Labeling preferences of diazirines with protein biomolecules

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Supporting Information

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Quantitative and binding site proteomics data are available in Supplementary Tables 1–5:

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Table S3: Quantitative proteomics for JN938 v JN1
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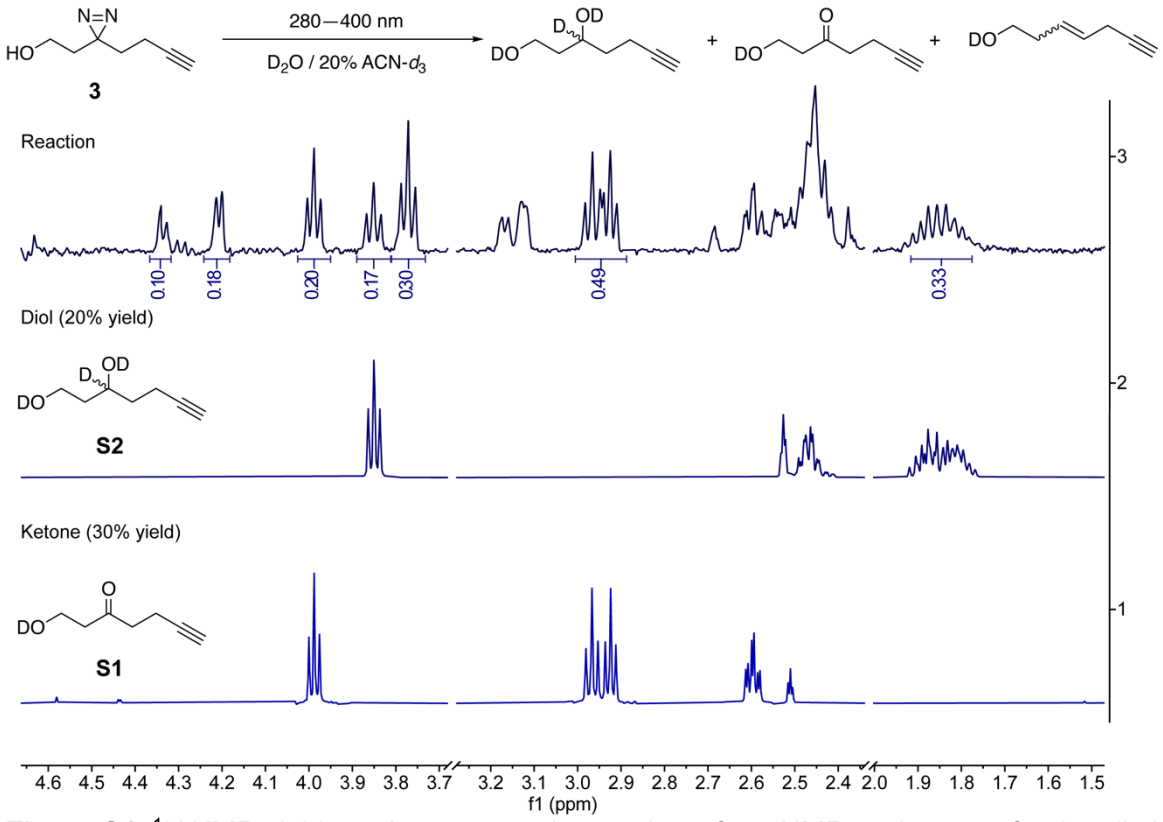


Figure S1. ^{1}H NMR yields and representative products from NMR assignment for the alkyl diazirine **3**.

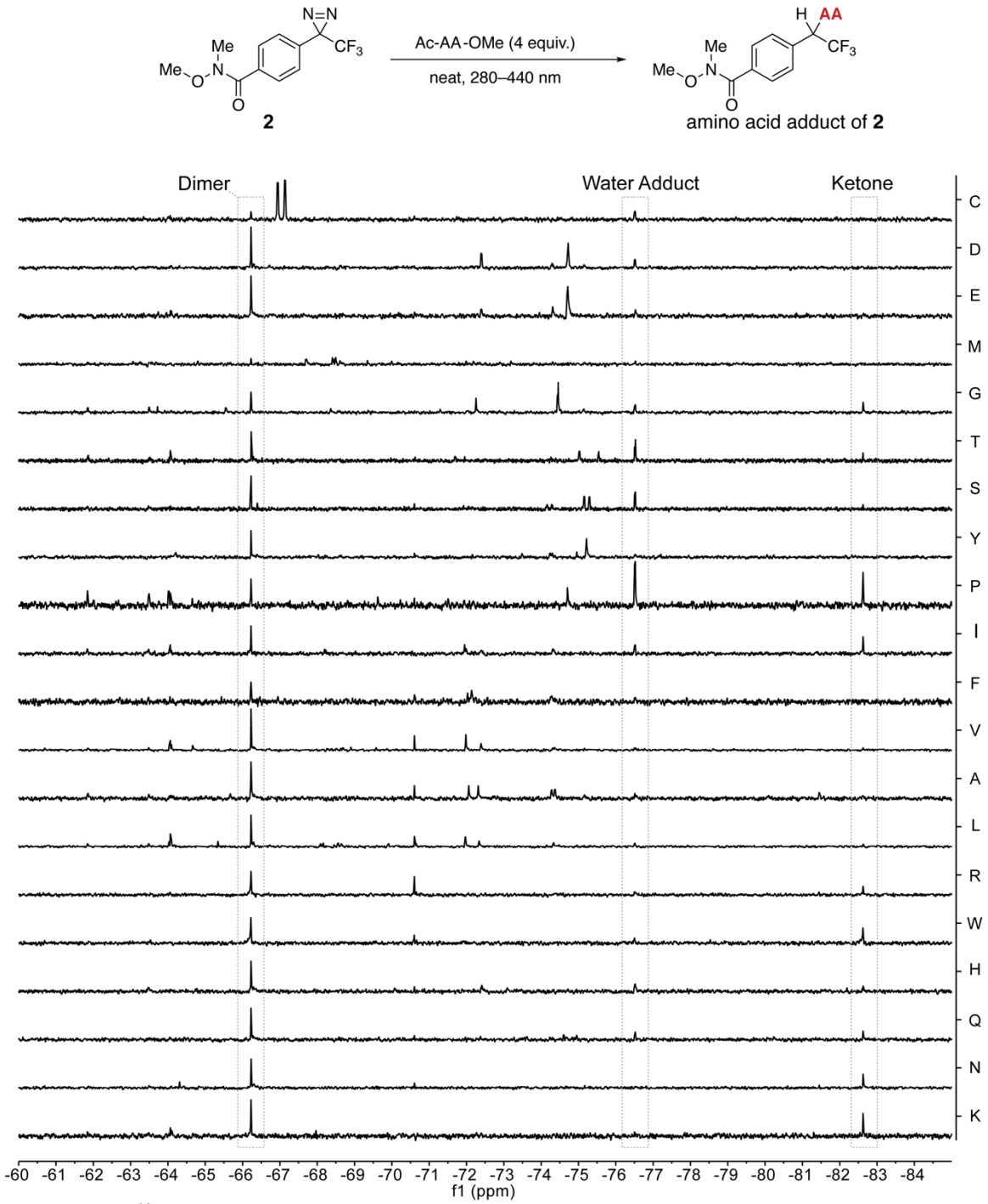


Figure S2. ^{19}F NMR spectra for neat reactions between the aryl-trifluorodiazirine **2** and the 20 protected amino acids. Common products are highlighted in the grey boxes. The integrals of the unique peaks were used to estimate the yield of amino acid adducts.

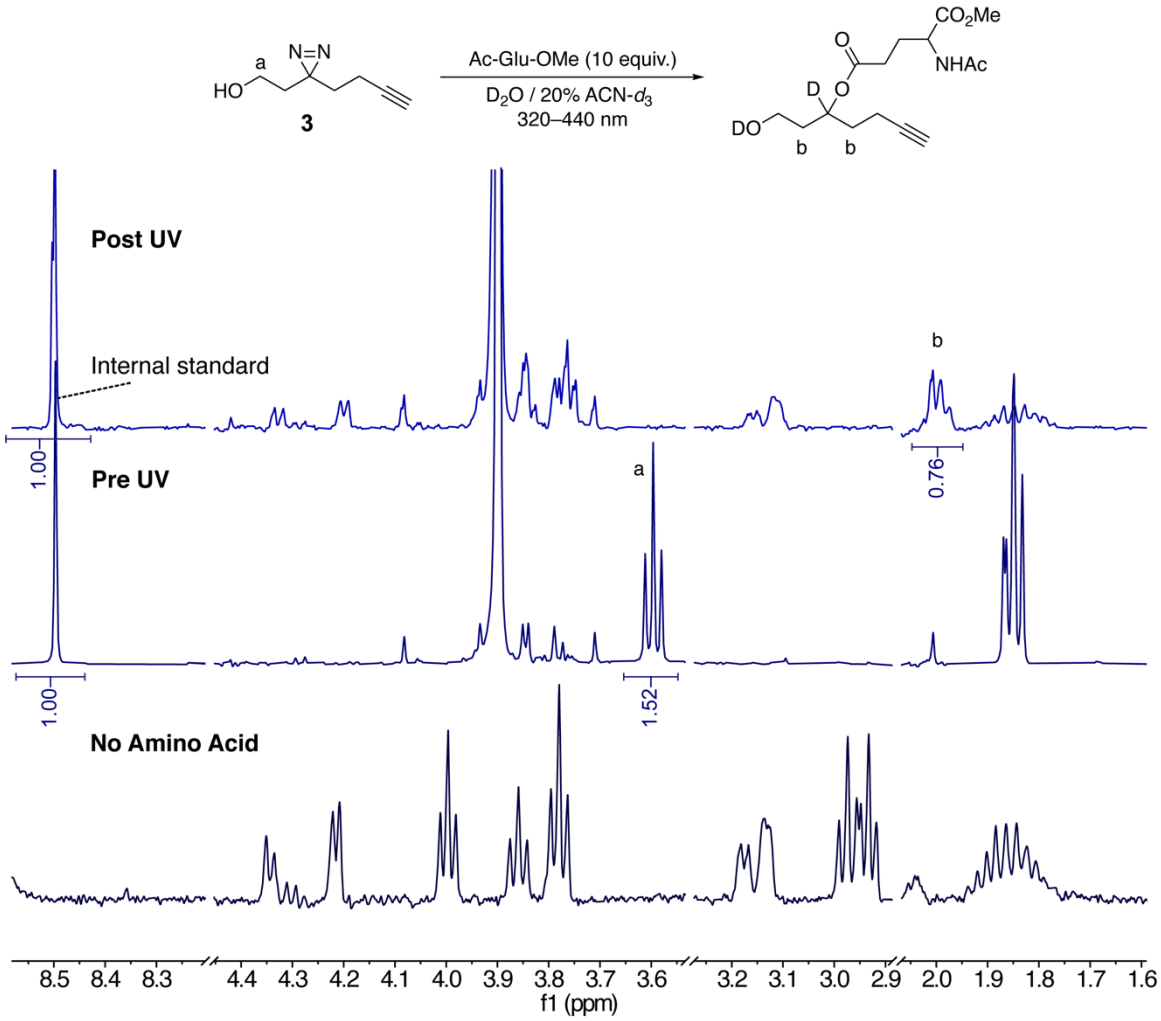


Figure S3. Representative ^{1}H NMR of aqueous reactions between the alkyl diazirine **3** and protected amino acids. Putative products are shown along with the integrated proton signals used to estimate their yields. Product formation was usually observed along with the loss of ketone formation.

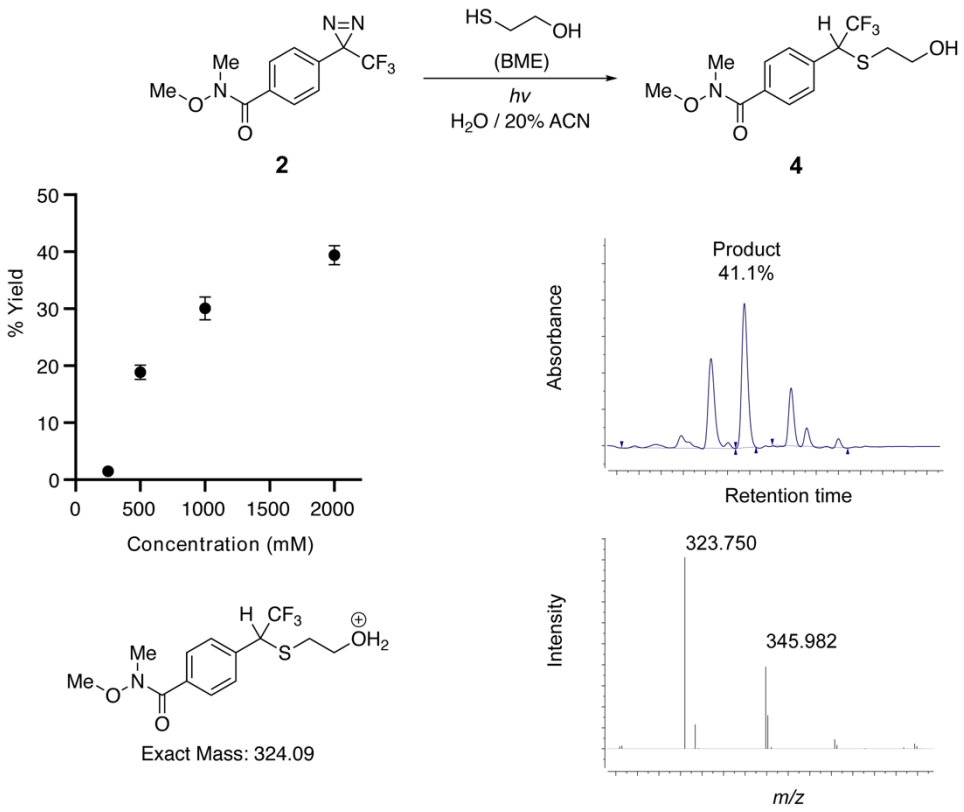


Figure S4. Reaction with aryl diazirine **2** and β-mercaptoethanol (BME) in aqueous conditions. LC-MS spectra from reaction with 2 M BME are shown. Detectible product formation begins at 250 mM concentration of BME. Yields were calculated using UV-Vis peak integration of the BME adduct peak relative to other products.

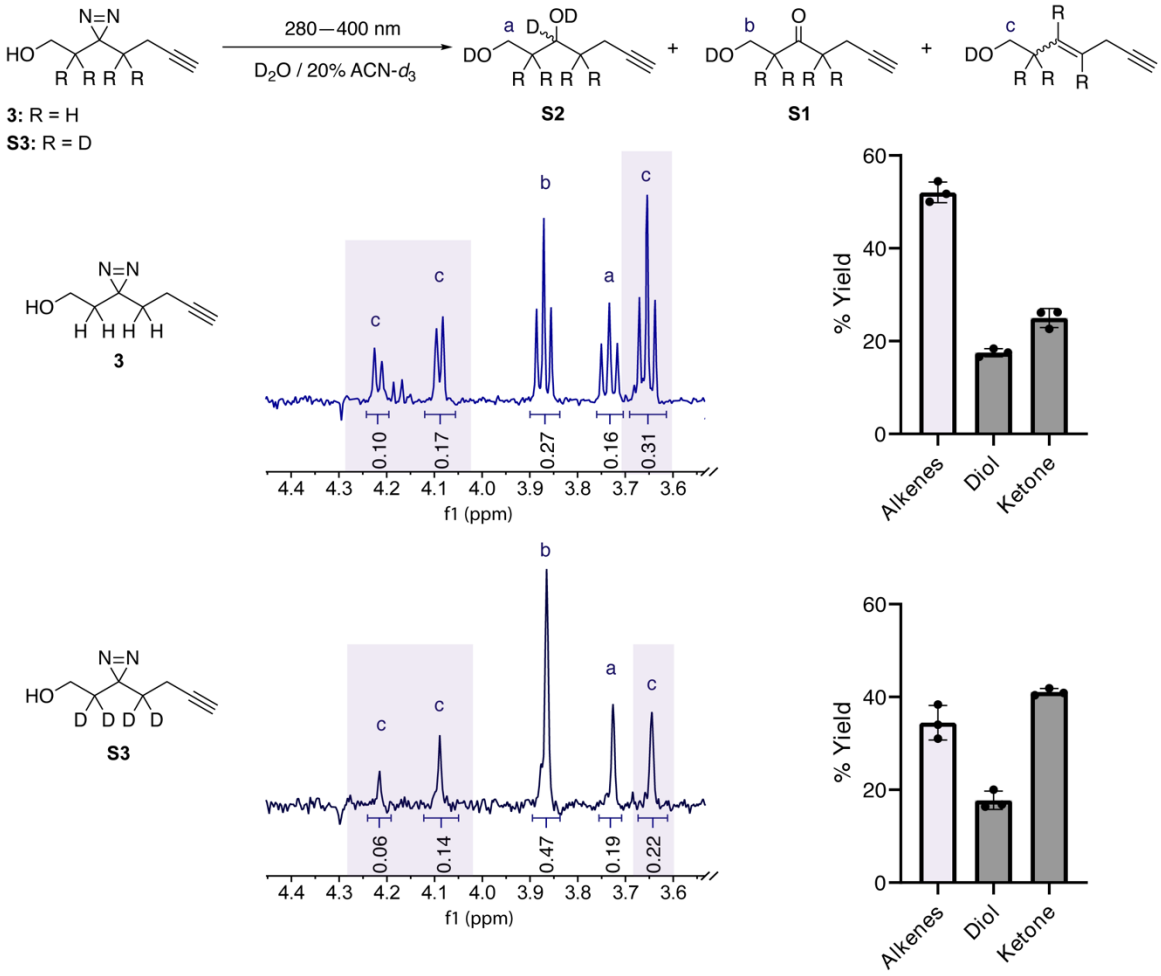


Figure S5. ^{1}H NMR yields of aqueous photolysis of the diazirine **3** and its tetra-deuterated analog **S3**. Peaks corresponding to alkene products are highlighted. Yields were calculated using ^{1}H NMR peak integration relative to an internal standard.

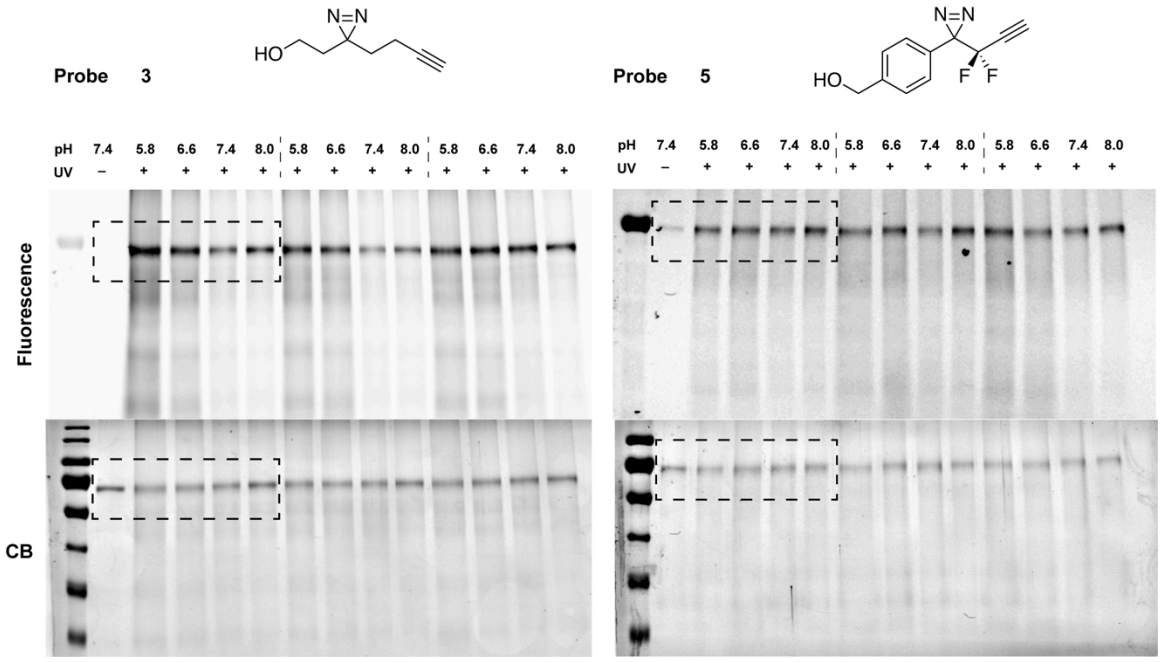


Figure S6. Full gels of single protein labeling of BSA with diazirine probes **3** and **5** in Tris buffer**.** Boxed regions are shown in **Fig 3.** Labeling was quantified by dividing Azide-fluor 488 fluorescence by coomassie blue signal (CB).

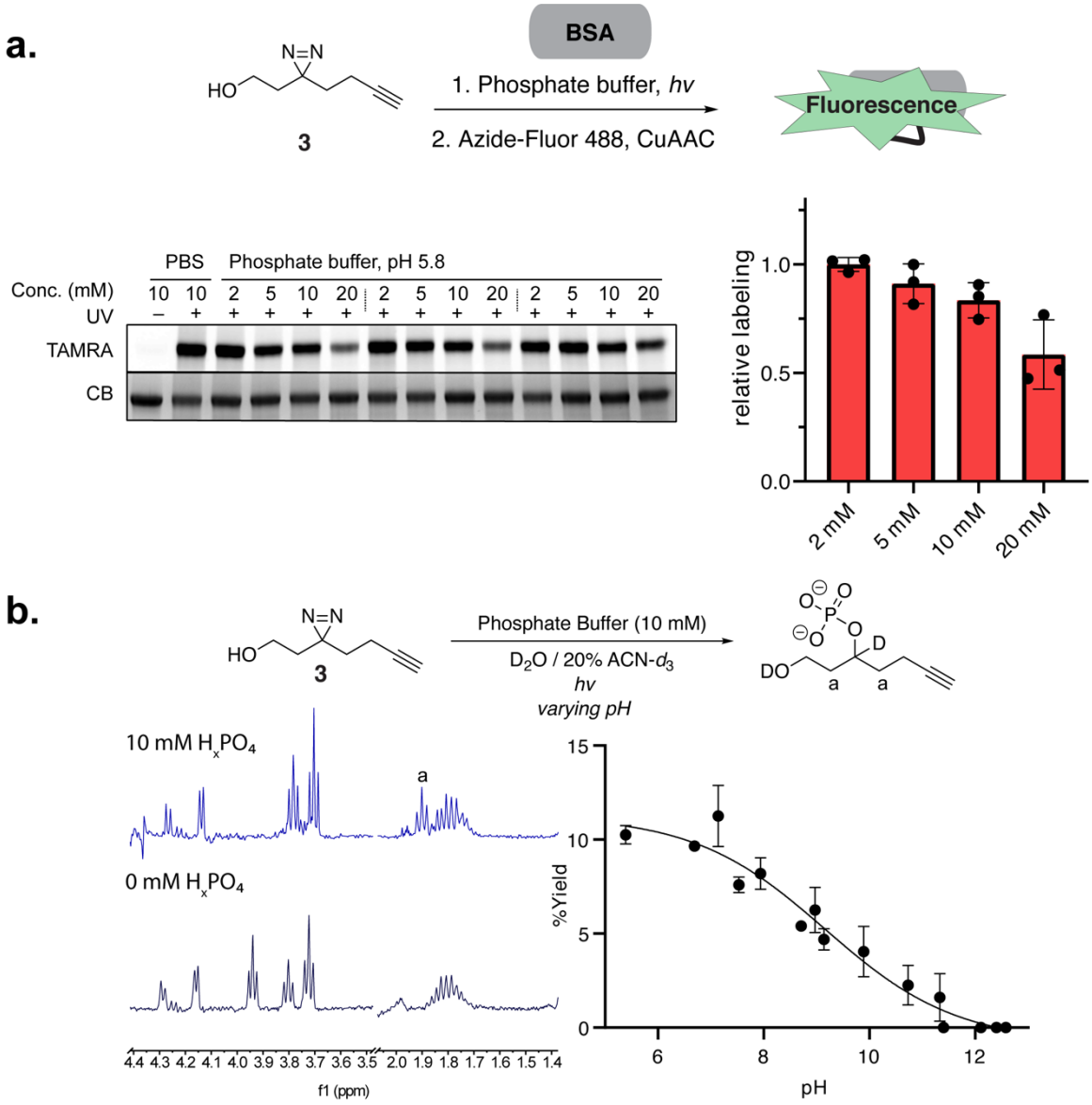


Figure S7. (a) Single protein labeling of BSA by the diazirine **3** in acidic phosphate buffer at varying concentration. Relative labeling was quantified with Azide-fluor 488 fluorescence relative to coomassie (CB) signal. **(b)** Photolysis of the diazirine **3** in phosphate buffer produces pH-dependent yields of a phosphate adduct. Yields were calculated using ^{1}H NMR peak integration relative to an internal standard.

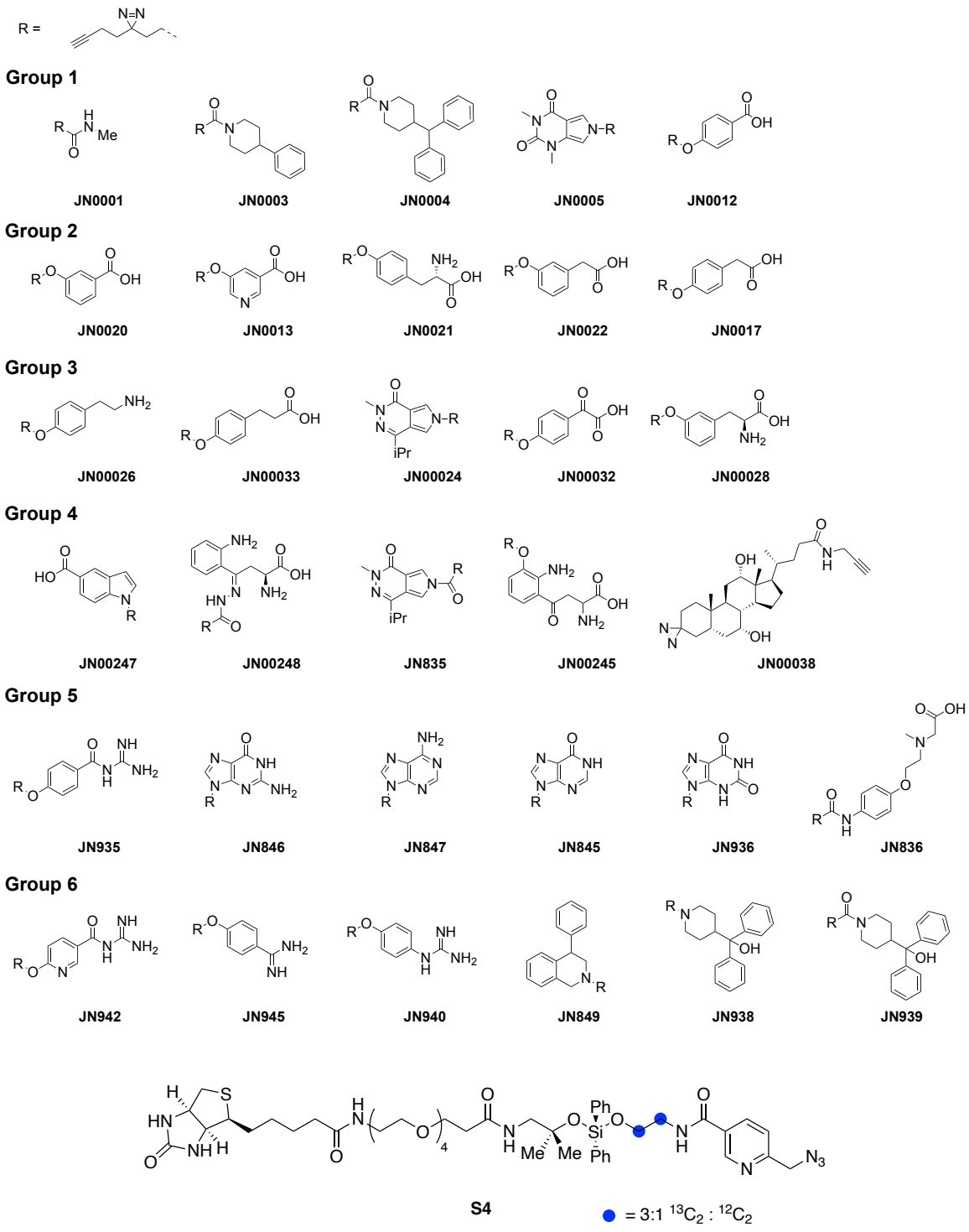


Figure S8. PAL probe structures and their corresponding dosing groups to SK-N-SH cells, and the isotopically-labeled cleavable picolyl biotin azide probe **S4**.[1]

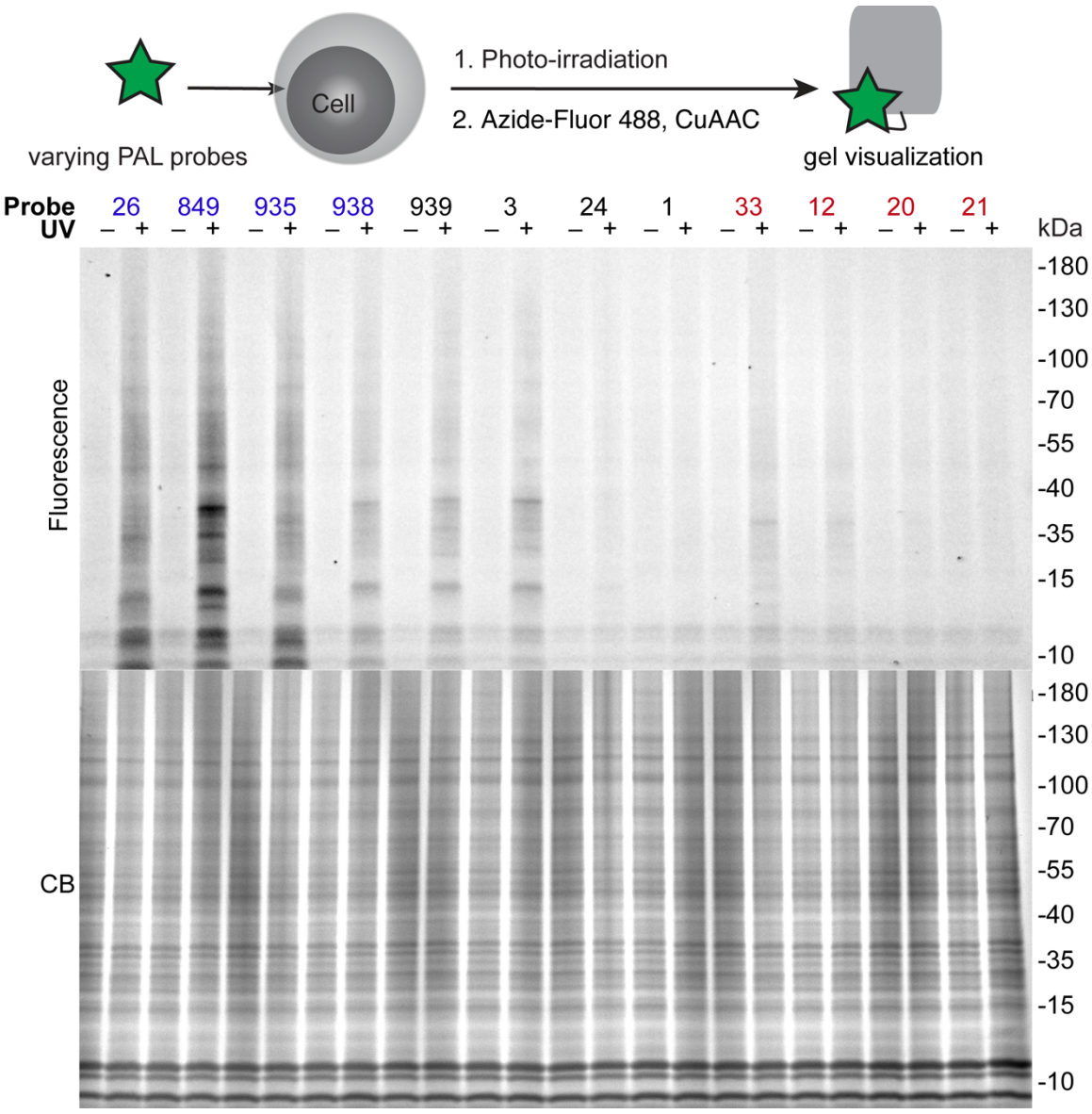


Figure S9. Photoaffinity labeling yields of 12 PAL probes in HEK293T cells visualized by Azide-fluor 488 and in-gel fluorescence. Top image is Azide-fluor 488 fluorescence, bottom is coomassie blue stain.

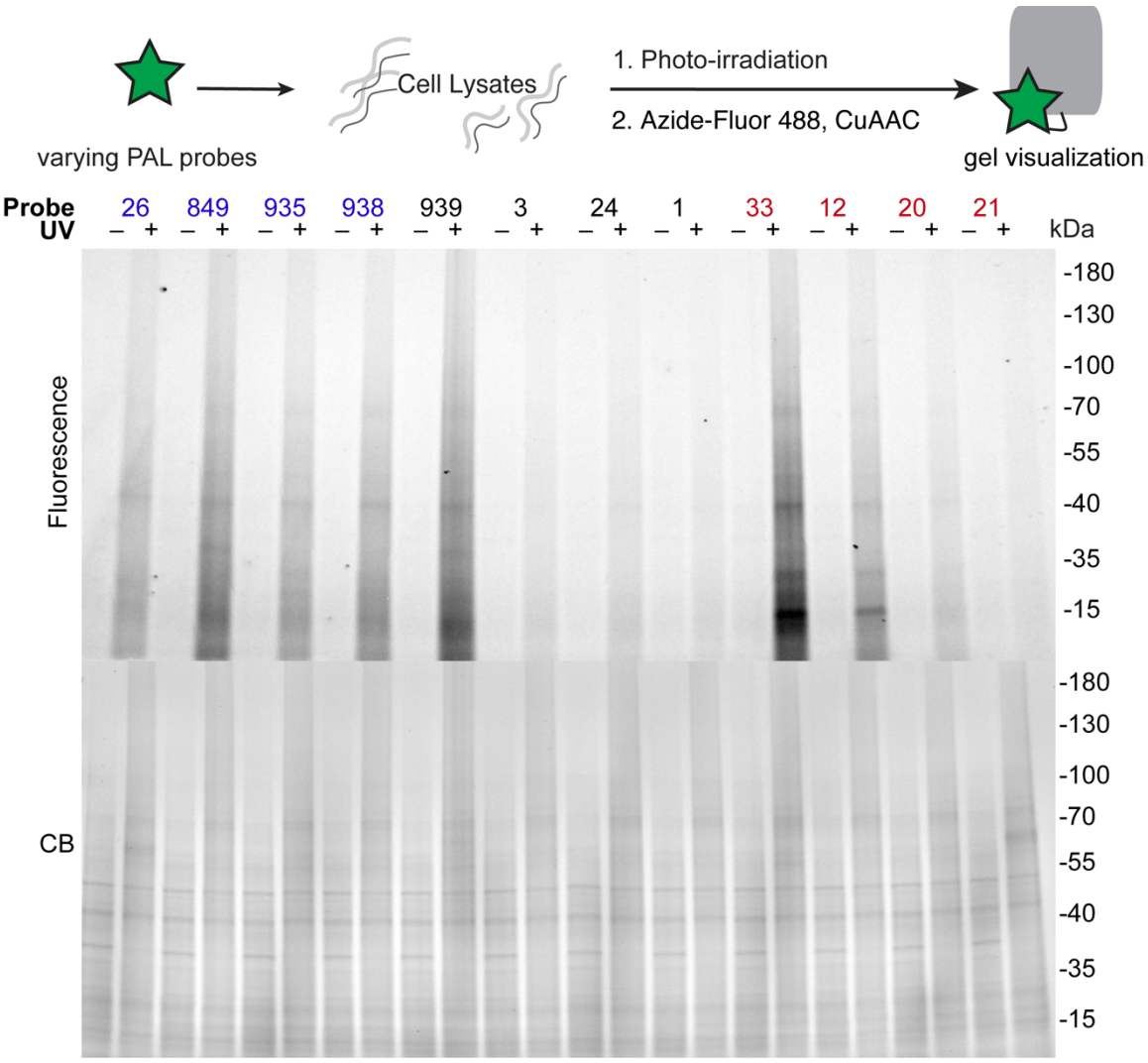


Figure S10. Photoaffinity labeling yields of 12 PAL probes in nondenatured HEK293T lysates visualized by Azide-fluor 488 and in-gel fluorescence. Top image is Azide-fluor 488 fluorescence, bottom is coomassie blue stain.

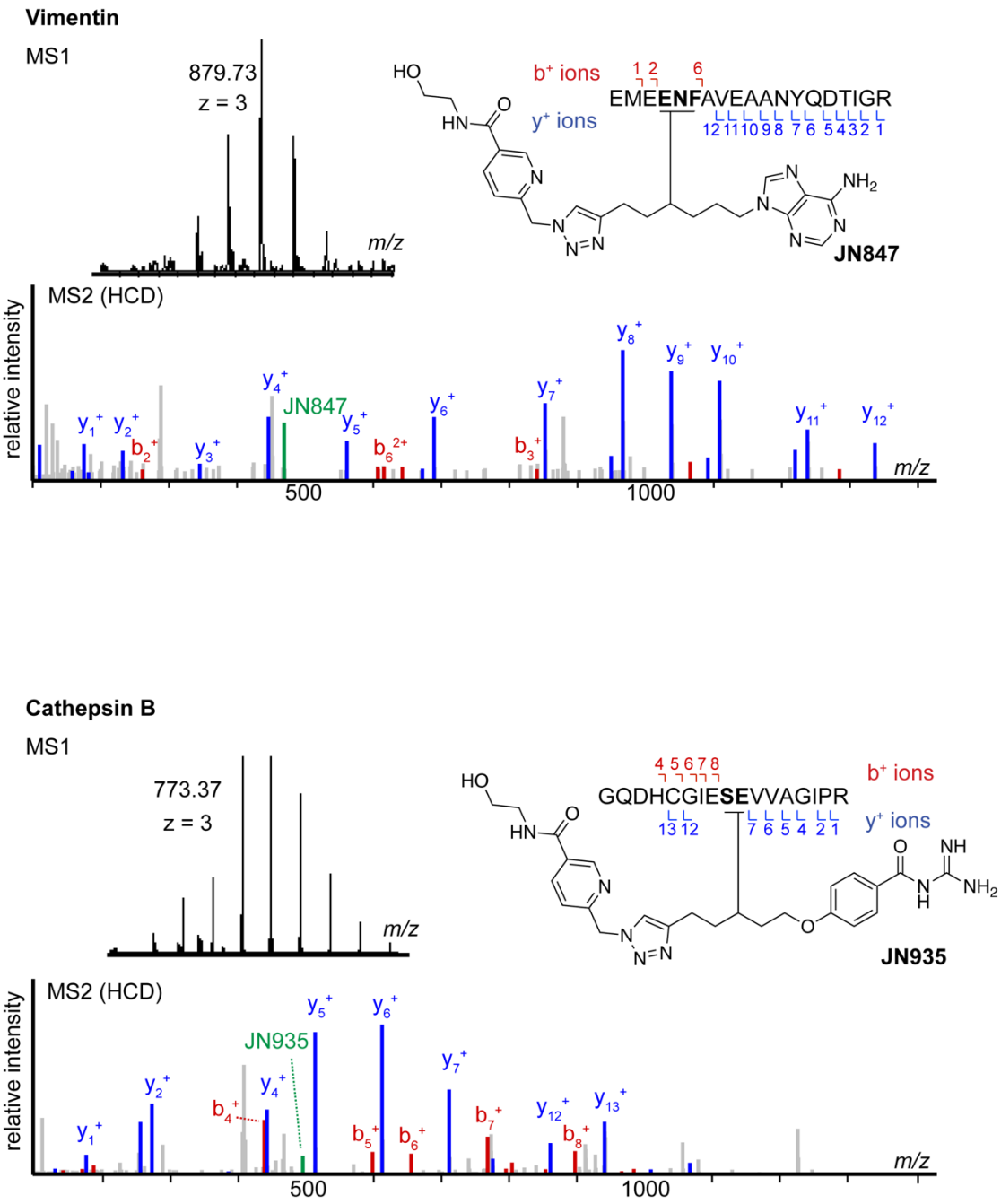
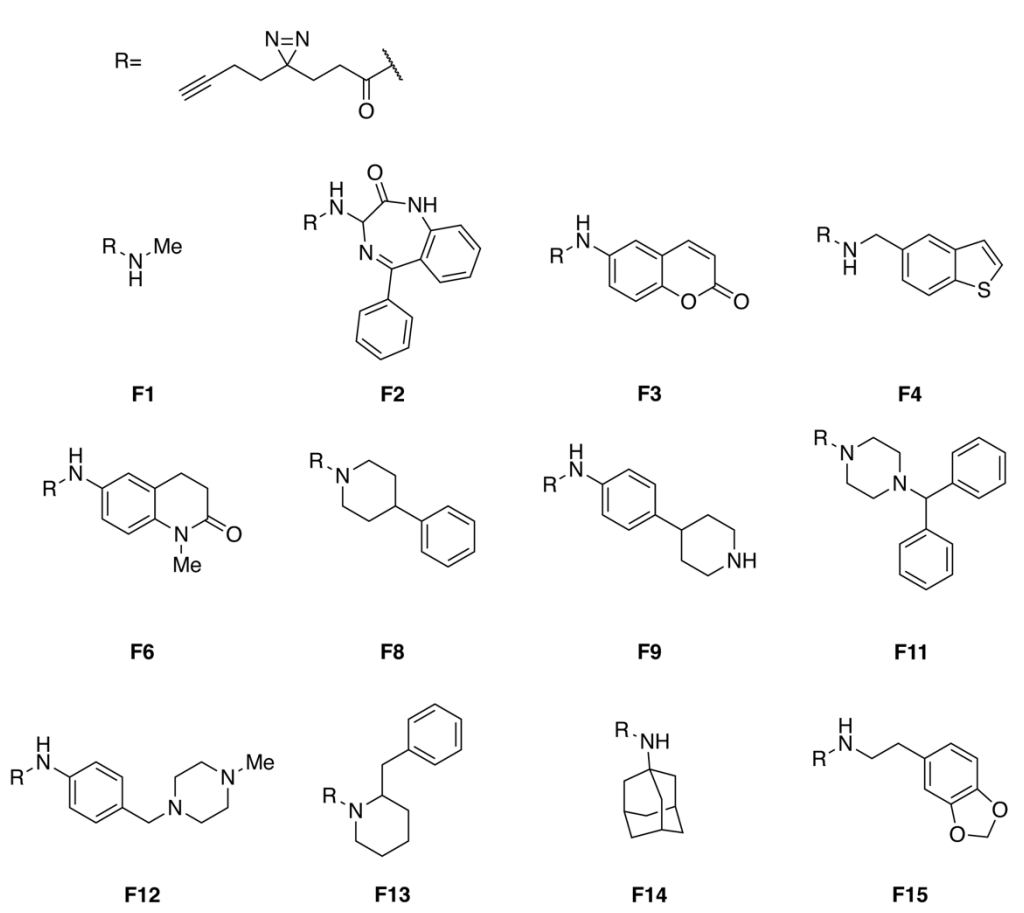


Figure S11. Annotated binding sites for Vimentin and Cathepsin B.

a.



b.

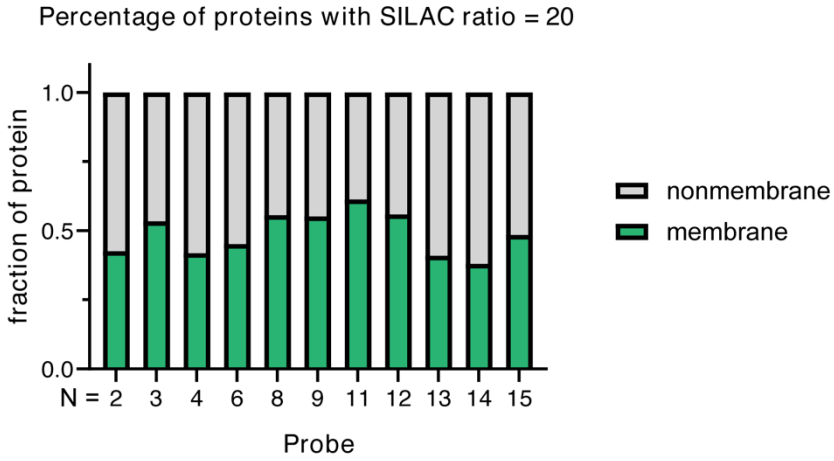


Figure S12. Meta-analysis of proteomics data in Parker *et al*.[2] **(a)** Structures of fully functionalized fragments (FFFs). **(b)** Analysis of data from FFFs showing the fraction of membrane proteins with SILAC ratios greater than or equal to 20. These data were collected from HEK293T cells at 200 μM dosing of FFF's.[2]

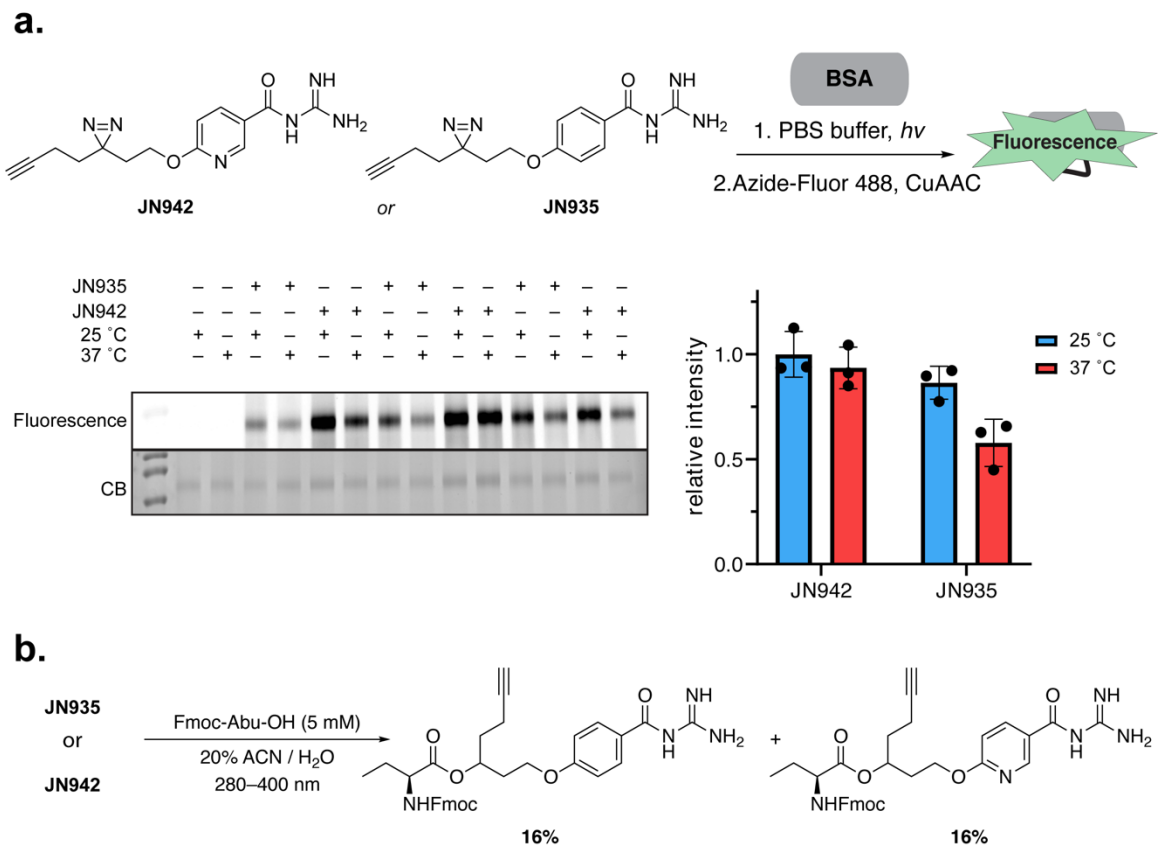


Figure S13. Single protein and amino acid labeling by **JN942** and **JN935. (a)** Labeling efficiency of BSA in PBS and 25 ˚C or 37 ˚C was calculated with in gel fluorescence Azide-fluor 488 signal relative to coomassie blue signal. Plotted values show the mean with error bars representing standard deviation with n =3. **(b)** Reactivity of the PAL probe with the amino acid Fmoc-Abu-OH. Amino acid reaction yields were calculated by UPLC-MS UV-Vis peak integration.

Supplementary Methods

General Synthetic Procedures. All reactions were performed in single-neck, oven-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless-steel cannula. Organic solutions were concentrated by rotary evaporation at 30–33 °C. Normal phase flash-column chromatography was performed as described by Still and co-workers.[3] Normal phase purifications employ silica gel (60 Å, 40–63 μm particle size) purchased from Silicycle (Quebec, Canada). Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV), iodine (I_2), and/or submersion in *p*-anisaldehyde or ninhydrin followed by brief heating with a heat gun (10–15 s).

Chemical Materials. Commercial solvents and reagents were used as received with the following exceptions. Dichloromethane (DCM) was purified according to the method of Grubbs and co-workers.[4] Acetonitrile was dried over calcium hydride for at least 24 h prior to use. Minimalist tag **3** was prepared according to the method of Yao and co-workers.[5] The cleavable biotin azide probe was synthesized according to the method of Woo and co-workers.[1] Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) was obtained from Sigma-Aldrich (#762342). Azide-fluor 488 was obtained from Sigma-Aldrich (#760765). Biotin-PEG_3-azide was obtained from Sigma-Aldrich (#762024). All diazirine probe concentrations are based on calculated concentration after dilution from a dimethylsulfoxide stock, acetonitrile or D_2O stock solutions as specified in their corresponding experimental sections.

Biological Materials. SK-N-SH and HEK293T cells were a gift from the Bertozzi lab (Stanford University) and were acquired from the ATCC. GelCode Blue Stain Reagent (Thermo Scientific, #24590) was used to image total protein in polyacrylamide gels. For Western blotting, IRDye 800CW Streptavidin (LICOR #926-32230), Streptavidin-HRP (Thermo Fisher, 21130) and BSA (Sigma-Aldrich #A7906) were used. Protease inhibitor tablets (Roche EDTA-free cOmplete tablets, Sigma-Aldrich, #11836170001) were resuspended as a 25x stock in 2 mL PBS and stored at −20 ˚C. For LC-MS/MS analysis, proteins were digested with Sequencing grade trypsin (Promega, #V5111) using S-Trap mini (Protifi, #C02-mini) or micro devices (Protifi, #C02-micro). C18 Ziptips (Millipore, #ZTC18S096) were used for sample desalting, or Mini Bio-Spin columns (Bio-Rad, #7326207). Triton X-114 (Thomas Scientific, #C987Q42) was used as a nondenaturing buffer for cell lysate experiments.

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 500 or 400 MHz at 24 °C. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent [$CHCl_3$, δ 7.26] or [D_2O, δ 4.79]. Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, coupling constant in Hertz, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 125 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent ($CDCl_3$, δ 77.0). ^{13}C NMR and data are represented as follows: chemical shift, carbon type. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane. Infrared (IR) spectra

were obtained using a Shimadzu 8400S FT-IR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Small molecule high-resolution mass spectrometry (HRMS) measurements were obtained at the Harvard University Chemistry and Chemical Biology Department Mass Spectrometry Facility using an Agilent 1260 UPLC-MS Bruker connected in line with a microTOF-Q II hybrid quadrupole-time of flight MS. Low-resolution mass spectrometry (LRMS) measurements were obtained on a Waters ACQUITY UPLC equipped with SQ Detector 2 mass spectrometer. Ultrapure water was obtained using a GenPure UV/UF xCAD plus system (Thermo Scientific). All UV irradiation was performed using a Dymax ECE 5000 UV Light-Curing Flood Lamp system (#41060). The lamp was warmed up for at least 15 minutes prior to use. Irradiation was performed in a 4 ˚C cold room for specified time. For irradiation, samples were placed either in 100 µL or 500 µL PPE PCR tubes (20170-010) or in quartz NMR tubes (528-PP-7QTZ). Transfer of proteins from SDS-PAGE gels to membranes was performed using a iBlot 2 dry blotting system (Invitrogen). In-gel fluorescence, GelCode Blue staining, IR blots, and Ponceau were imaged by an Azure Imager C600 (Azure Biosystems, Inc., Dublin, CA). Peptides were dried using a Vacufuge plus (Eppendorf). Proteomics mass spectrometry instrumentation is described in the "Proteomics Mass Spectrometry and Data Processing Procedures" section.

Software. Data was analyzed and visualized using Microsoft Excel (v16.22) and GraphPad Prism (v8.1.1), in addition to software listed by experiment. Pymol v2.1.0 (Schrodinger) was used to visualize protein structures. DNA and protein sequences were analyzed using Geneious (v10.0.7). Images were made using ImageJ (NIH, v1.52q), Adobe Photoshop (v19.1.5), and Adobe Illustrator (v23.1.1).

Experimental Procedures with Individual Amino Acids

Photolysis of the diazirine in aqueous conditions. A quartz NMR tube was charged with a solution of the diazirine **3** (1 mM) and calcium formate (1 mM) in 20% acetonitrile-d_3–deuterium oxide (500 µL). The resulting solution was analyzed via ^{1}H NMR and then irradiated (280–320 nm) for 2 x 90 sec at 4 ˚C. The irradiated sample was reanalyzed via ^{1}H NMR and the product distribution was integrated against calcium formate as the internal standard. For the diazirine **3**, the ketone **S1** and the diol **S2** products were analogous to synthetic standards.

General procedure for neat photolysis of the diazirine with N-acetyl O-methyl amino acids. Stock solutions of the 20 N-acetyl O-methyl amino acids and the diazirine probes **1** or **2** (100 mM) were prepared in acetonitrile and dried over activated 3 Å molecular sieves for 24 h immediately before use. The diazirine probe **1** or **2** (5.00 µL, 1 equiv) and the amino acid (20.0 µL, 4.00 equiv) were added in sequence to a PCR tube (Part number) in quadruplicate. The resulting solution was concentrated to dryness by vacufuge for 1 h at 30 ˚C to afford an oil emulsion, with the exception of mixtures prepared with Asn, Gln, and Trp, which afforded a white crystalline solid. The neat mixture was degassed, and the PCR tube was sealed under nitrogen in a glovebox. Three of the four sealed tubes were irradiated with a broadband UV lamp (280–400 nm) for 6 x 90 s at 4 ˚C. The remaining sealed tube was not irradiated and kept in the dark.

Note: Drying the stock solution and sealing the PCR tube under nitrogen is required to reduce UV products arising from residual water or oxygen. For Arg and Asn, methanol was used to prepare the stock solutions.

Analysis of the alkyl diazirine 1 photolysis by UPLC-MS. The irradiated samples were unsealed and dissolved in dimethylsulfoxide (DMSO) (100 µL). Half of the dissolved sample (50.0 µL) was diluted with a 4 mM solution of L-2-(Fmoc-amino)butyric acid (50 µL, 2 mM final) used as an internal standard. The resulting solution was analyzed via UPLC-MS (5–90% acetonitrile–water, 10 min), and monitored by UV-Vis absorbance over 250–270 nm. The product identity for each peak was determined by the *m/z* in the ES+ mass spectra. Product yields were determined using peak integrals relative to the internal standard.

Analysis of the aryl diazirine 2 photolysis by NMR. The irradiated samples were dissolved in 500 µL of DMSO-d_6 containing 1 mM fumaric acid and 1 mM hexafluorobenzene and transferred to a NMR tube (final concentration ~1 mM diazirine). The samples were analyzed via ^{1}H NMR and ^{19}F NMR. Product identities were inferred from unique peaks in the ^{19}F NMR spectra relative to the non-irradiated control. Product yields were calculated from ^{19}F NMR peak integration relative to hexafluorobenzene internal standard.

Aqueous photo-reactions with individual amino acids. Stock solutions of the 20 N-acetyl O-methyl amino acids (50 mM) and the diazirine probe **3** (10 mM) were prepared in 50% acetonitrile-d_3–D_2O prior to use. The amino acid was added at a equimolar ratio (10.0 µL, 1.00 mM, 1.00 equiv.) or 10-fold excess (100 µL, 10.0 mM, 10.0 equiv.) to a solution of the diazirine probe **3** (50.0 µL, 1.00 mM, 1 equiv.) and calcium formate (5.00 µL, 1.00 mM, 1.00 equiv.) in 20% acetonitrile-d_3–D_2O in a quartz NMR tube in quadruplicate. Three of the four samples were irradiated with either a broadband UV lamp (280–400 nm) for 2

x 90 seconds, or a filtered UV lamp (320–400 nm) for 4 x 90 seconds at 4 ˚C. The remaining sample was not irradiated and kept in the dark as a control. The photolyzed samples were analyzed by ^{1}H NMR and UPLC-MS.

For ^{1}H NMR, reactions were examined by comparing the spectra of irradiated samples to the spectra of the no-UV control and spectra from aqueous photolysis of diazirine probe **3** alone. Unique peaks not belonging to starting materials or diazirine **3** photolysis products were observed only with reactions with Glu, Asp, Tyr, and Cys (Figure **S3**). To calculate product yields, clearly-resolved peaks were integrated relative to the calcium formate internal standard. All samples were additionally analyzed without further dilution via UPLC-MS, (5–90% acetonitrile in water over 10 min). Product identities were determined from the *m/z* in the ES+ mass spectra. *m/z* matching expected XH insertion products were found for Glu, Asp, Tyr, and Cys but no other amino acids.

pH titration reactions with individual amino acids. Stock solutions of the N-acetyl O-methyl Tyr, Lys, His and Glu, and the Fmoc diazirine probe **1** were prepared (100 mM) in acetonitrile prior to use. The diazirine (5.00 µL, 1.00 mM, 1 equiv) and the amino acid (50.0 µL, 10.0 mM, 10.0 equiv) were added in sequence to 20% acetonitrile–water (445 µL). The pH of the solution was adjusted using KOH (100 mM in water) or HCl (100 mM in water) and measured. The resulting solution was aliquoted into 4 x 100 µL samples in PCR tubes. Three of the samples were irradiated (320–400 nm) for 4 x 90 s at 4 ˚C. The remaining sample was kept in the dark. After UV treatment, Fmoc-Ser(tBu)-OH (1 mM final) was added to each sample as an internal standard. The resulting solutions were analyzed via UPLC-MS, (5–90% acetonitrile–water, 10 min), and monitored by UV absorbance (250–270 nm). Product yields were determined using peak integrals relative to the internal standard.

pH titration reactions with phosphate. Stock solutions of monobasic phosphate (100 mM) in D_2O and alkyl diazirine probe **3** (10 mM) were prepared in 50% acetonitrile-d_3–D_2O prior to use. The phosphate solution was added at 10-fold excess (100 µL, 10.0 mM, 10.0 equiv.) to a solution of the diazirine probe **3** (50.0 µL, 1.00 mM, 1 equiv.) and calcium formate (5.00 µL, 1.00 mM, 1.00 equiv.) in 20% acetonitrile-d_3–D_2O. The solution was pH-adjusted with HCl (100 mM in D_2O) then partitioned into four quartz NMR tubes. Three of the four samples were irradiated (280–400 nm) for 2 x 90 seconds at 4 ˚C. The remaining sample was not irradiated and kept in the dark as a control. The photolyzed samples were analyzed by ^{1}H NMR. Reactions were examined by comparing the spectra of irradiated samples to the spectra of the no-UV control and spectra from aqueous photolysis of diazirine probe **3** alone. Unique peaks not belonging to starting materials or diazirine 3 photolysis products were observed and attributed to putative phosphate adduct**.**

Aqueous photo-reactions with β-mercapto ethanol. Solutions of β-mercapto ethanol (BME) in 50% acetonitrile–water were prepared via serial dilution (2 M, 1 M, 500 mM, 250 mM, 125 mM). A stock solution of aryl diazirine **2** (10 mM) in 50% acetonitrile was prepared, and 5 µL added to 45 µL aliquots of respective BME solutions in quadruplicate in PCR tubes. Three of the four samples per concentration were irradiated (280–400 nm) 2 x 90 sec at 4 ˚C. The samples were then analyzed via UPLC-MS (5-90% acetonitrile in water, 6 min). Product identities were determined from the *m/z* in the ES+ mass spectra. Yields were calculated using relative peak integration in the UV-Vis chromatogram.

Experimental Procedures with Purified Proteins

pH-dependent labeling of BSA with diazirine probes 3 and 5. Tris (pH 7.4 and 8.0) and bis-tris (pH 5.8 and 6.6) buffers were prepared at 100 mM with 1% SDS and pH adjusted. All experiments done with tris and bis-tris buffers were performed at 20 mM concentration. Phosphate buffers were prepared at 100 mM concentration with 1% SDS and pH adjusted to pH 5.8, 6.6 and 7.4, then diluted to 20 mM, 10 mM, 5 mM and 2 mM. 20x stock solutions of purified BSA (1 µL) were added to 18 µL (0.1 µg / mL) of variable buffer in a PCR tube. 20x solutions of diazirine probes **3** or **5** in DMSO (1 µL) were added to each sample (100 µM) and the samples incubated at 24 ˚C for 30 min. The samples were irradiated for 30 s at 4 ˚C on a bed of ice. Following irradiation, samples were diluted with acetone (100 µL) and transferred to 1.7 mL Eppendorf tubes. An additional 100 µL of acetone was used to rinse the samples into the new tubes. The protein was precipitated at –80 ˚C for 30 min, then pelleted via centrifugation (10 min, 21,000 g, 4 ˚C). The supernatant was discarded and the pellets were dried under air for 5 min. Samples were resuspended in 50 µL of 5x PBS–1%SDS and click reacted with a mixture of Azide-fluor 488 (25 µM), copper (II) sulfate (250 µM), THPTA (250 µM), sodium ascorbate (2.0 mM) for 60 min at 24 ˚C with rotation. The reaction was quenched with the addition of acetone (300 µL) followed by precipitation at –20 ˚C for 12 h. Proteins were pelleted by centrifugation (21,000 g, 10 min, 4 ˚C). The supernatant was discarded and the pellets dried under air for 5 min. The pellets were suspended in 1x SDS-PAGE gel loading buffer (30 µL) and stored at –20 ˚C. Thawed samples were heated for 5 min at 95 ˚C prior to separation via SDS-PAGE 12% gel. Probe labeling was visualized by analyzing fluorescence signal and protein loading visualized with coomassie blue staining.

Experimental Procedures with Whole Cells

Photo-labeling in HEK293T cells with photo probes for in-gel fluorescence imaging. Cells were grown to 80-90% confluency in 3.5 cm, six-well plates and incubated with 10 μM of each corresponding probe in FBS-free media for 1 h at 37 ˚C. Following incubation, half of the plates were irradiated for 60 s at 4 ˚C. The cells were harvested via scraping, collected in PBS (2 x 1 mL), pelleted (500 g, 5 min), and washed with PBS (1 mL). The pellets were resuspended in PBS (200 μL) with 1% SDS and proteasome inhibitor then sonicated with a probe tip sonicator to lyse the cells. The lysates were cleared via centrifugation and the concentration of protein adjusted to 1.8 mg / mL using the BCA assay. 50 μg of protein (60 μL) from each sample was taken and reacted with a mixture of Azide-Fluor 488 (25 μM), copper (II) sulfate (250 μM), THPTA (250 μM), sodium ascorbate (2.0 mM) for 90 min at 24 ˚C with rotation. The reaction was quenched with the addition of acetone (300 μL) followed by precipitation at –20 ˚C for 12 h. Proteins were pelleted by centrifugation (21,000 g, 10 min, 4 ˚C). The supernatant was discarded and the pellets dried under air for 5 min. The pellets were suspended in 1x SDS-PAGE gel loading buffer (50 μL) and stored at –20 ˚C. Thawed samples were heated for 5 min at 95 ˚C prior to separation on a 4%–15% Criterion TGX Precast gel (#5671085). Probe labeling was visualized by analyzing fluorescence signal and protein loading visualized with coomassie blue staining.

Photo-labeling in HEK293T lysates with photo probes for in-gel fluorescence imaging. Cells were grown to 80-90% confluency in 15 cm plates. The cells were washed with PBS (10 mL) then treated with trypsin (3 mL) and incubated at 37 ˚C to detach. The cells were collected in PBS (7 mL), pelleted (500 g, 5 min), and washed with PBS (10 mL). The pellets were resuspended in PBS (1000 μL) with 0.3% Triton and proteasome inhibitor then sonicated with a probe tip sonicator to lyse the cells. The lysates were cleared via centrifugation and the concentration of protein adjusted to 1.0 mg / mL using the BCA assay. 50 μg aliquots were prepared in PCR tubes, dosed with each corresponding probe and incubated at 24 ˚C for 30 min. Half of the samples were irradiated for 30 s at 4 ˚C. Following irradiation, 10% SDS–PBS (5 μL) was added to denature proteins and the samples were reacted with a mixture of Azide-fluor 488 (25 μM), copper (II) sulfate (250 μM), THPTA (250 μM), sodium ascorbate (2.0 mM) for 90 min at 24 ˚C with rotation. The reactions were transferred with acetone (100 μL) to 1.7 mL Eppendorf tubes and diluted with an additional 200 μL, followed by precipitation at –20 ˚C for 12 h. Proteins were pelleted by centrifugation (21,000 g, 10 min, 4 ˚C). The supernatant was discarded and the pellets dried under air for 5 min. The pellets were suspended in 1x SDS-PAGE gel loading buffer (50 μL) and stored at –20 ˚C. Thawed samples were heated for 5 min at 95 ˚C prior to separation on a 4%–15% Criterion TGX Precast gel (#5671085). Probe labeling was visualized by analyzing fluorescence signal and protein loading visualized with coomassie blue staining.

Photo-labeling of SK-N-SH cells with photo-probes for quantitative LC-MS/MS. Cells were grown to 90–100% confluency in 10 cm plates then incubated with 10 μM of each probe in the treatment group (or 0.5% DMSO) in FBS-free media for 2 h at 37 ˚C. Following incubation, the plates were irradiated for 60 s at 4 ˚C. The cells were harvested via scraping, collected in PBS (10 mL), pelleted (2000 g, 5 min), and washed with PBS (10 mL). The pellets were resuspended in PBS (500 μL) with 1% SDS and proteasome inhibitor then sonicated with a probe tip sonicator to lyse the cells. The lysates were cleared via centrifugation and the concentration of protein adjusted using the BCA assay (to concentrations of ~2 mg / mL). The lysates were reacted with a mixture of picolyl biotin

azide probe **S4** (100 μM), copper (II) sulfate (250 μM), THPTA (250 μM), sodium ascorbate (2.5 mM) for 90 min at 24 ˚C with rotation. The reaction was quenched with the addition of MeOH (1200 μL) followed by precipitation at –80 ˚C for 1 h. Proteins were pelleted by centrifugation (21,000 g, 10 min, 4 ˚C). The supernatant was discarded and the pellets were dried under air for 5 min.

Enrichment of photo-crosslinked proteins for quantitative LC-MS/MS. Protein pellets were resuspended in 1% SDS in PBS (500 μL) with brief sonication. 20 μL of sample was taken as the "load fraction." Streptavidin-agarose resin (200 μL, washed 3 x 1 mL with PBS) was added to each sample, which was then incubated for 18 h at 24 ˚C with rotation. The slurries were transferred to mini biospin column (#7326207) and centrifuged (1 min, 1000 *g*). The supernatant collected as the "supernatant fraction." The columns were transferred to a vacuum manifold and washed with 1% SDS in PBS (1 mL), 8 M fresh urea in PBS (5 x 1 mL) and PBS (5 x 1 mL). The beads were resuspended in PBS (200 μL), reduced with 5 mM dithiothreitol (DTT) for 30 min at 24 ˚C, then alkylated with 10 mM iodoacetamide for 30 min at 24 ˚C in the dark with rotation. The columns were transferred to the vacuum manifold and washed with PBS (1 mL) and 50 mM HEPES (1 mL, pH 8.5). The beads were centrifuged then washed with PBS (1 mL) and resuspended in 250 μL 0.5 M guanidine-HCl / 50 mM HEPES (pH 8.5). 2 μL of 0.5 mg / mL Trypsin was added to each sample. Samples were incubated at 37 ˚C for 12 h. The beads were pelleted via centrifugation and washed water (200 μL), and 50% acetonitrile / water (200 μL). The supernatant and washes were collected as part of the "trypsin fraction." The trypsin fraction was concentrated to dryness using a SpeedVac heated to 45 ˚C. Trypsin fractions were suspended in 25 μL water, the corresponding TMT reagent (2 μL, 20 μg/μL in ACN) was added, and the solutions rotated for 1 h at 24 ˚C. Hydroxyammonia (6 μL, 5%) was added to each sample, which was rotated an additional 15 min to quench the reactions. All trypsin fractions were combined and dried via SpeedVac at 45 ˚C. The trypsin fraction was fractionated using Thermo Pierce High pH Reversed-Phase Peptide Fractionation Kit (#84868). The fractions were dried and dissolved in 20 μL of 0.1% formic acid.

Multiplexed photo-labeling of SK-N-SH cells with photo-probes for binding site LC-MS/MS. Probes were sorted into six separate treatment groups according to **Figure S8**. Cells were grown to 90–100% confluency in 10 cm plates, then incubated with 10 μM of each probe in the treatment group, or 0.5% DMSO (two plates per treatment group) in FBS-free media for 2 h at 37 ˚C. Following incubation, the plates were irradiated for 60 s at 4 ˚C. The cells were harvested via scraping, collected in PBS (10 mL), pelleted (2000 g, 5 min), and washed with PBS (10 mL). The pellets were resuspended in PBS (500 μL) with 1% SDS and proteasome inhibitor then sonicated with a probe tip sonicator to lyse the cells. The lysates were cleared via centrifugation and the concentration of protein adjusted using the BCA assay (to concentrations of ~1-1 mg / mL). The lysates were reacted with a mixture of cleavable picolyl biotin azide probe **S4** (100 μM), copper (II) sulfate (250 μM), THPTA (250 μM), sodium ascorbate (2.5 mM) for 3 h at 24 ˚C with rotation. The reaction was quenched with the addition of MeOH (1 mL) followed by precipitation at –80 ˚C for 1 h. Proteins were pelleted by centrifugation (21,000 g, 10 min, 4 ˚C). The supernatant was discarded and the pellets were dried under air for 5 min.

Enrichment of photo-crosslinked proteins for binding site LC-MS/MS. Protein pellets were resuspended in 1% SDS in PBS (500 μL) with brief sonication. 20 μL of sample was taken as the "load fraction." Streptavidin-agarose resin (200 μL, washed 3 x 1 mL with PBS) was added to each sample, which was then incubated for 18 h at 24 ˚C with rotation. The slurries were transferred to mini biospin column (#7326207) and centrifuged (1 min,

1000 *g*). The supernatant collected as the "supernatant fraction." The columns were transferred to a vacuum manifold and washed with 1% SDS in PBS (1 mL), 8 M fresh urea in PBS (5 x 1 mL) and PBS (5 x 1 mL). The beads were resuspended in PBS (200 µL), reduced with 5 mM dithiothreitol (DTT) for 30 min at 24 ˚C, then alkylated with 10 mM iodoacetamide for 30 min at 24 ˚C in the dark with rotation. The columns were transferred to the vacuum manifold and washed with PBS (1 mL) and 50 mM HEPES (1 mL, pH 8.5). The beads were centrifuged then washed with PBS (1 mL) and resuspended in 250 µL 0.5 M guanidine-HCl / 50 mM HEPES (pH 8.5). 2 µL of 0.5 mg / mL Trypsin was added to each sample. Samples were incubated at 37 ˚C for 12 h. The beads were pelleted via centrifugation and washed water (200 µL), and 50% acetonitrile / water (200 µL). The supernatant and washes were collected as part of the "trypsin fraction." The beads were then dissolved in 2% formic acid / water (pH 2, 200 µL) for 30 min at 24 ˚C to hydrolyze the cleavable biotin probe **S1**. These samples were centrifuged and the supernatant collected as the "cleavage fraction." Both the cleavage fractions were concentrated to dryness using a SpeedVac heated to 45 ˚C. The cleavage fraction samples were desalted using a C18 Ziptip according to the previously described procedure with elution in 0.1% formic acid/75% acetonitrile/water.[6] The elutions were concentrated to dryness using a SpeedVac and resuspended in 20 µL 0.1% formic acid/water.

Mass Spectrometry Procedures.

Mass spectrometry procedures. The desalted samples were resuspended in 0.1% formic acid/water (20 µL). The sample (10.0 µL) was loaded onto a C18 trap column (3 cm, 3 µm particle size C10 Dr. Maisch 150 µm I.D) and then separated on an analytical column (Thermo Scientific Acclaim PepMap 100, 2 µm particle size, 250 mm length, 75 µm internal diameter) at 150 nL/min with a Thermo Scientific Ultimate 3000 system connected in line to a Thermo Scientific Orbitrap Fusion Tribrid. The column temperature was maintained at 50 ˚C. The tryptic peptides were separated via a step-wise gradient from 5% to 98% of 0.1% formic acid/acetonitrile over 120 min (0–1 min, 0–5%; 1–91 min, 5–27%; 91–115 min, 27–98%; 115–120 min, 98%–0%). The cleavage peptides were separated via the same gradient described above. Survey scans of peptide precursors were performed at 120K FWHM resolution (*m/z* = 200). Tandem MS was performed on the most abundant precursors exhibiting a charge state from 2 to 6 at a resolving power settings of 50K. HCD fragmentation was applied with 35% collision energy and resulting fragments accumulated for up to 100 ms.

Data Analysis Procedures.

Quantitative analysis of photolabeled SK-N-SH proteome. Analysis was performed in Thermo Scientific Proteome Discoverer version 2.3. HCD spectra with a signal-to-noise ratio greater than 1.5 were searched against a database containing the Uniprot 2016 annotated human proteome (Swissprot) and contaminant proteins using Sequest HT with a mass tolerance of 10 ppm for the precursor and 0.02 Da for fragment ions with specific trypsin digestion, 2 missed cleavages, variable oxidation on methionine residues (+15.995 Da), static carboxyamidomethylation of cysteine residues (+57.021 Da), and static TMT labeling at lysine residues and N-termini. Assignments were validated using Percolator. The resulting peptide spectral matches (PSMs) were filtered to include medium and high confidence matches, and TMT reporter ions were quantified using the Reporter Ions Quantifier. PSMs were filtered based on if a PSM is in only one protein group with an Isolation Interference under 70%. Empty abundances were filled in with minimum noise level computed by taking the minimum for each channel in Control and minimum for each channel in Treatment. 2000 centroids were generated at random from the absolute max in Control and Treatment and the absolute min in Control and Treatment, and a minimum noise level was generated using a K-means clustering method. If one abundance was missing, then the instance was filled with the geometric mean of the PSM for Control or Treatment. If all abundances were missing for Control and Treatment or the variance between existing abundances was above 30%, the PSM was removed. Any empty abundance missing completely at random, missing not at random, or missing at random any valid instances were filled with the appropriate method described above. P-values for enrichment ratios were calculated using the t-test (background) method.

Binding site analysis. Data analysis was performed with Proteome Discoverer version 2.3 using SEQUEST HT, allowing for variable modifications (methionine oxidation: +15.995 Da; cysteine carbamidomethylation: +57.021 Da; asparagine/glutamine deamidation: +0.984; and JN compound masses from **Table S6**), up to two missed cleavages and a mass tolerance of 10 ppm for the precursor ion and 0.02 Da for fragment ions from HCD. Searching was performed against the Swiss-Prot human database and a contaminant protein database. For binding sites of JN probes, MS/MS data from the cleavage fraction were searched by SEQUEST HT against the Uniprot 2016 annotated human proteome (Swissprot) and contaminant proteins. The high and medium confidence

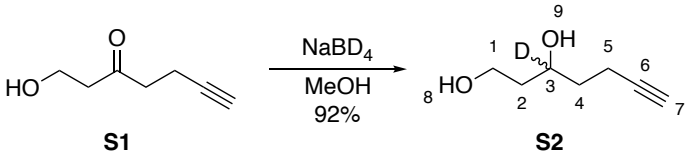
peptide assignments (false discovery rate < 5%) were analyzed using IsoStamp for the precursor isotope pattern and filtered based on manual validation.

Table S6. List of probes and modification masses

Probe	Treatment Group	JNXX_CBPA_si0	JNXX_CBPA_si2
JN12	1	451.1856	453.1923
JN5	1	492.2234	494.2301
JN4	1	592.3162	594.3229
JN3	1	502.2696	504.2759
JN1	1	372.1910	374.1977
JN22	2	494.2278	496.2345
JN21	2	452.1808	454.1875
JN20	2	451.1856	453.1923
JN17	2	465.2012	467.2079
JN13	2	465.2012	467.2079
JN24	3	518.2754	520.2821
JN33	3	479.2169	481.2236
JN32	3	479.1805	481.1872
JN28	3	494.2278	496.2345
JN26	3	450.2379	452.2446
JN835	4	546.2703	548.2770
JN248	4	563.2605	565.2672
JN247	4	474.2016	476.2083
JN245	4	537.2336	539.2403
JN38	4	648.3999	650.4066
JN936	5	465.1873	467.1940
JN935	5	492.2234	494.2301
JN847	5	448.2084	450.2151
JN846	5	464.2033	466.2100
JN845	5	449.1924	451.1991
JN836	5	565.2649	567.2716
JN945	6	449.2175	451.2242
JN942	6	493.2186	495.2253
JN940	6	464.2284	466.2351
JN939	6	608.3111	610.3178
JN938	6	580.3162	582.3229
JN849	6	522.2743	524.2810

Synthetic Procedures:

Ketone **S1** and amine **S6** were prepared according to Li et al.[5] Trifluoromethyl diazirine **S8** was synthesized following procedures adapted from Nassal, M.[7] Difluoro ester **S10** was prepared according to Fustero et al.[8]

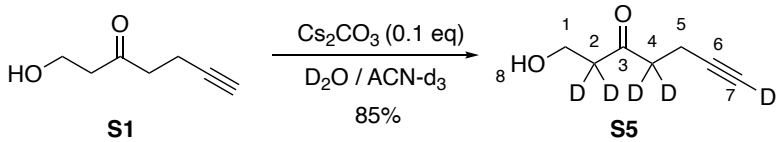


S1 **S2**

Synthesis of deuterated diol (S2):

Sodium borodeuteride (5.0 mg, 119 µmol, 1.50 equiv) was added to a solution of the ketone **S1** (9.9 mg, 79.0 µmol, 1 equiv) in MeOH (1.2 mL). The resulting solution was stirred at 24 ˚C for 30 min. The reaction was concentrated via N_2 flow, then diluted with water (1 mL) and extracted in situ with dichloromethane / *i*-PrOH (5 x 1 mL, 3:1 mixture). The organic layers were combined, washed with brine (1 mL) and dried over Na_2SO_4. The dried organic layer was filtered over a cotton plug and concentrated *in vacuo*. The crude material was purified via flash chromatography (0-5% MeOH in DCM) to yield **S2** (9.4 mg, 728 µmol, 93%) as a clear oil.

R_f = 0.3 (50% EtOAc in hexane, *p*-anisaldehyde stain) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.02 – 3.76 (m, 2H, H_1), 2.82 (s, 1H, O_8), 2.41 (s, 1H, O_9), 2.34 (tt, *J* = 6.8, 2.7 Hz, 2H, H_5), 1.98 (t, *J* = 2.7 Hz, 1H, H_7), 1.81 – 1.64 (m, 4H, H_2 and H_4). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 83.38 (s, C_6), 70.48 (t, *J* = 22.1 Hz, C_3), 67.71 (s, C_7), 61.61 (s, C_1), 38.64 (s, C_2), 34.97 (s, C_4), 14.80 (s, C_5). **IR** (ATR-FTIR) cm^{-1}: 3373.93, 3283.47, 2922.55, 1436.59, 1111.62, 1059.01. **HRMS-ESI** (*m/z*): $[M+H]^+$ calculated for $C_7H_{11}DO_2$ H^+ = 130.0973; found 130.0973



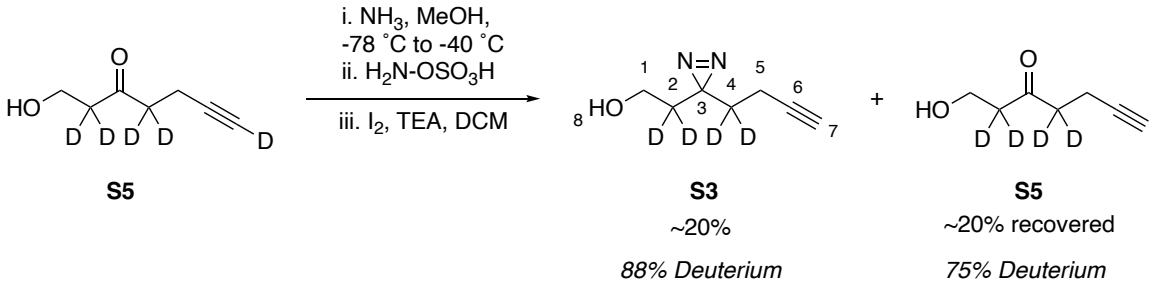
S1 **S5**

99% Deuterium

Synthesis of deuterated ketone (S5):

Cesium carbonate (24.0 mg, 74 µmol, 0.10 equiv) was added to a solution of the ketone **S1** (93.0 mg, 737 µmol, 1 equiv) in 20% acetonitrile-*d_3* in D_2O (2.9 mL). The solution was stirred at 24 ˚C under N_2 for 18 h. When the % deuterium was above 99% as determined by ^{1}H NMR, the reaction was extracted with dichloromethane (5 x 5 mL). The organic layers were combined, washed with brine (5 mL) and dried over Na_2SO_4. The dried organic layer was filtered over cotton and concentrated *in vacuo* to afford **S5** (82 mg, 628 µmol, 85%) as a clear oil.

R_f = 0.35 (50% EtOAc in hexane, *p*-anisaldehyde stain) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.86 (d, *J* = 7.9 Hz, 2H, H_1), 2.46 (s, 2H, H_5), 2.30 (t, *J* = 7.9 Hz, 1H, H_8). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 209.29 (s, C_3), 82.28 (t, *J* = 8.2 Hz, C_6), 68.73 (t, *J* = 38.0 Hz, C_7), 57.65 (s, C_1), 43.91 (t, *J* = 19.2 Hz, C_2), 41.14 (t, *J* = 20.0, 18.4 Hz, C_4), 12.62 (C_5). **IR** (ATR-FTIR) cm^{-1}: 3550.19, 2948.89, 2582.74, 1972.29, 1699.22, 1433.69, 1286.12, 1226.34, 1060.86, 1007.56. **HRMS-ESI** (*m/z*): $[M+H]^+$ calculated for $C_7H_5D_5O_2+H^+$: = 132.1067; found 132.1067



S5 **S3** **S5**

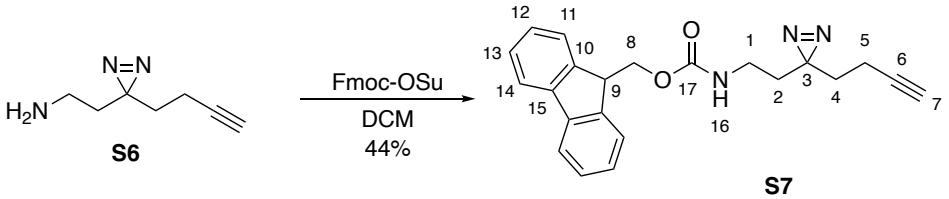
~20% ~20% recovered

88% Deuterium *75% Deuterium*

Synthesis of deuterated diazirine (S3):

A solution of deuterated ketone **S5** (55.0 mg, 420 µmol, 1 equiv) in MeOH (1.1 mL) was cooled to –78 ˚C under N_2 flow. Ammonia was condensed into the flask (~5 mL) and the reaction stirred for 30 min at –78 ˚C. The solution was warmed to –40 ˚C and stirred an additional 8 h. A solution of hydroxylamine-O-sulfonic acid (56.0 mg, 500 µmol, 1.20 equiv) in MeOH (500 µL) was added and the reaction warmed to 24 ˚C overnight with a bubbler to vent pressure. The reaction was concentrated *in vacuo* to afford a white film which was dissolved in dichloromethane / MeOH (0.1 M, 1:1 mixture) and cooled to 0 ˚C. Triethylamine (115 µL, 830 µmol, 1.50 equiv) was added followed by titration with a solution of I_2 in dichloromethane / MeOH (1 M, 2:1 mixture) until a brown color persists in solution. The reaction was stirred an additional 30 min, then quenched with sat sodium thiosulfate (1 mL). The solution was diluted with sat NH_4Cl (10 mL) and extracted with dichloromethane (3 x 10 mL). The organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude mixture was purified via flash chromatography to afford **S3** (12 mg, 80 µmol, 20%) as a clear oil and starting material **S5** without deuterium on the alkyne (11 mg, 80 µmol, 20%). Notably, the percentage of deuterium in product **S3** was 88% while the percentage in recovered starting material **S5** dropped to 75%.

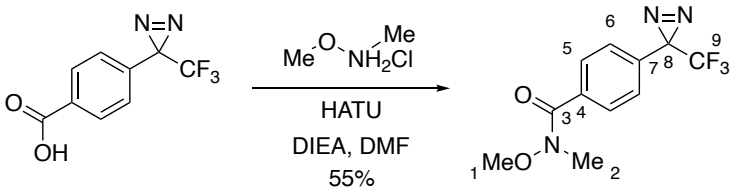
R_f = 0.6 (50% EtOAc in hexane, *p*-anisaldehyde stain). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.49 (d, *J* = 4.7 Hz, 2H, H_1), 2.03 (s, 2H, H_5), 2.00 (t, *J* = 2.7 Hz, 1H, H_7), 1.44 (t, *J* = 5.3 Hz, 1H, H_8). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 82.84 (s, C_6), 69.20 (s, C_7), 57.31 (s, C_1), 34.77 (t, *J* = 20.7 Hz, C_2), 32.27 (t, *J* = 20.5 Hz, C_3), 26.38 (s, C_3), 13.05 (s, C_5). **IR** (ATR-FTIR) cm^{-1}: 3294.79, 2921.87, 2888.40, 2587.17, 2214.59, 2118.45, 1694.02, 1584.84, 1470.99, 1433.20, 1378.32, 1314.45, 1046.86. **HRMS-ESI** (*m/z*): $[M+H]^+$ calculated for $C_7H_6D_4N_2O+H^+$ = 143.1117; found 143.1116



Synthesis of Fmoc diazirine (S7):

Fmoc-OSu (53.0 mg, 0.16 mmol, 1.20 equiv) was added to a solution of amine **S6** (18.0 mg, 0.13 mmol, 1 equiv) in DCM (1.6 mL). The reaction was stirred at 24 ˚C under N_2 for 2 hours. The reaction was diluted with sat. NH_4Cl (5 mL) and washed with dichloromethane (3 x 5 mL). The organic layers were combined, washed with brine (5 mL) and dried over Na_2SO_4 The dried organic layer was filtered through a cotton plug and concentrated *in vacuo*. The crude material was purified via column chromatography (0-30% EtOAc in hexane over 40 CV) to yield **S7** (25 mg, 70 µmol, 44%) as a clear oil.

R_f = 0.3 (20% EtOAc in hexane, UV) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H, H_{11}), 7.60 (d, *J* = 7.6 Hz, 2H, H_{14}), 7.41 (t, *J* = 7.5 Hz, 2H, H_{12}), 7.32 (t, *J* = 7.4 Hz, 2H, H_{13}), 4.85 (s, 1H, H_{16}), 4.41 (d, *J* = 7.0 Hz, 2H, H_8), 4.22 (t, *J* = 6.9 Hz, 1H, H_9), 3.06 (q, *J* = 6.5 Hz, 2H, H_1), 2.02 (td, *J* = 7.0, 2.1 Hz, 2H, H_5), 1.99 (t, *J* = 2.4 Hz, 1H, H_7), 1.70 (t, *J* = 6.7 Hz, 2H, H_2), 1.66 (t, *J* = 7.3 Hz, 2H, H_4). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.17 (s, C_{17}), 143.88 (s, C_{10}), 141.33 (s, C_{15}), 127.69 (s, C_{13}), 127.04 (s, C_{14}), 125.01 (s, C_{12}), 119.98 (s, C_{11}), 82.60 (s, C_6), 69.42 (s, C_7), 66.67 (s, C_8), 47.24 (s, C_1), 35.76 (s, C_2), 32.93 (s, C_4), 32.21 (s, C_9), 26.63 (s, C_3), 13.20 (s, C_5). **IR** (ATR-FTIR) cm^{-1}: 3295.13, 3064.69, 2943.49, 2117.31, 1698.29, 1520.51, 1472.58, 1443.92, 1370.08, 1321.89, 1241.86, 1137.97, 1021.76. **HRMS-ESI** (*m/z*): $[M+H]^+$ calculated for $C_{22}H_{21}N_3O_2+H^+$ = 360.1707; found 360.1708

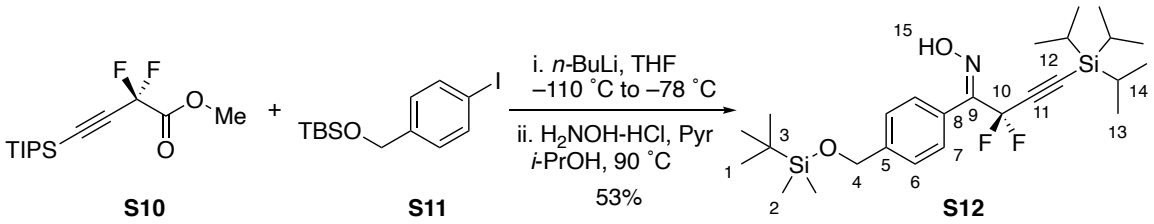


Synthesis of amide trifluoromethyl diazirine (S9):

(1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) (148 mg, 0.39 mmol, 1.50 equiv) diisopropylethylamine (68.0 µL, 0.39 mmol, 1.50 equiv) and Weinreb salt (38.0 mg, 0.39 mmol, 1.50 equiv) were added in sequence to a solution of the acid **S8** (60.0 mg, 0.26 mmol, 1 equiv) in DMF (1.3 mL). The reaction was stirred at 24 ˚C under Ar for 3 hours then concentrated *in vacuo*. The crude mixture was dissolved in dichloromethane (3 mL), washed with HCl (1 N, 3 mL),

sat. $NaHCO_3$ (1 mL) and brine (1 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude material was purified via column chromatography to afford diazirine **S9** (39 mg, 0.14 mmol, 55%).

R_f = 0.4 (20% EtOAc in hexane, UV). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.72 (d, 2H, H_6), 7.22 (d, 2H, H_5), 3.52 (s, 3H, H_1), 3.36 (s, 3H, H_2). **^{19}F NMR** (471 MHz, $CDCl_3$) δ -65.00 (s, C_9). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.56 (s, C_3), 135.26 (s, C_7), 131.38 (s, C_4), 128.70 (s, C_6), 126.04 (s, C_5), 121.96 (q, J = 274.9 Hz, C_9), 61.20 (s, C_1), 33.38 (s, C_2). **IR** (ATR-FTIR) cm^{-1}: 1643.41, 1418.99, 1378.66, 1285.49, 1150.80. **HRMS-ESI** (*m/z*): $[M+H]^+$ calculated for $C_{11}H_{10}F_3N_3O_2+H^+$ = 274.0798; found 274.0800

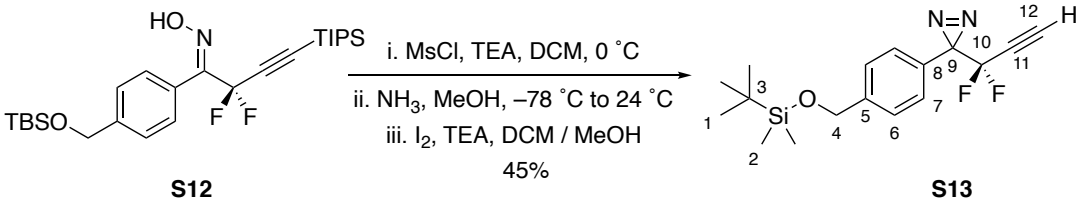


Synthesis of difluoro oxime (S12):

n-butyl lithium (2.03 M, 142 µL, 0.29 mmol, 1 equiv) in hexane was added to a solution of **S10** (84.0 mg, 0.29 mmol, 1 equiv) and **S11** (101 mg, 0.29 mmol, 1.00 equiv) in THF (5.8 mL) over 5 minutes, stirring at –110 ˚C under N_2. The reaction was warmed to –78 ˚C and stirred for 30 min. The reaction was quenched with sat. NH_4Cl (10 mL) and washed with ethyl acetate (EtOAc) (3 x 10 mL). The organic layers were combined, washed with brine (10 mL), dried over Na_2SO_4 and concentrated *in vacuo.* The crude oil was dissolved in *i*-PrOH (1.45 mL), H_2NOH-HCl (101 mg, 1.45 mmol, 5.00 equiv) and pyridine (233 µL, 299.4 mg, 2.9 mmol, 10.0 equiv) were added and the solution warmed to 90 ˚C stirring under N_2 for 2 h. The reaction was cooled to 0 ˚C, diluted with 1 N HCl and washed with dichloromethane. The organic layers were combined, washed with brine and dried over Na_2SO_4. The crude oil was purified via column chromatography (0-5% EtOAc in hexane over 30 CV) to yield 76 mg of **S12** (76 mg, 0.15 mmol, 53%) as an orange solid.

