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A Convenient Synthetic Route to Mannose 6-Phosphonate–Cholesteryl Conjugate

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ABSTRACT: *A multistep synthesis of a mannose 6-phosphonate-based glycolipid is described involving (1) a one-carbon chain elongation at the 6-position of mannose, followed by (2) phosphonation, using* tris*(trimethylsilyl)phosphite. This method was shown to be efficient and provides a general route to various mannose 6-phosphonate-based compounds for the design of drug delivery systems.* © 2003 Wiley Periodicals, Inc. Heteroatom Chem 14:241–246, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.10134

INTRODUCTION

Lectins are involved in a wide variety of biological functions such as cell–cell communication, virus–cell, toxin–cell, or bacterium–cell interactions, all of which occur through lectin–carbohydrate recognition at the cell surface [1]. As part of our ongoing work, we focus on one lectin target from the P-type family, namely, the Mannose 6-Phosphate/Insulin-like II Receptor (M6P/IGFIIR). This 300 kDa type I transmembrane glycoprotein was shown [2] to bind Mannose 6-Phosphate (M6P)-bearing glycoproteins with high affinity (μM–nM range) and then internalize them through endocytosis of the receptor–ligand complex [3]. We recently [4] demonstrated that an amphiphilic steroidal mannose 6-phosphonate can be incorporated into the liposome lipidic bilayer and that the resulting "functionalized" vesicle interacted strongly with MCF-7 breast cancer cells (overexpressing the M6P/IGFIIR), demonstrating the high potential of such system for drug delivery applications.

However, during the preparation of M6Pn/CSA [4] (where M6Pn and CSA stands for mannose 6-phosphonate and CholesterylSuccinylAnilinyl, respectively) used in that investigation, we observed (Fig. 1) partial degradation of this molecule, using Rabinowitz's procedure [5] for the deprotection of the diethylphosphonate (using Me_3SiBr), and partial cleavage of the anomeric bond and/or amide bond(s).

It is worth pointing out that similar degradation problems have been described previously for aminoacids [6] or carbohydrate [7] frameworks. We therefore decided to prepare a modified amphiphilic M6Pn compound in which the hydrophilic carbohydrate head will be linked to the hydrophobic steroidal tail through a triethyleneglycol (TEG) moiety (Fig. 1).

RESULTS AND DISCUSSION

We decided to use the Arbuzov phosphonation as the key step to introduce the phosphonate moiety at the very end of our synthetic scheme. This approach allowed us to use *tris*(trimethylsilyl)phosphite, which reacts with an alkyl halide derivative affording *bis*-silylated phosphonates, which are easily hydrolyzed

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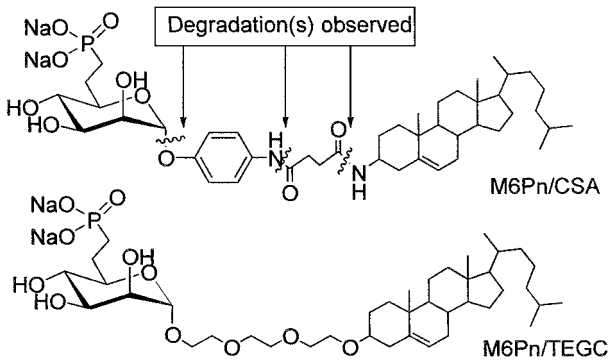
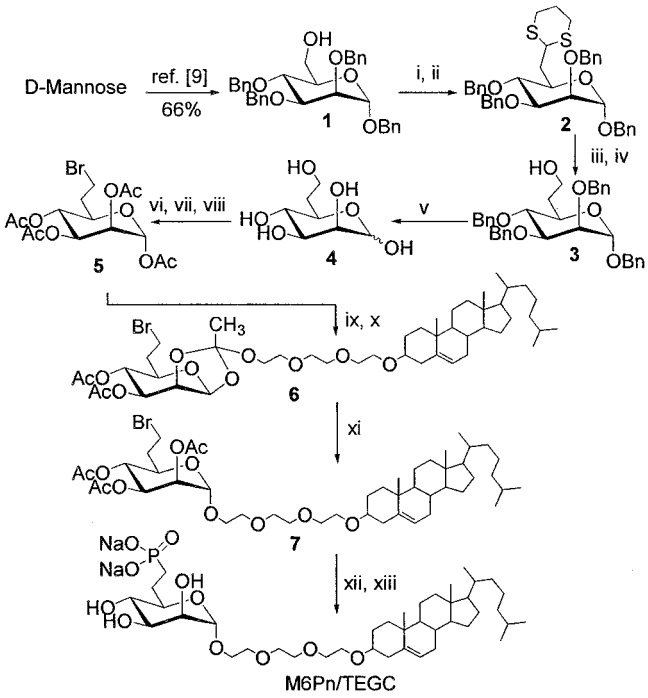


