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Supporting Information

Light-Activated Reactivity of Nitrones with Amino Acids and Proteins

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Supplementary Information

Light-activated nitrone bioconjugation reagents

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Materials and methods

All materials were used as received from vendors. An Agilent 6230 time-of-flight mass spectrometer and 1260 Infinity pump were used for the high-pressure liquid chromatography mass spectrometry (HPLC-MS). Protein samples were analyzed using an Agilent PLRP-S reverse-phase column (50mmX2.1mm 5 μm) kept at 75 °C and running a solvent gradient from 5% to 95% acetonitrile in water with 0.1% formic acid over 5 min. Small molecules samples were analyzed using an Agilent Zorbax RRHD Eclipse Plus C18 column (50 mmX2.1 mm 1.8 μm) kept at 50 °C and running a gradient 5% to 95% acetonitrile in water with 0.1% formic acid over 5 minutes. Irradiation was done using a 370 nm Kessil lamp, approximately 10 nm FWHM and used at the 100% intensity setting. Samples were typically irradiated in NMR tubes (Norell ST500, 5 mm) placed 5-15 cm away from the light source. Silver staining was done using the Pierce Silver Stain Kit from Thermo Scientific used according to manufacturer's protocol. Cells were obtained from UC Berkeley's Biosciences Divisional Services Cell Culture Facility and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% (v/v) fetal bovine serum and p/s and maintained at 37 °C with 5% CO_2. Description of amino acids "*N*-Ac" indicates α-amino acetylated and "*C*-OMe" indicates α-carboxy methyl esters.

Structures and names of referenced nitrones and oxaziridines

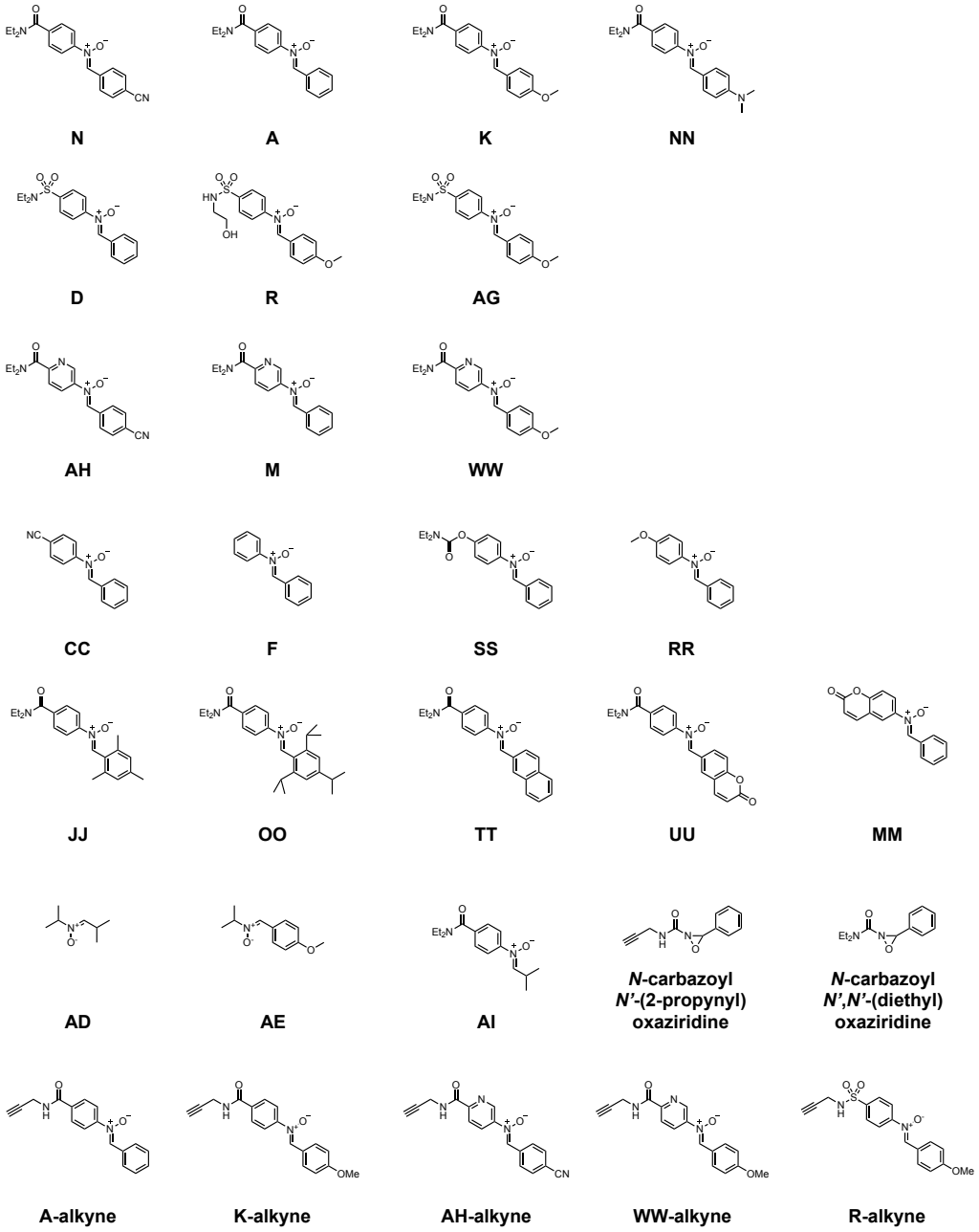


Figure S1. Chemical structures and names of the referenced nitrones and oxaziridines.

Nitrone NOE experiment

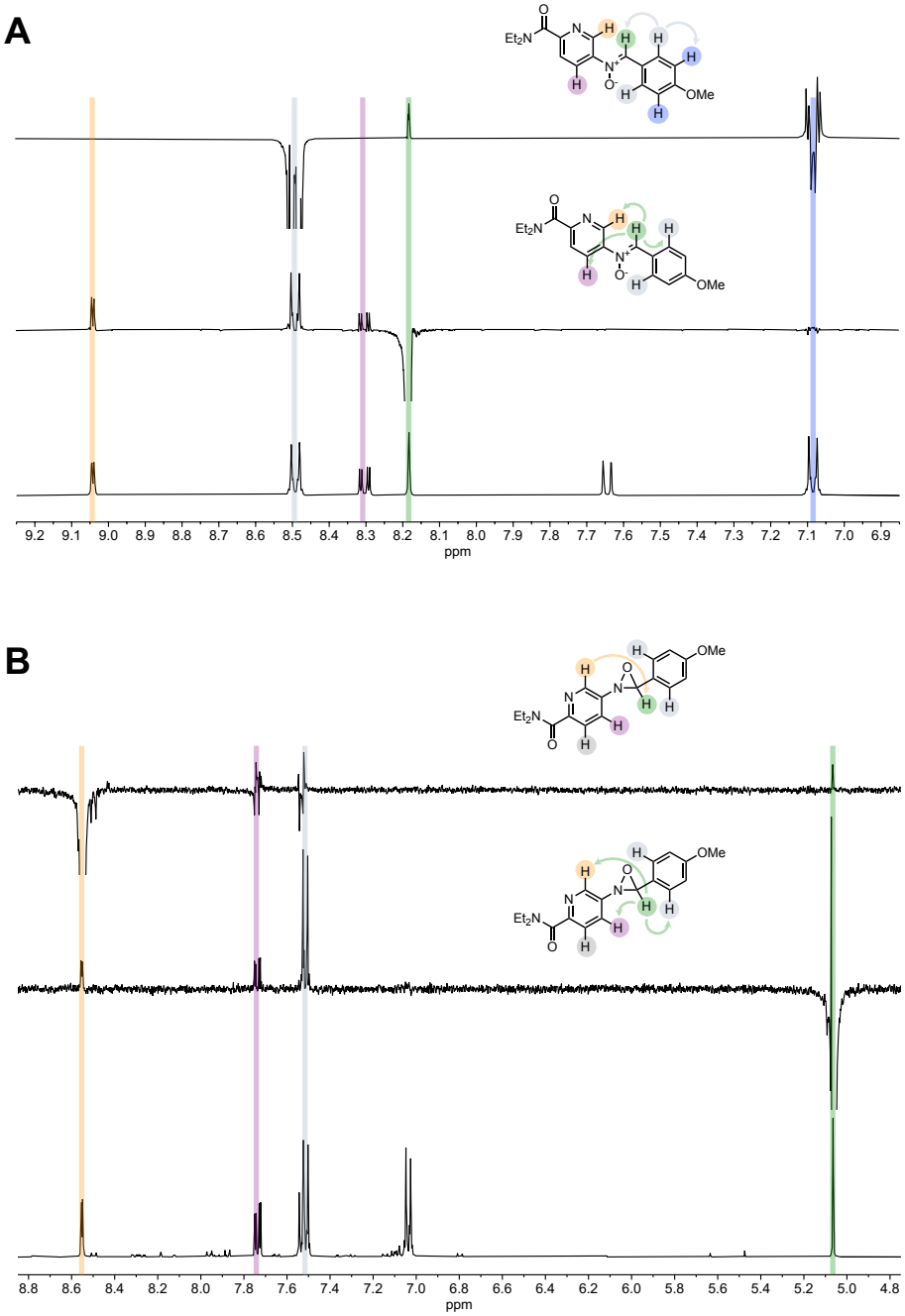


Figure S2. Selective NOESY experiments in CD_3CN used to determine *E/Z* isomerism of nitrone **WW** and oxaziridine **WW**. (**A**) ^1H NMR of nitrone **WW** (bottom trace), NOE of nitrone **WW** when the nitrone C-H is perturbed (middle trace, ~8.2 ppm), and NOE spectrum of nitrone **WW** when the aryl C-H are perturbed (top trace, ~8.5 ppm). (**B**) ^1H NMR of oxaziridine WW (bottom trace), NOE of oxaziridine **WW** when C*α*-H is perturbed (middle trace, ~5.1 ppm), and NOE when *N*-pyridyl C-H is perturbed (top trace, ~8.5 ppm).

Stability of N,α-diaryl nitrones

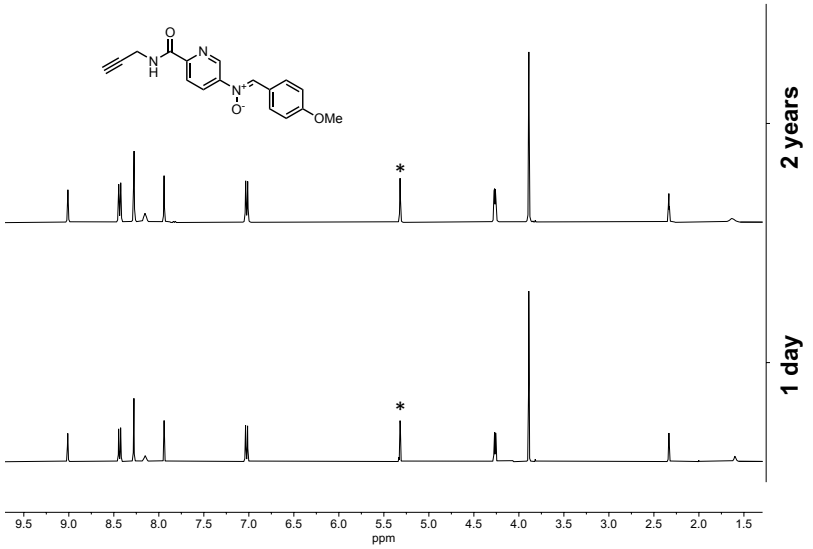


Figure S3. The ^{1}H NMR spectrum of nitrone **WW**-alkyne in CD_2Cl_2 remains unchanged between 1 day and 2 years after its synthesis, when stored as a solid at –20°C. (* indicates residual solvent)

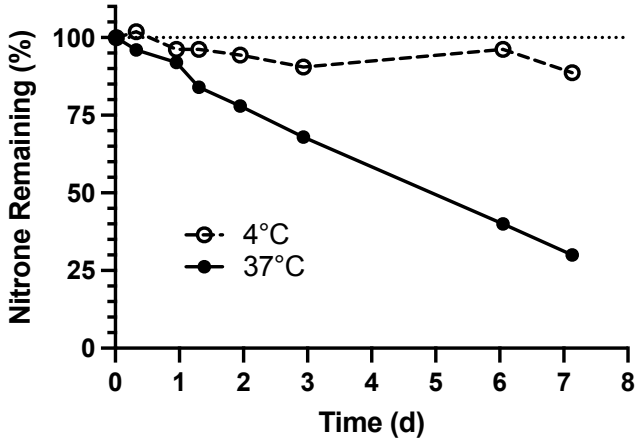


Figure S4. Stability of nitrone **WW** in a solution of 1:1 D_2O:CD_3OD buffered with phosphate at pD 7.0, kept in the dark at the indicated temperatures and probed by ^{1}H NMR.

The stability of nitrone **WW** in the presence of amino acids in buffered water was measured by HPLC-MS. A 5.00 mM stock solution of nitrone **WW** was prepared in acetonitrile. Stock solution of each amino acid (*N*-Ac and *C*-OMe protected) were prepared at 100 mM in a 1:1 mixture of acetonitrile to water. A separate reaction solution for each amino acid was made by combining 880 µL water, 100 µL sodium phosphate buffer (0.5 M at pH 7.0), 10.0 µL of the nitrone stock solution, and 10.0 µL of an amino acid stock solution. The nitrone to amino acid ratio in the screening reactions was 1:20 (50 µM in nitrone, 1 mM in amino acid) and the concentration of the phosphate buffer was 50 mM. This step was repeated to make a screening reaction for each amino acid and a control reaction without an amino acid. The reaction mixtures were shaken at 240 rev/min at rt. A sample of each reaction was collected at 0.5 h, 6 h, and 24 h, and diluted ten-fold in water to give a concentration of 5 µM in

nitrone. Diluted samples were assayed by HPLC-MS, the amount of nitrone remaining at each timepoint was calculated with respect to the amount of nitrone in the control sample without an amino acid added.

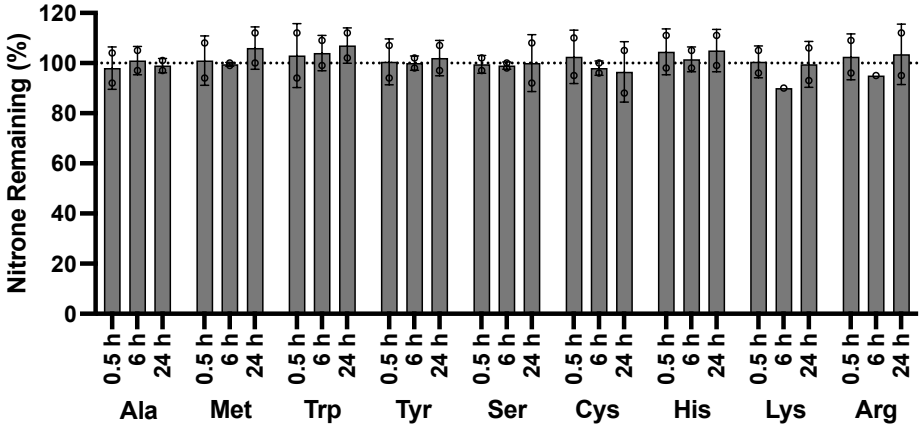


Figure S5. Stability of nitrone **WW** (50 μM) in the presence of amino acids (1 mM) in aqueous conditions (50 mM phosphate buffer, pH 7.0). The nitrone was **not** irradiated. Individual experimental replicates shown, error bars show standard deviation.

Photoisomerization of nitrones to oxaziridines

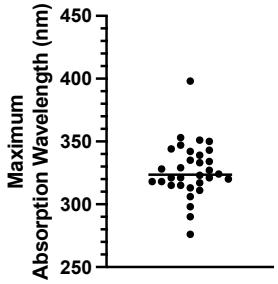


Figure S6. Distribution of maximum absorption wavelengths of the nitrones reported in this publication. UV-Vis spectra were taken in methanol.

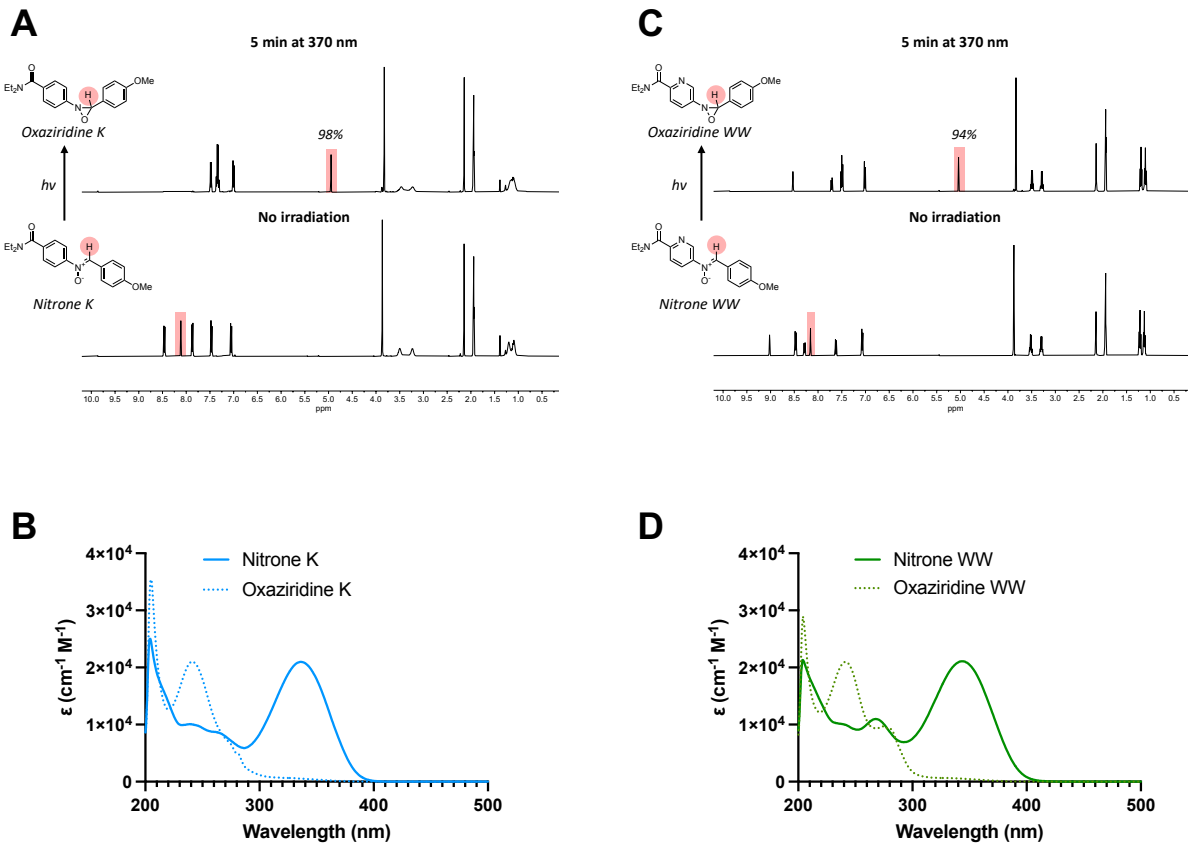


Figure S7. Isomerization of nitrones to oxaziridines. (**A**, **C**) 1H NMR spectra before and after irradiation of nitrones in CD_3CN, residual solvent peak was used as an internal standard for determining the yield shown. (**B**, **D**) UV-Vis spectra before and after irradiation of nitrones in acetonitrile. Panels **A, B** show nitrone **K** and panels **C**, **D** show nitrone **WW**.

