Synthesis and Biological Evaluation of Two Mannose 6-Phosphate Analogs

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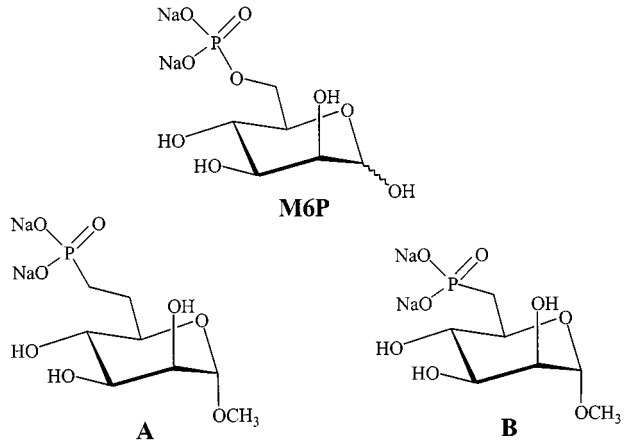
Two β-hydroxyphosphonate analogs of M6P have been prepared by condensation of the lithiated anion of methyldiethylphosphonate with the C-6 carbonyl of a mannose derivative. The diastereoisomers thus obtained have been separated and the absolute configuration at the 6-position has been determined by spectroscopic analysis in conjunction with theoretical calculations. Recognition phenomena by M6P/IGFIIR have been evaluated for both diastereoisomers.

Introduction

The cation-dependent (46 kDa) and cation-independent (275 kDa) mannose 6-phosphate receptors[1,2,3,4] (CD-MPR and CI-MPR, respectively) belong to the P-type lectin family. Their function is to sort and transport M6P-bearing glycoproteins on their N-linked oligosaccharides from the *trans*-Golgi network (TGN) to lysosomes. The receptor−enzyme complex accumulates in clathrin-coated vesicles and travels to an acidic pre-lysosomal compartment where low pH leads to dissociation of the complex. The receptor can then go back to the TGN and reinitiate another cycle of enzyme transport.

Our interest has been focused on CI-MPR as, according to its presence on the plasma membrane, this receptor is also responsible for the binding and the specific endocytosis of extracellular M6P-bearing glycoproteins. This receptor is a type I multifunctional transmembrane glycoprotein also called Mannose 6-Phosphate/Insulin-like Growth Factor II Receptor (M6P/IGFIIR) which, apart from M6P, also binds IGF II and retinoic acid[5] (RA) at different sites. M6P/IGFIIR is the first example of a receptor able to bind three different categories of ligands such as a saccharide (M6P), a peptide[6] (IGFII), and a lipid (RA). Moreover, this receptor has been shown to play a fundamental role in the control of cell growth in fetal development and carcinogenesis.[7]

The presence of this receptor on cell surfaces is particularly interesting since it offers a possible means for internalizing M6P-bearing compounds. However, the phosphomonoester bond of M6P is easily hydrolyzed by phosphatases. Thus, the transport of M6P-bearing molecules appears to be delicately balanced. A first study carried out in our laboratory led us to prepare phosphonate analogs of M6P in which the P−O bond was replaced by a P−C bond insensitive to phosphatase hydrolysis. Two analogs (**A** and **B**) were synthesized with the aim of determining their affinities towards M6P/IGFIIR[8] (Scheme 1).



Scheme 1. Phosphonate analogs of M6P

The isosteric compound **A** was shown to be as well recognized as natural M6P, whereas the non-isosteric compound **B** was weakly recognized by M6P/IGFIIR. These results suggest that such isosteric M6P analogs may have considerable potential in targeting therapy. Thus, replacement of the phosphate group by a phosphonate moiety (compound **A**) does not affect the recognition phenomenon. Moreover, when the chain between the phosphorus and the pyran ring was shortened by a methylene unit (compound **B**), only a weak recognition was observed. In the light of these results and in order to determine the structural factors that govern recognition phenomenon, two β-hydroxyphosphonates (**4a**,**b**) have been prepared and their affinities towards M6P/IGFIIR have been evaluated.

