Supporting Information

Synthesis of Oligosaccharides on Soluble High Molecular-Weight Branched Polymers in Combination with Purification by Nanofiltration

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General: 4-Arm branched polyethylene glycol (M.W. 10,000) and 8-arm branched polyethylene glycol (M.W. 40,000) were purchased from Shearwater Polymers Inc. All other chemicals were obtained from Aldrich, Acros, and Fluka and used without purification. Molecular sieves were activated at 350 °C *in vacuo* for 3 hours. All solvents were distilled from the appropriate drying agents. All reactions were performed under anhydrous conditions and monitored by TLC on Kieselgel 60 F₂₅₄ (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Column chromatography was performed on silica gel (Merck, mesh 70-230). Size exclusion column chromatography was performed on Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden. Gel Filtration Theory and Practice published by Pharmacia LKB Biotechnology). Size exclusion column: length 5 ft, internal diameter 25 mm, flow rate 15 drops/minute. Eluent: dichloromethane / methanol (1/1, v/v). Extracts were concentrated under reduced pressure at <40 °C (water bath). ¹H NMR and ¹³C NMR spectra were recorded on a Varian Mercury300, Varian Inova500, and a Varian Inova600 spectrometer equipped with Sun workstations. ¹H spectra recorded in CDCl₃ were referenced to residual CHCl₃ at 7.24 ppm, and ¹³C NMR spectra to the central peak of CDCl₃ at 77.0 ppm. Assignments were made by standard gCOSY, gHSQC, TOCSY, and gHMBC experiments. For polymer bound samples, the glycol peak of PEG was suppressed by saturation at δ = 3.64 ppm and the core methylene signal of bPEG (δ = 3.40 ppm) was used as an internal reference. Negative ion matrix assisted laser desorption ionization time of flight (MALDI - TOF) mass spectra were recorded on an

HP-MALDI instrument using gentisic acid as a matrix. High resolution mass spectra were recorded on an Applied Biosystems 4700 proteomics analyzer. A Millipore Stirred Cell in combination with a Biomax disc membrane with low molecular weight cut-off (Nominal Molecular Weight Limit, NMWL=5,000 Da) was used for purification by nanofiltration.

Synthesis of compound 3

Compound **27** was synthesized according to a procedure reported by Zieman and co-workers. (F. Weygand and H. Zieman, *Liebigs Ann. Chem.*, **1962**, 657, 179).

Ethyl 2,3,4-tri-O-benzoyl-6-O-levulinyl-1-thio-β-D-glucopyranoside (5). Ethyl 2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside **27** (2.24 g, 4.18 mmol) was dissolved in dichloromethane (30 mL) and placed under an atmosphere of nitrogen. Levulinic acid (970 mg, 8.35 mmol) was added and the resulting solution was stirred for five minutes. A mixture of DCC (1.72 g, 8.35 mmol) and DMAP (102 mg, 0.84 mmol) was added and stirring was continued for 16 hours. The reaction mixture was filtered to remove the precipitated dicyclohexyl urea. The filtrate was washed with aqueous NaHCO₃ (40 mL,

10%), dried (MgSO₄), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: EtOAc/Hexane, 3/7, v/v) to give **5** (3.72 g, 89%) as a colorless syrup. ¹H NMR (300 MHz, CDCl₃): δ 7.96-7.84 (m, 4H; Ar-*H*), 7.78 (d, *J*=6.0 Hz, 2H; Ar-*H*), 7.52-7.20 (m, 9H, Ar-*H*), 5.86 (t, *J*=9.6 Hz, 1H; H-3), 5.58-5.46 (m, 2H; H-2, H-4), 4.80 (d, *J*=10.2 Hz, 1H, H-1), 4.90 (m, 2H; H-6a, H-6b), 4.10-3.95 (m, 1H; H-5), 2.80-2.45 (m, 6H; CH₂CH₂ of Lev, CH₂ of SEt), 2.10 (s, 3H; COCH₃, Lev), 1.27 (t, *J*=7.2 Hz, 3H, CH₃ of SEt). HR MALDI-TOF MS: calcd for C₂₉H₂₇O₇S 519.1477, found *m/z* 519.1487.

***p*-(allyloxy)benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-levulinyl-β-*D*-glucopyranoside (28).** A suspension of compound **5** (819 mg, 1.29 mmol), 4-allyloxybenzyl alcohol (158.9 mg, 0.97 mmol) and activated molecular sieves 4 Å (100 mg) in dichloromethane (25 mL) was placed under an atmosphere of argon and stirred at room temperature for 10 min. The mixture was cooled (0°C) and subsequently, NIS (290.4 mg, 1.55 mmol) and TMSOTf (34 μL, 0.15 mmol) were added. After the glycosyl donor was consumed (monitored by TLC, ethyl acetate/hexane, 1/1, v/v), the reaction mixture was diluted with dichloromethane (100 mL) and molecular sieves were removed by filtration. The filtrate was washed with aqueous Na₂S₂O₃ (15%, 5 mL), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: EtOAc/Hexane, 7/13, v/v) to give **28** (520 mg, 72%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ 7.81 (d, *J*=7.3 Hz, 4H, Ar-*H*), 7.73 (d, *J*=7.3 Hz, 2H, Ar-*H*), 7.47-7.19 (m, 9H, Ar-*H*), 7.06 (d, *J*=5.8 Hz, 2H, Ar-*H*), 6.67 (d, *J*=8.3 Hz, 2H, Ar-*H*), 5.97 (m, 1H; H-vinylic), 5.71 (t, *J*=9.8 Hz, 1H, H-3), 5.49-5.44 (m, 2H, H-2, H-4), 5.34 (d, *J*=17.1 Hz,

