Total synthesis of alginate and zwitterionic SP1 oligosaccharides

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Chemical Synthesis of Guanosine Diphosphate Mannuronic Acid (GDP-ManA) and its C-4-*O*-Methyl and C-4-deoxy Congeners

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5.1 Introduction

Alginates are linear anionic polysaccharides, produced by brown algae and bacteria such as *Pseudomonas aeruginosa*.[1-3] Algal derived alginates have been widely used in food, cosmetic and medical industries because of their gelling properties.[4-6] In algae, they can be found in the cell wall,[1-3] while *P. aeruginosa* employs alginate to create a biofilm to protect the bacterium from its environment.[7] Alginates are composed of (1-4)-linked β-D-mannuronic acid (ManA, M) and α-L-guluronic acid (GulA, G) residues (See Figure 5.1),

which occur in poly-M, poly-G or alternating MG blocks. Algae produce all three types of alginate, whereas bacteria only produce poly-M and MG polymers.[8] The ManA-residues in these alginates can carry acetyl esters at the C-2 or C-3 positions.[8] The *P. aeruginosa* biomachinery used to construct alginate polysaccharides is schematically depicted in Figure 5.1. It comprises an ensemble of 10 proteins that together span the periplasmic space.[9-12] First, guanosine diphosphate mannuronic acid (GDP-ManA) is polymerized by the action of Alg8 to create a poly-ManA chain.[13] While this polysaccharide is transported through the periplasm, ManA residues can either be acetylated by the concerted action of Alg I, J, F and X,[14-16] or epimerized at C-5 by AlgG to create GulA residues.[17-21] Acetylation of GulA residues is not found, indicating that these modifications are mutually exclusive. Cleavage of the alginate chains is accomplished by AlgL.[22-25] The length and composition of the alginate chains dictate its properties and understanding and harnessing the biosynthesis enzymes may open the way for therapeutic intervention[26,27] as well as the generation of designer alginates with tailor made properties for medical applications.[28,29]

To be able to study the polymerase enzyme, Alg8, sufficient amounts of the GDP-ManA donor are required and therefore the development of an efficient route of synthesis for this mannuronic acid synthon was undertaken. Taking into account that ManA-polymerases may be used for the *in vitro* construction of alginate polysaccharides, it was reasoned that GDP-ManA donors that cannot be elongated at the C-4-OH, could potentially be used as "chain stoppers" to control the length of the growing alginate chain and thereby the properties of the polymer.[30] As potential capping GDP-ManA donors, it was therefore decided to target the C-4-OMe GDPManA **2** and C-4-deoxy GDP-ManA **3** alongside the natural donor GDP-ManA **1** (Scheme 5.1).

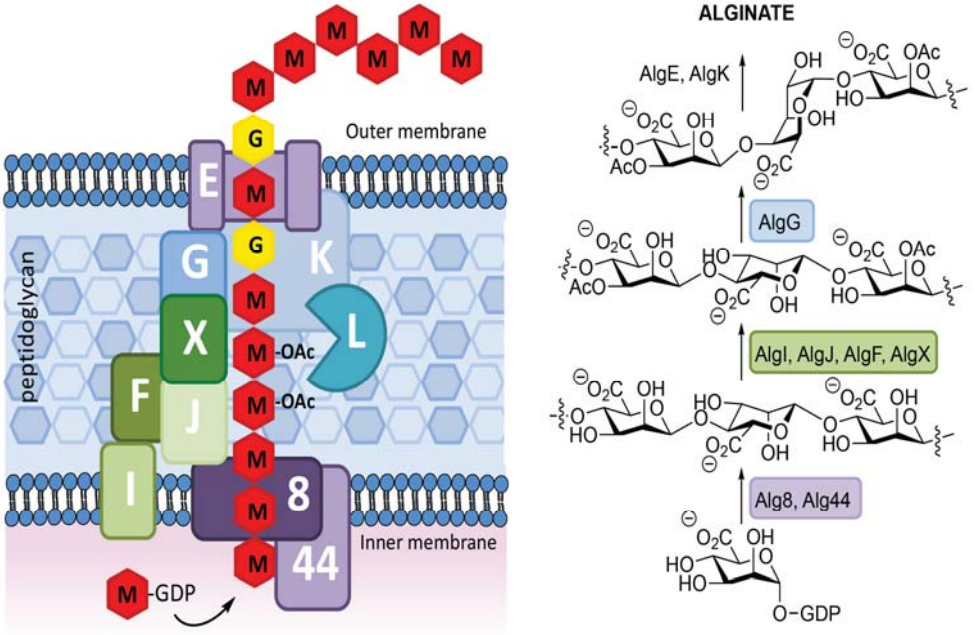
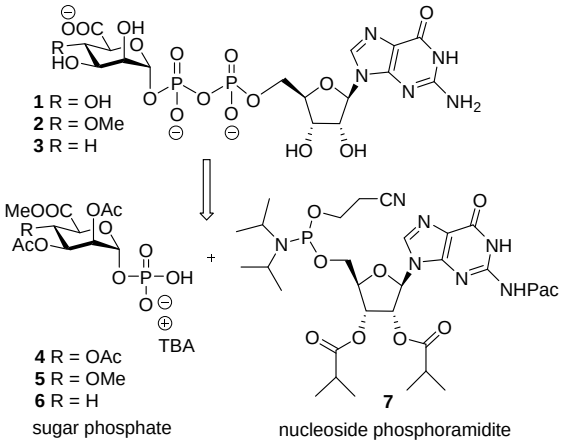


Figure 5.1 Biosynthesis of the alginate exopolysaccharide by *P. aeruginosa*.

The crucial synthetic step in the assembly of nucleotide diphosphate sugars is the union of the carbohydrate and the nucleoside through the construction of the pyrophosphate moiety. There are many different procedures reported to achieve the introduction of pyrophosphates[31,32] and the most commonly employed methods have in common the condensation of a phosphate monoester with an activated phosphate monoester.[31-39] It has been recently shown that the powerful phosphorylation capacity of phosphoramidites can be combined with phosphate monoesters for the effective construction of various types of pyrophosphate linkages.[40-44] Towards a nucleotide diphosphate sugar, a suitably protected sugar-1-phosphate is coupled to a nucleoside

phosphoramidite using an appropriate activator, such as dicyanoimidazole (DCI), to provide a phosphate-phosphite intermediate. This $P^{(V)}$-$P^{(III)}$ species can be oxidized to give the partially protected pyrophosphate.[40-44] This chapter describes the use of this method in the construction of GDP-ManA donors **1**, **2** and **3** (Scheme 5.1).

