Electronic Supplementary Information

Modular Automated Solid Phase Synthesis of Dermatan Sulfate Oligosaccharides

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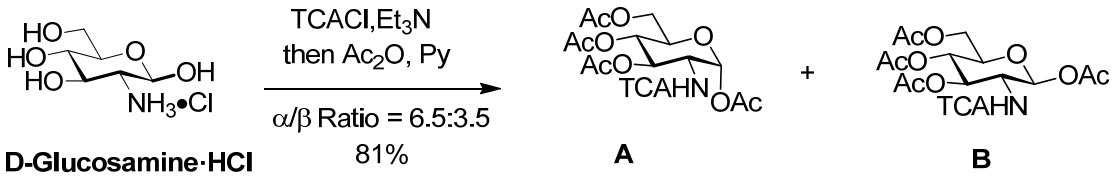
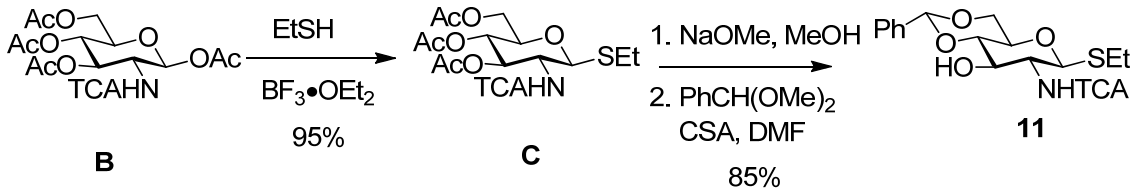
1. General Materials And Methods

All chemicals used were reagent grade and used as supplied except where noted. Anhydrous solvents used were taken from a dry solvent system (jcmeyer-solvent systems). Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). Purification by normal/reverse phase HPLC was performed using Agilent 1200 series. ^{1}H, ^{13}C spectra were recorded on a Varian 400-MR (400 MHz) and/or Varian 600-MR (600 MHz) spectrometer in $CDCl_3$ (δ, 7.24), methanol-D_4 (δ, 3.31), D_2O (δ, 4.80). NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hz. High resolution mass spectra were obtained with a 6210 ESI-TOF mass spectrometer (Agilent) with ES ionization (small organics) or an Amazon ETD ion trap mass spectrometer (Bruker; oligonucleotides). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using Perkin-Elmer 241 and Unipol L1000 polarimeters.

2. Synthesis of Building Blocks And Merrifield Supported Linker

2.1 Synthesis of Ethyl 4,6-*O*-benzyliden-2-deoxy-2-*N*-trichloroacetamido-thio-β-D-glucopyranoside, 10

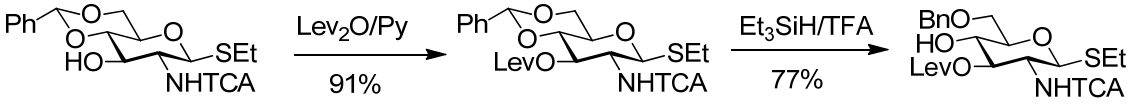




D-Glucosamine·HCl (20 g, 92.7 mmol) was stirred in MeOH (200 mL) at room temperature. Et_3N (30 mL) was added and cooled to 0 °C to which trichloroacetyl chloride was added dropwise and allowed to warm to room temperature. After 5 days, the reaction was filtered through a plug of Celite and evaporated to dryness. The crude residue was co-evaporated with pyridine (twice), dissolved in pyridine (250 mL), and cooled to 0^oC. Ac_2O (100 mL) was added dropwise to the reaction mixture and allowed to warm to room temperature. After 24h, the reaction mixture was diluted with EtOAc and washed with 0.1M HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, concentrated, and subjected to flash column chromatography (Silica: DCM/EtOAc) to obtain **A** (α-isomer, 24 g) and **B** (β-Isomer, 13 g) in 81% overall yield. The analytical data was in agreement with the literature data.[1] To a solution of **B** (12.0 g, 24.35 mmol) in CH_2Cl_2 (150 mL) were added ethanethiol (2.7 mL, 35.70 mmol) and boron trifluoride etherate ($BF_3.OEt_2$) (3.1 mL, 24.34 mmol) at 0 °C. The reaction was allowed to warm to room temperature and stirred for 3 hours. After that, the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, concentrated and subjected to a short flash column chromatography (Silica; Hexane/EtOAc) to obtain thioglycoside **C** in 95% yield (11.5 g). The analytical data was in agreement with the literature data.[2] Thioglycoside **C** (11.0 g, 22.2 mmol) was dissolved in a mixture of methanol and DCM (150 mL, 1:1), to which

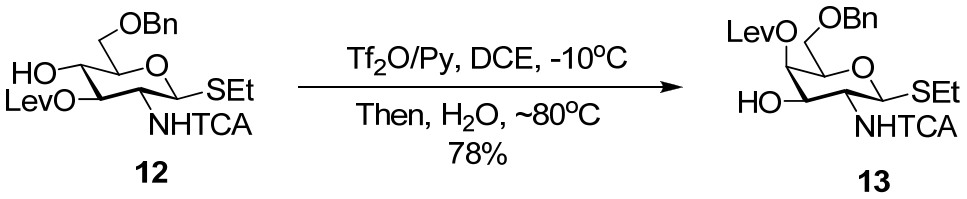
NaOMe (1.2 g, solid) was added, and stirred overnight. The reaction mixture was neutralized with Amberlite®IR120 (H+), filtered and evaporated to dryness. The crude triol was co-evaporated twice with toluene and dissolved in DMF (100 mL). Benzaldehyde dimethyl acetal (4.0 mL, 26.7 mmol) and CSA (500 mg) were added to the reaction mixture and stirred at 65°C. After complete conversion of the starting material (~4 h), the reaction was quenched by the addition of triethylamine (5.3 mL) and the volatiles were removed *in vacuo*. The remainder was dissolved in DCM and washed with brine. The organic layer was dried over $MgSO_4$, concentrated and subjected to flash column chromatography (Silica, EtOAc:Hexane) to obtain thioglycoside **11** in 85% yield (8.65 g, over two steps). The analytical data was in agreement with the literature data.[3]

2.2 Synthesis of Ethyl 6-*O*-benzyl-3-*O*-levulinoyl-2-deoxy-2-*N*-trichloroacetamido-thio-β-D-glucopyranoside, 12



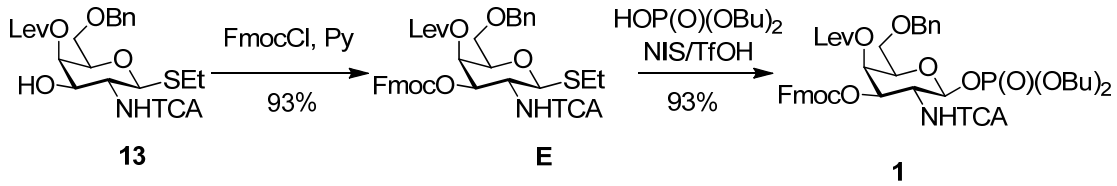
Thioglycoside **11** (8.0 g, 21.7 mmol) and levulinic anhydride (Lev_2O, 6.3 g, 29.6 mmol) were stirred in a mixture of dichloromethane (DCM) and pyridine (100 mL, 1:1) at room temperature and the progress was monitored by TLC. After completion (~ 18 h), the reaction mixture was diluted with DCM and washed with 0.1M HCl, saturated $NaHCO_3$ and brine. The organic layer was then dried over $MgSO_4$, concentrated and subjected to flash column chromatography to obtain Lev-protected thioglycoside **D** in 91% yield (8.84 g). The analytical data was in agreement with the literature data.[2] Lev-protected thioglycoside **D** (8 g, 14.4 mmol), triethylsilane (9.21 mL, 57.7 mmol), and hot gun-dried 4 Å molecular sieves (powdered, 2.5 g) were stirred in anhydrous DCM (25 mL) for 30 minutes at room temperature and cooled down to 0°C. Trifluoroacetic acid (4.4 mL, 57.7 mL) was added dropwise and the reaction mixture was allowed to warm to room temperature. After complete conversion of the starting material the reaction mixture was neutralized with Et_3N and diluted with DCM. Molecular sieves were filtered off and the filtrate was washed with H_2O, saturated $NaHCO_3$ and brine. The organic layer was then dried over $MgSO_4$, concentrated and subjected to flash column chromatography (Silica, Hexane/EtOAc) to obtain thioglycoside **12** in 77% yield (6.2 g). The analytical data was in agreement with the literature data.[2]

2.3 Synthesis of Ethyl 6-*O*-benzyl-4-*O*-levulinoyl-2-deoxy-2-*N*-trichloroacetamido-thio-β-D-galactopyranoside, 13



Thioglycoside **12** (5 g, 9.0 mmol) was stirred in a mixture of DCE and pyridine (30 mL, 10:1) at -15°C. Tf_2O (9.9 mL, 1M solution in DCM) was added dropwise to the reaction mixture and the progress was monitored by TLC. After complete conversion of the starting material, H_2O (4 mL) was added and the mixture was stirred at ~80°C for 5h. The reaction mixture was then cooled down to room temperature, diluted with DCM, and washed with 0.1M HCl, saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, concentrated and subjected to flash column chromatography to obtain title compound **13** in 78% yield (3.9 g). $[\alpha]_D^{20}$ -27.17 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35 – 7.25 (m, 5H), 7.05 – 6.80 (d, *J* = 8.1 Hz, 1H), 5.46 (d, *J* = 2.7 Hz, 1H), 4.76 (d, *J* = 10.2 Hz, 1H), 4.48 (q, *J* = 11.7 Hz, 2H), 4.07 (dd, *J* = 10.2, 3.2 Hz, 1H), 3.90 – 3.79 (m, 2H), 3.57 (dd, *J* = 9.8, 6.0 Hz, 1H), 3.50 (dd, *J* = 9.8, 6.5 Hz, 1H), 2.83 – 2.64 (m, 4H), 2.64 – 2.50 (m, 2H), 2.17 (s, 3H), 1.26 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 207.6, 172.5, 162.3, 137.7, 128.3, 127.9, 127.7, 92.4, 83.4, 76.4, 73.6, 71.0, 70.3, 68.2, 55.1, 38.2, 29.7, 28.0, 24.5, 15.0. IR (neat) ν_{max} = 3337, 2926, 1739, 1704, 1527 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{22}H_{28}Cl_3NNaO_7S$: 578.0550; Found: 578.0562.

2.4 Synthesis of Di-*O*-butyl 6-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-4-*O*-levulinoyl-2-deoxy-2-*N*-trichloroacetamido-thio-β-D-galactopyranosylphosphate, 1

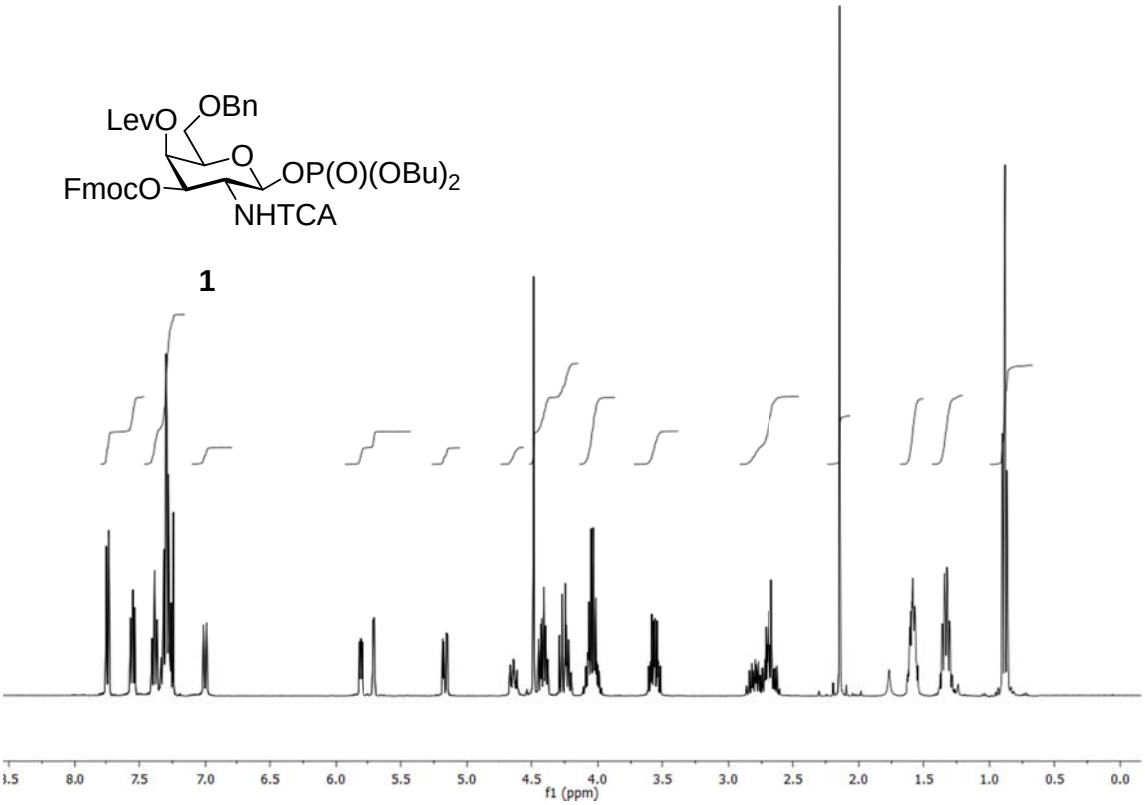


Thioglycoside **13** (3.9 g, 7.0 mmol) and FmocCl (3.6 g, 14.0 mmol) were stirred in DCM at 0°C and pyridine (2.5 mL) was added dropwise. After stirring for 4h at room temperature, the reaction mixture was concentrated and co-evaporated with toluene (twice) and subjected to

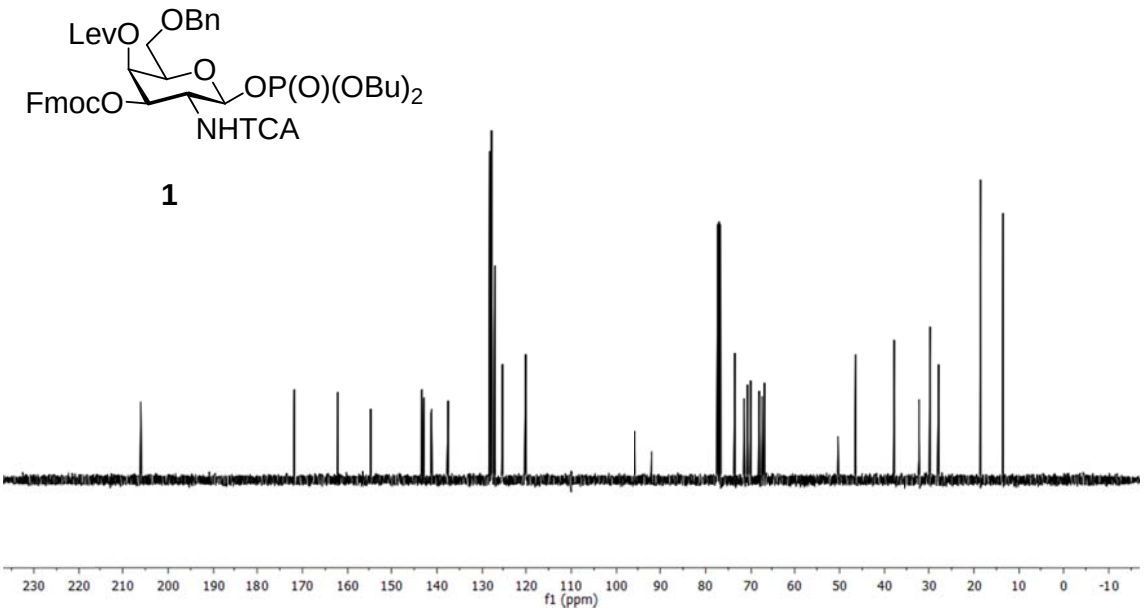
flash column chromatography using Hexane/EtOAc to afford Fmoc-protected thioglycoside **E** in 93% yield (5.1 g). $[\alpha]_D^{20}$ -8.20 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.73 (d, *J* = 7.6 Hz, 2H), 7.55 (dd, *J* = 10.2, 3.6 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 2H), 7.34 – 7.25 (m, 7H), 6.78 (d, *J* = 8.7 Hz, 1H), 5.67 (d, *J* = 3.1 Hz, 1H), 5.19 (dd, *J* = 10.8, 3.3 Hz, 1H), 4.85 (d, *J* = 10.3 Hz, 1H), 4.48 (d, *J* = 5.0 Hz, 2H), 4.43 (dd, *J* = 10.0, 6.8 Hz, 1H), 4.32 – 4.08 (m, 3H), 3.91 (m, 1H), 3.61 (dd, *J* = 9.6, 5.9 Hz, 1H), 3.52 (dd, *J* = 9.6, 6.9 Hz, 1H), 2.85 – 2.57 (m, 6H), 2.13 (s, 3H), 1.27 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 205.9, 171.7, 161.8, 154.3, 143.3, 142.9, 141.2, 141.1, 137.6, 128.3, 127.9 (2C), 127.8, 127.7, 127.1, 127.16, 125.2, 125.1, 120.0, 119.9, 92.1, 83.6, 76.1, 74.5, 73.5, 70.6, 67.7, 67.2, 52.3, 46.5, 37.9, 29.7, 27.8, 24.5, 14.9. IR (neat) ν_{max} = 1747, 1718, 1214 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{37}H_{38}Cl_3NNaO_9S$: 800.1231; Found: 800.1242.

