Supporting Information for

Tunable Heparan Sulfate Mimetics for Modulating Chemokine Activity

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1. Supporting Figures

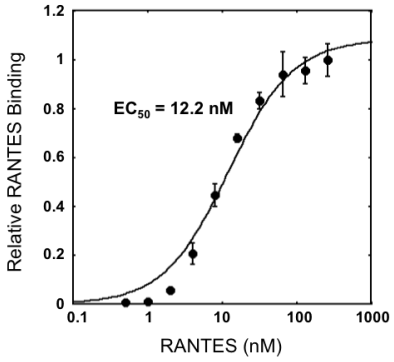


Figure S1. Binding of RANTES to heparin, as determined by ELISA. Heparin-binding 96-well plates were coated with heparin (25 μg/mL; 20-kDa). Human RANTES (1 – 1024 nM; ●) was serially diluted and then incubated in the heparin-coated plates. For ELISA analysis, RANTES was first incubated with a mouse anti-RANTES primary antibody and then with a goat anti-mouse IgG antibody conjugated to horseradish peroxidase (HRP). Levels of plate-bound RANTES were measured by detecting HRP activity at 450 nm. Data were fitted to a sigmoidal curve to determine the half-maximal effective concentration (EC_{50}) for RANTES binding to heparin. Experiments were conducted in quadruplicate, and the standard error is depicted.

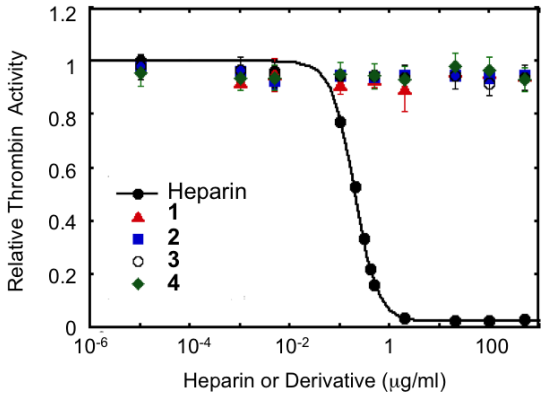


Figure S2. Glycopolymers **1** – **4** do not potentiate inhibition of thrombin in a purified system with antithrombin III. Human antithrombin III (1 IU/mL) was incubated with an excess of thrombin (24 IU/mL) in the presence of either heparin or glycopolymer (10^{-5} – 500 μg/mL). Heparin is known to bind to and induce a conformation change in the structure of antithrombin III, thereby increasing its inhibitory activity for thrombin (as observed in this assay). A chromogenic substrate (1.25 umol/mL) specific for the proteolytic activity of thrombin was added to measure residual levels of thrombin in the mixture. The absorbance was measured at 405 nm and is inversely proportional to the ability of heparin or glycopolymer to potentiate the inhibitory activity of antithrombin III. Data represent the mean ± standard error for quadruplicate assays.

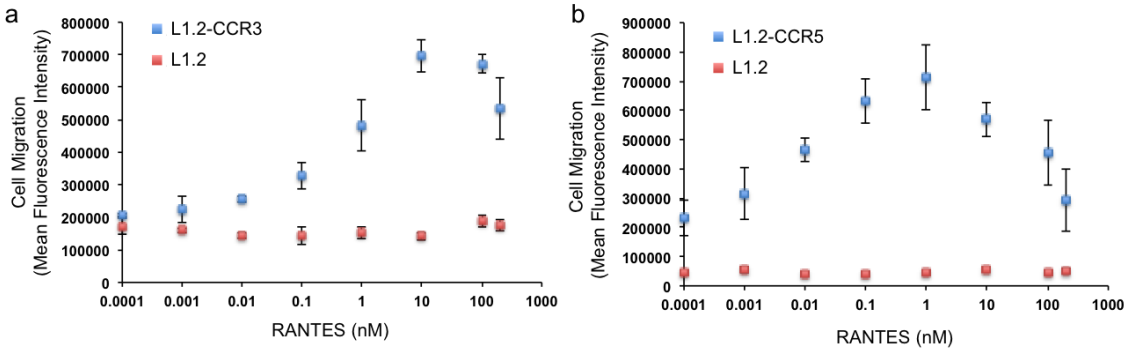


Figure S3. RANTES-induced migration of CCR3- and CCR5-expressing cells. Chemotactic response to RANTES is maximal at (a) 10 nM for CCR3-stably transfected L1.2 cells and (b) 1 nM for CCR5-stably transfected L1.2 cells. In both experiments, wild-type L1.2 cells did not migrate in response to RANTES as expected. Chemotaxis was measured using a 96-well modified Boyden chamber, and the relative number of migrated cells was determined using a fluorescent nucleic acid dye. Data represent the mean ± standard error for two independent experiments, each conducted in quadruplicate.

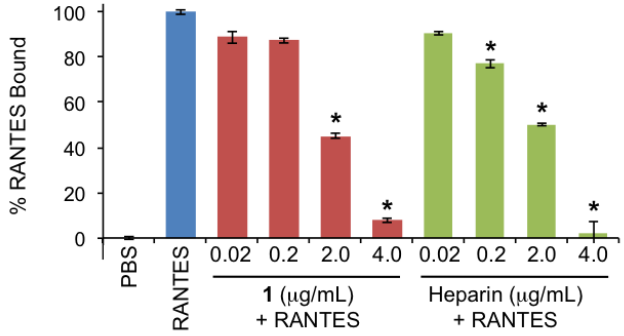


Figure S4. RANTES binding to CCR3-expressing cells. RANTES (100 nM) was preincubated with varying concentrations of heparin or glycopolymer **1** (0.02 – 2 μg/mL). Heparin or **1** inhibits the binding of RANTES to L1.2-CCR3 cells, as determined by flow cytometry (*, $P < 0.05$; means were compared to RANTES treatment alone using a Student's *t* test). Data represent the mean ± standard error for three independent experiments, each conducted in quadruplicate.

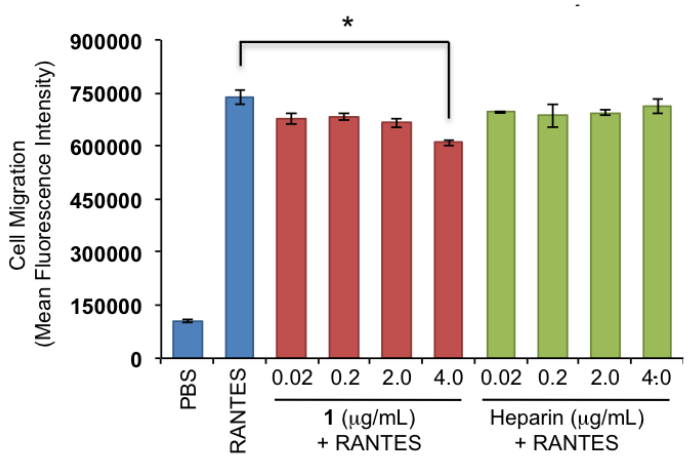


Figure S5. RANTES-induced migration of CCR5-expressing cells. RANTES (1 nM) was added to the bottom half of a 96-well modified Boyden chamber and pre-incubated with various concentrations of heparin or glycopolymer **1** prior to chemotaxis induction. The relative number of migrated cells was measured using a fluorescent nucleic acid dye. Heparin and **1** did not affect the migration of L1.2-CCR5 cells, except at the highest concentration of **1** (4 μg/mL; *, $P < 0.01$; means were compared to RANTES treatment alone using a Student's *t* test). Experiments were conducted in quadruplicate, and the standard error is depicted.

2. Supporting Tables

Table S1. **Properties of glycopolymers 1 – 4.**

Polymer	Monomer	mol% Catalyst	M_n[a] (g/mol)	PDI[a]	n (DP[b])
1	**22**	5	27,870	1.22	35
2	**23**	5	36,490	1.02	48
3	**24**	5	53,580	1.16	90
4	**25**	5	32,880	1.03	46

[a] Number average molecular weight (M_n) and polydispersity index (PDI) were determined by size exclusion chromatography multi-angle light scattering (SEC-MALS).

[b] Degree of polymerization (DP). Despite equal mol% of catalyst, the DP was notably higher for unsulfated monomer **24**. This was likely due to increased solubility of the unsulfated polymer (**3**) in the polymerization co-solvent (10:1 dichloroethane/methanol) compared to the sulfated polymers (**1**, **2**, and **4**).

Table S2. **Half maximal inhibitory concentration (IC_{50}) for antagonists of RANTES**

Antagonist	IC_{50} (μg/mL)[a]	IC_{50} (nM)[b]	IC_{50} (μg/mL of disac)[c]	IC_{50} (μM of disac)[d]
1	9.3 ± 1.1	334 ± 39	6.8 ± 0.80	11.7 ± 1.4
2	31.1 ± 6.2	852 ± 170	21.8 ± 4.4	40.3 ± 8.0
4	58.0 ± 5.7	1760 ± 170	40.6 ± 4.0	81.3 ± 8.0
Heparin	0.90 ± 0.03	45.0 ± 1.5	0.90 ± 0.03	1.50 ± 0.05

[a] Values were determined from Figure 1 using KaleidaGraph software. Data represent the mean ± standard error for quadruplicate assays.

[b] Values were calculated from Figure 1 based on molar concentration of antagonist (see Table S1 for molecular weights of polymers **1**-**4**).

[c] Values were corrected for ligand valence after taking into account the mass percentage of disaccharide contributing to each disaccharide-norbornyl linker unit.

[d] Values were corrected for ligand valence based on molar concentration of disaccharide.

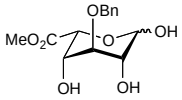
3. Experimental Methods

3-1. General Synthetic Procedures

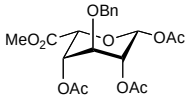
Unless stated otherwise, reactions were performed in flame-dried glassware under an argon atmosphere using freshly dried solvents. Solvents were dried via passage through an activated alumina column under argon. All other commercially obtained reagents were used as received unless otherwise noted. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 pre-coated plates (0.25 mm). Visualization of the chromatogram was accomplished by UV, cerium ammonium molybdate or ninhydrin staining as necessary. ICN silica gel (particle size 0.032 – 0.063 mm) was used for flash chromatography. Gel filtration chromatography was also used in order to achieve purification of the final products.

^{1}H and ^{13}C NMR experiments were recorded on Varian Mercury 300 (at 300 MHz), Varian Inova 500 (at 500 MHz), or Varian Inova 600 (600 MHz) spectrometers and are reported in parts per million (δ) relative to $CDCl_3$ (7.26 ppm) or CD_3OD (4.80 ppm). Data for ^{1}H are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hz, and integration. ^{13}C NMR spectra were obtained on Varian Inova 500 (at 125 MHz) or Varian Inova 600 (at 150 MHz) spectrometers and are reported in terms of chemical shift (77.2 ppm for $CDCl_3$; 49.0 for CD_3OD). When necessary, proton and carbon assignments were made by means of ^{1}H-^{1}H gCOSY, ^{1}H-^{1}H TOCSY, and ^{1}H-^{13}C gHSQCAD. Stereochemical assignments are supported by ^{1}H-^{1}H ROESY spectra. Mass spectra were obtained using a Perkin Elmer/Sciex API 365 triple quadrupole/electrospray tandem mass spectrometer or a Waters LCT Premier XE high resolution mass spectrometer.

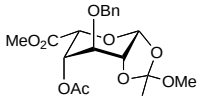
3-2. Synthesis of Compounds and Assignments



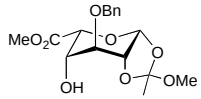
Methyl 3-*O*-benzyl-L-idopyranosyluronate (9). Compound **9** was prepared in six steps from the commercially available diacetone glucose (Sigma Aldrich) using previously reported procedures.[1] The analytical data were in agreement with the reported spectra.



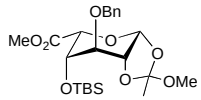
Methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-β-L-idopyranosyluronate (10). Compound **9** (0.30 g, 1.0 mmol) was added to CH_2Cl_2 (5.5 mL) at 0 °C, and the solution was cooled to -40 °C. 4-Dimethylaminopyridine (120 mg, 0.10 mmol) was added, followed by pyridine (700 μL, 10 mmol). Acetyl chloride (470 μL, 6.0 mmol) was then added dropwise to the reaction mixture, which was stirred for 10 h at -40 °C. The reaction was quenched with aqueous $NaHCO_3$ (50 mL), extracted with CH_2Cl_2 (2.0 x 50 mL), and subsequently washed with H_2O, 1M H_2SO_4, and then H_2O (50 mL for each wash). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel flash chromatography (3:1 hexanes:EtOAc) afforded compound **10** (0.40 g) in quantitative yield. The analytical data were in agreement with previously reported spectra.[3] ESI-TOF HRMS: *m/z* calcd for $C_{20}H_{23}O_{10}$ [M+H]-H_2 423.1286; found: 423.1286.



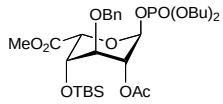
Methyl 4-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate 1,2-(methyl-orthoacetate) (11). $TiBr_4$ (8.1 g, 22 mmol) was added to a solution of compound **10** (6.9 g, 16 mmol) in CH_2Cl_2 (360 mL), and the reaction was stirred for 16 h at ambient temperature with exclusion of light. The reaction was quenched with ice-cold H_2O (2.0 x 500 mL), filtered through Celite, and concentrated under reduced pressure. The resulting brown oil was immediately used in the next reaction without further purification. The crude bromide intermediate (16 mmol) was dissolved in CH_2Cl_2 (220 mL). 2,4,6-Collidine (11 mL, 80 mmol) and methanol (8.0 mL) were added to this solution, and the reaction was stirred for 14 h at room temperature (rt). The reaction mixture was then diluted with CH_2Cl_2 (500 mL), washed with aqueous $NaHCO_3$ and H_2O (200 mL each), dried over $MgSO_4$, and concentrated under reduced pressure. Purification by silica gel flash chromatography (6:1 hexanes:EtOAc + 1% Et_3N) afforded **11** (7.6 g, 75% over 2 steps) as a light yellow oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.44 – 7.30 (m, 5H, OCH_2*Ph*), 5.57 (d, *J* = 2.7 Hz, 1H, H-1), 5.20 (dt, *J* = 2.7, 1.3 Hz, 1H, H-4), 4.82 (d, *J* = 11.7 Hz, 1H, OCH_2Ph), 4.69 (d, *J* = 11.7 Hz, 1H, OCH_2Ph), 4.56 (d, *J* = 1.4 Hz, 1H, H-5), 4.15 (dd, *J* = 2.7, 1.9 Hz, 1H, H-3), 4.09 (ddd, *J* = 2.9, 1.9, 1.2 Hz, 1H, H-2), 3.79 (s, 3H, CO_2CH_3), 3.26 (s, 3H, OCH_3), 2.05 (s, 3H, $OCOCH_3$), 1.74 (s, 3H, CH_3); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.1, 168.1, 136.8, 128.6, 128.4, 128.0, 96.6, 77.3, 76.1, 72.9, 71.3, 69.6, 68.9, 52.6, 49.1, 25.0, 20.1; ESI-TOF HRMS: *m/z* calcd for $C_{19}H_{23}O_9$ [M+H]-H_2 395.1342; found: 395.1354.



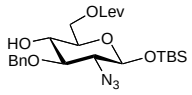
Methyl 3-*O*-benzyl-β-L-idopyranuronate 1,2-(methyl-orthoacetate) (12). Compound **11** (7.2 g, 18 mmol) was dissolved in methanol (90 mL) and cooled to -10 °C. A 0.5 M solution NaOMe (1.8 mL, 0.91 mmol) was added, and the reaction mixture was stirred at -10 °C for 2 h and at 5 °C overnight. The solution was diluted with CH_2Cl_2 (200 mL) at 5 °C, quenched with aqueous $NaHCO_3$ and H_2O (500 mL each), and then extracted with (3.0 x 250 mL). The organic fractions were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel flash chromatography (4:1 → 1:1 hexanes:EtOAc + 1% Et_3N) yielded **12** (9.5 g, 80%) as a clear oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36 – 7.34 (m, 5H, OCH_2*Ph*), 5.51 (d, *J* = 2.4 Hz, 1H, H-1), 4.72 (d, *J* = 11.7 Hz, 1H, OCH_2Ph), 4.62 (d, *J* = 11.7 Hz, 1H, OCH_2Ph), 4.52 (s, 1H, H-4), 4.15 – 4.08 (m, 3H, H-2, H-5), 3.81 (s, 3H, CO_2CH_3), 3.30 (s, 3H, OCH_3), 2.78 (d, *J* = 11.4 Hz, 1H, H-3), 1.76 (s, 3H, CH_3); ^{13}C NMR (125 MHz; $CDCl_3$): δ 168.3, 136.8, 128.7, 128.4, 127.9, 96.8, 75.8, 73.0, 72.9, 71.8, 67.0, 52.5, 50.3, 24.4; ESI-TOF HRMS: *m/z* calcd for $C_{17}H_{21}O_8$ [M+H]-H_2 353.1236; found: 353.1226.



