Transcriptome-wide mapping of small molecule RNA binding sites in cells informs an isoform-specific degrader of *QSOX1* mRNA

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Table S1: Sequences of oligonucleotides used in these studies.			
Oligonucleotide	**Sequence 5' → 3'**	**Experiment**	**Supplier**
QSOX1-F	CTT GCG TGT GGT GGT GAG C	qPCR	IDT
QSOX1-R	CAG CGG GTC GGA AGG CGA ATA GAG	qPCR	IDT
SQSTM1-F	TAT GGC GTC GCT CAC CGT GAA GG	qPCR	IDT
SQSTM1-R	CAG CCT CGG CTT CCG CCT CAG	qPCR	IDT
18S-F	GTA ACC CGT TGA ACC CCA TT	qPCR	IDT
18S-R	CCA TCC AAT CGG TAG TAG CG	qPCR	IDT
QSOX1-ASO[a]	**UGU** TGG TCT CCT C**AG C**	Cellular assays	IDT
Scrambled-ASO[a]	**GUG** AGG G**UC A**	Cellular assays	IDT
Cy5-QSOX1-WT[b]	Cy-5 GGU GCU UGC GUG UGG UGG UGA GCG CAG CGC C	*In vitro* assays	Dharmacon
Cy5-QSOX1-BP[b]	Cy-5 GGU GCU UGC GUG UGG UGG UGA GCG CAA GCG CC	*In vitro* assays	Dharmacon

[a] Linkages of all nucleotides are phosphorothioate.

[b] 2'-O-Methoxyelthyl (MOE) nucleotides are indicated in bold.

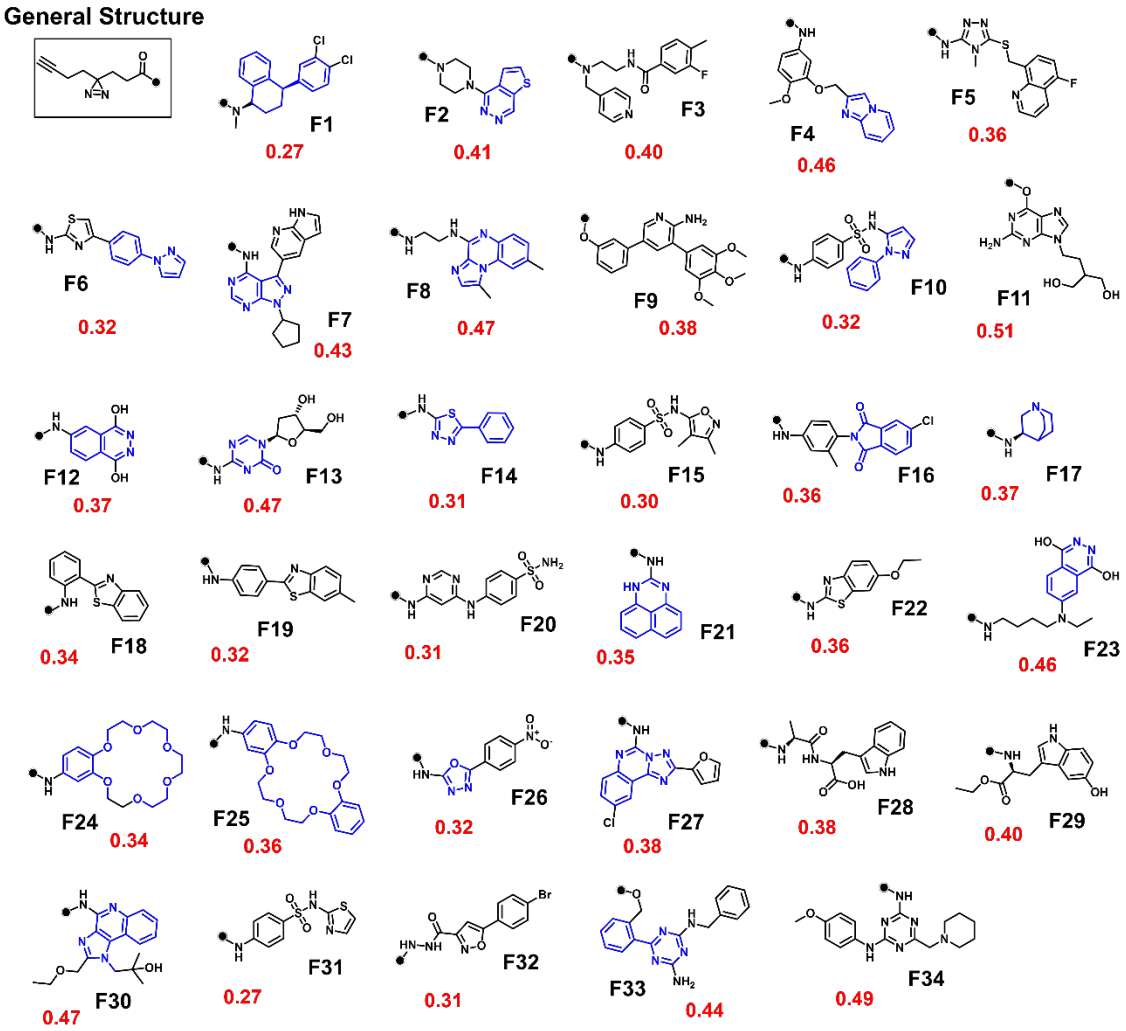


Figure S1. Chemical structures of 34 compounds synthesized for *in vitro* screening. The **red** number is the average Tanimoto score calculated for each structure compared to all known RNA binders.[1, 2] A Tanimoto score < 0.7 is typically considered as chemically dissimilar. The core structures highlighted in **blue** represent novel chemotypes that are not present in previously known RNA binders. This library was constructed based on the by UMAP (Uniform Manifold Approximation and Projection) analysis (Figure S2) to identify molecules that are similar to known RNA binders in a broad sense, as well as including novel chemotypes that are not previously reported to bind RNA in cells and commercial availability (F24/F25).

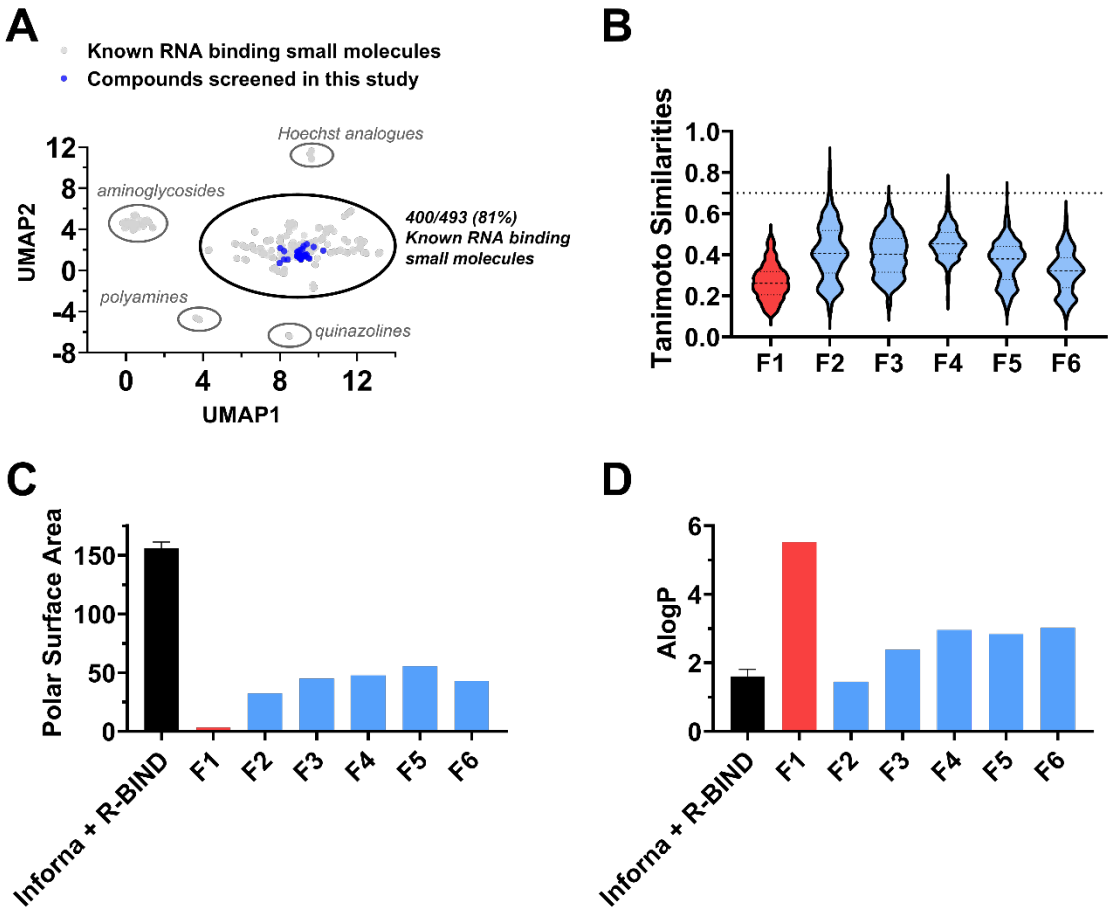


Figure S2. Comparison of screened compounds with known small molecules that bind RNA. (A) The panel of 34 structurally diverse compounds are clustered with ~80% of all known RNA binding small molecules[1, 2] as determined by UMAP (Uniform Manifold Approximation and Projection) analysis based on Morgan fingerprints[3] of structural similarities. (B) Tanimoto scores of hit compounds (F1 to F6) compared with each known RNA-binding small molecules suggest that they are overall dissimilar to known RNA binders (Tanimoto score < 0.7). Among these six hits, F1 is the most dissimilar compound with average Tanimoto score of only 0.27±0.09. The other five hits are also dissimilar to most RNA-binding compounds, although the range of values for F2 - F5 extend over the 0.7 cut-off. (C) All six hits showed lower polar surface area (PSA) than average known RNA binders, and F1 shows the lowest PSA. (D) F1 shows the highest atomic logP (AlopP) compared to other hits and the average known RNA binders.

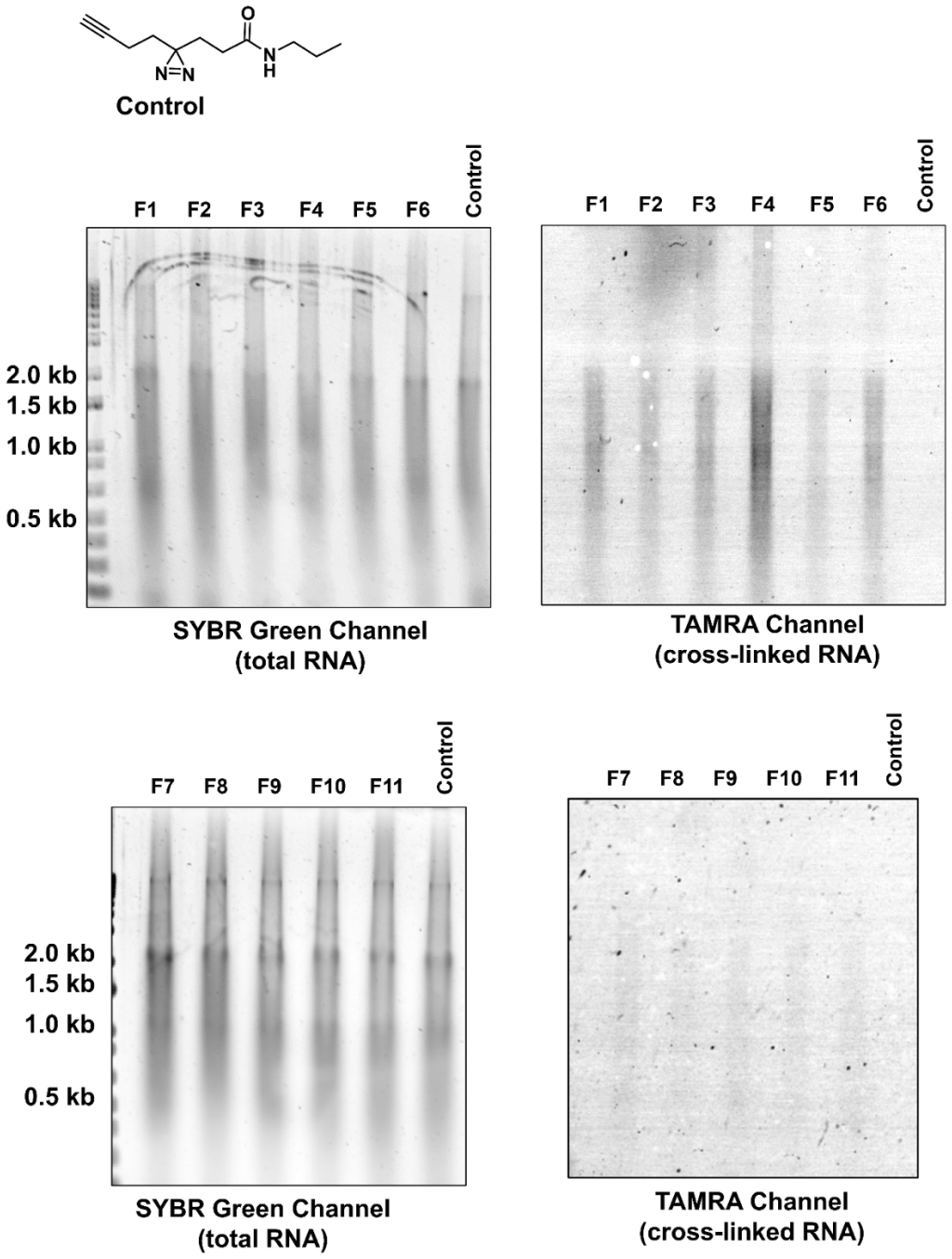


Figure S3. Representative image of *in vitro* Chem-CLIP screening of compounds cross-linked with purified total RNA from MDA-MB-231 cells. The general reactivity of compounds was studied using *in vitro* Chem-CLIP by cross-linking the compound to purified total RNA from MDA-MB-231 cells. Cross-linking was then visualized by a click reaction with azide-functionalized TAMRA (tetramethylrhodamine) dye, and the approximate sizes of the cross-linked RNA analyzed by agarose gel electrophoresis. After imaging TAMRA fluorescence, the gel was stained with SYBR Green to visualize total RNA. A fragment was deemed a hit if the resulting TAMRA signal was at least 3-fold above the background. "Control" indicates incubation of total RNA with the control diazirine probe shown at the top of the figure.

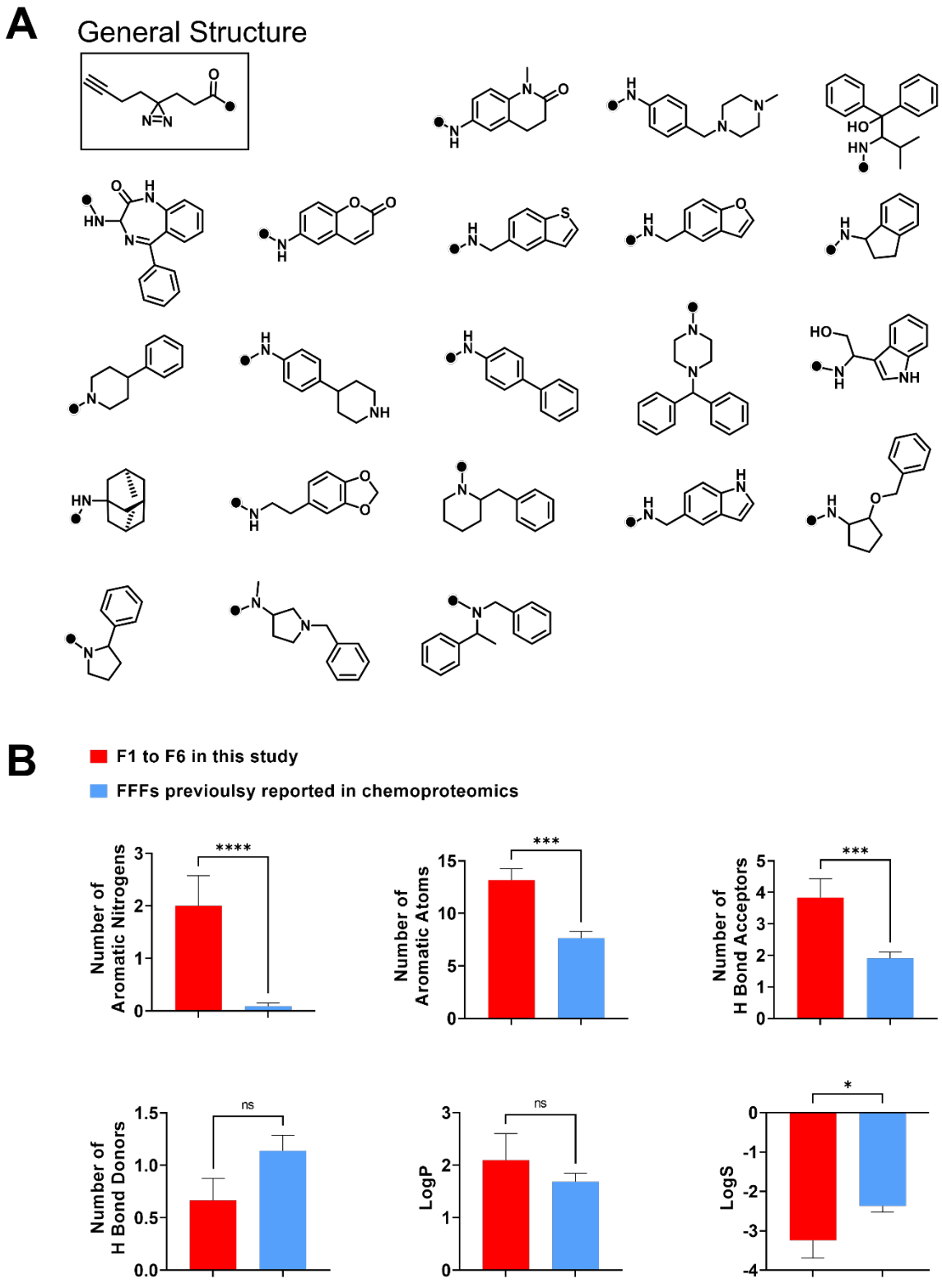
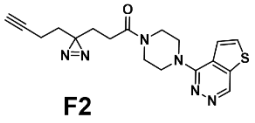
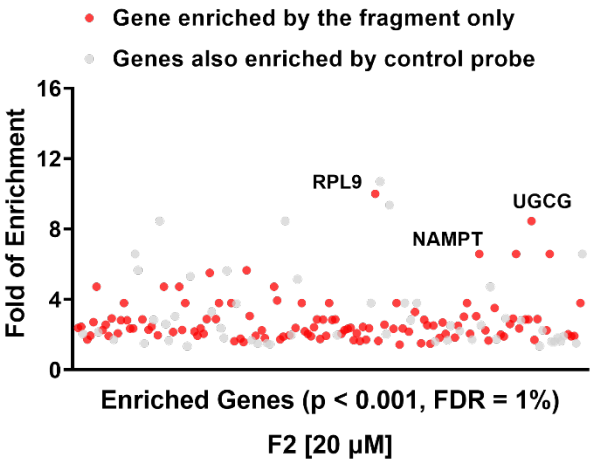
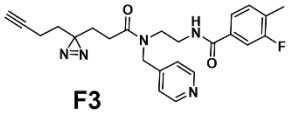


Figure S4. Comparison of F1 – F6 to molecules previously reported chemoproteomic studies that use fully functionalized fragments.[4, 5] (A) The chemical structures of fully functionalized fragments (FFFs) previously reported in chemoproteomic studies. (B) Comparison of physicochemical properties of F1 – F6 to those shown in panel A.

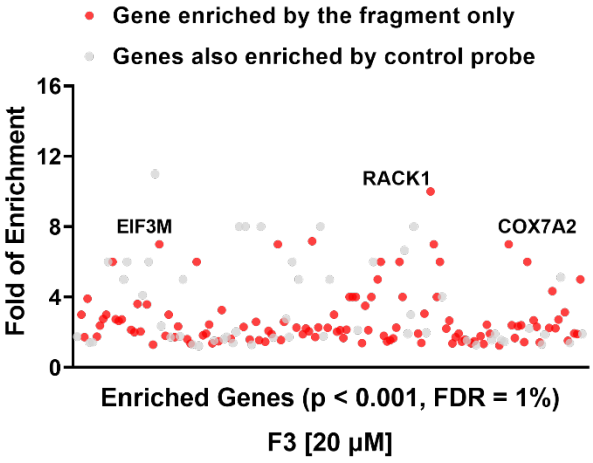


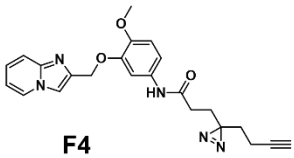
F2


Gene enriched by the fragment only
Genes also enriched by control probe
Fold of Enrichment
16
12
8
4
0
RPL9
UGCG
NAMPT
Enriched Genes (p < 0.001, FDR = 1%)
F2 [20 μM]

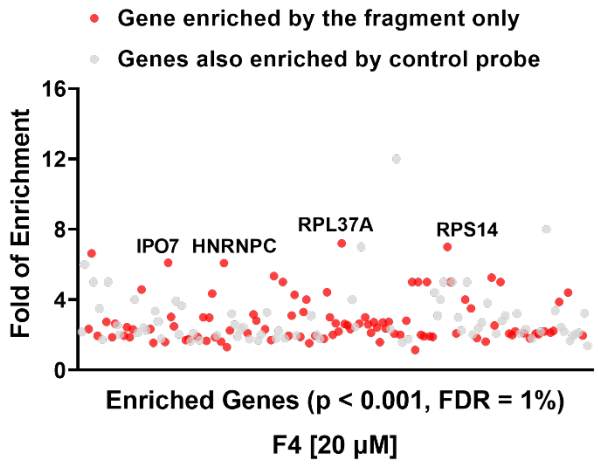


F3


Gene enriched by the fragment only
Genes also enriched by control probe
Fold of Enrichment
16
12
8
4
0
RACK1
EIF3M
COX7A2
Enriched Genes (p < 0.001, FDR = 1%)
F3 [20 μM]



F4


Gene enriched by the fragment only
Genes also enriched by control probe
Fold of Enrichment
16
12
8
4
0
IPO7
HNRNPC
RPL37A
RPS14
Enriched Genes (p < 0.001, FDR = 1%)
F4 [20 μM]

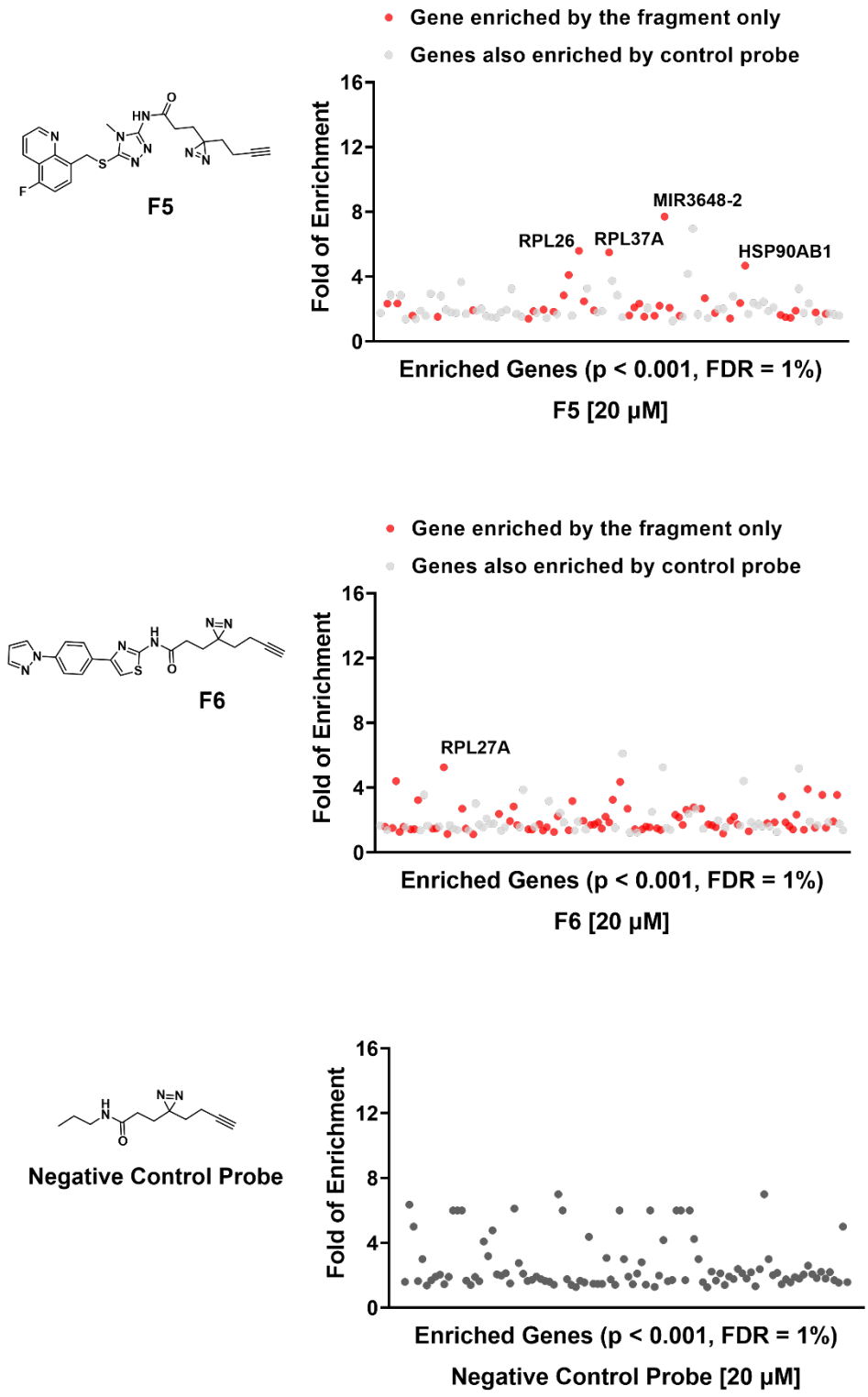
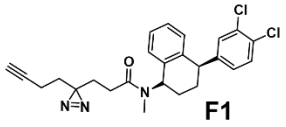
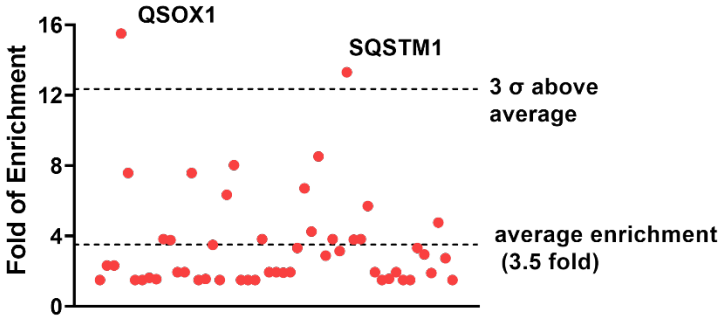


Figure S5. Transcripts enriched by F2 - F6 and the control diazirine probe using Chem-CLIP-seq in MDA-MB-231 cells. Genrich uses a null model with a log-normal distribution to calculate p values by comparing sequencing runs of the same RNA sample before and after pull-down. A minimum read count of 5 and fold enrichment of 1.5 were applied to filter out low-confidence peaks from the RNA-seq analysis. F2 enriched 166 transcripts, including 52 transcripts (31%) that were also enriched by the control probe. F3 enriched 163 transcripts,

including 53 transcripts (32%) that were also enriched by the control probe. F4 enriched 173 transcripts, including 45 transcripts (43%) that were also enriched by the control probe. F5 enriched 92 transcripts, including 57 transcripts (62%) that were also enriched by the control probe. F6 enriched 127 transcripts, including 55 transcripts (43%) that were also enriched by the control probe. The control probe enriched 102 transcripts.

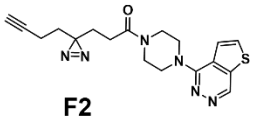


F1

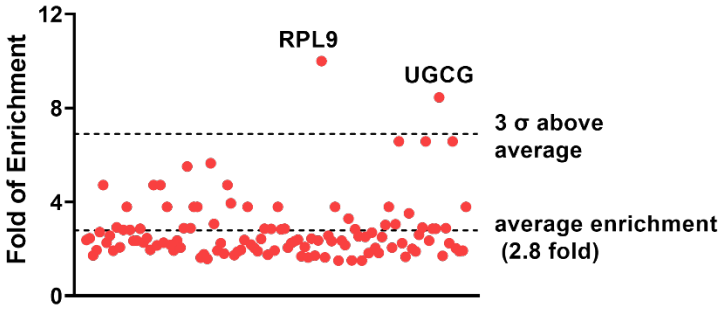


Enriched genes by F1 but not by control probe

(n = 51)

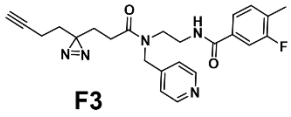


F2

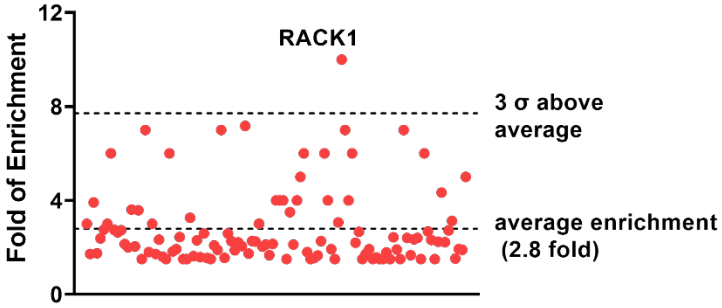


Enriched genes by F2 but not by control probe

(n = 114)



F3



Enriched genes by F3 but not by control probe

(n = 111)

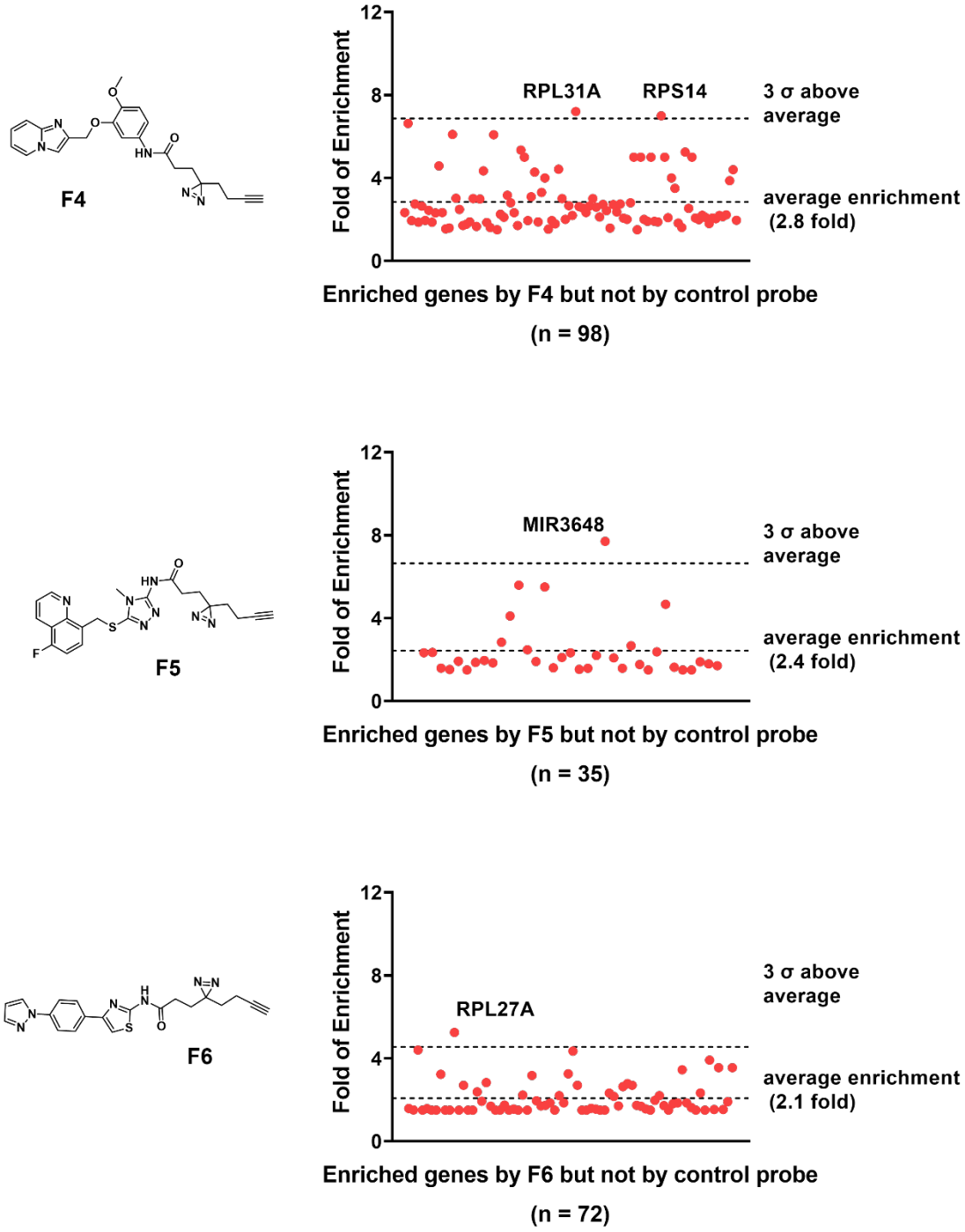


Figure S6. Transcripts enriched by F1 - F6 using Chem-CLIP-seq in MDA-MB-231 cells after excluding genes overlapping with the control diazirine probe.

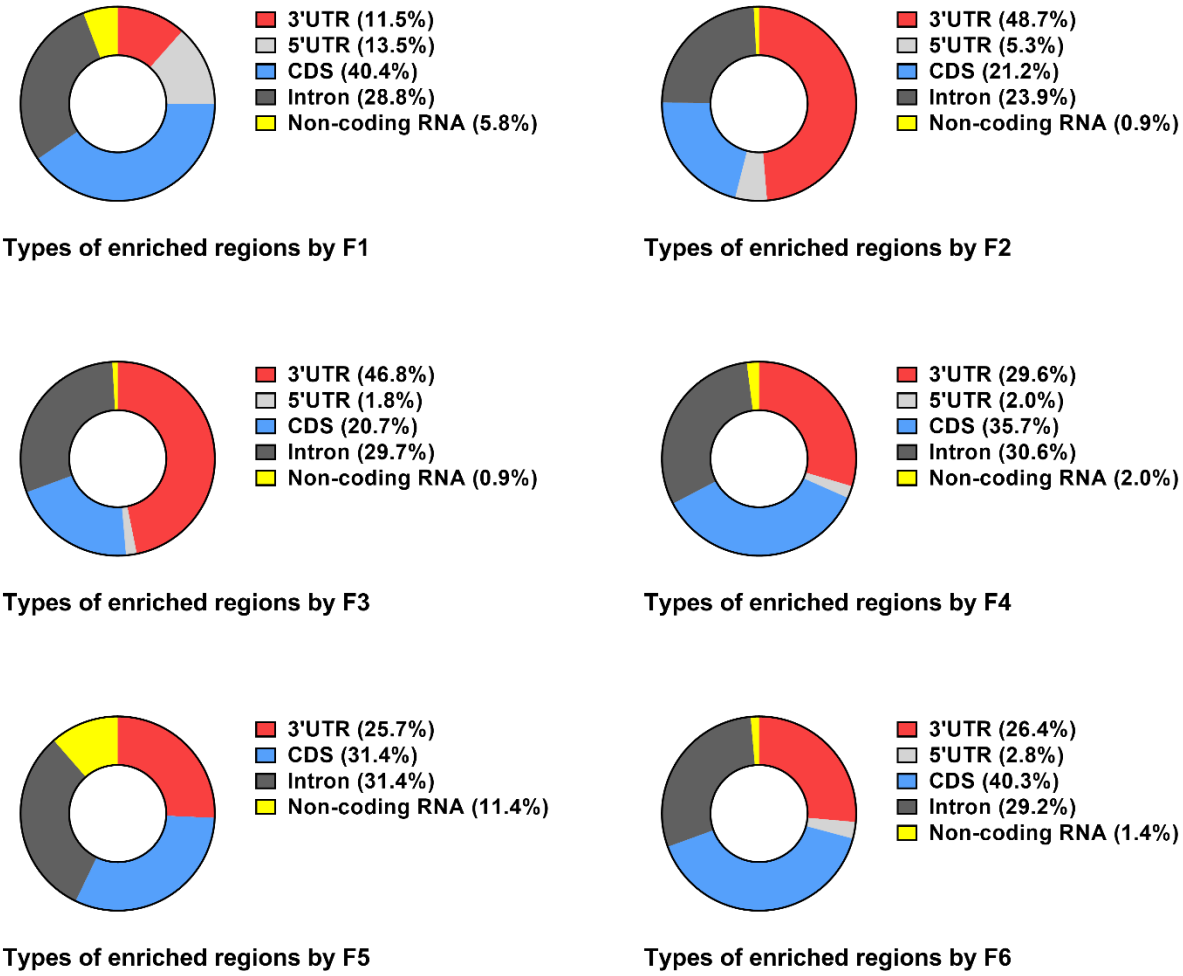


Figure S7. Distribution of binding sites within targeted transcripts by each small molecule. Regions are classified as 5' or 3' untranslated regions (UTRs), coding regions (CDS), introns, or non-coding RNAs.

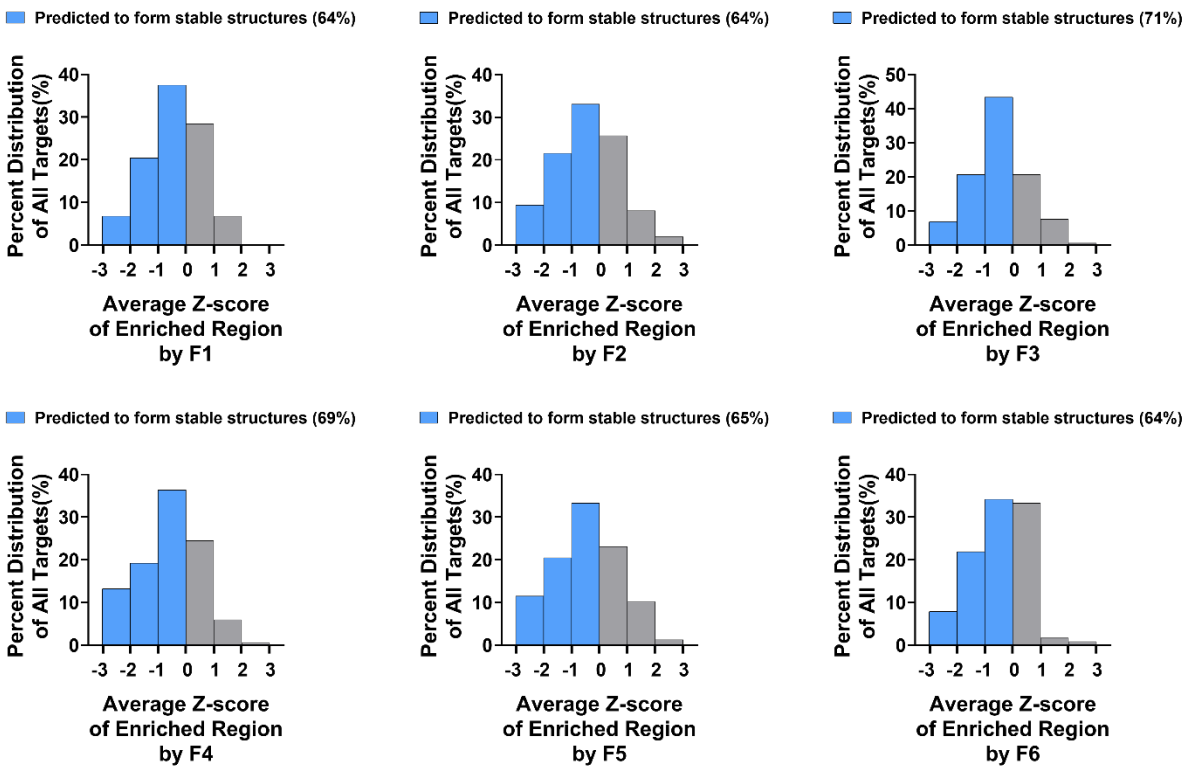


Figure S8. Studying the structures of the regions pulled down by each small molecule by ScanFold. ScanFold predicts the secondary structure of an RNA by using a scanning window. That is, the transcript is fold in 120-nucleotide increments, moving down the sequence in 1 bp increments (step size = 1). The stability of the resultant structures in each window is compared to the average stability of 100 random sequences of the same nucleotide composition. Thus, this program identifies unusually stable structures within an RNA. A Z-score < -1 indicates a structure that is 1 standard deviation more stable than a random sequence (1σ) while a Z-score < -2 indicates a structure that is 2σ.

