

Binding and Neurotoxicity Mitigation of Toxic Tau Oligomers by Synthetic Heparin Like Oligosaccharides

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Supporting Information

Table of Contents

Materials and Methods.....	S4
General Procedure for Preactivation Based Glycosylation	S4
General Procedure for TBS Removal.....	S4
General Procedure for Removal of Levulinoyl Esters.....	S4
General Procedure for Oxidation of 6-OH.....	S4
General Procedure for Methyl Ester Formation after Oxidation.....	S4
General Procedure for Benzyl Ester Formation after Oxidation.....	S5
General Procedure for Transesterification.....	S5
General Procedure for 1, 3-Propanedithiol Mediated Azide Reduction.....	S5
General Procedure for Selective N-Sulfation.....	S5
General Procedure for Simultaneous O, N-Sulfation.....	S5
General Procedure for Global Debenzylation.....	S5
General Procedure for Methyl Ester Saponification.....	S5
Preparation of TauO.....	S6
Preparation of TauO in the presence of heparin like oligosaccharides.....	S6
BLI Binding Assay of Heparin and Tau Oligomers.....	S6
Morphological analysis of TauO by AFM.....	S6
Cell Toxicity assays.....	S6
Immunofluorescence.....	S7
Product Preparation and Characterization Data.....	S8
Compound 3.....	S8
Compound 7.....	S8
Compound 8.....	S9
Compound 9.....	S10
Compound 10.....	S11
Compound 11.....	S11
Compound 12.....	S12
Compound 13.....	S13
Compound 14.....	S14
Compound 15.....	S15
Compound 16.....	S15
Compound 17.....	S16
Compound 18.....	S17
Compound 19.....	S18
Compound 20.....	S18
Compound 21.....	S19
Compound 22.....	S20
Compound 23.....	S21
Compound 24.....	S21

Compound 25.....	S22
Compound 26.....	S23
Compound 27.....	S23
Compound 28.....	S24
Reference	S25
Figure S1.....	S26
Product Characterization Spectra.....	S27
Compound 3.....	S27
Compound 7.....	S31
Compound 8.....	S35
Compound 9.....	S39
Compound 10.....	S43
Compound 11.....	S44
Compound 12.....	S48
Compound 13.....	S52
Compound 14.....	S56
Compound 15.....	S60
Compound 16.....	S64
Compound 17.....	S68
Compound 18.....	S72
Compound 19.....	S76
Compound 20.....	S80
Compound 21.....	S84
Compound 22.....	S88
Compound 23.....	S92
Compound 24.....	S94
Compound 25.....	S98
Compound 26.....	S103
Compound 27.....	S104
Compound 28.....	S105

Materials and Methods

General Procedure for Preactivation Based Glycosylation. A solution of donor (1.0 equiv) and freshly activated 4 Å molecular sieves (1 g per 20 mL of final solvent) in CH₂Cl₂ was stirred at room temperature for 10 min and then cooled to -78 °C. AgOTf (2.5 equiv) dissolved in Et₂O/CH₂Cl₂ (10/1) was added directly to the solution. After 10 min, the orange-colored promoter *p*-TolSCl (1.0 equiv) was added with a microsyringe directly to the flask to avoid freezing the promoter on the walls of the flask. The color of *p*-TolSCl disappeared rapidly, indicating the consumption of *p*-TolSCl. After TLC indicated that the donor was fully activated (about 5 min at -78 °C), a solution of acceptor (0.8-1.0 equiv) in CH₂Cl₂ along with TTBP (1.0 equiv) was slowly added along the walls of the flask. This was done to allow the acceptor solution to cool before mixing with the activated donor. The final ratio of Et₂O/CH₂Cl₂ was 1/1 after all reagents were added. The reaction mixture was slowly warmed to 0 °C over 2 h. The mixture was quenched with Et₃N, diluted with CH₂Cl₂ and filtered through Celite. After washing the Celite with CH₂Cl₂ until all organic compounds were removed, as verified by TLC, the CH₂Cl₂ fractions were combined and washed with sat. NaHCO₃ solution and brine. The organic layer was collected and dried over Na₂SO₄. After removal of the solvent, the product was purified by silica gel chromatography unless noted.

General Procedure for TBS Removal. The TBS-containing oligosaccharide was dissolved in pyridine (5 mL per 1 g oligosaccharide) and transferred to a 50 mL plastic centrifuge tube. The pyridine solution was cooled to 0 °C, followed by dropwise addition of HF·pyridine (2.5 mL per 1 g oligosaccharide) while stirring. The reaction was then allowed to warm to room temperature and kept overnight or 3 days. The reaction was diluted with CH₂Cl₂ and washed sequentially with sat. CuSO₄, sat. NaHCO₃, and 1M HCl. The organic layer was dried over Na₂SO₄, concentrated, and purified by silica gel chromatography.

General Procedure for Removal of Levulinoyl Esters. A solution of the oligosaccharide containing Lev esters (1 equiv) in pyridine/AcOH (3/2) was cooled to 0 °C. To this was added hydrazine hydrate (5 equiv per Lev ester). The reaction was stirred at 0 °C for 3 h or until TLC showed that the reaction was complete. To quench the reaction, excess acetone was added and the reaction was stirred at room temperature for 30 min. The reaction mixture was then diluted with ethyl acetate and washed with 1 M HCl, sat. NaHCO₃ and brine. The resulting organic layer was then dried over Na₂SO₄, concentrated, and purified by silica gel chromatography.

General Procedure for Oxidation of 6-OH. The desired compound to be oxidized (1 equiv) was dissolved in a solution of DCM/*t*-BuOH/H₂O (4/4/1). To this solution was added TEMPO (0.3 equiv per 6-OH), followed by BAIB (3 equiv per 6-OH). The reaction was then stirred at room temperature overnight. After ensuring that the reaction was complete by TLC, the reaction was quenched by addition of excess Na₂S₂O₃ solution and allowed to stir at room temperature for 15 min. The mixture was then diluted with DCM and washed with brine. The organic layers were combined, dried over Na₂SO₄, and concentrated. The crude product could then be protected as a methyl or benzyl ester.

General Procedure for Methyl Ester Formation after Oxidation. The crude product from oxidation was dissolved in DMF. To this solution was added K₂CO₃ (5 equiv per COOH), followed by CH₃I (2.5 equiv per COOH), and the reaction was allowed to stir overnight at room temperature.

After verifying that the reaction was complete by TLC, the reaction was diluted with ethyl acetate and water. The mixture was then washed with 1 M HCl and sat. NaHCO₃, dried over Na₂SO₄, concentrated, and purified by silica gel chromatography.

General Procedure for Benzyl Ester Formation after Oxidation. The crude product from oxidation was dissolved in DCM. To this was added phenyl diazomethane until a deep red color persisted. The reaction was allowed to stir overnight. After TLC indicated that the reaction was complete, the mixture was concentrated and purified by silica gel chromatography.

General Procedure for Transesterification. The ester containing oligosaccharide was dissolved in a mixture of DC/MeOH (1/1). NaOMe solution was added to the oligosaccharide solution until the pH reached 10. The reaction was maintained at pH 10 and stirred at room temperature. After the reaction was confirmed complete by TLC, it was quenched by adding H⁺ resin. The quenched reaction was filtered, concentrated and purified by silica gel chromatography.

General Procedure for 1, 3-Propanedithiol Mediated Azide Reduction. The starting oligosaccharide was dissolved in anhydrous MeOH (dried over 4 Å molecular sieves) and protected from light. To this solution were added triethylamine (30 equiv per N₃) and 1, 3-propanedithiol (30 equiv per N₃), and the reaction was stirred at room temperature for 72 h. The reaction was concentrated and purified by silica gel chromatography.

General Procedure for Selective N-Sulfation. To a solution of NH₂-containing compound (1 equiv) in MeOH was added 1 M aqueous NaOH solution at 0 °C until the pH reaches 10. SO₃·pyridine (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 purified by silica gel chromatography.

General Procedure for Simultaneous O, N-Sulfation. A compound (1 equiv) containing both free OH and NH₂ groups was dissolved in dry pyridine (1 mL per 5 mg compound, dried over 4 Å molecular sieves). To this mixture was added SO₃·pyridine (100 mg per 1mL pyridine), which had been previously washed with H₂O, 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/H₂O = 3/1/1).

General Procedure for Global Debenzylation. A mixture of the Bn-containing compound (for 6 mg of compound, 1 equiv), MeOH/H₂O (4 mL/2 mL), and Pd(OH)₂/C (100 mg) was stirred under H₂ at room temperature overnight and then filtered. The filtrate was concentrated to dryness under vacuum and then diluted with H₂O (15 mL). The aqueous phase was further washed with CH₂Cl₂ (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 H₂O (0.4 mL per 1 mg oligosaccharides) was cooled to 0 °C and 1 M LiOH (15 equiv per COOMe) was added dropwise, followed by addition of H₂O₂ (150 equiv per COOMe, 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 H₂O. 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.

Preparation of TauO. Recombinant tau protein (tau-441 (2N4R) MW 45.9 kDa) was expressed and purified as described.^{1, 2} Tau pellet was treated with 8M urea followed by overnight dialysis against 1X phosphate-buffered saline (PBS), pH 7.4. Tau concentration was measured using bicinchoninic acid protein assay (Micro BCA kit, Pierce) and diluted to 1 mg/ml using 1X PBS. Aliquots of tau monomer in PBS were stored at -20°C. Each 300 µl of tau stock (0.3 mg) was added to 700 µl of 1X PBS and incubated for 1 hour on an orbital shaker at room temperature. After shaking, the resulting TauO were purified by fast protein liquid chromatography (FPLC, Superdex 200HR 10/30 column, Amersham Biosciences).

Preparation of TauO in the presence of heparin like oligosaccharides. TauO (1 µg/µl, 100 µl) were incubated with heparin like oligosaccharides (1:5 molar ratio). Oligosaccharides were dissolved in ddH₂O at a final concentration of 50 mM and diluted in 1X PBS or cell culture medium for incubation or toxicity assays. TauO in the presence of oligosaccharides and controls were incubated without stirring for 16 hours under oligomerization conditions as previously described.³

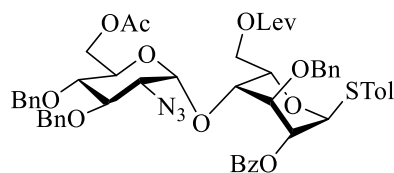
BLI Binding Assay of Heparin and Tau Oligomers. The heparin oligosaccharides were biotinylated by reaction with sulfo-*N*-hydroxysuccinimide long-chain biotin (ApexBio Tech LLC) following a previously reported method.³¹ The binding assay was performed on the Octet K2 System (Pall ForteBio). The biotinylated heparin oligosaccharides were absorbed to streptavidin (SA) sensor at a concentration of 50 µM for 2 min. The sensor was then balanced in the assay buffer (PBS containing 0.005% P20) and dipped into tau oligomer solution in assay buffer at different concentration (4.36, 2.18, 1.09, 0.545, 0.272, 0.136, 0.0681 µM). After 2 min of association, the sensor was brought back to the previous assay buffer for a 3-min dissociation step. At the end of the assay, the sensor was regenerated in 1 M NaCl to remove the bound tau oligomers. Each measurement was repeated 3 times on the same sensor. The control assay was done with another sensor loaded with saturated biotin solution.

Morphological analysis of TauO by AFM. Samples were prepared by adding 10 µl TauO in the absence or presence of heparin like oligosaccharides on freshly-cleaved mica and were allowed to adsorb to the surface.^{4, 5} Mica were then washed three times with distilled water to remove unbound protein and impurities followed by air-drying. Samples were then imaged with a multimode 8 AFM instrument (Veeco, CA) using a non-contact tapping method (ScanAsyst-Air).

Cell Toxicity assays. Human neuroblastoma SH-SY5Y cells were purchased from American Type Culture Collection, cultured and treated for measuring cytotoxicity using LDH release assay (Cytotoxicity Detection KitPLUS -LDH, Roche) following manufacturers' instructions as previously described.⁴⁻⁶ Briefly, cells were maintained in Dulbecco's modified Eagle's medium (DMEM) and grown to confluency in 96-well plates. Cells (≈10,000 cells /well) were treated for 24 hours with 2.0 µM TauO or 2.0 µM TauO incubated with 10 µM of heparin like oligosaccharides (**25**, **27**, and **28**) followed by assaying with LDH. Optical density (OD) was measured at 490 nm with POLARstar OMEGA microplate reader (BMG Labtech). All measurements were performed in triplicate and corrected by the vehicle background. Statistical analysis was based on one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test performed using GraphPad Prism 6.01.

Immunofluorescence. SH-SY5Y cells were maintained in Dulbecco's modified Eagle's medium (DMEM) and grown to confluence using poly-L-lysine coated coverslip in 24-well plates.⁷ Cells ($\approx 20,000$ cells /well) were treated for 1 hour with $0.5 \mu\text{M}$ TauO or a mixture of $0.5 \mu\text{M}$ TauO with $2.5 \mu\text{M}$ of oligosaccharides. After washing off unbound proteins, cells were stained with $5 \mu\text{g/mL}$ WGA (Wheat Germ Agglutinin) for 10 min followed by fixation in chilled methanol. After washing three times with 1X PBS, cells were permeabilized with 0.25% Triton-X 100, diluted in 1X PBS for 10 min. Cells were washed in 1X PBS for 10 min prior to blocking in 5% goat serum for 1 hour and then incubated with Tau 13 antibody (1:1000) overnight. The next day, cells were washed three times with 1X PBS and then incubated with goat anti-mouse IgM Alexa-568 (1:1000, Invitrogen) for 1 hour. After washing three times with PBS (10 min each), cells were then stained with DAPI (Vector Laboratories) and mounted using Vectashield mounting medium (Fluoromount-4',6-diamidino-2-phenylindole). Cells were imaged with confocal microscope Zeiss LSM880 using standard filters for DAPI, GFP and Texas Red channels. Images were analyzed with ImageJ and statistical analysis was performed by Student's T test, using GraphPad Prism 6.01.

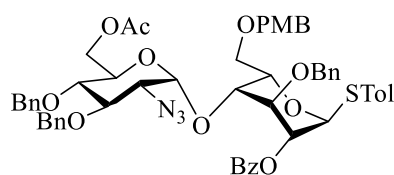
Product Preparation and Characterization Data



p-Tolyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl- 3-*O*-benzyl-6-*O*-levulinoyl-1-thio- α -L-idopyranoside (**3**)

Compound **3** was prepared from compound **7** in 2 steps. Firstly, compound **7** (1.04 g, 1.03 mmol) was dissolved in DCM/H₂O (45/5 mL), cooled to 0 °C and DDQ (467 mg, 2.06 mmol) was added. The reaction was allowed to warm up to room temperature and stirred overnight. Upon completion, the reaction was quenched with sat. NaHCO₃, diluted with DCM and washed sequentially with water and sat. NaHCO₃. The organic phase was dried over Na₂SO₄, concentrated, and purified through silica gel (Hex/EtOAc = 2/1). The product was then diluted in DCM (25 mL). To this solution was added EDC·HCl (522 mg, 2.77 mmol), DMAP (10 mg, 0.08 mmol) and levulinic acid (257 μ L, 2.52 mmol), and the reaction was stirred at room temperature overnight. The mixture was then diluted with DCM, washed with sat. NaHCO₃, dried over Na₂SO₄, concentrated, and purified by silica gel column to afford compound **3** (1.02 g, 89% yield) over 2 steps.

¹HNMR (500 MHz, CDCl₃): δ = 2.02 (s, 3H, Ac), 2.16 (s, 3H, Lev-CH₃), 2.35 (s, 3H, STol-CH₃), 2.56-2.62 (m, 2H, Lev-CH₂), 2.69-2.75 (m, 2H, Lev-CH₂), 3.29 (dd, 1H, *J* = 3.5, 10.0 Hz, Glu-H2), 3.38 (t, 1H, *J* = 9.5 Hz, Glu-H4), 3.56 (t, 1H, *J* = 9.5 Hz, Glu-H3), 3.64 (s, 1H, Ido-H4), 3.91 (d, 1H, *J* = 10.5 Hz, Bn-CH₂), 3.93-3.97 (m, 1H, Glu-H5), 4.17 (s, 1H, Ido-H2), 4.20-4.26 (m, 2H, Glu-H6, Bn-CH₂), 4.26-4.31 (m, 1H, Glu-H6), 4.33 (dd, 1H, *J* = 4.0, 11.5 Hz, Ido-H6), 4.42 (dd, 1H, *J* = 8.0, 12.0 Hz, Ido-H6), 4.50 (d, 1H, *J* = 10.5 Hz, Bn-CH₂), 4.56 (d, 1H, *J* = 4.0 Hz, Glu-H1), 4.73 (d, 1H, *J* = 10.5 Hz, Bn-CH₂), 4.78 (d, 1H, *J* = 11.5 Hz, Bn-CH₂), 4.94-4.98 (m, 1H, Ido-H5), 5.00 (d, 1H, *J* = 12.0 Hz, Bn-CH₂), 5.39 (s, 1H, Ido-H3), 5.58 (s, 1H, Ido-H1), 7.10-7.18 (m, 4H), 7.21-7.44 (m, 14H), 7.46-7.53 (m, 4H), 8.13-8.18 (m, 2H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.85, 21.22, 27.86, 29.91, 37.93, 62.79, 63.88, 63.94, 66.06, 69.55, 70.41, 71.34, 72.64, 75.04, 75.13, 76.08, 77.64, 80.82, 86.5, 99.26, 127.95, 128.06, 128.14, 128.16, 128.38, 128.46, 128.54, 128.58, 128.71, 129.8, 129.89, 130.05, 131.97, 132.23, 133.29, 137.29, 137.45, 137.47, 137.8, 165.73, 170.74, 172.4, 206.45. HRMS: *m/z* calc. for C₅₄H₆₁N₄O₁₃S: 1005.3956; found: 1005.3941 [M + NH₄]⁺

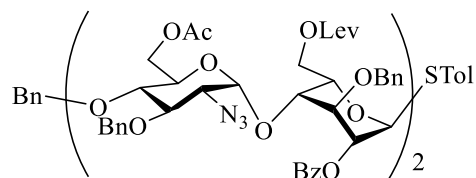


p-Tolyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl- 3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio- α -L-idopyranoside (**7**)

Compound **7** was prepared from compound **5** (533 mg, 1.0 mmol) and **6** (600 mg, 1.0 mmol) by following the general procedure for preactivation based glycosylation. The reaction was performed at -78 °C in DCM/Et₂O (15/15 mL) until quenched by Et₃N at the same temperature to avoid product decomposition under acidic conditions. Purification through silica gel column (Hex/EtOAc = 3/1) provided compound **7** (879 mg, 87% yield).

H1-C1 coupling constant (171.0 Hz) confirmed the stereochemistry.

¹HNMR (500 MHz, CDCl₃): δ = 2.02(s, 3H, Ac), 2.34 (s, 3H, STol-CH₃), 3.31 (dd, 1H, *J* = 3.5, 10.0 Hz, Glu-H2), 3.43 (dd, 1H, *J* = 8.5, 10.0 Hz, Glu-H4), 3.64 (dd, 1H, *J* = 9.0, 10.5 Hz, Glu-H3), 3.74 (t, 1H, *J* = 3.0 Hz, Ido-H4), 3.79 (d, 2H, *J* = 6.0 Hz, Ido-6), 3.82 (s, 3H, OCH₃), 3.98 (dt, 1H, *J* = 3.5, 10.0 Hz, Glu-H5), 4.14 (d, 1H, *J* = 10.5 Hz, Bn-CH₂), 4.17-4.21 (m, 3H, Glu-H6, Ido-H2), 4.32 (d, 1H, *J* = 10.5 Hz, Bn-CH₂), 4.50-4.55 (m, 3H, PMB-CH₂, Bn-CH₂), 4.72 (d, 1H, *J* = 3.5 Hz, Glu-H1), 4.76 (d, 1H, *J* = 3.5 Hz, Bn-CH₂), 4.78 (d, 1H, *J* = 5.0 Hz, Bn-CH₂), 4.94 (dd, 1H, *J* = 2.5, 6.5 Hz, Ido-5), 4.97 (d, 1H, *J* = 12.0 Hz, Bn-CH₂), 5.42 (s, 1H, Ido-H3), 5.57 (s, 1H, Ido-H1), 6.88 (d, 2H, *J* = 9.0 Hz, PMB), 7.07 (d, 2H, *J* = 7.5 Hz), 7.17-7.21 (m, 2H), 7.25-7.43 (m, 16H, PMB, Bz), 7.48 (d, 4H, *J* = 8.0 Hz), 8.13-8.16 (m, 2H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.9, 21.24, 55.35, 62.82, 64.06, 67.18, 69.13, 70.06, 70.12, 72.08, 72.67, 73.08, 75.12, 75.17, 77.72, 80.8, 86.52, 98.64, 113.84, 127.99, 128.02, 128.08, 128.17, 128.21, 128.5, 128.52, 128.52, 128.62, 128.69, 129.44, 129.74, 129.95, 130.01, 130.26, 131.92, 132.52, 133.27, 137.52, 137.56, 137.59, 137.66, 159.28, 165.76, 170.65. HRMS: *m/z* calc. for C₅₇H₆₃N₄O₁₂S: 1027.4163; found: 1027.4120 [M + NH₄]⁺



p-Tolyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl -3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl-1-thio- α -L-idopyranoside (**8**)

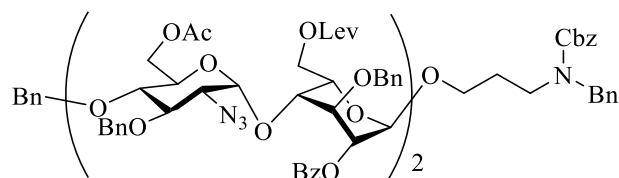
Compound **8** was prepared from compound **3** (412 mg, 0.42 mmol) and **1** (374 mg, 0.42 mmol) by following the general procedure for preactivation based glycosylation in DCM/Et₂O (10/10 mL). Purification through silica gel column (Hex/EtOAc = 1/1) provided compound **8** (629 mg, 85% yield).

H1-C1 coupling constants (170.0, 170.5, 173.0 Hz) confirmed the stereochemistry.

¹HNMR (500 MHz, CDCl₃): δ = 1.99 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.10 (s, 3H, Lev-CH₃), 2.15 (s, 3H, Lev-CH₃), 2.35 (s, 3H, STol-CH₃), 2.43-2.51 (m, 2H, Lev-CH₂), 2.51-2.58 (m, 2H, Lev-CH₂), 2.58-2.63 (m, 2H, Lev-CH₂), 2.65-2.75 (m, 2H, Lev-CH₂), 3.27 (ddd, 2H, *J* = 2.5, 4.0, 9.5 Hz, Glu-H2, Glu-H2), 3.45 (t, 1H, *J* = 9.5 Hz, Glu-H4), 3.48 (t, 1H, *J* = 9.5 Hz, Glu-H3), 3.62 (t, 1H, *J* = 2.5 Hz, Ido^R-H4), 3.66-3.73 (m, 4H, Ido-H4, Glu-H3, Bn-CH₂), 3.83-3.88 (m, 2H, Glu-H5), 4.02-4.07

(m, 2H, Ido-H2), 4.14-4.17 (m, 1H, Ido^R-H2), 4.20-4.40 (m, 9H, Ido^R-H6), 4.47-4.56 (m, 4H, Glu-H1, Bn-CH₂), 4.71 (d, 1H, *J* = 4.0 Hz, Glu-H1), 4.74-4.80 (m, 3H, Bn-CH₂), 4.83 (d, 1H, *J* = 11.5 Hz, Bn-CH₂), 4.94 (ddd, 1H, *J* = 1.5, 4.0, 7.0 Hz, Ido^R-H5), 4.98 (d, 1H, *J* = 12.0 Hz, Bn-CH₂), 5.08 (d, 1H, *J* = 3.5 Hz, Ido-H1), 5.12 (t, 1H, *J* = 4.0 Hz, Ido-H3), 5.38 (t, 1H, *J* = 2.0 Hz, Ido^R-H3), 5.59 (s, 1H, Ido^R-H1), 7.14 (d, 4H, *J* = 8.0 Hz), 7.20-7.53 (m, 31H), 8.10 (d, 2H, *J* = 7.0 Hz, Bz), 8.16 (d, 2H, *J* = 6.5 Hz, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.7, 20.8, 21.18, 27.76, 27.79, 29.83, 29.84, 37.76, 37.89, 62.18, 62.34, 62.56, 63.6, 63.67, 63.9, 65.94, 67.61, 69.53, 70.11, 70.15, 70.23, 71.19, 72.6, 73.43, 74.14, 74.73, 74.88, 75.17, 75.22, 75.29, 75.76, 77.62, 79.17, 80.46, 86.42, 97.8, 98.81, 98.91, 127.61, 127.99, 128.03, 128.07, 128.11, 128.13, 128.16, 128.25, 128.28, 128.33, 128.44, 128.49, 128.5, 128.62, 128.68, 129.61, 129.76, 129.84, 129.86, 129.92, 131.92, 132.15, 133.35, 133.42, 137.24, 137.31, 137.34, 137.5, 137.72, 137.74, 165.44, 165.73, 170.62, 170.74, 172.25, 172.26, 206.36, 206.39. HRMS: *m/z* calc. for C₉₄H₁₀₄N₇O₂₆S: 1778.6752; found: 1778.6780 [M + NH₄]⁺

(^R: reducing end)



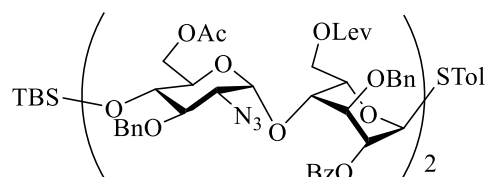
N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4) -2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranoside (**9**)

Compound **9** was prepared from compound **3** (153 mg, 0.16 mmol) and **2** (150 mg, 0.14 mmol) by following the general procedure for preactivation based glycosylation in DCM/Et₂O (7/7 mL). Purification through silica gel column (Hex/EtOAc = 1/1) provided compound **9** (239 mg, 88% yield).

H1-C1 coupling constants (169.5, 170.5, 172.0, 173.0 Hz) confirmed the stereochemistry.

¹HNMR (500 MHz, CDCl₃): δ = 1.80-1.91 (m, 2H, Linker-CH₂), 1.98 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.10 (s, 3H, Lev-CH₃), 2.14 (s, 3H, Lev-CH₃), 2.41-2.51 (m, 4H, Lev-CH₂), 2.60 (t, 2H, *J* = 7.0 Hz, Lev-CH₂), 2.64-2.73 (m, 2H, Lev-CH₂), 3.23-3.28 (m, 2H, Glu-H2, Glu-H2), 3.31-3.40 (m, 2H, Linker-CH₂), 3.41-3.52 (m, 2H, Glu-H4), 3.54-3.60 (m, 1H, Glu-H3), 3.60-3.77 (m, 5H, Glu-H3), 3.80-3.87 (m, 2H, Linker-CH₂), 3.93 (d, 1H, *J* = 10.0 Hz), 4.00-4.07 (m, 3H, Ido-H2), 4.15-4.22 (m, 1H), 4.22-4.30 (m, 4H), 4.30-4.40 (m, 5H), 4.43-4.54 (m, 4H, Linker-Bn-CH₂), 4.59-4.66 (m, 2H, Glu-H1), 4.68 (d, 1H, *J* = 3.5 Hz, Glu-H1), 4.69-4.74 (m, 1H), 4.74-4.86 (m, 4H), 4.88-4.98 (m, 1H, Ido-H1), 5.06-5.13 (m, 3H, Ido-H1, Ido-H3), 5.16 (d, 2H, *J* = 10.0 Hz), 7.10-7.20 (m, 3H), 7.20-7.50 (m, 38H), 8.07-8.11 (m, 2H), 8.12-8.15 (m, 2H). ¹³CNMR (125 MHz, CDCl₃): δ = 20.85, 20.87, 27.82, 29.9, 37.83, 62.33, 62.62, 63.75, 63.9, 65.41, 67.24, 67.49, 68.75, 70.01, 70.19, 70.21, 72.31, 73.43, 73.96, 74.93, 74.95, 75.18, 75.26, 75.34, 77.68, 79.19, 80.54, 97.91, 98.39, 98.87, 127.36,

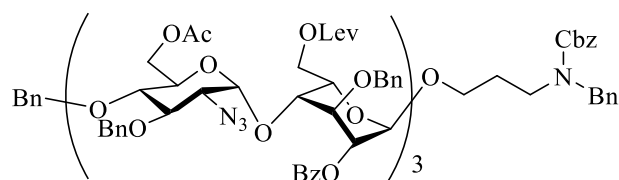
127.68, 127.94, 128.03, 128.08, 128.15, 128.22, 128.29, 128.33, 128.41, 128.48, 128.51, 128.55, 128.58, 128.6, 128.67, 128.72, 129.69, 129.87, 129.9, 133.4, 137.36, 137.38, 137.54, 137.79, 165.52, 165.76, 170.67, 170.77, 172.29, 172.35, 206.4. HRMS: m/z calc. for $C_{105}H_{117}N_8O_{29}$: 1953.7926; found: 1953.7886 $[M + NH_4]^+$



p-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl-1-thio- α -L-idopyranoside (**10**)

Compound **10** was prepared from compound **4** (93 mg, 0.092 mmol) and **1** (83 mg, 0.092 mmol) by following the general procedure for preactivation based glycosylation in DCM/Et₂O (5/5 mL). Purification through silica gel column (Hex/EtOAc = 2/1) provided compound **10** (118 mg, 72% yield).

¹HNMR (500 MHz, CDCl₃): δ = -0.07 (s, 3H), 0.00 (s, 3H), 0.88 (s, 9H), 1.99 (s, 3H), 2.01 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 2.33 (s, 3H), 2.45-2.55 (m, 4H), 2.60-2.75 (m, 4H), 3.24 (ddd, 2H, J = 3.5, 9.0, 10.0 Hz), 3.42-3.50 (m, 2H), 3.56 (dd, 1H, J = 8.5, 9.5 Hz), 3.60 (t, 1H, J = 2.5 Hz), 3.65 (dd, 1H, J = 8.5, 10.0 Hz), 3.69 – 3.76 (m, 3H), 3.82 (ddd, 1H, J = 2.0, 4.5, 10.0 Hz), 4.00-4.08 (m, 3H), 4.09-4.14 (m, 2H), 4.20 (dd, 1H, J = 2.0, 12.0 Hz), 4.24 (dd, 1H, J = 5.0, 11.5 Hz), 4.27-4.34 (m, 4H), 4.35-4.42 (m, 2H), 4.49 (t, 2H, J = 10.5 Hz), 4.54 (d, 1H, J = 4.0 Hz), 4.72 – 4.78 (m, 3H), 4.80 (d, 1H, J = 3.5 Hz), 4.90 (ddd, 1H, J = 2.0, 4.5, 7.5 Hz), 4.95 (d, 1H, J = 12.0 Hz), 5.05 (d, 1H, J = 4.0 Hz), 5.12 (t, 1H, J = 4.5 Hz), 5.35-5.37 (m, 1H), 5.55 (s, 1H), 7.10-7.14 (m, 4H), 7.20-7.26 (m, 6H), 7.25-7.33 (m, 8H), 7.35-7.51 (m, 12H), 8.05-8.09 (m, 2H), 8.11-8.14 (m, 2H). Comparison with literature data confirmed its identity.⁸

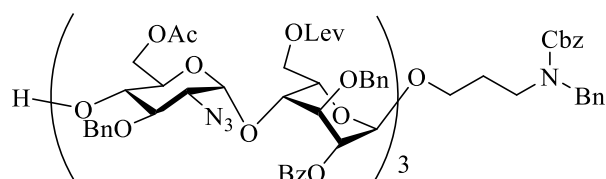


N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranoside (**11**)

Compound **11** was prepared from compound **8** (110 mg, 0.062 mmol) and **2** (67 mg, 0.062 mmol) by following the general procedure for preactivation based glycosylation in DCM/Et₂O (5/5 mL). Purification through silica gel column (Hex/DCM/EtOAc = 1/1/1) provided compound **11** (98 mg, 58% yield).

H1-C1 coupling constants (167.0, 167.5, 170.0, 170.5, 171.5, 173.0 Hz) confirmed the stereochemistry.

¹HNMR (500 MHz, CDCl₃): δ = 1.78-1.92 (m, 2H, Linker-CH₂), 1.98 (s, 3H, Ac), 2.02 (s, 6H, Ac), 2.10 (s, 6H, Lev-CH₃), 2.14 (s, 3H, Lev-CH₃), 2.39-2.74 (m, 12H, Lev-CH₂), 3.21-3.28 (m, 3H, Glu-H₂), 3.29-3.40 (m, 3H, Linker-CH₂), 3.43 (t, 2H, *J* = 9.5 Hz), 3.55 (t, 1H, *J* = 9.5 Hz), 3.58-3.72 (m, 6H, Ido-H₄), 3.73-3.78 (m, 2H, Linker-CH₂), 3.91 (d, 1H, *J* = 10.5 Hz), 3.97-4.09 (m, 5H, Ido-H₂), 4.14-4.30 (m, 8H, Glu-H₆), 4.30-4.39 (m, 6H), 4.41-4.55 (m, 5H, Linker-Bn-CH₂), 4.57-4.63 (m, 2H, Glu-H₁), 4.63-4.85 (m, 10H, Glu-H₁), 4.86-4.97 (m, 2H, Ido-H₁), 5.03-5.10 (m, 3H, Ido-H₁), 5.10-5.19 (m, 4H, Ido-H₃), 7.10-7.53 (m, 54H), 8.01-8.18 (m, 6H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.8, 20.85, 27.8, 27.82, 29.89, 37.8, 37.86, 43.93, 44.95, 50.83, 51.05, 62.04, 62.26, 62.32, 62.62, 62.96, 63.25, 63.43, 63.64, 63.74, 63.84, 65.4, 65.54, 65.74, 67.23, 67.44, 67.86, 68.73, 69.98, 70.15, 70.22, 70.43, 70.67, 71.31, 72.31, 72.37, 73.42, 73.58, 73.86, 74.36, 74.69, 74.9, 74.94, 75.11, 75.27, 75.35, 75.43, 77.67, 78.92, 79.07, 80.08, 80.56, 97.81, 97.97, 98.35, 98.47, 98.87, 127.36, 127.64, 127.73, 127.85, 127.9, 127.94, 127.96, 128.01, 128.04, 128.08, 128.11, 128.14, 128.19, 128.21, 128.26, 128.28, 128.3, 128.33, 128.41, 128.43, 128.46, 128.48, 128.53, 128.56, 128.58, 128.61, 128.68, 128.71, 128.75, 129.58, 129.68, 129.89, 129.92, 133.45, 133.5, 136.81, 136.91, 137.32, 137.35, 137.38, 137.53, 137.73, 137.78, 137.85, 137.98, 138.05, 156.22, 156.77, 165.52, 165.56, 165.75, 170.68, 170.72, 170.78, 171.95, 172.26, 172.31, 172.34, 206.42, 206.46. HRMS: *m/z* calc. for C₁₄₅H₁₆₄N₁₂O₄₂: 1372.5533; found: 1372.5518 [M + 2NH₄]²⁺

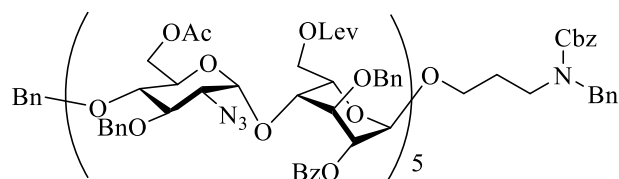


N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranoside (**12**)

Compound **12** was prepared from compound **10** (200 mg, 0.11 mmol) and **2** (96 mg, 0.09 mmol) by following the general procedure for preactivation based glycosylation in DCM/Et₂O (5/5 mL) and TBS removal in pyridine (3 mL). Purification through silica gel column (Hex/DCM/EtOAc = 1/1/2) provided compound **12** (198 mg, 84% yield) over 2 steps.

¹HNMR (500 MHz, CDCl₃): δ = 1.80-1.90 (m, 2H, Linker-CH₂), 2.01 (s, 3H, Ac), 2.02 (s, 3H, Ac),

2.04 (s, 3H, Ac), 2.10 (s, 3H, Lev-CH₃), 2.12 (s, 3H, Lev-CH₃), 2.13 (s, 3H, Lev-CH₃), 2.36-2.56 (m, 6H, Lev-CH₂), 2.57-2.73 (m, 6H, Lev-CH₂), 3.21 (dd, 1H, *J* = 3.5, 10.0 Hz, Glu-H2), 3.23-3.27 (m, 2H, Glu-H2), 3.30-3.50 (m, 5H, Linker-CH₂), 3.54 (t, 1H, *J* = 9.5 Hz), 3.57-3.65 (m, 3H, Glu-H3), 3.65-3.71 (m, 3H, Ido-H4), 3.72-3.85 (m, 5H, Linker-CH₂), 3.91 (d, 1H, *J* = 10.0 Hz), 3.98-4.07 (m, 5H, Ido-H2), 4.10-4.24 (m, 5H), 4.25-4.39 (m, 8H), 4.41 (d, 1H, *J* = 11.0 Hz), 4.43-4.47 (m, 2H, Linker-Bn-CH₂), 4.49 (d, 1H, *J* = 4.0 Hz), 4.51 (d, 1H, *J* = 4.0 Hz), 4.59 (d, 2H, *J* = 10.0 Hz), 4.61 (d, 1H, *J* = 7.5 Hz, Glu-H1), 4.66-4.84 (m, 9H, Glu-H1), 4.87-4.95 (m, 1H), 5.03-5.10 (m, 3H, Ido-H1), 5.10-5.18 (m, 4H, Ido-H3), 7.10-7.38 (m, 40H), 7.39-7.54 (m, 9H), 8.06-8.15 (m, 6H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.85, 20.86, 27.82, 28.41, 29.89, 29.92, 37.86, 37.9, 43.93, 44.95, 50.83, 51.05, 62.08, 62.21, 62.28, 62.4, 62.84, 63.1, 63.44, 63.72, 63.85, 65.41, 65.54, 65.75, 67.23, 67.63, 67.81, 68.73, 70.15, 70.21, 70.31, 70.38, 70.72, 71.26, 72.32, 72.45, 73.44, 73.56, 73.79, 74.16, 74.3, 74.7, 74.93, 75.07, 75.18, 75.3, 75.41, 78.84, 79.07, 79.81, 97.8, 97.86, 98.35, 98.44, 98.47, 98.52, 127.35, 127.63, 127.67, 127.84, 127.88, 127.93, 128.0, 128.1, 128.13, 128.16, 128.17, 128.24, 128.4, 128.46, 128.48, 128.6, 128.61, 128.68, 128.7, 128.73, 129.57, 129.88, 129.91, 133.42, 133.49, 133.54, 136.79, 136.89, 137.3, 137.37, 137.72, 137.78, 137.84, 137.89, 137.96, 137.98, 138.03, 156.2, 156.76, 165.51, 165.58, 165.75, 170.77, 170.8, 171.84, 172.25, 172.34, 172.43, 206.42, 206.48, 206.93. HRMS: *m/z* calc. for C₁₃₈H₁₅₄N₁₁O₄₂: 2637.0253; found: 2637.0212 [M + NH₄]⁺



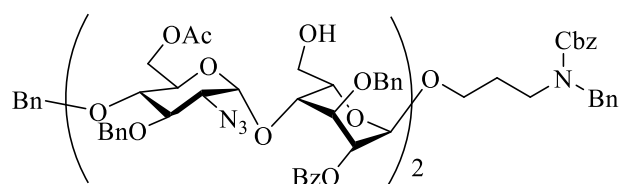
N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4) -2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinoyl- α -L-idopyranoside (**13**)

Compound **13** was prepared from compound **8** (808 mg, 0.46 mmol) and **12** (841 mg, 0.32 mmol) by following the general procedure for preactivation based glycosylation in DCM/Et₂O (30/10 mL). Purification through silica gel column (Hex/DCM/EtOAc = 1/1/1) provided compound **13** (1.145 g, 84% yield).

H1-C1 coupling constants (168.0, 168.5, 170.0, 171.0, 173.0, 173.0 Hz) confirmed the stereochemistry. No all H1-C1 were found due to the overlap of signals.

¹HNMR (500 MHz, CDCl₃): δ = 1.82-1.93 (m, 2H, Linker-CH₂), 1.99 (s, 3H, Ac), 2.02 (s, 6H, Ac), 2.04 (s, 6H, Ac), 2.11 (s, 12H, Lev-CH₃), 2.14 (s, 3H, Lev-CH₃), 2.40-2.73 (m, 20H, Lev-CH₂),

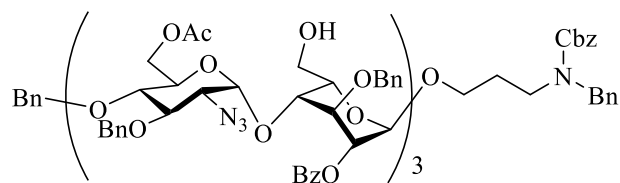
3.24-3.30 (m, 5H, Glu-H2), 3.32-3.41 (m, 3H, Linker-CH₂), 3.45 (t, 2H, *J* = 9.5 Hz, Glu-H4), 3.56 (t, 1H, *J* = 9.5 Hz, Glu-H3), 3.61-3.73 (m, 10H, Glu-H3), 3.73-3.81 (m, 6H, Linker-CH₂), 3.81-3.88 (m, 3H), 3.92 (d, 1H, *J* = 10.5 Hz), 3.99-4.09 (m, 9H, Ido-H2), 4.14-4.26 (m, 10H), 4.26-4.39 (m, 14H), 4.43-4.49 (m, 2H, Linker-Bn-CH₂), 4.49-4.55 (dd, 3H, *J* = 7.0, 10.5 Hz), 4.58-4.64 (m, 2H, Glu-H1), 4.68 (d, 1H, *J* = 3.5 Hz, Glu-H1), 4.71-4.85 (m, 15H, Glu-H1), 4.88-4.97 (m, 2H, Ido-H1), 5.05-5.12 (m, 5H, Ido-H1), 5.12-5.20 (m, 6H, Ido-H3), 7.11-7.55 (m, 80H), 8.07-8.17 (m, 10H). ¹³CNMR (125 MHz, CDCl₃): δ = 20.7, 20.78, 27.74, 29.81, 37.72, 37.77, 37.79, 43.86, 44.88, 50.76, 50.98, 61.96, 62.18, 62.26, 62.31, 62.55, 63.36, 63.61, 63.68, 63.76, 65.31, 65.45, 65.67, 67.15, 67.39, 67.75, 68.64, 69.93, 70.09, 70.12, 70.15, 70.34, 72.23, 72.33, 73.35, 73.49, 73.52, 73.81, 74.18, 74.22, 74.34, 74.56, 74.61, 74.81, 74.85, 75.02, 75.06, 75.19, 75.28, 75.32, 75.38, 77.6, 78.72, 78.75, 78.87, 78.99, 80.48, 97.72, 97.8, 97.9, 98.29, 98.39, 98.79, 127.29, 127.57, 127.62, 127.67, 127.78, 127.82, 127.87, 127.94, 127.97, 128.0, 128.03, 128.07, 128.1, 128.12, 128.15, 128.24, 128.26, 128.34, 128.38, 128.41, 128.48, 128.51, 128.54, 128.61, 128.66, 128.68, 129.48, 129.49, 129.6, 129.82, 133.38, 133.43, 133.49, 136.75, 136.84, 137.25, 137.28, 137.32, 137.47, 137.67, 137.78, 137.83, 137.95, 156.14, 156.68, 165.43, 165.46, 165.48, 165.68, 170.59, 170.64, 170.7, 172.17, 172.2, 172.24, 172.27, 206.34, 206.38. HRMS: *m/z* calc. for C₂₂₅H₂₄₆N₁₇O₆₈: 4273.6314; found: 4273.6372 [M + NH₄]⁺



N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside (**14**)

Compound **14** was prepared from compound **9** (1.0 g, 0.52 mmol) by following the general procedure for removal of levulinoyl esters in pyridine/AcOH (9/6 mL). Purification through silica gel column (Hex/DCM/EtOAc = 1/1/1) provided compound **14** (778 mg, 86% yield).

