Supporting information:

"2-Sulfonyl pyridines as tunable, cysteine-reactive electrophiles"

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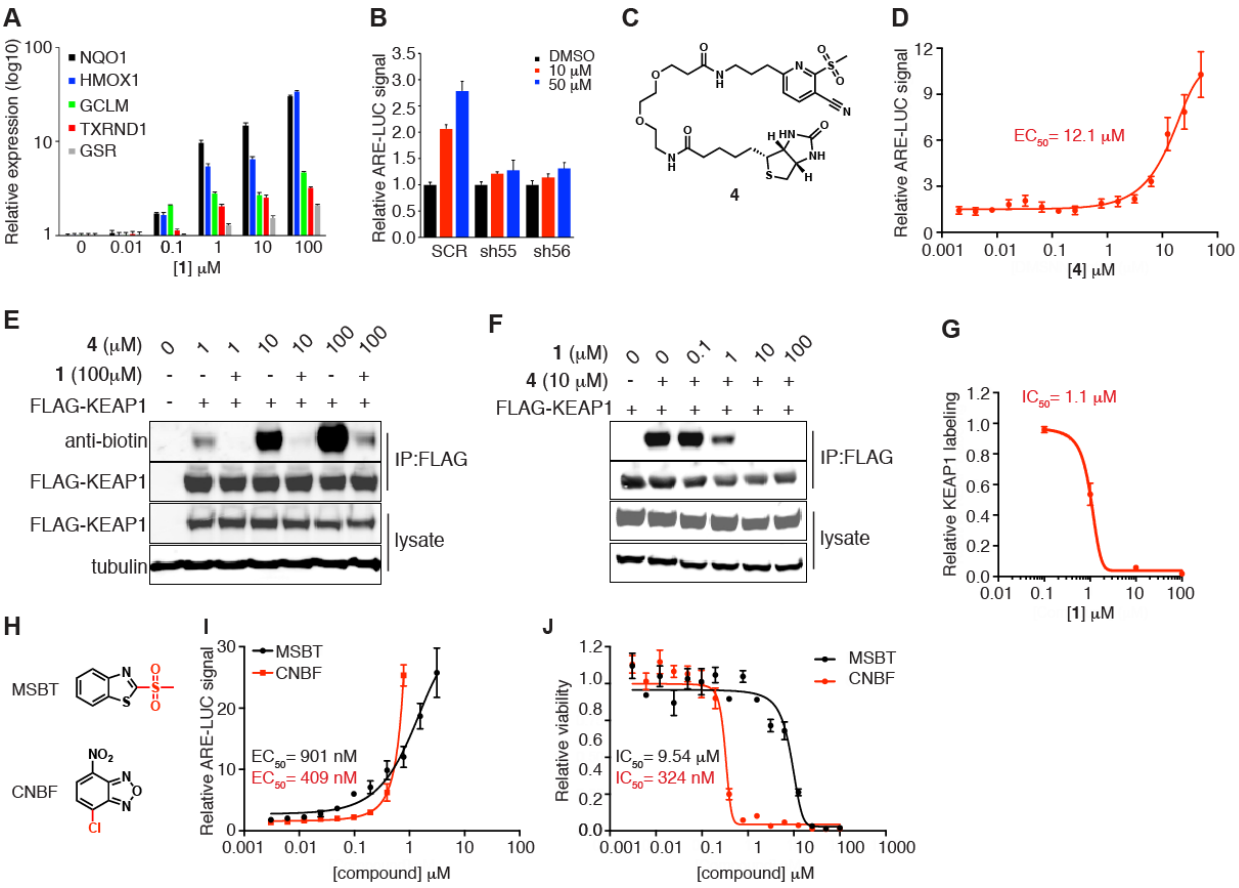
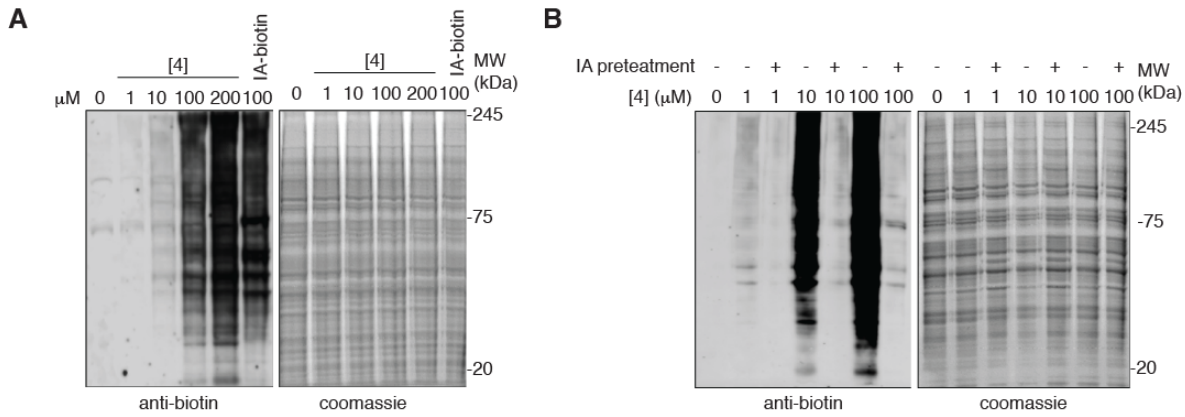
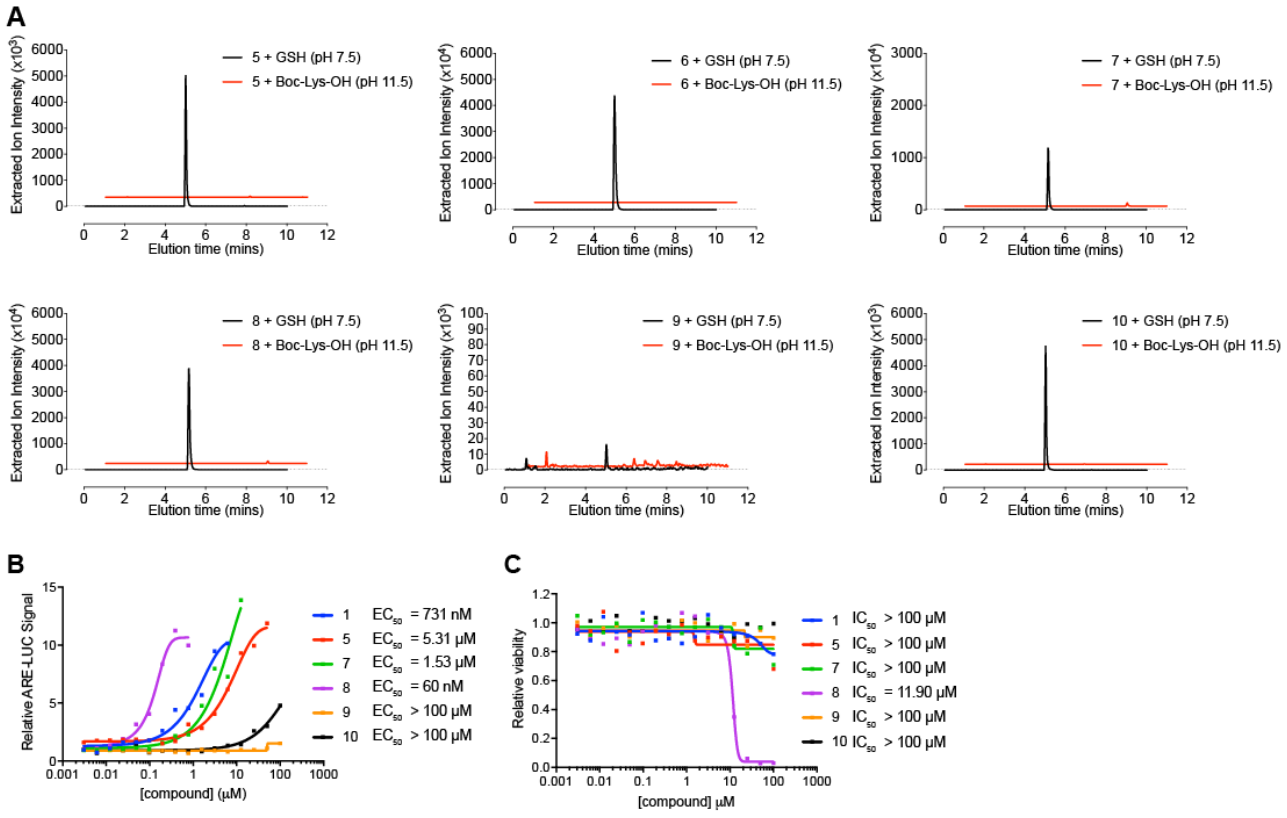


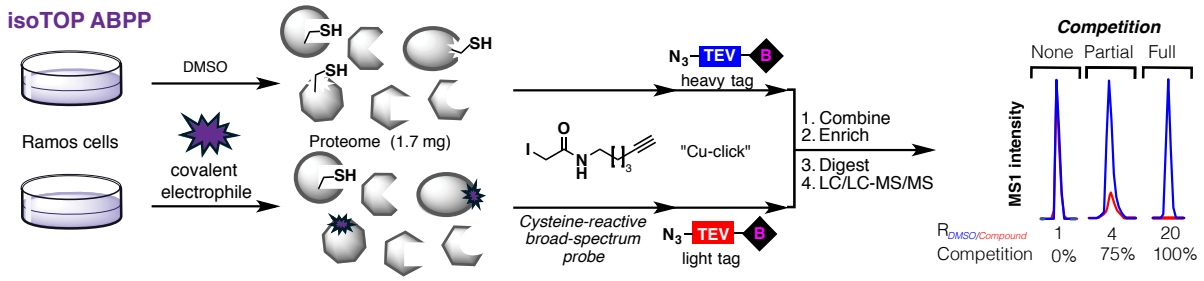
Supplementary Figure 1. Compound 1 and reported S_NAr-type cysteine-reactive fragments activate a NRF2-specific transcriptional program via covalent modification of KEAP1. (A) Relative expression (log_{10}) of the indicated NRF2-driven transcripts from IMR32 cells treated for 24 h with the indicated concentrations of **1** (mean and s.d., *n*=3). (B) Relative ARE-LUC luminance values from HEK293T cells transiently transfected with the indicated vectors encoding shRNAs to NRF2 (*NFE2L2* transcript, sh55 and sh56) and then treated with the indicated concentrations of **1** for 24 h (SCR=scramble, a non-targeting shRNA; mean and s.d.; *n*=3). (C, D) Structure and relative ARE-LUC luminance values from IMR32 cells treated with the indicated doses of biotin probe **4** for 24 h (mean and s.e.m.; *n*=3). (E) Western blotting analyses for biotinylation of anti-FLAG immunoprecipitated FLAG-KEAP1 content from HEK293T cells transfected with FLAG-KEAP1 plasmid and then treated with 100 μM of **1** and then the indicated dose of **4** for 1 h. (F, G) Western blotting analyses and quantification of biotinylation of anti-FLAG immunoprecipitated FLAG-KEAP1 protein content from HEK293T cells transfected with FLAG-KEAP1 plasmid and then treated with the indicated concentration response of **1** for 1 h and then treating with the 10 μM of biotin probe **4** for an additional 1 h (*n* =3; mean and s.e.m.). (H) Structures of reported S_NAr-type cysteine reactive molecules MSBT (Methylsulfonyl benzothiazole) and CNBF (4-Chloro-7-nitrobenzofurazan) with leaving groups denoted in red. (I, J) Relative ARE-LUC and viability measurements from IMR32 cells treated for 24 h with the indicated doses of MSBT and CNBF (mean and s.e.m.; *n*=3).



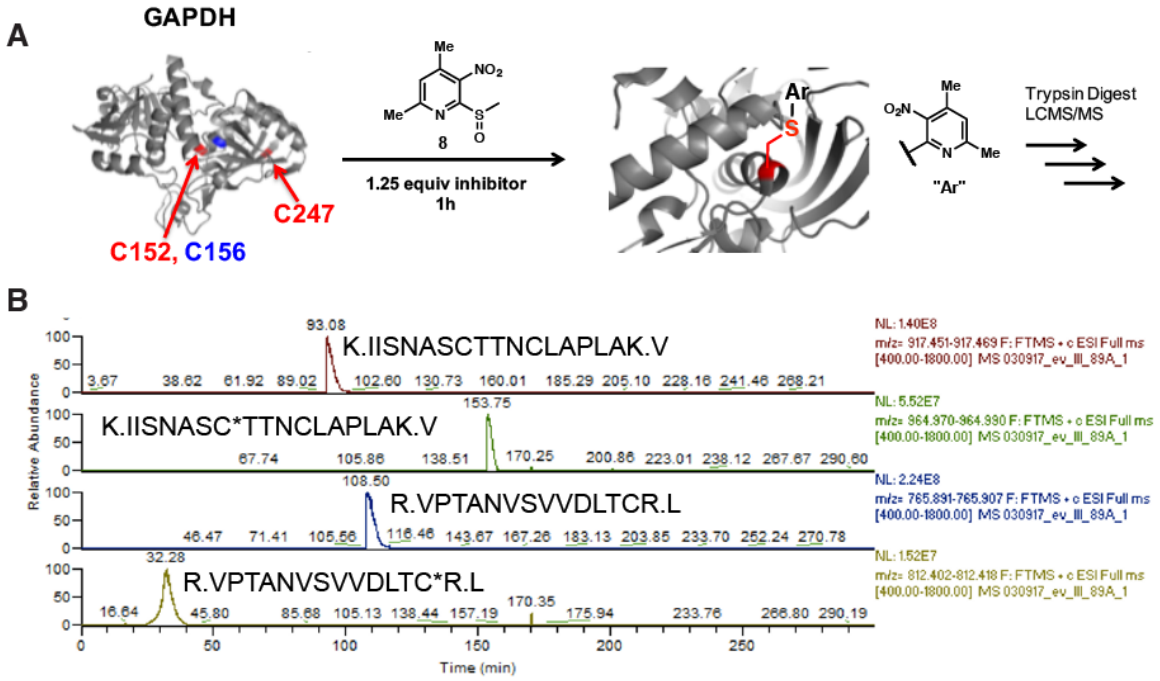
Supplementary Figure 2. Biotin probe 4 selectively labels reactive cysteines in the proteome. (A) Representative Western blot analyses and parallel Coomassie-stained SDS-PAGE gels of proteomes from IMR32 cells treated with the indicated concentrations of **4** or IA-biotin for 1 h. (B) Representative Western blot analyses and Coomassie-stained gels from live IMR32 cells pre-treated with 1 mM iodoacetamide or vehicle before labeling with the indicated concentrations of **4** for an additional h.



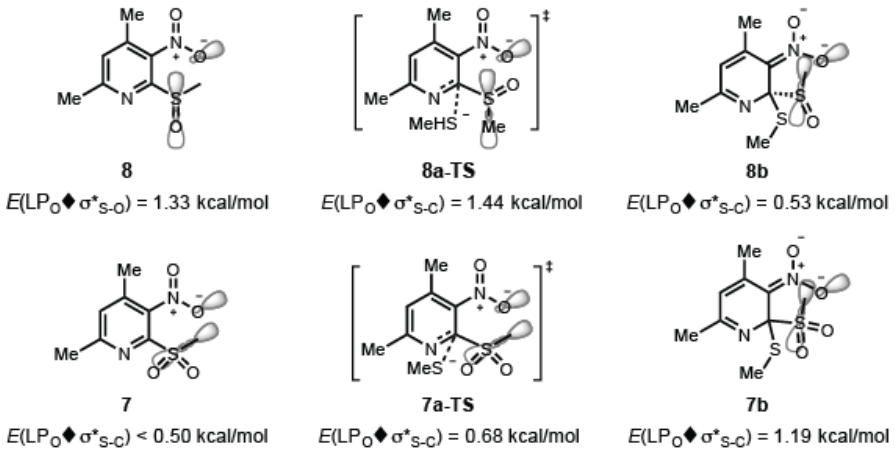
Supplementary Figure 3. Selective reactivity of 2-sulfonyl-pyridines with biological thiol nucleophiles. (A) Liquid chromatography traces for product formation from reactions in which the indicated compounds were incubated with GSH (at pH 7.5) or Boc-Lys-OH (at pH 11.5) for 1 hour. Relative ARE-LUC signal (B) and viability (C) measurements from IMR32 cells treated for 24 h with a concentration response to the indicated compounds.



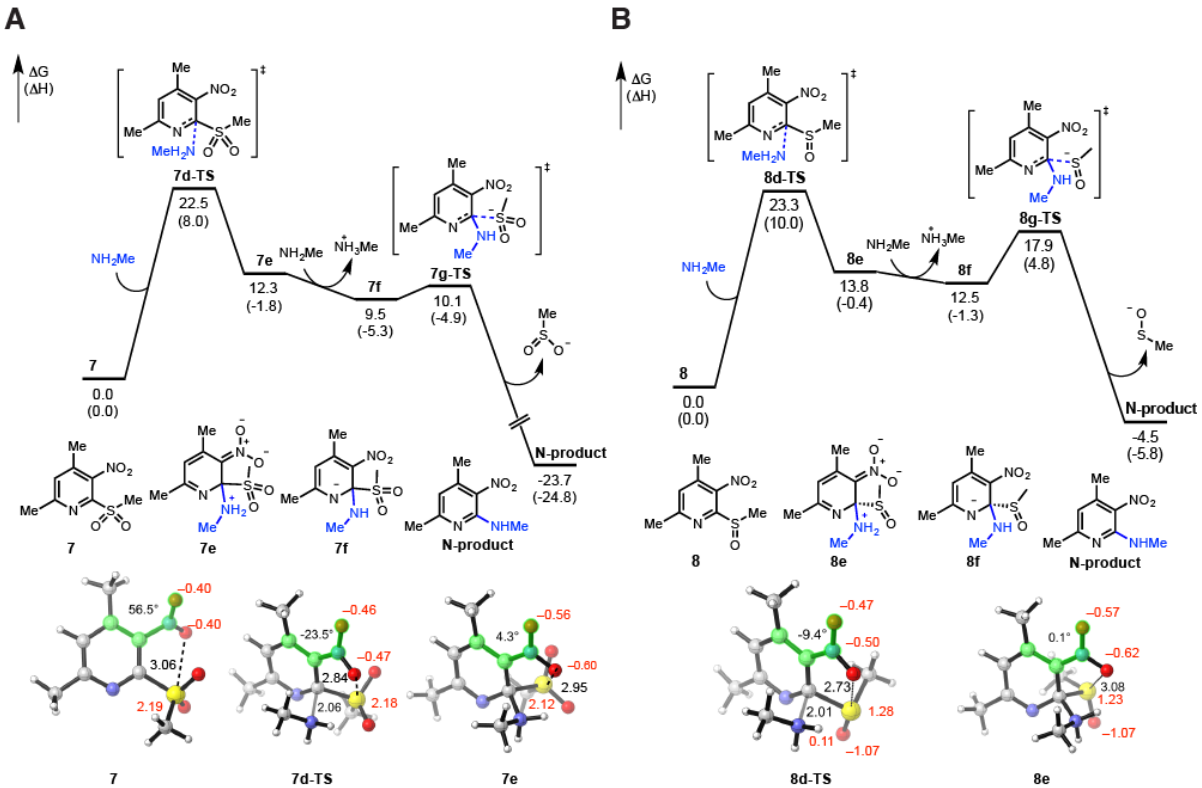
Supplementary Figure 4. isoTOP-ABPP, a quantitative chemical proteomics platform for defining cysteines liganded by electrophilic reactive groups. Schematic depicting the experimental workflow for isoTOP-ABPP experiments along with metric correlating representative *R* values to their fractional level of competition for labeling a given cysteine residue.



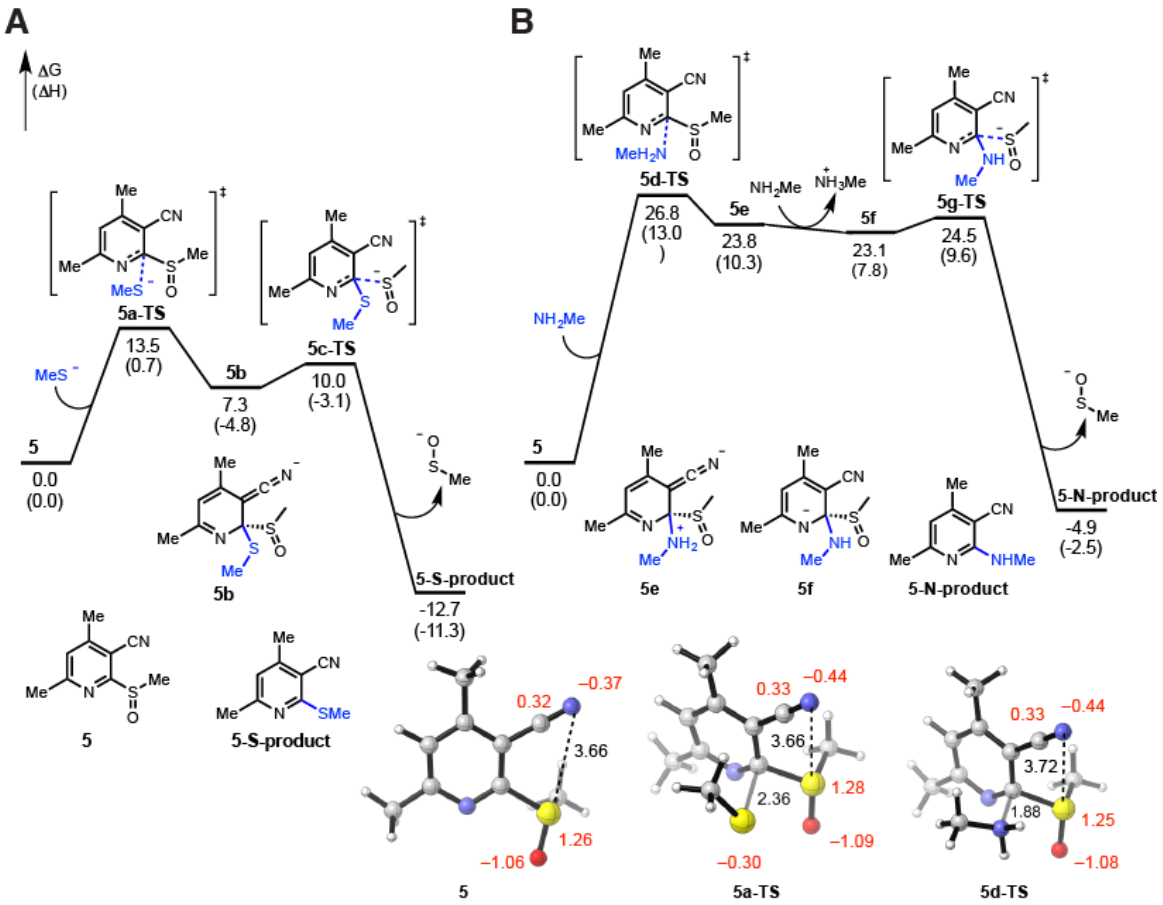
Supplementary Figure 5. Site of labeling study with GAPDH and 8. (A) Schematic depicting the *in vitro* reaction conditions and cysteine residues of recombinant GAPDH modified by **8**. (B) MS1 peak intensities from the indicated tryptic peptides of GAPDH unmodified or modified by pyridylation by **8**. A star next to cysteine residues indicates observed modification by **8**.



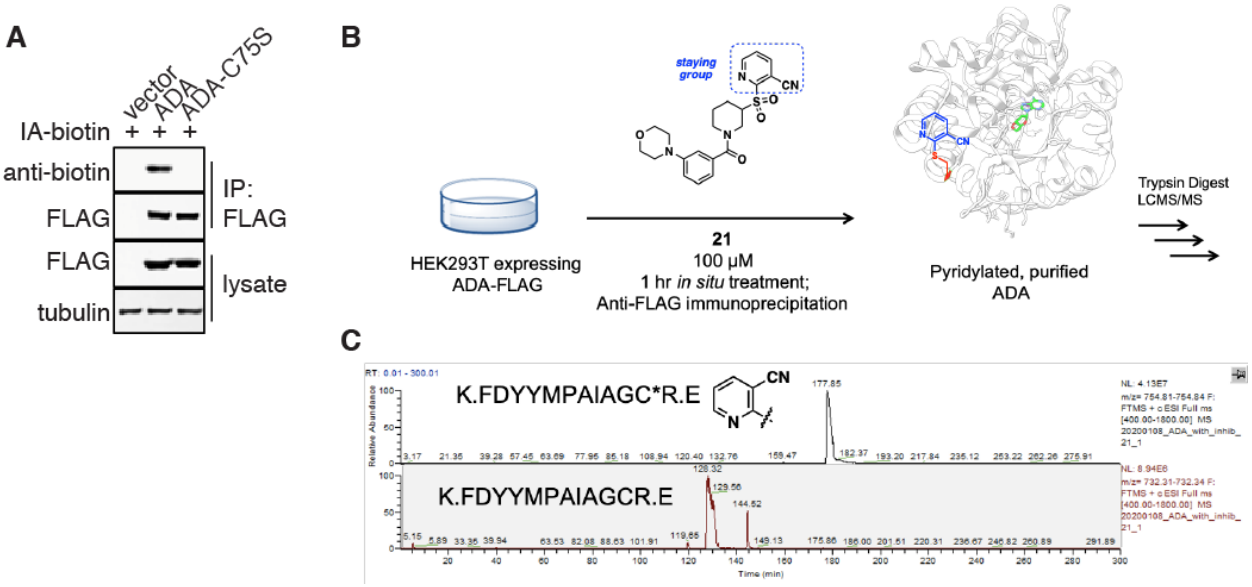
Supplementary Figure 6. Natural bond orbital (NBO) second order perturbation theory analysis on the nucleophilic addition steps depicted in this work. The donor/accepter interactions between the lone pair of the nitro oxygen and the σ*$_{(S–C)}$ or σ*$_{(S–O)}$ orbitals are relatively weak in both **8a-TS** and **7a-TS**.



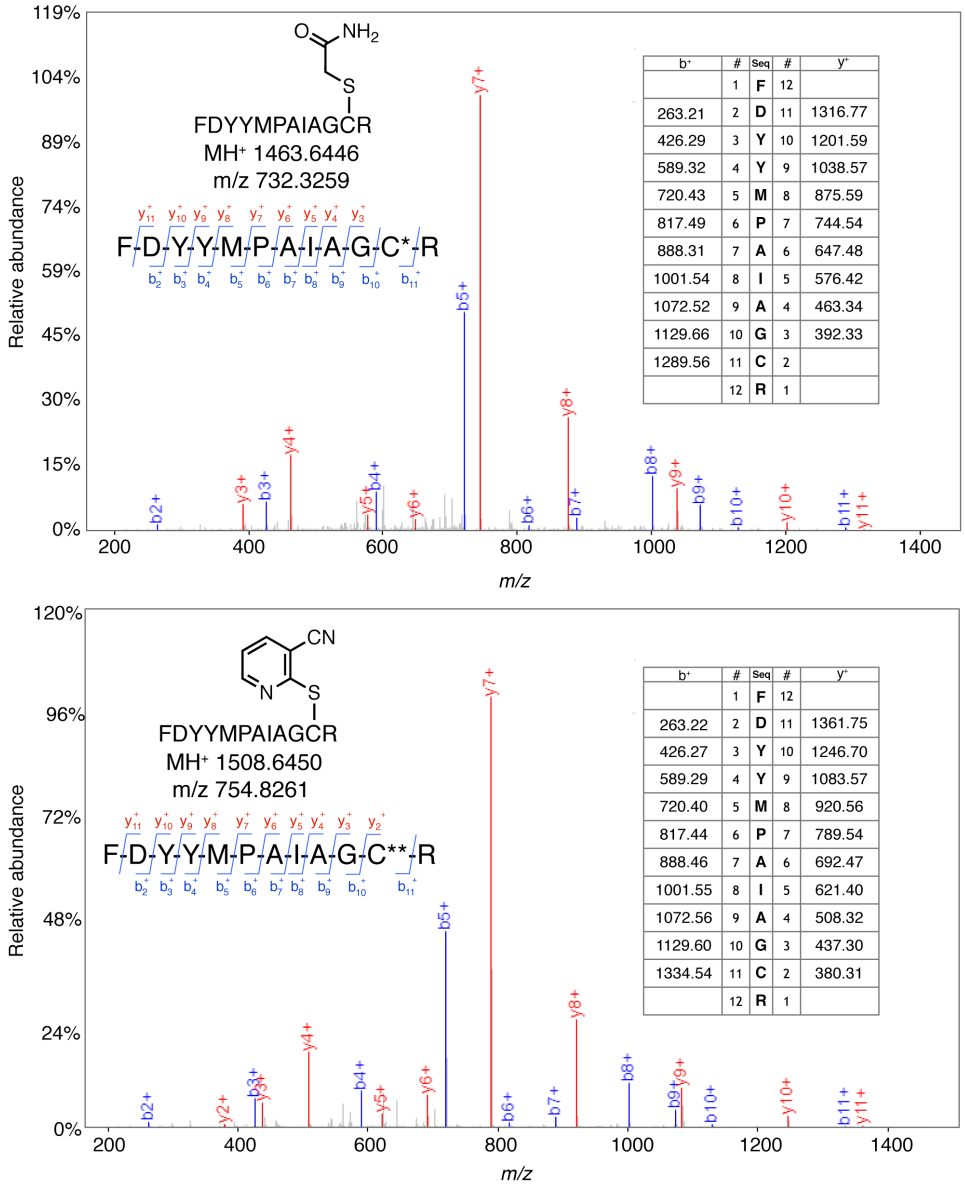
Supplementary Figure 7. DFT calculations predict the basis for selective reactivity of 2-sulfonyl pyridines with thiols. Computed energy profiles and transition states for the formation of the indicated product via reaction with **7** (A) and **8** (B) with methylamine (Gibbs free energies and enthalpies, kcal/mol; distances (in black), angstrom; red numbers, natural population analysis (NPA) charge of indicated atoms).



