Supporting Information

for

Studies on the substrate specificity of a GDP-mannose pyrophosphorylase from Salmonella enterica

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**General methods**

All reagents used were purchased from commercial sources and were used without further purification unless noted. The solvents used were purified by successive passage through columns of alumina and copper under an argon atmosphere. All reactions were carried out under a positive pressure of argon at room temperature unless indicated otherwise. The reactions were monitored by analytical TLC on silica gel 60-F254 (0.25 mm, Silicycle, Quebec, Canada), and the spots were visualized under UV light (254 nm) or stained by charring with acidified anisaldehyde solution in ethanol. Organic solvents were evaporated under reduced pressure and the products were purified by column chromatography on silica gel (230–400 mesh, Silicycle, Quebec, Canada) or SepPak C18 reverse phase cartridges (Waters). Before use, the cartridges were prewashed with 10 mL of MeOH followed by 20 mL of H2O. Optical rotations were measured on Perkin Elmer 241 polarimeter at ambient temperature in units of degree·mL/(g·dm). 1H NMR spectra were recorded at 400, 500 or 600 MHz and chemical shifts were referenced to CDCl3 (7.26 ppm), CD3OD (3.31 ppm), or D2O (4.79 ppm). 13C NMR spectra were recorded at 100 or 125 MHz and chemical shifts were referenced to CDCl3 (77.1 ppm) or CD3OD (49.0 ppm). 31P NMR spectra were recorded at 162 or 202 MHz and chemical shifts were referenced to external H3PO4 (0.0 ppm). Assignments of NMR spectra were based on two-dimensional experiments (1H,1H COSY or HSQC). The stereochemistry of the anomeric centres of the pyranose rings was confirmed by measuring the 1J_C-1,H-1 coupling constant of coupled HSQC spectra [1]. Electrospray mass spectra were recorded on an Agilent Technologies 6220 TOF.
2-O-Methyl-α-D-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (9)

The protected phosphate 18 (118 mg, 0.2 mmol) was dissolved in a mixture of toluene–Et₃N–pyridine (15:2:3, 2 mL). To this solution was added 20% Pd(OH)₂–C (11 mg) and the reaction mixture was stirred for 2 days under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated to afford a pale yellow syrup. Without further purification, this crude compound was dissolved in MeOH–H₂O–Et₃N (5:2:1, 4 mL) and the mixture was stirred for 2 days. The organic impurities were removed using a SepPak C₁₈ cartridge eluting with H₂O. The H₂O fraction was then lyophilized to afford 9 (80.2 mg, 92%) as a white solid. [α]D = +24.8 (c 0.3, H₂O); ¹H NMR (600 MHz, D₂O) δ 5.53 (dd, J = 8.1, 1.7 Hz, 1H, H-1), 3.93 (dd, J = 9.9, 3.5 Hz, 1H, H-3), 3.86 (dd, J = 12.2, 2.2 Hz, 1H, H-6a), 3.80 (ddd, J = 9.9, 6.0, 2.0 Hz, 1H, H-5), 3.71 (dd, J = 12.2, 6.0 Hz, 1H, H-6b), 3.61 (dd, J = 3.5, 1.9 Hz, 1H, H-2), 3.56 (app t, J = 10.0 Hz, 1H, H-4), 3.48 (s, 3H, OCH₃), 3.19 (q, J = 7.3 Hz, 9H, 1.5 × N(CH₂CH₃)₃), 1.26 (t, J = 7.3 Hz, 13.5H, 1.5 × N(CH₂CH₃)₃). ¹³C NMR (126 MHz, D₂O) δ 93.4 (d, ²J_C,P = 5.2 Hz, 1C, C-1, ¹J_C,H = 177.9 Hz), 81.6 (d, ³J_C,P = 7.9 Hz, 1C, C-2), 74.3 (C-5), 70.7 (C-3), 67.9 (C-4), 61.9 (C-6), 59.8 (OCH₃), 47.7 (N(CH₂CH₃)₃), 9.2 (N(CH₂CH₃)₃). ³¹P NMR (162 MHz, CD₃OD) δ 0.60. HRMS (ESI) m/z Calcd for C₇H₁₄O₉P [M − H]⁻: 273.0381. Found: 273.0381.
3-O-Methyl-\(\alpha\)-d-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (10)

The protected phosphate 23 (33 mg, 0.06 mmol) was dissolved in a mixture of toluene–Et\(_3\)N–pyridine (15:2:3, 2.8 mL). To this solution was added 20\% Pd(OH)\(_2\)–C (4 mg). The reaction mixture was stirred for 4 days under an atmosphere of hydrogen. The catalyst was then removed by filtration through Celite and the filtrate was concentrated to afford a pale yellow syrup. The syrup was dissolved in MeOH–H\(_2\)O–Et\(_3\)N (5:2:1, 4 mL) and the mixture was stirred for 3 days. Any organic impurities were removed using a SepPak C\(_{18}\) cartridge eluting with H\(_2\)O and the filtrate was lyophilized to afford 10 (16 mg, 67\%) as a white solid. \([\alpha]_D = +16.5\) (c 0.1, H\(_2\)O); \(^1\)H NMR (500 MHz, D\(_2\)O) \(\delta\) 5.42 (d, \(J = 7.8\) Hz, 1H, H-1), 4.21 (br. s, 1H, H-2), 3.87-3.82 (m, 2H, H-5, H-6a), 3.74 (dd, \(J = 11.8, 5.0\) Hz, 1H, H-6b), 3.69 (app t, \(J = 9.8\) Hz, 1H, H-4), 3.59 (dd, \(J = 9.6, 2.0\) Hz, 1H, H-3), 3.44 (s, 3H, OCH\(_3\)), 3.18 (q, \(J = 7.3\) Hz, 6H, N(CH\(_2\)CH\(_3\))\(_3\)), 1.26 (t, \(J = 7.2\) Hz, 9H, N(CH\(_2\)CH\(_3\))\(_3\)). \(^{13}\)C NMR (126 MHz, D\(_2\)O) \(\delta\) 96.8 (d, \(^2J_{C,P} = 5.2\) Hz, 1C, C-1), \(^1J_{C,H} = 178.5\) Hz), 80.27 (C-3), 74.4 (C-5), 67.3 (d, \(^3J_{C,P} = 8.2\) Hz, 1C, C-2), 66.5 (C-4), 61.8 (C-6), 57.2 (OCH\(_3\)), 47.7 (N(CH\(_2\)CH\(_3\))\(_3\)), 9.2 (N(CH\(_2\)CH\(_3\))\(_3\)). \(^{31}\)P NMR (162 MHz, D\(_2\)O) \(\delta\) −1.82. HRMS (ESI) \(m/z\) Calcd for C\(_7\)H\(_{14}\)O\(_9\)P [M − H\(^-\)]: 273.0381. Found: 273.0371.
**4-O-Methyl-α-D-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (11)**

The protected phosphate 30 (220 mg, 0.38 mmol) was dissolved in a mixture of toluene–Et$_3$N–pyridine (15:2:3, 4 mL). To the solution was added Pd(OH)$_2$–C (40 mg). The reaction mixture was stirred for 3 days under a hydrogen atmosphere. Then the catalyst was removed by filtration through Celite and the filtrate was concentrated to afford a pale yellow syrup. Without purification the crude compound was dissolved in MeOH–H$_2$O–Et$_3$N (5:2:1, 8 mL) and the mixture was stirred for 2 days. Organic impurities were removed using a SepPak C$_{18}$ cartridge eluting with H$_2$O. The H$_2$O fraction was lyophilized to yield 11 (101 mg, 70%) as a white solid. 

$\left[\alpha\right]_D = +28.8$ (c 0.3, H$_2$O); $^1$H NMR (500 MHz, D$_2$O) $\delta$ 5.33 (d, $J = 8.1$ Hz, 1H, H-1), 3.97 (dd, $J = 9.6, 3.2$ Hz, 1H, H-3), 3.94 (br. s, 1H, H-2), 3.85 (d, $J = 12.0$ Hz, 1H, H-6a), 3.83–3.78 (m, 1H, H-5), 3.73 (dd, $J = 12.0, 5.2$ Hz, 1H, H-6b), 3.53 (s, 3H, OCH$_3$), 3.42 (app t, $J = 9.8$ Hz, 1H, H-4), 3.18 (q, $J = 7.3$ Hz, 4.7H, 0.78 × N(CH$_2$CH$_3$)$_3$), 1.26 (t, $J = 7.3$ Hz, 7H, 0.78 × N(CH$_2$CH$_3$)$_3$).

