DISAL Glycosyl Donors for the Synthesis of a Linear Hexasaccharide under Mild Conditions

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SUPPORTING INFORMATION

EXPERIMENTAL PROCEDURES INCLUDING SPECTROSCOPIC AND ANALYTICAL DATA

General Procedures: Molecular sieves (4Å) were activated under high vacuum at 150°C for 24h. CH₃NO₂ was purified by vacuum distillation to remove the water/CH₃NO₂ azeotrop, followed by drying with CaSO₄, and stored in dark bottles over 4Å molecular sieves. Water contents (< 20 ppm) were measured by Karl-Fischer titration. All other solvents were distilled and/or stored over 3Å or 4Å molecular sieves as appropriate. ¹H NMR spectra were recorded on either a Varian Mercury 300 operating at 300.06 MHz equipped with a 4-nuclei probe or a Varian Unity Inova 500 operating at 499.87 MHz equipped with a z- (single axis) PFG inverse detection C-H-P probe. ¹³C-NMR were recorded on a Varian Mercury 300 operating at 75.46 MHz or a Varian Unity Inova 500 operating at 125 MHz. Chemical shift (δ) values are in ppm, coupling constants (*J*) are in Hz. All assignments were supported by 2D homonuclear chemical-shift correlation spectroscopy (gCOSY) and in some cases heteronuclear single quantum correlated spectroscopy (gHSQC) experiments. Vacuum liquid chromatography (VLC) was carried on Merck Silica Gel 60H. HPLC analyses were conducted with a Waters system (600 control unit, 996 photodiode array (PDA) detector, 717 Plus autosampler, Millenium32 control software) on a Waters Nova-Pak C₁₈ column (3.9×50 mm cartridge; 4 μm particle size) or a Waters Symmetry300 C₄ column (3.9×150 mm; 5 μm particle size) using a linear gradient of 0.1% aq. TFA (A) and 0.1% TFA in CH₃CN (B): 0 min: 0% B, 2 min: 0% B, 5 min: 50% B, 12 min: 95% B, 13 min: 95% B, 13.5 min: 0% B, 20 min: 0% B. Monitoring was from 200 to 400 nm, integrations were performed at 215 and 265 nm, and individual peaks were analyzed by their UV-spectra. The purity of compounds was determined from integrations at 215 nm. Preparative HPLC purifications were conducted with the above system on three consecutive Prep Nova-Pak C₁₈ columns (40×100 mm; 6 μm particle size) or a Waters PrepPak C₄ cartridge (Delta-PakTM, 25×100 mm; 15 μm particle size). MS analyses were performed on a Micromass LCT mass spectrometer.

Synthesis of donors 4 and 5: DISAL glycosyl donors were synthesized as previously describedⁱ with small changes in the work-up procedure. The crude reaction mixture was transferred to a packed VLC column of oven-dried silica gel (110°C for several days). Elution was performed using EtOAc and toluene (1:15) stored over 4Å molecular sieves. This procedure raised isolated yields of disaccharide donors from approx. 50-60% to almost 90% as discussed in the main text.

Data for 2-carboxymethyl-4,6-dinitrophenyl 2,3,6-tri-O-benzyl-4-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)- α -D-glucopyranoside 4 α

Colorless foam from CH₂Cl₂ stored at -10°C. ¹H NMR (500 MHz, CDCl₃): δ 8.68 (d, 1H, *J* = 3.0), 8.57 (d, 1H, *J* = 3.0), 7.32-6.93 (m, 35H), 5.63 (d, 1H, *J* = 3.8; H-1' α ; HSQC showed strong coupling to 97.34 in ¹³C), 5.41 (d, 1H, *J* = 3.0; H-1 α ; HSQC shows strong coupling to 104.14 in ¹³C), 4.96-4.28 (m, 14H), 4.21 (dd, 1H, *J* = 8.1 and 9.0), 4.14 (dd, 1H, *J* = 8.1 and 9.4), 4.10 (br. d, 1H, *J* ~ 9.4), 3.97 (dd, 1H, *J* = 9.4), 3.92 (dd, 1H, *J* = 3.0 and 11.1), 3.85 (s, 3H), 3.82 (br. d, 1H, *J* ~ 9.8), 3.71-3.63 (m, 3H), 3.59 (dd, 1H, *J* = 3.4 and 10.7), 3.54 (dd, 1H, *J* = 3.8 and 9.8), 3.48 (dd, 1H, *J* = 1.7 and 10.7). ¹³C NMR (75 MHz, CDCl₃): δ 163.87, 154.43, 144.98, 141.50, 138.95, 138.90, 138.68, 138.37, 138.19, 138.17, 137.08, 129.75, 129.25, 128.6-127.5 (m), 127.47, 127.12, 125.52, 123.58, 104.14, 97.34, 82.16, 81.23, 80.92, 79.86, 77.91, 75.75, 75.19, 74.87, 74.47, 73.90, 73.71, 73.47, 73.40, 72.64, 71.39, 68.61, 68.56, 53.59. **ESI-MS:** *m/z* calcd. for C₆₉H₇₂N₃O₁₇ (M+NH₄)⁺ 1214.49, found 1214.61; *m/z* calcd. for C₆₉H₆₈N₂NaO₁₇ (M+Na)⁺ *m/z* 1219.44, found 1219.55

