Synthesis and Biological Activity of Phosphonate Analogs of Mannose 6-Phosphate (M6P)

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Two phosphonate analogs of mannose 6-phosphate (M6P) have been synthesized. The isosteric analog **1** was obtained by a Wittig–Horner reaction at position 6 of a sugar aldehyde. The non-isosteric analog **2** was obtained by a Michaelis–Arbuzov rearrangement of a 6-bromo derivative. In contrast to the non-isosteric analog **2**, the isoster **1** was shown to bind to M6P receptors as effectively as does M6P itself, thus demonstrating the considerable potential of the system in drug design.

Mannose 6-phosphate (M6P) moieties are involved in the selective targeting of newly synthesized enzymes to lysosomes and in the activation of pro-TGFβ. Specific receptors recognize M6P residues that are added to the nascent enzyme molecule in the Golgi apparatus. Receptor−ligand complexes are sorted in a prelysosomal compartment where they dissociate due to the acidic pH. The ligands are delivered to lysosomes and the receptor can be recycled to the Golgi apparatus. Two different M6P receptors have been identified. The larger M6P receptor, also called the M6P/IGF II receptor since it binds IGF II growth factor, is a glycoprotein of 275 kDa. The smaller M6P receptor is a glycoprotein of 46 kDa, which requires divalent cations for optimal ligand binding. While both receptors cycle to the plasma membrane, only the M6P/IGF II receptor mediated the endocytosis of extracellular M6P-containing ligands (for a review, see Kornfeld[1][2]). Thus, the M6P/IGF II receptor on the cell surface can be used for targeting exogenous proteins, which possess the M6P signal, to endosomes and lysosomes. It has already been shown in lysosomal diseases that treatment with an exogenous enzyme could partially restore its deficiency in many organs.[3,4] In addition, the level of the M6P/IGF II receptor mRNA in vivo is increased in breast cancer tumors as compared to benign breast disease, a fact that also suggests a possible targeting of cytotoxic drugs into cancer cells.[5]

For these reasons, the M6P signal seems to be an interesting candidate to target bioactive molecules to endosomes and thereafter to lysosomes, but the major drawback with phosphates is their sensitivity to hydrolysis by phosphatases. In order to alleviate this hydrolysis, it seemed appropriate to use an M6-phosphonate in which a P−O bond is replaced by a P−C bond, which is stable toward hydrolases. Moreover, the possible attachment of drugs to sugar phosphonates is of potential interest. Several methods for the preparation of sugar phosphonic acids have been described in the literature.[6] In this article we describe the synthesis of phosphonates **1** and **2** in order to study their affinity toward M6P receptors.

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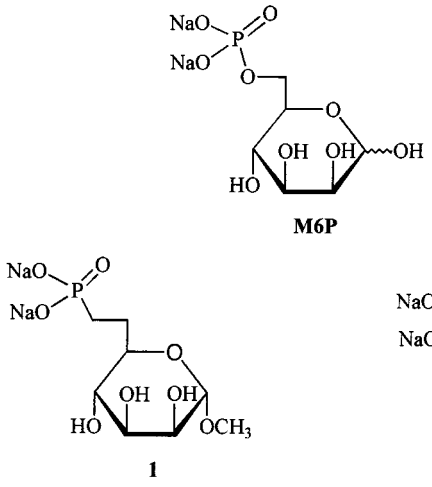
Results and Discussion

Synthesis of Phosphonate Analogs of M6P

The two phosphonate analogs of mannose 6-phosphate (M6P), shown in Scheme 1, were prepared by two different pathways.



Scheme 1. Phosphonate analogs of M6P

One pathway led to the isosteric compound **1**, while the other led to a non-isosteric analog **2**, which possesses one less carbon atom between its phosphorus atom and the sugar moiety. In both cases, the alcohol **3**[7] is the starting compound (Scheme 1).