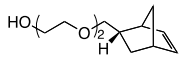
Methyl 3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-β-L-idopyranuronate 1,2-(methyl-orthoacetate) (13). Compound **12** (230 mg, 0.64 mmol) was dissolved in pyridine (7.8 mL) and the solution was cooled to -10 °C. TBSOTf (1.5 mL, 0.65 mmol) was added, and the reaction was stirred overnight at 0 °C. The reaction was diluted with CH_2Cl_2 (100 mL), quenched with aqueous $NaHCO_3$ (100 mL), and extracted with EtOAc (3.0 x 50 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel flash chromatography (7:1 hexanes:EtOAc + 1% Et_3N) yielded compound **32** (380 mg, 92%) as a clear oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.47 (m, 5H, OCH_2*Ph*), 5.62 (d, *J* = 2.5 Hz, 1H, H-1), 4.80 (d, *J* = 12 Hz, 1H, OCH_2Ph), 4.75 (d, *J* = 12 Hz, 1H, OCH_2Ph), 4.51 (s, 1H, H-4), 4.21 (s, 1H, H-5), 4.20 (d, *J* = 1 Hz, 1H, H-4), 3.98 (s, 1H, H-3), 3.89 (s, 3H, CO_2CH_3), 3.40 (s, 3H, OCH_3), 1.84 (s, 3H, CH_3), 0.94 (s, 9H, $SiC(CH_3)_3$), 0.08 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$); ^{13}C NMR (125 MHz; $CDCl_3$): δ 169.6, 137.0, 128.9, 128.6, 128.2, 124.6, 97.1, 76.3. 74.6, 72.8, 72.5, 67.9, 52.4, 49.5, 29.9, 25.7, 25.6, -4.4, -5.2; TOF HRMS ES *m/z* calcd for $C_{23}H_{36}O_8SiNa$ $[M+Na]^+$: 491.2077; found: 491.2070.



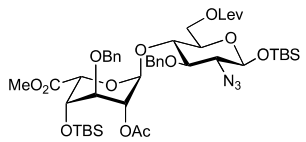
Methyl (dibutylphosphate-2-*O*-acetyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosid)uronate (6). Compound **13** (140 mg, 0.29 mmol) was dissolved in CH_2Cl_2 (7.3 mL) at rt. Freshly activated 4Å molecular sieves (290 mg) were added, and the solution was stirred for 15 min. Dibutylphosphate (0.54 mL, 2.9 mmol) was added slowly, and the reaction mixture was stirred overnight. After confirming that the reaction was complete by TLC, the reaction was quenched with triethylamine (2.0 mL) and concentrated under reduced pressure. Silica gel flash chromatography (5:1 → 3:1 hexanes:EtOAc + 1% Et_3N) afforded the desired product (170 mg) in quantitative yield. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36 – 7.35 (m, 5H, OCH_2*Ph*), 5.82 (d, *J* = 7.2 Hz, 1H, H-1), 4.97 (m, 1H, H-2), 4.86 (d, *J* = 2.7 Hz, 1H, H-5), 4.78 (d, *J* = 12 Hz, 1H, OCH_2Ph), 4.62 (d, *J* = 12 Hz, 1H, OCH_2Ph), 4.09 – 3.99 (m, 5H, H-4, $P(OCH_2CH_2CH_3)_2$), 3.77 (s, 3H, CO_2CH_3), 3.62 (m, 1H, H-3), 2.04 (s, 3H, $COCH_3$), 1.64 – 1.60 (m, 4H, $P(OCH_2CH_2CH_2CH_3)_2$), 1.40 – 1.25 (m, 4H, $P(OCH_2CH_2CH_2CH_3)_2$), 0.96 – 0.88 (m, 6H, $P(OCH_2CH_2CH_2CH_3)_2$), 0.81 (s, 9H, $SiC(CH_3)_3$), 0.07 (s, 3H, $SiCH_3$), 0.17 (s, 3H, $SiCH_3$); ^{13}C NMR (125 MHz; $CDCl_3$): δ 169.8, 169.2, 146.6, 137.3, 128.4, 128.0, 95.4, 73.8, 72.0, 68.0, 67.8, 67.0, 66.9, 52.1, 32.1, 25.4, 20.9, 18.6, 17.8, 13.5, -4.7, -5.7; ESI-TOF HRMS *m/z* calcd for $C_{30}H_{52}O_{11}PSi$ $[M+H]^+$: 647.3017; found: 647.3001.



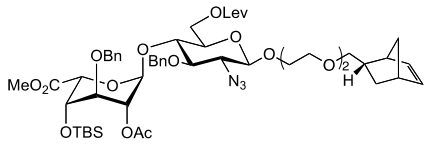
2-*O*-Azido-3-*O*-benzyl-6-*O*-levulinoyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside (7). Compound **7** was prepared from readily available D-glucosamine (Sigma Aldrich) using previously published procedures, and the analytical data were in agreement with previously reported spectra.[1]



2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethanol (8). Compound **8** was prepared using a previously published procedure, and the analytical data were in agreement with reported spectra.[2]

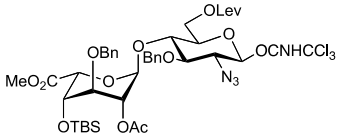


Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-*tert*-butyldimethylsilyl (2-azido-3-*O*-benzyl-6-*O*-levulinoyl-2-deoxy-β-D-glucopyranoside) (14). Compound **6** (92 mg, 0.14 mmol) and **7** (77 mg, 0.17 mmol) were co-evaporated with toluene (3.0 x 1.0 mL) and placed under vacuum overnight. The mixture was dissolved in CH_2Cl_2 (4.2 mL), and freshly activated 4Å molecular sieves (0.21 g) were added. After stirring at rt for 15 min, the temperature was lowered to -10 °C and the mixture stirred for an additional 15 min. TMSOTf (31 μL, 0.18 mmol) was added dropwise to the reaction mixture. The reaction was stirred at -30 °C for 30 min, quenched with Et_3N (1.0 mL), filtered through a silica pad, and concentrated under reduced pressure. Silica gel flash chromatography (5:1 → 4:1 hexanes:EtOAc) afforded the desired product (120 mg) in 93% yield. ^{1}H NMR (600 MHz; $CDCl_3$): δ 7.37 – 7.24 (m, 10H, OCH_2Ph), 5.20 (d, *J* = 4.2 Hz, 1H, H-1 of IdoA), 4.85 (t, *J* = 4.1 Hz, 1H, H-2 of IdoA), 4.82 (d, *J* = 10.6 Hz, 1H, OCH_2Ph), 4.74 (d, *J* = 11.8 Hz, 1H, OCH_2Ph), 4.71 (d, *J* = 10.8 Hz, 1H, OCH_2Ph), 4.69 (d, *J* = 3.9 Hz, 1H, H-5 of IdoA), 4.64 (d, *J* = 11.8 Hz, 1H, OCH_2Ph), 4.54 (dd, *J* = 11.8, 2.1 Hz, 1H, H-1 of GlcN), 4.51 – 4.45 (m, 1H, H-6), 4.20 – 4.03 (m, 1H, H-6), 3.99 (t, *J* = 4.3 Hz, 1H, H-4 of IdoA), 3.91 – 3.77 (m, 1H, H-4 of GlcN), 3.61 (t, *J* = 4.3 Hz, 1H, H-3 of IdoA), 3.54 (s, 3H, CO_2CH_3), 3.48 (ddd, *J* = 9.8, 5.9, 2.2 Hz, 1H, H-5 of GlcN), 3.37 – 3.24 (m, 2H, H-2 and H-3 of GlcN), 2.89 – 2.66 (m, 2H, $COCH_2CH_2COCH_3$), 2.61 (t, *J* = 6.7 Hz, 2H, $COCH_2CH_2COCH_3$), 2.19 (s, 3H, $COCH_2CH_2COCH_3$), 2.00 (s, 3H, $COCH_3$), 0.92 (s, 9H, $SiC(CH_3)_3$), 0.81 (s, 9H, $SiC(CH_3)_3$), 0.13 (d, *J* = 4.6 Hz, 6H, $SiCH_3$), -0.06 (s, 3H, $SiCH_3$), -0.12 (s, 3H, $SiCH_3$); ^{13}C NMR (125 MHz; $CDCl_3$): δ 206.5, 172.2, 170.2, 169.9, 138.2, 137.7, 128.5, 128.1, 128.1, 127.9, 127.4, 97.6, 97.1, 80.6, 76.8, 76.5, 75.4, 74.8, 73.4, 72.7, 71.5, 69.9, 68.9, 68.5, 62.6, 51.7, 38.0, 29.9, 28.0, 25.6, 25.5, 20.9, 18.0, 17.8, -4.3, -4.7, -5.2, -5.5; ESI-TOF HRMS *m/z* calcd for $C_{46}H_{69}N_3O_{14}Si_2$ $[M+Na]^+$: 966.4216; found: 966.4211.



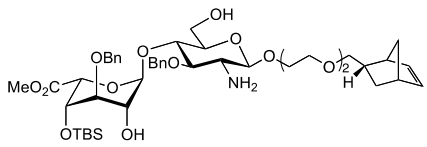
Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-1-*O*-(2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-yl-methoxy)ethoxy)ethyl)-6-*O*-levulinoyl-2-deoxy-α-D-glucopyranoside (5). Compound **15** (37 mg, 60 μmol) and **8** (33 mg, 70

μmol) were co-evaporated with toluene (3.0 x 1.0 mL) and placed under vacuum overnight. The mixture was dissolved in CH_2Cl_2 (1.7 mL) and freshly activated 4Å molecular sieves (80 mg) were added. After stirring at rt for 15 min, the temperature was lowered to -30 °C and the mixture stirred for an additional 15 min. TMSOTf (14 μL, 70 μmol) was added to dropwise to the reaction mixture. The reaction was stirred at -10 °C for 10 min, slowly raised to rt over 15 min, quenched with Et_3N (0.50 mL), filtered through a silica pad, and concentrated under reduced pressure. Silica gel flash chromatography (10:1 → 4:1 → 3:1 hexanes:EtOAc) afforded the desired product (35 mg) in 67% yield. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.38 – 7.25 (m, 10H, OCH_2*Ph*), 6.07 (ddd, *J* = 25.6, 5.7, 3.0 Hz, 2H, C*H*=C*H* of Nb), 5.19 (d, *J* = 4.2 Hz, 1H, H-1 of IdoA), 4.89 – 4.79 (m, 2H, H-2 of IdoA, OC*H*$_2$Ph), 4.72 – 4.68 (m, 3H, H-5 of IdoA, OC*H*$_2$Ph), 4.64 (d, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.52 (dd, *J* = 12.1, 2.2 Hz, 1H, H-6 of GlcN), 4.34 (d, *J* = 7.9 Hz, 1H, H-1 of GlcN), 4.12 (dd, *J* = 12.1, 2.2 Hz, 1H, H-6 of GlcN), 4.02 – 3.93 (m, 2H, H-4 of IdoA, OC*H*$_2$ of PEG linker), 3.87 (dd, *J* = 9.8, 8.9 Hz, 1H, H-4 of GlcN), 3.82 – 3.55 (m, 5H, H-3 of IdoA, OC*H*$_2$ of PEG linker), 3.54 (s, 3H, CO_2C*H*$_3$), 3.53 – 3.29 (m, 5H, H-5 of GlcN, H-2 of GlcN, H-3 of GlcN, OC*H*$_2$ of PEG linker), 2.88 – 2.67 (m, 4H, C*H*-CH=CH of Nb, COC*H*$_2CH_2COCH_3$), 2.67 – 2.56 (m, 2H, $COCH_2C$*H*$_2COCH_3$), 2.19 (s, 3H, $COCH_2CH_2COC$*H*$_3$), 2.00 (s, 3H, OCOC*H*$_3$), 1.75 – 1.66 (m, 1H, C*H* of Nb), 1.39 – 1.15 (m, 4H, C*H*$_2$ of Nb), 0.81 (s, 9H, SiC(C*H*$_3$)), -0.06 (s, 3H, SiC*H*$_3$), -0.11 (s, 3H, SiC*H*$_3$); ^{13}C NMR (125 MHz; $CDCl_3$): δ 136.6, 128.5, 128.2, 127.8, 102.2, 80.9, 76.8, 76.1, 75.0, 73.2, 72.9, 71.6, 70.7, 70.4, 66.0, 45.0, 43.6, 38.8, 38.0, 29.8, 28.1, 25.5; ESI-TOF HRMS *m/z* calcd for $C_{52}H_{73}N_3O_{16}Si$ $[M+Na]^+$: 1046.4658; found: 1046.4670



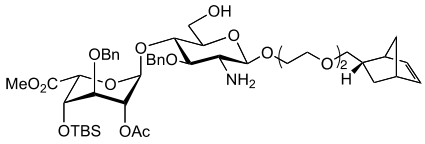
Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-6-*O*-levulinoyl-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (15). Compound **14** (840 mg, 0.89 mmol) was dissolved in THF (27 mL) and the solution was cooled to 0 °C. 1M TBAF (1.2 mL, 1.2 mmol) and AcOH (60 μL, 1.1 mmol) were added simultaneously, and the reaction was stirred for 30 min at 0 °C. The reaction was quenched with aqueous $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (2.0 x 10 mL), and subsequently washed with H_2O, 1M H_2SO_4, and then H_2O (10 mL for each wash). After concentrating under reduced pressure, the crude mixture (0.89 mmol) was dissolved in CH_2Cl_2 (27 mL) and cooled to 0 °C. To

the reaction mixture, trichloroacetonitrile (1.3 mL, 13 mmol) and DBU (26 μL, 0.18 mmol) were added. The reaction was stirred at 0 °C for 12 h, quenched with Et_3N (1.0 mL), and concentrated under reduced pressure. Silica gel flash chromatography (5:1 → 4:1 → 3:1 hexanes:EtOAc + 1% Et_3N) afforded the desired product (770 mg) in 89% yield over two steps. ^{1}H NMR (600 MHz; $CDCl_3$): δ 8.72 (s, 1H, OCN*H*CCl_3), 7.47 – 7.28 (m, 10H, OCH_2*Ph*), 6.37 (d, *J* = 3.6 Hz, 1H, H-1 of IdoA), 5.24 (d, *J* = 4.7 Hz, 1H, H-1 of GlcN), 4.96 (d, *J* = 10.5 Hz, 1H, OCH_2Ph), 4.90 (t, *J* = 4.4 Hz, 1H, H-2 of GlcN), 4.75 (d, *J* = 11.7 Hz, 1H, OCH_2Ph), 4.71 (d, *J* = 10.5 Hz, 1H, OCH_2Ph), 4.66 (d, *J* = 11.8 Hz, 1H, OCH_2Ph), 4.63 (d, *J* = 4.2 Hz, 1H, H-5 of GlcN), 4.52 (dd, *J* = 12.3, 1.8 Hz, 1H, H-6 of GlcN), 4.13 (dd, *J* = 12.3, 4.3 Hz, 1H, H-6 of GlcN), 4.08 – 3.98 (m, 3H, H-4 and H-5 of IdoA, H-4 of GlcN), 3.91 (dd, *J* = 10.2, 8.5 Hz, 1H, H-3 of IdoA), 3.69 (dd, *J* = 10.2, 3.6 Hz, 1H, H-2 of IdoA), 3.66 – 3.61 (m, 1H, H-3 of GlcN), 3.57 (s, 3H, CO_2CH_3), 2.89 – 2.69 (m, 2H, $COCH_2CH_2COCH_3$), 2.67 – 2.54 (m, 2H, $COCH_2CH_2COCH_3$), 2.18 (s, 3H, $COCH_2CH_2COCH_3$), 2.01 (s, 3H, $COCH_3$), 0.82 (d, *J* = 2.5 Hz, 9H, $SiC(CH_3)_3$), -0.05 (s, 3H, $SiCH_3$), -0.09 (s, 3H, $SiCH_3$); ^{13}C NMR (125 MHz; $CDCl_3$): δ 206.5, 172.1, 170.2, 170.0, 160.7, 137.7, 137.6, 128.7, 128.2, 128.1, 128.0, 127.9, 127.6, 97.7, 94.4, 78.2, 77.2, 76.7, 75.1, 75.0, 73.0, 72.0, 71.9, 70.2, 69.0, 62.8, 61.9, 51.7, 38.0, 29.9, 28.0, 25.5, 20.1, 17.8, 4.7, 5.4; ESI-TOF HRMS *m/z* calcd for $C_{42}H_{55}N_3O_{15}SiCl_3$ $[M+Na]^+$: 997.2366; found: 997.2415.



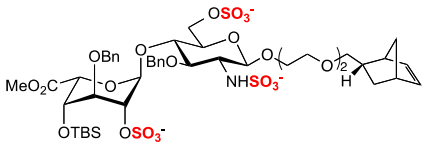
Methyl 3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-1-*O*-(2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl)-2-deoxy-α-D-glu-copyranoside (16). Compound **5** (17 mg, 20 μmol) was dissolved in anhydrous MeOH (0.80 mL), and 1,3-propanedithiol (0.14 mL, 60 μmol) and DIPEA (0.12 mL, 60 μmol) were added dropwise. Upon confirmation of partial disappearance of **5** by TLC, flame-dried K_2CO_3 (2.4 mg, 20 μmol) was added and the reaction mixture was stirred for 24 h at rt. The reaction was quenched with Dowex 5W-X8 (H^+ form), filtered through a pad of Celite, and concentrated under reduced pressure. Silica gel flash chromatography (1:1 hexanes:EtOAc) afforded the desired product (14 mg) in 93% yield. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.48 – 7.20 (m, 10H, OCH_2*Ph*), 6.17 – 5.88 (m, 2H, C*H*=C*H* of Nb), 5.25 (d, *J* = 4.5 Hz, 1H, H-1 of IdoA), 4.96 (d, *J* = 11.4 Hz, 1H, OCH_2Ph), 4.83 (d, *J* = 11.4 Hz, 1H, OCH_2Ph), 4.68 – 4.51 (m, 3H, OCH_2Ph, H-5 of IdoA), 4.32 (d, *J* = 8.0 Hz, 1H, H-1 of GlcN), 4.09 – 3.76 (m, 5H, H-6 of IdoA, H-4 of IdoA, H-2 of

IdoA, OCH_2 of PEG linker), 3.76 – 3.53 (m, 10H, H-2 of IdoA, H-6 of IdoA, OCH_2 of PEG linker, CO_2CH_3), 3.47 – 3.32 (m, 4H, H-4 of GlcN, OCH_2 of PEG linker, H-5 of GlcN, H-3 of GlcN), 2.84 – 2.68 (m, 3H, H-2 of GlcN, C*H*-CH=CH of Nb), 1.69 – 1.63 (m, 1H, C*H* of Nb), 1.40 – 1.03 (m, 4H, CH_2 of Nb), 0.82 (s, 9H, SiC(CH_3)), -0.03 (d, J = 7.3 Hz, 7H, SiCH_3); ^{13}C NMR (125 MHz; CD_3OD): δ 171.7, 140.1, 139.7, 137.7, 137.5, 129.2, 128.8, 128.4, 104.5, 102.3, 84.1, 79.5, 77.7, 77.0, 75.2, 74.4, 72.8, 71.5, 71.4, 61.8, 57.8, 52.4, 45.8, 44.9, 42.8, 40.1, 30.6, 26.1, 18.7, -4.5, -5.1; ESI-TOF HRMS: *m/z* calcd for $C_{45}H_{66}NO_{13}Si$ $[M-H]^-$ 856.4303; found: 856.4326.