TD-DFT modeling of nitrones

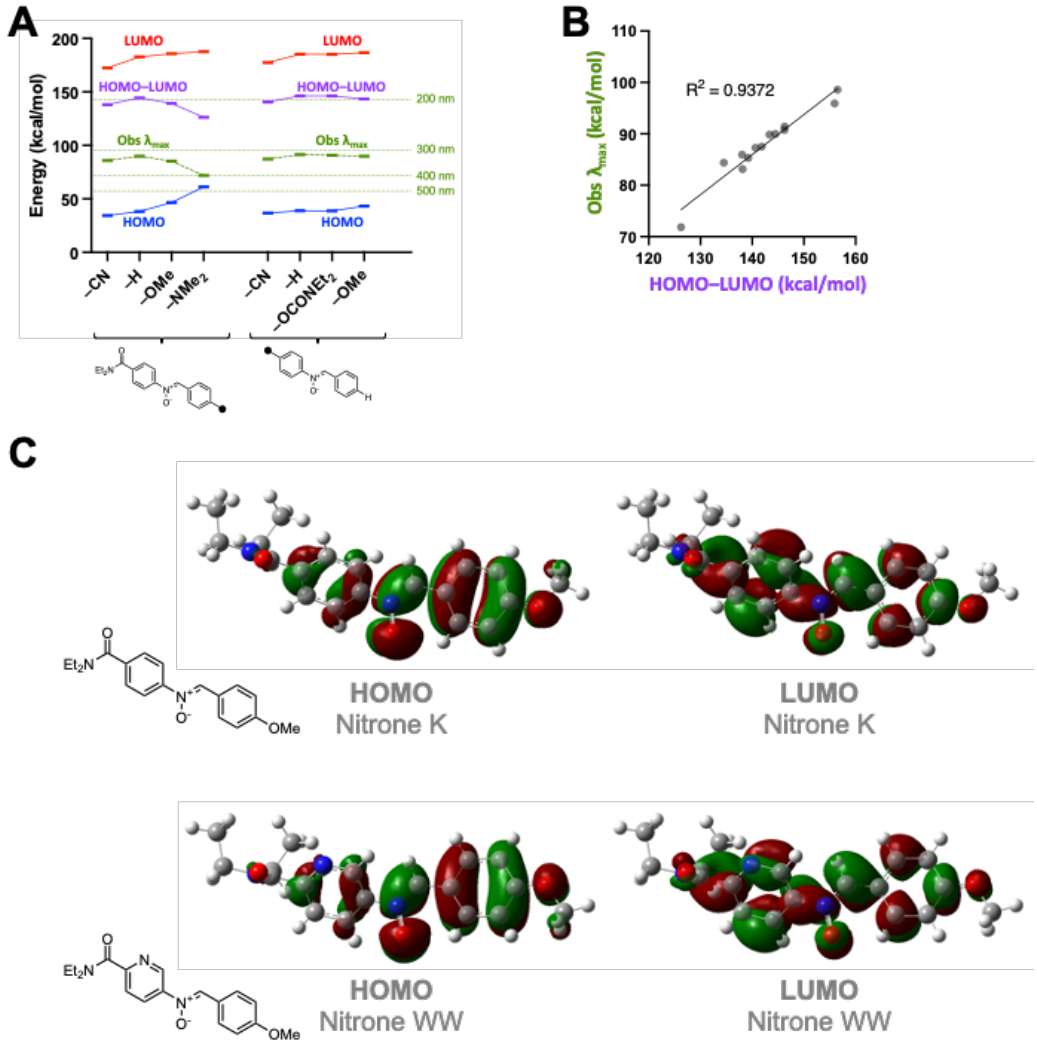


Figure S8. DFT modeling describes impact of electron density in aryl rings on light absorption by nitrones. (**A**) Impact of substitution pattern on the HOMO and LUMO energy levels and the HOMO-LUMO gap compared to the observed maximum absorption wavelength. (**B**) The HOMO-LUMO gap correlates well with the observed maximum absorption wavelength. (**C**) HOMO and LUMO orbitals for nitrones **K** and **WW**.

Crystal structure of nitrone WW

Single crystals of nitrone **WW** were obtained by vapor diffusion of benzene into a ~30 mg/mL solution of nitrone **WW** in diethyl ether. A suitable crystal was selected and data collected on a ROD, Synergy Custom DW system, Pilatus 200K diffractometer. The crystal was kept at 293(2) K during data collection. Using Olex2[1], the structure was solved with the SHELXT[2] structure solution program using Intrinsic Phasing and refined with the SHELXL[3] refinement package using Least Squares minimization.

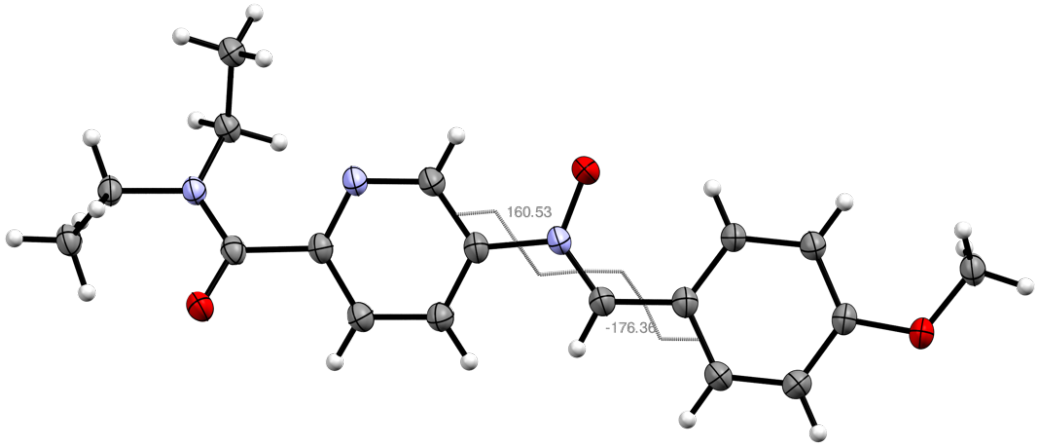


Figure S9. Nitrone **WW** structure based on single crystal X-ray diffraction. Ellipsoids indicate 50% probability level. Dihedral angles between the nitrone and the *α*- and *N*-aryl rings are marked and show that the *N*-aryl ring is twisted by approximately 20° out of the plane that holds the nitrone and *α*-aryl groups.

Crystal Data for $C_{18}H_{21}N_3O_3$ (*M* =327.38 g/mol): triclinic, space group P-1 (no. 2), *a* = 7.7344(3) Å, *b* = 7.8083(3) Å, *c* = 14.1227(9) Å, *α* = 77.647(4)°, *β* = 81.535(4)°, *γ* = 80.063(3)°, *V* = 815.25(7) Å^3, *Z* = 2, *T* = 293(2) K, μ(Cu Kα) = 0.751 mm^{-1}, *Dcalc* = 1.334 g/cm^3, 6010 reflections measured (6.45° ≤ 2Θ ≤ 149.054°), 6010 unique (R_{int} = ?, R_{sigma} = 0.0098) which were used in all calculations. The final R_1 was 0.0587 (I > 2σ(I)) and wR_2 was 0.1851 (all data).

Impact of geometry on light absorption by nitrones

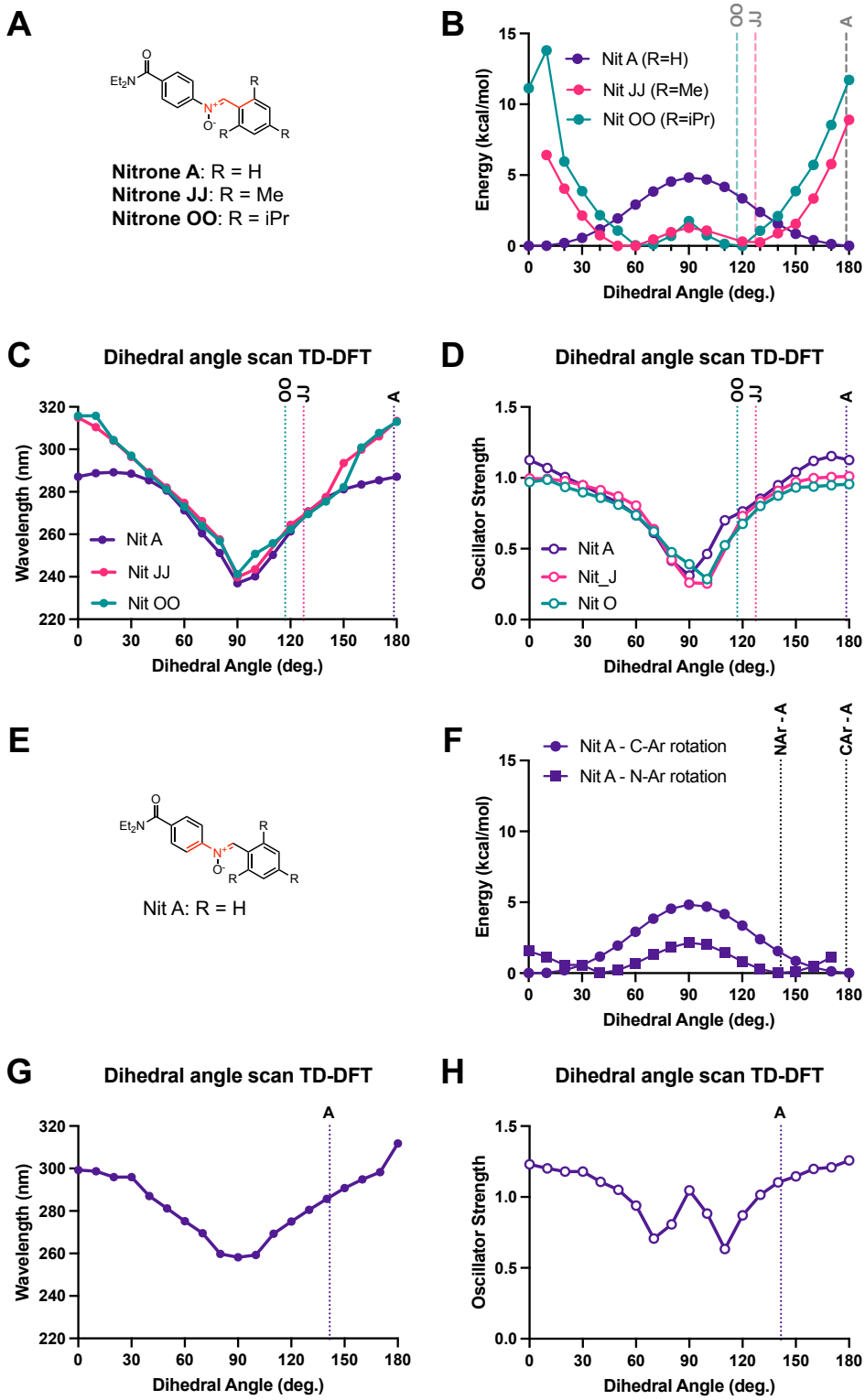


Figure S10. (**A**) Structure of nitrones **A**, **JJ**, and **OO** with the dihedral angle (indicated in red) varied in panels **B**–**D**. (**B, F**) The energy of the DFT optimized geometry while scanning the dihedral angle from 0 to 180°. (**C, G**) The maximum absorption wavelength of the strongest

absorption band as predicted by TD-DFT. (**D, H**) The oscillator strength of the band shown in (**C** and **G**). (**F**) Structure of nitrone **A** with the dihedral angle (indicated in red) varied in panels **F**–**H**. Vertical dashed lines indicate the dihedral angle of the minimum optimized geometry of the molecules without any constraints. All DFT models were carried out with the m06x level of theory and a def2tzvp basis set, with TD-DFT in the Tamm-Dancoff approximation.

Impact of *N*-aryl electron density on light absorption by nitrones

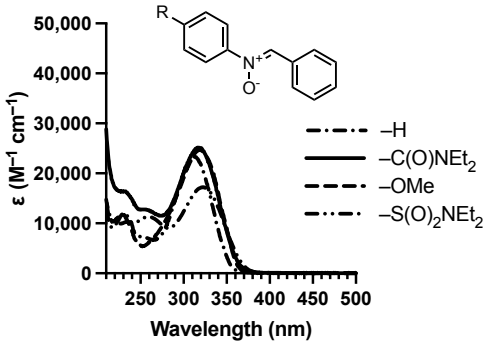


Figure S11. Impact of *N*-aryl substitutions on UV-Vis absorption spectra of nitrones. UV-Vis absorption spectra taken in methanol.

Impact of extended conjugation on light absorption by nitrones

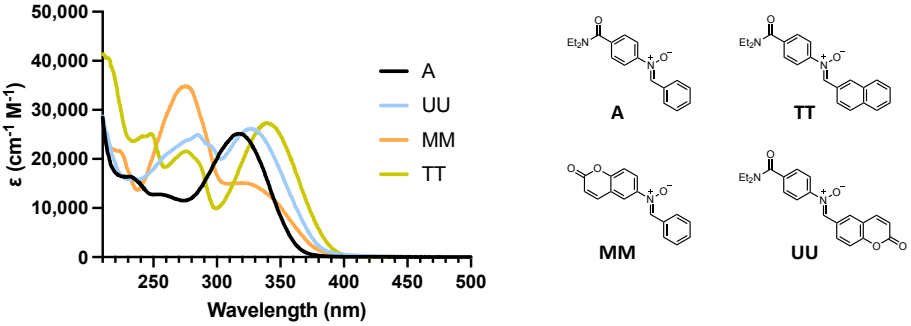


Figure S12. Impact of extended conjugation on *N*- and *α*-aryl systems on UV-Vis absorption spectra of nitrones (methanol solution).

Stability of *N*,α-diaryl oxaziridines

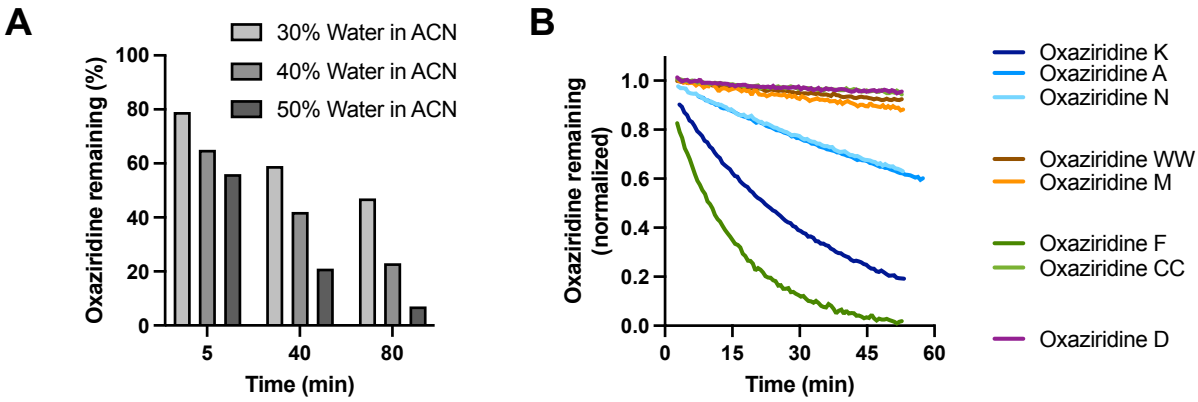


Figure S13. Solvent affects rate of oxaziridine decomposition. (**A**) The rate of oxaziridine **A** decomposition increases with higher ratios of water-d_2 to acetonitrile-d_3 and (**B**) with increasing electron density on the α-aryl ring in 30% water-d_2 in acetonitrile-d_3. Nitrones were irradiated at 370 nm for 10 min in CD_3CN then diluted with D_2O to the indicated percentage of total volume. The amount of oxaziridine remaining in solution was quantified with reference to methylsulfonylmethane as an internal standard.

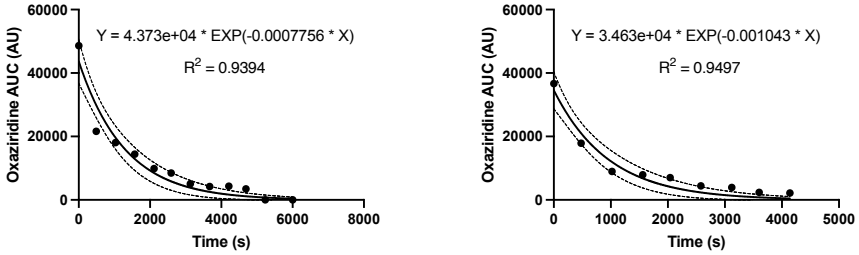


Figure S14. Stability of oxaziridine **WW** in buffered water. Nitrone **WW** irradiated in acetonitrile for 5 min at 370 nm, then added to a phosphate buffered (5 mM) solution of water at pH 7.0 to a final concentration of 2.5 μM of oxaziridine and 1% acetonitrile. The solution was sampled by LC-MS and the integrated oxaziridine peak was fit with a first order decay function to give the rate and half-life. Two replicates are shown. Average pseudo-first-order rate constants were (**A**) 0.78e-3 and (**B**) 1.0e-3 1/s (average of 0.00089 1/s).

Stabilities of all oxaziridines under the same conditions were measured by first irradiating a 15 mM solution of nitrone in acetonitrile-D_3 with 4.5 mM $DMSO_2$ as an internal standard for 5 min at 370 nm. Subsequently water-d_2 was added to a final ratio of 30/70 water-D_2/acetonitrile-D_3 and ^1H NMR scans were taken at regular intervals (approximately every 34 s) over 50 min. The ratio of the integrated oxaziridine C–H peak to the $DMSO_2$ internal standard peak was fit to a one phase decay model using a least squares regression method with a plateau value constrained to zero using GraphPad Prism software.

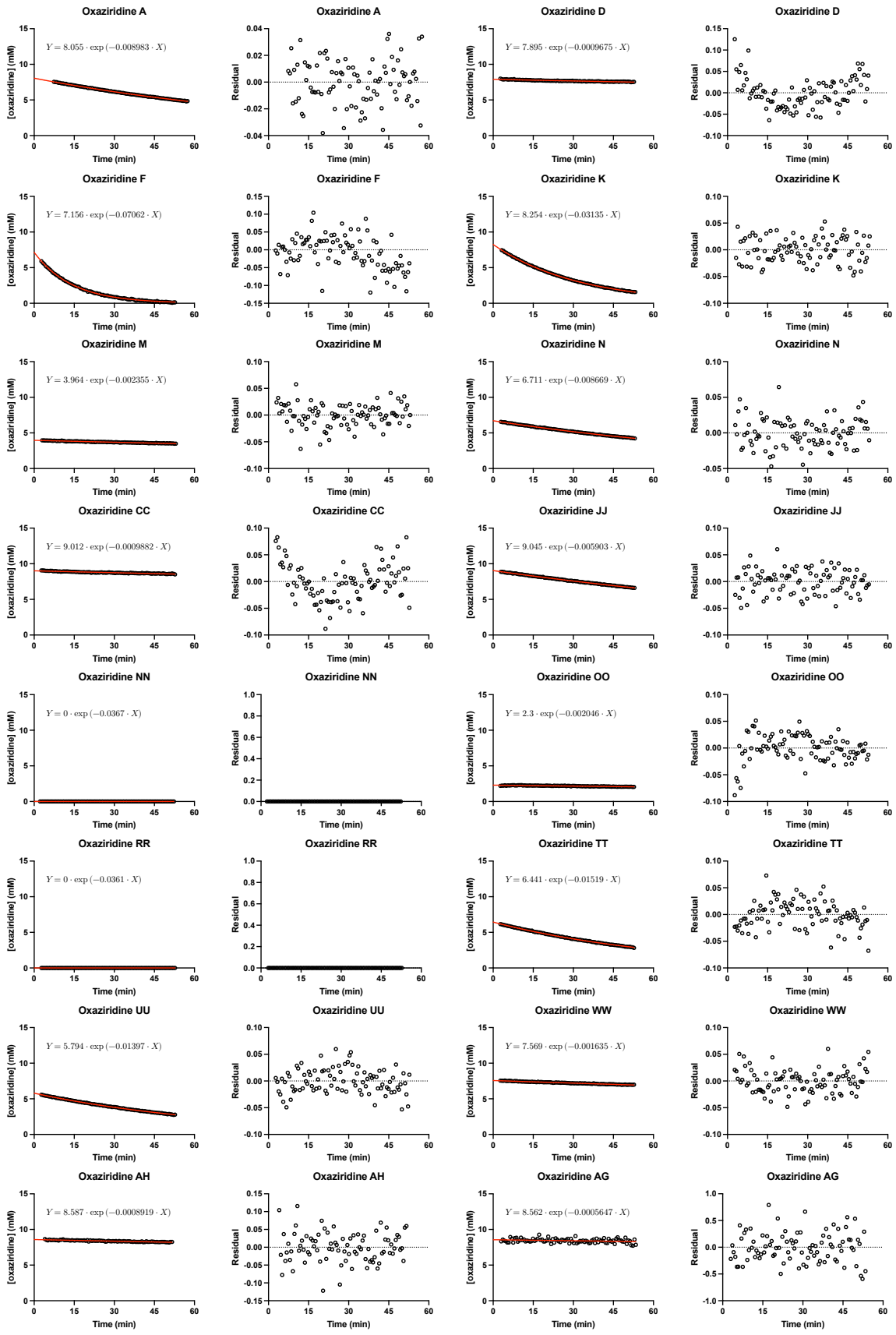

Oxaziridine A
$Y = 8.055 \cdot \exp(-0.008983 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine A
Residual
Time (min)
Oxaziridine D
$Y = 7.895 \cdot \exp(-0.0009675 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine D
Residual
Time (min)
Oxaziridine F
$Y = 7.156 \cdot \exp(-0.07062 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine F
Residual
Time (min)
Oxaziridine K
$Y = 8.254 \cdot \exp(-0.03135 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine K
Residual
Time (min)
Oxaziridine M
$Y = 3.964 \cdot \exp(-0.002355 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine M
Residual
Time (min)
Oxaziridine N
$Y = 6.711 \cdot \exp(-0.008669 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine N
Residual
Time (min)
Oxaziridine CC
$Y = 9.012 \cdot \exp(-0.0009882 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine CC
Residual
Time (min)
Oxaziridine JJ
$Y = 9.045 \cdot \exp(-0.005903 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine JJ
Residual
Time (min)
Oxaziridine NN
$Y = 0 \cdot \exp(-0.0367 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine NN
Residual
Time (min)
Oxaziridine OO
$Y = 2.3 \cdot \exp(-0.002046 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine OO
Residual
Time (min)
Oxaziridine RR
$Y = 0 \cdot \exp(-0.0361 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine RR
Residual
Time (min)
Oxaziridine TT
$Y = 6.441 \cdot \exp(-0.01519 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine TT
Residual
Time (min)
Oxaziridine UU
$Y = 5.794 \cdot \exp(-0.01397 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine UU
Residual
Time (min)
Oxaziridine WW
$Y = 7.569 \cdot \exp(-0.001635 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine WW
Residual
Time (min)
Oxaziridine AH
$Y = 8.587 \cdot \exp(-0.0008919 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine AH
Residual
Time (min)
Oxaziridine AG
$Y = 8.562 \cdot \exp(-0.0005647 \cdot X)$
[oxaziridine] (mM)
Time (min)
Oxaziridine AG
Residual
Time (min)

Figure S15. Rates of oxaziridine decomposition measured in 70:30 acetonitrile/water solvent system with dimethyl sulfone as an internal standard. Plots of oxaziridine concentration over time with single phase decay fit (red curves) constrained to plateau of zero, the corresponding residual plots shown at the right.

