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Synthesis and Biological Evaluation of New Mannose 6-Phosphate Analogues

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Abstract—Three new analogues of mannose 6-phosphate (M6P)—a sulphate and two carboxylates—have been synthesized and their affinity toward the M6P/IGFII receptor evaluated by affinity column chromatography. These compounds display strong binding to the receptor and therefore are new M6P analogues which may find some dermatological applications, for example healing of post-surgical scars.
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Introduction

Mannose 6-phosphate (M6P) is a recognition marker involved in the selective targeting of newly synthesized enzymes to lysosomes.[1] While two different mannose 6-phosphate receptors (M6PR) recognize specifically the M6P residues, only the larger M6P receptor mediates the endocytosis of extracellular M6P-containing ligands.[2] This receptor is a type I glycoprotein of 275 kDa, which apart from M6P residues, is also known to bind, through distinct binding sites, retinoic acid[3] (RA) and insulin-like growth factor II[4] (IGFII). This receptor (M6P/IGIIR) is the first example of a protein able to bind three different classes of ligands, that is a saccharide (M6P), a peptide (IGFII) and a lipid (RA). Moreover, the M6P/IGFIIR plays a fundamental role in the control of cell growth in fetal development and carcinogenesis.[5] In our ongoing research program, we focus on the ability of the M6P/IGFIIR to recognize and then internalize M6P analogues. We recently described[6] the synthesis of two mannose 6-phosphonate (M6Pn) analogues (**I** and **II**) of M6P (Fig. 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 non-isosteric compound **II** was only weakly recognized. We present herein the preparation and biological evaluation of three M6P analogues.

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Results and Discussion

Recognition requirements and previous results

As a continuation of our previous work, we decided to synthesize other analogues with a few modifications of the carbohydrate backbone in agreement with some observations reported in literature.[7–9] Some structural features were shown to be determinant in the binding of M6P to the receptor such as : (a) the hydroxyl group at the 2-position of the pyranose ring must be axial since glucose 6-phosphate (G6P) is weakly recognized by the receptor whereas fructose 1-phosphate (F1P) and M6P bind strongly to the receptor (Fig. 1). The absence of the hydroxyl group at the same position will also affect the recognition since 2-deoxy-glucose 6-phosphate (2dG6P) is as weakly recognized as G6P.[7] The substitution at the anomeric center (b) does not influence the interaction since F1P is as well recognized as M6P[7] (Fig. 1) and (c) the substitution at the 5-position of the hexopyranoside ring — anomeric position for the F1P — does not affect the interaction since F1P is as well recognized as M6P[7] (Fig. 2).

Another important structural feature was shown to play an important role in the recognition phenomenon, namely the distance between the negative charge and the pyranose ring. We demonstrated[6] that this distance must be in the same range as that in the M6P, that is four atoms from the 5-position (Fig. 2) since the isosteric M6Pn analogue is very well recognized by the M6P/IGFIIR whereas the non isosteric M6Pn analogue

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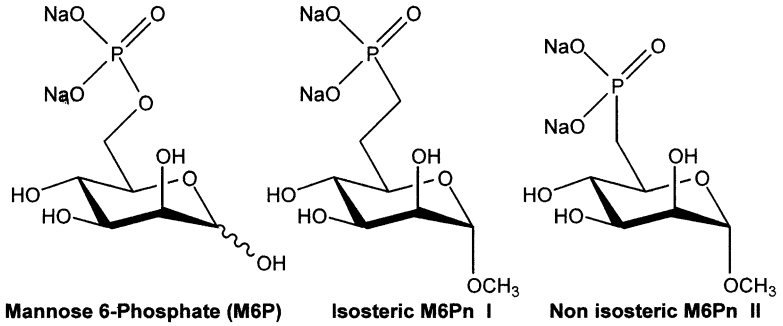


Figure 1. Relation of the distance between the negative charge and the pyranose ring.