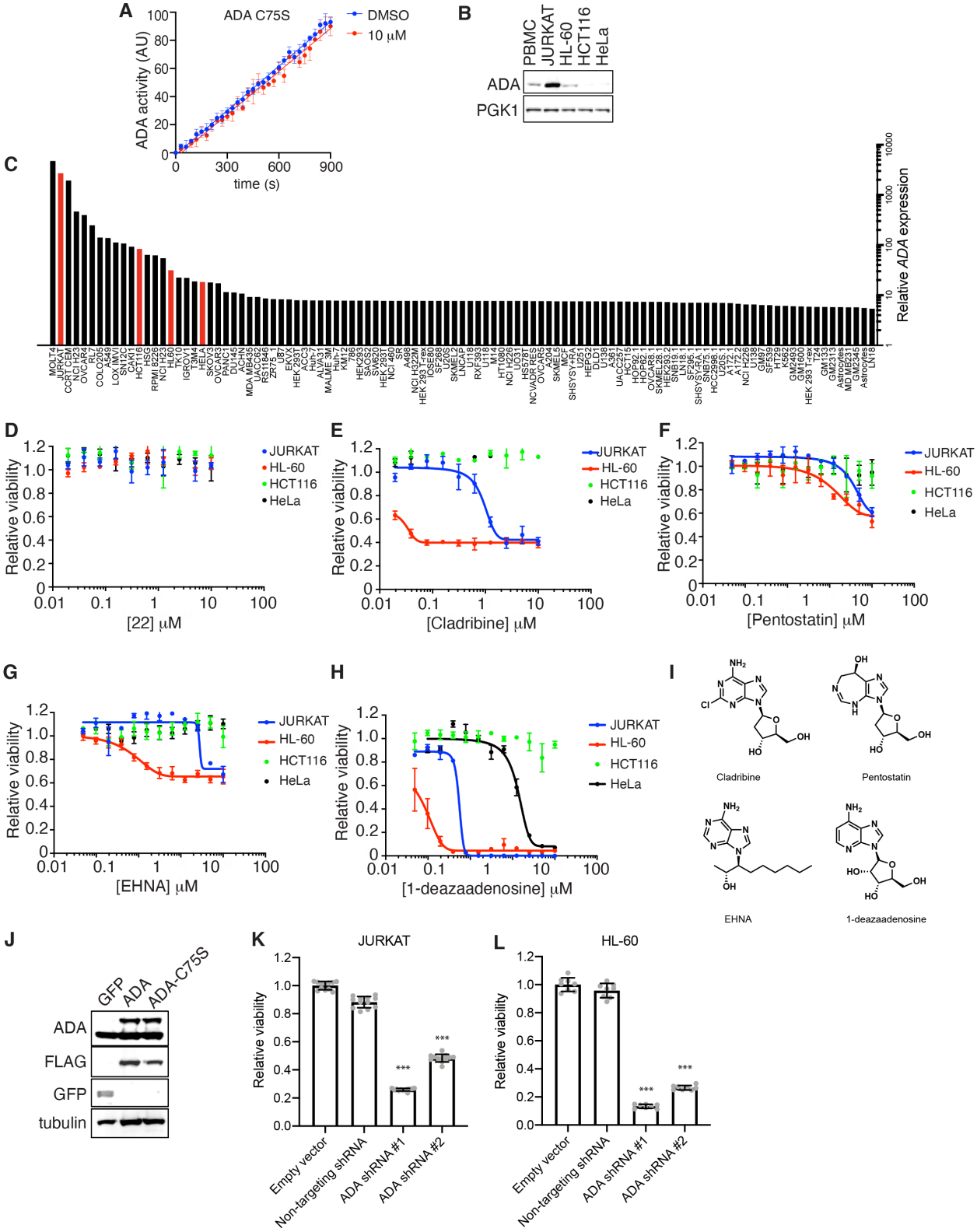
Supplementary Figure 8. Computational reaction modeling of S_NAr reactions with 5. Computed energy profiles and transition states in the reactions of **5** with methanethiolate (A) and methylamine (B), (Gibbs free energies and enthalpies, kcal/mol; distances (in black), angstrom; red numbers, natural population analysis (NPA) charge of indicated atoms).



Supplementary Figure 9. Compound 21 covalently modifies C75 of ADA. (A) Anti-biotin Western blot analysis of the indicated ADA-FLAG-immunoprecipitated transgene after *in situ* labeling with IA-biotin (100 μM). (B) Schematic depicting the site of labeling experiment performed with ADA and **21**. (C) MS1 peak intensities from the indicated tryptic peptide of immunoprecipitated ADA, unmodified or modified by pyridylation by *in situ* treatment of HEK293T cells with **21** (100 μM) for 1 hour. A star next to cysteine residues indicates observed modification by **21**.



Supplementary figure 10. MS/MS spectra for the ADA site-of-labeling experiment. MS/MS spectra of ADA peptide (amino acids 65-76) bearing an acetamide (+57.02146, top) or pyridinyl (+102.02180, bottom) modifications. The acetamide modification (a product of iodoacetamide treatment) occurs on cysteine residues that were not engaged with probe 21 in the experiment. The m/z 732.3259 and 754.8261 represent double-charged ADA peptides (65-76). The b and y ions are shown together with the corresponding peptide sequences.



Supplementary Figure 11. Inhibition of ADA activity is anti-proliferative in ADA-expressing lymphocyte-derived cell lines. (A) Enzymatic activity of ADA-C75S (AU = arbitrary fluorescent units) after a 1-hour pre-treatment with **21** (10 μM). (B)Western blotting analysis of ADA levels from the indicated cell lines. (C) Relative *ADA* transcript levels from NCI-

60 cell lines as retrieved from BioGPS (biogps.org). (D-I) Relative viability measurements of the indicated cell lines after treatment with concentration responses of **22**, Cladribine, Pentostatin, EHNA, and 1-deazaadenosine for 24 h (mean and s.e.m., *n*=3). **(**I) Structures of the indicated active site-directed inhibitors of ADA. (J) Western blot analyses from JURKAT-derived cell lines stably overexpressing GFP, ADA (a wildtype FLAG-tagged transgene), or ADA-C75S (a point mutant FLAG-tagged transgene) as in Figure 6G. (K, L) Relative viability measurements of JURKAT and HL-60 cells 96 h after transduction with lentiviruses encoding non-targeting or *ADA*-targeting shRNAs (*n*=3, mean and s.d.; ***P<0.0005, *t* test).

Cell sources

IMR32, HEK293T, JURKAT, HL-60, HCT116, Ramos and HeLa cells were purchased from American Type Culture Collection (ATCC). All cell types were propagated in DMEM (Corning) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% Penicillin-Streptomycin (Pen Strep, Gibco) with the exception of JURKAT, HL-60, Ramos, and T cells which were grown in RPMI-1640 (Gibco) supplemented with 10% FBS (Omega Scientific or Gibco) and 1% Pen Strep and 2 mM L-glutamine. PBMCs were isolated from healthy donors via standard Lymphoprep gradients (Sigma) and T cells isolated from this mixture using an EasySep Human T Cell Isolation Kit (Stemcell Technologies). Cell cultures were intermittently evaluated for the presence of mycoplasma contamination via an in-house ELISA-based detection service.

Miniaturized ARE-LUC assay and fragment library screen

For miniaturized reporter assays, 5,000 IMR32 cells were plated at 5 x 10^3 cells per well in white 384-well plates (Corning) in 40 µL of growth medium as above. 24 hours later, 100 ng of pTI-ARE-LUC reporter plasmid was delivered to cells in 10 µL of Optimem medium (Gibco), diluted such that per 1 µg of reporter plasmid 4 µL of FuGENE HD transfection reagent was supplied. The next day, 100 nL of DMSO solutions of compound was transferred per well using a PerkinElmer FX instrument such that the final concentration was that as reported. For high throughput evaluation of the fragment library, compounds were transferred via an Echo acoustic liquid handler (Labcyte). Screened fragments (Supplementary Data 1) were evaluated at 33, 16.7, 8.3, and 4.2 µM. After 24 hours of compound treatment, ARE-LUC luminance values were captured on an Envision plate reader after the administration of 30 µL of BrightGlo reagent (Promega, diluted 1:3 in water). For knockdown studies with ARE-LUC reporter vectors in HEK293T cells, 5 x 10^3 cells were plated in poly-D-lysine coated plates and transfected with 150 ng of shRNA vector (Sigma; scramble = SCH002; NRF2 shRNA55 = NM_006164.2-1987s1c1, NRF2 shRNA56 = NM_006164.2-1786s1c1) and 50 ng of pTI-ARE-LUC using Optimem and FuGENE HD reagent as above in 96-well plates. After 24-hour incubation, an additional 50 µL of growth medium containing compound concentrated such that the indicated concentration was added to each well. Luminance values were recorded after 24-hour incubation on an Envision instrument after 75 µL of BrightGlo solution as above.

Miniaturized viability assays

The indicated cell types were plated at 2 x 10^3 cells per well in white 384-well plates in 50 µL of their respective growth medium. 24 hours later, 100 nL of compound was transferred via pintool such that an assay concentration range from 40 nM to 100 µM was achieved. After 24 hours, viability was determined via luminance measurements on an Envision instrument after the addition of 30 µL of CellTiterGlo solution (Promega, diluted 1:6 in water). T cell viability was determined after 24-hour treatment with the indicated concentration of compound via fixable Near-IR live-dead staining (Invitrogen).

Western blotting

For Western blot analyses of total NRF2 levels, cells were first collected by trypsinization with TrypLE (Gibco) and brief centrifugation (500*g*). Pellets were sonicated in NRF2 loading buffer (50 mM TRIS-HCl (pH 6.8), 2% SDS, 10% glycerol, 100 mM DTT, and 0.1% bromophenol blue) and protein concentrations evaluated via absorbance measurements (Nanodrop instrument). 60 µg of lysate was separated on 8% Bis-Tris SDS PAGE gels (Invitrogen), transferred to low fluorescence PVDF membranes (Biorad) via a semi-dry transfer apparatus, and then blocked in 5% non-fat dry milk (Biorad) in TBST (Tris-buffered saline with 0.1% Tween 20) for one hour at room temperature. Primary antibody (anti-NFE2L2, H-300, Santa Cruz Biotechnology, 1:100) was incubated in 5% non-fat dry milk in TBST overnight at 4 degrees. After 5x TBST washes, fluorophore conjugated secondary antibodies (1:2000, LI-COR) were exposed in milk and TBST for 1 hour before visualizing on a LI-COR Odyssey fluorescent scanner. The relevant NRF2 band analyzed is ~100 kDa in mass, as has been reported widely in the literature as the correct molecular weight for this protein.[1] Western blots for other proteins were performed as above except cells were lysed in RIPA buffer directly after washing cells in culture with PBS. Centrifugally clarified lysates were loaded for separation of 4-12% Bis-Tris SDS PAGE gels (Invitrogen) with 4x loading buffer (200 mM Tris, 4% DSS, 1% BPB). Additional antibodies used in this study are anti-TUBG1 (Sigma, T6557, 1:2000), anti-PGK1 (SCBT, A-5, 1:1000), anti-FLAG (Sigma, M2, 1:2000), anti-Biotin (Abcam, ab1228, 1:500), and anti-NQO1 (Abcam, ab34173, 1:1000).

***In situ* labeling and immunoprecipitation of ectopically expressed ADA-FLAG and KEAP1-FLAG**

HEK293T cells were plated at 6 x 10^5 cells per well in 6-well plates in growth medium as above, and at the time of plating transfected with 1 µg of ADA-FLAG plasmid in 100 µL of Optimem medium with 4 µL of FuGENE HD transfection reagent. ADA-FLAG plasmid was constructed with a human codon-optimized gBlock from Integrated DNA Technologies which promotes ADA expression with a C-terminal FLAG tag separated from the CDS by a GGSGGS linker in the pCMV6-entry backbone (Origene). The C75S mutant plasmid was constructed using a Q5 Site-Directed Mutagenesis kit (NEB) according to supplied instructions using primers from IDT. Vector refers to empty pCMV6-Entry plasmid. 48 hours after transfection, cells were treated for 1 hour with the indicated doses of inhibitor 22 as 0.1% DMSO final solutions in growth medium followed by a 1-hour treatment with 100 µM of iodoacetamide ethylene biotin (IA-biotin, Biotium) also delivered as a DMSO solution. For KEAP1-FLAG transfection, HEK293T cells were transfected identically as above and incubated for 48 hours. The KEAP1-FLAG plasmid corresponds to Addgene plasmid 28023 (termed Flag-Keap1; RRID: Addgene_28023; a gift of Qing Zhong). Compound **4** was then delivered as a 0.1% DMSO final solution in growth medium for 1 hour at 37 degrees. After incubation, cells were washed twice with PBS, and then lysed directly with the addition of 250 µL ice-cold 1X RIPA buffer (Millipore) followed by tip sonication. After spinning at 12,000 rpm at 4 °C for 5 min to separate out the debris, lysate concentrations were quantified by absorbance measurements and 1 mg of lysate per condition was then incubated in 1 mL of RIPA buffer with 20 µL of magnetic M2 beads (M8823, Sigma-Aldrich) overnight with slow shaking at 4 °C. Beads were washed 3 times with 500 µL of RIPA buffer using magnetic separation and eluted by exposure of beads to 250 ng/mL FLAG-peptide (Sino Biological) for 30 minutes at room temperature. Lysates and immunoprecipitated material were separated by SDS-PAGE as above with the addition of either using anti-Biotin antibody (Abcam, 1228, 1:500) or IRDye 680 RD Streptavidin (1:1000 in 5% milk TBST overnight, LI-COR) to detect the competitive pyridylation of ADA C75.

Generation of stable cell lines.
Point mutations at position C75 in human ADA (expressed as a FLAG- and Myc-tagged transgene from Origene vector RC206679L3) were introduced using a site directed mutagenesis kit (NEB) and the identity of clones validated by Sanger sequencing. Lentiviruses encoding the overexpression of GFP (Addgene plasmid #19319, pLJM1-eGFP), ADA (wildtype, Origene vector RC206679L3), or ADA-C75S (derived from RC206679L) were generated in HEK293T cells using packaging plasmids pMD2.G (Addgene plasmid #12259) and pSPAX2 (Addgene plasmid #12260). Stable JURKAT cells were selected for 72 hours in 1 μg/mL puromycin and grown for 2 weeks to generate stable cell lines, the expression of appropriate transgene from which was validated using Western blotting as above.

Enzymatic assays of ADA activity.
Transgenes encoding C75S, C75F, C75Y, and C75W of ADA were introduced into vector RC206679L3 as above. 5 μg of plasmid was transiently transfected into HEK293T cells grown in 10 cm dishes and transgene allowed to express for 48 hours, at which time protein was isolated using magnetic anti-FLAG beads and the buffer exchanged into PBS without free FLAG peptide. 5 ng of the indicated enzyme per 100 μL in each well of a 96-well plate was evaluated in a fluorescent enzymatic activity assay from Sigma-Aldrich (catalog number EPI020) according to supplier's instructions. For assays involving covalent labeling with **21**, free enzyme in PBS was labeled for 1.5 hours at 37 C and then exposed to the enzymatic assay conditions. Fluorescent values of ADA activity over a linear portion of the reaction coordinate were normalized to the initial recorded values per condition and subtracted from background reactions devoid of ADA enzyme and reported as arbitrary fluorescent units (AU).

Quantitative RT-PCR
RNA from cells collected via trypsinization was isolated using RNeasy kits (Qiagen) and concentrations quantified using a Nanodrop instrument. 250 ng to 2 μg of RNA was then subjected to oligo dT-primed reverse transcription reactions (SuperScript III First Strand Synthesis Kit). Quantitative RT-PCR reactions were measured on a Viia7 Instrument (Thermo) using Clontech SYBR green-based master mix (TB Green Premix). Transcript specific primers are listed below. Reactions were normalized to *TUBG1* (Tubulin) levels. Ct values were determined by automatic thresholding within the Viia7 software and transcript abundance calculated using the standard comparative Ct method.

Transcript	Forward Primer Sequence	Reverse Primer Sequence
NQO1	GCCTCCTTCATGGCATAGTT	GGACTGCACCAGAGCCAT
HMOX1	GAGTGTAAGGACCCATCGGA	GCCAGCAACAAAGTGCAAG
GCLM	GCTTCTTGGAAACTTGCTTCA	CTGTGTGATGCCACCAGATT
TXNRD1	TCAGGGCCGTTCATTTTTAG	GATCTGCCCGTTGTGTTTG
GSR	TTGGAAAGCCATAATCAGCA	CAAGCTGGGTGGCACTTG
TUBG1	ATCTGCCTCCCGGTCTATG	TACCTGTCGGAACATGGAGG

***In vitro* reactions with GSH**
Stock solutions of 50 mM reduced Glutathione (GSH) in 0.2 M phosphate buffer pH = 7.4 were freshly prepared before the assay and kept on ice. Compounds were dissolved in DMSO at 50 mM and kept at – 20°C for long-term storage. The GSH stock solution was further diluted to 1 or 4 mM in 50 μL of 0.2 M phosphate buffer pH = 7.4. Electrophilic compounds (200 μM or 1 mM) were added by brief vortexing and then incubated at 37 °C for 1 hour in a water bath. Product

formation was evaluated by LC-MS by diluting 5 µL of the mixture into 45 µL of 1:1 CH_3CN/H_2O + 0.01% TFA and injecting either 5 or 1 µL depending on the concentration of the reaction.

Isolation of proteome and quantification of protein labeling

For proteomic analysis Ramos cells were grown to 1 x 10^6 cells/mL in media described above. The cells were then treated with DMSO or the compounds (50 µM) for 2h, collected by centrifugation (3 min at 1400 *g*), washed with cold PBS and lysed by sonication. The protein concentration was then normalized to 1.7 mg/mL using a standard DC protein assay (Bio-Rad) and the samples were analyzed by competitive isotopic tandem orthogonal proteolysis activity-based protein profiling (isoTOP ABPP) using a protocol from Gao D.-W. *et al.* (Supplementary Figure 4).[2]

isoTOP-ABPP *R* value calculation and processing

The heavy/light isoTOP-ABPP ratios (*R* values) for each unique peptide (DMSO/compound treated) were quantified as previously described with in-house CIMAGE software[3] using default parameters (3 MS1 acquisitions per peak and signal to noise threshold set to 2.5). Site-specific engagement of cysteine residues was assessed by blockade of IA-alkyne probe labeling. A maximal ratio of 20 was assigned for peptides that showed a ≥95% reduction in MS1 peak area in the compound treated proteome (light TEV tag) compared to the control DMSO-treated proteome (heavy TEV tag). Ratios for unique peptide sequences were calculated for each experiment; peptides with sequences encompassing the same cysteines were grouped together (e.g., different charge states, elution times or tryptic termini) and the median ratio for the experiment is reported as the final ratio (*R*). The reported sequences associated with each peptide entry represent the longest overlapping sequence between all constituent peptides, and the reported residue numbers are kept as originally annotated by ProLuCID search algorithm. The peptide ratios reported by CIMAGE were further filtered to ensure the removal or correction of low-quality ratios in each individual dataset. The quality filters applied were the following: removal of reverse and half-tryptic peptides; removal of non-unique peptides; removal of peptides with *R* = 20 and only a single MS2 event triggered during the elution of the parent ion; manual annotation of all the peptides with ratios of 20. Biological replicates of the same treatment were averaged if the standard deviation was below 60% of the mean; otherwise, for cysteines with at least one *R* value < 4 per treatment, the lowest value of the ratio set was taken. For peptides where all *R* values were > 4, the average was reported.

Within individual replicates and when aggregating ratios across replicates, peptides with R = 20 were discarded if the ratio set contained a single 20, and the minimum ratio in the set was less than 4. In order for a peptide to be reported it must be quantified in at least 2 replicates within a particular group. In order for a peptide to be considered “liganded” it must have the final value *R* ≥ 4. In order for the peptide to be included in the scatter plot in Supplementary Figure 4D, it must be quantified in at least 2 replicates for each of the groups (**KB02** and **8**).

Site of labeling experiments

For the site of labeling experiment with compound **8** recombinant GAPDH (50 µL, 50µM in PBS) was treated with compound **8** (1 µL, 3.125 mM, 1.25 equiv, final concentration 62.5 µM) for 80 min at room temperature in a 1.5 mL Eppendorf tube. Following this incubation period, acetone (200 µL) was added to precipitate the proteins, the tube was left on ice for 10 min and then spun down (maximum speed, 10 min). The supernatant was aspirated, the proteins were resuspended in PBS (50 µL) and treated with tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 1 µL of fresh 50 mM stock in water, final concentration = 1 mM) at 60 °C for 15 min. Iodoacetamide (2.5 µL of a 400 mM stock in water, final concentration = 20 mM) was then added and the final mixture was incubated at 37 °C for 30 min with shaking. Trypsin (Promega, sequencing grade; 2 µg in 6 µL of trypsin buffer containing 1 mM $CaCl_2$) was added to the

mixture and the digestion was allowed to proceed overnight at 37 °C with shaking. The samples were then acidified to a final concentration of 5% (v/v) formic acid and stored at –80 °C prior to analysis. The resulting tryptic peptides were analyzed by LC-MS/MS. The mass-spectra were extracted using the ProLuCID algorithm with the differential peptide modification for the S_NAr product on all cysteine residues (+93.085). For the site of labeling experiment with compound **21**, HEK293T cells overexpressing ADA-FLAG transgene were treated with **21** at 100 μM for 1 hr, at which time the cells were washed twice with PBS and thn lysed in RIPA buffer. Pyridylated ADA-FLAG transgene was isolated using anti-FLAG magnetic beads as described above and the modification determined using the same methodology above.

Chemicals

The fragment library was purchased through the chemical suppliers indicated in Supplementary Data 1. MSBT (2-Methylsulfonyl benzothiazole), CNBF (4-Chloro-7-Nitrobenzofurazan), and iodoacetamide were purchased from Sigma. IA-biotin (biotin ethylenediamine iodoacetamide) was from Biotium. Commercial compounds were diluted as powders into DMSO stocks and used without further purification.

Synthetic procedures

For chemical syntheses, solvents and reagents were purchased from Sigma-Aldrich and Combi-blocks and used directly without further purification. All reactions were carried out using anhydrous solvents under a N_2 atmosphere, unless otherwise noted. Analytical thin layer chromatography (TLC) was performed on 0.25 mm silica gel 60- F254. Visualization was carried out with UV light and vanillin or Ninhydrin staining. LC-MS analysis of reaction mixtures was carried out on an Agilent 6130 Quadrupole LC/MS, using an Agilent Poroshell 120 EC-C8 2.7 μm, (4.6 X 50 mm) column. High-resolution mass spectra (HRMS) were recorded on an Agilent Mass spectrometer using ESI-TOF (electrospray ionization-time of flight).

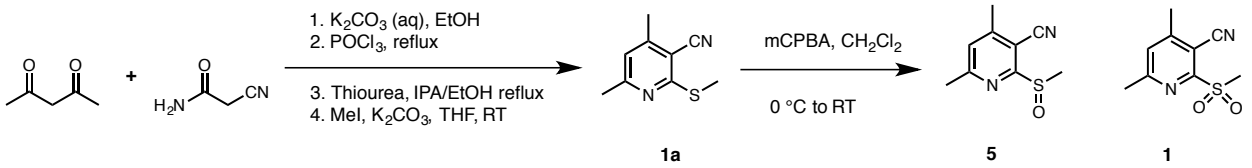
HPLC conditions for the purification of final compounds

Instrument – Agilent 1260 HPLC. Column – Luna 5 μm C18 100 Å 250 x 2mm; solvent A: milliQ water + 0.01% TFA; solvent B: acetonitrile + 0.01% TFA; flow rate – variable from 5 ml/min to 15 ml/min depending on scale. General gradient: 0 min – 95%A, 5 min – 85%A, 8 min – 70%A, 12 min – 50%A, from 14 to 25 min to reach 100%B, 18 to 24 min 95%A.

NMR analysis

$^1H/^{13}C$ and ^{19}F NMR spectra were recorded at ambient temperature on Bruker DMX 400 (400 MHz for ^1H NMR and 125 MHz for ^{13}C NMR), Bruker DRX 500 (500 MHz for 1H NMR and 125 MHz for 13C NMR), and DRX 600 CryoProbe (600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR) instruments in the specified deuterated solvents. Chemical shifts are given in ppm with respect to residual undeuterated solvent signal as internal standard. Coupling constants are reported as *J*-values in Hertz (Hz). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, m = multiplet, bs = broad singlet, br = broad.

Synthesis of 1 and 5



Compounds **1** and **5** were synthesized using variations of methods reported in the literature as summarized below. Advanced intermediate **1a**, is a commercially available compound for which full characterization is provided below.

Step 1
2-cyanoacetamide (4.2 g, 50 mmol, 1.0 equiv) was dissolved in 50 mL of absolute EtOH, and the resulting solution was treated with K_2CO_3 (2.0 g, 15 mmol, 0.3 equiv) delivered as a solution in 20 mL of H_2O. Acetyl acetone (5.0 g, 50 mmol, 1.0 equiv) was then added and the mixture was stirred overnight at room temperature. The white precipitate obtained was filtered through a fritted funnel, briefly washed with ice-cold EtOH and dried under vacuum for 3 hours (white powder, 5.3 g). LC-MS analysis indicated full conversion to the desired product that was brought to the next step without further purification.

Step 2
The above-mentioned product (5.3 g), was dissolved in 18 mL of anhydrous $POCl_3$ and refluxed until TLC and LC-MS analysis indicated that the starting material had been consumed. The orange thick mixture was then cooled down to room temperature and carefully poured into an Erlenmeyer flask containing 65 mL of ice-water (Note: this is an exothermic reaction and appropriate precautions should be taken). Upon pouring of the reaction into ice water a white precipitate formed, which was immediately filtered through a fritted funnel, briefly washed with ice-cold water and dried under vacuum for 3 hours (white powder, 3.0 g). LC-MS analysis confirmed the identity of the desired product that was brought to the next step without further purification.

Step 3
The above-mentioned product (3.0 g), was dissolved in a 1:1 mixture or EtOH/Isopropanol (or, alternatively, *n*-BuOH) to a final concentration of 0.1 M. To the resulting solution was added thiourea (4.1 g, 54 mmol, 3.0 equiv) and the mixture was refluxed until a yellow/orange precipitate appeared. TLC and LC-MS analysis indicated that the starting material had been consumed and converted to the desired product. The orange precipitate was then cooled down to room temperature and filtered through a fritted funnel, briefly washed with ice cold EtOH and dried under vacuum for 3 hours (bright yellow powder, 2.0 g). LC-MS analysis (negative mode) confirmed the identity of the desired product that was brought to the next step without further purification.

