Supporting Information:

A Cleavable Azide Resin for Direct Click Chemistry Mediated Enrichment of Alkyne-Labeled Proteins

Christian Sibbersen,[a,b] Lennart Lykke, [a] Niels Gregersen,[c] Karl Anker Jørgensen[a] and Mogens Johannsen*[b]

[a] *Center for Catalysis, Department of Chemistry, Aarhus University, Langelandsgade 140, 8000 Aarhus C, Denmark.*

[b] *Bioanalytical Unit, Department of Forensic Medicine, Aarhus University, Brendstrupgårdsvej 100, 8200 Aarhus N, Denmark.*

[c] *Research Unit for Molecular Medicine, Institute of Clinical Medicine, Aarhus University Hospital, Brendstrupgårdsvej 100, 8200 Aarhus N, Denmark.*

General

NMR spectra were acquired on a Bruker AVANCE III HD spectrometer, running at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm relative to residual solvent signals ($CHCl_3$, 7.26 ppm; D_2O, 4.79 ppm) for ^{1}H NMR spectra and relative to the central solvent resonance ($CDCl_3$, 77.16 ppm) for ^{13}C NMR spectra. The following abbreviations are used to indicate the multiplicity in ^{1}H NMR spectra: s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; dt, double triplet; quin, quintet; m, multiplet; bs, broad signal. ^{13}C NMR spectra were acquired in broadband decoupled mode. Mass spectra were recorded on a Bruker Maxis Impact spectrometer using electrospray (ES+) ionization. Analytical thin layer chromatography (TLC) was performed using pre-coated aluminium-backed plates (Merck Kieselgel 60 F254) and visualized by ultraviolet irradiation or $KMnO_4$ dip. Commercially available reagents and solvents for flash chromatography were used without further purification. CH_2Cl_2 and acetone were dried over activated 4 Å molecular sieves overnight. Dry THF was acquired from an MBraun SPS-800 solvent purification system and used immediately. Glassware was dried by warming with a heat gun under high vacuum and then flushed with N_2.

Water for biological assays was purified using a Milli-Q system (Millipore). Phosphate buffer saline (PBS) was Mg^{2+} and Ca^{2+} free and adjusted to pH = 7.4.

SDS loading buffer consisted of 2% SDS, 10% glycerol, 50 mM Tris-HCl, pH 6.8, 12.5 mM ethylene diamine tetraacetic acid (EDTA), 200 mM dithiothreitol (DTT, added immediately before heating) and 0.02 mg/mL bromophenol blue.

Synthesis

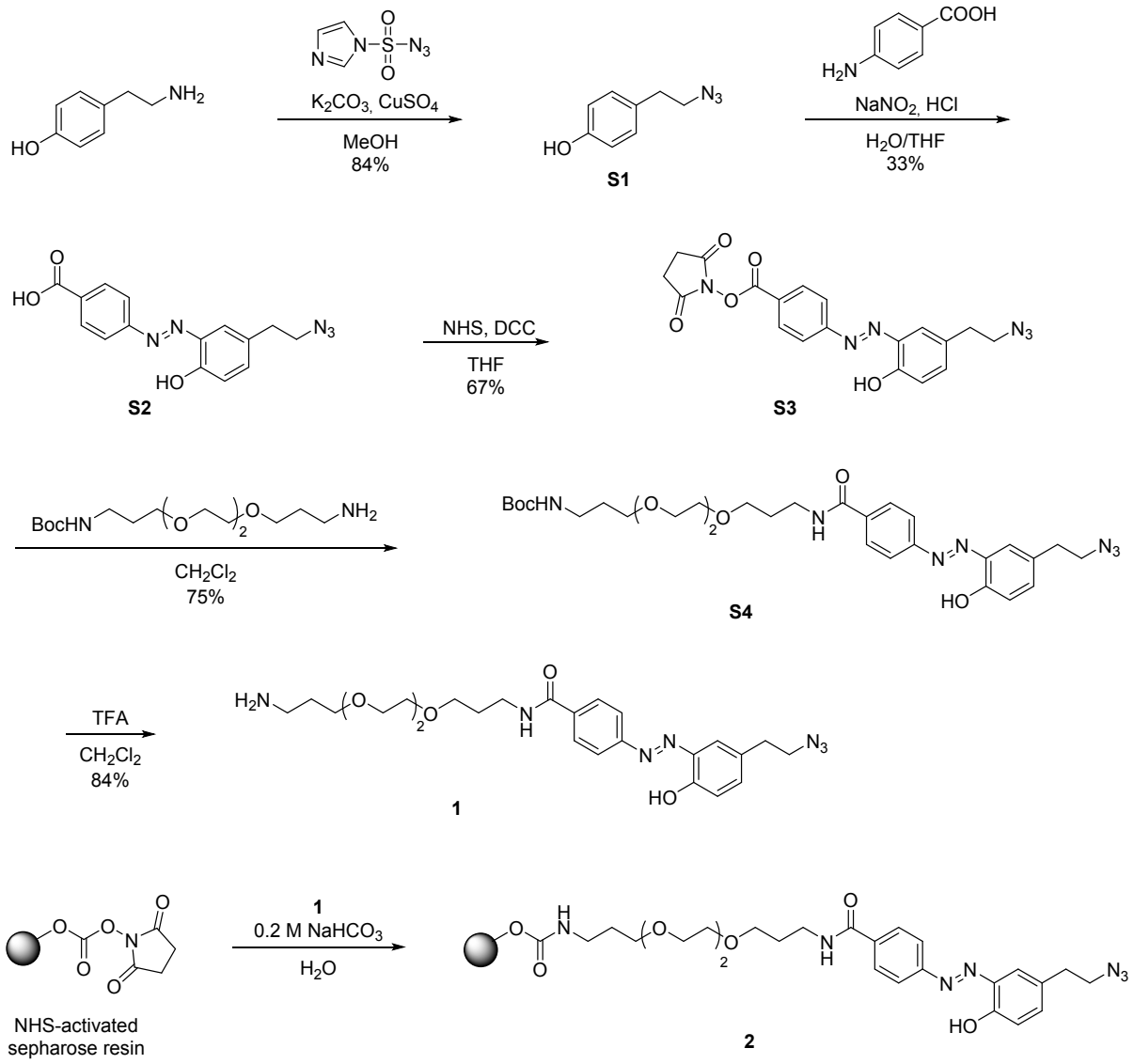
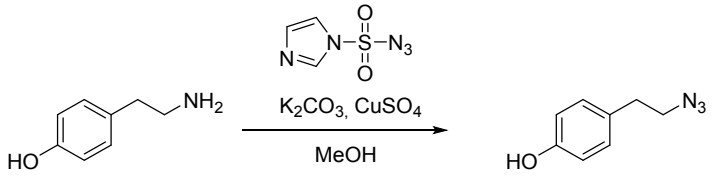


Fig. S1 - Synthesis of azido-azo beads

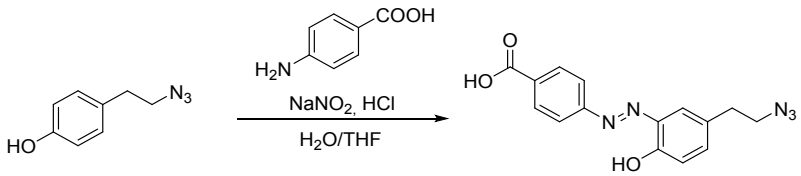
4-(2-Azidoethyl)phenol, **S1**



S1

Imidazole-1-sulfonyl azide hydrochloride[1] (2.00 g, 9.54 mmol, 1.2 eq) was added to tyramine (1.09 g, 7.95 mmol, 1 eq), K_2CO_3 (1.15 g, 8.35 mmol, 1.05 eq) and $CuSO_4 \cdot 5H_2O$ (20 mg, 0.08 mmol, 0.01 eq) in 40 mL MeOH and stirred at rt for 4 h. The reaction mixture was then concentrated *in vacuo*, dissolved in 20 mL H_2O and acidified with concentrated HCl. The solution was extracted with 3x20 mL Et_2O, dried over $MgSO_4$ and concentrated again. Flash chromatography in 1:4 EtOAc/pentane afforded the product as a clear oil (1.09 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 2H), 5.61 (bs, 1H), 3.44 (t, *J* = 7.2 Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 130.3, 130.1 (2C), 115.7 (2C), 52.8 (2C), 34.6 (2C).