For the synthesis of the mannose 6-phosphate isoster **1**, we followed the strategy described in Scheme 2. Intermedi-

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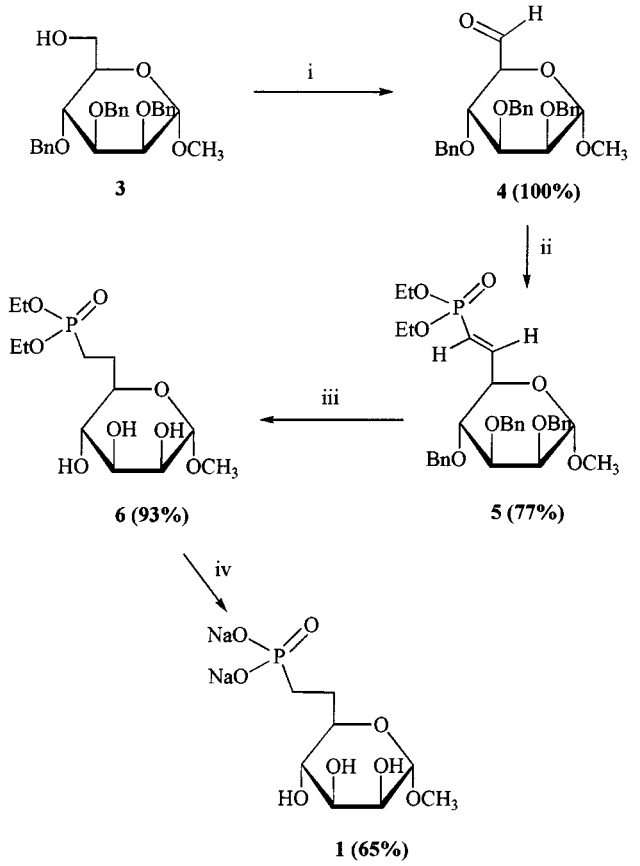
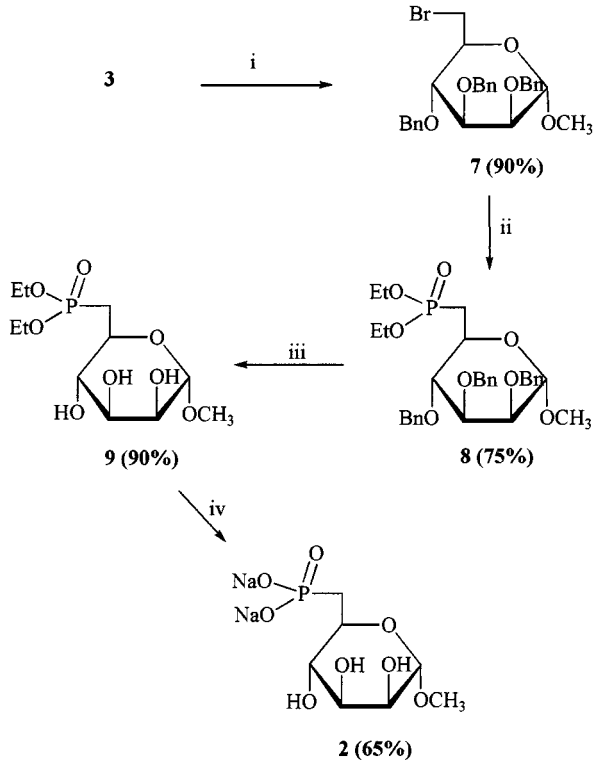
ate **3** was quantitatively oxidized to aldehyde **4**[7] by a Swern reaction.[8]



Scheme 2. i: $(COCl)_2$, DMSO, iPr_2NEt, THF, −60°C, 20 min. − ii: tetraethyl methylenediphosphonate (TEMDP), NaH, C_6H_6, 20°C, 30 min. − iii: $EtOH/H_2O$ (5:1), H_2/Pd/C, 20°C, 24 h. − iv: a) CH_3CN, pyridine, $(CH_3)_3SiBr$, 2 h, 20°C; b) H_2O, pyridine 0°C, then room temp., 2 h; cation exchange resin, 5 h, room temp.

