Supporting Information

Glycosyl Tricyclic Orthoesters as Versatile Intermediates for the Preparation of Glycosyl Phosphate Building Blocks

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General Information

All chemicals used were reagent grade and used as supplied except where noted. All reactions were performed in oven-dried glassware under an inert atmosphere unless noted otherwise. Reagent grade dichloromethane (CH₂Cl₂) was passed through activated neutral alumina column prior to use. Reagent grade N,N-dimethylformamide (DMF) and methanol (MeOH) were dried over activated molecular sieves prior to use. Pyridine, triethylamine and acetonitrile were distilled over CaH₂ prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F₂₅₄ plates (0.25mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh).

¹H, ¹³C and ³¹P NMR spectra were recorded on a Varian Mercury 300 (300 MHz), Varian Gemini 300 (300 MHz), Bruker DRX500 (500 MHz) in CDCl₃ with chemical shifts referenced to internal standards CDCl₃ (7.26 ppm ¹H, 77.0 ppm ¹³C) unless otherwise stated. ³¹P spectra are reported in δ value relative to H₃PO₄ (0.0 ppm) as an external reference. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for ¹H NMR data. High-resolution mass spectral (HRMS) analyses were performed by the MS-service at the Laboratory for Organic Chemistry (LOC) at ETH Zürich. High-resolution MALDI and ESI mass spectra were run on an IonSpec Ultra instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by the Microanalytical Laboratory of the LOC, ETH Zürich.
automated synthesis was performed on an ABI 431A peptide synthesizer with a custom-made jacketed glass reaction vessel.

**Preparation of Glycosyl Tricyclic Orthoester via Acid-Catalyzed Intramolecular Trans-orthoesterification of Glycosyl 1,2-Orthoester**

**3,4-O-Benzyl α-D-mannopyranose 1,2,6-orthobenzoate (5a):**

From mannosyl 1,2-orthoester 2a. To a solution of 2a [1] (195 mg, 0.65 mmol) in CH₃CN (4 mL) in the presence of activated 4Å MS (220 mg) was added CSA (35 mg, 0.15 mmol) at room temperature. The mixture was stirred for 12 h when additional amount of CSA (15 mg) was added. Further stirred for 12 h, the reaction was quenched by the addition of Et₃N (0.15 mL). Filtration through a pad of Celite, followed by removal of the solvents gave the crude tricyclic orthoester. This crude material was dissolved in DMF (4.5 mL), added BnBr (0.24 mL, 2.0 mmol) and NaH (48 mg, 2.0 mmol) at 0 °C. The mixture was gradually warmed up to room temperature during 12 h. Excess NaH was quenched by the addition of MeOH. Typical aqueous workup, followed by silica gel column chromatography gave 5a (244 mg, 85%) as a white solid.

From mannosyl 1,2-orthoester 2b. To a solution of 2b [2] (440 mg, 1.34 mmol) in CH₃CN (8 mL) in the presence of activated 4Å MS (220 mg) was added CSA (35 mg, 0.15 mmol) at room temperature. The mixture was stirred for 12 h before quenched with Et₃N (0.13 mL). Filtration through a pad of Celite, followed by removal of the solvents gave the crude tricyclic orthoester. This crude material was dissolved in DMF (7.5 mL), added BnBr (0.5 mL, 4.20 mmol) and NaH (90 mg, 3.9 mmol) at 0 °C. The mixture was gradually warmed up to room temperature and the reaction completed after 3 h. Excess NaH was quenched by the addition of MeOH. Typical aqueous workup, followed by silica gel column chromatography gave 5a (578 mg, 95%) as a white solid. Rf 0.40 (Hexanes/EtOAc = 4 : 1); [α]D = +2.1 (c = 1.2, CHCl₃); m.p.=110–111 °C; ¹H NMR (300 MHz, CDCl₃) δ 3.74 (dd, J =12.9, 3.6 Hz, 1H), 3.77 (dd, J = 7.5, 2.4 Hz, 1H), 4.18 (d, J = 12.9 Hz, 1H), 4.21 (dd, J =7.5, 1.3 Hz, 1H), 4.24 (dd, J = 3.6, 1.3 Hz 1H), 4.64 (dd, J =7.5, 1.3 Hz, 1H), 4.66 (d, J = 12.0 Hz, 1H), 4.80 (d, J = 12.0 Hz, 1H), 4.84 (app s, 2H), 5.86 (d, J = 5.7 Hz, 1H), 7.25-7.44 (m, 13H), 7.62-7.65 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 70.6,
72.4, 72.6, 73.2, 74.5, 77.4, 79.3, 99.7, 121.9, 126.0, 127.8, 127.9, 128.0, 128.4, 128.5, 129.3, 137.1, 138.0; Anal. Calcd for C$_{27}$H$_{26}$O$_6$; C, 72.63; H, 5.87; Found: C, 72.48; H, 5.86.

