Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

Automated access to well-defined ionic oligosaccharides

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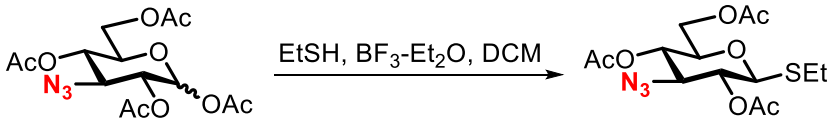
1. General Materials and Methods

All chemicals used were reagent grade and used as supplied unless otherwise noted. The automated syntheses were performed on a home-built synthesizer developed at the Max Planck Institute of Colloids and Interfaces. 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 p-anisaldehyde (PAA) solution. Flash column chromatography was carried out by using forced flow of the indicated solvent on Fluka Kieselgel 60 M (0.04 – 0.063 mm). Analysis and purification by normal and reverse phase HPLC was performed by using an Agilent 1200 series. Products were lyophilized using a Christ Alpha 2-4 LD plus freeze dryer. ^{1}H, ^{13}C and HSQC NMR spectra were recorded on a Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. Spectra were recorded in $CDCl_3$ by using the solvent residual peak chemical shift as the internal standard ($CDCl_3$: 7.26 ppm ^{1}H, 77.0 ppm ^{13}C) or in D_2O using the solvent as the internal standard in ^{1}H NMR (D_2O: 4.79 ppm ^{1}H). High resolution mass spectra were obtained using a 6210 ESI-TOF mass spectrometer (Agilent) and a MALDI-TOF autoflex™ (Bruker). MALDI and ESI mass spectra were run on IonSpec Ultima instruments. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured by using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

2. Synthesis of Building Blocks

2.1. Synthesis of BB-1

Ethyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-azido-1-thio-β-D-glucopyranoside, 1



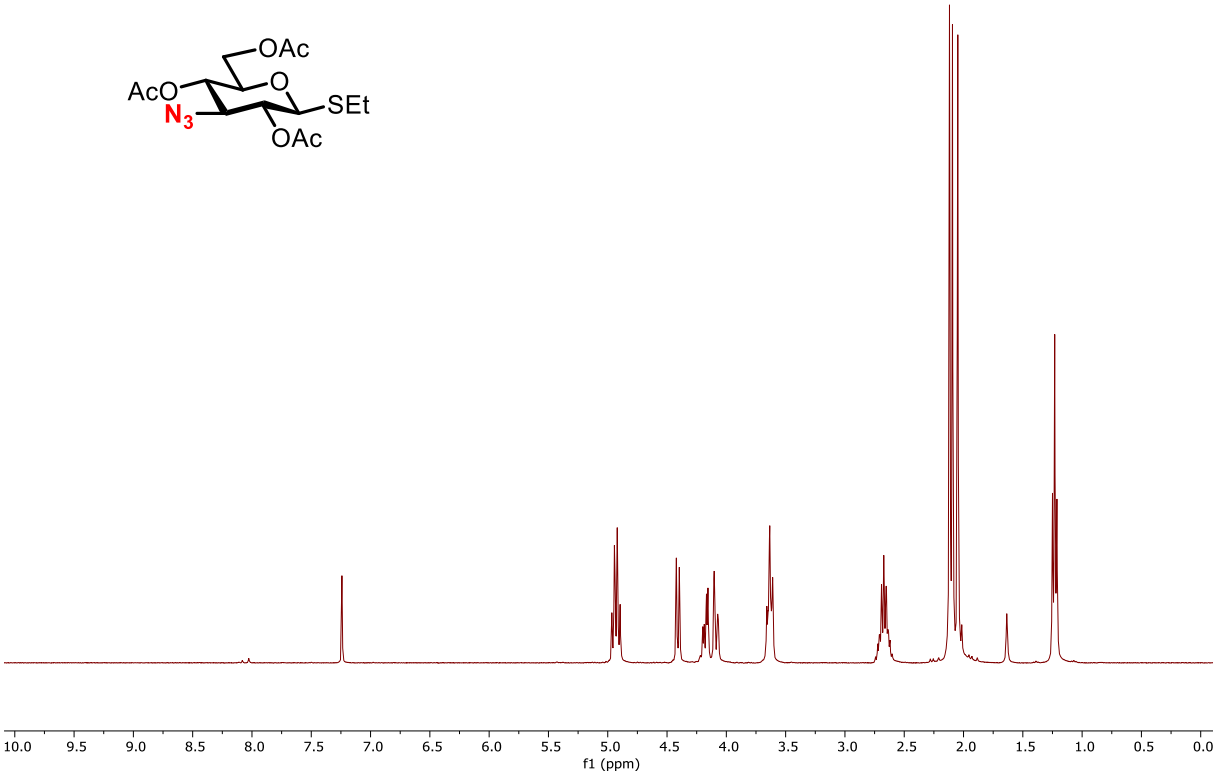
S1 **1**

S1 was prepared according to previously established procedures. [1]

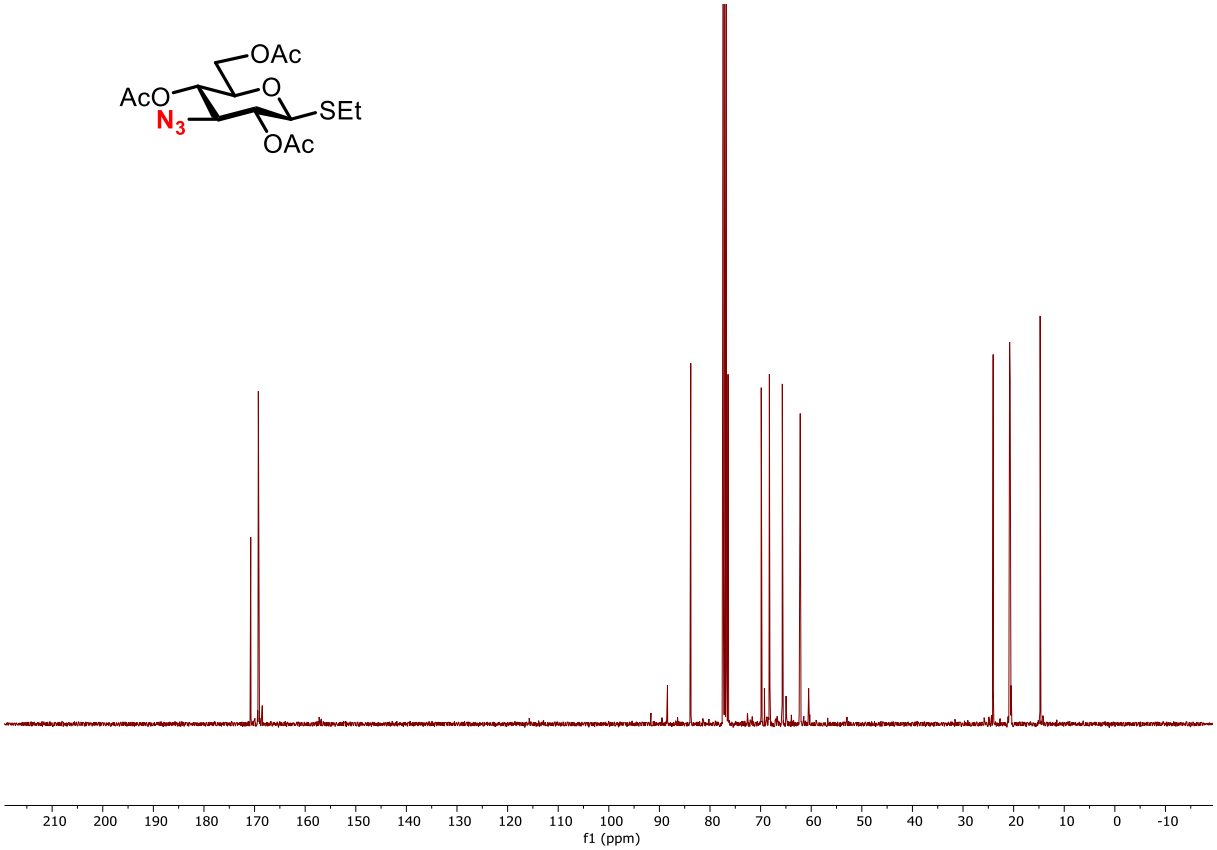
1,2,4,6-tetra-*O*-acetyl-3-deoxy-3-azido-D-glucopyranoside **S1** (22.0 g, 58.9 mmol) was dissolved in anhydrous DCM (150 mL). Ethanethiol (EtSH) (6.4 mL, 88.7 mmol) was added, and the reaction was cooled to 0°C. Boron trifluoride-diethyl ether (10.9 mL, 88.7 mmol) was slowly added under argon atmosphere. After 4 hours, the reaction was diluted with DCM (400 mL), washed with saturated aq. $NaHCO_3$ solution (500 mL), and the water layer extracted with additional DCM (200 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 4:1→Hexane : EtOAc = 2:1) to give **1** as a light yellow oil (9.2 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.96 (td, *J* = 9.9, 8.4 Hz, 2H), 4.45 (d, *J* = 9.9 Hz, 1H), 4.21 (dd, *J* = 12.4, 5.0 Hz, 1H), 4.12 (dd, *J* = 12.4, 2.5 Hz, 1H), 3.72 – 3.61 (m, 2H), 2.83 – 2.52 (m, 2H), 2.15 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 1.26 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ

170.76, 169.26, 83.81, 76.43, 69.87, 68.27, 65.71, 62.17, 24.05, 20.83, 20.79, 20.69, 14.76; $[\alpha]_D^{25}$ -36.04 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 2111, 1748, 1217 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 398.1004 ($C_{14}H_{21}N_3O_7SNa^+$ requires 398.0992).

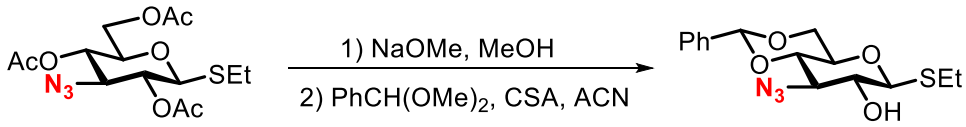
^{1}H NMR of 1 (400 MHz, $CDCl_3$)



^{13}C NMR of 1 (101 MHz, $CDCl_3$)



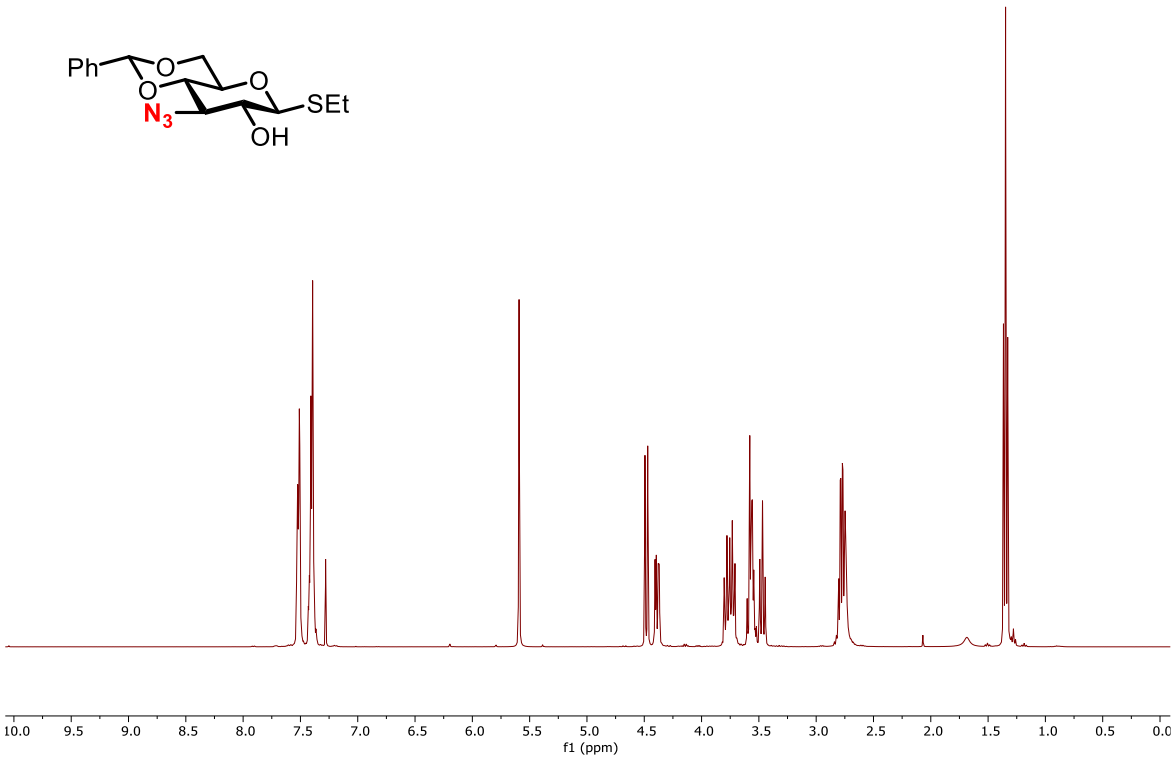
Ethyl 3-deoxy-3-azido-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside, 3



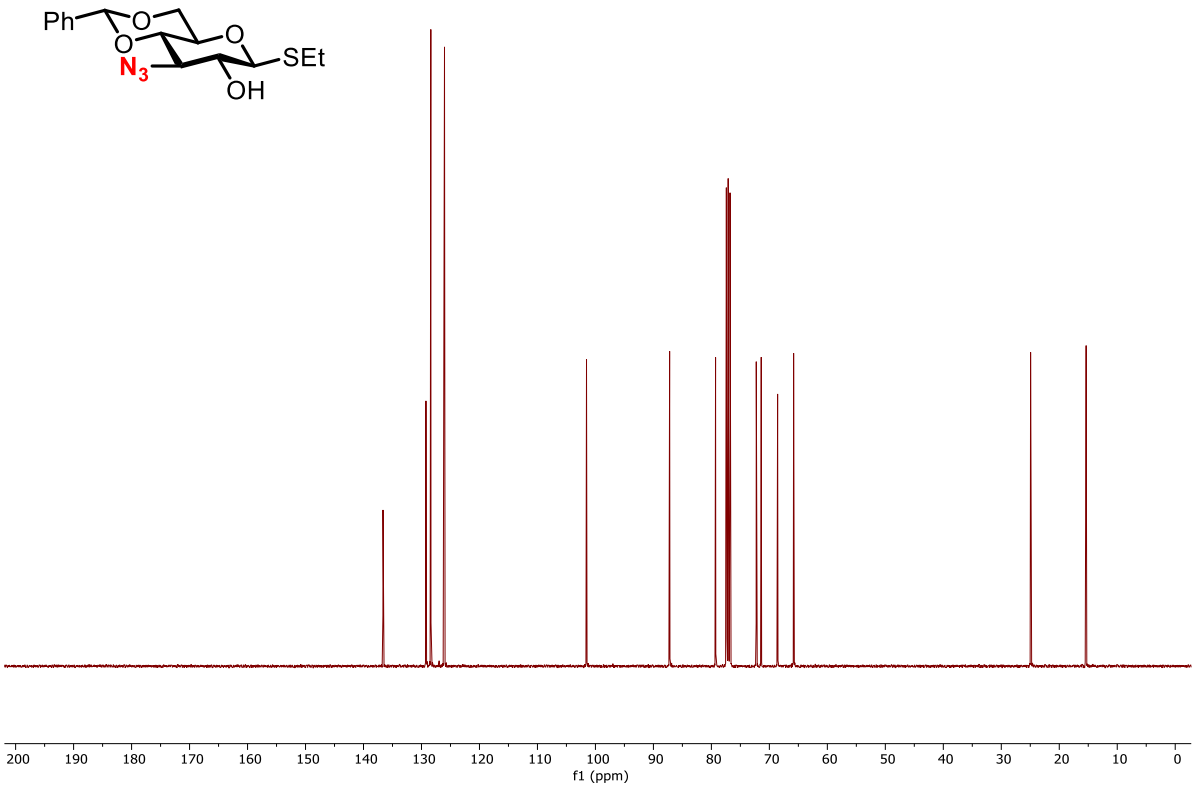
1 **3**

Ethyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-azido-1-thio-β-D-glucopyranoside **1** (9.20 g, 24.6 mmol) was dissolved in MeOH (100 mL). Sodium methoxide (0.5 M in methanol, 5 mL, 2.5 mmol) was added, and the reaction was stirred at room temperature for 18 h. The reaction was neutralized with Amberlite IR-120 (H^+ form), filtered, and concentrated under reduced pressure. The crude compound was dissolved in 50 ml acetonitrile. Camphorsulfonic acid (0.24 g, 1.0 mmol) and benzaldehyde dimethyl acetal (7.6 mL, 49.2 mmol) were added, and the reaction was stirred at room temperature overnight. Upon completion, TEA (1 mL) was added to quench the reaction. The solvent was evaporated and the resulting crude product was purified by column chromatography (Hexanes: EtOAc = 4:1 to Hexanes: EtOAc = 1:1) to give **3** as a white solid (6.65 g, 80% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.47 (m, 2H), 7.45 – 7.35 (m, 3H), 5.59 (s, 1H), 4.48 (d, *J* = 9.7 Hz, 1H), 4.39 (dd, *J* = 10.8, 3.9 Hz, 1H), 3.84 – 3.66 (m, 2H), 3.63 – 3.50 (m, 2H), 3.47 (t, *J* = 9.4 Hz, 1H), 2.83 – 2.66 (m, 3H), 1.35 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.64, 129.23, 128.39, 126.03, 101.54, 87.20, 79.28, 72.26, 71.40, 68.57, 65.82, 24.94, 15.36; $[\alpha]_D^{25}$ -48.01 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 2113, 1076, 985 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 360.0999 ($C_{15}H_{19}N_3O_4SNa^+$ requires 360.0988).

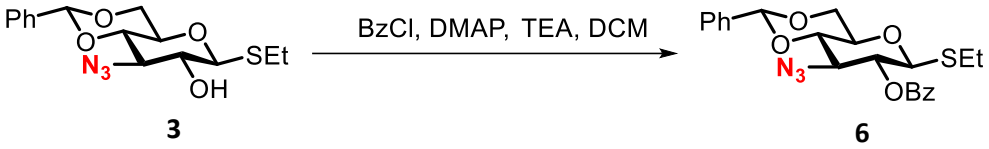
^{1}H NMR of 3 (400 MHz, $CDCl_3$)



^{13}C NMR of 3 (101 MHz, $CDCl_3$)



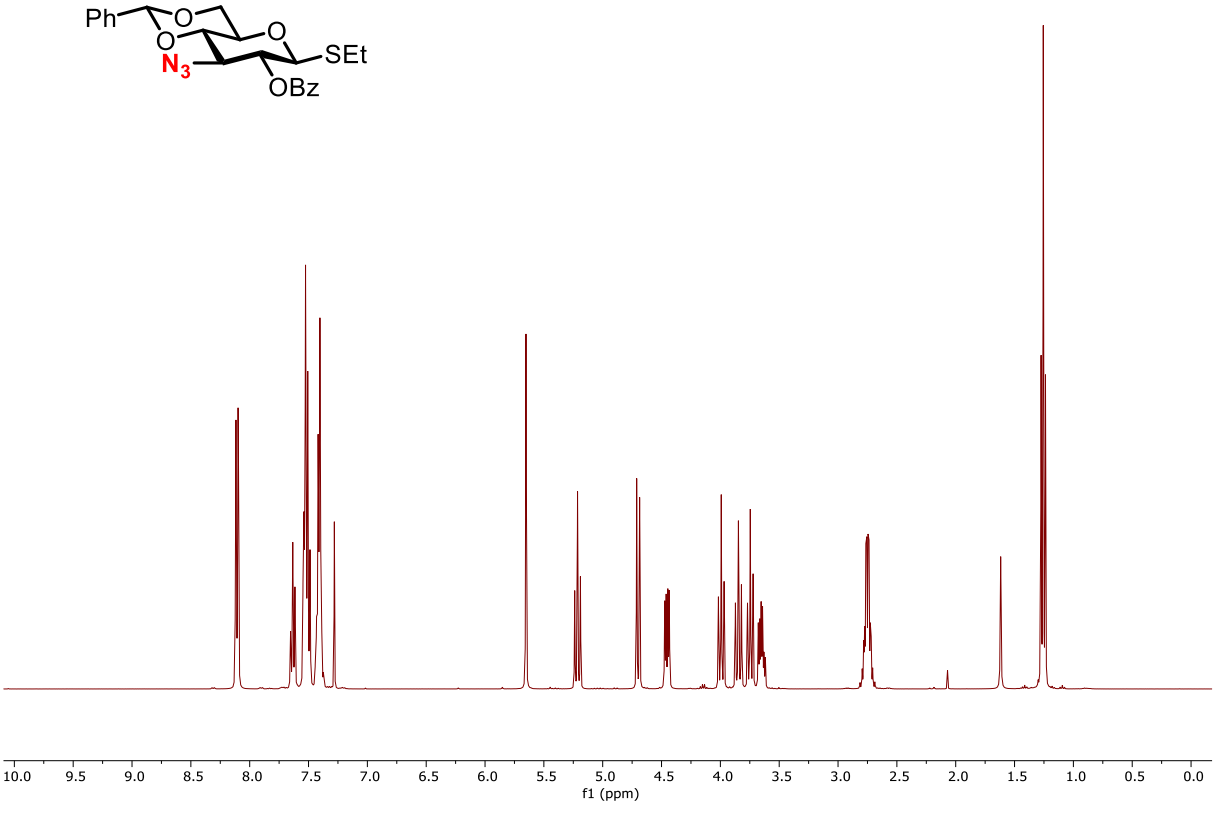
Ethyl 2-*O*-benzoyl-3-deoxy-3-azido-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside, 6



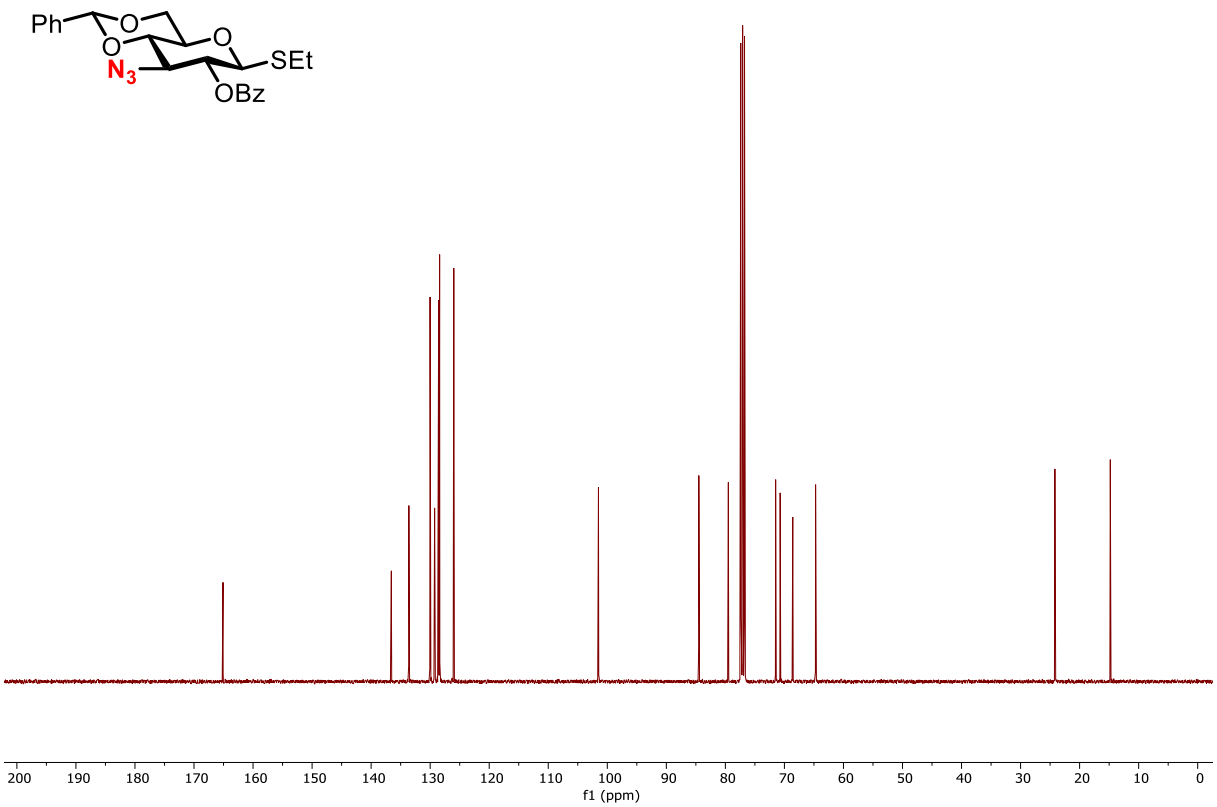
3 **6**

Ethyl 3-deoxy-3-azido-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside **3** (6.60 g, 19.6 mmol) was dissolved in anhydrous DCM (60 mL). TEA (8.6 mL, 61.7 mmol) and 4-dimethylaminopyridine (0.48 g, 3.9 mmol) were added. Benzoyl chloride (3.4 mL, 29.3 mmol) was added dropwise to the solution cooled with an ice bath. After 30 min, the system was allowed to room temperature and the reaction stirred for additional 10 h. The reaction mixture was washed with water (50 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→3:1) to give **6** as a white solid (8.22 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.04 (m, 2H), 7.66 – 7.60 (m, 1H), 7.57 – 7.47 (m, 4H), 7.45 – 7.36 (m, 3H), 5.65 (s, 1H), 5.21 (t, *J* = 9.7 Hz, 1H), 4.70 (d, *J* = 10.0 Hz, 1H), 4.45 (dd, *J* = 10.6, 4.9 Hz, 1H), 3.99 (t, *J* = 9.7 Hz, 1H), 3.85 (t, *J* = 10.2 Hz, 1H), 3.75 (t, *J* = 9.5 Hz, 1H), 3.65 (td, *J* = 9.6, 4.8 Hz, 1H), 2.75 (qd, *J* = 7.5, 3.3 Hz, 2H), 1.26 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.09, 136.58, 133.61, 130.01, 129.25, 129.18, 128.56, 128.40, 126.00, 101.49, 84.49, 79.50, 71.49, 70.72, 68.59, 64.72, 24.19, 14.81; $[\alpha]_D^{25}$ -11.71 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 2112, 1730, 1095 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 464.1260 ($C_{22}H_{23}N_3O_5SNa^+$ requires 464.1251).

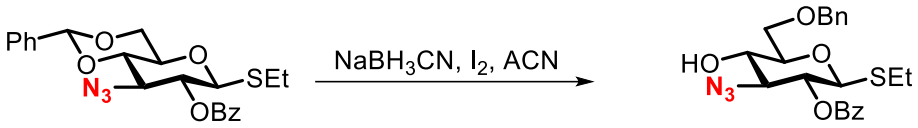
^{1}H NMR of 6 (400 MHz, $CDCl_3$)



^{13}C NMR of 6 (101 MHz, $CDCl_3$)



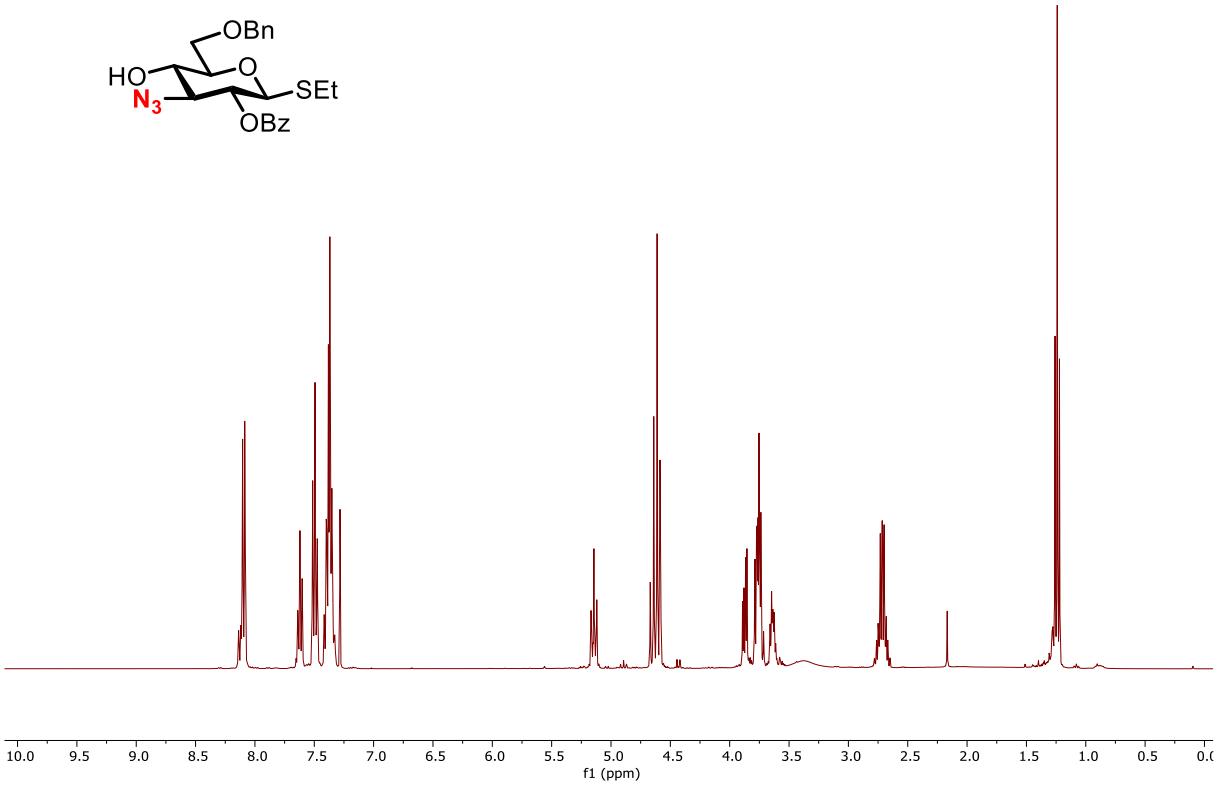
Ethyl 2-*O*-benzoyl-3-deoxy-3-azido-6-*O*-benzyl-1-thio-β-D-glucopyranoside, S2



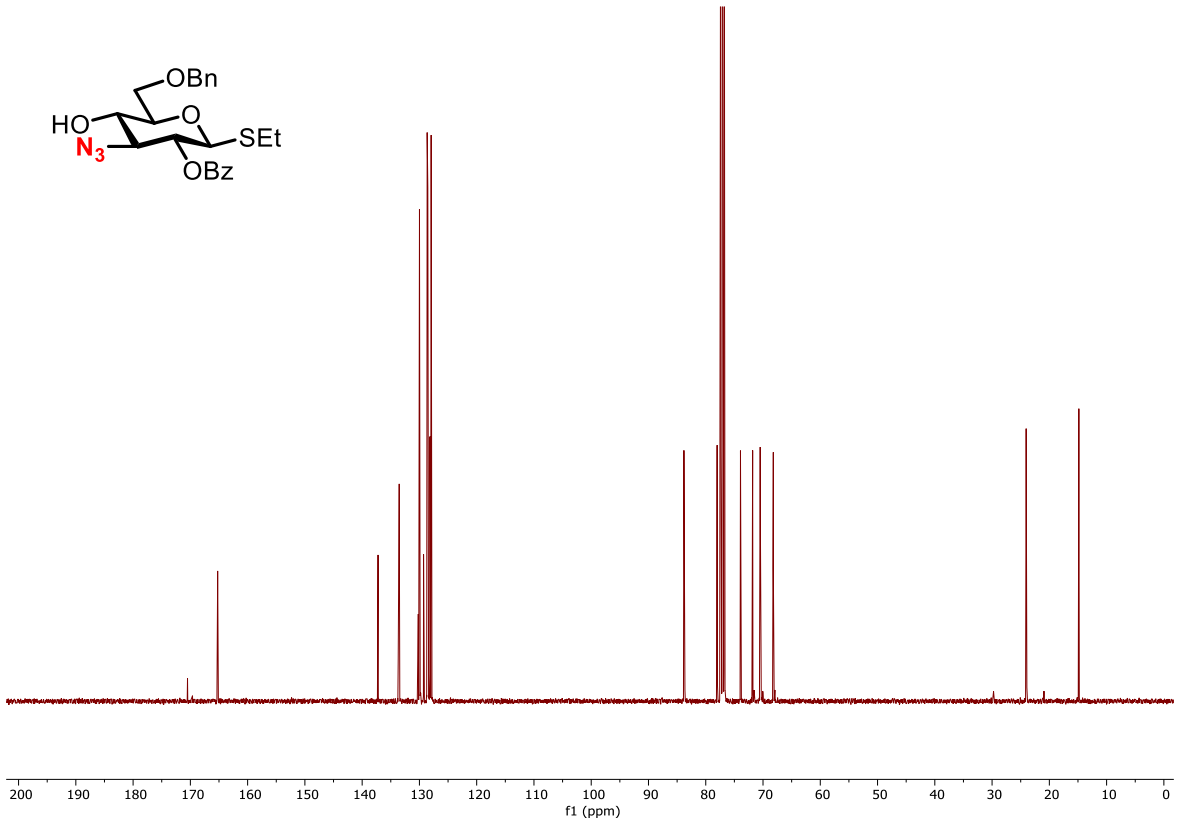
6 **S2**

Ethyl 2-*O*-benzoyl-3-deoxy-3-azido-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside **6** (8.20 g, 18.5 mmol) was dissolved in anhydrous ACN (150 mL). Sodium cyanoborohydride (5.89 g, 93.7 mmol) and 4Å molecular sieve were added. After 15 min, iodine (16.7 g, 65.8 mmol) was slowly added to the solution. Upon consumption of the starting material (monitored by TLC), DCM (300 mL) was added. The reaction mixture was washed with saturated aq. $NaHCO_3$-$Na_2S_2O_3$ (5%) solution (500 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→2:1) to give **S2** as a light yellow oil (2.83 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 8.06 (m, 2H), 7.62 (m, 1H), 7.49 (m, 2H), 7.42 – 7.33 (m, 5H), 5.19 – 5.09 (m, 1H), 4.69 – 4.54 (m, 3H), 3.87 (dd, *J* = 10.1, 4.7 Hz, 1H), 3.80 – 3.71 (m, 3H), 3.64 (dt, *J* = 9.2, 5.1 Hz, 1H), 3.41 (br. s, 1H), 2.72 (qd, *J* = 7.5, 5.4 Hz, 2H), 1.24 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.23, 137.22, 133.53, 129.99, 129.28, 128.65, 128.53, 128.16, 127.95, 83.81, 78.01, 73.94, 71.81, 70.50, 70.45, 68.20, 24.05, 14.84; $[\alpha]_D^{25}$ -13.85 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 2110, 1728, 1265 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 466.1411 ($C_{22}H_{25}N_3O_5SNa^+$ requires 464.1407).

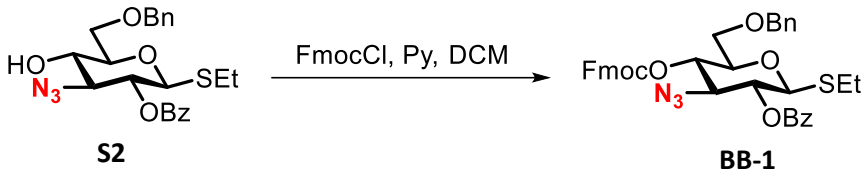
^{1}H NMR of S2 (400 MHz, $CDCl_3$)



^{13}C NMR of S2 (101 MHz, $CDCl_3$)



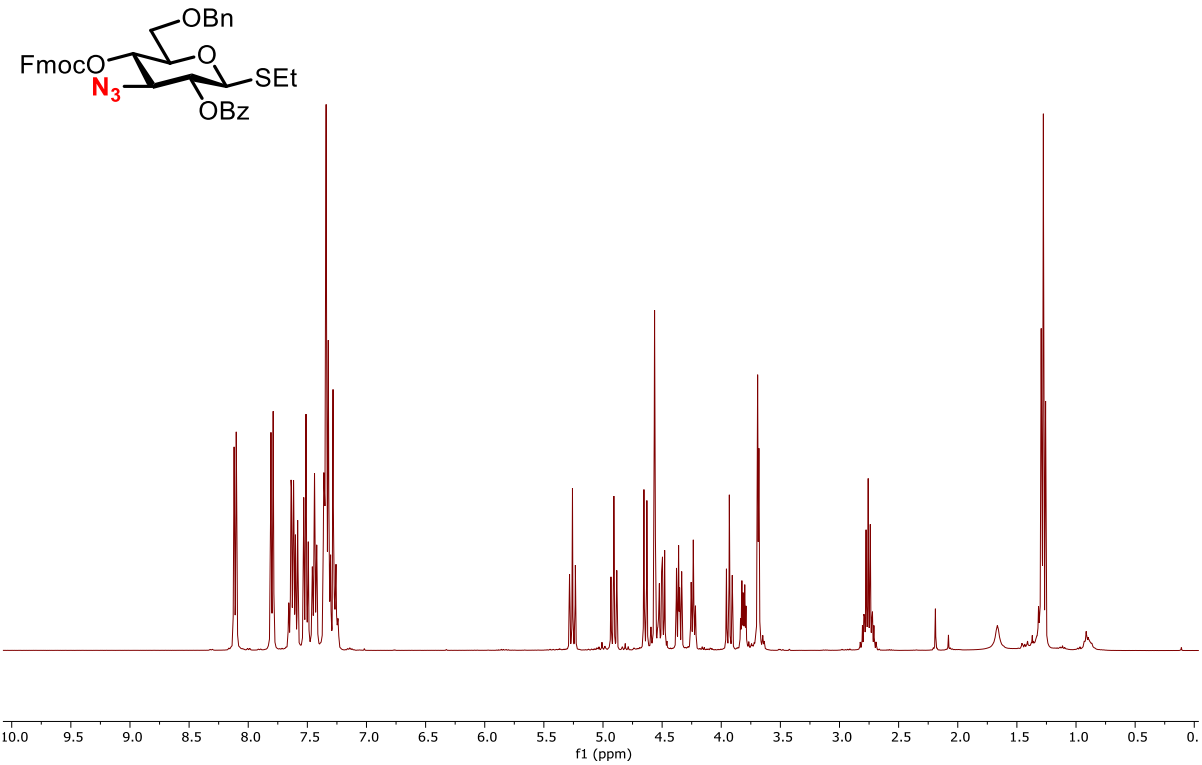
Ethyl 2-*O*-benzoyl-3-deoxy-3-azido-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-benzyl-1-thio-β-D-glucopyranoside, BB-1



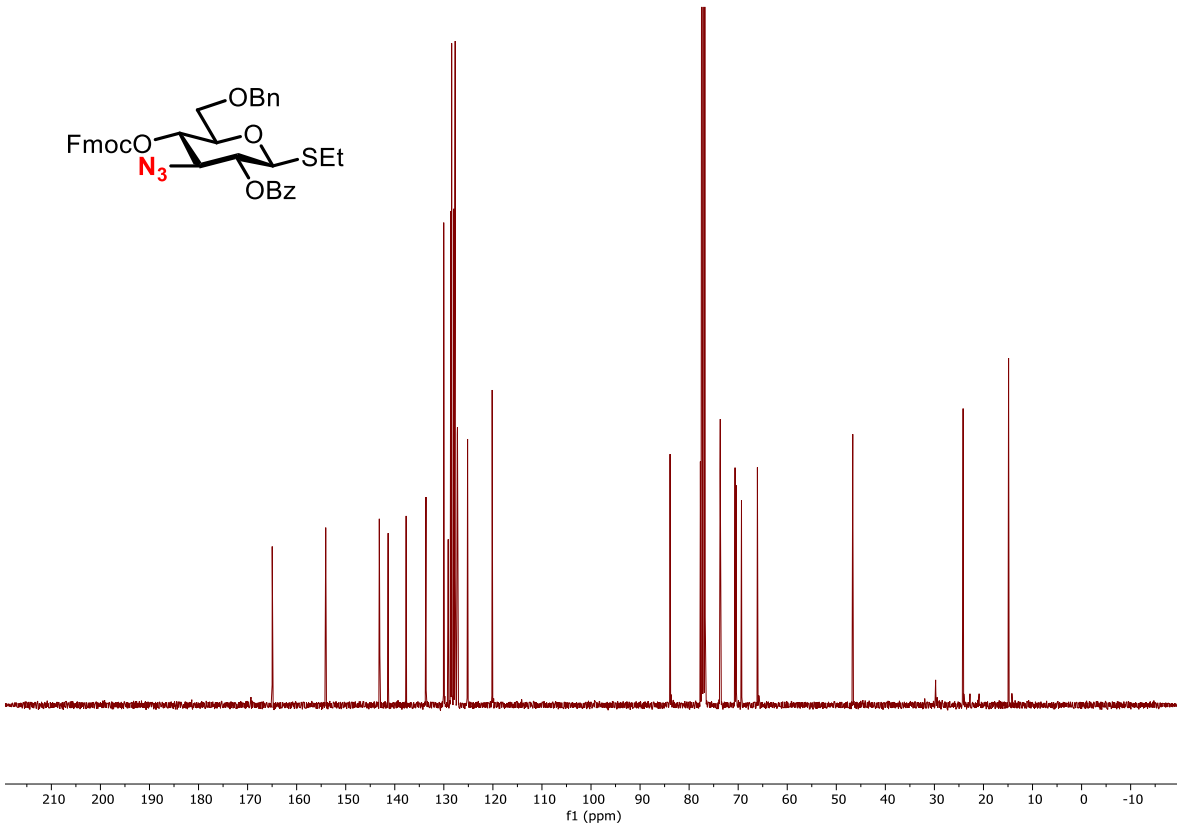
S2 **BB-1**

Ethyl 2-*O*-benzoyl-3-deoxy-3-azido-6-*O*-benzyl-1-thio-β-D-glucopyranoside **S2** (2.83 g, 6.40 mmol), was dissolved in anhydrous DCM (30 mL) and pyridine (10 mL) was added. The solution was cooled to 0˚C with an ice bath for 15 min, and fluorenylmethyloxycarbonyl chloride (3.30 g, 12.8 mmol) was added slowly. The reaction was allowed to room temperature and stirred for additional 6 h. Upon completion, DCM (50 mL) was added and the organic phase was washed with aqueous citric acid (0.5 M, 50 mL). After extracting the water phase with DCM (20 mL), the organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→3:1) to give **BB-1** as a white solid (3.83 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.08 (m, 2H), 7.80 (d, *J* = 7.5 Hz, 2H), 7.67 – 7.57 (m, 3H), 7.51 (t, *J* = 7.8 Hz, 2H), 7.47 – 7.40 (m, 2H), 7.38 – 7.29 (m, 6H), 7.30 – 7.23 (m, 1H), 5.26 (t, *J* = 9.8 Hz, 1H), 4.91 (t, *J* = 9.9 Hz, 1H), 4.64 (d, *J* = 9.9 Hz, 1H), 4.60 – 4.46 (m, 3H), 4.36 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.24 (t, *J* = 7.3 Hz, 1H), 3.93 (t, *J* = 9.9 Hz, 1H), 3.81 (dt, *J* = 9.4, 4.3 Hz, 1H), 3.69 (d, *J* = 4.3 Hz, 2H), 2.76 (p, *J* = 7.3 Hz, 2H), 1.28 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.98, 154.08, 143.15, 143.05, 141.35, 141.30, 137.68, 133.64, 130.02, 129.11, 128.58, 128.40, 128.00, 127.77, 127.69, 127.26, 127.22, 125.16, 125.13, 120.15, 120.13, 83.89, 77.71, 73.71, 73.65, 70.64, 70.40, 69.33, 66.08, 46.64, 24.15, 14.88; $[\alpha]_D^{25}$ 22.15 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 2110, 1755, 1247 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 688.2092 ($C_{37}H_{35}N_3O_7SNa^+$ requires 688.2088).

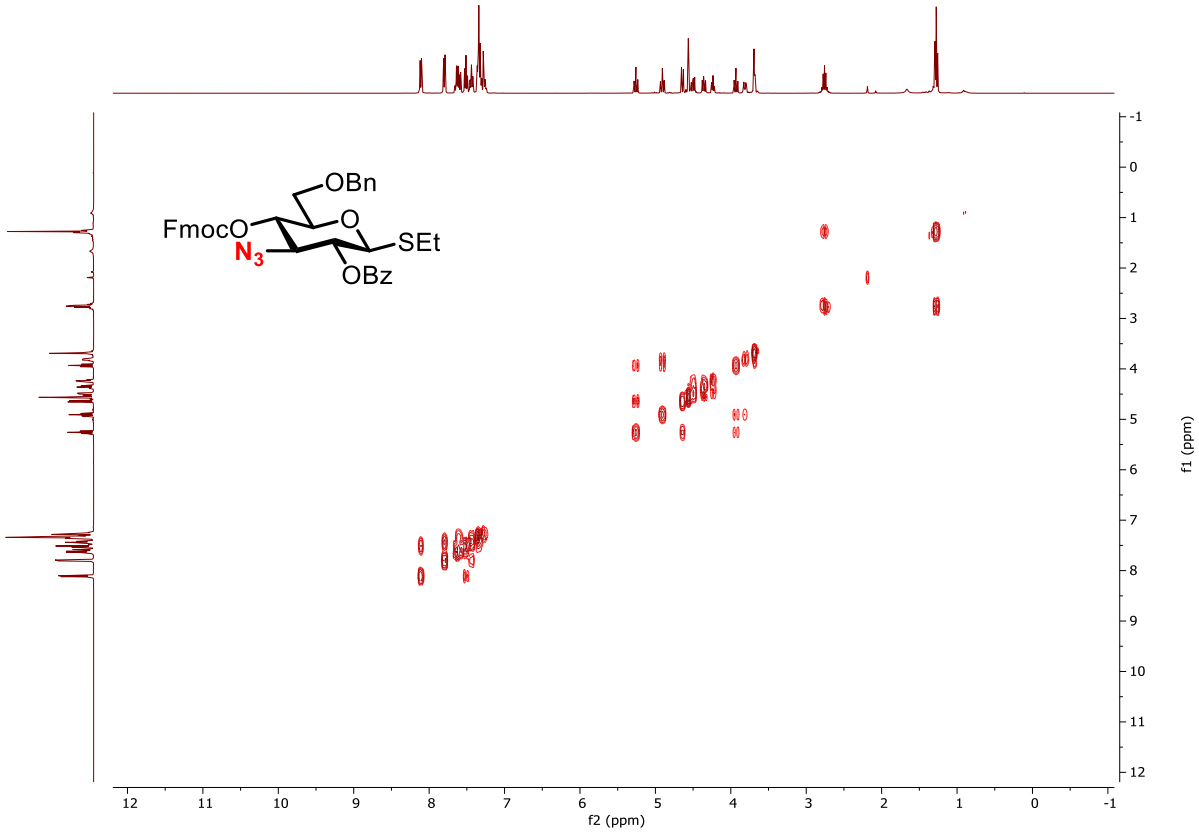
^{1}H NMR of BB-1 (400 MHz, $CDCl_3$)



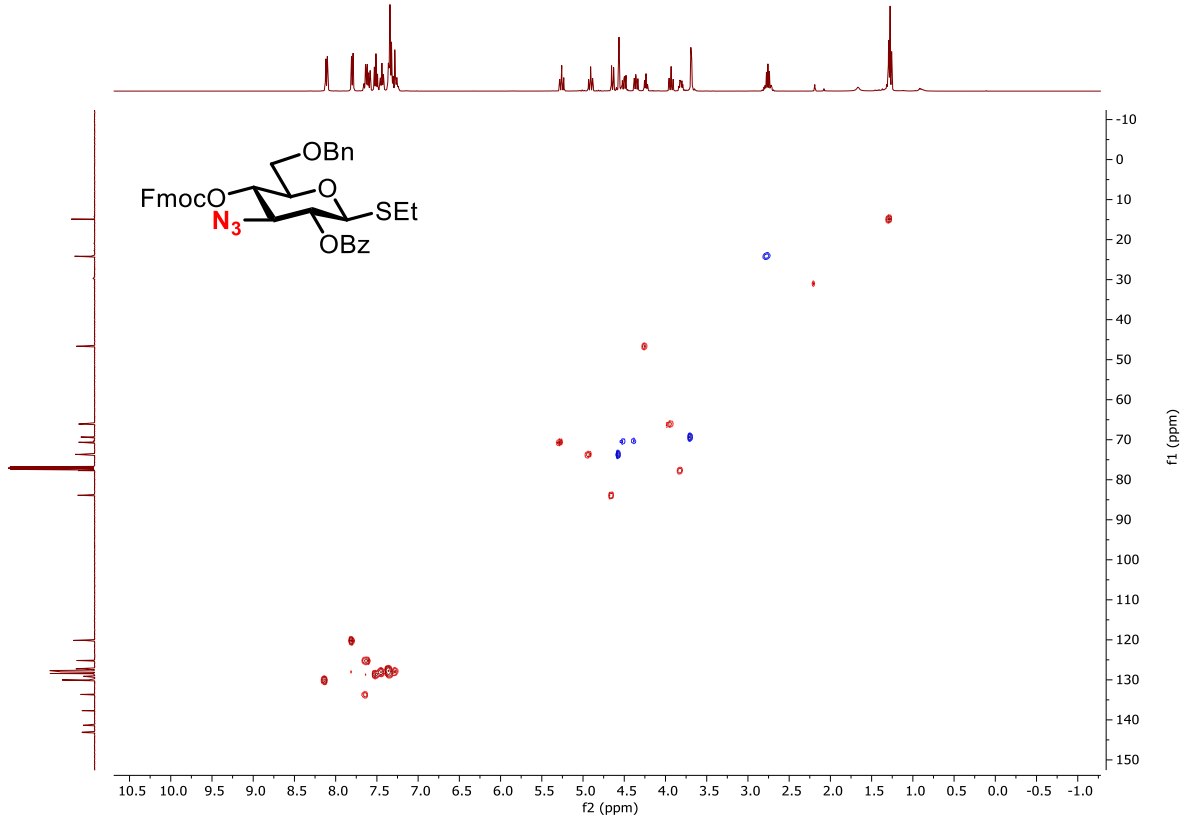
^{13}C NMR of BB-1 (101 MHz, $CDCl_3$)



COSY NMR of BB-1 ($CDCl_3$)

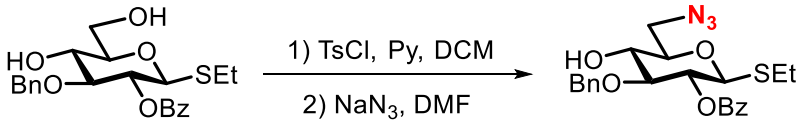


HSQC NMR of BB-1 ($CDCl_3$)



2.2. Synthesis of BB-2

Ethyl 2-*O*-benzoyl-3-*O*-benzyl-6-deoxy-6-azido-1-thio-β-D-glucopyranoside, S4

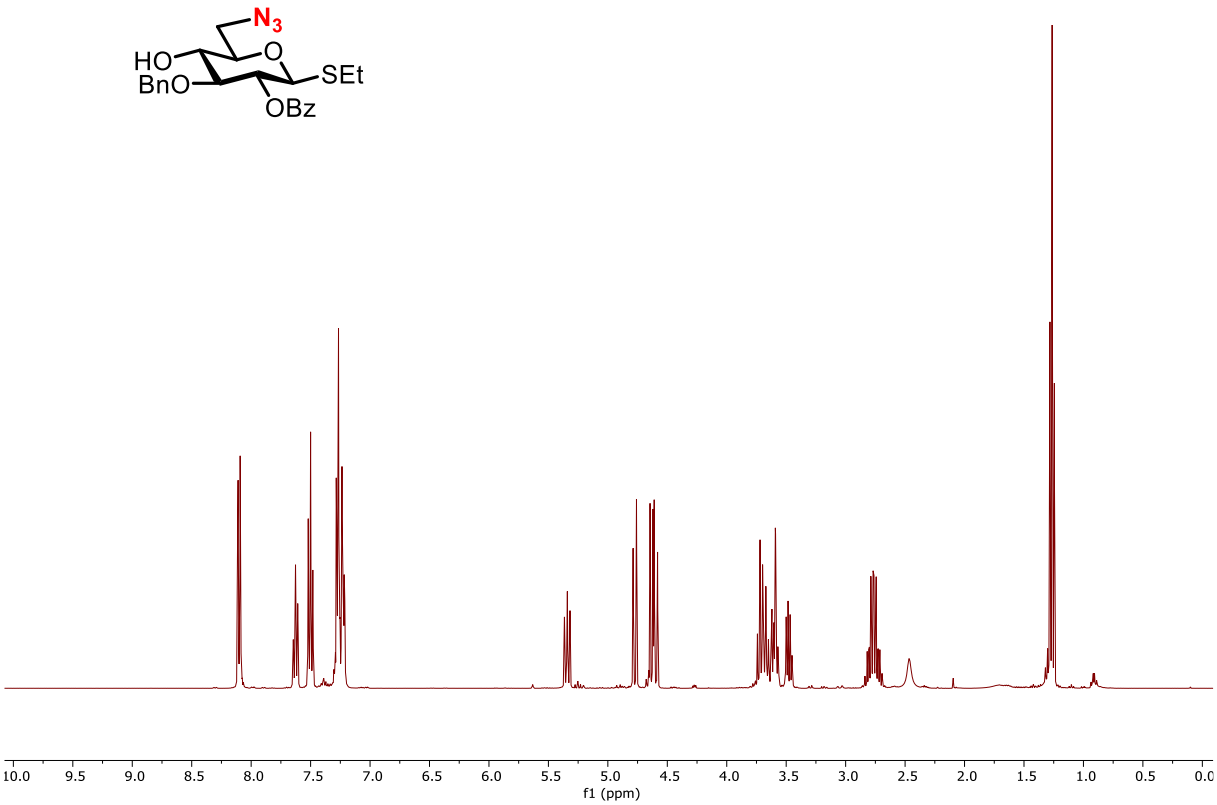


S3 **S4**

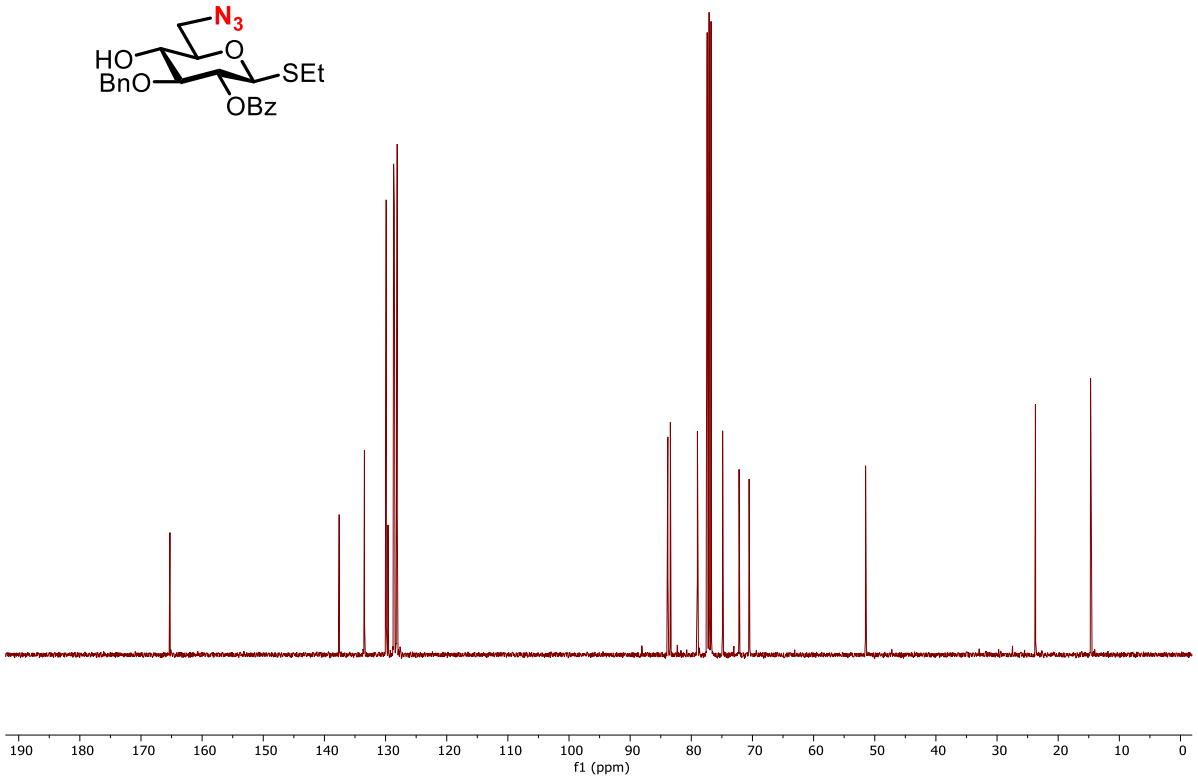
S3 was prepared according to previously established procedures. [2]

Ethyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-glucopyranoside **S3** (3.0 g, 7.2 mmol) was dissolved in anhydrous DCM (50 mL). Pyridine (10 mL) was added and the solution was cooled to 0°C. Toluenesulfonyl chloride (1.76 g, 9.2 mmol) was added slowly to the solution. After 30 min, the system was allowed to room temperature and the reaction stirred for additional 6 h. The reaction mixture was washed with citric acid (0.5 M in water, 200 mL) twice, and the water layer was extracted with additional DCM (50 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→Hexane : EtOAc = 2:1) to give the 6-*O*-toluenesulfonyl sugar as a white solid. This compound was dissolved in DMF (30 mL) and sodium azide (1.40 g, 21.6 mmol) was added. The reaction was heated to 80°C under argon atmosphere for 18 h. Upon completion, the reaction was cooled to room temperature and diluted with water (100 mL). Ethyl acetate (100 mL) was added, the organic layer separated, and the water phase extracted with additional ethyl acetate (50 mL). The organic layers were combined, washed with water (50 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 4:1→ Hexane : EtOAc = 2:1) to give **S4** as a colorless oil (2.2 g, 70% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.07 (m, 2H), 7.68 – 7.59 (m, 1H), 7.55 – 7.46 (m, 2H), 7.30 – 7.20 (m, 5H), 5.34 (dd, *J* = 10.0, 8.6 Hz, 1H), 4.77 and 4.60 (ABq, *J* = 11.4 Hz, 2H), 4.63 (d, *J* = 10.0 Hz, 1H), 3.75 – 3.64 (m, 2H), 3.63 – 3.56 (m, 2H), 3.52 – 3.41 (m, 1H), 2.86 – 2.68 (m, 2H), 2.47 (br. s, 1H), 1.26 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.26, 137.59, 133.46, 129.90, 129.61, 128.69, 128.58, 128.22, 128.10, 83.84, 83.42, 79.00, 74.88, 72.19, 70.54, 51.50, 23.72, 14.70; $[\alpha]_D^{25}$ -24.12 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 2100, 1725, 1269 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 466.1411 ($C_{22}H_{25}N_3O_5SNa^+$ requires 466.1407).

