hz, $CH_2CO_2Me$), 1.65–1.40 (m, 4 H, $-CH_2-$), and 1.2–1.3 (8 H, aliph.); $^{13}$C-n.m.r. ($CDCl_3$): $\delta$ 174.16 ($CO_2Me$), 150.71 and 150.63, 138.49, 138.36, 138.31, 138.11, 138.06 (quat. arom.), 129.75–120.14 (20 lines, tert. arom.), 99.37 (C-1'), 98.97 (C-1), 80.21, 79.69, 75.14 (2 C), 75.06, 74.36, 73.95, 72.55, 72.39, 72.22, 71.56 (d, $^3J_{C,P}$ 7.5 Hz, C-5'), 70.72 (d, $^3J_{C,P}$ 8.5 Hz, C-5), 68.19 (C-6,6', br.), 67.99 ($OCH_2CH_2$), 51.34 ($OCH_3$), 34.08 ($CH_2CO_2Me$), and 29.47, 29.23, 29.14, 29.09, 26.07, 24.94 (aliph.); $^{31}$P-n.m.r. ($CDCl_3$): $\delta$ −12.41 and −12.51.

*Anal.* Calc. for $C_{81}H_{88}O_{19}P_2$: C, 68.15; H, 6.21. Found: C, 68.24; H, 6.25.

*8-Methoxycarbonyloctyl 2-*O*-(α-*D*-mannopyranosyl 6-disodium phosphate)-α-*D*-mannopyranoside* (**3**). — Compound **17** (54 mg, 45 μmol) was dissolved in 95% ethanol (2 mL) containing 10% Pd–C (27 mg) and the mixture was stirred under $H_2$ gas (0.1 MPa) for 15 h, by which time t.l.c. showed a major product (~90%) having $R_F$ 0.74 (60:35:6 chloroform–methanol–water) and a more polar product (~10%) having $R_F$ 0.30 (same solvent). The catalyst was removed by filtration and washed with 95% ethanol (10 mL), the solvent evaporated, and the residue was redissolved in 95% ethanol (2 mL). Adams' catalyst ($PtO_2$, 5 mg) was then added and the mixture stirred under $H_2$ (0.1 MPa) for 3 h by which time only one non-u.v. absorb-

ing product ($R_F$ 0.62, 4:1 2-propanol–water) was detectable by t.l.c. The catalyst was removed by filtration, the solvent evaporated, and the residue passed through a column of Bio-Gel P-2 (200–400 mesh) with 10% aqueous ethanol as eluent. The carbohydrate-containing fractions were pooled, concentrated, and passed through Dowex 50-X8 (5 mL, $Na^+$) cation-exchange resin. The eluate was concentrated and lyophilized to provide **3** as a white powder (25.2 mg, 85%), $[\alpha]_D^{22}$ +36° (*c* 0.51, water); $^{13}$C-n.m.r. ($D_2O$): 178.66 (*C*$O_2$Me), 103.10 99.10, 79.51, 73.63, 73.09 (d, 7.5 Hz), 71.20, 70.95, 70.80, 69.03, 67.76, 67.03, 64.55 (d, *J* 4.5 Hz), 61.71, 52.86 ($OCH_3$), 34.52 (*C*$H_2CO_2$Me), 29.35, 29.01, 28.94, 28.89, 26.09, and 25.07; $^{31}$P-n.m.r. ($D_2O$): δ +3.70.

*Anal.* Calc. for $C_{22}H_{39}Na_2O_{16}P \cdot H_2O$: C, 40.37; H, 6.31. Found: C, 39.97; H, 6.00.