### 3,4-O-Benzyl α-L-mannopyranose 1,2,6-orthoacetate (5b):

To a solution of 2d [3] (500 mg, 1.91 mmol) in CH$_3$CN (10 mL) in the presence of activated 4Å MS (300 mg) was added CSA (44 mg, 0.19 mmol) at room temperature. The mixture was stirred for 12 h before quenched with Et$_3$N (0.20 mL). Filtration through a pad of Celite, followed by removal of the solvents gave the crude tricyclic orthoester. This crude material was dissolved in DMF (10 mL), added BnBr (0.68 mL, 5.70 mmol) and NaH (137 mg, 5.7 mmol, 60% in mineral oil) at 0 °C. The mixture was gradually warmed up to room temperature and the reaction completed after 3 h. Excess NaH was quenched by the addition of MeOH. Typical aqueous workup, followed by silica gel column chromatography gave 5b (700 mg, 95%) as a colorless syrup. R$_f$ 0.28 (Hexanes/EtOAc = 4 : 1); [α]$_D^{r,t}$ = –8.1 (c = 7.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 1.65 (s, 3H), 3.52 (dd, J = 13.0, 3.3 Hz, 1H), 3.66 (dd, J = 7.5, 2.4 Hz, 1H), 3.98 (app d, J = 13.0 Hz, 1H), 4.04 (dd, J = 7.5, 1.2 Hz, 1H), 4.06 (dd, J = 3.3, 1.2 Hz 1H), 4.45 (dd, J = 6.0, 2.4 Hz, 1H), 4.60 (d, J = 12.0 Hz, 1H), 4.76 (d, J = 12.0 Hz, 1H), 4.80 (app s, 2H), 5.68 (d, J = 6.0 Hz, 1H), 7.25-7.42 (m, 10H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 23.8, 69.8, 72.4, 73.1, 73.8, 77.2, 79.1, 99.4, 122.9, 127.7, 127.8, 127.9, 128.3, 128.4, 137.9, 138.0; HRMS-MALDI (m/z): [M+Na]$^+$ calcd for C$_{22}$H$_{24}$O$_6$, 407.1471; Found: 407.1459.

### 3-O-Benzyl α-L-xylpyranose 1,2,4-orthoacetate (5c):

To a solution of 2e [4] (348 mg, 1.50 mmol) in CH$_3$CN (40 mL) was added CSA (42 mg, 0.18 mmol) at room temperature. The mixture was stirred for 12 h before quenched with Et$_3$N (0.15 mL). Filtration through a pad of Celite, followed by removal of the solvents gave the crude tricyclic orthoester. Two-third portion of this crude material was dissolved in DMF (3 mL), added BnBr (0.13 mL, 1.1 mmol) and NaH (32 mg, 1.3 mmol, 60% in mineral oil) at 0 °C. The mixture was gradually warmed up to room temperature and the reaction completed after 5 h. Excess NaH was quenched by the addition of MeOH. Typical aqueous workup, followed by silica gel column chromatography gave 5c (194 mg, 73%) as a syrup. R$_f$ 0.53 (CH$_2$Cl$_2$/EtOAc = 1 : 1); [α]$_D^{r,t}$ = +44.0 (c = 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 1.63 (s, 3H), 3.91–3.96 (m,
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1H), 4.12–4.19 (m, 3H), 4.35–4.39 (m, 1H), 4.58–4.71 (m, 2H), 5.75 (d, J = 4.8 Hz, 1H), 7.28–7.40 (m, 5H); 13C NMR (75 MHz, CDCl3) δ 20.3, 65.5, 69.9, 71.4, 72.2, 72.3, 97.9, 118.2, 127.7 (2x), 128.1, 128.5 (2x), 137.4; HRMS-MALDI (m/z): [M+Na]+ Calcd for C14H16O5, 287.0889; Found: 287.0880.