1H, H-vinylic), 5.22 (d, $J=10.3$ Hz, 1H, H-vinylic), 4.78 (d, $J=12.2$ Hz, 1H, CHHPh), 4.71 (d, $J=7.8$ Hz, 1H, H-1), 4.57 (d, $J=12.2$ Hz, 1H, CHHPh), 4.42 (dd, $J=5.37$ Hz, 1H, H-6a), 4.26 (dd, $J=4.4$ Hz, 1H, H-6b), 3.91-3.87 (m, 1H, H-5), 2.26-2.66 (m, 2H, CH₂CH₂, Lev), 2.55-2.52 (m, 2H, CH₂CH₂, Lev), 2.10 (s, 3H, COCH₃, Lev). HR MALDI-TOF MS: calcd for C₃₇H₃₃O₉ 621.2125, found m/z 621.2134.

***p*-Hydroxybenzyl 2,3,4-tri-*O*-benzoyl-6-*O*-levulinyl- β -D-glucopyranoside (3).**

Compound **28** (500 mg, 0.678 mmol) was dissolved in a mixture of ethanol and H₂O (40 mL, 19/1, v/v) and (Ph₃P)₄Pd (78.34 mg, 0.0678 mmol) was added. After heating the reaction mixture under reflux for 6 hours, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent: ethyl acetate/hexane, 2/3, v/v) to give compound **3** (304.2 mg, 64 %) as colorless oil. ¹H NMR (300 MHz, CDCl₃): δ 7.87 (d, $J=8.1$ Hz, 4H; Ar-*H*), 7.78 (d, $J=8.4$ Hz, 2H; Ar-*H*), 7.50-7.22 (m, 9H; Ar-*H*), 7.08 (d, $J=8.4$ Hz, 2H; Ar-*H*), 6.64 (d, $J=8.4$ Hz, 2H; Ar-*H*), 5.77 (t, $J=9.6$ Hz, 1H; H-3), 5.55-5.48 (m, 2H; H-2, H-4), 4.80 (d, $J=12.3$ Hz, 1H; CHHPh), 4.77 (d, $J=7.81$ Hz, 1H; H-1), 4.62 (d, $J=12.3$ Hz, 1H; CHHPh), 4.30-4.29 (dd, $J=5.4$ Hz, 2H; H-6a, H-6b), 3.99-3.86 (m, 1H; H-5), 2.76-2.70 (m, 2H; CH₂CH₂, Lev), 2.61-2.55 (m, 2H; CH₂CH₂, Lev), 2.16 (s, 3H; COCH₃, Lev). HR MALDI-TOF MS: calcd for C₃₄H₂₉O₉ 581.1812, found m/z 581.1836.

Synthesis of compound 11

Ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-*t*-butyldimethylsilyl- β -D-1-thio-glucopyranoside (29).

Compound **27** (1.0 g, 2.95 mmol) was dissolved in dichloromethane (25 mL) and placed under an atmosphere of nitrogen. After the mixture was cooled (0°C), pyridine (15 mL) and *tert*-butyldimethylsilyl chloride (667 mg, 4.43 mmol) were added and the reaction mixture was stirred for 16 hours. The solvent was evaporated under reduced pressure and the residue was dissolved in dichloromethane (50 mL) and washed with water (30 mL), dried (MgSO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: EtOAc/Hexane, 1/49, v/v) to give compound **29** (1.84 g, 96%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3): δ 7.92 (t, $J=6.0$ Hz, 4H; Ar-*H*), 7.79 (d, $J=8.1$ Hz, 2H; Ar-*H*), 7.47-7.21 (m, 9H; Ar-*H*), 5.86 (t, $J=9.3$ Hz, 1H; H-3), 5.56-5.46 (m, 2H; H-2, H-4), 4.77 (d, $J=9.9$ Hz, 1H; H-1), 3.88-3.80 (m, 3H; H-6a, H-6b, H-5), 2.90-2.60 (m, 2H; CH_2 of SEt), 1.25 (t, 3H, $J=7.5$ Hz, CH_3 of SEt), 0.82 (s, 9H; *t*-Bu of TBDMS), 0.04 (s, 6H; CH_3 of TBDMS). HR MALDI-TOF MS: calcd for $\text{C}_{35}\text{H}_{42}\text{O}_8\text{SSi}$ 650.2370, found m/z 650.2375.