Fmoc-protected thioglycoside (5.0 g, 6.5 mmol), dibutyl hydrogen phosphate (6.5 mL, 32.7 mmol) and hot gun-dried 4 Å molecular sieves (3.0 g, powdered) were stirred in anhydrous DCM (15 mL) at room temperature. After for 30 minutes, N-iodosuccinimide (NIS, 1.76 g, 7.8 mmol) was added to the reaction mixture at once and cooled immediately to -5°C. After 3 minutes, a catalytic amount of TfOH (0.1 mL) was added and stirred for 1h at the same temperature. After complete conversion of the starting material the reaction mixture was neutralized with pyridine and diluted with DCM. Molecular sieves were filtered off and the filtrate was washed with saturated $Na_2S_2O_3$, $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$, concentrated and subjected for flash column chromatography (Silica; Hexane/EtOAc), to obtain phospho-glycoside **1** in 93% yield, 5.56 g. $[\alpha]_D^{20}$ 60.14 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 7.5 Hz, 2H), 7.55 (dd, *J* = 6.8, 5.9 Hz, 2H), 7.38 (dd, *J* = 10.7, 4.3 Hz, 2H), 7.34 – 7.25 (m, 7H), 7.00 (d, *J* = 9.1 Hz, 1H), 5.81 (dd, *J* = 5.9, 3.3 Hz, 1H), 5.70 (d, *J* = 3.0 Hz, 1H), 5.17 (dd, *J* = 11.3, 3.1 Hz, 1H), 4.63 (ddt, *J* = 12.1, 9.1, 3.1 Hz, 1H), 4.48 (s, 2H), 4.41 (dt, *J* = 11.4, 6.3 Hz, 2H), 4.29 – 4.19 (m, 2H), 4.12 – 3.97 (m, 4H), 3.56 (qd, *J* = 9.7, 6.3 Hz, 2H), 2.91 – 2.59 (m, 4H), 2.14 (s, 3H), 1.58 (ddd, *J* = 5.6, 4.8, 2.7 Hz, 4H), 1.33 (ddd, *J* = 15.2, 7.6, 2.3 Hz, 4H), 0.88 (td, *J* = 7.4, 1.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 206.0, 171.7, 162.2, 154.5, 143.3, 142.8, 141.2, 141.1, 137.5, 128.3, 127.9, 127.9, 127.9, 127.8, 127.1, 125.2, 125.1, 120.0, 120.0, 95.6, 95.6, 91.9, 73.5, 71.5, 70.7, 70.1, 68.3, 68.2, 68.1, 67.5, 67.0, 50.3, 50.2, 46.4, 37.9, 32.1, 32.1, 32.1, 32.0, 29.7, 27.8, 18.5, 13.5. IR (neat) ν_{max} = 1750, 1715, 1451 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{43}H_{51}Cl_3NNaO_{13}P$: 948.2061; Found: 948.2073.

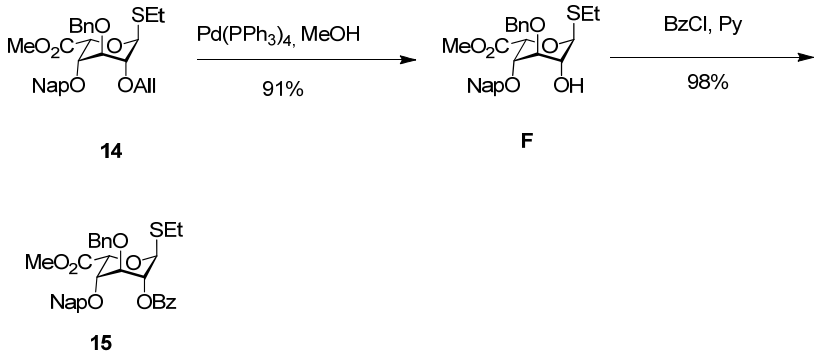
^{1}H NMR of Compound 1



^{13}C NMR of Compound 1



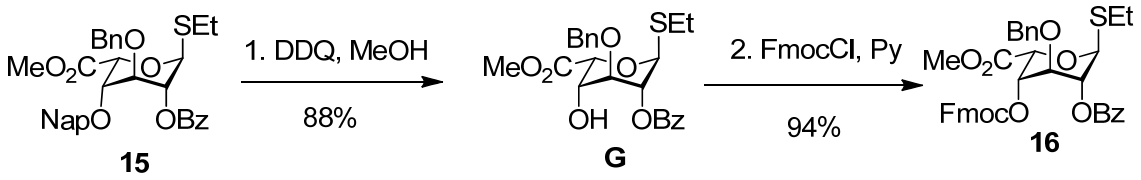
2.5 Synthesis of Methyl(ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-naphthyl)methyl-1-thio-α-L-idopyranosyl)urinate, 15



Thioglycoside **14**[4] (5.6 g, 10.7 mmol), 1,3-dimethylbarbituric acid (3.35, 21.4 mmol) and tetrakis(triphenylphosphine)palladium (1.23 g, 1.07 mmol) were stirred in methanol (25 mL) at 40°C under an argon atmosphere and the progress was monitored by TLC (10% EtOAc/Toluene). After completion (~ 5 h), the reaction mixture was cooled down to room temperature, diluted with ethyl acetate and washed with saturated $NaHCO_3$ and brine. The organic layer was then dried over $MgSO_4$, concentrated and subjected to flash column chromatography (Silica, Hexane/EtOAc) to obtain the de-allylated thioglycoside **F** in 91% yield (4.7 g). $[\alpha]_D^{20}$ -74.36 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.76 (m, 3H), 7.63 (s, 1H), 7.52 – 7.44 (m, 2H), 7.35 – 7.24 (m, 6H), 5.40 (s, 1H), 5.15 (d, *J* = 1.8 Hz, 1H), 4.72 (d, *J* = 11.8 Hz, 1H), 4.62 (t, *J* = 12.2 Hz, 2H), 4.45 (d, *J* = 12.0 Hz, 1H), 3.95 (m, 1H), 3.86 (s, 2H), 3.77 (s, 1H), 3.64 (s, 3H), 2.65 (m, 2H), 1.28 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 137.2, 133.9, 133.1, 133.0, 128.4, 128.3, 127.9, 127.90, 127.7, 127.6, 127.1, 126.3, 126.2, 125.8, 86.4, 75.0, 73.1, 72.0, 71.7, 68.6, 67.7, 52.1, 26.9, 15.0. IR (neat) ν_{max} = 1763, 1735 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{27}H_{30}NaO_6S$: 505,1661; Found: 505,1656. De-allylated thioglycoside **F** (4.6 g, 9.5 mmol) was stirred in pyridine (100 ml) at 0°C and benzoyl chloride (2.5 mL) was added dropwise. After stirring overnight at room temperature, the reaction mixture was diluted with EtOAc and washed with 0.1M HCl, saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, concentrated and subjected to flash column chromatography (Silica; Hexane/EtOAc) to afford title compound **15** in 98% yield (5.5 g). $[\alpha]_D^{20}$ -62.9 (*c* 1, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.84 – 7.74 (m, 1H), 7.67 (dd, *J* = 9.0, 3.3 Hz, 2H), 7.55 (s, 1H), 7.50 – 7.41 (m, 2H), 7.41 – 7.26 (m, 6H), 7.25 – 7.23 (m, 1H), 7.11 (dd, *J* = 8.3, 7.5 Hz, 2H), 5.56 (d, *J* = 1.8 Hz, 1H), 5.27 (dd, *J* = 4.1, 2.1 Hz, 1H), 5.20 (d, *J* = 1.8 Hz, 1H), 4.80 (d, *J* = 11.9

Hz, 1H), 4.57 (m, 3H), 3.94 (d, *J* = 2.0 Hz, 2H), 3.75 (s, 3H), 2.80 – 2.57 (m, 2H), 1.29 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.0, 165.6, 137.2, 134.8, 133.0, 133.0, 132.9, 129.9, 129.3, 128.4, 128.1, 127.9, 127.9, 127.8, 127.6, 126.4, 126.0, 125.9, 125.7, 82.4, 74.6, 72.6, 72.3, 71.3, 68.9, 68.5, 52.1, 26.5, 14.9. IR (neat) ν_{max} = 1765, 1718 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{34}H_{34}NaO_7S$: 609.1923; Found: 609.1918.

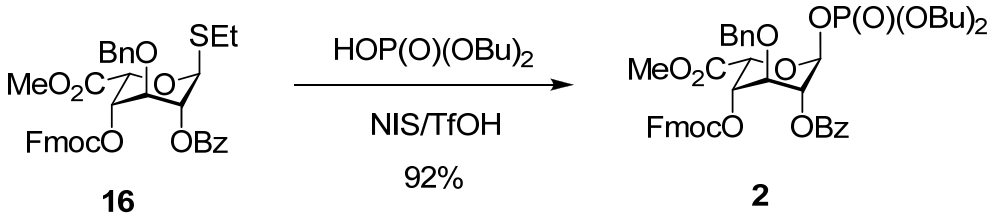
2.6 Synthesis of Methyl(ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-α-L-idopyranosyl)urinate, 16



Thioglycoside **15** (5.5 g, 9.37 mmol) was stirred in a mixture of dichloromethane (DCM) and methanol (50 mL, 4:1) at room temperature and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (4.26 g, 18.75 mmol) was added at once and the progress was monitored by TLC. After completion (~ 8 h), the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$, and brine. The organic layer was then dried over $MgSO_4$, concentrated and subjected to flash column chromatography (Silica, Hexane/EtOAc) to obtain de-naphthylated thioglycoside **G** in 88% yield (3.7 g). $[\alpha]_D^{20}$ -77.71 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (dd, *J* = 8.3, 1.1 Hz, 2H), 7.56 (m, 1H), 7.47 – 7.26 (m, 7H), 5.54 (s, 1H), 5.30 (m, 1H), 5.27 (d, *J* = 1.7 Hz, 1H), 4.82 (d, *J* = 11.9 Hz, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 4.08 (s, 1H), 3.85 (td, *J* = 3.0, 1.1 Hz, 1H), 3.81 (s, 3H), 2.68 (m, 2H), 1.30 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.8, 164.9, 137.0, 133.7, 129.7, 128.9, 128.6, 128.4, 127.9, 127.7, 83.29, 73.74, 72.25, 69.71, 68.48, 68.33, 52.37, 27.04, 14.93. IR (neat) ν_{max} = 1759, 1720 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{23}H_{26}NaO_7S$: 469.1297; Found: 469.1298. De-naphthylated thioglycoside **G** (3.7 g, 8.30 mmol) and FmocCl (4.29 g, 16.6 mmol) were stirred in DCM (50 mL) at 0^{o}C and pyridine (4 mL) was added dropwise. After stirring for 4h at room temperature, the reaction mixture was concentrated, co-evaporated with toluene (twice) and subjected to column chromatography (Silica, Hexane/EtOAc) to afford thioglycoside **16** in 94% yield (5.2 g). $[\alpha]_D^{20}$ -40.53 (*c* 1, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.13 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.74 (dd, *J* = 7.6, 0.7 Hz, 2H), 7.58 (dd, *J* = 7.5, 0.8 Hz, 1H), 7.49 – 7.33 (m, 8H), 7.28 (tdd, *J* = 7.8, 3.1, 0.9 Hz, 4H), 7.19 (td, *J* = 7.5, 1.1 Hz, 1H), 5.59 (s, 1H), 5.39 (d, *J* = 2.0 Hz, 1H), 5.31 (m, 1H), 5.17 (m, 1H), 4.85 (d, *J* = 11.8 Hz, 1H), 4.73

(d, *J* = 11.8 Hz, 1H), 4.32 (dd, *J* = 10.4, 8.1 Hz, 1H), 4.22 (dd, *J* = 10.3, 7.3 Hz, 1H), 4.10 (t, *J* = 7.6 Hz, 1H), 4.00 (td, *J* = 2.9, 1.1 Hz, 1H), 3.79 (s, 3H), 2.96 – 2.53 (m, 2H), 1.32 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 168.7, 165.3, 154.3, 143.2, 142.8, 141.2, 141.1, 136.8, 133.4, 130.0, 129.2, 128.4, 128.3, 127.9, 127.9, 127.9, 127.6, 127.2, 127.1, 125.2, 125.0, 120.0, 82.9, 72.6, 71.9, 71.2, 70.2, 68.4, 66.4, 52.57, 46.5, 26.9, 14.9. IR (neat) ν_{max} = 1748, 1719 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{38}H_{36}NaO_9S$: 691.1978; Found: 691.2006.

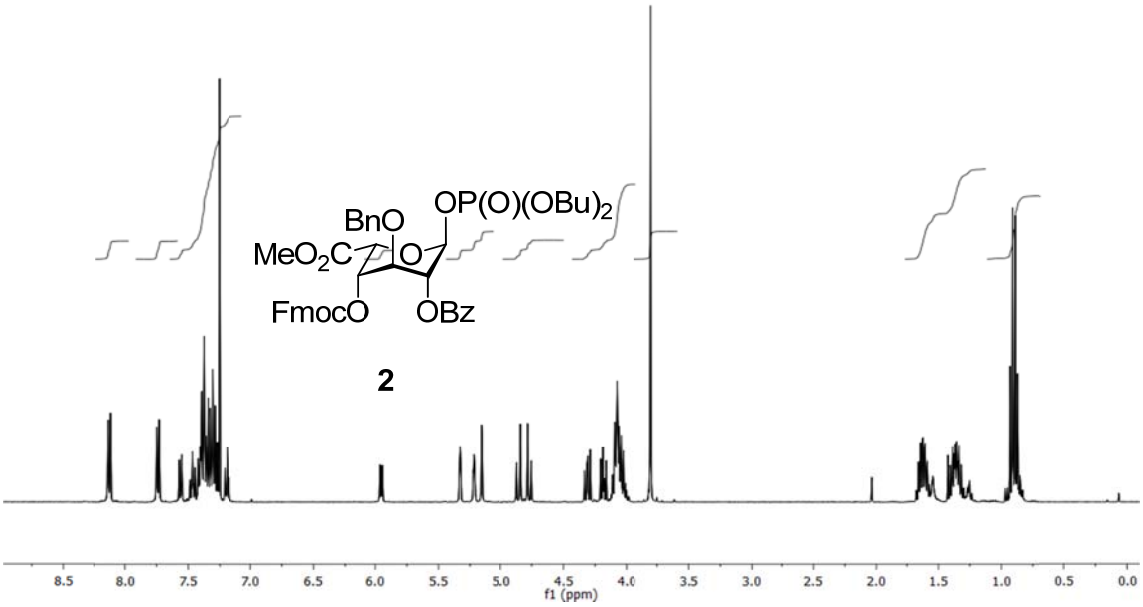
2.7 Synthesis of Methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-di-O-butylphosphatidyl-α-L- idopyranosyluronate, 2



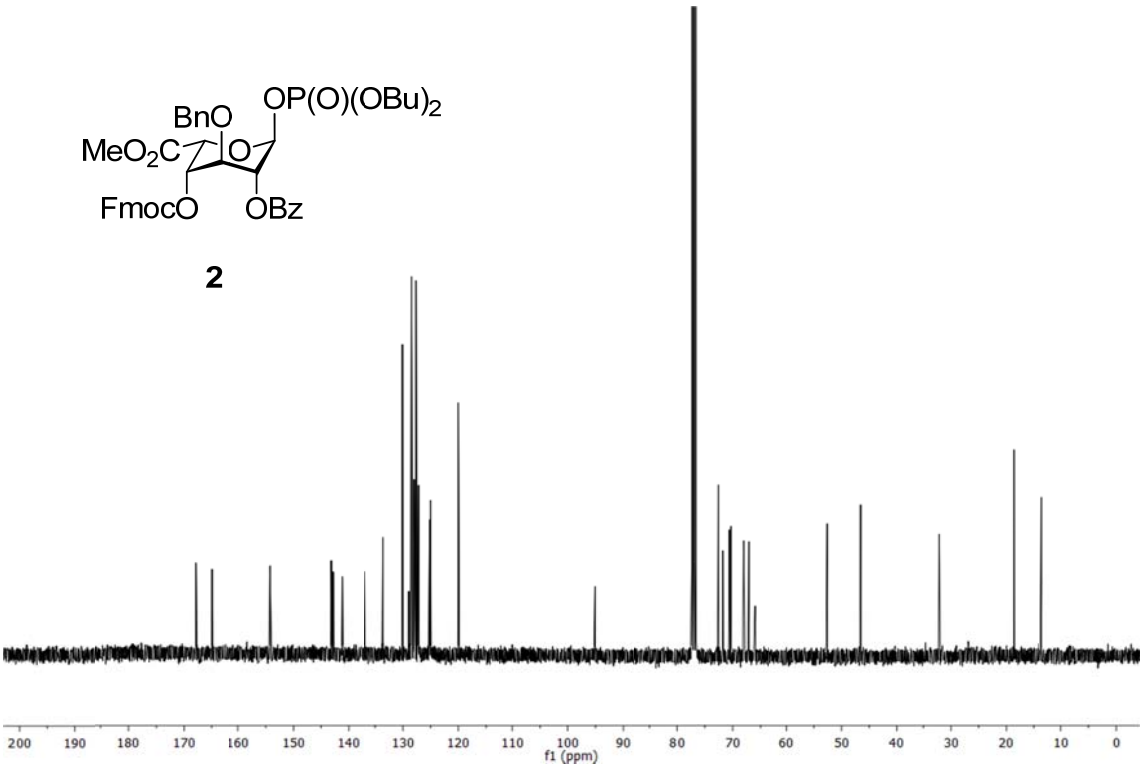
Thioglycoside **16** (5.5 g, 8.22 mmol), dibutyl hydrogen phosphate (8.24 mL, 41.1 mmol), and hot gun-dried 4 Å molecular sieves (powdered, 3.0 g) were stirred in anhydrous DCM (15 mL) at room temperature. After 30 minutes, NIS (2.2 g, 9.87 mmol) was added to the reaction mixture at once and immediately cooled to -5^{o}C. After 3 minutes, a catalytic amount of TfOH (0.1 mL) was added to the reaction and stirred for 1h at the same temperature and monitored by TLC. After complete conversion of the starting material the reaction mixture was neutralized with pyridine and diluted with DCM. Molecular sieves were filtered off and the filtrate was washed with saturated $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, concentrated and subjected for column chromatography (Silica; Hexane/Ethyl acetate) to obtain phospho-glycoside **2** in 92% yield (6.2 g). $[\alpha]_D^{20}$ -6.88 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 7.5 Hz, 1H), 7.47 (m, 1H), 7.44 – 7.26 (m, 11H), 7.19 (td, *J* = 7.5, 1.0 Hz, 1H), 5.95 (d, *J* = 6.8 Hz, 1H), 5.33 (d, *J* = 1.1 Hz, 1H), 5.22 (m, 1H), 4.86 (d, *J* = 11.6 Hz, 1H), 4.77 (d, *J* = 11.6 Hz, 1H), 4.31 (dd, *J* = 10.3, 8.0 Hz, 1H), 4.18 (dd, *J* = 10.4, 7.3 Hz, 1H), 4.12 – 3.98 (m, 6H), 3.80 (s, 3H), 1.61 (ddd, *J* = 14.8, 13.7, 6.8 Hz, 4H), 1.44 – 1.30 (m, 4H), 0.90 (dt, *J* = 8.7, 7.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.8, 164.9, 154.2, 143.1, 142.8, 141.2, 141.1, 136.9, 133.5, 130.0, 128.9, 128.4, 128.3, 127.9, 127.9, 127.9, 127.6, 127.2, 127.1, 125.1, 125.0, 120.0, 95.1, 95.0, 72.58, 71.76, 70.59, 70.33, 68.10, 68.04, 67.98, 67.09, 66.06,

65.97, 52.61, 46.54, 32.23, 32.17, 32.10, 18.57, 18.56, 13.52, 13.51. IR (neat) ν_{max} = 1750, 1726 cm^{-1}. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{44}H_{49}NaO_{13}P$: 839.2808; Found: 839.2830.