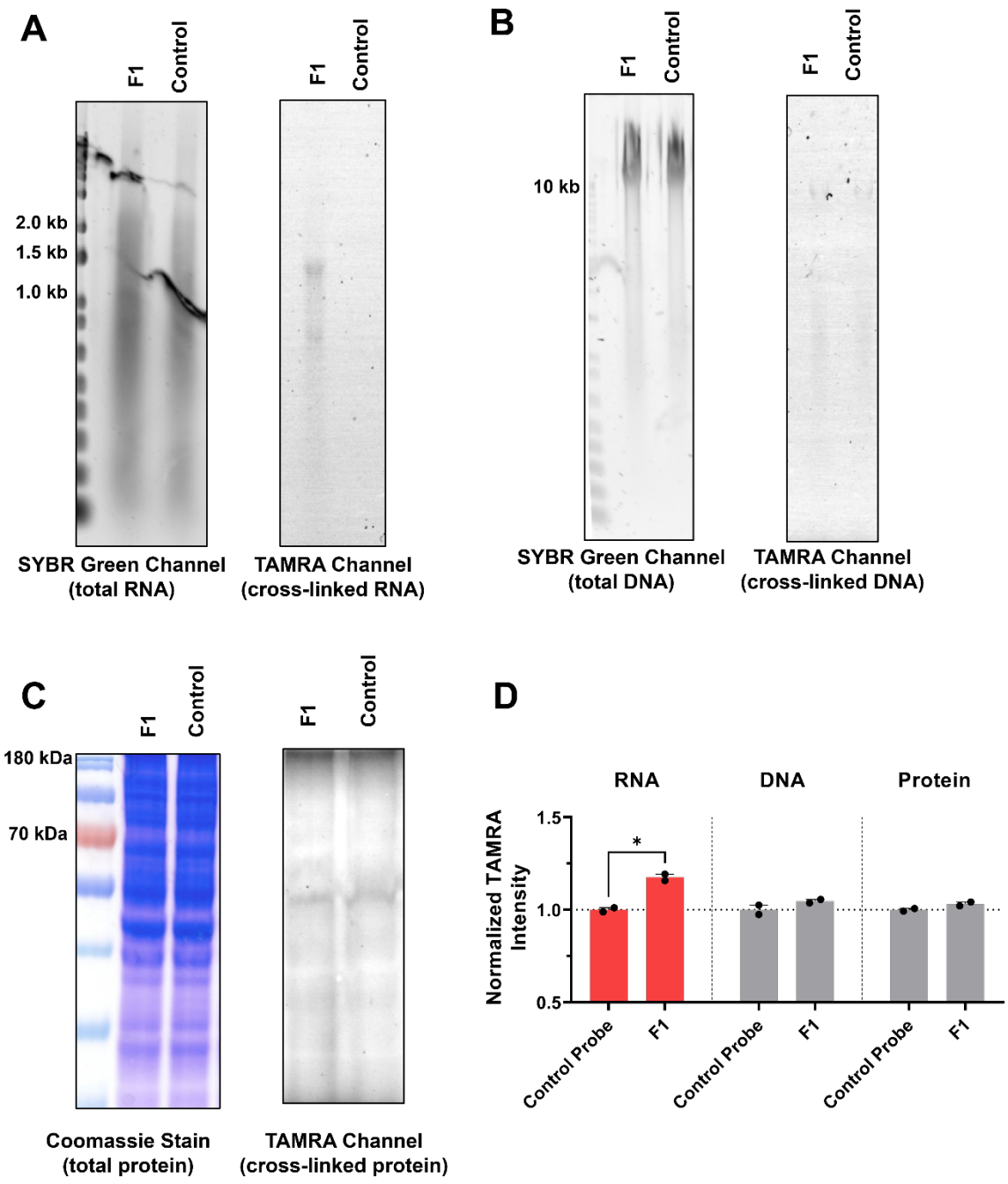
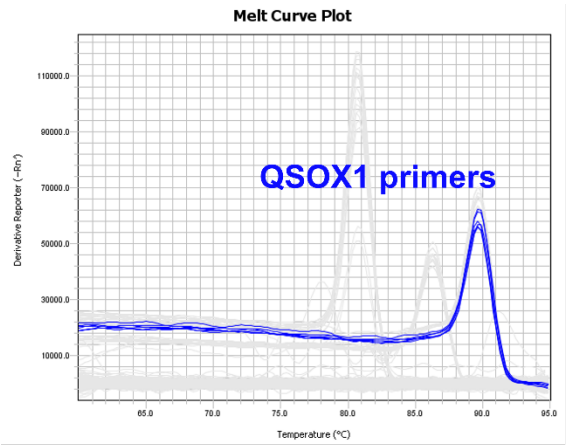
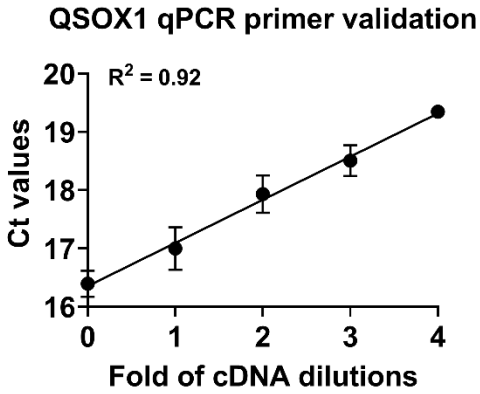
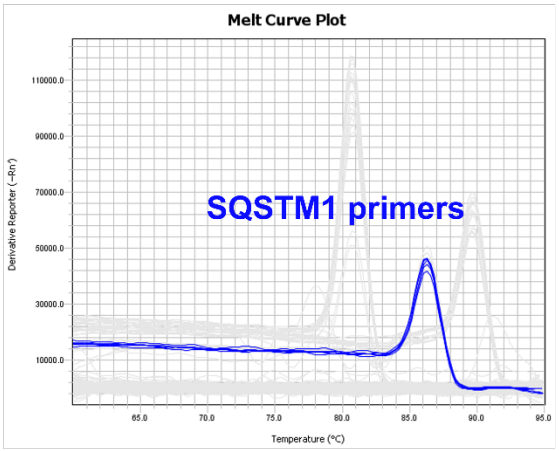
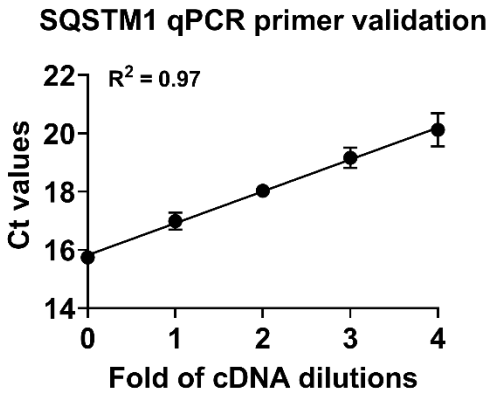


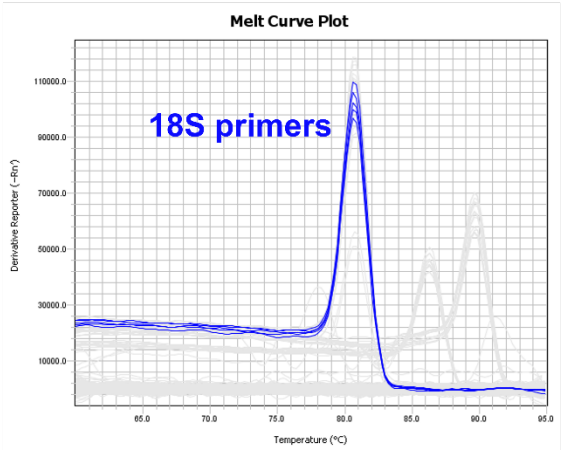
Figure S9. Evaluation of RNA, DNA, and proteins cross-linked by F1 in MDA-MB-231 cells. MDA-MB-231 cells were treated with 20 μM F1 or control probe lacking an RNA-binding module ("Control") for 16 h followed by UV irradiation and isolation of total RNA, DNA, and protein from the same batch of cells. All samples were clicked with TAMRA azide and analyzed by gel electrophoresis. TAMRA imaging was used to identify the crosslinked RNA (panel A), DNA (panel B), and proteins (panel C). SYBR green staining was used visualize total RNA or DNA, and Coomassie staining was used to visualize total proteins. Quantification shown in panel D (n = 2), where the total signal intensity in the "Control" lane was set to 1, and the TAMRA intensity in the F1 lanes as normalized accordingly. *$p < 0.05$ as measured by a Student's t test. Error bars are reported as SD.











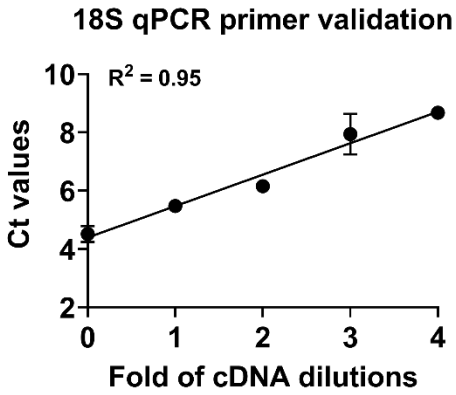


Figure S10. Validation of primers used in qPCR experiments. **Left:** melting curves of qPCR products. **Right:** linear correlations between the qPCR Ct values and the fold of input cDNA dilutions. Each experiment was performed with three biological replicates. Error bars are reported as SD.

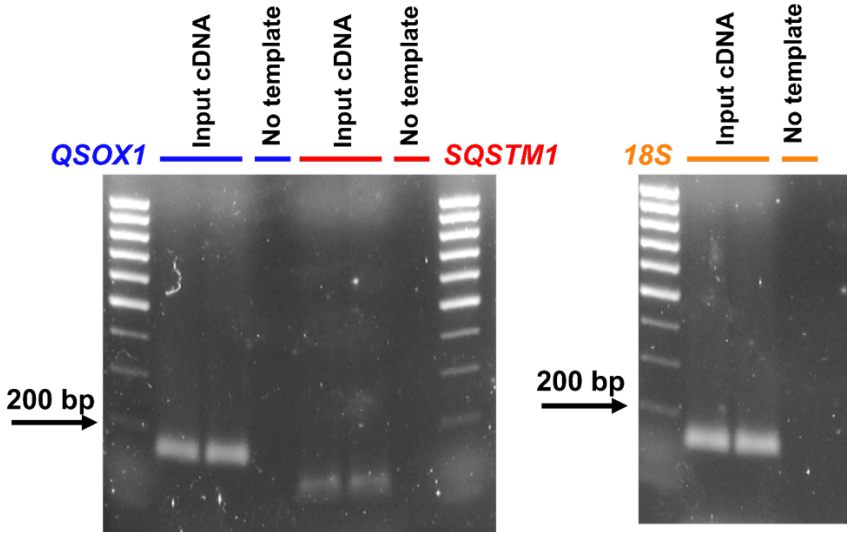


Figure S11. Gel visualization of qPCR products. A single band product with expected size was observed for all primer sets with input cDNA templates. No band was observed from qPCR amplification in the absence of cDNA template.

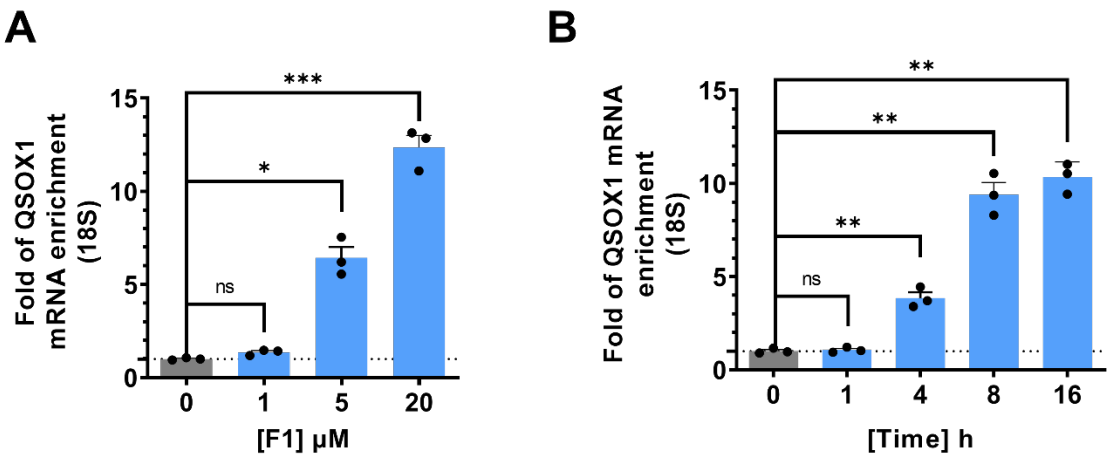


Figure S12. Concentration- and time-dependence of the pull-down of *QSOX1* mRNA by F1. (A) Enrichment of *QSOX1* mRNA levels by F1 at varying concentrations in MDA-MB-231 cells, as measured by RT-qPCR (n = 3). (B) Time course of F1 pull-down of *QSOX1* mRNA in MDA-MB-231 cells, as measured by RT-qPCR (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as measured by a Student's t test. Error bars are reported as SD.

Figure S13. Structural difference in the 5' UTR of the two isoforms of *QSOX1*. The mRNA transcript encoding *QSOX1-a* (75 kDa) contains the 5' UTR sequence that folds into the hairpin (shown on the left) targeted by F1. The red arrow indicates the U-bulge engaged by F1. The mRNA encoding *QSOX1-b* (60 kDa), however, has a truncated 5' UTR sequence that can no longer fold into the same hairpin structure.

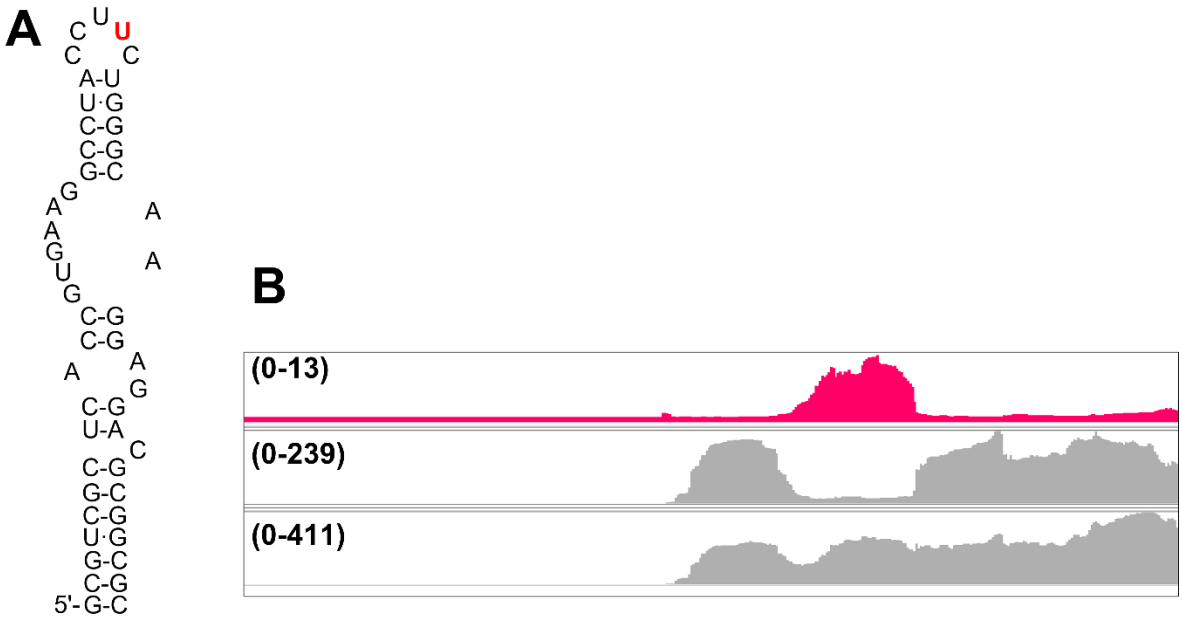


Figure S14. The predicted structure of *SQSTM1* binding site for F1. (A) The secondary structure of enriched sequence as predicted by using ScanFold. The red nucleotide indicates the mapped cross-linking site by F1 in cells. (B) The sequencing tracks showing the enrichment of *SQSTM1* mRNA by F1 in cells. **Top**: the ratio of sequencing reads after vs. before the pulldown. The range reported in the upper left corner indicates the scale of the y-axis, reported as Fold Enrichment; **Middle**: raw sequencing track before pulldown. The range reported in the upper left corner indicates the scale of the y-axis, reported as Read Count); **Bottom**: raw sequencing track after pulldown. The range shown in the upper left corner indicates the scale of the y-axis, reported as Read Count.

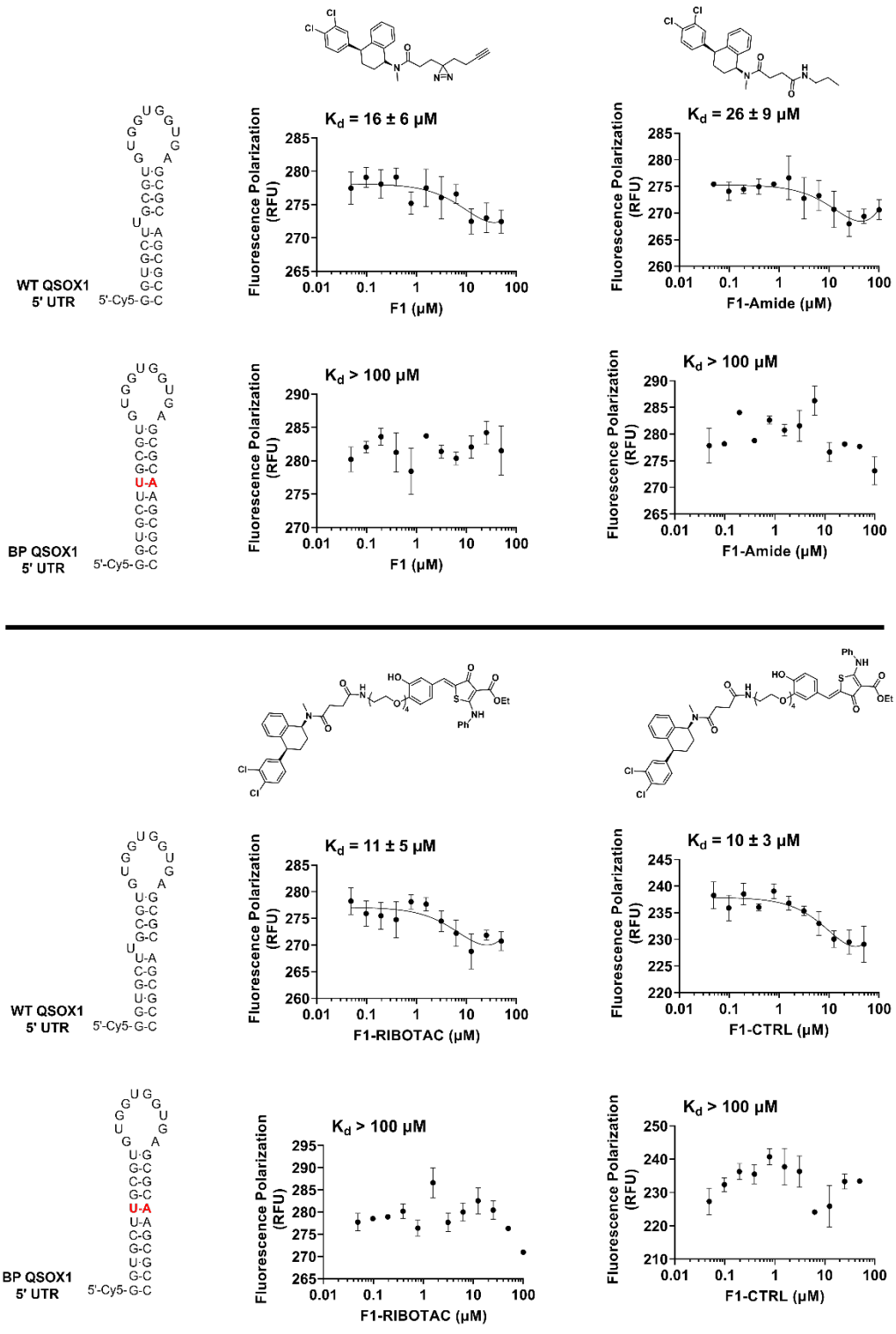


Figure S15. *In vitro* binding of F1 and F1 derivatives to the *QSOX1* 5'UTR hairpin. Cy5-labeled RNA constructs were used to measure changes in fluorescence polarization (Ex. 640 nm, Em. 680 nm) upon incubating with an increasing concentration of small molecule. Each experiment was performed with two independent replicates, and error bars are reported as SD.

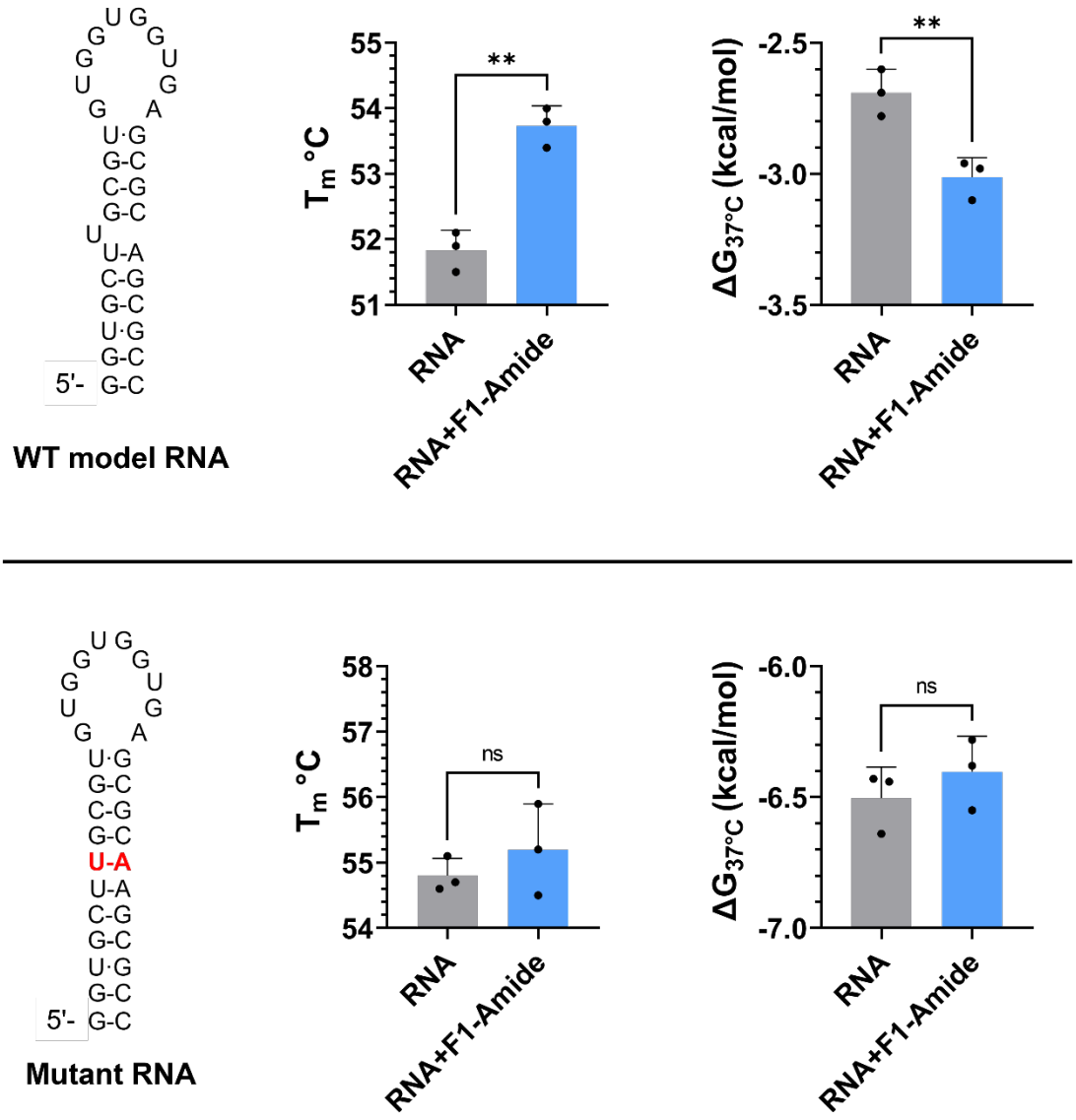


Figure S16. *In vitro* melting experiments with WT and mutant model of the QSOX1-a hairpin structure. Top: Addition of F1-Amide significantly increased the T_m of WT model RNA (from 51.8 to 53.7 °C) and decreased the $\Delta G_{37°C}$ from -2.7 to -3.0 kcal/mol (n = 3). Bottom: No significant effect on the T_m or $\Delta G_{37°C}$ was observed upon additional of F1-Amide to the mutant RNA, which the targeted U-bulge was mutated to the U/A base pair (n = 3). **p < 0.01 as measured by a Student's t test. Error bars are reported as SD.

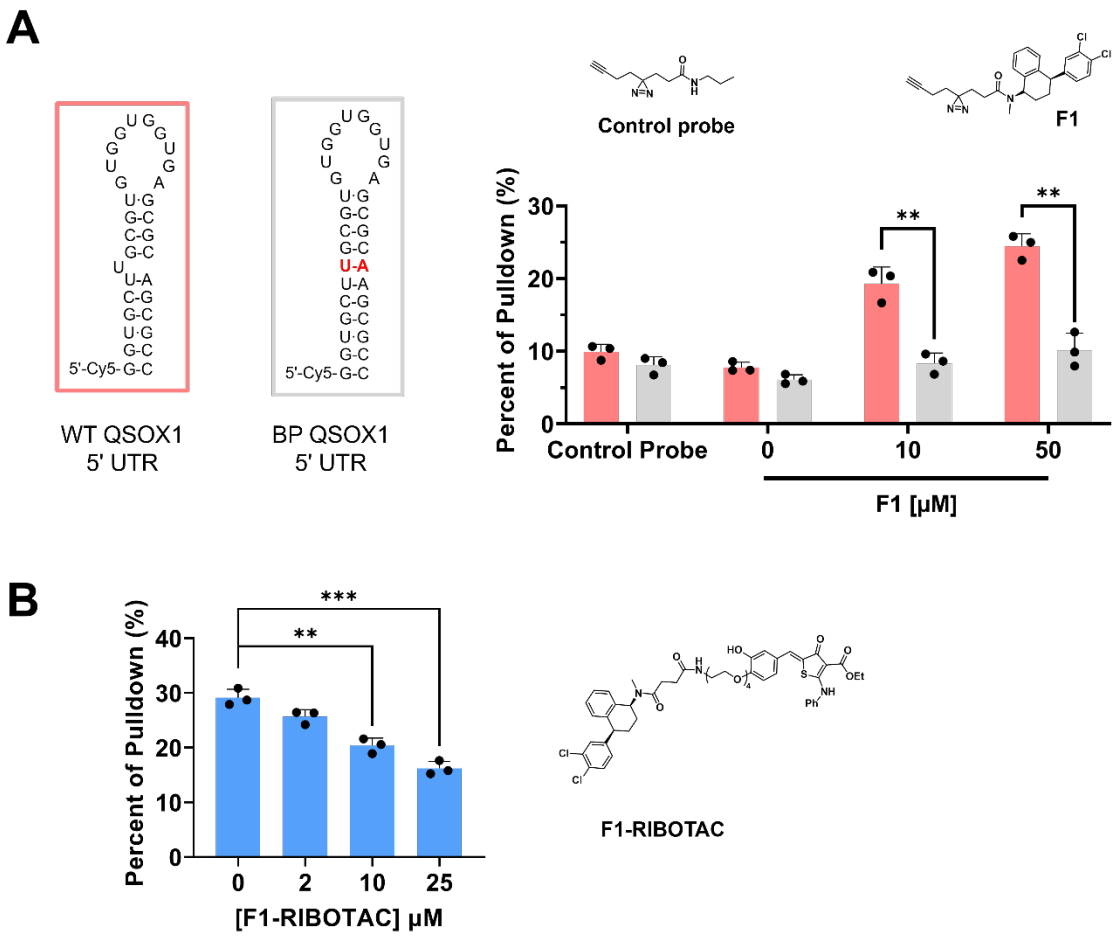


Figure S17. Target validation by *in vitro* Chem-CLIP with F1 and Cy5-labeled RNA constructs. (A) F1 significantly pulled down wild type (WT) QSOX1 5' UTR RNA hairpin but not the base paired (BP) mutant RNA lacking the U-bulge (n = 3). (B) Co-incubation of the QSOX1-a hairpin with an increasing concentration of F1-RIBOTAC and a constant concentration of F1 (20 μM) dose-dependently ablates the cross-linking of F1 to the RNA *in vitro* (n = 3). **p < 0.01, *** p < 0.001 as measured by a Student's t test. Error bars are reported as SD.

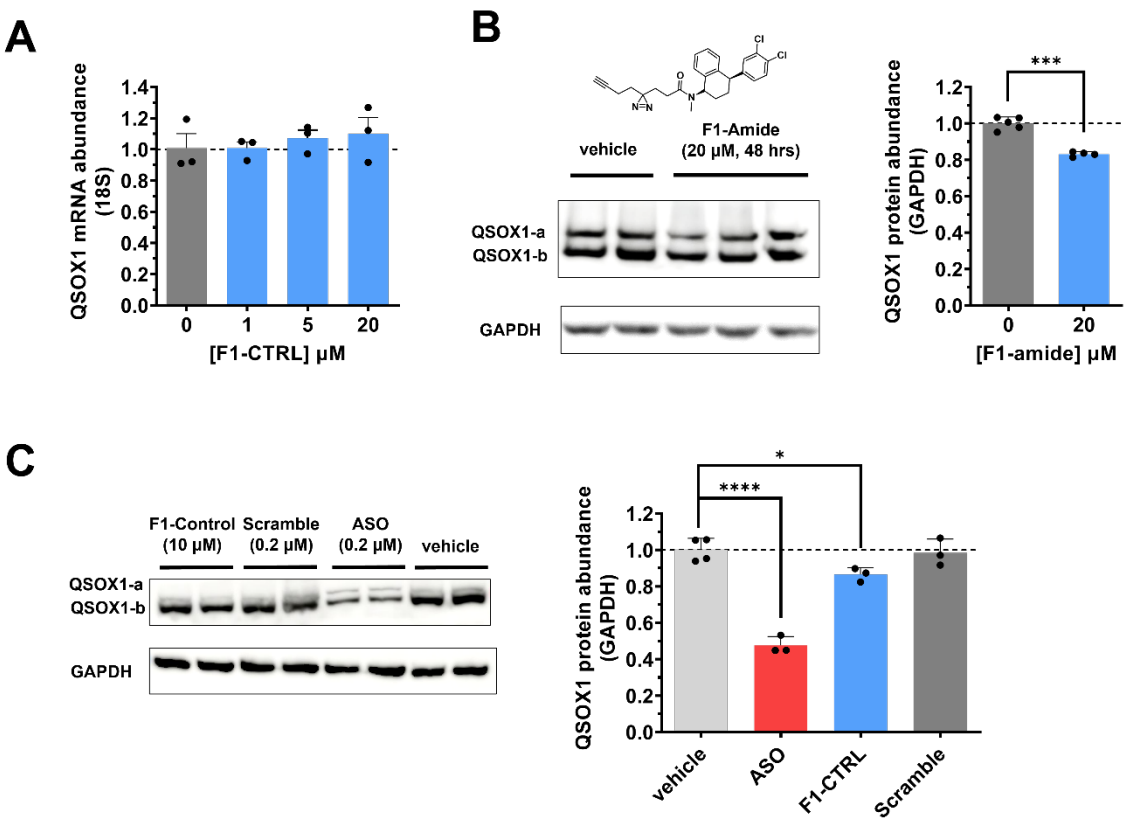


Figure S18. **Effect of F1-CTRL and F1-Amide on *QSOX1* mRNA and protein levels in MDA-MB-231 cells.** (A) Effect of F1-CTRL on *QSOX1* mRNA levels in MDA-MB-231 cells, as measured by RT-qPCR (n = 3). (B) Effect of F1-Amide on QSOX1 protein levels in MDA-MB-231 cells, measured by Western blot (n = 5 for vehicle; n = 4 for F1-Amide). (C) Effect of F1-Control and an antisense oligonucleotide (ASO) on QSOX1 protein levels in MDA-MB-231 cells, measured by Western blot (n = 4 for vehicle; n = 3 for ASO, F1-CTRL, and Scrambled ASO). *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$ as measured by a Student's t test. Error bars are reported as SD.

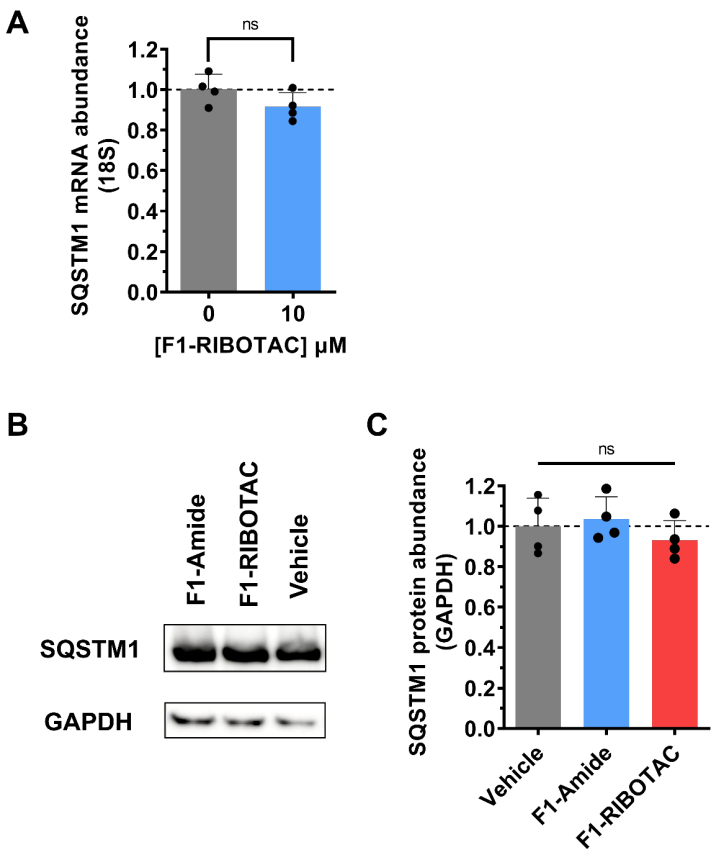


Figure S19. Effect of F1-Amide and F1-RIBOTAC on *SQSTM1* mRNA and protein levels. (A) Effect of F1-RIBOTAC on *SQSTM1* mRNA levels upon treatment of MDA-MB-231 cells for 48 h (n =4), as measured by RT-qPCR. (B) Representative Western blot image measuring the abundance of *SQSTM1* protein levels in MDA-MB-231 cells treated with F1-Amide (20 μM) and F1-RIBOTAC (10 μM) for 48 h. (C) Quantification of the abundance of *SQSTM1* protein levels normalized to *GAPDH* protein levels (n = 4).

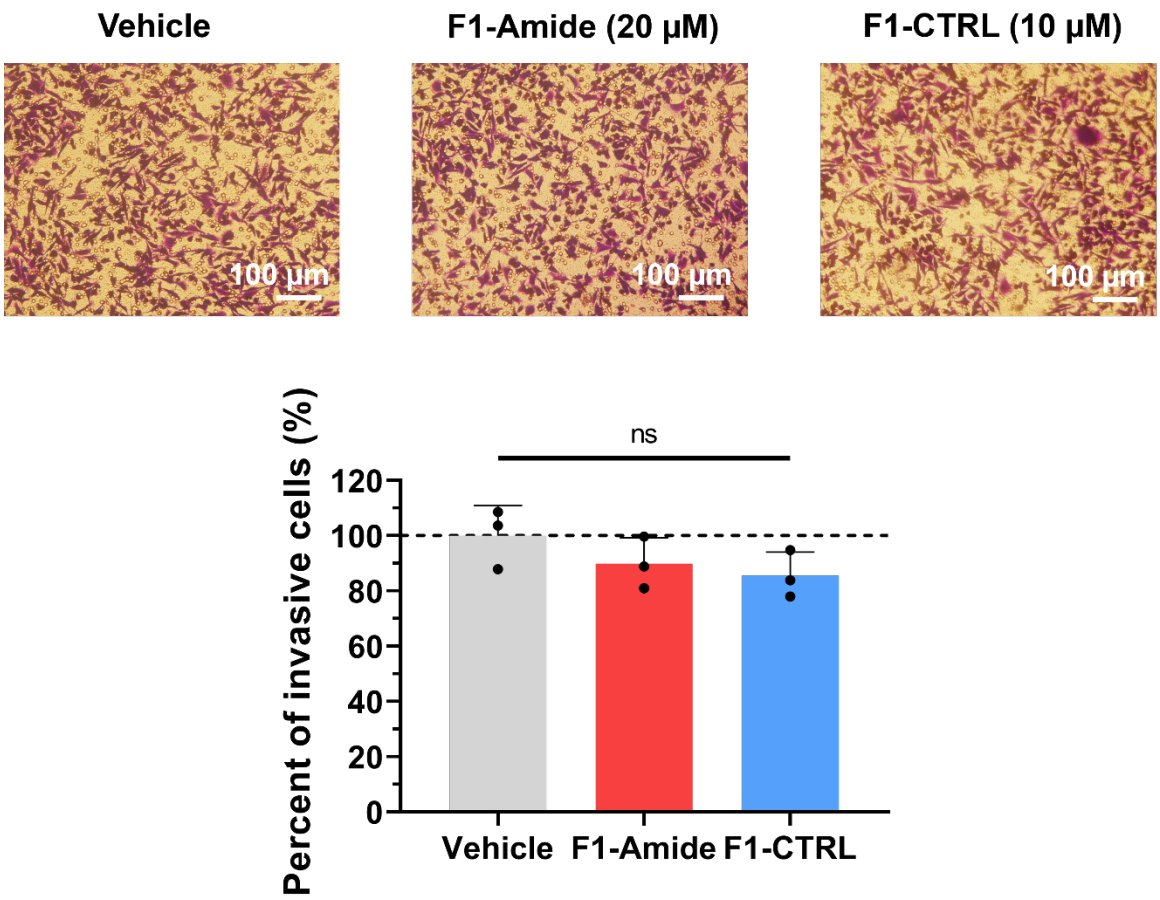


Figure S20. Effect of F1-Amide and F1-CTRL on invasion phenotype of MDA-MB-231 cells. **Top:** representative microscopic images showing invasive cells with the indicated treatment. **Bottom:** quantification of the number of invasive cells upon treatment as indicated for 48 h (n = 3 biological replicates).

MATERIALS & METHODS

General Methods. All DNA and RNA oligonucleotides were obtained from Integrated DNA Technologies (IDT, Inc.) and Dharmacon, respectively. DNA oligonucleotides were used directly. Fluorescently labeled RNAs were deprotected according to the manufacturer's protocol, desalted on PD-10 Desalting Columns (GE Healthcare Life Sciences) per the manufacturer's instructions, and quantified by UV/Vis spectroscopy on a DU800 UV/Vis spectrometer (Beckman Coulter) by measuring absorbance at 260 nm (90°C) and the corresponding extinction coefficient.