Data for 2-carboxymethyl-4,6-dinitrophenyl 2,3-di-O-benzyl-6-O-trityl-4-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)- α -D-glucopyranoside 5 α

¹H NMR (500 MHz, CDCl₃, see also Figure 1): 8.67 (d, 1H, *J* = 6), 8.57 (d, 1H, *J* = 6), 7.48-7.06 (41H), 6.95 (br. d, 2H, *J* = 7), 6.90 (br. d, 2H, *J* = 7), 5.62 (d, 1H, *J* = 3.84), 5.43 (d, 1H, *J* = 3.41), 5.08-4.28 (12H), 4.21 (m, 2H), 4.11 (m, 1H), 4.00 (1H, *J* = 11.1, *J* = 3.0), 3.97 (t, 1H, *J* = 9.4), 3.94 (dd, 1H, *J* = 8.1, *J* = 2.1), 3.85 (3H, CH₃), 3.79 (1H), 3.70 (dd, 1H, *J* = 8.5, *J* = 3.4), 3.64 (dd, 1H, *J* = 9.81, *J* = 3.4), 3.49 (dd, 1H, *J* = 10.2, *J* = 1.3), 3.13 (dd, 1H, *J* = 10.2, *J* = 3.8). ¹³C NMR (125 MHz, CDCl₃): δ 164.38, 154.93, 145.47, 144.65, 141.96, 139.63, 139.38, 138.96, 138.84, 138.58, 137.67, 130.3-127 (21 C), 124.07, 104.57, 97.20, 87.04, 82.68, 81.34, 81.26, 80.94, 78.66, 76.52, 75.68, 75.29, 74.92, 74.32, 73.88, 73.76, 72.06, 69.09, 63.09, 54.07. **ESI-MS:** *m/z* calcd. for C₈₁H₇₆N₂NaO₁₇ (M+Na)⁺ 1371.50, found 1371.38.

Optimization of glycosylation conditions (Table 1): Disaccharide glycosyl donor **4** or **5** (typically 0.01 mmol) and promoter(s) were dissolved in solvent (600 μ L), and cyclohexanol (stored over 4Å molecular sieves) was added followed by mixing. After shaking for the indicated period on an Eppendorf Thermomixer 5436 (combined heater/shaker), a sample (40 μ L) was diluted in acetonitrile (0.6 mL) and analyzed by analytical HPLC. Reported yields were based on integrated areas of products formed (215 nm). From a series of test reactions, the product was purified by preparative HPLC and characterized.

Cyclohexyl 2,3,6-tri-O-benzyl-4-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)- α -D-glucopyranoside 6 α