***p*-(Allyloxy)benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-*t*-butyldimethylsilyl- β -D-glucopyranoside**

(30). Compound **29** (2.07 g, 3.18 mmol), allyloxy benzyl alcohol (627 mg, 3.82 mmol) and activated molecular sieves (200 mg, 4Å) in dichloromethane (40 mL) were placed under an atmosphere of nitrogen and stirred at room temperature for 10 min. The mixture was cooled (0°C) and subsequently NIS (788.2 mg, 3.50 mmol) and TMSOTf (64 µL, 0.35 mmol) were added. After the glycosyl donor was consumed (monitored by TLC, ethyl acetate/hexane, 1/1, v/v), the reaction mixture was diluted with dichloromethane (40 mL) and the molecular sieves were removed by filtration. The filtrate was washed with aqueous Na₂S₂O₃ (15%, 10 mL), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: EtOAc/Hexane, v/v, 1/9) to give **30** (1.73 g, 69.5%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ 7.86 (d, *J*=8.1 Hz, 4H; Ar-*H*), 7.76 (d, *J*=7.2 Hz, 2H; Ar-*H*), 7.60-7.19 (m, 9H; Ar-*H*), 7.09 (d, *J*=8.7 Hz, 2H; Ar-*H*), 6.70 (d, *J*=8.4 Hz, 2H; Ar-*H*), 6.0 (m, 1H; H-vinylic), 5.73 (t, *J*=9.6 Hz, 1H; H-3), 5.52-5.38 (m, 2H; H-2, H-4), 5.37 (d, *J*=17.4 Hz, 1H; H-vinylic), 5.25(d, *J*= 11.7 Hz, 1H; H-vinylic), 4.81 (d, *J*= 12.0 Hz, 1H; CHHPh), 4.72 (d, *J*= 7.8 Hz, 1H; H-1), 4.61 (d, *J*= 12.0 Hz, 1H; CHHPh), 4.58-4.42 (m, 2H, CH₂ allylic), 3.88-3.66 (m, 3H; H-6a, H-6b, H-5), 0.82 (s, 9H; *t*-Bu of TBDMS), 0.04 (s, 6H; CH₃ of TBDMS). HR MALDI-TOF MS: calcd for C₄₃H₄₈O₁₀Si 752.3017, found *m/z* 752.3029.

***p*-Hydroxybenzyl 2,3,4-tri-*O*-benzoyl-6-*O*-*t*-butyldimethylsilyl- β -D-glucopyranoside (11).** Compound **30** (789 mg, 1.0 mmol) was dissolved in a mixture of ethanol and H₂O (45 mL, 19/1 v/v) and (Ph₃P)₄Pd (127.1 mg, 0.11 mmol) was added. The reaction mixture was heated under reflux for 6 hours after which the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: EtOAc/Hexane, v/v, 3/17) to give compound **11** (411.7 mg, 55.5%). ¹H NMR (300 MHz, CDCl₃): δ 7.88 (m, 4H; Ar-*H*), 7.79 (d, *J*=7.2 Hz, 2H; Ar-*H*), 7.52-7.22 (m, 9H; Ar-*H*), 7.05 (d, *J*=8.1 Hz, 2H; Ar-*H*), 6.64 (d, *J*=8.7 Hz, 2H; Ar-*H*), 5.77 (t, *J*=9.6 Hz, 1H; H-3), 5.61-5.40 (m, 2H; H-2, H-4), 5.24 (s, 1H; phenolic OH), 4.80 (d, *J*=12.3, 1H; CHHPh), 4.75 (d, *J*=8.1, 1H; H-1), 4.62 (d, *J*= 12.3 Hz, 1H; CHHPh), 4.30-4.29 (dd, *J*= 5.37 Hz, 2H; H-6a, H-6b), 3.88-3.65 (m, 3H; H-6a, H-6b, H-5), 0.88 (s, 9H; *t*-Bu of TBDMS), 0.04 (s, 6H; CH₃ of TBDMS). HR MALDI-TOF MS: calcd for C₄₀H₄₄O₁₀Si 712.2704, found *m/z* 712.2715.

Synthesis of compound 13

Ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-(9-fluorenyl methoxy carbonyl)-1-thio- β -D-glucopyranoside (13). Compound **27** (1.8 g, 3.36 mmol) was dissolved in a mixture of dichloromethane (25 mL) and pyridine (1.36 mL) and the solution was placed under an atmosphere of nitrogen. 9-Fluorenyl methyl chloroformate (1.74 g, 6.71 mmol) was added and the reaction mixture was stirred for 4 hours. The solvent was evaporated under

reduced pressure and the residue dissolved in dichloromethane (30 mL), washed with water (30 mL), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/Hexane, 1/9, v/v) to give **13** as a colorless oil (1.68 g, 66%). ¹H NMR (500 MHz, CDCl₃): δ 7.96-7.9 (m, 4H; Ar-*H*), 7.80 (d, *J*=7.5 Hz, 2H; Ar-*H*), 7.75 (d, *J*=7.5 Hz, 2H; Ar-*H*), 7.62-7.56 (m, 2H; Ar-*H*), 7.52-7.46 (m, 2H; Ar-*H*), 7.52-7.46 (m, 11H; Ar-*H*), 5.90 (t, *J*=10 Hz, 1H; H-3), 5.58-5.53 (m, 2H; H-2, H-4), 4.82 (d, *J*=10 Hz, 1H; H-1), 4.44-4.30 (m, 4H; H-6a, H-6b, CH₂ of Fmoc), 4.21 (t, *J*=10 Hz, 1H, CH of Fmoc), 4.10-4.05 (m, 1H; H-5), 2.84-2.72 (m, 2H; CH₂ of SEt), 1.27 (t, *J*=7 Hz, 3H; CH₃ of SEt). HR MALDI-TOF MS: calcd for C₄₄H₃₈O₁₀S 758.2186, found *m/z* 758.2197.