**Note: purification of the ketone from the initial Li-I exchange proved difficult due to very similar R_f between the ketone and both starting materials. The oxime formation could be performed on the crude product of the first reaction, and was much more amenable to purification.*

R_f = 0.4 (3% EtOAc in hexane, UV). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.26 (s, 1H, H_{15}), 7.45 (d, J = 8.3 Hz, 2H, H_7), 7.42 – 7.36 (m, 2H, H_6), 4.77 (s, 3H, H_4), 1.09 – 0.99 (m, 20H, H_{13}), 0.98 – 0.89 (m, 9H, H_1), 0.15 – 0.05 (m, 6H, H_2). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 152.77 (t, J = 29.9 Hz, C_9), 143.50 (s, C_5), 128.80 (s, C_6), 125.84 (s, C_7), 125.61 (s, C_8), 110.29 (t, J = 233.3 Hz, C_{10}), 96.74 (t, J = 37.5 Hz, C_{11}), 94.09 (t, C_{12}), 64.49 (s, C_4), 25.93 (s, C_1), 18.66 (s, C_2), 18.38 (s, C_{13}), 10.87 (s, C_{14}), -5.31 (s, C_2). **^{19}F NMR** (471 MHz, $CDCl_3$) δ -80.90 (s, F_{10}). **IR** (ATR-FTIR) cm^{-1}: 3276.67, 2945.83, 2928.29, 2892.67, 2865.44, 1702.95, 1686.50, 1625.42, 1613.35, 1462.74. **HRMS-ESI** (*m/z*): $[M+H]^+$ calculated for $C_{26}H_{43}F_2NO_2Si_2+H^+$ = 492.2873; found 496.2872



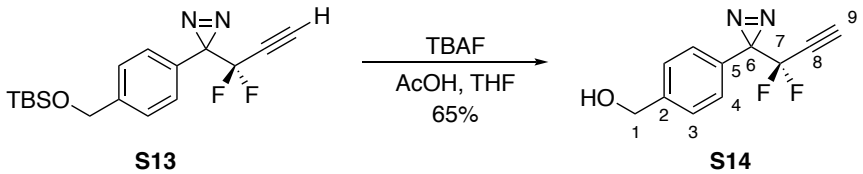
S12 **S13**

Synthesis of protected aryl difluoro diazirine (S13):

Mesyl chloride (9.84 µL, 14.6 mg, 0.127 mmol, 1.50 equiv) was added to a solution of **S12** (42.0 mg, 85.0 µmol, 1 equiv) in dichloromethane (850 µL) and triethylamine (22.5 µL, 17.2 mg, 0.169 mmol, 2.00 equiv) at 0 ˚C under N_2. The reaction was warmed to 24 ˚C over 2 hours, then diluted with sat NH_4Cl and washed with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to dryness *in vacuo.* The crude oil was redissolved in MeOH (1 mL) and cooled to –78 ˚C under N_2. Ammonia (~5 mL) was condensed into the reaction, which was allowed to warm to 24 ˚C gradually over 18 h with a light flow of N_2 and a bubbler to vent pressure. The reaction was concentrated *in vacuo,* then redissolved in dichloromethane and MeOH (2:1 mixture, 1.5 mL). Triethylamine (47.2 µL, 34.3 mg, 0.339 mmol, 4 equiv) was add to the reaction, stirring under N_2 at 0 ˚C. A solution of iodine in MeOH (1 M) was added dropwise to the reaction until a brown color persisted in solution. The reaction was stirred 30 min, then sat. sodium thiosulfate was added until the brown color of the reaction turned clear. The solution was diluted with sat NH_4Cl (3 mL) and washed with ethyl acetate (3 x 3 mL). The organic layers were combined, washed with brine (3 mL) and dried over Na_2SO_4. The crude oil was purified via column chromatography (0-10% EtOAc in hexane over 30 CV) to yield 14 mg of **S13** (14 mg, 0.040 mmol, 45%) as a yellow oil.

**Note: deprotection of the TIPS group was unintentional, and a small amount of the product with the TIPS still intact is formed and has a similar R_f to the protonated alkyne.*

R_f = 0.5 (5% EtOAc in hexane, UV). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.0 Hz, 2H, H_7), 7.24 (d, 2H, H_6), 4.72 (s, 2H, H_4), 2.94 (t, *J* = 5.1 Hz, 1H, H_{12}), 0.92 (s, 9H, H_1), 0.08 (s, 6H, H_2). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 142.91 (s, C_5), 129.03 (s, C_8), 127.01 (s, C_6), 126.06 (s, C_7), 110.47 (t, *J* = 241.0 Hz, C_{10}), 79.98 (t, *J* = 6.4 Hz, C_{12}), 73.97 (t, *J* = 40.6 Hz, C_{11}), 64.34 (s, C_4), 31.31 (t, *J* = 35.0 Hz, C_9), 25.91 (s, C_1), 17.98 (s, C_3), -5.31 (s, C_2). **^{19}F NMR** (471 MHz, $CDCl_3$) δ -82.47 (d, *J* = 4.8 Hz, F_{10}). **IR** (ATR-FTIR) cm^{-1}: 2953.98, 2929.23, 2895.80, 2858.01, 2137.55, 1462.44. **HRMS-ESI** (*m/z*): $[M+ NH_4]^+$ calculated for $C_{17}H_{26}F_2N_3OSi^+$ = 354.1808; found 354.1800



S13 **S14**

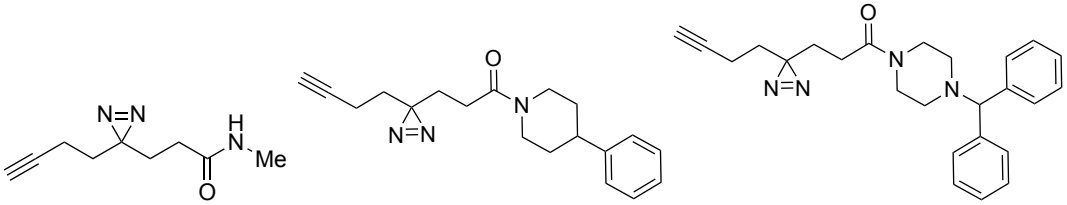
Synthesis of aryl difluoro diazirine (S14):

Tetrabutylammonium fluoride (1 M, 92.0 µL, 92.0 µmol, 2.20 equiv) was added to a solution of **S13** (14.0 mg, 0.42 mmol, 1 equiv) in tetrahydrofuran (830 µL) and acetic acid

(6 µL, 0.104 mmol, 2.50 equiv) at 0 ˚C under Ar. The reaction was warmed to 24 ˚C over 18 h, then diluted with water (1 mL) and extracted with ethyl acetate (3 x 1 mL). The combined organic layers were washed with brine (1 mL), dried over Na_2SO_4 and concentrated *in vacuo.* The crude oil was purified via column chromatography (20-50% EtOAc in hexane over 30 CV) to yield 6 mg of **S14** (6 mg, 0.027 mmol, 65%) as a clear oil.

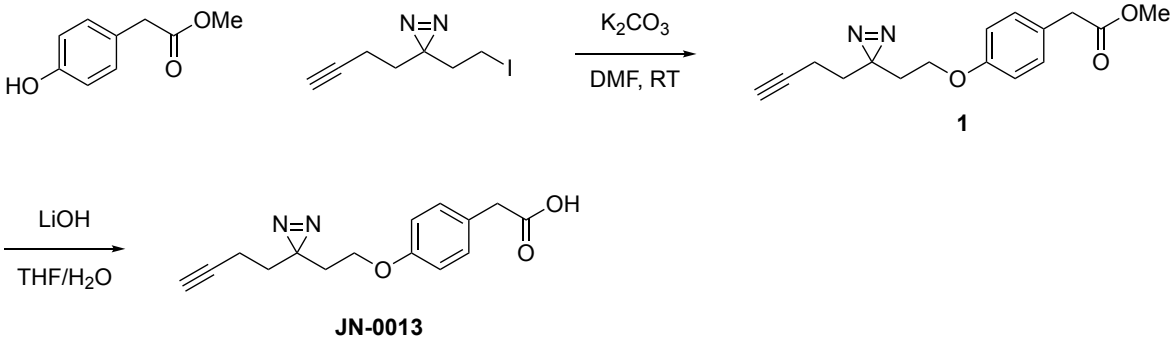
R_f = 0.3 (20% EtOAc in hexane, UV). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.41 – 7.31 (m, 2H, H_4), 7.26 (d, *J* = 8.6 Hz, 2H, H_3), 4.70 (s, 2H, H_1), 2.95 (t, *J* = 5.1 Hz, 1H, H_9). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 142.23 (s, C_5), 129.91 (s, C_2), 127.42 (s, C_4), 127.05 (s, C_3), 110.52 (t, *J* = 236.1 Hz, C_7), 80.19 (t, *J* = 6.4 Hz, C_9), 73.96 (t, *J* = 40.6 Hz, C_8), 64.72 (s, C_1), 31.35 (t, *J* = 34.8 Hz, C_6). **^{19}F NMR** (471 MHz, $CDCl_3$) δ -82.42 (d, *J* = 4.6 Hz, F_7). **IR** (ATR-FTIR) cm^{-1}: 3297.45, 2928.69, 2873.72, 2136.25, 1610.02 **HRMS-ESI** (*m/z*): $[M+H]^+$ calculated for $C_{11}H_8F_2N_2O_1+H^+$ = 221.0530; found 221.0532

Synthetic Procedures for PAL Probes:



Preparation of **JN-0001**, **JN-0003** and **JN-0004** was performed as reported in Parker et al.[2]

Synthesis of JN-0013



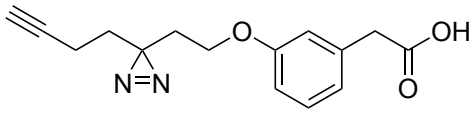
Step 1

3-but-3-ynyl-3-(2-iodoethyl)diazirine (26.9 mg, 108.3 µmol, 1.2 equiv) was added to a solution of Methyl 2-(4-hydroxyphenyl)acetate (15 mg, 90.3 µmol, 1.0 equiv) in dimethylformamide (DMF) (902.7 uL), and potassium carbonate (25.0 mg, 180.5 µmol, 2 equiv). The reaction mixture was stirred at 24 ˚C 12 h. The reaction mixture was filtered, diluted with DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure intermediate 1 as a white solid (26 mg, 23%).

Step 2

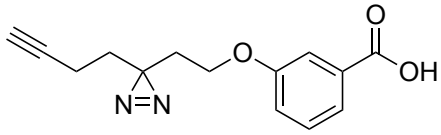
2M aq. LiOH (2.51 mg, 104.8 umol, 52 µL, 2.00 equiv) was added to a solution of intermediate 1 (6 mg, 21.0 µmol, 1 equiv) in a mixture of THF (293.4 µL) and water (125.7 µL). The reaction mixture was stirred at 24 ˚C overnight. 1M aq. HCl was slowly added and the resulting mixture was washed with dichloromethane (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-0013 as a white solid (3 mg, 50%). LC/MS (m/z): $[M+H]^+$ 273. ^1H NMR (400 MHz, DMSO-d_6) δ 7.16 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.0 Hz, 2H), 3.79 (t, J = 8.0 Hz, 2H), 3.48 (s, 2H), 2.84 (dd, J = 4.0, 4.0 Hz, 1H), 2.04 (td, J = 8.0, 4.0 Hz, 2H), 1.86 (t, J = 8.0 Hz, 2H), 1.65 (t, J = 8.0 Hz, 2H).

JN-0017, **JN-0020**, **JN-0021**, and **JN-0032** were prepared using the same procedure as **JN-0013**.



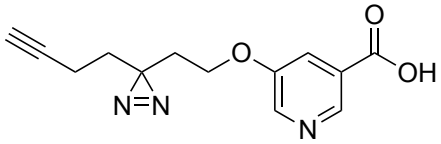
JN-0017

LC/MS (m/z): $[M+H]^+$ 273. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 6.89 (d, *J* = 7.8 Hz, 1H), 6.86 – 6.76 (m, 2H), 3.83 (t, *J* = 6.2 Hz, 2H), 3.64 (s, 2H), 2.11 – 2.02 (m, 2H), 1.99 (t, *J* = 2.7 Hz, 1H), 1.88 (t, *J* = 6.2 Hz, 2H), 1.74 (t, *J* = 7.5 Hz, 2H).



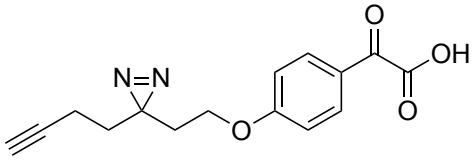
JN-0020

LC/MS (m/z): $[M+H]^+$ 259. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.44 – 7.37 (m, 2H), 7.17 (dd, *J* = 8.6, 2.6 Hz, 1H), 3.88 (t, *J* = 6.0 Hz, 2H), 2.84 (t, *J* = 2.6 Hz, 1H), 2.04 (td, *J* = 7.4, 2.7 Hz, 2H), 1.89 (t, *J* = 6.0 Hz, 2H), 1.67 (t, *J* = 7.4 Hz, 2H).



JN-0021

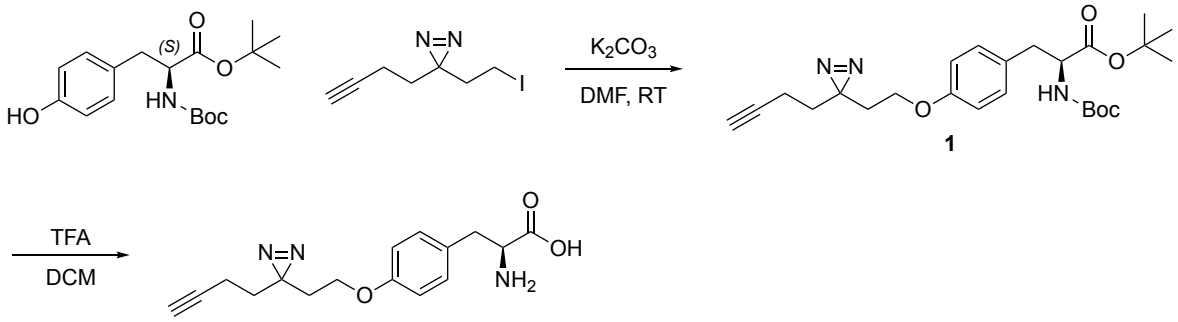
LC/MS (m/z): $[M+H]^+$ 260. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (d, *J* = 1.6 Hz, 1H), 8.46 (d, *J* = 2.9 Hz, 1H), 7.73 (dd, *J* = 3.0, 1.7 Hz, 1H), 3.99 (t, *J* = 6.1 Hz, 2H), 2.84 (t, *J* = 2.7 Hz, 1H), 2.04 (td, *J* = 7.3, 2.6 Hz, 2H), 1.91 (t, *J* = 6.1 Hz, 2H), 1.67 (t, *J* = 7.4 Hz, 2H).



JN-00032

LC/MS (m/z): $[M-H]^-$ 285. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (d, *J* = 8.0 Hz, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 3.96 (t, *J* = 6.1 Hz, 2H), 2.84 (t, *J* = 2.7 Hz, 1H), 2.04 (td, *J* = 7.3, 2.7 Hz, 2H), 1.91 (t, *J* = 6.0 Hz, 2H), 1.67 (t, *J* = 7.4 Hz, 2H).

Synthesis of JN-0022



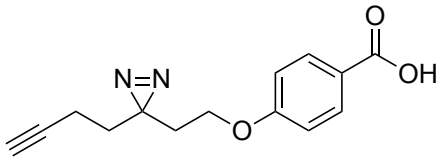
Step 1

3-but-3-ynyl-3-(2-iodoethyl)diazirine (12.1 mg, 48.9 µmol, 1.50 equiv) was added to a solution of tert-butyl (2S)-2-(tert-butoxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (11 mg, 32.6 µmol, 1 equiv) and potassium carbonate (6.76 mg 48.9 µmol, 1.50 equiv) in DMF (326 µL). The reaction mixture was stirred at 24 ˚C overnight. Water was added and the resulting mixture was washed with dichloromethane (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude intermediate 1 was used for next step without any further purification.

Step 2

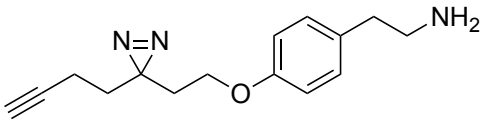
Crude intermediate 1 (15 mg, 32.78 µmol, 1 equiv) was dissolved in DCM (327.83 µL), then trifluoroacetic acid (TFA) (74.76 mg, 655.65 µmol, 50.51 µL, 20.4 equiv) was added and the reaction was stirred at 24 ˚C overnight. The reaction was concentrated under vacuum, then the crude was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-0022 as a white solid (2 mg, 15% yield over 2 steps) LC/MS (m/z): $[M+H]^+$ 302. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.17 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 3.94 (brs, 1H), 3.77 (t, J = 6.0 Hz, 2H), 3.07 (dd, J = 12.0 4.0 Hz, 1H), 2.85 (t, J = 2.5 Hz, 1H), 2.83 – 2.73 (m, 1H), 2.11 – 1.98 (m, 2H), 1.87 (t, J = 6.0 Hz, 2H), 1.67 (t, J = 7.3 Hz, 2H).

JN-0012, **JN-00026**, **JN-00028**, and **JN-00945** were prepared using the same procedure as JN-0022



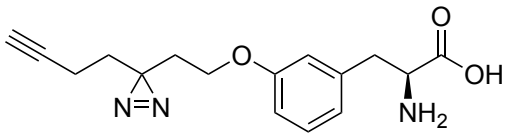
JN-0012

LC/MS (m/z): $[M+H]^+$ 259. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 8.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 3.90 (t, J = 6.1 Hz, 2H), 2.84 (t, J = 2.6 Hz, 1H), 2.04 (td, J = 7.3, 2.7 Hz, 2H), 1.90 (t, J = 6.1 Hz, 2H), 1.67 (t, J = 7.4 Hz, 2H).



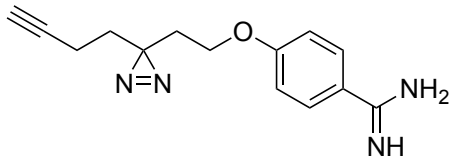
JN-00026

LC/MS (m/z): $[M+H]^+$ 258. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (brs, 1H), 7.15 (d, J = 8.2 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 3.78 (t, J = 6.0 Hz, 3H), 2.94 (t, J = 7.7 Hz, 2H), 2.84 (t, J = 2.7 Hz, 1H), 2.75 (t, J = 7.9 Hz, 2H), 2.03 (td, J = 7.4, 2.6 Hz, 2H), 1.87 (t, J = 6.0 Hz, 2H), 1.65 (t, J = 7.4 Hz, 2H).



JN-00028

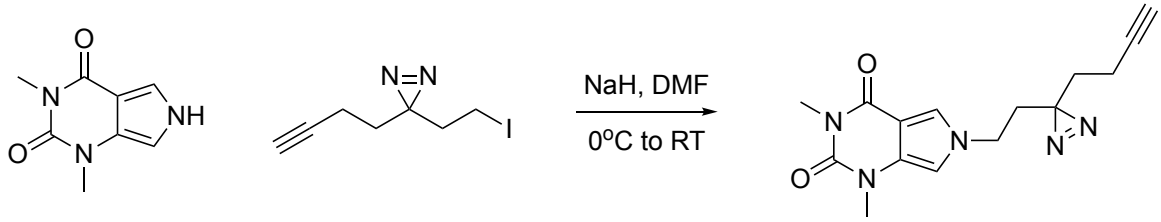
LC/MS (m/z): $[M+H]^+$ 302. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.19 (t, J = 7.9 Hz, 1H), 6.83 (d, J = 7.0 Hz, 2H), 6.79 – 6.73 (m, 1H), 3.79 (t, J = 6.1 Hz, 2H), 3.16 – 3.09 (m, 2H), 2.86 (t, J = 2.7 Hz, 1H), 2.78 (dd, J = 14.3, 8.6 Hz, 1H), 2.04 (td, J = 7.4, 2.7 Hz, 2H), 1.87 (t, J = 6.1 Hz, 2H), 1.66 (t, J = 7.4 Hz, 2H).



JN-00945

LC/MS (m/z): $[M+H]^+$ 257. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.80 (d, J = 8.8 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H), 3.94 (t, J = 6.1 Hz, 2H), 2.85 (t, J = 2.7 Hz, 1H), 2.04 (td, J = 7.4, 2.7 Hz, 2H), 1.92 (t, J = 6.0 Hz, 2H), 1.67 (t, J = 7.4 Hz, 2H).

Synthesis of JN-0005

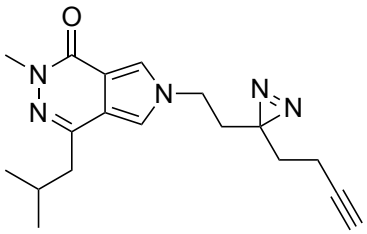


JN-0005

Sodium hydride (60% dispersion in mineral oil, 4.63 mg, 120.94 µmol, 1.50 equiv) was added to a solution of 1,3-dimethyl-6H-pyrrolo[3,4-d]pyrimidine-2,4-dione (21.67 mg, 120.94 µmol, 1.50 equiv) in dry dimethylformamide (806.25 µL) at 0 ˚C. The reaction mixture was stirred at 0 ˚C for 15 minutes, then 3-but-3-ynyl-3-(2-iodoethyl)diazirine (20 mg, 80.62 µmol, 1 equiv) was added and the reaction was slowly warmed to 24 ˚C and stirred until completion. 1M aq. HCl was added and the mixture was washed with dichloromethane (x3). The combined organic layers were dried over Na_2SO_4, filtered, and

concentrated under vacuum. The crude was then purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-0005 as a white solid (6mg, 25%) LC/MS (m/z): $[M+H]^+$ 300. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (s, 1H), 6.86 (s, 1H), 3.94 (t, *J* = 7.4 Hz, 2H), 3.26 (s, 3H), 3.19 (s, 3H), 2.85 (s, 1H), 1.98 (td, *J* = 7.0, 3.0 Hz, 2H), 1.95 – 1.85 (m, 2H), 1.59 – 1.49 (m, 2H).

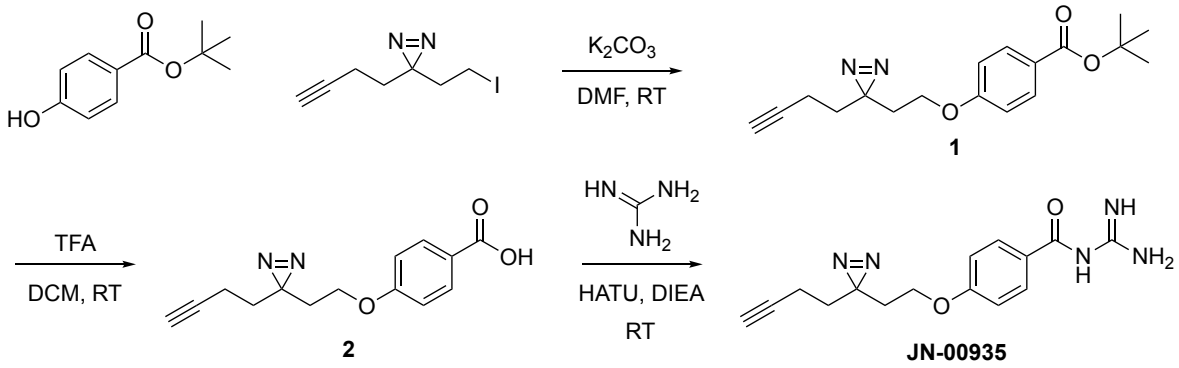
JN-00024 was prepared using the same procedure as JN-0005



JN-00024

LC/MS (m/z): $[M+H]^+$ 326. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (d, *J* = 2.0 Hz, 1H), 7.59 (d, *J* = 2.0 Hz, 1H), 4.11 (t, *J* = 7.2 Hz, 2H), 3.54 (s, 3H), 2.83 (t, *J* = 2.6 Hz, 1H), 2.10 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.96 (ddt, *J* = 7.5, 5.3, 2.4 Hz, 4H), 1.51 (t, *J* = 7.4 Hz, 2H), 0.91 (d, *J* = 6.6 Hz, 6H).

Synthesis of JN-00935



Step 1

3-but-3-ynyl-3-(2-iodoethyl)diazirine (38.32 mg, 154.46 µmol, 1.5 equiv) was added to a solution of tert-butyl 4-hydroxybenzoate (20 mg, 102.97 µmol, 1 equiv) in dimethylformamide (343.24 µL), and potassium carbonate (42.70 mg, 308.92 µmol, 18.64 µL, 3.00 equiv). The reaction mixture was stirred at 24 ˚C overnight. Brine was added and the resulting mixture was then washed with ethyl acetate (x3). The combined organic layers were dried on Na_2SO_4, filtered, and concentrated under vacuum. The crude was purified by flash column chromatography using heptane / ethyl acetate as eluent to give pure intermediate 1 as a white solid (24 mg, 74%)

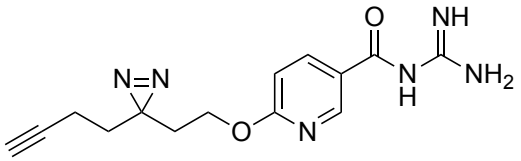
Step 2

Intermediate 1 (13 mg, 41.35 µmol, 1 equiv) was dissolved in dichloromethane (413.51 µL), then TFA (94.30 mg, 827.03 µmol, 63.71 µL, 20 equiv) was added and the reaction was stirred at 24 ˚C overnight. The reaction was then concentrated under vacuum and the crude product was azeotroped with toluene (x3) to remove the excess of TFA, and used for next step without any further purification.

Step 3

Intermediate 2 (3 mg, 11.62 µmol, 1 equiv) was dissolved in dimethylformamide (116.16 µL), then HATU (5.30 mg, 13.94 µmol, 1.20 equiv) was added followed by diisopropylethylamine (6.00 mg, 46.46 µmol, 8.09 µL, 4.00 equiv). The reaction was stirred at 24 ˚C for 10 min, then guanidine-HCl (2.22 mg, 23.23 µmol, 2.00 equiv) was added and the reaction mixture was stirred overnight. The reaction mixture was then purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00935 as a white solid (3mg, 70% yield over two steps) LC/MS (m/z): $[M+H]^+$ 300. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 2H), 8.01 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.8 Hz, 2H), 3.86 (t, *J* = 6.1 Hz, 3H), 2.85 (t, *J* = 2.6 Hz, 1H), 2.05 (td, *J* = 7.4, 2.8 Hz, 2H), 1.89 (t, *J* = 6.1 Hz, 2H), 1.67 (t, *J* = 7.4 Hz, 2H).

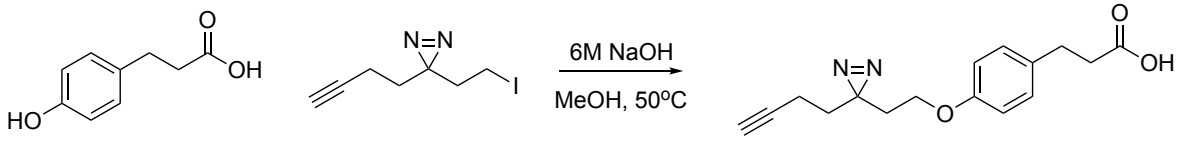
JN-00942 was prepared using same procedure as JN-00935.



JN-00942

LC/MS (m/z): $[M+H]^+$ 301**.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 2.5 Hz, 1H), 8.14 (s, 1H), 7.96 (dd, *J* = 9.5, 2.5 Hz, 1H), 6.55 (s, 1H), 6.37 (d, *J* = 9.5 Hz, 1H), 3.90 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 2.7 Hz, 1H), 2.02 (td, *J* = 7.4, 2.7 Hz, 2H), 1.73 (t, *J* = 7.5 Hz, 2H), 1.62 (t, *J* = 7.4 Hz, 2H).

Synthesis of JN-00033

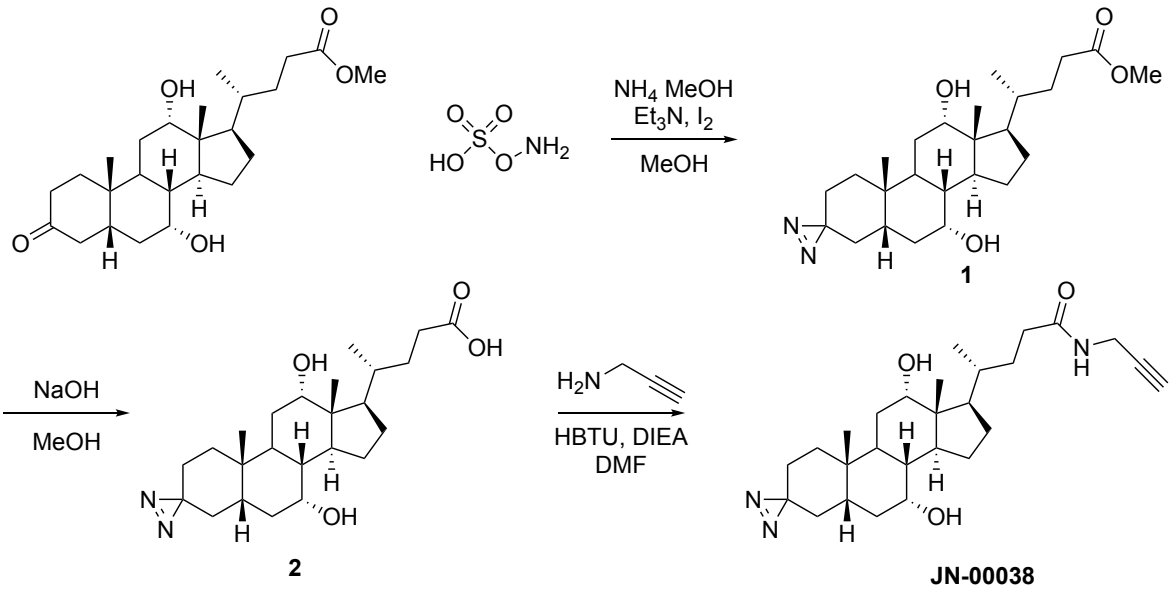


JN-00033

NaOH 6M (18.05 mg, 451.34 µmol, 75uL, 3.00 equiv) was added to a solution of 3-(4-hydroxyphenyl)propanoic acid (25 mg, 150.45 µmol, 1 equiv) in methanol (300.89 µL) followed by 3-but-3-ynyl-3-(2-iodoethyl)diazirine (55.98 mg, 225.67 µmol, 1.50 equiv). The reaction was then stirred at 50 ˚C overnight. 1M aq. HCl was added and the resulting acidic mixture was washed with ethyl acetate (x2). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude product was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00033 as a white solid (10mg, 23%

yield). LC/MS (m/z): $[M+H]^+$ 287. ^1H NMR (400 MHz, DMSO-d_6) δ 7.12 (d, J = 8.3 Hz, 2H), 6.82 (d, J = 8.4 Hz, 2H), 3.77 (t, J = 6.1 Hz, 2H), 2.84 (t, J = 2.6 Hz, 1H), 2.73 (t, J = 7.6 Hz, 2H), 2.45 (t, J = 7.6 Hz, 2H), 2.03 (td, J = 7.4, 2.6 Hz, 2H), 1.85 (t, J = 6.1 Hz, 2H), 1.65 (t, J = 7.4 Hz, 2H).

Synthesis of JN-00038



Step 1

Methyl (4R)-4-[(5R,7R,8R,10S,12S,13R,14S,17R)-7,12-dihydroxy-10,13-dimethyl-3-oxo-1,2,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]pentanoate (100 mg, 237.77 µmol, 1 equiv) was dissolved in ammonia 7.0 M in methanol (237.77 µmol, 1.25 mL, 1.00 equiv) at -20 ˚C and the resulting reaction mixture was stirred for 1 hour. The temperature was then raised to 24 ˚C and the reaction was stirred for additional 2 hours. The temperature was then lowered to -15 ˚C and amino hydrogen sulfate (32.5 mg, 287.37 µmol, 1.200 equiv - 6.5mg x 5 times were added every 30 minutes). When the addition was completed the reaction was warmed to 24 ˚C and stirred for additional 14 hours. The reaction was concentrated under reduced pressure, and the residue was dissolved in methanol (1.25 mL). Triethylamine (40.90 mg, 404.20 µmol, 56.34 µL, 1.50 equiv) was then added at 0 ˚C followed by Iodine (108.62 mg, 427.98 µmol, 1.80 equiv) portion wise. The resulting mixture was then warmed to 24 ˚C and stirred for additional 30 minutes. Saturated aq. $Na_2S_2O_3$ solution was added to quench the excess of Iodine, then the mixture was washed with ethyl acetate (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude product was purified by flash column chromatography using heptane / ethyl acetate as eluent to give pure intermediate 1 (68mg, 66%).

Step 2

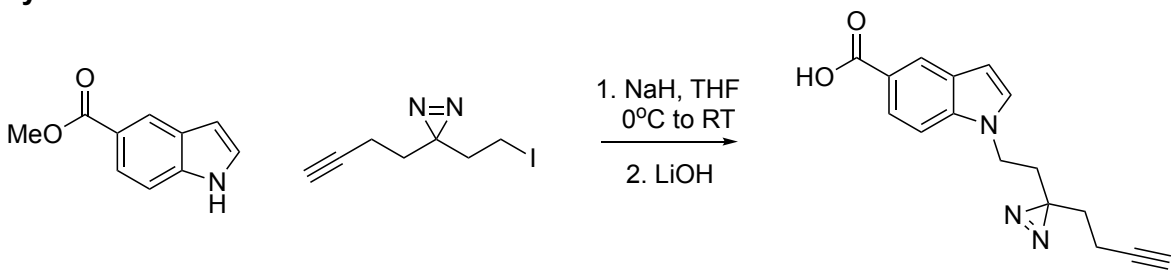
Intermediate 1 (68 mg, 157.19 µmol, 1 equiv) was suspended in methanol (1.57 mL) and NaOH (62.88 mg, 1.57 mmol, 10 equiv) and the reaction was stirred at 24 ˚C overnight. The reaction mixture was then acidified with 1M aq. HCl and washed with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated

under vacuum. The crude product was used for next step without any further purification (49 mg, 75%).

Step 3

Intermediate 2 (49 mg, 117.07 µmol, 1 equiv) was dissolved in dimethylformamide (1.17 mL), then HBTU (66.59 mg, 175.60 µmol, 1.50 equiv), prop-2-yn-1-amine (19.34 mg, 351.20 µmol, 22.49 µL, 3.00 equiv.) and diisopropylethylamine (45.39 mg, 351.20 µmol, 61.17 µL, 3.00 equiv) were added and the reaction was stirred at 24 ˚C for 18 h. Water was then added and the resulting mixture was washed with EtOAc (x3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude was then purified by flash column chromatography using DCM/DCM:MeOH 9:1 to give pure JN-00038 as a white solid (32mg, 61%) LC/MS (m/z): $[M+H]^+$ 456. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.68 (s, 1H), 4.05 (ddd, *J* = 5.3, 2.6, 0.8 Hz, 2H), 4.02 (dd, *J* = 2.8 Hz, 1H), 3.88 – 3.85 (m, 1H), 2.92 (dd, *J* = 15.0, 13.4 Hz, 1H), 2.32 – 2.08 (m, 4H), 1.98 – 1.86 (m, 4H), 1.85 – 1.53 (m, 10H), 1.52 – 1.10 (m, 7H), 1.00 - 0.98 (m, 5H), 0.72 (s, 3H), 0.32 – 0.16 (m, 2H).

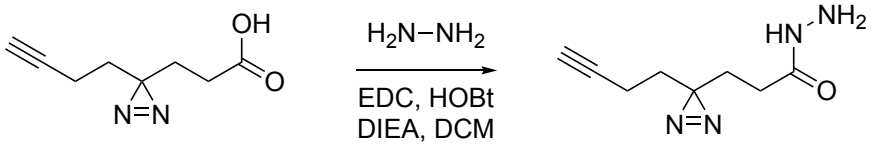
Synthesis of JN-00247



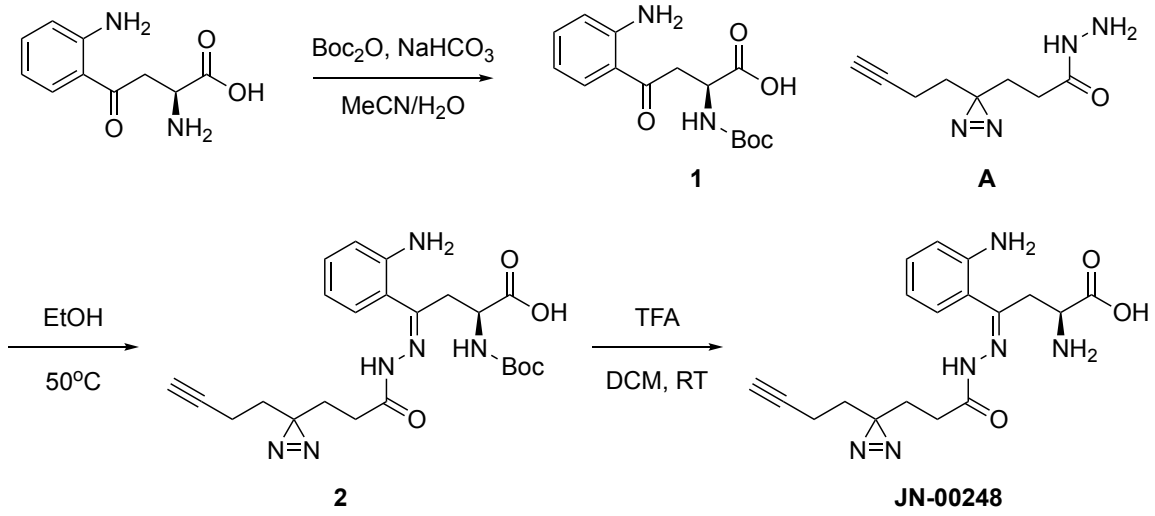
JN-00247

Sodium hydride (3.94 mg, 102.75 µmol, 60% dispersion in mineral oil, 1.20 equiv) was added to a solution of methyl 1H-indole-5-carboxylate (15 mg, 85.62 µmol, 1 equiv) in dry THF (856.24 µL) at 0 ˚C , and the reaction was stirred for 30 minutes. 3-but-3-ynyl-3-(2-iodoethyl)diazirine (25.49 mg, 102.75 µmol, 1.20 equiv) was then added and the reaction was slowly warmed to RT and stirred for 48 hours. Some ester hydrolysis was detected along with the desired product. 2M aq. LiOH (428.12 µmol, 0.214 mL) was added and the reaction was stirred at 50 ˚C for 18 h. 1M aq. HCl was added until the pH was 2-3, then the mixture was washed with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude product was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00247 as a white solid (6.7mg, 28%). LC/MS (m/z): $[M+H]^+$ 282. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, *J* = 1.5 Hz, 1H), 7.75 (dd, *J* = 8.7, 1.7 Hz, 1H), 7.54 (dd, *J* = 6.0, 2.8 Hz, 2H), 6.61 (d, *J* = 3.1 Hz, 1H), 4.15 (t, *J* = 7.2 Hz, 2H), 2.83 (t, *J* = 2.6 Hz, 1H), 1.90 (td, *J* = 7.3, 4.8 Hz, 4H), 1.49 (t, *J* = 7.3 Hz, 2H).

Synthesis of JN-00248



A



Synthesis of A

Hydrazine (19.28 mg, 601.77 µmol, 18.91 µL, 2.00 equiv), diisopropylethylamine (38.89 mg, 300.88 µmol, 52.41 µL, 1.00 equiv), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (86.52 mg, 451.33 µmol, 1.50 equiv), and HOBt (60.98 mg, 451.33 µmol, 1.50 equiv) were added in sequence to a solution of 3-[3-azidopropyl(methyl)amino]-6,6-dimethyl-5,7-dihydro-2H-indol-4-one (50 mg, 300.88 µmol, 1 equiv) in dichloromethane (5.01 mL), and the reaction was stirred at 24 ˚C for 12 h. The reaction was diluted with dichloromethane then washed with saturated aq. NH_4Cl and brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude intermediate A was used for next step without any further purification (54 mg, 74%). LC/MS (m/z): $[M+H]^+$ 181.

Step 1

(2S)-2-amino-4-(2-aminophenyl)-4-oxo-butanoic acid (200 mg, 960.55 µmol, 1 equiv) and Di-tert-butyl dicarbonate (440.24 mg, 2.02 mmol, 462.92 µL, 2.20 equiv) were dissolved in acetonitrile (2 mL) and water (4 mL). Sodium bicarbonate (338.91 mg, 4.03 mmol 4.00 equiv) was added and the reaction mixture was stirred at 24 ˚C for 60 hours. 5-10% of bis-protected product was observed along with the desired product. The reaction was acidified with 1 N HCl and washed with EtOAc (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude intermediate 1 was used for next step without any further purification (270 mg, 91%).

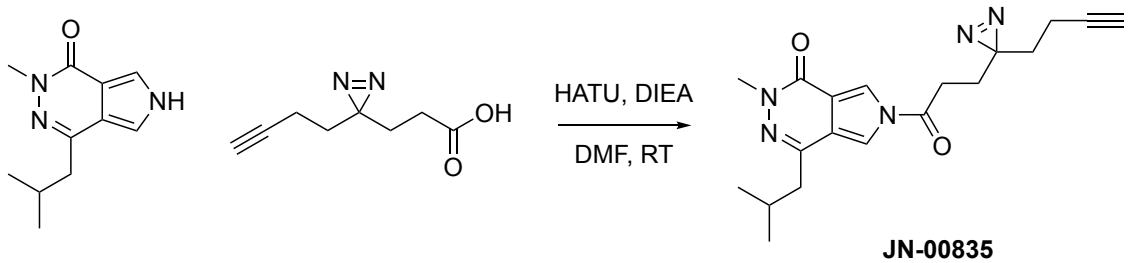
Step 2

Crude intermediate 1 (15 mg, 48.65 µmol, 1 equiv) and intermediate A (10.52 mg, 58.38 µmol, 1.2 equiv) were dissolved in ethanol (243.25 µL), and the reaction mixture was stirred at 50 ˚C 12 h. The solvent was then evaporated and the crude was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in Acetonitrile as eluent to give pure intermediate 2 as a white solid (7 mg, 31%).

Step 3

Intermediate 2 (7 mg, 14.88 µmol) was dissolved in DCM (148.77 µL) then TFA (33.93 mg, 297.54 µmol, 22.92 µL) was added and the reaction was stirred at 24 ˚C. The reaction was concentrated *in vacuo* then azeotroped with Toluene (x3) to remove the excess TFA. The crude material was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00248 as a light yellow solid (2 mg, 26%). LC/MS (m/z): $[M+H]^+$ 371. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, *J* = 8.1 Hz, 1H), 7.27 (s, 2H), 7.03 (t, *J* = 7.7 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 6.52 (t, *J* = 7.4 Hz, 1H), 2.89 (d, *J* = 14.5 Hz, 1H), 2.84 (t, *J* = 2.6 Hz, 1H), 2.11 (t, *J* = 7.8 Hz, 2H), 2.02 (td, *J* = 7.3, 2.8 Hz, 3H), 1.76 (t, *J* = 7.7 Hz, 2H), 1.62 (t, *J* = 7.3 Hz, 3H).

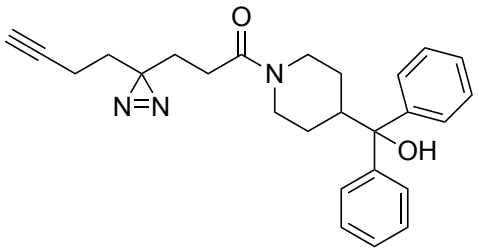
Synthesis of JN-00835



JN-00835

3-(3-but-3-ynyldiazirin-3-yl)propanoic acid (18.22 mg, 109.62 µmol, 1.50 equiv) and HATU (41.68 mg, 109.62 µmol, 1.50 equiv) were dissolved in dimethylformamide (365.40 µL) then diisopropylethylamine (23.61 mg, 182.70 µmol, 31.82 µL) was added and the reaction was stirred at 24 ˚C for 5-10 minutes. 1-isobutyl-3-methyl-6H-pyrrolo[3,4-d]pyridazin-4-one (15 mg, 73.08 µmol, 1 equiv) was then added and the reaction was stirred at 24 ˚C for 10 minutes. The reaction mixture was then diluted with DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00835 as a white solid (11.5 mg, 45%). LC/MS (m/z): $[M+H]^+$ 354. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, *J* = 4.8 Hz, 1H), 8.15 (d, *J* = 4.5 Hz, 1H), 3.55 (s, 3H), 3.17 (brs, 2H), 2.89 – 2.80 (m, 1H), 2.59 – 2.57 (brm, 2H), 2.16 – 1.95 (m, 4H), 1.83 – 1.77 (brm, 2H), 1.71 – 1.65 (brm, 2H), 0.97 – 0.90 (brm, 6H).

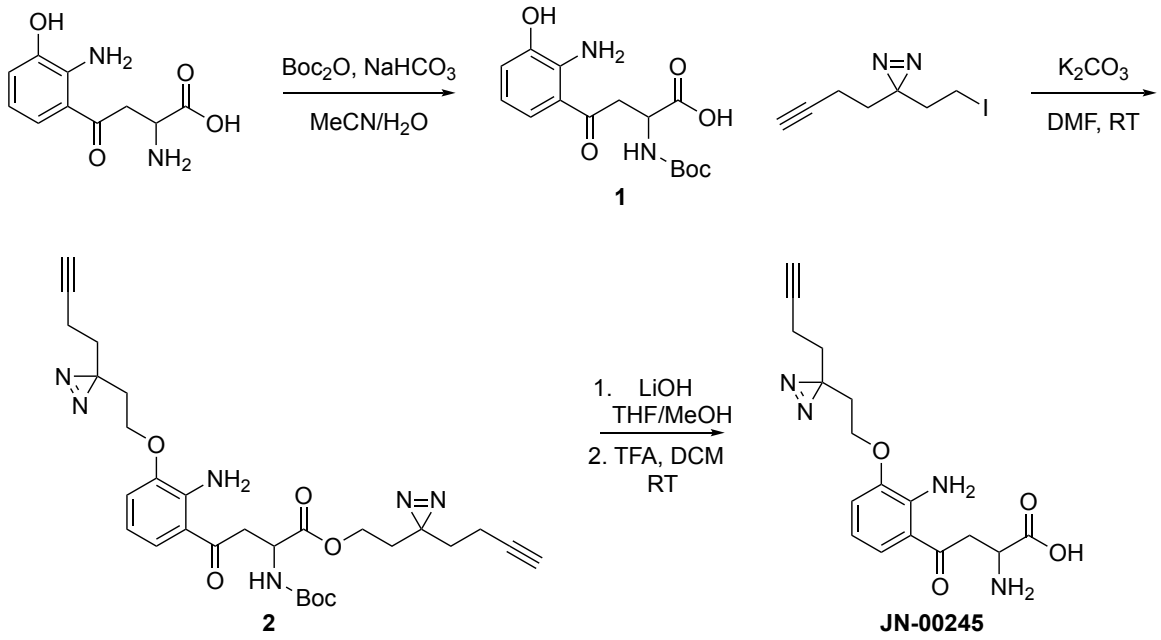
JN-00939 was prepared using same procedure as JN-00835



JN-00939

LC/MS (m/z): $[M+H]^+$ 416. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (dd, *J* = 7.7, 3.7 Hz, 4H), 7.27 (t, *J* = 7.6 Hz, 4H), 7.14 (t, *J* = 7.3 Hz, 2H), 5.32 (s, 1H), 4.39 (d, *J* = 12.8 Hz, 1H), 3.81 (d, *J* = 13.1 Hz, 1H), 3.02 – 2.89 (m, 1H), 2.85 – 2.73 (m, 2H), 2.10 (t, *J* = 7.6 Hz, 2H), 1.98 (td, *J* = 7.4, 2.7 Hz, 2H), 1.59 (q, *J* = 7.7 Hz, 4H), 1.39 – 1.18 (m, 4H).

Synthesis of JN-00245



Step 1

2-amino-4-(2-amino-3-hydroxy-phenyl)-4-oxo-butanoic acid (25 mg, 111.50 µmol, 1 equiv) and Di-tert-butyl dicarbonate (51.10 mg, 234.15 µmol, 53.74 µL, 2.00 equiv) were dissolved in a mixture of acetonitrile (0.5 mL) and water (0.5 mL), then sodium bicarbonate (39.34 mg, 468.31 µmol, 4.00 equiv) was added and the reaction mixture was stirred at RT for 3 hours. Some bis-Boc protected product was observed along with the desired product. 1M aq. HCl was added and the mixture was washed with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude intermediate 1 was used for next step without any further purification (10 mg, 28%).

Step 2

Intermediate 1 (10 mg, 30.83 µmol, 1 equiv) was dissolved in DMF (308.33 µL), then potassium carbonate (21.31 mg, 154.16 µmol, 5.00 equiv) was added followed by 3-but-3-ynyl-3-(2-iodoethyl)diazirine (19.12 mg, 77.08 µmol, 2.20 equiv). The reaction mixture was stirred at 24 ˚C overnight and the main product was the bis-propargylated derivative. Saturated aq. NaHCO3 solution was then added and the mixture was extracted with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude intermediate 2 was used for next step without any further purification.

Step 3

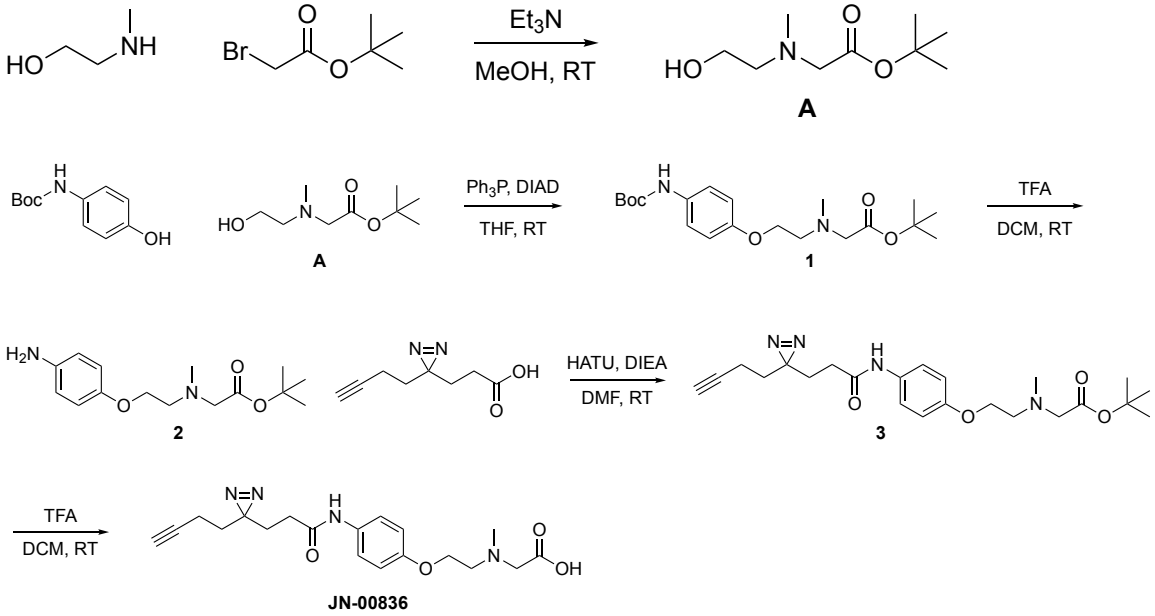
Intermediate 2 (17.5 mg, 30.99 µmol, 1 equiv) was dissolved in THF (247.95 µL) and methanol (61.99 µL), then 2M aq. LiOH (3.71 mg, 154.97 µmol, 78uL, 5.00 equiv) was added and the reaction mixture was stirred at 24 ˚C for 30 min. 1M aq. HCl was added and the mixture was washed with EtOAc (x3). The combined organic layers were dried

over Na_2SO_4, filtered, and concentrated under vacuum. The crude intermediate was used for next step without any further purification.

Step 4

The above crude intermediate (13.8 mg, 31.05 µmol, 1 equiv) was dissolved in dichloromethane, then TFA (70.80 mg, 620.95 µmol, 47.84 µL, 20 equiv) was added and the reaction was stirred at 24 ˚C for 1 h. The reaction was concentrated *in vacuo* and the crude product was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00245 as a white solid (2 mg, 15% yield over 4 steps). LC/MS (m/z): $[M+H]^+$ 345. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (d, *J* = 8.3 Hz, 1H), 6.91 (d, *J* = 7.7 Hz, 1H), 6.78 (s, 2H), 6.47 (t, *J* = 7.9 Hz, 1H), 3.79 (t, *J* = 6.0 Hz, 2H), 2.78 (t, *J* = 2.7 Hz, 1H), 1.97 (td, *J* = 7.3, 2.6 Hz, 2H), 1.88 (t, *J* = 6.1 Hz, 2H), 1.61 (t, *J* = 7.3 Hz, 2H).

Synthesis of JN-00836



Synthesis of Intermediate A

2-(methylamino)ethanol (88.57 mg, 1.18 mmol, 94.32 µL 1.10 equiv), tert-butyl 2-bromoacetate (200 mg, 1.03 mmol, 150.38 µL, 1.00 equiv), were added to a solution of triethylamine (103.76 mg, 1.03 mmol, 142.91 µL, 1.00 equiv) in methanol (2.05 mL), and stirred at 24 ˚C for 12 h. The concentrated *in vacuo* then the crude product was dissolved in water and mixture washed with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography using DCM / MeOH as eluent to give intermediate A as a white solid (134 mg, 69%). LC/MS (m/z): $[M+H]^+$ 190. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.40 (brs, 1H), 3.47 – 3.44 (brm, 2H), 3.20 (brs, 2H), 2.61 – 2.54 (m, 2H), 2.31 (s, 3H), 1.42 (s, 9H).

Step 1

tert-butyl N-(4-hydroxyphenyl)carbamate (25 mg, 119.48 µmol, 1 equiv) and Intermediate A (29.40 mg, 155.32 µmol, 1.40 equiv) were dissolved in dry THF (1.19 mL), then Ph_3P (47.01 mg, 179.22 µmol, 1.5 equiv) was added and the resulting solution was brought to 0 ˚C before diisopropyl azodicarboxylate (36.24 mg, 179.22 µmol, 35.18 µL) was added dropwise. The reaction was slowly warmed to 24 ˚C for 12 h. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography using heptane / EtOAc as eluent to give the pure intermediate 1 as a white solid (40 mg, 88%).

Step 2

TFA (119.87 mg, 1.05 mmol, 80.99 µL, 10 equiv) was added to a solution of intermediate 1 (40 mg, 105.13 µmol, 1 equiv) in dichloromethane (1.05 mL) and the reaction stirred at 24 ˚C for 6 hours. The solvent was removed *in vacuo* and the crude product was azeotroped with toluene (x3) to remove the excess of TFA. Intermediate 2 was used for next step without any further purification.

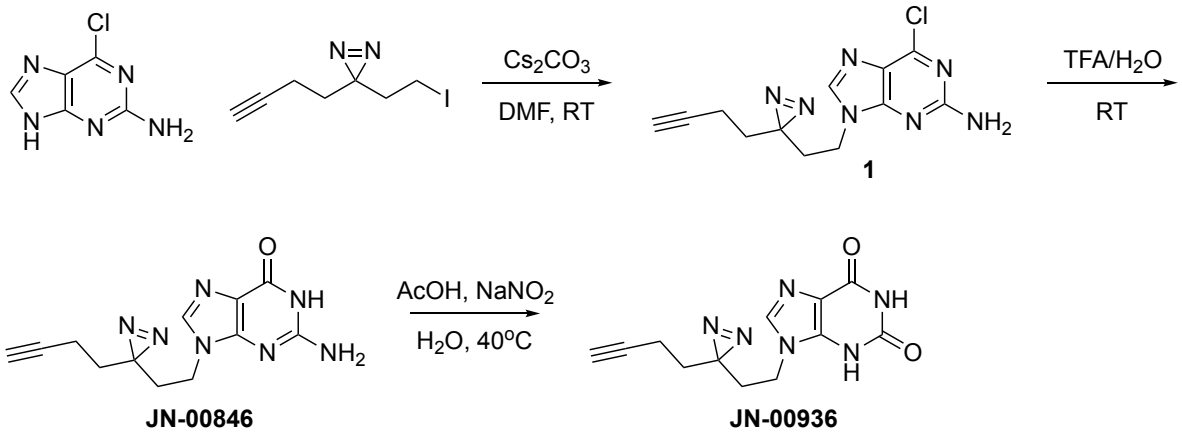
Step 3

3-(3-but-3-ynyldiazirin-3-yl)propanoic acid (9.48 mg, 57.05 µmol, 1.50 equiv) was added to a solution of HATU (20.25 mg, 53.25 µmol, 1.40 equiv) and diisopropylethylamine (24.58 mg, 190.17 µmol, 33.12 µL, 5.00 equiv) in DMF (190.17 µL), and the reaction was stirred at 24 ˚C for 10 min. The above crude intermediate 2 (15 mg, 38.03 µmol, TFA, 1 equiv) was then added and the reaction was complete in 5 min. The reaction mixture was purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure intermediate 3 as a white solid (6 mg, 40%).

Step 4

TFA (31.93 mg, 280.03 µmol, 21.57 µL, 20 equiv) was added to a solution of intermediate 3 (6 mg, 14.00 µmol, 1 equiv) in DCM (140.02 µL), and the reaction was stirred at 24 ˚C for 24 hours. The solvent was then removed *in vacuo*, and the crude azeotroped with toluene (x3) to remove the excess of TFA and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give JN-00836 as a white solid (1.1 mg, 20%). LC/MS (m/z): $[M+H]^+$ 373. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 7.46 (d, J = 8.1 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 4.06 – 4.02 (brm, 2H), 3.24 – 3.21 (brm, 2H), 3.00 - 2.92 (brm, 2H), 2.86 – 2.84 (brs, 1H), 2.43 (s, 3H), 2.11 – 2.07 (brm, 2H), 2.02 – 1.99 (brm, 2H), 1.77 – 1.72 (brm, 2H), 1.63 – 1.58 (brm, 2H).

Synthesis of JN-00846 and JN-00936



Step 1

Cesium carbonate (57.64 mg, 176.92 µmol, 1.50 equiv) was added to a solution of 6-chloro-9H-purin-2-amine (20 mg, 117.94 µmol, 1 equiv) in DMF (589.72 µL) followed by 3-but-3-ynyl-3-(2-iodoethyl)diazirine (35.11 mg, 141.53 µmol, 1.2 equiv). The reaction mixture was stirred at 24 ˚C for 12 h. The reaction was diluted with brine and the mixture was washed with DCM (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude intermediate 1 was used for next step without any further purification.

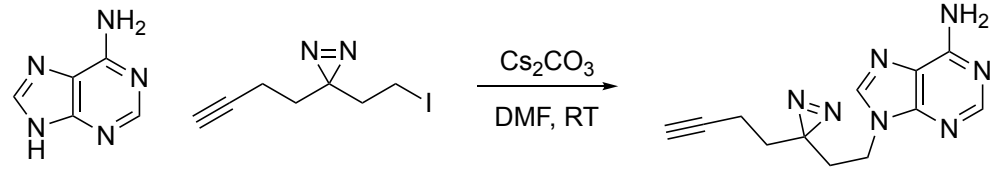
Step 2

Intermediate 1 (34 mg, 117.35 µmol, 1 equiv) was dissolved in a mixture of TFA (880.15 µL) and water (293.38 µL), and the reaction was stirred at 24 ˚C for 48 hours. The solvents were evaporated *in vacuo*, then the crude azeotroped with toluene (x3) to remove excess of TFA and water. The obtained solid was then washed with diethyl ether to give pure JN-00846 as a white precipitate that was filtered and dried under vacuum (12 mg, 38%). LC/MS (m/z): $[M+H]^+$ 272. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.22 (s, 1H), 6.71 (s, 2H), 3.93 (t, *J* = 7.3 Hz, 2H), 2.85 (dt, *J* = 4.6, 2.6 Hz, 1H), 1.99 (td, *J* = 7.3, 2.6 Hz, 2H), 1.93 (t, *J* = 7.3 Hz, 2H), 1.62 (t, *J* = 7.1 Hz, 3H).

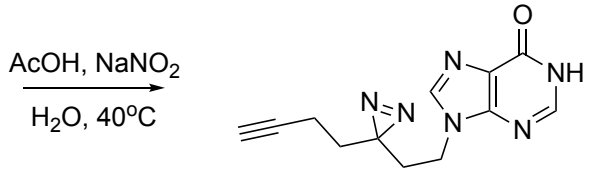
Synthesis of JN-00936

JN-00846 (20 mg, 73.73 µmol, 1 equiv) was resuspended in water (1.84 mL), then acetic acid (66.41 mg, 1.11 mmol, 63.25 µL, 15 eq) was added followed by Sodium Nitrite (45.78 mg, 663.53 µmol). The reaction mixture was stirred at 40 ˚C 12 h. Brine was added and the resulting mixture was washed with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to to give pure JN-00936 as a white solid (5 mg, 25%). LC/MS (m/z): $[M+H]^+$ 273. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 7.70 (s, 1H), 3.95 (t, *J* = 7.5 Hz, 2H), 2.85 (t, *J* = 2.7 Hz, 1H), 1.98 (td, *J* = 7.4, 2.6 Hz, 2H), 1.82 (dd, *J* = 8.2, 6.8 Hz, 2H), 1.62 (t, *J* = 7.4 Hz, 2H).

Synthesis of JN-00847 and JN-00845



JN-00847

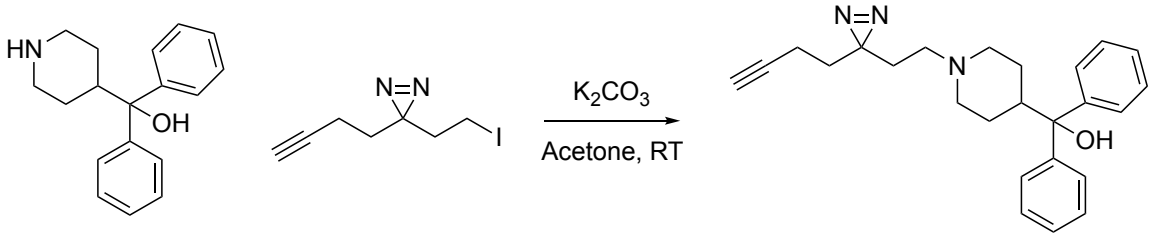


JN-00845

Cesium carbonate (54.25 mg, 166.51 µmol. 1.50 equiv) was added to a solution of 9H-purin-6-amine (15 mg, 111.01 µmol, 1 equiv) in DMF (222.01 µL), followed by 3-but-3-ynyl-3-(2-iodoethyl)diazirine (33.04 mg, 133.21 µmol, 1.20 equiv), then the reaction mixture was stirred at 24 ˚C 12 h. Water was added and the resulting mixture was washed with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was treated with DCM to give pure JN-00847 as a white solid (10 mg, 35%). LC/MS (m/z): $[M+H]^+$ 256. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 8.13 (s, 1H), 7.22 (s, 1H), 4.10 (t, *J* = 7.1 Hz, 2H), 2.83 (t, *J* = 2.7 Hz, 1H), 1.97 (td, *J* = 7.3, 2.9 Hz, 4H), 1.59 (d, *J* = 14.8 Hz, 2H).

Crude JN-00847 (22 mg, 86.18 µmol) was resuspended in water (2.15 mL), then sodium nitrite (53.51 mg, 775.63 µmol, 24.66 µL) was added, followed by acetic acid (77.63 mg, 1.29 mmol, 73.93 µL). The reaction mixture was stirred at 40 ˚C 12. The reaction mixture was concentrated *in vacuo*, then the crude was azeotroped with toluene (x3) to remove excess of water and acetic acid. The crude was then dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00845 as a white solid (8 mg, 36%). LC/MS (m/z): $[M+H]^+$ 257. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 8.04 (s, 1H), 4.11 (t, *J* = 7.2 Hz, 2H), 2.84 (t, *J* = 2.7 Hz, 1H), 2.01 – 1.91 (m, 4H), 1.59 (t, *J* = 7.4 Hz, 2H).

Synthesis of JN-00938

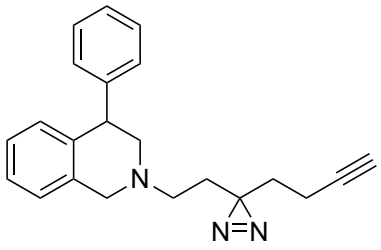


JN-00938

Potassium carbonate (11.63 mg, 84.15 µmol, 5.08 µL, 1.50 equiv) was added to a solution of diphenyl(4-piperidyl)methanol (15 mg, 56.10 µmol, 1 equiv) in acetone (561.03 µL), followed by 3-but-3-ynyl-3-(2-iodoethyl)diazirine (16.70 mg, 67.32 µmol, 1.20 equiv). The reaction was then stirred at 24 ˚C 12 h. The reaction was concentrated *in vacuo* and the

crude was dissolved in DMSO, filtered and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00938 as a white solid (9 mg, 37%). LC/MS (m/z): $[M+H]^+$ 388. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (d, *J* = 7.5 Hz, 4H), 7.26 (t, *J* = 7.6 Hz, 4H), 7.12 (t, *J* = 7.3 Hz, 2H), 2.83 – 2.77 (m, 3H), 2.06 (t, *J* = 7.5 Hz, 2H), 1.99 (td, *J* = 7.4, 2.7 Hz, 2H), 1.84 (t, *J* = 11.5 Hz, 2H), 1.60 – 1.39 (m, 6H), 1.26 – 1.15 (m, 2H).

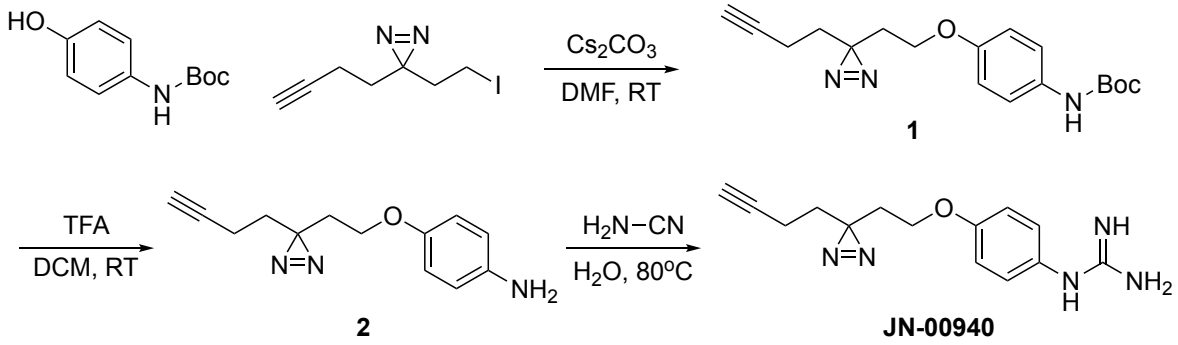
JN-00849 was prepared using the same procedure as JN-00938



JN-00849

LC/MS (m/z): $[M+H]^+$ 330. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.28 (m, 1H), 7.25 – 7.17 (m, 3H), 7.16 – 7.03 (m, 3H), 6.87 (d, *J* = 7.5 Hz, 1H), 4.29 – 4.17 (m, 1H), 3.79 – 3.61 (m, 2H), 3.00 (ddd, *J* = 11.4, 5.4, 1.2 Hz, 1H), 2.62 (dd, *J* = 11.5, 8.0 Hz, 1H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.00 – 1.91 (m, 3H), 1.68 – 1.55 (m, 4H).

Synthesis of JN-00940



Step 1

Cesium carbonate (58.39 mg, 179.22 µmol, 1.50 equiv) was added to a solution of tert-butyl N-(4-hydroxyphenyl)carbamate (25 mg, 119.48 µmol, 1 equiv) d in DMF (1.19 mL), followed by 3-but-3-ynyl-3-(2-iodoethyl)diazirine (35.57 mg, 143.37 µmol, 1.20 equiv). The resulting mixture was stirred at 24 ˚C 12 h. Brine was added and the mixture was washed with diethyl ether (x3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude was purified by flash column chromatography using heptane / EtOAc as eluent to give pure intermediate 1 as a white solid (15 mg, 38%).

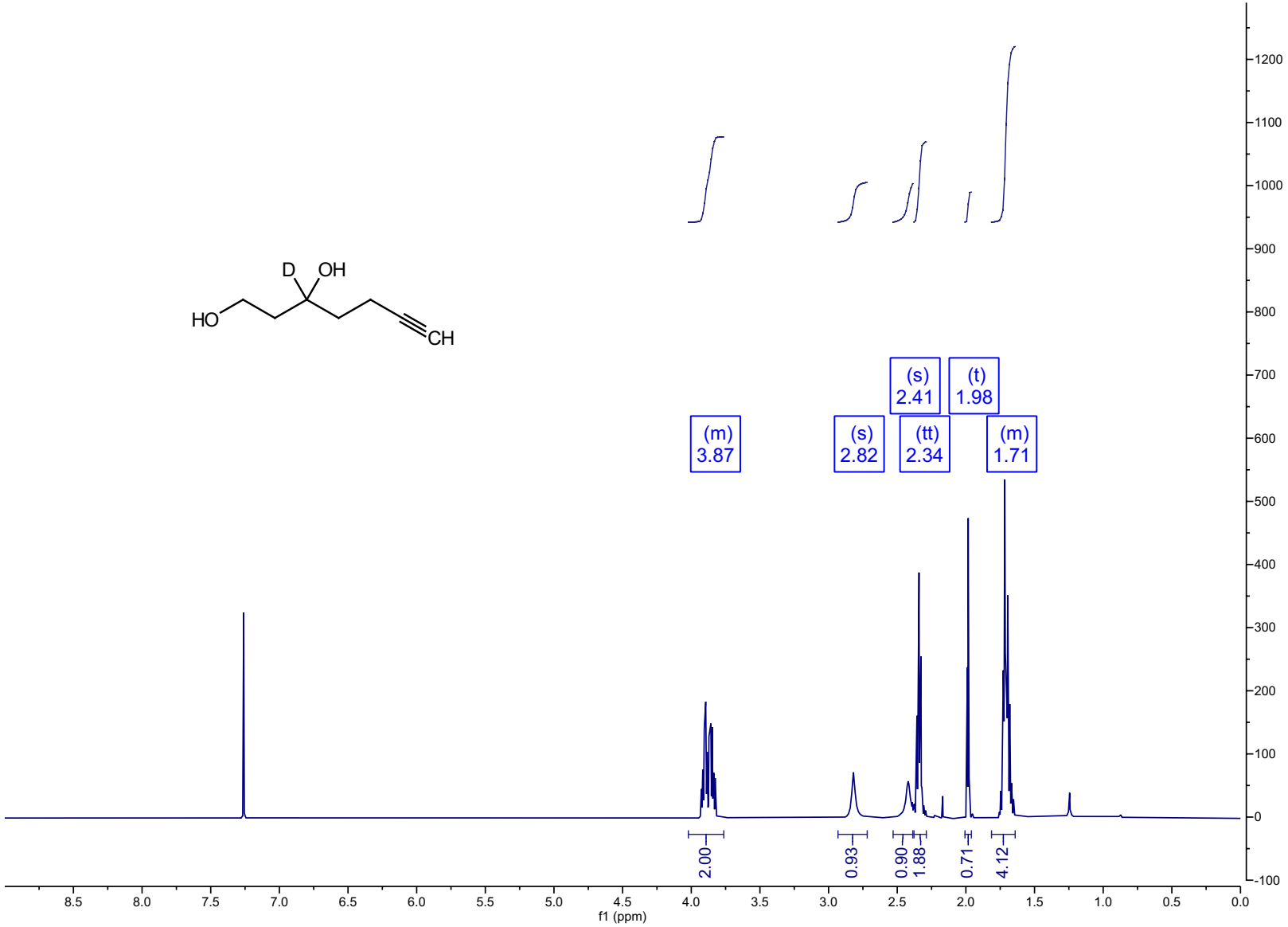
Step 2

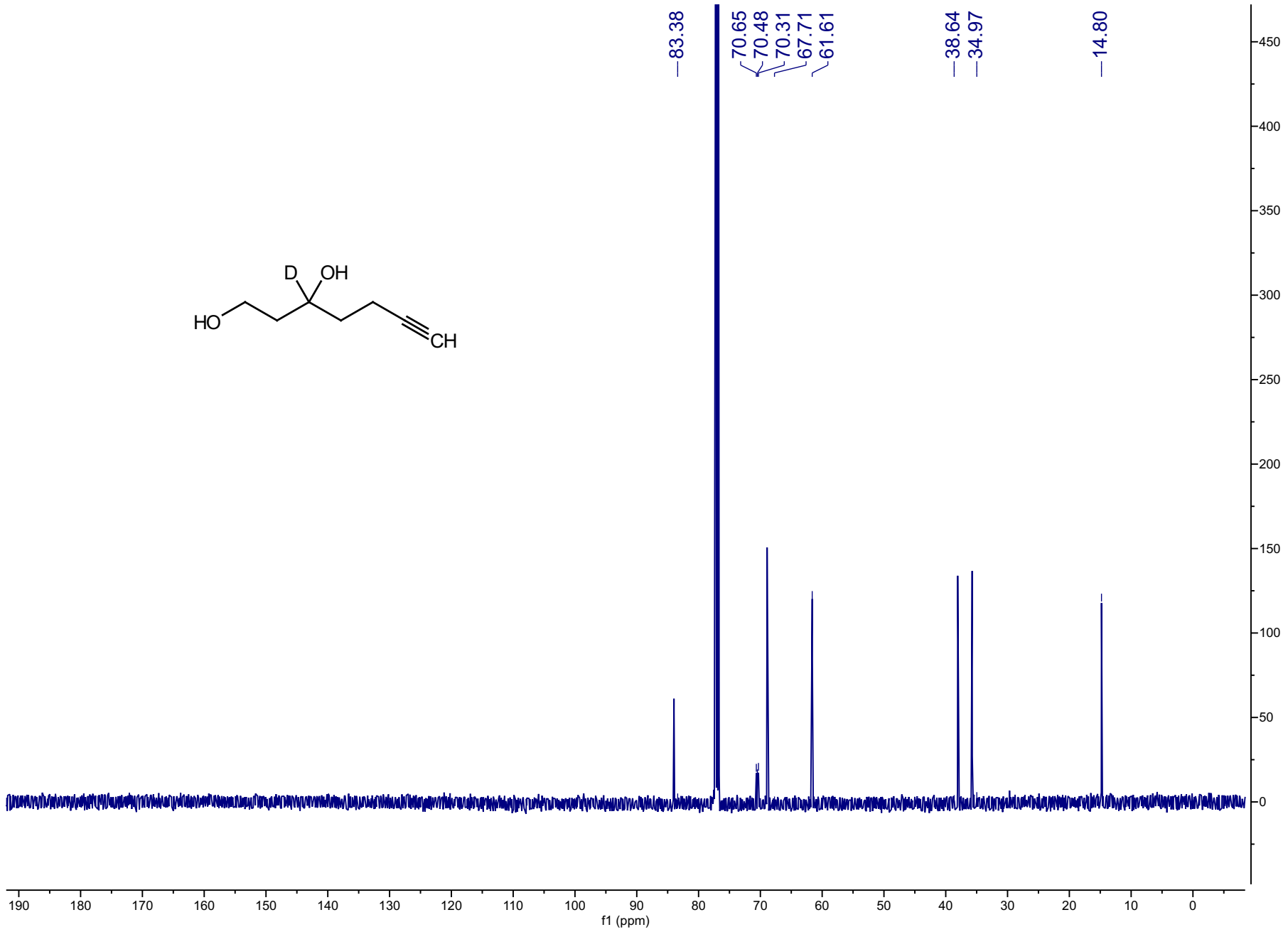
Intermediate 1 (15 mg, 45.54 µmol, 1 equiv) was dissolved in DCM (455.38 µL), then TFA (103.85 mg, 910.77 µmol, 70.17 µL, 20.0 equiv) was added and the reaction was stirred at 24 ˚C for 12 h. The reaction was concentrated *in vacuo*, then the crude was azeotroped with toluene (x3) to remove the excess of TFA. The crude intermediate 2 was used for next step without any further purification.

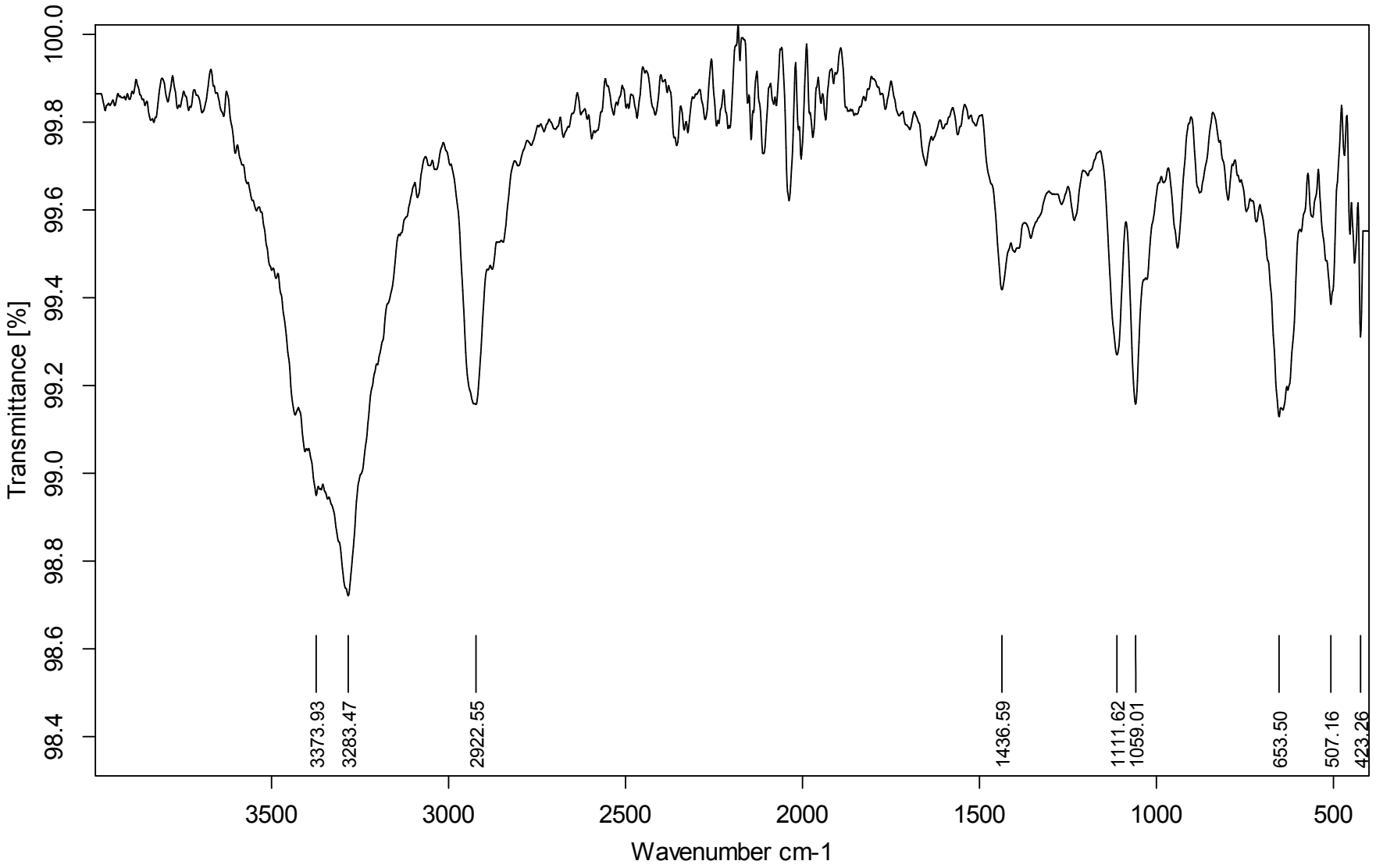
Step 3

Intermediate 2 (11 mg, 47.98 µmol) was dissolved in water (479.77 µL), then cyanamide (10.08 mg, 239.88 µmol, 7.88 µL) was added and the reaction mixture was stirred at 80 ˚C overnight. The reaction was concentrated *in vacuo,* then the crude product was dissolved in DMSO and purified by prep HPLC using 0.1% formic acid in water / 0.1% formic acid in acetonitrile as eluent to give pure JN-00940 as a white solid (3 mg, 20%). LC/MS (m/z): $[M+H]^+$ 272. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.55 (s, 4H), 7.12 (d, *J* = 9.2 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 3.80 (t, *J* = 6.0 Hz, 2H), 2.85 (t, *J* = 2.7 Hz, 1H), 2.04 (td, *J* = 7.4, 2.6 Hz, 2H), 1.89 (t, *J* = 6.0 Hz, 2H), 1.66 (t, *J* = 7.4 Hz, 2H).

Catalog of nuclear magnetic resonance and infrared spectra for synthesized compounds:




83.38
70.65
70.48
70.31
67.71
61.61
38.64
34.97
14.80
D
OH
HO
CH
f1 (ppm)



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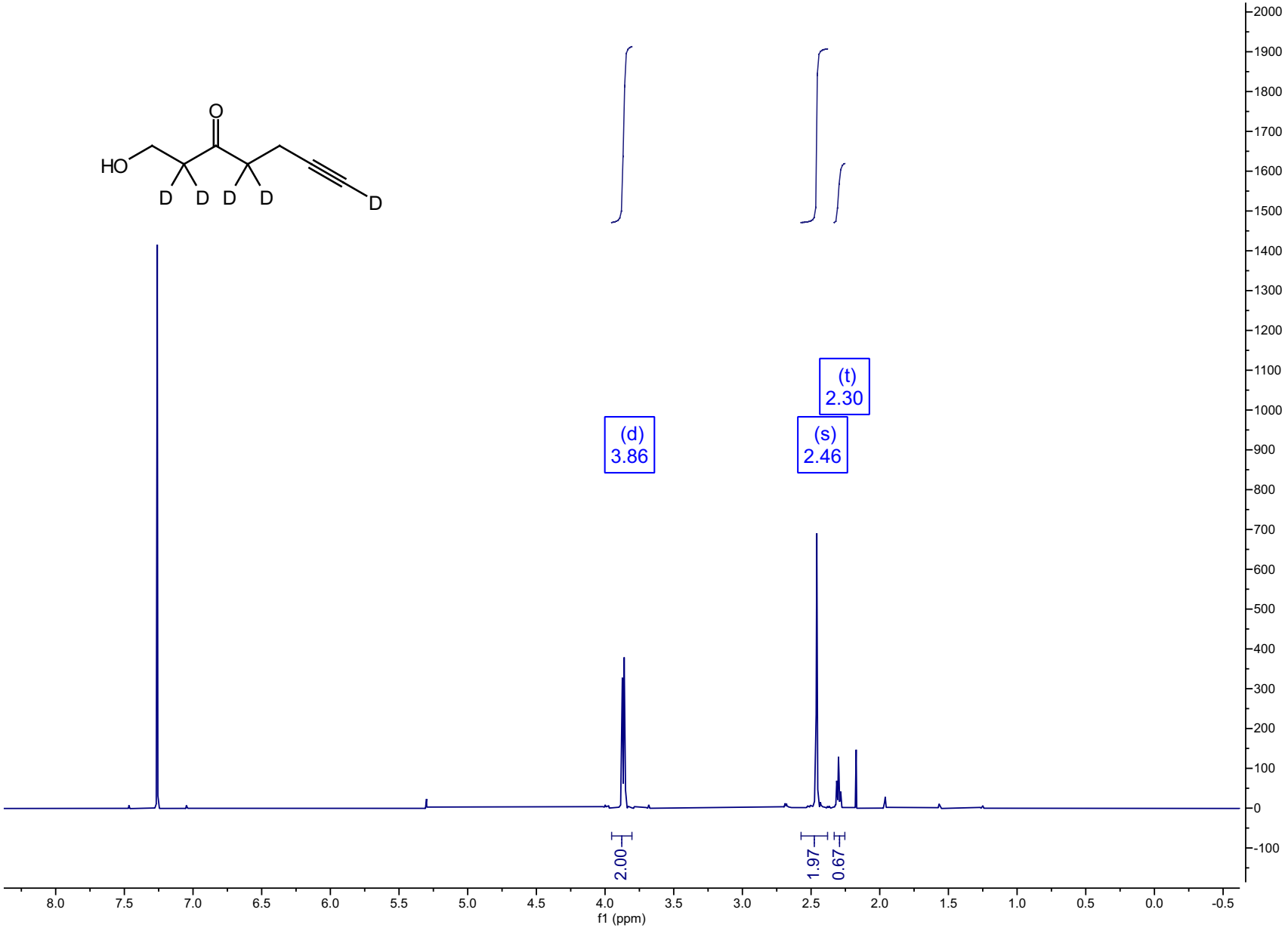
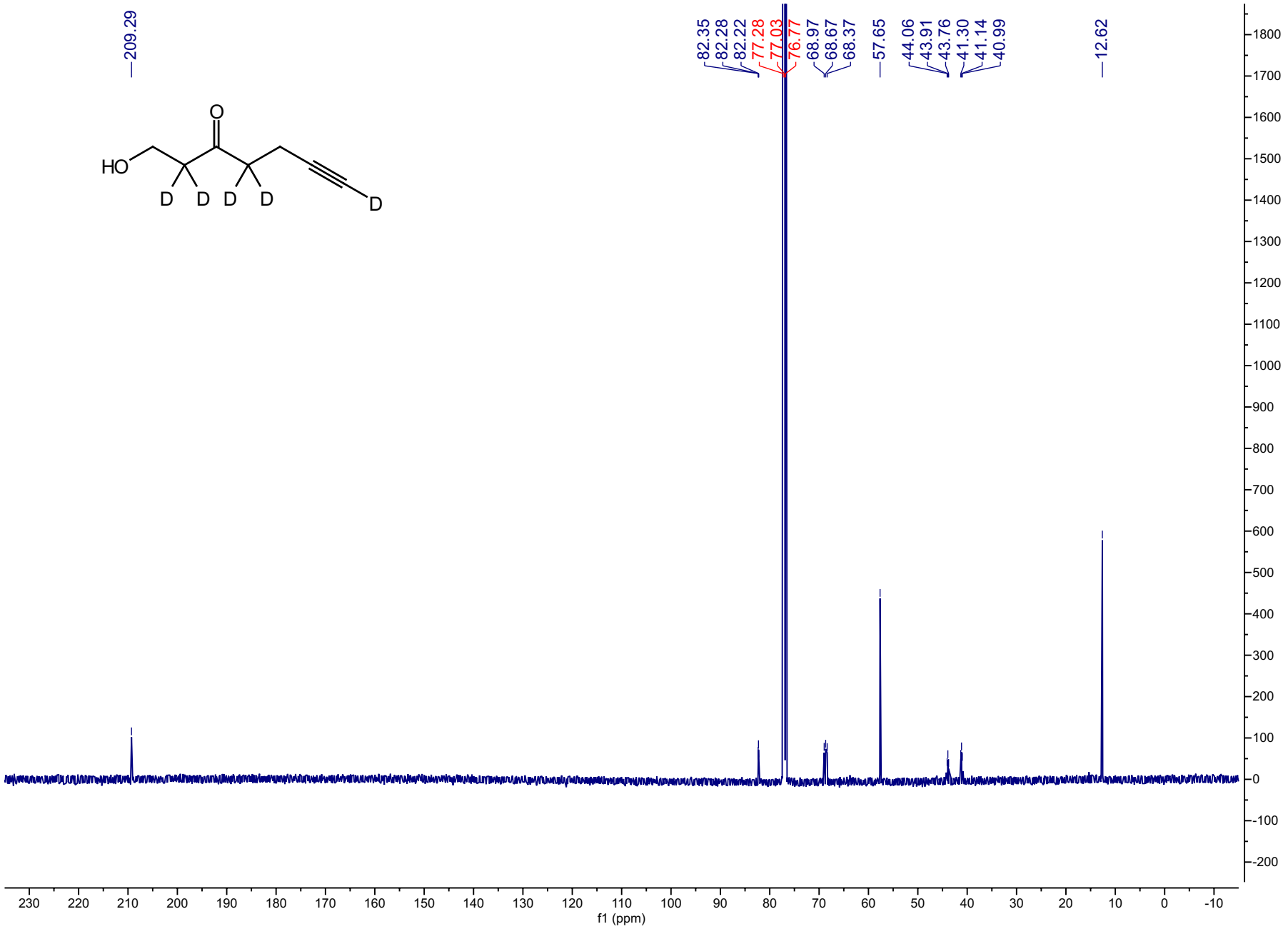

HO
O
D
D
D
D
D
(d)
3.86
(s)
2.46
(t)
2.30
2.00
1.97
0.67
f1 (ppm)


209.29
82.35
82.28
82.22
77.28
77.03
76.77
68.97
68.67
68.37
57.65
44.06
43.91
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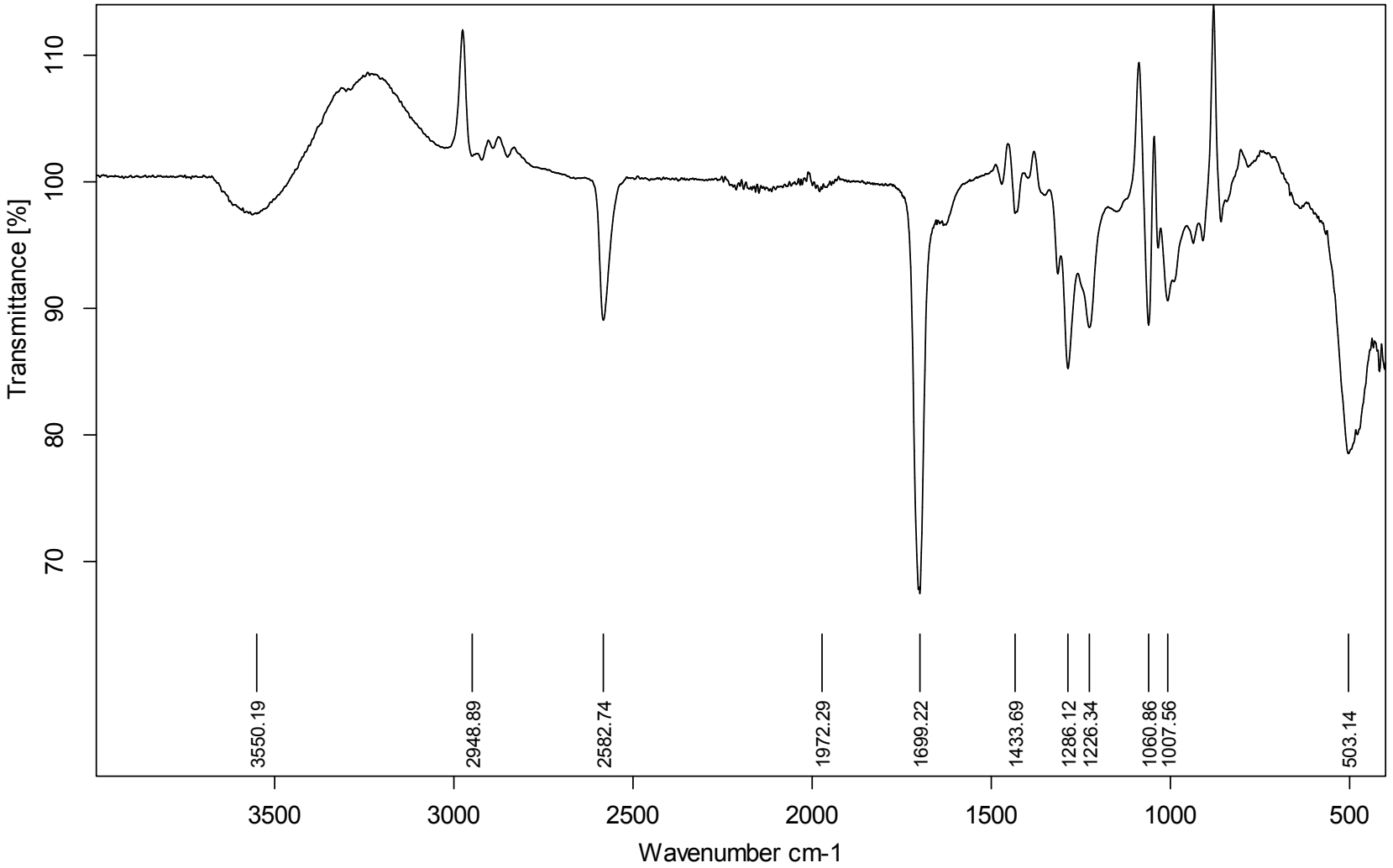
41.30
41.14
40.99
12.62
HO
O
D
D
D
D
D
f1 (ppm)



