Supporting information

Probing amyloid β interactions with synthetic heparan sulfate oligosaccharides

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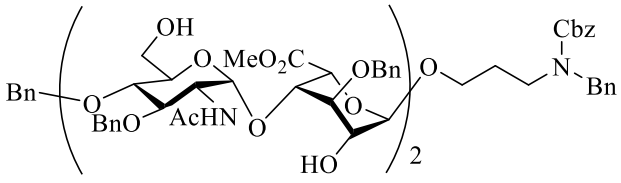
Materials and Methods

General Procedure for Global Debenzylation

A mixture of the Bn-containing compound (for 6 mg of compound, 1 equiv), MeOH/H_2O (4 mL/2 mL), and $Pd(OH)_2/C$ (100 mg) was stirred under H_2 at room temperature overnight and then filtered. The filtrate was concentrated to dryness under vacuum and then diluted with H_2O (15 mL). The aqueous phase was further washed with CH_2Cl_2 (3 × 5 mL) and EtOAc (3 × 5 mL), and then the aqueous phase was dried under vacuum. The crude product was further purified by a Sephadex G-15 column.

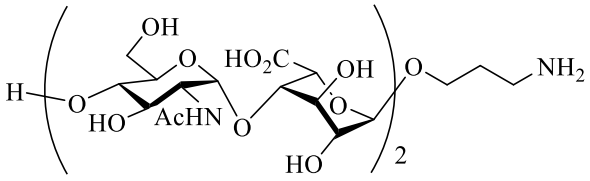
General Procedure for Methyl Ester Saponification

The solution of compound (1 equiv) in THF was cooled to 0 °C and 1 M LiOH (15 equiv per CO_2Me) was added dropwise, followed by addition of H_2O_2 (150 equiv per CO_2Me, 30%). Additional LiOH was added to adjust the pH to 9. The reaction was warmed up to room temperature and stirred overnight. Then the mixture was eluted from a Sephadex G-15 column with H_2O. To simplify mass spectrometry analysis, the product was then eluted from a column of Dowex 50WX4-Na^+ to convert the compound into the sodium salt form.



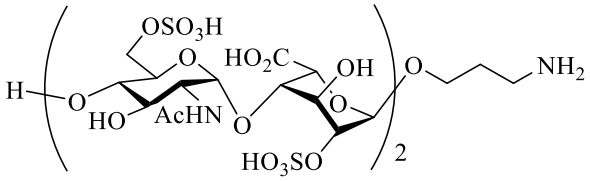
***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-acetamido-3, 4-di-*O*-benzyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-*α*-L-idopyranosyluronate-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-*α*-L-idopyranosyluronate**

(**8**). Compound **7**[1] (60 mg, 0.04 mmol) was dissolved in THF (4 mL), followed by the addition of Zn (104 mg, 1.6 mmol), AcOH (70 μL, 1.2 mmol) and Ac_2O (116 μL, 1.2 mmol) The reaction was stirred at room temperature overnight. Upon completion, the mixture was filtered, concentrated and purification through silica gel column (DCM/MeOH = 8/1) provided compound **8** in 99% yield. ^{1}HNMR (500 MHz, $CDCl_3$): δ = 1.60 (s, 3H), 1.74 (s, 3H), 1.76-1.84 (m, 2H), 3.20-3.39 (m, 4H), 3.44 (s, 3H), 3.48-3.61 (m, 4H), 3.65 (s, 3H), 3.67-3.87 (m, 8H), 3.90-4.19 (m, 7H), 4.34-4.56 (m, 6H), 4.57-4.70 (m, 5H), 4.72 (d, 1H, J = 11.0 Hz), 4.78 (d, 1H, J = 11.0 Hz), 4.82-5.02 (m, 5H), 5.08-5.20 (m, 2H), 5.31 (s, 1H), 5.96-6.18 (m, 2H), 6.73 (br, 1H), 7.05-7.45 (m, 35H). ^{13}CNMR (125 MHz, $CDCl_3$): δ =22.9, 22.99, 27.71, 29.73, 31.53, 36.63, 43.71, 44.26, 50.39, 50.77, 51.91, 52.17, 52.31, 52.87, 52.94, 60.95, 61.5, 66.2, 67.33, 67.35, 67.52, 67.88, 68.03, 71.59, 71.87, 72.02, 72.18, 72.33, 72.73, 72.89, 73.44, 74.36, 74.57, 74.81, 74.98, 77.41, 77.69, 78.38, 80.0, 96.18, 96.7, 100.75, 101.4, 127.2, 127.4, 127.54, 127.6, 127.69, 127.71, 127.78, 127.86, 127.88, 127.91, 127.97, 128.02, 128.05, 128.11, 128.19, 128.35, 128.44, 128.47, 128.52, 128.55, 128.6, 128.62, 136.52, 136.74, 137.39, 137.55, 137.57, 137.69, 137.71, 137.75, 138.02, 138.17, 138.24, 138.43, 156.37, 156.79, 162.97, 169.84, 170.06, 170.59, 170.82, 171.06. HRMS: m/z calc. for $C_{83}FeH_{97}N_3O_{25}$: 795.7880; found: 795.7868 $[M + Fe]^{2+}$.



3-Aminopropyl 2-acetamido-2-deoxy-*α*-D-glucopyranosyl-(1→4)-*α*-L-idopyranosyluronate-(1→4)-2-acetamido-2-deoxy-*α*-D-glucopyranosyl-(1→4)-*α*-L-idopyranosyluronate (1). Compound **1** was prepared from compound **8** (5 mg, 0.003 mmol) by following the general

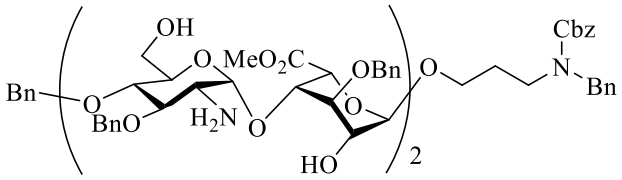
procedure for global debenzylation and methyl ester saponification, providing the final product in 65% yield over 2 steps. ^{1}HNMR (500 MHz, D_2O): δ =1.80-1.89 (m, 2H), 1.84 (s, 3H), 1.86 (s, 3H), 2.98 (dt, 2H, *J* = 2.5, 6.5 Hz), 3.31 (t, 1H, *J* = 9.5 Hz), 3.45-3.50 (m, 1H), 3.50-3.61 (m, 6H), 3.61-3.74 (m, 10H), 3.75 (d, 1H, *J* = 4.0 Hz), 3.76-3.83 (m, 3H), 3.87 (t, 1H, *J* = 3.0 Hz), 3.92 (t, 1H, *J* = 3.5 Hz), 4.33 (d, 1H, *J* = 3.0 Hz), 4.58 (d, 1H, *J* = 3.0 Hz), 4.73 (d, 1H, *J* = 3.0 Hz), 4.75 (d, 1H, *J* = 4.0 Hz), 4.97 (d, 1H, *J* = 4.0 Hz), 5.03 (d, 1H, *J* = 3.5 Hz), . ^{13}CNMR (125 MHz, D_2O): δ = 21.7, 21.79, 26.2, 38.05, 53.41, 53.57, 59.46, 60.02, 66.3, 67.21, 68.12, 68.48, 69.55, 69.83, 70.95, 71.01, 71.83, 73.25, 74.19, 76.43, 94.23, 94.31, 100.29, 101.56, 174.2, 174.28, 174.81, 174.99. HRMS: m/z calc. for $C_{31}H_{50}N_3O_{23}$: 832.2835; found: 832.2836 $[M - H]^-$.



3-Aminopropyl 2-acetamido-2-deoxy-6-*O*-sulfonato-*α*-D-glucopyranosyl-(1→4)-2-*O*-sulfonato-*α*-L-idopyranosyluronate-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfonato-*α*-D-glucopyranosyl-(1→4)-2-*O*-sulfonato-*α*-L-idopyranosyluronate (2). Compound **2** was prepared from compound **8** (5 mg, 0.003 mmol) was dissolved in dry pyridine (1 mL per 5 mg compound, dried over 4 Å molecular sieves). To this mixture was added SO_3·pyridine (100 mg per mL of pyridine), which had been previously washed with H_2O, MeOH, and DCM and dried under vacuum. The reaction was protected from light and stirred for 24 h at 55 °C. The reaction was diluted with 1:1 DCM:MeOH and eluted from a Sephadex LH-20 column, ensuring that all pyridine was removed. The fractions containing sugar were concentrated and further purified by

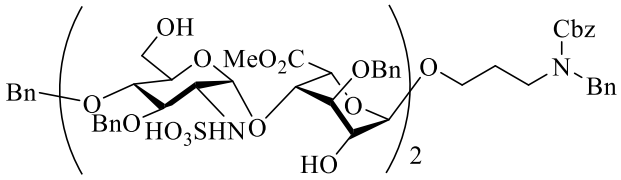
prep TLC (EtOAc/MeOH/H_2O = 3/1/1). The product was subjected to global debenzylation and methyl ester saponification, providing the final product in 83% yield over 3 steps.

^{1}HNMR (500 MHz, D_2O): δ = 1.83-1.89 (m, 2H), 1.90 (s, 3H), 1.92 (s, 3H), 3.01 (t, 2H, *J* = 6.5 Hz), 3.41 (t, 1H, *J* = 9.5 Hz), 3.49 (t, 1H, *J* = 9.0 Hz), 3.53-3.64 (m, 5H), 3.76-3.82 (m, 2H), 3.86 (dd, 2H, *J* = 3.5, 10.0 Hz), 3.91 (dd, 1H, *J* = 3.5, 10.5 Hz), 3.96 (t, 1H, *J* = 2.5 Hz), 3.98 (t, 1H, *J* = 2.5 Hz), 4.07-4.11 (m, 1H), 4.11-4.20 (m, 7H), 4.76 (d, 1H, *J* = 2.5 Hz), 4.99 (d, 2H, *J* = 3.5 Hz), 5.01 (s, 1H), 5.06-5.08 (m, 2H). ^{13}CNMR (125 MHz, D_2O): δ = 22.0, 22.1, 25.96, 38.19, 53.06, 53.21, 63.01, 64.61, 66.03, 66.41, 66.5, 66.72, 66.8, 69.03, 69.63, 69.78, 70.51, 70.79, 72.08, 73.0, 73.58, 76.6, 93.98, 95.11, 98.28, 99.08, 172.54, 172.65, 174.8. HRMS: m/z calc. for $C_{31}H_{46}N_3Na_3O_{35}S_4$: 608.5245; found: 608.5258 $[M + 3Na - 5H]^{2-}$.