***In Vitro* Screening of Functionalized Compounds with Clickable TAMRA Azide.** MDA-MB-231 cells were grown in 100 mm dishes. Upon reaching confluency, total RNA was harvested using a Quick-RNA Miniprep Kit (Zymo; R1054) per manufacturer's protocol. Total RNA (2 µg) was folded by heating to 95 °C in 1× Folding Buffer (8 mM Na_2HPO_4, pH 7.0, 185 mM NaCl, and 1 mM EDTA) for 2 min followed by cooling on ice. This solution was then mixed with the compound of interest at a final concentration of 100 μM, and the sample was incubated at room temperature for 30 min. After incubation, samples were irradiated with UV light (365 nm) for 15 min and then added to a "click reaction mix", composed of TAMRA azide (1 µL, 10 mM; Cat# AZ109, Click Chemistry Tools), $CuSO_4$ (1 µL, 10 mM), THPTA (1 µL, 50 mM; Cat# 1010, Click Chemistry Tools) and sodium ascorbate (1 µL, 250 mM). The samples were incubated at 37 °C for 3 h, and the RNA was purified by ethanol precipitation. The precipitated RNA was dissolved in Nanopure water, and 2 µg RNA was loaded on a 1% (w/v) agarose gel. The agarose gel was then imaged using a Typhoon FLA 9500 variable mode

imager (GE Healthcare Life Sciences). Following imaging of TAMRA fluorescence, the agarose gel was stained with SYBR green and imaged to visualize total RNA.

***In Vitro* Chemical-Crosslinking and Isolation by Pull-down (Chem-CLIP) with Fluorescently Labeled RNA.** Cy5-labeled RNA (100 µL, 2 µM) was folded by heating at 95 °C for 2 min in 1× Folding Buffer and cooled on ice. The RNA was then mixed with F1 at the indicated concentrations. The sample was irradiated with UV light (365 nm) for 15 min and then added to 100 µL of azide-disulfide agarose beads (Click Chemistry Tools, 1238-2) pre-washed with 200 µL of 25 mM HEPES, pH 7.0. A click reaction solution was prepared by mixing 30 µL of 250 mM sodium ascorbate, 30 µL of 10 mM $CuSO_4$, and 30 µL of 50 mM THPTA, which was added to the sample. The sample was incubated while rotating at 37 °C for 2 h, briefly centrifuged, and then the supernatant was decanted. The beads were washed six times with 1× Washing Buffer (10 mM Tris-HCl, pH 7, 4 M NaCl, 1 mM EDTA, and 0.05% (v/v) Tween-20) following by incubation with 1× Releasing Buffer (50 mM TCEP and 100 mM K_2CO_3) at 37 °C for 30 min. An equivalent of iodoacetamide (200 mM) was added to the sample, which was incubated at 37 °C for an additional 30 min. The sample was briefly centrifuged and supernatant containing RNA was carefully transferred to a clean tube. Cy-5 fluorescence in the supernatant was measured with a Molecular Devices SpectraMax M5 plate reader with an excitation wavelength of 640 nm and an emission wavelength of 680 nm. For competitive Chem-CLIP, F1-RIBOTAC was added to the folded the RNA and incubated at room temperature for 15 min prior to the addition of F1. Pull-down was then completed as described above.

***In Vitro* Binding by Measuring Changes in Fluorescence Polarization.** Cy5-labeled RNA was folded by heating at 95 °C for 2 min in 1× Folding Buffer and cooled on ice. Serial dilutions of F1 (200 to 0.4 μM, 1:1 dilutions) were prepared in 1× Folding Buffer with 4% (w/v) bovine serum albumin (BSA) with constant DMSO concentration (2% v/v). The compound solutions (10 μL) were added to a well of non-binding 384-well plate (Corning). An equal volume of folded RNA was adding to each well with a final concentration of 50 nM. The samples were incubated at room temperature for 30 min, and then fluorescence polarization was measured with a Molecular Devices SpectraMax M5 plate reader with an excitation wavelength of 640 nm and an emission wavelength of 680 nm.

Optical Melting Experiments. The thermal stability of WT and mutant model RNAs in the presence and absence of **F1-Amide** was measured by optical melting. The RNA (1 μM) was prepared in 1× Melting Buffer (8 mM Na_2HPO_4, pH 7, 10 mM NaCl, and 1 mM EDTA) and heated to 95 °C followed by cooling on ice. F1-Amide was added to the final concentration of 2 μM; an equal volume of DMSO was added to the vehicle sample. The absorbance of the solution at 260 nm was measured by a Beckman Coulter DU800 spectrophotometer as a function of temperature, from 12 °C to 85 °C at a rate of 1 °C/min. The background absorbance of buffer with DMSO or compound was subtracted before fitting the curve with MeltWin (v3.5)[6] to determine the melting temperature (T_m) and the Gibbs free energy ($\Delta G_{37°C}$).

Cell Culture. Cellular experiments were carried out in MDA-MB-231 cells (ATCC, HTB-26) ,

cultured at 37°C with 5% CO_2 in 1× DMEM (Dulbecco's Modified Eagles Medium; Sigma Aldrich) supplemented with 10% (v/v) fetal bovine serum (FBS; Sigma Aldrich), 2 mM L-alanyl-L-glutamine (Glutagro; Corning), 1× Penicillin/Streptomycin (Corning). Cells were used at a maximum passage number of 20 and checked for mycoplasma contamination (PromoKine, PK-CA91-1024) before performing experiments. Unless stated otherwise, compound treatment was performed by replacing the growth medium with fresh medium that includes compounds at the treatment concentration with 0.1% DMSO (v/v). Antisense oligonucleotides (ASO) were transfected by using RNAiMax (Thermo Fisher) per manufacturer's protocol.

Construction of RNase L Kock Out (KO) MDA-MB-231 cells

The CRISPR-edited MDA-MB-231 cell lines used in this study were the same as those previously reported and characterized.[7] Briefly, lentiviral constructs containing Cas9 and gRNA targeting RNase L mRNA (or a scramble control) were purchased (Transomic Tech) and transfected in HEK293T cells (ATCC CRL-11268) to harvest virus. Transduction to MDA-MB-231 cells were performed in the presence of 6 μg/mL polybrene (Millipore, Cat# TR-1003-G) followed by selection with puromycin.

Cross-linking to RNA, DNA, and Proteins in Live Cells with Clickable TAMRA Azide. MDA-MB-231 cells were seeded in 100 mm dishes and allowed to reach ~80% confluency. The cells were then treated with 20 μM of compound in growth medium and incubated for 16 h. Cells were washed with 1× DPBS and irradiated with UV light for 10 min. Total RNA was

harvested using a Zymo Quick-RNA Miniprep Kit per the manufacturer's protocol with DNase and proteinase treatment. Total DNA was harvested using a Zymo Quick-DNA Miniprep Kit per the manufacturer's protocol with RNase and proteinase treatment. Total protein was harvested by using Mammalian Protein Extraction Reagent (M-PER, Thermo Scientific) per the manufacturer's protocol with RNase and DNase treatment. Each sample (4 μg for RNA or DNA, 25 μg for proteins) was then incubated in a "click reaction mixture" with TAMRA azide as described in ***In Vitro*** **Screening of Functionalized Compounds with Clickable TAMRA Azide.** DNA and RNA samples were separated by using a 1.0% (w/v) and 1.5% (w/v) agarose gel in TBE buffer, respectively. Protein samples were loaded to 10% SDS-polyacrylamide gel. All samples were first imaged with TAMRA channel by using a Typhoon FLA 9500 variable mode imager (GE Healthcare Life Sciences). RNA and DNA were then visualized by SYBR green staining, and total proteins were visualized by a Coomassie Brilliant Blue staining (Bio-Rad) per manufacturer's protocols.

Chemical-Cross-linking and Isolation by Pull-down (Chem-CLIP) in Live Cells. MDA-MB-231 cells were seeded in 60 mm dishes and allowed to reach ~80% confluency. The cells were then treated with 20 μM of compound in growth medium and incubated for 16 h. Cells were washed with 1× DPBS and irradiated with UV light for 10 min. Total RNA was harvested using a Zymo Quick-RNA Miniprep Kit per the manufacturer's protocol with DNase treatment. Total RNA was chemically fragmented by using an NEBNext Magnesium RNA Fragmentation Module (E6150S) per manufacturer's protocol to achieve final lengths between 100 – 150 nucleotides. Pull-down of cross-linked RNAs was performed by adding 10 μg of

total RNA to 100 µL of azide-disulfide agarose beads (Click Chemistry Tools, 1238-2) pre-washed with 25 mM HEPES, pH 7.0. The click reaction and pull-down were completed as described in "***In Vitro* Chemical-Crosslinking and Isolation by Pull-down (Chem-CLIP) with Fluorescently Labeled RNA**". The pulled down RNA was purified by RNA CleanXP beads (Beckman, A66514) per manufacturer's protocol.

For dose response studies, cells were treated with varying concentration of F1 (1, 5, 20 µM) as described above for 16 h. For time course studies, cells were treated with 20 µM of for 1, 4, 8, or 16 h. With the exception of compound concentration or time at which total RNA was harvested, the remaining steps of the protocol were completed as described above for both concentration- and time-dependent studies.

RNA-seq Library Preparation and Data Analysis. The quality of the pulled down RNA was analyzed by using an Agilent 2100 Bioanalyzer RNA nanochip as well as to confirm fragment lengths. RNA concentration was quantified by Qubit 2.0 Fluorometer (Invitrogen). The input RNA (200 ng) was depleted of ribosomal RNA with NEBNext rRNA Depletion Module (E6310) according to manufacturer's recommendations. Library preparation was performed with NEBNext Ultra II Directional RNA kit (E7760) per manufacturer's protocols. Briefly, the rRNA-depleted samples were reverse transcribed with random hexamer primers to generate first strand cDNA, followed by second strand synthesis with dUTP instead of dTTP. The cDNA was end repaired and adenylated at their 3' ends, followed by adaptor ligation. The strand information of the RNA was preserved by using USER enzyme (Uracil-specific excision reagent) to degrade the second strand. The cDNA was then PCR amplified with barcoded

Illumina-compatible primers to generate the final libraries. These libraries were loaded into a NextSeq 500 v2.5 flow cell and sequenced with 2 x 40bp paired-end chemistry. All fastq files were aligned to the human genome by STAR.[8] The output bam files were processed by Genrich (v0.6.1, available at https://github.com/jsh58/Genrich) for peak calling to identify regions of enrichment ($p < 0.001$ and False Discovery Rate = 1%). Fold enrichment was calculated by Deeptool[9] and visualized by IGV browser.[10] A minimum read count of 5 and a minimum fold enrichment of 1.5 were applied filter to filter out low-confidence peaks.

Proteomic Sample Preparation and Analysis. MDA-MB-231 cells were seeded in 60 mm dishes and allowed to reach ~80% confluency. The cells were then treated with 20 μM of compound in growth medium and incubated for 16 h. Cells were washed with 1× DPBS and irradiated with UV light for 10 min. For each condition, two biological replicates were prepared, and each biological replicate was split into three technical replicates. Cells were then homogenized by sonication and resuspended in 1× DPBS (0.35 mL), followed by incubation with biotin azide (50 μM), tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (1 mM), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (100 μM), and copper(II) sulfate (1 mM) at room temperature for 1 h. Proteins were isolated by adding 1.4 mL of MeOH, 0.35 mL of chloroform, and 1.05 mL of water, followed by centrifugation at 14,000 × g at 4 °C for 5 min. Total proteins were resuspended in 2% (w/v) SDS in 1× PBS via sonication, followed by centrifugation at 4,700 × g at 4 °C for 5 min. The supernatant was transferred to a new tube and diluted with 1× PBS to afford a final SDS concentration as 0.2% (w/v). Streptavidin agarose beads (ProteoChem) were added for pull-down of cross-linked proteins, and the sample was incubated

at room temperature for 4 h, followed by washing with 1% SDS in 1× PBS (1 × 10 mL), 1× PBS (3 × 10 mL), and finally water (3 × 10 mL).

Pulled-down proteins were released from beads by resuspending in 6 M urea/1× PBS (0.5 mL) supplemented with 10 mM of TCEP at room temperature for 30 min. Iodoacetamide (25 mM final concentration) was added to the mixture, followed by incubation at room temperature for another 30 min in the dark. The beads were then diluted with 0.7 mL of 1× PBS, pelleted by centrifugation (1,400 × g, 2 mins), and resuspended in 150 μL of digestion solution (2 M urea, 1 mM $CaCl_2$, 3 μg/mL trypsin, pH = 8) overnight at 37 °C. To the samples was added 1 volume of isopropanol containing 1% (v/v) trifluoroacetic acid, followed by a styrenedivinylbenzene reverse-phase sulfonate (SDB-RPS) StageTip.[11] Peptides were resuspended in 0.1% (v/v) formic acid (FA) in water and analyzed using Proxeon EASY-nLC 1200 nano-UHPLC coupled to QExactive HF-X Quadrupole-Orbitrap mass spectrometer (Thermo Scientific). The chromatography column consisted of a 40 cm long, 75 μm microcapillary capped by a 5 μm tip and packed with ReproSil-Pur 120 C18-AQ 2.4 μm beads (Dr. Maisch GmbH). Peptides were eluted into the mass spectrometer at a flow rate of 300 nL/min with 0.1% FA in H2O (Buffer A) and 0.1% FA in MeCN (Buffer B) over a 90-minute linear gradient (5-35% Buffer B) at 50 °C. Data were acquired in data-dependent mode (top-20, NCE 28, R = 15,000) after full MS scan (R = 60,000, m/z 400-1,300). Dynamic exclusion was set to 30 s and isotope exclusion was enabled.

The MS data were analyzed with MaxQuant[12] (V1.6.1.0) and searched against the human proteome (Uniprot) and a common list of contaminants included in MaxQuant. The first peptide search tolerance was set at 20 ppm, and 10 ppm was used for the main peptide search. The fragment mass tolerance was set to 0.02 Da. The false discovery rate for peptides, proteins, and

sites identification was set to 1%. The minimum peptide length was set to six amino acids and peptide re-quantification was enabled with the minimal number of peptides per protein set to 2. Methionine oxidation and protein N-terminal acetylation were searched as variable modifications, and carbamidomethylation of cysteines was searched as a fixed modification.

Analysis of mRNA Abundance by RT-qPCR. Unless stated otherwise, all RT-qPCR experiments were performed to measure nascent RNA levels by using Click-it Nascent RNA Capture Kit (Cat# C10365, Thermo Fisher) per the manufacturer's protocols. Briefly, cells were treated with 0.2 mM of ethynyl uridine (EU) in growth medium for 16 h, after which the medium was replaced with fresh growth medium lacking EU. Compound or ASO treatment was performed at the same time of initial EU treatment, and compounds were replenished after replacing the medium to remove EU. Total RNA was extracted by using a Zymo Quick-RNA Miniprep Kit as per manufacturer's protocol with DNase treatment. The click reaction and pull-down of nascent RNA were performed as described in "***In Vitro* Chemical-Crosslinking and Isolation by Pull-down (Chem-CLIP) with Fluorescently Labeled RNA**". Reverse transcription (RT) was performed by using 300 ng total RNA and a QScript cDNA synthesis kit (QuantaBio) per manufacturer's protocols. qPCR was performed in 384-well plates by using Power SYBR Green Master Mix (Life Technologies) on a QuantStudio5TM Cycler (Applied Biosystems) using the "Comparative Ct with Melt" method.

Measuring Protein Abundance by Western Blotting. MDA-MB-231 cells were seeded in 6-well plates at ~50% confluency and treated with vehicle (DMSO) or compound as described

above but without EU treatment. After 16 h, the compound-containing growth medium was removed and replaced with fresh medium containing compound (re-dosing). After an additional 32 h incubation, total protein was harvested (48 h total treatment time). The cells were then washed twice with cold 1× DPBS, and total protein was harvested by using Mammalian Protein Extraction Reagent (M-PER, Thermo Scientific) per the manufacturer's protocol. Protein concentration was quantified by using BCA Protein Assay Kit (Pierce), and the proteins were separated by using 10% SDS-polyacrylamide gel (20 µg protein per lane). After gel electrophoresis in 1× Running Buffer (50 mM MOPS, 50 mM Tris-base, pH 7.5, 1 mM EDTA, and 0.1% (w/v) SDS) at 120 V for 1 h, the proteins were transferred to a PVDF membrane (0.45 μm, Cytiva) at 300 mA for 1 h in 1× Transfer Buffer (25 mM Bicine, 25 mM Tris-base, pH 7.5, 1:4 MeOH:H_2O). The membrane was blocked with 5% (w/v) non-fat dry milk in TBST (1× TBS containing 0.1% (v/v) Tween-20) for 1 h at room temperature. The primary antibody for QSOX1 protein (Cat# 10092-932, Proteintech) or SQSTM1 protein (Cat# ab109012, Abcam) was incubated with 1:1000 dilutions at 4 °C for 16 h. The membrane was then washed by TBST four times (5 min each) and then incubated with the secondary antibody (Cat# 7074S, Cell Signaling) with 1:5000 dilution at room temperature for 2 h. The membrane was washed with TBST four times (5 min each) and imaged by using a SuperSignal West Pico Chemiluminescent Substrate (Pierce) per manufacturer's protocol. The blot was stripped by washing the membrane in 1× Stripping Buffer (200 mM glycine, pH 2.2, 4 mM SDS, 1% (v/v) Tween 20) at room temperature for 30 min, followed by blocking again as described above. The antibody for GAPDH protein (Cat# 97166, Cell Signaling) was then applied with 1:2000 dilutions followed by the same procedure to image as described above. Expression

levels of proteins were quantified based on band intensity by using ImageJ. QSOX1 and SQSTM1 signals were normalized to GAPDH signal for each sample.

Invasion Assay. MDA-MB-231 cells were seeded in 6 mm dishes and treated with compound or transfected by ASO for 48 h as described in **Cell Culture**. After treatment for 48 h, the medium was replaced with fresh medium that contains same concentration of compound but lacking FBS for 8 h (serum starvation). ThinCert (GBO) 24-well inserts with 8 μm pores were coated with 100 μL of 0.5 mg/mL Matrigel (Fisher Scientific: CB40234) per well, and then cells were added into the insert (50,000 cells per well). Fresh growth medium with FBS was added to a 24-well plate (600 μL each well), and the inserts containing cells were placed on the top, allowing medium to cover the bottom of the insert. After 16 h, all medium was removed, and cells were washed by 1× DPBS twice. Cells were then fixed with 3% (w/v) paraformaldehyde (PFA) in 1× DPBS at room temperature for 30 min, followed by staining with Crystal Violet (10 mg/mL; 4:1 H_2O:MeOH) at room temperature for 20 min. A cotton swab was used to gently remove non-invasive cells on sitting on top of the Matrigel. The inserts were then imaged under a regular optical microscope (3 views per well), and the number of invasive cells were counted manually.

Proliferation Assay. MDA-MB-231 cells were seeded in 96-well plates (Corning) at ~40% confluency and treated with compound or transfected with ASO as described in **Cell Culture**. Compound was replenished every 16 h by replacing all the growth medium with freshly prepared medium containing same concentration of compounds. After 48 h total treatment

period, proliferation was measured by using CellTiter 96 AQueous One Solution Cell Proliferation Assay (Cat# G3582, Promega) per manufacturer's protocol.

Synthetic Methods and Characterization

Abbreviations:

DCM: Dichloromethane

DIPEA: Diisopropyl ethyl amine

DMF: *N, N*-Dimethylformamide

DMSO: Dimethyl sulfoxide

EtOAc: Ethyl acetate

HATU: Hexafluorophosphate azabenzotriazole tetramethyl uronium

HOAt, 3-hydroxytriazolo[4,5-*b*]pyridine

HPLC: High-performance liquid chromatography

MeOH: Methanol

TFA: Trifluoro acetic acid

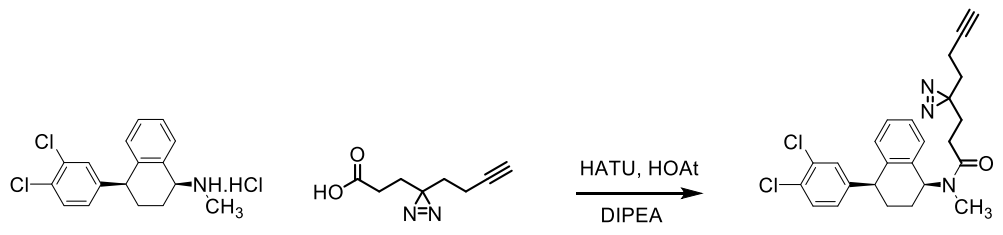
$POCl_3$: Phosphoryl chloride

General Synthetic Methods. Amine derivatives were purchased from Enamine. 3-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)propanoic acid was purchased from Sigma Aldrich. *N*, *N*-dimethylformamide (DMF, anhydrous) was purchased from EMD and used without further purification. Claricep S-series pre-packed silica columns were purchased from Agela-Technologies. Flash chromatography was carried out on the Biotage Isolera One automated flash chromatography system with pre-packed Claricep S-series (40-60 μm) normal-phase flash columns of various sizes. ^{1}H NMR spectra were collected on a Bruker 400 MHz NMR spectrometer; ^{13}C NMR spectra were collected on either Bruker 400 MHz NMR spectrometer

or Bruker 600 MHz NMR spectrometer.

Preparative HPLC was performed using a Waters 1525 Binary HPLC pump equipped with a Waters 2487 dual absorbance detector system and a Waters Sunfire C18 OBD 5 μm, 19 ×150 mm S-14 column. Absorbance was monitored at 254 and 345 nm. A linear gradient with a flowrate of 5 mL/min from 0-100% methanol in water with 0.1% (v/v) TFA over 100 min was used for small molecule purification. Purity was assessed by analytical HPLC using a Waters Symmetry C18 5 μm, 4.6 × 150 mm column with a flow rate of 1 mL/min and a linear gradient from 0-100% methanol in water with 0.1% (v/v) TFA over 60 min. Absorbance was monitored at 254 and 345 nm. Mass spectra were recorded on an Applied Biosystems 4800 plus MALDI-TOF/TOF analyzer using an α-cyano-4-hydroxycinnamic acid matrix.

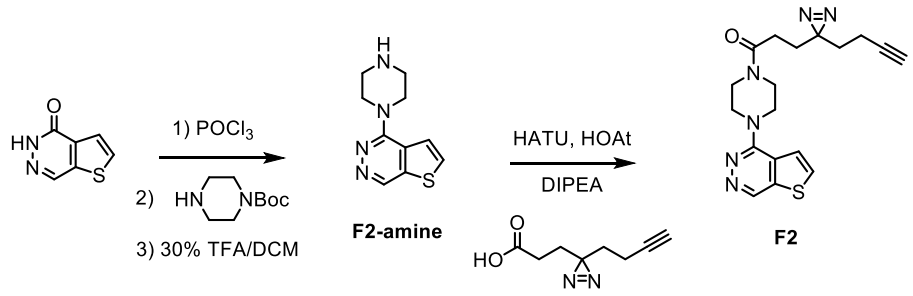
General Procedure for Diazirine Probe Synthesis and Purification: The following were added to a vial containing 1 mL DMF: 3-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)propanoic acid (1.5 equiv.), HATU (2 equiv.), HOAt (2 equiv.) and DIPEA (3 equiv.), and the reaction was stirred for 10 min at room temperature. After 10 min, an amine (1.0 equiv.) was added, and the sample was stirred at temperature for 12 h. Compounds were purified by flash chromatography or by HPLC as described in **General Synthetic Methods**.



F1

Scheme 1: Compound **F1** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

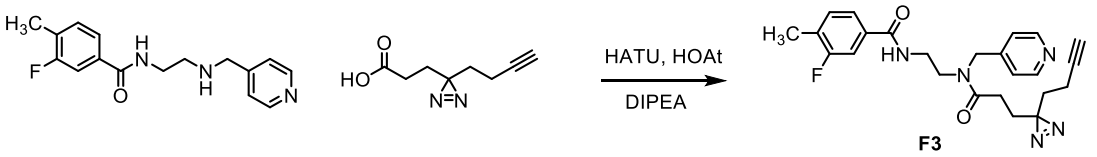
Characterization of F1. **F1** was obtained in 78% yield (15.5 mg, 34.1 μmol). ^{1}H NMR, $CDCl_3$ (400 MHz) δ (ppm): 1.68-1.73 (t, 4H, CH_2), 1.95-2.0 (m, 3H, CH, CH_2), 2.05-2.08 (m, 3H,CH_2), 2.11-2.24 (m, 3H, CH_2), 2.70 (s, 3H, CH_3), 4.20-4.24 (m, 1H, CH), 5.91-5.94 (m, 1H, CH), 6.77-6.85 (m, 1H, ArH), 6.96-7.01 (m, 1H. ArH), 7.08-7.14 (m, 2H, ArH), 7.17-7.20 (t, 1H, ArH), 7.22-7.27 (m, 1H, ArH), 7.33-7.34 (m, 1H, ArH). ^{13}C NMR, $CDCl_3$ (600 MHz) δ (ppm) = 13.39, 21.53, 22.66, 27.64, 27.81, 28.09, 30.17, 30.90, 32.74, 42.76, 43.01, 52.72, 69.34, 82.82, 126.64, 127.23, 127.41, 127.54, 128.01, 130.11, 130.61,130.87, 131.15, 132.30, 135.98, 138.29, 147.03, 171.95. HR-MS: Calculated for $[C_{25}H_{26}Cl_2N_3O]^+$, 454.1453; found 454.1450.



Scheme 2: Scheme for the synthesis of **F2**.

Synthesis of F2. Thieno[2,3-*d*]pyridazin-4(5*H*)-one 11 (30 mg, 197 µmol) was refluxed in $POCl_3$ (1 mL) for 12 h. The solvent was evaporated and carefully quenched by slowly adding it to warm water with constant stirring. The compound was then dried under vacuum for 12 h and resuspended in isopropanol (1 mL), to which *tert*-butyl piperazine-1-carboxylate was added. The reaction was heated at 120 ^{o}C for 2 h and purified by column chromatography

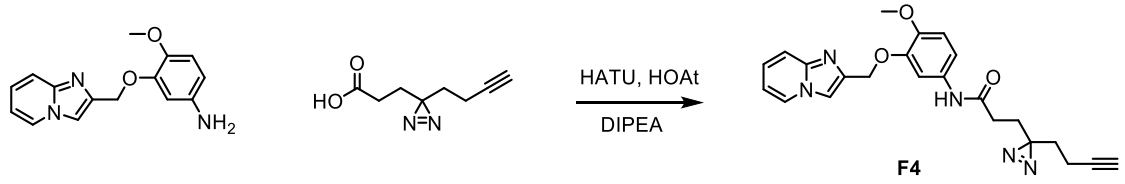
as described in **General Synthetic Methods**. The Boc protecting group was then removed by treatment with 30% (v/v) TFA in DCM and stirring the reaction at room temperature for 2 h. The resulting compound, **F2-amine**, was purified by HPLC as described in **General Synthetic Methods**. **F2** was then synthesized from **F2-amine** as described in **General Procedure for Diazirine Probe Synthesis and Purification**. **F2** was obtained in 40% yield (6.6 mg, 17.9 μmol). ^{1}H NMR, $CDCl_3$ (400 MHz) δ (ppm): 1.68-1.72 (t, 2H, J = 7.4 Hz, CH_2), 1.90-1.94 (t, 2H, J = 7.5 Hz , CH_2), 2.00-2.01 (t, 1H, J = 2.6 Hz, CH), 2.03-2.08 (td, 2H, J = 7.4, 2.5 Hz, CH_2), 2.12-2.16 (s, 2H, J = 7.5 Hz, CH_2), 3.70-3.73 (m, 2H, CH_2), 3.86-3.92 (m, 6H, CH_2), 7.71-7.73 (d, 1H, J = 5.4 Hz, ArH), 8.21-8.23 (d, 1H, J = 5.4 Hz, ArH), 9.67 (s, 1H, ArH). ^{13}C NMR, $CDCl_3$ (400 MHz) δ (ppm) = 13.38, 17.46, 18.66, 26.87, 27.94, 32.80, 41.36, 44.84, 45.70, 48.37, 49.71, 53.43, 69.15, 82.75, 122.01, 129.27, 132.92, 140.22, 140.93, 157.45, 169.99. HR-MS: Calculated for $[C_{18}H_{21}N_6OS]^+$, 369.1498; found 369.1492.



Scheme 3: Compound **F3** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

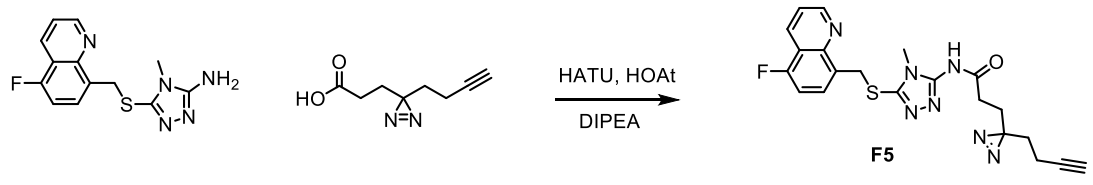
Characterization of F3 was obtained in 44.5% yield (8.1 mg, 18.6 µmol). ^{1}H NMR, MeOD (400 MHz) δ (ppm): 1.50-1.57 (m, 2H,), 1.73-1.78 (m, 4H), 1.93-1.97 (m, 2H), 2.07-2.11 (m, 2H), 2.25-2.27 (m, 2H), 2.32-2.37 (m, 1H), 3.57-3.62 (m, 4H), 4.76 (m, 2H), 7.34-7.37 (t, 1H), 7.45-7.55 (m, 2H), 7.80-7.89 (d, 2H), 8.71-8.73 (d, 2H). ^{13}C NMR, $CDCl_3$ (600 MHz) δ (ppm) = 12.42, 13.08, 26.30, 27.73, 32.21, 37.87, 68.97, 82.12, 113.58, 122.54, 129.15, 131.60,

133.32, 146.11, 148.15, 160.32, 162.08, 167.67, 173.24. HR-MS: Calculated for $[C_{24}H_{27}FN_5O_2]^+$, 436.2149; found 436.2151.



Scheme 4: Compound **F4** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

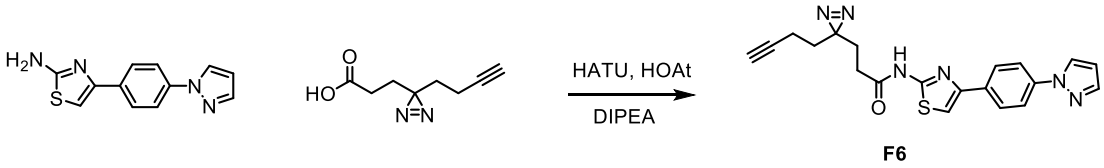
Characterization of F4 was obtained in 96% yield (17.8 mg, 42.6 µmol). ^{1}H NMR, $CDCl_3$ (600 MHz) δ (ppm): 1.63-1.66 (t, 2H, *J* = 7.4 Hz, CH_2), 1.85-1.88 (t, 2H, CH_2), 1.97-2.02 (m, 3H,CH_2, CH), 2.08-2.011 (t, 2H, CH_2), 3.83 (s, 3H, CH_3), 5.29 (s, 2H, CH_2), 6.79-6.80 (d, 1H, *J* = 8.7 Hz, ArH), 6.84-6.86 (t, 1H, *J* = 6.7 Hz, ArH), 7.13 (d, 1H, *J* = 2.2 Hz, ArH), 7.24-7.27 (m, 2H, ArH), 7.60-7.62 (d, 1H, *J* = 9.1 Hz, ArH), 7.69 (s, 1H, ArH), 8.10-8.11 (d, 2H, *J* = 6.9 Hz, ArH). ^{13}C NMR, $CDCl_3$ (600 MHz) δ (ppm): 13.41, 27.96, 28.29, 31.02, 32.34, 41.04, 56.21, 64.86, 69.22, 82.83, 106.74, 111.43, 111.88, 113.08, 113.27, 116.71, 126.12, 131.59, 141.44, 144.20, 146.22, 147.50, 169.56. HR-MS: Calculated for $[C_{23}H_{24}N_5O_3]^+$, 418.1879; found 418.1872.



Scheme F5: Compound **F5** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F5 was obtained in 49% yield (9.6 mg, 22 µmol). ^{1}H NMR, $CDCl_3$ (600

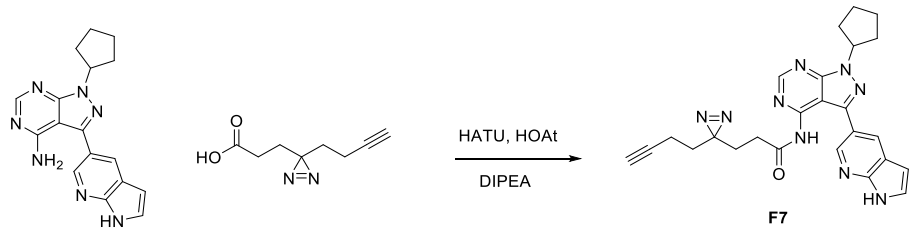
MHz) δ: 1.66-1.69 (t, 2H, *J* = 7.5 Hz, CH_2), 1.88-1.90 (t, 2H, *J* = 7.4 Hz, CH_2), 1.97-1.98 (t, 1H, J = 2.6 Hz, CH), 2.01-2.04 (td, 2H, *J* = 7.5, 2.6 Hz, CH_2), 2.33-2.36 (t, 2H, *J* = 7.4 Hz, CH_2), 3.33 (s, 3H, CH_3), 4.95 (s, 2H, CH_2), 7.14-7.17 (dd, 1H, *J* = 9.4, 8.0 Hz, ArH), 7.48-7.50 (dd, 1H, *J* = 8.4, 4.2 Hz, ArH), 7.68-7.70 (dd, 1H, *J* = 7.9, 6.0 Hz, ArH), 8.43-8.44 (dd, 1H, *J* = 8.4, 1.7 Hz, ArH), 8.96-8.97 (dd, 1H, *J* = 4.2, 1.7 Hz, ArH). ^{13}C NMR, $CDCl_3$ (600 MHz) δ (ppm): 13.26, 27.9, 30.74, 31.16, 32.24, 33.07, 40.94, 69.25, 82.71, 109.90, 119.32, 121.47, 129.72, 130.99, 146.50, 150.22, 150.61, 156.85, 158.55. HR-MS: Calculated for $[C_{21}H_{21}FN_7OS]^+$, 438.1512; found 438.1509.



F6

Scheme 6: Compound **F6** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

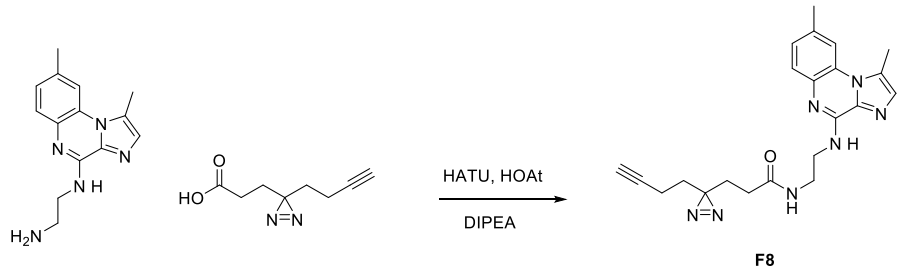
Characterization of F6 was obtained in 39% yield (6.9 mg, 17.7 μmol). ^{1}H NMR, $CDCl_3$ (400 MHz) δ (ppm): 1.70-1.74 (t, 2H, CH_2), 2.01-2.03 (m, 2H, CH_2), 2.05-2.09 (m, 3H, CH, CH_2), 2.35-2.39 (t, 2H, CH_2), 7.19 (s, 1H, ArH), 7.38 (s, 1H, ArH), 7.46-7.48 (d, 2H, ArH), 7.93-7.95 (d, 2H, ArH), 8.44 (s, 1H, ArH). ^{13}C NMR (600 MHz, DMSO-d6) δ (ppm): 174.86, 171.03, 158.43, 150.69, 139.60,135.02, 128.55, 127.44, 122.26, 121.00, 109.29, 82.40, 69.11, 54.52, 42.52, 32.04, 27.75, 17.43, 15.99, 11.89. HR-MS: Calculated for $[C_{20}H_{19}N_6OS]^+$, 391.1341; found 391.1337.



F7

Scheme 7: Compound **F7** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F7. **F7** was obtained in 53% yield (3.7 mg, 7.9 μmol). 1H NMR (400 MHz, CD3OD): δ= 8.74 (s, 1H), 8.65 (s, 1H), 8.58 (s, 1H), 7.64 (d, J=4 Hz, 1H), 6.79 (d, J=4 Hz, 1H), 5.48 (quint, J=7 Hz, 1H), 2.30-2.15 (m, 5H), 2.12 (m, 2H), 2.08-1.97 (m, 2H), 1.88 (td, J=8-2 Hz, 2H), 1.85-1.78 (m, 2H), 1.40 (t, J=7 Hz, 2H), 1.30 (t, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 173.36, 158.13, 152.84, 148.56, 148.09, 142.33, 128.1, 127.79, 127.43, 127.07, 119.76, 100.70, 100.31, 83, 29, 71.85, 71.81, 32.1, 31.95, 31.92, 31.47, 27.98, 27.45, 24.37, 24.34, 12.68. HR-MS: Calculated for $[C_{25}H_{26}N_9O]^+$, 468.2262; found 468.2259.

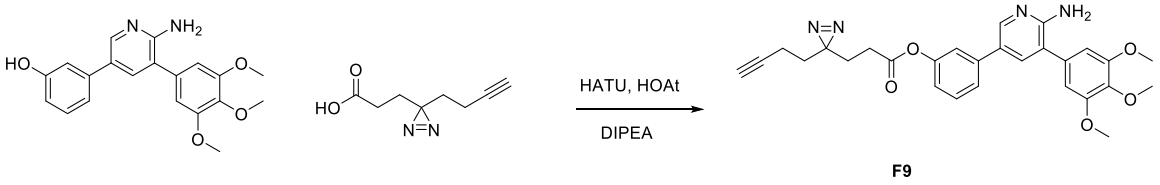


F8

Scheme 8: Compound **F8** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F8. **F8** was obtained in 95% yield (8.0 mg, 19.8 μmol). 1H NMR (400 MHz, DMSO-d6): δ= 8.00 (s, 1H), 7.59 (d, J=8 Hz, 1H), 7.30-7.22 (m, 2H), 3.77 (m, 2H), 3.52 (m, 2H), 2.88 (s, 3H), 2.48 (s, 3H), 2.21 (t, J=3 Hz, 1H), 1.98 (m, 2H), 1.91 (td, J=8-3 Hz, 2H),

1.66 (m, 2H), 1.50 (t, J=8 Hz, 2H); 13C NMR (125 MHz, DMSO-d_6). ^{13}C NMR (600 MHz, DMSO-d6): δ= 171.45, 158.04, 146.20, 132.50, 132.47, 131.79, 131.72, 129.77, 127.49, 125.54, 115.74, 83.28, 71.83, 53.68, 40.04, 31.44, 29.55, 28.29, 28.12, 21.09, 12.69, 8.72. HR-MS: Calculated for $[C_{22}H_{26}N_7O]^+$, 404.2199; found 404.2194.



