Diverse Redoxome Reactivity Profiles of Carbon Nucleophiles

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Supplementary Information

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Contents:

Reagents

Azido-tagged biotin with a photocleavable linker (biotin–ultraviolet (UV)–azide) was purchased from Kerafast. SCX spin columns were purchased from Nest group. Streptavidin sepharose was purchased from GE Healthcare Life Sciences. HPLC-grade water, ACN and methanol were purchased from J.T. Baker. Other chemicals and reagents were obtained from Sigma unless otherwise indicated.

Cell culture

Human RKO cells (ATCC) were maintained at 37 °C in a 5% CO_2, humidified atmosphere and were cultured in McCoy medium (Invitrogen) containing 10% FBS (Invitrogen). For qualitative *S*-sulphenylome analysis, cells were grown until 70–80% confluent, rinsed with PBS quickly and lysed.

Probe labeling and sample preparation

For proteomic analyses, cells were lifted with 0.25% trypsin-EDTA (Invitrogen), collected by centrifugation at 1,500*g* for 3 min, washed and resuspended in lysis buffer at a density of ~5 × 10^6 cells ml^{-1}. To one volume of cell pellet, 3 volumes of lysis buffer containing 5 mM probe and 200 unit/ml catalase was added. Cell pellets were lysed on ice in HEPES lysis buffer (50 mM HEPES, 150 mM NaCl, 1% Igepal, pH 7.5). The lysate was then incubated at 37 ^oC for 2 h. Reversibly oxidized cysteines in the lysate were reduced by adding 10 mM DTT and further incubating at RT for 1 h. Reduced cysteines were then alkylated with 32 mM iodoacetamide for 30 min in the dark. Protein concentrations of lysate samples were determined with the BCA assay (Pierce Thermo Fisher). For a typical qualitative *S*-sulphenylome analysis, ~5 mg protein was used. Protein precipitation was performed with a methanol-chloroform system (aqueous phase/methanol/chloroform, 4:4:1 (v/v/v). Proteins

were collected at the aqueous/organic phase interface as a solid disk after centrifugation at 1,400*g* for 20 min at 4 °C. Liquid layers were discarded and the protein was washed twice in methanol/chloroform (1:1, v/v), followed by centrifugation at 16,000*g* for 10 min at 4 °C to repellet the protein. The protein pellets were evaporated to dryness on a thermomixer and resuspended with 50 mM ammonium bicarbonate containing 0.2 M urea at a protein concentration of 2 mg ml^{-1}. Resuspended proteins were first digested with sequencing grade trypsin (Promega) at a 1:50 (enzyme/substrate) ratio overnight at 37 °C. A secondary digestion was performed by adding additional trypsin to a 1:100 (enzyme/substrate) ratio, followed by incubation at 37 °C for additional 4 h. The tryptic digests were desalted with HLB extraction cartridges (Waters). The desalted samples were then evaporated to dryness under vacuum. Desalted tryptic digests were reconstituted in an aqueous solution containing 30% ACN, 0.8 mM biotin–UV–azide, 8 mM sodium ascorbate, 1 mM TBTA and 8 mM $CuSO_4$, pH 6. The pH of the reaction mixture was adjusted using pH-indicator strips and the mixture was incubated for 2 h at room temperature within the dark rotation. The excess biotin reagents were removed by SCX chromatography. In brief, the sample was diluted into SCX loading buffer (5 mM KH_2PO_4, 25% ACN, pH 3.0), passed through the SCX spin columns and washed with several column volumes of loading buffer. The retained peptides were eluted with a series of high-salt buffers containing 100, 200 and 400 mM NaCl. Eluent was diluted 10 x with 50 mM sodium acetate buffer (NaAc, pH 4.5) and then allowed to interact with pre-washed streptavidin sepharose for 2 h at room temperature. Streptavidin sepharose then was washed with 50 mM NaAc, 50 mM NaAc containing 2 M NaCl and water twice each with votexing and/or rotation to remove non-specific binding peptides and resuspended in 25 mM ammonium bicarbonate. The suspension of streptavidin sepharose

was transferred to several glass tubes (VWR), irradiated with 365 nm UV light (Entela, Upland, CA) for 2 h at room temperature with stirring. The supernatant was collected, evaporated to dryness under vacuum and stored at −20 °C until analysis.

For western blotting analyses, cells were harvested and lysed in modified RIPA lysis buffer (vol = 0.25 ml/plate, 50 mM Triethanolamine, 150 mM NaCl, 1% NP-40, pH 7.4, 1% sodium deoxycholate, 0.1% SDS. EDTA-free protease inhibitor (1 tab/10 ml) and 200 units/ml catalase) for 20 min on ice. Cell lysis was aided by pipetting up and down. The lysate was centrifuged to remove the cell debris (20 min at 16,000 x g, 4 ^{o}C). Subsequently, the supernatants were combined and protein concentration was determined by BCA assay (conc. = 3 mg/ml). A western-blot was performed on the lysate by loading various concentrations (1 - 25 μg). The lysate was aliquot in eppendorf tubes (= 100 μl/tube), flash froze and stored at -80 ^{o}C for future use. The RKO cell lysate was thawed on ice and buffer exchanged to HEPES 50 mM, NaCl 100 mM (pH 7.4) using preequilibrated Micro Bio-Spin^{TM} P-30 gel columns (Bio-Rad, Catalog # 7326224) or Micro Bio-Spin^{TM} P-6 gel columns (Bio-Rad, Catalog # 7326222). The lysate recovered after filtration was aliquot in different eppendorf tubes (1.5 ml) to give effective concentration of 1.5 mg/ml. Appropriate amount of labeling buffer (HEPES 50 mM, NaCl 100 mM, pH 7.4) was added followed by the addition of probe (5 μl of 2 mM solution in 70:30 buffer:DMSO to give 100 μM effective concentration) to give a total reaction volume of 100 μl. Resulting reaction mixtures were allowed to shake at room temperature for 1 h. After the completion, the reaction was quenched by filtering using preequilibrated Micro Bio-Spin^{TM} P-30 gel columns (Bio-Rad, Catalog # 7326224) or Micro Bio-Spin^{TM} P-6 gel columns (Bio-Rad, Catalog # 7326222). To the filtered lysate recovered from following the above protocol was added biotin azide (2 μl of 5 mM stock solution to give effective concentration of 100 μM),

followed by a solution of $CuSO_4$ (2 μl of 15 mM solution to give effective concentration of 250 μM) and BTTP (5 μl of 10 mM solution to give effective concentration of 500 μM). Finally, a solution of sodium ascorbate (2 μl of 125 mM to give effective concentration of 1.5 mM) was added. The total reaction volume was adjusted to 100 μl by the addition of appropriate volume of buffer. Resulting reaction mixture was allowed to shake at room temperature for 1 h. The click reaction was quenched by the addition of EDTA solution (1 μl of 100 mM stock solution).

LC-MS/MS

LC-MS/MS analyses were performed on a Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) operated with an Easy-nLC1000 system (Thermo Fisher Scientific). Samples were reconstituted in 0.1% formic acid and pressure-loaded onto a 360 µm outer diameter × 75 µm inner diameter microcapillary precolumn packed with Jupiter C18 (5 µm, 300 Å, Phenomenex) and then washed with 0.1% acetic acid. The precolumn was connected to a 360 µm outer diameter × 50 µm inner diameter microcapillary analytical column packed with the ReproSil-Pur C18-AQ (3 µm, 120 Å, Dr Maisch) and equipped with an integrated electrospray emitter tip. The spray voltage was set to 1.5 kV and the heated capillary temperature to 250 °C. For qualitative analysis, two different LC gradient conditions were used. Gradient A consisted of 0–15 min, 2% B; 35 min, 15% B; 40 min, 20% B; 50 min, 30% B; 55 min, 35% B; 59–65 min, 90% B; 80–85 min, 2% B (A=water, 0.1% formic acid; B=ACN/0.1% formic acid) at a flow rate of 300 nl min^{-1}. Gradient B consisted of 0–10 min, 5% B; 100 min, 35% B; 120–130 min, 90% B; 140–150 min, 5% B at a flow rate of 300 nl min^{-1}. HCD MS/MS spectra were recorded in the data-dependent mode using a Top 12 method for qualitative analysis and Top 20 method for quantitative analysis, respectively. MS1 spectra were measured with a resolution of 70,000,

an AGC target of 3e6 and a mass range from *m/z*300 to 1,800. HCD MS/MS spectra were acquired with a resolution of 17,500, an AGC target of 2e5 and normalized collision energy of 28. Peptide *m/z* that triggered MS/MS scans were dynamically excluded from further MS/MS scans for 20 s.

Peptide identification and site localization

Raw data files were analyzed by using TagRecon algorithm (Version 1.4.47) and MS-GF+(Version 9517) against a decoy protein database consisting of forward and reversed human RefSeq database (Version 20130621). Precursor ion mass tolerance and fragmentation tolerance were both set as 10 ppm for the database search. The maximum number of modifications allowed per peptide was three, as was the maximum number of missed cleavages allowed. Methionine oxidation (15.9949 Da) and cysteine modifications either by iodoacetamide (carbamidomethyl, 57.0214 Da) or by *S*-sulfenylcysteine tagged with Probe-triazohexanoic acid group were searched as dynamic modifications (333.1689 for DYn-2, 356.1154 for TD, 320.1485 for PYD, 334.1641 for PRD, and 418.1311 for BTD, respectively). The maximum Q value of PSMs was adjusted to achieve either a peptide or a protein false discovery rate no greater than 2% using IDPicker software (Version 3.0.529). All the automatically identified peptides were manually evaluated as previously described.

MS1 filtering based quantification and standardization

Raw files then were directly imported into Skyline in their native file format. MS1 filtering was performed as previously described and graphical displays of chromatographic traces for the top three isotopic peaks were manually inspected for proper peak picking. Those with isotopic dot product scores lower than 0.8 were rejected to ensure that the correct peaks were identified. The mean peak areas obtained from two biological replicates were

measured and standardized with Z-score approach. Z-score for each modified peptide was calculated from the following formula. $z = (X - \mu) / \sigma$ where z is the z-score, X is the value of the element, μ is the population mean, and σ is the standard deviation.

Bioinformatics

The consensus sequence motifs of *S*-sulfenylated cysteines were visualized by pLogo or 2LogP, a linear sequence prediction algorithm based on their statistical significance ($P<0.05$). The crystal structures of proteins were visualized by Discovery Studio (Version 2.5, Accelrys) and the relative RSA values for cysteine residues were calculated with a default probe radius of 1.4 Å. The heat map was generated by Microsoft Excel.

Western blot. Protein samples were separated by SDS-PAGE using Mini-Protean TGX 4-15% Tris-Glycine gels (BioRad) and transferred to a polyvinylidene difluoride (PVDF) membrane (BioRad). After transfer, the membrane was washed with TBST (3 x 5 min.) and blocked with 3% BSA in TBST for 1 h at room temperature or overnight at 4 °C. The membrane was washed with TBST (3 x 5 min.) and immunoblotting was performed with the following primary and secondary antibodies at the indicated dilutions in TBST, unless otherwise noted: streptavidin-HRP (GE Healthcare, 1:80000), GAPDH (Santa Cruz Biotechnology, 1:200), rabbit anti-mouse IgG-HRP (Invitrogen, 1:30000). PVDF membrane was developed with chemiluminescence (GE Healthcare ECL Plus Western Blot Detection System) and imaged by film.

LC-MS Nucleophile Rate Assay - The LC/MS assay for measuring reaction rates of nucleophiles with a model dipeptide-SOH was performed as previously described [Gupta, V.; Carroll, K. S. *Chem. Sci.* **2016**, *7*, 400. Gupta, V.; Carroll, K. S. *Chem. Commun. (Camb)* **2016,** *52*, 3414-7. Gupta, V.; Paritala, H.; Carroll, K. S. *Bioconjug. Chem.* **2016,** *27* (5), 1411-8.].

Sample preparation - Stock solutions of nucleophile probes were prepared in DMSO (100 mM concentration). Stock solutions were then diluted to appropriate concentration in PBS (10 mM, pH = 7.4). The pH was checked for each stock solution and was found to be in range of 7.40 – 7.45. Similarly, a 100 mM stock solution of dipeptide sulfenamide was prepared in acetonitrile and diluted to appropriate concentration in acetonitrile.

Assay – To initiate the reaction for each rate study, to a 2 mL solution of the nucleophile, was added 1 mL solution of cyclic sulfenamide. Effective concentrations were: Cyclic sulfenamide – 25 or 50 or 100 μM; Nucleophile – 125 or 250 μM, or 1.0 mM. A 5 – 10 fold concentration difference was maintained between nucleophile and dipeptide-SOH to maintain pseudo 1st-order conditions. An aliquot (300 μL) of the reaction was removed and quenched at regular intervals by adding it to an LC-MS vial containing formic acid (100 μL) and analyzing it by LC-MS. At each time point, the area under the curve for product was obtained quantified and plotted versus time to obtain k_{obs}. KaleidaGraph (version 4.1.1) was used for graphing and further data analysis to obtain pseudo unimolecular 1st order rate constants. The following equation was used for the purpose of graphing: $m1 * (1 - \exp(-m2 * m0)); m1 = xx; m2 = yy$. 2nd order rate constants were obtained by dividing k_{obs} by nucleophile concentration, which presupposes that the same rate law applies throughout the entire concentration range.

LC/MS instrumentation and columns - The LC-MS used was Agilent technologies 1220 Infinity LC and Agilent Technologies 6120 quadrupole MS. The column used was Agilent Poroshell 120 SB C-18, 2.7 μM particle size and dimensions were 3.0 x 50 mm. LC-MS grade solvents were used which were buffered with 0.1% Formic acid (LC-MS grade). Data was analyzed by LC/MSD ChemStation (Rev. B.04.03-SP1).

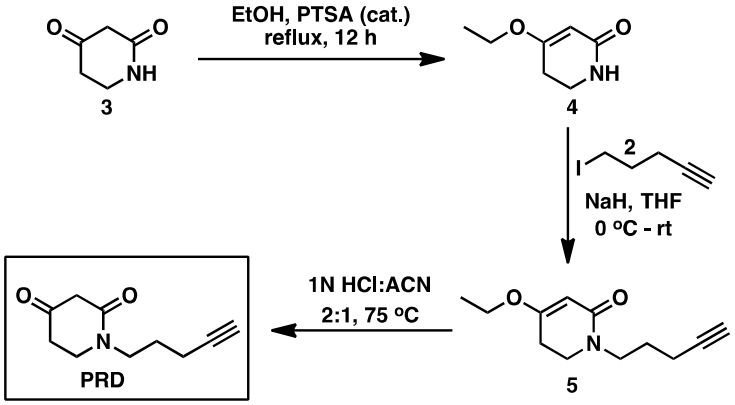
LC-MS method – The gradient was started at 95% H_2O – 5% acetonitrile (0 minutes) with a flow rate of 1 ml/min. The gradient was changed to 0% H_2O – 100% acetonitrile over 5 min. with same flow rate. This gradient was maintained for 2 min. Total run time was 7 minutes followed by a 1.4 min. of post-time. The LC trace was obtained by monitoring 190 nm wavelength.

Synthetic Materials and Methods.

All reactions were conducted in flame-dried glassware under nitrogen pressure with dry solvents, unless otherwise noted. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific or VWR and used as received. Silica gel P60 (Sorbent Technologies) was used for column chromatography. Reactions were monitored by thin layer chromatography (TLC) carried out using Analtech 60 F254 silica gel (precoated sheets, 0.25 mm thick) and LC/MS. ^{1}H-NMR and ^{13}C-NMR spectra were collected in DMSO-d_6 or $CDCl_3$ (Cambridge Isotope Laboratories, Cambridge, MA) at 400 (for ^{1}H-NMR) and 100 MHz (^{13}C-NMR) respectively, using a Bruker AM-400 instrument with chemical shifts relative to residual $CHCl_3$ (7.27 and 77.37 ppm). Low-resolution mass spectral analyses were carried out on an Agilent LC/MS system. All spectra are available upon request.

DYn-2 was prepared according to protocol reported in Gupta, V.; Carroll, K.S., *Chem. Sci.* **2016**, *7*(1), 400-415.

Scheme S1 Synthesis of small molecule sulfenic acid probe PRD



Synthesis of 4-ethoxy-5,6-dihydropyridin-2(1H)-one (4). To the slurry of 2,4-piperidinedione (1.1 g, 10 mmol) in absolute ethanol (75 ml) was added PTSA (0.15 g, 1 mmol) and resulting solution was refluxed for 18 h under nitrogen. After the completion of the reaction, ethanol was

removed and residue was partitioned between ethyl acetate and water. Organic layer was separated and aqueous layer was subjected to ethyl acetate extractions (2 x 25 ml). Combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and evaporated. This crude was purified by column chromatography using a gradient of 2% - 5% MeOH/DCM to recover the title product in 75% yield.

^{1}H-NMR (400 MHz, $CDCl_3$): 6.49 (bs, 1H), 5.01 (s, 1H), 3.88 (q, 2H, J = 8.0 Hz), 3.39 (t, 2H, J = 8.0 Hz), 2.42 (t, 2H, J = 8.0 Hz), 1.34 (t, 3H, J = 8.0 Hz); ^{13}C-NMR (in CDCl3, 100 MHz): 170.1, 169.5, 93.8, 64.0, 38.6, 27.9, 14.1.