Scheme 5.1 Retrosynthetic analysis towards target GDP-ManA compounds 1, 2 and 3.

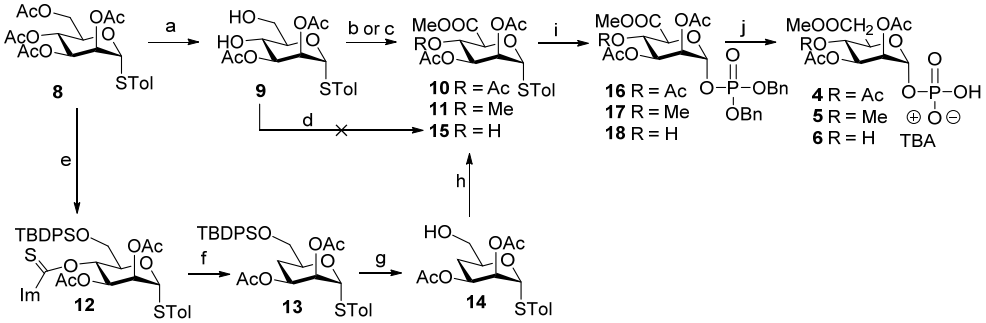


5.2 Results and discussion

As retrosynthetically depicted in Scheme 5.1, three protected ManA-1-phosphates (**4**-**6**) and a protected guanosine cyanoethyl phosphoramidite (**7**) were required. The latter building block was assembled according to well-established procedures[45] and the synthesis of the protected ManA-phosphate donors is presented in Scheme 5.2. The anomeric phosphate group in these building blocks was stereoselectively introduced by coupling of the mannuronic acid thioglycosides with dibenzyl phosphate and subsequent removal of the benzyl groups. The assembly of the required mannuronic acid building blocks started from 2,3-di-acetyl-*S*-tolyl mannoside **8**[46] by a regio- and chemoselective oxidation step to furnish the *S*-tolyl mannuronic acid.[47-51] The crude acid was immediately

esterified to give ManA methyl ester **9** in 68% over two steps. Acetylation of the remaining alcohol led to fully protected ManA **10**, while treatment of the alcohol with trimethylsilyldiazomethane and borontrifluoride-diethyl etherate provided the C-4-methyl ether **11** in 26% yield. Attempts to remove the alcohol group from **9** using a Barton-McCombie procedure failed, as the intermediate C-4-xanthate ester proved to be prone to elimination of the C-4-ester leading to the α,β-unsaturated ManA ester.[52,53] Therefore it has been decided to install the C-5-carboxylate after deoxygenation of C-4. To this end, the C-6-alcohol in 2,3-di-acetyl-*S*-tolyl mannoside **8** was masked as a silyl ether, after which the xanthate ester was installed at C-4 and subsequent radical reduction led to C-4-deoxy mannose **13**. Silyl removal then liberated the primary alcohol, which was oxidized to the corresponding acid. Treatment of the crude acid with trimethylsilyldiazomethane then furnished C-4 deoxy ManA **15** in excellent yield (95% over two steps). The three *S*-tolyl mannuronic acids were coupled with dibenzyl phosphoric acid under the agency of *N*-iodosuccinimide (NIS) to stereoselectively provide the α-ManA-phosphates **16**, **17** and **18** in good yields.[54] Cleavage of the benzyl esters through hydrogenation and treatment of the intermediate phosphate monoesters with tetrabutyl ammonium (TBA) hydroxide then gave the TBA-phosphates **4**, **5** and **6**, to be used in the crucial pyrophosphate forming step.

Scheme 5.2 Synthesis of ManA-1-phosphates **4**, **5** and **6**.

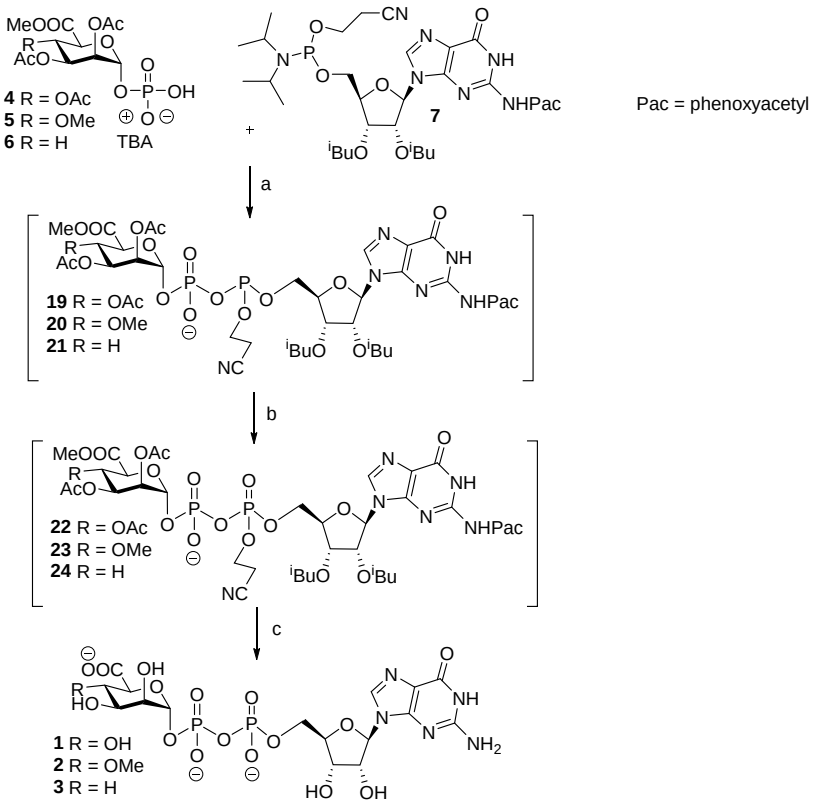


Reagents and conditions: a) i. TEMPO/BAIB, DCM/*t*BuOH/H_2O, ii. MeI, K_2CO_3, DMF, two steps yield 68%; b) for **10**: Ac_2O, pyridine, 88%, c) for **11**: trimethylsilyldiazomethane, $BF_3O{\bullet}Et_2$, DCM, 26%; d) thiocarbonyldiimidazole, toluene, 90°C. e) i. TBDPSCl, imidazole, DMF, 93%; ii. thiocarbonyldiimidazole, toluene, 90°C, quantitative yield; f) AIBN, Bu_3SnH, toluene, 90°C, 2 h, 84%; g) HF/Py, pyridine, THF, 99%; h) i. TEMPO/BAIB, DCM/*t*BuOH/H_2O, ii. Trimethylsilyldiazomethane, DCM, MeOH, two steps yield 95%; i) dibenzylphosphate, NIS, DCM, **16**: 77%, **17**: 69%, **18**: 79%. j) i. H_2, Pd/C, ii. tetrabutylammonium hydroxide, **4:** quantitative yield, **5**: 64%, **6**: 99%.

