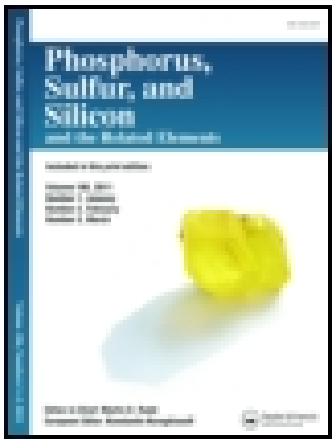

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A Flexible Route to Mannose 6-Phosphonate Functionalized Derivatives

Sébastien Vidal [a] , Jean-Louis Montero [a] , Alain Leydet [a] & Alain Morère [a]

[a] Laboratoire de Chimie Biomoléculaire , Université Montpellier II, Ecole Nationale Supérieure de Chimie de Montpellier , Montpellier, France

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A FLEXIBLE ROUTE TO MANNOSE 6-PHOSPHONATE FUNCTIONALIZED DERIVATIVES

Sébastien Vidal, Jean-Louis Montero, Alain Leydet, and Alain Morère
Laboratoire de Chimie Biomoléculaire, Université Montpellier II, Ecole Nationale Supérieure de Chimie de Montpellier, Montpellier, France

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A new approach for the synthesis of a mannose 6-phosphonate isosteric analog of mannose 6-phosphate is reported. The mannosylphosphonate has been prepared in a multistep synthesis involving an homologation reaction of the methyl α-D-mannopyranoside followed by an Arbuzov reaction between a bromohomomannosyl derivative and the tris*(trimethylsilyl)phosphite. This approach, avoiding the deprotection of dialkylphosphonate, allowed us to prepare the mannose 6-phosphonate in good yield. The described method was successfully extended to the preparation of a mannose 6-phosphonate linked to a cholesteryl moiety. This strategy affords a more general route for a wide range of functionalized mannose 6-phosphonate derivatives.*

Keywords: Glycolipid; lectin; mannose 6-phosphate; mannose 6-phosphate/insulin-like growth factor; mannose 6-phosphonate/triethyleneglycolcholesteryl; phosphonation; *tris*(trimethylsilyl)-phosphite

INTRODUCTION

Mannose 6-Phosphate (M6P) is a recognition marker involved in the selective targeting of newly synthesized enzymes to lysosomes.[1] While two different receptors recognize specifically the M6P residues, only the larger M6P receptor mediates the endocytosis of extracellular

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Address correspondence to Alain Morere, Laboratoire de Chimie Biomoléculaire (UMR 5032), Université Montpellier II, Ecole Nationale Supérieure de Chimie de Montpellier, 8 Rue de l'Ecole Normale, F-34296 Montpellier Cedex 05, France. E-mail: morere@univ-montp2.fr

M6P-containing ligands.[2] This receptor is a type I glycoprotein of 275 kDa, which apart from M6P residues, is also known to bind retinoic acid[3] through distinct binding sites (RA) and insulin-like growth factor II[4] (IGF-II). This receptor is the first example of a protein able to bind three different classes of ligands, that is, a saccharide (M6P), a peptide (IGF-II), and a lipid (RA). Moreover, the M6P/IGFIIR plays a fundamental role in the control of cell growth in fetal development and carcinogenesis.[6] In our ongoing research program, we focus on the ability of the M6P/IGF-IIR to recognize and then internalize M6P analogs. We recently described[7] the synthesis of two mannose 6-phosphonate (M6Pn) analogs (**I** and **II**) of M6P (Figure 1), and evaluated their affinity towards the M6P/IGFIIR.

Isosteric compound **I** has been shown to have an affinity to the M6P/IGFIIR similar to natural M6P, whereas nonisosteric compound **II** was only weakly recognized. In our previous syntheses[7,8] of the mannosyl phosphonate **I**, the key reaction in the synthetic scheme was the last step, involving the deprotection of the diethylphosphonate moiety according to Rabinowitz's procedure.[9] However, the conversion of diethylphosphonates into the corresponding free phosphonic acids using the method of transesterification by Me_3SiBr can be troublesome. Indeed, degradation problems, partial deprotection, or even complete failure of the deprotection has been described in several cases, such as with aminoacids or glycopyranose frameworks.[10–12] Recently, these difficulties were met with the preparation of a glycoconjugate we called