$^{13}$C NMR (126 MHz, D$_2$O) $\delta$ 96.5 (d, $^2J_{C,P} = 5.2$ Hz, 1C, C-1), 77.7 (C-4), 73.2 (C-5), 72.0 (d, $^3J_{C,P} = 8.0$ Hz, 1C, C-2), 70.8 (C-3), 61.7 (C-6), 61.1 (OCH$_3$), 47.7 (N(CH$_2$CH$_3$)$_3$), 9.2 (N(CH$_2$CH$_3$)$_3$). $^{31}$P NMR (162 MHz, D$_2$O) $\delta$ −0.29. HRMS (ESI) m/z Calcd for C$_{7}$H$_{14}$O$_{9}$P $[M - H]^-$: 273.0381. Found: 273.0377.
6-O-Methyl-α-D-mannopyranosyl phosphate disodium salt (12)

The protected α-D-mannopyranosyl phosphate 36 (181 mg, 0.25 mmol) was dissolved in MeOH (7.5 mL). A 1 M NaHCO₃ solution (0.75 mL) and 20% Pd(OH)₂–C (80 mg) were added to the mixture. The mixture was stirred for 6 days under a hydrogen atmosphere. Then the catalyst was removed by filtration through Celite and the filtrate was concentrated. Organic impurities were removed using a SepPak C₁₈ cartridge eluting with H₂O. The filtrate was lyophilized to afford 12 (72 mg, 91%) as a white amorphous solid [α]₅₀ = +30.3 (c 1.1, H₂O); ¹H NMR (400 MHz, D₂O) δ 5.30 (dd, J = 8.6, 1.1 Hz, 1H, H-1), 3.99–3.91 (m, 3H, H-2, H-3, H-5), 3.75 (dd, J = 10.9, 2.2 Hz, 1H, H-6a), 3.64 (dd, J = 10.9, 6.3 Hz, 1H, H-6b), 3.59 (dd, J = 10.0, 9.4 Hz, 1H, H-4), 3.40 (s, 3H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ 95.4 (d, ²J_C,P = 4.1 Hz, 1C, C-1, ¹J_C,H = 170.9 Hz), 72.1 (C-6), 71.9 (C-5), 71.5 (d, ³J_C,P = 6.4 Hz, 1C, C-2), 70.5 (C-3), 67.5 (C-4), 56.0 (OCH₃). ³¹P NMR (162 MHz, D₂O) δ 2.00. HRMS (ESI) m/z Calcd for C₇H₁₄O₉P [M – H]⁻: 273.0381. Found: 273.0380.
6-Deoxy-α-D-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (13)

The protected phosphate 45 (59 mg, 0.08 mmol) was dissolved in a mixture of toluene–Et₃N–pyridine (15:2:3, 2.8 mL). To the solution was added 20% Pd(OH)₂–C (12 mg). The reaction mixture was stirred for 2 days under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated to afford pale yellow syrup. Without further purification, the crude compound was dissolved in MeOH–H₂O–Et₃N (5:2:1, 8 mL) and the mixture was stirred for 6 days. The organic impurities were removed using a SepPak C₁₈ cartridge eluting with H₂O. The H₂O fraction was lyophilized to afford 13 (18 mg, 72%) as a white solid [α]D = +18.1 (c 0.2, H₂O); ¹H NMR (500 MHz, D₂O) δ 5.38 (d, J = 7.8 Hz, 1H, H-1), 4.04 (br. s, 1H, H-2), 4.01–3.92 (m, 2H, H-5, H-3), 3.50 (app t, J = 9.8 Hz, 1H, H-4), 3.27 (q, J = 7.3 Hz, 2H, 0.33 × N(CH₂CH₃)₃), 1.38–1.32 (m, 6H, H-6, 0.33 × N(CH₂CH₃)₃). ¹³C NMR (126 MHz, D₂O) δ 96.4 (d, ²J_C,P = 5.4 Hz, 1C, C-1), 73.2 (C-4), 71.8 (d, ³J_C,P = 8.3 Hz, 1C, C-2), 70.7 (C-3), 70.1 (C-5), 47.7 (N(CH₂CH₃)₃), 17.8 (C-6), 9.2 (N(CH₂CH₃)₃). ³¹P NMR (162 MHz, D₂O) δ −0.71. HRMS (ESI) m/z Calcd for C₆H₁₂O₈P [M – H]⁻: 243.0275. Found: 243.0275.
Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-methyl-α-D-mannopyranoside (15)

Methyl 3-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside 14 [2] (1.43 g, 3.8 mmol) was dissolved in DMF (15 mL) and the solution was cooled to 0 °C. After NaH (60% NaH in mineral oil, 274 mg, 6.9 mmol) was added, the mixture was stirred for 10 min before the addition of CH₃I (0.36 mL, 5.76 mmol). The reaction mixture was stirred overnight and then quenched by the addition of MeOH (1 mL). The solution was diluted with CH₂Cl₂, washed with distilled water and brine, and the organic layer was dried over Na₂SO₄. Evaporation of the solvent and chromatography (hexane–EtOAc 5:1) of the resulting residue gave 15 (1.17 g, 80%) as a colorless syrup. [α]D = +55.6 (c 0.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.60–7.26 (m, 10H, ArH), 5.67 (s, 1H, O₂C₇H₆), 4.92 (d, J = 12.3 Hz, 1H, CH₂Ph), 4.80 (br. s, 1H, H-1), 4.77 (d, J = 12.3 Hz, 1H, CH₂Ph), 4.31 (dd, J = 10.0, 4.3 Hz, 1H, H-6a), 4.22 (app t, J = 9.5 Hz, 1H, H-4), 4.00 (dd, J = 9.9, 2.7 Hz, 1H, H-3), 3.92 (app t, J = 10.5 Hz, 1H, H-6b), 3.85–3.81 (m, 1H, H-5), 3.65–3.64 (m, 1H, H-2), 3.61 (s, 1H, OCH₃), 3.39 (s, 1H, OCH₃). ¹³C NMR (101 MHz, CDCl₃) δ 138.7 (Ar), 137.7 (Ar), 128.8 (Ar), 128.3 (Ar), 128.2 (Ar), 127.6 (Ar), 127.5 (Ar), 126.1 (Ar), 101.5 (O₂C₇H₆), 100.1 (C-1), 79.5 (C-2), 79.2 (C-4), 76.2 (C-3), 73.1 (CH₂Ph), 68.9 (C-6), 64.2 (C-5), 60.1 (OCH₃), 54.9 (OCH₃). HRMS (ESI) m/z Calcd for C₂₂H₂₆O₆Na [M + Na]⁺: 409.1622. Found: 409.1616.
1,3,4,6-Tetra-O-acetyl-2-O-methyl-α-D-mannopyranose (16)

Monosaccharide 15 (1.17 g, 3 mmol) was dissolved in Ac₂O–HOAc–H₂SO₄ (35:15:1 v/v/v, 18.36 mL). After being stirred overnight the mixture was diluted with CH₂Cl₂, washed with distilled water, NaHCO₃ (satd aq soln) and brine. The organic layer was dried over Na₂SO₄ and concentrated, and the crude residue purified by chromatography (hexane–EtOAc 3:2) to afford 16 (894 mg, 81%) as a pale yellow syrup. [α]D = +56.2 (c 0.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 6.04 (d, J = 1.9 Hz, 1H, H-1), 5.23 (app t, J = 10.1 Hz, 1H, H-4), 5.08 (dd, J = 10.1, 3.3 Hz, 1H, H-3), 4.12 (dd, J = 12.4, 4.7 Hz, 1H, H-6a), 3.92 (dd, J = 12.4, 2.3 Hz, 1H, H-6b), 3.87 (ddd, J = 10.0, 4.6, 2.3 Hz, 1H, H-5), 3.50 (dd, J = 3.2, 2.1 Hz, 1H, H-2), 3.36 (s, 3H, OCH₃), 2.01 (s, 3H, C(O)CH₃), 1.95 (s, 3H, C(O)CH₃), 1.93 (s, 3H, C(O)CH₃), 1.90 (s, 3H, C(O)CH₃). ¹³C NMR (101 MHz, CDCl₃) δ 90.5 (C-1), 76.8 (C-2), 70.7 (C-3, C-5), 65.6 (C-4), 62.0 (C-6), 59.3 (OCH₃), 20.7 (C(O)CH₃), 20.6 (C(O)CH₃), 20.5 (C(O)CH₃), 20.4 (C(O)CH₃). HRMS (ESI) m/z Calcd for C₁₅H₂₂O₁₀Na [M + Na]⁺: 385.1105. Found: 385.1099.

Ethyl 3,4,6-tri-O-acetyl-2-O-methyl-1-thio-α-D-mannopyranoside (17)

Tetraacetate 16 (888 mg, 2.45 mmol) and ethanethiol (272 µL, 3.68 mmol) were dissolved in CH₂Cl₂ (12 mL) and cooled to 0 °C before BF₃·OEt₂ (615 µL, 4.9 mmol) was added. The reaction mixture was stirred for 18 h and then diluted with CH₂Cl₂, and washed with NaHCO₃ (satd aq soln), distilled water and brine. The organic layer was dried over Na₂SO₄ and concentrated, and the crude residue was purified by chromatography (hexane–EtOAc 3:1 → 2:1)
to give 17 (583 mg, 65%) a pale yellow syrup. $[\alpha]_D = +85.7 \, (c \, 0.5, \text{CHCl}_3)$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.34 (s, 1H, H-1), 5.29–5.18 (m, 1H, H-4), 5.07 (dd, $J = 9.8, 3.1$ Hz, 1H, H-3), 4.29–4.14 (m, 2H, H-5, H-6a), 3.98 (d, $J = 10.3$ Hz, 1H, H-6b), 3.65 (d, $J = 1.2$ Hz, 1H, H-2), 3.37 (s, 3H, OCH$_3$), 2.69–2.45 (m, 2H, SCH$_2$CH$_3$), 1.97 (d, $J = 13.8$ Hz, 9H, C(O)CH$_3$ x 3), 1.24 (t, $J = 7.4$ Hz, 3H, SCH$_2$CH$_3$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 81.3 (C-1), 79.3 (C-2), 71.6 (C-3), 68.8 (C-5), 66.5 (C-4), 62.4 (C-6), 58.6 (OCH$_3$), 25.3 (SCH$_2$CH$_3$), 20.7 (C(O)CH$_3$), 20.6 (C(O)CH$_3$), 20.6 (C(O)CH$_3$), 14.8 (SCH$_2$CH$_3$). HRMS (ESI) $m/z$ Calcd for C$_{15}$H$_{24}$O$_8$SNa [M + Na]$^+$: 387.1084. Found: 387.1081.

