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# Synthesis of octyl 2-*O*-$\alpha$-D-mannopyranosyl-$\alpha$-D-mannopyranoside, its 6′-phosphate and the corresponding monomethyl phosphodiester: intermediate structures in the biosynthesis of N-linked oligosaccharides in *Dictyostelium discoidium*

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## Abstract

Octyl 2-*O*-$\alpha$-D-mannopyranosyl-$\alpha$-D-mannopyranoside, its 6′-phosphate and the corresponding 6′-methyl phosphodiester have been chemically synthesized. The latter compound was prepared using an H-phosphonate intermediate. These structures are for use as enzyme substrates and reference standards in a study on the biosynthesis of phosphorylated N-linked glycoproteins in the amoebae *Dictyostelium discoideum*.

## 1. Introduction

Mannose-6-phosphate (Man-6-P) is required for the targeting of some mammalian acid-hydrolases to lysosomes [1,2]. The required Man-6-P residues are located on the high-mannose oligosaccharide chains of N-linked glycoproteins where they are produced via the biosynthetic pathway summarized in Fig. 1. Enzymes destined for transport to the lysosomes contain the $\alpha$-D-Man-(1 → 2)-$\alpha$-D-Man sequence (**A**) and are recognized by the enzyme Glc*p*NAc-1-phosphate transferase which adds an $\alpha$-D-Glc*p*NAc-1-phosphate grouping to form a phosphodiester: $\alpha$-D-Glc*p*NAc-1-yl D-Man*p*-6-phosphate (**B**, Fig. 1) [3]. Next, a specific $\alpha$-N-acetylphosphoglucosaminidase cleaves the $\alpha$-D-Glc*p*NAc residue from phos-

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phodiester **B** to generate oligosaccharide sequences carrying Man-6-P residues (**C**, Fig. 1) [4] which are recognized and sequestered by specific phosphomannosyl receptors involved in the lysosomal targeting.

The structure and biosynthesis of phosphorylated N-linked oligosaccharide chains in the cellular slime mold *Dictyostelium discoideum* shows a remarkable similarity to those of mammalian cells (Fig. 1) [5–7]. Moreover, phosphorylated glycoproteins from this organism also bind well to the mammalian phosphomannosyl receptors. However, in *Dictyostelium* Man-6-P occurs as a methylphosphodiester (**D**, Fig. 1) [7]. Protein, nucleic acid and carbohydrate methylation are well known, but phosphomethylation is rare. The recently identified S-adenosylmethionine-dependent *Dictyostelium* phosphate methyltransferase is the first enzyme of its kind and appears to be selective for Man-6-P [8]. The enzyme has a marked preference for terminal Man-6-P residues that are α-(1 → 2)-linked to an underlying Man residue.

We report here the chemical synthesis of three oligosaccharide fragments which are partial structures occurring in the biosynthetic pathway of phosphorylated glycoproteins in *Dictyostelium discoideum*. Molecules containing the sequences **A** [9–11], **B** [12] and **C** [13,14] (Fig. 1) have previously been prepared with various aglycons (R). In the present work, compounds with sequences **A**, **C** and **D** were synthesized as their octyl glycosides [Fig. 1, R = $(CH_2)_7CH_3$]. This aglycon was selected since it is chemically stable and confers on the product disaccharides a hydrophobicity sufficient to cause their adsorption from aqueous solution onto reversed-phase $C_{18}$ chromatography supports. The compounds are thus designed to function as enzyme acceptors and reference product standards in a study of the enzymes involved in the biosynthetic pathway summarized in Fig. 1, using crude enzyme preparations, tracer $^{32}P$ or $^{14}C/^{3}H$-labeled donors and Sep-Pak assays [15].