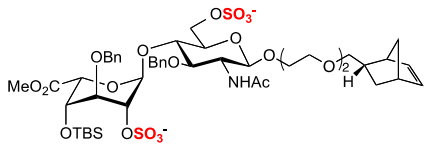


Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-2-acetylamido-3-*O*-benzyl-1-*O*-(2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl)-2-deoxy-α-D-glucopyranoside (17). Compound **5** (190 mg, 0.18 mmol) was dissolved in anhydrous MeOH (11 mL). 1,3-propanedithiol (1.1 mL, 5.4 mmol) and DIPEA (1.1 mL, 6.3 mmol) were added dropwise, and the reaction mixture was stirred for 24 h at rt. The reaction was quenched with Dowex 5W-X8 (H^+ form), filtered through a pad of Celite, and concentrated under reduced pressure. Silica gel flash chromatography (30:2:1 → 20:2:1 EtOAc:MeOH:H_2O) afforded the desired product (150 mg), and the resulting intermediate was dissolved in pyridine (2.8 mL). To this mixture, a solution of hydrazine monohydrate (1.2 mmol) and AcOH (9.9 mmol) in pyridine (17 mL) was added at rt. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with cold water (15 mL), saturated $NaHCO_3$ (15 mL), water (15 mL), and saturated brine (15 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel flash chromatography (20:2:1 EtOAc:MeOH:H_2O) afforded the desired product (140 mg) in 87% yield over two steps. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.46 – 7.27 (m, 10H, OCH_2Ph), 6.14 – 5.98 (m, 2H, C*H*=C*H* of Nb), 5.31 (d, J = 4.4 Hz, 1H, H-1 of IdoA), 4.99 (d, J = 11.2 Hz, 1H, OCH_2Ph), 4.88 (q, J = 3.6 Hz, 1H, H-2 of IdoA), 4.80 – 4.68 (m, 2H, OCH_2Ph, H-5 of IdoA), 4.67 – 4.55 (m, 2H, OCH_2Ph), 4.36 (dt, J = 8.0, 4.1 Hz, 1H, H-1 of GlcN), 4.05 – 3.85 (m, 4H, H-4 of IdoA, H-6 of GlcN, H-5 of GlcN, OCH_2 of PEG linker), 3.85 – 3.34 (m, 15H, OCH_2 of PEG linker, H-6 of GlcN, H-3 of IdoA, CO_2CH_3, H-3 of GlcN, H-4 of GlcN), 2.89 (dd, J = 10.0, 7.8 Hz, 1H, H-2 of GlcN), 2.81 – 2.67 (m, 2H, C*H*-CH=CH of Nb), 2.01 (s, 3H, OCOCH_3), 1.42 – 1.14 (m, 5H, C*H* and CH_2 of Nb), 0.82 (s, 9H, SiC(CH_3)), -0.08 (d, J = 7.3 Hz, 6H, SiCH_3); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.2, 138.7, 137.8, 136.7, 128.5, 128.1,

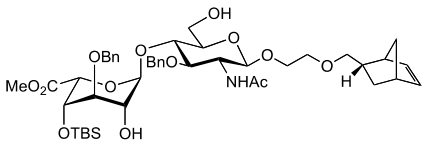
127.9, 127.6, 97.8, 76.8, 76.2, 75.7, 75.5, 74.2, 72.7, 72.0, 70.7, 70.4, 69.3, 69.0, 61.7, 56.6, 51.9, 45.1, 43.8, 41.7, 38.9, 38.6, 29.9, 25.7, 21.1, 17.9, -4.5, -5.4; ESI-TOF HRMS: *m/z* calcd for $C_{47}H_{70}NO_{14}Si$ $[M-H]^-$ 900.4565; found: 900.4568.



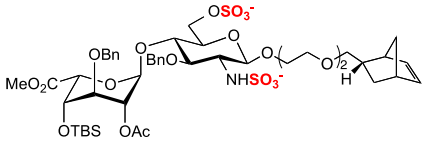
Methyl 3-*O*-benzyl-2-*O*-sulfonato-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-3-*O*-benzyl-1-*O*-(2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl)-2-deoxy-2-sulfonatamido-6-*O*-sulfonato-α-D-glucopyranoside (18). Compound **16** (9.2 mg, 10 μmol) was dissolved in freshly distilled pyridine (1.0 mL) and to this SO_3•Py (50 mg, 0.32 mmol) and Et_3N (0.20 mL) were added. The reaction mixture was stirred at rt for 24 h, refluxed at 50 °C for 24 h, quenched with MeOH (1.0 mL), and concentrated to afford a golden syrup. Purification by Sephadex LH-20 gel filtration (1:1 CH_2Cl_2:MeOH), followed by silica gel flash chromatography (15:2:1 → 10:2:1 → 8:2:1 EtOAc:MeOH:H_2O) gave the desired product (8.7 mg) in 78% yield. ^{1}H NMR (500 MHz; CD_3OD): δ 7.51 – 7.50 (m, 2H, OCH_2*Ph*), 7.43 – 7.41 (m, 2H, OCH_2*Ph*), 7.37 – 7.34 (m, 2H, OCH_2*Ph*), 7.30 – 7.26 (m, 3H, OCH_2*Ph*), 7.23 – 7.22 (m, 1H, OCH_2*Ph*), 6.11 – 6.04 (m, 2H, C*H*=C*H* of Nb), 5.30 (s, H-1 of IdoA), 4.98 (d, *J* = 12.5 Hz, 1H, OC*H*$_2$Ph), 4.87 (d, *J* = 11.5 Hz, 1H, OC*H*$_2$Ph), 4.78 (d, *J* = 6 Hz, 1H, H-1 of GlcN), 4.67 (d, *J* = 11.5 Hz, 1H, OC*H*$_2$Ph), 4.59 (d, *J* = 12.5 Hz, 1H, OC*H*$_2$Ph), 4.43 (s, 1H, H-2 of IdoA), 4.37 – 4.28 (m, 2H, H-6, H-6 of GlcN), 4.13 – 4.12 (m, 1H, H-4 of GlcN), 4.05 – 4.03 (m, 1H, H-5 of GlcN), 3.96 – 3.93 (m, 1H, H-4 of IdoA), 3.81 – 3.78 (m, 2H, H-3 of IdoA, OC*H*$_2$ of PEG linker), 3.73 – 3.71 (m, 2H, OC*H*$_2$ of PEG linker), 3.69 – 3.56 (m, 5H, OC*H*$_2$ of PEG linker), 3.54 – 3.51 (m, 1H, H-2 of GlcN), 3.45 – 3.35 (m, 2H, OC*H*$_2$ of PEG linker), 3.15 (s, 3H, CO_2CH_3), 2.77 (s, 1H, C*H*-CH=CH of Nb), 2.72 (s, 1H, C*H*-CH=CH of Nb), 2.11 (s, 3H, OCOC*H*$_3$), 1.98 (s, 3H, OCOC*H*$_3$), 1.72 – 1.68 (m, 1H, C*H* of Nb), 1.38 – 1.21 (m, 3H, C*H*$_2$ of Nb), 1.15 – 1.12 (m, 1H, C*H*$_2$ of Nb), 0.76 (s, 9H, SiC(C*H*$_3$)), -0.17 (s, 3H, SiC*H*$_3$), -0.24 (s, 3H, SiC*H*$_3$); ^{13}C NMR (125 MHz; CD_3OD): δ 172.8, 141.1, 140.4, 138.7, 131.6, 131.0, 130.6, 130.4, 130.3, 130.2, 130.0, 129.4, 104.3, 100.1, 80.9, 78.0, 77.0, 72.7, 72.4, 71.0, 70.7, 56.2, 53.5, 46.9, 46.0, 43.8, 41.1, 31.7, 27.4, 19.9, -3.0, -4.4; ESI-TOF HRMS: *m/z* calcd for $C_{45}H_{66}NO_{22}S_3Si$ $[M-H]^-$ 1016.2597; found: 1016.2583.



Methyl 2-*O*-sulfonato-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-2-acetylamido-3-*O*-benzyl-1-*O*-(2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy) ethyl)-2-deoxy-6-*O*-sulfonato-α-D-glucopyranoside (19). To a solution of compound **16** (130 mg, 0.15 mmol) in anhydrous MeOH (8.4 mL) at ambient temperature were added Ac_2O (0.30 mL, 3.0 mmol) and Et_3N (0.50 mL). Additional amounts of Ac_2O (0.30 mL, 3.0 mmol) were added every hour until complete conversion to the desire product was observed by TLC (at least 4 h). The reaction mixture was directly loaded onto a Sephadex LH-20 gel filtration column and eluted with 1:1 CH_2Cl_2:MeOH. The *N*-acetylated intermediate was dissolved in freshly distilled pyridine (8.1 mL), and SO_3•Py (450 mg, 3.3 mmol) and Et_3N (1.6 mL) were added. The reaction mixture was stirred at rt for 24 h, refluxed at 50 °C for 24 h, quenched with MeOH (5.0 mL), and concentrated to afford a golden syrup. Purification by Sephadex LH-20 gel filtration (1:1 CH_2Cl_2:MeOH), followed by silica gel flash chromatography (10:2:1 EtOAc:MeOH:H_2O) gave the desired product (130 mg) in 85% yield over two steps. ^{1}H NMR (500 MHz; CD_3OD): δ 7.53 – 7.10 (m, 10H, OCH_2Ph), 6.12 – 5.95 (m, 2H, C*H*=C*H* of Nb), 5.35 (s, 1H, H-1 of IdoA), 4.84 (m, 2H, H-5 of IdoA, OCH_2Ph), 4.73 (d, J = 11.2 Hz, 1H, OCH_2Ph), 4.60 (d, J = 11.6 Hz, 1H, OCH_2Ph), 4.55 (d, J = 8.3 Hz, 1H, H-1 of GlcN), 4.52 (dd, J = 2.1, 1.1 Hz, 1H, H-2 of IdoA), 4.47 (d, J = 11.2 Hz, 1H, OCH_2Ph), 4.41 (dd, J = 11.3, 2.2 Hz, 1H, OCH_2 of PEG linker), 4.29 (dd, J = 11.2, 5.0 Hz, 1H, OCH_2 of PEG linker), 4.00 – 3.87 (m, 4H, H-4 of IdoA, H-2 of GlcN, H-3 of GlcN, H-6 of GlcN), 3.86 (t, J = 1.8 Hz, 1H, H-3 of IdoA), 3.79 – 3.48 (m, 11H, H-6 of GlcN, OCH_2 of PEG linker, H-4 of GlcN, H-5 of GlcN), 3.46 – 3.36 (m, 1H, OCH_2 of PEG linker), 3.33 (s, 3H, CO_2CH_3), 2.74 (d, J = 31.6 Hz, 2H, C*H*-CH=CH of Nb), 1.85 (d, J = 1.2 Hz, 3H, $NHCOCH_3$), 1.67 (d, J = 4.5 Hz, 1H, C*H* of Nb), 1.39 – 1.04 (m, 4H, CH_2 of Nb), 0.78 (d, J = 1.2 Hz, 9H, $SiC(CH_3)$), -0.11 (dd, J = 47.6, 1.1 Hz, 6H, $SiCH_3$); ^{13}C NMR (125 MHz; CD_3OD): δ 173.3, 172.1, 139.7, 139.2, 137.7, 137.5, 129.9, 129.5, 129.1, 128.6, 102.6, 99.2, 82.3, 77.1, 76.2, 75.7, 75.3, 74.1, 72.8, 71.5, 71.4, 70.1, 69.7, 67.8, 56.5, 52.4, 45.9, 44.9, 42.8, 40.0, 30.7, 26.2, 23.0, 18.9, -4.2, -5.4; ESI-TOF HRMS: *m/z* calcd for $C_{47}H_{67}NO_{20}NaSiS_2$ $[M+Na]^+$ 1080.3365; found: 1080.3392.

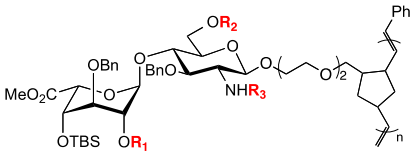


Methyl 3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-2-acetyl-amido-3-*O*-benzyl-1-*O*-(2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl)-2-deoxy-α-D-glucopyranoside (20). To a solution of compound **16** (130 mg, 0.15 mmol) in anhydrous MeOH (8.4 mL) at ambient temperature were added Ac_2O (0.30 mL, 3.0 mmol) and Et_3N (0.50 mL). Additional amounts of Ac_2O (0.30 mL, 3.0 mmol) were added every hour until complete conversion to the desired product was observed by TLC (at least 4 h). Purification by Sephadex LH-20 gel filtration (1:1 CH_2Cl_2:MeOH), followed by silica gel flash chromatography (20:2:1 EtOAc:MeOH:H_2O) gave the desired product (140 mg) in quantitative yield. ^{1}H NMR (600 MHz; $CDCl_3$): δ 7.36 – 7.09 (m, 10H, OCH_2Ph), 6.12 – 5.87 (m, 2H, C*H*=C*H* of Nb), 5.11 (s, 1H, H-1 of IdoA), 4.76 – 4.72 (m, 1H, H-1 of GlcN), 4.70 (t, J = 2.7 Hz, 1H, H-4 of IdoA), 4.68 – 4.56 (m, 2H, OCH_2Ph), 4.51 – 4.43 (m, 2H, OCH_2Ph), 4.00 – 3.92 (m, 1H, H-3 of IdoA), 3.91 – 3.80 (m, 2H, OCH_2 of PEG linker, H-6 of GlcN), 3.80 – 3.17 (m, 19H, H-6 of GlcN, H-2 of GlcN, H-3 of GlcN, H-4 of GlcN, H-5 of GlcN, H-2 of IdoA, H-5 of IdoA, OCH_2 of PEG linker, CO_2CH_3), 2.75 – 2.46 (m, 2H, C*H*-CH=CH of Nb), 1.67 (d, J = 4.2 Hz, 3H, $NHCOCH_3$), 1.64 – 1.53 (m, 1H, C*H* of Nb), 1.30 – 1.13 (m, 4H, CH_2 of Nb), 0.71 (s, 9H, $SiC(CH_3)$), -0.15 (d, J = 9.3 Hz, 6H, $SiCH_3$); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.4, 169.7, 138.6, 137.5, 136.8, 136.6, 128.8, 128.4, 128.2, 127.4, 107.3, 101.6, 100.9, 78.7, 77.0, 76.4, 75.7, 75.5, 72.6, 72.5, 71.0, 70.7, 70.4, 69.8, 69.1, 68.8, 67.2, 62.7, 52.1, 45.2, 43.8, 41.7, 38.8, 30.0, 29.9, 25.6, 23.3, 17.9, -4.7, -5.4; ESI-TOF HRMS: *m/z* calcd for $C_{47}H_{69}NaNO_{14}Si$ $[M+Na]^+$ 922.4380; found: 922.4385.



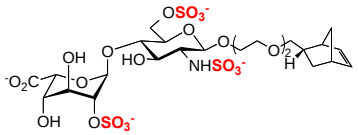
Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyluronate-(1→4)-3-*O*-benzyl-1-*O*-(2-(2-((2*S*)-bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl)-2-deoxy-2-sulfonatamido-6-*O*-sulfonato-α-D-glucopyranoside (21). To a solution of compound **17** (22 mg, 0.020 mmol) in freshly distilled pyridine (2.3 mL) were added SO_3•Py (110 mg, 0.60 mmol) and Et_3N (0.5 mL). The reaction mixture was stirred at rt for 24 h and refluxed at 50 °C for 24 h, quenched with MeOH (1.0 mL), and concentrated to afford a golden syrup. Purification by Sephadex LH-20 gel filtration (1:1 CH_2Cl_2:MeOH), followed by silica gel flash chromatography

(15:2:1 → 10:2:1 EtOAc:MeOH:H_2O) gave the desired product (26 mg) in 78% yield. ^{1}H NMR (600 MHz; CD_3OD): δ 7.79 – 7.03 (m, 10H, OCH_2Ph), 6.25 – 5.94 (m, 2H, C*H*=C*H* of Nb), 5.31 (d, *J* = 4.4 Hz, 1H, H-1 of IdoA), 5.05 – 4.76 (m, 4H, H-2 of IdoA, H-5 of IdoA, OCH_2Ph), 4.75 – 4.64 (m, 1H, OCH_2Ph), 4.65 – 4.47 (m, 2H, OCH_2Ph, H-1 of GlcN), 4.38 (m, 1H, H-6 of GlcN), 4.20 (m, 1H, H-6 of GlcN), 4.07 – 3.85 (m, 4H, H-2 of IdoA, H-2 of GlcN, H-2 of GlcN, OCH_2 of PEG linker), 3.86 – 3.71 (m, 1H, OCH_2 of PEG linker), 3.71 – 3.45 (m, H, 11H, H-3 of IdoA, H-3 of GlcN, H-5 of GlcN, OCH_2 of PEG linker), 3.40 (s, 3H, CO_2CH_3), 2.81 – 2.74 (m, 2H, C*H*-CH=CH of Nb), 2.07 (s, 3H, $OCOCH_3$), 1.52 – 1.10 (m, 5H, C*H* and CH_2 of Nb), 0.83 (s, 9H, $SiC(CH_3)$), -0.09 (s, 3H, $SiCH_3$), -0.13 (s, 3H, $SiCH_3$); ^{13}C NMR (125 MHz; $CDCl_3$): δ 173.3, 172.0, 171.8, 139.8, 139.1, 137.7, 137.5, 102.8, 99.2, 82.1, 79.8, 77.1, 76.6, 76.2, 75.9, 75.3, 75.2, 73.2, 71.5, 71.4, 71.3, 71.0, 70.1, 69.8, 69.4, 67.3, 56.3, 52.4, 45.9, 44.9, 42.8, 40.0, 37.4, 36.0, 30.7, 23.0, 21.2, 18.7, -4.3, -5.4; ESI-TOF HRMS: *m/z* calcd for $C_{47}H_{67}NO_{20}NaSiS_2$ $[M+Na]^+$ 1080.3365; found: 1080.3392.