Reactivity of *N*,*α*-diaryl oxaziridines

Nitrone stock solutions were made in acetonitrile at a 10 mM concentration and irradiated in an NMR tube for 10 min with 370 nm light. Amino acid (*N*-Ac *C*-OMe protected) stock solutions were dissolved in a small amount of acetonitrile (final concentration of 5%$_{v/v}$ acetonitrile in the amino acid stock solution), then diluted with water to a final concentration of 2 mM. Each reaction mixture was made by combining 920 µL of water, 20 µL of 0.5 M phosphate buffer (pH 7.0), 50 µL of amino acid stock solution, and 10 µL of irradiated nitrone stock solution. The final reaction mixture contained 100 µM nitrone, 100 µM amino acid, 10 mM phosphate buffer, and 1.3% acetonitrile. The reaction mixtures were allowed to react for 10 min at room temperature, then diluted 1-in-10 with 10 mM phosphate buffered water (pH 7.0) and interrogated by HPLC-MS. Repeated injections from the same vial were used to assess stability of products at room temperature. Amino acid conversion was calculated using the integral of the amino acid EIC peak between the first reaction timepoint and an identical reaction set-up without the oxaziridine addition ("amino acid only" samples).

HPLC analysis was run with one of two methods:

150mm column method: Agilent Poroshell 120 EC-C18 3x150mm, 2.7 µm particle reverse phase column at 40°C. A gradient of 0.1% formic acid in water to acetonitrile was used for separation with the following points (%ACN, time): (20%, 0.0 min), (60%,7.0 min), (95%, 8.0 min), (95%, 8.5 min), (20%, 9.0 min), (20%, 10.0 min). Mass spectra were obtained with Agilent 6230 TOF mass spectrometer between 0.5 and 10 min.

50mm column method: Agilent Zorbax Eclipse Plus C1 2.1x50mm, 1.8 µm particle reverse phase column at 50°C. A gradient of 0.1% formic acid in water to acetonitrile was used for separation with the following points (%ACN, time): (5%, 0.0 min), (60%, 4.0 min), (95%, 5.0 min), (95%, 6.0 min), (5%, 6.5 min), (5%, 8.0 min). Mass spectra were obtained with Agilent 6230 TOF mass spectrometer between 0.5 and 8 min.

Table S1. Percent amino acid conversion (ave±sd of three replicates) upon reaction with irradiated nitrones.

	N	A	K	AH	M	WW	D	AG
Cys	75±4	64±4	39±11	84±3	91±4	94±2	98±3	98±4
Met	32±2	16±0	10±1	65±2	65±1	57±1	49±3	37±1
Trp	31±4	20±4	11±4	52±5	52±5	54±5	42±4	42±3

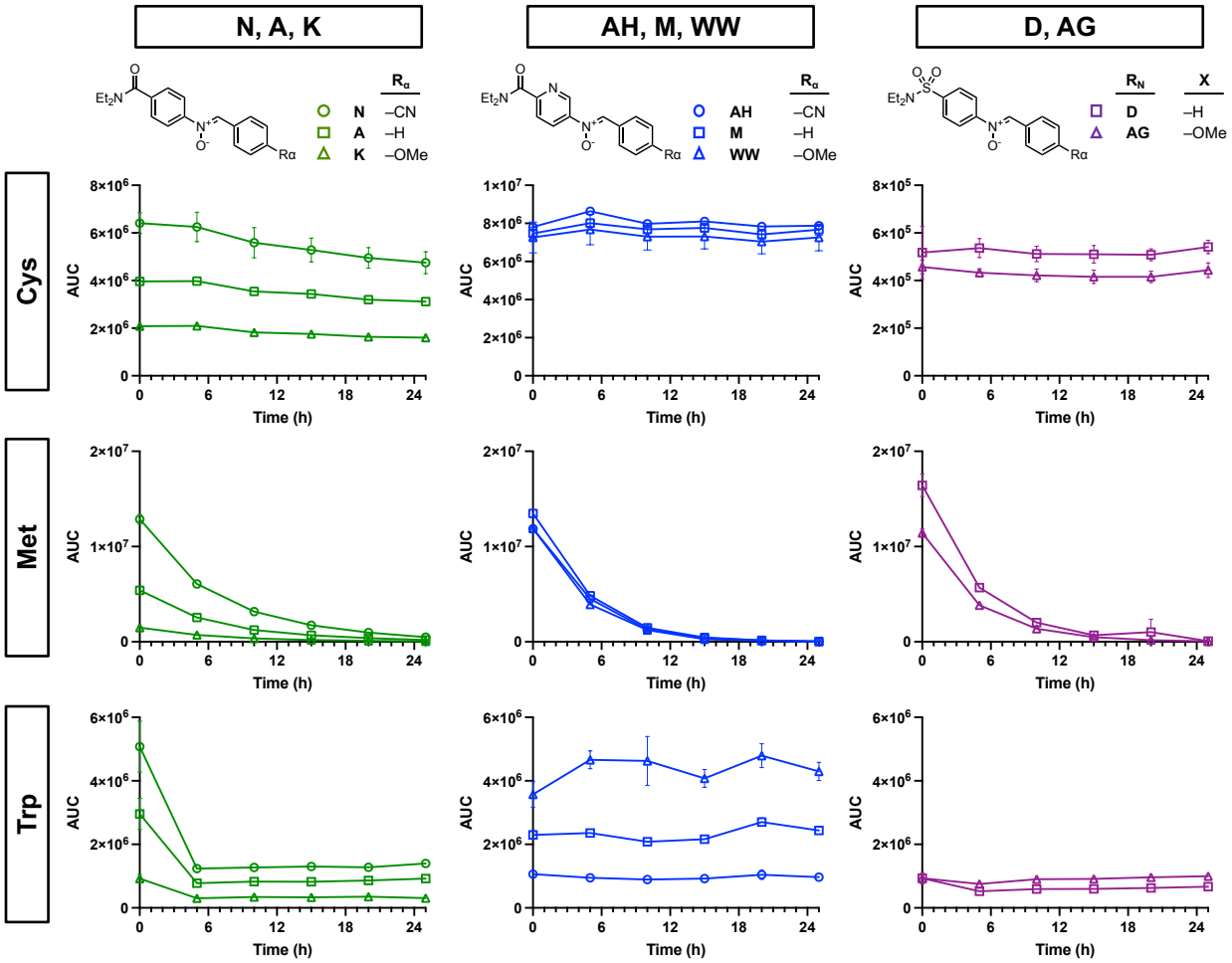


Figure S16. Stability of oxaziridine conjugates. Conjugates made with cysteine, methionine, and tryptophan. Within each graph, the products measured have identical structures.

Figure S17 through Figure S27, chromatograms show the TIC on top and the individual EIC of the mass indicated at the left. The EIC traces are normalized individually to the same maximum height, the actual value of this maximum for each trace is given on the right. Chromatography used the 15 mm column HPLC method outlined earlier. * Asterisk indicates peak caused by sodium phosphates originating from the buffer.

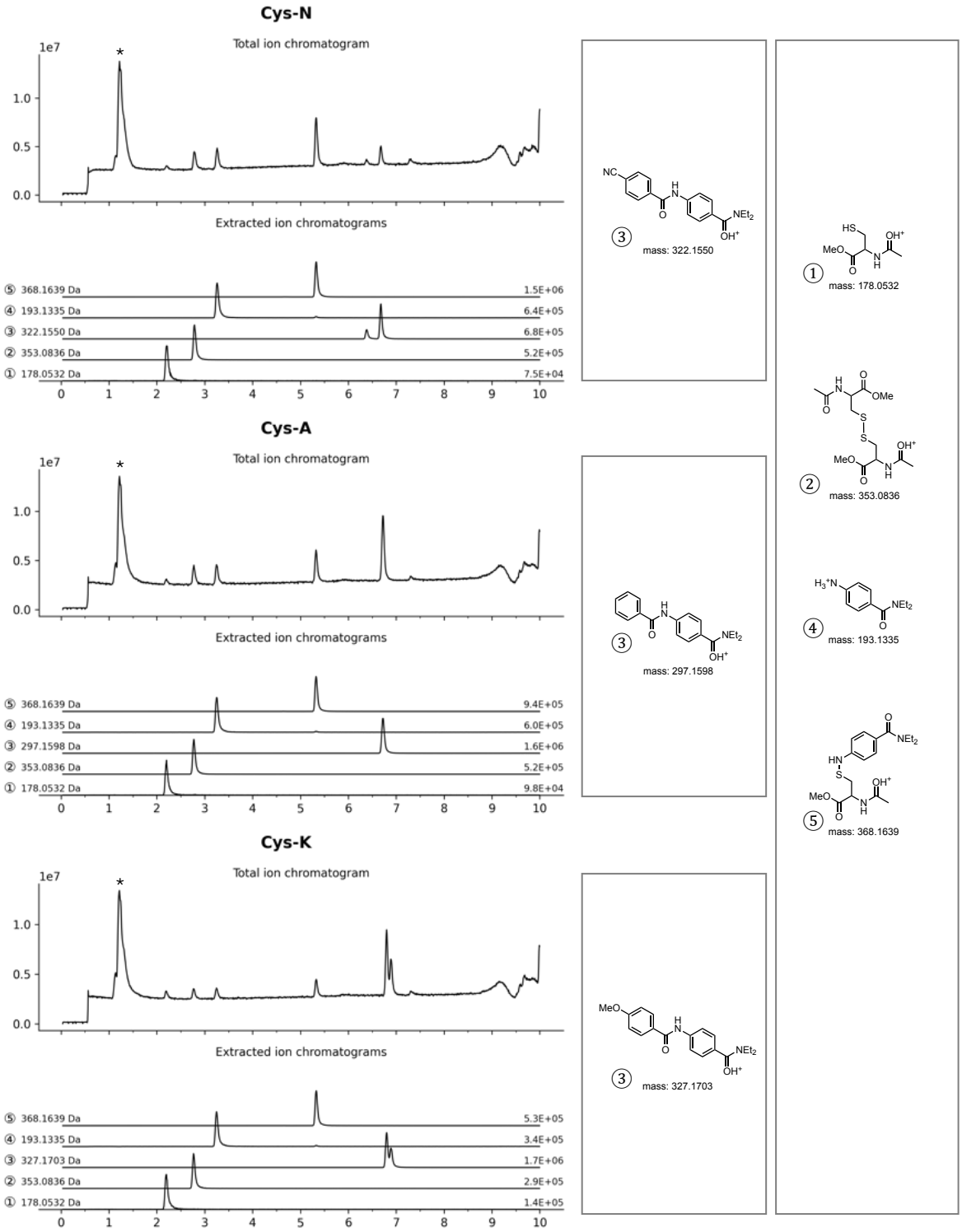


Figure S17. Reactions of Ac-Cys-OMe with oxaziridines **N**, **A**, and **K**.

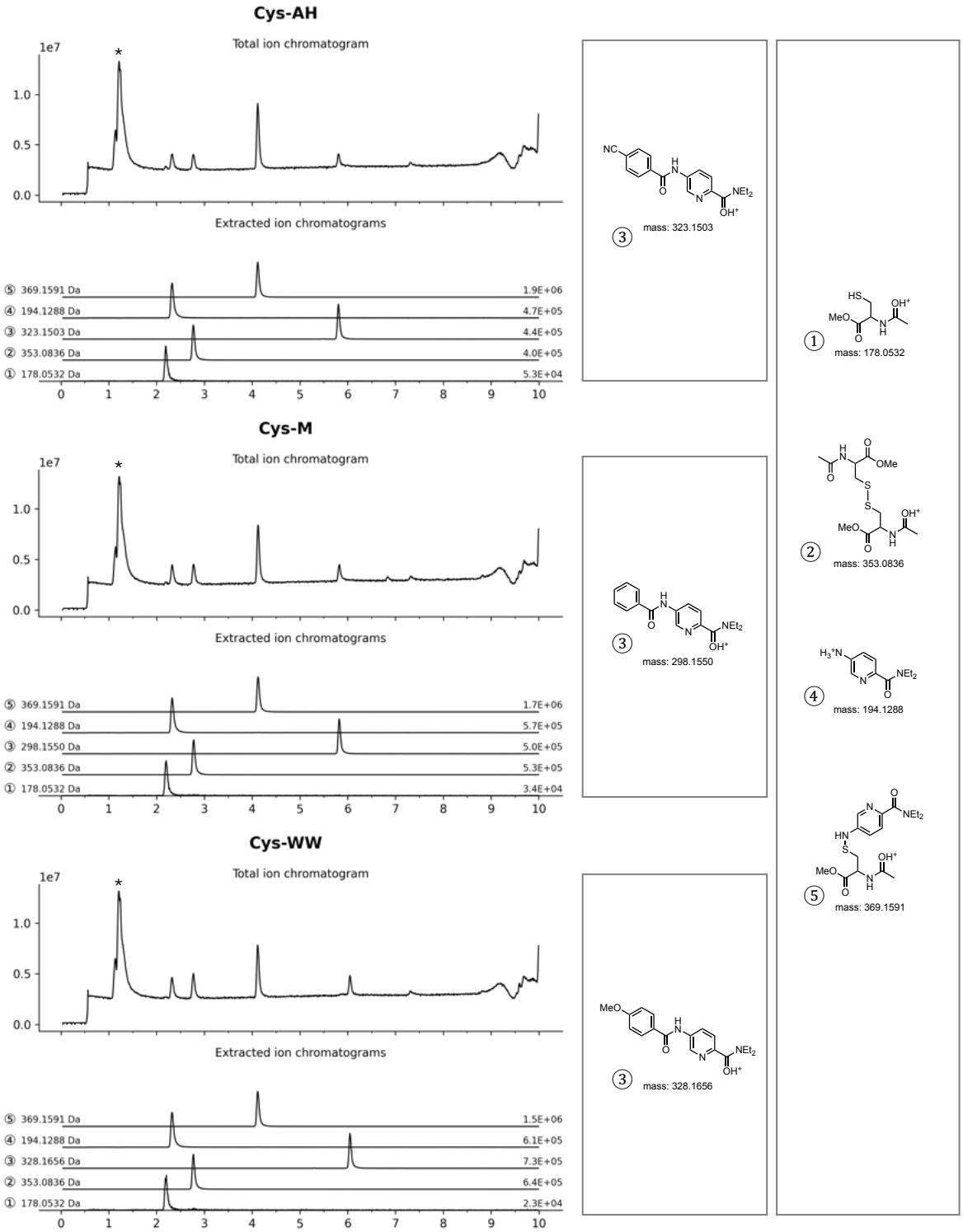


Figure S18. Reactions of Ac-Cys-OMe with oxaziridines **AH**, **M**, and **WW**.

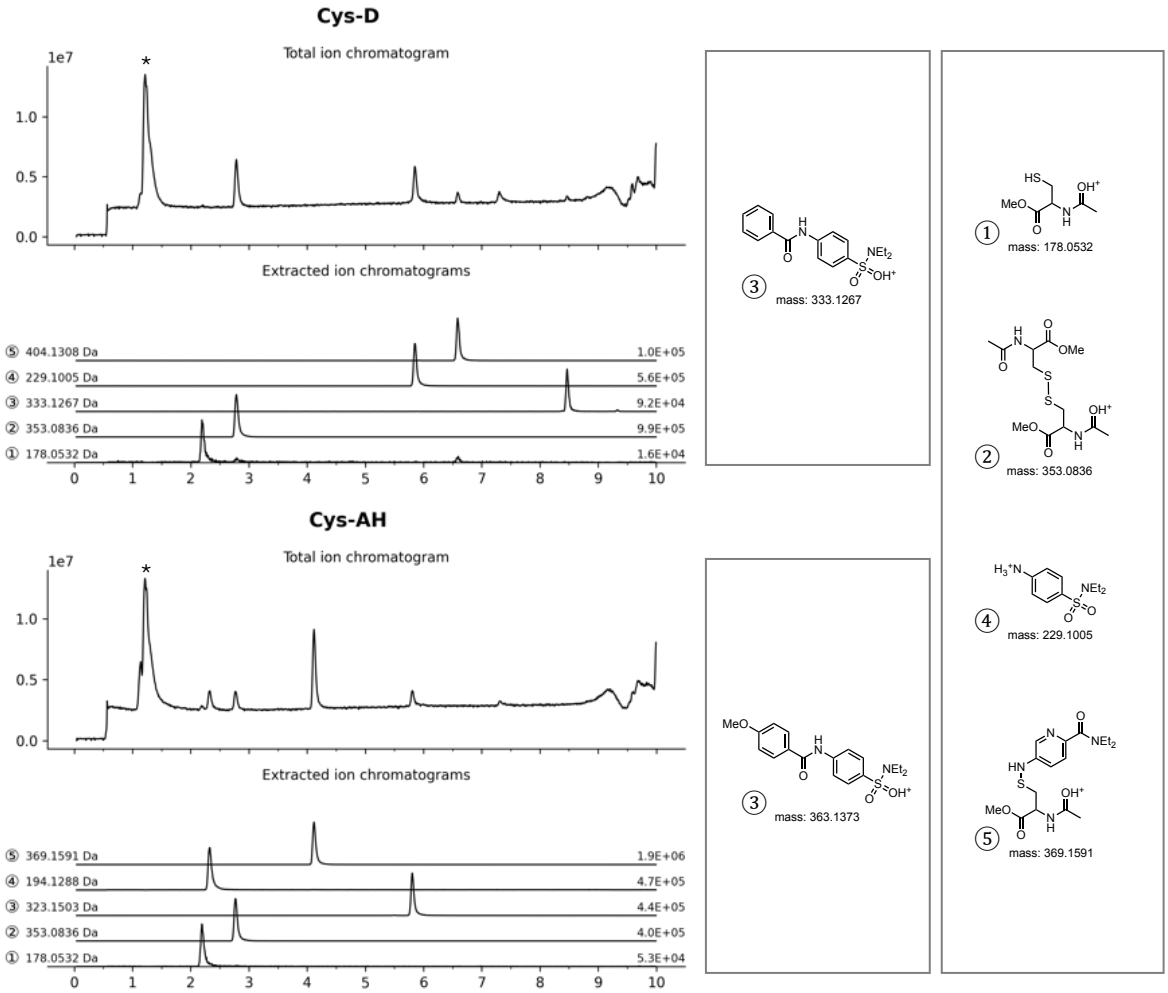


Figure S19. Reactions of Ac-Cys-OMe with oxaziridines **D** and **AH**.

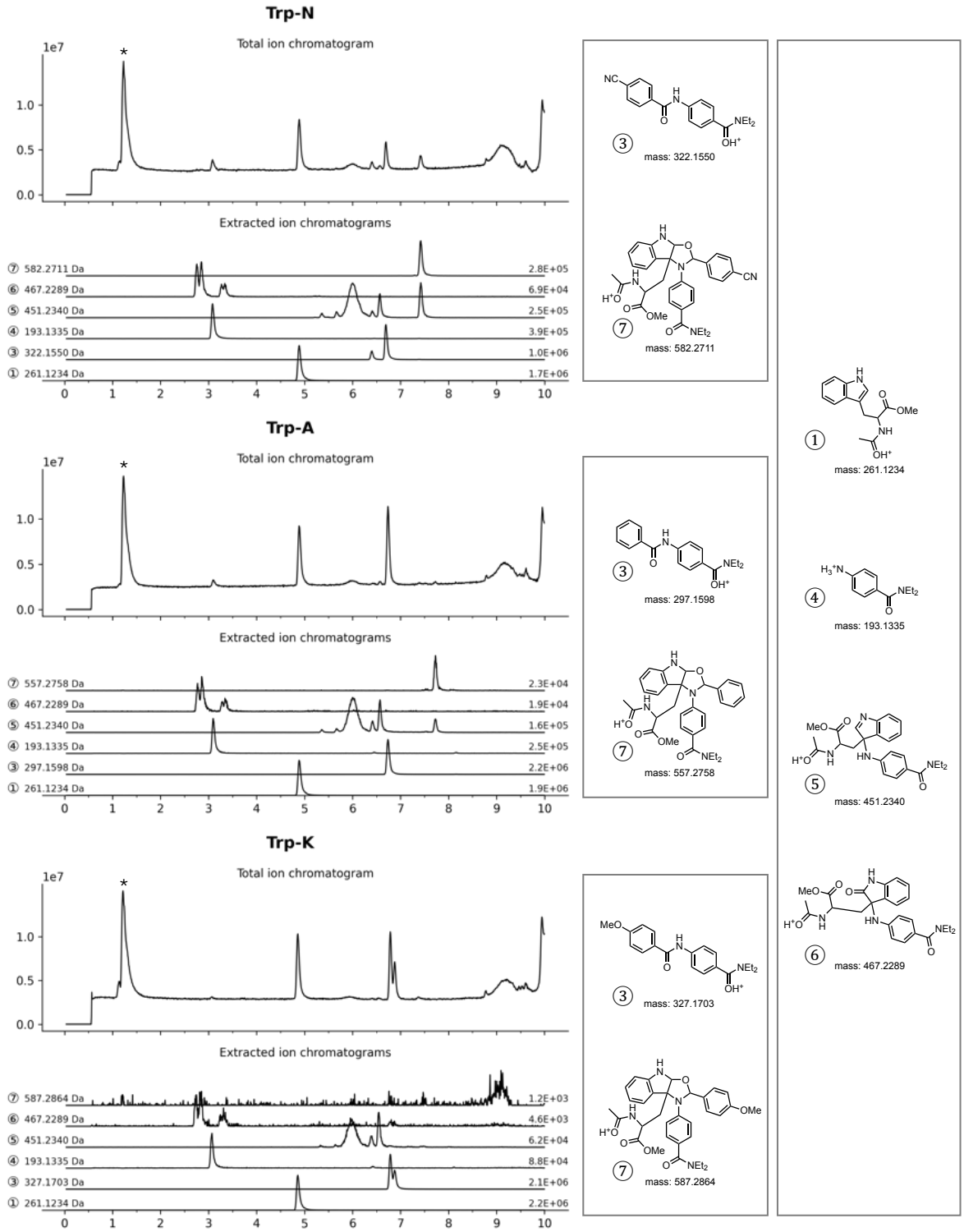


Figure S20. Reactions of Ac-Trp-OMe with oxaziridines **N**, **A**, and **K**.