The latter compound, on treatment with the tetraethyl methylenediphosphonate carbanion, led (by a Wittig−Horner[9] reaction in 77% yield) to the phosphonate **5**, which possesses an exocyclic double bond in position 6−7. Hydrogenation of phosphonate **5** using a Pd/C catalyst allowed not only the removal of the benzyl groups at positions 2, 3 and 4, but also the reduction of the double bond. Derivative **6** was transformed into phosphonate **1** after reaction with trimethylsilyl bromide[10] followed by treatment with a cation exchange resin.

The strategy described in Scheme 3 was chosen for the preparation of non-isosteric analog **2**. Derivative **3** was quantitatively mesylated in position 6 and then transformed into the bromide by heating in the presence of LiBr in butanone, thereby generating adduct **7**[11] in 90% yield. The conversion into the phosphonate **8** was effected by a Michaelis−Arbusov reaction in which the bromide derivative was heated at 160°C with triethyl phosphite.[12] The expected compound **8** was obtained in 75% yield. The phosphonate was then deprotected in two stages: The secondary alcohol functions at positions 2, 3 and 4 were first released by hydrogenolysis (H_2, Pd/C) to give compound **9**. The phosphonate **2**, a non-isosteric analog of M6P, was obtained after deprotecting phosphonate **9** using trimethylsilyl bromide followed by treatment with a cation exchange resin.



Scheme 3. i: a) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 30 min, 0°C; b) LiBr, butanone, 80°C, 1 h 30 min. − ii: $P(OEt)_3$, 160°C, 6 h. − iii: $EtOH/H_2O$ (5:1), H_2/Pd/C, 20°C, 24 h. − iv: a) CH_3CN, pyridine, $(CH_3)_3SiBr$, 2 h, 20°C; b) H_2O, pyridine, 0°C, then room temp., 2 h, cation exchange resin, 5 h, room temp.

Biological Activity of Phosphonate Analogs of M6P

The affinity of phosphonates **1** and **2** toward the M6P/IGF II receptor was assayed on pentamannose 6-phosphate Sepharose columns.[13] Retained receptors were eluted using increasing concentrations of the two compounds and analyzed on silver-stained polyacrylamide gels (Figure 1). The 270-kDa bands eluted from the column correspond to the M6P/IGF II receptor. The isosteric phosphonate **1** eluted the receptor in a mannose-dependent concentration with a maximum at 10 mM. This compound displayed a higher affinity than the non-isosteric derivative **2** since this last compound only partially eluted the receptor. Interestingly, the receptor remaining on the affinity column after addition of compound **2** could be further dissociated with 10 mM phosphonate **1** (Figure 1, column 7).

Phosphonate **1** was found to have a similar affinity toward the M6P/IGF II receptors as the mannose 6-phosphate routinely used at 10 mM to elute this receptor (data not shown). On the other hand, the non-isosteric analog of M6P, **2**, shows only a weak affinity toward those receptors. Thus, the replacement of the oxygen atom by a methylene

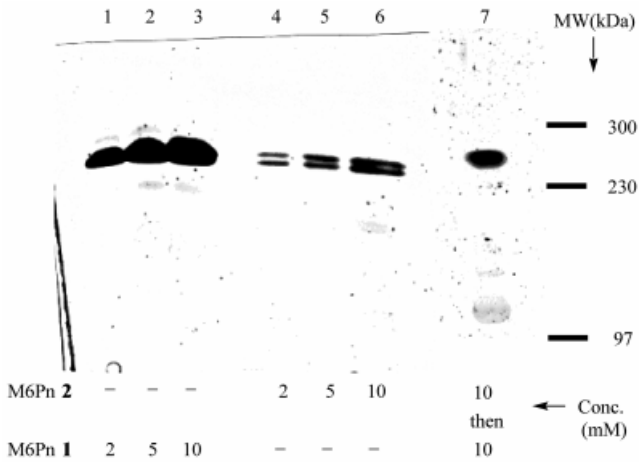


Figure 1. Analysis on electrophoresis gel of retained receptors eluted with compounds **1** and **2**; positions of standard molecular weights are indicated

group does not modify the recognition of the substrate by the receptor.