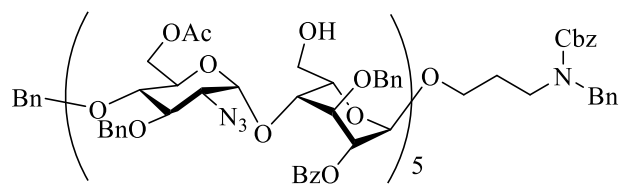
¹HNMR (500 MHz, CDCl₃): δ = 1.80-1.90 (m, 2H, Linker-CH₂), 2.01 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.60 (br, 1H, OH), 3.17-3.37 (m, 5H, Linker-CH₂, Glu-H2), 3.38-3.44 (m, 1H, Glu-H4), 3.50-3.61 (m, 3H, Ido-H4), 3.61-3.80 (m, 5H, Glu-H3), 3.81-4.00 (m, 5H, Linker-CH₂, Glu-H5), 4.03-4.14 (m, 3H, Ido-H2), 4.14-4.29 (m, 4H), 4.29-4.43 (m, 3H), 4.43-4.60 (m, 5H, Glu-H1, Linker-Bn-CH₂), 4.64-4.74 (m, 2H, Glu-H1), 4.76 (d, 2H, *J* = 10.5 Hz), 4.81-4.97 (m, 3H), 4.98-5.05 (m, 1H, Ido-H1), 5.05-5.21 (m, 4H, Ido-H1, Ido-H3), 7.10-7.50 (m, 41H), 8.08-8.17 (m, 4H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.89, 28.19, 28.35, 44.87, 50.61, 61.3, 62.72, 62.8, 63.79, 64.17, 66.33, 67.41, 68.9, 69.13, 70.01, 70.37, 72.14, 72.99, 74.03, 75.09, 75.24, 75.28, 77.74, 79.45, 80.7, 97.98, 98.22, 127.35, 127.42, 127.92, 127.95, 128.03, 128.07, 128.19, 128.22, 128.27, 128.39, 128.43, 128.5, 128.54, 128.57, 128.59, 128.65, 128.7, 129.73, 129.79, 129.92, 130.1, 133.26, 133.42, 137.4, 137.46, 137.52, 137.54, 137.87, 165.81, 170.58, 170.69. HRMS: *m/z* calc. for C₉₅H₁₀₂N₇O₂₅: 1740.6925; found: 1740.6924 [M + H]⁺



N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4) -2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside (**15**)

Compound **15** was prepared from compound **11** (500 mg, 0.18 mmol) by following the general procedure for removal of levulinoyl esters in pyridine/AcOH (9/6 mL). Purification through silica gel column (Hex/DCM/EtOAc = 1/1/1) provided compound **15** (404 mg, 93% yield).

^1H NMR (500 MHz, CDCl_3): δ = 1.81-1.89 (m, 2H, Linker- CH_2), 2.00 (s, 3H, Ac), 2.04 (s, 6H, Ac), 3.12-3.23 (m, 3H), 3.23-3.38 (m, 4H, Linker- CH_2 , Glu-H2, Glu-H3), 3.38-3.44 (m, 2H), 3.44-3.63 (m, 7H, Ido-H4), 3.63-3.72 (m, 2H), 3.72-3.82 (m, 4H, Linker- CH_2), 3.82-4.00 (m, 5H), 4.01-4.09 (m, 3H), 4.15-4.29 (m, 7H), 4.30-4.62 (m, 9H, Glu-H1, Linker-Bn- CH_2), 4.63-4.80 (m, 6H, Glu-H1), 4.81-4.97 (m, 4H, Ido-H1), 4.98-5.22 (m, 8H, Ido-H1, Ido-H3), 7.12-7.47 (m, 54H), 8.08-8.17 (m, 6H, Bz). ^{13}C NMR (125 MHz, CDCl_3): δ = 14.32, 20.88, 20.94, 21.18, 28.18, 50.59, 60.51, 61.24, 61.33, 62.41, 62.72, 62.79, 63.8, 64.02, 67.41, 67.67, 69.01, 70.0, 70.07, 70.42, 72.14, 72.91, 72.99, 73.14, 73.69, 74.03, 75.15, 75.18, 75.24, 75.27, 77.73, 79.35, 79.47, 80.73, 97.34, 97.89, 97.95, 98.22, 127.34, 127.43, 127.89, 127.93, 128.03, 128.07, 128.12, 128.23, 128.26, 128.29, 128.32, 128.4, 128.47, 128.54, 128.6, 128.66, 128.68, 129.66, 129.76, 129.88, 129.9, 130.1, 133.27, 133.41, 137.4, 137.48, 137.52, 137.86, 165.85, 165.91, 170.6, 170.7, 171.27. HRMS: m/z calc. for $\text{C}_{130}\text{H}_{139}\text{N}_{10}\text{O}_{36}$: 2415.9353; found: 2415.9319 $[\text{M} + \text{H}]^+$

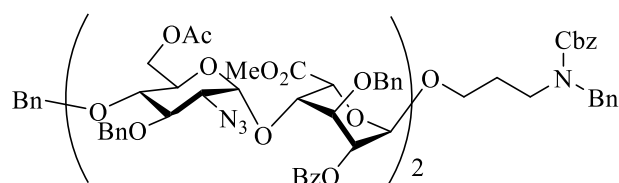


N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside (**16**)

Compound **16** was prepared from compound **13** (120 mg, 0.028 mmol) by following the general

procedure for removal of levulinoyl esters in pyridine/AcOH (6/4 mL). Purification through silica gel column (Hex/DCM/EtOAc = 1/1/1) provided compound **16** (88 mg, 83% yield).

¹HNMR (500 MHz, CDCl₃): δ = 1.80-1.89 (m, 2H, Linker-CH₂), 1.99 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.02 (s, 6H, Ac), 2.03 (s, 3H, Ac), 3.07-3.24 (m, 5H), 3.28 (dd, 1H, *J* = 3.5, 10.0 Hz), 3.28-3.37 (m, 5H, Linker-CH₂, Glu-H₂), 3.37-3.49 (m, 6H), 3.50-3.62 (m, 8H, Ido-H₄), 3.62-3.70 (m, 3H, Glu-H₃), 3.70-3.99 (m, 13H, Linker-CH₂), 3.99-4.08 (m, 5H, Ido-H₂), 4.08-4.37 (m, 18H), 4.37-4.60 (m, 9H, Glu-H₁, Linker-Bn-CH₂), 4.62-4.80 (m, 10H, Glu-H₁), 4.80-4.96 (m, 6H, Ido-H₁), 4.97-5.06 (m, 4H, Ido-H₁), 5.06-5.21 (m, 7H, Ido-H₃), 7.10-7.48 (m, 80H), 8.06-8.17 (m, 10H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.9, 20.96, 29.83, 61.31, 62.43, 62.74, 62.81, 63.82, 64.07, 67.45, 67.53, 67.7, 69.09, 70.01, 70.13, 70.45, 72.17, 72.8, 72.94, 73.03, 73.12, 73.17, 73.59, 73.7, 74.04, 75.2, 75.26, 75.3, 77.75, 79.4, 80.74, 97.34, 97.89, 97.98, 98.23, 127.44, 127.91, 127.94, 128.05, 128.09, 128.22, 128.24, 128.29, 128.31, 128.36, 128.42, 128.49, 128.53, 128.56, 128.59, 128.62, 128.68, 128.71, 128.74, 129.7, 129.77, 129.81, 129.88, 129.92, 133.47, 137.41, 137.43, 137.49, 137.53, 137.88, 165.87, 165.94, 165.97, 170.61, 170.72. HRMS: *m/z* calc. for C₂₀₀H₂₁₃N₁₆O₅₈: 3766.4209; found: 3766.4148 [M + H]⁺



N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyluronate (**17**)

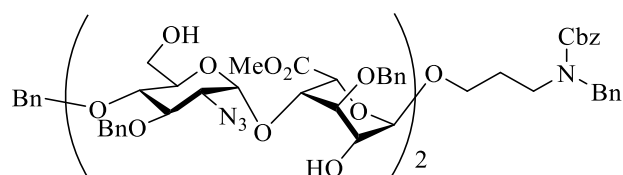
Compound **17** was prepared from compound **14** (696 mg, 0.40 mmol) by following the general procedure for oxidation of 6-OH in DCM/*t*-BuOH/H₂O (8/8/2 mL) and formation of methyl esters after oxidation in DMF (15 mL). Purification through silica gel column (Hex/DCM/EtOAc = 3/2/2) provided compound **14** (597 mg, 83% yield) over 2 steps.

¹HNMR (500 MHz, CDCl₃): δ = 1.77-1.88 (m, 2H, Linker-CH₂), 1.99 (s, 3H), 2.11 (s, 3H), 3.18-3.26 (m, 2H, Glu-H₂), 3.27-3.46 (m, 3H, Linker-CH₂), 3.47-3.57 (m, 3H, Glu-H₃), 3.60 (s, 3H, OMe), 3.65 (s, 3H, OMe), 3.73-3.84 (m, 3H, Linker-CH₂), 3.87 (d, 1H, *J* = 10.0 Hz), 3.93-4.00 (m, 2H), 4.03-4.17 (m, 3H, Ido-H₂), 4.22-4.35 (m, 4H), 4.35-4.54 (m, 5H, Linker-Bn-CH₂), 4.57 (d, 1H, *J* = 11.0 Hz), 4.65 (t, 2H, *J* = 3.5 Hz, Glu-H₁), 4.68-4.90 (m, 6H), 4.94 (d, 1H, *J* = 3.0 Hz, Glu-H₁), 5.02-5.20 (m, 5H, Ido-H₁, Ido-H₂), 5.48 (d, 1H, *J* = 4.0 Hz, Ido-H₁), 7.07-7.41 (m, 36H), 7.43-7.57 (m, 5H), 8.08-8.17 (m, 4H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.91, 20.95, 52.13, 52.27, 61.82, 62.29, 63.51, 63.61, 67.28, 67.45, 68.0, 69.71, 70.11, 70.25, 70.51, 72.28, 72.5, 74.09, 74.63, 74.86, 75.08, 75.11, 75.54, 75.71, 78.74, 80.03, 98.49, 99.1, 99.26, 127.69, 127.98, 128.03, 128.08, 128.1, 128.17, 128.19, 128.24, 128.43, 128.53, 128.57, 128.6, 128.62, 128.82, 128.85, 129.36, 129.98, 130.09, 133.53, 137.31, 137.57, 137.7, 137.78, 165.27, 169.51, 170.6, 170.79. HRMS: *m/z* calc. for C₉₇H₁₀₅N₈O₂₇: 1813.7089; found: 1813.7002 [M + NH₄]⁺

acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyluronate (19)

Compound **19** was prepared from compound **16** (80 mg, 0.021 mmol) by following the general procedure for oxidation of 6-OH in DCM/*t*-BuOH/H₂O (4/4/1 mL) and formation of benzyl esters after oxidation in DCM (10 mL). Purification through silica gel column (Hex/DCM/EtOAc = 3/2/1) provided compound **19** (73 mg, 81% yield) over 2 steps.

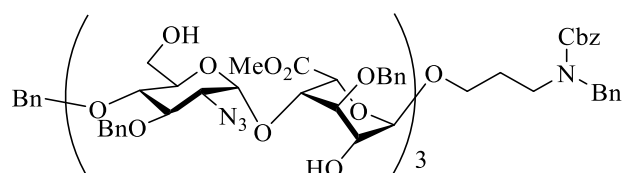
¹HNMR (500 MHz, CDCl₃): δ = 1.75-1.85 (m, 2H, Linker-CH₂), 1.98 (s, 3H), 2.01 (s, 6H), 2.03 (s, 3H), 2.09 (s, 3H), 3.10-3.24 (m, 6H, Linker-CH₂, Glu-H₂), 3.37-3.54 (m, 8H, Glu-H₃), 3.60 (d, 1H, *J* = 10.0 Hz), 3.65-3.72 (m, 2H, Linker-CH₂), 3.72-3.90 (m, 9H), 3.91-3.99 (m, 5H), 4.05-4.47 (m, 24H, Linker-Bn-CH₂, Ido-H₃), 4.47-4.63 (m, 7H, Glu-H₁), 4.63-4.80 (m, 13H), 4.80-4.88 (m, 4H, Glu-H₁), 4.92-5.01 (m, 7H, Glu-H₁), 5.01-5.10 (m, 4H, Ido-H₁), 5.10-5.22 (m, 8H, Ido-H₂), 5.45-5.49 (m, 3H, Ido-H₁), 5.54 (d, 1H, *J* = 10.0 Hz, Ido-H₁), 7.05-7.42 (m, 90H), 7.42-7.59 (m, 15H), 8.07-8.17 (m, 10H, Bz). ¹³CNMR (125 MHz, CDCl₃): δ = 20.8, 20.89, 26.6, 50.79, 51.05, 53.56, 61.67, 61.75, 61.86, 62.31, 63.11, 63.24, 63.48, 63.58, 65.46, 66.42, 67.12, 67.16, 67.29, 67.64, 67.78, 69.8, 70.03, 70.15, 70.88, 71.24, 71.6, 72.42, 73.17, 74.28, 74.33, 74.43, 74.62, 74.83, 75.06, 75.23, 75.72, 75.99, 76.09, 76.25, 76.91, 77.16, 77.36, 77.42, 77.53, 78.07, 78.14, 78.3, 78.44, 80.04, 84.05, 98.31, 98.36, 98.55, 99.12, 99.2, 100.16, 127.08, 127.65, 127.7, 127.74, 127.77, 127.8, 127.91, 127.94, 128.02, 128.04, 128.07, 128.11, 128.18, 128.2, 128.22, 128.24, 128.25, 128.33, 128.36, 128.41, 128.45, 128.47, 128.49, 128.51, 128.56, 128.58, 128.61, 128.68, 128.73, 128.77, 128.88, 128.95, 129.18, 129.24, 130.02, 130.07, 130.13, 133.7, 133.84, 134.88, 135.13, 135.18, 137.23, 137.28, 137.3, 137.33, 137.65, 137.67, 137.83, 137.88, 137.95, 165.23, 165.26, 165.29, 165.53, 169.0, 169.09, 169.11, 169.13, 170.58, 170.69, 170.71, 170.74. HRMS: *m/z* calc. for C₂₃₅H₂₃₄N₁₆O₆₃: 2143.7799; found: 2143.7791 [M + 2H]²⁺



N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate (20)

Compound **20** was prepared from compound **17** (400 mg, 0.22 mmol) by following the general procedure for transesterification in DCM/MeOH (10/10 mL). Purification through silica gel column (DCM/MeOH = 20/1) provided compound **20** (261 mg, 79% yield).

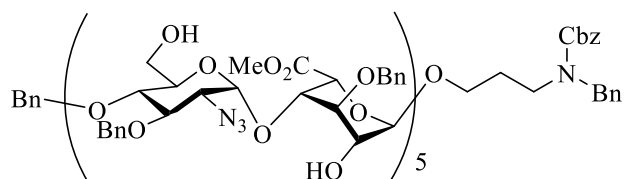
^1H NMR (500 MHz, CDCl_3): δ = 1.67 (br, 1H), 1.78-1.91 (m, 2H, Linker- CH_2), 2.22(br, 1H), 3.35-3.44 (m, 2H, Linker- CH_2), 3.46(s, 3H, OMe), 3.47-3.65 (m, 7H), 3.65-3.72 (m, 3H), 3.76 (s, 3H, OMe), 3.77-3.84 (m, 4H, Linker- CH_2), 3.84-3.90 (m, 3H), 3.94 (t, 1H, J = 9.5 Hz), 4.04 (t, 1H, J = 3.5 Hz), 4.13-4.18 (m, 1H), 4.43-4.54 (m, 3H, Linker-Bn- CH_2), 4.58 (d, 1H, J = 11.5 Hz), 4.63 (d, 1H, J = 6.5 Hz), 4.65 (d, 1H, J = 6.5 Hz), 4.70 (d, 1H, J = 4.5 Hz), 4.75 (d, 2H, J = 11.0 Hz), 4.80-4.86 (m, 4H), 4.95-5.05 (m, 3H, Glu-H1, Ido-H1), 5.17 (s, 2H), 5.28-5.30 (m, 1H, Ido-H1), 7.11-7.15 (m, 1H), 7.17-7.45 (m, 34H). ^{13}C NMR (125 MHz, CDCl_3): δ = 27.9, 28.36, 43.81, 44.53, 50.53, 50.86, 52.29, 52.52, 61.21, 61.26, 63.67, 64.16, 65.34, 65.36, 66.26, 66.62, 66.94, 67.34, 67.63, 68.05, 71.87, 71.95, 72.02, 72.22, 72.37, 72.64, 72.84, 73.06, 74.18, 74.81, 75.17, 75.86, 77.3, 79.35, 80.78, 95.55, 100.97, 101.84, 127.04, 127.23, 127.32, 127.39, 127.75, 127.94, 128.03, 128.06, 128.21, 128.24, 128.34, 128.46, 128.56, 128.57, 128.59, 128.64, 128.8, 136.71, 136.82, 137.14, 137.53, 137.69, 137.86, 141.04, 156.31, 156.78, 169.56, 170.24. HRMS: m/z calc. for $\text{C}_{79}\text{FeH}_{89}\text{N}_7\text{O}_{23}$: 779.7679; found: 779.7664 $[\text{M} + \text{Fe}]^{2+}$



N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate (**21**)

Compound **21** was prepared from compound **18** (89 mg, 0.033 mmol) by following the general procedure for transesterification in DCM/MeOH (5/5 mL). Purification through silica gel column (DCM/MeOH = 20/1) provided compound **21** (66 mg, 97% yield).

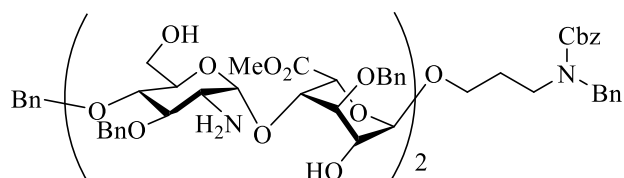
^1H NMR (500 MHz, CDCl_3): δ = 1.71-1.90 (m, 2H, Linker- CH_2), 2.00-2.35 (br, 5H), 3.27-3.38 (m, 2H, Linker- CH_2), 3.40 (s, 3H, OMe), 3.44 (s, 3H, OMe), 3.46-3.58 (m, 6H), 3.58-3.64 (m, 3H), 3.64-3.74 (m, 5H), 3.75 (s, 3H, OMe), 3.77-3.83 (m, 4H, Linker- CH_2), 3.83-3.97 (m, 6H), 4.00-4.05 (m, 2H), 4.12-4.17 (m, 1H), 4.44-4.59 (m, 5H, Linker-Bn- CH_2), 4.60-4.66 (m, 3H), 4.67-4.77 (m, 7H), 4.77-4.90 (m, 5H), 4.93-5.04 (m, 4H, Glu-H1, Ido-H1), 5.16 (s, 2H), 5.26 (s, 1H, Ido-H1), 5.29 (s, 1H, Ido-H1), 7.10-7.15 (m, 1H), 7.15-7.46 (m, 44H). ^{13}C NMR (125 MHz, CDCl_3): δ = 27.9, 28.36, 43.81, 44.54, 50.54, 50.86, 52.18, 52.29, 52.52, 61.21, 63.64, 64.05, 64.2, 65.35, 65.38, 66.26, 67.35, 67.64, 67.82, 68.08, 71.98, 72.15, 72.22, 72.39, 72.61, 72.67, 72.79, 72.87, 73.11, 73.94, 74.09, 74.82, 75.07, 75.85, 77.31, 79.16, 79.35, 80.75, 95.59, 100.89, 100.95, 101.84, 127.06, 127.28, 127.39, 127.7, 127.74, 127.76, 127.77, 127.94, 128.03, 128.07, 128.2, 128.21, 128.24, 128.32, 128.37, 128.39, 128.47, 128.5, 128.55, 128.57, 128.6, 128.63, 128.8, 128.82, 137.08, 137.12, 137.52, 137.7, 137.84, 140.99, 156.32, 169.55, 169.57. HRMS: m/z calc. for $\text{C}_{106}\text{H}_{120}\text{N}_{10}\text{Na}_2\text{O}_{33}$: 1053.3907; found: 1053.3929 $[\text{M} + 2\text{Na}]^{2+}$



N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate (**22**)

Compound **22** was prepared from compound **19** (25 mg, 0.0058 mmol) by following the general procedure for transesterification in DCM/MeOH (4/4 mL). Purification through silica gel column (DCM/MeOH = 8/1) provided compound **22** (17 mg, 90% yield).

^1H NMR (500 MHz, CDCl_3): δ = 1.75-1.88 (m, 2H, Linker- CH_2), 2.25 (br, 10H), 3.28-3.35 (m, 2H, Linker- CH_2), 3.38 (s, 3H, OMe), 3.39 (s, 3H, OMe), 3.43 (s, 3H, OMe), 3.45-3.53 (m, 7H), 3.53-3.63 (m, 6H), 3.63-3.68 (m, 4H), 3.69-3.81 (m, 10H), 3.74 (s, 3H, OMe), 3.81-3.97 (m, 9H, Linker- CH_2), 3.98-4.04 (m, 3H), 4.14 (s, 1H), 4.42-4.57 (m, 7H, Linker-Bn- CH_2), 4.57-4.65 (m, 5H), 4.65-4.75 (m, 14H), 4.75-4.85 (m, 6H), 4.90-5.02 (m, 5H, Glu-H1, Ido-H1), 5.15 (s, 2H), 5.23 (s, 2H, Ido-H1), 5.27 (s, 1H, Ido-H1), 7.09-7.13 (m, 1H), 7.14-7.45 (m, 64H). ^{13}C NMR (125 MHz, CDCl_3): δ = 17.1, 26.59, 39.83, 52.21, 52.32, 63.68, 64.06, 64.14, 64.24, 65.44, 66.29, 67.38, 67.69, 67.87, 68.13, 72.0, 72.21, 72.25, 72.66, 72.84, 72.91, 73.15, 73.95, 74.16, 74.85, 75.11, 75.88, 79.17, 79.37, 80.78, 83.98, 95.61, 95.68, 100.9, 100.97, 101.86, 127.1, 127.28, 127.31, 127.43, 127.76, 127.8, 127.98, 128.07, 128.1, 128.23, 128.26, 128.41, 128.44, 128.49, 128.53, 128.58, 128.6, 128.62, 128.65, 128.67, 128.82, 128.84, 137.08, 137.1, 137.14, 137.54, 137.72, 137.85, 140.97, 156.34, 169.58, 169.61. HRMS: m/z calc. for $\text{C}_{160}\text{H}_{182}\text{N}_{16}\text{Na}_2\text{O}_{53}$: 1610.5917; found: 1610.5923 [$\text{M} + 2\text{Na}$] $^{2+}$

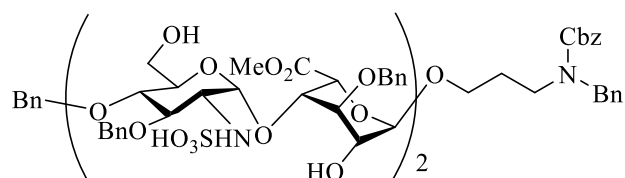


N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-amino-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-amino-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate (**23**)

Compound **20** (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 **23** (51 mg, 88% yield).

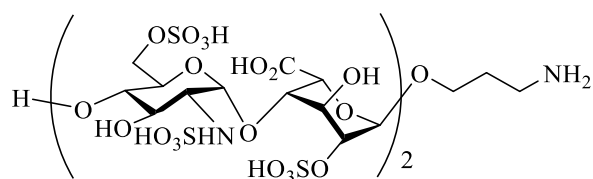
¹HNMR (500 MHz, CDCl₃): δ = 1.75-1.87 (m, 2H, Linker-CH₂), 3.11 (d, 1H, J = 10.5 Hz), 3.20 (d, 1H, J = 10.5 Hz), 3.25-3.44 (m, 3H, Linker-CH₂), 3.38 (s, 3H), 3.48 (d, 2H, J = 9.5 Hz), 3.57 (t, 2H, J = 9.5 Hz), 3.62-3.73 (m, 3H, Linker-CH₂), 3.65 (s, 3H), 3.79 (d, 2H, J = 11.5 Hz), 3.85 (d, 3H, J = 11.5 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). ¹³CNMR (125 MHz, CDCl₃): 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 C₇₉H₉₅N₃O₂₃: 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 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-3-*O*-benzyl-2-deoxy-2-sulfoamino- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate (**24**)

Compound **24** was prepared from compound **23** (15 mg, 0.010 mmol) by following the general procedure for selective N-sulfation in MeOH (5 mL). Purification through silica gel column (DCM/MeOH = 8/1) provided compound **24** (13 mg, 78% yield).

¹HNMR (500 MHz, CD₃OD): δ = 1.74-1.85 (m, 2H, Linker-CH₂), 3.23 (s, 2H), 3.32-3.34 (m, 1H), 3.35 (s, 3H), 3.42-3.52 (m, 5H, Linker-CH₂, Glu-H2), 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, Linker-CH₂), 3.73-3.79 (m, 1H), 3.81 (s, 2H), 3.83-3.90 (m, 2H), 3.98 (s, 1H), 4.07 (s, 1H, Ido-H2), 4.14 (s, 2H, Ido-H2), 4.20 (s, 1H), 4.37-4.44 (m, 3H, Linker-Bn-CH₂), 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, Ido-H1), 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, Ido-H1), 5.40-5.44 (m, 2H, Glu-H1), 7.06-7.12 (m, 1H), 7.14-7.36 (m, 28H), 7.37-7.45 (m, 4H), 7.46-7.50 (m, 2H). ¹³CNMR (125 MHz, CD₃OD): 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 C₇₉H₉₁N₃O₂₉S₂: 804.7590; found: 804.7593 [M - 2H]²⁻



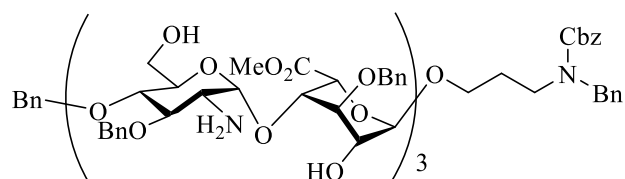
3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate (**25**)

Compound **25** was prepared from compound **24** (5 mg, 0.003 mmol) by following the general procedure for simultaneous O, N-sulfation, global debenzylation and methyl ester saponification, providing the final product (3 mg, 81% yield) over 3 steps.

H1-C1 coupling constants (174.0, 174.5, 174.5, 175.0 Hz) confirmed the stereochemistry.

^1H NMR (500 MHz, D_2O): δ = 1.80-1.90 (m, 2H, Linker- CH_2), 3.02 (t, 2H, J = 1.5 Hz, Linker- CH_2), 3.10 (dd, 1H, J = 3.5, 10.0 Hz, Glu-H2), 3.13 (dd, 1H, J = 3.5, 10.5 Hz, Glu-H2), 3.42 (t, 1H, J = 10.0 Hz, Glu-H4), 3.50 (t, 1H, J = 10.0 Hz, Glu-H3), 3.52-3.59 (m, 2H, Linker- CH_2 , Glu-H3), 3.62 (t, 1H, J = 9.0 Hz, Glu-H4), 3.80 (d, 2H, J = 7.5 Hz, Linker- CH_2 , Glu-H5), 3.87 (d, 1H, J = 10.0 Hz, Glu-H5), 3.93-3.99 (m, 2H, Ido^R-H4, Ido-H4), 4.02-4.15 (m, 5H, Glu-H6, Ido^R-H2, Ido^R-H3, Ido-H3), 4.18-4.27 (m, 3H, Glu-H6, Ido-H2), 4.40 (d, 1H, J = 3.0 Hz, Ido^R-H5), 4.78 (d, 1H, J = 2.5 Hz, Ido-H5), 4.97 (d, 1H, J = 3.5 Hz, Ido^R-H1), 5.08 (s, 1H, Ido-H1), 5.25 (d, 1H, J = 3.5 Hz, Glu-H1), 5.27 (d, 1H, J = 3.5 Hz, Glu-H1). ^{13}C NMR (125 MHz, D_2O): δ = 26.0, 38.31, 46.57, 57.84, 66.3, 66.57, 68.47, 68.58, 68.82, 68.87, 69.05, 69.18, 69.43, 69.93, 70.82, 75.25, 75.79, 75.92, 76.01, 76.4, 96.48, 97.34, 98.78, 99.07, 174.04, 174.26. HRMS: m/z calc. for $\text{C}_{27}\text{H}_{42}\text{N}_3\text{Na}_3\text{O}_{39}\text{S}_6$: 646.4708; found: 646.4703 [$\text{M} + 3\text{Na} - 5\text{H}$]²⁻

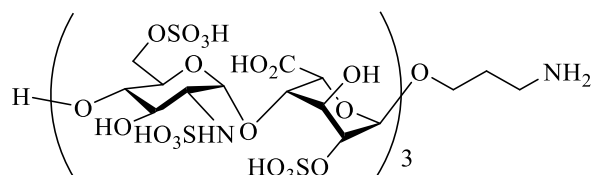
(^R: reducing end)



N-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-amino-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl-3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-amino-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl-3-*O*-benzyl- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-amino-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl 3-*O*-benzyl- α -L-idopyranosyluronate (**26**)

Compound **26** was prepared from compound **21** (15 mg, 0.007 mmol) by following the general procedure for 1, 3-propanedithiol mediated azide reduction in MeOH (5 mL). Purification through silica gel column (DCM/MeOH = 6/1 with 2% Et_3N) provided compound **26** (11 mg, 76% yield).

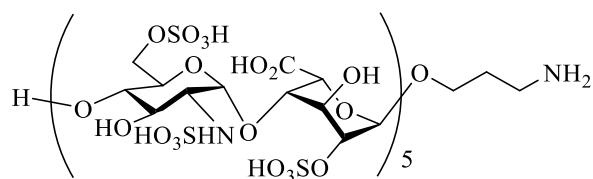
$^1\text{H NMR}$ (500 MHz, CDCl_3): δ = 1.78-1.88 (m, 2H), 2.83-2.91 (m, 2H), 3.23-3.32 (m, 2H), 3.34-3.51 (m, 10H), 3.53 (s, 3H), 3.55 (s, 3H), 3.65-3.68 (m, 2H), 3.70-3.73 (m, 2H), 3.74 (s, 3H), 3.75-3.82 (m, 5H), 3.82-3.91 (m, 4H), 3.94 (s, 3H), 3.96-4.02 (m, 2H), 4.14-4.22 (m, 3H), 4.41 (d, 2H, J = 11.5 Hz), 4.43-4.54 (m, 4H), 4.58 (d, 2H, J = 11.5 Hz), 4.64 (d, 2H, J = 11.0 Hz), 4.66 (s, 4H), 4.72 (dd, 2H, J = 3.5, 11.5 Hz), 4.81 (d, 2H, J = 11.5 Hz), 4.86 (d, 1H, J = 3.0 Hz), 4.87-4.91 (m, 3H), 4.92-4.97 (m, 5H), 4.99 (d, 1H, J = 12.0 Hz), 5.15 (s, 2H), 5.29 (dd, 2H, J = 3.5, 8.0 Hz), 7.10-7.13 (m, 1H), 7.17-7.38 (m, 44H). Comparison with literature data confirmed its identity.⁸



3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate (**27**)

Compound **27** was prepared from compound **26** (5 mg, 0.0025 mmol) by following the general procedure for simultaneous O, N-sulfation, global debenzylation and methyl ester saponification, providing the final product (3 mg, 64% yield) over 3 steps.

$^1\text{H NMR}$ (500 MHz, D_2O): δ = 1.92-2.01 (m, 2H), 3.11-3.15 (m, 1H), 3.21 (dd, 1H, J = 3.5, 10.5 Hz), 3.25 (dd, 2H, J = 3.0, 10.5 Hz), 3.54 (t, 2H, J = 10.0 Hz), 3.59-3.67 (m, 4H), 3.70-3.79 (m, 3H), 3.90-3.94 (m, 1H), 3.95-3.98 (m, 1H), 3.98-4.03 (m, 2H), 4.06-4.10 (m, 3H), 4.14-4.20 (m, 4H), 4.22-4.27 (m, 3H), 4.29-4.41 (m, 5H), 4.47 (d, 1H, J = 3.0 Hz), 4.81 (d, 1H, J = 3.0 Hz), 5.06 (d, 1H, J = 3.5 Hz), 5.19 (d, 2H, J = 3.0 Hz), 5.39 (d, 2H, J = 3.5 Hz), 5.42 (d, 1H, J = 3.5 Hz). Comparison with literature data confirmed its identity.⁸



3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate-(1 \rightarrow 4)-2-deoxy-2-sulfoamino-6-*O*-sulfonato- α -D-glucopyranosyl-(1 \rightarrow 4)-2-*O*-sulfonato- α -L-idopyranosyluronate (**28**)

Compound **28** was prepared from compound **22** (5 mg, 0.0016 mmol) by following the general procedure for 1, 3-propanedithiol mediated azide reduction, simultaneous O, N-sulfation, global

debenzylation and methyl ester saponification, providing the final product (2 mg, 42% yield) over 4 steps.

^1H NMR (500 MHz, D_2O): δ = 1.92-1.99 (m, 2H, Linker- CH_2), 3.09-3.15 (m, 2H, Linker- CH_2), 3.20 (dd, 2H, J = 3.0, 10.0 Hz, Glu-H2), 3.25 (dd, 4H, J = 3.5, 10.5 Hz, Glu-H2), 3.54 (t, 3H, J = 10.0 Hz), 3.58-3.66 (m, 5H, Linker- CH_2), 3.68-3.79 (m, 6H), 3.92-4.02 (m, 7H, Linker- CH_2), 4.08 (t, 4H, J = 3.5 Hz), 4.14-4.19 (m, 6H, Ido-H2, Glu-H6), 4.20-4.27 (m, 6H, Glu-H6), 4.29-4.34 (m, 5H, Glu-H6), 4.34-4.42 (m, 5H, Glu-H6), 4.79-4.83 (m, 2H), 5.18 (d, 5H, J = 3.0 Hz, Ido-H1), 5.39 (d, 5H, J = 3.0 Hz, Glu-H1). δ_{C} (values obtained from F1 dimension of HSQC spectrum) = 26.0, 38.24, 57.81, 57.82, 61.11, 65.54, 66.22, 66.23, 66.26, 66.29, 66.51, 66.52, 68.60, 69.12, 69.17, 69.24, 69.28, 69.40, 69.42, 69.66, 69.73, 70.87, 70.88, 75.66, 75.70, 75.77, 75.80, 75.81, 96.57, 99.18. HRMS: m/z calc. for $\text{C}_{63}\text{H}_{87}\text{N}_6\text{Na}_{13}\text{O}_{96}\text{S}_{15}$: 810.4149; found: 810.4116 $[\text{M} + 13\text{Na} - 17\text{H}]^{4-}$

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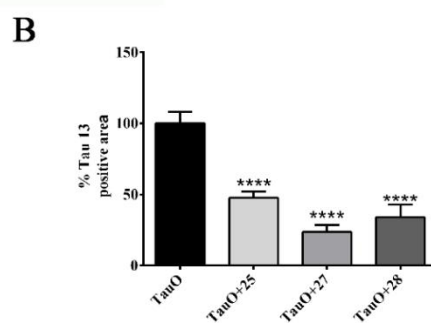
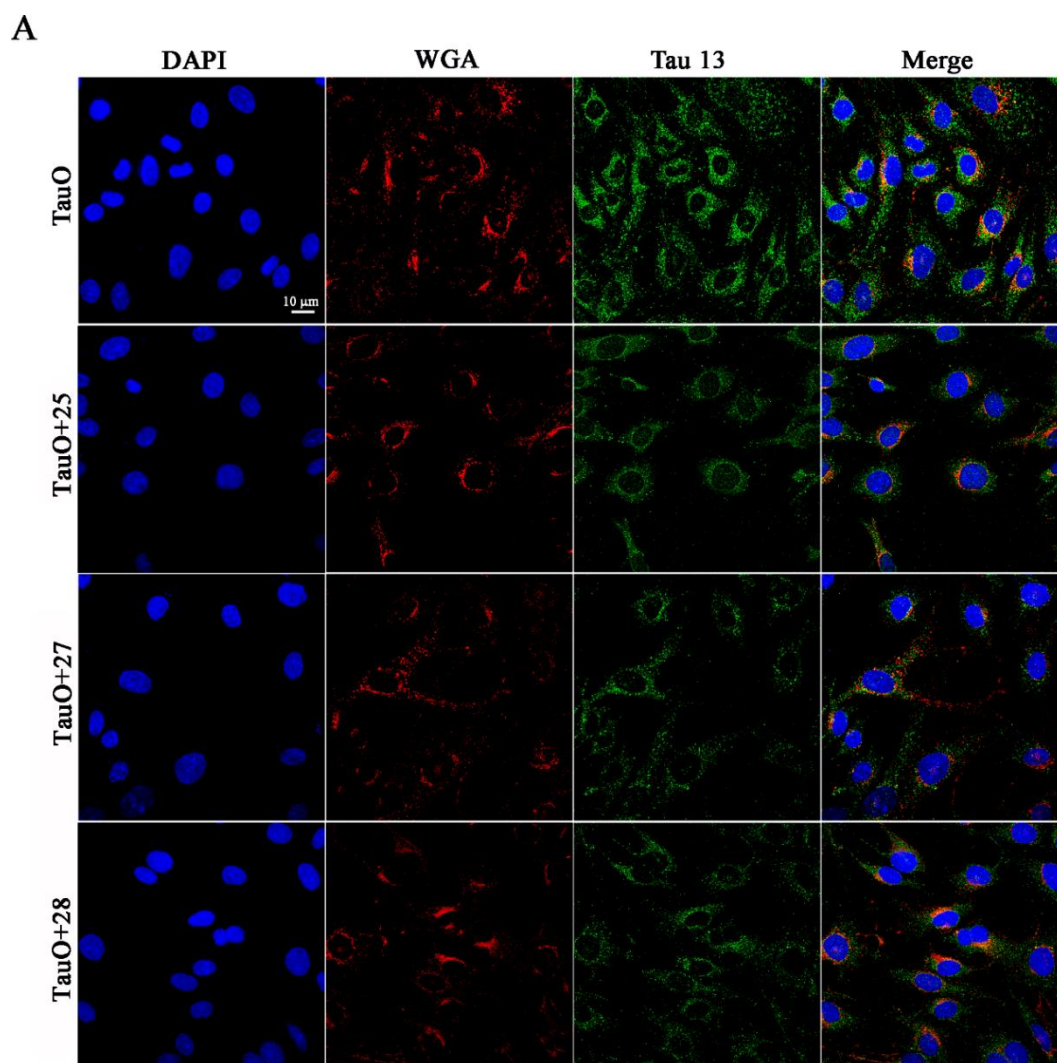
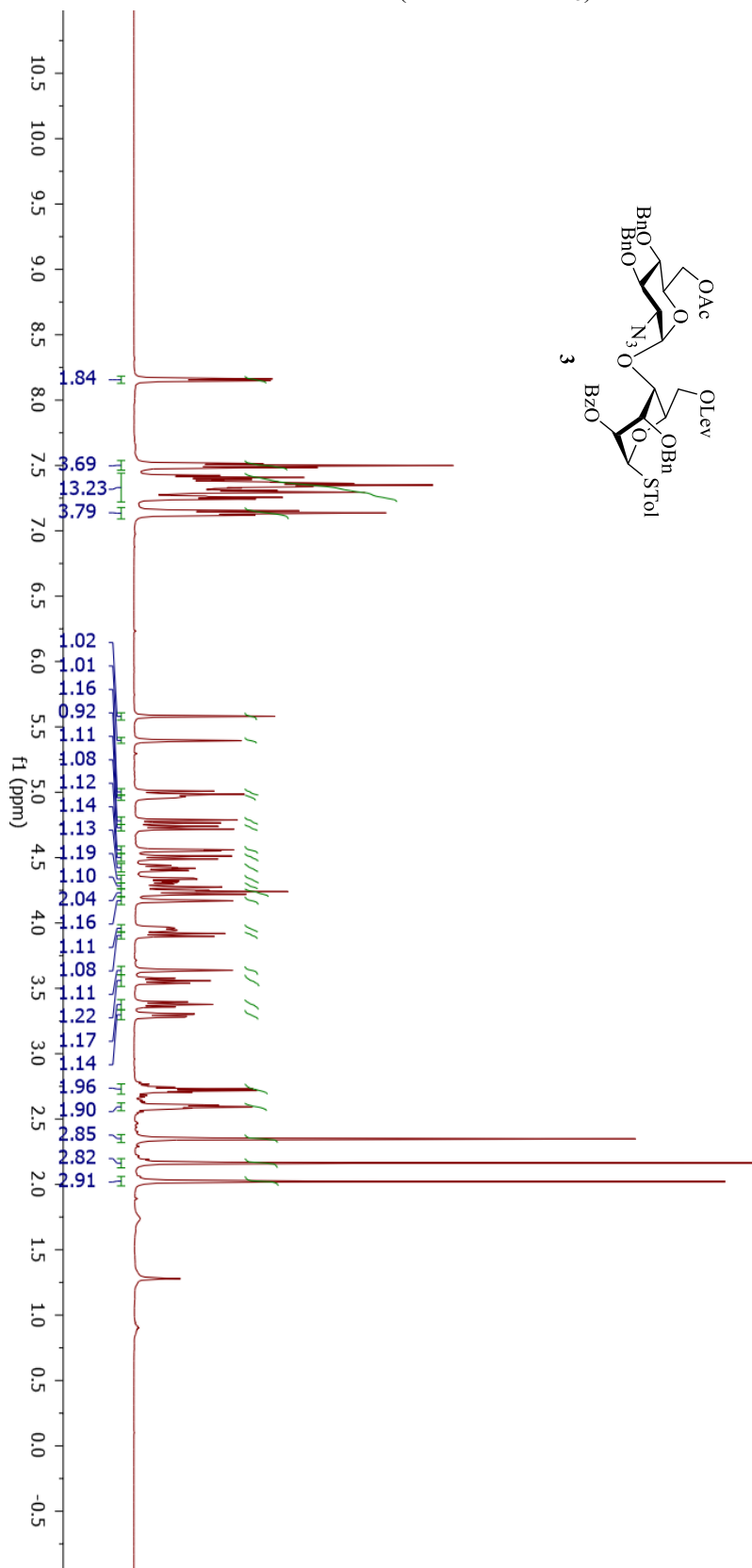