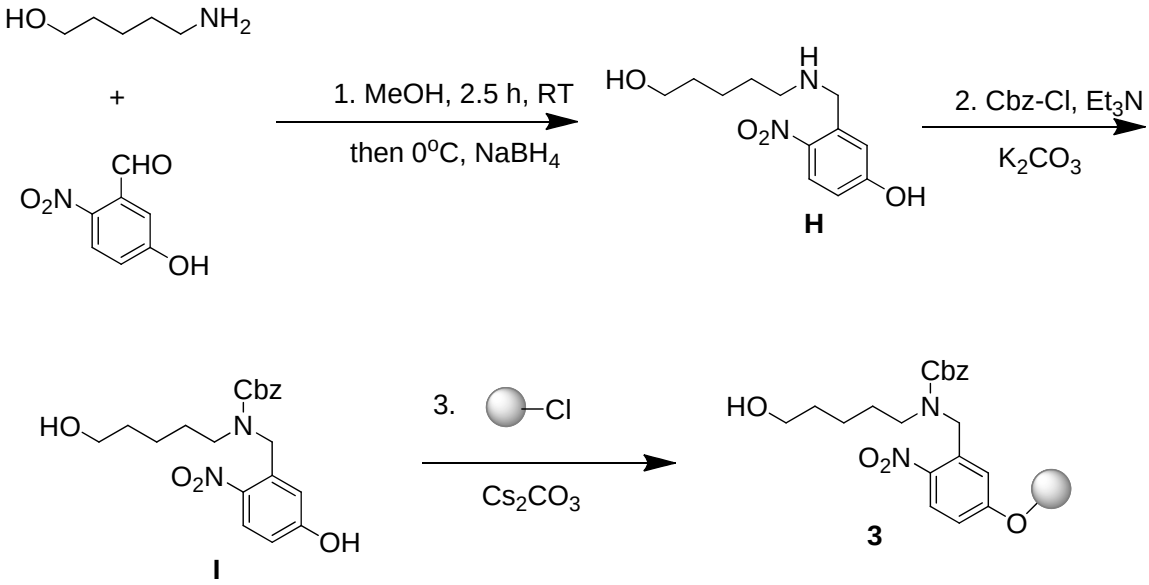
^{1}H NMR of Compound 2



^{13}C NMR of Compound 2



2.8 Synthesis of Merrifield-Supported Photo-cleavable Linker 3



5-Hydroxy-2-nitrobenzaldehyde (0.5 g, 3.0 mmol) and 5-aminopentanol (0.31 g, 3.0 mmol) were stirred in anhydrous methanol (10 mL) at room temperature for 2.5 h under argon atmosphere. Then, the reaction mixture was cooled to 0°C and $NaBH_4$ (0.12 g, 3.0 mmol) was added a portion-wise and allowed to warm to room temperature. After 1h, excess $NaBH_4$ was quenched by the addition of acetone (15 ml) and the solvents were evaporated to furnish the secondary amine **H**. Secondary amine **H** was re-dissolved and stirred in MeOH (80 ml) followed by the addition of triethylamine (1.25 mL, 8.9 mmol) and Cbz-Cl (1.68 mL, 7.48 mmol) at room temperature. After 1 h, K_2CO_3 (2.0 g) was added to the reaction mixture and stirred for an hour. The reaction mixture was then filtered through celite and the solvents were evaporated to dryness. The crude product was dissolved in DCM and washed with 0.1M HCl and water. The organic layer was dried over $MgSO_4$, filtered, concentrated and subjected to flash chromatography (Silica/ EtOAc:Hexane) to obtain photo-cleavable linker **I** in 85% yield (0.98 g).[5]

Merrifield resin (1.0 g, 0.5 mmol, loading 0.50 mmol/g) was swollen overnight in DCM (10 mL) and drained. The photo-cleavable linker **I** (0.8 g, 2.0 mmol) was dissolved in a minimum amount of DCM and transferred into the resin containing flask. Anhydrous DMF (10 mL) was added to the flask followed by Cs_2CO_3 (0.67 g, 2.0 mmol) and TBAI (0.76 g, 2.0 mmol). The solution was stirred overnight on the rotavap at ~60 °C and washed successively with

DMF/water (1/1), DMF, THF, MeOH, DCM, MeOH, and DCM (6 times each). The resin was again transferred into a flask containing DMF (10 mL) and CsOAc (0.3 g, 1.57 mmol) and stirred overnight on a rotavap at ~60 °C (capping process). The resin was then washed successively with DMF/water (1/1), DMF, THF, MeOH, DCM, MeOH, and DCM (6 times each) and dried under high vacuum to obtain photo-cleavable linker **3**. Loading (0.37 mmol/g) was determined as reported previously by us.[5]

3. Automated Synthesis Of Protected Dermatan Sulfate Oligosaccharides

3.1 General Materials and Methods: All solvents used were taken from a dry solvent system (jcmeyer-solvent systems). The building blocks were dried overnight in high vacuum before use. Activator, deprotection, sulfation and building block solutions were freshly prepared and kept under argon during the automation run. Modules were adopted from previous publication.[5]

3.2 Preparation of Stock Solutions

- **Building Block Solution:** 0.25 mmol of building block was dissolved in 2 mL of DCM
- **Activator Solution:** 480.0 µL TMSOTf was dissolved in 20 mL of DCM
- **Fmoc Deprotection Solution:** A solution of 25% Et_3N in DMF (v/v) was prepared
- **Acetylation:** Ac_2O was directly used
- **Lev deprotection:** Hydrazine Acetate (550 mg) was dissolved in a mixture of Pyridine:AcOH (40 mL; 4:1)
- **Sulfation:** 1.6 g of Pyridine-SO_3 complex was dissolved in a mixture of DMF:Py (20 mL; 1:1)

3.3 Modules for Automated Synthesis

For all compounds, automated synthesis was carried out on a 0.025 mmol scale using building blocks **1** and **2** and Merrifield supported photo-cleavable linker **3**.

3.3.1 Phosphate Glycosylation: The resin (70 mg, loading 0.37 mmol/g, 0.025 mmol) was swollen in 2 mL DCM and the temperature of the reaction vessel was adjusted to -10 °C. For the glycosylation reaction, the DCM is drained and a solution of phosphate building block (5 eq. in 1.0 mL DCM) was delivered to the reaction vessel. After the set temperature is reached, the reaction was started by the addition of TMSOTf in DCM (5 eq. in 1.0 mL DCM). The glycosylation was performed for 15 min at -10 °C and for 45 min at 0 °C. After completion of the reaction, the solution is drained and the resin was washed with 0.2 M acetic acid in DCM

and then DCM (six times each with 2 mL for 25 s). This procedure was repeated one time more.

3.3.2 Fmoc Deprotection: The resin was washed with DMF (six times with 2 mL for 25 s), swollen in 2 mL DMF and the temperature of the reaction vessel was adjusted to 25 °C. For Fmoc deprotection the DMF was drained and 2 mL of a solution of 25% Et_3N in DMF was delivered to the reaction vessel. After 5 minutes, the reaction solution was collected in the fraction collector of the oligosaccharide synthesizer and 2 mL of a solution of 25% Et_3N in DMF was delivered to the resin. This procedure was repeated for three times. For the next glycosylation the resin is washed with DMF (six times with 3 mL for 25 s), THF, 0.2 M acetic acid in DCM and DCM (six times each with 2 mL for 25 s).

3.3.3 Acetylation of Terminal Hydroxyl Group: After the last Fmoc deprotection, the resin was washed with DMF (six times with 3 mL for 25 s), DCM, and pyridine (six times each with 2 mL for 25 s), swollen in 2 mL pyridine, and the temperature of the reaction vessel is adjusted to 25 °C. The reaction was started by addition of 1 mL of acetic anhydride to the reaction vessel. After 60 min the reaction solution is drained and the resin is washed with pyridine (six times with 2 mL for 25 s). This acetylation procedure is performed three times.

3.3.4 Lev Deprotection: The resin is washed with DCM (six times with 2 mL for 25 s), swollen in 1.3 mL DCM, and the temperature of the reaction vessel is adjusted to 25 °C or 40 ^{o}C. For Lev deprotection 0.8 mL of a 0.15 M solution of hydrazine acetate in pyridine/acetic acid (1:1) is added. After 30 min the reaction solution is drained and the resin is washed with 0.2 M acetic acid in DCM and DCM (six times each with 2 mL for 25 s). The entire procedure is performed three times.

3.3.5 Sulfation of Free Hydroxyl Groups: The resin is washed with DMF and pyridine (six times each with 2 mL for 25 s), swollen in 2 mL pyridine, and the temperature of the reaction vessel is adjusted to 50 °C. For sulfation, 2 mL of a 0.5 M solution of sulfur trioxide pyridine complex in DMF/pyridine, (1:1) is added. After 3 h, the reaction solution is drained and the resin is washed with DMF and pyridine (six times each with 2 mL for 25 s). The entire procedure is performed for three times.

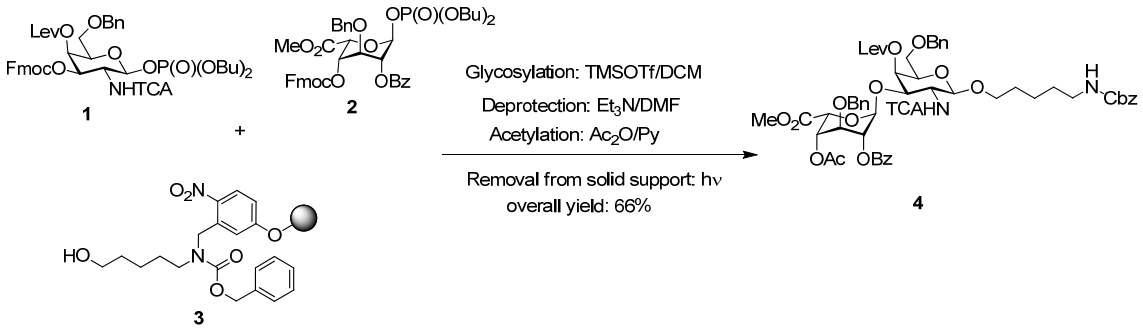
3.3.6 Finishing Automated Synthesis Process: Reaction temperature was brought to 25°C and the resin was subsequently washed with DMF and DCM (6 times each by delivering 2 ml) and drained to the waste.

3.4 Post-Synthesizer Manipulations

3.4.1 Cleavage from Solid Support: The resin is swollen in 2 mL DCM and taken up in a glass syringe. Photo-reactor FEP tubing is washed with 20 mL MeOH and 20 mL DCM using a flow rate of 4 mL per minute. For the cleavage, the resin is slowly injected from the glass syringe into the reactor and pushed through the tubing with 15 mL DCM (flow rate: 300 µL per minute). To shrink and wash out remaining resin, the tubing is washed with 15 mL DCM/MeOH, 1:1 (flow rate: 300 µL per min for 8 mL and 4 mL per minute for 7 mL), and finally with 15 mL MeOH (flow rate: 4 mL·min-1). The suspension leaving the reactor is directed into a filter (resin is filtered off) and washed with DCM/MeOH, 1:1, MeOH and DCM. The tubing is re-equilibrated with 20 mL DCM using a flow rate of 4 mL per minute. The entire procedure is performed three times.

3.4.2 Purification Solvent is evaporated in vacuo and the crude products were analyzed/purified using analytical/preparative HPLC (Agilent 1200 Series spectrometer) and/or size exclusion chromatography (LH 20-Sephadex).

3.5 Automated Synthesis of Protected Disaccharide 4



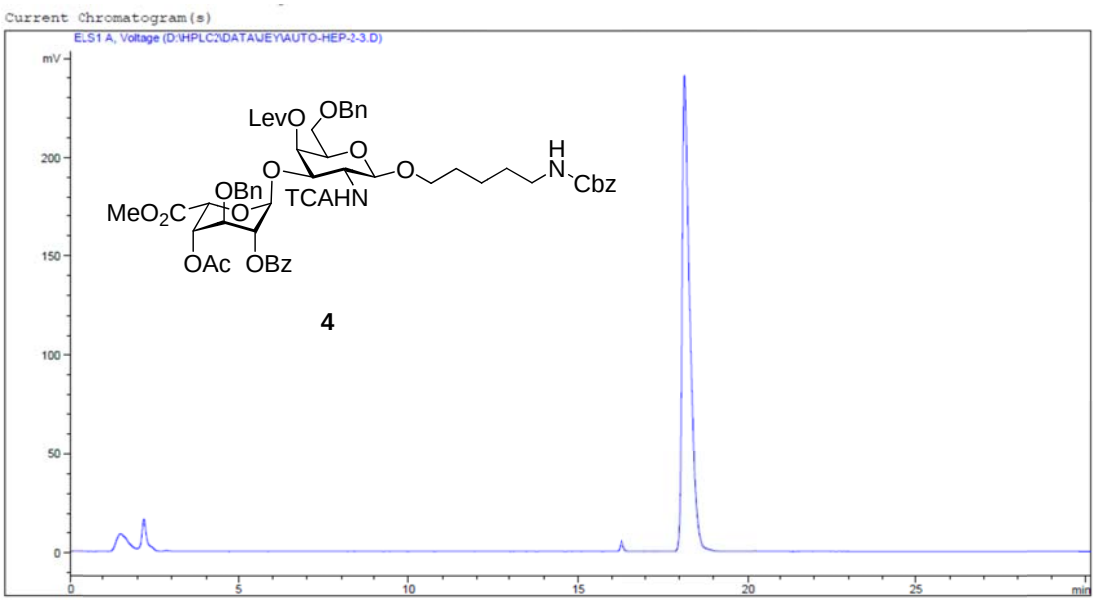
Functionalized resin **3** (70 mg, loading 0.37 mmol/g, 0.025 mmol) was subjected to glycosylation/deprotection followed by acetylation as described in Table 1.

Table 1. Automated synthesis protocol for disaccharide **4**

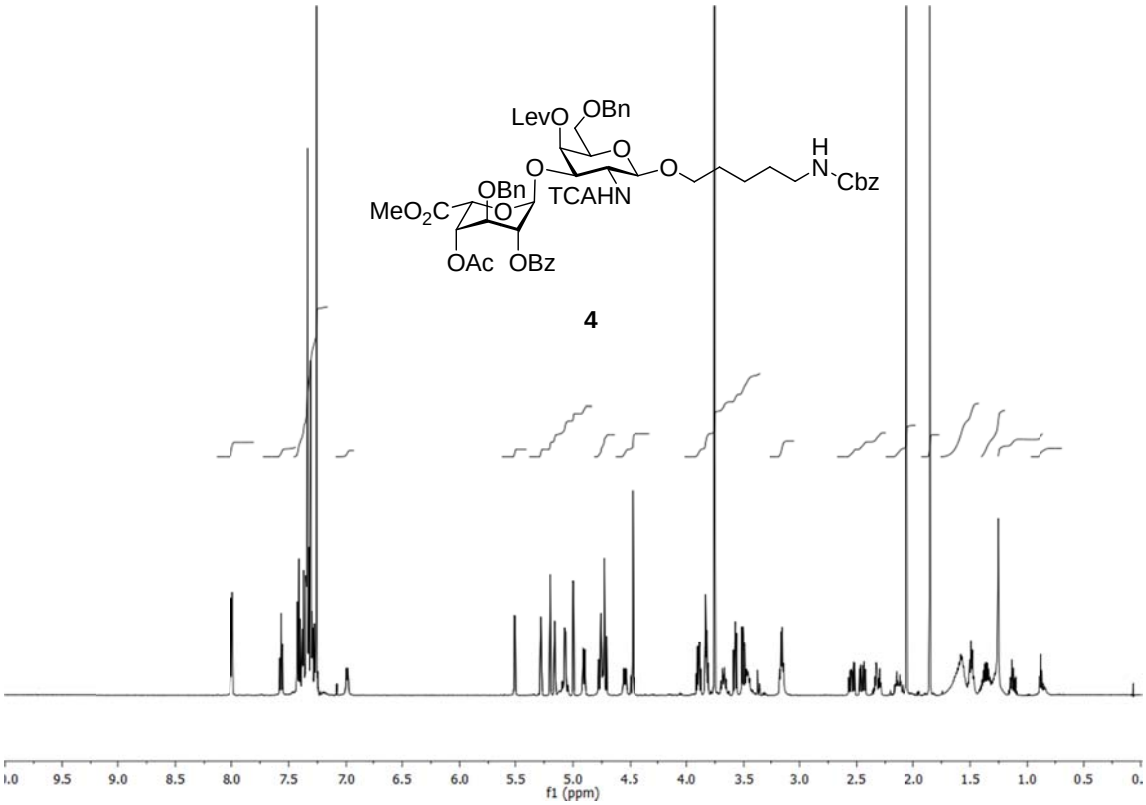
Steps	**Automated Process**	**Protocol Section**
1	Glycosylation: Donor **1**	3.3.1
2	Deprotection of Fmoc	3.3.2
3	Glycosylation: Donor **2**	3.3.1
4	Deprotection of Fmoc	3.3.2
5	Acetylation	3.3.3

Cleavage, Analysis and Purification: Disaccharide **4** was cleaved from the solid support as described in section 3.4.1. The crude product was analyzed using normal phase analytical HPLC (YMC-diol-300-NP; 5 µm, 150 mm, 4.6 mm; Linear gradient: Hexane/EtOAc; 20-100% at 40 mins) and purified using preparative HPLC (YMC-diol-300-NP; 5 µm, 150 mm, 20 mm; Linear gradient: Hexane/EtOAc; 20-100% at 40 mins) to obtain protected disaccharide **4** in 66% overall yield (19 mg). Analytical data for disaccharide **4**: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.00 (d, *J* = 7.2 Hz, 2H), 7.57 (ddd, *J* = 7.8, 2.5, 1.3 Hz, 1H), 7.41 (dd, *J* = 8.1, 7.6 Hz, 2H), 7.38 (d, *J* = 7.2 Hz, 2H), 7.36–7.27 (m, 13H), 6.99 (d, *J* = 7.4 Hz, 1H), 5.51 (d, *J* = 3.2 Hz, 1H), 5.28 (t, *J* = 2.5 Hz, 1H), 5.20 (s, 1H), 5.16 (m, 1H), 5.07 (d, *J* = 4.6 Hz, 1H), 5.00 (d, *J* = 2.0 Hz, 1H), 4.90 (d, *J* = 8.3 Hz, 1H), 4.81–4.65 (m, 2H), 4.55 (dd, *J* = 10.8, 3.0 Hz, 1H), 4.48 (d, *J* = 2.1 Hz, 2H), 3.89 (dt, *J* = 9.9, 6.1 Hz, 1H), 3.83 (dd, *J* = 10.6, 4.4 Hz, 2H), 3.75 (s, 3H), 3.73 – 3.63 (m, 1H), 3.58 (dd, *J* = 10.0, 6.3 Hz, 1H), 3.55 – 3.42 (m, 2H), 3.16 (dd, *J* = 13.4, 6.7 Hz, 2H), 2.54 (m, 1H), 2.45 (m, 1H), 2.31 (dt, *J* = 18.0, 5.8 Hz, 1H), 2.21–2.09 (m, 1H), 2.06 (s, 3H), 1.85 (s, 3H), 1.59 (m, 2H), 1.53–1.44 (m, 2H), 1.42–1.30 (m, 2H), 1.25 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.2, 171.9, 169.7, 168.7, 164.7, 162.1, 156.3, 137.9, 137.5, 136.5, 133.6, 129.8, 129.0, 128.5, 128.4, 128.4, 128.3, 128.0, 127.9, 127.8, 127.6, 100.8, 99.0, 75.4, 73.5, 72.8, 72.6, 72.5, 69.9, 69.4, 68.4, 67.8, 67.0, 66.6, 66.5, 56.3, 52.4, 40.9, 37.7, 29.7, 29.6, 28.9, 27.7, 23.3, 20.6. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{56}H_{63}Cl_3N_2NaO_{18}$: 1179.3039; Found: 1179.3032.