Dibenzyl 3,4,6-tri-O-acetyl-2-O-methyl-α-D-mannopyranosyl phosphate (18)

Thioglycoside 17 (547 mg, 1.5 mmol), dibenzyl phosphate (709 mg, 2.55 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH$_2$Cl$_2$ (14 mL) and stirred for 40 min. N–iodosuccinimide (507 mg, 2.25 mmol) and silver trifluoromethanesulfonate (116 mg, 0.45 mmol) were then added at $-30 \, ^{\circ}$C. The reaction mixture was stirred for 40 min from $-30 \, ^{\circ}$C to $-20 \, ^{\circ}$C, and then filtered through Celite. The filtrate was diluted with CH$_2$Cl$_2$, and washed with Na$_2$S$_2$O$_3$ (satd aq soln), NaHCO$_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na$_2$SO$_4$ then concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to yield 18 (729 mg, 84%) as a colorless syrup. $[\alpha]_D = +38.4 \, (c \, 0.4, \text{CHCl}_3)$; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.37–7.31 (m, 10H, ArH), 5.68 (dd, $J = 6.4, 2.0$ Hz, 1H, H-1), 5.31 (app t, $J = 10.2$ Hz, 1H, H-4), 5.18 (dd, $J = 10.1, 3.2$ Hz, 1H, H-3), 5.13–5.03 (m, 4H, CH$_2$Ph), 4.16 (dd, $J = 12.4, 4.6$ Hz, 1H, H-6a), 3.96 (ddd, $J = 10.1, 4.6, 2.1$ Hz, 1H, H-5), 3.90 (dd, $J = $
12.4, 2.3 Hz, 1H, H-6b), 3.50 (dd, J = 3.1, 2.2 Hz, 1H, H-2), 3.37 (s, 3H, OCH₃), 2.06 (s, 3H, C(O)CH₃), 2.00 (s, 3H, C(O)CH₃), 1.96 (s, 3H, C(O)CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 170.6 (C=O), 170.0 (C=O), 169.4 (C=O), 135.4(1) (d, 3J_{C,P} = 6.6 Hz, 1C, Ar), 135.3(5) (d, 3J_{C,P} = 6.6 Hz, 1C, Ar), 128.8 (Ar), 128.6(9) (Ar), 128.6(8) (Ar), 128.2 (Ar), 128.0 (Ar), 95.2 (d, 2J_{C,P} = 6.0 Hz, 1C, C-1, ¹J_{C,H} = 177.7 Hz), 77.5 (d, 3J_{C,P} = 9.5 Hz, 1C, C-2), 70.4(0) (C-5), 70.3(7) (C-3), 69.8 (d, 2J_{C,P} = 5.3 Hz, 1C, CH₂Ph), 69.7 (d, 2J_{C,P} = 5.3 Hz, 1C, CH₂Ph), 65.5 (C-4), 61.9 (C-6), 59.7 (OCH₃), 20.8 (C(O)CH₃), 20.6 (C(O)CH₃). ³¹P NMR (202 MHz, CDCl₃) δ −1.80. HRMS (ESI) m/z Calcd for C₂₇H₃₃O₁₂PNa [M + Na]⁺: 603.16019. Found: 603.16003.

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-methyl-α-D-mannopyranoside (20)

Methyl 2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside [2] 19 (1.29 g, 3.48 mmol) was dissolved in DMF (15 mL) and the solution was cooled to 0 °C. After the addition of NaH (60% NaH in mineral oil, 250 mg, 6.26 mmol), the mixture was stirred for 10 min before the addition of CH₃I (0.33 mL, 5.29 mmol). The reaction mixture was stirred overnight and quenched by the addition of MeOH (1 mL). The solution was then diluted with CH₂Cl₂, washed with distilled water and brine, and the organic layer was dried over Na₂SO₄. Evaporation of the solvent and chromatography (hexane–EtOAc 8:1) of the residue afforded 20 (1.025 g, 76%) as a colorless syrup. [α]D = +30.9 (c 2.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.54–7.26 (m, 10H, ArH), 5.64 (s, 1H, O₂CHPh), 4.84 (d, J = 12.2 Hz, 1H, CH₂Ph), 4.75–4.72 (m, 2H, H-1, CH₂Ph), 4.28 (dd, J = 10.1, 4.7 Hz, 1H, H-6a), 4.18 (app t, J = 9.6 Hz, 1H, H-4), 3.93–3.86 (m, 2H, H-6b, H-2), 3.83–3.80 (m, 1H, H-5), 3.74 (dd, J = 10.0, 3.2 Hz, 1H, H-3), 3.50 (s, 1H, OCH₃), 3.37 (s, 1H, OCH₃). ¹³C NMR (125 MHz, CDCl₃) δ 138.1 (Ar), 137.7 (Ar), 128.9 (Ar), 128.4 (Ar), 128.2
(Ar), 128.0 (Ar), 127.8 (Ar), 126.2 (Ar), 101.7 (O<sub>2</sub>CHPh), 100.3 (C-1), 79.0 (C-4), 78.1 (C-3), 75.6 (C-2), 73.6 (CH<sub>2</sub>Ph), 68.9 (C-6), 64.0 (C-5), 58.8 (OCH<sub>3</sub>), 54.9 (OCH<sub>3</sub>). HRMS (ESI) m/z Calcd for C<sub>22</sub>H<sub>26</sub>O<sub>6</sub>Na [M + Na]<sup>+</sup>: 409.1622. Found: 409.1613.

1,2,4,6-Tetra-<i>O</i>-acetyl-3-<i>O</i>-methyl-d-mannopyranose (21)

Monosaccharide 20 (1 g, 2.59 mmol) was dissolved in Ac<sub>2</sub>O–HOAc–H<sub>2</sub>SO<sub>4</sub> (35:15:1 v/v/v, 13.26 mL). After being stirred for 2 days, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with distilled water, NaHCO<sub>3</sub> (satd aq soln) and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated, and the crude residue was purified by chromatography (hexane–EtOAc 3:2 → 1:1) to afford 21 (610 mg, 65%) as a pale yellow syrup. [α]<sub>D</sub> = +16.7 (c 1.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.06 (d, J = 2.0 Hz, 1H, H-1), 5.30 (dd, J = 3.3, 2.1 Hz, 1H, H-2), 5.20 (app t, J = 10.0 Hz, 1H, H-4), 4.21 (dd, J = 12.4, 5.2 Hz, 1H, H-6a), 4.12–4.06 (m, 1H, H-6b), 3.93 (ddd, J = 10.1, 5.2, 2.4 Hz, 1H, H-5), 3.63 (dd, J = 9.8, 3.4 Hz, 1H, H-3), 3.34 (s, 3H, OCH<sub>3</sub>), 2.13 (s, 3H, C(O)CH<sub>3</sub>), 2.12 (s, 3H, C(O)CH<sub>3</sub>), 2.06 (s, 3H, C(O)CH<sub>3</sub>), 2.05 (s, 3H, C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 170.7 (C=O), 170.0 (C=O), 169.7 (C=O), 168.1 (C=O), 91.0 (C-1), <sup>1</sup>J<sub>C,H</sub> = 177.9 Hz), 76.8 (C-3), 70.8 (C-5), 67.0 (C-4), 66.6 (C-2), 62.4 (C-6), 57.9 (OCH<sub>3</sub>), 20.8(8) (C(O)CH<sub>3</sub>), 20.8(6) (C(O)CH<sub>3</sub>), 20.8(3) (C(O)CH<sub>3</sub>), 20.7(5) (C(O)CH<sub>3</sub>). HRMS (ESI) m/z Calcd for C<sub>15</sub>H<sub>22</sub>O<sub>10</sub>Na [M + Na]<sup>+</sup>: 385.1105. Found: 385.1104.
Ethyl 2,4,6-tri-O-acetyl-3-O-methyl-1-thio-α-D-mannopyranoside (22)

Tetraacetate 21 (592 mg, 1.63 mmol) and ethanethiol (182 µL, 2.45 mmol) were dissolved in dry CH₂Cl₂ (8 mL) and cooled to 0 °C before the addition of BF₃·OEt₂ (411 µL, 3.27 mmol). The reaction mixture was stirred overnight and then was diluted with CH₂Cl₂ and washed with NaHCO₃ (satd aq soln), distilled water and brine. The organic layer was dried over Na₂SO₄ and concentrated, and the crude residue was purified by chromatography (hexane–EtOAc 3:1 → 2:1) to afford 22 (307 mg, 52%) as a pale yellow syrup. [α]D = +71.2 (c 1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.40 (dd, J = 3.3, 1.8 Hz, 1H, H-2), 5.29 (d, J = 2.0 Hz, 1H, H-1), 5.18 (app t, J = 9.8 Hz, 1H, H-4), 4.30–4.25 (m, 2H, H-5, H-6a), 4.10–4.07 (m, 1H, H-6b), 3.56 (dd, J = 9.8, 3.2 Hz, 1H, H-3), 3.32 (s, 3H, OCH₃), 2.69–2.58 (m, 2H, SCH₂CH₃), 2.14 (s, 3H, C(O)CH₃), 2.08 (s, 3H, C(O)CH₃), 2.07 (s, 3H, C(O)CH₃), 1.30 (t, J = 7.5 Hz, 3H, SCH₂CH₃). ¹³C NMR (125 MHz, CDCl₃) δ 170.7 (C=O), 170.3 (C=O), 169.8 (C=O), 82.6 (C-1), 77.6 (C-3), 69.5 (C-2), 69.1 (C-5), 67.8 (C-4), 62.7 (C-6), 57.8 (OCH₃), 25.7 (SCH₂CH₃), 21.0 (C(O)CH₃), 20.9 (C(O)CH₃), 20.8 (C(O)CH₃), 14.9 (SCH₂CH₃). HRMS (ESI) m/z Calcd for C₁₅H₂₄O₈SNa [M + Na]⁺: 387.1084. Found: 387.1084.

