Progress toward developing a carbohydrate-conjugate vaccine against *Clostridium difficile* ribotype 027: Synthesis of the cell-surface polysaccharide PS-I repeating unit

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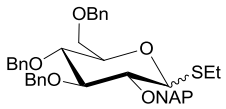
Supporting Information

General Experimental

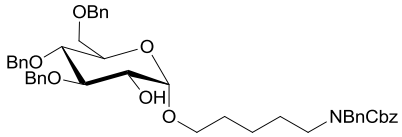
Commercial grade reagents and solvents were used without further purification, except as indicated below. All batch reactions were conducted under an Ar atmosphere. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Varian 400-MR or Varian 600 spectrometer. The proton signal of residual, non-deuterated solvent (δ 7.26 ppm for $CHCl_3$; δ 4.79 ppm for H_2O, 2.84 ppm for acetone) was used as an internal reference for ^{1}H spectra. For ^{13}C spectra, the chemical shifts are reported relative to the respective solvent (δ 77.16 ppm for $CDCl_3$, δ 29.84 ppm for acetone). For ^{13}C spectra in D_2O, MeOH (δ 49.50 ppm) was added as internal standard. Coupling constants are reported in Hertz (Hz). The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; m multiplet. Infrared (IR) spectra were recorded as thin films on a Perkin Elmer Spectrum 100 FTIR spectrophotometer. Optical rotations (OR) were measured with a Schmidt & Haensch UniPol L 1000 at 589 nm and a concentration (c) expressed in g/100 ml. High-resolution mass spectra (HRMS) were recorded with an Agilent 6210 ESI-TOF mass spectrometer at the Freie Universität Berlin, Mass Spectrometry Core Facility. MALDI-TOF spectra were recorded on a Bruker Daltonics Autoflex Speed, using a 2,4,6-trihydroxyacetophenone (THAP) matrix.
Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or a 1:1 mixture of H_2SO_4 (2N) and resorcine monomethylether (0.2%) in ethanol. Column chromatography was performed using silica gel 60 (230–400 mesh).

Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-(2-naphthalenylmethyl)-1-thio-D-glucopyranoside (9)



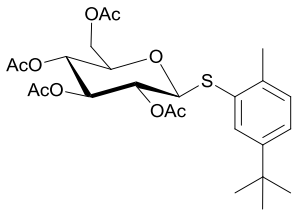
To a solution of **8**[1] (284 mg, 0.57 mmol) in anhydrous DMF (1 ml), NaH (20.7 mg, 0.86 mmol) was added followed by NAP-Br (228 mg, 1.03 mmol) at 0 °C. The mixture was warmed to room temperature over 1 h, cooled to 0 °C and quenched by the addition of MeOH (0.1 ml). Et_2O was added and the organic layer washed with 10 mM HCl solution and with saturated aqueous $NaHCO_3$ solution. The phases were separated and the organic layer was dried over $MgSO_4$ and concentrated. Column chromatography (cyclohexane/ethyl acetate) afforded **9** (335 mg, 0.53 mmol, 92%) as mixture of α/β-anomers as a white solid. Analytical data is reported for the β-anomer. $[\alpha]_D^{20}$ = +26.1 º (c = 5.3, $CHCl_3$), IR ν_{max} (film) 3061, 3030, 2864, 1949, 1808, 1603, 1497, 1453, 1360, 1065 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.82-7.69 (4H, m, Ar-H), 7.52-7.09 (18H, m, Ar-H), 5.08-5.02 (1H, m, -CH_2-Ar), 4.93-4.77 (4H, m, -CH_2-Ar), 4.60-4.50 (3H, m, -CH_2-Ar), 4.47 (1H, d, *J* 9.7, 1-H), 3.80-3.54 (4H, m), 3.52-3.41 (2H, m), 2.84-2.66 (2H, m, S-CH_2-), 1.31 (3H, t, *J* 7.3, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.7, 138.4, 138.2, 135.6, 133.4, 133.2, 128.56, 128.55, 128.5, 128.2, 128.1, 127.92, 127.87, 127.84, 127.80, 127.77, 127.7, 127.2, 126.5, 126.1, 126.0, 86.8, 85.2 (C-1), 82.0, 79.3, 78.2, 75.9, 75.7, 75.2, 73.6, 69.3, 25.2, 15.3; HRMS (ESI): Calcd for $C_{40}H_{42}O_5S$ $[M+Na]^+$ 657.2651, found 657.2651.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (2)



Thioglucoside **9** (335 mg, 0.53 mmol) and $HO(CH_2)_5NBnCbz$ (518 mg, 1.58 mmol) were coevaporated with toluene three times, dried *in vacuo*, then the compounds were dissolved in a solution of anhydrous toluene:dioxane=2:1 (4.5 ml). The solution was cooled to -40 °C, treated with NIS (131 mg, 0.58 mmol) and TfOH (4.7 µl, 53 µmol) and warmed to -20 °C over 1.5 h. The reaction was quenched with pyridine, diluted with DCM and washed with saturated aqueous $Na_2S_2O_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) gave a mixture of anomers which was dissolved in DCM (10 ml) and water (1 ml) and treated with DDQ (202 mg, 0.89 mmol) at 0 °C for 2 h. The mixture was diluted with DCM and the organic layer washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **2** (140 mg, 0.184 mmol, 35%) as a colorless oil. $[\alpha]_D^{20}$ = +53.3 ° (c = 5.5, $CHCl_3$), IR ν_{max} (film) 3458, 3031, 2927, 1952, 1876, 1808, 1454, 1421, 1360, 1229, 1129, 1067 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d6) δ 7.48-7.10 (25H, m, Ar-H), 5.15 (2H, bs), 4.99 (1H, d, *J* 11.4, -CH_2-Bn), 4.84 (1H, d, *J* 11.1, -CH_2-Bn). 4.79 (1H, d, *J* 11.4, -CH_2-Bn), 4.75 (1H, bs, 1-H), 4.62-4.49 (5H, m, -CH_2-Bn), 3.84-3.66 (6H, m), 3.62-3.47 (2H, m), 3.40 (1H, m), 3.31-3.18 (2H, m, linker-CH_2-), 1.67-1.50 (4H, m, linker-CH_2-), 1.43-1.29 (2H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, acetone-d6) δ 140.5, 139.8, 139.7, 139.5, 129.3, 129.1, 129.0, 128.9, 128.60, 128.58, 128.43, 128.41, 128.2, 128.0, 99.9 (C-1), 84.3, 78.7, 75.5, 75.4, 74.2, 73.7, 71.5, 70.2, 68.5, 67.4, 24.1; HRMS (ESI): Calcd for $C_{47}H_{53}NO_8$ $[M+Na]^+$ 782.3669, found 782.3633.