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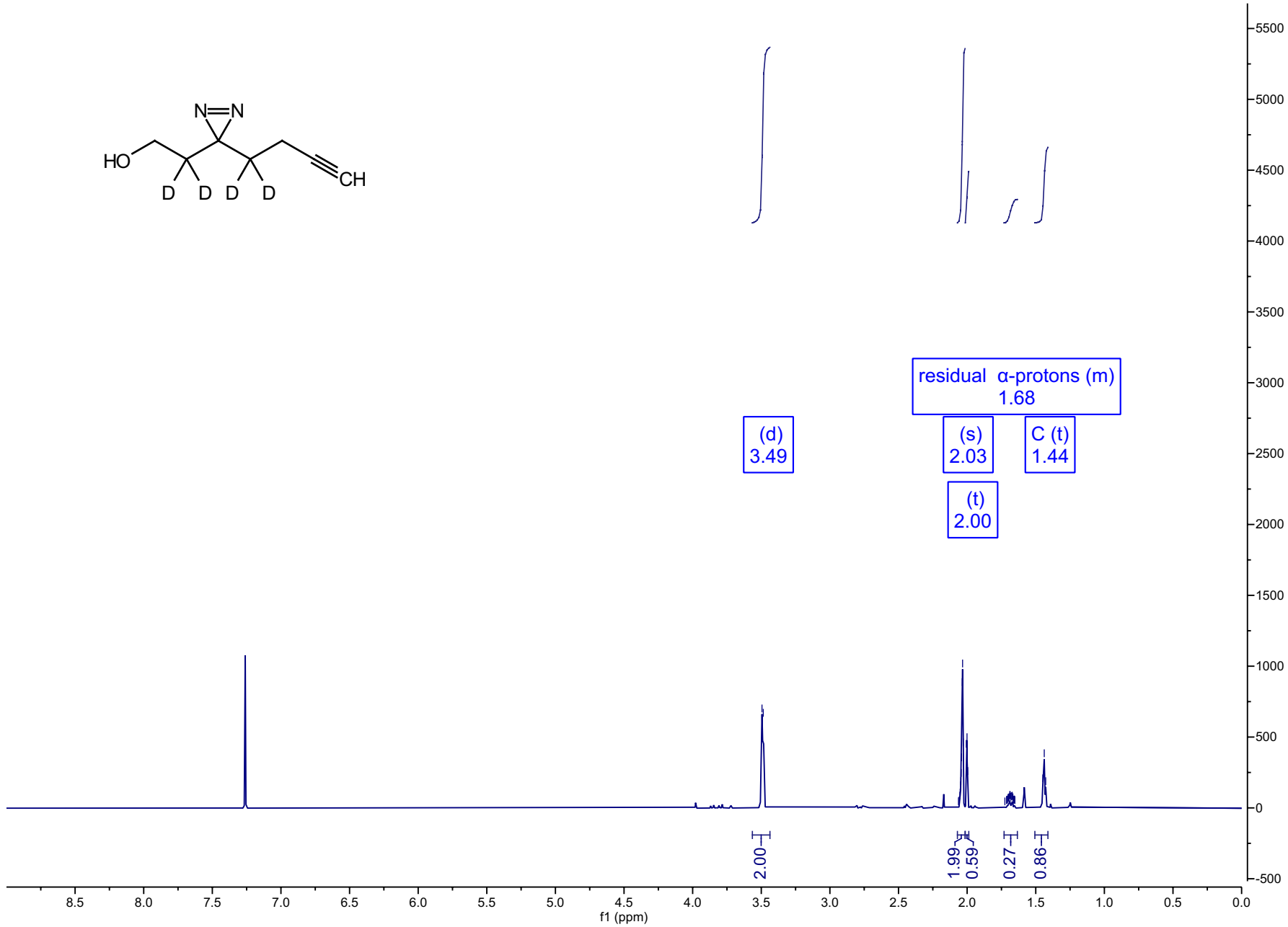
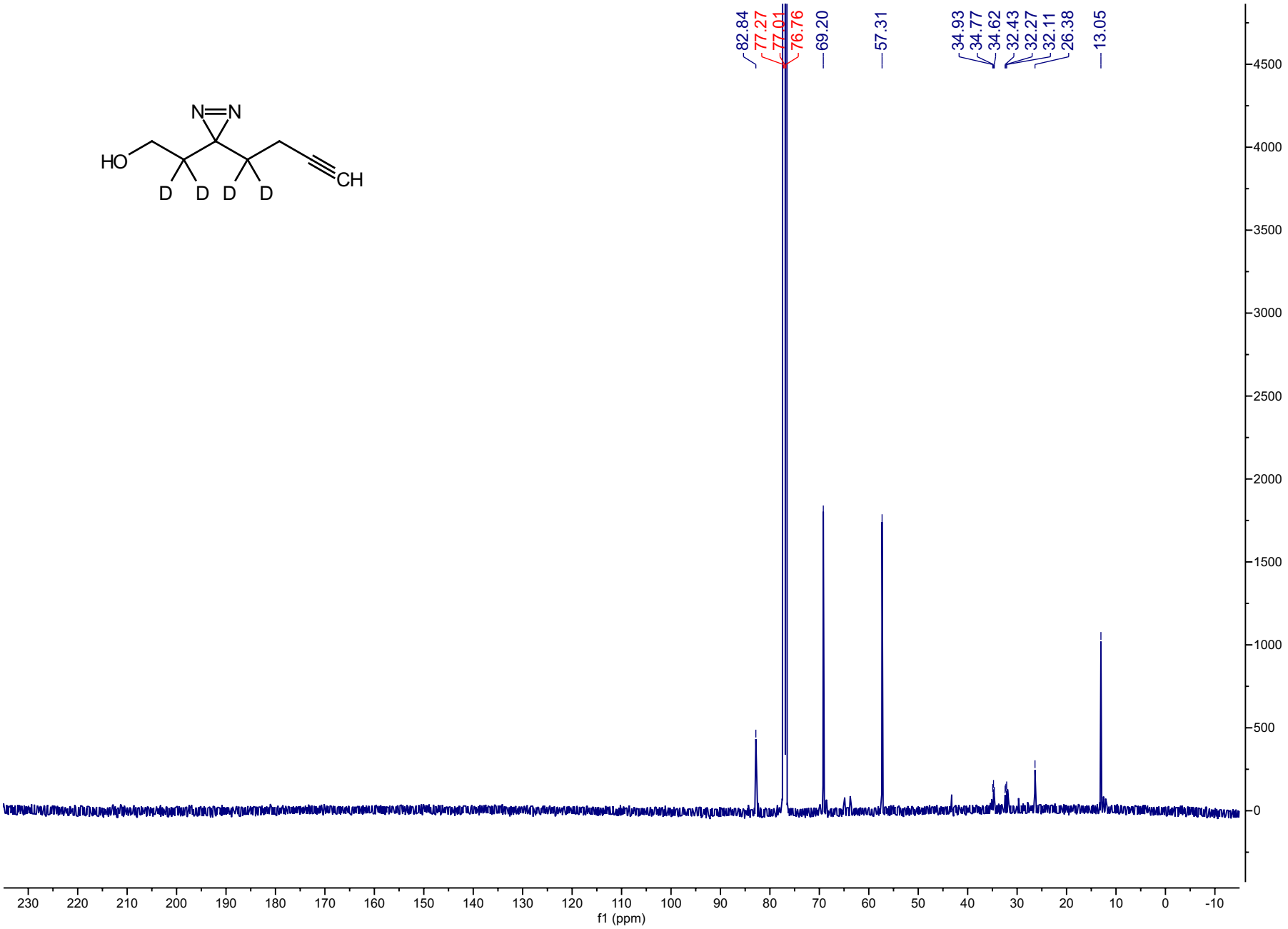
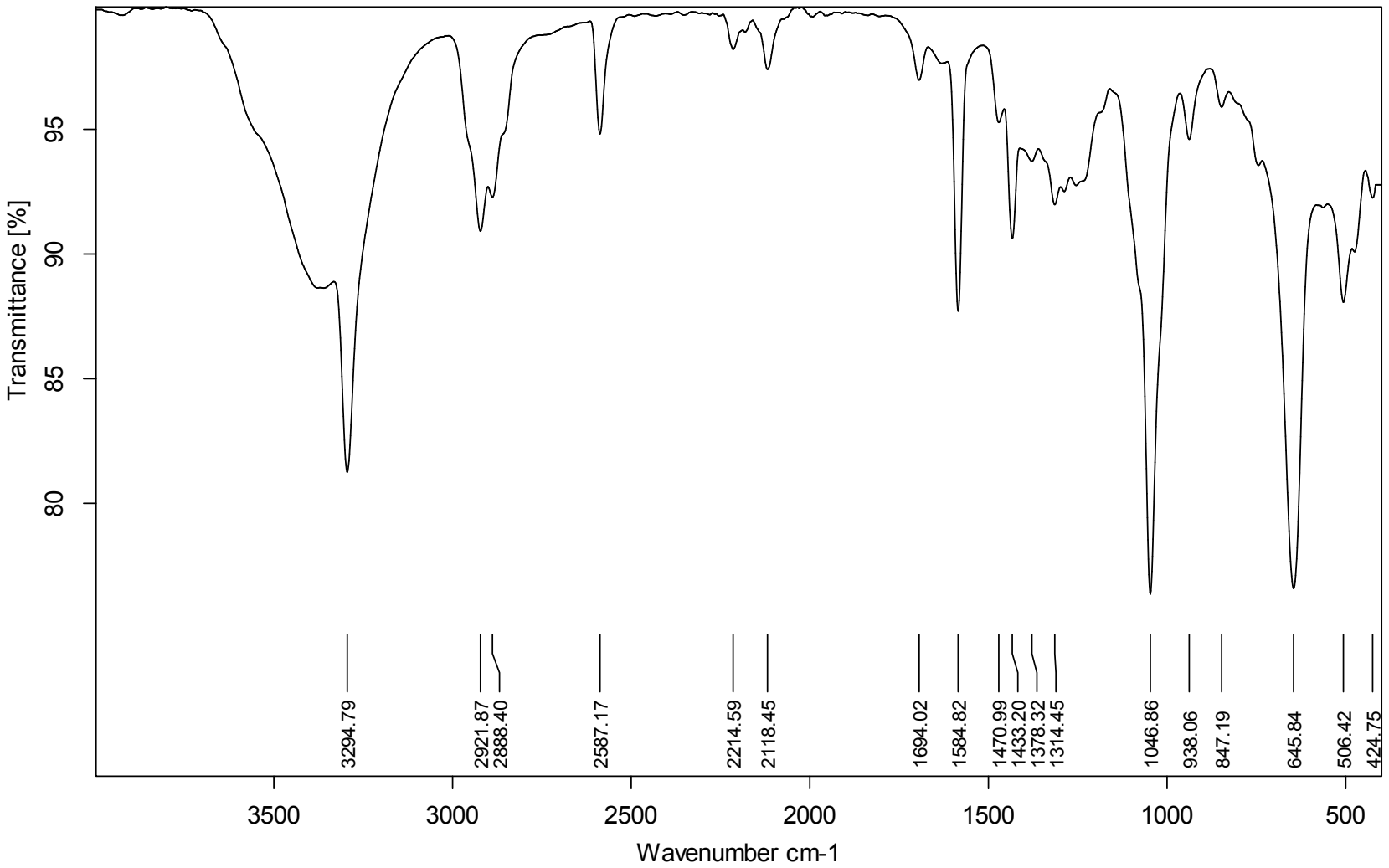

N=N
HO
D D D D
CH
residual α-protons (m)
1.68
(d)
3.49
(s)
2.03
C (t)
1.44
(t)
2.00
2.00
1.99
0.59
0.27
0.86
f1 (ppm)


82.84
77.27
77.01
76.76
69.20
57.31
34.93
34.77
34.62
32.43
32.27
32.11
26.38
13.05
HO
N=N
D D D D
CH
4500
4000
3500
3000
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



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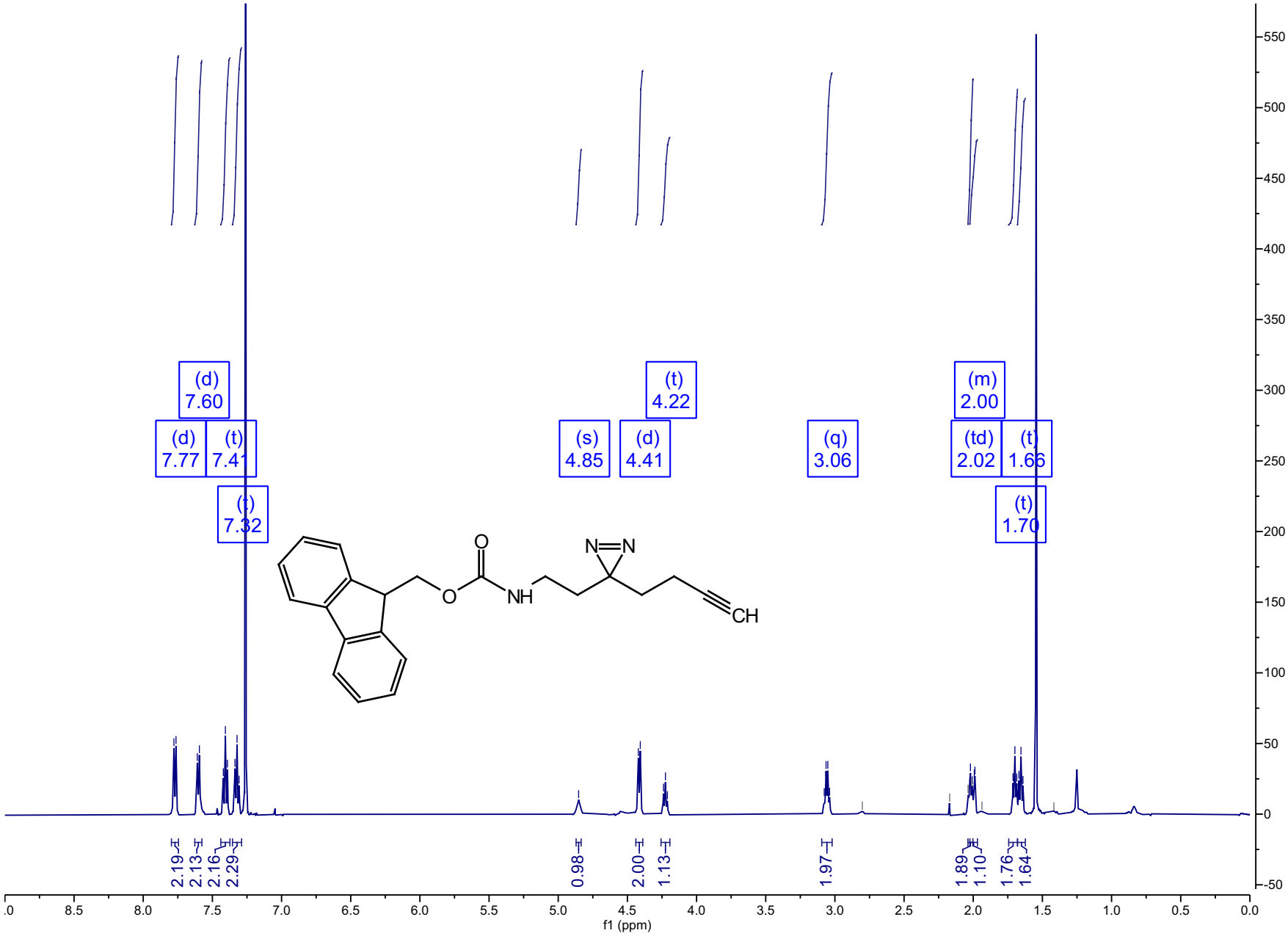
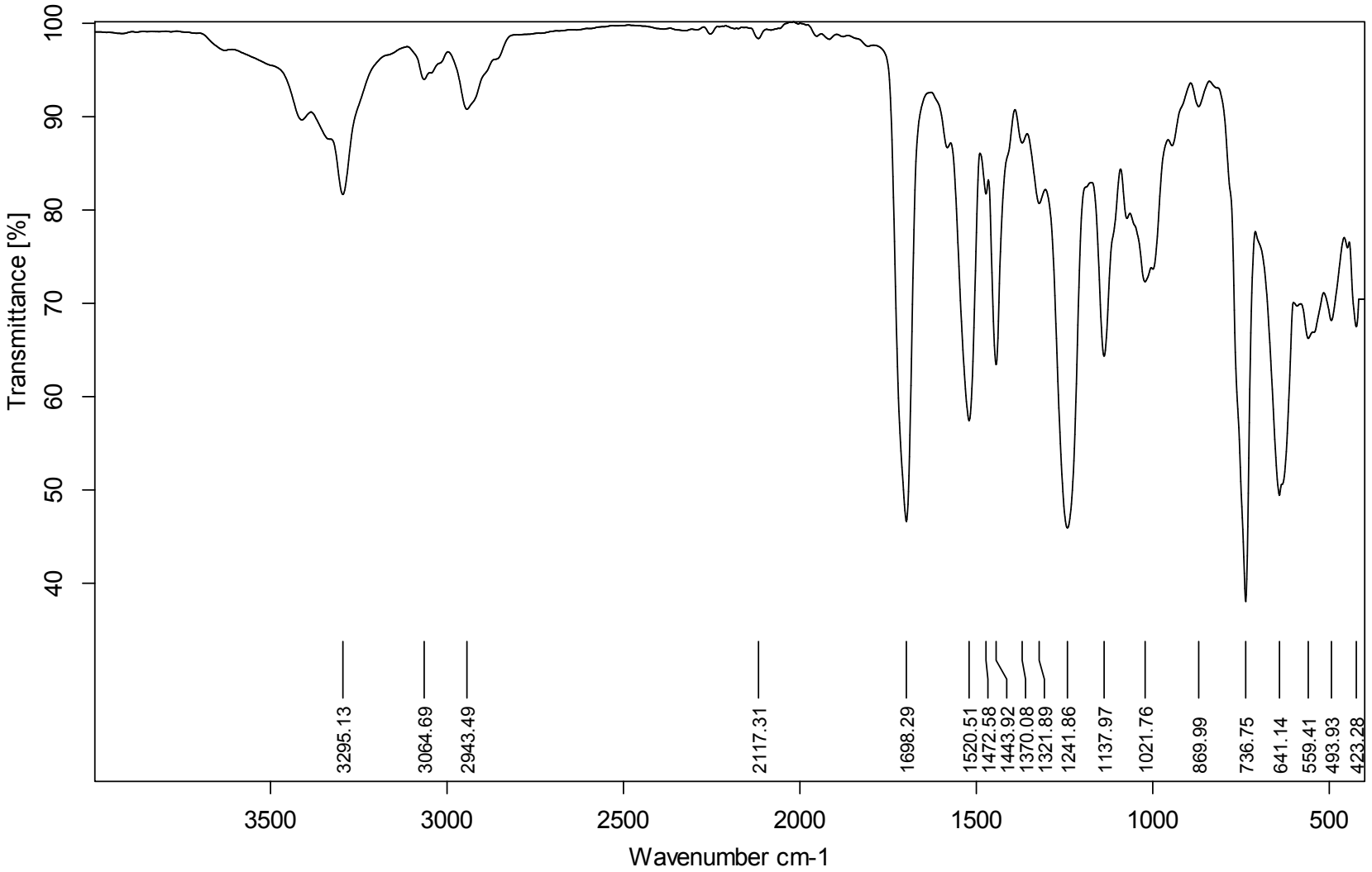

(d)
7.60
(d)
7.77
(t)
7.41
(t)
7.32
(s)
4.85
(t)
4.22
(d)
4.41
(q)
3.06
(m)
2.00
(td)
2.02
(t)
1.66
(t)
1.70
O
O
NH
N=N
CH
2.19
2.13
2.16
2.29
0.98
2.00
1.13
1.97
1.89
1.10
1.76
1.64
f1 (ppm)


156.17
143.88
141.33
127.69
127.04
125.01
119.98
82.60
69.42
66.67
47.24
35.76
32.93
32.21
26.63
13.20
O
O
NH
N=N
CH
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)



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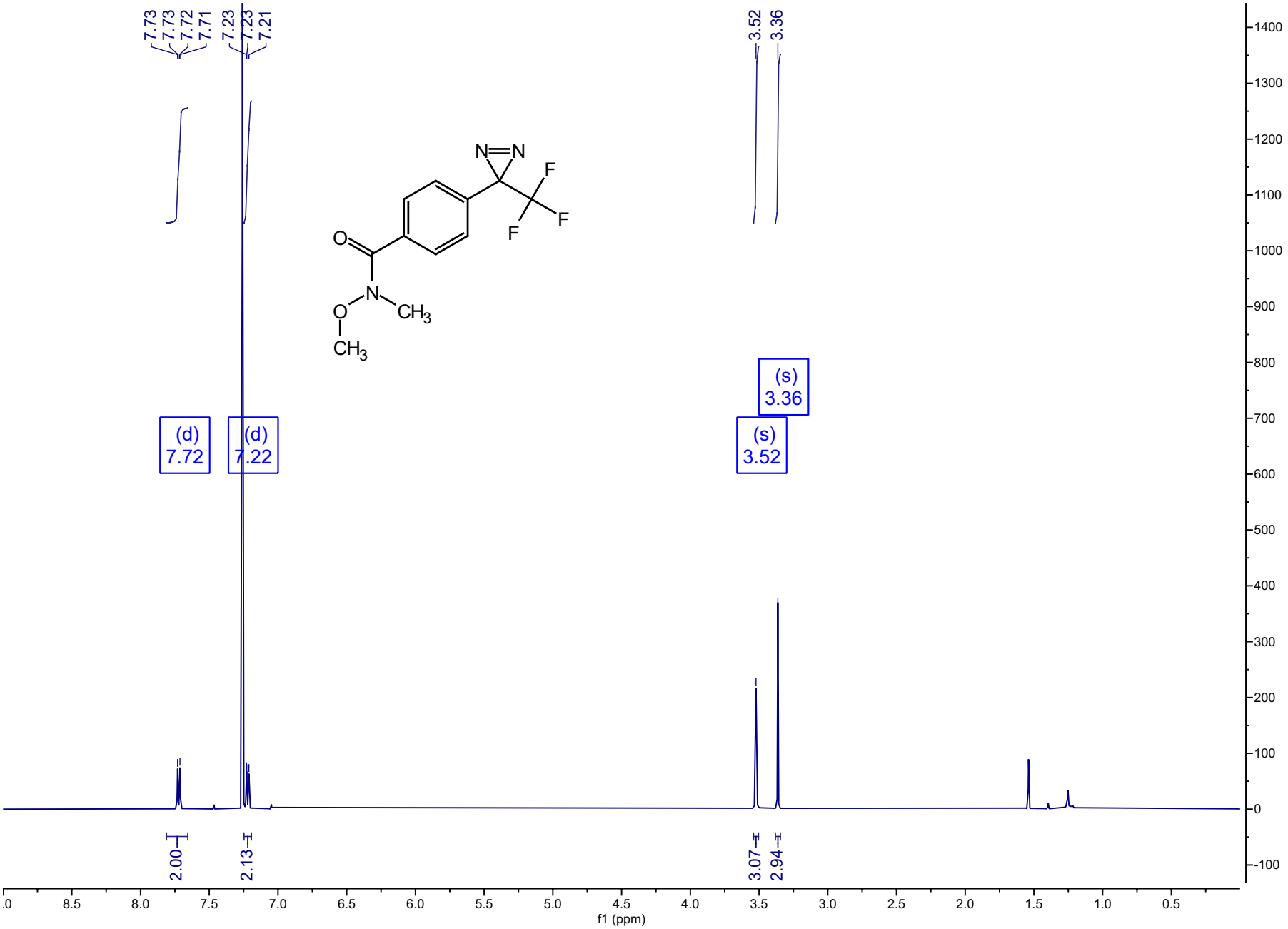
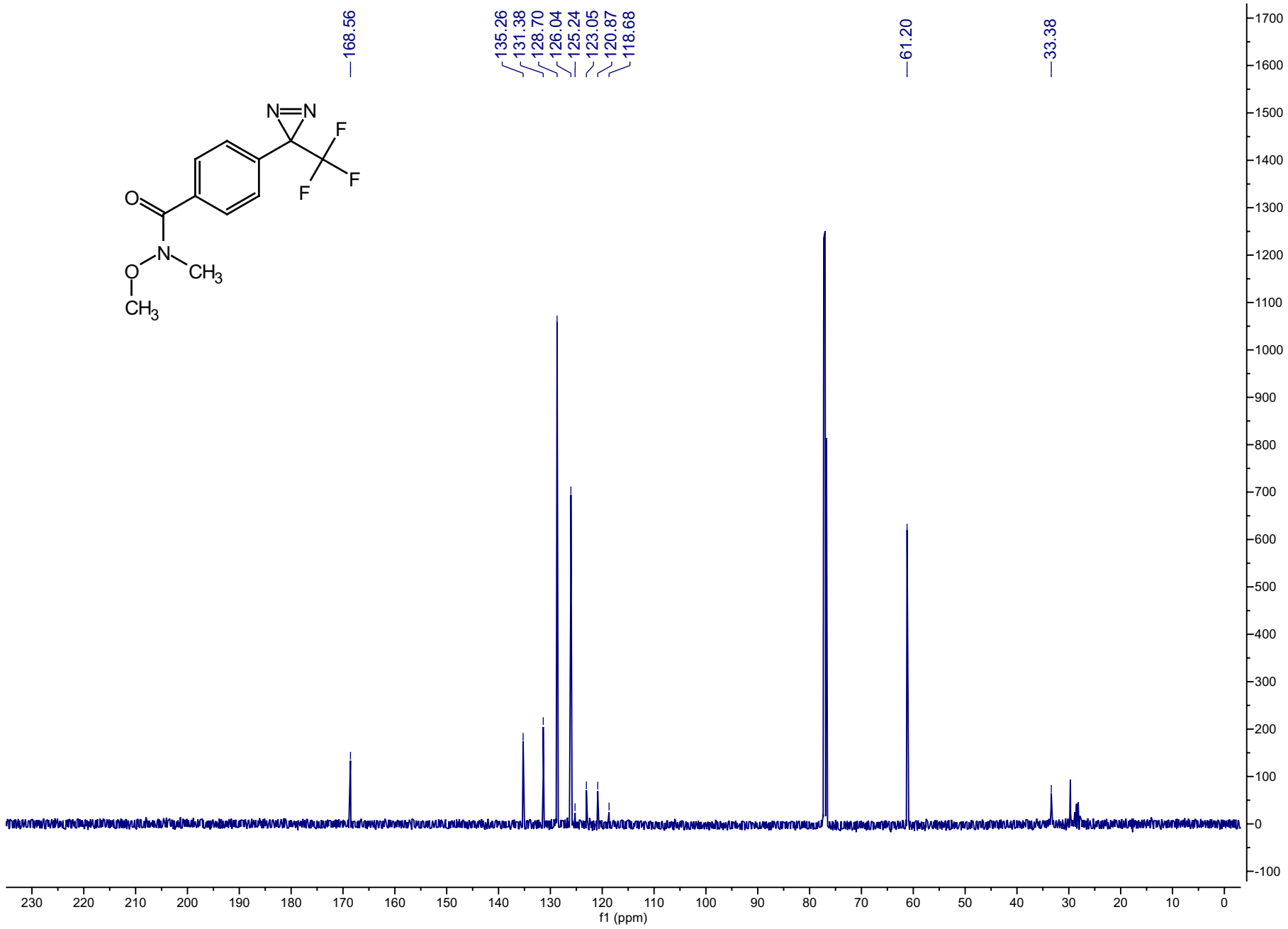

7.73
7.73
7.72
7.71
7.23
7.23
7.21
3.52
3.36
N
N
F
F
F
O
N
O
CH3
CH3
(d)
7.72
(d)
7.22
(s)
3.36
(s)
3.52
2.00
2.13
3.07
2.94
f1 (ppm)


168.56
135.26
131.38
128.70
126.04
125.24
123.05
120.87
118.68
61.20
33.38
N
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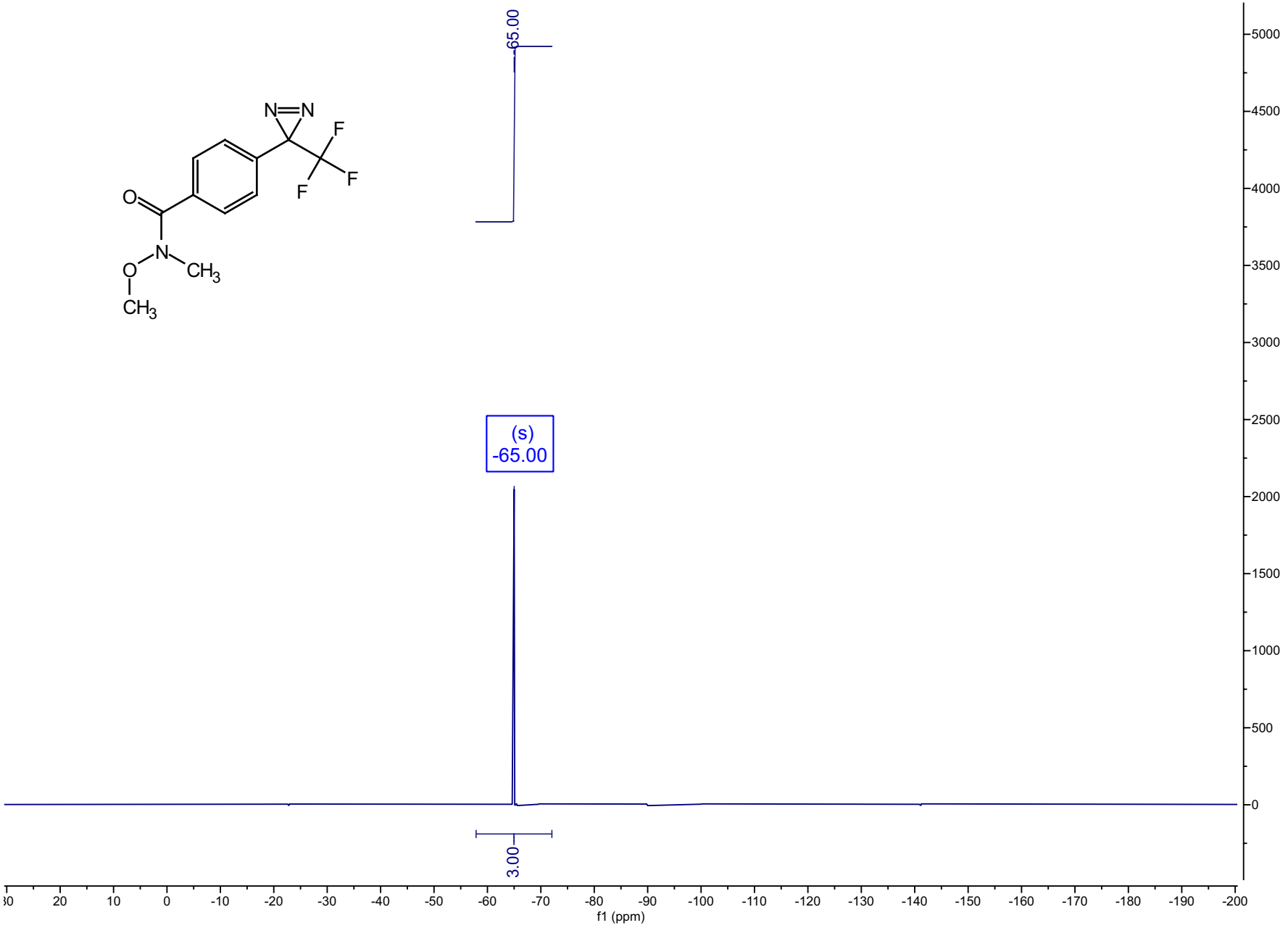
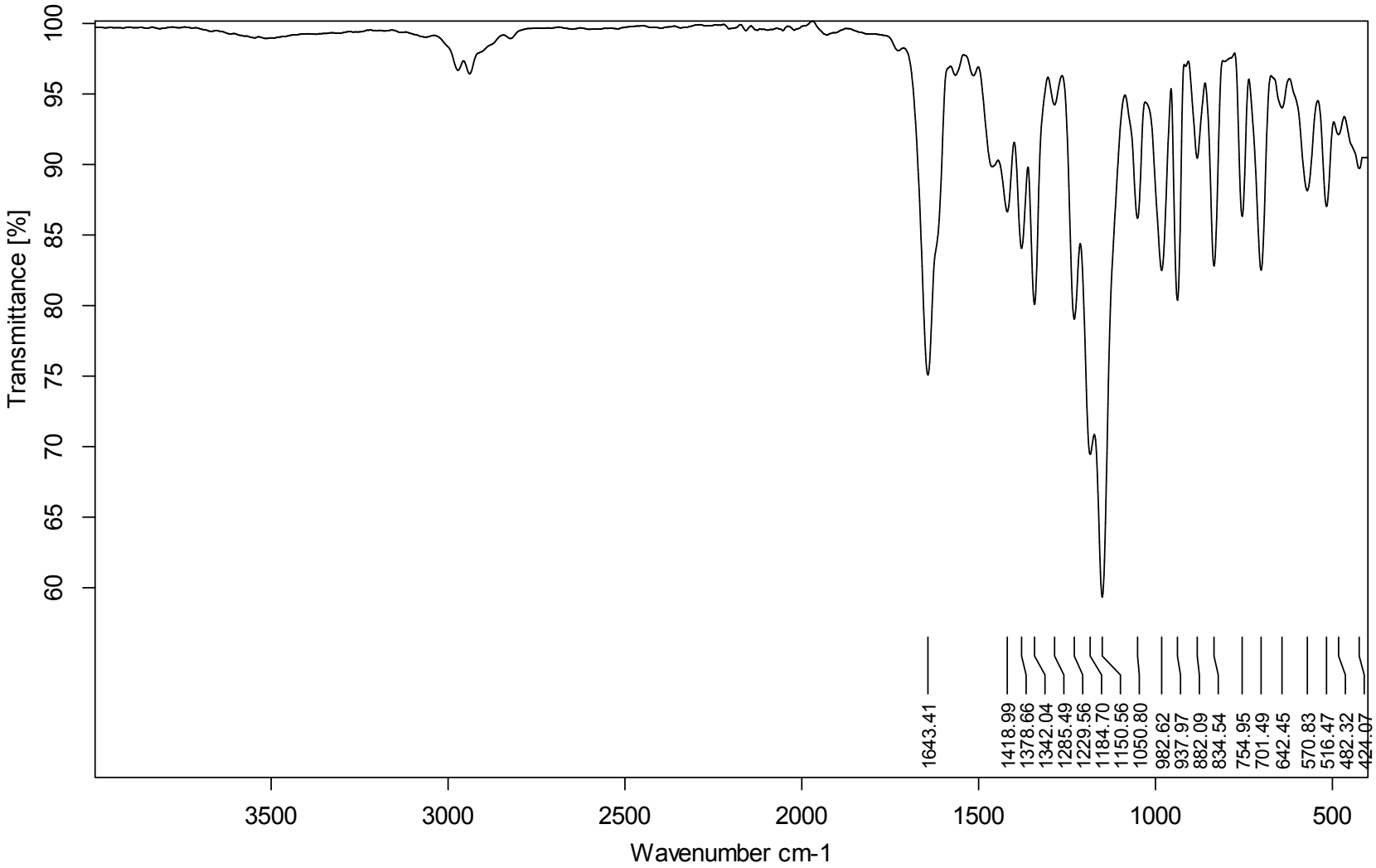
F
F
F
O
N
O
CH3
CH3
f1 (ppm)


N=N
F
F
F
O
N
O
CH3
CH3
-65.00
(s)
-65.00
3.00
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)



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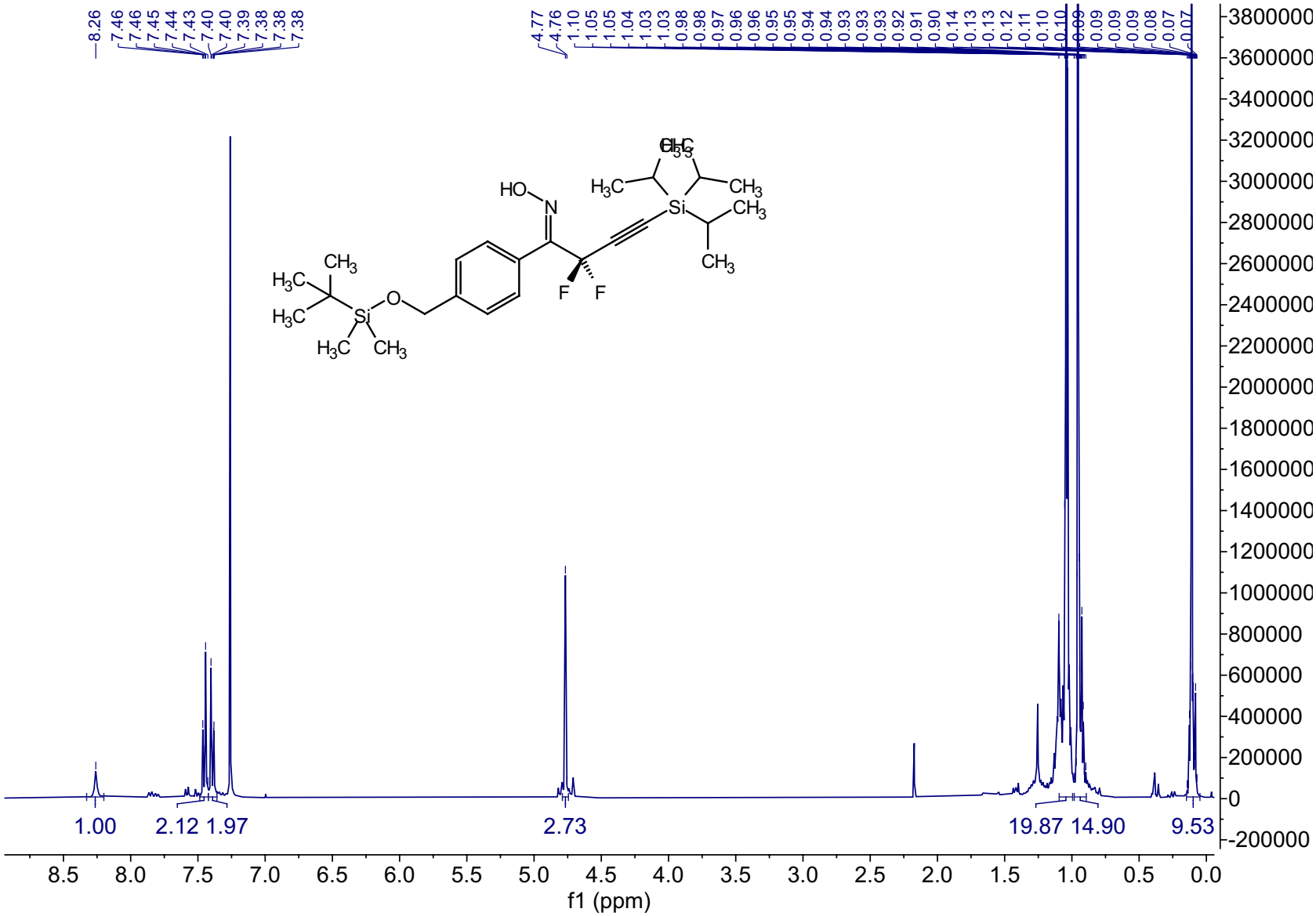
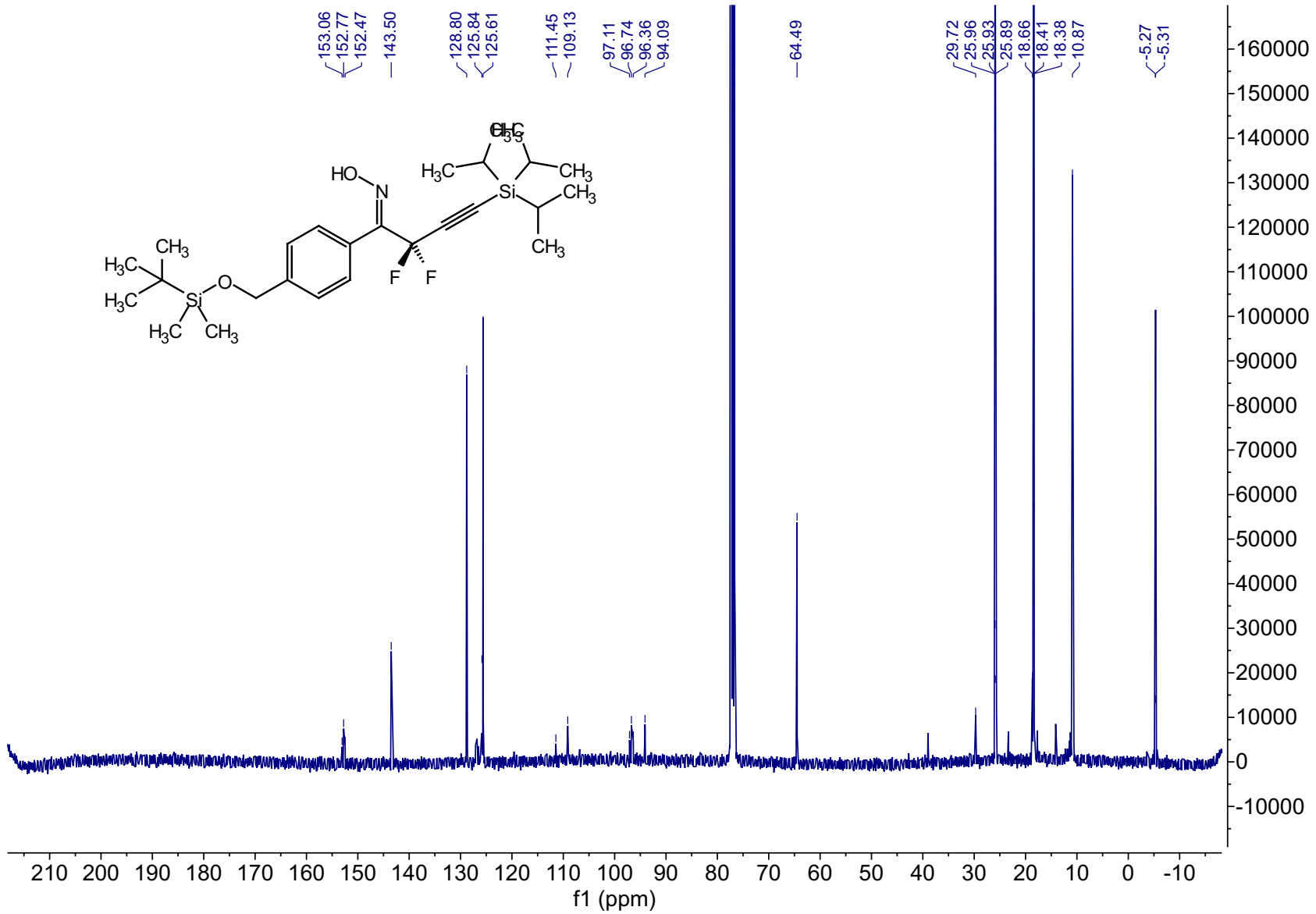
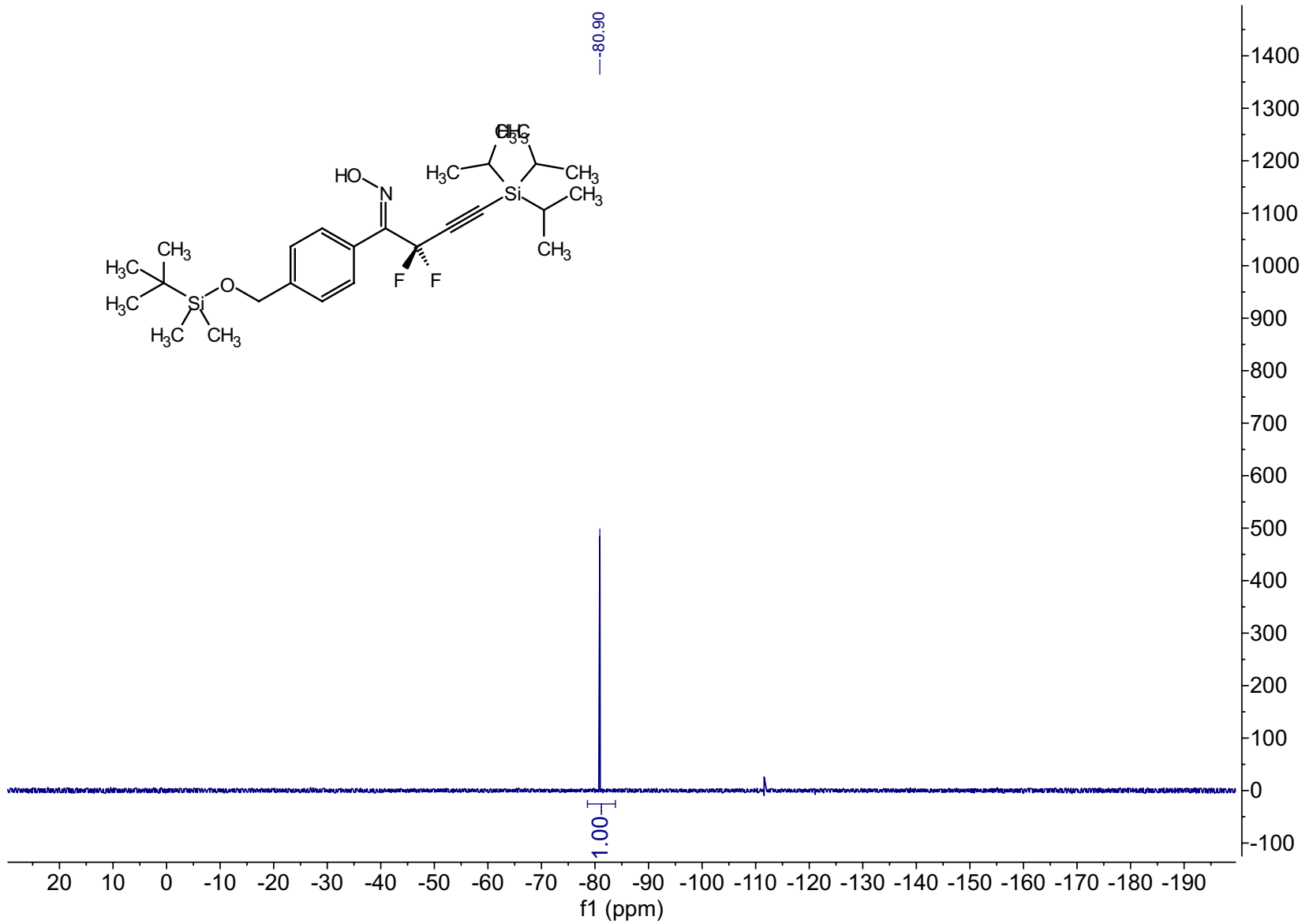
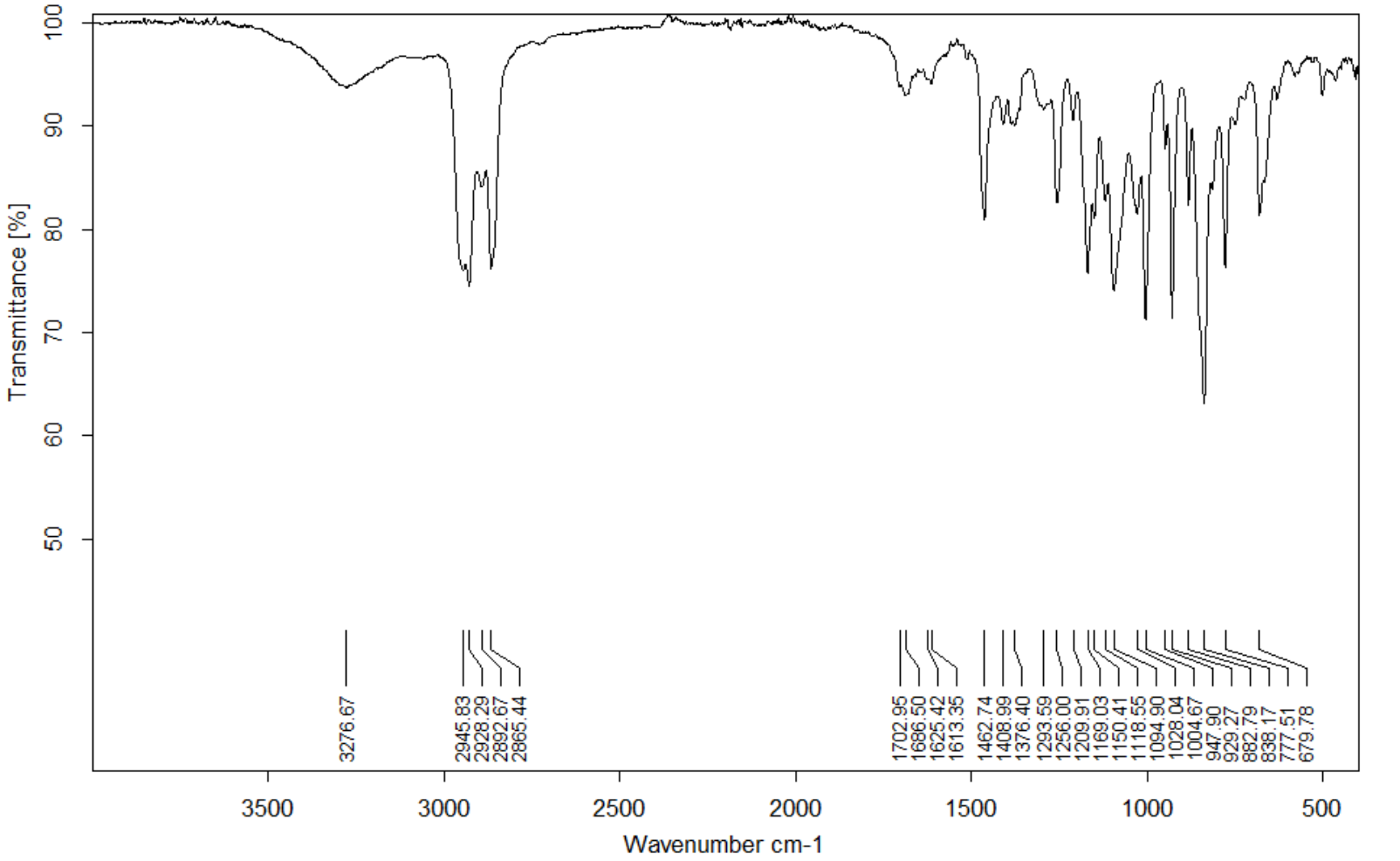

8.26
7.46
7.46
7.45
7.44
7.43
7.40
7.40
7.39
7.38
7.38
7.38
4.77
4.76
1.10
1.05
1.05
1.04
1.03
1.03
0.98
0.98
0.97
0.96
0.96
0.95
0.95
0.94
0.94
0.93
0.93
0.93
0.92
0.91
0.90
0.14
0.13
0.13
0.12
0.11
0.10
0.10
0.09
0.09
0.09
0.09
0.08
0.07
0.07
HO
N
F
F
Si
O
Si
1.00
2.12
1.97
2.73
19.87
14.90
9.53
f1 (ppm)


153.06
152.77
152.47
143.50
128.80
125.84
125.61
111.45
109.13
97.11
96.74
96.36
94.09
64.49
29.72
25.96
25.93
25.89
18.66
18.41
18.38
10.87
-5.27
-5.31
HO
N
F
F
Si
O
Si
f1 (ppm)


-80.90
HO
N
F
F
Si
O
Si
1.00
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190
f1 (ppm)
1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100 0 -100



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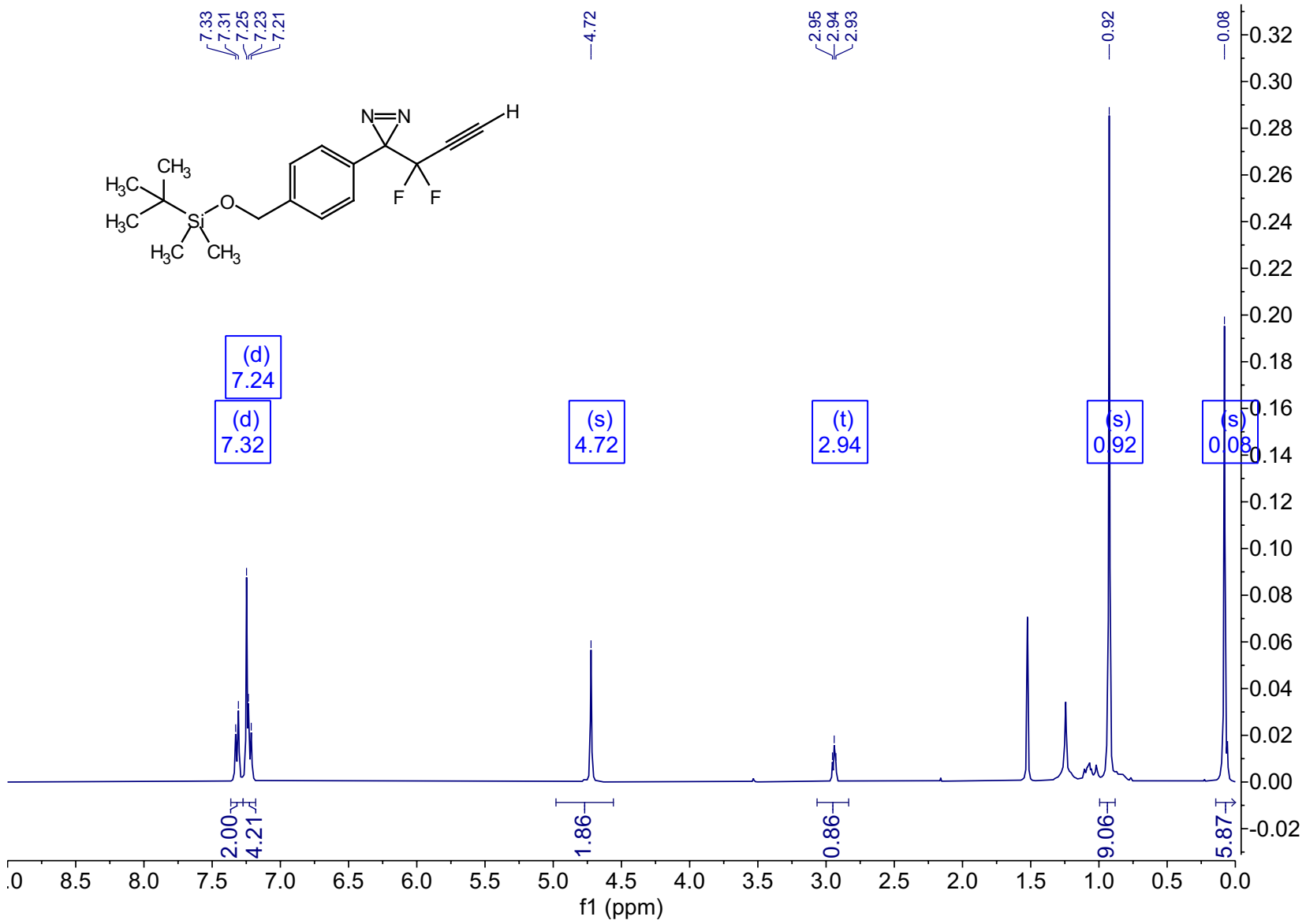
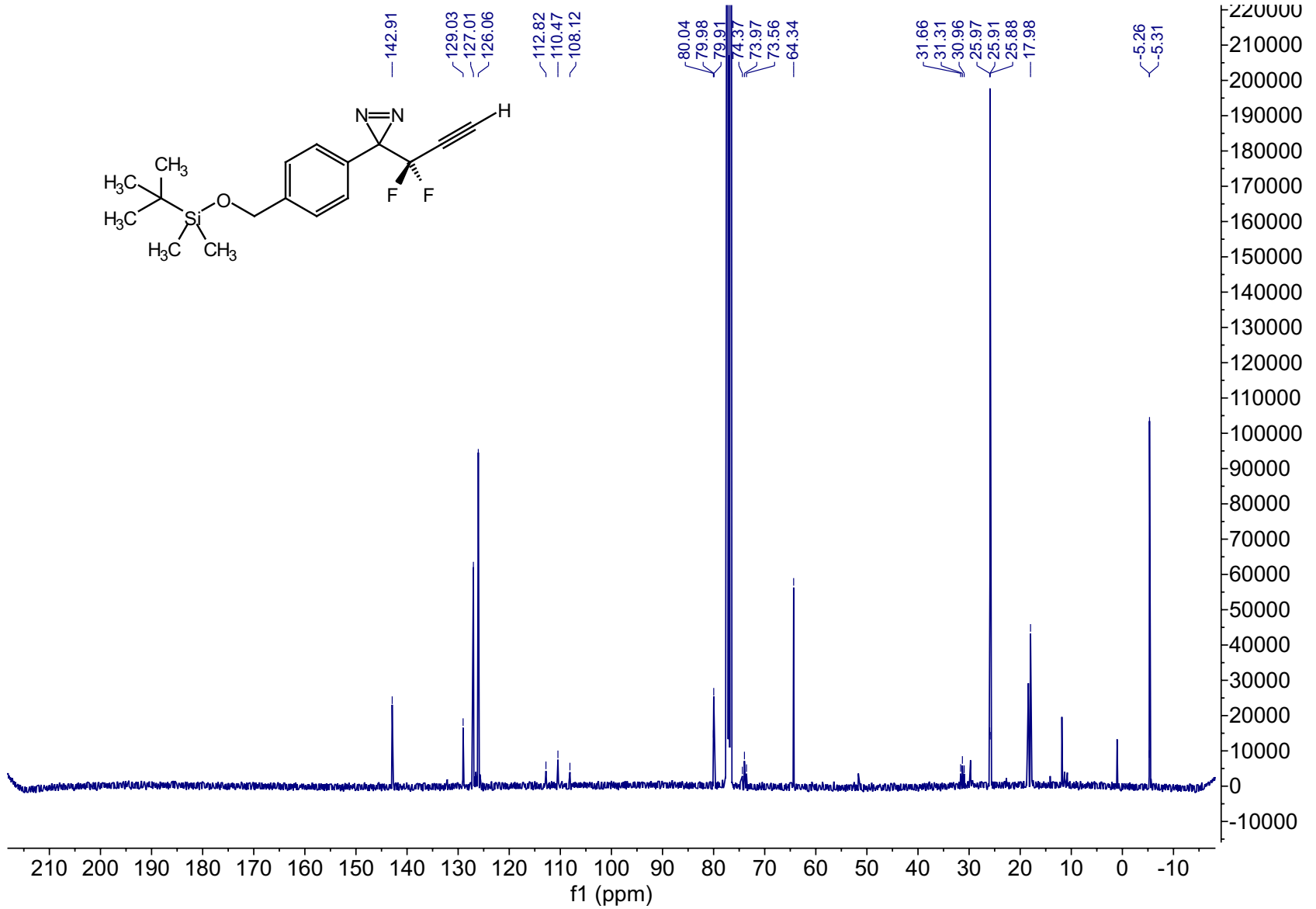
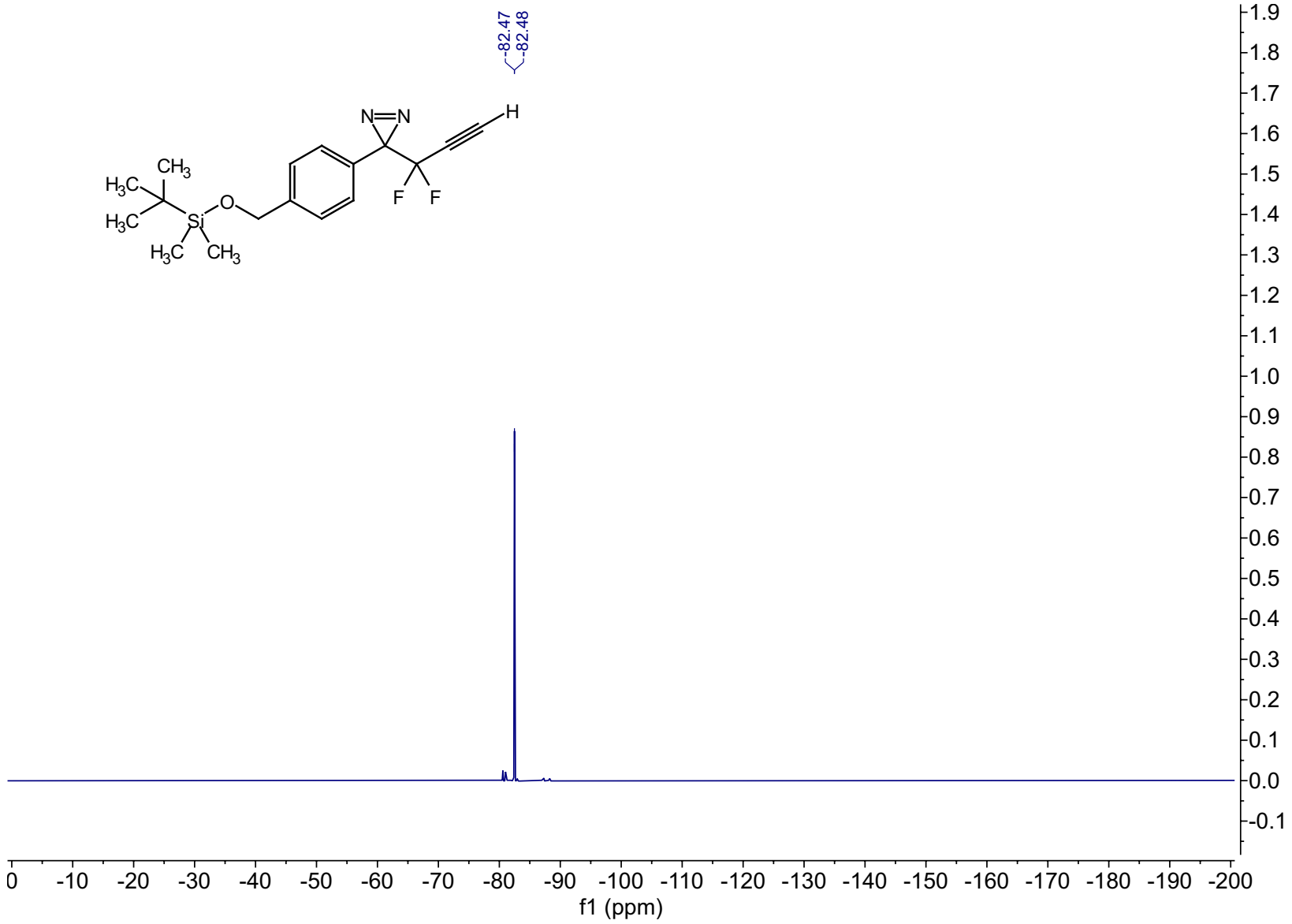
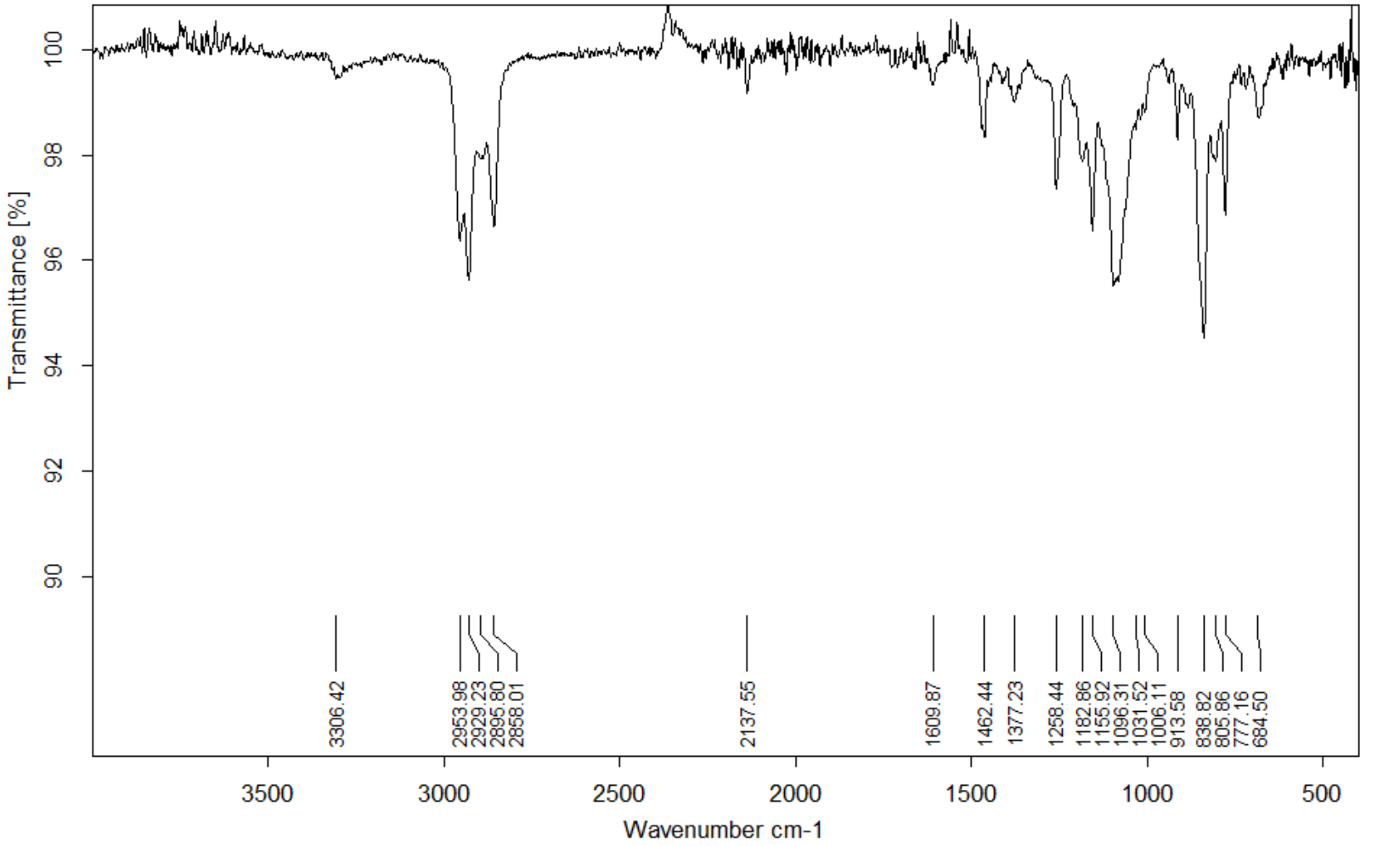

7.33
7.31
7.25
7.23
7.21
4.72
2.95
2.94
2.93
0.92
0.08
N=N
H
F
F
O
Si
H3C
CH3
(d)
7.24
(d)
7.32
(s)
4.72
(t)
2.94
(s)
0.92
(s)
0.08
2.00
4.21
1.86
0.86
9.06
5.87
f1 (ppm)


142.91
129.03
127.01
126.06
112.82
110.47
108.12
80.04
79.98
79.91
74.37
73.97
73.56
64.34
31.66
31.31
30.96
25.97
25.91
25.88
17.98
-5.26
-5.31
N
N
H
F
F
O
Si
H3C
CH3
f1 (ppm)


-82.47
-82.48
N
N
H
F
F
O
Si
CH3
H3C
H3C
H3C
CH3
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)



Z:\ALPHA FTIR DATA\MTP-04-020p.42 MTP-04-020p Diamond ATR 05/12/2020

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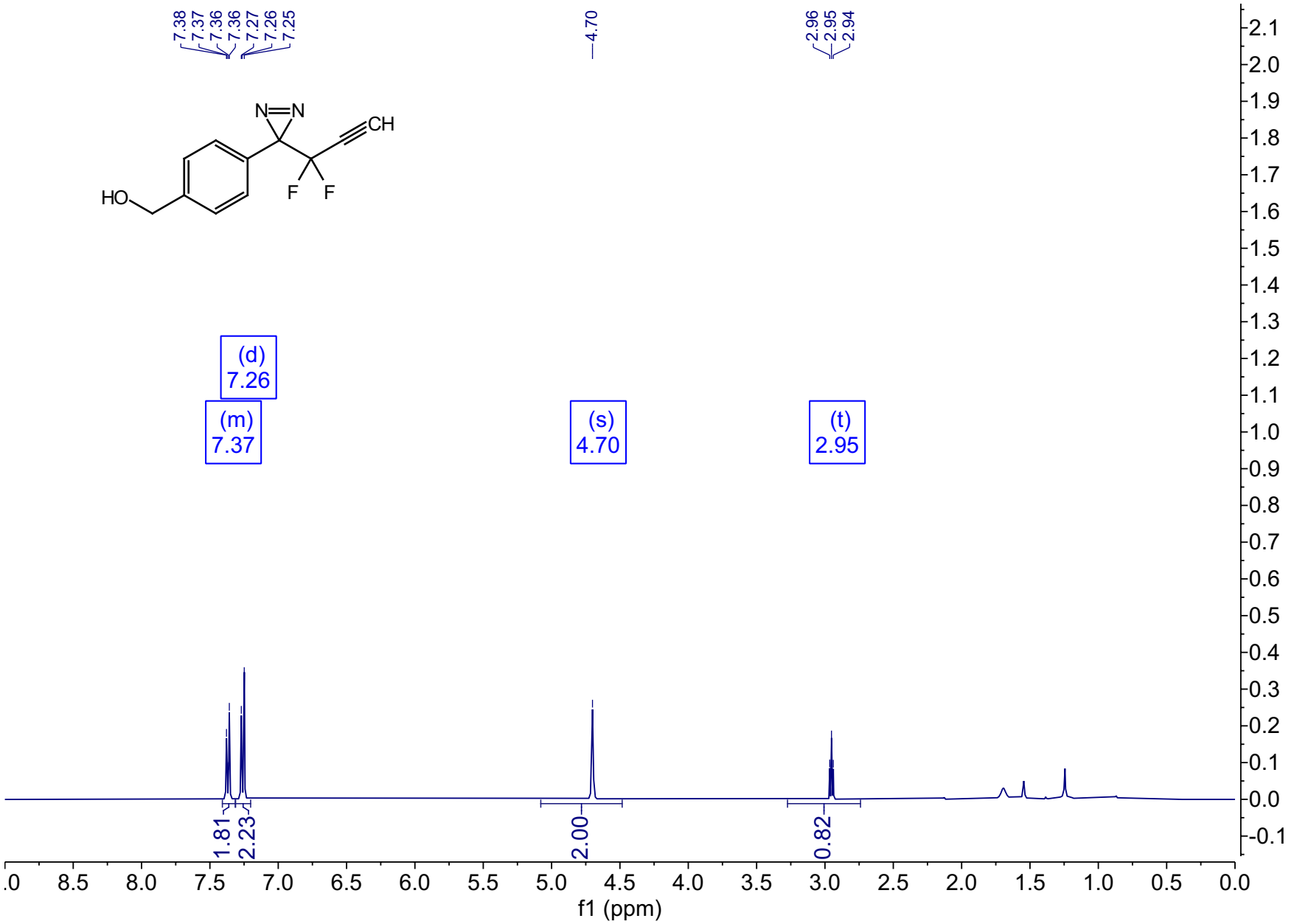
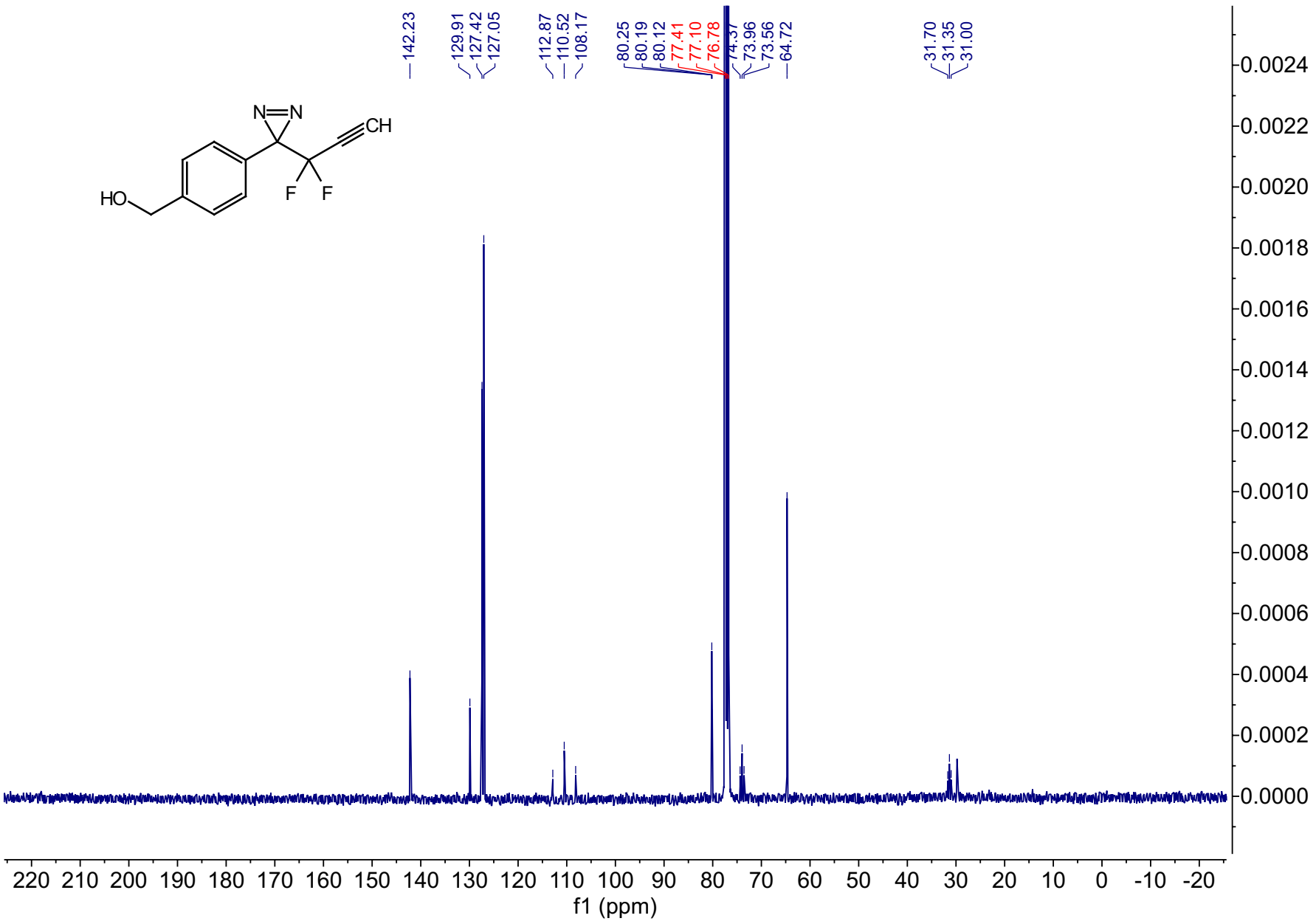
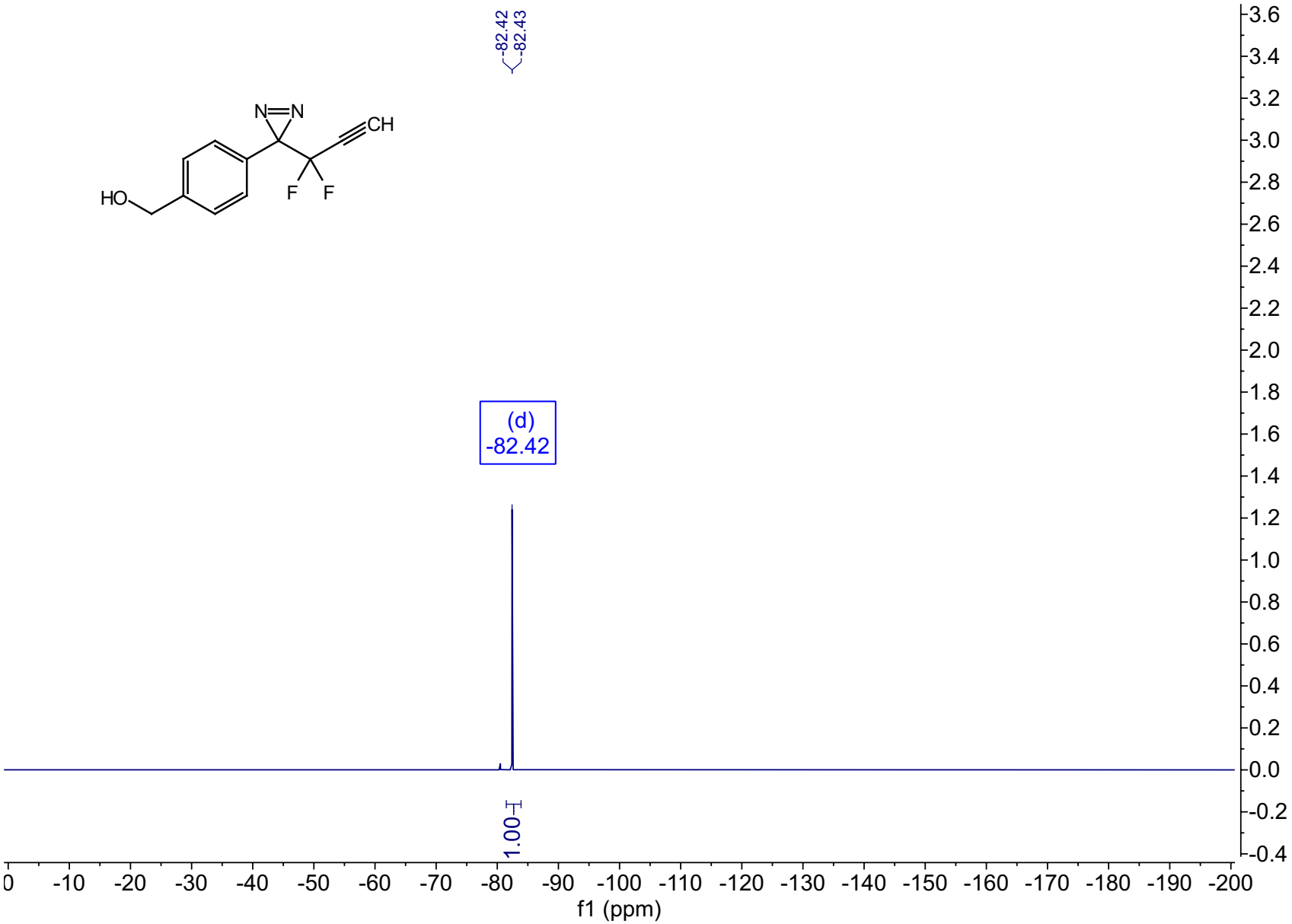
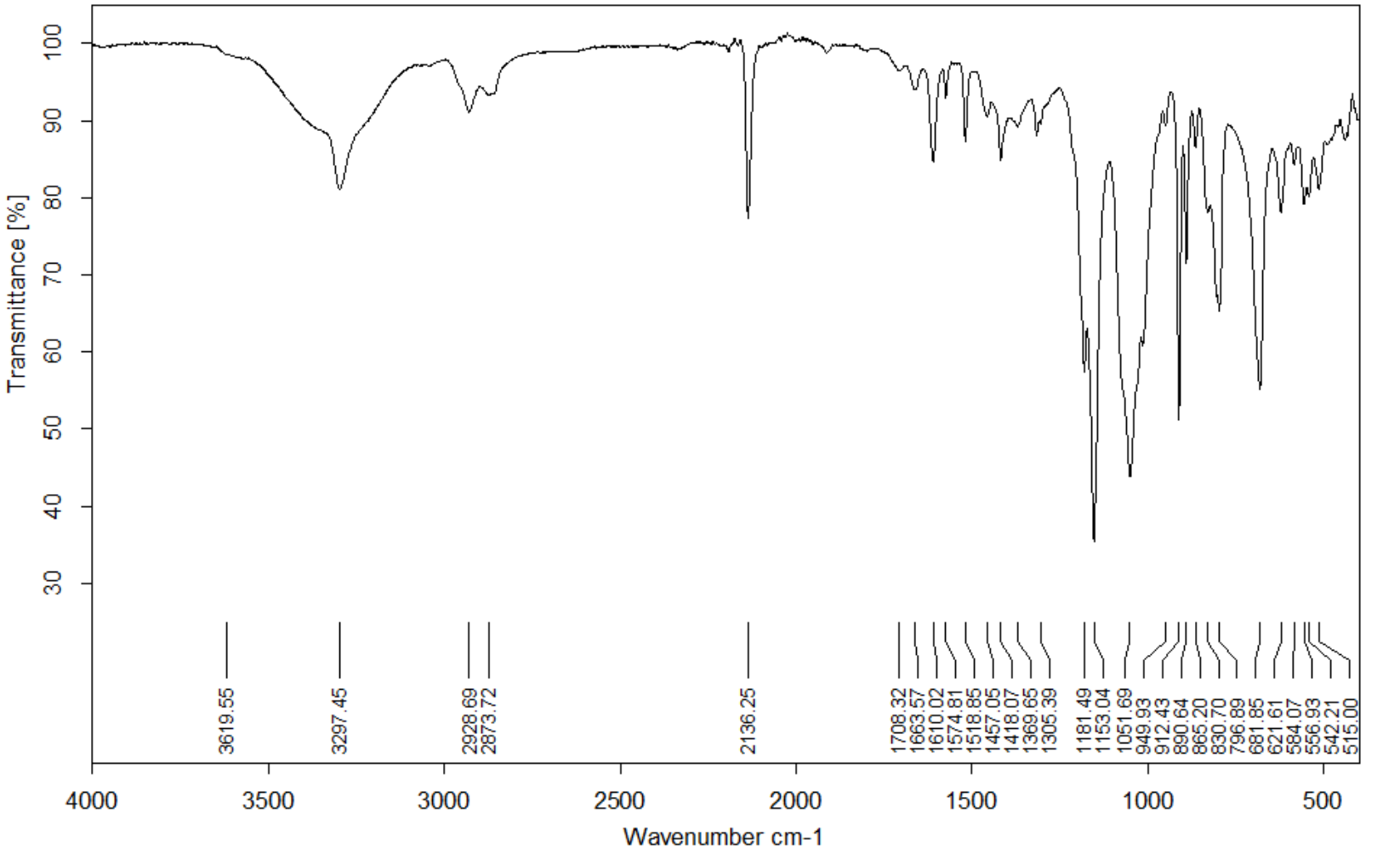

7.38
7.37
7.36
7.36
7.27
7.26
7.25
4.70
2.96
2.95
2.94
N=N
CH
HO
F
F
(d)
7.26
(m)
7.37
(s)
4.70
(t)
2.95
1.81
2.23
2.00
0.82
f1 (ppm)


142.23
129.91
127.42
127.05
112.87
110.52
108.17
80.25
80.19
80.12
77.41
77.10
76.78
74.37
73.96
73.56
64.72
31.70
31.35
31.00
N=N
CH
HO
F
F
0.0024
0.0022
0.0020
0.0018
0.0016
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


-82.42
-82.43
N=N
CH
HO
F
F
(d)
-82.42
1.00
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)



Z:\ALPHA FTIR DATA\MTP-04-020p.44 MTP-04-020p Diamond ATR 08/12/2020

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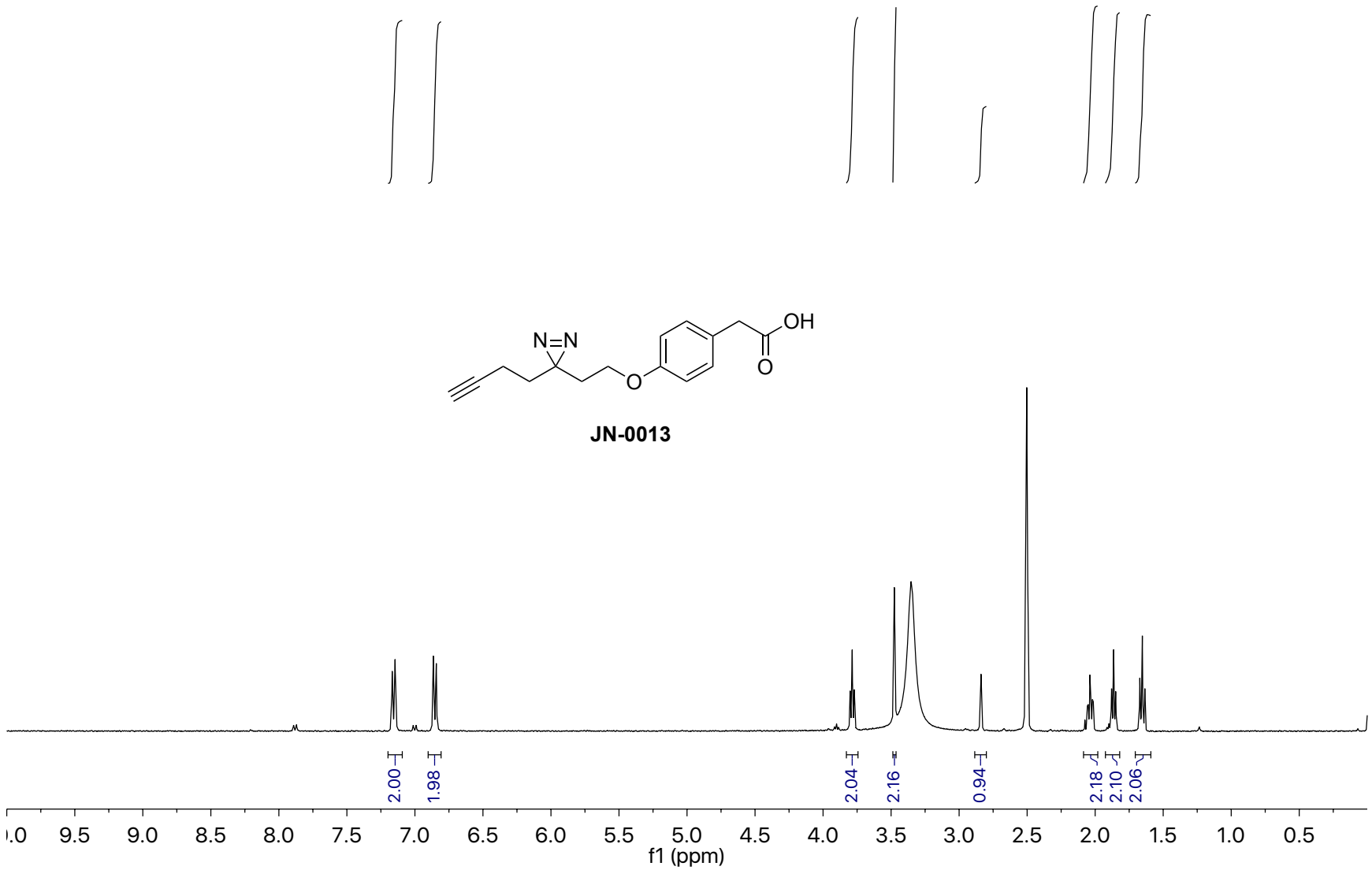
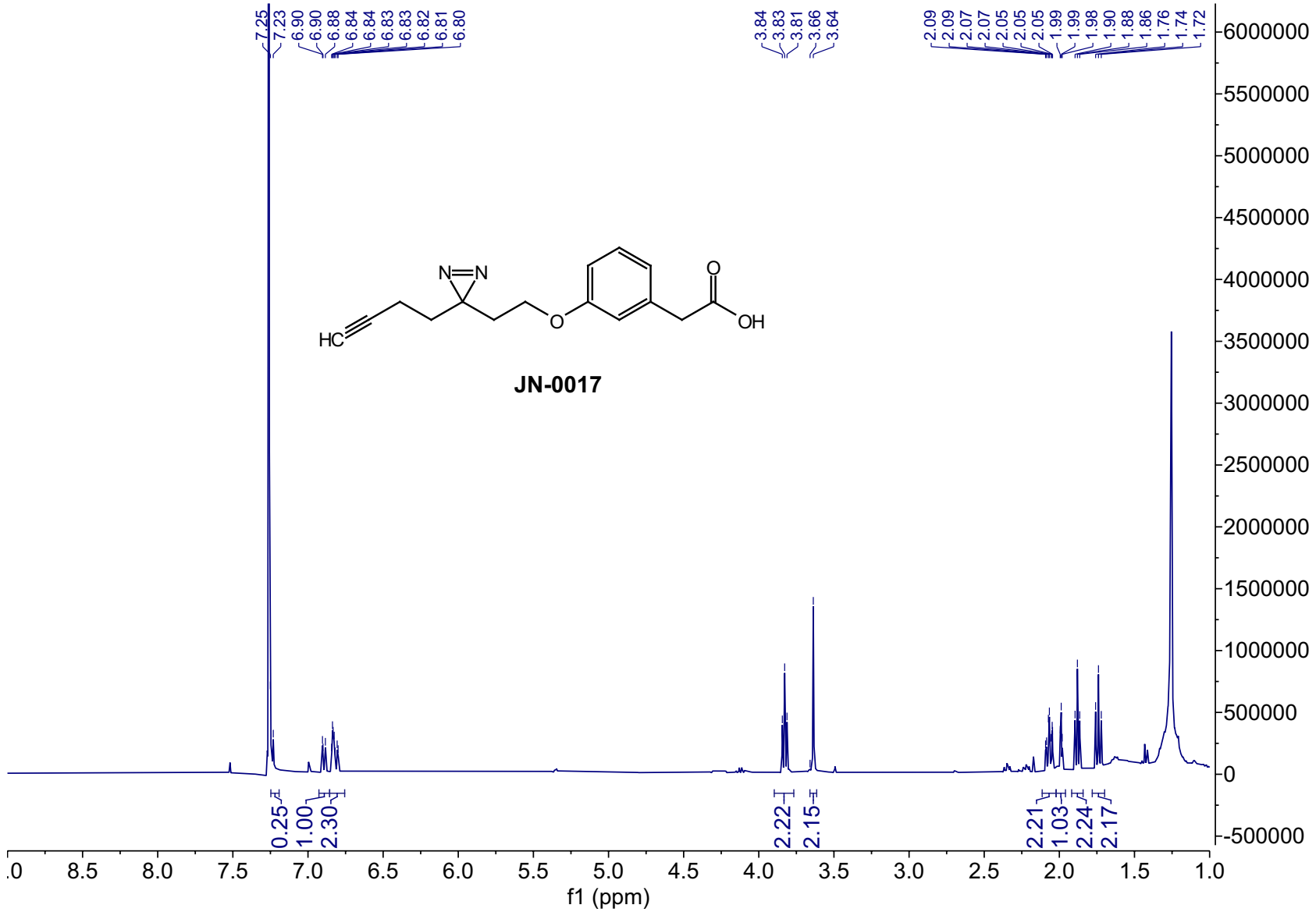
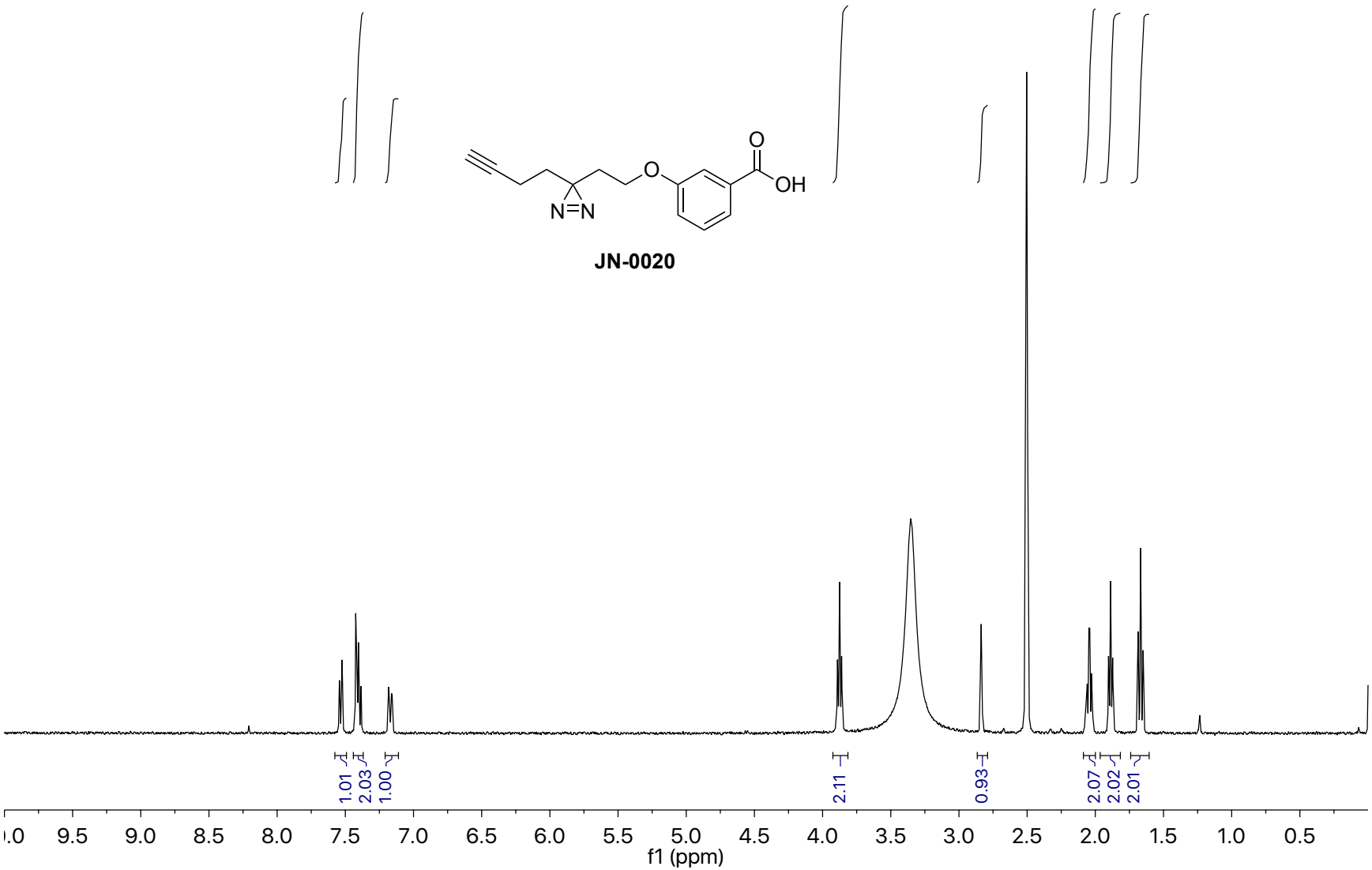
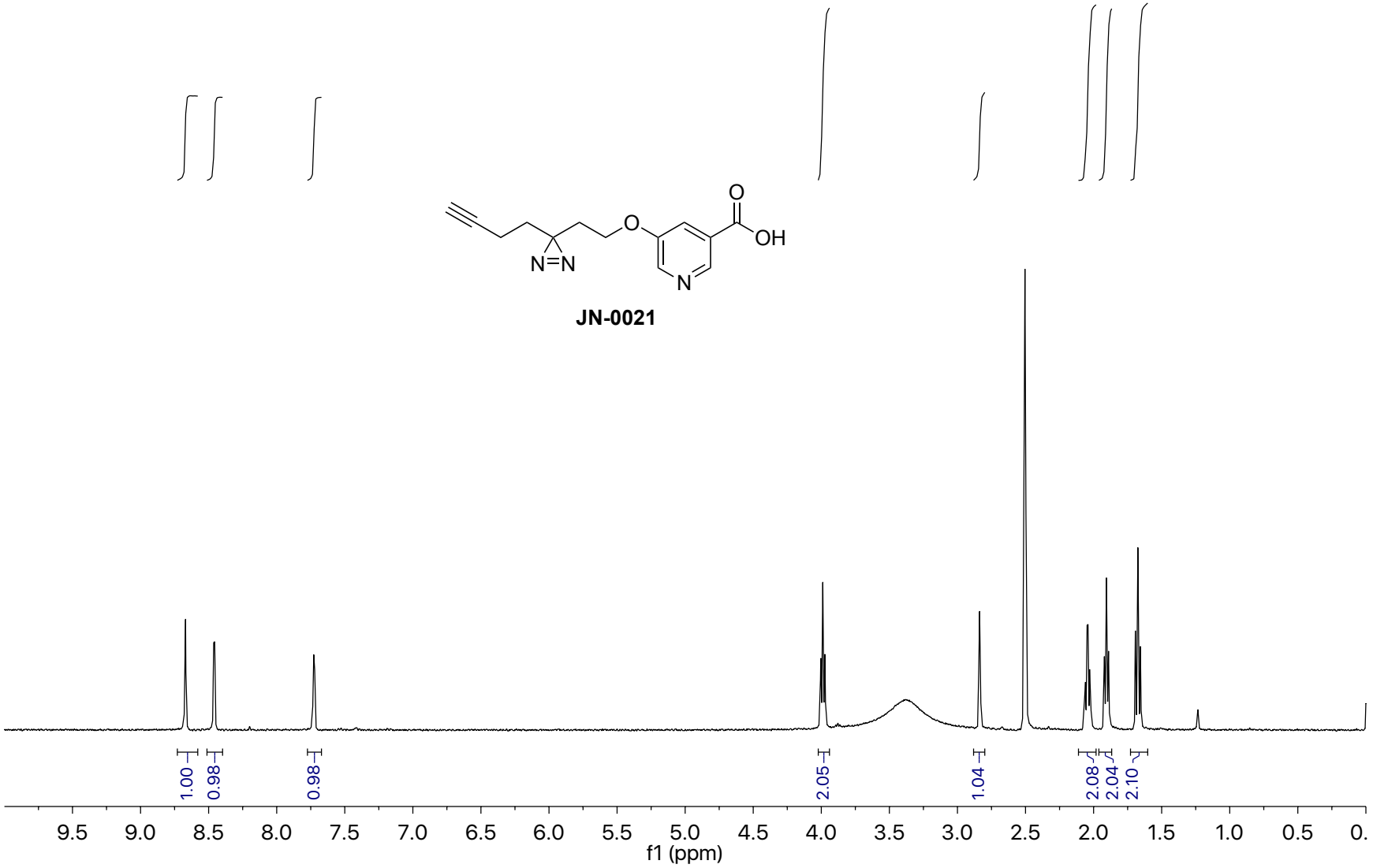
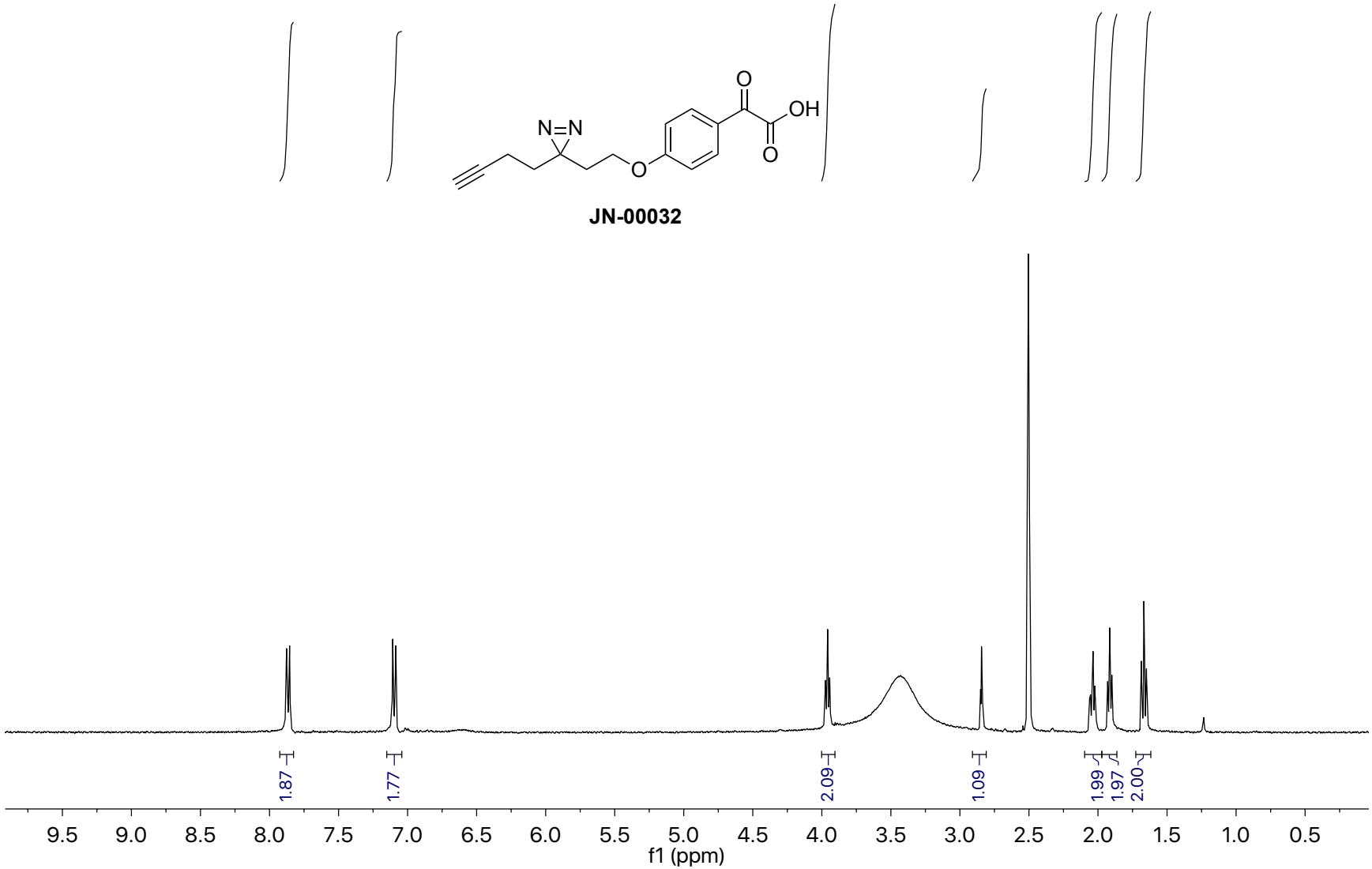
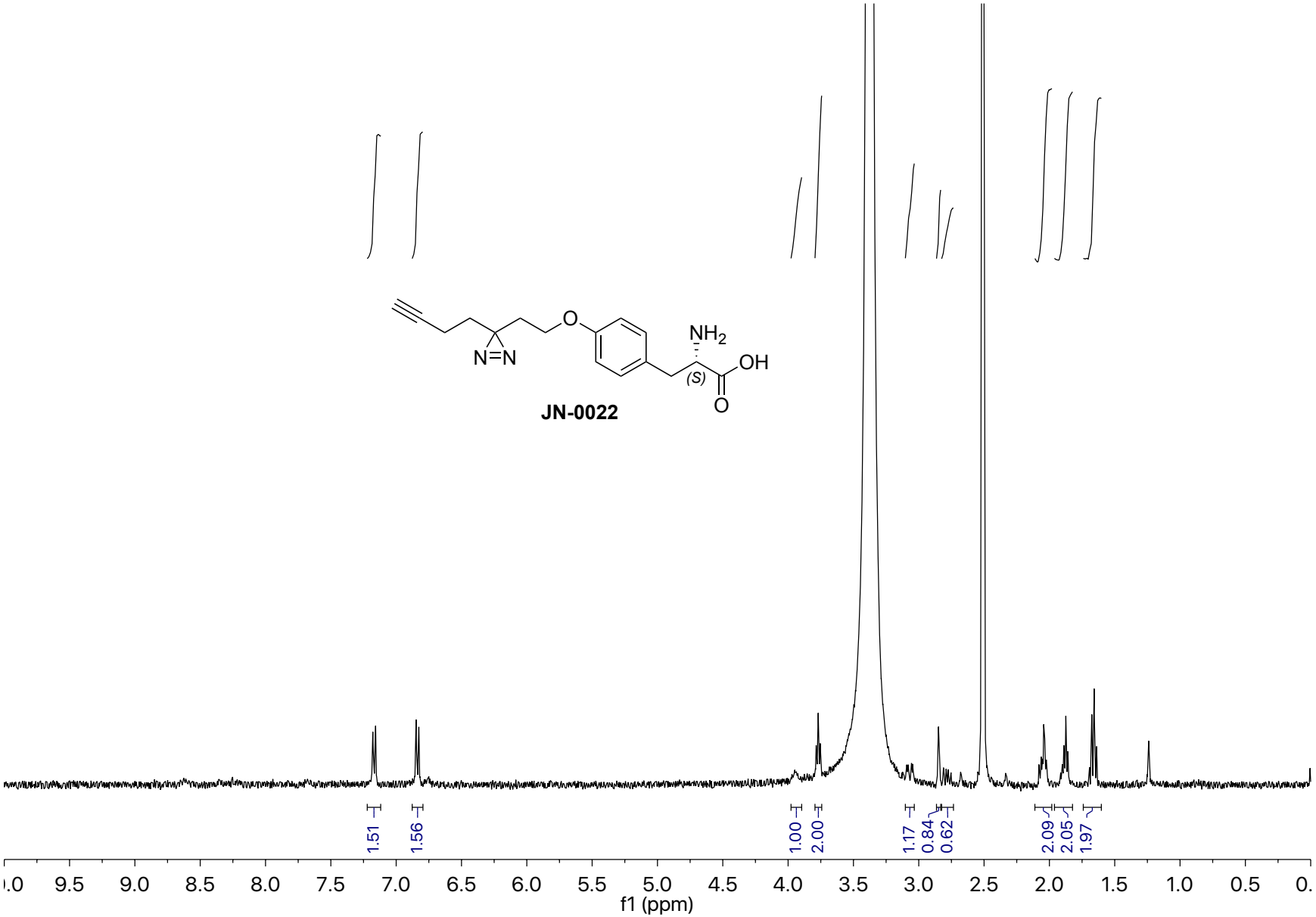