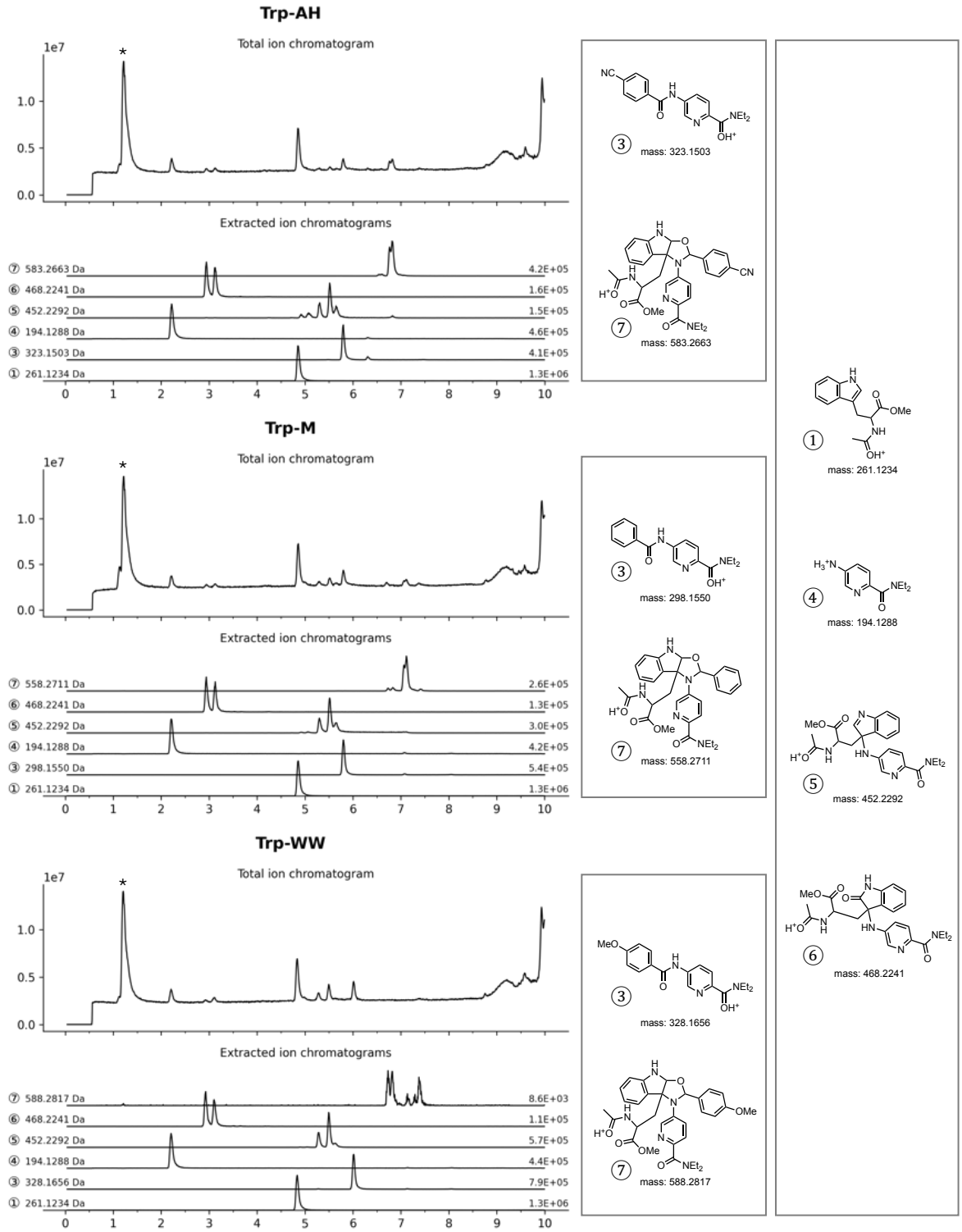


Figure S21. Reactions of Ac-Trp-OMe with oxaziridines **AH**, **M**, and **WW**.

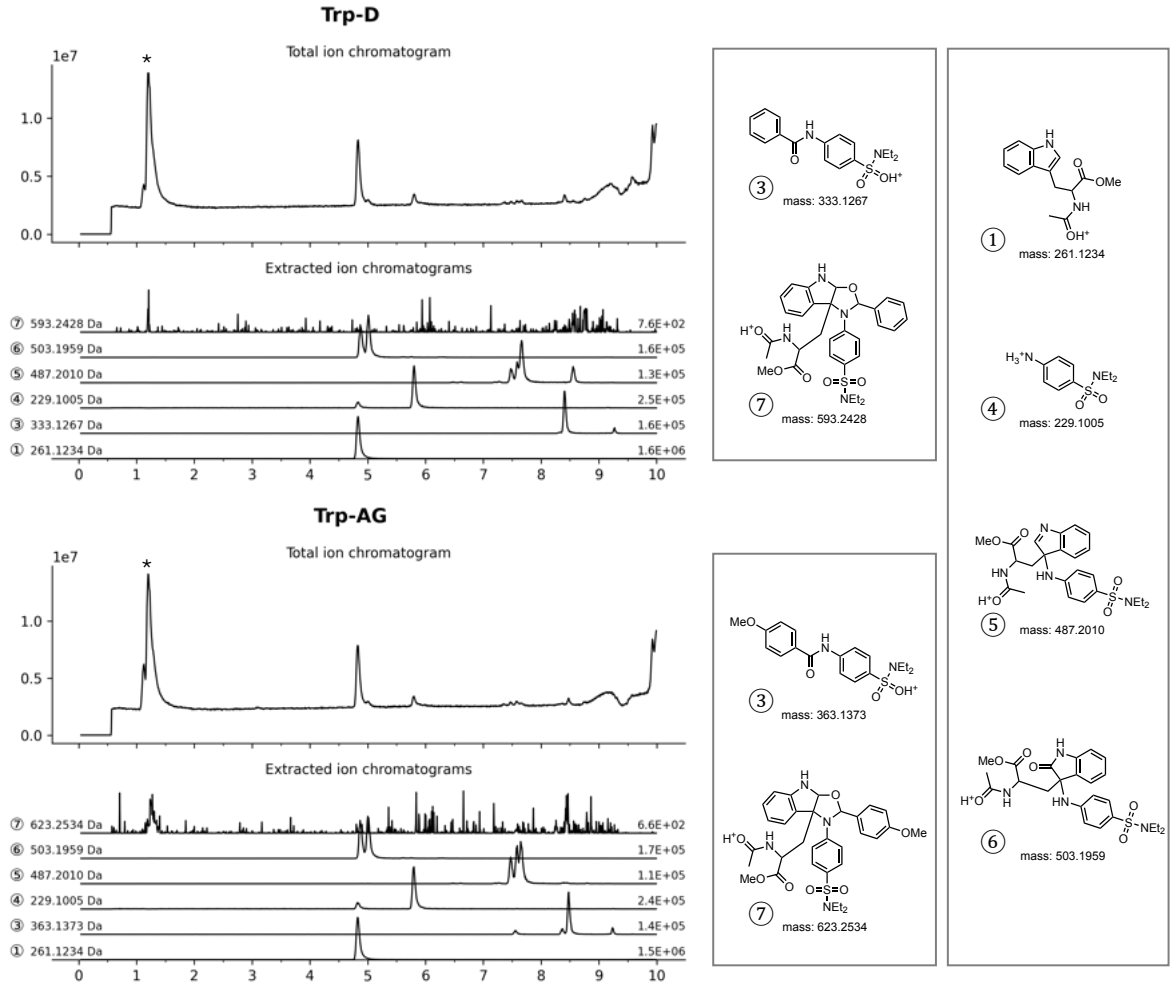


Figure S22. Reactions of Ac-Trp-OMe with oxaziridines **D** and **AG**.

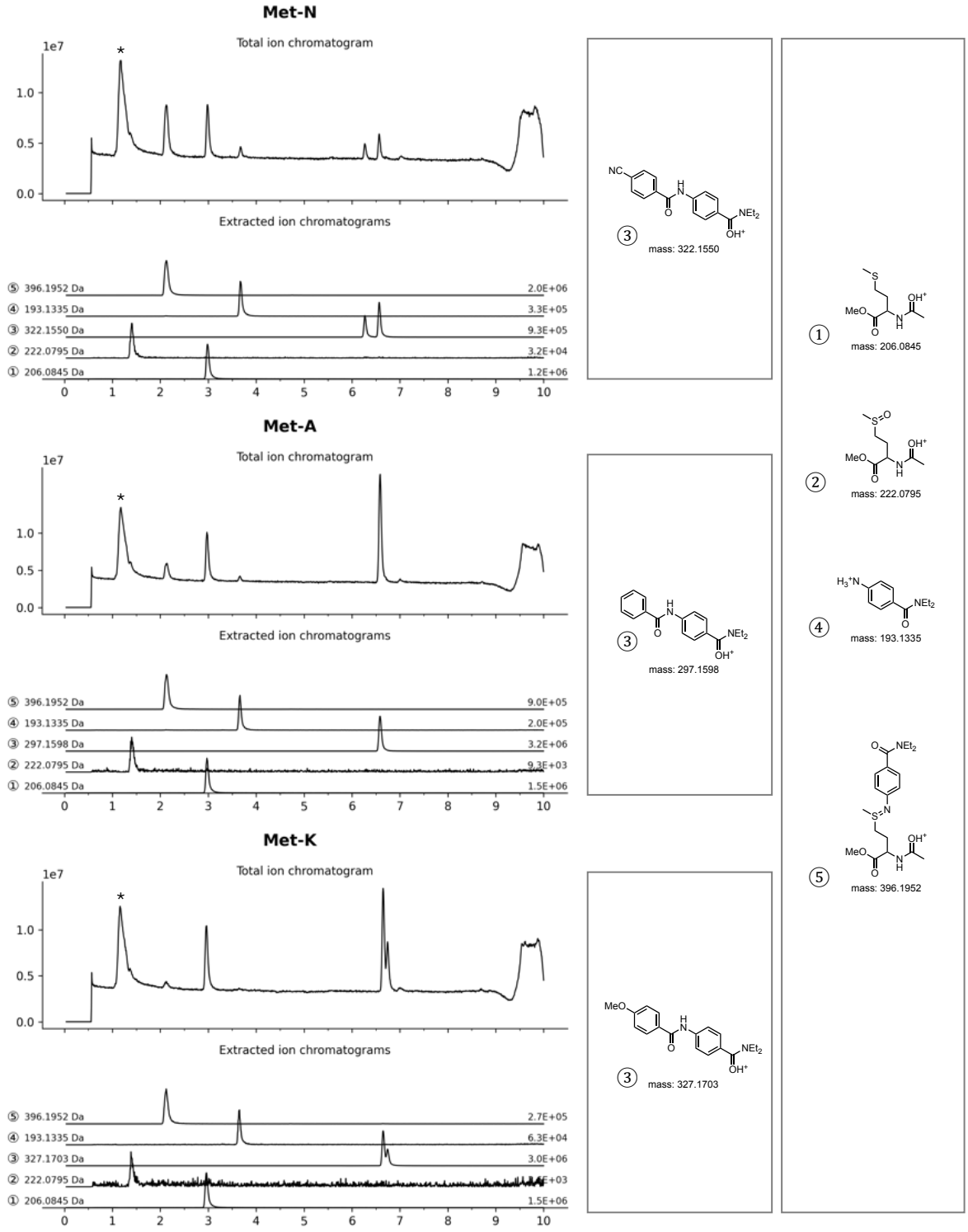


Figure S23. Reactions of Ac-Met-OMe with oxaziridines **N**, **A**, and **K**.

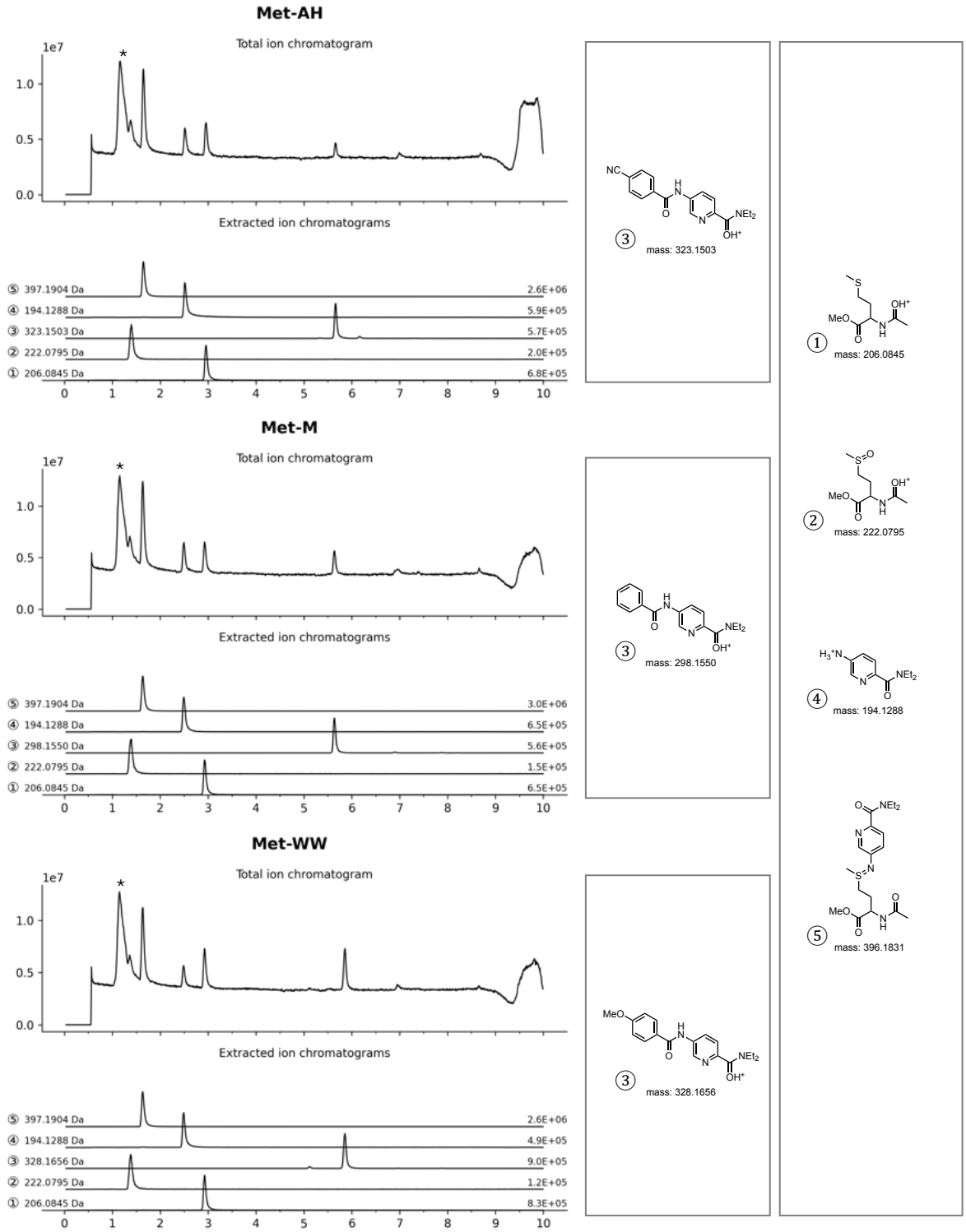


Figure S24. Reactions of Ac-Met-OMe with oxaziridines **AH**, **M**, and **WW**.

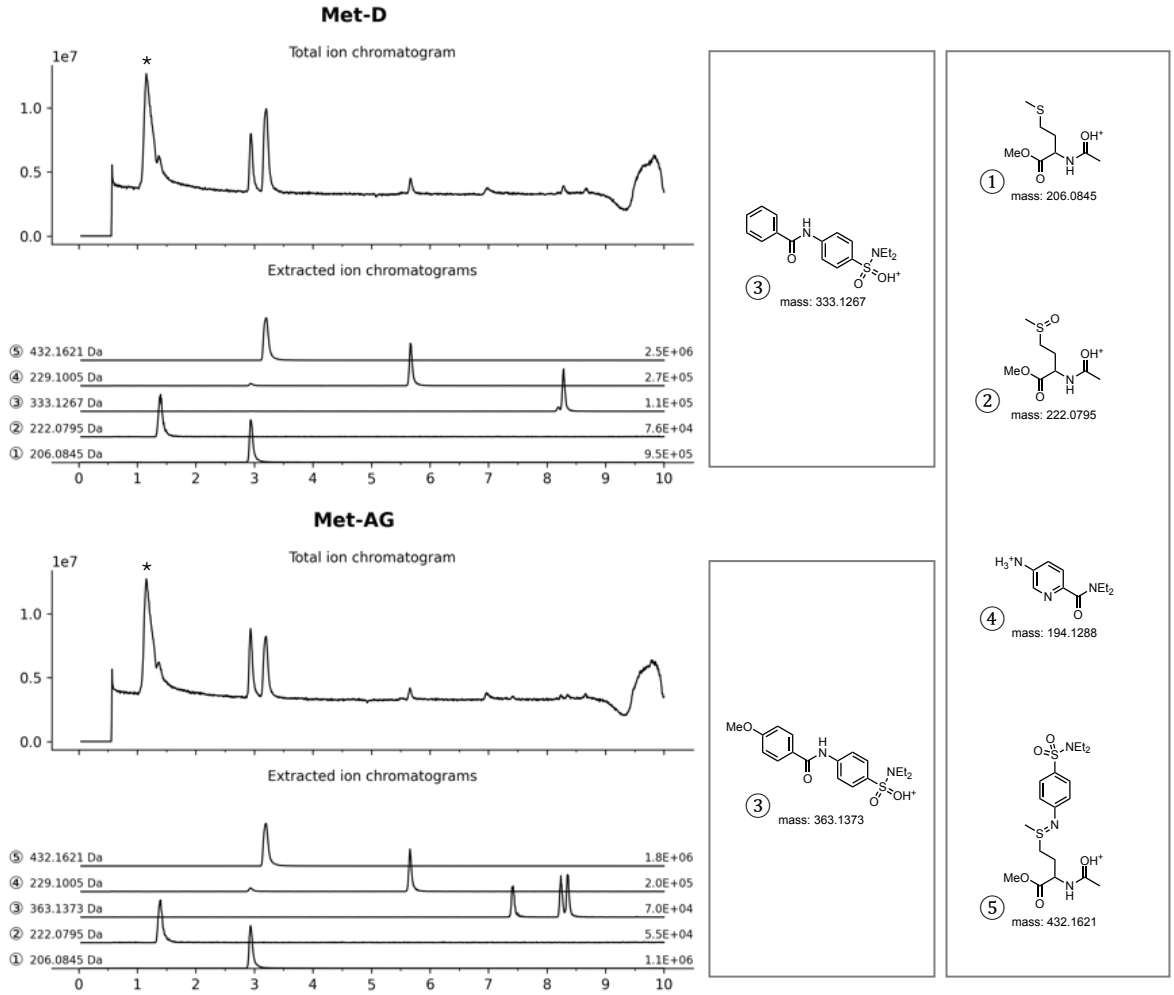


Figure S25. Reactions of Ac-Met-OMe with oxaziridines **D** and **AH**.