F9

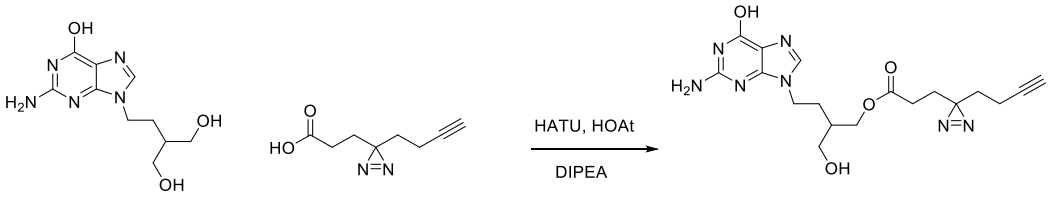
Scheme 9: F9 was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F9. F9 was obtained in 52% yield (2.6 mg, 5.2 μmol). 1H NMR (600 MHz, CD3OD): δ= 8.21 (br, 1H), 7.71 (m, 1H), 7.46 (m, 2H), 7.36 (m, 1H), 7.07 (m, 1H), 6.79 (s, 2H), 3.89 (s, 6H), 3.81 (s, 3H), 2.47 (t, J = 7, 2H), 2.27 (t, J=3 Hz, 1H), 2.06 (td, J=7-3 Hz, 2H), 1.91 (t, J=7 Hz, 2H), 1.68 (t, J=7 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.77, 166.98, 153.28, 131.09, 137.59, 131.77, 131.68, 130.24, 128.78, 124.14, 123.51, 122.11, 120.92, 119.29, 106.29, 83.28, 71.91, 60.13, 56.01, 53.66, 31.30, 29.74, 28.42, 27.47, 13.97. HR-MS: Calculated for $[C_{28}H_{29}N_4O_5]^+$, 501.2138; found 501.2135.

F10

Scheme 10: Compound **F10** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

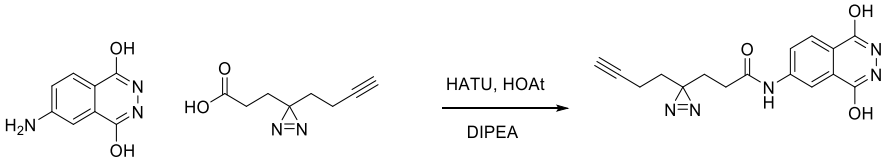
Characterization of F10. **F10** was obtained in 95% yield (3.6 mg, 7.8 μmol). 1H NMR (400 MHz, CD3OD): δ= 7.63-7.59 (m, 2H), 7.54-7.49 (m, 3H), 7.40-7.34 (m, 3H), 7.23-7.19 (m, 2H), 6.06 (d, J=4 Hz, 1H), 2.23-2.18 (m, 2H), 1.84 (t, J=8 Hz, 2H), 1.71 (t, J=8 Hz, 1H), 1.66-1.54 (m, 4H). 13C NMR (125 MHz, DMSO-d_6): δ= 173.45, 170.72, 143.33, 139.72, 138.34, 128.89, 128.16, 127.64, 124.51, 118.87, 112.62, 83.39, 72.00, 31.59, 31.48, 28.32, 27.99, 12.76. HR-MS: Calculated for $[C_{23}H_{23}N_6O_3S]^+$, 463.1552; found 463.1548.



F11

Scheme 11: Compound **F11** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

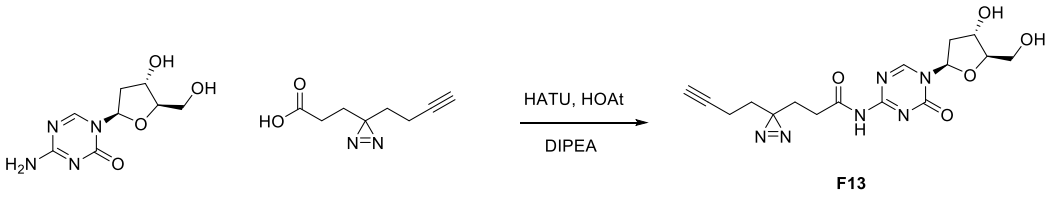
Characterization of F11. **F11** was obtained in 95% yield (2.8 mg, 7.0 μmol). 1H NMR (400 MHz, CD3OD): δ= 7.65-7.60 (m, 1H), 4.38-4.14 (m, 4H), 3.67-3.55 (m, 2H), 2.27 (t, J=4 Hz, 1H), 2.22-2.11 (m, 2H), 2.03 (m, 1H), 1.99 (m, 1H), 1.78 (td, J=4-8 Hz, 2H), 1.61 (t, J=8 Hz, 2H), 1.41-1.37 (m, 3H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 171.91, 156.98, 153.43, 151.24, 137.54, 116.75, 83, 71.78, 64.29, 63.53, 37.53, 31.32, 29.07, 27.91, 27.40, 22.16, 18.94, 12.66. HR-MS: Calculated for $[C_{18}H_{24}N_7O_4]^+$, 402.1890; found 402.1885.



F12

Scheme 12: Compound **F12** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

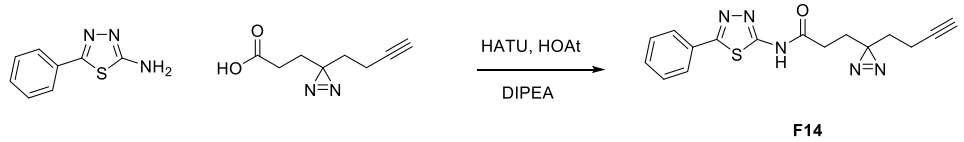
Characterization of F12. **F12** was obtained in 25% yield (1.2 mg, 3.7 μmol). 1H NMR (400 MHz, CD3OD): δ= 8.45 (d, J=4 Hz, 1H), 8.14 (d, J=8 Hz, 1H), 8.03 (dd, J=4-8 Hz, 1H), 2.31-2.26 (m, 3H), 2.06 (td, J=1.8-8 Hz, 2H), 1.89 (t, J=8 Hz, 2H), 1.67 (t, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.62, 159.68, 153.03, 145.63, 130.74, 125.33, 120.40, 113.72, 106.80, 83.28, 71.90, 31.56, 31.38, 30.68, 28.30, 12.74. HR-MS: Calculated for $[C_{16}H_{16}N_5O_3]^+$, 326.1253; found 326.1249.



F13

Scheme 13: Compound **F13** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F13. **F13** was obtained in 27% yield (1.8 mg, 4.8 μmol). 1H NMR (400 MHz, CD3OD): δ= 8.38 (s, 1H), 6.09 (t, J=8 Hz, 1H), 4.41-4.32 (m, 2H), 4.14 (q, J=4 Hz, 1H), 3.82 (t, J=4 Hz, 1H), 2.32-2.24 (m, 2H), 2.25-2.18 (m, 2H), 2.05-2.00(m, 2H), 1.84-1.75 (m, 2H), 1.66-1.58 (m, 3H). 13C NMR (125 MHz, DMSO-d_6): δ= 172.15, 171.95, 166.33, 156.55, 85.99, 84.60, 83.87, 72.43, 70.65, 61.68, 38.20, 31.70, 28.40, 28.20, 27.80, 13.06. HR-MS: Calculated for $[C_{16}H_{21}N_6O_5]^+$, 377.1573; found 377.1570.



F14

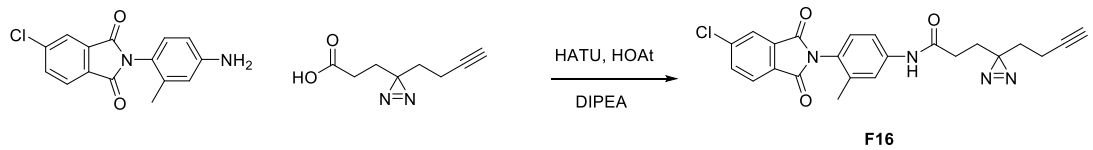
Scheme 14: Compound **F14** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F14. **F14** was obtained in 55% yield (3.2 mg, 9.8 μmol). 1H NMR (600 MHz, DMSO-d_6): δ= 12.66 (br, 1H), 7.96-7.91 (m, 2H), 7.55-7.50 (m, 3H), 2.83 (t, J=6 Hz, 2H), 2.36 (t, J=6 Hz, 2H), 2.02 (td, J=3-6, Hz, 2H), 1.81 (t, J=6 Hz, 1H), 1.61 (t, J=6 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 172.15, 171.95, 166.33, 156.55, 85.99, 84.60, 83.87, 72.43, 70.65, 61.68, 38.20, 31.70, 28.40, 28.20, 27.80, 13.06. HR-MS: Calculated for $[C_{16}H_{16}N_5OS]^+$, 326.1076; found 326.1073.

F15

Scheme 15: Compound **F15** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

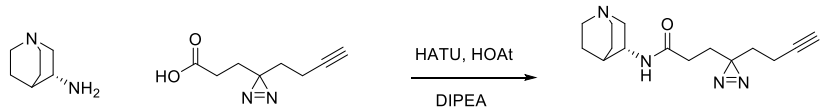
Characterization of F15. **F15** was obtained in 35% yield (1.2 mg, 2.9 μmol). 1H NMR (600 MHz, DMSO-d6): δ= 10.93 (br, 1H), 10.37 (s, 1H), 7.76 (m, 2H), 7.69 (m, 2H), 2.82 (t, J=3 Hz, 1H), 2.19 (t, J=8 Hz, 2H), 2.07 (s, 3H), 2.01 (td, J=3-7, 2H) 1.77 (t, J=8 Hz, 2H), 1.60 (m, 5H); ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.64, 161.52, 155.60, 143.45, 133.60, 128.02, 118.86, 105.21, 83.26, 71.86, 31.56, 30.64, 28.28, 27.57, 12.74, 10.40, 5.92. HR-MS: Calculated for $[C_{19}H_{22}N_5O_4S]^+$, 416.1393; found 416.1390.



F16

Scheme 16: Compound **F16** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

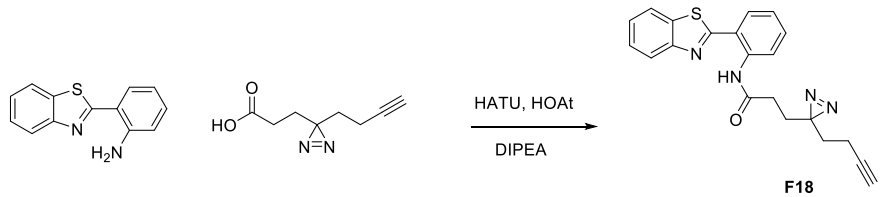
Characterization of F16. **F16** was obtained in 42% yield (2.1 mg, 4.8 μmol). 1H NMR (400 MHz, CD3OD): δ= 7.92-7.87 (m, 2H), 7.82 (dd, J = 8-2 Hz, 1 H) 7.63 (d, J=2 Hz, 1H), 7.51 (dd, J=8-2 Hz, 1H), 7.12 (d, J=8 Hz, 1H), 2.19 (m, 2H), 2.14 (s, 3H), 2.11 (t, J=4 Hz, 1H), 2.02 (td, J=6-2, 2H), 1.86 (m, 2H), 1.66 (t, J=6 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.02, 166.49, 166.10, 139.90, 139.62, 136.79, 134.58, 133.74, 130.31, 129.56, 125.52, 125.38, 123.75, 120.87, 117.33, 83.27, 71.87, 31.56, 30.58, 28.33, 27.80, 17.74, 12.75. HR-MS: Calculated for $[C_{23}H_{20}N_4O_3Cl]^+$, 435.1224; found 435.1228.



F17

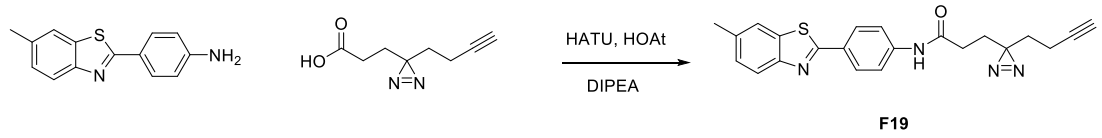
Scheme 17: Compound **F17** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F17. **F17** was obtained in 95% yield (3.1 mg, 11 μmol). 1H NMR (400 MHz, CDCl3): δ= 7.76 (d, J=8 Hz, 1H), 4.47 (s, 2H), 4.38 (dd, J=8-16 Hz, 1H), 3.62-3.52 (m, 1H), 3.52-3.41 (m, 2H), 3.31-3.11 (m, 3H), 2.37 (q, J=4 Hz, 1H), 2.30-2.19 (m, 1H), 2.07-1.99 (m, 6H), 1.85-1.80 (m, 2H), 1.64 (t, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 171.15, 83.24, 71.85, 53.62, 51.94, 45.71, 45.11, 43.79, 31.46, 29.41, 28.32, 27.99, 23.96, 21.43, 12.72. HR-MS: Calculated for $[C_{15}H_{23}N_4O]^+$, 275.1871; found 275.1874.



Scheme 18: Compound **F18** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

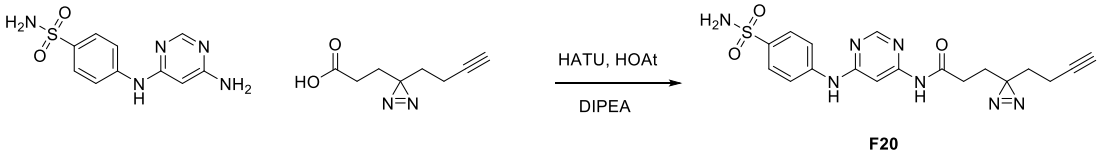
Characterization of F18. **F18** was obtained in 53% yield (4.0 mg, 10.7 μmol). 1H NMR (400 MHz, CDCl3): δ= 12.49 (s, 1H), 8.77 (m, 1H), 8.04 (m, 1H), 7.94 (m, 1H), 7.87 (m, 1H), 7.56 (m, 1H), 7.51-7.41 (m, 2H), 7.17 (m, 1H), 2.37 (m, 2H), 2.07 (td, J=8-3 Hz, 2H), 2.01 (m, 2H), 1.98 (t, J=3 Hz, 1H), 1.73 (t, J=7 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.42, 167.50, 152.33, 137.03, 133.38, 132.00, 130.19, 127.04, 126.17, 124.21, 122.72, 122.27, 121.86, 120.66, 83.23, 71.85, 31.58, 31.44, 28.26, 27.93, 12.75. HR-MS: Calculated for $[C_{21}H_{19}N_4OS]^+$, 375.1280; found 375.1277.



Scheme 19: Compound **F19** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F19. **F19** was obtained in 95% yield (8.5 mg, 21.9 μmol). 1H NMR (400 MHz, CDCl3): δ= 8.04 (m, 2H), 7.92 (d, J = 8, 1H), 7.68 (s, 1H), 7.64 (m, 2H), 7.29 (m, 1H), 2.50 (s, 3H), 2.16 (m, 2H), 2.06 (td, J=7-3 Hz, 2H), 2.00 (t, J=3 Hz, 1H), 1.97 (m, 2H), 1.70 (t, J=7 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.26, 165.94, 151.84, 141.83,

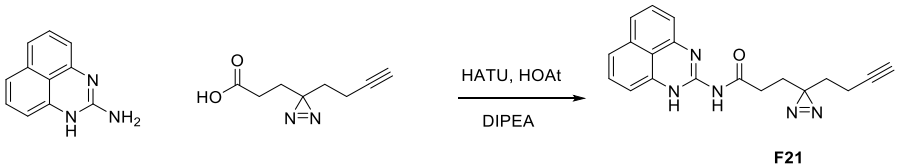
135.10, 134.48, 128.10, 127.92, 127.68, 122.21, 121.85, 119.29, 83.27, 71.87, 31.58, 30.65, 28.32, 27.71, 21.12, 12.75. HR-MS: Calculated for $[C_{22}H_{21}N_4OS]^+$, 389.1436; found 389.1439.



F20

Scheme 20: Compound **F20** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F20. **F20** was obtained in 9% yield (1.1 mg, 2.7 μmol). 1H NMR (400 MHz, CD3OD): δ= 8.49 (s, 1H), 7.88 (m, 2H), 7.81 (m, 2H), 7.27 (s, 1H), 2.32 (t, J=7 Hz, 2H), 2.28 (t, J=3, 1H), 2.05 (td, J=7-3 Hz, 2H), 1.86 (t, J=8 Hz, 2H), 1.65 (t, J=7 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 171.93, 161.25, 157.72, 156.92, 143.42, 136.76, 126.75, 118.64, 93.97, 83.28, 71.91, 31.58, 30.46, 28.27, 27.56, 12.73. HR-MS: Calculated for $[C_{18}H_{20}N_7O_3S]^+$, 414.1348; found 414.1344.



F21

Scheme 21: Compound **F21** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

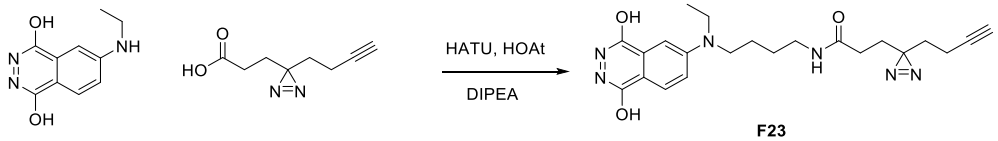
Characterization of F21. **F21** was obtained in 80% yield (3.1 mg, 9.4 μmol). 1H NMR (400

MHz, CD3OD): δ= 7.13 (t, J=4 Hz, 2H), 7.07 (d, J=4 Hz, 2H), 6.48 (d, J=4 Hz, 2H), 2.28 (t, J=2 Hz, 1H), 2.24 (t, J=4 Hz, 2H), 2.05 (td, J=4-2 Hz, 2H), 1.82 (t, J=5 Hz, 2H), 1.64(t, J=5 Hz, 2H). ^{13}C NMR (600 MHz, CD3OD): δ=178.90, 150.76, 136.69, 129.22, 121.47, 120.38, 108.66, 83.62, 70.34, 34.44, 32.56, 29.02, 28.82, 13.85. HR-MS: Calculated for $[C_{19}H_{18}N_5O_1]^+$, 332.1511; found 332.1508.

F22

Scheme 22: Compound **F22** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F22. **F22** was obtained in 73% yield (2.8 mg, 8.2 μmol). 1H NMR (400 MHz, CDCl3): δ= 7.63 (d, J=8 Hz, 1H), 7.28 (d, J=4 Hz, 1H), 7.06 (dd, J=8-4 Hz, 1H), 4.09 (q, J=8 Hz, 2H), 2.27 (t, J=8 Hz, 2H), 2.04-1.91 (m, 5H), 1.63 (t, J=8 Hz, 2H), 1.45 (t, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.66, 155.79, 155.39, 142.55, 132.77, 121.18, 115.30, 105.40, 83.24, 71.88, 63.66, 31.45, 29.44, 28.19, 27.39, 14.77, 12.72. HR-MS: Calculated for $[C_{17}H_{19}N_4O_2S]^+$, 343.1229; found 343.1232.

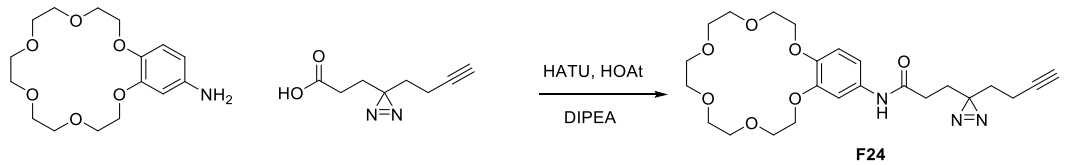


F23

Scheme 23: Compound **F23** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F23. **F23** was obtained in 42% yield (1.8 mg, 4.2 μmol). ^{1}H NMR (600

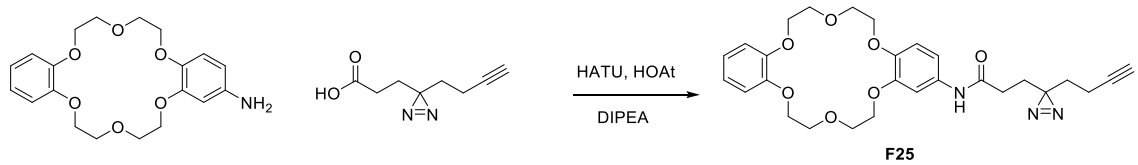
MHz, DMSO-d6): δ= 7.88 (t, J=4 Hz, 1H), 7.82 (d, J=8 Hz, 1H), 7.16 (dd, J=4-8 Hz, 1H), 7.01 (br, 1H), 3.47 (peak under solvent, 2H), 3.38 (t peak under solvent, J=8 Hz, 2H), 3.07 (q, J=4 Hz, 2H), 2.80 (t, J=4 Hz, 1H), 1.96 (td, J=4-8 Hz, 2H), 1.87 (dd, J=2-8 Hz, 2H), 1.63 (dd, J=2-8 Hz, 2H), 1.55 (m, 4H), 1.45 (quint, J=7 Hz, 2H), 1.12 (t, J=4 Hz, 2H); ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.66, 158.44, 158.20, 150.38, 128.70, 127.10, 116.68, 115.12, 103.27, 83.25, 71.79, 49.39, 44.52, 40.43, 38.30, 31.51, 29.58, 28.34, 28.19, 26.63, 24.28, 12.72, 11.90. HR-MS: Calculated for $[C_{22}H_{29}N_6O_3]^+$, 425.2301; found 425.2305.



F24

Scheme 24: Compound **F24** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F24. **F24** was obtained in 54% yield (1.2 mg, 2.5 μmol). 1H NMR (400 MHz, CD3OD): δ= 7.28 (d, J=2 Hz, 1H), 6.99 (dd, J=9-2 Hz, 1H), 6.88 (d, J=9 Hz, 1H), 4.16-4.07 (m, 4H), 3.90-3.81 (m, 4H), 3.74-3.70 (m, 4H), 3.70-3.66 (m, 4H), 3.66-3.60 (m, 4H), 2.27 (t, J=3 Hz, 1H), 2.18 (m, 2H), 2.04 (td, J=8-3 Hz, 2H), 1.83 (m, 2H), 1.64 (t, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 169.28, 148.00, 144.13, 132.94, 113.55, 111.31, 105.54, 83.27, 71.85, 69.92, 69.90, 60.87, 69.85, 68.88, 68.75, 68.42, 68.10, 31.56, 30.48, 28.34, 27.90, 12.74. HR-MS: Calculated for $[C_{24}H_{34}N_3O_7]^+$, 476.2397; found 476.2392.



F25

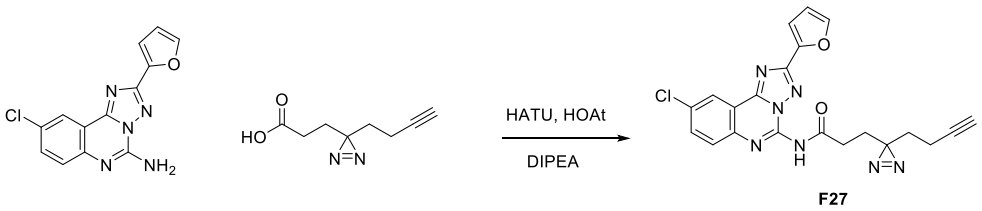
Scheme 25: Compound **F25** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F25. **F25** was obtained in 8% yield (1.6 mg, 3.1 μmol). 1H NMR (400 MHz, CD3OD): δ= 7.27 (d, J=2 Hz, 1H), 6.98 (dd, J=9-2 Hz, 1H), 6.95-6.90 (m, 2H), 6.90-6.85 (m, 3H), 4.18-4.09 (m, 8H), 4.00-3.91 (m, 8H), 2.27 (t, J=3 Hz, 1H), 2.18 (m, 2H), 2.04 (td, J=8-3 Hz, 2H), 1.82 (m, 2H), 1.64 (t, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 169.27, 147.98, 147.62, 143. 88, 132.70, 120.79, 112.45, 111.04, 104.75, 83.33, 71.90, 69.04, 69.00, 68.96, 68.93, 67.85, 67.68, 67.63, 67.60, 56.09, 31.55, 30.49, 28.32, 27.94, 12.74. HR-MS: Calculated for $[C_{28}H_{34}N_3O_7]^+$, 524.2397; found 524.2390.

F26

Scheme 26: Compound **F26** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

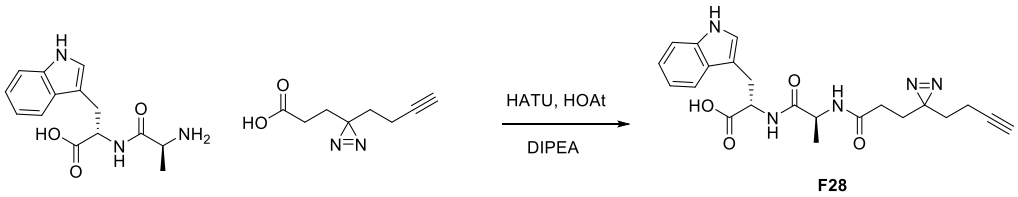
Characterization of F26. **F26** was obtained in 83% yield (1.3 mg, 3.7 μmol). 1H NMR (400 MHz, CDCl3): δ= 8.39 (m, 2H), 8.25 (m, 2H), 2.48 (m, 2H), 2.08 (m, 2H), 2.05-1.98 (m, 3H), 1.74 (t, J=7 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 173.35, 159.01, 158.14, 148.93, 129.02, 127.28, 124.80, 83.25, 71.81, 31.48, 28.12, 27.98, 27.46, 12.68. HR-MS: Calculated for $[C_{16}H_{15}N_6O_4]^+$, 355.1155; found 355.1158.



F27

Scheme 27: Compound **F27** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F27. **F27** was obtained in 25% yield (4.3 mg, 9.9 μmol). 1H NMR (400 MHz, CDCl3): δ= 8.98 (br, 1H), 8.50 (m, 1H), 7.90 (d, J=9 Hz, 1H), 7.76 (dd, J=9-2 Hz, 1H), 7.68 (dd, J=2-1 Hz, 1H), 7.31 (dd, J=4-1 Hz, 1H), 6.64 (dd, J=4-2 Hz, 1H), 2.94 (t, J=8 Hz, 2H), 2.10 (td, J=8-3 Hz, 2H), 2.07-1.98 (m, 3H), 1.77 (t, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.71, 155.66, 151.33, 145.75, 144.90, 141.30, 137.87, 132.88, 131.40, 129.44, 122.42, 116.87, 113.14, 112.46, 83.30, 71.89, 31.54, 30.60, 28.26, 27.46, 12.78. HR-MS: Calculated for $[C_{21}H_{17}N_7O_2Cl]^+$, 434.1132; found 434.1128.

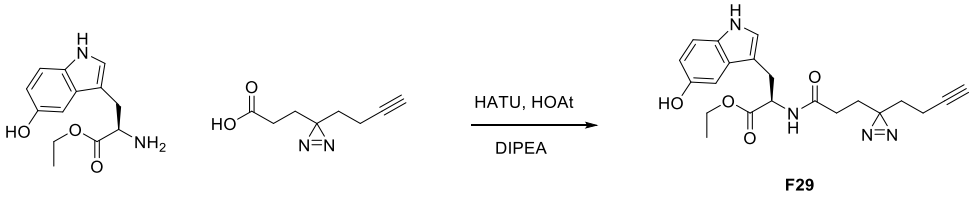


F28

Scheme 28: Compound **F28** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F28. **F28** was obtained in 61% yield (9.6 mg, 22.7 μmol). 1H NMR (400 MHz, CD3OD): δ= 7.58-7.52 (m, 1H), 7.35-7.30 (m, 1H), 7.13-7.05 (m, 2H), 7.03-6.96 (m, 1H), 4.75-4.67 (m, 1H), 4.38-4.30 (m, 1H), 3.39-3.32 (m, 1H), 3.26-3.17 (m, 1H), 2.25 (t, J=3 Hz, 1H), 2.02-1.92 (m, 4H), 1.69-1.62 (m, 2H), 1.60-1.52 (m, 2H), 1.28 (d, J=7 Hz, 2H),

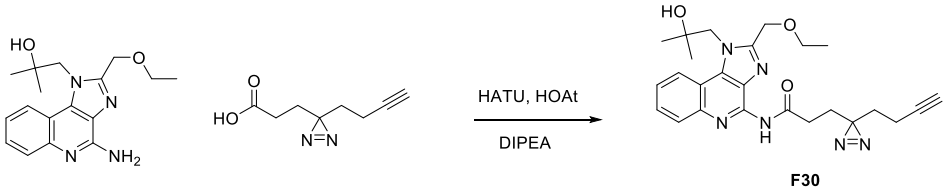
1.17 (d, J=7, 1H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.51, 136.09, 127.26, 123.74, 120.98, 118.45, 118.22, 111.43, 109.65, 83.28, 71.79, 52.93, 47.91, 31.40, 30.77, 29.35, 28.32, 28.20, 18.23, 12.72. HR-MS: Calculated for $[C_{22}H_{26}N_5O_4]^+$, 424.1985; found 424.1981.



F29

Scheme 29: Compound **F29** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

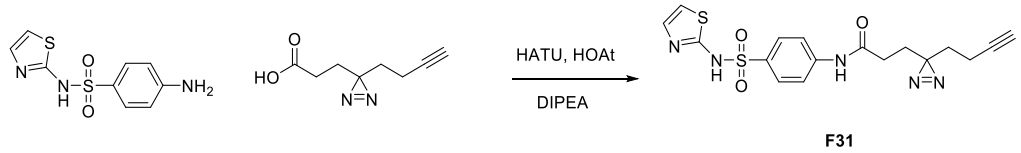
Characterization of F29. **F29** was obtained in 95% yield (4.5 mg, 11.4 μmol). 1H NMR (400 MHz, DMSO-d_6): δ= 10.51 (s, 1H), 8.60 (s, 1H), 8.27 (d, J=8 Hz, 1H), 7.10 (d, J=8 Hz, 1H), 7.01 (s, 1H), 6.76 (s, 1H), 6.57 (d, J=8 Hz, 1H), 4.41 (q, J= 8 Hz, 1H), 4.00 (td, J=4-8 Hz, 2H), 3.00 (dd, J=8-16 Hz, 1H), 2.89 (dd, J=8-12 Hz, 1H), 2.82-2.76 (m, 1H), 1.98-1.89 (m, 4H), 1.60-1.47 (m, 4H), 1.07 (t, J=8 Hz, 3H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 172.05, 170.96, 150.37, 130.70, 127.80, 124.21, 111.80, 111.36, 108.46, 101.99, 83.26, 71.78, 60.44, 53.10, 31.34, 30.77, 29.20, 28.26, 28.15, 13.99, 12.70. HR-MS: Calculated for $[C_{21}H_{25}N_4O_4]^+$, 397.1876; found 397.1879.



F30

Scheme 30: Compound **F30** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

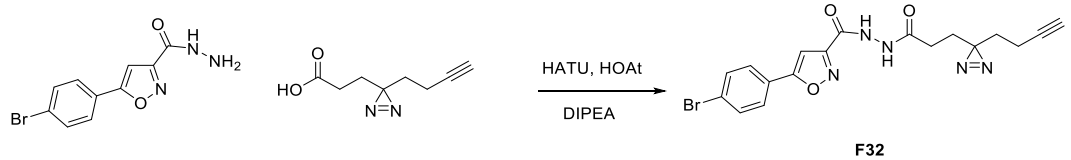
Characterization of F30. **F30** was obtained in 41% yield (7.5 mg, 16.2 μmol). 1H NMR (400 MHz, CD3OD): δ= 8.48 (m, 1H), 8.10 (m, 1H), 7.65 (m, 1H), 7.58 (m, 1H), 5.05 (br, 2H), 4.86 (br, 2H, overlapped with residual water), 3.63 (q, J=7 Hz, 2H), 2.61 (m, 2H), 2.28 (t, J=3 Hz, 1H), 2.08 (td, J = 7-3 Hz, 2H), 1.94 (m, 2H), 1.71 (t, J=8, 2H), 1.27 (m, 9H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 173.34, 154.00, 152.63, 149.02, 144.19, 136.58, 127.58, 124.87, 122.07, 116.96, 113.41, 83.31, 71.86, 70.75, 65.61, 64.89, 55.09, 31.59, 30.52, 28.38, 27.98, 27.80, 27.45, 15.06, 12.80. HR-MS: Calculated for $[C_{25}H_{31}N_6O_3]^+$, 463.2458; found 463.2452.



F31

Scheme 31: Compound **F31** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F31. **F31** was obtained in 22% yield (2.5 mg, 6.2 μmol). 1H NMR (400 MHz, CD3OD): δ= 7.81 (d, J=8 Hz, 2H), 7.69 (d, J=8 Hz, 2H), 7.08 (d, J=8 Hz, 1H), 6.70 (d, J=8 Hz, 1H), 2.22 (t, J=8 Hz, 2H), 2.11 (t, J=8 Hz, 2H), 1.81 (t, J=8 Hz, 1H), 1.75 (t, J=8 Hz, 2H), 1.63 (t, d, J=8 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 173.39, 170.36, 142.33, 136.33, 127.00, 118.62, 112.53, 108.20, 83.19, 71.69, 31.53, 31.48, 30.57, 27.99, 12.69. HR-MS: Calculated for $[C_{17}H_{18}N_5O_3S_2]^+$, 404.0851; found 404.0855.

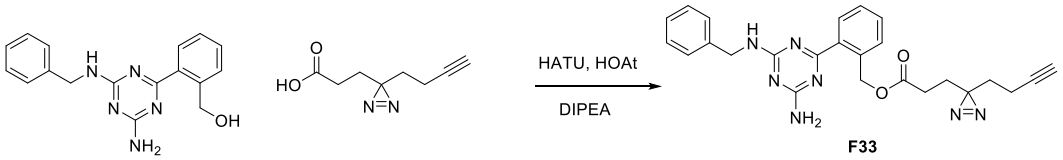


F32

Scheme 32: Compound **F32** was synthesized as described in **General Procedure for**

Diazirine Probe Synthesis and Purification.

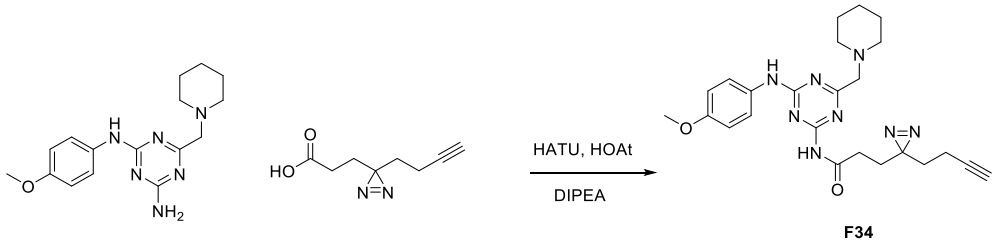
Characterization of F32. **F32** was obtained in 32% yield (5.9 mg, 13.8 μmol). 1H NMR (400 MHz, DMSO-d6): δ= 10.71 (s, 1H), 10.06 (s, 1H), 7.91 (m, 2H), 7.78 (m, 2H), 7.48 (s, 1H), 2.85 (t, J=3 Hz, 1H), 2.08-1.98 (m, 4H), 1.69 (m, 2H), 1.62 (t, J=7 Hz, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 170.04, 169.56, 158.33, 157.71, 132.48, 127.90, 125.39, 124.54, 100.65, 83.28, 71.87, 31.35, 28.24, 27.99, 27.45, 12.75. HR-MS: Calculated for $[C_{18}H_{17}N_5O_3Br]^+$, 430.0514; found 430.0510.



F33

Scheme 33: Compound **F33** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

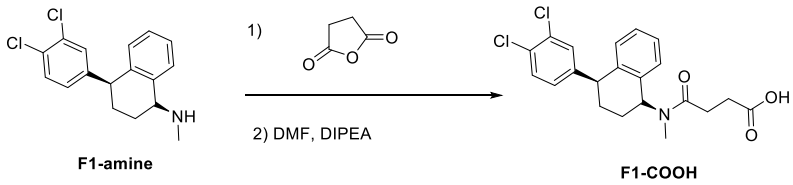
Characterization of F33. **F33** was obtained in 32% yield (4.3 mg, 9.5 μmol). 1H NMR (600 MHz, CD3OD): δ= 7.78 (m, 1H), 7.55-7.39 (m, 3H), 7.39-7.27 (m, 4H), 7.23 (m, 1H), 5.54-5.36 (m, 2H), 4.63 (s, 2H), 2.25 (m, 1H), 2.07 (m, 2H), 1.96 (m, 2H), 1.65 (m, 2H), 1.54 (m, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ= 173.41, 171.61, 158.21, 158.00, 140.16, 139.48, 135.41, 128.32, 127.20, 127.01, 126.89, 126.65, 115.87, 83.32, 71.86, 53.64, 45.79, 31.32, 27.98, 27.88, 27.40, 12.67. HR-MS: Calculated for $[C_{25}H_{26}N_7O_2]^+$, 456.2147; found 456.2142.



F34

Scheme 34: Compound **F34** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**.

Characterization of F34. **F34** was obtained in 28% yield (5.2 mg, 11.3 μmol). ^{1}H NMR (400 MHz, CD3OD) δ (ppm): 7.75-7.40 (m, 2H), 7.05-6.76 (m, 2H), 3.79 (s, 3H), 3.55 (s, 2H), 2.78-2.57 (m, 4H), 2.56-2.34 (m, 2H), 2.26 (m, 1H), 2.10-1.96 (m, 2H), 1.90-1.23 (m, 10H). 13C NMR (125 MHz, DMSO-d_6): δ= 173.35, 171.05, 163.73, 163.15, 155.02, 129.71, 121.49, 113.35, 82.27, 71.82, 55.38, 55.22, 54.17, 35.17, 31.56, 31.13, 28.25, 27.38, 25.17, 12.74. HR-MS: Calculated for $[C_{24}H_{31}N_8O_2]^+$, 463.2570; found 463.2568.

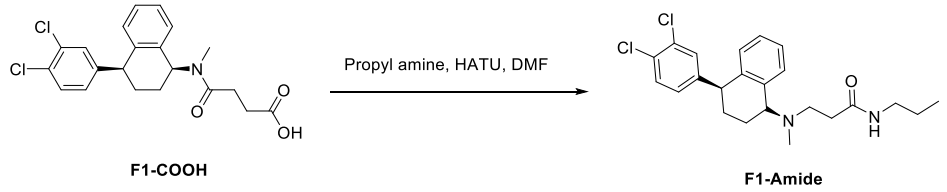


F1-amine F1-COOH

Scheme 35: Compound **F1-COOH** was synthesized by mixing the starting **F1-amine** (31 mg, 0.1 mmol) with succinic anhydride (20 mg, 0.2 mmol) in DMF (1 mL) followed by DIPEA (20 µL, 0.2 mmol) and stirred at room temperature overnight. The reaction was evaporated under vacuum to afford a crude oil. The crude oil was washed with diethyl ether twice to obtain **F1-COOH** as white solids.

Characterization of F1-COOH. **F1-COOH** was obtained in quantitative yield (38 mg, 0.1 mmol). ^{1}H NMR (400 MHz, CD3OD) δ (ppm): 7.42 (m, 1H), δ= 7.27-7.21 (m, 2H), 7.15 (m, 2H), 6.97 (m, 2H), 5.83 (t, 1H), 4.30 (s, H), δ= 2.84 (s, 3H), δ= 2.72-2.65 (m, 4H), δ= 2.27 (m, 1H), δ= 2.06 (m, 1H), δ= 1.74-1.69 (m, 2H). ^{13}C NMR (600 MHz, CD3OD): δ= 175.24, 173.76,

147.67, 138.35, 135.61, 131.67, 130.80, 130.44, 129.91, 128.30, 127.56, 127.38, 126.91, 126.67, 53.21, 42.75, 29.83, 28.75, 28.10, 22.08, 21.12 HR-MS: Calculated for $[C_{21}H_{22}NO_3Cl_2]^+$, 406.0977; found 406.0972.

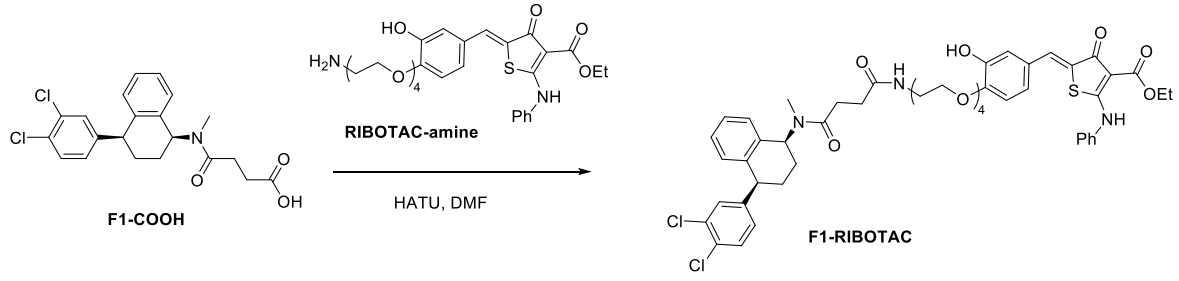


F1-COOH F1-Amide

Scheme 36: Compound **F1-Amide** was synthesized as described below.