Synthesis of polymer bound linker, *p*-bPEG-oxy-benzoic acid (4a,b). Cs₂CO₃ (1.12 g, 3.45 mmol) was added to the solution of 4-arm bPEG mesylate (2.22 g, 0.22 mmol, 0.88 mmol reaction sites for a 4-arm polymer) and methyl *p*-hydroxybenzoate (0.52 g, 3.45 mmol, 3.92 eqv. w.r.t reaction sites) in DMF (30 mL). After stirring the reaction mixture for 24 hours at 70°C, it was cooled to room temperature, excess of DOWEX-50 H⁺ resin added and stirring was continued for 0.5 hours. The resin was removed by filtration, the filtrate concentrated to dryness under reduced pressure, and the residue redissolved in dichloromethane (50 mL). The mixture was filtered through celite and washed with dichloromethane (30 mL). The combined filtrate and washings were concentrated under reduced pressure to a small volume (4 mL), cooled to 0°C, followed by addition of ice-cold anhydrous diethyl ether (200 mL) with vigorous stirring. The precipitate was collected by filtration and washed with ice-cold diethyl ether. The residue was dissolved

in a mixture of THF and MeOH (15 mL, 1/1, v/v) and 2(M) aqueous NaOH (20 mL) was added. The reaction mixture was stirred at room temperature for 18 hours after which aqueous 3(M)HCl (20 mL) was added. The aqueous mixture was concentrated to dryness under reduced pressure and the residue was dissolved in dichloromethane (50 mL), dried (MgSO_4) and filtered. The filtrate was concentrated under reduced pressure to a small volume (5 mL), cooled (0°C), and precipitated by addition of ice-cold diethyl ether (200 mL). The resulting solid was filtered-off, washed with diethyl ether and dried in *vacuo* to give the four-arm polymer **4a** as a white solid (0.98 g). ^1H NMR (300MHz, CDCl_3): δ 7.96 (d, $J=8.7$ Hz, 2H; Ar-*H*), 6.91 (d, $J=9.0$ Hz, 2H; Ar-*H*), 4.16 (t, 2H, $J=4.8$ Hz, bPEG- $\text{CH}_2\text{OC}_6\text{H}_4\text{COOH}$), 3.85-3.50 (m, bPEG- CH_2). In case of the eight arm polymer **4b**, the filtrate was concentrated to dryness under reduced pressure, dissolved in water and added to a Millipore stirred cell fitted with a Biomax disc membrane of NMWL=5,000 Da. The solution was magnetically stirred for one hour under 50 psi pressure of nitrogen to give the *p*-bPEG-oxy-benzoic acid **4b** as the residue which was dried in *vacuo*. ^1H NMR (300MHz, CDCl_3): δ 7.96 (d, $J=8.7$ Hz, 2H; Ar-*H*), 6.91 (d, $J=9.0$ Hz, 2H; Ar-*H*), 4.16 (t, 2H, $J=4.8$ Hz, bPEG- $\text{CH}_2\text{OC}_6\text{H}_4\text{COOH}$), 3.85-3.50 (m, bPEG- CH_2).

Synthesis of polymer bound linker, *p*-bPEG-oxy-succinic acid (12). A mixture of eight-arm bPEG (3.0 g, 0.075 mmol, 0.6 mmol reaction sites for an 8-arm polymer) and succinic anhydride (300 mg, 3.0 mmol, 5 eqv. w.r.t reaction sites) was dissolved in dichloromethane (70 mL) and stirred at 0°C under an atmosphere of nitrogen. Pyridine (2.4 mL) and DMAP (73.3 mg, 0.60 mmol) were added, and the reaction mixture was

stirred for 24 hours. The reaction mixture was filtered to remove dicyclohexyl urea (DCU) and the filtrate was concentrated under reduced pressure. The residue was redissolved in water (50 mL) and filtered to remove the remaining DCU. The filtrate was added through the inlet at the top of a Millipore stirred cell fitted with a Biomax membrane of NMWL=5,000 Da. The solution was magnetically stirred for one hour under 50 psi pressure of nitrogen to give bPEG-oxy-succinic acid **12** as the residue (2.97 g). ¹H NMR (300MHz, CDCl₃): δ 4.23 (t, 2H, *J*=4.8 Hz, bPEG-CH₂OC₆H₄COOH), 3.88-3.30 (m, bPEG-CH₂), 2.66-2.50 (m, 4H, COCH₂CH₂CO).

***p*-(p-bPEG-oxy-benzoyloxy)benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-levulinyl-β-D-glucopyranoside (6a, n=4).** A solution of **3** (104 mg, 0.164 mmol) and *p*-bPEG-oxy-benzoic acid **4a** (221 mg, 0.021 mmol, 0.084 mmol reaction sites for a 4-arm polymer) was stirred at room temperature under an atmosphere of nitrogen. DCC (34.81 mg, 0.168 mmol) and a catalytic amount of DMAP (2.05 mg, 0.017 mmol) were added and stirring was continued for 24 hours. The white precipitate (DCU) was filtered off and the solvent was evaporated under reduced pressure. The residue was redissolved in minimal amount of dichloromethane (4 mL), cooled (0°C) and ice cold diethyl ether (200 mL) added with vigorous stirring. The resulting precipitate was filtered off and washed with ice cold diethyl ether to give after drying *in vacuo* **6a** (n=4, n^l=0) (278 mg) as a white amorphous material. ¹H NMR (300 MHz, CDCl₃): δ 8.10 (d, *J*=9.0 Hz, 2H; Ar-*H*), 7.91-7.86 (m, 4H; Ar-*H*), 7.79 (d, *J*=8.1 Hz, 2H; Ar-*H*) 7.51-7.35 (m, 10H; Ar-*H*) 7.03-6.64 (m, 5H; Ar-*H*), 5.79 (t, *J*=9.6 Hz, 1H; H-3), 5.58-5.50 (m, 2H; H-2, H-4), 4.92 (d, *J*=12.6 Hz, 1H; CHHPh), 4.83 (d, *J*=7.7 Hz, 1H; H-1), 4.71 (d, *J*= 12.6 Hz, 1H; CHHPh), 4.32-4.31 (dd,