Results and Discussion

Synthesis of β-Hydroxyphosphonate Analogs of M6P

The starting alcohol **1** (Scheme 2) was prepared according to classical protection-deprotection reactions as de-

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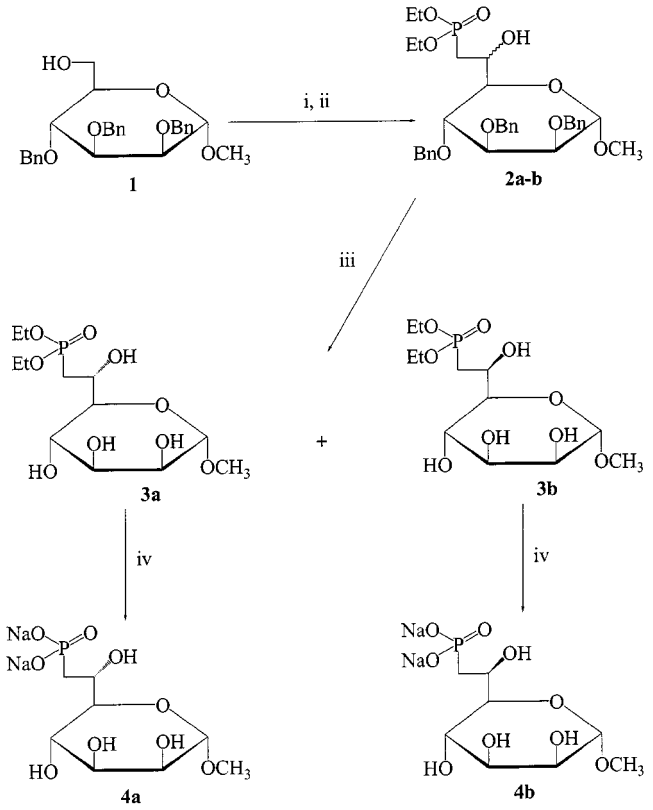
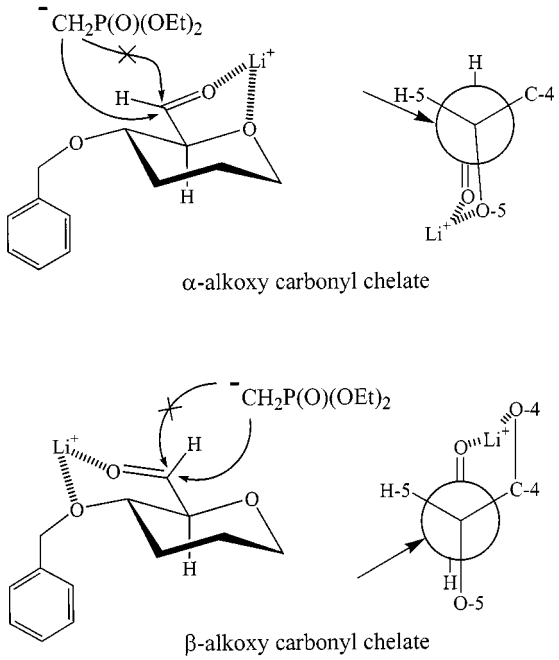
scribed in the literature.[9] The key step of this synthesis involves attack of the lithiated methyldiethylphosphonate anion on the aldehyde obtained from **1** by Swern oxidation.[10] The two diastereoisomers **2a**,**b** were obtained in 40% overall yield with relative proportions of 45% and 55%, respectively. Separation of these two diastereoisomers was accomplished after removal of the benzyl groups by hydrogenolysis. Deprotection of the diethylphosphonates **3a**,**b** was accomplished by well-known procedures[11] to give **4a**,**b** in 65% yield (Scheme 2).



Scheme 2. Preparation of β-hydroxyphosphonate analogs of M6P (i) $(COCl)_2$, DMSO, $i Pr_2NEt$, THF, −60 °C, 20 min; (ii) methyldiethylphosphonate, *n*BuLi, THF, −78 °C; (iii) EtOH/H_2O (1:1), H_2/10% Pd−C, 20 °C, 4 days; (iv) (a) CH_3CN, pyridine, Me_3SiBr, 20 °C, 2 h; (b) H_2O, pyridine, 0 °C then 20 °C, 2 h then cation-exchange resin, 20 °C, 24 h

The observed absence of diastereoselectivity is in accordance with the Cram chelate rule[12,13] when applied to α,β-dialkyloxy carbonyl compounds. Indeed, there are two possibilities for chelate formation. The chelation may either occur between the lithium counterion and the oxygen atoms of both the carbonyl and α-alkyloxy moieties leading to a five-membered ring (Scheme 3), or a six-membered ring may be generated by chelation of the lithium counterion and the oxygen atoms of both the carbonyl and β-alkyloxy moieties (Scheme 3).