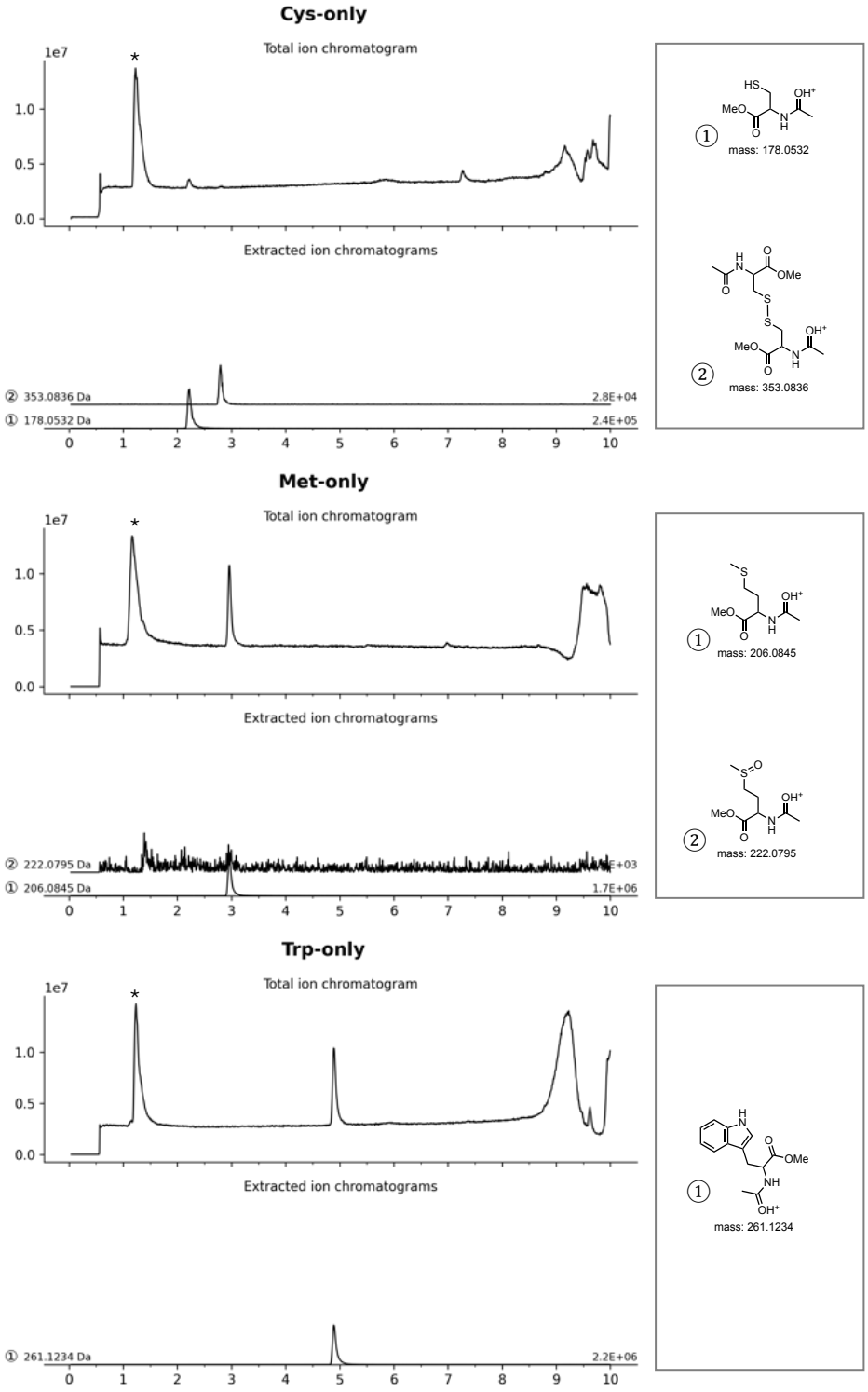


Figure S26. Solutions of amino acids only.

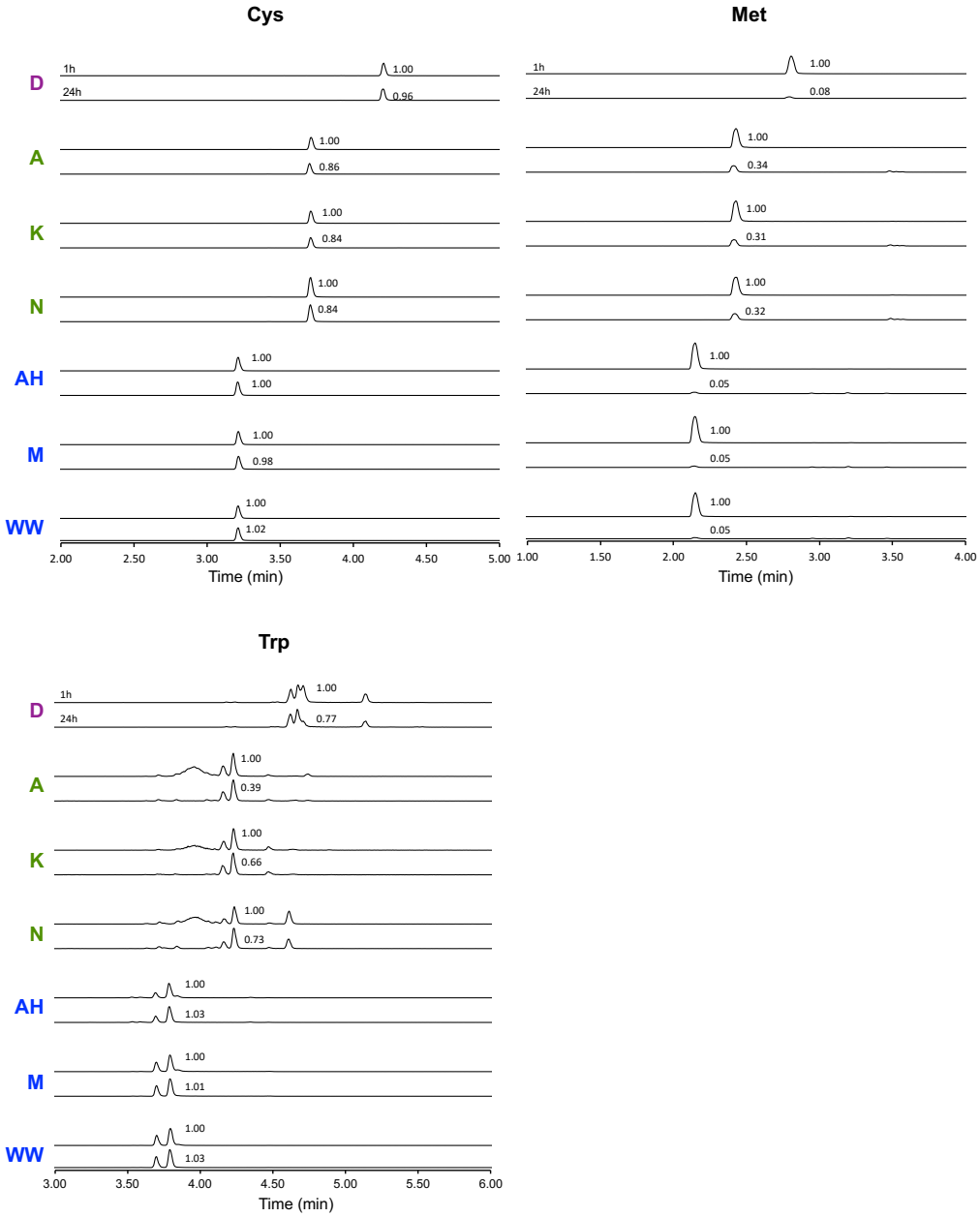


Figure S27. HPLC-MS traces of nitrone reactions with free amino acids. Traces are extracted chromatograms of ions corresponding to the N-transfer modification products. Two timepoints are given for each nitrone, the ~1 h (top) and ~24 h (bottom). The fractions are the sum of the integrals of all the major peaks in that trace, normalized to the ~1 h timepoint. Chromatography used the 50 mm column HPLC method outlined earlier.

Stability of *N*-pyridyl sulfenamide product

Low concentration protocol (10 eq, 0.5 mM): *N*-Ac *C*-OMe cysteine (50 μM) was treated with irradiated nitrone **WW** (50 μM) for 30 min at rt in phosphate buffered water (pH 7.0), then reductants (500 μM) were added and the reaction mixtures were incubated at rt for the indicated amount of time prior to LC-MS analysis.

High concentration protocol (20 eq, 10 mM): *N*-Ac *C*-OMe cysteine (500 µM) was treated with irradiated nitrone **WW** (500 µM) for 45 min at rt in phosphate buffered water (100 mM, pH 7.0), then DTT (10 mM) was added and the reaction mixture was incubated at 37 °C for 1 h, prior to being diluted in water 1:100 and analyzed by LC-MS.

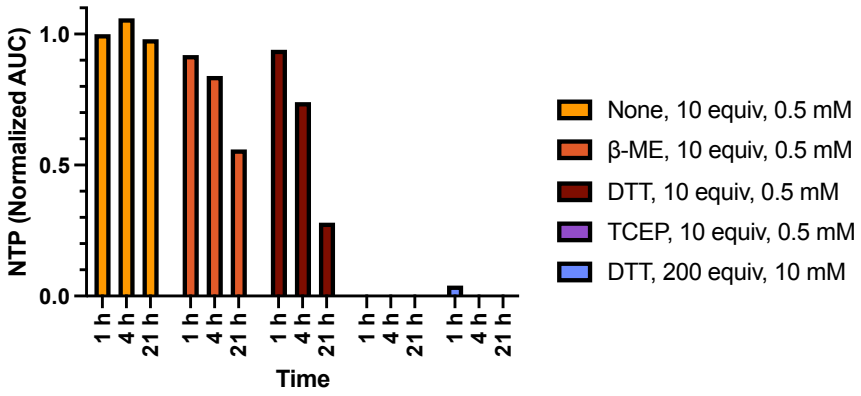


Figure S28. Stability of sulfenamide product over time at rt with 10 or 200 equiv of reducing agents.

Reaction of oxaziridine WW with Cys at varying ratios

Oxaziridine WW was added from a 2.0 mM stock solution of the nitrone in ACN that was irradiated for 10 min at 370 nm. Cys was added from a 2.0 mM stock solution in water. Final reactions contained nitrone and cysteine at concentrations indicated, 10 mM sodium phosphate buffer at pH 7.0. After addition of reagents the reaction was incubated at room temperature for 10 min, then diluted 1-in-10 in 10 mM sodium phosphate buffer (pH 7.0) and injected on an HPLC-MS. All water used for the experiment was purged with nitrogen gas for at least 15 min. Three EIC were integrated: cysteine starting materials (CysS-H at 178.0532 Da), oxidized cystine disulfide ((CysS)2 at 353.0836 Da), and conjugation product of cysteine and oxaziridine (CysS-(NHAr) at 369.1591 Da).

Table S2. Impact of reagent molar ratios on the conjugation of Cys with oxaziridine WW.

	Concentration (µM)		EIC Peak AUC			
Row	Cys	Nit WW	CysS-H	(CysS)2	CysS-(NHAr)	Cys Conv.
1	10	10	6,718	899,117	656,885	94%
2	10	50	0	360,766	1,583,302	100%
3	10	100	0	248,463	1,694,622	100%
4	50	10	450,687	1,781,492	493,924	24%
5	100	10	689,235	2,019,634	763,380	11%
6	10	0	120,659	60,483	0	-
7	10	0	105,852	93,312	0	-
8	10	0	107,318	122,483	465	-
9	50	0	595,871	217,554	319	-
10	100	0	772,304	212,906	0	-

Impact of electron density on N-/O-transfer ratio

N-/O-transfer ratios to methionine were quantified by ^{1}H NMR. A stock solution of *N*-Ac *C*-OMe methionine and dimethyl sulfone ($DMSO_2$, internal standard) in methanol-d_4 was used to dissolve each nitrone. An equivalent volume of potassium phosphate buffer (60 mM, pD 7.0 in D_2O) was added to the solution. The final reaction conditions were: 600µL total volume of 50/50 methanol-d_4/water-d_2, 30 mM PB, pD 7.0, 10 mM methionine, 10 mM nitrone, 3 mM $DMSO_2$. The solution was then irradiated at 370 nm for 5 min prior to quantification of N-/O-transfer ratios by ^{1}H NMR.

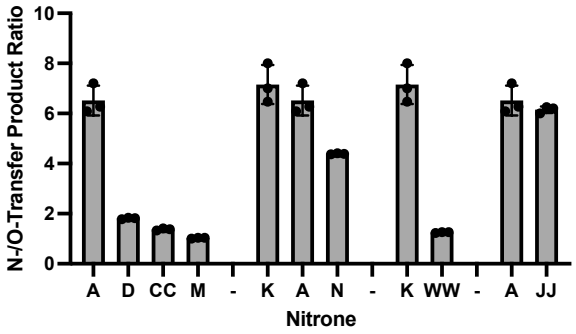


Figure S29. Measured N-/O-transfer ratios to methionine for nitrones measured by ^{1}H NMR in 50:50 methanol-d_4/water-d_2 solvent phosphate buffered to pD 7.0. Three replicates of each measurement are shown, error bars indicate standard deviations.

N-transfer product structures

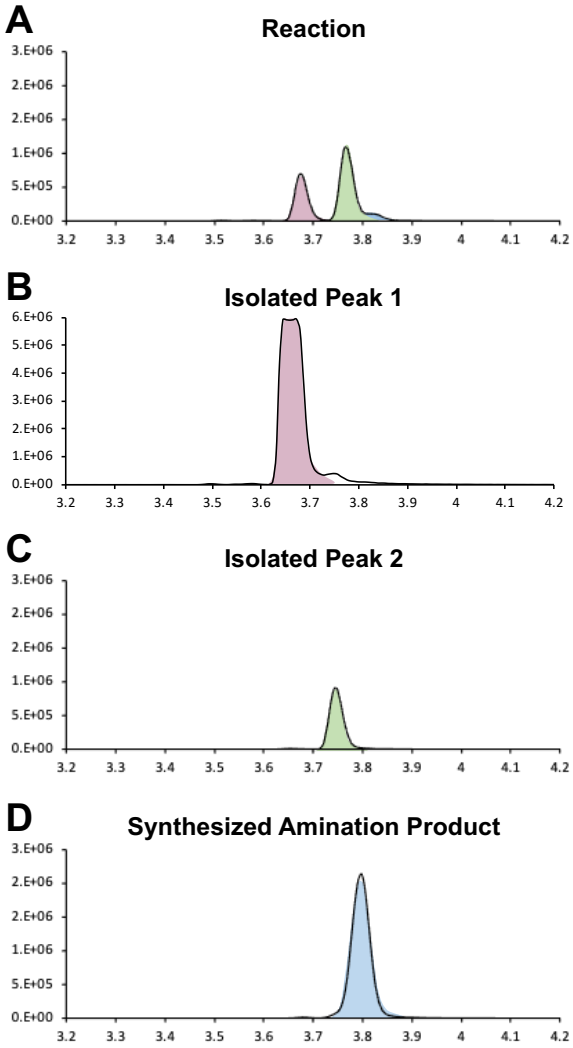


Figure S30. HPLC traces of the tryptophan labeling with nitrone **WW**. (**A**) Reaction mixture, (**B**) Isolated product peak 1, (**C**) isolated product peak 2, (**D**) synthesized amination product. All traces are EIC of 452.2292 Da ± 20 ppm corresponding to the $[M+H]^+$ masses.

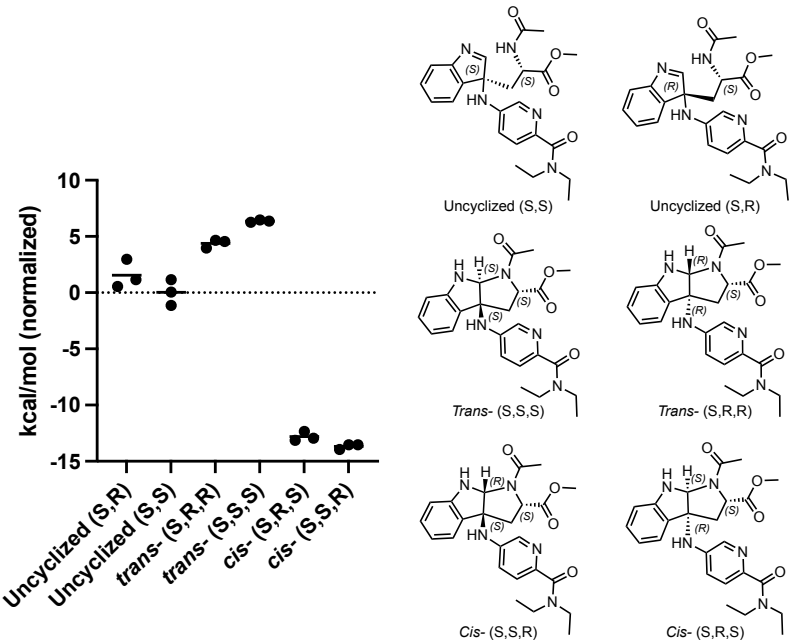
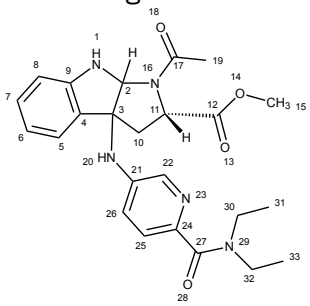
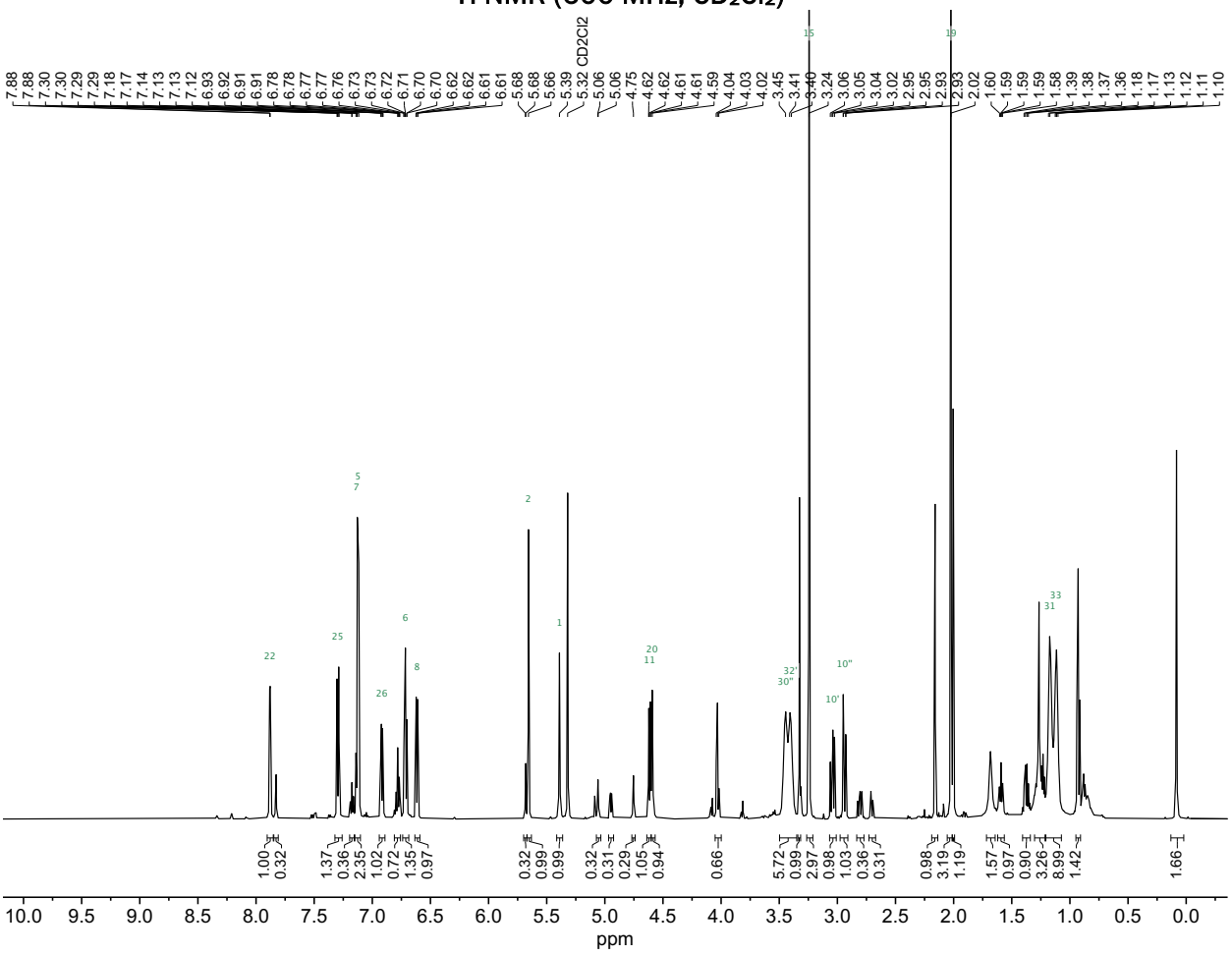


Figure S31. DFT calculated energies of possible uncyclized and cyclized product structures. Three lowest energy conformers (Maestro) were geometry optimized by DFT calculations (M062x/def2tzvp level of theory with SCRF SMD water solvent).