FIGURE 1 Partial degradations of M6Pn/CSA observed and structure of the target glycolipid M6Pn/TEGC.



SCHEME 1 Preparation of the M6Pn/TEGC. Reagents and conditions: (i) Tf_2O, CH_2Cl_2, 2,6-di-*t*-butyl-4-methylpyridine, −10°C to r.t., 5 min; (ii) 1,3-dithiane, THF, *n*-BuLi, HMPA, −78°C to r.t., 10 min, 80% over both steps; (iii) CH_3I, CH_3CN/H_2O (11:2), $CaCO_3$, 50°C, 3 h; (iv) $NaBH_4$, $EtOH/H_2O$ (1:1), r.t., 10 min, 90% over both steps; (v) H_2, $THF/H_2O/AcOH$ (50:50:1), $Pd(OH)_2/C$ (20%), r.t., 24 h, 98%; (vi) TsCl, C_5H_5N, r.t., 5 h; (vii) Ac_2O, C_5H_5N, 0°C to r.t., 12 h; (viii) LiBr, butanone, 85°C, 1 h, 45% over three steps; (ix) HBr/AcOH, CH_2Cl_2, r.t., 16 h; (x) 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-ol, CH_2Cl_2, AgOTf, *sym*-collidine, 0°C to r.t., 15 min, 45% over both steps; (xi) TMSOTf, CH_2Cl_2, molecular sieves (3 Å), −5°C, 5 min, 40%; (xii) $P(OSiMe_3)_3$, 60°C, 16 h then $NaHCO_{3(aq)}$; (xiii) NaOMe, MeOH, r.t., 16 h, 55% over both steps.

to the corresponding phosphonic acids through mild basic hydrolysis with saturated $NaHCO_{3(aq)}$ during the reaction work-up.

This new strategy requires a one-carbon chain elongation at the 6-position of mannose to obtain the alkyl halide intermediate. This mannose derivative was therefore prepared through (1) a cautiously selected one-carbon chain elongation method and (2) a glycosylation reaction.

The synthesis of the M6Pn/TEGC was accomplished as outlined in Scheme 1. Many studies have been performed concerning the one-carbon chain elongation of various alcohols [8]. From these methods, we decided to use a one-carbon chain elongation at the 6-position of benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside [9] (**1**) through triflate activation of the primary alcohol followed by substitution [10] with 2-lithio-1,3-dithiane affording compound **2** in 80% yield over both steps.