Step 4
The above-mentioned product (500 mg, 3.05 mmol, 1.0 equiv) was dissolved in 8 mL of anhydrous THF, treated with K_2CO_3 (992 mg, 6.7 mmol, 2.2 equiv), followed by dropwise addition of methyl iodide (265 µL, 4.27 mmol, 1.4 equiv). The mixture was stirred at room temperature overnight. TLC and LC-MS analysis indicated that the starting material had been consumed and converted to the desired product. The mixture was then filtered through a cotton

plug, the volatiles removed *in vacuo*, and product was purified by column chromatography on silica gel (10 to 30% ethyl acetate in hexanes) to obtain 250 mg of compound **1a**.

Spectral characterization of **1a**
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (s, 1H), 2.63 (s, 3H), 2.53 (s, 3H), 2.47 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 162.75, 161.31, 151.62, 119.55, 115.33, 104.79, 24.77, 20.10, 13.21 ppm.

Compounds **1** and **5** were obtained as follows: **4** (70 mg, 0.39 mmol, 1.0 equiv) was dissolved in 5 mL of anhydrous CH_2Cl_2, followed by portion wise addition of mCPBA (130 mg, 0.60 mmol, 1.5 equiv) at room temperature. TLC and LC-MS analysis indicated that the starting material had been fully converted to the desired sulfone and sulfoxide. The reaction was quenched with 10 mL of $NaHCO_3$ (aq, sat), diluted with additional 20 mL of CH_2Cl_2 and the organic layer was further washed (2x) with 10 mL of $NaHCO_3$ (aq, sat), dried *in vacuo* and the crude mixture was purified via column chromatography on silica gel (MeOH 0 to 5% in CH_2Cl_2) to obtain 20 mg of sulfoxide **5**, and 15 mg of sulfone **1**.

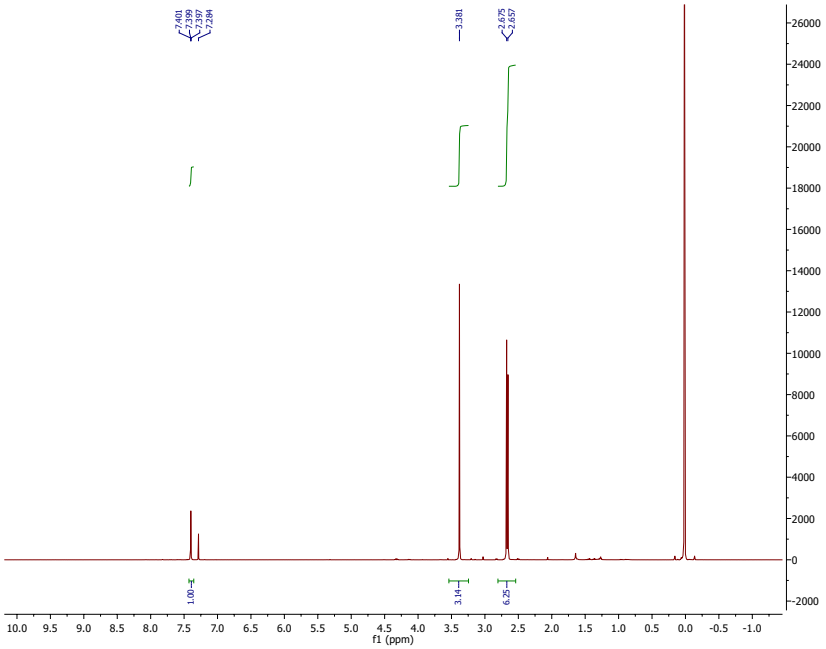
Spectral characterization of **1**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 3.38 (s, 3H), 2.67 (s, 3H), 2.65 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 161.73, 159.24, 154. 81, 127.51, 112.47, 104.36, 39.87, 24.52, 20.63 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_9H_{10}N_2O_2S$: 210.0463, expected: 211.0536, found: 211.0536.

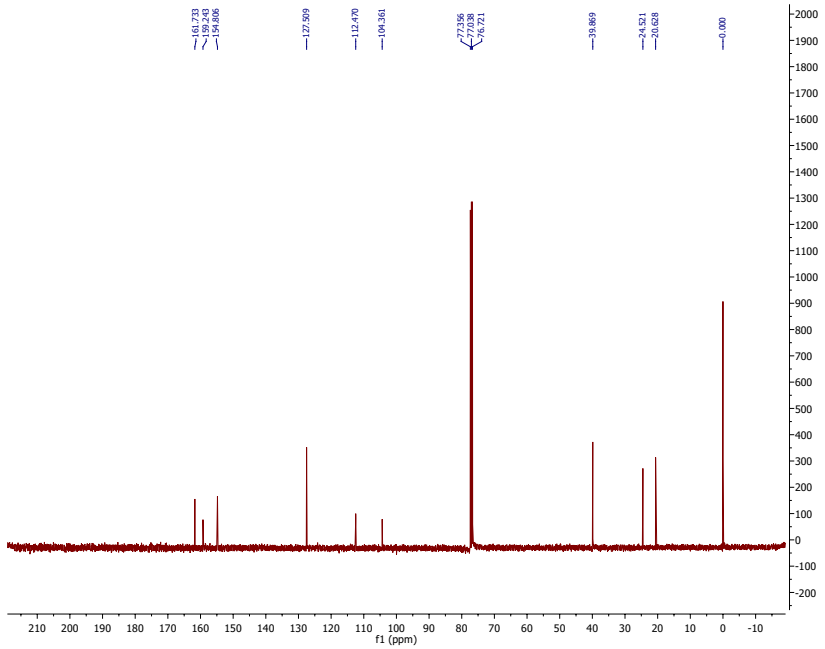
Spectral characterization of **5**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 1H), 2.95 (s, 3H), 2.70 (s, 3H), 2.61 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 165.71, 163.20, 153. 67, 126.15, 112.91, 105.71, 40.31, 24.71, 20.23 ppm. Dichloromethane impurity present.

HRMS High accuracy (ESI-TOF) Calcd. for $C_9H_{10}N_2OS$: 194.0510, expected: 195.0587, found: 195.0588.

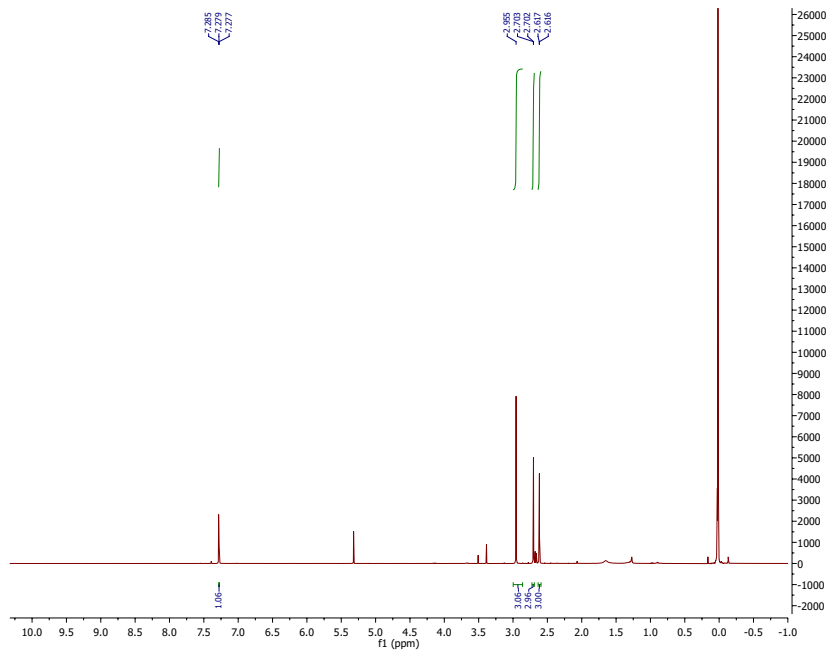
^{1}H NMR (400 MHz, $CDCl_3$) of **1**



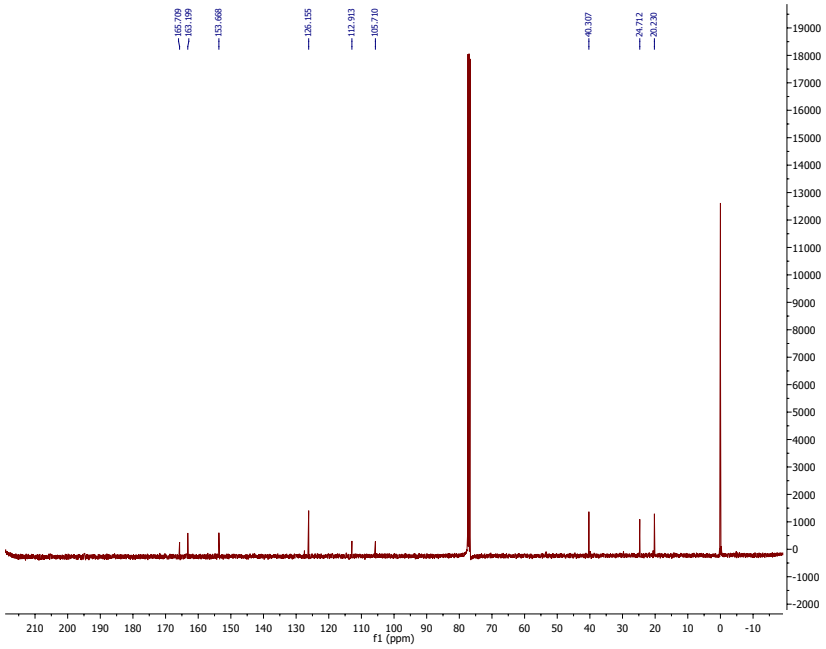
^{13}C NMR (125 MHz, $CDCl_3$) of **1**



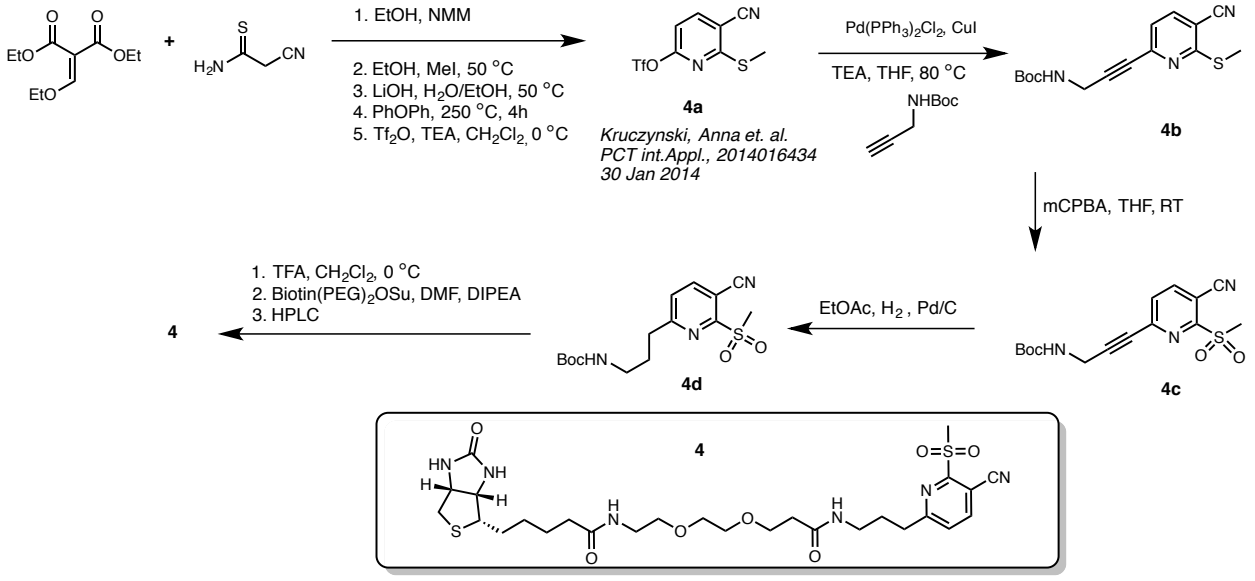
^{1}H NMR (400 MHz, $CDCl_3$) of **5**



^{13}C NMR (125 MHz, $CDCl_3$) of **5**



Synthesis of 4



Compound **4a** was obtained following a 5-steps sequence that involved two chromatography purifications, after step 4 and 5 respectively. All synthetic procedures were slight modifications of the reported route as it appears in *PCT int. Appl. 2014016434* by Kruczynski *et.al.*[4] Triflic acid pyridine ester **4a** was obtained as a yellow oil that solidified upon standing at -20 °C.

Spectral characterization of 4a.

^{1}H NMR (400 MHz, d_6-DMSO) δ 8.55 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 2.50 (s, 3H); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 164.53, 156.65, 147.91, 120.05 (plus satellite peaks from CF3 fluorine coupling), 114.87, 110.95, 107.53, 13.46 ppm. LC-MS (ESI): m/z $[M + H]^+$ calcd. for $C_{15}H_{17}N_3O_2S$: 303.10, found: 304.11

Anhydrous THF (15 mL, 0.11 M) was added to **4a** (500 mg, 1.7 mmol, 1.0 equiv), followed by TEA (652 µL, 5.1 mmol, 3.0 equiv), *N*-Boc-propargylamine (293 mg, 1.87 mmol, 1.1 equiv) dissolved in 3 mL of THF, $Pd(PPh_3)_2Cl_2$ (60 mg, 5 mol %), and finally a catalytic amount of CuI. The mixture was stirred at room temperature for 15 minutes, then brought to reflux and stirred for 2 h. TLC and LC-MS analysis indicated that the starting material had disappeared and desired Sonogashira coupling product formed in good yield. The mixture was then cooled to room temperature and filtered through a pad of Celite. Volatiles were evaporated *in vacuo* and the crude mixture was purified by column chromatography on silica gel (25% ethyl acetate in hexanes) to afford 450 mg of desired material **4b** as a yellow oil that was used directly in the next step.

LC-MS (ESI) for **4b**: m/z $[M + H]^+$ calcd. for $C_{15}H_{17}N_3O_2S$: 303.10, found: 304.11

Anhydrous THF (5 mL, 0.13 M) was added to **4b** (200 mg, 0.63 mmol, 1.0 equiv), followed by portion wise addition of mCPBA (331 mg, 1.90 mmol, 3.0 equiv) at room temperature. TLC and LC-MS analysis indicated that the starting material had fully converted to the desired sulfone. At this point 10 mL of $NaHCO_3$ (aq, sat) was added to the mixture along with 30 mL of Et_2O and the organic layer was further washed (2x) with 10 mL of $NaHCO_3$ (aq, sat), passed through a short pad of silica gel and concentrated *in vacuo*. The crude material was then dissolved in 10 mL of ethyl acetate, Pd/C was added to the mixture that was then submitted to five 3-minute cycles of H_2/vacuum using a Schlenk line. A hydrogen balloon was then applied, and the reaction stirred for 2h at room temperature. LC-MS analysis indicated full conversion to desired reduced material **4c**, and at this point the catalyst was removed by filtering through Celite, volatiles were evaporated *in vacuo* and the crude mixture was purified by column chromatography on silica gel (40% to 60% ethyl acetate in hexanes) to afford 50 mg of desired material **4c** as a yellow oil.

Spectral characterization of 4c.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 3.39 (s, 3H), 3.22 (m, 2H), 3.00 (m, 2H), 1.99 (m, 2H), 1.45 (s, 9H) ppm. LC-MS (ESI): m/z $[M + H]^+$ calcd. for $C_{339}H_{21}N_3O_4S$: 339.41, found: 340.50

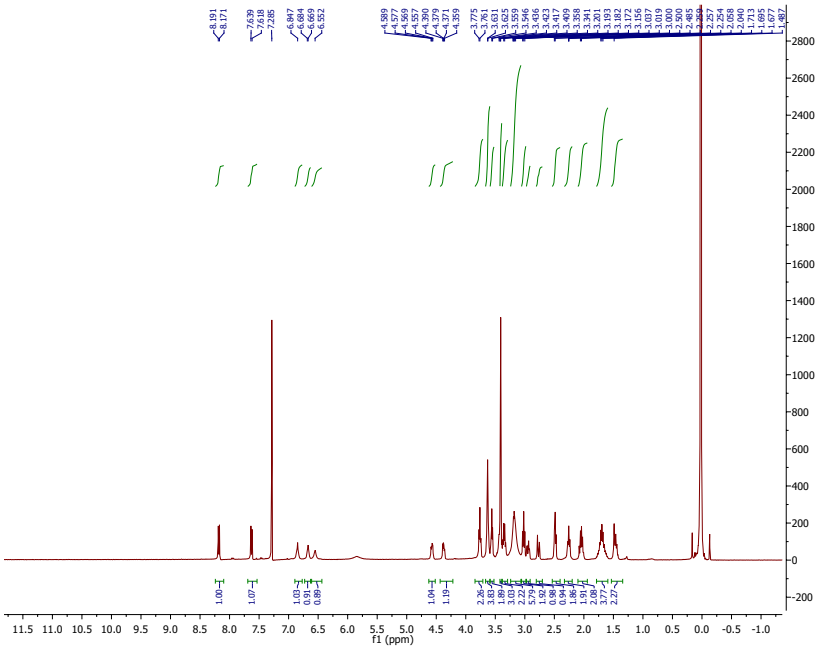
Anhydrous CH_2Cl_2 (3 mL) was added to **4c** (30 mg, 0.088 mmol, 1.0 equiv) and the mixture was cooled with an ice bath. TFA (2 mL) was added dropwise to the solution and the reaction was stirred at room temperature for 1h. After this time, LC-MS analysis indicated that the starting material was fully deprotected. The volatiles were then removed *in vacuo,* the crude mixture was taken up in anhydrous DMF (3 mL), Hunig's base (120 µL, 0.88 mmol, 10.0 equiv) was added followed by Biotin-PEG_2-NHS ester (PurePEG, CAS#596820-83-6) (62 mg, 0.125 mmol, 1.1 equiv). The mixture was left stirring overnight at room temperature and LC-MS analysis indicated that the reaction had proceeded to completion. The crude mixture was then filtered through a 0.45-micron filter and injected directly for HPLC purification (semi-preparative column).
Purification of the final biotin probe **4** was carried out on an Agilent 1260 HPLC, with a Luna 10 µm C18, (250 x 30 mm) preparative column using a gradient from 90:10 to 0:100 of H_2O/CH_3CN containing 0.01% TFA. Method specification: method length 25 minutes with a flow rate of 5

mL/min, monitoring UV absorbance at 210, 254 and 280 nm. Fractions containing the desired product, as confirmed by LC-MS analysis, were pooled, freeze-dried and lyophilized obtaining 5 mg of desired material **4**, which was characterized by ^{1}H, ^{13}C NMR, and HRMS as described and shown below.

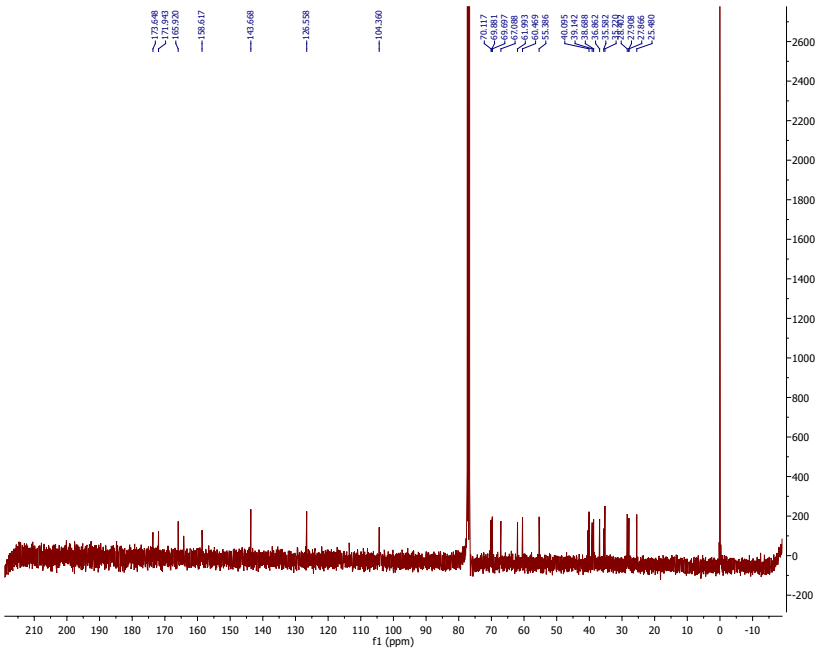
Spectral characterization of 4.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 6.84 (bs, 1H), 6.66 (bs, 1H), 6.50 (bs, 1H), 4.57 (dd, J = 8.0, 4.8 Hz, 1H), 4.38 (dd, J = 8.0, 4.8 Hz, 1H), 3.75 (t, *J* = 6.0 Hz, 2H), 3.63-3.57 (m, 4H), 3.56 (t, *J* = 5.2 Hz, 2H), 3.41-3.33 (m, 8H), 3.19-3.18 (m, 2H), 3.06-2.93 (brm, 10H), 2.76 (d, *J* = 13.2 Hz, 1H), 2.49 (t, *J* = 6.0 Hz, 2H), 2.26 (t, *J* = 6.0 Hz, 2H), 2.08-2.02 (m, 2H), 1.73-1.65 (m, 4H), 1.48-1.44 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.65, 171.94, 165.92, 158.62, 143.67, 126.56, 104.36, 70.11, 69.88, 69.69, 67.08, 61.99, 60.47, 55.38, 40.44, 40.09, 39.14, 38.68, 35.58, 35.22, 28.40, 27.91, 27.87, 25.48 ppm

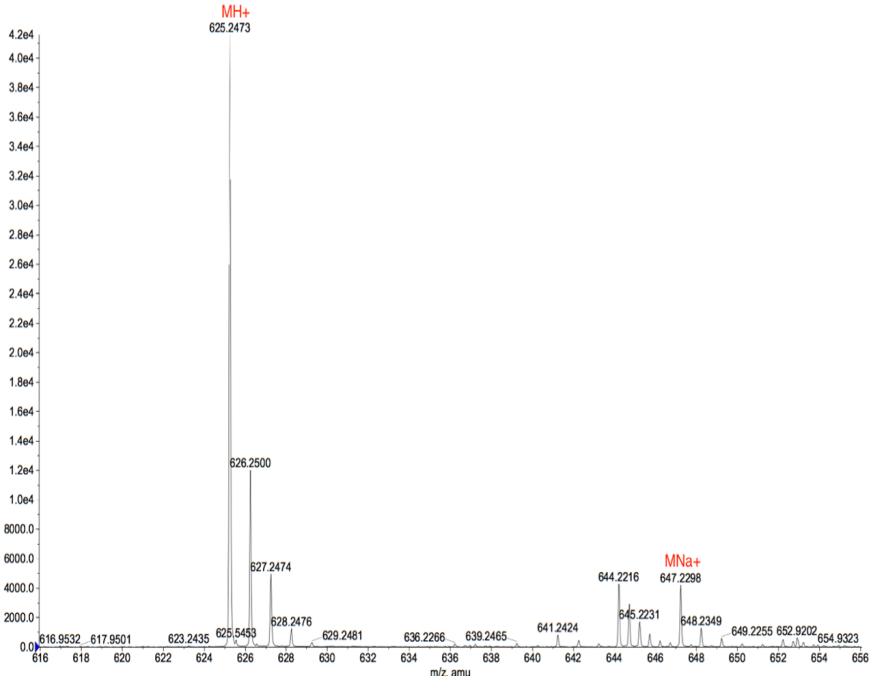
^{1}H NMR (400 MHz, $CDCl_3$) of **4**



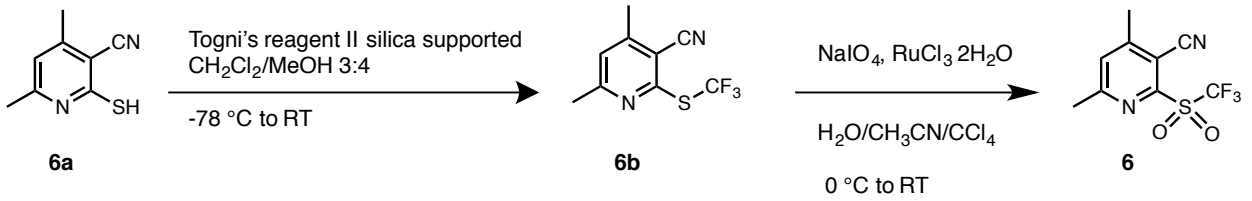
^{13}C NMR (125 MHz, $CDCl_3$) of **4**



High accuracy (ESI-TOF) of **4**.
Calcd. for $C_{27}H_{40}N_6O_7S_2$: 624.2400, expected: 625.2473, found: 625.2473



Synthesis of 6



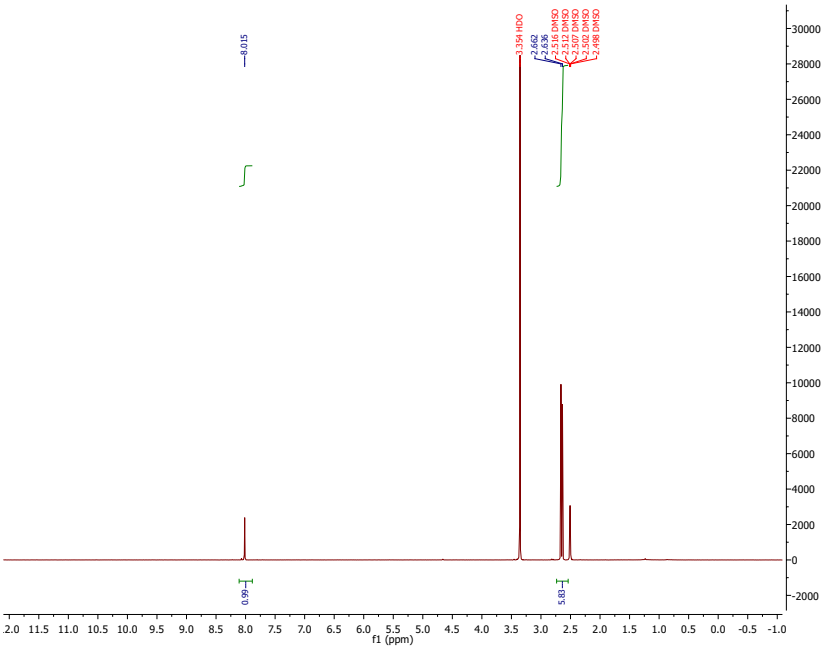
Trifluoromethylation using Togni's reagent II (1-Trifluoromethyl-1,2-benziodoxol-3-(1H)-one
Compound **6a**, which is commercially available, (100 mg, 0.61 mmol, 1.0 equiv) was resuspended, under inert atmosphere with sonication, in a 3:4 mixture of anhydrous MeOH/CH_2Cl_2 and cooled to -78 °C with a dry-ice/acetone bath. Togni's reagent II, 60% weight containing 40% Celatom (368 mg, 0.67 mmol, 1.1 equiv), was slowly added and the mixture was stirred at room temperature for 2h. TLC and LC-MS analysis indicated an acceptable conversion to the desired product, the reaction was therefore quenched with a spoon of silica gel, diluted with CH_2Cl_2, the slurry was then brought to dryness and dry-loaded for purification via silica gel (EtOAc 5 to 15-20% in Hexanes) to obtain 50 mg of **6b**, which was then brought directly to the final oxidation.