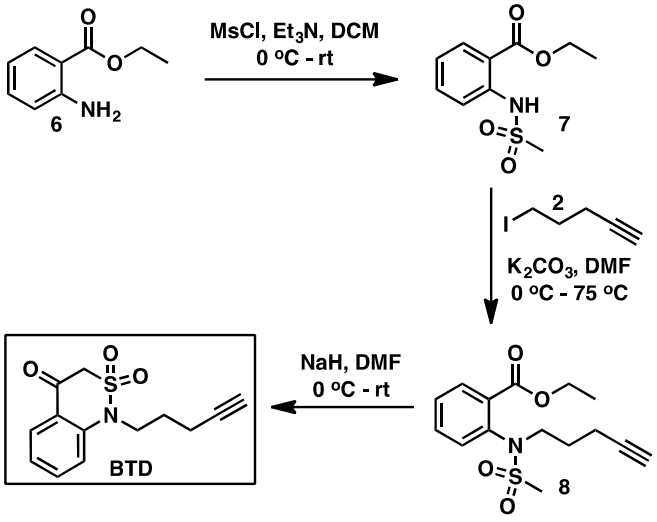
Synthesis of 4-ethoxy-1-(pent-4-yn-1-yl)-5,6-dihydropyridin-2(1*H*)-one (5). To a slurry of NaH (0.36 g, 60% w/w, 9.0 mmol) in anhydrous THF (5 ml), was added a solution of **4** (1.06 g, 7.5 mmol) in anhydrous THF (10 ml) at 0 ^{o}C under nitrogen. The resulting reaction mixture was stirred at rt for 15 minutes and then again cooled to 0 ^{o}C. To this anionic reaction mixture was added dropwise, a solution of 5-iodopent-1-yne **(2)** (1.41, 8.3 mmol) in THF (5 ml). Resulting RM was stirred at rt for 6 h. After completion, the reaction mixture was quenched with water. THF was removed under reduced pressure and residue was partitioned between ethyl acetate and water. Organic layer was separated and aqueous layer was extracted with ethyl acetate (2 x 25 ml). Combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and evaporated. This crude product was purified by column chromatography using a gradient of 2% - 5% MeOH/DCM to recover the title product in 88% yield as off-white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 5.02 (s, 1H), 3.84 (q, 2H, J = 7.0 Hz), 3.43 (t, 2H, J = 7.1 Hz), 3.39 (t, 2H, J = 7.2 Hz), 2.42 (t, 2H, J = 7.1 Hz), 2.20 (dt, 2H, J1 = 7.2 Hz, J2 = 2.6 Hz), 1.94 (t, 1H, J = 2.6 Hz), 1.74 (p, 2H, J = 7.1 Hz), 1.31 (t, 3H, J = 7.0 Hz); ^{13}C-NMR (in CDCl3, 100 MHz): 167.7, 167.3, 94.6, 83.7, 68.8, 64.1, 45.5, 44.9, 28.1, 26.7, 16.0, 14.1.

Synthesis of 1-(pent-4-yn-1-yl)piperidine-2,4-dione (PRD). To a solution of **5** (0.75 g, 3.5 mmol) in acetonitrile (15 ml) was added 1N HCl (30 ml). Resulting yellow solution was stirred at 75 ^{o}C with monitoring. The reaction was complete after 5 h. At this stage, the reaction mixture was concentrated and the product was directly purified by prep-HPLC.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.61 (t, 2H, J = 6.2 Hz), 3.58 (t, 2H, J = 7.1 Hz), 3.32 (s, 2H), 2.62 (t, 2H, J = 6.2 Hz), 2.24 (dt, 2H, J1 = 6.9 Hz, J2 = 2.7 Hz), 1.98 (t, 1H, J = 2.7 Hz), 1.80 (p, 2H, J = 7.0 Hz); ^{13}C-NMR (in CDCl3, 100 MHz): 203.6, 166.4, 83.3, 69.3, 49.0, 46.5, 43.8, 38.7, 26.4, 16.2.

Scheme S2 Synthesis of small molecule sulfenic acid probe BTD



Preparation of ethyl 2-(methylsulfonamido)benzoate (7). In a modified literature procedure, ethyl 2-aminobenzoate (3.3 g, 20 mmol) was taken in anhydrous DCM (100 ml) and triethylamine (2.43 g , 24 mmol) was added to it. Resulting reaction mixture was cooled to 0 ^{o}C and methanesulfonyl chloride (2.75 g, 24 mmol) was added directly. The reaction mixture became cloudy after which it was allowed to stir at room temperature for 3 h under nitrogen. After the completion of reaction as indicated by TLC and LC-MS, reaction mixture was further diluted by dichloromethane to 150 ml and washed with water (3 x 50 ml) and brine. Organic

layer was then dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. Crude liquid was purified by column chromatography using 25% EtOAc/Hexane gradient to obtain pure product (pale yellow crystalline solid) in 91% yield.

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 10.53 (s, 1H), 8.08 (dd, 1H, J1 = 8.0 Hz, J2 = 1.6 Hz), 7.76 (dd, 1H, J1 = 8.0 Hz, J2 = 0.9 Hz), 7.56 (dt, 1H, J1 = 8.0 Hz, J2 = 1.6 Hz), 7.13 (dt, 1H, J1 = 8.0 Hz, J2 = 1.1 Hz), 4.40 (q, 2H, J = 8.0 Hz), 3.06 (s, 3H), 1.42 (t, 3H, J = 8.0 Hz); ^{13}C-NMR (in $CDCl_3$, 100 MHz): δ 168.0, 140.9, 134.8, 131.5, 122.8, 118.1, 115.7, 61.8, 40.0, 14.2.

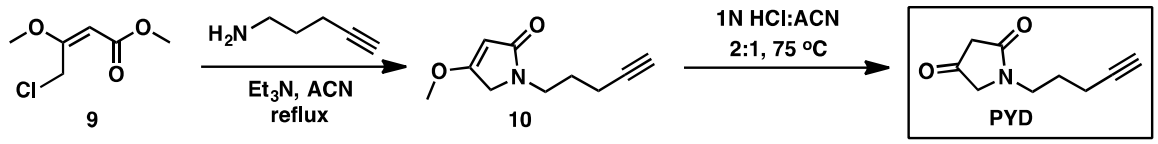
Preparation of ethyl 2-(*N*-(pent-4-yn-1-yl)methylsulfonamido)benzoate (8). Sulfonamide **7** prepared above (2.43 g, 10 mmol) was taken in anhydrous DMF (10 ml) and anhydrous potassium carbonate (2.8 g, 20 mmol, 2 eq) was added to it. Resulting reaction mixture was stirred for 15 minutes followed by the addition of 5-iodopent-1-yne **(2)** (2.13 g, 15 mmol). Resulting reaction mixture was heated and stirred at 75 ^{o}C for 5 h under nitrogen. After completion, the reaction mixture was neutralized by the addition of water and aqueous layer was extracted with ethyl acetate (5 x 25 ml). Combined organic layers were washed with water (2 x 50 ml) and brine (1 x 50 ml). Organic layer was then dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. Crude liquid was purified by column chromatography using 25% - 40% EtOAc/Hexane gradient to obtain pure product in quantitative yield.

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 7.90 – 7.94 (m, 1H), 7.52 – 7.57 (m, 1H), 7.39 – 7.44 (m, 2H), 4.37 (q, 2H, J = 7.1 Hz), 2.95 (s, 3H), 2.24 (dt, 2H, J1 = 7.0 Hz, J2 = 2.5 Hz), 1.92 (t, 1H, J = 2.7 Hz), 1.78 (p, 2H, J = 7.2 Hz), 1.39 (t, 3H, J = 7.1 Hz); ^{13}C-NMR (in $CDCl_3$, 100 MHz): δ 165.9, 138.2, 132.7, 131.7, 131.5, 131.1, 128.4, 83.1, 69.1, 61.5, 50.6, 39.0, 27.8, 15.8, 14.2.

Preparation of 1-(pent-4-yn-1-yl)-1*H*-benzo[*c*][1,2]thiazin-4(3*H*)-one 2,2-dioxide (BTD). To a stirred solution of NaH (60% in mineral oil, 0.6 g, 15 mmol, 2 eq.) in dry DMF (5 ml) was added dropwise a solution of **8** (1.9 g, 7.5 mmol) in DMF (10 ml). Resulting reaction mixture was allowed to stir under nitrogen with monitoring. The reaction mixture color changed from white to red-brown. After the completion (7 h), the reaction mixture was acidified to pH 3 with 2N HCl. Aqueous layer was extracted with ethyl acetate (5 x 25 ml). Combined organic layers were washed with water (2 x 50 ml) and brine (1 x 50 ml). Organic layer was then dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. Crude liquid was purified by column chromatography using 20% EtOAc/Hexane gradient followed by 100% DCM gradient to obtain pure product as a cream solid in 70% yield.

^{1}H-NMR (in $CDCl_3$, 400 MHz): 8.16 (dd, 1H, J1 = 7.9 Hz, J2 = 1.6 Hz), 7.68 (dt, 1H, J1 = 8.3 Hz, J2 = 1.7 Hz), 7.20 – 7.31 (m, 2H), 4.35 (s, 2H), 4.12 (t, 2H, J = 7.6 Hz), 2.36 (dt, 2H, J1 = 6.7 Hz, J2 = 2.7 Hz), 2.00 – 2.10 (m, 3H); ^{13}C-NMR (in $CDCl_3$, 100 MHz): δ 184.1, 143.2, 136.6, 129.8, 123.9, 123.5, 117.9, 82.5, 69.8, 61.8, 44.8, 27.1, 15.9.

Scheme S3 Synthesis of small molecule sulfenic acid probe PYD



Preparation of 4-methoxy-1-(pent-4-yn-1-yl)-1,5-dihydro-2*H*-pyrrol-2-one (10). To a refluxing solution of 5-aminopentyne (2.1 g, 25 mmol) in acetonitrile, was added dropwise a solution of methyl-(E)-4-chloro-3-methoxy-2-butenoate (3.3 g, 20 mmol) in acetonitrile and triethylamine (3.1 ml, 22 mmol) in acetonitrile in parallel. Resulting reaction mixture was allowed to reflux with monitoring. After successful completion of the reaction (14 h), reaction

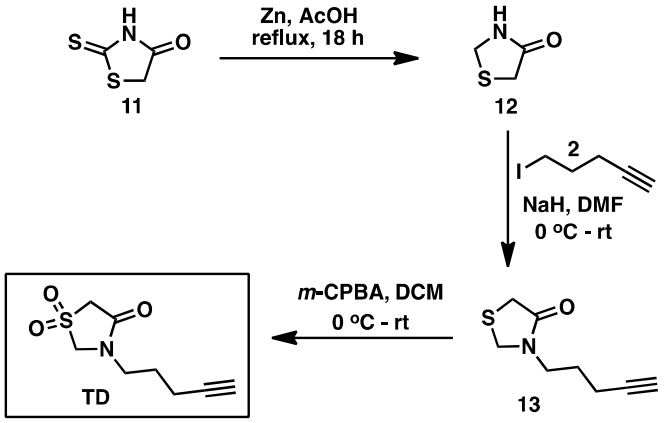
mixture was cooled to RT and acetonitrile was removed under reduced pressure. Residue was partitioned between DCM and water. Organic layer was removed and aqueous layer was extracted by DCM (50 ml x 2). Combined organic layer was washed with 1N HCl, water and brine and dried over anhydrous $MgSO_4$, filtered, and concentrated on a rotary evaporator. The crude residue was further purified by flash chromatography (DCM/MeOH) to give the product **(10)** in good yield (74%) as a yellow solid.

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 5.02 (s, 1H), 3.84 (bs, 2H), 3.74 – 3.78 (m, 3H), 3.45 (t, 2H, J = 7.2 Hz), 2.20 (tt, 2H, J1 = 7.1 Hz, J2 = 2.0 Hz), 1.95 (dt, 1H, J1 = 3.4 Hz, J2 = 0.8 Hz), 1.76 (p, 2H, J = 7.1 Hz); ^{13}C-NMR (in $CDCl_3$, 100 MHz): δ 173.1, 172.1, 94.5, 83.3, 68.9, 58.1, 50.8, 40.6, 27.4, 15.9.

Preparation of 1-(pent-4-yn-1-yl)pyrrolidine-2,4-dione (PYD). To a solution of **10** (0.5 g, 2.8 mmol) in acetonitrile (10 ml), was added 1N HCl (20 ml). Resulting reaction mixture was heated to 60 ^{o}C and stirred with monitoring. After the completion, the reaction mixture was cooled to RT and evaporated to dryness. After workup, the crude was purified by column chromatography using a gradient of 30% EtOAc/DCM to 50% EtOAc/DCM to recover the pure product as a pale yellow solid (78% yield).

^{1}H-NMR - (in $CDCl_3$, 400 MHz): δ 3.87 (t, 2H, J = 1.2 Hz), 3.53 (t, 2H, J = 7.1 Hz), 3.00 (s, 2H), 2.23 (dt, 2H, J1 = 6.8 Hz, J2 = 2.7 Hz), 1.92 (t, 1H, J = 2.7 Hz), 1.76 (p, 2H, J1 = 6.9 Hz); ^{13}C-NMR (in $CDCl_3$, 100 MHz): δ 203.5, 169.1, 83.0, 69.2, 57.6, 41.7, 41.4, 25.6, 16.2.

Scheme S4 Synthesis of small molecule sulfenic acid probe TD



Preparation of 3-(pent-4-yn-1-yl)thiazolidin-4-one (13). To the slurry of NaH (0.480 g, 12.0 mmol, 60%) in anhydrous DMF was added a solution of thiazolidin-4-one **12** (1.03 g, 10.0 mmol) in DMF at 0 ^{o}C under nitrogen. This pale yellow reaction mixture was allowed to stir at RT for 30 minute. At this stage, a solution of 5-iodopentyne (1.94 g, 10.0 mmol) in anhydrous DMF was added dropwise to the reaction mixture at RT. Resulting reaction mixture was then allowed to stir at same temperature with monitoring. After 5 h, complete consumption of 12 was observed so the reaction was quenched by the addition of water. Aqueous layer was extracted with EtOAc (5 x 25 ml). Combined EtOAc layers were washed with water (2 x 50 ml) and brine. Organic layer was then dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. Crude was purified by column chromatography using EtOAc/DCM gradient (0% to 20%). Pure product was recovered in 61% yield.

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 4.40 (t, 2H, J = 1.4 Hz), 3.53 (t, 2H, J = 1.4 Hz), 3.45 (t, 2H, J = 7.1 Hz), 2.22 (dt, 2H, J1 = 7.0 Hz, J2 = 2.7 Hz), 1.98 (t, 1H, J = 2.7 Hz), 1.78 (p, 2H, J = 7.0 Hz); ^{13}C-NMR (in $CDCl_3$, 100 MHz): δ 171.2, 82.9, 69.3, 47.8, 43.7, 32.4, 25.9, 16.0.

Preparation of 1-(pent-4-yn-1-yl)piperidine-2,4-dione (TD). To an ice-cooled solution of **13** (1.0 g, 5.9 mmol) in DCM, was added m-chloroperoxybenzoic acid (mCPBA, 70%, 5.83 g, 23.6 mmol, 4 eq) in several batches while keeping the reaction < 4 ^{o}C. After complete additions, the

reaction mixture was allowed to stir at rt with monitoring. The reaction was complete after 12 h and at this stage DMSO was added to quench unreacted mCPBA. The reaction mixture was then diluted with DCM and washed with saturated sodium bicarbonate solution (3 x 50 ml), water (2 x 50 ml) and brine. Organic layer was then dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. Crude was purified by column chromatography using EtOAc/DCM gradient (0% to 25%) followed by prep-HPLC.

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 4.76 (s, 2H), 4.12 (s, 2H), 3.47 (t, 2H, J = 7.0 Hz), 2.81 (1H, s, J = 2.7 Hz), 2.17 (dt, 2H, J1 = 7.2 Hz, J2 = 2.6 Hz), 1.68 (p, 2H, J = 7.2 Hz); ^{13}C-NMR (in $CDCl_3$, 100 MHz): δ 163.6, 83.6, 71.6, 67.1, 52.0, 41.9, 25.6, 15.1.