The dithioacetal **2** was subsequently unmasked to the aldehyde [11] (not isolated), which was reduced to furnish the homologated mannoheptose derivative **3** in 90% yield over both steps. This four-step procedure could be performed rapidly since each reaction in the synthetic scheme only takes 5–10 min and only one purification step is necessary, i.e., purification of the 1,3-dithianyl derivative **2**. Hydrogenolysis of **3**, using a catalytic amount of acetic acid [12] to aid in the deprotection of the anomeric center, afforded the deprotected 6-deoxy-D-mannoheptose **4**. Next, we had to functionalize the pyranose ring to allow (1) the introduction of the steroidal moiety via glycosylation and (2) the phosphonation reaction to obtain the final amphiphilic phosphonate. Thus, compound **4** was first activated at the 7-position, using tosyl chloride in pyridine followed by *in situ* peracetylation of the pyranose ring [13] and subsequent bromination utilizing lithium bromide in refluxing butanone [14], affording compound **5** in 45% yield over three steps. The poor yield obtained for this one-pot synthesis is probably a consequence of the low solubility of compound **4** in pyridine. Koenigs–Knorr glycosylation [15] of **5**, using known 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-ol [16] as the glycosyl acceptor, affords the orthoester derivative **6** as the major product, which upon subsequent treatment with a Lewis acid underwent rearrangement [17] to furnish the desired glycoside **7**.

The final step in our synthesis involved an Arbuzov reaction between the alkyl bromide derivative **7** and *tris*(trimethylsilyl)phosphite, followed by deprotection of the acetate under Zemplén conditions affording the final M6Pn/TEGC in good yields.

CONCLUSION

In summary, we have demonstrated that a new route to steroidal M6Pn is possible through (1) one-carbon chain elongation at the 6-position of mannose, followed by (2) phosphonation using the *tris*(trimethylsilyl)phosphite under Arbuzov conditions. Preparation of liposomes incorporating M6Pn/TEGC and evaluation of their interactions with MCF-7 cells are now being investigated in our group with the aim of developing a new drug delivery system. Although the overall yield is still low (1.9% over 18 steps starting from D-mannose), this strategy provides a 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

General Methods

Analytical TLC were performed using aluminum-coated TLC plates 60-F_{254} (Merck). Plates were developed with (1) UV light (254 nm), and (2) immersion in a 10% H_2SO_4/EtOH solution followed by charring or (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. IR Spectra were obtained on Perkin–Elmer FT-1600 spectrometer in CH_2Cl_2 solutions. Fast Atom Bombardment (FAB) mass spectra were recorded on a Jeol JMS-DX300 spectrometer in either positive (>0) or negative (<0) modes and using either 3-nitrobenzylic alcohol (NBA) or glycerol/thioglycerol (1:1) mixture (G/T). ^{1}H NMR Spectra were recorded on a Brüker DRX 400 (400 MHz), at 25°C. Chemical shifts (δ) are given in ppm and referenced using residual solvent signals (7.24 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 Brüker 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 Brüker DPX 200 (81.0 MHz). Chemical shifts (δ) are given in ppm relative to phosphoric acid (85%) as an external reference.

*Benzyl 6-Deoxy-6-(2′-dithianyl)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (**2**)*