3-O-Benzyl α-D-xylopyranose 1,2,4-orthobenzoate (5d):

To a solution of 2f [5] (2.65 g, 9.89 mmol) in CH3CN (50 mL) was added silica gel (10 g, Fluka Kieselgel 50), and the mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through a short pad of Celite and the solvent was removed in vacuo. Purification by silica gel chromatography gave tricyclic orthoester α-D-xylopyranose 1,2,4-orthobenzoate (1.33 g, 60%) as a colorless solid. Rf 0.31 (CH2Cl2/EtOAc = 1 : 1). [α]D r.t. = +71.5 (c = 1.0, CHCl3); m.p. = 145–147 °C; 1H NMR (300 MHz, CDCl3) δ 2.34 (d, J = 10.2 Hz, 1H), 4.24-4.29 (m, 1H), 4.28 (dd, J = 12.0, 4.5 Hz, 1H), 4.44 (app d, J = 12.0 Hz, 1H), 4.49 (td, J = 4.5, 2.0 Hz, 1H), 4.57 (app dt, J = 4.5, 2.0 Hz, 1H), 5.96 (d, J = 4.8 Hz, 1H), 7.36-7.44 (m, 3H), 7.66-7.70 (m, 2H); 13C NMR (75 MHz, CDCl3) δ 63.8, 65.9, 73.1, 74.5, 98.0, 117.7, 125.9, 128.1, 129.7, 133.5; Anal. Calcd for C12H12O5; C, 61.02; H, 5.12; Found: C, 60.98; H, 5.05.

To a solution of α-D-xylopyranose 1,2,4-orthobenzoate (777 mg, 3.29 mmol) in DMF (12 mL) was added BnBr (0.78 mL, 6.58 mmol) and NaH (158 mg, 6.58 mmol, 60% in mineral oil) at 0 °C. The mixture was allowed to warm up to room temperature gradually and stirred overnight. Excess NaH was quenched by the addition of MeOH. Typical aqueous workup, followed by silica gel column chromatography gave 5d (1.11 g, quant.) as a white solid. Rf 0.49 (Hexanes/EtOAc = 4 : 1). [α]D r.t. = +43.8 (c = 1.4, CHCl3); m.p. = 54–55 °C; 1H NMR (300 MHz, CDCl3) δ 4.10-4.12 (m, 1H), 4.27 (dd, J = 12.0, 6.9 Hz, 1H), 4.34 (dd, J = 12.0, 1.0 Hz, 1H), 4.38 (td, J = 4.2, 2.4 Hz, 1H), 4.58 (dt, J = 4.8, 2.0 Hz, 1H), 4.67 (d, J = 12.1 Hz, 1H), 4.74 (d, J = 12.1 Hz, 1H), 5.94 (d, J = 5.1 Hz, 1H), 7.37-7.42 (m, 8H), 7.65-7.68 (m, 2H); 13C NMR (75 MHz, CDCl3) δ 65.7, 70.0, 72.2, 72.4, 72.7, 98.1, 117.3, 127.8, 128.1, 128.2, 128.6, 129.8, 133.7, 137.4; Anal. Calcd for C19H18O5; C, 69.93; H, 5.56; Found: C, 69.66; H, 5.57.

3,6-Di-O-benzyl α-D-glucopyranose 1,2,4-orthoacetate (5e):

To a solution of 2g [6] (89.9 mg, 0.381 mmol) in acetonitrile (1.9 mL) was added 4Å AW 300 MS, and the mixture was stirred at 40 °C for 14 h, then at 60 °C for 3 h. The reaction mixture was
filtered through a short pad of Celite and the solvent was removed in vacuo. In a separate flask, NaH (116 mg, 60% in mineral oil) was washed with hexane twice and DMF (0.45 mL) was added. To this suspension of NaH in DMF was added the crude tricyclic orthoester mixture in DMF (1 mL) at 0°C, and BnBr (345 µL, 2.9 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 13 h, and H2O was added to quench the reaction. After extraction with diethyl ether, combined organic layer was washed with brine, and dried over magnesium sulfate. Filtration, evaporation, and purification by silica gel chromatography gave 5e as a colorless syrup (73.0 mg, 50%). [α]D = +21.1 (c = 0.82, CHCl3) 1H NMR (300 MHz, CDCl3) δ 1.63 (s, 3H), 3.72 (dd, J = 9.6, 7.8 Hz, 1H), 3.81 (dd, J = 9.6, 6.6 Hz, 1H), 3.97 (dt, J = 4.5 Hz, 1H), 4.26 (dd, J = 4.5, 2.1 Hz, 1H), 4.40 (dt, J = 4.8, 2.1 Hz, 1H), 4.48 (d, J = 12.3 Hz, 1H), 4.53 (brs, 2H), 4.63 (d, J = 12.0 Hz, 1H), 4.65 (t, J = 6.9 Hz, 1H), 5.80 (d, J = 4.8 Hz, 1H); 13C NMR (75 MHz, CDCl3) δ 20.4, 69.9, 71.1, 71.4, 72.0, 72.3, 73.2, 75.8, 97.8, 118.8, 127.5, 127.6, 127.7, 128.0, 128.3, 128.5, 137.2, 137.9; HRMS-MALDI (m/z): [M+Na]+ Calcd for C22H24O6, 407.1471; Found: 407.1467.