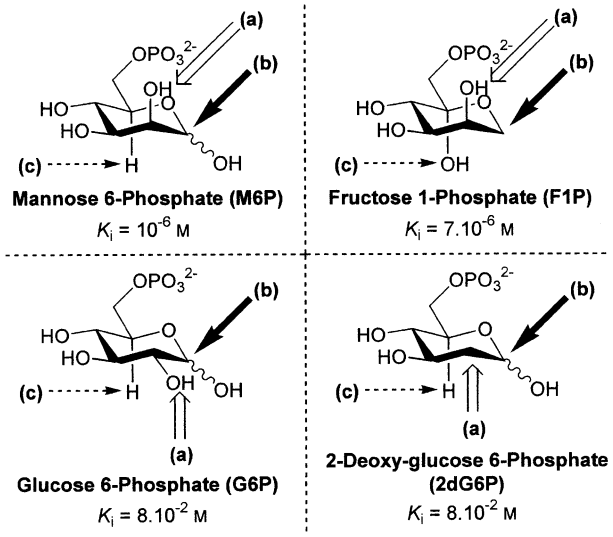


Figure 2. Three main structural requirements for reaching high affinity toward the M6P/IGFIIR. Influence of the substitution at the (a) 2-position, (b) anomeric center and (c) 5-position.

was very weakly recognized. We recently prepared two β-hydroxyphosphonate analogues of M6P and demonstrated that they were not recognized by the M6P/IGFIIR.[10] While the replacement of the phosphomonoester linkage by a methylene group does not affect the recognition phenomenon,[6] further substitution at the 6-position lessens the interaction of the analogues obtained with the receptor, probably due to steric hindrance.[10]

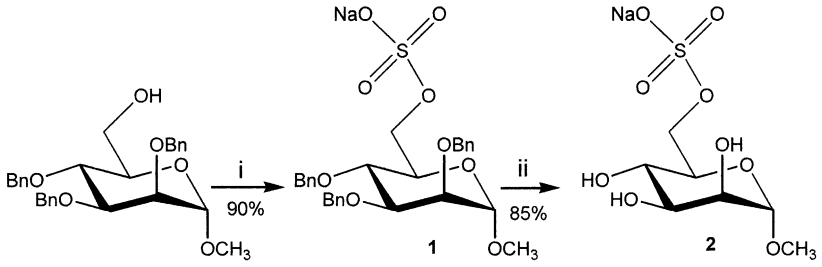
Concerning the negative charge, it has been shown[8] that a singly charged phosphodiester analogue of M6P (with one methyl ester on the phosphate moiety) displayed very similar affinity toward the receptor than M6P. This result demonstrates that only a single negative charge is necessary for the binding to the M6P/IGFIIR.

Preparation of the three new M6P analogues

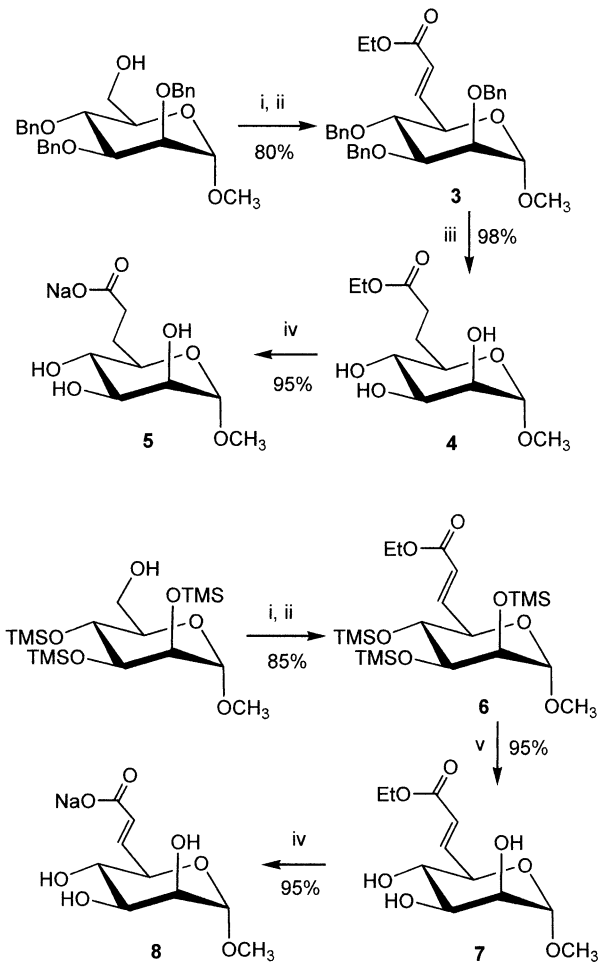
According to these observations, we decided to synthesize three different M6P analogues which will display only one negative charge and respect the geometrical/functional requirements discussed above. The first of these compounds will replace the M6P phosphorus atom for a sulfur atom to obtain the sulphate monoester derivative **2**. This compound could be prepared easily by a two steps synthesis (Scheme 1) starting from known methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside[11] by treatment with sulfur trioxide/pyridine complex[12] affording **1** in 90% yield. Subsequent hydrogenolysis[13] of the benzyl protecting groups furnished **2** in 85% yield. It is worth mentioning that purification of this compound could be achieved by quenching the reaction with Na_2CO_3 (1 M) then washing off the crude solid using methanol in which Na_2CO_3 is insoluble. This purification method will allow for the preparation in bulk quantities.