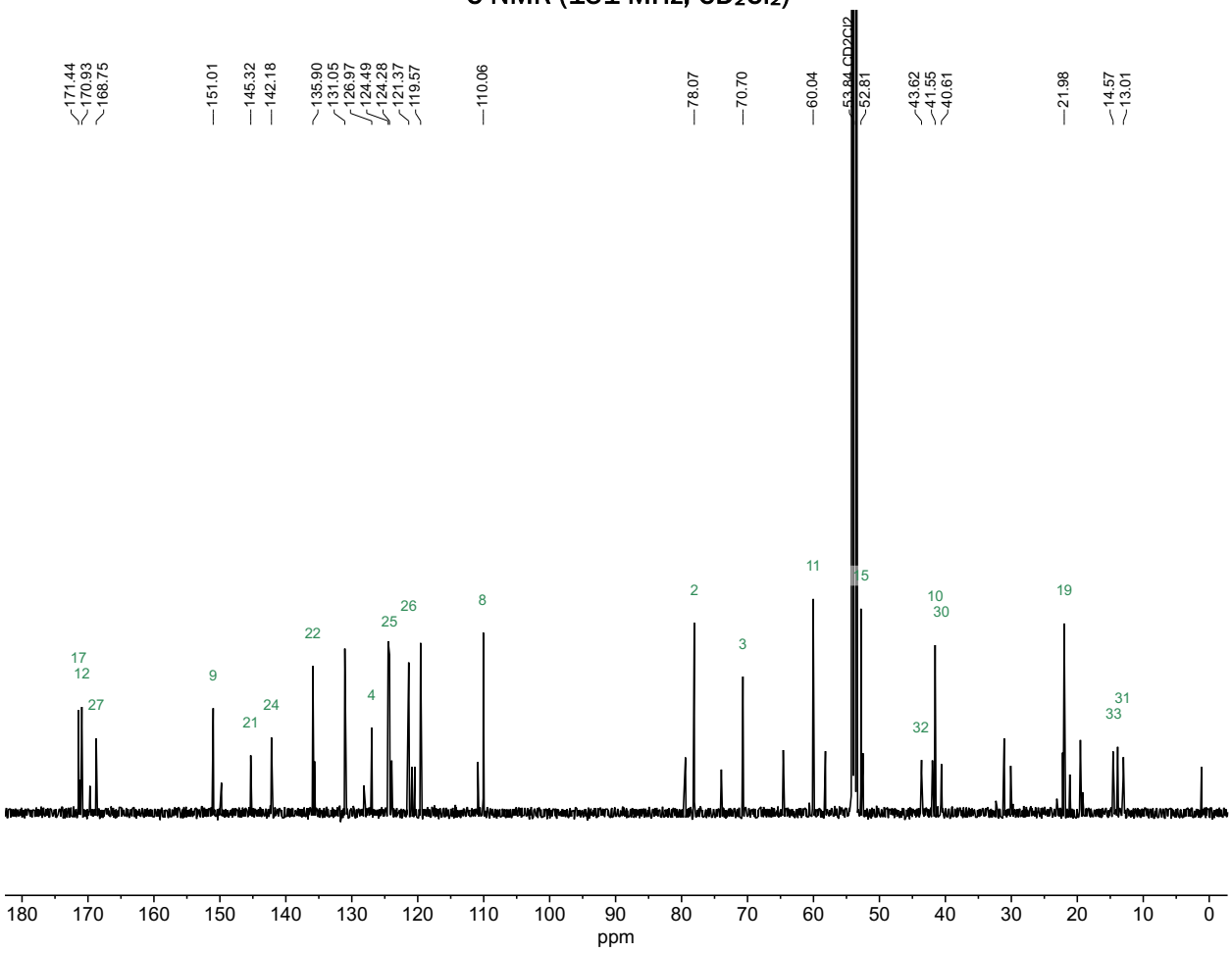
Numbering scheme for tryptophan N-transfer product isolated peak 1 and corresponding NMRs:



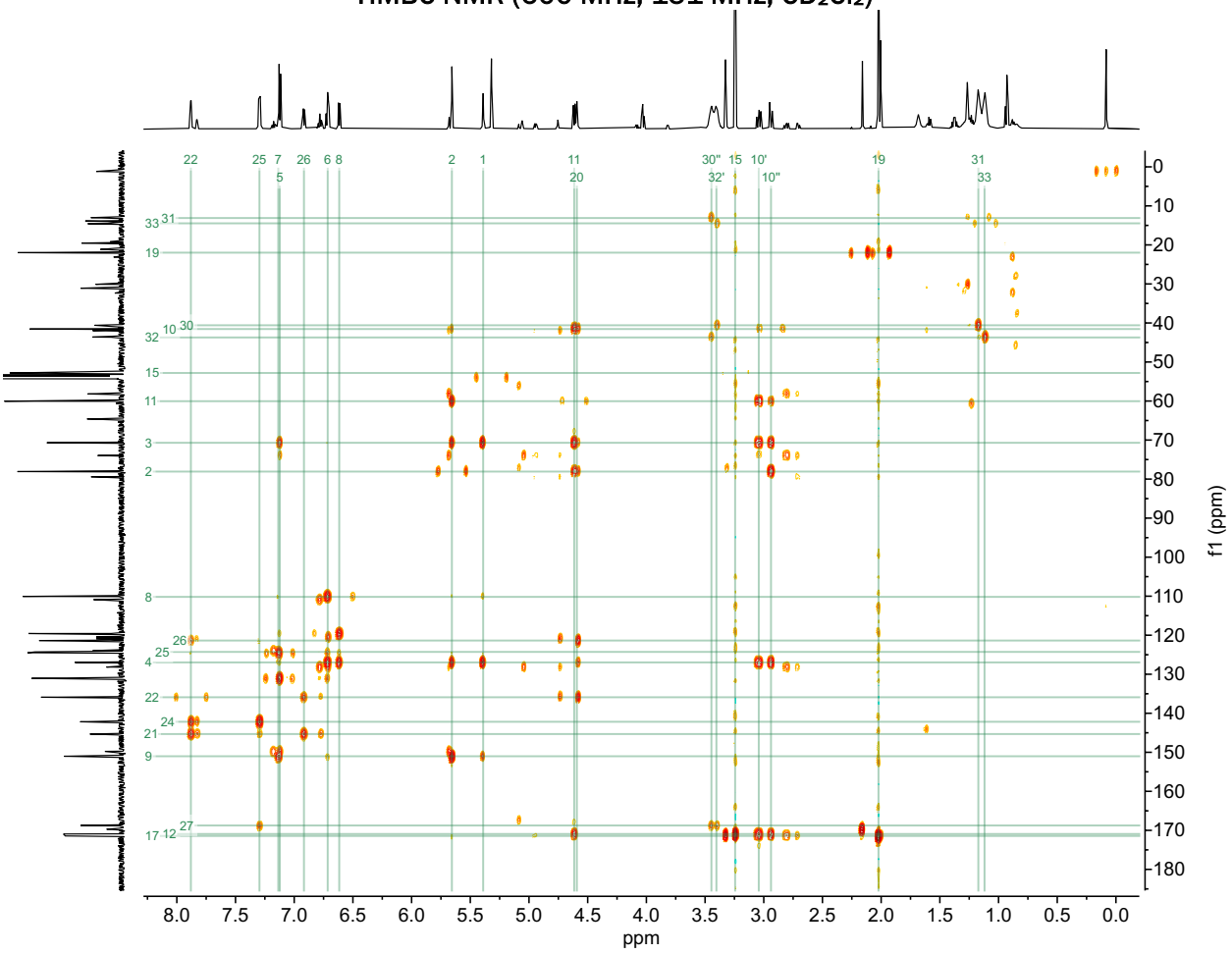
^{1}H NMR (600 MHz, CD_2Cl_2)



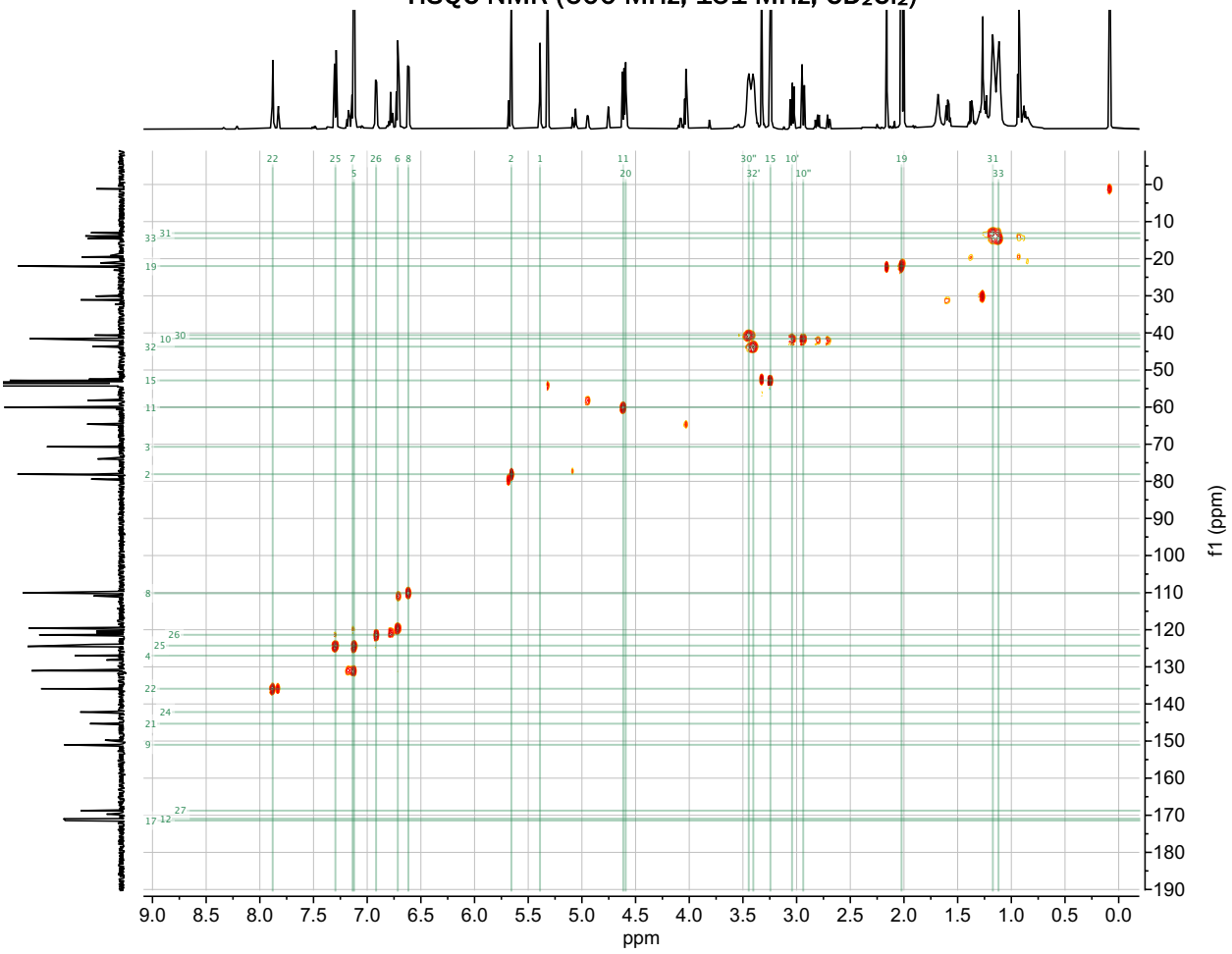
^{13}C NMR (151 MHz, CD_2Cl_2)



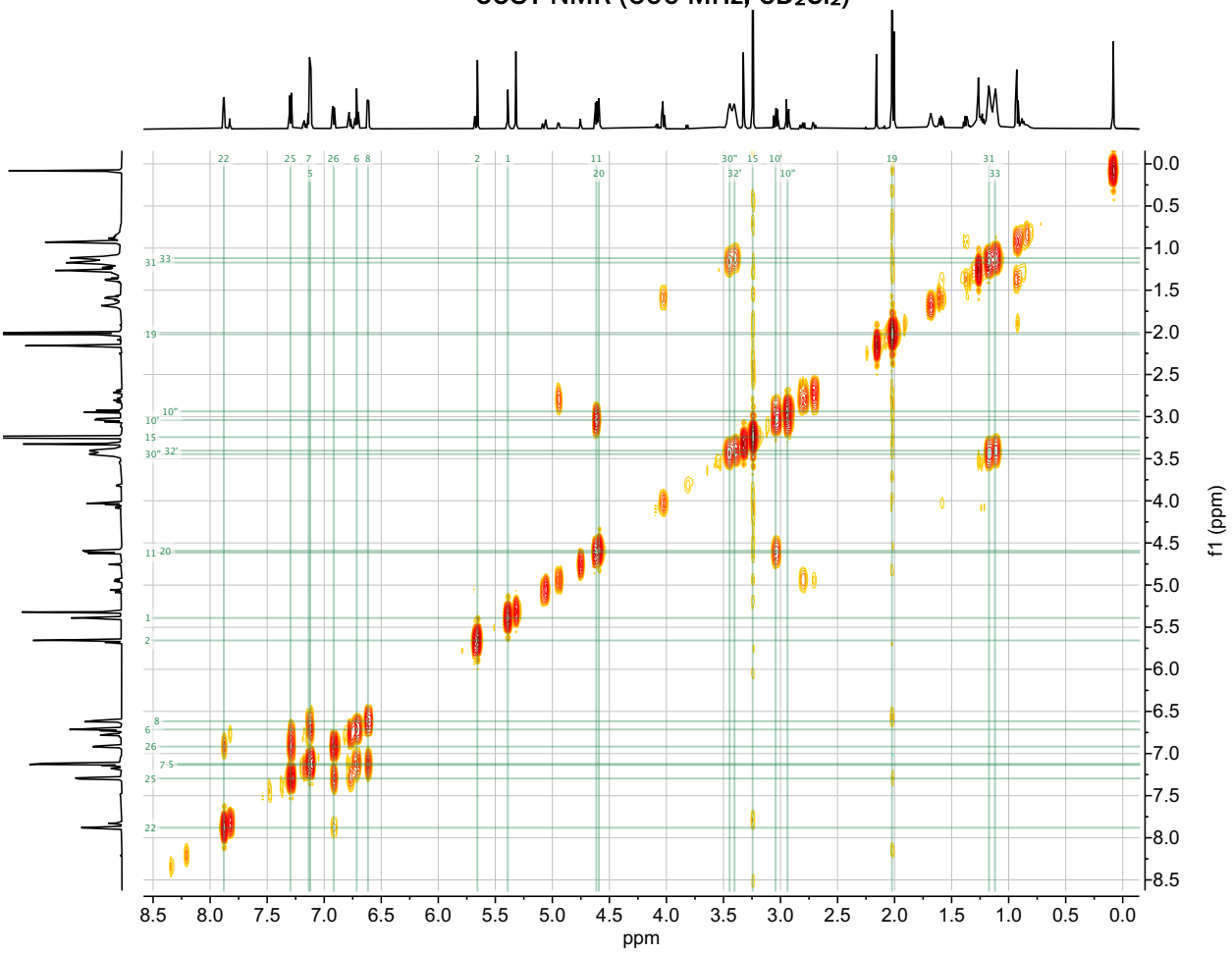
HMBC NMR (600 MHz, 151 MHz, CD_2Cl_2)



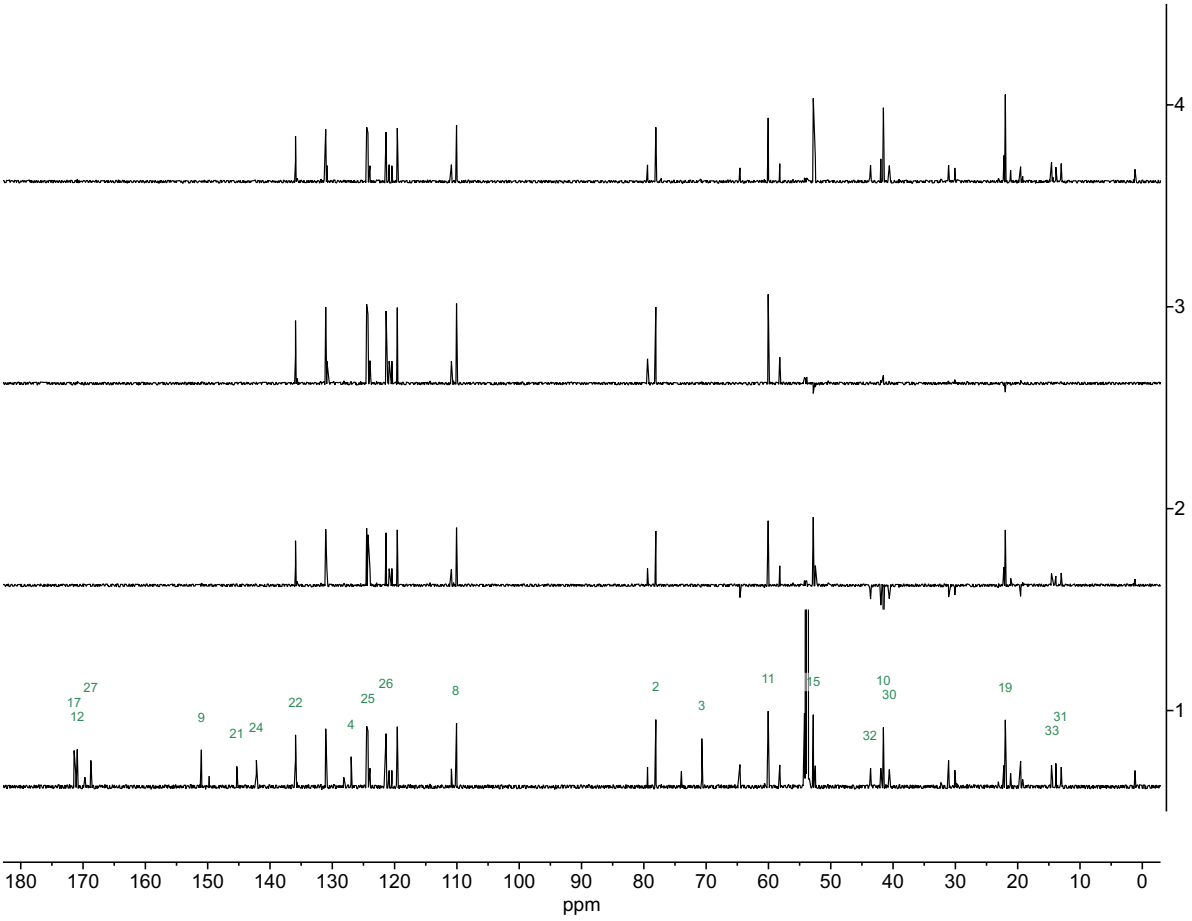
HSQC NMR (600 MHz, 151 MHz, CD_2Cl_2)



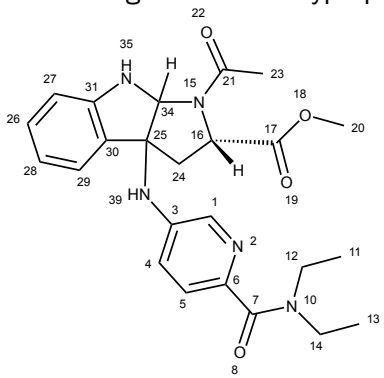
COSY NMR (600 MHz, CD_2Cl_2)



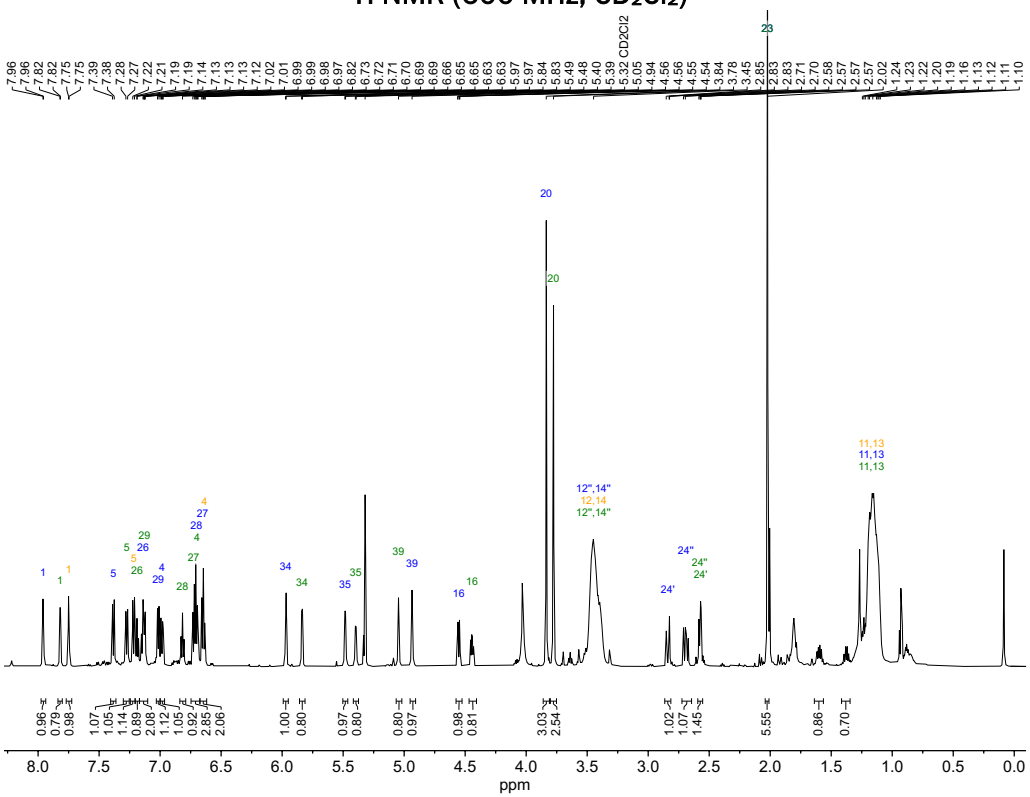
DEPT-45, DEPT-90, DEPT-135 NMR (151 MHz, CD_2Cl_2)