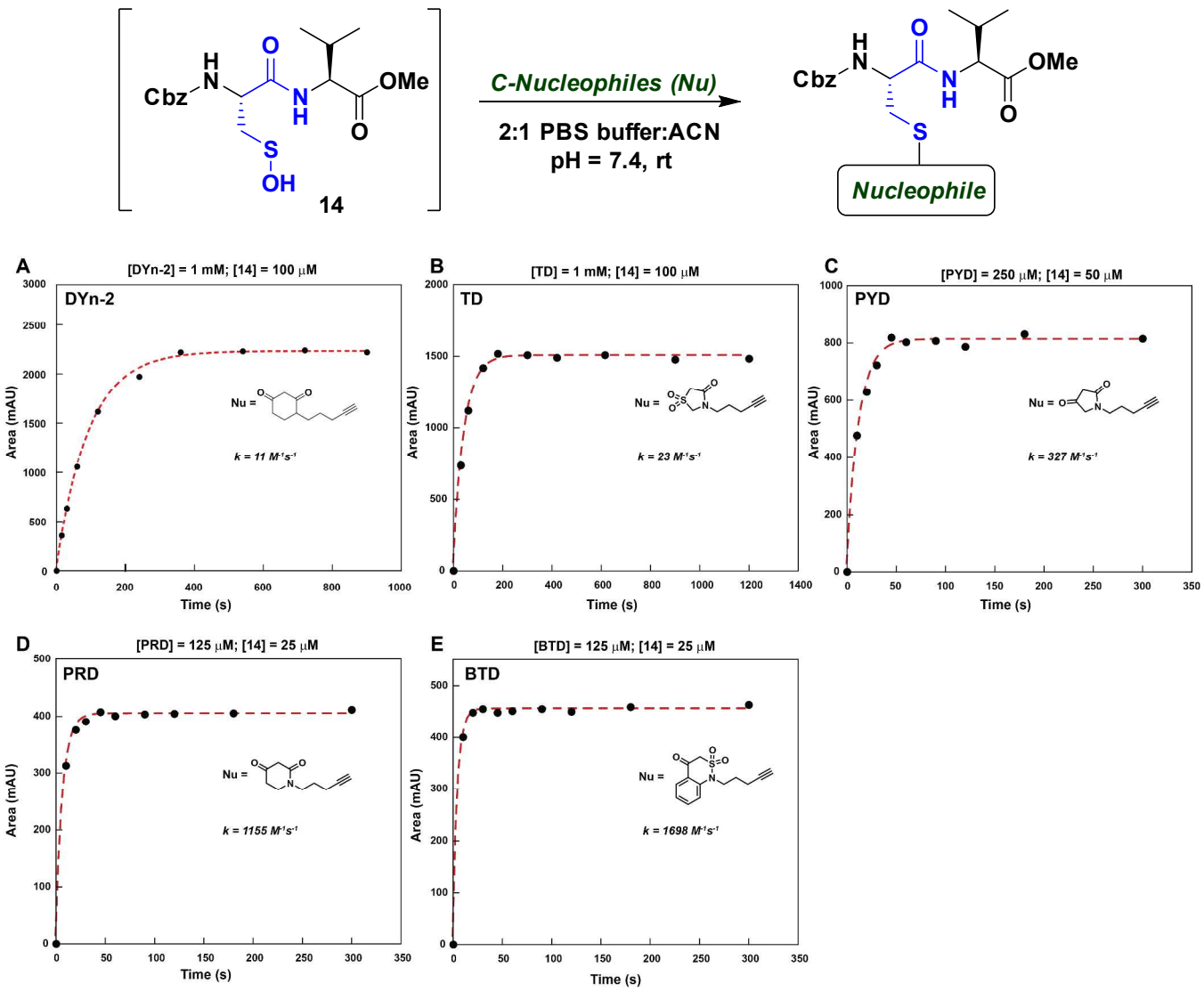


Fig. S1. Representative rate plots for the reaction of C-nucleophiles used in the present study with model dipeptide-SOH. Observed rate constants (k_{obs}) were obtained under pseudo first-order conditions and determined by a plot of thioether product formation versus time. The initial concentration of each reactant is indicated above each plot. Additional details pertaining to the LC/MS assay and kinetic analyses are provided in the Supporting Information above.

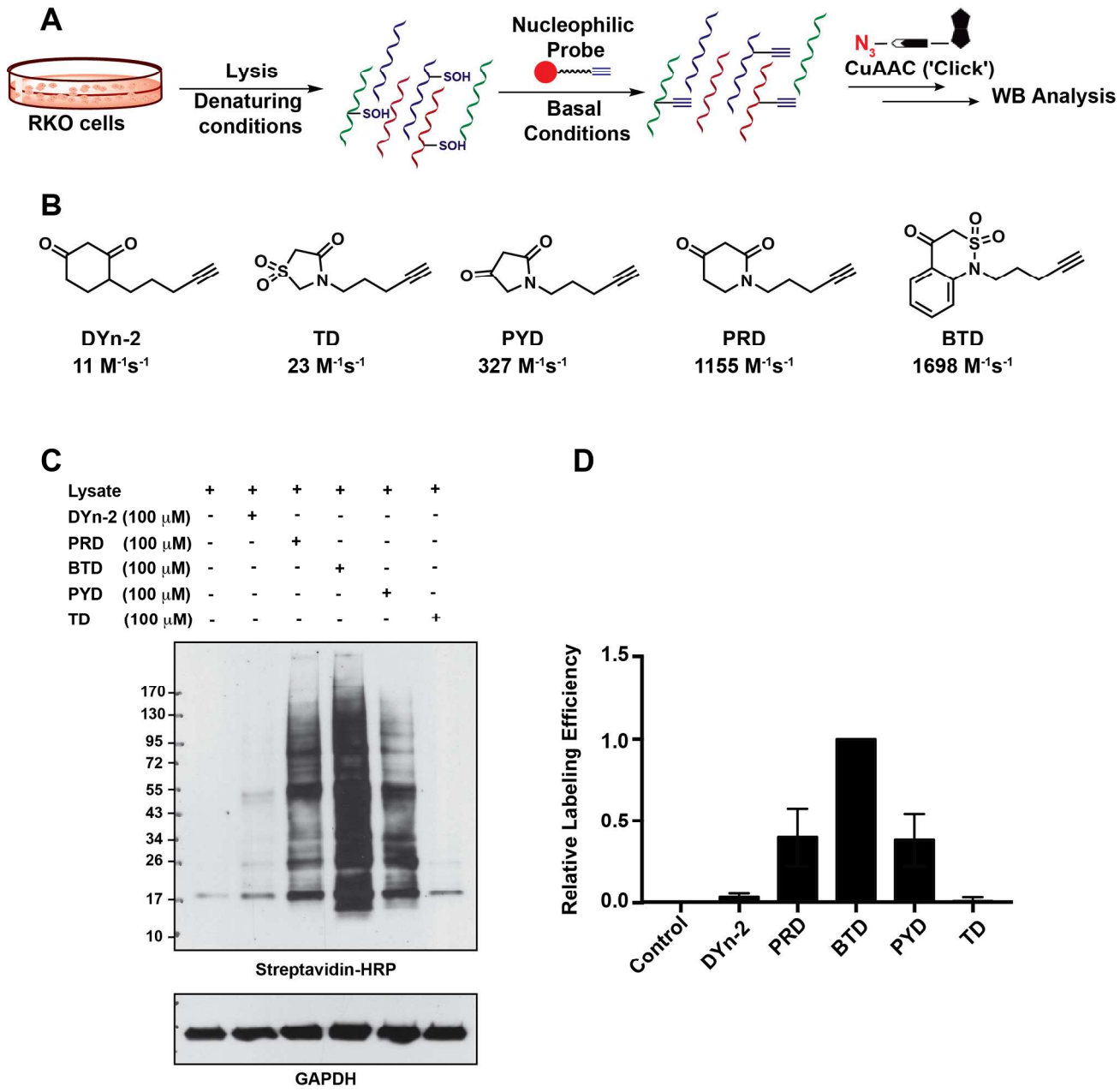


Fig. S2. (A) Scheme outlining the labeling of RKO cells by nucleophile probes. **(B)** Rank order of the reactivity of probes by rate constants. **(C)** Western blot showing the comparative capture of protein sulfenic acid by different probes and total GAPDH in RKO cell lysate. Lysate was incubated with 100 μM probe or vehicle for 1 h at RT. **(D)** Densitometric quantification of western blot using ImageJ.

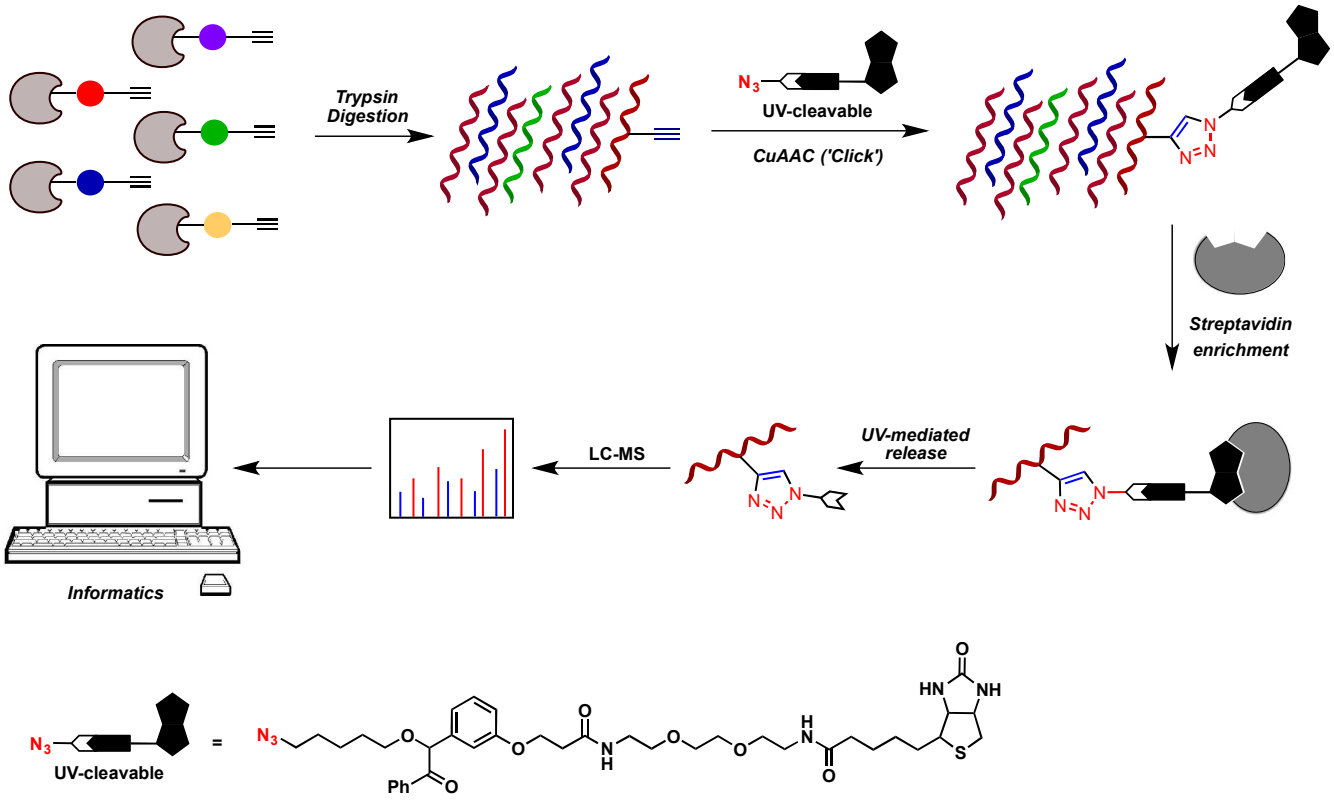


Fig. S3. MS workflow

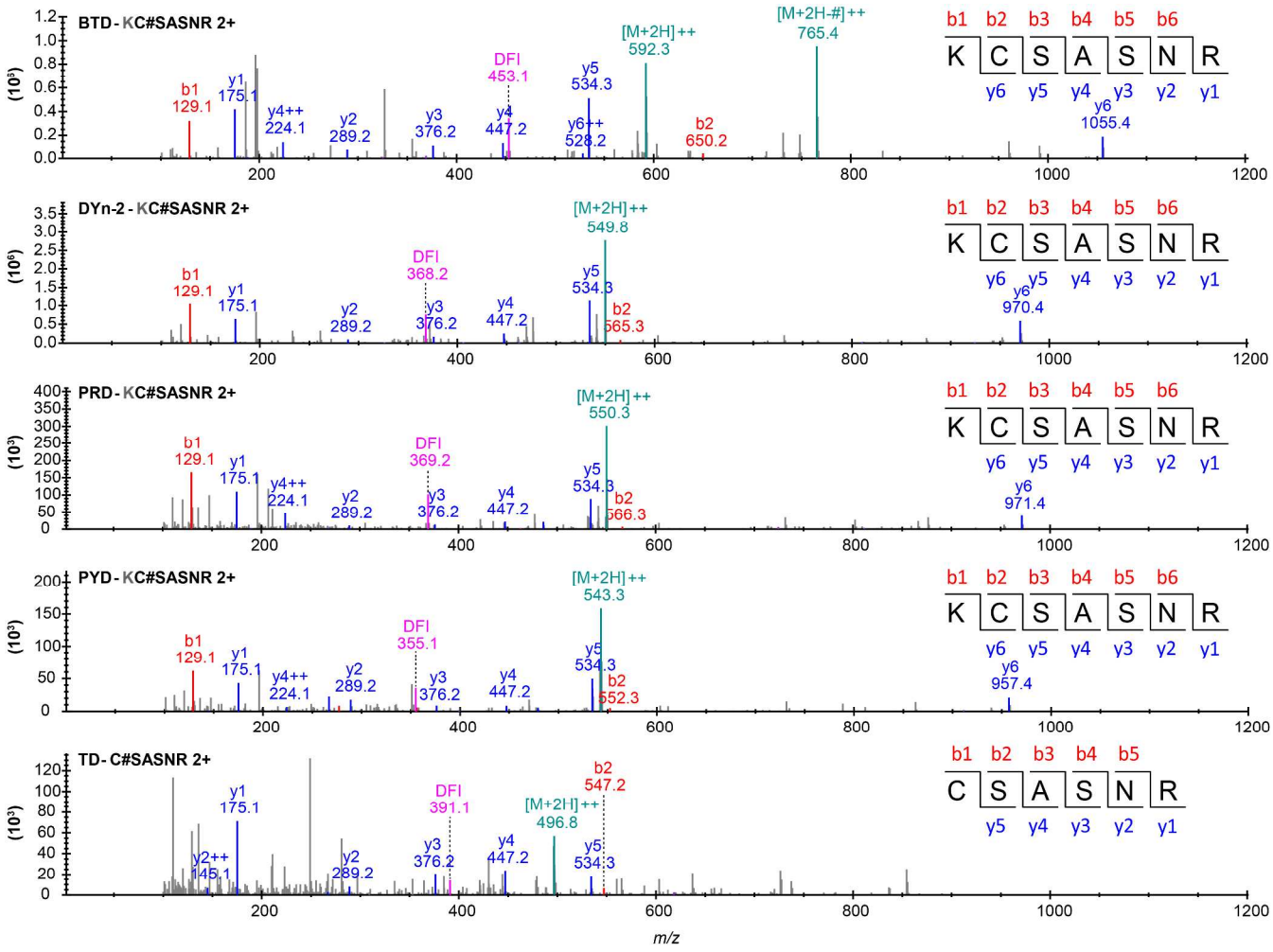


Fig. S4. ID of Cys17 of RPS21.

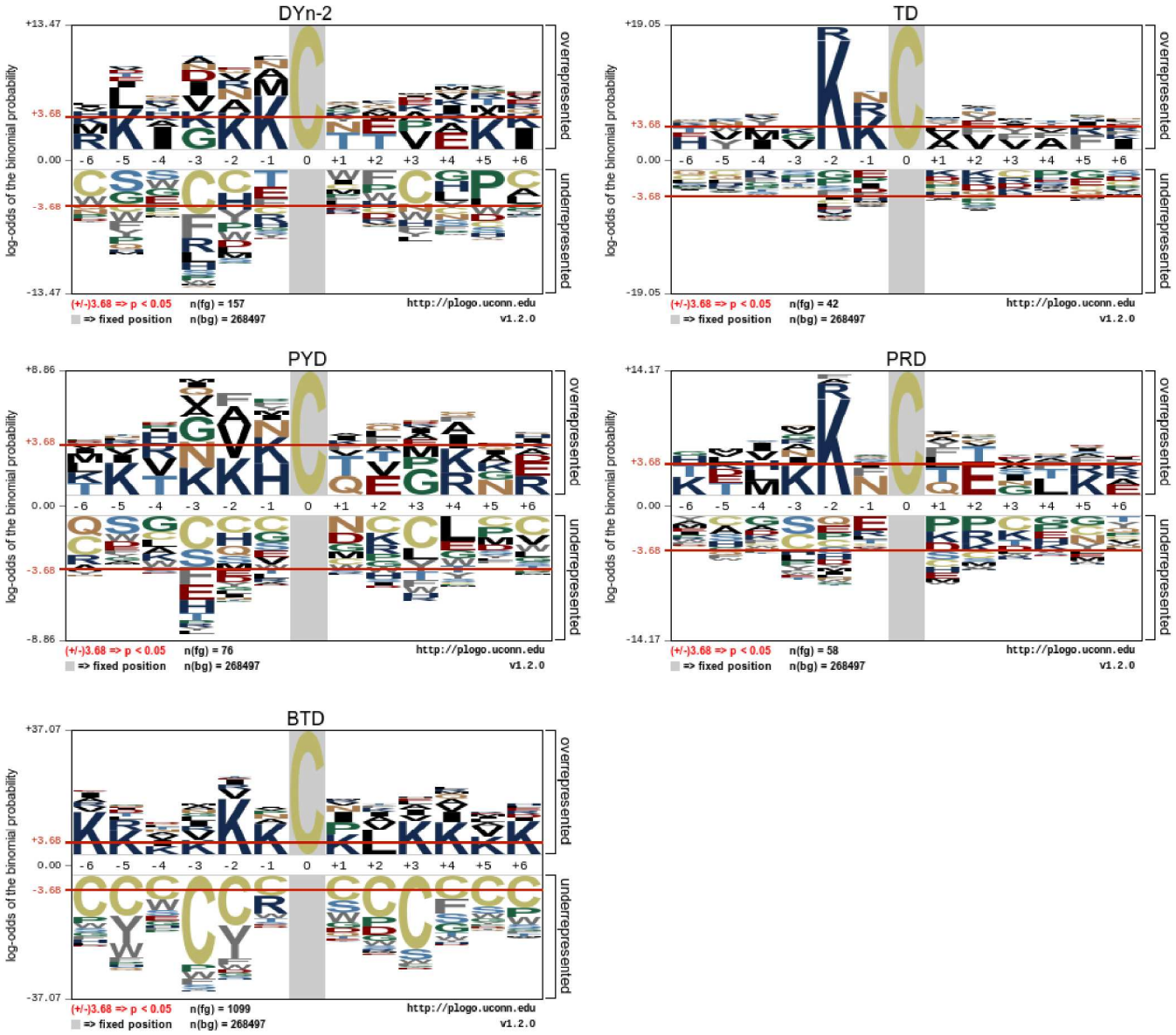


Fig. S5. Sequence motif analysis of peptides labeled by each probe.