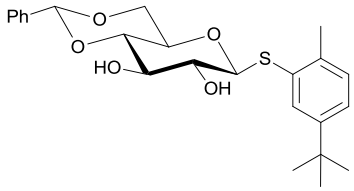
(2-Methyl-5-*tert*-butylphenyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (11)



Commercially purchased 1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose **10** (30 g, 77 mmol) was dissolved in anhydrous DCM (34 ml). 2-Methyl-5-*tert*-butyl thiophenol (17 ml, 92 mmol) was added with constant stirring. $BF_3{\cdot}OEt_2$ (13.6 ml, 108 mmol) was added dropwise and the resulting yellow solution was stirred overnight. After completion, the solution was diluted with DCM and extracted with saturated aqueous $NaHCO_3$ and H_2O, and the organic layer was dried over $MgSO_4$. The solvent was evaporated and the residue was dried *in vacuo*. The resulting yellow solid was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **11** (33.4 g, 65.4 mmol, 85%). $[\alpha]_D^{20}$ = -8.0 ° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2961, 1747, 1366, 1211, 1034, 912 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 (1H, d, *J* 2.0, Ar-H), 7.25-7.10 (2H, m, Ar-H), 5.19 (1H, dd, J_1J_2 9.4, 1-H), 5.10-4.98 (2H, m, 4-H, 2-H), 4.64 (1H, d, *J* 10.6, 1-H), 4.23 (1H, dd, J_1 12.2, J_2 5.0, 6-Ha), 4.10 (1H,

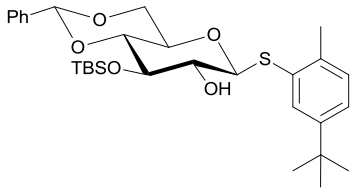
dd, J_1 12.2, J_2 1.9, 6-Hb), 3.71-3.63 (1H, m, 5-H), 2.34 (3H, s, CH_3), 2.07-2.03 (6H, m, OAc), 2.00-1.96 (6H, m, OAc), 1.29 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 170.3, 169.5, 169.4 (C=O OAc), 149.8, 137.51, 131.47, 130.53, 130.2, 125.8, 87.0 (C-1), 75.9 (C-5), 74.2 (C-3), 70.3 (C-3), 68.3(C-4), 62.4 (C-6), 31.4 (*t*Bu), 20.89, 20.88, 20.74, 20.70 (OAc), 20.5 (CH_3); HRMS (ESI): Calcd for $C_{25}H_{34}O_9S$ $[M+Na]^+$ 533.1816, found 533.1832.

(2-Methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (12)



Thioglycoside **11** (1.5 g, 2.94 mmol) was dissolved in methanol (12 ml). Sodium methoxide (58 mg, 1.07 mmol) was added and the reaction was stirred overnight. After completion, the solution was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The remainder was dried *in vacuo* to give (2-Methyl-5-*tert*-butylphenyl) 1-thio-β-D-glucopyranoside (1.0 g), which was used for the next reaction step without further purification. (2-Methyl-5-*tert*-butylphenyl) 1-thio-β-D-glucopyranoside (1.0 g) was dissolved in anhydrous acetonitrile (11.3 ml) at room temperature under argon atmosphere and benzaldehyde dimethylacetal (880 µL, 5.84 mmol) and camphorsulfonic acid (7 mg, 0.029 mmol) were added. After 2.5 h, the reaction was quenched with triethylamine, and the solvents were evaporated to give 1.5 g of colorless oil. The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **12** (1.09 g, 2.53 mmol, 87%). $[\alpha]_D^{20}$ = -49.4 º (c = 1.0, CH_2Cl_2); IR ν_{max} (film) 3410, 2963, 2870, 1384, 1264, 1082, 1072, 1029, 1003, 972 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (1H, d, *J* 2.0 Hz, Ar-H), 7.51-7.46 (2H, m, Ar-H), 7.39-7.35 (m, 3H, Ar-H), 7.29-7.23 (m, 2H, Ar-H), 7.16 (1H, d, *J* = 8.0, Ar-H), 5.54 (1H, s, benzylidene-H), 4.64 (1H, d, *J* 10.0, 1-H), 4.36 (1H, dd, J_1 10.3, J_2 4.5, 6-Ha), 3.90-3.73 (2H, m, 3-H, 6-Hb), 3.59-3.47 (3H, m, 2-H, 4-H, 5-H), 2.86 (1H, d, *J* 2.2, OH), 2.69 (1H, d, *J* 2.4, OH), 2.42 (3H, s, CH_3), 1.32 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 137.1, 137.0, 131.0, 130.3, 130.2, 129.4, 128.5, 126.4, 125.5 (C-aromatic), 102.0 (C-benzylidene), 88.8 (C-1), 80.4 (C-2), 74.8 (C-3), 73.0 (C-4), 70.5 (C-5), 68.7 (C-6), 31.4 (*t*Bu), 20.6 (CH_3); HRMS (ESI): Calcd for $C_{24}H_{30}O_5S$ $[M+Na]^+$ 453.1706, found 453.1714.

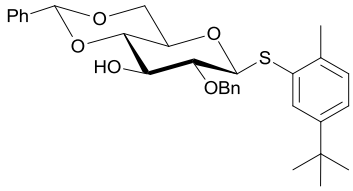
(2-Methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (13)



Compound **12** (658 mg, 1.53 mmol) and imidazole (208 mg, 3.06 mmol) were dissolved in anhydrous DMF (880 µL). TBSCl (346 mg, 2.29 mmol) was gradually added while stirring. After 4 h, the solvent was evaporated and the resulting oil was dissolved in DCM. The

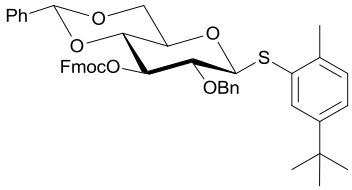
solution was extracted with 1 M HCl and saturated aqueous $NaHCO_3$ solution, the organic layer was dried over $MgSO_4$ and the solvent was evaporated. The colorless solid was dried *in vacuo* and the crude product (820 mg) was purified using flash column chromatography (cyclohexane/ethyl acetate) to afford **13** (573 mg, 1.05 mmol, 69 %). $[\alpha]_D^{20}$ = -49.1 º (c = 1.0, CH_2Cl_2); IR ν_{max} (film) 3559, 2957, 2928, 2858, 1631, 1383, 1259, 1110, 1086, 1067, 1009 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (1H, d, *J* 2.1, Ar-H), 7.51-7.46 (2H, m, Ar-H), 7.39-7.33 (3H, m, Ar-H), 7.26-7.22 (1H, m, Ar-H), 7.15 (1H, d, *J* 8.0, Ar-*H*), 5.52 (1H, s, benzylidene-H), 4.65 (1H, d, *J* 9.8, 1-H), 4.34 (1H, dd, J_1 10.4, J_2 4.4, 6-Ha), 3.84-3.74 (2H, m, 6-Hb, 3-H), 3.54-3.45 (3H, m, 4-H, 5-H, 2-H), 2.42 (3H, s, CH_3), 1.31 (9H, s, *t*Bu), 0.88 (9H, s, *t*Bu), 0.11 (3H, s, CH_3), 0.04 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 137.3, 137.0, 131.4, 130.1, 130.1, 129.1, 128.3, 126.3, 125.3 (C-aromatic), 101.8 (C-benzylidene), 89.0 (C-1), 81.2 (C-4), 76.2 (C-3), 74.0 (C-2), 70.8 (C-5), 68.8 (C-6), 31.4 (*t*Bu), 26.0 (*t*Bu), 20.6 (CH_3), -4.2 (CH_3), -4.6 (CH_3); HRMS (ESI): Calcd for $C_{30}H_{44}O_5SSi$ $[M+Na]^+$ 567.2571, found 567.2584.