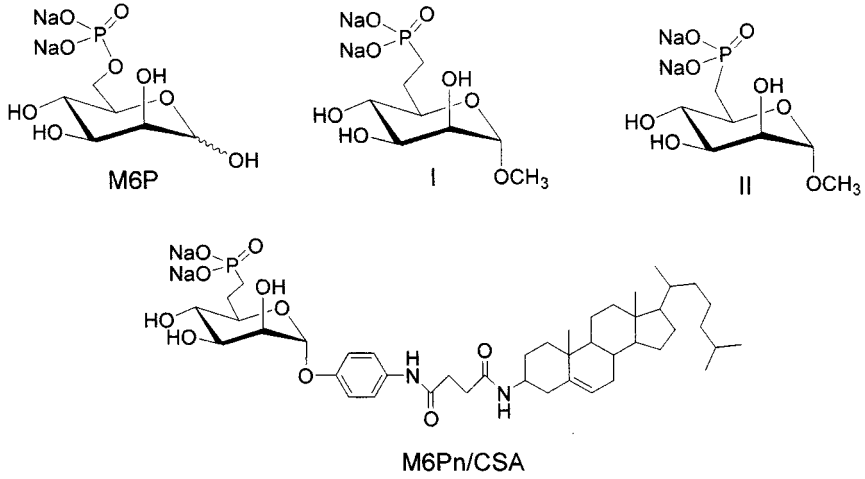


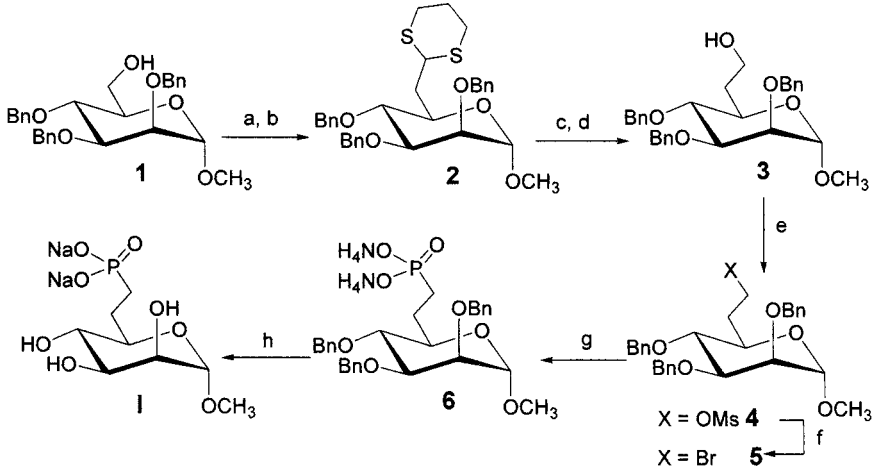
FIGURE 1 Phosphonate analogs of M6P.

M6Pn/CSA,[13] where M6Pn and CSA stand for mannose 6-phosphonate and cholesteryl-succinyl-anilinyl respectively (Figure 1). Partial deprotection of the diethylphosphonate of this molecule and partial cleavage of the anomeric bond and/or amide bonds were observed under Rabinowitz's procedure. It is worth pointing out that this amphiphilic steroidal mannose 6-phosphonate incorporated into the lipidic bilayer of a liposome[13] constitute an efficient drug delivery system in selective targeting of breast cancer cells.

RESULTS AND DISCUSSION

In order to circumvent the Rabinowitz deprotection step in the synthesis of M6Pn **I** and its conjugates, we decided to introduce the phosphonate moiety by an Arbuzov reaction between *tris*(trimethylsilyl)phosphite and a halogenohomomannosyl derivative. This attractive approach could furnish the *bis*(trimethylsilyl)ester which can be easily hydrolyzed with saturated aqueous $NaHCO_3$ solution.[14] The key step of this alternative synthesis involves the preparation of a mannoheptose derivative by a one carbon chain extension at the C-6 position. Various procedures are described in the literature, and many reactants can be used such as cyanide anion,[15–20] triphenylmethoxymethylphosphonium salt,[21] or iron-carbonyl-cyclopentadienyl complexes.[22,23] Homologation can be also accomplished via alkyne[24–28] or epoxysilane[29,30] derivatives. Another method is possible using a cyclic sulfate intermediate.[31]

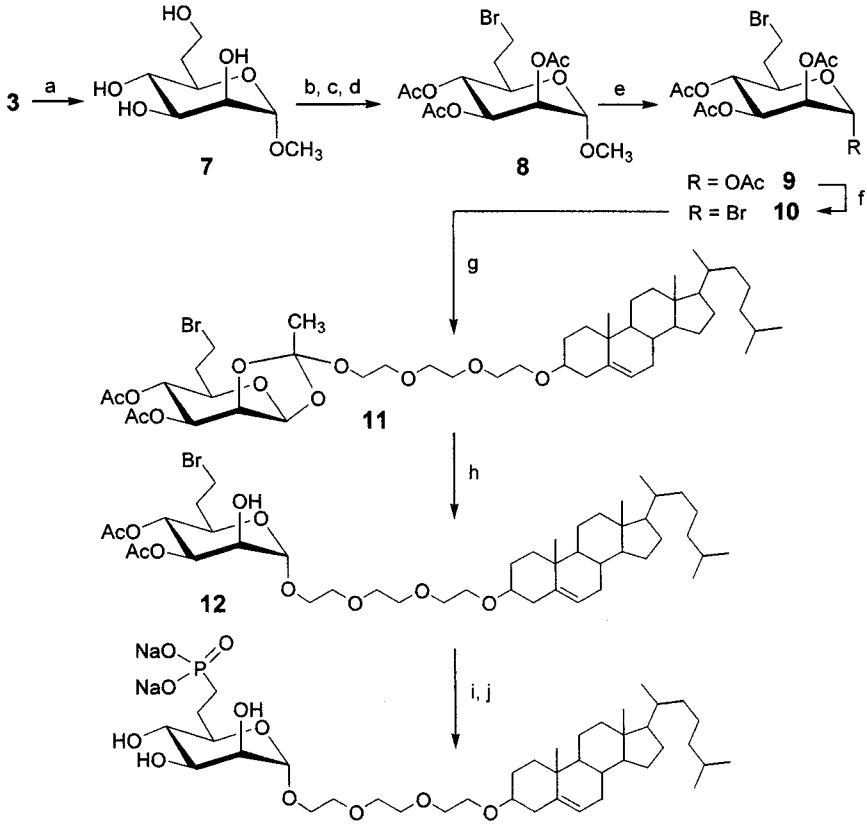
The preparation of the mannose 6-phosphonate was realized (Scheme 1) via an homologation reaction involving condensation of the 2-lithio-1,3-dithiane[32] with the triflate derivative of alcohol **1**.[33] Compound **2** was then reacted with ceric ammonium nitrate (CAN)[34–36] in wet acetonitrile affording a crude aldehyde (^{1}H NMR analysis gave satisfactory data showing the resonance of the aldehydic proton at 9.75 ppm) which was reduced by sodium borohydride to furnish the homologated alcohol **3** in a one-pot procedure. Reactions proceed quickly affording expected compounds in good yields. Conversion of homoalcohol **3** to homoalkylbromide **5** was carried out by reaction of **3** with methanesulfonylchloride (affording **4** in 95% yield) followed by bromination with lithium bromide[37,38] in butanone, affording **5** (95%) without further purification. This two-step procedure was preferred to the one-step PPh_3/CBr_4[39] method, in order to avoid problems with the removal of triphenylphosphine oxide byproduct by column chromatography. The phosphonate moiety was introduced by an Arbuzov reaction between *tris*(trimethylsilyl)phosphite and **5**, affording