**α-D-Glucopyranose 1,2,4-orthoacetate**

α-D-Glucopyranose 1,2,4-orthoacetate can be purified with silica gel chromatography, before being subjected to benzylation conditions. Spectral data are as follows. 
[α]D = +110 (c = 0.5, MeOH); m.p. = 119-120 °C; 1H NMR (300 MHz, CDCl3) δ 1.65 (s, 3H), 3.86 (dd, J = 12.0, 3.3 Hz, 1H), 4.02 (dd, J = 12.0, 4.2 Hz, 1H), 4.17 (d, J = 3.3 Hz, 1H), 4.28 (dd, J = 4.5, 2.1 Hz, 1H), 4.38 (dt, J = 4.8, 2.1 Hz, 1H), 4.59 (t, J = 3.6 Hz, 1H), 5.85 (d, J = 4.8 Hz, 1H); 13C NMR (75 MHz, CDCl3) δ 20.3, 62.3, 63.8, 74.2, 75.1, 76.7, 97.6, 119.0; HRMS-ESI (m/z): [M]– calcd for C8H11O6, 203.0556; Found: 203.0559.

**Opening of Glycosyl Tricyclic Orthoester with Dibutyl Phosphate: General Procedure**

To a solution of suitably protected glycosyl tricyclic orthoester (1.0 equiv) in either CH2Cl2 or CH3CN was added freshly activated 4Å molecular sieves. The mixture was stirred for 15 min stirring at room temperature, before added dibutyl phosphate (6 equiv.) in one portion. After completion of the reaction (TLC analysis), the reaction was cooled to 0°C and triethylamine (10 equiv) was added. The solution was warmed to room temperature and filtered off through a pad
of Et3N-deactivated silica gel. The resulting mixture was purified by flash silica column chromatography.

**Dibutyl-(2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranosyl) phosphate (6a):**

General procedure with mannosyl tricyclic orthoester 5a (51 mg, 0.11 mmol), dibutyl phosphate (136 µL, 0.68 mmol), 4Å MS (30 mg), CH2Cl2 (1.5 mL), room temperature, 24 h, gave 6a (62 mg, 83%) as a colorless syrup. Rf 0.32 (Hexanes/EtOAc = 1:1); [α]D r.t. = +0.44 (c = 2.5, CHCl3); 1H NMR (300 MHz, CDCl3) δ 0.92-0.98 (m, 6H), 1.35-1.49 (m, 4H), 1.63-1.73 (m, 4H), 2.16 (t, J = 6.9 Hz, 1H), 3.81-3.92 (m, 2H), 3.94-4.16 (m, 7H), 4.60 (d, J = 11.7 Hz, 1H), 4.68 (d, J = 10.8 Hz, 1H), 4.80 (d, J = 11.7 Hz, 1H), 4.92 (d, J = 10.8 Hz, 1H), 5.42 (app t, J = 2.4 Hz, 1H), 5.77 (dd, J = 6.3, 2.0 Hz, 1H), 7.24-7.37 (m, 10H), 7.45-7.51 (m, 2H), 7.58-7.64 (m, 1H), 8.06-8.09 (m, 2H); 13C NMR (75 MHz, CDCl3) δ 13.7, 18.8, 32.3, 61.6, 68.1, 68.6, 71.9, 73.3, 73.9, 75.4, 77.3, 95.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 129.4, 129.9, 133.4, 137.6, 137.9, 165.1; 31P NMR (121MHz, CDCl3): δ -2.19. HRMS-MALDI (m/z): [M+Na]+ Calcd for C35H45O10P, 679.2643; Found: 679.2639.