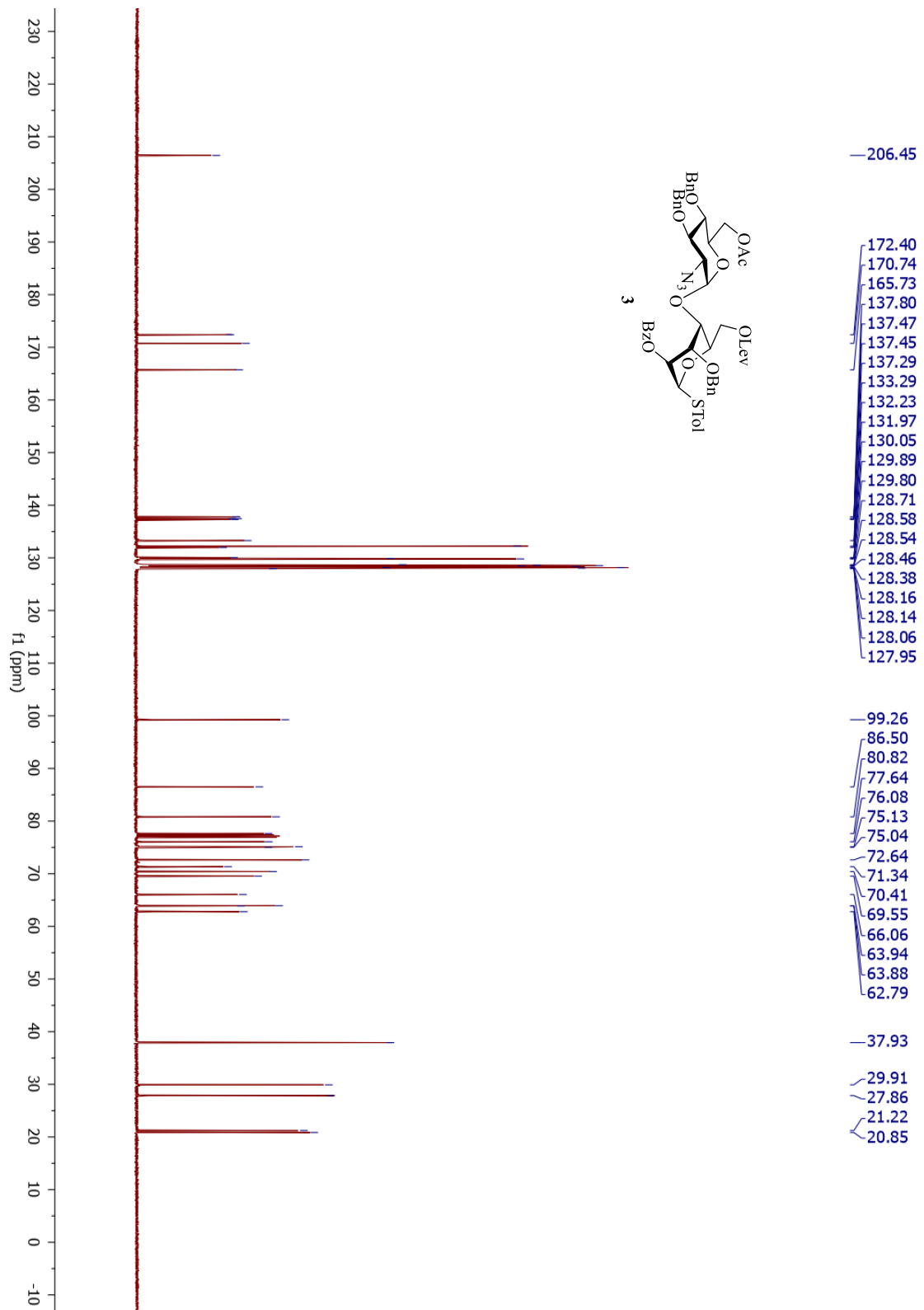
Fig. S1. Representative confocal images of human SH-SY5Y neuroblastoma cells after treatment with TauO or TauO in the presence of oligosaccharides. (A) SH-SY5Y after 1 hour of treatment with sub-lethal concentration (0.5 μ M) of TauO in the absence and presence of oligosaccharides (**25**, **27** and **28**). Cells were stained with DAPI (nuclei – blue), WGA (plasma membranes – red) and anti-Tau antibody, Tau 13 (green). (B) Analysis of the percentage of Tau 13 positive area was performed on selected regions of interest characterized by same size and comparable number of cells. Cells treated with TauO in the presence of oligosaccharides (**25**, **27**, and **28**) show a significant reduction of % area positive for tau oligomers as compared to the cells exposed to TauO alone, suggesting reduction of TauO uptake. Bars and error bars represent means and standard deviations, **** p <0.0001. (Magnification: 63X and scale bar = 10 μ m)

Product Characterization Spectra

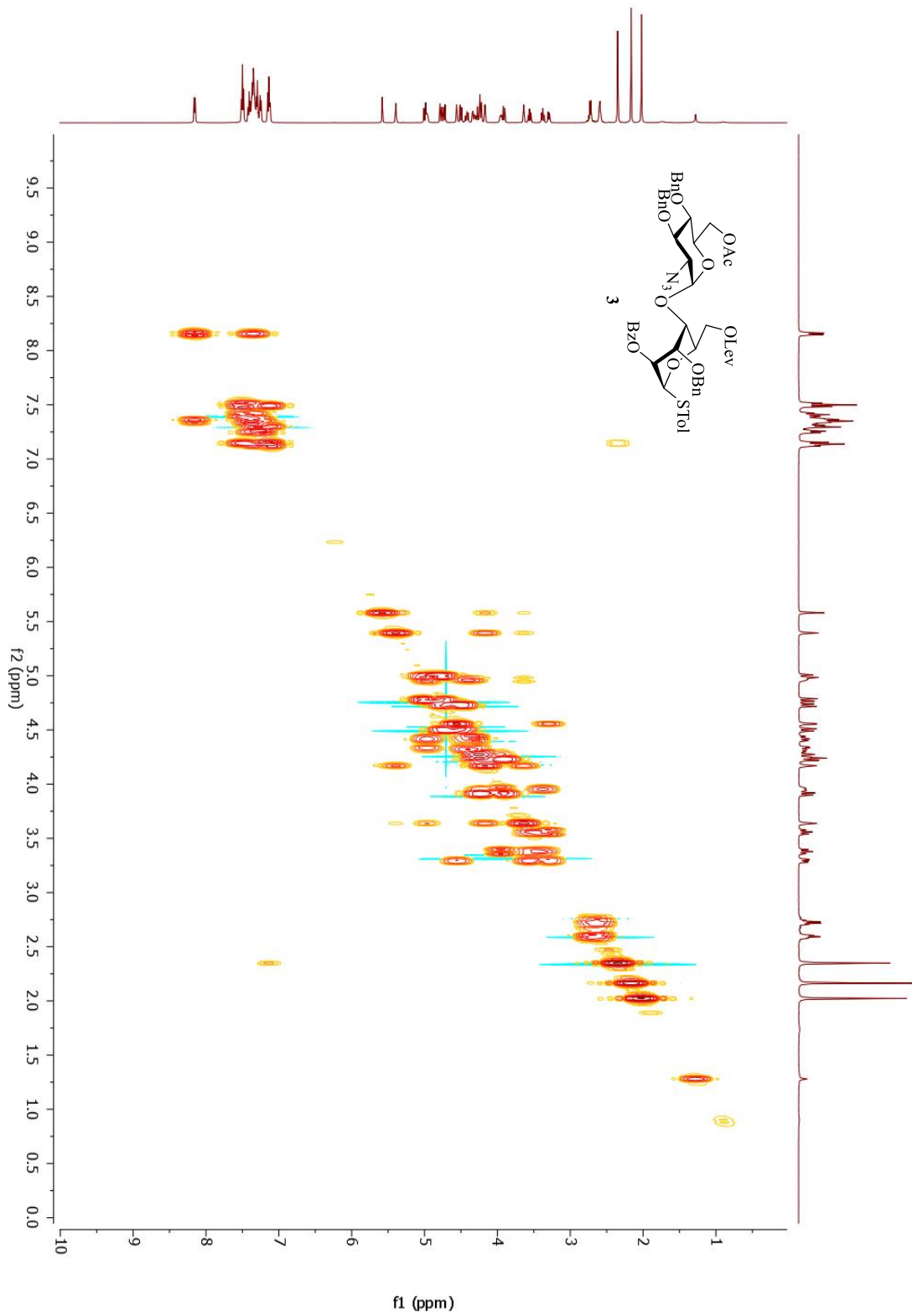
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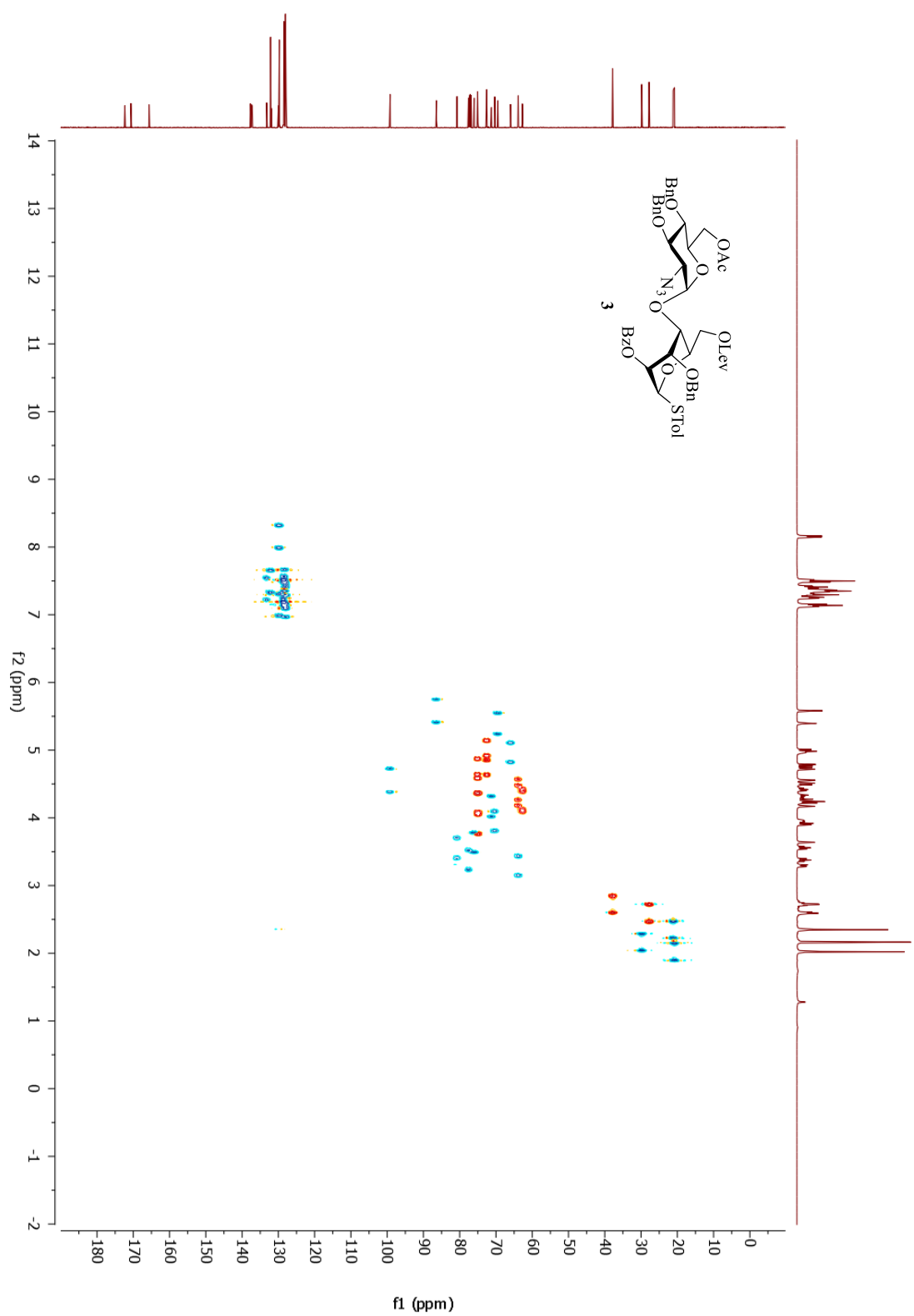
¹³C-NMR of **3** (125 MHz CDCl₃)



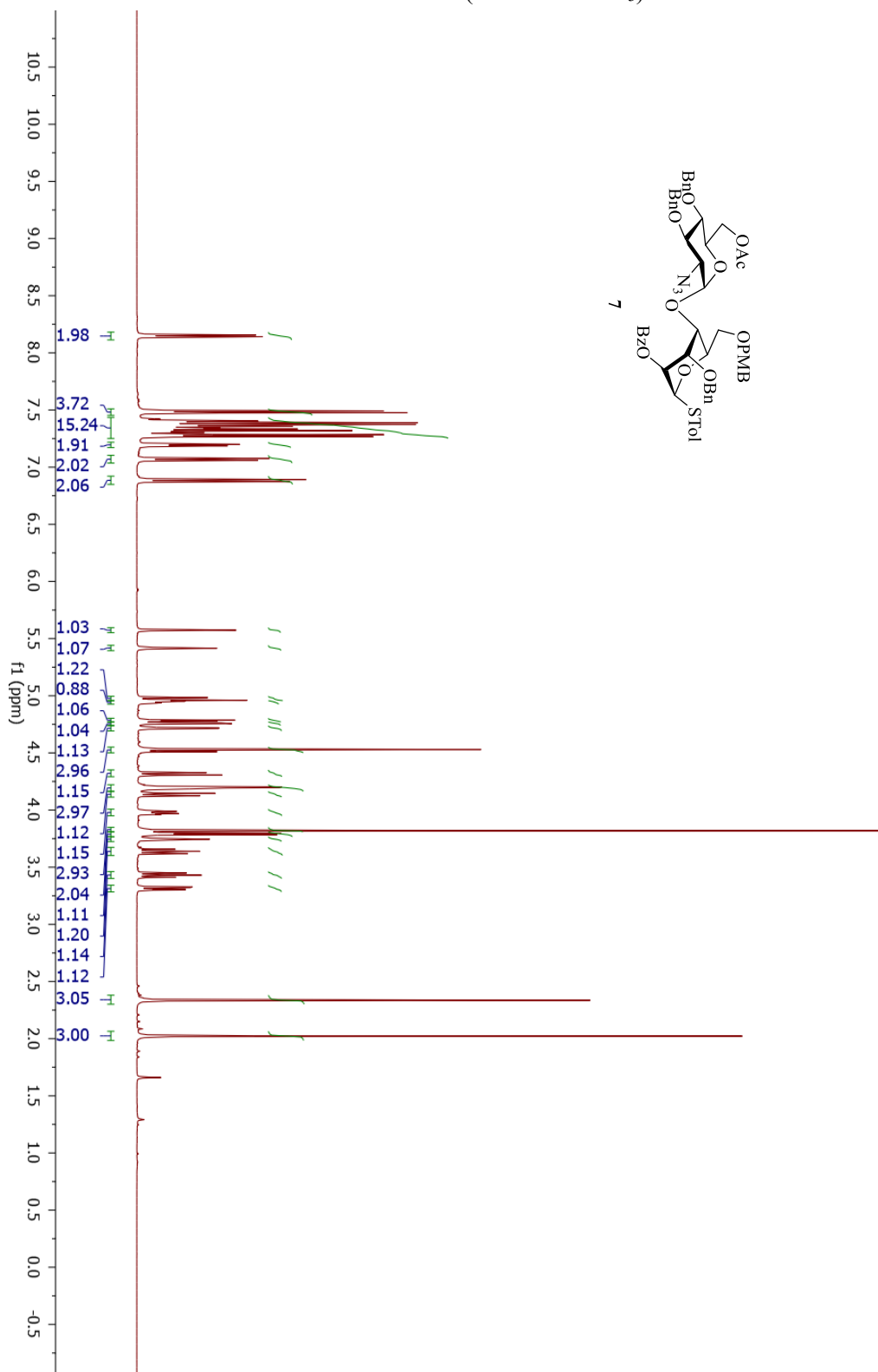
^1H - ^1H gCOSY of **3** (500 MHz CDCl_3)