SCHEME 1 Reagents and conditions: (a) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, −40°C, 10 min (85%). (b) 1,3-dithiane, *n*-BuLi, HMPA, THF, −78°C, 10 min (85%). (c) CAN, wet CH_3CN, rt, 15 min. (d) $NaBH_4$, EtOH/H_2O, rt, 5 min (75% for two steps). (e) MsCl, DIEA, CH_2Cl_2, rt, 15 min (95%). (f) LiBr, butanone, 85°C, 1 h (95%). (g) $P(OSiMe_3)_3$, 160°C, 16 h (85%). (h) H_2/Pd-C 10%, EtOH/H_2O, rt, 17 h then DOWEX 50WX2 (Na^+ form) (90%).

a *bis*(trimethylsilyl)phosphonate, which was hydrolyzed with saturated aqueous $NaHCO_3$ solution, giving the salt of the corresponding phosphonic acid **6**. Benzyl protecting groups were removed by hydrogenolysis over Pd-C (10%) to obtain the title compound **I** in good overall yield (29%).

We therefore decided to use the same strategy to prepare the M6Pn-based glycolipid **13** (Scheme 2) in which the hydrophilic carbohydrate head is linked to the hydrophobic steroidal tail through a triethyleneglycol (TEG) spacer arm. Compound **3** was deprotected to afford the mannoheptose derivative **7** which was activated at the 6-position followed by peracetylation[32,33,40] and subsequent bromination to afford **8** in 55% yield over three steps. The methyl glycoside **8** was then converted to the 1-*O*-acyl glycoside **9** then activated to provide a glycosyl donor intermediate **10**. Koenigs-Knorr glycosylation[41,42] of **10**, using known 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-ol[43] as the glycosyl acceptor, affords the orthoester derivative **11** as the major product, which upon subsequent treatment with a Lewis acid, underwent rearrangement[44–49] to furnish the desired *O*-glycoside **12**. These two isomers could easily be differentiated according to their respective NMR data (Figure 2 and Table I) and also by TLC.



SCHEME 2 Preparation of M6Pn/TEGC **13**—reagents and conditions: (a) H_2/Pd-C 10%, EtOH/H_2O, rt, 16 h, (95%). (b) TsCl, C_5H_5N, rt, 5 h. (c) Ac_2O, C_5H_5N, 0°C to rt, 1 h. (d) LiBr, 2-butanone, 85°C, 1 h, (55% over three steps). (e) Ac_2O/AcOH/H_2SO_4(cat.), rt, 24 h, (85%). (f) HBr/AcOH, CH_2Cl_2, rt, 16 h. (g) 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-ol, CH_2Cl_2, AgOTf, *sym*-collidine, 0°C to rt, 15 min, (45% over both steps). (h) TMSOTf, CH_2Cl_2, molecular sieves (3Å), −5°C, 5 min, (40%). (i) $P(OSiMe_3)_3$, 160°C, 16 h then $NaHCO_{3(aq)}$. (j) NaOMe, MeOH, rt, 16 h, (50% over both steps).

The final step in our synthesis involved an Arbuzov reaction between the alkyl bromide derivative **12** and *tris*(trimethylsilyl)phosphite, followed by deprotection of the acetate under Zemplén conditions affording the final M6Pn/TEGC **13** in good yields. Characterization of this glycolipid was troublesome but evidence of the structure was provided by NMR spectroscopy (^1H and ^{31}P) and also by mass spectrometric analyses.

TABLE I Comparison of Compounds **11** and **12** NMR Data

Compound	J_{1-2}[a]	$\delta_{H\text{-}1}$[b]	δ_C[c]	δ_{CH3}[d]	δ_{CH3}[e]
Orthoester **11**	2.6	5.44	124.7	—	1.71
Glycoside **12**	1.5	4.75	—	21.5	1.91

[a]Coupling constant, in Hz.
[b]^{1}H NMR chemical shift, in ppm.
[c]^{13}C NMR chemical shift for the quaternary carbon (see* in Figure 2), in ppm.
[d]^{13}C NMR chemical shift for the methyl (see † in Figure 2), in ppm.
[e]^{1}H NMR chemical shifts for the methyl groups, in ppm.