We also decided to prepare two carboxylic acids in order to further investigate the structure–activity relationships of the M6P/IGFIIR. These derivatives can be obtained by using a Swern oxidation[14] at the 6-position of mannose affording an aldehyde function which was then reacted under Wittig–Horner conditions[15] to furnish the corresponding conjugated esters. Another approach is the condensation of a malonate diester with a 6-deoxy-6-halogeno carbohydrate derivative under basic conditions.[16] Although both of these methods have been extensively described in literature for the preparation of various octoglycosuronic acids,[17] none of these focused on the synthesis of the deprotected acids and their subsequent biological evaluation. We therefore chose to use the Wittig–Horner method and selected two different protecting groups — namely benzyl and trimethylsilyl ethers — in order to obtain both the aliphatic carboxylic acid derivative **5** and the conjugated carboxylic acid derivative **8** respectively, as outlined in Scheme 2.

The Swern oxidation[14] of methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside[11] afforded the corresponding aldehyde (not isolated), which was then reacted with triethylphosphonoacetate sodium salt affording the conjugated ester **3** in 80% yield over both steps. The hydrogenolysis of benzyl protecting groups afforded **4** in 98% yield and subsequent saponification of the ester moiety furnished **5** in 95% yield. In the same fashion, methyl 2,3,4-tri-*O*-trimethylsilyl-α-D-mannopyranoside[18] was oxidized[14] to the corresponding aldehyde and reacted under the same Wittig–Horner conditions to obtain **6** in 85% over both steps. Mild acid hydrolysis of the trimethylsilyl protecting groups afforded **7** in 95% yield and saponification of the ester moiety furnished **8** in 95% yield.



Scheme 1. Reagents and conditions: (i) SO_3/C_5H_5N, C_5H_5N, 4 h; (ii) H_2, $Pd(OH)_2/C$ (20%), $EtOH/H_2O$ (1:1), 24 h.



Scheme 2. Reagents and conditions: (i) $(COCl)_2$, DMSO, *i*-Pr_2NEt, −60 °C, THF; (ii) $(EtO)_2(O)PCH_2CO_2Et$, NaH, THF; (iii) H_2, Pd/C (10%), $EtOH/H_2O$ (1:1), 4 h; (iv) 2 M NaOH, 1 h; (v) HCl (1 M in THF), 5 min.

Biological evaluation of the three new M6P analogues

With these three new M6P analogues in our hands, we needed to determine their relative affinities toward the M6P/IGFIIR. These biological assays were based on the method we described before.[10] Pentamannose 6-phosphate Sepharose columns[19] were loaded with the receptor then eluted with analogues **2**, **5** and **8**. The results obtained are presented in Figure 3.

We observed that both the sulphate monoester **2** and the conjugated carboxylic acid **8** displayed similar affinities (2.5 mM) to the M6P (Table 1) while **5** displayed a slightly lower affinity. The difference in affinities between the carboxylic derivatives **5** (5 mM) and **8** (2.5 mM) might be related to a better defined geometry of the double bond present in **8**, in comparison with the single bond present in **5**.

Conclusions

In conclusion, three new M6P analogues have been synthesized in excellent yields and these compounds are now being prepared on the 100 g scale for in vivo biological evaluation. The synthesis of both carboxylic acids (**5** and **8**) is now being adapted for the preparation of new amphiphilic M6P steroidal analogues in order to generate new drug delivery systems as powerful as the M6Pn cholesteryl conjugate previously described in a recent paper.[20] Another exciting potential application of these compounds is in dermatological treatments, since M6P and M6Pn were shown[21] to reduce the size of scars. The carboxylic acid analogues **5** and **8** are expected to display the same property and the preparation described herein presents a very efficient synthesis of these compounds for large-scale applications in various dermatological treatments (post-surgery scars, wrinkles, etc.).