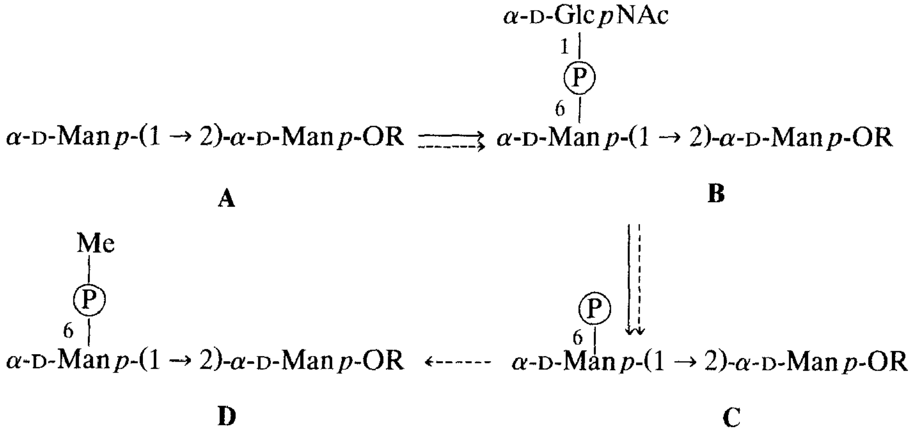
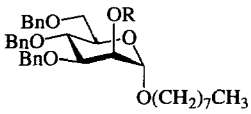


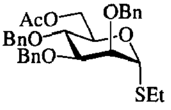
Fig. 1. Comparison of the pathways for the biosynthesis of phosphorylated oligosacccharides in mammalian cells (solid arrows) and *Dictoyostelium discoidium* (dashed arrows). R represents an N-linked, high-mannose oligosaccharide chain, and P represents a phosphate residue.

## 2. Results and discussion

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride [16] was condensed with 1-octanol at −20°C using silver trifluoromethanesulfonate (triflate) [17] as promoter to give the desired α-linked octyl glycoside **1** in 73% yield ($^3J_{\text{H-1,H-2}}$ 1.8 Hz and $^1J_{\text{C-1,H-1}}$ 170 Hz). If the temperature was allowed to rise above −20°C, the 2-*O*-acetyl group was lost giving a complex mixture of **1**, **2**, and some unidentified di- to tetra-saccharides. Zemplén *O*-deacetylation of **1** gave **2** in almost quantitative yield. The thioglycoside (**3**) of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranose [18] was prepared using ethanethiol and boron trifluoride etherate (81%). Attempts to make the corresponding glycosyl bromide failed due to cleavage of some of the benzyl ethers when 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranose was treated with hydrogen bromide in acetic acid. Compound **2** was glycosylated with the thioglycoside donor **3** using dimethyl(methylthio)sulfonium triflate [19] as promoter and gave disaccharide **4** in 65% yield ($^3J_{\text{H-1',H-2'}}$ 1.5 Hz and $^1J_{\text{C-1',H-1'}}$ 170 Hz). No β-linked disaccharide could be detected in the reaction mixture. The 6′-*O*-acetyl group of **4** was selectively removed giving the key



1) R = Ac
2) R = H



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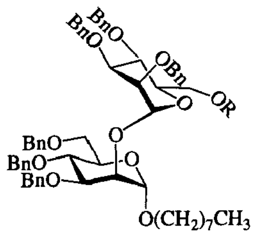
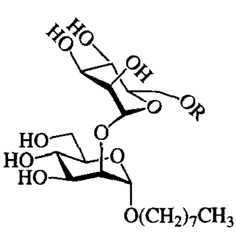


4) R = Ac
5) R = H
6) R = $\overset{\text{O}}{\overset{\|}{\text{P}}}\text{-OPh}$ with $\underset{\text{OPh}}{|}$
7) R = $\overset{\text{O}}{\overset{\|}{\text{P}}}\text{-OMe}$ with $\underset{\text{O}^-}{|}$



8) R = H
9) R = $\text{PO}_3^{2-}$
10) R = $\overset{\text{O}}{\overset{\|}{\text{P}}}\text{-OMe}$ with $\underset{\text{O}^-}{|}$

synthetic intermediate compound **5** (99%) with OH-6′ free for further modification.

Phosphorylation of alcohol **5** using chlorodiphenylphosphate in pyridine gave **6** (91%). Alternatively, treatment of **5** with triethylammonium methylphosphonate [20] in the presence of pivaloyl chloride gave the corresponding H-phosphonate diester [21], which was directly oxidized to the phosphate diester using iodine in pyridine–water and then converted to the sodium form **7** (59%). The benzyl groups of compounds **5** and **7** were hydrogenolyzed using palladium-on-charcoal in acetic acid and 95:5 ethanol–water, respectively, giving the octyl glycosides **8** and **10** (each in 90% yield). Compound **6** was deprotected via two consecutive hydrogenolysis reactions, the first using palladium-on-charcoal to remove the benzyl ethers and the second using Adams' catalyst in 95:5 ethanol–water to remove the phosphorous protecting groups to give **9** (74%). The results of enzyme assays using **8–10** will be reported in due course.

