Rapid assembly of the complex, doubly-branched pentasaccharide domain of the immunoadjuvant jujuboside A via convergent B(C₆F₅)₃-catalyzed glycosylation of sterically-hindered precursors

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A. MATERIALS AND METHODS

Reactions were performed in flame-dried sealed tubes or modified Schlenk (Kjeldahl shape) flasks fitted with a glass stopper under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe. The appropriate carbohydrate and sulfoxide reagents were dried via azeotropic removal of water with toluene. Molecular sieves were activated at 350 °C and were crushed immediately prior to use, then flame-dried under vacuum. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography was performed using glass plates pre-coated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm) and visualized under UV light (254 and 360 nm), or stained with Ceric Ammonium Molybdate in conc. H₂SO₄.

Dichloromethane, tetrahydrofuran, diethyl ether, and toluene were purified by passage through two packed columns of neutral alumina under an argon atmosphere.¹ Methanol was distilled from magnesium at 760 Torr. Trifluoromethanesulfonic anhydride was distilled from phosphorus pentoxide at 760 Torr. Boron trifluoride diethyl etherate was distilled from calcium hydride at 760 Torr. All other chemicals were obtained from commercial vendors and were used without further purification unless otherwise noted.

Automated flash chromatography was performed with an Isco Combiflash medium-pressure liquid chromatography with Redisep silica columns (47-60 µm). Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX or a Bruker Tensor 27 spectrophotometer. Data are presented as the frequency of absorption (cm⁻¹). Proton and carbon-13 nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were recorded on a Bruker Avance III instrument; chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvent (d-chloroform: δ 7.26 for ¹H NMR, δ 77.16 for ¹³C NMR; d₆-benzene: δ 7.16 for ¹H NMR, δ 128.06 for ¹³C NMR; d₄-methanol: δ 3.31 for ¹H NMR, δ 49.00 for ¹³C NMR; d₃-acetonitrile: δ 1.94 for ¹H NMR, δ 1.32 for ¹³C NMR; deuterium oxide: δ 4.79 for ¹H NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration, assignment.

**B. SYNTHESIS OF DISACCHARIDE 3**


Tribenzoyl rhamnose 5 (290 mg, 0.608 mmol, 2.8 equiv), phenyl sulfoxide (351 mg, 1.74 mmol, 8.0 equiv) and 2,4,6-tri-tert-butylpyridine (591 mg, 2.39 mmol, 11.0 equiv) were dissolved in CH2Cl2/PhMe (4 ml/10 mL) and cooled to –78 ºC. Tf2O (102 µl, 0.608 mmol, 2.8 equiv) was added and after 15 min reaction mixture was warmed to –45 ºC and stirred for additional 1 hour. In a separate flask arabinose 6 (50.0 mg, 0.217 mmol, 1 equiv) was dissolved in CH2Cl2 (5 mL) and cooled to –45 ºC. This solution of arabinose 6 was added to the reaction mixture via cannula. The reaction was stirred at this temperature for 30 min then at 0 ºC for another 30 min. The reaction mixture was diluted with 200 mL of CH2Cl2 and washed with saturated solution of NaHCO3 (50 mL). Organic layer was dried over Na2SO4. Solids were removed by filtration and obtained solution was concentrated under reduced pressure. The residue was purified by automated SiO2 column chromatography (10 g solid loading cartridge, 40 g column, 1-100% EtOAc gradient in hexanes) to yield disaccharide 7 (120 mg, 80%) as a white foamy solid.

**TLC** \( R_f \) 0.14 (hexanes:ethyl acetate 5:1); \([\alpha]_D^{19} +141.8 \) (c 1.00, C6H6); **FTIR** (NaCl film) 2985, 1730, 1601, 1452, 1264, 1106, 811, 803, 744, 729, 721, 676, 654, 643, 611; **1H NMR** (600 MHz, CDCl3) δ 8.12 – 8.09 (m, 2H), 7.96 (dd, \( J = 8.3, 1.4 \) Hz, 2H), 7.80 (dd, \( J = 8.4, 1.4 \) Hz, 2H), 7.64 – 7.59 (m, 1H), 7.54 – 7.47 (m, 3H), 7.40 (dt, \( J = 17.5, 7.6 \) Hz, 3H), 7.26 – 7.22 (m, 2H), 6.02 (dddd, \( J = 16.9, 10.7, 6.0, 5.0 \) Hz, 1H), 5.88 (dd, \( J = 10.1, 3.4 \) Hz, 1H), 5.80 (dd, \( J = 3.4, 1.8 \) Hz, 1H), 5.64 (t, \( J = 9.9 \) Hz, 1H), 5.44 (dd, \( J = 17.2, 1.6 \) Hz, 1H), 5.33 (d, \( J = 1.8 \) Hz, 1H), 5.30 (dd, \( J = 10.4, 1.5 \) Hz, 1H), 4.99 (d, \( J = 3.3 \) Hz, 1H), 4.44 (dd, \( J = 7.9, 5.6 \) Hz, 1H), 4.33 – 4.20 (m, 3H), 4.05 (dd, \( J = 13.2, 6.0 \) Hz, 1H), 4.01 (d, \( J = 2.1 \) Hz, 2H), 3.87 (dd, \( J = 7.9, 3.4 \) Hz, 1H), 1.56 (s, 3H), 1.37 (s, 3H), 1.34 (d, \( J = 6.2 \) Hz, 3H); **13C NMR** (151 MHz, CDCl3) δ 165.85, 165.54, 165.53, 133.71, 133.56, 133.45, 133.17, 130.07, 129.83, 129.80, 129.58, 129.39, 129.35, 128.69, 128.54, 128.37, 117.88, 109.24, 98.45, 97.05, 77.37, 77.18, 77.16, 76.95, 74.91, 73.73, 72.05, 70.83, 69.91, 68.71, 67.09, 58.95, 28.45, 26.46, 17.91; **HRMS** (ESI) m/z Caled for C38H40O12Na [M+Na]+ 711.2417, found 711.2430.