N=N
OH
O
O
JN-0013
2.00
1.98
2.04
2.16
0.94
2.18
2.10
2.06
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

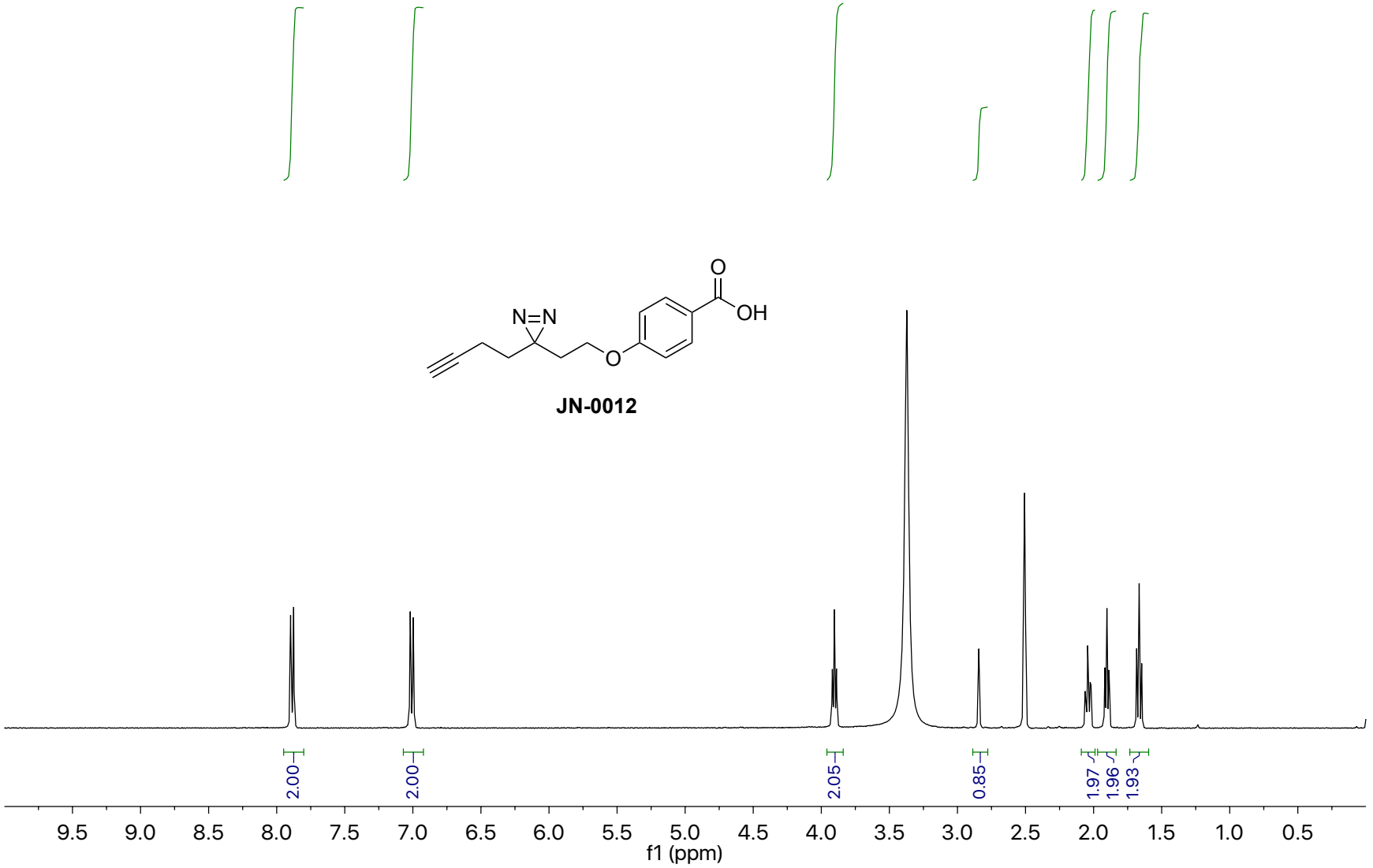

7.25
7.23
6.90
6.90
6.88
6.84
6.84
6.83
6.83
6.82
6.81
6.80
3.84
3.83
3.81
3.66
3.64
2.09
2.09
2.07
2.07
2.05
2.05
2.05
1.99
1.99
1.98
1.90
1.88
1.86
1.76
1.74
1.72
N=N
O
HC
O
OH
JN-0017
0.25
1.00
2.30
2.22
2.15
2.21
1.03
2.24
2.17
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
.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
f1 (ppm)

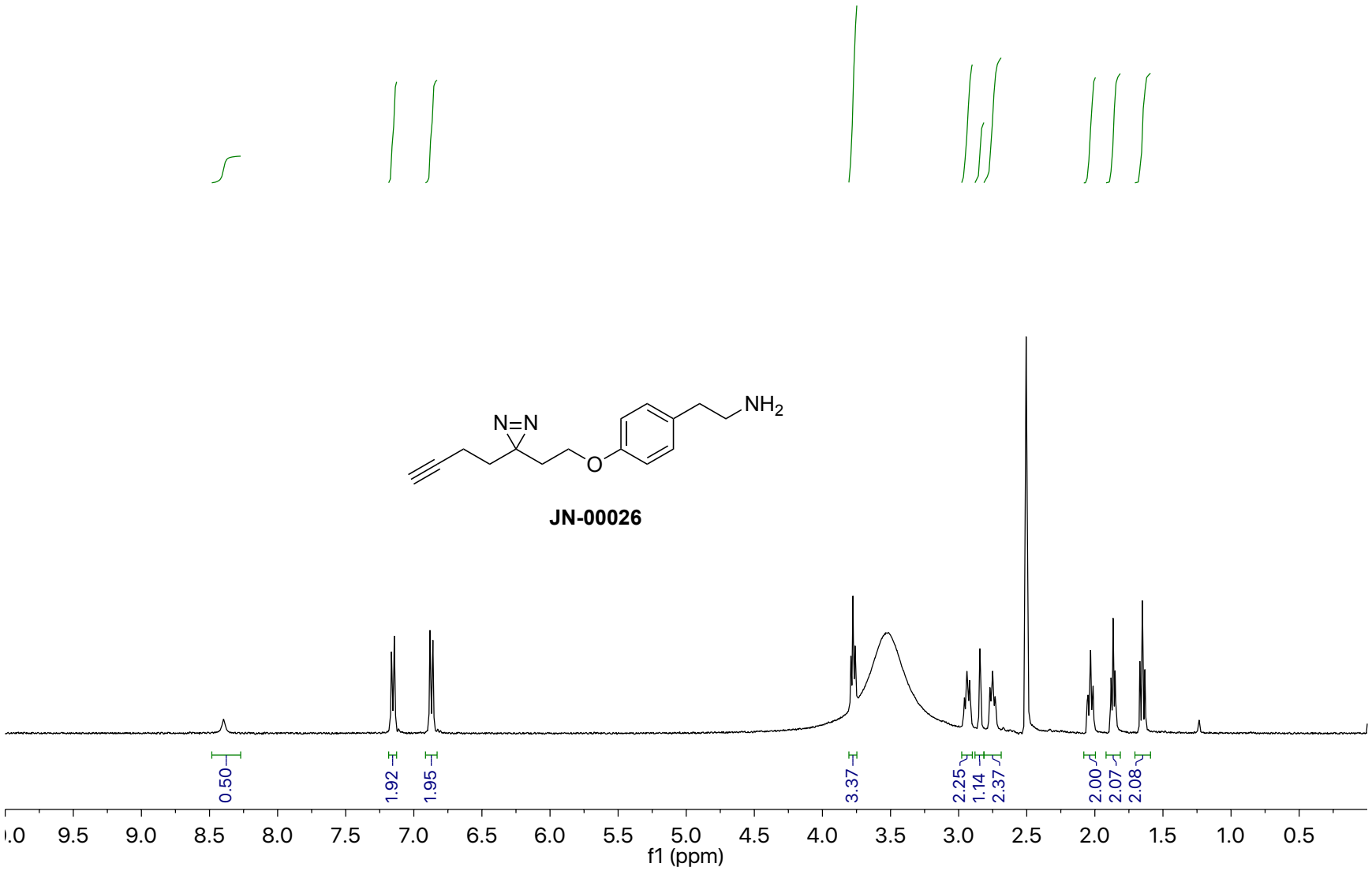

O
OH
O
N=N
JN-0020
1.01
2.03
1.00
2.11
0.93
2.07
2.02
2.01
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

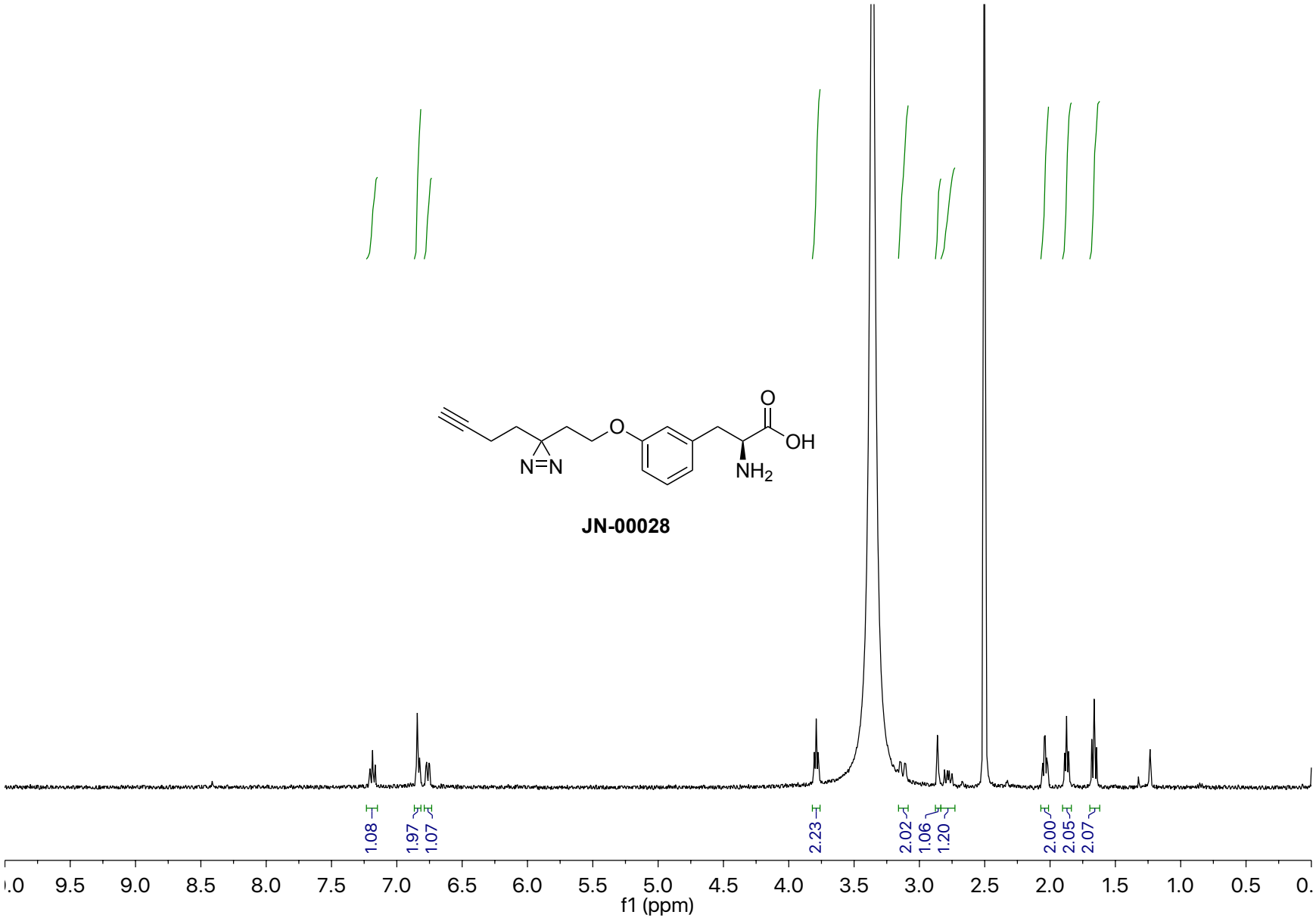

O
OH
O
N=N
N
JN-0021
1.00
0.98
0.98
2.05
1.04
2.08
2.04
2.10
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

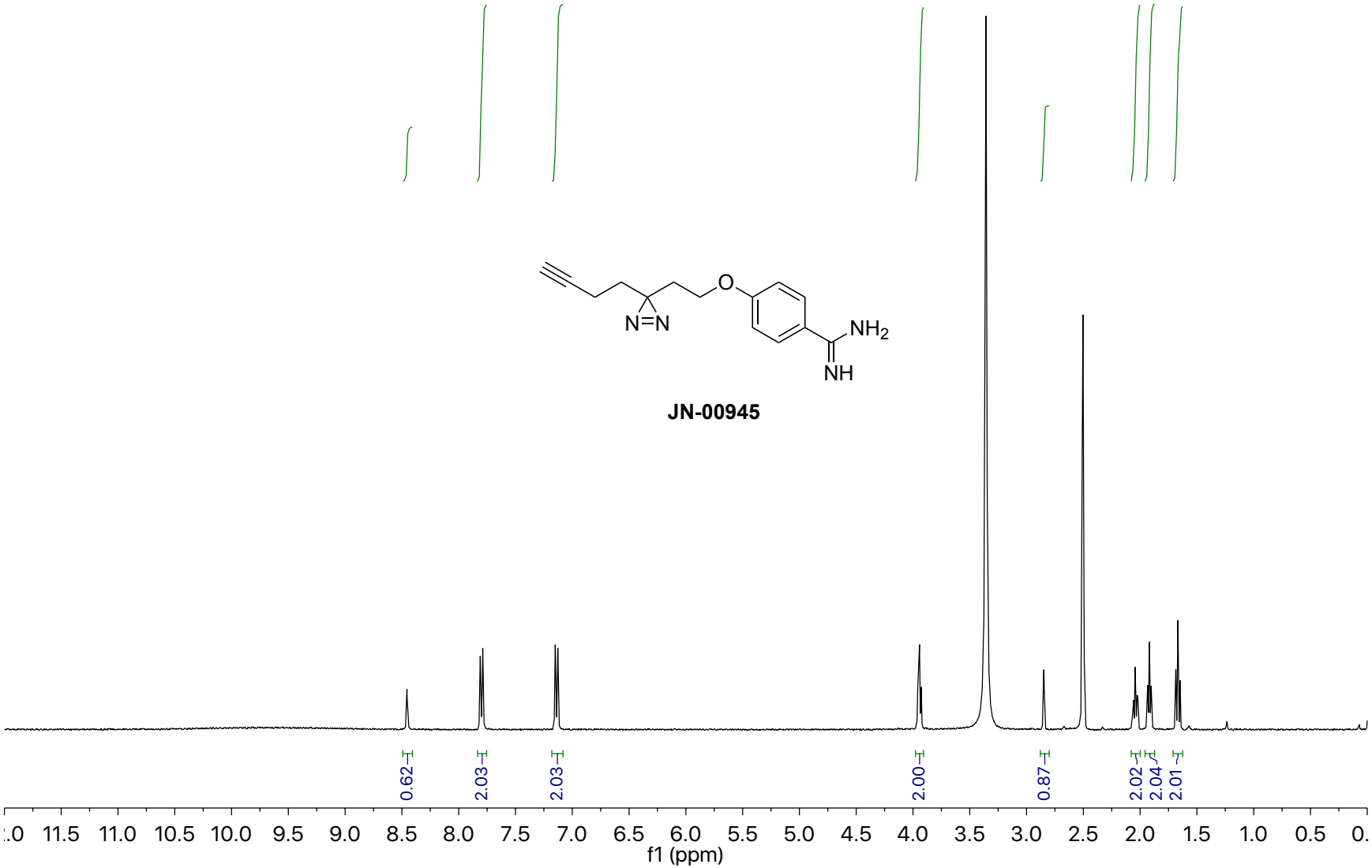

N=N
O
OH
O
O
JN-00032
1.87
1.77
2.09
1.09
1.99
1.97
2.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

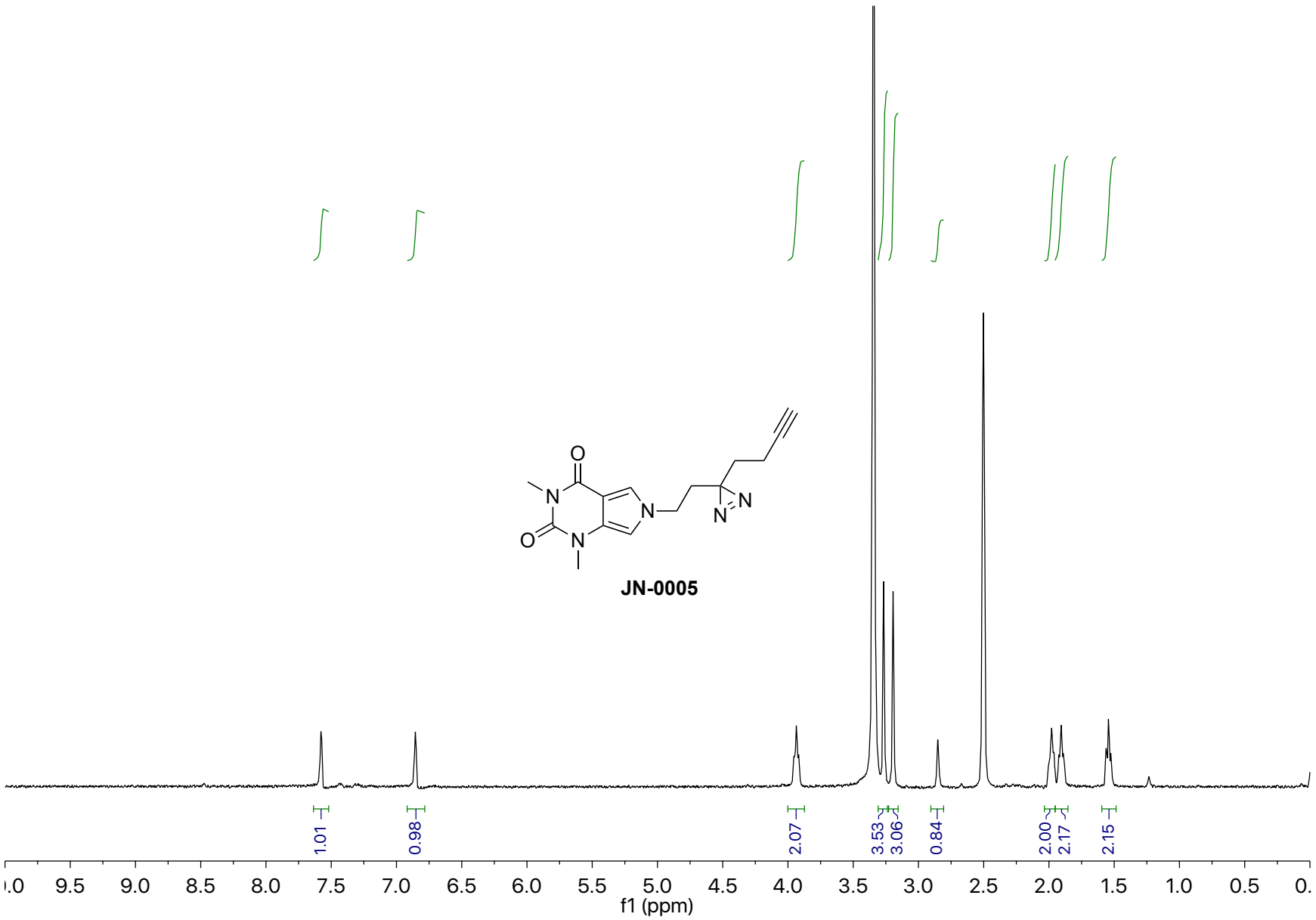

NH2
OH
O
N=N
O
(S)
JN-0022
1.51
1.56
1.00
2.00
1.17
0.84
0.62
2.09
2.05
1.97
f1 (ppm)

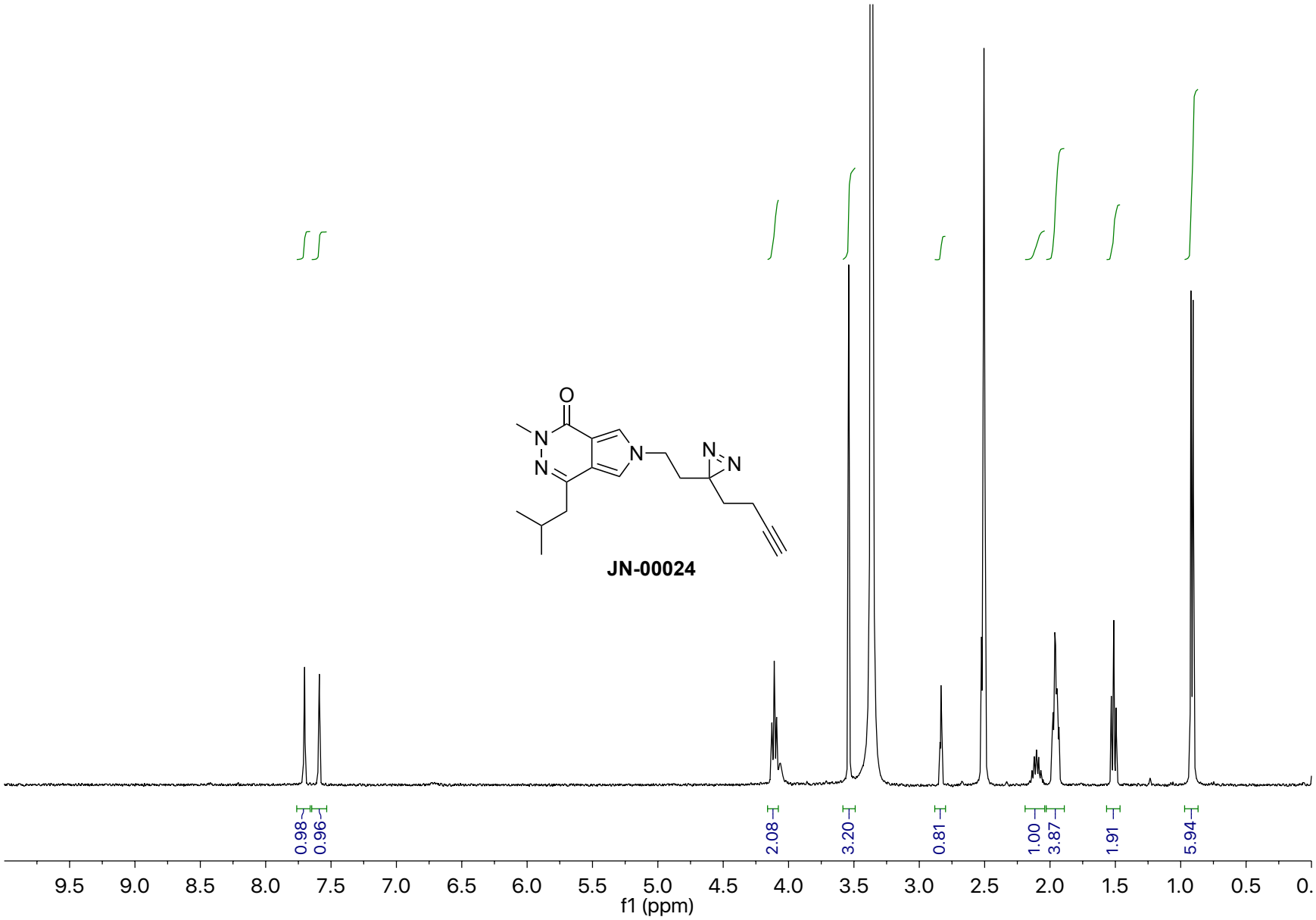

O
OH
N=N
O
JN-0012
2.00
2.00
2.05
0.85
1.97
1.96
1.93
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

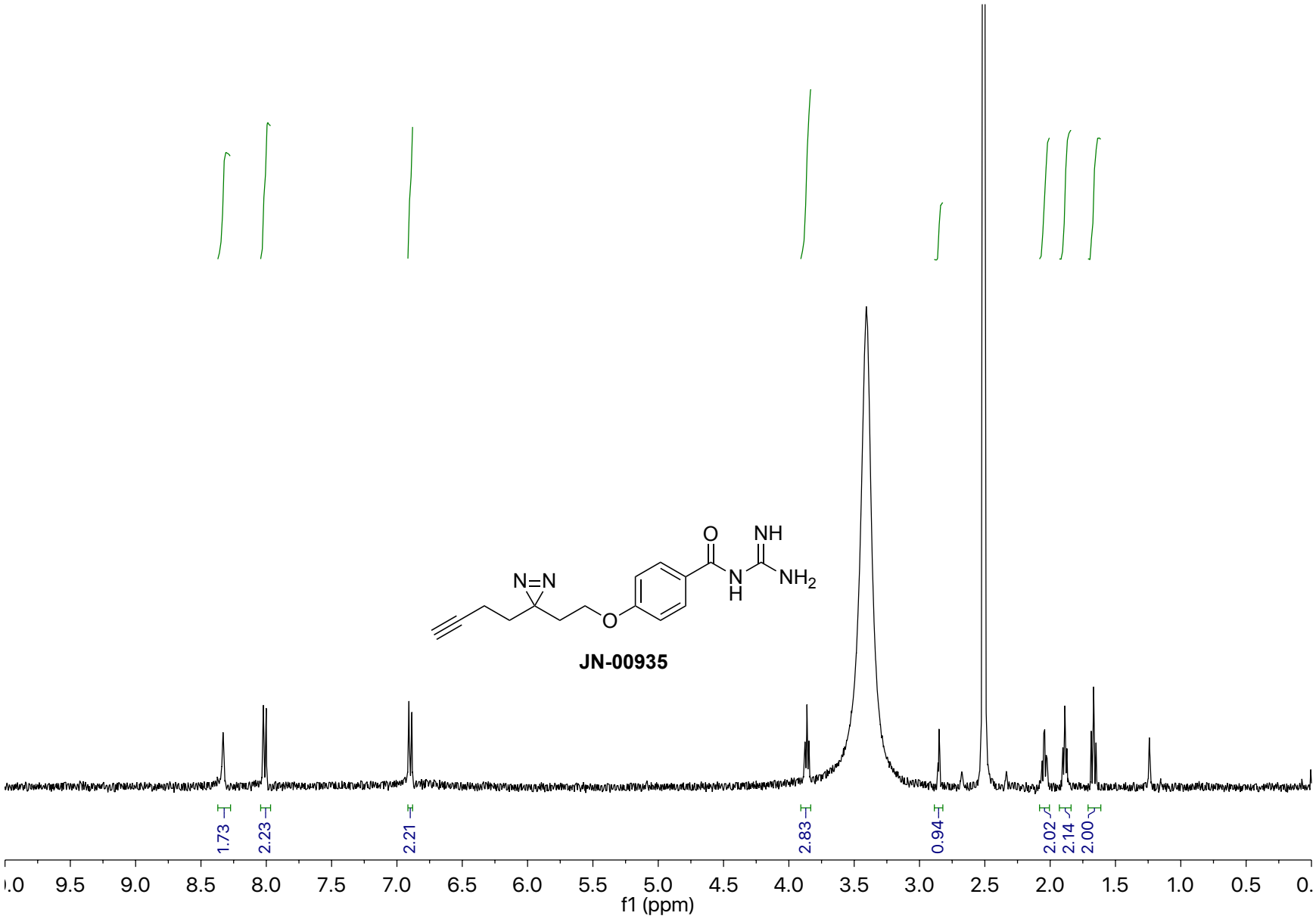

N=N
NH2
O
JN-00026
0.50
1.92
1.95
3.37
2.25
1.14
2.37
2.00
2.07
2.08
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

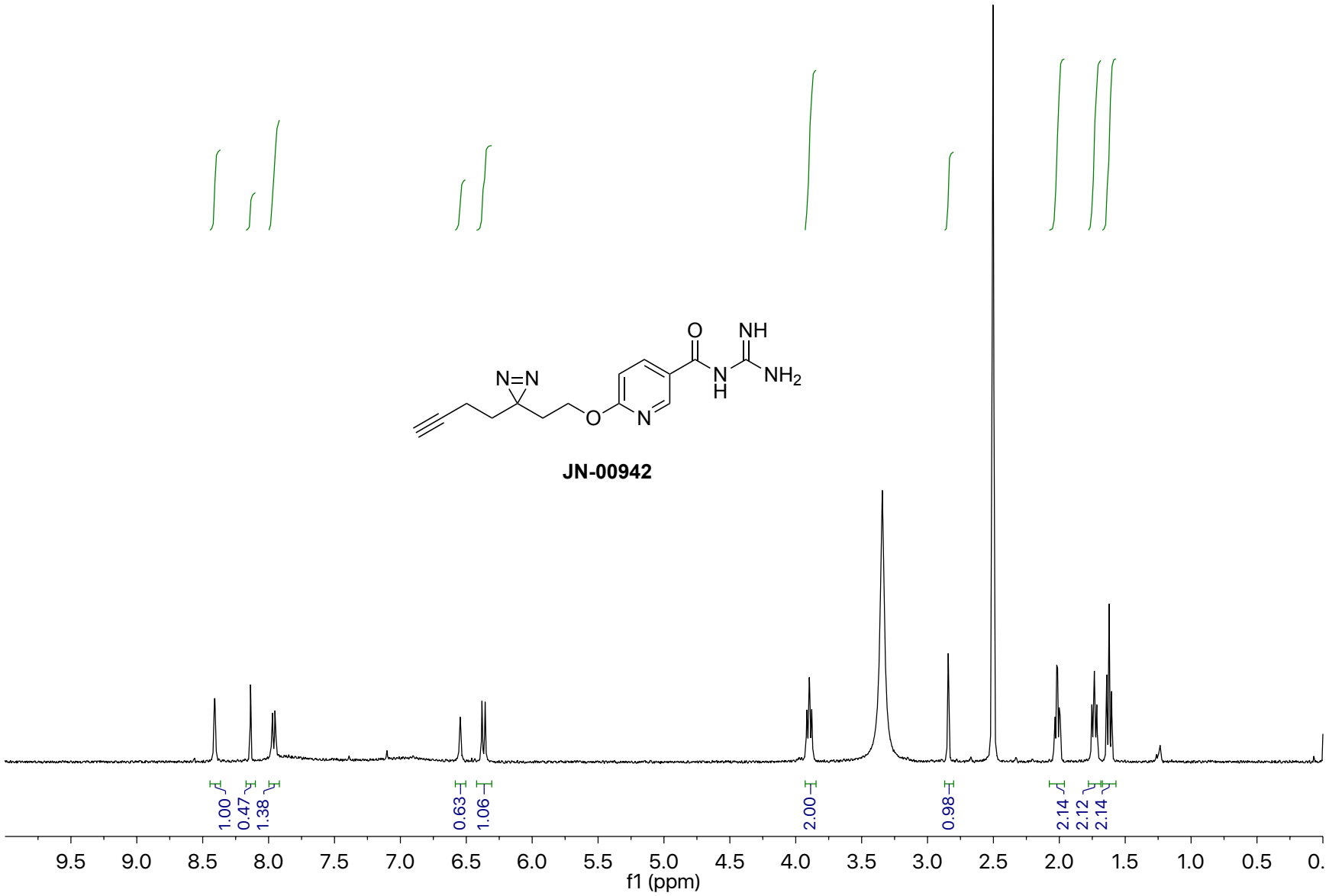

O
OH
N=N
NH2
JN-00028
1.08
1.97
1.07
2.23
2.02
1.06
1.20
2.00
2.05
2.07
f1 (ppm)

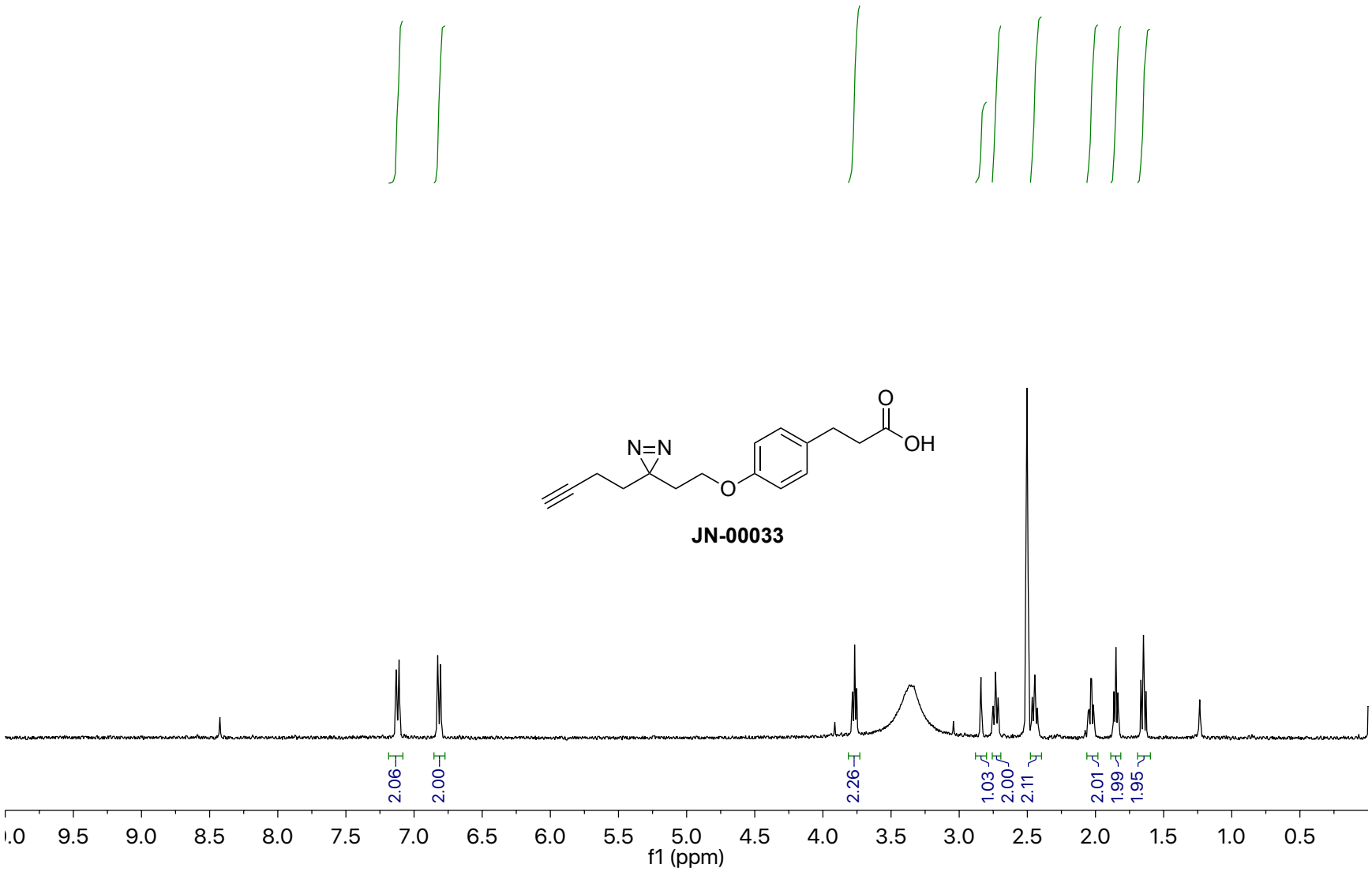

O
N=N
NH2
NH
JN-00945
0.62
2.03
2.03
2.00
0.87
2.02
2.04
2.01
f1 (ppm)

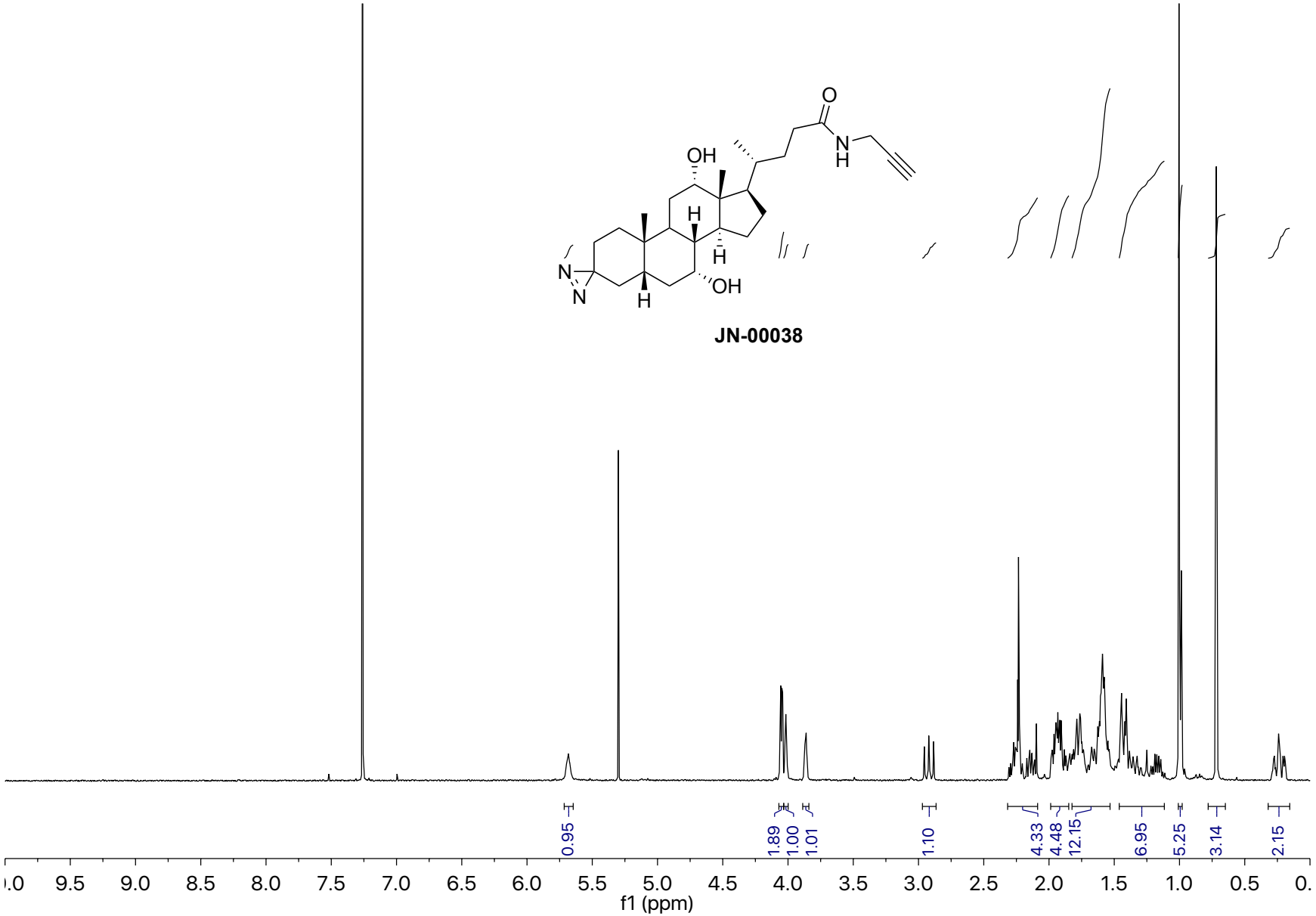

JN-0005
1.01
0.98
2.07
3.53
3.06
0.84
2.00
2.17
2.15
f1 (ppm)

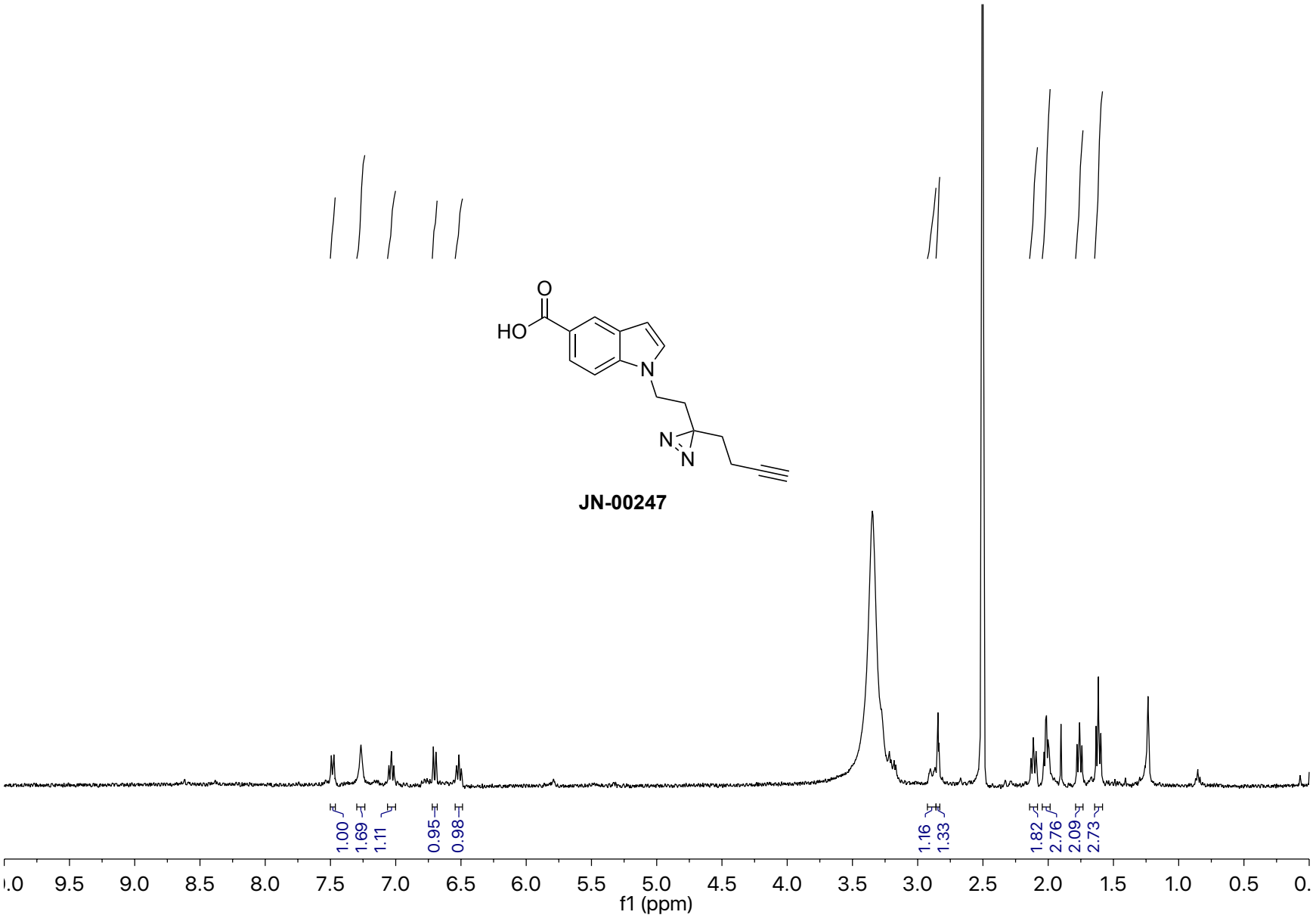

O
N
N
N
N
N
JN-00024
0.98
0.96
2.08
3.20
0.81
1.00
3.87
1.91
5.94
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

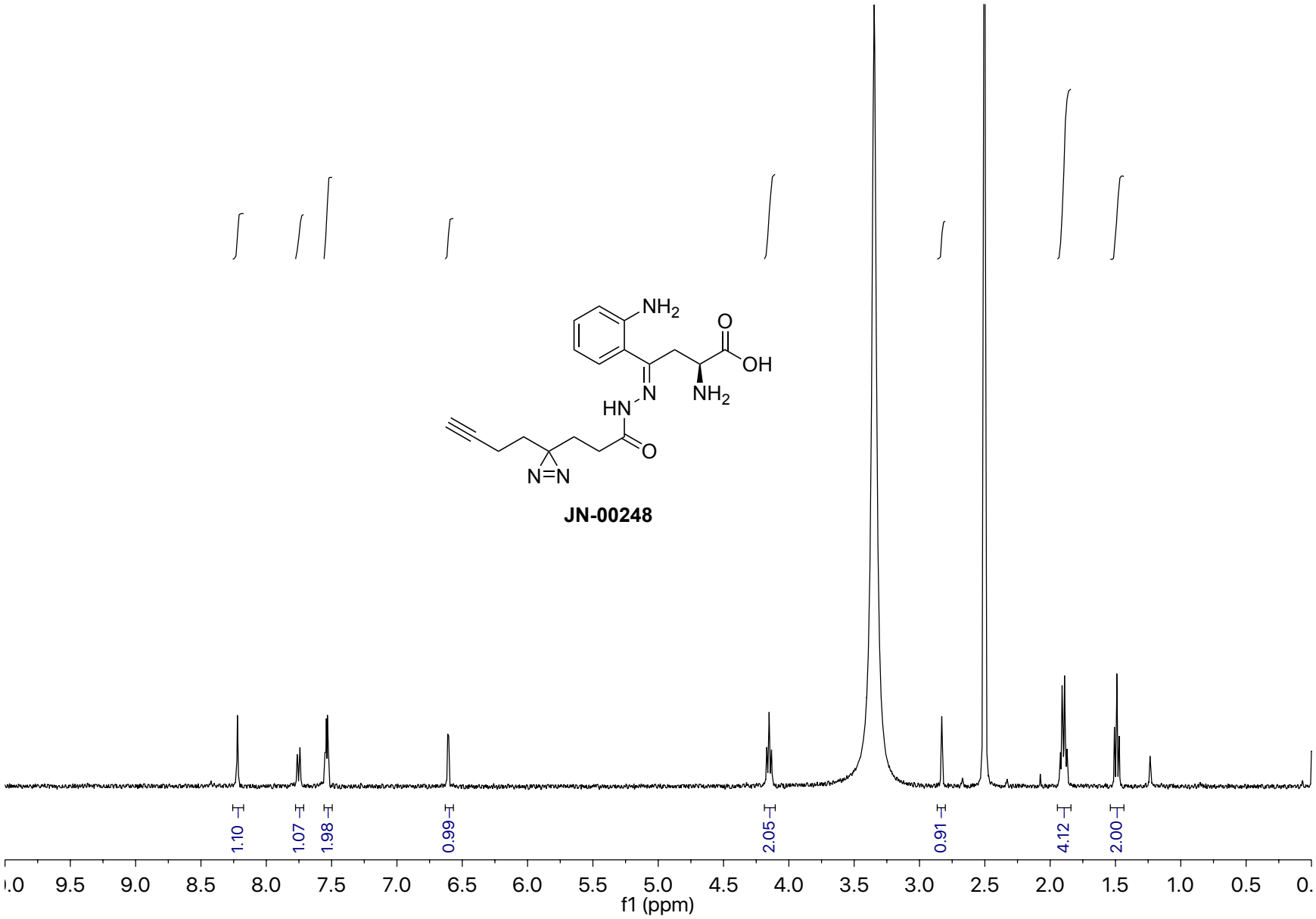

O
NH
N=N
N
H
NH2
O
JN-00935
1.73
2.23
2.21
2.83
0.94
2.02
2.14
2.00
f1 (ppm)

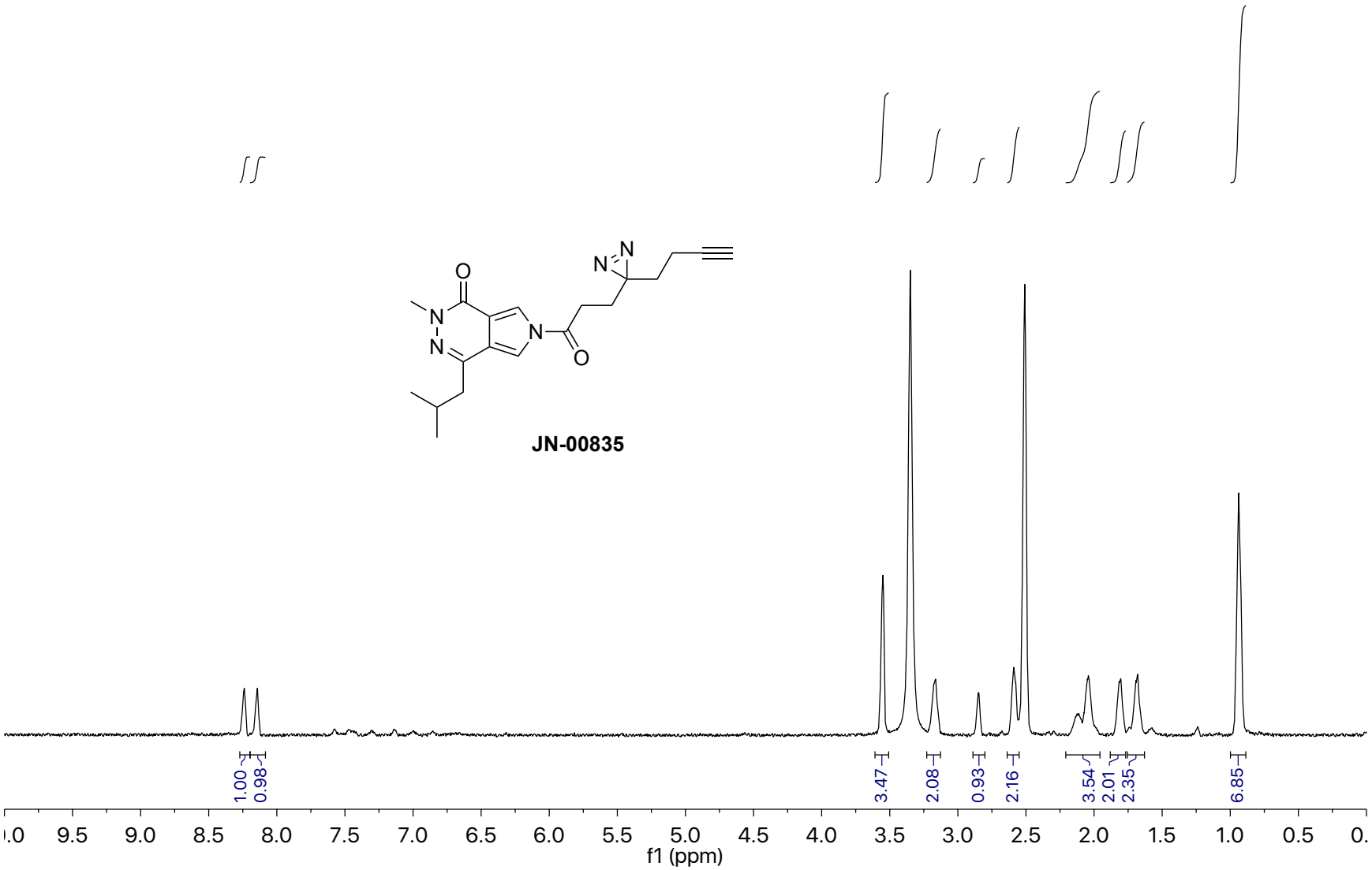

O
NH
N=N
N
H
NH2
O
N
JN-00942
1.00
0.47
1.38
0.63
1.06
2.00
0.98
2.14
2.12
2.14
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

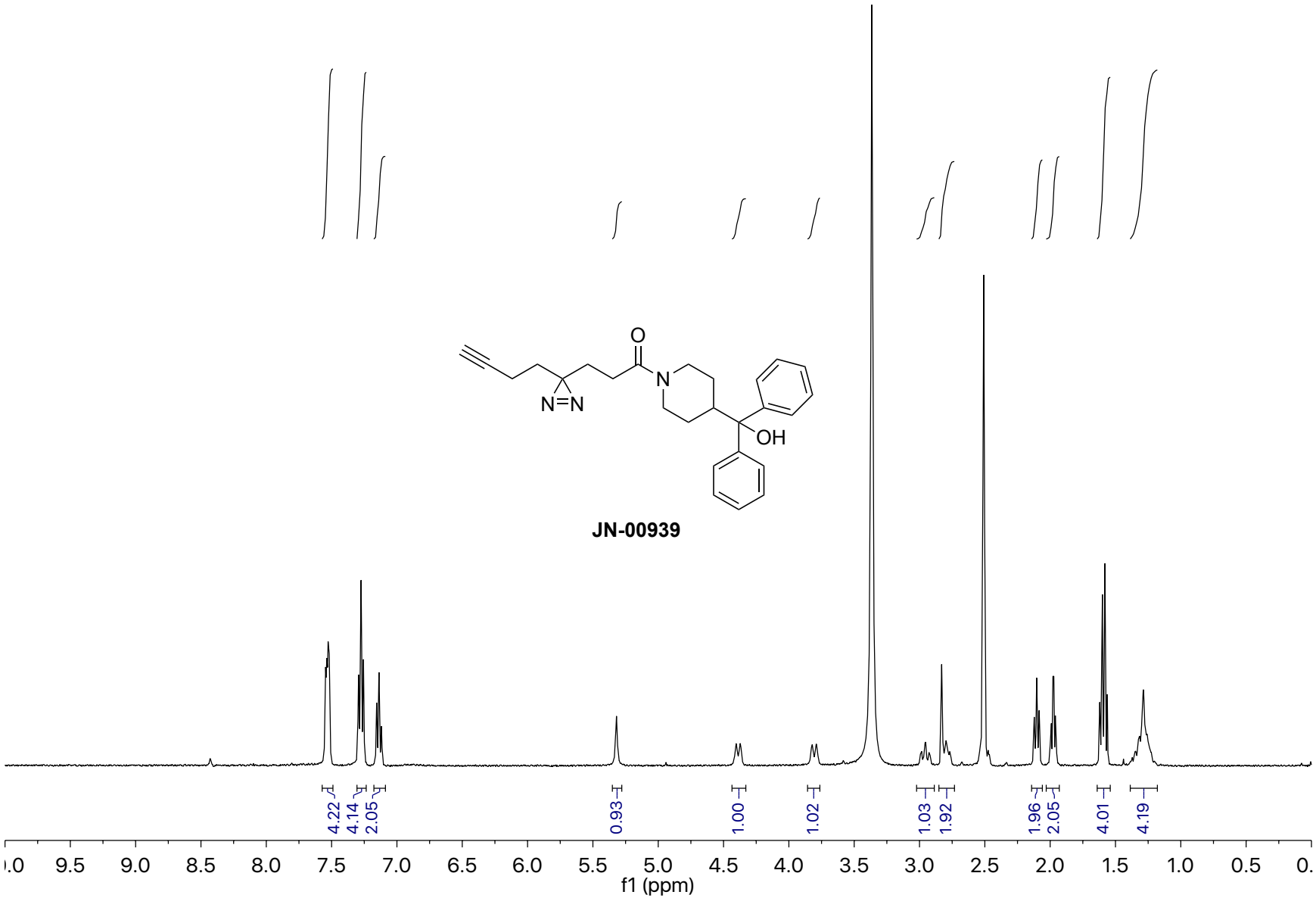

N=N
O
O
OH
JN-00033
2.06
2.00
2.26
1.03
2.00
2.11
2.01
1.99
1.95
f1 (ppm)

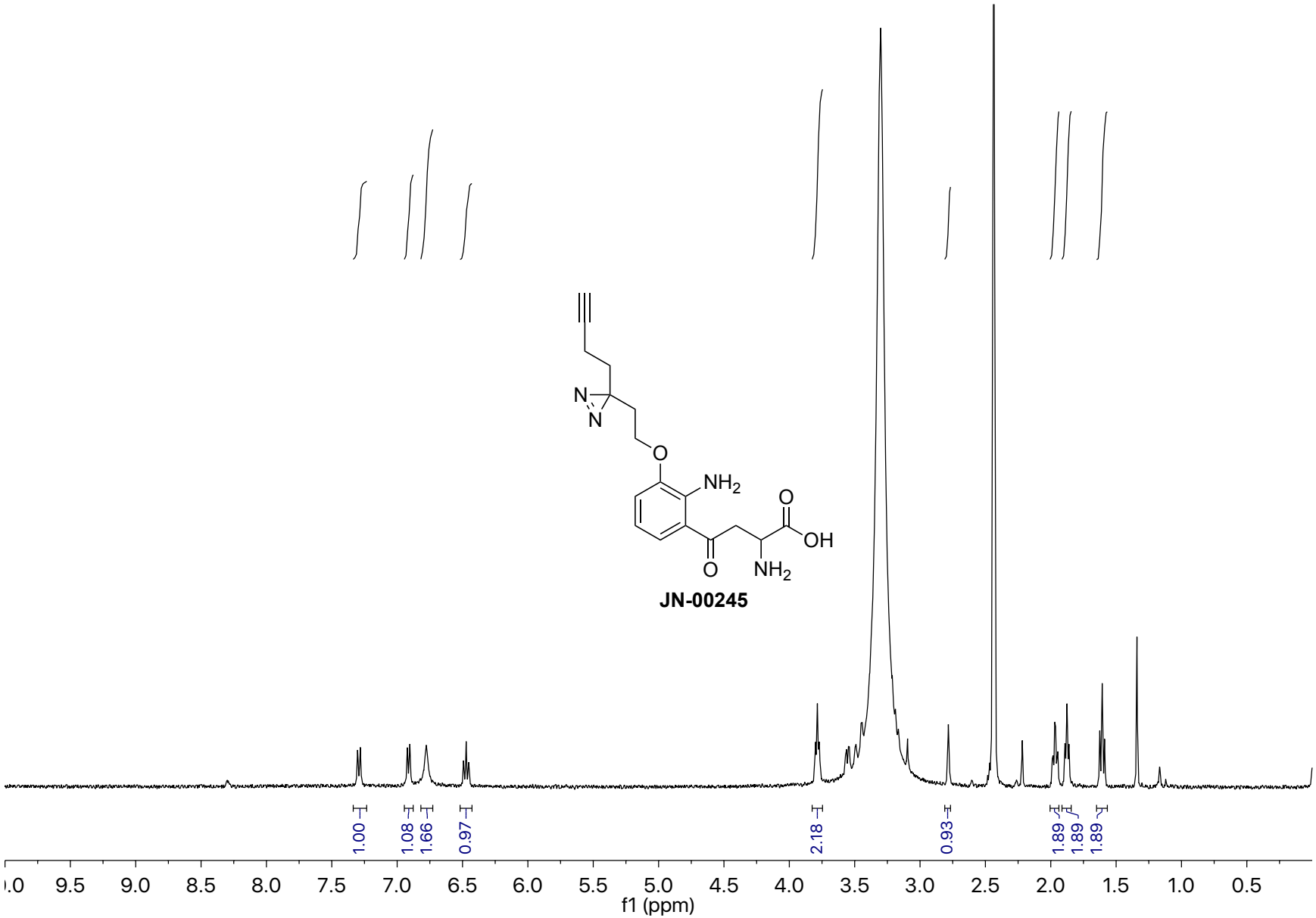

O
N
H
OH
H
H
N
N
H
OH
JN-00038
0.95
1.89
1.00
1.01
1.10
4.33
4.48
12.15
6.95
5.25
3.14
2.15
f1 (ppm)

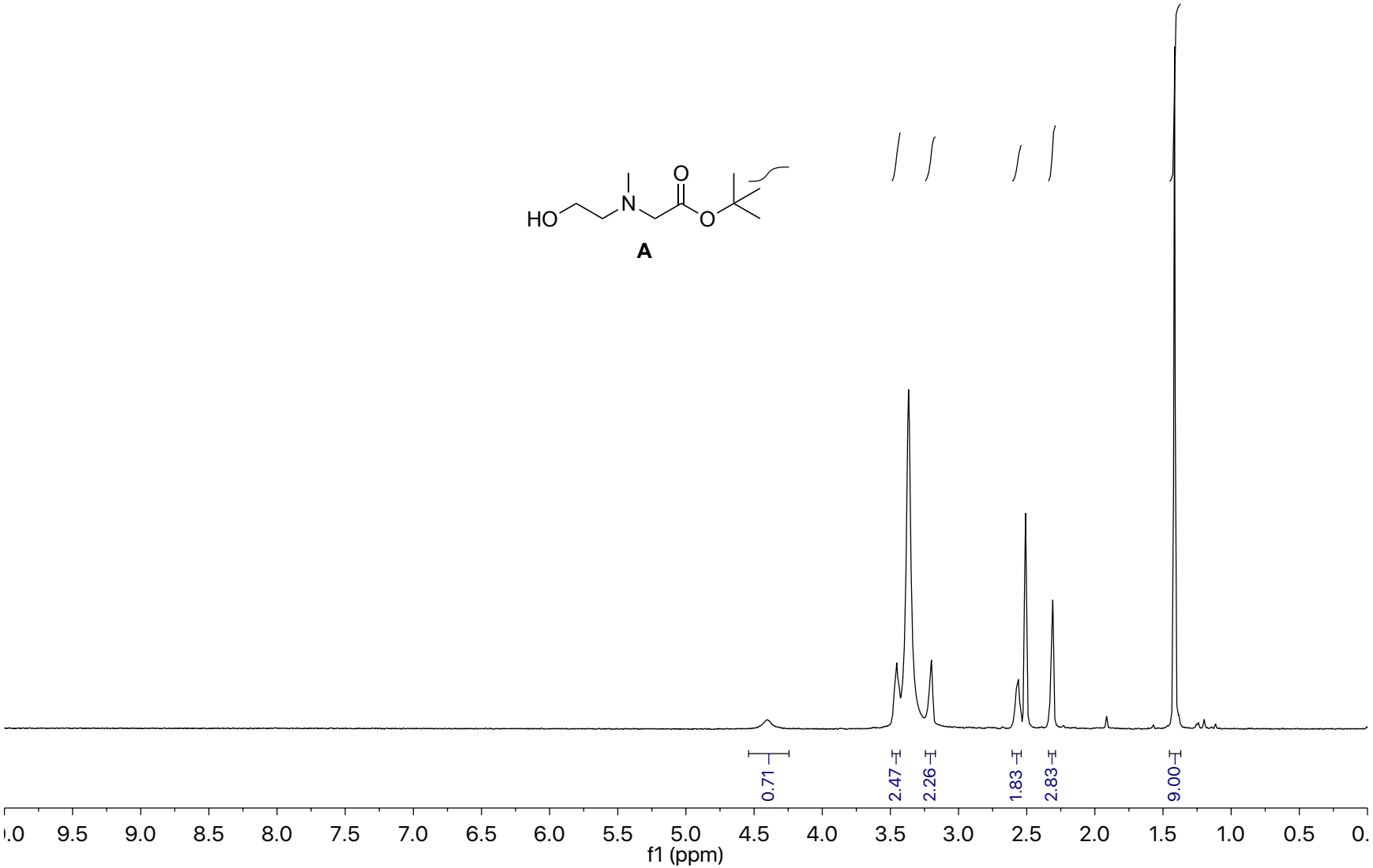

O
HO
N
N
N
JN-00247
1.00
1.69
1.11
0.95
0.98
1.16
1.33
1.82
2.76
2.09
2.73
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

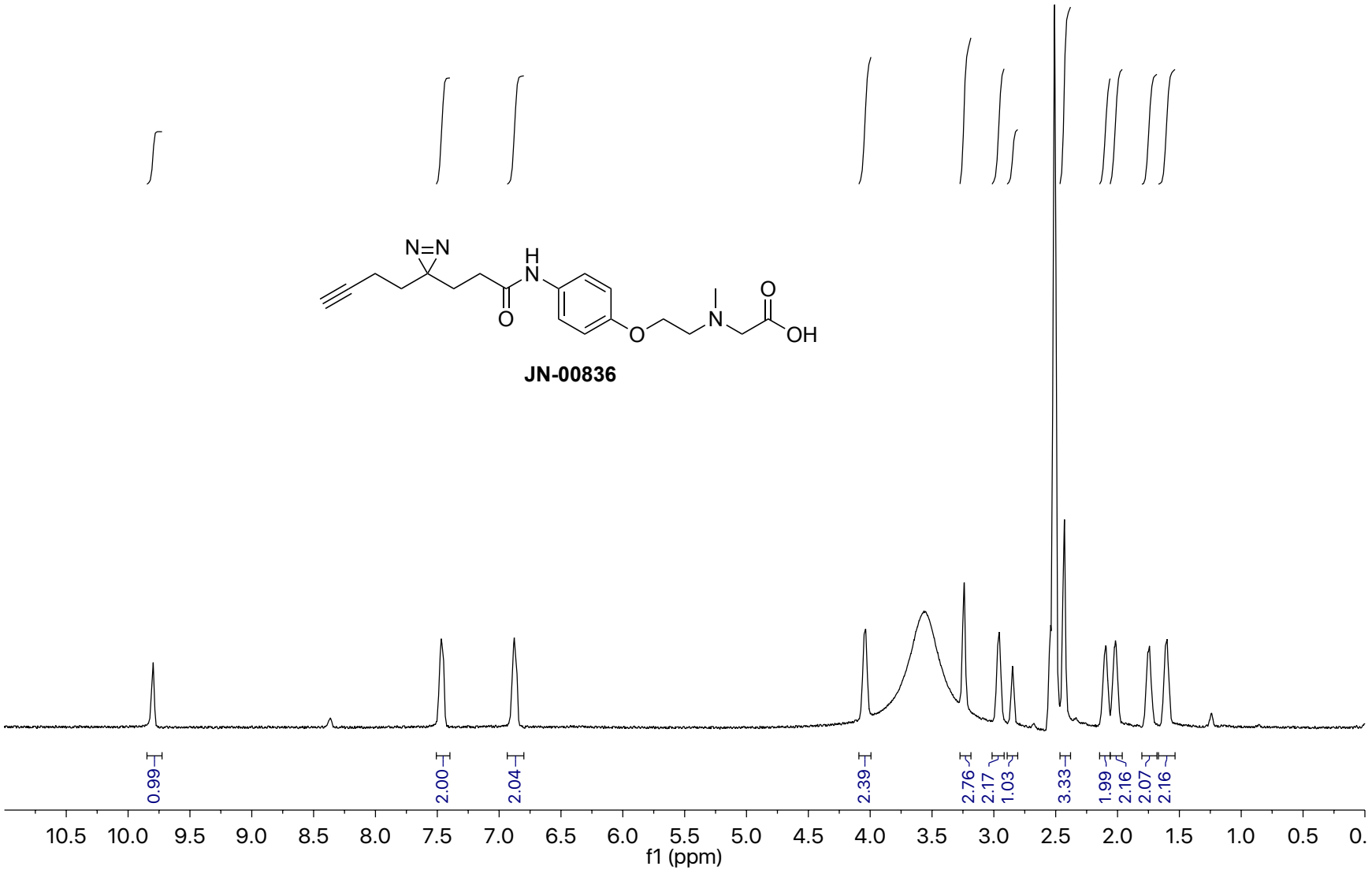

NH2
O
OH
N
NH2
HN
O
N=N
JN-00248
1.10
1.07
1.98
0.99
2.05
0.91
4.12
2.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

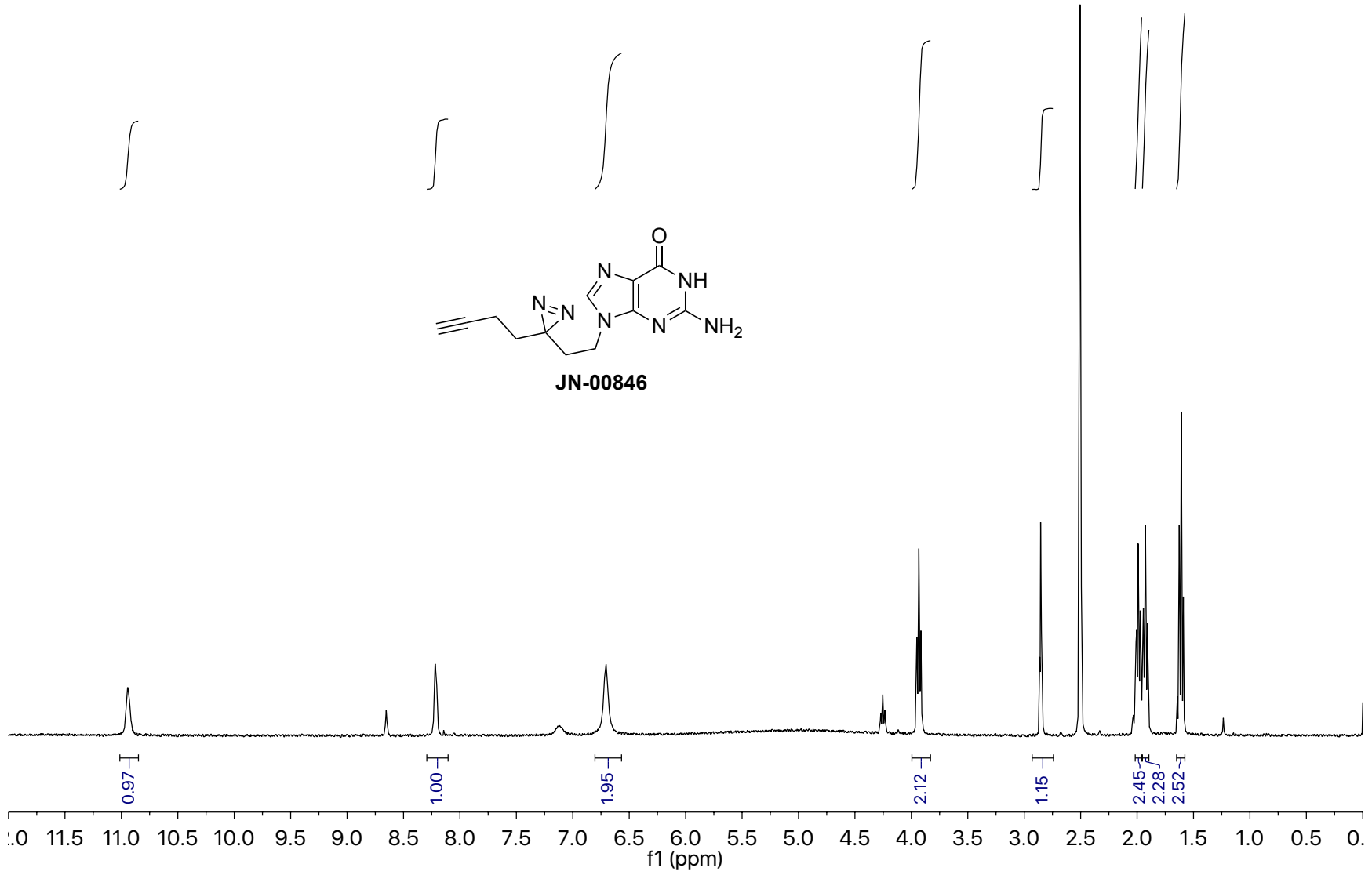

JN-00835
1.00
0.98
3.47
2.08
0.93
2.16
3.54
2.01
2.35
6.85
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

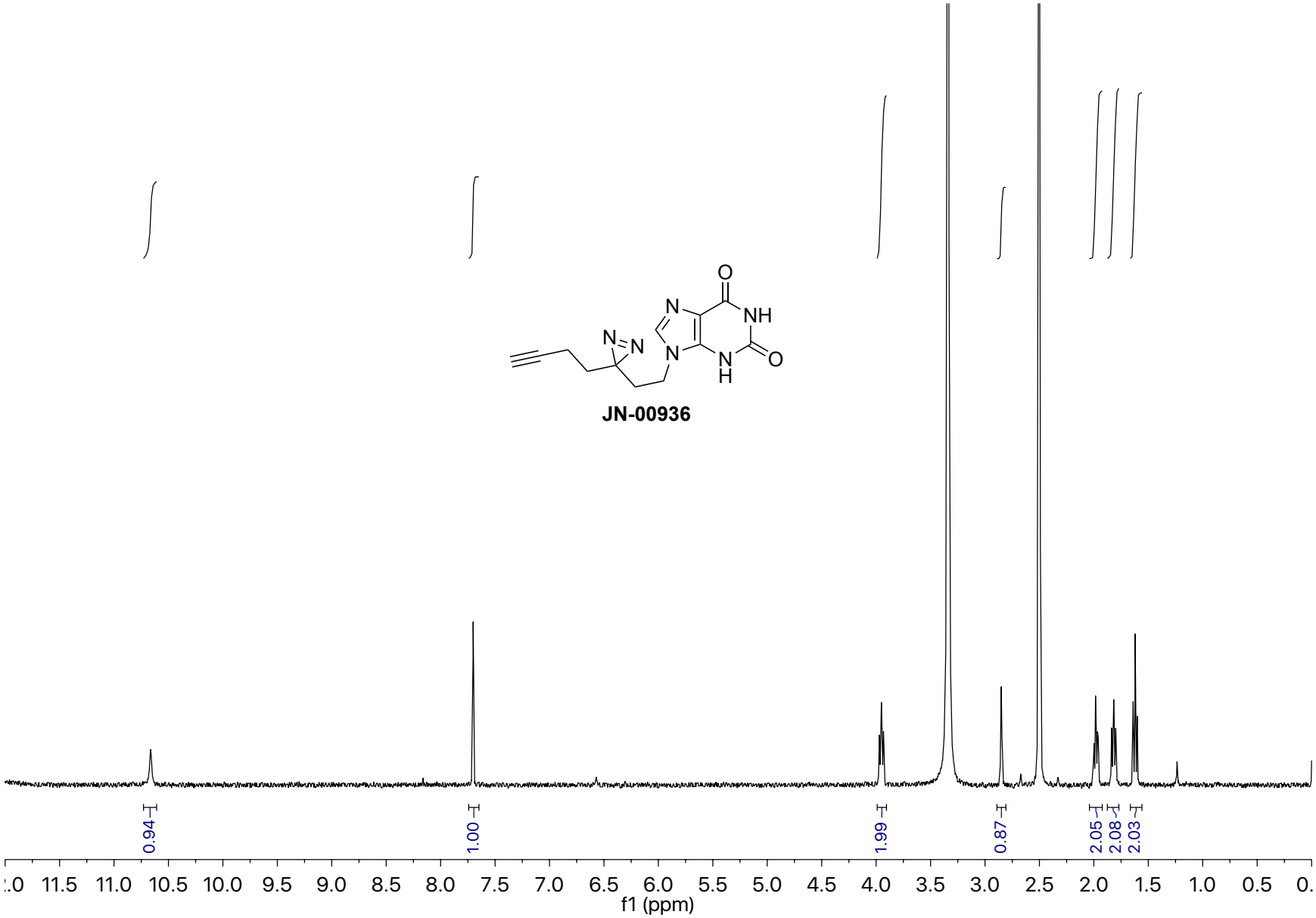

O
N
N=N
OH
JN-00939
4.22
4.14
2.05
0.93
1.00
1.02
1.03
1.92
1.96
2.05
4.01
4.19
f1 (ppm)

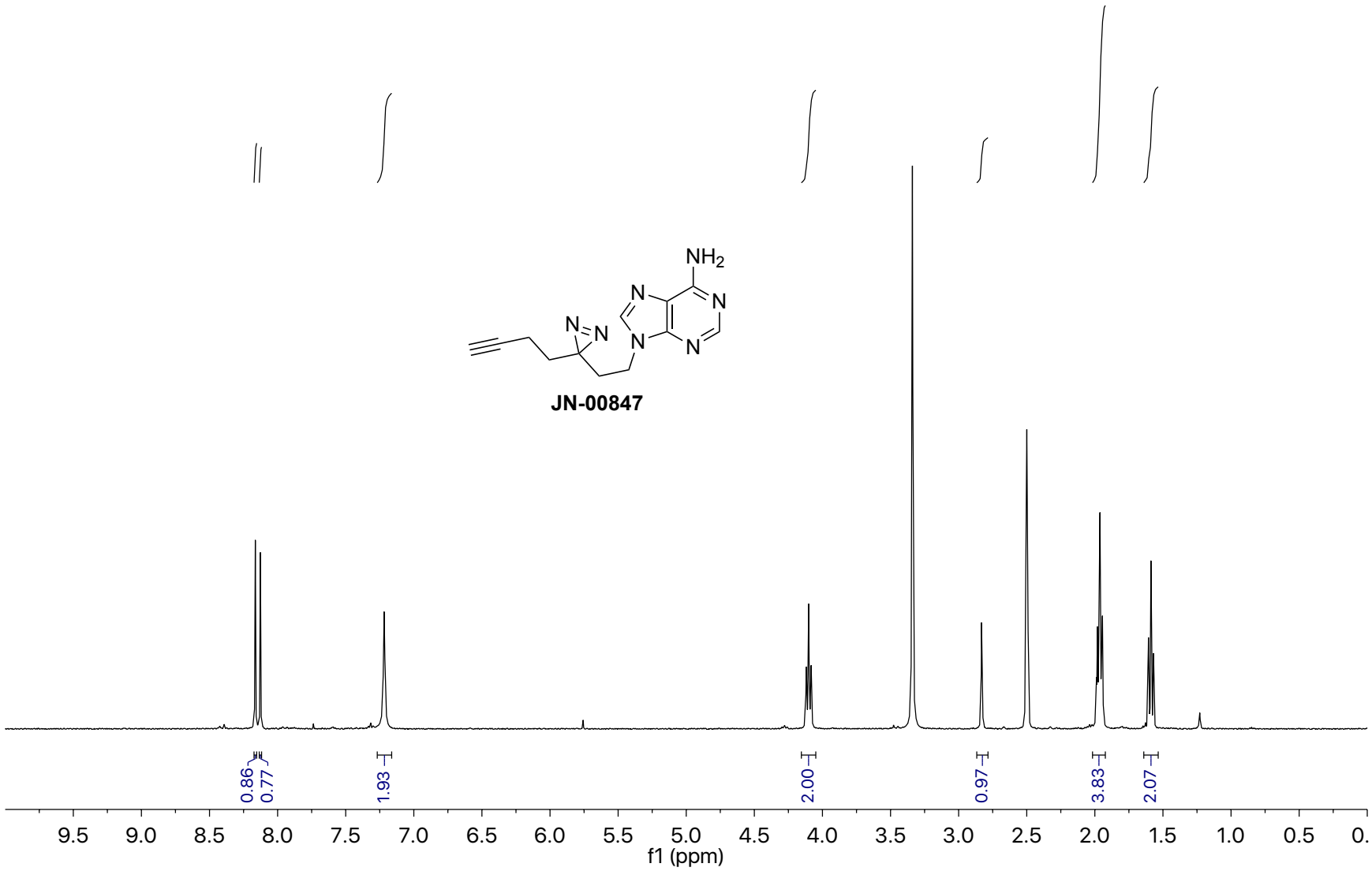

JN-00245
1.00
1.08
1.66
0.97
2.18
0.93
1.89
1.89
1.89
f1 (ppm)

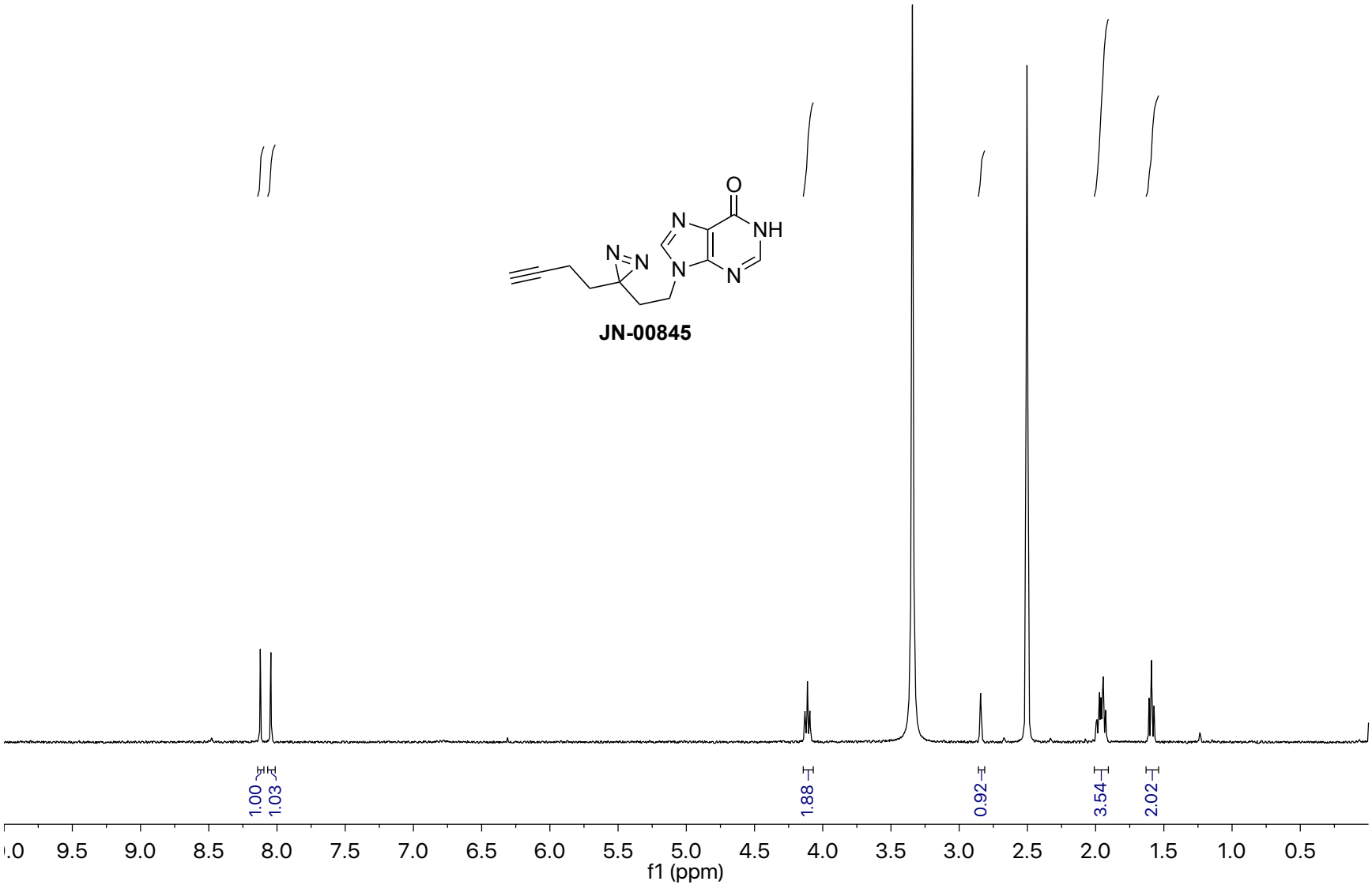

HO
N
O
O
A
0.71
2.47
2.26
1.83
2.83
9.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

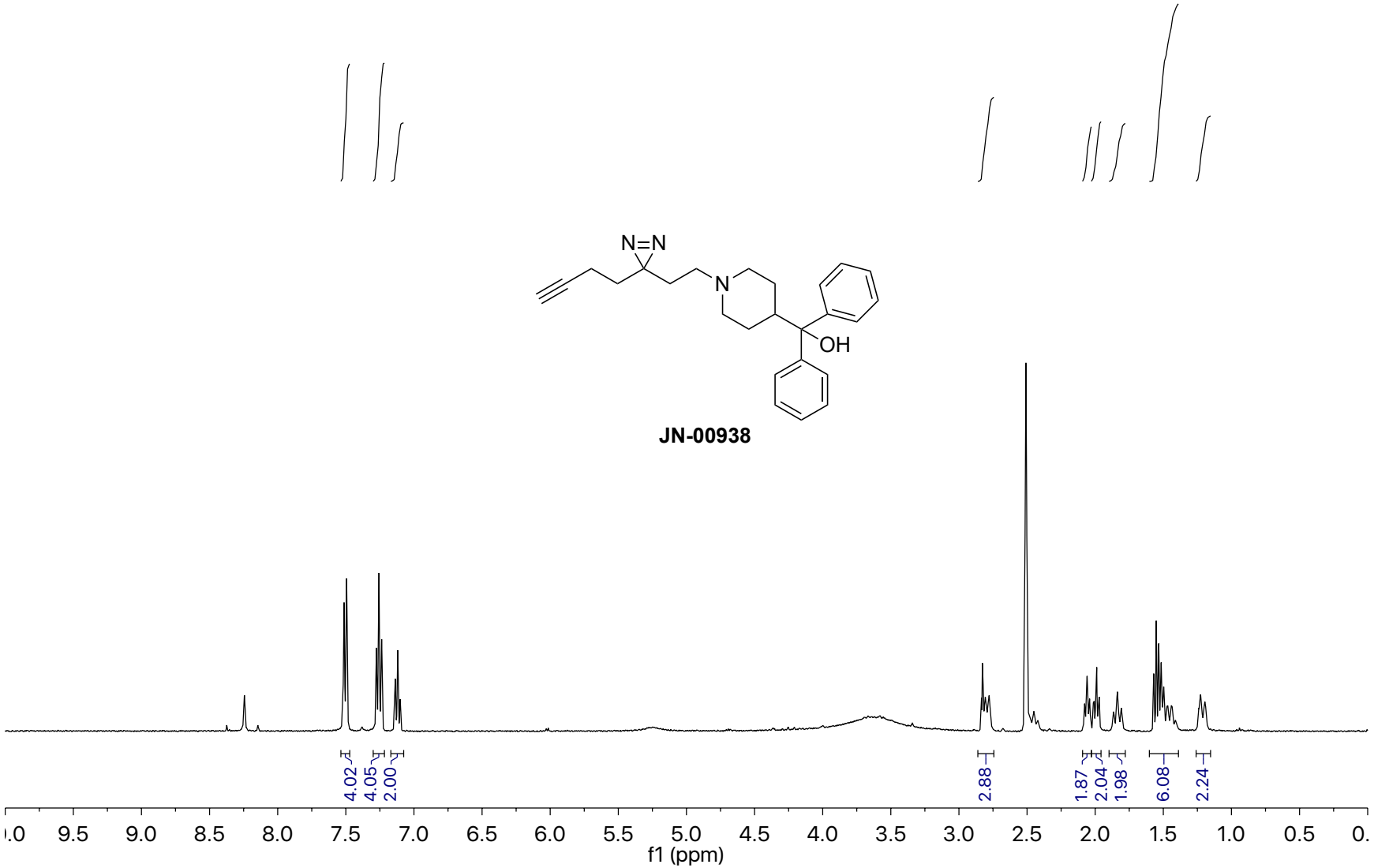

N=N
H
N
O
O
N
O
OH
JN-00836
0.99
2.00
2.04
2.39
2.76
2.17
1.03
3.33
1.99
2.16
2.07
2.16
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