## 3. Experimental

**General methods.**—Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ± 2°C. Analytical TLC was performed on Silica Gel 60-$F_{254}$ (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with $H_2SO_4$. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (40–60 $\mu$m, E. Merck, Darmstadt). $C_{18}$ Silica Gel was from Toronto Research Chemicals Inc. (35–70 $\mu$m), Millex-GV (0.22 $\mu$m) filter units were from Millipore (Missisuaga, ON), and $C_{18}$ Sep-Pak sample preparation cartridges were from Waters Associates (Missisuaga, ON). $^1$H NMR were recorded at 360 MHz (Bruker WM 360) or 300 MHz (Bruker AM 300) with either internal $(CH_3)_4Si$ ($\delta$ 0, $CDCl_3$, $CD_3OD$) or DOH ($\delta$ 4.80, $D_2O$). $^{13}$C NMR were recorded at 75.5 MHz (Bruker AM 300) with internal $(CH_3)_4Si$ ($\delta$ 0, $CDCl_3$, $CD_3OD$) or external acetone ($\delta$ 31.0, $D_2O$). $^{31}$P NMR were recorded at 81.015 MHz (Bruker WH 200) or 161.978 MHz (Bruker WH 400) with external $H_3PO_4$ ($\delta$ 0, $CDCl_3$, $CD_3OD$, $D_2O$). $^1$H data are reported as though they were first order. All $^{13}$C shifts assignments are tentative. Unless otherwise stated, all reactions were carried out at room temperature and in the processing of mixtures, solutions of organic solvents were washed with half the amounts of aqueous solutions. Organic solutions were dried ($Na_2SO_4$) prior to concentration under vacuum at < 40°C (bath). Microanalyses were carried out by the analytical services at this department and all samples submitted for elemental analyses were dried overnight under vacuum with $P_2O_5$ at 56°C (refluxing acetone). Mass spectra were recorded on samples suspended in a Clelland's matrix (1:5 1,4-dithiothreitol-1,4-dithioerythritol) using a Kratos AEIMS9 instrument with Xe as the bombarding gas.

*Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside* (**1**).—Silver triflate (91 mg, 0.355 mmol) was added at −40°C to a stirred mixture of 2-*O*-acetyl-3,4,6-tri-

*O*-benzyl-α-D-mannopyranosyl chloride [16] (145.6 mg, 0.284 mmol), 1-octanol (54 μL, 0.341 mmol), and 2,6 di-*tert*-butyl-4-methylpyridine (DTBMP, 47 mg, 0.227 mmol) in $\mathrm{CH_2Cl_2}$ (10 mL) containing ground 4A molecular sieves under $\mathrm{N_2}$ and the temperature was slowly raised to −20°C. When TLC (5:2 hexane–EtOAc) indicated complete reaction (1 h), 10% aq sodium thiosulfate was added. The mixture was allowed to attain room temperature, and then it was filtered through Celite. The organic layer was separated, washed with water, dried ($\mathrm{Na_2SO_4}$), filtered, and concentrated. Column chromatography (8:1 hexane–EtOAc) gave **1** (124 mg, 73%); $[\alpha]_{578}$ + 21° (*c* 1.0, $\mathrm{CHCl_3}$). $^{13}$C NMR ($\mathrm{CDCl_3}$): δ 14.1 (octyl $\mathrm{CH_3}$), 21.1 (acetate $\mathrm{CH_3}$), 22.6, 26.1, 29.2, 29.3, 29.4, 31.8 (octyl $\mathrm{CH_2}$), 68.0, 68.9, 70.0, 71.4, 71.8, 73.4, 74.4, 75.2, 78.3 (ring CH, $\mathrm{Ph}C\mathrm{H_2}$, $\mathrm{O}C\mathrm{H_2CH_2}$), 97.7 (C-1, $^1J_{\mathrm{C,H}}$ 170 Hz), 127.5–128.4 (quat aromatic), 138.0, 138.3, 138.4 (aromatic CH), 170.5 (acetate C = O); $^1$H NMR ($\mathrm{CDCl_3}$): δ 0.88 (t, 3 H, octyl $\mathrm{CH_3}$), 1.27 (10 H, octyl $\mathrm{CH_2}$), 1.55 (2 H, $\mathrm{OCH_2C}H_2$), 3.39 (ddd, 1 H, $^2J_{\mathrm{H,H}}$ 9.5, $3J_{\mathrm{H,H}}$ 6.5, $3J_{\mathrm{H,H}}$ 3.0 Hz, $\mathrm{O\text{-}C}H_2$), 3.89 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.99 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 4.83 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.37 (dd, 1 H, H-2). Anal. Calcd for $\mathrm{C_{37}H_{48}O_7}$: C, 73.48; H, 8.00. Found: C, 73.43; H, 8.04.

*Octyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside* (**2**).—Methanolic NaOMe (1.0 M, 4 mL) was added at room temperature to a solution of **1** (251 mg, 0.415 mmol) in $\mathrm{CH_2Cl_2}$ (4 mL). After 1.5 h, Dowex 50W-X8 ($\mathrm{H^+}$ form) resin was added and the mixture was stirred for another 5 min. The mixture was filtered and concentrated to give crude **2** (227 mg, 97%); $[\alpha]_{578}$ +42° (*c* 0.93, $\mathrm{CHCl_3}$). $^{13}$C NMR ($\mathrm{CDCl_3}$): δ 14.1 (octyl $\mathrm{CH_3}$), 22.7, 26.1, 29.2, 29.4, 29.4, 31.8 (octyl $\mathrm{CH_2}$), 67.8, 68.5, 69.0, 71.0, 72.0, 73.4, 74.4, 75.1, 80.4 (ring CH, $\mathrm{Ph}C\mathrm{H_2}$, $\mathrm{O}C\mathrm{H_2CH_2}$), 99.2 (C-1), 127.5–128.5 (quat aromatic), 138.0, 138.3, 138.4 (aromatic CH); $^1$H NMR ($\mathrm{CDCl_3}$): δ 0.88 (t, 3 H, octyl $\mathrm{CH_3}$), 1.27 (m, 10 H, octyl $\mathrm{CH_2}$), 1.54 (2 H, $\mathrm{OCH_2C}H_2$), 3.41 (ddd, 1 H, $\mathrm{O\text{-}C}H_2$), 3.67 (ddd, 1 H, $\mathrm{O\text{-}C}H_2$), 3.84 (dd, 1 H, $J_{3,4} = J_{4,5} = 8.7$ Hz, H-4), 3.89 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-3), 4.03 (dd, 1 H, $J_{1,2}$ 1.5 Hz, H-2), 4.89 (d, 1 H, H-1). Anal. Calcd for $\mathrm{C_{35}H_{46}O_6}$: C, 74.70; H, 8.24. Found: C, 74.52; H, 8.22.

*Ethyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio-α-D-mannopyranoside* (**3**).—Boron trifluoride etherate (7.86 mL, 63.9 mmol) was added at room temperature to a stirred mixture of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranose [18] (20.1 g, 37.6 mmol) and ethanethiol (3.62 mL, 48.9 mmol) in $\mathrm{CH_2Cl_2}$ (250 mL). After 10 min the mixture was partitioned between $\mathrm{CH_2Cl_2}$ and satd aq $\mathrm{NaHCO_3}$. The organic layer was dried ($\mathrm{Na_2SO_4}$), filtered, and concentrated. Column chromatography (5:1 hexane–EtOAc) gave **3** (16.27 g, 81%); $[\alpha]_{578}$ +76° (*c* 0.71, $\mathrm{CHCl_3}$). $^{13}$C NMR ($\mathrm{CDCl_3}$): δ 15.0 ($\mathrm{SCH_2}C\mathrm{H_3}$), 20.8 (acetate $\mathrm{CH_3}$), 25.4 ($\mathrm{S}C\mathrm{H_2CH_3}$), 63.5 (C-6), 70.3, 72.0, 72.0, 74.6, 75.1, 76.2, 80.3 (ring CH, $\mathrm{Ph}C\mathrm{H_2}$, $\mathrm{O}C\mathrm{H_2CH_2}$), 82.0 (C-1), 127.7–128.4 (quat aromatic), 138.0, 138.0, 138.2 (aromatic CH), 170.8 (acetate C = O); $^1$H NMR ($\mathrm{CDCl_3}$): δ 1.25 (t, 3 H, $\mathrm{SCH_2C}H_3$), 2.04 (s, 3 H, acetate $\mathrm{CH_3}$), 2.58 (m, 2 H, $\mathrm{SC}H_2\mathrm{CH_3}$), 3.85 (2 H, H-2 and H-3), 3.94 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.17 (ddd, 1 H, $J_{5,6a}$ 2.4 Hz, $J_{5,6b}$ 4.9 Hz, H-5), 4.30 (dd, 1 H, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.36 (dd, 1 H, H-6b), 5.36 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1). Anal. Calcd for $\mathrm{C_{31}H_{36}O_6S}$: C, 69.38; H, 6.76. Found: C, 69.35; H, 6.86.