LC-MS (ESI): m/z $[M + H]^+$ calcd. for **6b** $C_9H_7F_3N_2S$: 232.03, found: 233.20

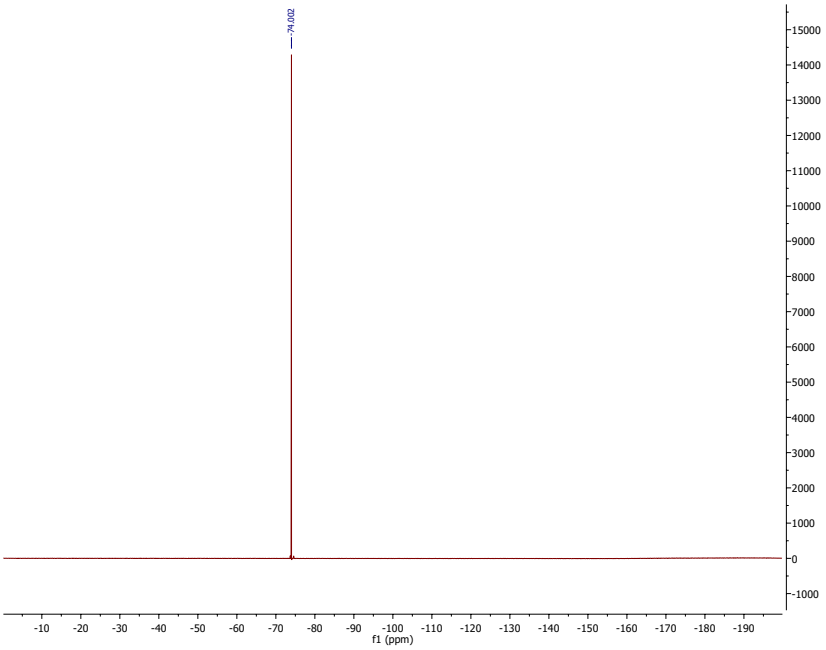
Oxidation using catalytic Ruthenium (III)
6b (25 mg, 0.1 mmol, 1.0 equiv) was dissolved in a mixture of CCl_4 (0.5 mL), H_2O (0.5 mL) and CH_3CN (1 mL), followed by addition of sodium metaperiodate (60 mg, 0.30 mmol, 3.0 equiv) and a catalytic amount of ruthenium (III) chloride hydrate. The mixture immediately turned dark red/brown and was stirred for 24h. TLC and LC-MS analysis indicated that the starting material had been fully converted to the desired sulfone. The reaction was quenched with a spoon of silica gel, diluted with CH_2Cl_2, the slurry was then brought to dryness and dry-loaded for purification via silica gel (10 to 30% EtOAc in Hexanes) to obtain 20 mg of **6**

Spectral characterization of **6**
^{1}H NMR (400 MHz, d_6-DMSO) δ 8.01 (s, 1H), 2.66 (s, 3H), 2.63 (s, 3H); **^{13}C NMR** (125 MHz, d^6-DMSO) δ 163.42, 156.67, 150.45, 131.16, 121.53, 118.26, 112.44, 109.23, 24.39, 20.81 ppm.
^{19}F NMR (400 MHz, d_6-DMSO) δ -74.0 ppm.
Note: ^{13}C spectra shows satellite peaks arising from C-F coupling.
HRMS High accuracy (ESI-TOF) Calcd. for $C_9H_7F_3N_2O_2S$: expected 265.0253, 287.0073 (M+Na) found: 287.0066

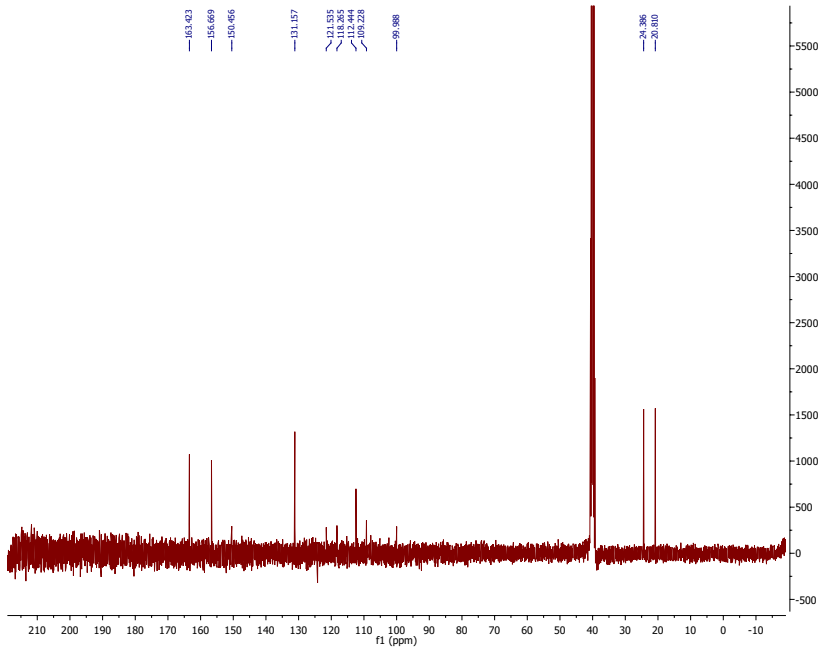
^{1}H NMR (400 MHz, d_6-DMSO) of **6**



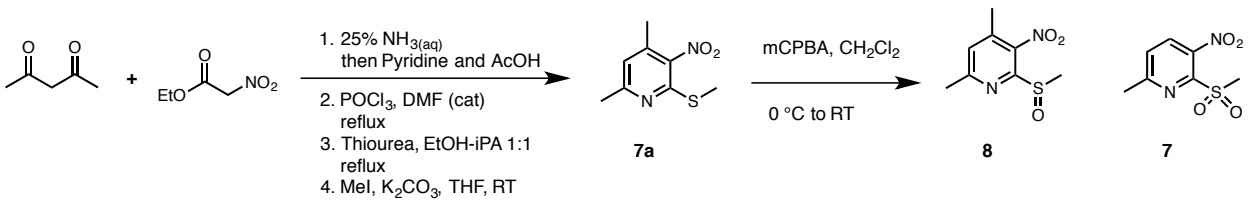
^{19}F NMR (400 MHz, d_6-DMSO) of **6**



^{13}C NMR (125 MHz, d_6-DMSO) of **6**



Synthesis of 7 and 8



Compounds **7** and **8** were synthesized using slight variations of methods known in the literature and are summarized below.

Step 1 to 4.
Advanced material 2,4-dimethyl-5-nitro-6-chloropyridine (product after step 1 and 2) is commercially available. The intermediate was carried through a 2-step sequence involving thiourea-mediated S_NAr and S-methylation that was previously described. Product identity was confirmed by ^{1}H NMR and LC-MS.

Spectral characterization of intermediate **7a**
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (s, 1H),2.58 (s,3H), 2.55 (s, 3H), 2.42(s, 3H).

Oxidation using mCPBA
Compounds **7** and **8** were obtained as follow: **7a** (86 mg, 0.43 mmol, 1.0 equiv) was dissolved in 5 mL of anhydrous CH_2Cl_2, followed by portionwise addition of mCPBA (120 mg, 0.52 mmol, 1.2 equiv) at room temperature. TLC and LC-MS analysis indicated that the starting material had been fully converted to the desired sulfone and sulfoxide. The reaction was quenched with 10 mL of $NaHCO_3$ (aq, sat), along with 20 mL of CH_2Cl_2 and the organic layer was further washed

(2x) with 10 mL of $NaHCO_3$ (aq, sat). The organic layer was then dried *in vacuo* and the crude mixture was purified via column chromatography on silica gel (0 to 5% MeOH in CH_2Cl_2) to obtain 30 mg of sulfoxide **8**, and 15 mg of sulfone **7**.

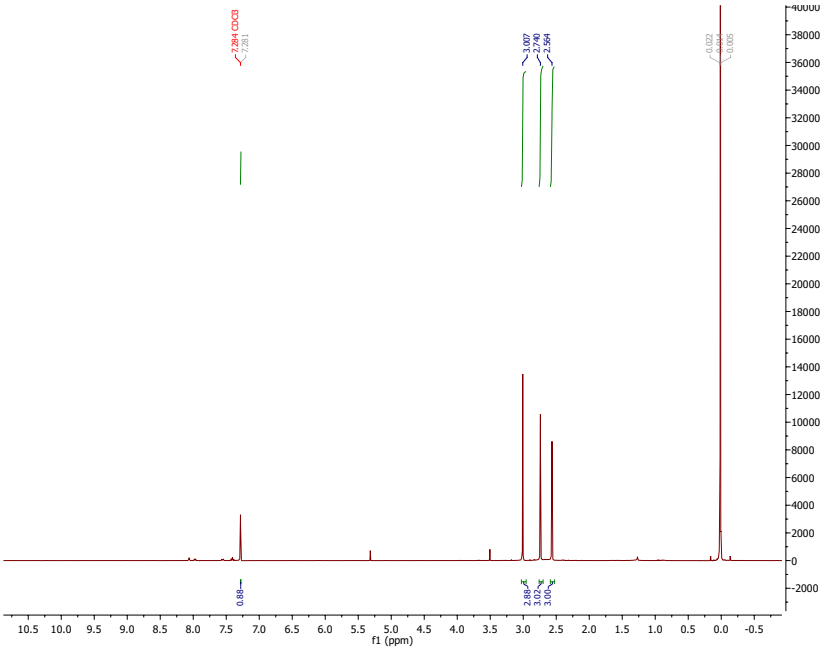
Spectral characterization of **8**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 1H), 3.00 (s, 3H), 2.74 (s, 3H), 2.56 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 163.18, 158.87, 143. 41, 128.29, 128.24, 41.05, 24.47, 18.83 ppm. *Note 1*: the aromatic peak is underneath $CDCl_3$ peak, as visible in the attached spectra. *Note 2*: one quaternary carbon is not visible under this condition.

HRMS High accuracy (ESI-TOF) Calcd. for $C_8H_{10}N_2O_3S$: expected 215.0485, found: 215.0484

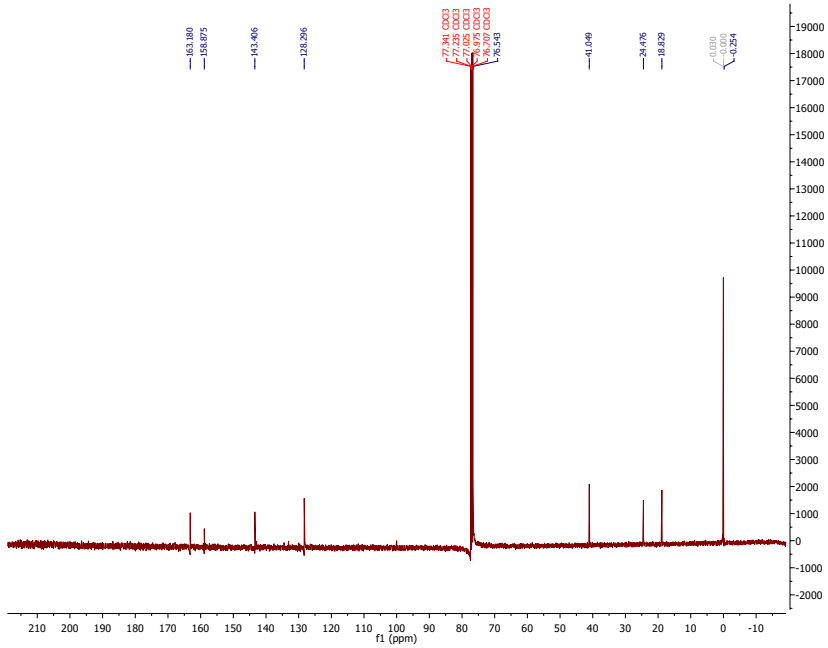
Spectral characterization of **7**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 3.33 (s, 3H), 2.68 (s, 3H), 2.42 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 160.45, 147.61, 142.36, 129.71, 41.26, 24.11, 17.06 ppm. *Note*: one quaternary carbon is not visible under this condition.

HRMS High accuracy (ESI-TOF) Calcd. for $C_8H_{10}N_2O_4S$: expected 231.0434, found: 231.0433

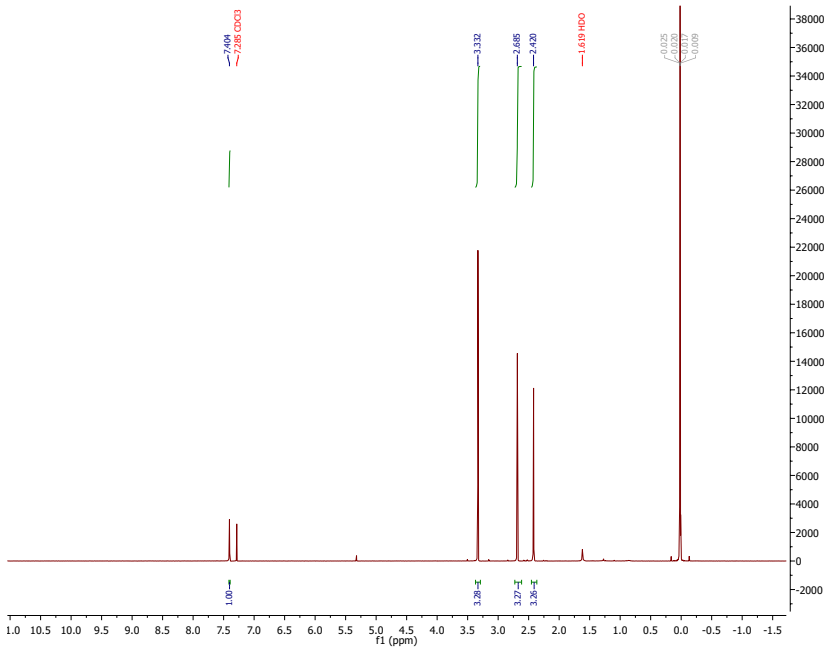
^{1}H NMR (400 MHz, $CDCl_3$) of **8**



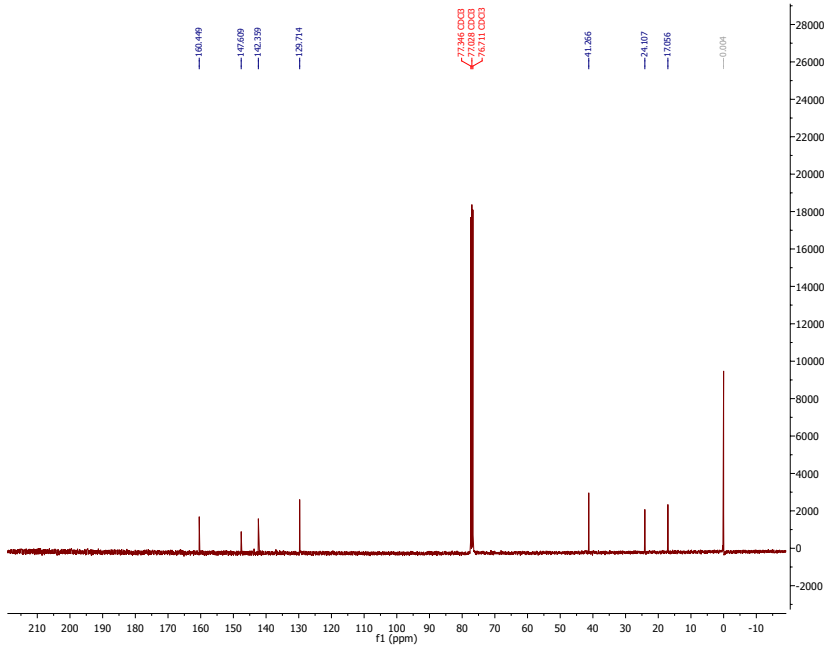
^{13}C NMR (125 MHz, $CDCl_3$) of **8**



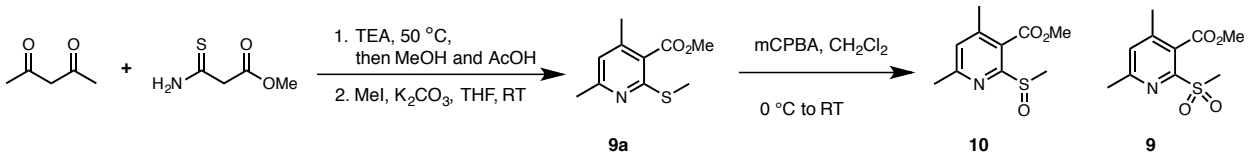
^{1}H NMR (400 MHz, $CDCl_3$) of **7**



^{13}C NMR (125 MHz, $CDCl_3$) of **7**



Synthesis of 9 and 10



Compounds **9** and **10** were synthesized using slight variations of methods known in the literature and are summarized below.

Step 1.
Pyridine condensation is described in: *Russian Chemical Bulletin*, *57* (10), 2139-2145, **2008**.[5] Product identity was confirmed by ^{1}H NMR and LC-MS.

Step 2
The abovementioned pyridine condensation product (100 mg, 0.51 mmol, 1.0 equiv) was dissolved in 3 mL of anhydrous THF, treated with K_2CO_3 (166 mg, 1.12 mmol, 2.2 equiv) and finally methyl iodide (50 µL, 0.71 mmol, 1.4 equiv) was added. The mixture was stirred at room temperature overnight. TLC and LC-MS analysis indicated that the starting material had been consumed and converted to the desired product. The mixture was then filtered through a cotton plug, the volatiles removed *in vacuo*, and the crude mixture was purified by column chromatography on silica gel (10 to 30% ethyl acetate in hexanes) to obtain 60 mg of compound **9a**.

Compound **9a** spectral characterization
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (s, 1H),3.95 (s,3H), 2.56 (s, 3H), 2.49 (s, 3H), 2.32 (s, 3H).

Oxidation

Compounds **9** and **10** were obtained as follows: **7** (60 mg, 0.28 mmol, 1.0 equiv) was dissolved in 3 mL of anhydrous CH_2Cl_2, followed by portionwise addition of mCPBA (96 mg, 0.42 mmol, 1.5 equiv) at room temperature. TLC and LC-MS analysis indicated that the starting material had been fully converted to the desired sulfone and sulfoxide. The reaction was quenched with 10 mL of $NaHCO_3$ (aq, sat), along with 20 mL of CH_2Cl_2, and the organic layer was further washed (2x) with 10 mL of $NaHCO_3$ (aq, sat). The organic layer was then dried *in vacuo* and the crude mixture was purified by column chromatography via silica gel (0 to 5% MeOH in CH_2Cl_2) to obtain 15 mg of sulfoxide **10** and 15 mg of sulfone **9**.

Spectral characterization of **10**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 3.97(s, 3H), 2.92 (s, 3H), 2.63 (s, 3H), 2.463 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 166.34, 159.61, 153. 48, 147.64, 128.24, 126.33, 53.23, 41.01, 24.09, 19.03 ppm. *Note*: CH_2Cl_2 residual peak at 5.32 ppm.

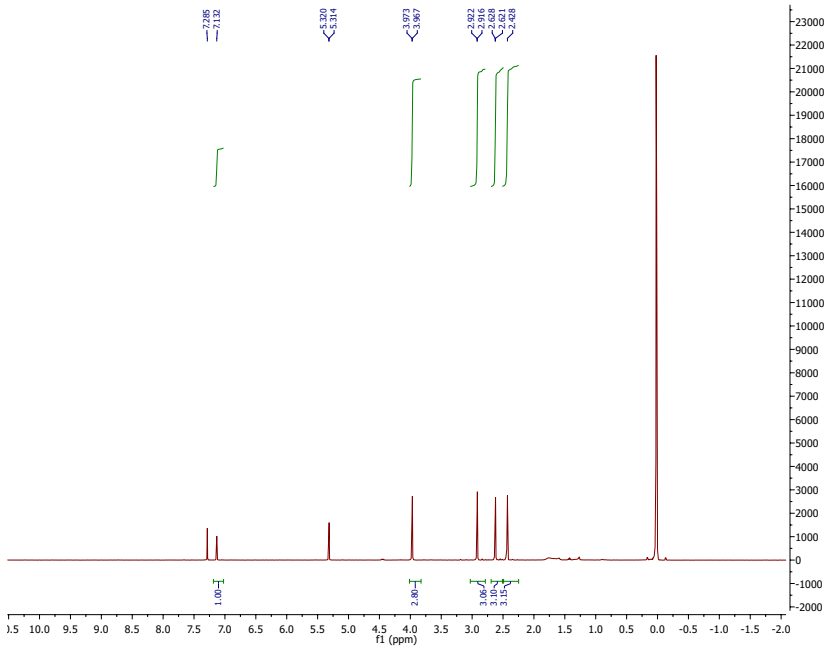
HRMS High accuracy (ESI-TOF) Calcd. for $C_{10}H_{13}NO_3S$: expected 228.0689, found: 228.0690

Spectral characterization of **9**

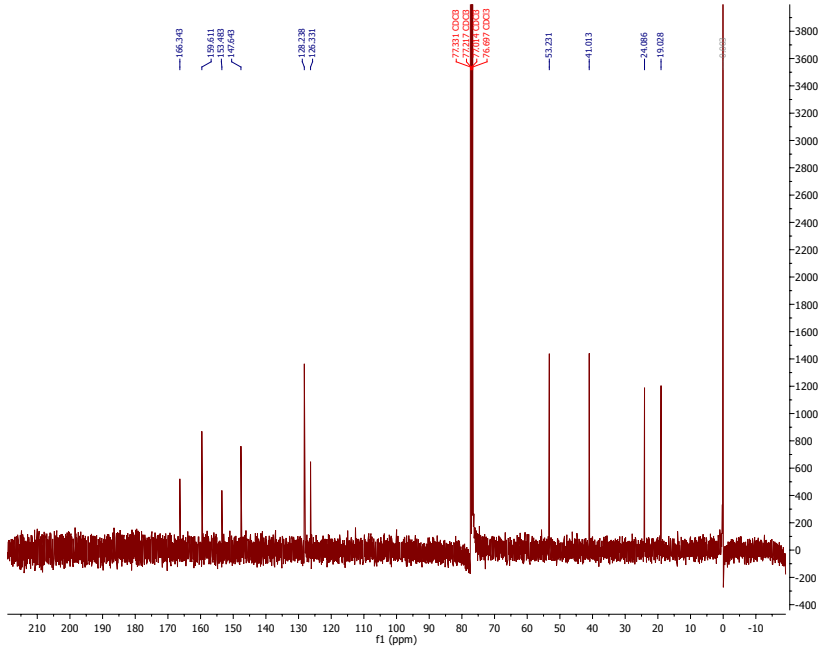
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 1H), 3.99 (s, 3H), 3.27 (s, 3H), 2.63 (s, 3H, 2.42 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 165.71, 163.20, 153. 67, 126.15, 112.91, 105.71, 40.31, 24.71, 20.23 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{10}H_{13}NO_4S$: expected 244.0638, found: 244.0639.

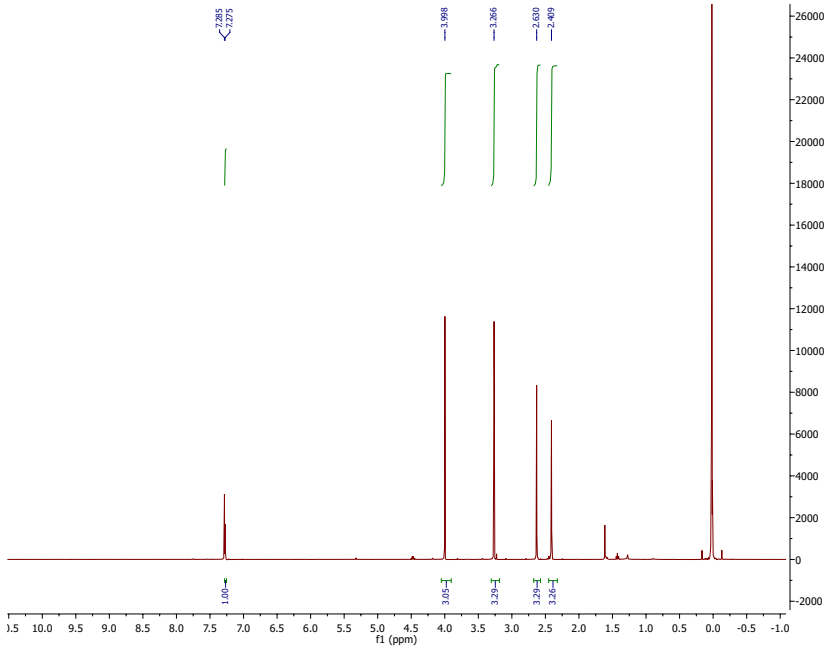
^{1}H NMR (400 MHz, $CDCl_3$) of **10**



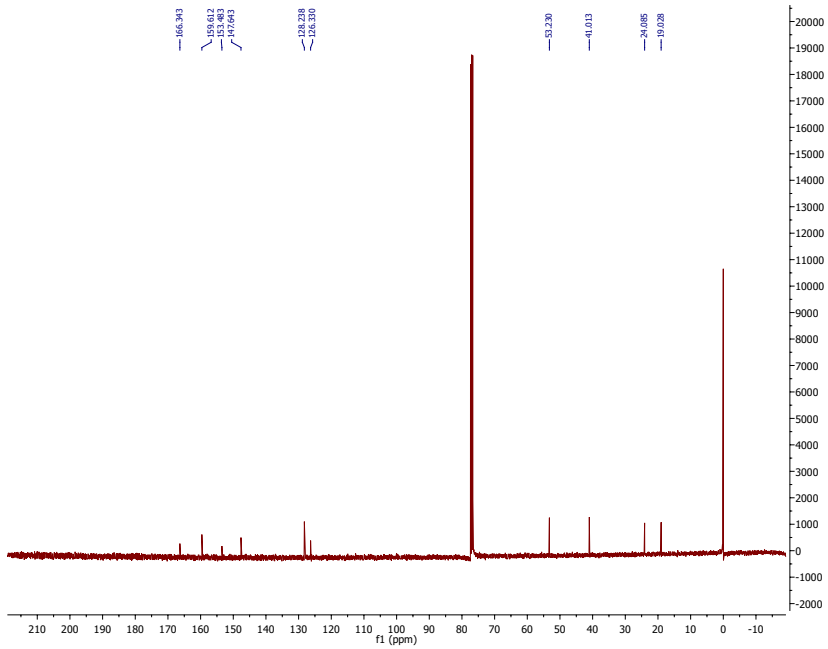
^{13}C NMR (125 MHz, $CDCl_3$) of **10**



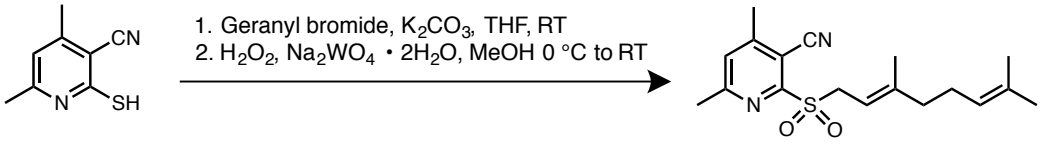
^{1}H NMR (400 MHz, $CDCl_3$) of **9**



^{13}C NMR (125 MHz, $CDCl_3$) of **9**



Synthesis of 11



6a **11**

Compound **6a** (170 mg, 1.02 mmol, 1.0 equiv) and K_2CO_3 (210 mg, 1.53 mmol, 1.5 equiv) were resuspended in 7 mL of a 1:1 mixture of anhydrous EtOH/DMF and stirred for 5 minutes at room temperature. Geranyl bromide (222 µL, 1.12 mmol, 1.1 equiv) was added dropwise, and the mixture was stirred until TLC and LC-MS analysis indicated complete consumption of the starting materials. The reaction mixture was then diluted with 20 mL of CH_2Cl_2, filtered through a cotton plug, and then washed with water (2 x 20 mL) and brine (2 x 20 mL). Organic phase was then dried over Na_2SO_4 and solvent was evaporated *in vacuo*. The crude mixture was then brought directly to the final oxidation step.