Synthesis of F1-Amide. Compound **F1-Amide** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**, except instead of 3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanoic acid, propyl amine (18 µmol, 1.5 equiv.) was used.

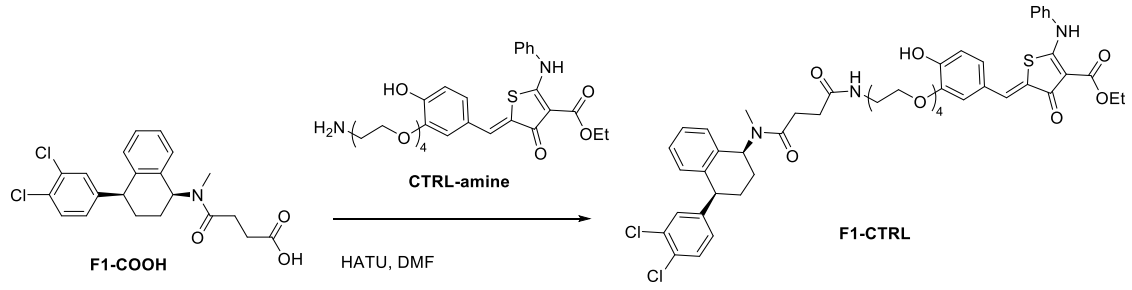
Characterization of F1-Amide. **F1-Amide** was obtained in 73% yield (3.7 mg, 8.7 µmol). ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 7.82 (m, 1H), 7.55 (d, 1H), 7.29-7.20 (m, 3H), 7.06-6.95 (m, 2H), 7.82 (m, 1H), 5.70 (s, 1H), 4.32 (m, 1H), 3.00 (m, 2H), 2.73 (s, 3H), 2.40 (m, 2H), 2.23 (m, 1H), 1.97 (m, 1H), 1.57 (m, 2H), 1.40 (m, 2H), 0.42 (m, 3H). ^{13}C NMR (600 MHz, DMSO-d6) δ (ppm): 172.77, 171.76, 148.48, 138.67, 136.77, 131.30, 131.02, 130.93, 130.90 130.77, 129.34, 129.07, 127.68, 127.60, 55.91, 49.07, 42.46, 31.06, 30.07, 28.97, 22.91, 22.60, 21.63, 11.91. HR-MS: Calculated for $[C_{24}H_{29}N_2O_2Cl_2]^+$, 447.1606; found 447.1610.



F1-COOH F1-RIBOTAC

Scheme 37: Compound **F1-RIBOTAC** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**, except instead of 3-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)propanoic acid, **RIBOTAC-amine**[13] (6.3 µmol, 1.5 equiv.) was used.

Characterization of F1-RIBOTAC. **F1-RIBOTAC** was obtained in 43% yield (1.7 mg, 1.8 µmol). ^{1}H NMR (600 MHz, DMSO-d6) δ (ppm): 11.24 (s, 1H), 9.43 (s, 1H), 7.91 (m, 1H), 7.54 (m, 5H), 7.49 (m, 1H), 7.44 (m, 1H), 7.28-7.19 (m, 3H), 7.06 (m, 1H), 7.02 (m, 1H), 6.98 (m, 2H), 6.95-6.89 (m, 2H), 5.69 (s, 1H), 4.28 (m, 3H), 4.11 (t, 2H), 3.74 (t, 2H), 3.57 (m, 2H), 3.50, (m, 6H), 3.36 (2H, overlap with water), 3.20 (m, 2H), 2.71-2.65 (m, 4H), 2.43 (m, 3H), 2.21 (m, 1H), 1.95 (m, 1H), 1.72-1.56 (m, 2H), 1.29 (t, 3H). ^{13}C NMR (600 MHz, DMSO-d6) δ (ppm): 180.32, 174.71, 171.63, 171.18, 170.98, 170.96, 164.25, 148.12, 147.38, 147.33, 146.41, 136.88, 129.83, 129.42, 129.01, 127.56, 126.60, 125.00, 124.01, 115.23, 113.12, 96.25, 69.31, 69.18, 69.12, 68.97, 68.53, 68.22, 67.32, 58.89, 41.37, 41.24, 37.97, 29.86, 28.97, 27.79, 21.51, 20.55, 13.79. HR-MS: Calculated for $[C_{49}H_{54}N_3O_{10}Cl_2S]^+$, 946.2907; found 946.2901.



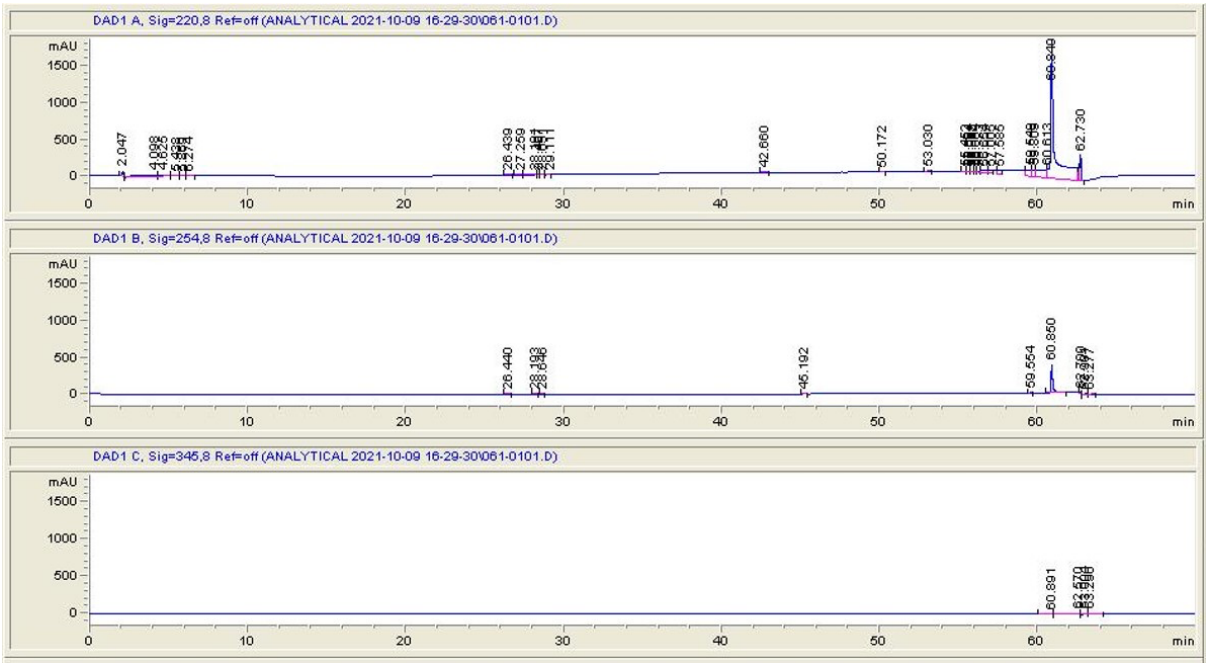
Scheme 38: Compound **F1-CTRL** was synthesized as described in **General Procedure for Diazirine Probe Synthesis and Purification**, except instead of 3-(3-(but-3-yn-1-yl)-3H-

diazirin-3-yl)propanoic acid, **CTRL-amine**[13] (6.3 μmol, 1.5 equiv.) was used.

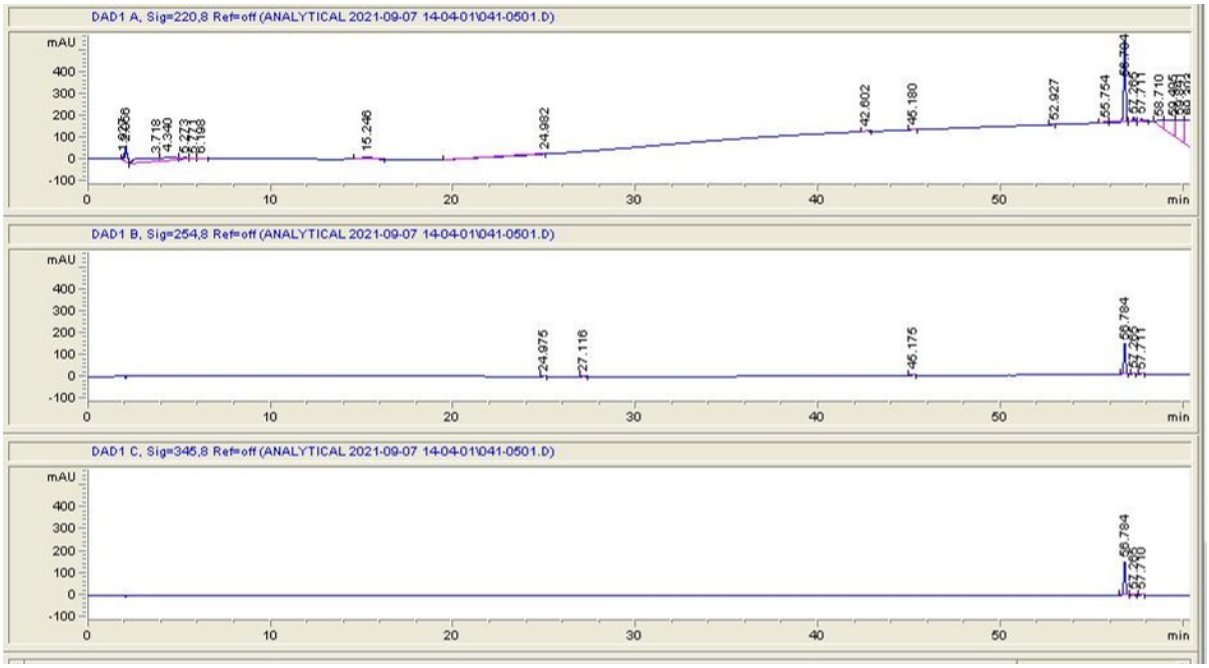
Characterization of F1-CTRL. **F1-CTRL** was obtained in 38% yield (1.5 mg, 1.6 μmol). ^{1}H NMR (600 MHz, DMSO-d6) δ (ppm): ^{1}H NMR (600 MHz, DMSO-d6) δ (ppm): 11.24 (s, 1H), 9.43 (s, 1H), 7.91 (m, 1H), 7.54 (m, 5H), 7.49 (m, 1H), 7.44 (m, 1H), 7.28-7.19 (m, 3H), 7.13 (m, 1H), 7.02 (m, 1H), 6.98 (m, 1H), 6.95-6.89 (m, 2H), 6.76 (m, 1H), 5.69 (s, 1H), 4.28 (m, 3H), 4.11 (t, 2H), 3.74 (t, 2H), 3.57 (m, 2H), 3.50, (m, 6H), 3.36 (m, 2H), 3.20 (m, 2H), 2.71-2.65 (m, 4H), 2.43 (m, 3H), 2.21 (m, 1H), 1.95 (m, 1H), 1.72-1.56 (m, 2H), 1.29 (t, 3H). ^{13}C NMR (600 MHz, DMSO-d6) δ (ppm): 180.82, 175.21, 171.48, 171.18, 170.98, 170.96, 164.25, 148.62, 147.38, 146.91, 146.41, 137.38, 129.91, 129.42, 129.01, 127.01, 126.60, 125.00, 124.01, 115.23, 113.12, 96.25, 69.31, 69.18, 69.12, 68.97, 68.53, 68.22, 67.32, 58.89, 41.37, 41.24, 37.97, 29.86, 28.97, 27.79, 21.51, 20.55, 14.29. HR-MS: Calculated for $[C_{49}H_{54}N_3O_{10}Cl_2S]^+$, 946.2907; found 946.2902.

Analytical HPLC Spectra

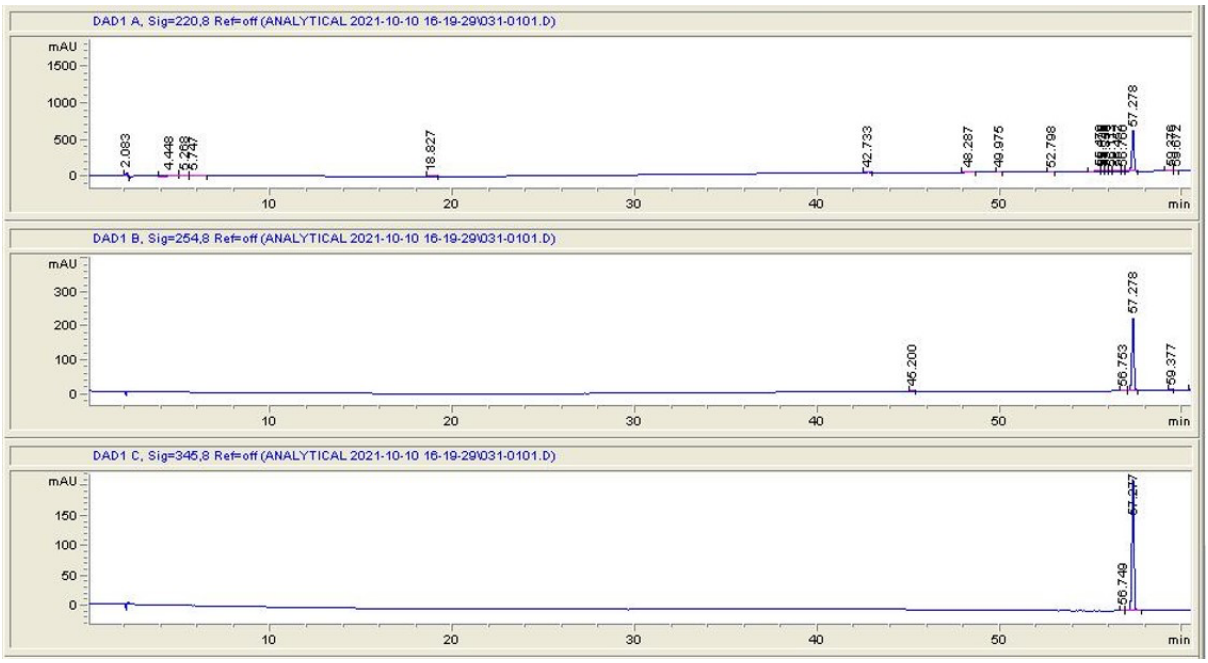
F1-Amide



F1-RIBOTAC



F1-CTRL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mAU
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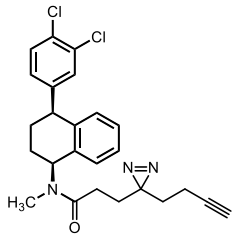
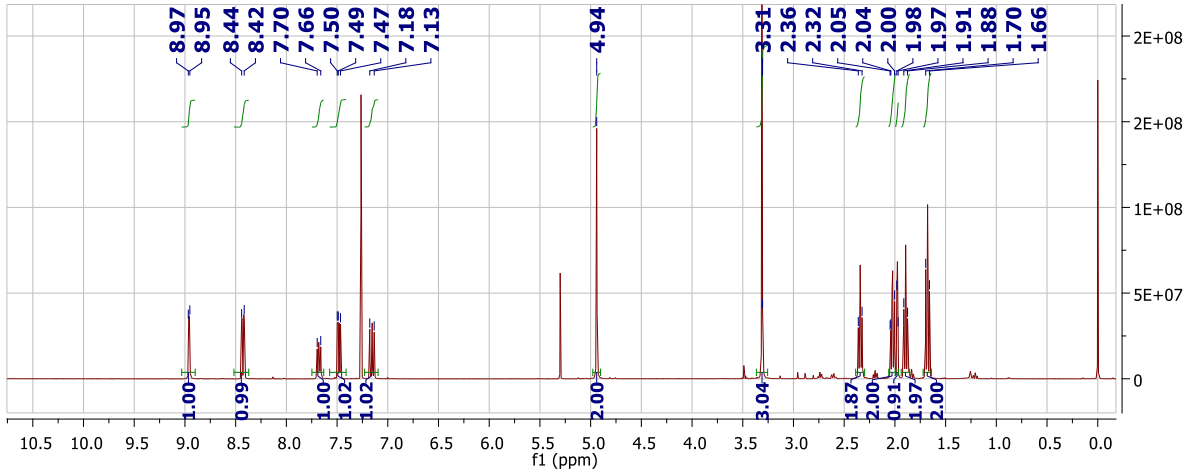
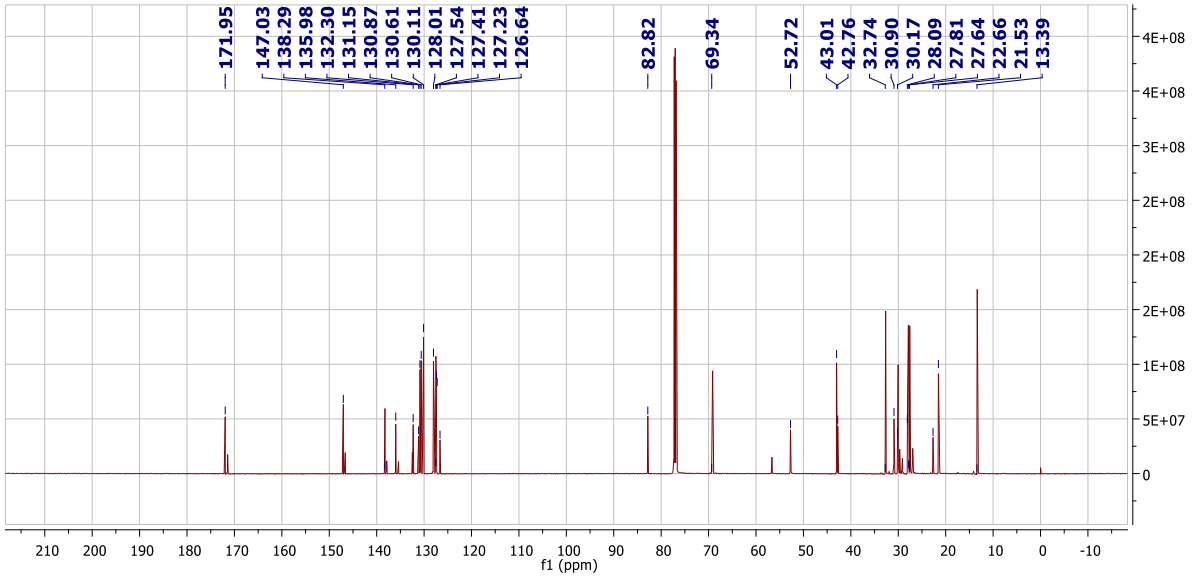
1000
500
0
2.083
4.448
18.827
42.733
48.287
49.975
52.798
57.278
10
20
30
40
50
min
DAD1 B, Sig=254,8 Ref=off (ANALYTICAL 2021-10-10 16-19-29\031-0101.D)
mAU
300
200
100
0
46.200
56.753
57.278
59.377
10
20
30
40
50
min
DAD1 C, Sig=345,8 Ref=off (ANALYTICAL 2021-10-10 16-19-29\031-0101.D)
mAU
150
100
50
0
56.749
57.277
10
20
30
40
50
min

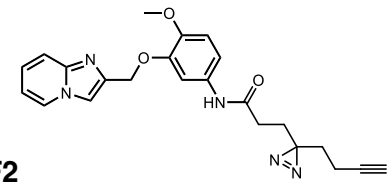
NMR Spectra



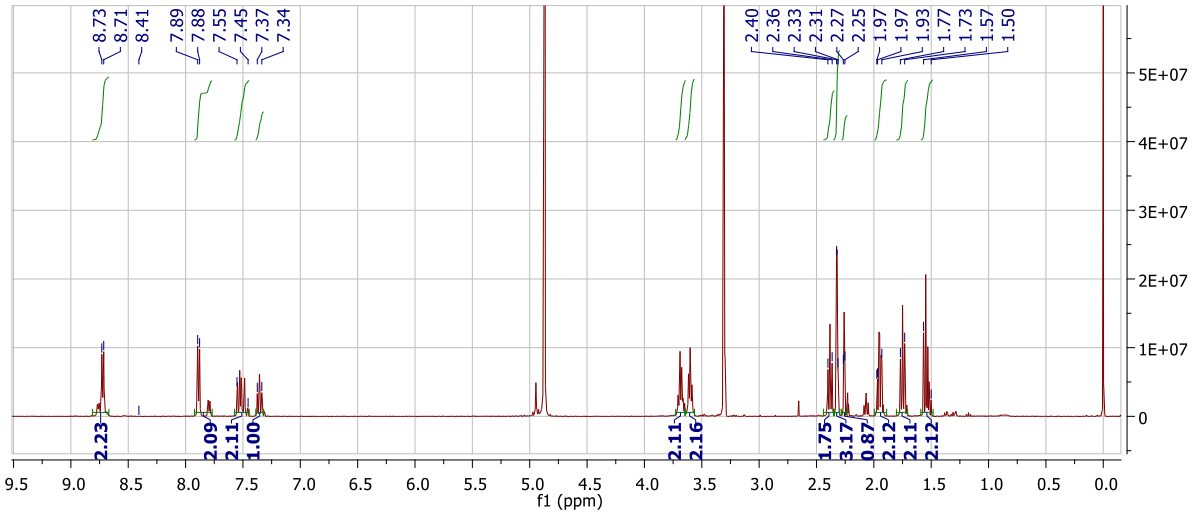
F1

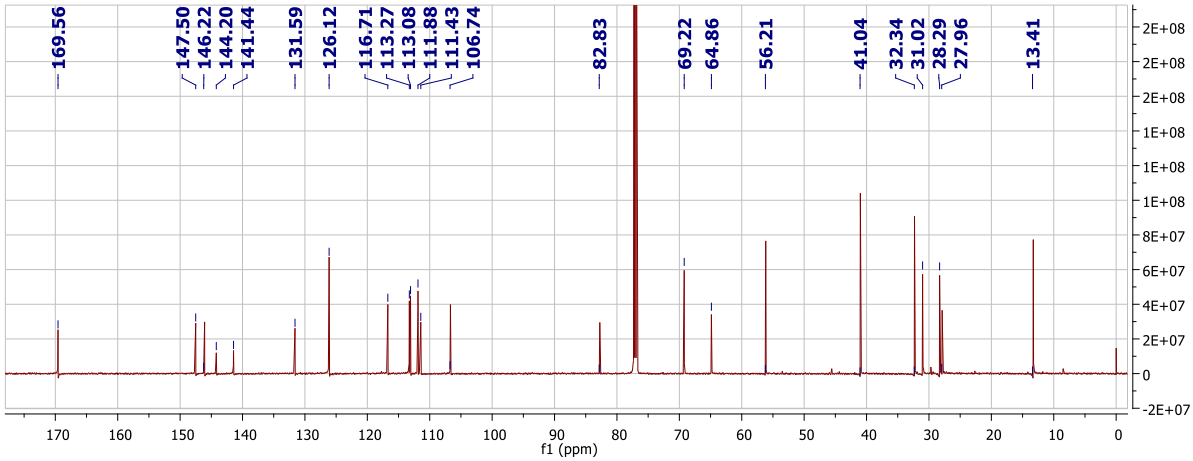


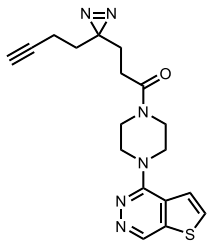




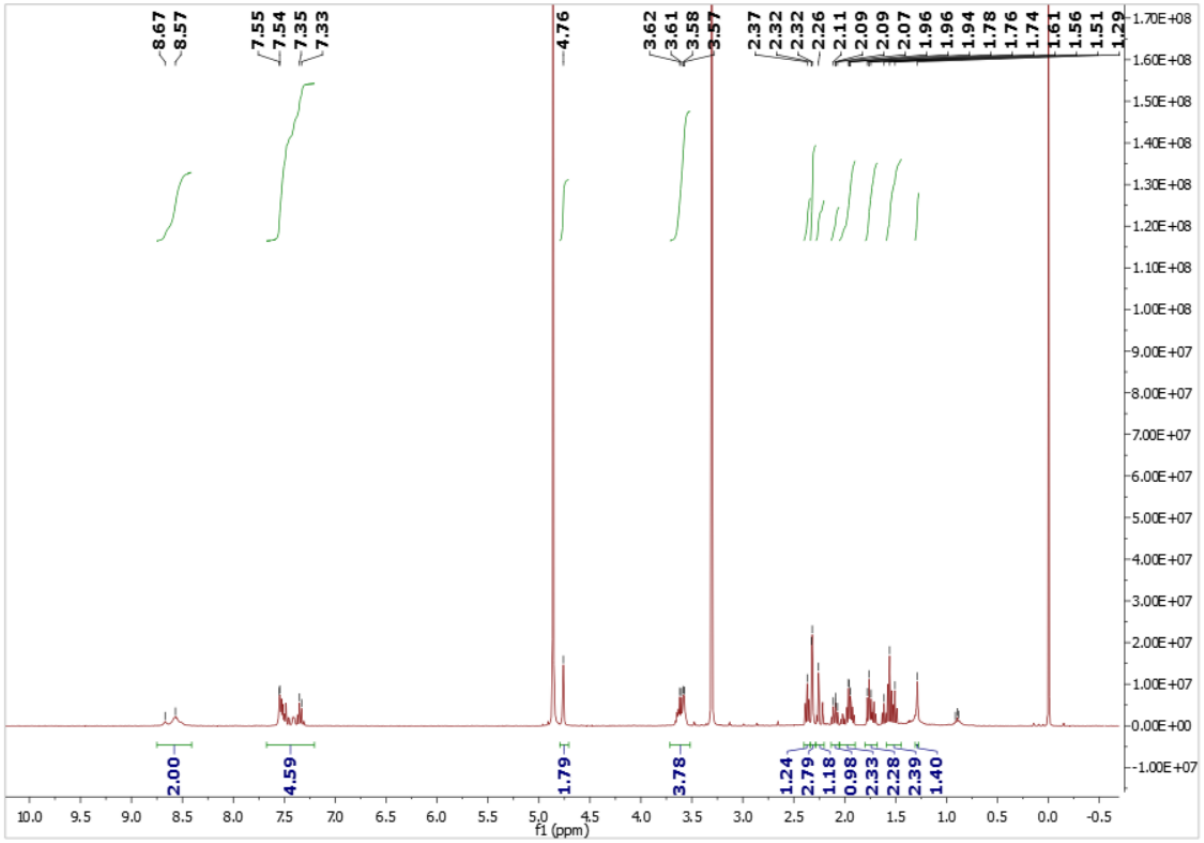
F2


8.73
8.71
8.41
7.89
7.88
7.55
7.45
7.37
7.34
2.40
2.36
2.33
2.31
2.27
2.25
1.97
1.97
1.93
1.77
1.73
1.57
1.50
2.23
2.09
2.11
1.00
2.11
2.16
1.75
3.17
0.87
2.12
2.11
2.12
f1 (ppm)


169.56
147.50
146.22
144.20
141.44
131.59
126.12
116.71
113.27
113.08
111.88
111.43
106.74
82.83
69.22
64.86
56.21
41.04
32.34
31.02
28.29
27.96
13.41
f1 (ppm)



F3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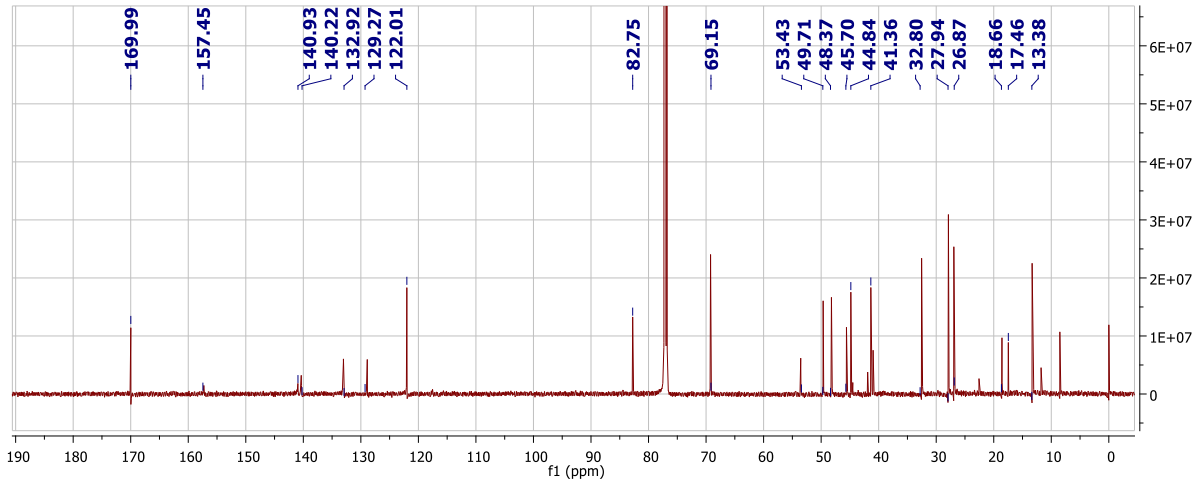
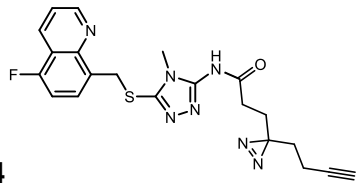
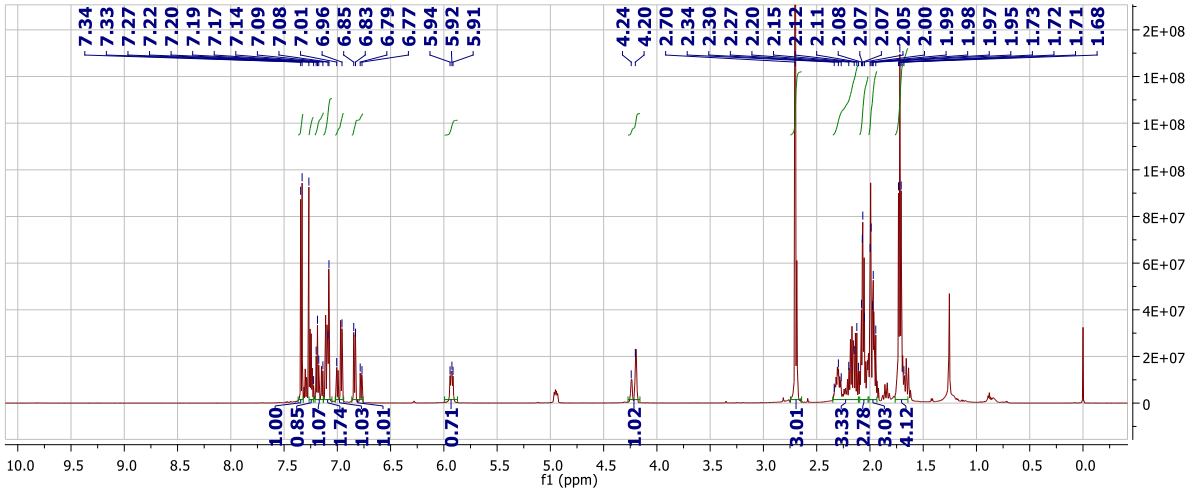
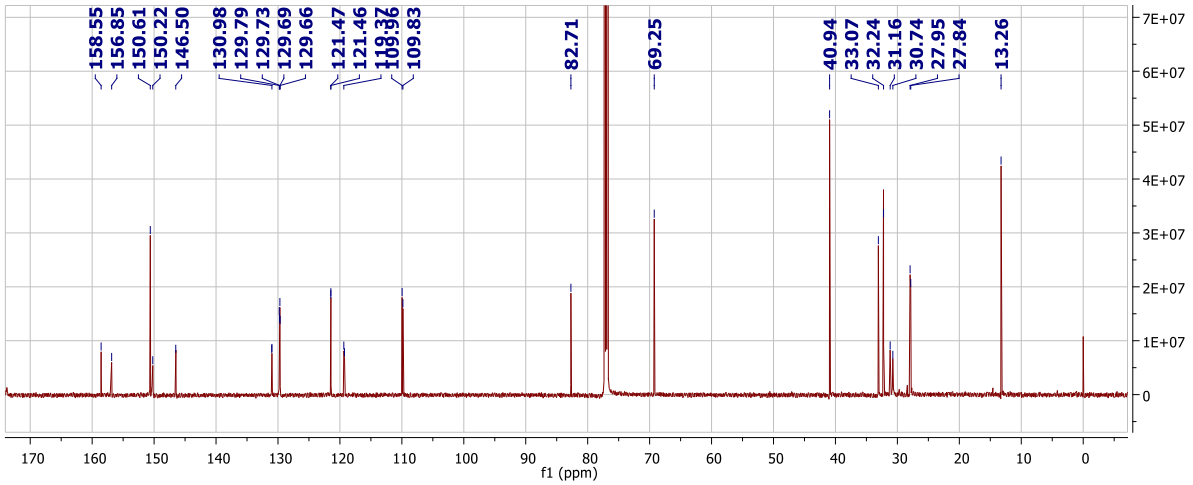
8.57
7.55
7.54
7.35
7.33
4.76
3.62
3.61
3.58
3.57
2.37
2.32
2.32
2.26
2.11
2.09
2.09
2.07
1.96
1.96
1.94
1.78
1.76
1.74
1.61
1.56
1.51
1.29
2.00
4.59
1.79
3.78
1.24
2.79
1.18
0.98
2.33
2.28
2.39
1.40
f1 (ppm)

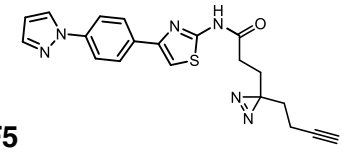

169.99
157.45
140.93
140.22
132.92
129.27
122.01
82.75
69.15
53.43
49.71
48.37
45.70
44.84
41.36
32.80
27.94
26.87
18.66
17.46
13.38
f1 (ppm)



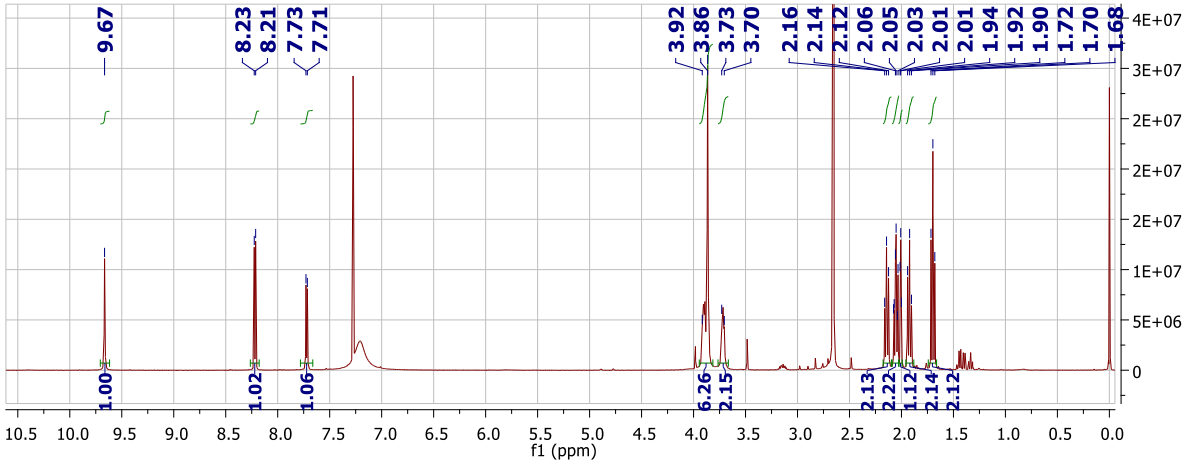
F4

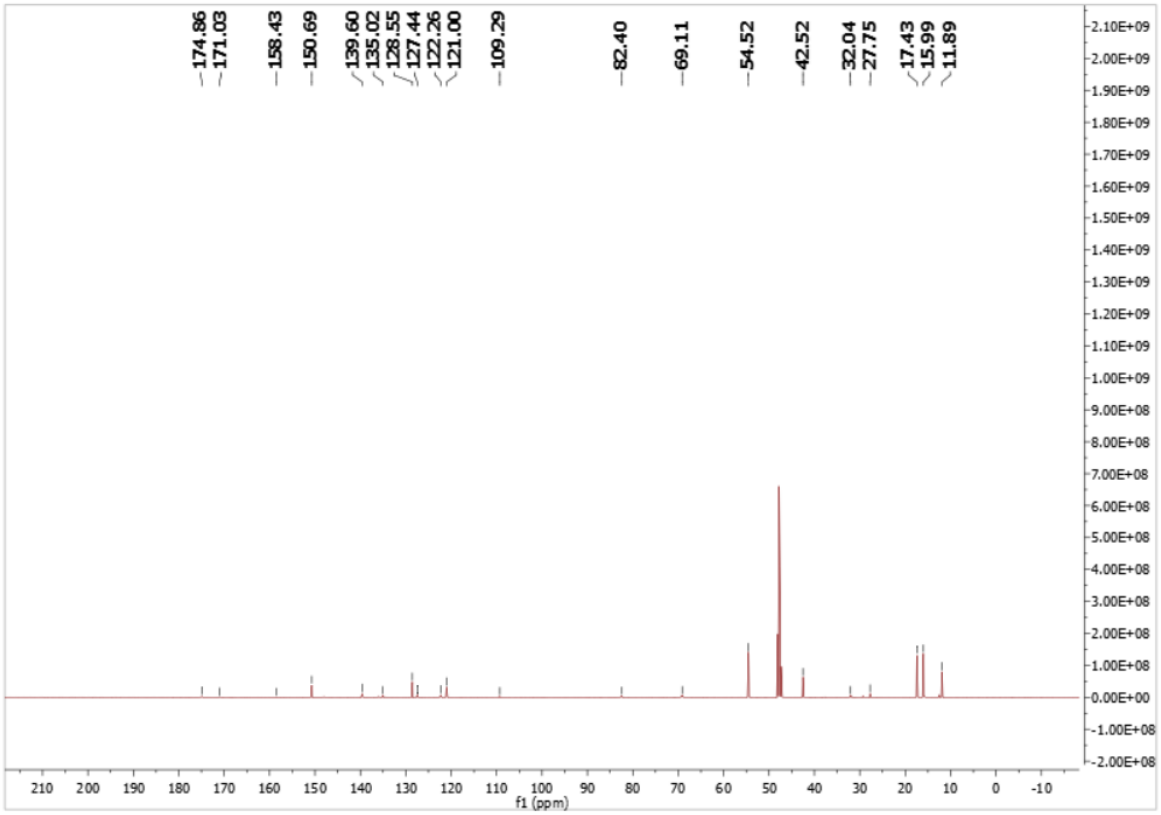

7.34 7.33 7.27 7.22 7.20 7.19 7.17 7.14 7.09 7.08 7.01 6.96 6.85 6.83 6.79 6.77 5.94 5.92 5.91
4.24 4.20 2.70 2.34 2.30 2.27 2.20 2.15 2.12 2.11 2.08 2.07 2.07 2.05 2.00 1.99 1.98 1.97 1.95 1.73 1.72 1.71 1.68
1.00 0.85 1.07 1.74 1.03 1.01 0.71 1.02 3.01 3.33 2.78 3.03 4.12
f1 (ppm)

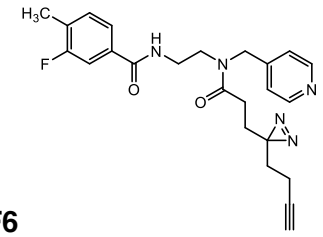

158.55 156.85 150.61 150.22 146.50 130.98 129.79 129.73 129.69 129.66 121.47 121.46 82.71 69.25 40.94 33.07 32.24 31.16 30.74 27.95 27.84 13.26
f1 (ppm)