*Octyl 2-O-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzyl-*

*α-*D*-mannopyranoside* (**4**).—DMTST (230 mg, 0.888 mmol) was added at room temperature to a stirred mixture of **2** (252 mg, 0.448 mmol) and **3** (301 mg, 0.560 mmol) in $CH_2Cl_2$ (30 mL) containing ground 4A molecular sieves under $N_2$. When TLC (5 : 2 hexane–EtOAc) indicated complete reaction (15 min), $Et_3N$ (5 mL) was added and the mixture was stirred for an additional 5 min. Toluene (10 mL) was added and the mixture was concentrated. Column chromatography (6 : 1 hexane–EtOAc) gave **4** (304 mg, 65%); $[\alpha]_{578}$ +20° (*c* 1.0, $CHCl_3$). $^{13}C$ NMR ($CDCl_3$): $\delta$ 14.1 (octyl $CH_3$), 20.8 (acetate $CH_3$), 22.6, 26.1, 29.2, 29.3, 29.4, 31.8 (octyl $CH_2$), 63.8, 67.8, 69.3, 70.5, 71.8, 72.1, 72.5, 73.3, 74.6, 74.7, 74.8, 74.9, 75.0, 75.1, 79.7, 80.0 (ring CH, Ph$CH_2$, O$CH_2$$CH_2$), 98.8, 99.3 (C-1, C-1′, $^1J_{C,H}$ 170 Hz for both), 127.3–12.8 (quat aromatic), 138.3, 138.4, 138.4, 138.5 (aromatic CH), 170.7 (acetate C = O); $^1H$ NMR ($CDCl_3$): $\delta$ 0.88 (t, 3 H, octyl $CH_3$), 1.27 (10 H, octyl $CH_2$), 1.53 (2 H, $OCH_2CH_2$), 2.05 (s, 3 H, acetate $CH_3$), 3.36 (ddd, 1 H, O-C$H_2$), 3.64 (ddd, 1 H, O-C$H_2$), 3.80 (1 H, $J_{1',2'}$ 1.5 Hz, H-2′), 4.01 (1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.29 (dd, 1 H, $J_{6a,6b}$ 11.7, $J_{5,6a}$ 4.9 Hz, H-6a), 4.35 (dd, 1 H, $J_{5,6b}$ 1.7 Hz, H-6b), 4.81 (d, 1 H, H-1), 5.16 (d, 1 H, H-1′). Anal. Calcd for $C_{64}H_{76}O_{12}$: C, 74.11; H, 7.38. Found: C, 73.91; H, 7.40.

*Octyl 3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**5**).—Methanolic NaOMe (1.0 M, 4 mL) was added at room temperature to a solution of **4** (3.60 g, 3.47 mol) in 1 : 1 $CH_2Cl_2$–MeOH (40 mL). After 1.5 h, Dowex 50W-X8 ($H^+$ form) resin was added and the mixture was stirred for an additional 5 min. The mixture was filtered and concentrated to give crude **5** (3.44 g, 99%); $[\alpha]_{578}$ +20° (*c* 1.0, $CHCl_3$). $^{13}C$ NMR ($CDCl_3$): $\delta$ 14.0 (octyl $CH_3$), 22.5, 26.0, 29.1, 29.2, 29.3, 31.7 (octyl $CH_2$), 62.2, 67.6, 69.1, 71.7, 72.1, 72.4, 72.7, 73.2, 74.3, 74.8, 74.9, 75.0, 75.0, 79.6, 80.0 (ring CH, Ph$CH_2$, O$CH_2$$CH_2$), 98.6, 99.4 (C-1, C-1′), 127.3–128.8 (quat aromatic), 138.1, 138.2 138.3, 138.4, 138.5 (aromatic CH); $^1H$ NMR ($CDCl_3$): $\delta$ (*inter alia*) 0.88 (t, 3 H, octyl $CH_3$), 1.27 (10 H, octyl $CH_2$), 1.53 (2 H, $OCH_2CH_2$), 3.37 (ddd, 1 H, O-C$H_2$), 3.64 (ddd, 1 H, O-C$H_2$), 3.91 (1 H, $J_{1',2'}$ 1.4 Hz, H-2′), 4.00 (1 H, $J_{1,2}$ 1.4 Hz, H-2), 4.83 (d, 1 H, H-1), 5.18 (d, 1 H, H-1′).