Conclusion

In summary, we have prepared two phosphonate analogs of M6P. The isosteric analog binds to the M6P/IGF II receptor with an affinity higher than the non-isosteric derivative and similar to M6P. This suggests the considerable potential of such compounds in targeting therapy. Thus, the addition of isosteric mannose 6-phosphonate on recombinant enzymes could be used in substitutive therapy of lysosomal disease. The synthesis of isosteric M6P analogs conjugated to drugs and other moieties of biological interest is now being considered in relation to targeted therapy in tissues rich in M6P/IGF II receptors.

Experimental Section

General Aspects: Reactions were monitored by TLC using aluminium-coated plates with silica gel 60 F_{254} (Merck) and visualized by UV light and/or by charring with H_2SO_4 (aqueous 10% spray solution). Aldehydes were developed by spraying with a 5% rhodanine solution in ethanol. Molybdenum blue was used to develop phosphorus-containing compounds. – Column chromatography was performed with Merck silica gel 60 H (Art. 9385). – ^{1}H- and ^{31}P-NMR spectra were recorded with an AC-250 Bruker spectrometer and ^{13}C-NMR spectra with a WP-200-SY Bruker spectrometer. Chemical shifts are given on the δ scale using the residual solvent peaks as a reference relative to TMS. – Specific rotations were measured with sodium-D light at 20°C using a Perkin–Elmer spectrometer. – Microanalyses were performed in the microanalysis laboratory of ENSCM (Montpellier). – Mass spectra were measured with a DX 300 JEOL spectrometer in the FAB^+ ion mode. – Compounds **3**, **4** and **7** were prepared according to published procedures.[7,11] – The cation exchange resin (50WX2 H^+ Dowex) was washed with a 1 M NaOH solution and then with distilled water before use. – The M6P/IGF II receptor was purified from fetal calf serum as previously described.[14] The pentamannose 6-phosphate was functionalized with β-(*p*-aminophenol)ethylamine, then reduced with sodium tetrahydroborate, and finally coupled on active Sepharose to lead to pentamannan 6-phosphate Sepharose.[13] – SDS polyacrylamide gel electrophoresis was performed according to Laemmli[15] and proteins visualized using a silver-staining kit (Biorad).

Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-6-diethoxyphosphinylmethylene-α-D-mannopyranoside (5): 95% NaH (0.087 g, 3.46 mmol) was added to 24 mL of anhydrous benzene under nitrogen. 1.07 mL (4.32 mmol) of tetraethyl methylenediphosphonate was added dropwise and 30 min later 0.8 g (1.73 mmol) of **4** in 7.2 mL of anhydrous benzene was added dropwise at 20°C. After stirring at room temp. for 30 min, the benzene was removed. The residue was dissolved in CH_2Cl_2 and water was added. The organic layer was extracted, dried and concentrated. Compound **5** was obtained after chromatography on silica gel (hexane/AcOEt, 6:4, then AcOEt) in 77% yield. – $[\alpha]_D$ = +67.3 (*c* = 1.07, $CHCl_3$). – ^{1}H NMR ($CDCl_3$): δ = 1.36 (t, 6 H, *J* = 7.1 Hz, 2 × C*H*$_3$CH$_2$OP), 3.33 (s, 3 H, OCH$_3$), 3.71 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, 4-H), 3.83 (dd, 1 H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, 2-H), 3.94 (dd, 1 H, 3-H), 4.04–4.25 (m, 5 H, 2 × CH$_3$C*H*$_2$OP and 5-H), 4.63 and 4.93 (AM$_q$, 2 H, J_{AM} = 10.6 Hz, C*H*$_2$Ph), 4.67 (s, 2 H, C*H*$_2$Ph), 4.74 and 4.82 (AB$_q$, J_{AB} = 12.4 Hz, C*H*$_2$Ph), 4.77 (d, 1 H, 1-H), 6.17 (ddd, 1 H, $J_{5,7}$ = 1.7 Hz, $J_{6,7}$ = 17.3 Hz, $J_{7,P}$ = 21.3 Hz, 7-H), 7.01 (ddd, 1 H, $J_{5,6}$ = 4.3 Hz, $J_{6,P}$ = 21.6 Hz, 6-H), 7.32–7.42 (m, 15 H, 3 C$_6$H$_5$). – ^{13}C NMR ($CDCl_3$): δ = 16.4 (m, 2 × *C*H$_3$CH$_2$OP), 55.0 (OCH$_3$), 61.8 (m, 2 × CH$_3$*C*H$_2$OP), 71.3 (d, $J_{C,P}$ = 21.3 Hz, C-5), 72.4, 73.0, 75.4 (3 × *C*H$_2$Ph), 74.8, 78.3, 80.2 (C-2,3,4), 99.4 (C-1), 118.1 (d, $J_{C,P}$ = 188.3 Hz, C-7), 127.7–128.4 (CH arom.), 138.1, 138.3, 138.4 (3 × C$_q$), 148.1 (d, $J_{C,P}$ = 5.8 Hz, C-6). – ^{31}P NMR ($CDCl_3$): δ = 14.51. – MS (FAB^+); *m/z* (%): 619 (1) $[M + Na]^+$, 597 (7) $[M + H]^+$. – $C_{33}H_{41}O_8P$ (596.65): calcd. C 66.43, H 6.93; found C 66.41, H 6.95.