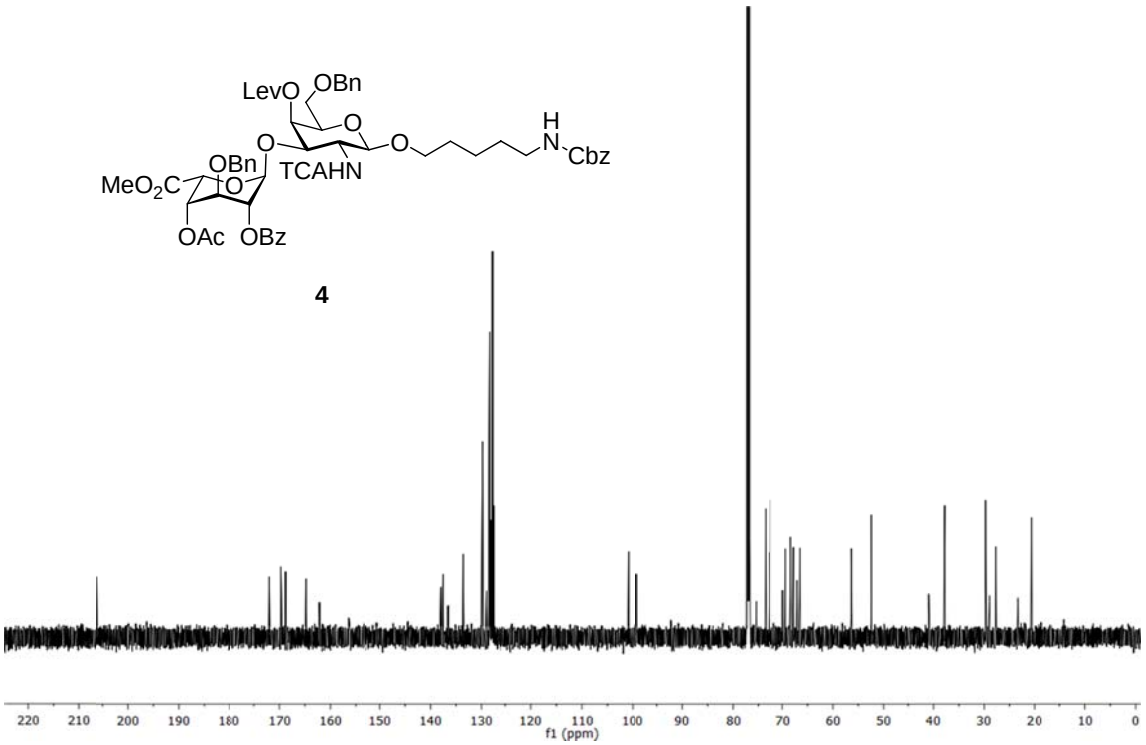
Analytical NP-HPLC YMC-Diol-300 of Crude Disaccharide 4 (ELSD trace)



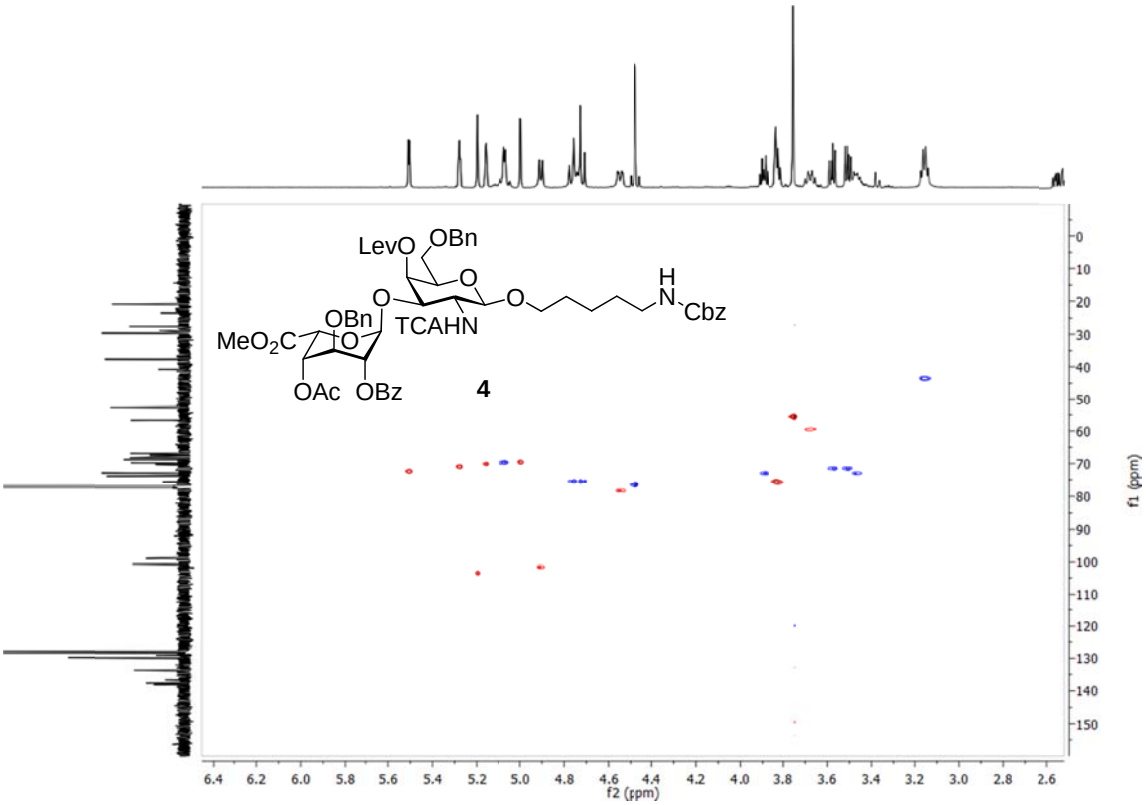
^{1}H NMR of Disaccharide 4



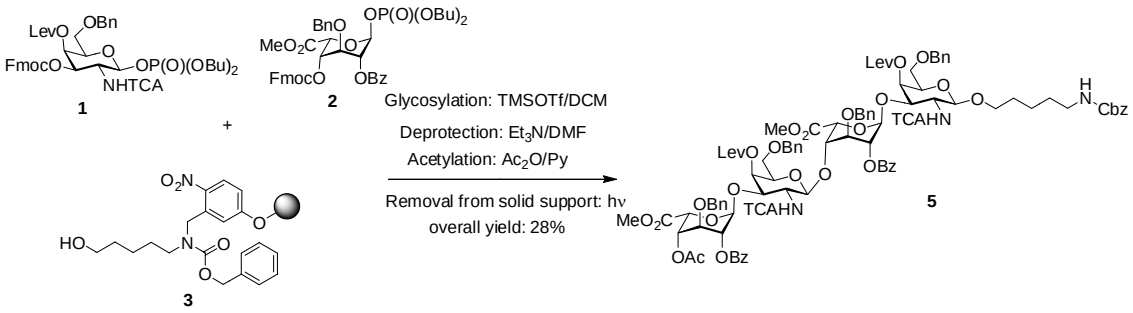
^{13}C NMR of Disaccharide 4



HSQC NMR of Disaccharide 4



3.6 Automated Synthesis of Protected Tetrasaccharide 5



Functionalized resin **3** (70 mg, loading 0.37 mmol/g, 0.025 mmol) was subjected to glycosylation/deprotection followed by acetylation as described in Table 2.

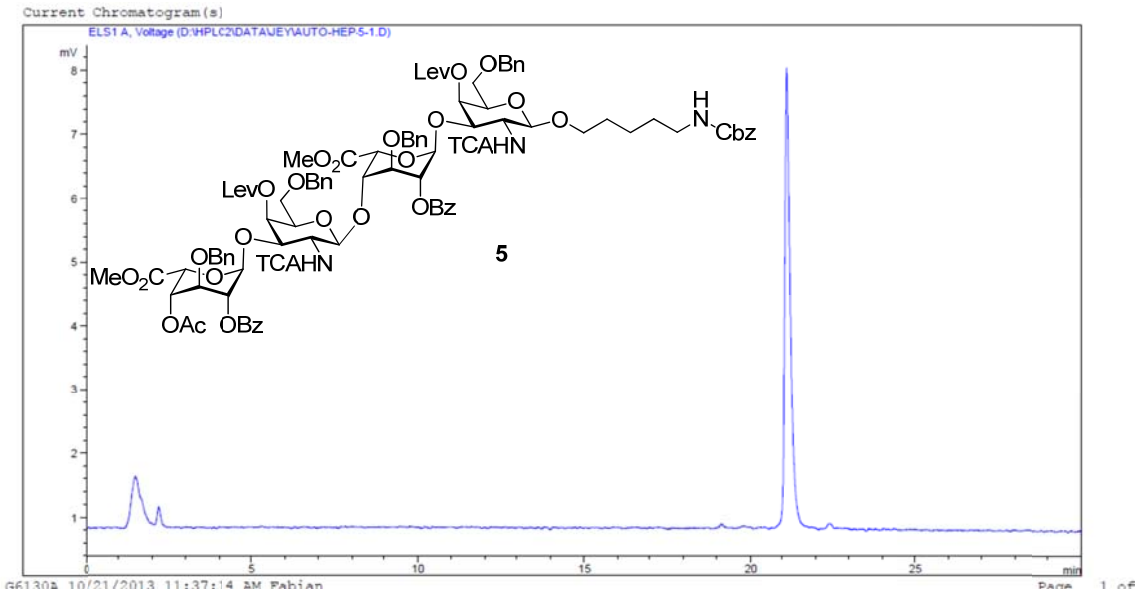
Table 2. Automated synthesis protocol for disaccharide **5**

Steps	Automated Process	Protocol Section

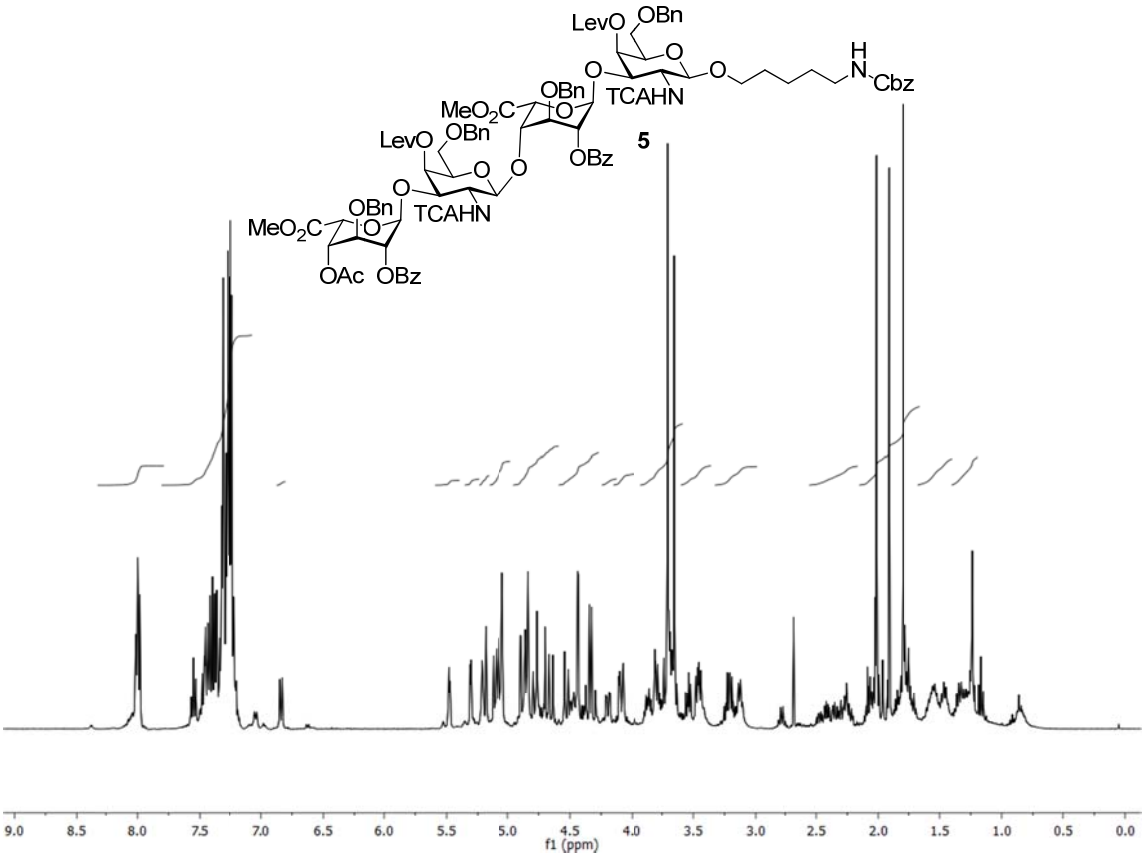
1	Glycosylation: Donor 1	3.3.1
2	Deprotection of Fmoc	3.3.2
3	Glycosylation: Donor 2	3.3.1
4	Deprotection of Fmoc	3.3.2
5	Glycosylation: Donor 1	3.3.1
6	Deprotection of Fmoc	3.3.2
7	Glycosylation: Donor 2	3.3.1
8	Deprotection of Fmoc	3.3.2
9	Acetylation	3.3.3

Cleavage, Analysis and Purification: Tetra-saccharide **5** was cleaved from the solid support as described in section 3.4.1. The crude product was analyzed using normal phase analytical HPLC (YMC-diol-300-NP; 5 µm, 150 mm, 4.6 mm; Linear gradient: Hexane/EtOAc; 20-100% at 40 mins) and purified by preparative HPLC (YMC-diol-300-NP; 5 µm, 150 mm, 20 mm; Linear gradient: Hexane/EtOAc; 20-100% at 40 mins) to obtain protected tetrasaccharide **5** in 28% overall yield (14 mg). Analytical Data: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19–7.84 (m, 3H), 7.54 – 7.22 (m, 32H), 7.04 (d, *J* = 7.4 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 5.48 (d, *J* = 3.1 Hz, 1H), 5.31 (d, *J* = 3.2 Hz, 1H), 5.20 (m, 2H), 5.14 – 5.02 (m, 4H), 4.96–4.82 (m, 4H), 4.81 – 4.61 (m, 4H), 4.56 – 4.41 (m, 3H), 4.34 (q, *J* = 11.6 Hz, 3H), 4.20 (dd, *J* = 10.7, 3.3 Hz, 1H), 4.09 (dd, *J* = 9.0, 5.0 Hz, 2H), 3.86 (dd, *J* = 9.8, 5.8 Hz, 1H), 3.83 – 3.74 (m, 2H), 3.71 (s, 3H), 3.73 – 3.61 (m, 5H), 3.65 (s, 3H), 3.54 (dd, *J* = 9.9, 6.1 Hz, 1H), 3.46 (dd, *J* = 9.9, 6.4 Hz, 2H), 3.18 (m, 3H), 2.35–2.17 (m, 2H), 2.11 – 2.02 (m, 2H), 2.01 (s, 3H), 1.91 (s, 3H), 1.82-1.70 (m, 3H),1.80 (s, 3H), 1.52 (m, 3H), 1.45 (m, 2H), 1.30 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 206.0, 171.9, 171.8, 169.7, 169.6, 168.7, 165.0, 164.6, 162.1, 162.0, 156.3, 137.9, 137.9, 137.7, 137.6, 136.5, 133.5, 133.3, 129.8, 129.7, 129.6, 129.0, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.5, 101.0, 101.0, 100.7, 99.2, 92.2, 92.2, 77.2, 76.3, 75.2, 74.1, 73.8, 73.5, 73.4, 72.8, 72.7, 72.5, 72.3, 69.8, 69.4, 68.9, 68.3, 68.2, 67.8, 66.9, 66.8, 66.7, 66.5, 66.4, 56.1, 55.3, 54.3, 52.4, 52.3, 40.8, 37.7, 37.5, 29.6, 29.5, 28.8, 27.6, 27.2, 23.2, 20.5. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{97}H_{105}C_{l6}N_3NaO_{32}$: 2056.4710; Found: 2056.4733.