**Dibutyl-(2-O-acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl) phosphate (6b):**

General procedure with microwave irradiation at 120 °C, using mannose tricyclic orthoester 5b (41 mg, 0.11 mmol), 4Å MS (50 mg), dibutyl phosphate (0.38 mL, 0.64 mmol), CH3CN (4 mL), 5 min, gave 6b as a colorless oil (38.2 mg, 60%), Rf 0.18 (Hexanes/EtOAc = 1:1); [α]D r.t. = +22.7 (c = 1, CHCl3); 1H NMR (300 MHz, CDCl3) δ 0.88–0.99 (m, 6H), 1.32–1.48 (m, 4H), 1.59–1.72 (m, 4H), 2.01 (brs, 1H), 2.15 (s, 3H), 3.73–3.91 (m, 4H), 3.95–4.13 (m, 5H), 4.48–4.77 (m, 3H), 4.88–4.95 (m, 1H), 5.42 (dd, J = 3.0, 2.4 Hz, 1H), 5.62 (dd, J = 6.2, 1.8 Hz, 1H), 7.27–7.38 (m, 10H); 13C NMR (75 MHz, CDCl3) δ 13.7, 18.7, 21.0, 32.2, 32.3, 61.6, 68.0, 68.1, 68.3, 72.0, 73.4, 73.7, 75.3, 77.1, 95.5, 95.6, 127.7 (2x), 127.9 (2x), 128.0 (2x), 128.3 (2x), 128.4 (2x), 137.5, 137.9, 169.7; 31P NMR (121MHz, CDCl3): δ –2.55; IR (film) νmax: 3372, 2961, 2875, 1748, 1456, 1368, 1233, 1029, 956 cm⁻¹; HRMS-MALDI (m/z): [M+Na]+ Calcd for C30H43O10P, 617.2486; Found: 617.2484.

**Dibutyl-(2-O-acetyl-3-O-benzyl-α-D-xylopyranosyl) phosphate (6c):**
General procedure with xylose tricyclic orthoester 5c (51 mg, 0.19 mmol), dibutyl phosphate (0.23 mL, 1.16 mmol), 4Å MS (30 mg), CH2Cl2 (1 mL), room temperature, 24 h, gave 6c as a colorless syrup (69 mg, 65%). Rf 0.22 (Hexanes/EtOAc = 1:1); $\left[\alpha\right]_D^{\text{r.t.}} = +63.2 \ (c = 1, \text{CHCl}_3); \ ^1H \text{NMR (300 MHz, CDCl}_3) \ \delta 0.89–1.00 \ (m, 6H), 1.32–1.49 \ (m, 4H), 1.60–1.74 \ (m, 4H), 2.05 \ (s, 3H), 2.32 \ (brs, 1H), 3.68–3.88 \ (m, 4H), 3.97–4.17 \ (m, 4H), 4.67–4.90 \ (m, 3H), 5.73 \ (dd, J = 6.6, 3.3 Hz, 1H), 7.23–7.41 \ (m, 5H); \ ^13C \text{NMR (75 MHz, CDCl}_3) \ \delta 13.5, 13.5, 18.5, 18.5, 20.7, 32.0, 32.1, 63.0, 67.7, 67.8, 69.1, 72.2, 72.3, 74.9, 79.1, 94.5, 94.5, 127.6 \ (2x), 127.9, 128.5 \ (2x), 138.1, 169.9; \ ^{31}P \text{NMR (121MHz, CDCl}_3): \ \delta –2.07; \ \text{HRMS-MALDI (m/z): [M+Na]^{+} Calcd for C}_{22}\text{H}_{35}\text{O}_{9}; 497.1911; \text{Found: 497.1918.}

**Dibutyl-(2-O-benzoyl-3-O-benzyl-\alpha-D-xylopyanosyl) phosphate (6d):**

General procedure with xylosyl tricyclic orthoester 5d (56 mg, 0.17 mmol), dibutyl phosphate (204 µL, 1.03 mmol), 4Å MS (30 mg), CH2Cl2 (2.0 mL), room temperature, 24 h gave 6d (82 mg, 90%) as a colorless solid. Rf 0.51 (Hexanes/EtOAc = 1:2); m.p. = 103–104 °C; $\left[\alpha\right]_D^{\text{r.t.}} = +80.7 \ (c = 2.2, \text{CHCl}_3); \ ^1H \text{NMR (300 MHz, CDCl}_3) \ \delta 0.79–0.88 \ (m, 6H), 1.20–1.32 \ (m, 4H), 1.42–1.56 \ (m, 4H), 2.69 \ (s, 1H), 3.80–4.00 \ (m, 8H), 4.73 \ (d, J = 11.7 Hz, 1H), 4.85 \ (d, J = 11.7 Hz, 1H), 5.11 \ (app dt, J = 9.6, 3.3 Hz, 1H), 5.86 \ (dd, J = 6.9, 3.3 Hz, 1H), 7.23–7.27 \ (m, 5H), 7.42–7.46 \ (m, 2H), 7.56–7.61 \ (m, 1H), 8.03–8.06 \ (m, 2H); \ ^13C \text{NMR (75 MHz, CDCl}_3) \ \delta 13.7, 18.6, 32.2, 63.2, 68.0, 69.6, 73.1, 75.4, 79.5, 94.9, 128.1, 128.2, 128.7, 129.5, 130.0, 133.7, 138.1, 165.9; \ ^{31}P \text{NMR (121MHz, CDCl}_3): \ \delta –1.83; \ \text{Anal. Calcd for C}_{27}\text{H}_{37}\text{O}_{9}P; \text{C, 60.44; H, 6.95; Found: C, 60.55; H, 6.75.}