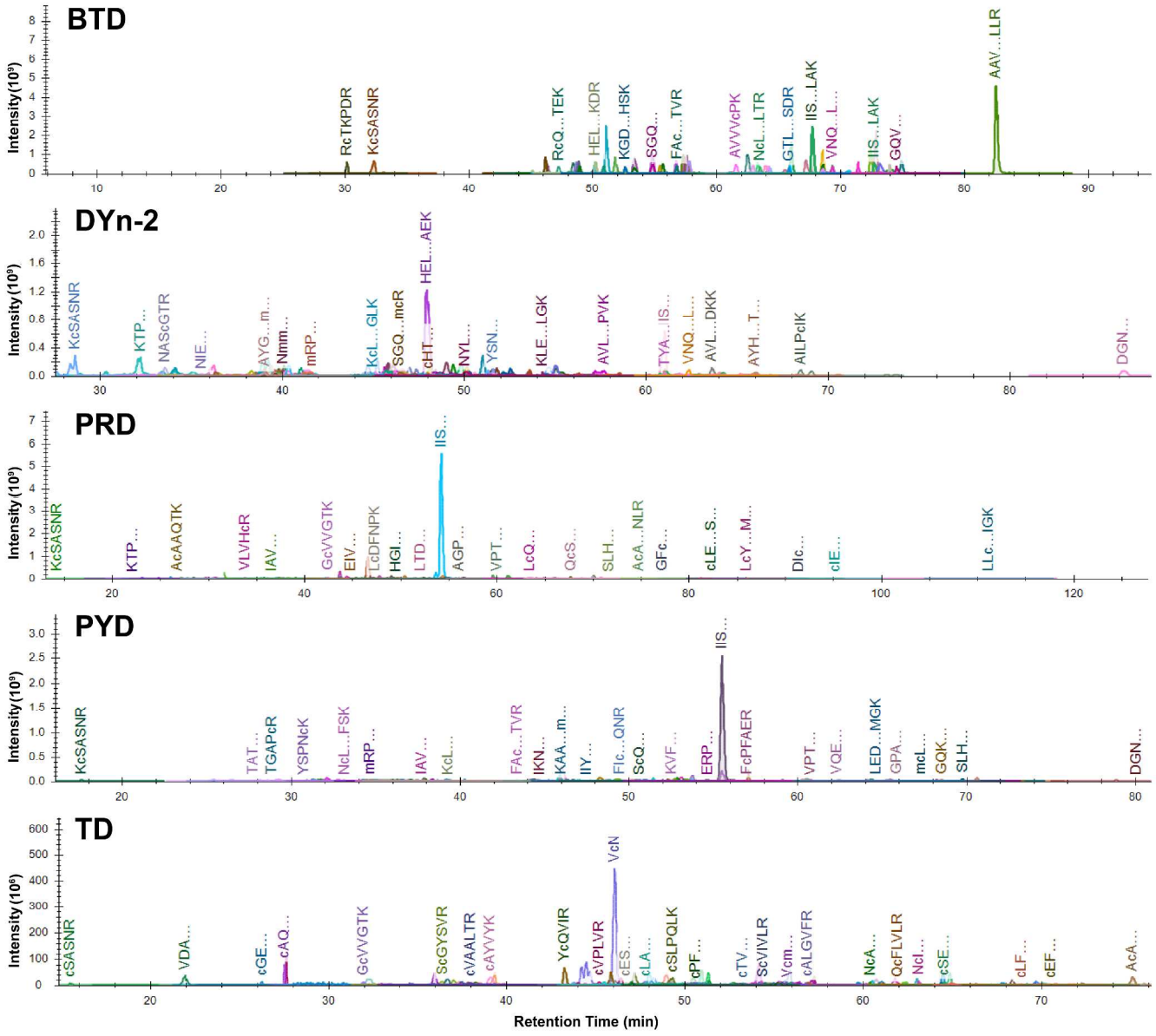


Fig. S6. MS1 filtered LC chromatograms

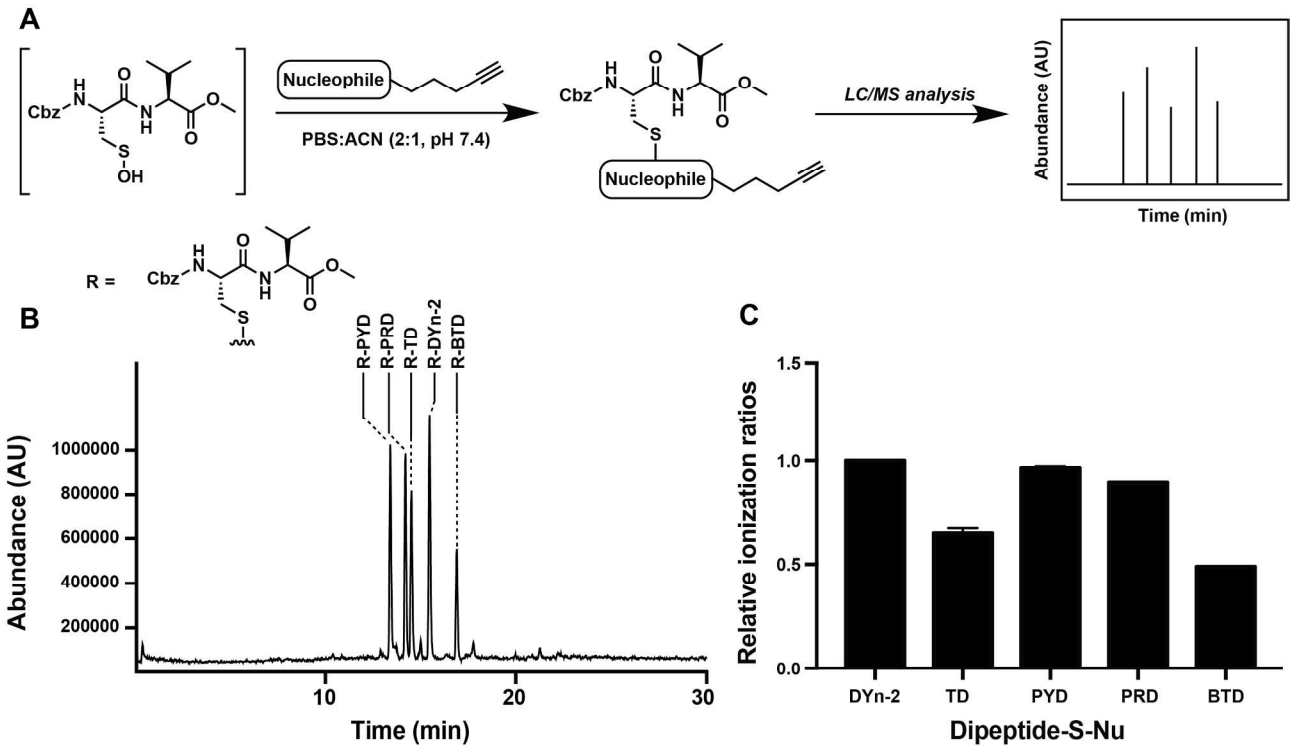


Fig. S7. Comparative ionization of dipeptide-probe adducts. **(A)** Synthetic scheme to prepare the dipeptide-probe thioether adducts. **(B)** MS-trace of dipeptide-probe adducts mixture. **(C)** Average relative intensity ratio of different probe modified peptide taking dipeptide-S-DYn-2 as reference. Error bars, mean ± s.e.m.

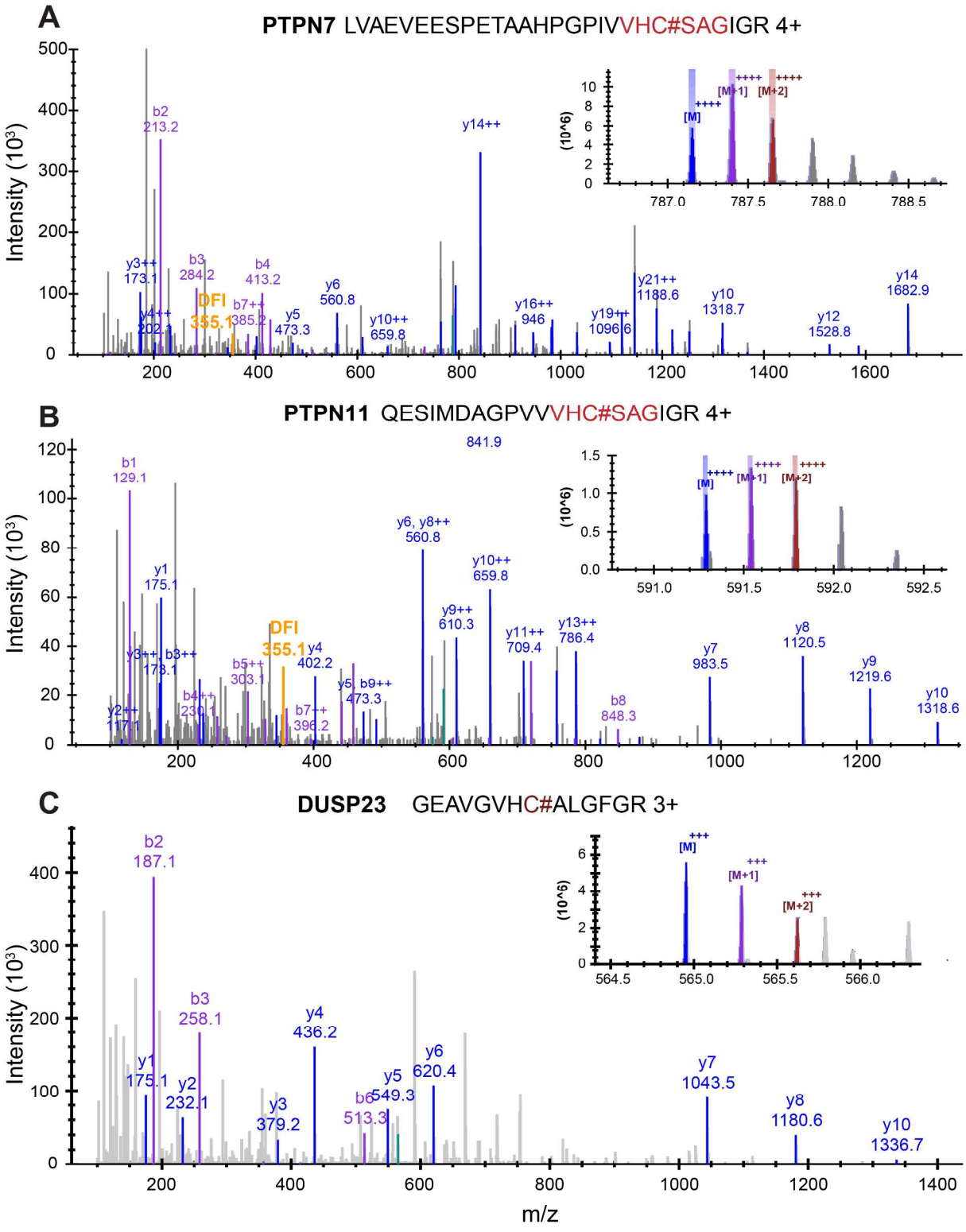
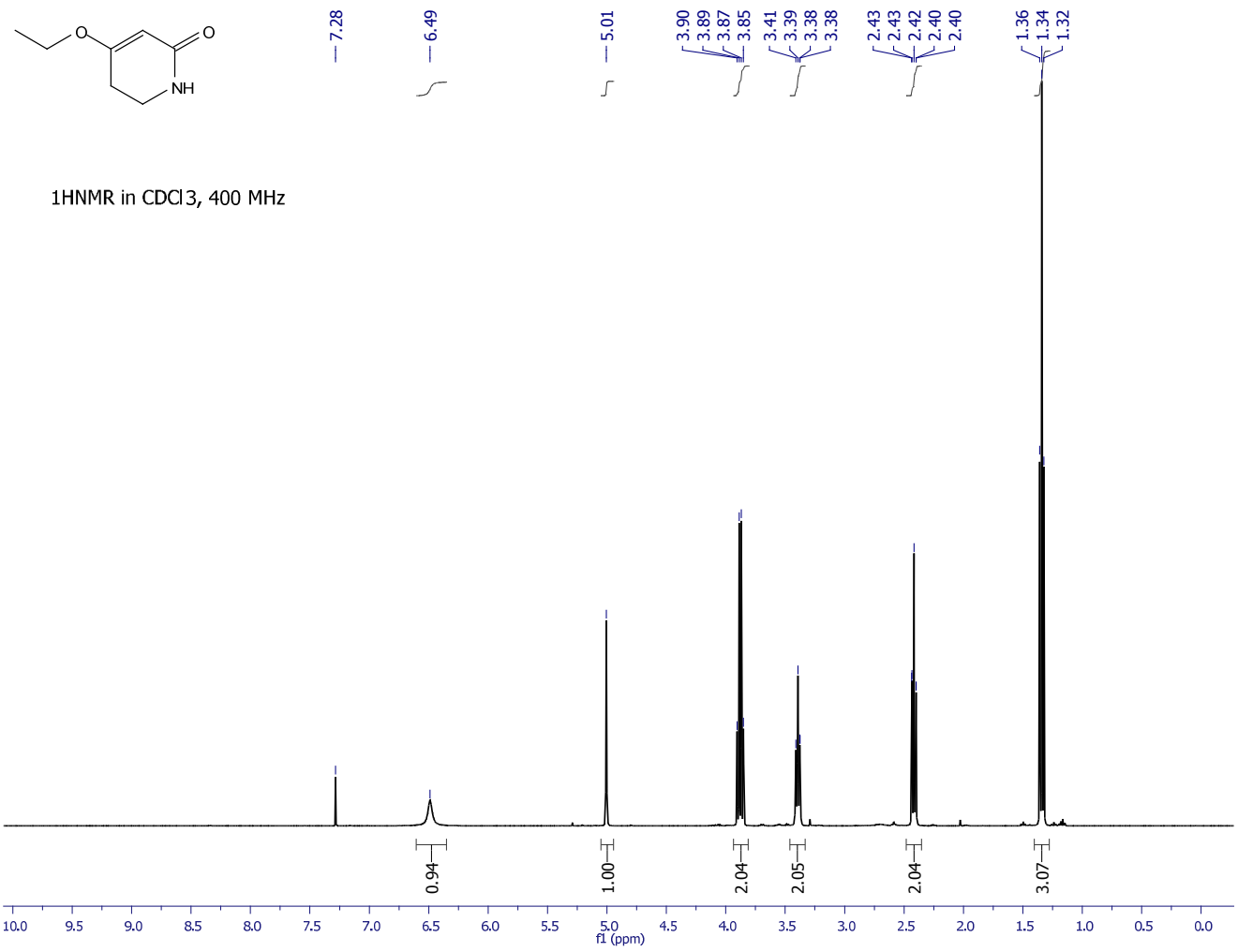
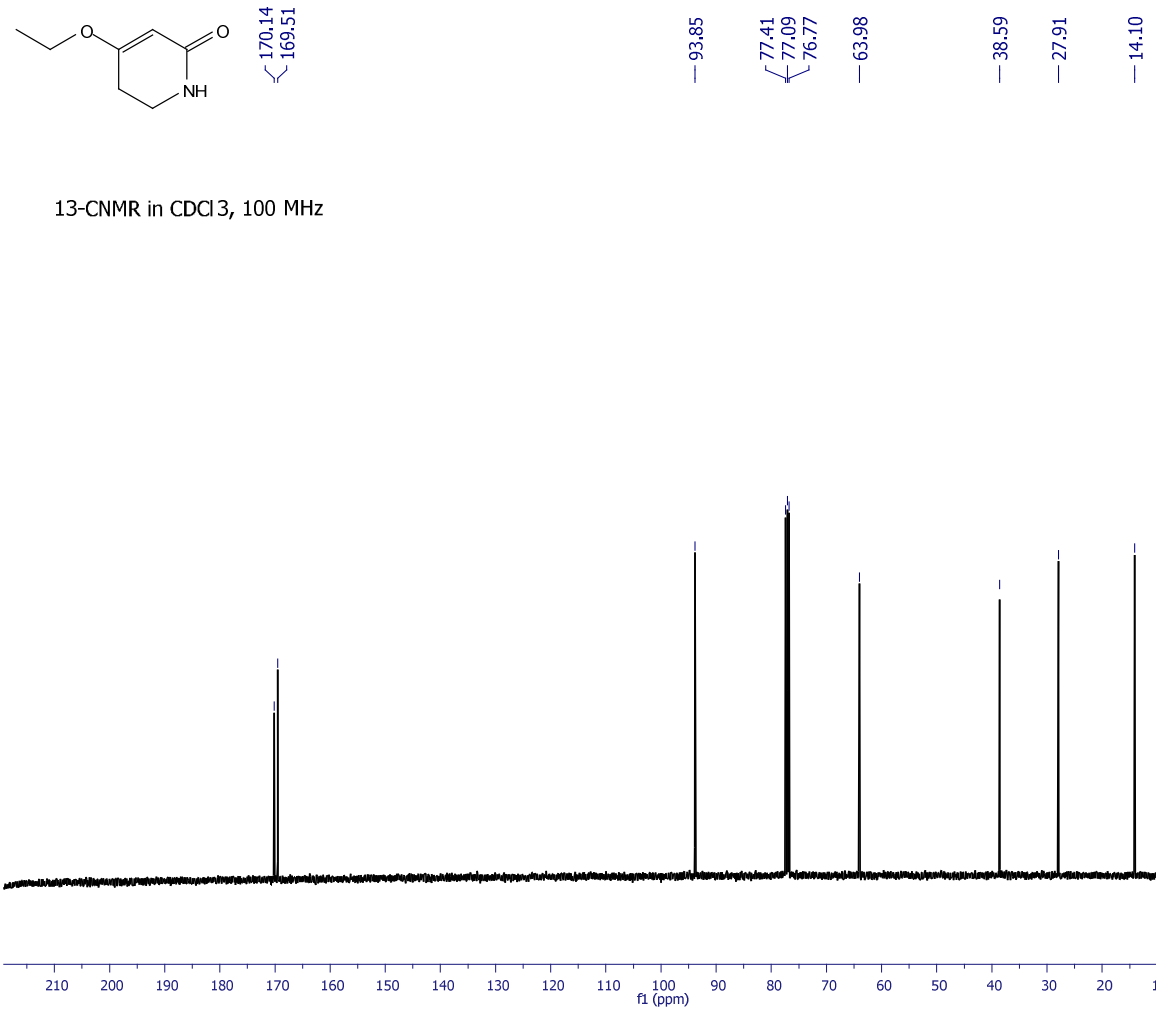