(2-Methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-1-thio-β-D-glucopyranoside (14)



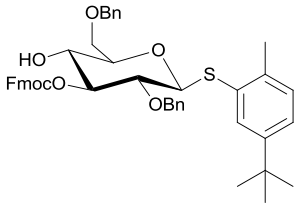
To a solution of **13** (2.00 g, 3.67 mmol) in anhydrous DMF (20 ml), NaH (0.21 g, 8.81 mmol) and BnBr (1.31 ml, 11.01 mmol) were added at 0 °C. The mixture was warmed to room temperature and stirred over night. Then cooled to 0 °C, quenched with MeOH and diluted with Et_2O. The organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded crude (2-methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.4 g), which was taken directly to the next step. Crude (2-methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.4 g) was dissolved in THF (30 ml), cooled to 0 °C and treated with a solution of TBAF (1 M in THF, 7.24 ml, 7.24 mmol). The mixture was warmed to room temperature over night and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **14** (1.77 g, 3.40 mmol, 93%). $[\alpha]_D^{20}$ = -11.4 º (c = 3.7, $CHCl_3$), IR ν_{max} (film) 3463, 3033, 2962, 1810, 1670, 1602, 1488, 1455, 1384, 1264, 1215, 1088 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.64-7.61 (1H, m, Ar-H), 7.51-7.20 (11H, m, Ar-H), 7.17-7.12 (1H, m, Ar-H), 5.55 (1H, s, benzylidene-H), 4.99 (1H, d, A of AB, J_{AB} 10.9, -CH_2-Bn) , 4.84 (1H, d, B of AB, J_{AB} 10.9, -CH_2-Bn), 4.75 (1H, d, *J* 9.8, 1-H), 4.34 (1H, dd, J_1 10.5, J_2 5.0, 6-Ha), 3.97-3.89 (1H, m, 3-H), 3.81 (1H, dd, J_1 J_2 10.3, 6-Hb), 3.60 (1H, dd, J_1 J_2 9.4, 4-H), 3.55-3.42 (2H, m, 2-H, 5-H), 2.52 (1H, d, *J* 2.4, 3-OH), 2.42 (3H, s, CH_3), 1.31 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 138.1, 137.1, 136.3, 132.8, 130.1, 129.4, 129.1, 128.7, 128.5, 128.4, 128.2, 126.4, 125.0, 101.9, 88.2 (C-1), 81.1 (C-2), 80.5 (C-4), 75.7, 75.6 (C-3), 70.1 (C-5), 68.8 (C-6), 34.6, 31.5, 20.5; HRMS (ESI): Calcd for $C_{31}H_{36}O_5S$ $[M+Na]^+$ 543.2181, found 543.2181.

(2-Methyl-5-tert-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (15)



To a solution of **14** (415 mg, 0.80 mmol) and pyridine (129 µl) in DCM (5 ml), Fmoc-Cl (309 mg, 1.20 mmol) was added and the mixture was stirred over night, diluted with DCM and the organic layers were washed with a 10 mM HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **15** (561 mg, 0.76 mmol, 95%) as a white solid. $[\alpha]_D^{20}$ = -0.3 ° (c = 5.9, $CHCl_3$), IR ν_{max} (film) 3033, 2961, 1955, 1754, 1605, 1451, 1385, 1251, 1077 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.79-7.73 (2H, m, Fmoc-H), 7.65-7.13 (19H, m, Ar-H), 5.55 (1H, s, benzylidene-H), 5.29-5.22 (1H, m), 4.98 (1H, A of AB, J_{AB} 10.7, -CH_2-Bn), 4.82 (1H, d, *J* 9.8, H-1), 4.72 (1H, B of AB, *J* 10.7, -CH_2-Bn), 4.49-4.42 (1H, m), 4.40-4.28 (2H, m), 4.24-4.18 (1H, m), 3.88-3.67 (3H, m), 3.60-3.52 (1H, m), 2.42 (3H, s, CH_3), 1.31 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.6, 149.8, 143.5, 143.3, 141.4, 137.5, 136.9, 136.6, 130.2, 129.6, 129.2, 128.4, 128.3, 128.2, 128.00, 127.97, 127.30, 127.27, 126.3, 126.2, 125.2, 120.1, 101.6, 88.7 (C-1), 79.5, 79.3, 78.5, 75.7, 70.33, 70.27, 68.8, 46.8, 34.6, 31.4, 20.5; HRMS (ESI): Calcd for $C_{46}H_{46}O_7S$ $[M+Na]^+$ 765.2862, found 765.2886.

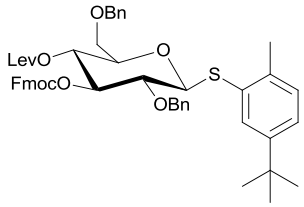
(2-Methyl-5-*tert*-butylphenyl) 2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (16)



To a solution of **15** (100 mg, 0.14 mmol) in anhydrous DCM (3 ml) freshly activated molecular sieves (4 Å) were added. The mixture was cooled to -78 °C, TES (64 µl, 0.40 mmol) and TfOH (41 µl, 0.46 mmol) were added. After stirring for 3 hours at -78 °C, the reaction was quenched by the addition of pyridine, diluted with DCM and washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was then dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **16** (73 mg, 0.10 mmol, 73%). $[\alpha]_D^{20}$ = +10.5 ° (c = 4.9, $CHCl_3$), IR ν_{max} (film) 3486, 3031, 2959, 1951, 1750, 1604, 1451, 1387, 1254, 1054 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80-7.74 (2H, m, Fmoc-H), 7.66-7.56 (3H, m, Ar-H), 7.44-7.09 (16H, m, Ar-H), 4.95 (1H, dd, J_1 J_2 9.2 , 3-H), 4.92 (1H, d, *J* 10.7, -CH_2-Bn), 4.69 (1H, d, *J* 9.8, 1-H), 4.68 (1H, d, *J* 10.8, -CH_2-Bn), 4.61 (1H, A of AB, J_{AB} 12.0, -CH_2-Bn), 4.55 (1H, B of AB, J_{AB} 12.0, -CH_2-Bn), 4.50-4.43 (1H, m, Fmoc-CH_2), 4.40-4.31 (1H, m, Fmoc-CH_2), 4.26-4.20 (1H, m, Fmoc-CH), 3.84 (1H, ddd, J_1 J_2 9.5, J_3 3.6, 4-H), 3.81-3.74 (2H, m, 6-H), 3.61 (1H, dd, J_1 J_2 9.5, 2-H), 3.56-3.49 (1H, m, 5-H), 2.97 (1H, d, *J* 3.6, 4-OH), 2.40 (1H, s, CH_3), 1.26 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.7, 149.8, 143.5, 143.4, 141.4, 137.7, 137.6, 136.5, 132.8,

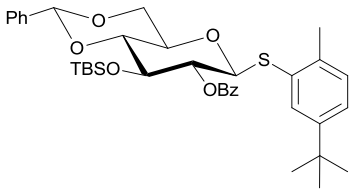
130.1, 129.5, 128.6, 128.4, 128.2 128.04, 127.98, 127.9, 127.3, 125.3, 125.2, 125.0, 120.2, 88.1 (C-1), 83.2 (C-3), 78.5 (C-2), 77.8 (C-5), 75.4, 73.9, 71.0 (C-4), 70.4, 70.3 (C-6), 46.9, 34.6, 31.4, 20.5; HRMS (ESI): Calcd for $C_{46}H_{48}O_7S$ $[M+Na]^+$ 767.3018, found 767.3038.