*Octyl 3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-6-O-diphenoxyphosphoryl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**6**).—Chlorodiphenylphosphate (58 μL, 0.282 mmol) was added at room temperature to a stirred solution of **5** (239 mg, 0.235 mmol) in pyridine (10 mL). After 1 h, water (240 μL) was added and the solution was stirred for an additional 5 min and then concentrated. Column chromatography (6 : 1 hexane–EtOAc) gave **6** (263 mg, 91%); $[\alpha]_{578}$ +16° (*c* 0.9, $CHCl_3$). $^{13}C$ NMR ($CDCl_3$): $\delta$ 14.1 (octyl $CH_3$), 22.6, 26.1, 29.2, 29.3, 29.5, 31.8 (octyl $CH_2$), 67.8 (ring CH), 68.1 (d, $^2J_{C,P}$ 5.2 Hz, C-6′), 69.3 (ring CH), 71.3 (d, $^3J_{C,P}$ 7.8 Hz, C-5′), 71.8, 72.1, 72.2, 72.4, 73.3, 74.2, 74.3, 74.9, 74.9, 75.1, 79.6, 80.1 (ring CH, Ph$CH_2$, O$CH_2$$CH_2$), 98.8, 99.2 (C-1, C-1′), 120.2–129.7 (quat aromatic), 138.2, 138.4, 138.5 (aromatic CH), 150.5–150.7 (aromatic C); $^1H$ NMR ($CDCl_3$): $\delta$ (*inter alia*) 3.89 (1 H, H-2′), 4.11 (1 H, H-2), 4.94 (1 H, H-1), 5.28 (1 H, H-1′)$^{31}P$ NMR ($CDCl_3$): $\delta$ 24.4. Anal. Calcd for $C_{74}H_{83}O_{14}P$: C, 72.41; H, 6.82. Found: C, 72.50; H, 6.85.

*Sodium octyl 3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-6-O-methoxyphosphoryl-*

*α-D-mannopyranosyl)-α-D-mannopyranoside* (**7**).—Ammonium methylphosphonate [20] (41 mg, 362 μmol) was concentrated from 9 : 1 $Et_3N$–pyridine (10 mL) three times in order to convert it to the corresponding triethylammonium salt. Compound **5** (180 mg, 181 μmol) dissolved in dry pyridine (5 mL) was added, and the mixture was concentrated twice from dry pyridine (5 mL), then dissolved in dry pyridine (5 mL), and cooled in an ice-bath. Pivaloyl chloride (89 μL, 724 μmol) was added, and the solution was stirred for 15 min. A solution of iodine (46 mg, 362 μmol) in 96 : 4 pyridine–$H_2O$ (5 mL) was added and stirring was continued for 15 min. The mixture was partitioned between $CHCl_3$ and satd aq sodium hydrogensulfite solution. The organic phase was washed with 1 M aq $H_2SO_4$, satd aq $NaHCO_3$ and water, dried ($Na_2SO_4$), and concentrated. The residue was purified by silica gel chromatography (100 : 15 : 1 : 1 $CHCl_3$–MeOH–$H_2O$–$Et_3N$), the appropriate fractions were concentrated and taken up in and $CHCl_3$–MeOH and passed through a Dowex 50 ($Na^+$ form) column to give **7** (179 mg, 59%); $[\alpha]_{578}$ +14° (*c* 1.0, 1 : 1 $CHCl_3$–MeOH). $^{13}C$ NMR: δ (1 : 1 $CDCl_3$–$CD_3OD$) 13.2 (octyl $CH_3$), 22.0, 25.6, 28.6, 28.7, 28.9, 31.2 (octyl $CH_2$), 53.1 (d, $^2J_{C,P}$ 6.0 Hz, OMe), 65.8 (d, $^2J_{C,P}$ 4.0 Hz, C-6′), 67.3, 68.6, 70.9, 71.0, 71.2, 71.5, 71.7, 71.9, 72.7, 73.6, 74.2, 74.6, 78.9, 79.5 (ring CH, Ph*C*$H_2$, O*C*$H_2CH_2$), 98.3, 98.8 (C-1, C-1′), 126.0–129.1 (quat aromatic), 137.4, 137.6, 137.7, 137.8 (aromatic CH); $^1H$ NMR (1 : 1 $CDCl_3$–$CD_3OD$): δ 0.87 (t, 3 H, octyl $CH_3$), 1.26 (10 H, octyl $CH_2$), 1.52 (m, 2 H, $OCH_2CH_2$), 3.36 (ddd, 1 H, $^2J_{H,H}$ 12.9, $^3J_{H,H}$ 6.5 and 2.8 Hz, O-C$H_2$), 3.78 (1 H, $J_{1',2'}$ 1.6 Hz, H-2′), 3.90 (m, 2 H, H-3 and H-3′), 3.99 (1 H, $J_{1,2}$ 1.7 Hz, H-2), 4.83 (d, 1 H, H-1), 5.13 (d, 1 H, H-1′); $^{31}P$ NMR (1 : 1 $CDCl_3$-$CD_3OD$): δ 0.4. Anal. Calcd for $C_{63}H_{76}NaO_{14}P$: C, 68.09; H, 6.89. Found: C, 68.12; H, 7.29.