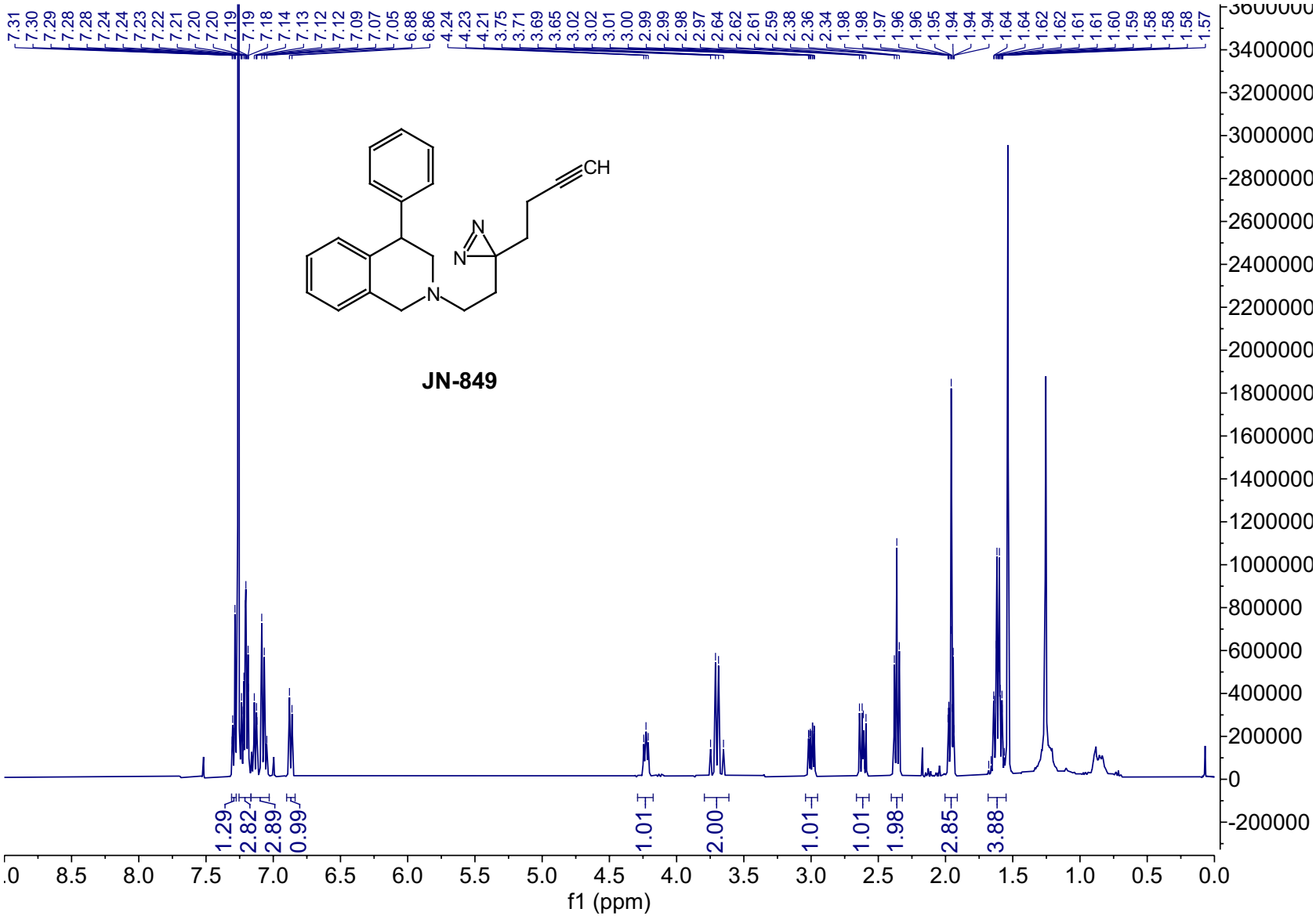

O
N
NH
N
N
N
N
NH2
JN-00846
0.97
1.00
1.95
2.12
1.15
2.45
2.28
2.52
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

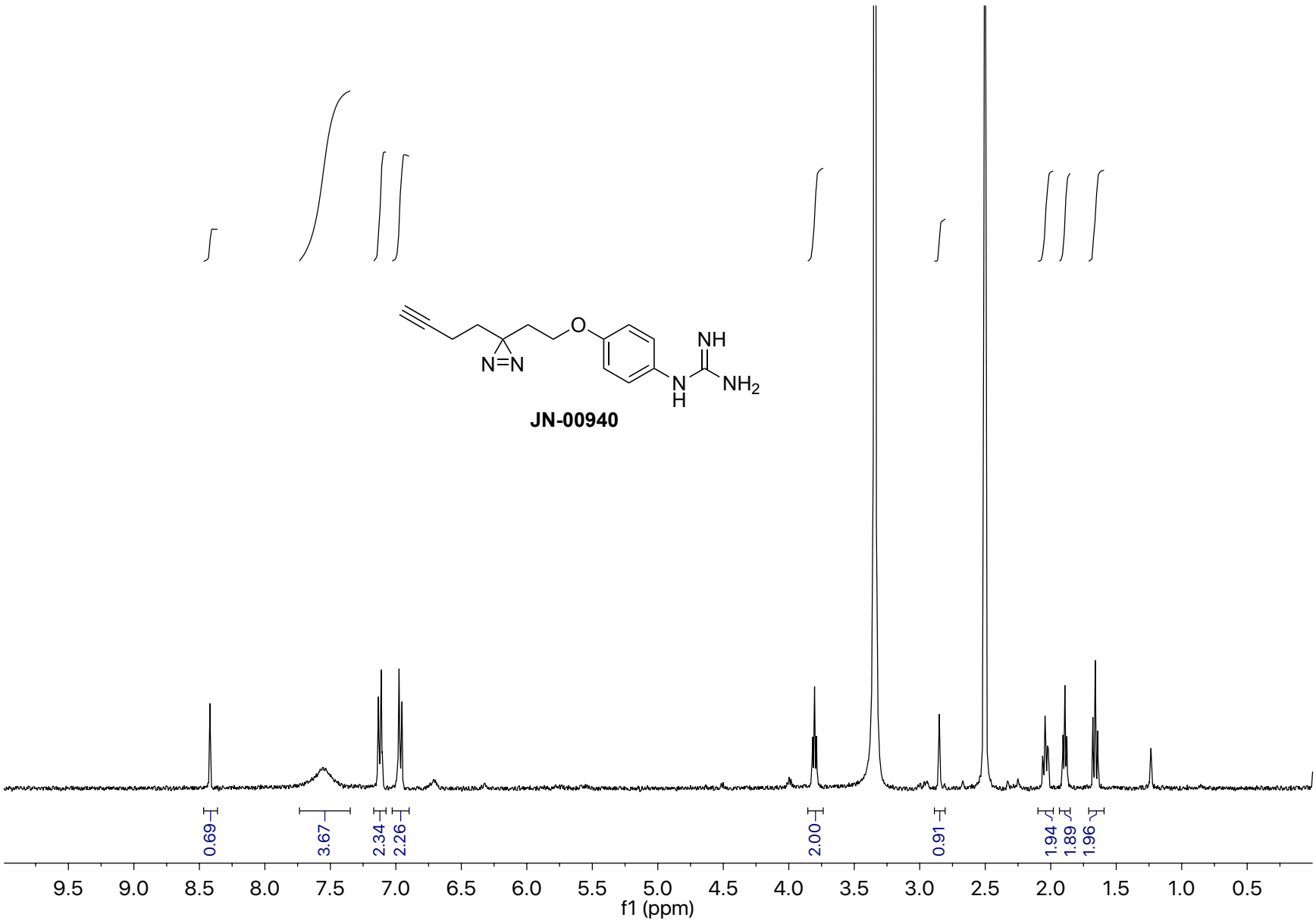

JN-00936
0.94
1.00
1.99
0.87
2.05
2.08
2.03
f1 (ppm)


NH2
N
N
N
N
N
N
JN-00847
0.86
0.77
1.93
2.00
0.97
3.83
2.07
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.
f1 (ppm)

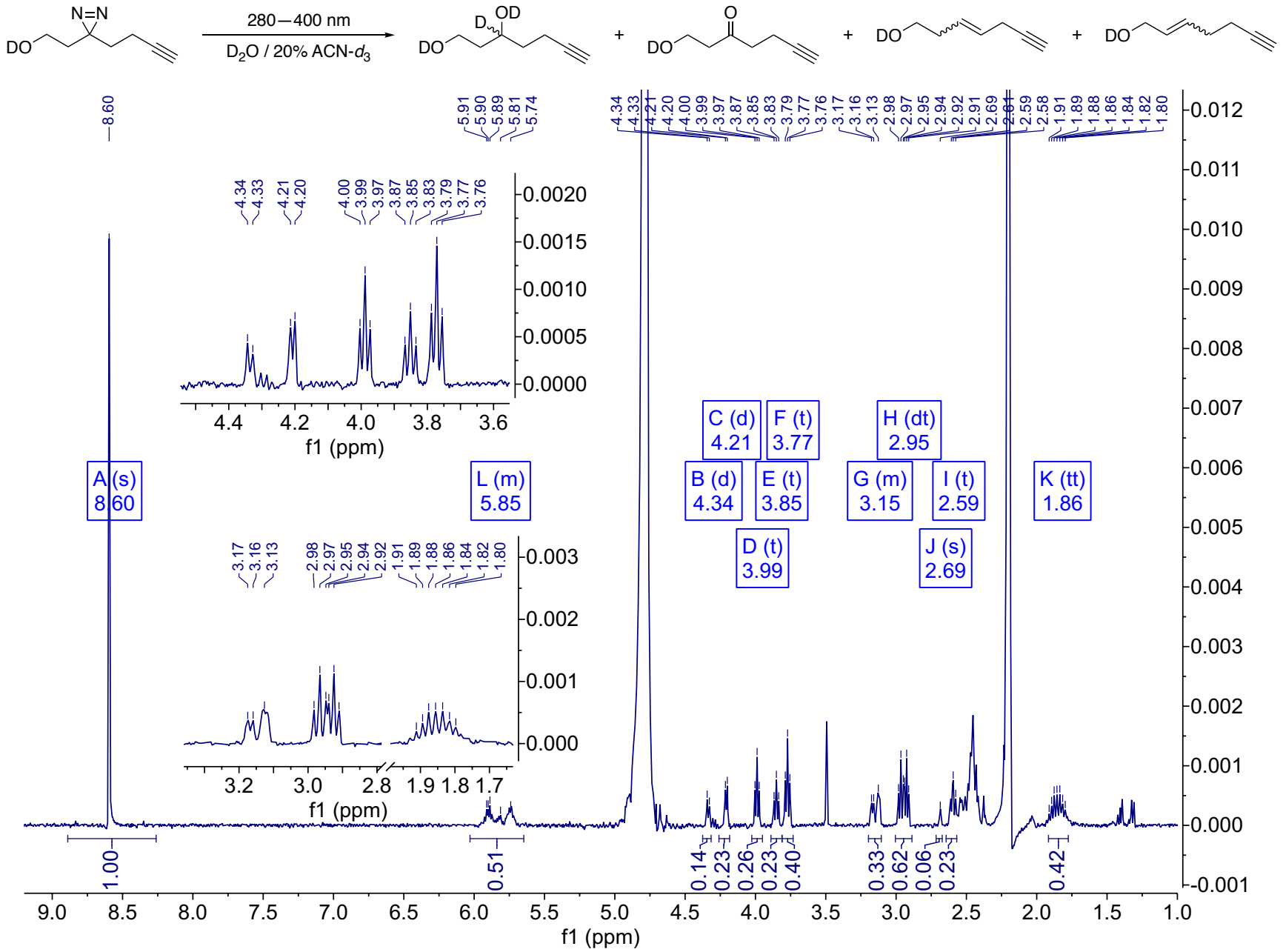

O
N
NH
N
N
N
N
JN-00845
1.00
1.03
1.88
0.92
3.54
2.02
f1 (ppm)

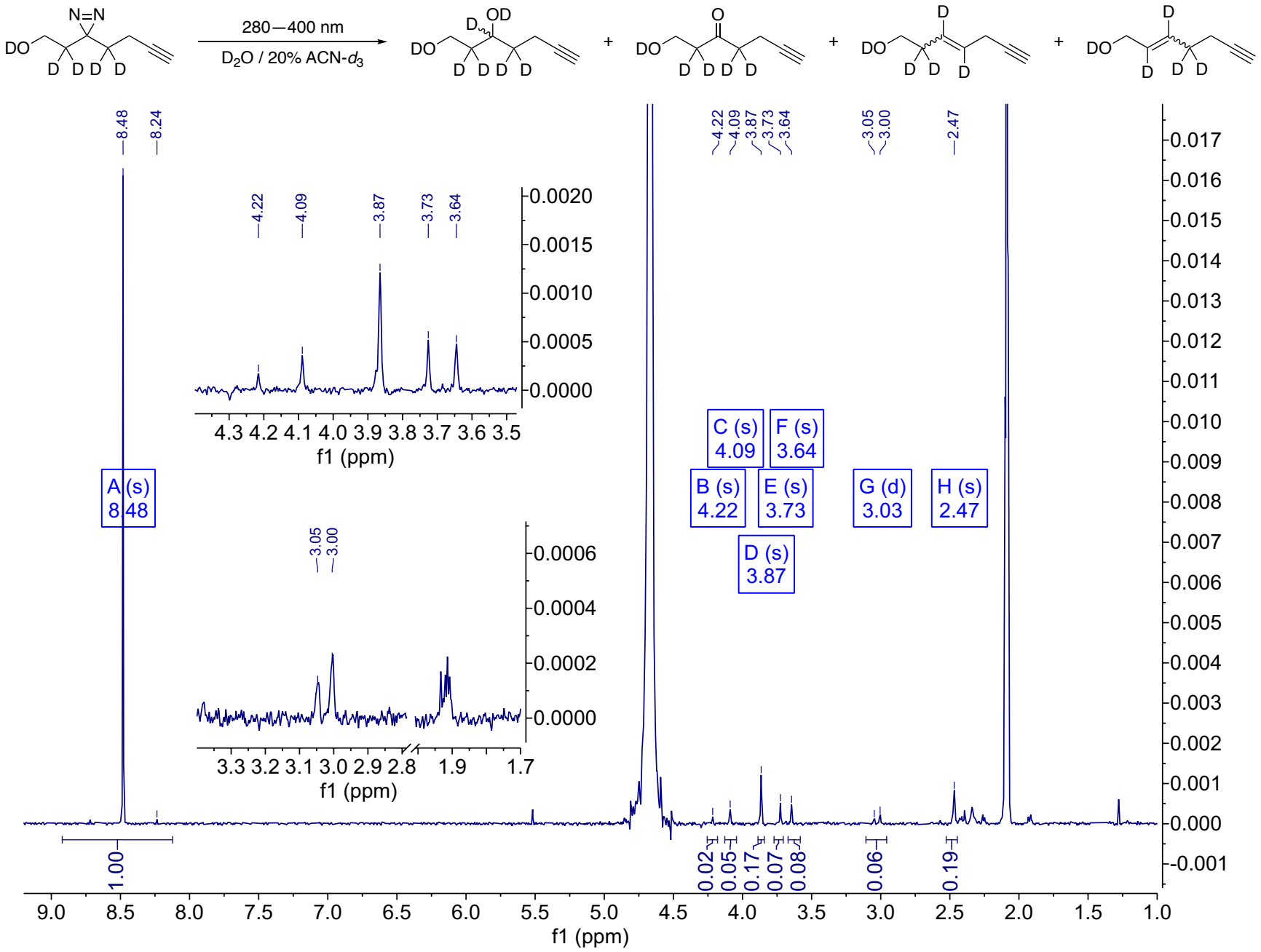

N=N
N
OH
JN-00938
4.02
4.05
2.00
2.88
1.87
2.04
1.98
6.08
2.24
f1 (ppm)

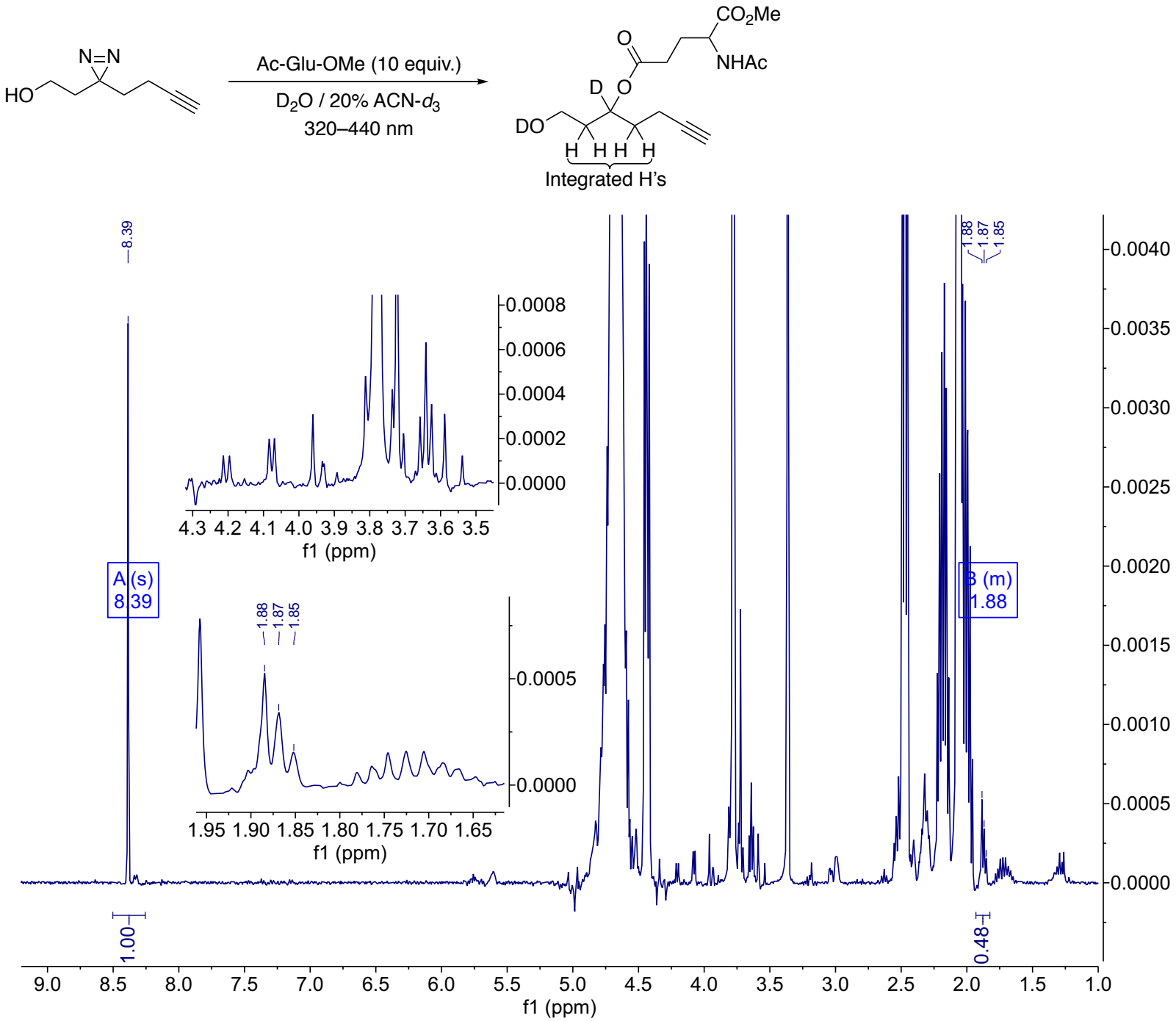

JN-849
CH
N
N
N
f1 (ppm)

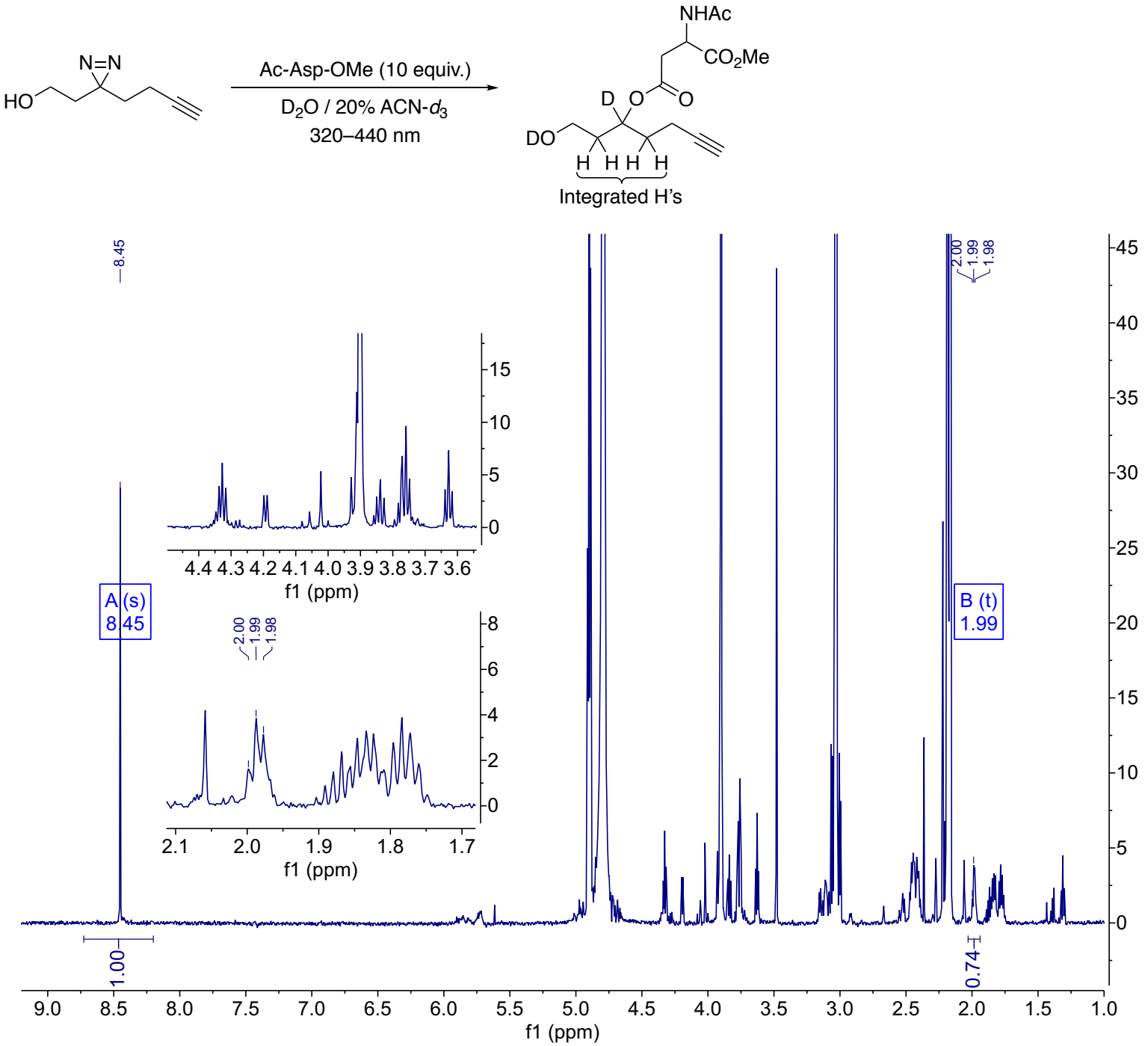

JN-00940
NH
NH2
N
H
N=N
O
0.69
3.67
2.34
2.26
2.00
0.91
1.94
1.89
1.96
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

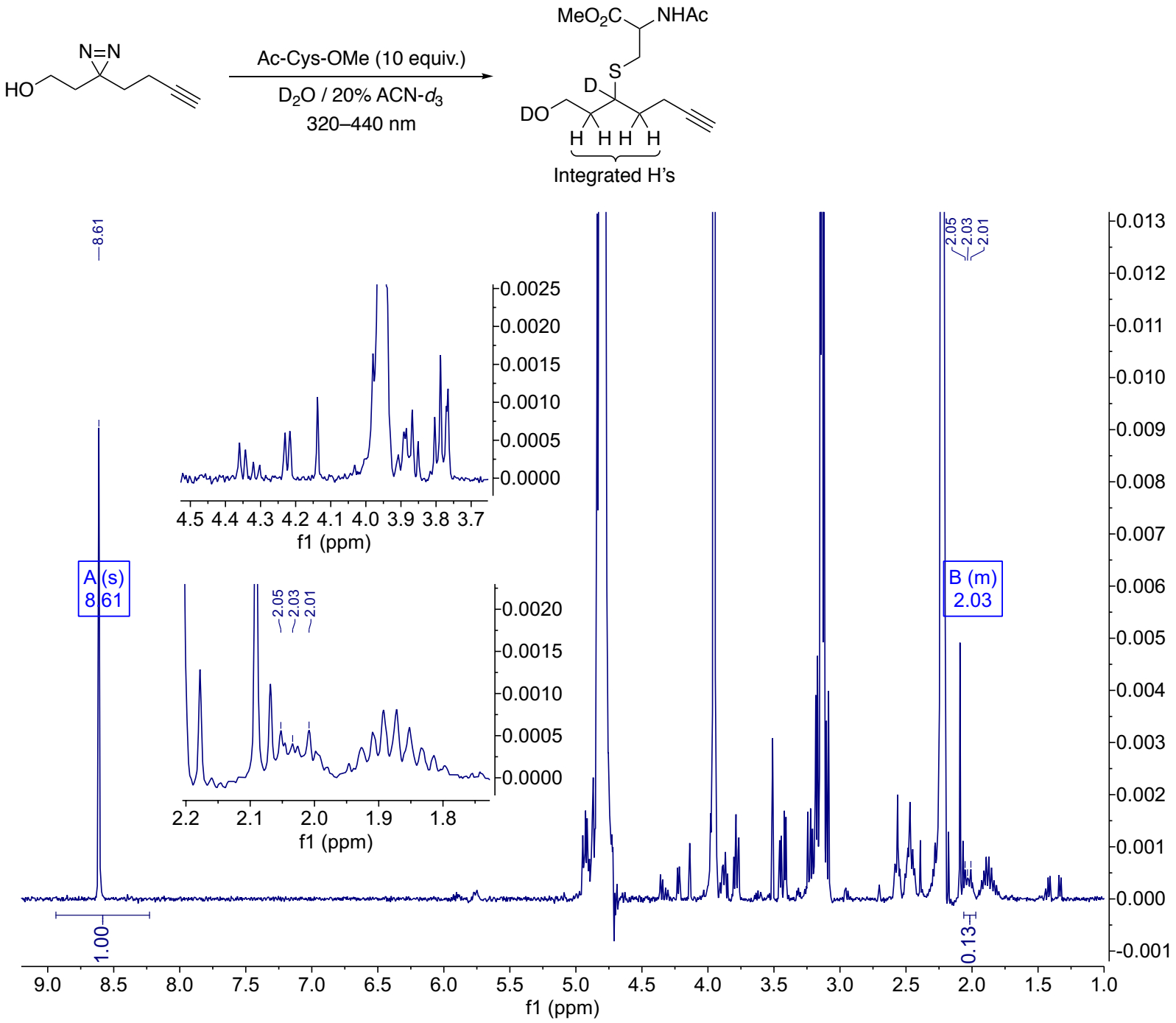
Catalog of nuclear magnetic resonance and liquid chromatograms for analytical reactions:

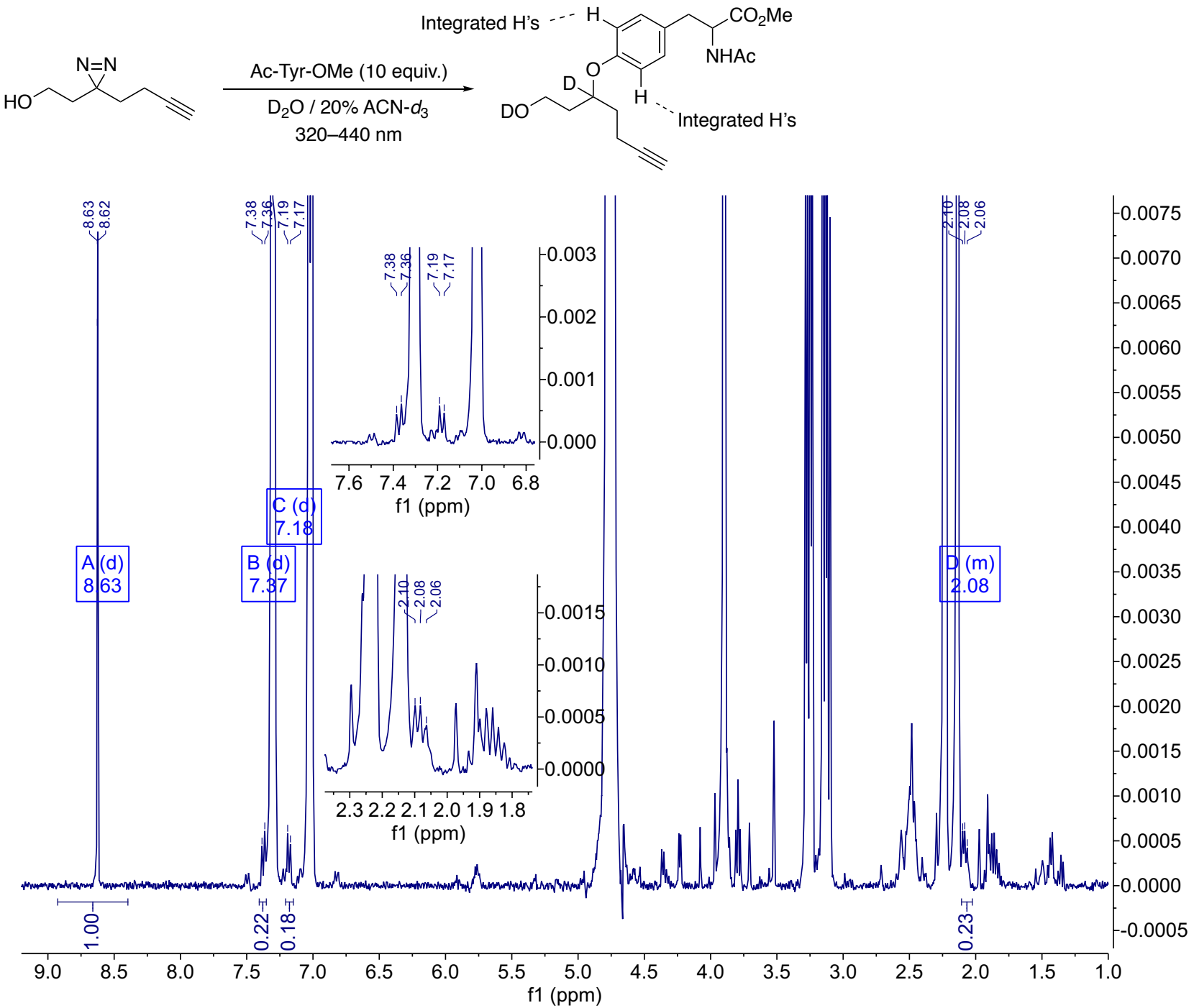


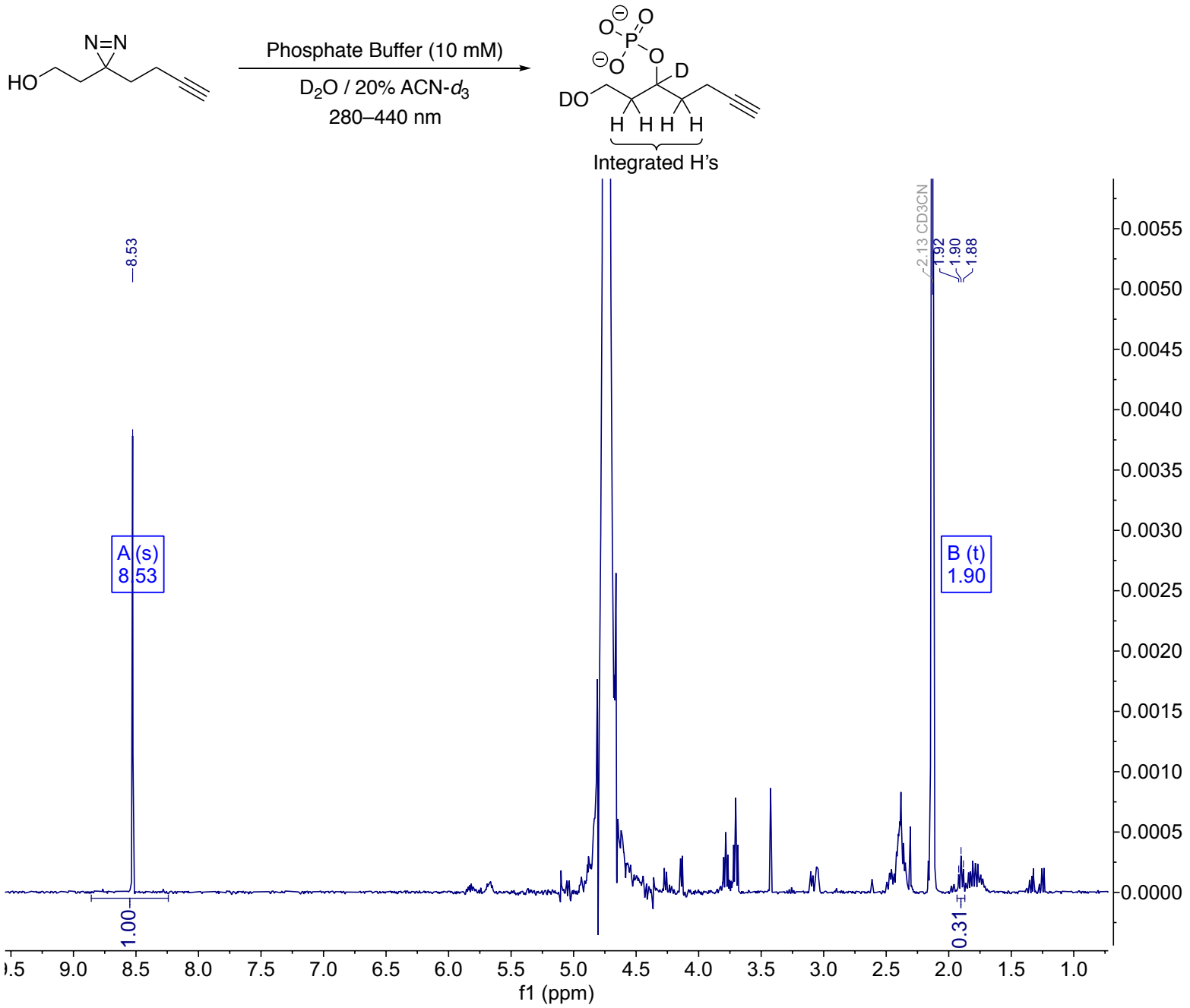

N=N
DO
D D D D
280—400 nm
D2O / 20% ACN-d3
D OD
O
D
8.48
8.24
4.22
4.09
3.87
3.73
3.64
3.05
3.00
2.47
A (s) 8.48
B (s) 4.22
C (s) 4.09
D (s) 3.87
E (s) 3.73
F (s) 3.64
G (d) 3.03
H (s) 2.47
1.00
0.02
0.05
0.17
0.07
0.08
0.06
0.19
f1 (ppm)

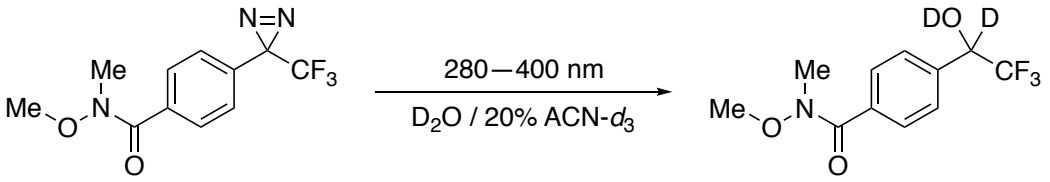

N=N
HO
Ac-Glu-OMe (10 equiv.)
D2O / 20% ACN-d3
320–440 nm
CO2Me
O
NHAc
D
O
DO
H H H H
Integrated H's
8.39
1.88
1.87
1.85
A (s)
8.39
B (m)
1.88
1.00
0.48
f1 (ppm)

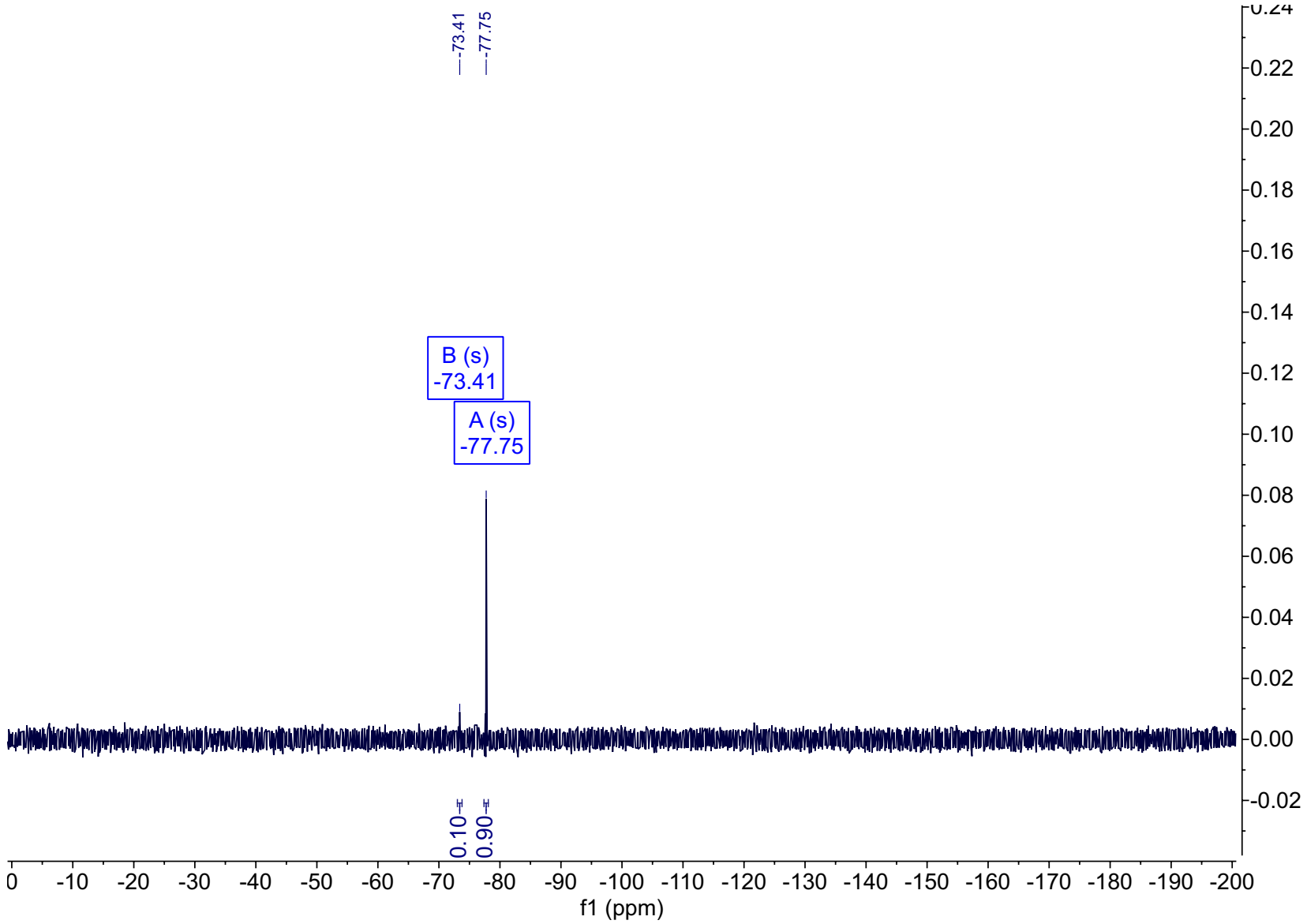

N=N
HO
Ac-Asp-OMe (10 equiv.)
D2O / 20% ACN-d3
320–440 nm
NHAc
CO2Me
D
O
O
DO
H H H H
Integrated H's
8.45
2.00
1.99
1.98
A (s)
8.45
B (t)
1.99
1.00
0.74
f1 (ppm)

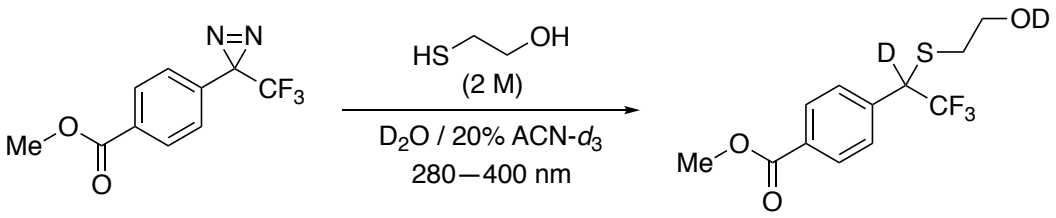

N=N
HO
Ac-Cys-OMe (10 equiv.)
D2O / 20% ACN-d3
320–440 nm
MeO2C
NHAc
S
D
DO
H H H H
Integrated H's
8.61
A (s)
8.61
1.00
2.05
2.03
2.01
B (m)
2.03
0.13
f1 (ppm)

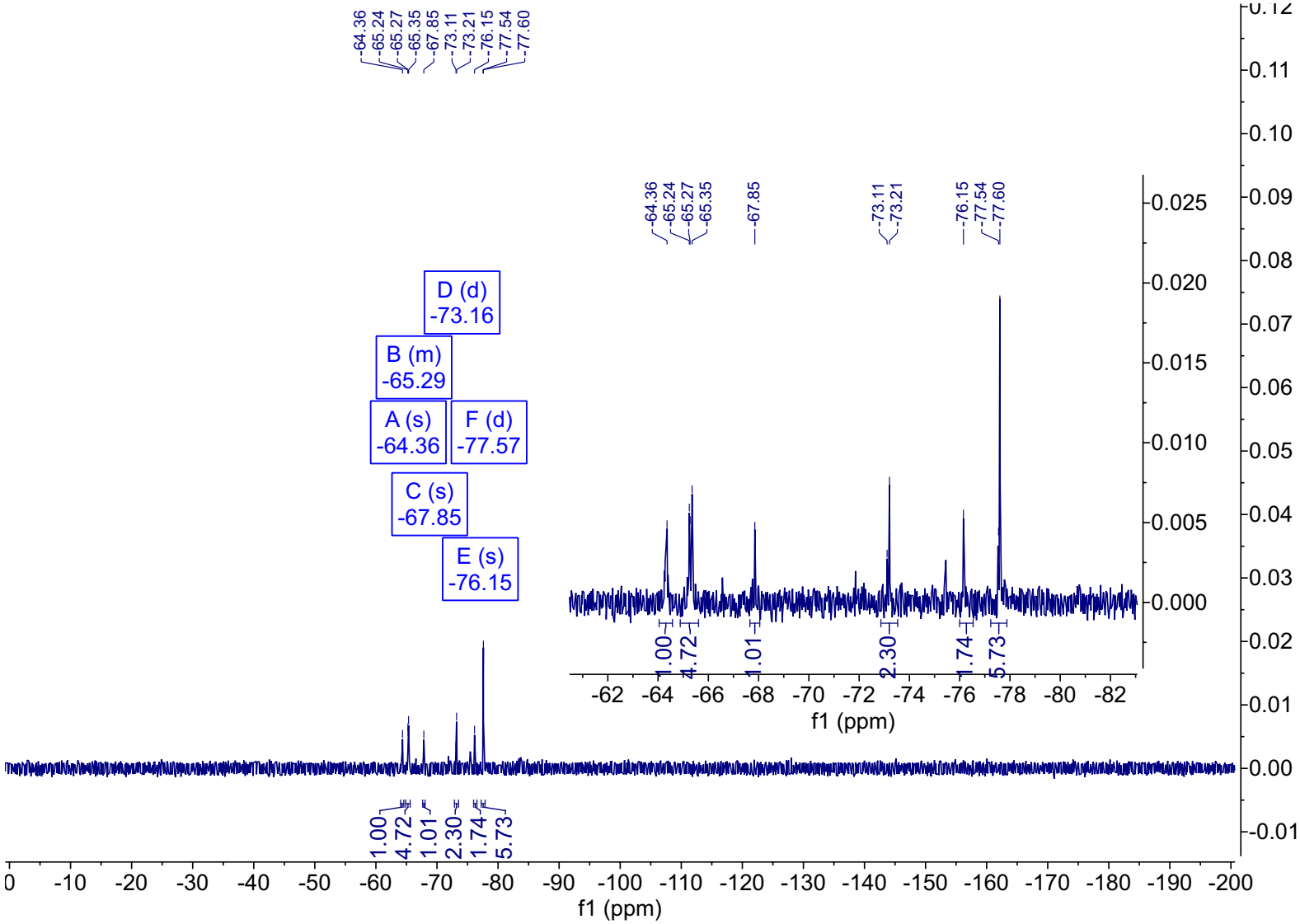

Integrated H's
H
CO2Me
NHAc
N=N
HO
Ac-Tyr-OMe (10 equiv.)
D2O / 20% ACN-d3
320–440 nm
O
D
DO
H
Integrated H's
8.63
8.62
7.38
7.36
7.19
7.17
2.10
2.08
2.06
A (d)
8.63
B (d)
7.37
C (d)
7.18
D (m)
2.08
1.00
0.22
0.18
0.23
f1 (ppm)

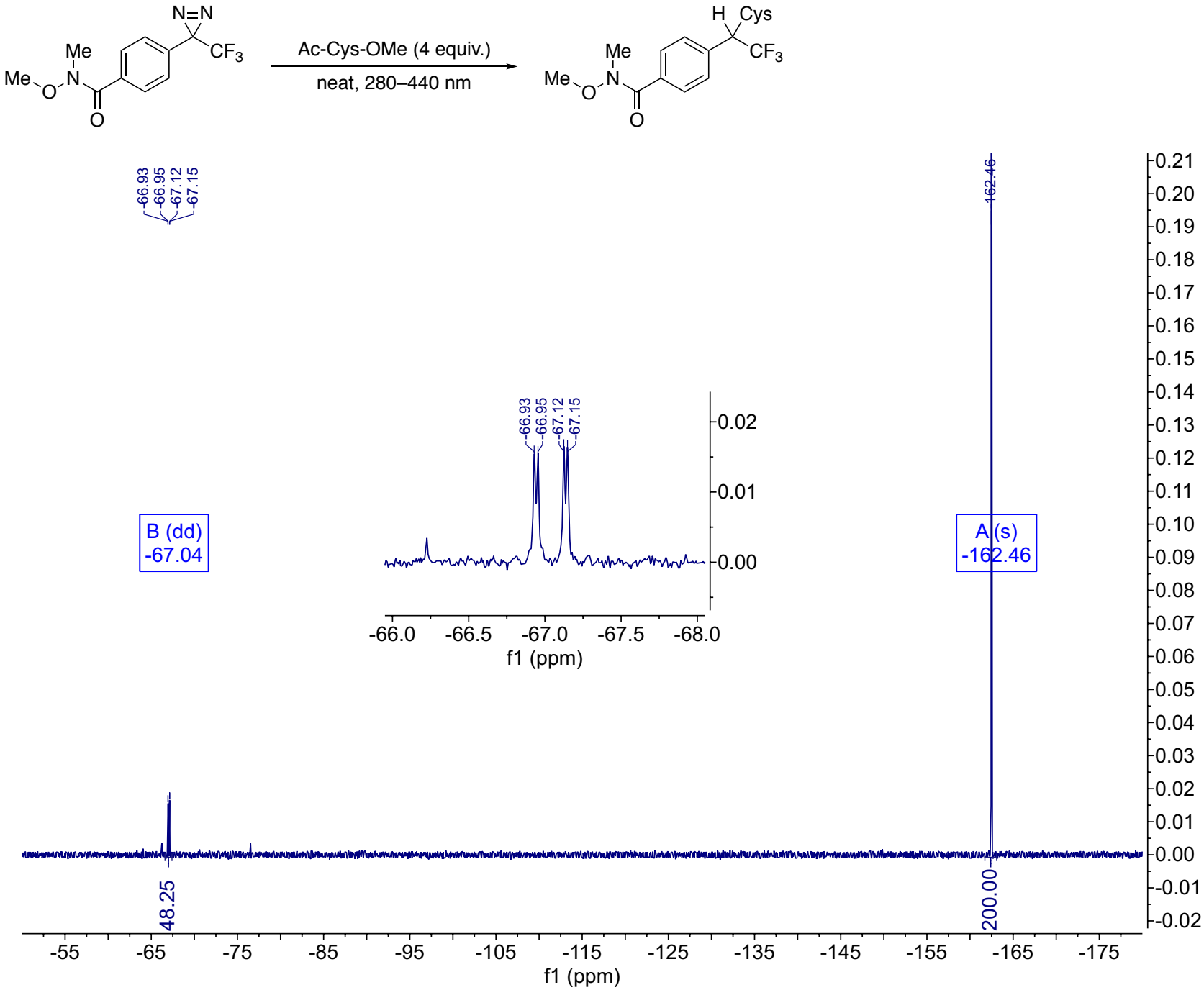

N=N
HO
Phosphate Buffer (10 mM)
D2O / 20% ACN-d3
280–440 nm
O
P
O
D
DO
H H H H
Integrated H's
8.53
2.13 CD3CN
1.92
1.90
1.88
A (s)
8.53
B (t)
1.90
1.00
0.31
0.0055
0.0050
0.0045
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

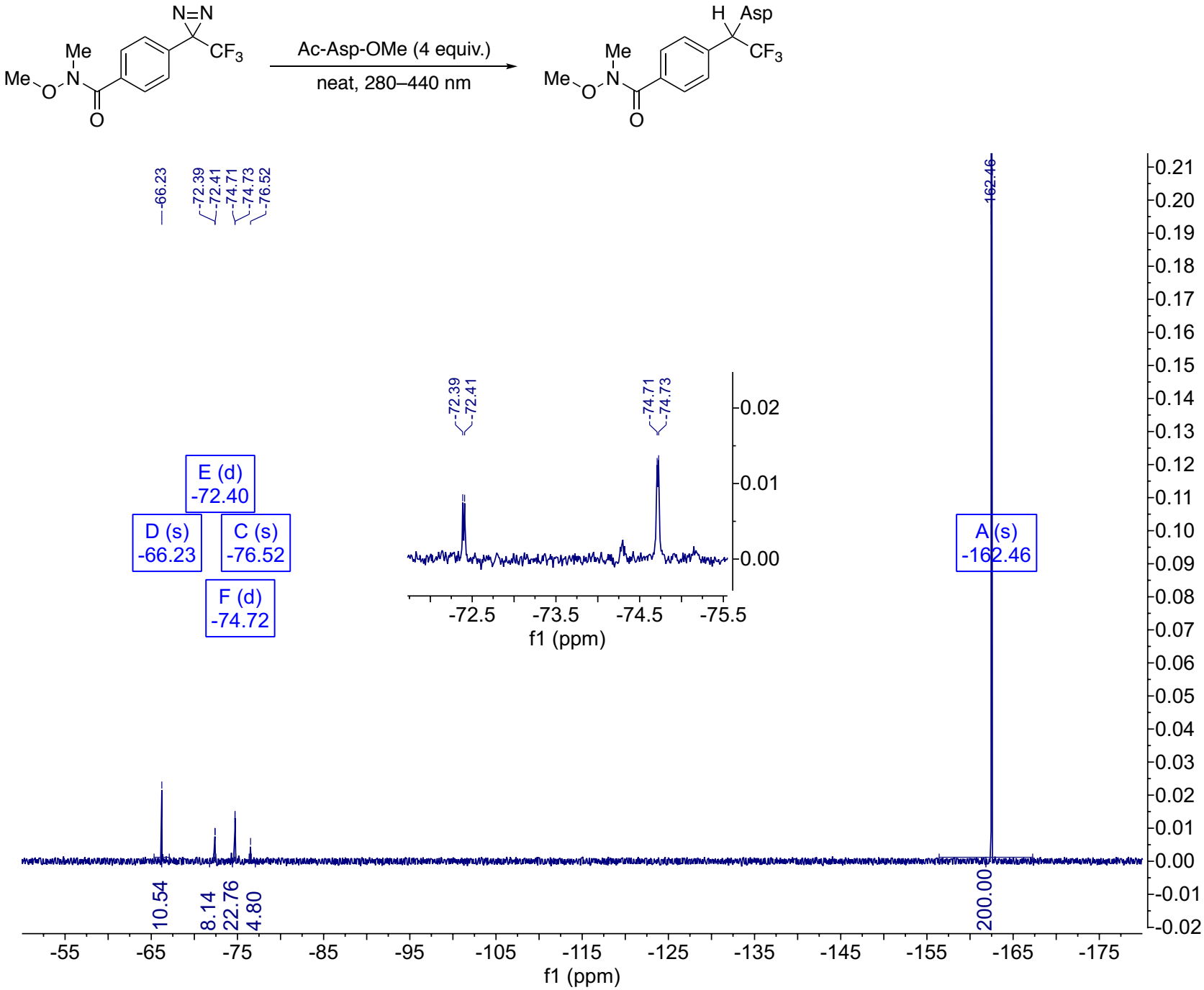

N=N
Me
CF3
Me
N
O
O
280—400 nm
D2O / 20% ACN-d3
DO
D
Me
CF3
Me
N
O
O

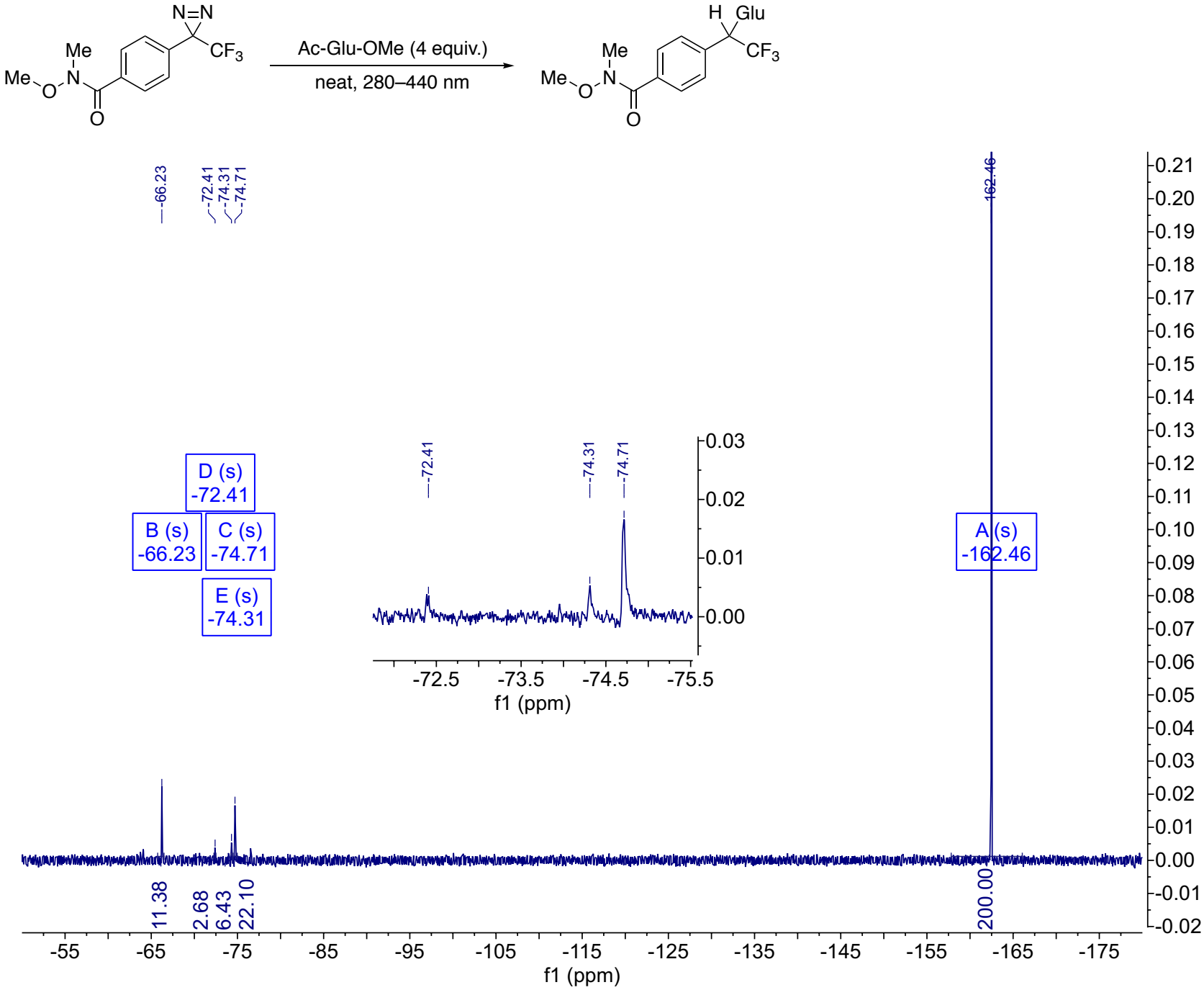

-73.41
-77.75
B (s)
-73.41
A (s)
-77.75
0.10
0.90
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

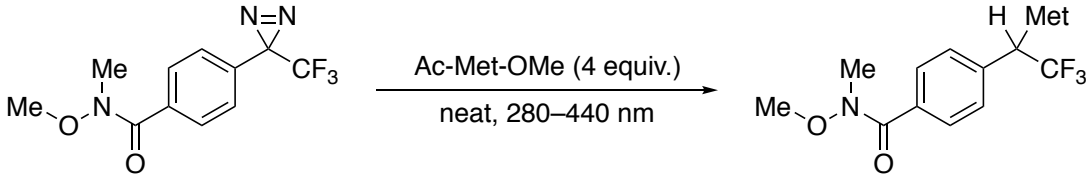

N=N
CF3
Me
O
O
HS
OH
(2 M)
D2O / 20% ACN-d3
280—400 nm
D
S
OD
CF3
Me
O
O

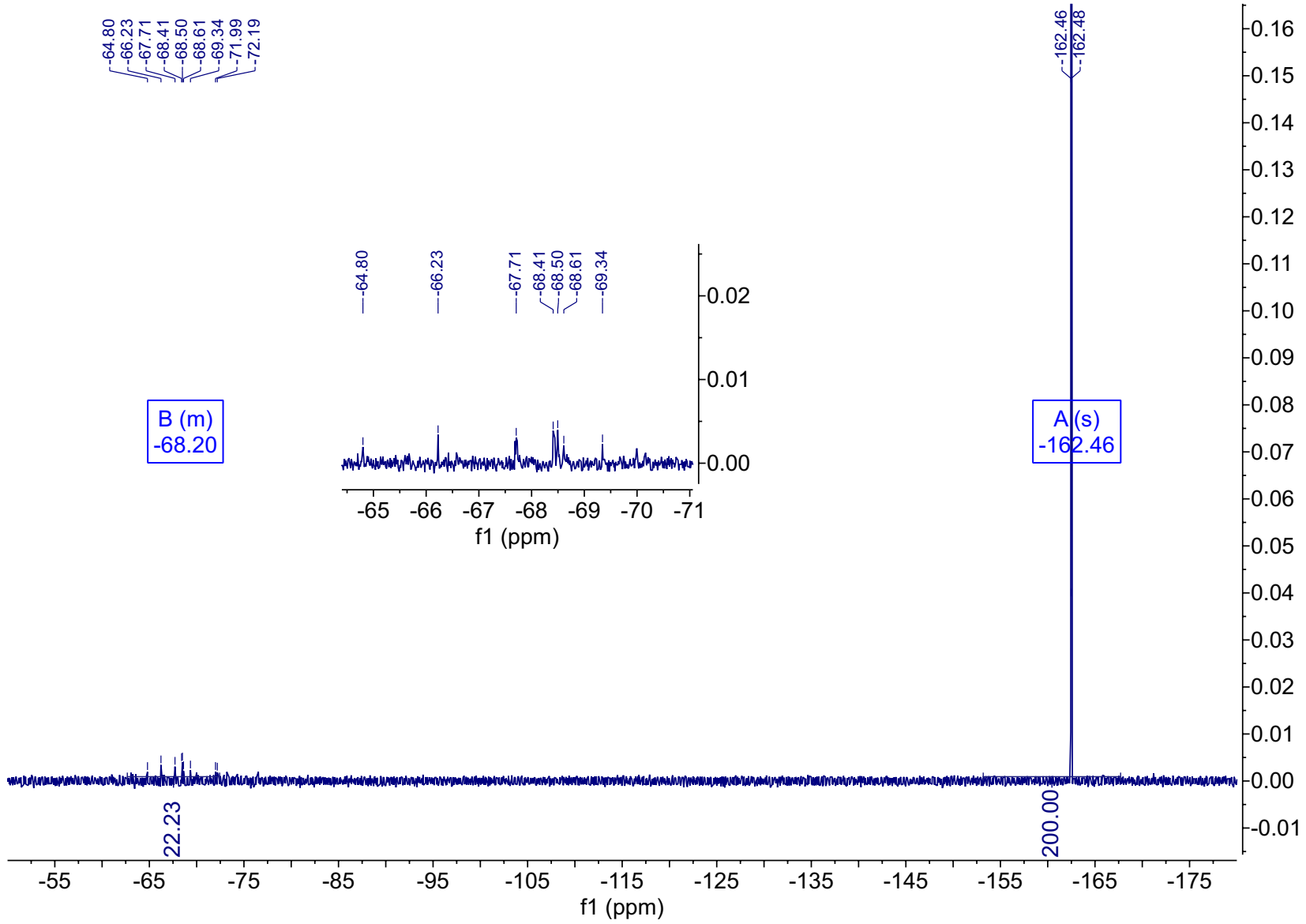

-64.36
-65.24
-65.27
-65.35
-67.85
-73.11
-73.21
-76.15
-77.54
-77.60
D (d)
-73.16
B (m)
-65.29
A (s)
-64.36
F (d)
-77.57
C (s)
-67.85
E (s)
-76.15
1.00
4.72
1.01
2.30
1.74
5.73
f1 (ppm)

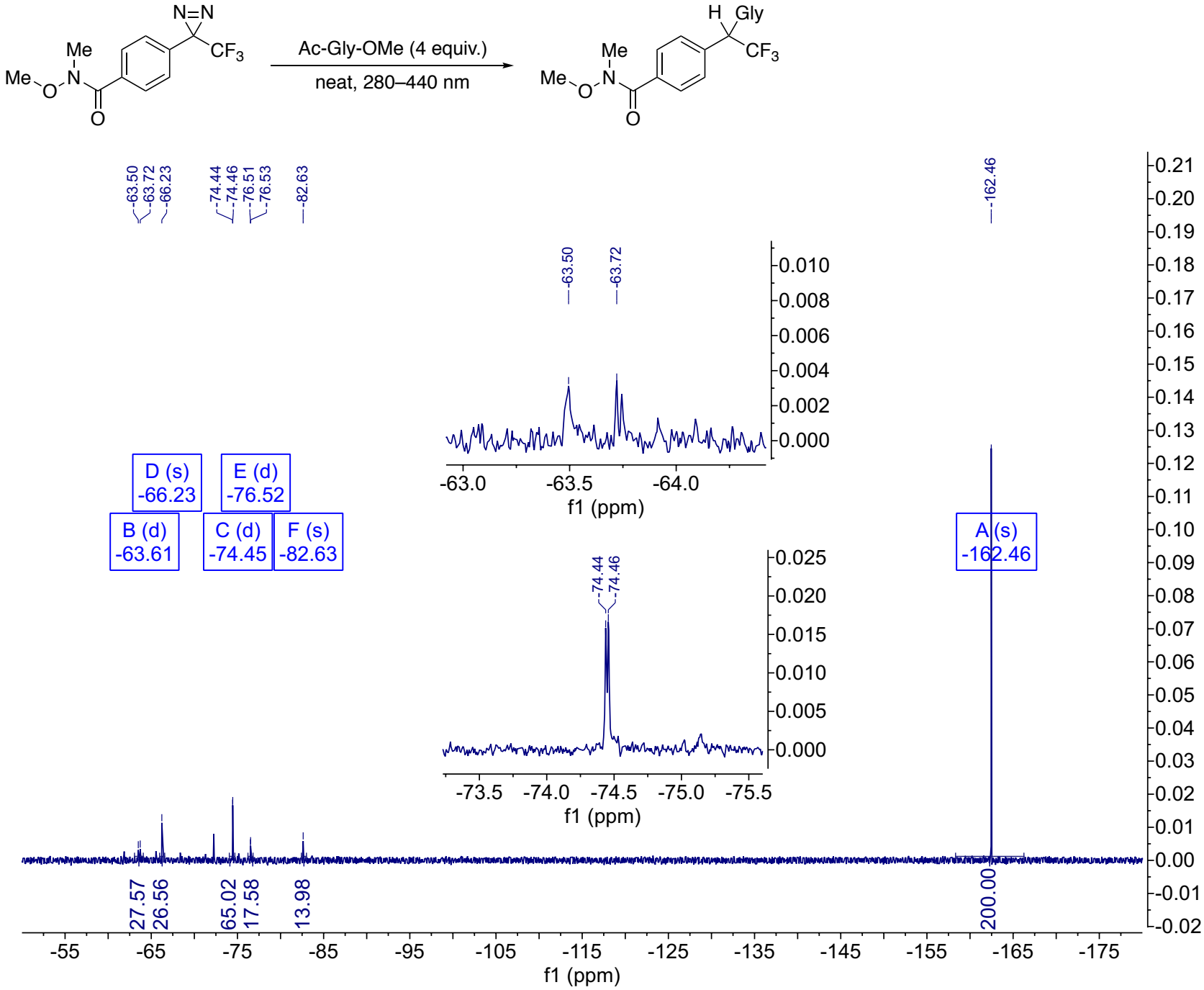

N=N
Me
CF3
Me
N
O
O
Ac-Cys-OMe (4 equiv.)
neat, 280–440 nm
H
Cys
Me
CF3
Me
N
O
O
-66.93
-66.95
-67.12
-67.15
162.46
B (dd)
-67.04
A (s)
-162.46
0.02
0.01
0.00
-66.0
-66.5
-67.0
-67.5
-68.0
f1 (ppm)
48.25
200.00
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

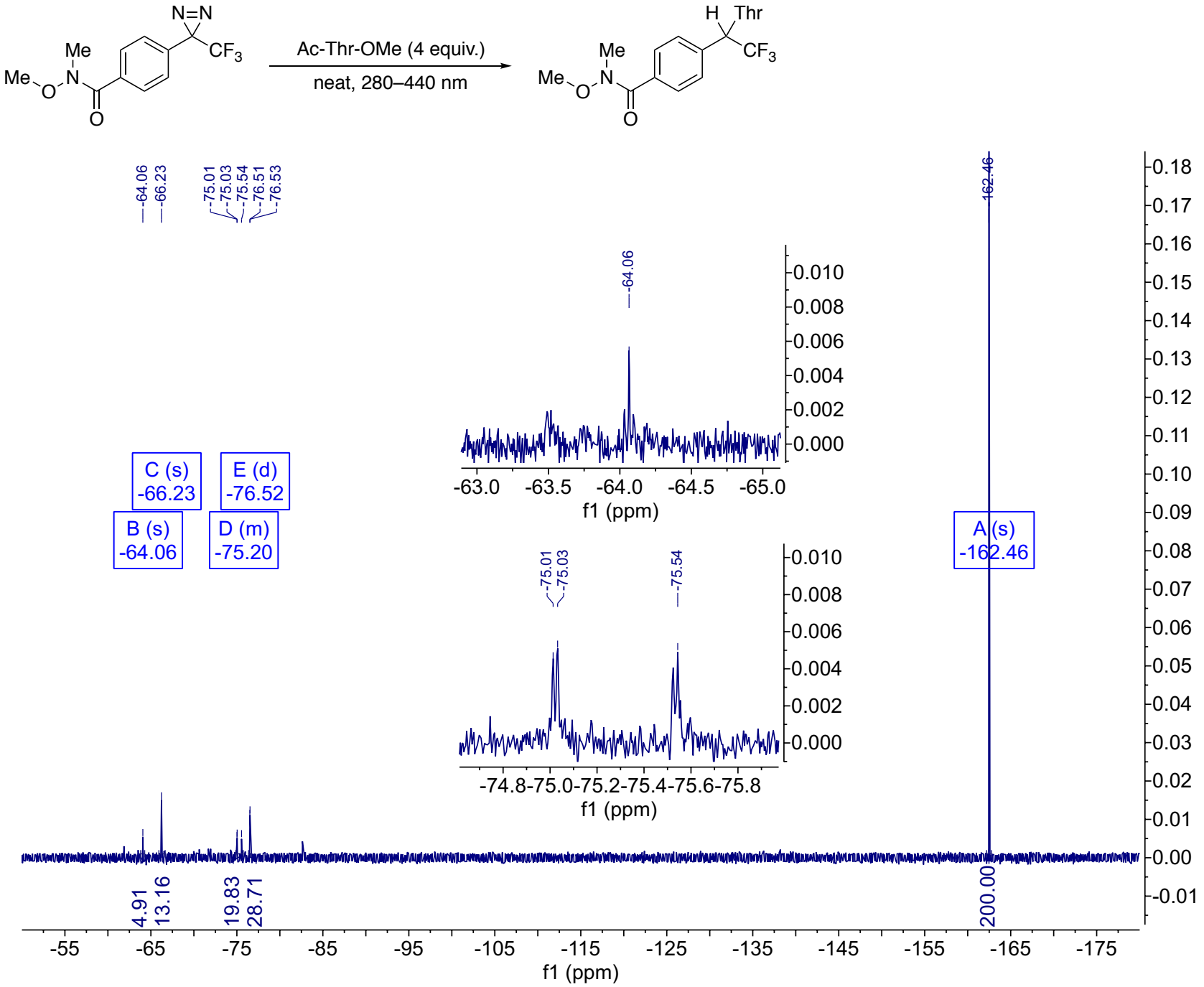

N=N
Me
CF3
Me
N
O
O
Ac-Asp-OMe (4 equiv.)
neat, 280–440 nm
H
Asp
Me
CF3
Me
N
O
O
-66.23
-72.39
-72.41
-74.71
-74.73
-76.52
162.46
E (d)
-72.40
D (s)
-66.23
C (s)
-76.52
F (d)
-74.72
A (s)
-162.46
-72.39
-72.41
-74.71
-74.73
0.02
0.01
0.00
-72.5
-73.5
-74.5
-75.5
f1 (ppm)
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
10.54
8.14
22.76
4.80
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

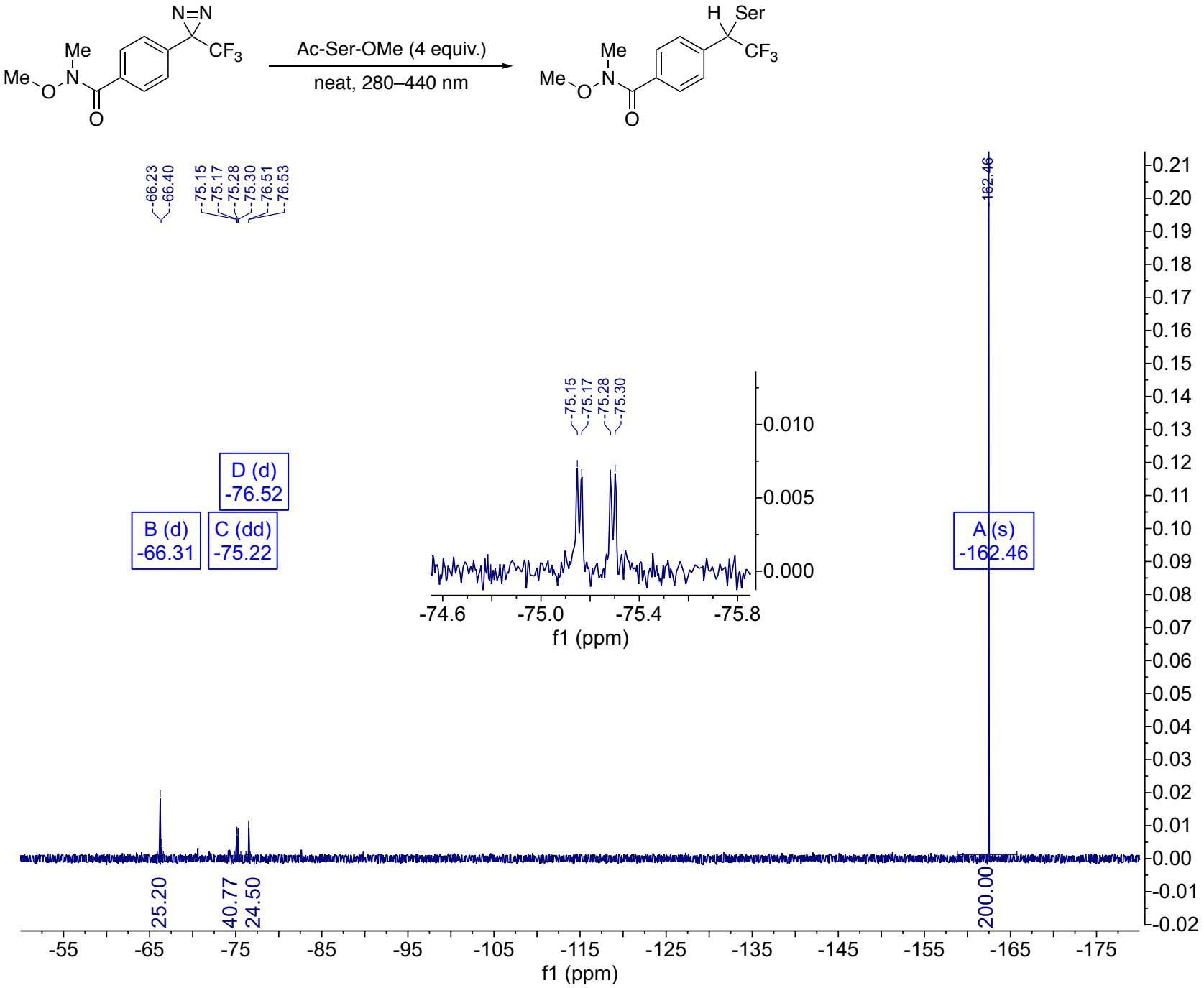

N=N
Me
CF3
Me
N
O
O
Ac-Glu-OMe (4 equiv.)
neat, 280–440 nm
H
Glu
Me
CF3
Me
N
O
O
-66.23
-72.41
-74.31
-74.71
162.46
D (s)
-72.41
B (s)
-66.23
C (s)
-74.71
E (s)
-74.31
A (s)
-162.46
-72.41
-74.31
-74.71
0.03
0.02
0.01
0.00
-72.5
-73.5
-74.5
-75.5
f1 (ppm)
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
11.38
2.68
6.43
22.10
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

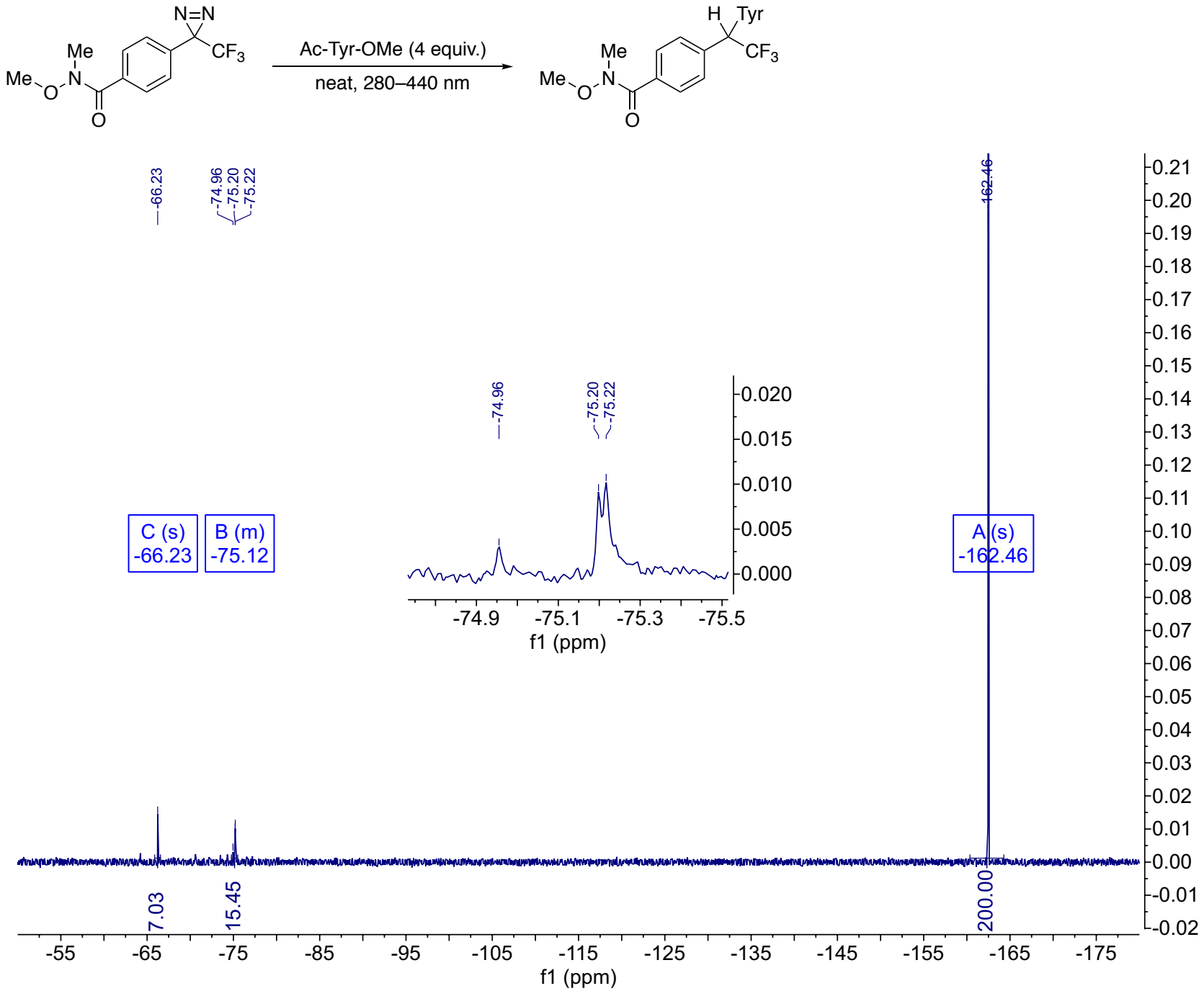

N=N
Me
CF3
Me
N
O
O
Ac-Met-OMe (4 equiv.)
neat, 280–440 nm
H
Met
Me
CF3
Me
N
O
O

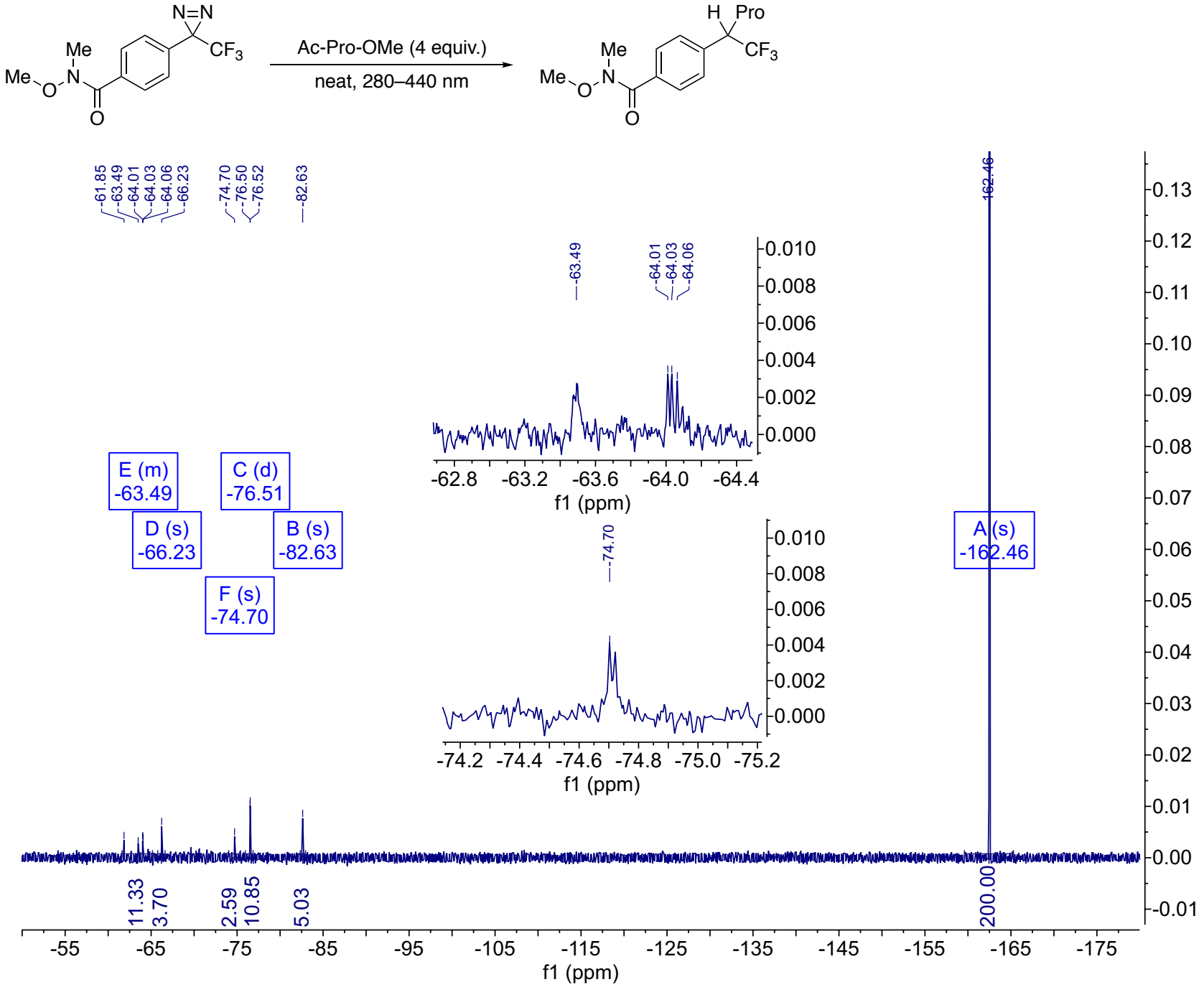

-64.80
-66.23
-67.71
-68.41
-68.50
-68.61
-69.34
-71.99
-72.19
-162.46
-162.48
B (m)
-68.20
A (s)
-162.46
0.02
0.01
0.00
-65
-66
-67
-68
-69
-70
-71
f1 (ppm)
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
22.23
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

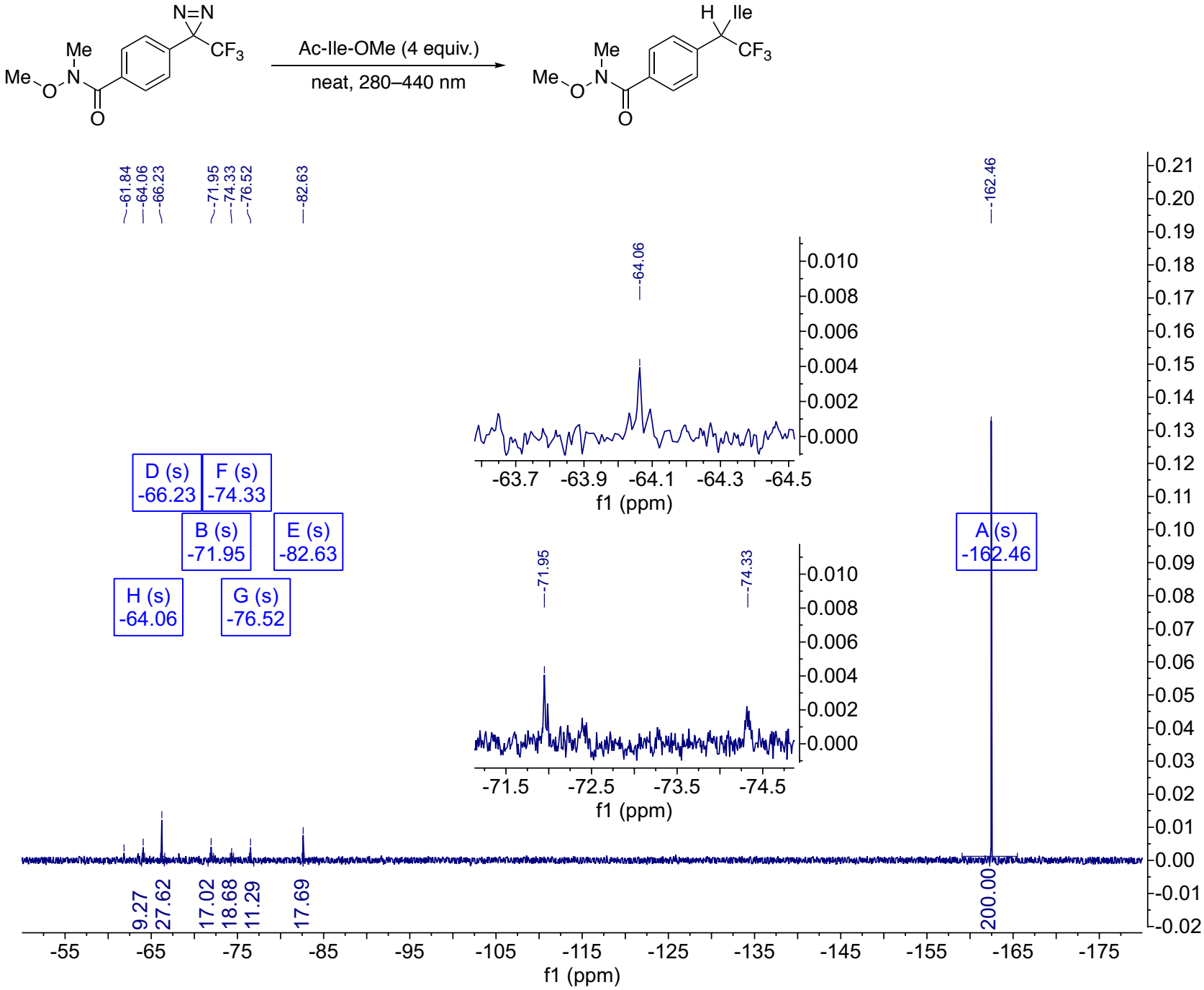

N=N
Me
CF3
Me
N
O
O
Ac-Gly-OMe (4 equiv.)
neat, 280–440 nm
H
Gly
CF3
-63.50
-63.72
-66.23
-74.44
-74.46
-76.51
-76.53
-82.63
-162.46
D (s)
-66.23
E (d)
-76.52
B (d)
-63.61
C (d)
-74.45
F (s)
-82.63
A (s)
-162.46
f1 (ppm)
27.57
26.56
65.02
17.58
13.98
200.00

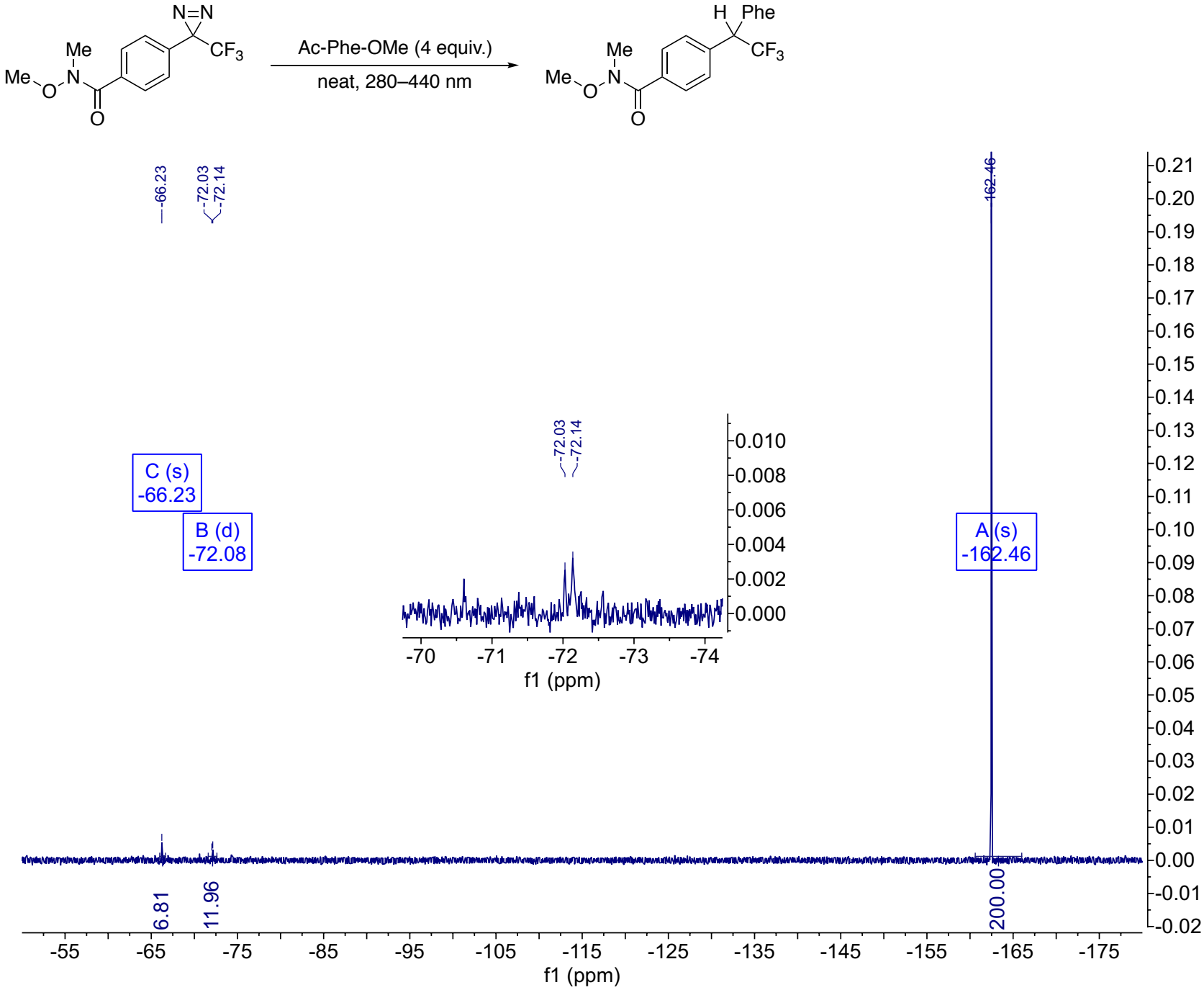

N=N
Me
CF3
Me
N
O
O
Ac-Thr-OMe (4 equiv.)
neat, 280–440 nm
H
Thr
Me
CF3
Me
N
O
O
-64.06
-66.23
-75.01
-75.03
-75.54
-76.51
-76.53
162.46
C (s)
-66.23
E (d)
-76.52
B (s)
-64.06
D (m)
-75.20
A (s)
-162.46
-64.06
-75.01
-75.03
-75.54
4.91
13.16
19.83
28.71
200.00
f1 (ppm)
f1 (ppm)
f1 (ppm)

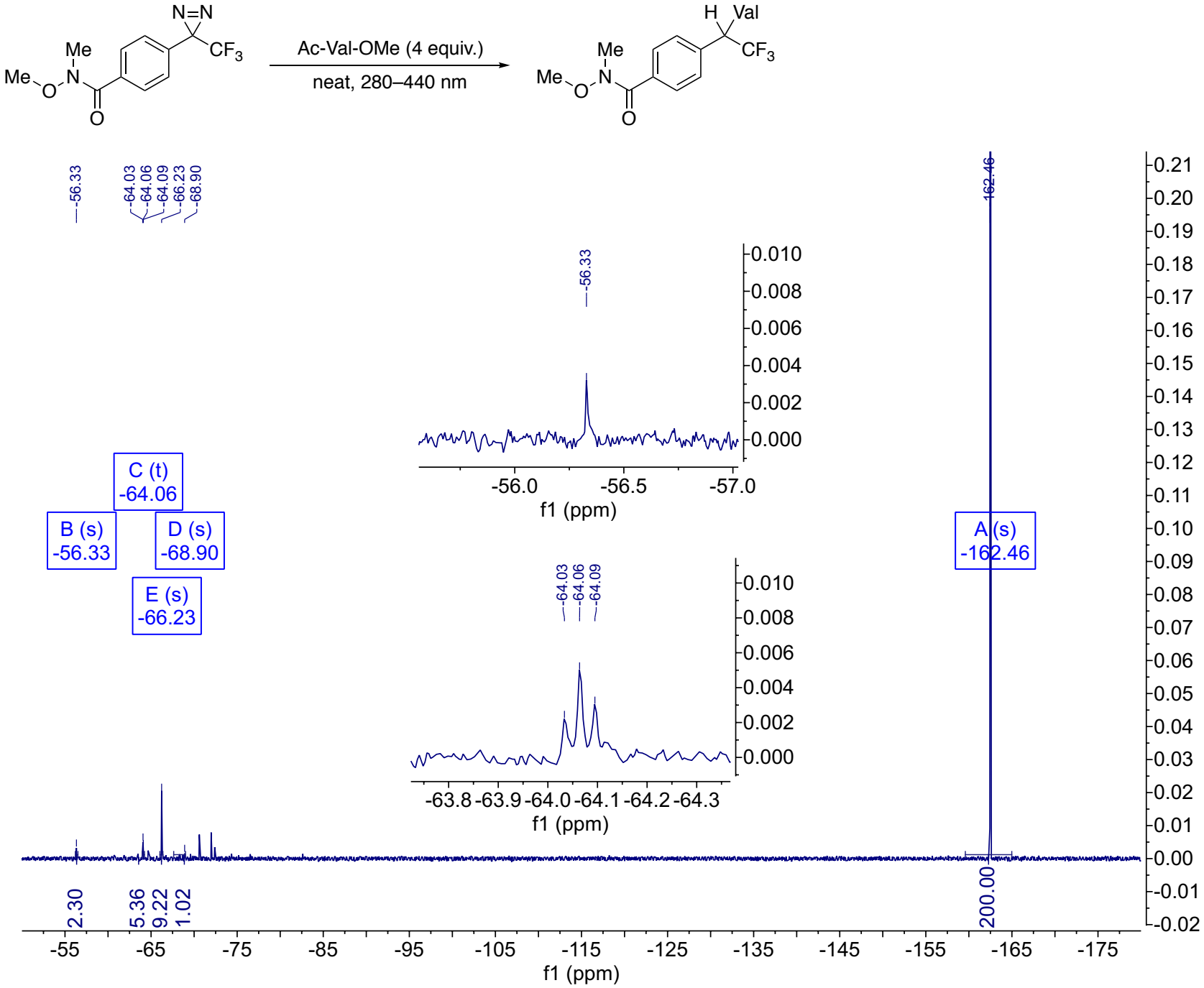

N=N
Me
CF3
Me
N
O
O
Ac-Ser-OMe (4 equiv.)
neat, 280–440 nm
H
Ser
Me
CF3
Me
N
O
O
-66.23
-66.40
-75.15
-75.17
-75.28
-75.30
-76.51
-76.53
162.46
D (d)
-76.52
B (d)
-66.31
C (dd)
-75.22
A (s)
-162.46
f1 (ppm)
f1 (ppm)
25.20
40.77
24.50
200.00