(*E*)-4-((5-(2-Azidoethyl)-2-hydroxyphenyl)diazenyl)benzoic acid, **S2**



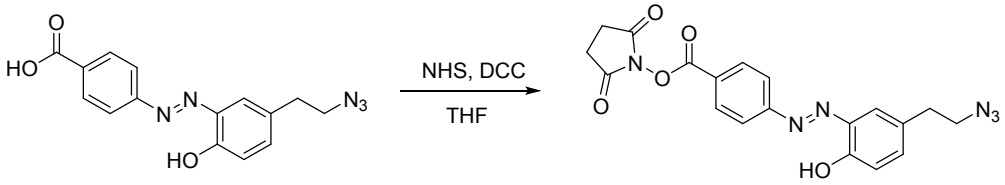
S1 **S2**

4-Aminobenzoic acid (840 mg, 6.13 mmol, 2 eq) was suspended in 20 mL 6 M HCl and cooled on an ice bath. $NaNO_2$ (1.06 g, 15.32 mmol, 5 eq) was added slowly (gas evolution was observed) and stirred at 0 °C for 40 min. The solution turned yellow. 4-(2-Azidoethyl)phenol (**S1**) (500 mg, 3.06 mmol, 1 eq) was dissolved in 30 mL 1:1 THF/saturated aqueous $NaHCO_3$ and cooled on an ice bath. The diazonium salt solution was added portion wise while stirring at 0 °C and the pH was kept basic by adding K_2CO_3 as needed. The reaction mixture was allowed to warm to rt and stirred overnight, turning dark red. The THF was removed in vacuo and the pH was adjusted to ~2 by addition of conc. HCl. The reaction mixture was extracted with EtOAc (3x50 mL) and the extracts were combined and washed with 1 M HCl (30 mL) and brine (30 mL) before drying over $MgSO_4$ and concentrating in vacuo. The residue was purified by column chromatography in 1:1 EtOAc/pentane and then 10% MeOH in CH_2Cl_2 and concentrated to afford the product as an orange solid (316 mg, 33%). The product in its acid form is insoluble in D_2O and only slightly soluble in $CDCl_3$ and CD_3OD, so in order to obtain a good NMR spectrum 20 mg of the compound (**S2**) was dissolved in 1 M KOH in D_2O

[1] Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797–3800.

and characterized in its basic form, which is dark red in color. ^{1}H NMR (400 MHz, D_2O) δ 7.98 (d, *J* = 8.5 Hz, 2H), 7.87 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 2.3 Hz, 1H), 7.21 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 3.50 (t, *J* = 6.8 Hz, 2H), 2.75 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, D_2O) δ 177.6, 169.8, 157.1, 145.9, 139.5, 138.0, 132.5, 126.7, 126.3, 124.3, 119.0, 54.8, 35.9. HRMS: Calculated for $[C_{15}H_{14}N_5O_3]^+$: 312.1091 found: 312.1091.

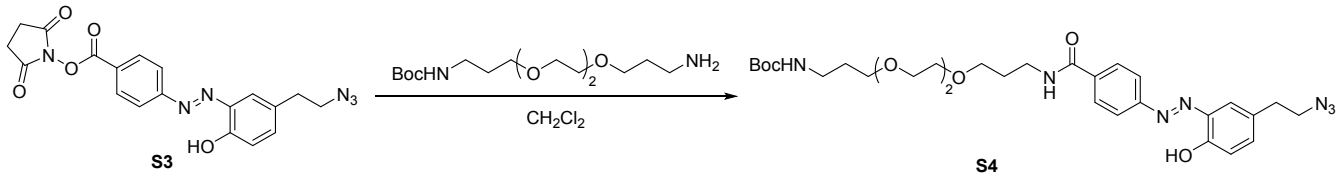
2,5-Dioxopyrrolidin-1-yl (*E*)-4-((5-(2-azidoethyl)-2-hydroxyphenyl)diazenyl)benzoate, **S3**



S2 **S3**

Compound **S2** (140 mg, 0.45 mmol, 1 eq), *N*,*N*'-dicyclohexylcarbodiimide (102 mg, 0.49 mmol, 1.1 eq) and *N*-hydroxysuccinimide (57 mg, 0.49 mmol, 1.1 eq) were dissolved in dry THF (10 mL) and stirred overnight at rt. The THF was removed *in vacuo* and the residue was redissolved in 10 mL chilled EtOAc and filtered to remove urea. The filtrate was concentrated and purified by column chromatography in 1:2 EtOAc/pentane to afford the product as a bright orange powder (123 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.50 (s, 1H), 8.23 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 8.8 Hz, 2H), 7.79 (d, *J* = 2.2 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 3.53 (t, *J* = 7.1 Hz, 2H), 2.95-2.83 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 161.2, 154.3, 151.8, 137.5, 135.3, 133.5, 131.9, 130.1, 126.7, 122.5, 118.7, 52.4, 34.2, 25.7. HRMS: Calculated for $[C_{19}H_{17}N_6O_5]^+$: 409.1255 found: 409.1257.

tert-Butyl (*E*)-(3-(2-(3-(4-((5-(2-azidoethyl)-2-hydroxyphenyl)diazenyl)benzamido)propoxy)ethoxy)propyl)carbamate, **S4**



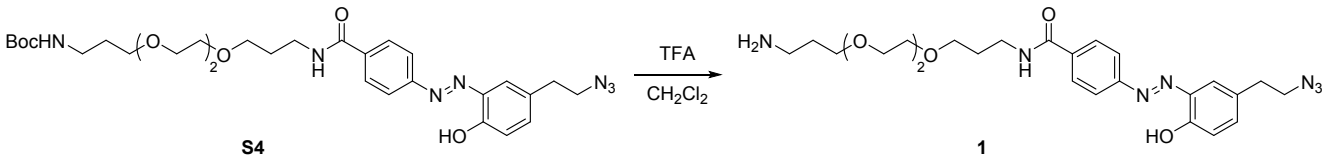
S3 **S4**

Tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate[2] (118 mg, 0.37 mmol, 1.5 eq) and **S3** (100 mg, 0.24 mmol, 1 eq) were stirred overnight in 4 mL dry CH_2Cl_2 at rt. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography first in 1:9 MeOH/CH_2Cl_2, then in 1:1 EtOAc/pentane to afford the product as an orange residue (124 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1H), 7.96 (d, *J* = 8.6 Hz, 2H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.80 (d, *J* = 2.2 Hz, 1H), 7.33 (bs, 1H), 7.21 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 4.89 (bs, 1H),

[2] Trester-Zedlitz, M.; Kamada, K.; Burley, S. K.; Fenyö, D.; Chait, B. T.; Muir, T. W. *J. Am. Chem. Soc.* **2003**, *125*, 2416–2425.

3.72-3.38 (m, 16H), 3.15 (m, 2H), 2.91 (t, *J* = 7.1 Hz, 2H), 1.90 (quin, *J* = 5.7 Hz, 2H), 1.67 (quin, *J* = 6.3 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 156.0, 152.1, 151.6, 137.3, 136.9, 134.4, 133.2, 129.8, 128.4 (2C), 122.2 (2C), 118.6, 78.9, 70.9, 70.4 (2C), 70.3, 70.1, 69.5, 52.4, 39.3, 38.5, 34.3, 29.7, 28.7, 28.4 (3C). HRMS: Calculated for $[C_{30}H_{44}N_7O_7]^+$: 614.3297 found: 614.3300.

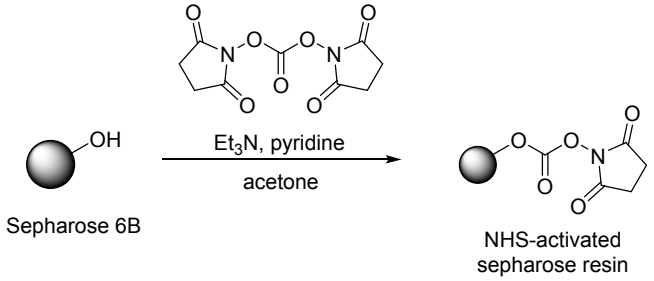
(*E*)-*N*-(3-(2-(2-(3-Aminopropoxy)ethoxy)ethoxy)propyl)-4-((5-(2-azidoethyl)-2-hydroxyphenyl)diazenyl)benzamide, **1**



S4 **1**

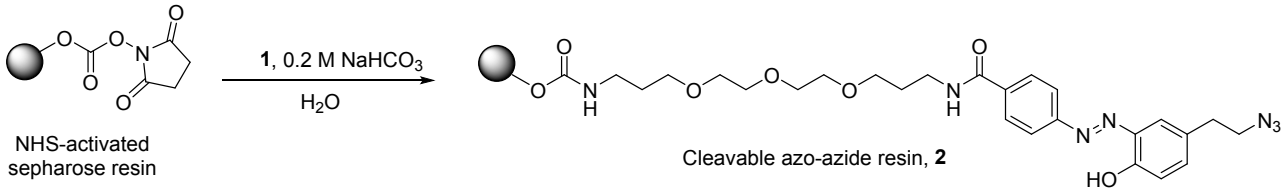
Compound **S4** (111 mg, 0.18 mmol, 1 eq) was dissolved in 5 mL CH_2Cl_2 and TFA (1 mL, 13 mmol, 72 eq) was added at rt. The color instantly changed from bright orange to dark red. Stirring was continued at rt and the reaction was monitored by TLC in 1:1 EtOAc/pentane until full conversion was observed (~30 min). The reaction was concentrated and excess TFA was removed under high vacuum to give the crude TFA-salt of the product as a crimson residue. The residue was taken up in 30 mL conc. aqueous ammonium hydroxide and extracted with 3x30 mL CH_2Cl_2 (be careful as overpressure is generated in the separation funnel). The combined extracts were washed with brine (30 mL) and dried over $MgSO_4$, then concentrated and purified by column chromatography in 1:1 MeOH/CH_2Cl_2 with 0.5% conc. aqueous ammonium hydroxide to afford the product as a crimson residue (78 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.6 Hz, 2H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.76 (d, *J* = 2.1 Hz, 1H), 7.69 (bs, 1H), 7.18 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 4.34 (bs, 2H), 3.68-3.34 (m, 16H), 2.87 (t, *J* = 6.9 Hz, 2H), 2.69 (t, J = 6.6 Hz, 2H), 1.87 (quin, *J* = 5.8 Hz, 2H), 1.61 (quin, *J* = 6.4 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 152.1, 151.8, 137.4, 137.0, 134.4, 132.8, 129.8, 128.4, 122.2, 118.6, 70.5, 70.5, 70.5, 70.3, 70.1, 69.6, 52.5, 39.6, 38.9, 34.3, 33.1, 28.8. HRMS: Calculated for $[C_{25}H_{36}N_7O_5]^+$: 514.2772 found: 514.2782.