Dibenzyl 2,4,6-tri-O-acetyl-3-O-methyl-α-D-mannopyranosyl phosphate (23)

Thioglycoside 22 (292 mg, 0.8 mmol), dibenzyl phosphate (378 mg, 1.36 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH₂Cl₂ (8 mL) and stirred at room temperature for
30 min. N-iodosuccinimide (270 mg, 1.2 mmol) and silver trifluoromethanesulfonate (62 mg, 0.24 mmol) were then added at −30 °C. The reaction mixture was stirred for 2 h between −30 °C to −20 °C, and then was filtered through Celite and diluted with CH₂Cl₂. The resulting solution was washed with Na₂S₂O₃ (satd aq soln), NaHCO₃ (satd aq soln), distilled water and brine. The organic layer was dried over Na₂SO₄ and concentrated, and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to give 23 (346 mg, 75%) as a colorless syrup. [α]D = +21.0 (c 1.1, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.35–7.26 (m, 10H, ArH), 5.59 (dd, J = 6.5, 2.0 Hz, 1H, H-1), 5.21 (dd, J = 3.3, 2.3 Hz, 1H, H-2), 5.13 (app t, J = 9.9 Hz, 1H, H-4), 5.11–5.02 (m, 4H, CH₂Ph), 4.13 (dd, J = 12.5, 5.0 Hz, 1H, H-6a), 3.97–3.94 (m, 1H, H-5), 3.92 (dd, J = 12.5, 2.5 Hz, 1H, H-6b), 3.50 (dd, J = 10.0, 3.5 Hz, 1H, H-3), 3.24 (s, 3H, OCH₃), 2.08 (s, 3H, C(O)C₃H₃), 2.03 (s, 3H, C(O)CH₃), 1.94 (s, 3H, C(O)CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 170.6 (C=O), 169.7 (C=O), 169.6 (C=O), 135.4 (Ar), 135.3 (Ar), 128.8 (Ar), 128.8 (Ar), 128.7 (Ar), 128.2 (Ar), 128.1 (Ar), 95.6 (d, ²JCP = 5.7 Hz, 1C, C-1, ¹JC,H = 183.1 Hz), 76.1 (C-3), 70.5 (C-5), 69.9 (d, ²JC,P = 5.1 Hz, 1C, CH₂Ph), 69.8 (d, ²JC,P = 5.5 Hz, 1C, CH₂Ph), 67.0 (d, ³JC,P = 11.2 Hz, 1C, C-2), 66.6 (C-4), 62.1 (C-6), 57.9 (OCH₃), 20.8 (1C(O)CH₃), 20.8 (C(O)CH₃), 20.6 (C(O)CH₃). ³¹P NMR (202 MHz, CDCl₃) δ −1.91. HRMS (ESI) m/z Calcd for C₂₇H₃₃O₁₂PNa [M + Na]⁺: 603.1602. Found: 603.1595.

**Methyl 6-O-trityl-α-D-mannopyranoside (25)**

To a solution of methyl α-D-mannopyranoside 24 (3.9 g, 0.02 mol) in dry pyridine (100 mL) was added trityl chloride (6.7 g, 0.024 mol) and DMAP (0.47 g, 4 mmol). The solution was stirred at 40 °C overnight and then cooled. Evaporation of the solvent, followed by chromatography of the
residue (hexane–EtOAc 1:1 → 1:3) afforded 25 (7.4 g, 85%) as a colorless syrup $[\alpha]_D = +21.9 (c 0.6, CHCl_3)$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.71–7.08 (m, 15H, ArH), 4.72 (d, $J = 1.3$ Hz, 1H, H-1), 3.96–3.87 (m, 1H, H-2), 3.79 (dd, $J = 8.9, 3.4$ Hz, 1H, H-3), 3.73 (app t, $J = 9.3$ Hz, 1H, H-4), 3.69–3.62 (m, 1H, H-5), 3.46 (d, $J = 4.8$ Hz, 1H, H-6a), 3.42 (dd, $J = 9.8, 5.3$ Hz, 1H, H-6b), 3.38 (s, 3H, OCH$_3$). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 143.6 (Ar), 128.6 (Ar), 128.0 (Ar), 127.2 (Ar), 100.6 (C-1), 87.4 (OC(Ph)$_3$), 71.6 (C-3), 70.4 70.3, 69.8 (C-2, C-4, C-5), 64.9 (C-6), 55.0 (OCH$_3$). HRMS (ESI) $m/z$ Calcd for C$_{26}$H$_{28}$O$_6$Na [M + Na]$^+$: 459.1778. Found: 459.1782.

Methyl 2,3-O-isopropylidene-6-O-trityl-$\alpha$-D-mannopyranoside (26)

Monosaccharide 25 (4.2 g, 9.6 mmol) was dissolved in 2,2-dimethoxypropane (20 mL). To this solution was added $p$-TsOH (183 mg, 0.96 mmol) and the reaction mixture was stirred overnight. Then, the solution was quenched by the addition of NaHCO$_3$ (satd aq soln). The solvent was evaporated and the residue was diluted with CH$_2$Cl$_2$, washed with distilled water and brine. The organic layer was dried over Na$_2$SO$_4$, concentrated and the residue was purified by chromatography (hexane–EtOAc 4:1) to give 26 (3.47 g, 76%) as a colorless oil. $[\alpha]_D = −1.3 (c 1.3, CHCl$_3$)$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.49–7.22 (m, 15H, ArH), 4.92 (s, 1H, H-1), 4.16–4.08 (m, 2H, H-2, H-3), 3.76–3.63 (m, 1H, H-4), 3.71–3.67 (m, 1H, H-5), 3.45–3.41 (m, 4H, H-6a, OCH$_3$), 3.38 (dd, $J = 10.0, 5.2$ Hz, 1H, H-6b), 2.45 (d, $J = 3.8$ Hz, 1H, OH), 1.50 (s, 3H, CH$_3$), 1.35 (s, 3H, CH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 143.7 (Ar), 128.7 (Ar), 127.9 (Ar), 127.2 (Ar), 109.5 (CO$_2$(CH$_3$)$_2$), 98.3 (C-1), 87.1 (OC(Ph)$_3$), 78.0 (C-3), 75.3 (C-2), 70.7 (C-4),
68.8 (C-5), 64.3 (C-6), 55.0 (OCH₃), 27.9 (CH₃), 26.1 (CH₃). HRMS (ESI) m/z Calcd for C₉H₃₂O₆Na [M + Na]⁺: 499.2091. Found: 499.2095.

Methyl 2,3-O-isopropylidene-4-O-methyl-6-O-trityl-α-D-mannopyranoside (27)

Monosaccharide 26 (272 mg, 0.57 mmol) was dissolved in DMF (6 mL) and the solution was cooled to 0 °C. After the addition of NaH (60% NaH in mineral oil, 41 mg, 1.03 mmol), the mixture was stirred for 10 min before the addition of CH₃I (53 µL, 0.86 mmol). The reaction mixture was stirred overnight and quenched by the addition of MeOH (1 mL). The solution was then diluted with CH₂Cl₂ and washed with distilled water and brine. The organic layer was dried over Na₂SO₄. Evaporation of the solvent and chromatography (hexane–EtOAc 10:1) of the residue afforded 27 (253 mg, 91%) as a colorless syrup. ¹H NMR (500 MHz, CDCl₃) δ 7.54–7.20 (m, 15H, ArH), 4.99 (s, 1H, H-1), 4.19–4.10 (m, 2H, H-3, H-2), 3.67-3.63 (m, 1H, H-5), 3.47 (s, 3H, OCH₃), 3.41 (dd, J = 9.9, 2.0 Hz, 1H, H-6a), 3.38 (dd, J = 10.2, 6.5 Hz, 1H, H-4), 3.32 (s, 1H, OCH₃), 3.20 (dd, J = 9.9, 5.4 Hz, 1H, H-6b), 1.58 (s, 1H, CH₃), 1.37 (s, 1H, CH₃). The ¹H spectral data was consistent with those previously reported [3]. HRMS (ESI) m/z Calcd for C₃₀H₃₄O₆Na [M + Na]⁺: 512.2248. Found: 513.2254.

1,2,3,6-Tetra-O-acetyl-4-O-methyl-α-D-mannopyranose (28)

Monosaccharide 27 (247 mg, 0.5 mmol) was dissolved in Ac₂O–HOAc–H₂SO₄ (35:15:1 v/v/v, 3.06 mL). After being stirred overnight, the mixture was diluted with CH₂Cl₂ and washed with
distilled water, NaHCO₃ (satd aq soln) and brine. After drying over Na₂SO₄, the organic layer was concentrated and the crude residue purified by chromatography (hexane–EtOAc 2:1 → 1:1) to afford 28 (96 mg, 55%) as a pale yellow syrup. [α]D = +42.5 (c 0.6, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 6.03 (d, J = 1.8 Hz, 1H, H-1), 5.24 (dd, J = 9.7, 3.5 Hz, 1H, H-3), 5.22 (dd, J = 3.5, 2.0 Hz, 1H, H-2), 4.36–4.28 (m, 2H, H-6a, H-6b), 3.91-3.87 (m, 1H, H-5), 3.56 (app t, J = 9.7 Hz, 1H, H-4), 3.47 (s, 3H, OCH₃), 2.16 (s, 3H, C(O)CH₃), 2.15 (s, 3H, C(O)CH₃), 2.12 (s, 3H, C(O)CH₃), 2.08 (s, 3H, C(O)CH₃). ¹³C NMR (125 MHz, CDCl₃) δ 171.7 (C=O), 169.8 (C=O), 169.7 (C=O), 168.3 (C=O), 90.7 (C-1, ¹J_C,H = 177.6 Hz), 74.3 (C-4), 71.8 (C-5), 71.3 (C-3), 68.7 (C-2), 62.9 (C-6), 60.7 (OCH₃), 20.9 (C(O)CH₃ × 2), 20.8 (C(O)CH₃), 20.8 (C(O)CH₃). HRMS (ESI) m/z Calcd for C₁₅H₂₁O₁₀Na [M + Na]⁺: 385.1105. Found: 385.1104.