Oxidation using catalytic sodium tungstate and hydrogen peroxide

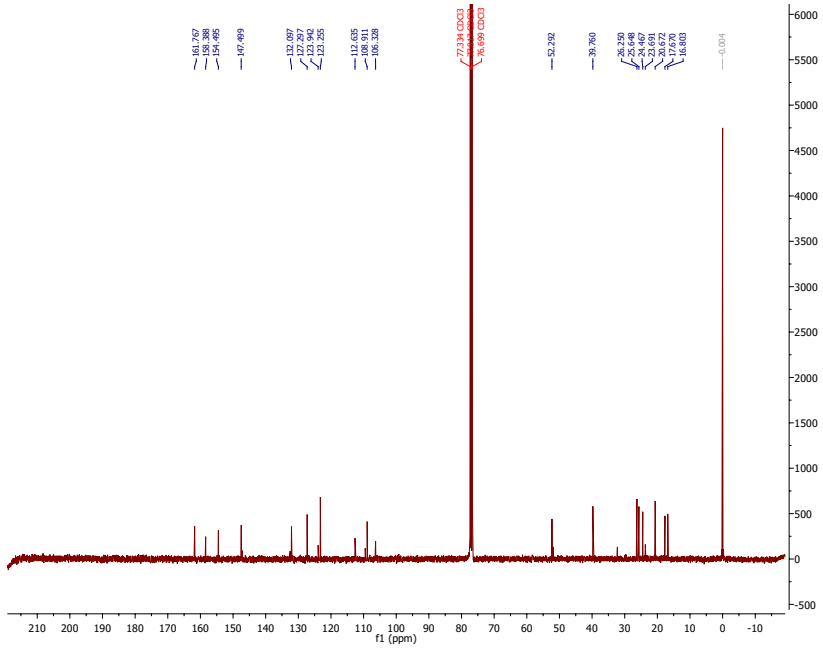
The abovementioned intermediate (300 mg, 1.0 mmol, 1.0 equiv) was dissolved in 10 mL of HPLC-grade MeOH and then cooled on an ice-bath. Sodium tungstate dihydrate (85 mg, 0.25 mmol, 0.25 equiv) was added to the solution, followed by slow addition of a 30% wt solution of H_2O_2 (1.2 mL, 10 mmol, 10 equiv), and the resulting mixture was stirred at room temperature for 24h. TLC and LC-MS analysis indicated that the starting material had been almost fully converted to the desired sulfone. The reaction was then diluted with 20 mL of CH_2Cl_2, washed with water (2 x 20 mL), and the organic phase was evaporated *in vacuo*. The crude reaction mixture was purified via column chromatography on silica gel (20 to 50% EtOAc in hexanes) to obtain 160 mg of **11**.

Spectral characterization of **11**

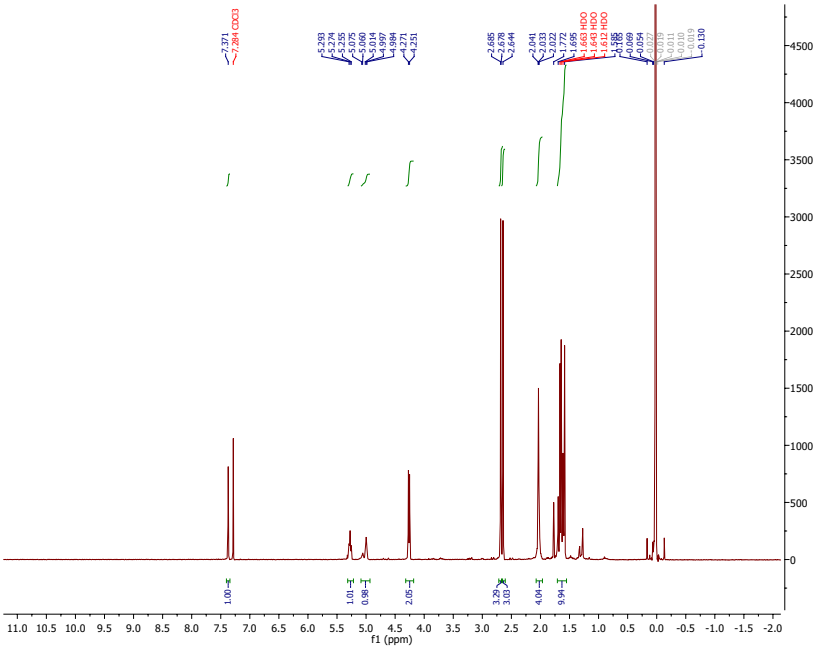
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 5.27 (t, *J* = 7.6 Hz, 1H), 5.05-4.98 (m, 1H), 4.27 (d, *J* = 8Hz, 2H), 2.68 (s, 3H), 2.64 (s, 3H), 2.04 (s, 4H), 1.69-1.58 (m, 9H) ; **^{13}C NMR** (125 MHz, $CDCl_3$) δ 161.76, 158.38, 154.49, 147.49, 132.10, 127.29, 123.25, 112.63, 108.91, 106.33, 52.29, 39.76,26.25, 26.65, 24.47, 20.67, 17.67, 16.80 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{18}H_{24}N_2O_2S$: expected 333.1631, found: 333.1637

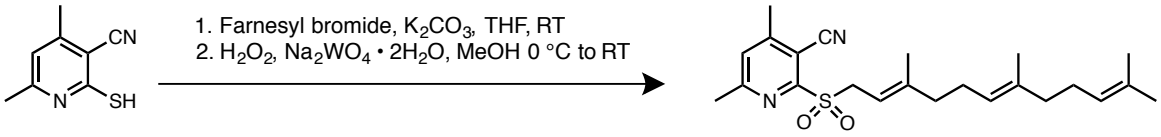
^{1}H NMR (400 MHz, $CDCl_3$) of **11**



^{13}C NMR (125 MHz, $CDCl_3$) of **11**



Synthesis of 12



6a **12**

Compound **6a** (160 mg, 0.97 mmol, 1.0 equiv), and K_2CO_3 (207 mg, 1.45 mmol, 1.5 equiv) were resuspended in 5 mL of anhydrous THF and stirred for 5 minutes at room temperature. Farnesyl bromide (290 µL, 1.1 mmol, 1.1 equiv) was added dropwise, and the mixture was stirred until TLC and LC-MS analysis indicated complete consumption of the starting materials. The reaction mixture was then diluted with 20 mL of Et_2O, filtered through a cotton plug, and evaporated *in vacuo*. The crude mixture was then purified via column chromatography on silica gel (0 to 15% EtOAc in Hexanes) to obtain 250 mg of S-farnesylated pyridine, which was then brought directly to the final oxidation step.

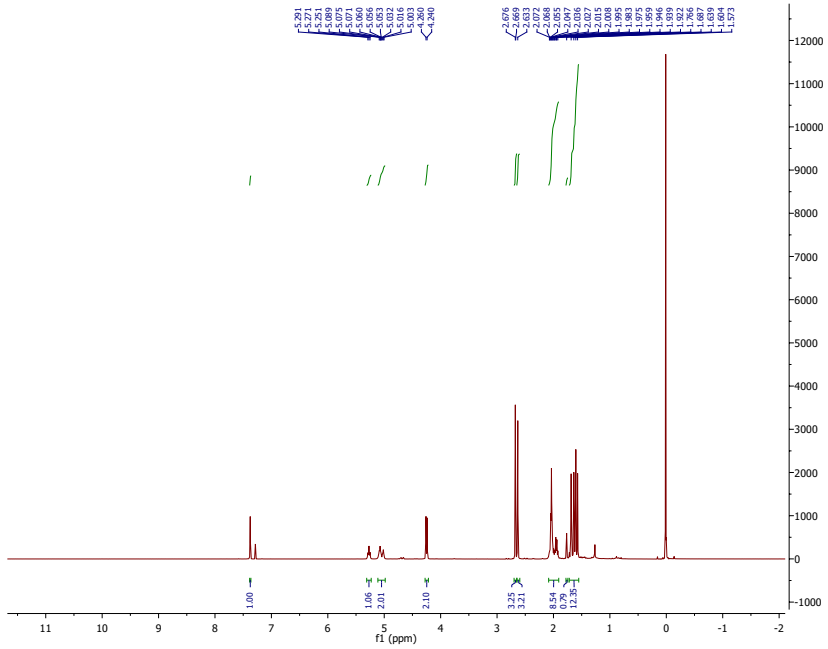
Oxidation using catalytic sodium tungstate and hydrogen peroxide

The abovementioned intermediate (50 mg, 0.14 mmol, 1.0 equiv) was dissolved in 3.5 mL of HPLC-grade MeOH and cooled on an ice-bath. Sodium tungstate dihydrate (10 mg, 0.035mmol, 0.25 equiv), was then added to the solution, followed by slow addition of a 30% wt solution of H_2O_2 (180 µL, 1.4 mmol, 10 equiv), and the mixture stirred for 24h. TLC and LC-MS analysis indicated that the starting material had been fully converted to the desired sulfone. The reaction was brought to dryness and loaded directly on silica gel for final purification (10 to 50% EtOAc in hexanes) to obtain 30 mg of **12**.

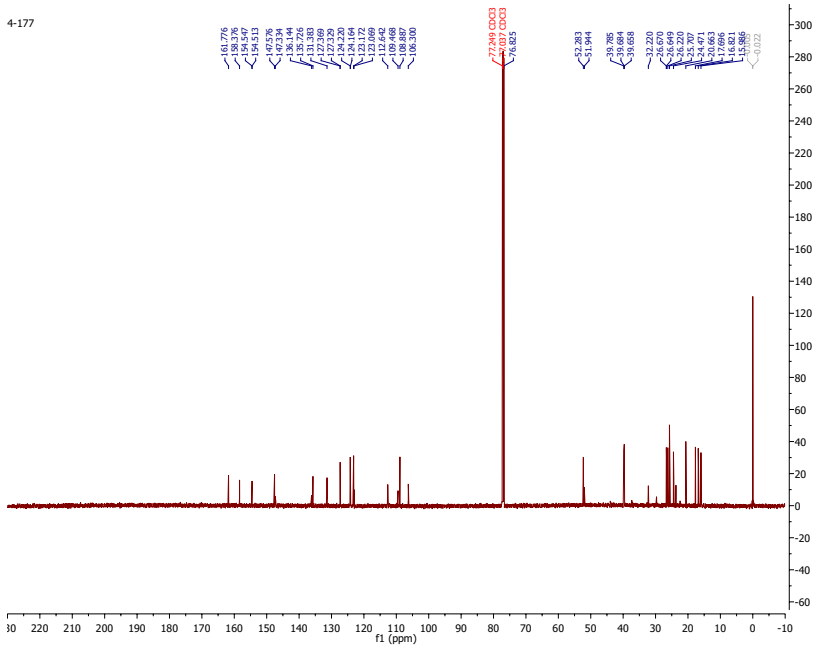
Spectral characterization of **12**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 5.27 (t, *J* = 8Hz, 1H), 5.1-5.00 (m, 2H), 4.26 (d, *J* = 8Hz, 2H), 2.67 (s, 3H), 2.63 (s, 3H), 2.04-1.80 (m, 8H), 1.68-1.57 (m, 12H) ; **^{13}C NMR** (125 MHz, $CDCl_3$) δ 161.77 158.37, 154.51, 147.57, 136.14, 131.38, 127.32, 124.22, 123.17, 112.64, 108.88, 106.30, 52.28, 39.78, 39.65, 26.67, 26.65, 26.16, 25.70, 23.71, 16.83, 16.04, 15.99 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{23}H_{32}N_2O_2S$: expected 401.2257, 423.2077 (M+Na) found: 423.2084

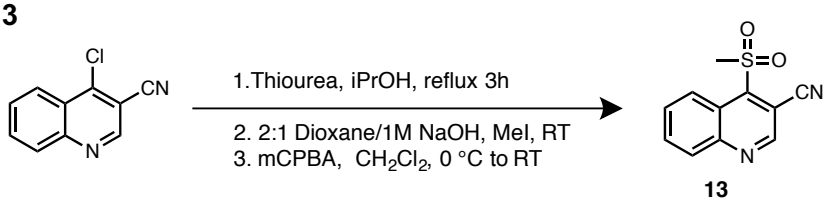
^{1}H NMR (400 MHz, $CDCl_3$) of **12**



^{13}C NMR 600MHz cryoprobe (125 MHz, $CDCl_3$) of **12**



Synthesis of 13

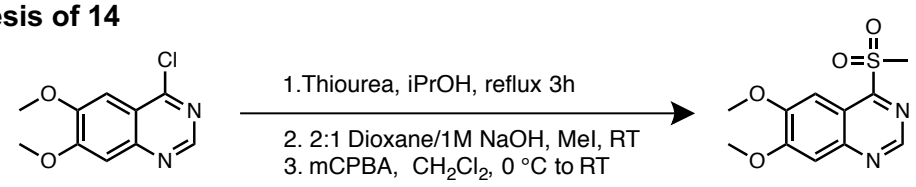


13

Commercially available 4-chloroquinoline-3-carbonitrile (500 mgs, 2.65 mmol, 1.0 equiv), and Thiourea (1.6 grams, 21.1 mmol, 3.0 equiv) were suspended in 8 mL of isopropanol and stirred for 10 minutes at room temperature. The suspension was then brought to reflux and turned into a clear solution over time; the formation of a bright orange precipitate denoted reaction completion. The mixture was cooled to room temperature and then cooled further in the freezer for 4 to 12 hours. The solid was filtered through a fritted funnel, washed with cold EtOH and let dry under air to obtain the crude thiol that was used without further purification in the next step, LCMS in negative mode and lack of chlorine isotope signature confirmed intermediate identity. Compound from the previous step, 4-thio-6,7-dimethoxyquinazoline, was dissolved in 6 mL of a 2:1 mixture of Dioxane/1M NaOH and then treated with methyl Iodide (181 µL, 2.91 mmol, 1.1 equiv) at room temperature for 3 hours. Upon reaction completion, as confirmed by LCMS, the reaction was then diluted with 40 mL of Et_2O, washed with water (2 x 20 mL), and the organic phase evaporated *in vacuo*. Compound from the previous step, 4-Methylthio-6,7-dimethoxyquinazoline, was dissolved in 7 mL of anhydrous CH_2Cl_2, followed by portion wise addition of mCPBA (683 mg, 3.97 mmol, 1.5 equiv) at room temperature. TLC and LC-MS analysis indicated that the starting material had been fully converted to the desired sulfoxide. The reaction was quenched with 20 mL of $NaHCO_{3(aq,\ sat)}$, along with 50 mL of Et_2O and the organic layer further washed (2x) with 20 mL of $NaHCO_{3(aq,\ sat)}$, the organic layer was then dried *in vacuo* and the crude mixture purified via silica gel (MeOH 0 to 5% in CH_2Cl_2) to obtain 150 mg sulfone **13**.

Spectral characterization of **13**
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 9.10 (d, *J* = 8.8 Hz, 1H), 8.37 (d, *J* = 9.2 Hz, 1H), 8.04 (t, *J* = 7.2 Hz, 1H), 7.94 (t, *J* = 8.4 Hz, 1H), 3.40 (s, 3H).
LC-MS Calcd. For $C_{11}H_8N_2O_2S$: expected 232.03, found: 233. 10

Synthesis of 14



14

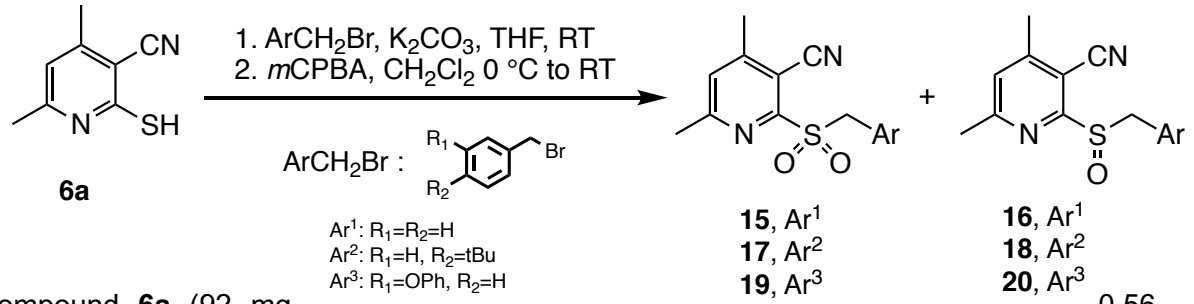
Commercially available 4-chloro-6,7-dimethoxyquinazoline (100 mgs, 0.45 mmol, 1.0 equiv), and Thiourea (101 mgs, 1.34 mmol, 3.0 equiv) were suspended in 3 mL of isopropanol and stirred for 10 minutes at room temperature. The suspension was then brought to reflux and turned into a clear solution over time; the formation of a canary yellow precipitate denoted reaction completion. The mixture was cooled to room temperature and then cooled further in the freezer for 4 to 12 hours. The solid was filtered through a fritted funnel, washed with cold EtOH and let dry under air to obtain the crude thiol that was used without further purification in the next step, LCMS in negative mode confirmed intermediate identity. Compound from the previous step, 4-thio-6,7-dimethoxyquinazoline, was dissolved in 5 mL of a 2:1 mixture of Dioxane/1M NaOH and then treated with methyl Iodide (32 µL, 0.5 mmol, 1.1 equiv) at room temperature for 3 hours. Upon reaction completion, as confirmed by LCMS, the mixture was then diluted with 20 mL of Et_2O, washed with water (2 x 10 mL), and the organic phase evaporated in vacuo. Compound from the previous step, 4-Methylthio-6,7-dimethoxyquinazoline, was dissolved in 5 mL of anhydrous CH_2Cl_2, followed by portion wise addition of mCPBA (77 mg, 0.67 mmol, 1.5 equiv) at room temperature. TLC and LC-MS analysis indicated that the starting material had been fully converted to the desired sulfoxide. The reaction was quenched with 10 mL of $NaHCO_3$ (aq, sat), along with 20 mL of CH_2Cl_2 and the organic layer further washed (2x) with 10 mL of $NaHCO_3$ (aq, sat), the organic layer was then dried in vacuo and the crude mixture purified via silica gel (MeOH 0 to 5% in CH_2Cl_2) to obtain 15 mg sulfone **14**.

Spectral characterization of **14**

Note: Compound **14** was found to be very reactive in our cell-based cysteine labeling studies and is chemically unstable, giving early signs of decomposition by NMR spectroscopy.

LC-MS Calcd. For $C_{11}H_{12}N_2O_4S$: expected 268.05, found: 269. 10

Synthesis of 15 – 20



Compound **6a** (92 mg, 0.56 mmol, 1.0 equiv), and K_2CO_3 (155 mg, 1.12 mmol, 2.0 equiv) were resuspended in 5 mL of anhydrous THF and stirred for 5 minutes at room temperature. Benzyl bromide (1.12 mmol, 2.0 equiv, Ar^1CH_2Br: 133µL, Ar^2CH_2Br: 206µL, Ar^3CH_2Br: 206µL) was then added dropwise, and the mixture was stirred overnight. After this time, the reaction mixture was filtered through a cotton plug, and the solvent was removed *in vacuo*. The crude mixture was then purified via preparative TLC on silica gel (15% EtOAc in Hexanes) to obtain S-benzylated pyridine (Ar^1CH_2SPy: 111.6 mg, Ar^2CH_2SPy: 147.9 mg, Ar^3CH_2SPy: 120.2 mg), which was then brought directly to the final oxidation step.

Oxidation using mCPBA

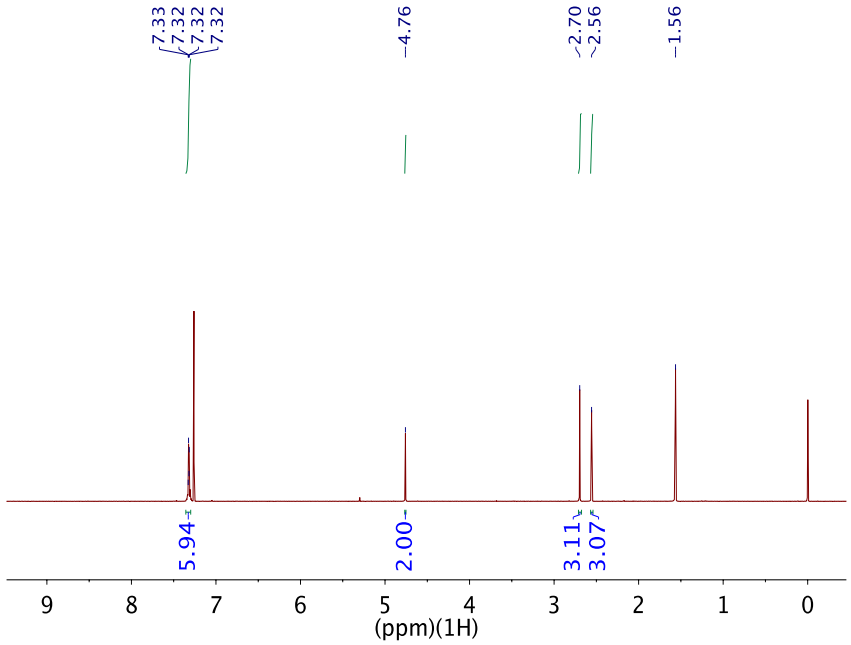
The abovementioned intermediates (0.35 mmol, 1.0 equiv) were dissolved in 3 mL of CH_2Cl_2 and cooled to 0 °C in an ice-bath. *m*CPBA (0.525mmol, 1.5 equiv) was added portionwise to the solution and the mixture was stirred for 3h with the progress monitored by TLC (2:1 CH_2Cl_2 in hexanes). Upon completion, the reaction was diluted with 20 mL of EtOAc, washed with $NaHCO_3$ (2 x 10 mL), and the organic phase was evaporated *in vacuo*. The crude reaction mixture was purified via prep. TLC on silica gel (2:1 CH_2Cl_2 in hexanes) to afford both sulfone and sulfoxide products **15–20**.

Spectral characterization of sulfone **15**

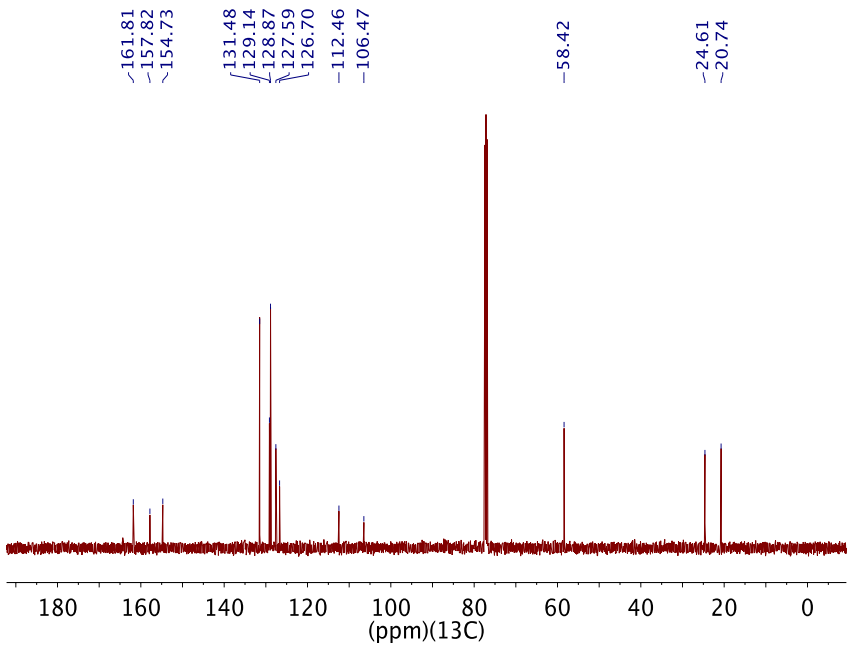
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 6H), 4.76 (s, 2H), 2.70 (s, 3H), 2.56 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 161.81, 157.82, 154.73, 131.48, 129.14, 128.87, 127.59, 126.70, 112.46, 106.47, 58.42, 24.61, 20.74 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{15}H_{14}N_2O_2S$: expected $[M+H]^+$ 287.0849 found: 287.0850

^{1}H NMR (500 MHz, $CDCl_3$) of **15**



^{13}C NMR (125 MHz, $CDCl_3$) of **15**

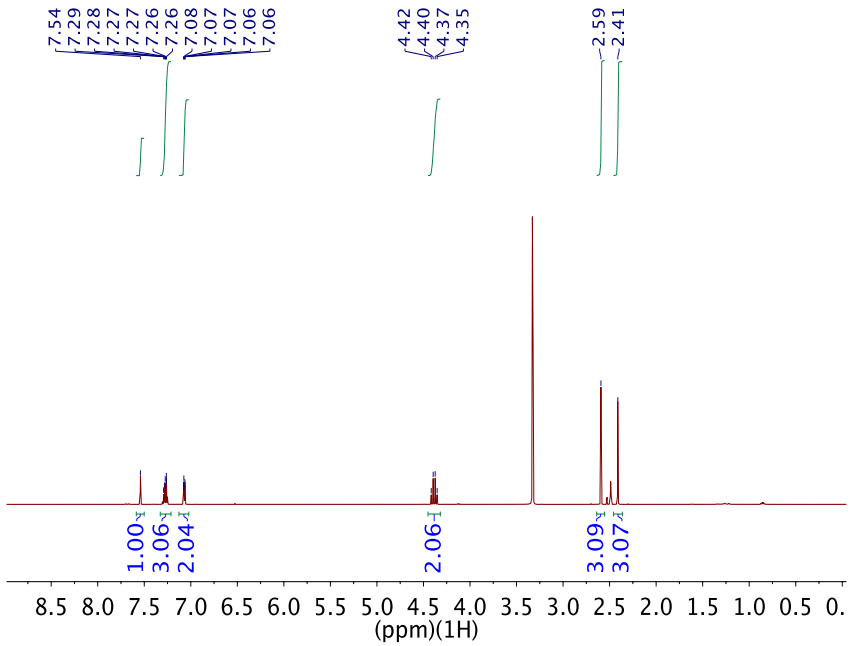


Spectral characterization of sulfoxide **16**

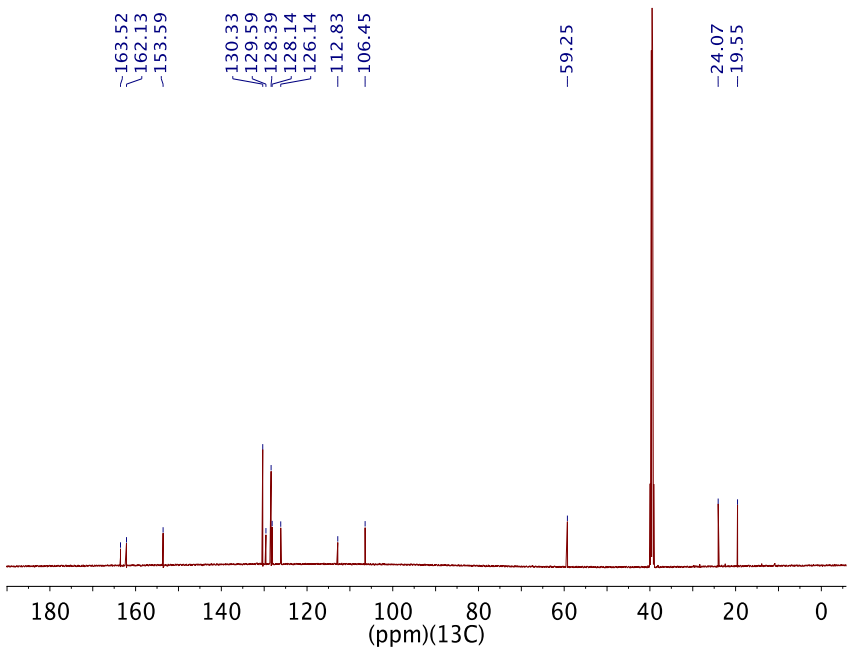
^{1}H NMR (600 MHz, DMSO-d_6) δ 7.54 (s, 1H), 7.31 – 7.24 (m, 3H), 7.09 – 7.05 (m, 2H), 4.38 (q, J = 12.6 Hz, 2H), 2.59 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 163.52, 162.13, 153.59, 130.33, 129.59, 128.39, 128.14, 126.14, 112.83, 106.45, 59.25, 24.07, 19.55.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{15}H_{14}N_2OS$: expected $[M+H]^+$ 271.09, found: 271.0905