Activation of sepharose resin



Sepharose 6B resin (Sigma Aldrich) was activated according to the procedure published by Wilchek & Miron;[3] Sepharose resin (10 g from an aqueous suspension) was placed in a Büchner funnel with sintered filter and dehydrated by washing with 50 mL of 3:1 water/acetone, followed by 1:1 water/acetone, then 1:3 and finally dry acetone. The dry resin was suspended in 10 mL dry acetone. The reaction was cooled on an ice bath and *N*,*N*'-disuccinimidyl carbonate (800 mg, 3.12 mmol, 1 eq), triethylamine (0.87 mL, 6.25 mmol, 2 eq) and pyridine (1 mL, 12.49 mmol, 4 eq) was added. The reaction mixture was stirred gently (150 rpm) at 4 °C for 2 h and then filtered on the Büchner funnel again. The resin was washed successively with cold acetone, 5% AcOH in dioxane, MeOH and *i*-PrOH, 50 mL of each. The activated resin was stored as a suspension in *i*-PrOH at 4 °C until it was used.

Coupling of azo-azide **1** to sepharose resin



3 g of the activated resin filtered from *i*-PrOH (containing 25-35 µmol/g active hydroxysuccinimide carbonates according to Wilchek & Miron) was suspended in 25 mL 0.2 M aqueous $NaHCO_3$ and the mixture was cooled to 4 °C. Compound **1** (75 mg, 0.15 mmol, ~1.6 eq) was added and the suspension was stirred carefully (150 rpm) at 4 °C overnight. The reaction mixture was then placed in a Büchner funnel with a sintered filter and washed with 3x30 mL H_2O, 3x30 mL MeOH and 3x30 mL acetone, yielding ~4 g bright yellow resin (**2**) (filtered from acetone) that swells to ~4.5 g in water, turning orange. After settling in water, the volume of the resin was ~5 mL. We noted that the color of the resin varies from dark brown-orange to bright yellow, depending on solvent and pH. Unreacted amine **1** can be recovered by combining and concentrating the colored washing aliquots *in vacuo*. The residue is dissolved in conc. aqueous ammonium hydroxide (30 mL) and extracted with 3x30 mL CH_2Cl_2. The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield compound **1**. 23 mg starting material was recovered in this experiment (45 µmol, 30%), indicating a binding of 23 µmol/g swelled resin.

[3] Wilchek, M.; Miron, T. *Appl. Biochem. Biotechnol.* **1985**, *11*, 191–193.

The beads can be stored in PBS with 0.02% sodium azide as a preservative or in 10% MeOH. They are stable for months when kept at 4 °C.

Probes **3** and **4** and linkers **5** and **6** were synthesized according to literature procedures.[4]

On-bead enrichment of proteins – General procedure

Cell lysis

HEK-293 cells were grown to 90% confluency in Dulbecco's modified eagle medium (Invitrogen) in T150 flasks before they were harvested using trypsin/EDTA in PBS. Cells were pelleted by centrifugation (5 min, 500 g, rt) in a 15 mL BD Falcon tube (Fisher Scientific) and resuspended in 1 mL in lysis buffer (1% Triton-X 100, 150 mM NaCl, 50 mM triethanolamine, pH = 7.6) with protease inhibitor cocktail (Sigma, "mammalian" type without EDTA) and benzonase. Probe (**4** or **3** + 100 µM H_2O_2) or spike proteins (100 µg in PBS) were added at this point. Samples were vortexed, incubated 30 min on ice, then vortexed again and centrifuged (16000 g, 15 min, °4 C) before transferring to new Eppendorf tubes. Protein levels were measured by Bradford assay (Bio-Rad). Aliquots were taken out, 200 µg protein is used for conjugation to rhodamine-azide and approximately 5 mg is used for conjugation to azido-azo beads **2**. If excess probe is believed to be present, this can be removed by $CHCl_3$/MeOH precipitation. [5] The protein concentration is adjusted to 2 mg/mL for rhodamine conjugation and 2-5 mg/mL for azido-azo beads **2** conjugation with 4% SDS buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine, pH = 7.6).

Rhodamine conjugation

200 µg sample in 100 µL 4% SDS buffer was treated with 3 µL 5 mM rhodamine-azide, 3 µL 80 mM TBTA in DMSO, 3 µL 50 mM $CuSO_4$, 5 µL 100 mM α-aminoguanidine and 5 µL 100 mM sodium ascorbate (freshly dissolved). The tubes were vortexed and incubated at room temperature for 1 h and precipitated by addition of 1 mL cold MeOH. Samples were incubated 1 h at -20 °C and centrifuged (16000 g, 5 min, 4 °C). The supernatant was removed and the pellets were washed by addition of 1 mL cold MeOH, vortexed and recovered by centrifugation as before. Supernatant was removed and the pellets were air dried 10 min and redissolved by bath sonication for 10 min in 100 µL sample buffer containing 200 mM freshly added DTT, heated for 5 min at 95 °C, vortexed and centrifuged (2 min, 10000 g, rt) before loading on a Criterion "Any kD" TGX gel. 10 µL (~20 µg protein) was loaded.

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[4] **3** - Qian, J.; Wani, R.; Klomsiri, C.; Poole, L. B.; Tsang, A. W.; Furdui, C. M. *Chem. Commun.* **2012**, *48*, 4091-4093. **4** - Sibbersen, C.; Palmfeldt, J.; Hansen, J.; Gregersen, N.; Jørgensen, K. A.; Johannsen, M. *Chem. Commun.* **2013**, *49*, 4012-4014. **5** - Fusz, S.; Srivatsan, S. G.; Ackermann, D.; Famulok, M. *J. Org. Chem.* **2008**, *73*, 5069-5077. **6** - Yang, Y.-Y.; Grammel, M.; Raghavan, A. S.; Charron, G.; Hang, H. C. *Chemistry & Biology* **2010**, *17*, 1212-1222.

[5] Wessel, D.; Flügge, U. I. *Anal. Biochem.* **1984**, *138*, 141–143.

Azido-azo-beads enrichment

100 µL of a 50% suspension of azido-azo beads **2** were washed with twice with 4% SDS buffer and transferred to Eppendorf tubes containing the cell ysates (2-5 mg protein in ~1 mL), followed by addition of 20 µL 80 mM TBTA in DMSO, 20 µL 50 mM $CuSO_4$, 50 µL 100 mM α-aminoguanidine and 50 µL 100 mM sodium ascorbate (freshly dissolved). The tubes were vortexed, then incubated for 1 h with rocking at rt. 100 µL 0.1 M EDTA was then added, the samples were vortexed again and centrifuged (1000 g, 1 min, rt) to pellet beads. The supernatant was removed (save if there is doubt about the conjugation) and the beads were incubated with rocking for 30 min in 1 mL 4% SDS buffer at rt, then washed 3 times with 4% SDS buffer, then 3 times with lysis buffer (1% Triton-X 100, 150 mM NaCl, 50 mM triethanolamine, pH = 7.6). Washing was done by adding 1 mL buffer, vortexing, centrifuging (1000 g, 1 min, rt) and carefully removing the supernatant without disturbing the beads. The bound proteins were then eluted by addition of 100 µL elution buffer (50 mM $Na_2S_2O_4$ and 1% SDS in PBS, pH = 7.4), vortexing and incubation at rt for 30 min. The beads were centrifuged (1000 g, 1 min, rt) and the supernatant carefully transferred to a new Eppendorf tube. This step was repeated, the supernatants combined and salts were removed by $CHCl_3$/MeOH precipitation and redissolved by bath sonication for 10 min in 30 µL sample buffer containing 200 mM freshly added DTT, heated for 5 min at 95 °C, vortexed and centrifuged (2 min, 10000 g, rt) before loading on a Criterion "Any kD" TGX gel. 20 µL was loaded initially, however for most samples this is too much and 10 µL is sufficient. The gel was run in a Tris-glycine running buffer (0.025 M Tris/0.192 M glycine/0.1% SDS, pH 8.5) at 300 V, then destained 1 h in 4:1:5 MeOH/AcOH/H_2O, then analyzed for in-gel fluorescence using an ImageQuant LAS 4000 (GE Healthcare). The gel was then stained with Coomassie brilliant blue for 1 h and destained overnight before scanning.