¹H NMR (500 MHz, CDCl₃): δ 7.31-7.11 (m, 35H), 5.72 (d, 1H, *J* = 3.4), 5.07 (d, 1H, *J* = 11.5), 4.98 (d, 1H, *J* = 3.4), 4.91 (d, 1H, *J* = 10.7), 4.84-4.78 (m, 3H), 4.67-4.48 (m, 7H), 4.45 (d, 1H, *J* = 10.7), 4.31 (d, 1H, *J* = 12.4), 4.12 (dd, 1H, *J* = 8.5 and 9.0), 4.05 (dd, 1H, *J* = 8.5 and 9.8), 4.01 (ddd, 1H, *J* ~ 2 and 9.8), 3.94 (dd, 1H, *J* = 9.4), 3.87 (dd, 1H, *J* = 3.8 and 10.7), 3.77 (ddd, 1H, *J* ~ 2 and 9.8), 3.69-3.64 (m, 2H), 3.62 (dd, 1H, *J* = 3.8 and 9.4), 3.60-3.53 (m, 1H), 3.53-3.49 (m, 2H), 3.43 (dd, 1H, *J* = 1.7 and 10.7), 1.96-1.89 (m, 2H), 1.84-1.75 (m, 2H), 1.60-1.20 (m, 6H). ¹³C NMR (75 MHz, CDCl₃, 30 mg): δ 139.39, 139.07, 138.76, 138.57, 138.36, 138.24, 138.20, 128.6-126.93 (m), 96.81, 94.68, 82.23, 82.06, 80.60, 79.78, 77.98, 75.96, 75.73, 75.14, 74.34, 73.69, 73.34, 73.09, 72.89, 71.20, 69.84, 69.48, 68.54, 33.70, 31.86, 25.89, 24.75, 24.49. RP-HPLC (*R*_t 21.09 min, C₁₈ column, 215 nm) > 99%. **ESI-MS:** *m/z*

calcd. for $C_{67}H_{78}NO_{11}$ ($M+NH_4$)⁺ 1072.56, found 1072.55 (100%); m/z calcd. for $C_{67}H_{74}NaO_{11}$ ($M+Na$)⁺ m/z 1077.51, found 1077.52 (35%).

Cyclohexyl 2,3,6-tri-O-benzyl-4-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)- β -D-glucopyranoside 6 β
¹H NMR (500 MHz, $CDCl_3$): δ 7.32-7.11 (m, 35H), 5.68 (d, 1H, J = 3.7), 4.98-4.93 (m, 2H), 4.87 (d, 1H, J = 11.5), 4.79 (d, 1H, J ~ 8), 4.77 (d, 1H, J ~ 7), 4.76 (d, 1H, J = 11.5), 4.61-4.45 (m, 8H), 4.36 (d, 1H, J = 11.5), 4.03 (dd, 1H, J = 8.6 and 9.3), 3.90 (dd, 1H, J = 9.2 and 9.5), 3.81-3.69 (m, 5H), 3.66-3.46 (m, 6H), 2.04-1.20 (m, 10H). **¹³C NMR** (75 MHz, $CDCl_3$; selected data): δ 139.62, 139.51, 139.21, 139.19, 139.10, 138.72, 102.66, 97.42, 34.55, 32.80, 30.39, 26.36, 24.72. RP-HPLC (R_t 21.32 min, C_{18} column, 215 nm): > 99%. **ESI-MS**: m/z calcd. for $C_{67}H_{78}NO_{11}$ ($M+NH_4$)⁺ 1072.56, found 1072.55 (100%); m/z calcd. for $C_{67}H_{74}NaO_{11}$ ($M+Na$)⁺ m/z 1077.51, found 1077.52 (45%).

General procedure for glycosylation reactions A 5 mL cryotube was charged with glycosyl donor (0.075 mmol), glycosyl acceptor (0.050 mmol), activators and additives if required, a magnet, and crushed 4Å molecular sieves. The tubes were mounted with a suitable rubber septum, which was then pierced with a needle. The closed tube was then evacuated under high vacuum; Ar was let in followed by re-evacuation. This cycle was repeated twice and then left to dry for 1-2h. Ar was let in, the needle was removed, and finally dry solvent (1 mL) was added by means of a syringe. The reaction mixture was then stirred magnetically while heating by means of an oil bath if required. Work-up consisted of filtration (0.45 μ m) and purification by preparative HPLC followed by evaporation of appropriate fractions. In cases where the reaction mixture was poorly filterable due to the crushed molecular sieves and additives, it was eluted through a short plug of silica gel using a CH_2Cl_2 -MeOH mixture. The residue upon concentration was then purified by preparative HPLC.