To a cooled (−10°C) solution of benzyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-D-mannopyranoside (**1**) [9] (3.29 g, 6.08 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (1.56 g, 7.6 mmol) in CH_2Cl_2 (20 ml) was added triflic anhydride (1.79 ml, 6.7 mmol). The reaction mixture was stirred at r.t. for 10 min then neutralized with $NaHCO_{3(aq)}$ (200 ml, 0.1 g/l) and the aqueous layer was extracted with CH_2Cl_2 (3 × 300 ml). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude triflate was used for next step without further purification. To a cooled (−78°C) solution of 1,3-dithiane (2.56 g, 21.29 mmol) in THF (20 ml) was added HMPA (1.06 ml, 6.08 mmol). Next, *n*-BuLi (5 ml, 21.29 mmol, 1.6 M in hexanes) was added dropwise. After 10 min, a solution of triflate dissolved in THF (30 ml) was added dropwise, and after a further 10 min, the reaction was neutralized with $NH_4Cl_{(aq)}$ (200 ml, 0.1 g/l) and the aqueous layer extracted with CH_2Cl_2 (2 × 300 ml). Organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (light petroleum/ether 4:1) affording **2** (2.87 g, 80%). $R_f = 0.50$ (light petroleum/ether 7:3). $[\alpha]^{20}_D + 31.0°$ (*c* 0.72, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.80–2.20 (m, 3H, H-6a and CH_2CH_2S), 2.35–2.55 (m, 1H, H-6b), 2.65–2.90 (m, 4H, CH_2CH_2S), 3.76 (t, 1H, J = 9.4 Hz, H-4), 3.86 (dd, 1H, J = 1.9, 3.0 Hz, H-2), 4.00 (dd, 1H, H-3), 4.10 (td, 1H, J = 2.3, 9.4 Hz, H-5), 4.38 (dd, 1H, J = 4.0, 11.1 Hz, H-7), 4.45 (d, 1H, J = 11.5 Hz, CH_2Ph$_{anom}$), 4.65 (s, 2H, CH_2Ph), 4.69 (d, 1H, J = 10.9 Hz, CH_2Ph), 4.73 (d, 1H, J = 12.4 Hz, CH_2Ph), 4.81 (d, 1H, CH_2Ph), 4.90 (d, 1H, CH_2Ph$_{anom}$), 4.92 (d, 1H, H-1), 5.00 (d, 1H, CH_2Ph), 7.30–7.45 (m, 20H, H-ar). ^{13}C NMR ($CDCl_3$): δ = 26.5 (*C*H_2CH_2S), 29.7, 30.3 (CH_2*C*H_2S), 38.2 (C-6), 43.8 (C-7), 68.6–80.7 (C-2, C-3, C-4, and C-5), 69.4 (*C*H_2Ph$_{anom}$), 72.7, 73.3, 75.9 (*C*H_2Ph), 97.4 (C-1), 128.0–129.4 (CH-ar), 137–137.5 (C_{quat}-ar). MS FAB > 0 (NBA): m/z = 589 $(M)^+$, 551 $(M\text{-}CH_2Ph)^+$, 535 $(M\text{-}OCH_2Ph)^+$, 443 $(M\text{-}OCH_2Ph\text{-}CH_2Ph)^+$, 91 $(CH_2Ph)^+$. Anal for $C_{38}H_{42}O_5S_2$: Calcd: C 71.00, H 6.59; Found: C 71.28, H 6.64.

*Benzyl 6-Deoxy-2,3, 4-tri-O-benzyl-α-D-manno-heptopyranoside (**3**)*

To a solution of **2** (1 g, 1.56 mmol) dissolved in CH_3CN/H_2O (52 ml, 11:2) was added calcium carbonate (470 mg, 4.67 mmol) and methyl iodide

(2.15 ml, 34.3 mmol). The reaction was heated (50°C) for 3 h then neutralized with saturated $Na_2S_2O_{3(aq)}$ (100 ml). The aqueous layer was extracted with EtOAc (2 × 100 ml), and the organic layers combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude product was dissolved in absolute ethanol (25 ml) and a solution of sodium borohydride (148 mg, 3.89 mmol), dissolved in EtOH/H_2O (25 ml, 1:1) was added dropwise. The reaction was neutralized with 1 M HCl to pH ≈ 6. The reaction mixture was then diluted with H_2O (150 ml), and the aqueous layer extracted with EtOAc (5 × 100 ml). The organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (light petroleum/ether 3:7) affording **3** (778 mg, 90%). $R_f = 0.44$ (light petroleum/ether 3:7). $[\alpha]^{20}{}_D +$ 54.9° (c 1.02, $CHCl_3$). IR (NaCl): $\nu = 3460$. ^{1}H NMR ($CDCl_3$): $\delta = 1.80$–2.00 (m, 1H, H-6a), 2.10–2.30 (m, 1H, H-6b), 3.75–4.05 (m, 6H, H-2, H-3, H-4, H-5, H-7a, and H-7b), 4.46 (d, 1H, $J =$ 11.8 Hz, C*H*$_2$Ph), 4.66 (s, 2H, C*H*$_2$Ph), 4.67 (d, 1H, $J = 10.8$ Hz, C*H*$_2$Ph$_{anom}$), 4.71 (d, 1H, $J = 12.4$ Hz, C*H*$_2$Ph), 4.72 (d, 1H, C*H*$_2$Ph), 4.80 (d, 1H, C*H*$_2$Ph) 4.89 (d, 1H, $J = 1.6$ Hz, H-1), 5.01 (d, 1H, C*H*$_2$Ph$_{anom}$), 7.20–7.45 (m, 20H, H-ar). ^{13}C NMR ($CDCl_3$): $\delta =$ 34.2 (C-6), 61.5 (C-7), 69.4 (*C*H$_2$Ph$_{anom}$), 72.2–80.6 (C-2, C-3, C-4, and C-5), 72.6, 73.3, 75.8 (*C*H$_2$Ph), 97.5 (C-1), 128.0–128.9 (CH-ar), 137.5–138.8 (C$_{quat}$-ar). MS FAB > 0 (NBA): $m/z = 577$ $(M + Na)^+$, 553 $(M\text{-}H)^+$, 447 $(M\text{-}OCH_2Ph)^+$, 91 $(CH_2Ph)^+$. Anal for $C_{35}H_{38}O_6$: Calcd: C 75.79, H 6.91; Found: C 75.96, H 6.95.