2H; H-6a, H-6b), 4.31-3.36 (m, bPEG-CH₂, H-5), 2.78-2.69 (m, 2H; CH₂CH₂, Lev), 2.61-2.55 (m, 2H; CH₂CH₂, Lev), 2.15 (s, 3H; COCH₃, Lev).

***p*-(bPEG-oxy-succinoyloxy) benzyl-2,3,4-tri-*O*-benzoyl-6-*O*-*t*-butyldimethylsilyl-β-D-glucopyranoside (14).** DCC (111 mg, 0.538 mmol) and DMAP (13.1 mg, 0.108 mmol) were added to a solution of **11** (300 mg, 0.404 mmol) and **12** (1.37 g, 0.034 mmol, 0.272 mmol reaction sites for an 8-arm polymer) in dichloromethane (20 mL). After stirring the reaction mixture for 24 hours, the white precipitate (DCU) was filtered off and the filtrate was concentrated under reduced pressure. The residue was redissolved in a mixture of water and methanol (50 mL, 9/1, v/v) and filtered to remove remaining DCU. The filtrate was added to a Millipore stirred cell fitted with a Biomax disc membrane of NMWL= 5,000 Da. The solution was magnetically stirred for 2 hours under 50 psi pressure of nitrogen after which the residue was dried in *vacuo* to give compound **14** (1.47 g) as a white amorphous material. ¹H NMR data (300 MHz, CDCl₃): δ 7.88 (d, *J*=7.5 Hz, 2H; Ar-*H*), 7.78 (d, *J*=7.5 Hz, 2H; Ar-*H*), 7.60-7.10 (m, 13H; Ar-*H*), 6.90 (d, *J*=8.1 Hz, 2H; Ar-*H*), 5.76 (t, *J*=9.9 Hz, 1H; H-3), 5.56-5.40 (m, 2H; H-2, H-4), 4.87 (d, *J*=12.9 Hz, 1H; CHHPh), 4.77 (d, *J*=7.8 Hz, 1H; H-1), 4.67 (d, *J*= 12.6 Hz, 1H; CHHPh), 4.30-4.15 (m, 2H; bPEG-CH₂CH₂OCO), 3.90-3.40 (H-5, H-6a, H-6b, m, bPEG-CH₂CH₂), 2.90-2.60 (m, 4H; CH₂CH₂ of Succinate), 0.86 (s, 9H; CH₃, *t*-Bu of TBDMS), 0.02 (s, 6H; CH₃ of TBDMS).

General procedure for NIS/TMSOTf-mediated glycosylation on branched Polymer (bPEG). A suspension of glycosyl donor (0.075 mmol, 1.5 eqv. w.r.t reaction sites on

polymer-bound acceptor), 4-arm bPEG-bound glycosyl acceptor (0.0125 mmol, 0.05 mmol reaction sites for a 4-arm polymer) and activated molecular sieves 4Å (30 mg) in dichloromethane (5 mL) was stirred under an atmosphere of argon for 30 minutes. The mixture was cooled (0°C), followed by addition of NIS (23 mg, 0.1 mmol) and TMSOTf (2 µL, 0.01 mmol). After the glycosyl donor was consumed (monitored by TLC), the reaction mixture was diluted with dichloromethane (60 mL) and the molecular sieves removed by filtration. Aqueous Na₂S₂O₃ (15%, 2 mL) was added to the filtrate and the organic layer was separated, dried (MgSO₄) and concentrated under reduced pressure. Purification was accomplished by selective precipitation with ice-cold diethyl ether (150 mL).

General procedure for Lev (levulinyl) deprotection: 4-arm bPEG-bound levulinyl protected sugar (0.016 mmol, 0.064 mmol reaction sites for a 4-arm polymer) was dissolved in dichloromethane (10 mL) and stirred under an atmosphere of nitrogen. A solution of NH₂NH₂.CH₃COOH (8.73 mg, 0.095 mmol, 1.5 eqv. w.r.t the polymer-bound reaction sites) in CH₃OH (2 mL) was added, and stirring was continued for 2 hours to give after purification by selective precipitation with ice-cold diethyl ether (100 mL), bPEG-bound levulinyl deprotected sugar.

General method for TBDMS (*tert*-butyl dimethyl silyl) deprotection. Compound **14** (410 mg, 8.8 µmol, 70.4 µmol reaction sites for an 8-arm polymer) was dissolved in acetonitrile (10 mL) and stirred under an atmosphere of nitrogen. A solution of aqueous HBF₄ (48%, 9.8 mL) was added and stirring was continued for 2 hours. Et₃N was added

to neutralize the excess acid and the solvents were evaporated under reduced pressure. The residue was dissolved in a mixture of water and methanol (50 mL, 9/1, v/v) and the resulting solution was added to a Millipore stirred cell fitted with a Biomax disc membrane of NMWL=5,000 Da. The solution was magnetically stirred for one hour under 50 psi pressure of nitrogen to give compound **12** (374 mg) as the residue.