Comparison with streptavidin beads

Lysate treated with either 15 mM 2-oxohex-5-ynal (**4)** or PBS (control) was divided into 3 aliquots of 500 µL (2 mg protein/mL). The azide-containing reagents (linkers **5** and **6** or azido-azo beads **2**) were now added to the samples. One pair of 500 µL samples (one control and one alkynylated lysate) were added azido-biotin (**5**) (11.3 µL of a 5 mM solution in DMSO), one pair was added azido-azo-biotin (**6**) (11.3 µL of a 5 mM solution in DMSO) and one pair was added azido-azo beads (**2**) (100 µL of a 50% slurry in lysis buffer).

The remaining "click chemistry" reagents were now added one at a time, vortexing gently after each addition; 11.3 µL 50 mM TCEP, 34.0 µL 1.7 mM TBTA in 1:4 DMSO/tBuOH and 11.3 µL 50 mM $CuSO_4$,[6] followed by vortexing and incubation for 1 h at rt, vortexing again after 30 min.

Proteins from the azido-biotin (**5**) and azido-azo-biotin (**6**) samples were then precipitated by addition of 4 mL MeOH, incubated for 2 h at -20°C and pelleted by ultracentrifugation (10 min, 16000 g). Excess reagents were removed by addition of 1 mL cold MeOH, sonication (3x10 strokes at 30% power) and ultracentrifugation (5 min, 16000 g), upon which the MeOH supernatant was discarded. This washing step was repeated twice. Proteins pellets were now redissolved in 1 mL 1% SDS in PBS by vortexing and diluted to a total volume of 8 mL with PBS. 100 µL of a 50%

[6] Speers, A. E.; Cravatt, B. F. In *Current Protocols in Chemical Biology*; Arkin, A. P.; Mahal, L.; Romesberg, F.; Shah, K.; Shamu, C.; Thomas, C., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2009.

slurry of Pierce high capacity streptavidin beads (Thermo Scientific, pre-washed twice in PBS) was now added and the samples were incubated for 1 h on an end-over-end rotator. The samples were then centrifuged (2 min, 1000 g, rt) and the supernatant discarded.

All the samples (now immobilized on beads) were then transferred into empty micro bio-spin columns (Bio-Rad) and washed thrice with 6 M urea/2 M thiourea/25 mM HEPES, thrice with 1% SDS in PBS and thrice with PBS.

Proteins from the azido-azo-biotin (**6**) and azido-azo beads (**2**) samples were released using elution buffer as described above.

Beads from the azido-biotin (**5**) samples were transferred to an eppendorf tube and incubated in 200 µL 3% SDS at 95 °C for 10 minutes with shaking. They were then transferred back into a bio-spin column and centrifuged (5 min, 1000 g, rt) to elute proteins. Remaning beads in the eppendorf tube were washed out with 100 µL 1% SDS in PBS, transferred to the bio-spin column and centrifuged again to yield a total eluate volume of 300 µL, which was precipitated by chloroform/MeOH precipitation.

All samples were then redissolved and separated on SDS-PAGE as described above.

SDS-PAGE of commercial albumin with impurities

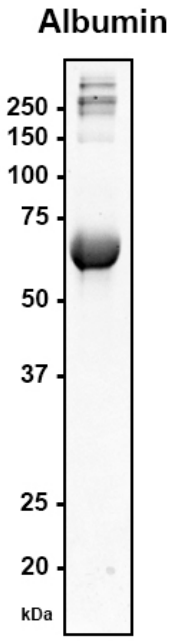


Fig. S2 – Commercial bovine serum albumin (Sigma) loaded on SDS-PAGE to show impurities (upper bands)