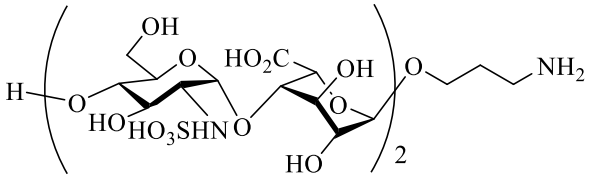
***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-amino-3, 4-di-*O*-benzyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-*α*-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-*α*-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-*α*-L-idopyranosyluronate** (**9**). Compound **7** (60 mg, 0.04 mmol) was dissolved in THF (4 mL), followed by the addition of Zn (104 mg, 1.6 mmol) and AcOH (70 μL, 1.2 mmol). The reaction was stirred at room temperature for 3h. Upon completion, the mixture was filtered, concentrated and purification through silica gel column (DCM/MeOH = 6/1) provided compound **9** in 88% yield. ^{1}HNMR (500 MHz, $CDCl_3$): δ = 1.75-1.87 (m, 2H), 3.11 (d, 1H, *J* = 10.5 Hz), 3.20 (d, 1H, *J* = 10.5 Hz), 3.25-3.44 (m, 3H), 3.38 (s, 3H), 3.48 (d, 2H, *J* = 9.5 Hz), 3.57 (t, 2H, *J* = 9.5 Hz), 3.62-3.73 (m, 3H), 3.65 (s, 3H), 3.79 (d, 2H, *J* = 11.5 Hz), 3.85 (d, 3H, *J* = 11.5 Hz), 3.93-4.05 (m, 4H), 4.14 (s, 1H), 4.35-4.51 (m, 4H),

4.54 (d, 1H, *J* = 11.5 Hz), 4.58-4.75 (m, 4H), 4.75-4.99 (m, 6H), 5.14 (s, 3H), 5.27 (s, 2H), 7.07-7.15 (m, 4H), 7.15-7.40 (m, 31H). ^{13}CNMR (125 MHz, $CDCl_3$): δ = 22.1, 29.76, 43.88, 44.76, 50.65, 50.93, 52.12, 52.28, 53.98, 54.33, 60.37, 60.83, 65.38, 65.53, 66.04, 66.23, 66.9, 67.02, 67.29, 67.79, 70.09, 71.04, 71.74, 72.04, 72.3, 72.53, 72.98, 74.63, 74.79, 75.17, 77.84, 78.75, 93.09, 94.4, 100.88, 101.33, 127.22, 127.28, 127.54, 127.61, 127.71, 127.83, 127.89, 127.95, 128.01, 128.12, 128.14, 128.4, 128.44, 128.47, 128.52, 128.59, 128.64, 136.68, 136.8, 137.33, 137.72, 137.83, 137.88, 156.25, 156.76, 170.19. HRMS: m/z calc. for $C_{79}H_{95}N_3O_{23}$: 726.8178; found: 726.8185 $[M + 2H]^{2+}$.



***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 3, 4-di-*O*-benzyl-2-deoxy-2-sulfoamino-*α*-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-*α*-L-idopyranosyluronate-(1→4)-3-*O*-benzyl-2-deoxy-2-sulfoamino-*α*-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-*α*-L-idopyranosyluronate** (**10**). To a solution of compound **9** (15 mg, 0.010 mmol, 1 equiv) in MeOH was added 1 M aqueous NaOH solution at 0 °C until the pH reached 10. SO_3·pyridine (16 mg, 0.10 mmol, 10 equiv) was added to the solution at the same temperature followed by NaOH to adjust the pH back to 10. The solution was allowed to warm up to room temperature and stirred overnight. The reaction was concentrated and the desired product **10** was purified by silica gel chromatography (DCM/MeOH = 8/1) in 78% yield. ^{1}HNMR (500 MHz, CD_3OD): δ = 1.74-1.85 (m, 2H), 3.23 (s, 2H), 3.32-3.34 (m, 1H), 3.35 (s, 3H), 3.42-3.52 (m, 5H), 3.53 (t, 1H, *J* = 10.0 Hz), 3.63 (t, 1H, *J* = (9.0 Hz), 3.69 (s, 3H), 3.70-3.71 (m, 2H), 3.73-3.79 (m, 1H), 3.81 (s, 2H),

3.83-3.90 (m, 2H), 3.98 (s, 1H), 4.07 (s, 1H), 4.14 (s, 2H), 4.20 (s, 1H), 4.37-4.44 (m, 3H), 4.59 (d, 2H, J = 11.5 Hz), 4.66 (t, 3H, J = 10.0 Hz), 4.74 (d, 1H, J = 10.0 Hz), 4.77 (d, 2H, J = 10.5 Hz), 4.87-4.94 (m, 2H), 5.03 (d, 1H, J = 1.5 Hz), 5.06 (d, 1H, J = 10.5 Hz), 5.09-5.14 (m, 2H), 5.18 (s, 1H), 5.40-5.44 (m, 2H), 7.06-7.12 (m, 1H), 7.14-7.36 (m, 28H), 7.37-7.45 (m, 4H), 7.46-7.50 (m, 2H). ^{13}CNMR (125 MHz, CD_3OD): δ = 24.7, 45.2, 46.05, 48.49, 48.66, 48.83, 49.0, 49.17, 49.34, 49.51, 49.85, 51.58, 52.73, 52.97, 59.42, 59.65, 61.42, 61.74, 66.9, 67.21, 68.33, 68.54, 72.75, 72.86, 73.02, 73.16, 73.38, 73.48, 74.08, 74.4, 74.63, 75.68, 76.12, 76.25, 78.48, 79.36, 81.47, 97.4, 97.54, 101.6, 102.69, 128.13, 128.25, 128.38, 128.51, 128.58, 128.66, 128.87, 128.9, 129.07, 129.13, 129.21, 129.27, 129.39, 129.54, 129.57, 137.97, 137.99, 139.07, 139.25, 139.41, 139.6, 139.76, 139.9, 140.29, 157.91, 158.36, 171.89, 172.2. HRMS: m/z calc. for $C_{79}H_{91}N_3O_{29}S_2$: 804.7590; found: 804.7593 $[M - 2H]^{2-}$.



3-Aminopropyl 2-deoxy-2-sulfoamino-*α*-D-glucopyranosyl-(1→4)-*α*-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-*α*-D-glucopyranosyl-(1→4)-*α*-L-idopyranosyluronate (3). Compound **3** was prepared from compound **10** (5 mg, 0.003 mmol) by following the general procedure for global debenzylation and methyl ester saponification, providing the final product in 83% yield over 2 steps.

^{1}HNMR (500 MHz, D_2O): δ = 1.76-1.91 (m, 2H), 2.96-3.01 (m, 1H), 3.02-3.10 (m, 2H), 3.30 (t, 1H, J = 9.5 Hz), 3.47 (t, 1H, J = 10.0 Hz), 3.50-3.60 (m, 5H), 3.60-3.76 (m, 8H), 3.85-3.89 (m,

1H), 3.90-3.96 (m, 2H), 4.01 (s, 1H), 4.31 (d, 1H, *J* = 2.0 Hz), 4.62 (d, 1H, *J* = 2.5 Hz), 4.74 (s, 1H), 4.79 (d, 1H, *J* = 3.0 Hz), 5.16 (d, 1H, *J* = 4.0 Hz), 5.23 (d, 1H, *J* = 3.5 Hz). ^{13}CNMR (125 MHz, D_2O): δ = 26.1, 38.24, 57.78, 57.89, 59.61, 60.11, 66.36, 67.02, 67.73, 68.1, 68.74, 69.29, 69.64, 69.66, 70.85, 71.17, 71.57, 74.41, 74.72, 76.85, 95.29, 95.72, 100.27, 101.48, 175.24, 175.29. HRMS: m/z calc. for $C_{31}H_{50}N_3O_{35}S_4$: 908.1760; found: 908.1729 $[M - H]^-$.

Procedure for SPR experiments

Sensor SA chips were acquired from BIAcore (GE healthcare, Uppsala, Sweden). SPR measurements were performed on a BIAcore 3000 (GE healthcare, Uppsala, Sweden) operated using BIAcore 3000 control and BIAevaluation software (version 4.0.1).

The biotinylated heparin was prepared by reacting sulfo-*N*-hydroxysuccinimide long-chain biotin (EZ-Link™ Sulfo-NHS-LC-Biotin, ThermoFisher) with free amino groups of unsubstituted glucosamine residues in the heparin polysaccharide (16 kDa, Celsus Laboratories) following a published procedure.[2] The biotinylated heparin was immobilized to streptavidin (SA) chip based on the manufacturer's protocol. In brief, 20 µl solution of the heparin-biotin conjugate (0.1 mg/mL) in HBS-EP running buffer was injected over the flow cell 2 (FC2) of the SA chip at a flow rate of 10 µL/min. The successful immobilization of heparin was confirmed by the observation of a ~150 resonance unit (RU) increase in the sensor chip. The control flow cell (FC1) was prepared by 1 min injection with saturated biotin.

The $A\beta_{40}$ peptide was pre-dissolved in an aqueous NaOH solution (10 mM) in a 1mg:1ml ratio with sonication for 1 min. The peptide was then diluted in HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4). Various dilutions of samples were injected at a flow rate of 30 µL/min. At the end of the sample injection, the same buffer was

flowed over the sensor surface to facilitate dissociation. After a 3 min dissociation time, the sensor surface was regenerated by injecting with 30 μL of 2M NaCl for FGF2 or SDS (0.25 % in water) for Aβ40 to get fully regenerated surface. The response was monitored as a function of time (sensorgram) at 25 °C.

For the competition assay between heparin on chip surface and heparin like oligosaccharides in solution using SPR, Aβ1-40 peptide mixed with different concentrations of HS oligos in HBS-EP buffer were injected over the heparin chip at a flow rate of 30 μL/min, respectively. After each run, the dissociation and the regeneration were performed as described above. For each set of competition experiments on SPR, a control experiment (only protein without any heparin or oligosaccharides) was performed to make sure the surface was completely regenerated and that the results obtained between runs were comparable. IC_{50} was calculated based on the protein binding signals from the sensorgrams with the addition of HS like oligosaccharides in various concentrations. Solution based affinities (K_i) were calculated from IC_{50} measured from SPR competition experiments using the equation: $K_i = IC_{50}/1+[C]/K_D$; [C] = protein concentration used in the competition SPR; K_D is protein-heparin binding affinity.