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(2S,3R,4R,5S,6S)-2-(((2S,3R,4S,5S)-2-(allyloxy)-4,5-dihydroxytetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8) Disaccharide 7 (500 mg, 0.726 mmol, 1 equiv) was dissolved in CH$_2$Cl$_2$/MeOH (10 mL/20 mL) at ambient temperature. TsOH·H$_2$O (96.6 mg, 0.508 mmol, 0.7 equiv) was added and the reaction mixture was stirred for 4 hours. Et$_3$N (200 µl) was added to quench the reaction and concentrated. The residue was purified by automated SiO$_2$ column chromatography (10 g solid loading cartridge, 40 g column, 1-100% EtOAc in hexanes) to yield diol 8 (359 mg, 78%).

**TLC** $R_f$ 0.12 (hexanes:ethyl acetate 3:2); [α]$_D^{19}$ +90.7 (c 1.00, C$_6$H$_6$); **FTIR** (NaCl film) 3424, 2923, 1723, 1601, 1451, 1315, 1264, 1177, 1109, 1070, 1027, 709, 665; **$^1$H NMR** (500 MHz, CDCl$_3$) δ 8.02 (dd, $J = 8.3$, 1.4 Hz, 2H), 7.97 (dd, $J = 8.4$, 1.4 Hz, 2H), 7.84 – 7.77 (m, 2H), 7.59 – 7.54 (m, 1H), 7.51 (td, $J = 7.3$, 1.4 Hz, 1H), 7.45 – 7.35 (m, 5H), 7.26 – 7.21 (m, 2H), 6.05 – 5.95 (m, 1H), 5.86 (dd, $J = 10.0$, 3.4 Hz, 1H), 5.83 (dd, $J = 3.5$, 1.7 Hz, 1H), 5.66 (t, $J = 9.9$, 1H), 5.40 (dd, $J = 17.2$, 1.6 Hz, 1H), 5.26 (d, $J = 1.8$ Hz, 1H), 5.25 – 5.20 (m, 1H), 5.11 (d, $J = 3.4$ Hz, 1H), 4.40 (dq, $J = 9.9$, 6.2 Hz, 1H), 4.29 (ddt, $J = 13.1$, 5.2, 1.5 Hz, 1H), 4.22 (dt, $J = 9.8$, 3.8 Hz, 1H), 4.15 – 4.07 (m, 1H), 4.04 (dd, $J = 9.7$, 3.5 Hz, 1H), 3.97 (ddt, $J = 13.0$, 6.1, 1.5 Hz, 1H), 3.91 – 3.84 (m, 1H), 3.76 (dd, $J = 12.5$, 1.8 Hz, 1H), 3.56 (bs, 1H), 3.11 (bs, 1H), 3.13 (d, $J = 6.3$ Hz, 3H); **$^{13}$C NMR** (151 MHz, CDCl$_3$) δ 165.90, 165.81, 165.74, 165.74, 133.81, 133.68, 133.51, 133.51, 133.06, 129.86, 129.39, 129.34, 129.19, 128.71, 128.58, 128.46, 117.59, 117.59, 99.90, 98.00, 79.21, 77.37, 77.16, 76.95, 71.77, 70.70, 70.20, 69.51, 68.75, 68.14, 67.27, 62.05, 17.97; **HRMS** (ESI) $m/z$ Calcd for C$_{35}$H$_{56}$O$_{12}$Na [M+Na]$^+$ 671.2104, found 671.2097.

(2S,3R,4R,5S,6S)-2-(((2S,3R,4S,5S)-2-(allyloxy)-5-(benzoyloxy)-4-hydroxytetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl tribenzoate (3) i. Diol 8 (1.413 g, 2.178 mmol, 1 equiv.) and Ph(OEt)$_2$ (3.0 mL, 13 mmol, 6 equiv.) were dissolved in PhMe (30 mL) at ambient temperature. TsOH·H$_2$O (112.5 mg, 0.6534 mmol, 0.3 equiv.) was added and the reaction mixture was stirred at this temperature for 90 min. The reaction was quenched by addition of Et$_3$N (0.5 mL), then diluted with CH$_2$Cl$_2$ (100 mL) and washed with H$_2$O (2 x 40 mL). Organic layer was dried over Na$_2$SO$_4$. Solids were removed by filtration and the obtained solution was concentrated. The residue obtained was used in the subsequent step.

ii. The residue from step i was dissolved in AcOH/H$_2$O (16 ml/4mL) and stirred for 90 min. At this point AcOH and H$_2$O were azeotropically removed with the aid of PhMe and the
residue was purified by automated SiO$_2$ column chromatography (10 g solid loading cartridge, 40 g column, 1-100% EtOAc in hexanes) to yield disaccharide 3 (1.125 g, 69%) as a white solid.