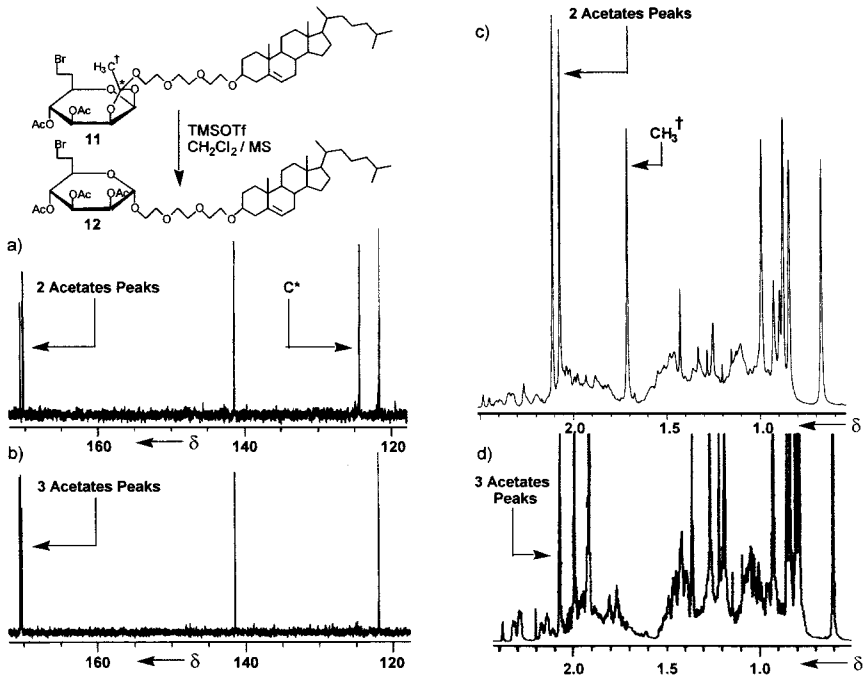


FIGURE 2 Partial NMR analyses for compounds **11** and **12**. a) ^{13}C NMR in $CDCl_3$ shows the signal corresponding to the quaternary carbon (C*) from the orthoester derivative **11** while b) does not display this peak for **12**. c) ^{1}H NMR in $CDCl_3$ shows two signals for the acetates and one more singlet corresponding to the orthoester-methyl ($CH_{3\dagger}$) group for **11** while d) displays the three signals corresponding to each acetyl groups for **12**.

CONCLUSION

The compound **I** was obtained in good overall yield (29%), demonstrating the usefulness of the Arbuzov phosphonation—using the *tris*(trimethylsilyl)phosphite—for the preparation of glycopyranosylphosphonic acids, such as the isosteric analog (**I**) of mannose 6-phosphate. This strategy was successfully applied to the preparation of a steroidal derivative M6Pn/TEGC **13**. Preparation of liposomes incorporating M6Pn/TEGC **13** and evaluation of their interactions with MCF7 cells now are being investigated in our group with the aim of developing drug delivery systems. Although the overall yield is still very low (1.75% over 16 steps starting from methyl α-D-mannopyranoside) for the preparation of M6Pn/TEGC **13**, this strategy provides a more general route to a wide variety of functionalized M6Pn where the anomeric center will be substituted with any biologically active molecule during the glycosylation.

EXPERIMENTAL

Analytical TLC were performed using aluminum-coated TLC plates 60-F_{254} (Merck). Plates were developed with: (1) UV light (254 nm), (2) immersion in a 10% H_2SO_4/EtOH solution followed by charring, (3) immersion in a 5% rhodanine/EtOH solution followed by charring (for aldehydes) or (4) immersion in a phosphomolybdic solution (for phosphorus containing compounds). Silica gel column chromatography was performed with silica gel 60A (Carlo Erba). Optical rotations were measured at the sodium D-line with a Perkin-Elmer-241 polarimeter. Fast Atom Bombardment (FAB) mass spectra were recorded on a Jeol JMS-DX300 spectrometer in either positive (FAB+) or negative (FAB−) modes and using either 3-nitrobenzylic alcohol (NBA) or glycerol/thioglycerol (1:1) mixture (G/T). ^{1}H NMR spectra were recorded on a Bruker DRX 400 (400 MHz), at 25°C. Chemical shifts (δ) are given in ppm and referenced using residual solvent signals (7.26 ppm for $CHCl_3$ and 4.79 ppm for HOD). The following abbreviations were used to explain the signal multiplicities or characteristics: s (singlet), d (doublet), dd (double doublet), t (triplet), td (triplet doublet), m (multiplet). ^{13}C NMR spectra were recorded on a Bruker DRX 400 (100.6 MHz). Chemical shifts (δ) are given in ppm relative to TMS as an external reference. ^{31}P NMR spectra were recorded on a Bruker DPX 200 (81.0 MHz). Chemical shifts (δ) are given in ppm relative to phosphoric acid (85%) as an external reference.

Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-6-(1,3-dithianyl)-α-D-mannopyranoside 2