**Ethyl 2,3,6-tri-O-acetyl-4-O-methyl-1-thio-α-D-mannopyranoside (29)**

Tetraacetate 28 (259 mg, 0.72 mmol) and ethanethiol (80 µL, 1.08 mmol) were dissolved in CH₂Cl₂ (4 mL) and cooled to 0 °C before the addition of BF₃·OEt₂ (180 µL, 1.44 mmol). The reaction mixture was stirred overnight and then was diluted with CH₂Cl₂, and washed with NaHCO₃ (satd aq soln), distilled water and brine. The organic layer was dried over Na₂SO₄, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1 → 1:1) to afford 29 (183 mg, 70%) as a pale yellow syrup. [α]D = +75.0 (c 0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.32 (dd, J = 3.4, 1.6 Hz, 1H, H-2), 5.23 (d, J = 1.5 Hz, 1H, H-1), 5.19 (dd, J = 9.6, 3.4 Hz, 1H, H-3), 4.41–4.30 (m, 2H, H-6a, H-6b), 4.25 (ddd, J = 9.9, 5.1, 2.3 Hz, 1H, H-5), 3.54 (app t, J = 9.7 Hz, 1H, H-4), 3.45 (s, 3H, OCH₃), 2.77–2.53 (m, 2H, SCH₂CH₃), 2.15 (s, 3H, C(O)CH₃), 2.12 (s, 3H, C(O)CH₃), 2.06 (s, 3H, C(O)CH₃), 1.29 (t, J = 7.4 Hz, 3H, SCH₂CH₃).
Dibenzyl 2,3,6-tri-O-acetyl-4-O-methyl-α-D-mannopyranosyl phosphate (30)

Thioglycoside 29 (178 mg, 0.49 mmol), dibenzyl phosphate (231 mg, 0.83 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH$_2$Cl$_2$ and stirred for 30 min. Then, N–iodosuccinimide (165 mg, 0.74 mmol) and silver trifluoromethanesulfonate (38 mg, 0.15 mmol) were added at −40 °C. The reaction mixture was stirred for 3 h at −40 °C to −30 °C, and was then filtered through Celite and diluted with CH$_2$Cl$_2$. The solution was washed with Na$_2$S$_2$O$_3$ (satd aq soln), NaHCO$_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na$_2$SO$_4$, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to give 30 (226 mg, 80%) as a colorless oil. [α]$_D$ = +37.4 (c 0.6, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.40–7.35 (m, 10H, ArH), 5.63 (dd, $J = 6.6$, 1.6 Hz, 1H, H-1), 5.29–5.26 (m, 2H, H-2, H-3), 5.14–5.06 (m, 4H, CH$_2$Ph), 4.26 (dd, $J = 12.1$, 4.8 Hz, 1H, H-6a), 4.21 (dd, $J = 12.1$, 2.3 Hz, 1H, H-6b), 3.98 (ddd, $J = 9.6$, 4.6, 2.3 Hz, 1H, H-5), 3.58–3.51 (m, 1H, H-4), 3.47 (s, 3H, OCH$_3$), 2.16 (s, 3H, C(O)CH$_3$), 2.09 (s, 3H, C(O)CH$_3$), 2.03 (s, 3H, C(O)CH$_3$). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 170.6 (C=O), 169.6 (C=O), 169.5 (C=O), 135.4 (d, $^3$J$_{C,P}$ = 6.9 Hz, 1C, Ar), 135.3 (d, $^3$J$_{C,P}$ = 6.8 Hz, 1C, Ar), 128.7(3) (Ar), 128.7(1) (Ar), 128.6(8) (Ar), 128.2 (Ar), 128.0 (Ar), 95.3 (d, $^2$J$_{C,P}$ = 5.3 Hz, 1C, C-1, $^1$J$_{C,H}$ = 178.7 Hz), 74.0 (C-4), 71.4 (C-5), 70.7 (C-3), 71.6 (C-2), 70.0 (C-5), 63.2 (C-6), 60.4 (OCH$_3$), 25.4 (SCH$_2$CH$_3$), 21.0 (C(O)CH$_3$), 20.9 (C(O)CH$_3$), 20.8 (C(O)CH$_3$), 14.8 (SCH$_2$CH$_3$). HRMS (ESI) m/z Calcd for C$_{15}$H$_{24}$O$_8$SNa [M + Na]$^+$: 387.1084. Found: 387.1084.
69.9 (d, $^2J_{C,P} = 5.5$ Hz, 1C, CH$_2$Ph), 69.7 (d, $^2J_{C,P} = 5.5$ Hz, 1C, CH$_2$Ph), 69.2 (d, $^3J_{C,P} = 11.2$ Hz, 1C, C-2), 62.7 (C-6), 60.6 (OCH$_3$), 20.9 (C(O)CH$_3$), 20.8 (C(O)CH$_3$), 20.7 (C(O)CH$_3$). HRMS (ESI) $m$/z Calcd for C$_{27}$H$_{33}$O$_2$PNa [M + Na]$^+$: 603.1602. Found: 603.1603.

**Ethyl 6-O-t-butyldiphenylsilyl-1-thio-$\alpha$-D-mannopyranoside (32)**

Thioglycoside 31 (5 g, 12.7 mmol) was dissolved in methanol (100 mL) and 1.0 M NaOMe (2 mL) was added dropwise. After stirring overnight, the reaction mixture was neutralized with Amberlite IR120 H$^+$ ion exchange resin and then concentrated. *t*ert-Butylchlorodiphenylsilane (5.6 mL, 21.7 mmol) was added to a solution of the crude residue and imidazole (3.9 g, 57.3 mmol) in dry DMF (24 mL). The reaction mixture was stirred overnight and then quenched by the addition of MeOH (5 mL). The solvent was then evaporated and the resulting residue purified by chromatography (hexane–EtOAc 3:1 → 1:1) to provide 32 (4.6 g, 78%) as a colorless syrup. $[\alpha]_D = +88.0$ (c 1.1, CH$_3$OH); $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.77–7.67 (m, 4H, ArH), 7.47–7.33 (m, 6H, ArH), 5.30 (d, $J = 1.0$ Hz, 1H, H-1), 4.10–4.04 (m, 1H, H-5), 4.02 (dd, $J = 10.9$, 2.0 Hz, 1H, H-6a), 3.90 (dd, $J = 3.4$, 1.4 Hz, 1H, H-2), 3.84 (dd, $J = 10.9$, 7.0 Hz, 1H, H-6b), 3.68 (dd, $J = 9.4$, 3.4 Hz, 1H, H-3), 3.59 (app t, $J = 9.6$ Hz, 1H, H-4), 2.78–2.50 (m, 2H, SCH$_2$CH$_3$), 1.28 (t, $J = 7.4$ Hz, 3H, SCH$_2$CH$_3$), 1.03 (s, 9H, Si(Ph)$_2$C(CH$_3$)$_3 \times 3$). $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$ 136.8(4) (Ar), 136.8(0) (Ar), 134.9 (Ar), 134.8 (Ar), 130.7(9) (Ar), 130.7(5) (Ar), 128.7(3) (Ar), 128.7(0) (Ar), 85.4 (C-1), 75.4 (C-5), 73.5 (C-2), 73.4 (C-3), 69.2 (C-4), 65.3 (C-6), 27.3 (Si(Ph)$_2$C(CH$_3$)$_3$), 25.4 (SCH$_2$CH$_3$), 20.1 (Si(Ph)$_2$C(CH$_3$)$_3$), 15.2 (SCH$_2$CH$_3$). HRMS (ESI) $m$/z Calcd for C$_{24}$H$_{34}$O$_5$SSiNa [M + Na]$^+$: 485.1788. Found: 485.1780.
Ethyl 2,3,4-tri-\(O\)-benzyl-6-\(O\)-(\textit{tert}-butyldiphenylsilyl)-1-thio-\(\alpha\)-D-mannopyranoside (33)

Thioglycoside 32 (4.6 g, 9.9 mmol) was dissolved in dry THF (40 mL) and cooled to 0 °C. Then NaH (60% NaH in mineral oil, 2.3 g, 59.4 mmol) was added. The reaction mixture was stirred for 0.5 h before the addition of BnBr (5.3 mL, 44.6 mmol) and tetra-\(n\)-butylammonium iodide (0.37 g, 1 mmol). The reaction mixture was stirred overnight and then was quenched by the addition of MeOH. The solution was then diluted with CH\(_2\)Cl\(_2\), washed with distilled water, NaHCO\(_3\) (satd aq soln) and brine. The organic layer was dried over Na\(_2\)SO\(_4\), and concentrated and the crude residue purified by chromatography (hexane–EtOAc 18:1) to provide 33 (6.1 g, 84%) as a pale yellow syrup. \([\alpha]_D = +33.8\) (c 1.2, CHCl\(_3\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.87–7.12 (m, 25H, ArH), 5.39 (d, \(J = 1.3\) Hz, 1H, H-1), 4.93–4.47 (m, 6H, CH\(_2\)Ph), 4.13–3.97 (m, 3H, H-4, H-5, H-6a), 3.90 (m, 2H, H-6b, H-3), 3.86–3.83 (m, 1H, H-2), 2.68–2.49 (m, 2H, SCH\(_2\)CH\(_3\)), 1.24 (t, \(J = 7.4\) Hz, 3H, SCH\(_2\)CH\(_3\)), 1.07 (s, 9H, Si(Ph)\(_2\)C(CH\(_3\))\(_3\)). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 138.6 (Ar), 138.4(0) (Ar), 138.3(8) (Ar), 136.0 (Ar), 135.7 (Ar), 133.9 (Ar), 133.4 (Ar), 129.5(1) (Ar), 129.4(9) (Ar), 128.3(9) (Ar), 128.3(5) (Ar), 128.3(1) (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7(4) (Ar), 127.6(6) (Ar), 127.6(5) (Ar), 127.6(3) (Ar), 127.5(7) (Ar), 127.5(2) (Ar), 81.4 (C-1), 80.6 (C-3), 77.0 (C-2), 75.2 (CH\(_2\)Ph), 75.0 (C-4), 73.4 (C-5), 72.2(4) (CH\(_2\)Ph), 72.2(0) (ArCH\(_2\)), 63.3 (C-6), 26.8 (Si(Ph)\(_2\)C(CH\(_3\))\(_3\)), 25.0 (SCH\(_2\)), 19.3 (Si(Ph)\(_2\)C(CH\(_3\))\(_3\)), 14.8 (SCH\(_2\)CH\(_3\)). HRMS (ESI) \(m/z\) Calcd for C\(_{45}\)H\(_{52}\)O\(_5\)SSiNa [M + Na]\(^+\): 755.3197. Found: 755.3196.
Ethyl 2,3,4-tri-O-benzyl-1-thio-α-D-mannopyranoside (34)