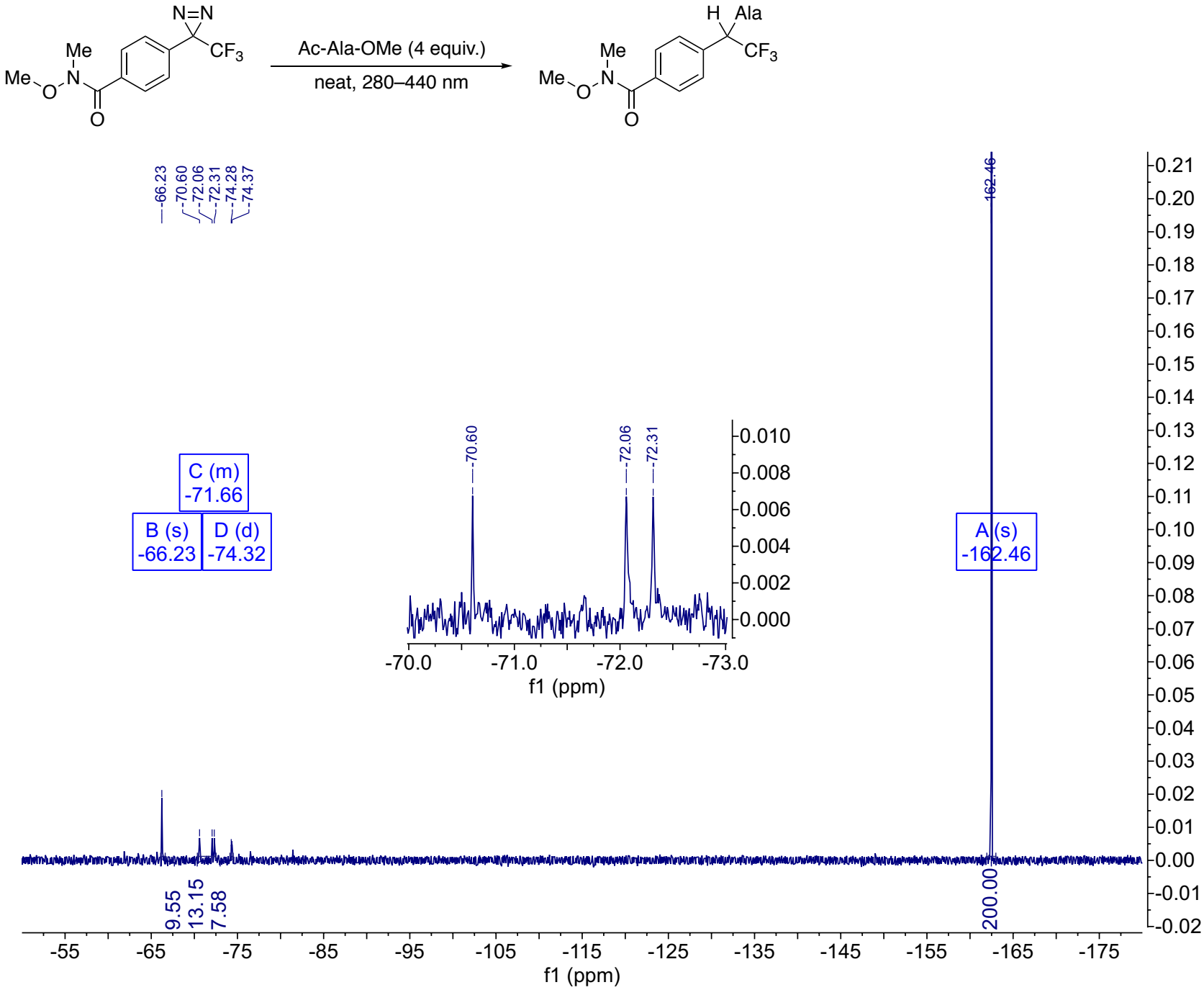

N=N
Me
CF3
Me
N
O
O
Ac-Tyr-OMe (4 equiv.)
neat, 280–440 nm
H
Tyr
Me
CF3
Me
N
O
O
-66.23
-74.96
-75.20
-75.22
162.46
C (s)
-66.23
B (m)
-75.12
A (s)
-162.46
f1 (ppm)
f1 (ppm)
7.03
15.45
200.00

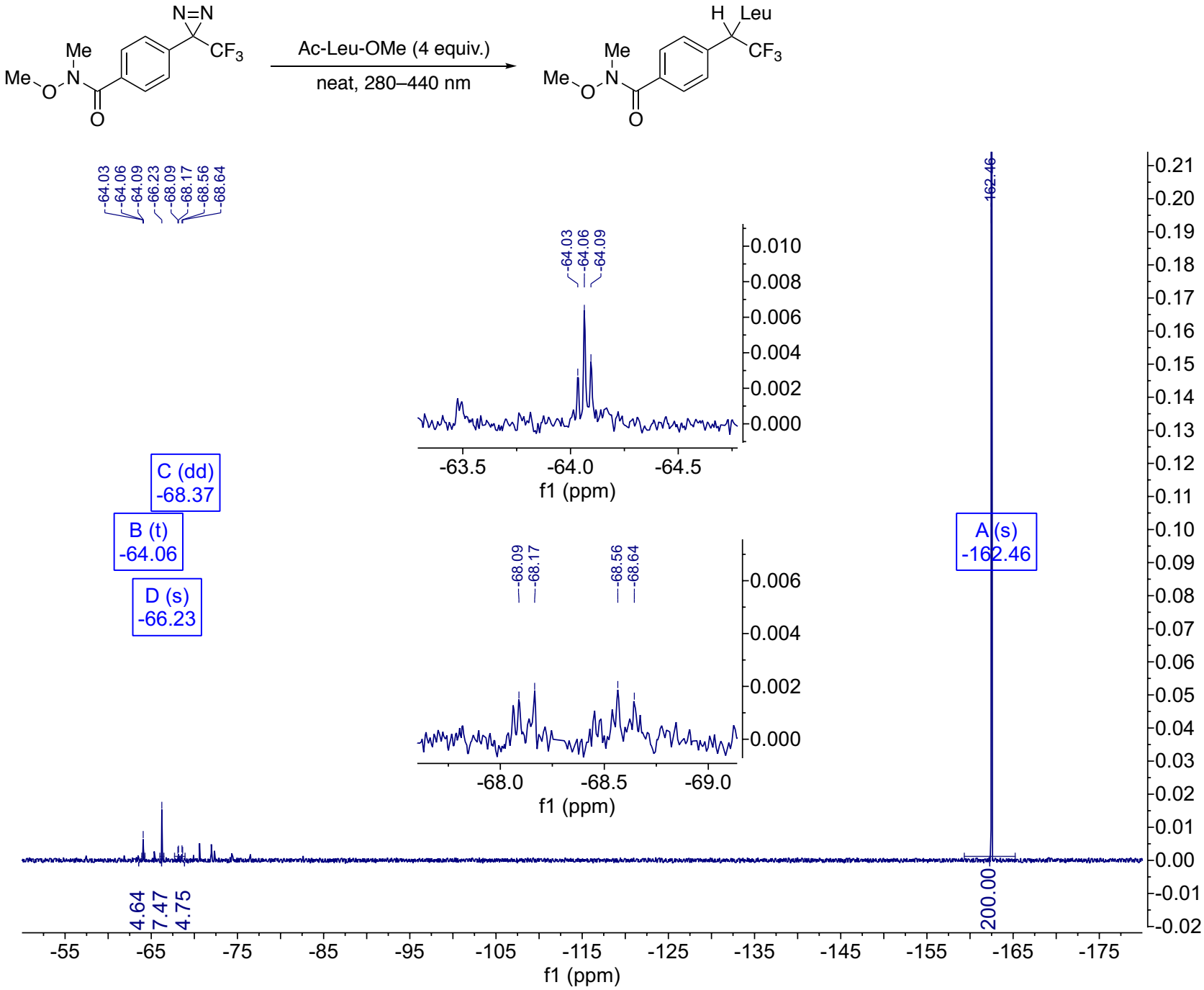

N=N
Me
CF3
Me
N
O
O
Ac-Pro-OMe (4 equiv.)
neat, 280–440 nm
H
Pro
Me
CF3
Me
N
O
O
-61.85
-63.49
-64.01
-64.03
-64.06
-66.23
-74.70
-76.50
-76.52
-82.63
162.46
E (m)
-63.49
C (d)
-76.51
D (s)
-66.23
B (s)
-82.63
F (s)
-74.70
A (s)
-162.46
11.33
3.70
2.59
10.85
5.03
200.00
f1 (ppm)
f1 (ppm)
f1 (ppm)

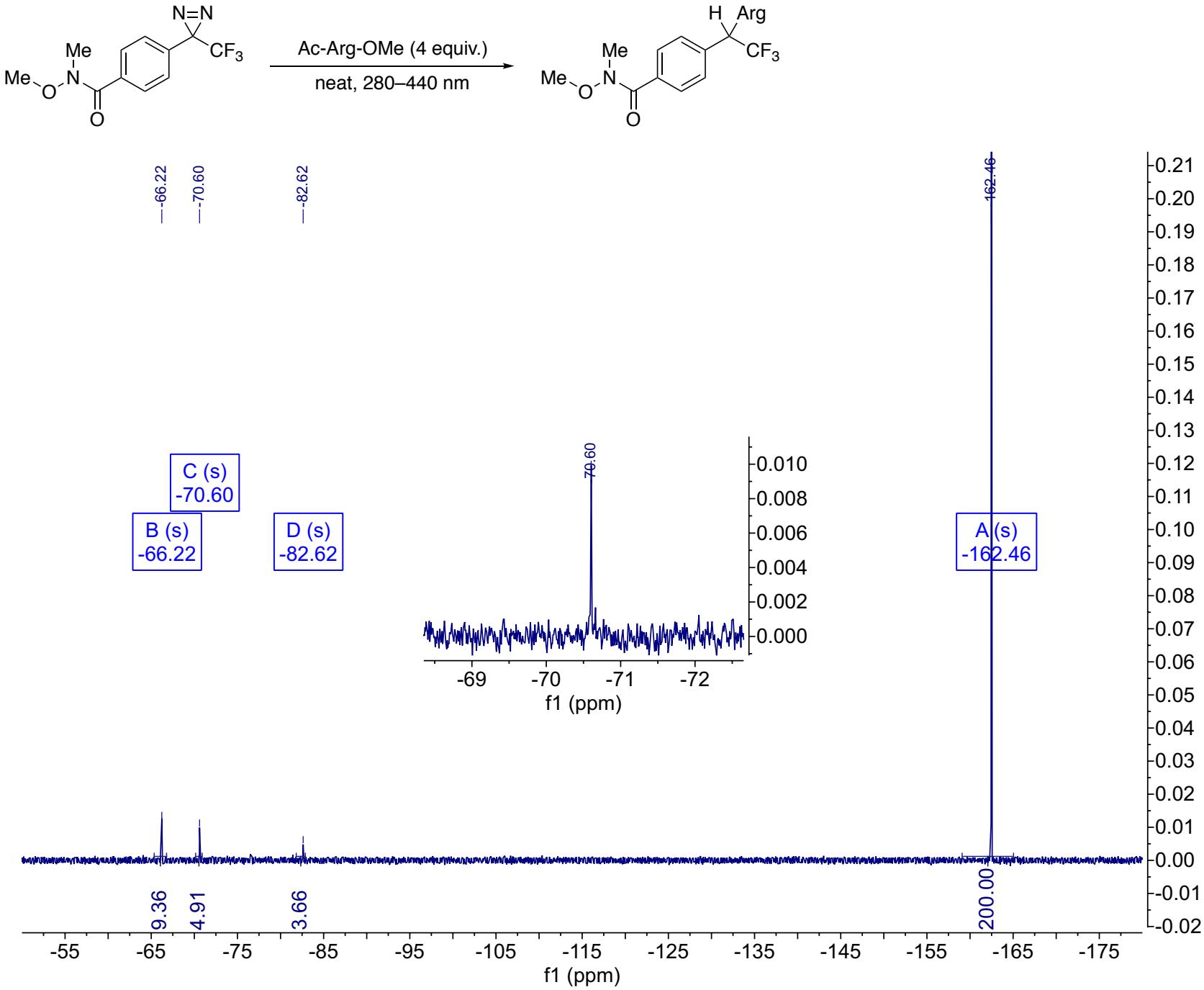

N=N
Me
CF3
Me
N
O
O
Ac-Ile-OMe (4 equiv.)
neat, 280–440 nm
H
Ile
-61.84
-64.06
-66.23
-71.95
-74.33
-76.52
-82.63
-162.46
D (s)
-66.23
F (s)
-74.33
B (s)
-71.95
E (s)
-82.63
H (s)
-64.06
G (s)
-76.52
A (s)
-162.46
f1 (ppm)
9.27
27.62
17.02
18.68
11.29
17.69
200.00

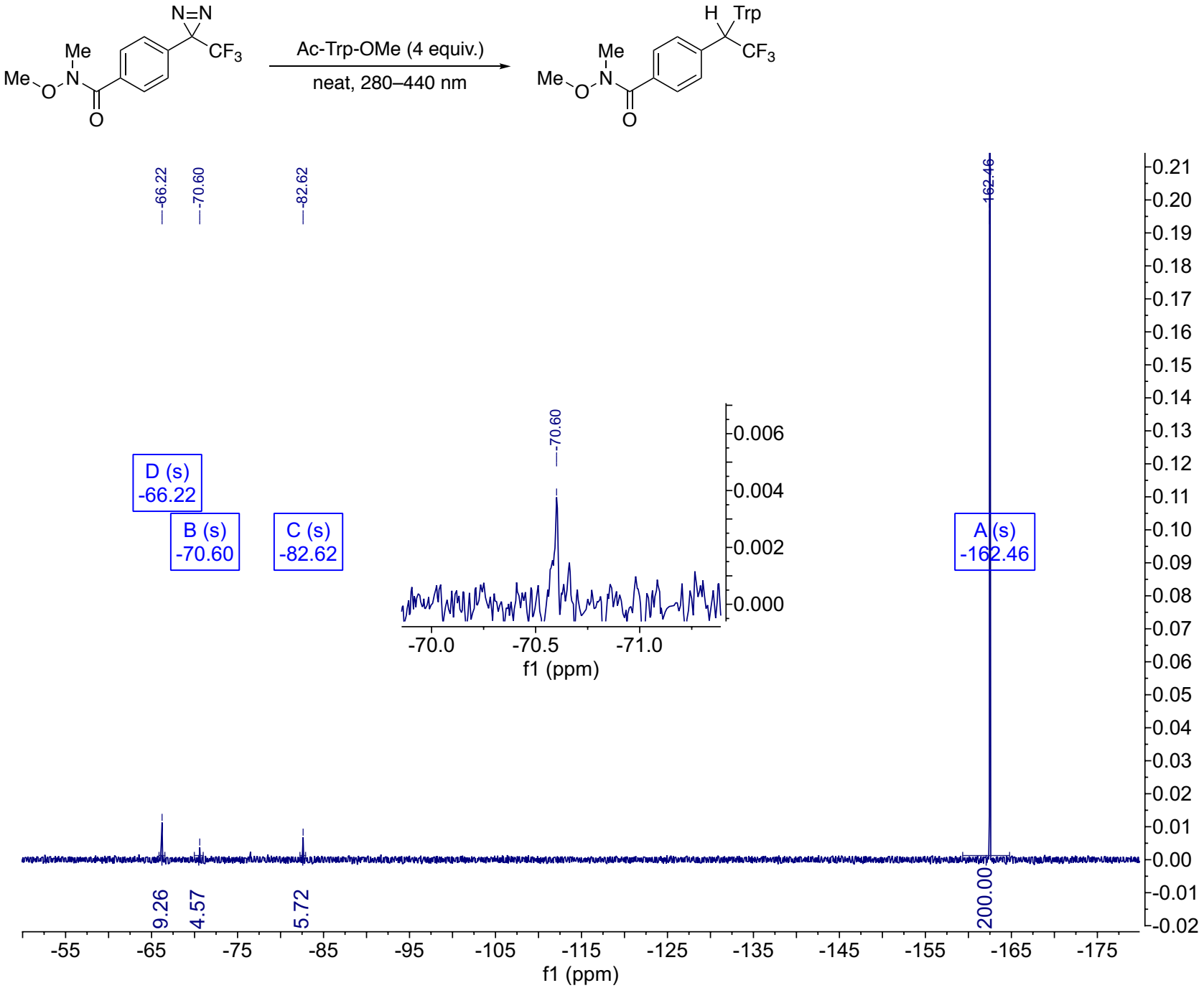

N=N
Me
CF3
Me
N
O
O
Ac-Phe-OMe (4 equiv.)
neat, 280–440 nm
H
Phe
Me
CF3
Me
N
O
O
-66.23
-72.03
-72.14
162.46
C (s)
-66.23
B (d)
-72.08
A (s)
-162.46
-72.03
-72.14
0.010
0.008
0.006
0.004
0.002
0.000
-70
-71
-72
-73
-74
f1 (ppm)
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
6.81
11.96
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

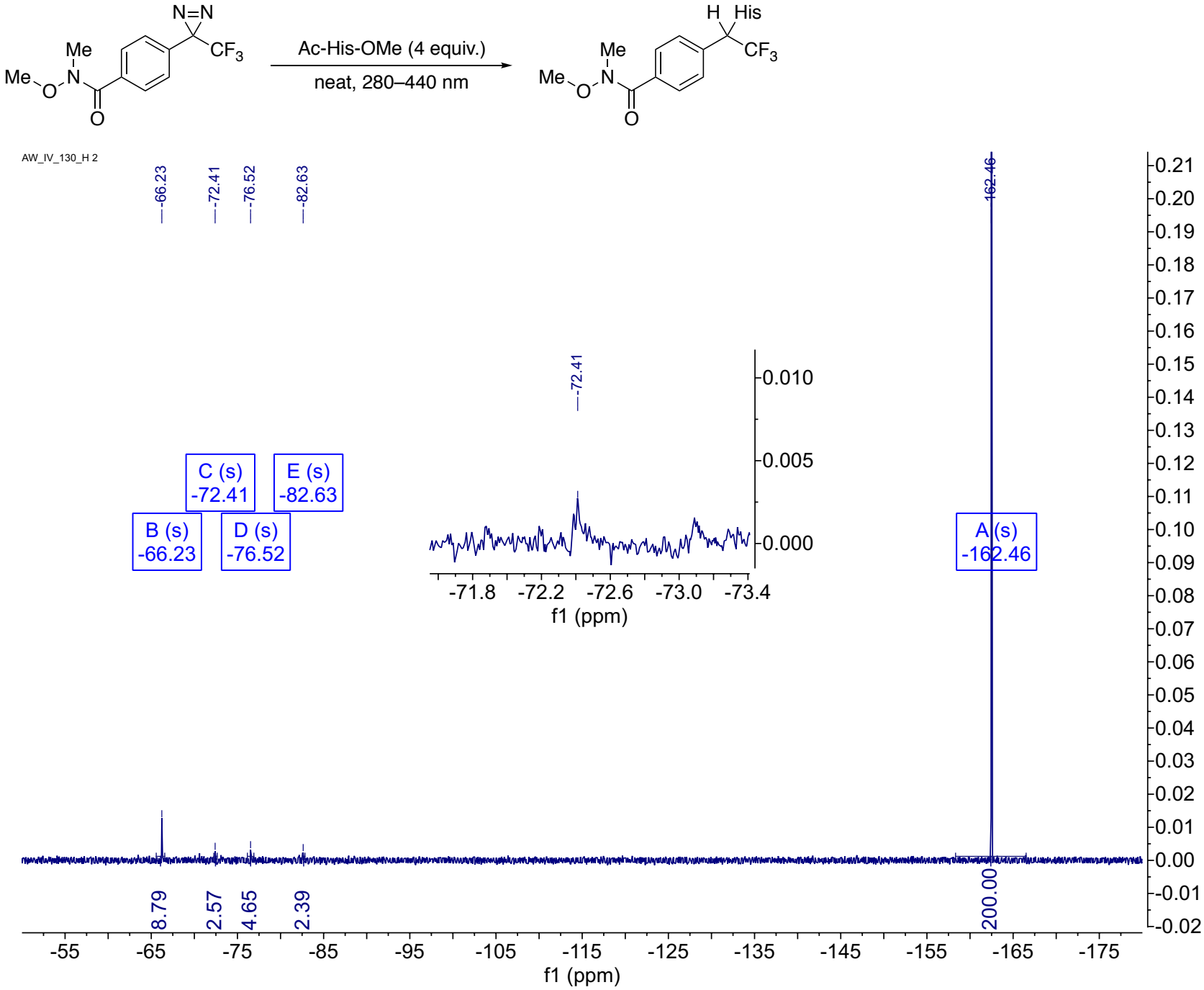

N=N
Me
CF3
Me
N
O
O
Ac-Val-OMe (4 equiv.)
neat, 280–440 nm
H
Val
Me
CF3
Me
N
O
O
-56.33
-64.03
-64.06
-64.09
-66.23
-68.90
162.46
C (t)
-64.06
B (s)
-56.33
D (s)
-68.90
E (s)
-66.23
A (s)
-162.46
f1 (ppm)
2.30
5.36
9.22
1.02
200.00

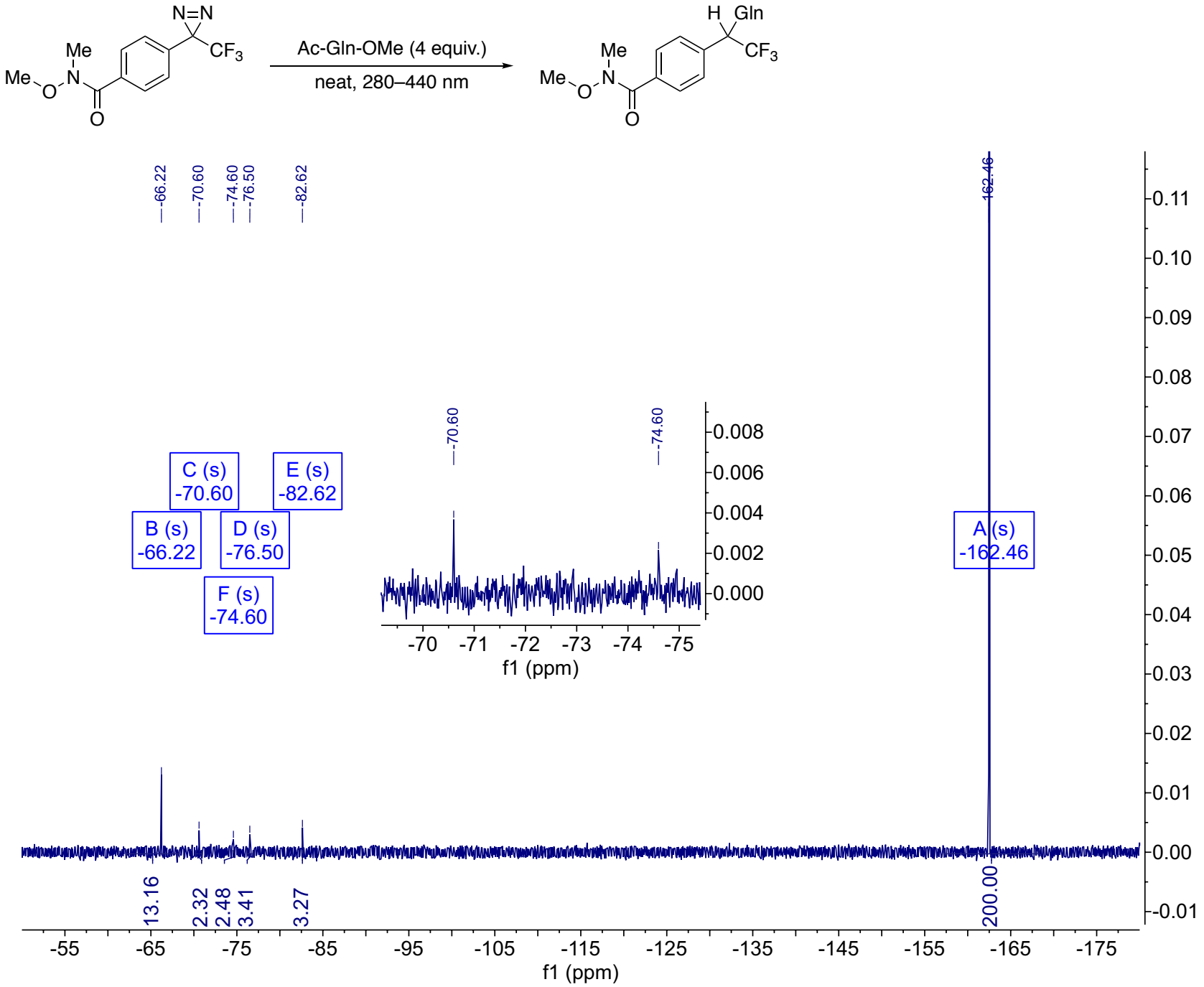

N=N
Me
CF3
Me
N
O
O
Ac-Ala-OMe (4 equiv.)
neat, 280–440 nm
H
Ala
-66.23
-70.60
-72.06
-72.31
-74.28
-74.37
162.46
C (m)
-71.66
B (s)
-66.23
D (d)
-74.32
A (s)
-162.46
9.55
13.15
7.58
200.00
f1 (ppm)
f1 (ppm)

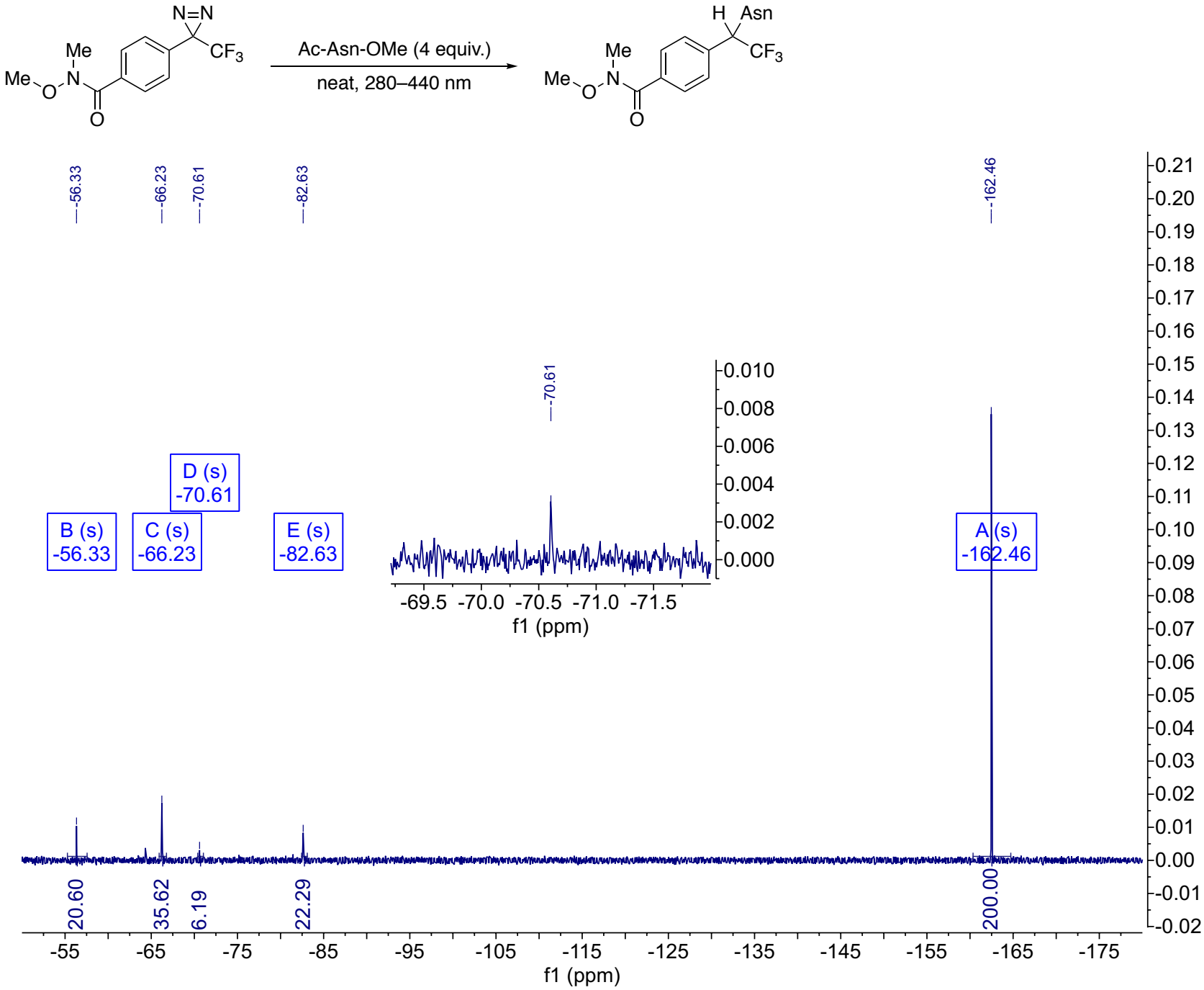

N=N
Me
CF3
Me
N
O
O
Ac-Leu-OMe (4 equiv.)
neat, 280–440 nm
H
Leu
Me
CF3
Me
N
O
O
-64.03
-64.06
-64.09
-66.23
-68.09
-68.17
-68.56
-68.64
C (dd)
-68.37
B (t)
-64.06
D (s)
-66.23
A (s)
-162.46
162.46
200.00
4.64
7.47
4.75
f1 (ppm)
f1 (ppm)
f1 (ppm)

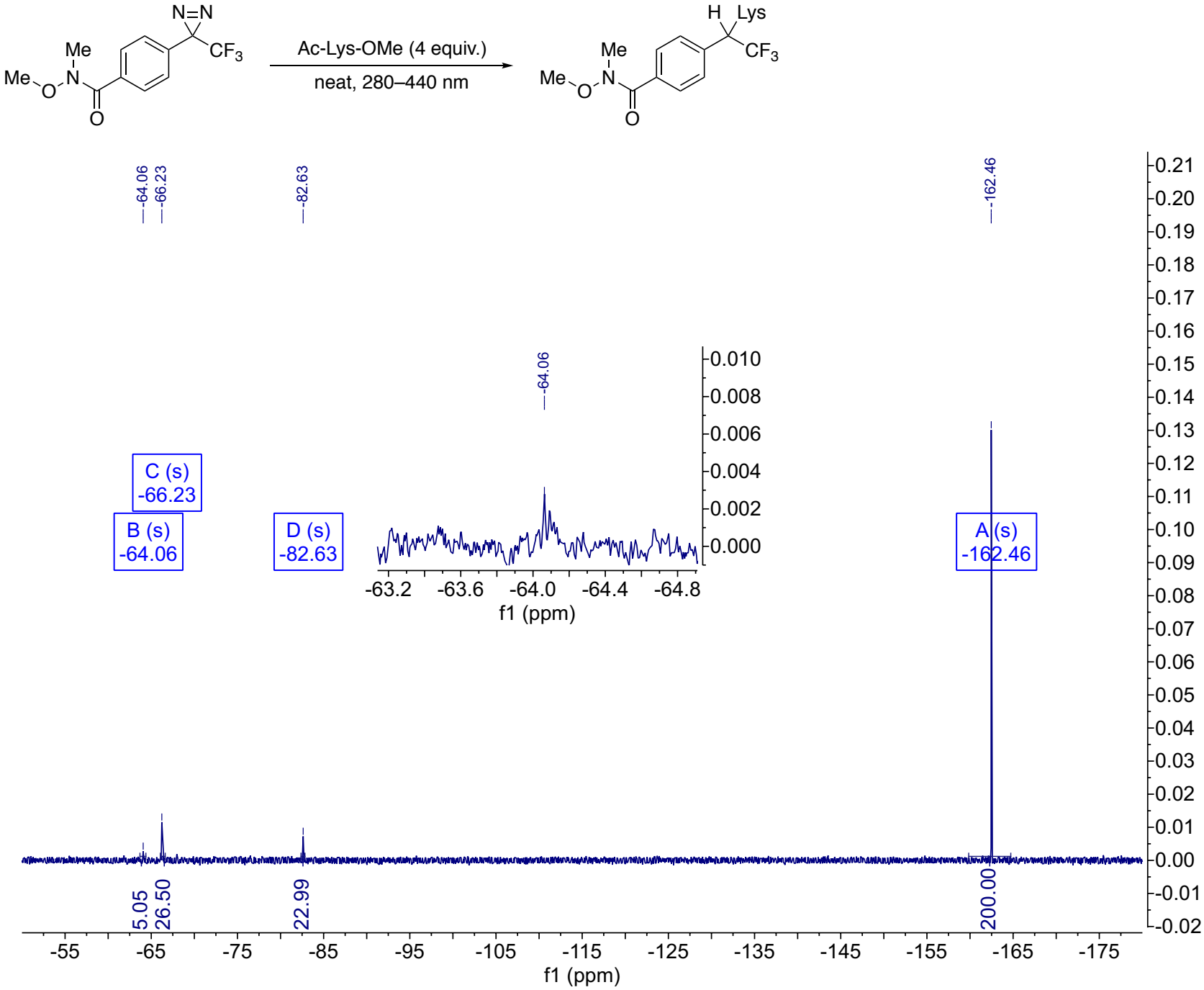

N=N
Me
CF3
Me
N
O
O
Ac-Arg-OMe (4 equiv.)
neat, 280–440 nm
H
Arg
Me
CF3
Me
N
O
O
-66.22
-70.60
-82.62
162.46
C (s)
-70.60
B (s)
-66.22
D (s)
-82.62
A (s)
-162.46
70.60
0.010
0.008
0.006
0.004
0.002
0.000
-69
-70
-71
-72
f1 (ppm)
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
9.36
4.91
3.66
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

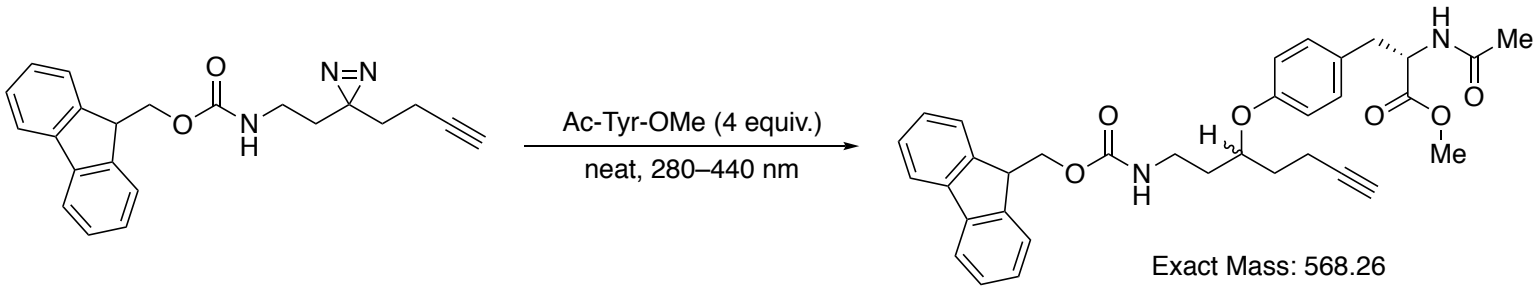

N=N
Me
CF3
Me
N
O
O
Ac-Trp-OMe (4 equiv.)
neat, 280–440 nm
H
Trp
Me
CF3
Me
N
O
O
-66.22
-70.60
-82.62
162.46
D (s)
-66.22
B (s)
-70.60
C (s)
-82.62
A (s)
-162.46
-70.60
0.006
0.004
0.002
0.000
-70.0
-70.5
-71.0
f1 (ppm)
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
9.26
4.57
5.72
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

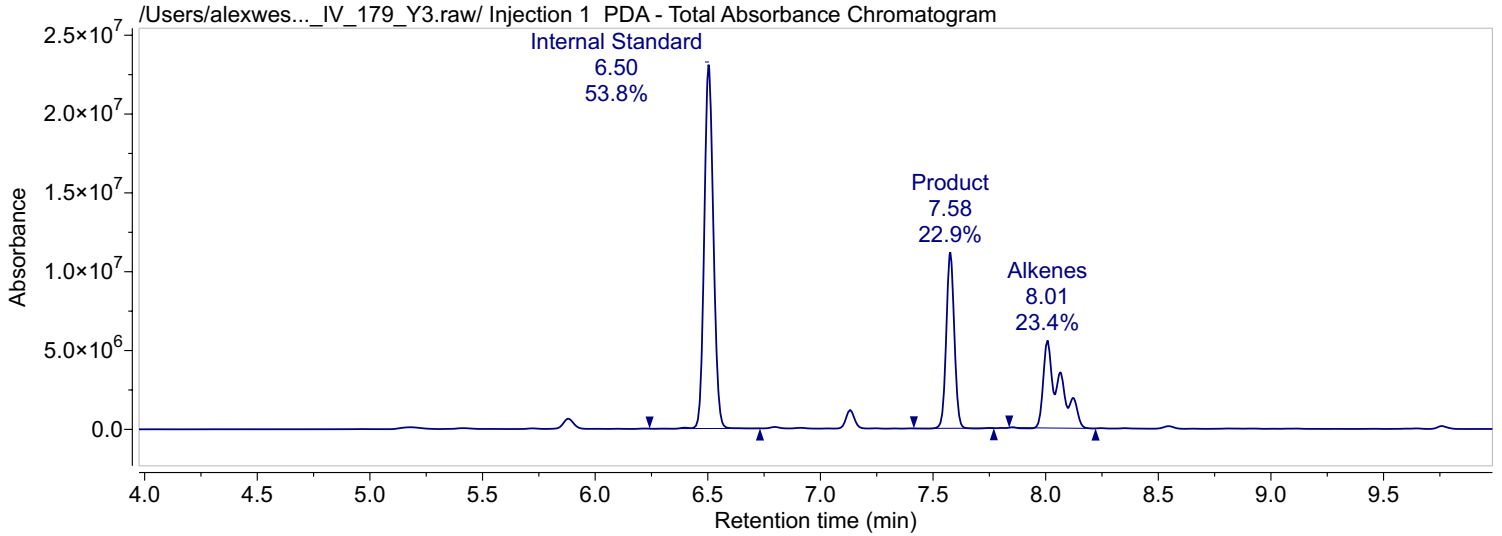

N=N
Me
CF3
Me
N
O
O
Ac-His-OMe (4 equiv.)
neat, 280–440 nm
H
His
Me
CF3
Me
N
O
O
AW_IV_130_H 2
-66.23
-72.41
-76.52
-82.63
162.46
C (s)
-72.41
E (s)
-82.63
B (s)
-66.23
D (s)
-76.52
A (s)
-162.46
-72.41
0.010
0.005
0.000
-71.8
-72.2
-72.6
-73.0
-73.4
f1 (ppm)
8.79
2.57
4.65
2.39
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02

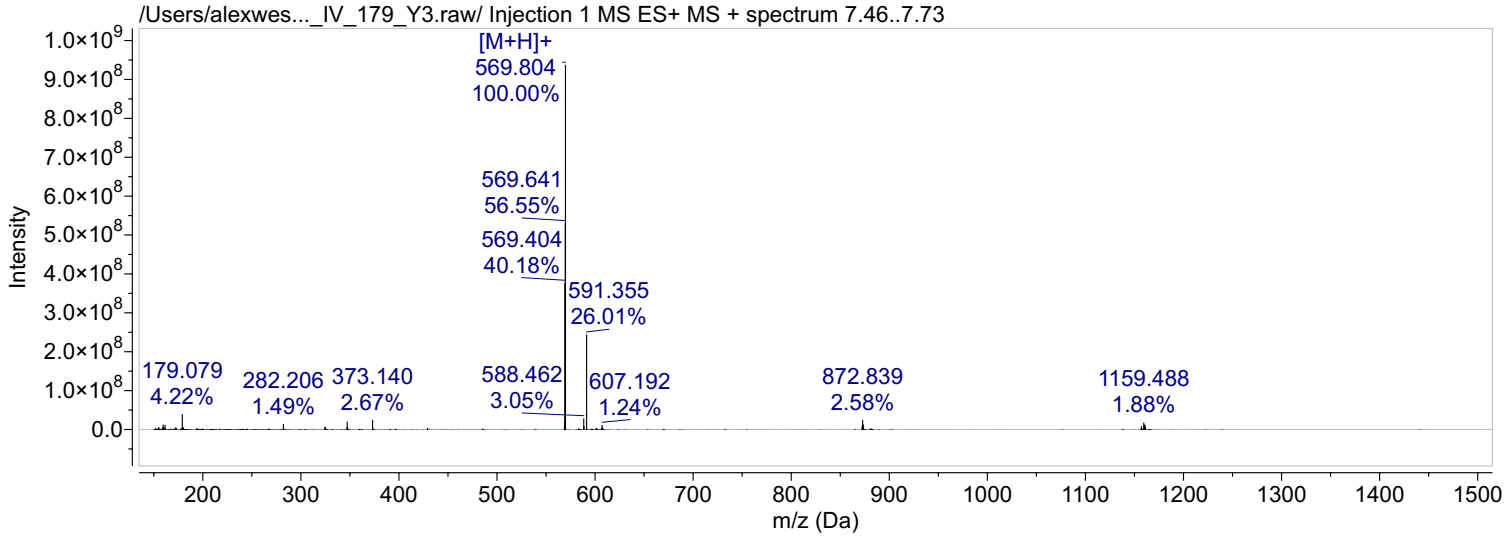

N=N
Me
CF3
Me
N
O
O
Ac-Gln-OMe (4 equiv.)
neat, 280–440 nm
H
Gln
Me
CF3
Me
N
O
O
-66.22
-70.60
-74.60
-76.50
-82.62
162.46
C (s)
-70.60
E (s)
-82.62
B (s)
-66.22
D (s)
-76.50
F (s)
-74.60
A (s)
-162.46
-70.60
-74.60
0.008
0.006
0.004
0.002
0.000
-70
-71
-72
-73
-74
-75
f1 (ppm)
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
13.16
2.32
2.48
3.41
3.27
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

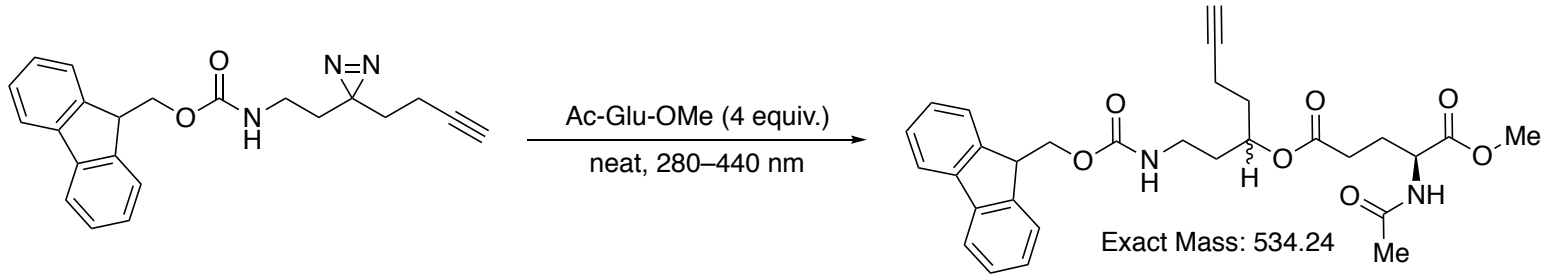

N=N
Me
CF3
Me
N
O
O
Ac-Asn-OMe (4 equiv.)
neat, 280–440 nm
H
Asn
Me
CF3
Me
N
O
O
-56.33
-66.23
-70.61
-82.63
-162.46
B (s)
-56.33
C (s)
-66.23
D (s)
-70.61
E (s)
-82.63
A (s)
-162.46
-70.61
f1 (ppm)
20.60
35.62
6.19
22.29
200.00
f1 (ppm)

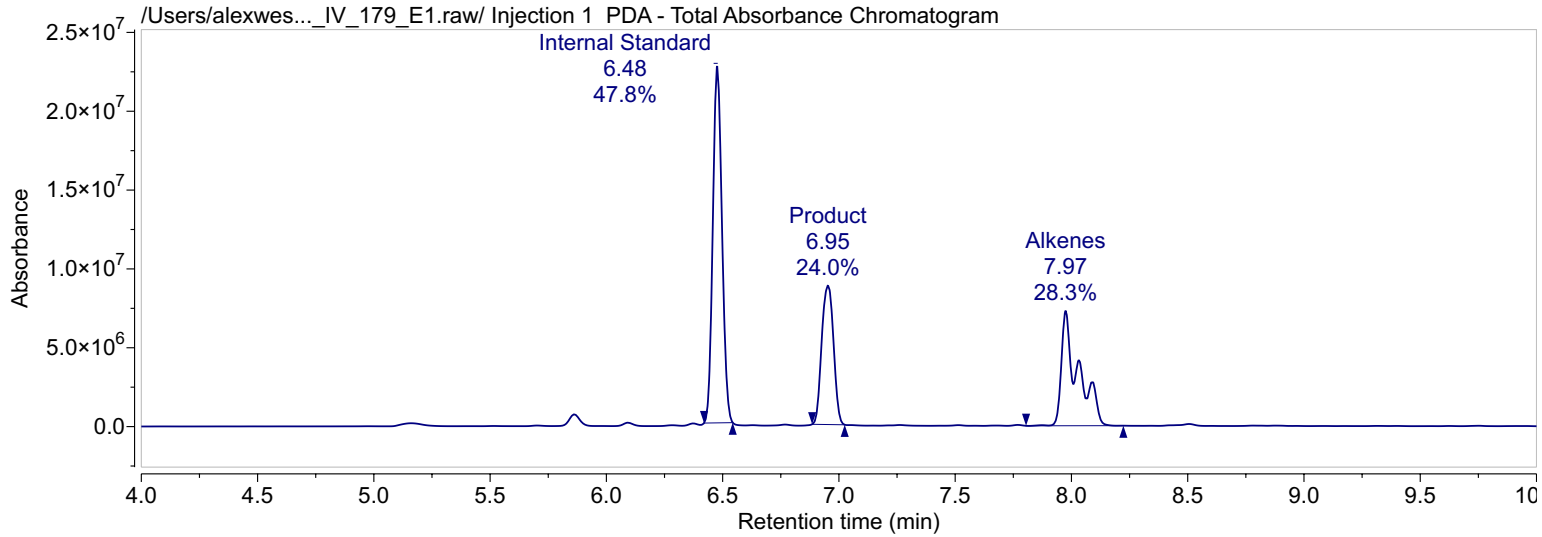

N=N
Me
CF3
Me
N
O
O
Ac-Lys-OMe (4 equiv.)
neat, 280–440 nm
H
Lys
Me
CF3
Me
N
O
O
-64.06
-66.23
-82.63
-162.46
C (s)
-66.23
B (s)
-64.06
D (s)
-82.63
A (s)
-162.46
-64.06
0.010
0.008
0.006
0.004
0.002
0.000
-63.2
-63.6
-64.0
-64.4
-64.8
f1 (ppm)
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
5.05
26.50
22.99
200.00
-55
-65
-75
-85
-95
-105
-115
-125
-135
-145
-155
-165
-175
f1 (ppm)

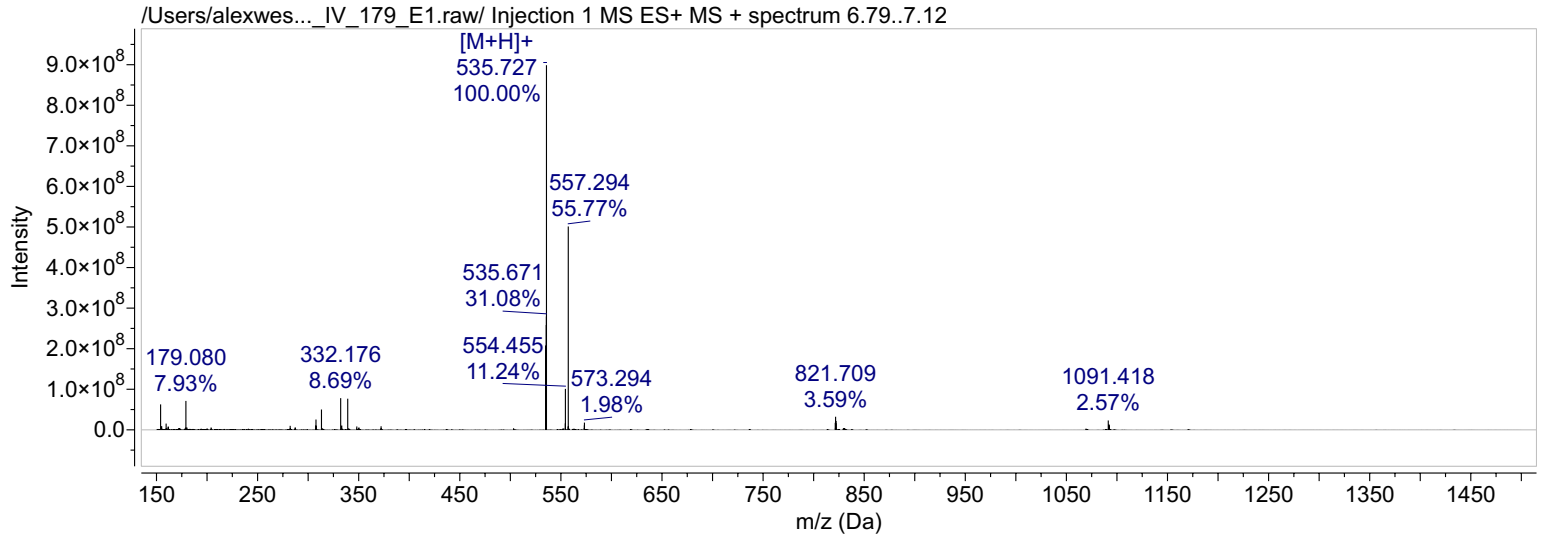

Ac-Tyr-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 568.26

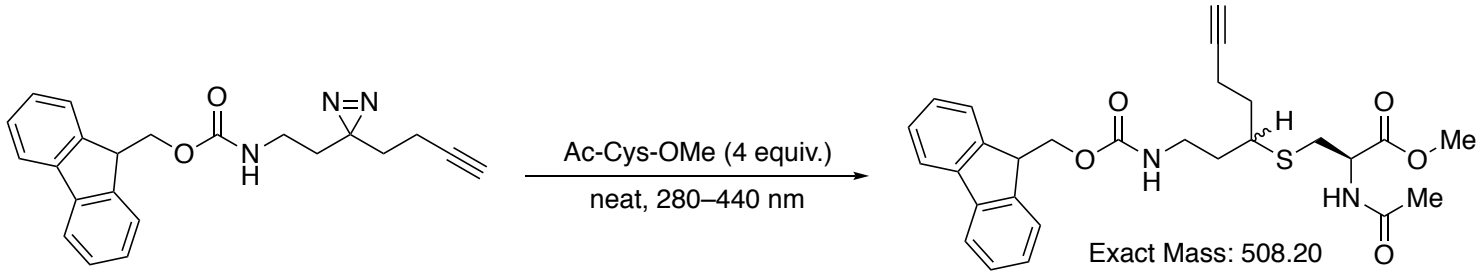

/Users/alexwes..._IV_179_Y3.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.50
53.8%
Product
7.58
22.9%
Alkenes
8.01
23.4%
Absorbance
Retention time (min)

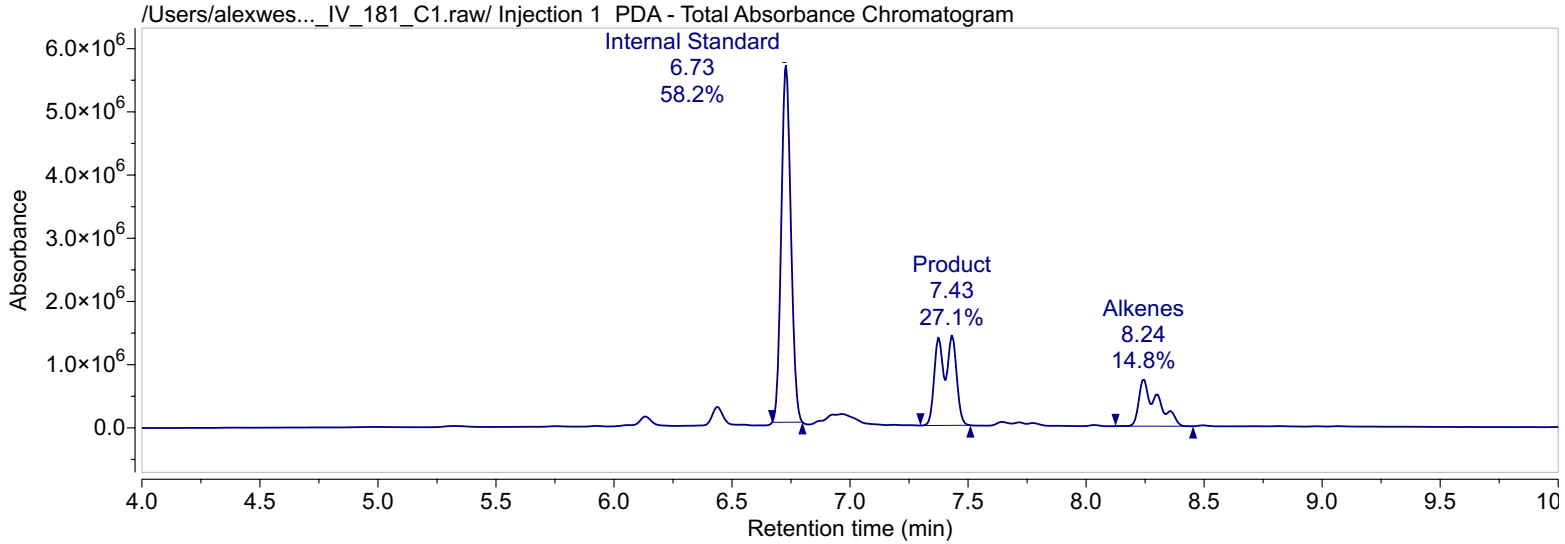

/Users/alexwes..._IV_179_Y3.raw/ Injection 1 MS ES+ MS + spectrum 7.46..7.73
[M+H]+
569.804
100.00%
569.641
56.55%
569.404
40.18%
591.355
26.01%
179.079
4.22%
282.206
1.49%
373.140
2.67%
588.462
3.05%
607.192
1.24%
872.839
2.58%
1159.488
1.88%
Intensity
m/z (Da)

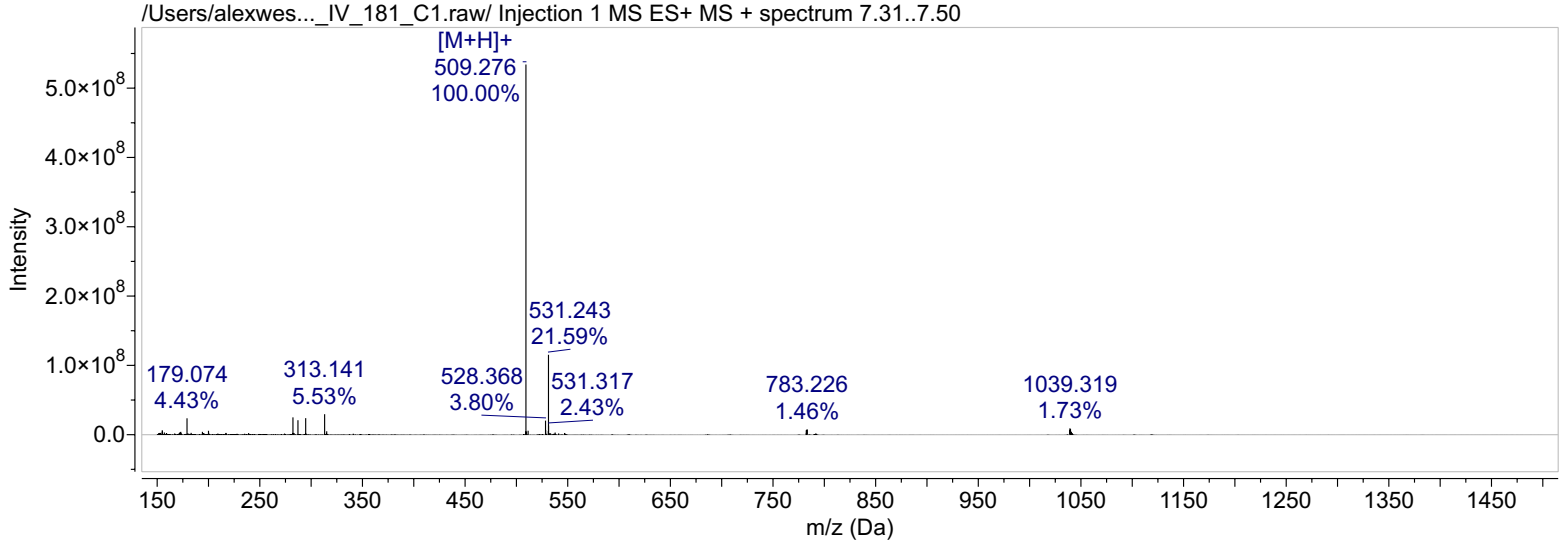

Ac-Glu-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 534.24

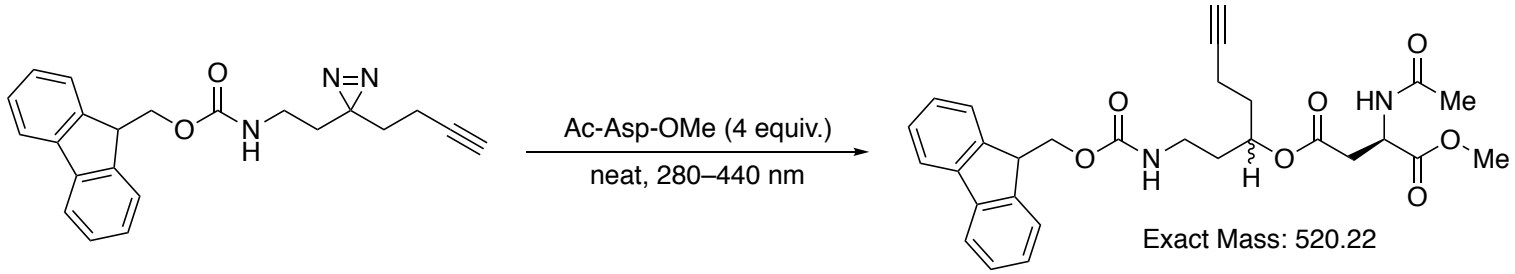

/Users/alexwes..._IV_179_E1.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.48
47.8%
Product
6.95
24.0%
Alkenes
7.97
28.3%
Absorbance
Retention time (min)

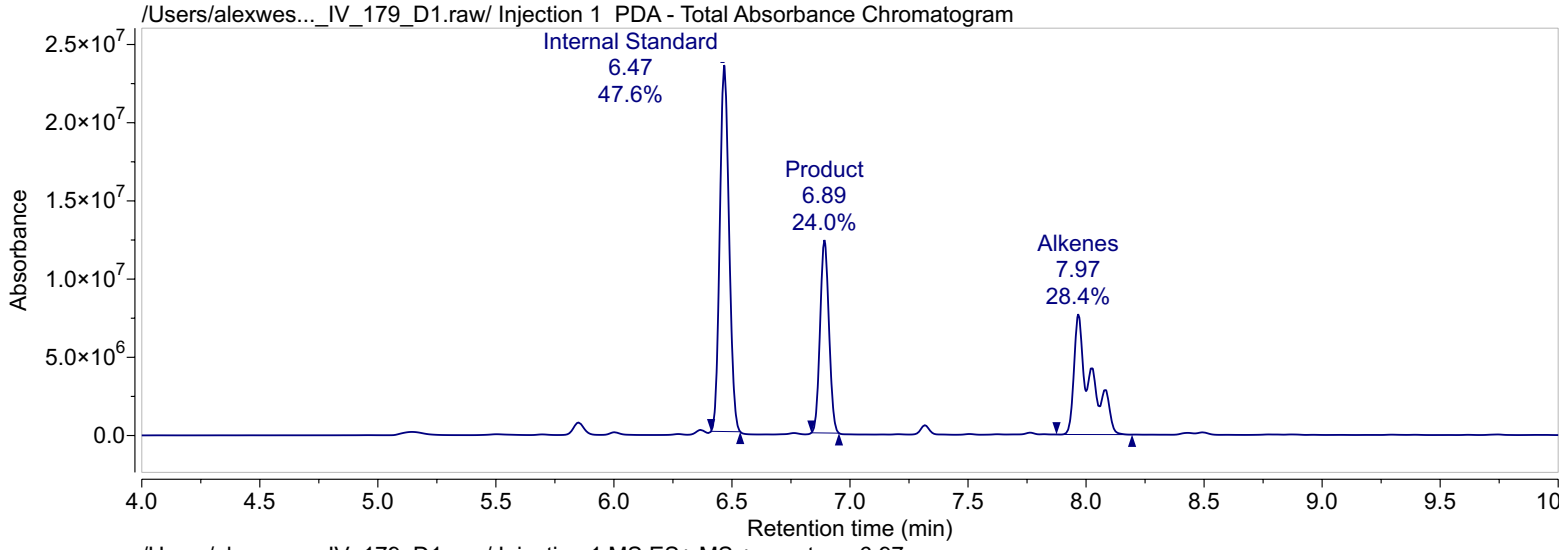

/Users/alexwes..._IV_179_E1.raw/ Injection 1 MS ES+ MS + spectrum 6.79..7.12
[M+H]+
535.727
100.00%
557.294
55.77%
535.671
31.08%
554.455
11.24%
573.294
1.98%
179.080
7.93%
332.176
8.69%
821.709
3.59%
1091.418
2.57%
Intensity
m/z (Da)

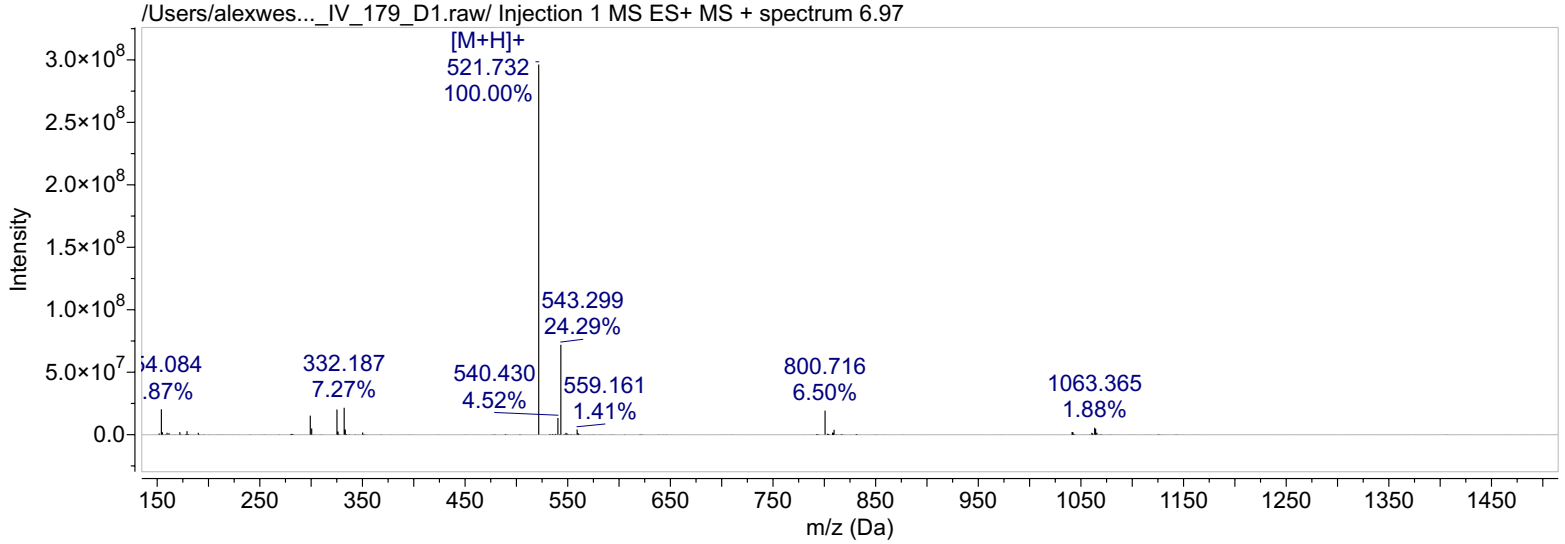

Ac-Cys-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 508.20

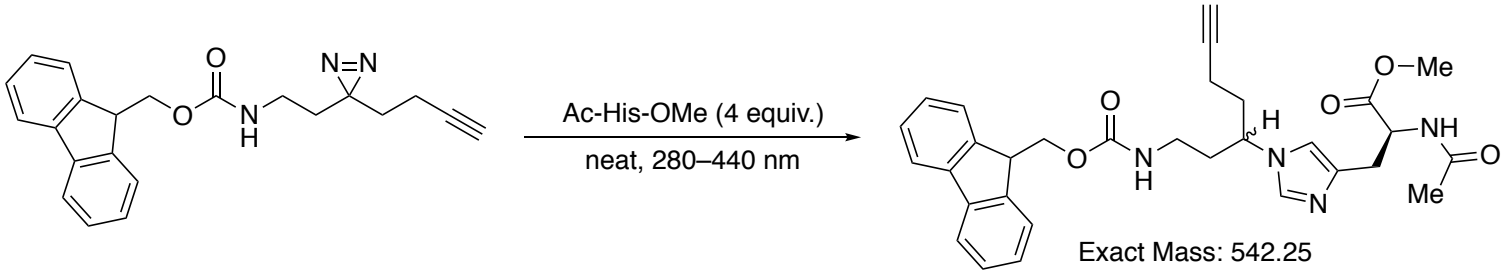

/Users/alexwes..._IV_181_C1.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.73
58.2%
Product
7.43
27.1%
Alkenes
8.24
14.8%
Absorbance
Retention time (min)

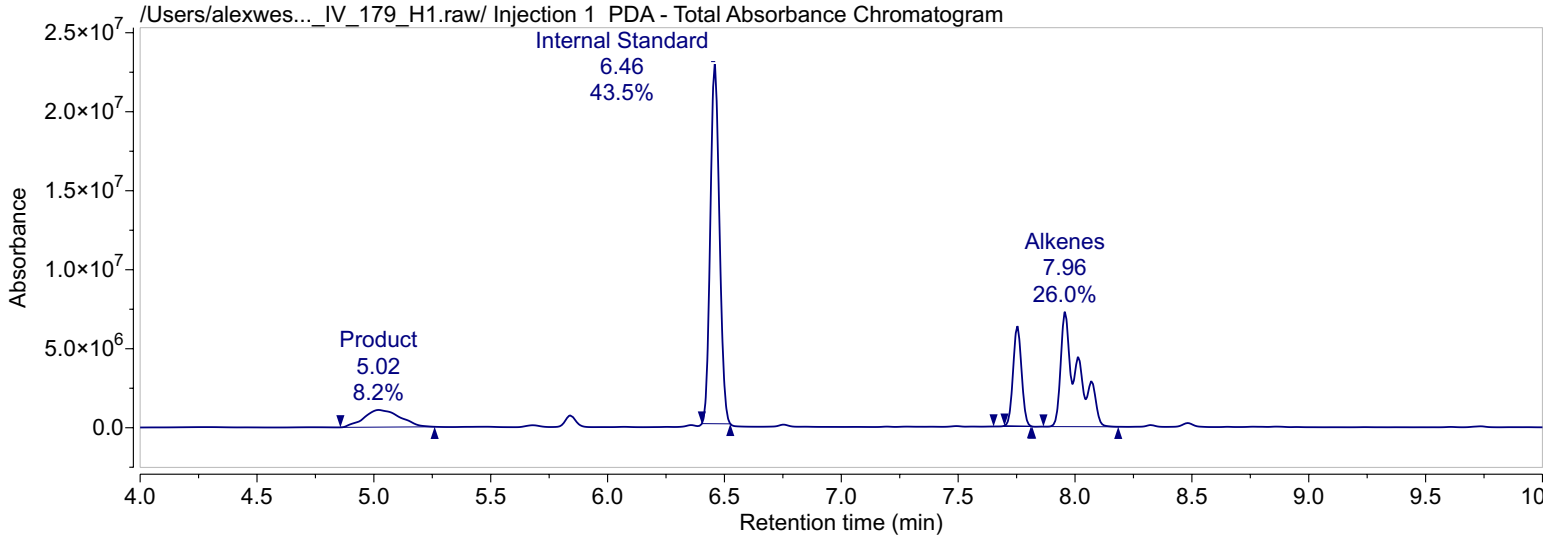

/Users/alexwes..._IV_181_C1.raw/ Injection 1 MS ES+ MS + spectrum 7.31..7.50
[M+H]+
509.276
100.00%
531.243
21.59%
179.074
4.43%
313.141
5.53%
528.368
3.80%
531.317
2.43%
783.226
1.46%
1039.319
1.73%
Intensity
m/z (Da)

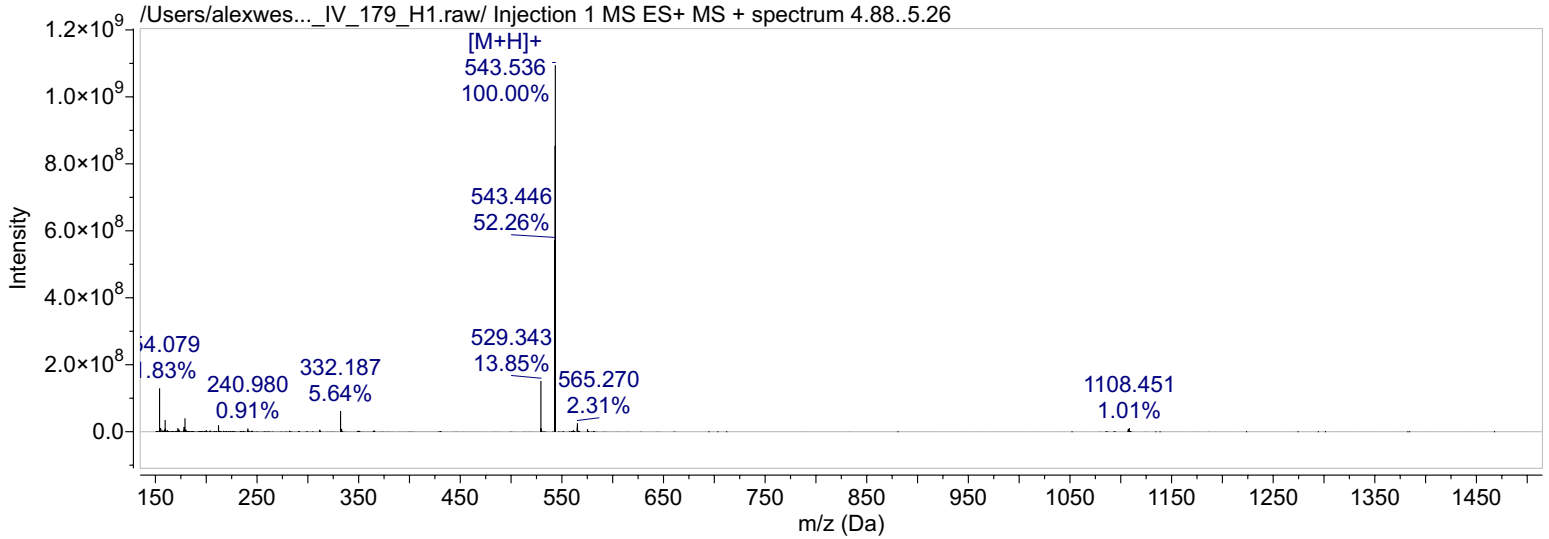

Ac-Asp-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 520.22

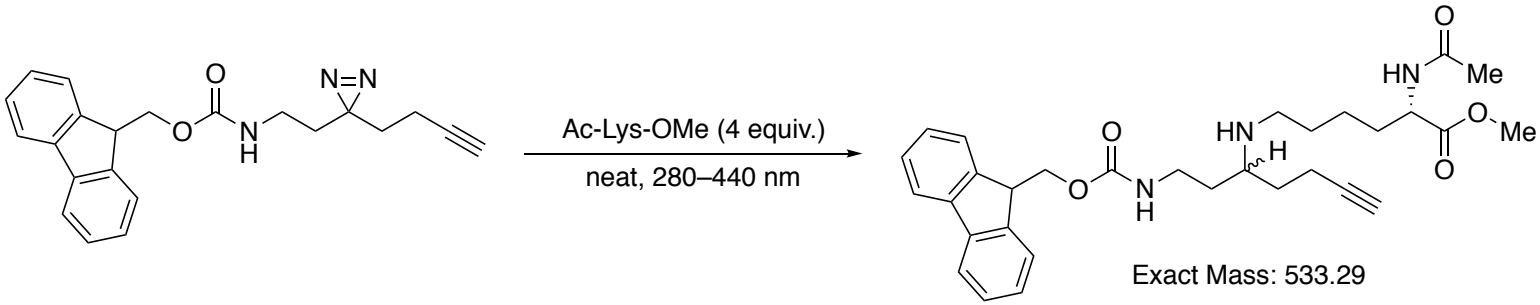

/Users/alexwes..._IV_179_D1.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.47
47.6%
Product
6.89
24.0%
Alkenes
7.97
28.4%
Absorbance
Retention time (min)

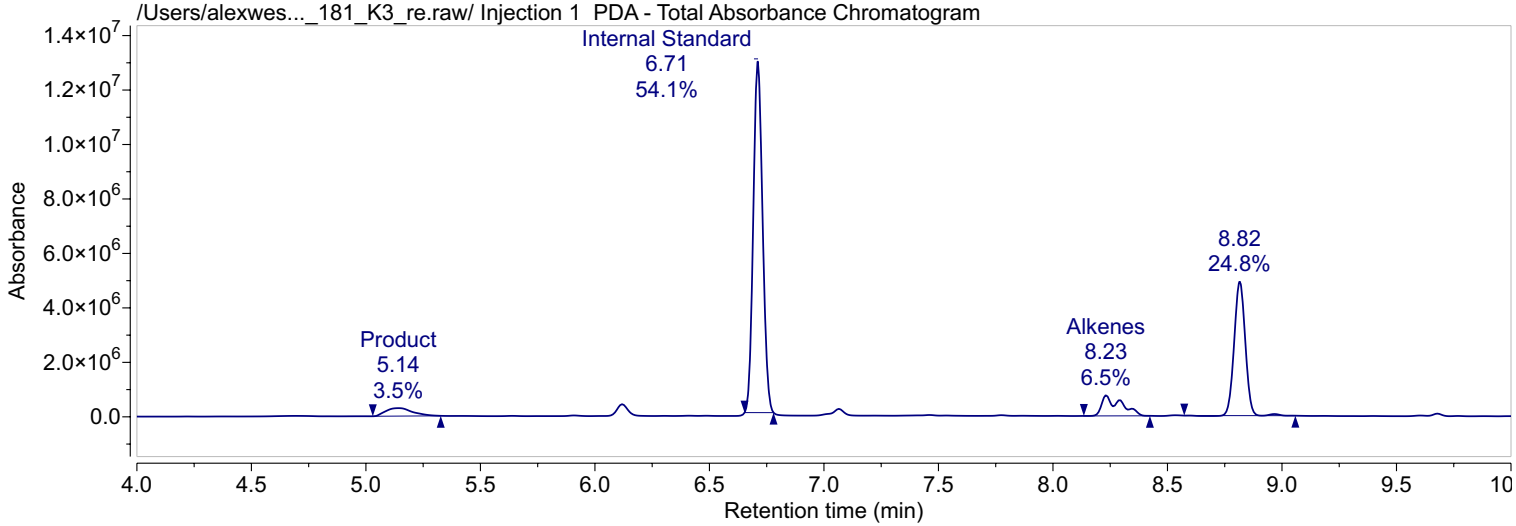

/Users/alexwes..._IV_179_D1.raw/ Injection 1 MS ES+ MS + spectrum 6.97
[M+H]+
521.732
100.00%
543.299
24.29%
332.187
7.27%
540.430
4.52%
559.161
1.41%
800.716
6.50%
1063.365
1.88%
Intensity
m/z (Da)

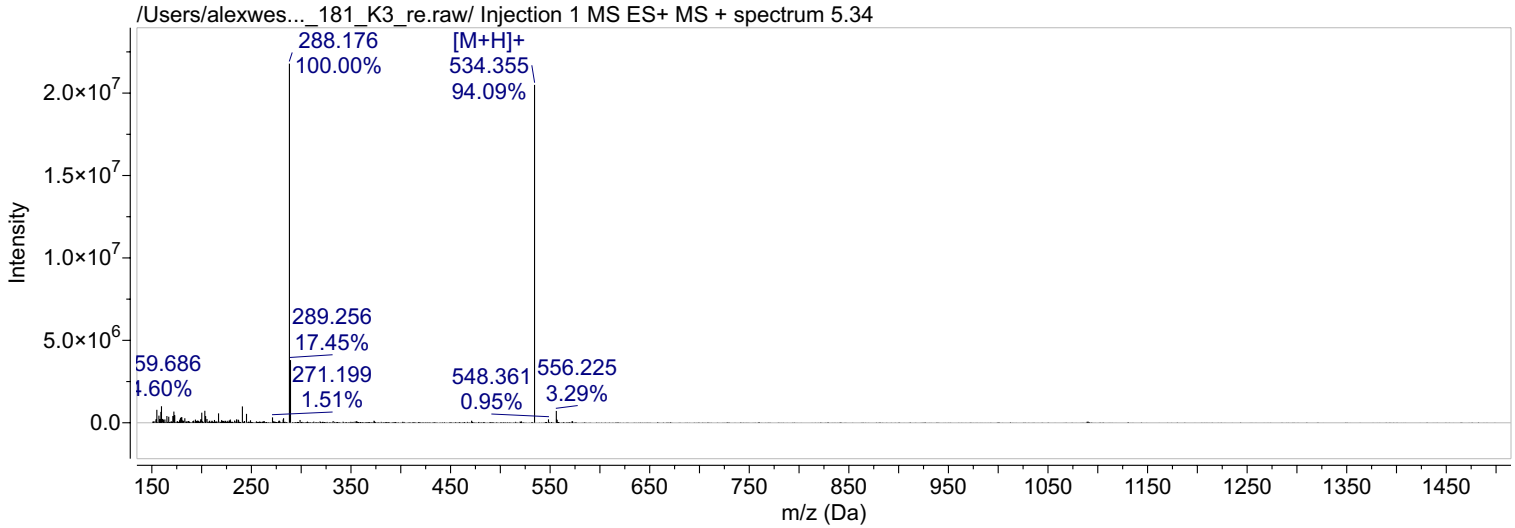

Ac-His-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 542.25

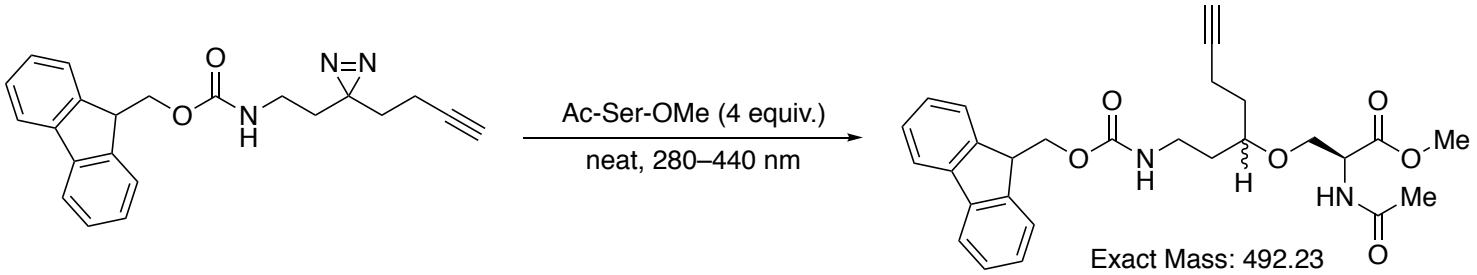

/Users/alexwes..._IV_179_H1.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.46
43.5%
Alkenes
7.96
26.0%
Product
5.02
8.2%
Absorbance
Retention time (min)

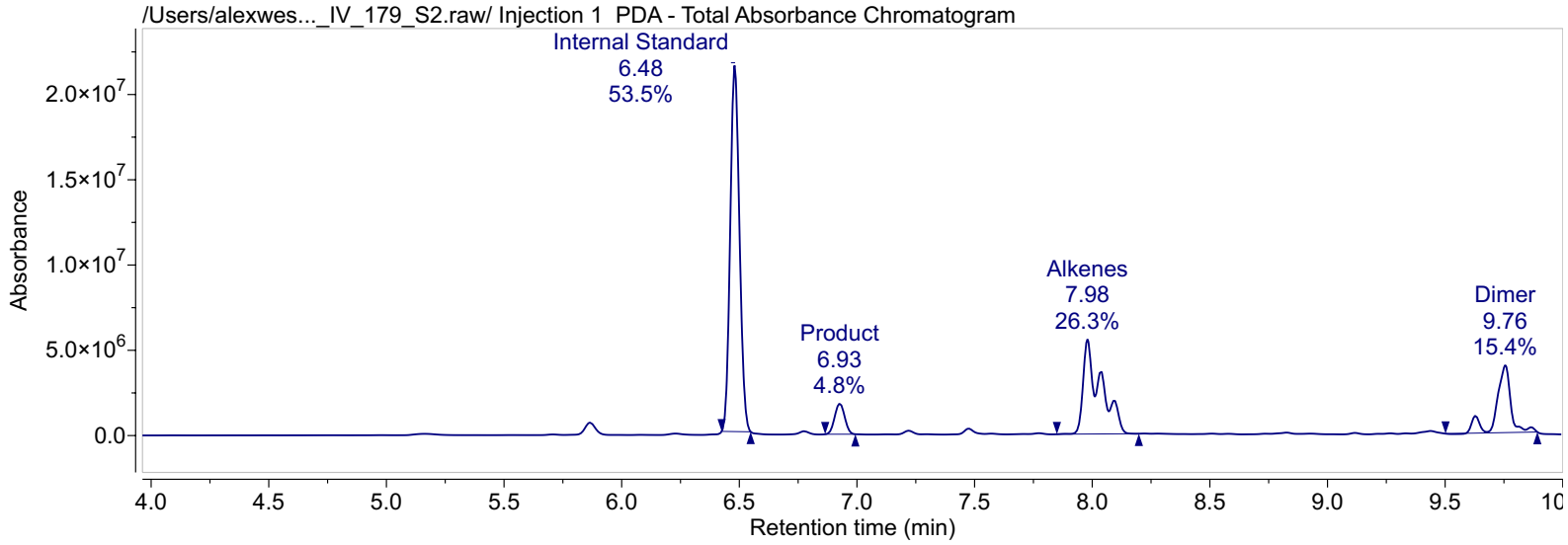

/Users/alexwes..._IV_179_H1.raw/ Injection 1 MS ES+ MS + spectrum 4.88..5.26
[M+H]+
543.536
100.00%
543.446
52.26%
529.343
13.85%
565.270
2.31%
332.187
5.64%
240.980
0.91%
1108.451
1.01%
Intensity
m/z (Da)

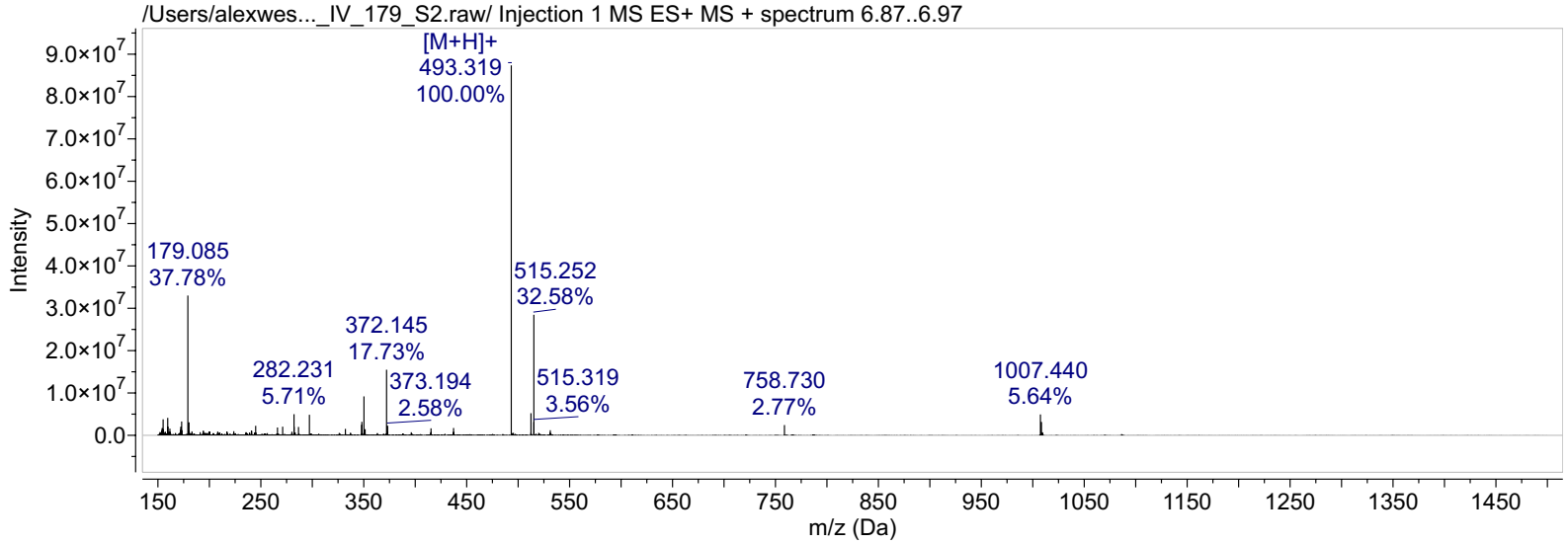

Ac-Lys-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 533.29

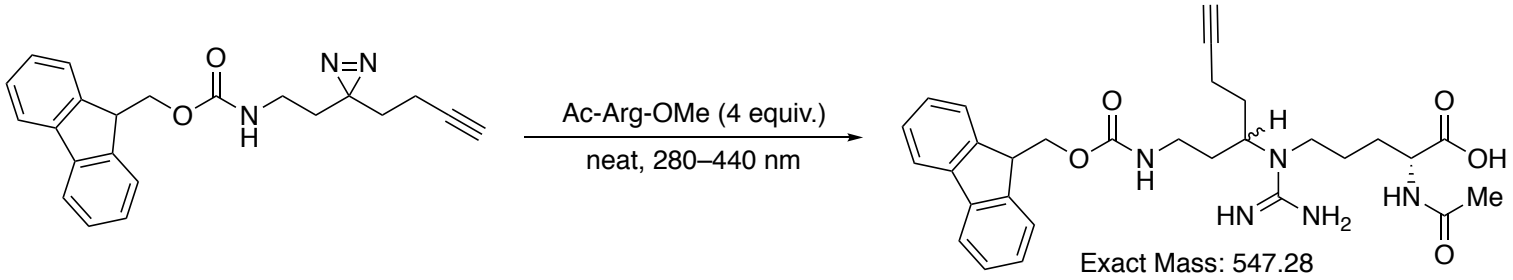

/Users/alexwes..._181_K3_re.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.71
54.1%
Product
5.14
3.5%
Alkenes
8.23
6.5%
8.82
24.8%
Absorbance
Retention time (min)

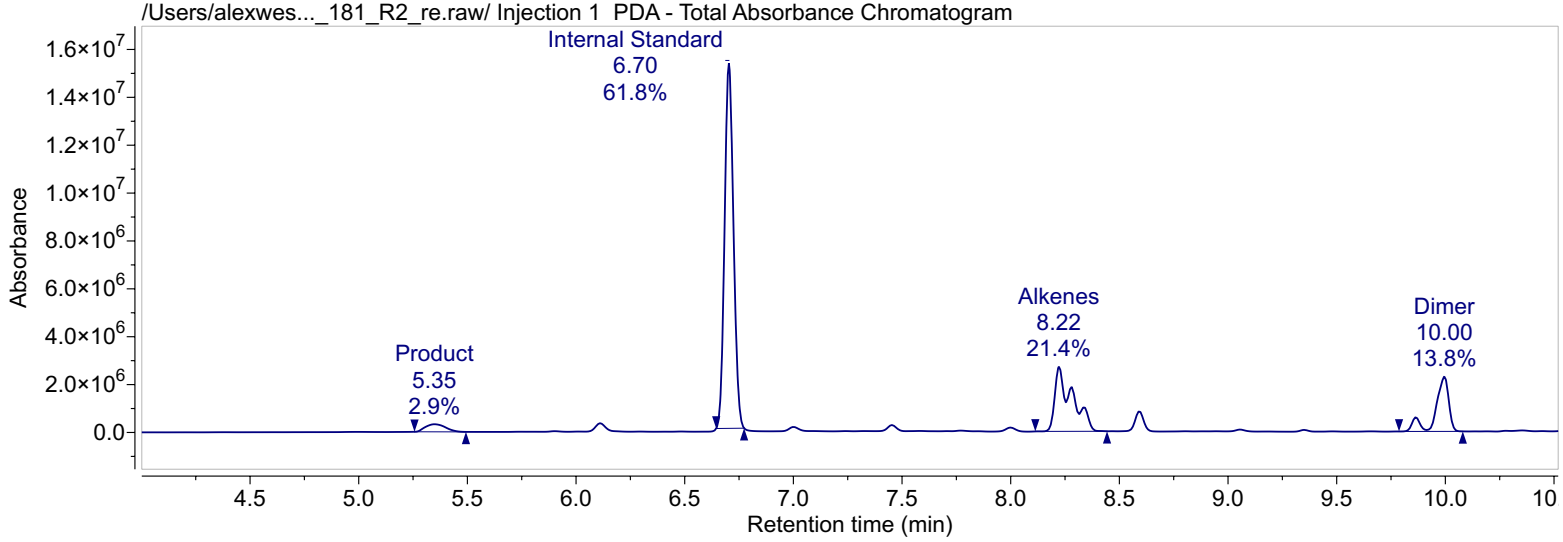

/Users/alexwes..._181_K3_re.raw/ Injection 1 MS ES+ MS + spectrum 5.34
288.176
100.00%
[M+H]+
534.355
94.09%
289.256
17.45%
271.199
1.51%
548.361
0.95%
556.225
3.29%
Intensity
m/z (Da)

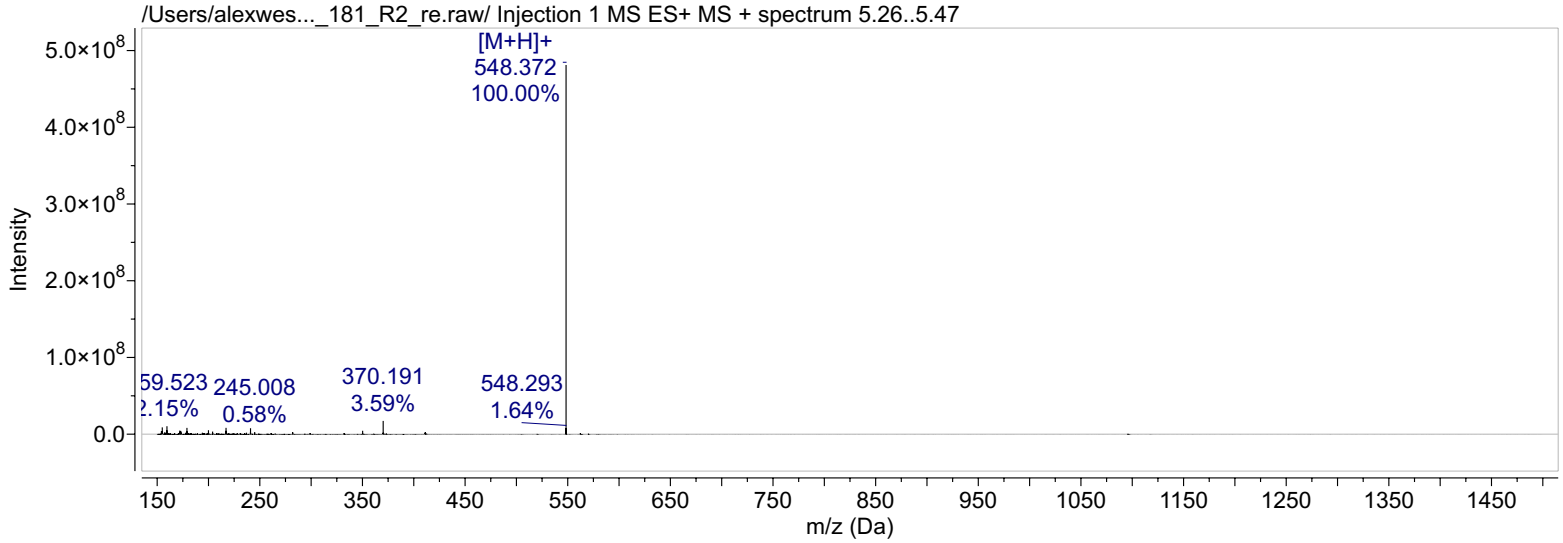

Ac-Ser-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 492.23

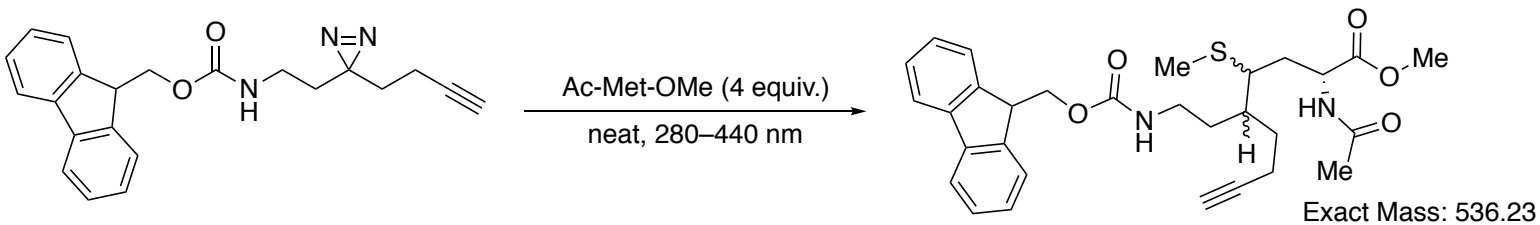

/Users/alexwes..._IV_179_S2.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.48
53.5%
Product
6.93
4.8%
Alkenes
7.98
26.3%
Dimer
9.76
15.4%
Absorbance
Retention time (min)