(2-Methyl-5-*tert*-butylphenyl) 2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-4-*O*-levulinoyl-1-thio-β-D-glucopyranoside (3)



To a solution of **16** (480 mg, 0.64 mmol) in DCM (8 ml) and pyridine (0.3 ml) Lev_2O (55 mg, 0.26 mmol) was added and stirred for three days. The mixture was diluted with DCM and washed with a 1 M HCl solution and with saturated aqueous $NaHCO_3$ solution. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **3** (428 mg, 0.51 mmol, 79%). $[\alpha]_D^{20}$ = +19.2 º (c = 1.0, $CHCl_3$), IR ν_{max} (film) 3065, 2955, 1754, 1719, 1604, 1488, 1452, 1363, 1259, 1152, 1070, 1039 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80-7.74 (2H, m, Ar-H), 7.68-7.58 (3H, m, Ar-H), 7.44-7.17 (15H, m, Ar-H), 7.15-7.11 (1H, m, Ar-H), 5.20 (1H, dd, J_1 J_2 9.7, 4-H), 5.15-5.07 (1H, m, 3-H), 4.95 (1H, A of AB, J_{AB} 10.8, -CH_2-Bn), 4.71 (1H, d, J 9.8, 1-H), 4.69 (1H, B of AB, J_{AB} 10.4, -CH_2-Bn), 4.56-4.41 (3H, m), 4.29-4.20 (2H, m), 3.74-3.55 (4H, m, 2-H, 4-H, 6-H), 2.60-2.52 (2H, m, Lev-CH_2), 2.42 (3H, s, Lev-CH_3), 2.41-2.32 (2H, m, Lev-CH_2), 2.02 (3H, s, CH_3), 1.26 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.0, 171.6, 154.8, 149.9, 143.7, 143. 5, 141.4, 141.3, 138.0, 137.6, 136.6, 132.7, 130.1, 129.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 127.4, 127.3, 125.5, 125.4, 125.0, 120.1, 88.2, 80.5, 78.9, 77.3, 75.6, 73.7, 70.6, 69.4, 69.1, 46.7, 37.8, 34.6, 31.4, 29.7, 28.0, 20.5; HRMS (ESI): Calcd for $C_{51}H_{54}O_9S$ $[M+Na]^+$ 865.3386 found 865.3412.

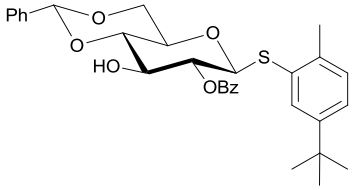
(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (17)



Thioglycoside **13** (1.00 g, 1.84 mmol) was dissolved under argon in anhydrous pyridine (4 ml). DMAP (67 mg, 0.55 mmol) was added and the solution was cooled to 0 °C. BzCl (639 µL, 5.51 mmol) was added dropwise and the solution was heated to 70 °C and stirred for 12 h. After completion, the reaction was quenched with methanol. The suspension was diluted with DCM and extracted with 1 M HCl and H_2O. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **17** (1.05 g, 1.62 mmol, 88%). $[\alpha]_D^{20}$ = +22.9 º (c = 1.0, CH_2Cl_2); IR ν_{max} (film) 2959, 2929, 2858, 1732, 1384, 1266, 1096, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.08 (2H, dd, J 8.3, Ar-H), 7.56 (1H, d, J 1.8, Ar-H), 7.52-7.43 (5H, m, Ar-H), 7.37 (3H, dd, J_1 5.2, J_2 2.0, Ar-H), 7.20 (1H, dd, J_1 8.0, J_2 2.1, Ar-H), 7.07 (1H, d, J 8.0,

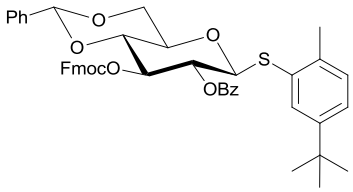
Ar-*H*), 5.58 (1H, s, benzylidene-H), 5.35 (1H, dd, J_1 10.3, J_2 8.6, 2-H), 4.84 (1H, d, J 10.3, 1-H), 4.38 (1H, dd, J_1 10.5, J_2 5.0, 6-Ha), 4.06 (1H, dd, J_1 J_2 8.9, 3-H), 3.88 (1H, dd, J_1 10.3, J_2 5.0, 6-Hb), 3.69 (1H, dd, J_1 J_2 9.1 Hz, 4-H), 3.60-3.52 (1H, m, 5-H), 2.18 (3H, s, CH_3), 1.28 (9H, s, *t*Bu), 0.70 (9H, s, *t*Bu), -0.05 (3H, s, CH_3), -0.14 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 133.1, 129.9, 129.8, 129.4, 129.1, 128.3, 128.1, 126.2, 125.1 (C-Ar), 101.9 (C-benzylidene), 88.1 (C-1), 81.3 (C-4), 74.3 (C-3), 73.6 (C-2), 70.6 (C-5), 68.7 (C-6), 31.3 (*t*Bu), 25.5 (*t*Bu), 20.2 (CH_3), -4.2 (CH_3), -5.0 (CH_3); HRMS (ESI): Calcd for $C_{37}H_{48}O_6SSi$ $[M+Na]^+$ 671.2833, found 671.2852.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (18)



To a solution of **17** (200 mg, 0.31 mmol) in DMF (1 ml) a solution of TBAF·3H_2O (683 mg, 1.85 mmol) and glacial acetic acid (124 μL, 2.16 mmol) in DMF (1 ml) were added. The mixture was warmed to 35 °C for 9 h, diluted with Et_2O and washed with a 10 mM HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **18** (150 mg, 0.28 mmol, 91%). $[\alpha]_D^{20}$ = -5.5 º (c = 0.8, $CHCl_3$); IR ($CHCl_3$): 3455, 2963, 2870, 1729, 1268, 1100, 1071 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11 (2H, d, J 7.4, Ar-H), 7.64-7.33 (9H, m, Ar-H), 7.27-7.20 (1H, m, Ar-H), 7.10 (1H, d, J 8.0, Ar-H), 5.59 (1H, s, benzylidene-H), 5.25 (1H, dd, $J_1$10.1, J_2 8.7, 2-H), 4.88 (1H, d, J 10.1, 1-H), 4.40 (1H, dd, J_1 10.5, J_2 5.0, 6-Ha), 4.09 (1H, dd, J_1 9.0, J_2= 8.7, 3-H), 3.87 (1H, dd, J_1 10.4, J_2 5.0, 6-Hb), 3.71 (1H, dd, J_1 9.0, J_2 9.7, 4-H), 3.57 (1H, td, J_1 9.7, J_2 5.0, 5-H), 2.83 (1H, br, 3-OH), 2.23 (3H, s, CH_3), 1.29 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 166.1 (C=O benzoyl), 149.7, 137.32, 136.9, 133.6, 131.9, 130.4, 130.2, 129.5, 128.6, 128.5, 126.4, 125.6 (aromatics), 102.1 (C-benzylidene), 87.5 (C-1), 80.9 (C-4), 74.0 (C-3), 73.6 (C-2), 70.5 (C-5), 68.7 (C-6), 34.6, 31.4 (*t*Bu), 20.4 (CH_3); HRMS (ESI): Calcd for $C_{31}H_{34}O_6S$ $[M+Na]^+$ 557.1968, found 557.1975.