Washing experiment using various buffers

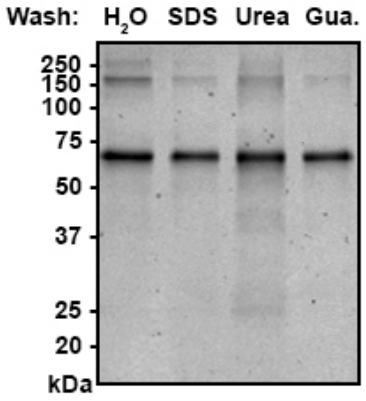
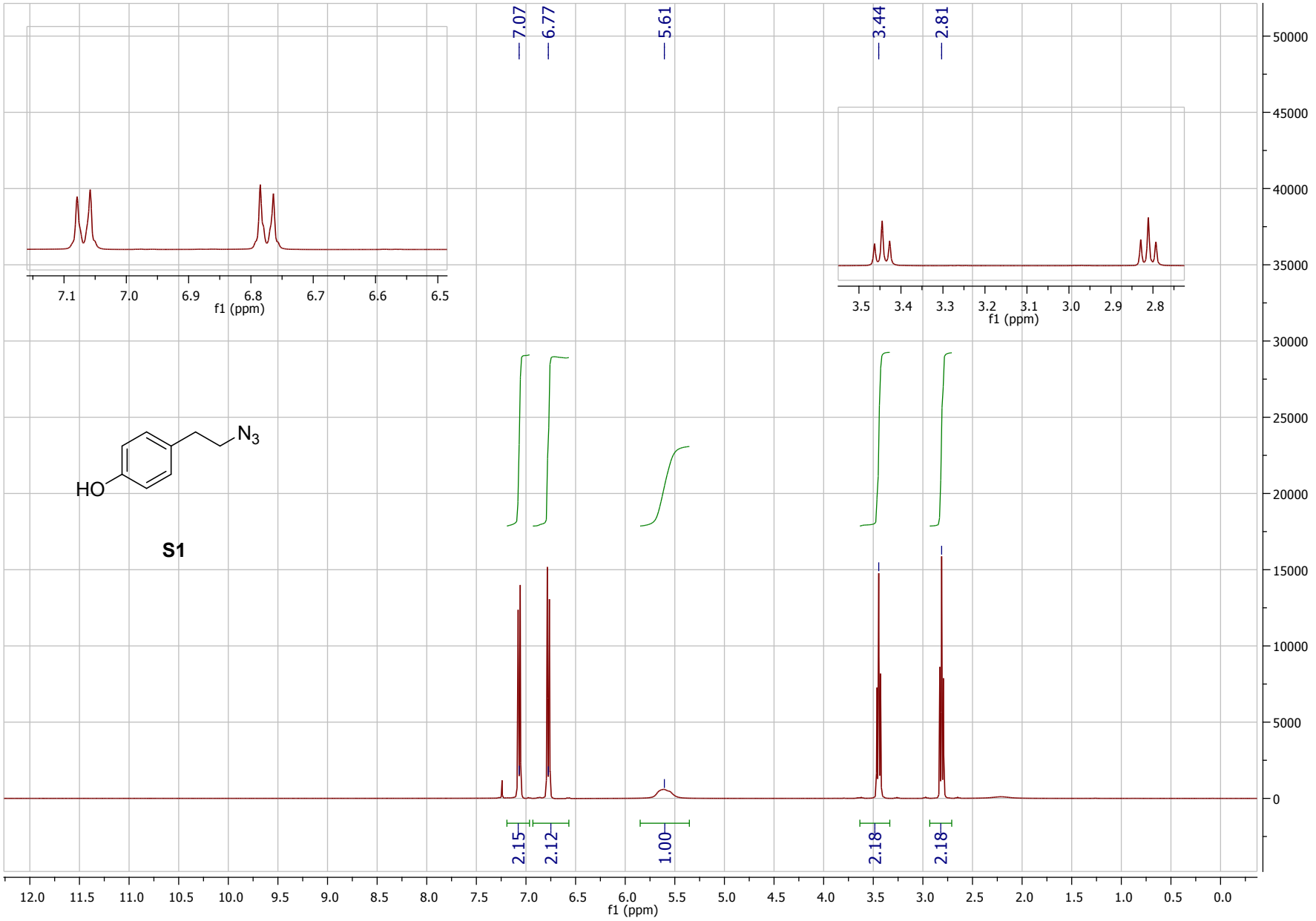
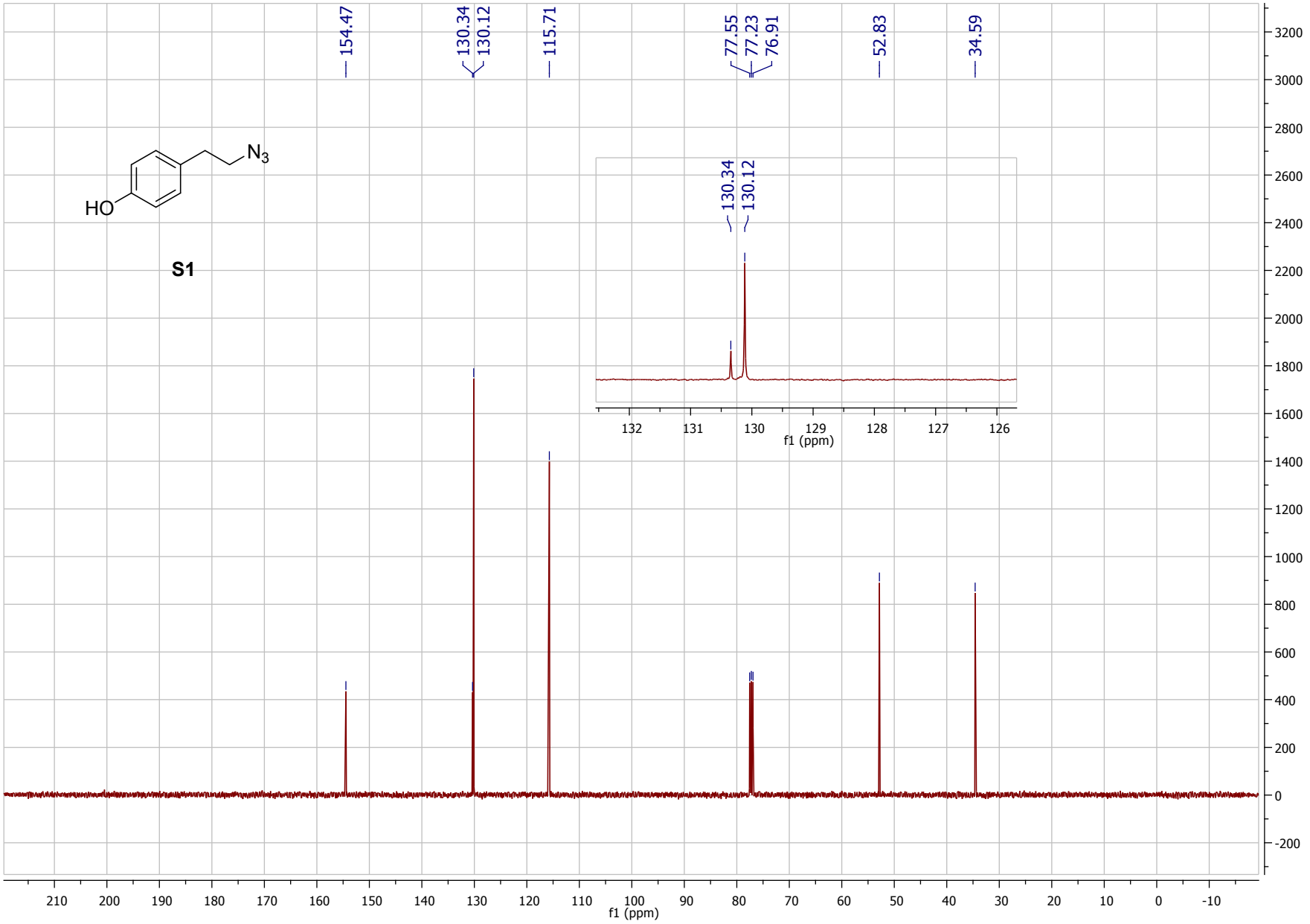
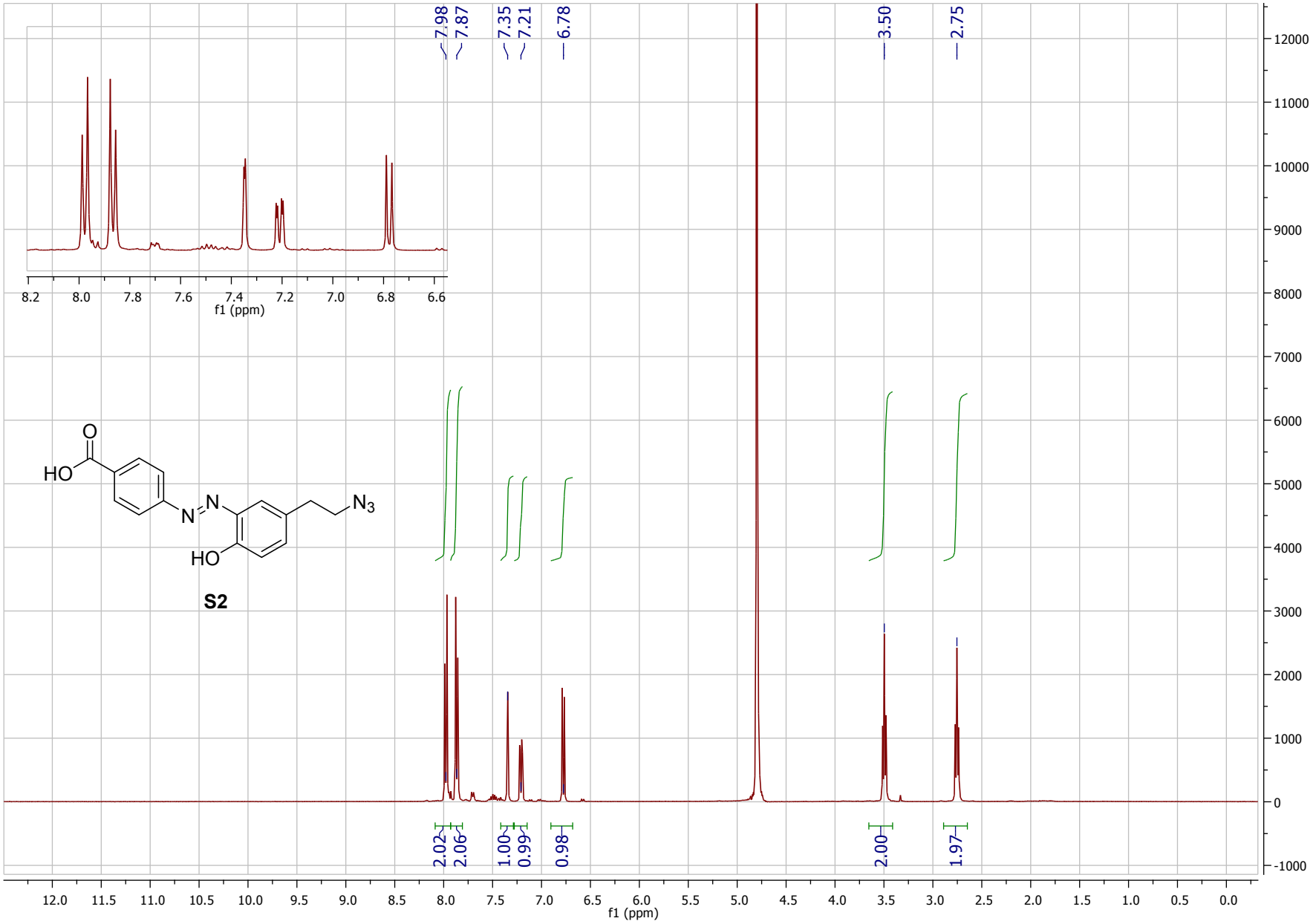


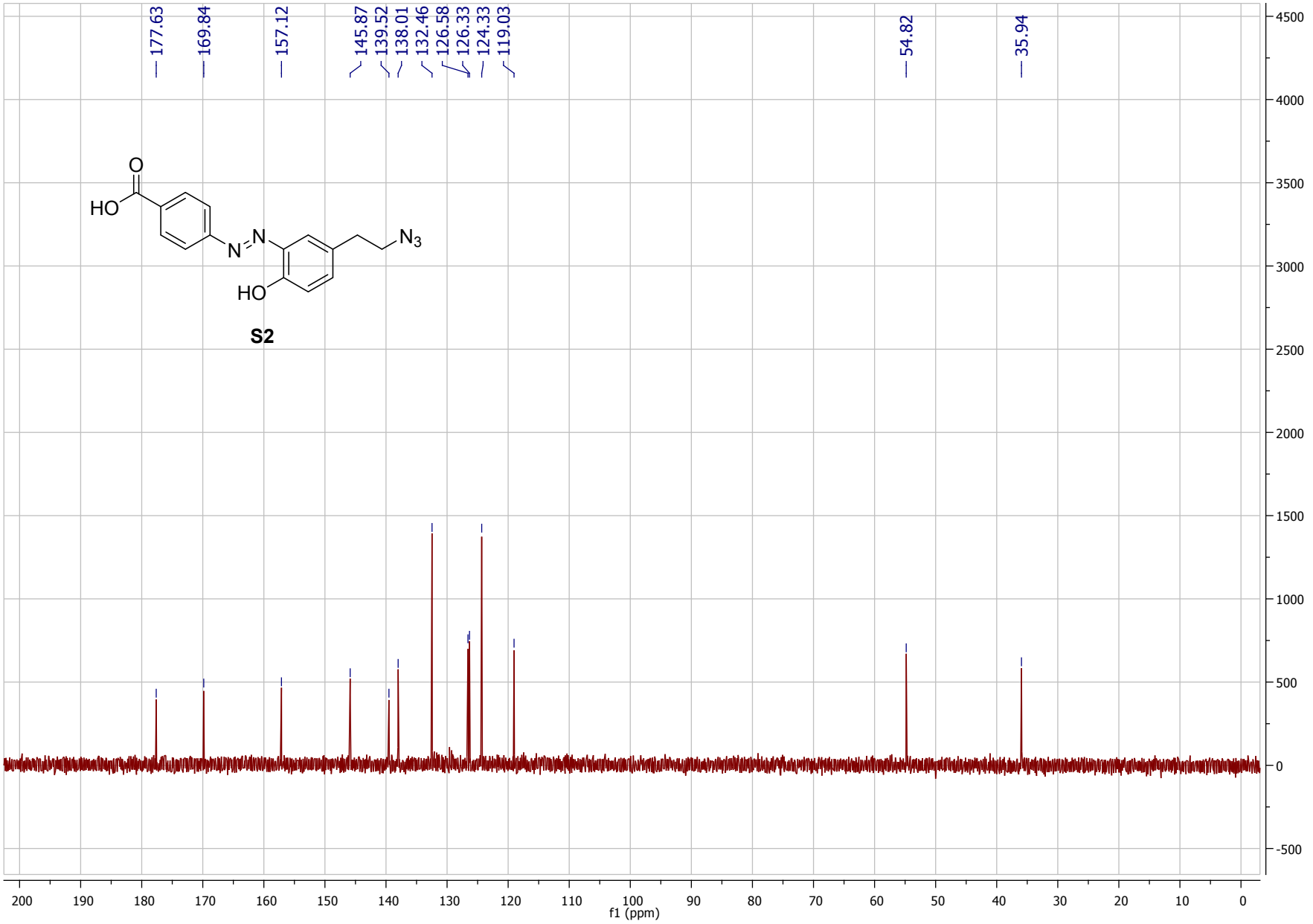
Fig. S3 – Coomassie stain of albumin conjugated to azido-azo beads 2 and washed with either water, 10% SDS, 8 M urea or 8 M guanidine HCl.