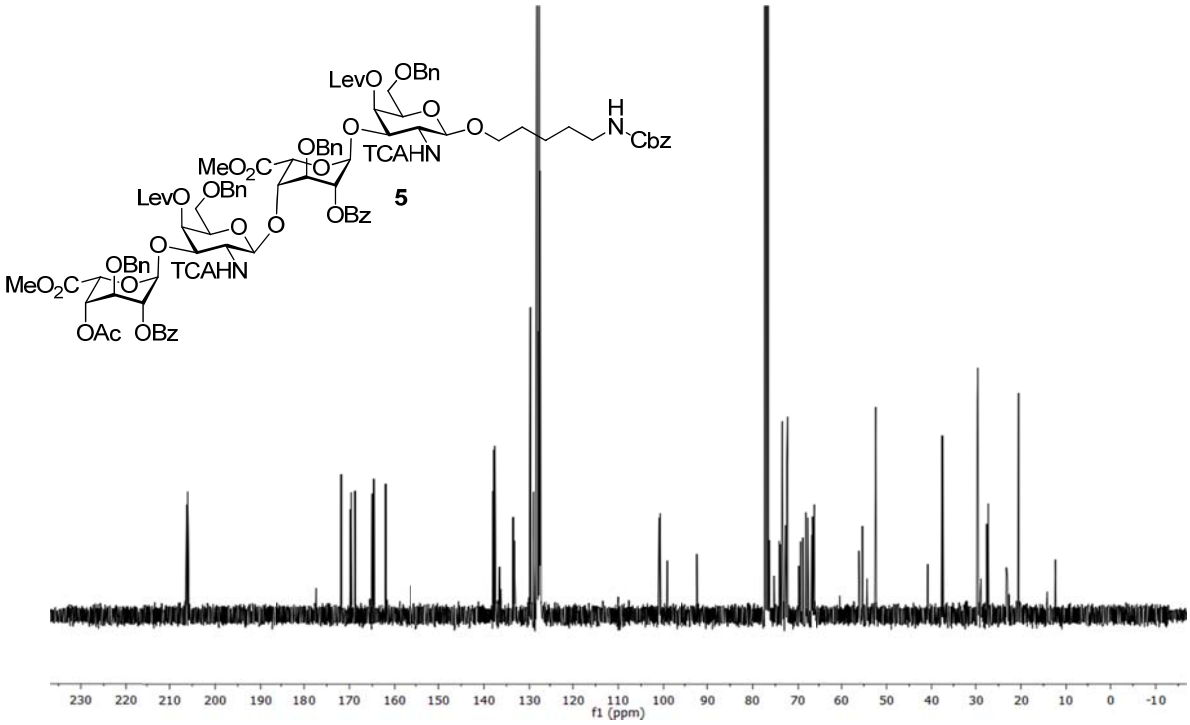
Analytical NP-HPLC YMC-Diol-300 of Crude Tetrasaccharide 5 (ELSD trace)



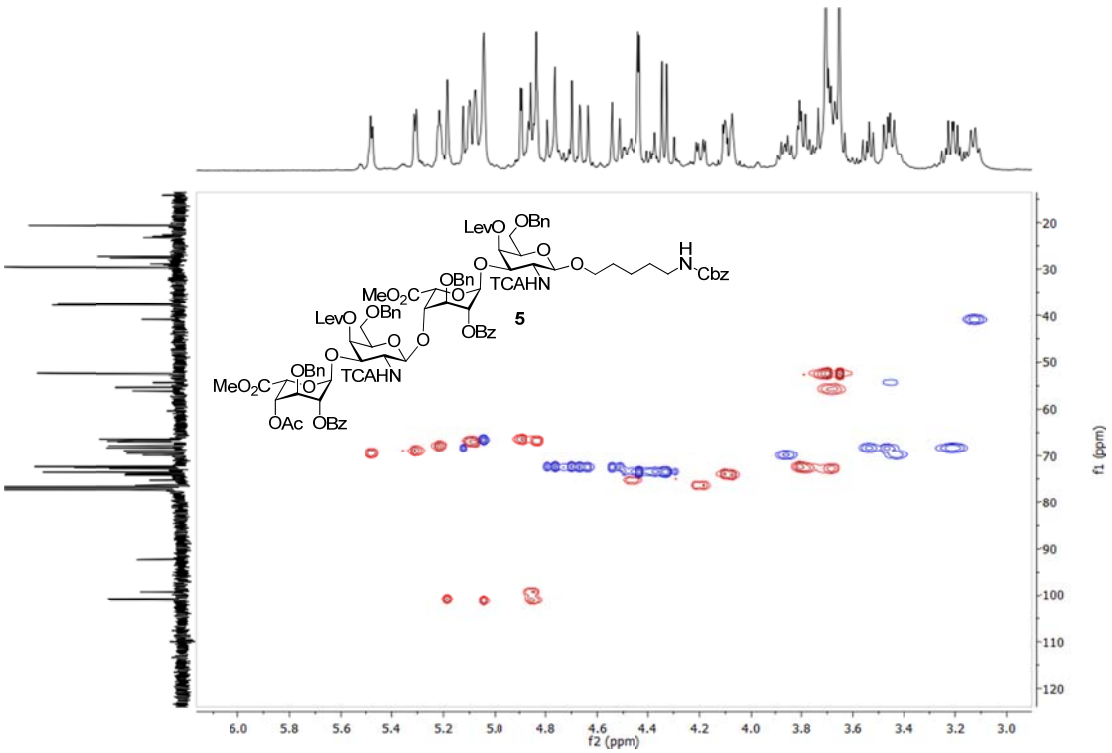
^{1}H NMR of Tetrasaccharide 5



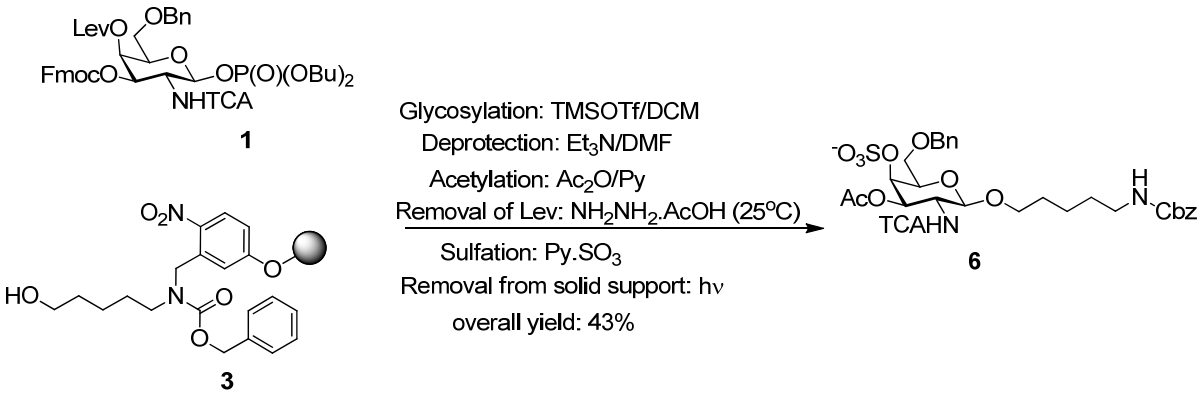
^{13}C NMR of Tetrasaccharide 5



HSQC NMR of Tetrasaccharide 5



3.7 Automated Synthesis of Sulfated Monosaccharide 6



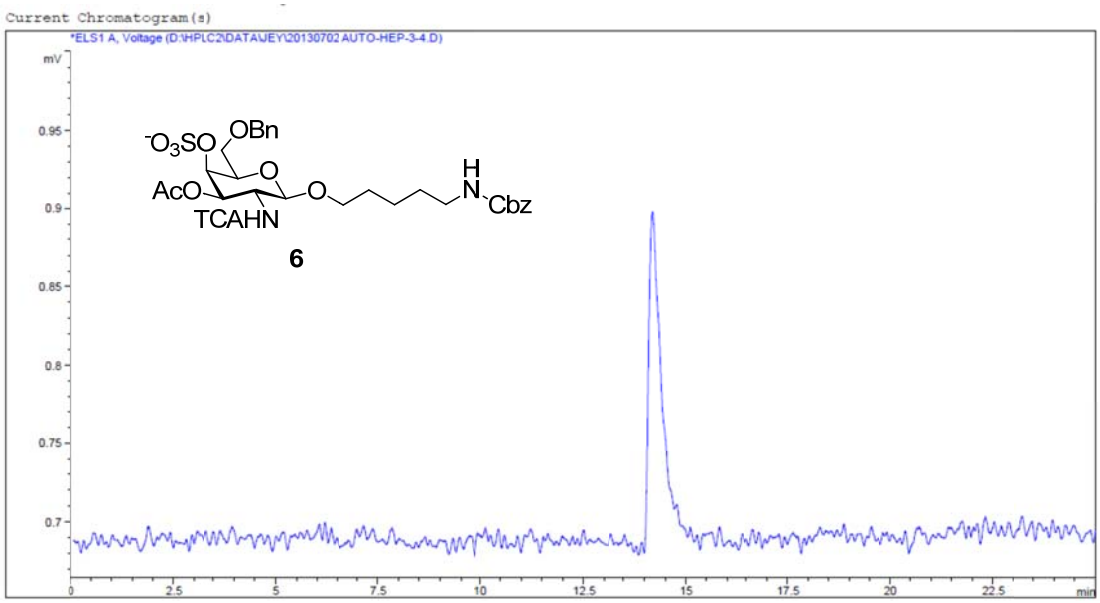
Functionalized resin **3** (70 mg, loading 0.37 mmol/g, 0.025 mmol) was subjected to glycosylation, deprotection, acetylation, and sulfation as described in Table 3.

Table 3. Automated synthesis protocol for sulfated monosaccharide **6**

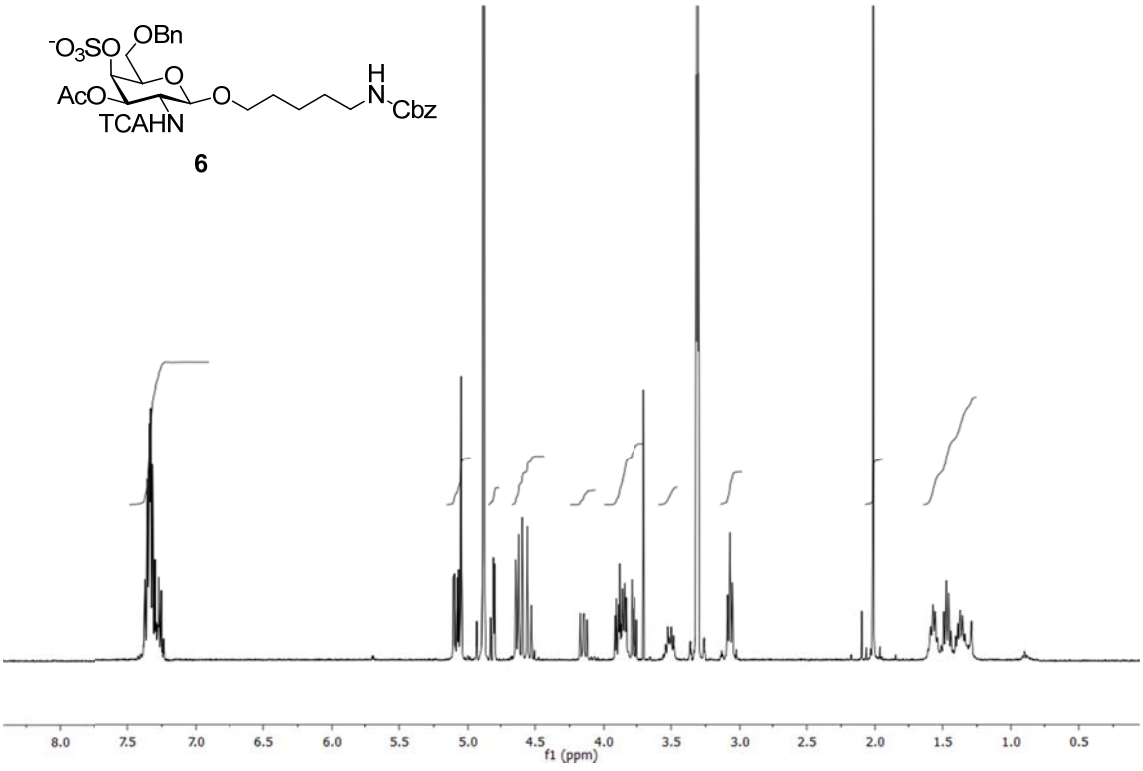
Steps	Automated Process	Protocol Section
1	Glycosylation: Donor 1	3.3.1
2	Deprotection of Fmoc	3.3.2
3	Acetylation	3.3.3
4	Removal of Lev at 25°C	3.3.4
5	Sulfation	3.3.5

Analysis and Purification: Sulfated monosaccharide **5** was cleaved from the solid support as described in section 3.4.1. The crude product was analyzed using analytical HPLC and was recorded by ELSD using a flow of 1 mL/min on a NUCLEOSIL® 120-5 RP-C4 column (5 µm, 250 mm, 4.6 mm, 110 Å). Eluents A (0.1% FA in TDW) and B (100% ACN) were used in a linear gradient (10% to 95% B in 40 min). The purification was performed using size-exclusion column chromatography (Sephadex-LH-20, eluent-methanol) to obtain sulfated monosaccharide **6** in 43% overall yield (8 mg). Analytical Data: ^{1}H NMR (400 MHz, CD_3OD) δ: 7.44 – 7.12 (m, 10H), 5.09 (dd, *J* = 11.4, 3.3 Hz, 1H), 5.05 (s, 2H), 4.81 (d, *J* = 3.3 Hz, 1H), 4.66 – 4.47 (m, 3H), 4.15 (dd, *J* = 11.4, 8.4 Hz, 1H), 3.88 (ddd, *J* = 19.0, 10.7, 4.4 Hz, 3H), 3.77 (dd, *J* = 9.9, 6.8 Hz, 1H), 3.51 (dt, *J* = 10.3, 6.7 Hz, 1H), 3.07 (t, *J* = 7.0 Hz, 2H), 2.01 (s, 3H),1.73 – 1.20 (m, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ: 172.63, 164.47, 139.88, 129.59, 129.49, 129.07, 128.90, 128.80, 102.31, 74.85, 74.51, 73.54, 72.32, 71.28, 70.90, 67.44, 53.68, 41.88, 30.71, 30.47, 24.48, 21.13. ESI HR-MS: m/z $[M]^-$ calcd. for $C_{30}H_{36}Cl_3N_2O_{12}S^-$: 753.1060; Found: 753.1083.

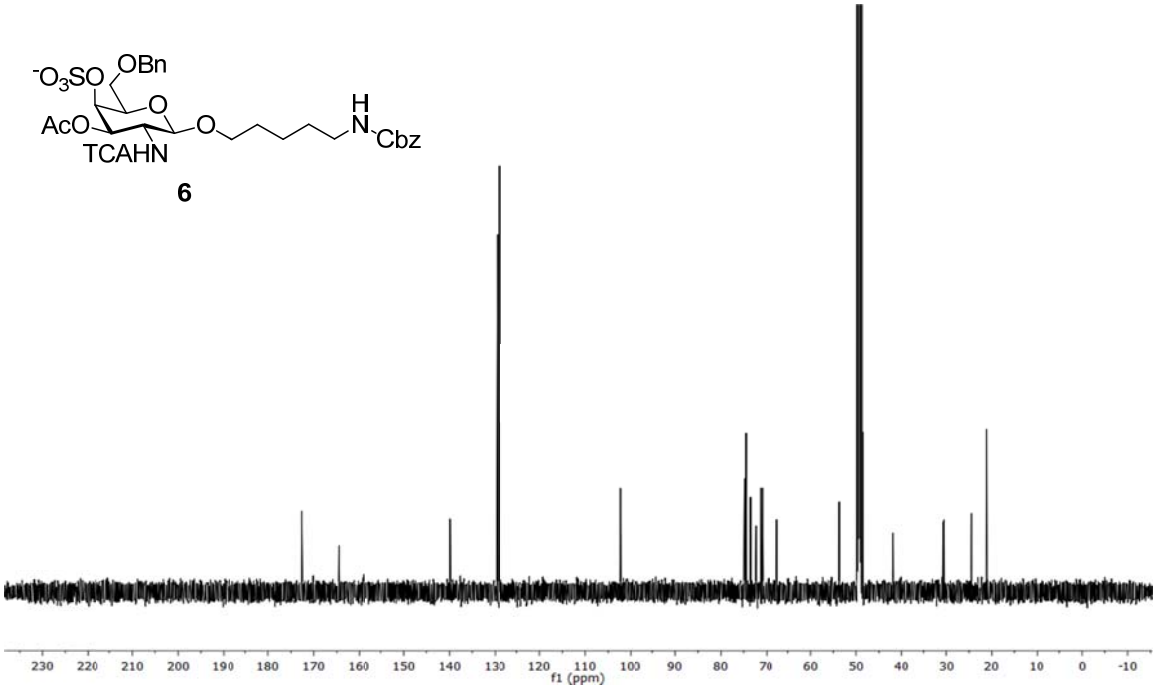
Analytical RP-HPLC C-4 of Sulfated Monosaccharide 6 (ELSD trace)