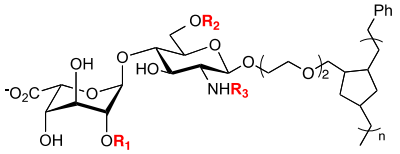


Protected HS glycopolymers (22 – 25). Monomers **18** – **21** were converted into polymers **22** – **25**, which contain the following functional groups: $R_1 = SO_3^-$, $R_2 = SO_3^-$, $R_3 = SO_3^-$ (**1**); $R_1 = SO_3^-$, $R_2 = SO_3^-$, $R_3 = Ac^-$ (**2**); R_1 = H, R_2 = H, R_3 = Ac (**3**); R_1 = H, $R_2 = SO_3^-$, $R_3 = SO_3^-$ (**4**). In a typical polymerization, a small vial was charged with monomer (**18** – **21**; 6.0 mg, 5.0 μmol) and a small stir bar under the flow of argon. To this was added degassed dichloroethane (DCE)/MeOH (10:1, 0.025 M) and *bis*-pyridine Grubbs catalyst $((H_2IMes)(Py)_2(Cl)_2Ru=CHPh)$[4] in DCE (5 mg/mL stock solution, 24 μL, 0.11 μmol) by syringe at rt. The reaction mixture was stirred at rt for 1 h, quenched with ethyl vinyl ether (0.10 mL), and diluted with diethyl ether (1.0 mL) and hexanes (0.50 mL) to obtain a white precipitate. The mixture was centrifuged to remove the organic layer, and the resulting white solid (83 – 98% conversion) was dried *in vacuo*. ^{1}H NMR confirmed disappearance of the norbornene olefinic peaks at 6.04 – 6.11 ppm. The protected polymers were characterized by size exclusion chromatography multi-angle light scattering (SEC-MALS) using a system equipped with an MZ-Gel SDplus organic column (10E5Å, MZ Analysentechnik), a light scattering detector (miniDAWN, Wyatt Technology), and a refractive index detector (TREOS, Wyatt Technology), and 0.2 M LiBr in DMF as the mobile phase. ^{1}H NMR (500 MHz; D_2O): δ 7.49 – 7.15 (m, 10H), 5.42 (br, 1H), 5.18 (br, 1H), 4.75 (br, 1H), 4.65 – 4.51 (m, 2H), 4.38 (br, 1H), 4.05 – 3.84 (m, 4H), 3.86 (br, 1H), 3.79 – 3.50 (m, 13H), 3.46 (br,

1H), 3.31 (br, 3H) 3.30 – 3.25 (m, 2H), 2.49 (br, 2H), 1.94 (br, 3H), 1.79 – 1.07 (m, 5H), 0.77 (br, 9H), -0.07 (br, 3H), -0.17 (br, 3H).



2-*O*-Sulfonato-α-L-idopyranosyluronate-(1→4)-1-*O*-(2-(2-((2*S*)bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl)-2-deoxy-2-sulfonatamido-6-*O*-sulfonato-α-D-glucopyranoside (26). Compound **18** (14 mg, 0.013 mmol) was dissolved in THF (1.3 mL), and TMSOK (33 mg, 0.26 mmol) was added to the reaction mixture. The reaction was stirred for 24 h at rt and quenched with MeOH (1.0 mL). The crude reaction mixture was loaded directly onto a Sephadex G-25 gel filtration column and eluted with 100% H_2O, and fractions were combined, lyophilized, and subjected to hydrogenation. The intermediate was dissolved in a 3:2 mixture of 80 mM phosphate buffered saline (2.4 mL, pH = 7.0) and MeOH (0.80 mL). To this, $Pd(OH)_2$/charcoal (80 mg, 8x by weight of starting material) was added, and the reaction was carried out under 1 atm H_2 gas for 3 d. The reaction mixture was filtered using a vacuum filtration system (0.45 μm PES membrane, VWR) and desalted on a Sephadex G-25 column in 100% H_2O to obtain the desired product 60% yield after lyophilization. ^{1}H NMR (500 MHz, D_2O) δ 5.24 (s, 1H, H-1 of IdoA), 4.83 (d, *J* = 2.7 Hz, 1H, H-5 of IdoA), 4.73 (d, *J* = 8.2 Hz, 1H, H-1 of GlcN), 4.44 (m, 1H, H-6 of GlcN), 4.34 (m, 2H, H-6 of GlcN, H-2 of IdoA), 4.10 (m, 2H, H-3 of IdoA, H-4 of IdoA), 4.01 – 3.71 (m, 11H, OCH_2 of PEG linker, H-3 of GlcN, H-4 of GlcN, H-5 of GlcN), 3.44 – 3.31 (m, 2H, OCH_2 of PEG linker), 3.16 (m, 1H, H-2 of GlcN), 2.28 – 2.20 (m, 2H, bridgehead CH_2 of Nb), 1.82 (s, 2H, CH_2 of Nb), 1.59 (s, 2H, CH_2 of Nb), 1.48 – 1.36 (m, 2H, C*H* of Nb), 1.27 – 1.19 (m, 2H, CH_2 of Nb), 1.08 (s, 1H, C*H* of Nb); ESI-TOF HRMS: *m/z* calcd for $C_{24}H_{37}Na_3NO_{22}S_3$ $[M+3Na-H]^{2+}$ 856.0662; found: 856.0624.



Deprotected HS glycopolymers (1 – 4). Polymers **22** – **25** were deprotected to obtain final polymers **1** – **4**, which contain the following functional groups: $R_1 = SO_3^-$, $R_2 = SO_3^-$, $R_3 = SO_3^-$ (**1**); $R_1 = SO_3^-$, $R_2 = SO_3^-$, $R_3 = Ac^-$ (**2**); $R_1 = H$, $R_2 = H$, $R_3 = Ac$ (**3**); $R_1 = H$, $R_2 = SO_3^-$, $R_3 = SO_3^-$ (**4**). In a typical reaction, polymer (11 mg, 10 μmol per unit) was dissolved in THF (1.0 mL), and

TBAI (7.0 mg, 20 μmol) and TMSOK (25 mg, 0.20 mmol) were added. The reaction was stirred for 24 h at rt and quenched with MeOH (1.0 mL). The crude reaction mixture was loaded directly onto a Sephadex G-25 gel filtration column and eluted with 100% H_2O. The polymer-containing fractions were combined, lyophilized, and subjected to hydrogenation. In a typical hydrogenation reaction, the polymer from the previous reaction was dissolved in a 3:2 mixture of 80 mM phosphate buffered saline (0.9 mL, pH = 7.0) and MeOH (0.60 mL). To this, $Pd(OH)_2$/charcoal (84 mg, 8x weight of polymer) was added, and the reaction was carried out under 1 atm H_2 gas for 3 d. Samples were filtered using a vacuum filtration system (0.45 μm PES membrane, VWR) and desalted on a Sephadex G-25 column in 100% H_2O to obtain the desired polymers in 35 – 55% yield after lyophilization. ^{1}H NMR showed disappearance of the benzyl and methyl ester peaks at 7.79 – 7.03 ppm and 3.40 ppm, respectively. Deprotected polymers were characterized by SEC-MALS using a system equipped with an OHpak water column (SB-804 HQ, Shodex), a light scattering detector (miniDAWN, Wyatt Technology), and a refractive index detector (TREOS, Wyatt Technology), and 3 mM NaN_3 and 6 mM $NaNO_3$ in H_2O as the mobile phase. ^{1}H NMR (500 MHz; D_2O): δ 5.03 (br, 1H), 4.44 (br, 1H), 4.25 – 4.20 (m, 1H), 4.18 – 4.11 (m, 2H), 3.92 (br, 1H), 3.84 (br, 2H), 3.75 – 3.40 (m, 12H), 3.34 (br, 1H), 3.19 (br, 1H), 1.91 (br, 3H), 1.72 (br, 1H), 1.48 – 0.88 (m, 6H).

3-3. Direct and Competitive Enzyme-Linked Immunosorbent Assay (ELISA)

A 96-well heparin-binding plate (BD Biosciences) was coated with 25 μg/mL of heparin (Neoparin) for 12 h at rt. Wells were rinsed with phosphate-buffered saline (PBS) and blocked with 10% fetal bovine serum (FBS) in PBS for 1 h at 37 °C. For the direct ELISA, various concentrations of RANTES (0.50 – 1024 nM; R&D Systems) were serially diluted in 1% BSA in PBS and incubated in each well for 1.5 h at 37 °C. For the competitive ELISA, RANTES (at 12 nM, the pre-determined EC_{50}; Figure S1) was pre-incubated (3 h, 37 °C) with various concentrations of heparin (0.010 – 40 μg/mL) or glycopolymers **1** – **4** (0.10 – 180 μg/mL), and the co-mixture was added to the 96-well plate for 1.5 h at 37 °C. Wells were washed three times with PBST (PBS + 0.1% Tween-20), incubated with a mouse anti-RANTES antibody (R&D Systems) for 1 h at 37 °C, washed three times with PBST, and incubated with a horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibody (GE Healthcare Life Sciences) for 1 h at 37 °C. After three washes with PBST, RANTES binding was detected using a 3,3',5,5'-tetramethylbenzidine (TMB) substrate kit (Thermo Scientific) according to the manufacturer's instructions. Fluorescence was measured at 450 nm using a Victor 3 plate reader (PerkinElmer). The half-maximal effective concentration (EC_{50}) and half maximal inhibitory concentration (IC_{50})

were calculated using KaleidaGraph software (Synergy). IC_{50} values reported in the paper are for both the mass and molar concentrations of antagonist. IC_{50} values were also corrected for ligand valence (Table S2) by calculating the mass percentage of the disaccharide epitope contributing to each disaccharide-norbornyl linker unit, and then dividing by the molecular weight of the disaccharide epitope.

3-4. Cell Culture

L1.2 cells (mouse pre-B lymphocytes) stably transfected with CCR3, CCR5, or vector only, were kindly provided by Dr. Osamu Yoshie (Kinki University, School of Medicine, Japan). Cells were maintained in RPMI 1640 (Invitrogen) supplemented with 10% FBS, 100 μg/mL penicillin/streptomycin (Invitrogen), and 50 μM 2-mercaptoethanol (Sigma Aldrich). Cells were routinely analyzed by flow cytometry (FACSCalibur, Beckman Dickenson; see section 2-6) to verify that cultures expressed adequate levels of chemokine receptor (>90%) for migration and cell binding assays.

3-5. Cell Migration Assay

Experiments were performed using ChemoTx chambers (Neuroprobe). L1.2 cells (wild-type or stably-transfected with CCR3 or CCR5) were harvested and washed twice in flow cytometry buffer (Hank's Balanced Salt Solution (HBSS) with 2.5 mg/mL bovine serum albumin (BSA) and 10 mM HEPES). Human RANTES (R&D Systems) was serially diluted in flow cytometry buffer (0.5 – 1024 nM), and 30 μL of each dilution was added to the bottom wells of the ChemoTx chamber. Alternatively, in competitive migration assays, 1 or 10 nM of RANTES was pre-incubated with various concentrations of heparin or glycopolymer **1** (0.020 – 4.0 μg/mL) for 30 min at rt, and the same volume of each solution was added to the bottom wells. The sample plate was fitted with a 5-μm pore filter, and 10^6 cells (50 μL) were placed on top of each well. Cells were allowed to migrate through the filter for 4 h at 37 °C and 5% CO_2. Subsequently, non-migrating cells were removed from the top of the filter by manual scraping; cells adhering to the filter were dislodged using 20 μL of 2.5 mM EDTA for 30 min at rt. Migrated cells were transferred (500 x *g*, 5 min) to a 96-well black-walled clear-bottomed plate (Corning) using a funnel plate (Neuroprobe). Cells were lysed at -80 °C and stained with CyQUANT dye (Invitrogen) as described in the product literature. Fluorescence was measured at 535 nm using a Victor 3 plate reader (PerkinElmer).

3-6. Chemokine Cell Binding Assay

3 x 10^6 L1.2 cells (wild-type or stably-transfected with CCR3) were washed twice with flow cytometry buffer and incubated with RANTES (100 nM in flow cytometry buffer) for 45 min at rt. Alternatively, cells were incubated with RANTES (100 nM in flow cytometry buffer) previously treated with various concentrations of heparin or glycopolymer **1** (0.02 – 2 μg/mL) for 30 min at rt. Cells were spun twice (500 x g, 5 min) through 100% FBS (1.0 mL) to remove excess reagent and stained with phycoerythrin (*PE*)-conjugated anti-RANTES (1 test) in FACS buffer (100 μL) for 1 h at 4 ℃. Cells were again spun twice through 100% FBS (1.0 mL) and resuspended in flow cytometry buffer (500 μL) for flow cytometry analysis. Immediately before analysis, 7-amino-actinomycin-D (7-AAD, 5 μL, eBioscience) was added to evaluate cell viability. Cells were analyzed for PE intensity on a FACSCalibur flow cytometer (Beckman Dickenson, Caltech Flow Cytometry Facility) with 10,000 cell events per sample. Data analysis was performed using FlowJo (Tree Star Inc.).

3-7. Chromogenic Assay for the Measurement of Antithrombin Activity

Factor Xa Activity: The BIOPHEN Heparin Anti-Xa (2 stages) USP/EP kit (Aniara) was used to determine factor Xa activity. This chromogenic anti-Xa method for measuring homogeneous heparin in purified systems is in compliance with Pharmacopoeias (USP, EP) and FDA guidelines. All reagents were prepared according to manufacturer's instructions and incubated at 37 °C for 15 min. Varying concentrations of heparin (Neoparin) or glycopolymers **1** – **4** (40 uL) and antithrombin (40 uL) were added to a microcentrifuge tube, mixed, and incubated at 37 °C for 2 min. Factor Xa (40 uL) was added to the solution, incubated at 37 °C for exactly 2 min, and then the factor Xa chromogenic substrate (40 μL) was added. After 2 min, the reaction was quenched with citric acid (20 g/L, 240 μL), and the absorbance was measured at 405 nm. The sample blank was obtained by mixing the reagents in reverse order, and the resulting value was deducted from the absorbance values measured in the assay.

Thrombin (Factor IIa) Activity: The BIOPHEN Heparin Anti-IIa (2 stages) USP/EP kit (Aniara) was used to determine factor IIa activity. This chromogenic anti-IIa method was conducted according to the same procedure used for factor Xa.

4. References

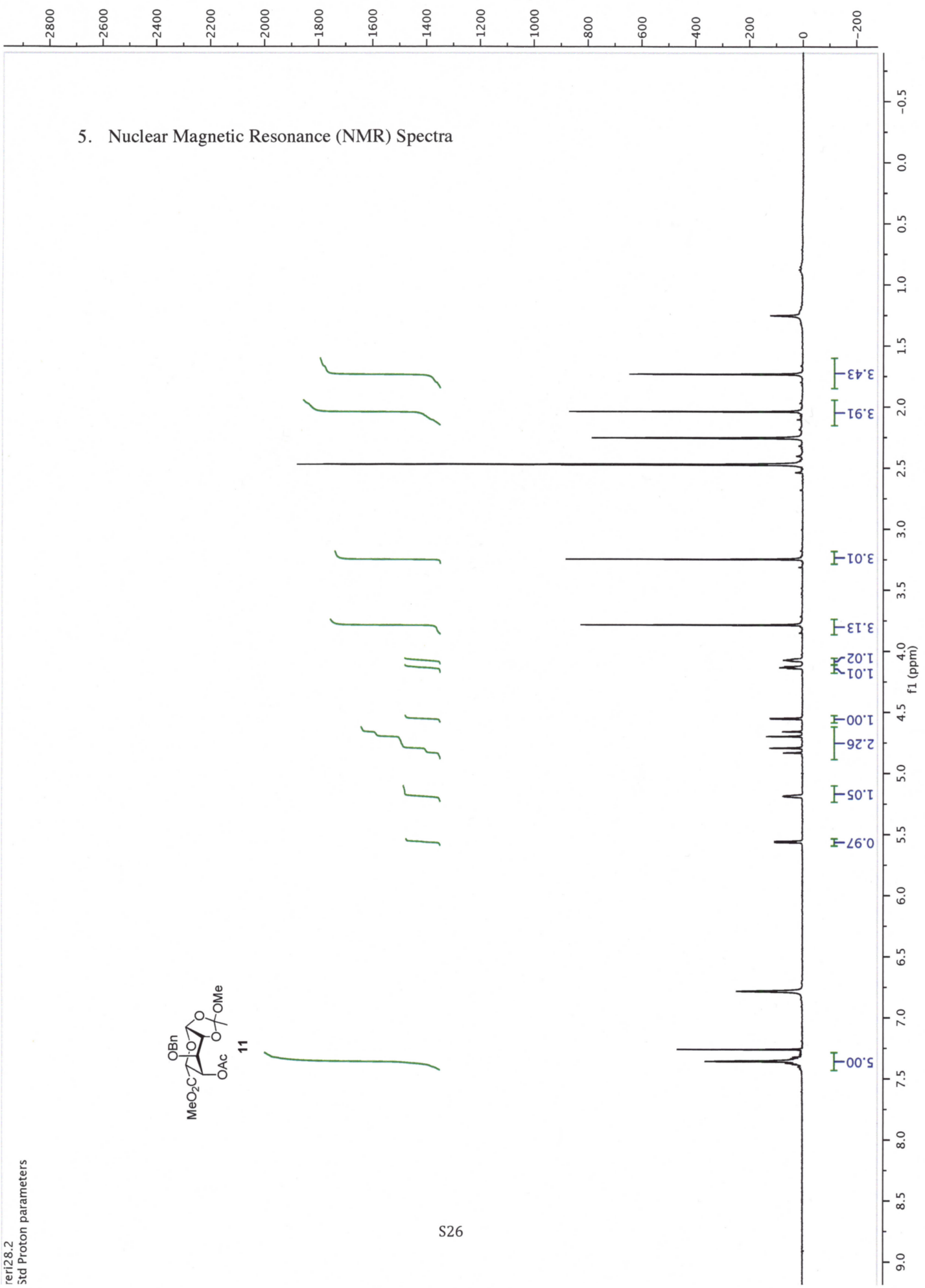
(1) (a) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem. Eur. J.* **2003**, *9*, 140. (b) Lohman, G. J. S.; Hunt, D. K.; Hogermeier, J. A.; Seeberger, P. H. *J. Org. Chem.* **2003**, *68*, 7559.