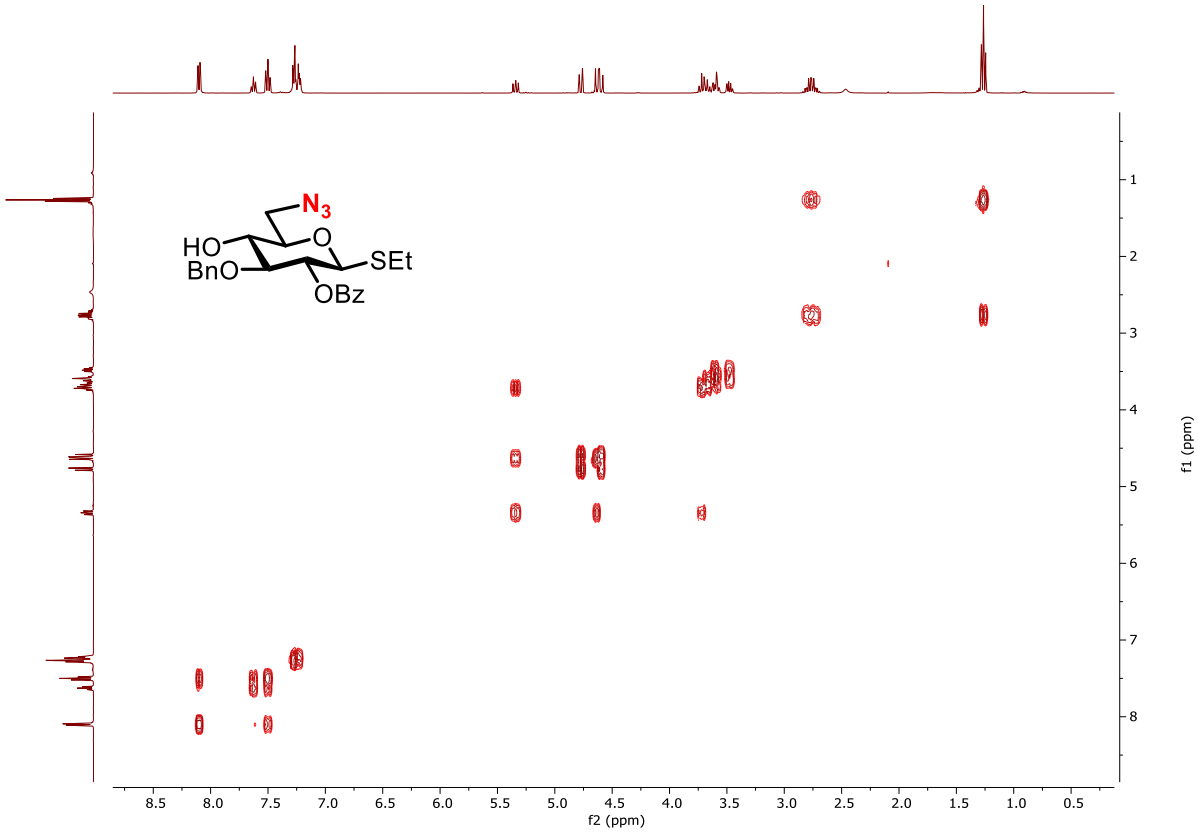
^{1}H NMR of S4 (400 MHz, $CDCl_3$)



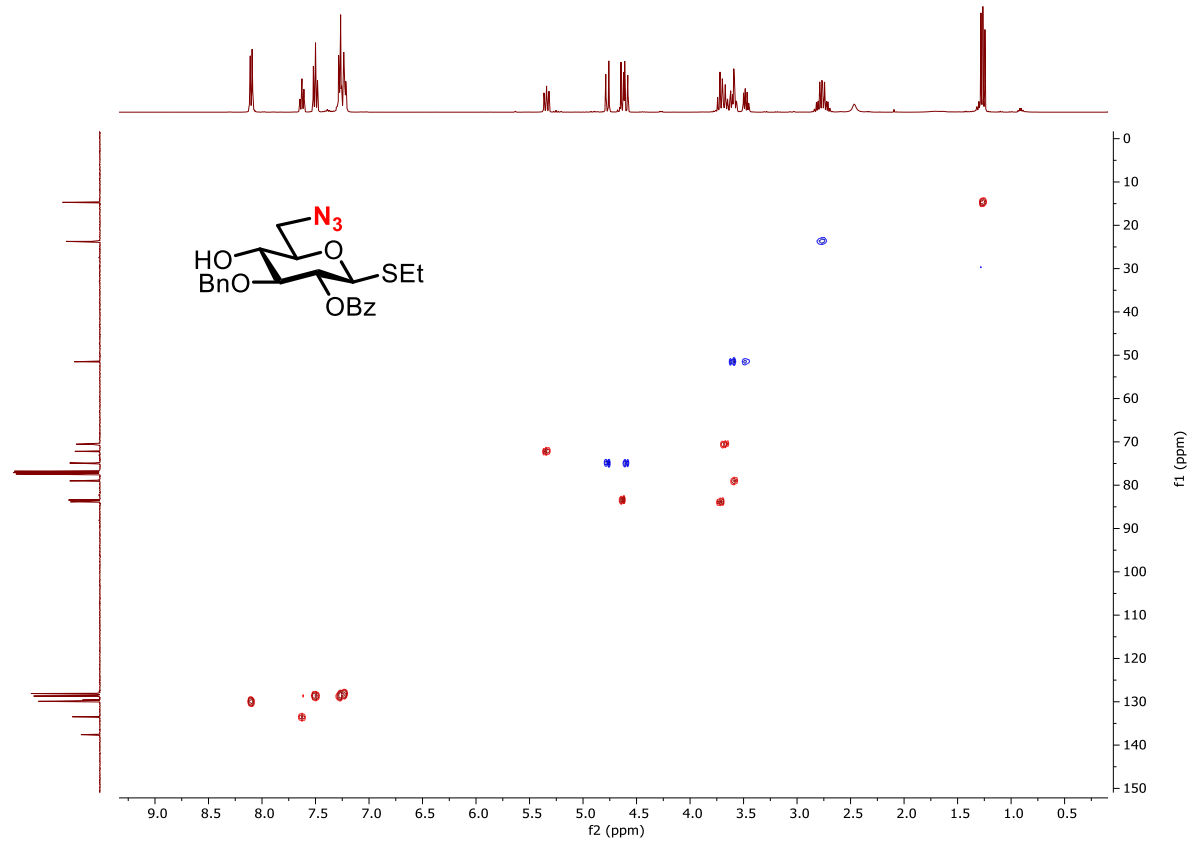
^{13}C NMR of S4 (101 MHz, $CDCl_3$)



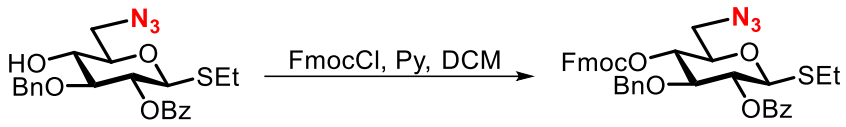
COSY NMR of S4 ($CDCl_3$)



HSQC NMR of S4 ($CDCl_3$)



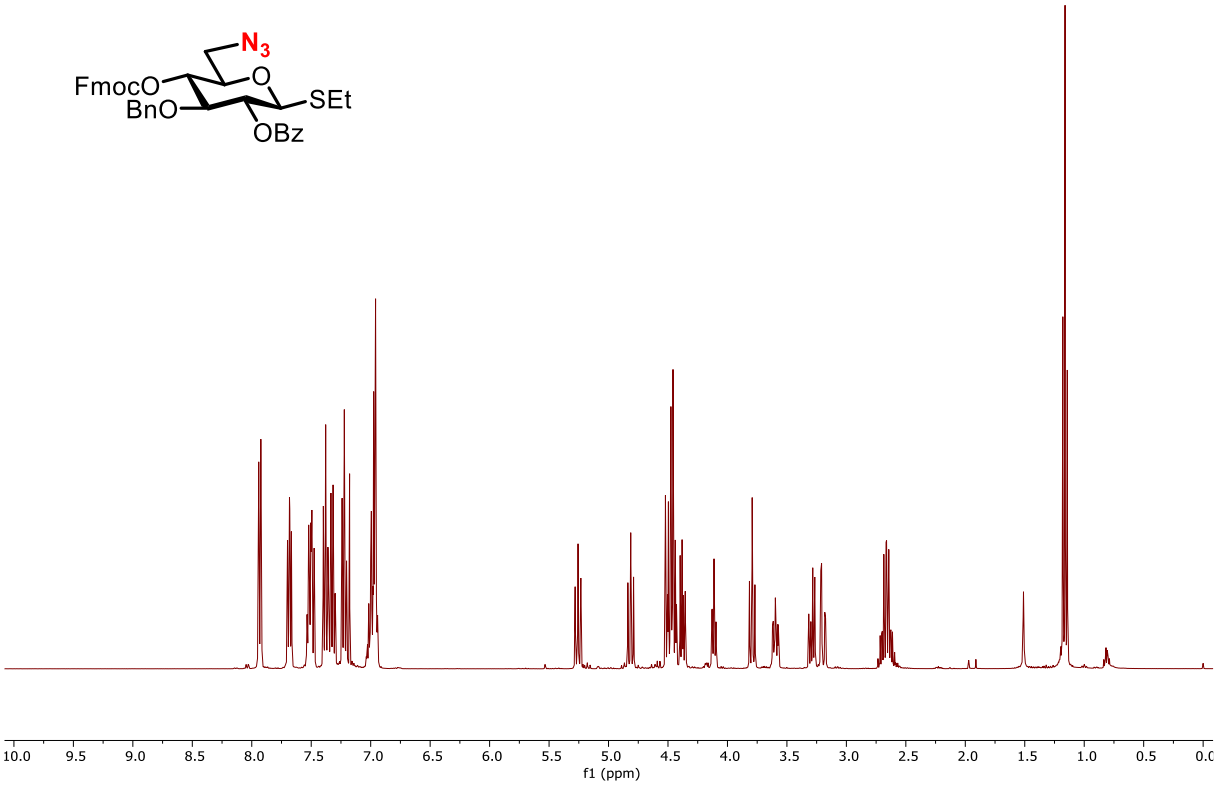
Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-deoxy-6-azido-1-thio-β-D-glucopyranoside, BB-2



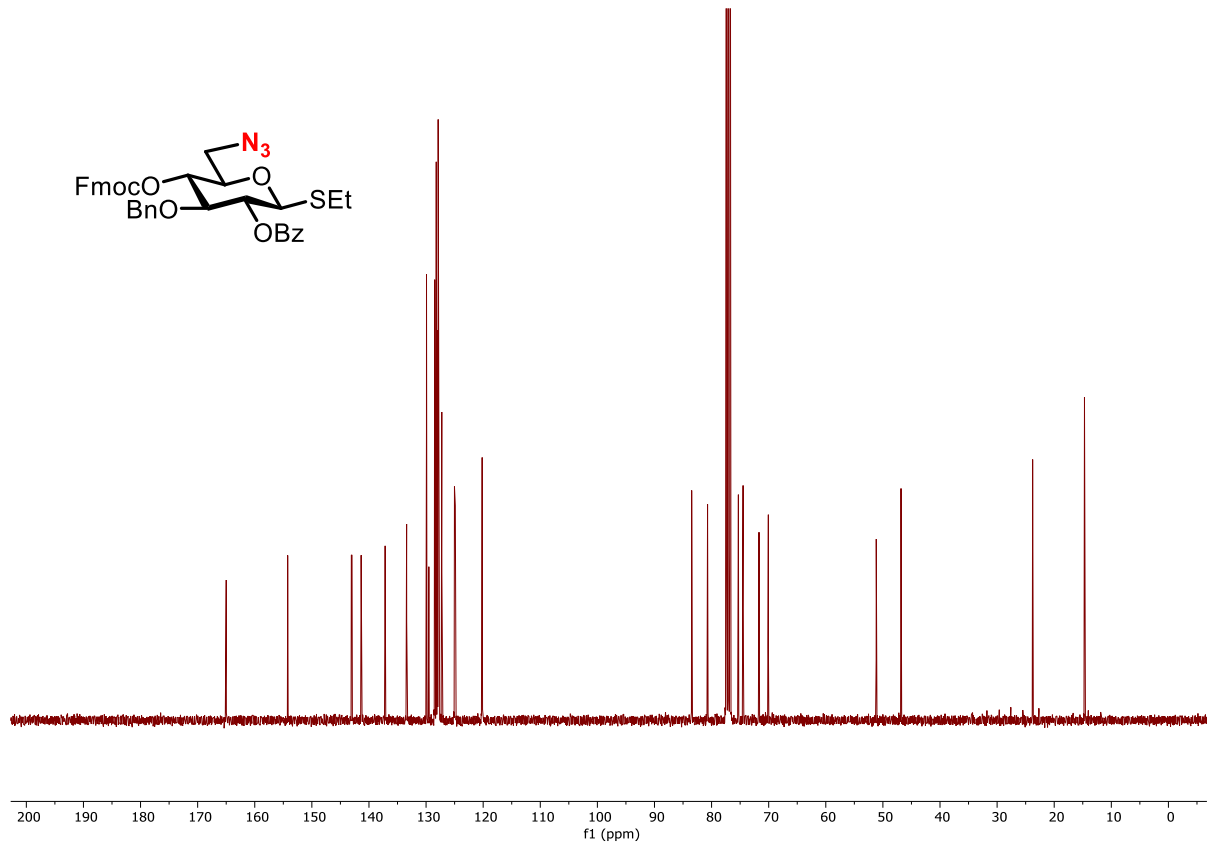
S4 **BB-2**

Ethyl 2-*O*-benzoyl-3-*O*-benzyl-6-deoxy-6-azido-1-thio-β-D-glucopyranoside **S4** (2.2 g, 5.0 mmol) was dissolved in anhydrous DCM (40 mL) and pyridine (10 mL) was added. The solution was cooled to 0°C with an ice bath for 15 min, and fluorenylmethyloxycarbonyl chloride (3.41 g, 13.2 mmol) was added slowly. The reaction was allowed to room temperature and stirred for 6 h. Upon completion, DCM (50 mL) was added and the organic phase was washed with aqueous citric acid (0.5 M, 50 mL). After extracting the water phase with DCM (30 mL), the organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→3:1) to give **BB-2** as a white solid (2.95 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (m, 2H), 7.68 (m, 2H), 7.55 – 7.45 (m, 3H), 7.41 – 7.26 (m, 4H), 7.22 (tt, *J* = 7.5, 1.1 Hz, 2H), 7.05 – 6.90 (m, 5H), 5.26 (dd, *J* = 10.0, 9.1 Hz, 1H), 4.81 (dd, *J* = 10.0, 9.2 Hz, 1H), 4.54 – 4.42 (m, 4H), 4.38 (dd, *J* = 10.6, 6.6 Hz, 1H), 4.11 (t, *J* = 6.6 Hz, 1H), 3.79 (t, *J* = 9.2 Hz, 1H), 3.68 – 3.51 (m, 1H), 3.29 (dd, *J* = 13.4, 6.9 Hz, 1H), 3.19 (dd, *J* = 13.4, 2.7 Hz, 1H), 2.77 – 2.56 (m, 2H), 1.16 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.99, 154.23, 143.09, 142.96, 141.40, 141.37, 137.17, 133.40, 129.92, 129.51, 128.49, 128.22, 128.03, 127.88, 127.75, 127.25, 127.24, 125.03, 124.88, 120.21, 120.18, 83.48, 80.70, 75.31, 74.49, 71.70, 70.06, 51.16, 46.83, 23.77, 14.70; $[\alpha]_D^{25}$ 27.75 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 2102, 1752, 1245 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 688.2097 ($C_{37}H_{35}N_3O_7SNa^+$ requires 688.2088).

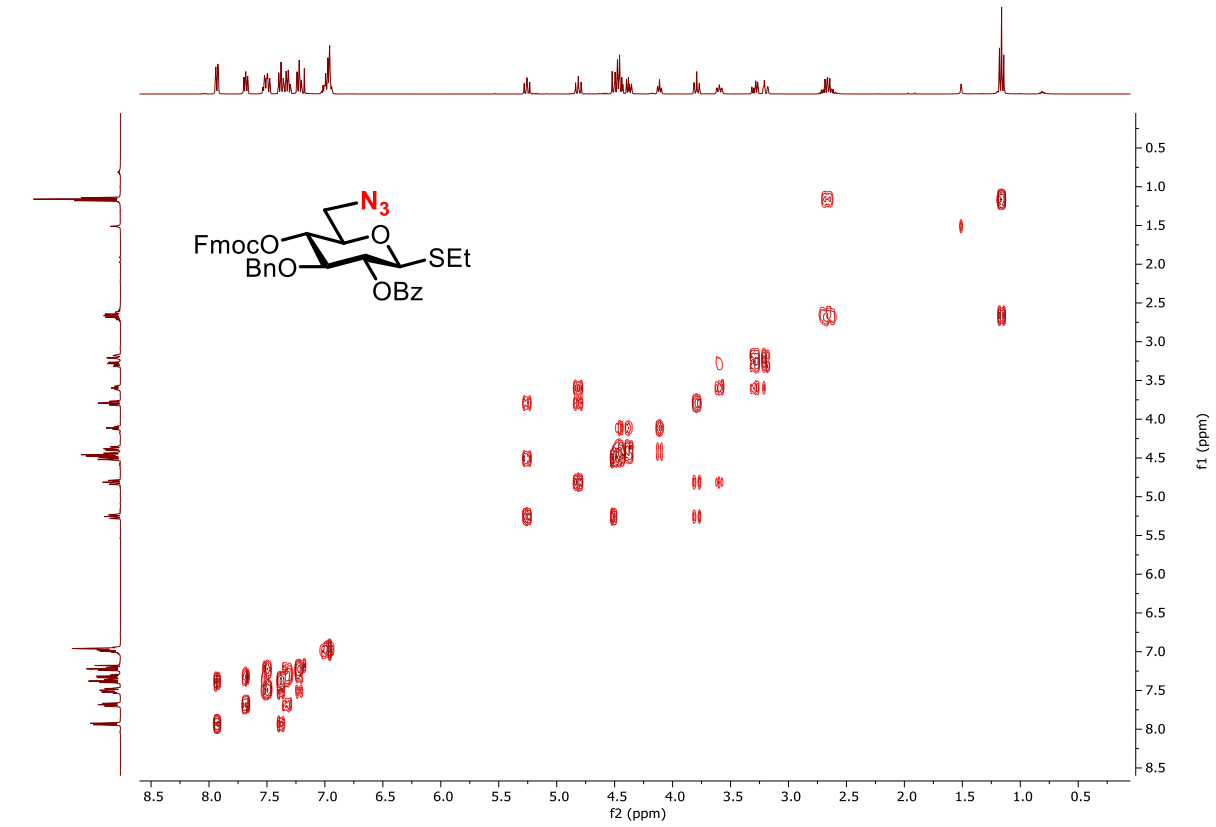
^{1}H NMR of BB-2 (400 MHz, $CDCl_3$)



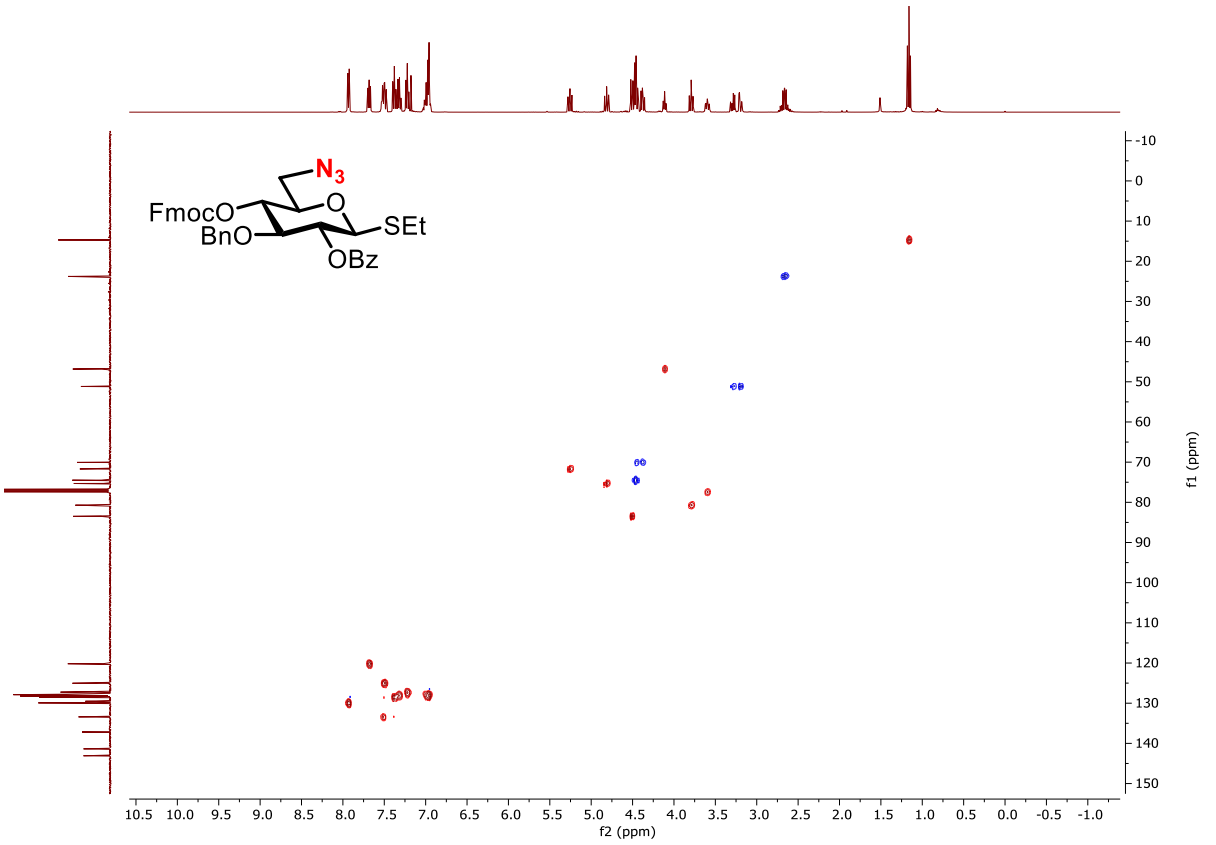
^{13}C NMR of BB-2 (101 MHz, $CDCl_3$)



COSY NMR of BB-2 ($CDCl_3$)



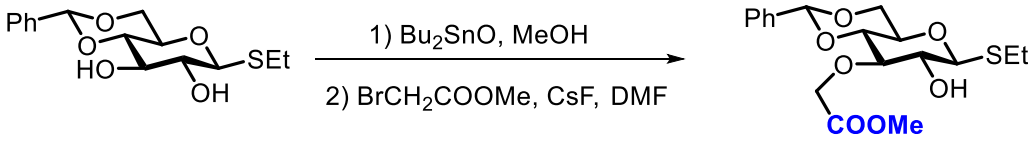
HSQC NMR of BB-2 ($CDCl_3$)



2.3. Synthesis of BB-3

BB-3 was prepared according literature procedure [3]

Ethyl 3-*O*-methoxycarbonylmethyl-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside, S5

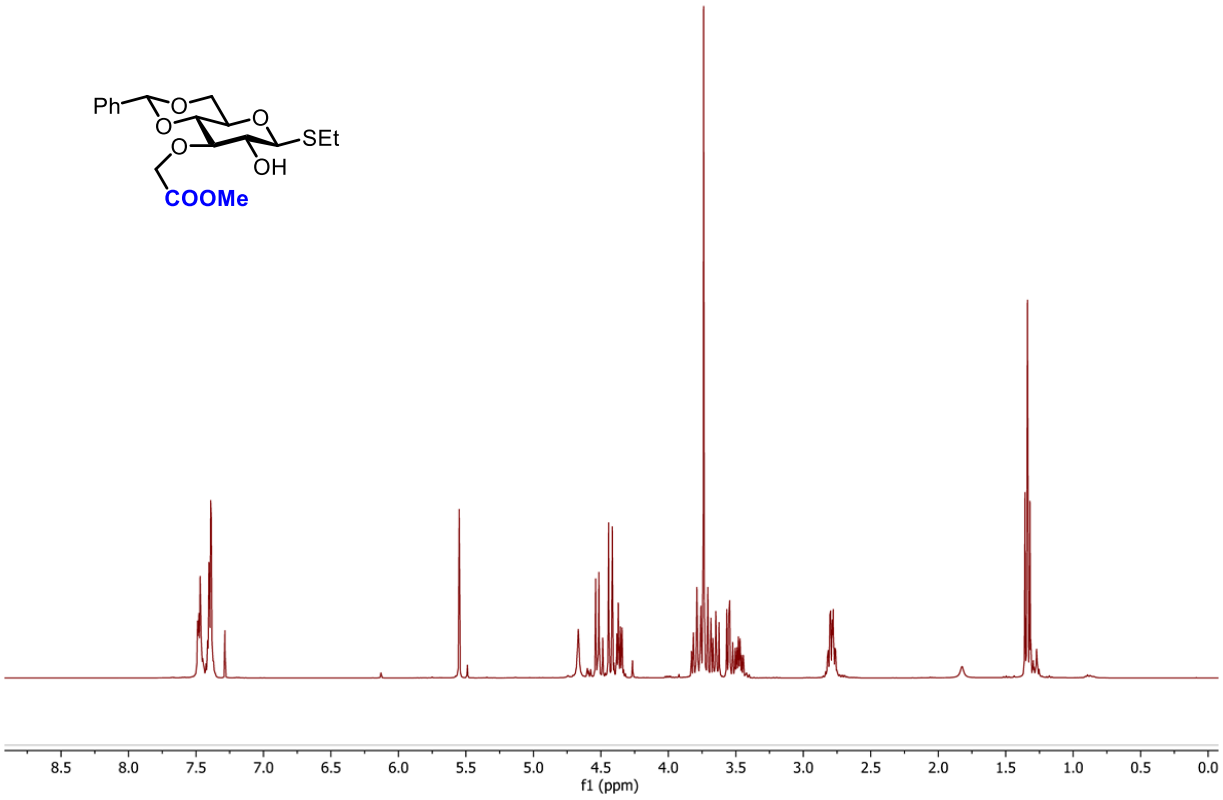


4 **S5**

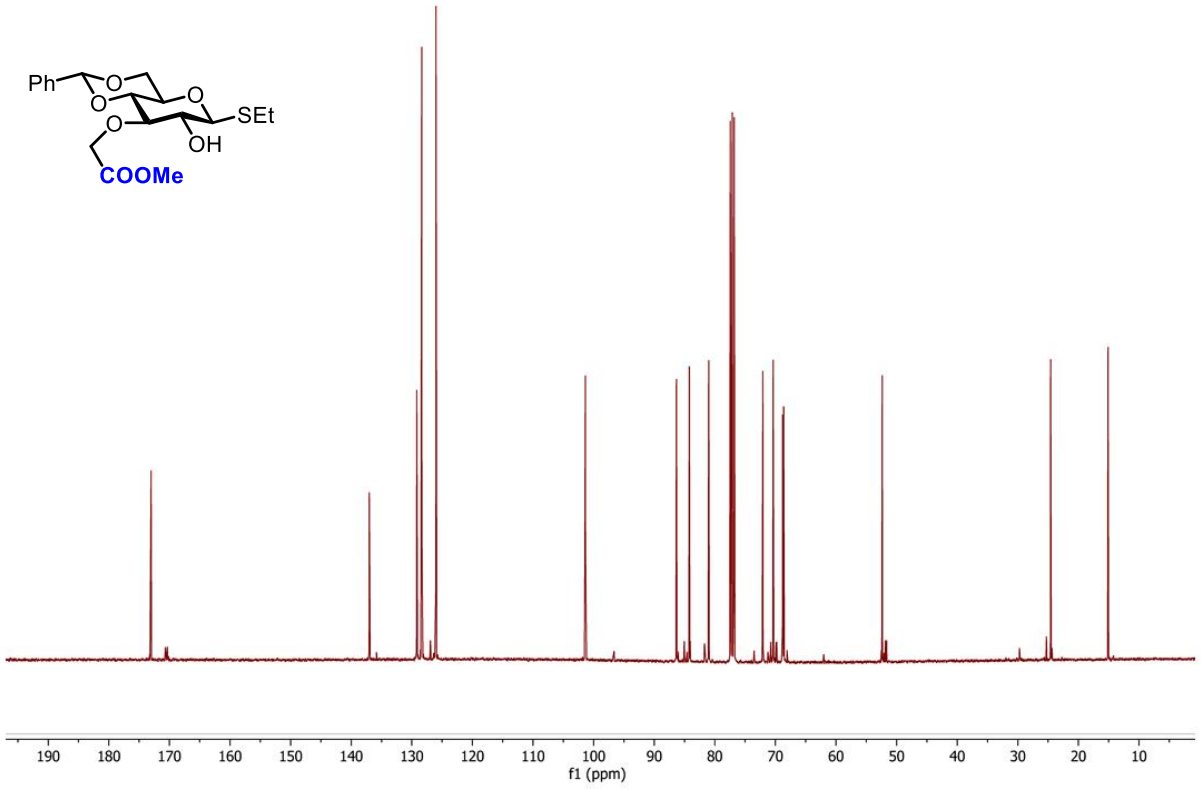
4 was prepared according to previously established procedures. [2, 4]

Ethyl 4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside **4** (5.20 g, 16.6 mmol) was dissolved in MeOH (150 mL). Dibutyltin oxide (5.15 g, 20.7 mmol) was added. The reaction mixture was charged with nitrogen and refluxed for 18 h. The methanol was removed under reduced pressure and DMF (100 mL) was added. Cesium fluoride (3.40 g, 22.4 mmol) and methyl 2-bromoacetate (1.87 mL, 20.0 mmol) were added, and the reaction was stirred at room temperature for 24 h. DMF was removed under reduced pressure and DCM (100 mL) was added. KF aq. Solution (1 M, 100 mL) was used to wash the organic layer. The water layer was extracted with additional DCM (50 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 4:1→2:1) to give **S5** as a colorless oil (4.0 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (m, 2H), 7.40 (m, *J* = 5.1, 2.1 Hz, 3H), 5.55 (s, 1H), 4.53 (d, *J* = 9.6 Hz, 1H), 4.50 – 4.33 (m, 3H), 3.89 – 3.61 (m, 6H), 3.59 – 3.53 (m, 1H), 3.53 – 3.42 (m, 1H), 2.79 (qd, *J* = 7.4, 2.3 Hz, 2H), 1.34 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.00, 137.01, 129.17, 128.34, 126.00, 101.34, 86.32, 84.19, 80.99, 72.06, 70.37, 68.81, 68.62, 52.37, 24.58, 15.11; $[\alpha]_D^{25}$ -10.23 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1736, 1083, 700 cm^{-1}; m/z (HRMS$^+$) 407.1143 $[M + Na]^+$ ($C_{18}H_{24}O_7SNa^+$ requires 407.1135).

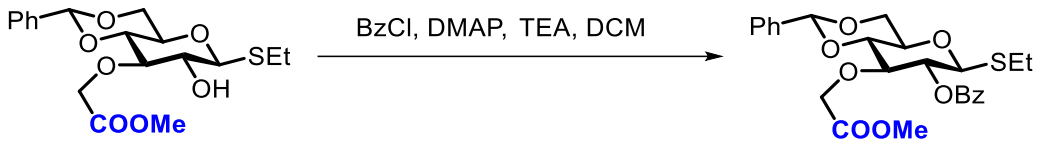
^{1}H NMR of S5 (400 MHz, $CDCl_3$)



^{13}C NMR of S5 (101 MHz, $CDCl_3$)



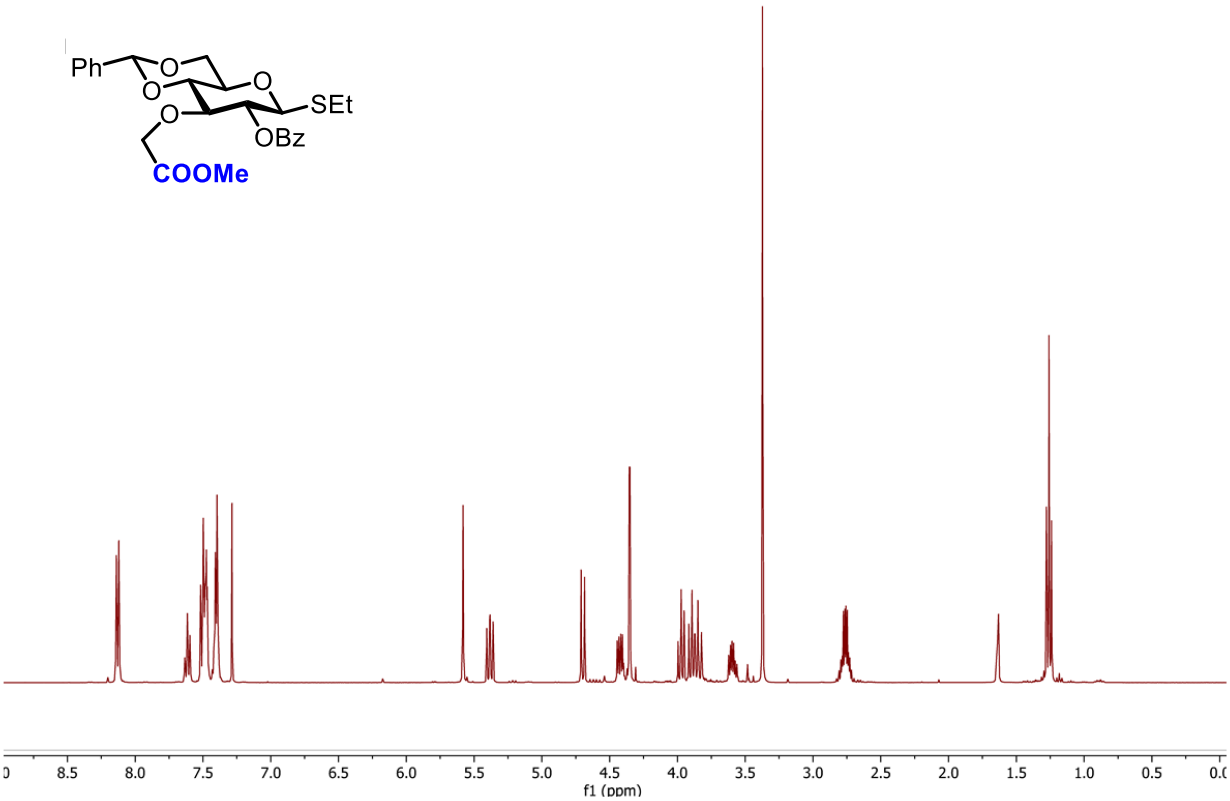
Ethyl 2-*O*-benzoyl-3-*O*-methoxycarbonylmethyl-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside, 8



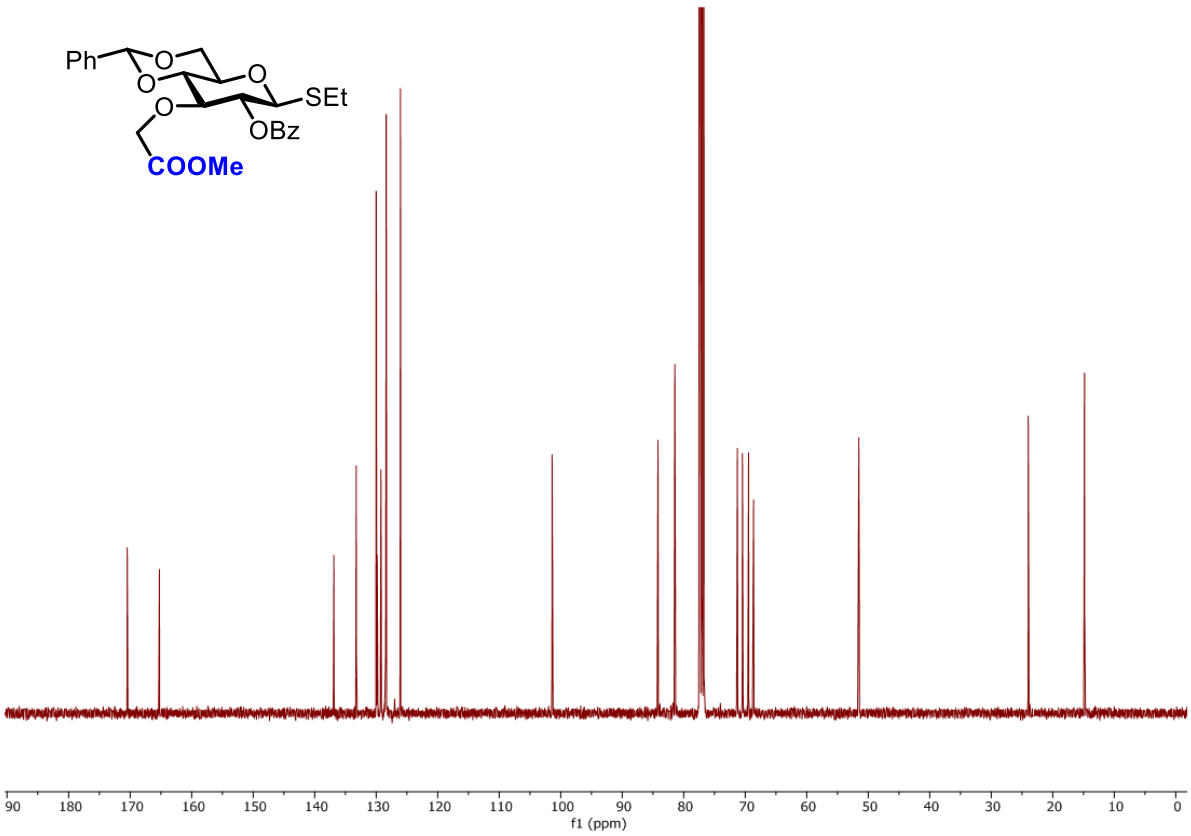
S5 **8**

Ethyl 3-*O*-methoxycarbonylmethyl-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside **S5** (4.00 g, 10.4 mmol) was dissolved in anhydrous DCM (80 mL). TEA (3.8 mL, 26.3 mmol) and 4-dimethylaminopyridine (0.25 g, 2.0 mmol) were added. Benzoyl chloride (1.9 mL, 16.3 mmol) was added dropwise to the solution cooled with an ice bath. After 30 min, the system was allowed to room temperature and the reaction stirred for additional 10 h. The reaction mixture was washed with water (50 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 5:1→3:1) to give **8** as a white solid (3.92 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.09 (m, 2H), 7.66 – 7.57 (m, 1H), 7.54 – 7.44 (m, 4H), 7.40 (dd, *J* = 5.0, 2.0 Hz, 3H), 5.58 (s, 1H), 5.38 (dd, *J* = 10.1, 8.5 Hz, 1H), 4.70 (d, *J* = 10.1 Hz, 1H), 4.43 (dd, *J* = 10.5, 5.0 Hz, 1H), 4.35 (d, *J* = 2.3 Hz, 2H), 3.97 (t, *J* = 8.9 Hz, 1H), 3.89 (t, *J* = 8.3 Hz, 1H), 3.85 (t, *J* = 9.4 Hz, 1H), 3.59 (td, *J* = 9.7, 5.0 Hz, 1H), 3.37 (s, 3H), 2.76 (qd, *J* = 7.5, 4.4 Hz, 2H), 1.26 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.46, 165.22, 136.86, 133.22, 129.96, 129.82, 129.21, 128.42, 128.34, 126.03, 101.38, 84.17, 81.44, 81.42, 71.27, 70.44, 69.45, 68.63, 51.52, 23.98, 14.82; $[\alpha]_D^{25}$ -26.67 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1728, 1267, 1094 cm^{-1}; m/z (HRMS$^+$) 511.1403 $[M + Na]^+$ ($C_{25}H_{28}O_8SNa^+$ requires 511.1397).

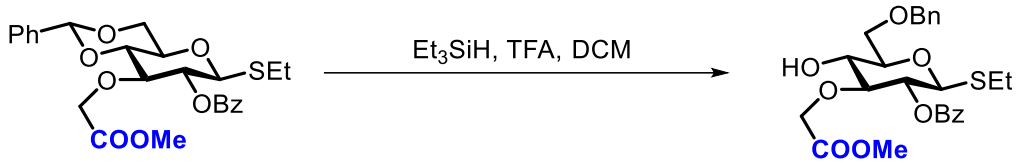
^{1}H NMR of 8 (400 MHz, $CDCl_3$)



^{13}C NMR of 8 (101 MHz, $CDCl_3$)



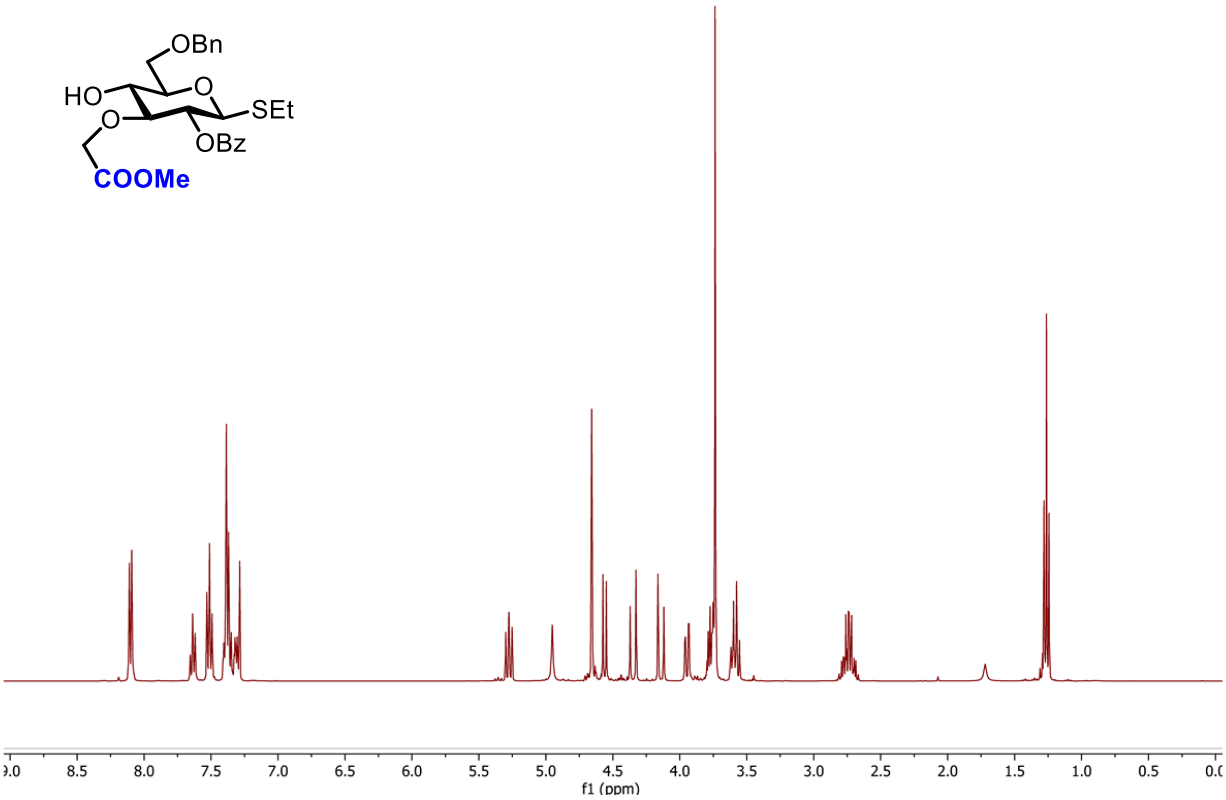
Ethyl 2-*O*-benzoyl-3-*O*-methoxycarbonylmethyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside, S6



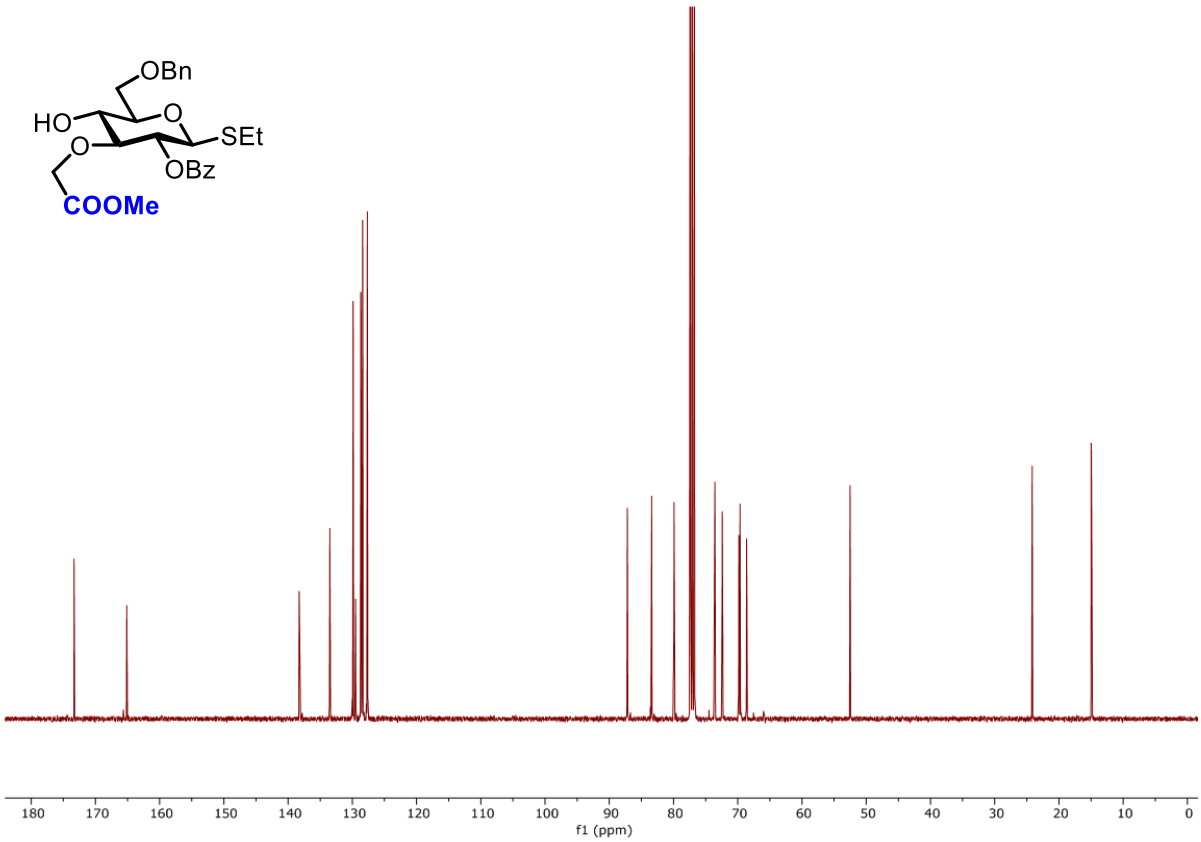
8 **S6**

Ethyl 2-*O*-benzoyl-3-*O*-methoxycarbonylmethyl-4,6-*O*-phenylmethylene-1-thio-β-D-glucopyranoside **8** (3.92 g, 8.0 mmol) was dissolved in anhydrous DCM (70 mL). 4Å molecular sieves were added and the system was charged with nitrogen. Triethylsilane (13 mL, 81.4 mmol) was added and the mixture was stirred at room temperature for 30 min, and then cooled to 0°C. TFA (6.5 mL, 83.2 mmol) was added and the mixture was stirred for 1 h at 0°C. Upon completion, molecular sieves were filtered and the organic phase was washed with saturated $NaHCO_3$ aq. solution (2 x 100 mL) and water (50 mL). The water layers were combined and extracted with DCM (50 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 4:1→3:1) to give **S6** as a colorless oil (3.31 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 – 8.03 (m, 2H), 7.69 – 7.59 (m, 1H), 7.56 – 7.45 (m, 2H), 7.42 – 7.34 (m, 4H), 7.33 – 7.29 (m, 1H), 5.28 (dd, *J* = 10.1, 9.0 Hz, 1H), 4.95 (s, 1H), 4.66 (s, 2H), 4.56 (d, *J* = 10.0 Hz, 1H), 4.35 and 4.14 (ABq, *J* = 17.5 Hz, 2H), 3.95 (dd, *J* = 10.9, 2.2 Hz, 1H), 3.82 – 3.71 (m, 5H), 3.64 – 3.53 (m, 2H), 2.85 – 2.65 (m, 2H), 1.26 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.30, 165.11, 138.26, 133.49, 129.86, 129.47, 128.63, 128.38, 127.66, 127.59, 87.18, 83.39, 79.92, 73.55, 72.41, 69.83, 69.64, 68.62, 52.51, 24.16, 14.96; $[\alpha]_D^{25}$ -32.08 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1728, 1268, 1069 cm^{-1}; m/z (HRMS$^+$) 513.1559 $[M + Na]^+$ ($C_{25}H_{30}O_8SNa^+$ requires 513.1554).

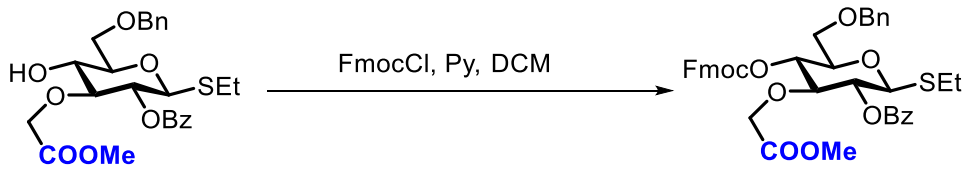
^{1}H NMR of S6 (400 MHz, $CDCl_3$)



^{13}C NMR of S6 (101 MHz, $CDCl_3$)



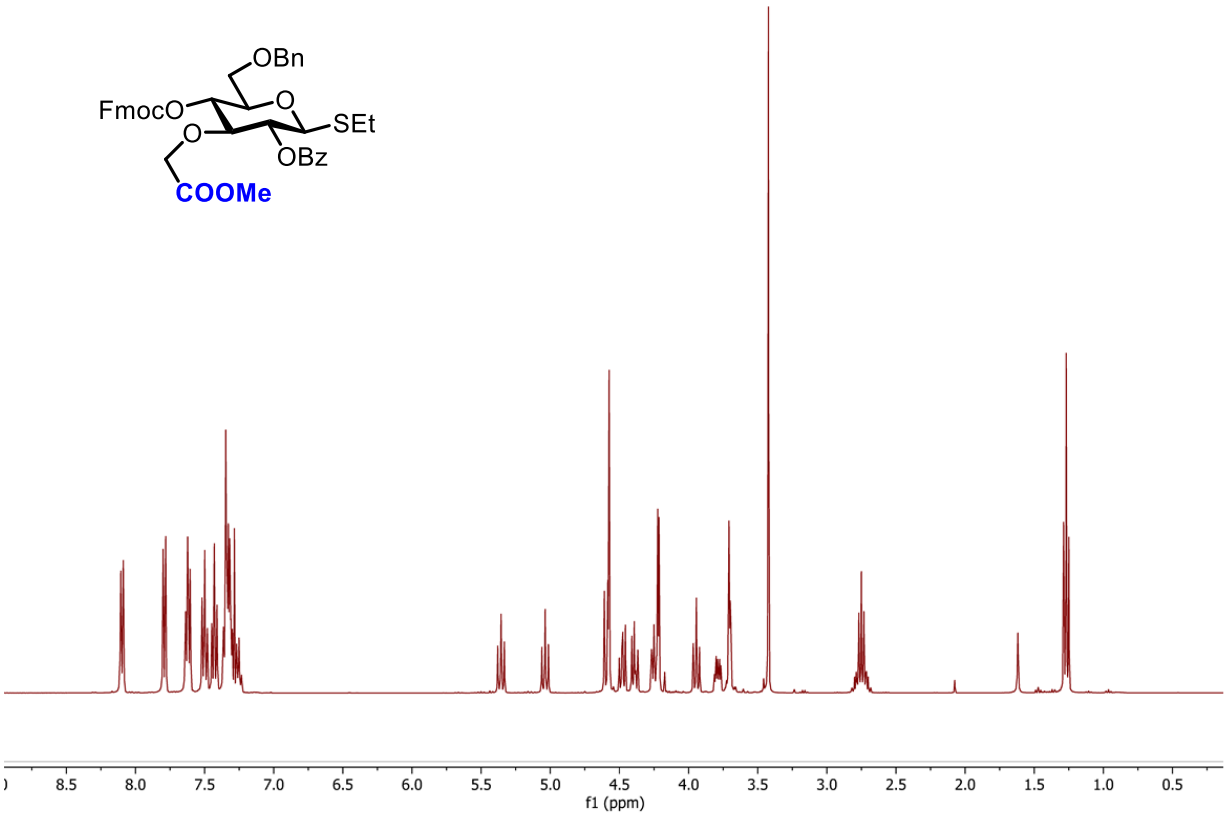
Ethyl 2-*O*-benzoyl-3-*O*-methoxycarbonylmethyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-benzyl-1-thio-β-D-glucopyranoside, BB-3



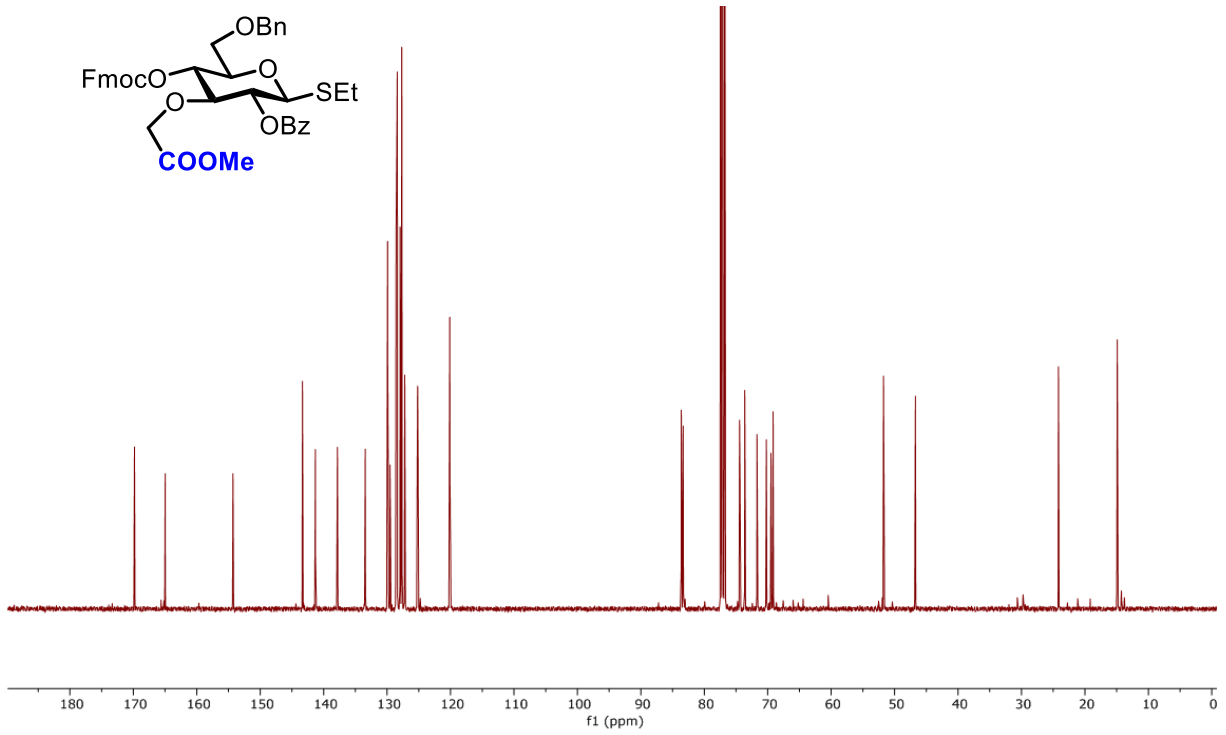
S6 **BB-3**

Ethyl 2-*O*-benzoyl-3-*O*-methoxycarbonylmethyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside **S6** (3.31 g, 6.7 mmol) was dissolved in anhydrous DCM (50 mL) and pyridine (2 mL, 24.8 mmol) was added. The solution was cooled with an ice bath for 15 min, after which time fluorenylmethyloxycarbonyl chloride (3.47 g, 13.4 mmol) was added slowly. The reaction was allowed to warm to room temperature and stirred for 6 h. After the reaction was finished, DCM (50 mL) was added and the organic phase was washed with aqueous citric acid (50 mL). After extracting the water phase with DCM (50 mL), the organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography. (Hexane : EtOAc = 5:1→3:1) to give **BB-3** as a white solid (3.80 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.97 (m, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.59 – 7.50 (m, 3H), 7.40 (dd, *J* = 8.4, 7.1 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.27 – 7.20 (m, 6H), 7.18 – 7.12 (m, 1H), 5.30 – 5.22 (m, 1H), 4.94 (t, *J* = 9.6 Hz, 1H), 4.50 (d, *J* = 9.9 Hz, 1H), 4.48 (s, 2H), 4.38 (dd, *J* = 10.4, 7.3 Hz, 1H), 4.29 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.19 – 4.09 (m, 3H), 3.85 (t, *J* = 9.2 Hz, 1H), 3.69 (ddd, *J* = 9.6, 5.1, 3.7 Hz, 1H), 3.65 – 3.57 (m, 2H), 3.33 (s, 3H), 2.65 (p, *J* = 7.4 Hz, 2H), 1.17 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.81, 164.96, 154.28, 143.33, 141.30 (d, *J* = 1.5 Hz), 137.81, 133.44, 129.90, 129.52, 128.55, 128.38, 127.92, 127.70, 127.67, 127.21, 127.18, 125.17, 125.15, 120.10, 83.61, 83.32, 74.43, 73.62, 71.68, 70.20, 69.48, 69.14, 51.74, 46.72, 24.16, 14.90; $[\alpha]_D^{25}$ 10.18 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1756, 1248, 1070 cm^{-1}; m/z (HRMS$^+$) 735.2244 $[M + Na]^+$ ($C_{40}H_{40}O_{10}SNa^+$ requires 735.2234).