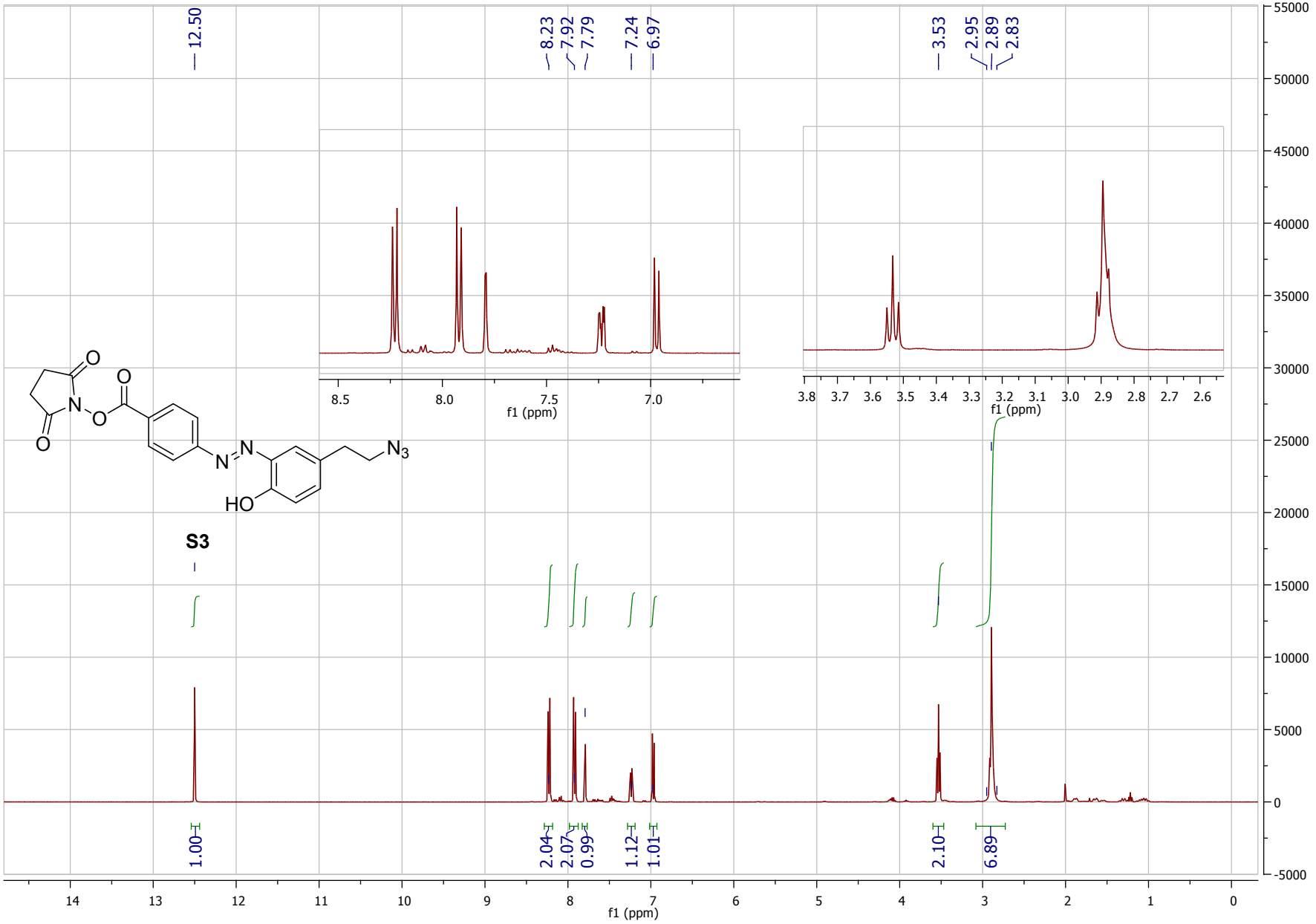
200 µg albumin pre-treated with 2-oxohex-5-ynal **4** was dissolved in 500 µL 4% SDS buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine, pH = 7.6) and conjugated to 25 µL azido-azo beads **2** (50 µL of a 50% suspension, pre-washed twice in 4% SDS buffer) using the conditions described above. The beads were then washed once with water to remove excess click reagents, then washed with either water, 10% SDS, 8 M urea or 8 M guanidine HCl. This was done by suspending the beads in 500 µL of the solution and shaking 10 min at rt before centrifugation (1000 g, 1 min, rt) and discarding the supernatant. The washing step was repeated and the beads were then washed twice in water to remove excess washing reagent. Proteins were released as described above and dissolved in 50 µL loading buffer. 10 µL was loaded on the SDS-PAGE (Fig. S3).

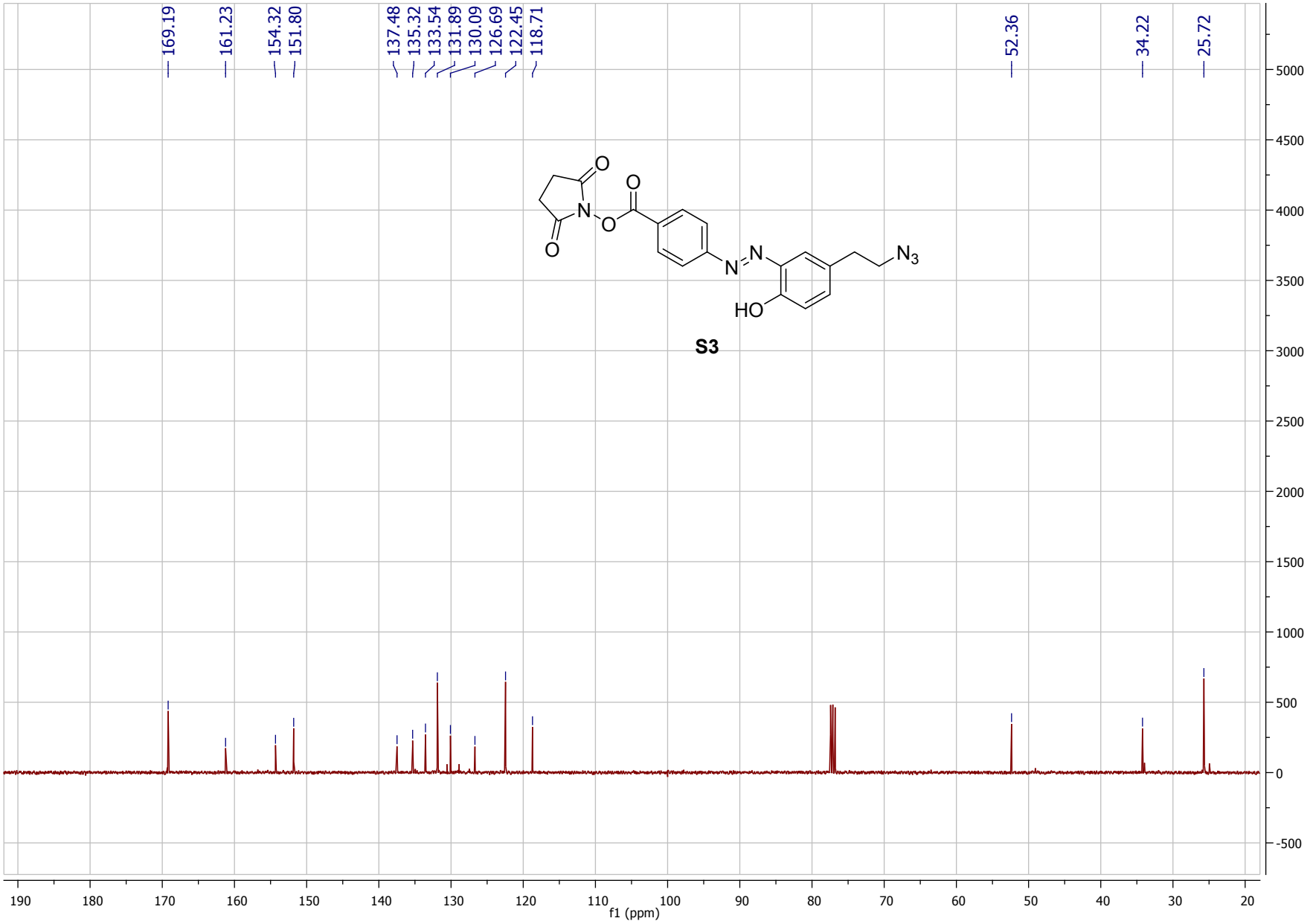

7.07
6.77
5.61
3.44
2.81
HO
N3
S1
2.15
2.12
1.00
2.18
2.18
f1 (ppm)