**Dibutyl-(2-O-acetyl-3,4-di-O-benzyl-\beta-D-glucopyanosyl) phosphate (6e):**

General procedure with glucosyl tricyclic orthoester 5e (46 mg, 0.12 mmol), dibutyl phosphate (142 µL, 0.72 mmol), 4Å MS (23 mg), CH3CN (1.2 mL), room temperature, 5 h gave 6e (69 mg, 90%) as a colorless syrup. $\left[\alpha\right]_D^{\text{r.t.}} = +61.0 \ (c = 1, \text{CHCl}_3); \ ^1H \text{NMR (300 MHz, CDCl}_3) \ \delta 0.84–0.93 \ (m, 6H), 1.25-1.42 \ (m, 4H), 1.53-1.64 \ (m, 4H), 1.97 \ (s, 3H), 3.51-3.59 \ (m, 2H), 3.63-3.79 \ (m, 3H), 3.89–4.06 \ (m, 4H), 4.51 \ (d, J = 12.0 Hz, 1H), 4.56 \ (d, J = 12.0 Hz, 1H), 4.69 \ (d, J = 12.0 Hz, 1H), 4.79 \ (d, J = 12.0 Hz, 1H), 5.03 \ (app t, J = 9.0 Hz, 1H), 5.18 \ (app t, J = 7.2 Hz, 1H), 7.24–7.41 \ (m, 10H); \ ^13C \text{NMR (75 MHz, CDCl}_3) \ \delta 13.6, 18.7, 20.9, 32.1, 68.0, 69.6, 71.3, 72.7, 73.6, 74.6, 75.0, 81.8, 96.5, 127.5, 127.7, 128.3, 128.4, 137.5, 138.0, 169.3; \ ^{31}P \text{NMR (121MHz, CDCl}_3): \ \delta –2.07; \ \text{HRMS-MALDI (m/z): [M+Na]^{+} Calcd for C}_{22}\text{H}_{35}\text{O}_{9}P; \text{C, 60.44; H, 6.95; Found: C, 60.55; H, 6.75.}

Dibutyl 2-O-benzoyl-3,4-di-O-benzyl-6-O-(9-fluorenylmethoxycarbonyl)-α-D-manno pyranosyl phosphate (4a):

Following the general procedure for the selective opening of glycosyl tricyclic orthoester, mannosyl orthoester 5a (131 mg, 0.29 mmol) was treated with dibutyl phosphate (291 µL, 1.47 mmol) in CH₂Cl₂ (3.0 mL) at room temperature for 24 h. To this mixture at 0 °C was added pyridine (0.47 mL, 5.8 mmol) and FmocCl (150 mg, 0.58 mmol). The mixture was stirred for 2 h before subjected to typical aqueous workup. The crude material was purified by silica gel column chromatography to give the target compound 4a (236 mg, 85%) as a syrup. Rf 0.55 (Hexanes/EtOAc = 2 : 1). [α]D = +6.4 (c = 1.8, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 0.92-0.98 (m, 6H), 1.36-1.50 (m, 4H), 1.65-1.73 (m, 4H), 4.00-4.21 (m, 7H), 4.27 (t, J = 7.5 Hz, 1H), 4.37-4.48 (m, 4H), 4.62 (d, J = 11.1 Hz, 1H), 4.64 (d, J = 10.8 Hz, 1H), 4.84 (d, J = 11.1 Hz, 1H), 4.94 (d, J = 10.8 Hz, 1H), 5.72 (app t, J = 2.4 Hz, 1H), 5.80 (dd, J = 6.3, 1.8 Hz, 1H), 7.26-7.45 (m, 15H), 7.52-7.65 (m, 3H), 7.78 (d, J = 7.2 Hz, 2H), 8.13 (d, J = 7.2 Hz, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 13.7, 18.9, 32.4, 46.8, 66.4, 68.1, 68.3, 70.1, 71.5, 71.8, 73.1, 75.4, 77.3, 95.4, 120.0, 125.1, 127.2, 127.8-128.4, 129.4, 129.9, 133.4, 137.4, 137.6, 141.2, 143.1, 143.3, 155.0, 165.1; ³¹P NMR (121 MHz, CDCl₃) δ -2.23; Anal. Calcd for C₅₀H₅₅O₁₂P; C, 68.33; H, 6.31; Found: C, 68.23; H, 6.21.