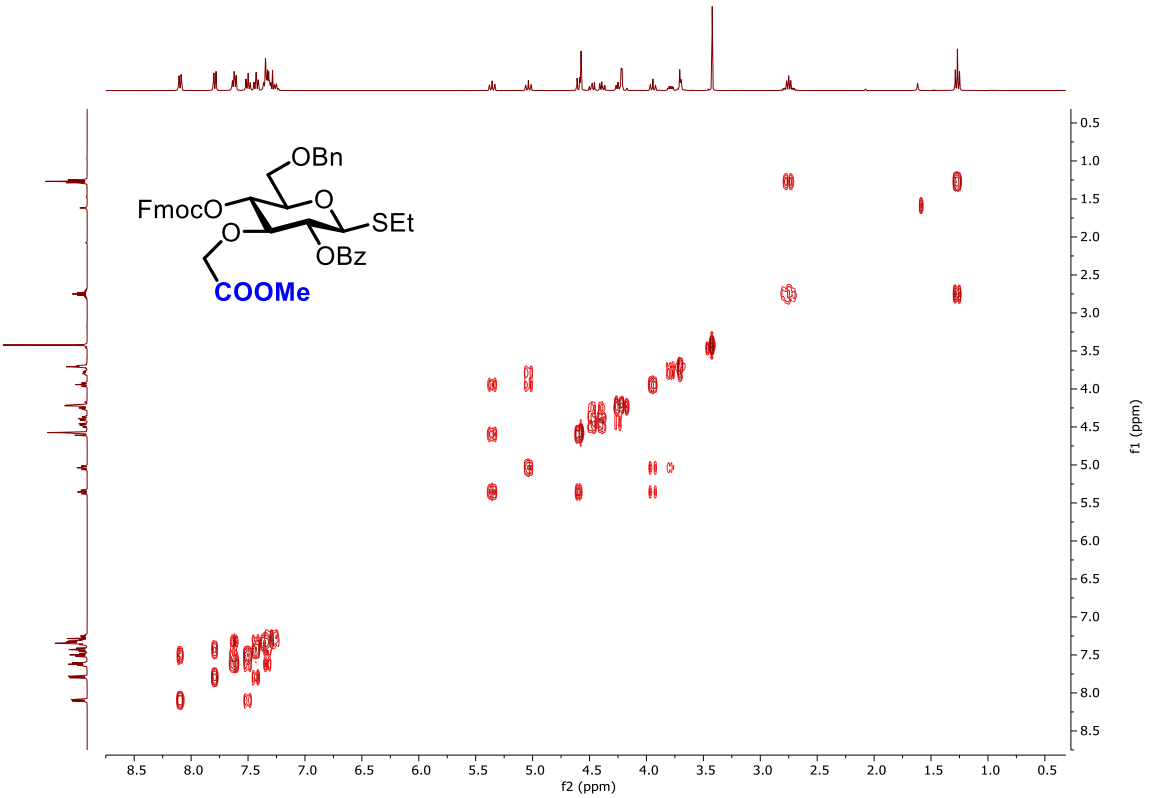
^{1}H NMR of BB-3 (400 MHz, $CDCl_3$)



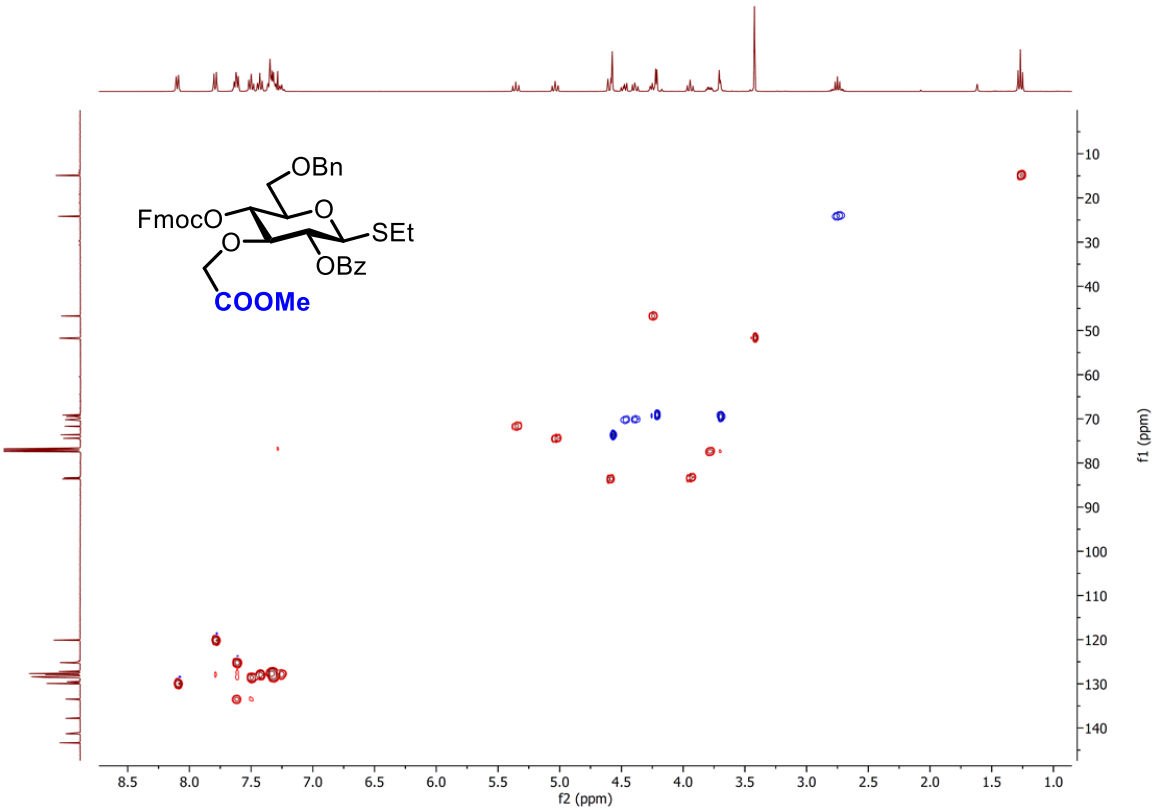
^{13}C NMR of BB-3 (101 MHz, $CDCl_3$)



COSY NMR of BB-3 ($CDCl_3$)

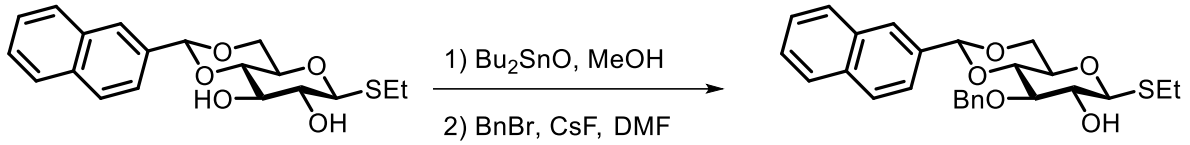


HSQC NMR of BB-3 ($CDCl_3$)



2.4. Synthesis of BB-4

Ethyl 3-*O*-benzyl-4,6-*O*-naphthylmethylene-1-thio-β-D-glucopyranoside, S7

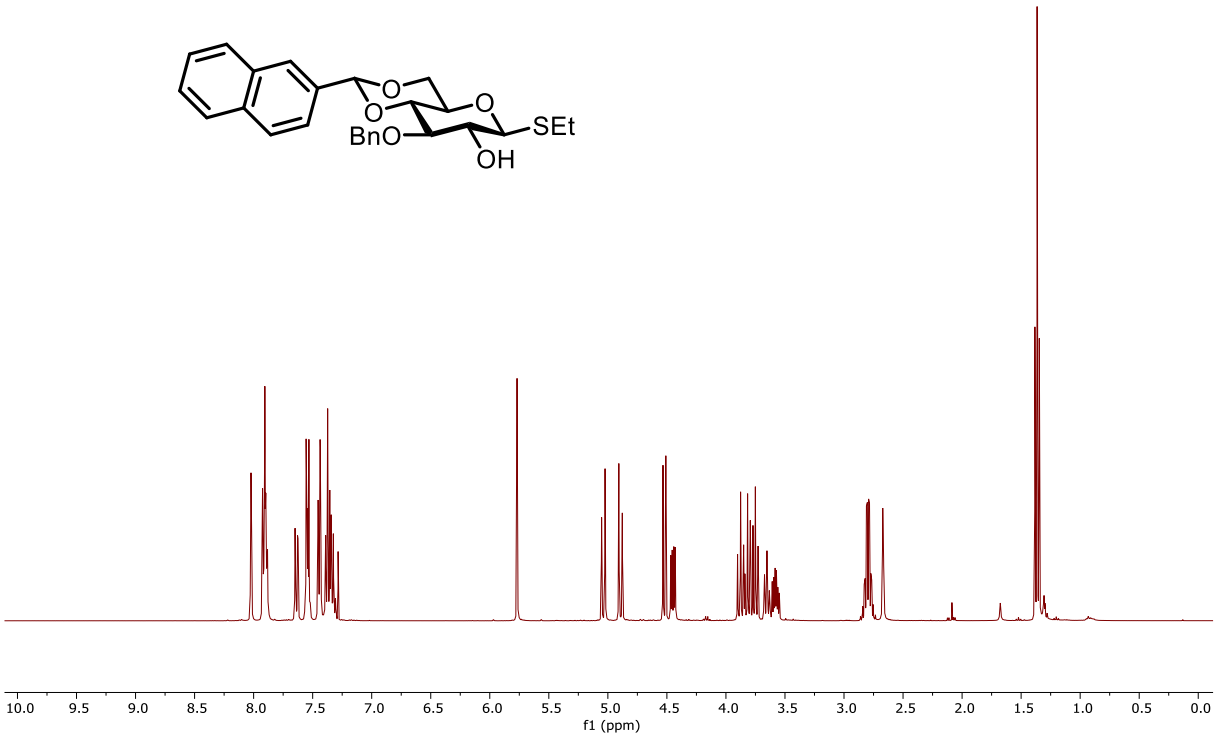


5 **S7**

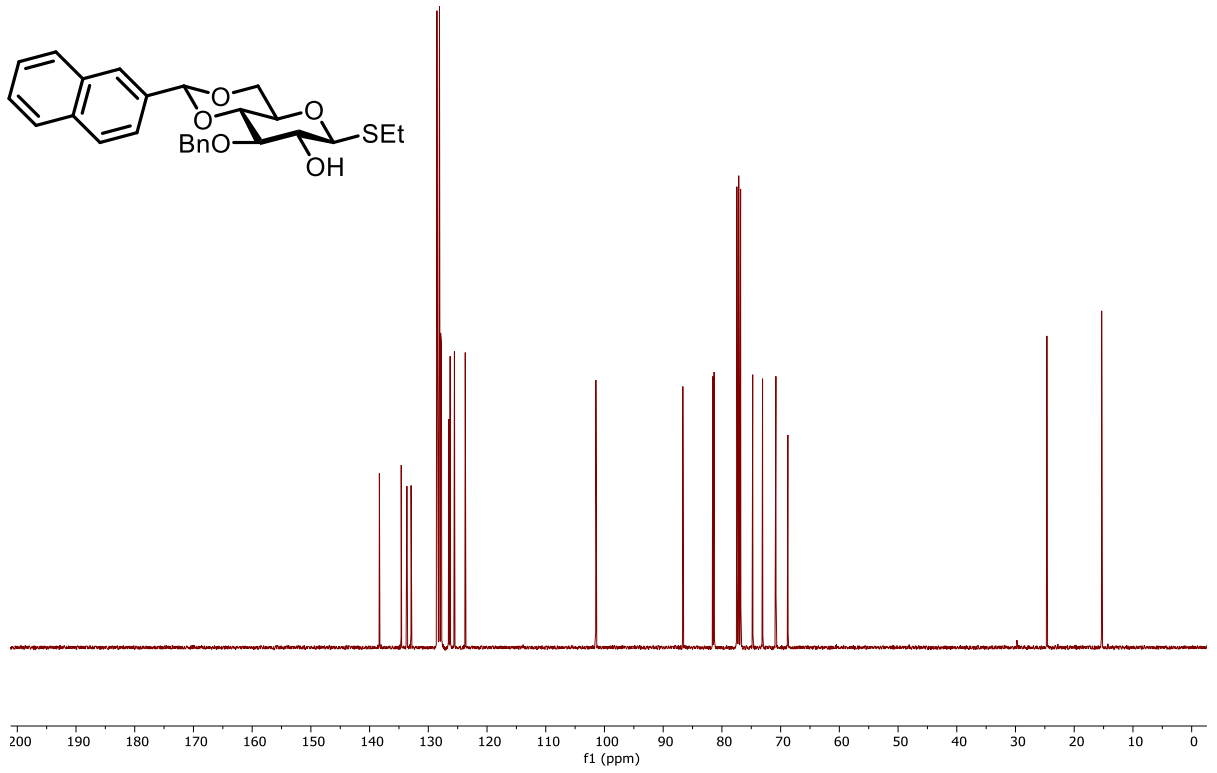
5 was prepared according to previously established procedures. [5]

Ethyl 4,6-*O*-naphthylmethylene-1-thio-β-D-glucopyranoside **5** (5.00 g, 13.8 mmol) was dissolved in MeOH (150 mL). Dibutyltin oxide (4.15 g, 16.7 mmol) was added. The reaction mixture was charged with nitrogen and refluxed for 18 h. The solvent was removed under reduced pressure and DMF (100 mL) was added. Cesium fluoride (3.15 g, 20.7 mmol) and benzyl bromide (2.2 mL, 18.0 mmol) were added, and the reaction was stirred at room temperature for 24 h. DMF was removed under reduced pressure and DCM (100 mL) was added. KF aq. solution (1M, 100 mL) was used to wash the organic layer. The water layer was extracted with additional DCM (50 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 4:1→ Hexane : EtOAc :DCM = 3:1:1) to give **S7** as a white solid (4.50 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.95 – 7.85 (m, 3H), 7.64 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.58 – 7.50 (m, 2H), 7.47 – 7.42 (m, 2H), 7.41 – 7.30 (m, 3H), 5.77 (s, 1H), 5.04 and 4.89 (ABq, *J* = 11.7 Hz, 2H), 4.52 (d, *J* = 9.6 Hz, 1H), 4.45 (dd, *J* = 10.4, 5.0 Hz, 1H), 3.88 (t, *J* = 10.3 Hz, 1H), 3.82 (t, *J* = 9.1 Hz, 1H), 3.77 – 3.73 (m, 1H), 3.65 (ddd, *J* = 9.8, 8.0, 1.7 Hz, 1H), 3.58 (ddd, *J* = 10.1, 9.0, 5.0 Hz, 1H), 2.80 (qd, *J* = 7.5, 3.2 Hz, 2H), 2.67 (s, 1H), 1.36 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.33, 134.62, 133.65, 132.94, 128.53, 128.45, 128.18, 128.10, 127.91, 127.77, 126.53, 126.28, 125.55, 123.71, 101.46, 86.66, 81.57, 81.33, 74.76, 73.08, 70.80, 68.77, 24.66, 15.33; $[\alpha]_D^{25}$ -52.18 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1062, 742, 697 cm^{-1}; m/z (HRMS$^+$) 475.1554 $[M + Na]^+$ ($C_{26}H_{28}O_5SNa^+$ requires 475.1550).

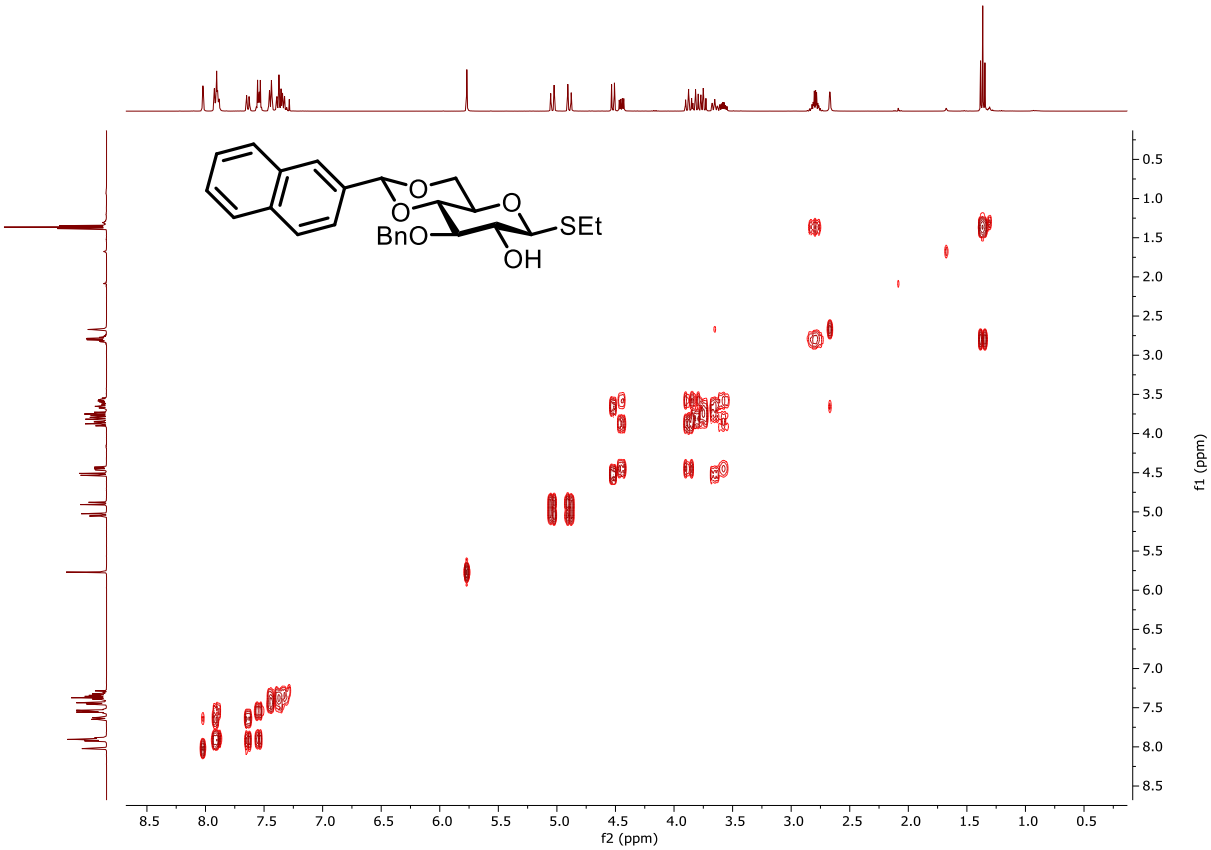
^{1}H NMR of S7 (400 MHz, $CDCl_3$)



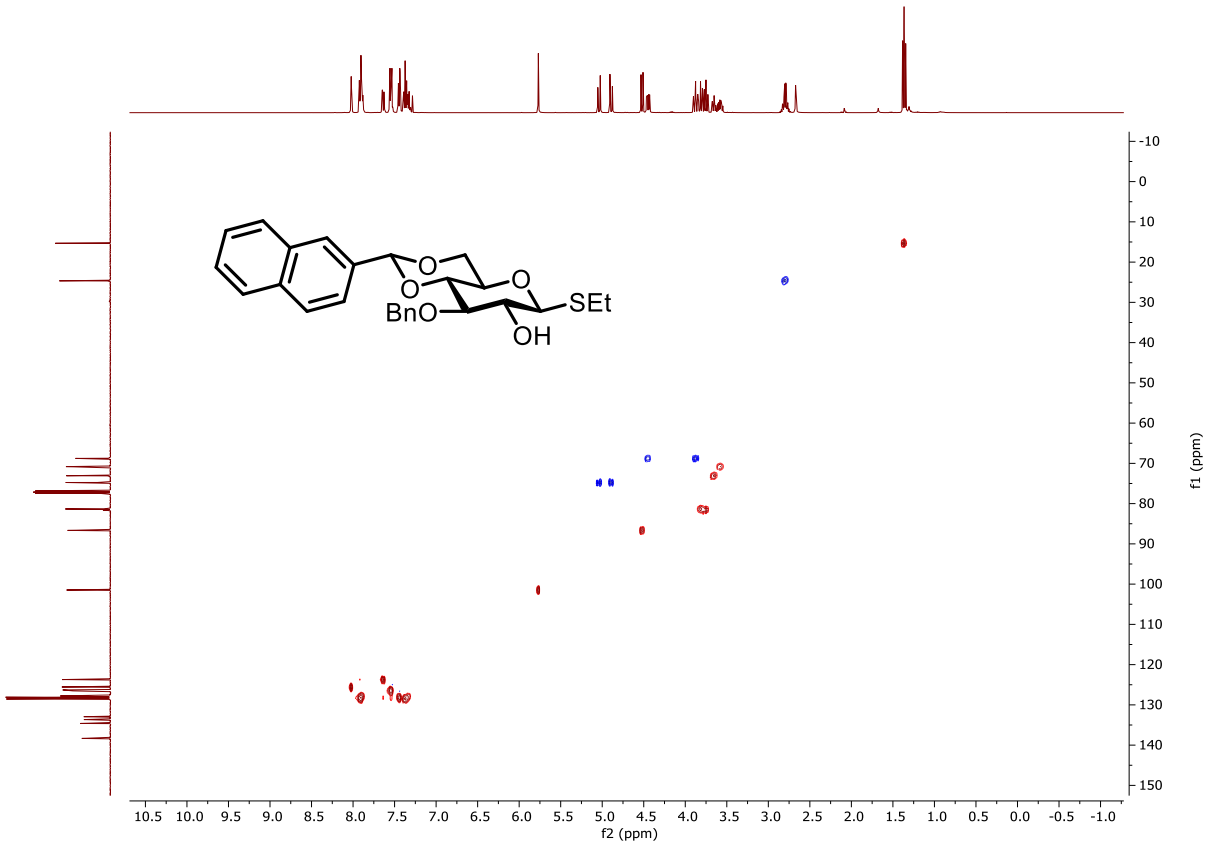
^{13}C NMR of S7 (101 MHz, $CDCl_3$)



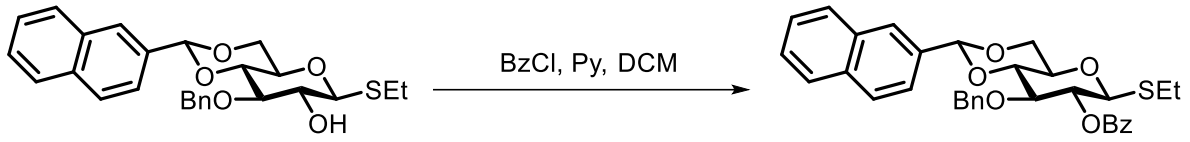
COSY NMR of S7 ($CDCl_3$)



HSQC NMR of S7 ($CDCl_3$)



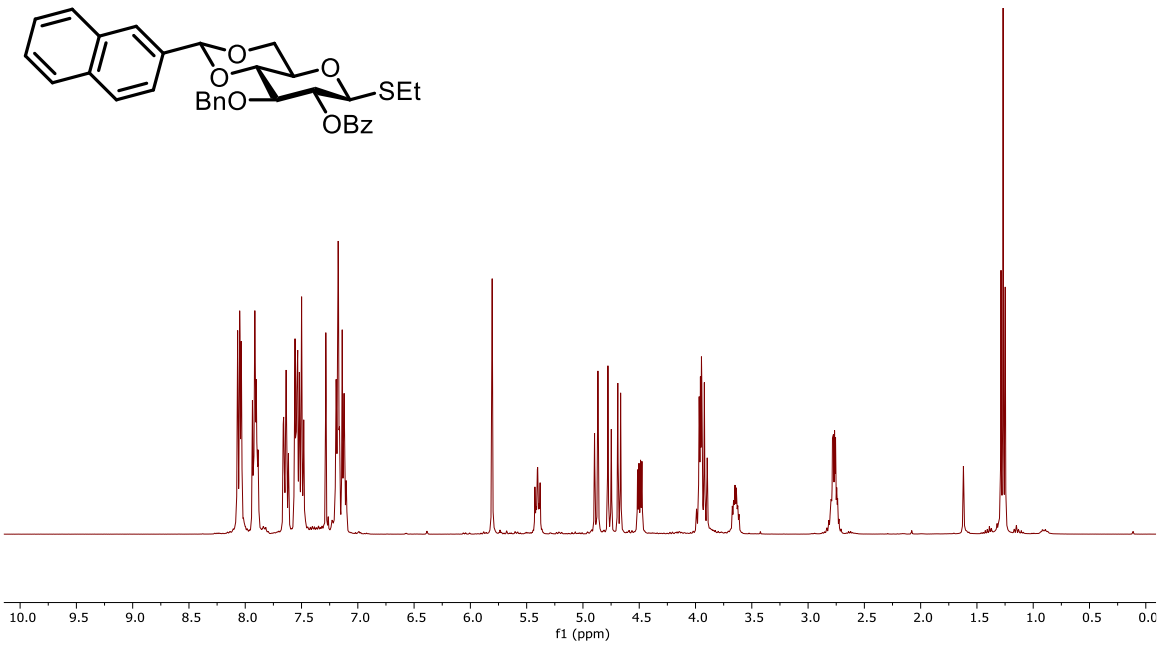
Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-naphthylmethylene-1-thio-β-D-glucopyranoside, 9



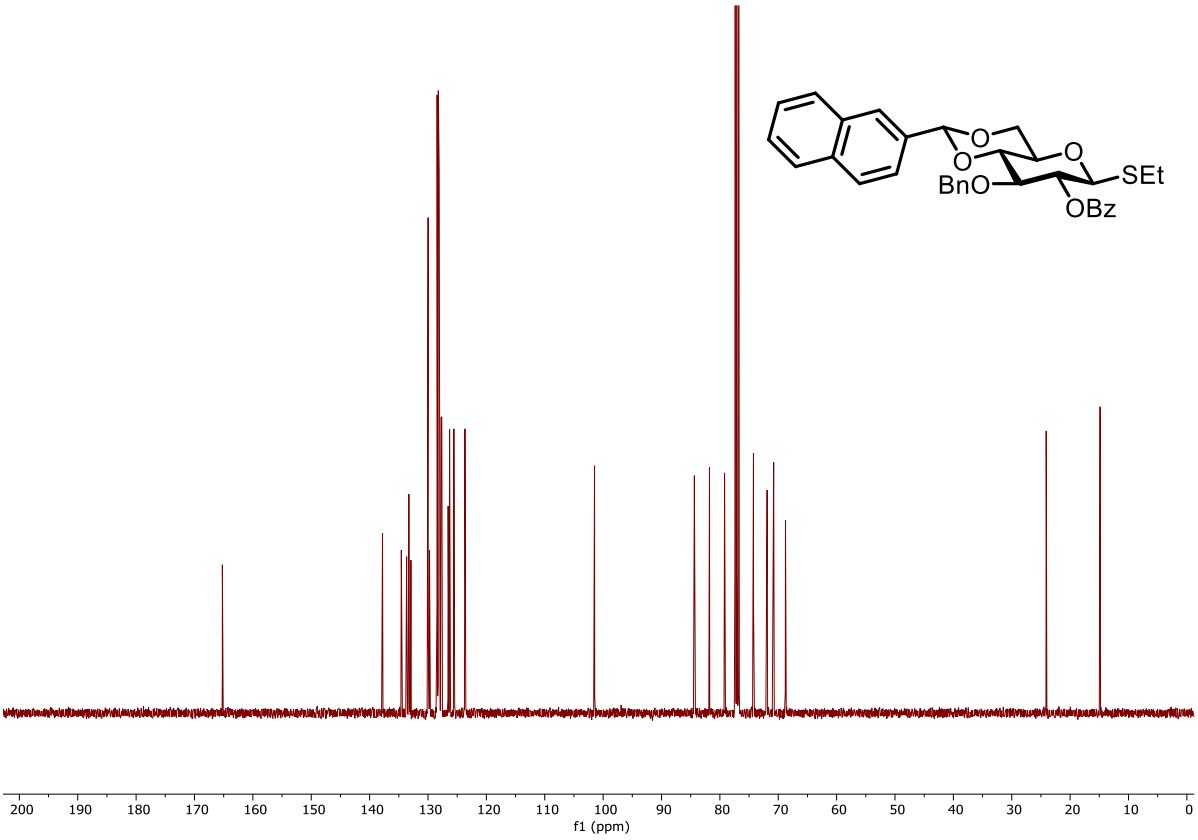
S7 **9**

Ethyl 3-*O*-benzyl-4,6-*O*-naphthylmethylene-1-thio-β-D-glucopyranoside **S7** (4.48 g, 9.9 mmol) was dissolved in anhydrous DCM (50 mL). Pyridine (10 mL) was added. Benzoyl chloride (2.3 mL, 19.8 mmol) was added dropwise to the solution cooled with an ice bath. After 30 min, the system was allowed to room temperature and the reaction stirred for additional 6 h until completion. The reaction mixture was washed with citric acid (0.5 M in water, 2 x 200 mL), and the water layer was extracted with additional DCM (50 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→ Hexane : EtOAc :DCM = 5:1:1) to give **9** as a white solid (5.01 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.01 (m, 3H), 7.96 – 7.87 (m, 3H), 7.68 – 7.61 (m, 2H), 7.58 – 7.47 (m, 4H), 7.21 – 7.15 (m, 3H), 7.15 – 7.10 (m, 2H), 5.81 (s, 1H), 5.45 – 5.35 (m, 1H), 4.88 and 4.76 (ABq, *J* = 12.0 Hz, 2H), 4.68 (d, *J* = 10.1 Hz, 1H), 4.50 (dd, *J* = 10.5, 5.0 Hz, 1H), 4.00 – 3.87 (m, 3H), 3.71 – 3.52 (m, 1H), 2.76 (qt, *J* = 8.2, 4.1 Hz, 2H), 1.27 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.22, 137.79, 134.57, 133.66, 133.28, 132.93, 129.98, 129.74, 128.44, 128.23, 128.20, 128.09, 127.76, 127.64, 126.55, 126.30, 125.56, 123.67, 101.47, 84.37, 81.78, 79.20, 74.24, 71.90, 70.78, 68.76, 24.10, 14.87; $[\alpha]_D^{25}$ 6.56 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1728, 1269, 1094 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 579.1808 ($C_{33}H_{32}O_6SNa^+$ requires 579.1812).

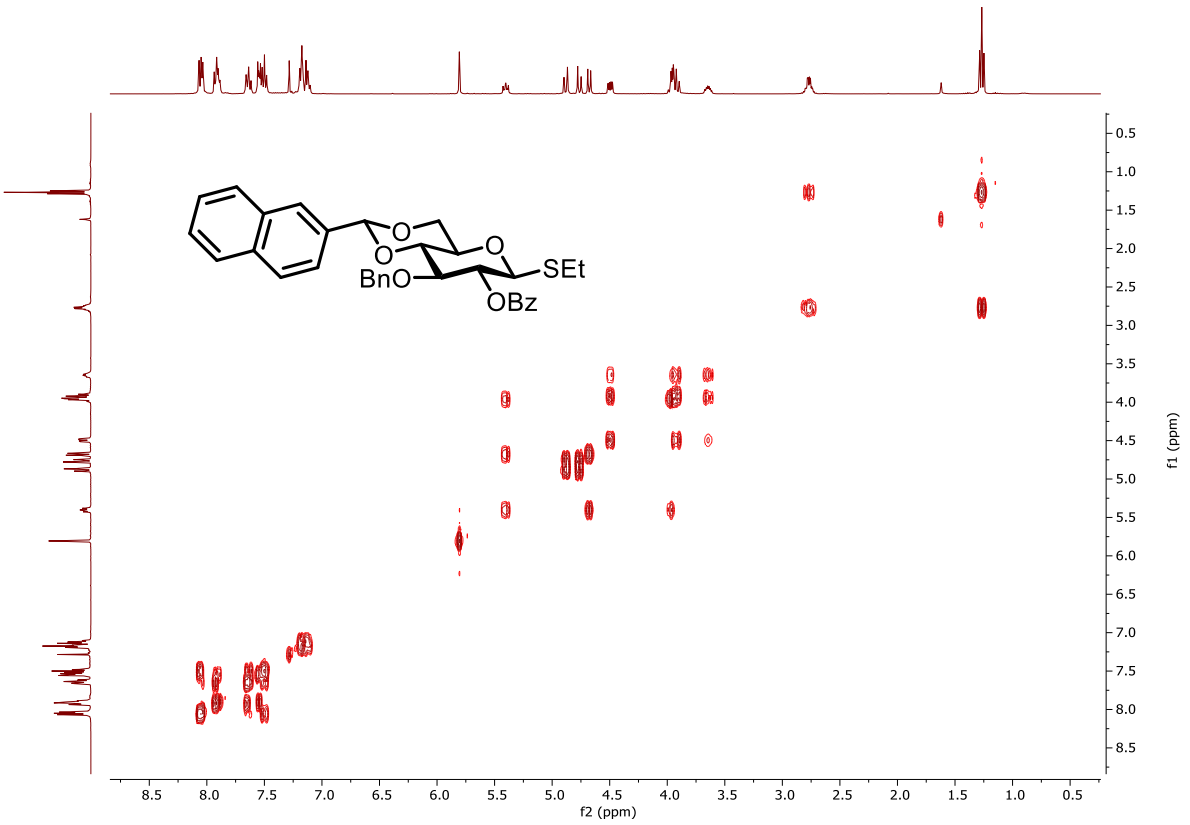
^{1}H NMR of 9 (400 MHz, $CDCl_3$)



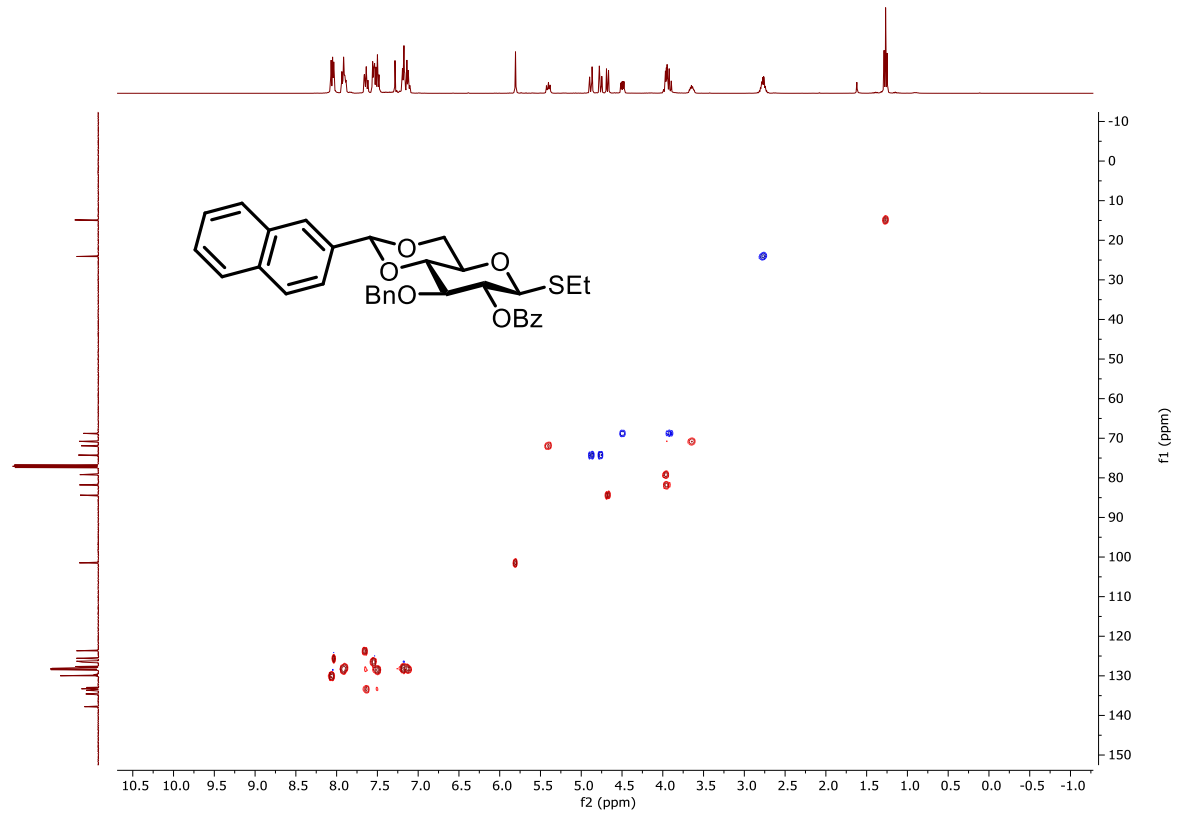
^{13}C NMR of 9 (101 MHz, $CDCl_3$)



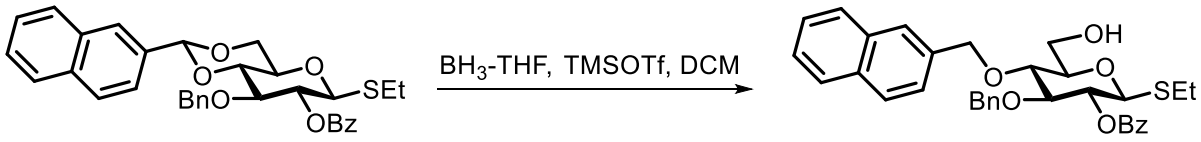
COSY NMR of 9 ($CDCl_3$)



HSQC NMR of 9 ($CDCl_3$)



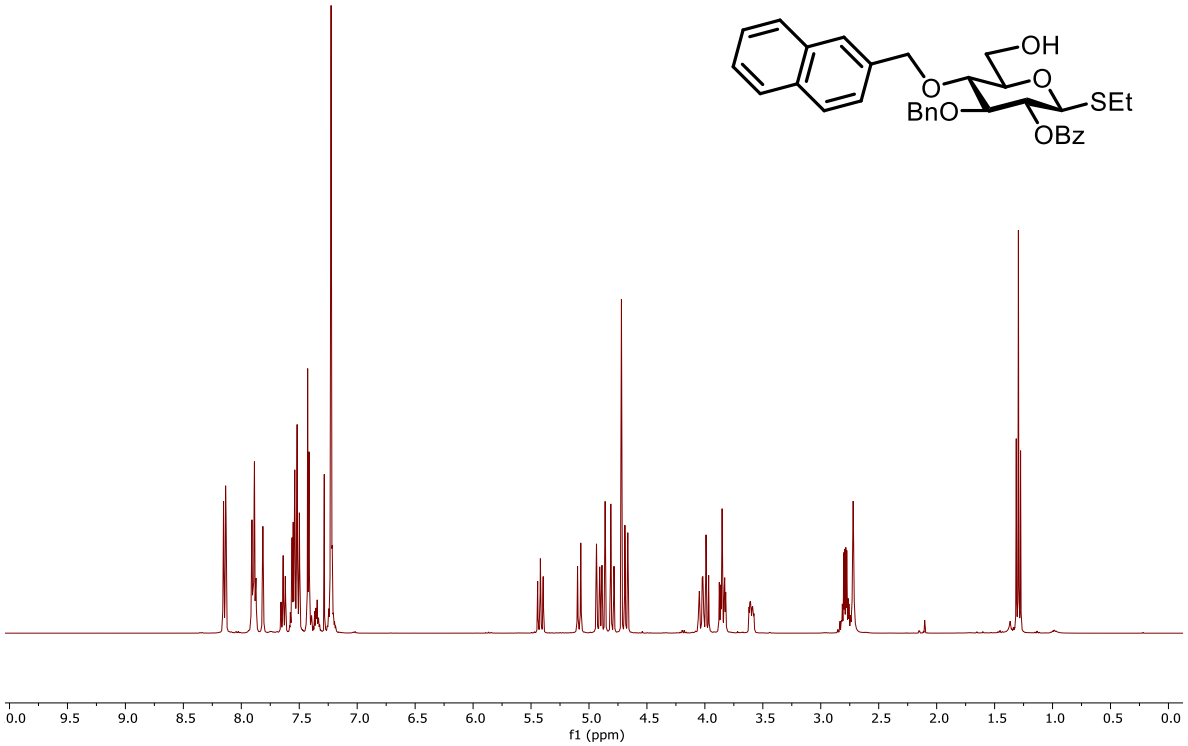
Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-naphthyl)methyl-1-thio-β-D-glucopyranoside, S8



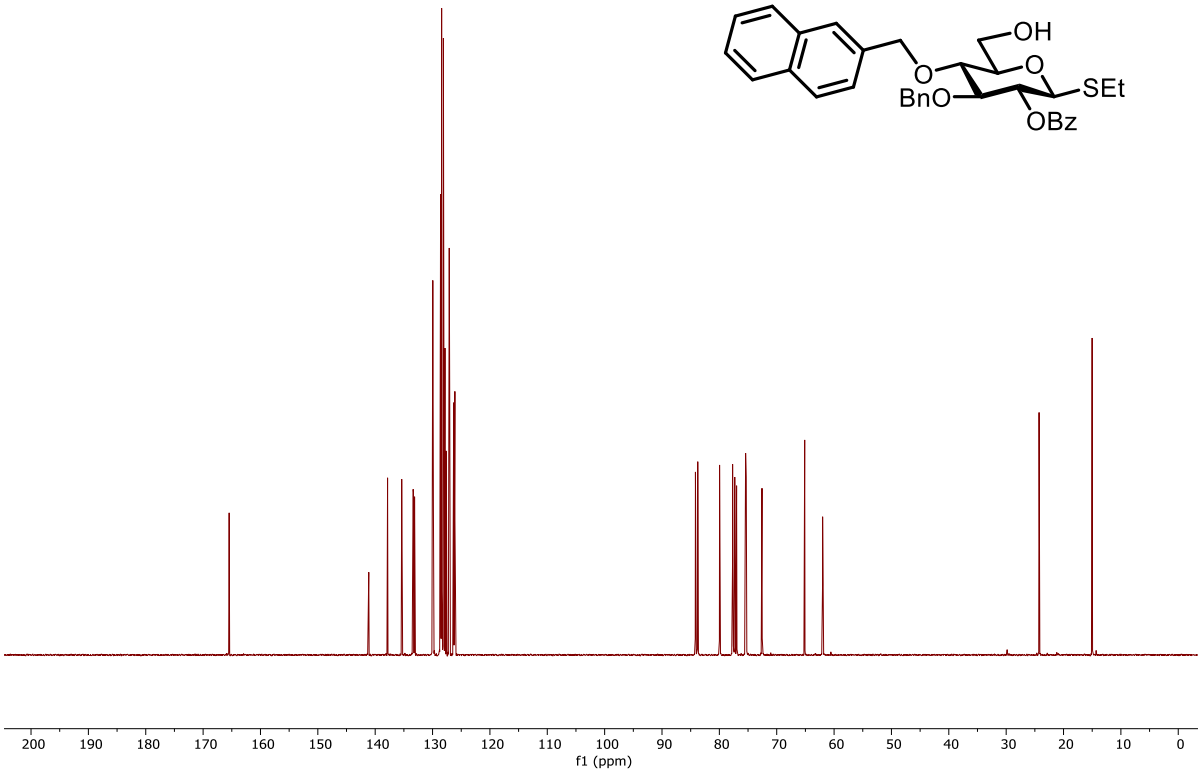
9 **S8**

Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-naphthylmethylene-1-thio-β-D-glucopyranoside **9** (5.00 g, 9.0 mmol) was dissolved in anhydrous DCM (50 mL). Borane-tetrahydrofuran (BH_3-THF) complex (1.0 M in THF, 18.0 mL, 18.0 mmol) was added under argon atmosphere. Trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.24 mL, 1.35 mmol) was added dropwise to the solution. The reaction was kept at room temperature for around 3 h. Upon completion, the reaction was carefully quenched (ice bath) by addition of saturated aq. $NaHCO_3$ solution (100 mL). The water layer was extracted with DCM (50 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→ Hexane : EtOAc = 3:1) to give **S8** as a white solid (4.72 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (m, 2H), 7.93 – 7.86 (m, 3H), 7.81 (d, *J* = 1.7 Hz, 1H), 7.66 – 7.61 (m, 1H), 7.57 – 7.49 (m, 5H), 7.25 – 7.20 (m, 5H), 5.42 (dd, *J* = 10.1, 9.0 Hz, 1H), 5.08 and 4.92 (ABq, *J* = 11.1 Hz, 2H), 4.87 and 4.80 (ABq, *J* = 11.1 Hz, 2H), 4.68 (d, *J* = 10.1 Hz, 1H), 4.06 – 4.00 (m, 1H), 3.98 (d, *J* = 9.1 Hz, 1H), 3.89 – 3.81 (m, 2H), 3.60 (ddd, *J* = 9.7, 4.8, 2.4 Hz, 1H), 2.79 (qd, *J* = 7.5, 3.3 Hz, 2H), 2.72 (s, 1H), 1.29 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.46, 137.84, 135.37, 133.39, 133.35, 133.14, 129.96, 129.85, 128.60, 128.58, 128.42, 128.11, 128.09, 127.84, 127.83, 127.60, 127.09, 127.01, 126.32, 126.18, 126.11, 84.17, 83.76, 79.96, 77.69, 75.42, 75.31, 72.57, 62.00, 24.26, 15.01; $[\alpha]_D^{25}$ 9.52 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1725, 1267, 1068 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 581.1971 ($C_{33}H_{34}O_6SNa^+$ requires 581.1968).