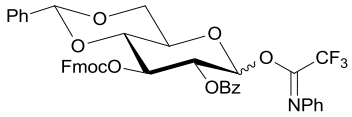
(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (4)



To a solution of **4** (277 mg, 0.52 mmol) and pyridine (130 μl) in DCM (4 ml), Fmoc-Cl (268 mg, 1.04 mmol) was added and the mixture stirred overnight, diluted with DCM and the organic layers were washed with a 10 mM HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column

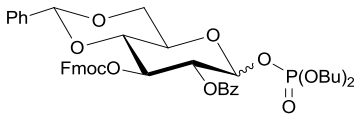
chromatography on silica gel (cyclohexane/ethyl acetate) afforded **4** (378 mg, 0.50 mmol, 96%). $[\alpha]_D^{20}$ = +50.2 º (c = 4.5, $CHCl_3$), IR ν_{max} (film) 3066, 2961, 1752, 1732, 1602, 1488, 1450, 1385, 1316, 1268, 1250, 1093 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06-7.99 (2H, m, Ar-H), 7.73-7.67 (2H, m, Ar-H), 7.61-7.07 (19H, m, Ar-H), 5.60 (1H, s, benzylidene-H), 5.51-5.36 (2H, m, 2-H, 3-H), 4.95 (1H, d, *J* 9.9, 1-H), 4.46-4.39 (1H, m, 6-*H*), 4.27-4.16 (2H, m, Fmoc-CH_2), 4.06-4.00 (1H, m, Fmoc-CH), 3.98-3.88 (2H, m, 4-H, 6-H), 3.72-3.63 (1H, m, 5-H) 2.23 (1H, s, CH_3), 1.29 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.3, 154.6, 149.8, 143.4, 143.2, 141.3, 141.2, 137.4, 136.8, 133.6, 131.7, 130.5, 130.2, 130.1, 129.3, 129.2, 128.6, 128.4, 127.9, 127.27, 127.25, 126.3, 125.8, 125.3, 125.2, 120.00, 119.99, 101.8, 88.0 (C-1), 78.3 (4-H), 77.3 (C-3), 71.4 (C-2), 70.8 (C-5), 70.5, 68.7 (C-6), 46.6, 34.6, 31.7, 31.4, 20.4, 14.3; HRMS (ESI): Calcd for $C_{46}H_{44}O_8S$ $[M+Na]^+$ 779.2655, found 779.2649.

2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethoxycarbonyl-D-glucopyranoside *N*-phenyltrifluoroacetimidate (5)



To a solution of **4** (110 mg, 0.15 mmol), TTBP (144 mg, 0.58 mmol) and NIS (98 mg, 0.44 mmol) in acetonitrile (5 ml) and H_2O (0.1 ml), AgOTf (112 mg, 0.44 mmol) was added and stirred for 4 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution, and extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded the lactol which was taken directly to the next step. A solution of the lactol in DCM (8 ml) was cooled to 0 °C, $CF_3C(NPh)Cl$ (41 mg, 0.20 mmol) and Cs_2CO_3 (65 mg, 0.20 mmol) were added and the resulting solution was stirred for 2 h at room temperature, diluted with DCM, filtered through a plug of celite and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **5** (75 mg, 0.10 mmol, 67%) as an inseparable mixture of α/β anomers. $[\alpha]_D^{20}$ = +46.3 º (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3069, 2925, 1755, 1734, 1600, 1451, 1379, 1322, 1270, 1212, 1165, 1096 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07-8.00 (2H, m, Ar-H), 7.75-7.68 (2H, m, Ar-H), 7.58-7.28 (14H, m, Ar-H), 7.20-7.09 (2H, m, Ar-H), 6.83-6.70 (2H, m, Ar-H), 6.13 (1H, bs, 1-H), 5.70-5.55 (2H, m, benzylidene-H), 5.45-5.35 (1H, m), 4.55-3.83 (8H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.5, 143.3, 143.1, 141.3, 136.6, 133.8, 130.1, 129.4, 128.9, 128.8, 128.7, 128.4, 127.9, 127.3, 126.4, 125.3, 125.2, 120.0, 119.3, 102.0, 78.0, 77.4, 75.8, 71.8, 70.6, 68.5, 67.3, 46.6; HRMS (MALDI-TOF): Calcd for $C_{43}H_{34}F_3NO_9$ $[M+Na]^+$ 788.2083, found 788.1952.

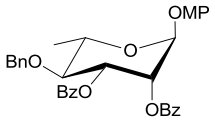
Dibutyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethoxycarbonyl-D-gluco-pyranoside phosphate (6)



Thioglucoside **4** (690 mg, 0.91 mmol) was coevaporated with toluene three times and dried *in vacuo*, then dissolved in anhydrous DCM (10 ml). Freshly activated molecular sieves (4 Å) and dibutyl hydrogen phosphate (542 µl, 2.73 mmol) were added and the solution cooled to

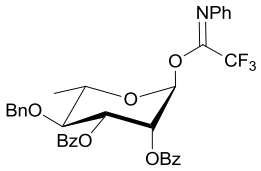
0 °C. NIS (246 mg, 1.09 mmol), followed by TfOH (10 µl, 0.11 mmol) was added and stirred at 0 °C for one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **6** (583 mg, 0.74 mmol, 81%) in a mixture of α/β-anomers (α/β=1:4). NMR data are reported for the β-anomer. $[\alpha]_D^{20}$ = +8.9 º (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3067, 2961, 1755, 1733, 1602, 1451, 1268, 1096, 1026 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06-7.99 (2H, m, Ar-H), 7.72-7.66 (2H, m, Ar-H), 7.55-7.29 (12H, m, Ar-H), 7.18-7.11 (2H, m, Ar-H), 5.60-5.54 (2H, m, benzylidene-H, 1-H), 5.50 (1H, dd, J_1 J_2 9.4, 2-H), 5.36 (1H, dd, J_1 J_2 9.4, 3-H), 4.49-4,41 (1H, m, 6-H), 4.30-4.18 (2H, m, Fmoc-CH_2), 4.10-4.01 (3H, m, Fmoc-H, phosphate-CH_2), 4.00-3.94 (1H, m, 4-H), 3.90-3.86 (1H, m, 6-H), 3.82-3.67 (3H, m, phosphate-CH_2, 5-*H*), 1.67-1.60 (2H, m, phosphate-CH_2), 1.42-1.25 (4H, m, phosphate-CH_2), 1.10-1.01 (2H, m, phosphate-CH_2), 0.92 (3H, t, *J* 7.4, phosphate-CH_3), 0.70 (3H, t, *J* 7.4, phosphate-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.1, 154.5, 143.3, 143.1, 141.3, 136.6, 133.8, 130.1, 129.4, 128.9, 128.6, 128.4, 127.9, 127.3, 127.2, 126.4, 126.3, 125.3, 125.2, 120.0, 101.9, 96.90, 96.85, 78.0, 75.8, 72.6, 70.6, 68.4, 68.1, 67.1, 46.6, 32.2, 32.1, 32.0, 31.9, 18.7, 18.4, 13.7, 13.5; δ_P (160 MHz, $CDCl_3$) -2.95; HRMS (ESI): Calcd for $C_{43}H_{47}O_{12}P$ $[M+Na]^+$ 809.2703, found 809.2690.