Our results have shown that the β-alkyloxy carbonyl chelate is slightly favored, leading to a slight predominance of the (6*S*) diastereoisomer **3b** (55%). The ratio 55:45 is in accordance with data for the acyclic series reported in the literature.[12,13] It is worth pointing out that the absolute configuration at the 6-position has been determined by ^1H



Scheme 3. Two possibilities for the addition of nucleophiles to α-chiral carbonyl compounds

NMR analysis and that these data have been confirmed by a conformational study. The conformational behavior was conveniently analyzed by means of a conformation map obtained by plotting the relative energy of the conformation vs. the dihedral angle. The relative energies of the conformations were calculated using the CFF91 force field as implemented in the Discover program of the Insight II program (MSI, San Diego), systematically varying the dihedral angles in increments of 10°. The lowest-energy rotamers thus obtained for **3a** (6*R*) and **3b** (6*S*) are shown in Figure 1 and Figure 2, respectively.

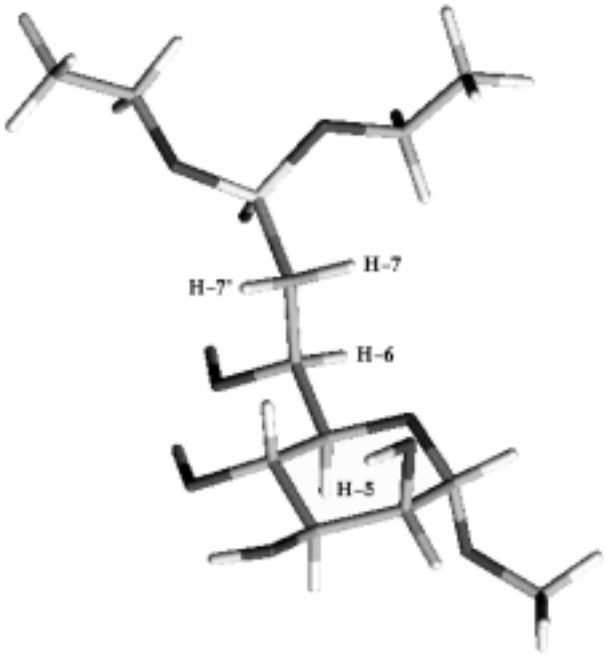


Figure 1. Stick representation of the minimum-energy conformation of **3a** (6*R*)

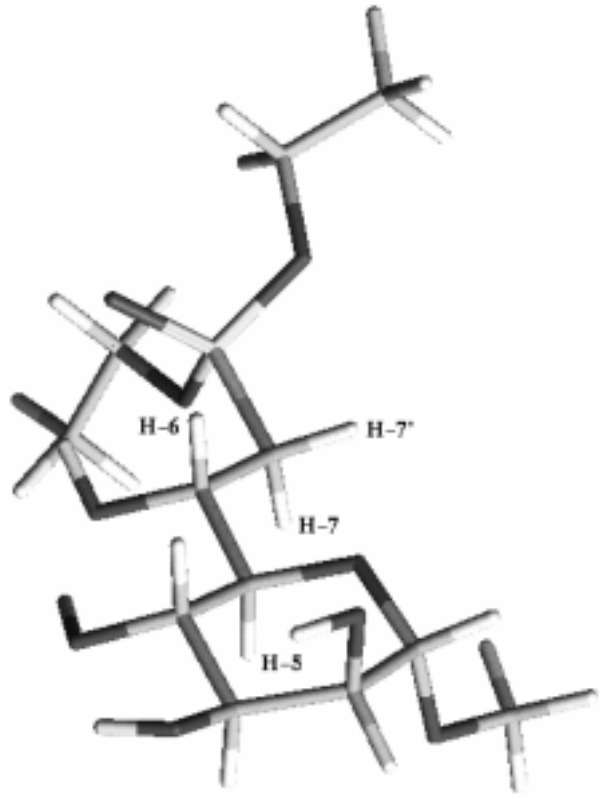


Figure 2. Stick representation of the minimum-energy conformation of **3b** (6*S*)

The coupling constant values calculated by means of the Karplus equation from the dihedral angles in **3a** (6*R*) and **3b** (6*S*) are listed in Table 1.