The assembly of the GDP-ManA pyrophospates is depicted in Scheme 5.3. Tri-acetyl ManA phosphate **4** was coupled with protected guanosine phosphoramidite **7** under the agency of DCI to generate phosphate-phosphite adduct **19**. This species was oxidized in the same reaction flask with *tert*-butyl hydroperoxide (*t*BuOOH) to generate the pyrophosphate, of which the cyanoethyl group was removed using dry 1,5-diazabicyclo[4.3.0]non-5-ene (DBU) to give the pyrophosphate dianion. Initially we tried to saponify the methyl ester, three acetyl groups, two *iso*-butyl esters and phenoxyacetyl group using lithium hydroxide in a mixture of THF and water, but this led to cleavage of the anomeric phosphate ester to give guanosine diphosphate. We therefore switched to a milder saponification protocol using triethyl amine/water/methanol to remove all labile protecting groups. Gratifyingly, this procedure did not jeopardize the anomeric phosphate

linkage and the GDP-ManA trisodium salt **1** could be obtained after ion exchange purification and Dowex-Na^+ treatment in 40% yield (from phosphoramidite **7**). Application of the same sequence of reactions to ManA-phosphates **5** and **6** furnished C-4-Methyl GDP ManA **2** in similar yield (45%) and C-4-deoxy GDP-ManA **3** in excellent yield (80%). All three syntheses could be accomplished on multi-milligram scale yielding 27, 42 and 53 mg of the target GDP-ManA donors **1**, **2** and **3** respectively.

Scheme 5.3 Synthesis of GDP-ManA **1**, **2** and **3** using a phosphoramidite coupling approach.



Reagents and conditions: a) dicyanoimidazole, **4**, **5** or **6**, MeCN, rt, 30 min; b) *t*BuOOH, rt, 30 min; c) i. DBU, 30 min, ii. $Et_3N/MeOH/H_2O$, rt, overnight, iii. ion-exchange purification, Dowex-Na^+, lyophilization; **1**: 40%, **2**: 45%, **3**: 80% (from **7**).

5.3 Conclusion

In conclusion, the assembly of a triad of guanosine diphosphate mannuronic acids using a phosphoramidite coupling strategy was successfully completed. Key features in our syntheses are the chemo- and regioselective oxidation of a partially protected mannose thioglycoside to generate the corresponding mannuronic acids, the stereoselective introduction of the anomeric phosphates and the construction of the pyrophosphate moieties. The latter functionality was created by coupling the tetrabutylammonium ManA phosphates with a protected cyanoethyl guanosine phosphoramidite. Oxidation and global deprotection of the intermediates then effectively provided the target compounds that were each generated in multi-milligram quantities. The GDP-ManA donors will be employed to fuel the mannuronic polymerase for the enzymatic assembly of polymannuronic acids. The generated C-4-capped and C-4-deoxygenated GDP-ManA donors will be explored as "chain stoppers" to gain control over the length of the growing polymannuronic acid chains.

5.4 Experimental Section

General methods and materials

Commercially available reagents were used as received, except where noted. DCM and THF were dried over 4Å molecular sieves. Acetonitrile (DNA reagent grade) was stored over 4Å molecular sieves prior to use. Analytical TLC was performed on aluminium sheets, pre-coated with silica gel (Merck silica gel 60 F_{254}) and visualized with UV or spraying with either 20% H_2SO_4 in ethanol or Ammonium molybdate/Cerium sulphate solution [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_6{\cdot}2H_2O$ (10 g/L), 10% sulphuric acid in ethanol], followed by charring. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a 400MHz spectrometer at 400.2, 100.6 and 162.0 MHz respectively. Chemical shifts are reported as δ values (ppm) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or indirectly referenced to H_3PO_4 (0.00 ppm) in D_2O via the solvent residual signal. As a result of chair interconversion between the $^4C_1$ and $^1C_4$ conformers, the NMR spectra of the mannuronic acid esters show significant line broadening for some signals in the ^{13}C spectra, as well as ^3J coupling constants in the ^1H spectra that are an average of the ^3J coupling constants

from both chair conformers [55-58]. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture.

General experimental procedure for synthesis of D-mannopyranosyl uronate dibenzylphosphate 16, 17 and 18.

After coevaporation with toluene three times, the thioglycoside donor **10**, **11** or **15** (2 mmol) and dibenzylphosphate (4 mmol) were dissolved in dry DCM (9 ml). NIS (3 mmol) and TfOH (0.28 mmol) were added to the reaction solution at 0 $^{\circ}$C, the mixture was stirred for 1h and monitored by TLC analysis. The reaction mixture was quenched by the addition of 5% aq. $Na_2S_2O_3$-solution (30 ml). The aqueous layer was separated and extracted with DCM. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. Purification by column chromatograpy (silica gel, pentane/DCM/EtOAc, 3/1/1, v/v/v) yielded **16**, **17** or **18**.

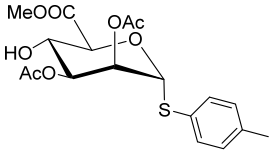
General experimental procedure for synthesis of D-mannopyranosyl uronate phosphate, mono-tetrabutylammonium salt 4, 5 and 6.

Dibenzylphostriester **16**, **17** or **18** (1 mmol) was dissolved in MeOH (10 ml) and purged with argon. Palladium on charcoal (Pd/C, 10%) (106 mg) was added and the reaction was purged with hydrogen gas and then stirred under a hydrogen atmosphere for 4 h at room temperature. The mixture was then filtered over Whatmann paper and the filter was rinsed with MeOH. Concentration of the organic solvent under reduced pressure followed by addition of 40% aq. solution of tetrabutylammonium hydroxide (0.67 ml, 1 mmol) and concentration afforded compound **4**, **5** or **6.**

General experimental procedure for synthesis of sugar nucleotides 1, 2 and 3.