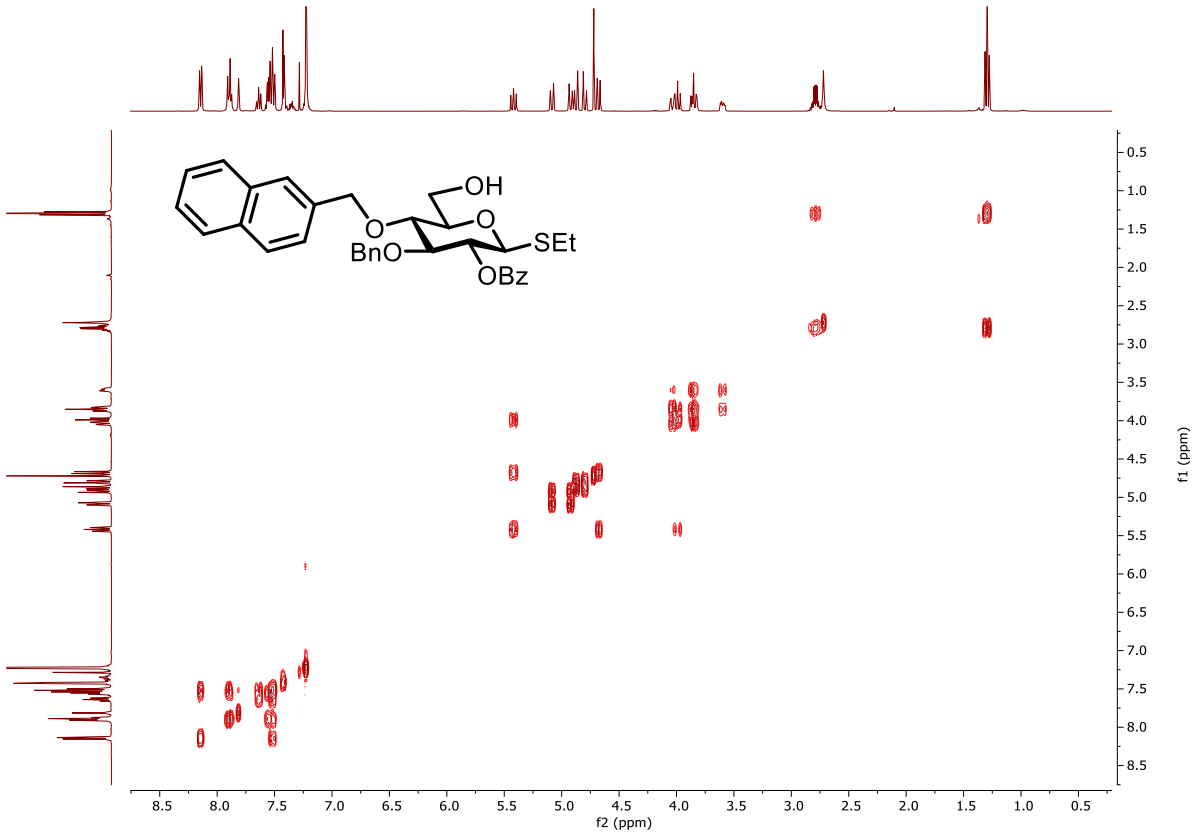
^{1}H NMR of S8 (400 MHz, $CDCl_3$)



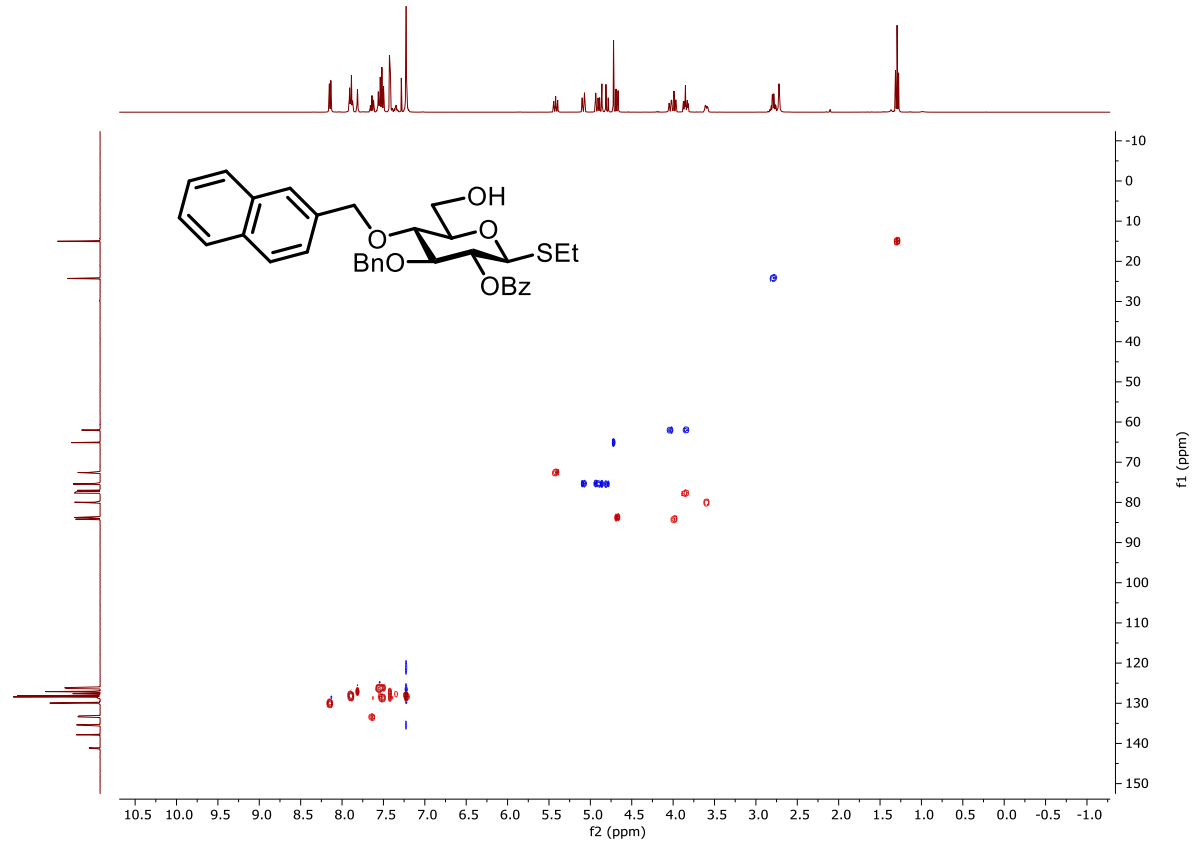
^{13}C NMR of S8 (101 MHz, $CDCl_3$)



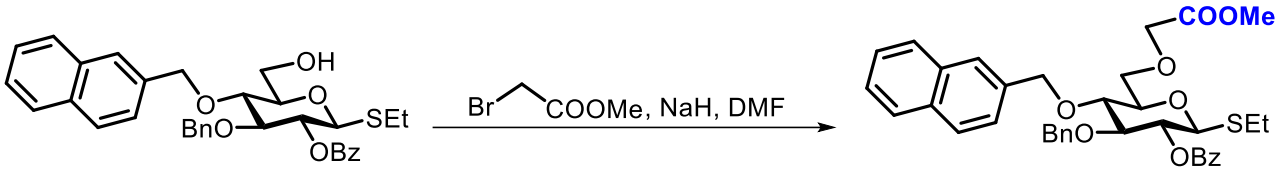
COSY NMR of S8 ($CDCl_3$)



HSQC NMR of S8 ($CDCl_3$)



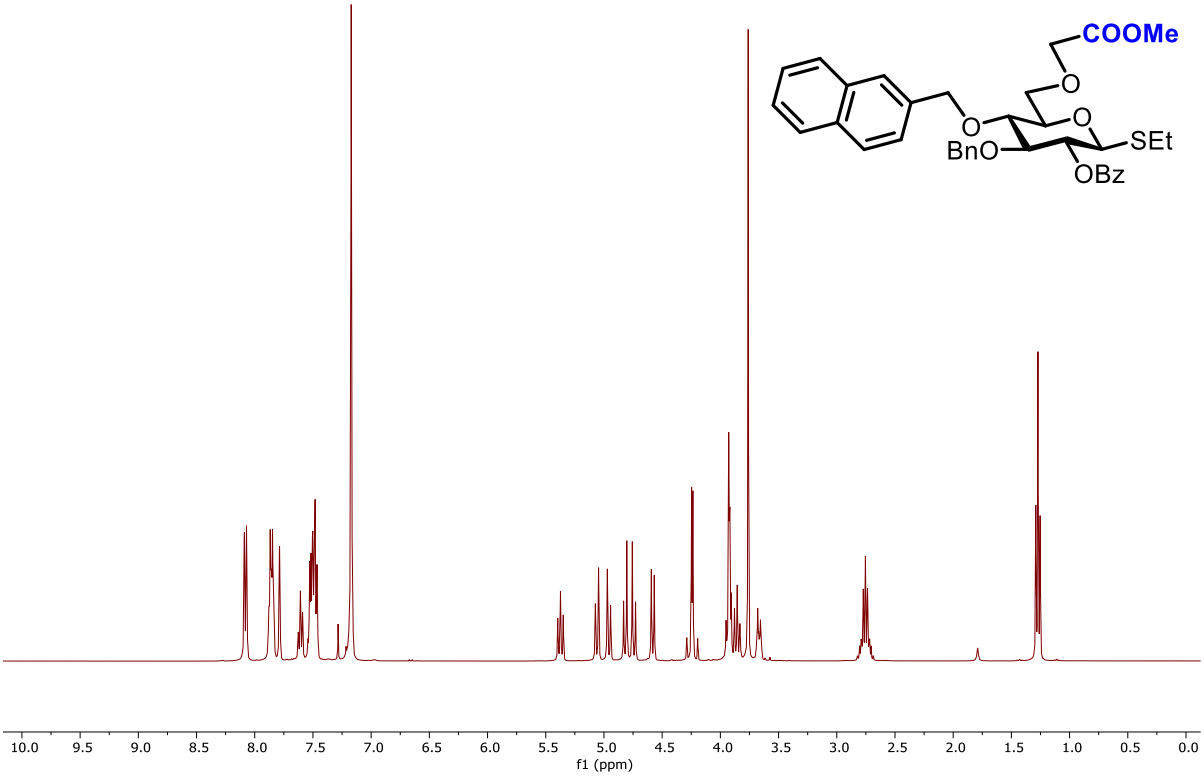
Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-naphthyl)methyl-6-*O*-methoxycarbonylmethyl-1-thio-β-D-glucopyranoside, S9



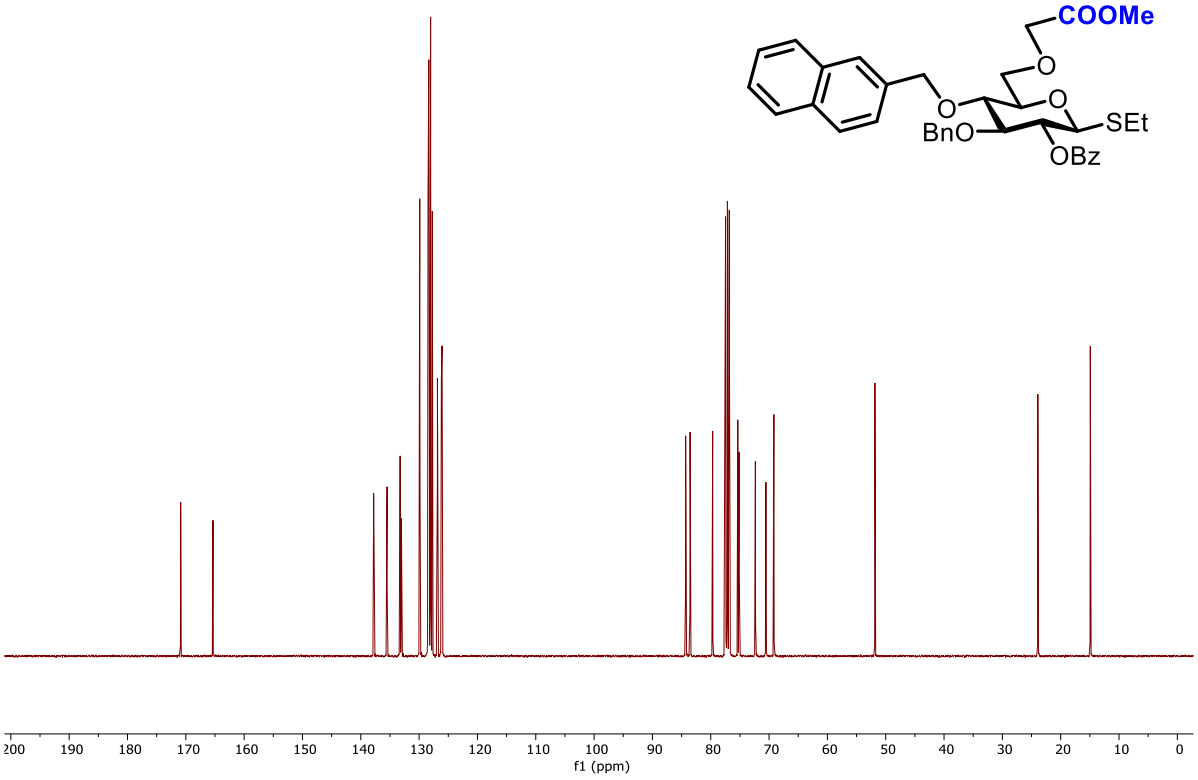
S8 **S9**

Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-naphthyl)methyl-1-thio-β-D-glucopyranoside **S8** (4.7 g, 8.4 mmol) was dissolved in anhydrous DMF (45 mL), and methyl 2-bromoacetate (2.4 mL, 25.6 mmol) was added. 4Å molecular sieves were added to the solution that was stirred for 30 min at room temperature. After this time, the mixture was cooled to 0°C and sodium hydride (60% dispersion in mineral oil, 840 mg, 21.0 mmol) was added. The reaction was allowed to room temperature, and stirred for additional 2 h. After this time, a second addition of methyl 2-bromoacetate (2.4 mL, 25.6 mmol) followed by sodium hydride (60% dispersion in mineral oil, 840 mg, 21.0 mmol) was performed at 0°C and . After further 2 h incubation at room temperature, the reaction was cooled with an ice bath and carefully quenched with citric acid solution (0.5 M in water, 200 mL). Ethyl acetate (200 mL) was added and the organic layer separated. The aqueous phase was extracted with ethyl acetate (50 mL), the organic layers were combined, washed with water (50 mL), and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→ Hexane : EtOAc = 4:1) to give **S9** as a white solid (2.82 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.03 (m, 2H), 7.85 (dd, *J* = 8.0, 4.4 Hz, 3H), 7.79 (s, 1H), 7.64 – 7.57 (m, 1H), 7.55 – 7.45 (m, 5H), 7.17 (s, 5H), 5.37 (dd, *J* = 10.0, 8.9 Hz, 1H), 5.06 and 4.96 (ABq, *J* = 11.1 Hz, 2H), 4.82 and 4.74 (ABq, *J* = 11.0 Hz, 2H), 4.58 (d, *J* = 10.0 Hz, 1H), 4.31 – 4.14 (m, 2H), 3.97 – 3.90 (m, 3H), 3.86 (t, *J* = 9.3 Hz, 1H), 3.76 (s, 3H), 3.67 (dt, *J* = 9.7, 3.3 Hz, 1H), 2.75 (p, *J* = 7.3 Hz, 2H), 1.27 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.88, 165.34, 137.79, 135.49, 133.30, 133.25, 133.04, 129.89, 129.85, 128.48, 128.33, 128.25, 128.03, 128.01, 127.74, 126.86, 126.19, 126.07, 126.05, 84.30, 83.55, 79.67, 77.59, 75.36, 75.14, 72.35, 70.54, 69.17, 51.86, 23.92, 14.93; $[\alpha]_D^{25}$ 20.08 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1727, 1268, 1090 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 653.2178 ($C_{36}H_{38}O_8SNa^+$ requires 653.2180).

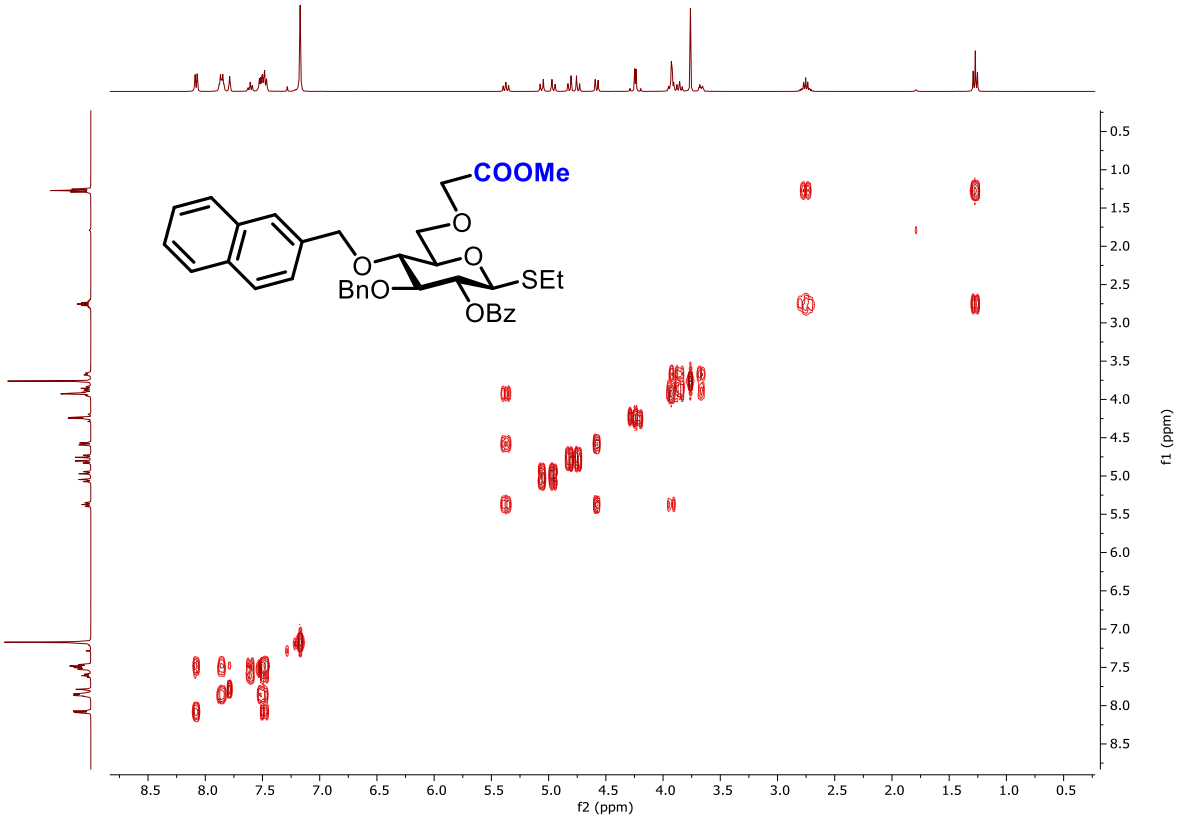
^{1}H NMR of S9 (400 MHz, $CDCl_3$)



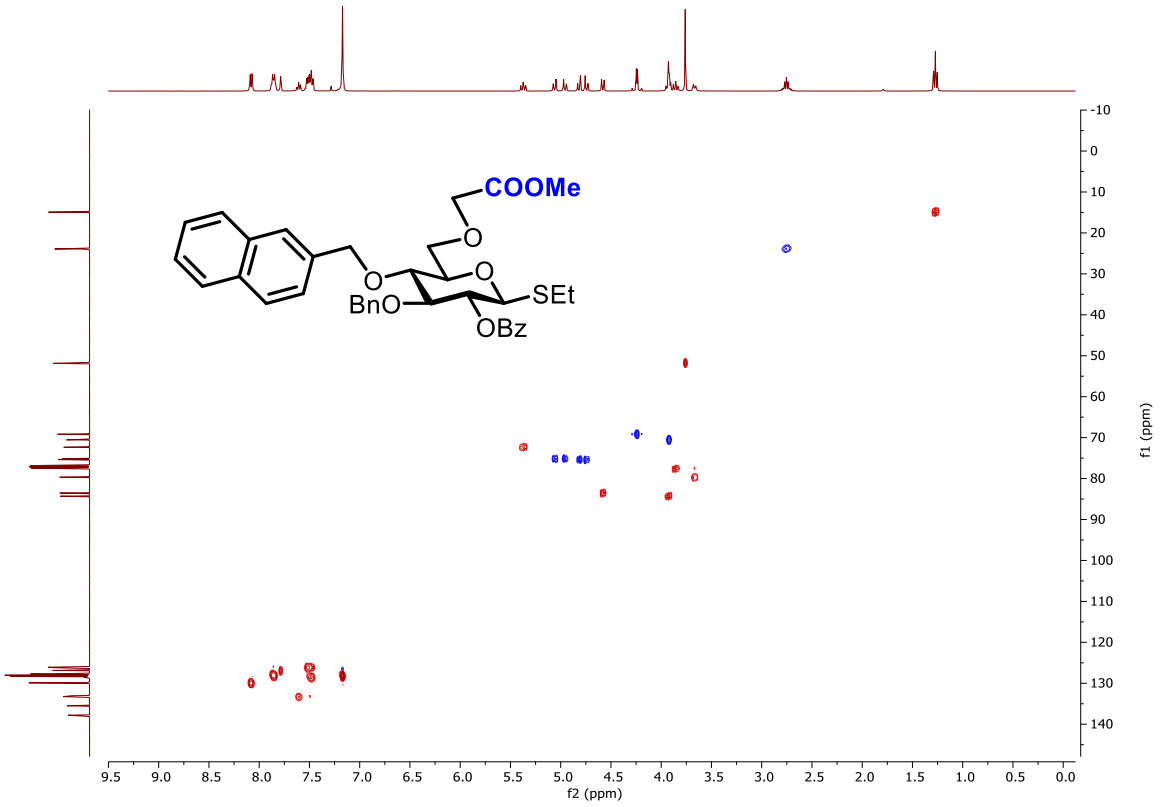
^{13}C NMR of S9 (101 MHz, $CDCl_3$)



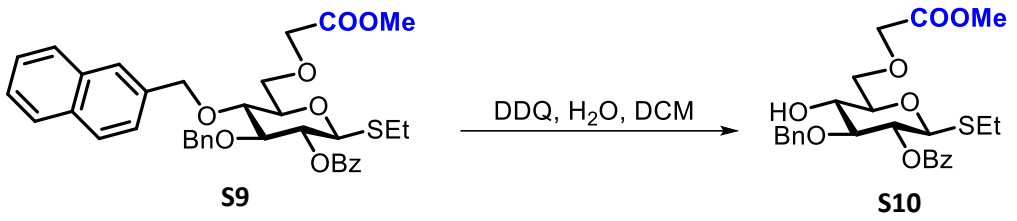
COSY NMR of S9 ($CDCl_3$)



HSQC NMR of S9 ($CDCl_3$)



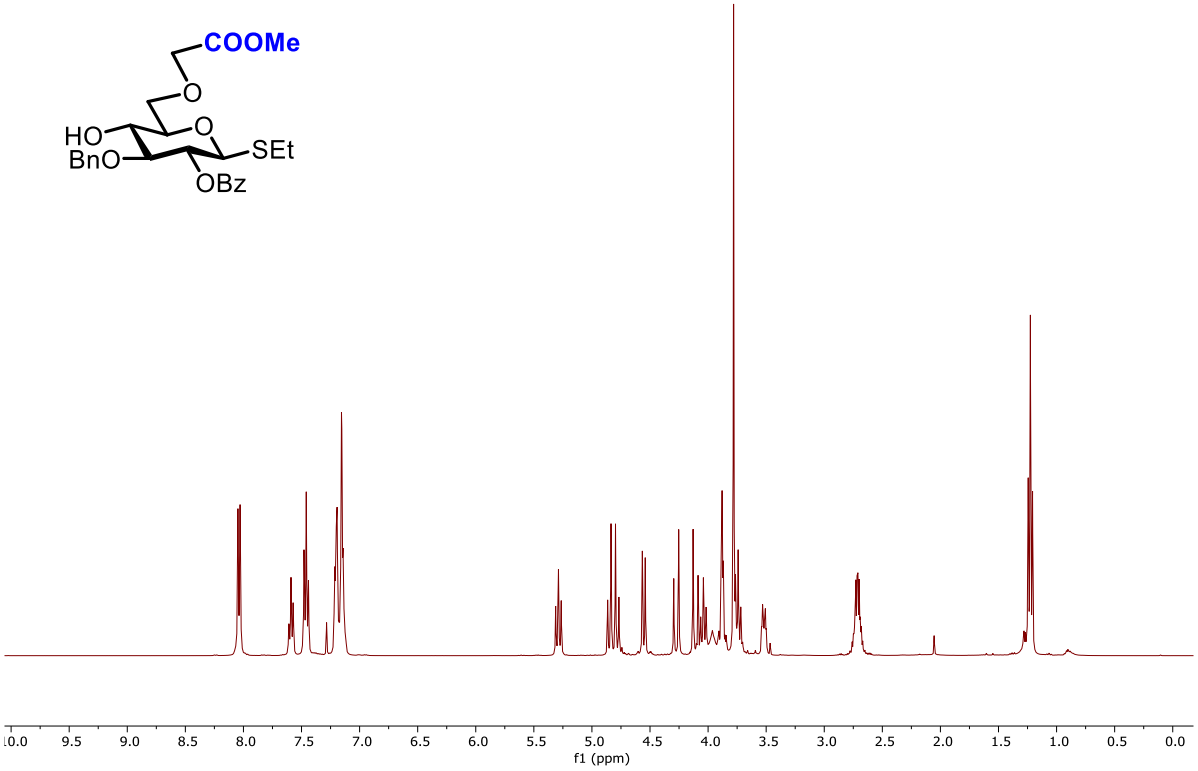
Ethyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-methoxycarbonylmethyl-1-thio-β-D-glucopyranoside, S10



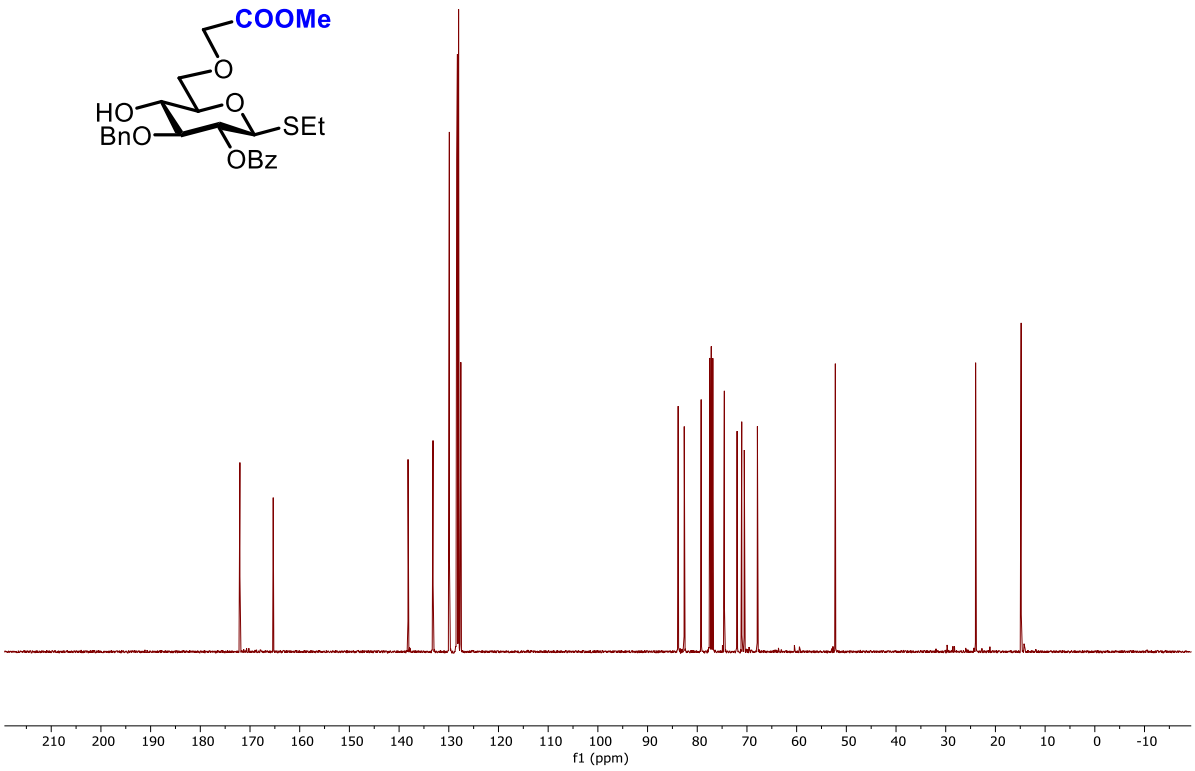
S9 **S10**

Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-naphthyl)methyl-6-*O*-methoxycarbonylmethyl-1-thio-β-D-glucopyranoside **S9** (356 mg, 0.56 mmol) was dissolved in DCM (5 mL) and water (250 µL) was added. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (240 mg, 1.06 mmol) was added, and the reaction was stirred for 4 h at room temperature. DCM (20 mL) was added to dilute the reaction mixture and the solution was washed with $Na_2S_2O_3$ (aq) (5%, 50 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated. The resulting crude product was purified by column chromatography (Hexane : EtOAc = 6:1→ Hexane : EtOAc = 3:1) to give **S10** as a colorless oil (200 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 8.00 (m, 2H), 7.63 – 7.55 (m, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.24 – 7.10 (m, 5H), 5.29 (t, *J* = 9.6 Hz, 1H), 4.90 – 4.74 (m, 2H), 4.55 (d, *J* = 10.1 Hz, 1H), 4.27 and 4.11 (ABq, *J* = 17.0 Hz, 2H), 4.04 (t, *J* = 9.4 Hz, 1H), 3.98 – 3.94 (br. s, 1H), 3.92 – 3.84 (m, 2H), 3.76 (d, *J* = 15.7 Hz, 4H), 3.52 (dt, *J* = 9.7, 3.4 Hz, 1H), 2.71 (qd, *J* = 7.4, 4.3 Hz, 2H), 1.23 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.03, 165.33, 138.20, 133.17, 129.91, 129.89, 128.41, 128.26, 128.02, 127.58, 83.84, 82.62, 79.21, 74.55, 71.98, 71.05, 70.54, 67.90, 52.22, 23.99, 14.82; $[\alpha]_D^{25}$ 27.75 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1727, 1068, 710 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 513.1555 ($C_{25}H_{30}O_8SNa^+$ requires 513.1554).

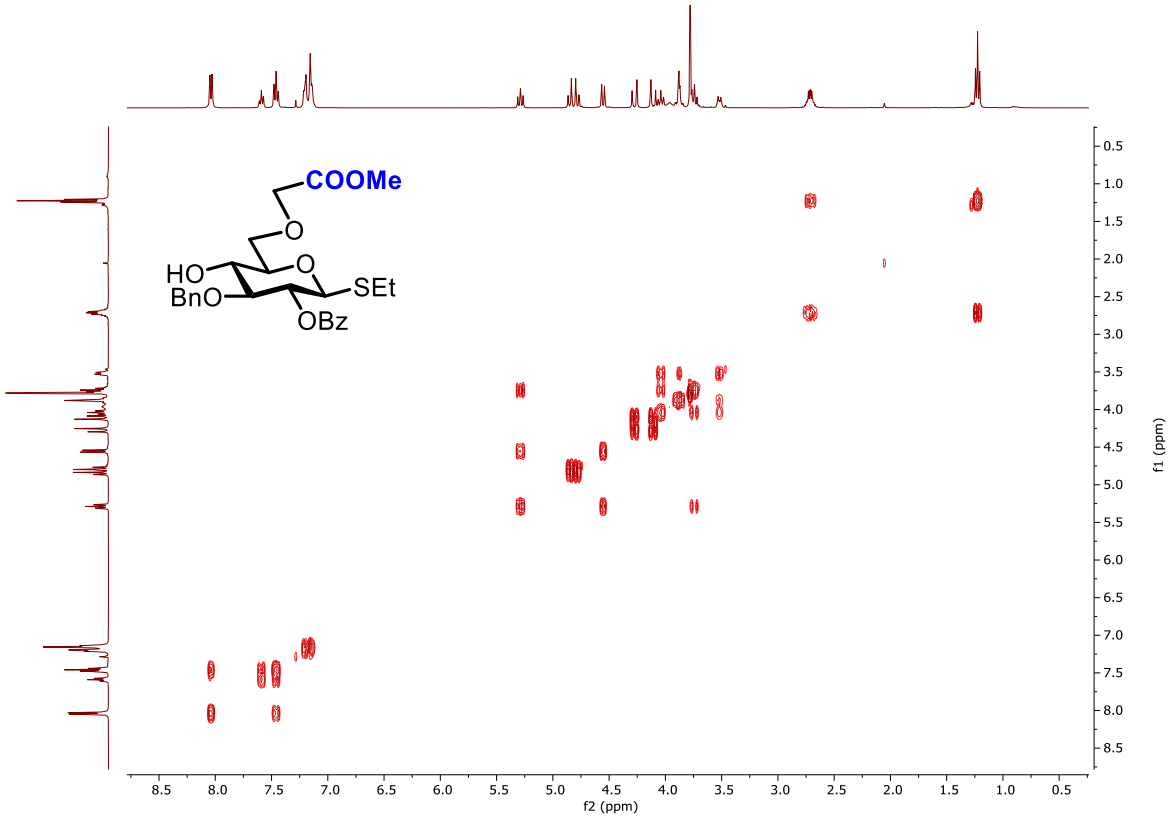
^{1}H NMR of S10 (400 MHz, $CDCl_3$)



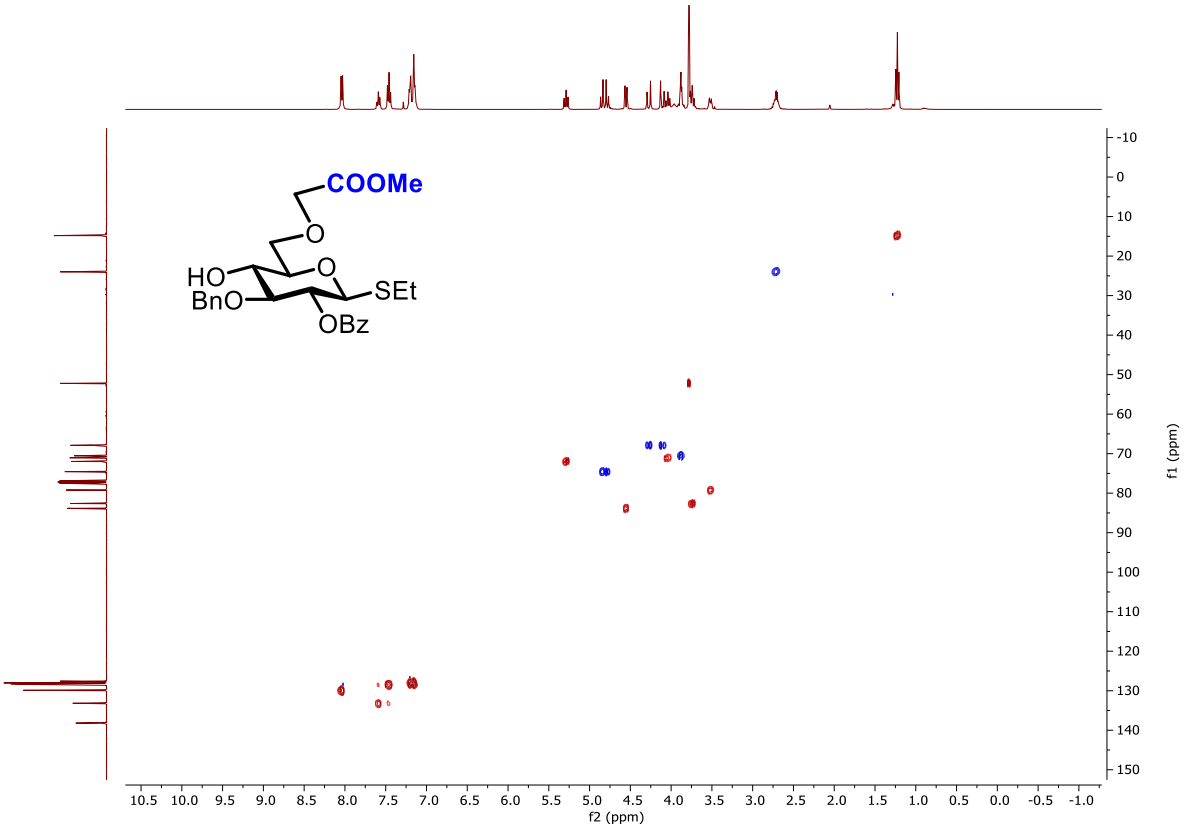
^{13}C NMR of S10 (101 MHz, $CDCl_3$)



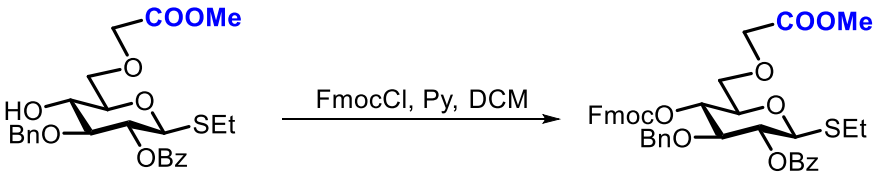
COSY NMR of S10 ($CDCl_3$)



HSQC NMR of S10 ($CDCl_3$)



Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-methoxycarbonylmethyl-1-thio-β-D-glucopyranoside, BB-4

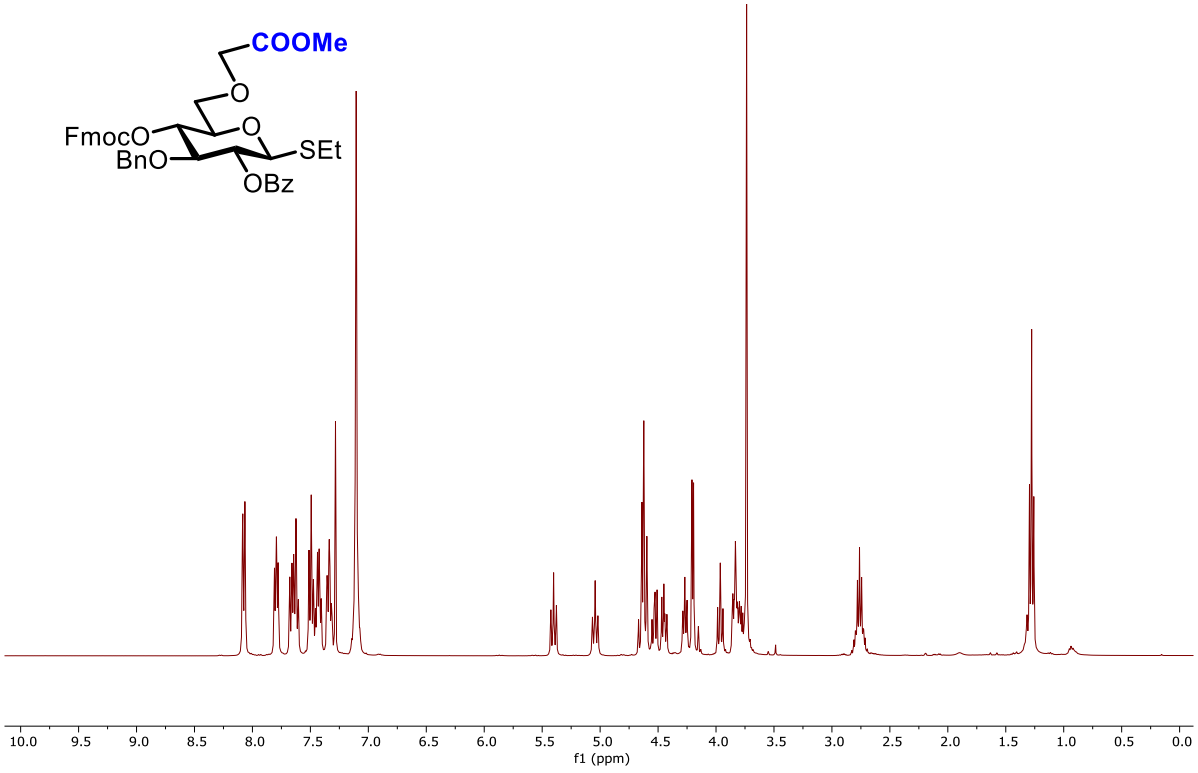


S10 **BB-4**

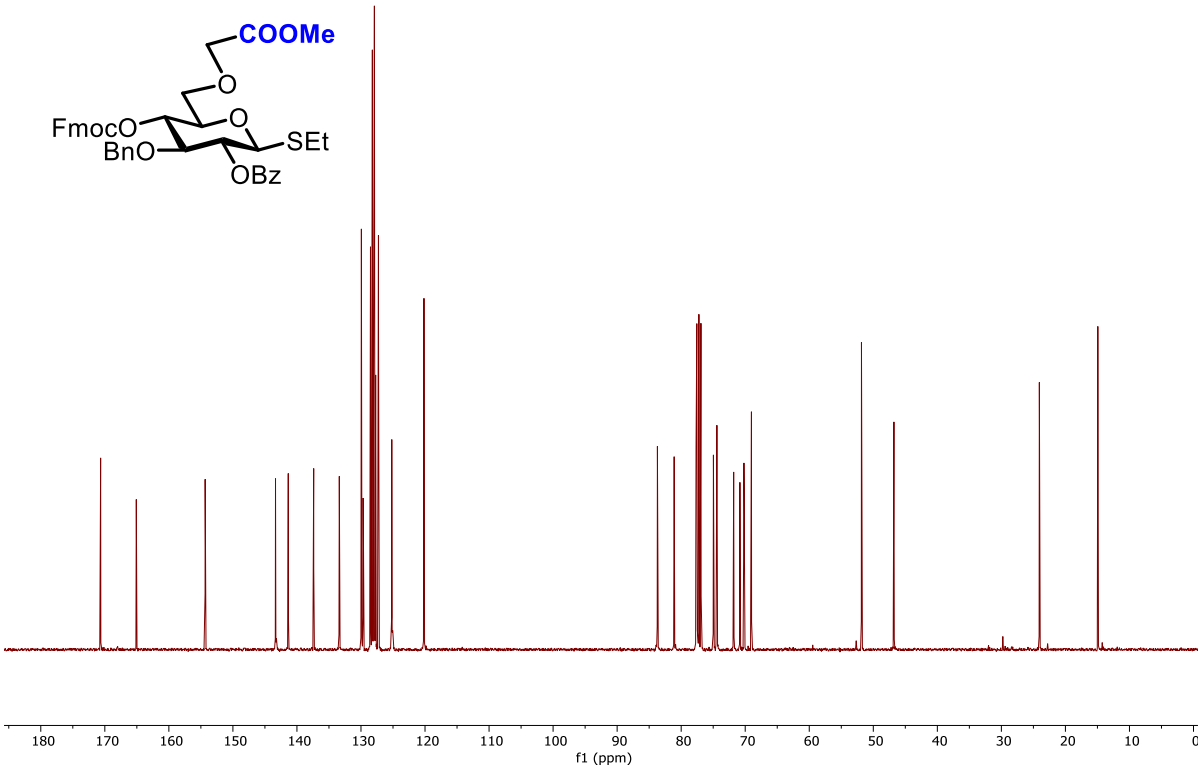
Ethyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-methoxycarbonylmethyl-1-thio-β-D-glucopyranoside **S10** (200 mg, 0.41 mmol) was dissolved in anhydrous DCM (2 mL) and pyridine (0.5 mL) was added. The solution was cooled with an ice bath for 15 min, and fluorenylmethyloxycarbonyl chloride (263 mg, 1.02 mmol) was added slowly. The reaction was allowed to room temperature and stirred for 6 h. Upon completion, DCM (20 mL) was added and the organic phase was washed with aqueous citric acid (0.5 M, 20 mL). After extracting the water phase with DCM (10 mL), the organic layers were combined and dried over Na_2SO_4, filtered, and evaporated. The resulting crude product purified by column chromatography (Hexane : EtOAc = 6:1→3:1) to give **BB-4** as a white solid (255 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 8.1, 1.5 Hz, 2H), 7.85 – 7.75 (m, 2H), 7.71 – 7.58 (m, 3H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.43 (td, *J* = 7.3, 5.1 Hz, 2H), 7.34 (tdd, *J* = 7.5, 2.5, 1.2 Hz, 2H), 7.11 (m, 5H), 5.40 (t, *J* = 9.5 Hz, 1H), 5.04 (t, *J* = 9.4 Hz, 1H), 4.69 – 4.58 (m, 3H), 4.53 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.45 (dd, *J* = 10.6, 7.1 Hz, 1H), 4.27 (t, *J* = 7.6 Hz, 1H), 4.20 (d, *J* = 5.6 Hz, 2H), 3.96 (t, *J* = 9.1 Hz, 1H), 3.89 – 3.58 (m, 6H), 2.76 (p, *J* = 7.3 Hz, 2H), 1.28 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.65, 165.09,

154.33, 143.35, 143.23, 141.36, 141.33, 137.38, 133.39, 129.94, 129.65, 128.52, 128.22, 128.00, 127.91, 127.71, 127.27, 125.19, 125.08, 120.17f; $[\alpha]_D^{25}$ 29.44 (c = 1, $CHCl_3$); IR (neat) ν_{max} = 1753, 1247, 1027 cm^{-1}; m/z (HRMS$^+$) $[M + Na]^+$ 735.2238 ($C_{40}H_{40}O_{10}SNa^+$ requires 735.2234).

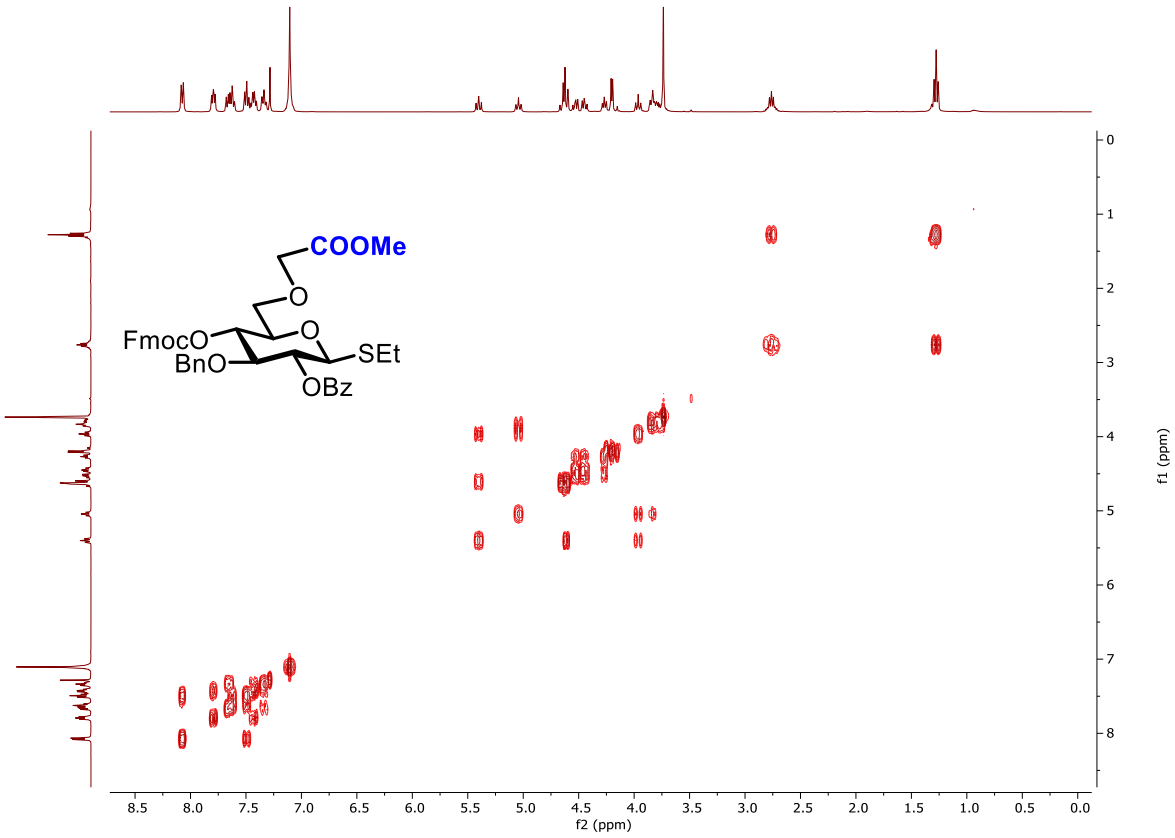
^{1}H NMR of BB-4 (400 MHz, $CDCl_3$)



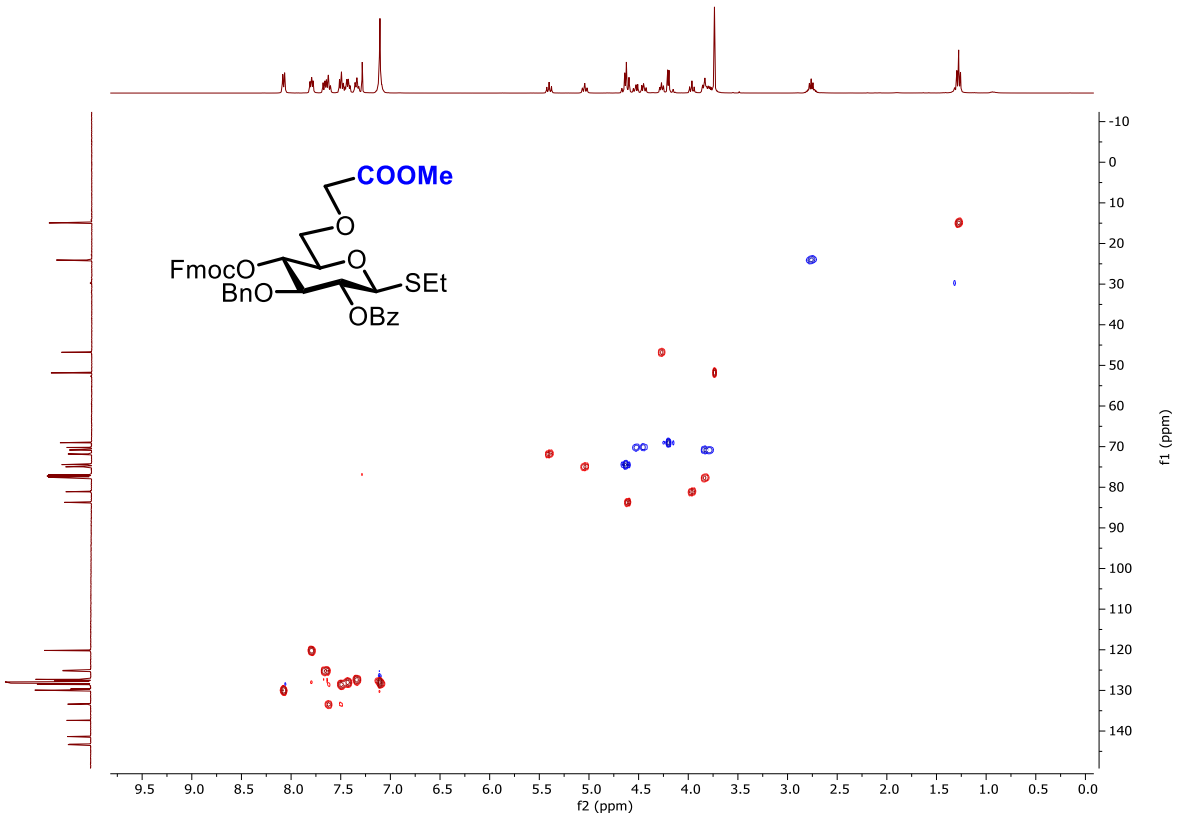
^{13}C NMR of BB-4 (101 MHz, $CDCl_3$)

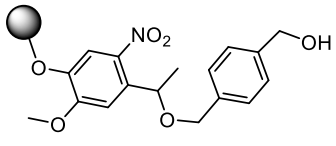


COSY NMR of BB-4 ($CDCl_3$)

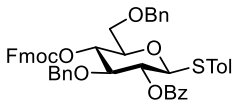


HSQC NMR of BB-4 ($CDCl_3$)





S0



BB-0

Building block 0 (**BB-0**) is commercially available. Photo-cleavable linker **S0** is prepared according to previously established procedures.[6]

3. Automated Glycan Assembly

3.1. General materials and methods

All solvents used were HPLC-grade. The solvents used for the building block, activator, TMSOTf and capping solutions were taken from an anhydrous solvent system (jcmeyer-solvent systems). The building blocks were co-evaporated three times with toluene and dried for 1 h on high vacuum before use. Activator, capping, deprotection, acidic wash and building block solutions were freshly

prepared and kept under argon during the automation run. All yields of products obtained by AGA were calculated on the basis of resin loading. Resin loading was determined following previously established procedures.[7]

3.2. Preparation of stock solutions

- **Building Block**: between 0.06 and 0.08 mmol of building block (depending on BB) was dissolved in DCM (1 mL).
- **Activator solution**: 1.35 g of recrystallized NIS was dissolved in 40 mL of a 2:1 mixture of anhydrous DCM and anhydrous dioxane. Then triflic acid (55 µL) was added. The solution is kept at 0°C for the duration of the automation run.
- **Fmoc deprotection solution**: A solution of 20% triethylamine in DMF (v/v) was prepared.
- **TMSOTf solution**: TMSOTf (0.45 mL) was added to DCM (40 mL).
- **Capping solution**: A solution of 10% acetic anhydride and 2% methanesulfunic acid in DCM (v/v) was prepared.

3.3. Modules for automated synthesis

Module A: Resin Preparation for Synthesis (20 min)

The automated synthesis were performed on 0.0125 mmol scale for 3,7 and 8,[3] and 0.0146 mmol scale for the other compounds. Resin was placed in the reaction vessel and swollen in DCM for 20 min at room temperature prior to synthesis. During this time, all reagent lines needed for the synthesis were washed and primed. Before the first glycosylation, the resin was washed with the DMF, THF, and DCM (three times each with 2 mL for 25 s).

Module B: Acidic Wash with TMSOTf Solution (20 min)

The resin was swollen in 2 mL DCM and the temperature of the reaction vessel was adjusted to -20°C. Upon reaching the low temperature, TMSOTf solution (1 mL) was added drop wise to the reaction vessel. After bubbling for 3 min, the acidic solution was drained and the resin was washed with 2 mL DCM for 25 s.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	-20	(15 min)*
Deliver	1	DCM	2 mL	-20	-
Deliver	1	TMSOTf solution	1 mL	-20	3 min
Wash	1	DCM	2 mL	-20	25 sec

*Time required to reach the desired temperature.

Module C: Thioglycoside Glycosylation (35 min)

The building block solution (0.08 mmol of BB in 1 mL of DCM per glycosylation) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by drop wise addition of the activator solution (1.0 mL, excess). The glycosylation conditions are building block dependent (we report the most common set of conditions). After completion of the reaction, the solution is drained and the resin was washed with DCM, DCM : dioxane (1:2, 3 mL for 20 s) and DCM (two times, each with 2 mL for 25 s). The temperature of the reaction vessel is increased to 25°C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	-20	-
Deliver	1	BB solution	1 mL	-20	-
Deliver	1	Activator solution	1 mL	-20	-
Reaction time (BB dependent)	1			-20 to 0	5 min 20 min
Wash	1	DCM	2 mL	0	5 sec
Wash	1	DCM : Dioxane (1:2)	2 mL	0	20 sec
Heating	-	-	-	25	-
Wash	2	DCM	2 mL	> 0	25 sec

Module D: Capping (30 min)

The resin was washed with DMF (two times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25°C. 2 mL of Pyridine solution (10% in DMF) was delivered into the reaction vessel. After 1 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s). 4 mL of capping solution was delivered into the reaction vessel. After 20 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s).

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Heating	-	-	-	25	(5 min)*
Wash	2	DMF	2 mL	25	25 sec
Deliver	1	10% Pyridine in DMF	2 mL	25	1 min
Wash	3	DCM	2 mL	25	25 sec
Deliver	1	Capping Solution	4 mL	25	20 min
Wash	3	DCM	2 mL	25	25 sec

*Time required to reach the desired temperature.

Module E: Fmoc Deprotection with TEA (20 min)

The resin was washed with DMF (three times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25°C. 2 mL of Fmoc deprotection solution 2 was delivered to the reaction vessel. After 5 min, the reaction solution was drained. The deprotection process was repeated for 3 times. The resin was washed with DMF (three times with 3 mL for 25 s) and DCM (five times each with 2 mL for 25 s). The temperature of the reaction vessel is decreased to -20°C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Wash	3	DMF	2 mL	25	25 sec
Deliver	3	Fmoc depr. Solution 2	2 mL	25	5 min
Wash	1	DMF	2 mL		
Cooling	-	-	-	-20	-
Wash	3	DMF	2 mL	< 25	25 sec
Wash	5	DCM	2 mL	< 25	25 sec

3.4. Post-synthesizer manipulations

Module F: On-resin Staudinger Reduction

The resin was suspended in THF (5 mL). 0.5 mL of triethylphosphine solution (1.0 M in THF) and 0.2 mL of ammonia solution (30% in water) were added and the suspension was gently shaken at room temperature. After micro-cleavage (see **Module H1**) indicated the complete reduction of azido groups (around 24 hours), the resin was repeatedly washed with THF (2mL x 5).

Module G: On-resin Methanolysis

The resin was suspended in THF (5 mL). 0.5 mL of NaOMe in MeOH (0.5 M) was added and the suspension was gently shaken at room temperature. This condition also hydrolyzes the methyl esters due to the trace amount of water in the reaction mixture. After micro-cleavage (see **Module H1**) indicated the complete removal of benzoyl groups and methyl esters (4 to 48 hours), the resin was repeatedly washed with MeOH (2mL x 3) and THF (2mL x 3).

Module H: Cleavage from Solid Support

The oligosaccharides were cleaved from the solid support using a continuous-flow photoreactor as described previously.[8]

Module H1: Micro-cleavage from Solid Support

Trace amount of resin (around 20 beads) was dispersed in DCM (0.1 mL) and irradiated with a UV lamp (6 watt, 356 nm) for 10 minutes. ACN (10 µL) was then added to the resin and the resulting solution analyzed by MALDI.

Module I: Solution-phase Methanolysis

The protected oligosaccharide was dissolved in THF (1.5 mL). NaOMe in MeOH (0.5 M, 3 equiv. per benzoyl ester) was added and the solution was stirred at room temperature for 12 h, neutralized with HOAc and concentrated *in vacuo.* The crude compound was used for hydrogenolysis without further purification. This condition also hydrolyzes the methyl esters due to the trace amount of water in the reaction mixture.

Module J: Hydrogenolysis at Ambient Pressure

The crude compound was dissolved in 2 mL of EA: *t*BuOH: H_2O (1:0.5:0.5). 100% by weight Pd-C (10%) was added and the reaction was stirred under H_2–atmosphere for 6 h. The reaction was filtered through celite and washed with *t*BuOH and H_2O. The filtrates were concentrated *in vacuo*, and dissolved in 3.5 mL water.

Module K: Purification

Purification was conducted at different stage of the synthesis as reported for the individual procedures. The products were analyzed using analytical HPLC (Agilent 1200 Series spectrometer, **Method A** and **Method C**). The purification was conducted using preparative HPLC (Agilent 1200 Series spectrometer).

- **Method A:** (YMC-Diol-300 column, 150 x 4.6 mm) flow rate of 1.0 mL / min with Hex – 20% EtOAc as eluent [isocratic 20% EtOAc (5 min), linear gradient to 55% EtOAc (35 min), linear gradient to 100% EtOAc (5 min)].

- **Method B:** (YMC-Diol-300 column, 150 x 20 mm) flow rate of 15 mL / min with Hex – 20% EtOAc as eluents [isocratic 20% EtOAc (5 min), linear gradient to 55% EtOAc (35 min), linear gradient to 100% EtOAc (5 min)].

- **Method C:** (Synergi Hydro RP18 column, 250 x 4.6 mm) flow rate of 1.0 mL / min with H_2O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 30% ACN (30 min), linear gradient to 100% ACN (5 min)].

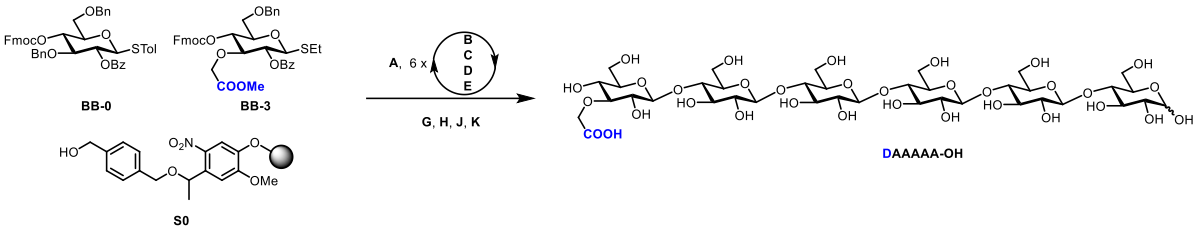
- **Method D:** (Synergi Hydro RP18 column, 250 x 10 mm) flow rate of 4.0 mL / min with H_2O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 20% ACN (30 min), linear gradient to 100% ACN (5 min)].

- **Method E:** (Manual normal phase silica gel column chromatography): Hexanes: EtOAc = 2:1 to Hexanes: EtOAc = 1:2.

Following final purification, all deprotected products were lyophilized on a Christ Alpha 2-4 LD plus freeze dryer prior to characterization.