Data for thiophenyl 2,3,6-tri-O-benzyl-4-(2,3,4-tri-O-benzyl-6-[2,3,6-tri-O-benzyl-4-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)- α -D-glucopyranosyl]- α -D-glucopyranosyl)- β -D-glucopyranoside

Glc- α (1-4)-Glc- α (1-6)-Glc- α (1-4)-Glc- β SPh 8 α **¹H NMR** (500 MHz, $CDCl_3$, see also Figure 2): δ 7.64-7.59 (m, 2H), 7.38-7.08 (m, 68H), 5.73 (d, 1H, J = 3.4; H-1 α), 5.56 (d, 1H, J = 3.4; H-1 α), 5.17 (d, 1H, J = 3.8; H-1 α); all three α -anomeric signals shows strong coupling to ~ 97 ppm in ¹³C), 5.05-3.20 (m, 54H). **¹³C NMR** (75 MHz, $CDCl_3$): δ 139.25, 139.08, 139.05, 138.90, 138.85, 138.82, 138.73, 138.60, 138.43, 138.31, 138.26, 138.24, 138.09, 134.11, 133.88, 132.26, 132.11, 139.14, 128.7-126.5 (m), 97.05, 96.98, 96.88, 87.48, 87.10, 82.31, 82.13, 81.87, 81.09, 80.38, 79.73, 78.95, 77.95, 77.47, 75.70, 75.60, 75.47, 75.28, 75.14, 75.08, 74.35, 74.19, 73.71, 73.68, 73.52, 73.47, 73.21, 72.66, 72.50, 72.19, 71.80, 71.21, 70.11, 69.35, 69.27, 68.53, 65.18. **ESI-MS**: m/z calcd. for $C_{121}H_{124}KO_{20}S$ ($M+K$)⁺ m/z 1967.80, found 1967.81 (65%); m/z calcd. for $C_{121}H_{124}NaO_{20}S$ ($M+Na$)⁺ 1951.83, found 1951.69 (50%).

Data for thiophenyl 2,3,6-tri-O-benzyl-4-(2,3,4-tri-O-benzyl-6-[2,3,6-tri-O-benzyl-4-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)- β -D-glucopyranosyl]- α -D-glucopyranosyl)- β -D-glucopyranoside

Glc- α (1-4)-Glc- β (1-6)-Glc- α (1-4)-Glc- β SPh 8 β **¹H NMR** (500 MHz, $CDCl_3$; selected data): δ 5.68 (br. d, 2H, J ~ 3.4; 2 $\times$ H-1 α , HSQC showed strong coupling to 97.4 in ¹³C). **¹³C NMR** (125 MHz, $CDCl_3$; selected data): δ 104.41, 97.44, 88.10, 87.48, 85.61, 82.74, 82.70, 82.52, 81.77, 80.13, 79.78, 79.37, 78.71, 78.50, 76.16, 76.07, 75.87, 75.65, 75.55, 75.52, 75.50, 74.86, 74.51, 74.19, 74.13, 73.98, 73.92, 73.30, 73.17, 71.73, 71.47, 70.07, 69.78, 69.03. **ESI-MS**: m/z calcd. for $C_{121}H_{124}KO_{20}S$ ($M+K$)⁺ m/z 1967.80, found 1967.81 (65%); m/z calcd. for $C_{121}H_{124}NaO_{20}S$ ($M+Na$)⁺ 1951.83, found 1951.69 (50%).

Deprotection of tritylated tetrasaccharide, thiophenyl 2,3,6-tri-O-benzyl-4-(2,3,4-tri-O-benzyl-6-[2,3,6-tri-O-benzyl-4-(2,3,4-tri-O-benzyl-6-O-trityl- α -D-glucopyranosyl)- α/β -D-glucopyranosyl]- α -D-glucopyranosyl)- β -D-glucopyranoside 10 The crude product from a glycosylation reaction using tritylated donor **5** and acceptor **7** after elution through a plug of silica gel using CH_2Cl_2 -MeOH (98:2) and concentration was dissolved in a mixture of glacial acetic acid, water and acetonitrile (approx.