**TLC** $R_f$ 0.40 (hexanes:ethyl acetate 2:1); $[\alpha]_D^{19}$ +188.3 (c 1.00, C$_6$H$_6$); **FTIR** (NaCl film) 3486, 2982, 1728, 1602, 1451, 1267, 1177, 1069, 1026, 928, 813, 804, 799, 791, 753, 739, 701, 664, 647, 633, 615; **$^1$H NMR** (600 MHz, CDCl$_3$) $\delta$ 8.12 – 8.08 (m, 2H), 8.08 – 8.05 (m, 2H), 7.98 (dd, $J$ = 8.3, 1.4 Hz, 2H), 7.82 (dd, $J$ = 8.3, 1.4 Hz, 2H), 7.63 – 7.58 (m, 1H), 7.58 – 7.51 (m, 2H), 7.50 – 7.38 (m, 7H), 7.29 – 7.23 (m, 2H), 6.04 (dddd, $J$ = 16.9, 10.8, 6.1, 5.0 Hz, 1H), 5.89 (dd, $J$ = 10.1, 3.4 Hz, 1H), 5.78 (dd, $J$ = 3.4, 1.8 Hz, 1H), 5.68 (t, $J$ = 9.9 Hz, 1H), 5.54 – 5.48 (m, 1H), 5.44 (dq, $J$ = 17.3, 1.6 Hz, 1H), 5.32 (d, $J$ = 1.8 Hz, 1H), 5.29 (dd, $J$ = 10.5, 1.4 Hz, 1H), 5.17 (d, $J$ = 3.5 Hz, 1H), 4.44 (dt, $J$ = 10.1, 4.0 Hz, 1H), 4.40 (dd, $J$ = 9.8, 6.2 Hz, 1H), 4.32 (ddt, $J$ = 13.0, 5.1, 1.5 Hz, 1H), 4.12 (dd, $J$ = 10.0, 3.5 Hz, 1H), 4.05 – 4.02 (m, 1H), 4.02 – 3.99 (m, 1H), 3.88 (dd, $J$ = 13.1, 1.9 Hz, 1H), 2.65 (d, $J$ = 4.4 Hz, 1H), 1.36 (d, $J$ = 6.3 Hz, 3H). **$^{13}$C NMR** (151 MHz, CDCl$_3$) $\delta$ 166.49, 165.82, 165.70, 165.58, 133.66, 133.63, 133.51, 133.36, 133.27, 130.02, 129.99, 129.96, 129.85, 129.82, 129.41, 129.37, 129.25, 128.70, 128.58, 128.55, 128.41, 117.90, 99.80, 97.87, 79.04, 77.37, 77.16, 76.95, 72.31, 71.85, 70.69, 70.01, 68.88, 67.33, 67.02, 60.84, 17.98; **HRMS** (ESI) $m/z$ Caled for C$_{42}$H$_{40}$O$_{13}$Na [M+Na]$^+$ 775.2367, found 775.2332.
C. SYNTHESIS OF TRISACCHARIDE 2

(2S,3R,4S,5R,6R)-6-(((2R,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (9) β-gentiobiose (350 mg, 0.892 mmol, 1 equiv.) was suspended in mixture of Py/Ac₂O (30 mL/ 45 mL) and heated to 60 ºC for 24 hrs. Reaction mixture was cooled to room temperature, diluted with EtOAc (500 mL), and washed sequentially with H₂O (2 x 100 mL), 1M HCl (2 x 150 mL), saturated solution of NaHCO₃ (150 mL) and brine (150 mL). Organic layer was dried on MgSO₄. Solids were removed by filtration and obtained solution was concentrated. The residue was purified by automated SiO₂ column chromatography (10 g solid loading cartridge, 40 g column, 1-100% EtOAc in hexanes) to yield β-gentiobiose octaacetate 9 (617.5 mg, 95%) as white solid.