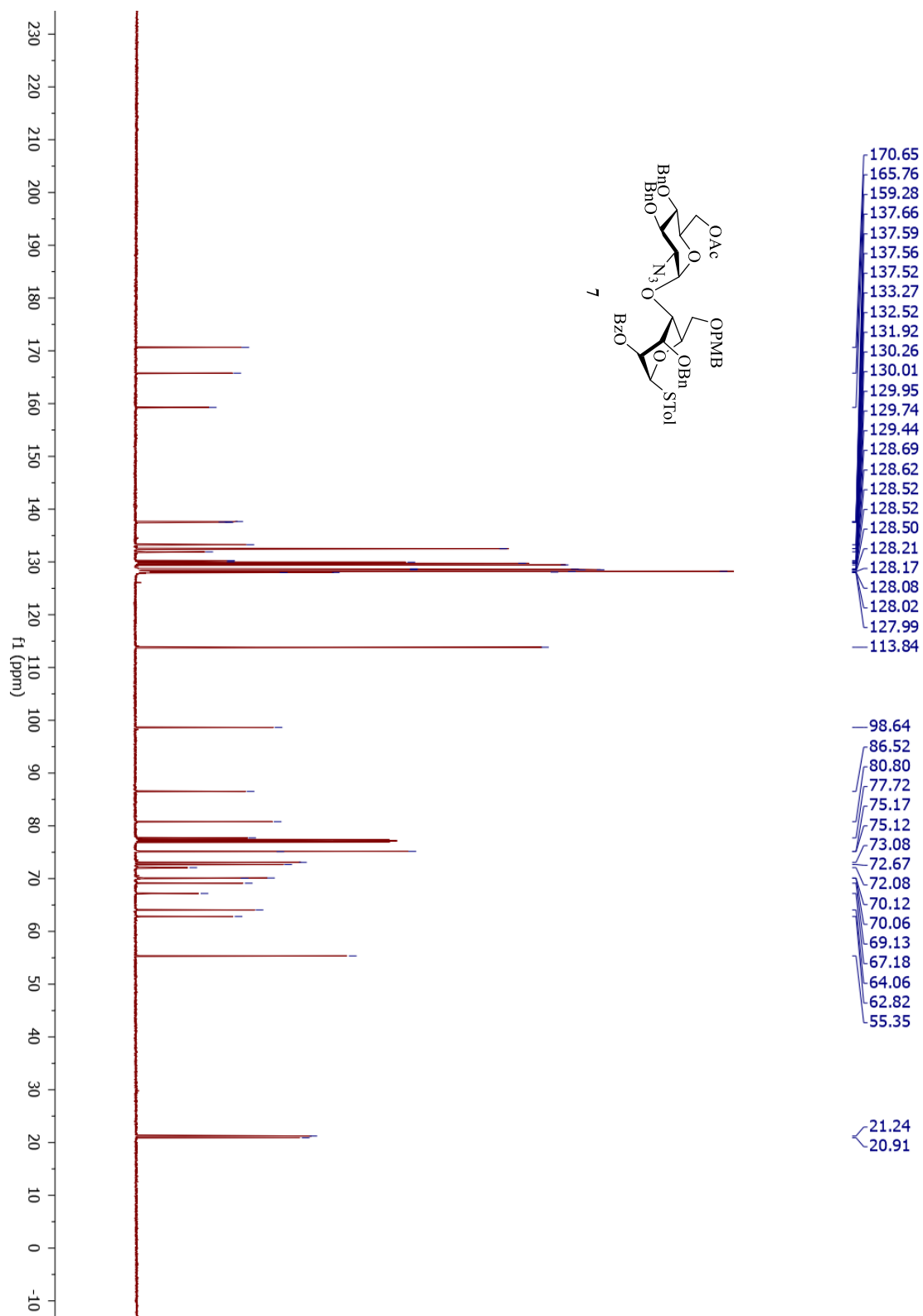
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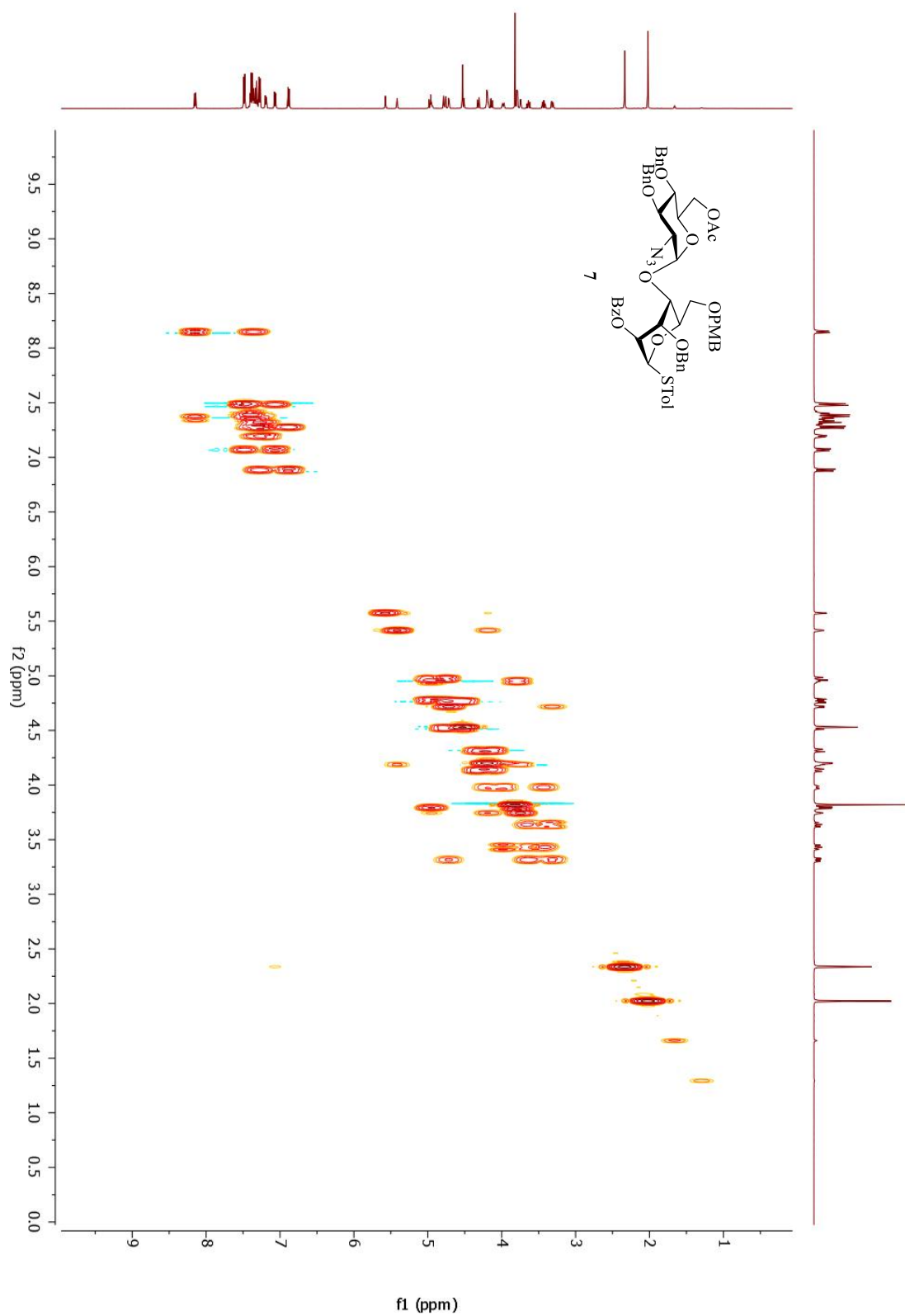
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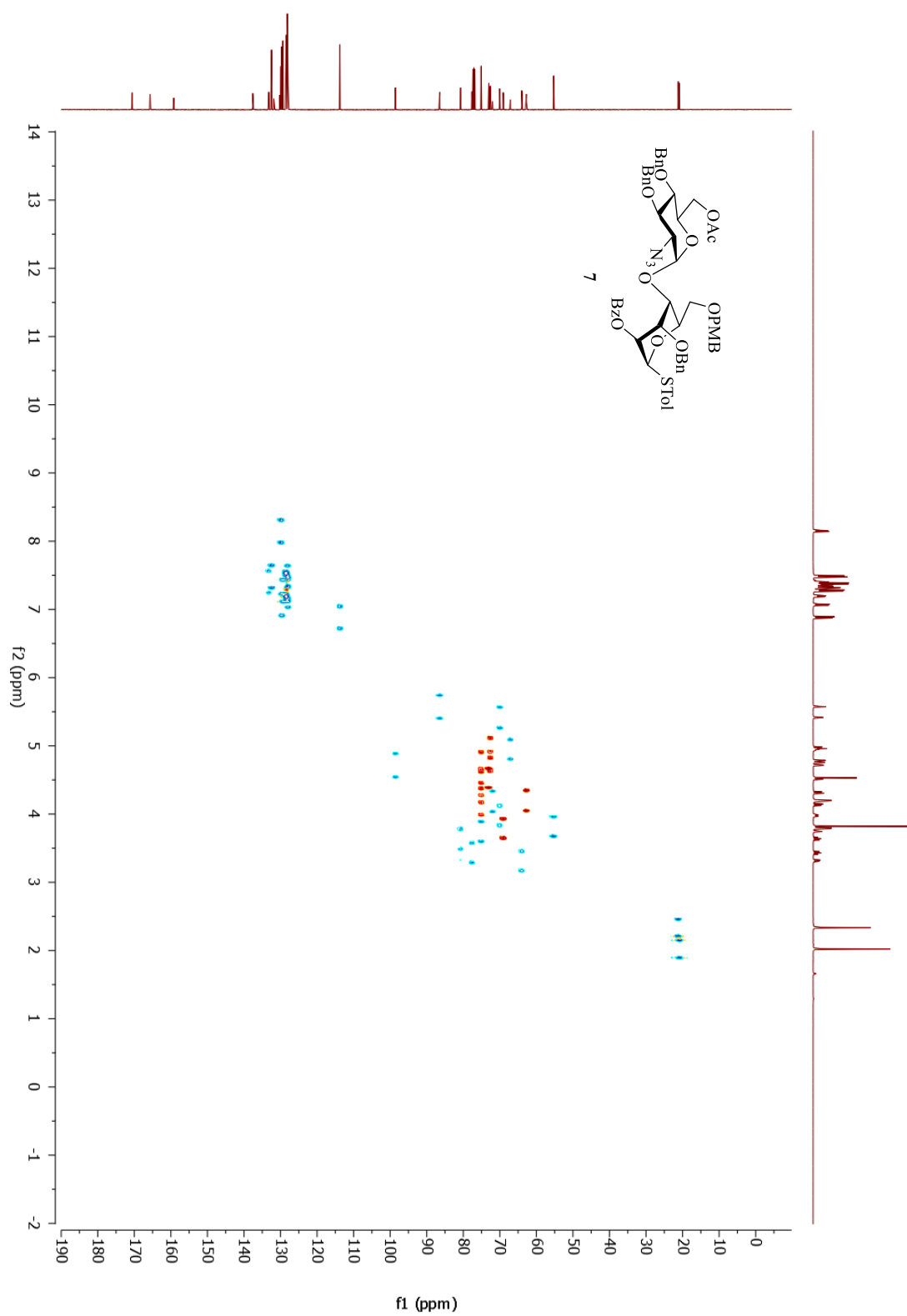
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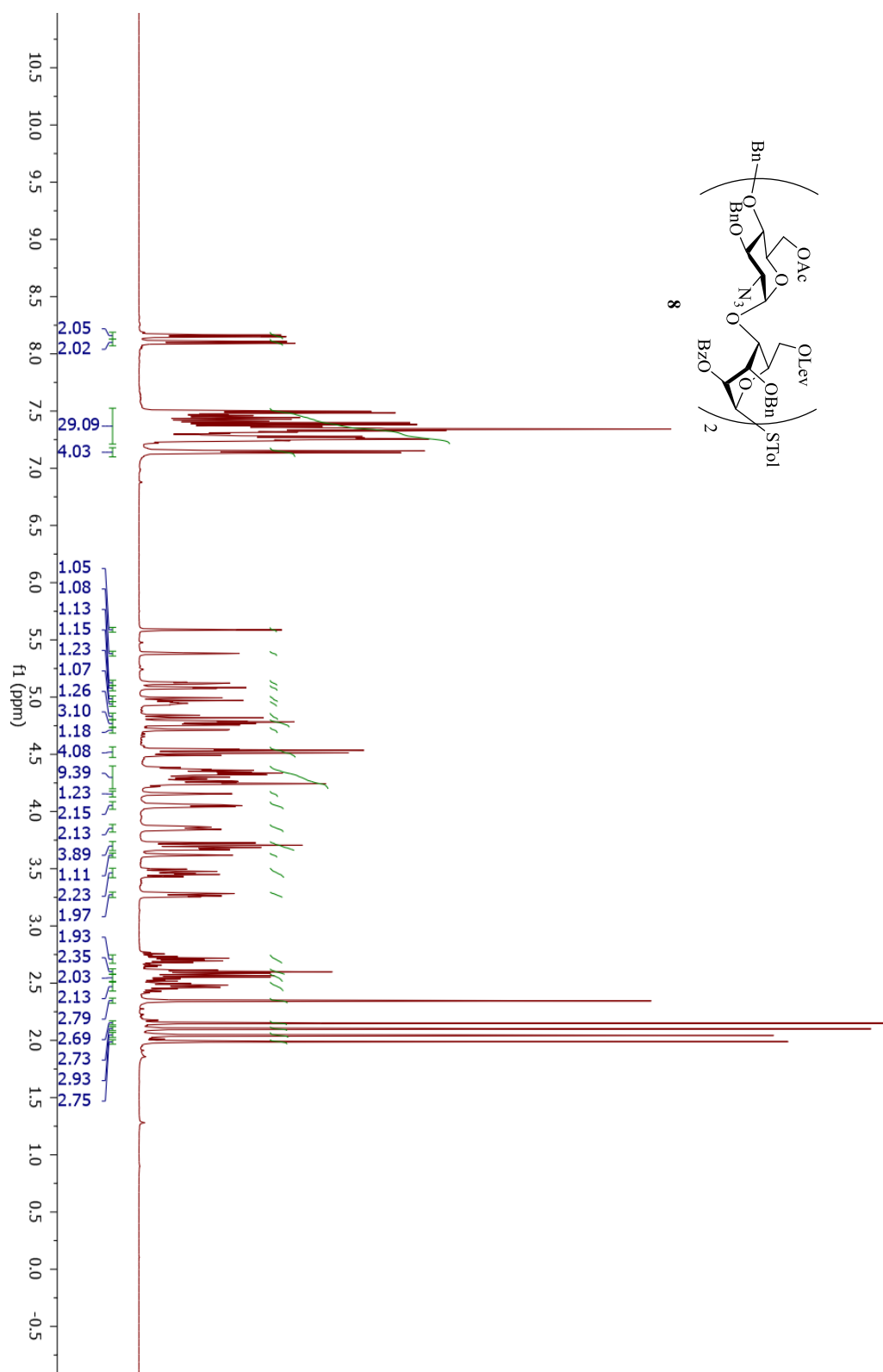
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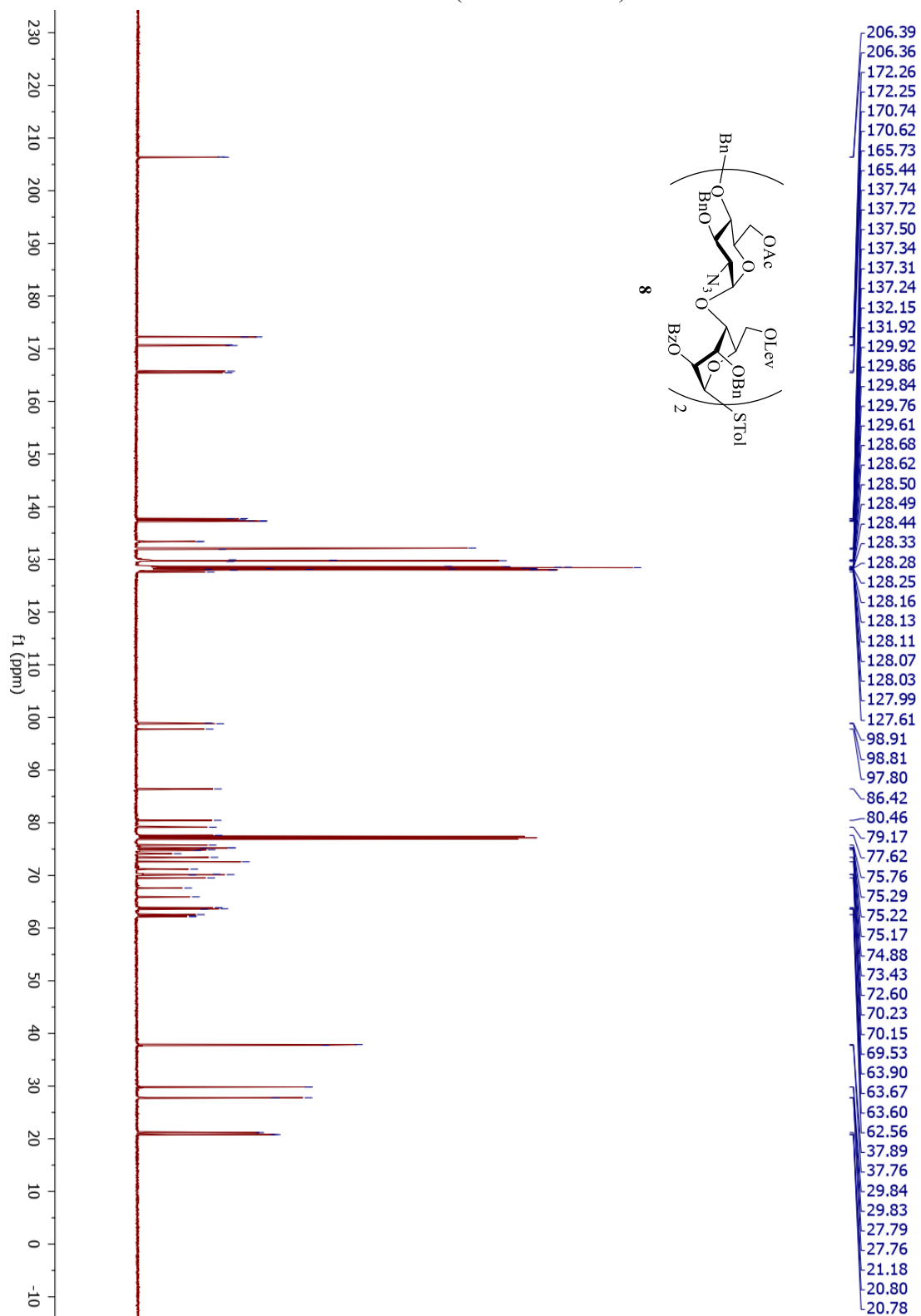
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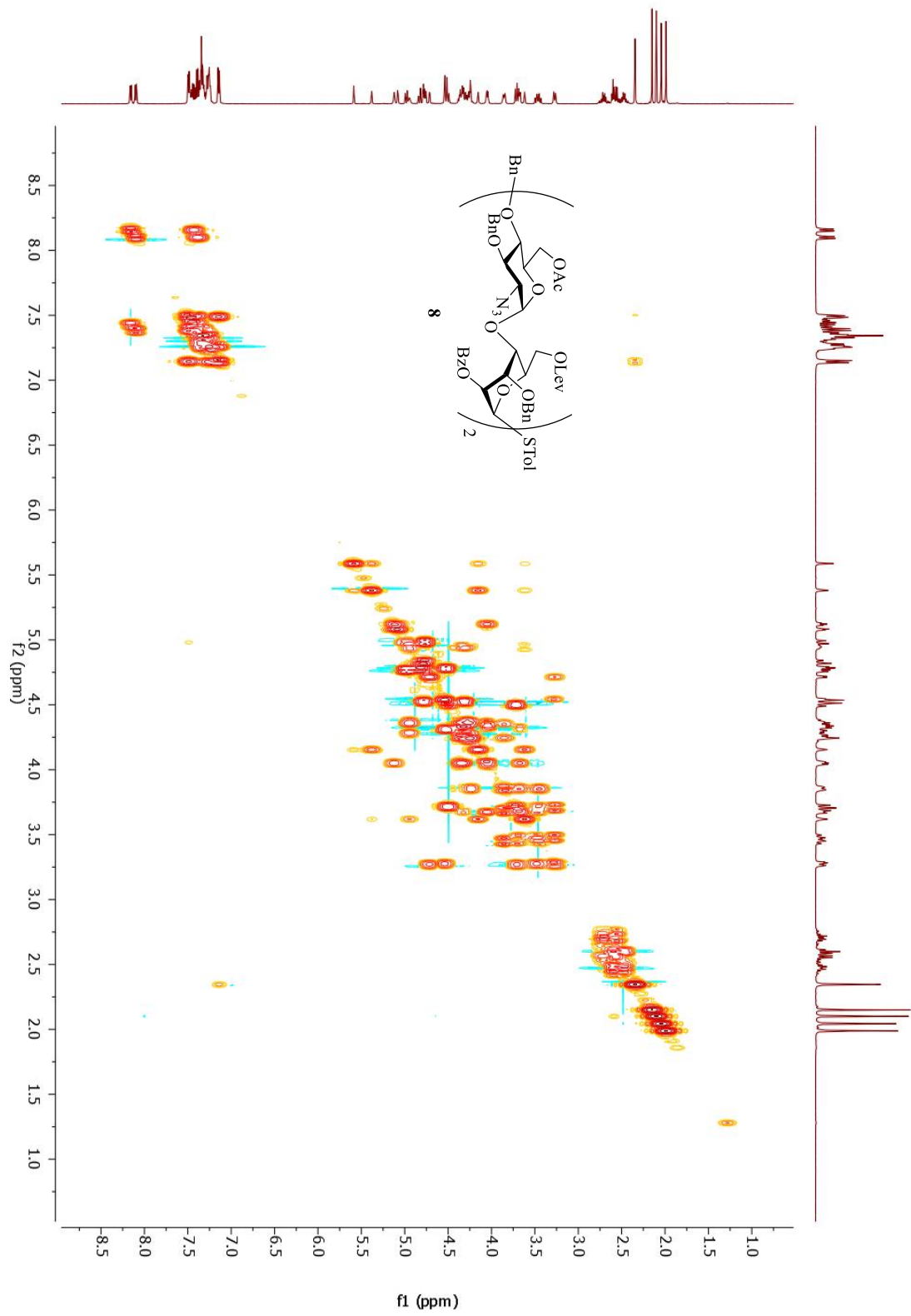
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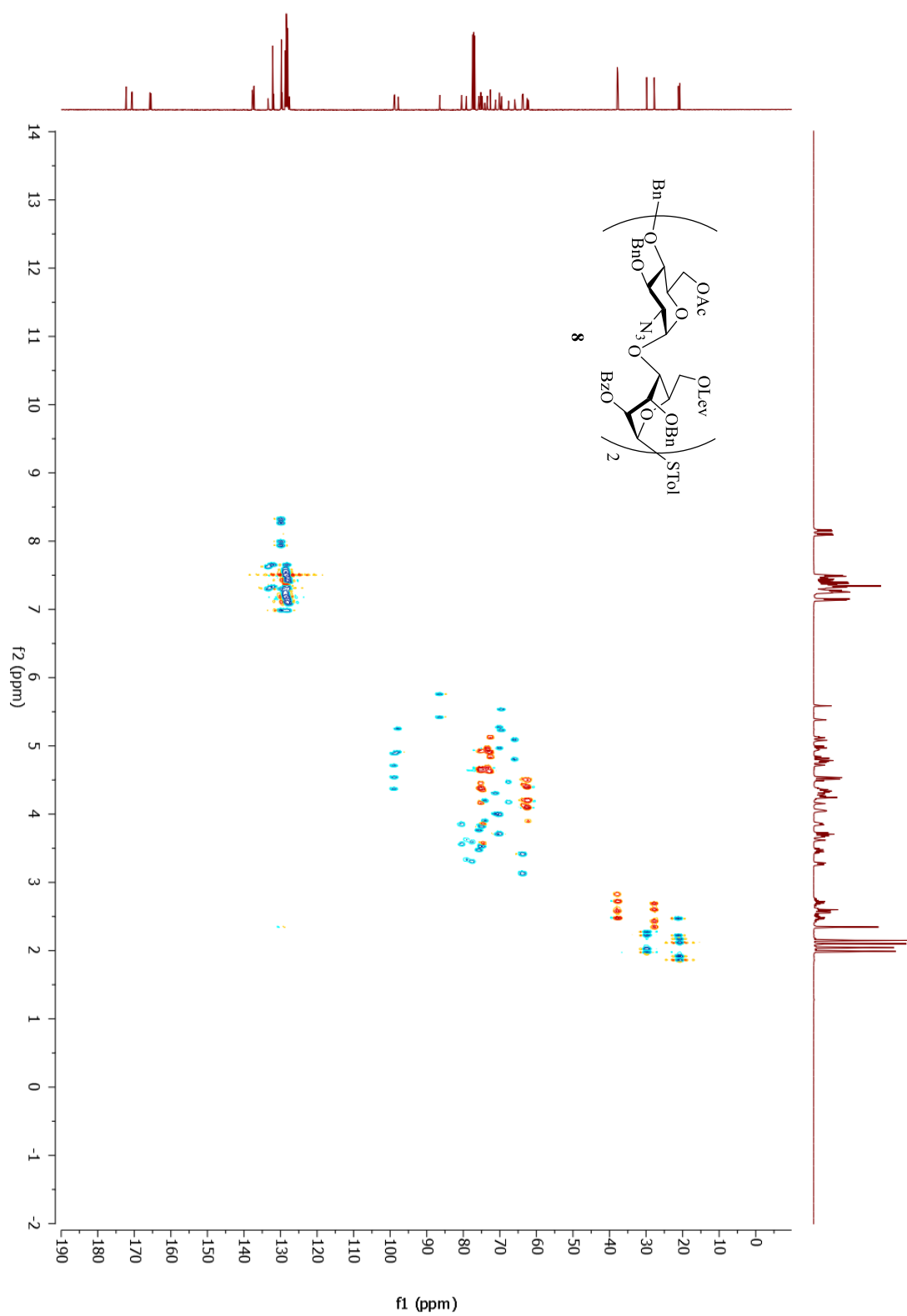
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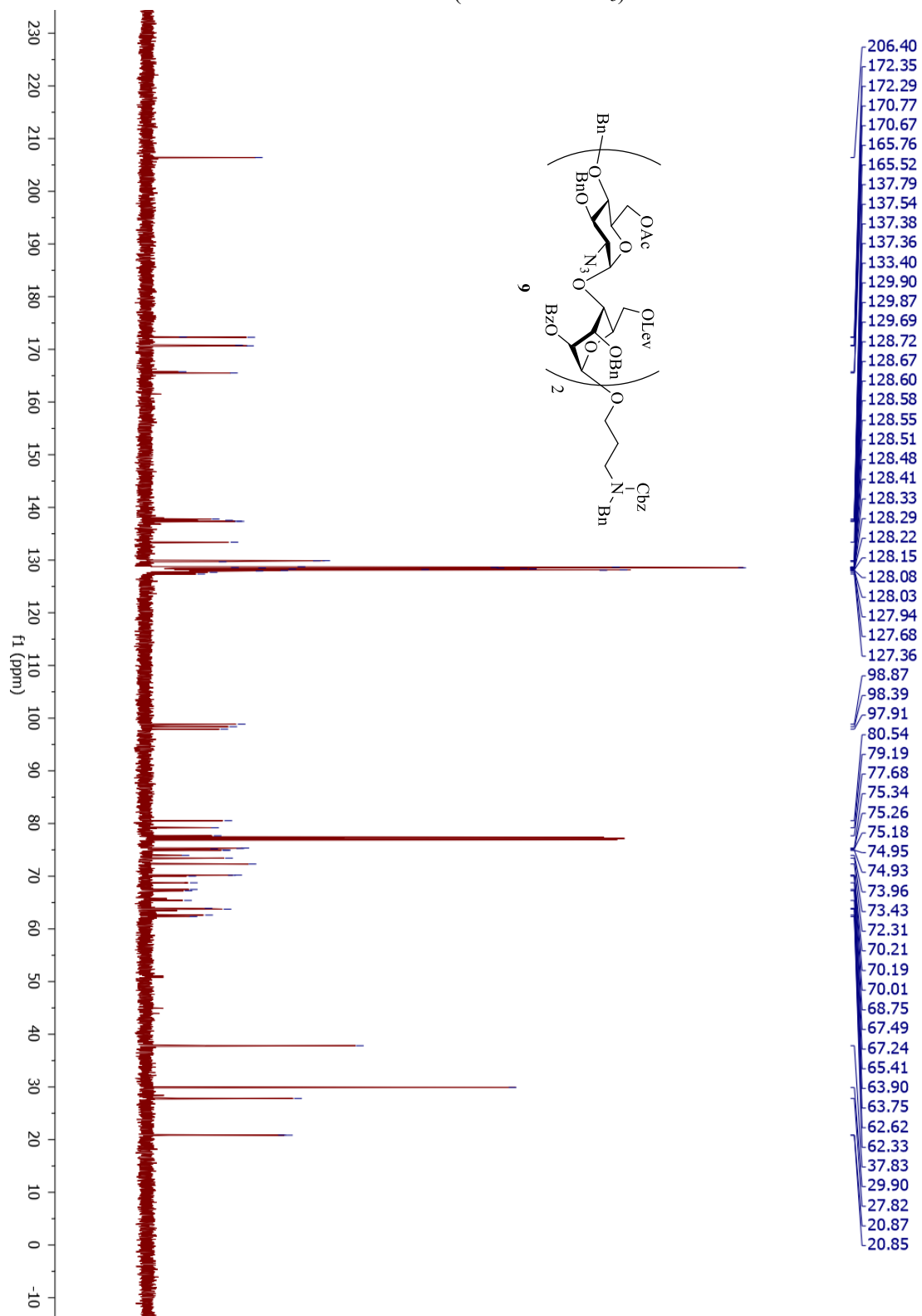
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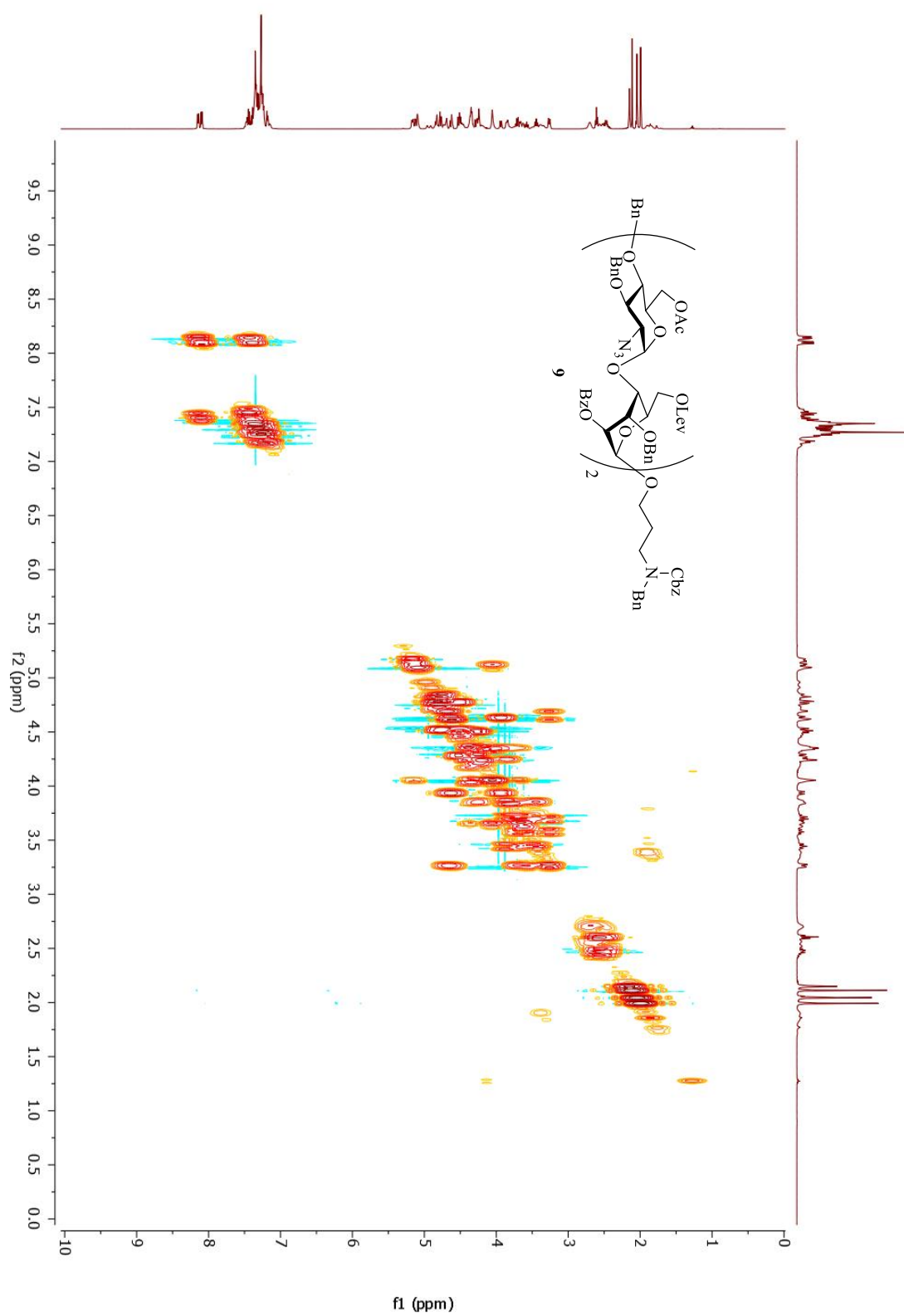
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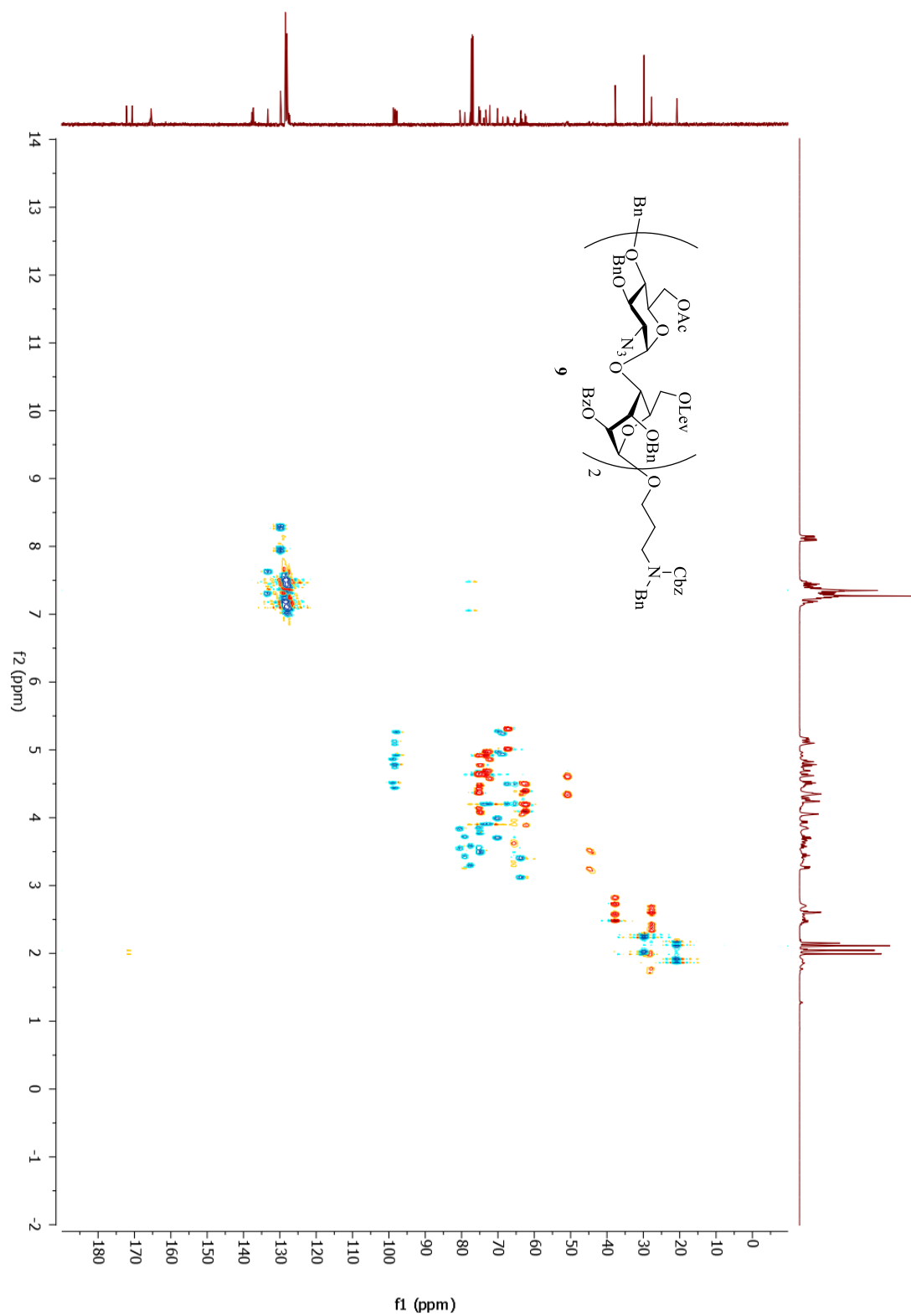
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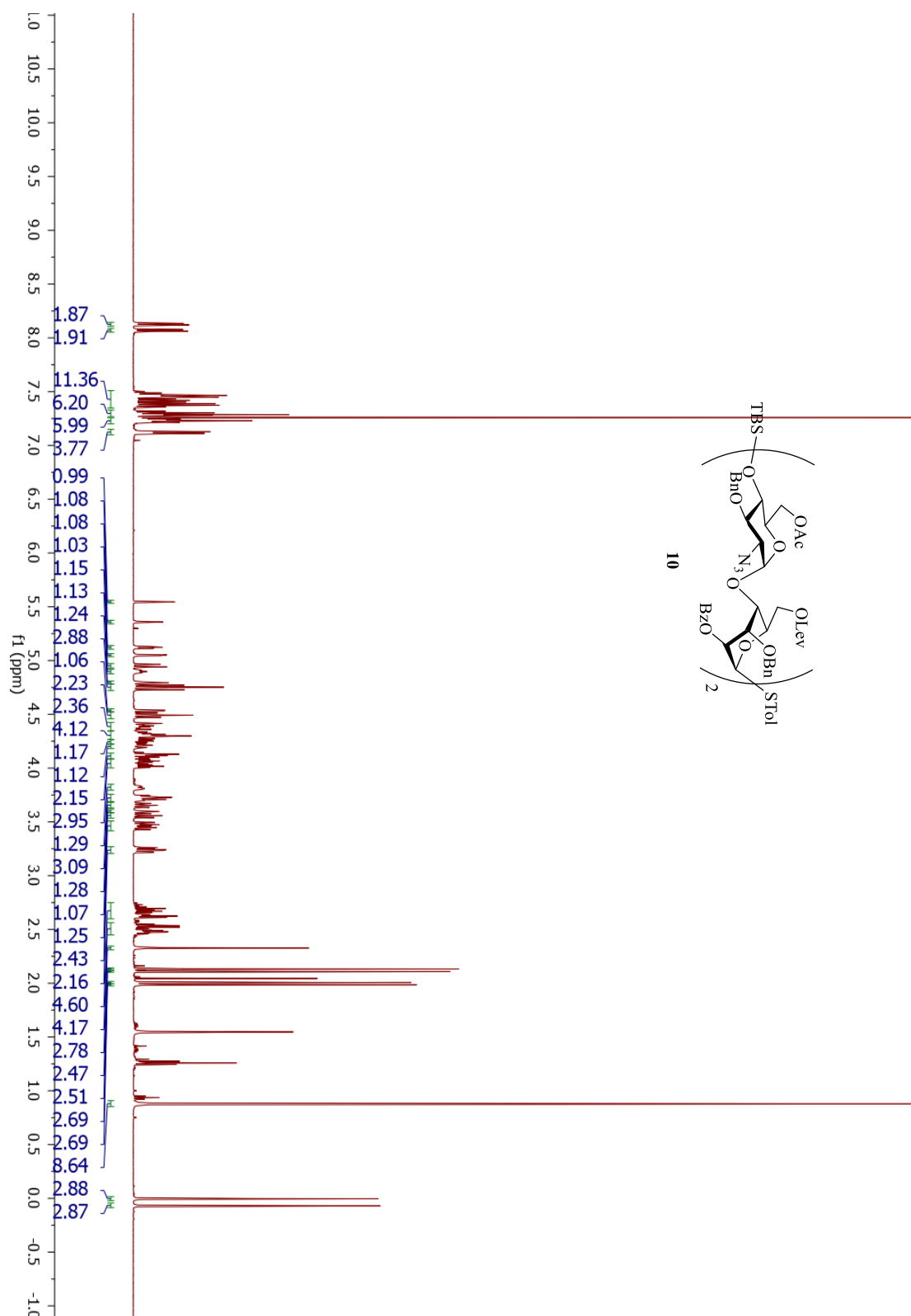
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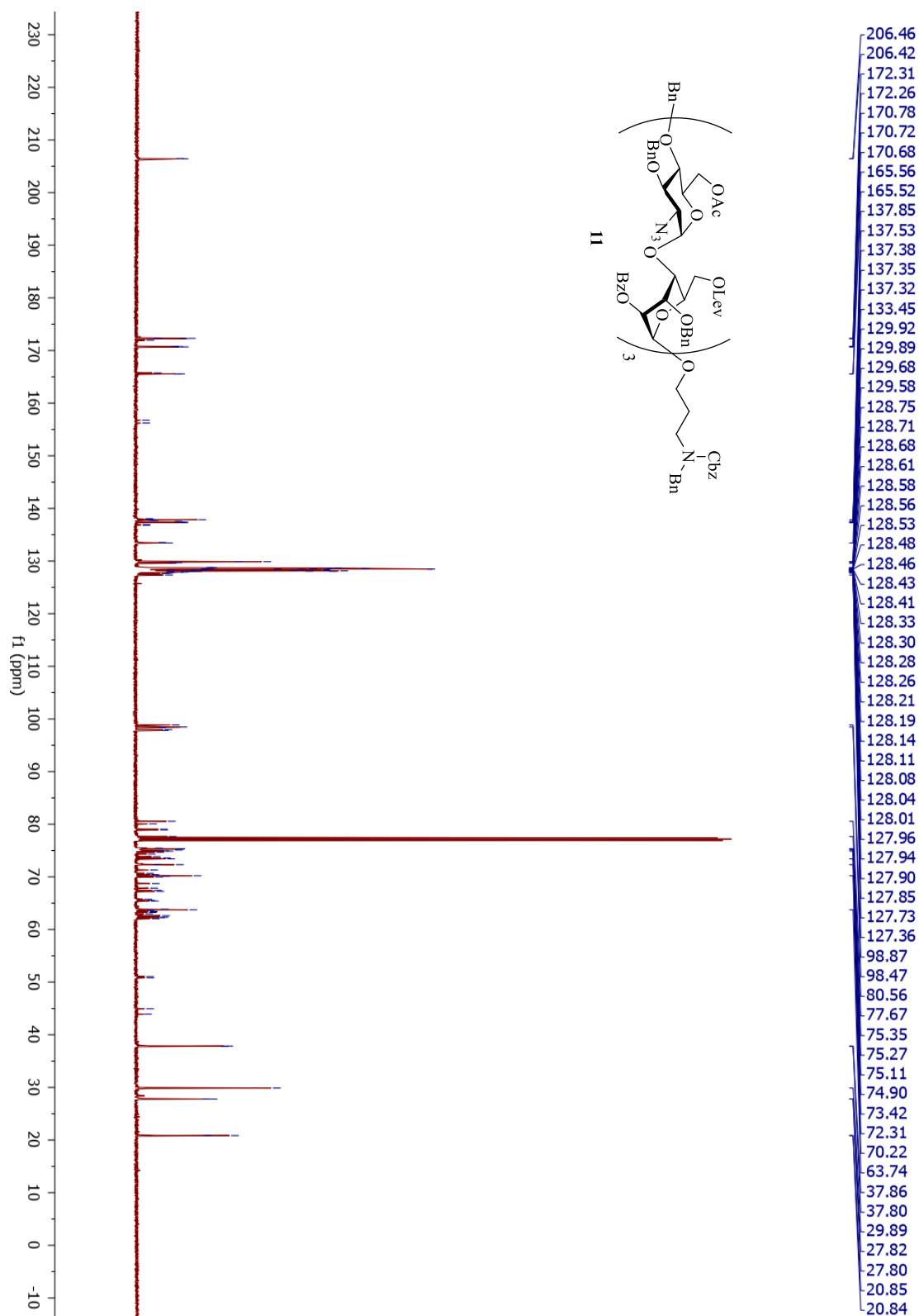
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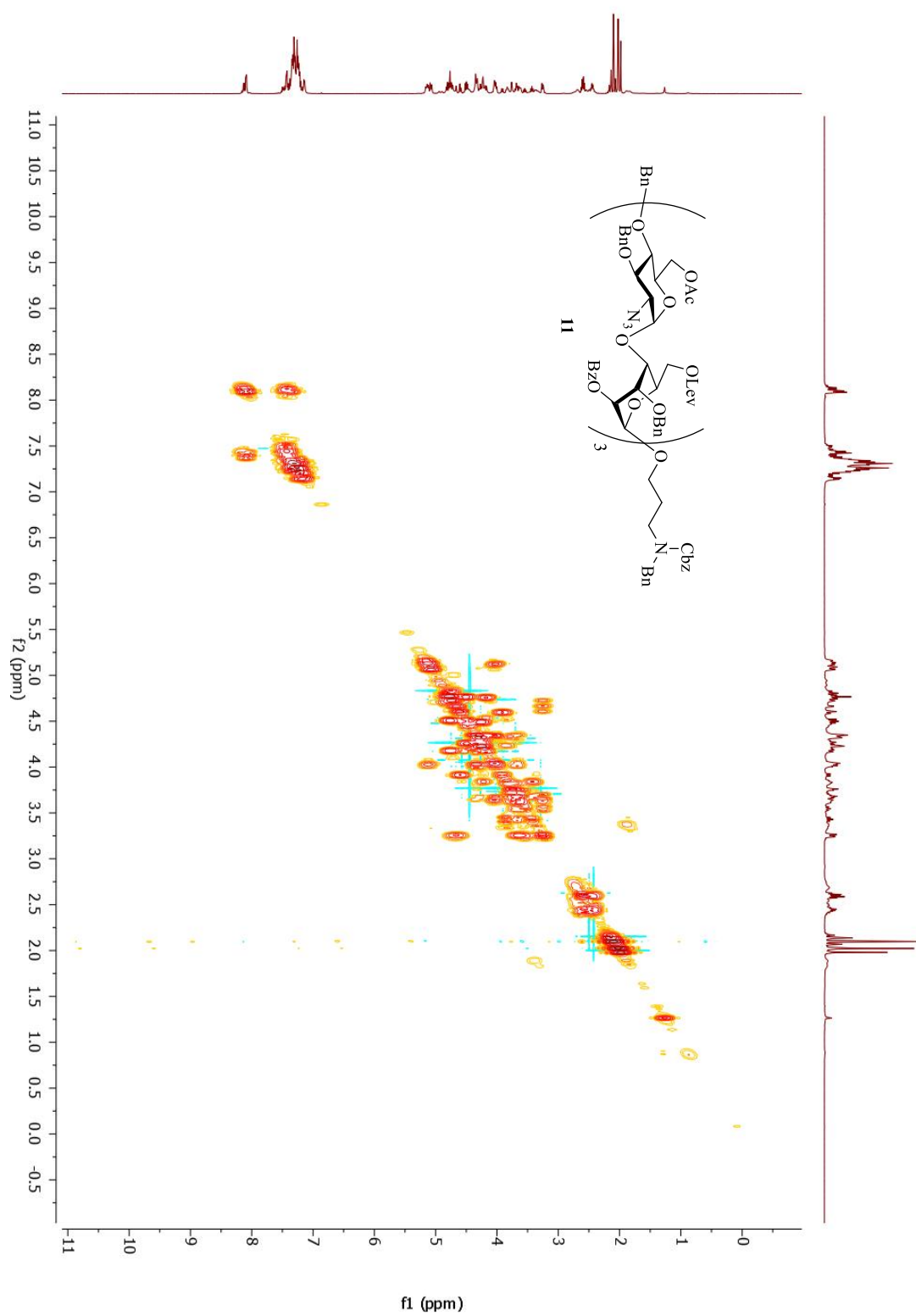
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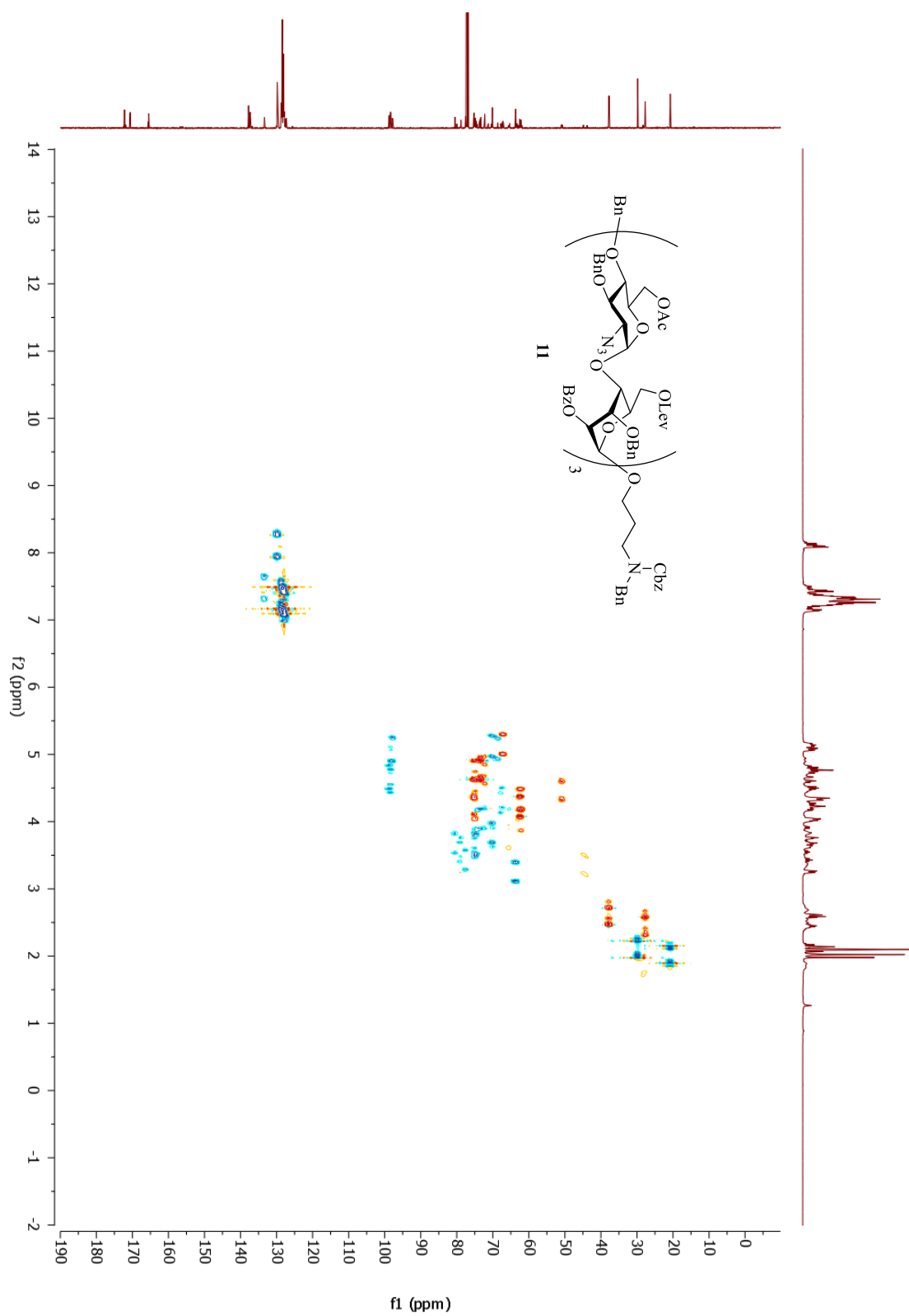
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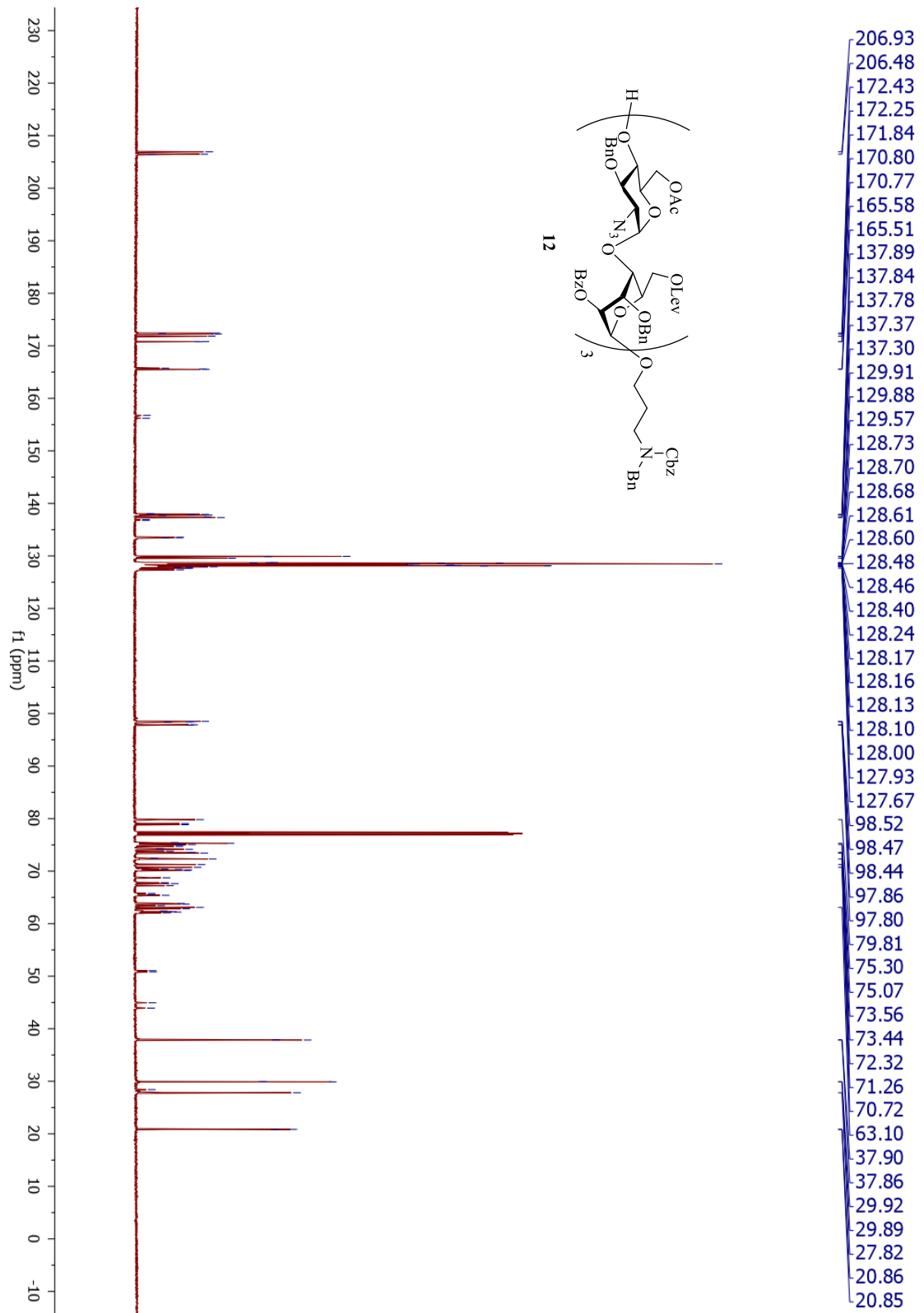
^1H - ^1H gCOSY of **11** (500 MHz CDCl_3)



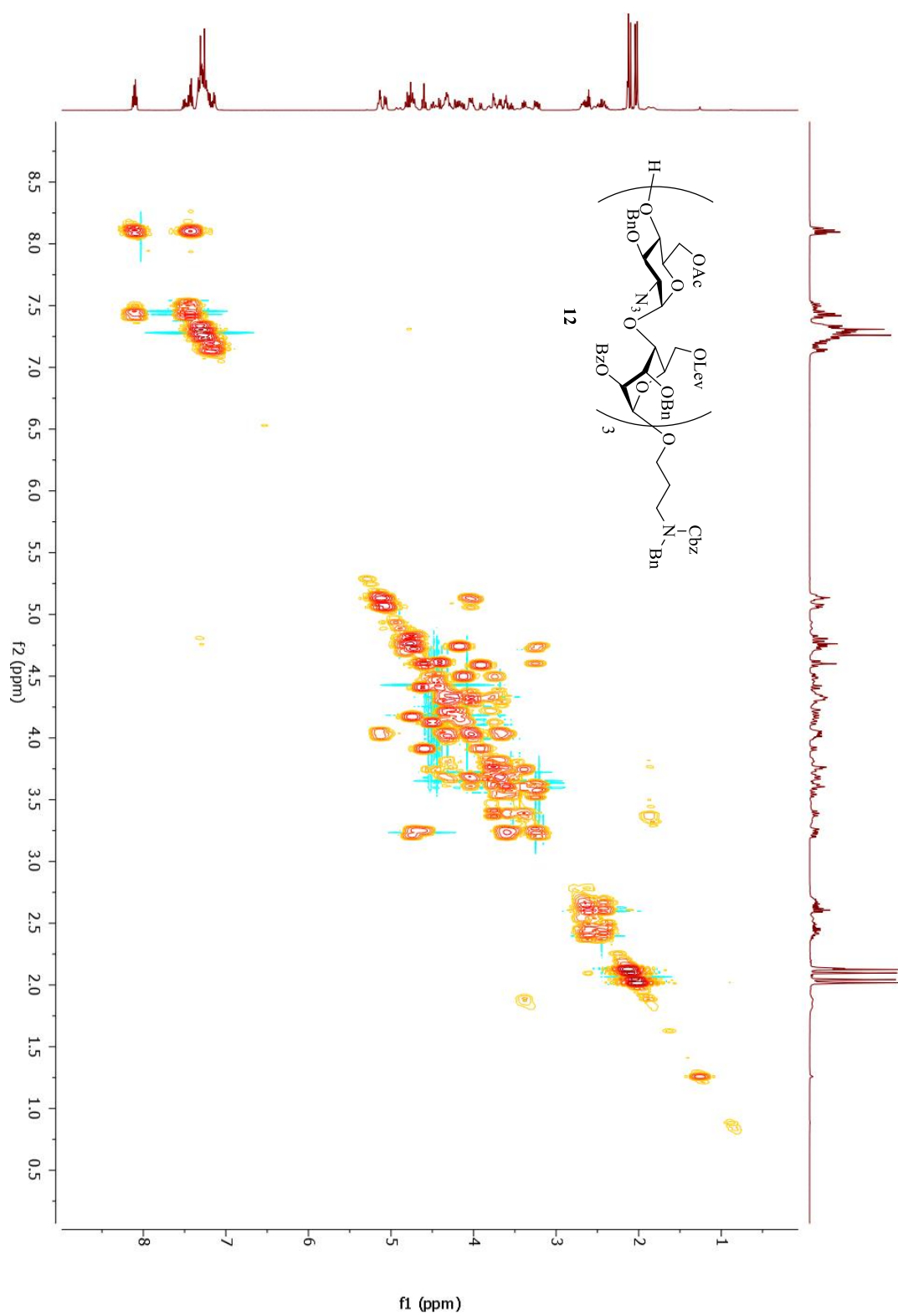
^1H - ^{13}C gHSQCAD of **11** (500 MHz CDCl_3)



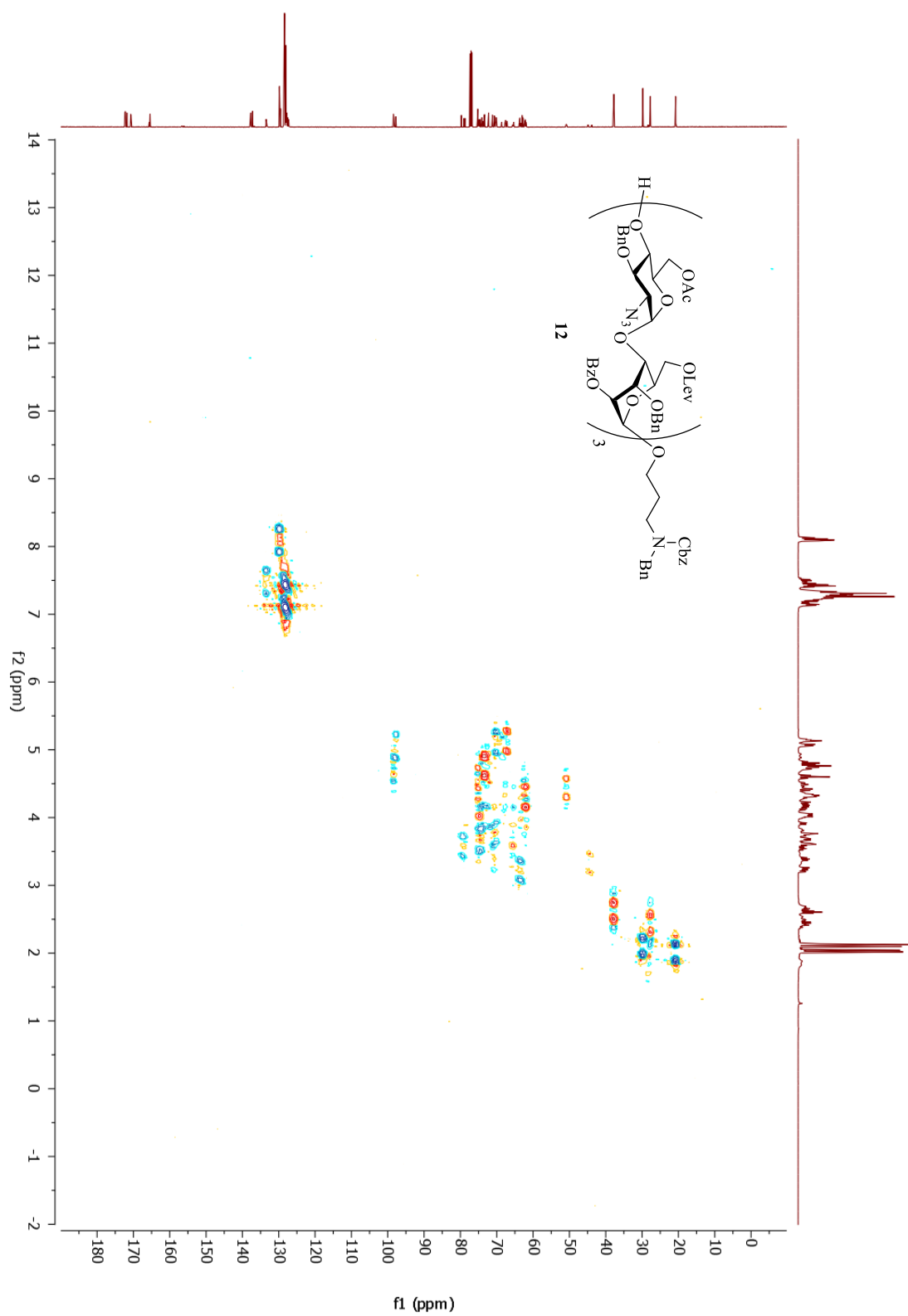
¹³C-NMR of **12** (125 MHz CDCl₃)



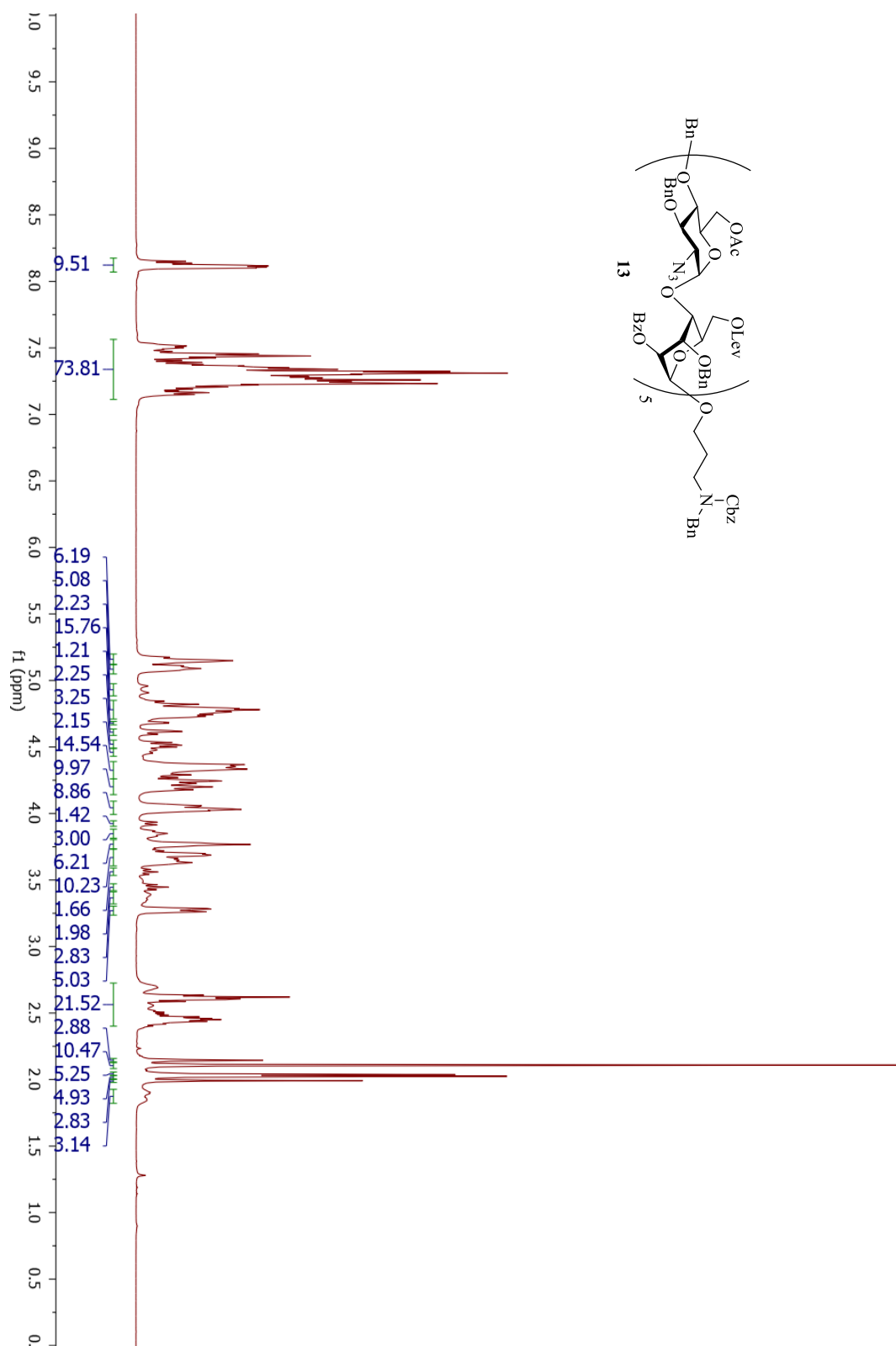
^1H - ^1H gCOSY of **12** (500 MHz CDCl_3)



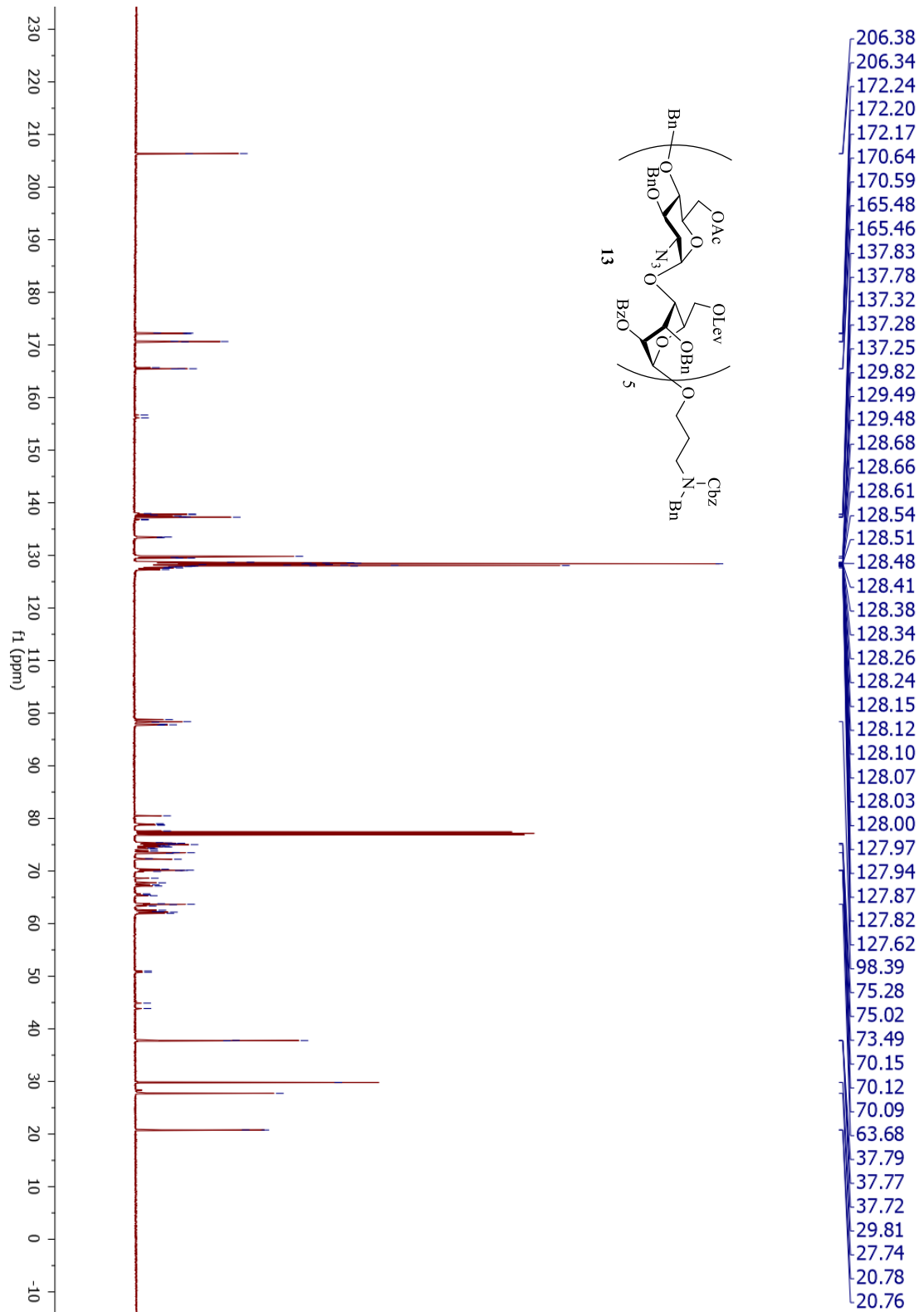
^1H - ^{13}C gHSQCAD of **12** (500 MHz CDCl_3)