To a solution of **1**[33] (1 g, 2.16 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (555 mg, 2.7 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise triflic anhydride (633 μL, 2.37 mmol), at −40°C. After stirring for 30 min, the reaction was neutralized with $NaHCO_3$ (50 mL, 1 g/100 mL H_2O) and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane then hexane/ether 4:1) affording a pale pink oil (1.09 g, 85%). To a solution of 1,3-dithiane (706 mg, 5.87 mmol) dissolved in THF (2 mL) was added dropwise HMPA (300 μl, 1.67 mmol). The mixture was cooled to −78°C before addition of *n*-BuLi (5 mL, 5.87 mmol, 1.6 M in hexane). After 5 min, a solution of the triflate derivative previously prepared (1 g, 1.67 mmol) in THF (5 mL) was added dropwise at −78°C. After 10 min, the reaction mixture was quenched with NH_4Cl (50 mL, 1 g/100 mL H_2O) and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ether 95:5 then 3:1) affording **2** as pale yellow oil (807 mg, 85%). $R_f = 0.67$ (hexane/ether 2:3). $[\alpha]_D^{20} = +20.8^\circ$ (*c* 0.72/$CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 1.80-2.20$ (m, 3H, H-6 CH_2CH$_2$S), 2.30−2.50 (m, 1H, H-6′), 2.70−3.00 (m, 4H, CH_2S), 3.41 (s, 3H, OCH_3), 3.73 (t, 1H, $J = 9.3$ Hz, H-4), 3.84 (dd, 1H, $J = 1.9$ and 2.8 Hz, H-2), 3.90−4.05 (m, 2H, H-3 H-5), 4.32 (dd, 1H, $J = 3.7$ and 11.0 Hz, H-7), 4.65 (s, 2H, CH_2Ph), 4.69 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.72 (d, 1H, H-1), 4.76 (d, 1H, $J = 12.5$ Hz, CH_2Ph), 4.83 (d, 1H, CH_2Ph), 5.01 (d, 1H, CH_2Ph), 7.20−7.50 (m, 15H, 15H-ar). ^{13}C NMR ($CDCl_3$): $\delta = 24.0$, 24.3, 29.0, 36.5, 42.1, 54.0, 67.0, 70.0–73.0 (3C), 73.7, 77.7, 79.2, 98.1, 125.0–127.0 (15C), 137.0–137.5 (3C). MS (FAB+, NBA): *m/z*: 589 $[M + Na]^+$, 567 $[M + H]^+$, 535 $[M{-}OCH_3]^+$, 475 $[M{-}PhCH_2]^+$, 91 $[PhCH_2]^+$.

Methyl 2,3,4-Tri-O-benzyl-6-deoxy-α-D-*manno*-heptopyranoside 3

To a solution of **2** (440 mg, 0.78 mmol) dissolved in CH_3CN/H_2O (15:1, 16 mL) was added ceric ammonium nitrate (1.285 g, 2.34 mmol). After stirring at rt for 15 min, the reaction mixture was evaporated under reduced pressure before diluting with EtOAc (100 mL). The organic layer was washed with H_2O (2 × 75 mL) and the organic layer extracted with EtOAc (2 × 100 mL). Organic layers were combined, dried (Na_2SO_4),

filtered, and evaporated under reduced pressure. The crude oil was dissolved in absolute ethanol (10 mL) before addition of a solution of $NaBH_4$ (75 mg, 1.95 mmol) in EtOH/H_2O (1:1, 20 mL). The reaction mixture was stirred at rt for 5 min before being neutralized with HCl (**1** M) and then diluted with H_2O (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ether 7:3 then 1:1) affording **3** as pale yellow oil (278 mg, 75%). $R_f = 0.44$ (hexane/ether 1:4). $[\alpha]_D^{20} = +21.5^\circ$ (*c* 0.65/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.80–2.30 (m, 2H, H-6 H-6′), 3.35 (s, 3H, OC*H*$_3$), 3.75–3.95 (m, 6H, H-2 H-3 H-4 H-5 H-7 H-7′), 4.65 (s, 2H, C*H*$_2$Ph), 4.66 (d, 1H, *J* = 10.9 Hz, C*H*$_2$Ph), 4.70 (d, 1H, *J* = 1.7 Hz, H-1), 4.73 (d, 1H, *J* = 12.4 Hz, C*H*$_2$Ph), 4.82 (d, 1H, C*H*$_2$Ph), 5.05 (d, 1H, C*H*$_2$Ph), 7.30–7.50 (m, 15H, 15H-ar). ^{13}C NMR ($CDCl_3$): δ = 34.2, 55.2, 61.6, 71.9, 72.5, 73.4, 74.9, 75.7, 78.6, 80.6, 99.5, 128.0–129.0 (15C), 138.6–138.9 (3C). MS (FAB+, NBA): *m/z*: 501 $[M + Na]^+$, 479 $[M + H]^+$, 447 $[M{-}OCH_3]^+$, 91 $[PhCH_2]^+$.

Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-7-*O*-methanesulfonyl-α-D-*manno*-heptopyranoside 4

To a solution of **3** (250 mg, 0.52 mmol) dissolved in CH_2Cl_2 (5 mL) was added DIEA (116 μL, 0.68 mmol) and then methanesulfonyl chloride (50 μL, 0.63 mmol). After stirring for 15 min at rt, the reaction mixture was diluted with CH_2Cl_2 (150 mL) and the organic layer was washed with $NaHCO_3$ (10% aqueous, 2 × 100 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure affording **4** as a white oil (275 mg, 95%). $R_f = 0.55$ (hexane/ether 1:4). ^{1}H NMR ($CDCl_3$): δ = 1.80–2.05 (m, 1H, H-6), 2.25–2.50 (m, 1H, H-6′), 2.95 (s, 3H, C*H*$_3$SO$_2$), 3.34 (s, 3H, OC*H*$_3$), 3.60–4.00 (m, 4H, H-2 H-3 H-4 H-5), 4.35–4.50 (m, 2H, H-7 H-7′), 4.65 (s, 2H, C*H*$_2$Ph), 4.66 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.71 (d, 1H, *J* = 1.8 Hz, H-1), 4.73 (d, 1H, *J* = 12.3 Hz, C*H*$_2$Ph), 4.81 (d, 1H, C*H*$_2$Ph), 5.01 (d, 1H, C*H*$_2$Ph), 7.30–7.50 (m, 15H, 15H-ar). ^{13}C NMR ($CDCl_3$): δ = 31.7, 37.8, 55.3, 67.0, 67.8, 72.5, 73.4, 75.0, 75.7, 78.7, 80.7, 99.5, 128.0–129.0 (15C), 138.0–139.0 (3C). MS (FAB+, NBA): *m/z*: 579 $[M + Na]^+$, 557 $[M + H]^+$, 525 $[M{-}OCH_3]^+$, 91 $[PhCH_2]^+$.