*6-Deoxy-α-D-manno-heptopyranose (**4**)*

A solution of **3** (4.44 g) and $Pd(OH)_2$/C (20%, 2.5 g) in THF/H_2O (100 ml, 1:1) and glacial acetic acid (5 ml) was vigorously stirred under a hydrogen atmosphere for 24 h. The reaction was then filtered through celite and the filtrate concentrated under reduced pressure then freeze-dried from H_2O affording **4** (1.52 g, 98%). $R_f = 0.50$ (CH_2Cl_2/MeOH 7:3). ^{1}H NMR (D_2O): $\delta = 1.40$–2.10 (m, 2H, H-6a and H-6b), 3.20–3.85 (m, 6H, H-2, H-3, H-4, H-5, H-7a, and H-7b), 4.70–4.75 (m, 1H, H-1β), 4.98–5.02 (m, 1H, H-1α). ^{13}C NMR (D_2O): $\delta = 33.5$, 33.6 (C-6α and C-6β), 58.8, 58.6 (C-7α and C-7β), 69.4–73.6 (C-2α, C-2β, C-3α, C-3β, C-4α, C-4β, C-5α, and C-5β), 94.0, 94.3 (C-1α and C-1β). MS FAB > 0 (G/T): $m/z = 195$ $(M + H)^+$, 177 $(M\text{-}OH)^+$. Anal for $C_7H_{14}O_6$: Calcd: C 43.30, H 7.27; Found: C 43.13, H 7.32.