Experimental

General aspects

All solvents were dried prior to use according to standard methods.[22] 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). 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 solution. 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). ^1H 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

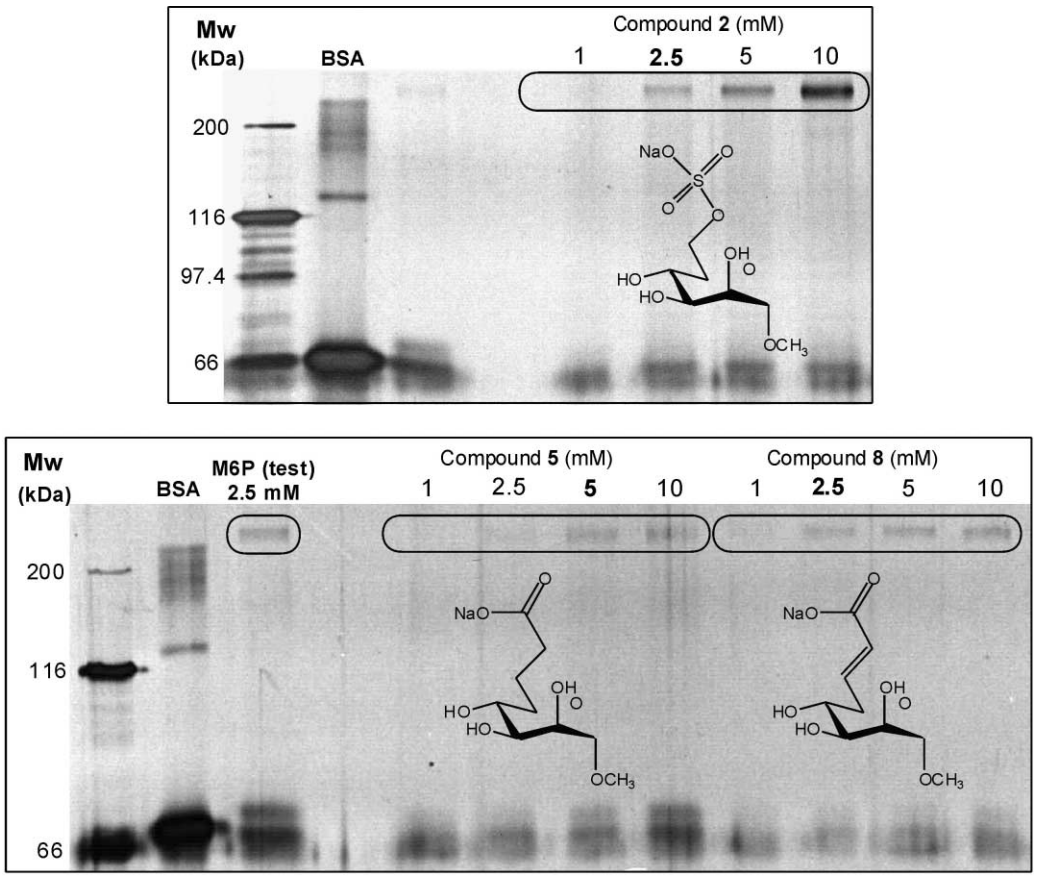


Figure 3. Affinities of M6P analogues toward M6P/IGFIIR. M6P/IGFIIR from calf serum was retained on phosphomannan Sepharose columns and eluted with the indicated concentrations of mannose 6-phosphate (M6P test) and compounds **2**, **5** and **8**. The eluted protein was analyzed in a 10% SDS polyacrylamide gel followed by silver-staining. Mw, molecular weight markers; boxes indicate the eluted M6P/IGFIIR.

Table 1. Relative affinities observed for compounds **2**, **5** and **8**

Compd	Minimum concentration needed to observe the eluted M6P/IGFIIR (mM)
M6P	2.5
M6Pn I	1[a]
2	2.5
5	5
8	2.5

[a]Result previously described.[6]

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), ddd (double double doublet), t (triplet), td (triplet doublet), q (quartet), 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. ^{29}Si NMR spectra were recorded on a Brüker DPX 200 (39.8 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. The pentamannose 6-phosphate was functionalized with β-(*p*-aminophenyl)ethylamine, then reduced with sodium tetrahydroborate, and finally coupled on CNBr activated Sepharose leading to phosphomannan Sepharose.[19] The M6P/IGFIIR was purified from fetal calf serum on a phosphomannan–Sepharose affinity column, according to the method previously described.[23] SDS polyacrylamide gel electrophoresis was performed according to Laemmli[24] and proteins visualized using a silver-staining kit (Biorad).