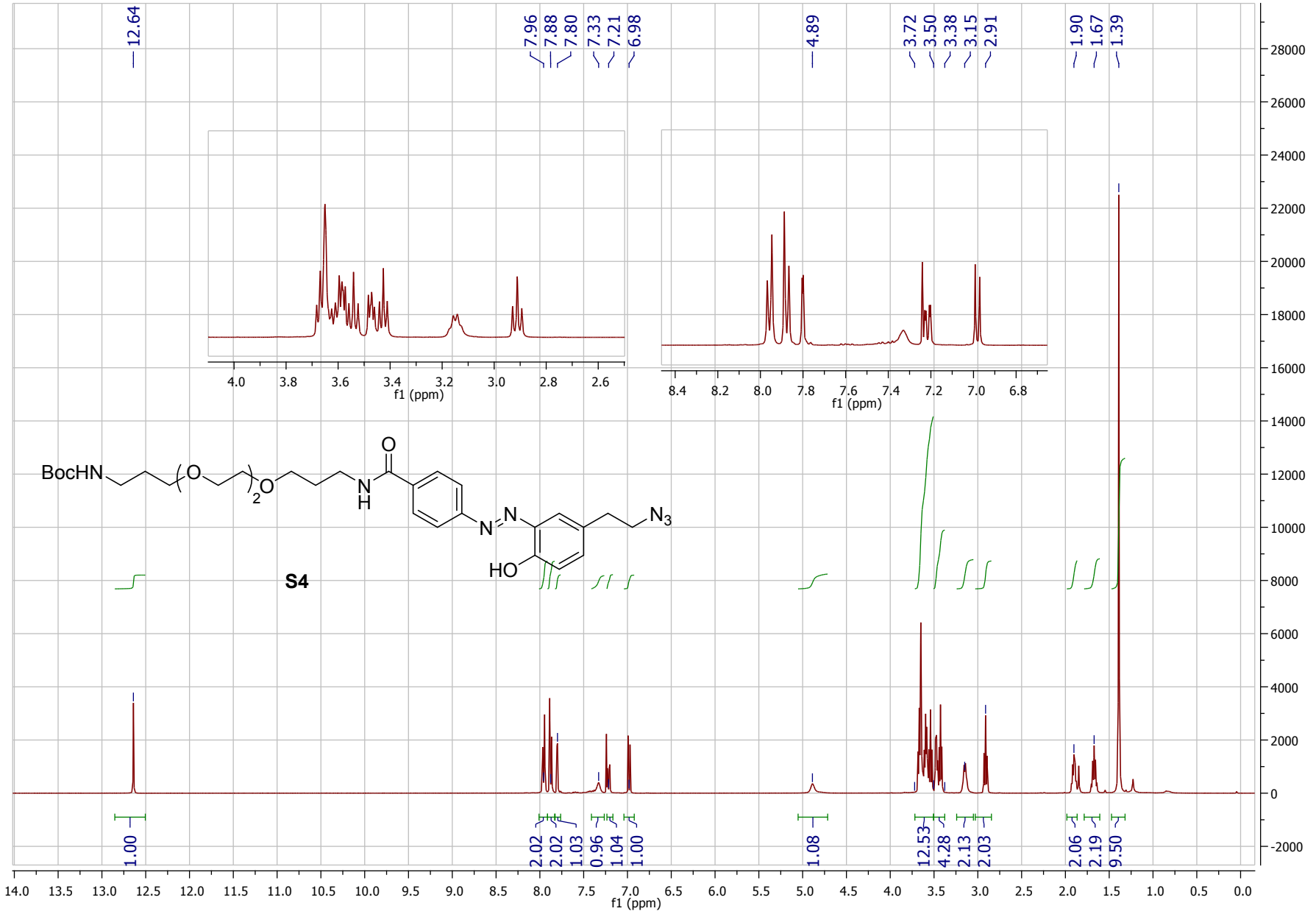

154.47
130.34
130.12
115.71
77.55
77.23
76.91
52.83
34.59
HO
N3
S1
f1 (ppm)
f1 (ppm)

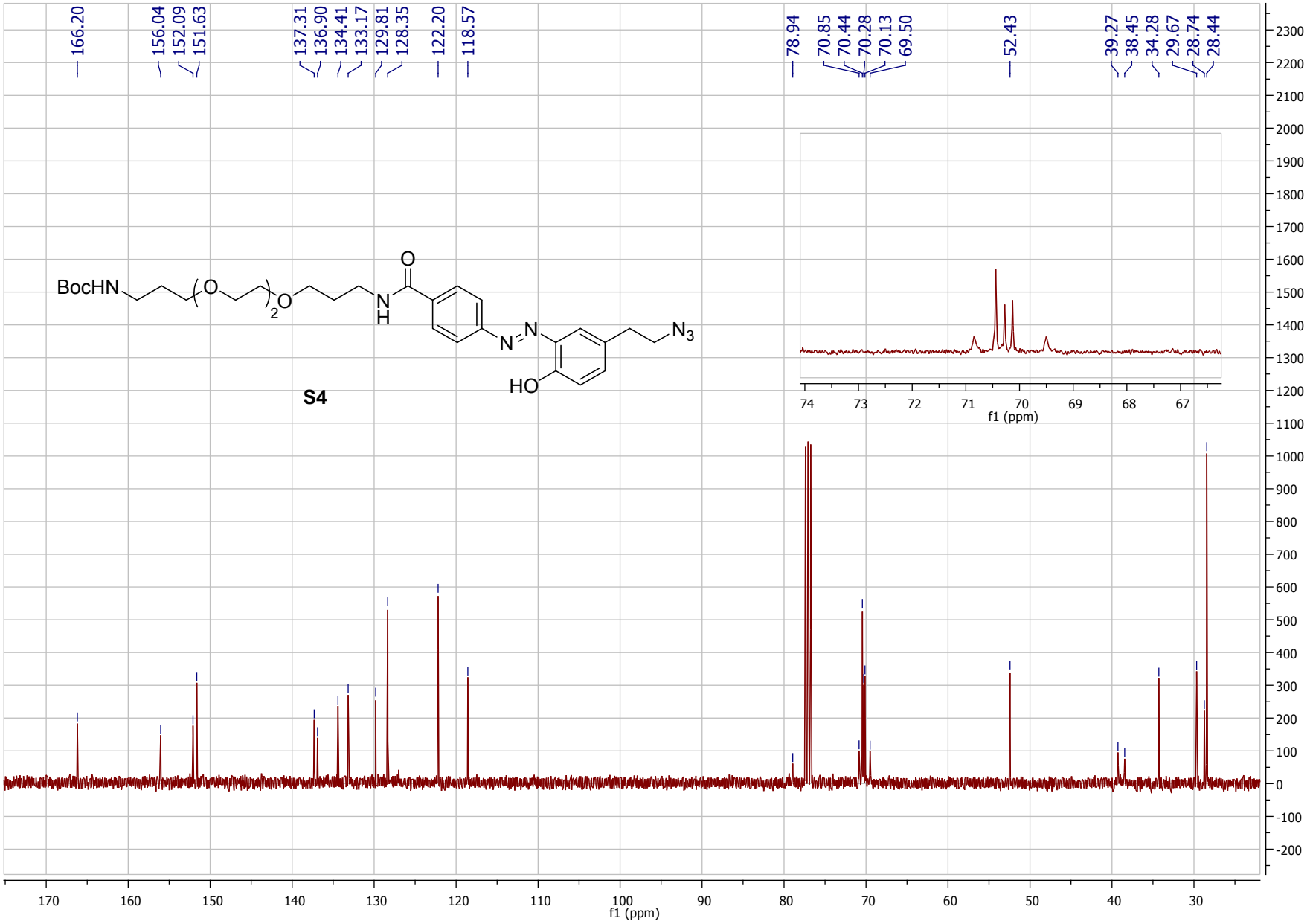

7.98
7.87
7.35
7.21
6.78
3.50
2.75
2.02
2.06
1.00
0.99
0.98
2.00
1.97
f1 (ppm)
HO
O
N
N
HO
N3
S2