For compound **3b** (6*S*), the H^6-H^5 dihedral angle approaches 180° and the calculated coupling constant value is 9.6 Hz, as compared with the experimental value of 5.4 Hz. On the other hand, for the diastereoisomer **3a** (6*R*), the calculated coupling constant value is 2.1 Hz whereas no coupling constant is observed experimentally.

Biological Evaluation of the Two β-Hydroxyphosphonate Analogs of M6P

The affinities of the β-hydroxyphosphonate analogs **4a** (6*R*) and **4b** (6*S*) for M6P/IGFIIR were compared to that of M6Pn **A** by affinity chromatography on a phosphomannan Sepharose gel (Figure 3). M6P/IGFIIR was eluted with maximum efficacy using 5 or 10 mM isosteric phosphonate **A**, whereas similar concentrations of the β-hydroxyphosphonates **4a** and **4b** were totally inactive. Therefore, irrespective of the stereochemistry at the 6-position of compounds **4a**,**b**, the presence of hydroxyl groups prevents binding to M6P/IGFIIR.

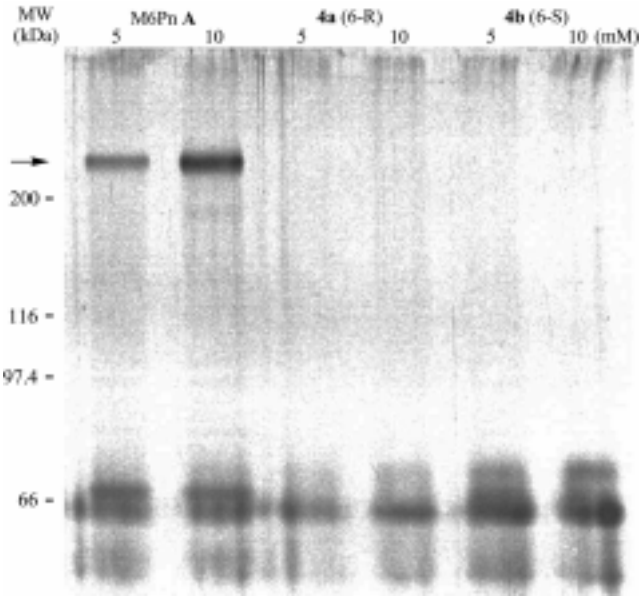


Figure 3. Affinity of mannose 6-phosphate analogs for M6P/IGFII receptors; M6P/IGFII receptors from calf serum were retained on phosphomannan Sepharose columns and eluted with the indicated concentrations of mannose 6-phosphonate **A** (M6Pn) and β-hydroxyphosphonates **4a** (6*R*) or **4b** (6*S*) as described in the Experimental Section; the eluted proteins were analyzed on 10% SDS polyacrylamide gel with visualization by silver-staining; MW, molecular weight markers, arrow indicates the eluted M6P/IGFIIR; this experiment was repeated and similar results were obtained

Conclusion

In summary, two β-hydroxyphosphonate analogs of M6P have been prepared. The diastereoisomers have been separated and the absolute configuration at the 6-position has been determined by spectroscopic analysis in conjunction with theoretical calculations. However, no recognition phenomena have been observed between the β-hydroxyphosphonates **4a**,**b** and M6P/IGFIIR. It has already been established that replacement of the phosphomonoester linkage by a methylene group does not affect the recognition phenomenon. On the other hand, further substitution at the 6-position seems to be critical with regard to the recognition phenomenon and the ineffectiveness of the β-hydroxyphos-

Table 1. Comparison of ^3J coupling constant values

	3a (6-*R*) Dihedral angle[a] (φ°)	Calculated ^3J[b] (Hz)	Experimental ^3J (Hz)	3b (6-*S*) Dihedral angle[a] (φ°)	Calculated ^3J[b] (Hz)	Experimental ^3J (Hz)
H5−H6	−62.4	2.1	−	171.2	9.6	5.4
H6−H7	−61.2	2.3	3.7	175.9	9.7	9.8
H6−H7′	−177.9	9.8	9.3	59.4	2.5	2.7

[a] According to Insight II measurements. − [b] According to Karplus equations.

phonates **4a,b** could be due to steric hindrance. Thus, in the hope of gaining further insight into the molecular basis of the interaction of M6P/IGFIIR with M6P, we are currently developing new M6P mimetics.