*Octyl 2-O-α-D-mannopyranosyl-α-D-mannopyranoside* (**8**).—Compound **5** (228 mg, 229 μmol) was hydrogenolyzed in AcOH (5 mL) over 10% Pd–C at 100 kPa overnight. The mixture was filtered through Celite and concentrated. The residue was dissolved in water (5 mL) and loaded on a column of reversed-phase $C_{18}$ silica gel (10 g). The column was washed with water (20 mL) and **8** was eluted with 1 : 1 MeOH–$H_2O$. The carbohydrate-containing fractions were pooled, concentrated, re-dissolved in water (5 mL), and filtered through a Millipore filter. Lyophilization provided **8** (94 mg, 90%) as a white powder; $[\alpha]_{578}$ +64° (*c* 1.0, $H_2O$). $^{13}C$ NMR ($D_2O$): δ 14.5 (octyl $CH_3$), 23.2, 26.7, 29.9, 30.0, 30.1, 32.5 (octyl $CH_2$), 61.3, 61.6 (C-6, C-6′), 67.2 (ring C), 68.5 (octyl $CH_2$), 70.7, 71.0, 71.3, 73.4, 73.8, 79.6 (ring CH), 99.2, 103.0 (C-1, C-1′, $^1J_{C,H}$ 170 and 172 Hz, respectively); $^1H$ NMR ($D_2O$): δ (*inter alia*) 0.87 (t, 3 H, octyl $CH_3$), 1.29 (10 H, octyl $CH_2$), 1.60 (m, 2 H, $OCH_2CH_2$), 3.55 (ddd, 1 H, O-C$H_2$), 3.85 (dd, 1 H, $J_{2',3'}$ 3.3, $J_{3',4'}$ 9.6 Hz, H-3′), 3.95 (dd, 1 H, $J_{1,2}$ 1.3, $J_{2,3}$ 3.1 Hz, H-2), 4.08 (dd, 1 H, $J_{1',2'}$ 1.5 Hz, H-2′), 5.02 (d, 1 H, H-1′), 5.10 (d, 1 H, H-1). Positive-ion FABMS: *m/z* 455 $(M+H)^+$ and 477 $(M+Na)^+$.