Methyl 2,3,4-Tri-*O*-benzyl-7-bromo-6,7-dideoxy-α-D-*manno*-heptopyranoside 5

A solution of **4** (200 mg, 0.36 mmol) and LiBr (625 mg, 7.2 mmol) in 2-butanone (10 mL) was stirred at 85°C for 1 h. The reaction mixture

was cooled to rt then diluted with CH_2Cl_2 (130 mL) and the organic layer was washed with H_2O (2 × 100 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure affording **5** as a pale yellow oil (185 mg, 95%). $R_f = 0.68$ (hexane/ether 2:3). $[\alpha]_D^{20} = +28.6^\circ$ (*c* 0.21/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 2.00–2.25 (m, 1H, H-6), 2.35–2.50 (m, 1H, H-6′), 3.41 (s, 3H, OC*H*$_3$), 3.86 (dd, 1H, *J* = 1.8 and 3.1 Hz, H-2), 3.96 (dd, 1H, *J* = 8.9 Hz, H-3), 3.55–3.90 (m, 4H, H-4 H-5 H-7 H-7′), 4.67 (s, 2H, C*H*$_2$Ph), 4.69 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.74 (d, 1H, H-1), 4.77 (d, 1H, *J* = 12.4 Hz, C*H*$_2$Ph), 4.85 (d, 1H, C*H*$_2$Ph), 5.02 (d, 1H, C*H*$_2$Ph), 7.30–7.50 (m, 15H, 15H-ar). ^{13}C NMR ($CDCl_3$): δ = 28.7, 34.0, 53.9, 67.9, 71.1, 71.9, 73.5, 74.1, 77.2, 79.3, 98.0, 126.5–128.0 (15C), 137.0–137.5 (3C). MS (FAB +, NBA): *m/z*: 563 $[M + Na]^+$, 509 $[M{-}OCH_3]^+$, 449 $[M{-}PhCH_2]^+$, 417 $[M{-}PhCH_2{-}OCH_3]^+$, 91 $[PhCH_2]^+$.

Diammonium Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-6-dihydroxyphosphonylmethylene-α-D-mannopyranoside 6

A solution of **5** (130 mg, 0.24 mmol) dissolved in *tris*(trimethylsilyl) phosphite (50 eq, 11.96 mmol) was stirred at 160°C for 16 h. The reaction mixture was then diluted with CH_2Cl_2 (150 mL) and poured into satd. $NaHCO_3$ (100 mL) at 0°C. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (*i*-PrOH/NH_4OH/H_2O 6:3:1) affording **6** as white foam after freeze drying from H_2O (118 mg, 85%). $R_f = 0.50$ (*i*-PrOH/NH_4OH/H_2O 6:3:1). ^{1}H NMR ($CDCl_3$): δ = 1.50–2.30 (m, 4H, H-6 H-6′ H-7 H-7′), 3.17 (s, 3H, OC*H*$_3$), 3.40–3.85 (m, 4H, H-2 H-3 H-4 H-5), 4.40–4.90 (m, 7H, H-1 3C*H*$_2$Ph), 6.70–7.00 (m, 8H, 2N*H*$_4$), 7.10–7.40 (m, 15H, 15H-ar). ^{13}C NMR ($CDCl_3$): δ = 23.3 (d, *J* = 138.6 Hz), 24.3 (d, *J* = 2.3 Hz), 53.7, 70.6–79.3 (3C), 70.7 (d, *J* = 15.5 Hz), 70.8, 71.0, 74.1, 97.7, 126.4–127.9 (15C), 137.1–137.7 (3C). ^{31}P NMR ($CDCl_3$): δ = 27.2 (s, *P*(O)$(ONH_4)_2$). MS (FAB +, NBA): *m/z*: 565 $[M{-}2NH_4{+}Na]^+$, 543 $[M{-}2NH_4{+}H]^+$, 511 $[M{-}2NH_4{-}OCH_3]^+$, 91 $[PhCH_2]^+$.

Disodium Methyl 6-Deoxy-6-dihydroxyphosphonylmethylene-α-D-mannopyranoside I

A suspension of **6** (100 mg, 0.24 mmol) and Pd-C 10% (250 mg) in EtOH/H_2O (1:1, 15 mL) was stirred at rt under H_2 atmosphere for 16 h. The reaction mixture was filtered through celite and the filtrate evaporated under reduced pressure. The residue was dissolved in H_2O (30 mL) and ion exchange resin (Dowex 50WX2, Na^+ form, 10 g) was

added and the solution was kept at rt for 24 h before filtering-off the resin and freeze drying from H_2O to afford **I** as a white foam (48 mg, 90%). $R_f = 0.16$ (*i*-PrOH/NH_4OH/H_2O 6:3:1). ^1H NMR (D_2O): $\delta = 1.20$–2.10 (m, 4H, H-6 H-6′ H-7 H-7′), 3.26 (s, 3H, OCH_3), 3.30–3.70 (m, 3H, H-3 H-4 H-5), 3.79 (dd, 1H, $J = 1.4$ and 3.2 Hz, H-2), 4.59 (d, 1H, H-1). ^{13}C NMR (D_2O): $\delta = 24.2$ (d, $J = 134.4$ Hz), 25.4 (d, $J = 2.2$ Hz), 55.0, 70.2–70.8 (3C), 72.7 (d, $J = 17.1$ Hz), 101.1 (C-1). ^{31}P NMR (D_2O): $\delta =$ 26.7 (s, *P*(O)$(ONa)_2$). MS (FAB+, NBA): *m/z*: 273 $[M-2Na+3H]^+$. MS (FAB−, NBA): *m/z*: 271 $[M-2Na-H]^-$.