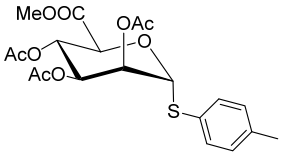
Phosphoramidite **7** (0.1 mmol, 1 eq) (coevaporated once with 5 mL anhydrous MeCN) was dissolved in 1.5 mL anhydrous MeCN under an atmosphere of argon. Sugar phosphate **4**, **5**, or **6** (0.12 mmol, 1.2 eq) and DCI (0.2 mmol, 2 eq) (coevaporated in 5 mL dry MeCN) were dissolved in 2 mL anhydrous MeCN and added to the phosphoramidite 7 at ambient temperature. The reaction mixture was stirred for 30 minutes at ambient temperature, after which t-BuOOH (80 ul, 0.4 mmol, 4 eq) was added. After 30 minutes of reaction time DBU (75 ul, 0.5 mmol, 5 eq) was added and the reaction was stirred for an additional 30 minutes. Et_3N/MeOH/H_2O (3 mL/3 ml/1.5 ml) was added and the reaction was stirred for overnight. The mixture was concentrated *in vacuo* at no more than 30 °C. The crude product was applied to a strong anion exchange column and eluted with a gradient of ammonium acetate [0.05M (pH 7.0) - 0.5M (pH 7.1)] at 4mL per minute. The fractions containing the product were collected and concentrated under reduced pressure. Repeated lyophilization (to remove residual ammonium acetate), followed by filtration over dowex-Na^+ form, produced the desired sugar nucleotides in good yields.

Methyl (tolyl 2,3-di-O-acetyl -1-thio-α-D-mannopyranosyl uronate) (9): The starting material **8** (2.93 g, 7.91 mmol)



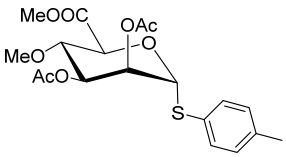
was dissolved in DCM/t-BuOH/H_2O (54 ml, 4/4/1, v/v/v) and the mixture was cooled to 0 °C and treated with TEMPO (247 mg, 1.58 mmol) and BAIB (6.37 g, 19.77 mmol). After stirring overnight at 4 °C, sat. aq. $Na_2S_2O_3$ was added and the mixture was stirred for 30 minutes, diluted with EtOAc, washed with sat. aq. NaCl and the organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DMF (20 ml), followed by the addition of K_2CO_3 (1.09 g, 7.9 mmol) and MeI (0.98 ml, 15.82 mmol) at 0 °C. The mixture was allowed to stir overnight at 4 °C, and then diluted with EtOAc, washed with sat. aq. NaCl and the organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (silica gel, pentane/EtOAc, 2/1, v/v) yielded methyl ester **9** (2.13 g, yield: 68%). TLC: R_f = 0.39 (pentane/EtOAc, 1/1, v/v); $[\alpha]^{20}_D$ = +86° (c = 0.84, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.39 (d, J = 8.2 Hz, 2H), 7.12 (d, J = 7.9 Hz, 2H), 5.46 (dd, J = 3.2, 2.3 Hz, 1H, H-2), 5.44 (d, J = 2.3 Hz, 1H, H-1), 5.21 (dd, J = 9.5, 3.3 Hz, 1H, H-3), 4.80 (d, J = 9.1 Hz, 1H, H-5), 4.27 (td, J = 9.4, 4.0 Hz, 1H, H-4), 3.83 (s, 3H), 3.54 (d, J = 4.1 Hz, 1H, C4-OH), 2.32 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.20, 169.93, 138.38, 132.56, 129.92, 128.75, 86.37 (C-1), 72.20 (C-5), 70.67 (C-3), 70.32 (C-2), 66.87 (C-4), 52.84, 21.08, 20.76, 20.74. HRMS: [M+H$^+$] calculated for $C_{18}H_{22}O_8S$: 399.11081; found: 399.11072.

Methyl (tolyl 2,3,4-tri-O-acetyl -1-thio-α-D-mannopyranosyl uronate) (10):



Compound **9** (1.95 g, 4.9 mmol) was dissolved in pyridine (5 ml) and Ac_2O (1 ml) and DMAP (60 mg, 0.49 mmol) were added to the solution at 0 °C. Then the mixture was allowed to stir overnight at room temperature after which it was concentrated under reduced pressure. Purification by column chromatography (silica gel, pentane/EtOAc, 3/1, v/v) yielded **10** as a colourless foam (1.91 g, yield: 88%). TLC: R_f = 0.47 (pentane/EtOAc, 2/1, v/v); $[\alpha]^{20}_D$ = +78° (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.40 (d, J = 8.2 Hz, 2H), 7.20 – 7.06 (m, 2H), 5.52 (d, J = 3.7 Hz, 1H, H-1), 5.45 (m, J = 8.6 Hz, 1H, H-4), 5.42 (m, J = 3.5 Hz, 1H, H-2), 5.33 (dd, J = 8.7, 3.3 Hz, 1H, H-3), 4.81 (d, J = 8.1 Hz, 1H, H-5), 3.77 (s, 3H), 2.33 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.66, 167.99, 138.42, 132.41, 129.99, 128.55, 84.96 (C-1), 70.98 (C-5), 69.40 (C-2), 68.40 (C-3), 67.47 (C-4), 52.81, 21.16, 20.87, 20.73, 20.65. HRMS: [M+H$^+$] calculated for $C_{20}H_{24}O_9S$: 441.12138; found: 441.12148.

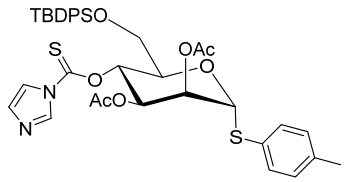
Methyl (tolyl 2,3-di-O-acetyl -4-O-methyl-1-thio-α-D-mannopyranosyl urinate) (11): Compound **9** (698 mg, 1.75



mmol) was dissolved in DCM (7.5 ml) and trimethylsilyldiazomethane (2.63 ml, 5.26 mmol, 2 M in hexane) and BF_3•OEt_2 (0.63 ml, 5.25 mmol) were added to the solution in -40 °C. Then the mixture was allowed to stir for 2 h, after which additional trimethylsilyldiazomethane (2.63 ml, 5.26 mmol, 2 M in hexane) was

added and stirred was continued for another 3 hours and quenched with AcOH (1 mL). The mixture was poured into 100 mL EtOAc and washed with sat. aq. $NaHCO_3$ and brine. The water layers were extracted with 100mL EtOAc and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (10-20% EtOAc in petroleum ether) produced the title compound (187 mg, 0.45 mmol, yield: 26%). TLC: R_f = 0.61 (pentane/EtOAc, 2/1, v/v); $[\alpha]^{20}_D$ = +70° (c = 0.96, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.42 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 7.9 Hz, 3H), 5.44 (d, J = 4.2 Hz, 1H, H-1), 5.38 (dd, J = 4.2, 3.2 Hz, 1H, H-2), 5.26 (dd, J = 8.1, 3.2 Hz, 1H, H-3), 4.70 (d, J = 7.4 Hz, 1H, H-5), 3.91 (t, J = 7.8 Hz, 1H, H-4), 3.82 (s, 3H), 3.48 (s, 3H), 2.33 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.84, 169.58, 169.37, 138.30, 132.63, 129.94, 128.97, 85.04 (C-1), 76.08 (C-4), 72.18 (C-5), 70.44 (C-3), 69.58 (C-2), 59.84, 52.67, 21.23, 20.93. HRMS: [M+H$^+$] calculated for $C_{19}H_{24}O_8S$: 413.12646; found: 413.12648.