Procedure for ssNMR measurements.

Synthesis of isotopically labeled $A\beta_{40}$ peptides

All isotopically labeled amino acids were purchased from Cambridge Isotope Laboratory Inc. Three $A\beta_{40}$ sequences with isotope-labeling at selective residues were synthesized manually using routine solid-phase peptide synthesis protocols with FMOC chemistry. Crude peptides were cleaved from resin using a cocktail containing 82.5% trifluoroacetic acid (TFA), 5% ddH_2O (v/v),

2.5% phenol (m/m), 5% thioanisole (m/m), 2.5% 1,2-ethanedithiol (v/v), and 2.5% Me_2S (v/v), which was followed by the addition of 1.5% NH_4I (m/m). The peptides were purified using a reversed-phase HPLC with C18 column (Aglient HP1200 Series), and the identity and purity were confirmed by LC-MS (Shimadzu, purity > 95%). All peptides were lyophilized and stored at -20°C until usage.

Preparation of free and HS-bound 3Q $A\beta_{40}$ fibrils

The 3Q $A\beta_{40}$ fibrils were prepared using a modified protocol.[3-4] To prepare free 3Q $A\beta_{40}$ fibrils, lyophilized $A\beta_{40}$ peptides (2.0 mg/ml) were dissolved into 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 1 mL) and bath-sonicated for 5 minutes on ice. The solution was incubated at ambient temperature overnight to break pre-formed aggregates. The organic solvent was then removed using N_2 flow and the remaining films were further dried under vacuum for at least 8 hours. The dried films were re-dissolved in dimethyl sulfoxide (DMSO) to 2.0 mM, sonicated on ice for 3 minutes, and centrifuged at 14, 000 rpm for 30 minutes. The DMSO solution (95% of the supernatant after centrifugation) was then diluted into 10 mM phosphate buffer (pH 7.4, 0.05% NaN_3) to the final Aβ concentration 50 μM. The solution was incubated at 37 °C quiescently for two weeks to produce the parent fibril (denoted as G_0). Thioflavin-T fluorescence spectroscopy was utilized to confirm the formation of fibrils.

To perform the generation seeding protocols, the G_0 fibrils were sonicated on ice using a probe sonicator for 30 seconds three times (with continuous power output, 20% duty cycle) with a 30 second interval between each sonication. 10 mol% seeds (i.e. sonicated G_0 fibrils) were added into freshly dissolved 50 μM $A\beta_{40}$ peptides in 10 mM phosphate buffer. The mixture of seeds and monomers were incubated quiescently at 37 °C for 72 hours with one-minute brief sonication

(using a water-bath sonicator) every 24 hours. This protocol produced the first-generation fibrils (denoted as G_1). For the second through the eighth generation seeding (G_2-G_8 fibrils), the fibrils from previous generation were sonicated using the same procedure and added to freshly dissolved $A\beta_{40}$ peptides with 1:10 molar ratio. The incubation time was kept at 24 hours for each generation. The last generation (G_8) fibrils were utilized as the 3Q $A\beta_{40}$ fibrils for ssNMR measurements. To prepare HS-bound 3Q $A\beta_{40}$ fibrils, the HS hexasaccharide and decasaccharide were added to pre-formed 3Q $A\beta_{40}$ fibrils in 4:1 (*m/m*) fibril:HS ratios, and the mixture was further incubated for 24 hours at ambient temperature.

The ssNMR spectroscopy

Both free and HS-bound $A\beta_{40}$ fibrils were ultra-centrifuged at 432,000x g (Beckmann Benchtop Ultracentrifuge with a TLA 100.4 rotor) for 30 minutes to collect the pellets, which were then lyophilized and packed into 2.5 mm magic-angle-spinning (MAS) ssNMR rotors. The samples were then re-hydrated with 1 μL/mg deionized water in the rotors.

All ssNMR spectra were collected on a 600 MHz Bruker spectrometer installed with a 2.5 mm TriGamma MAS probe. Two-dimension (2D) ^{13}C-^{13}C spin-diffusion spectra were obtained using the following pulse parameters: a 75 kHz $\pi/2$ ^{1}H pulse, a cross-polarization block on ^{1}H and ^{13}C channels with 65 kHz and 50 kHz (with 30% linear ramp) radiofrequency fields respectively, a 20 ms mixing time period with 10 kHz ^{1}H radiofrequency field and a 95 kHz ^{1}H decoupling field through acquisition. All spectra were processed with 100 Hz Gaussian line broadening in both dimensions. The MAS frequency was set as 10 kHz and the sample temperature was kept at ~ 280 K during measurements, monitored using the H_2O ^{1}H chemical shifts. Typical signal averaging time was ~ 24-48 hours for each individual 2D spectroscopy.

Table S1. Summary of the residue-specific $^{13}C\alpha/^{13}C\beta$ chemical shifts in free and HS-bound 3Q Aβ fibrils.

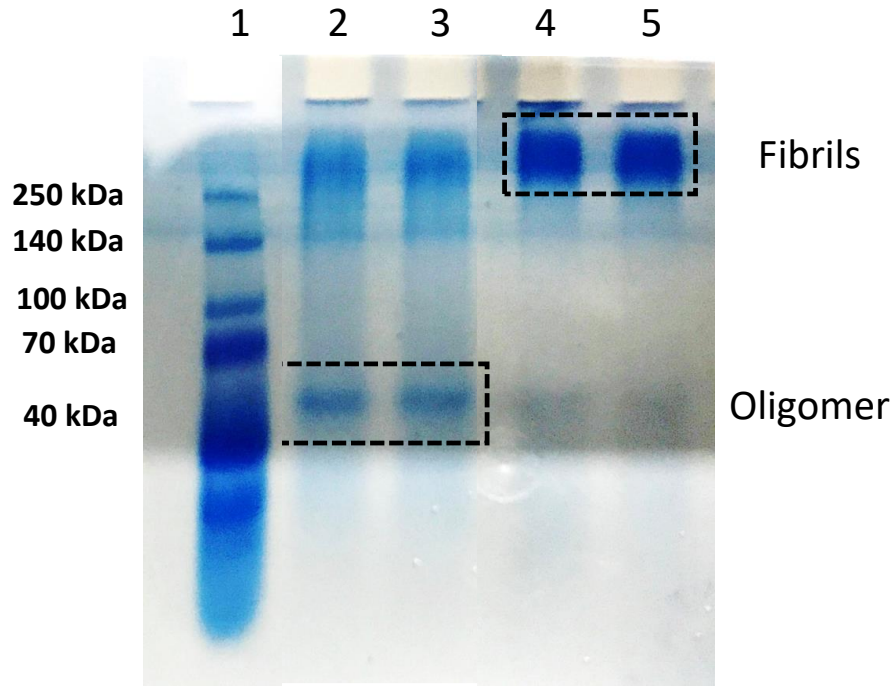
Residue	free 3Q $A\beta_{40}$ fibril		fibril + HS hexamer **5**		fibril + HS decamer **6**	
	Cα (ppm)	Cβ (ppm)	Cα (ppm)	Cβ (ppm)	Cα (ppm)	Cβ (ppm)
F19	55.7	40.9	55.4	43.5	54.5	41.1
A21	49.2	22.4	49.1	22.1	49.1	22.4
D23	50.0	40.3	50.0	40.1	50.1	40.3
V24	58.6	32.4	58.3	32.4	58.4	32.3
S26-1*	55.5	64.4	55.2	64.5	55.4	64.4
S26-2*	57.6	62.9	57.4	62.9	57.0	62.9
G29	42.6		42.8		42.7	
A30	49.6	20.7	49.6	20.1	49.3	20.4
I31	56.6	39.6	55.9	40.2	56.4	40.0
L34	52.0	44.8	54.2	44.5	53.0	44.7
M35	53.2	35.0	52.5	34.2	52.0	33.8
G38	44.2		42.5		42.6	
V39	62.7	33.0	59.7	33.3	60.1	33.2

*S26 shows two major conformers.

MTS cell viability assay

SH-SY5Y neuroblastoma cell line is a common model for neuronal screening.[5] As $A\beta_{42}$ is known to exhibit substantially higher cellular toxicity than $A\beta_{40}$, $A\beta_{42}$ was utilized in cell protection studies. $A\beta_{42}$ peptide (1 mg) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 3mL) and sonicated for 15 minutes. Then, HFIP was removed by freeze drying for 72 hours and a thin film of $A\beta_{42}$ peptide was formed. This film was dissolved in an aqueous NaOH solution (10 mM, 250 µL) and incubated without stirring. Under such conditions after 24 hours, $A\beta_{42}$ mainly existed in the fibril form as confirmed by SDS-PAGE analysis (**Figure S1**).

Figure S1. SDS-PAGE gel analysis showed that $A\beta_{42}$ mainly existed as oligomers after incubation for 2 hours, and as fibrils after overnight incubation. Lane 1: Molecular weight marker; Lane 2: Aβ after 2 hour incubation; Lane 3: repeat of lane 2; Lanes 4: Aβ after overnight incubation; Lane 5: repeat of lane 4.



A solution of Aβ fibril (10 mM, 250 µL) was diluted to 1000 µL by adding FBS free DMEM solution containing Pen-Strep 1% (Aβ concentration: 221 µM). To each well of a 96 well plate, the Aβ solution (27 µL) was added, and the total volume was brought to 100 µL by adding

FBS free DMEM. SH-SY5Y cells (2 x 10^4) were suspended in DMEM containing 2% FBS (100 μL) and added to each well. The final concentration of Aβ was 30 μM per well. The 96 well plate was kept in an incubator at 37 °C containing CO_2 (5%) for 48 hours. Finally, the medium was replaced with DMEM medium (180 μL) containing the MTS reagent (20 μL). It was kept in the incubator for 1 hour until the brown color developed in each well. Then absorbance was measured at 490 nm to quantify the number of viable cells in wells incubated with Aβ and that in wells without the addition of Aβ. The changes of the number of cells due to Aβ were calculated. The toxicity of Aβ was calculated as: (1 - the number of cells in the presence of Aβ / the number of cells in the absence of Aβ) x 100%. Hence, the relative toxicity of Aβ was 100%.