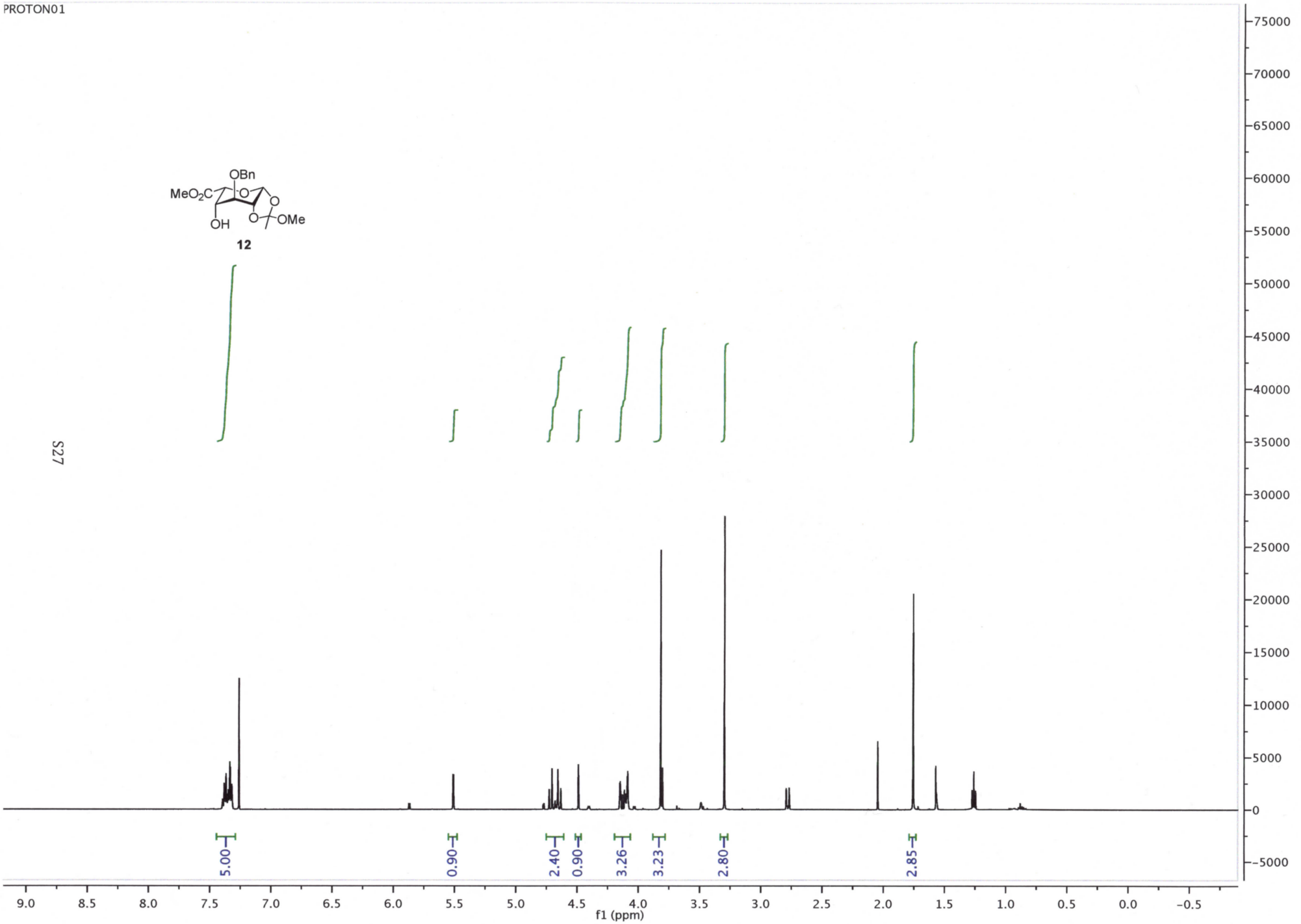
(2) Lee, S. G.; Brown, J. M.; Rogers, C. J.; Matson, J. B.; Krishnamurthy, C.; Rawat, M.; Hsieh-Wilson, L. C. *Chem. Sci.* **2010**, *1*, 322.

(3) Gavard, O.; Hersant, Y.; Alais, J.; Duverger, V.; Dilhas, A.; Bascou, A.; Bonnaffe, D. *Eur. J. Org. Chem.* **2003**, *2003*, 3603.

(4) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.