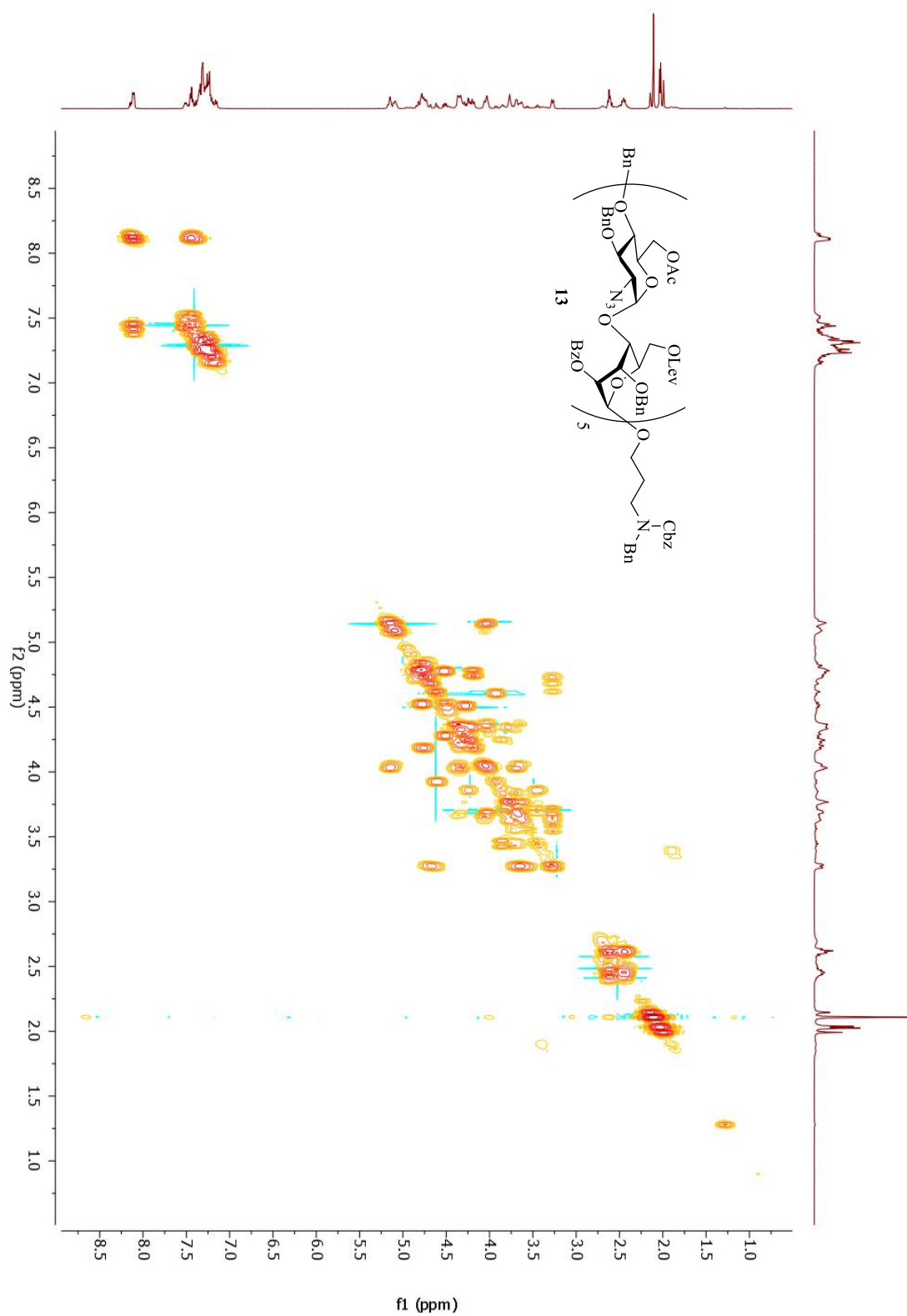
¹H-NMR of **13** (500 MHz CDCl₃)



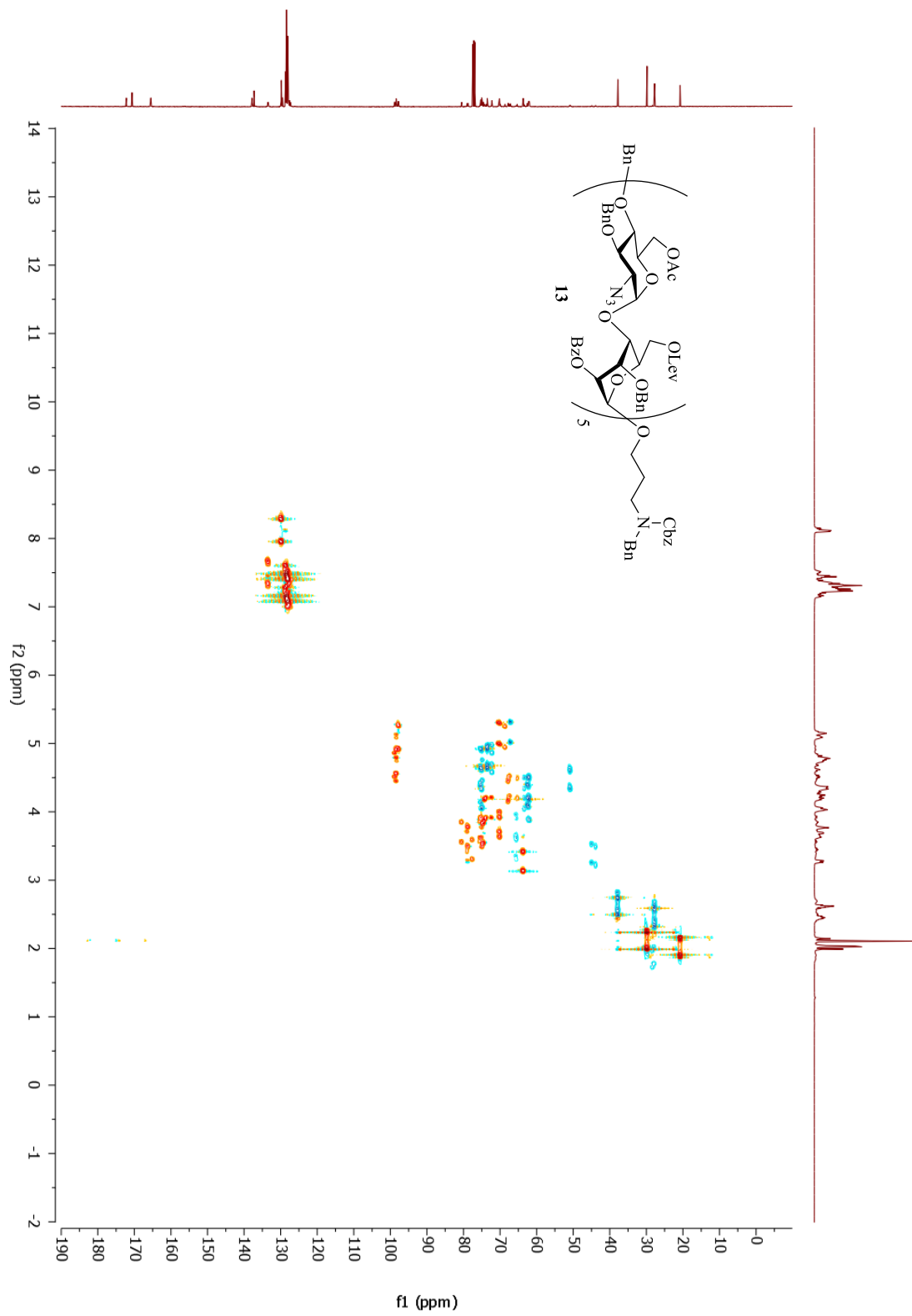
¹³C-NMR of **13** (125 MHz CDCl₃)



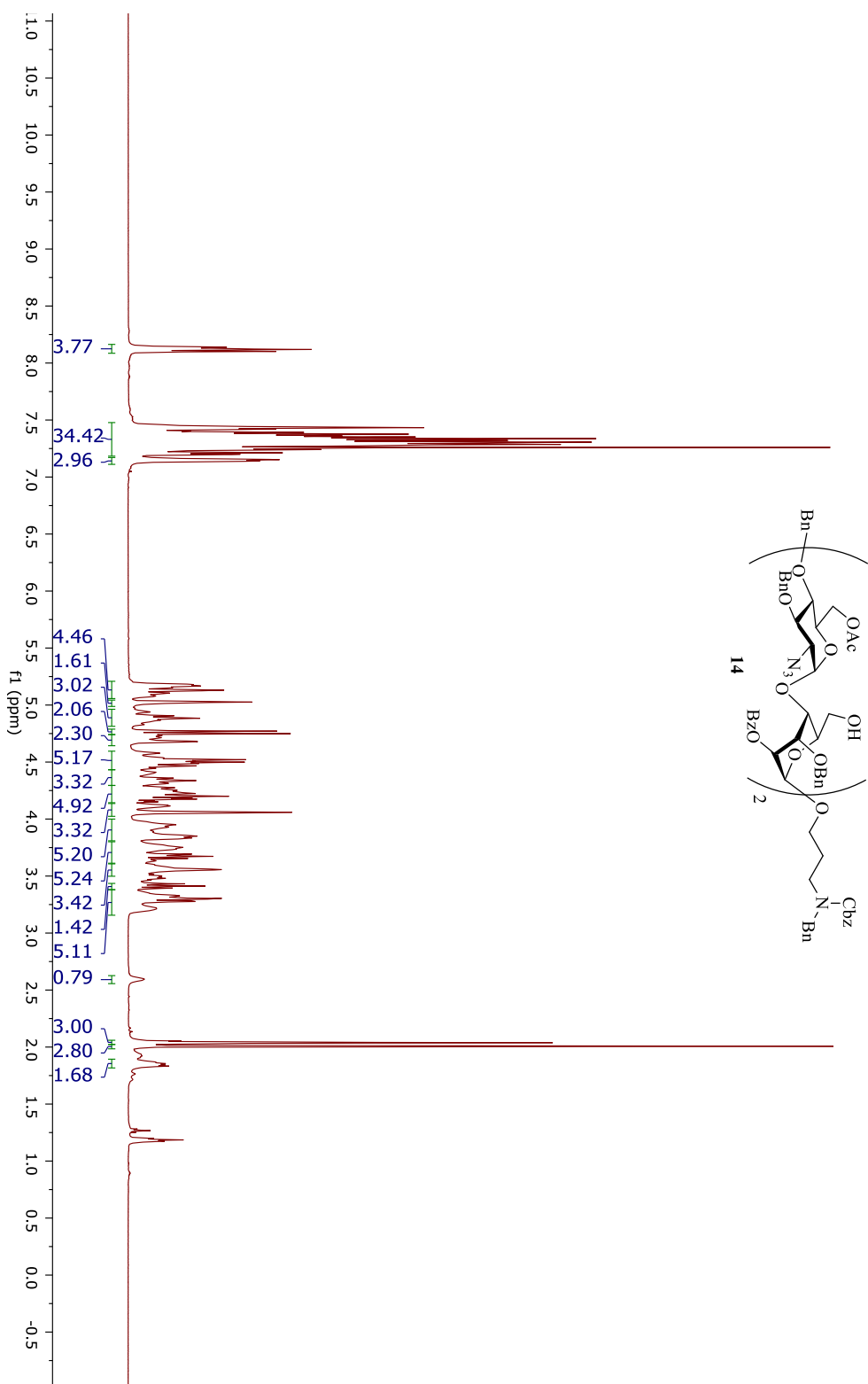
^1H - ^1H gCOSY of **13** (500 MHz CDCl_3)



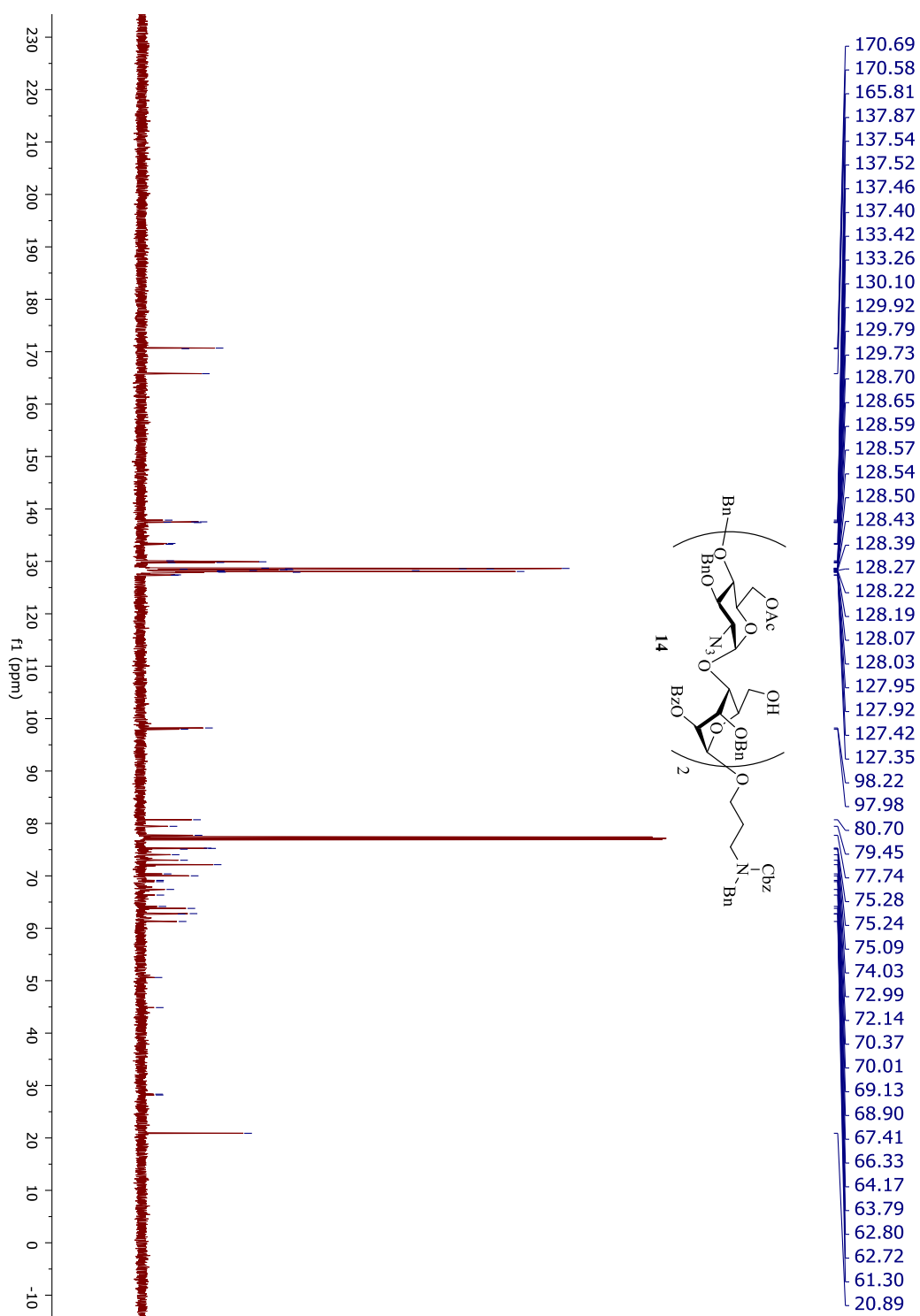
^1H - ^{13}C gHSQCAD of **13** (500 MHz CDCl_3)



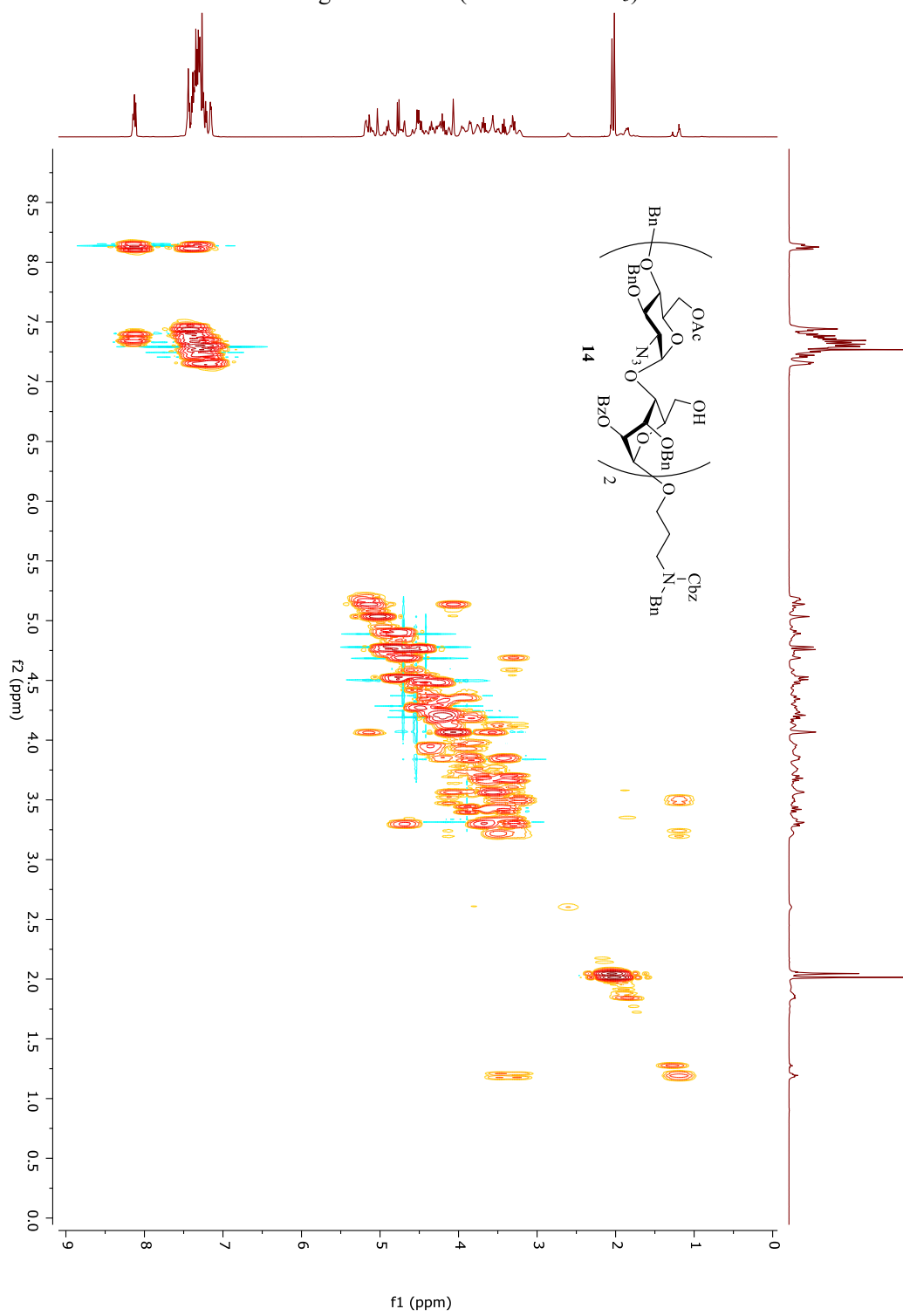
¹H-NMR of **14** (500 MHz CDCl₃)



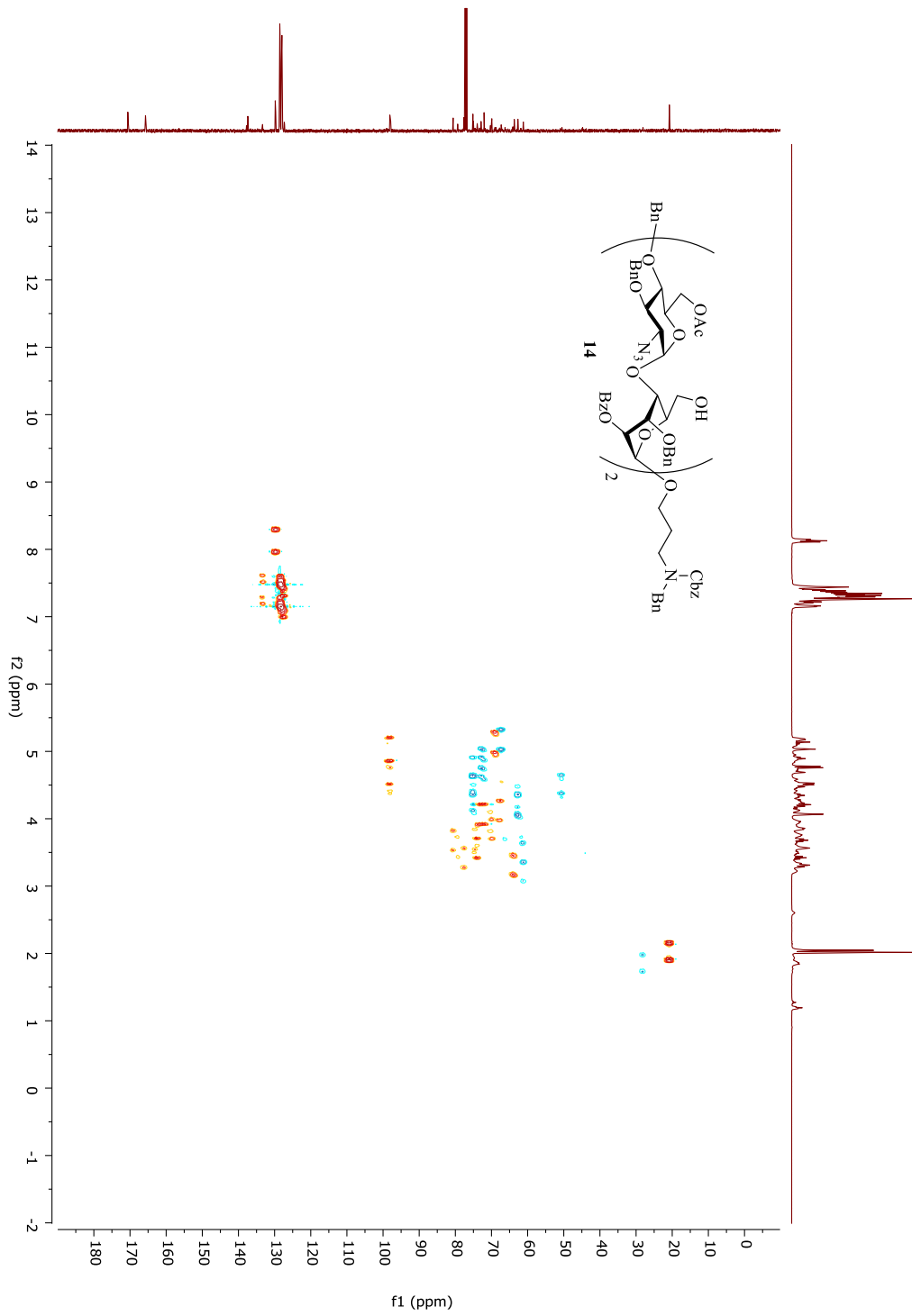
¹³C-NMR of **14** (125 MHz CDCl₃)



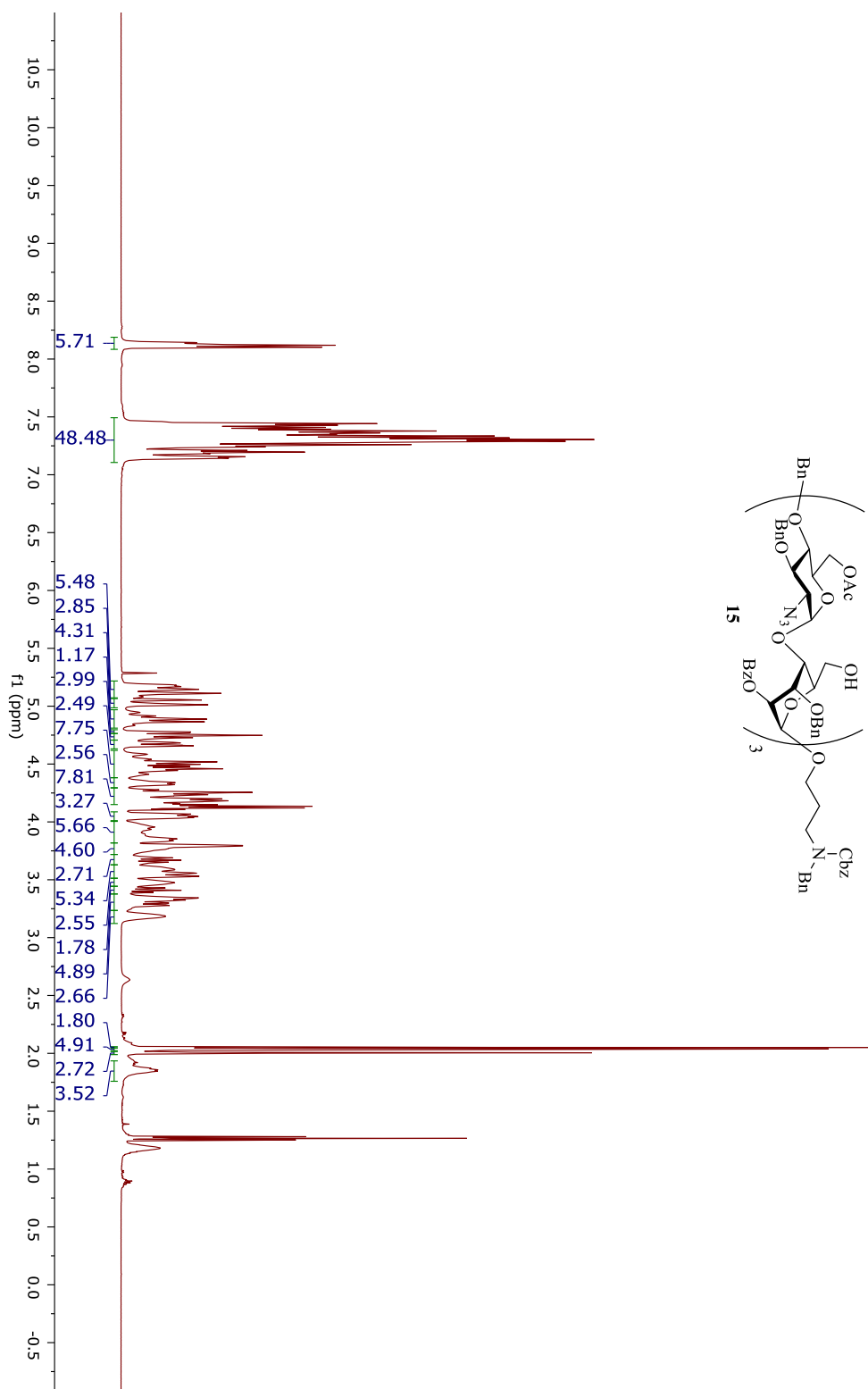
^1H - ^1H gCOSY of **14** (500 MHz CDCl_3)



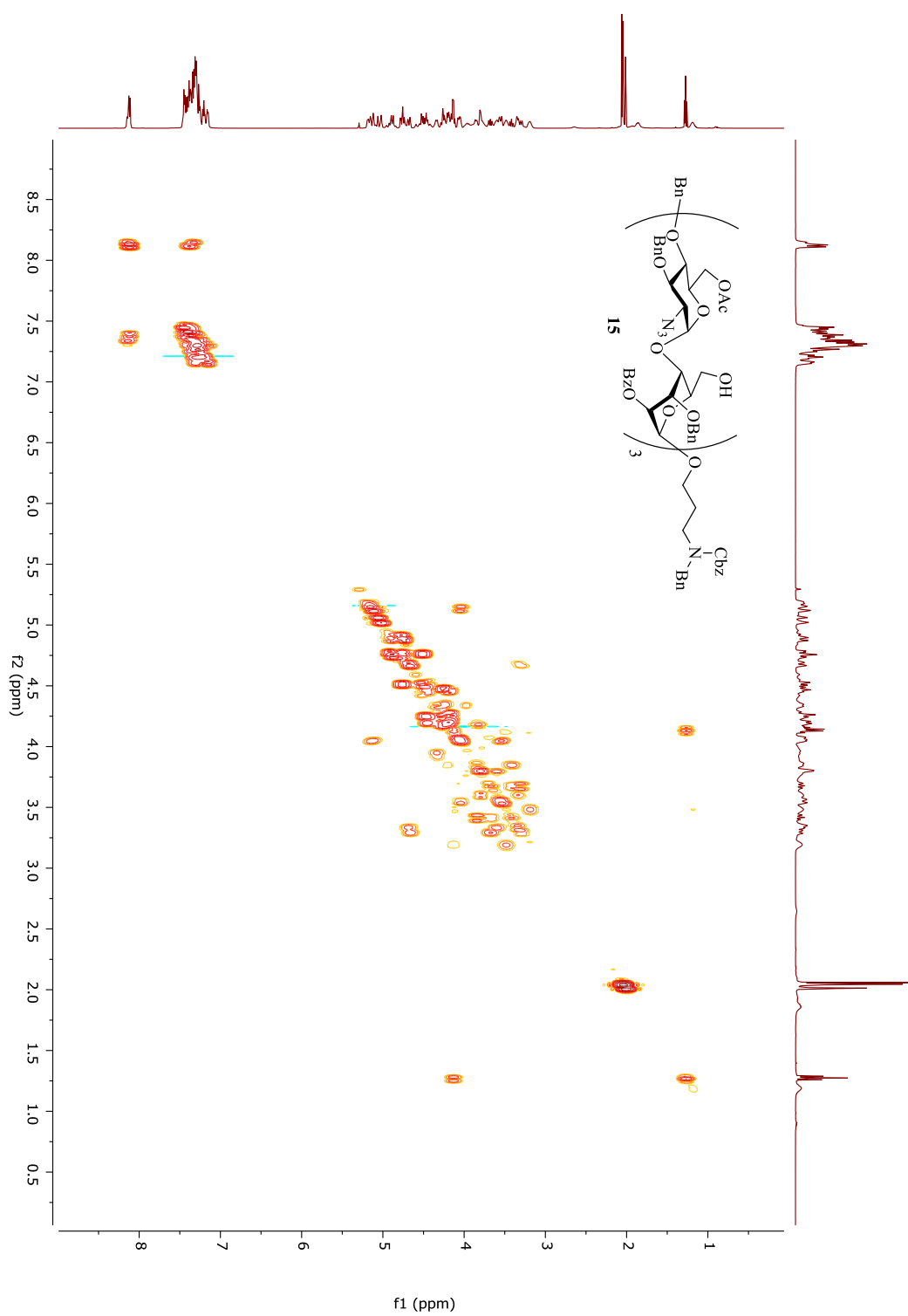
^1H - ^{13}C gHSQCAD of **14** (500 MHz CDCl_3)



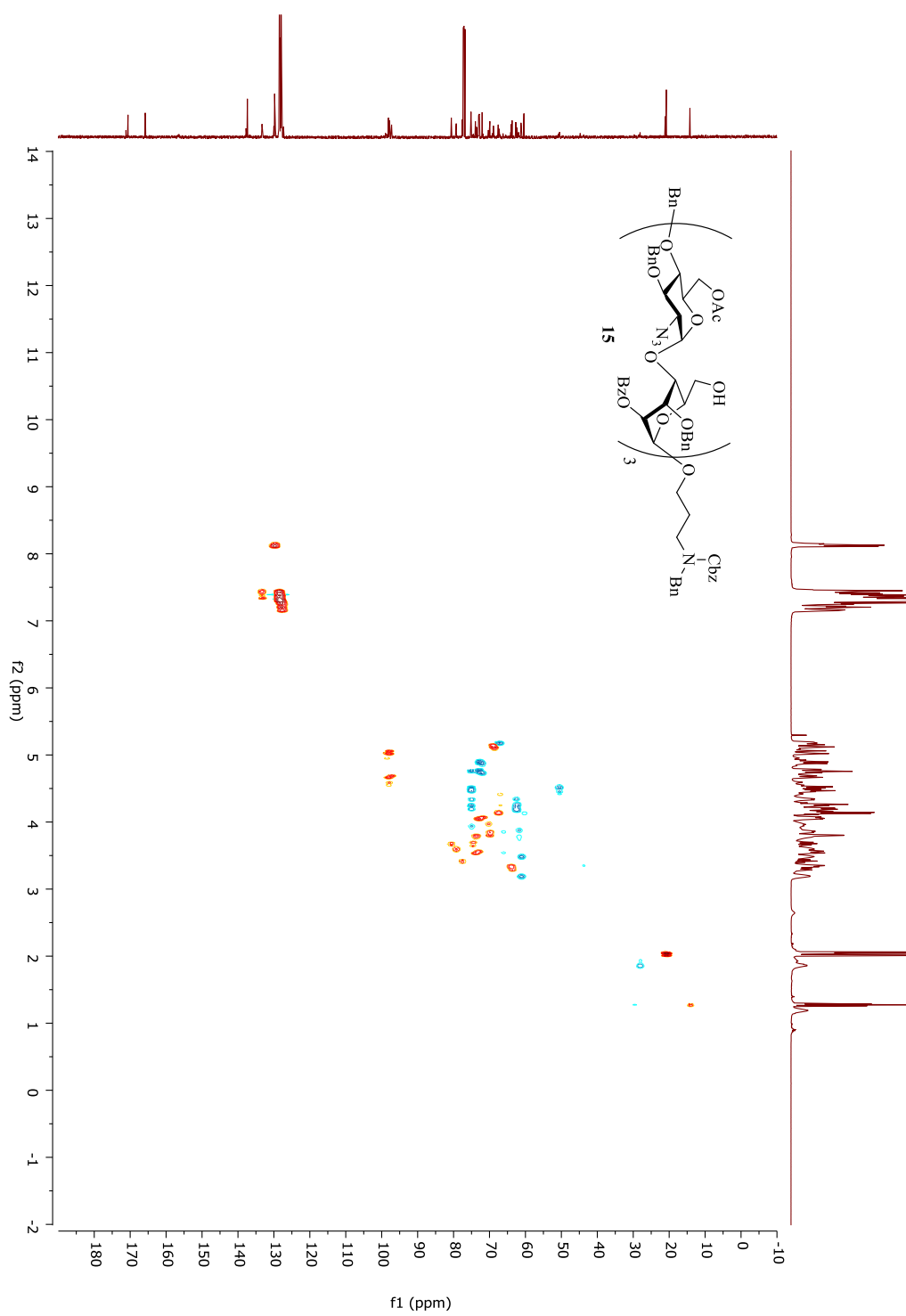
¹H-NMR of **15** (500 MHz CDCl₃)



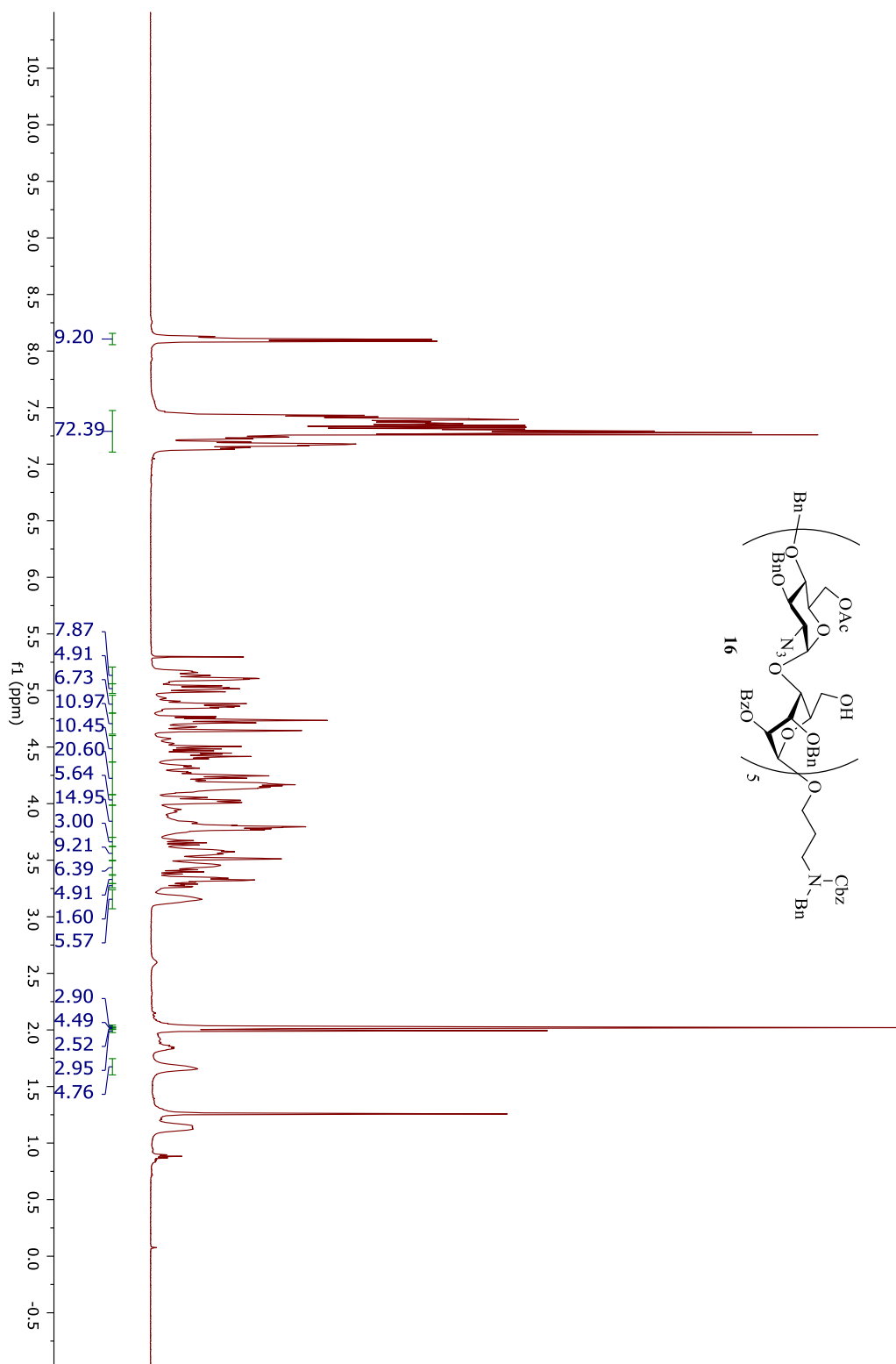
^1H - ^1H gCOSY of **15** (500 MHz CDCl_3)



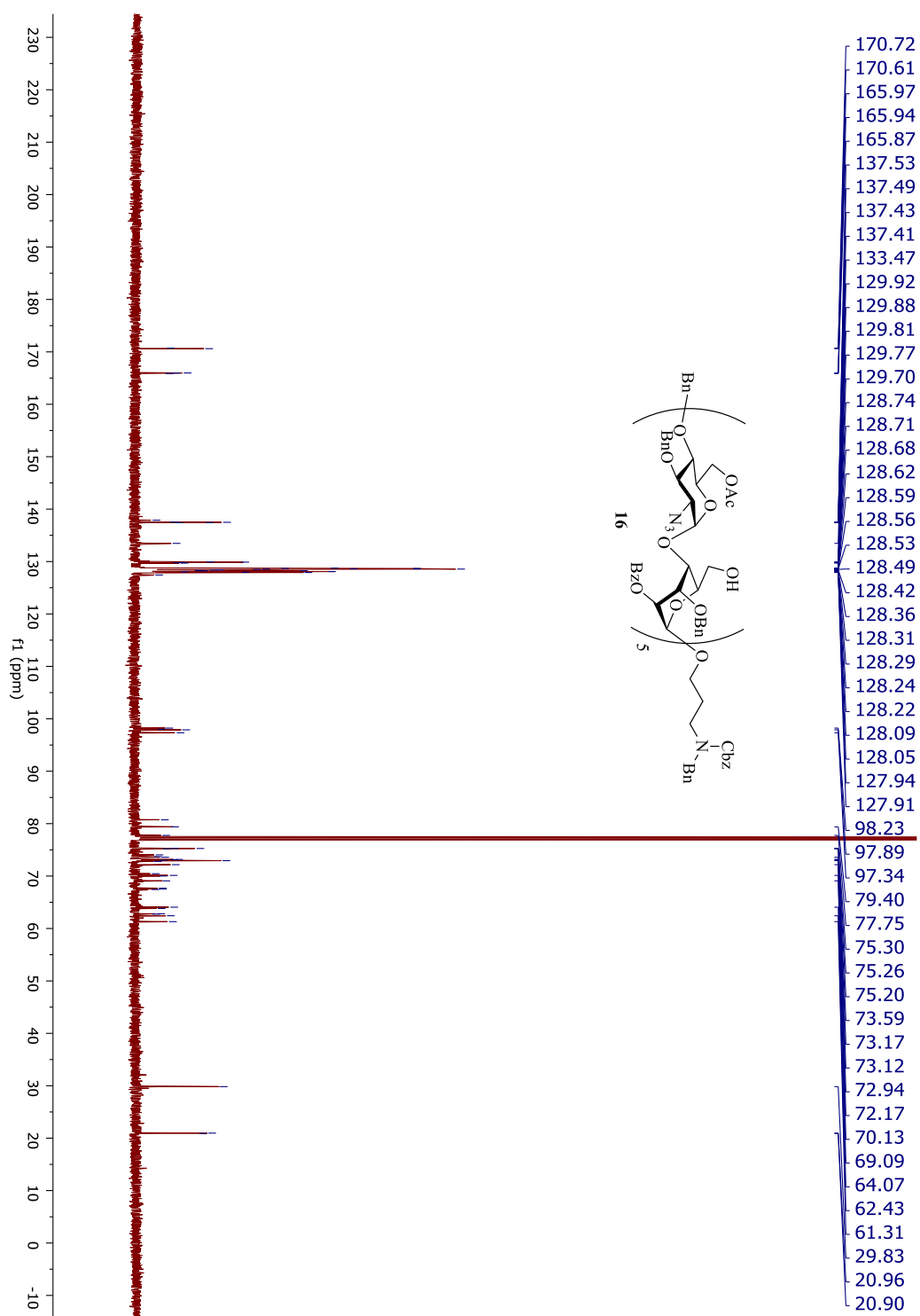
^1H - ^{13}C gHSQCAD of **15** (500 MHz CDCl_3)