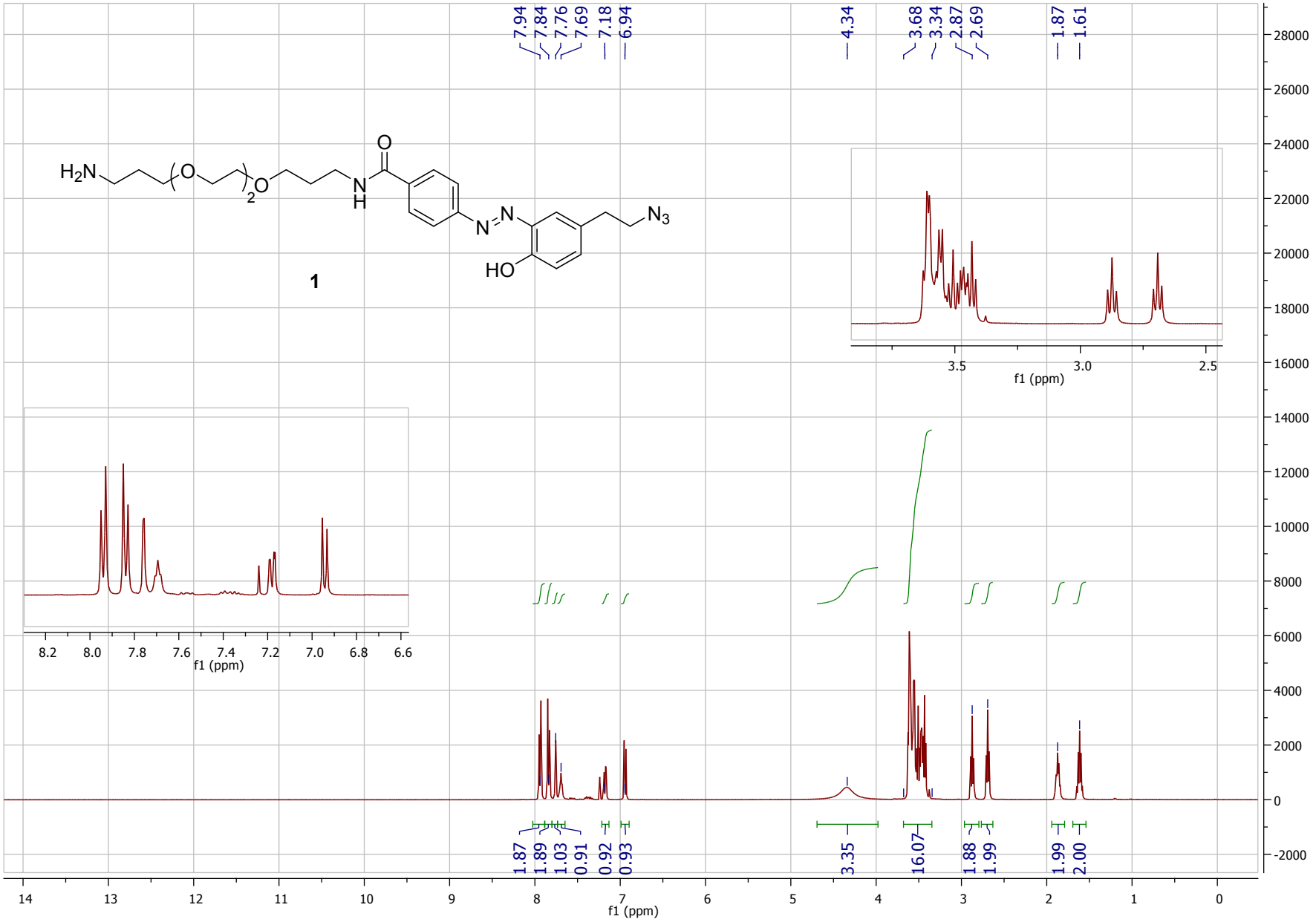

177.63
169.84
157.12
145.87
139.52
138.01
132.46
126.58
126.33
124.33
119.03
54.82
35.94
HO
O
N
N
HO
N3
S2
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

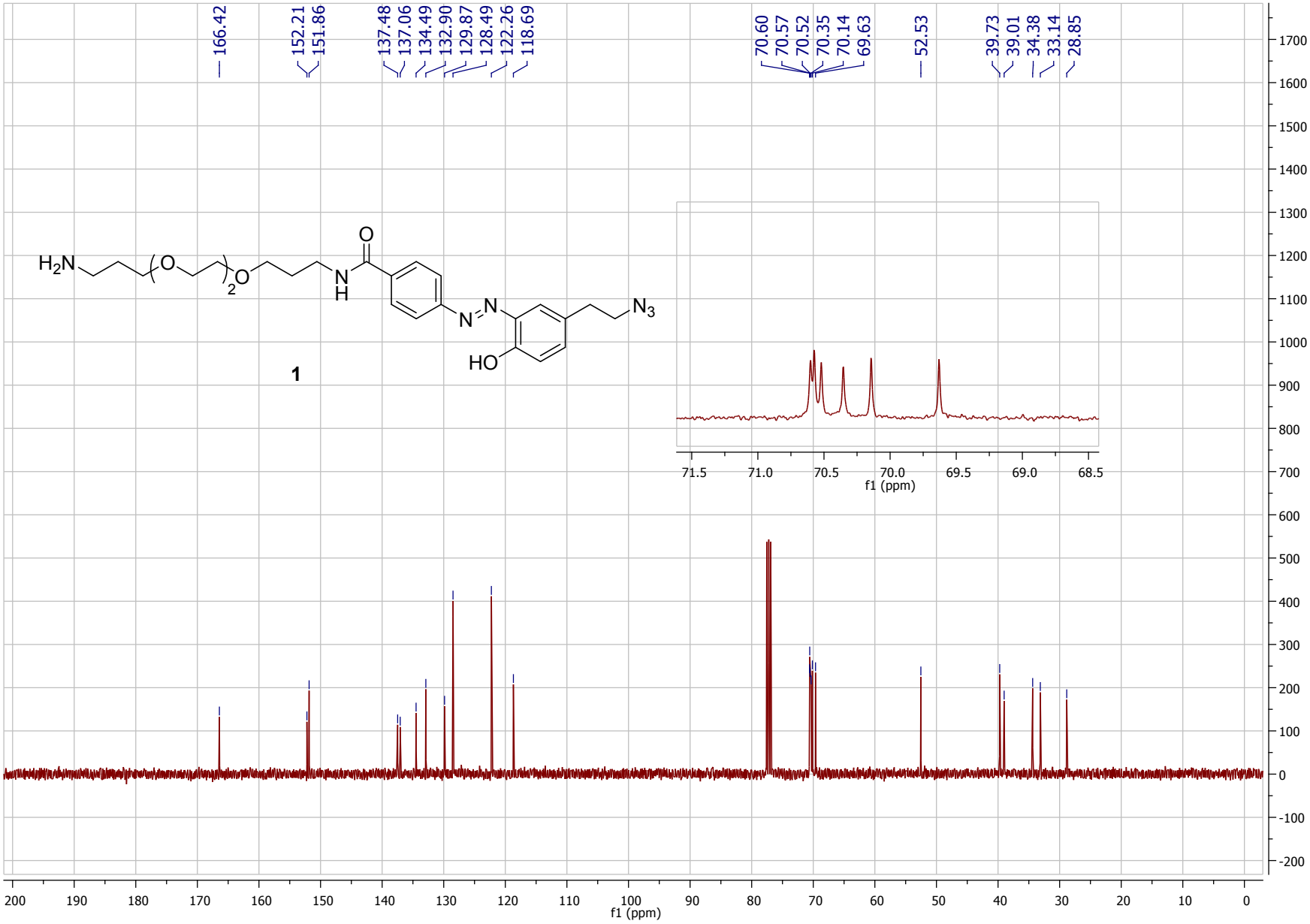

12.50
8.23
7.92
7.79
7.24
6.97
3.53
2.95
2.89
2.83
S3
1.00
2.04
2.07
0.99
1.12
1.01
2.10
6.89
f1 (ppm)


169.19
161.23
154.32
151.80
137.48
135.32
133.54
131.89
130.09
126.69
122.45
118.71
52.36
34.22
25.72
O
O
N
O
O
N
N
HO
N3
S3
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


12.64
7.96
7.88
7.80
7.33
7.21
6.98
4.89
3.72
3.50
3.38
3.15
2.91
1.90
1.67
1.39
BocHN
O
O
2
O
N
H
O
N
N
HO
N3
S4
f1 (ppm)
1.00
2.02
2.02
1.03
0.96
1.04
1.00
1.08
12.53
4.28
2.13
2.03
2.06
2.19
9.50


166.20
156.04
152.09
151.63
137.31
136.90
134.41
133.17
129.81
128.35
122.20
118.57
78.94
70.85
70.44
70.28
70.13
69.50
52.43
39.27
38.45
34.28
29.67
28.74
28.44
BocHN
O
O
2
O
N
H
N
N
HO
N3
S4
f1 (ppm)
f1 (ppm)


7.94
7.84
7.76
7.69
7.18
6.94
4.34
3.68
3.34
2.87
2.69
1.87
1.61
H2N
O
O
2
O
N
H
N
N
N3
HO
1
f1 (ppm)
f1 (ppm)
f1 (ppm)
1.87
1.89
1.03
0.91
0.92
0.93
3.35
16.07
1.88
1.99
1.99
2.00


166.42
152.21
151.86
137.48
137.06
134.49
132.90
129.87
128.49
122.26
118.69
70.60
70.57
70.52
70.35
70.14
69.63
52.53
39.73
39.01
34.38
33.14
28.85
H2N
O
2
O
N
H
O
N
N
N3
HO
1
71.5
71.0
70.5
70.0
69.5
69.0
68.5
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200