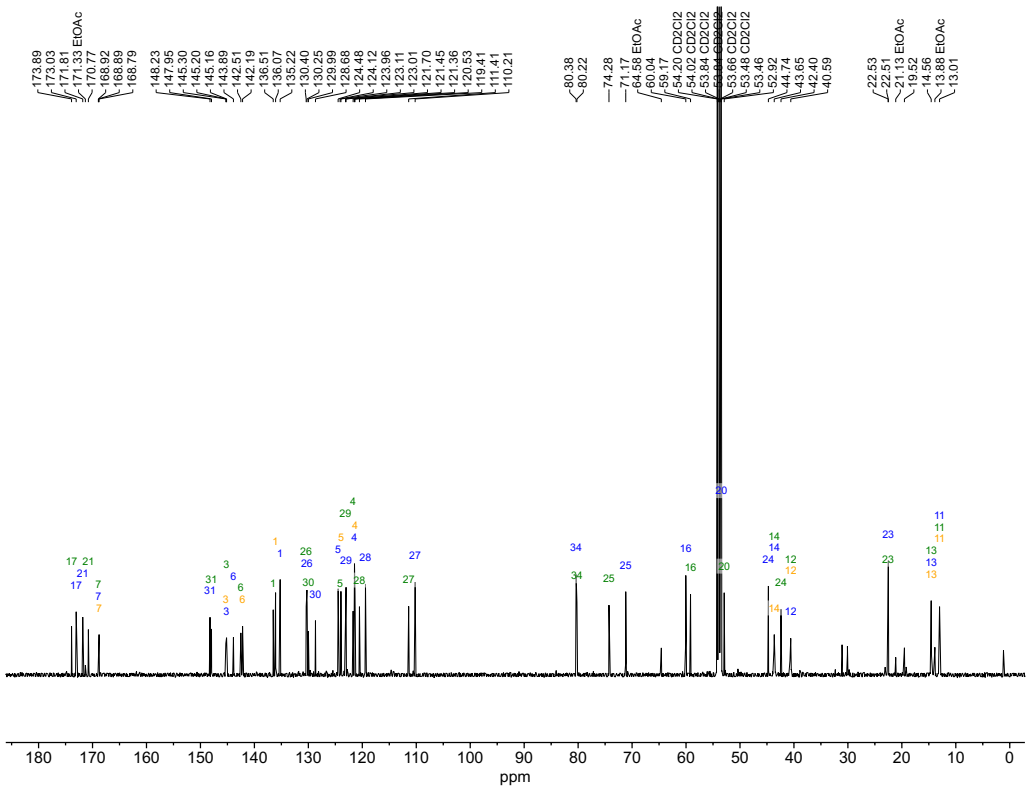
Numbering scheme for tryptophan N-transfer product isolated peak 2 and corresponding NMRs:



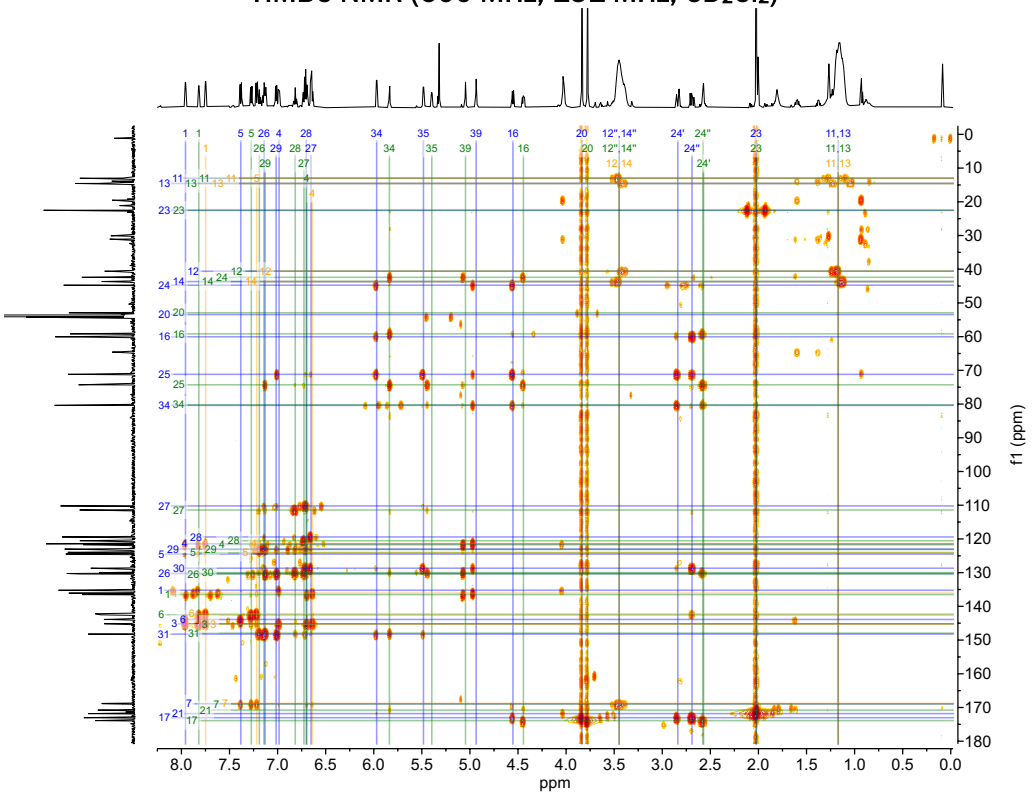
^{1}H NMR (600 MHz, CD_2Cl_2)



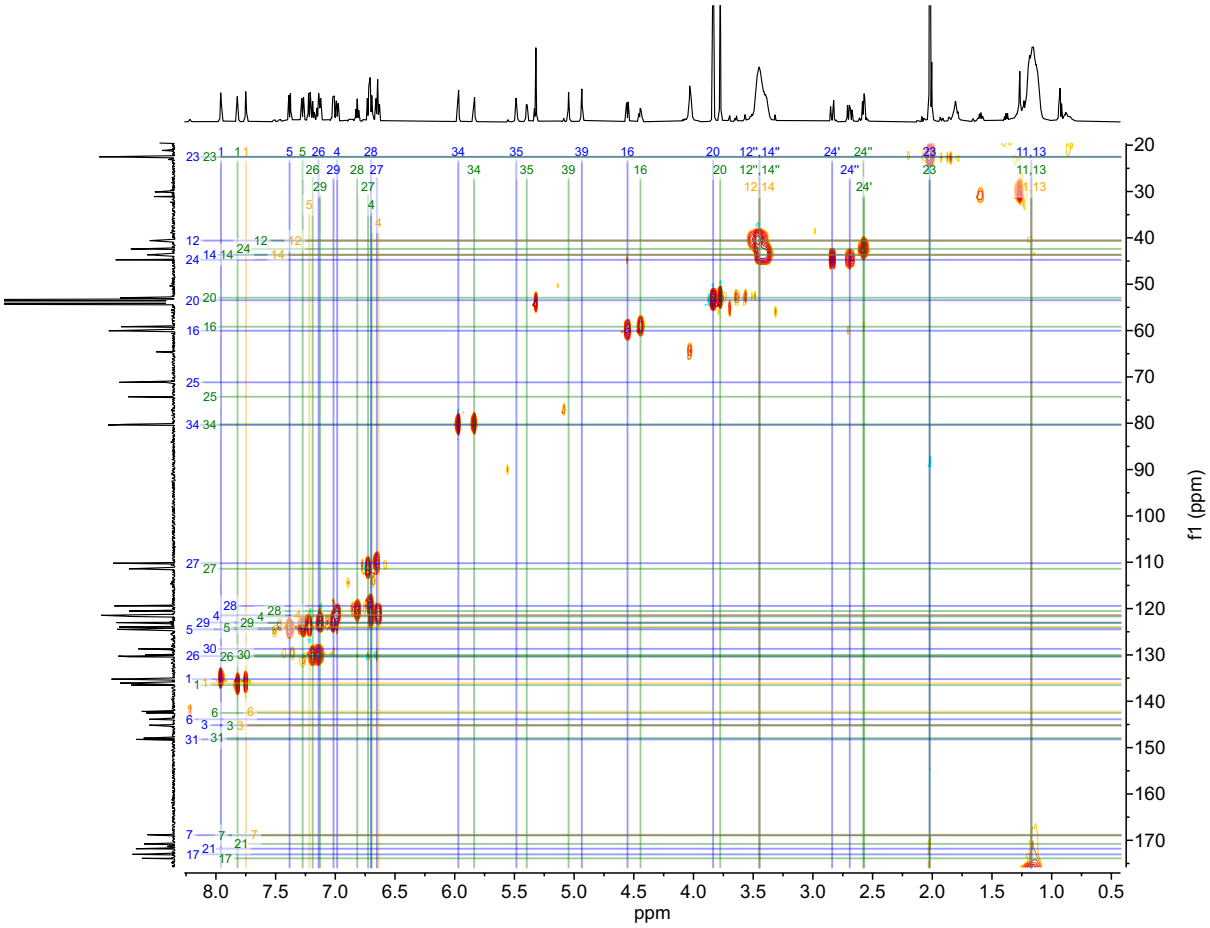
^{13}C NMR (151 MHz, CD_2Cl_2)



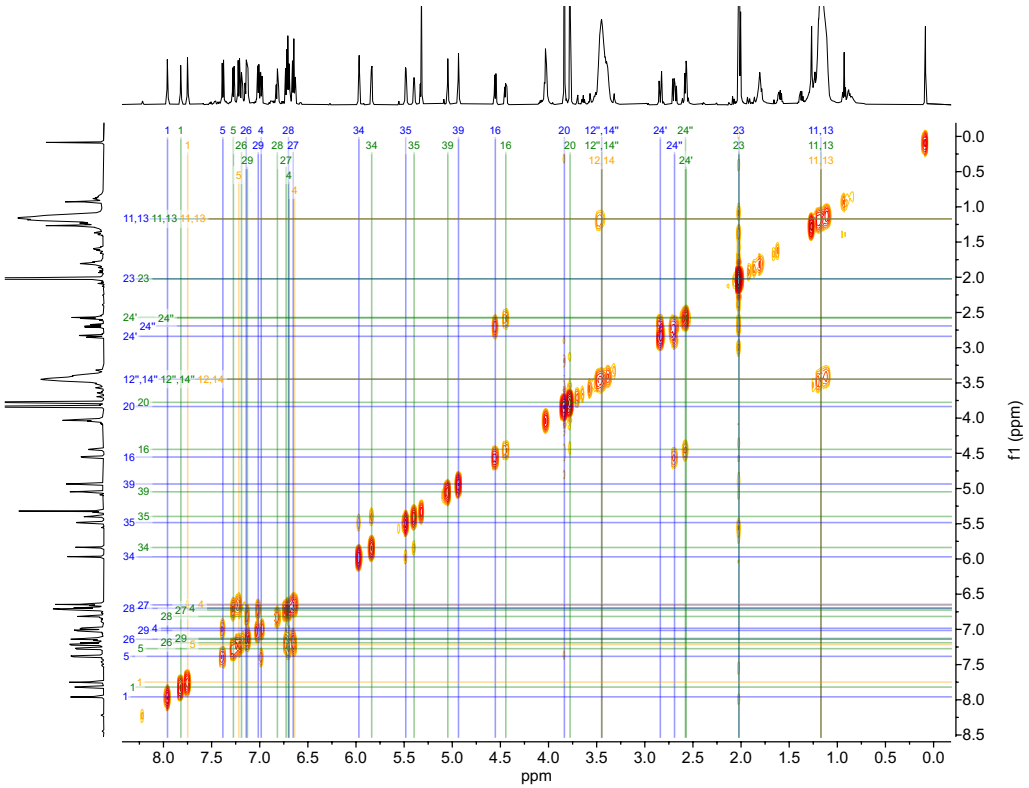
HMBC NMR (600 MHz, 151 MHz, CD_2Cl_2)



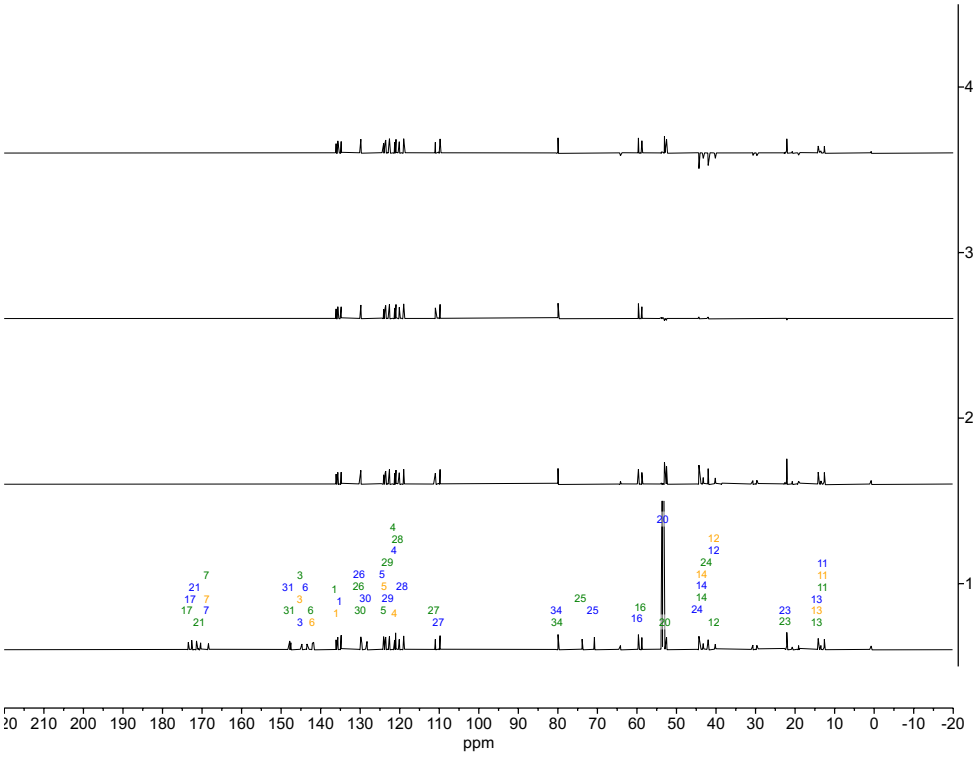
HSQC NMR (600 MHz, 151 MHz, CD_2Cl_2)

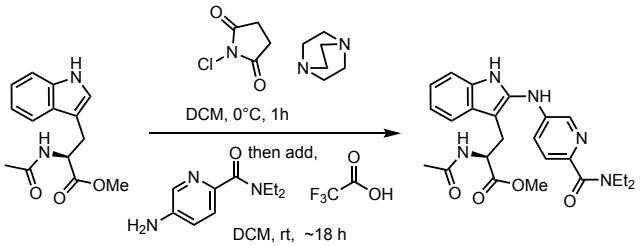


COSY NMR (600 MHz, CD_2Cl_2)



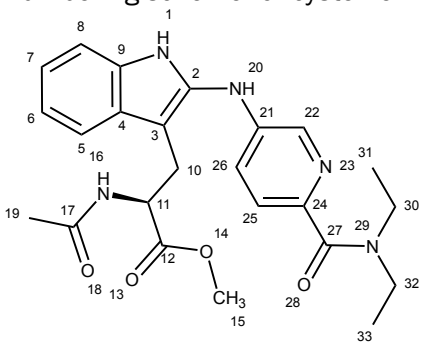
DEPT-45, DEPT-90, DEPT-135 NMR (151 MHz, CD_2Cl_2)



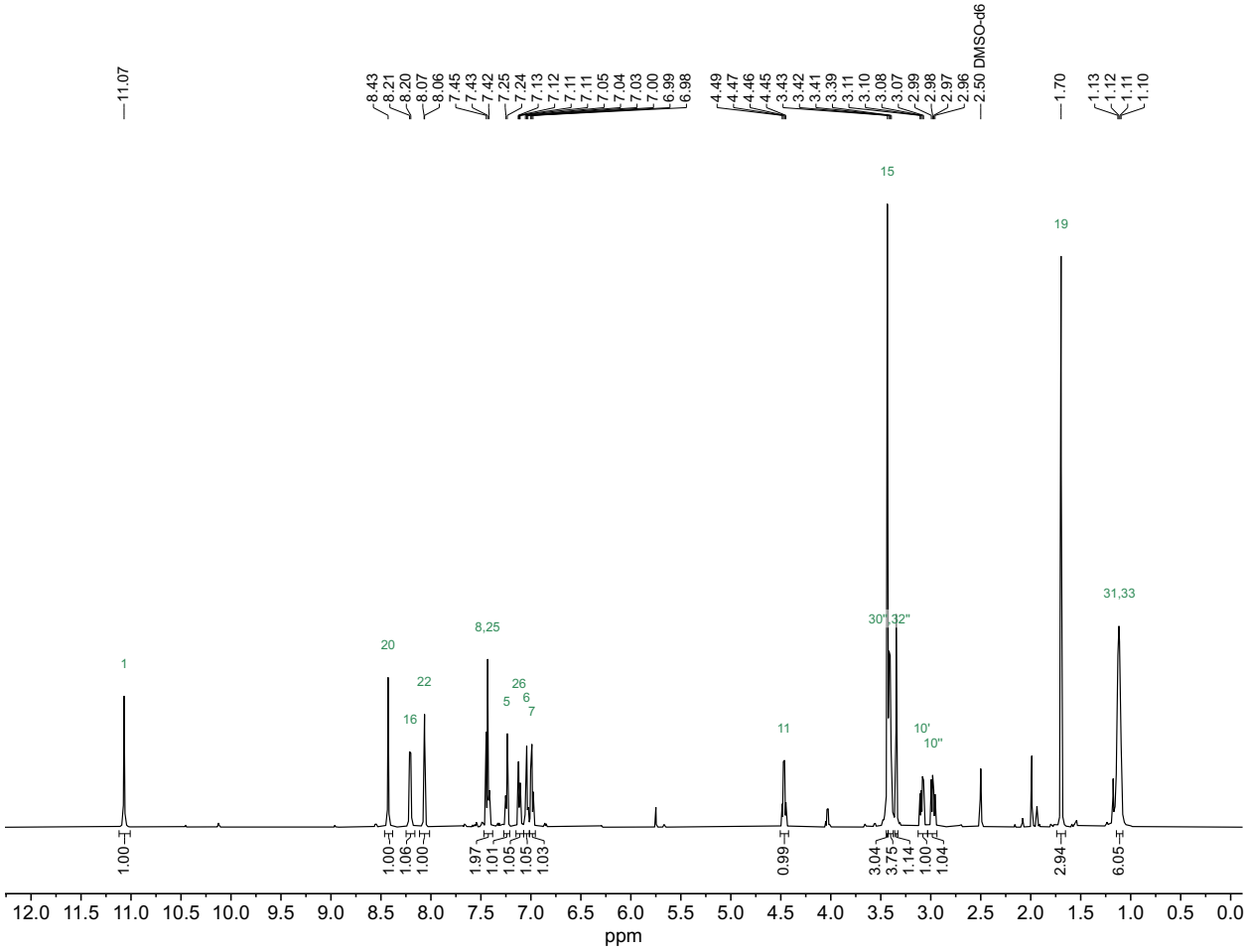


Authentic *C2*-amination product was synthesized based on a reported procedure.[4] *N*-Ac *C*-OMe tryptophan (150 mg, 0.58 mmol, 1.0 equiv) and DABCO (36 mg, 0.32 mmol, 0.55 equiv) were dissolved in anhydrous dichloromethane (10 mL), the solution was purged with nitrogen gas and cooled to 0 °C in an ice bath. *N*-Chlorosuccinimide (85 mg, 0.63 mmol, 1.1 equiv) was added as a solid and the resulting solution was stirred at 0 °C for 1 h, when full consumption of starting material was confirmed by TLC (5/45/45 methanol/ethyl acetate/dichloromethane, visualize with UV, PMA or Seebach stains). 5-Amino-*N*,*N*-diethylpicolinamide (110 mg, 0.56 mmol, 1.0 equiv) and trichloroacetic acid (24 mg, 0.14 mmol, 0.25 equiv) were added as a solution in 3 mL of anhydrous dichloromethane and the resulting reaction mixture was allowed to warm to rt and stirred overnight. The crude reaction product was purified by silica column chromatography (MeOH/EtOAc/DCM: 1/49.5/49.5 to 10/45/45). Product was obtained as a white foam (148 mg, 0.58 mmol, 57% yield).