Methyl 6-*O*-Sulfonato-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (1). To a solution of SO_3/C_5H_5N complex (335 mg, 2.09 mmol) in C_5H_5N (5 mL), a solution of methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside[11] (540 mg, 1.16 mmol) in C_5H_5N (5 mL) was added. Reaction was stirred for a period of 4 h after which it was neutralized with $Na_2CO_{3(aq)}$ (1 M, 10 mL) and concentrated under reduced pressure. The salts were washed with anhydrous MeOH (50 mL) and filtered. The filtrate was concentrated under reduced pressure and the residue purified by silica gel column chromatography (EtOAc/MeOH 9:1) affording **1** (590 mg, 90%) as a white foam. R_f 0.70 (EtOAc/MeOH 3:1). $[\alpha]_D$ +58.3 ($c=0.9$/$CHCl_3$). ^1H NMR ($CDCl_3$), δ: 3.21 (s, 3H, OCH_3); 3.62–3.75 (m, 1H, H-5); 3.72–3.78 (m, 1H, H-2); 3.82 (dd, 1H, $J=2.1$, 9.3 Hz, H-3); 4.11 (t, 1H, H-4); 4.38–4.47 (m, 2H, H-6 and H-6′); 4.52 (s, 2H, CH_2Ph); 4.62–5.00 (m, 5H, CH_2Ph and H-1); 7.10–7.40 (m, 15H, H-ar). ^{13}C NMR ($CDCl_3$), δ: 55.5 (OCH_3); 66.7–77.7 (C-6 and CH_2Ph); 70.5–80.3 (C-2, C-3, C-4 and C-5); 99.7 (C-1); 127.9–129.1 (CH–Ar); 137.8–138.7 (C_{ar}^{IV}). MS

(FAB > 0, NBA), *m/z*: 589 $[M+Na]^+$, 567 $[M+H]^+$, 487 $[M-SO_3+H]^+$, 455 $[M-SO_3-OCH_3]^+$, 91 $[CH_2Ph]^+$. MS (FAB < 0, NBA): *m/z*: 543 $[M-Na]^-$.

Methyl 6-*O*-sulfonato-α-D-mannopyranoside (2). A suspension of **1** (530 mg) and $Pd(OH)_2/C$ (20%, 350 mg) in $EtOH/H_2O$ (1:1, 50 mL) was vigorously stirred under an H_2 atmosphere for 24 h. The reaction was filtered through a Celite pad and concentrated under reduced pressure affording pure **2** (240 mg, 85%) as a white powder. Mp 156–159 °C. R_f 0.50 (CH_2Cl_2/MeOH 2:1). ^1H NMR (D_2O), δ: 3.29 (s, 3H, OCH_3); 3.56 (t, 1H, *J* = 9.4 Hz, H-4); 3.65 (dd, 1H, *J* = 2.9 Hz, H-3); 3.71 (ddd, 1H, *J* = 2.2, 5.3 Hz, H-5); 3.81 (dd, 1H, *J* = 1.7 Hz, H-2); 4.10 (dd, 1H, *J* = 11.2 Hz, H-6); 4.23 (dd, 1H, H-6′); 4.64 (d, 1H, H-1). ^{13}C NMR (D_2O), δ: 55.2 (OCH_3); 66.7 (C-4); 67.6 (C-6); 70.2 (C-2); 70.7,70.8 (C-3 and C-5); 101.3 (C-1). MS (FAB < 0, G/T), *m/z*: 295 $[M-H]^-$, 273 $[M-Na]^-$. $C_7H_{13}NaO_9S$ (296.02): calcd C 28.38, H 4.42; found C 27.85, H 4.58.