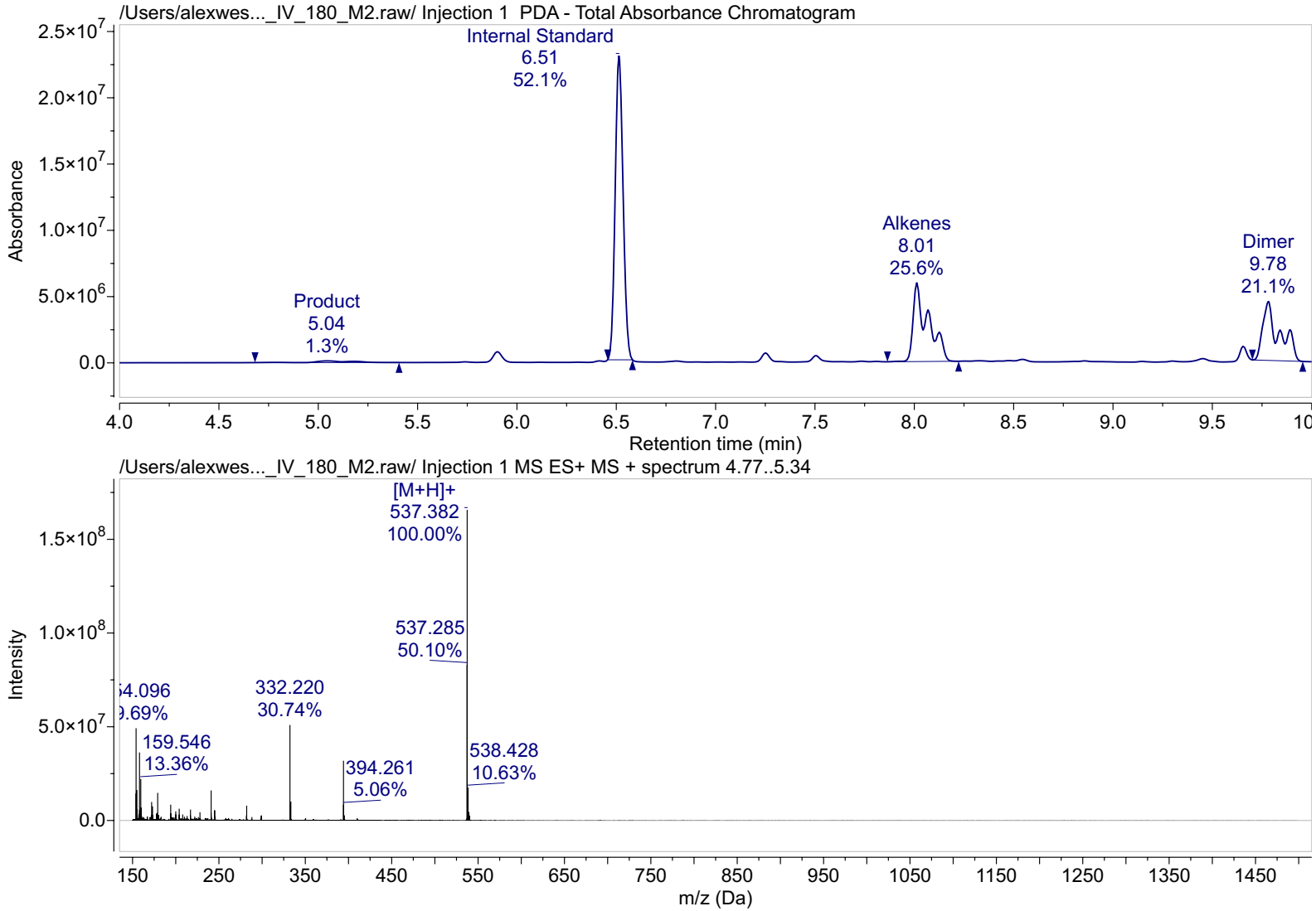

/Users/alexwes..._IV_179_S2.raw/ Injection 1 MS ES+ MS + spectrum 6.87..6.97
[M+H]+
493.319
100.00%
179.085
37.78%
515.252
32.58%
372.145
17.73%
282.231
5.71%
373.194
2.58%
515.319
3.56%
758.730
2.77%
1007.440
5.64%
Intensity
m/z (Da)

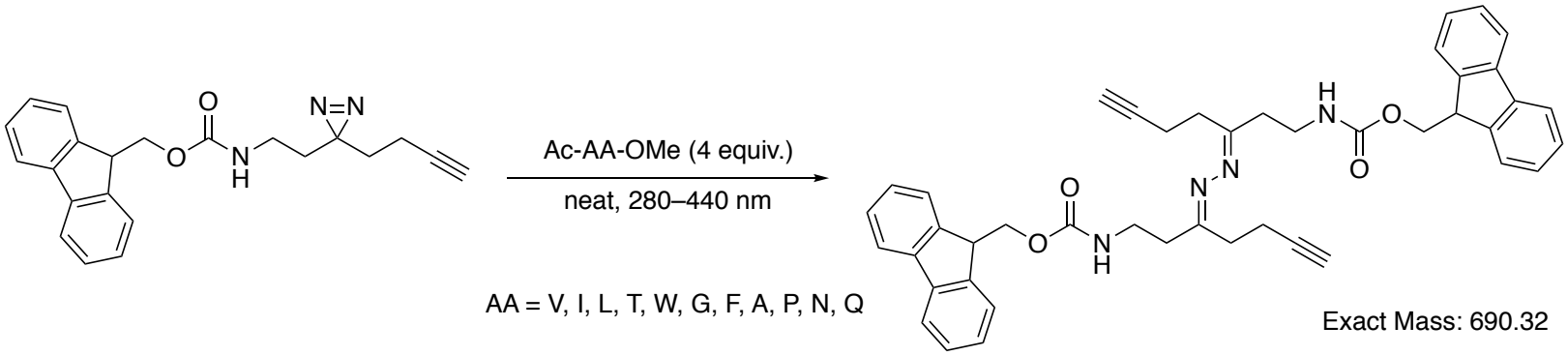

Ac-Arg-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 547.28

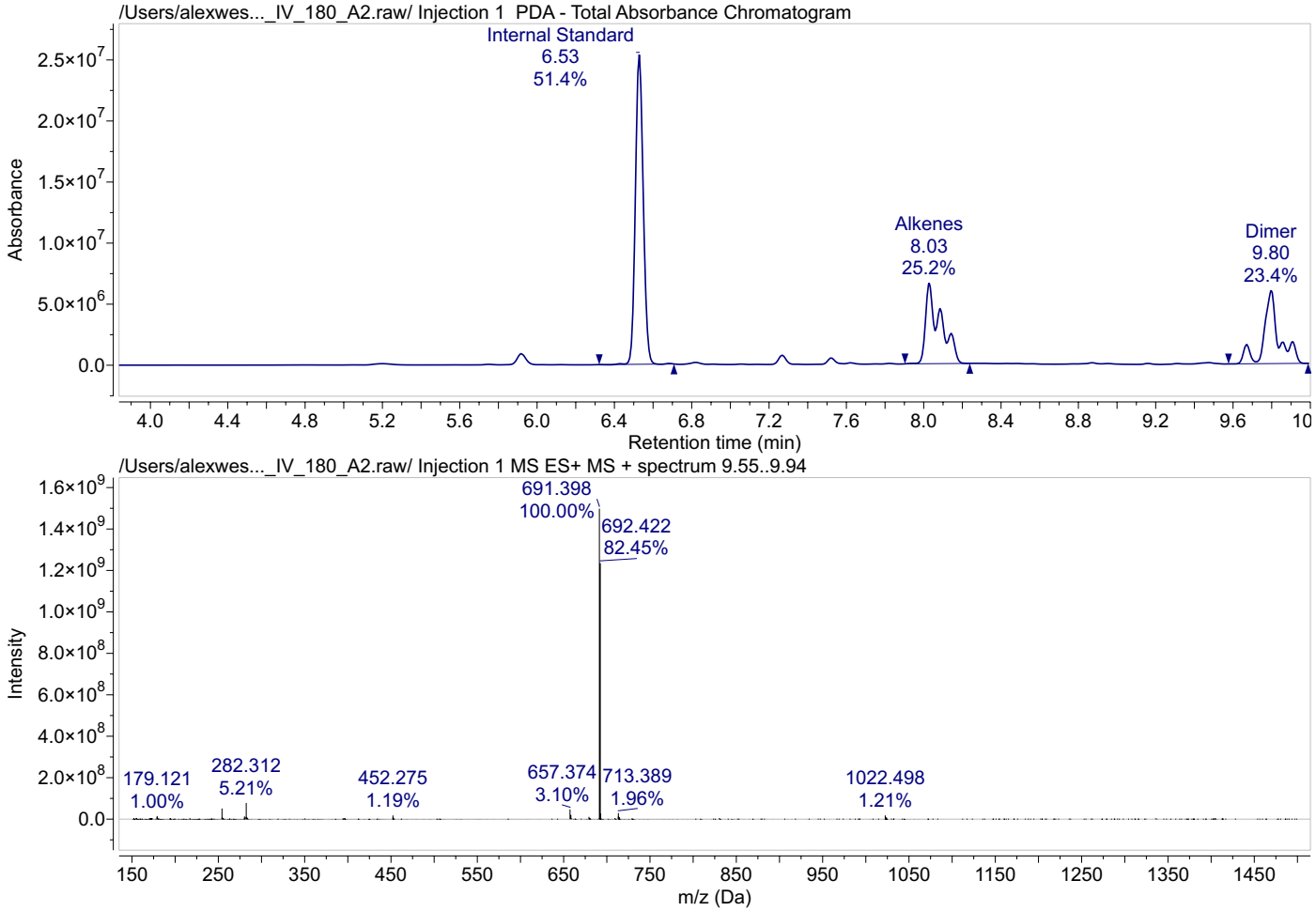

/Users/alexwes..._181_R2_re.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.70
61.8%
Product
5.35
2.9%
Alkenes
8.22
21.4%
Dimer
10.00
13.8%
Absorbance
Retention time (min)

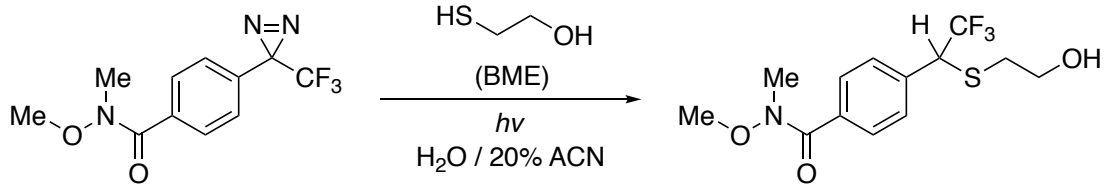

/Users/alexwes..._181_R2_re.raw/ Injection 1 MS ES+ MS + spectrum 5.26..5.47
[M+H]+
548.372
100.00%
370.191
3.59%
548.293
1.64%
245.008
0.58%
Intensity
m/z (Da)


Ac-Met-OMe (4 equiv.)
neat, 280–440 nm
Exact Mass: 536.23

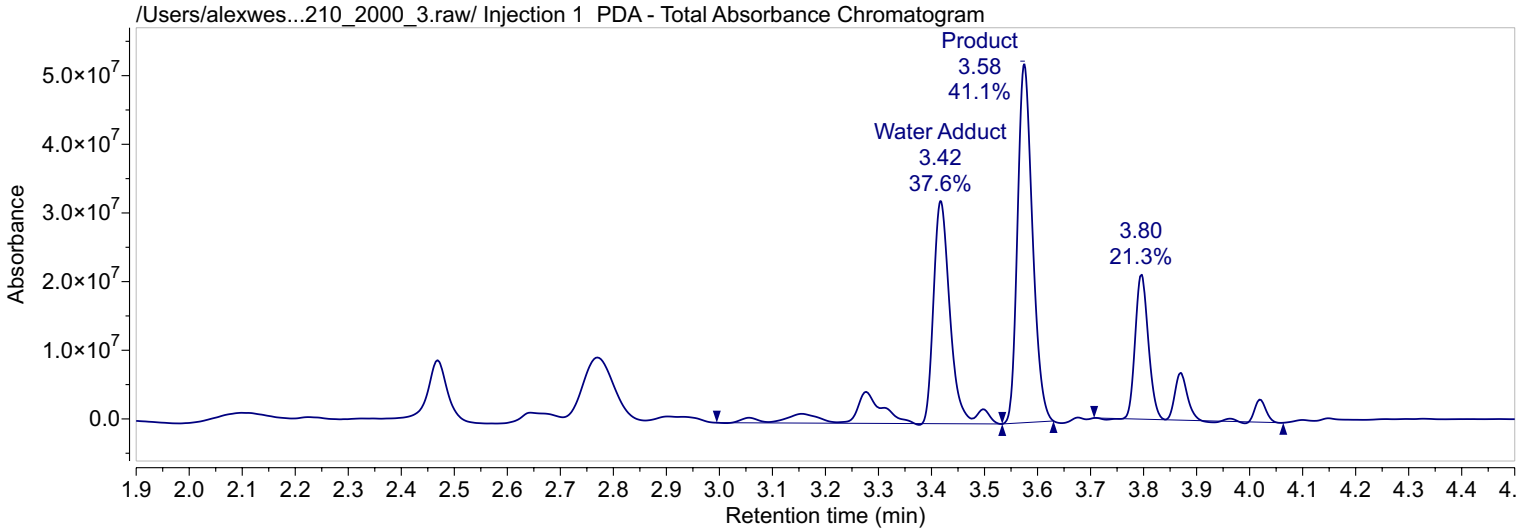

/Users/alexwes..._IV_180_M2.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.51
52.1%
Alkenes
8.01
25.6%
Dimer
9.78
21.1%
Product
5.04
1.3%
Absorbance
Retention time (min)
/Users/alexwes..._IV_180_M2.raw/ Injection 1 MS ES+ MS + spectrum 4.77..5.34
[M+H]+
537.382
100.00%
537.285
50.10%
332.220
30.74%
159.546
13.36%
394.261
5.06%
538.428
10.63%
Intensity
m/z (Da)

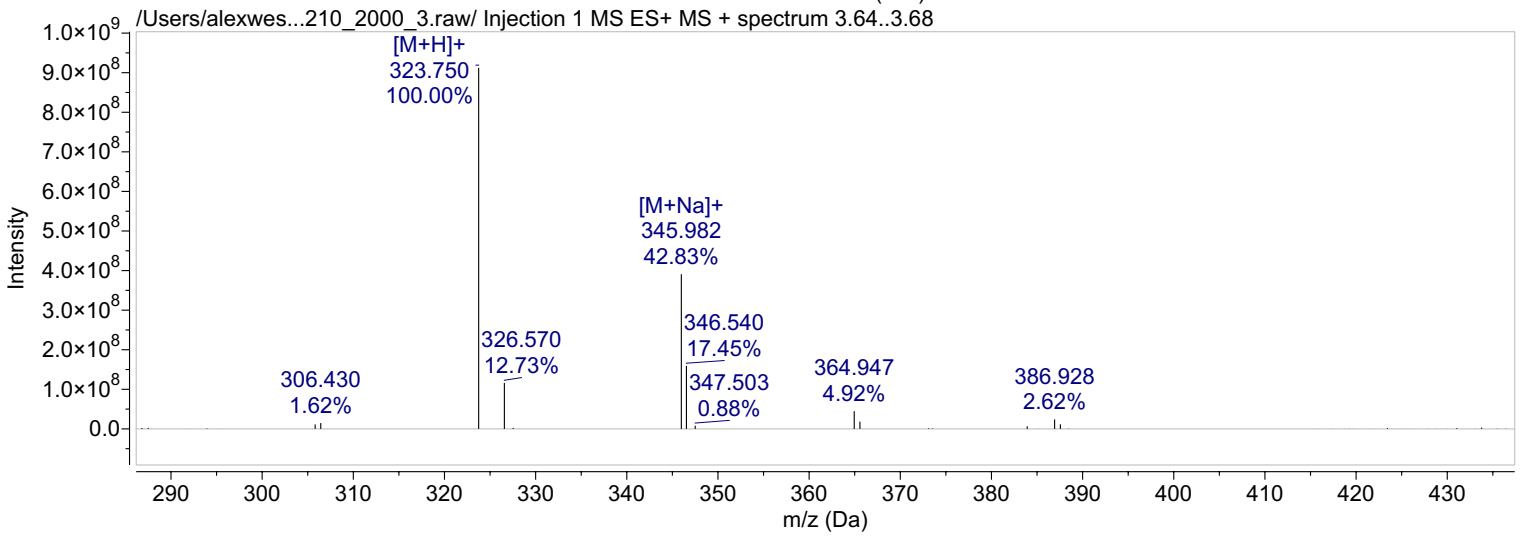

Ac-AA-OMe (4 equiv.)
neat, 280–440 nm
AA = V, I, L, T, W, G, F, A, P, N, Q
Exact Mass: 690.32


/Users/alexwes..._IV_180_A2.raw/ Injection 1 PDA - Total Absorbance Chromatogram
Internal Standard
6.53
51.4%
Alkenes
8.03
25.2%
Dimer
9.80
23.4%
Absorbance
Retention time (min)
/Users/alexwes..._IV_180_A2.raw/ Injection 1 MS ES+ MS + spectrum 9.55..9.94
691.398
100.00%
692.422
82.45%
179.121
1.00%
282.312
5.21%
452.275
1.19%
657.374
3.10%
713.389
1.96%
1022.498
1.21%
Intensity
m/z (Da)

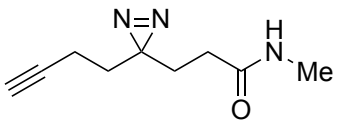


Exact Mass: 323.08

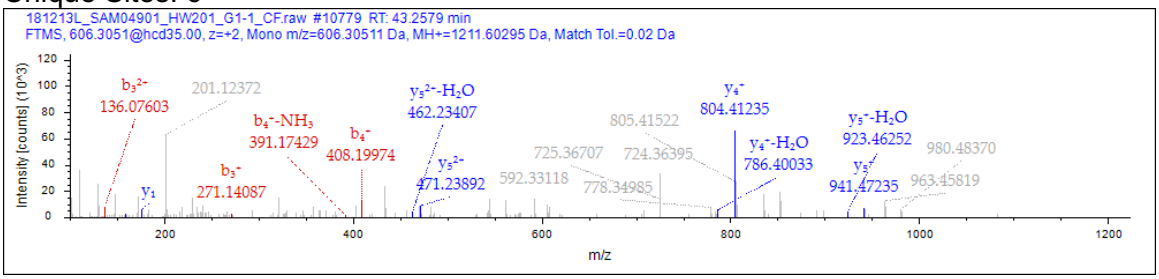




Catalog of binding site spectra
Group 1:

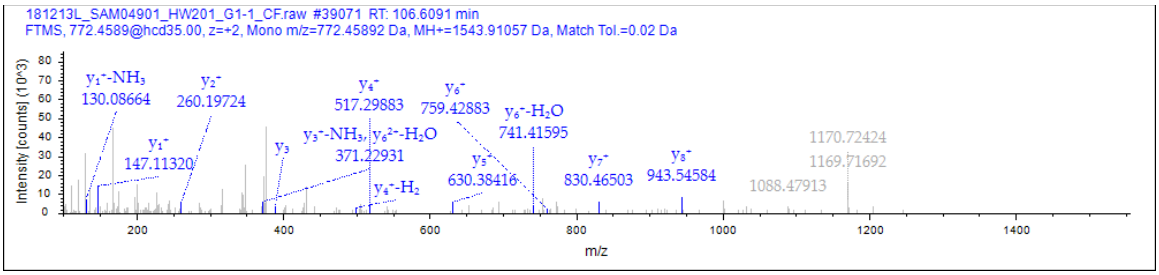


JN Mod: JN-0001-NX-1
PSMs: 22
Unique Sites: 6

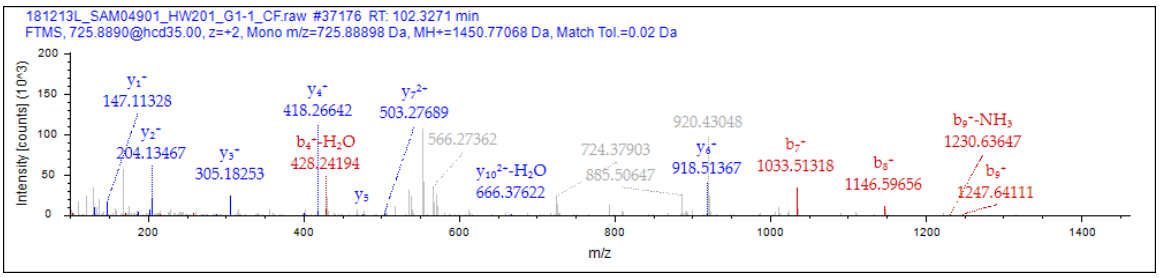


Sequence: AQAHAADR
Charge: +2,
Monoisotopic m/z: 606.30511 Da (+0.06 mmu/+0.09 ppm),
MH+: 1211.60295 Da,
RT: 43.2579 min,
Identified with: Sequest HT (v1.17);
XCorr:1.21,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

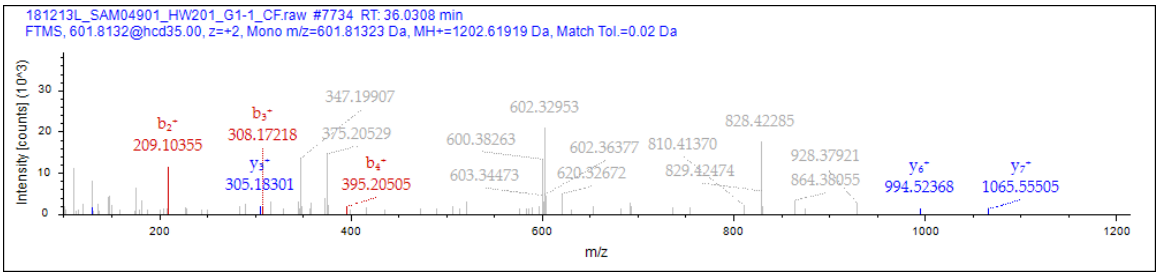
- Putative uncharacterized protein C15orf54 OS=Homo sapiens OX=9606 GN=C15orf54 PE=2 SV=1
-



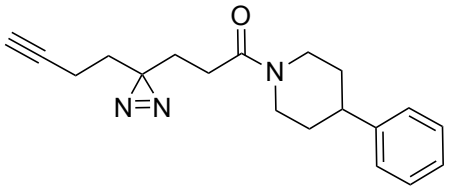
Sequence: ILLAELEQLK
Charge: +2,
Monoisotopic m/z: 772.45892 Da (-0.57 mmu/-0.73 ppm),
MH+: 1543.91057 Da,
RT: 106.6091 min,
Identified with: Sequest HT (v1.17);
XCorr:1.57,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4



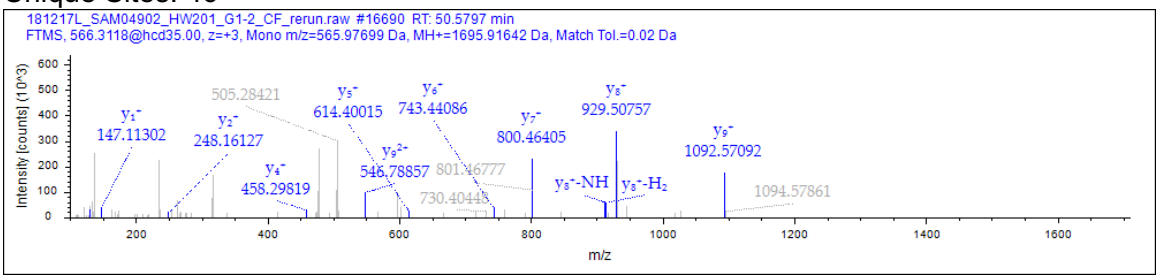
Sequence: TTRSSAGLTGK
Charge: +2,
Monoisotopic m/z: 725.88898 Da (-2.72 mmu/-3.75 ppm),
MH+: 1450.77068 Da,
RT: 102.3271 min,
Identified with: Sequest HT (v1.17);
XCorr:1.98,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Mucin-19 OS=Homo sapiens OX=9606 GN=MUC19 PE=1 SV=3



Sequence: HAVSEGTK
Charge: +2,
Monoisotopic m/z: 601.81323 Da (+0.24 mmu/+0.4 ppm),
MH+: 1202.61919 Da,
RT: 36.0308 min,
Identified with: Sequest HT (v1.17);
XCorr:1.55,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (15):
- Histone H2B type 2-E OS=Homo sapiens OX=9606 GN=HIST2H2BE PE=1 SV=3
- Histone H2B type 2-F OS=Homo sapiens OX=9606 GN=HIST2H2BF PE=1 SV=3
- Histone H2B type 1-M OS=Homo sapiens OX=9606 GN=HIST1H2BM PE=1 SV=3
- Histone H2B type 1-B OS=Homo sapiens OX=9606 GN=HIST1H2BB PE=1 SV=2
- Histone H2B type 1-D OS=Homo sapiens OX=9606 GN=HIST1H2BD PE=1 SV=2
- Histone H2B type 1-A OS=Homo sapiens OX=9606 GN=HIST1H2BA PE=1 SV=3
- Histone H2B type 1-H OS=Homo sapiens OX=9606 GN=HIST1H2BH PE=1 SV=3
- Histone H2B type 1-K OS=Homo sapiens OX=9606 GN=HIST1H2BK PE=1 SV=3
- Histone H2B type 1-L OS=Homo sapiens OX=9606 GN=HIST1H2BL PE=1 SV=3

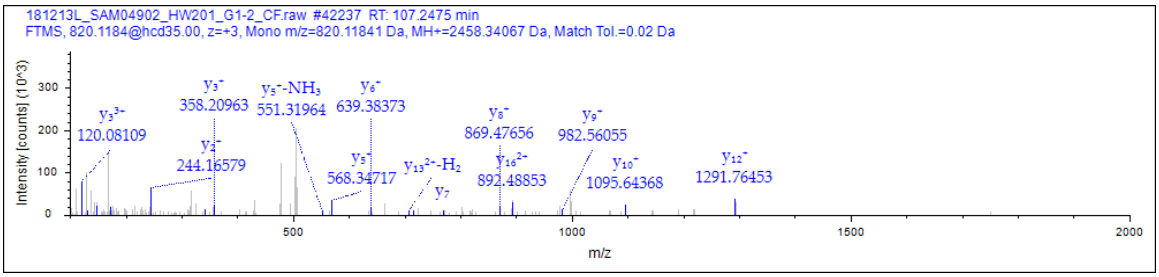


JN Mod: JN-0003
PSMs: 267
Unique Sites: 46

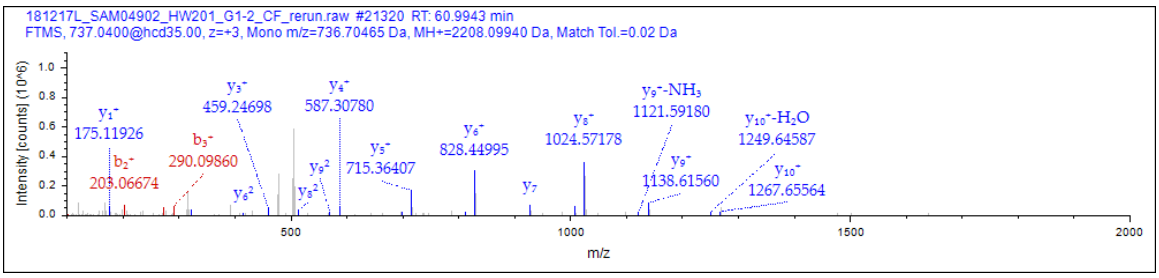


Sequence: VYEGERPLTK
Charge: +3, Monoisotopic m/z: 565.97699 Da (+1.24 mmu/+2.18 ppm),
MH+: 1695.91642 Da,
RT: 50.5797 min,
Identified with: Sequest HT (v1.17);
XCorr:1.35,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

- Endoplasmic reticulum chaperone BiP OS=Homo sapiens OX=9606 GN=HSPA5 PE=1 SV=2

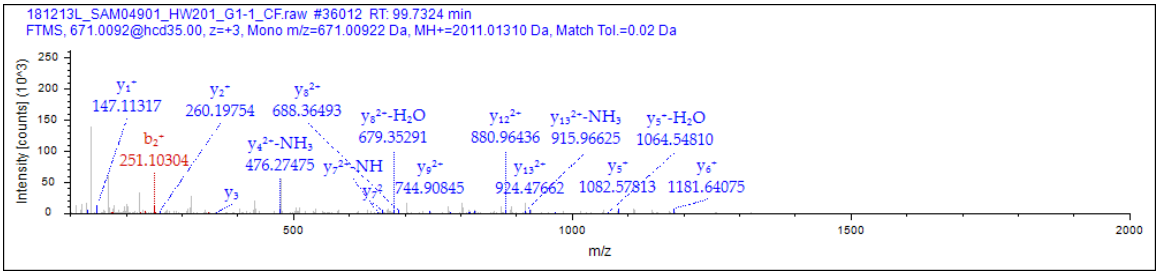


Sequence: VAPEEHPVLLTEAPLNPK
Charge: +3,
Monoisotopic m/z: 820.11841 Da (+0.12 mmu/+0.15 ppm),
MH+: 2458.34067 Da,
RT: 107.2475 min,
Identified with: Sequest HT (v1.17);
XCorr:2.67,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (2):
- Actin, cytoplasmic 1 OS=Homo sapiens OX=9606 GN=ACTB PE=1 SV=1
- Actin, cytoplasmic 2 OS=Homo sapiens OX=9606 GN=ACTG1 PE=1 SV=1

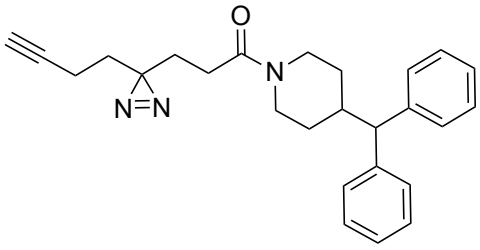


Sequence: SDSFENPVLQQHFR
Charge: +3, Monoisotopic m/z: 736.70465 Da (+3.3 mmu/+4.48 ppm), MH+: 2208.09940 Da, RT: 60.9943 min,
Identified with: Sequest HT (v1.17); XCorr:2.41,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

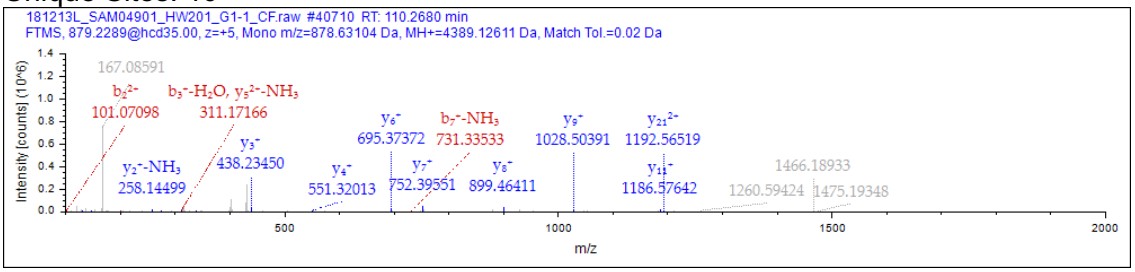
- X-ray repair cross-complementing protein 6 OS=Homo sapiens OX=9606 GN=XRCC6 PE=1 SV=2



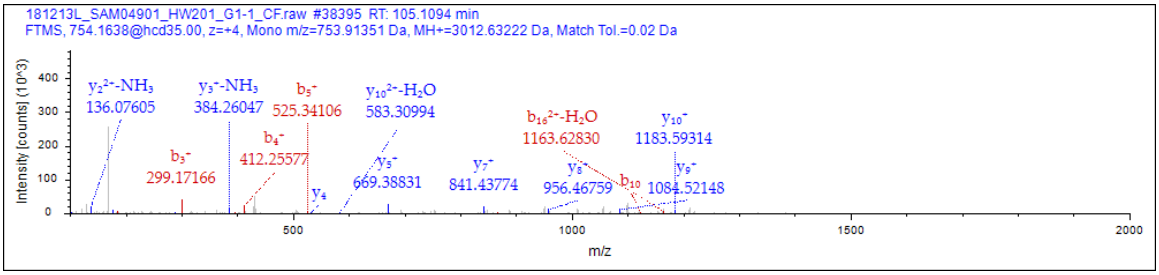
Sequence: YSNSALGHVNCTIK
Charge: +3,
Monoisotopic m/z: 671.00922 Da (+0.09 mmu/+0.13 ppm),
MH+: 2011.01310 Da,
RT: 99.7324 min,
Identified with: Sequest HT (v1.17);
XCorr:2.46,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Reticulon-4 OS=Homo sapiens OX=9606 GN=RTN4 PE=1 SV=2



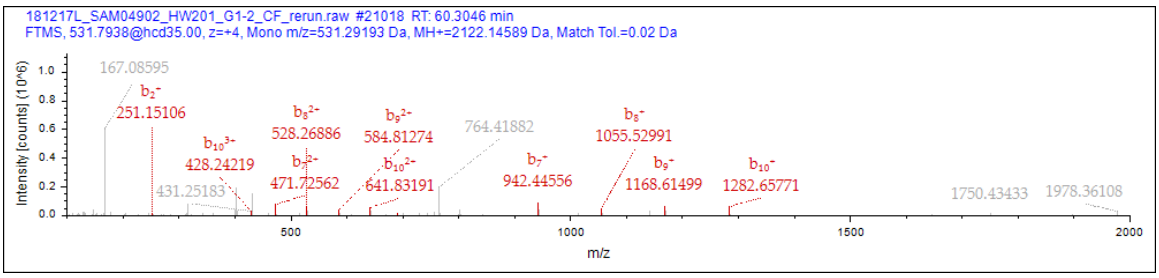
JN Mod: JN-0004
PSMs: 19
Unique Sites: 10



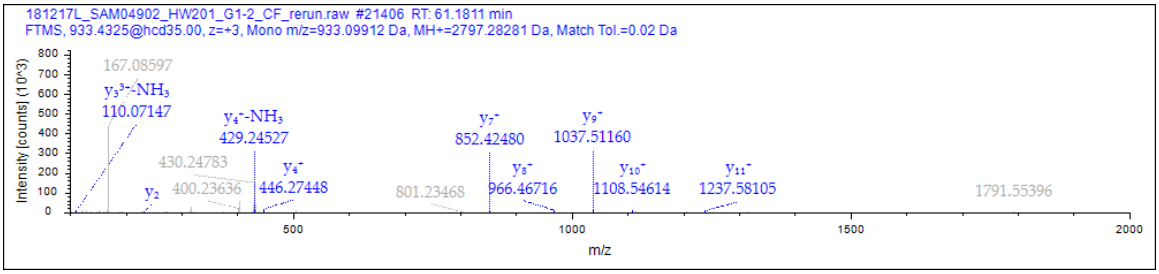
Sequence: VTQSNFAVGYKTDEFQLHTNVNDGTEFGGSIYQK
Charge: +5,
Monoisotopic m/z: 878.63104 Da (+1.99 mmu/+2.26 ppm),
MH+: 4389.12611 Da,
RT: 110.2680 min,
Identified with: Sequest HT (v1.17); XCorr:2.90,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens OX=9606 GN=VDAC1 PE=1 SV=2



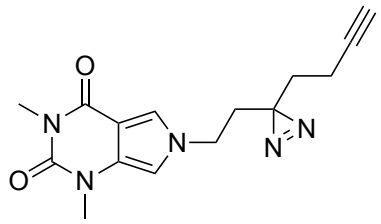
Sequence: IANLLKPDKEIVQDGDHMIIR
Charge: +4,
Monoisotopic m/z: 753.91351 Da (-2.01 mmu/-2.66 ppm),
MH+: 3012.63222 Da,
RT: 105.1094 min,
Identified with: Sequest HT (v1.17);
XCorr:2.74,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Retinol-binding protein 1 OS=Homo sapiens OX=9606 GN=RBP1 PE=1 SV=2



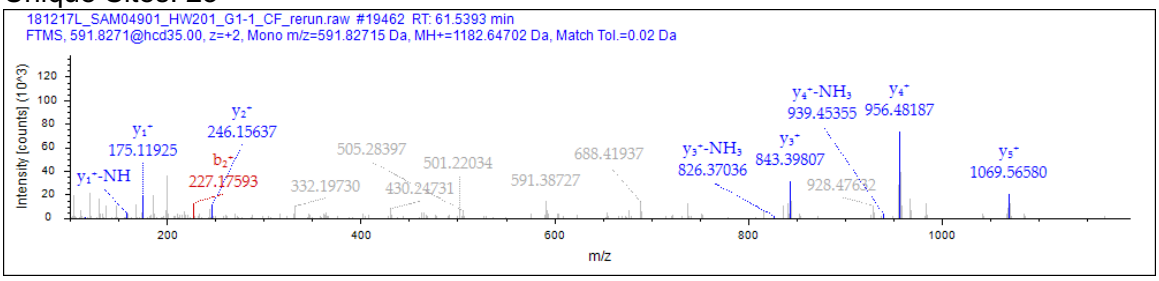
Sequence: HLREYQDLLNVK
Charge: +4,
Monoisotopic m/z: 531.29193 Da (-1.2 mmu/-2.27 ppm),
MH+: 2122.14589 Da,
RT: 60.3046 min,
Identified with: Sequest HT (v1.17);
XCorr:1.91,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (4):
- Desmin OS=Homo sapiens OX=9606 GN=DES PE=1 SV=3
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4
- Neurofilament medium polypeptide OS=Homo sapiens OX=9606 GN=NEFM PE=1 SV=3
- Alpha-internexin OS=Homo sapiens OX=9606 GN=INA PE=1 SV=2



Sequence: EMEENFAVEAANYQDTIGR
Charge: +3,
Monoisotopic m/z: 933.09912 Da (-0.3 mmu/-0.32 ppm),
MH+: 2797.28281 Da,
RT: 61.1811 min,
Identified with: Sequest HT (v1.17);
XCorr:1.27,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4

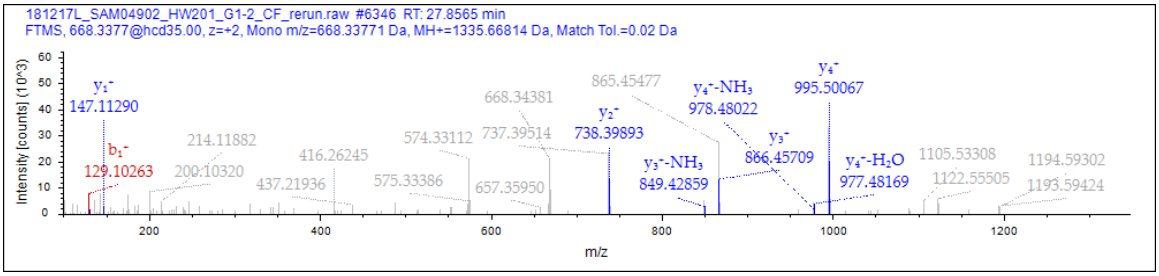


JN Mod: JN-0005-NX-1
PSMs: 102
Unique Sites: 23



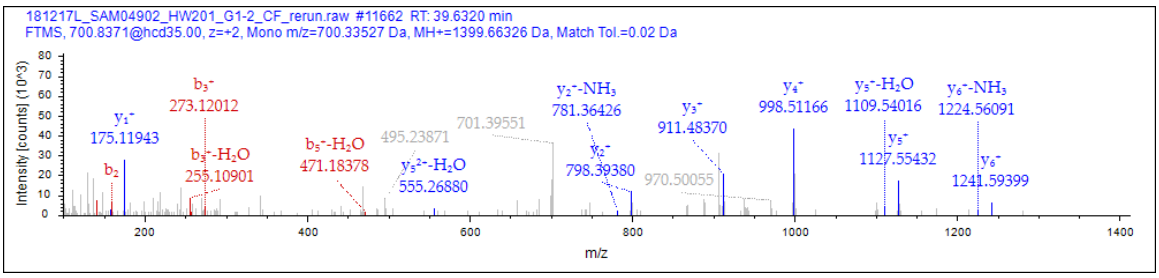
Sequence: LLLCAR
Charge: +2,
Monoisotopic m/z: 591.82715 Da (-0.26 mmu/-0.44 ppm),
MH+: 1182.64702 Da,
RT: 61.5393 min,
Identified with: Sequest HT (v1.17);
XCorr:1.82,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -NH_3; y; b; y
Proteins (1):

- Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens OX=9606 GN=TGM2 PE=1 SV=2

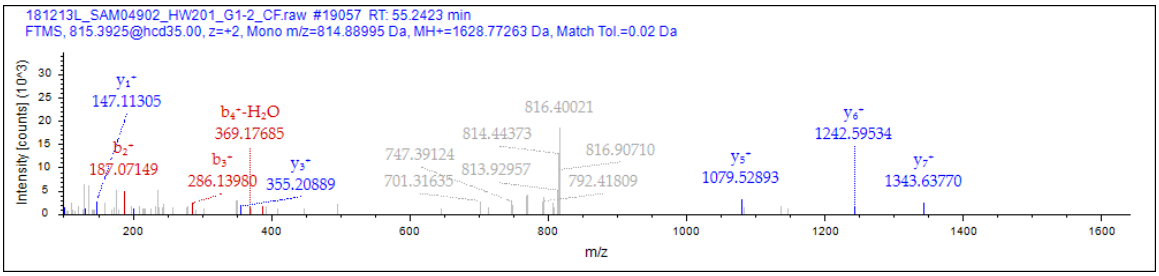


Sequence: KPDEQPK
Charge: +2,
Monoisotopic m/z: 668.33771 Da (-1.69 mmu/-2.52 ppm),
MH+: 1335.66814 Da,
RT: 27.8565 min,
Identified with: Sequest HT (v1.17);
XCorr:1.66,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

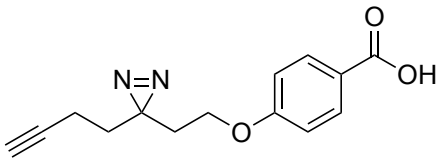
- Sodium channel protein type 8 subunit alpha OS=Homo sapiens OX=9606 GN=SCN8A PE=1 SV=1



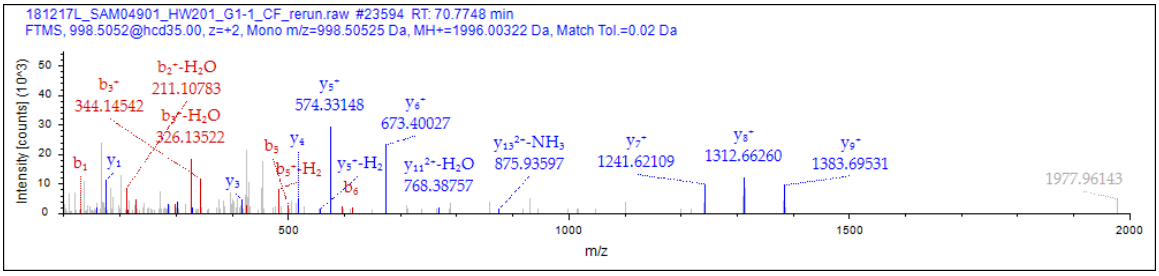
Sequence: GTNESLER
Charge: +2,
Monoisotopic m/z: 700.33527 Da (+0.43 mmu/+0.61 ppm),
MH+: 1399.66326 Da,
RT: 39.6320 min,
Identified with: Sequest HT (v1.17);
XCorr:2.06,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (2):
- Glial fibrillary acidic protein OS=Homo sapiens OX=9606 GN=GFAP PE=1 SV=1
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4



Sequence: DAVTYTEHAK
Charge: +2,
Monoisotopic m/z: 814.88995 Da (-0.02 mmu/-0.03 ppm),
MH+: 1628.77263 Da,
RT: 55.2423 min,
Identified with: Sequest HT (v1.17);
XCorr:1.61,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Histone H4 OS=Homo sapiens OX=9606 GN=HIST1H4A PE=1 SV=2

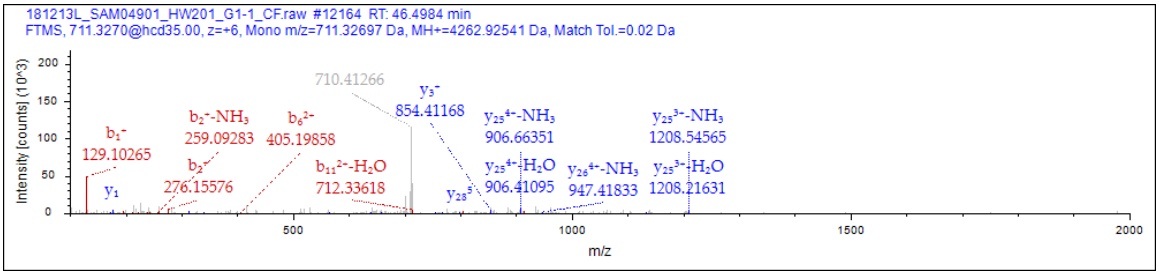


JN Mod: JN-0012-NX-1
PSMs: 39
Unique Sites: 12

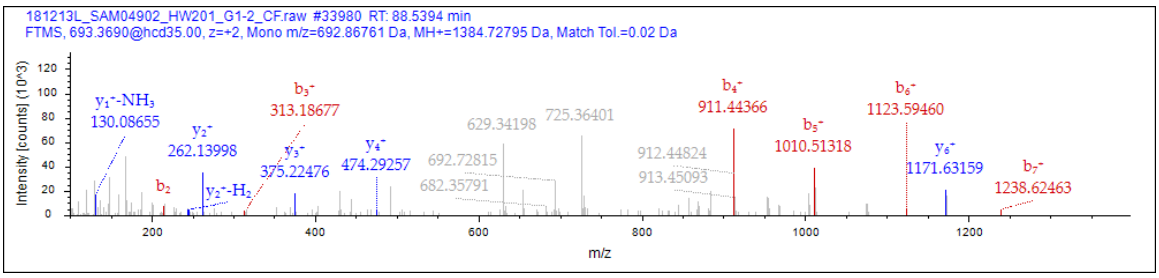


Sequence: EVDVGLAADVGTLQR
Charge: +2,
Monoisotopic m/z: 998.50525 Da (-0.65 mmu/-0.65 ppm),
MH+: 1996.00322 Da,
RT: 70.7748 min,
Identified with: Sequest HT (v1.17);
XCorr:2.95,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

- Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial OS=Homo sapiens OX=9606 GN=ECH1 PE=1 SV=2

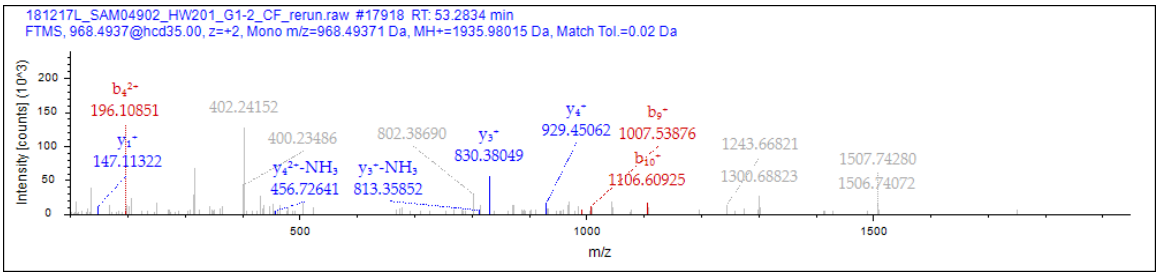


Sequence: KMLAYWMLEVCEEQRCEEEVFPLAMNYLDR
Charge: +6,
Monoisotopic m/z: 711.32697 Da (+1.69 mmu/+2.37 ppm),
MH+: 4262.92541 Da,
RT: 46.4984 min,
Identified with: Sequest HT (v1.17);
XCorr:2.02,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- G1/S-specific cyclin-D3 OS=Homo sapiens OX=9606 GN=CCND3 PE=1 SV=2



Sequence: NVVFVIDK
Charge: +2,
Monoisotopic m/z: 692.86761 Da (+0.98 mmu/+1.41 ppm),
MH+: 1384.72795 Da,
RT: 88.5394 min,
Identified with: Sequest HT (v1.17);
XCorr:1.78,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

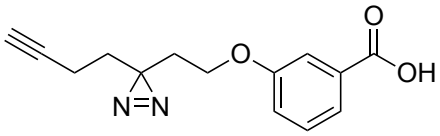
- Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens OX=9606 GN=ITIH4 PE=1 SV=4



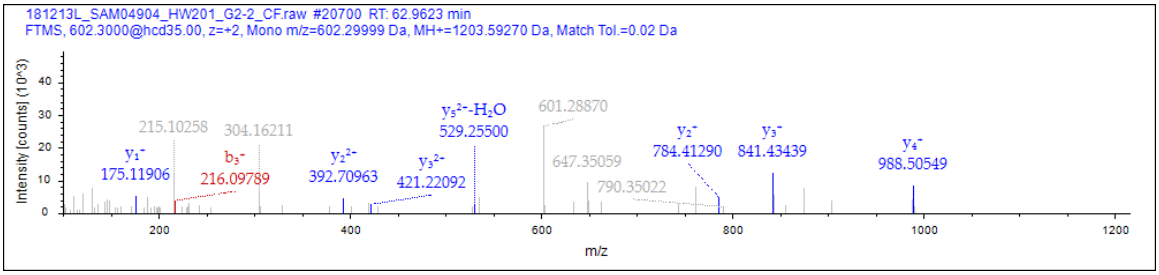
Sequence: IGGYTKKVCVMTK
Charge: +2,
Monoisotopic m/z: 968.49371 Da (-0.91 mmu/-0.94 ppm),
MH+: 1935.98015 Da,
RT: 53.2834 min,
Identified with: Sequest HT (v1.17);
XCorr:1.41,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y

Proteins (2):
- Schlafen family member 12 OS=Homo sapiens OX=9606 GN=SLFN12 PE=2 SV=2
- Schlafen family member 12-like OS=Homo sapiens OX=9606 GN=SLFN12L PE=2 SV=4

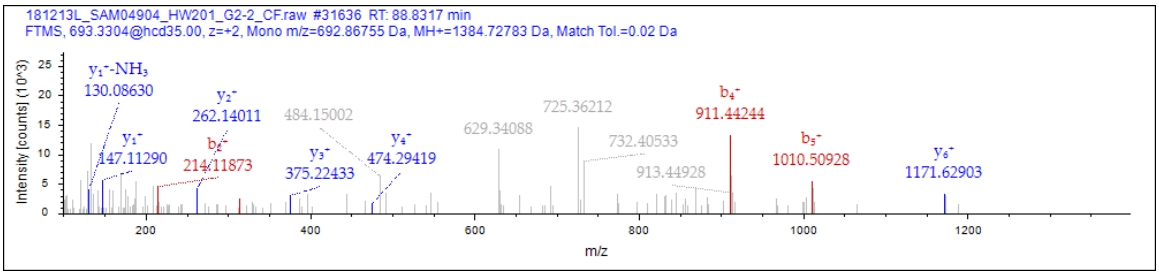
Group 2:



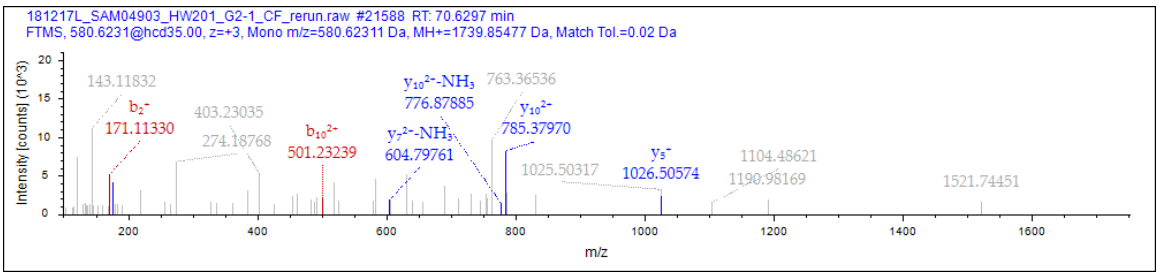
JN Mod: JN-0020
PSMs: 9
Unique Sites: 4



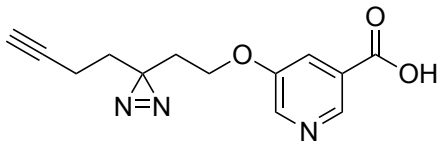
Sequence: AGSFGRR
Charge: +2,
Monoisotopic m/z: 602.29999 Da (-0.07 mmu/-0.12 ppm),
MH+: 1203.59270 Da,
RT: 62.9623 min,
Identified with: Sequest HT (v1.17); XCorr:1.14,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: $-H_2O$; y; $-NH_3$; y; b; b; $-H_2O$; y
Proteins (1):
- Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit OS=Homo sapiens OX=9606 GN=DDOST PE=1 SV=4



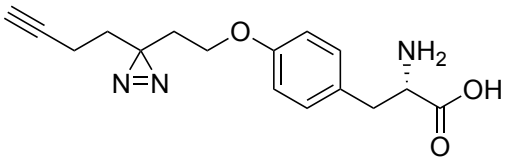
Sequence: NVVFVIDK
Charge: +2, Monoisotopic m/z: 692.86755 Da (+0.92 mmu/+1.33 ppm), MH+: 1384.72783 Da, RT: 88.8317 min,
Identified with: Sequest HT (v1.17); XCorr:2.19,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: $-H_2O$; y; $-NH_3$; y; b; b; $-H_2O$; b; $-NH_3$; y
Proteins (1):
- Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens OX=9606 GN=ITIH4 PE=1 SV=4



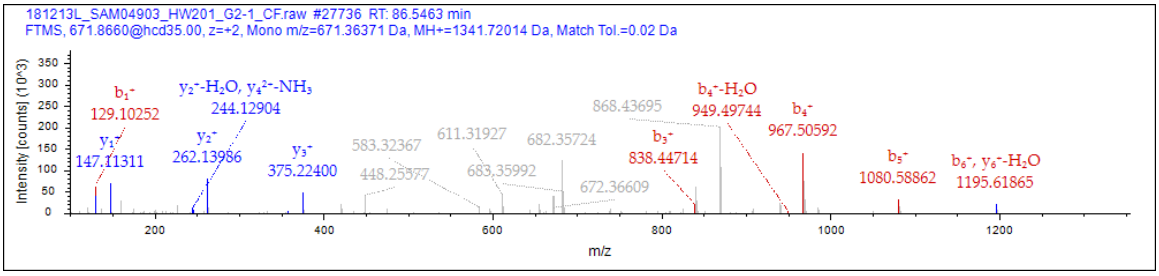
Sequence: GLCAIAQAESLR
Charge: +3,
Monoisotopic m/z: 580.62311 Da (+0.46 mmu/+0.79 ppm),
MH+: 1739.85477 Da,
RT: 70.6297 min,
Identified with: Sequest HT (v1.17); XCorr:1.18,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: $-H_2O$; y; $-NH_3$; y; b; b; $-H_2O$; b; $-NH_3$; y
Proteins (1):
- 40S ribosomal protein S3 OS=Homo sapiens OX=9606 GN=RPS3 PE=1 SV=2



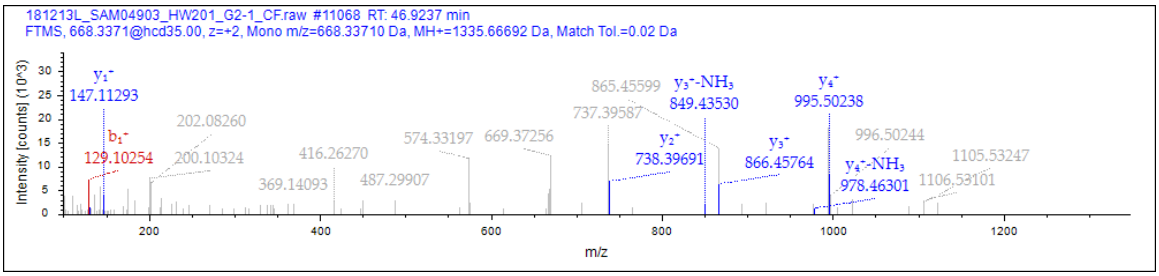
JN Mod: JN-0021
PSMs: 0
Unique Sites: 0



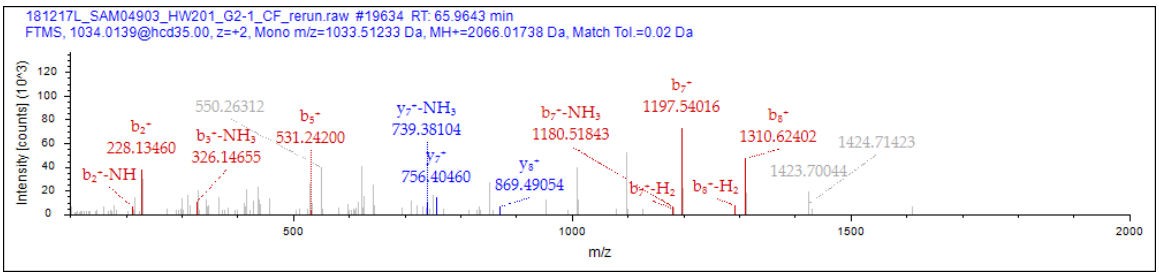
JN Mod: JN-0022
PSMs: 19
Unique Sites: 6



Sequence: KSKEIDK
Charge: +2,
Monoisotopic m/z: 671.36371 Da (+1.99 mmu/+2.97 ppm),
MH+: 1341.72014 Da,
RT: 86.5463 min,
Identified with: Sequest HT (v1.17); XCorr:2.11,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Guanine nucleotide-binding protein subunit alpha-13 OS=Homo sapiens OX=9606 GN=GNA13 PE=1 SV=2

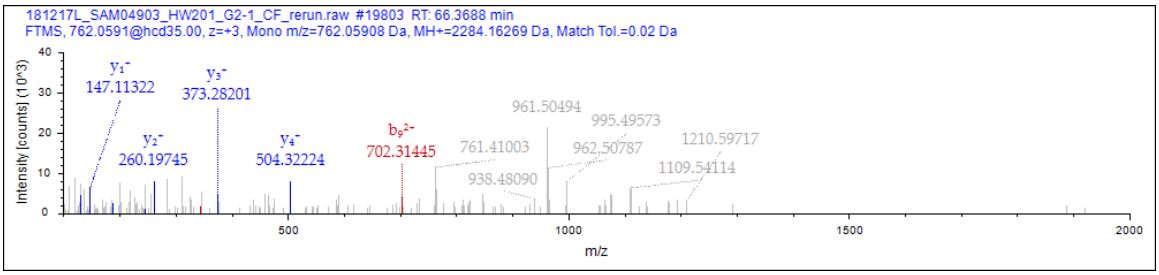


Sequence: KPDEQPK
Charge: +2,
Monoisotopic m/z: 668.33710 Da (-1.15 mmu/-1.71 ppm),
MH+: 1335.66692 Da,
RT: 46.9237 min,
Identified with: Sequest HT (v1.17); XCorr:1.36,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Sodium channel protein type 8 subunit alpha OS=Homo sapiens OX=9606 GN=SCN8A PE=1 SV=1

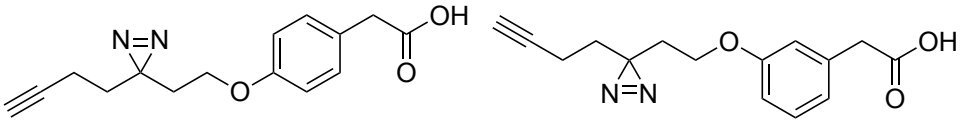


Sequence: ARDGMTALHKAACAR
Charge: +2,
Monoisotopic m/z: 1033.51233 Da (+0.13 mmu/+0.12 ppm),
MH+: 2066.01738 Da,
RT: 65.9643 min,
Identified with: Sequest HT (v1.17); XCorr:1.13,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

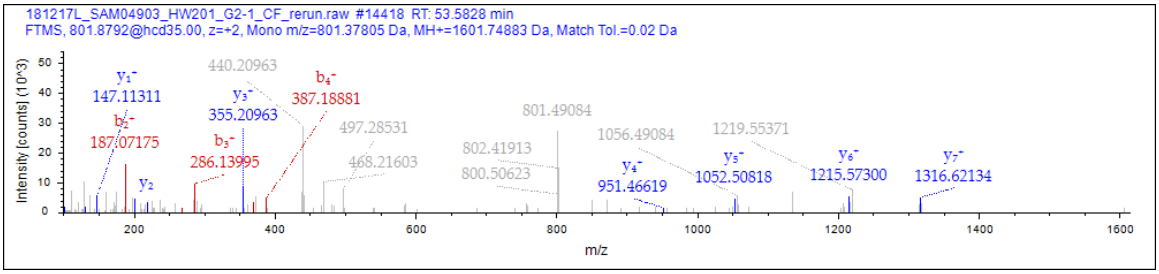
- SH3 and multiple ankyrin repeat domains protein 1 OS=Homo sapiens OX=9606 GN=SHANK1 PE=1 SV=2



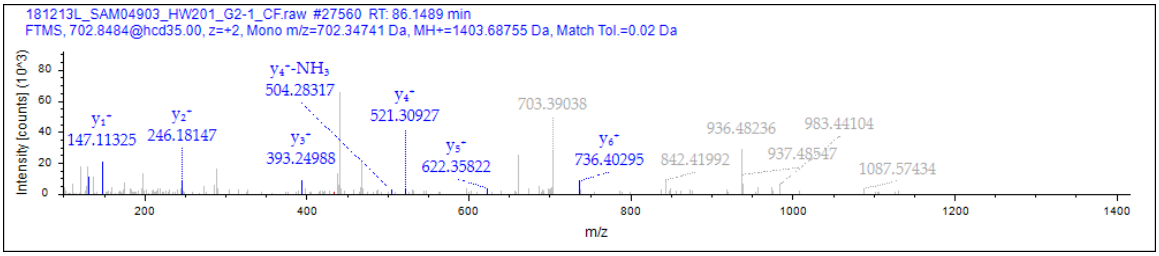
Sequence: AEAAGHRDTLYTMLIK
Charge: +3,
Monoisotopic m/z: 762.05908 Da (+2.79 mmu/+3.66 ppm),
MH+: 2284.16269 Da,
RT: 66.3688 min,
Identified with: Sequest HT (v1.17); XCorr:1.42,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Tumor necrosis factor receptor superfamily member 10B OS=Homo sapiens OX=9606 GN=TNFRSF10B PE=1 SV=2



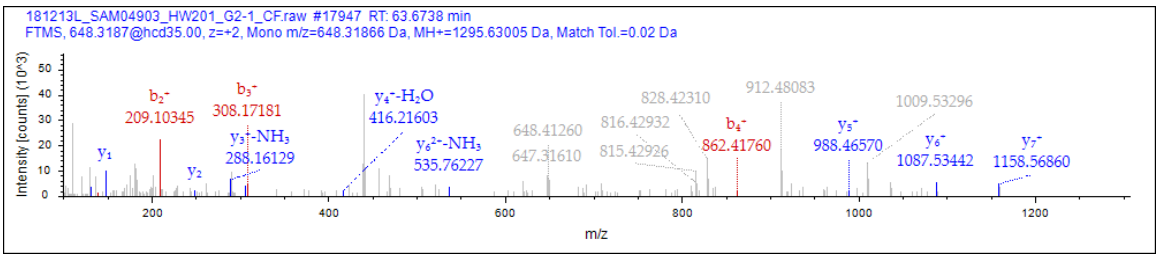
JN Mod: JN-0013 and JN-0017
PSMs: 26
Unique Sites: 7



Sequence: DAVTYTEHAK
Charge: +2,
Monoisotopic m/z: 801.37805 Da (-0.83 mmu/-1.03 ppm),
MH+: 1601.74883 Da,
RT: 53.5828 min,
Identified with: Sequest HT (v1.17); XCorr:2.10,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Histone H4 OS=Homo sapiens OX=9606 GN=HIST1H4A PE=1 SV=2



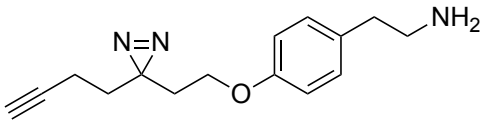
Sequence: AENTQFVK
Charge: +2, Monoisotopic m/z: 702.34741 Da (+0.56 mmu/+0.8 ppm),
MH+: 1403.68755 Da,
RT: 86.1489 min,
Identified with: Sequest HT (v1.17); XCorr:1.05,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Heme oxygenase 2 OS=Homo sapiens OX=9606 GN=HMOX2 PE=1 SV=2



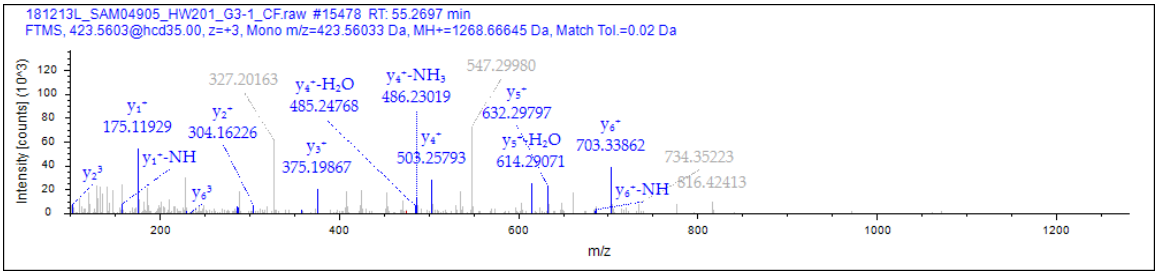
Sequence: HAVSEGTK
Charge: +2,
Monoisotopic m/z: 648.31866 Da (+0.57 mmu/+0.88 ppm),
MH+: 1295.63005 Da,
RT: 63.6738 min,
Identified with: Sequest HT (v1.17); XCorr:1.95,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y

Proteins (15):
- Histone H2B type 2-E OS=Homo sapiens OX=9606 GN=HIST2H2BE PE=1 SV=3
- Histone H2B type 2-F OS=Homo sapiens OX=9606 GN=HIST2H2BF PE=1 SV=3
- Histone H2B type 1-M OS=Homo sapiens OX=9606 GN=HIST1H2BM PE=1 SV=3
- Histone H2B type 1-B OS=Homo sapiens OX=9606 GN=HIST1H2BB PE=1 SV=2
- Histone H2B type 1-D OS=Homo sapiens OX=9606 GN=HIST1H2BD PE=1 SV=2
- Histone H2B type 1-A OS=Homo sapiens OX=9606 GN=HIST1H2BA PE=1 SV=3
- Histone H2B type 1-H OS=Homo sapiens OX=9606 GN=HIST1H2BH PE=1 SV=3
- Histone H2B type 1-K OS=Homo sapiens OX=9606 GN=HIST1H2BK PE=1 SV=3
- Histone H2B type 1-L OS=Homo sapiens OX=9606 GN=HIST1H2BL PE=1 SV=3
- Histone H2B type 1-C/E/F/G/I OS=Homo sapiens OX=9606 GN=HIST1H2BC PE=1 SV=4
- Histone H2B type F-S OS=Homo sapiens OX=9606 GN=H2BFS PE=1 SV=2
- Histone H2B type 3-B OS=Homo sapiens OX=9606 GN=HIST3H2BB PE=1 SV=3
- Histone H2B type 1-J OS=Homo sapiens OX=9606 GN=HIST1H2BJ PE=1 SV=3
- Histone H2B type 1-O OS=Homo sapiens OX=9606 GN=HIST1H2BO PE=1 SV=3
- Histone H2B type 1-N OS=Homo sapiens OX=9606 GN=HIST1H2BN PE=1 SV=3

Group 3:

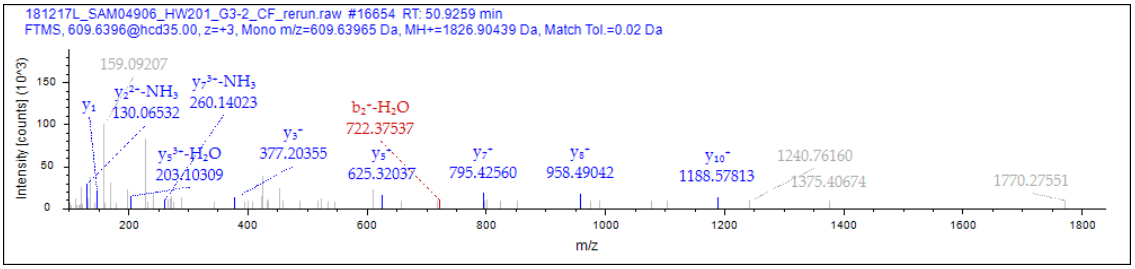


JN Mod: JN-00026
PSMs: 719
Unique Sites: 68



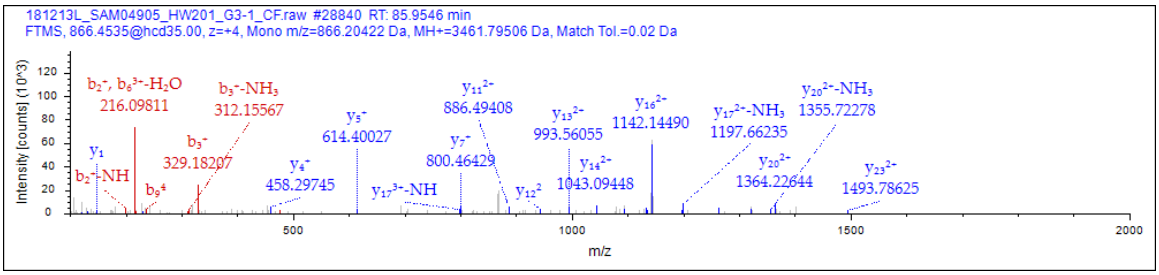
Sequence: LAEQAER
Charge: +3,
Monoisotopic m/z: 423.56152 Da (+1.47 mmu/+3.48 ppm),
MH+: 1268.67002 Da,
RT: 41.0711 min,
Identified with: Sequest HT (v1.17);
XCorr:1.80,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y

Proteins (7):
- 14-3-3 protein zeta/delta OS=Homo sapiens OX=9606 GN=YWHAZ PE=1 SV=1
- 14-3-3 protein sigma OS=Homo sapiens OX=9606 GN=SFN PE=1 SV=1
- 14-3-3 protein beta/alpha OS=Homo sapiens OX=9606 GN=YWHAB PE=1 SV=3
- 14-3-3 protein eta OS=Homo sapiens OX=9606 GN=YWHAH PE=1 SV=4
- 14-3-3 protein gamma OS=Homo sapiens OX=9606 GN=YWHAG PE=1 SV=2
- 14-3-3 protein theta OS=Homo sapiens OX=9606 GN=YWHAQ PE=1 SV=1
- 14-3-3 protein epsilon OS=Homo sapiens OX=9606 GN=YWHAE PE=1 SV=1

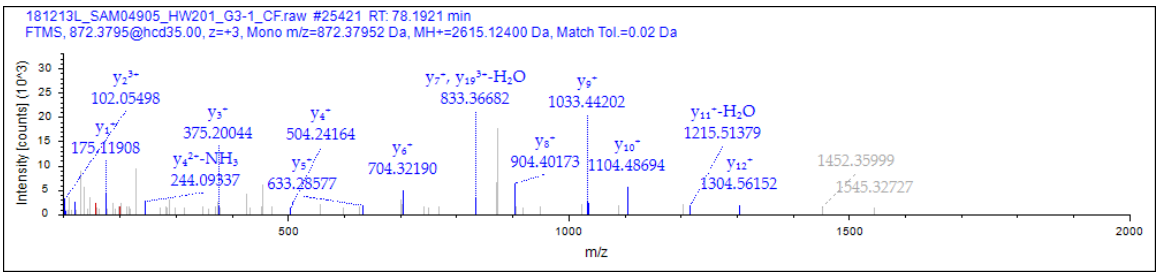


Sequence: WTEYGLTFTEK
Charge: +3,
Monoisotopic m/z: 609.63965 Da (+0.73 mmu/+1.2 ppm), MH+: 1826.90439 Da,
RT: 50.9259 min,
Identified with: Sequest HT (v1.17);
XCorr:1.05,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):

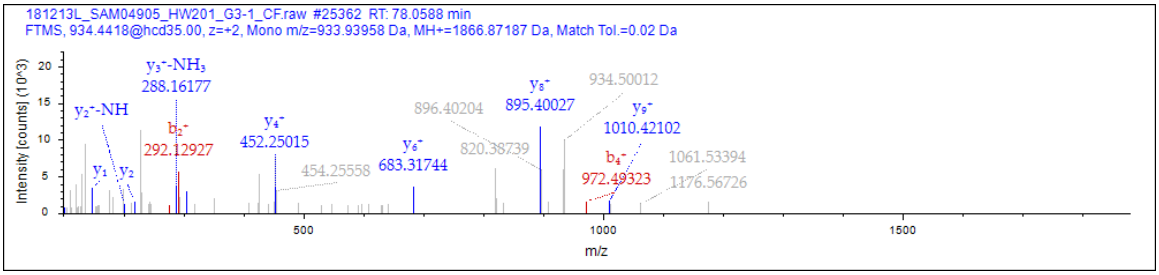
- Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens OX=9606 GN=VDAC1 PE=1 SV=2



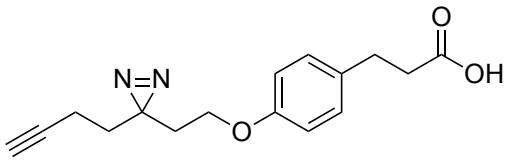
Sequence: SQIFSTASDNQPTVTIKVYEGERPLTK
Charge: +4,
Monoisotopic m/z: 866.20422 Da (-0.57 mmu/-0.66 ppm),
MH+: 3461.79506 Da,
RT: 85.9546 min,
Identified with: Sequest HT (v1.17);
XCorr:3.83,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Endoplasmic reticulum chaperone BiP OS=Homo sapiens OX=9606 GN=HSPA5 PE=1 SV=2



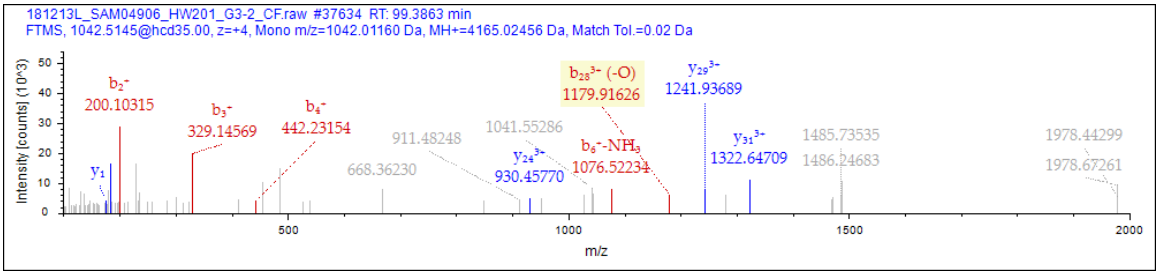
Sequence: VGEEDEEAAEAEAEAEEAER
Charge: +3,
Monoisotopic m/z: 872.37952 Da (-1.66 mmu/-1.91 ppm),
MH+: 2615.12400 Da,
RT: 78.1921 min,
Identified with: Sequest HT (v1.17); XCorr:2.43,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Neurosecretory protein VGF OS=Homo sapiens OX=9606 GN=VGF PE=1 SV=2



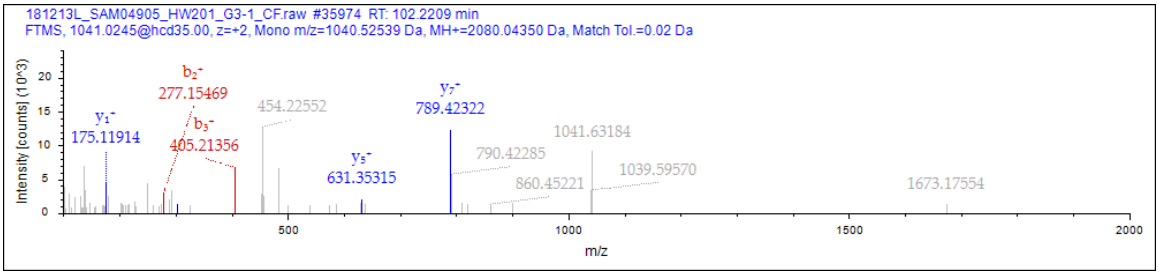
Sequence: YQIDPDACFSAK
Charge: +2,
Monoisotopic m/z: 933.93958 Da (-1.74 mmu/-1.86 ppm),
MH+: 1866.87187 Da,
RT: 78.0588 min,
Identified with: Sequest HT (v1.17); XCorr:2.05,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens OX=9606 GN=VDAC1 PE=1 SV=2



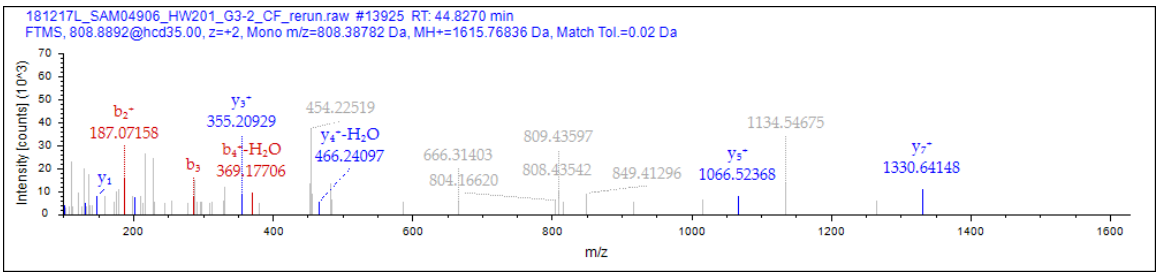
JN Mod: JN-00033
PSMs: 25
Unique Sites: 13



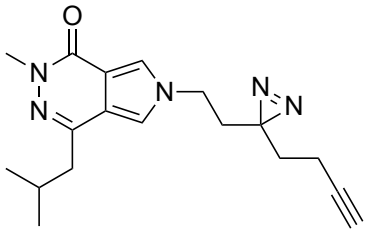
Sequence: AQELGLAPDMFFCLRLLEETGICVVPGSGFGQR
Charge: +4,
Monoisotopic m/z: 1042.01160 Da (-0.23 mmu/-0.22 ppm),
MH+: 4165.02456 Da,
RT: 99.3863 min,
Identified with: Sequest HT (v1.17); XCorr:1.29,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Alanine aminotransferase 1 OS=Homo sapiens OX=9606 GN=GPT PE=1 SV=3



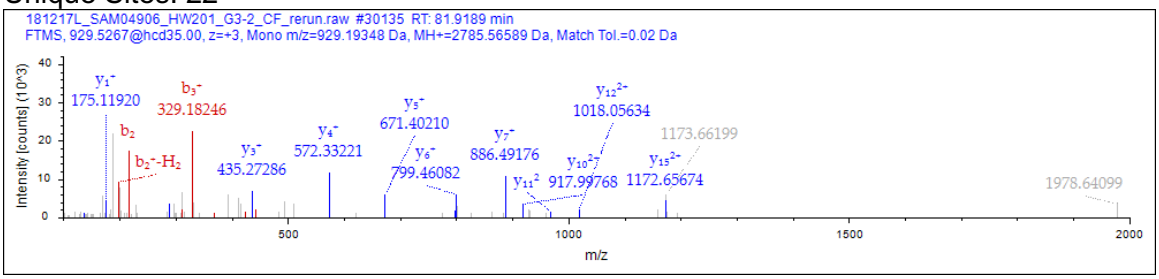
Sequence: IYQIYEGTSQIQR
Charge: +2,
Monoisotopic m/z: 1040.52539 Da (+1.3 mmu/+1.25 ppm),
MH+: 2080.04350 Da,
RT: 102.2209 min,
Identified with: Sequest HT (v1.17); XCorr:1.45,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Medium-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Homo sapiens OX=9606 GN=ACADM PE=1 SV=1



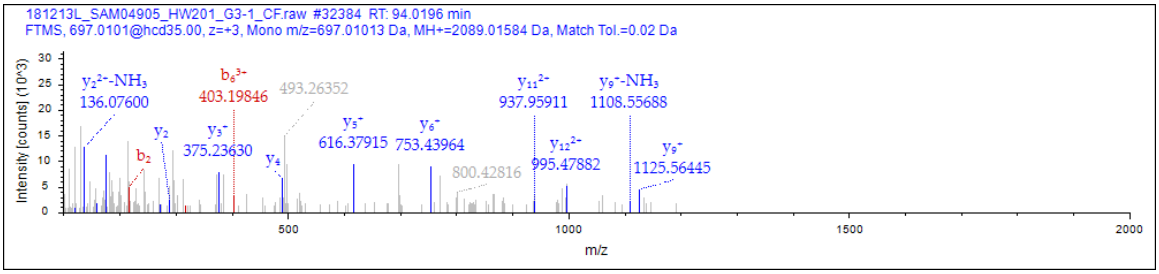
Sequence: DAVTYTEHAK
Charge: +2,
Monoisotopic m/z: 808.38782 Da (+1.09 mmu/+1.35 ppm),
MH+: 1615.76836 Da,
RT: 44.8270 min,
Identified with: Sequest HT (v1.17); XCorr:1.33,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Histone H4 OS=Homo sapiens OX=9606 GN=HIST1H4A PE=1 SV=2



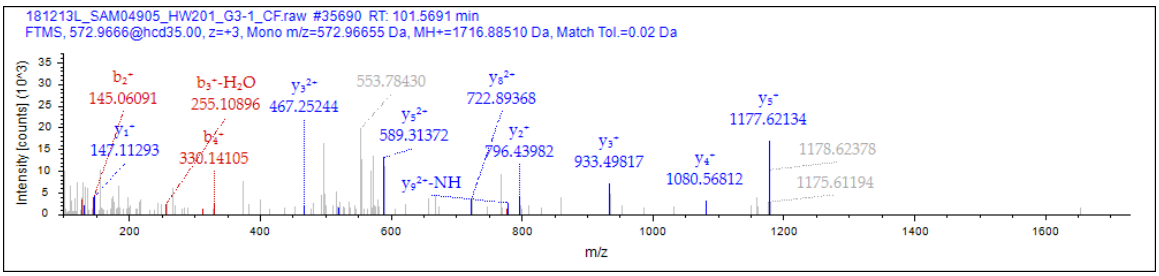
JN Mod: JN-0024
PSMs: 98
Unique Sites: 22



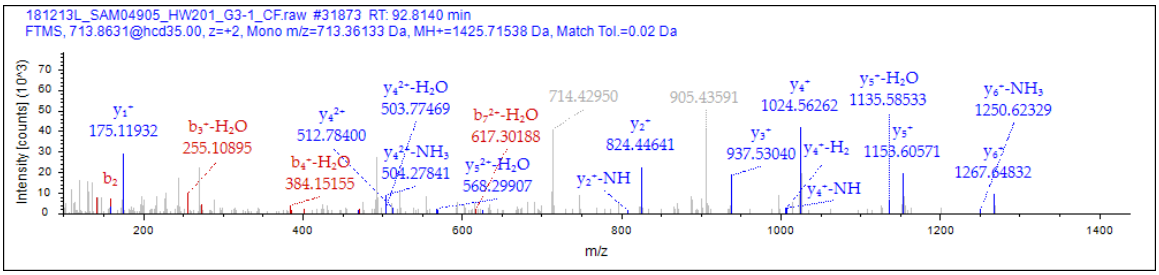
Sequence: TLNIPVLTVIEWSQVHFLR
Charge: +3,
Monoisotopic m/z: 929.19348 Da (+2.8 mmu/+3.01 ppm),
MH+: 2785.56589 Da,
RT: 81.9189 min,
Identified with: Sequest HT (v1.17); XCorr:3.04,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Lysosome membrane protein 2 OS=Homo sapiens OX=9606 GN=SCARB2 PE=1 SV=2



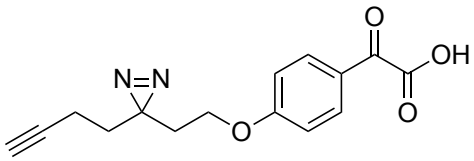
Sequence: VDNDENEHQLSLR
Charge: +3,
Monoisotopic m/z: 697.01013 Da (+1.27 mmu/+1.82 ppm),
MH+: 2089.01584 Da,
RT: 94.0196 min,
Identified with: Sequest HT (v1.17); XCorr:2.80,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Nucleophosmin OS=Homo sapiens OX=9606 GN=NPM1 PE=1 SV=2



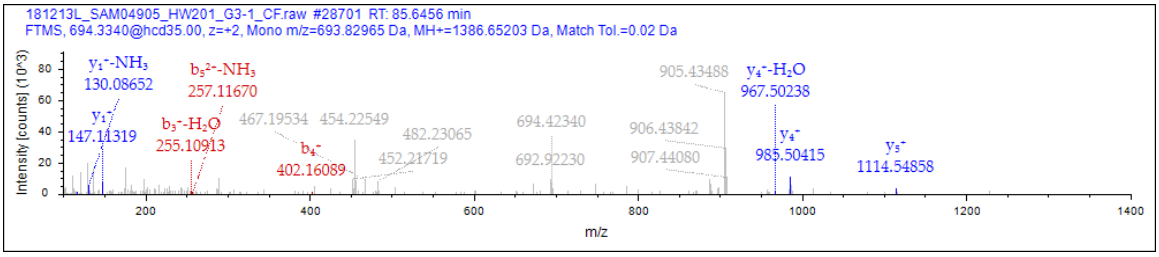
Sequence: GSQGPLPFHEK
Charge: +3,
Monoisotopic m/z: 572.96655 Da (-0.95 mmu/-1.66 ppm),
MH+: 1716.88510 Da,
RT: 101.5691 min,
Identified with: Sequest HT (v1.17); XCorr:2.42,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Mitochondrial dicarboxylate carrier OS=Homo sapiens OX=9606 GN=SLC25A10 PE=1 SV=2



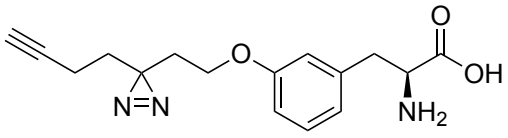
Sequence: GTNESLER
Charge: +2,
Monoisotopic m/z: 713.36133 Da (+0.49 mmu/+0.69 ppm),
MH+: 1425.71538 Da,
RT: 92.8140 min,
Identified with: Sequest HT (v1.17); XCorr:2.12,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (2):
- Glial fibrillary acidic protein OS=Homo sapiens OX=9606 GN=GFAP PE=1 SV=1
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4



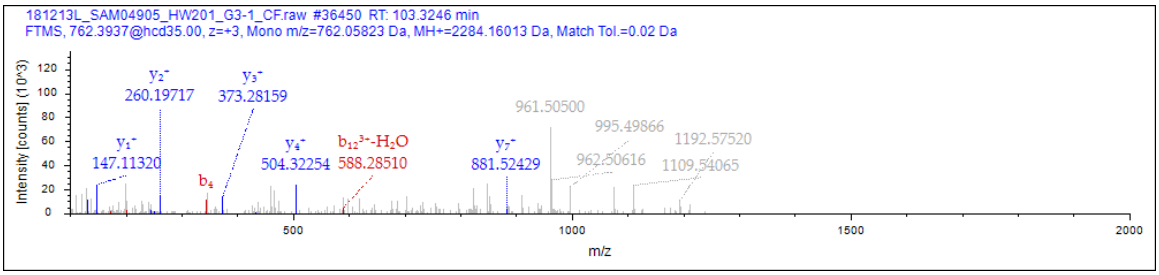
JN Mod: JN-00032
PSMs: 2
Unique Sites: 1



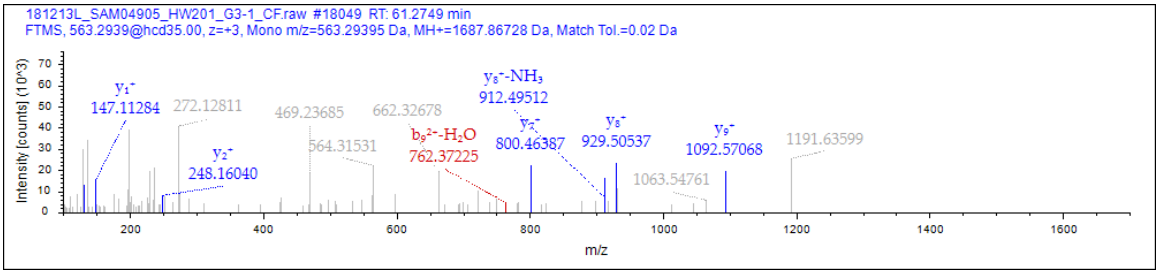
Sequence: GSQEKQTK
Charge: +2,
Monoisotopic m/z: 693.82965 Da (-1.93 mmu/-2.78 ppm),
MH+: 1386.65203 Da,
RT: 85.6456 min,
Identified with: Sequest HT (v1.17); XCorr:1.02,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Bcl-2-associated transcription factor 1 OS=Homo sapiens OX=9606 GN=BCLAF1 PE=1 SV=2



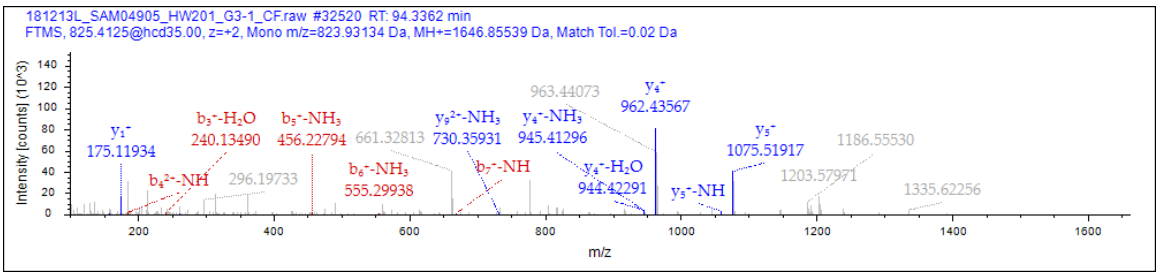
JN Mod: JN-00028
PSMs: 29
Unique Sites: 7



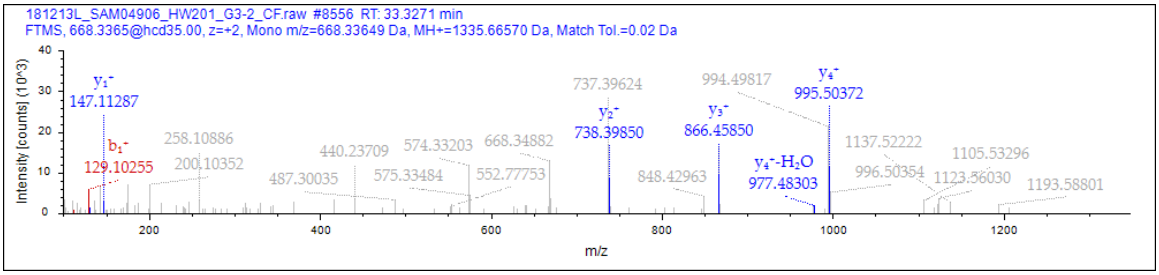
Sequence: AEAAGHRDTLYTMLIK
Charge: +3,
Monoisotopic m/z: 762.05823 Da (+1.93 mmu/+2.54 ppm),
MH+: 2284.16013 Da,
RT: 103.3246 min,
Identified with: Sequest HT (v1.17); XCorr:2.13,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Tumor necrosis factor receptor superfamily member 10B OS=Homo sapiens OX=9606 GN=TNFRSF10B PE=1 SV=2



Sequence: VYEGERPLTK
Charge: +3, Monoisotopic m/z: 563.29395 Da (-1.35 mmu/-2.39 ppm),
MH+: 1687.86728 Da,
RT: 61.2749 min,
Identified with: Sequest HT (v1.17); XCorr:1.32,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Endoplasmic reticulum chaperone BiP OS=Homo sapiens OX=9606 GN=HSPA5 PE=1 SV=2

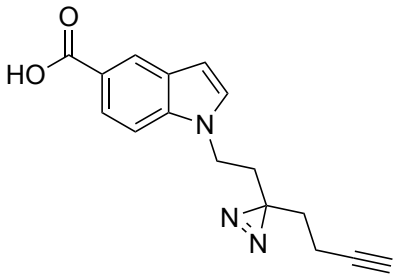


Sequence: LGSKSVLSTCR
Charge: +2,
Monoisotopic m/z: 823.93134 Da (-1.99 mmu/-2.41 ppm),
MH+: 1646.85539 Da,
RT: 94.3362 min,
Identified with: Sequest HT (v1.17); XCorr:1.02,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Reelin OS=Homo sapiens OX=9606 GN=RELN PE=1 SV=3