5. Nuclear Magnetic Resonance (NMR) Spectra

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OH
OMe
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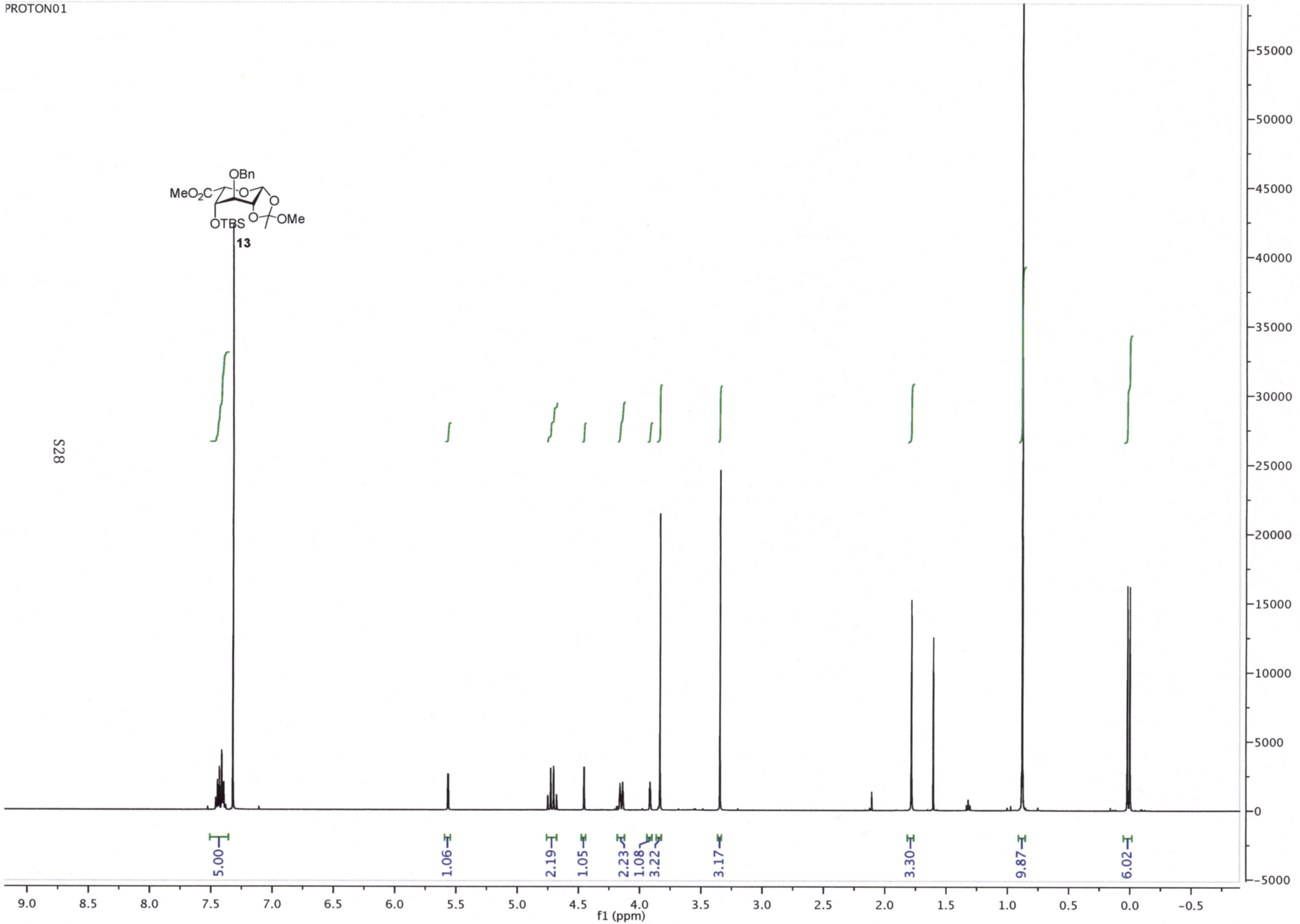
0.90
3.26
3.23
2.80
2.85
f1 (ppm)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OBn
MeO2C
OTBS
OMe
13
S28
5.00
1.06
2.19
1.05
2.23
1.08
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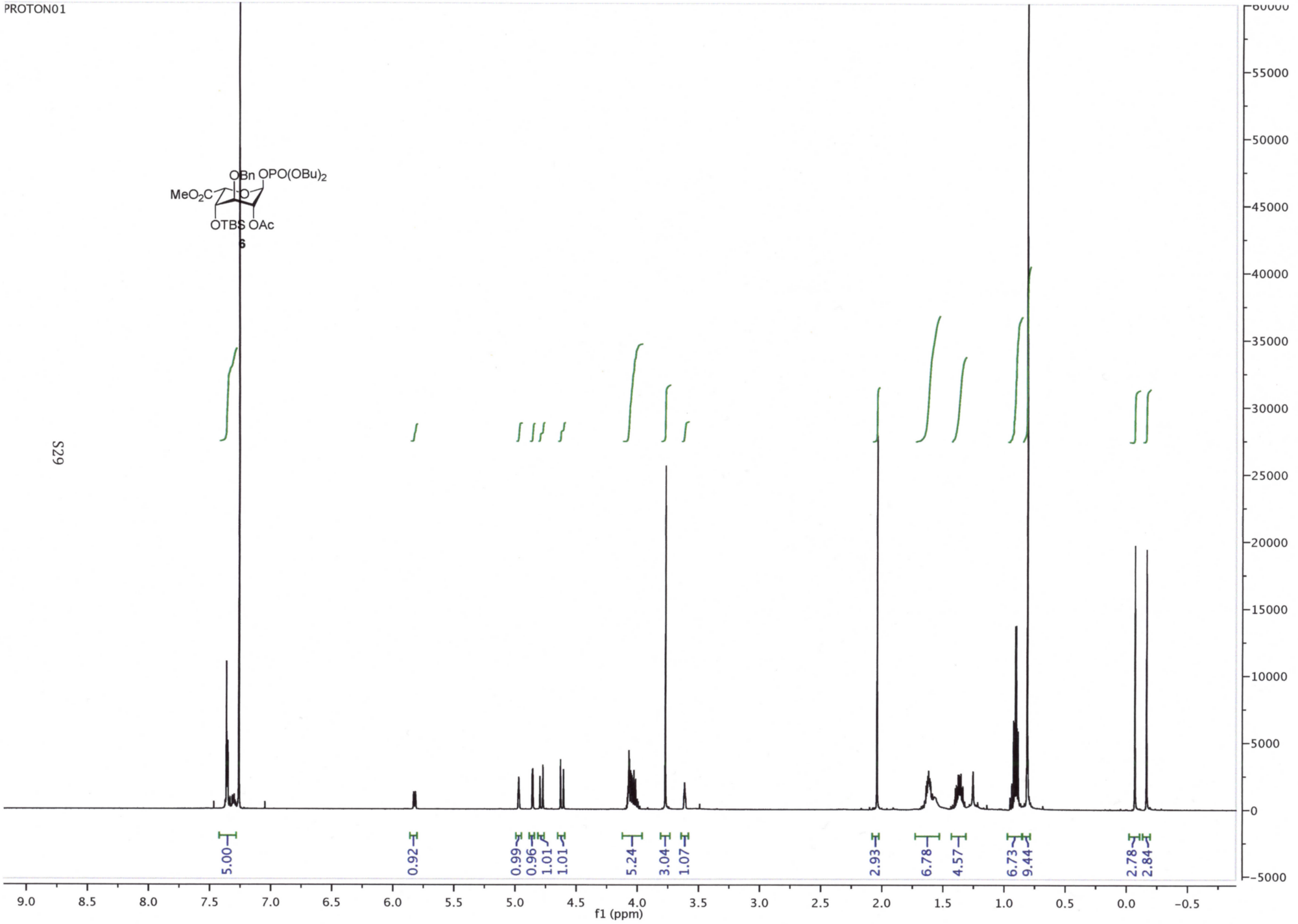
3.17
3.30
9.87
6.02
f1 (ppm)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OTBS OAc
6
S29
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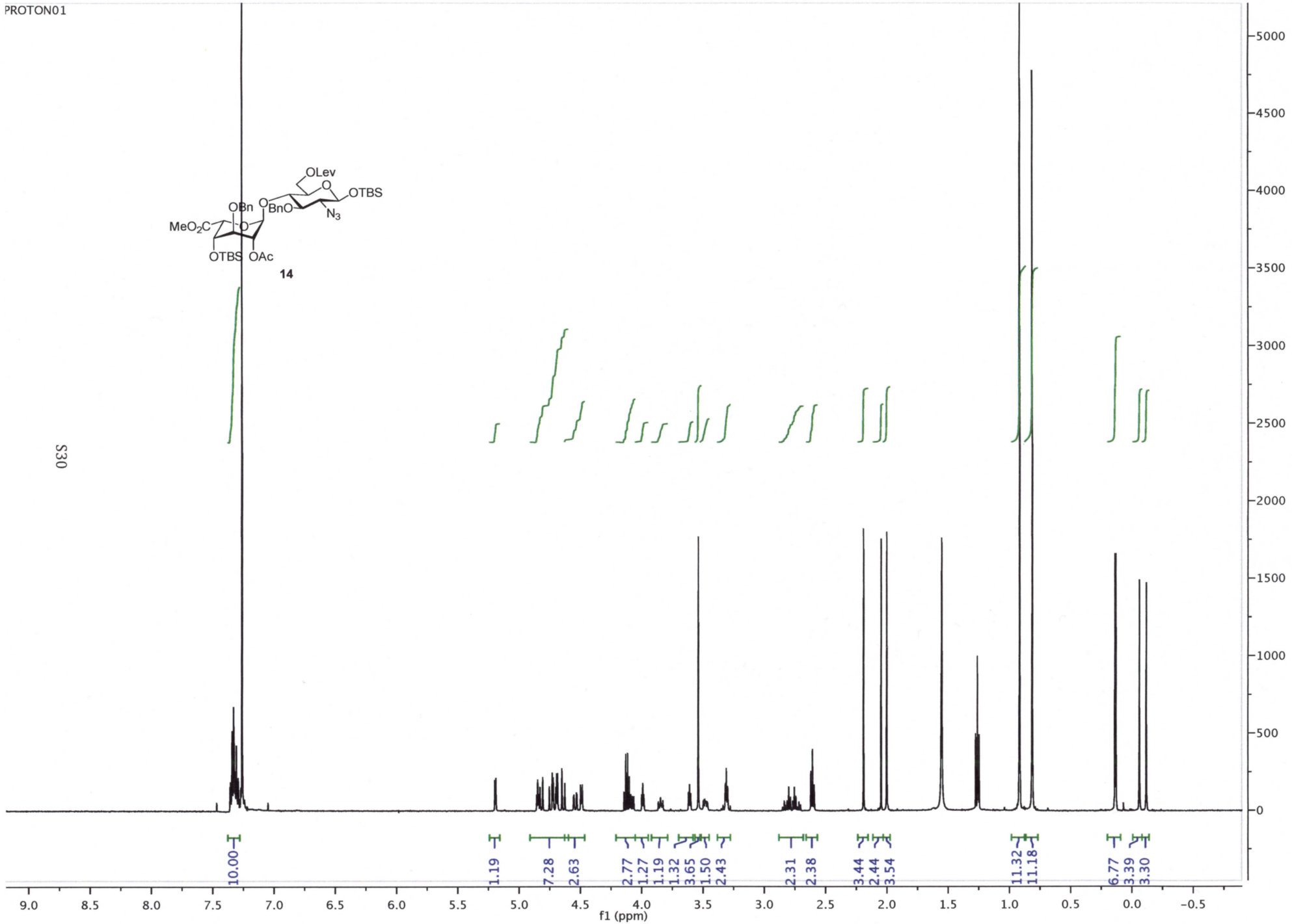
0.92
0.99
0.96
1.01
1.01
5.24
3.04
1.07
2.93
6.78
4.57
6.73
9.44
2.78
2.84
f1 (ppm)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OBn
BnO
N_3
MeO_2C
OTBS
OAc
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S30
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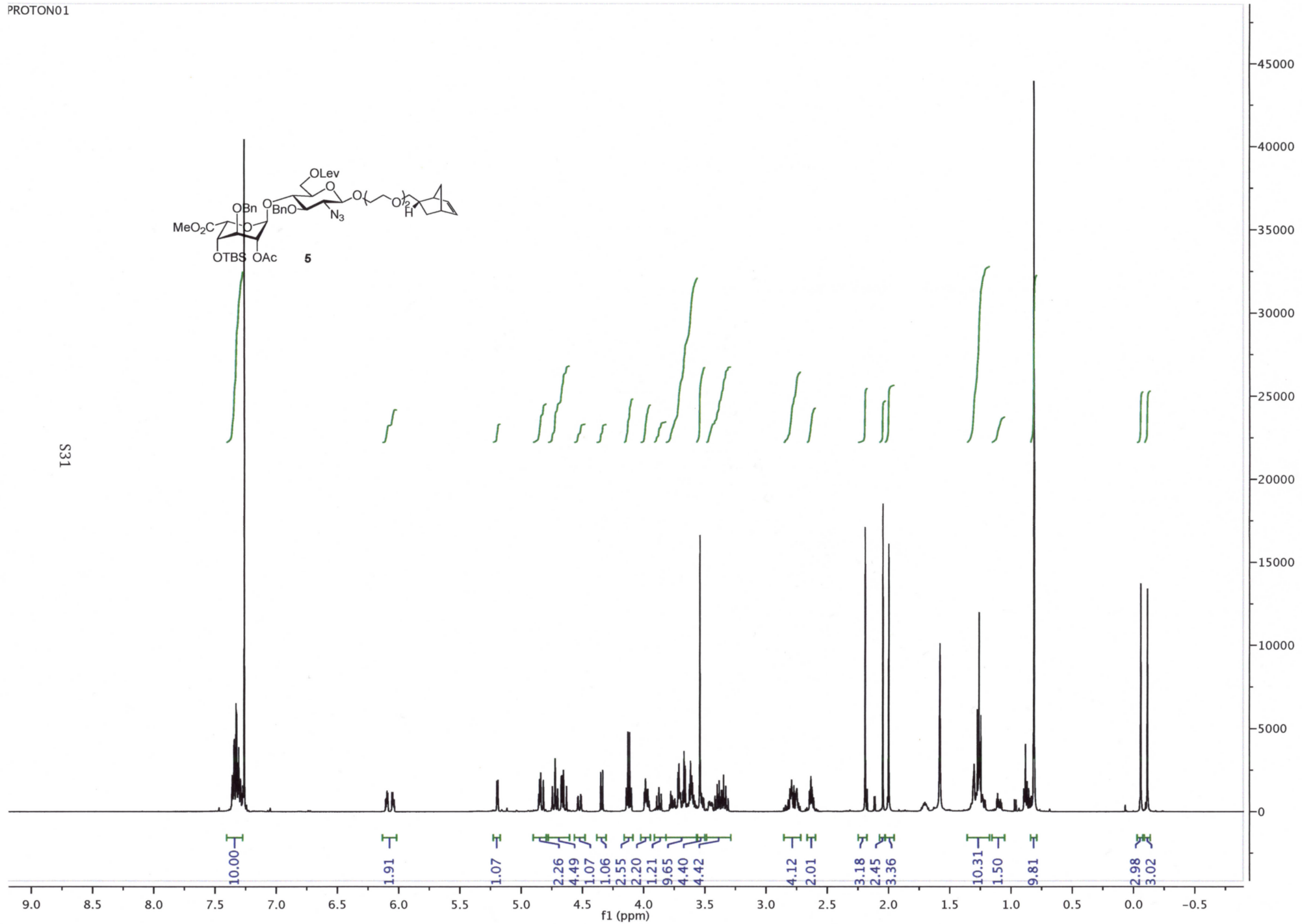
1.19
7.28
2.63
2.77
1.27
1.19
1.32
3.65
1.50
2.43
2.31
2.38
3.44
2.44
3.54
11.32
11.18
6.77
3.39
3.30
f1 (ppm)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BnO
N_3
MeO_2C
OTBS
OAc
5
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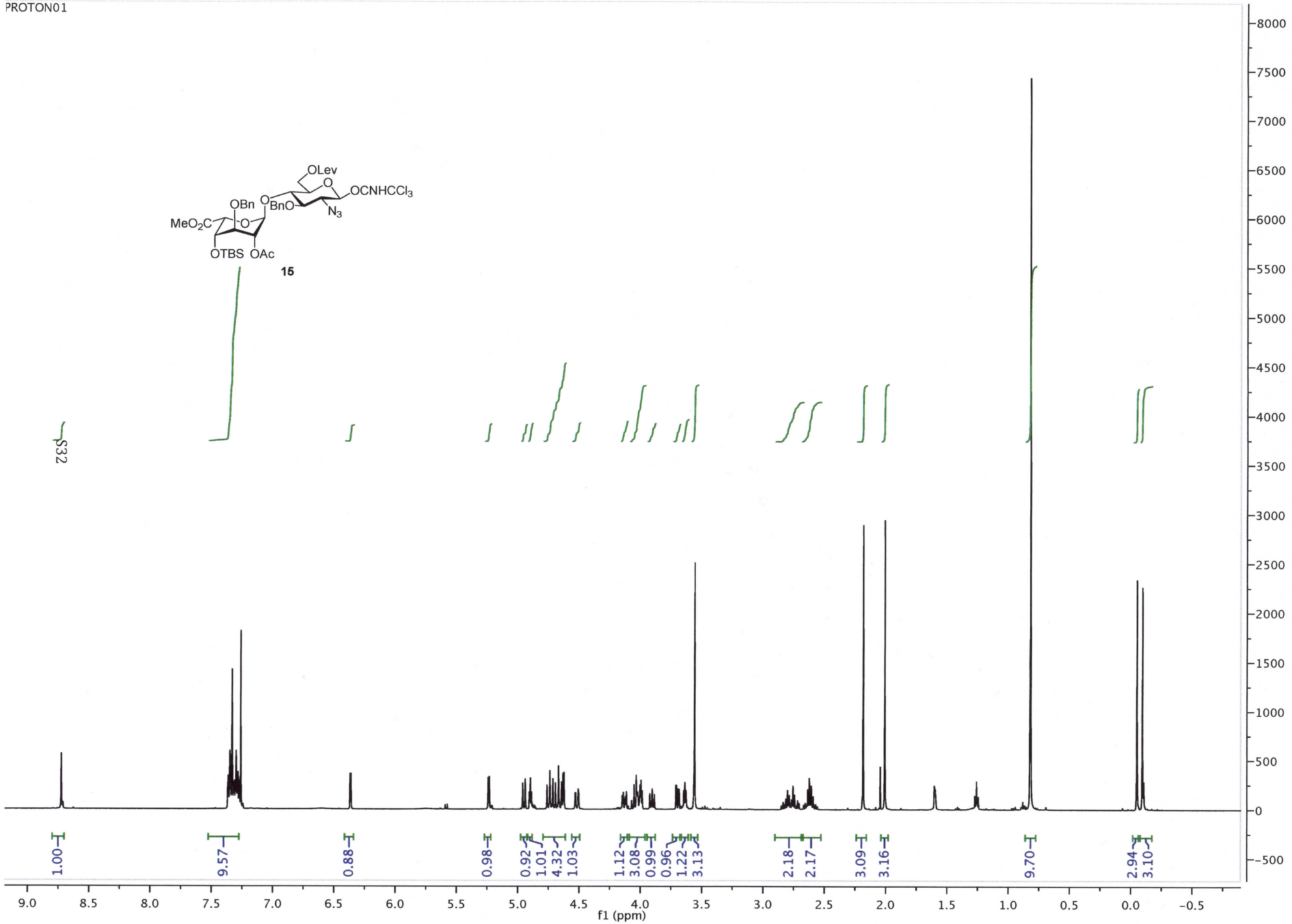
40000
35000
30000
25000
20000
15000
10000
5000
0
10.00
1.91
1.07
2.26
4.49
1.07
1.06
2.55
2.20
1.21
9.65
4.40
4.42
4.12
2.01
3.18
2.45
3.36
10.31
1.50
9.81
2.98
3.02
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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OLev
OCNHCCl3
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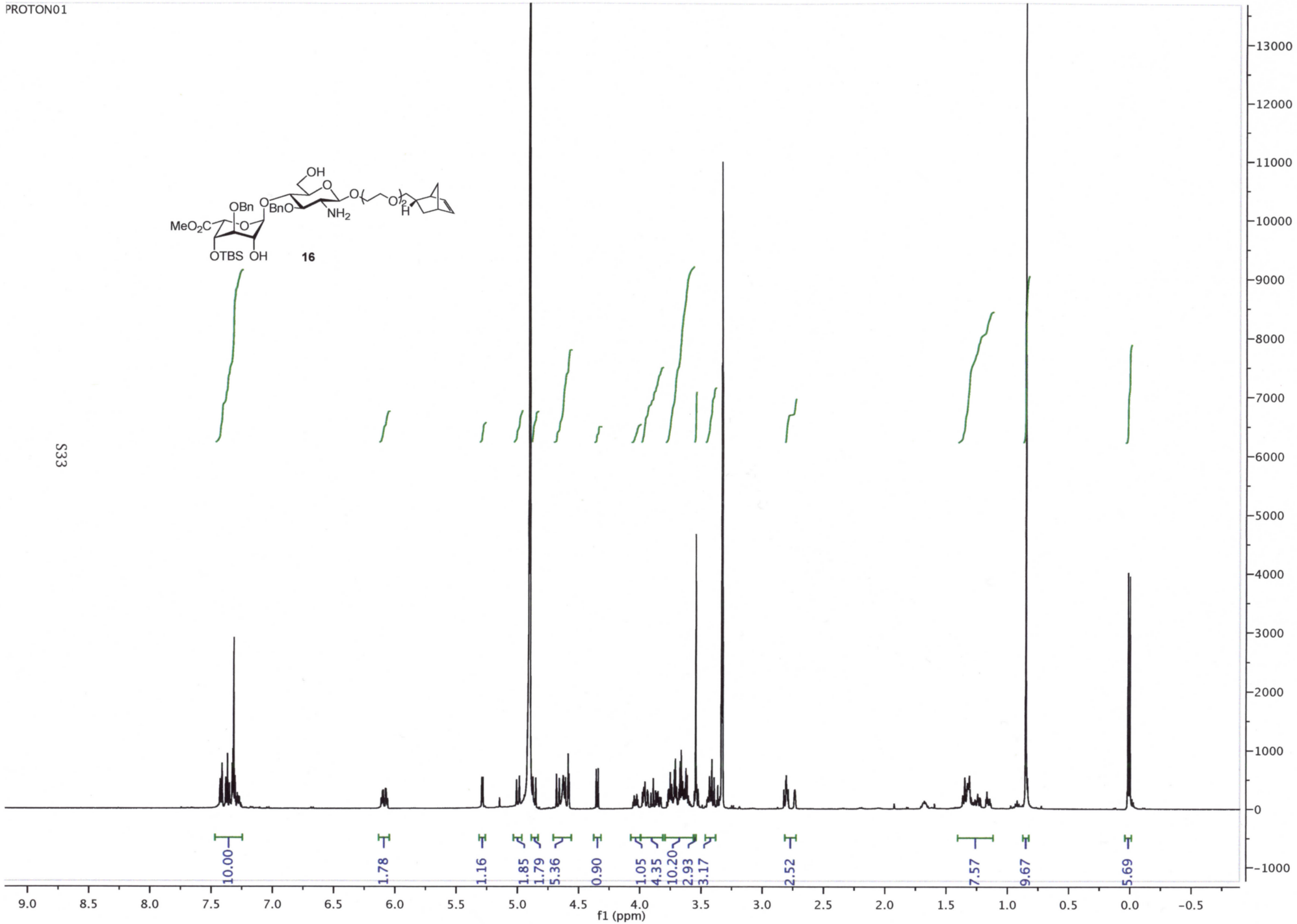
BnO
N3
MeO2C
OTBS OAc
15
S32
1.00
9.57
0.88
0.98
0.92
1.01
4.32
1.03
1.12
3.08
0.99
0.96
1.22
3.13
2.18
2.17
3.09
3.16
9.70
2.94
3.10
f1 (ppm)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MeO2C
OTBS OH
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S33
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1.16
1.85
1.79
5.36
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4.35
10.20
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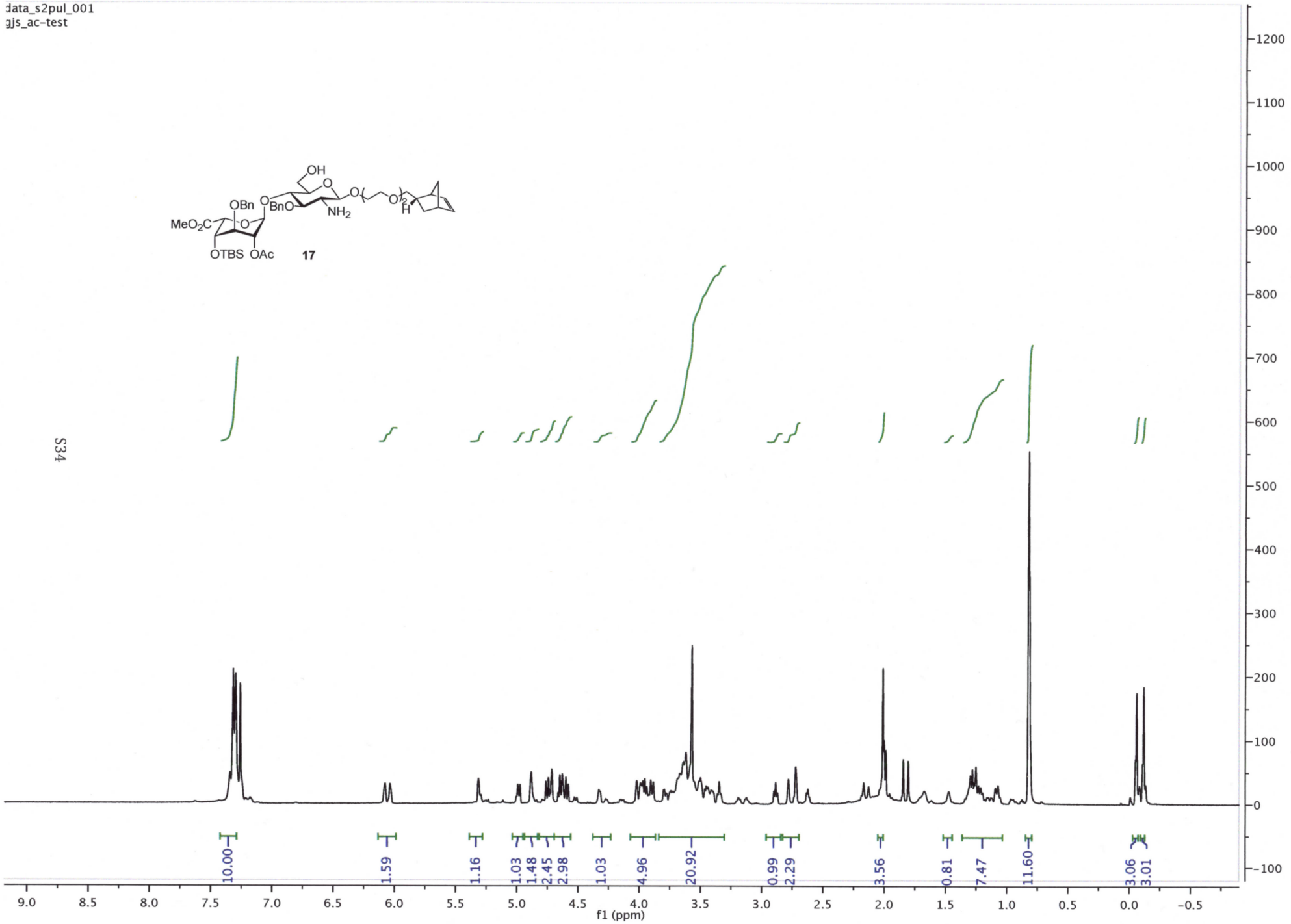
2.52
7.57
9.67
5.69
f1 (ppm)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gjs_ac-test
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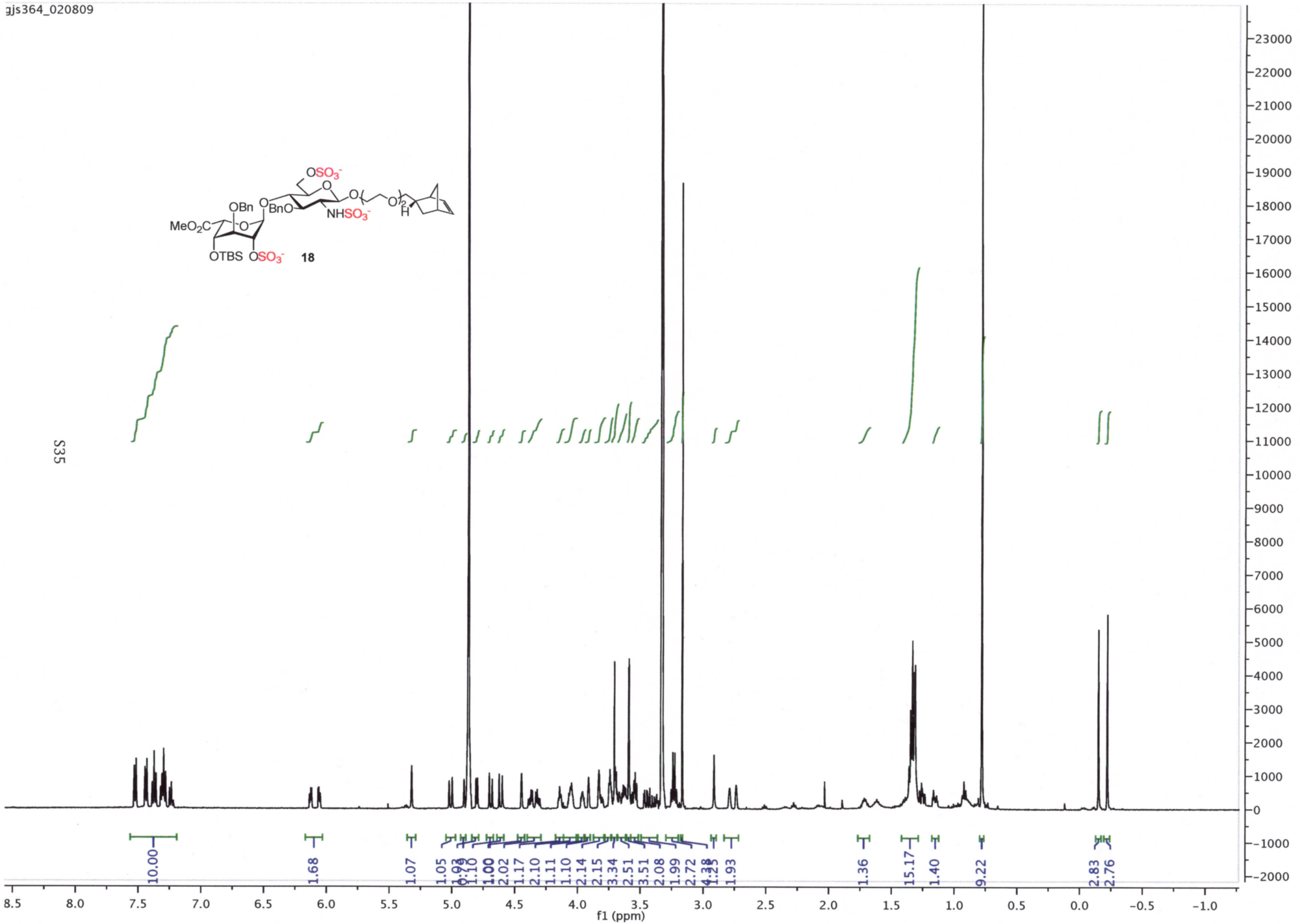
OBn
O
BnO
NH2
O
O
2
H
MeO2C
O
OTBS OAc
17
10.00
1.59
1.16
1.03
1.48
2.45
2.98
1.03
4.96
20.92
0.99
2.29
3.56
0.81
7.47
11.60
3.06
3.01
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100