Ethyl [methyl (E)-2,3,4-tri-*O*-benzyl-6,7-dideoxy-α-D-*manno*-oct-6-enopyranoside]uronate (3). To a cooled (−60 °C) solution of oxalyl chloride (106 μL, 1.23 mmol) in THF (2 mL), DMSO (190 μL, 2.69 mmol) was added dropwise. After 10 min, a THF (4 mL) solution of methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside[11] (520 mg, 1.12 mmol) was added dropwise. After an additional 20 min, *i*-Pr_2NEt (960 μL, 5.6 mmol) was added dropwise. Reaction was allowed to reach rt, stirred for 2 h and then concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (130 mL) and the organic layer washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude aldehyde was used for the next step without further purification.

To a suspension of NaH (95%, 57 mg, 2.24 mmol) in THF (2 mL) was added triethylphosphonoacetate (540 μL, 2.69 mmol). Reaction was stirred for 10 min, then a solution of the crude aldehyde in THF (5 mL) was added dropwise. The mixture was stirred for 30 min before concentrating under reduced pressure. The residue was diluted with CH_2Cl_2 (130 mL) and the organic layer washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ether 3:2→1:1) to afford **3** (477 mg, 80%) as a yellow oil. R_f 0.62 (hexane/ether 1:1). $[\alpha]_D$ +77.9 (*c* 0.95/$CHCl_3$). IR (NaCl film), ν: 1661 cm^{-1}, 1712 cm^{-1}. ^1H NMR ($CDCl_3$), δ: 1.33 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3); 3.35 (s, 3H, OCH_3); 3.82 (t, 1H, *J* = 9.3 Hz, H-4); 3.85–3.90 (m, 1H, H-2); 3.99 (dd, 1H, *J* = 2.9 Hz, H-3); 4.23–4.34 (m, 1H, H-5); 4.28 (q, 2H, OCH_2CH_3); 4.60–5.00 (m, 7H, CH_2Ph and H-1); 6.31 (dd, 1H, *J* = 1.1, 15.7 Hz, H-7); 7.22 (dd, 1H, *J* = 4.7 Hz, H-6); 7.23–7.50 (m, 15H, H-ar). ^{13}C NMR ($CDCl_3$), δ: 13.2 (OCH_2CH_3); 53.8 (OCH_3); 59.3 (OCH_2CH_3); 69.4–79.0 (C-2, C-3, C-4 and C-5); 71.3, 71.8, 74.4 (CH_2Ph); 98.2 (C-1); 121.1 (C-7); 126.5–127.7 (CH-Ar); 136.9–137.4 (C_{ar}^{IV}); 143.1 (C-6); 165.3 (CO_2Et). MS (FAB > 0, NBA), *m/z*: 531 $[M-H]^+$, 501 $[M-OCH_3]^+$, 91 $[CH_2Ph]^+$.

Ethyl (methyl 6,7-dideoxy-α-D-*manno*-octopyranoside) uronate (4). A suspension of **3** (80 mg) and Pd/C (10%, 100 mg) in $EtOH/H_2O$ (1:1, 20 mL) was vigorously stirred under an H_2 atmosphere for 4 h. The reaction was filtered through a Celite pad and concentrated under reduced pressure affording pure **4** (39 mg, 98%) as a yellow oil. R_f 0.55 (EtOAc/MeOH 9:1). $[\alpha]_D$ +63.6 (*c* 1.1/$CHCl_3$). IR (CH_2Cl_2): ν: 1728 cm^{-1}, 3407 cm^{-1}. ^1H NMR ($CDCl_3$), δ: 1.26 (t, 3H, *J* = 7.1 Hz, OCH_2CH_3); 1.65–1.95 (m, 1H, H-6); 2.10–2.30 (m, 1H, H-6′); 2.30–2.65 (m, 2H, H-7 and H-7′); 3.30–3.45 (m, 1H, H-5); 3.33 (s, 3H, OCH_3); 3.51 (t, 1H, *J* = 8.9 Hz, H-4); 3.71 (dd, 1H, *J* = 3.2 Hz, H-3); 3.89 (dd, 1H, *J* = 1.3 Hz, H-2); 4.14 (q, 2H, OCH_2CH_3); 4.64 (d, 1H, H-1). ^{13}C NMR ($CDCl_3$), δ: 13.1 (OCH_2CH_3); 25.5 (C-6); 28.7 (C-7); 53.9 (OCH_3); 59.6 (OCH_2CH_3); 69.4–70.2 (C-2 C-3 C-4 and C-5); 99.9 (C-1); 173.5 (CO_2Et). MS (FAB > 0, NBA), *m/z*: 287 $[M+Na]^+$, 265 $[M+H]^+$, 233 $[M-OCH_3]^+$.