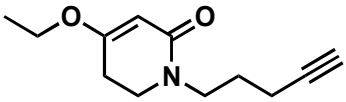
Fig. S8. MS/MS spectrum of PYD labeled PTPs. **(A)** PTPN7 was labled by PYD at Cys396. **(B)** PTPN11 was labeled by PYD at Cys459. **(C)** DUSP23 was labeled by PYD at Cys95.

NMR Spectra

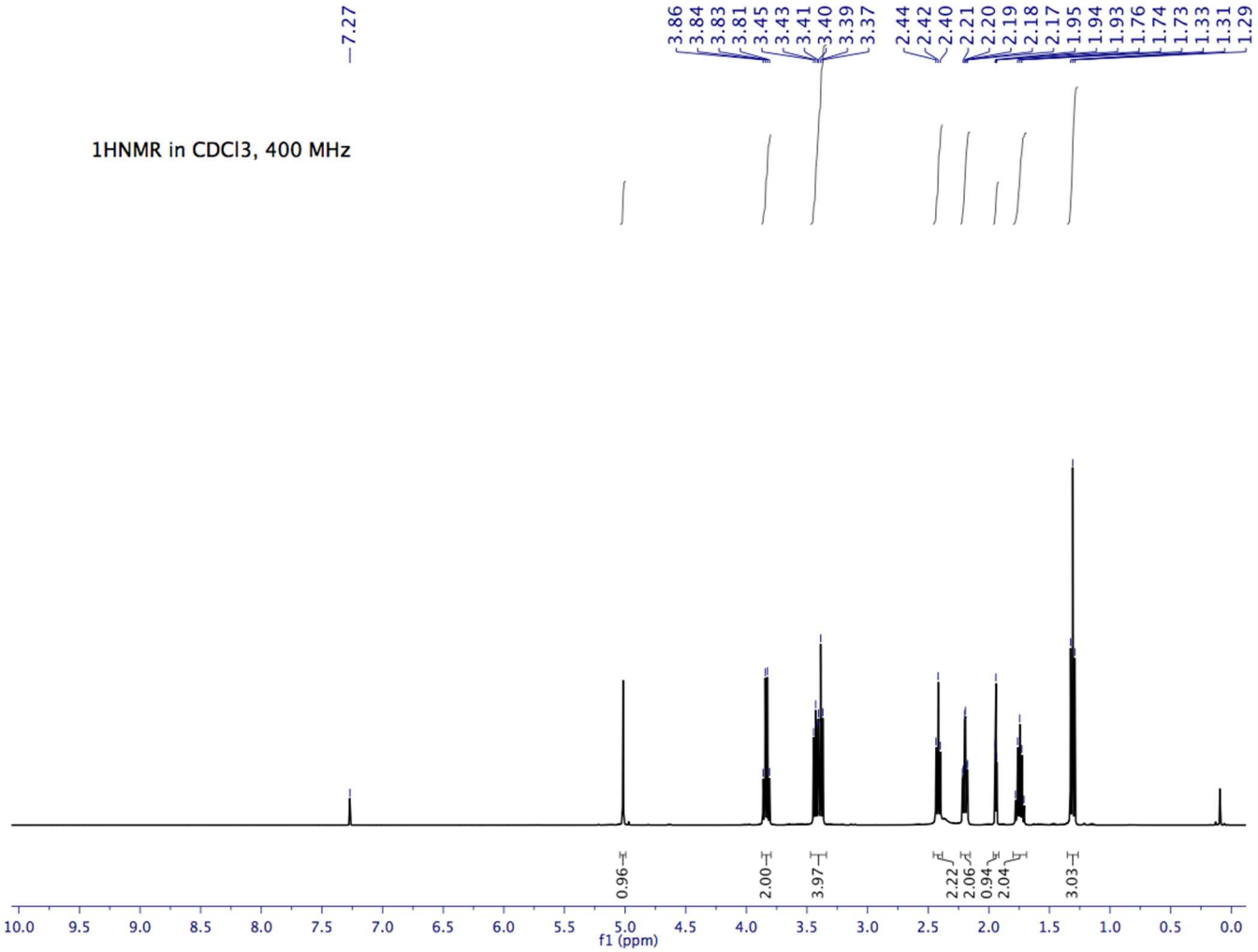

O
O
NH
7.28
6.49
5.01
3.90
3.89
3.87
3.85
3.41
3.39
3.38
3.38
2.43
2.43
2.42
2.40
2.40
1.36
1.34
1.32
1HNMR in CDCl3, 400 MHz
0.94
1.00
2.04
2.05
2.04
3.07
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

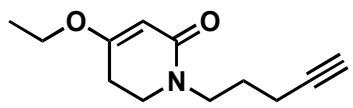


13-CNMR in CDCl3, 100 MHz

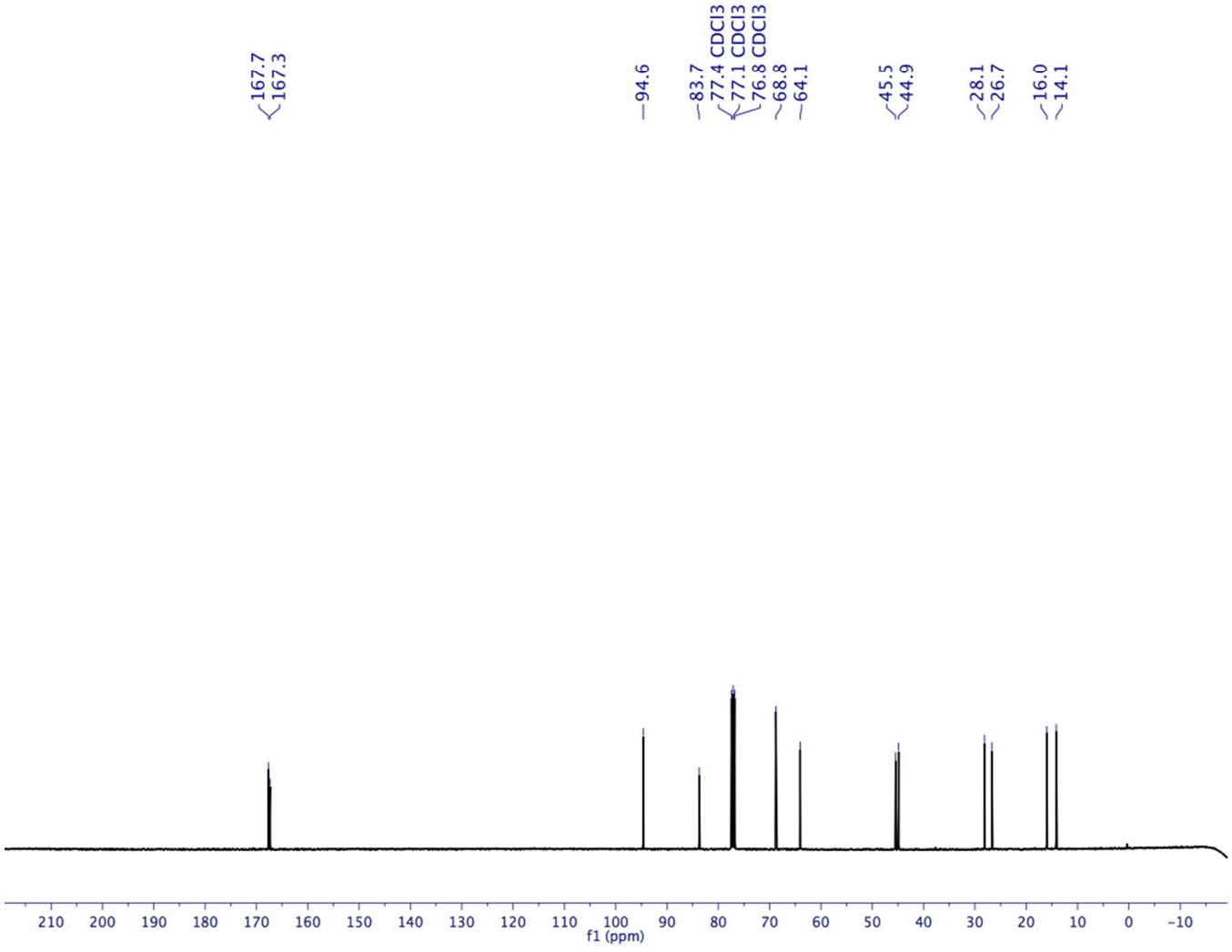


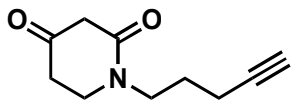
1HNMR in CDCl3, 400 MHz



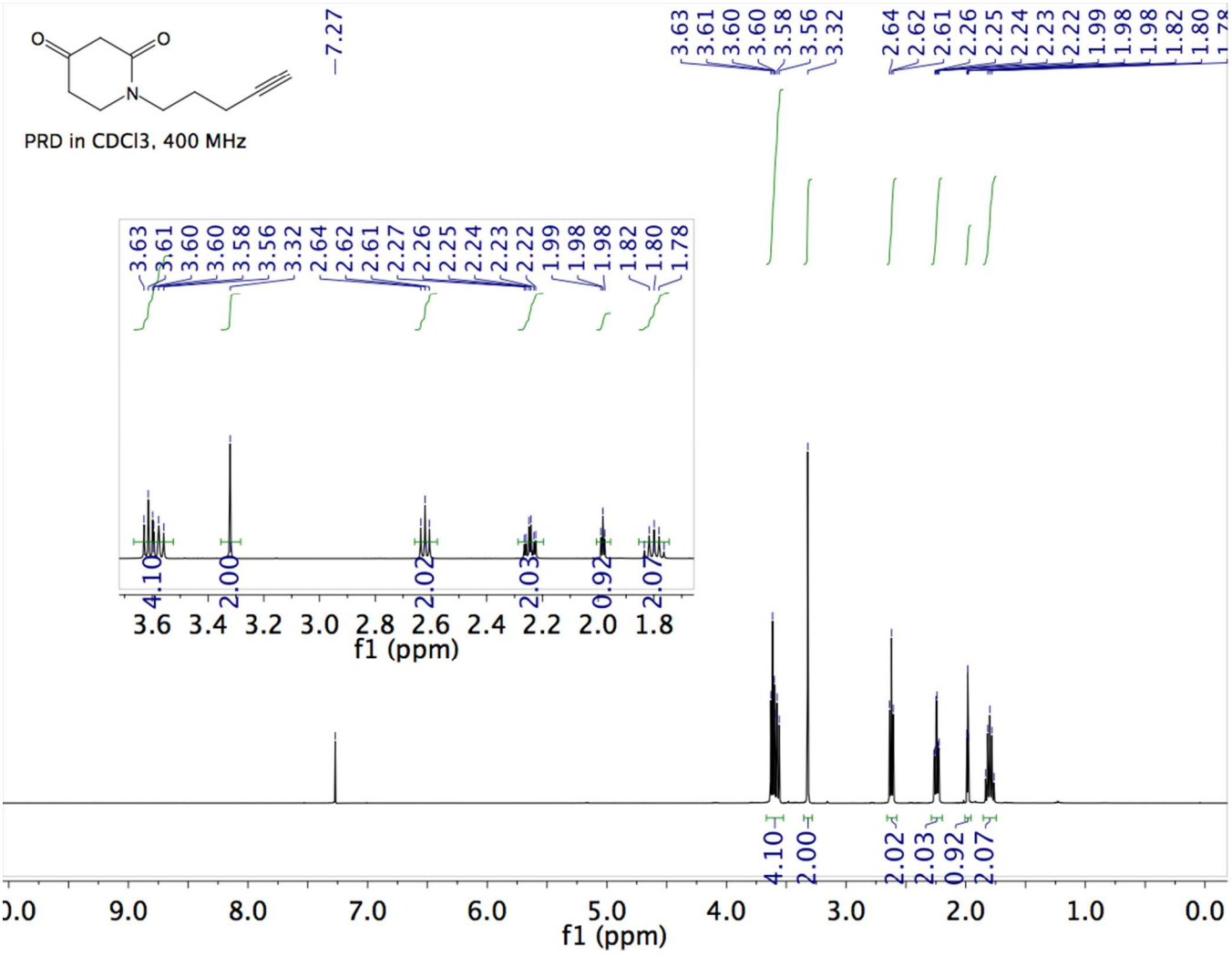


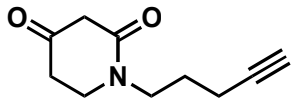
^{13}C-NMR (in $CDCl_3$, 100 MHz)