3.5. Oligosaccharides synthesis

1. ***Synthesis of DAAAAA-OH***

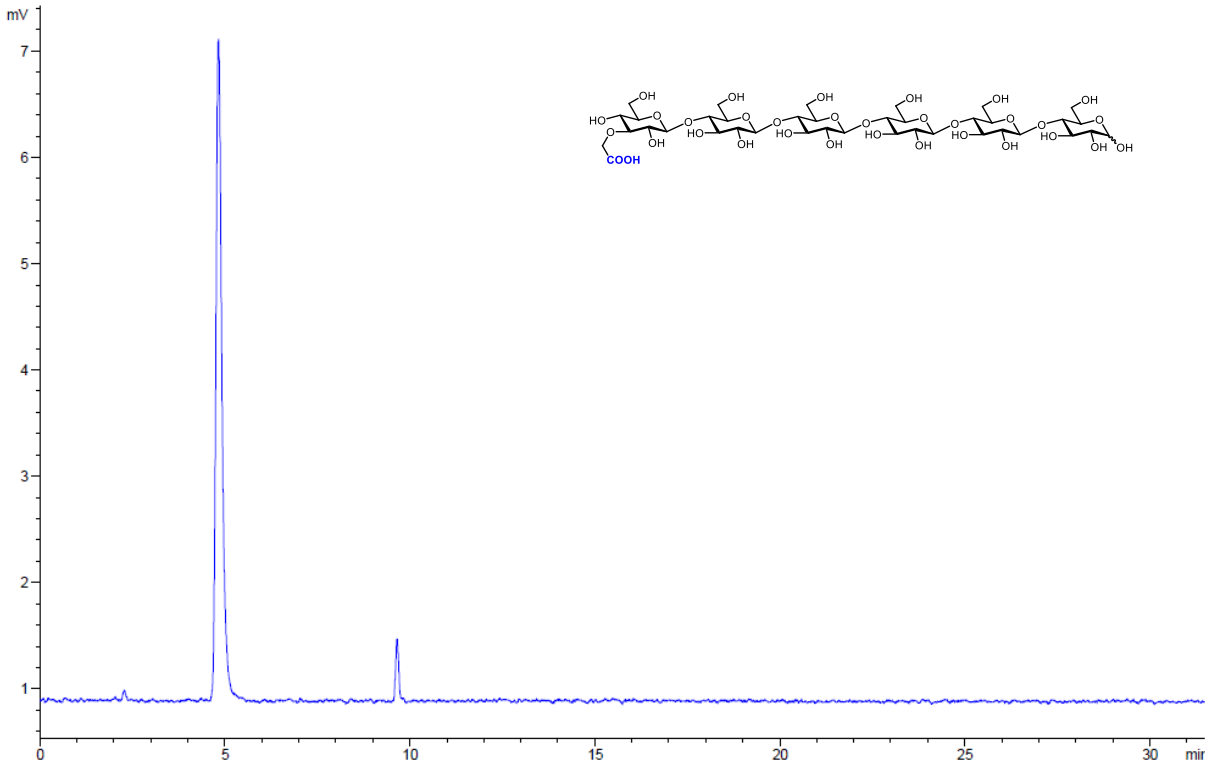


	Module	Conditions
	A: Resin Preparation for Synthesis	
5	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	G: On-resin Methanolysis	
	H: Cleavage from Solid Support	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 18.8 min**

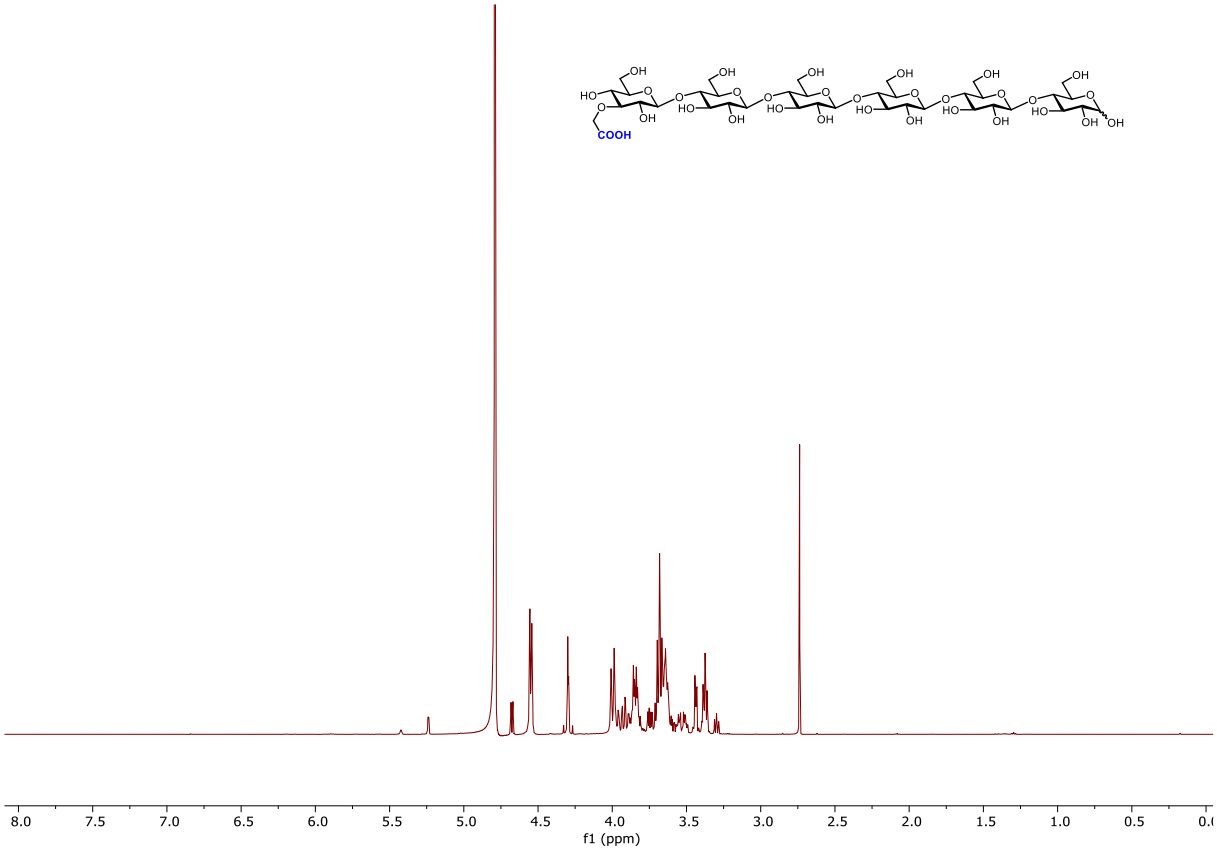
Automated synthesis, global deprotection, and purification afforded **DAAAAA-OH** as white solid (3.5 mg, 23% overall yield).

Analytical data for **DAAAAA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 0.31 H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.69 H, β-H1), 4.55 (dd, *J* = 7.8, 1.8 Hz, 5H), 4.30 (d, *J* = 2.9 Hz, 2H), 4.05 – 3.77 (m, 13H), 3.75 (dd, *J* = 12.5, 5.7 Hz, 1H), 3.72 – 3.57 (m, 14H), 3.57 – 3.48 (m, 2H), 3.46 – 3.41 (m, 2H), 3.41 – 3.34 (m, 3.3H), 3.30 (dd, *J* = 9.0, 7.9 Hz, 0.7H); ^{13}C NMR (151 MHz, D_2O) δ 177.99, 102.27, 95.68 (β-C1), 91.74 (α-C1), 85.45, 78.56, 78.41, 78.21, 78.19, 75.70, 74.75, 74.18, 73.94, 73.93, 73.82, 72.88, 72.87, 72.85, 72.76, 71.23, 71.15, 70.80, 70.05, 68.94, 60.42, 59.94, 59.79; m/z (HRMS$^+$) 1049.341 $[M + H]^+$ ($C_{38}H_{65}O_{33}^+$ requires 1049.340).

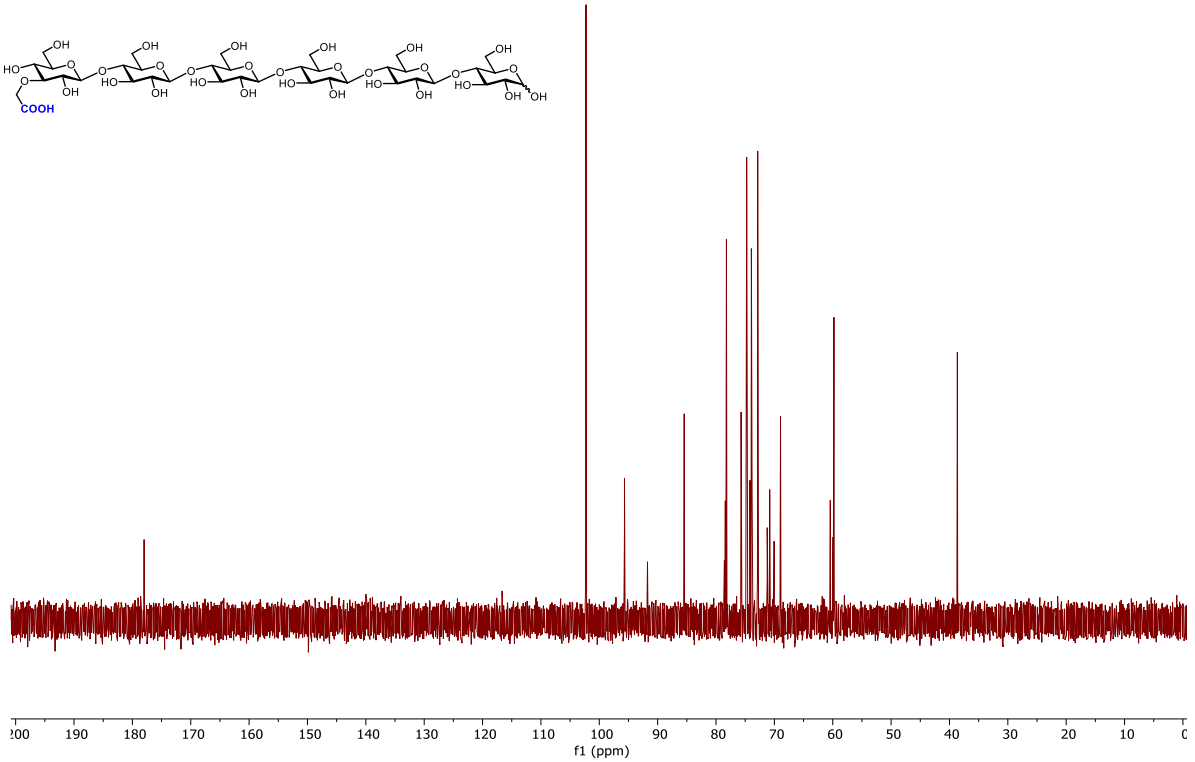
RP-HPLC of DAAAAA-OH (ELSD trace, Method C, t_R = 4.9 min)



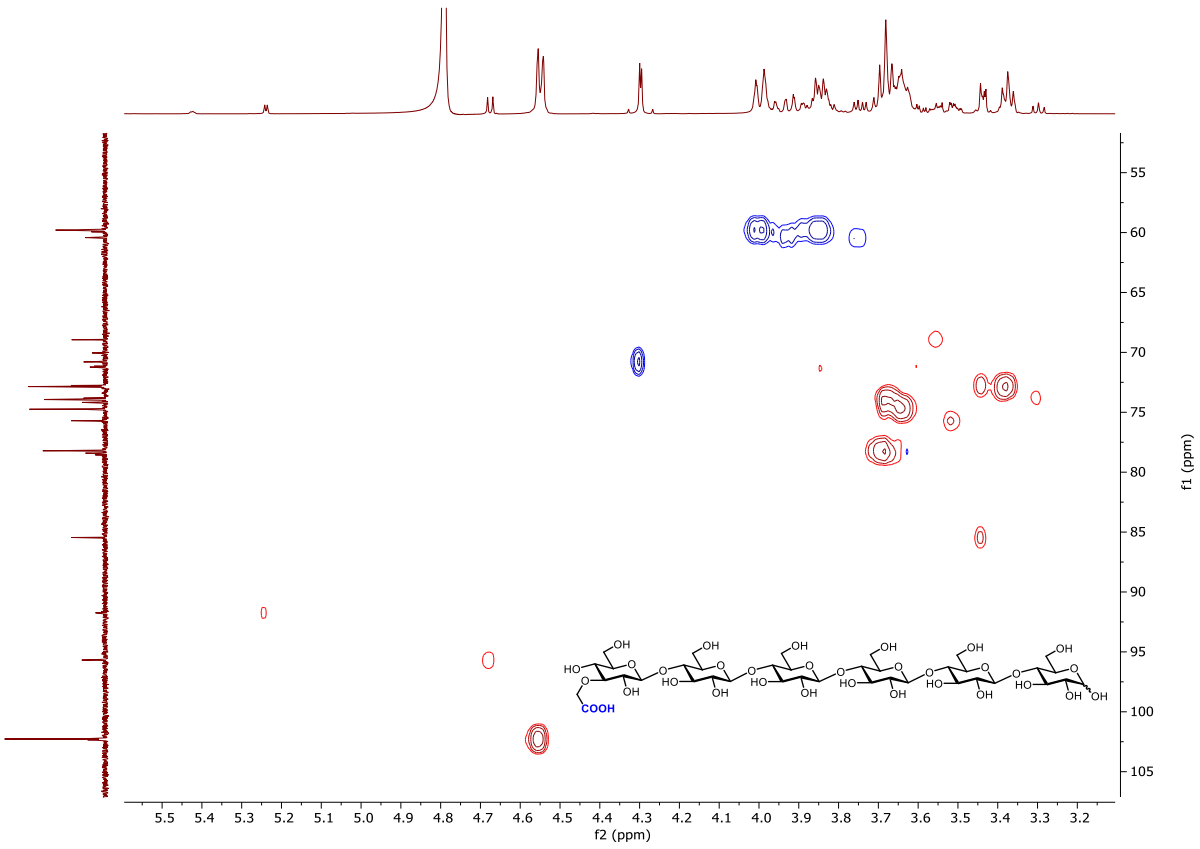
^{1}H NMR of DAAAAA-OH (600 MHz, D_2O)



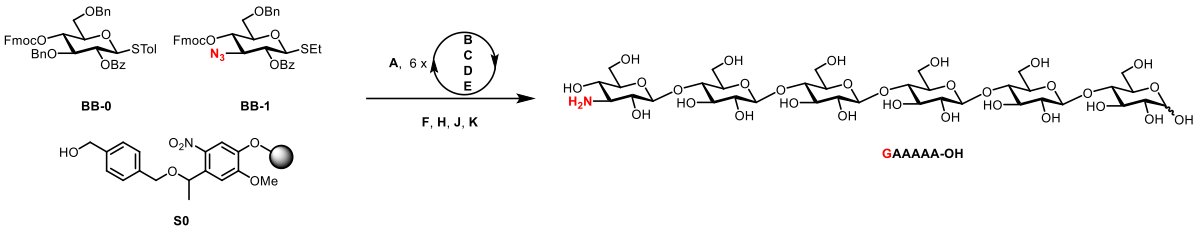
^{13}C NMR of DAAAAA-OH (151 MHz, D_2O)



HSQC NMR of DAAAAA-OH (D_2O)



2. *Synthesis of GAAAAA-OH*

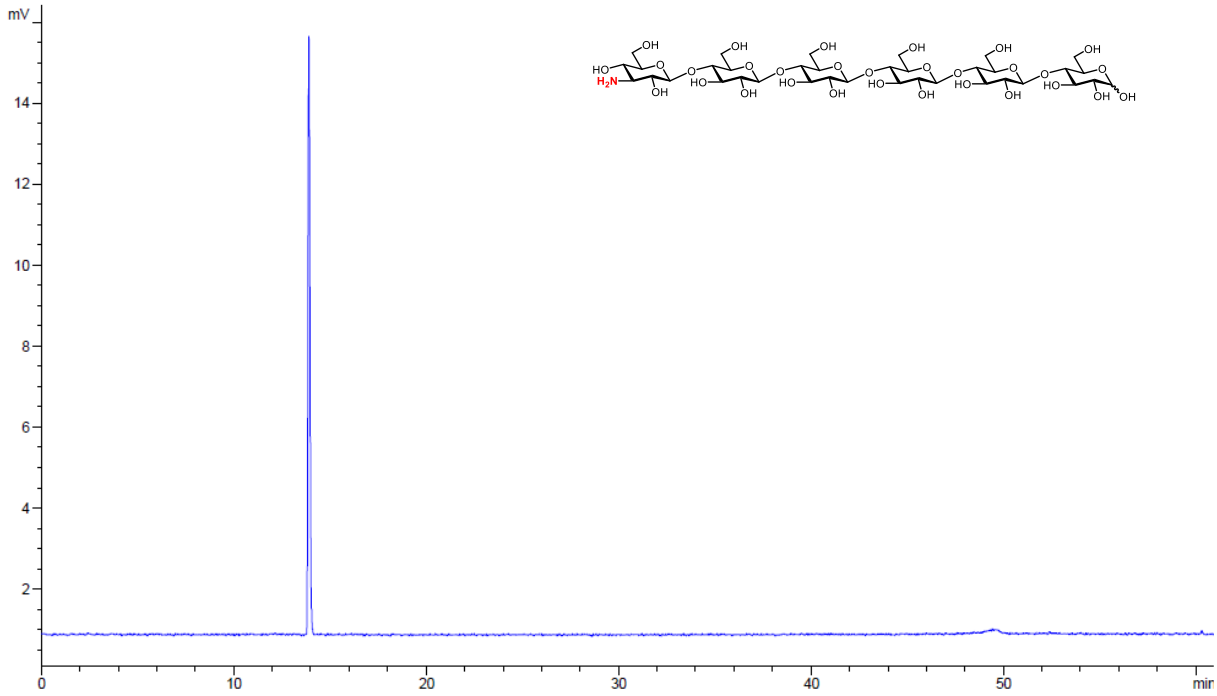


	Module	Conditions
	A: Resin Preparation for Synthesis	
5	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-1**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	F: On-resin Staudinger Reduction **H:** Cleavage from Solid Support **J**: Hydrogenolysis at Ambient Pressure **K**: Purification	**Method D, t_R = 14.5 min**

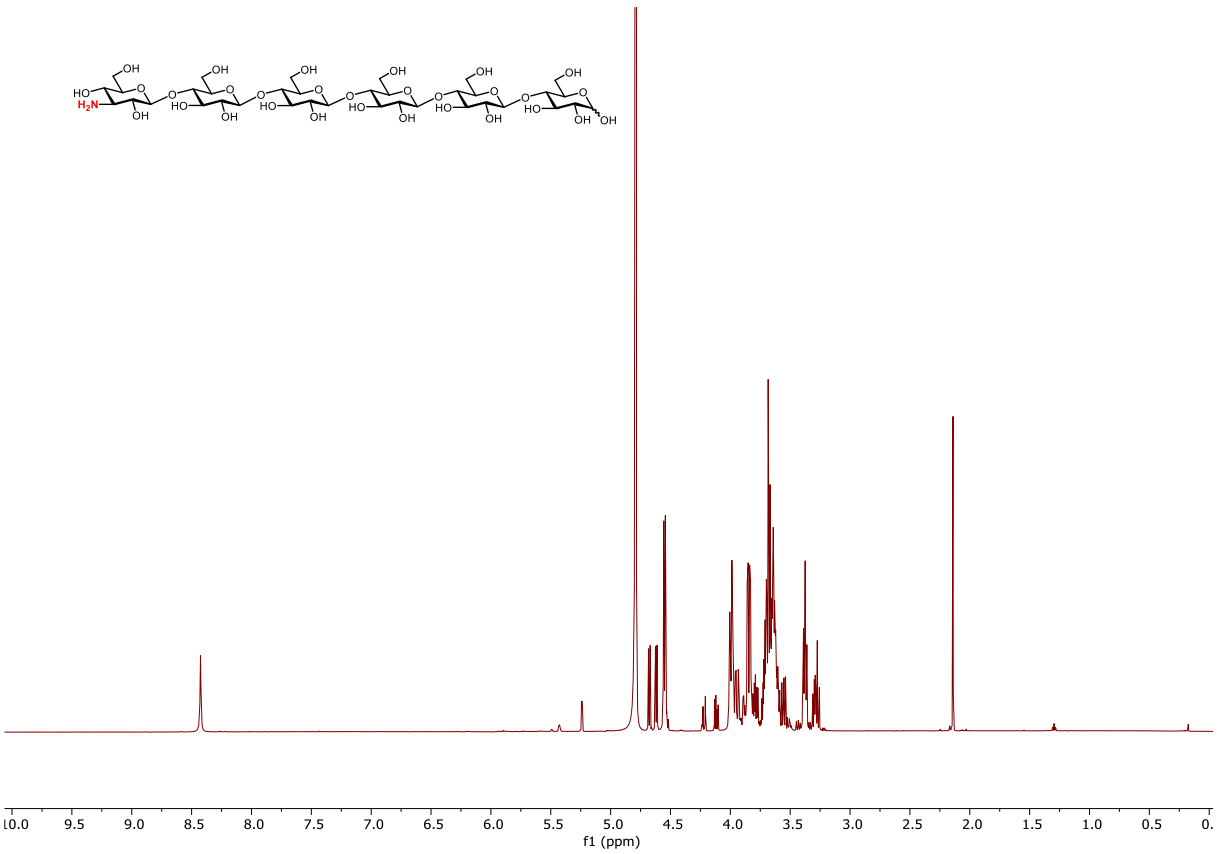
Automated synthesis, global deprotection, and purification afforded **GAAAAA-OH** as white solid (3.4 mg, 24% overall yield).

Analytical data for **GAAAAA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 0.32 H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.68 H, β-H1), 4.62 (d, *J* = 7.7 Hz, 1H), 4.55 (dd, *J* = 7.9, 1.5 Hz, 4H), 4.05 – 3.91 (m, 6H), 3.90 – 3.76 (m, 7H), 3.76 – 3.47 (m, 17H), 3.46 – 3.34 (m, 4H), 3.33 – 3.20 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 102.42, 102.27, 95.68 (β-C1), 91.75 (α-C1), 78.56, 78.40, 78.18, 78.14, 76.66, 74.73, 74.17, 73.93, 73.91, 73.88, 73.82, 72.88, 71.23, 71.16, 70.05, 69.48, 69.34, 65.72, 65.45, 62.08, 59.93, 59.90, 59.77, 59.73, 57.53; m/z (HRMS$^+$) 990.3554 $[M + H]^+$ ($C_{36}H_{64}NO_{30}^+$ requires 990.3508).

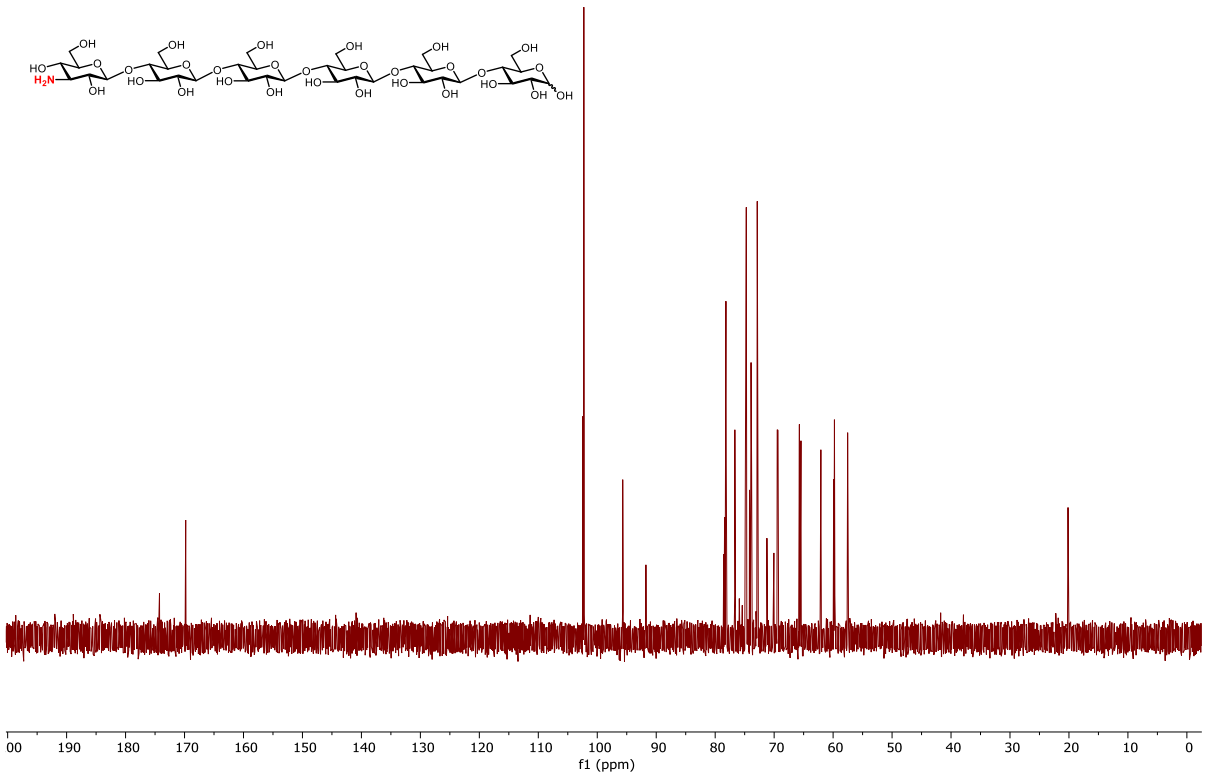
RP-HPLC of GAAAAA-OH (ELSD trace, Method C, t_R = 14.1 min)



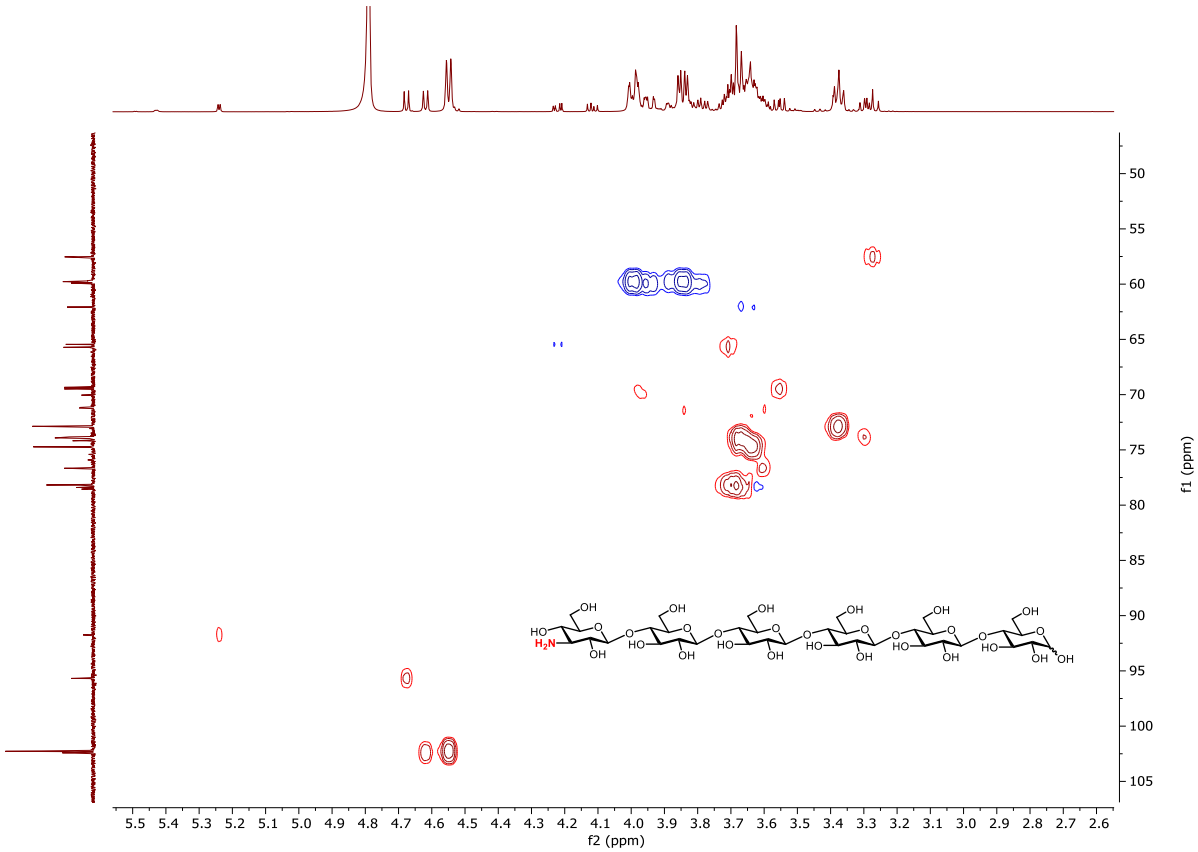
^{1}H NMR of GAAAAA-OH (600 MHz, D_2O)



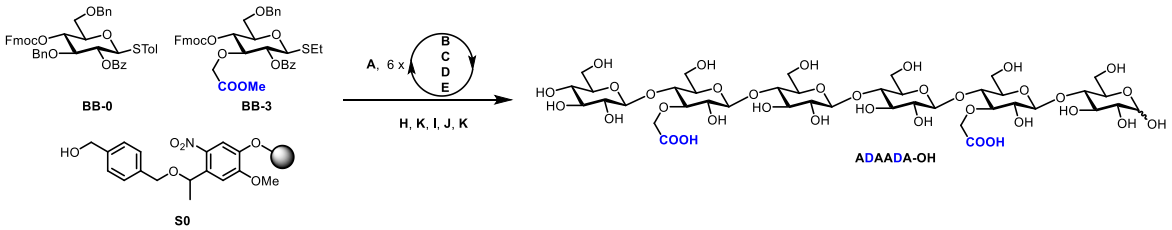
^{13}C NMR of GAAAAA-OH (151 MHz, D_2O)



HSQC NMR of GAAAAA-OH (D_2O)



3. *Synthesis of ADAADA-OH* [3]

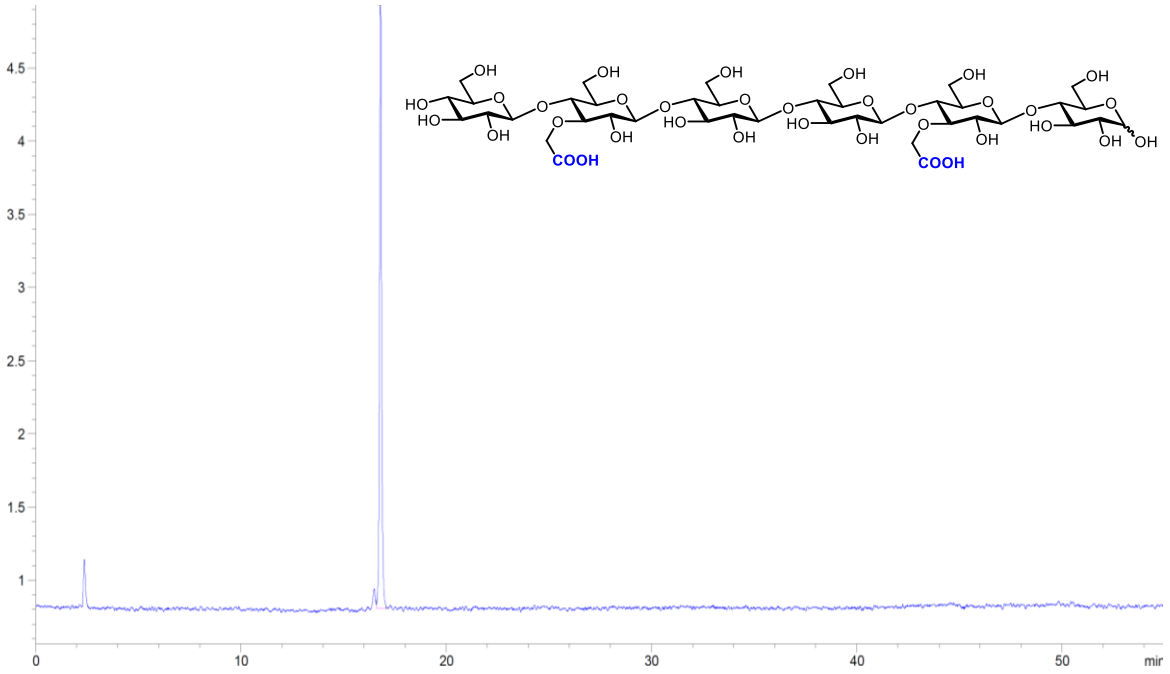


	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	H: Cleavage from Solid Support	
	K: Purification	**Method E and Method B, t_R = 34.2 min**
	I: Solution-phase Methanolysis	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 16.3 min**

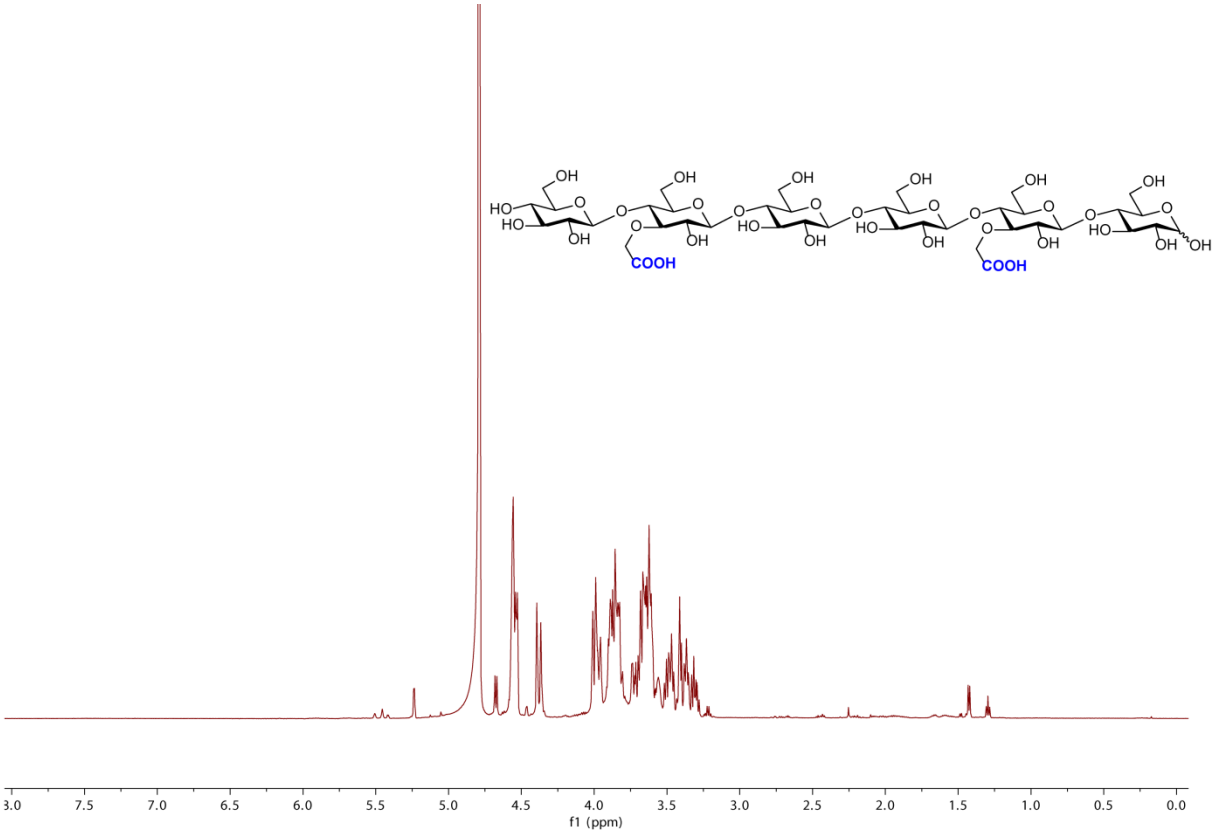
Automated synthesis, global deprotection, and purification afforded **ADAADA-OH** as white solid (3.5 mg, 25% overall yield).

Analytical data for **ADAADA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 0.31 H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.69H, β-H1), 4.62 – 4.46 (m, 7H), 4.42 – 4.35 (m, 2H), 4.05 – 3.94 (m, 5H), 3.94 – 3.77 (m, 9H), 3.77 – 3.53 (m, 13H), 3.53 – 3.45 (m, 3H), 3.45 – 3.35 (m, 4H), 3.34 – 3.20 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 175.93, 166.68, 102.33, 102.28, 102.24, 102.16, 95.68 (β-C1), 91.75 (α-C1), 82.94, 78.48, 78.43, 78.31, 78.08, 76.18, 76.01, 75.49, 74.90, 74.89, 74.84, 74.71, 74.15, 74.01, 73.89, 73.81, 73.58, 73.32, 72.87, 72.42, 71.21, 71.15, 70.03, 69.97, 69.48, 60.81, 60.07, 59.90, 59.75, 59.68; m/z (HRMS$^+$) 1129.341 $[M + Na]^+$ ($C_{40}H_{66}O_{35}Na^+$ requires 1129.327).

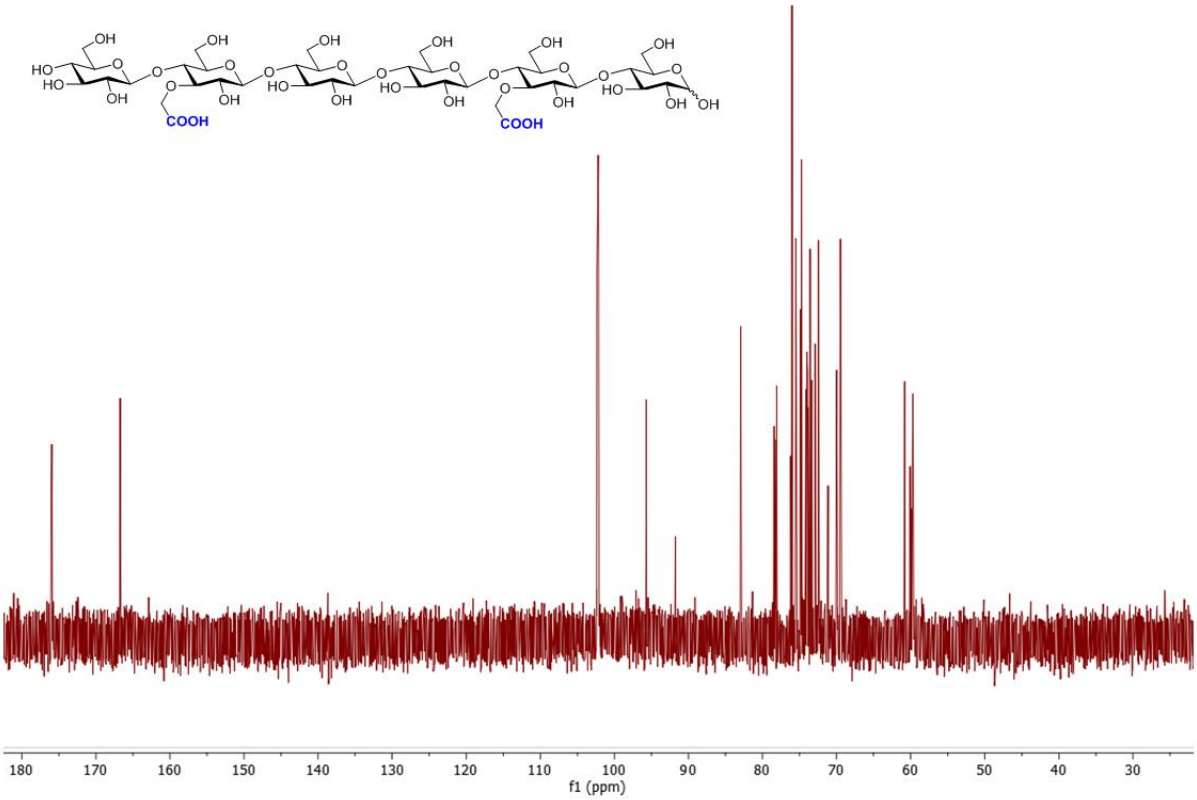
RP-HPLC of ADAADA-OH (ELSD trace, Method C, t_R = 16.8 min)



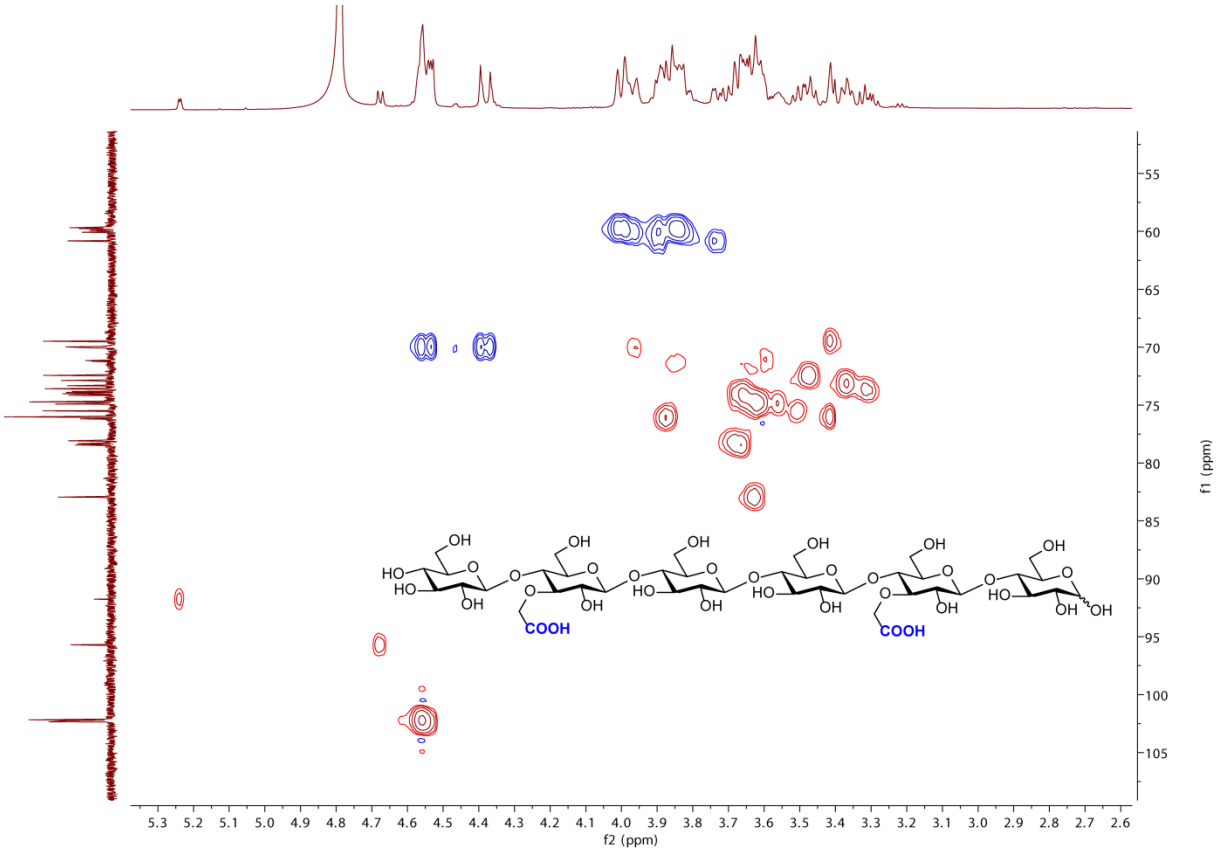
^{1}H NMR of ADAADA-OH (600 MHz, D_2O)



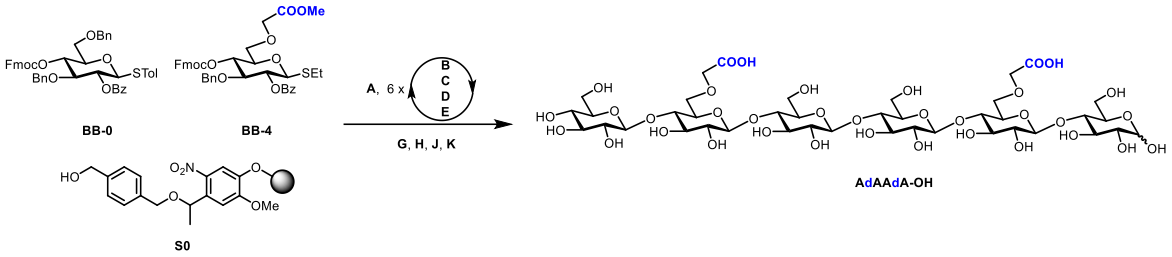
^{13}C NMR of ADAADA-OH (151 MHz, D_2O)



HSQC NMR of ADAADA-OH (D_2O)



4. *Synthesis of AdAAdA-OH*

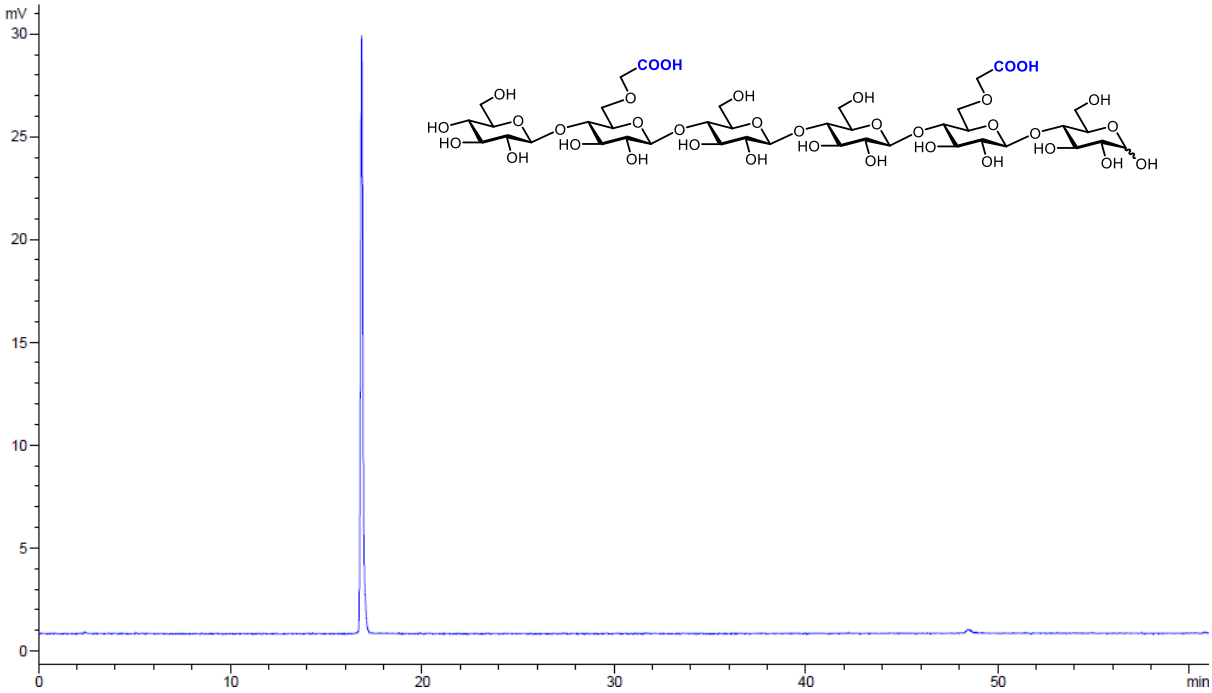


	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-4**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	G: On-resin Methanolysis	
	H: Cleavage from Solid Support	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 18.6 min**

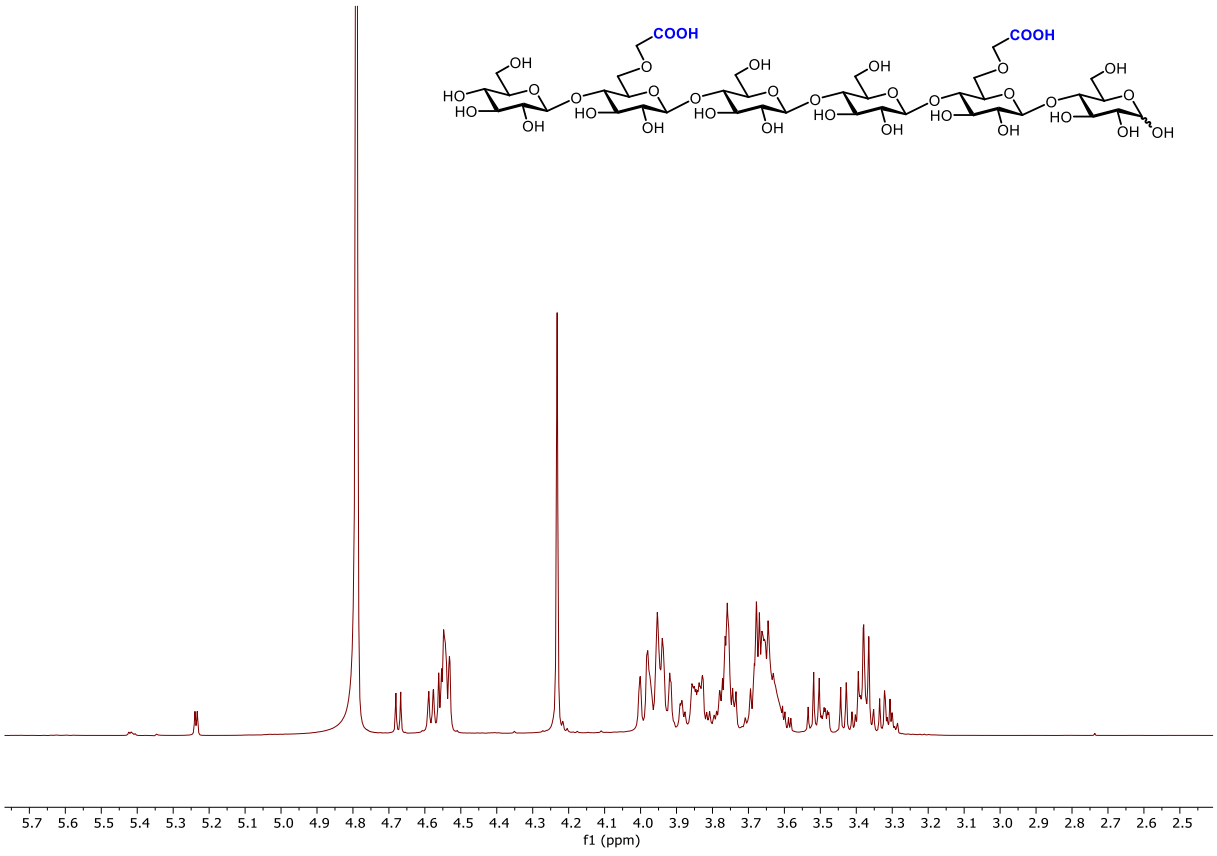
Automated synthesis, global deprotection, and purification afforded **AdAAdA-OH** as white solid (5.6 mg, 35% overall yield).

Analytical data for **AdAAdA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.7 Hz, 0.32H, α-H1), 4.67 (d, *J* = 8.0 Hz, 0.68H, β-H1), 4.61 – 4.50 (m, 5H), 4.23 (s, 4H), 4.03 – 3.90 (m, 8H), 3.89 – 3.80 (m, 4H), 3.79 – 3.73 (m, 5H), 3.72 – 3.57 (m, 11H), 3.55 – 3.46 (m, 2H), 3.43 (t, *J* = 9.4 Hz, 1H), 3.40 – 3.28 (m, 5H); ^{13}C NMR (151 MHz, D_2O) δ 174.79, 174.75, 102.44, 102.41, 102.39, 102.26, 102.24, 95.66 (β-C1), 91.73 (α-C1), 78.91, 78.78, 78.53, 78.21, 78.03, 77.94, 77.91, 75.88, 75.42, 74.72, 74.70, 74.68, 74.20, 73.94, 73.88, 73.85, 73.77, 73.47, 73.04, 72.80, 72.78, 71.24, 71.11, 69.99, 69.39, 69.06, 69.04, 69.02, 68.36, 68.32, 60.49, 59.95, 59.77; m/z (HRMS$^+$) 1129.333 $[M + Na]^+$ ($C_{40}H_{66}O_{35}Na^+$ requires 1129.327).