Sodium (methyl 6,7-dideoxy-α-D-*manno*-octopyranoside)uronate (5). Compound **4** (30 mg) was dissolved in 2 M NaOH (3 mL) and the mixture was stirred for 1 h and neutralized with ion exchange resin (Dowex 50WX2, H^+ form, 7 g) until the pH was 4–5. The resin was washed wih H_2O (100 mL) and filtered off. The filtrate was freeze dried from H_2O affording **5** (28 mg, 95%) as a light brown foam. R_f 0.36 (*i*-PrOH/NH_4OH/H_20 8:1:1). $[\alpha]_D$ +43.3 (*c*0.3/H_2O). ^1H NMR (DMSO-d_6), δ: 1.40–1.60 (m, 1H, H-6); 1.85–2.45 (m, 3H, H-6′ H-7 and H-7′); 3.15–3.45 (m, 2H, H-3H-4 and H-5); 3.21 (s, 3H, OCH_3); 3.30–3.45 (m, 1H, H-3); 3.58 (dd, 1H, *J* = 1.5, 3.2 Hz, H-2); 4.46 (d, 1H, H-1). ^{13}C NMR (DMSO-d_6), δ: 27.7 (C-6); 31.1 (C-7); 54.8 (OCH_3); 71.0–71.9 (C-2 C-3 C-4 and C-5); 101.8 (C-1); 175.6 (CO_2Na). MS (FAB > 0, NBA), *m/z*: 259 $[M+H]^+$, 237 [M-Na]$^+$. MS (FAB < 0, NBA), *m/z*: 235 $[M-Na]^-$. $C_9H_{15}NaO_7$ (258.07): calcd C 41.87, H 5.86; found C 41.44, H 5.99.

Ethyl [methyl (E)-2,3,4-tri-*O*-trimethylsilyl-6,7-dideoxy-α-D-*manno*-oct-6-enopyranoside]uronate (6). To a cooled (−60 °C) solution of oxalyl chloride (340 μL, 4.02 mmol) in THF (2 mL), DMSO (620 μL, 8.78 mmol) was added dropwise, after 10 min, a THF (5 mL) solution of methyl 2,3,4-tri-*O*-trimethylsilyl-α-D-mannopyranoside[18] (1.5 g, 3.66 mmol) was added dropwise. After an additional 20 min, *i*-Pr_2NEt (3.13 mL, 18.3 mmol) was added dropwise. The reaction mixture was kept at −60 °C for 10 min then allowed to reach rt, stirred for 3 h and diluted with CH_2Cl_2 (180 mL). The organic layer was washed with brine (75 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude aldehyde was used for next step without further purification. To a suspension of NaH (95%, 185 mg, 7.32 mmol) in THF (2 mL) was added triethyl phosphonoacetate (1.76 mL, 8.78 mmol). After 10 min, a THF (10 mL) solution of the crude aldehyde was added dropwise. Reaction was stirred for 10 min, solvent was evaporated and the residue diluted with CH_2Cl_2 (180 mL). The organic layer was washed with brine (75 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (light petroleum/ether 1:1) affording **6** (1.48 g, 85%) as a colorless oil. R_f 0.41 (light petroleum/ether 4:1). $[\alpha]_D$ +57.5 (*c*

1.07/$CHCl_3$). IR ($CHCl_3$), ν: 1660 cm^{-1}, 1728 cm^{-1}. ^1H NMR ($CDCl_3$), δ: 0.10, 0.12, 0.15 (3s, 27H, $Si(CH_3)_3$); 1.25 (t, 3H, $J=7.1$ Hz, OCH_2CH_3); 3.32 (s, 3H, OCH_3); 3.65–3.85 (m, 3H, H-2H-3 and H-4); 4.09 (ddd, 1H, $J=1.7$, 4.8, 8.5 Hz, H-5); 4.21 (q, 2H, OCH_2CH_3); 4.53 (d, 1H, $J=1.4$ Hz, H-1); 6.16 (dd, 1H, $J=15.7$ Hz, H-7); 7.07 (dd, 1H, H-6). ^{13}C NMR ($CDCl_3$), δ: 0.4–1.4 ($Si(CH_3)_3$); 14.6 (OCH_2CH_3); 55.1 (OCH_3); 60.6 (OCH_2CH_3); 71.8–73.8 (C-2, C-3, C-4 and C-5); 102.4 (C-1); 122.0 (C-7); 145.7 (C-6); 166.7 (CO_2Et). ^{29}Si NMR ($CDCl_3$), δ: 17.6, 18.3, 19.9 (3s, $Si(CH_3)_3$). MS (FAB > 0, NBA), *m/z*: 551 $[M+SiMe_3]^+$, 501 $[M+Na]^+$, 478 $[M]^+$, 447 $[M-OCH_3]^+$, 73 $[SiMe_3]^+$.