TLC  \( R_f \) 0.20 (hexanes:ethyl acetate 1:1); \([\alpha]_D^{19}+1.5 \) (c 1.00, C₆H₆); FTIR (NaCl film) 2945, 1753, 1433, 1369, 1220, 1038, 908, 734, 699; ¹H NMR (600 MHz, CDCl₃) δ 5.68 (d, \( J = 8.3 \) Hz, 1H), 5.22 (t, \( J = 9.4 \) Hz, 1H), 5.18 (t, \( J = 9.5 \) Hz, 1H), 5.10 – 5.03 (m, 2H), 5.01 – 4.95 (m, 2H), 4.53 (d, \( J = 8.0 \) Hz, 1H), 4.25 (dd, \( J = 12.3, 4.9 \) Hz, 1H), 4.11 (dd, \( J = 12.3, 2.3 \) Hz, 1H), 3.92 (d, \( J = 11.4, 5.7 \) Hz, 1H), 2.10 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 170.68, 170.27, 170.11, 169.54, 169.41, 169.40, 169.25, 168.83, 100.62, 91.58, 77.24, 77.03, 76.82, 73.88, 72.85, 72.73, 71.90, 70.86, 70.22, 68.39, 68.29, 67.49, 61.82, 20.82, 20.77, 20.64, 20.62, 20.60, 20.59, 20.58; HRMS (ESI) m/z Calcd for C₂₈H₃₈O₁₉Na [M+Na]^+ 701.1905, found 701.1882.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(((2R,3S,4R)-3,4-diacetoxy-3,4-dihydro-2H-pyran-2-yl) methoxy)tetrahydro-2H-pyran-2,3,4,5-tetrayl triacetate (11) TiBr₄ (302 mg, 0.822, 0.6 equiv.) was suspended in CH₂Cl₂ (10 mL) at ambient temperature. AcOH (1 drop) was added and stirred for 5 min. Solution of β-gentiobiose octaacetate 9 (930.0 mg, 1.371 mmol, 1 equiv.) in CH₂Cl₂ (10 mL) was added dropwise. The addition funnel was rinsed with additional CH₂Cl₂ (10 mL) and added to the reaction mixture. Reaction mixture was stirred for 24 hrs and quenched by adding solid NaHCO₃ in 4 portions (500 mg each). Each portion was followed by addition of 30 drops of water. Additional water (50 mL) was added and extracted with CH₂Cl₂ (3 x 40 mL), dried over Na₂SO₄, filtered, concentrated to give crude glycosyl bromide as off white solid, which was carried to next step without further purification.

Glycosyl bromide (940 mg, 1.34 mmol, 1 equiv.), freshly activated zinc dust (439 mg, 6.72 mmol, 5 equiv.) and NH₄Cl (359 mg, 6.72 mmol, 5 equiv.) were combined in MeOH (1.6
mL) at ambient temperature. To this mixture V(O)Salen (10)\(^4\) (13 mg, 0.040 mmol, 0.03 equiv.) was added. Reaction mixture was stirred for 30 min, filtered through a pad of Celite and concentrated. The residue was dissolved in EtOAc (100 mL), washed with saturated solution of NaHCO\(_3\) (50 mL) and brine (50 mL), dried over MgSO\(_4\). Solids were removed by filtration and obtained solution was concentrated. The residue was purified by automated SiO\(_2\) column chromatography (10 g solid loading cartridge, 40 g column, 1-100% EtOAc in hexanes) to yield glucal 11 (553 mg, 73% over 2 steps) as a white solid.

TLC \(R_f\) 0.31 (hexanes:ethyl acetate 1:1); [\(\alpha\)]\(_{D}\)\(^19\) -28.9 (c 1.00, C\(_6\)H\(_6\)); FTIR (NaCl film) 2959, 2891, 1751, 1650, 1433, 1371, 1224, 1039, 906, 820, 735, 701; \(^1\)H NMR (600 MHz, C\(_6\)D\(_6\)) \(\delta\) 6.14 (dd, \(J = 6.2, 1.2\) Hz, 1H), 5.43 (dt, \(J = 5.1, 1.2\) Hz, 1H), 5.40 (t, \(J = 9.6\) Hz, 1H), 5.35 (dd, \(J = 6.8, 5.1\) Hz, 1H), 5.30 (dd, \(J = 9.6, 7.9\) Hz, 1H), 5.25 (dd, \(J = 10.1, 9.3\) Hz, 1H), 4.70 (dd, \(J = 6.0, 3.5\) Hz, 1H), 4.29 (d, \(J = 7.9\) Hz, 1H), 4.24 (dd, \(J = 12.3, 4.4\) Hz, 1H), 4.17 (tdd, \(J = 6.6, 4.3, 1.0\) Hz, 1H), 4.00 (dd, \(J = 12.4, 2.3\) Hz, 1H), 3.97 (dd, \(J = 11.2, 4.4\) Hz, 1H), 3.65 (dd, \(J = 11.2, 6.6\) Hz, 1H), 3.18 (ddd, \(J = 10.1, 4.4, 2.3\) Hz, 1H), 1.89 (s, 3H), 1.70 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H), 1.67 (s, 3H), 1.63 (s, 3H); \(^{13}\)C NMR (151 MHz, C\(_6\)D\(_6\)) \(\delta\) 170.07, 169.99, 169.88, 169.26, 169.09, 169.08, 145.83, 101.09, 98.91, 74.82, 73.26, 72.25, 71.63, 68.63, 67.92, 66.96, 66.92, 61.67, 20.57, 20.44, 20.37, 20.28, 20.25, 20.18; HRMS (ESI) \(m/z\) Calcd for C\(_{24}\)H\(_{32}\)O\(_{15}\)Na [M+Na]\(^+\) 583.1639, found 583.1630.

Glucal 11 (1.00 g, 1.78 mmol, 1 equiv.) was dissolved in CH\(_2\)Cl\(_2\) (40 mL) and cooled to –78 ºC. Solution of dimethyldioxirane (0.065 M, 41.2 mL, 2.67 mmol, 1.5 equiv.) was added and the reaction mixture was stirred for 30 min at –78 ºC, then warmed to 0 ºC and stirred for another 20 min and concentrated under vacuum (at 0 ºC). The residue was dissolved in allyl alcohol (40 mL) and stirred for 5 hrs at ambient temperature. Excess allyl alcohol was removed by rotary evaporation and the residue was purified by automated SiO\(_2\) column chromatography (10 g solid loading cartridge, 80 g column, 1-100% MTBE in PhMe) to yield disaccharide 12 (827 mg, 73%) as a white solid.