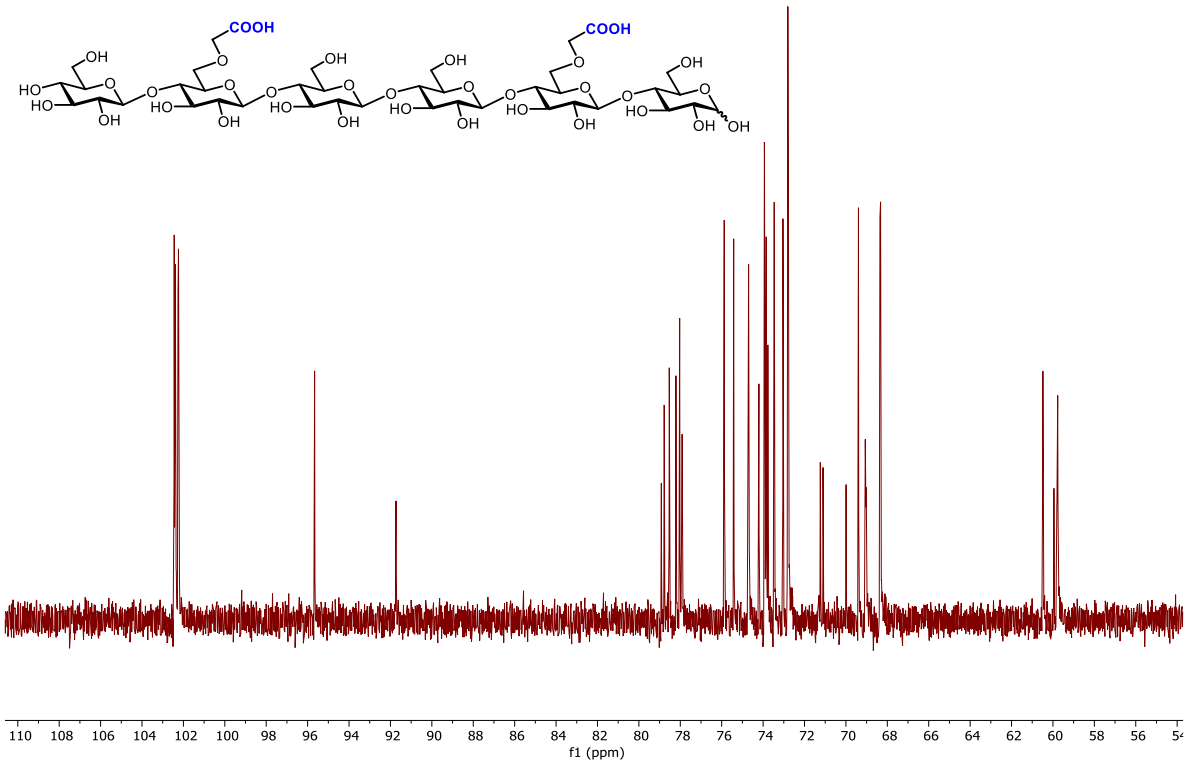
RP-HPLC of AdAAdA-OH (ELSD trace, Method C, t_R = 16.9 min)



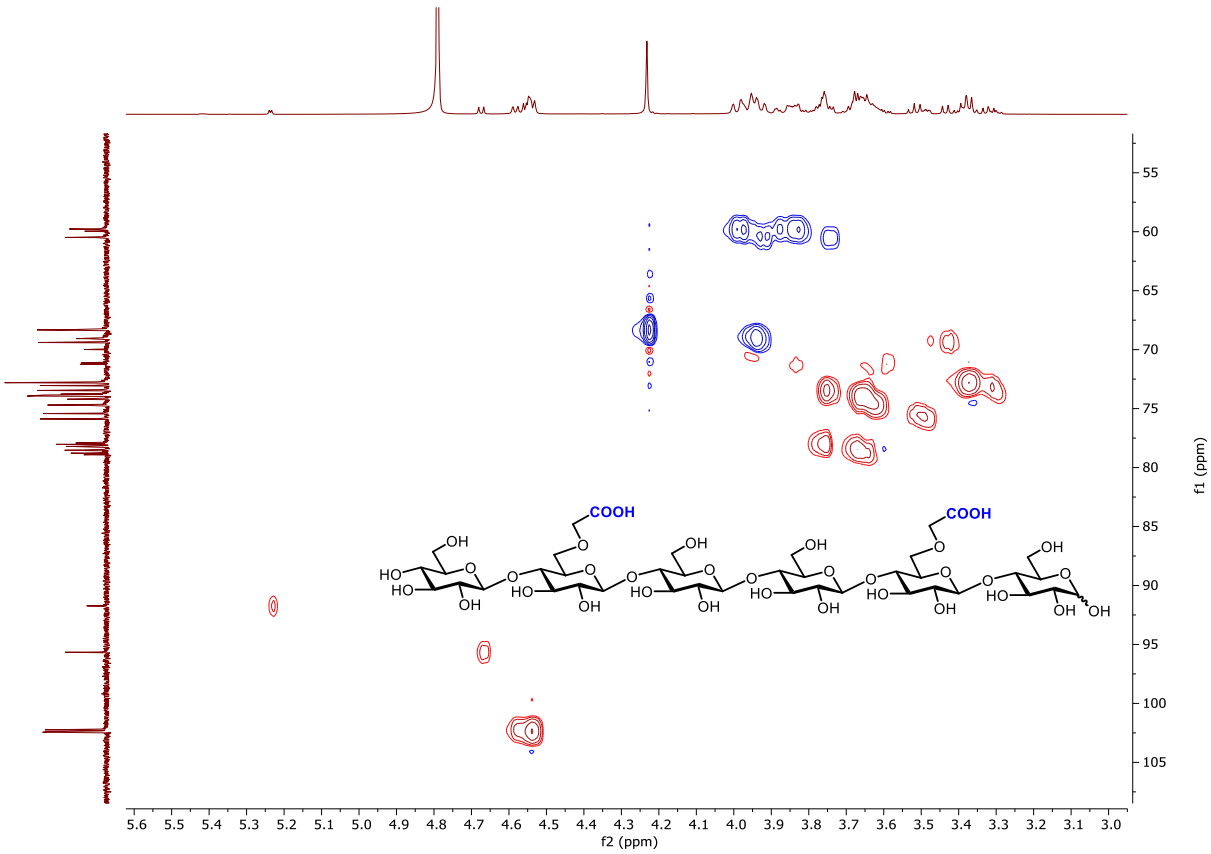
^{1}H NMR of AdAAdA-OH (600 MHz, D_2O)



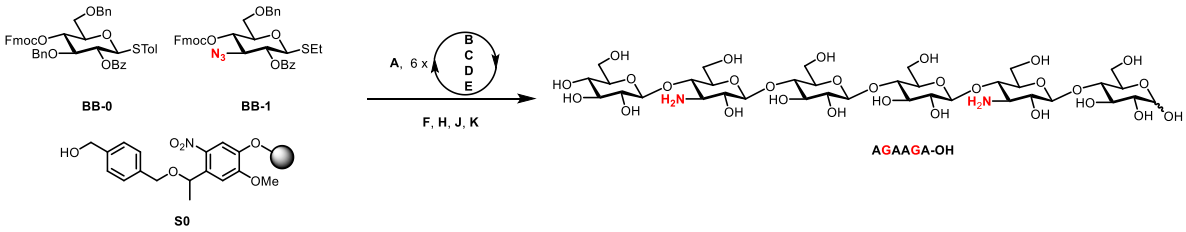
^{13}C NMR of AdAAdA-OH (151 MHz, D_2O)



HSQC NMR of AdAAdA-OH (D_2O)



5. *Synthesis of AGAAGA-OH*

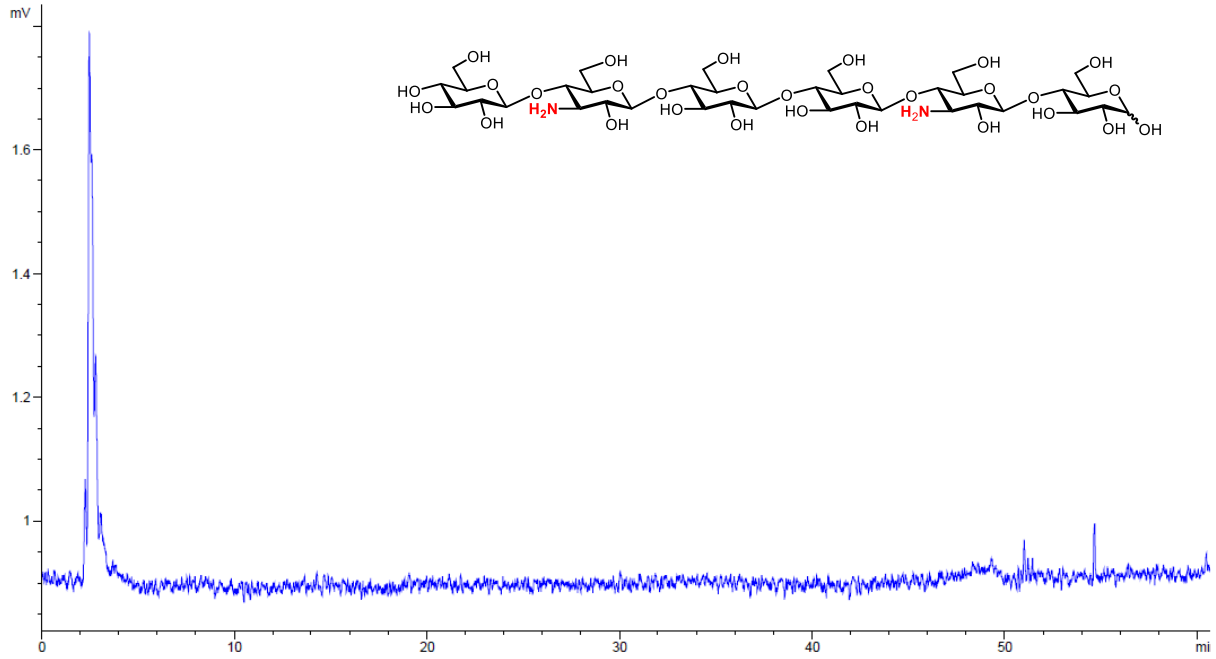


	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-1**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	F: On-resin Staudinger Reduction	
	H: Cleavage from Solid Support	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 4.7 min**

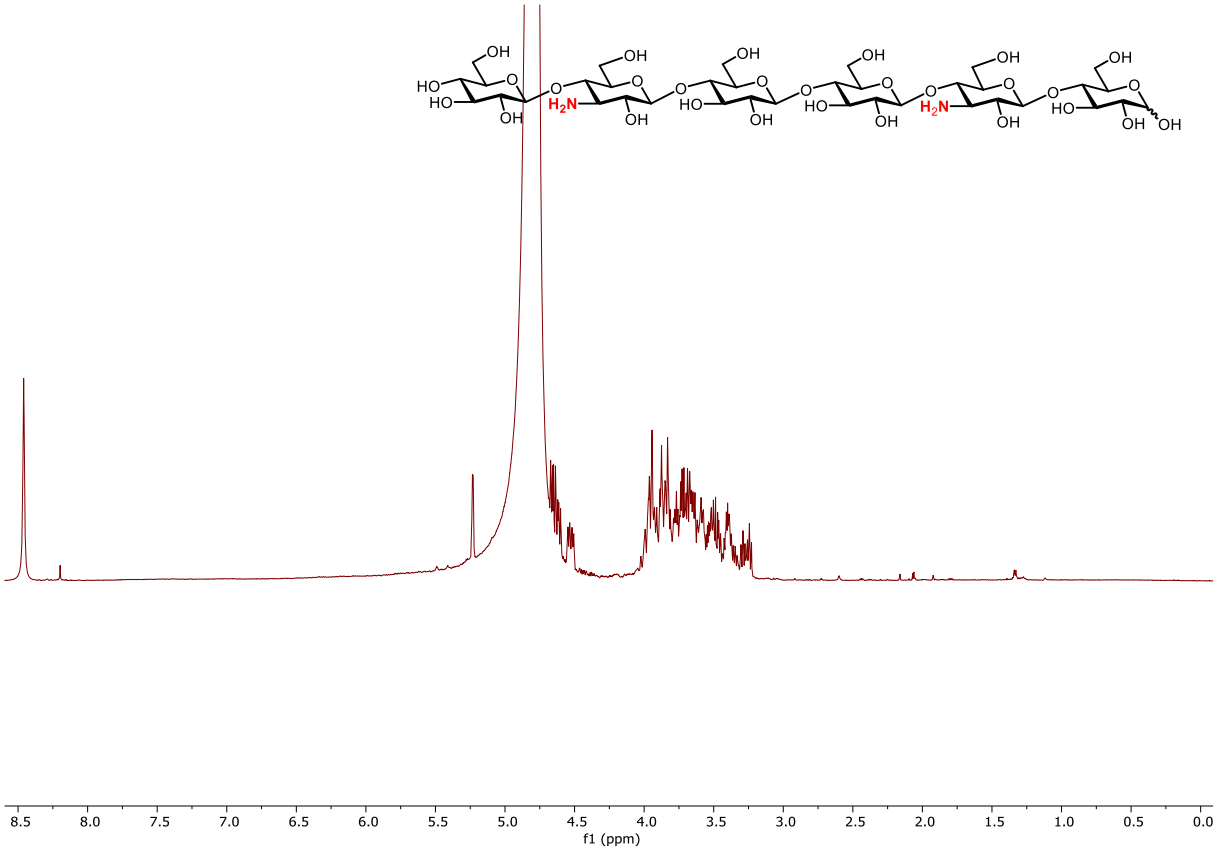
Automated synthesis, global deprotection, and purification afforded **AGAAGA-OH** as white solid (2.2 mg, 15% overall yield).

Analytical data for **AGAAGA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.23 (d, *J* = 3.7 Hz, 0.35H, α-H1), 4.67 (d, *J* = 7.9 Hz, 0.65H, β-H1), 4.64 – 4.59 (m, 3H), 4.56 – 4.50 (m, 2H), 4.05 – 3.80 (m, 11H), 3.80 – 3.63 (m, 8H), 3.63 – 3.44 (m, 8H), 3.44 – 3.31 (m, 5H), 3.31 – 3.20 (m, 4H); ^{13}C NMR (151 MHz, D_2O) δ 102.39, 102.41, 102.28, 102.26, 102.16, 102.14, 95.80, 95.67 (β-C1), 91.99, 91.73 (α-C1), 78.50, 78.36, 78.27, 76.65, 75.95, 75.85, 75.65, 74.68, 74.03, 73.83, 72.55, 72.67, 71.33, 71.14, 70.03, 69.60, 60.52, 69.49, 69.14, 65.83, 60.64, 60.48, 60.38, 59.91, 56.95, 57.53, 55.96; m/z (HRMS$^+$) 989.3672 $[M + H]^+$ ($C_{36}H_{65}N_2O_{29}^+$ requires 989.3668).

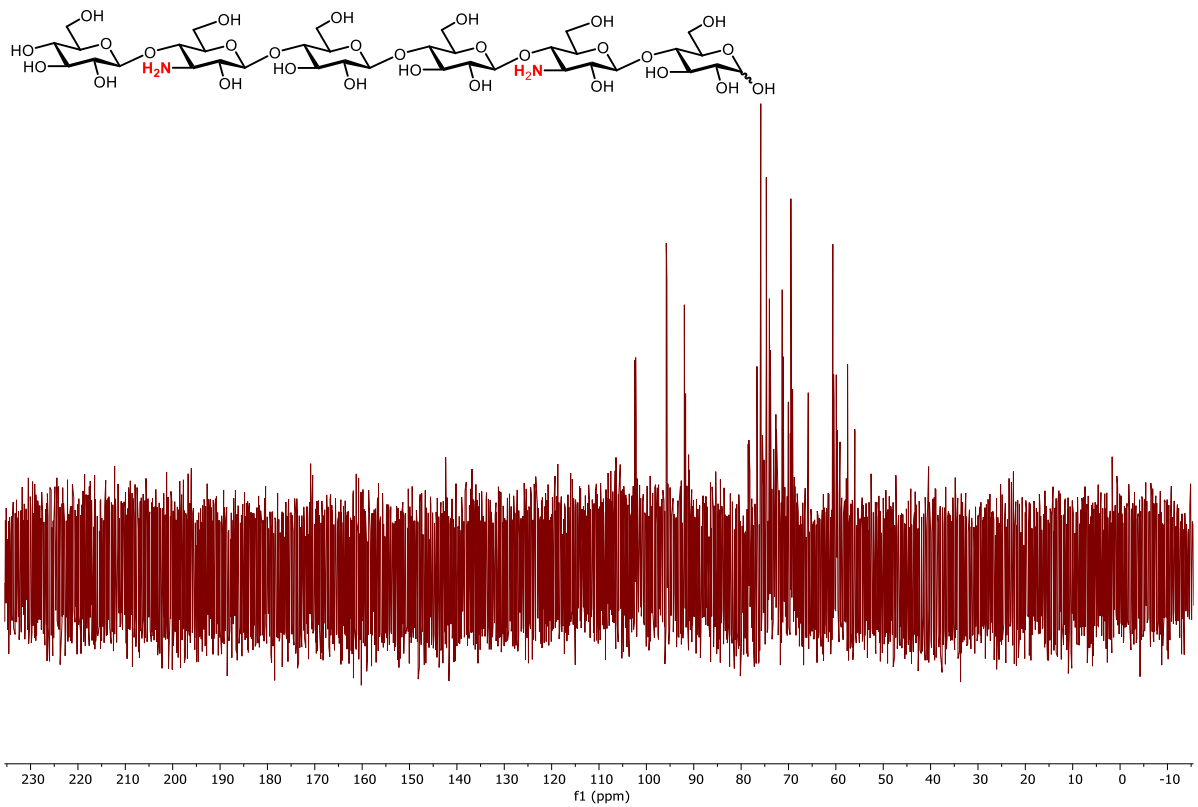
RP-HPLC of AGAAGA-OH (ELSD trace, Method C, t_R = 2.6 min)



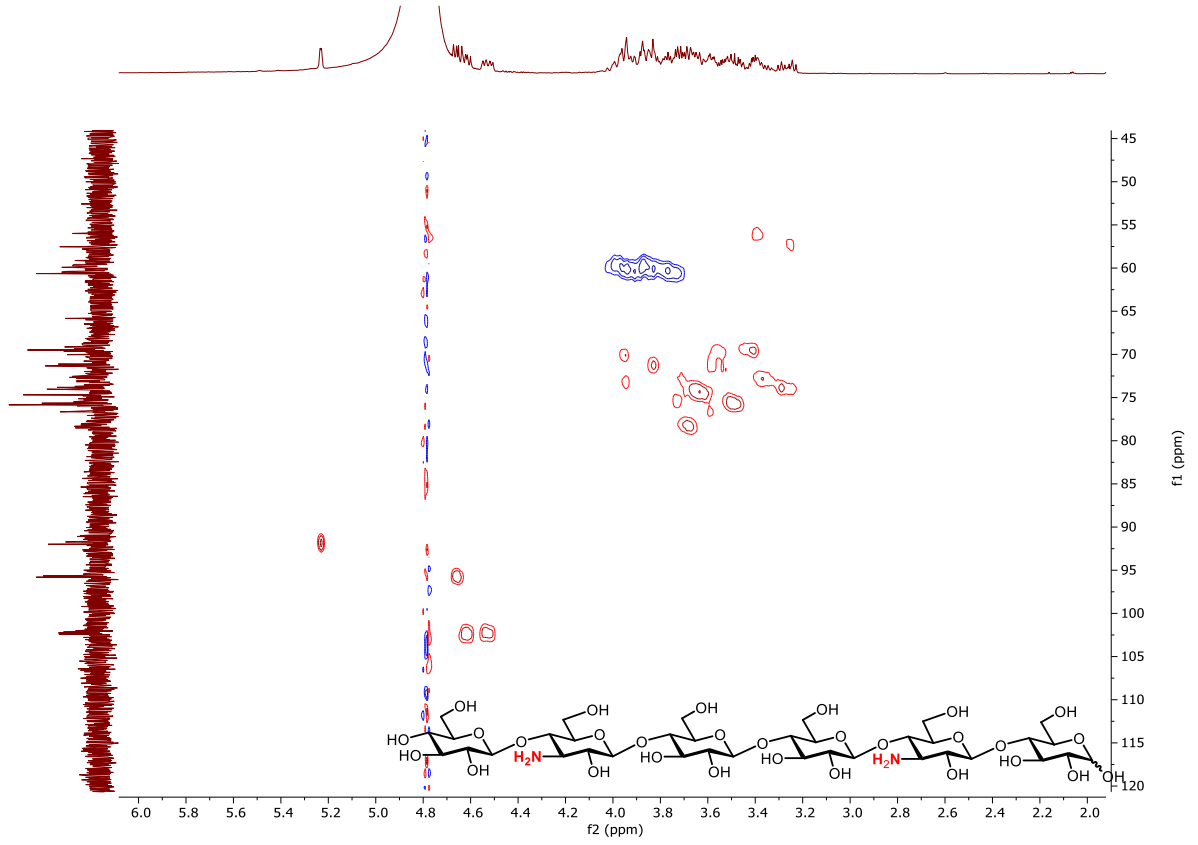
^{1}H NMR of AGAAGA-OH (600 MHz, D_2O)



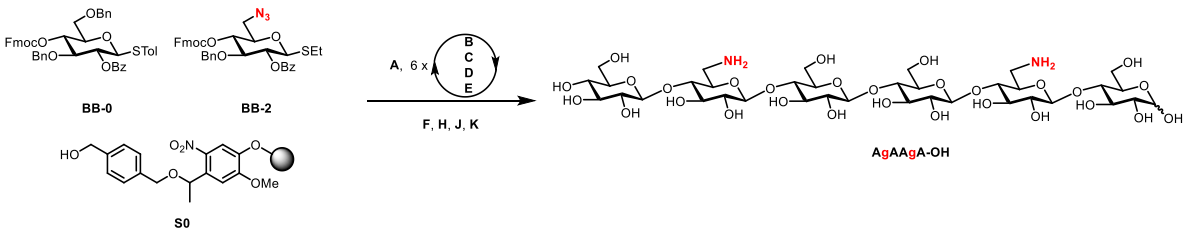
^{13}C NMR of AGAAGA-OH (151 MHz, D_2O)



HSQC NMR of AGAAGA-OH (D_2O)



6. *Synthesis of AgAAgA-OH*

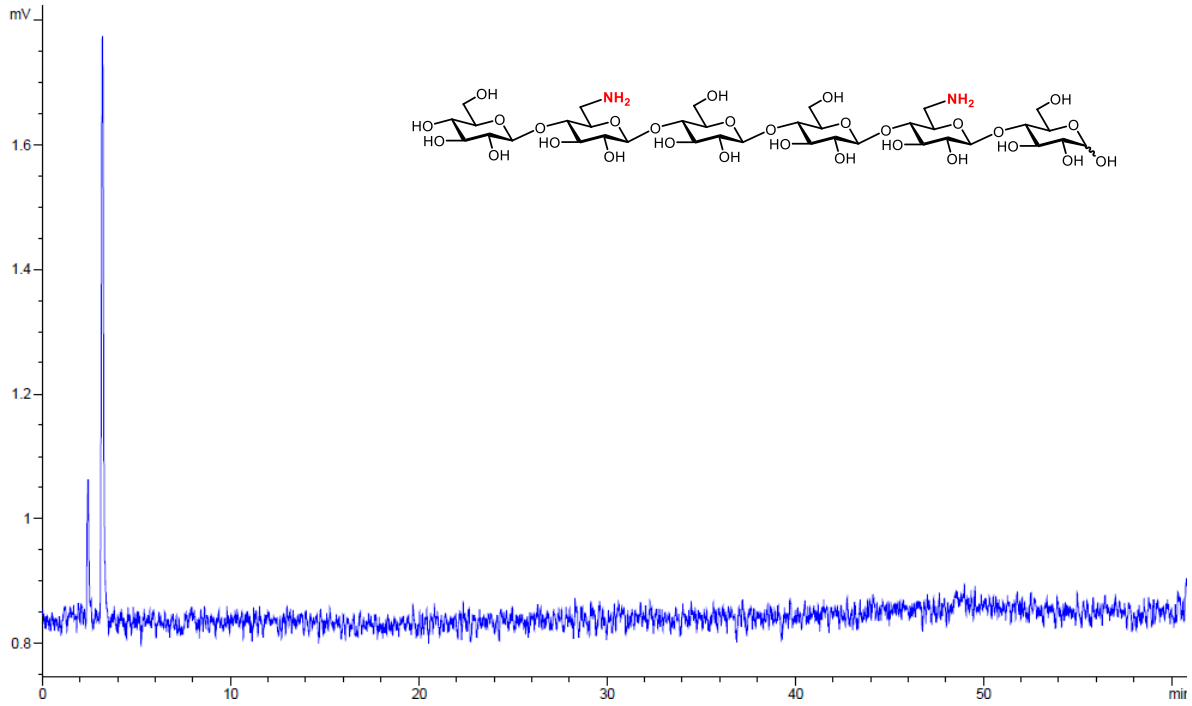


	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-2**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	F: On-resin Staudinger Reduction	
	H: Cleavage from Solid Support	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 4.3 min**

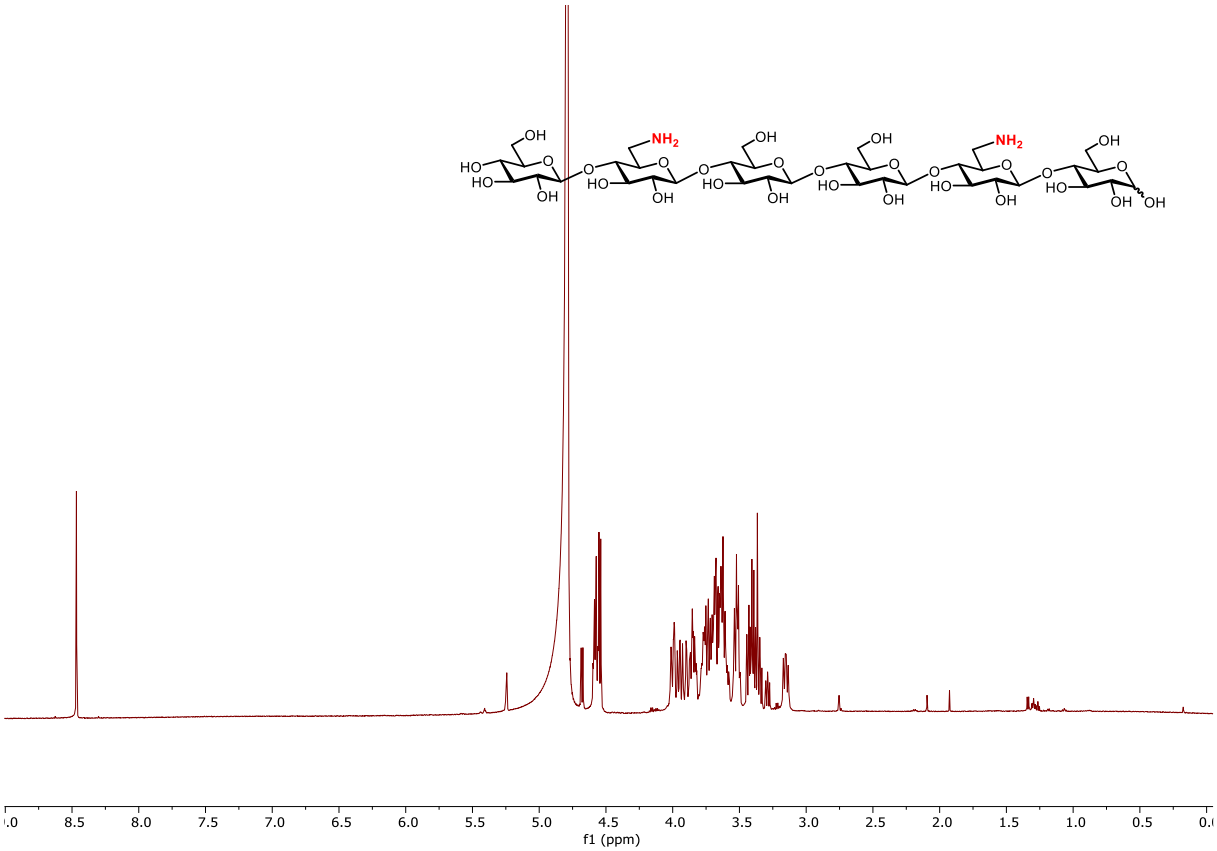
Automated synthesis, global deprotection, and purification afforded **AgAAgA-OH** as white solid (3.5 mg, 24% overall yield).

Analytical data for **AgAAgA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.25 (d, *J* = 3.8 Hz, 0.39H, α-H1), 4.68 (d, *J* = 7.9 Hz, 0.61H, β-H1), 4.61 – 4.52 (m, 5H), 4.04 – 3.88 (m, 5H), 3.88 – 3.80 (m, 5H), 3.80 – 3.56 (m, 13H), 3.55 – 3.49 (m, 4H), 3.45 – 3.33 (m, 5H), 3.29 (dd, *J* = 9.5, 7.9 Hz, 1H), 3.15 (dd, *J* = 13.5, 8.7 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 102.56, 102.40, 102.38, 101.85, 101.80, 95.77 (β-C1), 91.79 (α-C1), 80.39, 78.12, 76.80, 76.61, 76.56, 76.02, 75.42, 74.87, 74.84, 74.00, 73.98, 73.95, 73.77, 73.68, 73.66, 73.00, 72.98, 72.80, 72.76, 71.30, 71.07, 71.01, 70.24, 69.32, 60.48, 59.76, 59.66, 59.53, 40.07, 40.05; m/z (HRMS$^+$) 1011.327 $[M + Na]^+$ ($C_{36}H_{64}N_2O_{29}Na^+$ requires 1011.348).

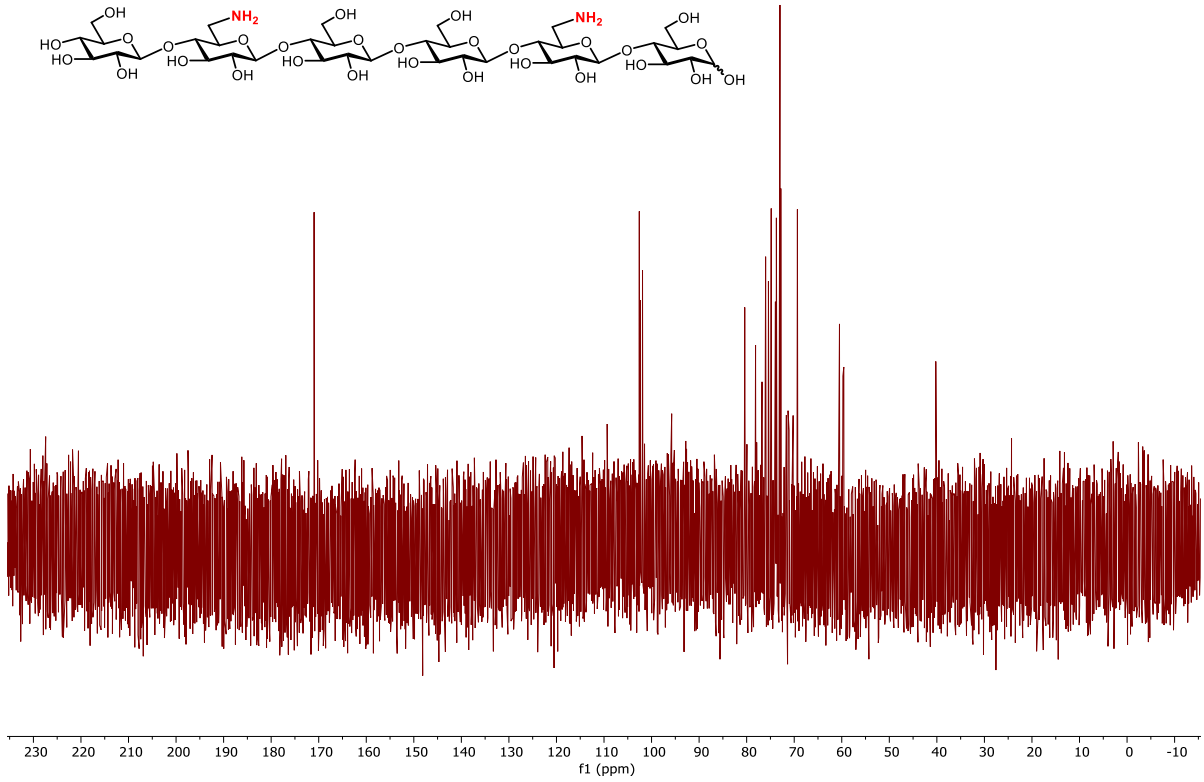
RP-HPLC of AgAAgA-OH (ELSD trace, Method C, t_R = 3.3 min)



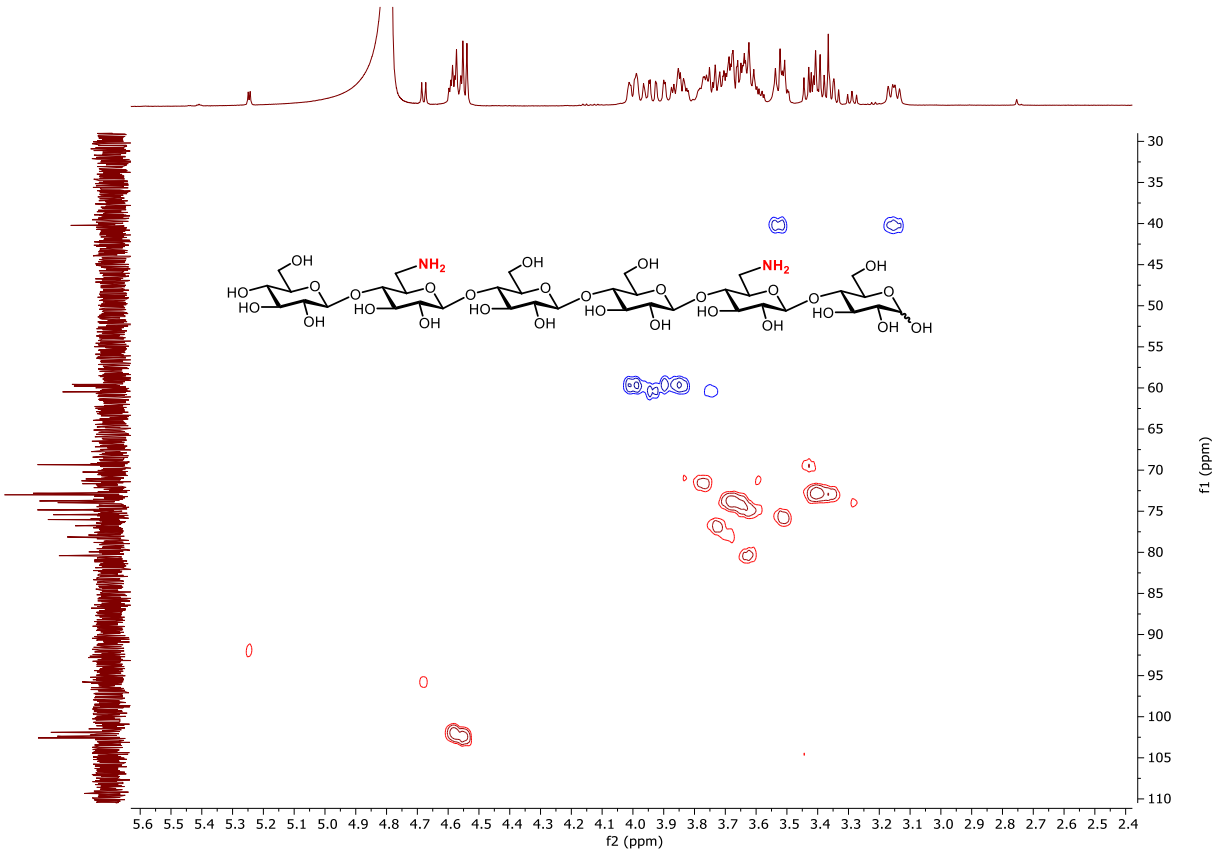
^{1}H NMR of AgAAgA-OH (600 MHz, D_2O)



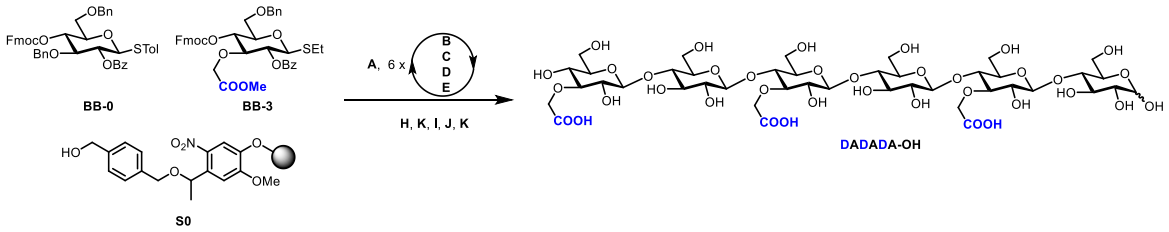
^{13}C NMR of AgAAgA-OH (151 MHz, D_2O)



HSQC NMR of AgAAgA-OH (D_2O)



7. *Synthesis of DADADA-OH* [3]

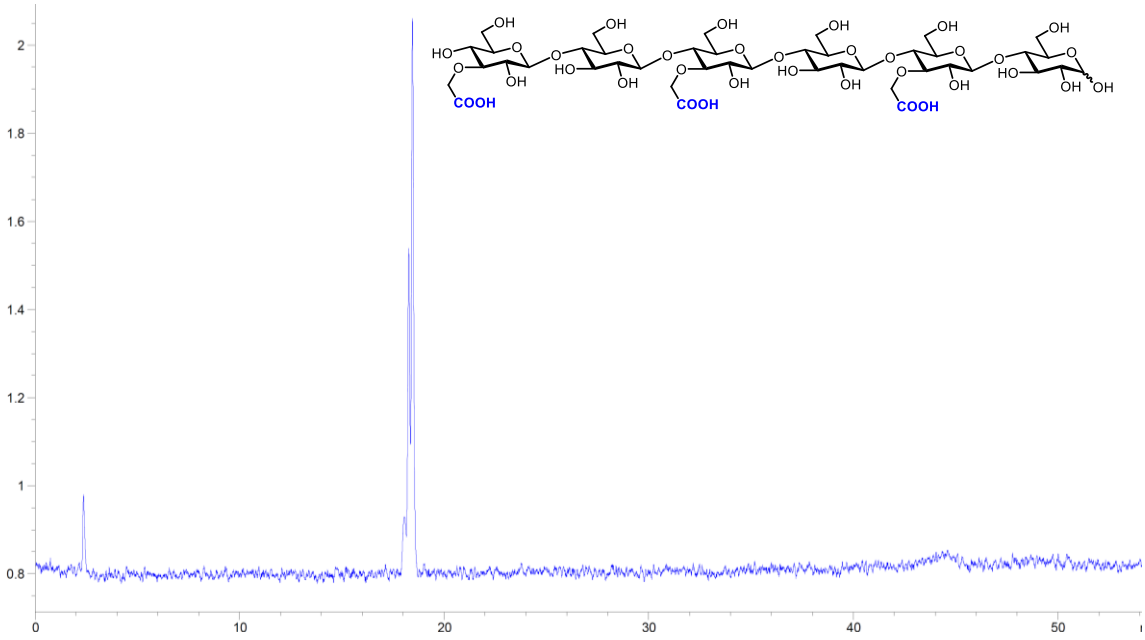


	Module	Conditions
	A: Resin Preparation for Synthesis	
3	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	H: Cleavage from Solid Support	
	K: Purification	**Method E and Method B, t_R = 36.4 min**
	I: Solution-phase Methanolysis	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 17.8 min**

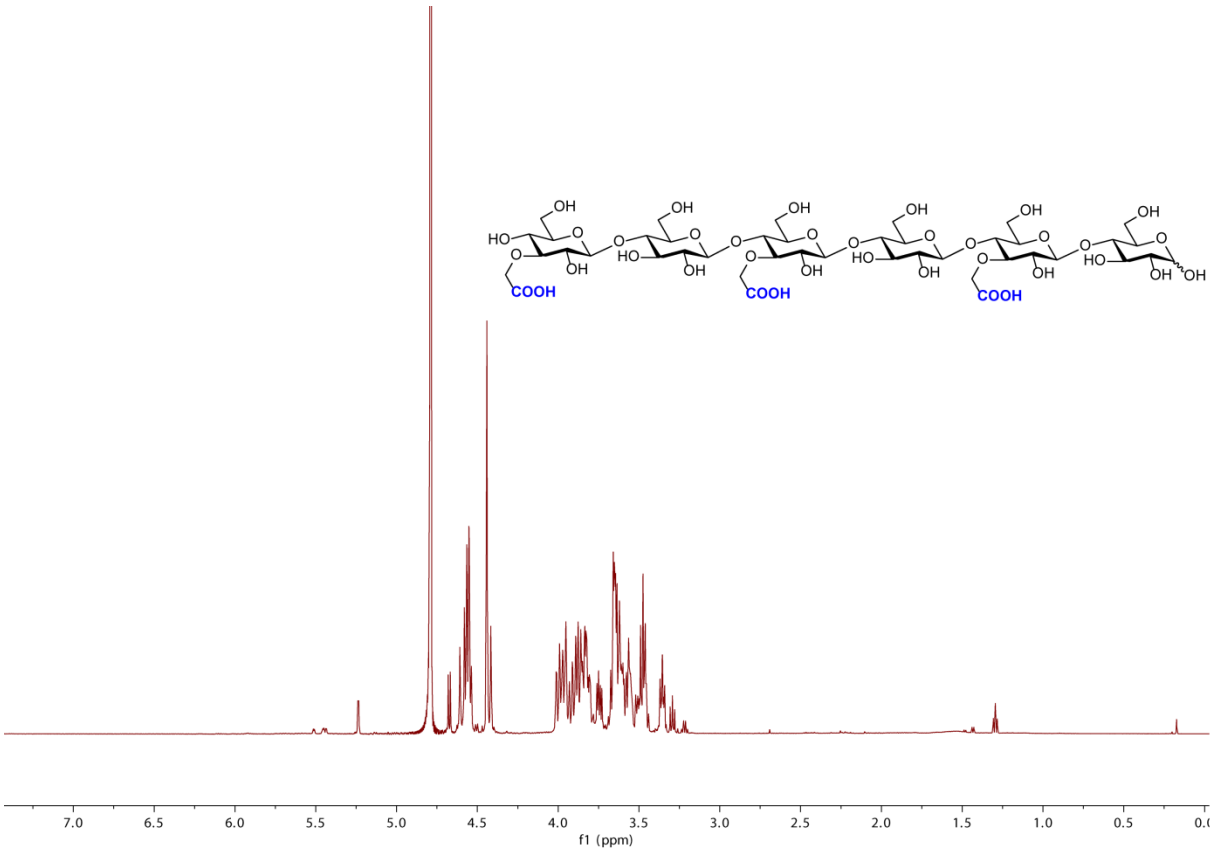
Automated synthesis, global deprotection, and purification afforded **DADADA-OH** as white solid (3.4 mg, 24 % overall yield).

Analytical data for **DADADA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 0.35H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.65H, β-H1), 4.66 – 4.48 (m, 7H), 4.48 – 4.37 (m, 4H), 4.04 – 3.80 (m, 14H), 3.80 – 3.71 (m, 2H), 3.70 – 3.59 (m, 9H), 3.59 – 3.53 (m, 3H), 3.53 – 3.44 (m, 5H), 3.36 (ddd, *J* = 10.1, 7.4, 5.0 Hz, 2H), 3.32 – 3.20 (m, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.75, 175.24, 175.21, 175.17, 166.06, 102.28, 102.25, 102.24, 102.15, 102.12, 95.68 (β-C1), 91.74 (α-C1), 85.22, 82.89, 82.87, 78.50, 78.44, 78.35, 76.13, 75.61, 74.84, 74.71, 74.15, 74.01, 73.98, 73.81, 73.31, 72.74, 72.46, 71.21, 71.15, 70.03, 69.82, 69.63, 68.89, 60.36, 60.08,60.05, 59.92, 59.79, 59.67; m/z (HRMS$^+$) 1187.332 $[M + Na]^+$ ($C_{42}H_{68}O_{37}Na^+$ requires 1187.333).

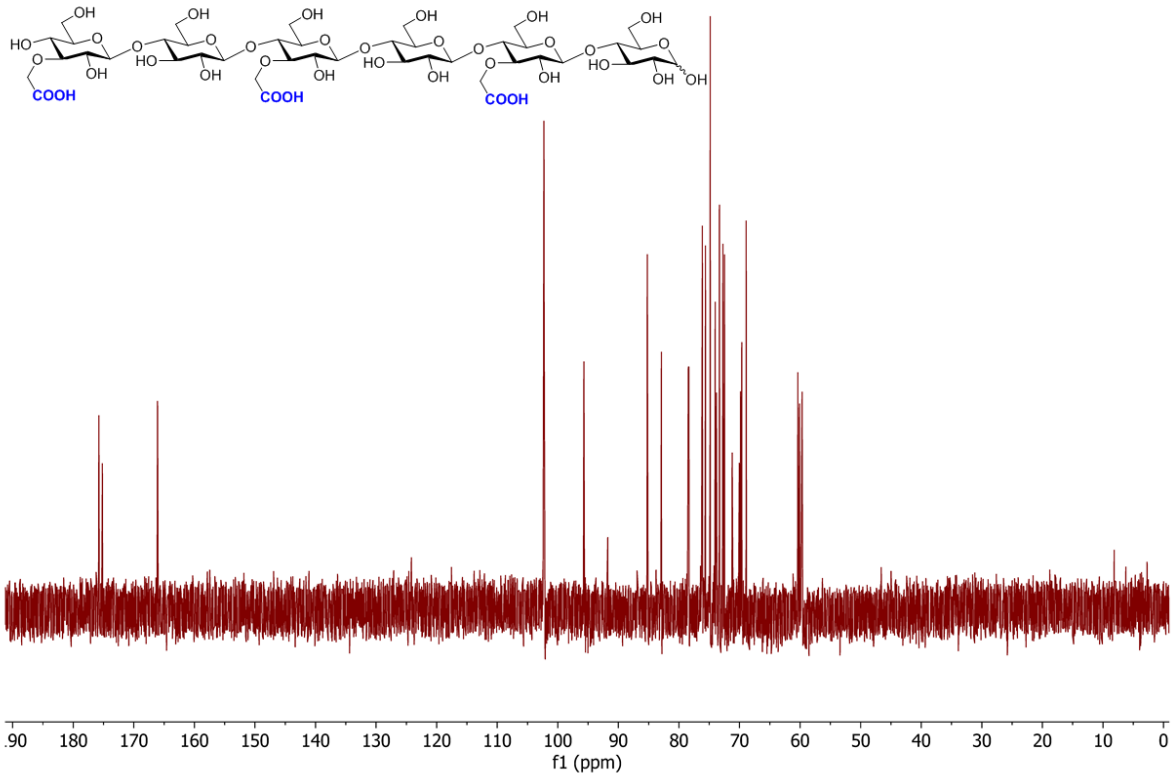
RP-HPLC of DADADA-OH (ELSD trace, Method C, t_R = 18.1, 18.3 min)



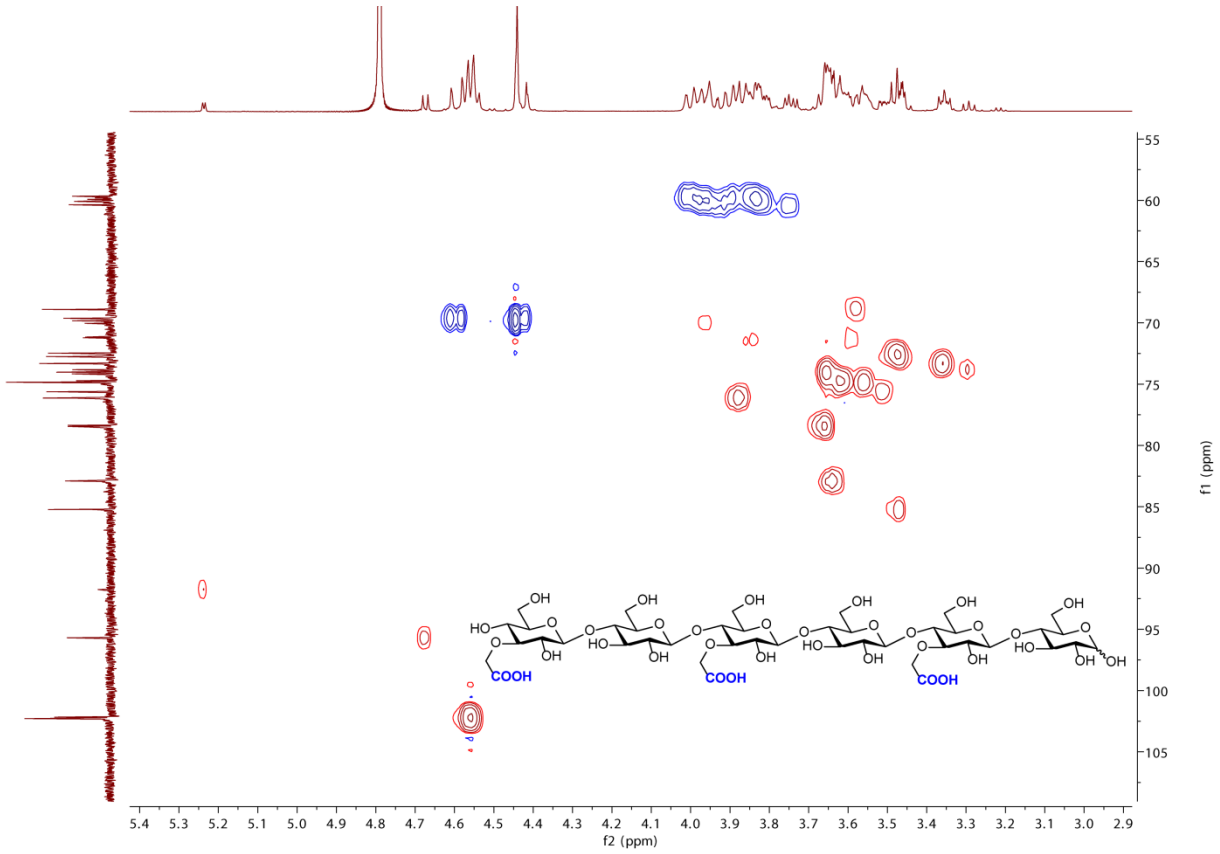
^{1}H NMR of DADADA-OH (600 MHz, D_2O)



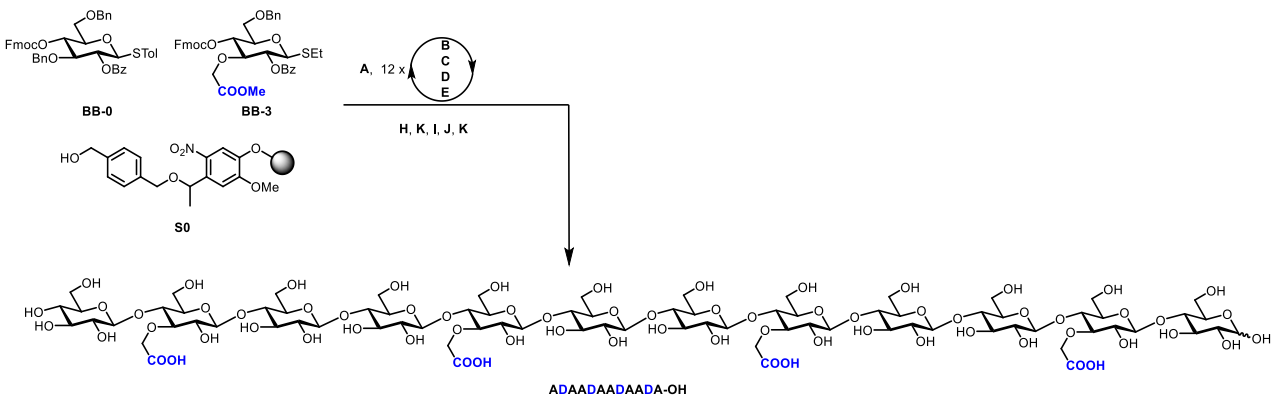
^{13}C NMR of DADADA-OH (151 MHz, D_2O)



HSQC NMR of DADADA-OH (D_2O)



8. *Synthesis of ADAADAADAAD-OH* [3]



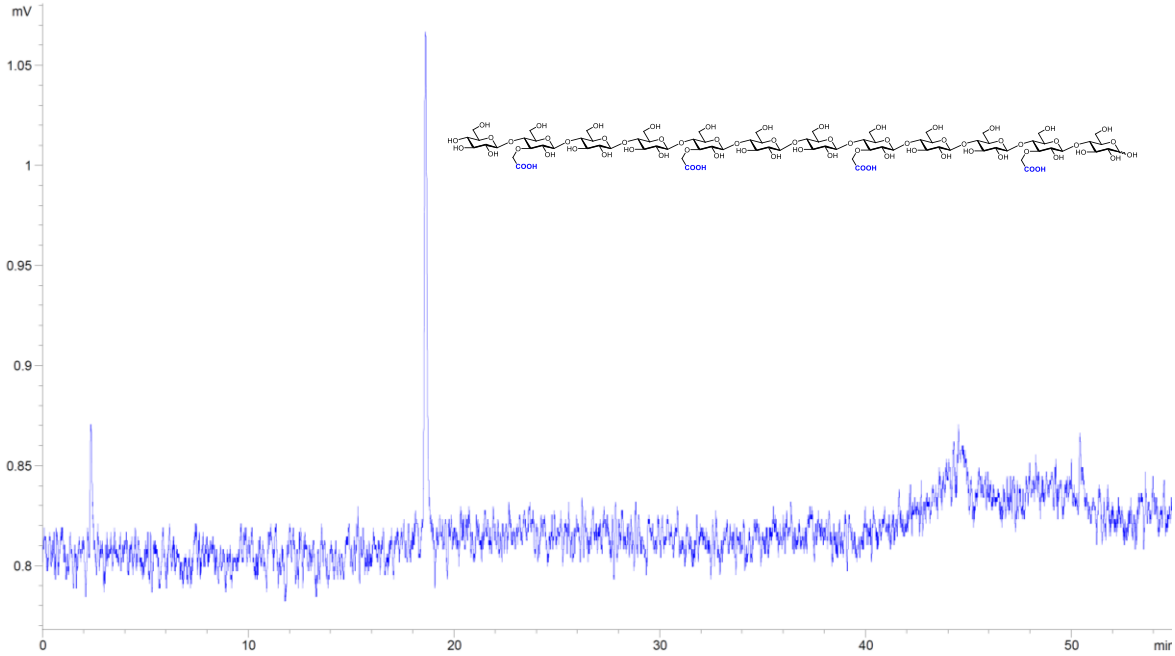
	Module	Conditions
	A: Resin Preparation for Synthesis	
4	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	H: Cleavage from Solid Support **K**: Purification	**Method E and Method B, t_R = 36.3 min**
	I: Solution-phase Methanolysis **J**: Hydrogenolysis at Ambient Pressure **K**: Purification	**Method D, t_R = 17.9 min**

Automated synthesis, global deprotection, and purification afforded **ADAADAADAADA-OH** as white solid (4.8 mg, 17 % overall yield).

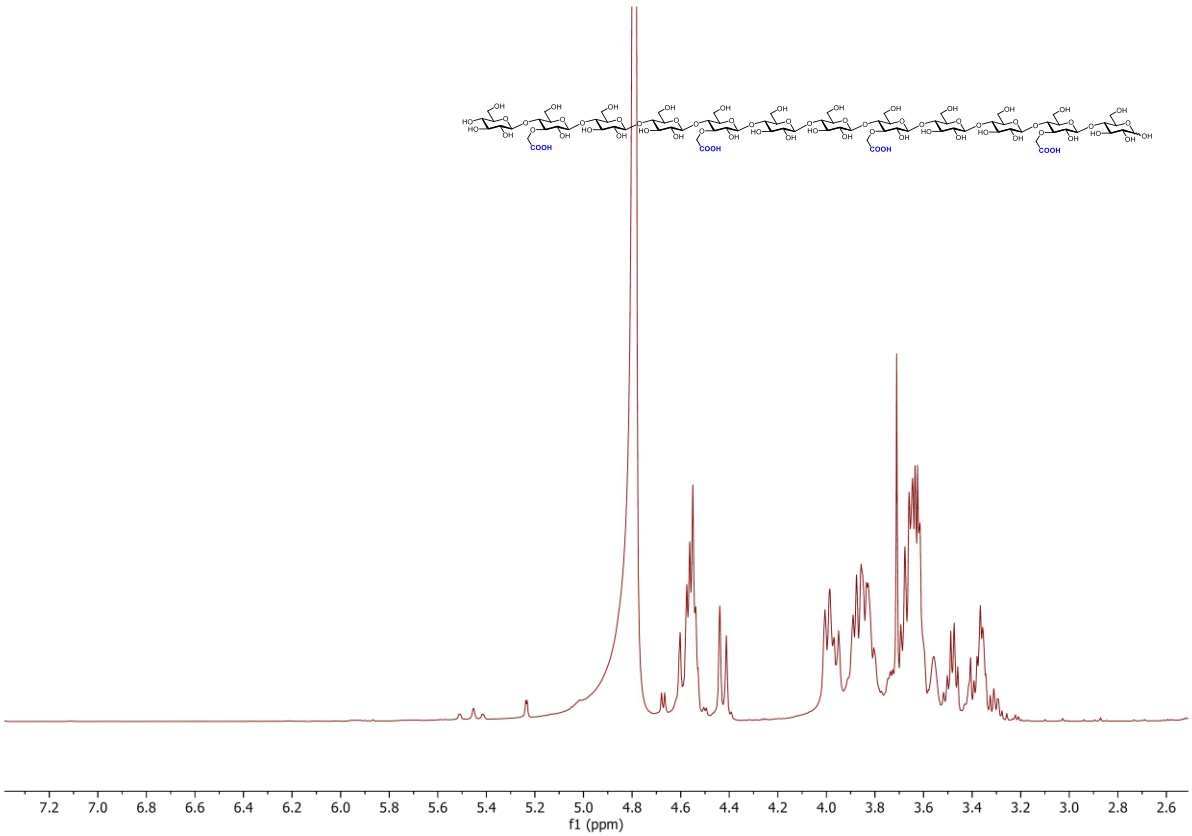
Analytical data for **ADAADAADAADA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.7 Hz, 0.31H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.69H, β-H1), 4.64 – 4.50 (m, 9H), 4.43 (d, *J* = 16.4 Hz, 4H), 4.07 – 3.93 (m, 12H), 3.93 – 3.77 (m, 19H), 3.76 – 3.54 (m, 30H), 3.53 – 3.45 (m, 6H), 3.45 – 3.25 (m, 11H); ^{13}C NMR (151 MHz, D_2O) δ 175.27, 166.10, 102.34, 102.29, 102.24, 102.14, 95.69 (β-C1), 91.75 (α-C1), 82.92, 82.89, 78.50, 78.43, 78.33, 78.11, 76.13, 76.04, 75.96, 75.48, 74.85, 74.72, 74.16, 74.00, 73.90, 73.81, 73.57,

73.31, 72.87, 72.47, 71.21, 71.15, 70.03, 69.66, 69.49, 60.82, 60.67, 60.08, 59.76, 59.67; m/z (HRMS$^+$) 2217.641 $[M + Na]^+$ ($C_{80}H_{130}O_{69}Na^+$ requires 2217.655).