gjs364_020809
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BnO
NHSO3-
MeO2C
OTBS
OSO3-
18
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9.22
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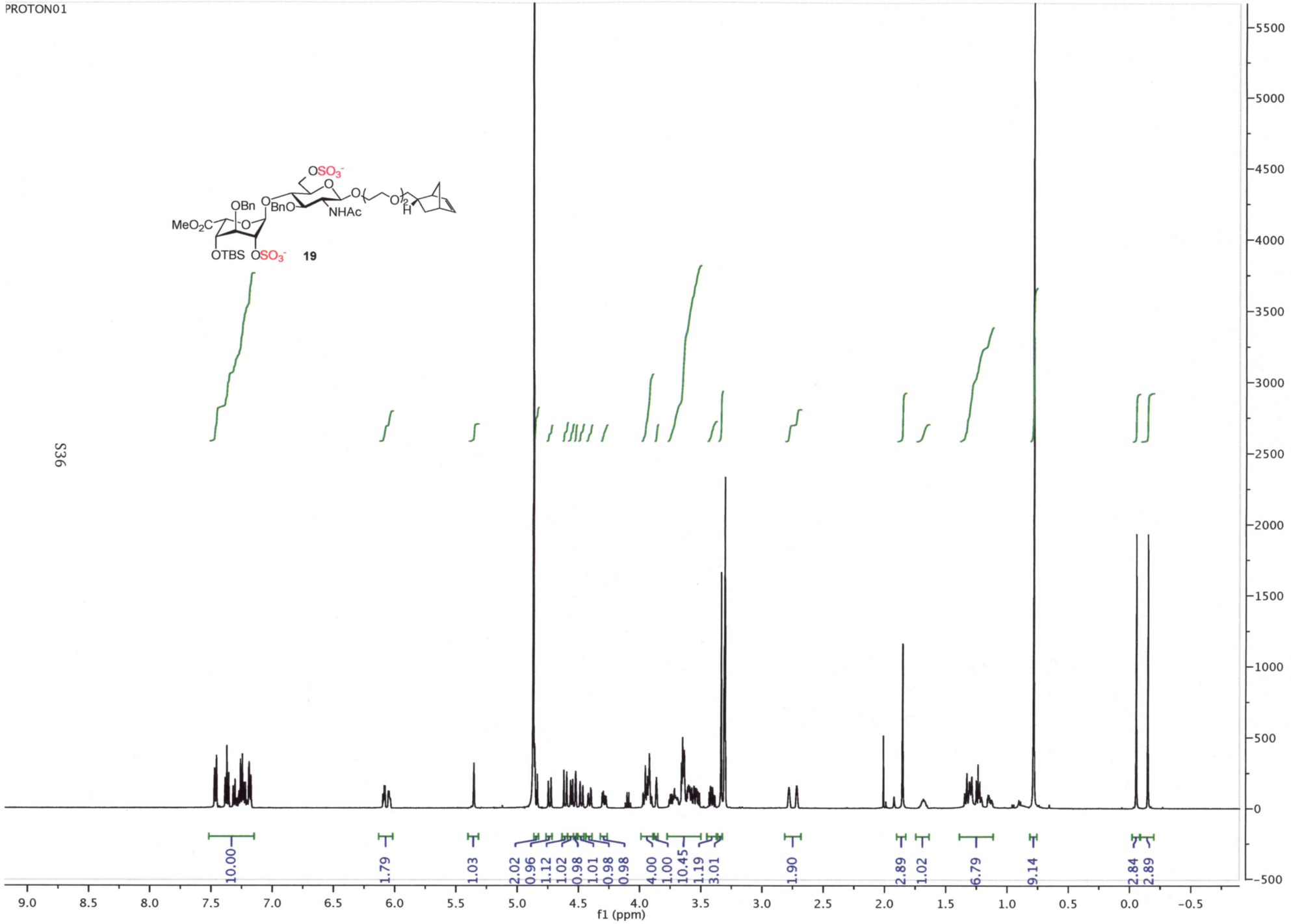
2.76
f1 (ppm)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OSO3-
OBn
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NHAc
MeO2C
OTBS
OSO3-
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S36
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2.02
0.96
1.12
1.02
0.98
1.01
0.98
0.98
4.00
1.00
10.45
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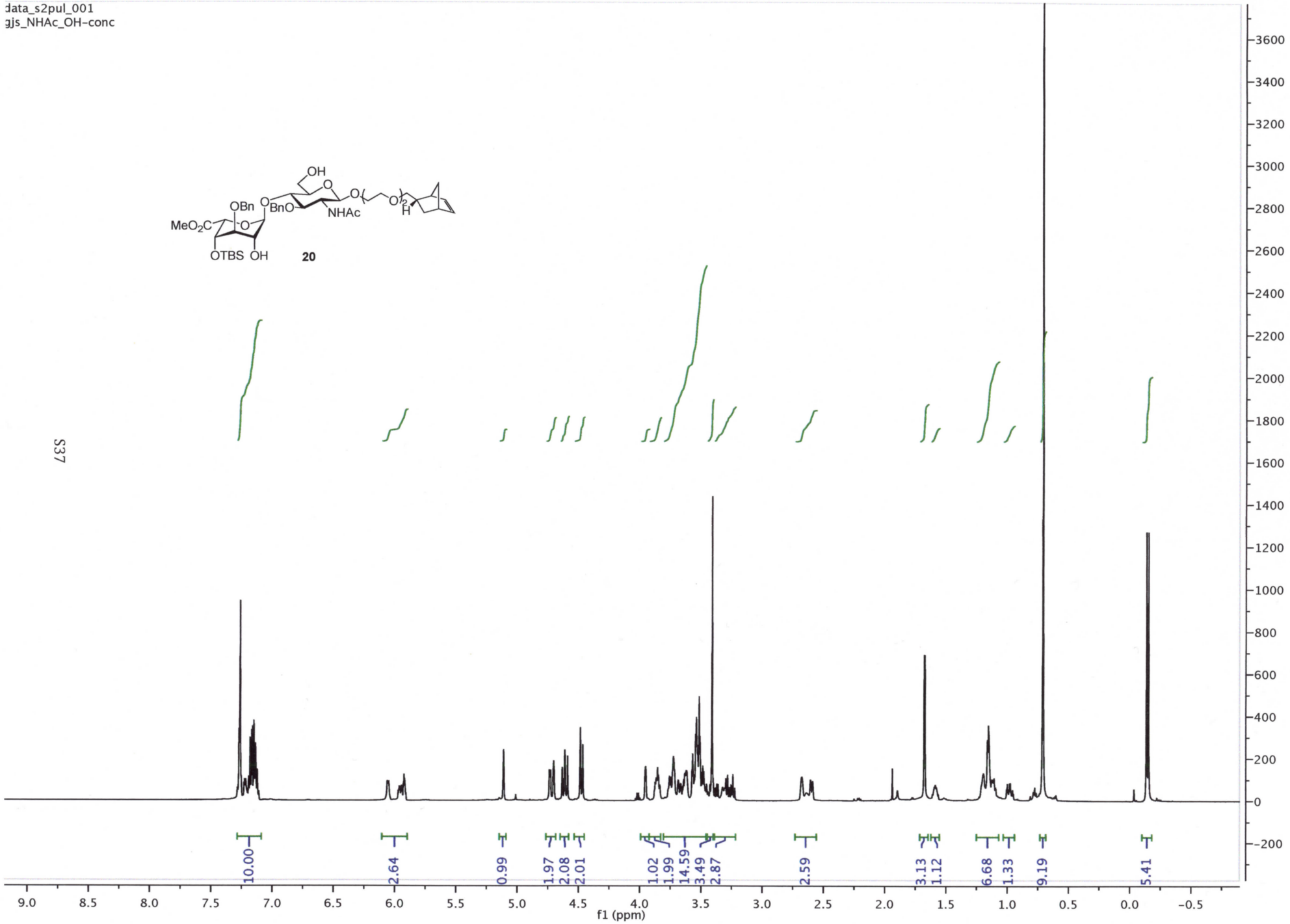
3.01
1.90
2.89
1.02
6.79
9.14
2.84
2.89
f1 (ppm)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NHAc
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OTBS OH
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2.64
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1.97
2.08
2.01
1.02
1.99
14.59
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2.87
2.59
3.13
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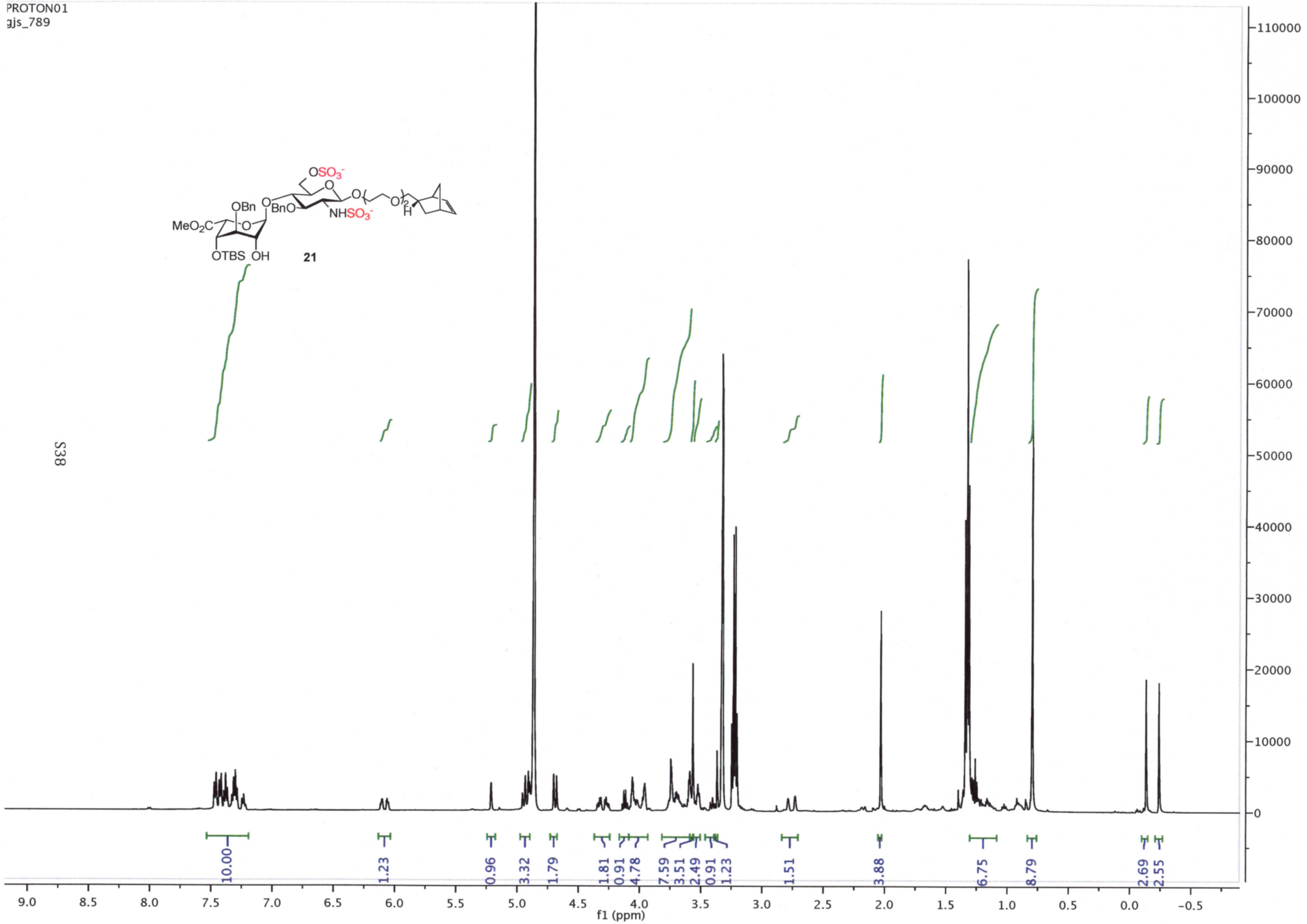
6.68
1.33
9.19
5.41
f1 (ppm)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BnO
NHSO3-
MeO2C
OTBS OH
21
S38
10.00
1.23
0.96
3.32
1.79
1.81
0.91
4.78
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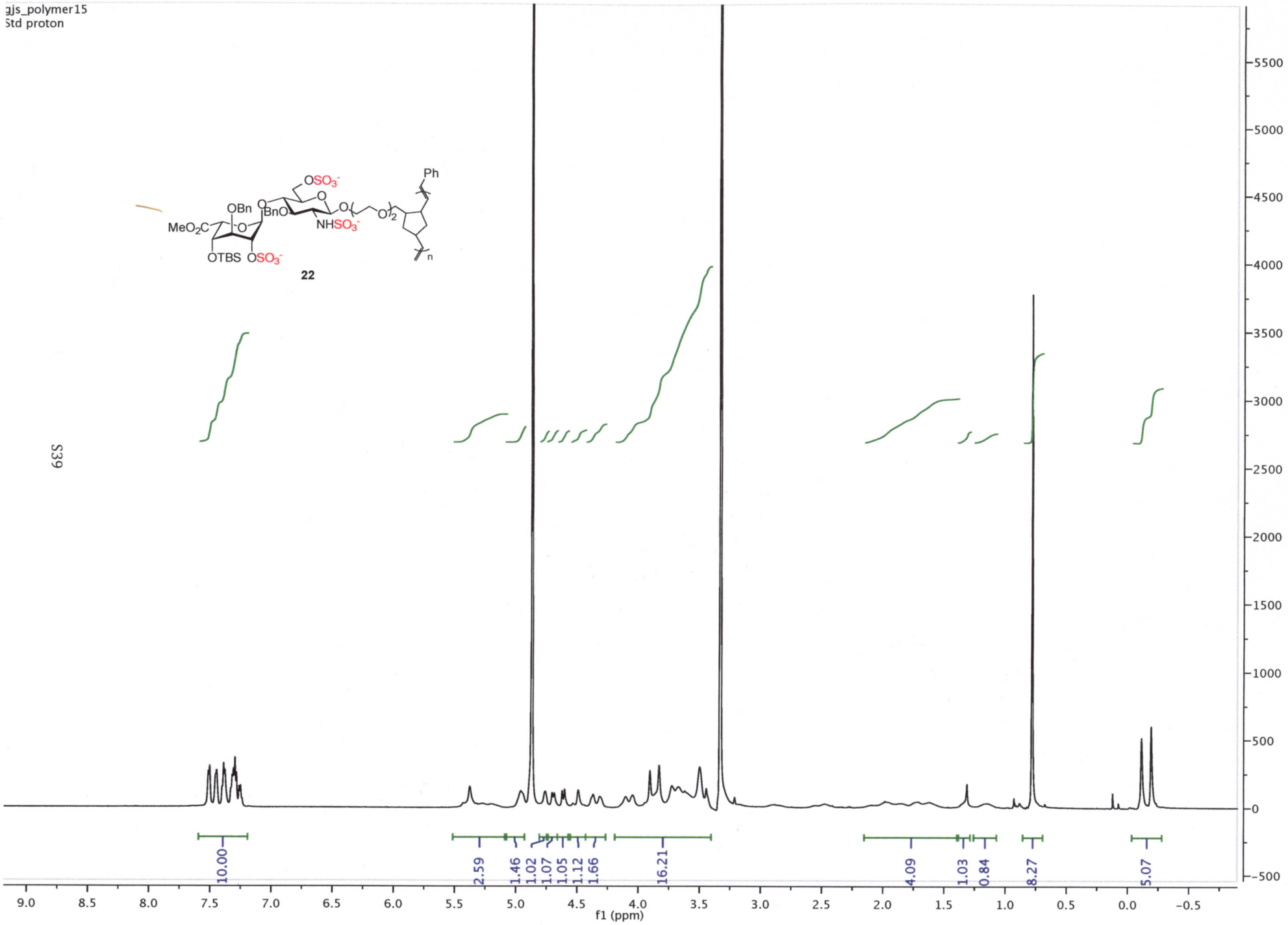
8.79
2.69
2.55
f1 (ppm)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Std proton
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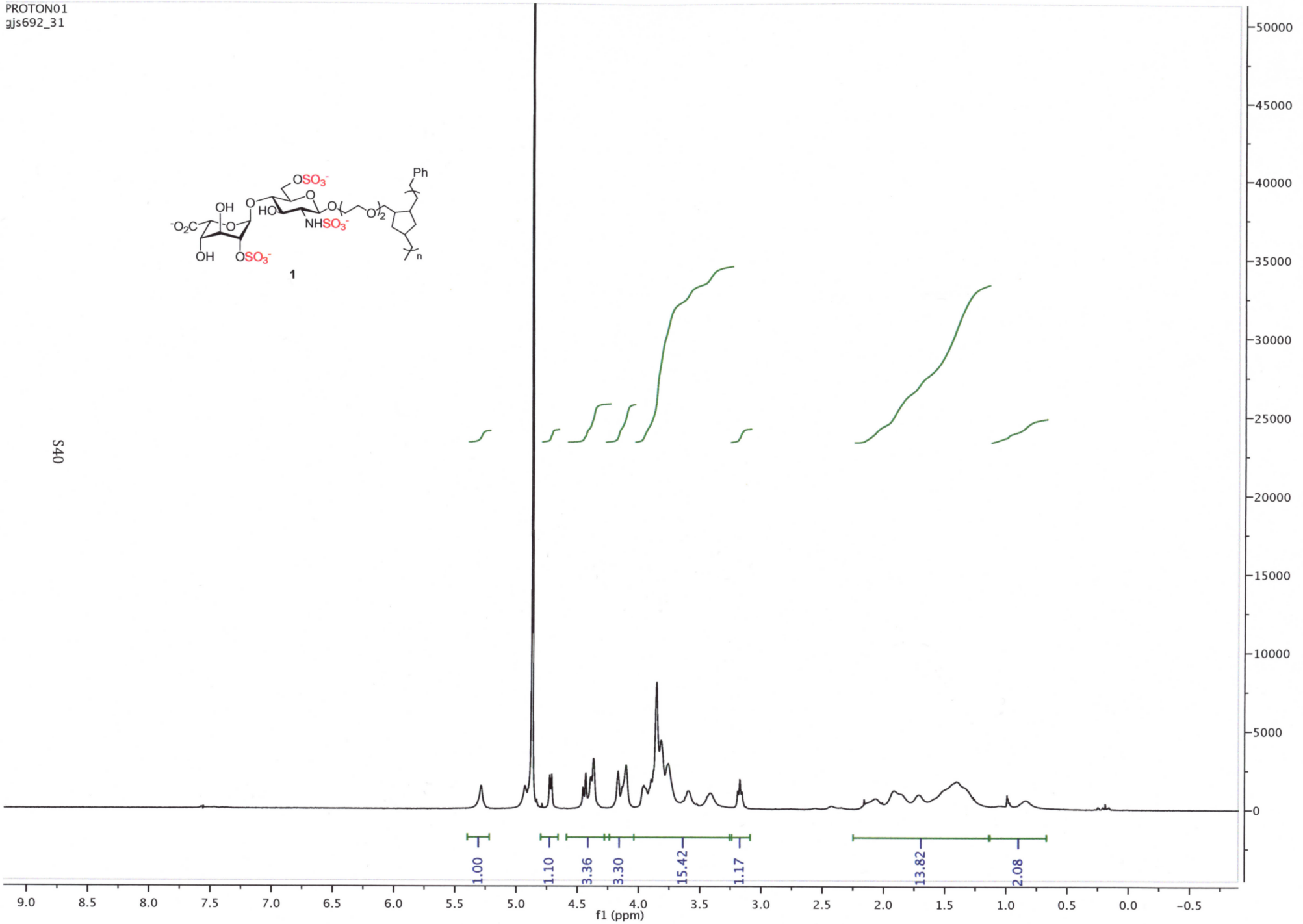
Ph
OBn
BnO
NHSO3-
MeO2C
OTBS
OSO3-
22
S39
10.00
2.59
1.46
1.02
1.07
1.05
1.12
1.66
16.21
4.09
1.03
0.84
8.27
5.07
f1 (ppm)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gjs692_31
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Ph
OH
O
HO
O
O
O2
NHSO3-
-O2C
O
OH
OSO3-
n
1
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
1.00
1.10
3.36
3.30
15.42
1.17
13.82
2.08
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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3.0
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2.0
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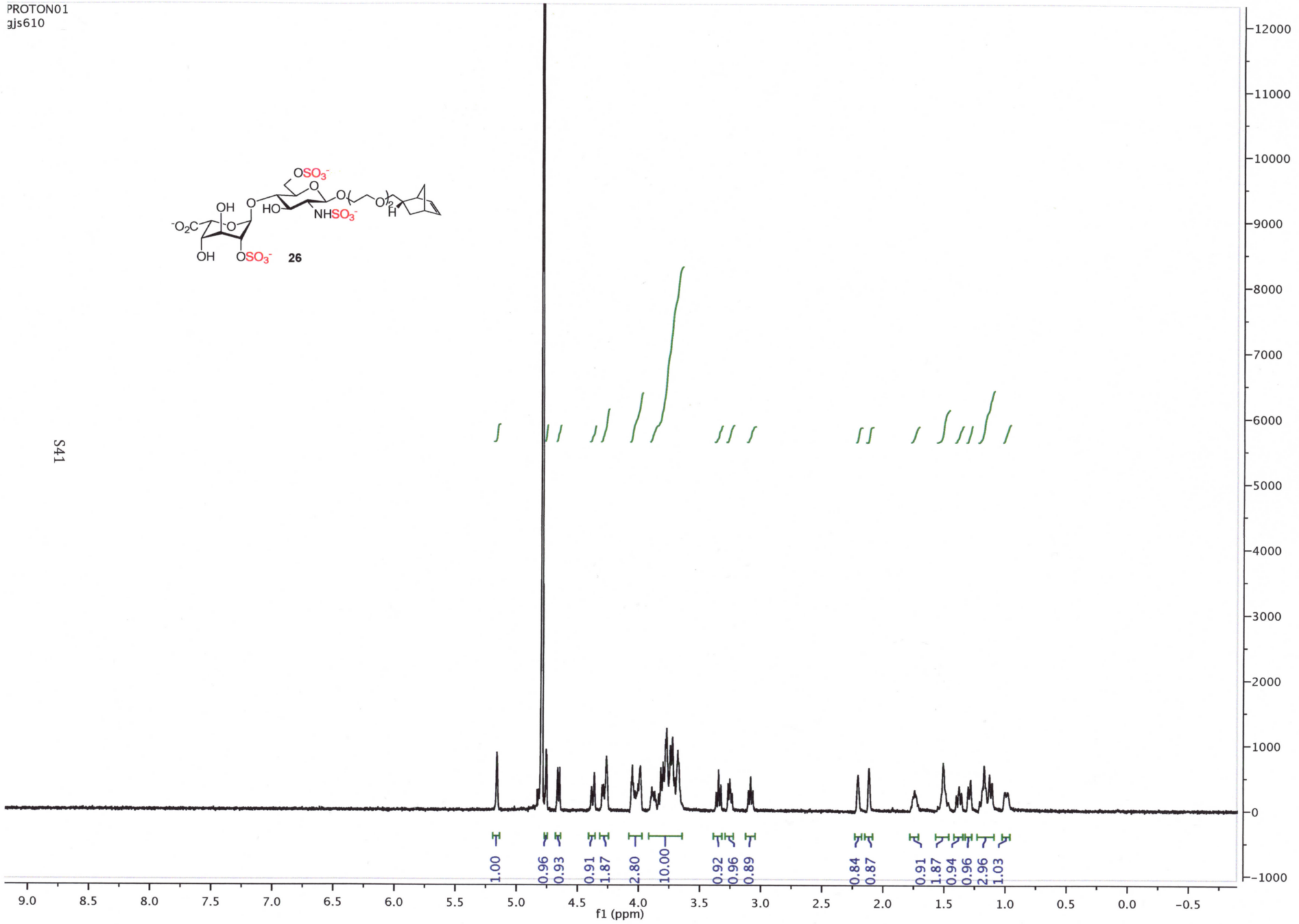
1.0
0.5
0.0
-0.5
f1 (ppm)