^{1}H NMR (600 MHz, DMSO-d_6) of **16**



^{13}C NMR (125 MHz, DMSO-d_6) of **16**

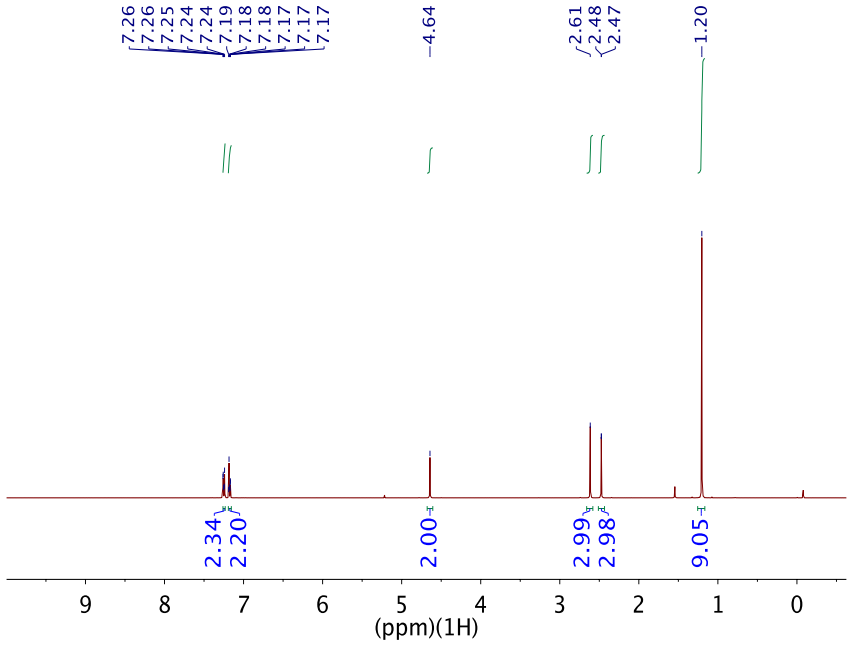


Spectral characterization of sulfone **17**

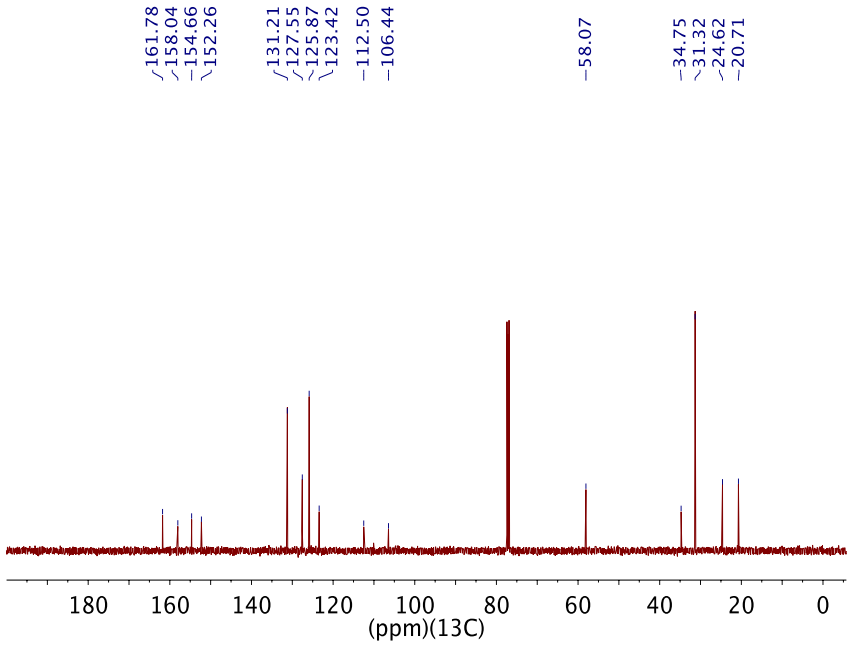
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.23 (m, 2H), 7.19 – 7.16 (m, 2H), 4.64 (s, 2H), 2.61 (s, 3H), 2.54 (d, *J* = 0.8 Hz, 3H), 1.20 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 161.78, 158.04, 154.66, 152.26, 131.21, 127.55, 125.87, 123.42, 112.50, 106.44, 58.07, 34.75, 31.32, 24.62, 20.71 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{19}H_{22}N_2O_2S$: expected $[M+H]^+$ 343.1475, found: 343.1478

^{1}H NMR (500 MHz, $CDCl_3$) of **17**



^{13}C NMR (125 MHz, $CDCl_3$) of **17**

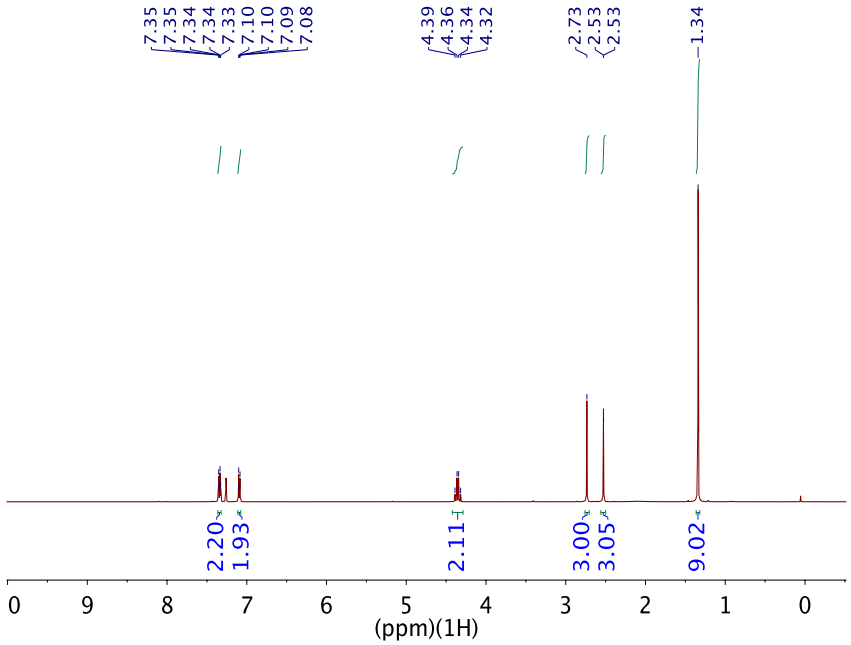


Spectral characterization of sulfoxide **18**

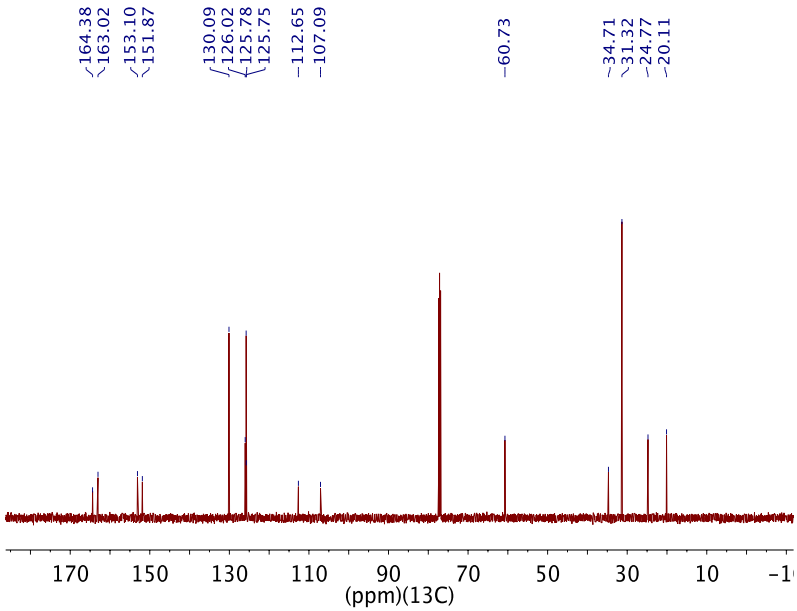
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.30 (m, 2H), 7.15 – 7.05 (m, 2H), 4.40 – 4.31 (m, 2H), 2.73 (s, 3H), 2.53 (d, *J* = 0.7 Hz, 3H), 1.34 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.38, 163.02, 153.10, 151.87, 130.09, 126.02, 125.78, 125.75, 112.65, 107.09, 60.73, 34.71, 31.32, 24.77, 20.11ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{19}H_{22}N_2OS$: expected $[M+H]^+$ 327.1526, found: 327.1532

^{1}H NMR (500 MHz, $CDCl_3$) of **18**



^{13}C NMR (125 MHz, $CDCl_3$) of **18**

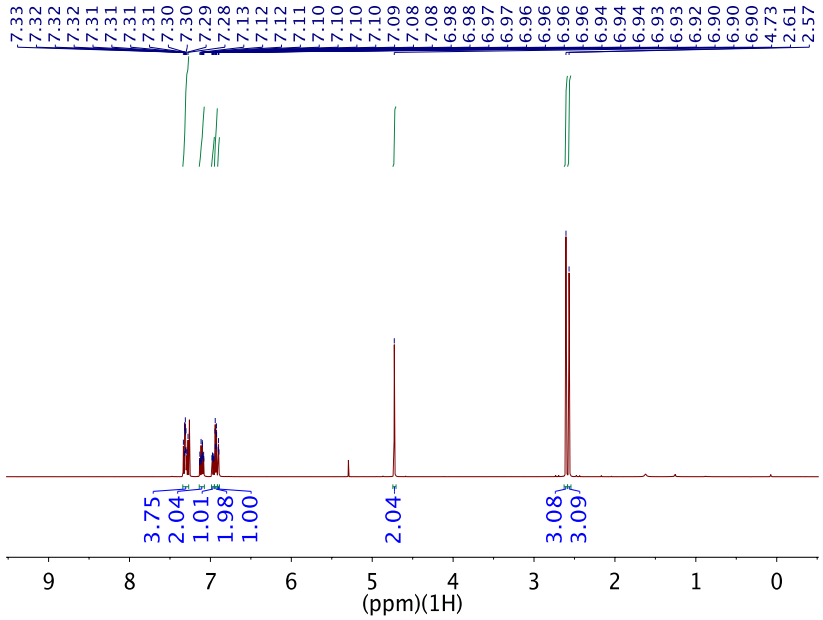


Spectral characterization of sulfone **19**

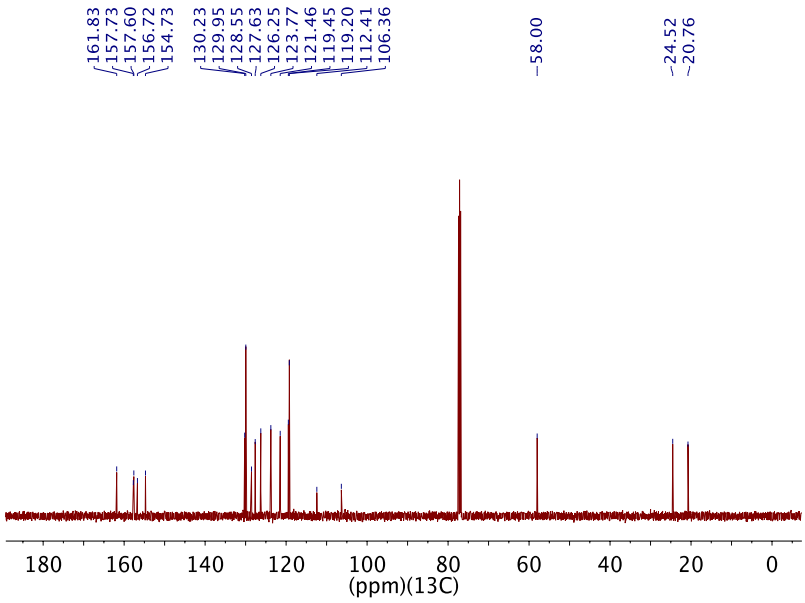
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.26 (m, 4H), 7.14 – 7.08 (m, 2H), 6.99 – 6.96 (m, 1H), 6.95 – 6.92 (m, 2H), 6.90 (t, J = 2.0 Hz, 1H), 4.73 (s, 2H), 2.61 (s, 3H), 2.57 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 161.83, 157.73, 157.60, 156.72, 154.73, 130.23, 129.95, 128.55, 127.63, 126.25, 123.77, 121.46, 119.45, 119.20, 112.41, 106.36, 58.00, 24.52, 20.76 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{21}H_{18}N_2O_3S$: expected $[M+H]^+$ 379.1111, found: 379.1114

^{1}H NMR (500 MHz, $CDCl_3$) of **19**



^{13}C NMR (125 MHz, $CDCl_3$) of **19**

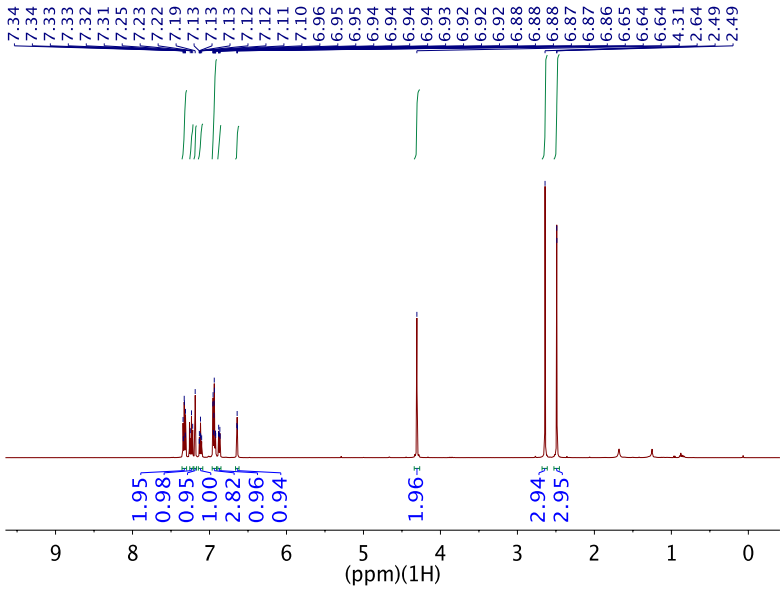


Spectral characterization of sulfoxide **20**

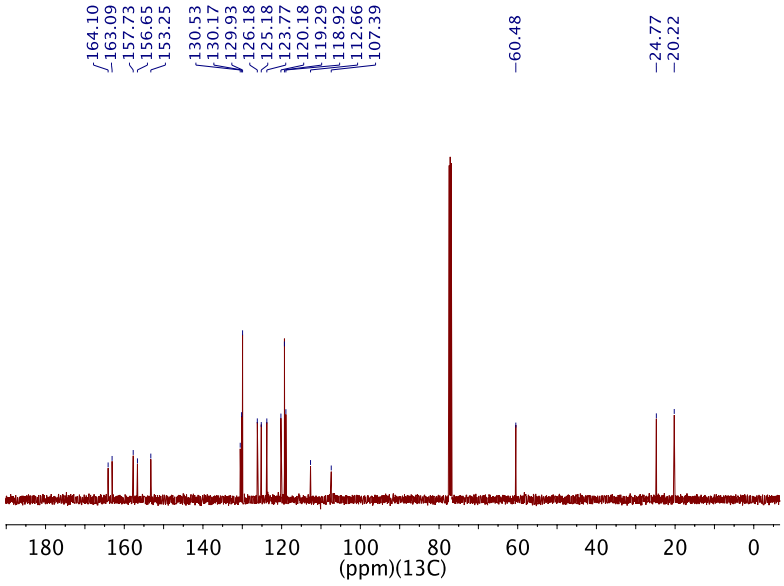
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 2H), 7.23 (t, *J* = 7.9 Hz, 1H), 7.19 (s, 1H), 7.14 – 7.09 (m, 1H), 6.97 – 6.91 (m, 3H), 6.87 (d, *J* = 7.6, 1H), 6.64 (t, *J* = 2.1 Hz, 1H), 4.31 (s, 2H), 2.64 (s, 3H), 2.49 (d, *J* = 0.7 Hz, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 164.10, 163.09, 157.73, 156.65, 153.25, 130.53, 130.17, 129.93, 126.18, 125.18, 123.77, 120.18, 119.29, 118.92, 112.66, 107.39, 60.48, 24.77, 20.22 ppm.

HRMS High accuracy (ESI-TOF) Calcd. for $C_{21}H_{18}N_2O_2S$: expected $[M+H]^+$ 363.1162, found: 363.1169

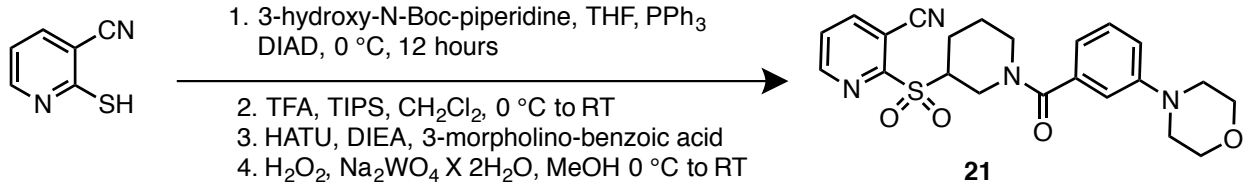
^{1}H NMR (500 MHz, $CDCl_3$) of **20**



^{13}C NMR (125 MHz, $CDCl_3$) of **20**



Synthesis of 21



21

Step 1.
2-thio-3-pyridinecarbonitrile (330 mg, 2.42 mmol, 1.0 equiv), *N*-Boc-3-hydroxy-piperidine (783 mg, 3.02 mmol, 1.6 equiv), and PPh_3 (1 g, 3.90 mmol, 1.6 equiv) were dissolved in 10 mL of anhydrous THF and cooled to 0 °C using an ice bath. DIAD (780 µL, 3.90 mmol, 1.6 equiv) was slowly added over the course of about 10 minutes and upon addition the mixture was left stirring overnight. TLC and LC-MS analysis indicated that the starting material had been consumed and converted to the desired product. The volatiles were removed *in vacuo* and the crude mixture was purified using column chromatography on silica gel (5 to 30% EtOAc in hexanes) to obtain 900 mg of the desired compound.

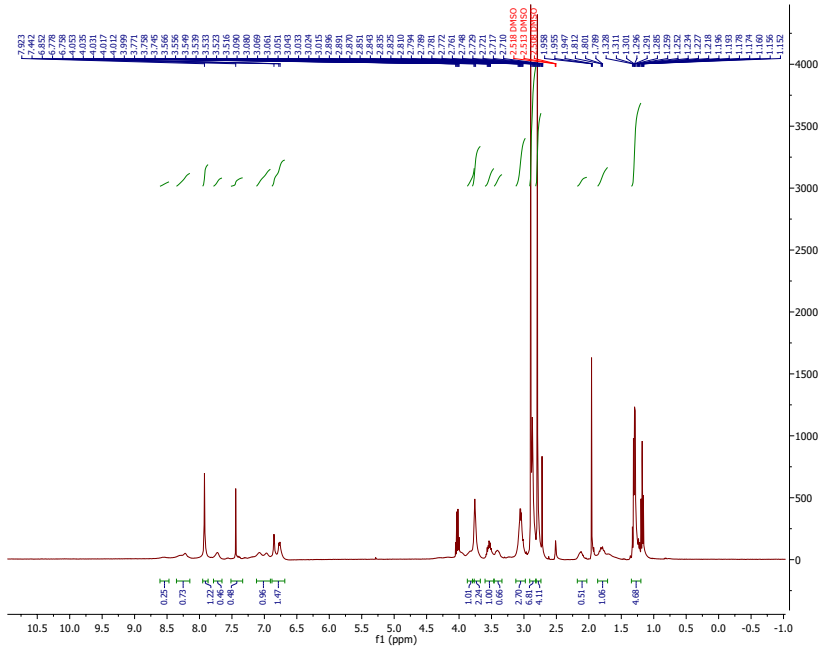
LC-MS (ESI): m/z $[M + H]^+$ calcd. for $C_{16}H_{21}N_3O_2S$: 319.14, found: 342.20 $[M + Na]^+$

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 4.4 Hz, 1H), 7.81 (d, *J* = 8.26 Hz, 1H), 7.08 (dd, *J* = 4.50, *J* = 8.30 Hz, 1H), 4.12-4.01 (m, 2H), 3.69-3.64 (m, 1H), 3.22-3.17 (m, 2H), 2.16-2.11 (m, 1H), 1.83-1.63 (m, 3H), 1.46 (s, 9H) ppm.

Steps 2 and 3.
The abovementioned product (400 mg, 1.25 mmol, 1.0 equiv) was dissolved in 15 mL of CH_2Cl_2 together with 200 µL of triisopropylsilane and the mixture was brought to 0 °C using an ice bath. TFA (4 mL) was then added dropwise and the mixture left stirring at room temperature. The Boc-deprotection was monitored by TLC and LC-MS and, upon reaction completion, the mixture was co-evaporated with toluene (2 x 30 mL). The crude material was then redissolved in 7 mL of anhydrous DMF and added dropwise to a pre-activated mixture of HATU (617 mg, 1.63 mmol, 1.3 equiv), Hunig's base (651 µL, 3.75 mmol, 3.0 equiv), and 3-morpholinobenzoic acid (337 mg, 1.631 mmol, 1.3 equiv). The mixture was left stirring until LC-MS analysis showed complete conversion to the desired, coupled product. The crude mixture was then evaporated *in-vacuo* and loaded directly on silica gel (50 to 100% EtOAc in hexanes) to afford 600 mg of the desired material. The complexity of the ^{1}H-NMR of the desired product (room temperature) is likely due to slow interconversion through the piperidine C-N amide bond. ^{1}H NMR is attached to this document and contains residual DMF.

LC-MS (ESI): m/z $[M + H]^+$ calcd. for $C_{22}H_{24}N_4O_2S$: 408.16, found: 409.20

^1H NMR (400 MHz, $CDCl_3$/d_6-DMSO 6:1)

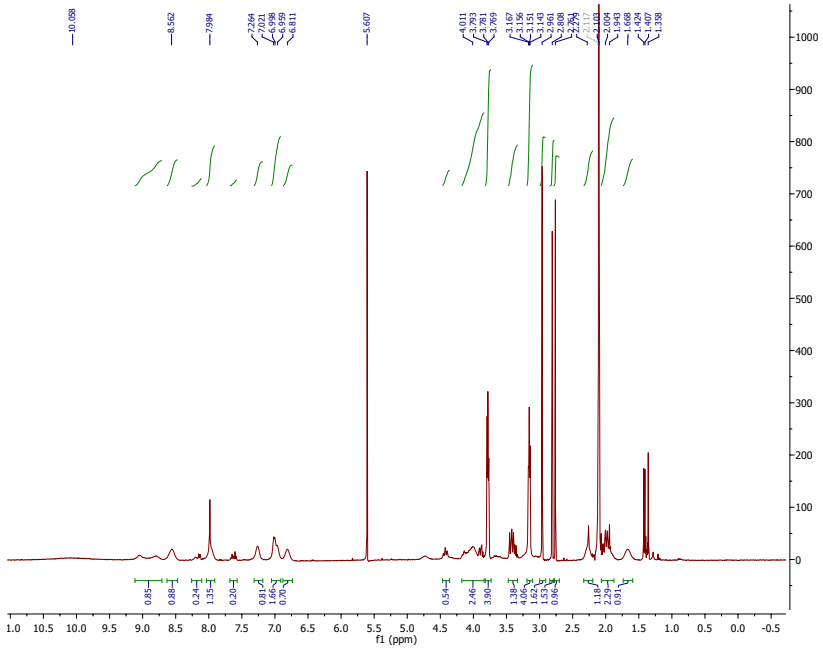


Step 4.
The abovementioned intermediate (600 mg, 1.46 mmol, 1.0 equiv) was dissolved in 10 mL of HPLC-grade MeOH and then cooled in an ice-bath. Sodium tungstate dihydrate (120 mg, 0.36 mmol, 0.25 equiv) was added to the solution, followed by slow addition of a 30% wt solution of H_2O_2 (1.5 mL, 10 mmol, 10 equiv), and the final mixture was stirred for 24h. After this time, LC-MS analysis indicated that the starting material had been almost fully converted to the desired sulfone. The reaction was then diluted with 40 mL of CH_2Cl_2, washed with water (2 x 30 mL), and the organic phase was evaporated *in vacuo*. The crude reaction mixture was purified via column chromatography on silica gel (the desired product eluted at 10% MeOH in EtOAc). *Note:* NMR spectra contain signals of residual dichloromethane. One dimensional, bi-dimensional and variable temperature NMR analysis confirmed that compound **21** (and synthetic non-oxidized predecessor) exist as a mixture of rotationally impaired isomers at room temperature. Attached ^1H and ^{13}C spectra of **21** taken both at room temperature and 70 °C

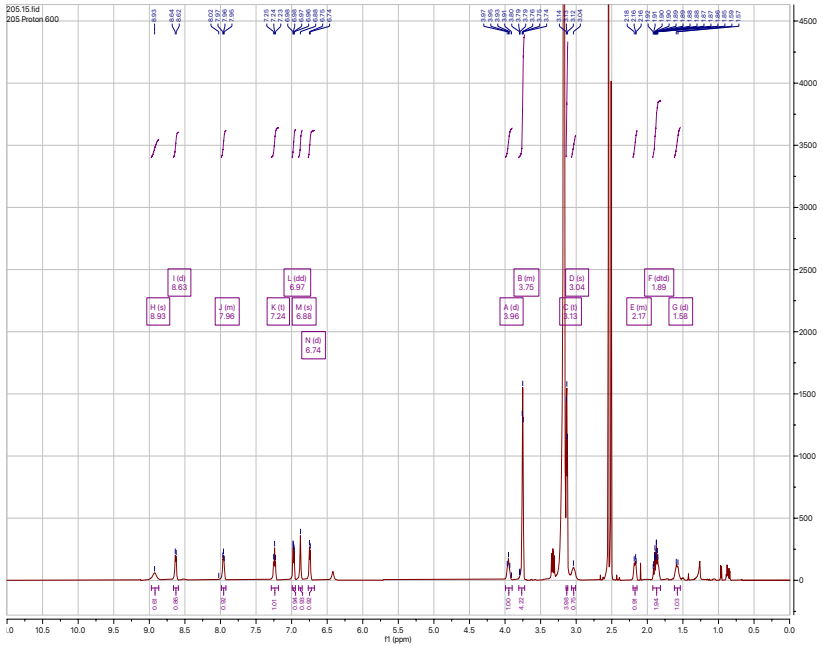
Spectral characterization of **21**

^{1}H NMR (600 MHz, d_6-DMSO, 70 ºC) δ 8.90 (bs, 1H), 8.62 (bd, 1H), 7.95 (m, 1H), 7.24 (t, *J* = 6Hz, 1H), 6.97 (d, *J* = 6Hz, 1H), 6.87 (s, 1H), 6.74 (d, *J* = 6Hz, 1H), 3.95 (m, 1H), 3.75 (m, 4H), 3.33 (m, 1H), 2.55 (m, 5H), 2.24 (bd, 1H), 1.88 (m, 2H), 1.55 (bd, 1H) ; **^{13}C NMR** (125 MHz, d_6-DMSO) δ 171.11, 158.59, 152.62, 150.85, 150.47, 137.68, 128.99, 127.41, 117.37, 115.79, 113.72, 112.89, 108.67, 67.45 (2C), 57.06, 48.21 (2C), 40.72, 40.34, 23.22, 22.80 ppm.