Cells were then incubated with Aβ (30 μM) in the presence of HS **4**, **5**, and **6** (60 μM) respectively. The numbers of viable cells were determined by the MTS cell viability assay after 48 h. The changes in cell numbers were calculated by subtracting from the number of cells cultured in media only. The relative cytotoxicity values were calculated based on the following formula: (changes of the number of cells with HS/Aβ) / (changes of the number of cells with Aβ only) x 100%.

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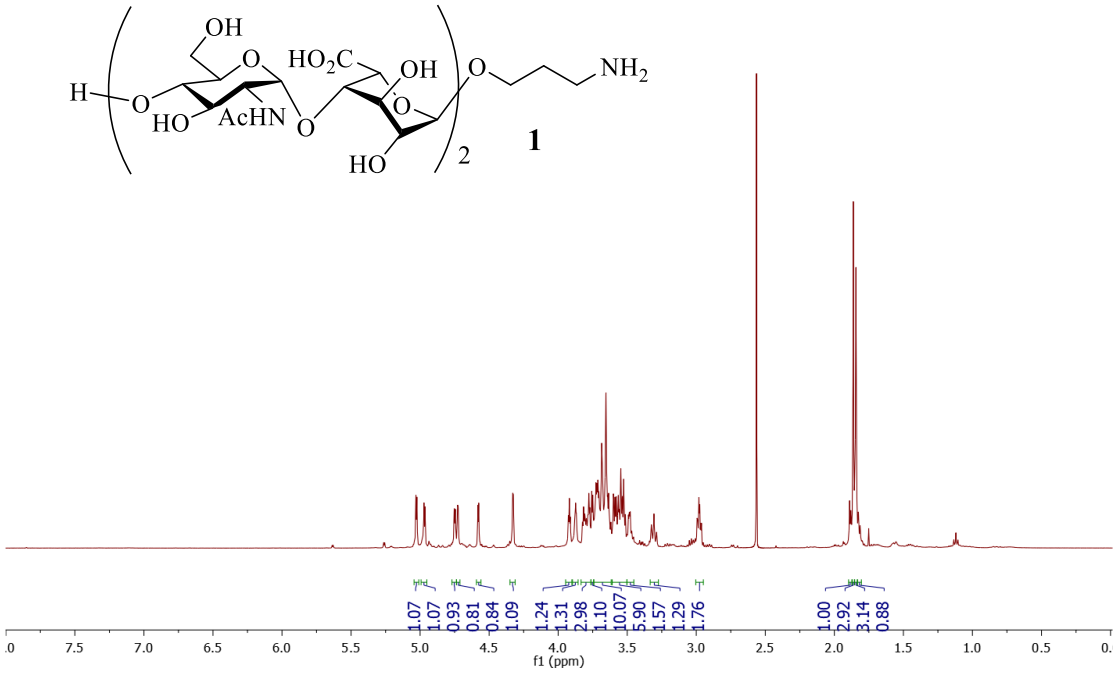
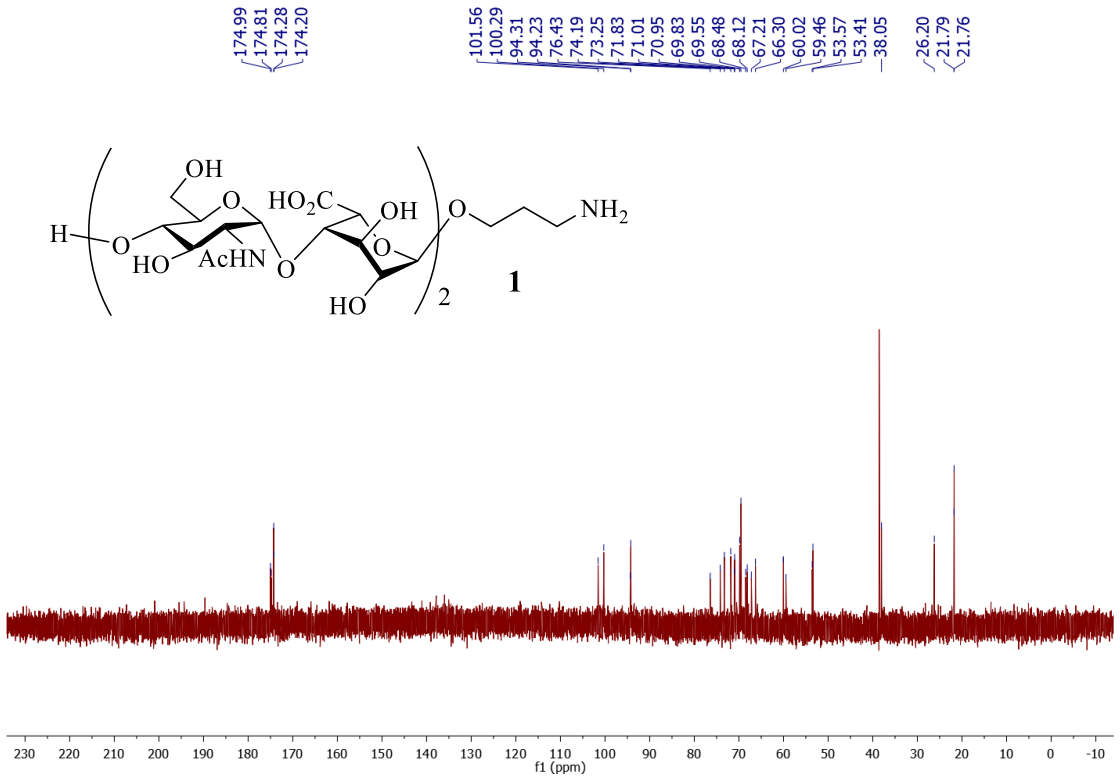
^{1}H-NMR of **1** (500 MHz D_2O)



^{13}C-NMR of **1** (125 MHz D_2O)



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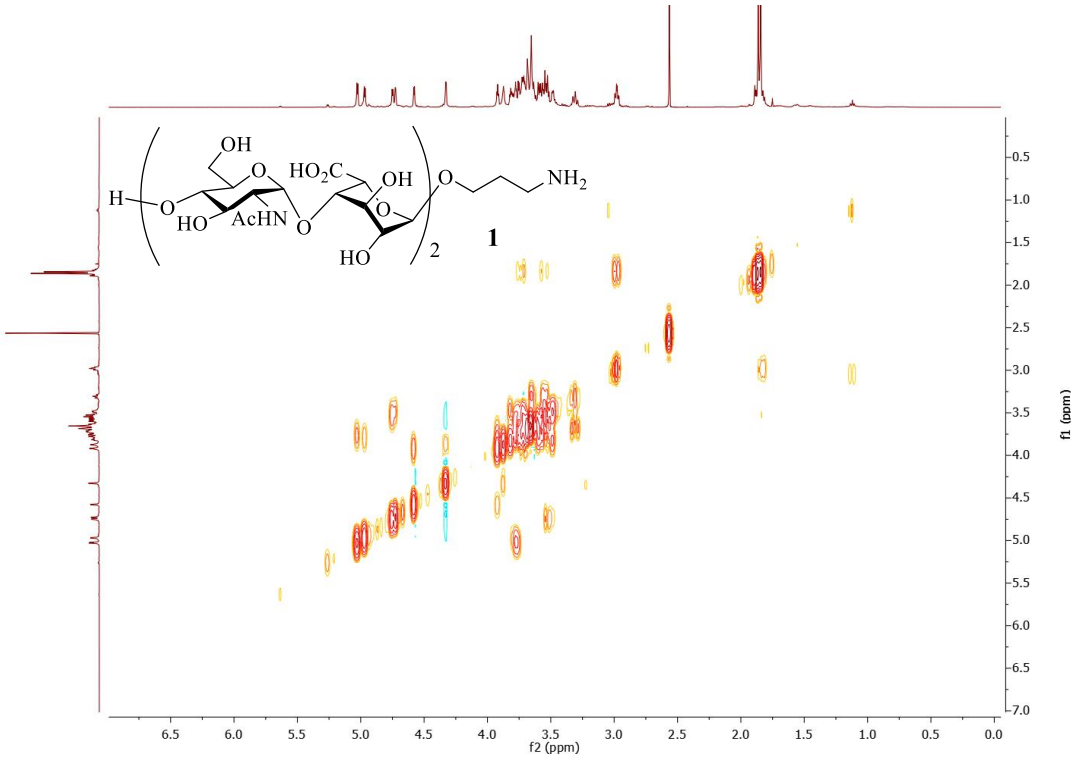

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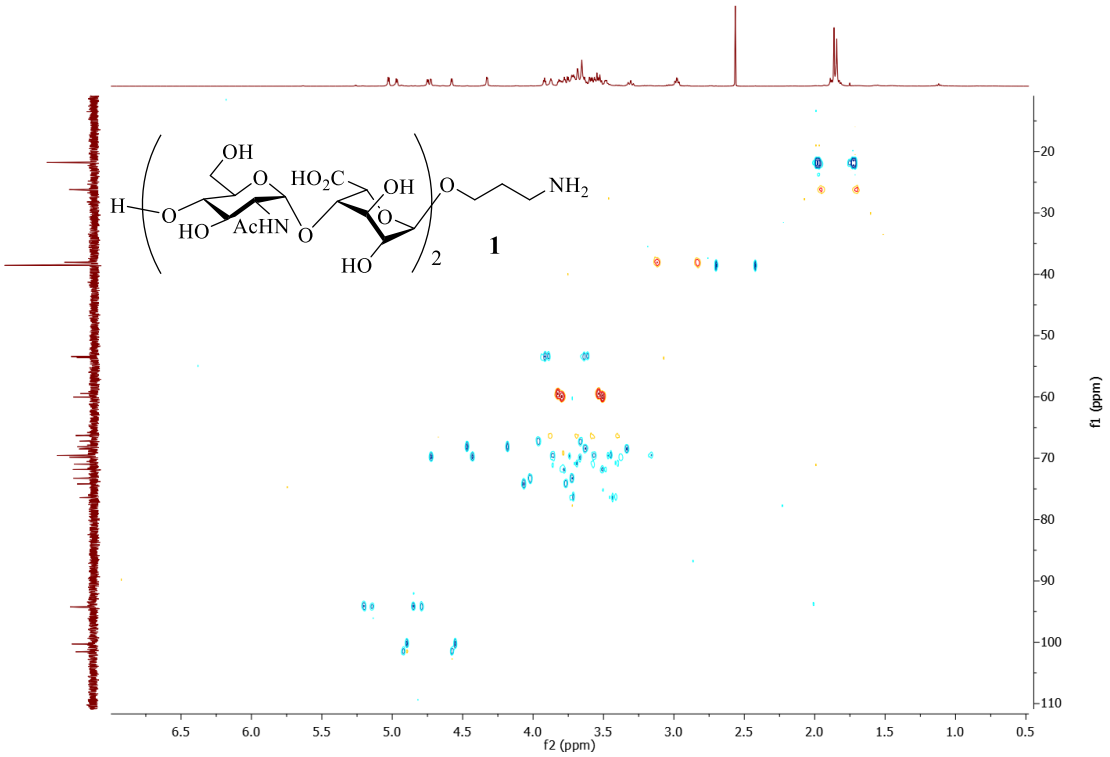