*Disodium octyl 2-O-(α-D-mannopyranosyl-6-phosphate)-α-D-mannopyranoside* (**9**).—The benzyl groups of **6** (106 mg, 86 mmol) were hydrogenolyzed in 95 : 5 EtOH–$H_2O$ (5 mL) over 10% Pd–C (50 mg) at 100 kPa for 15 h when TLC showed one major component (~95%) with $R_f$ 0.60 (50 : 15 : 1 $CHCl_3$–MeOH–$H_2O$) and a more polar component ($R_f$ 0.11 in the same solvent), presumably the monophenylester. The catalyst was removed by filtration and washed with 95:5 EtOH–$H_2O$ and the filtrate was evaporated. The residue was dissolved in 95:5 EtOH–$H_2O$ (5 mL) and stirred with Adams' catalyst ($PtO_2$, 50 mg) for another 16 h under $H_2$ (100 kPa) when TLC showed the presence of a single, non-UV absorbing component with $R_f$ 0.5 (60:35:6 $CHCl_3$–MeOH–$H_2O$). The catalyst was removed by filtration and washed with water. Saturated aq $NaHCO_3$ (500 $\mu$L) was added and the solution was concentrated. The residue was dissolved in water (5 mL) and loaded on a $C_{18}$ silica gel column (10 g). The column was washed with water (20 mL) and **9** was eluted with 1:1 MeOH–$H_2O$. The carbohydrate-containing fractions were pooled, concentrated, re-dissolved in water (5 mL), filtered through a Millipore filter, and then converted into the sodium salt by passage through Dowex 50-X8 ($Na^+$ form, 5 mL) resin. Lyophilzation provided **9** (36.7 mg, 74%) as a white powder; $[\alpha]_{578}$ +38° (*c* 1.0, $H_2O$). $^{13}C$ NMR ($D_2O$): $\delta$ 16.0 (octyl $CH_3$), 24.6, 28.4, 31.0, 31.0, 31.2, 33.7 (octyl $CH_2$), 63.4 (C-6), 65.1 (d, $^2J_{C,P}$ 4.5 Hz, C-6′), 68.5, 69.5 (ring CH), 70.8 (octyl $CH_2$), 72.5, 72.7, 73.0 (ring CH), 75.3 (d, $^3J_{C,P}$ 7.5 Hz, C-5′), 75.4 (ring CH) 100.0, 104.9 (C-1, C-1′); $^1H$ NMR ($D_2O$): $\delta$ (*inter alia*) 3.95 (dd, 1 H, $J_{1',2'}$ 1.4, $J_{2',3'}$ 3.2 Hz, H-2′), 4.05 (dd, 1 H, $J_{1,2}$ 1.2, $J_{2,3}$ 3.2 Hz, H-2), 4.07–4.12 (m, 2 H, H-6′), 5.01 (d, 1 H, H-1′), 5.04 (d, 1 H, H-1); $^{31}P$ NMR ($D_2O$): $\delta$ 4.4. Positive-ion FABMS: *m/z* 579 $(M + H)^+$ and 601 $(M + Na)^+$.

*Sodium octyl 2-O-(α-D-mannopyranosyl-6-methylphosphate-α-D-mannopyranoside* (**10**).—Compound **7** (17.3 mg, 15.6 $\mu$mol) was hydrogenolyzed in EtOH (3 mL) over 10% P–C at 100 kPa overnight. The mixture was filtered through Celite and concentrated. The residue was dissolved in water (5 mL) and loaded on two consecutive Sep-Pac $C_{18}$ cartridges. The cartridges were washed with water (15 mL) and **7** was eluted with MeOH (10 mL), concentrated, re-dissolved in water (5 mL), filtered through a Millipore filter, and then converted into the sodium salt by passage through Dowex 50-X8 ($Na^+$ form, 5 mL) resin. Lyophilization of the carbohydrate-containing fractions provided **7** (8.0 mg, 90%) as a white powder; $[\alpha]_{578}$ +30° (*c* 0.50, $H_2O$). $^{13}C$ NMR ($D_2O$): $\delta$ 14.2 (octyl $CH_3$), 22.8, 26.3, 29.2, 29.3, 29.4, 31.9 (octyl $CH_2$), 53.8 (d, $^2J_{C,P}$ 4.5 Hz, OMe), 61.7 (C-6), 65.4 ($^2J_{C,P}$ 4.5 Hz C-6′), 67.2, 67.7 (ring CH), 69.0 (octyl $CH_2$), 70.7, 71.1, 71.2 (ring CH), 73.0 ($^3J_{C,P}$ 7.9 Hz C-5′), 73.6, 79.6 (ring CH), 99.1, 103.2 (C-1, C-1′, $^1J_{C,H}$ 172 and 171 Hz, respectively); $^1H$ NMR ($D_2O$): $\delta$ (*inter alia*) 0.86 (t, 3 H, octyl $CH_3$), 1.28 (m, 10 H, octyl $CH_2$), 1.61 (2 H, $OCH_2CH_2$), 3.61 (d, 1 H, $J_{H,P}$ 10.7 Hz, $OCH_3$), 3.95 (dd, 1 H, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.3 Hz, H-2′) 4.04 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 3.5 Hz, H-2), 5.03 (d, 1 H, H-1′), 5.06 (d, 1 H, H-1); $^{31}P$ NMR ($D_2O$): d 2.2. Positive-ion FABMS: *m/z* 571 $(M + H)^+$ and 593 $(M + Na)^+$.

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