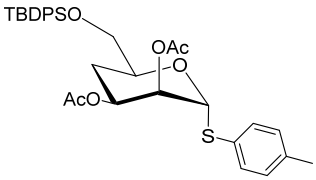
Tolyl 2,3-di-O-acetyl-4-O-imidazole-thiocarbonyl-6-O-TBDPS-1-thio-α-D-mannopyranoside (12): Compound **8** (1.384



g, 3.74 mmol) and imidazole (0.51 g, 7.5 mmol) were dissolved in DMF (10 ml), then TBDPSCl (1.22 ml, 4.86 mmol) was added to the reaction mixture at 0 °C. The mixture was allowed to stir overnight at room temperature. The reaction was quenched with MeOH, then diluted with EtOAc and washed with sat. aq. $NaHCO_3$ and brine. The water layers were extracted with EtOAc and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (10-20% EtOAc in petroleum ether) produced tolyl 2,3-di-O-acetyl-6-O-TBDPS-1-thio-α-D-mannopyranoside (2.124 g, 3.49 mmol, yield: 93%). TLC: R_f = 0.70 (pentane/EtOAc, 2/1, v/v). $[\alpha]^{20}_D$ = +57° (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.70 – 7.67 (m, 4H), 7.53 – 7.32 (m, 6H), 7.30 (d, J = 8.1 Hz, 2H), 7.04 (d, J = 7.9 Hz, 2H), 5.48 (dd, J = 3.4, 1.6 Hz, 1H, H-2), 5.35 (d, J = 1.5 Hz, 1H, H-1), 5.19 (dd, J = 9.6, 3.3 Hz, 1H, H-3), 4.30 – 4.15 (m, 2H, H-5, H-4), 4.04 – 3.86 (m, 2H, H-6), 2.30 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.74, 170.09, 135.86, 135.66, 133.21, 132.80, 132.66, 129.97, 129.72, 127.94, 86.39 (C-1), 72.90 (C-5), 72.23 (C-3), 71.31 (C-2), 67.44 (C-4), 64.28 (C-6), 26.93, 21.26, 21.07, 20.97, 19.41. HRMS: [M+H$^+$] calculated for $C_{33}H_{40}O_7SSi$: 609.23368; found: 609.23351. Then tolyl 2,3-di-O-acetyl-6-O-TBDPS-1-thio-α-D-mannopyranoside (1.82 g, 3 mmol) and thiocarbonyldiimidazole (0.896 g, 5.04 mmol) were dissolved in anhydrous toluene (30 ml) and the reaction mixture was allowed to stir for 7 h at 90 °C. After cooling to room temperature, the reaction mixture was washed with sat. aq. $NaHCO_3$ and brine. The organic layers were dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. Purification by column chromatography (silica gel, pentane/DCM/EtOAc, 4/1/1, v/v/v) yielded **12** (2.19 g, quantitative yield). TLC: R_f = 0.36 (pentane/DCM/EtOAc, 3/1/1, v/v); $[\alpha]^{20}_D$ = +63° (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.29 (t, J = 1.1 Hz, 1H), 7.68 – 7.54 (m, 5H), 7.43 – 7.33 (m, 4H), 7.33 – 7.23 (m, 5H), 7.11 – 7.03 (m, 3H), 6.34 (t, J = 9.5 Hz, 1H, H-4), 5.66 – 5.51 (m, 2H, H-3, H-2), 5.46 (d, J = 1.3 Hz, 1H, H-1), 4.55 (dt, J = 9.8, 3.2 Hz, 1H, H-5), 3.81 (d, J = 3.2 Hz, 2H, H-6), 2.33 (s, 3H), 2.16 (s, 3H), 1.96 (s, 3H), 1.03 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.95, 169.93, 169.79, 138.29, 135.79, 135.47,

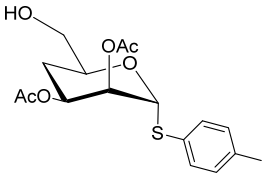
133.07, 132.53, 132.35, 131.03, 130.01, 129.77, 129.20, 127.68, 127.57, 117.91, 86.06 (C-1), 75.27 (C-4), 71.89 (C-5), 71.43 (C-2), 69.46 (C-3), 62.37 (C-6), 26.70, 21.20, 20.89, 20.74, 19.22. HRMS: $[M+H^+]$ calculated for $C_{37}H_{42}N_2O_7S_2Si$: 719.22755; found: 719.22754.

Tolyl 2,3-di-O-acetyl-4-deoxy-6-O-TBDPS-1-thio-α-D-mannopyranoside (13):



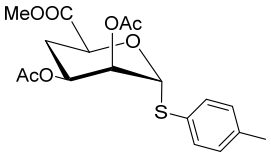
Barton-MaCombie precursor **12** (2.07 g, 2.88 mmol) was coevaporated with anhydrous toluene two times and was dissolved in anhydrous toluene (35 ml). Bu_3SnH (1.91 ml, 7.29 mmol) and AIBN (71 mg, 0.43 mmol) were added at 90 °C. The reaction was stirred at this temperature for 2 h and was then cooled down before being washed with sat. aq. $NaHCO_3$ and brine. The organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuo. Purification by column chromatography (silica gel, pentane/DCM/EtOAc, 6/1/1, v/v/v) yielded **13** (1.44 g, 2.43 mmol, yield: 84%). TLC: R_f = 0.86 (pentane/DCM/EtOAc, 3/1/1, v/v); $[\alpha]^{20}_D$ = +72° (c = 0.66, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.68 (dq, J = 7.0, 1.3 Hz, 4H), 7.50 – 7.31 (m, 8H), 7.12 – 6.96 (m, 2H), 5.43 (d, J = 1.6 Hz, 1H, H-1), 5.35 (brs, H-2), 5.25 (m, 1H, H-3), 4.46 (m, 1H, H-5), 3.84 – 3.64 (m, 2H, H-6), 2.29 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.03-1.85 (m, 2H, H-4), 1.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.14, 169.95, 137.75, 135.75, 135.60, 133.54, 133.21, 132.40, 130.17, 129.80, 129.71, 127.71, 127.68, 86.94 (C-1), 69.87 (C-2), 69.62 (C-5), 67.24 (C-3), 66.20 (C-6), 28.40 (C-4), 26.82, 21.14, 21.03, 20.97, 19.32. HRMS: $[M+H^+]$ calculated for $C_{33}H_{40}O_6SSi$: 593.23876; found: 593.23840.