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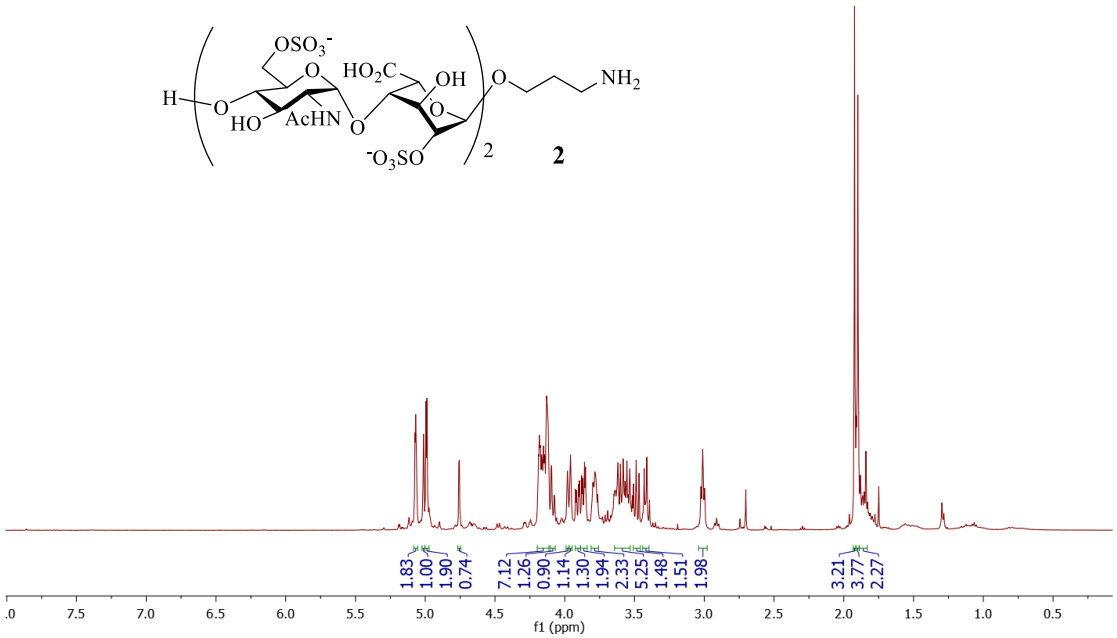

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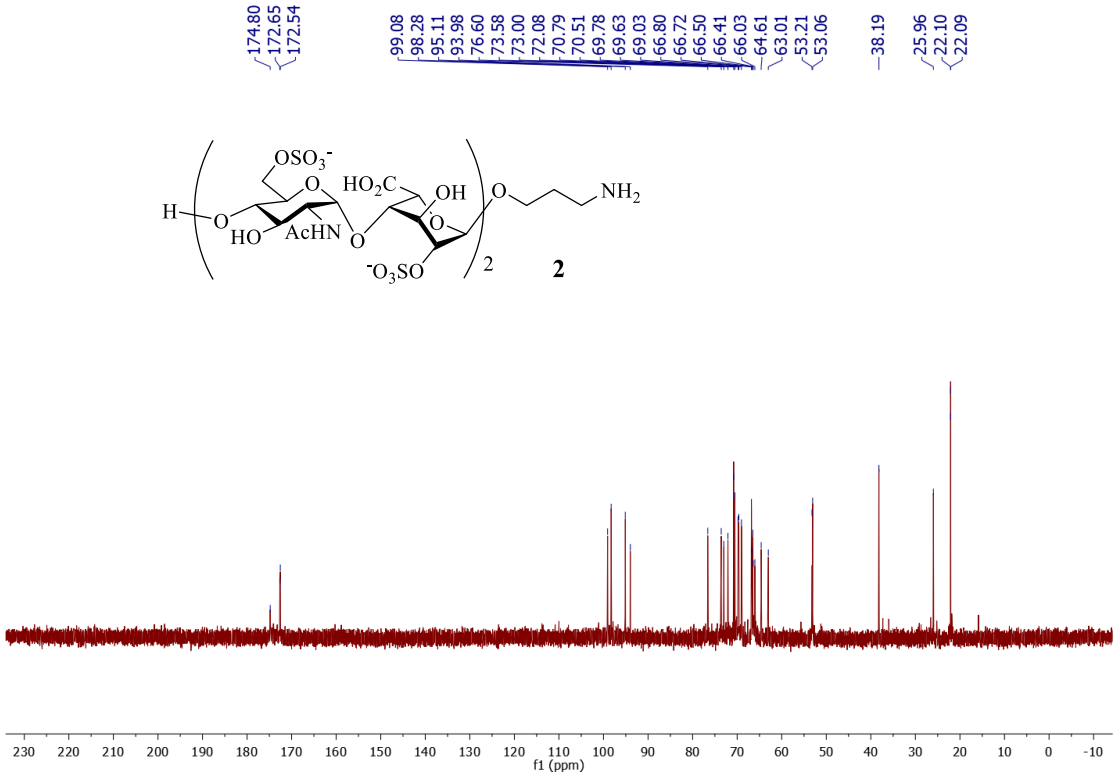

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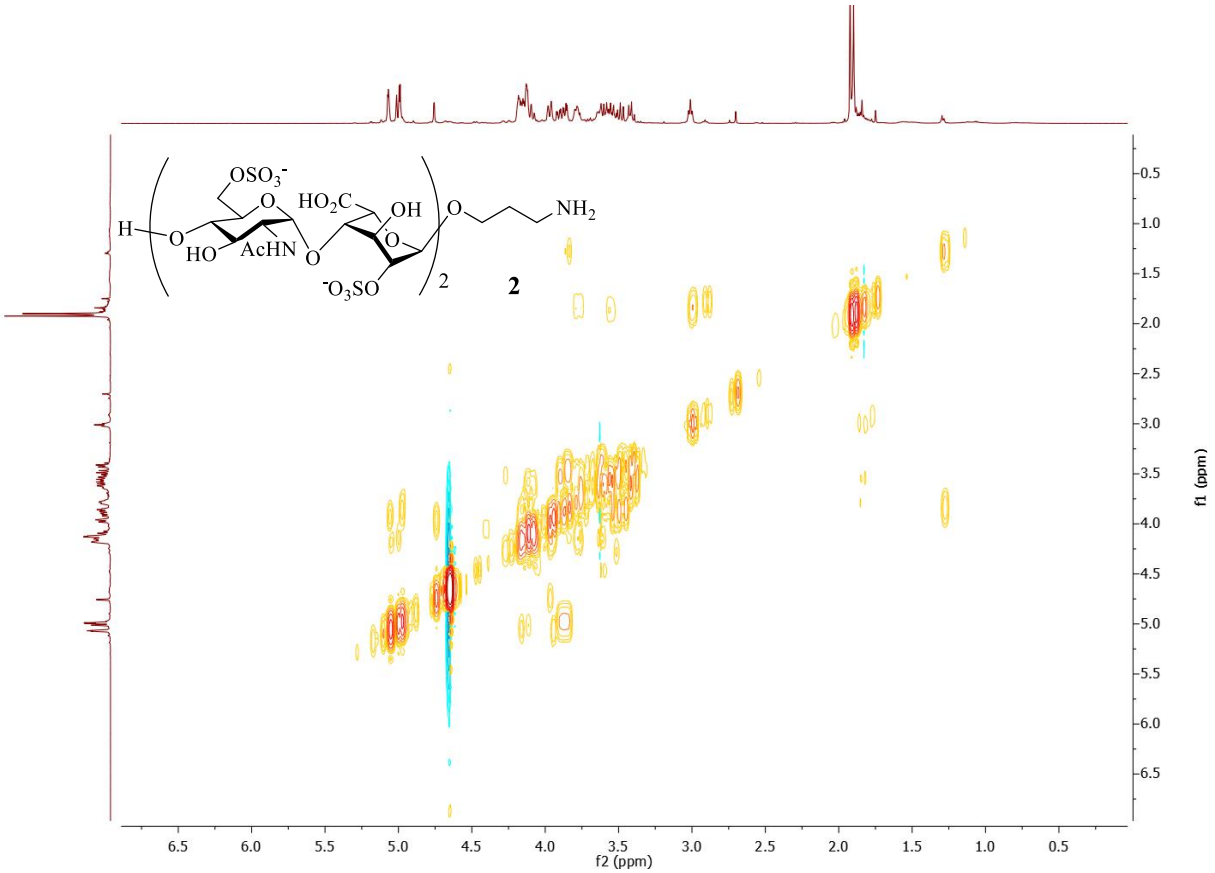