General procedure for Fmoc (9-fluorenyl methoxy carbonyl) deprotection. Eight arm bPEG-bound Fmoc-protected sugar (7.44 μmol , 59.52 μmol reaction sites for an 8-arm polymer) was dissolved in dichloromethane (15 mL) and placed under an atmosphere of nitrogen. The solution was cooled (0°C) and Et_3N in dichloromethane (70%, 6 mL) was added. After stirring the reaction mixture for 16 hours, the solvents were evaporated under reduced pressure, and the residue dissolved in acetonitrile (50 mL) and added to a Millipore stirred cell fitted with a Biomax disc membrane of NMWL=5,000 Da. The solution was magnetically stirred for 16 hours under 50 psi pressure of nitrogen to give the bPEG-bound Fmoc-deprotected sugar as the residue, which was dried in *vacuo*.

General procedure for the cleavage of *p*-hydroxybenzoate linker: Four arm bPEG-bound sugar (0.02 mmol, 0.08 mmol reaction sites for a 4-arm polymer) was dissolved in THF (6 mL) and Et_3N (0.8 mL) and H_2O_2 (48% in water, 0.2 mL) were added. After stirring at room temperature for 20 hours, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane (3 mL), cooled (0°C) and ice-cold diethyl ether (60 mL) was added. The white precipitate was filtered off and

the filtrate was concentrated under reduced pressure. The residue was purified by Sephadex LH-20 size exclusion column chromatography.

General procedure for the cleavage of *p*-hydroxysuccinate linker. Eight arm bPEG-bound sugar (300 mg, 5.12 μ mol, 40.96 μ mol reaction sites for an 8-arm polymer) was dissolved in dichloromethane (12 mL) and placed under an atmosphere of nitrogen. Hydrazine acetate (37.71 mg, 0.409 mmol, 10 eqv. w.r.t the polymer-bound reaction sites) in methanol (5 mL) was added and the reaction mixture was stirred for 3 days. The reaction mixture was concentrated under reduced pressure, the residue dissolved in acetonitrile (50 mL) and the solution added to a Millipore stirred cell fitted with a Biomax disc membrane with NMWL= 5,000 Da. The solution was magnetically stirred for 16 hours under 50 psi pressure of nitrogen. The filtrate was concentrated to dryness under reduced pressure and purified by Sephadex LH-20 size exclusion column chromatography to give pure oligosaccharide.

4-hydroxybenzyl 2,3,4-tri-*O*-benzoyl- β -D-(1-6)-glucopyranosyl-2,3,4-tri-*O*- β -D-(1-6)-glucopyranosyl-2,3,4-tri-*O*-benzoyl- β -D-(1-6)-glucopyranosyl-2,3,4-tri-*O*-benzoyl- β -D-glucopyranoside (10). ^1H NMR (500 MHz, CDCl_3): δ 8.15-7.7 (m, 24H; Ar-*H*), 7.60-7.12 (m, 38H; Ar-*H*, *p*-hydroxybenzyl), 6.79 (d, J = 8.5 Hz, 2H; Ar-*H* of *p*-hydroxybenzyl), 5.98 (t, J = 10.0 Hz, 1H; H-3), 5.78-5.70 (m, 2H; H-3', H-3'''), 5.57-5.48 (m, 2H; H-3'', H-2'), 5.38-5.28 (m, 5H; H-2, H-2'', H-4, H-4', H-4''), 5.19-5.14 (dd, J = 8.0 Hz, J = 10.0 Hz, 1H; H-2'''), 5.06 (t, J = 10.0 Hz, 1H; H-4'''), 4.95 (d, J = 7.5 Hz, 1H, H-1), 4.65-4.58 (m, 5H; H-1', H-1'', H-1''', CH_2Ph), 3.96-3.7 (m, 8H; H-5, H-

5', H-5'', H-5''', H-6a, H-6a', H-6a'', H-6b'''), 3.66-3.61 (m, 2H; H-6b, H-6b'), 3.52-3.45 (m, 2H; H-6a'', H-6b''). HR-MALDI MS: (m/z); Calculated mass 2043.57 [$M + \text{Na}$]⁺, mass obtained 2043.5670 [$M + \text{Na}$]⁺.

***p*-(bPEG-oxy-succinoyloxy)benzyl 6-*O*-benzyl-2-deoxy-2-[[*(2,2,2*-trichloroethoxy)-carbonyl]amino]-3-*O*-(9-fluorenylmethoxycarbonyl)-4-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside (22).** A suspension of thioglycoside **18** (102.1 mg, 0.144 mmol) and bPEG bound compound **19** (505 mg, 0.012 mmol, 0.096 mmol reaction sites for an 8-arm polymer) and activated molecular sieves (4 Å) in dichloromethane (8 mL) was stirred under an atmosphere of argon for 30 minutes. The mixture was cooled (0°C) and subsequently NIS (38.7 mg, 0.172 mmol) and TMSOTf (3.1 μL, 0.017 mmol) were added. After the glycosyl donor was consumed (monitored by TLC, ethyl acetate: hexanes, 1/2, v/v), compound **21** (74.32 mg, 0.144 mmol) and NIS (38.92 mg, 0.173 mmol) and TMSOTf (3.1 μL, 0.017 mmol) were added and the reaction mixture was stirred at 0°C for 0.5h. The reaction mixture was diluted with dichloromethane (100 mL) and molecular sieves were removed by filtration. Aqueous Na₂S₂O₃ (15%, 4 mL) was added to the filtrate and the organic layer was dried (MgSO₄) and concentrated under reduced pressure. The residue was dissolved in acetonitrile (40 mL) and added to the Millipore stirred cell fitted with a Biomax disc membrane of NMWL= 5,000 Da. The solution was magnetically stirred for 16 hours under 50 psi pressure of nitrogen to give the bPEG-bound sugar **22** as residue (585.3 mg).