Experimental Section

General Aspects: Reactions were monitored by TLC using aluminum-backed plates coated with silica gel 60 F_{254} (Merck); spots were visualized with UV light (254 nm) or by charring with H_2SO_4 (10% aqueous spray solution). Aldehyde spots were developed by spraying with 5% rhodanine solution in ethanol followed by heating. Molybdenum blue was used to develop phosphorus-containing compounds. − Column chromatography was performed on Carlo Erba silica gel 60A (35−70 μm). − ^{1}H and ^{31}P NMR spectra were recorded on an AC-250 Bruker spectrometer and ^{13}C NMR spectra on a WP-200-SY Bruker spectrometer. Chemical shifts are given on the δ scale using residual solvent peaks as a reference relative to TMS. − Specific rotations were measured with sodium D light at 20 °C using a Perkin−Elmer polarimeter. − Mass spectra were measured on a DX 300 JEOL spectrometer in the FAB^+ (NBA) or FAB^- (GT) ion modes. − The cation-exchange resin (Dowex 50WX2 H^+) was washed with 1 M NaOH solution and then with distilled water prior to use. − The pentamannose 6-phosphate was functionalized with β-(*p*-aminophenyl)ethylamine, then reduced with sodium tetrahydroborate, and finally coupled on CNBr-activated Sepharose to give phosphomannan Sepharose.[14] − The M6P/IGFII receptor was purified from fetal calf serum on a phosphomannan-Sepharose affinity column according to the method described previously.[15] − SDS polyacrylamide gel electrophoresis was performed according to Laemmli[16] and proteins were visualized using a silver-staining kit (Biorad).

Methyl 7-Deoxy-7-diethyloxyphosphinyl-2,3,4-tri-*O*-benzyl-(D,L)-*glycero*-α-D-*manno*-heptopyranoside (2a,b): In a two-necked round-bottomed flask, oxalyl chloride (0.41 mL, 4.74 mmol) was dissolved in THF (5.2 mL) and then DMSO (0.73 mL, 10.34 mmol) was added dropwise at −60 °C. The mixture was stirred for 15 min and then a solution of methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **(1)** (2.0 g, 4.31 mmol) in THF (4.3 mL) was added dropwise by means of a cannula, also at −60 °C. The resulting mixture was stirred for a further 15 min. Then, *i*Pr_2NEt (3.7 mL, 21.55 mmol) was added dropwise at −60 °C and the mixture was stirred at room temp. for 1 h. It was then concentrated in vacuo, diluted with CH_2Cl_2, and the organic phase was washed with water, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude aldehyde thus obtained was used for the next reaction without further purification.

In a two-necked round-bottomed flask, 1.6 M butyllithium in hexane (2.15 mL, 3.46 mmol) was diluted with anhydrous THF (7.8 mL). The solution was cooled to −78 °C, whereupon methyldiethylphosphonate (0.5 mL, 3.46 mmol) was added. The resulting mixture was stirred at −78 °C for 30 min and then a solution of the aldehyde (1.0 g, 2.16 mmol) in anhydrous THF (9 mL) was added dropwise by means of a cannula. The reaction was subsequently quenched by the addition of 1 M NH_4Cl solution (10 mL) and then CH_2Cl_2 was added. The organic layer was washed with 1 M NH_4Cl solution then twice with water, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether/AcOEt, 7:3) to afford compounds **2a,b** (1.06 g, 40%) as a mixture of diastereoisomers. − ^{31}P NMR ($CDCl_3$): δ = 31.14 (45%), 32.46 (55%) [2 s, $PO(OEt)_2$].