Methyl 6-Deoxy-α-D-*manno*-heptopyranoside 7

A suspension of **3** (1.84 g, 3.84 mmol) and Pd-C 10% (250 mg) in EtOH/H_2O (1:1, 20 mL) was stirred at rt under H_2 atmosphere for 16 h. The reaction mixture was filtered through celite and the filtrate evaporated under reduced pressure affording **7** as a white foam (760 mg, 95%). $R_f = 0.50$ (CH_2Cl_2/MeOH 4:1). ^1H NMR (D_2O): $\delta = 1.50$–1.70 (m, 1H, H-6), 1.90–2.10 (m, 1H, H-6′), 3.27 (s, 3H, OCH_3), 3.38 (t, 1H, $J =$ 9.5 Hz, H-4), 3.45–3.75 (m, 4H, H-3 H-5 H-7 H-7′), 3.81 (dd, 1H, $J = 1.6$ and 3.4 Hz, H-2), 4.58 (d, 1H, H-1).

Methyl 2,3,4-Tri-*O*-acetyl-7-bromo-6,7-dideoxy-α-D-*manno*-heptopyranoside 8

To a solution of **7** (760 mg, 3.65 mmol) in C_5H_5N (20 mL) was added a solution of TsCl (1.05 g, 5.48 mmol) in C_5H_5N (10 mL). After stirring for 5 h at rt, acetic anhydride (5 mL) was added at 0°C. The reaction was stirred at rt for 1 h then poured into satd. $NaHCO_3$ (150 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure and C_5H_5N was coevaporated with toluene (3 × 100 mL). The residue was dissolved in 2-butanone (50 mL) and LiBr (6.35 g, 73.07 mmol) was added. The reaction was stirred at 85°C for 1 h then cooled to rt and diluted with H_2O (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (light petroleum/ether 9:1 then 2:3) to afford **8** (800 mg, 55%). $R_f = 0.57$ (light petroleum/ether 2:3). ^1H NMR ($CDCl_3$): $\delta = 1.80$–2.05 (m, 2H, H-6 H-6′), 1.87 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 3.34 (s, 3H, OCH_3), 3.40–3.50 (m, 2H, H-7 H-7′), 3.88 (td, 1H, $J = 3.3$ and 9.8 Hz, H-5), 4.58 (d, 1H, $J = 1.7$ Hz, H-1), 4.99 (t, 1H, H-4), 5.11 (dd, $J = 3.4$ Hz ,H-2), 5.18 (dd, 1H, H-3).

1,2,3,4-Tetra-*O*-acetyl-7-bromo-6,7-dideoxy-D-*manno*-heptopyranoside 9

A solution of **8** (100 mg) in AcOH/Ac_2O (4:1, 10 mL) and two drops of conc. H_2SO_4 was stirred at rt for 24 h. The reaction mixture was neutralized with satd. $NaHCO_3$ (100 mL) and the aqueous layer was extracted with EtOAc (3×100 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (light petroleum/ether 95:5 then 3:7) affording **9** as a pale yellow oil (91 mg, 85%) and a portion of **8** (15 mg) was recovered. $R_f = 0.61$ (light petroleum/ether 3:7). ^{1}H NMR ($CDCl_3$): $\delta = 1.80$–2.00 (m, 2H, H-6 H-6′), 2.00 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), 3.40–3.70 (m, 2H, H-7 H-7′), 4.08 (td, 1H, $J = 3.0$ and 9.9 Hz, H-5), 5.16 (t, 1H, H-4), 5.26 (dd, 1H, $J = 1.8$ and 3.5 Hz, H-2), 5.34 (dd, 1H, H-3), 5.99 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): $\delta = 20.9$, 21.1, 21.2, 29.2, 33.9, 68.4–69.6 (4C), 90.6, 170.1, 170.2, 170.3, 170.4. MS (FAB+, NBA): *m/z*: 447 $[M + Na]^+$, 365 $[M{-}OAc]^+$, 345 $[M{-}Br]^+$, 245 $[M{-}2OAc{-}2H]^+$, 203 $[M{-}2OAc{-}CH_3CO{-}H]^+$, 43 $[CH_3CO]^+$.