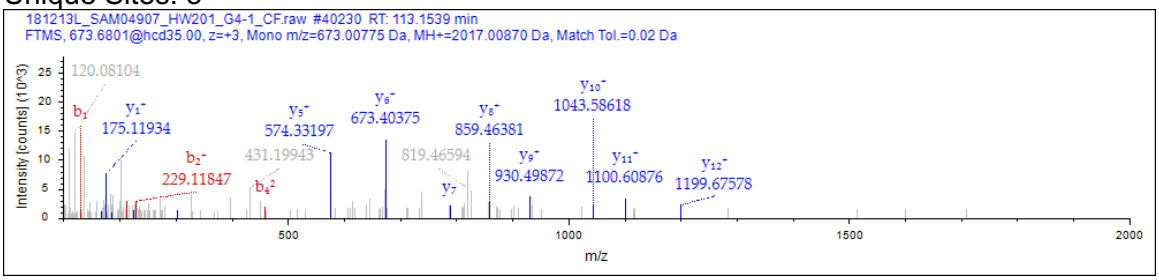


Sequence: KPDEQPK
Charge: +2,
Monoisotopic m/z: 668.33649 Da (-1.76 mmu/-2.63 ppm),
MH+: 1335.66570 Da,
RT: 33.3271 min,
Identified with: Sequest HT (v1.17); XCorr:1.46,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Sodium channel protein type 8 subunit alpha OS=Homo sapiens OX=9606 GN=SCN8A PE=1 SV=1

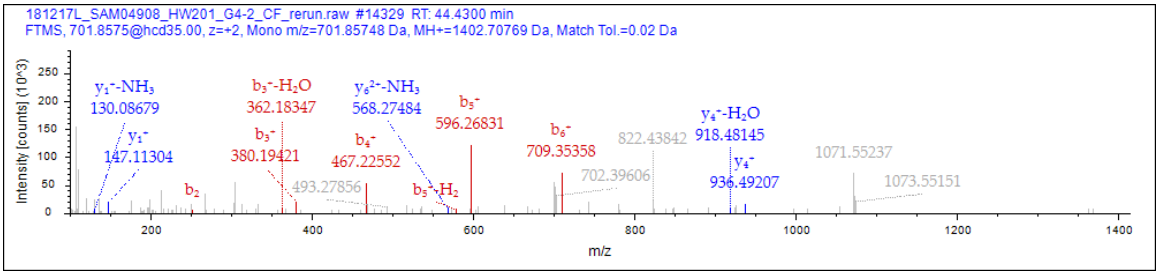
Group 4:



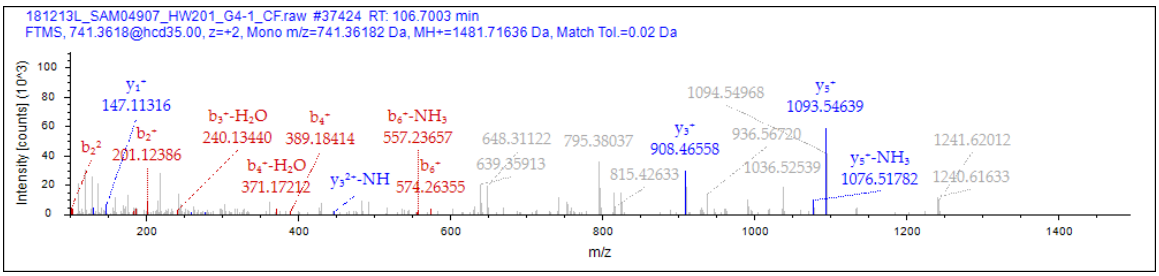
JN Mod: JN-00247
PSMs: 16
Unique Sites: 5



Sequence: EVDVGLAADVGTLQR
Charge: +3,
Monoisotopic m/z: 673.00775 Da (-1.71 mmu/-2.53 ppm),
MH+: 2017.00870 Da,
RT: 113.1539 min,
Identified with: Sequest HT (v1.17); XCorr:2.47,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial OS=Homo sapiens OX=9606 GN=ECH1 PE=1 SV=2

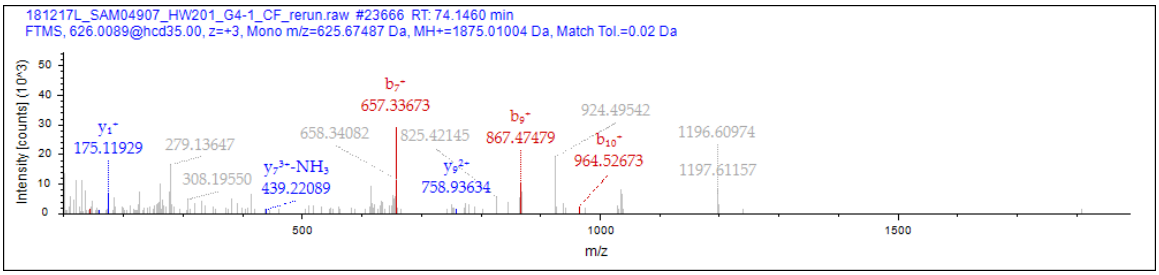


Sequence: HLESELAK
Charge: +2, Monoisotopic m/z: 701.85748 Da (+2.61 mmu/+3.72 ppm),
MH+: 1402.70769 Da,
RT: 44.4300 min,
Identified with: Sequest HT (v1.17); XCorr:1.51,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Protein Aster-B OS=Homo sapiens OX=9606 GN=GRAMD1B PE=1 SV=1

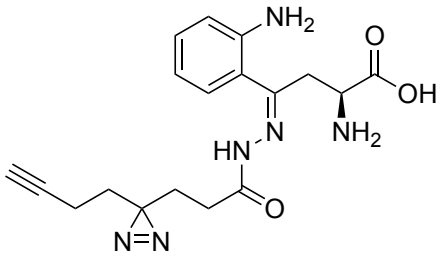


Sequence: TVGMANREK
Charge: +2,
Monoisotopic m/z: 741.36182 Da (-3.27 mmu/-4.41 ppm),
MH+: 1481.71636 Da,
RT: 106.7003 min,
Identified with: Sequest HT (v1.17); XCorr:1.92,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

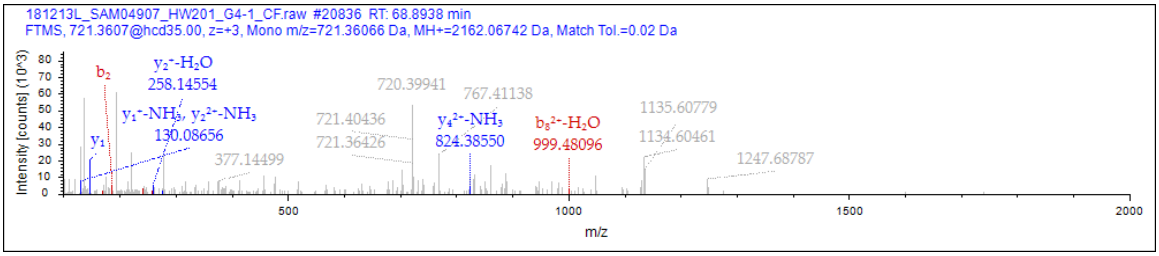
- Vacuolar protein sorting-associated protein 13D OS=Homo sapiens OX=9606 GN=VPS13D PE=1 SV=2



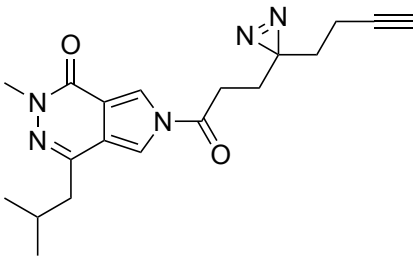
Sequence: AALCALNPLPFLR
Charge: +3,
Monoisotopic m/z: 625.67487 Da (+3.05 mmu/+4.88 ppm),
MH+: 1875.01004 Da,
RT: 74.1460 min,
Identified with: Sequest HT (v1.17); XCorr:1.07,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -NH_3; y; b; b; -NH_3; y
Proteins (1):
- Photoreceptor cilium actin regulator OS=Homo sapiens OX=9606 GN=PCARE PE=1 SV=1



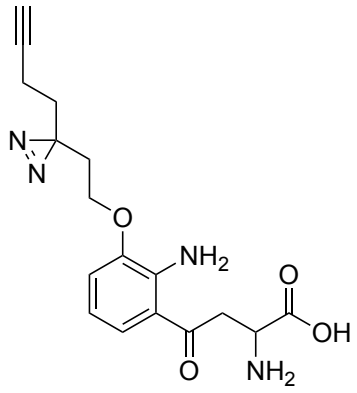
JN Mod: JN-00248
PSMs: 1
Unique Sites: 1



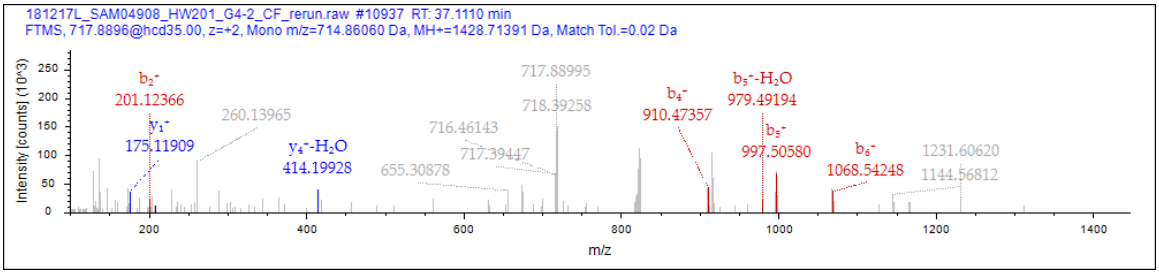
Sequence: QGAQLYVEK
Charge: +3,
Monoisotopic m/z: 721.36066 Da (-0.17 mmu/-0.24 ppm),
MH+: 2162.06742 Da,
RT: 68.8938 min,
Identified with: Sequest HT (v1.17); XCorr:1.34,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Ubiquitin carboxyl-terminal hydrolase 24 OS=Homo sapiens OX=9606 GN=USP24 PE=1 SV=3



JN Mod: JN-835
PSMs: 0
Unique Sites: 0

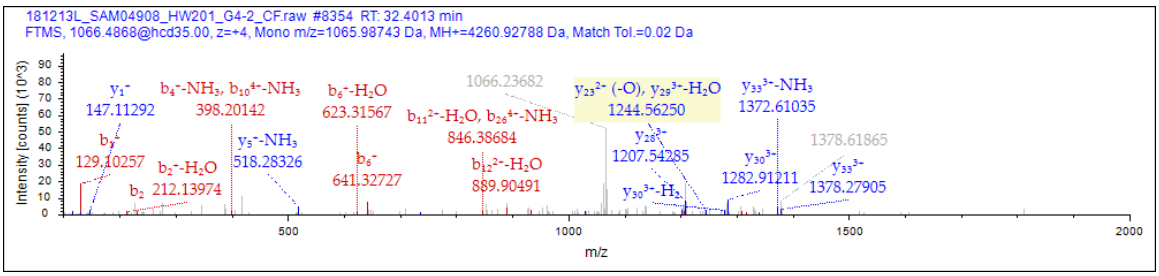


JN Mod: JN-00245
PSMs: 10
Unique Sites: 4

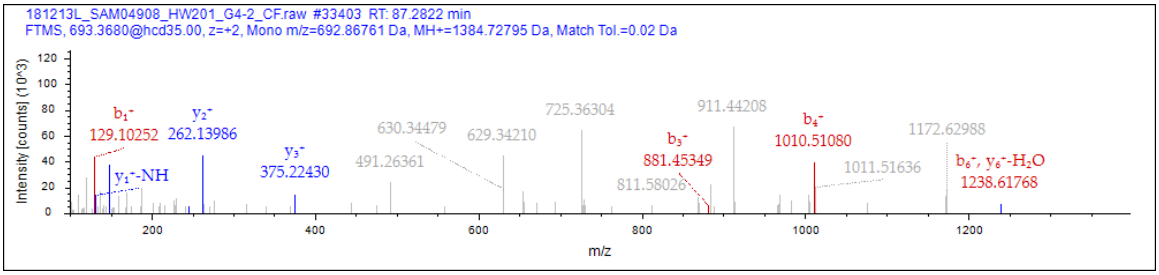


Sequence: LSLGSAGER
Charge: +2,
Monoisotopic m/z: 714.86060 Da (-0.08 mmu/-0.11 ppm),
MH+: 1428.71391 Da,
RT: 37.1110 min,
Identified with: Sequest HT (v1.17); XCorr:1.29,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y

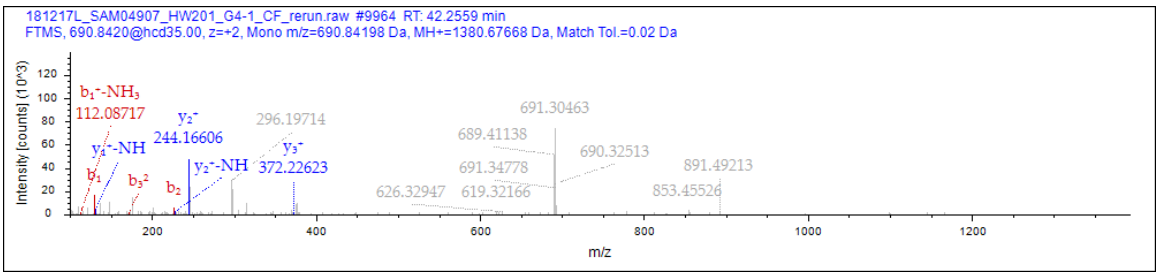
Proteins (1):
- Disrupted in schizophrenia 1 protein OS=Homo sapiens OX=9606 GN=DISC1 PE=1 SV=3



Sequence: KTGQPEELVSCSDCGRSGHPSCLQFTPVMMAAVK
Charge: +4,
Monoisotopic m/z: 1065.98743 Da (-2.83 mmu/-2.66 ppm),
MH+: 4260.92788 Da,
RT: 32.4013 min,
Identified with: Sequest HT (v1.17); XCorr:2.58,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Zinc finger protein ubi-d4 OS=Homo sapiens OX=9606 GN=DPF2 PE=1 SV=2

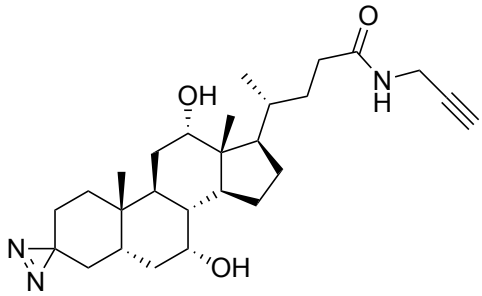


Sequence: KSKEIDK
Charge: +2,
Monoisotopic m/z: 692.86761 Da (+3 mmu/+4.33 ppm),
MH+: 1384.72795 Da,
RT: 87.2822 min,
Identified with: Sequest HT (v1.17); XCorr:1.17,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Guanine nucleotide-binding protein subunit alpha-13 OS=Homo sapiens OX=9606 GN=GNA13 PE=1 SV=2

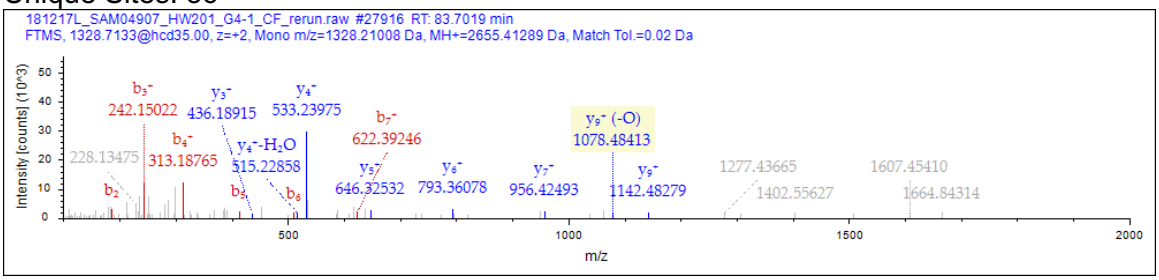


Sequence: KPDEQPK
Charge: +2,
Monoisotopic m/z: 690.84198 Da (-2.51 mmu/-3.64 ppm),
MH+: 1380.67668 Da,
RT: 42.2559 min,
Identified with: Sequest HT (v1.17); XCorr:1.18,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y

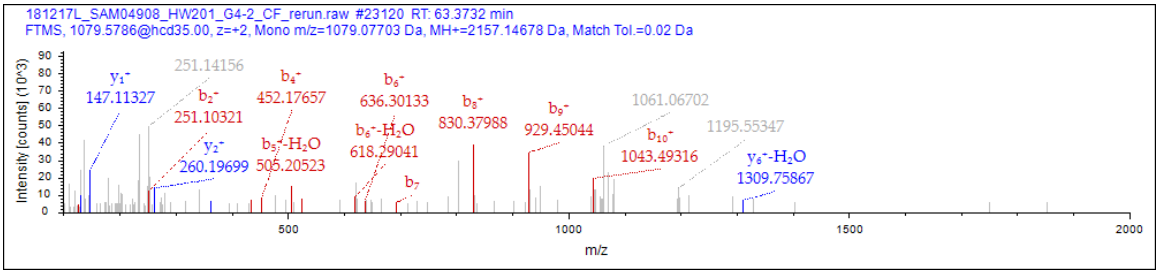
Proteins (1):
- Sodium channel protein type 8 subunit alpha OS=Homo sapiens OX=9606 GN=SCN8A PE=1 SV=1



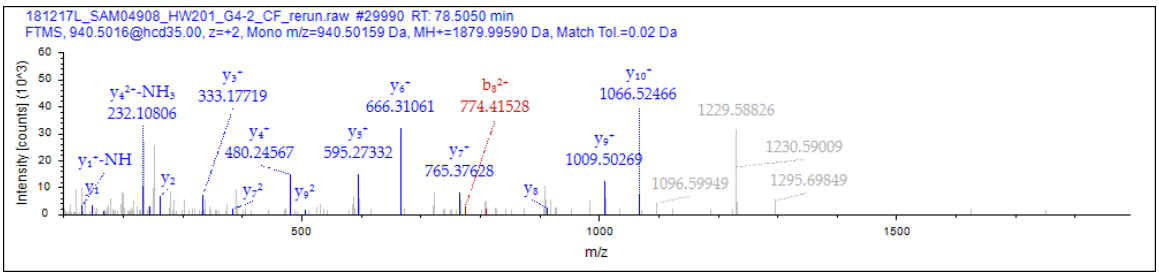
JN Mod: JN-00038
PSMs: 223
Unique Sites: 36



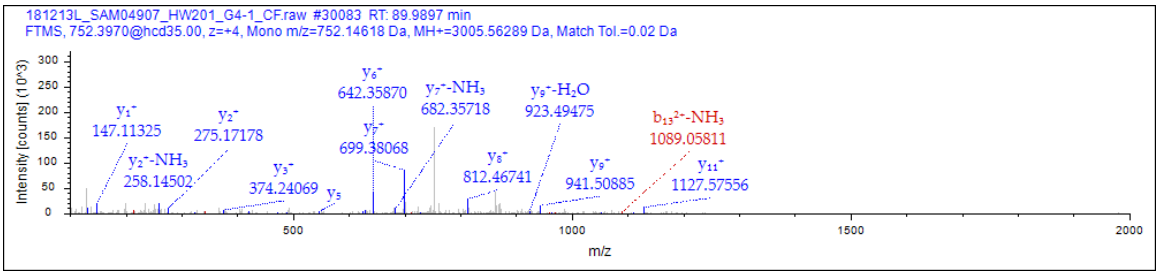
Sequence: AIGAVPLIQGEYMIPCEK
Charge: +2,
Monoisotopic m/z: 1328.21008 Da (-3.5 mmu/-2.64 ppm),
MH+: 2655.41289 Da,
RT: 83.7019 min,
Identified with: Sequest HT (v1.17);
XCorr:2.18,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Cathepsin D OS=Homo sapiens OX=9606 GN=CTSD PE=1 SV=1



Sequence: YSNSALGHVNCTIK
Charge: +2,
Monoisotopic m/z: 1079.07703 Da (+1.62 mmu/+1.5 ppm),
MH+: 2157.14678 Da, RT: 63.3732 min,
Identified with: Sequest HT (v1.17); XCorr:2.26,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Reticulon-4 OS=Homo sapiens OX=9606 GN=RTN4 PE=1 SV=2

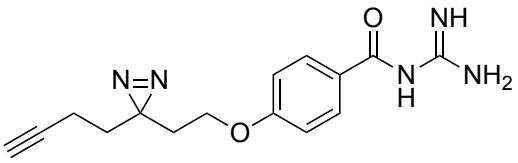


Sequence: YGPFVADFADK
Charge: +2,
Monoisotopic m/z: 940.50159 Da (+2.79 mmu/+2.97 ppm),
MH+: 1879.99590 Da,
RT: 78.5050 min,
Identified with: Sequest HT (v1.17); XCorr:2.07,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- ATP synthase F(0) complex subunit B1, mitochondrial OS=Homo sapiens OX=9606 GN=ATP5PB PE=1 SV=2

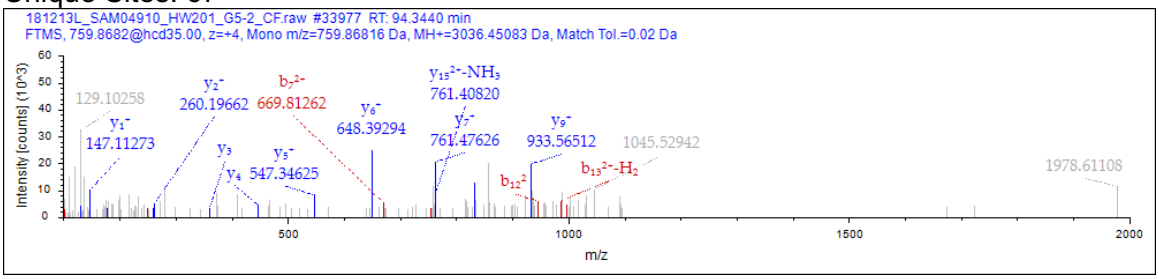


Sequence: VLQHYQESDKGEELGPGNVQK
Charge: +4,
Monoisotopic m/z: 752.14618 Da (-0.31 mmu/-0.41 ppm),
MH+: 3005.56289 Da,
RT: 89.9897 min,
Identified with: Sequest HT (v1.17); XCorr:3.16,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Mitochondrial carrier homolog 2 OS=Homo sapiens OX=9606 GN=MTCH2 PE=1 SV=1

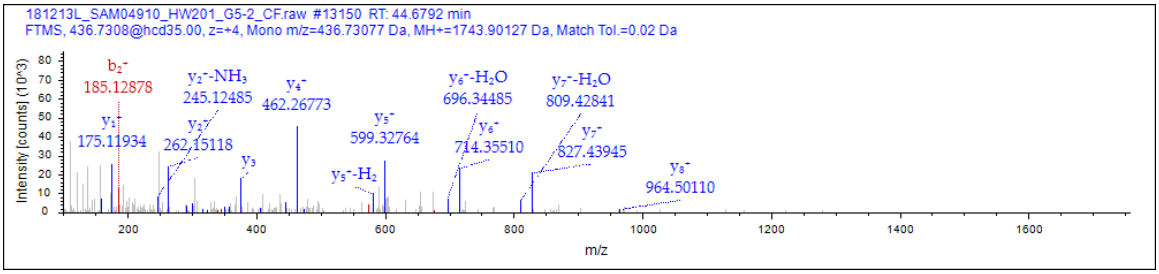
Group 5:



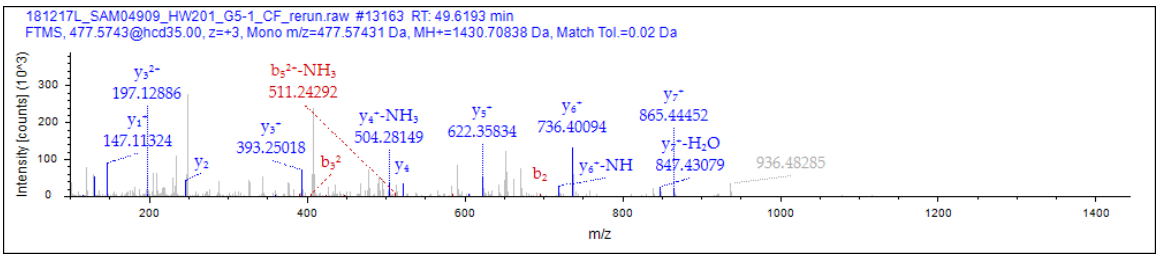
JN Mod: JN-935
PSMs: 589
Unique Sites: 67



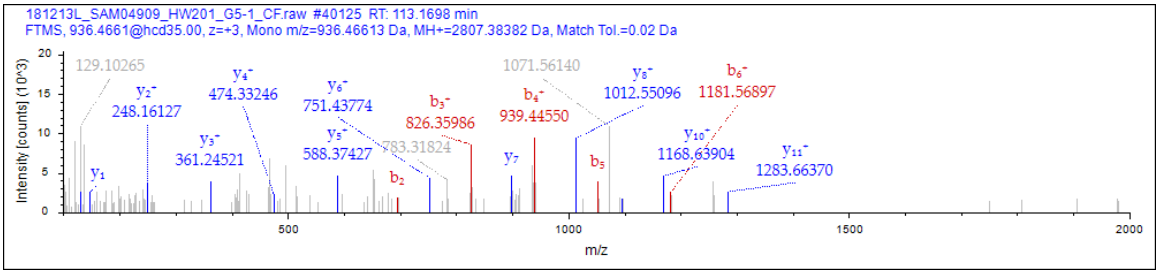
Sequence: TFHETLDCCGSSTLTALTTSVLK
Charge: +4,
Monoisotopic m/z: 759.86816 Da (+1.09 mmu/+1.44 ppm),
MH+: 3036.45083 Da,
RT: 94.3440 min,
Identified with: Sequest HT (v1.17);
XCorr:2.85,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- CD81 antigen OS=Homo sapiens OX=9606 GN=CD81 PE=1 SV=1



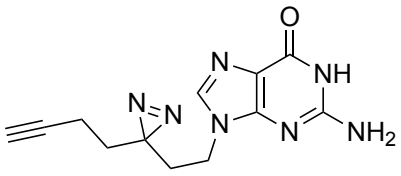
Sequence: ALTHIDHSLSR
Charge: +4,
Monoisotopic m/z: 436.73077 Da (+1.6 mmu/+3.67 ppm),
MH+: 1743.90127 Da,
RT: 44.6792 min,
Identified with: Sequest HT (v1.17); XCorr:3.00,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Methionine--tRNA ligase, cytoplasmic OS=Homo sapiens OX=9606 GN=MARS PE=1 SV=2



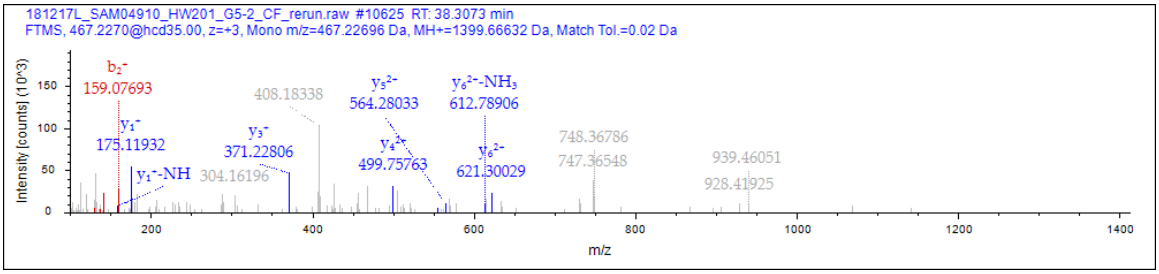
Sequence: AENTQFVK
Charge: +3,
Monoisotopic m/z: 477.57431 Da (-0.08 mmu/-0.17 ppm),
MH+: 1430.70838 Da,
RT: 49.6193 min,
Identified with: Sequest HT (v1.17); XCorr:1.98,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Heme oxygenase 2 OS=Homo sapiens OX=9606 GN=HMOX2 PE=1 SV=2



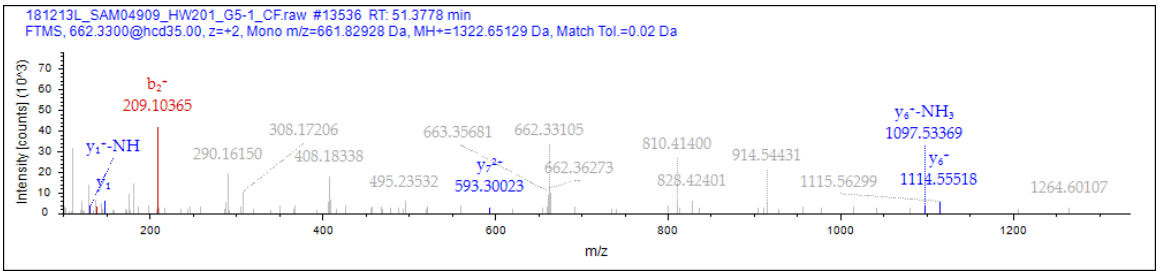
Sequence: AEMIIEQNTDGVNFYNILTK
Charge: +3,
Monoisotopic m/z: 936.46613 Da (+3.54 mmu/+3.78 ppm),
MH+: 2807.38382 Da,
RT: 113.1698 min,
Identified with: Sequest HT (v1.17); XCorr:3.42,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Retinoid-inducible serine carboxypeptidase OS=Homo sapiens OX=9606 GN=SCPEP1 PE=1 SV=1



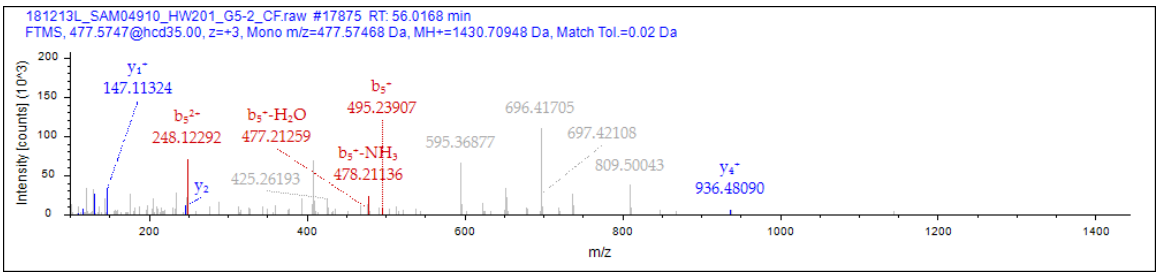
JN Mod: JN-846
PSMs: 55
Unique Sites: 10



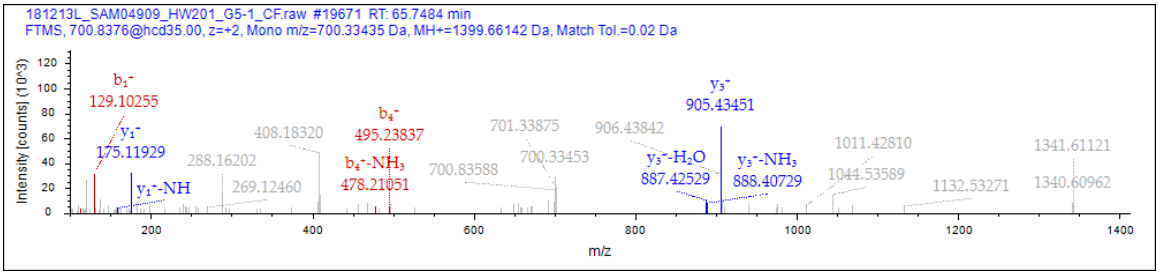
Sequence: GTNEYVPR
Charge: +3,
Monoisotopic m/z: 467.22696 Da (+1.64 mmu/+3.5 ppm),
MH+: 1399.66632 Da,
RT: 38.3073 min,
Identified with: Sequest HT (v1.17); XCorr:1.41,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Protocadherin Fat 4 OS=Homo sapiens OX=9606 GN=FAT4 PE=1 SV=2



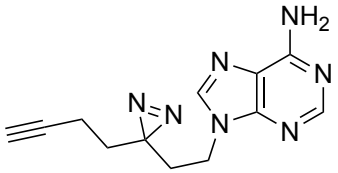
Sequence: HACSSIIK
Charge: +2,
Monoisotopic m/z: 661.82928 Da (-1.1 mmu/-1.67 ppm),
MH+: 1322.65129 Da,
RT: 51.3778 min,
Identified with: Sequest HT (v1.17); XCorr:0.94,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Exportin-5 OS=Homo sapiens OX=9606 GN=XPO5 PE=1 SV=1



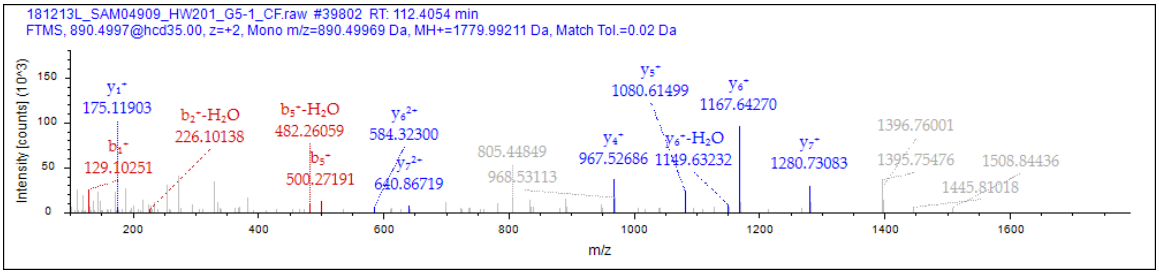
Sequence: GHDGKEPVK
Charge: +3,
Monoisotopic m/z: 477.57468 Da (+1.95 mmu/+4.09 ppm),
MH+: 1430.70948 Da,
RT: 56.0168 min,
Identified with: Sequest HT (v1.17); XCorr:1.34,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Fatty-acid amide hydrolase 2 OS=Homo sapiens OX=9606 GN=FAAH2 PE=2 SV=1



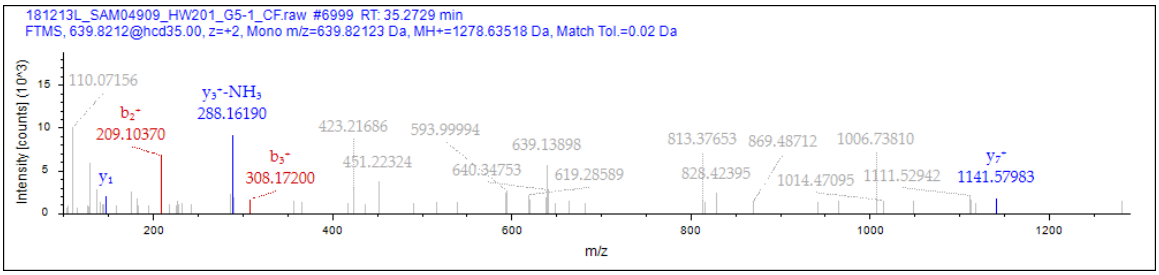
Sequence: KNDHTYR
Charge: +2,
Monoisotopic m/z: 700.33435 Da (-1.14 mmu/-1.63 ppm),
MH+: 1399.66142 Da,
RT: 65.7484 min,
Identified with: Sequest HT (v1.17); XCorr:1.00,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- 5-aminolevulinate synthase, nonspecific, mitochondrial OS=Homo sapiens OX=9606 GN=ALAS1 PE=1 SV=2



JN Mod: JN-847
PSMs: 43
Unique Sites: 14

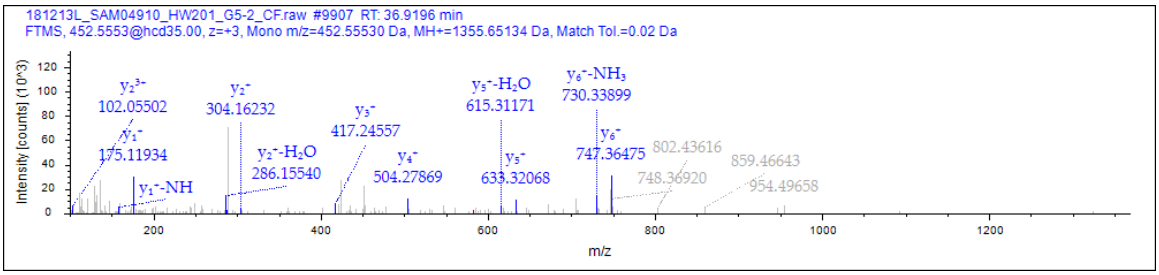


Sequence: KDAKGISIQTLR
Charge: +2,
Monoisotopic m/z: 890.49969 Da (-3.93 mmu/-4.42 ppm),
MH+: 1779.99211 Da,
RT: 112.4054 min,
Identified with: Sequest HT (v1.17); XCorr:1.30,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Zinc finger protein DZIP1L OS=Homo sapiens OX=9606 GN=DZIP1L PE=1 SV=2

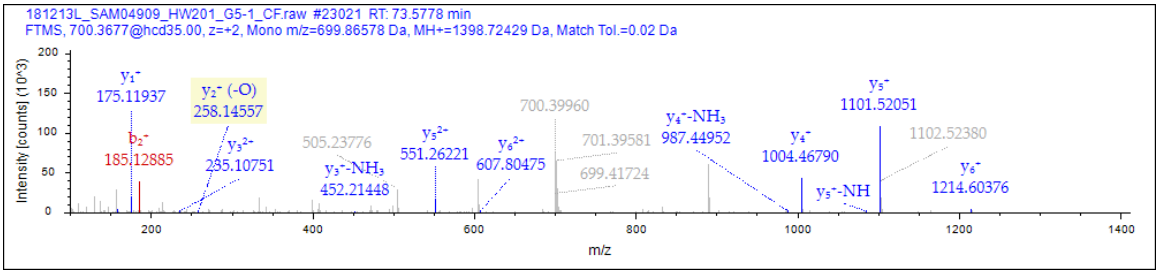


Sequence: HAVSEGTK
Charge: +2,
Monoisotopic m/z: 639.82123 Da (-0.46 mmu/-0.73 ppm),
MH+: 1278.63518 Da,
RT: 35.2729 min,
Identified with: Sequest HT (v1.17); XCorr:0.87,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (15):
- Histone H2B type 2-E OS=Homo sapiens OX=9606 GN=HIST2H2BE PE=1 SV=3
- Histone H2B type 2-F OS=Homo sapiens OX=9606 GN=HIST2H2BF PE=1 SV=3
- Histone H2B type 1-M OS=Homo sapiens OX=9606 GN=HIST1H2BM PE=1 SV=3
- Histone H2B type 1-B OS=Homo sapiens OX=9606 GN=HIST1H2BB PE=1 SV=2
- Histone H2B type 1-D OS=Homo sapiens OX=9606 GN=HIST1H2BD PE=1 SV=2

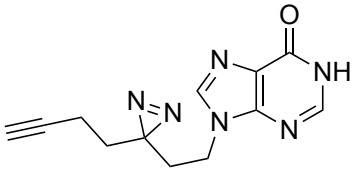
- Histone H2B type 1-A OS=Homo sapiens OX=9606 GN=HIST1H2BA PE=1 SV=3
- Histone H2B type 1-H OS=Homo sapiens OX=9606 GN=HIST1H2BH PE=1 SV=3
- Histone H2B type 1-K OS=Homo sapiens OX=9606 GN=HIST1H2BK PE=1 SV=3
- Histone H2B type 1-L OS=Homo sapiens OX=9606 GN=HIST1H2BL PE=1 SV=3
- Histone H2B type 1-C/E/F/G/I OS=Homo sapiens OX=9606 GN=HIST1H2BC PE=1 SV=4
- Histone H2B type F-S OS=Homo sapiens OX=9606 GN=H2BFS PE=1 SV=2
- Histone H2B type 3-B OS=Homo sapiens OX=9606 GN=HIST3H2BB PE=1 SV=3
- Histone H2B type 1-J OS=Homo sapiens OX=9606 GN=HIST1H2BJ PE=1 SV=3
- Histone H2B type 1-O OS=Homo sapiens OX=9606 GN=HIST1H2BO PE=1 SV=3
- Histone H2B type 1-N OS=Homo sapiens OX=9606 GN=HIST1H2BN PE=1 SV=3



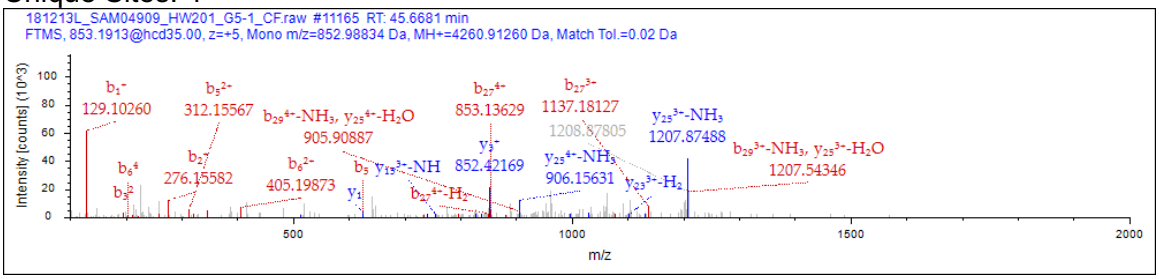
Sequence: GTNESLER
Charge: +3,
Monoisotopic m/z: 452.55530 Da (+1.32 mmu/+2.91 ppm),
MH+: 1355.65134 Da,
RT: 36.9196 min,
Identified with: Sequest HT (v1.17); XCorr:1.30,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (2):
- Glial fibrillary acidic protein OS=Homo sapiens OX=9606 GN=GFAP PE=1 SV=1
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4



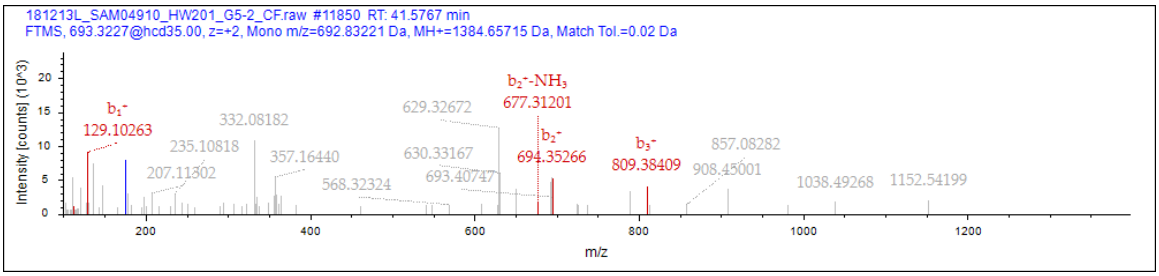
Sequence: AILPSFMR
Charge: +2,
Monoisotopic m/z: 699.86578 Da (+1.55 mmu/+2.21 ppm),
MH+: 1398.72429 Da, RT: 73.5778 min,
Identified with: Sequest HT (v1.17); XCorr:1.19,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -NH_3; y; b; y
Proteins (1):
- Protein SSX3 OS=Homo sapiens OX=9606 GN=SSX3 PE=1 SV=2



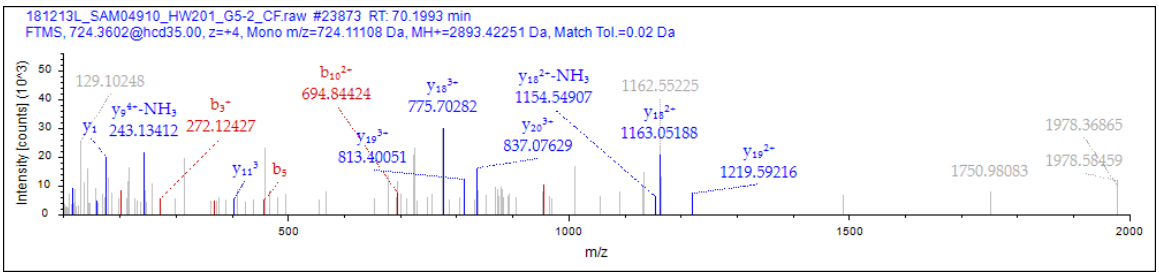
JN Mod: JN-845
PSMs: 6
Unique Sites: 4



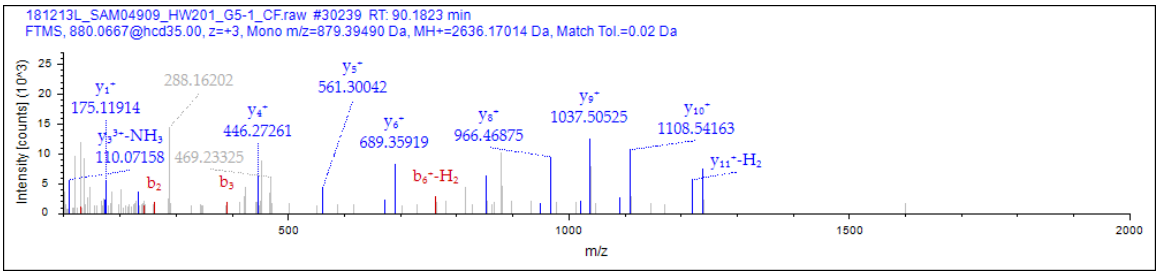
Sequence: KMLAYWMLEVCEEQRCEEEVFPLAMNYLDR
Charge: +5,
Monoisotopic m/z: 852.98834 Da (-1.9 mmu/-2.23 ppm),
MH+: 4260.91260 Da,
RT: 45.6681 min,
Identified with: Sequest HT (v1.17); XCorr:2.64,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- G1/S-specific cyclin-D3 OS=Homo sapiens OX=9606 GN=CCND3 PE=1 SV=2



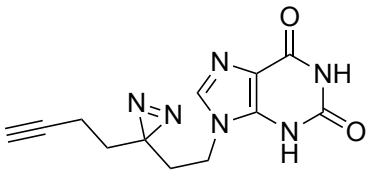
Sequence: KNDHTYR
Charge: +2,
Monoisotopic m/z: 692.83221 Da (+2.18 mmu/+3.14 ppm),
MH+: 1384.65715 Da,
RT: 41.5767 min,
Identified with: Sequest HT (v1.17); XCorr:1.07,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- 5-aminolevulinate synthase, nonspecific, mitochondrial OS=Homo sapiens OX=9606 GN=ALAS1 PE=1 SV=2



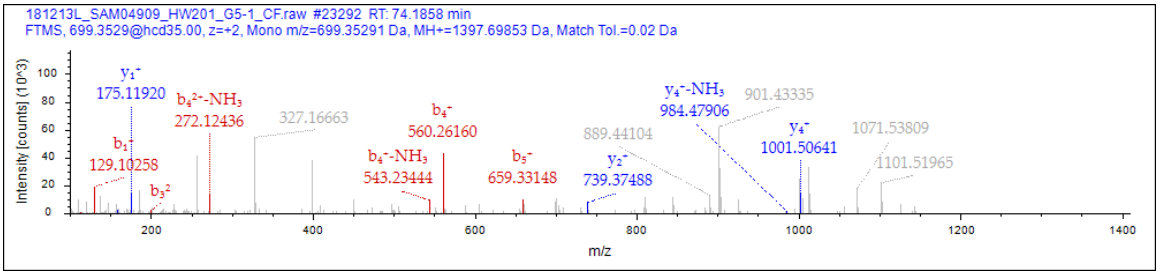
Sequence: AEALALLPCGLGMAFSQSHVMAAR
Charge: +4,
Monoisotopic m/z: 724.11108 Da (+1.35 mmu/+1.87 ppm),
MH+: 2893.42251 Da,
RT: 70.1993 min,
Identified with: Sequest HT (v1.17); XCorr:2.92,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Metallophosphoesterase domain-containing protein 1 OS=Homo sapiens OX=9606 GN=MPPED1 PE=2 SV=3



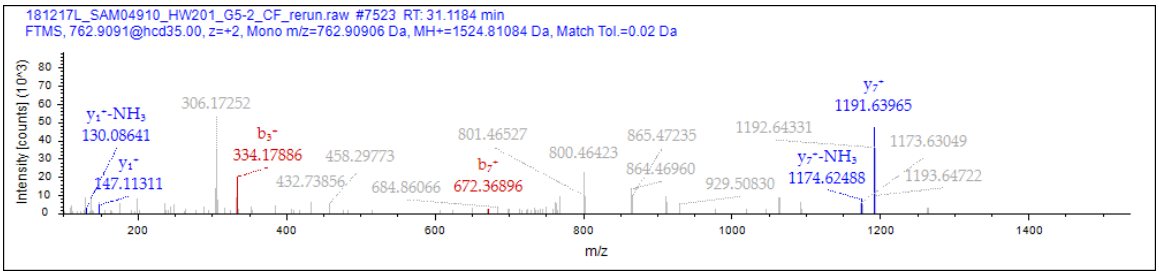
Sequence: EMEENFAVEAANYQDTIGR
Charge: +3,
Monoisotopic m/z: 879.39490 Da (+3.95 mmu/+4.5 ppm),
MH+: 2636.17014 Da,
RT: 90.1823 min,
Identified with: Sequest HT (v1.17); XCorr:2.95,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4



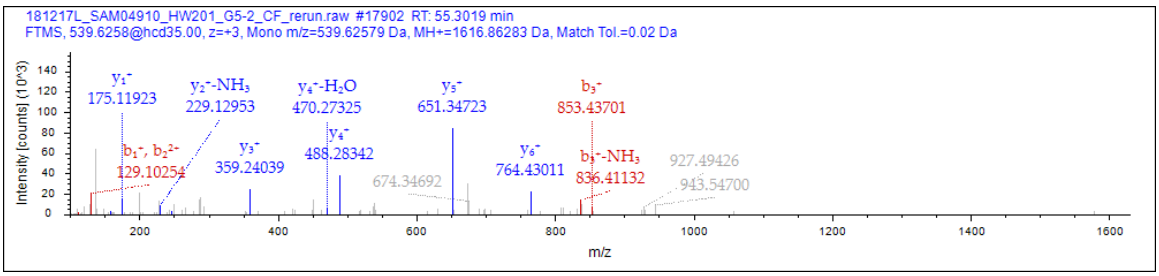
JN Mod: JN-936
PSMs: 32
Unique Sites: 6



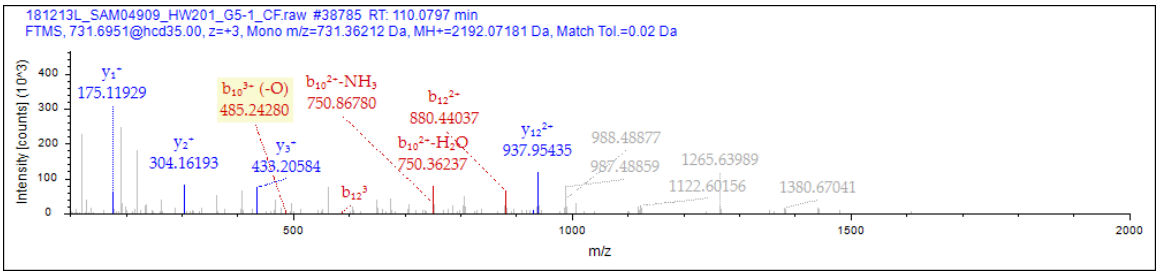
Sequence: KHMYVVR
Charge: +2,
Monoisotopic m/z: 699.35291 Da (-1.12 mmu/-1.6 ppm),
MH+: 1397.69853 Da,
RT: 74.1858 min,
Identified with: Sequest HT (v1.17); XCorr:1.93,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -NH3; y; b; b; -NH3; y
Proteins (1):
- Protein mono-ADP-ribosyltransferase PARP15 OS=Homo sapiens OX=9606 GN=PARP15 PE=1 SV=2



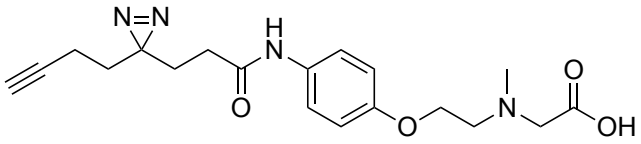
Sequence: YGLPGLAQLK
Charge: +2,
Monoisotopic m/z: 762.90906 Da (+1.92 mmu/+2.51 ppm),
MH+: 1524.81084 Da,
RT: 31.1184 min,
Identified with: Sequest HT (v1.17); XCorr:1.06,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -NH3; y; b; y
Proteins (1):
- Endonuclease G, mitochondrial OS=Homo sapiens OX=9606 GN=ENDOG PE=1 SV=4



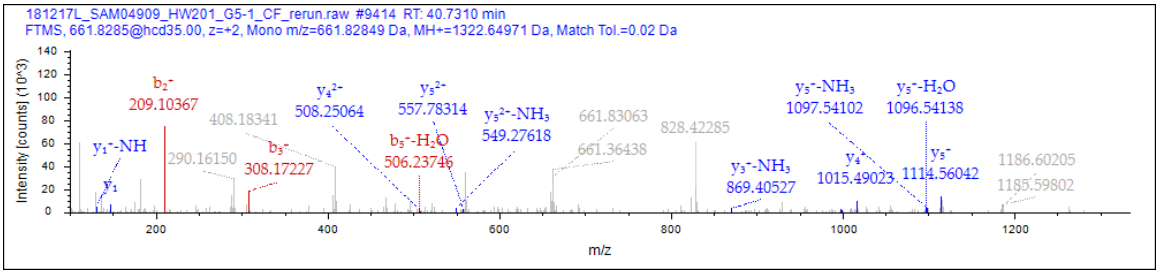
Sequence: KKEIYELAR
Charge: +3,
Monoisotopic m/z: 539.62579 Da (+2.07 mmu/+3.83 ppm),
MH+: 1616.86283 Da,
RT: 55.3019 min,
Identified with: Sequest HT (v1.17); XCorr:1.74,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial OS=Homo sapiens OX=9606 GN=AADAT PE=1 SV=2



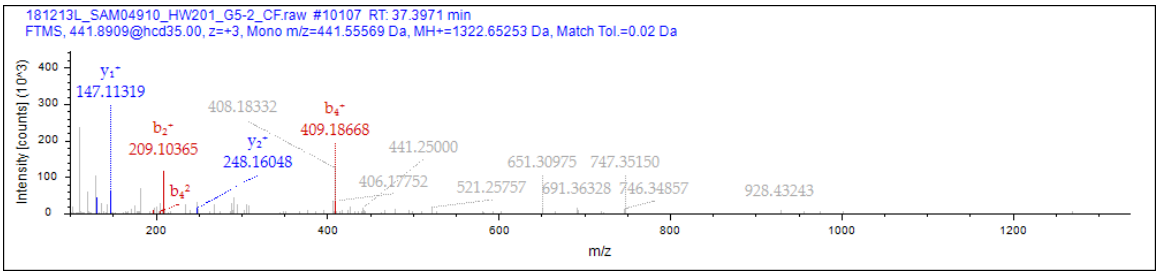
Sequence: VMARSLSPPPELEER
Charge: +3,
Monoisotopic m/z: 731.36212 Da (+1.76 mmu/+2.4 ppm),
MH+: 2192.07181 Da,
RT: 110.0797 min,
Identified with: Sequest HT (v1.17); XCorr:1.40,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Tuberin OS=Homo sapiens OX=9606 GN=TSC2 PE=1 SV=2



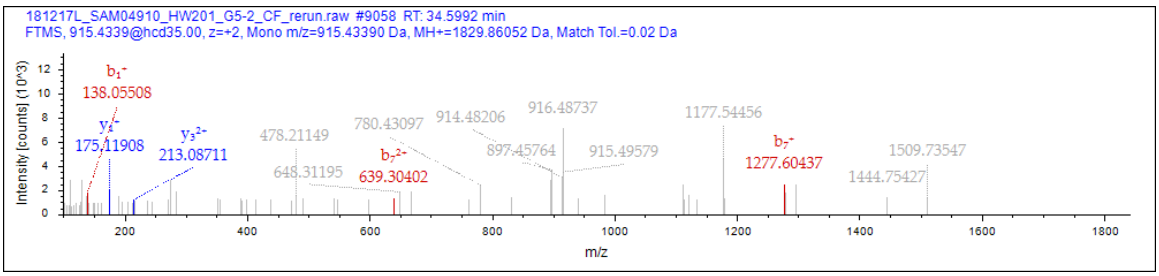
JN Mod: JN-836
PSMs: 16
Unique Sites: 4



Sequence: AHVESSK
Charge: +2,
Monoisotopic m/z: 661.82849 Da (+0.46 mmu/+0.69 ppm),
MH+: 1322.64971 Da,
RT: 40.7310 min,
Identified with: Sequest HT (v1.17); XCorr:1.22,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Melanoma-associated antigen H1 OS=Homo sapiens OX=9606 GN=MAGEH1 PE=1 SV=1

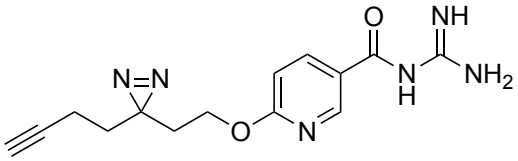


Sequence: AHAETTK
Charge: +3,
Monoisotopic m/z: 441.55569 Da (+1.25 mmu/+2.83 ppm),
MH+: 1322.65253 Da,
RT: 37.3971 min,
Identified with: Sequest HT (v1.17); XCorr:1.22,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Putative MAGE domain-containing protein MAGEA13P OS=Homo sapiens OX=9606 GN=MAGEA13P PE=5 SV=1

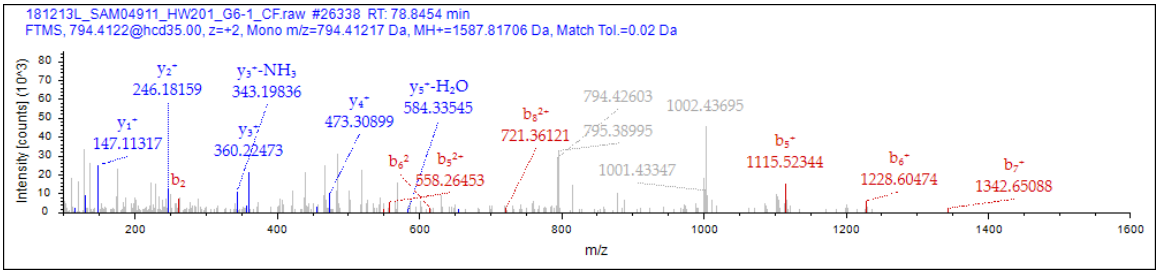


Sequence: HACSSIIKMCR
Charge: +2,
Monoisotopic m/z: 915.43390 Da (-0.14 mmu/-0.15 ppm),
MH+: 1829.86052 Da,
RT: 34.5992 min,
Identified with: Sequest HT (v1.17); XCorr:0.93,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Exportin-5 OS=Homo sapiens OX=9606 GN=XPO5 PE=1 SV=1

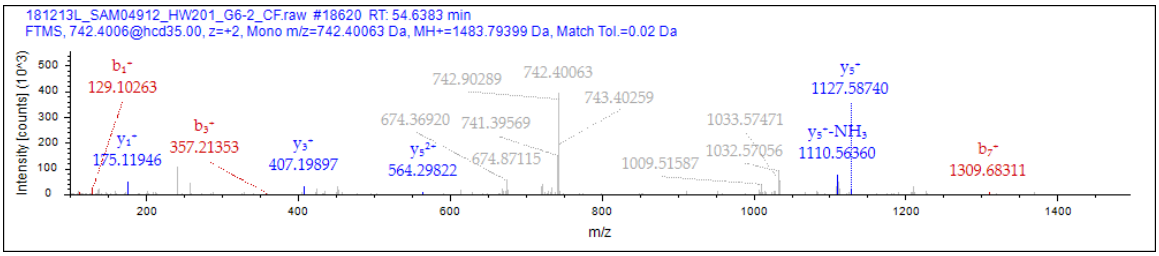
Group 6:



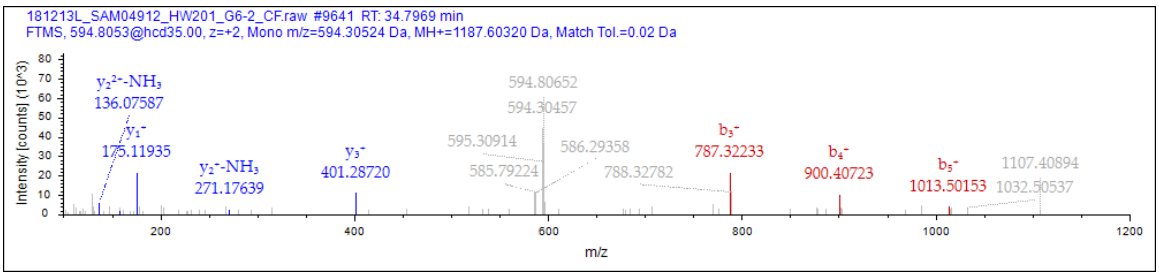
JN Mod: JN-942
PSMs: 21
Unique Sites: 11



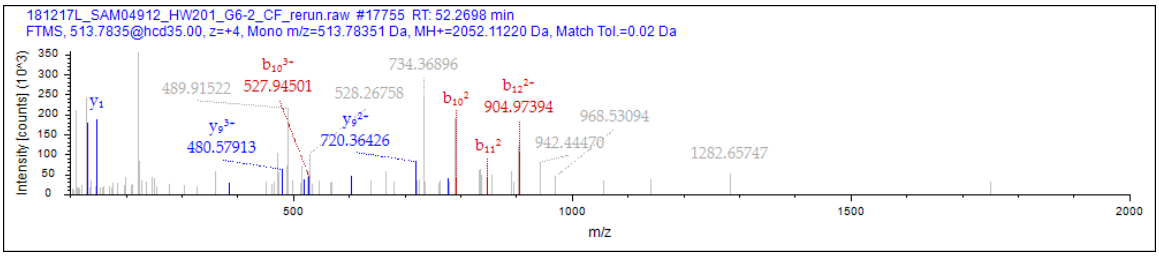
Sequence: FLTEEINVK
Charge: +2,
Monoisotopic m/z: 794.41217 Da (-0.89 mmu/-1.13 ppm),
MH+: 1587.81706 Da,
RT: 78.8454 min,
Identified with: Sequest HT (v1.17); XCorr:1.96,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Protocadherin gamma-A7 OS=Homo sapiens OX=9606 GN=PCDHGA7 PE=2 SV=1



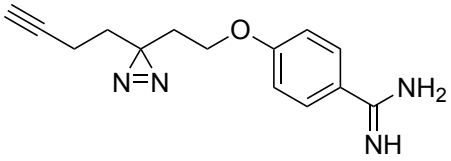
Sequence: KDLVKTMR
Charge: +2,
Monoisotopic m/z: 742.40063 Da (-0.53 mmu/-0.72 ppm),
MH+: 1483.79399 Da,
RT: 54.6383 min,
Identified with: Sequest HT (v1.17); XCorr:1.34,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Acetyl-CoA carboxylase 1 OS=Homo sapiens OX=9606 GN=ACACA PE=1 SV=2



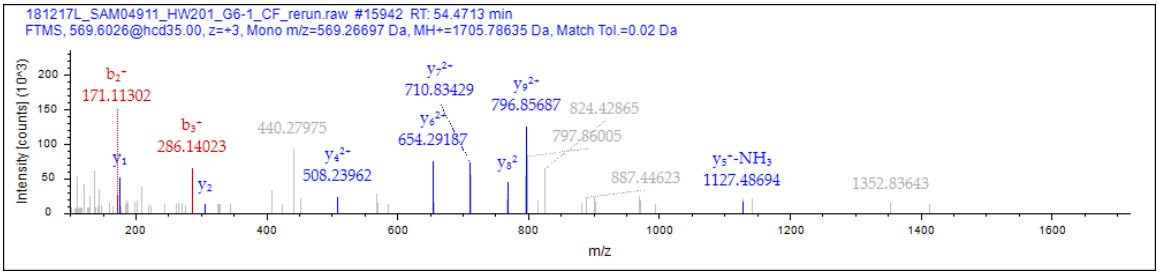
Sequence: GMSILR
Charge: +2,
Monoisotopic m/z: 594.30524 Da (+0.96 mmu/+1.62 ppm),
MH+: 1187.60320 Da,
RT: 34.7969 min,
Identified with: Sequest HT (v1.17); XCorr:1.40,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Cerebellar degeneration-related protein 2-like OS=Homo sapiens OX=9606 GN=CDR2L PE=1 SV=2



Sequence: MLRVIVESASNIPK
Charge: +4,
Monoisotopic m/z: 513.78351 Da (+1.01 mmu/+1.96 ppm),
MH+: 2052.11220 Da,
RT: 52.2698 min,
Identified with: Sequest HT (v1.17); XCorr:1.60,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Myoferlin OS=Homo sapiens OX=9606 GN=MYOF PE=1 SV=1

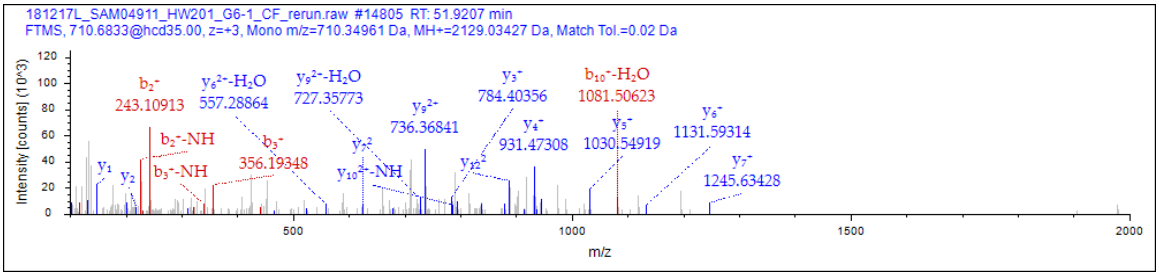


JN Mod: JN-945
PSMs: 441
Unique Sites: 48

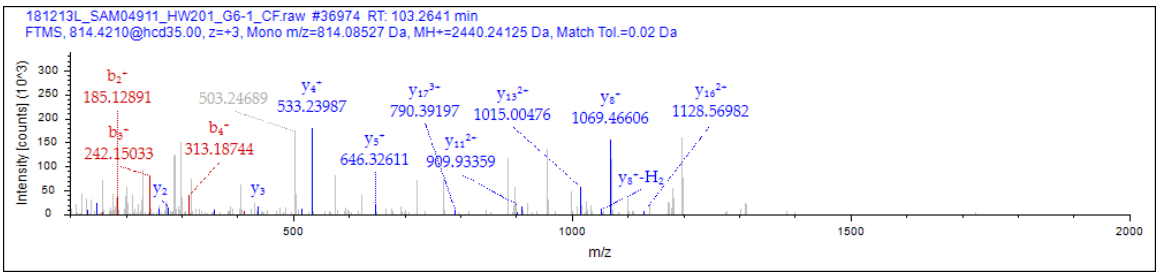


Sequence: LGDLYEEEMR
Charge: +3,
Monoisotopic m/z: 569.26697 Da (-1.64 mmu/-2.89 ppm),
MH+: 1705.78635 Da,
RT: 54.4713 min,
Identified with: Sequest HT (v1.17); XCorr:2.31,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):

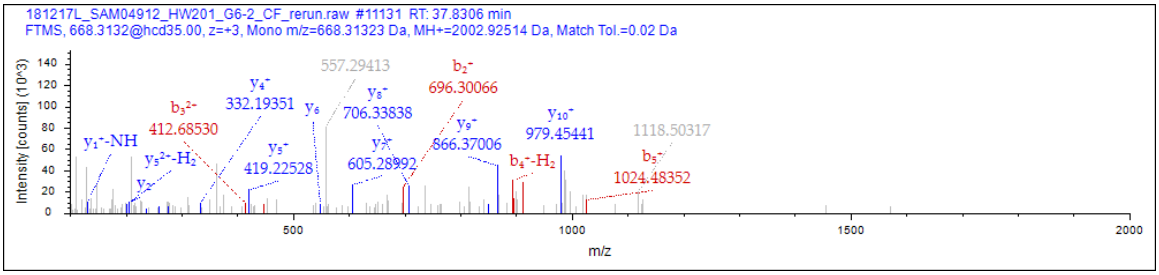
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4



Sequence: NQLTSNPENTVFDAK
Charge: +3,
Monoisotopic m/z: 710.34943 Da (+0.56 mmu/+0.79 ppm),
MH+: 2129.03373 Da,
RT: 41.4659 min,
Identified with: Sequest HT (v1.17); XCorr:3.08,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Endoplasmic reticulum chaperone BiP OS=Homo sapiens OX=9606 GN=HSPA5 PE=1 SV=2

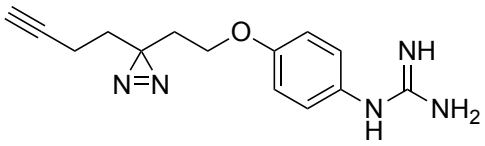


Sequence: AIGAVPLIQGEYMIPCEK
Charge: +3,
Monoisotopic m/z: 814.08527 Da (-0.45 mmu/-0.55 ppm),
MH+: 2440.24125 Da,
RT: 103.2641 min,
Identified with: Sequest HT (v1.17); XCorr:1.87,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Cathepsin D OS=Homo sapiens OX=9606 GN=CTSD PE=1 SV=1

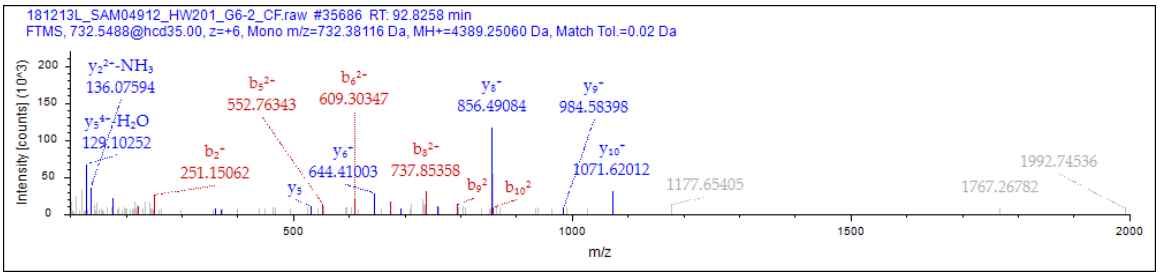


Sequence: EDQSILCTGESGAGK
Charge: +3,
Monoisotopic m/z: 668.31323 Da (+1.81 mmu/+2.7 ppm),
MH+: 2002.92514 Da,
RT: 37.8306 min,
Identified with: Sequest HT (v1.17); XCorr:2.80,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y

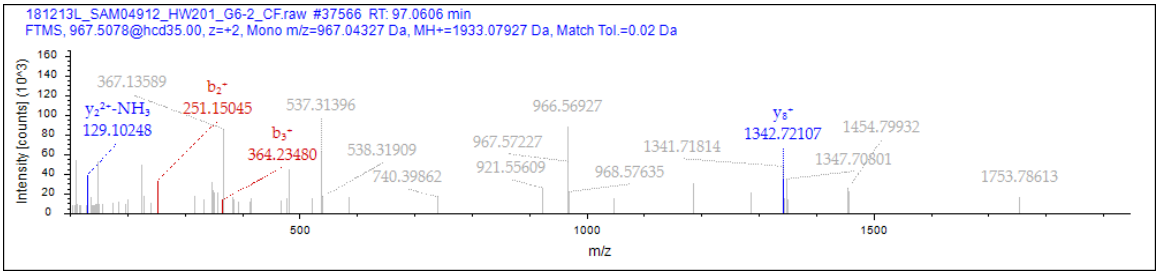
Proteins (4):
- Myosin-10 OS=Homo sapiens OX=9606 GN=MYH10 PE=1 SV=3
- Myosin-11 OS=Homo sapiens OX=9606 GN=MYH11 PE=1 SV=3
- Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4
- Myosin-14 OS=Homo sapiens OX=9606 GN=MYH14 PE=1 SV=2



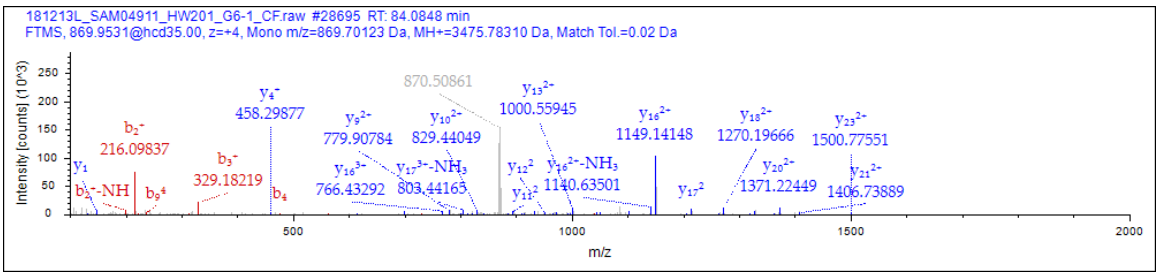
JN Mod: JN-940
SI number: 37
PSMs: 333
Unique Sites: 45



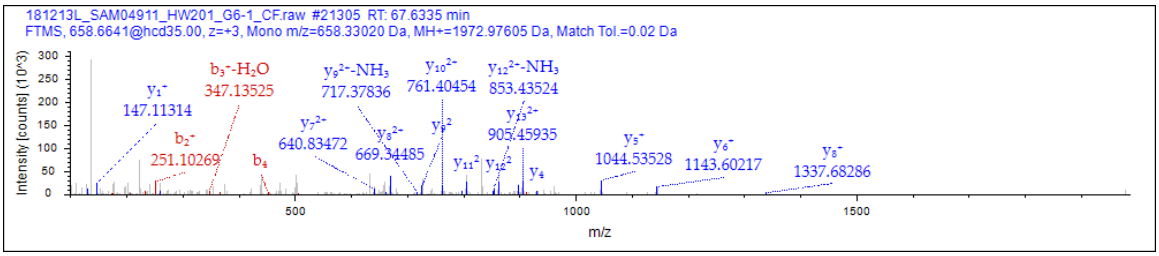
Sequence: LHEEEIQELQAQIQEQHVQIDVDVSKPDLTAALR
Charge: +6,
Monoisotopic m/z: 732.38116 Da (+0.17 mmu/+0.23 ppm),
MH+: 4389.25060 Da,
RT: 92.8258 min,
Identified with: Sequest HT (v1.17); XCorr:2.91,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4



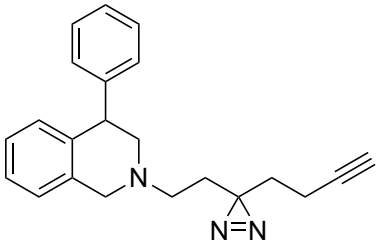
Sequence: HLIQVDFGVLAVR
Charge: +2,
Monoisotopic m/z: 967.04327 Da (-1.82 mmu/-1.88 ppm),
MH+: 1933.07927 Da,
RT: 97.0606 min,
Identified with: Sequest HT (v1.17); XCorr:0.83,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- E3 ubiquitin-protein ligase RNF213 OS=Homo sapiens OX=9606 GN=RNF213 PE=1 SV=3



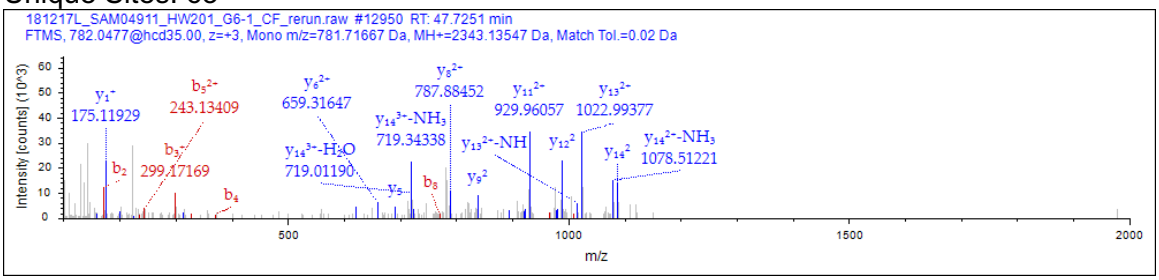
Sequence: SQIFSTASDNQPTVTIKVYEGERPLTK
Charge: +4,
Monoisotopic m/z: 869.70123 Da (-1.19 mmu/-1.37 ppm),
MH+: 3475.78310 Da,
RT: 84.0848 min,
Identified with: Sequest HT (v1.17); XCorr:4.87,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Endoplasmic reticulum chaperone BiP OS=Homo sapiens OX=9606 GN=HSPA5 PE=1 SV=2



Sequence: YSNSALGHVNCTIK
Charge: +3,
Monoisotopic m/z: 658.33020 Da (+1.34 mmu/+2.03 ppm),
MH+: 1972.97605 Da,
RT: 67.6335 min,
Identified with: Sequest HT (v1.17); XCorr:3.43,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Reticulon-4 OS=Homo sapiens OX=9606 GN=RTN4 PE=1 SV=2

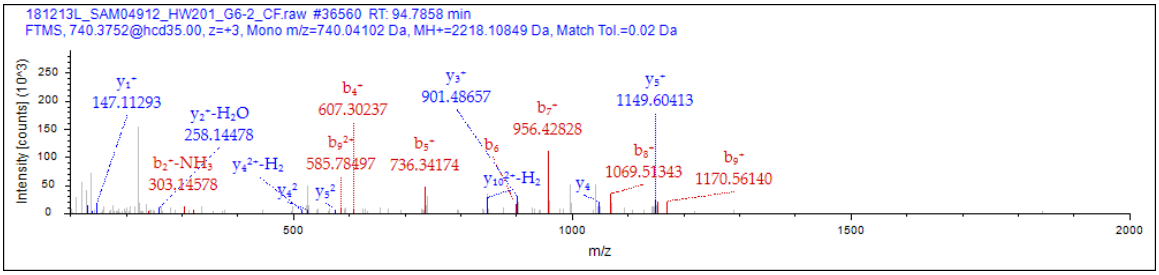


JN Mod: JN-849
PSMs: 570
Unique Sites: 63



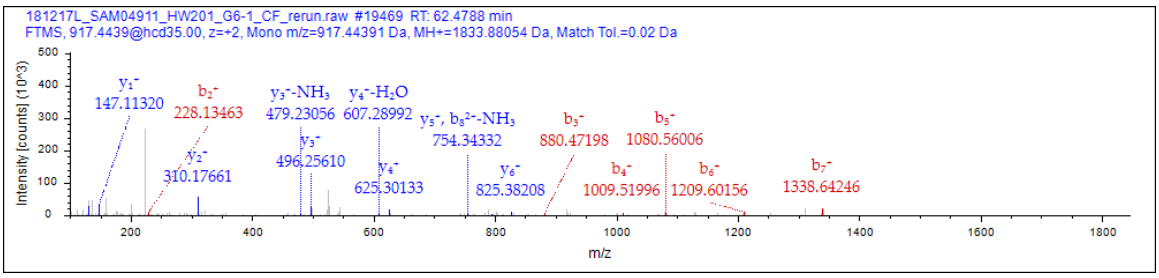
Sequence: AVQADGQVKECYQSHR
Charge: +3,
Monoisotopic m/z: 781.71667 Da (-0.23 mmu/-0.29 ppm),
MH+: 2343.13547 Da,
RT: 47.7251 min,
Identified with: Sequest HT (v1.17); XCorr:3.07,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

- Programmed cell death 6-interacting protein OS=Homo sapiens OX=9606 GN=PDCD6IP PE=1 SV=1

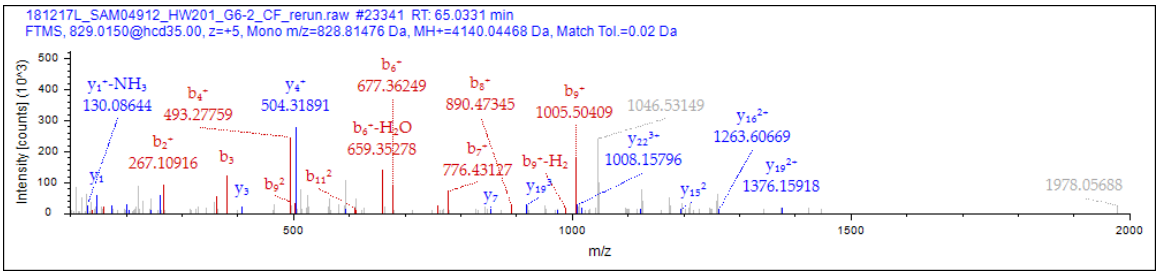


Sequence: YRWTEYGLTFTEK
Charge: +3,
Monoisotopic m/z: 740.04102 Da (+1.82 mmu/+2.46 ppm),
MH+: 2218.10849 Da,
RT: 94.7858 min,
Identified with: Sequest HT (v1.17); XCorr:2.93,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):

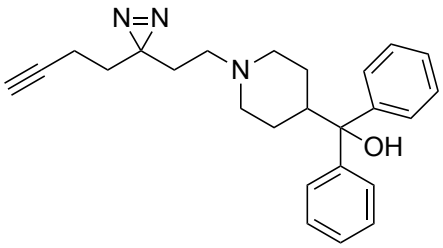
- Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens OX=9606 GN=VDAC1 PE=1 SV=2



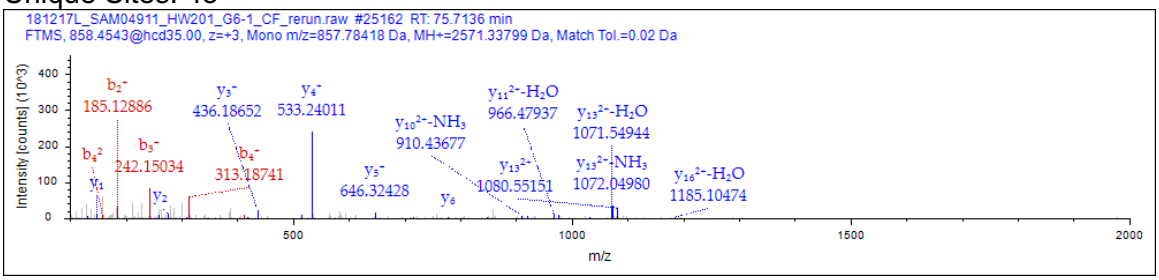
Sequence: NLQEAEEWYK
Charge: +2,
Monoisotopic m/z: 917.44391 Da (-3.18 mmu/-3.47 ppm),
MH+: 1833.88054 Da,
RT: 62.4788 min,
Identified with: Sequest HT (v1.17); XCorr:2.77,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (2):
- Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4
- Peripherin OS=Homo sapiens OX=9606 GN=PRPH PE=1 SV=2



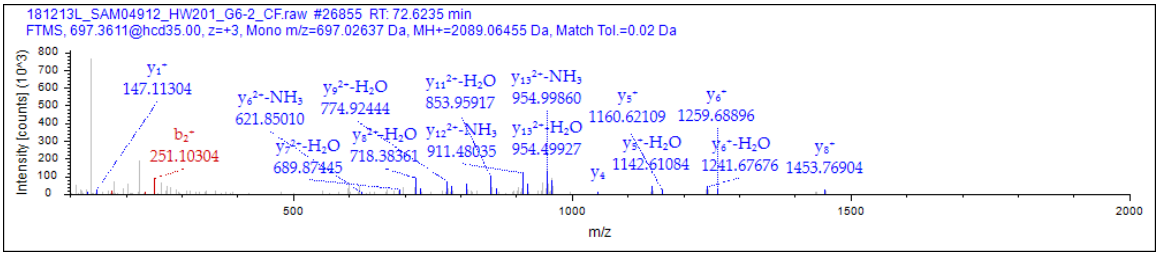
Sequence: HELLPSVNDITAVGPAHFYATNDHYFSDPFLK
Charge: +5,
Monoisotopic m/z: 828.81476 Da (+1.88 mmu/+2.26 ppm),
MH+: 4140.04468 Da,
RT: 65.0331 min,
Identified with: Sequest HT (v1.17); XCorr:4.86,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Serum paraoxonase/arylesterase 2 OS=Homo sapiens OX=9606 GN=PON2 PE=1 SV=4



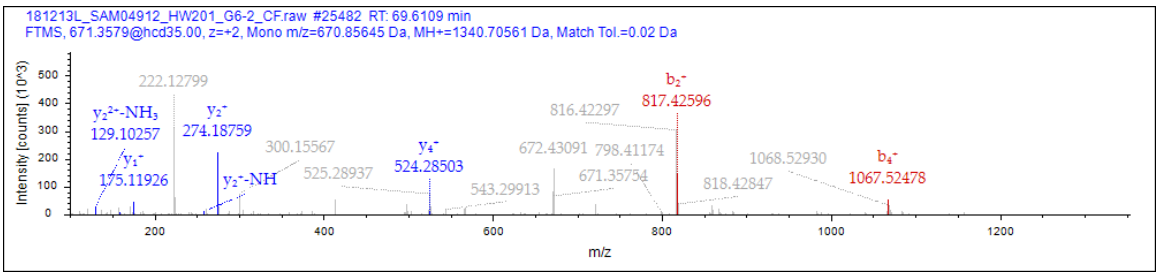
JN Mod: JN-938
PSMs: 244
Unique Sites: 45



Sequence: AIGAVPLIQGEYMIPCEK
Charge: +3,
Monoisotopic m/z: 857.78418 Da (-1.1 mmu/-1.28 ppm),
MH+: 2571.33799 Da,
RT: 75.7136 min,
Identified with: Sequest HT (v1.17); XCorr:2.91,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Cathepsin D OS=Homo sapiens OX=9606 GN=CTSD PE=1 SV=1

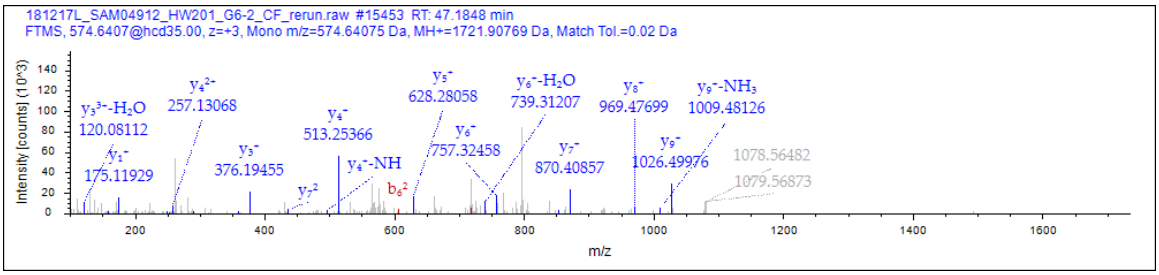


Sequence: YSNSALGHVNCTIK
Charge: +3,
Monoisotopic m/z: 697.02637 Da (+1.57 mmu/+2.25 ppm),
MH+: 2089.06455 Da,
RT: 72.6235 min,
Identified with: Sequest HT (v1.17); XCorr:2.60,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Reticulon-4 OS=Homo sapiens OX=9606 GN=RTN4 PE=1 SV=2

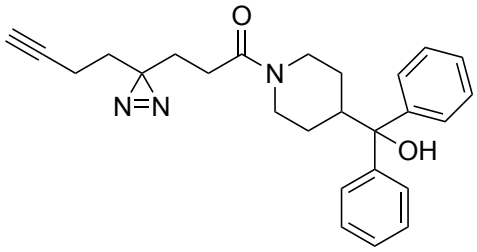


Sequence: YAYSVR
Charge: +2,
Monoisotopic m/z: 670.85645 Da (-0.23 mmu/-0.34 ppm),
MH+: 1340.70561 Da,
RT: 69.6109 min,
Identified with: Sequest HT (v1.17); XCorr:1.23,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):

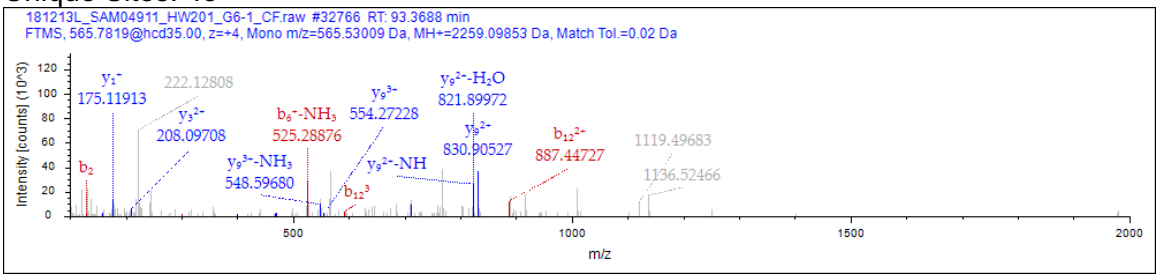
- Probable ATP-dependent DNA helicase HFM1 OS=Homo sapiens OX=9606 GN=HFM1 PE=1 SV=2



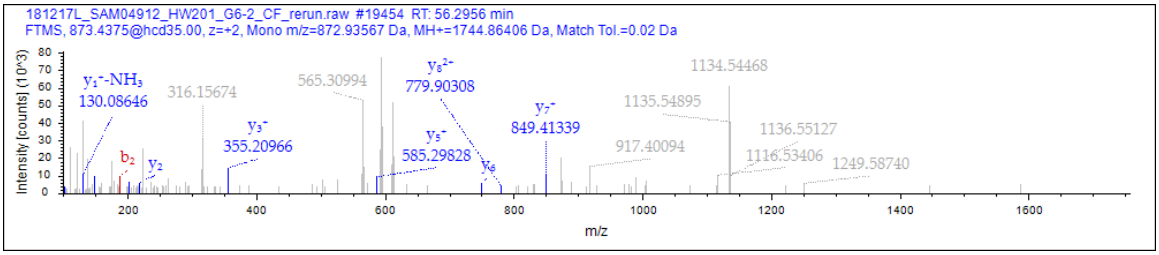
Sequence: LGVIEDHSNR
Charge: +3,
Monoisotopic m/z: 574.64075 Da (+1.48 mmu/+2.57 ppm),
MH+: 1721.90769 Da,
RT: 47.1848 min,
Identified with: Sequest HT (v1.17); XCorr:2.05,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (2):
- Endoplasmin OS=Homo sapiens OX=9606 GN=HSP90B1 PE=1 SV=1
- Putative endoplasmin-like protein OS=Homo sapiens OX=9606 GN=HSP90B2P PE=5 SV=1



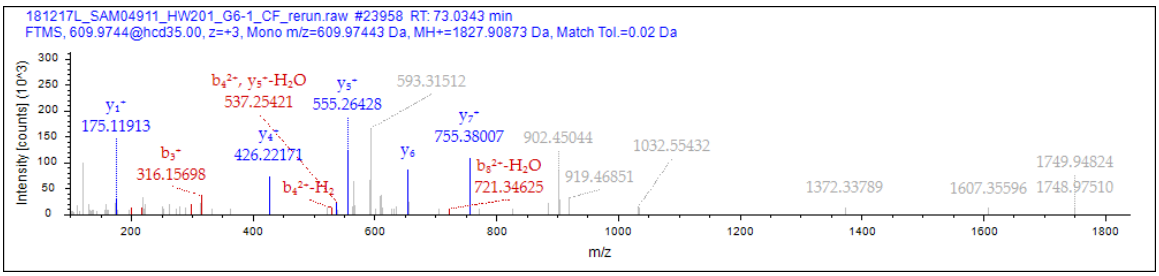
JN Mod: JN-939
PSMs: 208
Unique Sites: 43



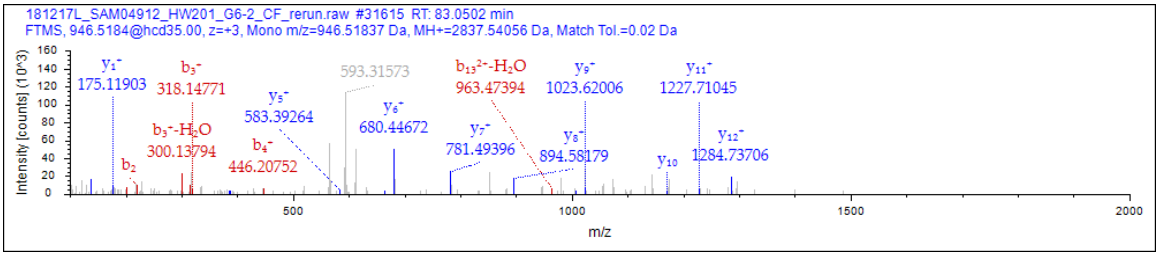
Sequence: AGRSAVGTKMTCAHCR
Charge: +4,
Monoisotopic m/z: 565.53009 Da (-0.53 mmu/-0.94 ppm),
MH+: 2259.09853 Da, RT: 93.3688 min,
Identified with: Sequest HT (v1.17); XCorr:1.50,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Zinc finger MYM-type protein 3 OS=Homo sapiens OX=9606 GN=ZMYM3 PE=1 SV=2



Sequence: DAVTYTEHAK
Charge: +2,
Monoisotopic m/z: 872.93567 Da (+1.84 mmu/+2.11 ppm),
MH+: 1744.86406 Da,
RT: 56.2956 min,
Identified with: Sequest HT (v1.17); XCorr:1.24,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Histone H4 OS=Homo sapiens OX=9606 GN=HIST1H4A PE=1 SV=2



Sequence: ESVFTVEGGHR
Charge: +3,
Monoisotopic m/z: 609.97443 Da (0 mmu/0 ppm),
MH+: 1827.90873 Da,
RT: 73.0343 min,
Identified with: Sequest HT (v1.17); XCorr:1.84,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; y
Proteins (1):
- Prohibitin-2 OS=Homo sapiens OX=9606 GN=PHB2 PE=1 SV=2



Sequence: MSVQPTVSLGGFEITPPVVLR
Charge: +3,
Monoisotopic m/z: 946.51837 Da (+2.42 mmu/+2.56 ppm),
MH+: 2837.54056 Da,
RT: 83.0502 min,
Identified with: Sequest HT (v1.17); XCorr:2.53,
Fragment match tolerance used for search: 0.02 Da
Fragments used for search: -H_2O; y; -NH_3; y; b; b; -H_2O; b; -NH_3; y
Proteins (1):
- Nucleophosmin OS=Homo sapiens OX=9606 GN=NPM1 PE=1 SV=2

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