Methyl 6-Deoxy-6-diethoxyphosphinylmethyl-α-D-mannopyranoside (6): To a solution of **5** (1.38 g, 2.31 mmol) in 350 mL of EtOH/H_2O (5:1) was added 0.138 g of 10% Pd/C. After stirring under hydrogen for 24 h, the mixture was filtered through Celite and then concentrated under vacuum. The residue was purified by chromatography on silica gel (AcOEt/MeOH, 9:1) to give **6** in 93% yield. – $[\alpha]_D$ = +54.4 (*c* = 1.47, $CHCl_3$). – ^{1}H NMR ($CDCl_3$): δ = 1.35 (t, 6 H, *J* = 7.2 Hz, 2 × C*H*$_3$CH$_2$OP), 1.71–2.25 (m, 4 H, 6,6′,7,7′-H), 2.41 (s, 1 H, OH), 3.35 (s, 3 H, OCH$_3$), 3.41–3.50 (m, 1 H, 2-H), 3.51 (t, 1 H, $J_{3,4} = J_{4,5}$ = 8.9 Hz, 4-H), 3.75 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, 3-H), 3.88–3.98 (m, 1 H, 5-H), 4.03–4.21 (m, 4 H, 2 × CH$_3$C*H*$_2$OP), 4.45–4.65 (m, 2 H, 2 × OH), 4.72 (d, 1 H, $J_{1,2}$ = 1.4 Hz, 1-H). – ^{13}C NMR ($CDCl_3$): δ = 16.4 (m, 2 × *C*H$_3$CH$_2$O), 21.0 (d, $J_{C,P}$ = 142.2 Hz, C-7), 24.2 (C-6), 54.9 (OCH$_3$), 62.0 (m, 2 × CH$_3$*C*H$_2$OP), 70.6, 70.7, 71.8 (C-2,3,4), 71.3 (d, $J_{C,P}$ = 15.7 Hz, C-5), 101.0 (C-1). – ^{31}P NMR ($CDCl_3$): δ = 34.05. – MS (FAB^+); *m/z* (%): 351 (45) $[M + Na]^+$, 329 (85) $[M + H]^+$. – $C_{12}H_{25}O_8P$ (328.29): calcd. C 43.90, H 7.68; found C 43.91, H 7.66.