*8-Methoxycarbonyloctyl 2-O-α-D-mannopyranosyl-α-D-mannopyranoside 6-disodium phosphate* (**4**). — Compound **20** (37 mg, 30 μmol) was dissolved in 95% ethanol (2 mL) and 10% Pd–C (19 mg) was added. The resulting mixture was stirred under $H_2$ (0.1 MPa) for 40 h. The catalyst was removed by filtration and washed with 95% ethanol, and the solvent evaporated. The residue was dissolved in 95% ethanol (2 mL), Adam's catalyst (5 mg) was added, and the mixture stirred under $H_2$ (0.1 MPa) for 3 h by which time t.l.c. showed only u.v.-absorbing product ($R_F$ 0.33, 5:1 2-propanol–water). Following catalyst removal by filtration and solvent evaporation, the residue was redissolved in anhydrous methanol containing a trace of sodium methoxide, and kept for 4 h when complete conversion to a product of lower mobility ($R_F$ 0.25, 5:1 2-propanol–water) was observed by t.l.c. After neutralization with IRC-50 ($H^+$) cation-exchange resin and solvent evaporation, the residue was passed through a column of Bio-Gel P-2 with 10% aqueous ethanol as eluent. The carbohydrate-containing fractions were pooled, concentrated, and converted into the sodium salt by passage through Dowex 50-X8 ($Na^+$) cation-exchange resin. Lyophilization of the eluate provided **4** (16.2 mg, 81%), $[\alpha]_D^{22}$ +37° (*c* 0.43, water); $^{13}$C-n.m.r. ($D_2O$): δ 178.68 (*C*$O_2$Me), 103.10 (C-1′), 99.24 (C-1), 79.22 (C-2), 74.19, 73.24 (d, $^3J_{C,P}$ 7 Hz, C-5), 71.11, 70.97, 70.94, 68.92, 67.90, 67.19, 63.61 (d, $^2J_{C,P}$ 4.5 Hz, C-6), 61.97 (C-6′), 52.90 ($OCH_3$), 34.56 (*C*$H_2CO_2$Me), 29.19, 29.06, 28.96, 28.93, 26.05, and 25.10; $^{31}$P-n.m.r. ($D_2O$): δ +4.38.

*Anal.* Calc. for $C_{22}H_{39}Na_2O_{16}P \cdot 1.5\ H_2O$: C, 39.82; H, 6.38. Found: C, 39.71; H, 6.08.

*8-Methoxycarbonyloctyl 2-O-(α-D-mannopyranosyl 6-disodium phosphate)-α-D-mannopyranoside 6-disodium phosphate* (**5**). — Compound **22** (37 mg, 26 μmol) was deprotected, as described for the preparation of **3**, with 10% Pd–C (19 mg) and Adam's catalyst (10 mg). The final sodium salt (16.7 mg, 85%; $R_F$ 0.56, 3:1 2-propanol–water) was obtained as a white, lyophilized powder, $[\alpha]_D^{22}$ +22.5° (*c* 0.64, water); $^{13}$C-n.m.r. ($D_2O$): δ 178.71 (*C*$O_2$Me), 103.09, 99.44, 78.84, 73.26 (d, *J* 7.5 Hz), 72.99 (d, *J* 7.0 Hz), 71.10, 70.95, 70.85, 69.13, 67.22, 66.98, 64.20 (d, $J \approx 4$ Hz) and 64.25 (d, $J \approx 4$ Hz), 52.89 ($OCH_3$), 34.56 (*C*$H_2CO_2$Me), and 29.34, 29.07, 28.96, 28.92, 26.10, 25.10 (aliph.); $^{31}$P-n.m.r. ($D_2O$): δ 2.72 and 2.43.

*Anal.* Calc. for $C_{22}H_{38}Na_4O_{16}P_2$: C, 34.75; H, 5.04. Found: C, 35.21; H, 5.02.