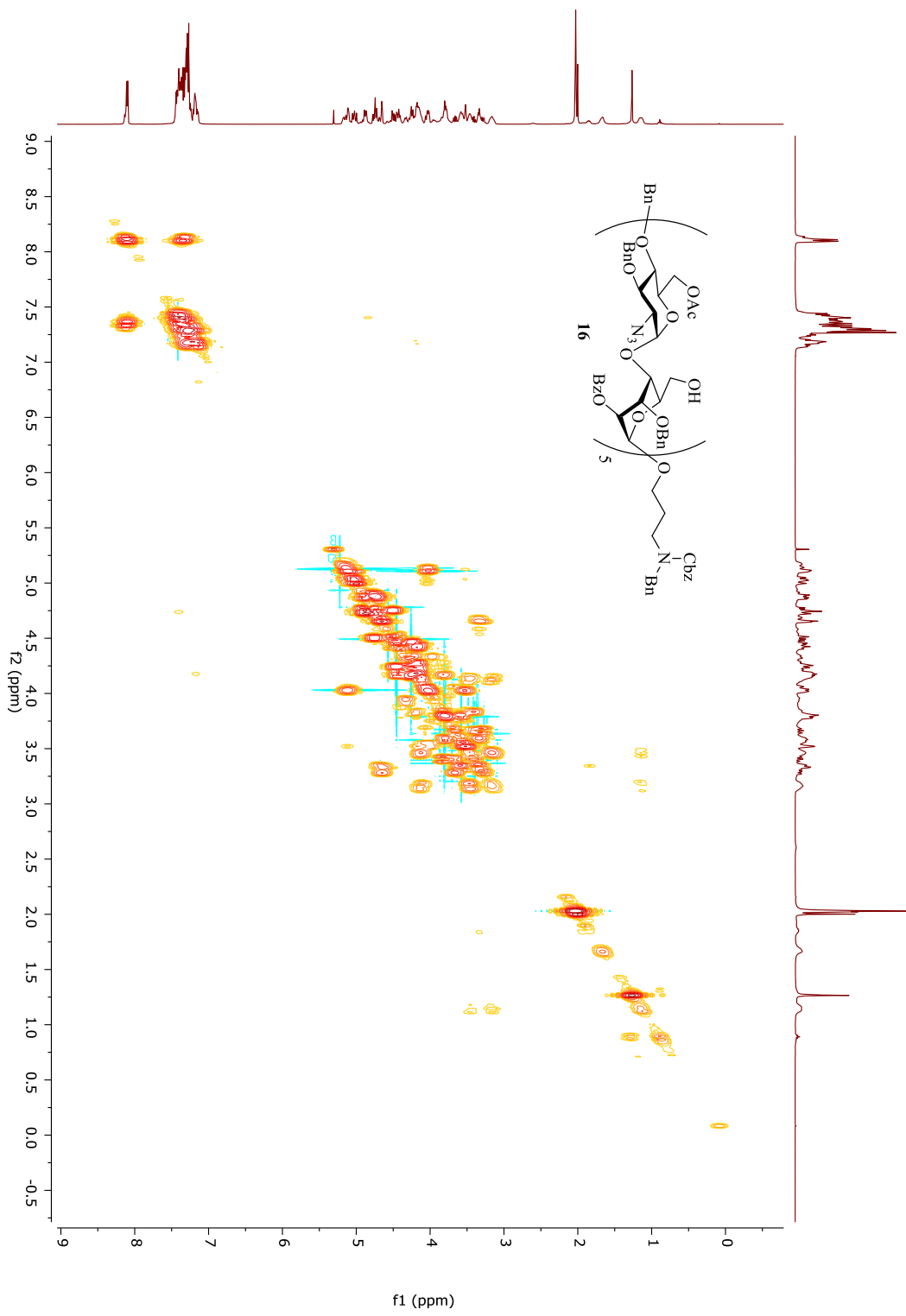
¹H-NMR of **16** (500 MHz CDCl₃)



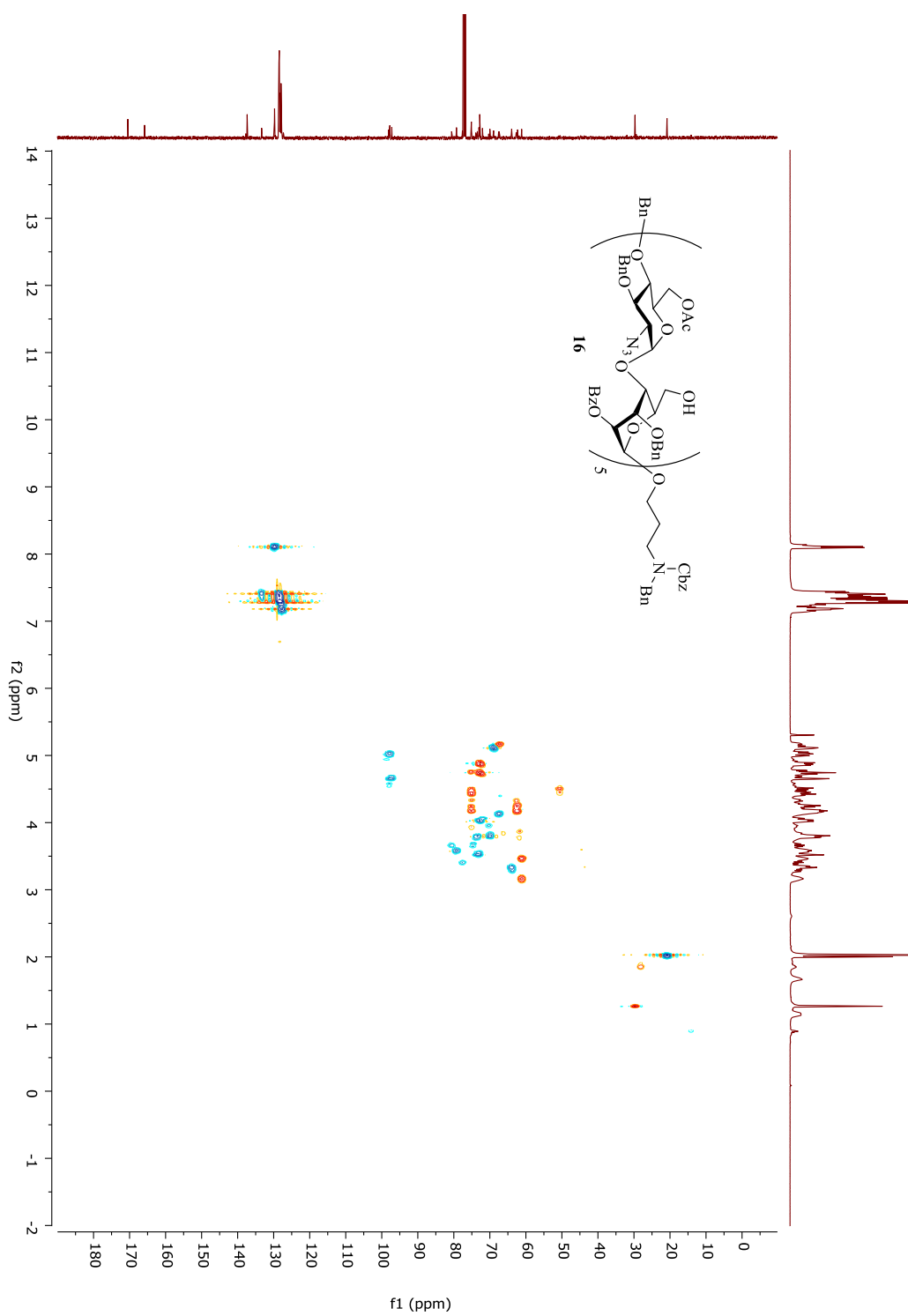
¹³C-NMR of **16** (125 MHz CDCl₃)



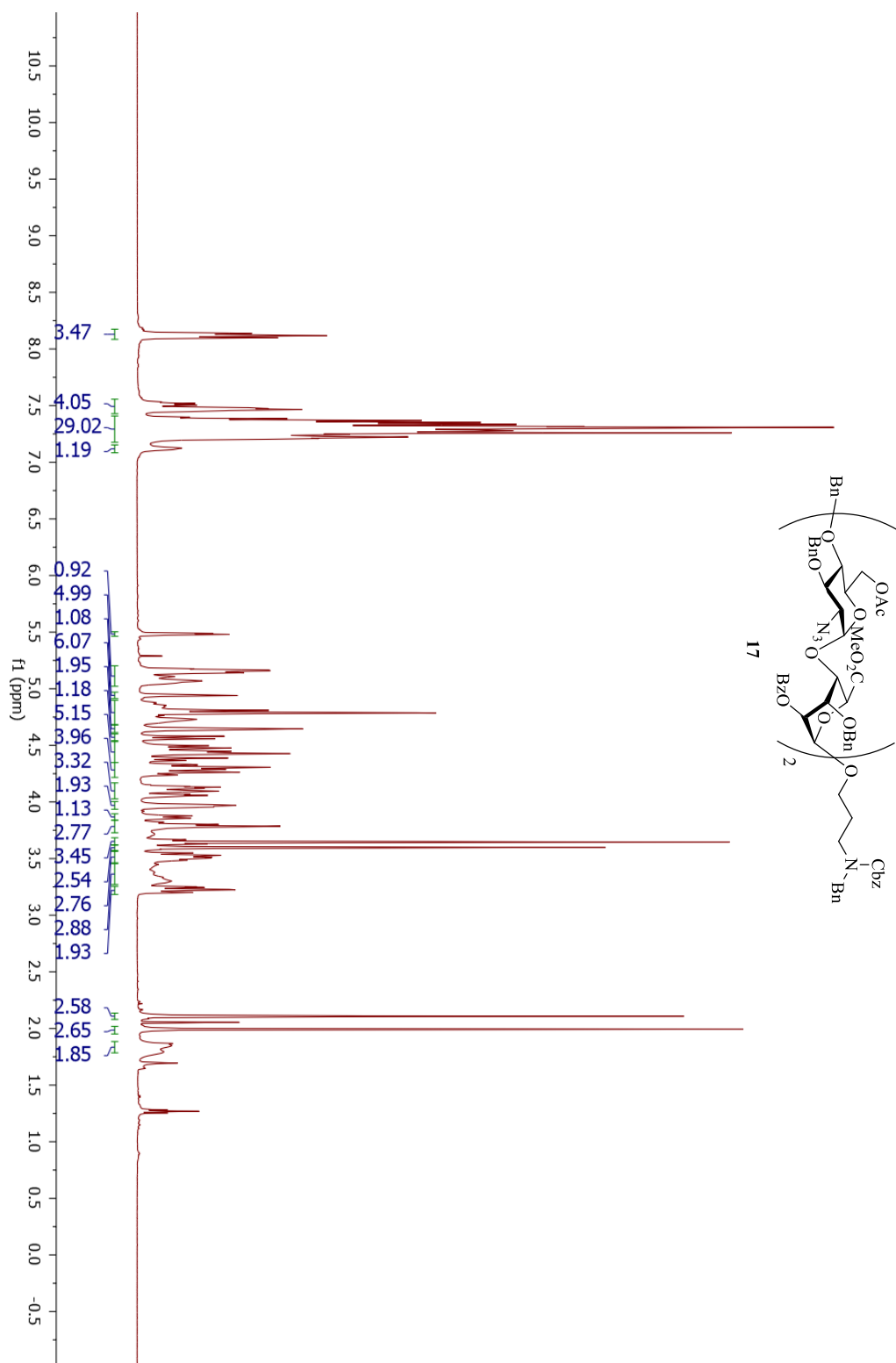
^1H - ^1H gCOSY of **16** (500 MHz CDCl_3)



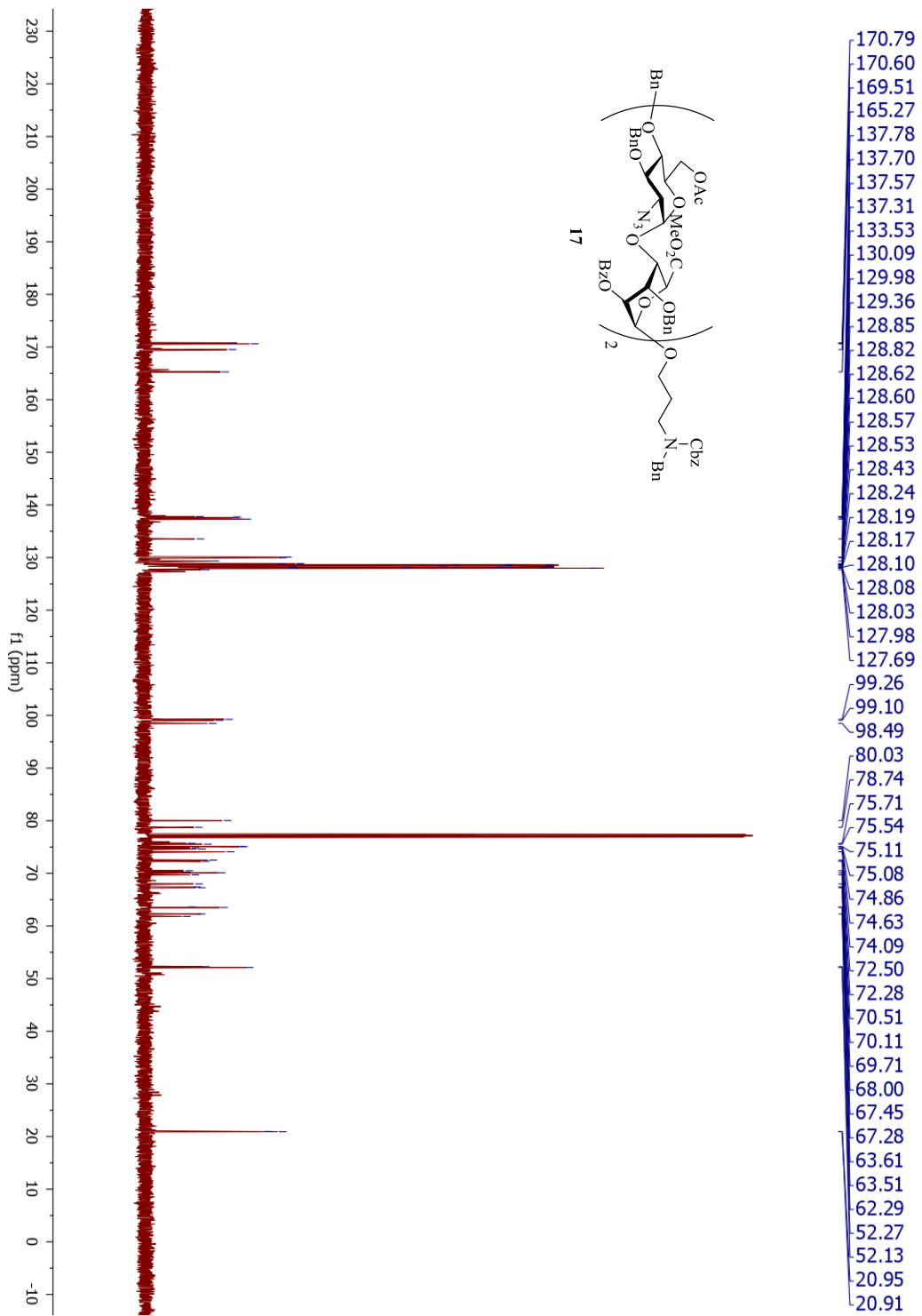
^1H - ^{13}C gHSQCAD of **16** (500 MHz CDCl_3)



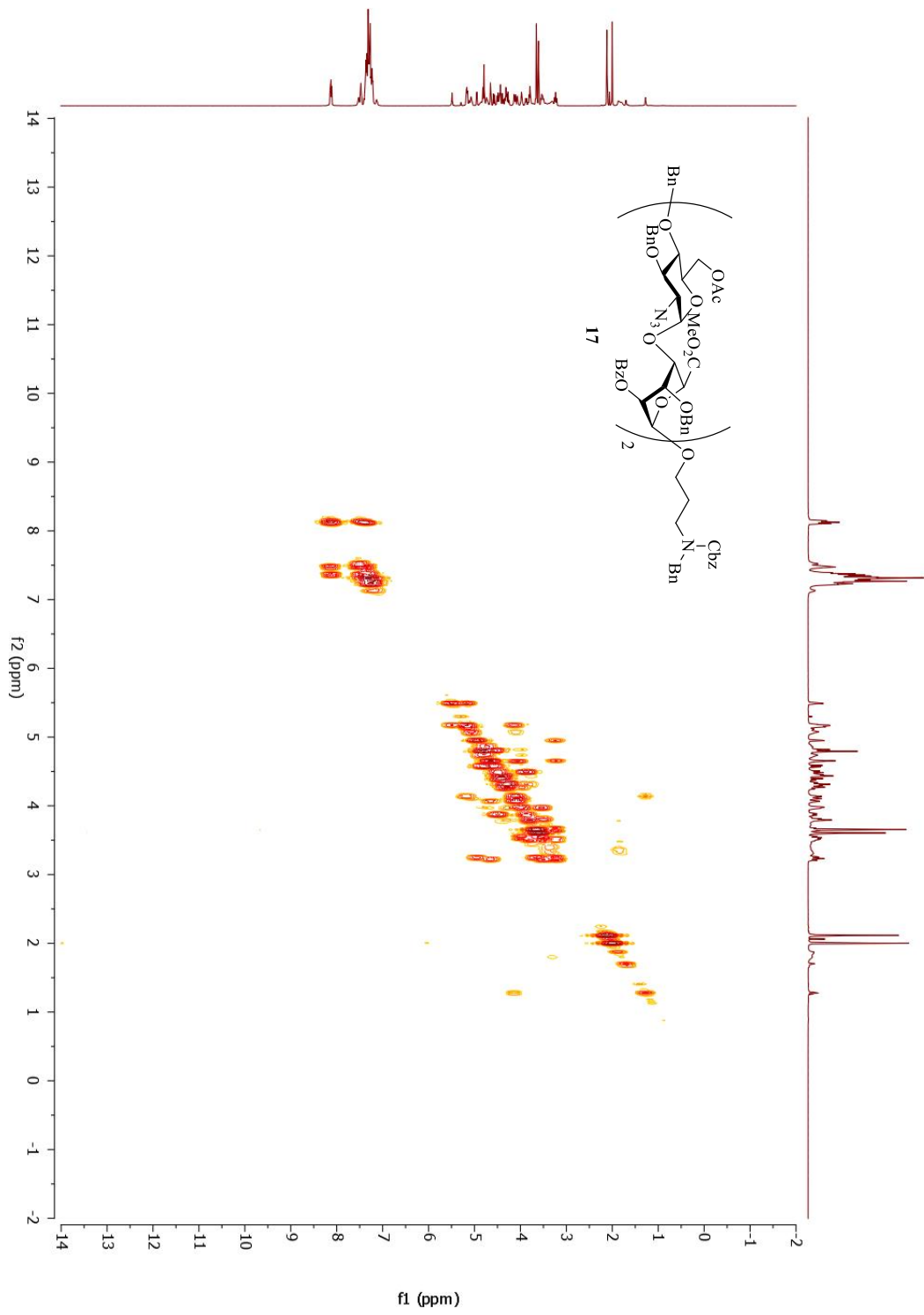
¹H-NMR of **17** (500 MHz CDCl₃)



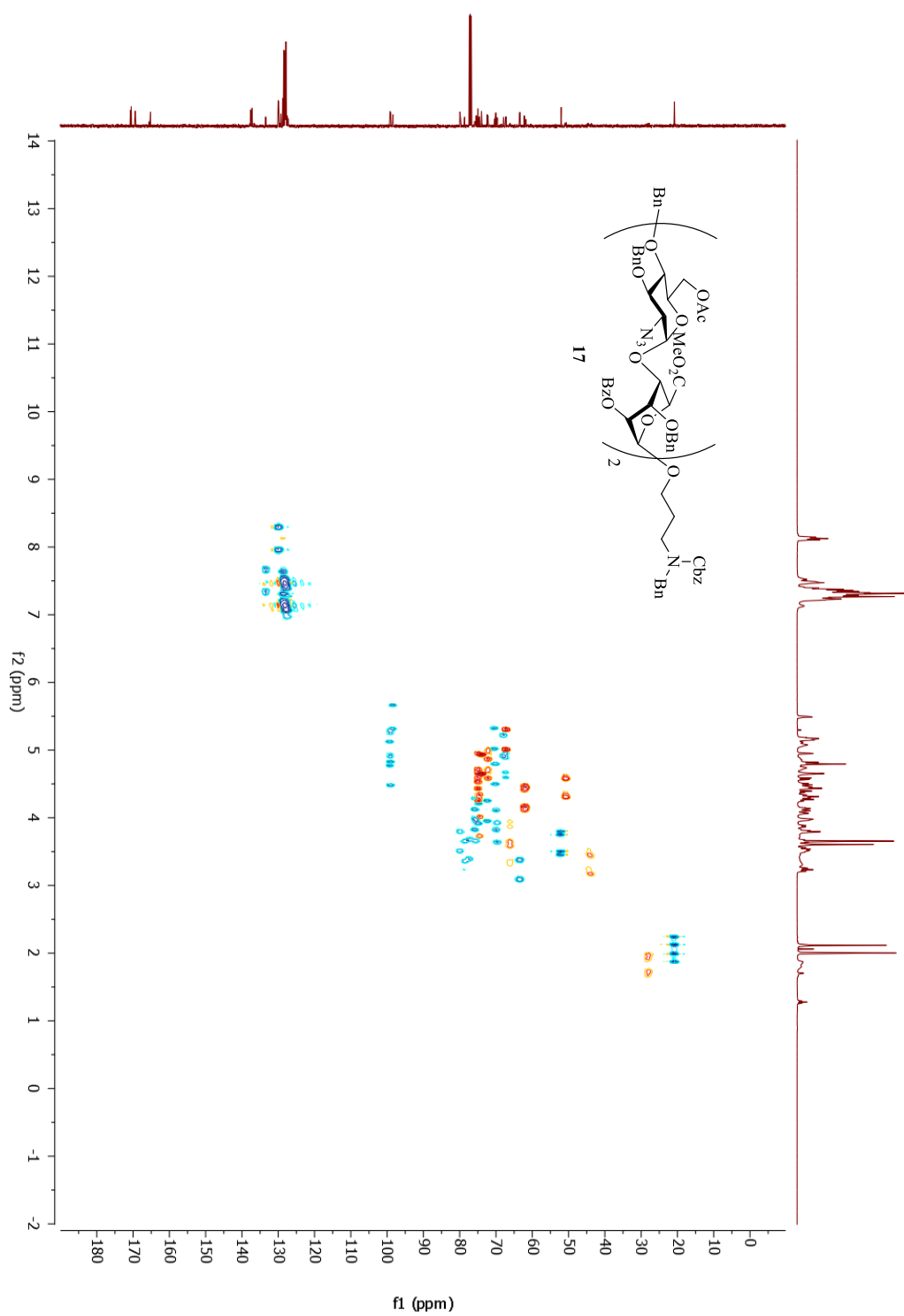
¹³C-NMR of 17 (125 MHz CDCl₃)



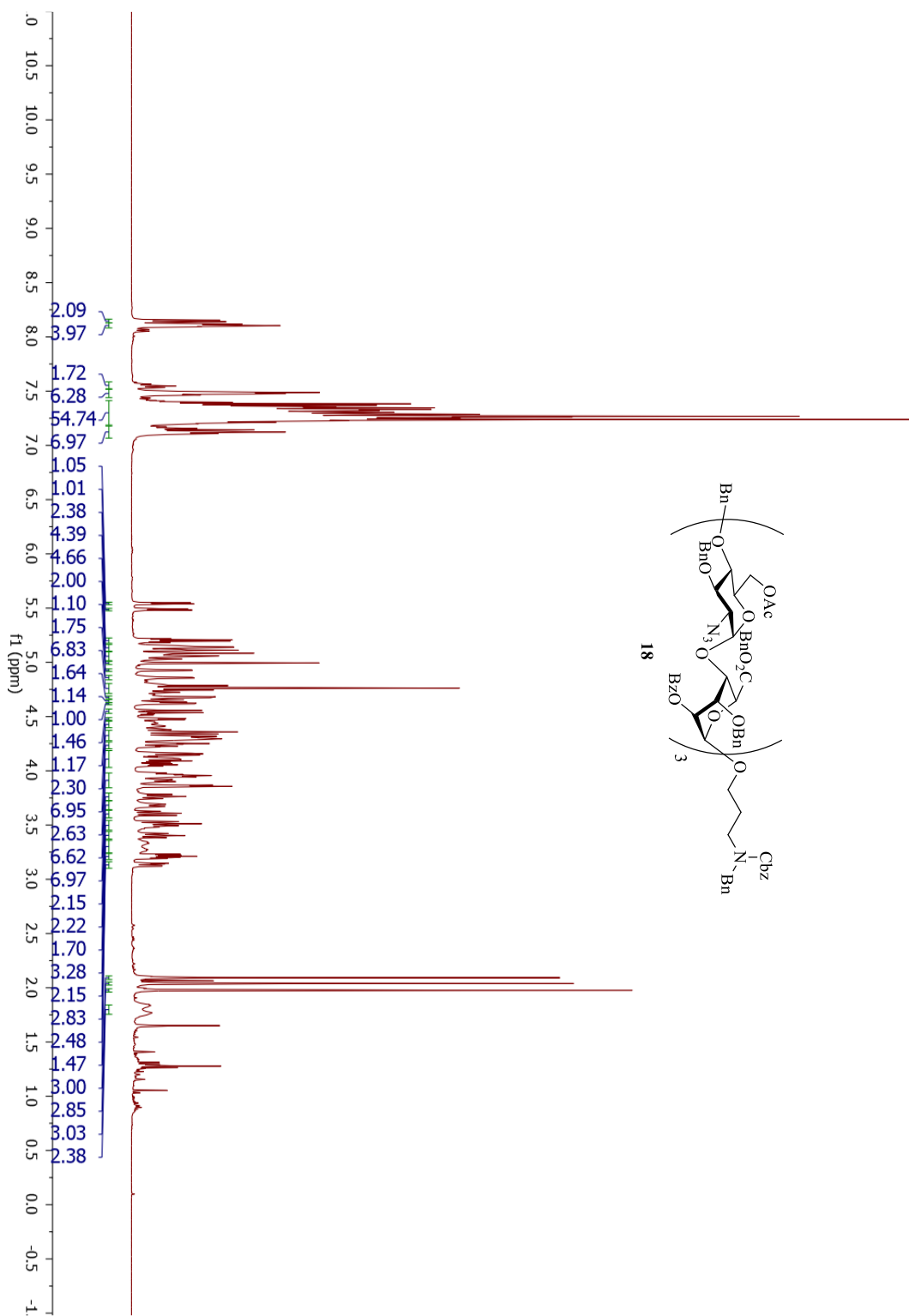
^1H - ^1H gCOSY of **17** (500 MHz CDCl_3)



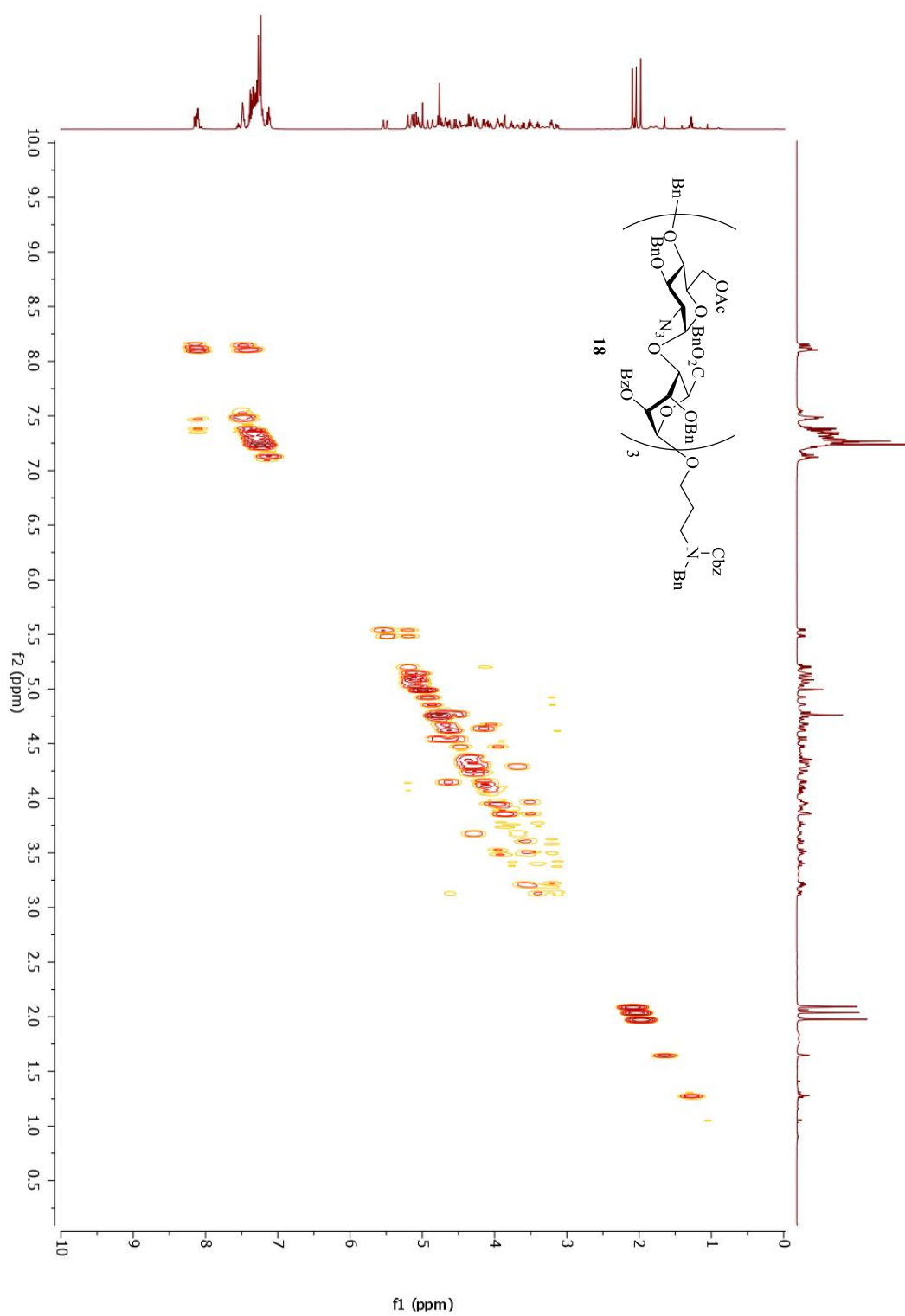
^1H - ^{13}C gHSQCAD of **17** (500 MHz CDCl_3)



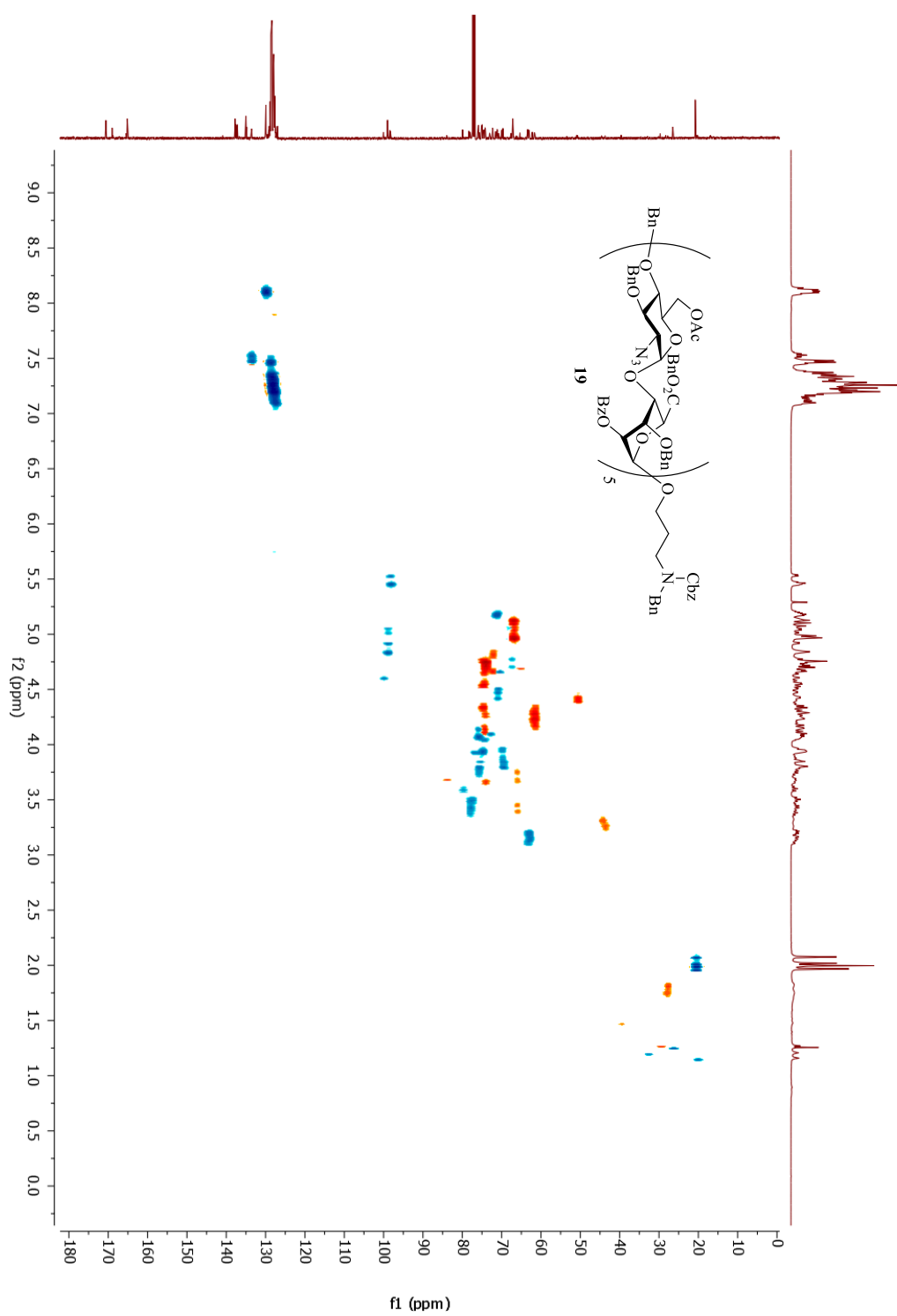
¹H-NMR of **18** (500 MHz CDCl₃)



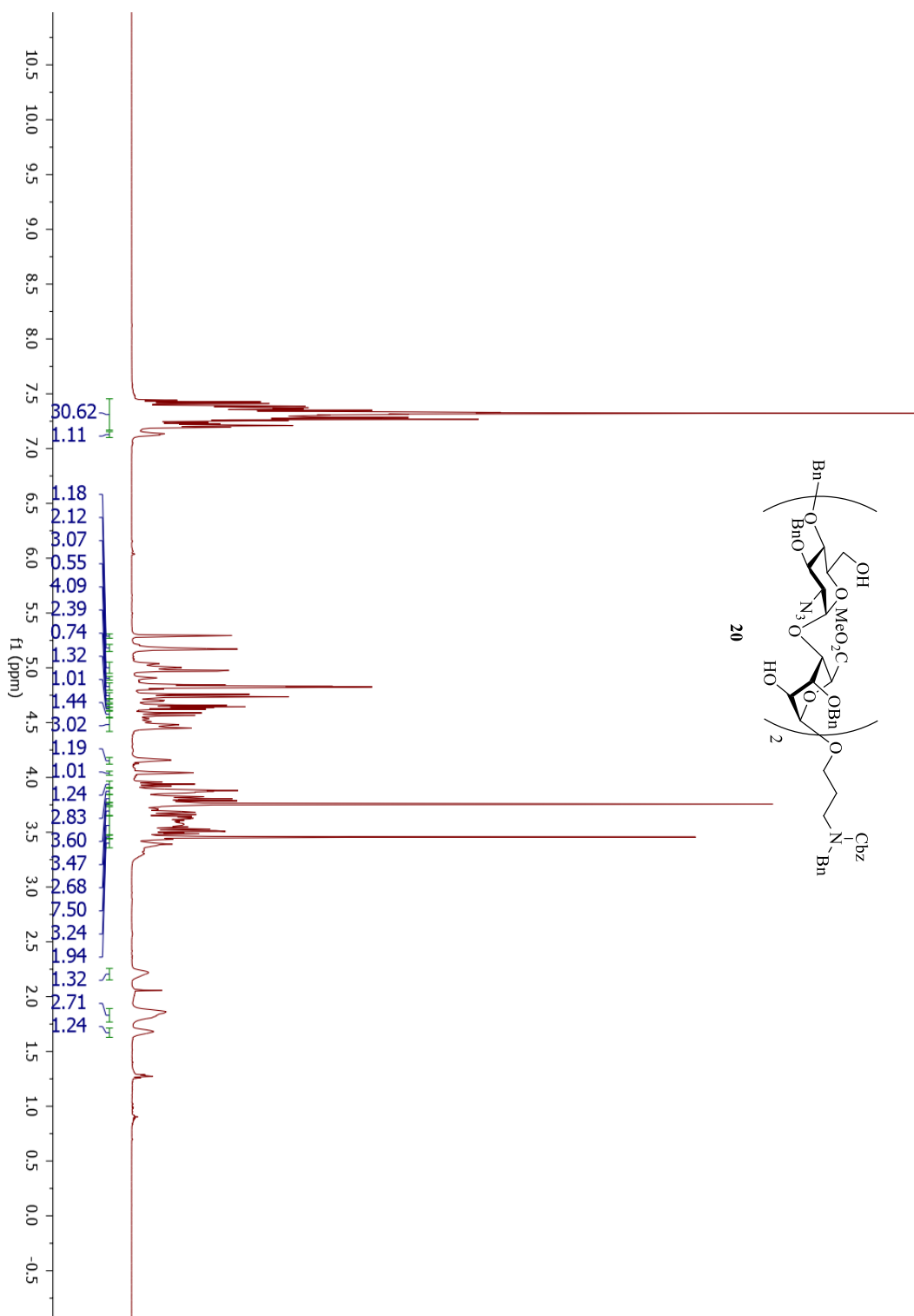
^1H - ^1H gCOSY of **18** (500 MHz CDCl_3)



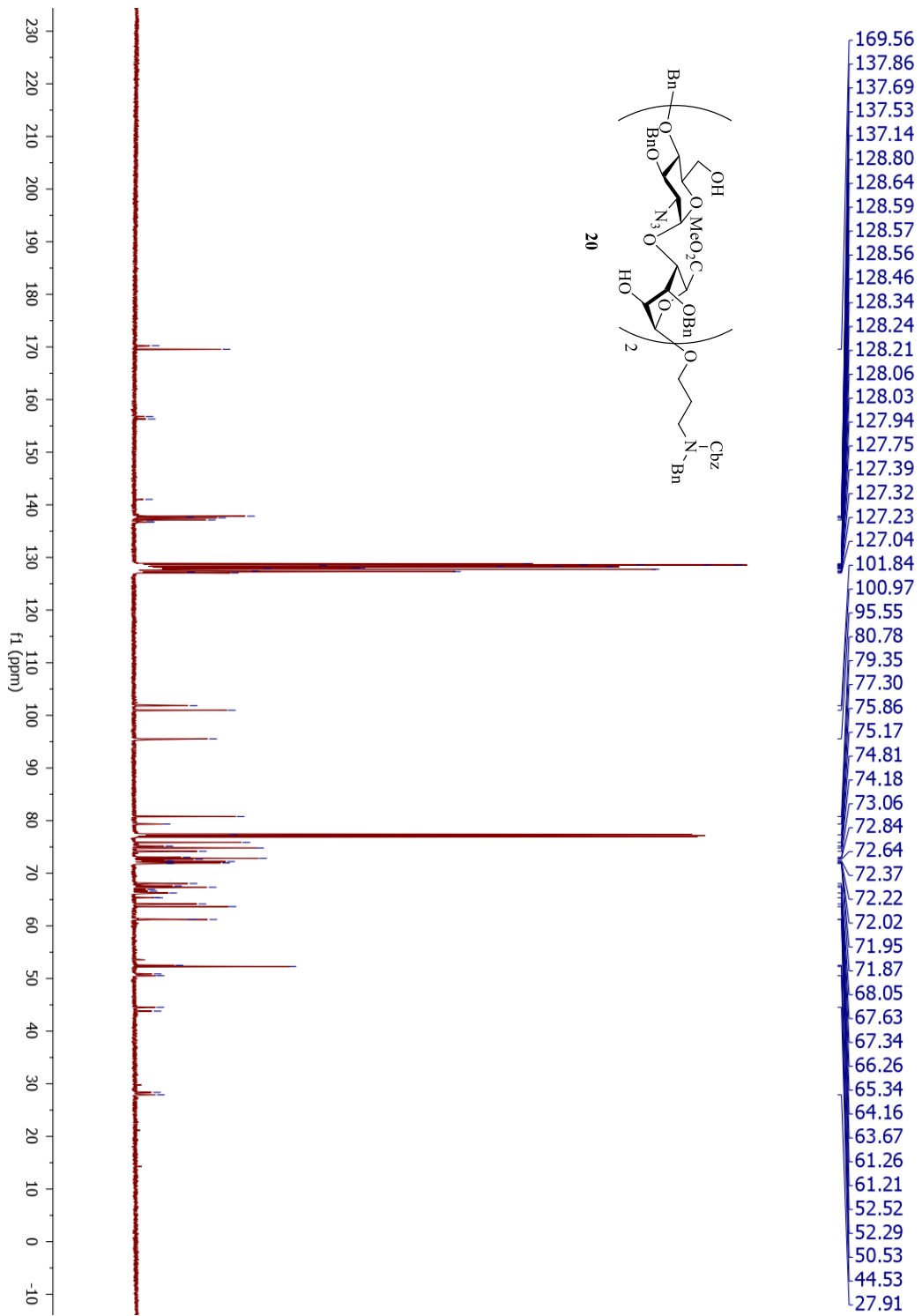
^1H - ^{13}C gHSQCAD of **19** (500 MHz CDCl_3)