Multigram synthesis of building block 5a from D-mannose (Scheme 3):

A solution of benzoyl chloride (193 mL, 1.665 mol) in pyridine (600 mL) was cooled to 0 °C in an ice-water bath. D-mannose (50 g, 0.278 mol) was added to the cooled reaction solution in small portions over a period of 30 min. The reaction mixture was allowed to warm to room temperature and the solution was kept stirring at room temperature for 12 h. The solvent was removed in vacuo and the residual material was extracted by EtOAc and 1 N HCl (aq.). The combined organic layer was washed with saturated NaHCO₃ (aq.) and concentrated in vacuo. The
crude product was recrystallized in hot EtOH to yield the perbenzoylated D-mannose as a white solid.

Perbenzoylated D-mannopyranoside (65.5 g, 99.32 mmol) was treated with acetic anhydride (14 mL, 150 mmol) and a solution of HBr in AcOH (100 mL of 33% solution, 579 mmol). The reaction was stirred at room temperature for 24 h, then poured in ice-cold water in a 500 mL separatory funnel, and extracted by CH₂Cl₂. The combined organic layer was concentrated in vacuo at room temperature to afford 1-bromo-2,3,4,6-O-benzoyl-α-D-mannopyranoside. The product obtained from this step was directly treated with 2,6-lutidine (46.27 mL, 397 mmol) and allyl alcohol (135 mL, 1986 mmol). The reaction was stirred at room temperature for 20 h and concentrated in vacuo. The crude product was co-evaporated with toluene (3x), dried under high vacuum for 12 h, dissolved in CH₂Cl₂, washed with water, and concentrated in vacuo to obtain a semi-solid product which was used in the next step without further purifications.

The following reactions were carried out in 15 to 50 mmol scales. The protected bicyclic orthoester crude product (ca 50 mmol) was dissolved in 1:1 THF/MeOH and treated with a freshly prepared solution of NaOMe in MeOH (metal Na in MeOH, 0.05 eq.) at reflux temperature for 8 h. The reaction mixture was concentrated in vacuo, re-dissolved in CH₂Cl₂ and filtered to a silica gel plug to remove methyl benzoate. Without further purifications the triol-bicyclic orthoester crude product was dissolved in CH₃CN and treated with camphor sulfonic acid (0.15 eq.) and 4Å molecular sieves (1x the mass of the crude product) at room temperature for 24 h. The reaction mixture was neutralized by addition of Et₃N and filtered to remove 4Å molecular sieves and concentrated in vacuo. The tricyclic orthoester crude product was dissolved in DMF and cooled to 0 °C before BnBr (3 eq.) and NaH (3 eq.) were added to the solution consecutively. The reaction mixture was allowed to warm to room temperature and kept stirring at room temperature for 12 h. The reaction mixture was extracted with Et₂O and water and washed with brine. The combined organic layer was concentrated in vacuo, and recrystallized in EtOAc and hexanes to obtain the title compound as a white solid (70% - 80%).

Automated Modules:

**Module A:** The resin is washed 6 times with THF for 15 sec. each.

**Module B:** The resin is washed with DCM for 15 sec. followed by hexanes. Repeated 6 times.
Module C: The resin is washed 6 times with DCM for 15 sec. each.

Module D: The building block (5 eq., 0.125 mmol in 1.0 mL DCM) is delivered to the reaction vessel containing the resin. The mixture is allowed to cool for 3 min. (with vortex for 30 sec. followed by standing for 30 sec.). TMSOTf (5 eq., 0.125 mmol, in 1.0 mL DCM) is added to the reaction vessel with vortex in two portions, with 2 min. interval. The reaction mixture is then left for 45 min. (with vortex for 30 sec. followed by standing for 30 sec.). After that time, the solution is drained and the resin is washed once with DCM.

Module E: The resin is submitted to piperidine (20% v/v in DMF, 2 mL) for 5 min. (with vortex for 30 sec., followed by standing for 30 sec.). After that time, the solution is drained and the resin is submitted to the same conditions twice.

Module F: The resin is washed 6 times with acetic acid (0.2 M in DCM) for 15 sec. each.