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REFERENCES

1 G. G. SAHAGIAN, *Biol. Cell*, 51 (1984) 207–214.
2 K. E. CREEK AND W. S. SLY, *Lysosomes Biol. Pathol.*, 7 (1984) 63–82.
3 L. LANG, M. REITMAN, J. TANG, R. M. ROBERTS, AND S. KORNFELD, *J. Biol. Chem.*, 259 (1984) 14 663–14 671.
4 A. KAPLAN, D. T. ACHORD, AND W. S. SLY, *Proc. Natl. Acad. Sci. U.S.A.*, 74 (1977) 2026–2030.
5 A. R. ROBINS, R. MYEROWITZ, R. J. YOULE, G. J. MURRAY, AND D. M. NEVILLE, JR., *J. Biol. Chem.*, 256 (1981) 10 618–10 622.
6 M. C. WILLINGHAM, I. H. PASTAN, G. G. SAHAGIAN, G. W. JOURDIAN, AND E. F. NEUFELD, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 6967–6971.
7 V. L. SHEPHERD, H. H. FREEZE, A. L. MILLER, AND P. D. STAHL, *J. Biol. Chem.*, 259 (1984) 2257–2261.
8 C. A. GABEL AND S. KORNFELD, *J. Biol. Chem.*, 257 (1982) 10 605–10 612.
9 K. VON FIGURA, A. WAHEED, AND A. HASILIK, *J. Biosci.*, 5 (Suppl. 1) (1983) 19–23.
10 W. S. SLY, in M. I. HOROWITZ (Ed.), *The Glycoconjugates*, Vol. 4, Academic Press, 1982, pp. 3–25.
11 A. VARKI AND S. KORNFELD, *J. Biol. Chem.*, 255 (1980) 10 847–10 858.
12 A. VARKI AND S. KORNFELD, *J. Biol. Chem.*, 258 (1983) 2808–2818.
13 M. NATOWICZ, J. U. BAENZIGER, AND W. S. SLY, *J. Biol. Chem.*, 257 (1982) 4412–4420.
14 R. U. LEMIEUX, D. R. BUNDLE, AND D. A. BAKER, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.
15 R. U. LEMIEUX, D. A. BAKER, AND D. R. BUNDLE, *Can. J. Biochem.*, 55 (1977) 507–512.
16 A. KAPLAN, D. FISCHER, AND W. S. SLY, *J. Biol. Chem.*, 253 (1978) 647–650.
17 H. D. FISCHER, M. NATOWICZ, W. S. SLY, AND R. K. BRETTHAUER, *J. Cell. Biol.*, 84 (1980) 77–86.
18 E. M. KARSON, E. F. NEUFELD, AND G. N. SANDO, *Biochemistry*, 19 (1980) 3856–3860.
19 G. J. MURRAY AND D. M. NEVILLE, *J. Biol. Chem.*, 255 (1980) 11 942–11 948.
20 Y. C. LEE, R. R. TOWNSEND, M. R. HARDY, J. LÖNNGREN, J. ARNARP, M. HARALDSSON, AND H. LONN, *J. Biol. Chem.*, 258 (1983) 199–202.
21 M. C. WILLINGHAM, I. M. PASTAN, AND G. G. SAHAGIAN, *J. Histochem. Cytochem.*, 31 (1983) 1–11.
22 W. J. BROWN AND M. G. FARQUHAR, *Cell*, 36 (1984) 663–667.
23 L. Y. W. BOURGUIGNON, K. BALAZOVICH, S. J. SUCHARD, O. HINDSGAUL, AND M. PIERCE, *J. Cell. Physiol.*, 127 (1986) 146–161.
24 M. M. PONPIPOM, *Carbohydr. Res.*, 59 (1977) 311–317.
25 M. GEORGES AND B. FRASER-REID, *Carbohydr. Res.*, 127 (1984) 162–164.
26 S. J. SONDHEIMER, R. EBY, AND C. SCHUERCH, *Carbohydr. Res.*, 60 (1978) 187–192.
27 H. PAULSEN AND O. LOCKHOFF, *Chem. Ber.*, 114 (1981) 3102–3114.
28 H. PAULSEN, R. LEBUHN, AND O. LOCKHOFF, *Carbohydr. Res.*, 103 (1982) c7–c11.
29 T. OGAWA, H. YAMAMOTO, T. NUKADA, T. KITAJIMA, AND M. SUGIMOTO, *Pure Appl. Chem.*, 56 (1984) 779–795.
30 T. OGAWA AND T. NUKADA, *Carbohydr. Res.*, 136 (1985) 135–152.
31 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
32 K. BOCK AND H. THØGERSEN, *Annu. Rep. NMR Spectrosc.*, 13 (1982) 1–57.
33 R. U. LEMIEUX, K. BOCK, L. T. J. DELBAERE, S. KOTO, AND V. S. RAO, *Can. J. Chem.*, 58 (1980) 631–653.
34 K. BOCK, *Pure Appl. Chem.*, 55 (1983) 605–622.
35 N. K. KOCHETKOV, O. S. CHIZOV, AND A. S. SHASHKOV, *Carbohydr. Res.*, 133 (1984) 173–185.
36 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.
37 R. RICHARZ AND K. WÜTHRICH, *J. Magn. Reson.*, 30 (1978) 147.
38 R. U. LEMIEUX AND K. BOCK, *Arch. Biochem. Biophys.*, 221 (1983) 125–134.