A 1 M solution of tetra-n-butylammonium fluoride in THF (13.4 mL, 13.4 mmol) was added dropwise to a solution of 33 (6.07 g, 8.3 mmol) in THF (29 mL), and the mixture was stirred overnight. Evaporation of the solvent and chromatography (hexane–EtOAc 7:2) of the crude residue provided the alcohol 34 (3.4 g, 83%) as colorless oil. [α]D = +81.0 (c 0.8, CHCl3); 1H NMR (400 MHz, CDCl3) δ 7.42–7.27 (m, 15H, ArH), 5.31 (d, J = 1.2 Hz, 1H, H-1), 4.95–4.57 (m, 6H, CH2), 4.04–3.98 (m, 2H, H-4, H-5), 3.89–3.79 (m, 4H, H-2, H-3, H-6a, H-6b), 2.67–2.48 (m, 2H, SCH2CH3), 1.93 (app t, J = 6.5 Hz, 1H, OH), 1.24 (t, J = 7.4 Hz, 3H, SCH2CH3). 13C NMR (101 MHz, CDCl3) δ 138.4 (Ar), 138.2 (Ar), 138.1 (Ar), 128.4 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7(2) (Ar), 127.6(8) (Ar), 82.3 (C-1), 80.4 (C-3), 76.6 (C-2), 75.2 (CH2Ph), 75.0 (C-4), 72.4(1) (C-5), 72.3(9) (CH2Ph), 72.2 (CH2Ph), 62.4 (C-6), 25.4 (SCH2), 14.9 (SCH2CH3). The 1H and 13C NMR spectral data were consistent with those previously reported [4,5]. HRMS (ESI) m/z Calcd for C29H34O5SNa [M + Na]+: 517.2019. Found: 517.2012.

Ethyl 2,3,4-tri-O-benzyl-6-O-methyl-1-thio-α-D-mannopyranoside (35)

Monosaccharide 34 (148.4 mg, 0.3 mmol) was dissolved in DMF (3 mL) and the solution was cooled to 0 °C. After the addition of NaH (60% NaH in mineral oil, 20.4 mg, 5.1 mmol), the mixture was stirred for 10 min before the addition of CH3I (26 µL, 0.42 mmol). The reaction mixture was stirred overnight and quenched by the addition of MeOH (1 mL). The solution was then diluted with CH2Cl2, washed with distilled water and brine. The organic layer was dried
over Na$_2$SO$_4$. Evaporation of the solvent and chromatography (hexane–EtOAc 8:1) of the residue afforded 35 (133 mg, 87%) as a colorless syrup $[\alpha]_D = +82.1$ (c 0.6, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.42–7.26 (m, 15H, ArH), 5.39 (s, 1H, H-1), 4.94–4.50 (m, 6H, CH$_2$Ph), 4.09 (ddd, $J = 9.8$, 4.7, 1.8 Hz, 1H, H-5), 4.01 (app t, $J = 9.3$ Hz, 1H, H-4), 3.86–3.81 (m, 2H, H-2, H-3), 3.70 (dd, $J = 10.6$, 4.8 Hz, 1H, H-6), 3.60 (dd, $J = 10.6$, 2.0 Hz, 1H, H-6), 3.39 (s, 3H, OC$_3$H$_3$), 2.69–2.51 (m, 2H, SCH$_2$CH$_3$), 1.25 (t, $J = 7.4$ Hz, 1H, SCH$_2$CH$_3$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 138.6 (Ar), 138.3 (Ar), 138.1 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7(2) (Ar), 127.6(5) (Ar), 127.6 (Ar), 82.0 (C-1), 80.3 (C-3), 76.3 (C-2), 75.1(4) (CH$_2$Ph), 75.0(5) (C-4), 72.0(1) (CH$_2$Ph), 71.9(9) (CH$_2$Ph), 71.7 (C-5), 71.6 (C-6), 59.2 (OCH$_3$), 25.3 (SCH$_2$CH$_3$), 14.9 (SCH$_2$CH$_3$). HRMS (ESI) m/z Calcd for C$_{30}$H$_{36}$O$_5$SNa [M + Na]$^+$: 531.2176. Found: 531.2169.

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**Dibenzyl 2,3,4-tri-O-benzyl-6-O-methyl-\textalpha-d-mannopyranosyl phosphate (36)**

Thioglycoside 35 (50 mg, 0.1 mmol), dibenzyl phosphate (47.3 mg, 0.17 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH$_2$Cl$_2$ (3 mL) and stirred for 40 min. Then, N-iodosuccinimide (34 mg, 0.15 mmol) and silver trifluoromethanesulfonate (8 mg, 0.03 mmol) were added at $-30^\circ$C. The reaction mixture was stirred for 40 min from $-30^\circ$C to $-20^\circ$C, and then was filtered through Celite and diluted with CH$_2$Cl$_2$. The solution was washed with Na$_2$S$_2$O$_3$ (satd aq soln), NaHCO$_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na$_2$SO$_4$, and concentrated. The crude residue was purified by chromatography (hexane–EtOAc 5:2) to afford 36 (50.4 mg, 70%) as a colorless oil. $[\alpha]_D = +24.8$ (c 1.3, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.48–7.27 (m, 25H, ArH), 5.86 (dd, $J = 6.1$, 1.9 Hz, 1H, H-1), 5.21–4.47
Methyl 2,3,4-tri-O-benzoyl-α-D-mannopyranoside (37)

Monosaccharide methyl 2,3,4-tri-O-benzoyl-6-O-trityl-α-D-mannopyranoside [6] (749 mg, 1 mmol) was dissolved in 1:1 CH₂Cl₂–MeOH (10 mL). To this solution was added 10% HCl in MeOH (2 mL, HCl: MeOH v/v = 1:9). The solution was stirred overnight and then the solvent was evaporated. The residue was purified by chromatography (hexane–EtOAc 2:1 → 1:2) to provide 37 (410 mg, 81%) as a colorless syrup [α]D = −156.7 (c 0.5, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.17–7.22 (m, 15H, ArH), 5.98 (dd, J = 10.1, 3.4 Hz, 1H, H-3), 5.86 (app t, J = 10.1 Hz, 1H, H-4), 5.69 (dd, J = 3.3, 1.8 Hz, 1H, H-2), 5.02 (d, J = 1.5 Hz, 1H, H-1), 4.09 (dd, J = 10.0, 3.9, 2.2 Hz, 1H, H-5), 3.86 (dd, J = 12.7, 2.2 Hz, 1H, H-6a), 3.80 (dd, J = 12.7, 4.0 Hz, 1H, H-6b), 3.53 (s, 3H, OCH₃), 2.62 (dd, J = 8.4, 5.8 Hz, 1H, OH). ¹³C NMR (126 MHz, CDCl₃) δ 166.5 (C=O), 165.6 (C=O), 165.5 (C=O), 133.7 (Ar), 133.6 (Ar), 133.2 (Ar), 129.9(4) (Ar),

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129.9(2) (Ar), 129.7(2) (Ar), 129.3(3) (Ar), 129.2 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.3 (Ar), 98.8 (C-1), 70.9 (C-5), 70.6 (C-2), 69.6 (C-3), 67.3 (C-4), 61.4 (C-6), 55.5 (OCH$_3$). HRMS (ESI) $m/z$ Calcd for C$_{28}$H$_{26}$O$_9$Na [M + Na]$^+$: 529.1469. Found: 529.1461.

**Methyl 2,3,4-tri-O-benzoyl-6-O-methyl-α-D-mannopyranoside (38)**

Monosaccharide 37 (112.8 mg, 0.22 mmol) was dissolved in CH$_3$I (2.5 mL). To this solution was added Ag$_2$O (155 mg, 0.67 mmol) and CaSO$_4$ (121 mg, 0.89 mmol). The reaction mixture was stirred for 3 days, and then filtered through Celite. The filtrate was concentrated and the crude residue was purified by chromatography (hexane–EtOAc 4:1) to afford 38 (60 mg, 52%) as a colorless syrup, as well as the side product methyl 2,3,6-tri-O-benzoyl-4-O-methyl-α-D-mannopyranoside (35 mg, 31%) as a colorless syrup. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.15–7.20 (m, 15H, ArH), 5.91–5.85 (m, 2H, H-4, H-3), 5.66 (dd, $J = 2.8, 1.8$ Hz, 1H, H-2), 5.00 (d, $J = 1.7$ Hz, 1H, H-1), 4.24–4.23 (m, 1H, H-5), 3.64 (m, 2H, H-6a, H-6b), 3.54 (s, 3H, OCH$_3$), 3.40 (s, 3H, OCH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 165.5(7) (C=O), 165.5(6) (C=O), 165.4 (C=O), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.3 (Ar), 129.2 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 98.6 (C-1), 71.6 (C-6), 70.5 (C-2), 70.0 (C-5), 69.9, 67.4 (C-4, C-3), 59.6 (OCH$_3$), 55.5 (OCH$_3$). The $^1$H and $^{13}$C NMR spectral data were consistent with those previously reported [7]. HRMS (ESI) $m/z$ Calcd for C$_{29}$H$_{28}$O$_9$Na [M + Na]$^+$: 543.1626. Found: 543.1618.
1-O-Acetyl-2,3,4-tri-O-benzoyl-6-O-methyl-α-D-mannopyranose (39)