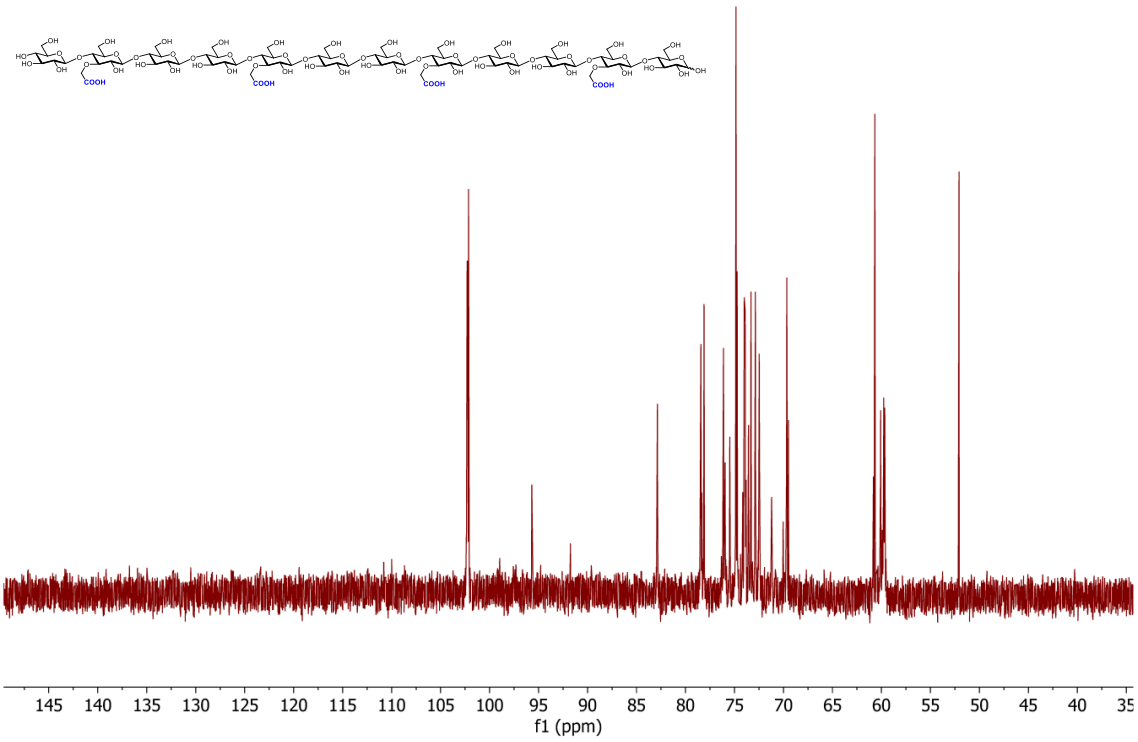
RP-HPLC of ADAADAADAADA-OH (ELSD trace, Method C, t_R = 18.5 min)



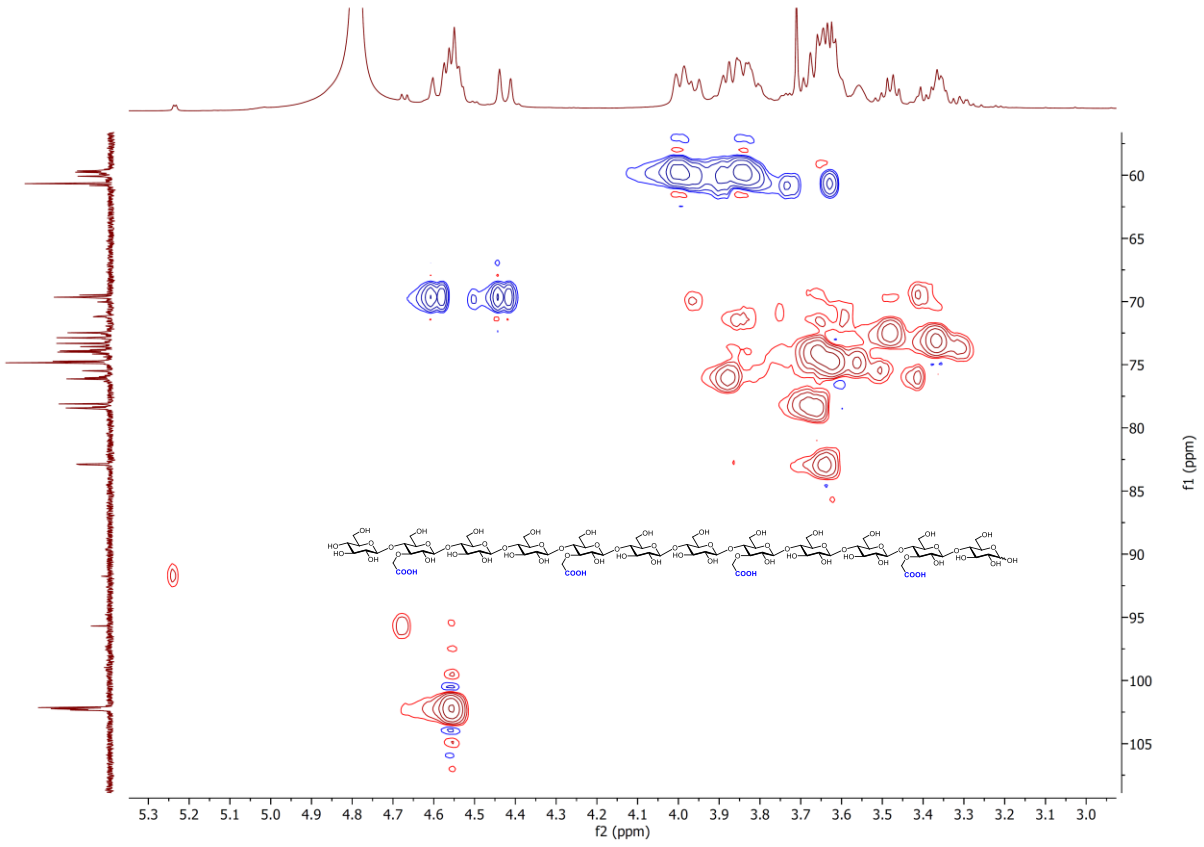
^{1}H NMR of ADAADAADAADA-OH (600 MHz, D_2O)



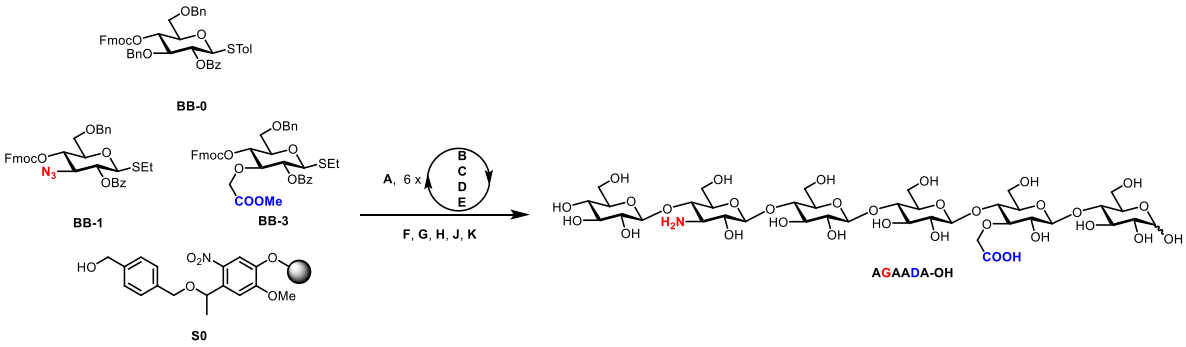
^{13}C NMR of ADAADAADAADA-OH (151 MHz, D_2O)



HSQC NMR of ADAADAADAADA-OH (D_2O)



9. *Synthesis of AGAADA-OH*

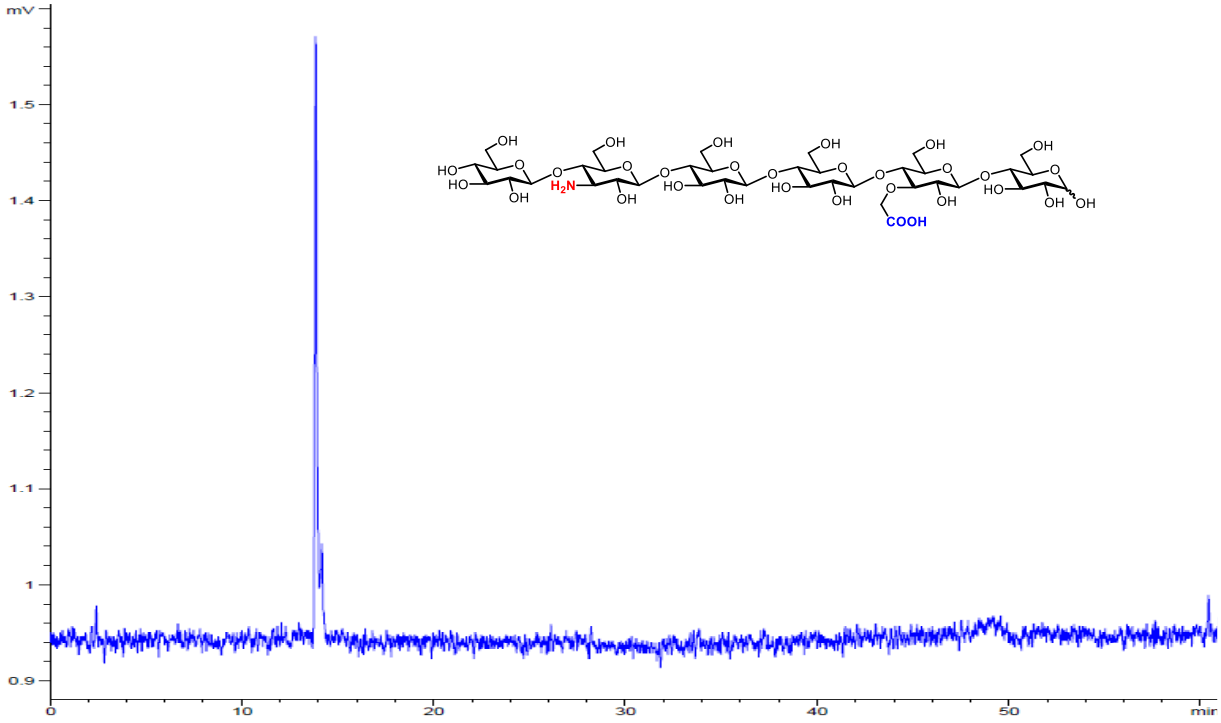


	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-1**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	F: On-resin Staudinger Reduction **G**: On-resin Methanolysis **H:** Cleavage from Solid Support **J**: Hydrogenolysis at Ambient Pressure **K**: Purification	**Method D, t_R = 10.3 min**

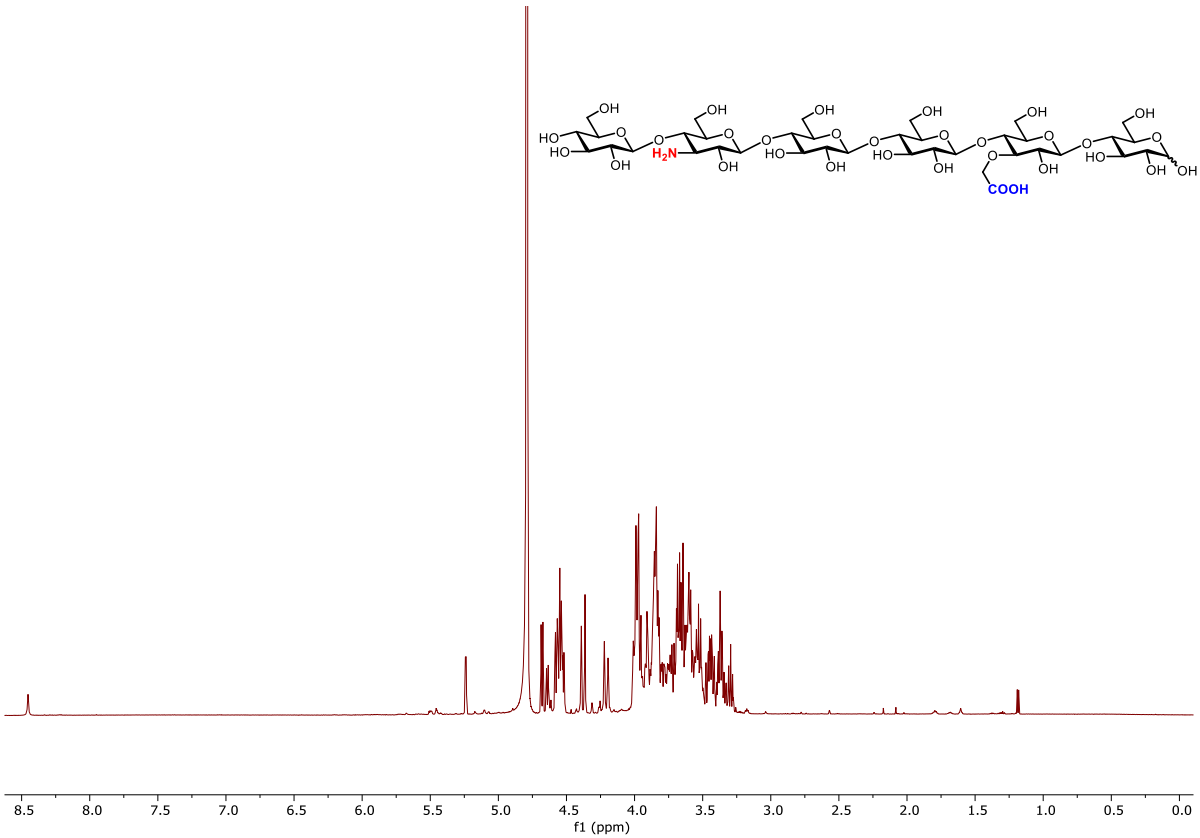
Automated synthesis, global deprotection, and purification afforded **AGAADA-OH** as white solid (3.2 mg, 21% overall yield).

Analytical data for **AGAADA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 0.38H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.62H, β-H1), 4.65 – 4.50 (m, 5H), 4.38 and 4.21 (ABq, *J* = 16.2 Hz, 2H), 4.04 – 3.81 (m, 13H), 3.81 – 3.72 (m, 3H), 3.72 – 3.48 (m, 13H), 3.48 – 3.41 (m, 3H), 3.40 – 3.32 (m, 3H), 3.32 – 3.27 (m, 1H); ^{13}C NMR (151 MHz, D_2O) δ 178.59, 102.49, 102.27, 102.24, 102.17, 95.70 (β-C1), 91.76 (α-C1), 83.02, 82.93, 78.53, 78.45, 78.26, 78.08, 76.32, 75.96, 75.86, 75.49, 75.40, 75.13, 75.00, 74.79, 74.73, 74.68, 74.14, 74.01, 73.82, 73.40, 73.36, 73.10, 73.03, 72.89, 72.78, 72.25, 71.21, 71.17, 71.15, 70.03, 69.47, 69.40, 69.38, 69.16, 60.48, 60.40, 60.09, 60.01, 59.89, 59.76, 59.69, 59.13, 56.01; m/z (HRMS^{+}) 1048.354 $[M + H]^{+}$ ($C_{38}H_{66}NO_{32}^{+}$ requires 1048.356).

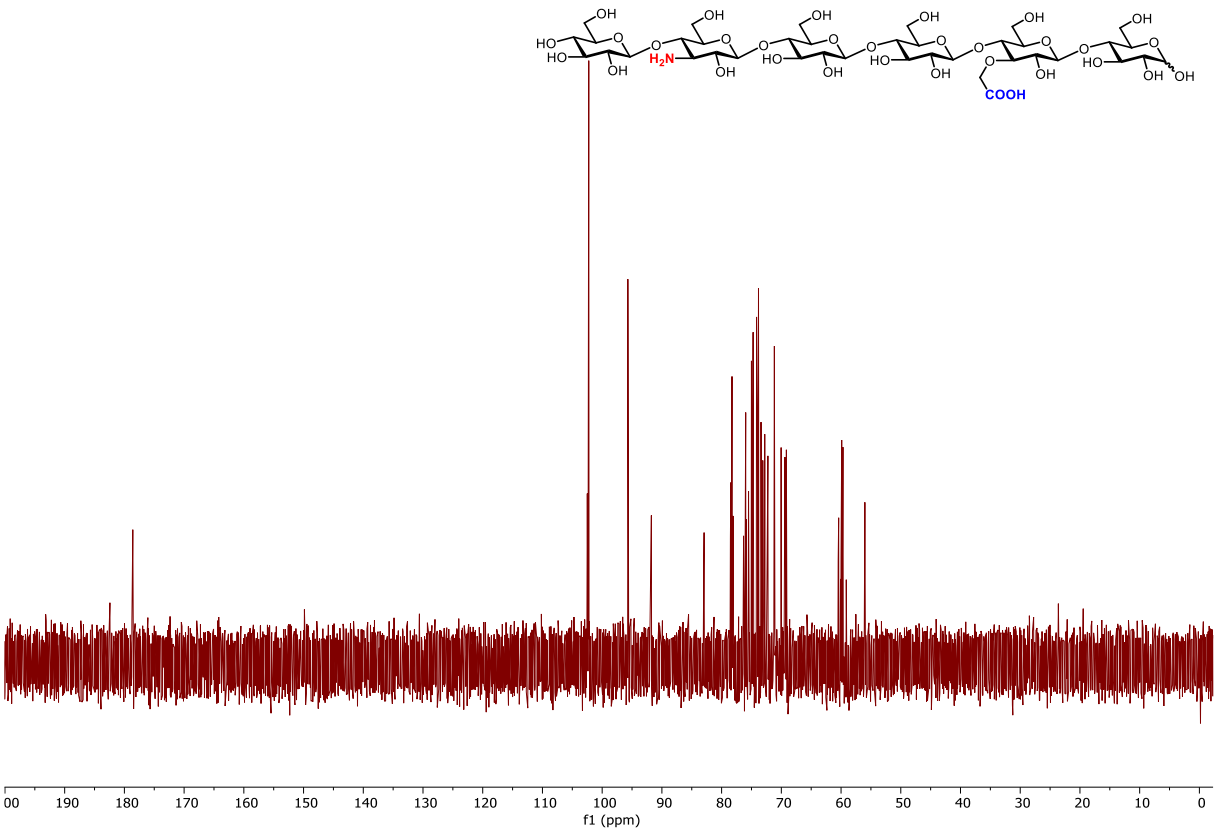
RP-HPLC of AGAADA-OH (ELSD trace, Method C, t_R = 13.8 min)



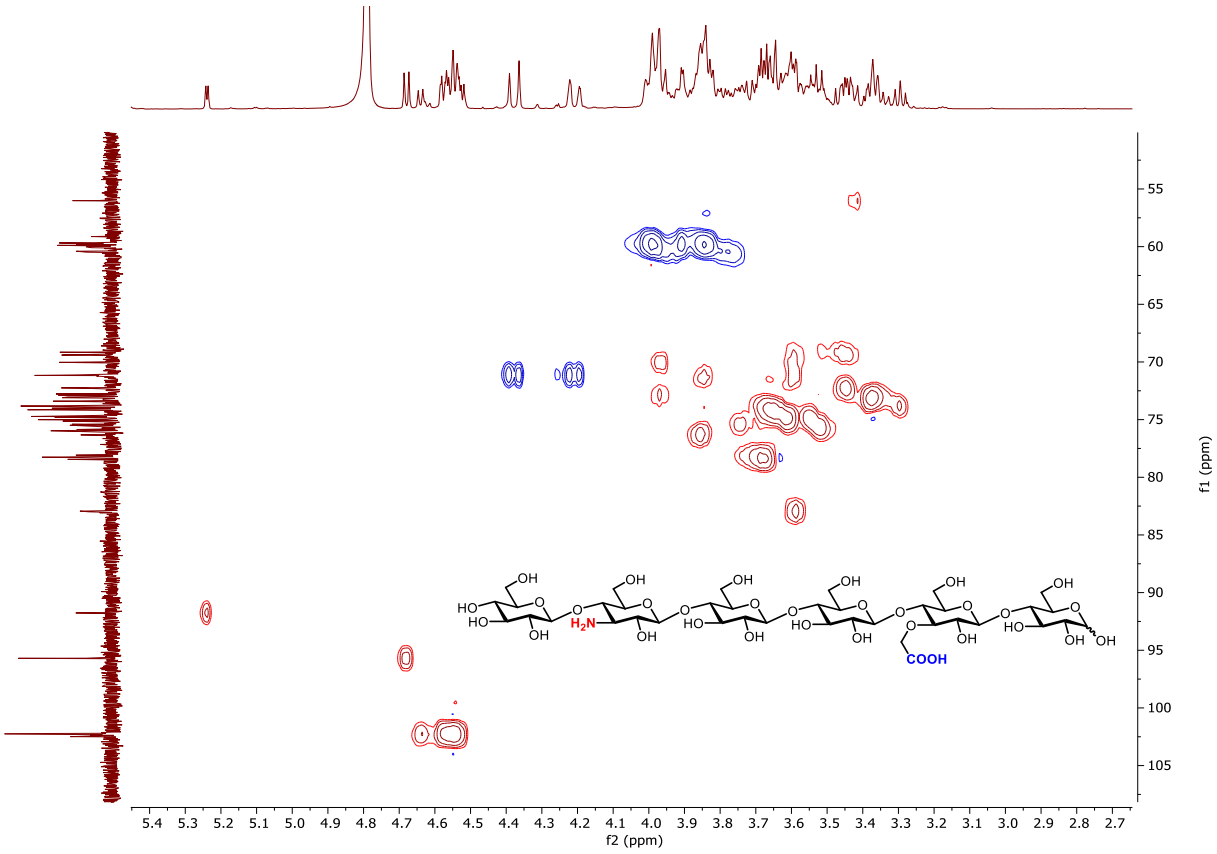
^{1}H NMR of AGAADA-OH (600 MHz, D_2O)



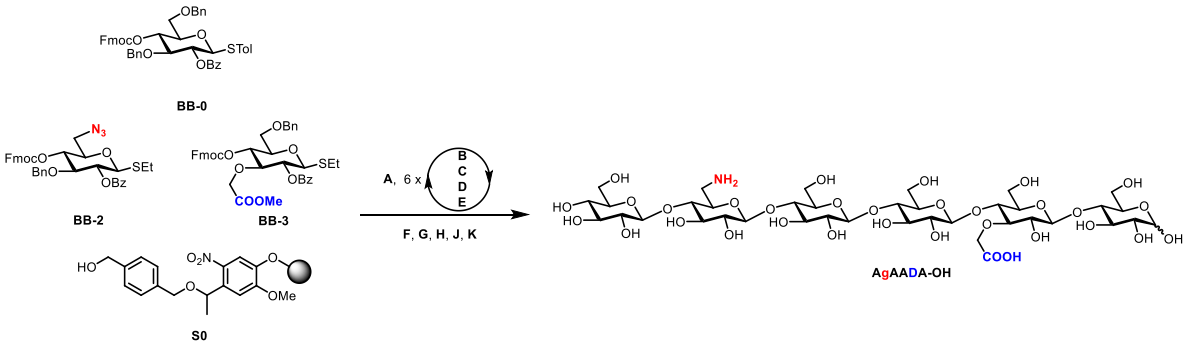
^{13}C NMR of AGAADA-OH (151 MHz, D_2O)



HSQC NMR of AGAADA-OH (D_2O)



10. *Synthesis of AgAADA-OH*



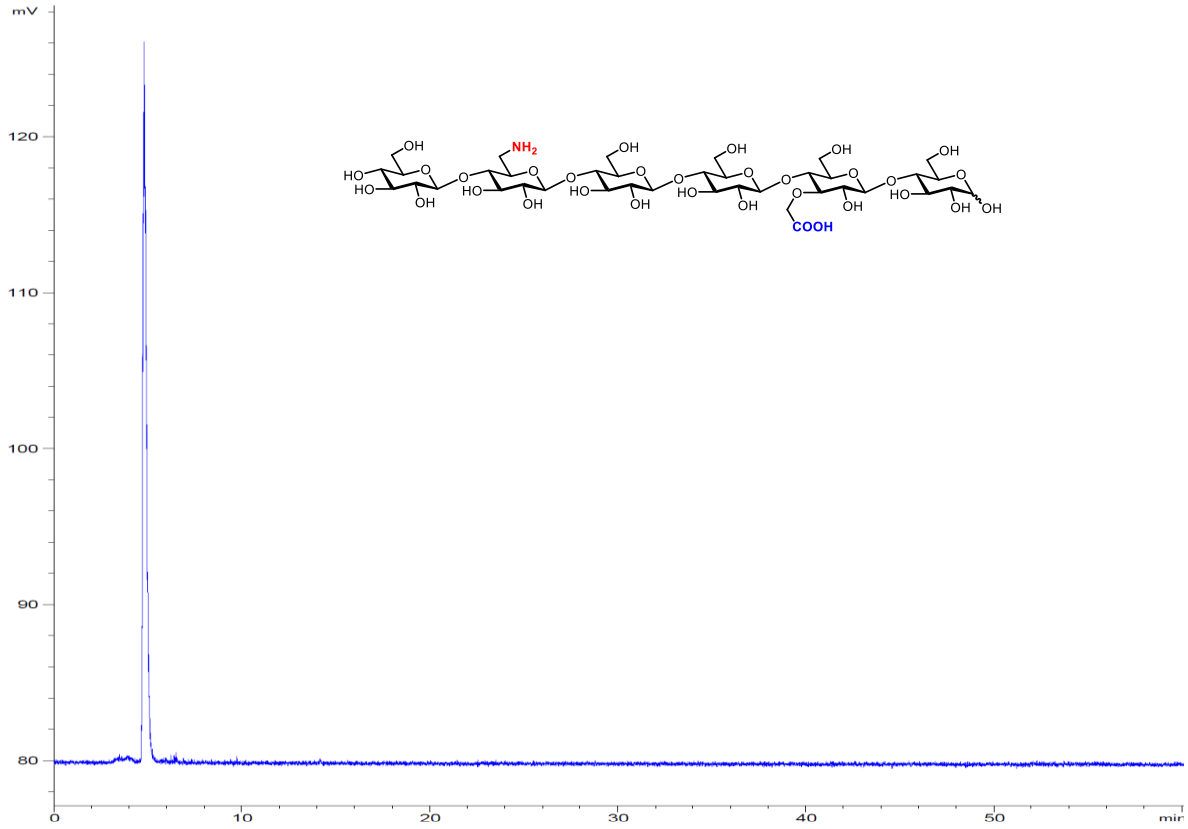
Module	Conditions
A: Resin Preparation for Synthesis	
B: Acidic Wash with TMSOTf Solution	
C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
D: Capping	
E: Fmoc Deprotection	
B: Acidic Wash with TMSOTf Solution	

	C: Thioglycoside Glycosylation	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
2	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-2**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	B: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	F: On-resin Staudinger Reduction	
	G: On-resin Methanolysis	
	H: Cleavage from Solid Support	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 4.8 min**

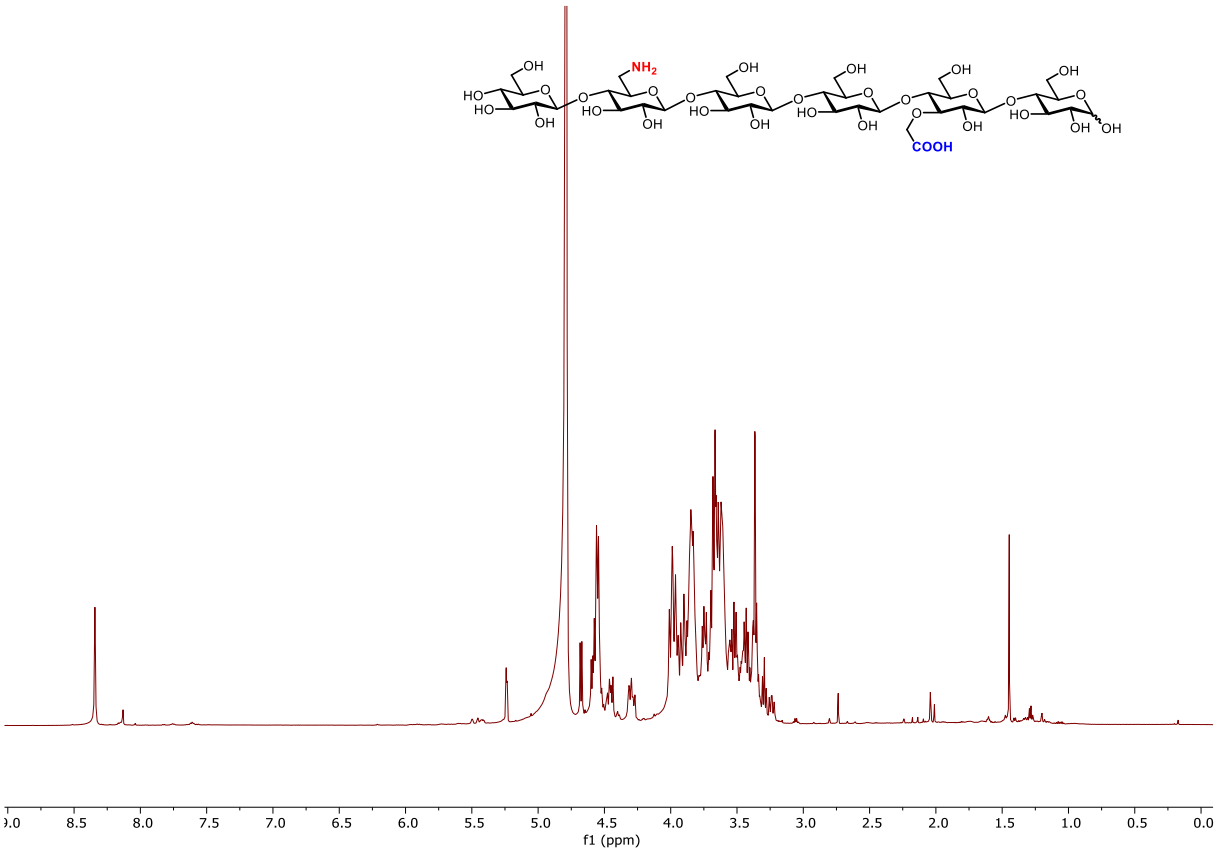
Automated synthesis, global deprotection, and purification afforded **AgAADA-OH** as white solid (4.3 mg, 28% overall yield).

Analytical data for **AgAADA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 0.34H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.66H, β-H1), 4.63 – 4.50 (m, 5H), 4.49 – 4.42 (m, 1H), 4.33 – 4.26 (m, 1H), 4.08 – 3.79 (m, 12H), 3.75 (dd, *J* = 12.0, 6.6 Hz, 3H), 3.71 – 3.57 (m, 10H), 3.57 – 3.49 (m, 3H), 3.49 – 3.40 (m, 3H), 3.40 – 3.32 (m, 3H), 3.32 – 3.27 (m, 1H), 3.24 (dd, *J* = 13.3, 8.9 Hz, 1H); ^{13}C NMR (151 MHz, Deuterium Oxide) δ 167.89, 102.54, 102.36, 102.24, 102.21, 101.77, 95.70, 91.76, 82.95, 80.39, 78.47, 78.35, 78.29, 78.28, 76.40, 76.28, 76.02, 75.42, 74.94, 74.84, 74.82, 74.73, 74.15, 74.03, 73.83, 73.73, 73.64, 73.39, 73.35, 73.04, 72.98, 72.75, 72.36, 72.34, 71.21, 71.17, 70.78, 70.63, 70.04, 69.37, 69.32, 60.48, 60.06, 59.90, 59.78, 59.69, 59.52, 40.02; m/z (HRMS$^+$) 1048.358 $[M + H]^+$ ($C_{38}H_{66}NO_{32}^+$ requires 1048.356).

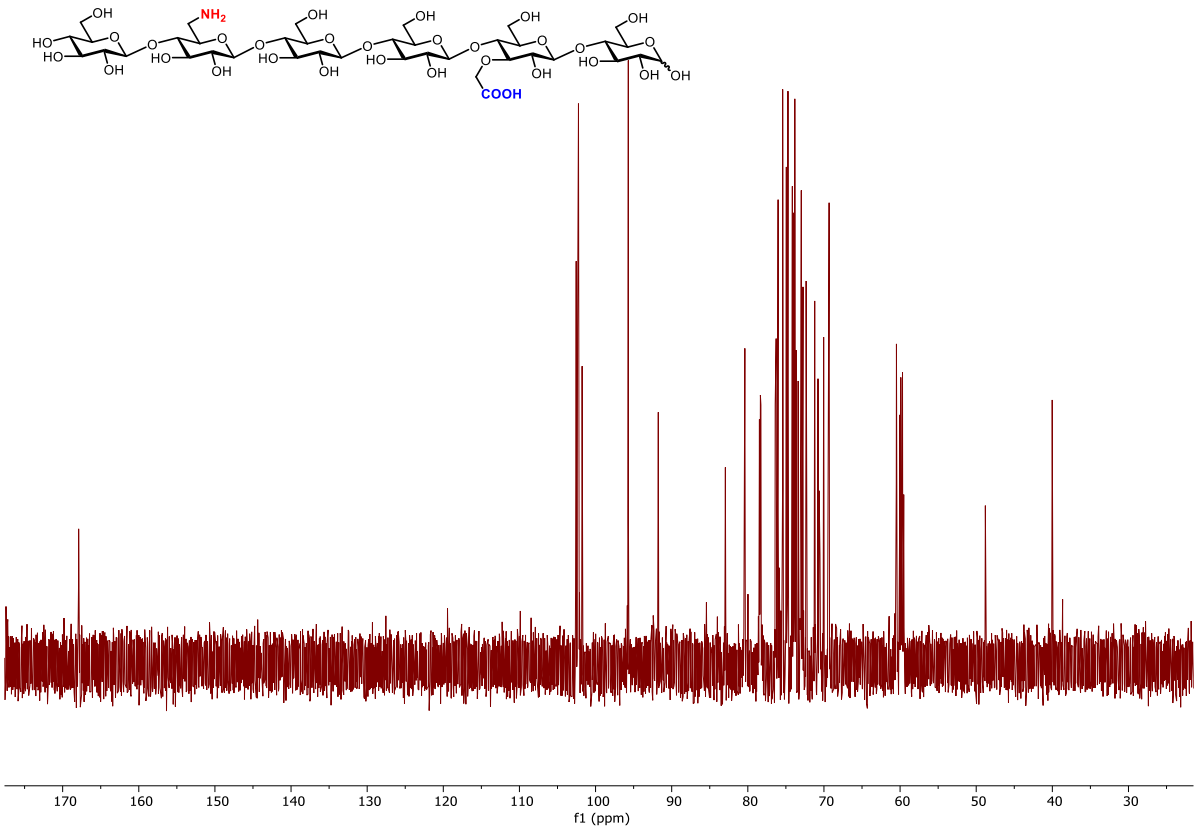
RP-HPLC of AgAADA-OH (ELSD trace, Method D, t_R = 4.8 min)



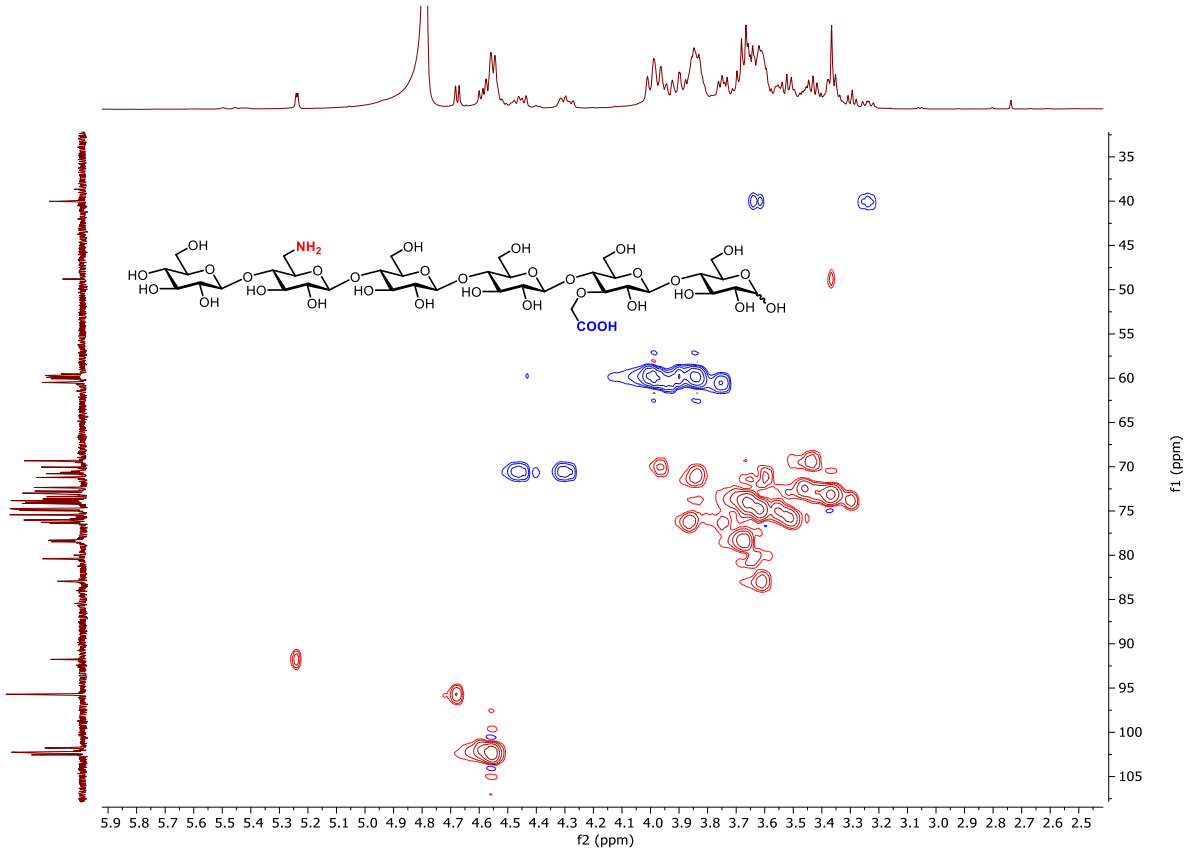
^{1}H NMR of AgAADA-OH (600 MHz, D_2O)



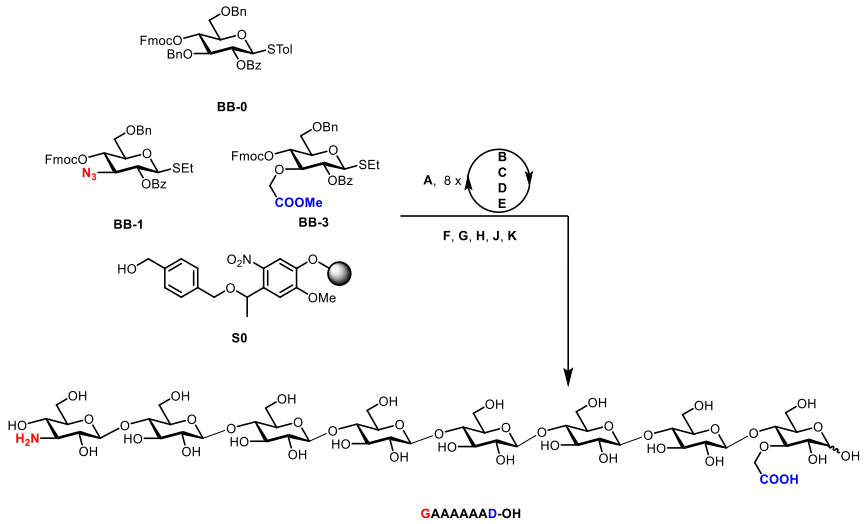
^{13}C NMR of AgAADA-OH (151 MHz, D_2O)



HSQC NMR of AgAADA-OH (D_2O)



11. *Synthesis of GAAAAAAD-OH*



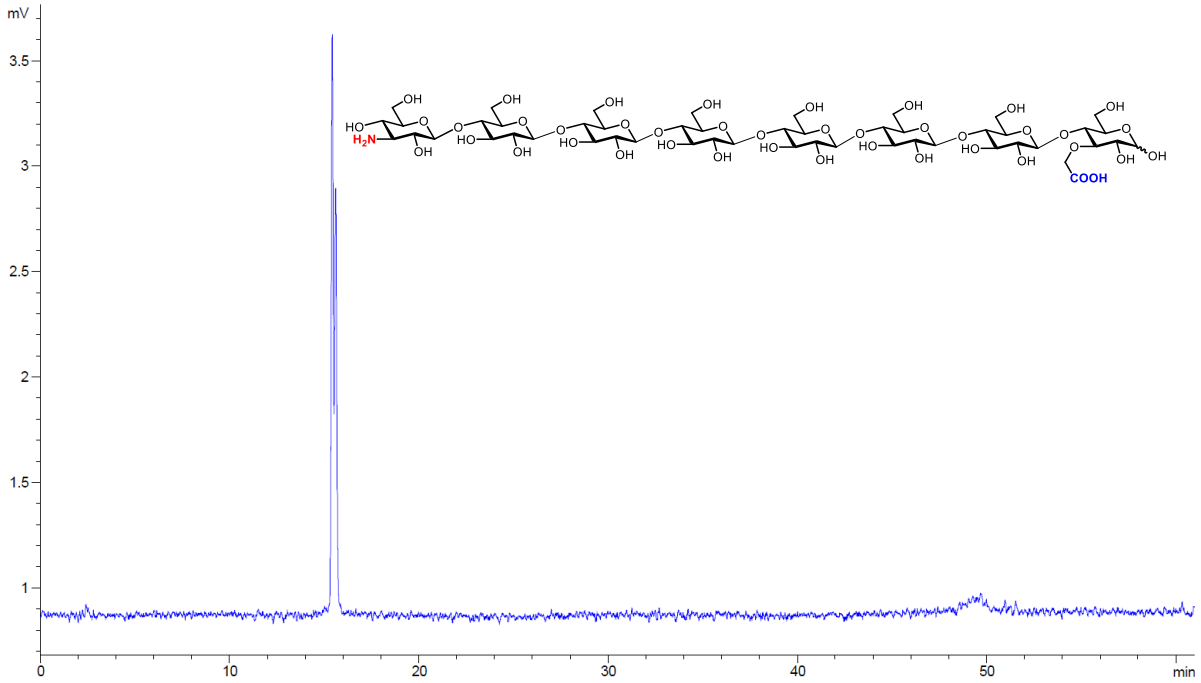
	Module	Conditions
	A: Resin Preparation for Synthesis	
6	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
6	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
1	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	**BB-1**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	D: Capping	
	E: Fmoc Deprotection	
	F: On-resin Staudinger Reduction	
	G: On-resin Methanolysis	
	H: Cleavage from Solid Support	
	J: Hydrogenolysis at Ambient Pressure	
	K: Purification	**Method D, t_R = 16.7 min**

Automated synthesis, global deprotection, and purification afforded **GAAAAAAD-OH** as white solid (2.4 mg, 12% overall yield).

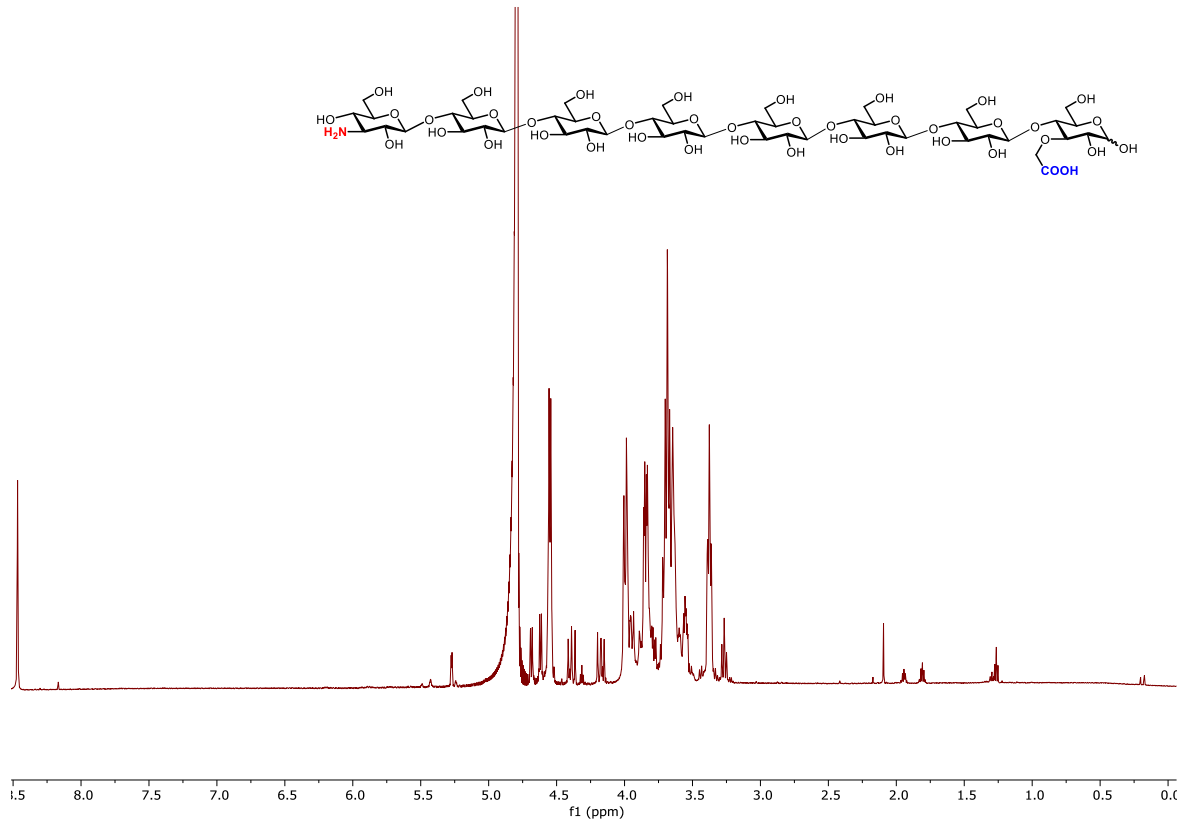
Analytical data for **GAAAAAAD-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.27 (d, *J* = 3.4 Hz, 0.32H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.68H, β-H1), 4.62 (d, *J* = 7.7, 1H), 4.59 – 4.51 (m, 6H), 4.42 – 4.35 (m, 1H), 4.21 – 4.14 (m, 1H), 4.05 – 3.91 (m, 9H), 3.91 – 3.76 (m, 10H), 3.75 – 3.50 (m, 21H), 3.44 – 3.30 (m, 7H), 3.27 (t, *J*

= 10.4 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 170.94, 102.42, 102.27, 95.77 (β-C1), 91.42 (α-C1), 80.61, 78.39, 78.17, 78.15, 76.67, 76.51, 74.81, 74.99, 74.73, 74.06, 73.90, 73.40, 73.07, 72.89, 72.87, 71.30, 71.14, 70.55, 69.58, 65.77, 59.91, 59.81, 59.76, 58.53, 58.20, 57.53; m/z (HRMS$^+$) 1372.472 $[M + H]^+$ ($C_{50}H_{86}NO_{42}^+$ requires 1372.462).

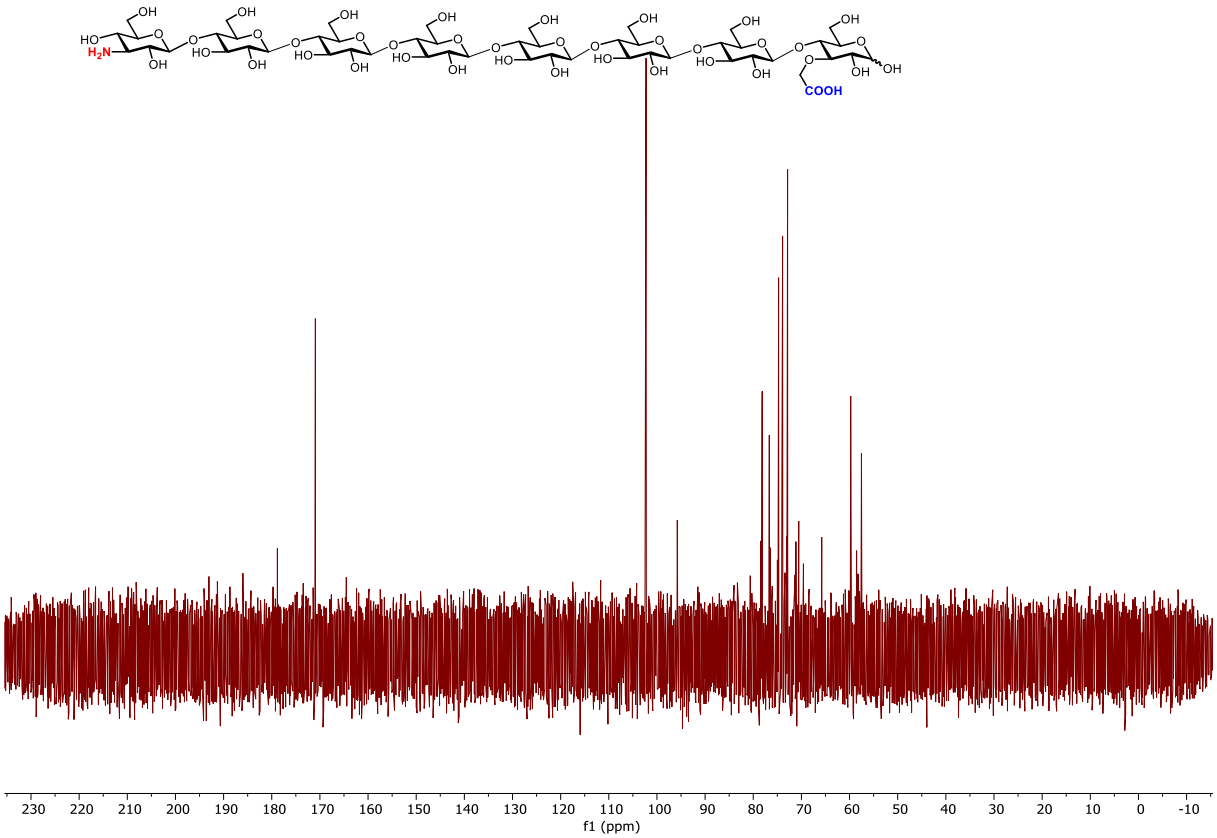
RP-HPLC of GAAAAAAD-OH (ELSD trace, Method C, t_R = 15.4, 15.6 min)



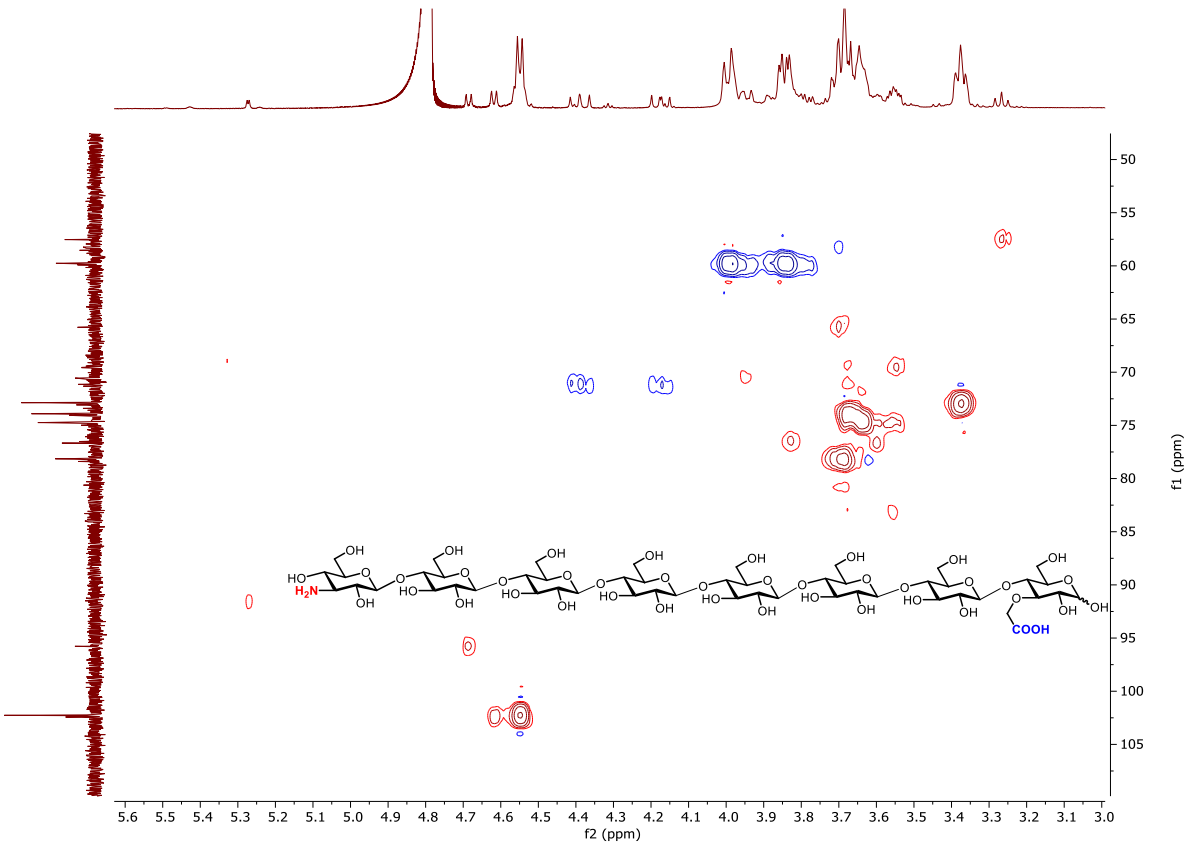
^{1}H NMR of GAAAAAAD-OH (600 MHz, D_2O)



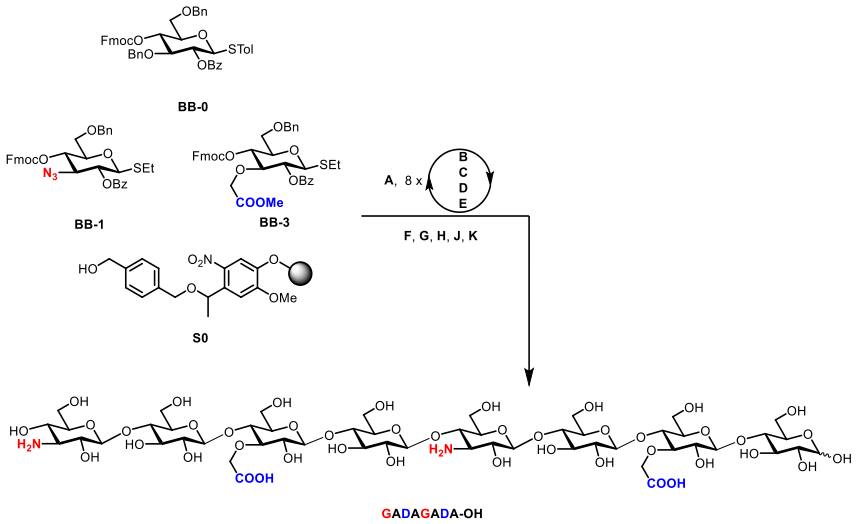
^{13}C NMR of GAAAAAAD-OH (151 MHz, D_2O)



HSQC NMR of GAAAAAAD-OH (D_2O)



12. *Synthesis of GADAGADA-OH*

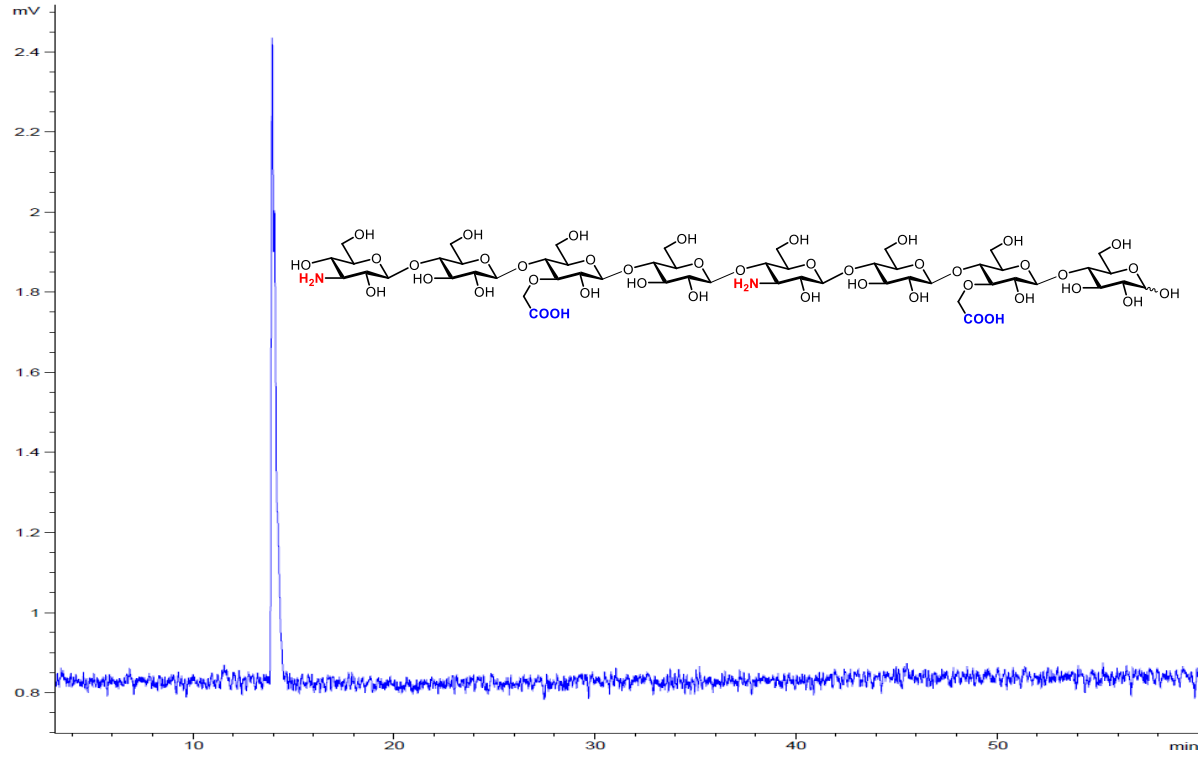


	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-3**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-0**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	B: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Capping **E**: Fmoc Deprotection	**BB-1**, 6.5 equiv.(-20°C for 5 min, 0°C for 20 min)
	F: On-resin Staudinger Reduction **G**: On-resin Methanolysis **H:** Cleavage from Solid Support **J**: Hydrogenolysis at Ambient Pressure **K**: Purification	**Method D, t_R = 15.3 min**

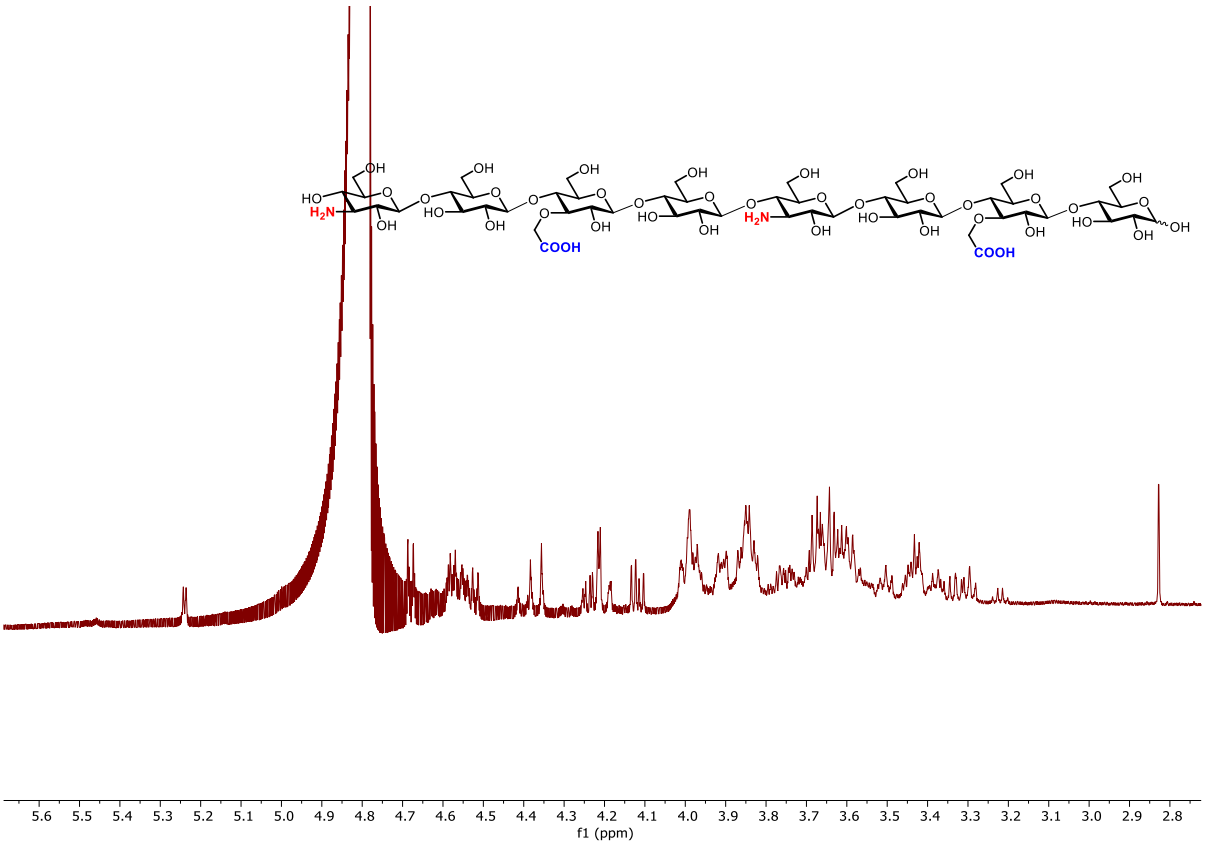
Automated synthesis, global deprotection, and purification afforded **GADAGADA-OH** as white solid (0.1 mg, 1% overall yield).