PROTON01
gjs610
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10.00
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0.96
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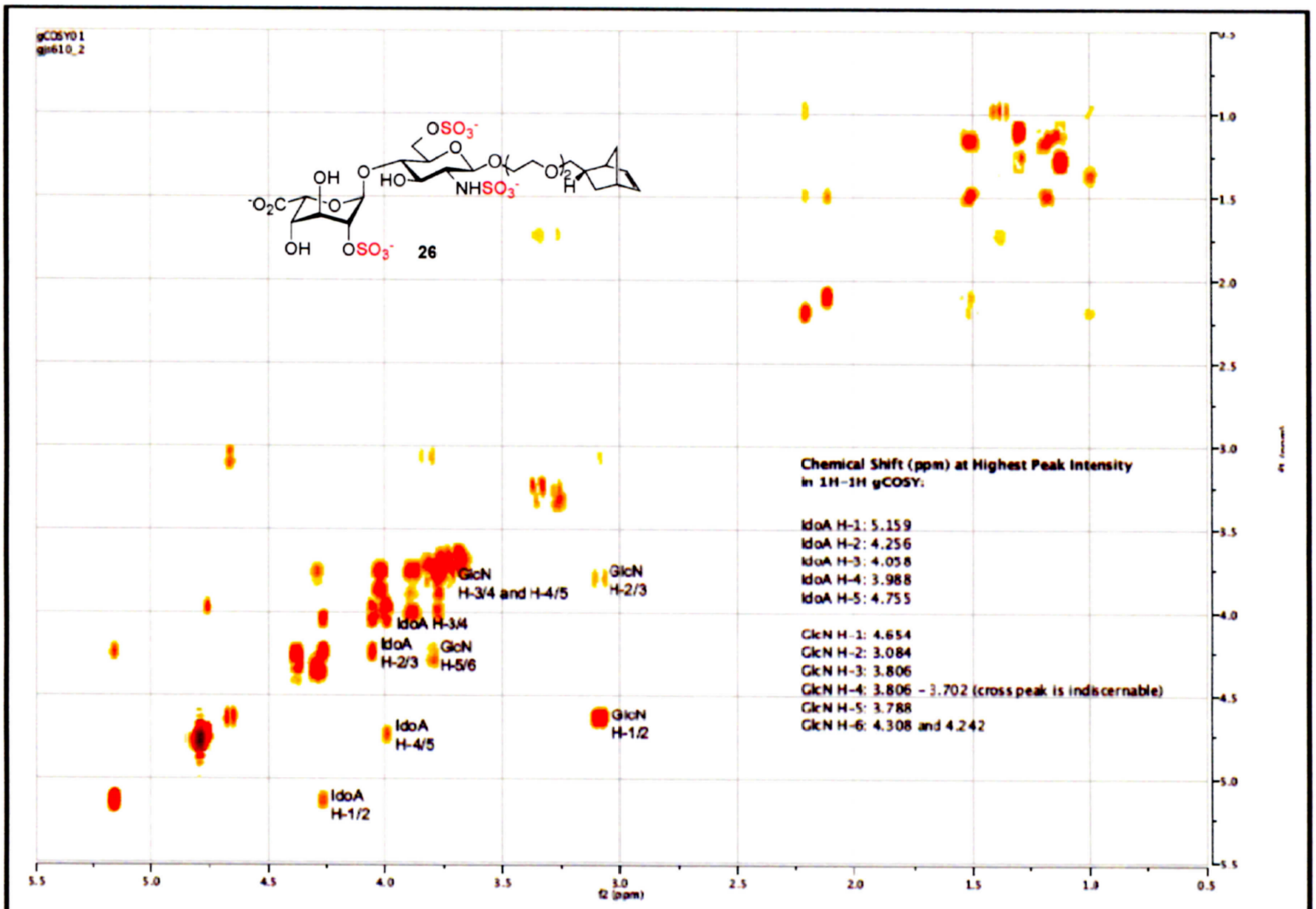
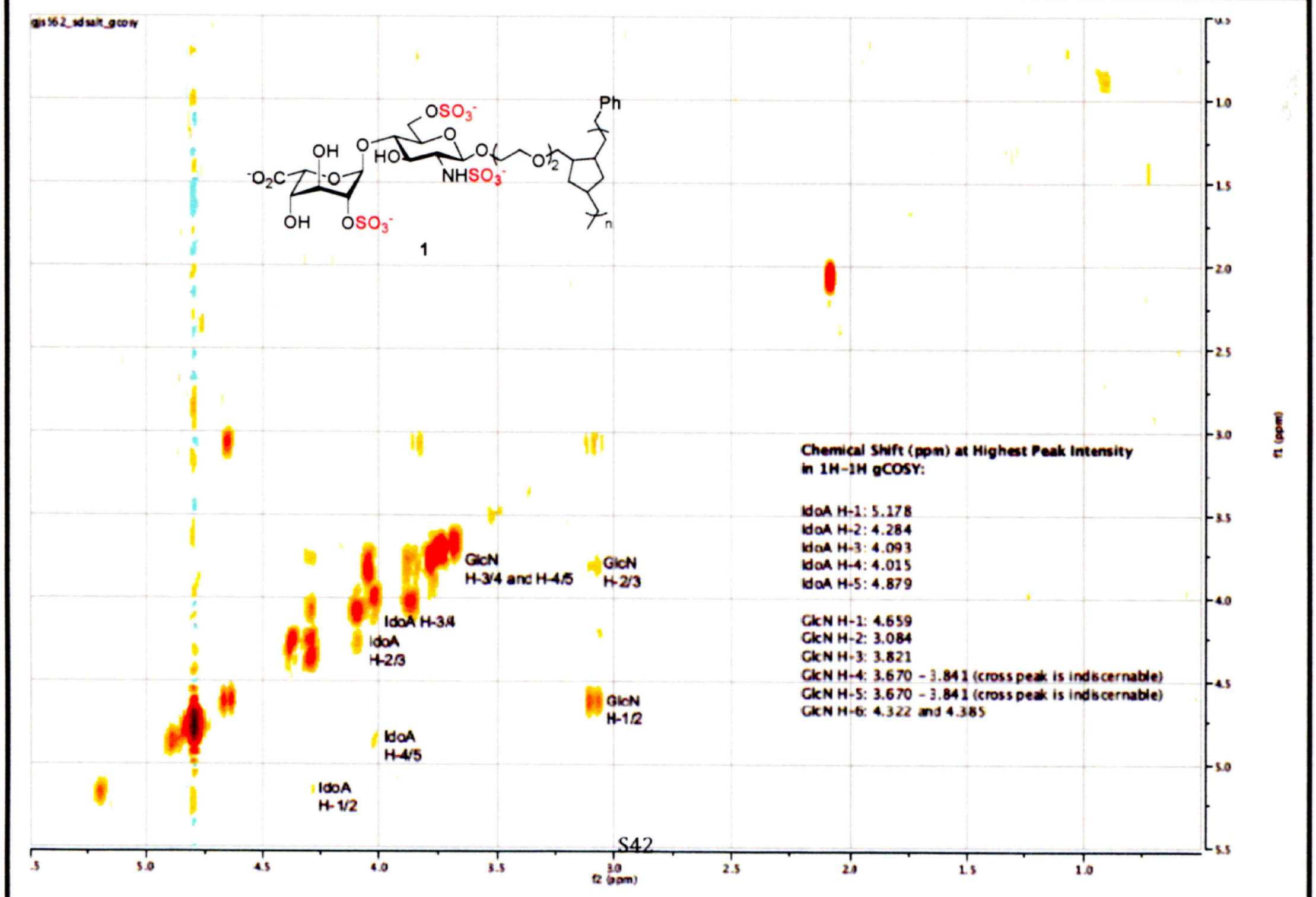
0.87
0.91
1.87
0.94
0.96
2.96
1.03
f1 (ppm)


gCOSY01
qis610_2
OSO3-
O
OH
O
HO
O
O
O
2
H
NHSO3-
-O2C
O
OH
OSO3-
26
Chemical Shift (ppm) at Highest Peak Intensity in 1H-1H gCOSY:
IdoA H-1: 5.159
IdoA H-2: 4.256
IdoA H-3: 4.058
IdoA H-4: 3.988
IdoA H-5: 4.755
GlcN H-1: 4.654
GlcN H-2: 3.084
GlcN H-3: 3.806
GlcN H-4: 3.806 - 3.702 (cross peak is indiscernable)
GlcN H-5: 3.788
GlcN H-6: 4.308 and 4.242
GlcN H-3/4 and H-4/5
GlcN H-2/3
IdoA H-3/4
IdoA H-2/3
GlcN H-5/6
GlcN H-1/2
IdoA H-4/5
IdoA H-1/2
f2 (ppm)
f1 (ppm)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OSO3-
O
OH
O
HO
O
O
O
2
Ph
n
NHSO3-
-O2C
O
OH
OSO3-
1
Chemical Shift (ppm) at Highest Peak Intensity in 1H-1H gCOSY:
IdoA H-1: 5.178
IdoA H-2: 4.284
IdoA H-3: 4.093
IdoA H-4: 4.015
IdoA H-5: 4.879
GlcN H-1: 4.659
GlcN H-2: 3.084
GlcN H-3: 3.821
GlcN H-4: 3.670 - 3.841 (cross peak is indiscernable)
GlcN H-5: 3.670 - 3.841 (cross peak is indiscernable)
GlcN H-6: 4.322 and 4.385
GlcN H-3/4 and H-4/5
GlcN H-2/3
IdoA H-3/4
IdoA H-2/3
GlcN H-1/2
IdoA H-4/5
IdoA H-1/2
f2 (ppm)
f1 (ppm)