TLC \(R_f\) 0.13 (hexanes:ethyl acetate 1:1); [\(\alpha\)]\(_{D}\)\(^19\) +9.0 (c 1.00, C\(_6\)H\(_6\)); FTIR (NaCl film) 3487, 2944, 1752, 1431, 1368, 1223, 1171, 1037, 907, 735, 699, 600; \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 5.94 (dddd, \(J = 17.0, 10.4, 6.4, 5.1\) Hz, 1H), 5.38 (dq, \(J = 17.3, 1.6\) Hz, 1H), 5.28 (dq, \(J = 10.4, 1.3\) Hz, 1H), 5.20 (t, \(J = 9.5\) Hz, 1H), 5.13 (t, \(J = 9.6\) Hz, 1H), 5.09 (t, \(J = 9.5\) Hz, 1H), 5.02 (dd, \(J = 9.6, 8.0\) Hz, 1H), 4.87 (dd, \(J = 10.1, 9.3\) Hz, 1H), 4.61 (d, \(J = 8.0\) Hz, 1H), 4.44 – 4.39 (m, 2H), 4.28 (dd, \(J = 12.3, 4.8\) Hz, 1H), 4.20 – 4.12 (m, 2H), 3.89 (dd, \(J = 11.0, 2.1\) Hz, 1H), 3.73 – 3.65 (m, 2H), 3.65 – 3.55 (m, 2H), 2.48 (d, \(J = 3.0\) Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 170.81, 170.77.

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170.37, 169.95, 169.53, 169.39, 133.31, 118.58, 101.48, 100.84, 77.37, 77.16, 76.95, 74.50, 73.46, 72.87, 72.27, 72.03, 71.20, 70.45, 69.15, 68.35, 61.93, 20.95, 20.89, 20.82, 20.75, 20.74; HRMS (ESI) m/z Calcd for C_{27}H_{38}O_{17}Na \ ([M+Na]^+) 657.2007, found 657.2029.

(2S,3R,4S,5R)-2-(((2R,3R,4S,5R,6R)-4,5-diacteoxy-2-(allyloxy)-6-((2R,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (14) β-gentiobiose derivative 12 (160 mg, 0.252 mmol, 1 equiv.) and xylose trichloroimidate 13 (306 mg, 0.504 mmol, 2 equiv.) were azeotropically dried with PhMe (3 mL), then dissolved in CH$_2$Cl$_2$ (15 mL) and cooled to –20 ºC. Powdered 4A mol. sieves (100 mg) was added and the mixture was stirred for 10 min. TMSOTf (4.2 µl, 0.025 mmol, 0.12 equiv.) was added and the reaction mixture was stirred at – 20 ºC for 40 min, and then quenched by adding Et$_3$N (250 µl). The reaction mixture was allowed to warm to room temperature, filtered through a pad of celite and the obtained solution was concentrated. The residue was purified by automated SiO$_2$ column chromatography (10 g solid loading cartridge, 40 g column, 1-100% EtOAc in hexanes) to yield trisaccharide 14 (225 mg, 83%) as a white solid.

TLC R$_f$ 0.29 (hexanes:ethyl acetate 1:1); [α]$_D^{19}$ -46.9 (c 1.00, C$_6$H$_6$); FTIR (NaCl film) 3063, 2944, 2881, 1753, 1601, 1584, 1491, 1452, 1367, 1260, 1176, 1039, 906, 803, 736, 713, 599; $^1$H NMR (600 MHz, CDCl$_3$) δ 7.99 – 7.94 (m, 2H), 7.91 – 7.85 (m, 2H), 7.83 – 7.76 (m, 2H), 7.50 – 7.38 (m, 3H), 7.33 – 7.28 (m, 4H), 7.22 – 7.16 (m, 2H), 5.84 (dddd, $J$ = 16.9, 10.4, 6.3, 5.1 Hz, 1H), 5.55 (t, $J$ = 5.9 Hz, 1H), 5.26 (dd, $J$ = 17.2, 1.6 Hz, 1H), 5.19 – 5.05 (m, 5H), 5.02 (d, $J$ = 4.0 Hz, 1H), 4.98 (t, $J$ = 9.7 Hz, 1H), 4.90 (dd, $J$ = 9.6, 7.9 Hz, 1H), 4.67 (t, $J$ = 9.7 Hz, 1H), 4.51 (dd, $J$ = 12.9, 3.6 Hz, 1H), 4.48 (d, $J$ = 8.0 Hz, 1H), 4.42 (d, $J$ = 7.7 Hz, 1H), 4.38 – 4.32 (m, 1H), 4.16 (dd, $J$ = 12.3, 4.8 Hz, 1H), 4.04 (dddd, $J$ = 12.3, 9.2, 5.2, 1.8 Hz, 2H), 3.75 (dd, $J$ = 11.1, 2.2 Hz, 1H), 3.70 (dd, $J$ = 9.5, 7.7 Hz, 1H), 3.62 (dd, $J$ = 12.8, 4.9 Hz, 1H), 3.57 (dd, $J$ = 10.0, 4.8, 2.5 Hz, 2H), 3.48 (dd, $J$ = 11.0, 7.3 Hz, 1H), 1.98 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.87 (s, 3H), 1.77 (s, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 170.74, 170.36, 170.10, 169.97, 169.53, 169.39, 165.76, 165.21, 165.17, 133.57, 133.52, 133.49, 133.33, 130.09, 129.96, 129.94, 129.51, 129.29, 129.23, 128.57, 128.52, 128.50, 128.47, 128.46, 128.44, 118.35, 100.84, 100.64, 99.16, 77.37, 77.16, 76.95, 76.76, 74.42, 73.11, 72.89, 72.05, 71.20, 70.53, 69.83, 69.57, 69.29, 68.70, 68.39, 68.34, 61.91, 60.64, 20.89, 20.83, 20.77, 20.75, 20.74, 20.70; HRMS (ESI) m/z Calcd for C$_{53}$H$_{58}$O$_{24}$Na [M+Na]$^+$ 1101.3216, found 1101.3182.
Deprotection of the trisaccharide (14) To the solution of the trisaccharide 14 (6.0 mg, 0.0067 mmol, 1 equiv) in MeOH (1 mL) was added MeONa (3.6 mg, 0.067 mmol, 10 equiv). The reaction mixture was stirred at ambient temperature for 16 hrs and concentrated. Residue was dissolved in D$_2$O and analyzed by $^1$H NMR.