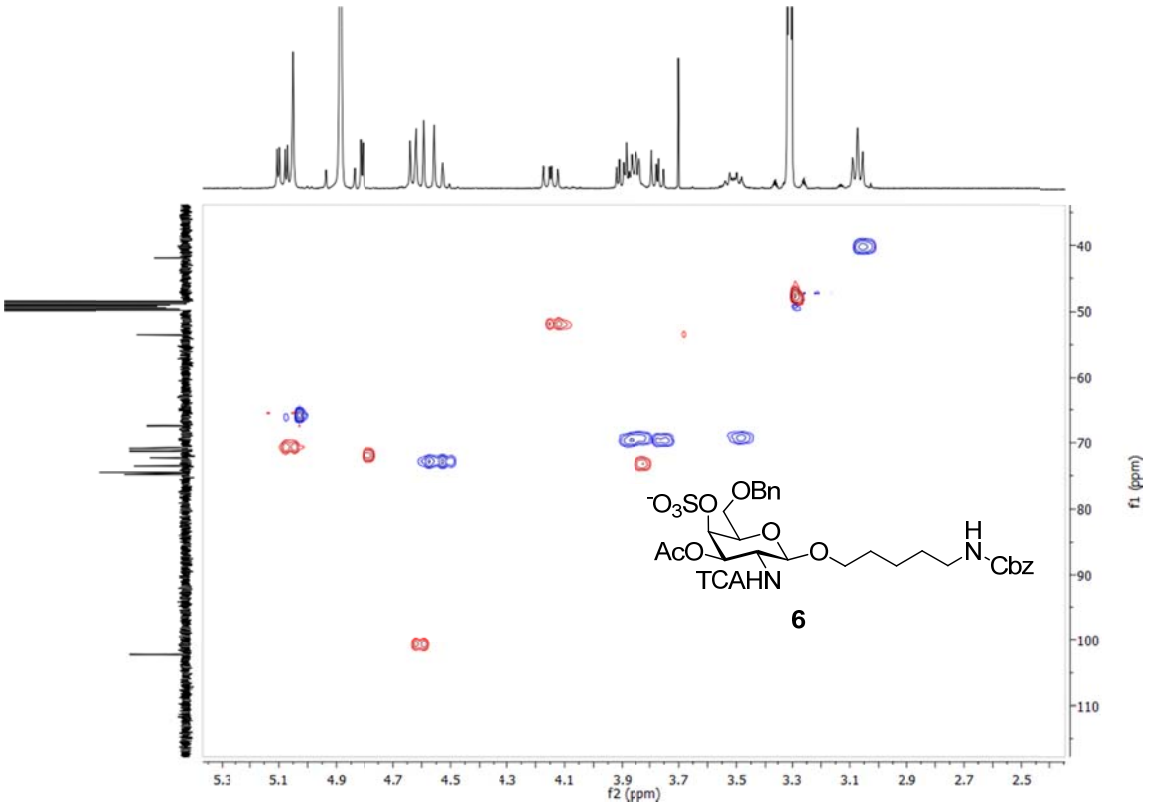
^{1}H NMR of Sulfated Monosaccharide 6



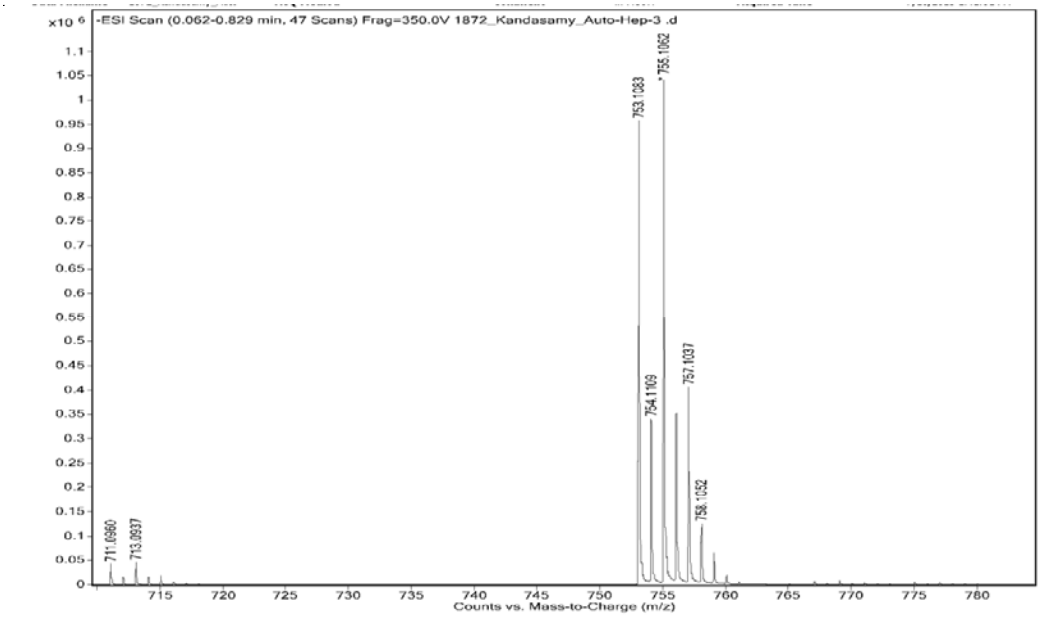
^{13}CNMR of Sulfated Monosaccharide 6



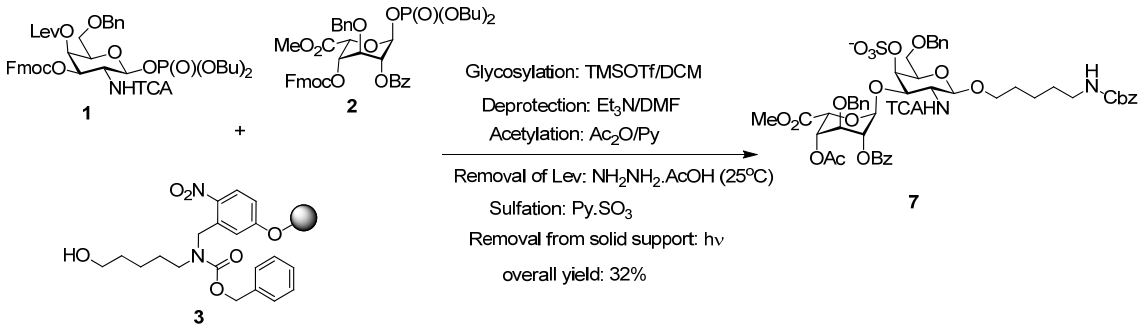
HSQC NMR of Sulfated Monosaccharide 6



ESI-MS of Sulfated Monosaccharide 6



3.8 Automated Synthesis of Sulfated Disaccharide 7



Functionalized resin **3** (70 mg, loading 0.37 mmol/g, 0.025 mmol) was subjected to glycosylation, deprotection, acetylation and sulfation as described in Table 4.

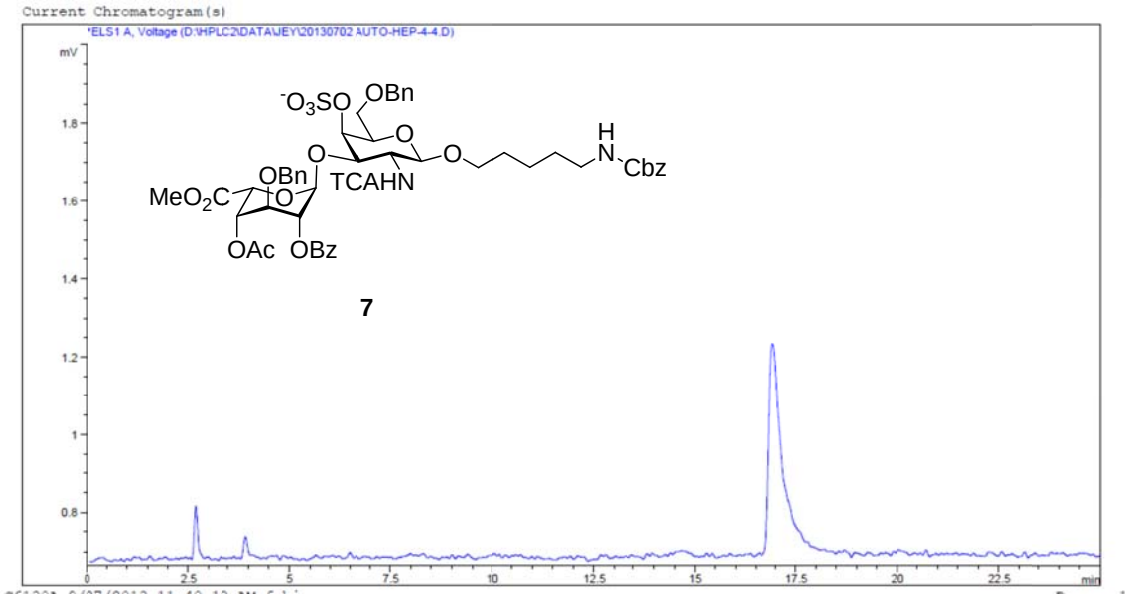
Table 4. Automated synthesis protocol for sulfated disaccharide **7**

Steps	**Automated Process**	**Protocol Section**
1	Glycosylation: Donor **1**	3.3.1
2	Deprotection of Fmoc	3.3.2
3	Glycosylation: Donor **2**	3.3.1
4	Deprotection of Fmoc	3.3.2
5	Acetylation	3.3.3
6	Removal of Lev at 25°C	3.3.4

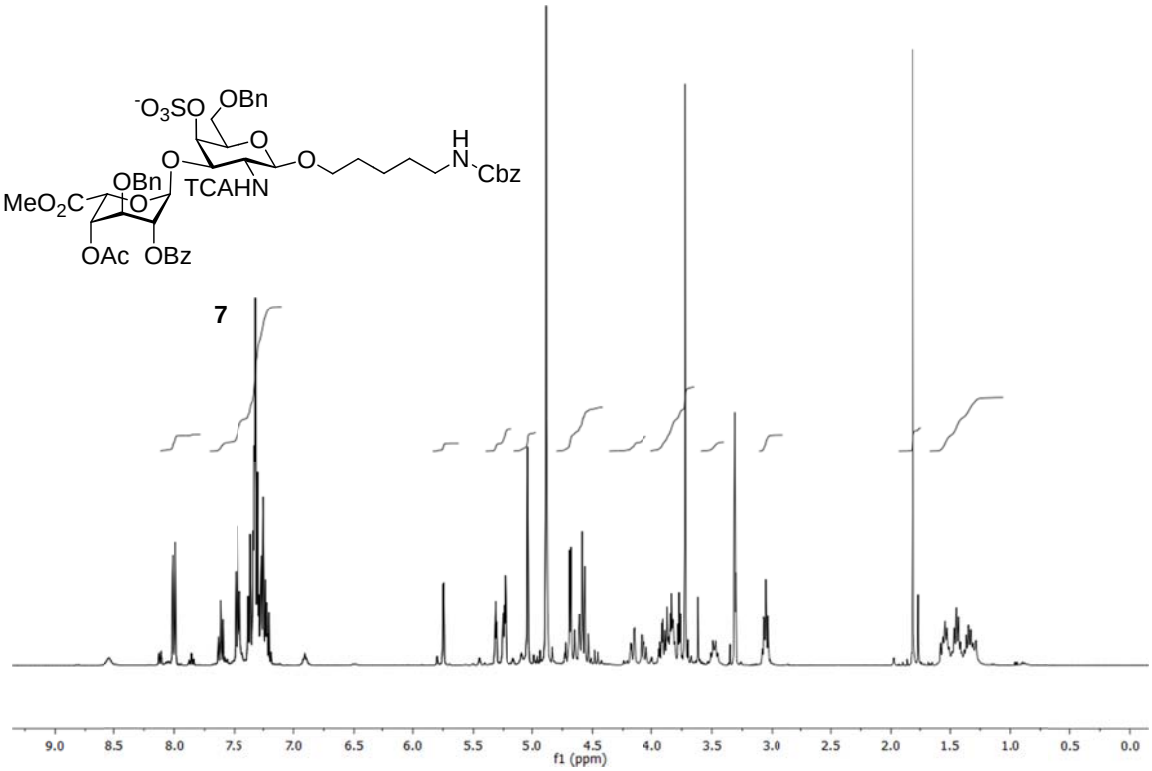
7	Sulfation	3.3.5

Analysis and Purification: Sulfated disaccharide **7** was cleaved from the solid support as described in section 3.4.1. The crude product was analyzed using analytical HPLC and was recorded by ELSD using a flow of 1 mL/min on a NUCLEOSIL® 120-5 RP-C4 column (5 µm, 250 mm, 4.6 mm, 110 Å). Eluents A (0.1% FA in TDW) and B (100% ACN) were used in a linear gradient (10% to 100% B in 40 min). The purification was performed using size-exclusion column chromatography (Sephadex-LH-20, eluent-methanol) to obtain sulfated disaccharide **7** in 32% overall yield (9 mg). Analytical data: ^{1}H NMR (400 MHz, CD_3OD) δ: 8.00 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.64 – 7.58 (m, 1H), 7.47 (t, *J* = 7.8 Hz, 3H), 7.40 – 7.15 (m, 14H), 5.75 (d, *J* = 2.6 Hz, 1H), 5.31 (m, 1H), 5.24 (m, 2H), 5.04 (s, 2H), 4.68 (d, *J* = 4.5 Hz, 2H), 4.62 – 4.51 (m, 3H), 4.17 (dd, *J* = 11.1, 2.8 Hz, 1H), 4.07 (dd, *J* = 10.9, 8.3 Hz, 1H), 3.95 – 3.80 (m, 5H), 3.77 (t, *J* = 4.0 Hz, 1H), 3.72 (s, 3H), 3.48 (dt, *J* = 9.6, 6.5 Hz, 1H), 3.06 (dd, *J* = 9.7, 4.2 Hz, 2H), 1.60-1.20 (m, 8H). ^{13}C NMR (101 MHz, CD_3OD) δ: 171.2, 171.1, 166.2, 164.4, 158.9, 139.9, 138.9, 138.7, 134.9, 131.2, 131.0, 130.7, 129.8, 129.5, 129.4, 129.4, 129.3, 129.0, 128.8, 128.8, 128.7, 103.2, 102.3, 94.1, 79.8, 77.7, 75.3, 74.5, 74.4, 73.1, 71.6, 70.9, 70.2, 69.2, 67.4, 55.4, 53.0, 41.8, 30.7, 30.4, 24.4, 20.7. ESI HR-MS: m/z $[M]^-$ calcd. for $C_{51}H_{56}Cl_3N_2O_{19}S^-$: 1137.2269; Found: 1137.2272.

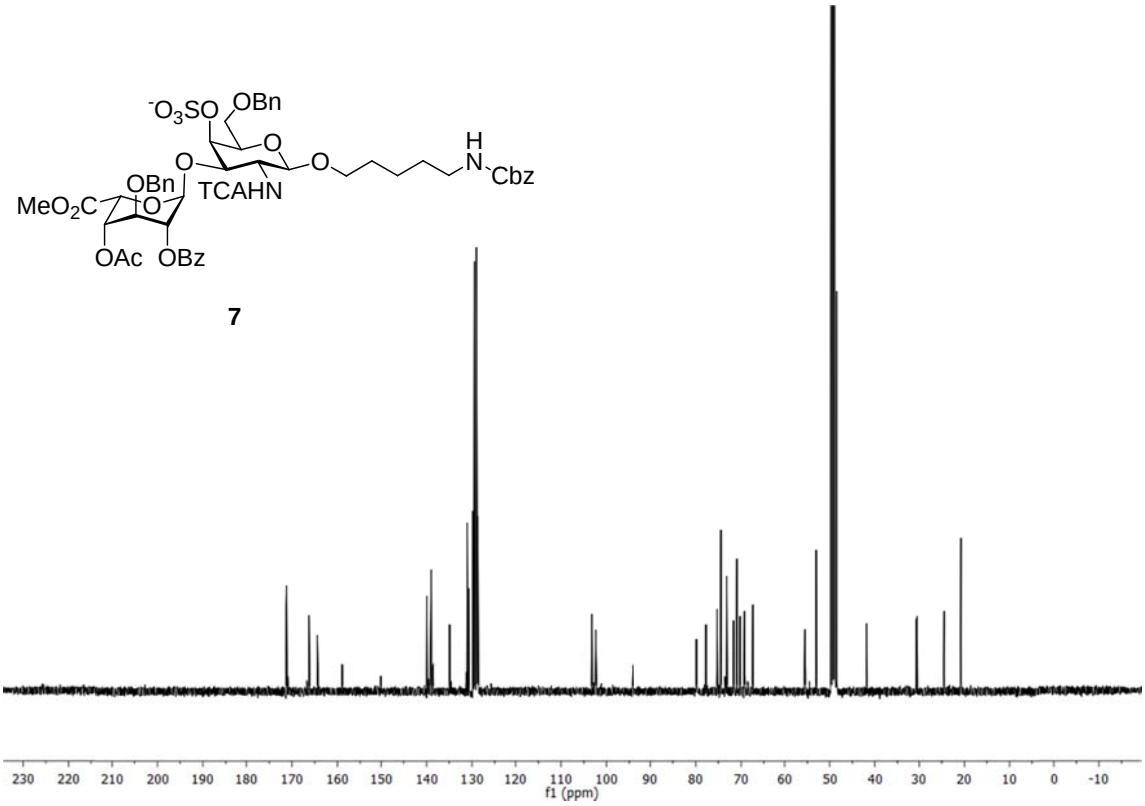
Analytical RP-HPLC C-4 of Sulfated Disaccharide 7 (ELSD trace)