Tolyl 2,3-di-O-acetyl-4-deoxy-1-thio-α-D-mannopyranoside (14): Compound **13** (1.38 g, 2.32 mmol) was dissolved in



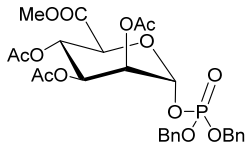
THF (6 ml) and pyridine (6 ml), and then HF/Py (70% HF in pyridine, 598 ul, 23 mmol) was added to reaction mixture at 0 °C. The reaction mixture was allowed to stir at room temperature and monitored by TLC analysis. The reaction mixture was poured into a mixture of EtOAc and sat. aq. $NaHCO_3$. The organic layer was washed with bine, dried over Na_2SO_4, filtrated and the solvent was removed *in vacuo*. Purification by column chromatography (silica gel, pentane/ EtOAc, 2/1, v/v) yielded **14** (0.815 g, 2.3 mmol, yield: 99%). TLC: R_f = 0.21 (pentane/ EtOAc, 2/1, v/v); $[\alpha]^{20}_D$ = +103° (c = 0.88, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.45 – 7.35 (m, 2H), 7.14 (d, J = 7.9 Hz, 2H), 5.44 (d, J = 1.7 Hz, 1H, H-1), 5.38 (brs, 1H, H-2), 5.30 (m, 1H, H-3), 4.49 (m, 1H, H-5), 3.68 (m, 2H, H-6), 2.34 (s, 3H), 2.14 (s, 3H), 2.05 (s, 3H), 1.99 (q, J = 12.2 Hz, 1H, H-4), 1.82 (m, 1H, H-4); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.06, 169.92, 138.31, 132.99, 129.95, 129.30, 86.79 (C-1), 69.77 (C-2), 69.32 (C-5), 66.96 (C-3), 65.08 (C-6), 27.79 (C-4), 21.14, 20.94. HRMS: $[M+H^+]$ calculated for $C_{17}H_{22}O_6S$: 355.12099; found: 355.12100.

Methyl (tolyl 2,3-di-O-acetyl-4-deoxy-1-thio-α-D-mannopyranosyl uronate) (15):



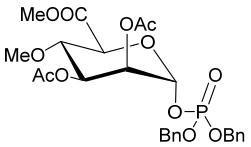
As described the synthesis of **9**, compound **15** was obtained (0.74 g, 1.94 mmol, yield: 95%). TLC: R_f = 0.26 (pentane/ EtOAc, 4/1, v/v); $[\alpha]^{20}_D$ = +97° (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.42 – 7.32 (m, 2H), 7.11 (d, J = 7.9 Hz, 2H), 5.55 (d, J = 2.5 Hz, 1H, H-1), 5.26 (q, J = 4.6 Hz, 2H, H-2, H-3), 4.94 (dd, J = 10.5, 3.6 Hz, 1H, H-5), 3.79 (s, 3H), 2.32 (s, 3H), 2.30 – 2.14 (m, 2H, H-4), 2.11 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.28, 169.99, 169.72, 138.17, 132.32, 129.95, 129.16, 86.15 (C-1), 69.03 (C-2 or C-3), 68.35 (C-5), 66.59 (C-2 or C-3), 52.49, 29.14 (C-4), 21.14, 20.92, 20.88. HRMS: [M+H$^+$] calculated for $C_{18}H_{22}O_7S$: 383.11590; found: 383.11596.

(Methyl 2,3,4-tri-O-acetyl-α-D-mannopyranosyl uronate) dibenzylphosphate (16):



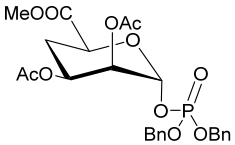
Compound **16** was obtained as described by the general experimental procedure for the synthesis of D-mannopyranosyl uronate dibenzylphosphate (1.29 g, 2.17 mmol, yield: 77%). TLC: R_f = 0.31 (pentane/ DCM/EtOAc, 2/1/1, v/v/v); $[\alpha]^{20}_D$ = +33° (c = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.41 – 7.30 (m, 10H), 5.71 (dd, J = 6.6, 2.5 Hz, 1H, H-1), 5.44 – 5.28 (m, 2H, H-4, H-3), 5.24 (t, J = 2.8 Hz, 1H, H-2), 5.09 (dd, J = 8.6, 3.5 Hz, 4H), 4.39 (d, J = 8.9 Hz, 1H, H-5), 3.68 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.46, 169.41, 169.37, 167.08, 135.18, 135.11, 135.07, 135.00, 128.70, 128.60, 128.55, 128.08, 127.94, 94.65 and 94.59 (C-1), 70.60 (C-5), 69.97, 69.92, 69.84, 69.79, 68.16 and 68.05 (C-2), 67.43 (C-3), 66.21 (C-4), 52.75, 20.59, 20.49. HRMS: [M+H$^+$] calculated for $C_{27}H_{31}O_{13}P$: 595.15750; found: 595.15766.

(Methyl 2,3-di-O-acetyl-4-O-methyl-α-D-mannopyranosyl uronate) dibenzylphosphate (17): Compound **17** was



obtained as described by the general experimental procedure for synthesis of D-mannopyranosyl uronate dibenzylphosphate (0.41 g, 0.724 mmol, yield: 69%). TLC: R_f = 0.17 (pentaneEtOAc, 2/1, v/v); $[\alpha]^{20}_D$ = +29° (c = 0.58, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.37 (p, J = 2.0 Hz, 10H), 5.71 (dd, J = 6.8, 2.4 Hz, 1H, H-1), 5.34 – 5.20 (m, 2H, H-2, H-3), 5.11 (dd, J = 8.3, 6.1 Hz, 4H), 4.37 (d, J = 9.0 Hz, 1H, H-5), 3.87 (t, J = 8.8 Hz, 1H, H-4), 3.75 (s, 3H), 3.46 (s, 3H), 2.74 (s, 1H), 2.14 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.63, 169.61, 168.63, 135.48, 135.41, 135.31, 135.24, 128.77, 128.72, 128.65, 128.16, 128.03, 95.12 and 95.07 (C-1), 75.22 (C-4), 72.43 (C-5), 70.00 (C-3), 69.94, 69.83, 69.78, 68.78 and 68.67 (C-2), 60.35, 52.79, 20.91, 20.81. HRMS: [M+H$^+$] calculated for $C_{26}H_{31}O_{12}P$: 567.16259; found: 567.16270.