(2S,3R,4S,5R)-2-(((2S,3R,4S,5R,6R)-4,5-diacetoxy-6-(((2R,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxyethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)-2-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (2) Trisaccharide 14 (182 mg, 0.169 mmol, 1 equiv) was dissolved in MeOH (10 mL). PdCl$_2$ (15.0 mg 0.0844 mmol, 0.5 equiv) was added and the reaction mixture was stirred at ambient temperature for 4 hrs and concentrated. The residue was then dissolved in CH$_2$Cl$_2$ (10 mL) and filtered through a pad of Celite. The Celite layer was washed with additional CH$_2$Cl$_2$ (3x 10 mL). Combined CH$_2$Cl$_2$ layers were concentrated and carried to the next step without further purification.

The residue from the previous step was dissolved in CH$_2$Cl$_2$ (10 mL) at ambient temperature. K$_2$CO$_3$ (233 mg, 1.68 mmol, 10 equiv.), followed by Cl$_3$CCN (169 µL, 1.68 mmol, 10 equiv.) were added. Reaction mixture was stirred at ambient temperature for 24 hours. Solids were filtered off and the obtained solution was concentrated. The residue was purified by automated SiO$_2$ column chromatography (10 g solid loading cartridge, 40 g column, 1-100% EtOAc in hexanes) to yield trichloroimidate imidate 2 (132 mg, 66%) as a white solid.

**TLC** $R_f$ 0.21 (hexanes:ethyl acetate 1:1); [α]$_D^{19}$ +8.5 (c 1.00, C$_6$H$_6$); **FTIR** (NaCl film) 3320, 3064, 2986, 2886, 2360, 1755, 1677, 1432, 1368, 1177, 1096, 1069, 971, 712; **$^1$H NMR** (500 MHz, CDCl$_3$) δ 8.75 (s, 1H), 8.03 – 7.97 (m, 2H), 7.92 (ddd, $J = 10.9, 8.2, 1.4$ Hz, 4H), 7.59 – 7.44 (m, 3H), 7.43 – 7.31 (m, 6H), 6.59 (d, $J = 3.5$ Hz, 1H), 5.70 (t, $J = 7.2$ Hz, 1H), 5.48 (t, $J = 9.8$ Hz, 1H), 5.32 – 5.24 (m, 2H), 5.17 (t, $J = 9.5$ Hz, 1H), 5.10 – 4.93 (m, 4H), 4.53 (d, $J = 7.9$ Hz, 1H), 4.39 (dd, $J = 12.4, 4.2$ Hz, 1H), 4.25 (dd, $J = 12.3, 4.5$ Hz, 1H), 4.16 – 4.07 (m, 3H), 3.96 (dd, $J = 10.3, 3.1$ Hz, 2H), 3.73 – 3.63 (m, 2H), 3.52 (dd, $J = 11.1, 4.5$ Hz, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.71 (s, 3H); **$^{13}$C NMR** (151 MHz, CDCl$_3$) δ 170.81, 170.36, 169.70, 169.67, 169.62, 169.49, 165.62, 165.60, 164.87, 160.85,
133.57, 133.55, 133.44, 130.08, 130.07, 130.01, 129.90, 129.25, 129.23, 128.91, 128.56, 128.53, 128.51, 128.46, 101.90, 100.54, 94.68, 90.96, 77.37, 77.29, 77.16, 76.95, 72.89, 71.92, 71.04, 70.98, 70.79, 70.22, 70.14, 68.96, 68.35, 68.31, 67.25, 61.86, 61.36, 20.89, 20.82, 20.77, 20.74, 20.73, 20.38; \textbf{HRMS (ESI)} m/z \textit{Calcd for C}_{52}\text{H}_{54}\text{NO}_{24}\text{Na} [\text{M+Na}]^{+} 1204.1999, \textit{found} 1204.2045.
D. SYNTHESIS OF PENTASACCHARIDE 16