Methyl 6-Deoxy-6-dihydroxyphosphinylmethyl-α-D-mannopyranoside Sodium Salts 1: To a solution of **6** (0.65 g, 2.0 mmol) in 25 mL of anhydrous CH_3CN under nitrogen was added 0.25 mL (0.42 mol) of pyridine and 2.6 mL (20 mmol) of Me_3SiBr. After stirring at room temp. for 2 h, 15 mL of distilled water and 0.42 mL of pyridine (5.25 mmol) were added at 0°C. Stirring was continued at room temp. for 2 h, then the aqueous phase was washed with CH_2Cl_2. The organic layer was then concentrated before the addition of 60 mL of distilled water and 30 g of cation exchange resin. After stirring at room temp. for 5 h, the resin was filtered off and washed several times with water. The aqueous phase was concentrated and the residue was purified by chromatography on reversed

phase (RP-18, H_2O). After lyophylization, **1** was obtained in 65% yield. − $[\alpha]_D$ = +61.9 (*c* = 1.05, CH_3OH). − ^{1}H NMR (D_2O): δ = 1.50−2.10 (m, 4 H, 6,6′,7,7′-H), 3.28 (s, 3 H, OCH_3), 3.30−3.50 (m, 2 H, 4,5-H), 3.65 (dd, 1 H, $J_{3,2}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, 3-H), 3.79 (dd, 1 H, $J_{1,2}$ = 1.6 Hz, 2-H), 4.60 (d, 1 H, 1-H). − ^{13}C NMR (D_2O): δ = 23.6 (d, $J_{C,P}$ = 136.6 Hz, C-7), 25.2 (d, $J_{C,P}$ = 3.8 Hz, C-6), 55.4 (OCH_3), 70.6, 70.8, 71.2 (C-2,3,4), 72.6 (d, $J_{C,P}$ = 17.1 Hz, C-5), 101.6 (C-1). − ^{31}P NMR (D_2O): δ = 31.04. − MS (FAB^-); *m/z* (%): 271 (55) $[M - 2\ Na + H]^-$. − $C_8H_{15}Na_2O_8P$ (316.15): calcd. C 30.39, H 4.78; found C 30.41, H 4.83.

Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-6-diethoxyphosphinyl-α-D-mannopyranoside (8): Compound **7** (2.50 g, 4.75 mmol) was dissolved in 35 mL of $P(OEt)_3$ under nitrogen. After stirring at 160°C for 6 h, $P(OEt)_3$ was removed by distillation and the product was purified by chromatography on silica gel (hexane/AcOEt, 6:4, then AcOEt) to give compound **8** in 75% yield. − $[\alpha]_D$ = +21.6 (*c* = 1.11, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 1.36 (td, 6 H, *J* = 7.1 Hz, J_{CH3-P} = 1 Hz, 2 C*H*$_3$CH$_2$OP), 2.01 (ddd, 1 H, $J_{5,6'}$ = 10.4 Hz, $J_{6,6'} = J_{6',P}$ = 15.4 Hz, 6′-H), 2.38 (ddd, 1 H, $J_{5,6}$ = 1.8 Hz, $J_{6,6'}$ = 15.4 Hz, $J_{6,P}$ = 20.1 Hz, 6-H), 3.41 (s, 3 H, OCH_3), 3.68 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.4 Hz, 4-H), 3.81 (dd, 1 H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.0 Hz, 2-H), 3.90 (dd, 1 H, 3-H), 3.98−4.00 (m, 1 H, 5-H), 4.01−4.21 (m, 4 H, 2 × CH$_3$C*H*$_2$OP), 4.61 (s, 2 H, C*H*$_2$Ph), 4.65 and 5.05 (AM_q, 2 H, J_{AM} = 11.2 Hz, C*H*$_2$Ph), 4.68 (d, 1 H, 1-H), 4.71 and 4.79 (AB_q, 2 H, J_{AB} = 12.5 Hz, C*H*$_2$Ph), 7.21−7.52 (m, 15 H, 3 C_6H_5). − ^{13}C NMR ($CDCl_3$): δ = 16.3 (m, 2 × *C*H$_3$CH$_2$OP), 28.4 (d, $J_{C,P}$ = 142.3 Hz, C-6), 55.1 (OCH_3), 61.6 (m, 2 × CH$_3$*C*H$_2$OP), 67.2 (d, $J_{C,P}$ = 6.7 Hz, C-5), 74.9 (C-2), 78.7 (d, $J_{C,P}$ = 45.6 Hz, C-4), 80.3 (d, $J_{C,P}$ = 3.3 Hz, C-3), 72.2, 73.0, 74.9 (3 × *C*H$_2$Ph), 99.1 (C-1), 127.6−128.4 (CH arom.). − ^{31}P NMR ($CDCl_3$): δ = 30.11. − MS (FAB^+); *m/z* (%): 585 (6) $[M^+ + H]$. − $C_{32}H_{41}O_8P$ (584.64): calcd. C 65.74, H 7.07; found C 65.80, H 7.06.