4-Methoxyphenyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (20)



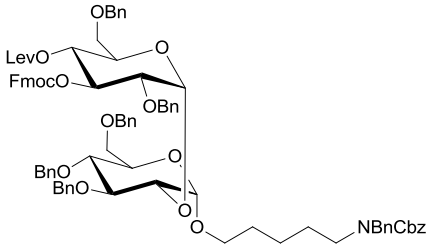
Rhamnoside **19**[2] (500 mg, 1.39 mmol) was dissolved in a solution of DCM (1 ml) and pyridine (1 ml). DMAP (68 mg, 0.56 mmol) was added and the mixture cooled to 0 °C, then BzCl (780 mg, 5.56 mmol) was added and the reaction warmed to room temperature over night. The reaction was quenched with MeOH, diluted with DCM and the organic layer was washed with a 10 mM HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **20** (768 mg, 1.35 mmol, 97%). $[\alpha]_D^{20}$ = +17.6 º (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3064, 2934, 1725, 1602, 1506, 1452, 1363, 1273, 1213, 1094, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11-8.05 (2H, m, Ar-H), 7.98-7.93 (2H, m, Ar-H), 7.67-7.61 (1H, m, Ar-H), 7.56-7.49 (3H, m, Ar-H), 7.40-7.35 (2H, m, Ar-H), 7.25-7.16 (5H, m, Ar-H), 7.08-7.03 (2H, m, Ar-H), 6.87-6.82 (2H, m, Ar-H), 5.94 (1H, dd, J_1 9.6, J_2 3.4, 3-H), 5.79 (1H, dd, J_1 3.4, J_2 1.9, 2-H), 5.54 (1H, d, *J* 1.8, 1-H), 4.75 (1H, A of AB, J_{AB} 10.9, -CH_2-Bn), 4.68 (1H, B of AB, J_{AB} 10.9, -CH_2-Bn), 4.20-4.11 (1H, m, 5-H), 3.88 (1H, dd, J_1 J_2 9.6, 4-H), 3.78 (3H, s, -CH_3), 1.41 (3H, d, *J* 6.2, 6-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.58, 165.55, 155.21, 150.20, 137.7, 133.6, 133.3, 130.0, 129.9, 129.8, 129.71, 128.69, 128.53, 128.48, 128.2, 128.0, 117.9, 114.7, 96.6 (C-1), 79.1 (C-4), 75.3, 72.3 (C-3), 71.2 (C-2), 68.5 (C-5), 55.8, 18.3 (C-6); HRMS (ESI): Calcd for $C_{34}H_{32}O_8$ $[M+Na]^+$ 591.1995, found 591.1985.

2,3-Di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside *N*-phenyltrifluoroacetimidate (7)



CAN (2.17 g, 3.96 mmol) was added to a mixture of **20** (750 mg, 1.32 mmol) in MeCN (12 ml) and H_2O (12 ml) and stirred vigorously for 2 h. H_2O and EtOAc were added, the layers separated, the organic layer washed with H_2O and brine, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded the lactol as an orange solid (548 mg). A solution of the lactol (548 mg) in DCM (10 ml) was cooled to 0 °C, $CF_3C(NPh)Cl$ (438 mg, 2.11 mmol) and Cs_2CO_3 (688 mg, 2.11 mmol) were added and the resulting solution was stirred overnight at room temperature, diluted with DCM, filtered through a plug of celite and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **7** (619 mg, 0.98 mmol, 74%). $[\alpha]_D^{20}$ = +41.2 º (c = 4.8, $CHCl_3$), IR ν_{max} (film) 3065, 2981, 1727, 1600, 1490, 1452, 1270, 1208, 1164, 1091 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07-8.02 (2H, m, Ar-H), 7.96-7.89 (2H, m, Ar-H), 7.66-7.60 (1H, m, Ar-H), 7.57-7.46 (3H, m, Ar-H), 7.40-7.19 (9H, m, Ar-H), 7.14-7.07 (1H, m, Ar-H), 6.91-6.82 (2H, m, Ar-H), 6.35 (1H, bs, 1-H), 5.84 (1H, s, 2-H), 5.77 (1H, dd, J_1 9.4, J_2 3.3, 3-H), 4.76 (1H, A of AB, J_{AB} 10.9, -CH_2-Bn), 4.68 (1H, B of AB, J_{AB} 10.9, -CH_2-Bn), 4.21-4.08 (1H, m, 5-H), 3.87 (1H, dd, J_1 J_2 9.5, 4-H), 1.48 (3H, d, J 6.1, 6-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.5, 165.3, 143.4, 137.4, 133.7, 133.4, 130.0, 129.8, 129.7, 129.4, 128.9, 128.7, 128.58, 128.57, 128.3, 128.2, 124.6, 119.6, 94.1 (C-1), 78.5 (C-4), 75.5, 72.0 (C-3), 70.7 (C-3), 69.6 (C-2), 18.4 (C-6); HRMS (ESI): Calcd for $C_{35}H_{30}F_3NO_7$ $[M+Na]^+$ 656.1872, found 656.1852.

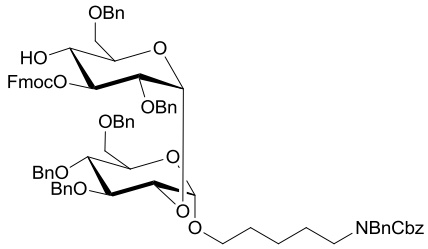
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-4-*O*-levulinoyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (21)**



Glucosides **3** (326 mg, 0.34 mmol) and **2** (262 mg, 0.35 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in anhydrous Et_2O (3 ml), NIS (93 mg, 0.41 mmol) was added and cooled to -35°C. TfOH (3.7 µl, 41 µmol) was added and the mixture was stirred and warmed up to -10 °C in one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **21** (343 mg, 0.24 mmol, 70%). $[\alpha]_D^{20}$ = +64.4 º (c = 5.9, $CHCl_3$), IR ν_{max} (film) 3032, 2932, 1755, 1700, 1605, 1497, 1452, 1362, 1259 cm^{-1}; ^{1}H-NMR

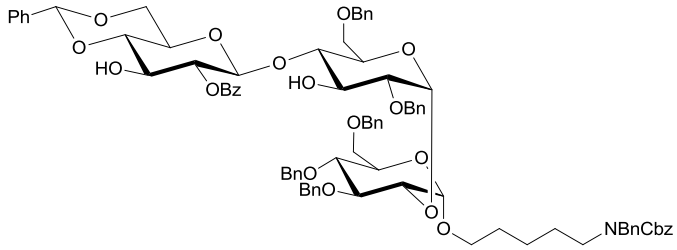
(400 MHz, $CDCl_3$) δ 8.00-6.90 (43H, m, Ar-H), 5.41 (1H, dd, J_1 J_2 9.7), 5.26 (1H, dd, J_1 J_2 9.8), 5.18-5.10 (2H, m), 5.06 (1H, bs, anomeric-H), 5.03-4.96 (2H, m, anomeric-H), 4.88 (1H, app d, *J* 11.0), 4.82 (1H, app d, *J* 10.8), 4.68-4.58 (3H, m), 4.52-4.41 (5H, m), 4.39-4.30 (2H, m), 4.26 (1H, app t, *J* 7.5), 4.14-4.08 (1H, m), 4.07-4.01 (1H, m), 3.82 (1H, dd, J_1 9.9, J_2 3.4), 3.80-3.56 (6H, m), 3.34-3.31 (2H, m), 3.28-3.06 (4H, m), 2.54-2.42 (2H, m), 2.32-2.17 (2H, m), 2.00 (1H, s, Lev-CH_3), 1.65-1.50 (4H, m, linker-CH_2-), 1.30-1.23 (4H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.0, 171.5, 154.9, 143.7, 143.6, 141.40, 141.35, 138.0, 137.8, 128.6, 128.54, 128.53, 128.39, 128.37, 128.2, 128.1, 128.0, 127.94, 127.87, 127.74, 127.66, 127.5, 127.3, 126.3, 125.5, 120.1, 120.0, 95.6 (C-anomeric), 94.0 (C-anomeric), 80.7, 78.2, 77.4, 77.0, 76.8, 76.2, 75.9, 75.4, 73.7, 73.5, 72.4, 70.5, 70.3, 68.8, 68.6, 68.4, 67.3, 46.8, 37.8, 31.4, 29.8, 27.9, 23.7; HRMS (ESI): Calcd for $C_{87}H_{91}NO_{17}$ $[M+Na]^+$ 1444.6179, found 1444.6128.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-_O_-benzyl-3-_O_-fluorenylmethoxycarbonyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (22)