Monosaccharide 38 (286 mg, 0.55 mmol) was dissolved in Ac₂O–HOAc–H₂SO₄ (70:30:1 v/v/v, 5.05 mL). After being stirred overnight, the mixture was diluted with CH₂Cl₂ and washed with distilled water, NaHCO₃ (satd aq soln) and brine. The organic layer was dried over Na₂SO₄ and concentrated, and the crude residue was purified by chromatography (hexane–EtOAc 2:1) to afford 39 (289 mg, 96%) as a pale yellow syrup $\left[\alpha\right]_D = -130.2$ (c 0.9, CHCl₃); $^1$H NMR (400 MHz, CDCl₃) δ 8.24–7.15 (m, 15H, ArH), 6.39 (d, $J = 2.0$ Hz, 1H, H-1), 5.98 (app t, $J = 10.0$ Hz, 1H, H-4), 5.89 (dd, $J = 10.2$, 3.3 Hz, 1H, H-3), 5.71 (dd, $J = 3.3$, 2.0 Hz, 1H, H-2), 4.31 (app dt, $J = 9.9$, 3.9 Hz, 1H, H-5), 3.65 (d, $J = 4.0$ Hz, 2H, H-6a, H-6b), 3.38 (s, 3H, OCH₃), 2.26 (s, 3H, C(O)CH₃). $^{13}$C NMR (126 MHz, CDCl₃) δ 168.2 (C=O), 165.6 (C=O), 165.4 (C=O), 165.3 (C=O), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 130.0 (Ar), 129.7 (8) (Ar), 129.7 (6) (Ar), 129.0 (8) (Ar), 129.0 (6) (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 90.9 (C-1, $^1$J_C,H = 178.5 Hz), 72.4 (C-5), 71.4 (C-6), 69.8 (C-3), 69.3 (C-2), 66.9 (C-4), 59.7 (OCH₃), 21.0 (C(O)CH₃). HRMS (ESI) $m$/z Calcd for C₃₀H₂₈O₁₀Na [M + Na]$^+$: 571.1575. Found: 571.1579.

Ethyl 2,3,4-tri-O-benzoyl-6-O-methyl-1-thio-α-D-mannopyranoside (40)

Monosaccharide 39 (280 mg, 0.51 mmol) and ethanethiol (57 µL, 0.77 mmol) were dissolved in CH₂Cl₂ (4 mL) and cooled to 0 °C before the addition of BF₃·OEt₂ (128 µL, 1.02 mmol). The reaction mixture was stirred overnight and then diluted with CH₂Cl₂, and washed with NaHCO₃ (satd aq soln), distilled water, and brine. The organic layer was dried over Na₂SO₄, concentrated
and the crude residue purified by chromatography (hexane–EtOAc 5:1 → 4:1) to afford 40 (211 mg, 75%) as a pale yellow syrup $[\alpha]_D = -49.3$ (c 1.4, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.12–8.10 (m, 2H, ArH), 8.00–7.98 (m, 2H, ArH), 7.84–7.82 (m, 2H, ArH), 7.61–7.57 (m, 1H, ArH), 7.53–7.45 (m, 3H, ArH), 7.43–7.36 (m, 3H, ArH), 7.27–7.23 (m, 2H, ArH), 5.96 (app. t, $J = 10.0$ Hz, 1H, H-4), 5.81 (dd, $J = 9.9, 3.3$ Hz, 1H, H-3), 5.78 (dd, $J = 3.3, 1.6$ Hz, 1H, H-2), 5.58 (d, $J = 1.4$ Hz, 1H, H-1), 4.62 (ddd, $J = 9.9, 4.8, 3.0$ Hz, 1H, H-5), 3.69–3.61 (m, 2H, H-6a, H-6b), 3.39 (s, 3H, OCH$_3$), 2.83–2.68 (m, 2H, SCH$_2$CH$_3$), 1.37 (t, $J = 7.4$ Hz, 3H, SCH$_2$CH$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 165.5(3) (C=O), 165.5(1) (C=O), 165.4 (C=O), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 82.2 (C-1), 72.3 (C-2), 71.4 (C-6), 70.6 (C-3), 70.4 (C-5), 67.6 (C-4), 59.6 (OCH$_3$), 25.5 (SCH$_2$CH$_3$), 14.9 (SCH$_2$CH$_3$). HRMS (ESI) $m/z$ Calcd for C$_{30}$H$_{30}$O$_8$SNa [M + Na]$^+$: 573.1554. Found: 573.1544.

**Dibenzyl 2,3,4-tri-O-benzoyl-6-O-methyl-α-D-mannopyranosyl phosphate (41)**

Thioglycoside 40 (103 mg, 0.18 mmol), dibenzyl phosphate (85 mg, 0.31 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH$_2$Cl$_2$ and stirred for 30 min. Then, N-iodosuccinimide (61 mg, 0.27 mmol) and silver trifluoromethanesulfonate (14 mg, 0.054 mmol) were added at –30 °C. The reaction mixture was stirred for 2 h, and then was filtered through Celite. The filtrate was diluted with CH$_2$Cl$_2$, and washed with Na$_2$S$_2$O$_3$ (satd aq soln), NaHCO$_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na$_2$SO$_4$, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 3:2) to afford 41 (123 mg, 89%) as a colorless oil $[\alpha]_D = -87.4$ (c 2.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23–7.18
(m, 25H, ArH), 5.97 (app t, J = 10.2 Hz, 1H, H-4), 5.95 (dd, J = 6.2, 2.3 Hz, 1H, H-1), 5.86 (dd, J = 10.1, 3.3 Hz, 1H, H-3), 5.69 (dd, J = 3.2, 2.1 Hz, 1H, H-2), 5.24–5.17 (m, 4H, CH₂Ph × 2), 4.37 (app dt, J = 10.0, 3.6 Hz, 1H, H-5), 3.57–3.53 (m, 2H, H-6a, H-6b), 3.34 (s, 3H, OCH₃). ¹³C NMR (126 MHz, CDCl₃) δ 165.3(5) (C=O), 165.3(2) (C=O), 165.1 (C=O), 135.6 (d, 3J_C,P = 7.1 Hz, 1C, Ar), 135.4 (d, 3J_C,P = 6.8 Hz, 1C, Ar), 133.6 (Ar), 133.4 (Ar), 133.2 (Ar), 130.0 (Ar), 129.8(1) (Ar), 129.7(6) (Ar), 129.1 (Ar), 129.0 (Ar), 128.7(4) (Ar), 128.7(0) (Ar), 128.6(6) (Ar), 128.6 (Ar), 128.5 (Ar), 128.3(2) (Ar), 128.2(7) (Ar), 128.1 (Ar), 127.9 (Ar), 95.4 (d, 2J_C,P = 5.5 Hz, 1C, C-1, 1J_C,H = 179.9 Hz), 71.9 (C-5), 70.8 (C-6), 70.0 (d, 2J_C,P = 5.3 Hz, 1C, CH₂Ph), 69.9 (d, 2J_C,P = 5.4 Hz, 1C, CH₂Ph), 69.8 (d, 3J_C,P = 11.3 Hz, 1C, C-2), 69.3 (C-3), 66.6 (C-4), 59.5 (OCH₃). ³¹P NMR (162 MHz, CDCl₃) δ –2.97. HRMS (ESI) m/z Calcd for C₄₂H₄₀O₁₂PNa [M + Na]⁺: 789.2071. Found: 789.2063.

**Methyl 2,3,4-tri-O-benzoyl-6-deoxy-6-iodo-α-D-mannopyranoside (42)**

To a solution of 37 (3.25 g, 6.4 mmol) in dry CH₂Cl₂ (34 mL) was added PPh₃ (3.37 g, 12.8 mmol), imidazole (874 mg, 12.8 mmol) and I₂ (3.26 g, 12.8 mmol) at 0 °C. The solution was stirred overnight, diluted with CH₂Cl₂, and then washed with Na₂S₂O₃ (satd aq soln) and brine. The organic layer was dried over Na₂SO₄ and concentrated, and the residue was purified by chromatography (hexane–EtOAc 4:1) to provide 42 (2.56 g, 65%) as a pale yellow syrup. ¹H NMR (500 MHz, CDCl₃) δ 8.20–7.20 (m, 15H, ArH), 5.89 (dd, J = 10.0, 3.4 Hz, 1H, H-3), 5.77 (app t, J = 9.8 Hz, 1H, H-4), 5.68 (dd, J = 3.4, 1.8 Hz, 1H, H-2), 5.02 (d, J = 1.7 Hz, 1H, H-1), 4.11–4.05 (m, 1H, H-5), 3.60 (s, 3H, OCH₃), 3.49 (dd, J = 10.9, 2.6 Hz, 1H, H-6a), 3.38 (dd, J = 10.9, 8.1 Hz, 1H, H-6b). ¹³C NMR (125 MHz, CDCl₃) δ 165.6 (C=O), 165.5 (C=O), 165.4
(C=O), 133.6(1) (Ar), 133.5(5) (Ar), 133.2 (Ar), 132.3 (Ar), 132.2 (Ar), 131.6 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.3 (Ar), 129.1 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 98.8 (C-1), 70.7, 70.5, 70.2, 69.6 (C-2, C-3, C-4, C-5), 55.8 (OCH$_3$), 4.7 (C-6). The $^1$H and $^{13}$C NMR spectral data were consistent with those previously reported [8]. HRMS (ESI) $m/z$ Calcd for C$_{28}$H$_{25}$O$_8$Na [M + Na]$^+$: 639.0486. Found: 639.0492.