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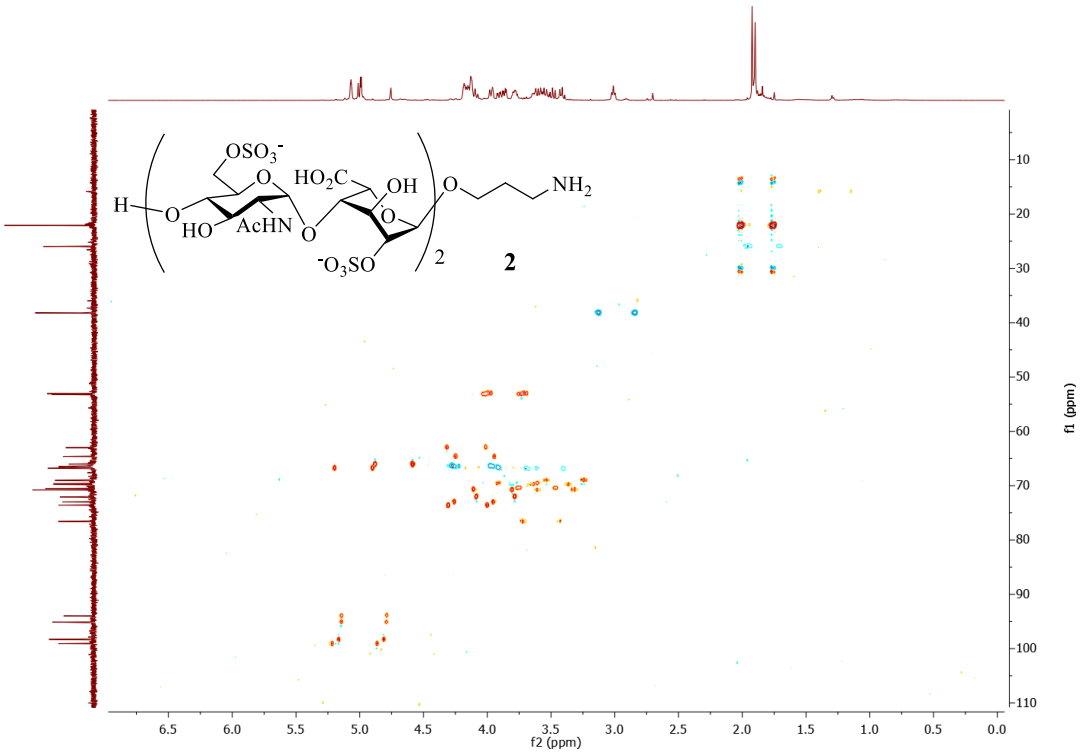

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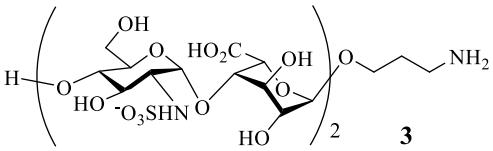

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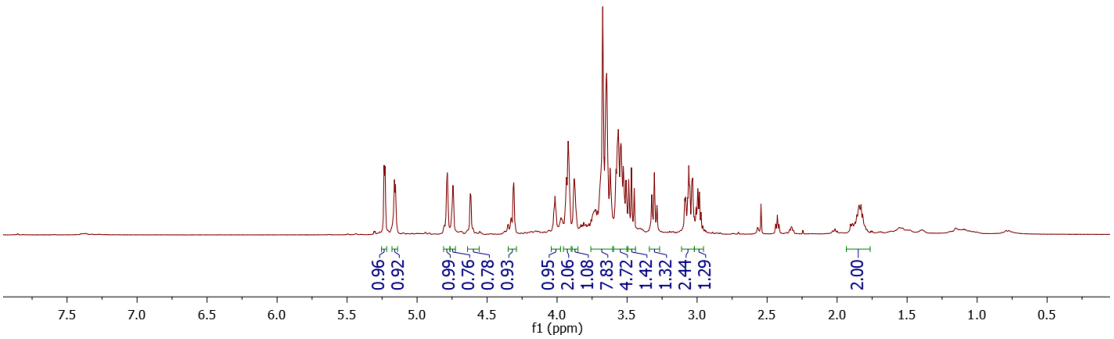

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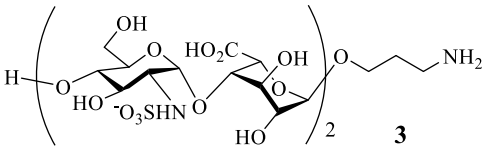

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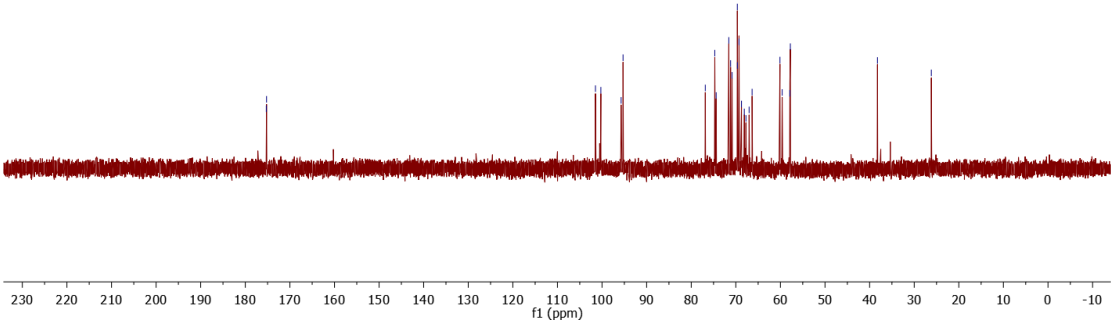
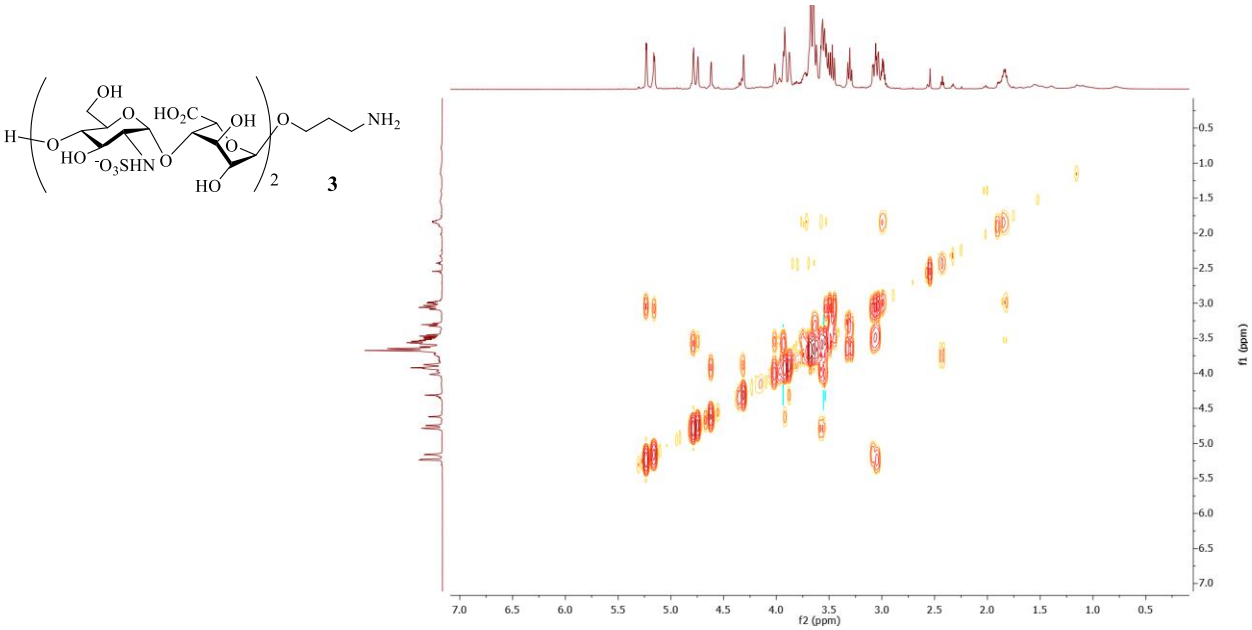


^{1}H-^{1}H gCOSY of **3** (500 MHz D_2O)