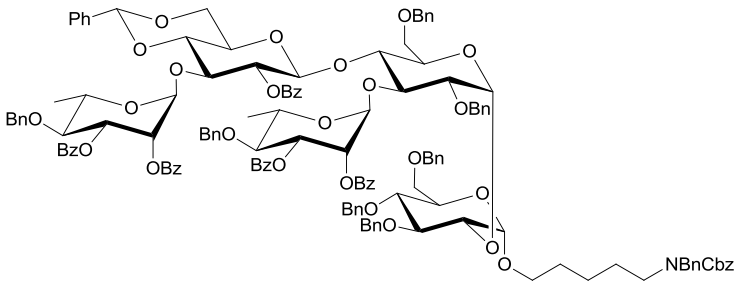
To a solution of **21** (224 mg, 0.16 mmol) in DCM (4.5 ml) hydrazine hydrate (31 µl, 0.63 mmol) dissolved in AcOH (0.4 ml) and pyridine (0.6 ml) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **22** (196 mg, 0.15 mmol, 94%). $[\alpha]_D^{20}$ = +57.7 º (c = 1.7, $CHCl_3$), IR ν_{max} (film) 3423, 3031, 2926, 1753, 1697, 1605, 1586, 1497, 1452, 1422, 1362, 1255, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d6) δ 7.92-7.84 (2H, m, Ar-H), 7.78-7.64 (2H, m, Ar-H), 7.56-7.14 (35H, m, Ar-H), 5.44-5.37 (2H, m), 5.20-5.10 (3H, m), 5.07 (1H, d, *J* 10.7), 4.89-4.77 (3H, m), 4.66-4.47 (8H, m), 4.46-4.39 (2H, m), 4.27 (1H, app t, *J* 6.9), 4-20-4.14 (1H, m), 3.99 (1H, app t, *J* 9.3), 3.89-3.80 (2H, m), 3.78-3.59 (7H, m), 3.59-3.52 (1H, m), 3.49-3.42 (1H, m), 3.25-3.15 (2H, m), 2.82-2.79 (1H, m), 1.60-1.44 (4H, m, linker-CH_2-), 1.33-1.25 (2H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, acetone-d6) δ 155.9, 144.7, 144.6, 142.2, 142.1, 139.9, 139.8, 139.74, 139.68, 139.5, 139.4, 129.34, 129.26, 129.1, 129.02, 129.00, 128.9, 128.7, 128.62, 128.55, 128.5, 128.4, 128.20, 128.16, 128.14, 128.05, 128.0, 127.9, 126.1, 126.0, 120.88, 120.86, 96.3, 94.2, 81.8, 80.0, 79.2, 78.0, 76.5, 75.5, 73.8, 73.5, 72.1, 71.9, 71.5, 70.2, 70.08, 70.06, 69.5, 68.6, 67.4, 47.6, 27.5, 24.2; HRMS (ESI): Calcd for $C_{82}H_{85}NO_{15}$ $[M+Na]^+$ 1346.5817, found 1346.5784.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_O_-benzoyl-4,6-_O_-benzylidene-β-D-glucopyranosyl-(1→4)-2,6-di-_O_-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (24)



Glycosyl phosphate **6** (74 mg, 94 μmol) and **22** (48 mg, 36 μmol) were coevaporated with toluene three times, dried *in vacuo* and then dissolved in anhydrous DCM (1.0 ml). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to –30 °C. TMSOTf (18 μl, 98 μmol) was added and then warmed to –7 °C over 1.5 h. The reaction was quenched with pyridine and concentrated. Column chromatography on silica gel (toluene/acetone) afforded crude **23**. 20% NEt_3 in DCM (1 ml) was added to crude **23** and stirred for 4 h, the mixture was concentrated. Column chromatography on silica gel (toluene/acetone) afforded **24** (20 mg, 14 μmol, 38 %). $[\alpha]_D^{20}$ = +8.1 ° (c = 1.6, $CHCl_3$), IR ν_{max} (film) 3462, 3032, 2924, 1732, 1699, 1603, 1497, 1453, 1364, 1268, 1093 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05-7.92 (2H, m, Ar-H), 7.63-7.06 (43H, m, Ar-H), 5.56 (1H, s, benzylidene-H), 5.24 (1H, app t, *J* 8.5), 5.20-5.11 (2H, m), 5.09-4.98 (2H, m, anomeric-H), 4.88 (1H, app d, *J* 10.7), 4.79-4.66 (4H, m, anomeric-H), 4.62-4.54 (1H, m), 4.49-4.36 (5H, m), 4.19-4.05 (2H, m), 4.03-3.91 (2H, m), 3.89-3.44 (14H, m), 3.39-3.04 (4H, m), 1.57-1.36 (4H, m, linker-CH_2-), 1.32-1.14 (2H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 138.51, 138.46, 138.4, 138.1, 136.8, 133.7, 130.1, 129.6, 129.4, 128.7, 128.6, 128.54, 128.52, 128.47, 128.45, 128.4, 127.98, 127.9, 127.84, 127.78, 127.7, 127.4, 126.4, 102.1, 101.7 (C-anomeric), 95.8 (C-anomeric), 94.5 (C-anomeric), 81.1, 80.8, 80.6, 78.2, 77.9, 77.4, 76.1, 75.2, 74.7, 73.6, 73.4, 72.8, 72.1, 71.6, 70.4, 69.5, 68.7, 68.4, 68.2, 67.8, 67.3, 66.4, 29.8, 29.4, 23.6; HRMS (ESI): Calcd for $C_{87}H_{93}NO_{19}$ $[M+Na]^+$ 1478.6239, found 1478.6136.