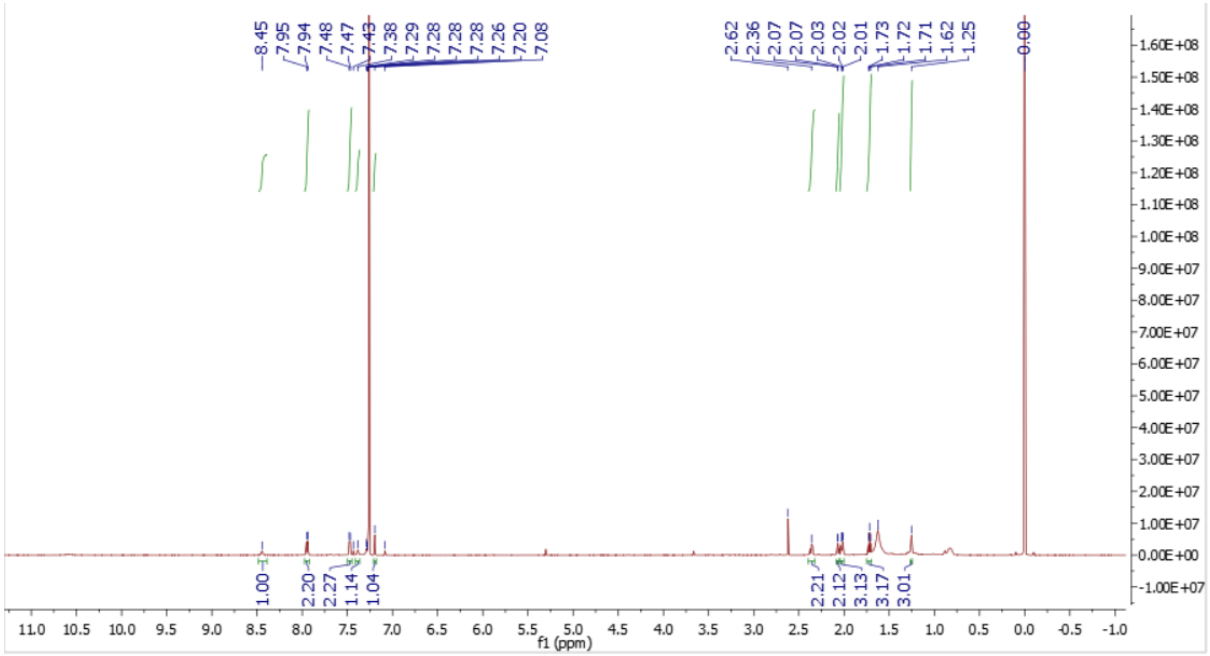
F5


9.67
8.23
8.21
7.73
7.71
3.92
3.86
3.73
3.70
2.16
2.14
2.12
2.06
2.05
2.03
2.01
2.01
1.94
1.92
1.90
1.72
1.70
1.68
1.00
1.02
1.06
6.26
2.15
2.13
2.22
1.12
2.14
2.12
f1 (ppm)


174.86
171.03
158.43
150.69
139.60
135.02
128.55
127.44
122.26
121.00
109.29
82.40
69.11
54.52
42.52
32.04
27.75
17.43
15.99
11.89
f1 (ppm)



F6


8.45
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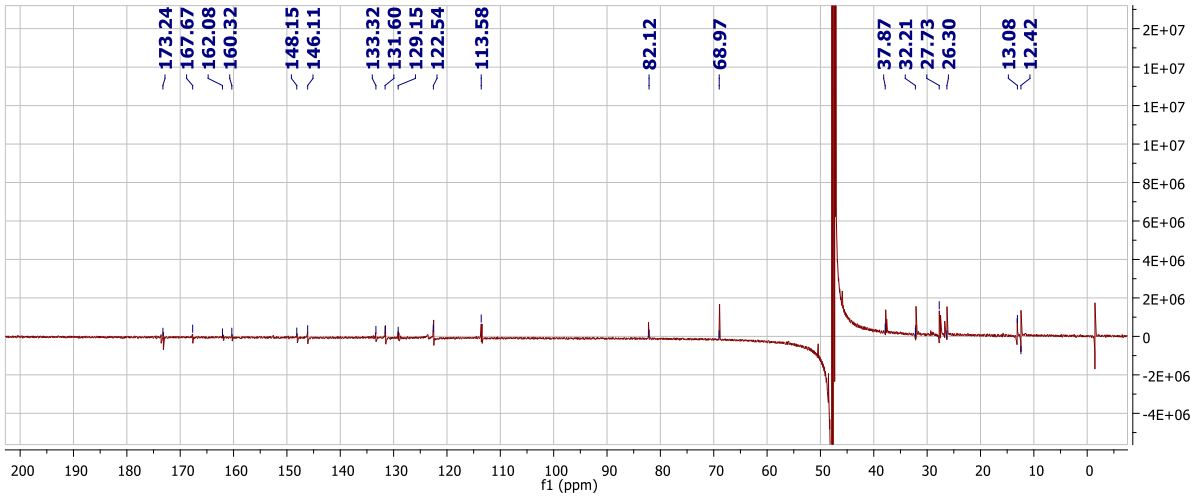
7.94
7.48
7.47
7.43
7.38
7.29
7.28
7.28
7.28
7.26
7.20
7.08
2.62
2.36
2.07
2.07
2.03
2.02
2.01
1.73
1.72
1.71
1.62
1.25
0.00
1.00
2.20
2.27
1.14
1.04
2.21
2.12
3.13
3.17
3.01
f1 (ppm)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160.32
148.15
146.11
133.32
131.60
129.15
122.54
113.58
82.12
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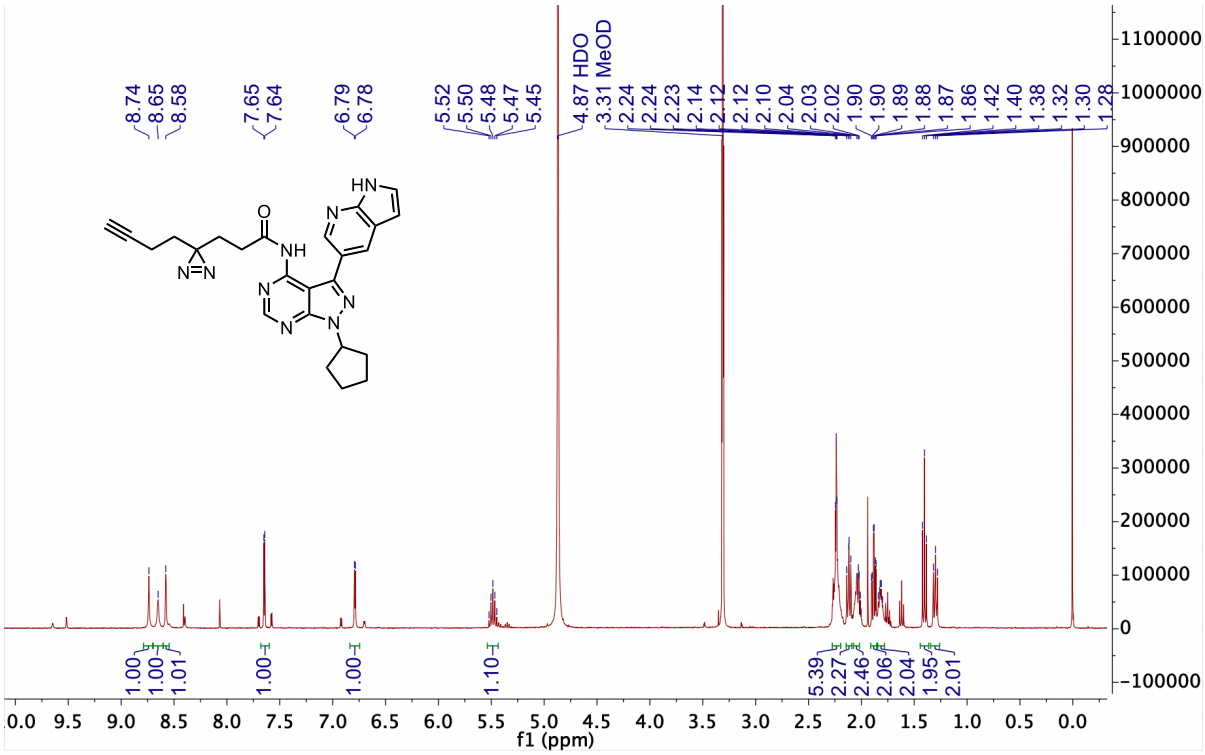
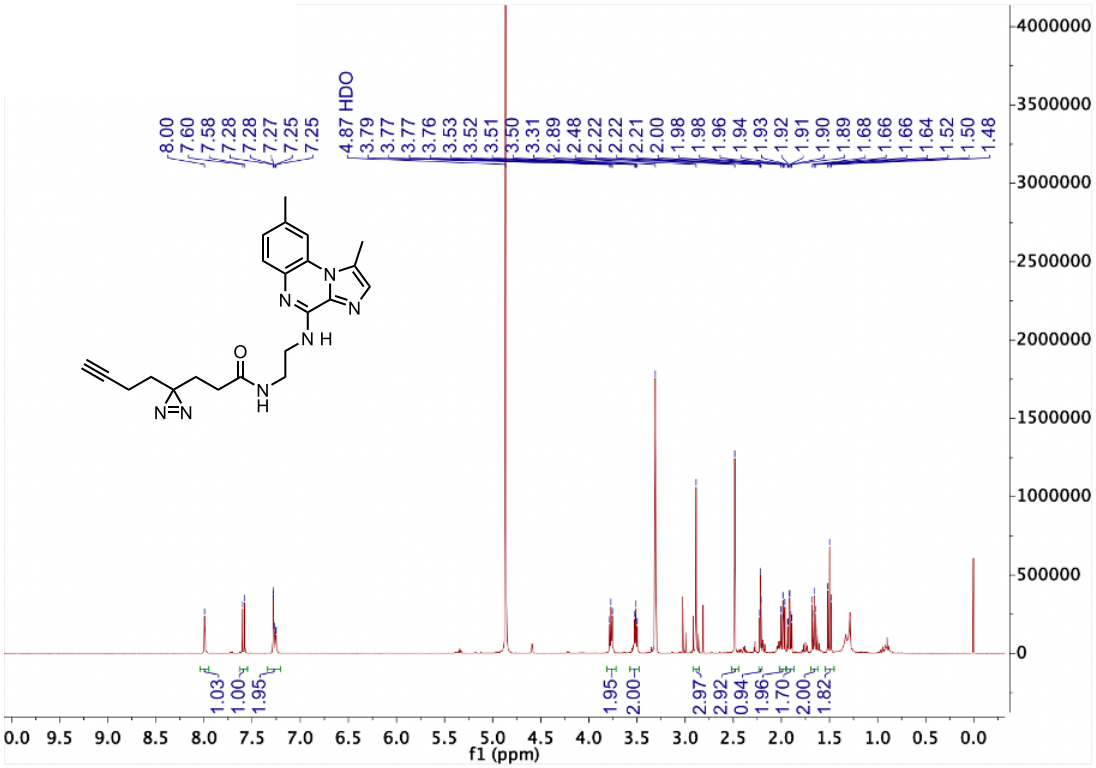
27.73
26.30
13.08
12.42
f1 (ppm)

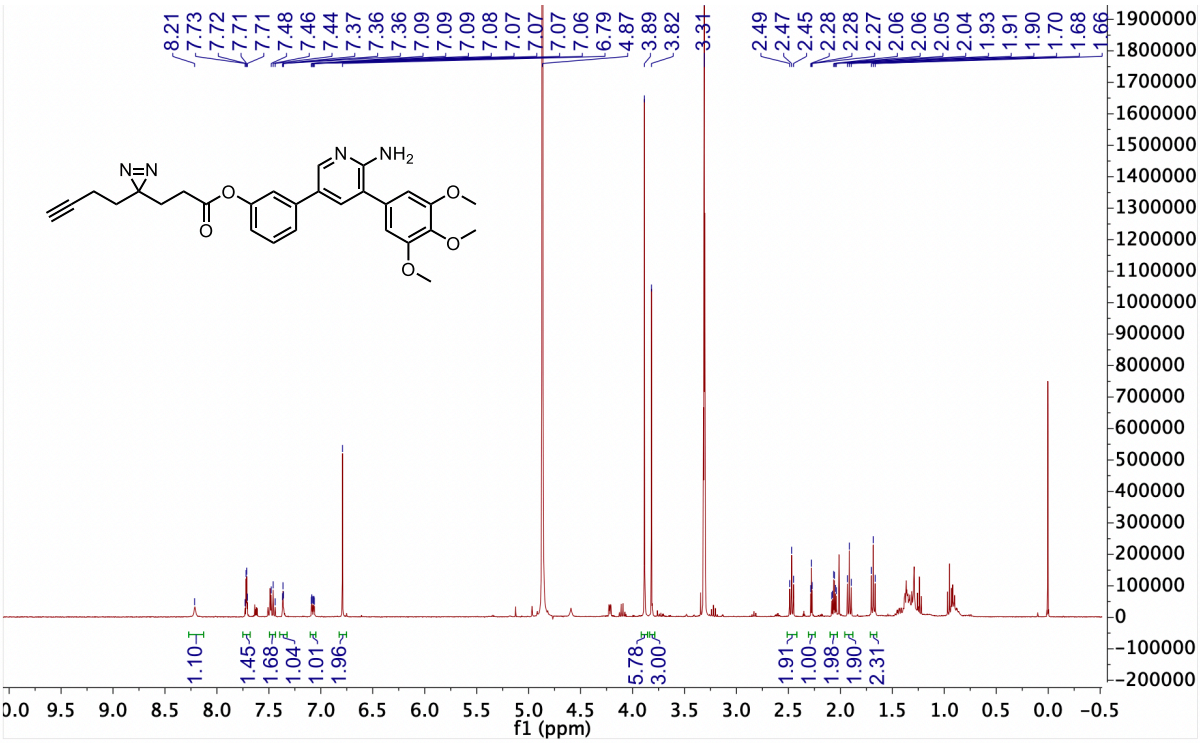
F7


8.74
8.65
8.58
7.65
7.64
6.79
6.78
5.52
5.50
5.48
5.47
5.45
4.87 HDO
3.31 MeOD
2.24
2.24
2.23
2.14
2.12
2.12
2.10
2.04
2.03
2.02
1.90
1.90
1.89
1.88
1.87
1.86
1.42
1.40
1.38
1.32
1.30
1.28
1.00
1.00
1.01
1.00
1.00
1.10
5.39
2.27
2.46
2.06
2.04
1.95
2.01
f1 (ppm)

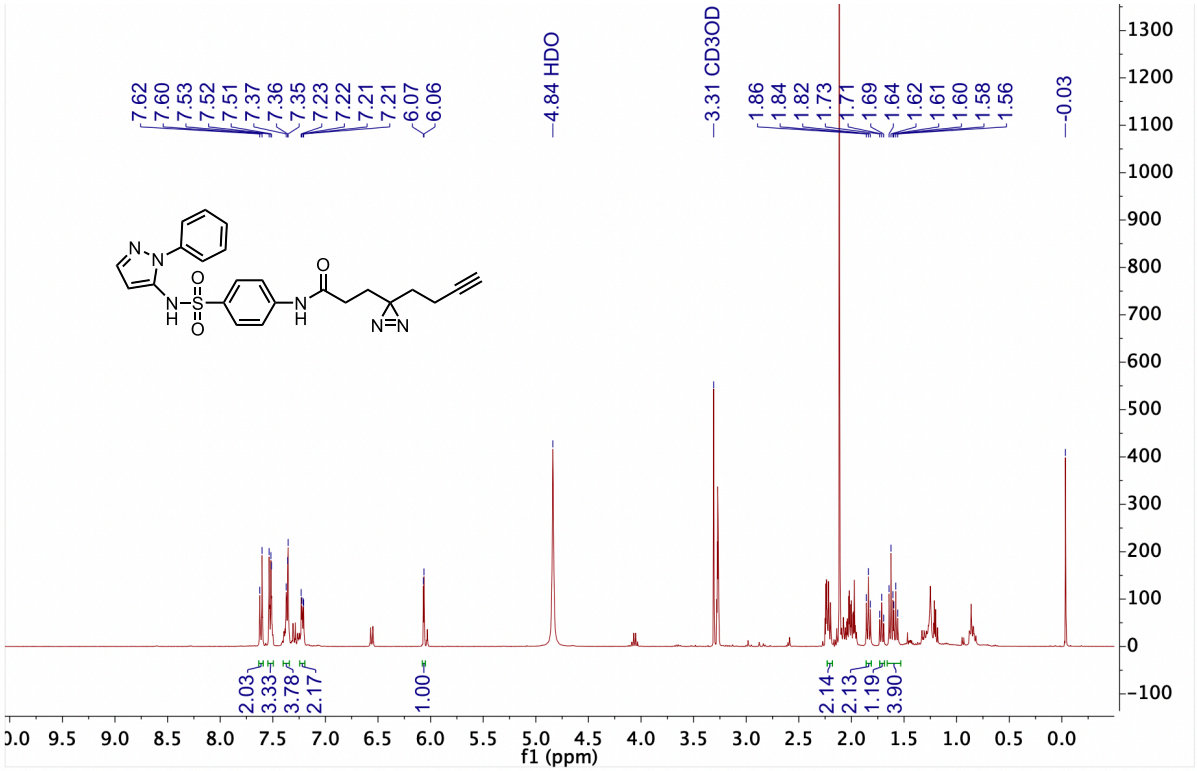
F8


8.00
7.60
7.58
7.28
7.28
7.27
7.25
7.25
4.87 HDO
3.79
3.77
3.77
3.76
3.53
3.52
3.51
3.50
3.31
2.89
2.48
2.22
2.22
2.21
2.00
1.98
1.98
1.96
1.94
1.93
1.92
1.91
1.90
1.89
1.68
1.66
1.66
1.64
1.52
1.50
1.48
1.03
1.00
1.95
1.95
2.00
2.97
2.92
0.94
1.96
1.70
2.00
1.82
f1 (ppm)

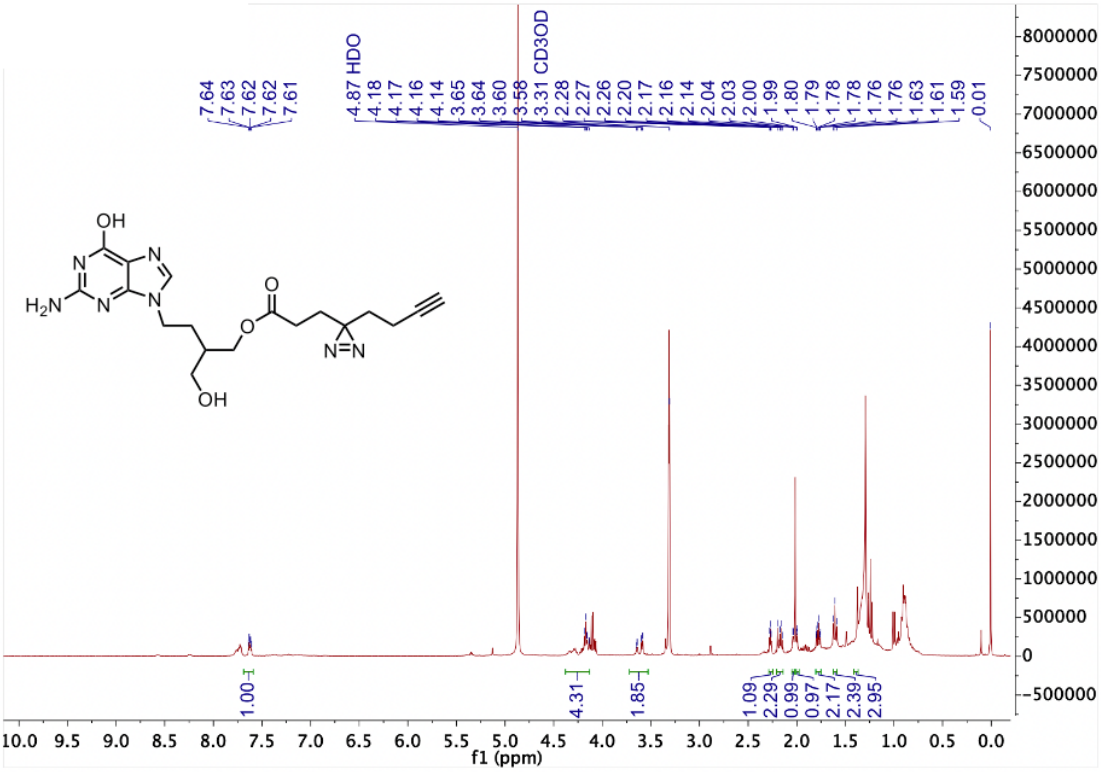
F9


8.21 7.73 7.72 7.71 7.71 7.48 7.46 7.44 7.37 7.36 7.36 7.09 7.09 7.09 7.08 7.07 7.07 7.07 7.06 6.79 4.87 3.89 3.82 3.31 2.49 2.47 2.45 2.28 2.28 2.27 2.06 2.06 2.05 2.04 1.93 1.91 1.90 1.70 1.68 1.66
1.10 1.45 1.68 1.04 1.01 1.96 5.78 3.00 1.91 1.00 1.98 1.90 2.31
f1 (ppm)

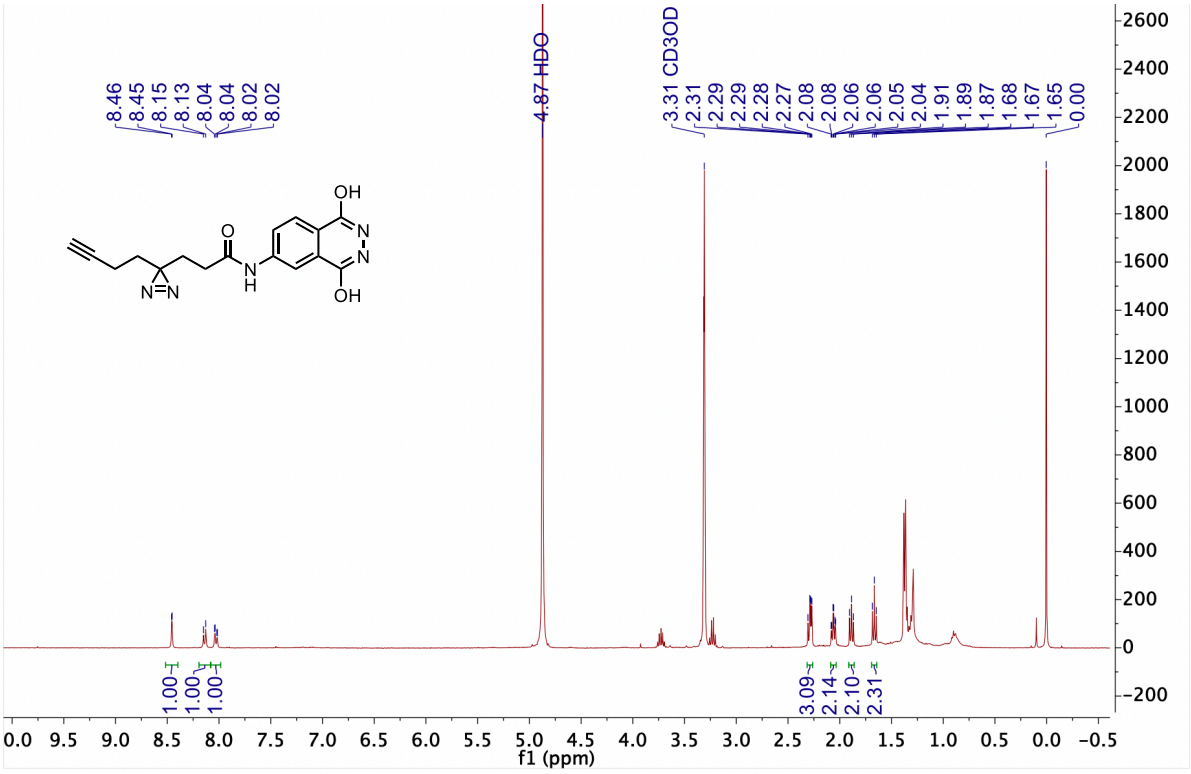
F10


7.62 7.60 7.53 7.52 7.51 7.37 7.36 7.35 7.23 7.22 7.21 7.21 6.07 6.06
4.84 HDO
3.31 CD3OD
1.86 1.84 1.82 1.73 1.71 1.69 1.64 1.62 1.61 1.60 1.58 1.56
0.03
2.03 3.33 3.78 2.17 1.00 2.14 2.13 1.19 3.90
f1 (ppm)

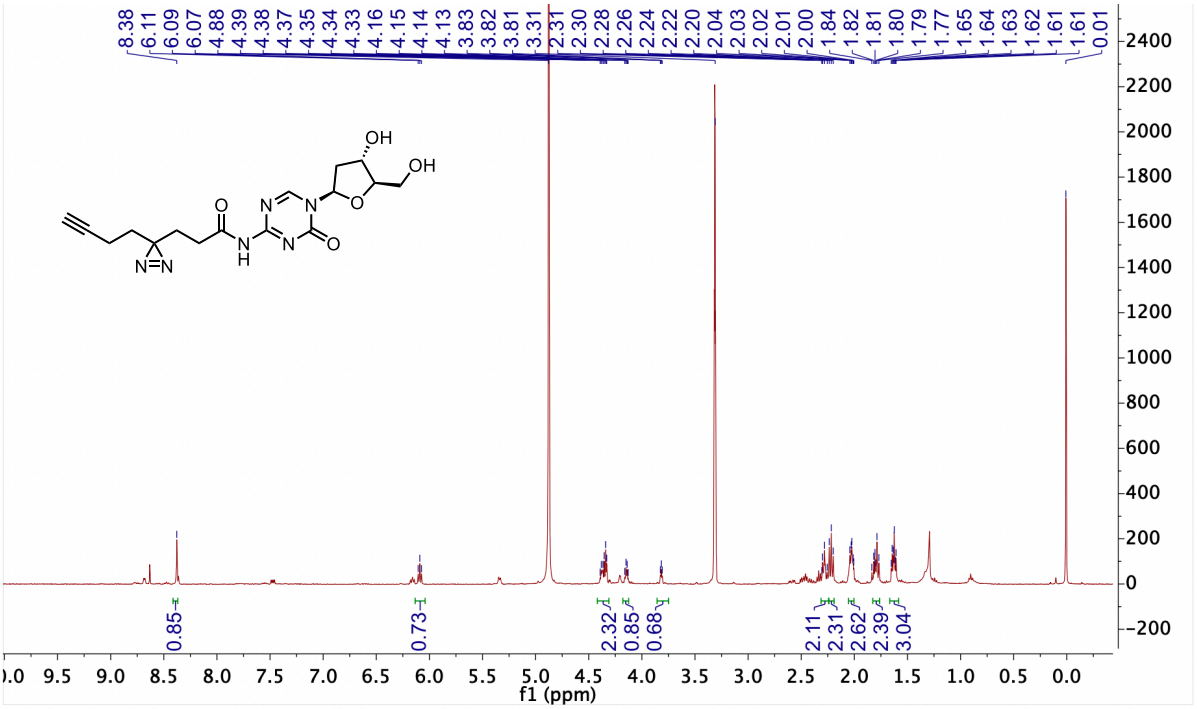
F11


7.64
7.63
7.62
7.62
7.61
4.87 HDO
4.18
4.17
4.16
4.14
3.65
3.64
3.60
3.58
3.31 CD3OD
2.28
2.27
2.26
2.20
2.17
2.16
2.14
2.04
2.03
2.00
1.99
1.80
1.79
1.78
1.78
1.76
1.76
1.63
1.61
1.59
0.01
OH
N
N
N
N
H2N
O
O
N=N
OH
1.00
4.31
1.85
1.09
2.29
0.99
0.97
2.17
2.39
2.95
f1 (ppm)

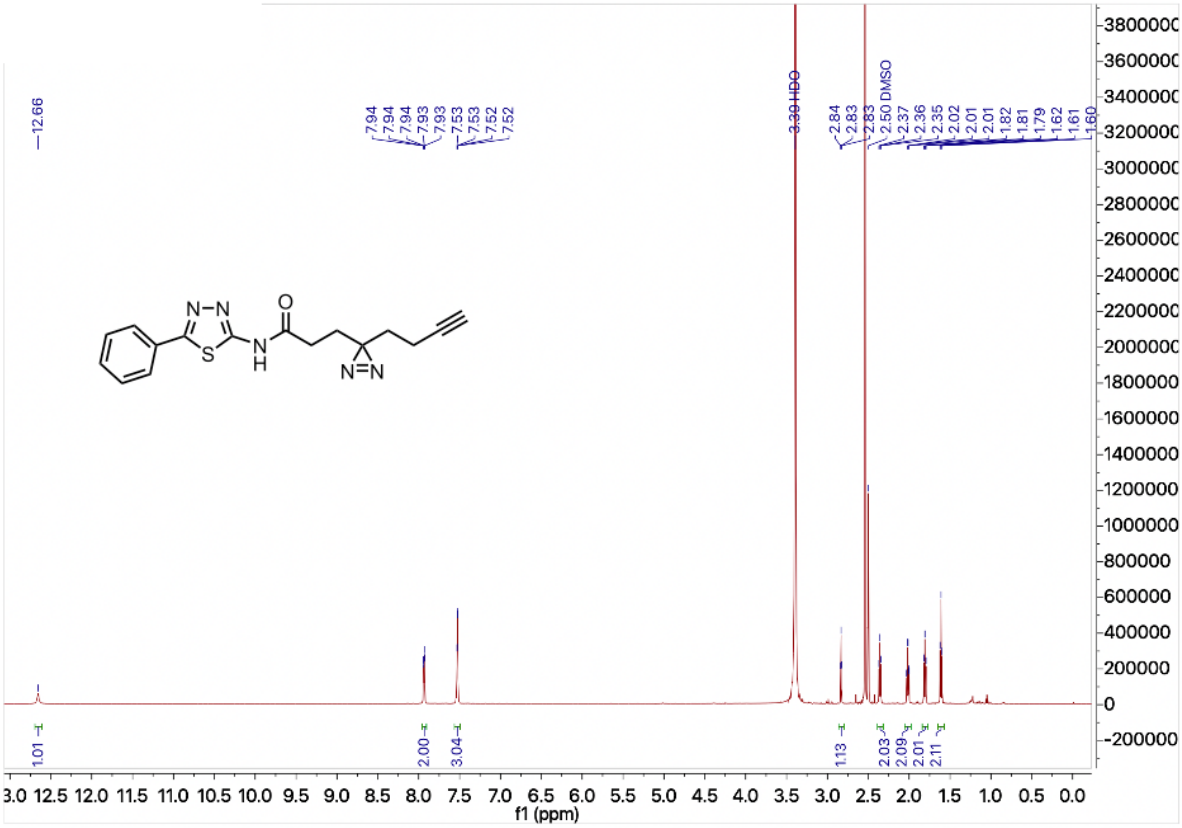
F12


8.46
8.45
8.15
8.13
8.04
8.04
8.02
8.02
4.87 HDO
3.31 CD3OD
2.31
2.29
2.29
2.28
2.27
2.08
2.08
2.06
2.06
2.05
2.04
1.91
1.89
1.87
1.68
1.67
1.65
0.00
OH
O
N
N
N
H
OH
1.00
1.00
1.00
3.09
2.14
2.10
2.31
f1 (ppm)

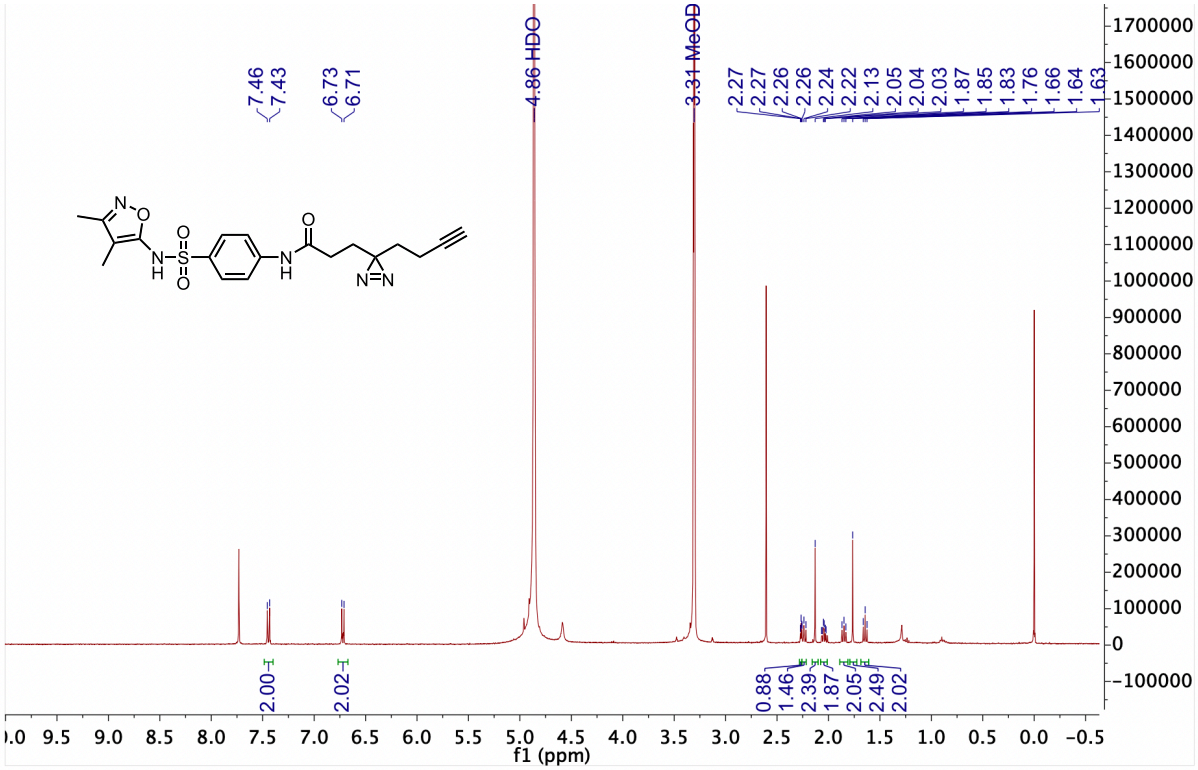
F13



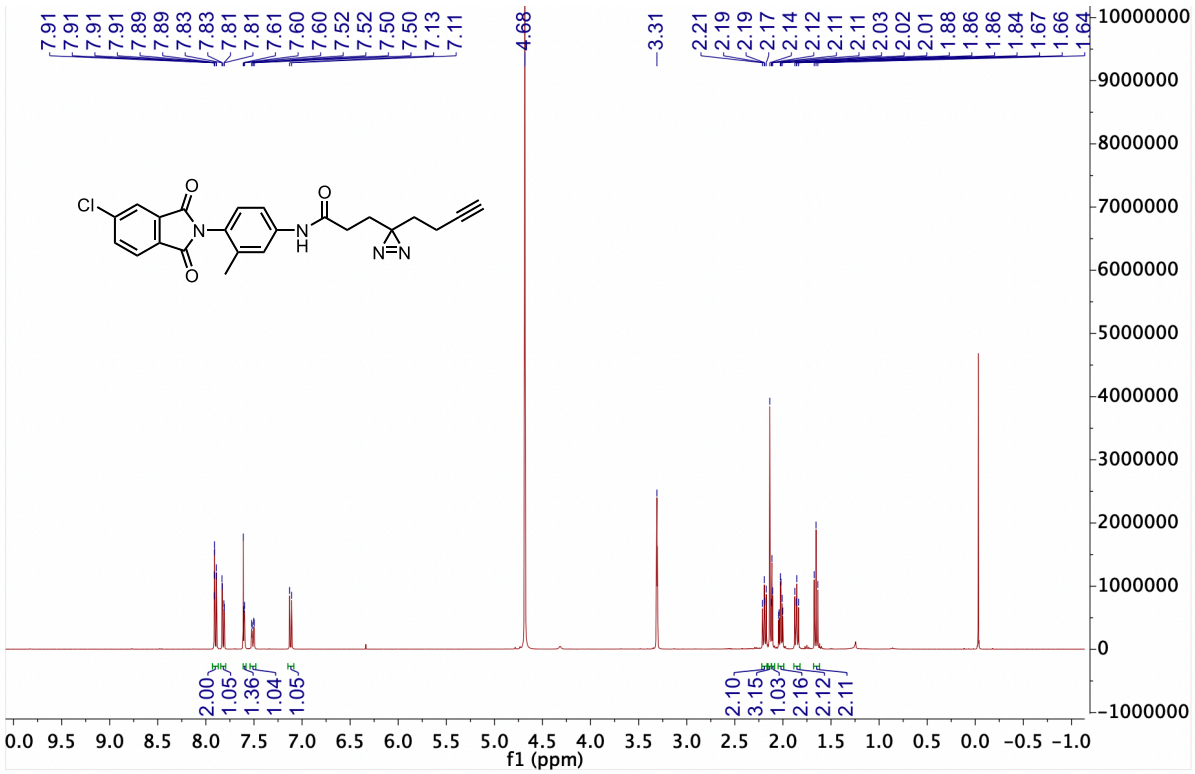
F14



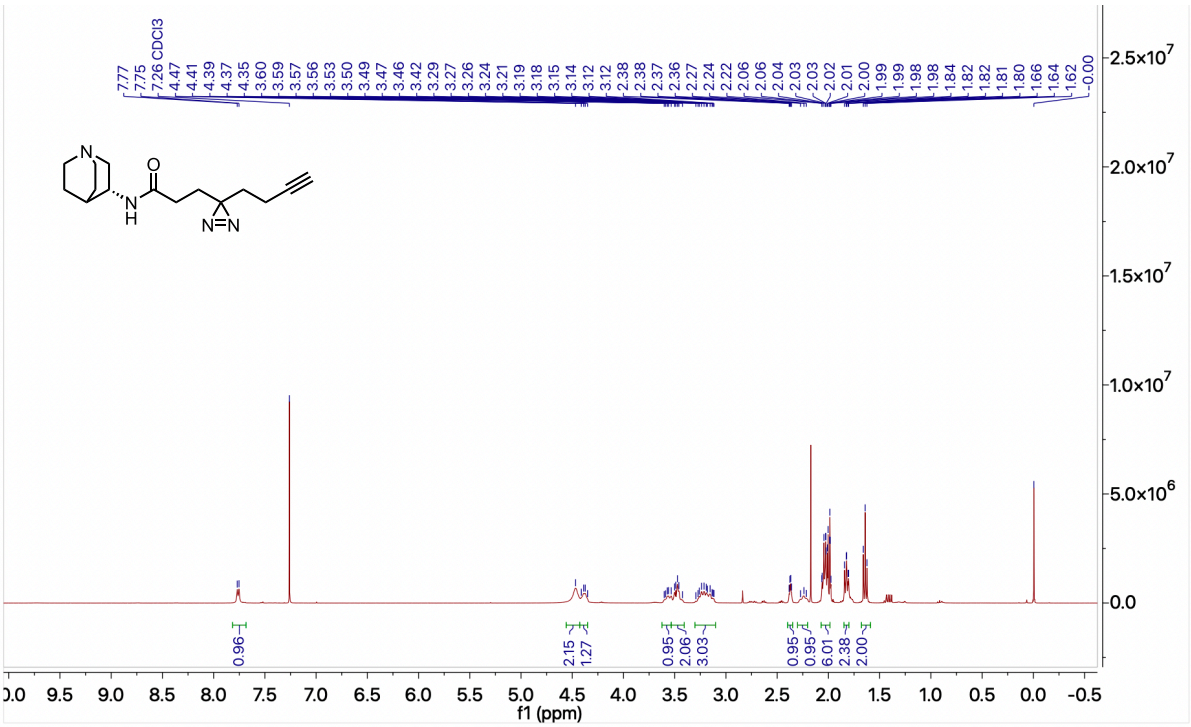
F15


7.46
7.43
6.73
6.71
4.86 HDO
3.31 MeOD
2.27
2.27
2.26
2.26
2.24
2.22
2.13
2.05
2.04
2.03
1.87
1.85
1.83
1.76
1.66
1.64
1.63
2.00
2.02
0.88
1.46
2.39
1.87
2.05
2.49
2.02
f1 (ppm)