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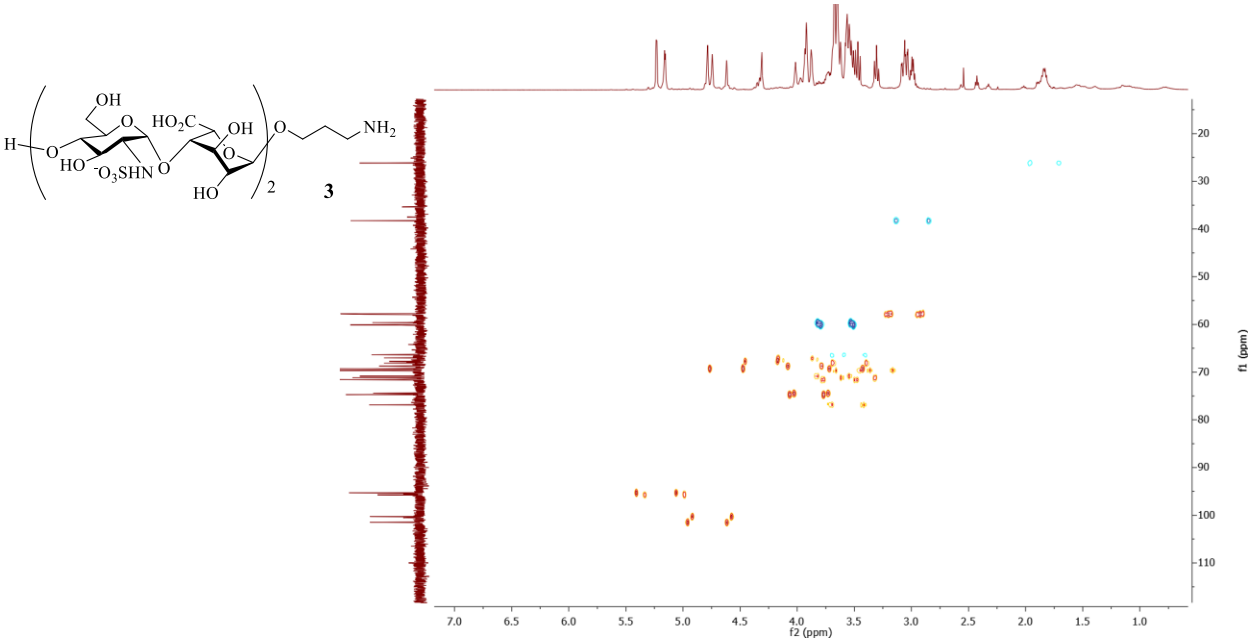
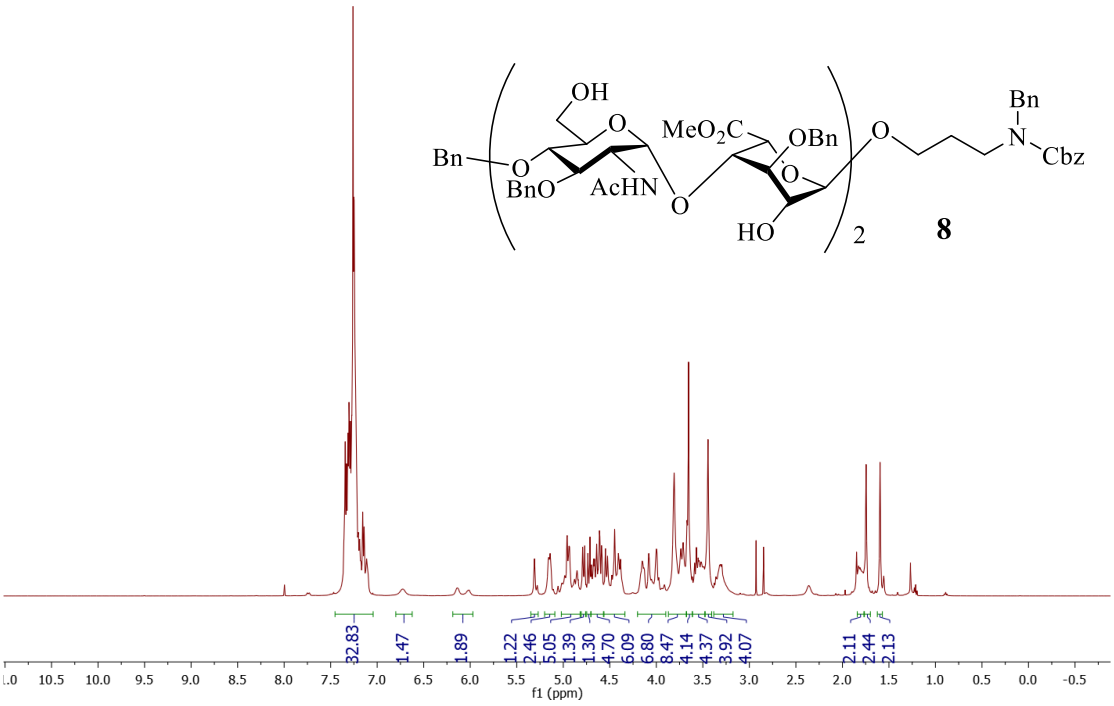
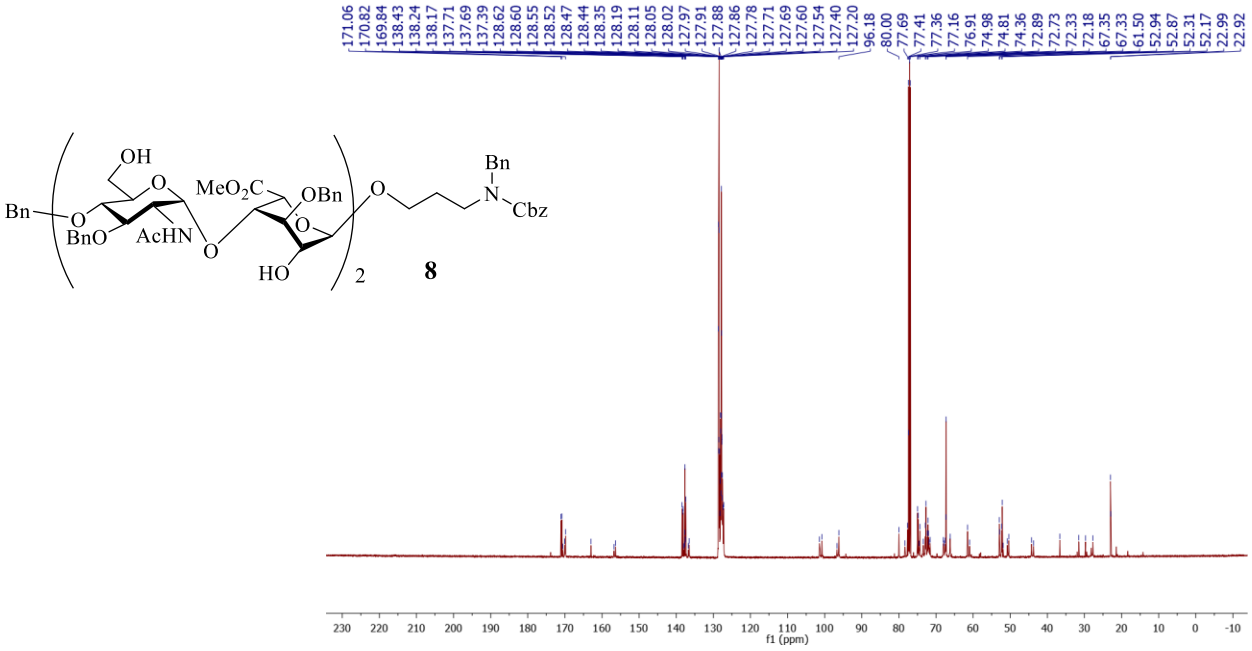


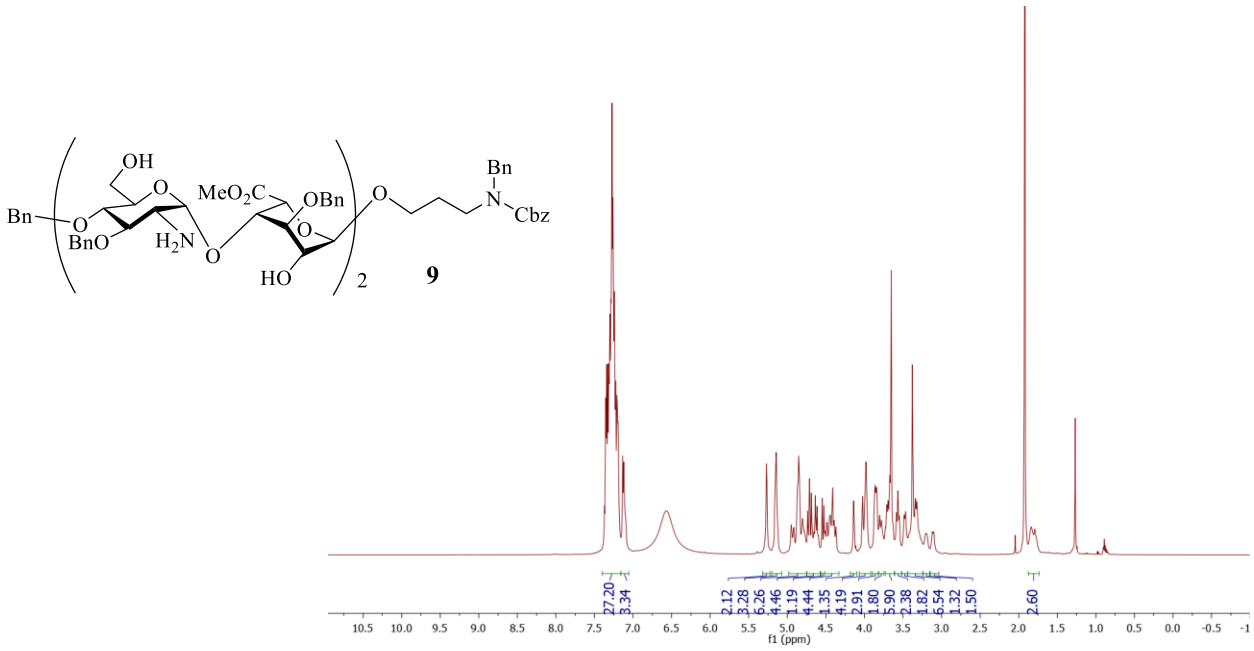
^{1}H-NMR of **8** (500 MHz $CDCl_3$)



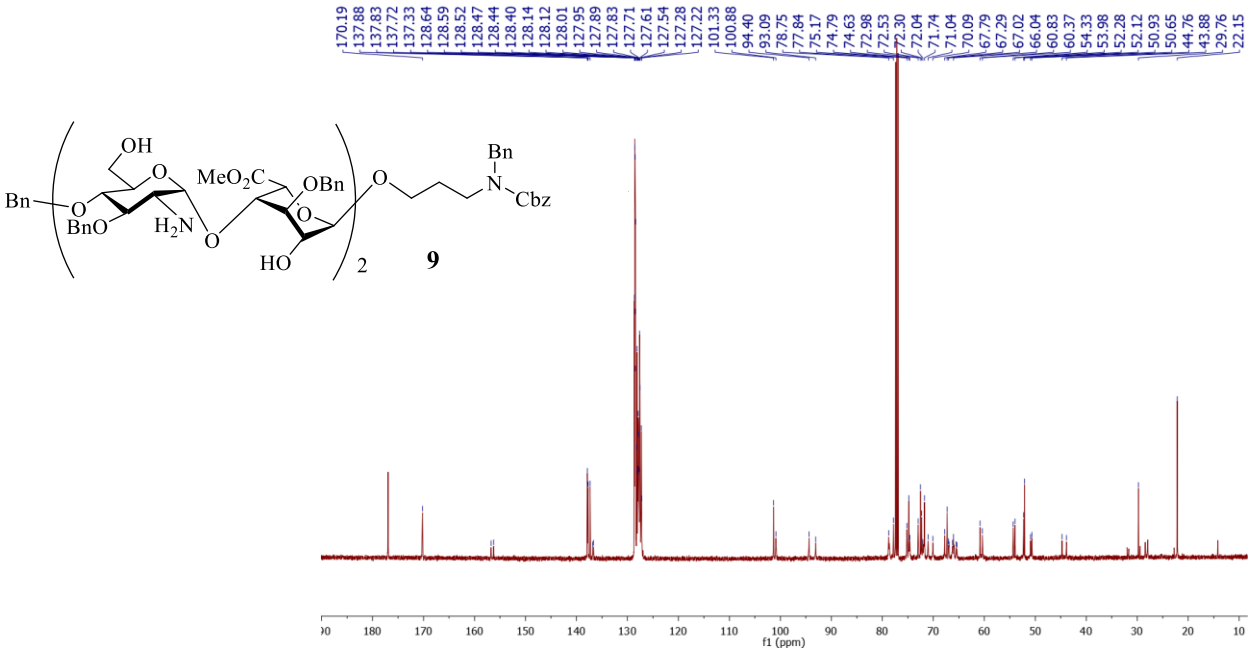
^{13}C-NMR of **8** (125 MHz $CDCl_3$)