90:7:3) was heated at 60°C by means of an oil bath for 3h. A sample was withdrawn from the reaction mixture and analyzed by analytical HPLC, which showed no presence of trityl-protected tetrasaccharide. The reaction mixture was concentrated and once reconcentrated from toluene. The residue was dissolved in acetonitrile, filtered, and purified by preparative HPLC using a C₄-column. Concentration of appropriate fractions yielded 34.5 mg of deprotected material, which amounted to 38% yield for the glycosylation step and deprotection (2 steps). ¹H NMR (500 MHz, CDCl₃, selected data): δ 5.69 (d, J = 3.4; minor isomer), 5.66 (d, J = 3.8; major isomer), 5.65 (d, J = 3.8; minor isomer), 5.56 (d, J = 3.4; major isomer), 5.14 (d, J = 3.4; major isomer). Ratio major-minor ~ 1.4:1. As three anomeric protons with α-configuration were present in the major isomer, it was assigned an anomeric configuration of α,α,α,βSPh and the minor isomer was assigned to α,β,α,βSPh.

Hexasaccharide thiophenyl 2,3,6-tri-O-benzyl-4-(2,3,4-tri-O-benzyl-6-[2,3,6-tri-O-benzyl-4-(2,3,4-tri-O-benzyl-6-[2,3,6-tri-O-benzyl-4-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-α/β-D-glucopyranosyl]-α-D-glucopyranosyl)-β-D-glucopyranoside 11.

Tetrasaccharide acceptor **10** (19 mg, 10 μmol, 1 equiv), disaccharide DISAL donor **4** (19 mg, 1.5 equiv), LiClO₄ (7 mg, 6 equiv), Li₂CO₃ (5 mg, 6 equiv.) and crushed 4Å molecular sieves (20 mg) were placed in plastic tube with magnet and sealed with a septum. The reagents were dried in high-vacuum for min. 1h. After Ar inlet, 1,2-dichloroethane (250 μL, kept over 4Å MS, dry, <5ppm H₂O) was added and the reaction was stirred for 0.5 h at room temperature. The temperature was raised to 35°C and stirring was continued for 1 d. Additionally, 1.5 equiv of disaccharide DISAL donor **4** (0.075 mmol) was added and the stirring was continued for 2 days. Additionally, 6 equiv of LiClO₄ was added and the stirring was continued for 1 d. Additionally, 1.5 equiv of disaccharide DISAL donor **4** (0.075 mmol) was added and the stirring was continued for 2 days. The reaction mixture was filtered and loaded onto a preparative C₄ HPLC column and eluted with a gradient of CH₃CN and water from 10% to 95% CH₃CN over 70 min. Hereby, hexasaccharide **11** (α/β ~3:2) was isolated as a colorless oil (11 mg, 38%) and the acceptor (8 mg, 50%) was recovered. The different diastereomeric hexasaccharide products were not perfectly separable on an analytical C₄ HPLC column (R_t 25.07 min) but NMR analysis and an estimate based on HPLC indicated an α/β ratio of the last formed 1,6-linkage of ~3:2. ¹H NMR (500MHz, CDCl₃, see also Figure 3): δ 7.6-7.55 (m, 2H), 7.35-7.02 (m, 98H), 5.78 (d, 1H, minor isomer), 5.72-5.62 (br. d, 1H, major isomer), 5.58 (d, 1H), 5.53 (d, 1H), 5.51 (d, 1H), 5.16-5.12 (br. d, 1H, major isomer), 5.07 (d, 1H, minor isomer), 5.0-4.2 (m, 35H), 4.14-3.16 (m, 41H). **ESI-MS**: *m/z* calcd. for C₁₇₅H₁₈₀NaO₃₀S (M+Na)⁺ 2816.2, found: 2816.9.

compound 5
Pulse Sequence: s2pu1
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
File: LP4-6A_1h1
INOVA-500 "nmr500"
PULSE SEQUENCE
Pulse 60.0 degrees
Acq. time 2.345 sec
Width 6988.1 Hz
8 repetitions
OBSERVE H1, 499.8677732 MHz
DATA PROCESSING
FT size 32768
Total time 1 minute

Figure 1: 500 MHz ¹H NMR spectra of compound 5.

Figure 3: 500 MHz ^1H NMR spectra of hexasaccharide **11**.

ⁱ (a) L. Petersen and K. J. Jensen, *J. Org. Chem.*, 2001, **66**, 6268; (b) L. Petersen and K. J. Jensen, *J. Chem. Soc. Perkin Trans. I*, 2001, 2175; (c) J. B. Laursen, L. Petersen and K. J. Jensen, *Org. Lett.*, 2001, **3**, 687.