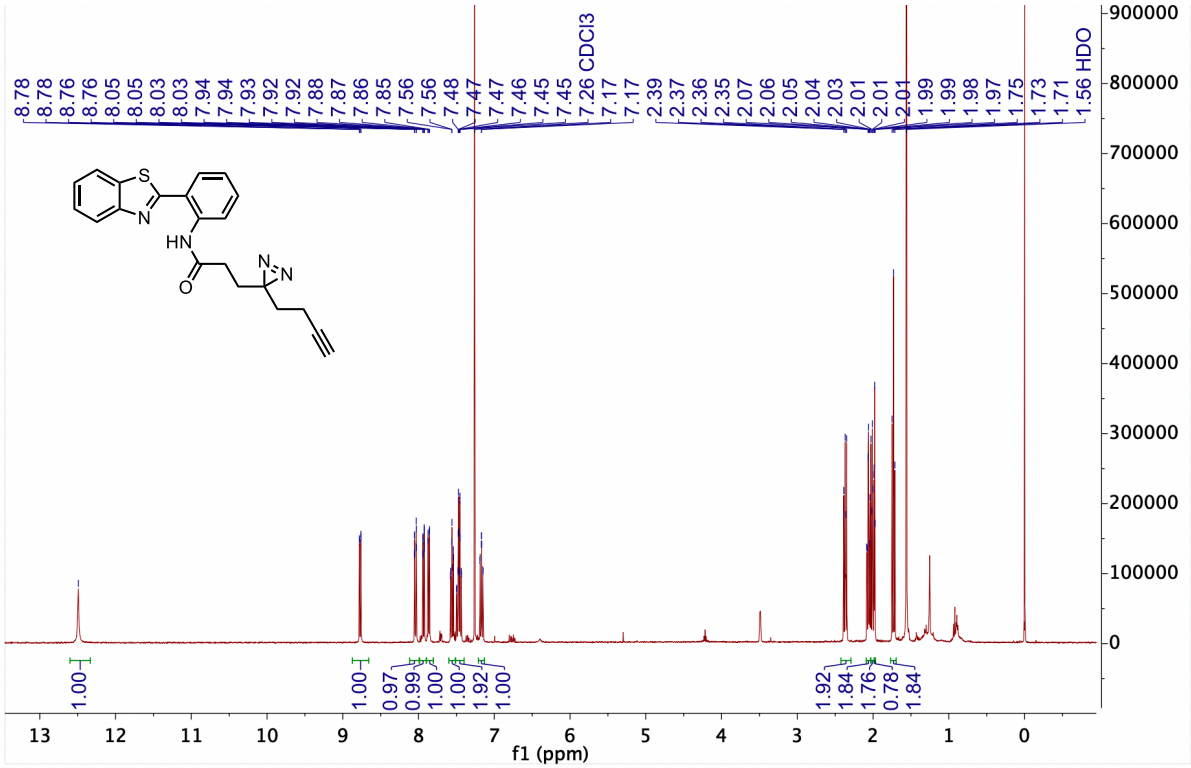
F16


7.91
7.91
7.91
7.91
7.89
7.89
7.83
7.83
7.81
7.81
7.61
7.60
7.60
7.52
7.52
7.50
7.50
7.13
7.11
4.68
3.31
2.21
2.19
2.19
2.17
2.14
2.12
2.11
2.11
2.03
2.02
2.01
1.88
1.86
1.86
1.84
1.67
1.66
1.64
2.00
1.05
1.36
1.04
1.05
2.10
3.15
1.03
2.16
2.12
2.11
f1 (ppm)

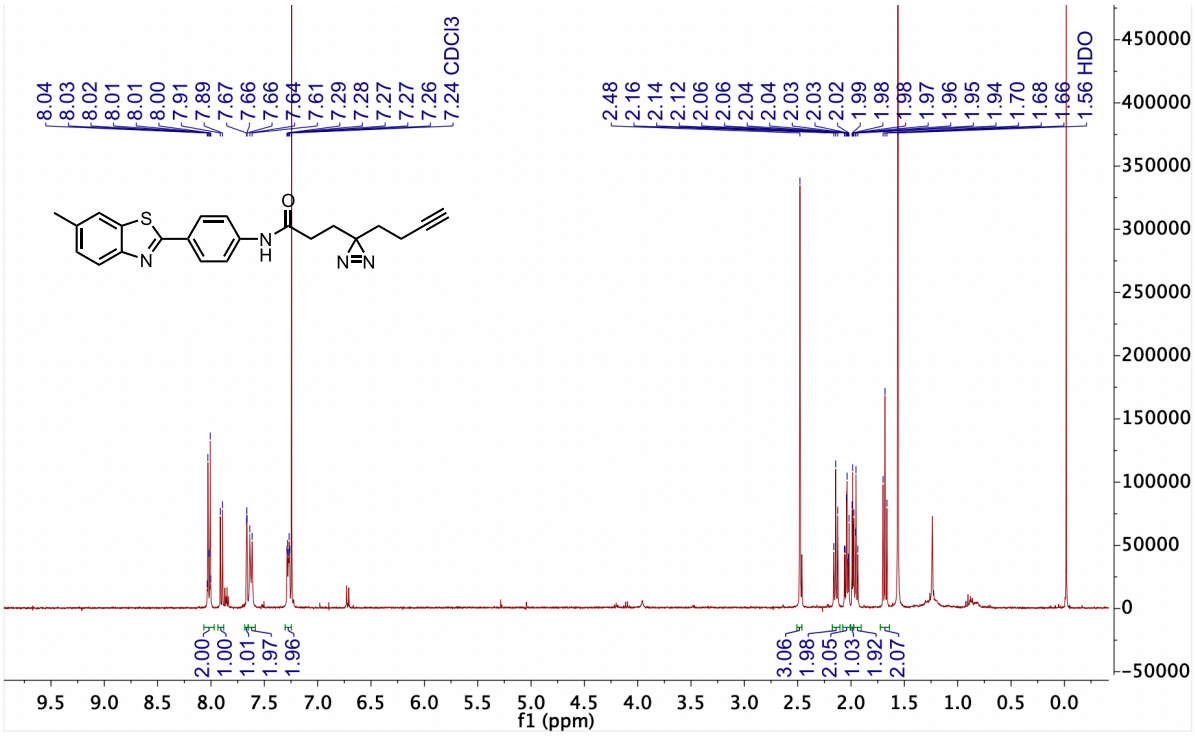
F17


7.77
7.75
7.26 CDCl3
4.47
4.41
4.39
4.37
4.35
3.60
3.59
3.57
3.56
3.53
3.50
3.49
3.47
3.46
3.42
3.29
3.27
3.26
3.24
3.21
3.19
3.18
3.15
3.14
3.12
3.12
2.38
2.38
2.37
2.36
2.27
2.24
2.22
2.06
2.06
2.04
2.03
2.03
2.02
2.01
2.00
1.99
1.99
1.98
1.84
1.82
1.82
1.81
1.80
1.66
1.64
1.62
-0.00
2.5x10^7
2.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
0.0
0.96
2.15
1.27
0.95
2.06
3.03
0.95
0.95
6.01
2.38
2.00
f1 (ppm)

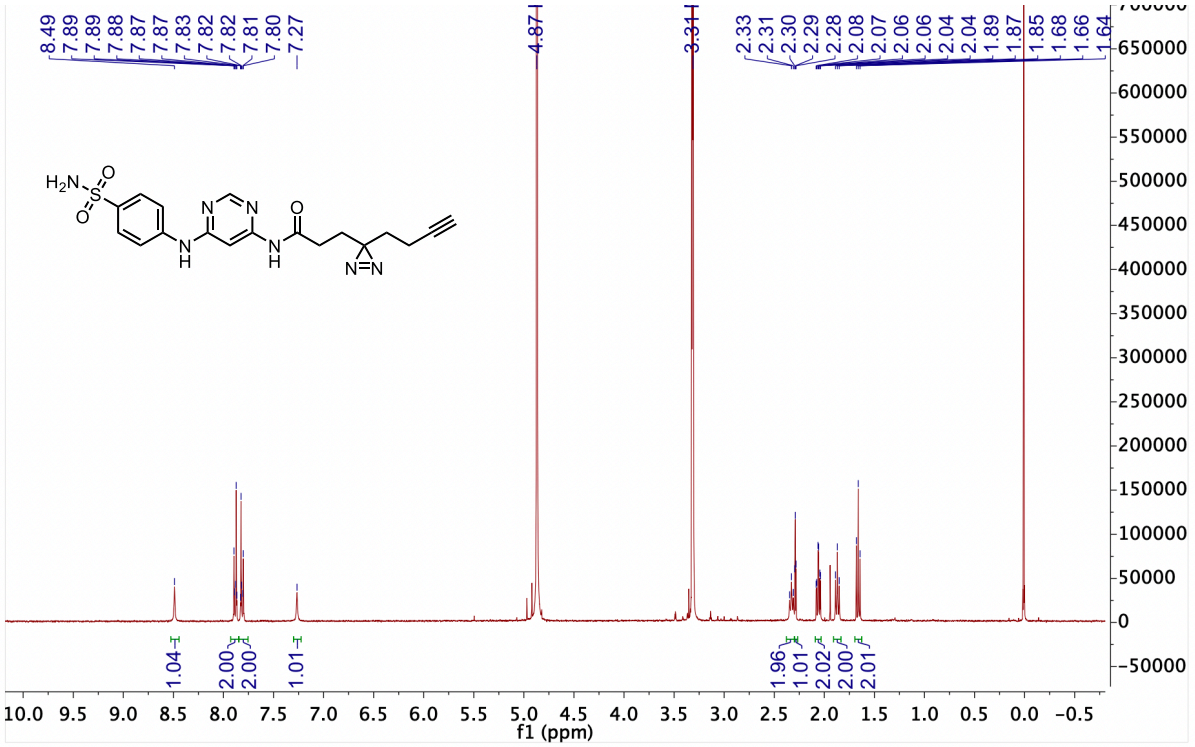
F18


8.78
8.78
8.76
8.76
8.05
8.05
8.03
8.03
7.94
7.94
7.93
7.92
7.92
7.88
7.87
7.86
7.85
7.56
7.56
7.48
7.47
7.47
7.46
7.45
7.45
7.26 CDCl3
7.17
7.17
2.39
2.37
2.36
2.35
2.07
2.06
2.05
2.04
2.03
2.01
2.01
2.01
1.99
1.99
1.98
1.97
1.75
1.73
1.71
1.56 HDO
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
1.00
1.00
0.97
0.99
1.00
1.00
1.92
1.00
1.92
1.84
1.76
0.78
1.84
f1 (ppm)

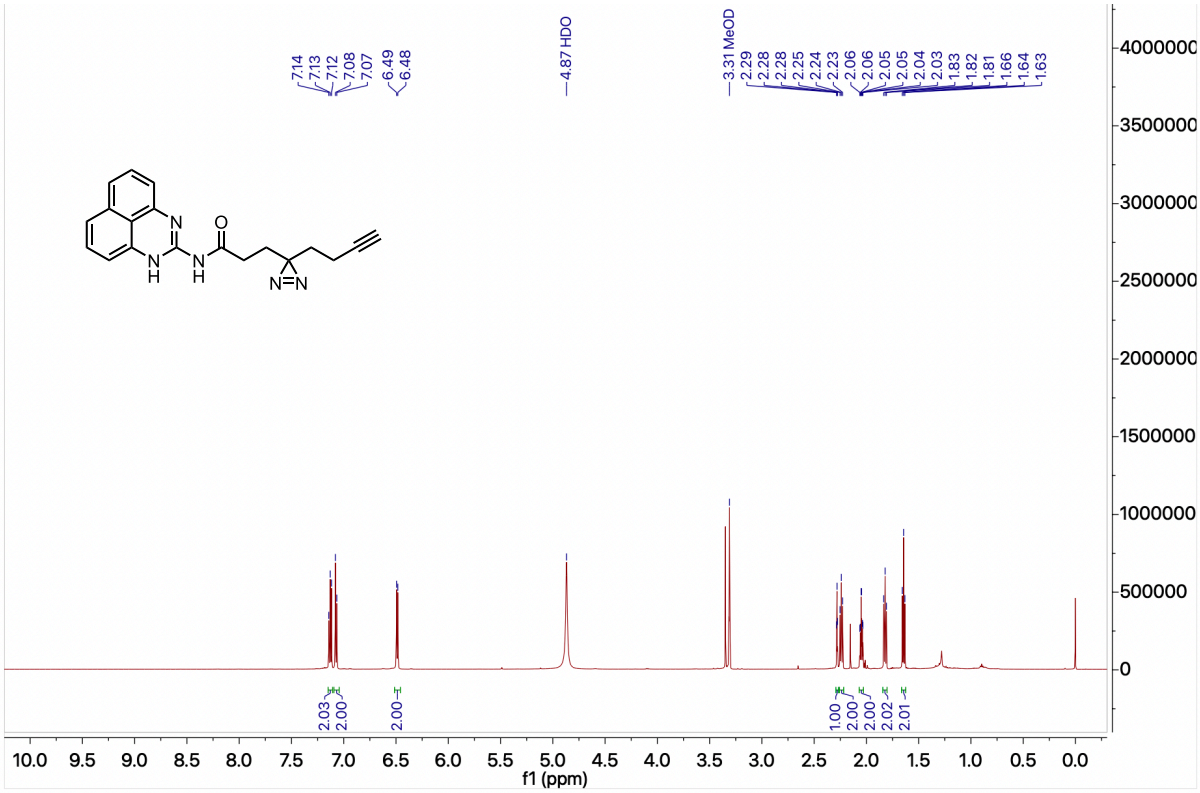
F19


8.04
8.03
8.02
8.01
8.01
8.00
7.91
7.89
7.67
7.66
7.66
7.64
7.61
7.29
7.28
7.27
7.26
7.24 CDCl3
2.48
2.16
2.14
2.12
2.06
2.06
2.04
2.04
2.03
2.03
2.02
1.99
1.98
1.97
1.96
1.95
1.94
1.70
1.68
1.66
1.56 HDO
2.00
1.00
1.01
1.97
1.96
3.06
1.98
2.05
1.03
1.92
2.07
f1 (ppm)

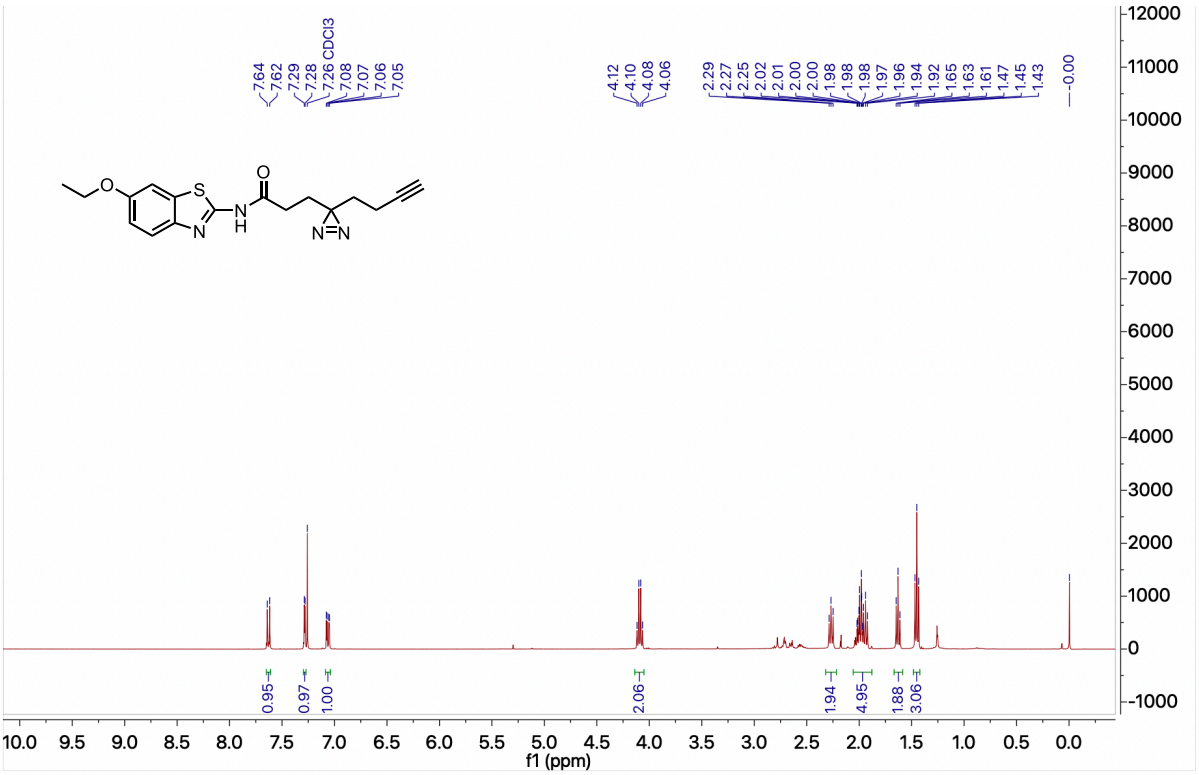
F20


8.49
7.89
7.89
7.88
7.87
7.87
7.83
7.82
7.82
7.81
7.80
7.27
4.87
3.31
2.33
2.31
2.30
2.29
2.28
2.08
2.07
2.06
2.06
2.04
2.04
1.89
1.87
1.85
1.68
1.66
1.64
1.04
2.00
2.00
1.01
1.96
1.01
2.02
2.00
2.01
f1 (ppm)

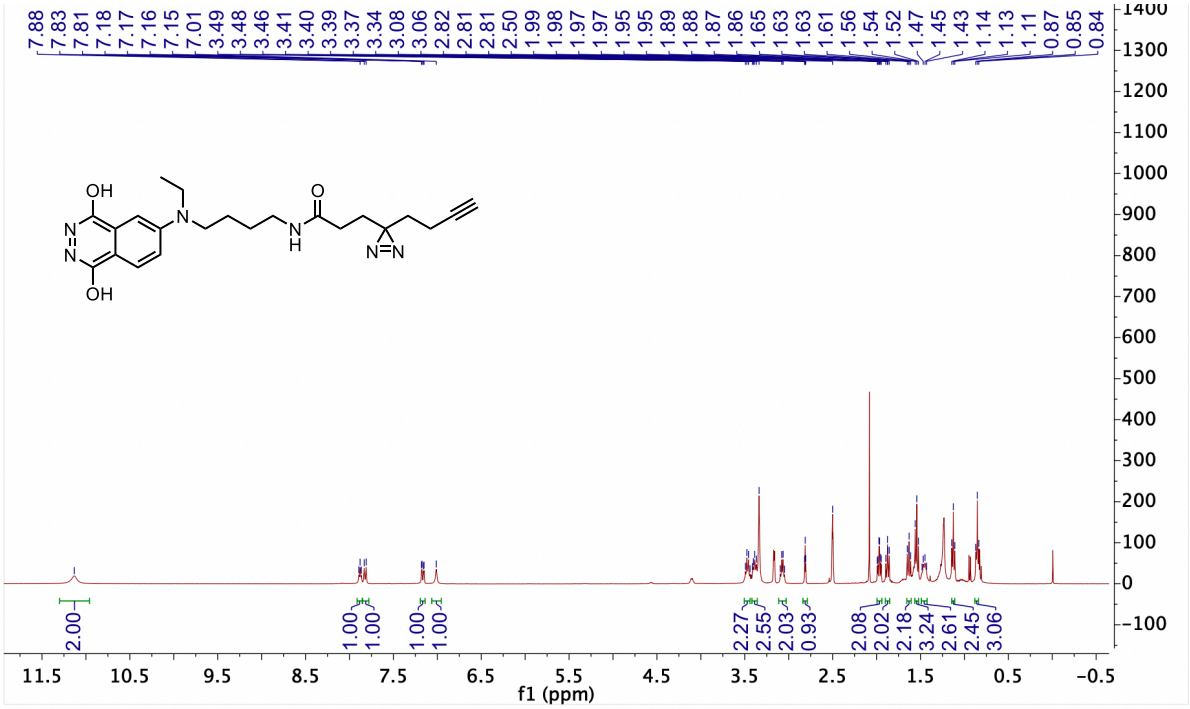
F21


7.14
7.13
7.12
7.08
7.07
6.49
6.48
4.87 HDO
3.31 MeOD
2.29
2.28
2.28
2.25
2.24
2.23
2.06
2.06
2.05
2.05
2.04
2.03
1.83
1.82
1.81
1.66
1.64
1.63
2.03
2.00
2.00
1.00
2.00
2.00
2.02
2.01
f1 (ppm)

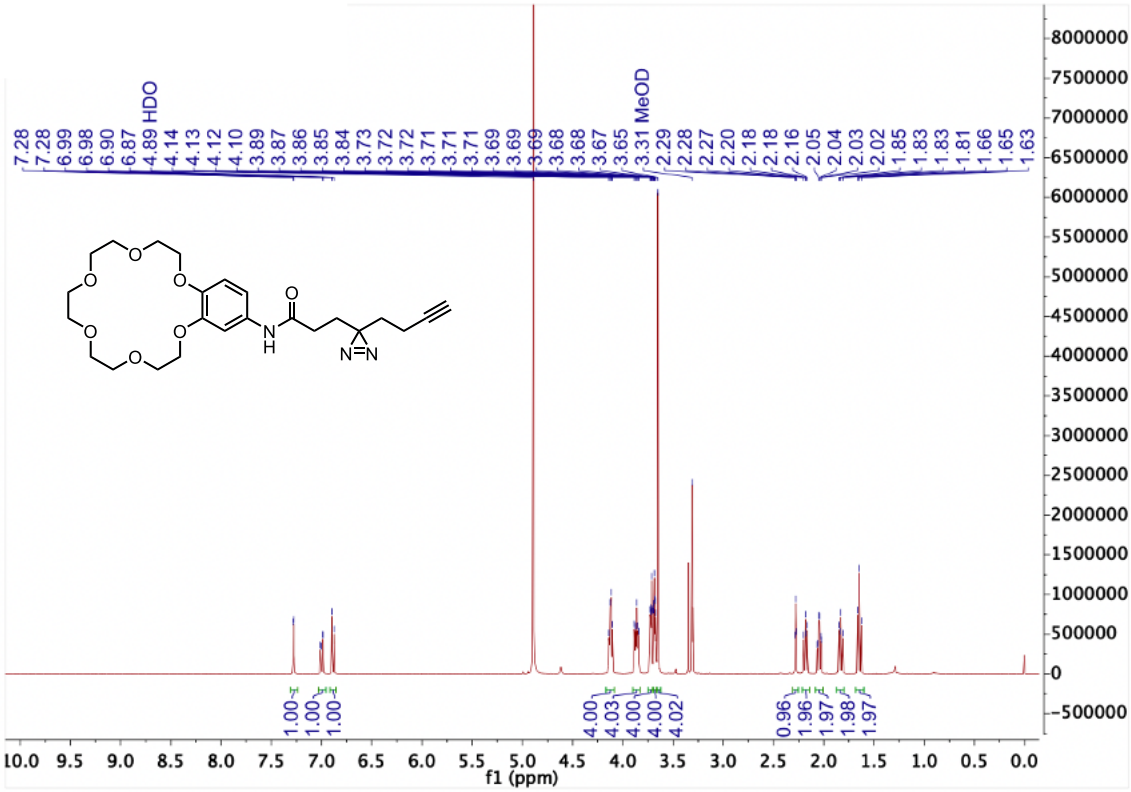
F22


7.64
7.62
7.29
7.28
7.26 CDCl3
7.08
7.07
7.06
7.05
4.12
4.10
4.08
4.06
2.29
2.27
2.25
2.02
2.01
2.00
2.00
1.98
1.98
1.98
1.97
1.96
1.94
1.92
1.65
1.63
1.61
1.47
1.45
1.43
0.00
0.95
0.97
1.00
2.06
1.94
4.95
1.88
3.06
f1 (ppm)

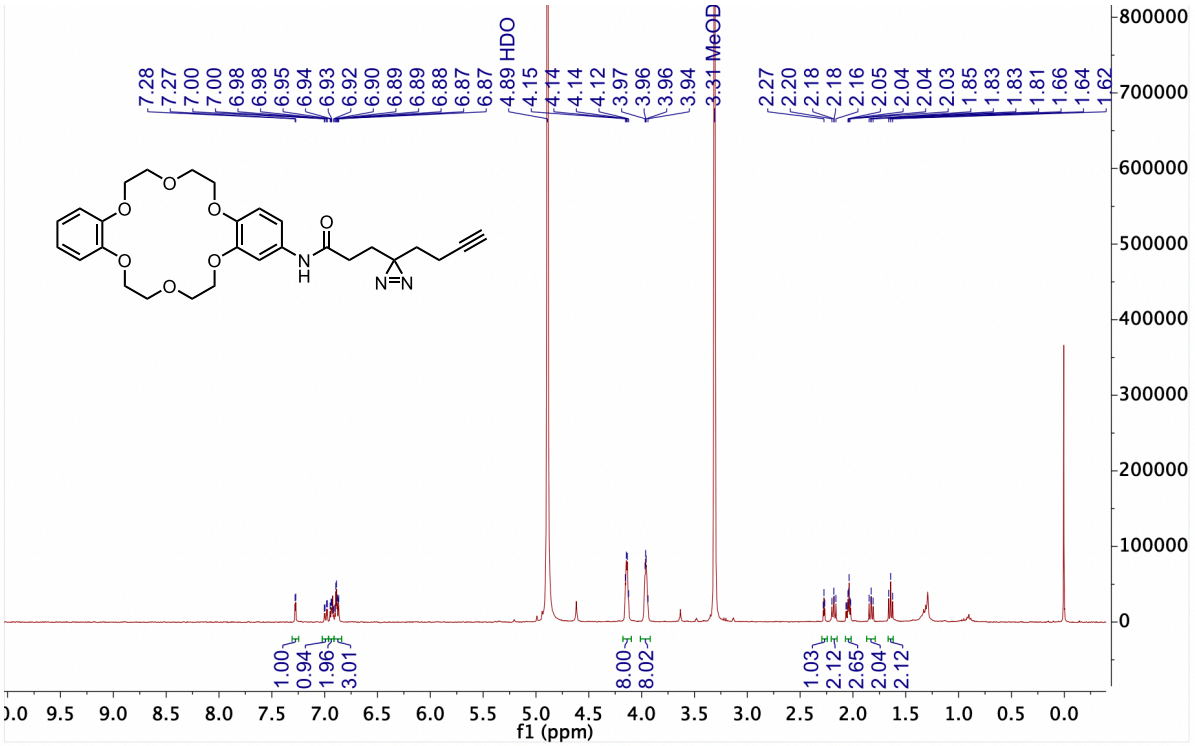
F23


7.88 7.83 7.81 7.18 7.17 7.16 7.15 7.01 3.49 3.48 3.46 3.41 3.40 3.39 3.37 3.34 3.08 3.06 2.82 2.81 2.81 2.50 1.99 1.98 1.97 1.97 1.95 1.95 1.89 1.88 1.87 1.86 1.65 1.63 1.63 1.61 1.56 1.54 1.52 1.47 1.45 1.43 1.14 1.13 1.11 0.87 0.85 0.84
OH
N
N
OH
N
O
N
H
N=N
2.00 1.00 1.00 1.00 1.00 2.27 2.55 2.03 0.93 2.08 2.02 2.18 3.24 2.61 2.45 3.06
f1 (ppm)

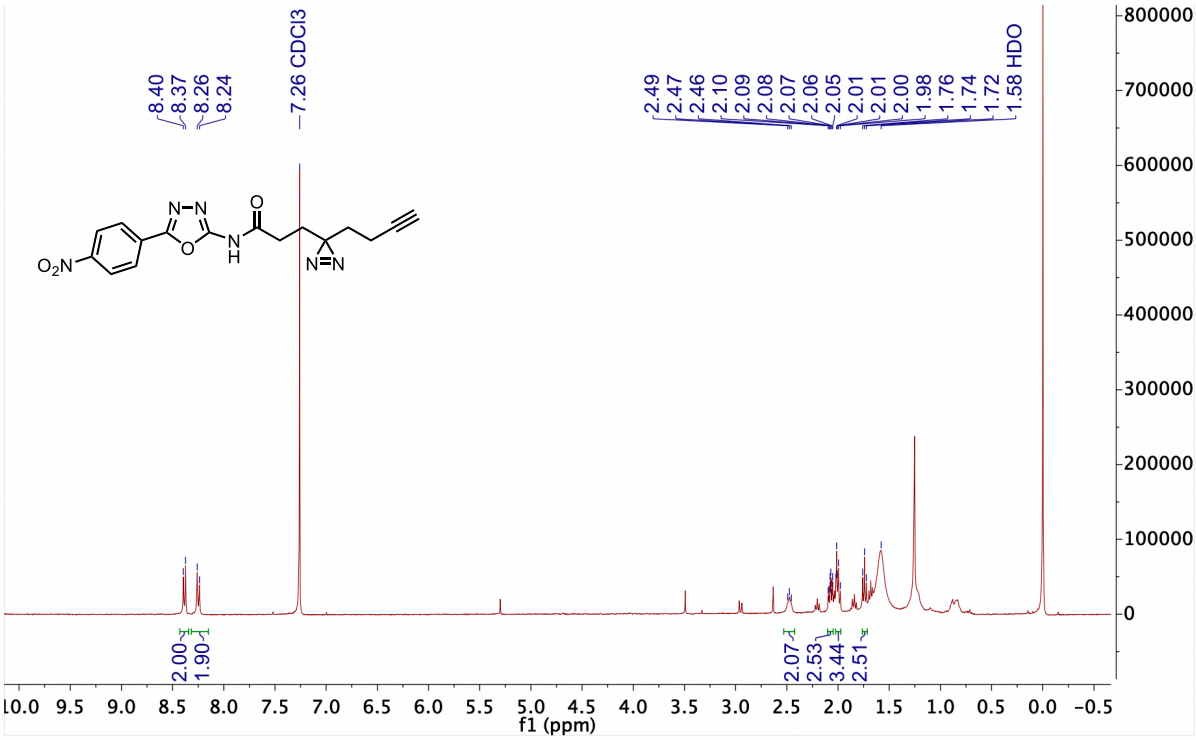
F24


7.28 7.28 6.99 6.98 6.90 6.87 4.89 HDO 4.14 4.13 4.12 4.10 3.89 3.87 3.86 3.85 3.84 3.73 3.72 3.72 3.71 3.71 3.71 3.69 3.69 3.69 3.68 3.68 3.67 3.65 3.31 MeOD 2.29 2.28 2.27 2.20 2.18 2.18 2.16 2.05 2.04 2.03 2.02 1.85 1.83 1.83 1.81 1.66 1.65 1.63
O
O
O
O
O
O
N
H
O
N=N
1.00 1.00 1.00 4.00 4.03 4.00 4.00 4.02 0.96 1.96 1.97 1.98 1.97
f1 (ppm)

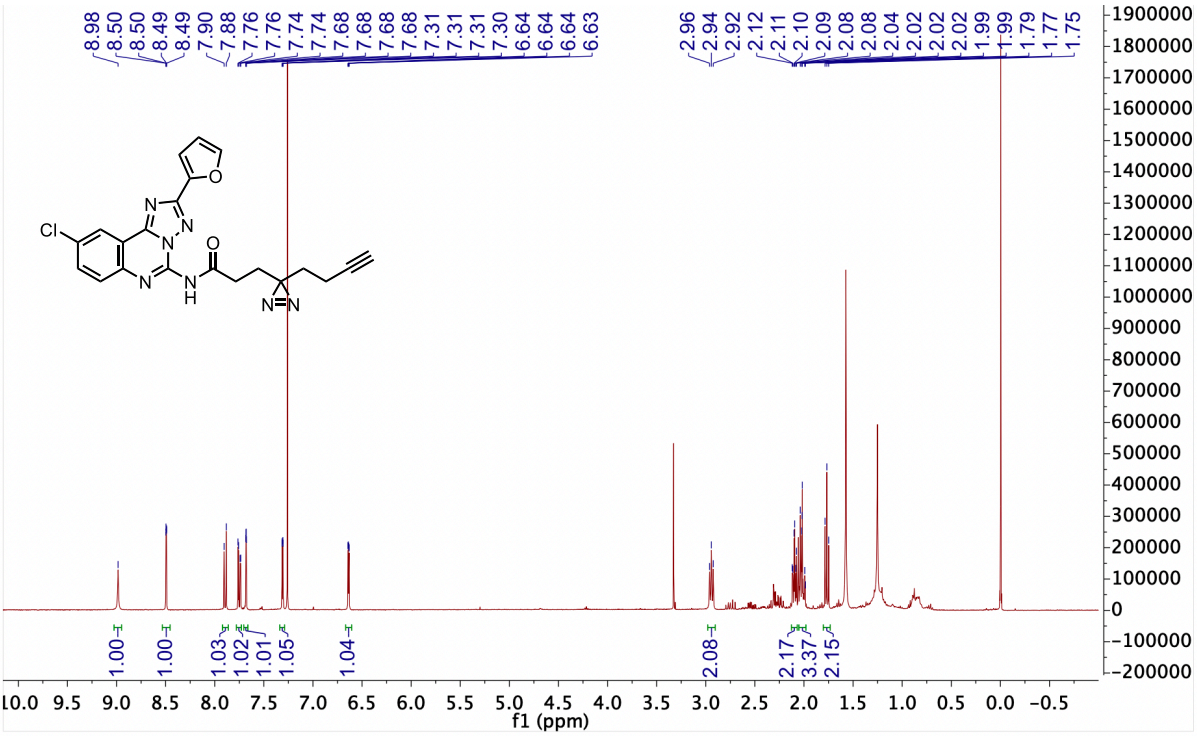
F25


7.28
7.27
7.00
7.00
6.98
6.98
6.95
6.94
6.93
6.92
6.90
6.89
6.89
6.88
6.87
6.87
4.89 HDO
4.15
4.14
4.14
4.12
3.97
3.96
3.96
3.94
3.31 MeOD
2.27
2.20
2.18
2.18
2.16
2.05
2.04
2.04
2.03
1.85
1.83
1.83
1.81
1.66
1.64
1.62
1.00
0.94
1.96
3.01
8.00
8.02
1.03
2.12
2.65
2.04
2.12
f1 (ppm)

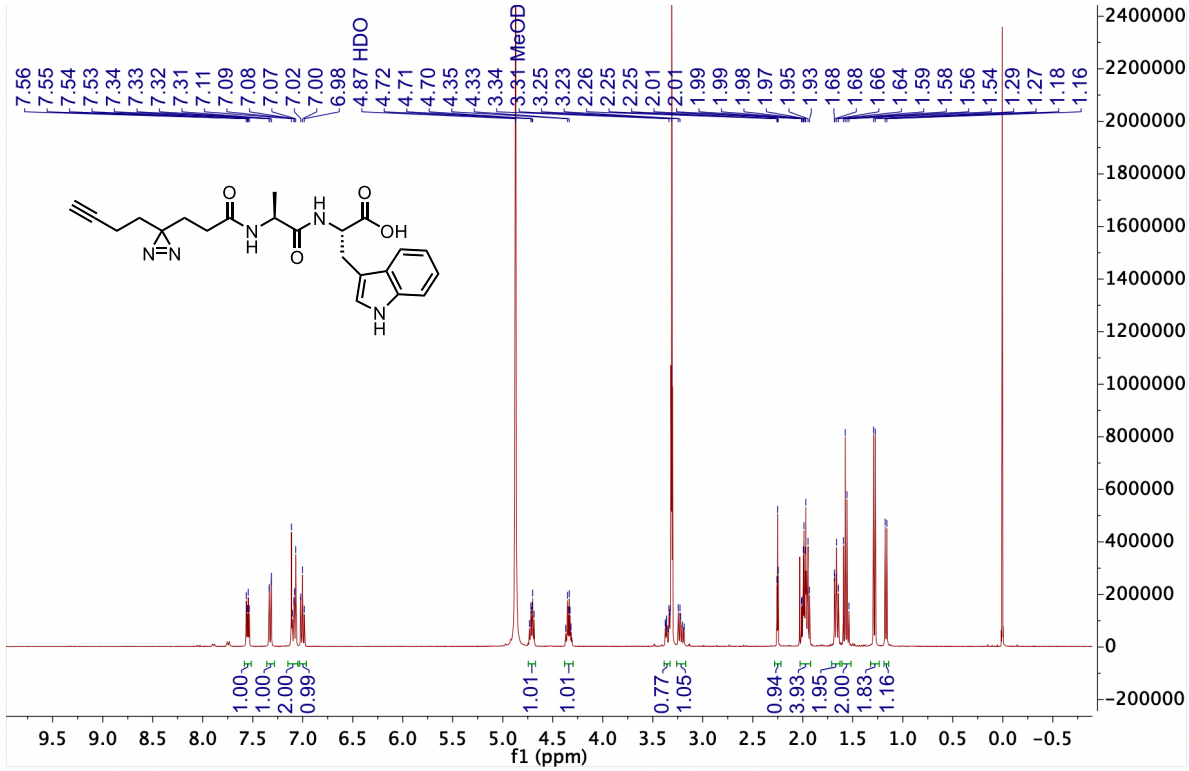
F26


8.40
8.37
8.26
8.24
7.26 CDCl3
2.49
2.47
2.46
2.10
2.09
2.08
2.07
2.06
2.05
2.01
2.01
2.00
1.98
1.76
1.74
1.72
1.58 HDO
2.00
1.90
2.07
2.53
3.44
2.51
f1 (ppm)

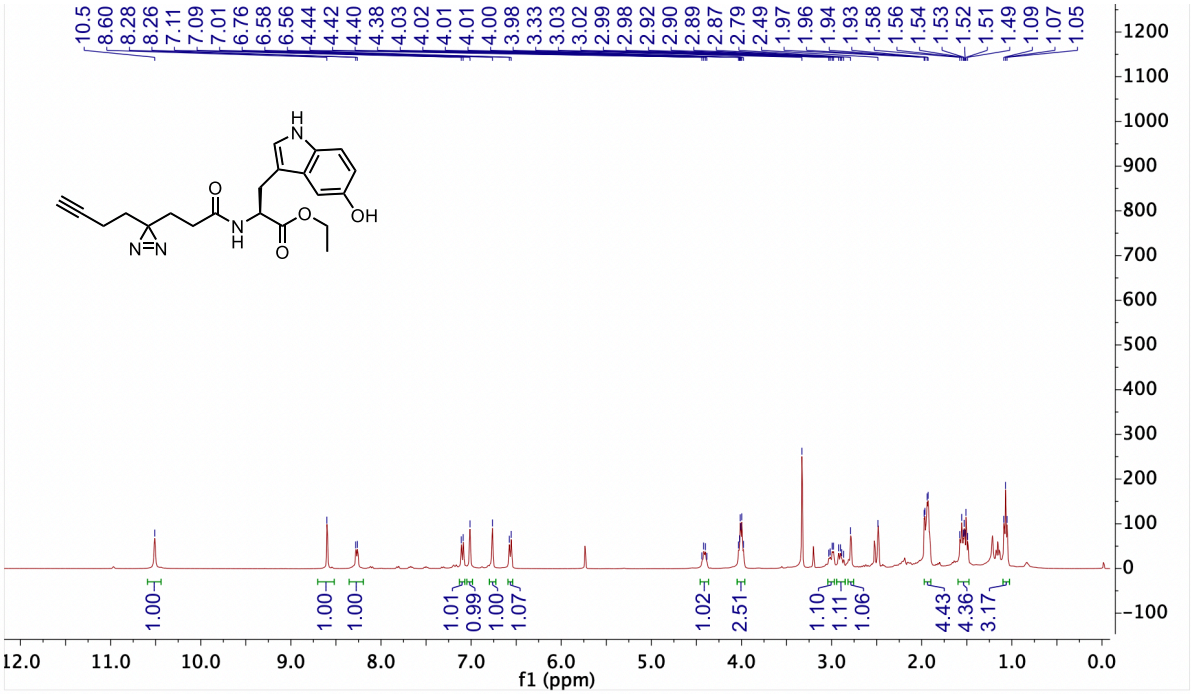
F27


8.98 8.50 8.50 8.49 8.49 7.90 7.88 7.76 7.76 7.74 7.74 7.68 7.68 7.68 7.31 7.31 7.31 7.30 6.64 6.64 6.63
2.96 2.94 2.92 2.12 2.11 2.10 2.09 2.08 2.08 2.04 2.02 2.02 2.02 1.99 1.99 1.79 1.77 1.75
1.00 1.00 1.03 1.02 1.01 1.05 1.04 2.08 2.17 3.37 2.15
f1 (ppm)