Methyl 7-Deoxy-7-diethyloxyphosphinyl-L-*glycero*-α-D-*manno*-heptopyranoside (3a) and Methyl 7-Deoxy-7-diethyloxyphosphinyl-D-*glycero*-α-D-*manno*-heptopyranoside (3b): The diastereoisomeric mixture **2a,b** (1.0 g, 1.63 mmol) was dissolved in ethanol/H_2O (1:1, *v*/*v*, 60 mL), 10% Pd/C (0.3 g) was added, and the mixture was stirred under hydrogen atmosphere for 4 days. It was then filtered through Celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt/MeOH, 9:1, *v*/*v*) to afford the separated diastereoisomers **3a** and **3b** (total yield 0.45 g, 80%) in relative amounts of 45% and 55%, respectively.

3a: $[\alpha]_D^{20}$ = +38.0 (*c* = 0.85, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, *J* = 7.0 Hz, 6 H, 2 × C*H*$_3$CH$_2$OP), 1.90 (ddd, J_{7-6} = 3.7 Hz, $J_{7-7'}$ = 16.1 Hz, $J_{7\text{-}P}$ = 19.1 Hz, 1 H, 7-H), 2.29 (td, $J_{7'\text{-}6}$ = 9.3 Hz, $J_{7'\text{-}7}$ = $J_{7'\text{-}P}$ = 16.1 Hz, 1 H, 7′-H), 3.25 (s, 3 H, OC*H*$_3$), 3.31 (d, J_{5-4} = 9.6 Hz, 1 H, 5-H), 3.71 (dd, J_{3-2} = 3.0 Hz, J_{3-4} = 9.6 Hz, 1 H, 3-H), 3.84 (t, 1 H, 4-H), 3.80−3.85 (m, 1 H, 2-H), 3.95−4.10 (m, 4 H, 2 × CH$_3$C*H*$_2$OP), 4.40 (m, 1 H, 6-H), 4.66 (s, 1 H, 1-H). − ^{13}C NMR ($CDCl_3$): δ = 16.7 (d, *J* = 2.2 Hz, *C*H$_3$CH$_2$OP), 16.8 (d, *J* = 2.7 Hz, *C*H$_3$CH$_2$OP), 31.1 (d, *J* = 141.4 Hz, C-7), 55.4 (OCH_3), 62.1 (d, *J* = 6.5 Hz, CH$_3$*C*H$_2$OP), 62.5 (d, *J* = 6.2 Hz, CH$_3$*C*H$_2$OP), 64.5 (d, *J* = 2.6 Hz, C-6), 67.1 (C-2), 71.1 (C-4), 71.9 (C-3), 74.5 (d, *J* = 13.4 Hz, C-5), 101.8 (C-1). − ^{31}P NMR ($CDCl_3$): δ = 31.94. − MS (FAB^+): *m*/*z* (%) (NBA) = 711 (5) $[2M + Na]^+$, 367 (100) $[M + Na]^+$, 345 (10) $[M + H]^+$. − $C_{12}H_{25}O_9P$ (344.3): calcd. C 41.86, H 7.32; found C 41.73, H 7.38.

3b: $[\alpha]_D^{20}$ = +58.0 (*c* = 0.6, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, *J* = 7.0 Hz, 6 H, 2 × C*H*$_3$CH$_2$OP), 2.06 (td, J_{7-6} = 9.8 Hz, $J_{7-7'}$ = $J_{7\text{-}P}$ = 15.9 Hz, 1 H, 7-H), 2.29 (ddd, $J_{7'\text{-}6}$ = 2.7 Hz, $J_{7'\text{-}P}$ = 18.8 Hz, 1 H, 7′-H), 3.27 (s, 3 H, OCH_3), 3.39 (dd, J_{4-5} = 9.3 Hz, J_{5-6} = 5.4 Hz, 1 H, 5-H), 3.72 (dd, J_{2-3} = 3.2 Hz, J_{3-4} = 9.3 Hz, 1 H, 3-H), 3.81 (t, 1 H, 4-H), 3.83 (dd, J_{1-2} = 1.3 Hz, 1 H, 2-H), 4.00−4.10 (m, 4 H, 2 × CH$_3$C*H*$_2$OP), 4.24 (m, 1 H, 6-H), 4.62 (d, 1 H, 1-H). − ^{13}C NMR ($CDCl_3$): δ = 16.7 (d, *J* = 1.8 Hz, *C*H$_3$CH$_2$OP), 16.8 (d, *J* = 2.2 Hz, *C*H$_3$CH$_2$OP), 29.9 (d, *J* = 140.8 Hz, C-7), 55.4 (OCH_3), 62.3 (d, *J* = 6.4 Hz, CH$_3$*C*H$_2$OP), 62.6 (d, *J* = 6.2 Hz, CH$_3$*C*H$_2$OP), 69.2 (d, *J* = 4.8 Hz, C-6), 70.4 (C-2), 70.8 (C-4), 71.9 (C-3), 73.5 (d, *J* = 15.1 Hz, C-5), 101.5 (C-1). − ^{31}P NMR ($CDCl_3$): δ = 32.54. − MS (FAB^+): *m*/*z* (%) (NBA) = 367 (90) $[M + Na]^+$, 345 (12) $[M + H]^+$. − $C_{12}H_{25}O_9P$ (344.3): calcd. C 41.86, H 7.32; found C 41.78, H 7.35.