***p*-(bPEG-oxy-succinoyloxy)benzyl 6-*O*-benzyl-2-deoxy-2-[[*(2,2,2*-trichloroethoxy)-carbonyl]amino]-4-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside (23):** bPEG bound compound **22** (550 mg, 0.011 mmol) was dissolved in dichloromethane (8 mL) and triethylamine (3 mL) was added. The reaction mixture was stirred at room temperature for 18 hours and then concentrated under reduced pressure. The residue was redissolved in acetonitrile (50 mL) and added to a Millipore stirred cell fitted with a Biomax disc membrane of NMWL= 5,000 Da. The solution was magnetically stirred for 16 hours under 50 psi pressure of nitrogen to give the bPEG-bound compound **23** (498.7 mg) as residue.

***p*-hydroxybenzyl 6-*O*-benzyl-2-deoxy-2-[[*(2,2,2*-trichloroethoxy)carbonyl]amino]-3-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl- α -L-fucopyranosyl)-4-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzoyl- β -D-galactopyranosyl)- β -D-glucopyranoside (**24**):** A solution of compound **24** (37.68 mg, 0.0984 mmol) and polymer bound compound **23** (400 mg, 0.0082 mmol, 0.0656 mmol reaction sites for an 8-arm polymer) in dichloromethane (14 mL) was stirred in the presence of molecular sieves 4Å for 30 minutes. The mixture was cooled (0°C) and NIS (26.56 mg, 0.118 mmol) and TMSOTf (2.0 μ L, 0.011 mmol) were added. After 30 minutes, TLC analysis (ethyl acetate/hexanes, 1/3, v/v) indicated that all the thioglycoside **24** had been consumed. The reaction mixture was diluted with dichloromethane (60 mL) and the molecular sieves were removed by filtration. The filtrate was washed with aqueous sodium thiosulfate (15%, 4 mL), dried (MgSO₄) and concentrated to dryness under reduced pressure. The residue was dissolved in acetonitrile (50 mL) and added to the Millipore stirred cell fitted with a Biomax disc membrane of NMWL= 5,000 Da. The solution was magnetically stirred for 16 hours under 50 psi pressure of nitrogen to give the bPEG-bound sugar **25** as the residue. This residue was retreated with thioglycoside **24** in the presence of NIS/TMSOTf as described above to afford compound **25** (384 mg), which was dissolved in THF (8 mL) and Et₃N (0.6 mL) and H₂O₂ (50% in water, 0.12 mL) were added. After being stirred at room temperature for 24 hours, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in acetonitrile (50 mL) and added to a Millipore stirred cell fitted with a Biomax membrane of NMWL= 5,000 Da. The solution was magnetically stirred for 16 hours under 50 psi pressure of nitrogen. This filtrate was concentrated and the crude product was purified by Sephadex LH-20 size exclusion column chromatography (eluent: methanol/dichloromethane, 1/1, v/v) to give **24** as a colorless syrup (40.8 mg). ¹H NMR (500 MHz, CDCl₃): δ 8.07-7.25 (m, 20H; Ar-H, 2 x Bn, 2 x Bz), 7.11 (d, 2H; Ar-H, *p*-hydroxybenzyl), 6.74 (d, 2H; Ar-H, *p*-hydroxybenzyl), 5.47 (d, *J* = 3.5 Hz, 1H; H-4'), 5.41 (d, *J* = 3.5 Hz, 1H; H-4''), 5.32-5.28 (m, 2H, H-3'', H-2'), 5.25 (d, *J* = 3.5 Hz, 1H; H-1''), 5.20 (d, *J* = 9.5 Hz, 1H, NH), 5.05 (q, *J* = 6.5 Hz, 1H; H-5''), 4.98 (dd, *J* = 10.0, 3.5 Hz, 1H; H-3'), 4.81-4.32 (m, 12H; H-1, 2 x OCH₂Ph, OCH₂PhOH, OCH₂CCl₃, H-1', H-6'a, H-6'b), 4.18 (t, *J* = 10.0 Hz, 1H; H-3), 4.02 (t, 1H; H-4), 3.88 (dd, *J* = 10.5, 3.5

Hz, 1H; H-2''), 3.73-3.68 (m, 2H; H-6a, H-5'), 3.52 (d, $J = 10.0$ Hz, 1H; H-6b), 3.26-3.13 (m, 2H, H-5, H-2), 2.22, 2.14, 1.94, 1.88 (4s, 4 x CH_3CO), 1.29 (d, $J = 6.5$ Hz, 3H; H-6''); ^{13}C NMR (125 MHz, CDCl_3): δ 170.6, 170.1, 169.9, 169.8 (4C; 2 x CH_3CO), 166.4, 164.7 (2C, 2 x PhCO), 155.7 (Ar-C), 153.4 (NHCO), 133.5-115.5 (Ar-C), 99.9 (C-1'), 98.5 (C-1), 97.3 (C-1''), 95.5 (CCl_3), 74.9 (C-5), 74.5, 74.0, 70.8, 70.6 (4C; 2 x OCH_2Ph , OCH_2CCl_3 , OCH_2PhOH), 74.1 (C-2''), 73.9 (C-3), 73.8 (C-4), 72.2 (C-4''), 71.1 (C-3'), 71.0 (C-5'), 70.4 (C-3'', C-2'), 67.7 (C-6), 67.3 (C-4'), 64.4 (C-5''), 61.2 (C-6'), 59.5 (C-2), 20.8, 21.0, 20.7 (4 x COCH_3), 16.1 (C-6''); MALDI-TOF MS: m/z (%): 1346 (100) [$M + \text{Na}$] $^+$.