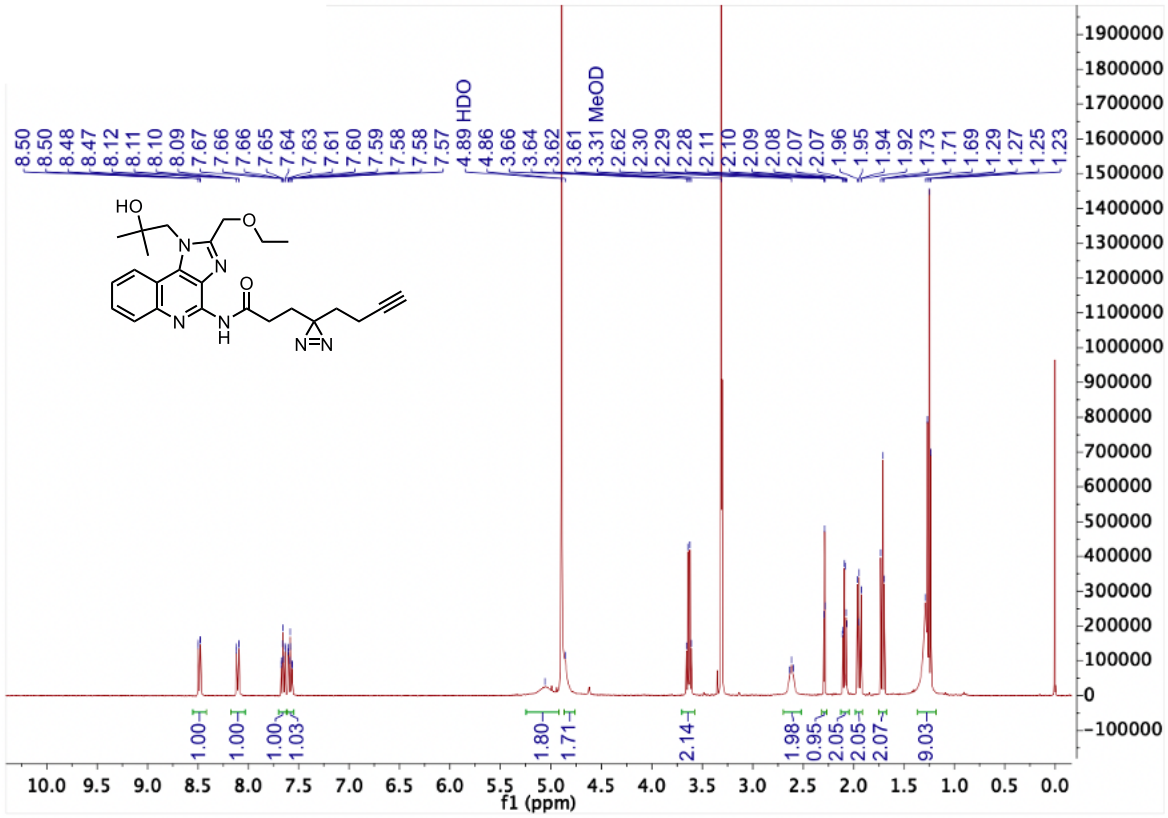
F28


7.56 7.55 7.54 7.53 7.34 7.33 7.32 7.31 7.11 7.09 7.08 7.07 7.02 7.00 6.98
4.87 HDO 4.72 4.71 4.70 4.35 4.33 3.34 3.31 MeOD 3.25 3.23 2.26 2.25 2.25 2.01 2.01 1.99 1.99 1.98 1.97 1.95 1.93 1.68 1.68 1.66 1.64 1.59 1.58 1.56 1.54 1.29 1.27 1.18 1.16
1.00 1.00 2.00 0.99 1.01 1.01 0.77 1.05 0.94 3.93 1.95 2.00 1.83 1.16
f1 (ppm)

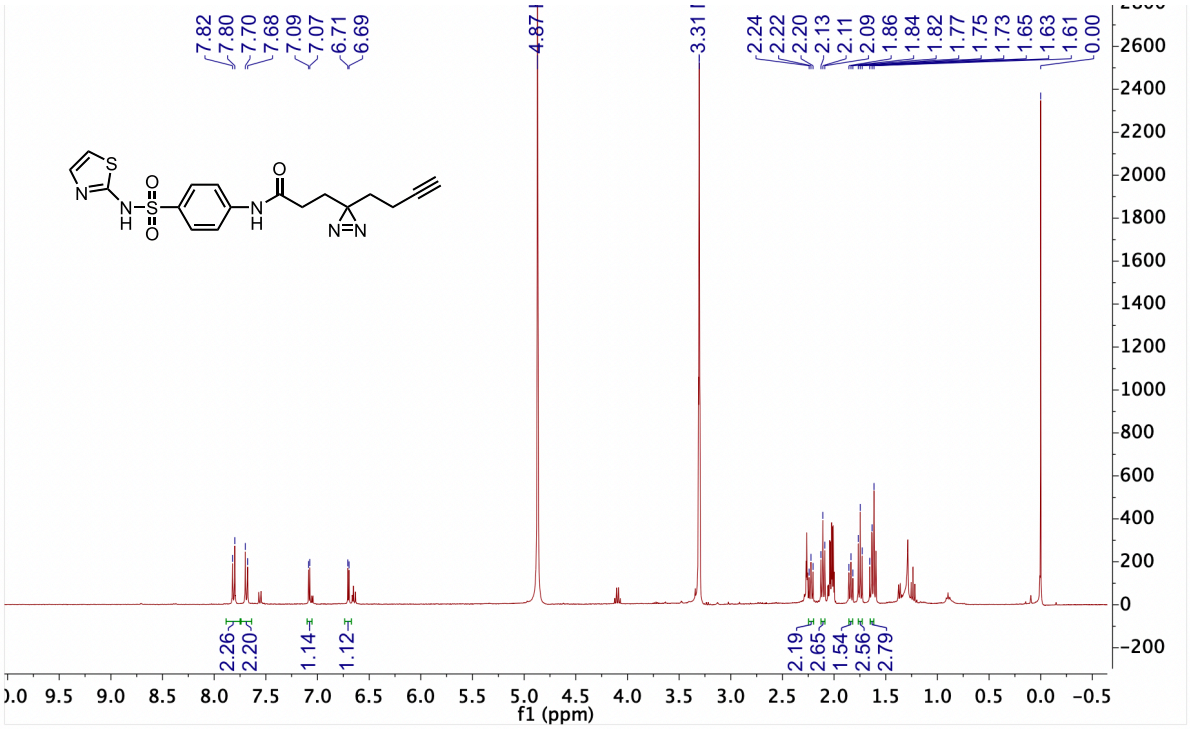
F29


10.5 8.60 8.28 8.26 7.11 7.09 7.01 6.76 6.58 6.56 4.44 4.42 4.40 4.38 4.03 4.02 4.01 4.01 4.00 3.98 3.33 3.03 3.02 2.99 2.98 2.92 2.90 2.89 2.87 2.79 2.49 1.97 1.96 1.94 1.93 1.58 1.56 1.54 1.53 1.52 1.51 1.49 1.09 1.07 1.05
1.00 1.00 1.00 1.01 0.99 1.00 1.07 1.02 2.51 1.10 1.11 1.06 4.43 4.36 3.17
f1 (ppm)

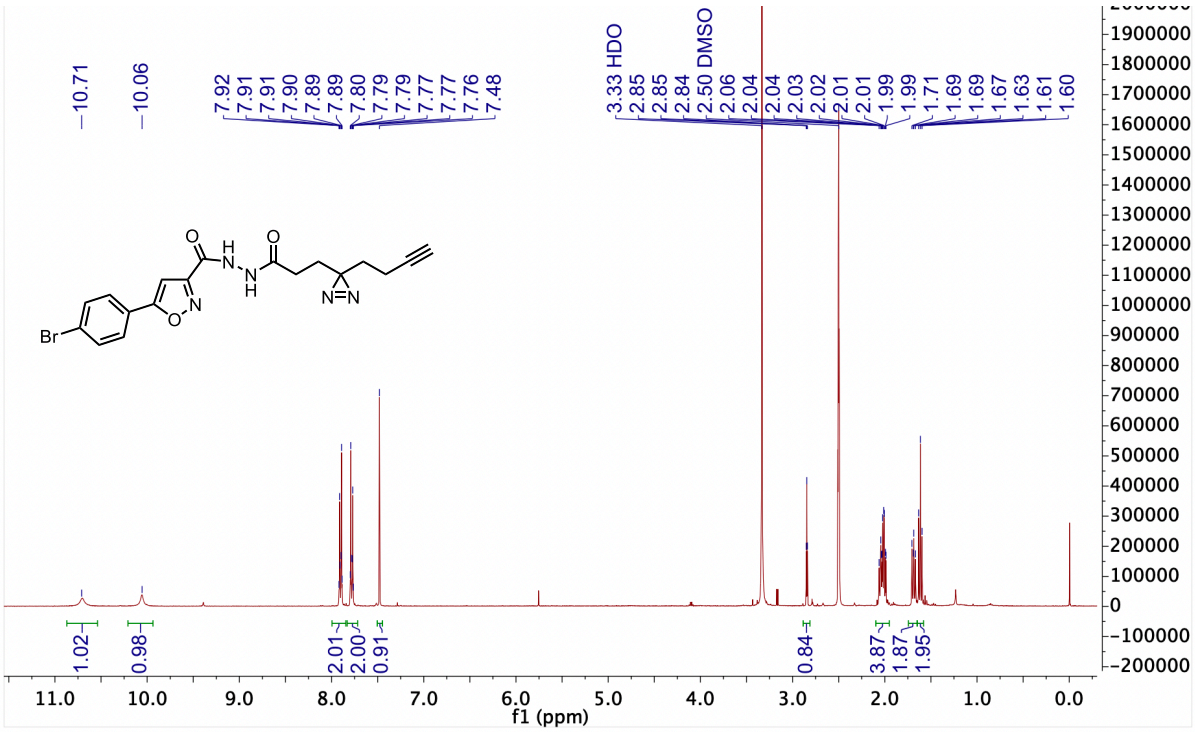
F30


8.50 8.50 8.48 8.47 8.12 8.11 8.10 8.09 7.67 7.66 7.66 7.65 7.64 7.63 7.61 7.60 7.59 7.58 7.58 7.57 4.89 HDO 4.86 3.66 3.64 3.62 3.61 3.31 MeOD 2.62 2.30 2.29 2.28 2.11 2.10 2.09 2.08 2.07 2.07 1.96 1.95 1.94 1.92 1.73 1.71 1.69 1.29 1.27 1.25 1.23
1.00 1.00 1.00 1.03 1.80 1.71 2.14 1.98 0.95 2.05 2.05 2.07 9.03
f1 (ppm)

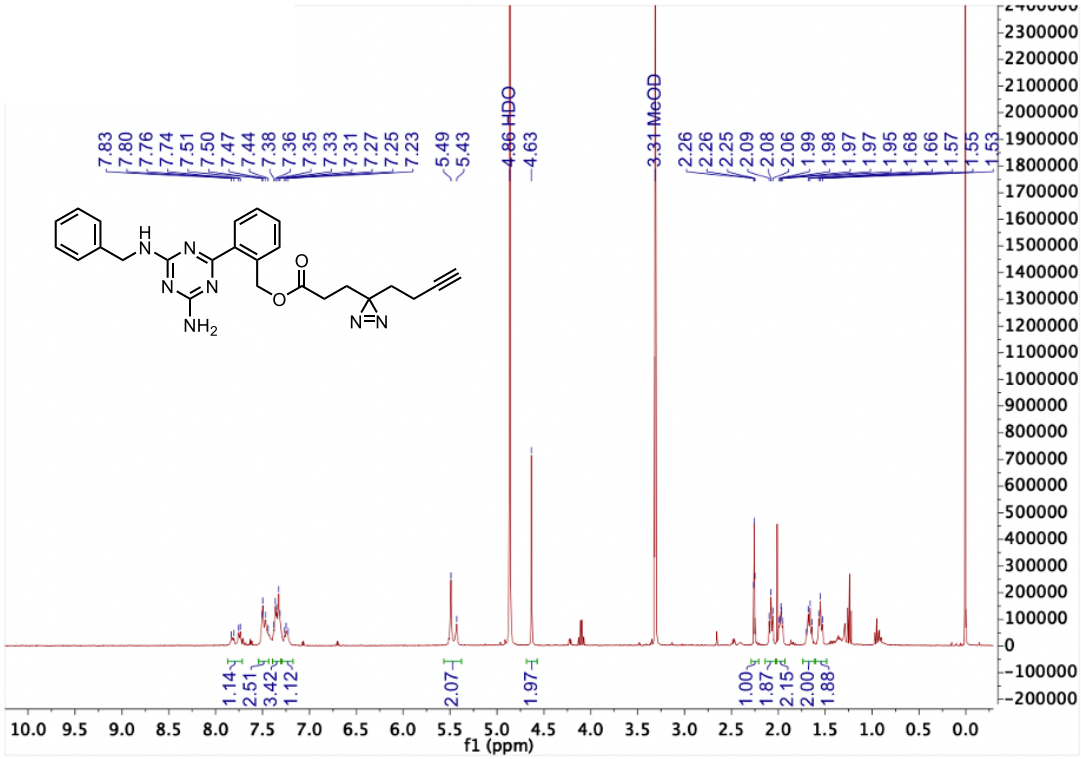
F31


7.82
7.80
7.70
7.68
7.09
7.07
6.71
6.69
4.87
3.31
2.24
2.22
2.20
2.13
2.11
2.09
1.86
1.84
1.82
1.77
1.75
1.73
1.65
1.63
1.61
0.00
2.26
2.20
1.14
1.12
2.19
2.65
1.54
2.56
2.79
f1 (ppm)

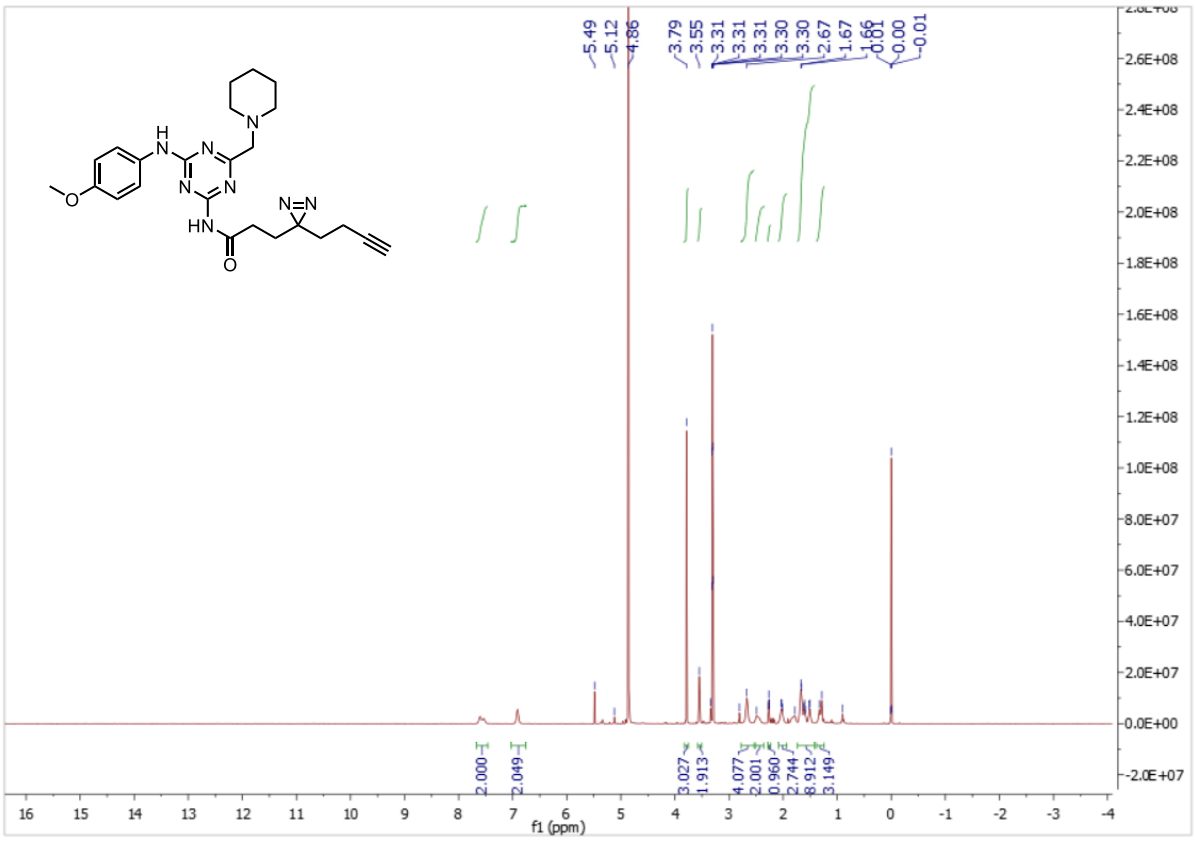
F32

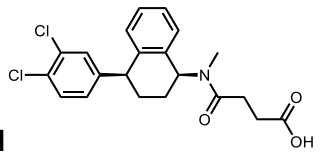

10.71
10.06
7.92
7.91
7.91
7.90
7.89
7.89
7.80
7.79
7.79
7.77
7.77
7.76
7.48
3.33 HDO
2.85
2.85
2.84
2.50 DMSO
2.06
2.04
2.04
2.03
2.02
2.01
2.01
1.99
1.99
1.71
1.69
1.69
1.67
1.63
1.61
1.60
Br
1.02
0.98
2.01
2.00
0.91
0.84
3.87
1.87
1.95
f1 (ppm)

F33

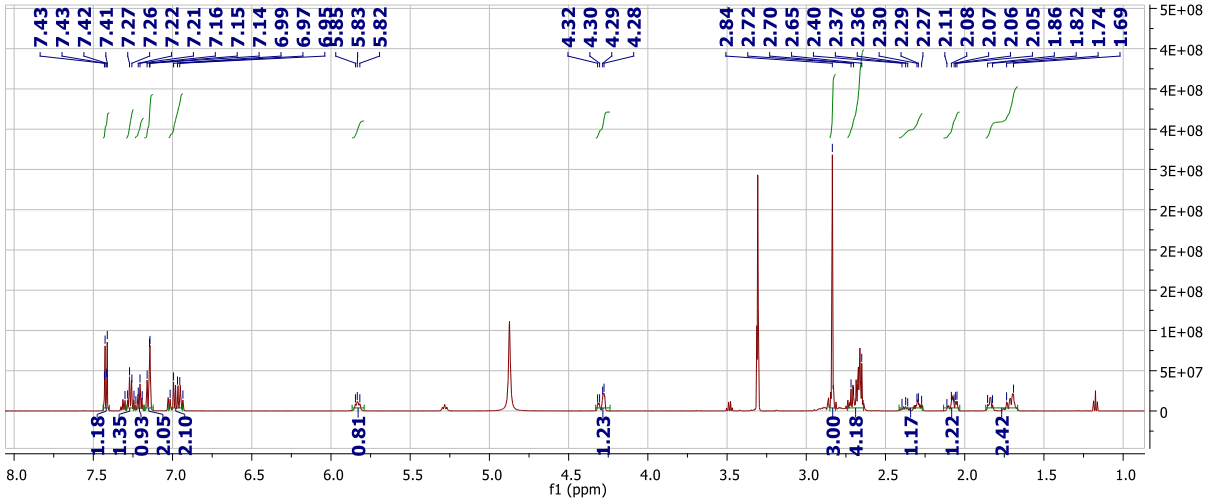

7.83
7.80
7.76
7.74
7.51
7.50
7.47
7.44
7.38
7.36
7.35
7.33
7.31
7.27
7.25
7.23
5.49
5.43
4.86 HDO
4.63
3.31 MeOD
2.26
2.26
2.25
2.09
2.08
2.06
1.99
1.98
1.97
1.97
1.95
1.68
1.66
1.57
1.55
1.53
1.14
2.51
3.42
1.12
2.07
1.97
1.00
1.87
2.15
2.00
1.88
f1 (ppm)

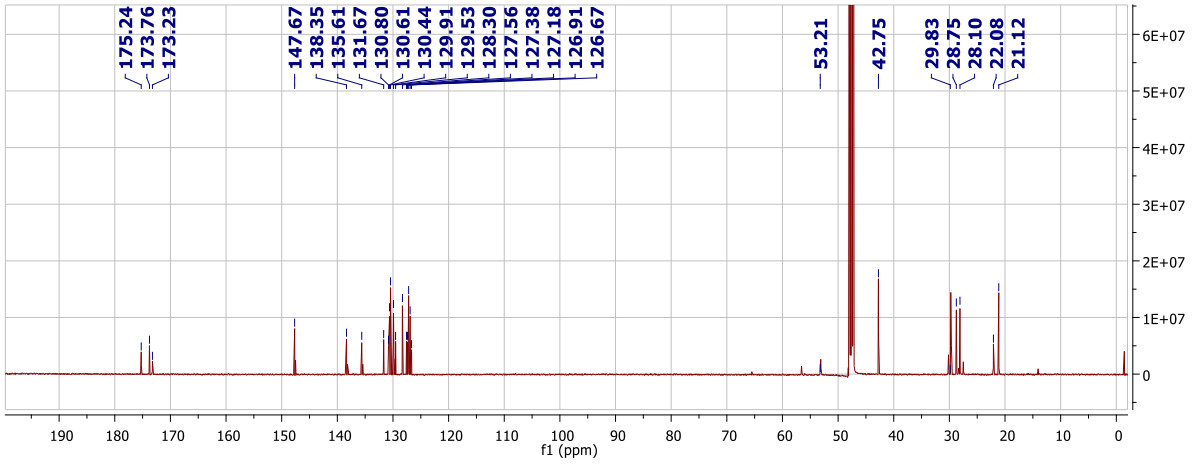
F34

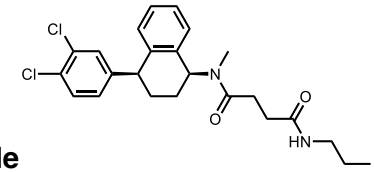

5.49
5.12
4.86
3.79
3.55
3.31
3.31
3.31
3.30
3.30
2.67
1.67
1.66
0.01
0.00
-0.01
2.000
2.049
3.027
1.913
4.077
2.001
0.960
2.744
8.912
3.149
f1 (ppm)


Cl
Cl
N
O
O
O
OH

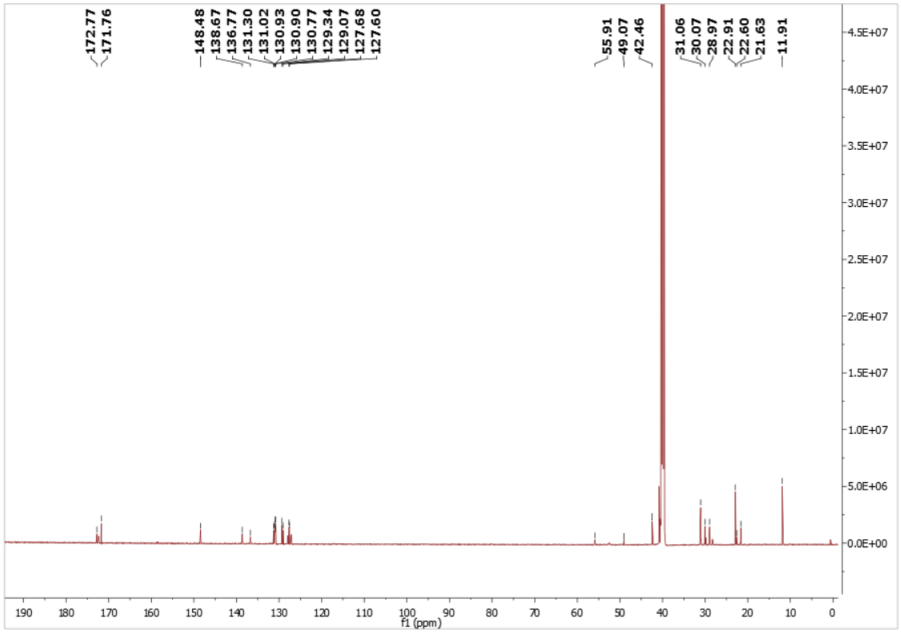
F1-COOH

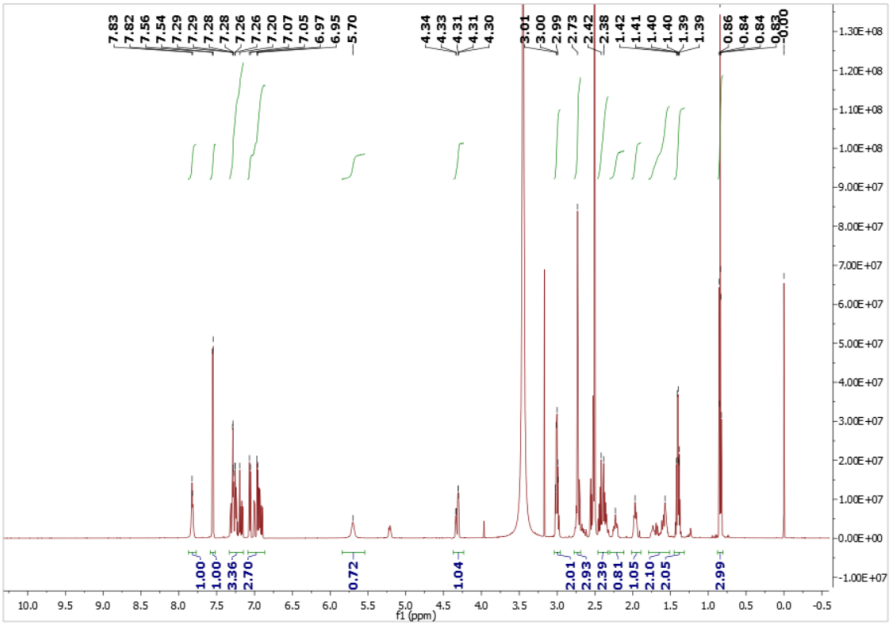

7.43
7.43
7.42
7.41
7.27
7.26
7.22
7.21
7.16
7.15
7.14
6.99
6.97
5.85
5.83
5.82
4.32
4.30
4.29
4.28
2.84
2.72
2.70
2.65
2.40
2.37
2.36
2.30
2.29
2.27
2.11
2.08
2.07
2.06
2.05
1.86
1.82
1.74
1.69
1.18
1.35
0.93
2.05
2.10
0.81
1.23
3.00
4.18
1.17
1.22
2.42
f1 (ppm)

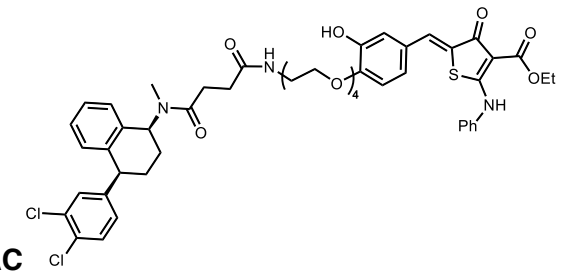

175.24
173.76
173.23
147.67
138.35
135.61
131.67
130.80
130.61
130.44
129.91
129.53
128.30
127.56
127.38
127.18
126.91
126.67
53.21
42.75
29.83
28.75
28.10
22.08
21.12
f1 (ppm)


Cl
Cl
N
O
O
HN

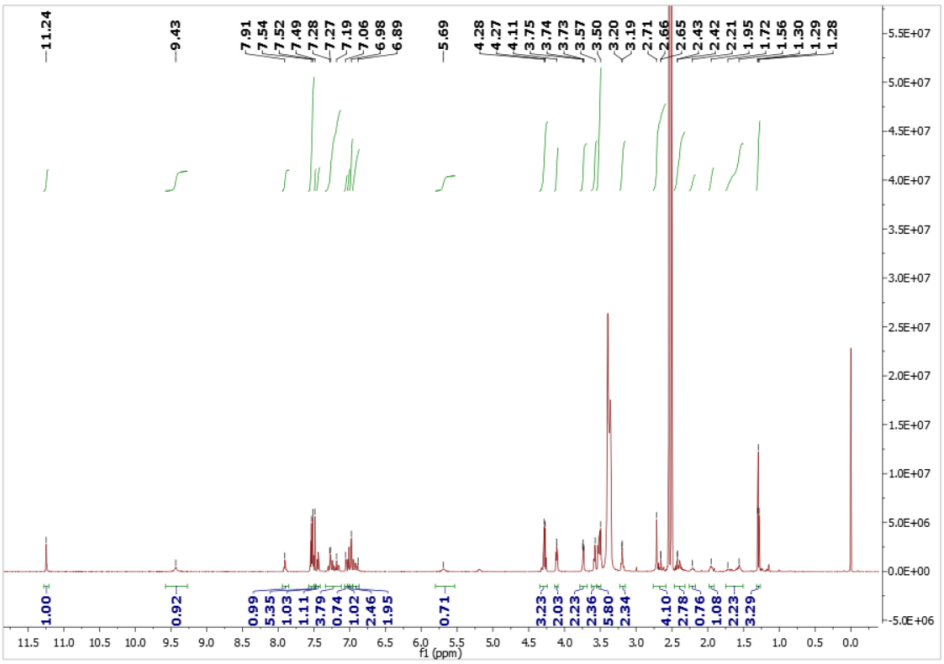
F1-Amide

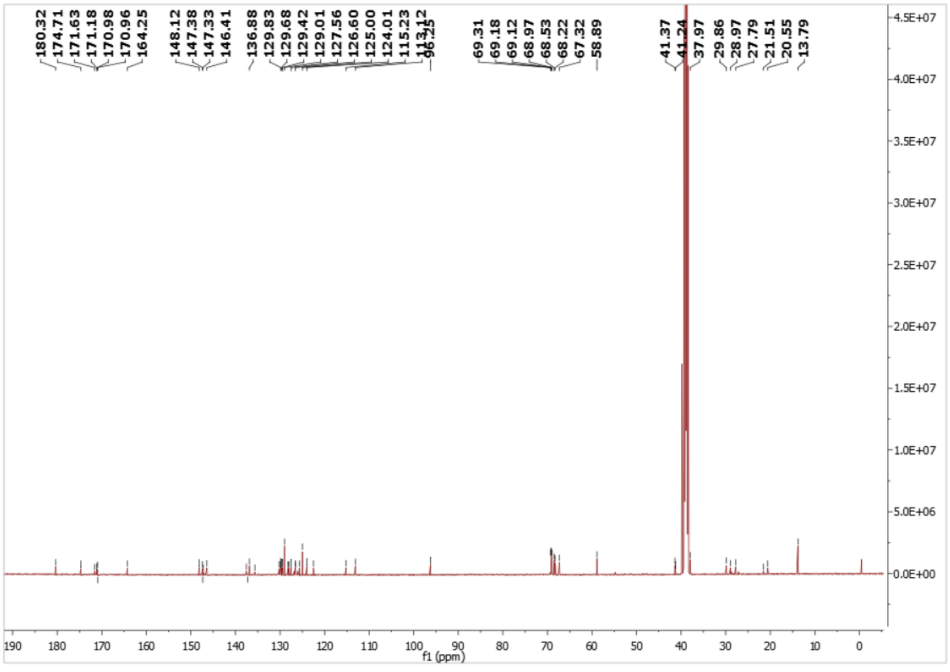

172.77
171.76
148.48
138.67
136.77
131.30
131.02
130.93
130.90
130.77
129.34
129.07
127.68
127.60
55.91
49.07
42.46
31.06
30.07
28.97
22.91
22.60
21.63
11.91
f1 (ppm)

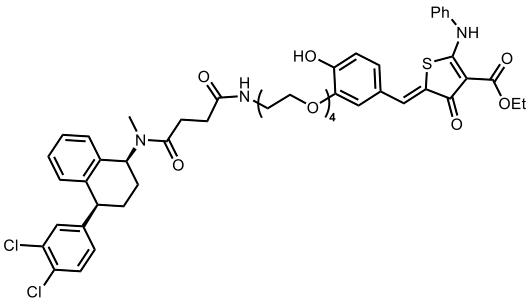

7.83
7.82
7.56
7.54
7.29
7.29
7.28
7.28
7.26
7.26
7.20
7.07
7.05
6.97
6.95
5.70
4.34
4.33
4.31
4.31
4.30
3.01
3.00
2.99
2.73
2.42
2.38
1.42
1.41
1.40
1.40
1.39
1.39
0.86
0.84
0.84
0.83
0.00
1.00
1.00
3.36
2.70
0.72
1.04
2.01
2.93
2.39
0.81
1.05
2.10
2.05
2.99
f1 (ppm)


HO
O
O
OEt
S
NH
Ph
O
H
N
O
N
O
4
Cl
Cl

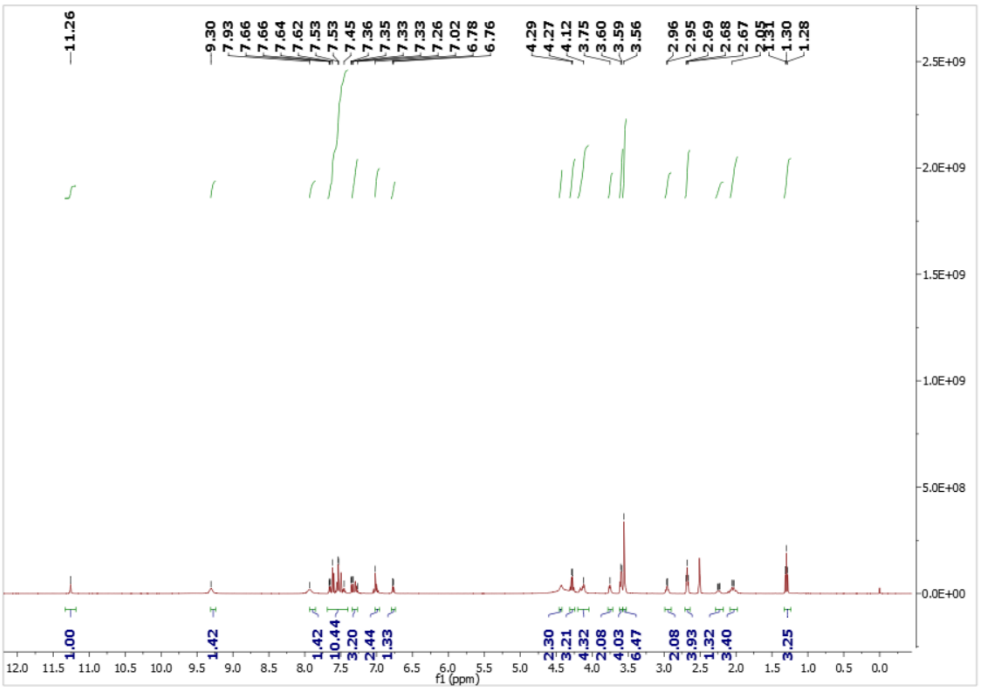
F1-RIBOTAC

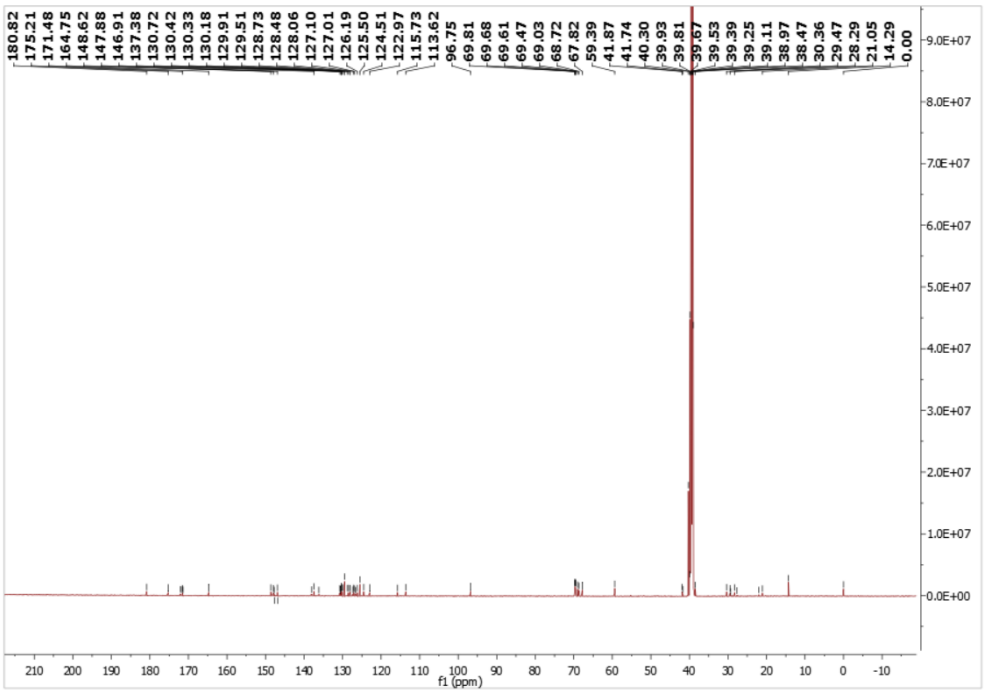

11.24
9.43
7.91
7.54
7.52
7.49
7.28
7.27
7.19
7.06
6.98
6.89
5.69
4.28
4.27
4.11
3.75
3.74
3.73
3.57
3.50
3.20
3.19
2.71
2.66
2.65
2.43
2.42
2.21
1.95
1.72
1.56
1.30
1.29
1.28
1.00
0.92
0.99
5.35
1.03
1.11
3.79
0.74
1.02
2.46
1.95
0.71
3.23
2.03
2.23
2.36
5.80
2.34
4.10
2.78
0.76
1.08
2.23
3.29
f1 (ppm)


180.32
174.71
171.63
171.18
170.98
170.96
164.25
148.12
147.38
147.33
146.41
136.88
129.83
129.68
129.42
129.01
127.56
126.60
125.00
124.01
115.23
113.12
96.25
69.31
69.18
69.12
68.97
68.53
68.22
67.32
58.89
41.37
41.24
37.97
29.86
28.97
27.79
21.51
20.55
13.79
f1 (ppm)


Ph
NH
HO
S
O
OEt
O
H
N
O
O
N
O
4
Cl
Cl

F1-CTRL


11.26
9.30
7.93
7.66
7.64
7.62
7.53
7.53
7.45
7.36
7.35
7.33
7.33
7.26
7.02
6.78
6.76
4.29
4.27
4.12
3.75
3.60
3.59
3.56
2.96
2.95
2.69
2.68
2.67
2.05
1.31
1.30
1.28
2.5E+09
2.0E+09
1.5E+09
1.0E+09
5.0E+08
0.0E+00
1.00
1.42
1.42
10.44
3.20
2.44
1.33
2.30
3.21
4.32
2.08
4.03
6.47
2.08
3.93
1.32
3.40
3.25
f1 (ppm)


180.82
175.21
171.48
164.75
148.62
147.88
146.91
137.38
130.72
130.42
130.33
130.18
129.91
129.51
128.73
128.48
128.06
127.10
127.01
126.19
125.50
124.51
122.97
115.73
113.62
96.75
69.81
69.68
69.61
69.47
69.03
68.72
67.82
59.39
41.87
41.74
40.30
39.93
39.81
39.67
39.53
39.39
39.25
39.11
38.97
38.47
30.36
29.47
28.29
21.05
14.29
0.00
9.0E+07
8.0E+07
7.0E+07
6.0E+07
5.0E+07
4.0E+07
3.0E+07
2.0E+07
1.0E+07
0.0E+00
f1 (ppm)

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