1-O-Acetyl 2,3,4-tri-O-benzoyl-6-deoxy-$\alpha$-D-mannopyranose (43)

Monosaccharide 42 (867 mg, 1.4 mmol) was dissolved in Ac$_2$O–HOAc–H$_2$SO$_4$ (35:15:1 v/v/v, 9.2 mL). After being stirred overnight, the mixture was diluted with CH$_2$Cl$_2$ and washed with distilled water, NaHCO$_3$ (saturated aq soln) and brine. The organic layer was dried over Na$_2$SO$_4$, concentrated and then dissolved in EtOAc (10 mL). To this solution was added 10% Pd–C (100 mg) and Et$_3$N (1.5 mL). The reaction mixture was stirred for 2 days under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated. The residue was purified by chromatography (hexane–EtOAc 4:1 → 3:1) to afford 43 (525 mg, 72%) as a pale yellow syrup. [$\alpha$]$_D$ = −133.5 (c 0.6, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.14–7.24 (m, 15H, ArH), 6.32 (d, $J = 1.6$ Hz, 1H, H-1), 5.86 (dd, $J = 10.2$, 3.4 Hz, 1H, H-3), 5.76–5.70 (m, 2H, H-2, H-4), 4.28 (dq, $J = 10.0$, 6.0 Hz, 1H, H-5), 2.26 (s, 3H, C(O)CH$_3$), 1.40 (d, $J = 6.2$ Hz, 3H, H-6). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 168.4 (C=O), 165.6 (C=O), 165.3 (C=O) 133.7 (Ar), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 130.0 (Ar), 129.7(4) (Ar), 129.7(1) (Ar), 129.1(4) (Ar), 129.0(9) (Ar), 128.9(6) (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 90.8 (C-1, $^1$J$_{C,H}$ = 177.2 Hz), 71.2 (C-4), 69.7 (C-2), 69.6 (C-3), 69.1 (C-5), 21.0 (C(O)CH$_3$), 17.7 (C-6). HRMS (ESI) $m/z$ Calcd for C$_{29}$H$_{26}$O$_9$Na [M + Na]$^+$: 541.1469. Found: 541.1465.
Ethyl 2,3,4-tri-O-benzoyl-6-deoxy-1-thio-α/β-D-mannopyranoside (44)

Monosaccharide 43 (361 mg, 0.70 mmol) and ethanethiol (78 µL, 1.05 mmol) were dissolved in CH₂Cl₂ (6 mL) and cooled to 0 °C before the addition of BF₃·OEt₂ (176 µL, 1.40 mmol). The reaction mixture was stirred overnight and then was diluted with CH₂Cl₂, and washed with NaHCO₃ (satd aq soln), distilled water and brine. The organic layer was dried over Na₂SO₄ and concentrated, and the crude residue was purified by chromatography (hexane–EtOAc 6:1 → 5:1) to afford 44 (324 mg, 89%, α/β 4:1) as a pale yellow syrup. Data for α isomer: ¹H NMR (500 MHz, CDCl₃) δ 8.26–7.17 (m, 15H, ArH), 5.85–5.78 (m, 2H, H-2, H-3), 5.78–5.71 (m, 1H, H-4), 5.52 (s, 1H, H-1), 4.59 (app dq, J = 9.5, 6.2 Hz, 1H, H-5), 2.87–2.59 (m, 2H, SCH₂CH₃), 1.41 (d, J = 6.3 Hz, 3H, H-6), 1.38 (t, J = 7.4 Hz, 3H, SCH₂CH₃). ¹³C NMR (126 MHz, CDCl₃) δ 165.8 (C=O), 165.6 (C=O), 165.4 (C=O), 133.5 (Ar), 133.4 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.5 (Ar), 129.3 (Ar), 129.1 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 82.2 (C-1), 72.7, 72.1, 70.5 (C-2, C-3, C-4), 67.4 (C-5), 25.7 (SCH₂CH₃), 17.6 (C-6), 15.0 (SCH₂CH₃). ¹H and ¹³C NMR spectral data were consistent with those previously reported [9]. HRMS (ESI) m/z Calcd for C₂₉H₂₈O₇SNa [M + Na]⁺: 543.1448. Found: 543.1448.

Dibenzyl 2,3,4-tri-O-benzoyl-6-deoxy-α-D-mannopyranosyl phosphate (45)

Thioglycoside 44 (64 mg, 0.12 mmol), dibenzyl phosphate (58 mg, 0.21 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH₂Cl₂ (2 mL) and stirred at room temperature for 30
min. N–iodosuccinimide (42 mg, 0.19 mmol) and silver trifluoromethanesulfonate (10 mg, 0.039 mmol) were then added at −30 °C. The reaction mixture was stirred for 2 h at −30 °C, and then filtered through Celite and diluted with CH₂Cl₂. The solution was washed with Na₂S₂O₃ (satd aq soln), NaHCO₃ (satd aq soln), distilled water and brine. The organic layer was dried over Na₂SO₄, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1) to afford 45 (59 mg, 67%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 8.08 (m, 2H, ArH), 7.97 (m, 2H, ArH), 7.83 (m, 2H, ArH), 7.66–7.25 (m, 19H, ArH), 5.85 (dd, J = 6.3, 1.9 Hz, 1H, H-1), 5.80 (dd, J = 10.2, 3.4 Hz, 1H, H-3), 5.69–5.64 (m, 2H, H-2, H-4), 5.23–5.14 (m, 4H, CH₂Ph), 4.29 (dq, J = 9.9, 6.2 Hz, 1H, H-5), 1.28 (d, J = 6.2 Hz, 3H, H-6). ³¹P NMR (202 MHz, CD₃Cl₃) δ −1.76. HRMS (ESI) m/z Calcd for C₄₁H₅₇O₁₁PNa [M + Na]⁺: 759.1966. Found: 759.1960.

**Enzymatic synthesis of GDP-Manp analogues**

GDP-α-D-mannose pyrophosphorylase from *Salmonella enterica* [10] was cloned, expressed in *E.coli* BL21(DE3)plySs, and purified. The enzymatic synthesis of GDP-Manp and GDP-Manp analogues were carried out in MOPS buffer (50 mM, pH 7.6) containing MgCl₂ (5 mM), DTT (1 mM), GTP (5 mM), Manp-1P or its analogues (5 mM), GDP-ManPP (200 µg), and inorganic pyrophosphatase (1.25 U) in a total reaction volume of 100 µL. The mixture was incubated at room temperature overnight with shaking and the reactions were stopped by removing the extra enzymes though centrifugal filtration when HPLC analysis of the spin-filtered (Amicon YM10) solution indicated that the reaction was complete.
Characterization of GDP-Manp analogues

HPLC analysis was carried out by using a Waters 600E HPLC equipped with a photodiode array (PDA) detector, with monitoring at 262 nm; the system was controlled by Empower chromatography software. Monitoring the conversion of Manp-1P analogues into corresponding GDP-Manp analogues was done by analytical HPLC on a Phenomenex C18 column (4.6 × 250 mm). A gradient elution using two buffers, buffer A (200 mM Et3N–HOAc, pH 6.6) and buffer B (200 mM Et3N–HOAc pH 6.6 containing 5% CH3CN), was used. The gradient conditions employed used 96% buffer A and 4% buffer B for 10 min (isocratic), followed by a gradient of 4→100% buffer B over 15 min, 100% buffer B for 10 min. The column was re-equilibrated with a gradient of 100→4% buffer B for 15 min, followed by 96% buffer A and 4% buffer B for 10 min. At the end of the reaction, the enzymes were removed by passing the incubation mixture through an empty 10 mL BioRad cartridge, equipped with a filter. The filtrate was then collected by passage through Amicon Ultra-15 centrifugal filter with a molecular-weight cut off of 10,000 Daltons; this filtrate was centrifuged to remove soluble proteins. Following centrifugation, the filtrate was collected and was applied to a Sephadex G-15 gel filtration column to remove salts.
Figure S1. GDP-Manp HPLC traces

Figure S2. GDP-Manp analogue HPLC traces
GDP-ManPP activity assay

The malachite green dye reagent was prepared as previously reported by MacMillan and coworkers [11]. Briefly, to a 100 mL volumetric flask were added 34.0 mg of malachite green oxalate salt, 1.236 g of ammonium molybdate, 3.4 mL of absolute ethanol, 80 mL of deionised water, 8.6 mL of concentrated HCl (37%), 1 mL of Tween 20, and deionised water up to 100 mL total volume. The resulting mixture was stirred for 1 h, then was filtered (0.2 μm) and stored at 4 °C for 7 days. The mixture was filtered again and calibrated by using a standard series of KH₂PO₄ solution at variable concentrations prior to use. In a 96-well plate, 25 μL of each standard was added to a well before the addition of 100 μL malachite reagent. The plate was incubated at 37 °C with mixing, and the absorbance was read at 650 nm over 10 min. The absorbance values at 10 mins were plotted against the phosphate concentration, and from linear fitting an equation was obtained, from which the slope gave the conversion factor.

Assays for GDP-ManPP activity were prepared containing Manp-1P or Manp-1P analogues (500 μM), GTP (2 mM), GDP-ManPP (50 ng), iPPase (0.5 U), MgCl₂ (8 mM), DTT (1 mM), in 25 μL of TRIS 50 mM pH 7.6. The reactions were incubated for 10 minutes at 37 °C in a 96–well plate, after which time the reactions were quenched by the addition of 100 μL of malachite reagent. The colorimetric activity assay was carried out in a plate reader at 37 °C, with mixing, for 10 minutes and the absorbance at 650 nm was then read. Kinetic analyses were carried out under the same conditions as the activity assay, except variable concentrations of the Manp-1P or Manp-1P analogue substrates were used as listed in Table S1.
Table S1: Variable concentrations of six substrates used for kinetics analysis.

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References

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