(2S,3R,4R,5S,6S)-2-(((2S,3R,4S,5S)-2-(allyloxy)-5-(benzoyloxy)-4-(((2S,3R,4S,5R,6R)-4,5-diacectoxy-6-(((2R,3R,4S,5R,6R)-3,4,5-triacetoxo-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)-3-(((2S,3R,4S,5R)-3,4,5-tris(benzoyloxy)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl tribenzoate (β-16) Trichloroimidate 2 (12 mg, 0.010 mmol, 1 equiv) and disaccharide 3 (9 mg, 0.01 mmol, 1.2 equiv) were azeotropically dried with PhMe (0.5 mL) and dissolved in t-BuCN/CF3Ph (150 µL /750 µL) solvent mixture. B(C6F5)3 (0.5 mg, 0.001 mmol, 0.1 equiv.) was added and the reaction mixture was stirred at ambient temperature for 40 min, then quenched by adding 25 µL of Et3N. The reaction mixture was concentrated and purified by automated SiO2 column chromatography (5 g solid loading cartridge, 4 g column, 1-100% EtOAc in benzene) yielded pentasaccharides β-16 (7.5 mg, 42%) and α-16 (7 mg, 39%).

For β-16: TLC Rf 0.30 (benzene:ethyl acetate 2:1) [α]D19 +27.2 (c 0.70, C6H6); FTIR (NaCl film) 2924, 1727, 1602, 1451, 1367, 1265, 1069, 710, 665; 1H NMR (600 MHz, CDCl3) δ 8.21 (dd, J = 8.3, 1.4 Hz, 2H), 8.10 (dt, J = 8.2, 1.1 Hz, 4H), 8.04 (ddt, J = 17.7, 7.1, 1.4 Hz, 4H), 7.65 – 7.59 (m, 1H), 7.59 – 7.56 (m, 1H), 7.56 – 7.46 (m, 6H), 7.46 – 7.40 (m, 6H), 7.29 (tt, J = 7.5, 1.6 Hz, 4H), 7.25 – 7.22 (m, 1H), 7.16 (tt, J = 6.4, 2.4 Hz, 1H), 6.94 (ddt, J = 7.4, 6.1, 1.5 Hz, 3H), 6.88 – 6.83 (m, 4H), 6.03 – 5.95 (m, 2H), 5.92 (dd, J = 10.5, 9.4 Hz, 1H), 5.77 (q, J = 3.7, 3.0 Hz, 2H), 5.74 (d, J = 2.2 Hz, 1H), 5.73 – 5.69 (m, 2H), 5.63 (t, J = 9.2 Hz, 1H), 5.60 (d, J = 3.8 Hz, 1H), 5.35 (dd, J = 17.3, 1.5 Hz, 1H), 5.30 (d, J = 3.4 Hz, 1H), 5.26 (d, J = 7.7 Hz, 1H), 5.23 (dd, J = 10.4, 1.4 Hz, 1H), 5.19 (d, J = 9.5 Hz, 1H), 5.15 – 5.07 (m, 2H), 4.91 (d, J = 5.6 Hz, 1H), 4.81 – 4.71 (m, 2H), 4.57 (dd, J = 10.0, 3.8 Hz, 1H), 4.46 (dq, J = 9.4, 6.2 Hz, 1H), 4.41 – 4.34 (m, 2H), 4.34 – 4.27 (m, 2H), 4.24 (d, J = 13.2 Hz, 1H), 4.18 – 4.08 (m, 1H), 4.03 (dd, J = 10.3, 8.0, 2.3 Hz, 1H), 3.98 (ddt, J = 12.2, 7.3, 1.2 Hz, 1H), 3.87 (td, J = 12.7, 12.1, 2.1 Hz, 2H), 3.82 – 3.72 (m, 3H), 3.66 (dd, J = 9.4, 7.7 Hz, 1H), 3.13 (dd, J = 13.2, 3.2 Hz, 1H), 2.33 (s, 3H), 2.11 (s, 3H), 2.04 (d, J = 2.3 Hz, 4H), 2.02 (s, 3H), 1.93 (s, 3H), 1.46 (s, 3H), 1.35 (d, J = 6.2 Hz, 3H); 13C NMR (151 MHz, CDCl3) δ 170.89, 170.30, 170.25, 169.79, 169.65, 168.84, 166.44, 166.13, 166.06, 166.02, 165.90, 165.86, 164.20, 133.94, 133.50, 133.43, 133.22, 133.07, 132.88, 132.78, 132.29, 130.54, 130.30, 130.19, 130.18, 130.03, 129.92, 129.77, 129.73, 129.66, 129.60, 129.59, 129.44, 129.20, 129.18, 128.71, 128.67, 128.59, 128.55, 128.35, 128.11, 127.60, 118.42, 102.13, 101.51, 101.30, 99.00, 98.09, 81.22, 77.37, 77.16, 76.95, 76.76, 74.97, 73.61, 73.53, 73.14, 72.72, 72.15, 72.01, 71.65, 71.23, 71.21, 70.83, 70.44, 69.74, 69.14, 68.85, 68.63, 68.06, 63.57, 62.03, 60.84, 21.47, 20.97, 20.89, 20.85, 20.81, 20.55, 18.43; HRMS (ESI) m/z Calcd for C92H92O36Na [M+Na]+ 1795.5266, found 1795.5338.