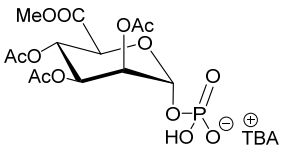
(Methyl 2,3-di-O-acetyl-4-deoxy-α-D-mannopyranosyl uronate) dibenzylphosphate (18): Compound **18** was obtained



as described by the general experimental procedure for synthesis of D-mannopyranosyl uronate dibenzylphosphate, (0.714 g, 1.332 mmol, yield: 79%). TLC:

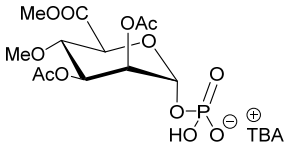
R_f = 0.18 (pentane/EtOAc, 2/1, v/v); $[\alpha]^{20}_D$ = +41° (c = 1, $CHCl_3$). ^1H NMR (400 MHz, Chloroform-d) δ 7.35 (dd, J = 9.0, 4.8 Hz, 10H), 5.76 (dd, J = 6.4, 2.2 Hz, 1H, H-1), 5.24 (m, 1H, H-3), 5.16 – 5.00 (m, 5H, H-2), 4.51 (dd, J = 12.0, 3.0 Hz, 1H, H-5), 3.71 (s, 3H), 2.21 – 2.13 (m, 1H, H-4), 2.11 (s, 3H), 2.06 (d, J = 12.2 Hz, 1H, H-4), 2.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.69, 169.39, 135.47, 135.40, 135.36, 135.30, 128.75, 128.70, 128.65, 128.20, 128.00, 95.91 and 95.86 (C-1), 69.97, 69.92, 69.81, 69.76, 68.74 (C-5), 66.74 and 66.63 (C-2), 65.31 (C-3), 52.55, 28.20 (C-4), 20.88, 20.83; ^{31}P NMR (162 MHz, $CDCl_3$) δ -2.79. HRMS: $[M+H^+]$ calculated for $C_{25}H_{29}O_{11}P$: 537.15202; found: 537.15217.

(Methyl 2,3,4-tri-O-acetyl-α-D-mannopyranosyl uronate) phosphate mono-tetrabutylammonium salt (4): Compound



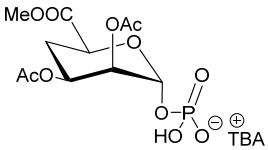
4 was obtained as by the described general experimental procedure for synthesis of D-mannopyranosyl uronate phosphate, mono-tetrabutylammonium salt (1 mmol, quantitative yield). ^1H NMR (400 MHz, Chloroform-d) δ 5.63 (dd, J = 7.8, 1.9 Hz, 1H, H-1), 5.47 (dd, J = 10.1, 3.5 Hz, 1H, H-3), 5.40 – 5.20 (m, 2H, H-2, H-4), 4.72 (d, J = 10.2 Hz, 1H, H-5), 3.67 (s, 3H), 3.34 (m, CH_2-TBA), 2.12 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.68 (m, CH_2-TBA), 1.45 (m, CH_2-TBA), 1.00 (t, m, CH_3-TBA); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.81, 169.69, 169.37, 168.68, 93.29 and 93.25 (C-1), 69.54 and 69.45 (C-2), 68.92 and 68.71 (C-3, C-5), 67 (C-4).05, 58.50, 52.03, 23.78, 20.68, 20.52, 20.46, 19.46, 13.51; ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.78. HRMS: $[M+H^+]$ calculated for $C_{13}H_{19}O_{13}P$: 415.06360; found: 415.06328.

(Methyl 2,3-di-O-acetyl-4-O-methyl-α-D-mannopyranosyl uronate) phosphate, mono-tetrabutylammonium salt (5):



Compound **5** was obtained as described by the general experimental procedure for synthesis of D-mannopyranosyl uronate phosphate, mono-tetrabutylammonium salt (99 mg, 0.158 mmol, yield: 64%). ^1H NMR (400 MHz, Chloroform-d) δ 5.63 – 5.51 (m, 1H, H-1), 5.38 – 5.20 (m, 2H, H-2, H-3), 4.51 (d, J = 9.9 Hz, 1H, H-5), 3.77 (s, 3H), 3.72 (d, J = 9.5 Hz, 1H, H-4), 3.38 (s, 3H), 3.33 (m, CH_2-TBA), 2.12 (s, 3H), 2.00 (s, 3H), 1.75 – 1.57 (m, CH_2-TBA), 1.44 (m, CH_2-TBA), 0.99 (t, CH_3-TBA); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.90, 169.76, 169.42, 93.72 and 93.70(C-1), 75.86 (C-4), 71.09 and 70.82 (C-3, C-5), 70.11 and 70.02 (C-2), 59.95, 58.51, 52.06, 23.80, 20.79, 20.76, 19.49, 13.54; ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.56. HRMS: $[M+H^+]$ calculated for $C_{12}H_{19}O_{12}P$: 387.06869; found: 387.06848.

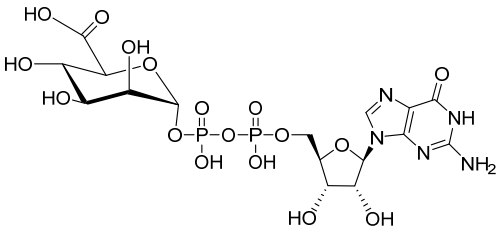
(Methyl 2,3-di-O-acetyl-4-deoxy-α-D-mannopyranosyl uronate) phosphate, mono-tetrabutylammonium salt (6):



Compound **6** was obtained as described by the general experimental procedure for synthesis of D-mannopyranosyl urinate phosphate, mono-tetrabutylammonium salt (177 mg, yield: 99%). ^1H NMR (400 MHz, Chloroform-d) δ 5.64 (dd, J = 7.6, 2.0 Hz, 1H, H-1), 5.40 (m, 1H, H-3), 5.18 (brs, 1H, H-2), 4.83

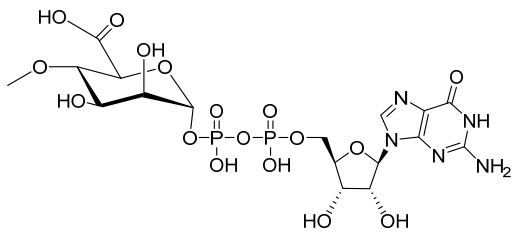
(dd, J = 12.4, 2.7 Hz, 1H, H-5), 3.72 (s, 3H), 3.33 (m, CH_2-TBA), 2.16 – 1.90 (m, 8H, 2xCH_3CO, H-4), 1.68 (m, CH_2-TBA), 1.44 (m, CH_2-TBA), 0.99 (t, CH_3-TBA); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.45, 169.23, 169.00, 93.55 and 93.50 (C-1), 67.47 and 67.38 (C-2), 66.63 (C-5), 66.01 (C-3), 58.05, 51.34, 28.22 (C-4), 23.38, 20.35, 19.08, 13.14; ^{31}P NMR (162 MHz, $CDCl_3$) δ -2.02. HRMS: [M+Na^+] calculated for $C_{11}H_{17}O_{11}P$: 379.04007; found: 379.04006.