Ethyl [methyl (E)-6,7-dideoxy-α-D-*manno*-oct-6-enopyranoside]uronate (7). Compound **6** (400 mg) was dissolved in a solution of HCl (1 M in THF, 20 mL) and the mixture was stirred for 5 min. The reaction was quenched with saturated $NaHCO_{3(aq)}$ until the pH was 8–9. The aqueous layer was extracted with CH_2Cl_2 (3×70 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. Compound **7** (230 mg, 95%) was obtained pure as a colorless oil. R_f 0.65 (EtOAc/MeOH 9:1). $[\alpha]_D$ +65.0 (*c* 0.6/CH_2Cl_2). IR (CH_2Cl_2), ν: 1716 cm^{-1}, 3410 cm^{-1}. ^1H NMR ($CDCl_3$), δ: 1.25 (m, 3H, OCH_2CH_3); 3.34 (s, 3H, OCH_3); 3.58 (t, 1H, $J=9.4$ Hz, H-4); 3.78 (dd, 1H, H-3); 3.92 (dd, 1H, $J=1.4$, 3.2 Hz, H-2); 4.05–4.25 (m, 3H, H-5 and OCH_2CH_3); 4.45 (m, 1H, OH); 4.73 (d, 1H, H-1); 4.83 (m, 1H, OH); 4.91 (m, 1H, OH); 6.19 (dd, 1H, $J=1.7$, 15.8 Hz, H-7); 7.11 (dd, 1H, $J=4.5$ Hz, H-6). ^{13}C NMR ($CDCl_3$), δ: 13.1 (OCH_2CH_3); 54.0 (OCH_3); 59.7 (OCH_2CH_3); 69.0–70.2 (C-2, C-3, C-4 and C-5); 100.2 (C-1); 120.9 (C-7); 143.5 (C-6); 166.2 (CO_2Et). MS (FAB > 0, NBA), *m/z*: 285 $[M+Na]^+$, 263 $[M+H]^+$, 231 $[M-OCH_3]^+$.

Sodium [methyl (E)-6,7-dideoxy-α-D-*manno*-oct-6-enopyranoside]uronate (8). Compound **7** (60 mg) was dissolved in 2 M NaOH (6 mL). The mixture was stirred for 1 h and neutralized with ion exchange resin (Dowex 50WX2, H^+ form, 14 g) until the pH was 4–5. The resin was washed with H_2O (100 mL) and filtered off. The filtrate was freeze dried from H_2O affording **8** (56 mg, 95%) as a light brown foam. R_f 0.54 (*i*-PrOH/NH_4OH/H_2O 8:1:1). $[\alpha]_D$ +52.0 (*c*1.0/H_2O). ^1H NMR (D_2O), δ: 3.28 (s, 3H, OCH_3); 3.47 (t, 1H, $J=9.6$ Hz, H-4); 3.67 (dd, 1H, $J=3.4$ Hz, H-3); 3.84 (dd, 1H, $J=1.7$ Hz, H-2); 3.95–4.10 (m, 1H, H-5); 4.66 (d, 1H, H-1); 6.04 (dd, 1H, $J=0.9$, 15.7 Hz, H-7); 6.54 (dd, 1H, $J=6.7$ Hz, H-6). ^{13}C NMR (D_2O), δ: 54.0 (OCH_3); 68.8–70.8 (C-2, C-3, C-4 and C-5); 100.2 (C-1); 128.0 (C-7); 138.7 (C-6); 172.6 (CO_2Na). MS (FAB > 0, NBA), *m/z*: 257 $[M+H]^+$. MS (FAB < 0, NBA), *m/z*: 233 $[M-Na]^-$. $C_9H_{13}NaO_7$ (256.06): calcd C 42.19, H 5.11; found C 41.75, H 5.29.

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