Automated synthesis of α(1-6) hexamannoside 7:
The octenediol resin (104 mg, 26 µmol) was loaded in the reaction vessel and the modules A, B, and C were performed at room temperature. The reaction vessel was cooled to -15°C and modules D and C were performed. The reaction vessel was warmed to room temperature and modules A, C, E, C, F, A, B, and C were performed to complete the first cycle. This cycle was executed six times to furnish the resin-bound hexasaccharide. The resin was washed manually with alternating DCM and Methanol and dried under high vacuum overnight. The resin was swelled in 2 mL DCM and treated with 2 mg of Grubb’s first generation catalyst. The flask was put under an atmosphere of ethylene and gently stirred overnight. The resin was washed six times with 2 mL of DCM. The washing solution was concentrated and loaded on a silica gel column. Elution with 20-30% ethyl acetate/hexanes furnished the desired hexamannoside 7 (57.5 mg, 80%). $[\alpha]_D^{25} = +44.8$ (c = 0.83, CHCl3); $^1$H NMR (300 MHz, CDCl3) δ 8.18-8.07 (m, 12H), 7.55-7.47 (m, 18H), 7.35-7.09 (m, 60H), 5.85-5.72 (m, 6H), 5.65-5.64 (m, 1H), 5.11-4.70 (m, 19H), 4.58 (t, $J$=10.5 Hz, 2H), 4.46-4.32 (m, 9H), 4.13-3.38 (m, 34H), 2.18-2.06 (m, 2H), 1.69-1.57 (m, 2H); $^{13}$C NMR (75 MHz, CDCl3) δ 165.9, 165.6, 165.6, 165.5, 138.6, 138.6, 138.5, 138.4, 138.0, 137.9, 137.7, 137.6, 137.6, 133.3, 130.0, 129.9, 128.7, 128.6, 128.5, 128.4, 128.2, 128.2,
128.2, 128.1, 128.1, 127.7, 127.4, 127.3, 127.2, 127.2, 115.1, 98.5, 98.5, 98.4, 98.3, 98.2, 98.0, 78.7, 78.4, 78.3, 78.2, 78.0, 77.7, 77.3, 75.2, 75.1, 75.1, 74.2, 74.0, 73.8, 73.7, 72.1, 71.7, 71.4, 71.2, 71.0, 70.8, 69.1, 68.6, 68.4, 67.4, 66.1, 65.8, 65.5, 61.9, 30.3, 28.6;

HRMS-MALDI (m/z): [M+Na]^+ Calcd for C_{167}H_{166}O_{37}, 2786.100; Found: 2786.092.

Reference:
Sample directory: d2o
Pulse Sequence: s2pul

Supplementary Material (ESI) for Chemical Communications
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Pulse Sequence: s2puf
STANDARD 1H OBSERVE

Sample directory: rw050906_5
File: /export/home/ioonmr/vnmrsys/exp1/text

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: preiko
Mercury-300BB "gem5oc"

Relax. delay 1.000 sec
Pulse 58.2 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
8 repetitions
OBSERVE H1, 299.7729174 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 25 sec
13C OBSERVE

Pulse Sequence: s2pul
Solvent: CDC13
Ambient temperature
User: pefiko
File: nr150906_1C
UNITYplus-300 "nmroc"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 20000.0 Hz
512 repetitions
OBSERVE C13, 75.4911544 MHz
DECOUPLE H1, 300.2242455 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 19 min, 42 sec
STANDARD 1H OBSERVE

Sample directory: rw070906_5
File: /export/home/locnmr/mmrsys/exp1.txt

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: preiko
Mercury-300BB "gem5oc"

Relax. delay 1.000 sec
Pulse 56.2 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
8 repetitions
OBSERVE H1, 299.7729174 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 25 sec
13C OBSERVE

File: CARBON

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
User: preiko
Mercury-300BB "gem5oc"

Relax, delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 20000.0 Hz
128 repetitions
OBSERVE C13, 75.4911544 MHz
DECOUPLE H1, 300.2242455 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 85536
Total time 5 min, 24 sec

ppm
13C OBSERVE

Sample directory: we
Pulse Sequence: s2pul

HO-BnO
BzO
O-P-OBu
OBu

6d

180  160  140  120  100  80  60  40  20  ppm
P-31 STANDARD PARAMETERS
PHOSPHATE REGION

Pulse Sequence: s2pul
Solvent: CDC13
Ambient temperature
User: preiko
File: rw301006_4p
Mercury-300BB "gem6oc"

Pulse 45.0 degrees
Acq. time 1.002 sec
Width 31948.9 Hz
64 repetitions
OBSERVE P31, 121.5324811 MHz
DECOUPLE H1, 300.2242455 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 1 min, 22 sec
XL13-6-pure 1H
File: PROTON
Pulse Sequence: s2pul

FmocO

OBz

BnO

BnO

O

P

OBu

4a

OBu

2.05 3.47 18.67
2.10 1.03 1.94 4.22 2.05 8.87 4.81 4.19 5.66
Pulse Sequence: s2pul