Guanosine diphosphate mannuronic acid (1):

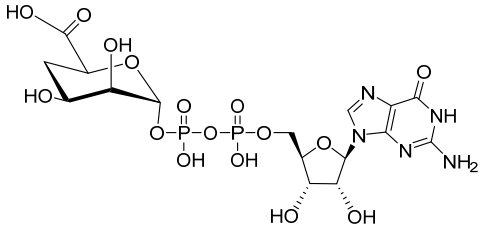


Compound **1** was obtained as described by the general experimental procedure for synthesis of sugar nucleotides (27 mg, 39.8 umol, 40%). ^1H NMR (400 MHz, Deuterium oxide) δ 8.09 (s, 1H), 5.91 (d, J = 6.0 Hz, 1H, H-1_{Rib}), 5.55 (dd, J = 8.1, 2.0 Hz, 1H, H-1_{Mann}), 4.74 (t, J = 5.6 Hz, 1H, H-2_{Rib}), 4.50 (dd, J = 5.2, 3.4 Hz, 1H, H-3_{Rib}), 4.35 (brs, 1H, H-4_{Rib}), 4.20 (m, 1H, H-5_{Rib}), 4.11 (d, J = 9.9 Hz, 1H, H-5_{Mann}), 4.08 – 4.02 (m, 1H, H-2_{Mann}), 3.94 (dd, J = 9.7, 3.4 Hz, 1H, H-3_{Mann}), 3.79 (t, J = 9.8 Hz, 1H, H-4_{Mann}); ^{13}C NMR (101 MHz, D_2O) δ 176.02, 158.57, 153.83, 151.46, 137.27, 115.68, 96.33 and 96.27 (C-1_{Mann}), 86.94 (C-1_{Rib}), 83.68 and 83.59 (C-4_{Rib}), 73.78 (C-2_{Rib}), 73.51(C-5_{Mann}), 70.24 (C-3_{Rib}), 70.03 and 69.94 (C-2_{Mann}), 69.50 (C-3_{Mann}), 68.41 (C-4_{Mann}), 65.21 and 65.15 (C-5_{Rib}); ^{31}P NMR (162 MHz, D_2O) δ -10.84, -13.15. HRMS: [M+H^+] calculated for $C_{16}H_{23}N_5O_{17}P_2$: 620.06369; found: 620.06338.

Guanosine diphosphate 4-O-methyl-mannuronic acid (2): Compound **2** was obtained as described by the general experimental procedure for synthesis of sugar nucleotides (42 mg, 60 umol, 45%). ^1H NMR (400 MHz, Deuterium oxide) δ 8.10 (s, 1H), 5.90 (d, J = 5.8 Hz, 1H, H-1_{Rib}), 5.52 (dd, J = 8.1, 2.1 Hz, 1H, H-1_{Mann}), 4.71 (t, J = 5.5 Hz, 1H, H-2_{Rib}), 4.49 (dd, J = 5.1, 3.6 Hz, 1H, H-3_{Rib}), 4.34 (m, 1H, H-4_{Rib}), 4.20 (m, 1H, H-5_{Rib}), 4.09 (d, J = 10.0 Hz, 1H, H-5_{Mann}), 4.04 (dd, J = 3.4, 2.1 Hz, 1H, H-2_{Mann}), 3.95 (dd, J = 9.8, 3.4 Hz, 1H, H-3_{Mann}), 3.55 (t, J = 9.8 Hz, 1H, H-4_{Mann}), 3.46 (s, 3H); ^{13}C NMR (101 MHz, D_2O) δ 176.21, 158.69, 153.84, 151.54, 137.37, 115.91, 96.36 and 96.30 (C-1_{Mann}), 86.97 (C-1_{Rib}), 83.77 and 83.68 (C-4_{Rib}), 78.96 (C-4_{Mann}), 73.90 (C-2_{Rib}), 73.64 (C-5_{Mann}), 70.33 (C-3_{Rib}), 70.26 (C-2_{Mann}), 69.23 (C-3_{Mann}), 65.29 and 65.23 (C-5_{Rib}), 59.91; ^{31}P NMR (162 MHz, D_2O) δ -10.80, -13.31. HRMS: [M+H^+] calculated for $C_{17}H_{25}N_5O_{17}P_2$: 634.07934; found: 634.08193.



Guanosine diphosphate 4-deoxy-mannuronic acid (3): Compound **3** was obtained as described by the general experimental procedure for synthesis of sugar nucleotides (53 mg, 80 umol, 80%). ^1H NMR (400



MHz, Deuterium Oxide) δ 8.11 (s, 1H), 5.95 (d, J = 5.7 Hz, 1H, H-1_{Rib}), 5.65 (dd, J = 7.9, 2.0 Hz, 1H, H-1_{Mann}), 4.75 (t, J = 5.5 Hz, 1H, H-2_{Rib}), 4.53 (dd, J = 5.2, 3.8 Hz, 1H, H-3_{Rib}), 4.44 (dd, J = 12.6, 2.7 Hz , 1H, H-5_{Mann}), 4.39-4.37 (m, 1H, H-4_{Rib}), 4.22 (m, 2H, H-5_{Rib}, H-3_{Mann}), 3.93 (t, J = 2.6 Hz, 1H, H-2_{Mann}), 2.21 – 2.05 (m, 1H), 1.81 (q, J = 12.4 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 178.24, 158.86, 153.85, 151.59, 137.41, 116.17, 96.98 and 96.93 (C-1_{Mann}), 86.98 (C-1_{Rib}), 83.67 and 83.58 (C-4_{Rib}), 73.92 (C-2_{Rib}), 70.31 (C-3_{Rib}), 70.26(C-5_{Mann}), 68.10 and 68.02 (C-2_{Mann}), 65.30 and 65.24 (C-5_{Rib}), 64.59 (C-3_{Mann}), 30.61; ^{31}P NMR (162 MHz, D_2O) δ -11.21, -13.52. HRMS: [M+H^+] calculated for $C_{16}H_{23}N_5O_{16}P_2$: 604.06878; found: 604.06864.

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