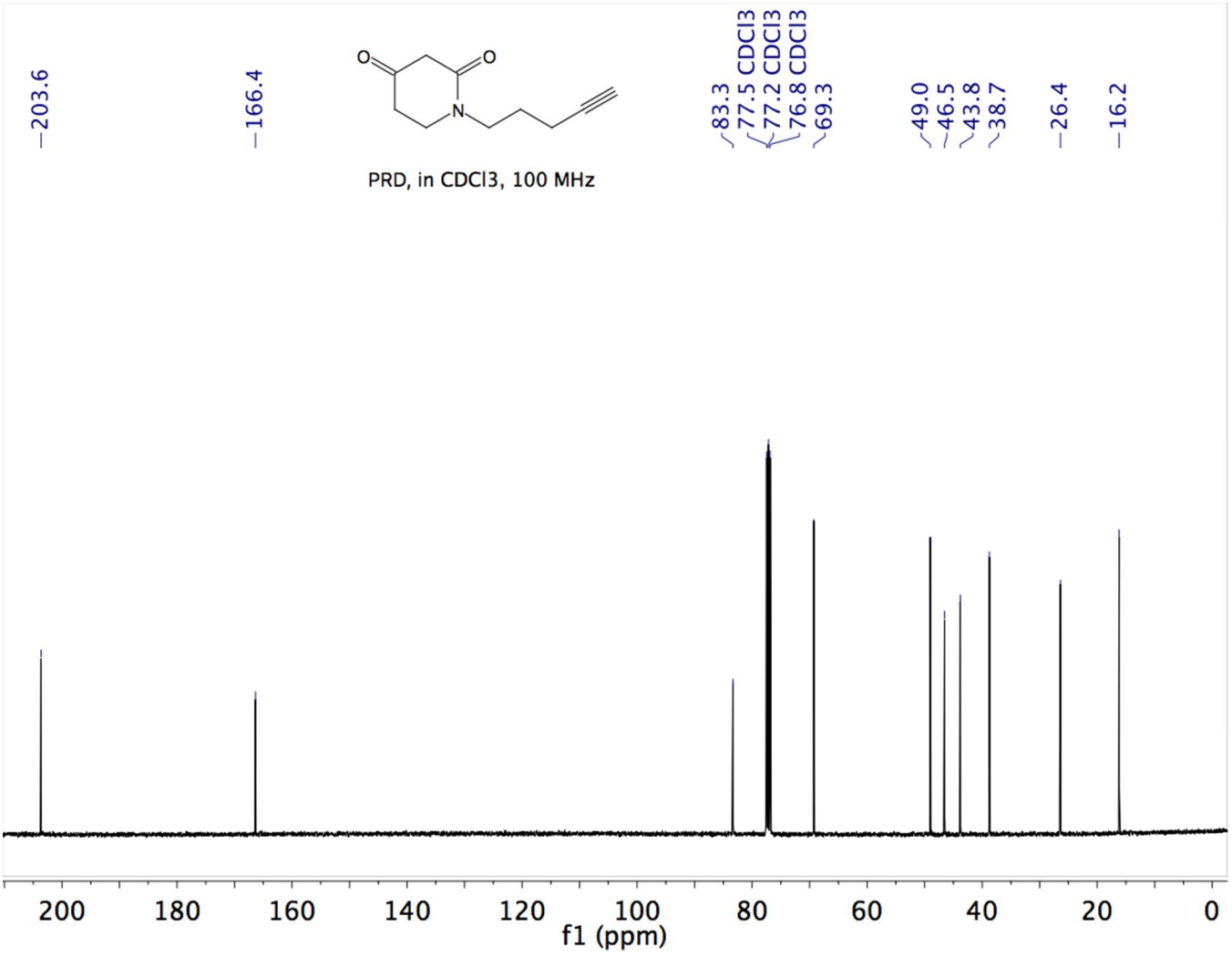


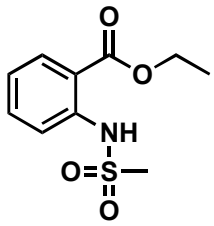
^{1}H-NMR (in $CDCl_3$, 400 MHz)



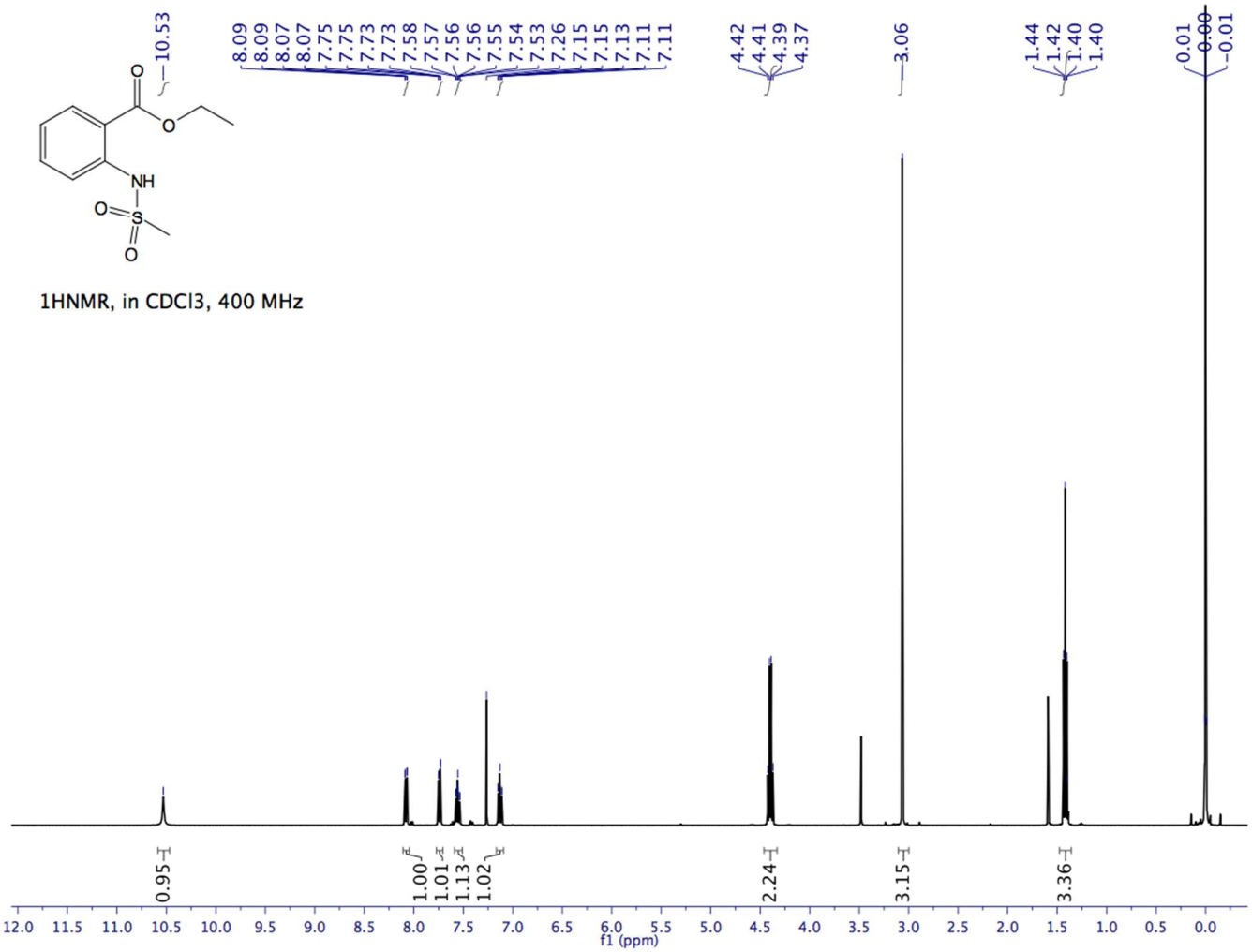


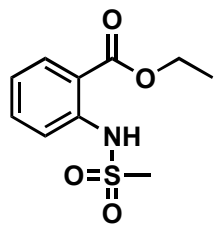
^{13}C-NMR (in $CDCl_3$, 100 MHz)



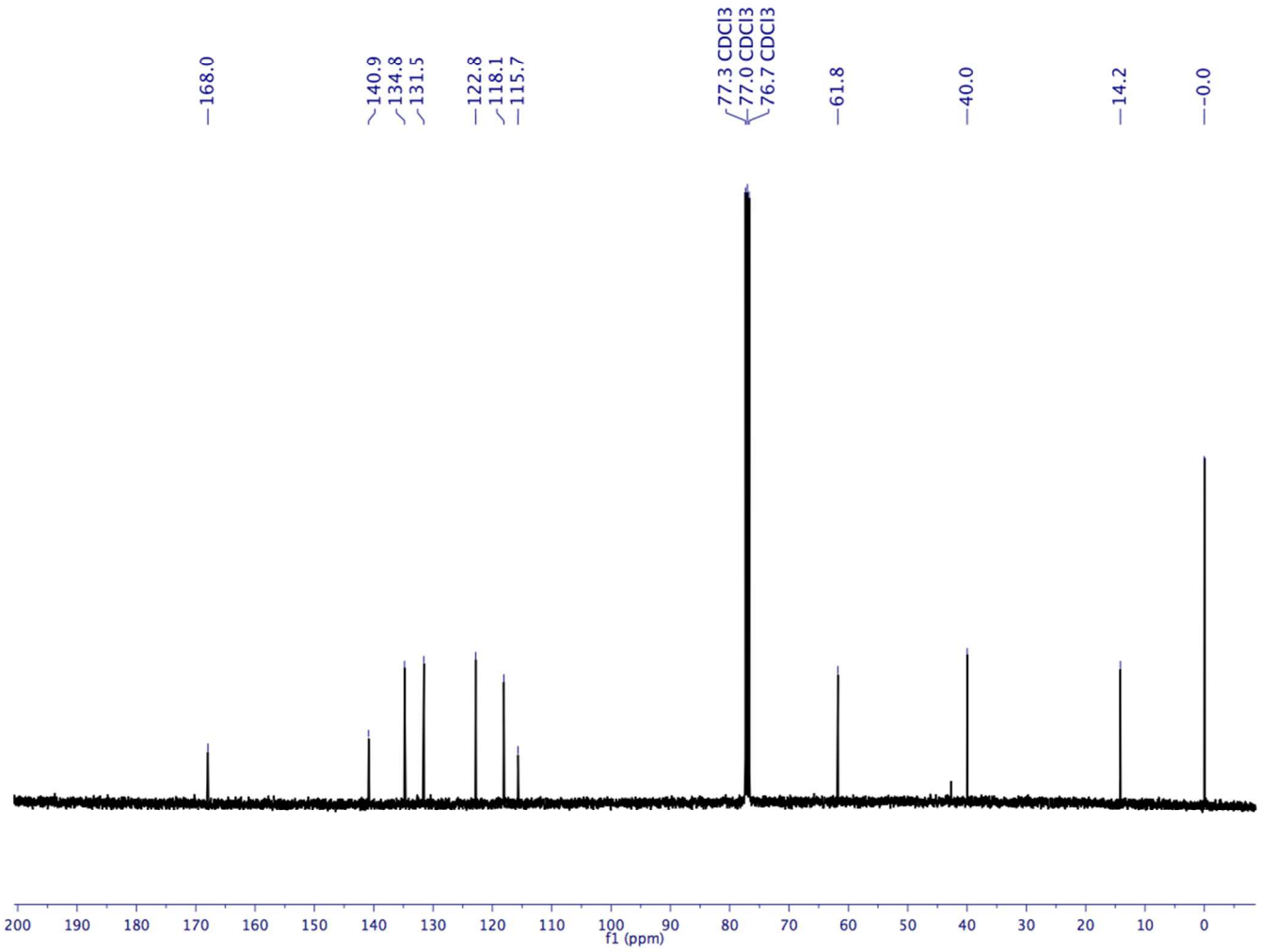


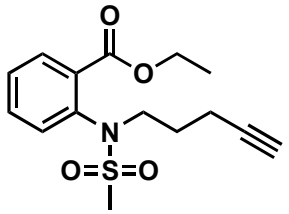
^{1}H-NMR (in $CDCl_3$, 400 MHz)



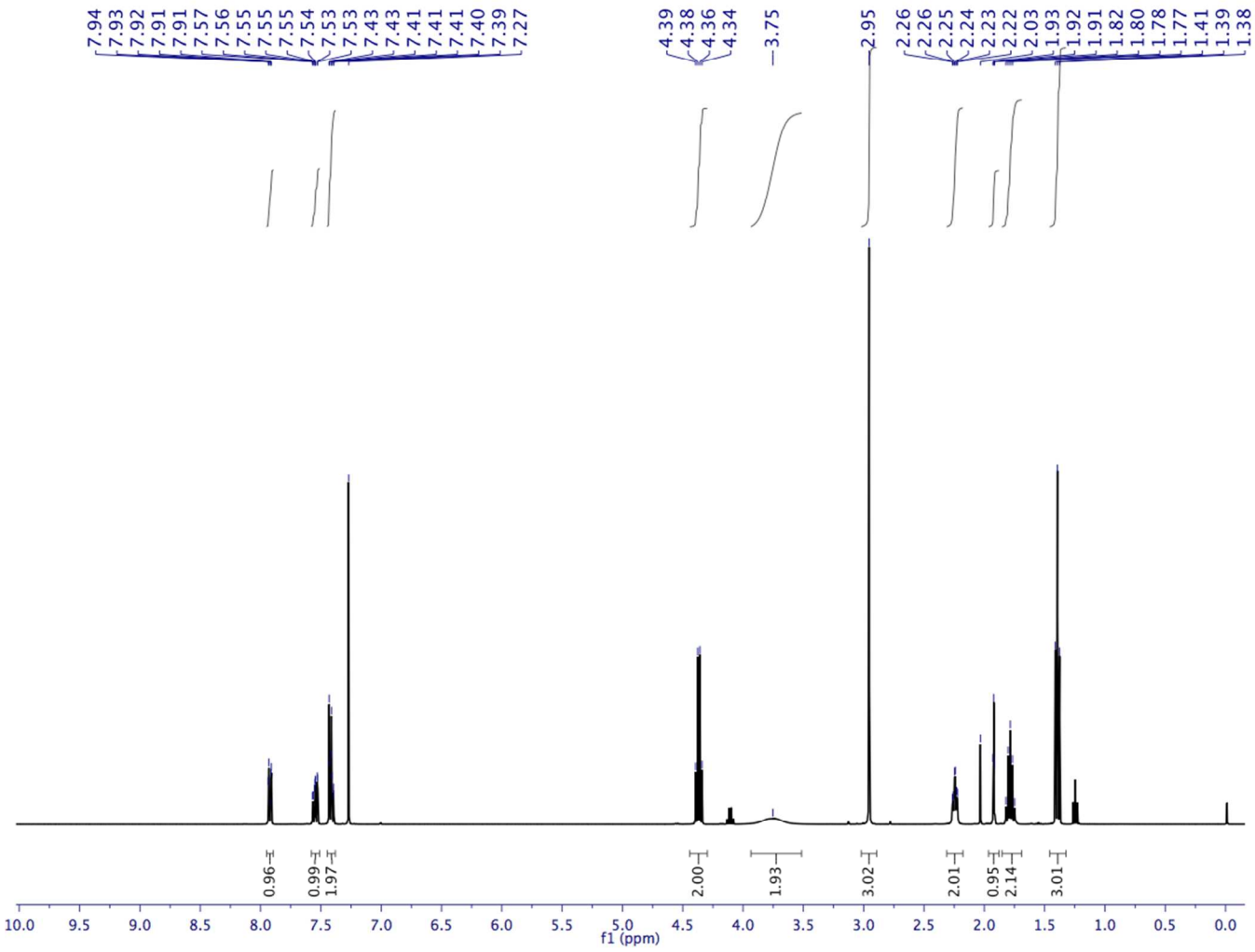


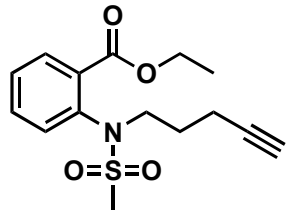
^{13}C-NMR (in $CDCl_3$, 100 MHz)





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





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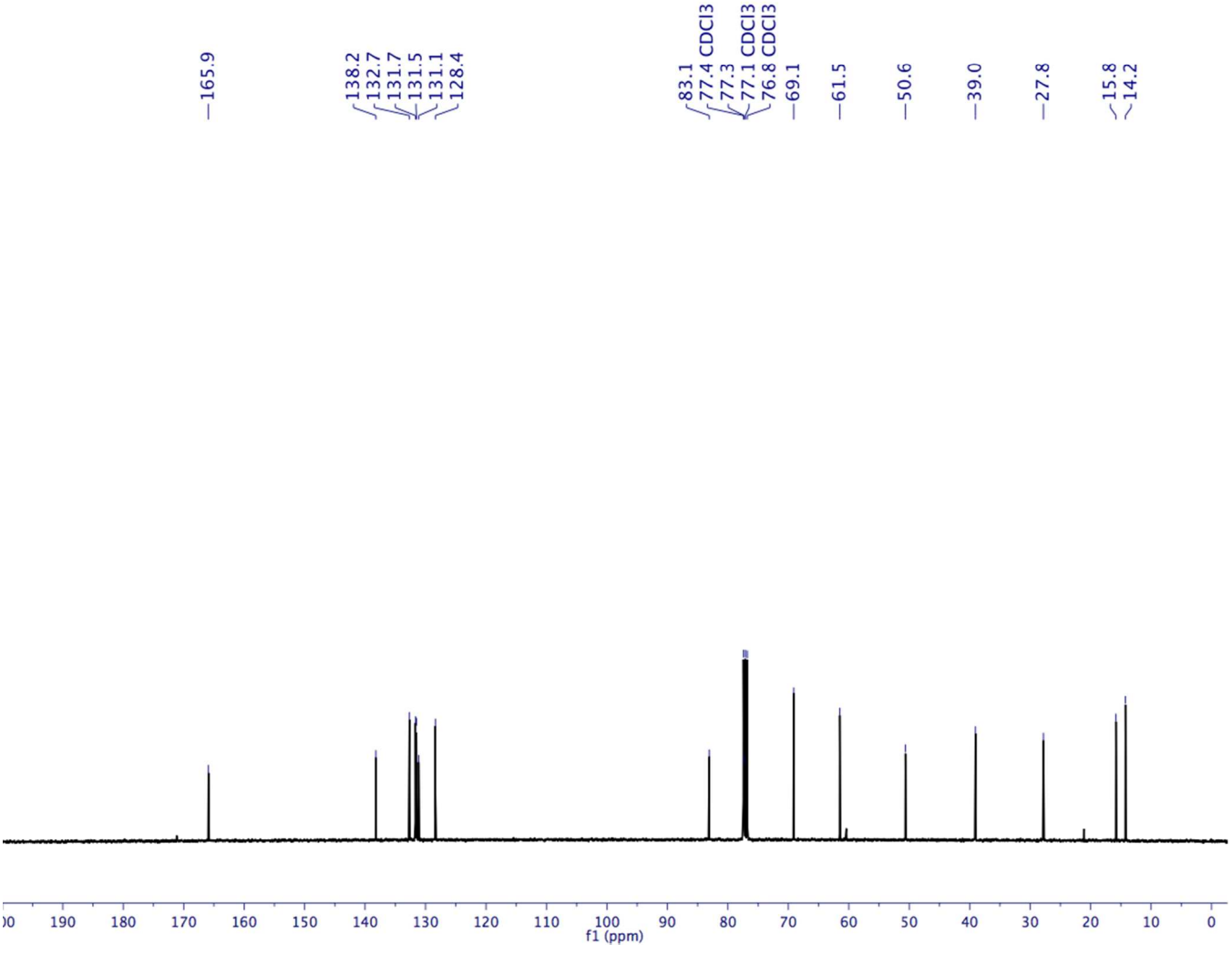