Methyl 7-Deoxy-7-dihydroxyphosphinyl-L-*glycero*-α-D-*manno*-heptopyranoside Disodium Salt (4a): To a solution of **3a** (0.088 g, 0.256 mmol) in anhydrous CH_3CN (3.2 mL) under nitrogen atmosphere was added pyridine (0.21 mL, 2.56 mmol) and Me_3SiBr (0.33 mL, 2.56 mmol). After stirring at room temperature for 2 h, distilled water (5 mL) and pyridine (0.21 mL, 2.56 mmol) were added at 0 °C. The organic layer was concentrated and then distilled water (75 mL) and cation-exchange resin (35 g) were added. After stirring at room temperature for 24 h, the resin was filtered off and washed several times with water. The filtrate was concentrated in vacuo and the residue was purified by reversed-phase chromatography (RP-18/H_2O) to afford **4a** (0.055 g, 65%). − $[\alpha]_D^{20}$ = −6.0 (*c* = 1.00, MeOH). − ^{1}H NMR (D_2O): δ = 1.90−2.25 (m, 2 H, 7,7′-H), 3.50 (s, 3 H, OCH_3), 3.51−4.07 (m, 4 H, 2,3,4,5-H), 4.30−4.47 (m, 1 H, 6-H), 4.88 (d, J_{1-2} = 1.4 Hz, 1 H, 1-H). − ^{13}C NMR (D_2O): δ = 32.7 (d, *J* = 132.0 Hz, C-7), 55.1 (OCH_3), 65.2 (d, *J* = 4.3 Hz, C-6), 66.8, 67.6, 70.4 (C-2,3,4), 74.4 (d, *J* = 11.3 Hz, C-5), 101.5 (C-1). − ^{31}P NMR (D_2O): δ = 23.57. − MS (FAB^-): *m*/*z* (%) (GT) = 287 (95) $[M - 2\,Na + H]^-$. − $C_8H_{15}Na_2O_9P$ (332.1): calcd. C 28.93, H 4.55; found C 28.68, H 4.65.

Methyl 7-Deoxy-7-dihydroxyphosphinyl-D-*glycero*-α-D-*manno*-heptopyranoside Disodium Salt (4b): Using the same procedure as for **4a**, compound **4b** was obtained in 65% yield. − $[\alpha]_D^{20}$ = +8.0 (*c* = 1.02, MeOH). − ^{1}H NMR (D_2O): δ = 1.85−2.20 (m, 2 H, 7,7′-H), 3.49 (s, 3 H, OCH_3), 3.66−4.09 (m, 4 H, 2,3,4,5-H), 4.38−4.42 (m, 6-H), 4.89 (d, J_{1-2} = 1.4 Hz, 1 H, 1-H). − ^{13}C NMR (D_2O): δ = 29.4 (d, *J* = 131.6 Hz, C-7), 55.1 (OCH_3), 67.2 (d, *J* = 4.2 Hz, C-6), 67.6, 70.1, 71.0 (C-2,3,4), 74.7 (d, *J* = 13.3 Hz, C-5), 101.2 (C-1). − ^{31}P NMR (D_2O): δ = 25.18. − MS (FAB^-): *m*/*z* (%) (GT) = 287 (90) $[M - 2\,Na + H]^-$. − $C_8H_{15}Na_2O_9P$ (332.1): calcd. C 28.93, H 4.55; found C 28.57, H 4.62.

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