2,3,4-Tri-*O*-acetyl-7-bromo-6,7-dideoxy-1,2-*O*-[8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-yloxyethylidene]-β-D-*manno*-heptopyranose 11

A solution of **9** (220 mg, 0.52 mmol) and HBr (5.7 M in AcOH, 2.28 mL, 13 mmol) in CH_2Cl_2 (15 mL) was stirred at rt for 16 h. The reaction mixture was poured into satd. $NaHCO_3$ (50 mL) and the aqueous layer was extracted with CH_2Cl_2 (3×70 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure affording **10** used for next step as the crude product. To a solution of 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-ol[43] (287 mg, 0.57 mmol) and AgOTf (146 mg, 0.57 mmol) dissolved in CH_2Cl_2 (5 mL) was added *sym*-collidine (76 μL, 0.57 mmol) and a solution of **10** in CH_2Cl_2 (15 mL). After stirring at rt for 15 min, the reaction mixture was filtered through celite and the filtrate evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (light petroleum/ether 4:1 then ether) affording **11** as a white gum (206 mg, 45%). $R_f = 0.41$ (light petroleum/ether 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 0.67$ (s, 3H, H-18$_{Chol}$), 0.86 (d, 6H, $J = 6.6$ Hz, H-26$_{Chol}$), 0.91 (d, 3H, $J = 6.4$ Hz, H-21$_{Chol}$), 0.99 (s, 3H, H-19$_{Chol}$), 0.80–2.50 (m, 30H, 28H-Chol H-6 H-6′), 1.71 (s, 3H, C$H_{3\dagger}$), 2.07 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 3.10–3.30 (m, 1H, H-3$_{Chol}$), 3.45–3.70 (m, 15H, OCH_2CH_2O H-5 H-7 H-7′), 4.59 (dd, 1H, $J = 2.6$ and 3.5 Hz, H-2), 5.00–5.20 (m, 2H, H-3

H-4), 5.30–5.38 (m, 1H, H-6_{Chol}), 5.44 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): $\delta = 12.2$, 15.6–41.7 (C-Chol $CH_{3\dagger}$ C-6), 21.1, 21.2, 29.1, 50.6, 56.5, 57.2, 62.4, 66.2, 67.7, 69.0, 70.3, 70.9, 71.0, 71.1, 71.3, 76.8, 79.9, 97.9, 124.7 (C*), 141.4, 170.2, 170.7.

8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-yl-2,3,4-tri-*O*-acetyl-7-bromo-6,7-dideoxy-α-D-*manno*-heptopyranoside 12

To a suspension of **11** (160 mg, 0.18 mmol) and molecular sieves (3Å) in CH_2Cl_2 (5 mL) was added dropwise TMSOTf (3.3 μL, 18 μmol) between -5°C and 0°C (ice-salt bath). The suspension was stirred for 5 min before neutralizing with Et_3N (50 μL) and diluting with H_2O (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3×50 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (light petroleum/ether 9:1 then ether) affording **12** as a white gum (64 mg, 40%). $R_f = 0.58$ (light petroleum/ether 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 0.60$ (s, 3H, H-18_{Chol}), 0.79 (d, 3H, J = 6.6 Hz, H-26_{Chol}), 0.80 (d, 3H, H-26_{Chol}), 0.84 (d, 3H, $J = 6.5$ Hz, H-21_{Chol}), 0.92 (s, 3H, H-19_{Chol}), 0.70–2.35 (m, 28H, 28H-Chol), 1.85–1.95 (m, 1H, H-6), 1.91 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.00–2.10 (m, 1H, H-6′), 3.05–3.15 (m, 1H, H-3_{Chol}), 3.40–3.50 (m, 1H, H-7), 3.50–3.70 (m, 13H, OCH_2CH_2O H-7′), 3.97 (td, 1H, $J = 2.5$ and 9.9 Hz, H-5), 4.75 (d, 1H, $J = 1.5$ Hz, H-1), 5.04 (t, 1H, H-4), 5.21 (dd, 1H, $J =$ 3.5 Hz, H-2), 5.25–5.30 (m, 1H, H-6_{Chol}), 5.27 (dd, 1H, H-3). ^{13}C NMR ($CDCl_3$): $\delta = 12.3$, 19.1–42.7 (C-Chol), 21.1, 21.2, 21.5, 29.2, 34.9, 50.6, 56.6, 57.2, 62.6, 67.7, 67.9, 69.5, 69.8, 70.1, 70.5, 71.0, 71.2, 71.3, 79.9, 97.8, 121.9, 141.4, 170.2, 170.3, 170.5.

Disodium 8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-yl-6-deoxy-6-dihydroxyphosphonylmethylene-α-D-mannopyranoside M6Pn/TEGC 13

A solution of **12** (67 mg, 76 μmol) dissolved in *tris*(trimethylsilyl) phosphite (5 mL) was stirred at 160°C for 16 h. The reaction mixture was then diluted with CH_2Cl_2 (15 mL) and poured into satd. $NaHCO_3$ (25 mL) at 0°C. The aqueous layer was extracted with CH_2Cl_2 (3×50 mL). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude mixture (showing a single signal by ^{31}P NMR spectroscopy at 27 ppm) was dissolved in MeOH (5 mL) and NaOMe (35 mg, 610 μmol) was added.

After 24 h, the reaction was neutralized with **1** M HCl and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (*i*-PrOH/NH_4OH/H_2O 6:3:1), then treated with ion exchange resin (Dowex 50WX2, Na^+ form, 5 g) affording M6Pn/TEGC **13** (31 mg, 50%). $R_f = 0.52$ (*i*-PrOH/NH_4OH/H_2O 6:3:1). ^{1}H NMR (D_2O): $\delta = 0.70$–2.30 (m, 47H, H-Chol H-6 H-6′ H-7 H-7′), 3.20–4.00 (m, 17H, OCH_2CH_2O H-2 H-3 H-4 H-5 H-3_{Chol}), 5.30–5.40 (m, 1H, H-6_{Chol}). ^{31}P NMR (D_2O): $\delta = 23.0$ (s, P(O)$(ONa)_2$). -MS (FAB+, G/T): *m/z*: 803 $[M + H]^+$, 781 $[M{-}Na + 2H]^+$. MS (FAB−, G/T): *m/z*: 757 $[M{-}2Na + H]^-$.

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