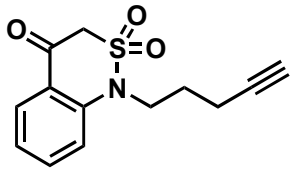

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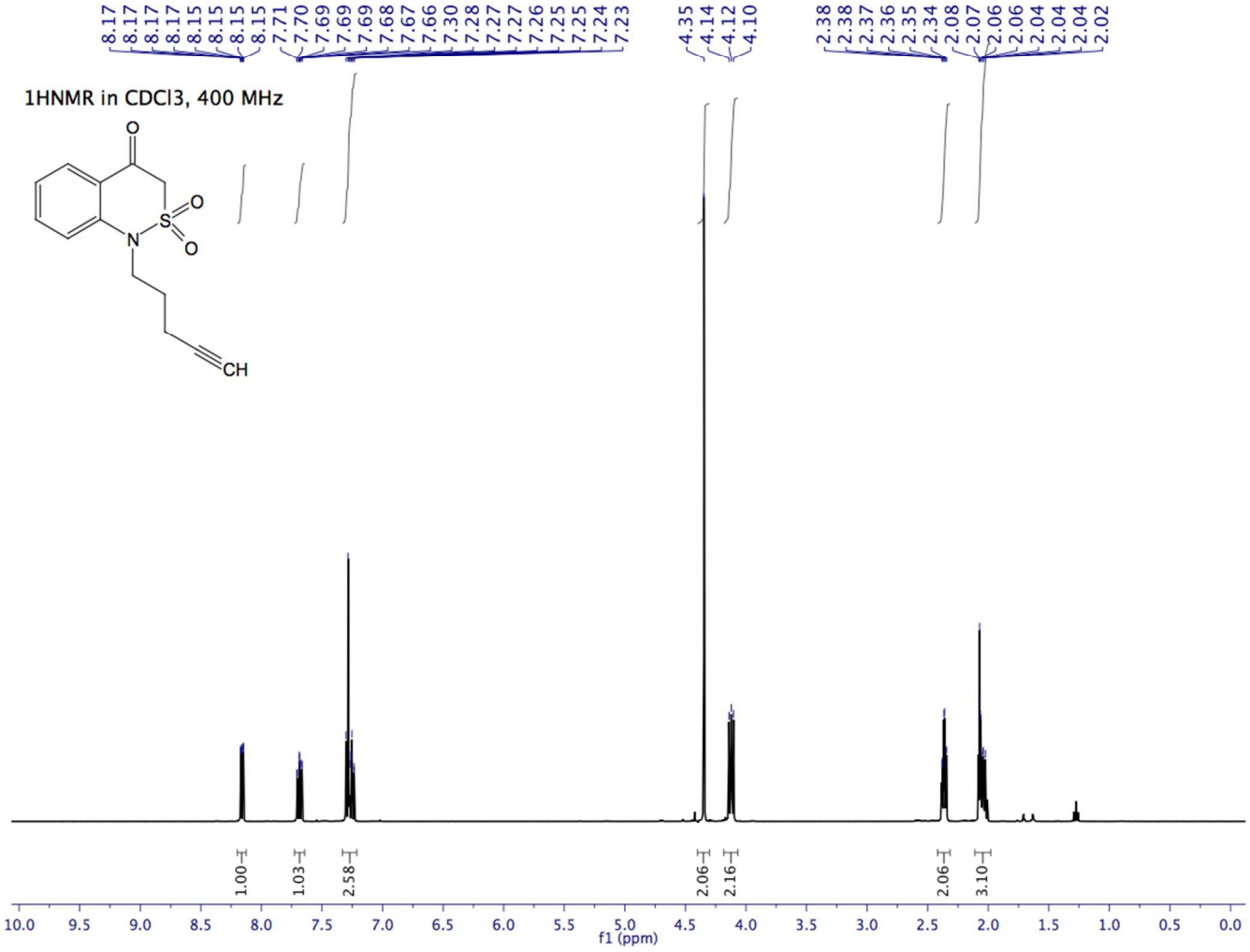
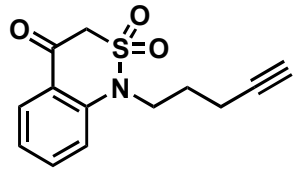
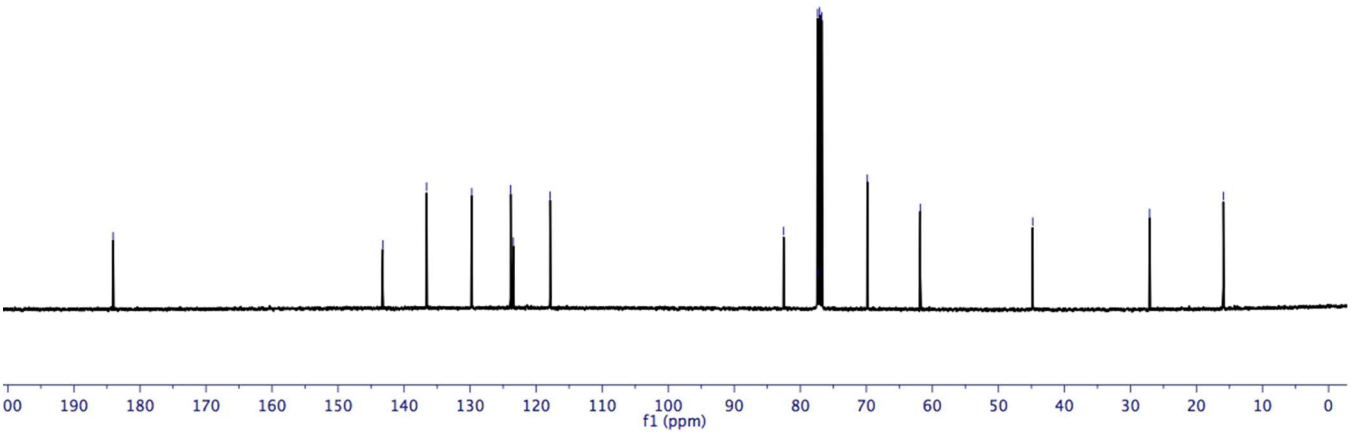
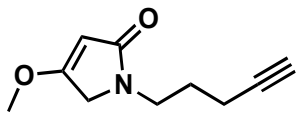




^{13}C-NMR (in $CDCl_3$, 100 MHz)

13-CNMR, in CDCl3, 100 MHz





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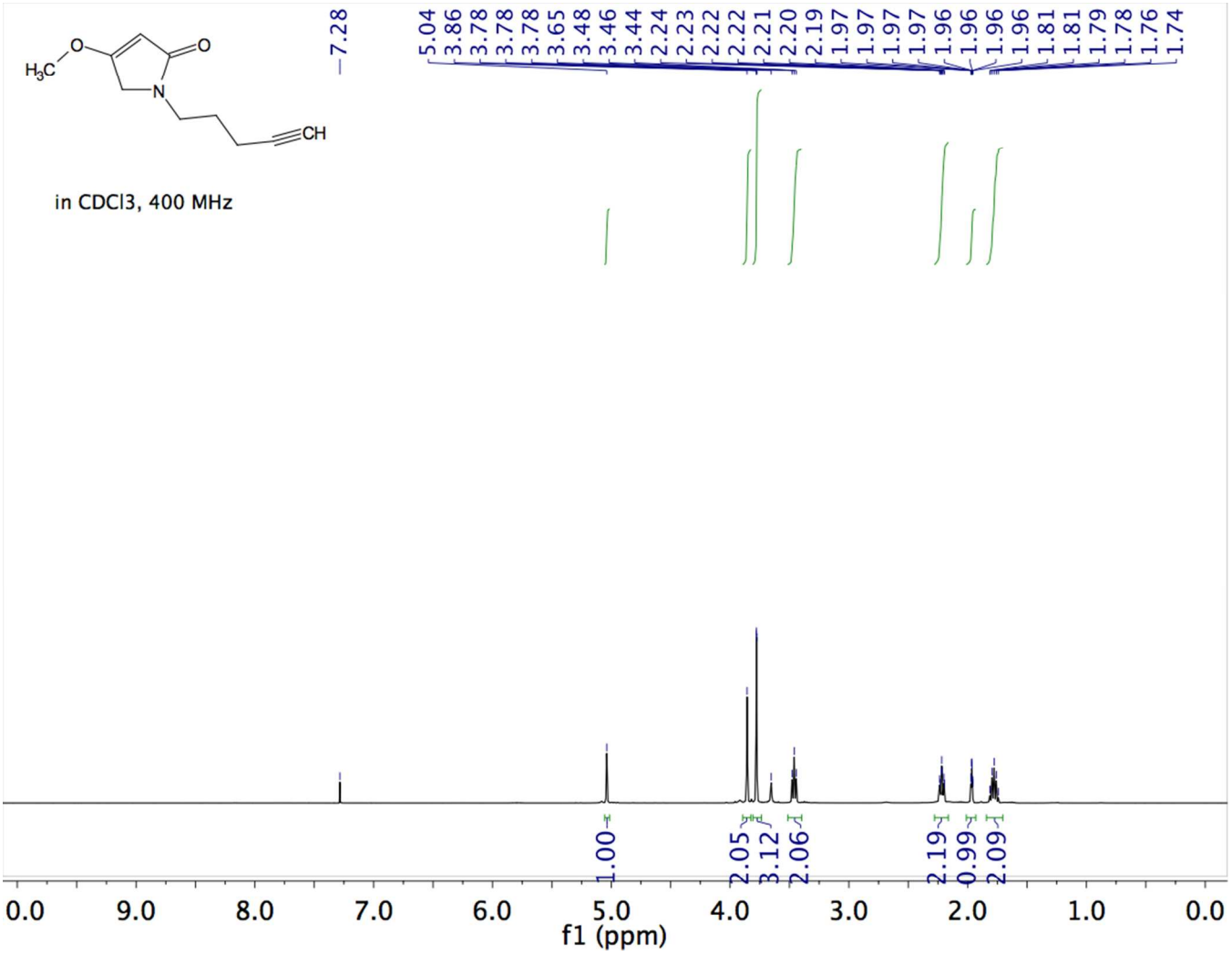
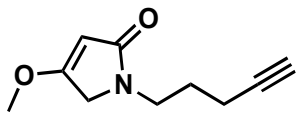
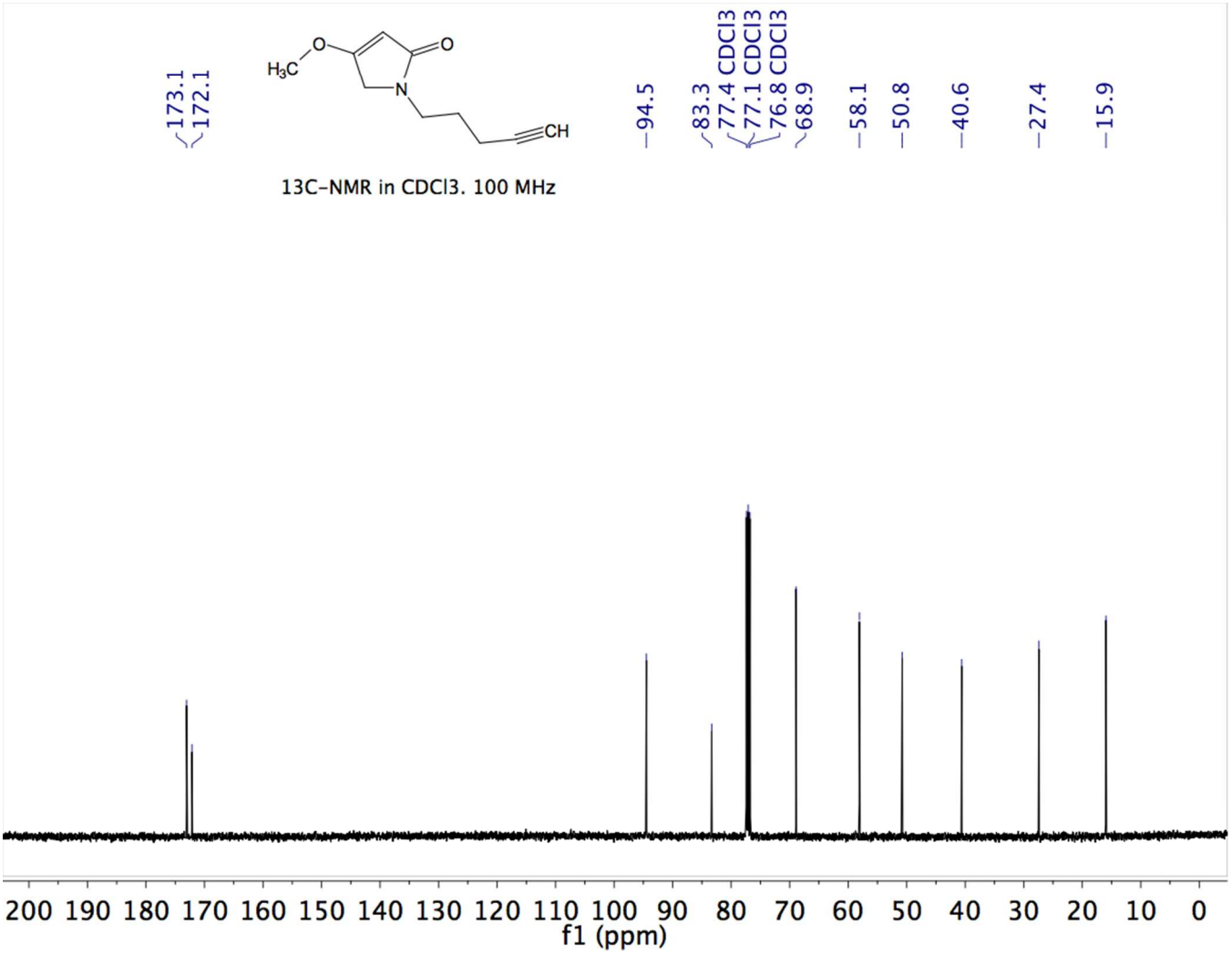
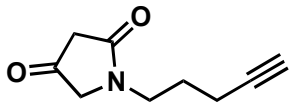




^{13}C-NMR (in $CDCl_3$, 100 MHz)





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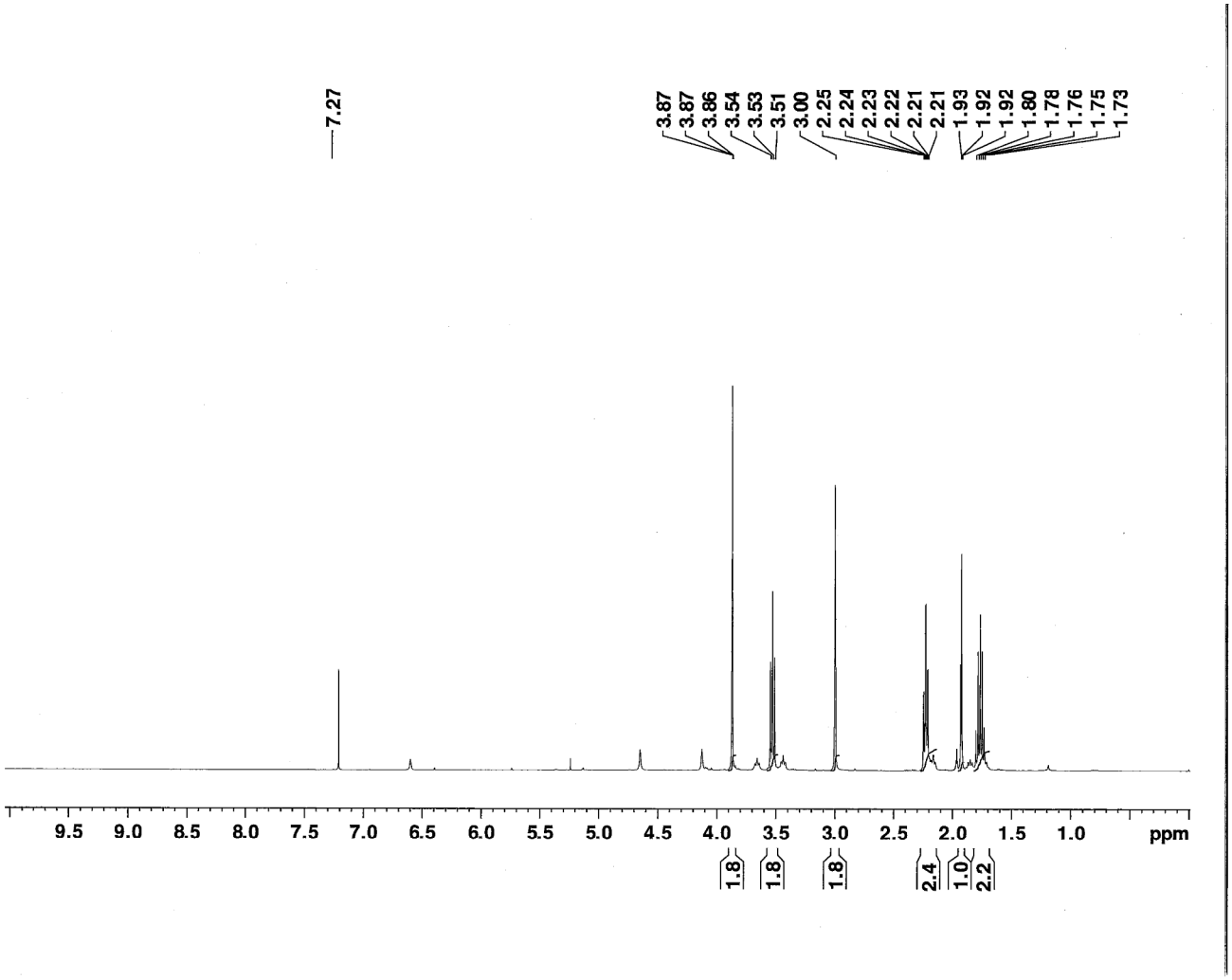