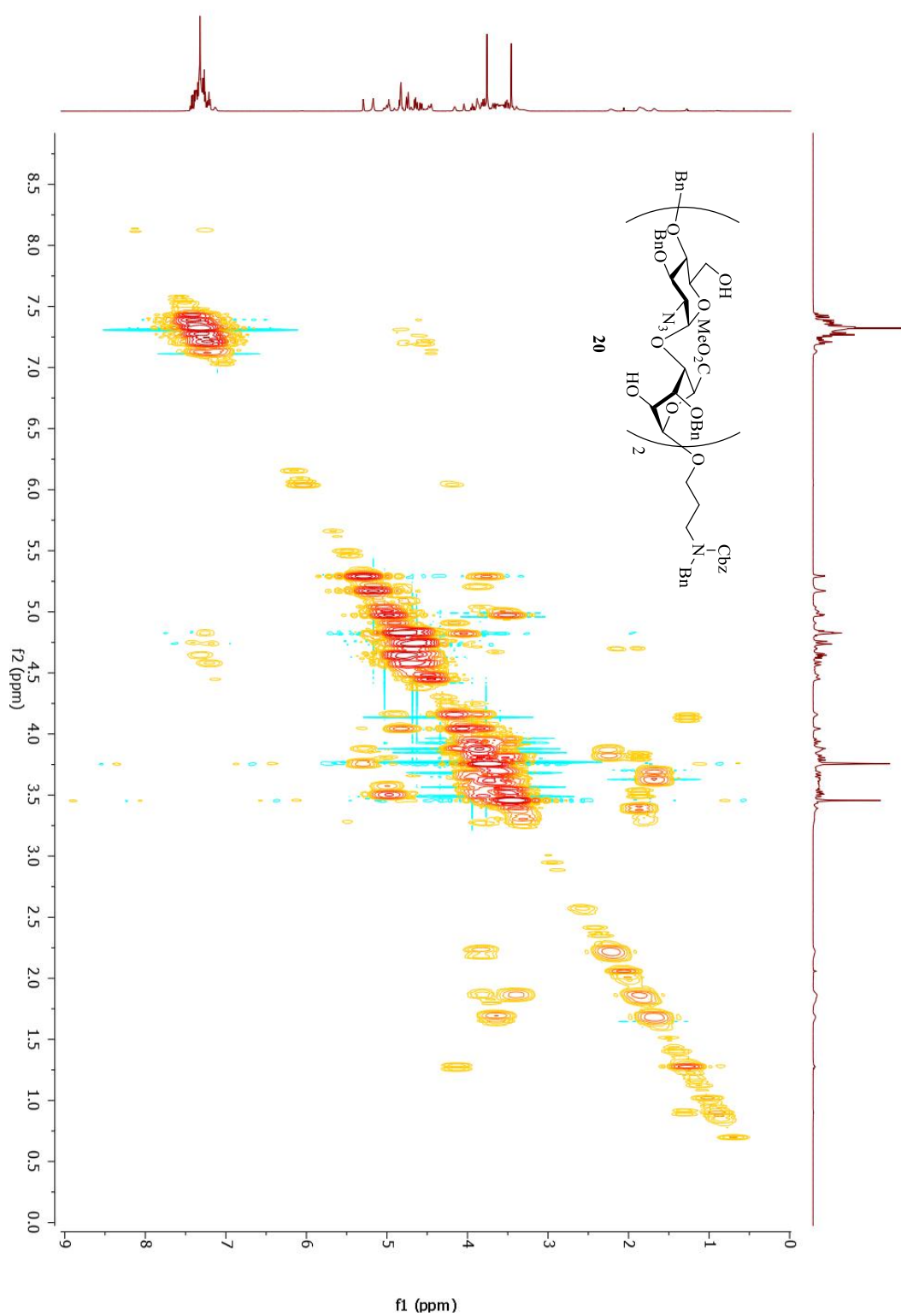
¹H-NMR of **20** (500 MHz CDCl₃)



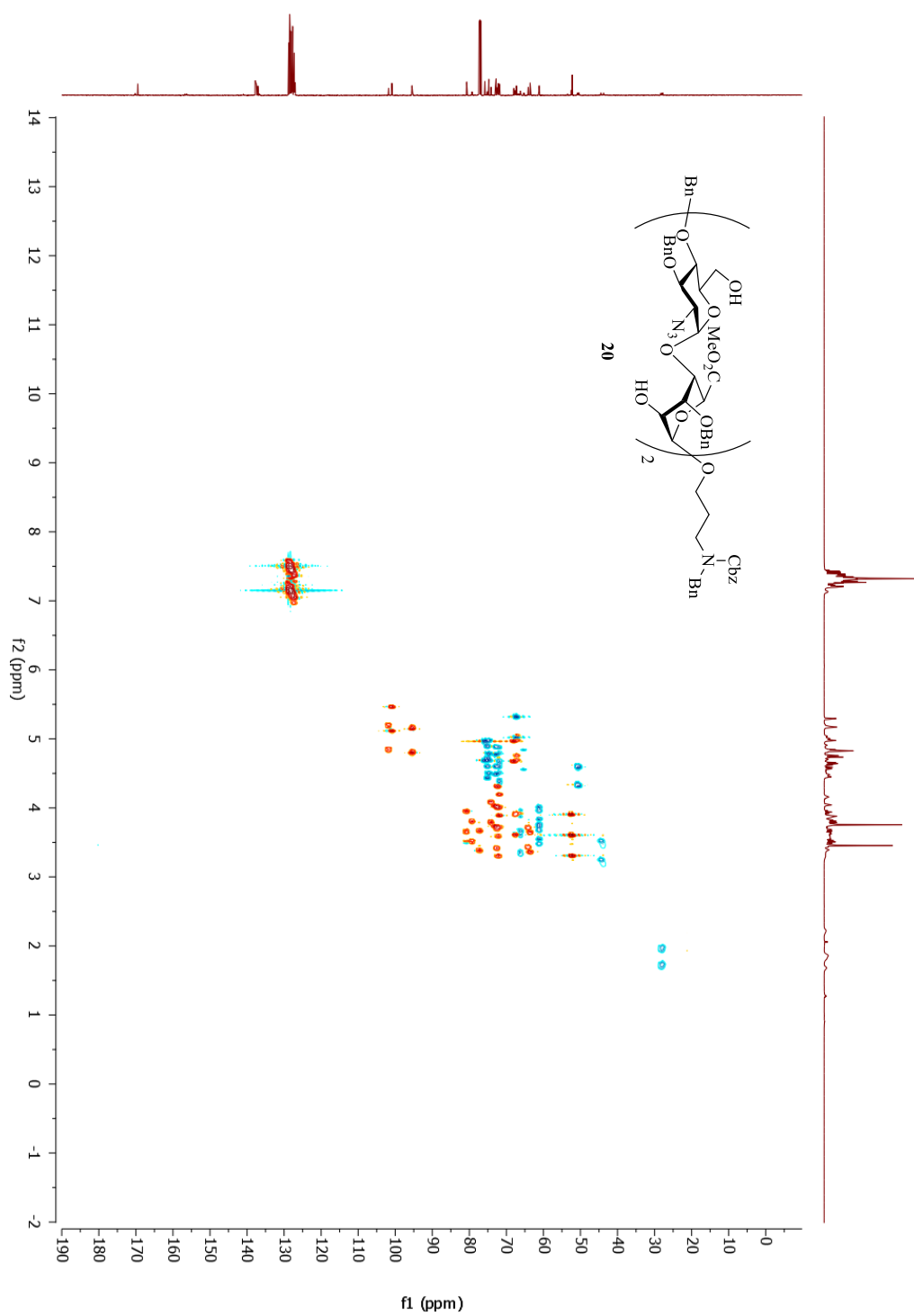
¹³C-NMR of **20** (125 MHz CDCl₃)



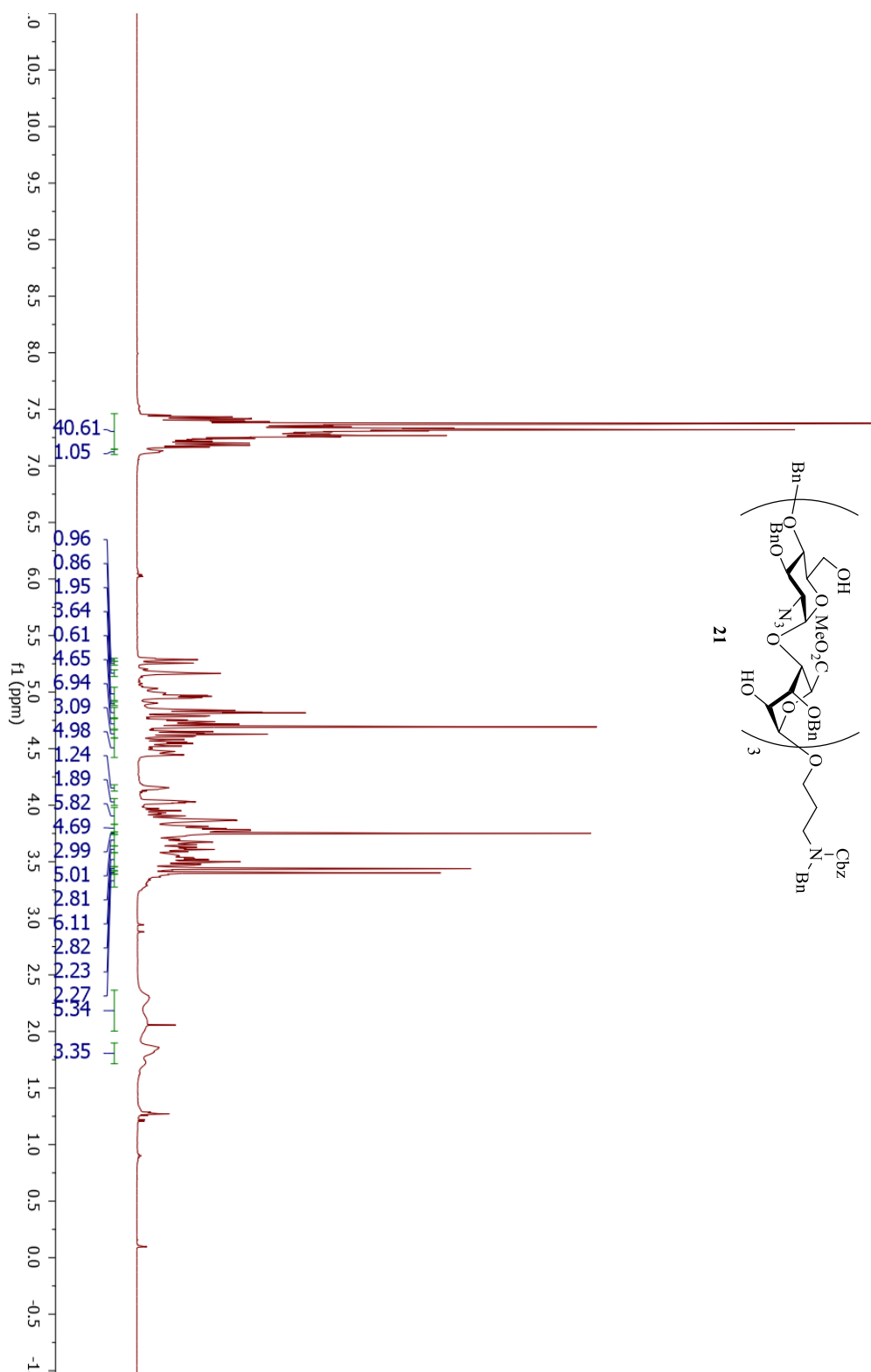
^1H - ^1H gCOSY of **20** (500 MHz CDCl_3)



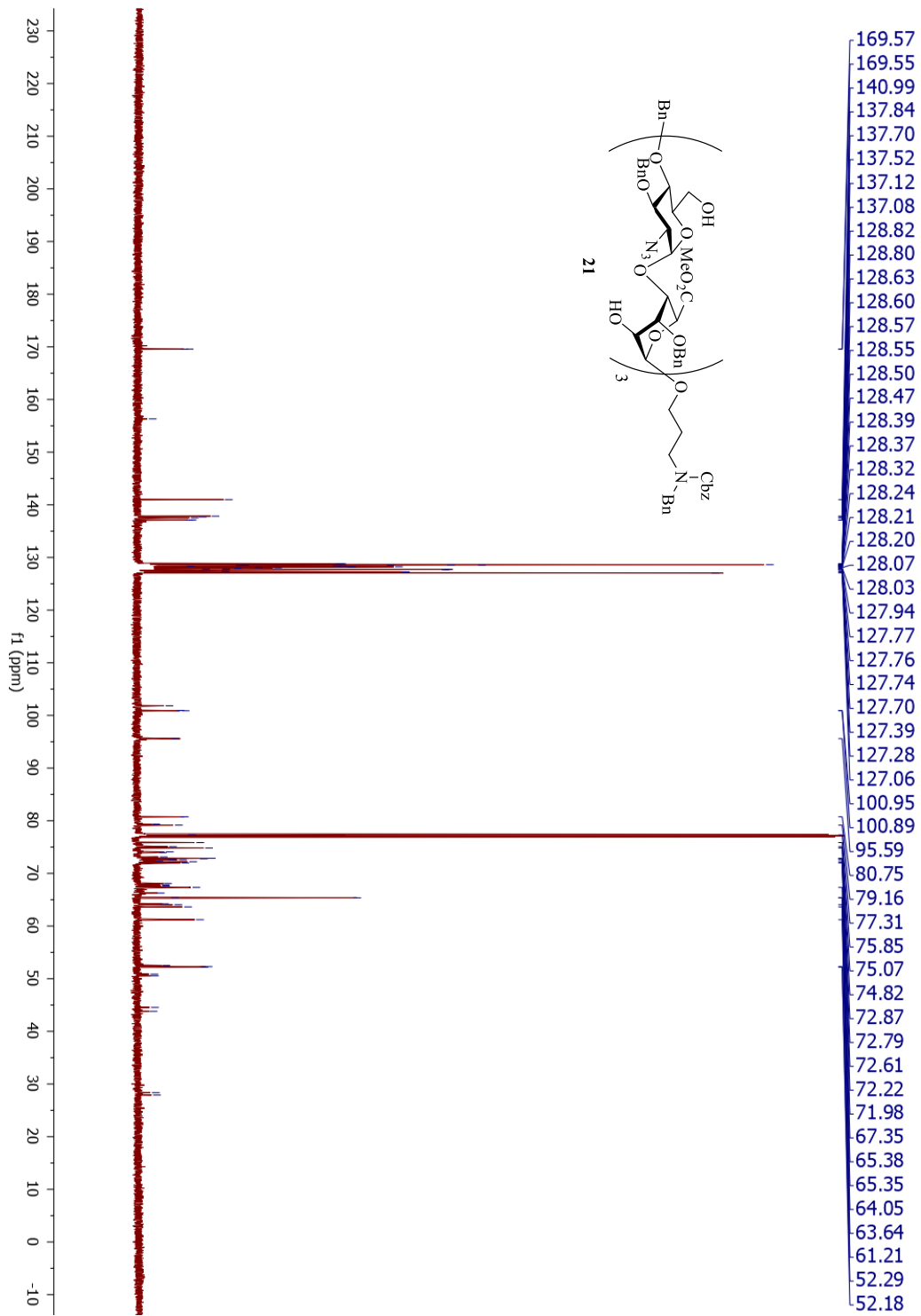
^1H - ^{13}C gHSQCAD of **20** (500 MHz CDCl_3)



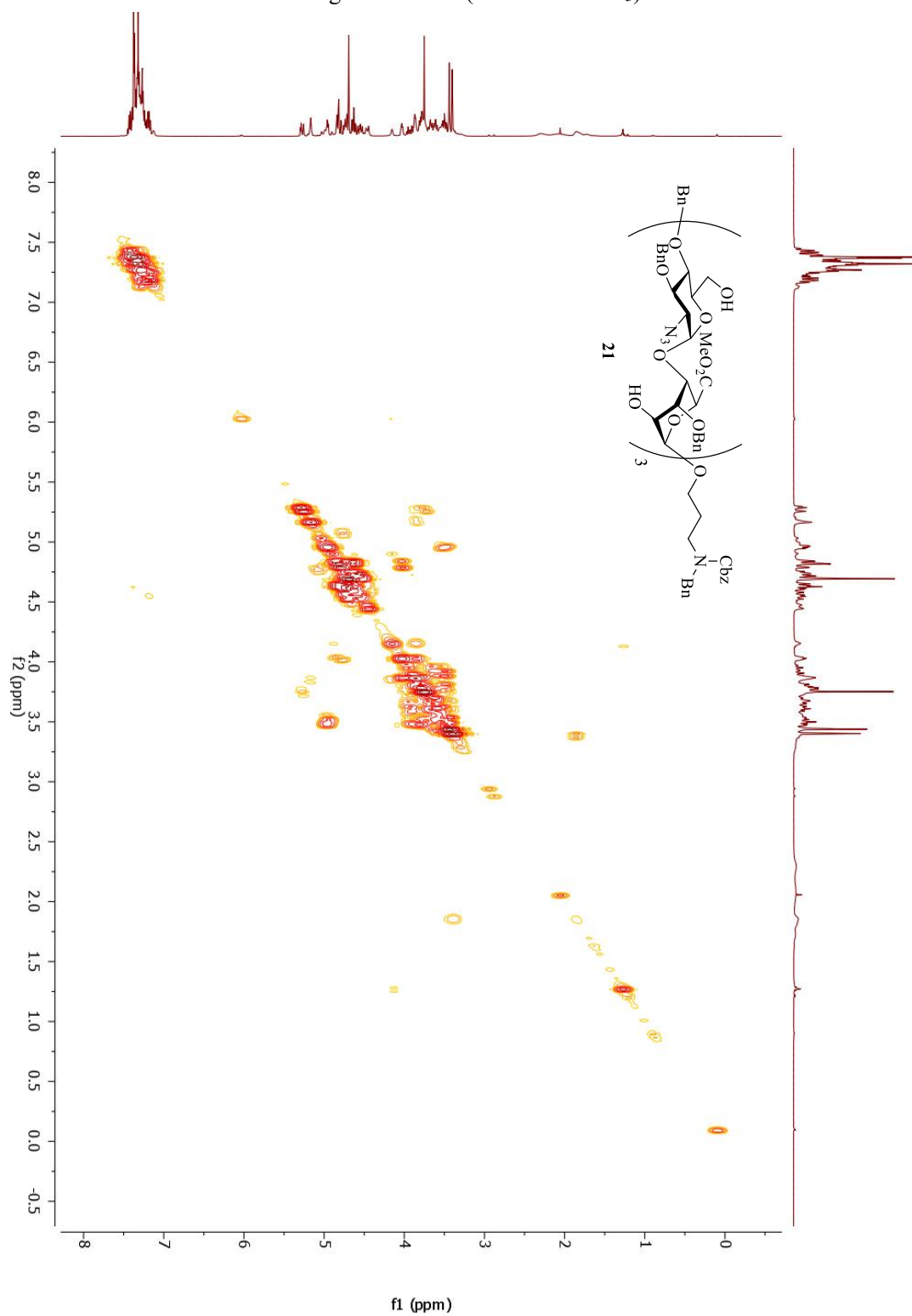
¹H-NMR of **21** (500 MHz CDCl₃)



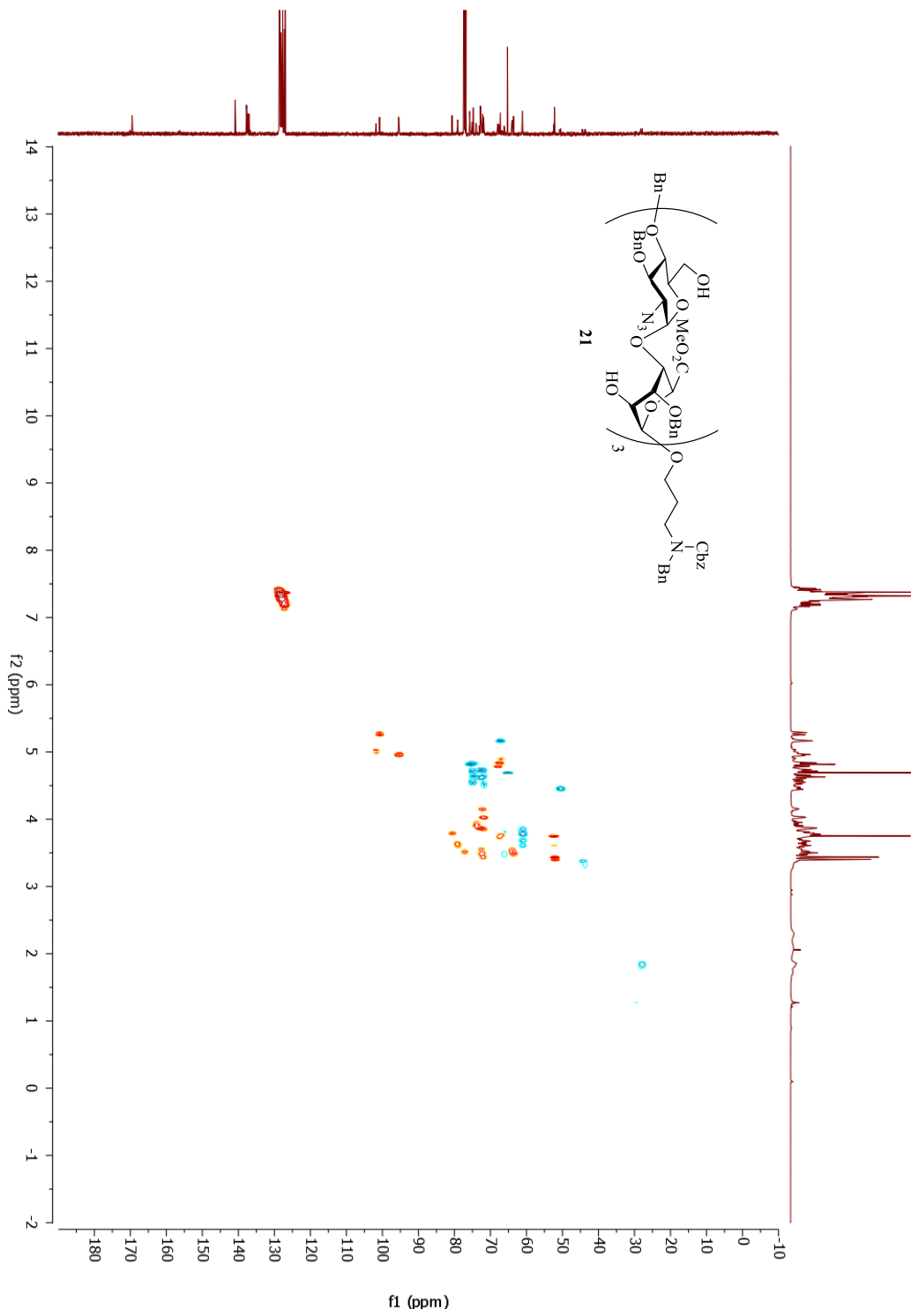
¹³C-NMR of **21** (125 MHz CDCl₃)



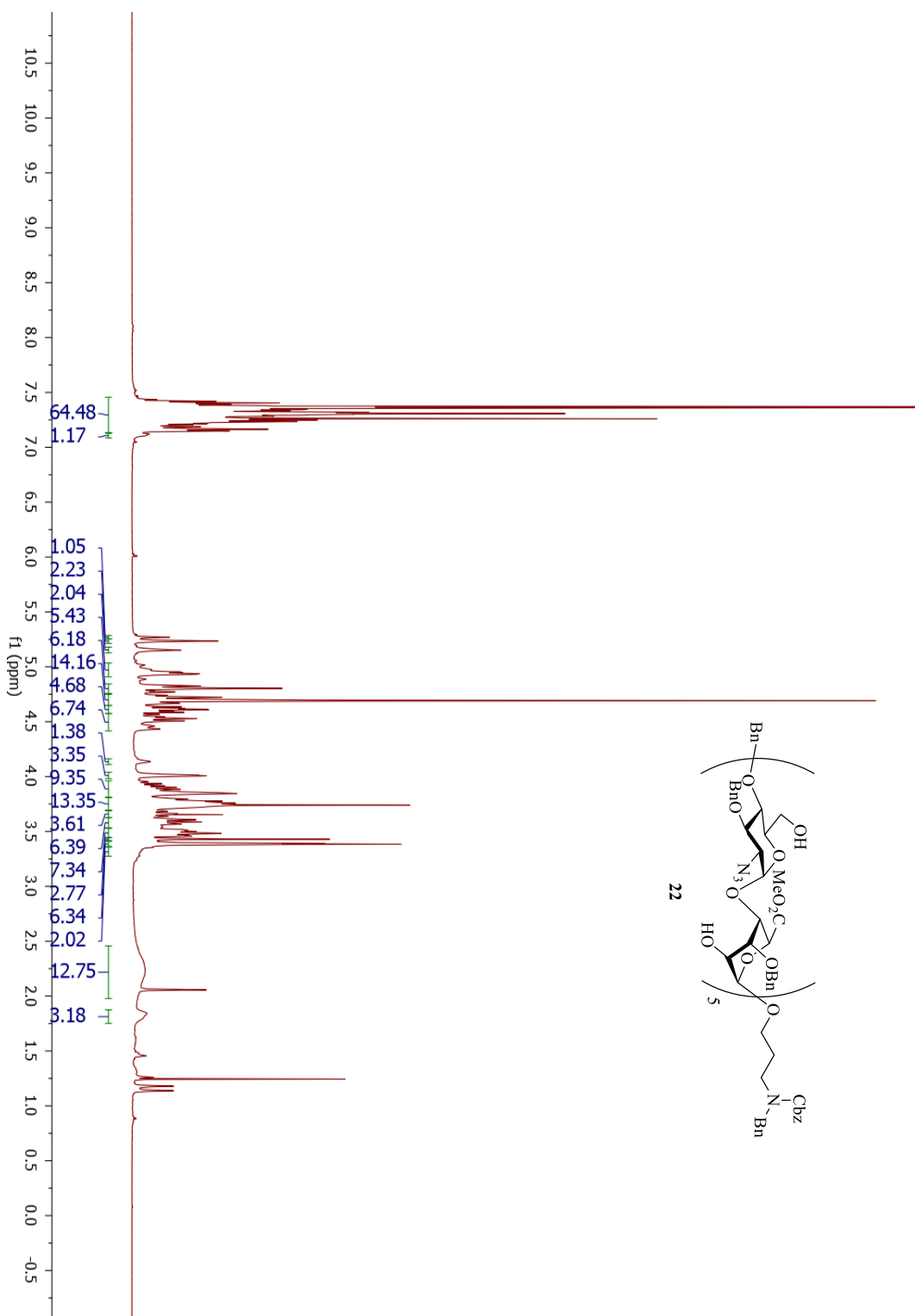
^1H - ^1H gCOSY of **21** (500 MHz CDCl_3)



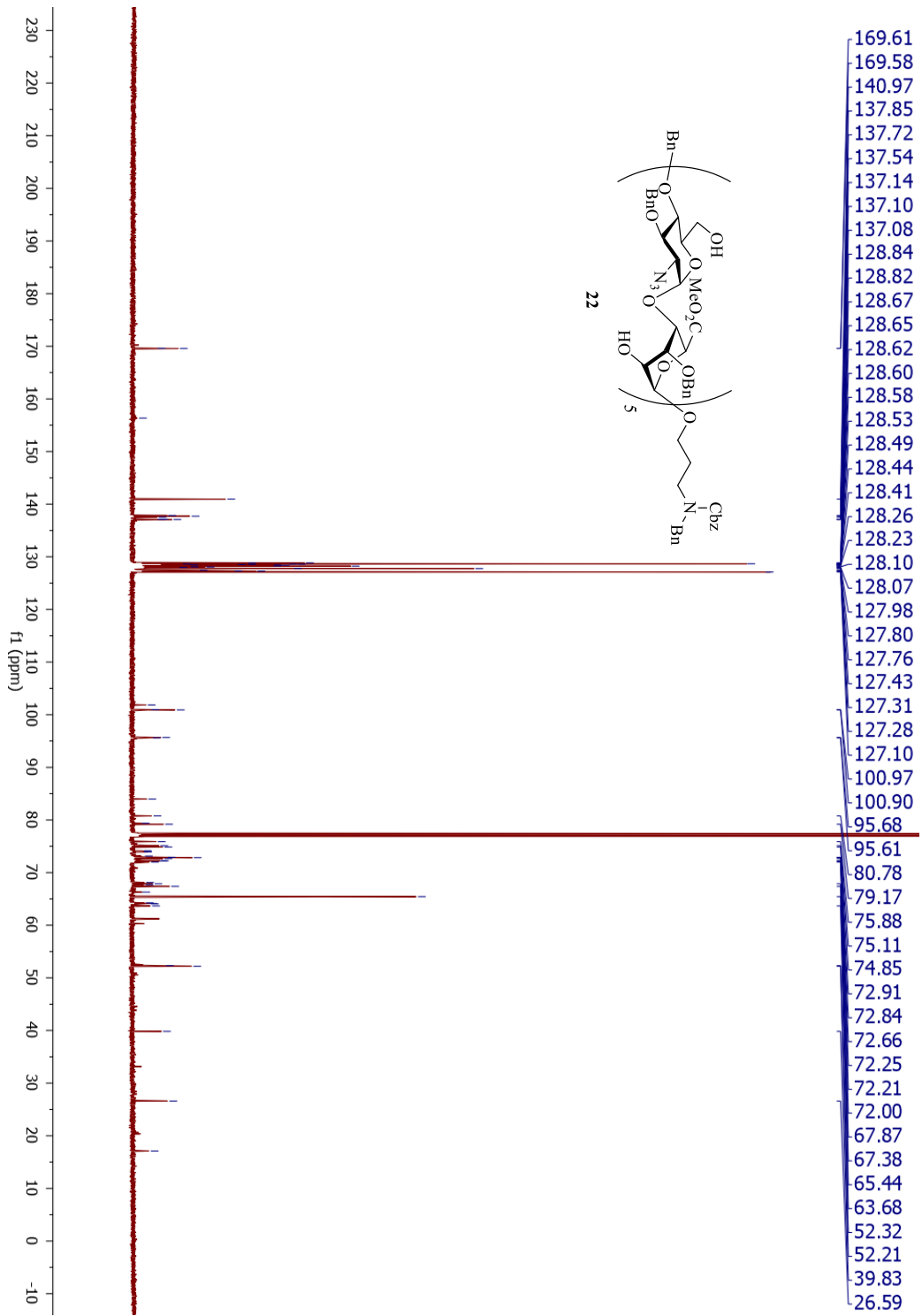
^1H - ^{13}C gHSQCAD of **21** (500 MHz CDCl_3)



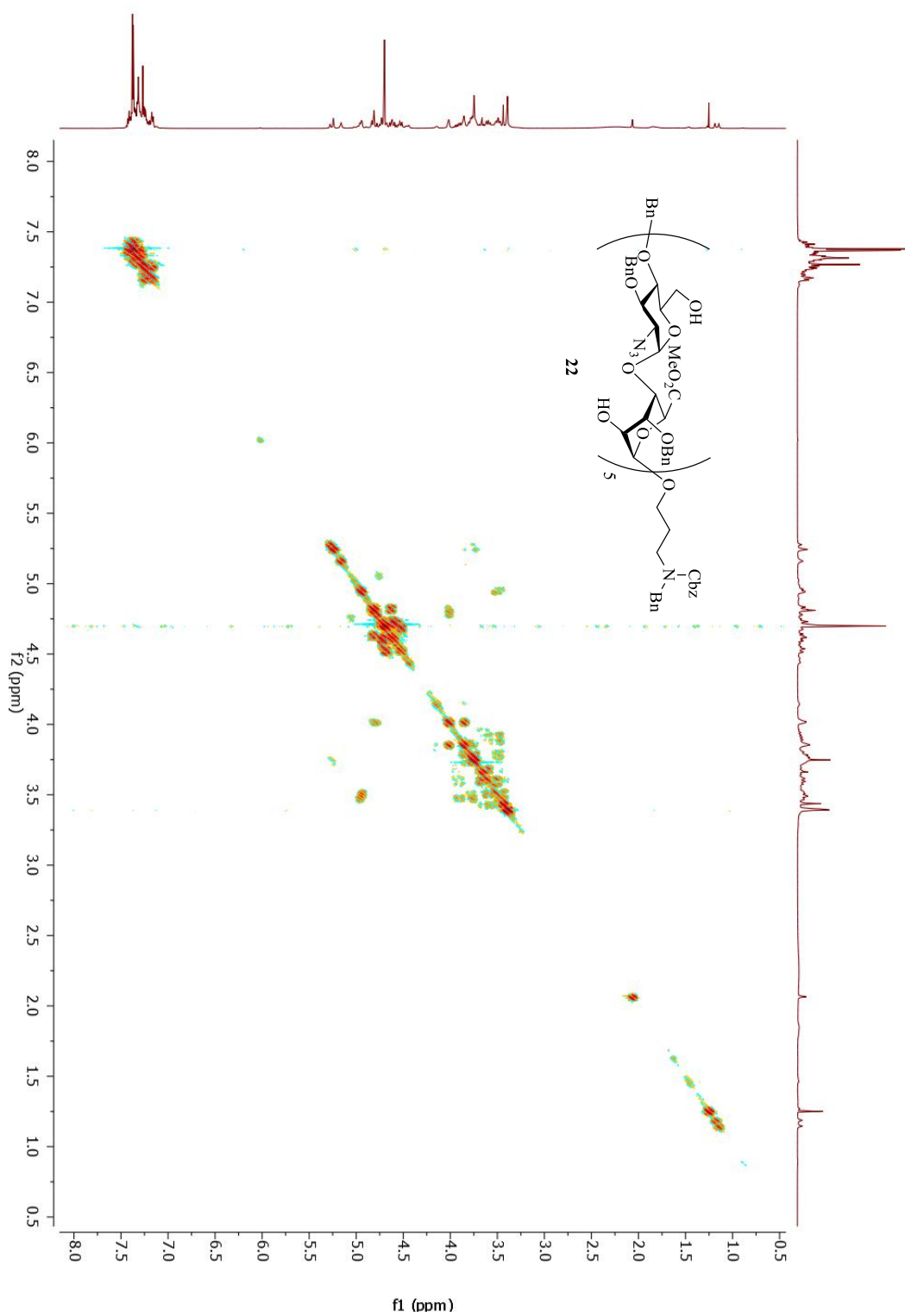
¹H-NMR of **22** (500 MHz CDCl₃)



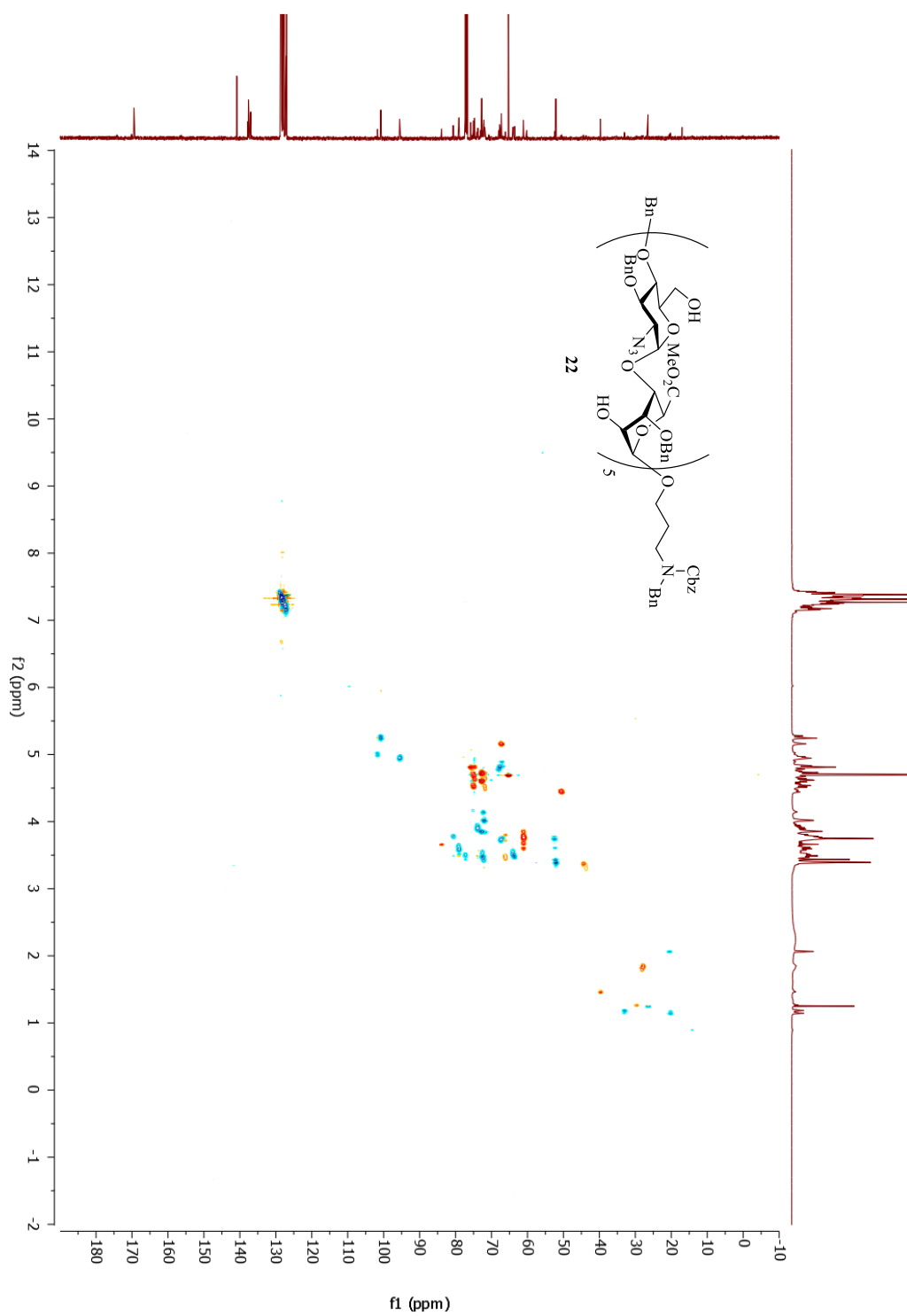
¹³C-NMR of **22** (125 MHz CDCl₃)



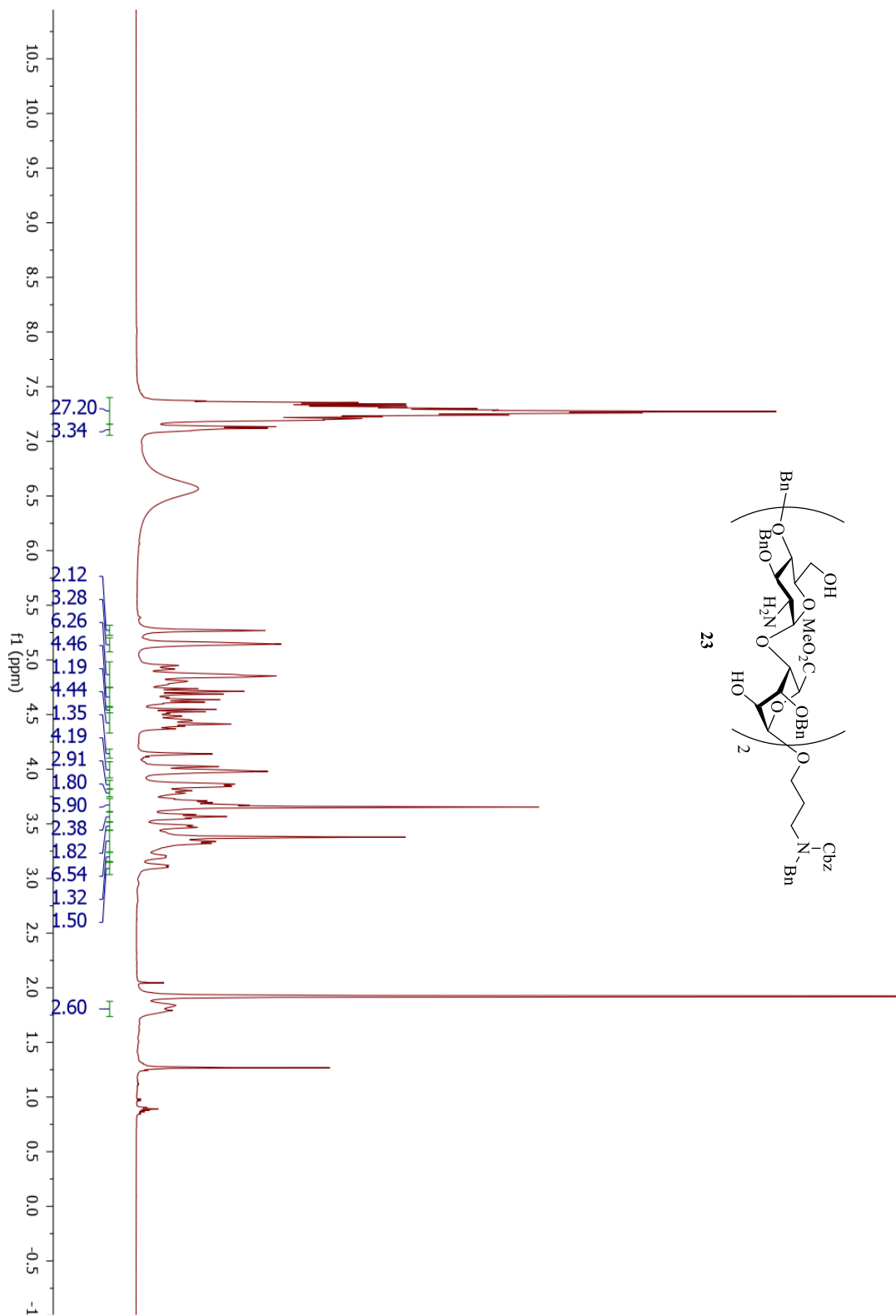
^1H - ^1H gCOSY of **22** (500 MHz CDCl_3)