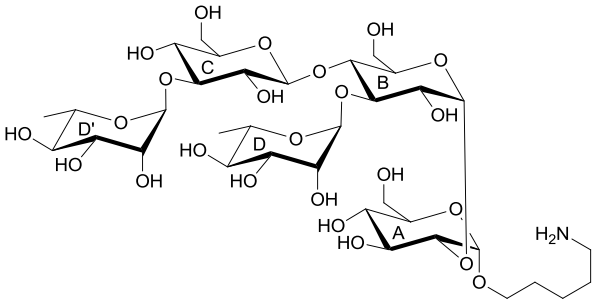
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)-2-_O_-benzoyl-4,6-_O_-benzylidene-3-β-D-glucopyranosyl-(1→4)-[2,3-Di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)]-2,6-di-_O_-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (25)



Compounds **7** (26 mg, 41 μmol) and **24** (10 mg, 6.9 μmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (1.0 ml). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to –30 °C. TMSOTf (10 μl of a

solution of 7.4 µl TMSOTf in 93 µl DCM, 4.1 µmol) was added and the reaction was stirred at –30 °C for 1.5 h. The reaction was quenched with pyridine and concentrated *in vacuo*. Column chromatography on silica gel (toluene/acetone) afforded **25** (14 mg, 5.5 µmol, 81 %). $[\alpha]_D^{20}$ = + 5.2° (c =0.7, $CHCl_3$), IR ν_{max} (film) 3032, 2933, 1728, 1602, 1585, 1496, 1452, 1363, 1263, 1094, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20-6.90 (75H, m, Ar-H), 5.79-5.67 (3H, m), 5.46 (1H, s, benylidene-H), 5.33-5.29 (1H, m), 5.28-5.21 (1H, m), 5.17-5.08 (3H, m, anomeric-H), 5.02 (1H, bs, anomeric-H), 4.92-4.78 (4H, m, anomeric-H), 4.74-4.60 (4H, m), 4.59-4.49 (4H, m, anomeric-H), 4.48-4.44 (1H, m), 4.43-4.31 (4H, m), 4.29-4.13 (4H, m, anomeric-H), 4.03-3.88 (3H, m), 3.83-3.45 (13H, m), 3.40-3.02 (7H, m), 1.65 (1H, d, *J* 6.2, Rha-CH_3), 1.53-1.32 (4H, m, linker-CH_2-), 1.24-1.10 (2H, m, linker-CH_2-), 0.90 (1H, d, *J* 6.1, Rha-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 165.48, 165.5, 164.5, 164.2, 138.3, 137.8, 137.6, 133.1, 130.1, 130.0, 129.94, 129.85, 129.7, 129.4, 129.1, 129.0, 128.9, 128.83, 128.76, 128.7, 128.6, 128.51, 128.47, 128.45, 128.42, 128.36, 128.32, 128.29, 128.23, 128.17, 128.04, 128.00, 127.94, 127.88, 127.8, 127.7, 126.5, 126.4, 100.6 (C-anomeric), 100.5 (C-anomeric), 97.9 (C-anomeric), 97.5, 95.8 (C-anomeric), 93.5 (C-anomeric), 80.2, 79.2, 78.1, 77.5, 77.4, 77.2, 76.8, 76.2, 76.1, 76.0, 74.2, 74.0, 73.6, 72.9, 72.1, 71.6, 71.2, 70.9, 68.7, 67.4, 67.2, 50.6, 47.2, 46.2, 29.9, 23.6, 18.4, 17.5; HRMS (ESI): Calcd for $C_{141}H_{141}NO_{31}$ $[M+Na]^+$ 2366.9385, found 2366.9440.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (1)



Fully protected pentasaccharide **25** (10 mg, 4.3 µmol) was dissolved in a solution of NaOMe in THF/MeOH (1:1, 0.5 M, 1 ml) and heated to 50 °C for 12 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 ($CHCl_3$/MeOH=1:1) afforded the de-benzoylated pentasaccharide (5.6 mg), which was dissolved in a mixture of MeOH (0.9 ml), H_2O (0.1 ml) and AcOH (25 µl). The solution was purged with Ar, 10% Pd/C (10 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 (MeOH) afforded **1** (2.3 mg, 2.6 µmol, 61%). NMR data are reported in Table 1, comparison with the data from native PS-I[3] is reported in Table 2. HRMS (MALDI-TOF): Calcd for $C_{35}H_{63}NO_{24}$ $[M+Na]^+$ 904.3632, found 904.3606.

Table 1: ^{1}H NMR δ (600 MHz, D_2O) and ^{13}C NMR δ (150 MHz, D_2O) of pentasaccharide **1**.[a]

	α-Glc **(A)**	*α-Glc* **(B)**	*β-Glc* **(C)**	*α-Rha* **(D)**	*α-Rha* **(D')**	*Linker*
H-1	5.18	5.09	4.53	5.24	5.14	
C-1	96.1	96.8	102.4	101.8	102.0	
H-2	3.70	3.73	3.38	4.06	4.06	
C-2	72.7	73.4	75.3	71.4	71.2	
H-3	3.70	4.03	3.61	3.88	3.81	
C-3	76.1	77.0	83.2	71.1	71.2	
H-4	3.48	3.86	3.46	3.47	3.47	
C-4	70.5	73.8	69.1	73.0	73.0	
H-5	3.82	4.05	3.45	4.43	4.03	
C-5	72.5	72.3	77.2	69.5	69.8	
H-6 a/b	3.88/3.78	3.92	3.80/3.96	1.27	1.27	
C-6	61.6	60.3	62.2	17.5	17.5	
H-1' a/b						3.79/3.59
C-1'						68.7
H-2'						1.70
C-2'						29.0
H-3'						1.49
C-3'						23.5
H-4'						1.70
C-4'						27.7
H-5'						3.01
C-5'						40.4

[a] ^{1}H and ^{13}C NMR resonances were assigned based on HSQC, HMBC, COSY and TOCSY experiments.

Table 2: Comparison of ^{1}H and ^{13}C NMR δ between **1** and the native PS-I repeating unit.[a]

	α-Glc **(A)**	*α-Glc* **(B)**	*β-Glc* **(C)**	*α-Rha* **(D)**	*α-Rha* **(D')**
H-1	5.18	5.09	4.53	5.24	5.14
	5.75	*5.13*	*4.53*	*5.23*	*5.17*
C-1	96.1	96.8	102.4	101.8	102.0
	93.5	*98.0*	*102.4*	*101.9*	*101.4*
H-2	3.70	3.73	3.38	4.06	4.06
	3.68	*3.70*	*3.38*	*4.07*	*4.09*
C-2	72.7	73.4	75.3	71.4	71.2
	77.3	*73.6*	*75.2*	*71.1*	*71.2*
H-3	3.70	4.03	3.61	3.88	3.81
	3.89	*4.01*	*3.62*	*3.85*	*3.97*
C-3	76.1	77.0	83.2	71.1	71.2
	72.1	*77.5*	*83.0*	*71.0*	*70.9*
H-4	3.48	3.86	3.46	3.47	3.47
	3.53	*3.86*	*3.46*	*3.46*	*4.07*
C-4	70.5	73.8	69.1	73.0	73.0
	70.1	*73.6*	*69.1*	*73.0*	*78.9*
H-5	3.82	4.05	3.45	4.43	4.03
	3.91	*4.06*	*3.45*	*4.44*	*4.12*
C-5	72.5	72.3	77.2	69.5	69.8
	73.8	*72.4*	*77.1*	*69.4*	*68.6*
H-6 a/b	3.88/3.78	3.92	3.80/3.96	1.27	1.27
	n.d.	*n.d.*	*3.80/3.95*	*1.27*	*1.33*
C-6	61.6	60.3	62.2	17.5	17.5
	n.d.	*n.d.*	*62.2*	*17.5*	*17.8*

[a] data of native PS-I reported in italic taken from: J. Ganeshapillai *et al.*, *Carbohydr. Res.*, 2008, **343**, 703.

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3. J. Ganeshapillai, E. Vinogradov, J. Rousseau, J. S. Weese and M. A. Monteiro, *Carbohydr. Res.*, 2008, **343**, 703.