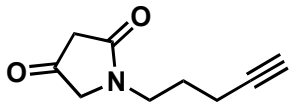

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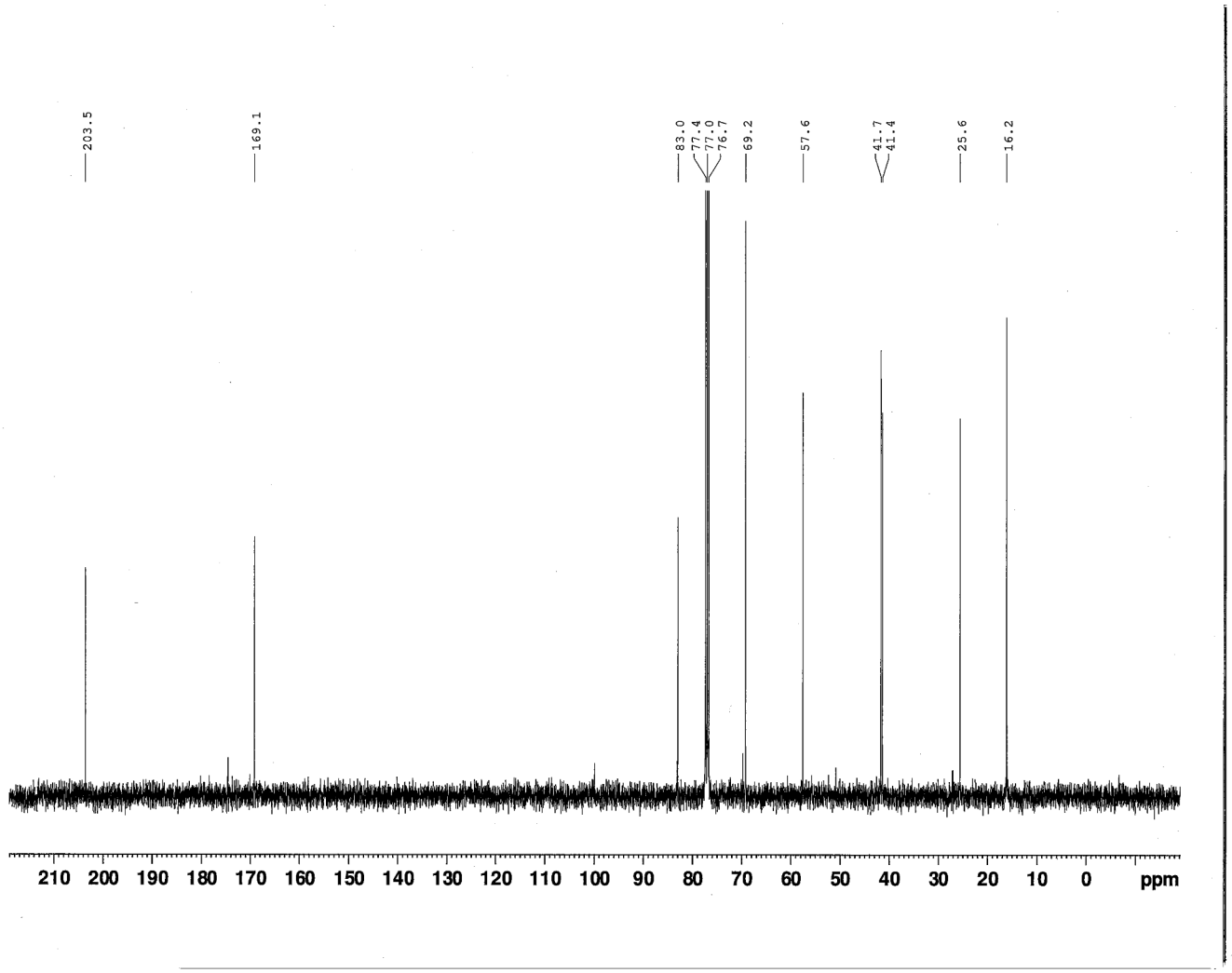
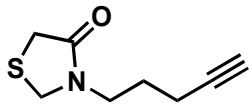
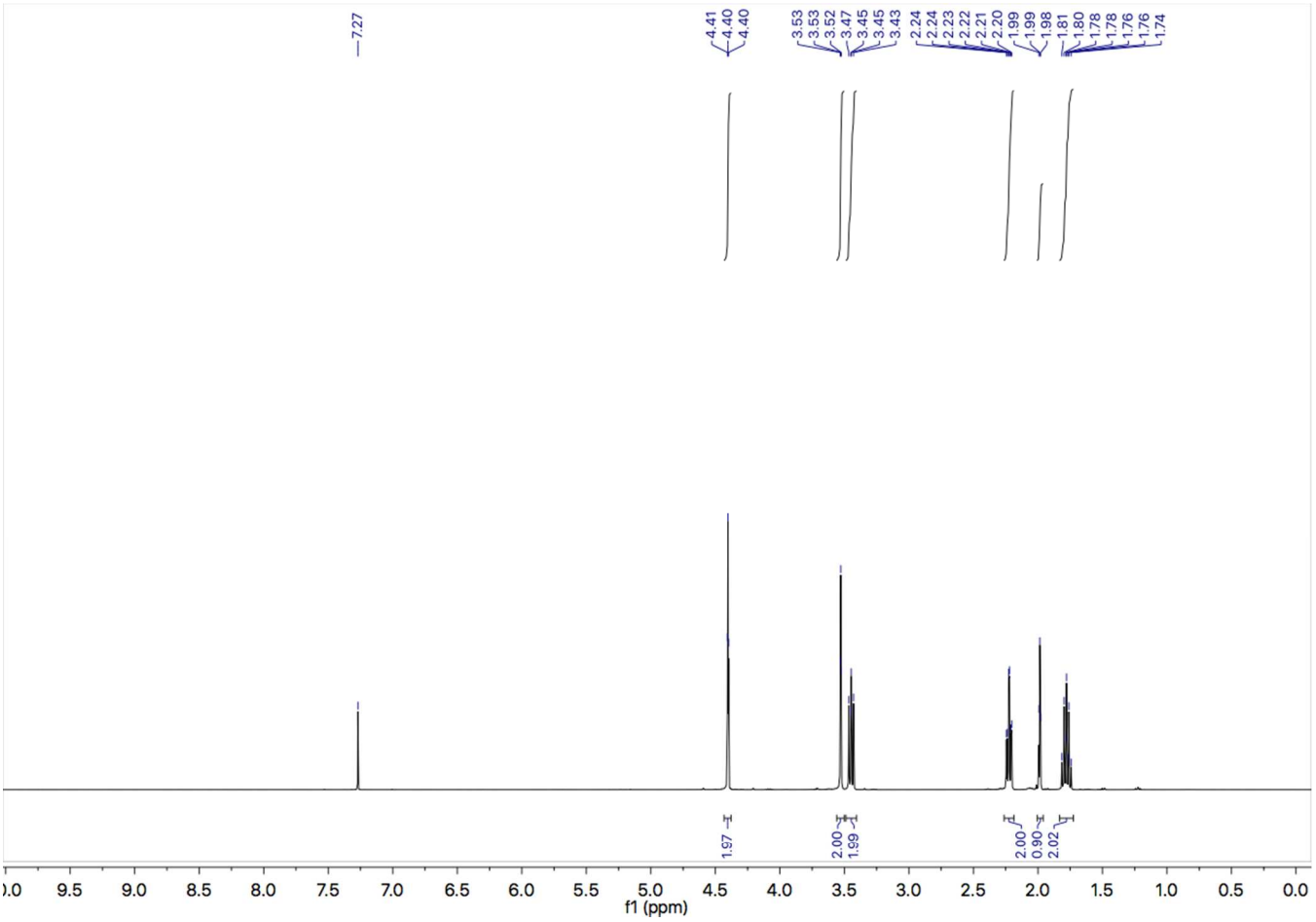
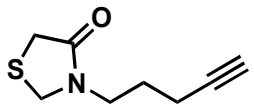




^{1}H-NMR (in $CDCl_3$, 400 MHz)





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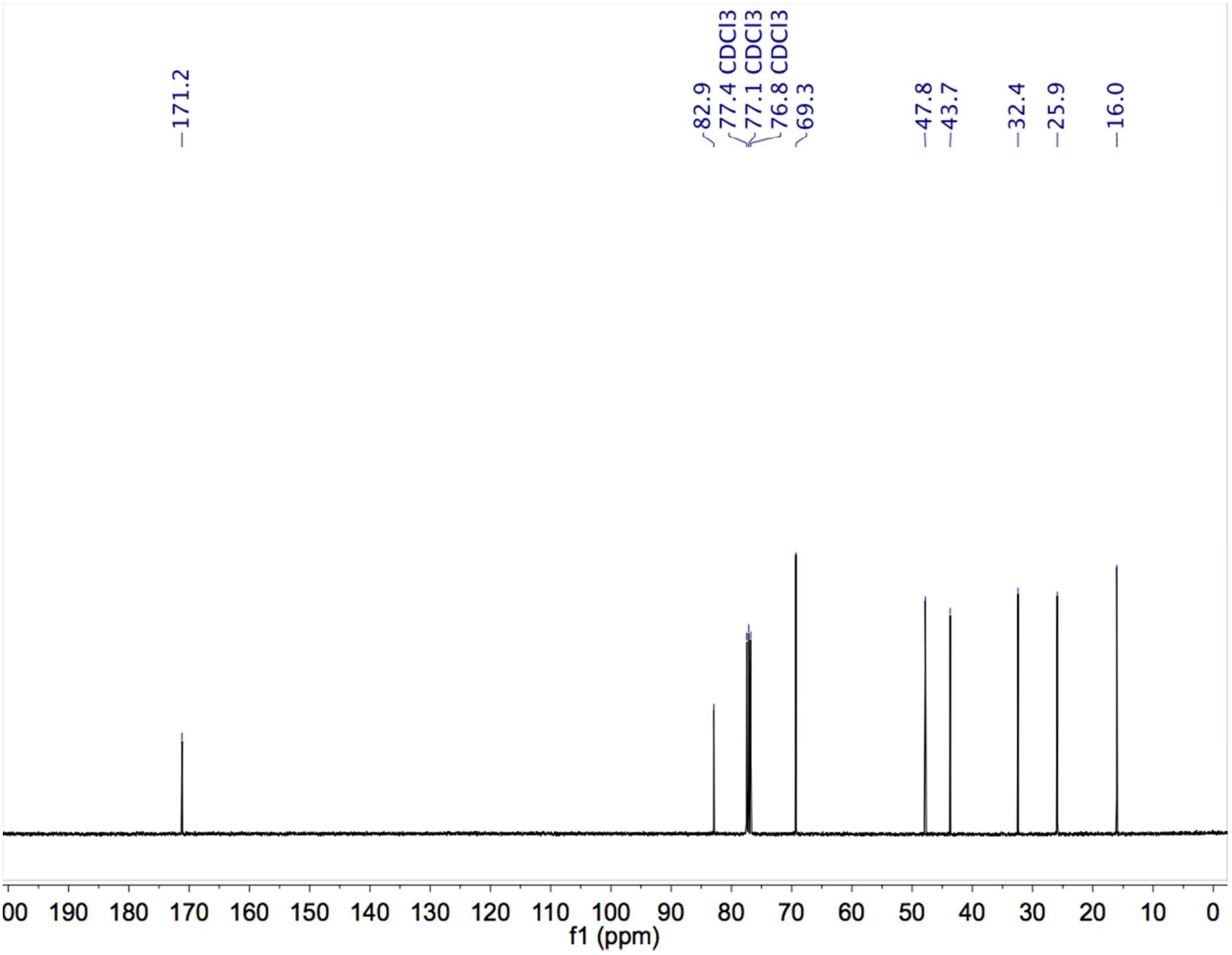

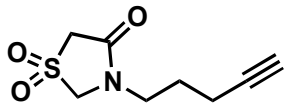

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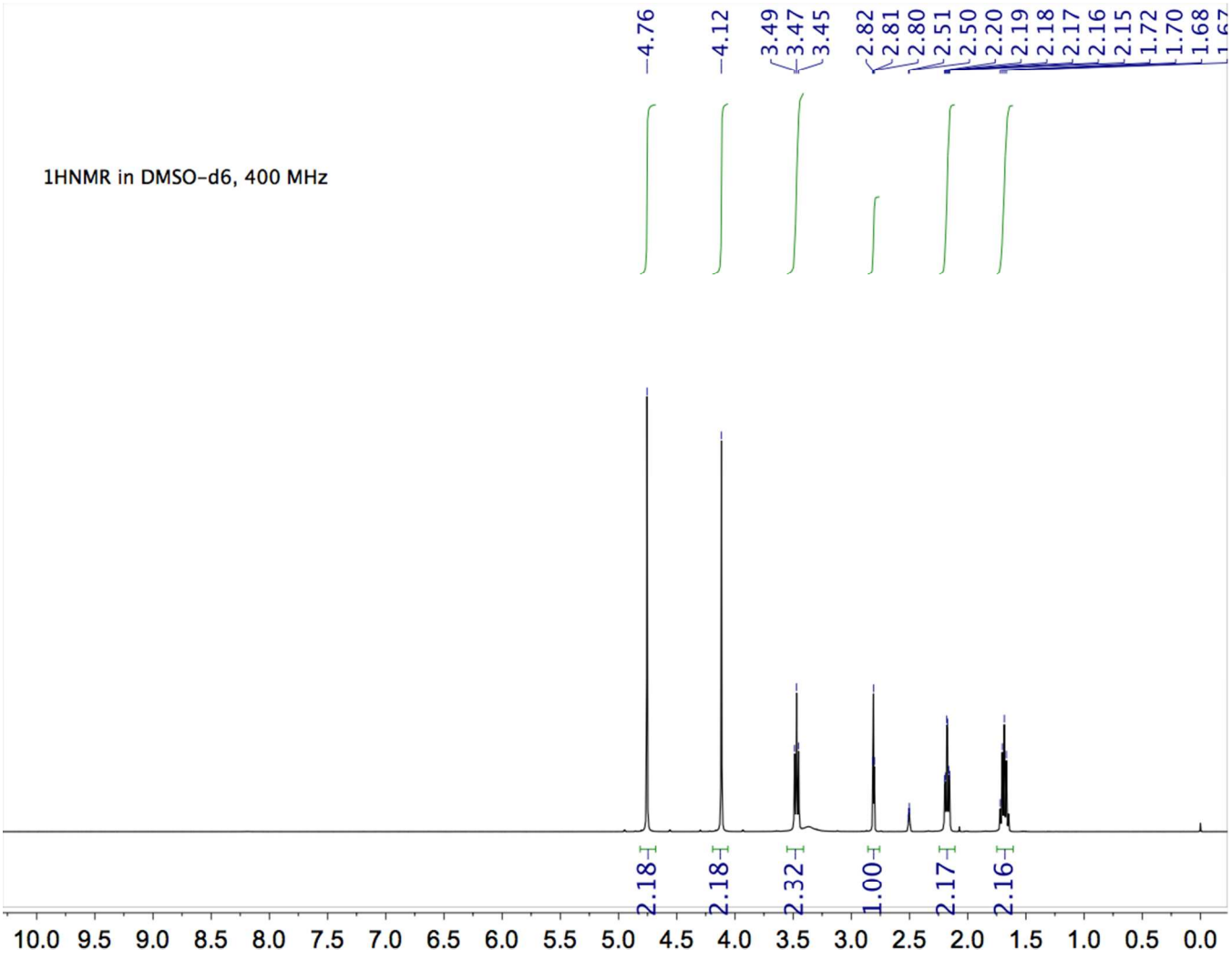

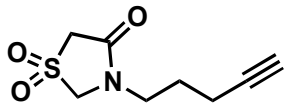

^{13}C-NMR (in DMSO-d6, 100 MHz)