Analytical data for **GADAGADA-OH**: ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 3.8 Hz, 0.39H, α-H1), 4.68 (d, *J* = 8.0 Hz, 0.61H, β-H1), 4.60 – 4.51 (m, 7H), 4.42 – 4.34 (m, 2H), 4.27 – 4.18 (m, 2H), 4.12 (m, 2H), 4.03 – 3.94 (m, 5H), 3.94 – 3.88 (m, 4H), 3.88 – 3.80 (m, 6H), 3.79 – 3.71 (m, 4H), 3.70 – 3.53 (m, 12H), 3.53 – 3.47 (m, 3H), 3.44 (m, 3H), 3.41 – 3.27 (m, 7H), 3.22 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 102.27 (C1), 95.66 (β-C1), 91.58 (α-C1), 71.09 (***C***H_2COOH); m/z (HRMS$^+$) 715.239 $[M + 2H]^{2+}$ ($C_{52}H_{90}N_2O_{43}^{2+}$ requires 715.245).

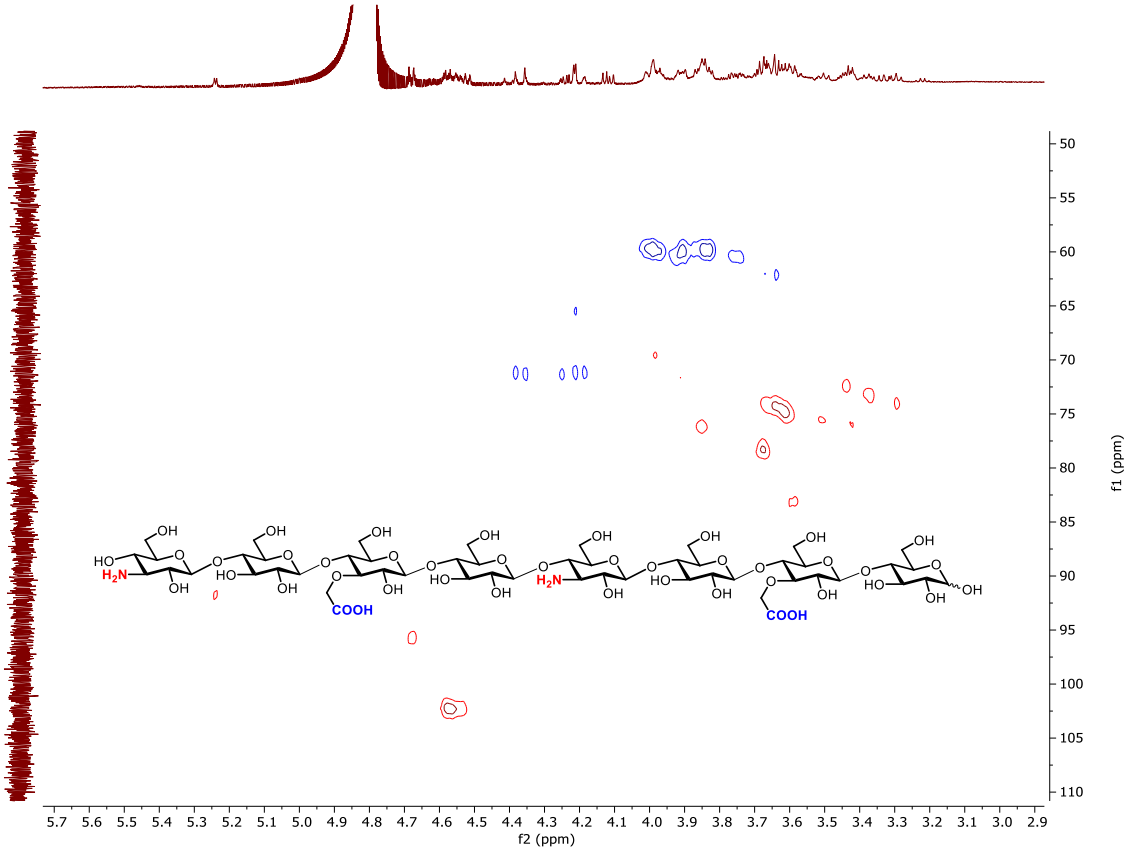
RP-HPLC of GADAGADA-OH (ELSD trace, Method C, t_R = 14.1min)



^{1}H NMR of GADAGADA-OH (600 MHz, D_2O)



HSQC NMR of GADAGADA-OH (D_2O)



4. Molecular Dynamics Simulations

All-atom molecular dynamics (MD) simulations were performed using gromacs 5.1.2[9]. The oligosaccharides were modeled using the modified GLYCAM06$_{OSMO,r14}$ force field[10, 11], and the system was solvated with TIP5P[12] water molecules to avoid excessive interactions between the monomers. Partial charges for non-standard monomers were derived using the R.E.D. tools scripts[13] following the GLYCAM06 protocol. Structure optimization for the charge derivation was performed using Gaussian[14] at the HF/6-31G* level of theory for neutral and cationic groups, and the HF/6-31++G** level of theory for anions.

Initial conformations of single hexamers were constructed with tleap. Stoichiometric amount of Na^+ or Cl^- ions was added to neutralize the ionic oligosaccharide. The topology was converted to gromacs format using the glycam2gmx.pl script and solvated with 2100 water molecules using gromacs tools. The systems were kept at a constant temperature of 303 K using a Nosé-Hoover thermostat[15, 16] and at constant pressure of 1 bar with the Parrinello-Rahman barostat[17, 18]. Non-bonded interactions were cut-off at 1.4 nm, long range electrostatics were calculated using the particle mesh Ewald method[19]. Bonds involving hydrogens were constrained using the LINCS[20] to allow a 2 fs time step algorithm; water molecules were kept rigid with SETTLE[21].

After energy minimization (steepest descent algorithm) and before the production run, the systems were equilibrated at 300 K for 50 ns in a canonical (NVT) ensemble (constant number of particles, volume and temperature) and subsequently at 300 K and 1 atm for 50 ns in an isothermal-isobaric (NPT) ensemble. All hexamers were simulated for 500 ns.

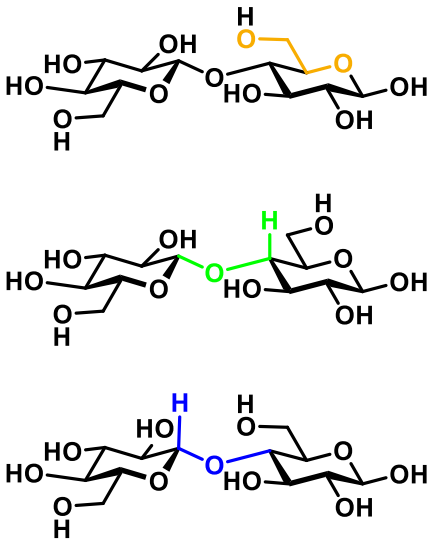
Definition of dihedrals exemplified for a β-1,4-glucose disaccharide

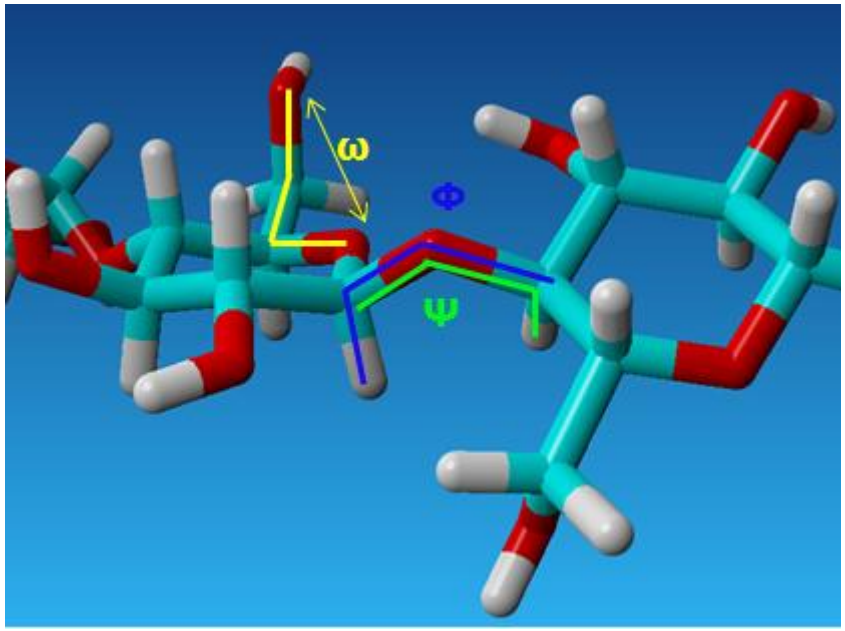
ω = O5C5C6O6

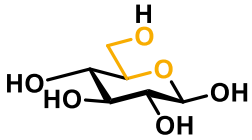
Ψ = C1O4C4H4

Φ = H1C1O4C4

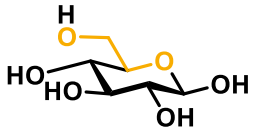
(The atoms in red belong to the following residue)



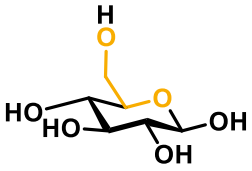




gt (ω = 60°)



tg (ω = 180°)

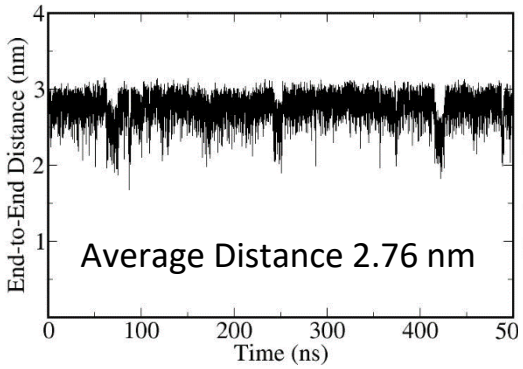


gg (ω = - 60°)

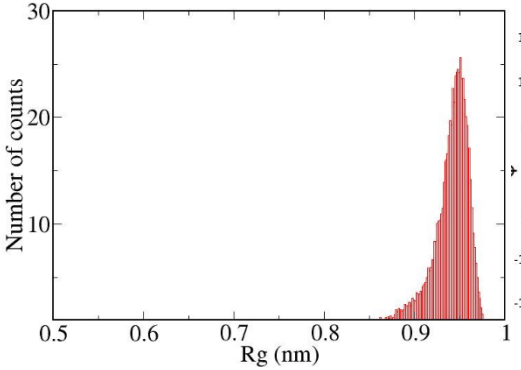
S0

Hexamer of glucose A_6 (for comparison)[3]

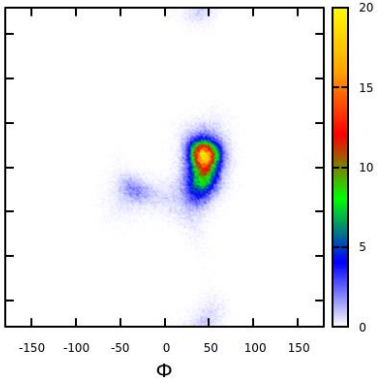
End-to-End Distance



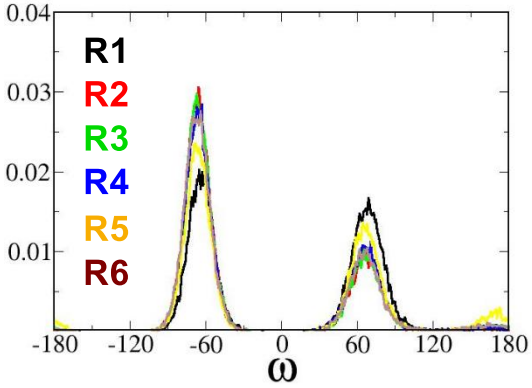
Radius of gyration



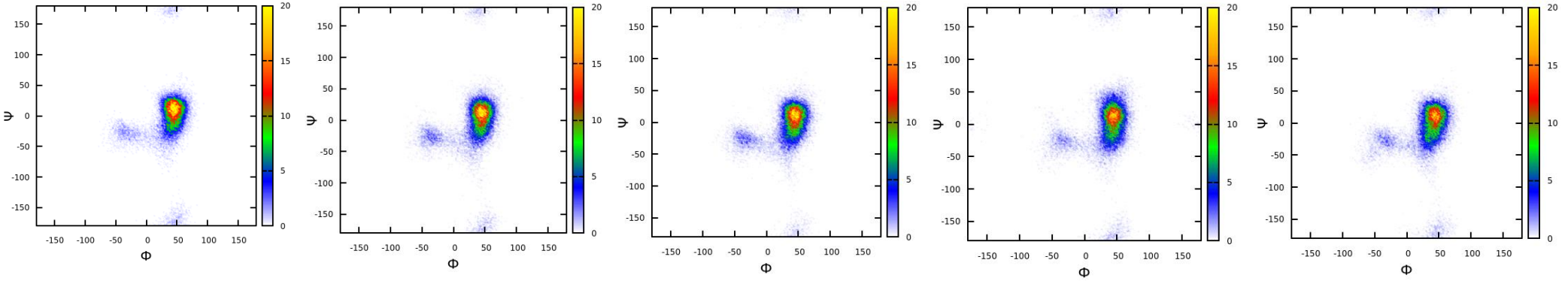
Ramachandran plot



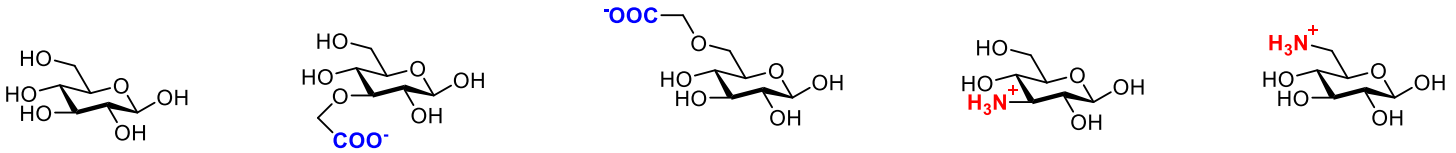
Omega dihedrals superimposed



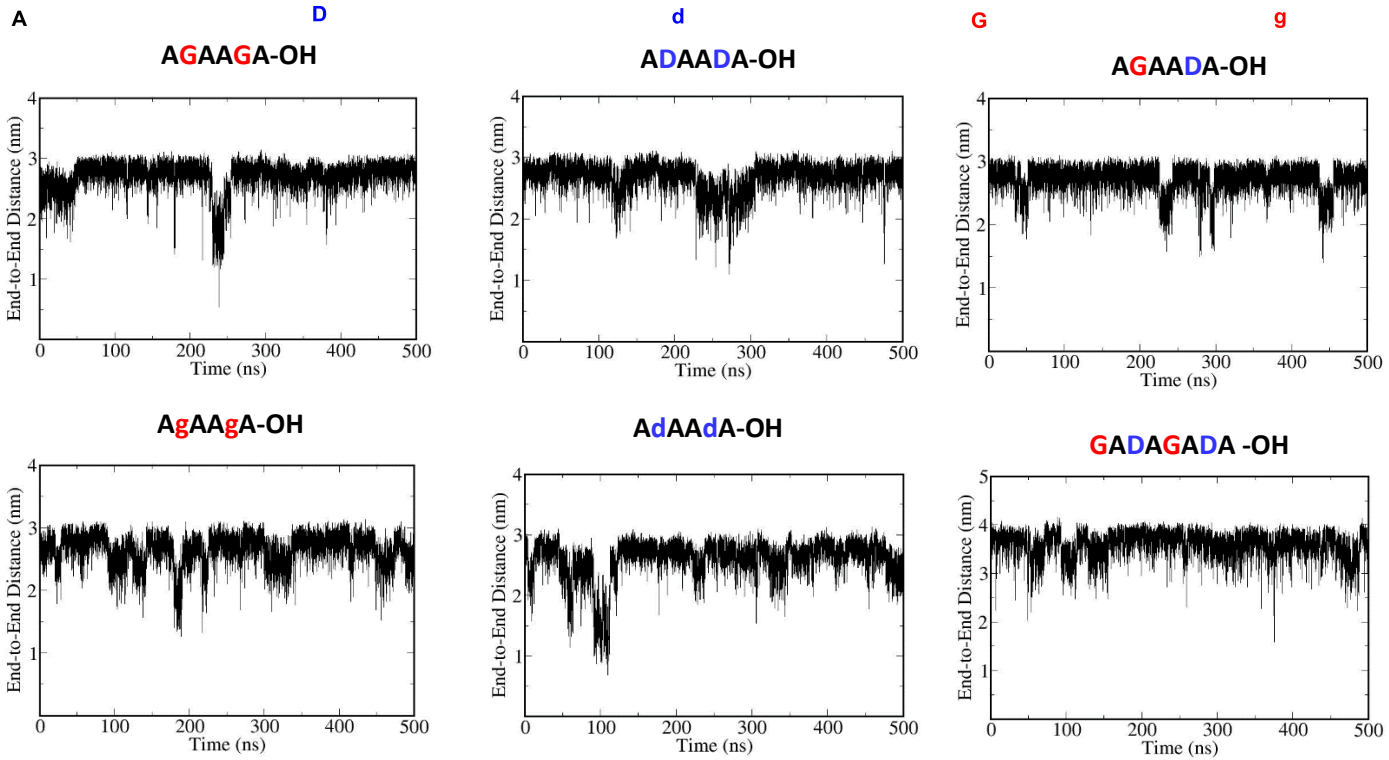
Representative snapshot



S1



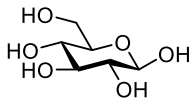
End-to-end distance as a function of time



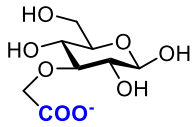
Hexamer	AGAAGA-OH	AgAAgA-OH	ADAADA-OH	AdAAdA-OH	AGAADA-OH	GADAGADA-OH
Average Distance (nm)	2.69	2.65	2.68	2.59	2.71	3.59
Standard Deviation	0.25	0.25	0.22	0.32	0.20	0.26

S2

Histograms of radius of gyration (**RoG**)



A



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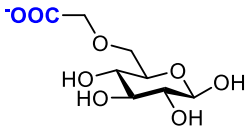

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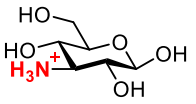
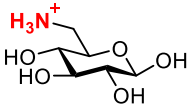
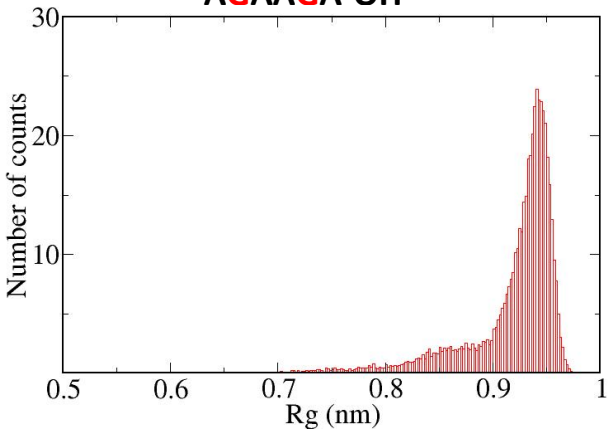


G

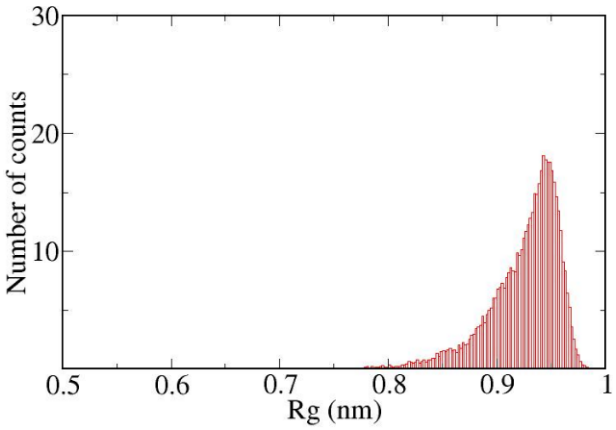


g

AGAAGA-OH



ADAADA-OH



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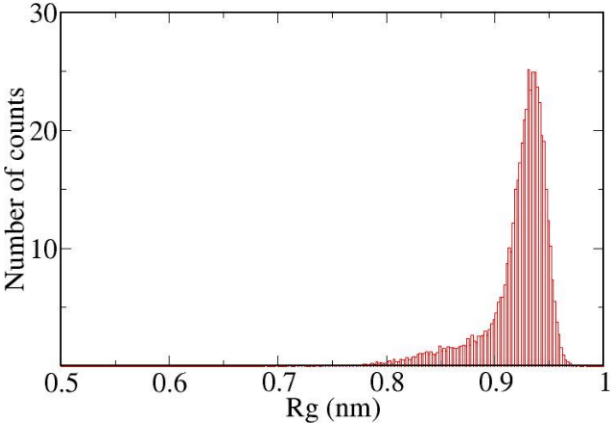

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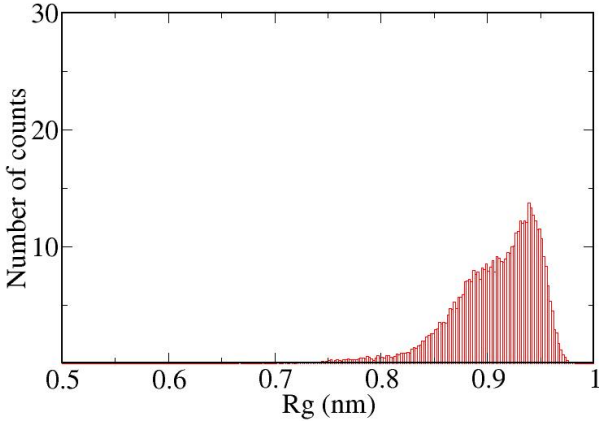
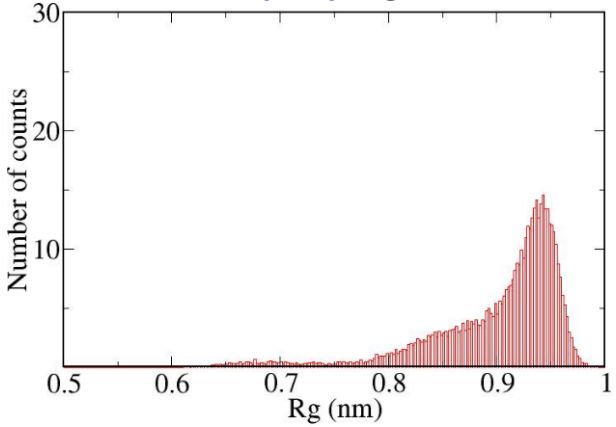
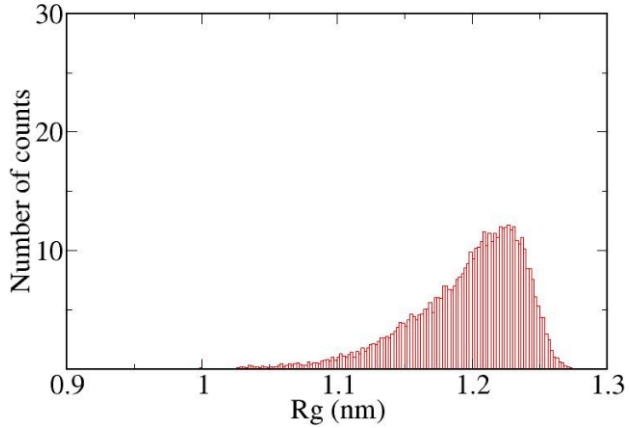


AdAAdA-OH



GADAGADA -OH



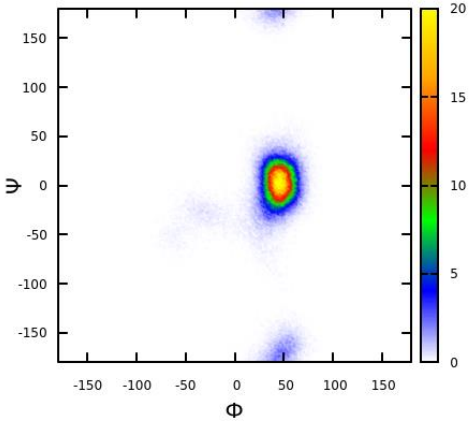
S3

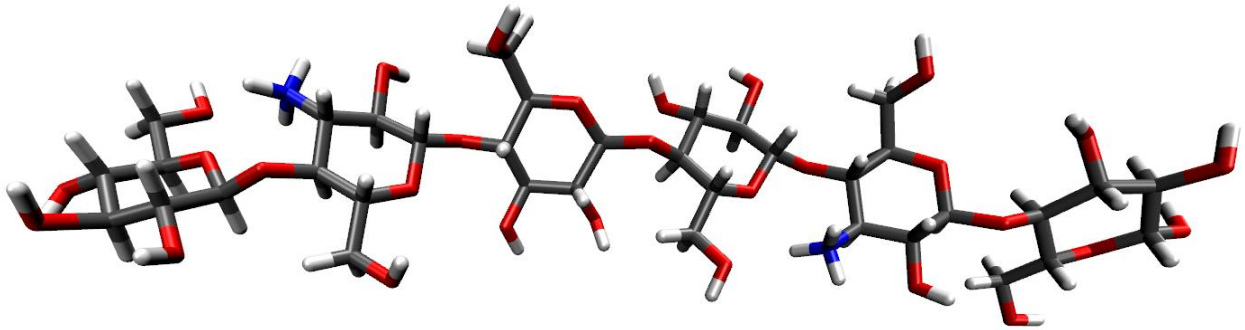
Ramachandran plots

A

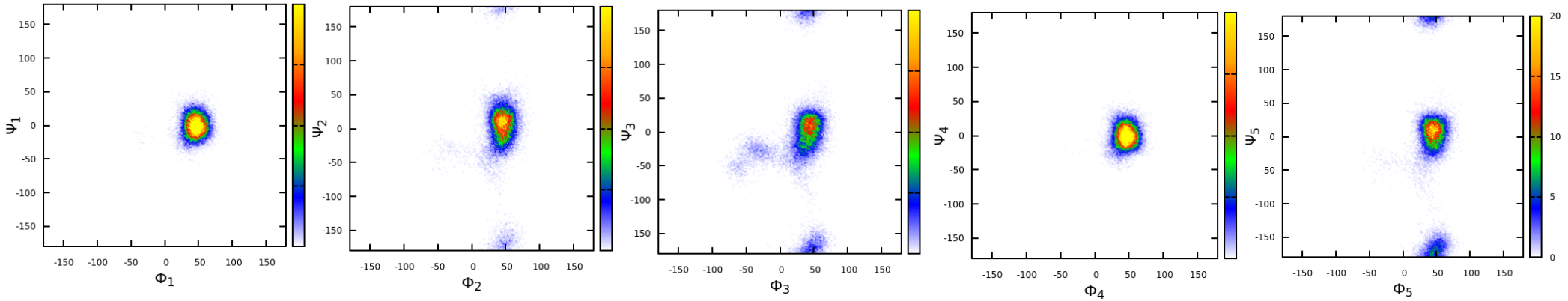
G

AGAAGA-OH





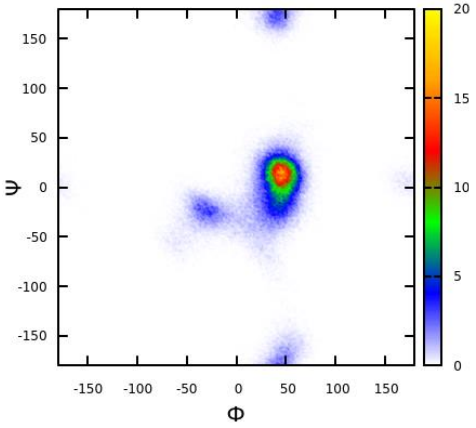
Representative snapshot



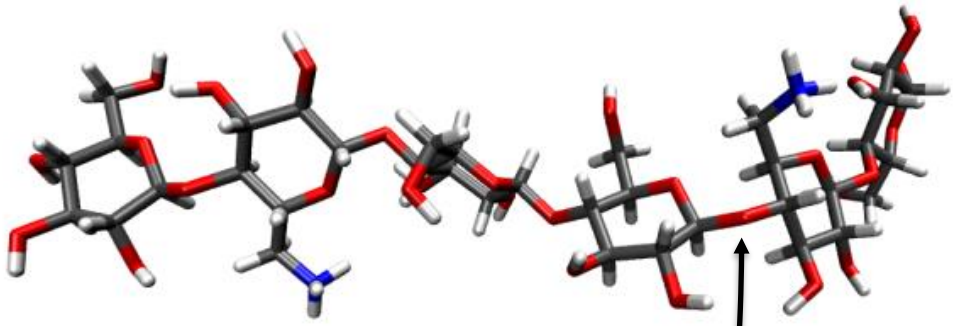
Ramachandran plots

A g

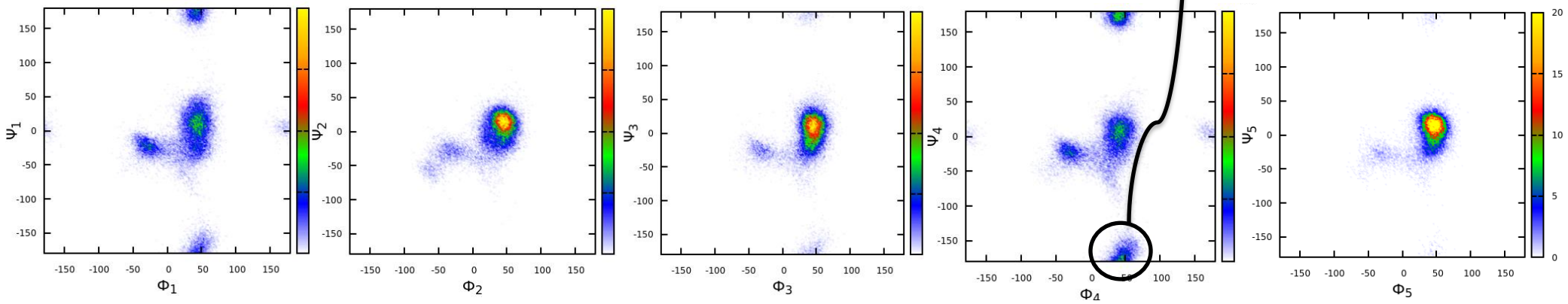
AgAAgA-OH



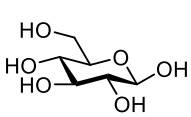
Representative snapshots



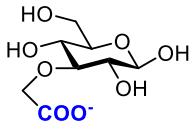
Ψ_4 = -172 °



Ramachandran plots



A



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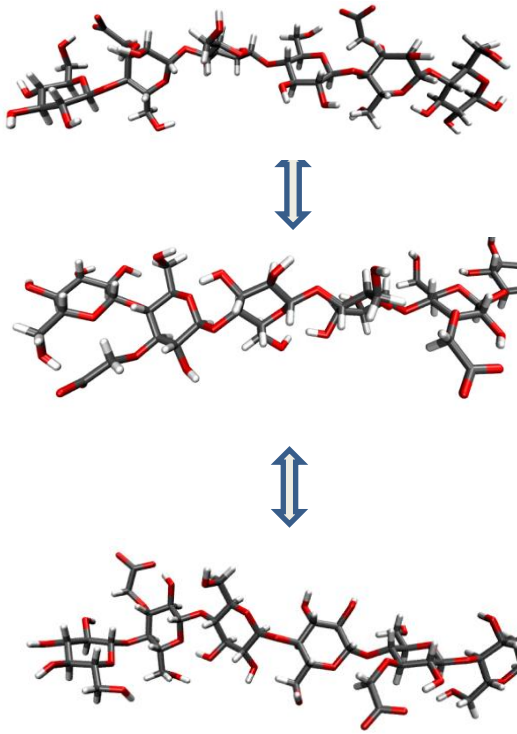
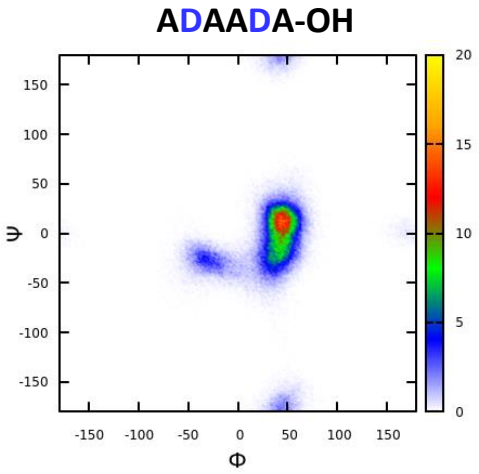
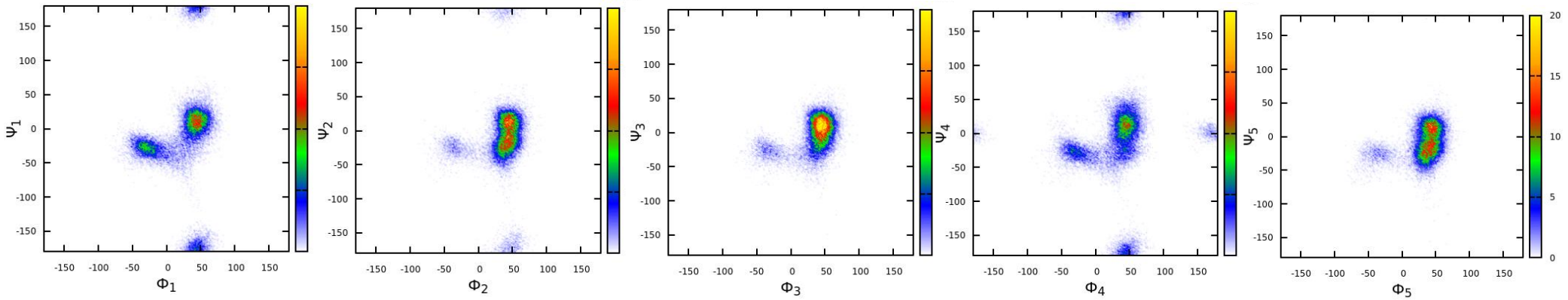
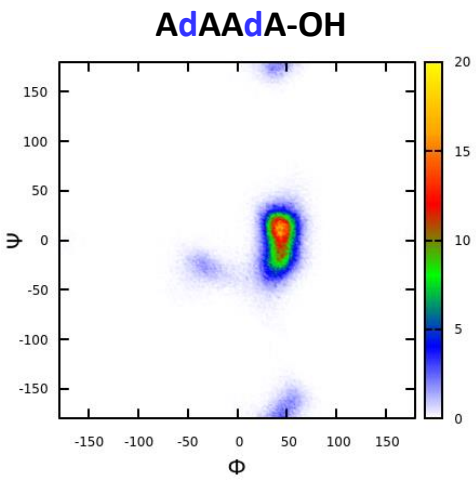


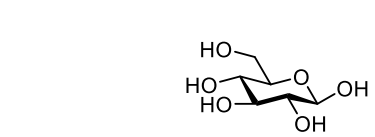
Representative snapshots





Ramachandran plots





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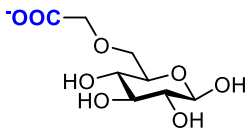

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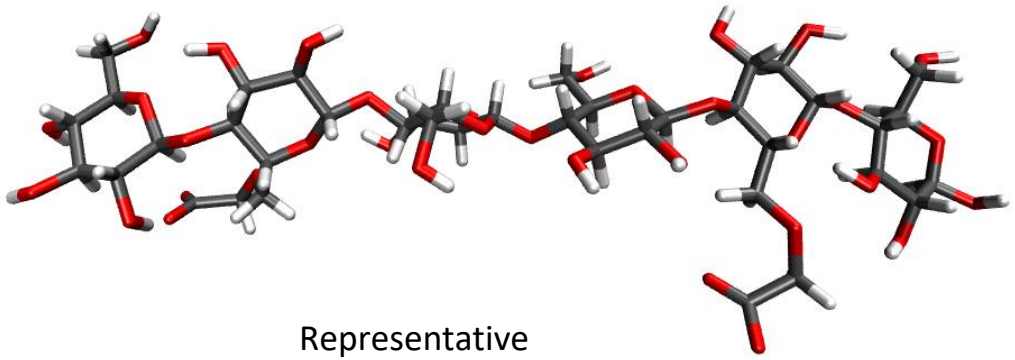
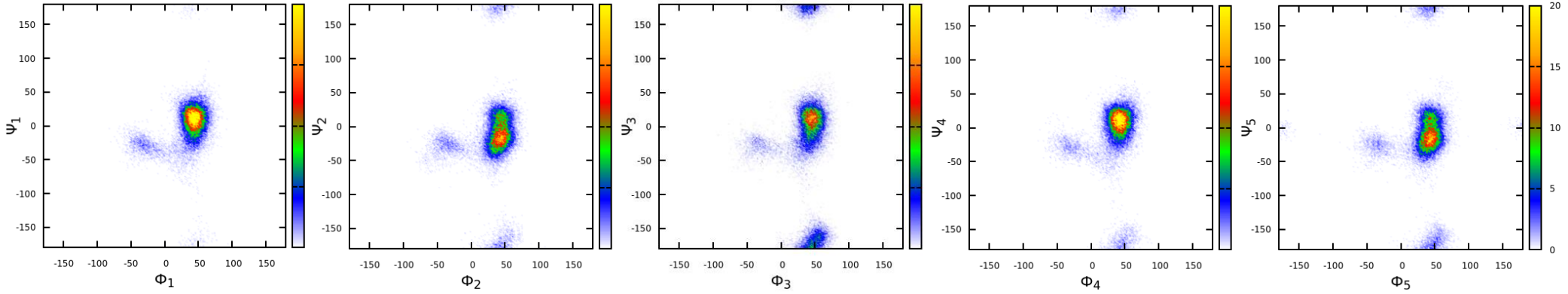
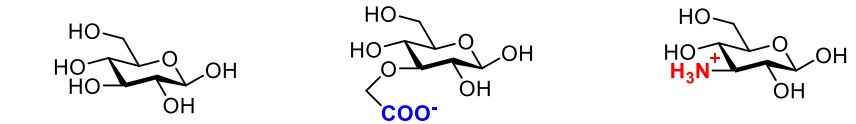


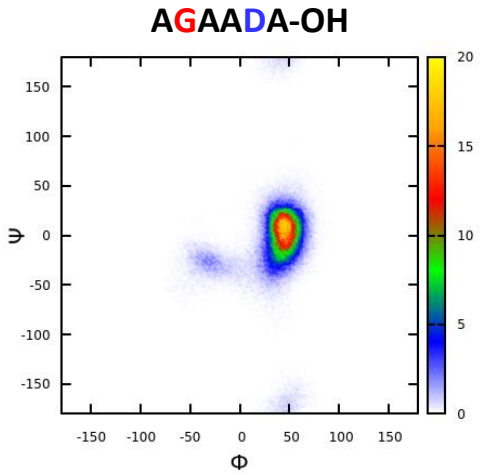
Representative snapshot

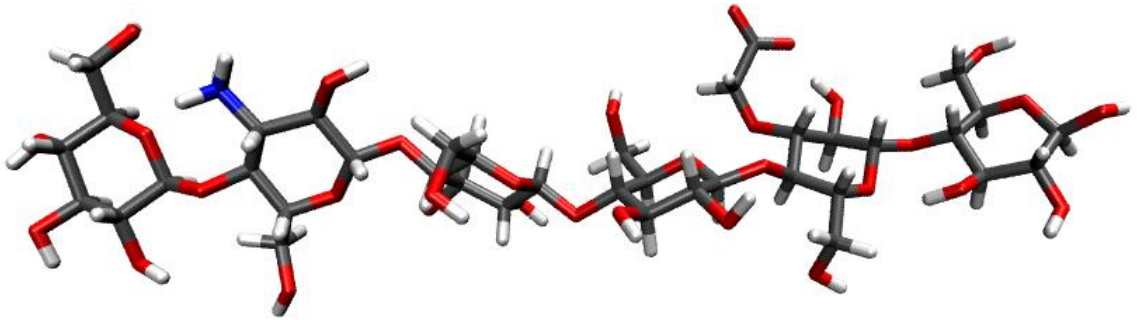


Ramachandran plots

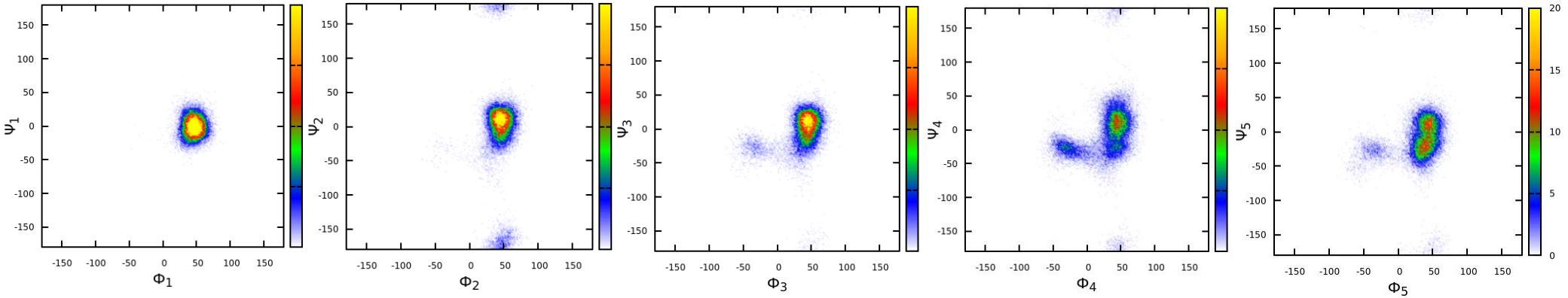


A D G

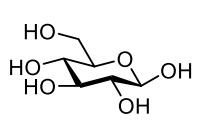




Representative snapshot



Ramachandran plots



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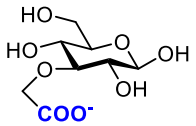

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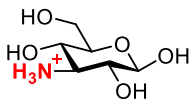
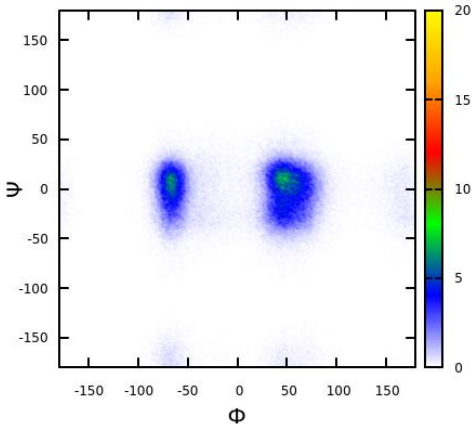
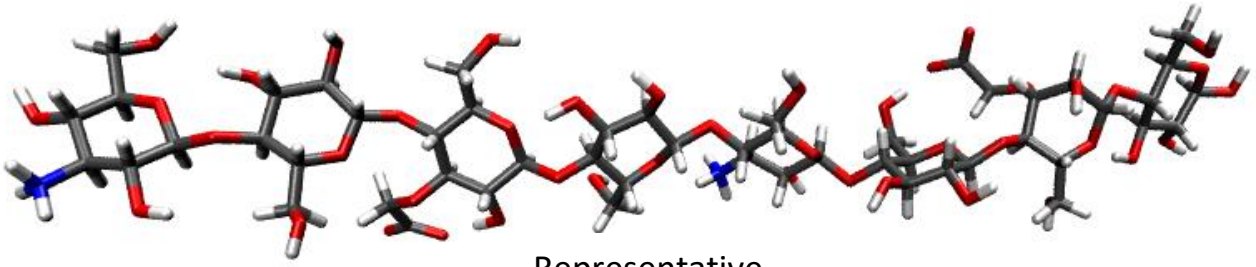


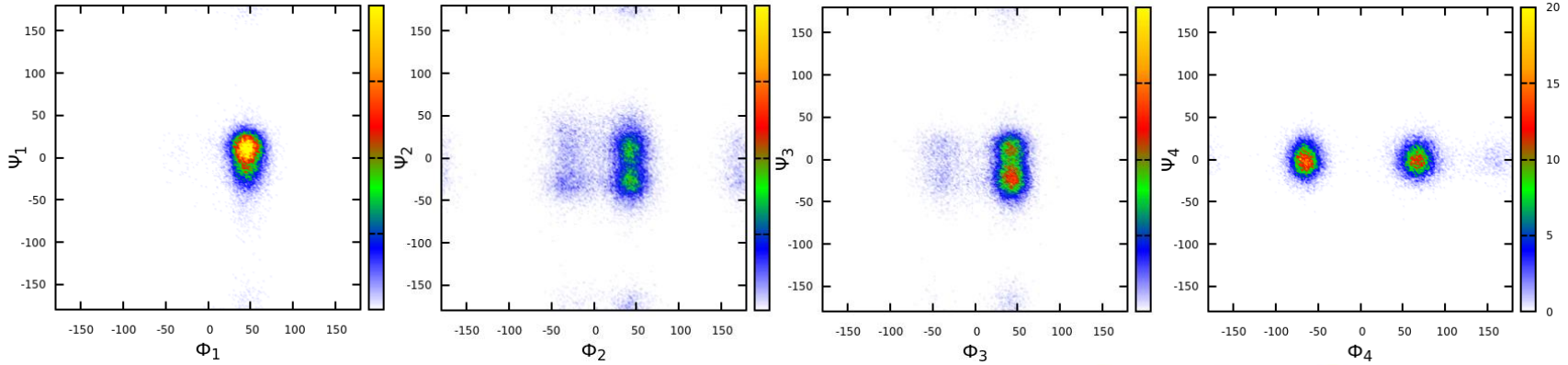
G

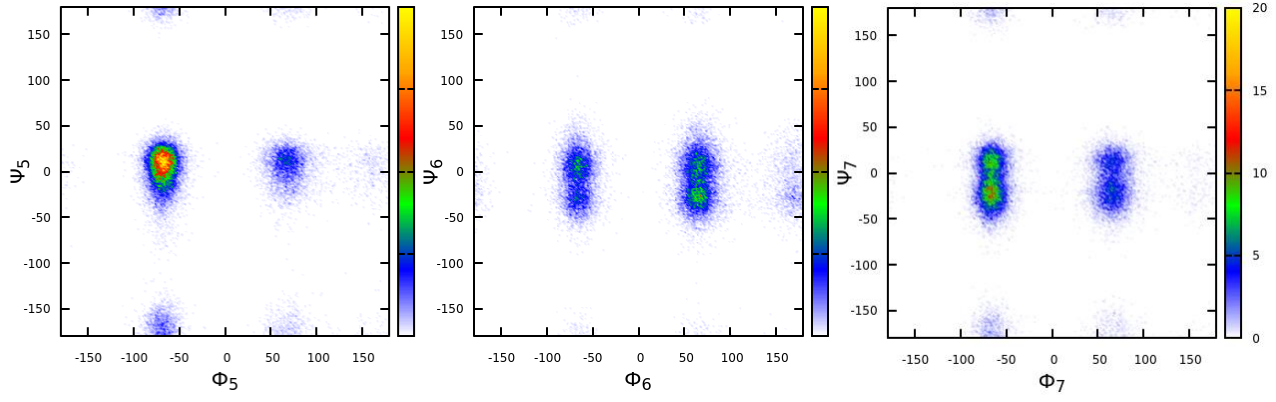
GADAGADA -OH





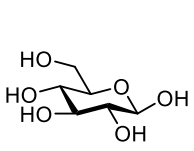
Representative snapshot



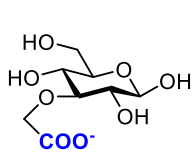


S4

Superimposed Omega (**ω**) torsion angles.



A



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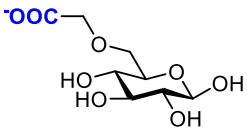

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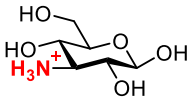
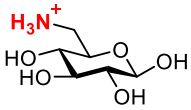


G

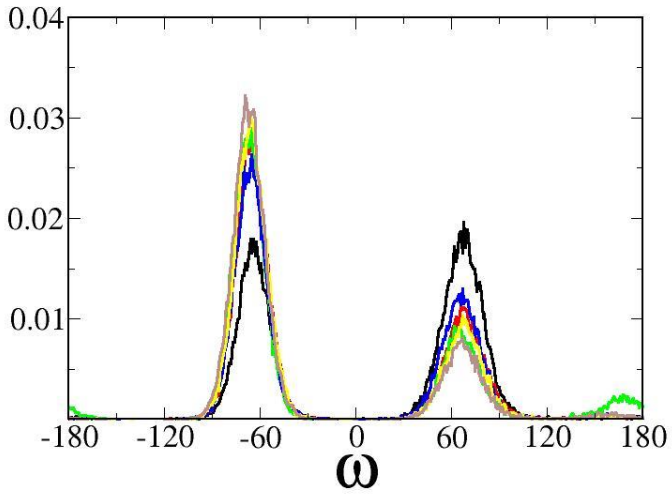


g

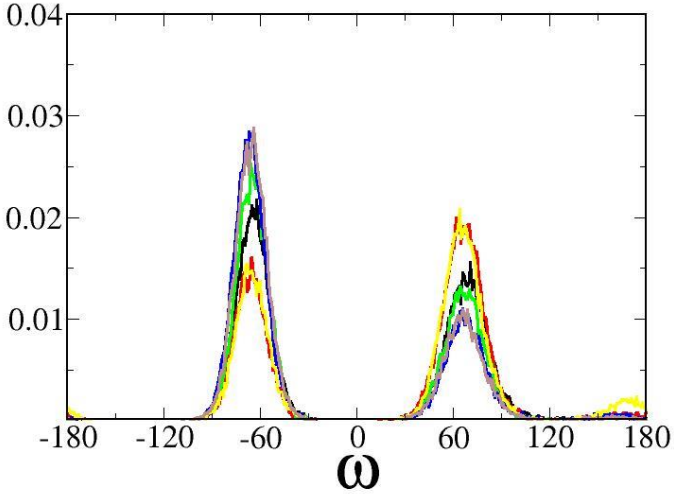
Non-reducing end

Reducing end

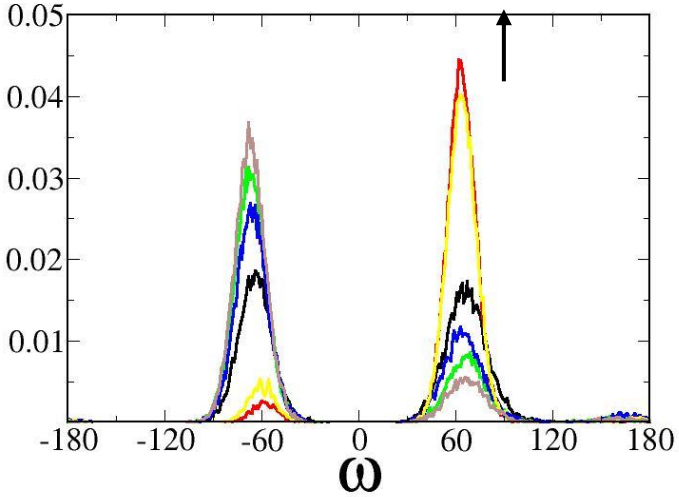
AGAAGA-OH



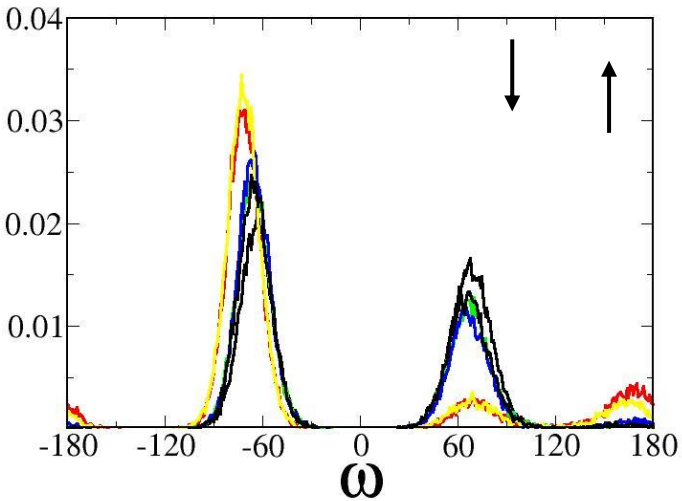
ADAADA-OH



AgAAgA-OH



AdAAdA-OH



5. Supplementary References

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