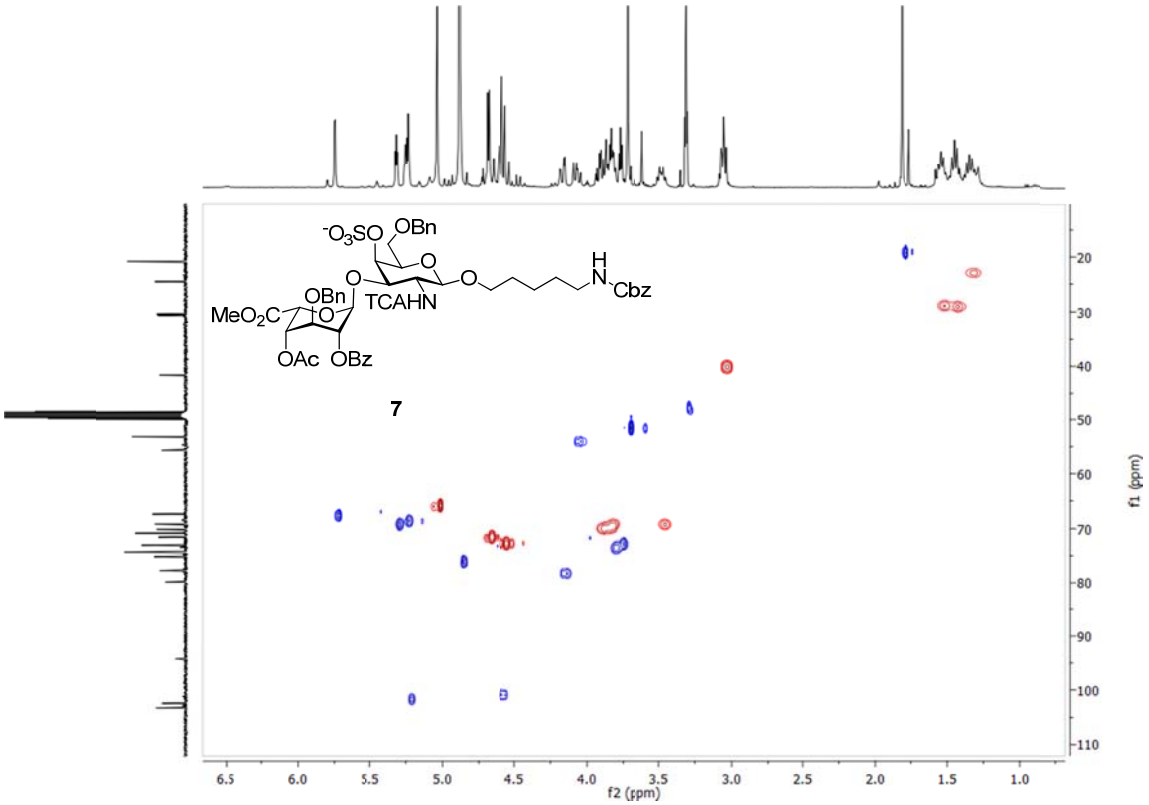
^{1}H NMR of Sulfated Disaccharide 7



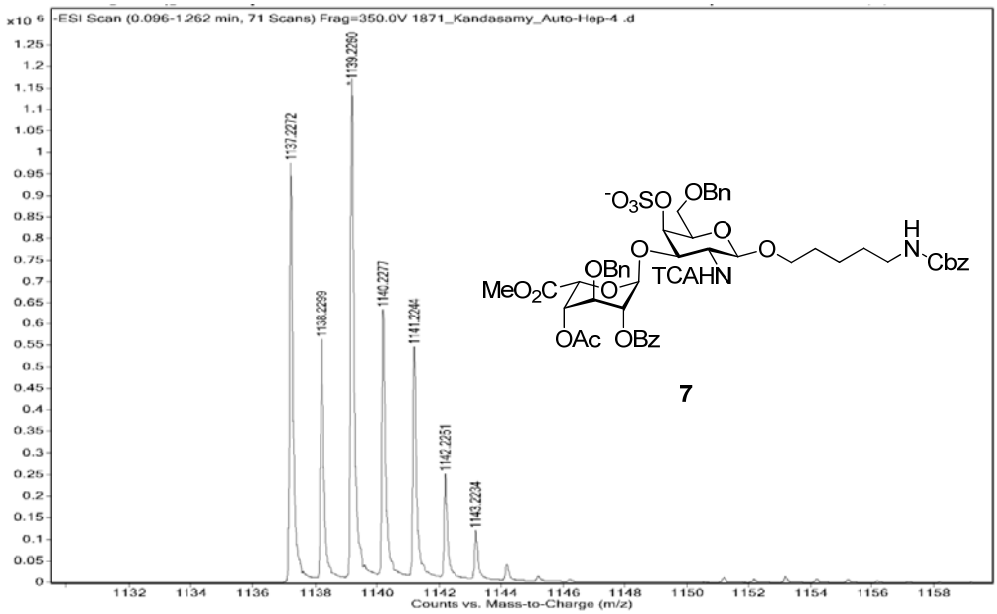
^{13}C NMR of Sulfated Disaccharide 7



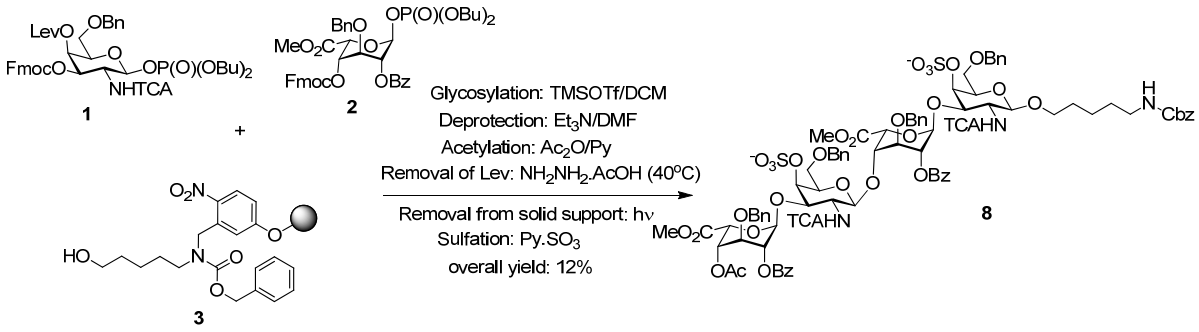
HSQC NMR of Sulfated Disaccharide 7



ESI-MS of Sulfated Disaccharide 7



3.9 Automated Synthesis of Sulfated Tetrasaccharide 8

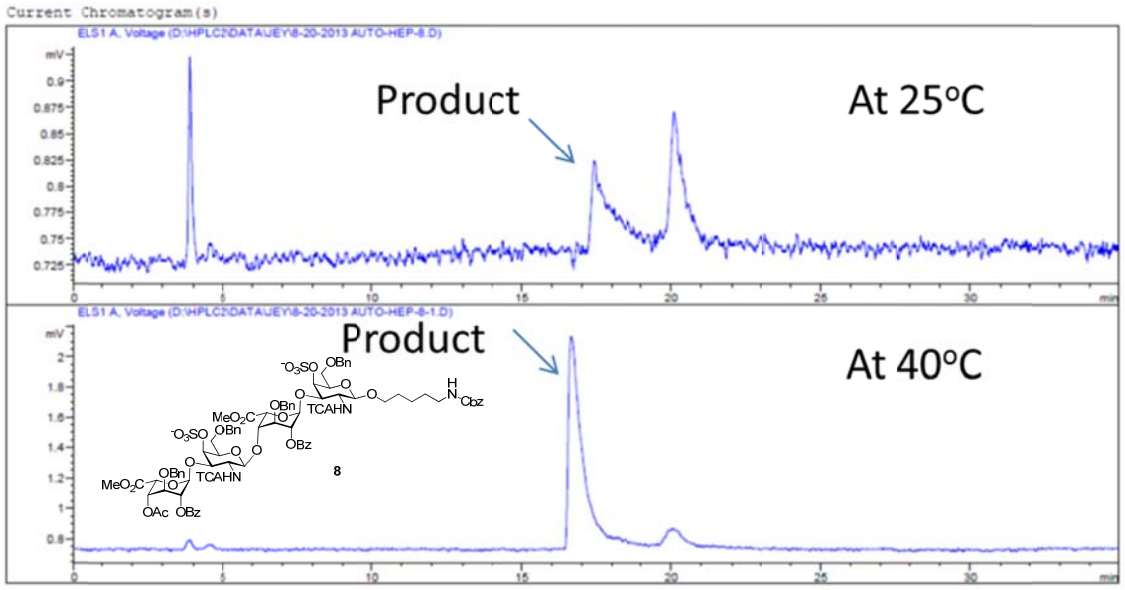


Two separate runs were carried out: **Run 1)** Functionalized resin **3** (70 mg, loading 0.37 mmol/g, 0.025 mmol) was subjected for glycosylation, Fmoc-deprotection, acetylation, **Lev-deprotection at 25°C** and sulfation as described in Table 5. **Run 2)** Functionalized resin **3** (70 mg, loading 0.37 mmol/g, 0.025 mmol) was subjected for glycosylation, Fmoc-deprotection, acetylation, **Lev-deprotection at 40°C** and sulfation as described in Table 5.

Table 5. Automated synthesis protocol for sulfated tetrasaccharide **8.**

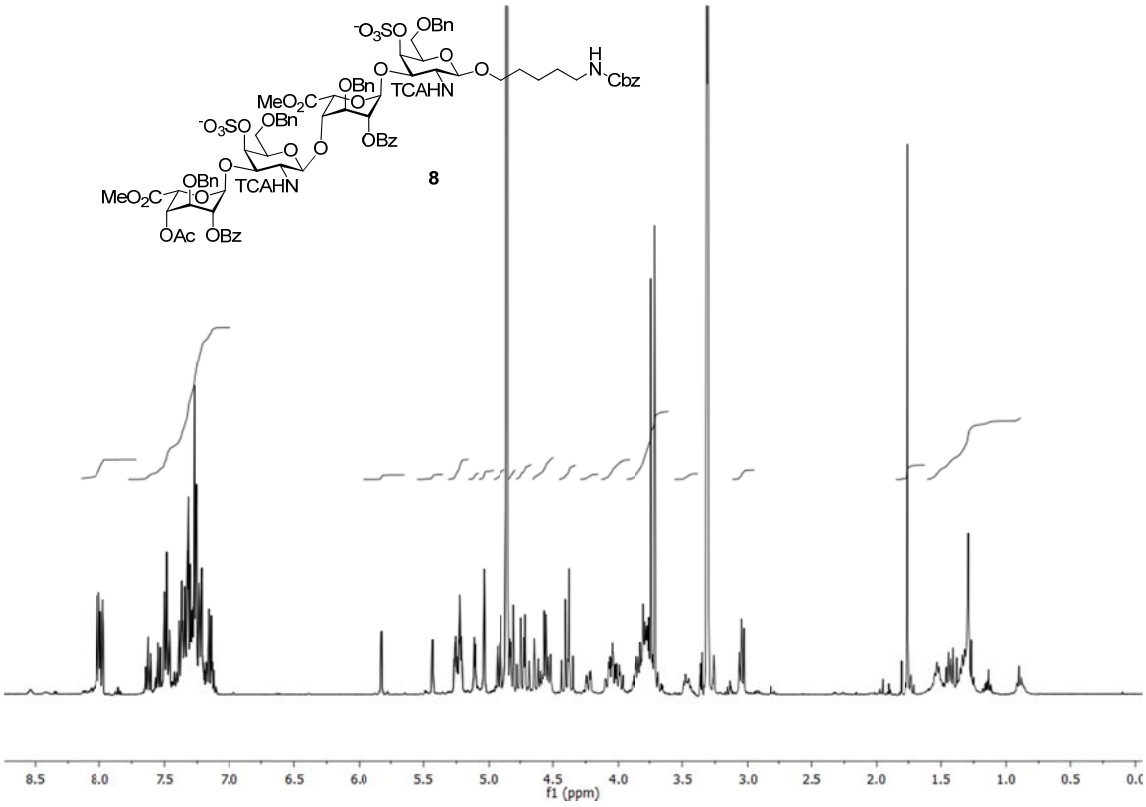
Steps	Automated Process	Protocol Section
1	Glycosylation: Donor 1	3.3.1
2	Deprotection of Fmoc	3.3.2
3	Glycosylation: Donor 2	3.3.1
4	Deprotection of Fmoc	3.3.2
5	Glycosylation: Donor 1	3.3.1
6	Deprotection of Fmoc	3.3.2
7	Glycosylation: Donor 2	3.3.1
8	Deprotection of Fmoc	3.3.2
9	Acetylation	3.3.3
10	**Removal of Lev at 25°C and 40°C**	3.3.4
11	Sulfation	3.3.5

Analytical RP-HPLC on C-8 column: Crude Sulfated Tetrasaccharide 8; Lev deprotection at 25°C and 40°C (ELSD trace)

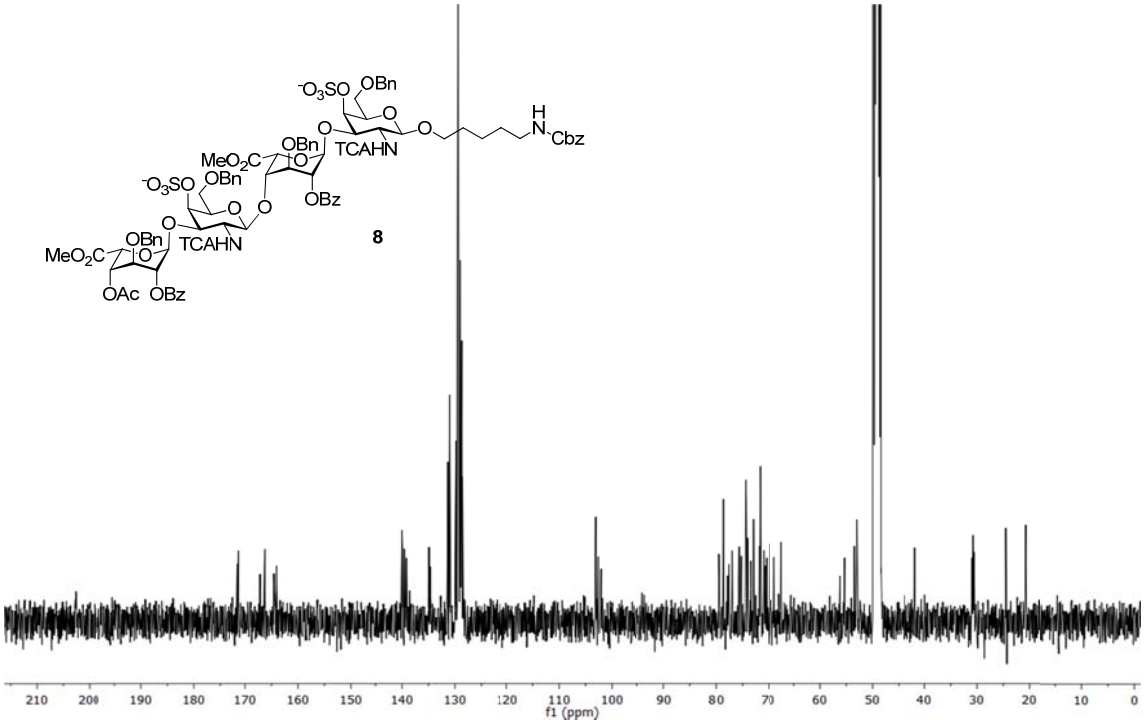


Analysis and Purification: Sulfated tetrasaccharide **8** was cleaved from the solid support as described in section 3.4.1. The crude product was analyzed using analytical HPLC and was recorded by ELSD using a flow of 1 mL/min on a NUCLEOSIL® 120-5 RP-C8 column (5 µm, 250 mm, 4.6 mm, 110 Å). Eluents A (0.1% FA in TDW) and B (100% ACN) were used in a linear gradient (10% to 100% B in 40 min). The purification was performed using size-exclusion column chromatography (Sephadex-LH-20, eluent-methanol) to obtain sulfated tetrasaccharide **8** in 12% overall yield (6 mg). Analytical data: ^{1}H NMR (400 MHz, CD_3OD) δ: 8.00 (m, 4H), 7.62 (m, 1H), 7.54 (m, 1H), 7.48 (td, *J* = 7.1, 3.5 Hz, 4H), 7.41 – 7.10 (m, 25H), 5.82 (d, *J* = 2.3 Hz, 1H), 5.43 (d, *J* = 2.6 Hz, 1H), 5.31 – 5.18 (m, 4H), 5.11 (d, *J* = 3.2 Hz, 1H), 5.04 (s, 2H), 4.95 – 4.88 (m, 2H), 4.82 (d, *J* = 2.6 Hz, 1H), 4.73 (dt, *J* = 16.1, 11.2 Hz, 3H), 4.57 (m, 4H), 4.39 (dt, *J* = 11.8, 10.7 Hz, 3H), 4.23 (dd, *J* = 10.9, 3.0 Hz, 1H), 4.12 – 3.96 (m, 4H), 3.90 – 3.69 (m, 8H), 3.74 (s, 3H), 3.71 (s, 3H), 3.51 – 3.44 (m, 1H), 3.08 – 3.02 (m, 2H), 1.76 (s, 3H), 1.58 – 1.49 (m, 2H), 1.47 – 1.42 (m, 2H), 1.33 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ: 171.5, 171.4, 171.2, 167.1, 166.2, 164.5, 164.0, 140.0, 139.9, 139.6, 139.1, 134.9, 134.6, 131.3, 131.0, 130.8, 129.9, 129.8, 129.5, 129.4, 129.4, 129.3, 129.3, 129.3, 129.0, 129.0, 128.8, 128.7, 128.6, 128.5, 103.1, 102.6, 102.1, 79.5, 78.6, 77.8, 77.6, 76.9, 75.6, 75.2, 74.4, 74.3, 74.1, 73.4, 72.9, 71.8, 71.6, 70.9, 70.7, 70.4, 69.7, 68.7, 67.4, 56.2, 55.3, 53.4, 52.9, 41.8, 30.9, 30.7, 30.5, 28.5, 24.5, 24.2, 20.6. ESI HR-MS: m/z $[M]^{2-}$ calcd. for $C_{87}H_{91}Cl_6N_3O_{34}S_2^{2-}$: 998.6519; Found: 998.6499.

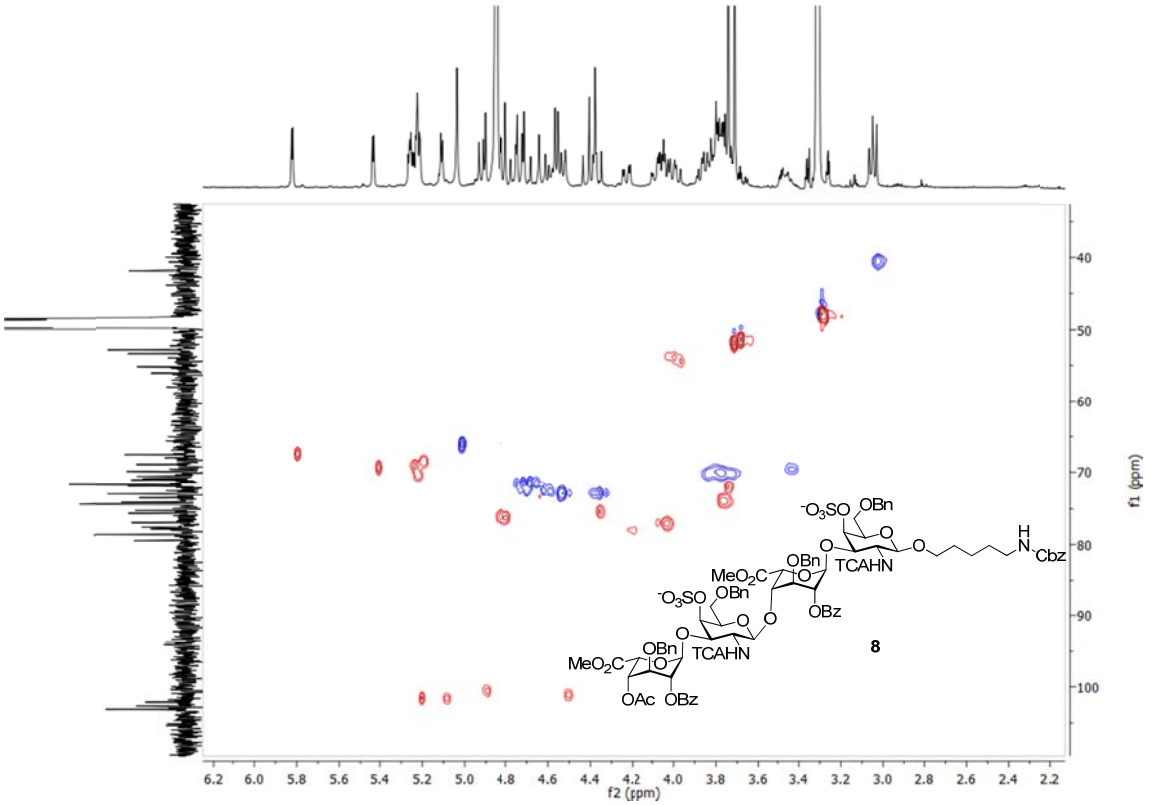
^{1}H NMR of Sulfated Tetrasaccharide 8



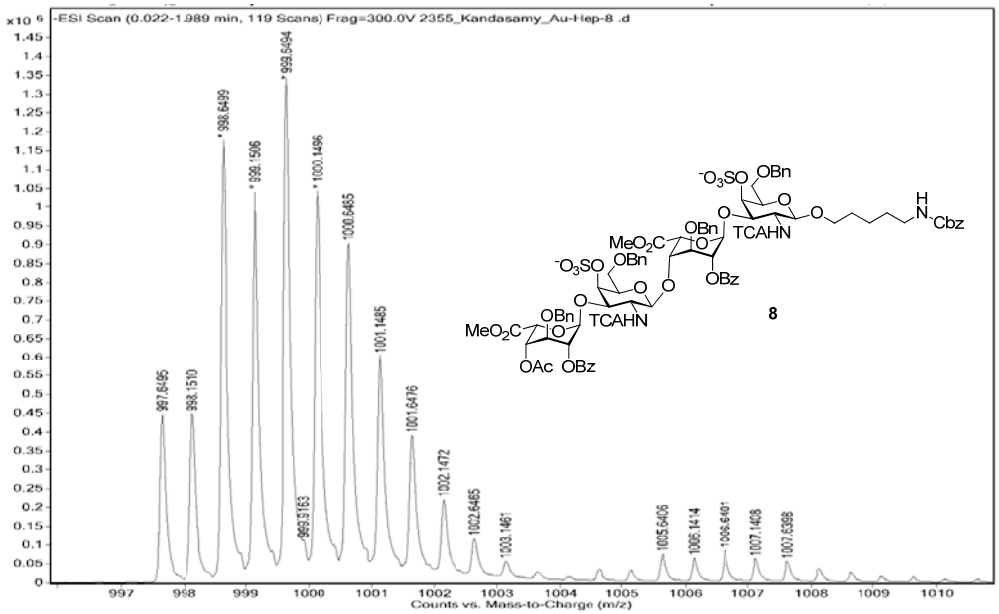
^{13}C NMR of Sulfated Tetrasaccharide 8