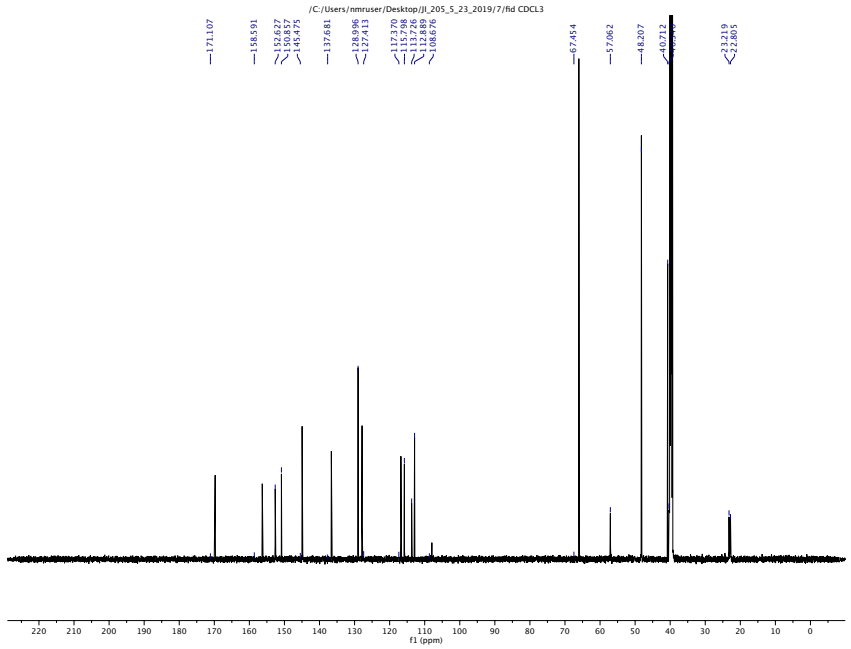
^{1}H NMR (400 MHz, Acetone-d_6) of **21 (295 K)**



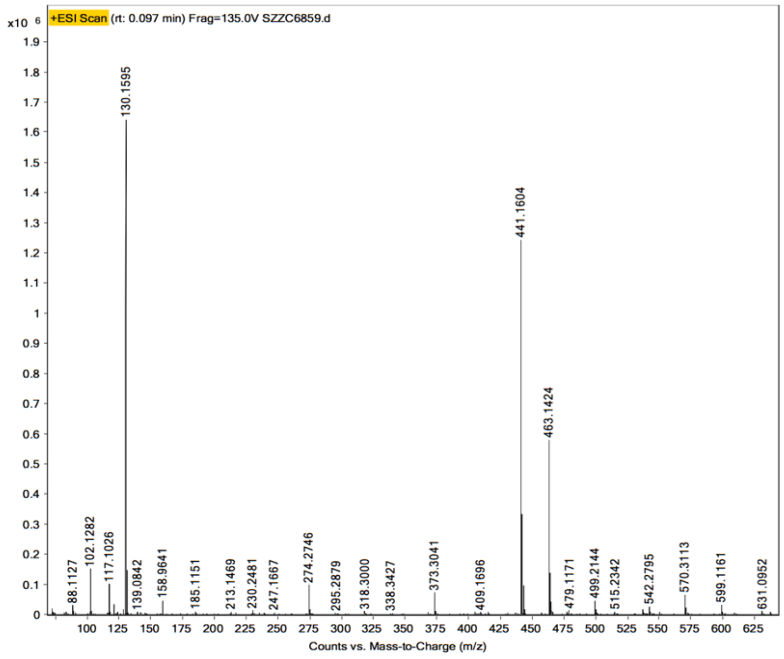
^{1}H NMR (600 MHz, d_6-DMSO) of **21 (343 K)**



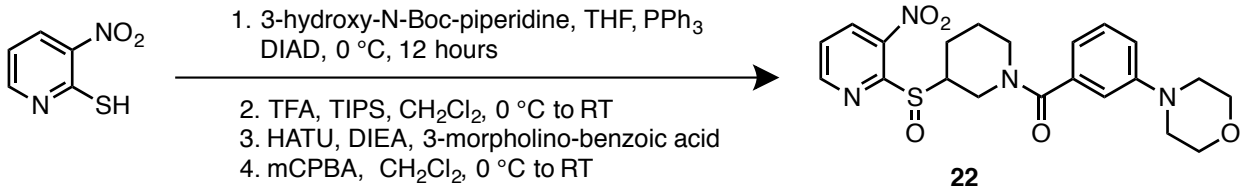
^{13}C NMR (150 MHz, d_6-DMSO) of **21 (343 K)**



High accuracy (ESI-TOF) of **21** Calcd. for $C_{22}H_{24}N_4O_4S$: expected 441.1591, found: 441.160



Synthesis of 22



22

Step 1.
2-thio-3-nitropyridine (110 mg, 0.71 mmol, 1.0 equiv), *N*-Boc-3-hydroxy-piperidine (285 mg, 1.42 mmol, 2.0 equiv), and PPh_3 (372 mg, 1.42 mmol, 2.0 equiv) were dissolved in 7 mL of anhydrous THF and cooled to 0 °C using an ice bath. DIAD (280 µL, 1.42 mmol, 2.0 equiv) was slowly added over the course of 5 minutes, and the mixture was left stirring overnight. TLC and LC-MS analysis indicated that the starting material had been consumed and converted to the desired product. The volatiles were removed *in vacuo*, and the crude mixture was purified using column chromatography on silica gel (5 to 30% EtOAc in hexanes) to obtain 200 mg of the desired compound.

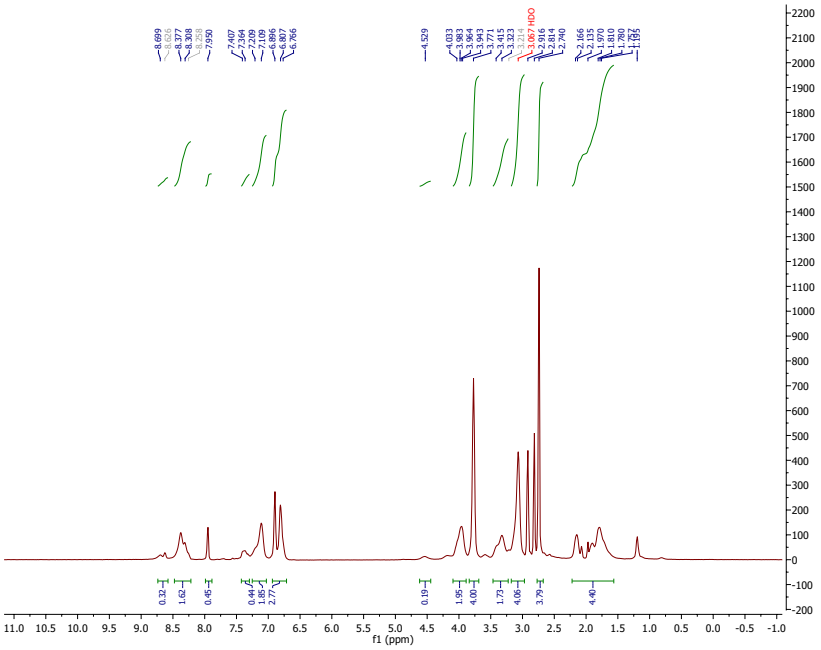
LC-MS (ESI): m/z $[M + H]^+$ calcd. for $C_{15}H_{21}N_3O_4S$: 339.13, found: 362.10 $[M + Na]^+$

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 4.46 Hz, 1H), 8.48 (d, *J* = 8.26 Hz, 1H), 7.21 (dd, *J* = 4.55, *J* = 8.27 Hz, 1H), 4.12-4.09 (m, 2H), 3.76 (dt, *J* = 4.44, *J* = 13.21 Hz, 1H), 3.20-3.14 (m, 2H), 2.16- 2.11 (m, 1H), 1.83- 1.63 (m, 3H), 1.46 (s, 9H) ppm.

Steps 2 and 3.
The abovementioned product (150 mg, 0.44 mmol, 1.0 equiv) was dissolved in 5 mL of CH_2Cl_2 together with 200 µL of triisopropylsilane and cooled to 0 °C with an ice bath. TFA (2 mL) was then added dropwise and the mixture was left stirring at room temperature. The Boc-deprotection was monitored by TLC and LC-MS and, upon reaction completion, the mixture was co-evaporated with toluene (2 x 30 mL). The crude material was then dissolved in 4 mL of anhydrous DMF and added dropwise to a pre-activated mixture of HATU (240 mg, 0.62 mmol, 1.4 equiv), Hunig's base (215 µL, 1.20 mmol, 2.8 equiv), and 3-morpholinobenzoic acid (130 mg, 0.62 mmol, 1.4 equiv). The mixture was left stirring until LC-MS analysis showed complete conversion to the desired, coupled product. The crude mixture was then evaporated *in-vacuo* and loaded directly on silica gel (50 to 100% EtOAc in hexanes) to afford 180 mg of the desired material. The complexity of the ^{1}H-NMR of the desired product (room temperature) is due to slow interconversion through the piperidine C-N amide bond. Given the complexity of the product, ^{1}H NMR is attached to this document.

LC-MS (ESI): m/z $[M + H]^+$ calcd. for $C_{21}H_{24}N_4O_4S$: 428.15, found: 429.20

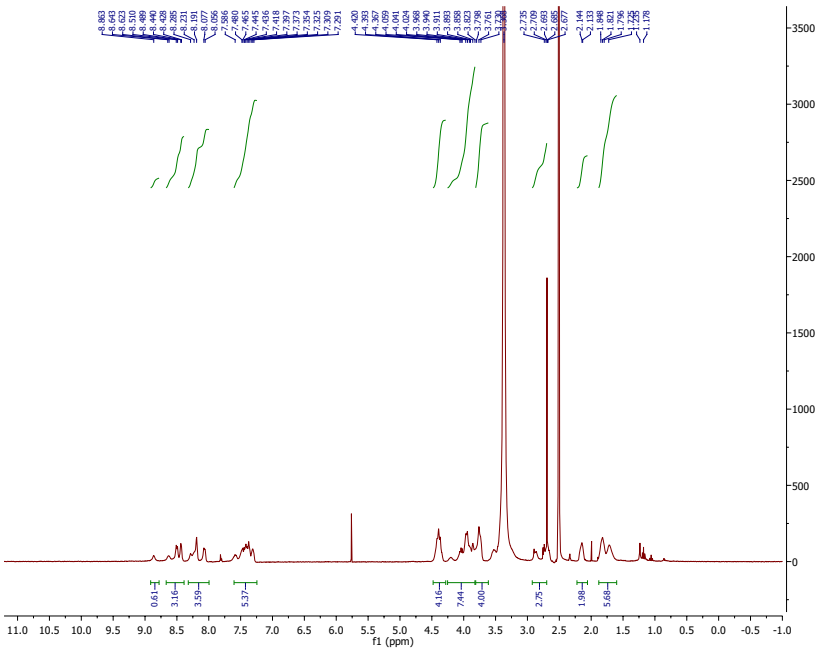
^{1}H NMR (400 MHz, $CDCl_3$/d_6-DMSO 6:1)



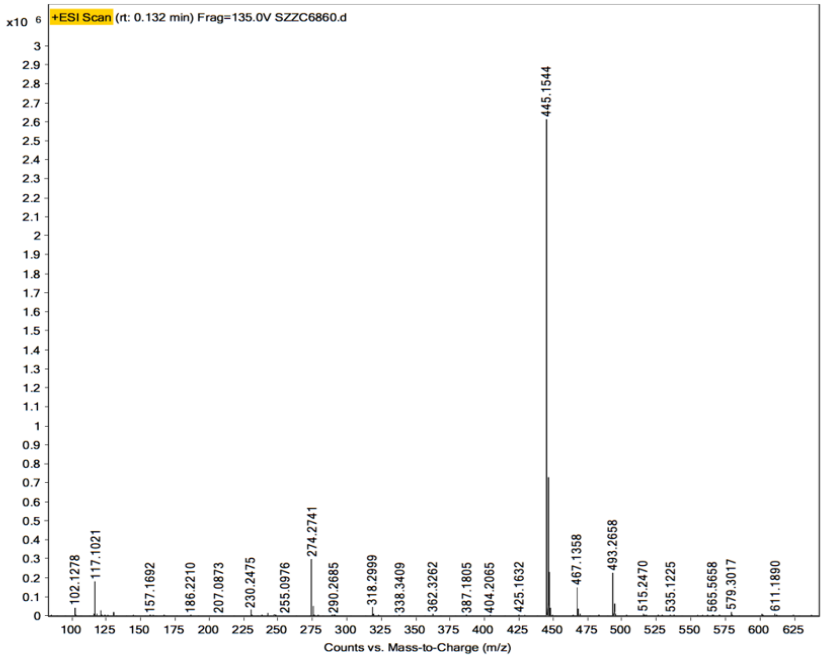
Step 4.
The abovementioned intermediate (160 mg, 0.373 mmol, 1.0 equiv) was dissolved in 6 mL of a 5:1 CH_2Cl_2-EtOAc mixture and cooled to 0 °C in an ice-bath. *m*CPBA (90 mg, 0.41 mmol, 1.1 equiv) was added portionwise to the solution and the mixture was stirred for 30 min. LC-MS analysis indicated that the starting material had been fully converted to the desired sulfoxide and partially overoxidated to the sulfone. The reaction was then diluted with 20 mL of EtOAc, washed with $NaHCO_3$ (2 x 10 mL), and the organic phase was evaporated *in vacuo*. The crude reaction mixture was purified via column chromatography on silica gel (the desired compound eluted at 10% MeOH in EtOAc).

The compound appears as a mixture of slowly interconverting isomers (through N-C bond of piperidine amide bond) and also a mixture of diastereoisomers given the chirality of both the sulfur atom and its vicinal stereogenic center.

^{1}H NMR (400 MHz, d_6-DMSO) of **22**



High accuracy (ESI-TOF) of **22** Calcd. for $C_{21}H_{24}N_4O_5S$: expected 445.1540, found: 445.1544



Computational methods

Geometry optimizations and single-point energy calculations were carried out using Gaussian 09.[6] The geometries of intermediates and transition states were optimized in water using the M06-2X functional[7] with the 6-311+G(3df,2p) basis set. The SMD continuum solvation model[8] was employed to include the solvation effect during geometry optimization and energy calculations. Vibrational frequency calculations were performed for all the stationary points to confirm if each optimized structure is a local minimum or a transition state structure. The calculation of natural population analysis (NPA) charge and natural bond orbital (NBO) second order perturbation theory analysis were all performed at the M06-2X/6-311+G(3df,2p)/SMD(water) level of theory.

Cartesian coordinates and energies of optimized structures

5

M06-2X SCF energy in solution: -931.83275395 a.u.
M06-2X enthalpy in solution: -931.643104 a.u.
M06-2X free energy in solution: -931.699179 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	1.586903	-1.514172	0.030919
C	-0.308313	-0.270671	-0.156428
C	0.395508	0.930161	-0.115515
C	1.788750	0.891278	0.019526
C	2.368344	-0.365450	0.093739
H	3.441697	-0.455826	0.194433
C	2.588337	2.151694	0.068336
H	3.649483	1.933993	0.155539
H	2.416167	2.740141	-0.834723
H	2.278512	2.761911	0.918637
C	2.193589	-2.875815	0.104052
H	3.276664	-2.822441	0.178322
H	1.802289	-3.407255	0.972675
H	1.922400	-3.451375	-0.781618
S	-2.116872	-0.226012	-0.341896
O	-2.558215	-1.613893	-0.673119
C	-2.464339	0.011462	1.401033
H	-2.051149	0.968330	1.718332
H	-3.547869	0.020585	1.502877
H	-2.023717	-0.814892	1.956214
N	0.250473	-1.453226	-0.095662
C	-0.284226	2.183480	-0.205699
N	-0.824206	3.196419	-0.270793

5a-TS

M06-2X SCF energy in solution: -1370.03743464 a.u.
M06-2X enthalpy in solution: -1369.807281 a.u.
M06-2X free energy in solution: -1369.869526 a.u.
Imaginary frequency: -217.1556 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	-1.281981	-1.865068	-0.094323
C	0.348400	-0.247173	-0.043524
C	-0.565777	0.718261	-0.567135
C	-1.922939	0.387878	-0.646612
C	-2.285568	-0.911830	-0.343324
H	-3.320826	-1.218658	-0.398304
C	-2.930952	1.418416	-1.052055
H	-3.923979	0.982010	-1.131052
H	-2.958995	2.228257	-0.319423
H	-2.663038	1.861867	-2.012702
C	-1.647616	-3.308928	0.076795
H	-1.648011	-3.560820	1.139809
H	-2.636704	-3.520387	-0.324241
H	-0.913015	-3.947479	-0.411852
S	2.105066	0.059599	-0.434381
O	2.918198	-0.971060	0.296495
C	2.053647	-0.520575	-2.130484
H	1.400628	0.134226	-2.707205
H	3.070523	-0.460365	-2.513927
H	1.694026	-1.547594	-2.147613
N	-0.000049	-1.558415	-0.012409
C	-0.776357	1.353963	2.448001
H	-1.630455	0.729316	2.708950
H	-0.612708	2.071230	3.251987
H	-1.027803	1.924103	1.551235
S	0.724631	0.372728	2.200372
C	-0.136681	2.031138	-0.874188
N	0.203556	3.106849	-1.123645

5b

M06-2X SCF energy in solution: -1370.04673180 a.u.
M06-2X enthalpy in solution: -1369.815992 a.u.
M06-2X free energy in solution: -1369.879376 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-1.490234	-1.394660	0.700239
C	0.425581	-0.092309	0.270432
C	-0.473974	0.927907	-0.347399
C	-1.842837	0.676737	-0.473725
C	-2.357102	-0.507762	0.012819
H	-3.412751	-0.729058	-0.056562
C	-2.734743	1.697733	-1.117933
H	-3.766930	1.354049	-1.132890
H	-2.689422	2.645165	-0.577317
H	-2.416726	1.897377	-2.143017
C	-2.092364	-2.638144	1.291864
H	-2.930089	-2.384756	1.943355
H	-2.486950	-3.270447	0.493648
H	-1.357313	-3.205501	1.856885
S	1.583853	-0.673659	-1.150688
O	2.640949	-1.614798	-0.615746
C	0.396890	-1.730583	-1.967962
H	-0.479027	-1.144217	-2.242138
H	0.881288	-2.114935	-2.863203
H	0.132831	-2.542615	-1.292190
N	-0.207469	-1.209429	0.869319
C	0.533175	1.552160	2.489734
H	-0.207076	0.875391	2.913351
H	1.125998	1.979129	3.296702
H	0.038660	2.354553	1.945298
S	1.675325	0.649613	1.427246
C	0.051438	2.151025	-0.791854
N	0.464340	3.171765	-1.160487

5c-TS

M06-2X SCF energy in solution: -1370.04342881 a.u.
M06-2X enthalpy in solution: -1369.813345 a.u.
M06-2X free energy in solution: -1369.875018 a.u.
Imaginary frequency: -173.9918 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	-1.683419	-1.067893	0.914865
C	0.311671	0.023513	0.437281
C	-0.435054	1.013828	-0.319658
C	-1.802904	0.848430	-0.528757
C	-2.428525	-0.235337	0.071710
H	-3.489498	-0.397625	-0.055643
C	-2.571422	1.827658	-1.363525
H	-3.627314	1.567965	-1.390691
H	-2.468791	2.838128	-0.963790
H	-2.186731	1.843820	-2.385029
C	-2.360274	-2.193742	1.640084
H	-1.964770	-3.149144	1.289115
H	-2.160235	-2.131995	2.710076
H	-3.435950	-2.182481	1.478474
S	1.409237	-1.045866	-1.260124
O	2.526919	-2.016522	-0.820507
C	0.024165	-2.120286	-1.599523
H	-0.829192	-1.516312	-1.908114
H	0.304100	-2.799504	-2.403736
H	-0.220963	-2.685326	-0.698570
N	-0.385681	-0.915600	1.143225
C	1.129352	1.690868	2.449283
H	0.459286	1.151944	3.115605
H	1.958639	2.095262	3.027026
H	0.600074	2.509909	1.964180
S	1.842700	0.574482	1.220190
C	0.221990	2.132107	-0.884479
N	0.725897	3.060572	-1.354596

5d-TS

M06-2X SCF energy in solution: -1027.66014513 a.u.
M06-2X enthalpy in solution: -1027.400884 a.u.
M06-2X free energy in solution: -1027.462271 a.u.
Imaginary frequency: -350.4173 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	-1.356905	-1.608234	-0.380470
C	0.366573	-0.157610	0.129014
C	-0.492827	0.967053	-0.143440
C	-1.840748	0.743177	-0.430832
C	-2.280511	-0.568093	-0.523908
H	-3.313362	-0.786156	-0.754655
C	-2.770394	1.899166	-0.629950
H	-3.753994	1.556832	-0.942336
H	-2.873132	2.461608	0.300736
H	-2.377886	2.587981	-1.379714
C	-1.794068	-3.031915	-0.539823
H	-1.157547	-3.543825	-1.261674
H	-1.691243	-3.553158	0.414019
H	-2.829624	-3.097034	-0.865390
S	2.147944	0.120124	-0.317793
O	2.913072	-1.054968	0.221593
C	1.948006	-0.162508	-2.067151
H	1.280338	0.598835	-2.469763
H	2.934213	-0.067759	-2.516917
H	1.542090	-1.161884	-2.214451
N	-0.068056	-1.414810	-0.122152
C	-0.544880	-0.349111	2.683991
H	-0.973450	-1.296586	2.364896
H	-0.386109	-0.373060	3.760574
H	-1.224671	0.465708	2.441651
N	0.717065	-0.137744	1.972320
H	1.385076	-0.884822	2.153090
H	1.136392	0.754905	2.231094
C	-0.002275	2.288862	-0.020310
N	0.385191	3.371335	0.090984

5e

M06-2X SCF energy in solution: -1027.66692179 a.u.
M06-2X enthalpy in solution: -1027.405293 a.u.
M06-2X free energy in solution: -1027.466981 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-0.974979	-1.796879	0.396877
C	0.418321	0.074970	0.375125
C	-0.718173	0.886488	-0.104037
C	-1.922007	0.255065	-0.427525
C	-2.039571	-1.105565	-0.221694
H	-2.955959	-1.626955	-0.457511
C	-3.056082	1.063047	-0.980970
H	-3.929414	0.438439	-1.153456
H	-3.326029	1.866546	-0.293123
H	-2.765643	1.530885	-1.923634
C	-1.145162	-3.257185	0.691318
H	-2.133908	-3.457082	1.103232
H	-1.060195	-3.821943	-0.240363
H	-0.381891	-3.611135	1.379992
S	1.833110	0.152718	-0.975103
O	3.077144	-0.439661	-0.351955
C	1.138677	-1.107793	-2.025155
H	0.138731	-0.796825	-2.328754
H	1.783115	-1.189639	-2.897800
H	1.110230	-2.044198	-1.470289
N	0.167471	-1.247763	0.739917
C	0.352979	0.873737	2.762483
H	0.019841	-0.111978	3.067949
H	0.974177	1.321525	3.531674
H	-0.496201	1.513504	2.540193
N	1.171474	0.732500	1.531680
H	1.992555	0.146417	1.719406
H	1.513380	1.656296	1.248332
C	-0.555093	2.272364	-0.261909
N	-0.399396	3.416757	-0.362404

5f

M06-2X SCF energy in solution: -1027.20559012 a.u.
M06-2X enthalpy in solution: -1026.959518 a.u.
M06-2X free energy in solution: -1027.018970 a.u.
Imaginary frequency: -21.9110 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	0.373994	1.951366	0.556305
C	-0.416699	-0.264174	0.486015
C	0.902901	-0.644059	-0.111984
C	1.835044	0.333263	-0.464930
C	1.545454	1.654514	-0.187014
H	2.242275	2.443496	-0.432859
C	3.130645	-0.060654	-1.111334
H	3.747715	0.813037	-1.311045
H	3.689876	-0.743562	-0.468543
H	2.947602	-0.582926	-2.052610
C	0.144847	3.383394	0.950714
H	1.059876	3.833138	1.336794
H	-0.148742	3.957609	0.068318
H	-0.642928	3.469535	1.695036
S	-1.710774	-0.562044	-0.965427
O	-3.131576	-0.280189	-0.508853
C	-1.280730	0.853754	-1.961709
H	-0.230376	0.800932	-2.240705
H	-1.904143	0.820255	-2.853056
H	-1.489930	1.755818	-1.386993
N	-0.515064	1.072073	0.938718
C	-0.081158	-1.291309	2.662980
H	-0.049365	-0.335608	3.182619
H	-0.504520	-2.030734	3.340145
H	0.946314	-1.585671	2.423424
N	-0.945126	-1.185110	1.486425
H	-1.004977	-2.107642	1.062556
C	1.192027	-2.004034	-0.293599
N	1.415670	-3.136367	-0.424871

5g-TS

M06-2X SCF energy in solution: -1027.20309389 a.u.
M06-2X enthalpy in solution: -1026.956692 a.u.
M06-2X free energy in solution: -1027.016740 a.u.
Imaginary frequency: -151.1346 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	2.102066	-0.355311	0.291872
C	-0.086451	0.275960	0.754945
C	-0.541550	-0.865133	-0.028815
C	0.363868	-1.611153	-0.785810
C	1.709678	-1.313974	-0.659755
H	2.453694	-1.865340	-1.216947
C	-0.117939	-2.721047	-1.670828
H	0.714942	-3.209900	-2.171567
H	-0.663316	-3.467743	-1.090283
H	-0.805951	-2.335948	-2.426477
C	3.563492	-0.136312	0.561634
H	4.185711	-0.786132	-0.050251
H	3.829396	0.901296	0.353782
H	3.783157	-0.325712	1.613302
S	-0.347236	2.108912	-0.518214
O	0.889253	2.065272	-1.441160
C	-1.695862	1.544873	-1.544584
H	-2.602017	1.500749	-0.938307
H	-1.839851	2.250504	-2.361755
H	-1.461509	0.555712	-1.945243
N	1.259716	0.352355	1.018859
C	-0.967601	-0.270335	2.932354
H	0.017236	-0.358140	3.386836
H	-1.662789	0.077246	3.693939
H	-1.283937	-1.265686	2.602962
N	-0.920029	0.718448	1.848853
H	-1.864654	0.848044	1.496971
C	-1.910904	-1.202923	-0.032184
N	-3.035566	-1.478171	-0.016174

5-N-product

M06-2X SCF energy in solution: -513.80795321 a.u.
M06-2X enthalpy in solution: -513.606964 a.u.
M06-2X free energy in solution: -513.658195 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-1.695547	-0.843511	-0.001698
C	-0.137370	0.849680	0.000055
C	0.916470	-0.102537	-0.002519
C	0.622481	-1.464665	-0.005382
C	-0.716179	-1.830414	-0.004804
H	-0.996426	-2.874445	-0.006911
C	1.721741	-2.479815	-0.001633
H	1.318995	-3.487732	-0.062575
H	2.396838	-2.316465	-0.843180
H	2.313872	-2.391307	0.911335
C	-3.146941	-1.207835	0.006136
H	-3.651179	-0.752369	-0.846975
H	-3.287415	-2.285468	-0.030194
H	-3.620608	-0.819830	0.909314
N	-1.416126	0.463958	-0.001915
C	-0.911285	3.180150	-0.006184
H	-1.493248	3.144006	-0.927654
H	-1.591294	3.058250	0.835718
H	-0.432548	4.152079	0.068795
N	0.125565	2.170249	0.010973
H	1.085785	2.471843	-0.025220
C	2.265116	0.339910	0.001866
N	3.354320	0.716602	0.005590

5-S-product

M06-2X SCF energy in solution: -856.63660992 a.u.
M06-2X enthalpy in solution: -856.451947 a.u.
M06-2X free energy in solution: -856.505812 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	0.689729	1.888844	0.001409
C	-0.594158	-0.009776	-0.003245
C	0.553978	-0.822738	-0.002356
C	1.819597	-0.233479	-0.000794
C	1.867094	1.152494	0.001709
H	2.820634	1.662339	0.005302
C	3.057005	-1.072001	0.000496
H	3.946471	-0.446962	-0.000134
H	3.077804	-1.716993	0.880707
H	3.078090	-1.719423	-0.877891
C	0.713858	3.383665	0.000939
H	0.266390	3.761391	-0.919810
H	0.122003	3.766468	0.832788
H	1.729820	3.762449	0.079976
N	-0.521022	1.310013	-0.002637
C	-3.295074	0.591249	0.003861
H	-3.170790	1.191603	-0.892491
H	-4.290530	0.152055	0.014303
H	-3.155009	1.196390	0.894634
S	-2.161574	-0.804043	-0.001841
C	0.431152	-2.242832	-0.000048
N	0.335483	-3.389611	0.002673

7

M06-2X SCF energy in solution: -1119.31782288 a.u.
M06-2X enthalpy in solution: -1119.117120 a.u.
M06-2X free energy in solution: -1119.175403 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-1.718738	1.768952	0.065758
C	0.134478	0.440687	0.055151
C	-0.632524	-0.710676	0.015102
C	-2.019364	-0.632589	-0.036332
C	-2.544006	0.653081	-0.003443
H	-3.617351	0.785904	-0.022810
C	-2.904312	-1.837123	-0.104000
H	-3.927638	-1.554652	0.128746
H	-2.876830	-2.263166	-1.107233
H	-2.579162	-2.609501	0.592791
C	-2.274515	3.153515	0.116297
H	-3.361049	3.139153	0.132744
H	-1.908617	3.668222	1.004991
H	-1.939845	3.718662	-0.754342
N	0.007465	-2.026819	0.084472
O	-0.244840	-2.824377	-0.787423
O	0.734696	-2.236233	1.028930
S	1.936072	0.384698	0.000251
O	2.297706	-0.733872	-0.838662
C	2.387476	1.871113	-0.814586
H	1.888096	1.911480	-1.779280
H	2.135693	2.723100	-0.191858
H	3.467347	1.790117	-0.943820
N	-0.383302	1.647641	0.077125
O	2.437699	0.412981	1.352305