Methyl 6-Deoxy-6-diethoxyphosphinyl-α-D-mannopyranoside (9): The procedure described for the preparation of **6** was employed to obtain **9** in 90% yield. − $[\alpha]_D$ = +55.3 (*c* = 1.03, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 1.32 (t, 6 H, *J* = 7.1 Hz, 2 × C*H*$_3$CH$_2$OP), 1.88 (s, 2 H, 2 × OH), 2.13−2.51 (m, 2 H, CH_2P), 3.42 (s, 3 H, OCH_3), 3.70 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, 4-H), 3.75−4.00 (m, 4 H, 2,3,4-H, OH), 4.04−4.20 (m, 4 H, 2 × CH$_3$C*H*$_2$OP), 4.69 (s, 1 H, $J_{1,2}$ = 1.2 Hz, 1-H). − ^{13}C NMR ($CDCl_3$): δ = 16.4 (m, 2 × *C*H$_3$CH$_2$O), 28.7 (d, $J_{C,P}$ = 141.6 Hz, C-6), 55.2 (OCH_3), 62.1 (m, 2 × CH$_3$*C*H$_2$OP), 67.5 (d, $J_{C,P}$ = 4.2 Hz, C-5), 70.6, 71.6 (C-2,3), 71.7 (d, $J_{C,P}$ = 8.4 Hz, C-4), 101.0 (C-1). − ^{31}P NMR ($CDCl_3$): δ = 31.40. − MS (FAB^+); *m/z* (%): 337 (97) $[M + Na]^+$, 315 (100) $[M + H]^+$. − $C_{11}H_{23}O_8P$ (314.27): calcd. C 42.04, H 7.38; found C 42.02, H 7.39.

Methyl 6-Deoxy-6-dihydroxyphosphinyl-α-D-mannopyranoside Sodium Salts 2: The procedure described for the preparation of **1** was employed to obtain **2** in 65% yield. − $[\alpha]_D$ = +19.2 (*c* = 1.51, MeOH). − ^{1}H NMR (D_2O): δ = 1.75 (ddd, 1 H, $J_{5,6'}$ = 9.5 Hz, $J_{6,6'}$ = 15.5 Hz, $J_{6',P}$ = 15.5 Hz, 6′-H), 2.10 (ddd, 1 H, $J_{5,6}$ = 2.9 Hz, $J_{6,P}$ = 18.5 Hz, 6-H), 3.35 (s, 3 H, OCH_3), 3.38 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, 4-H), 3.63 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, 3-H), 3.72 (dd, 1 H, 5-H), 3.80 (dd, 1 H, $J_{1,2}$ = 1.7 Hz, 2-H), 4.60 (d, 1 H, 1-H). − ^{13}C NMR (D_2O): δ = 31.3 (d, $J_{C,P}$ = 134.7 Hz, C-6), 55.8 (OCH_3), 69.4 (d, $J_{C,P}$ = 4.7 Hz, C-5), 70.7(C-2), 71.1 (d, $J_{C,P}$ = 2.1 Hz, C-3), 72.2 (d, $J_{C,P}$ = 12.9 Hz, C-4), 101.4 (C-1). − ^{31}P NMR (D_2O): δ = 24.46. − MS (FAB^-); *m/z* (%): 257 (60) $[M - 2\ Na + H]^-$. − $C_7H_{13}Na_2O_8P$ (302.12): calcd. C 27.83, H 4.34; found C 27.78, H 4.37.

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