HSQC NMR of Sulfated Tetrasaccharide 8

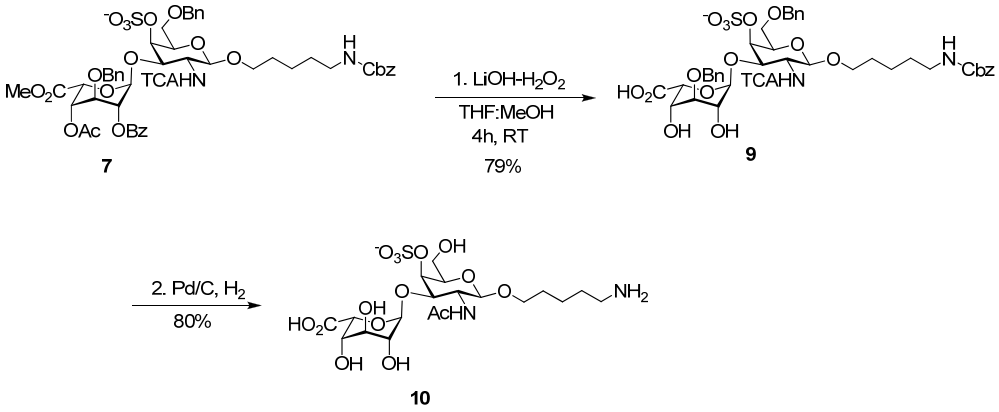


ESI-MS of Sulfated Tetrasaccharide 8



4. DEPROTECTION STRATEGY

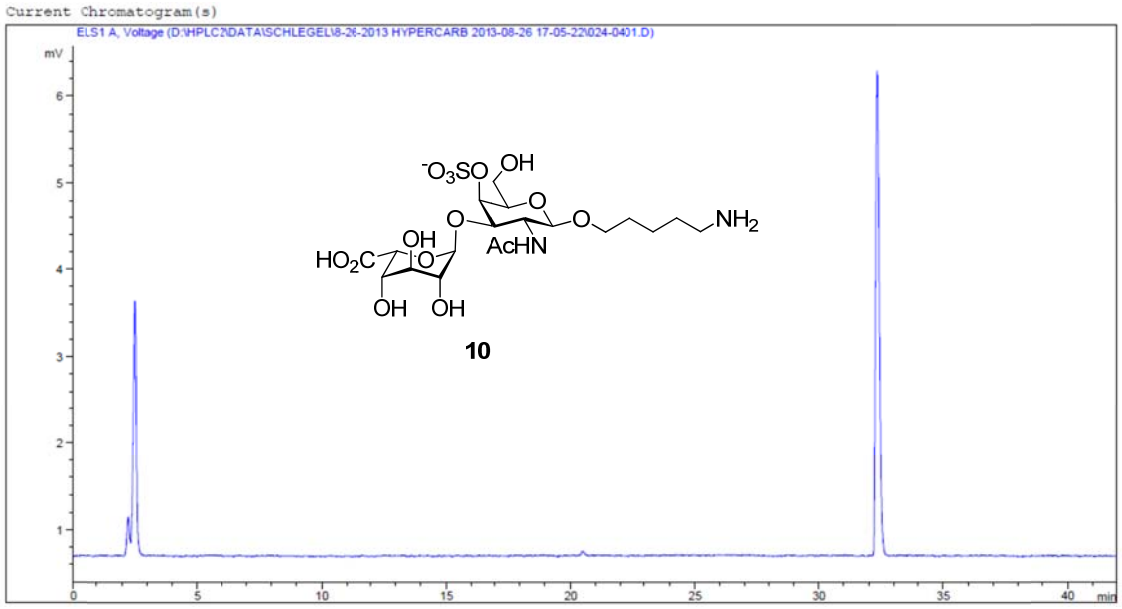
4.1 Deprotection of Sulfated Disaccharide 7



Sulfated disaccharide **7** (9 mg, 7.9 µmol) was stirred in a mixture of THF and methanol (2 mL, 4:1) at 0°C to which premixed solution of 1 M LiOH-35%H_2O_2 (150 µL, 2:1) was added and allowed to warm to room temperature. The reaction was monitored by LC-MS. After competition (~ 4 h), the reaction mixture was concentrated purified by Sephadex-LH-20 to obtain partially protected compound **9** in 79% yield (6.1 mg). Analytical Data:^{1}H NMR (400 MHz, CD_3OD) δ: 7.54 – 7.06 (m, 15H), 5.05 (s, 2H), 4.85-4.70 (m, 4H), 4.57 (m, 3H), 4.28 – 4.10 (m, 2H), 3.87 (m, 5H), 3.50 (m, 2H), 3.42 (t, *J* = 8.3 Hz, 1H), 3.07 (t, *J* = 6.9 Hz, 2H), 1.63 – 1.31 (m, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ: 164.4, 159.0, 140.5, 140.0, 129.5, 129.4, 129.2, 129.1, 129.0, 129.0, 128.9, 128.6, 128.4, 102.7, 94.5, 78.0, 75.4, 74.4, 72.0, 70.8, 67.4, 55.5, 41.8, 30.7, 30.5, 24.5. ESI HR-MS: m/z $[M]^-$ calcd. for $C_{41}H_{48}Cl_3N_2O_{17}S^-$: 977.1745; Found: 977.1754. Compound **9** was stirred in a mixture of solvents, H_2O:MeOH:EtOAc:AcOH (2:2:1:0.2) with 5% Pd/C (W/V). This mixture was exposed to 50 psi hydrogen gas at room temperature for 24h. Then, the reaction mixture was filtered through celite and evaporated to provide fully deprotected disaccharide **10** in 80% yield (2.8 mg). The purity was assessed by analytical HPLC using Thermo-HYPERCARB-RP-column (3 µm, 150 mm, 4.6 mm). Eluents A (0.1% FA in TDW) and B (100% ACN) were used in a linear gradient (0% to 50% B in 50 min). Analytical Data: ^{1}H NMR (600 MHz, D_2O) δ: 4.84 (d, *J* = 8.9 Hz, 1H), 4.74 (s, 1H), 4.65 (d, *J* = 3.0 Hz, 1H), 4.61 (d, *J* = 7.8 Hz, 1H), 4.11 – 4.00 (m, 2H), 3.98 – 3.91 (m, 2H), 3.91 – 3.76 (m, 3H), 3.70 – 3.59 (m, 2H), 3.53 (dd, *J* = 7.7, 4.7 Hz, 1H), 3.02 (m, 2H), 1.94 (s, 3H), 1.71 (m, 2H), 1.64 (m, 2H), 1.44 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 177.2, 105.4, 103.6, 78.9, 78.0, 77.1, 75.0, 74.1, 73.1, 72.6, 63.6, 54.5, 41.9,

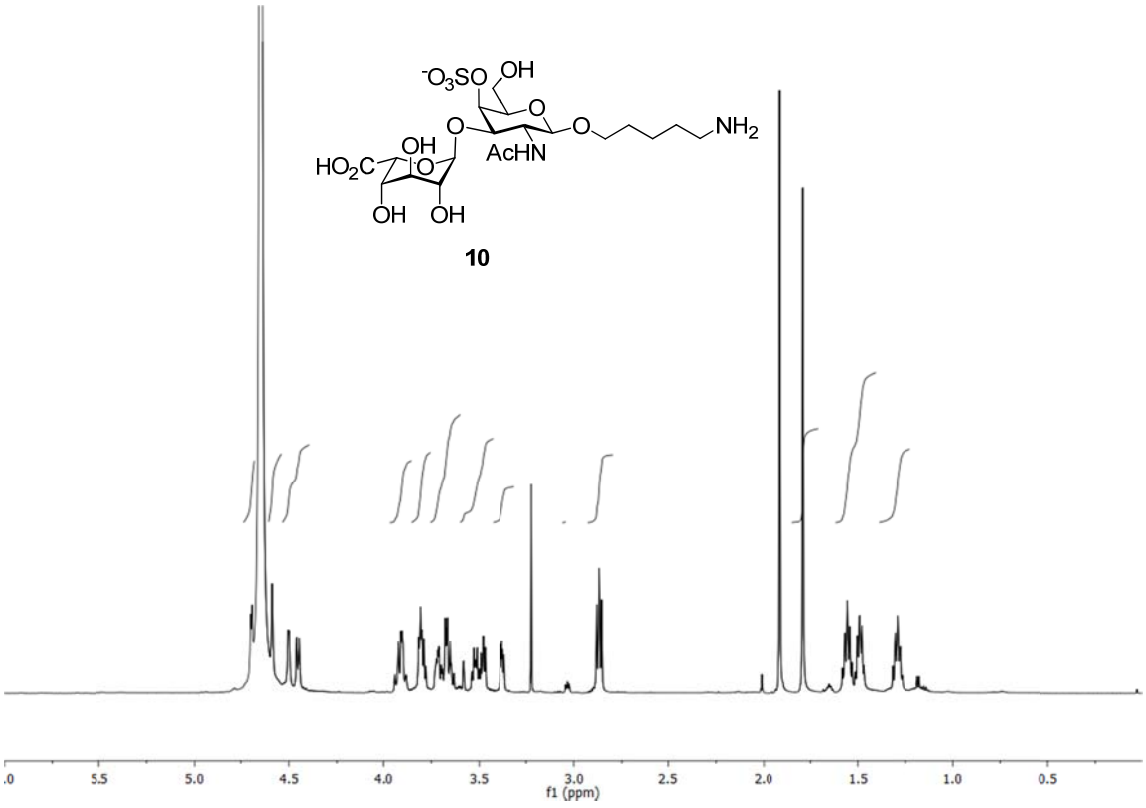
30.6, 28.9, 24.7, 24.7. ESI HR-MS: m/z $[M]^-$ calcd. for $C_{19}H_{33}N_2O_{15}S^-$: 561.1607; Found: 561.1626.

Analytical RP-HPLC (HYPERCARB) of Deprotected Disaccharide 10 (ELSD trace)



10

^{1}H NMR of Deprotected Disaccharide 9



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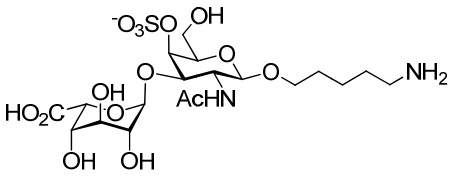
^{13}C NMR of Deprotected Disaccharide 9



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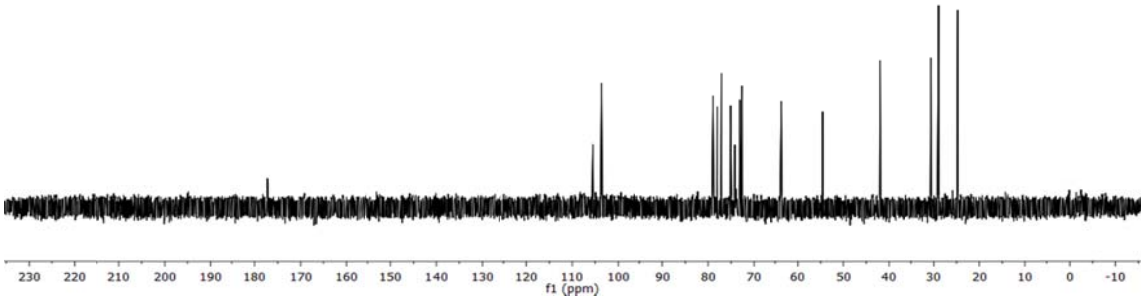
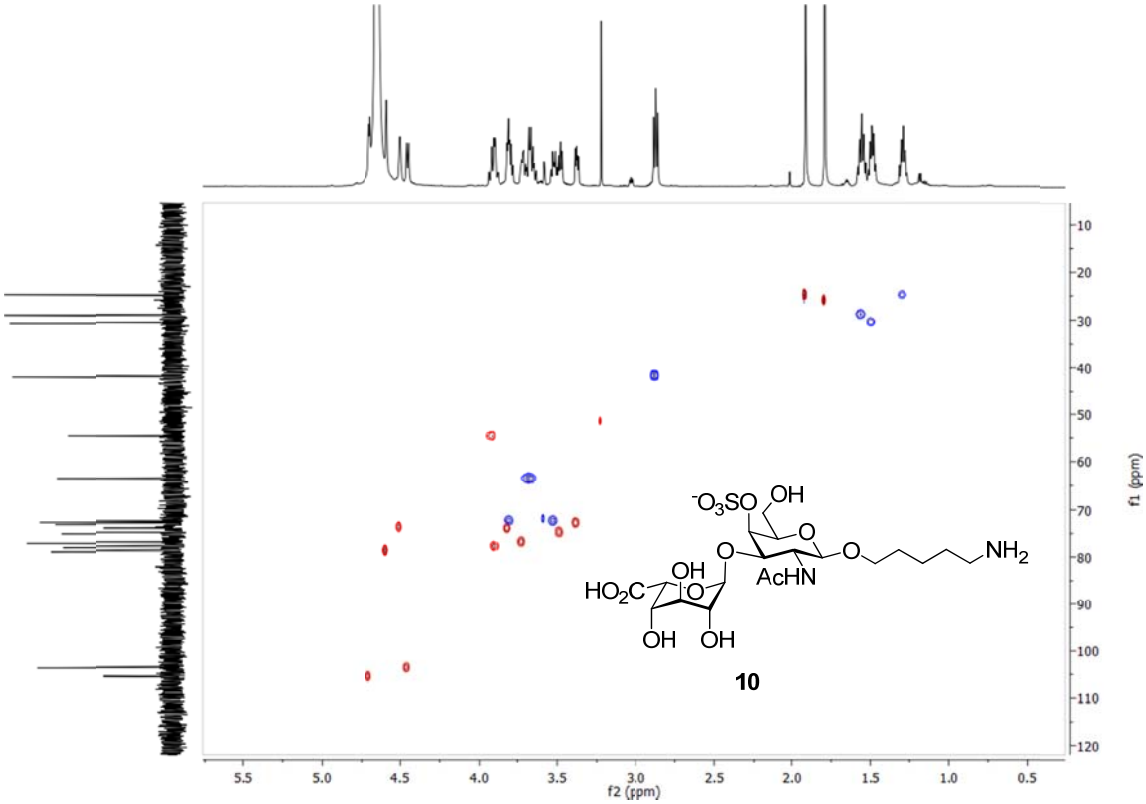


HSQC NMR of Deprotected Disaccharide 9



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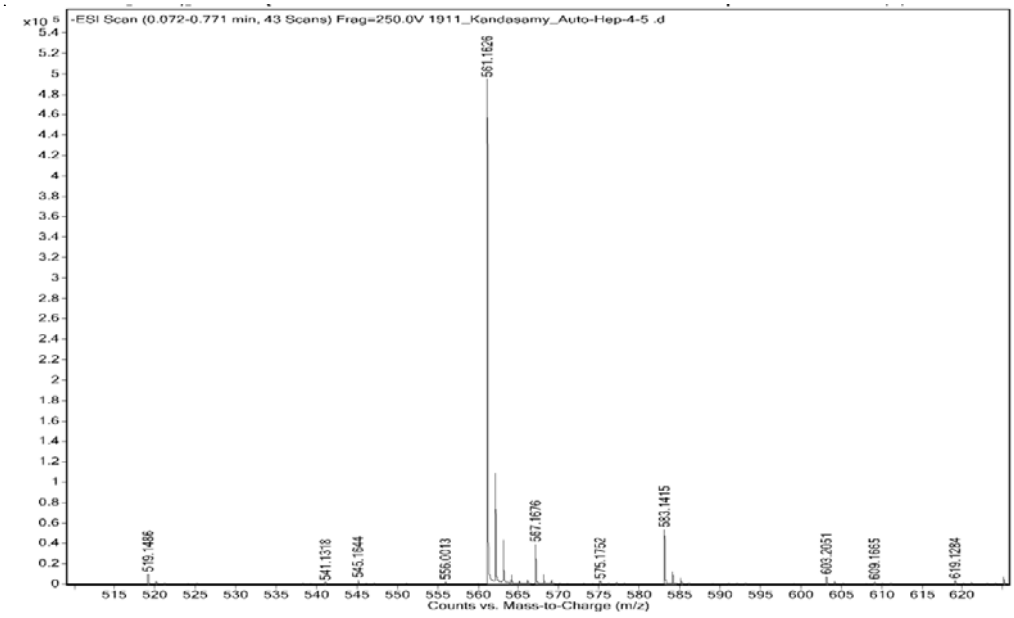
ESI-MS of Deprotected Disaccharide 9



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