7a-TS

M06-2X SCF energy in solution: -1557.52572607 a.u.
M06-2X enthalpy in solution: -1557.284009 a.u.
M06-2X free energy in solution: -1557.349678 a.u.
Imaginary frequency: -146.5630 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	1.429365	1.872732	-0.583492
C	-0.208135	0.288922	-0.203281
C	0.751210	-0.741763	-0.386501
C	2.090981	-0.440120	-0.592402
C	2.418569	0.907256	-0.703226
H	3.440218	1.196864	-0.905015
C	3.169962	-1.472480	-0.738530
H	4.024219	-1.027800	-1.244000
H	3.492887	-1.821391	0.242742
H	2.834520	-2.340915	-1.302565
C	1.755151	3.328985	-0.693387
H	2.783232	3.481114	-1.013416
H	1.081391	3.815312	-1.398548
H	1.617571	3.809060	0.277702
N	0.351709	-2.117564	-0.242424
O	1.062826	-2.866512	0.403332
O	-0.686964	-2.471995	-0.767733
S	-1.999207	0.102678	-0.565470
O	-2.682255	-0.899619	0.217220
C	-2.688925	1.666988	-0.129413
H	-2.462285	1.868343	0.913448
H	-2.310204	2.438781	-0.787944
H	-3.759381	1.521703	-0.274324
N	0.146286	1.561341	-0.385026
O	-2.073068	-0.041534	-2.009593
C	1.053140	0.657799	2.591534
H	1.228962	0.794888	3.658674
H	1.798468	-0.047170	2.217691
H	1.210874	1.622908	2.105419
S	-0.629459	0.047899	2.302261

7b

M06-2X SCF energy in solution: -1557.55042501 a.u.
M06-2X enthalpy in solution: -1557.308278 a.u.
M06-2X free energy in solution: -1557.372770 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-1.353165	-1.856169	-0.448475
C	0.234286	-0.262938	0.256495
C	-0.733704	0.811723	-0.144766
C	-2.063527	0.462998	-0.551800
C	-2.359633	-0.857668	-0.667766
H	-3.347573	-1.174035	-0.971933
C	-3.144812	1.459698	-0.856593
H	-4.052737	0.922076	-1.123404
H	-3.348439	2.101751	-0.001447
H	-2.862475	2.110357	-1.682437
C	-1.731658	-3.283155	-0.704906
H	-2.569761	-3.559845	-0.062050
H	-2.069450	-3.400369	-1.736009
H	-0.899141	-3.955495	-0.516507
N	-0.319903	2.096726	-0.026041
O	-1.076407	3.077335	-0.254969
O	0.866097	2.316751	0.314807
S	1.915453	-0.058817	-0.632162
O	2.941768	0.498922	0.227577
C	2.457575	-1.699131	-1.014413
H	1.769263	-2.172216	-1.704826
H	3.431594	-1.543028	-1.478496
H	2.560034	-2.270112	-0.096541
N	-0.150492	-1.601054	-0.059572
C	-0.945572	-0.726026	2.691727
H	-1.204937	-1.727512	2.355198
H	-0.860479	-0.726271	3.777165
H	-1.716860	-0.015022	2.399278
S	0.665080	-0.212638	2.066330
O	1.645599	0.605404	-1.895215

7c-TS

M06-2X SCF energy in solution: -1557.54528768 a.u.
M06-2X enthalpy in solution: -1557.304970 a.u.
M06-2X free energy in solution: -1557.369562 a.u.
Imaginary frequency: -186.9871 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	-0.004747	2.163460	-0.747974
C	0.288640	-0.139763	-0.576441
C	-1.166242	-0.297716	-0.414870
C	-1.951009	0.862720	-0.139179
C	-1.335259	2.076263	-0.248766
H	-1.876478	2.985691	-0.027448
C	-3.381028	0.836259	0.318038
H	-3.668402	1.838350	0.629566
H	-4.048885	0.509923	-0.477032
H	-3.515959	0.148906	1.151876
C	0.561984	3.520136	-1.024326
H	1.530128	3.450568	-1.512910
H	-0.120551	4.109045	-1.637562
H	0.681735	4.050505	-0.075645
N	-1.711489	-1.558413	-0.346689
O	-2.945836	-1.729323	-0.336681
O	-0.957049	-2.548193	-0.306003
S	1.009092	-0.275183	1.448066
O	2.284409	-0.993420	1.554224
C	1.343478	1.378444	2.029726
H	0.420046	1.952724	2.019440
H	1.716442	1.261584	3.047300
H	2.099456	1.832642	1.393931
N	0.747691	1.126170	-0.963340
C	2.739880	-0.621129	-1.818762
H	3.194002	-0.193945	-0.927823
H	3.385961	-1.413686	-2.196292
H	2.618911	0.139129	-2.582944
S	1.198750	-1.458988	-1.415597
O	-0.077708	-0.800446	2.286155

7d-TS

M06-2X SCF energy in solution: -1215.15338256 a.u.
M06-2X enthalpy in solution: -1214.882930 a.u.
M06-2X free energy in solution: -1214.945245 a.u.
Imaginary frequency: -326.1591 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	1.540076	1.855158	-0.245494
C	-0.184924	0.365898	0.091096
C	0.682337	-0.721902	-0.228031
C	2.031875	-0.490026	-0.518092
C	2.447543	0.832186	-0.510193
H	3.481467	1.070562	-0.714615
C	3.041630	-1.563086	-0.790108
H	4.031881	-1.116725	-0.836648
H	3.027009	-2.324417	-0.010769
H	2.833402	-2.063783	-1.734280
C	1.976050	3.284549	-0.230145
H	1.324271	3.883695	-0.865995
H	1.894435	3.678790	0.784688
H	3.004780	3.390019	-0.565320
N	0.155884	-2.042220	-0.113932
O	-0.858624	-2.184777	0.556523
O	0.714867	-2.977874	-0.662766
S	-1.968901	0.224465	-0.443746
O	-2.866568	-0.048361	0.655198
C	-2.360126	1.828048	-1.059073
H	-1.680145	2.087589	-1.864229
H	-3.377752	1.717663	-1.434481
H	-2.325897	2.552019	-0.251450
N	0.249197	1.624615	-0.012396
C	0.718085	-0.045531	2.743593
H	1.430211	0.710743	2.412698
H	0.651144	-0.008669	3.831637
H	1.085596	-1.029788	2.454849
N	-0.569325	0.191885	2.106327
H	-0.956011	1.099951	2.345146
H	-1.247283	-0.530540	2.319009
O	-1.974706	-0.689023	-1.567907

7e

M06-2X SCF energy in solution: -1215.17135877 a.u.
M06-2X enthalpy in solution: -1214.898476 a.u.
M06-2X free energy in solution: -1214.961588 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	1.346824	1.918181	0.160783
C	-0.237713	0.239181	0.524643
C	0.734906	-0.764610	0.024490
C	1.980998	-0.325388	-0.517714
C	2.251860	1.008439	-0.469892
H	3.182354	1.391546	-0.864023
C	2.990392	-1.247189	-1.132508
H	3.853171	-0.666907	-1.452307
H	3.311861	-2.009403	-0.424534
H	2.569239	-1.768069	-1.991333
C	1.748752	3.353879	0.263326
H	0.999566	3.934333	0.794447
H	2.709833	3.437524	0.772634
H	1.880763	3.763624	-0.740146
N	0.302677	-2.055024	-0.023846
O	-0.850122	-2.308826	0.414919
O	0.998683	-2.978930	-0.479256
S	-1.766950	0.272077	-0.692437
O	-2.962277	-0.089648	0.048975
C	-1.923838	1.953023	-1.206365
H	-1.001099	2.267641	-1.687225
H	-2.745771	1.938001	-1.922496
H	-2.165194	2.577688	-0.351945
N	0.203964	1.570983	0.663566
C	0.115348	-0.484578	2.890697
H	0.892550	0.273455	2.919141
H	-0.401650	-0.536332	3.843503
H	0.531962	-1.454207	2.636589
N	-0.880432	-0.110781	1.852166
H	-1.544867	-0.877354	1.717140
O	-1.416086	-0.508758	-1.858881
H	-1.391749	0.722652	2.155523

7f

M06-2X SCF energy in solution: -1214.71331783 a.u.
M06-2X enthalpy in solution: -1214.454429 a.u.
M06-2X free energy in solution: -1214.516981 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	0.847004	2.157429	0.104584
C	-0.311452	0.151515	0.581378
C	0.881434	-0.592378	0.029026
C	2.016615	0.112391	-0.488508
C	1.967405	1.469257	-0.472933
H	2.784499	2.053789	-0.872402
C	3.230928	-0.548075	-1.075854
H	3.912784	0.220765	-1.434071
H	3.743570	-1.165093	-0.340148
H	2.963205	-1.201371	-1.904962
C	0.895922	3.654910	0.107762
H	0.052977	4.080445	0.645193
H	1.828288	4.001036	0.556279
H	0.883488	4.016668	-0.922918
N	0.793693	-1.941778	-0.016453
O	-0.307909	-2.490712	0.260048
O	1.763026	-2.676798	-0.335459
S	-1.796271	-0.145118	-0.635332
O	-2.791339	-1.058729	-0.095127
C	-2.607386	1.416265	-0.822929
H	-1.926112	2.138178	-1.261710
H	-3.440346	1.205761	-1.493525
H	-2.971346	1.751219	0.144077
N	-0.180020	1.576433	0.622302
C	0.191048	-0.248224	2.907390
H	0.572693	0.766209	3.012842
H	-0.261045	-0.541325	3.852538
H	1.036617	-0.914610	2.706836
N	-0.833850	-0.289046	1.863575
H	-1.140813	-1.247913	1.751600
O	-1.217026	-0.486221	-1.926933

7g-TS

M06-2X SCF energy in solution: -1214.70947561 a.u.
M06-2X enthalpy in solution: -1214.453826 a.u.
M06-2X free energy in solution: -1214.516044 a.u.
Imaginary frequency: -206.0497 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	0.574290	2.246988	0.235632
C	-0.168903	0.112370	0.797389
C	1.015000	-0.441587	0.141992
C	1.901203	0.398718	-0.576620
C	1.637905	1.744042	-0.548522
H	2.262340	2.433397	-1.099617
C	3.053074	-0.084419	-1.408331
H	3.470525	0.756469	-1.957833
H	3.832886	-0.525225	-0.789926
H	2.734411	-0.849950	-2.115421
C	0.370470	3.728889	0.297637
H	-0.369856	3.989765	1.049206
H	1.310001	4.237775	0.514441
H	0.027261	4.088013	-0.675586
N	1.122918	-1.827680	0.052477
O	0.117696	-2.519749	0.281603
O	2.188865	-2.368340	-0.264395
S	-1.738405	-0.358349	-0.725705
O	-2.771224	-1.356317	-0.370804
C	-2.637507	1.158415	-1.010089
H	-1.940195	1.942399	-1.296074
H	-3.334711	0.946425	-1.820314
H	-3.173221	1.429380	-0.103284
N	-0.244036	1.486948	0.916347
C	0.016817	-0.375915	3.136970
H	0.091518	0.676478	3.402283
H	-0.486537	-0.897322	3.948307
H	1.029102	-0.781699	3.035228
N	-0.784710	-0.528301	1.918322
H	-0.887520	-1.513661	1.715616
O	-1.033439	-0.635648	-1.996669

8

M06-2X SCF energy in solution: -1044.08522669 a.u.
M06-2X enthalpy in solution: -1043.890423 a.u.
M06-2X free energy in solution: -1043.947256 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-1.169474	1.993725	0.036157
C	0.378191	0.319790	-0.094565
C	-0.615559	-0.654321	-0.037760
C	-1.960614	-0.293417	0.062234
C	-2.205842	1.072590	0.105288
H	-3.224561	1.423745	0.198321
C	-3.102017	-1.258334	0.153045
H	-3.994126	-0.727480	0.475787
H	-3.294227	-1.711125	-0.819273
H	-2.888502	-2.065768	0.851875
C	-1.422121	3.463589	0.085741
H	-2.486781	3.681920	0.097311
H	-0.964274	3.886038	0.981503
H	-0.962637	3.947843	-0.776093
N	-0.226488	-2.054242	-0.083044
O	-0.981290	-2.853143	-0.589799
O	0.857275	-2.348128	0.385991
S	2.155503	-0.057886	-0.362573
O	2.738153	1.183428	-0.954478
C	2.614075	-0.011439	1.371877
H	2.097539	-0.801346	1.910042
H	3.690880	-0.165847	1.404167
H	2.353640	0.973527	1.755310
N	0.108211	1.602001	-0.074611

8a-TS

M06-2X SCF energy in solution: -1482.29352738 a.u.
M06-2X enthalpy in solution: -1482.058078 a.u.
M06-2X free energy in solution: -1482.123145 a.u.
Imaginary frequency: -123.2066 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	-0.039400	2.129729	-0.800671
C	0.251996	-0.096683	-0.306595
C	-1.150276	-0.247691	-0.100100
C	-1.983557	0.876796	-0.115585
C	-1.378812	2.086465	-0.428813
H	-1.967999	2.992838	-0.432363
C	-3.446236	0.881295	0.218950
H	-3.780950	1.909826	0.332344
H	-3.648181	0.336107	1.139763
H	-4.027410	0.407203	-0.570513
C	0.602867	3.411759	-1.225317
H	1.320032	3.726898	-0.463815
H	-0.132387	4.201451	-1.360258
H	1.153903	3.266818	-2.154289
N	-1.683756	-1.524805	0.247644
O	-2.878799	-1.745067	0.092118
O	-0.921672	-2.371173	0.693028
S	1.309805	-1.560939	-0.615796
N	0.733047	1.046062	-0.792542
C	1.854521	1.534062	1.973661
H	2.275719	1.691078	0.978135
H	2.648473	1.703546	2.701556
H	1.081247	2.287114	2.131713
S	1.197193	-0.149365	2.128193
O	0.883071	-2.025935	-1.995709
C	2.864300	-0.724364	-0.902684
H	2.786651	-0.104367	-1.790152
H	3.580312	-1.530188	-1.061183
H	3.140366	-0.143060	-0.027567

8b

M06-2X SCF energy in solution: -1482.31187326 a.u.
M06-2X enthalpy in solution: -1482.075471 a.u.
M06-2X free energy in solution: -1482.137389 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-0.526785	2.094195	-0.584112
C	0.463441	0.115409	0.222102
C	-0.812333	-0.607238	-0.118823
C	-1.977558	0.147876	-0.495182
C	-1.823000	1.481245	-0.691164
H	-2.668425	2.097641	-0.964886
C	-3.354183	-0.434994	-0.651197
H	-4.059947	0.373382	-0.833189
H	-3.657903	-0.979847	0.241262
H	-3.398678	-1.136097	-1.482438
C	-0.415994	3.540586	-0.959603
H	-1.054681	4.139987	-0.307206
H	-0.773404	3.690618	-1.979806
H	0.609075	3.892387	-0.878485
N	-0.841871	-1.950408	0.024733
O	-1.868255	-2.639166	-0.234311
O	0.192287	-2.552464	0.418015
S	1.964620	-0.806404	-0.537158
N	0.532297	1.478351	-0.188320
C	-0.423548	1.134357	2.644419
H	-0.326407	2.132096	2.221813
H	-0.293883	1.190486	3.723769
H	-1.408111	0.722782	2.427913
S	0.868198	0.033561	2.036374
C	3.076070	0.565518	-0.794206
H	3.986935	0.116366	-1.189137
H	2.654926	1.273468	-1.501104
H	3.287146	1.039781	0.162388
O	1.514547	-1.196480	-1.923286

8c-TS

M06-2X SCF energy in solution: -1482.30217680 a.u.
M06-2X enthalpy in solution: -1482.067616 a.u.
M06-2X free energy in solution: -1482.130805 a.u.
Imaginary frequency: -224.6933 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	-1.097410	1.913568	-0.440964
C	-0.453541	-0.312483	-0.443760
C	0.932563	0.089722	-0.559848
C	1.274873	1.466762	-0.565137
C	0.241008	2.363546	-0.495543
H	0.441649	3.426120	-0.489796
C	2.675561	2.003749	-0.603792
H	2.639482	3.090605	-0.568552
H	3.199755	1.692986	-1.505267
H	3.254960	1.639424	0.244149
C	-2.199153	2.924661	-0.361493
H	-2.090395	3.671426	-1.149143
H	-2.143702	3.454186	0.592274
H	-3.172433	2.447820	-0.445960
N	1.903022	-0.903286	-0.509022
O	3.107951	-0.649111	-0.676232
O	1.537073	-2.066945	-0.291775
S	-0.294601	-0.937728	1.907266
N	-1.424353	0.646204	-0.467371
C	-2.710071	-1.540945	-1.557684
H	-2.812501	-0.727145	-2.269302
H	-3.304012	-1.339348	-0.670496
H	-3.063778	-2.464386	-2.016804
S	-0.991556	-1.885143	-1.152369
O	1.103457	-0.379674	2.252843
C	-1.441759	0.296062	2.507358
H	-2.448436	-0.010337	2.220909
H	-1.375022	0.353387	3.593712
H	-1.218028	1.268759	2.067172

8d-TS

M06-2X SCF energy in solution: -1139.91693200 a.u.
M06-2X enthalpy in solution: -1139.653004 a.u.
M06-2X free energy in solution: -1139.715812 a.u.
Imaginary frequency: -350.4037 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	1.162964	1.936128	-0.387842
C	-0.401401	0.317767	0.118962
C	0.570201	-0.705706	-0.158234
C	1.889011	-0.358860	-0.511239
C	2.167497	0.989522	-0.612605
H	3.162029	1.315954	-0.879501
C	2.997135	-1.335704	-0.765967
H	3.913066	-0.787542	-0.973291
H	3.154055	-1.984485	0.095018
H	2.766006	-1.980009	-1.612699
C	1.456789	3.397123	-0.507806
H	0.740705	3.865347	-1.183374
H	1.344432	3.872809	0.468318
H	2.465953	3.572734	-0.871704
N	0.176848	-2.047926	0.031085
O	0.917682	-2.975020	-0.266999
O	-0.942705	-2.248333	0.508484
S	-2.236326	0.038960	-0.213212
O	-2.810976	1.416274	-0.348265
C	-2.026091	-0.536516	-1.900576
H	-1.580594	-1.528920	-1.907570
H	-3.028927	-0.584763	-2.321323
H	-1.416236	0.180755	-2.447528
N	-0.083063	1.603322	-0.083116
C	0.623955	0.285408	2.748413
H	1.171198	1.151464	2.376884
H	0.540717	0.360538	3.832634
H	1.175744	-0.621193	2.503613
N	-0.684910	0.241177	2.104293
H	-1.229848	1.074376	2.309631
H	-1.211575	-0.584780	2.372051

8e

M06-2X SCF energy in solution: -1139.93643326 a.u.
M06-2X enthalpy in solution: -1139.669591 a.u.
M06-2X free energy in solution: -1139.730926 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-0.918611	1.958893	0.396133
C	-0.402437	-0.315371	0.367851
C	1.009323	0.014685	-0.000962
C	1.345434	1.380917	-0.294183
C	0.374445	2.313664	-0.121186
H	0.575148	3.355586	-0.328048
C	2.692187	1.826124	-0.781117
H	2.669408	2.900564	-0.949633
H	3.471502	1.594837	-0.056896
H	2.960417	1.322564	-1.708422
C	-1.889493	3.068673	0.638552
H	-1.456117	3.799170	1.323562
H	-2.081494	3.588411	-0.302502
H	-2.826793	2.697879	1.043411
N	1.893901	-1.007865	-0.110633
O	3.074923	-0.836429	-0.475169
O	1.519328	-2.184433	0.178974
S	-1.162787	-1.349129	-1.079059
O	-2.459119	-1.893956	-0.541767
C	-1.580690	0.031031	-2.124250
H	-0.667856	0.574974	-2.369288
H	-2.004905	-0.397229	-3.030792
H	-2.306403	0.666555	-1.621511
N	-1.279327	0.749355	0.668885
C	-0.003468	-0.753425	2.799189
H	-0.523690	0.166424	3.043367
H	-0.175038	-1.502607	3.565674
H	1.061798	-0.572296	2.683428
N	-0.530699	-1.284793	1.517512
H	-1.526438	-1.514333	1.602296
H	0.006735	-2.123919	1.263483

8f

M06-2X SCF energy in solution: -1139.47392203 a.u.
M06-2X enthalpy in solution: -1139.221390 a.u.
M06-2X free energy in solution: -1139.284068 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-0.705727	2.092076	-0.166998
C	-0.508218	-0.159386	0.510245
C	0.929419	-0.116093	0.020574
C	1.511092	1.117971	-0.438207
C	0.690172	2.192307	-0.521519
H	1.066037	3.145441	-0.868419
C	2.951257	1.285635	-0.830140
H	3.124222	2.324873	-1.104051
H	3.618944	1.016962	-0.013182
H	3.209588	0.647692	-1.673795
C	-1.541249	3.323551	-0.342315
H	-1.154043	4.122266	0.293906
H	-1.477472	3.677549	-1.372752
H	-2.579937	3.130417	-0.087358
N	1.622537	-1.263606	0.069195
O	2.844415	-1.353440	-0.240935
O	1.020760	-2.330588	0.413736
S	-1.526579	-1.532942	-0.406089
O	-2.935915	-1.008862	-0.526948
C	-0.830210	-1.284672	-2.036830
H	0.185416	-1.672186	-2.075556
H	-1.462652	-1.846229	-2.721879
H	-0.859648	-0.221705	-2.279158
N	-1.261624	1.036049	0.305466
C	0.018846	0.366272	2.815358
H	-0.438911	1.351929	2.747430
H	-0.094685	0.008585	3.836588
H	1.089036	0.467029	2.604478
N	-0.661647	-0.560629	1.909185
H	-0.221869	-1.471092	1.996407

8g-TS

M06-2X SCF energy in solution: -1139.46267882 a.u.
M06-2X enthalpy in solution: -1139.211640 a.u.
M06-2X free energy in solution: -1139.275348 a.u.
Imaginary frequency: -195.6520 cm-1

Cartesian coordinates

ATOM	X	Y	Z
C	-2.005788	0.967111	0.333418
C	-0.154444	-0.315984	0.863543
C	0.691187	0.628098	0.162939
C	0.114026	1.701823	-0.562547
C	-1.248560	1.831427	-0.487109
H	-1.751498	2.610096	-1.044366
C	0.871023	2.637675	-1.459449
H	0.161595	3.226378	-2.036945
H	1.506537	3.311706	-0.887913
H	1.518408	2.088611	-2.143536
C	-3.481976	1.190529	0.442290
H	-3.690808	2.218167	0.743556
H	-3.947456	1.041993	-0.534285
H	-3.928990	0.504047	1.156299
N	2.066440	0.384503	0.083523
O	2.851286	1.288466	-0.232529
O	2.494972	-0.753875	0.325237
S	-0.421159	-2.104025	-0.820360
O	-1.830491	-1.745080	-1.363546
C	0.706977	-1.556303	-2.099070
H	1.695880	-1.956853	-1.879120
H	0.357052	-1.945619	-3.055008
H	0.755854	-0.465200	-2.147790
N	-1.473472	-0.015402	1.018820
C	0.568920	-0.371740	3.137824
H	-0.370089	0.047833	3.494136
H	0.959207	-1.036671	3.905573
H	1.281586	0.448110	2.993079
N	0.333670	-1.149648	1.914408
H	1.210469	-1.556270	1.620485

MeS-

M06-2X SCF energy in solution: -438.20582851 a.u.
M06-2X enthalpy in solution: -438.165277 a.u.
M06-2X free energy in solution: -438.191823 a.u.

Cartesian coordinates

ATOM	X	Y	Z
S	0.000000	0.000000	0.707269
C	0.000000	0.000000	-1.124464
H	0.000000	1.015757	-1.523174
H	0.879672	-0.507879	-1.523174
H	-0.879672	-0.507879	-1.523174

MeSO-

M06-2X SCF energy in solution: -513.41919002 a.u.
M06-2X enthalpy in solution: -513.374387 a.u.
M06-2X free energy in solution: -513.405392 a.u.

Cartesian coordinates

ATOM	X	Y	Z
S	0.194099	-0.546289	-0.000016
O	1.275565	0.639074	-0.000005
C	-1.369790	0.345683	-0.000021
H	-2.177919	-0.387979	0.001098
H	-1.455981	0.971530	0.890004
H	-1.457458	0.970380	-0.890690

N-product

M06-2X SCF energy in solution: -626.06028006 a.u.
M06-2X enthalpy in solution: -625.854124 a.u.
M06-2X free energy in solution: -625.905712 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-1.903139	-0.916304	0.015077
C	-0.389643	0.829683	-0.021074
C	0.693618	-0.107124	-0.022796
C	0.433261	-1.486923	-0.064386
C	-0.894098	-1.873449	-0.057184
H	-1.148089	-2.922414	-0.109495
C	1.479615	-2.557984	-0.168049
H	1.001666	-3.486669	-0.470762
H	2.251065	-2.298807	-0.890861
H	1.971208	-2.715074	0.790967
C	-3.340516	-1.324448	0.074009
H	-3.892724	-0.859055	-0.743013
H	-3.451488	-2.404285	0.013733
H	-3.782013	-0.973334	1.008113
N	2.045001	0.357692	0.044596
O	2.910901	-0.383475	0.477964
O	2.308034	1.499903	-0.322686
N	-1.656340	0.387303	0.028097
C	-1.347633	3.081926	0.045811
H	-2.022765	2.960939	-0.800359
H	-1.916394	2.942728	0.963851
H	-0.943262	4.089529	0.031324
N	-0.232082	2.159843	-0.031159
H	0.696481	2.528897	-0.134905

NH2Me

M06-2X SCF energy in solution: -95.84728549 a.u.
M06-2X enthalpy in solution: -95.778535 a.u.
M06-2X free energy in solution: -95.805740 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	0.705268	-0.000021	-0.016596
H	1.120550	0.878470	0.473868
H	1.120651	-0.878053	0.474498
H	1.046931	-0.000209	-1.054858
N	-0.751900	-0.000014	0.130718
H	-1.128144	-0.807119	-0.354573
H	-1.128294	0.807141	-0.354383

NH3Me

M06-2X SCF energy in solution: -96.31087948 a.u.
M06-2X enthalpy in solution: -96.227228 a.u.
M06-2X free energy in solution: -96.254823 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	0.786566	-0.000022	-0.000141
H	1.129447	-0.996496	-0.254622
H	1.129349	0.719322	-0.735231
H	1.128604	0.277413	0.990529
N	-0.700773	0.000065	-0.000020
H	-1.067066	0.923316	0.236629
H	-1.066612	-0.666606	0.681412
H	-1.067704	-0.257267	-0.917730

product

M06-2X SCF energy in solution: -968.88701733 a.u.
M06-2X enthalpy in solution: -968.697240 a.u.
M06-2X free energy in solution: -968.750785 a.u.

Cartesian coordinates

ATOM	X	Y	Z
C	-0.865065	-2.005058	-0.030522
C	-0.609929	0.283456	-0.064898
C	0.788290	0.129996	-0.036904
C	1.379213	-1.132515	-0.025214
C	0.506325	-2.210994	-0.035234
H	0.900330	-3.217678	-0.049930
C	2.857732	-1.380090	-0.042567
H	3.042665	-2.401310	-0.367253
H	3.375340	-0.693374	-0.710005
H	3.278560	-1.250688	0.954225
C	-1.822831	-3.152350	-0.028534
H	-2.422306	-3.135486	-0.939943
H	-1.299785	-4.103448	0.032856
H	-2.508352	-3.065350	0.814781
N	1.634641	1.308406	0.003248
O	2.643031	1.274945	0.678645
O	1.287583	2.284098	-0.633312
N	-1.400354	-0.779007	-0.043804
C	-3.080000	1.419114	0.303938
H	-3.524833	0.846714	-0.503945
H	-3.139687	0.867379	1.237838
H	-3.600833	2.369869	0.406472
S	-1.381778	1.868493	-0.074641

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