^{1}H-NMR of **9** (500 MHz $CDCl_3$)



^{13}C-NMR of **9** (125 MHz $CDCl_3$)



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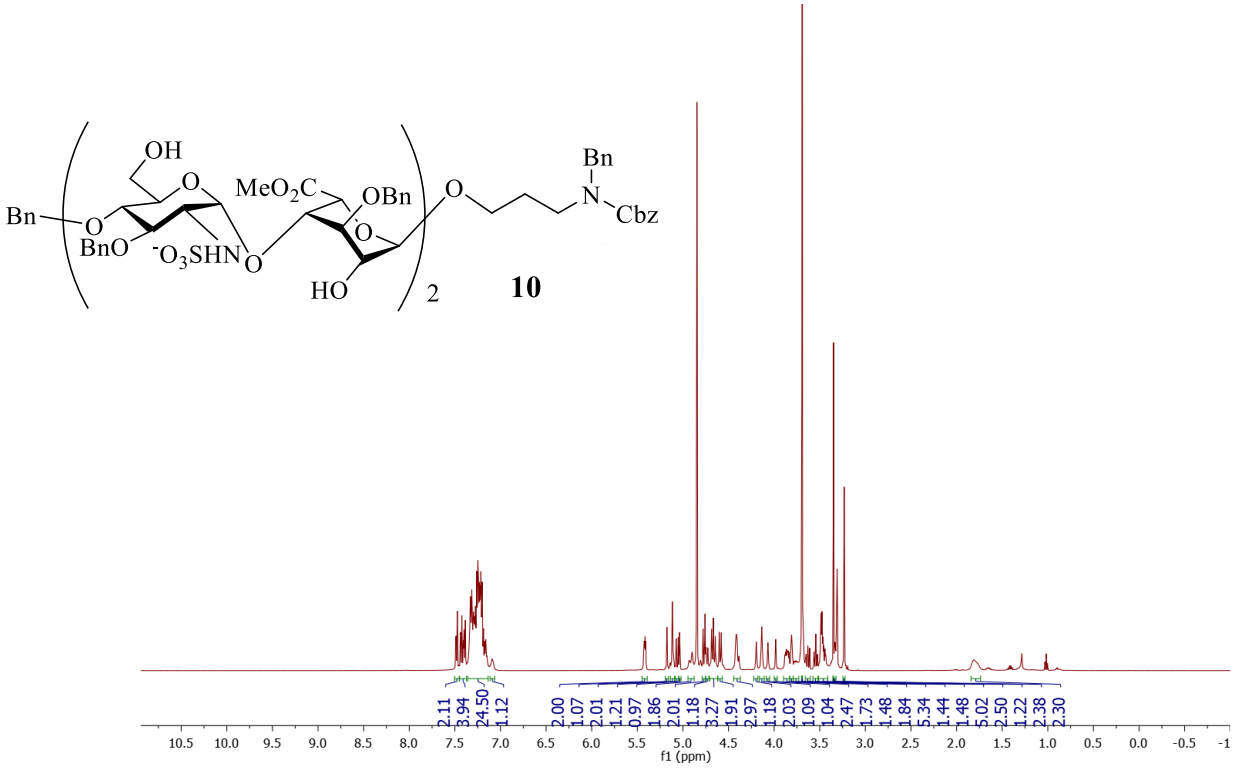

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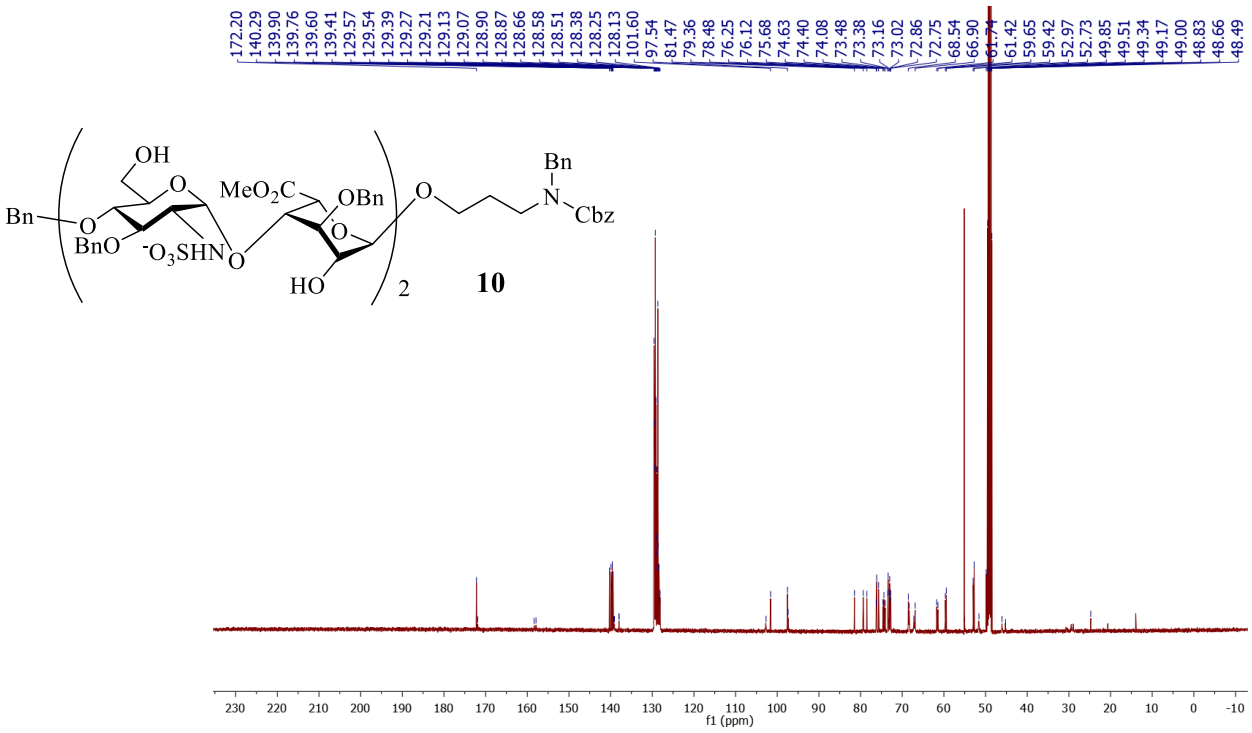
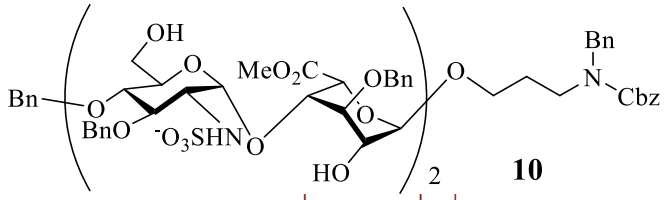


^{1}H-^{1}H gCOSY of **10** (500 MHz CD_3OD)



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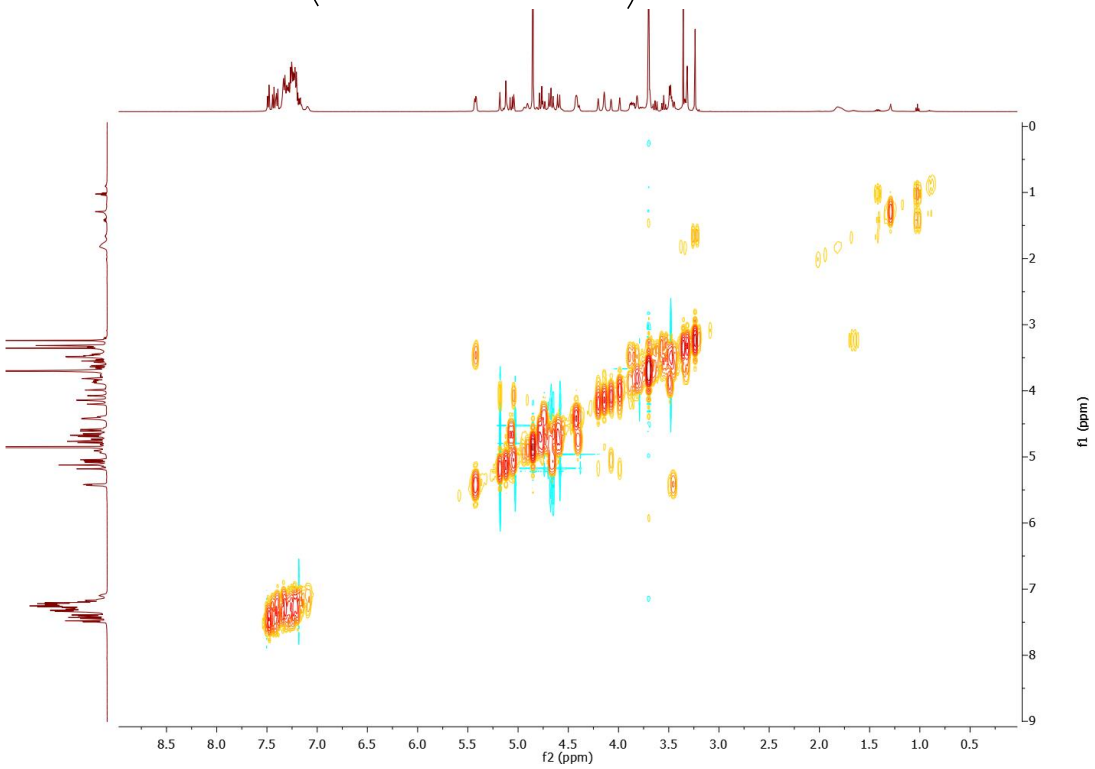

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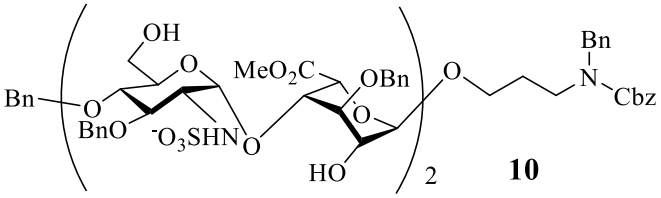

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