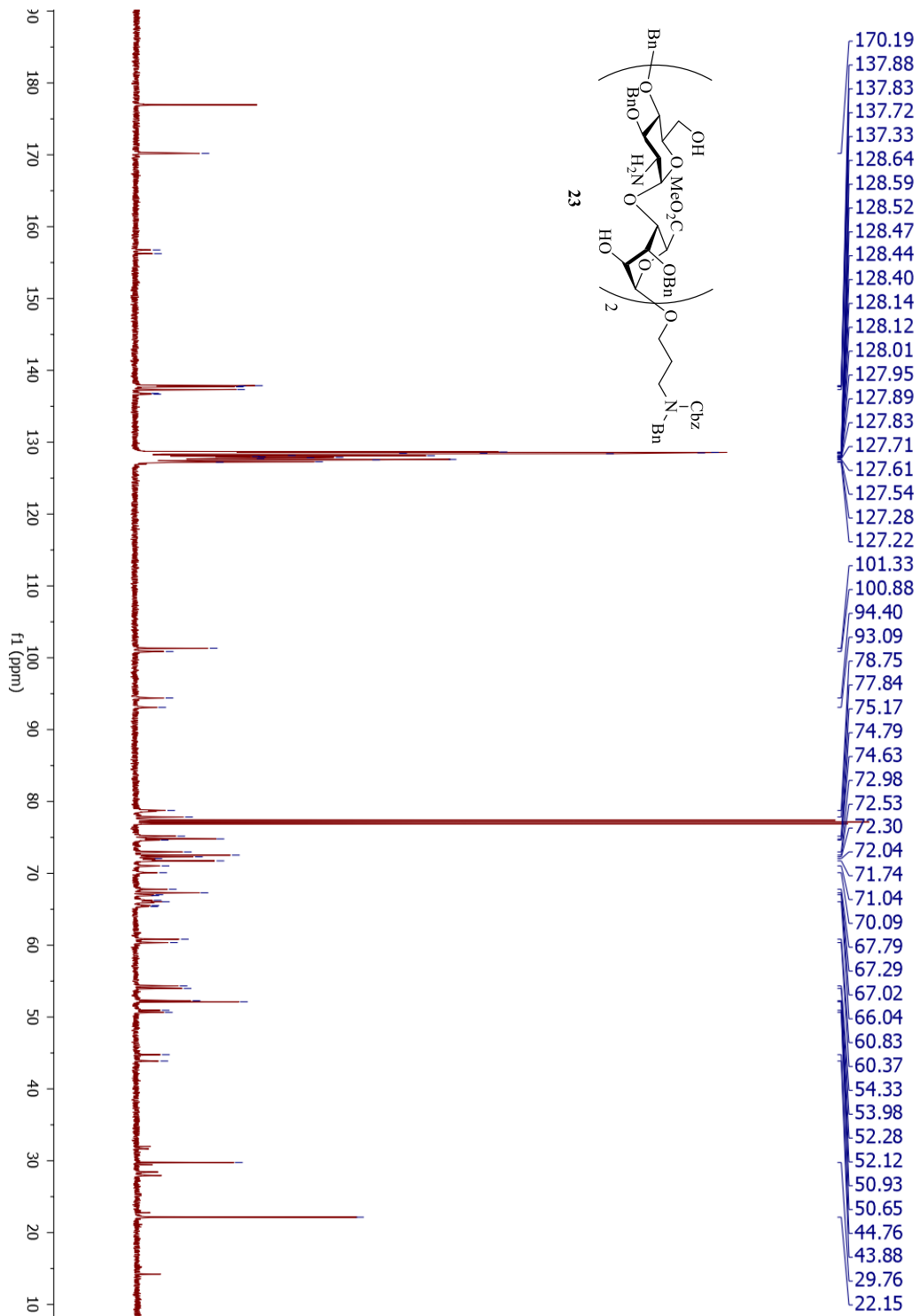
^1H - ^{13}C gHSQCAD of **22** (500 MHz CDCl_3)



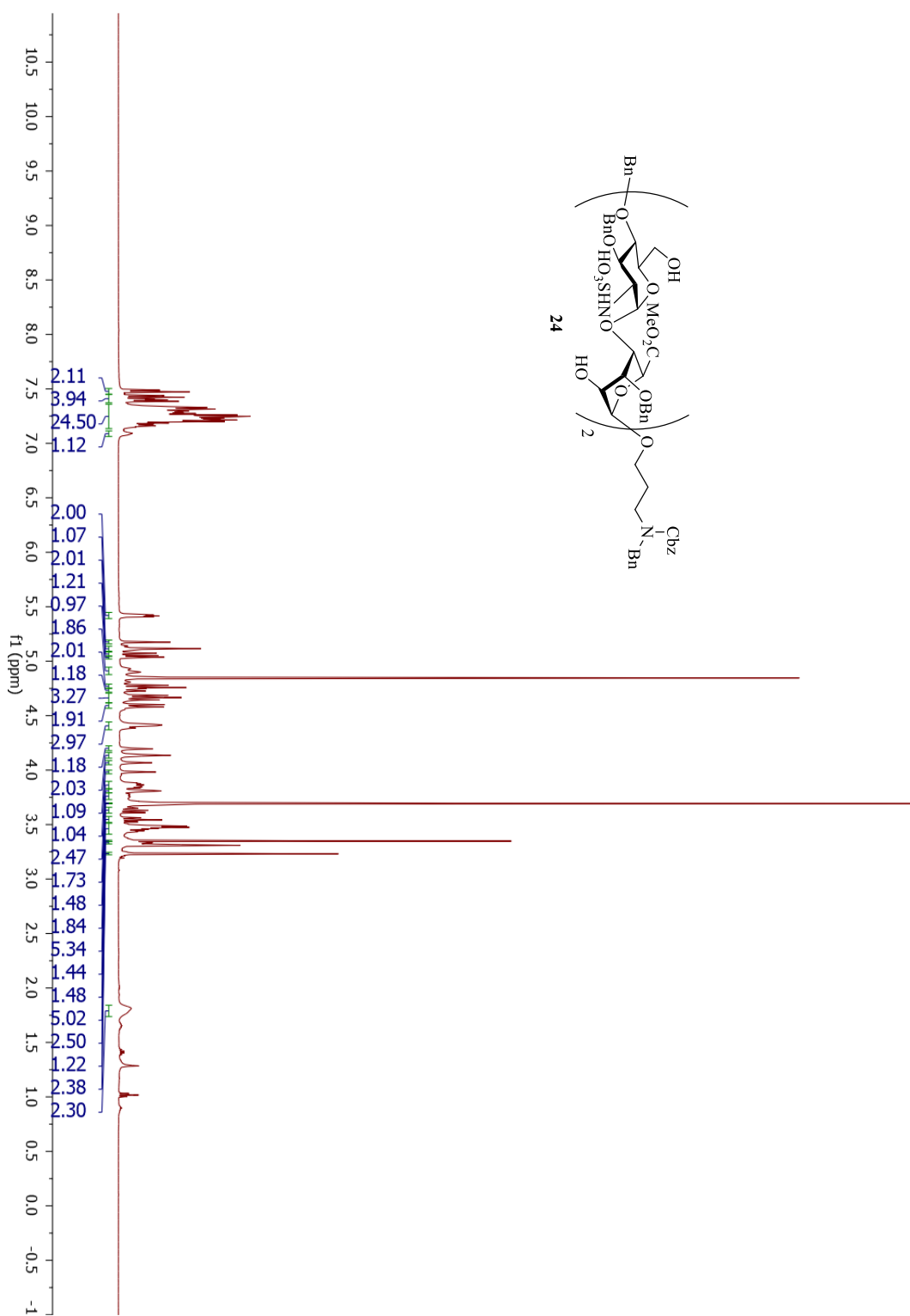
¹H-NMR of **23** (500 MHz CDCl₃)



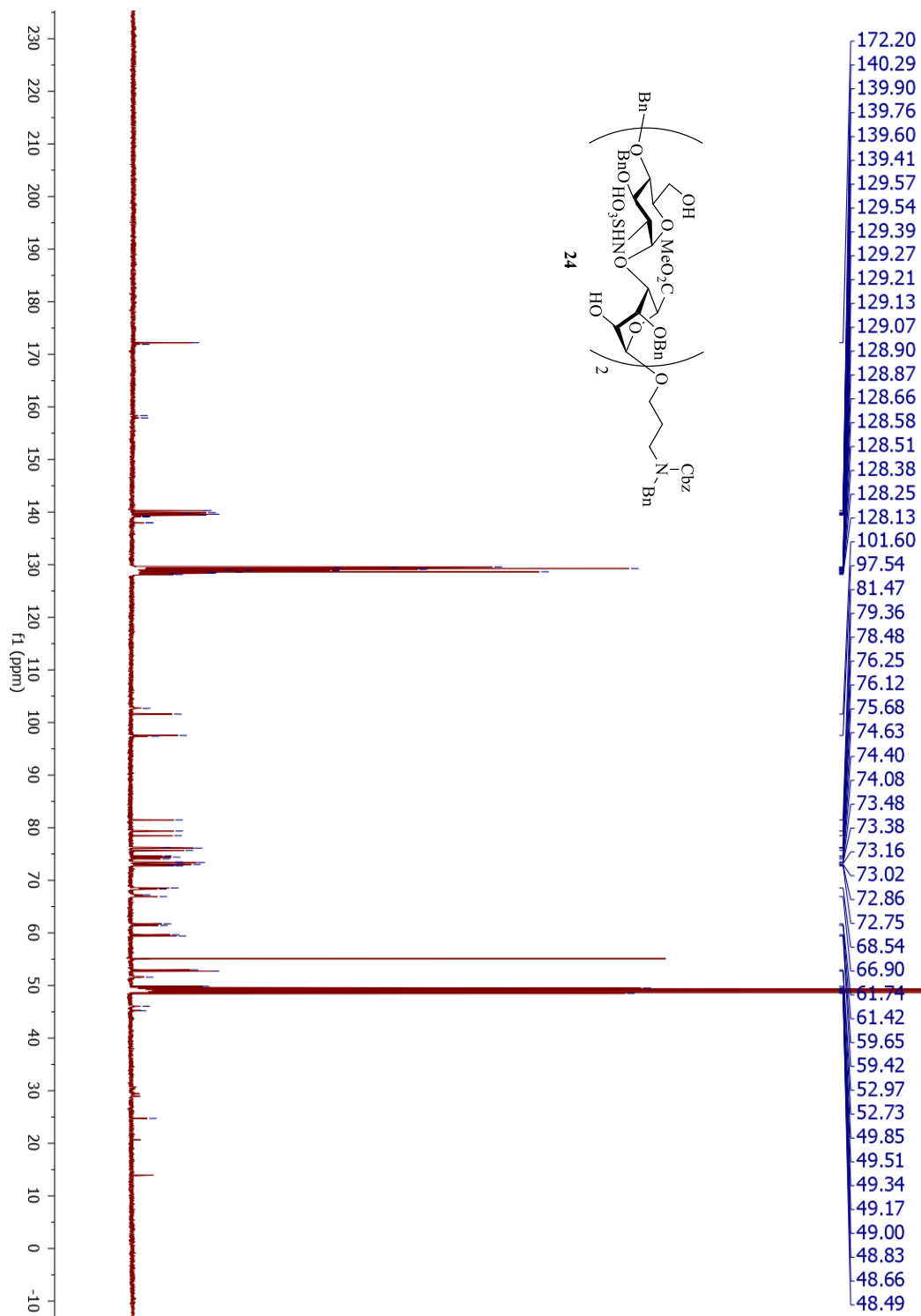
¹³C-NMR of **23** (125 MHz CDCl₃)



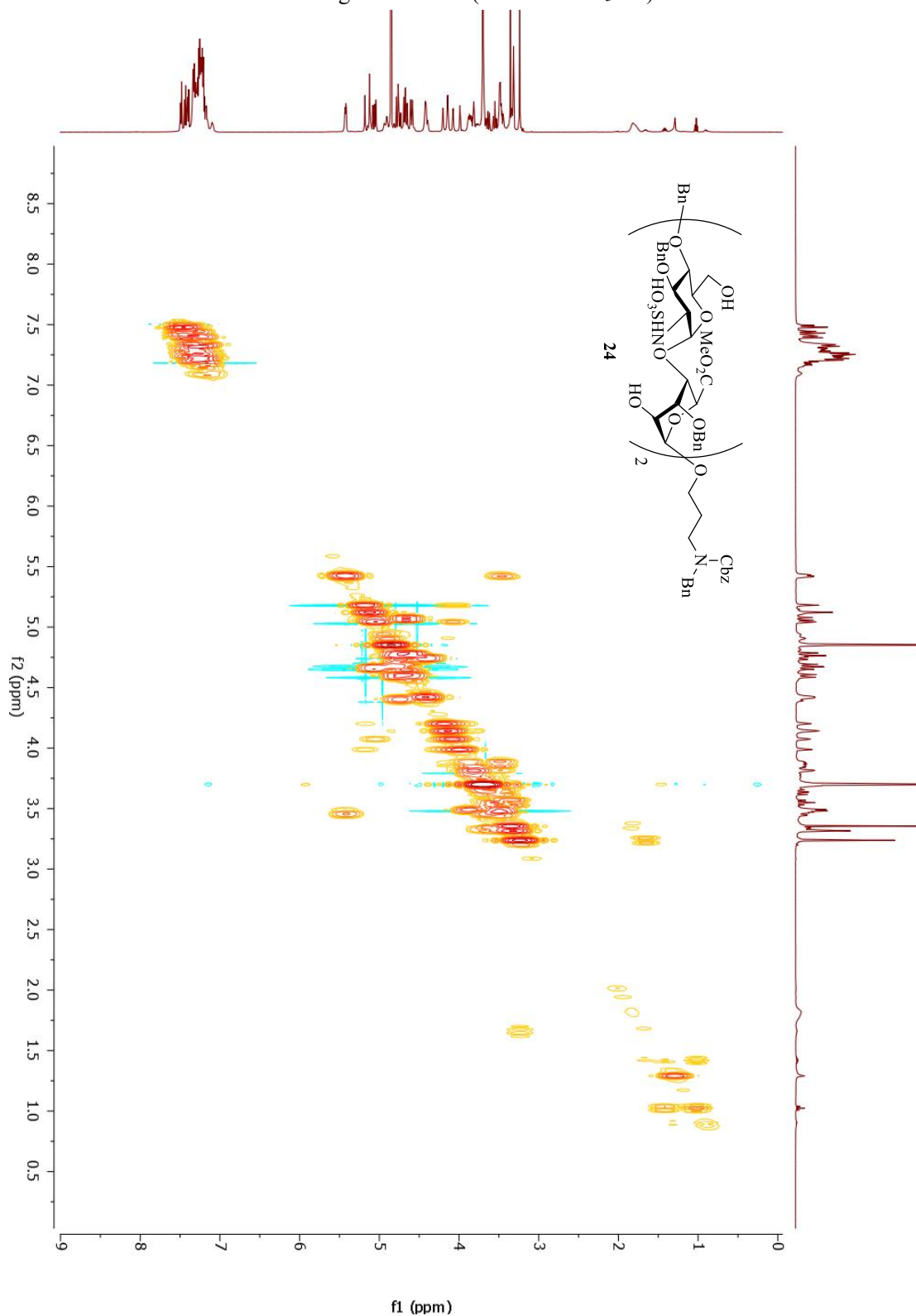
¹H-NMR of **24** (500 MHz CD₃OD)



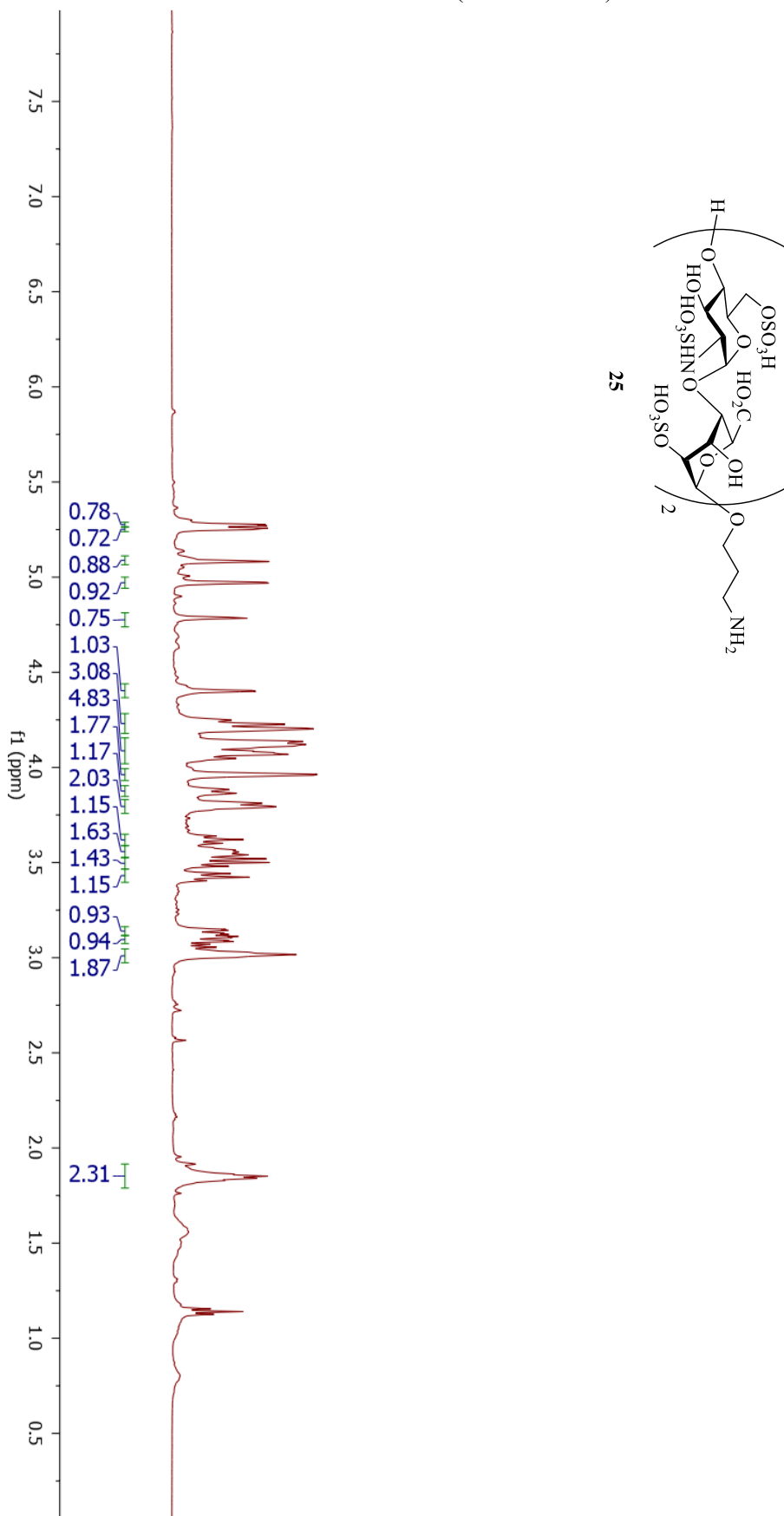
¹³C-NMR of **24** (125 MHz CD₃OD)



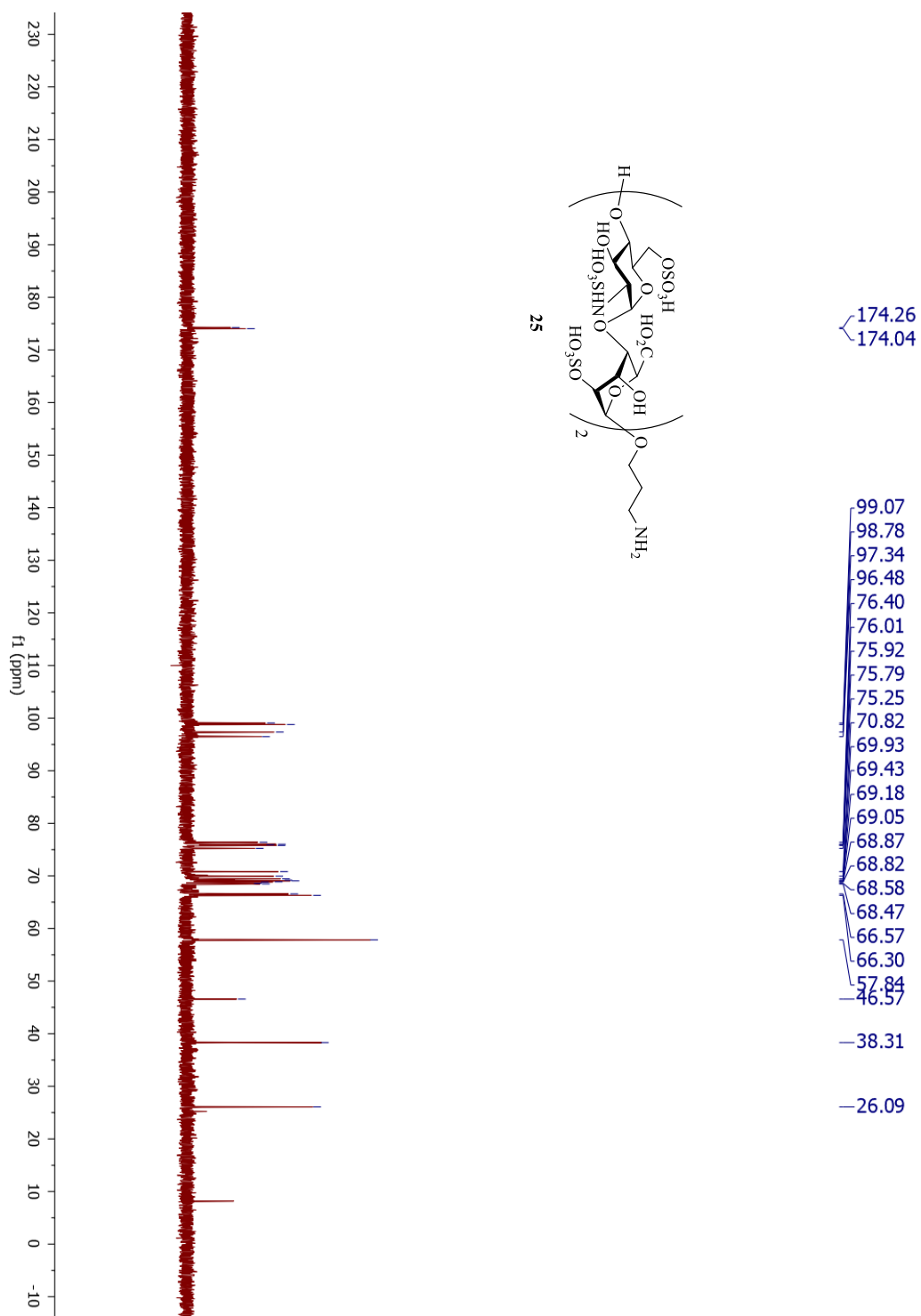
^1H - ^1H gCOSY of **24** (500 MHz CD_3OD)



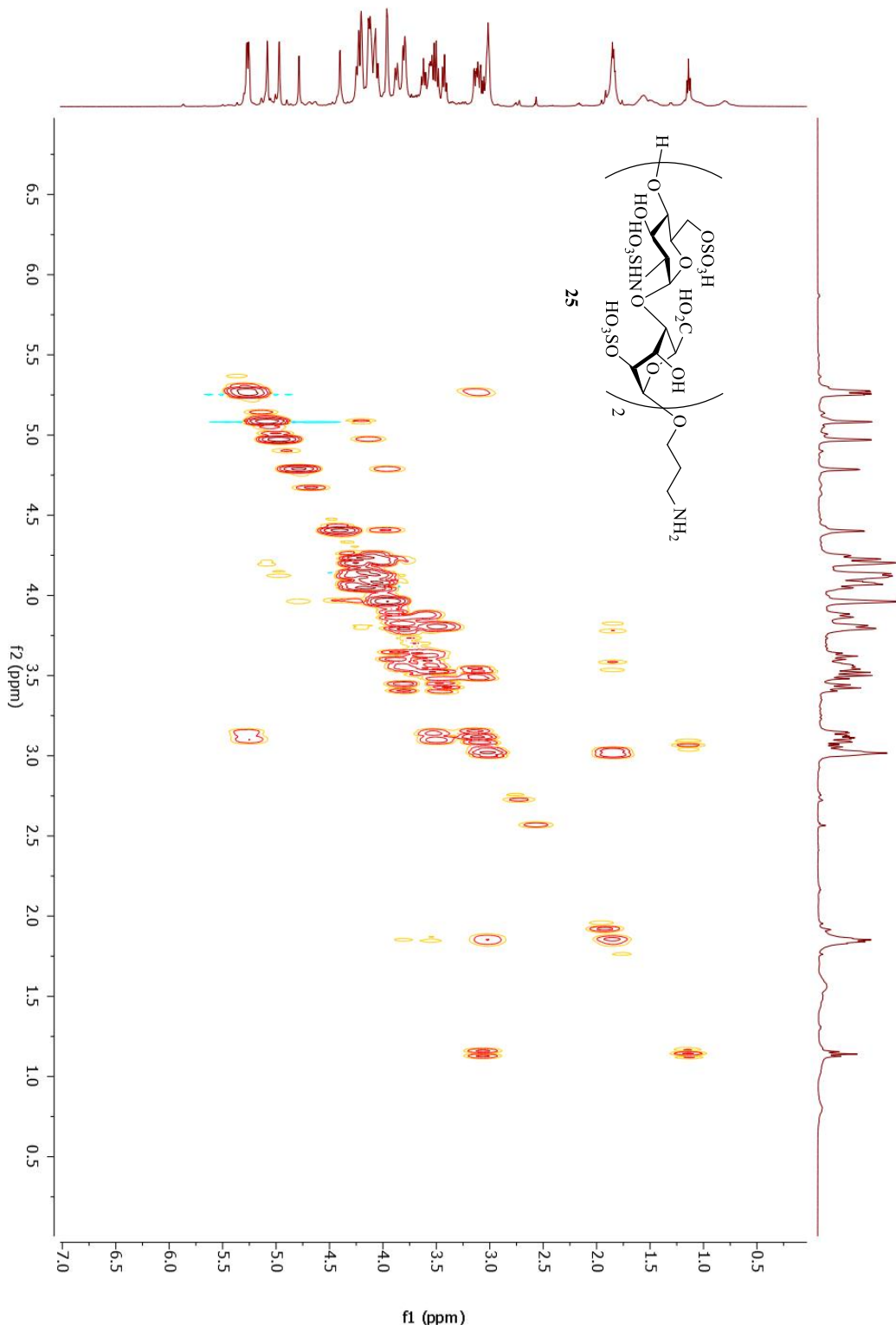
¹H-NMR of **25** (500 MHz D₂O)



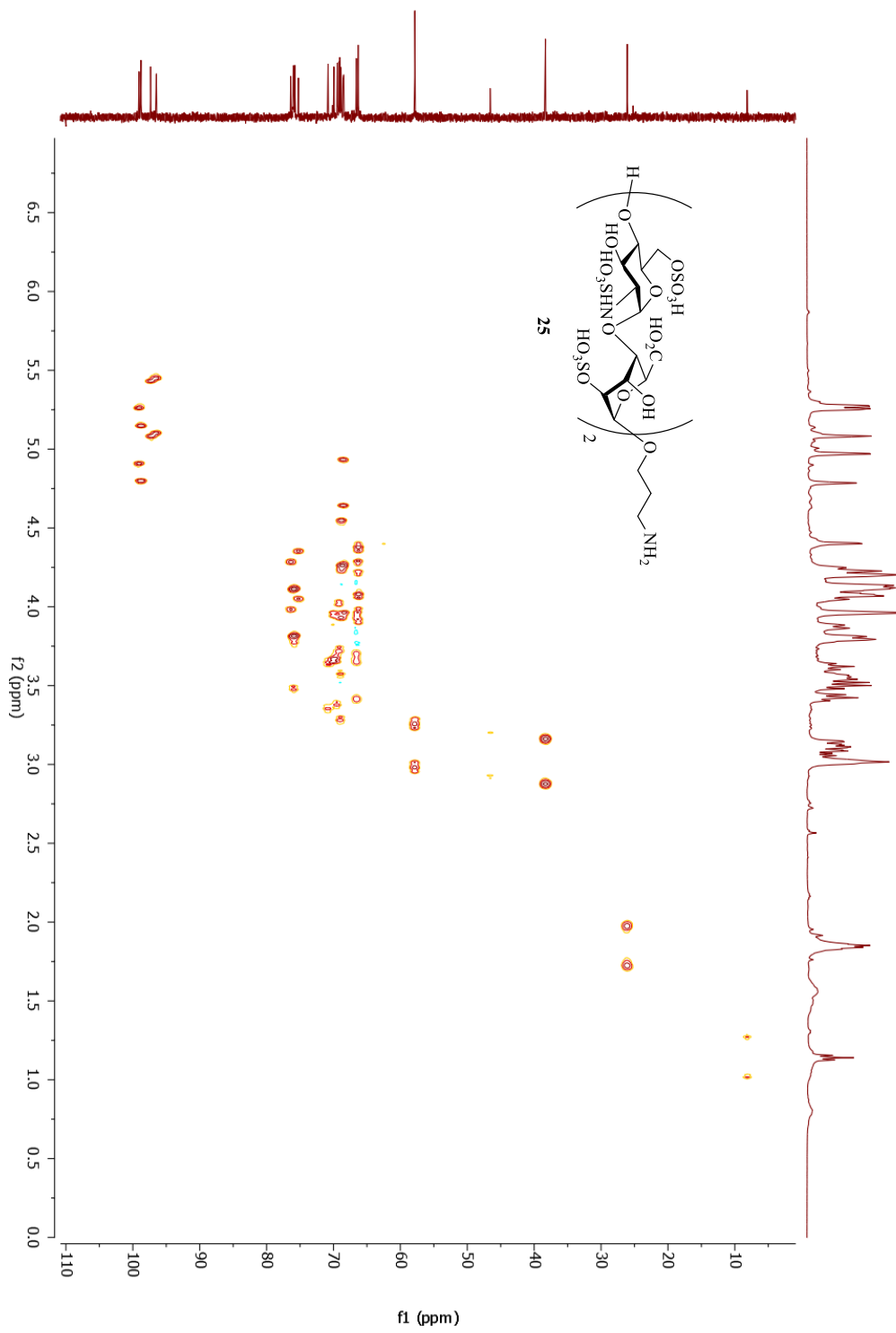
^{13}C -NMR of **25** (125 MHz D_2O)



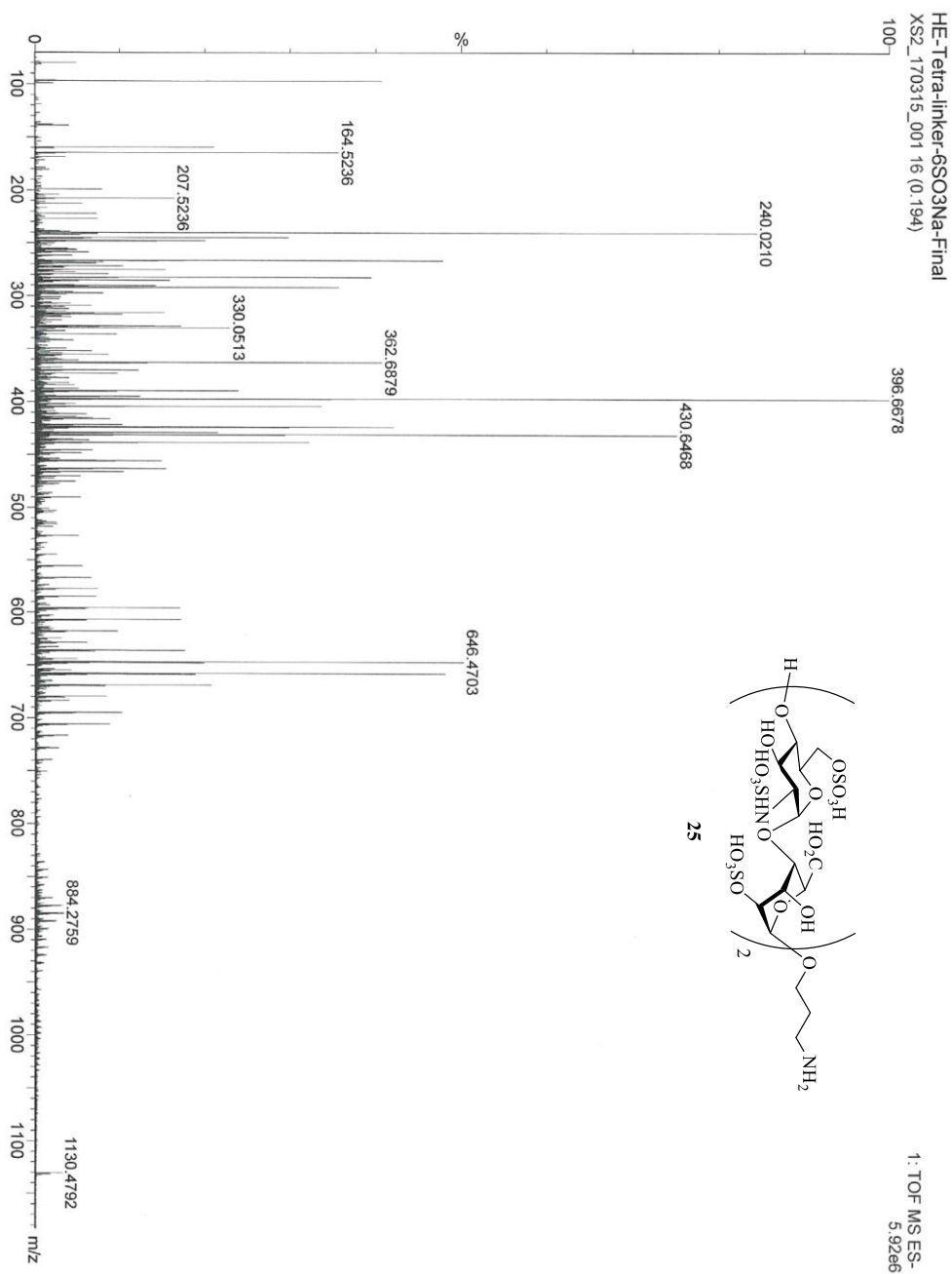
^1H - ^1H gCOSY of **25** (500 MHz D_2O)



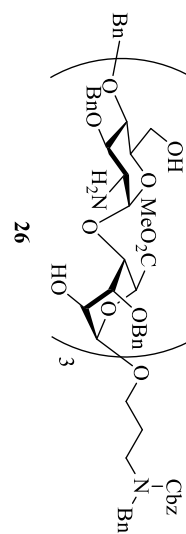
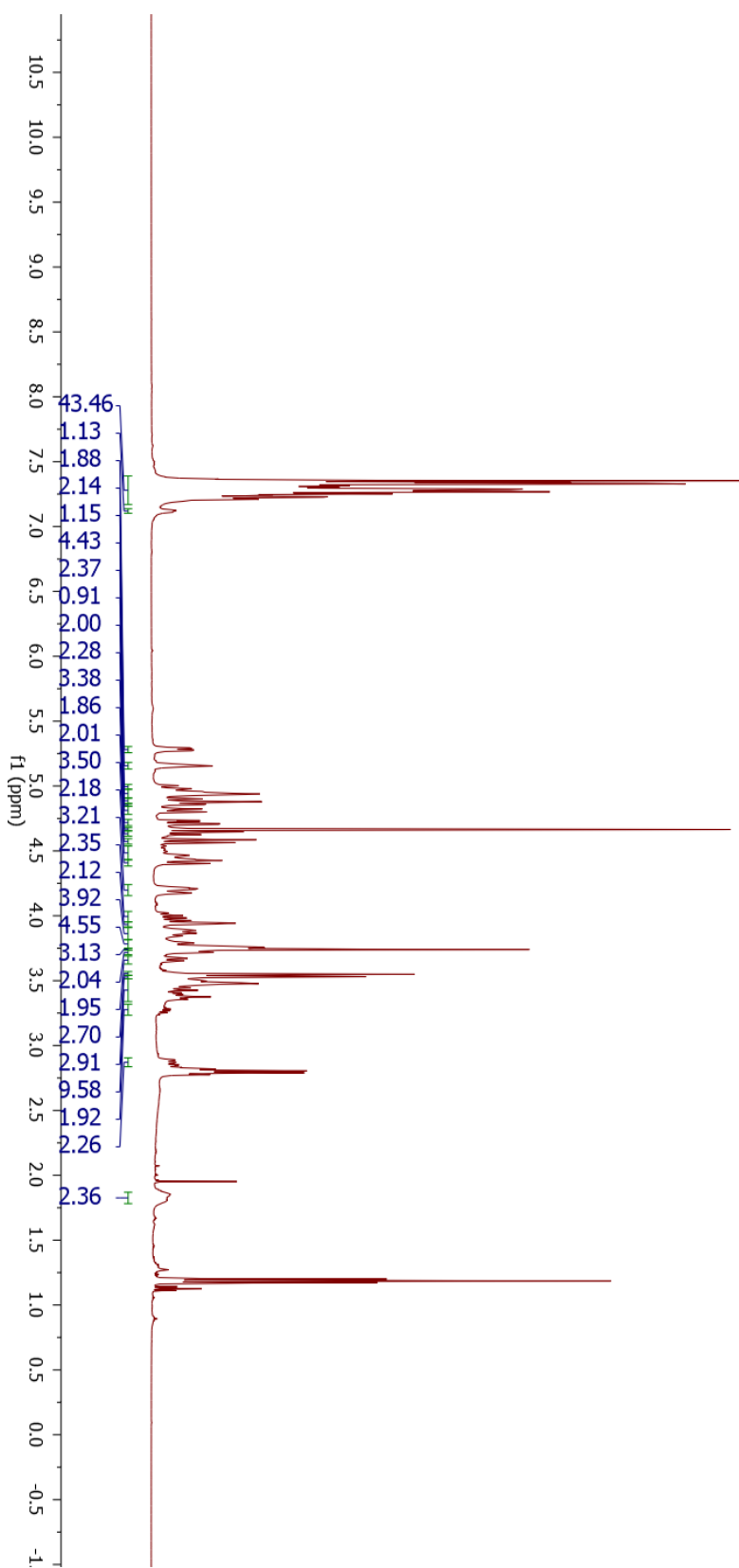
^1H - ^{13}C gHSQCAD of **25** (500 MHz D_2O)



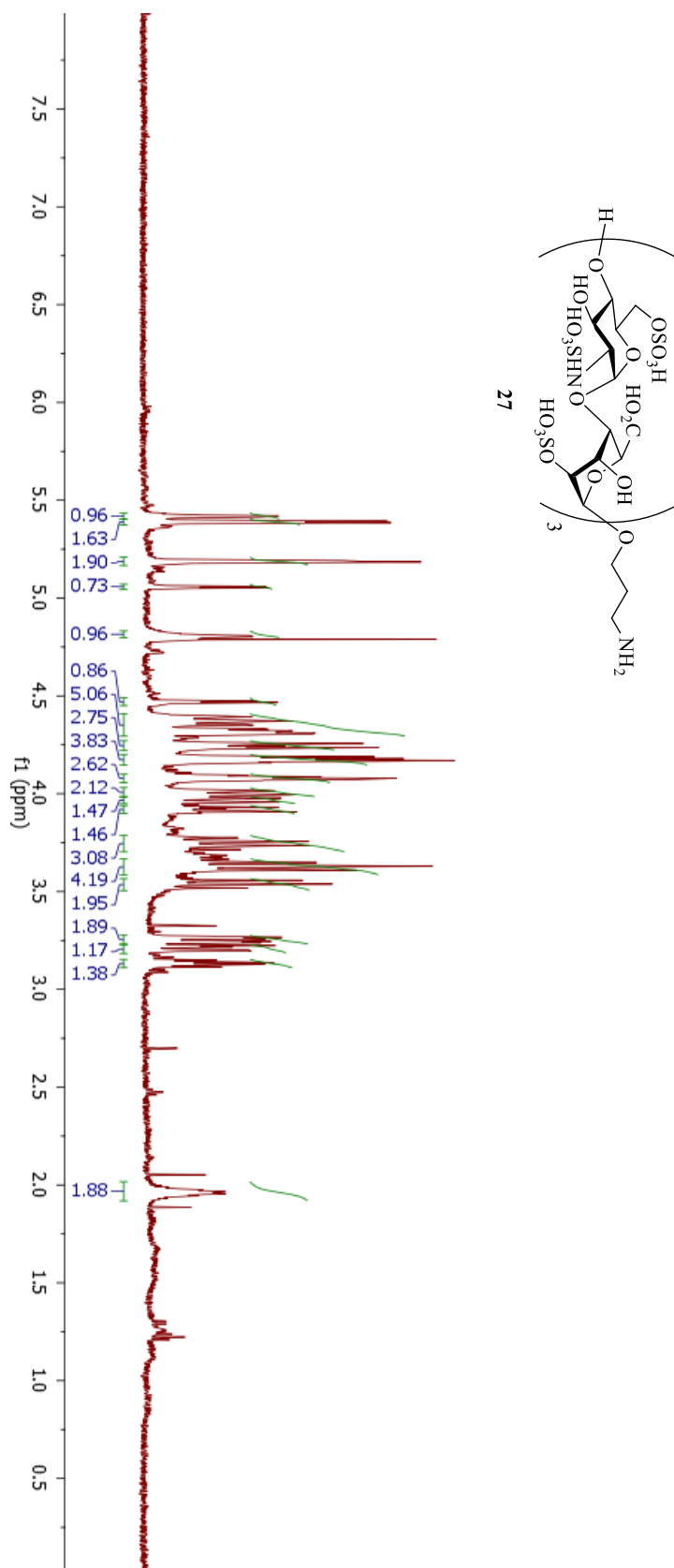
ESI-MS of 25



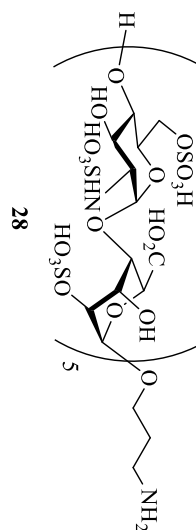
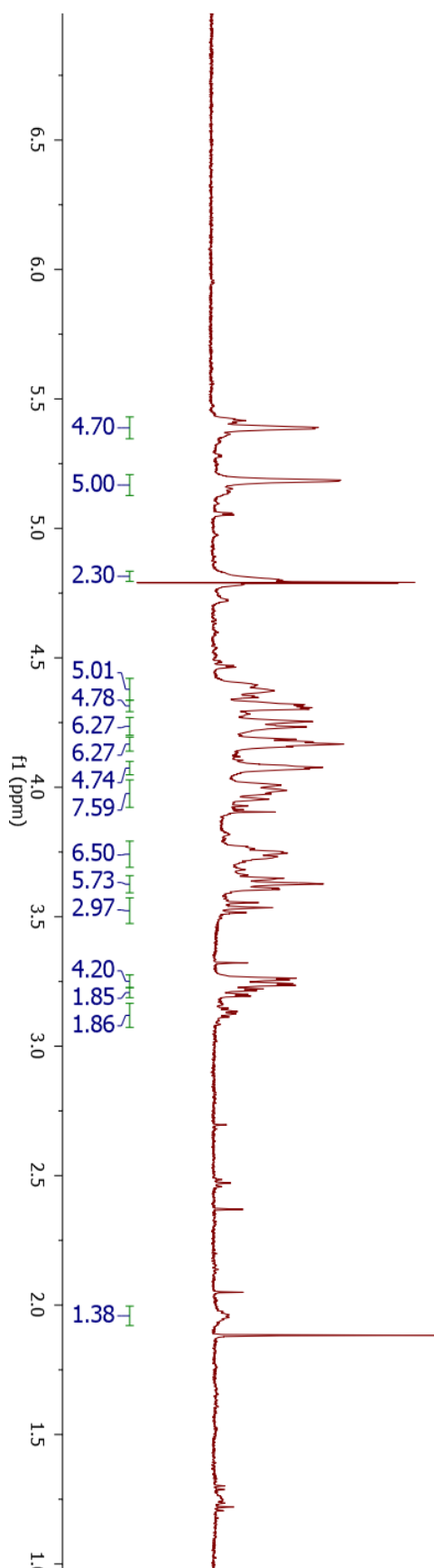
¹H-NMR of **26** (500 MHz CDCl₃)



¹H-NMR of 27 (500 MHz D₂O)



¹H-NMR of **28** (500 MHz D₂O)



^1H - ^{13}C gHSQCAD of **28** (900 MHz D_2O)