*7-Bromo-6,7-dideoxy-1,2,3,4-tri-O-acetyl-α-D-manno-heptopyranoside (**5**)*

To a solution of **4** (730 mg, 3.76 mmol) in C_5H_5N (50 ml) was added tosyl chloride (1.08 g, 5.64 mmol). Reaction was stirred for 5 h, then Ac_2O (7.2 ml, 75.26 mmol) was added to the cooled (0°C) reaction mixture. After 12 h, the reaction was diluted with brine (125 ml) and the aqueous layer extracted with CH_2Cl_2 (3 × 150 ml). The organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure, utilizing toluene (3 × 100 ml) to coevaporate C_5H_5N. The crude product was dissolved in butanone (20 ml) and lithium bromide (1 g, 11.5 mmol) added. The reaction was heated (85°C) for 1 h then diluted with brine (100 ml). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 ml) and the 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) affording **5** (716 mg, 45%). $R_f = 0.61$ (light petroleum/ether 3:7) ^{1}H NMR ($CDCl_3$): $\delta = 1.80$–2.00 (m, 2H, H-6a and H-6b), 2.00 (s, 3H, C*H*$_3$CO), 2.07 (s, 3H, C*H*$_3$CO), 2.15 (s, 3H, C*H*$_3$CO), 3.40–3.70 (m, 2H, H-7a and H-7b), 4.08 (td, 1H, $J = 3.0$, 9.9 Hz, H-5), 5.16 (t, 1H, H-4), 5.26 (dd, 1H, $J = 1.8$, 3.5 Hz, H-2), 5.34 (dd, 1H, H-3), 5.99 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): $\delta = 20.9$–21.2 (*C*H$_3$CO), 29.2 (C-7), 33.9 (C-6), 68.4–69.6 (C-2, C-3, C-4, and C-5), 90.6 (C-1), 170.1, 170.2, 170.3, 170.4 (CH$_3$*C*O). MS FAB > 0 (NBA): $m/z = 447$ $(M + Na)^+$, 365 $(M\text{-}OAc)^+$, 345 $(M\text{-}Br)^+$, 245 $(M\text{-}2OAc\text{-}2H)^+$, 203 $(M\text{-}2OAc\text{-}CH_3CO\text{-}H)^+$, 43 $(CH_3CO)^+$. Anal for $C_{15}H_{21}BrO_9$: Calcd: C 42.37, H 4.95; Found: C 42.29, H 4.92.

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

To a solution of **5** (220 mg, 0.52 mmol) in CH_2Cl_2 (15 ml) was added HBr (2.28 ml, 13 mmol, 5.7 M in acetic acid). The reaction was stirred at r.t. for 16 h and then neutralized with saturated $NaHCO_{3(aq)}$ (50 ml). The aqueous layer was extracted with CH_2Cl_2 (3 × 70 ml), and the organic layers combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude product dissolved in CH_2Cl_2 (15 ml) was added dropwise to a solution of 8-(cholest-5en-3β-yloxy)-3,6-dioxaoctan-1-ol (287 mg, 0.57 mmol), silver trifluoromethanesulfonate (146 mg, 0.57 mmol) and *sym*-collidine (76 μl, 0.57 mmol) in CH_2Cl_2 (5 ml). The reaction was stirred at RT for 15 min before filtering through celite. The filtrate was concentrated under reduced pressure,

and the residue purified by silica gel column chromatography (light petroleum/ether 4:1 → ether) affording **6** (210 mg, 45%). $R_f = 0.41$ (light petroleum/ether 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 0.67$ (s, 3H, H-18′), 0.86 (d, 6H, $J = 6.6$ Hz, H-26′), 0.91 (d, 3H, $J = 6.4$ Hz, H-21′), 0.99 (s, 3H, H-19′), 0.80–2.50 (m, 30H, *H*-Chol H-6a and H-6b), 1.71 (s, 3H, Orthoester-C*H*$_3$), 2.07 (s, 3H, C*H*$_3$CO), 2.11 (s, 3H, C*H*$_3$CO), 3.10–3.30 (m, 1H, H-3′), 3.45–3.70 (m, 15H, OC*H*$_2$C*H*$_2$O, H-5, H-7a, and H-7b), 4.59 (dd, 1H, $J = 2.6$, 3.5 Hz, H-2), 5.00–5.20 (m, 2H, H-3 and H-4), 5.30–5.38 (m, 1H, H-6′), 5.44 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$): $\delta = 12.2$ (C-18′), 15.6–41.7 (*C*-Chol, Orthoester-*C*H$_3$ and C-6), 21.1, 21.2 (*C*H$_3$CO), 29.1 (C-7), 50.6, 56.5, 57.2 (C-9′, C-14′, and C-17′), 62.4, 66.2 (OC*H*$_2$C*H*$_2$O), 67.7 (C-4), 69.0, 70.3 (OC*H*$_2$C*H*$_2$O), 70.9 (C-3), 71.0 (OC*H*$_2$CH$_2$O), 71.1 (C-5), 71.3 (OC*H*$_2$CH$_2$O), 76.8 (C-2), 79.9 (C-3′), 97.9 (C-1), 121.9 (Orthoester-C_{quat}), 141.4 (C-5′), 170.2, 170.7 (CH$_3$*C*O).