/export/home/debatosh/vnmrSYS/data/Superpuretetra

Archive directory: /export/home/debatosh/vnmrSYS/data

Sample directory: /export/home/debatosh/vnmrSYS/data/Superpuretetra

Pulse Sequence: s2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

File: superpuretetrasac

INOVA-500 "nmr2"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

128 repetitions

OBSERVE H1, 499.6391331 MHz

DATA PROCESSING

FT size 32768

Total time 6 min, 17 sec

8.0

7.5

7.0

6.5

6.0

5.5

5.0

4.5

4.0

3.5

3.0

ppm

cosy

cosy

compound # 10

HSQC

HSQC

COMPOUND NO. 10

TOCSY

TOCSY

COMPOUND NO.10

/export/home/debatosh/vnmrSYS/data/DM-4-x-52

Sample directory: /export/home/debatosh/vnmrSYS/data/DM-4-x-52

File: PROTON

Pulse Sequence: s2pul

Solvent: cdcl3
Temp. 29.0 C / 302.1 K
Mercury-300BB "merc300"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
16 repetitions

OBSERVE H1 300.0803996 MHz

DATA PROCESSING

Line broadening 0.3 Hz

FT size 32768

Total time 0 min, 49 sec

/export/home/debatosh/vnmrsys/data/DM-3-117-50

Sample directory: /export/home/debatosh/vnmrsys/data/DM-3-117-50
File: PROTON

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 29.0 C / 302.1 K
Mercury-300BB "merc300"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
32 repetitions

OBSERVE H1, 300.0804038 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 0 min, 0 sec

/export/home/debatosh/vnmrSYS/data/DW-4- -79

Sample directory: /export/home/debatosh/vnmrSYS/data/DW-4- -79
File: PROTON

Pulse Sequence: s2pul

Solvent: cdcl3
Temp. 29.0 C / 302.1 K
Mercury-300BB "merc300"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
32 repetitions

OBSERVE H1 300.0804075 MHZ
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 3 min, 18 sec

/export/home/debatosh/vnmrsys/data/DN-3-119-80

Sample directory: /export/home/debatosh/vnmrsys/data/DN-3-119-80

File: PROT0N

Pulse Sequence: s2pul

Solvent: cdcl3

Temp. 29.0 C / 302.1 K

Mercury-300BB "merc300"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.995 sec

Width 4506.5 Hz

8 repetitions

OBSERVE H1, 300.0804020 MHz

DATA PROCESSING

Line broadening 0.3 Hz

FT size 32768

Total time 0 min, 0 sec

/export/home/debatosh/vnmrsys/data/FmocprotectedThiogluconide

Archive directory: /export/home/debatosh/vnmrsys/data

Sample directory: /export/home/debatosh/vnmrsys/data/FmocprotectedThiogluconide

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

File: FmocProtectedThiogluconide

INOVA-500 "nmr1"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

128 repetitions

OBSERVE H1, 499.6261027 MHz

DATA PROCESSING

FT size 32768

Total time 6 min, 17 sec

Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
 INOVA-500 "inova500"
 PULSE SEQUENCE
 Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 1.831 sec
 Width 4085.0 Hz
 16 repetitions
 OBSERVE H1, 499.6834172 MHz
 DATA PROCESSING
 FT size 16384
 Total time 0 min, 52 sec

Pulse Sequence: gCOSY

Solvent: CDCl₃

Ambient temperature

INNOVA-500 "Inova500"

PULSE SEQUENCE: gCOSY

Relax. delay 1.000 sec

Acq. time 0.251 sec

Width 4085.0 Hz

2D Width 4085.0 Hz

2 repetitions

1024 increments

OBSERVE H1, 499.6834172 MHz

DATA PROCESSING

Sine bell 0.125 sec

F1 DATA PROCESSING

Sine bell 0.125 sec

FT size 2048 x 2048

Total time 47 min, 44 sec

COSY

COMPOUND NO.26

Pulse Sequence: gHSQC

Solvent: CDCl₃
Ambient temperature
INOVA-500 "Inova500"
PULSE SEQUENCE: gHSQC
Relax delay 1.000 sec
Acq time 0.125 sec
Width 4085.0 Hz
2D Width 20105.6 Hz
8 repetitions
2 x 256 increments
OBSERVE H1, 489.8634172 MHz
DECOUPLE C13, 125.8553168 MHz
Power 48 dB
on during acquisition
off during delay
GARP-1 modulated
DATA PROCESSING
Gauss apodization 0.058 sec
F1 DATA PROCESSING
Gauss apodization 0.006 sec
FT size 2048 x 2048
Total time 1 hr, 19 min, 39 sec