For α-16: TLC $R_f$ 0.41 (benzene:ethyl acetate 2:1); $[\alpha]_D^{18} +53.4$ (c 0.44, C$_6$H$_5$); FTIR (NaCl film) 2935, 1730, 1611, 1368, 1262, 1177, 1069, 1028, 710; $^1$H NMR (600 MHz, CDCl$_3$) δ 8.41 (dd, $J = 6.7$, 3.0 Hz, 2H), 8.04 (dd, $J = 8.1$, 1.4 Hz, 2H), 7.93 (ddd, $J = 10.0$, 8.4, 1.5 Hz, 4H), 7.79 (dd, $J = 8.1$, 1.4 Hz, 2H), 7.77 - 7.72 (m, 2H), 7.69 (td, $J = 5.6$, 4.7, 1.4 Hz, 5H), 7.60 (d, $J = 7.4$ Hz, 1H), 7.55 - 7.41 (m, 7H), 7.41 - 7.34 (m, 5H), 7.33 - 7.27 (m, 5H), 6.07 (ddt, $J = 16.3$, 10.7, 5.5 Hz, 1H), 5.81 (t, $J = 2.0$ Hz, 1H), 5.68 - 5.60 (m, 2H), 5.52 - 5.38 (m, 6H), 5.30 - 5.23 (m, 2H), 5.20 (d, $J = 3.6$ Hz, 1H), 5.14 - 5.09 (m, 2H), 5.07 (dd, $J = 10.1$, 9.0 Hz, 1H), 4.60 (td, $J = 9.9$, 5.7 Hz, 1H), 4.55 (t, $J = 7.9$ Hz, 2H), 4.52 - 4.48 (m, 1H), 4.48 - 4.44 (m, 1H), 4.41 (dq, $J = 10.5$, 6.1 Hz, 1H), 4.34 (dd, $J = 10.2$, 5.1, 2.6 Hz, 3H), 4.32 - 4.27 (m, 2H), 4.23 - 4.18 (m, 2H), 4.10 (dd, $J = 13.1$, 2.0 Hz, 1H), 3.99 - 3.94 (m, 1H), 3.92 (d, $J = 12.8$ Hz, 1H), 3.86 (dd, $J = 11.2$, 1.7 Hz, 1H), 3.74 (dd, $J = 10.1$, 4.5, 2.5 Hz, 1H), 3.67 (dd, $J = 10.1$, 3.5 Hz, 1H), 3.29 (t, $J = 10.8$ Hz, 1H), 2.10 (s, 3H), 2.02 (d, $J = 1.5$ Hz, 9H), 1.68 (s, 3H), 1.37 (d, $J = 6.2$ Hz, 3H), 0.96 (s, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 170.93, 170.53, 170.45, 170.02, 169.59, 169.32, 166.72, 165.63, 165.59, 165.41, 165.24, 164.75, 164.16, 133.72, 133.53, 133.36, 133.33, 133.28, 133.24, 132.91, 132.61, 132.40, 130.06, 129.84, 129.94, 129.91, 129.80, 129.71, 129.69, 129.65, 129.41, 129.33, 129.26, 129.22, 129.12, 128.61, 128.57, 128.54, 128.51, 128.36, 128.30, 117.63, 103.77, 101.27, 100.87, 98.03, 93.58, 79.49, 77.90, 77.37, 77.16, 76.95, 73.01, 72.36, 71.87, 71.66, 71.28, 70.96, 70.70, 69.93, 69.23, 69.00, 68.68, 68.65, 68.12, 68.09, 67.73, 67.30, 67.05, 62.64, 62.12, 59.92, 20.88, 20.84, 20.82, 20.79, 20.43, 19.52, 18.00; HRMS (ESI) m/z Calcd for C$_{92}$H$_{136}$O$_3$Na [M+Na]$^+$ 1795.5266 found 1795.5287.
E. Deprotection of Trisaccharide 13 and Pentasaccharides $\alpha$-16 and $\beta$-16

Deprotection of pentasaccharides $\alpha$-16 and $\beta$-16. To the solution of the pentasaccharide $\alpha$-16 or $\beta$-16 (8.5 mg, 0.0048 mmol, 1 equiv) in MeOH (1 mL) was added MeONa (4.4 mg, 0.082 mmol, 17 equiv). The reaction mixture was stirred at ambient temperature for 16 hrs and concentrated. Residue was dissolved in D$_2$O and analyzed by $^1$H NMR.

Figure S1. $^1$H NMR analysis of deprotected di-, tri- and pentasaccharides.
F. $^1$H AND $^{13}$C NMR SPECTRA
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α-17

$^1$H-$^1$C HSQC correlations of anomic protons and carbons

β-17

$^1$H-$^1$C HSQC correlations of anomic protons and carbons
$^1$H–$^1$H COSY spectrum of pentasaccharide $\alpha$-16

$\alpha$-16
\( ^1H-\text{HSQC spectrum of pentasaccharide alfa-16} \)
$\beta$-16

$^1$H–$^1$H COSY spectrum of pentasaccharide $\text{beta}–16$
β-16

$^1$H–$^1$C HSQC spectrum of pentasaccharide α-16

$^1$H–$^1$C HSQC correlations of anomeric protons and carbons