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

To a cooled (−5°C) solution of **6** (160 mg, 0.18 mmol) and molecular sieves (3 Å) in CH_2Cl_2 (5 ml) was added trimethylsilyl trifluoromethanesulfonate (3.3 μl, 18 μmol). The reaction was stirred for 5 min then neutralized with Et_3N (50 μl) before being diluted with H_2O (50 ml). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 ml), and the organic layers 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 → ether) affording **7** (65 mg, 40%). $R_f = 0.58$ (light petroleum/ether 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 0.60$ (s, 3H, H-18′), 0.79 (d, 3H, $J =$ 6.6 Hz, H-26′), 0.80 (d, 3H, H-26′), 0.84 (d, 3H, $J = 6.5$ Hz, H-21′), 0.92 (s, 3H, H-19′), 0.70–2.35 (m, 28H, *H*-Chol), 1.85–1.95 (m, 1H, H-6a), 1.91 (s, 3H, C*H*$_3$CO), 1.99 (s, 3H, C*H*$_3$CO), 2.07 (s, 3H, C*H*$_3$CO), 2.00–2.10 (m, 1H, H-6b), 3.05–3.15 (m, 1H, H-3′), 3.40–3.50 (m, 1H, H-7a), 3.50–3.70 (m, 13H, OC*H*$_2$C*H*$_2$O and H-7b), 3.97 (td, 1H, $J = 2.5$, 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′), 5.27 (dd, 1H, H-3). ^{13}C NMR ($CDCl_3$): $\delta = 12.3$ (C-18′), 19.1–42.7 (*C*-Chol), 21.1, 21.2, 21.5 (*C*H$_3$CO), 29.2 (C-7), 34.9 (C-6), 50.6, 56.6, 57.2 (C-9′, C-14′, and C-17′), 62.6, 67.7 (OC*H*$_2$CH$_2$O), 67.9 (C-5), 69.5 (C-3), 69.8 (C-4), 70.1 (C-2), 70.5, 71.0, 71.2, 71.3 (OC*H*$_2$CH$_2$O), 79.9 (C-3′), 97.8 (C-1), 121.9 (C-6′), 141.4 (C-5′), 170.2, 170.3, 170.5 (CH$_3$*C*O). Anal for $C_{46}H_{75}BrO_{11}$: Calcd: C 62.50, H 8.55; Found: C 62.77, H 8.63.

8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-yl-6-Deoxy-6-dihydroxyphosphinylmethylene-α-D-mannopyranoside Disodium Salt (M6Pn/TEGC)

A solution of **7** (67 mg, 76 μmol) in *tris*-(trimethylsilyl)phosphite (5 ml) was refluxed (160°C) for 16 h. The reaction mixture was poured into saturated $NaHCO_{3(aq)}$ (50 ml). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 ml), and the 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 concentrated under reduced pressure. The 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) affording M6Pn/TEGC (31 mg, 55%). $R_f = 0.52$ (*i*-PrOH/NH_4OH/H_2O 6:3:1). ^{1}H NMR (D_2O): $\delta = 0.70$–2.30 (m, 47H, H-Chol, H-6a, H-6b, H-7a, and H-7b), 3.20–4.00 (m, 17H, OC*H*$_2$C*H*$_2$O, H-2, H-3, H-4, H-5, and H-3′), 5.30–5.40 (m, 1H, H-6′). ^{31}P NMR (D_2O): $\delta = 23.0$ (s, $P(O)(ONa)_2$). MS FAB > O (G/T): $m/z = 803$ $(M + H)^+$, 781 $(M\text{-}Na + 2H)^+$. MS FAB < 0 (G/T): $m/z = 757$ $(M\text{-}2Na + H)^-$.

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