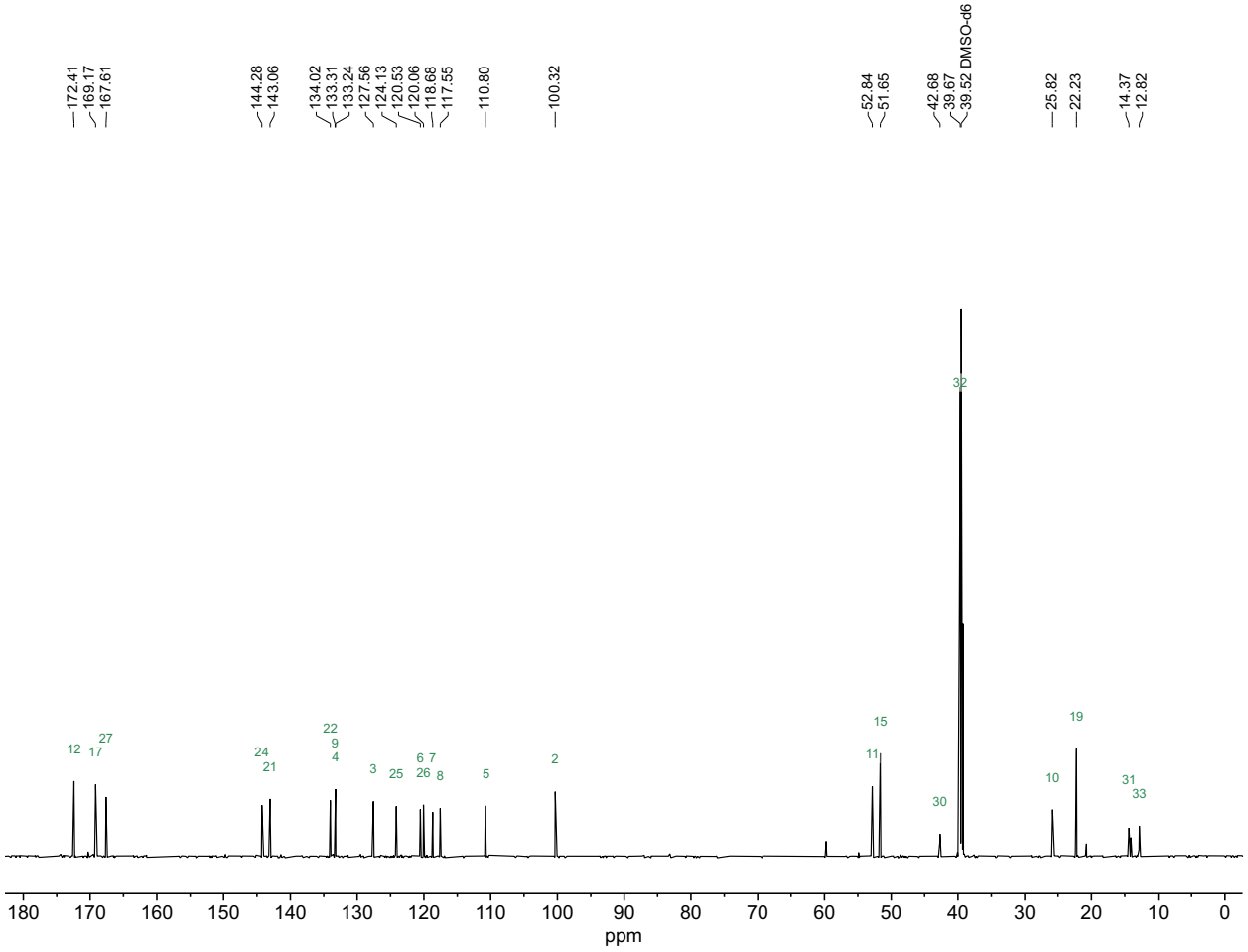
Numbering scheme for cysteine N-transfer product and corresponding NMRs:



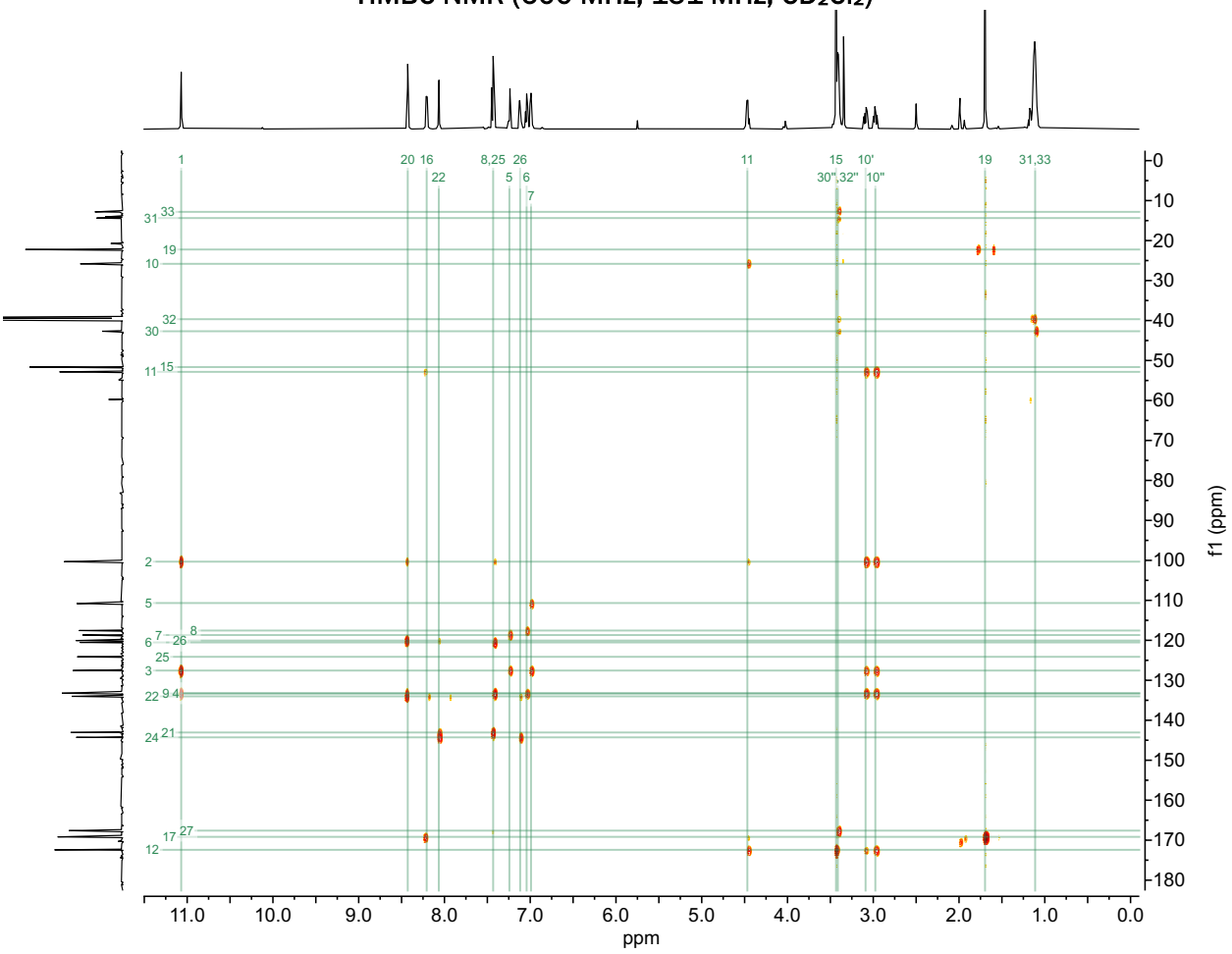
^{1}H NMR (600 MHz, CD_2Cl_2)



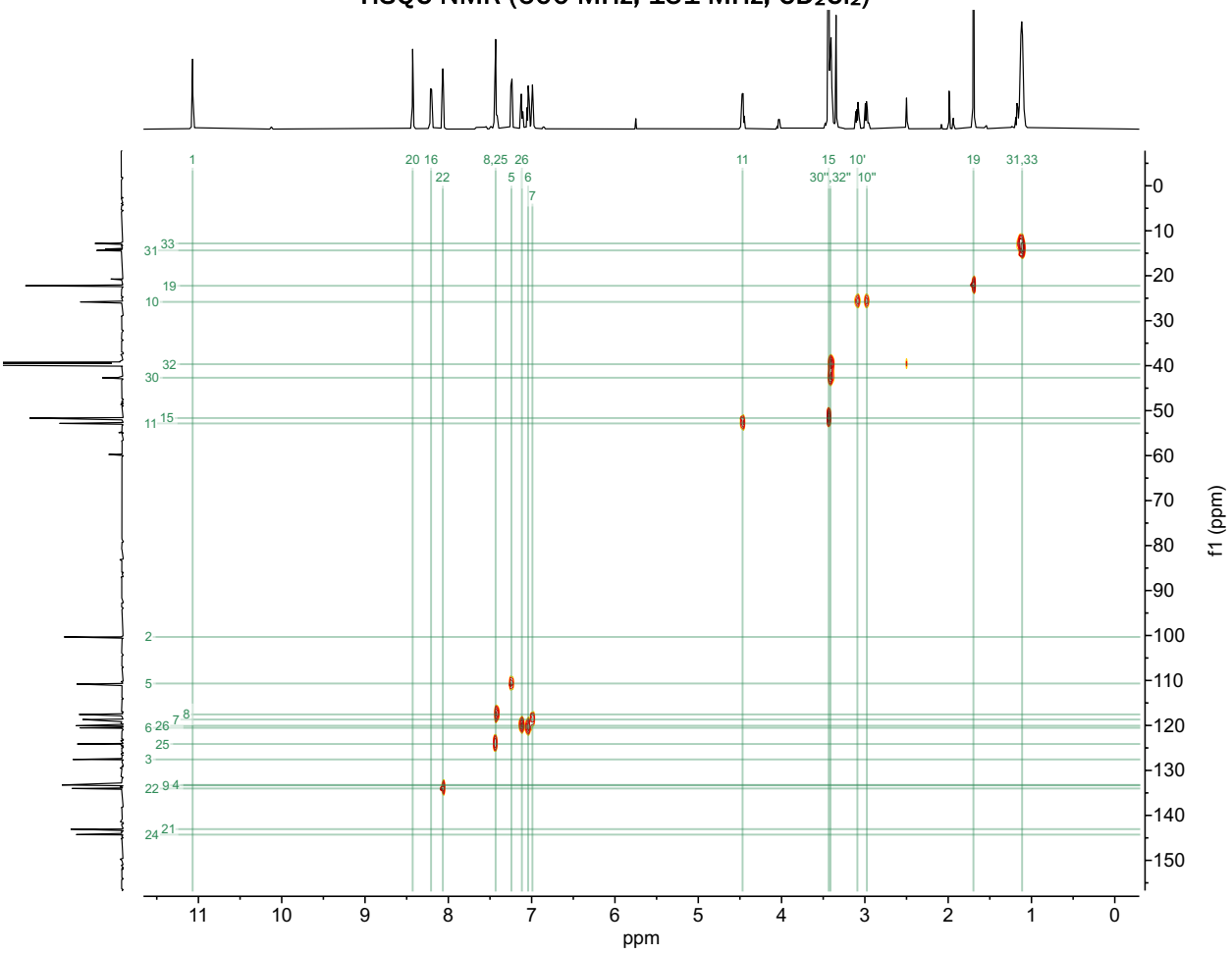
^{13}C NMR (151 MHz, CD_2Cl_2)



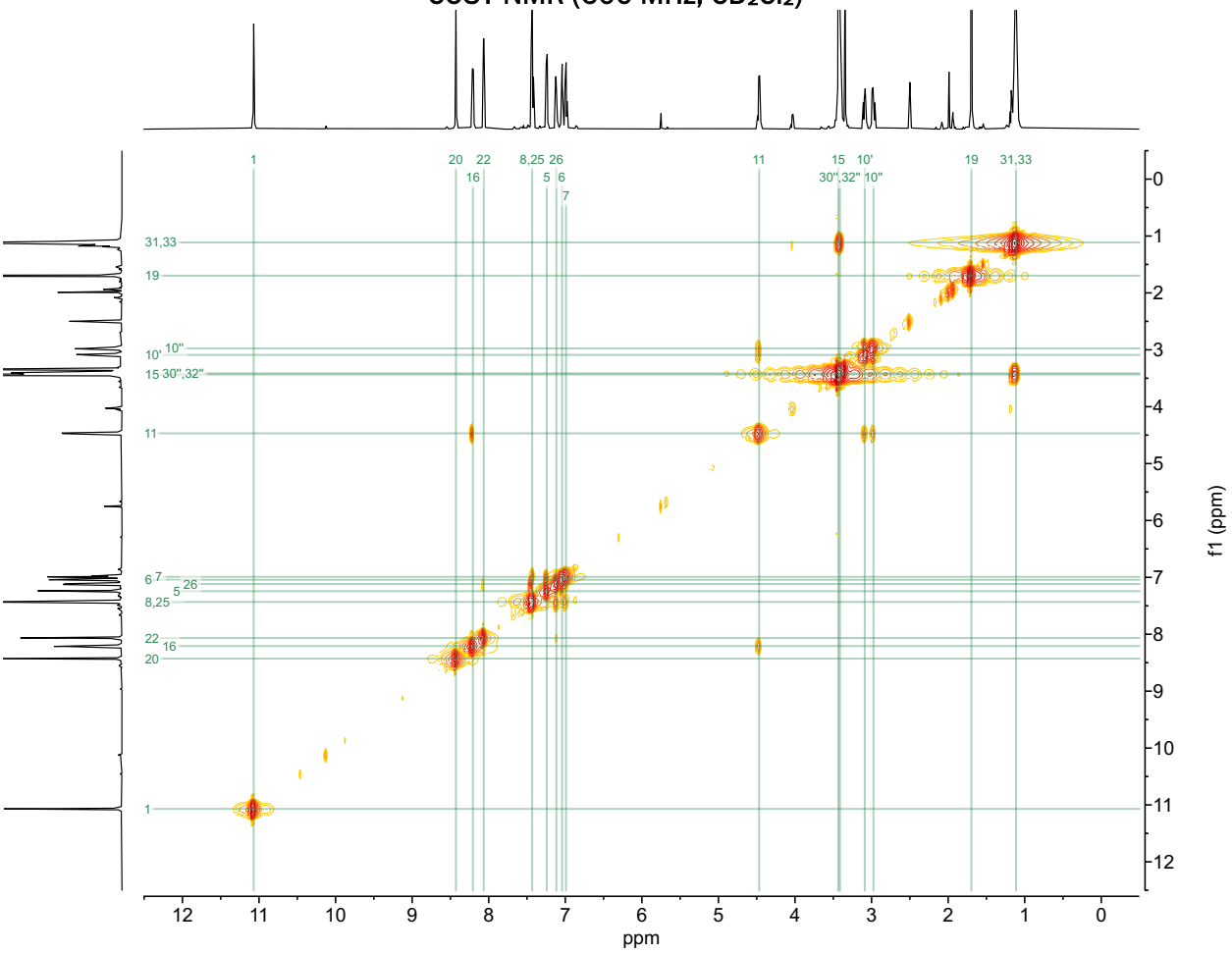
HMBC NMR (600 MHz, 151 MHz, CD_2Cl_2)



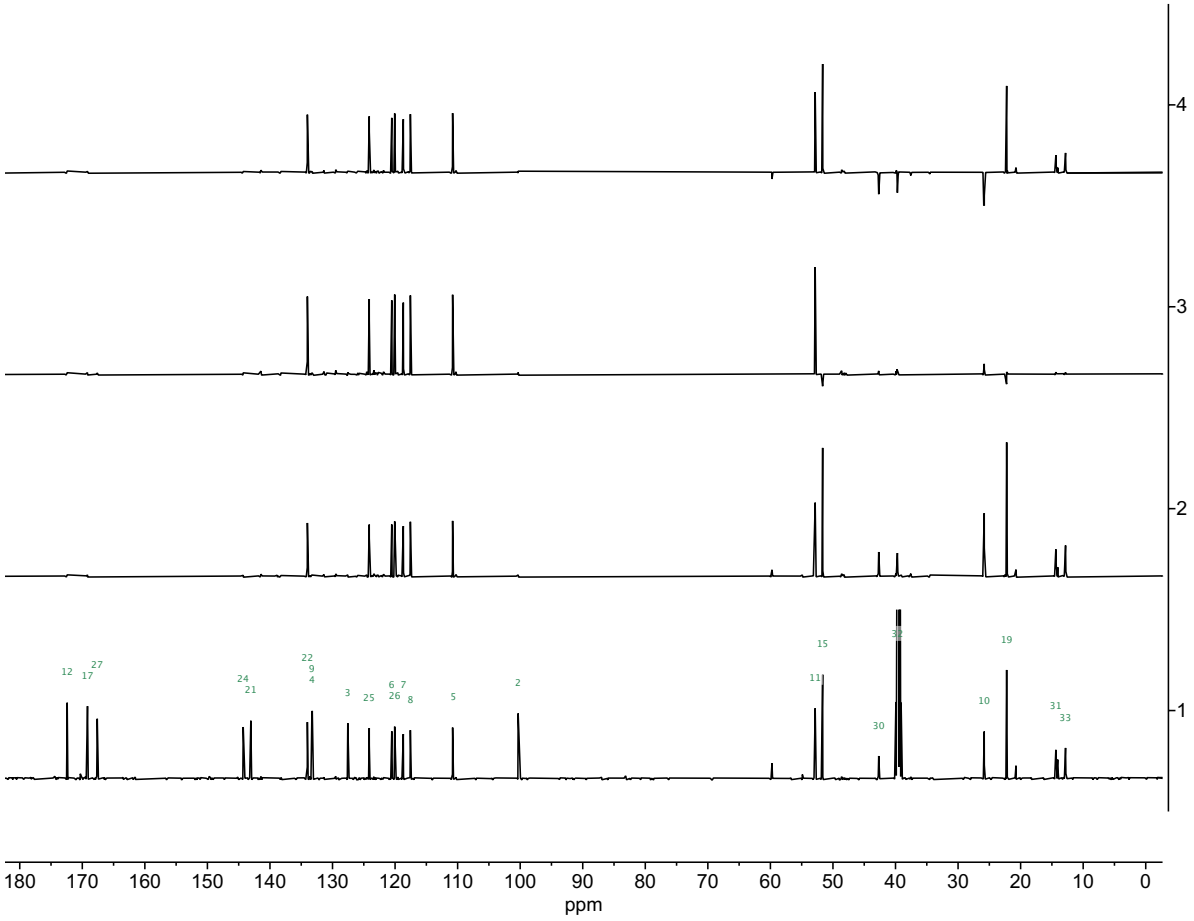
HSQC NMR (600 MHz, 151 MHz, CD_2Cl_2)



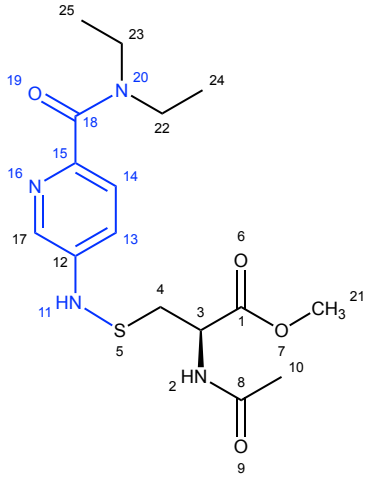
COSY NMR (600 MHz, CD_2Cl_2)



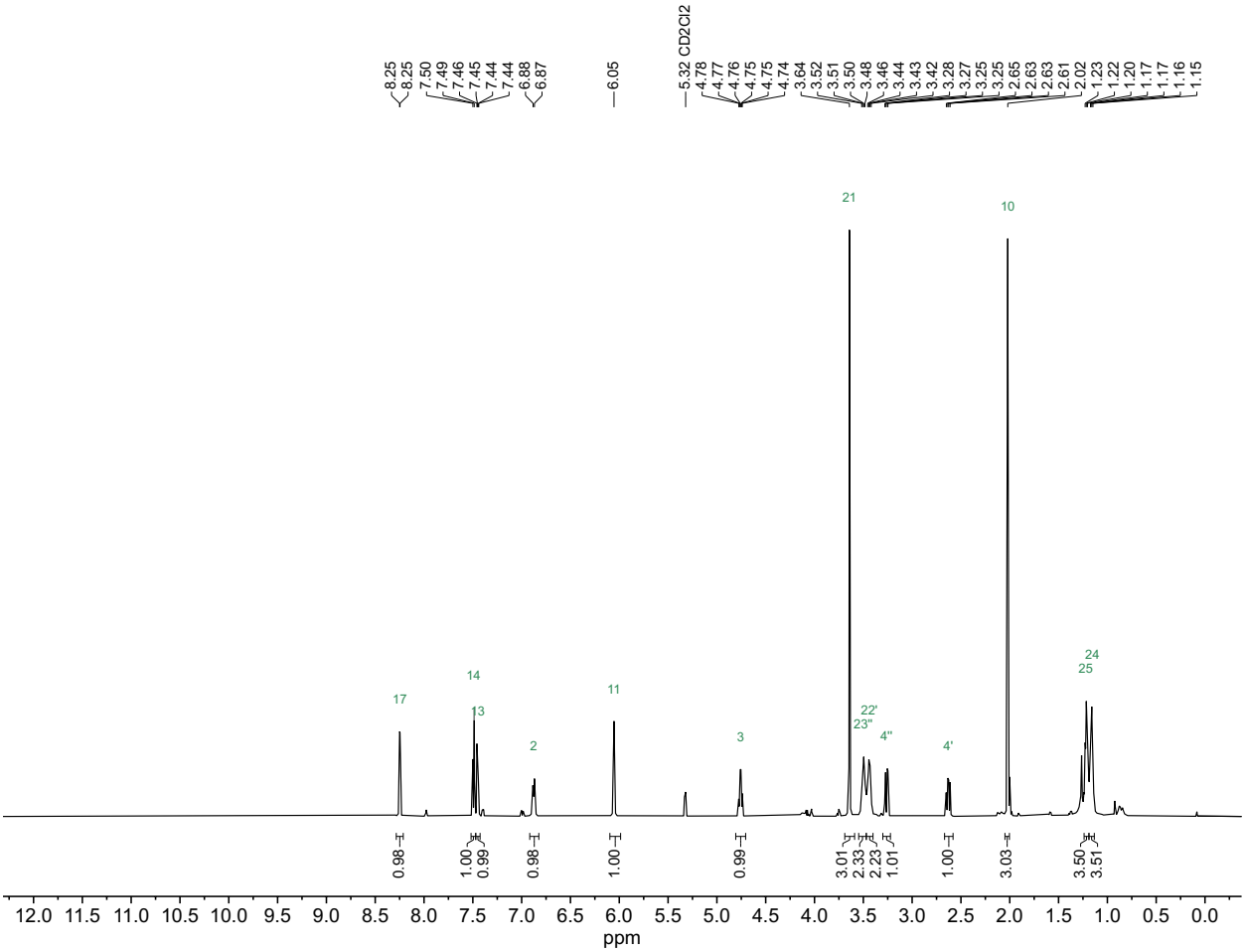
DEPT-135, DEPT-90, DEPT-45, ^{13}C NMR (151 MHz, CD_2Cl_2)



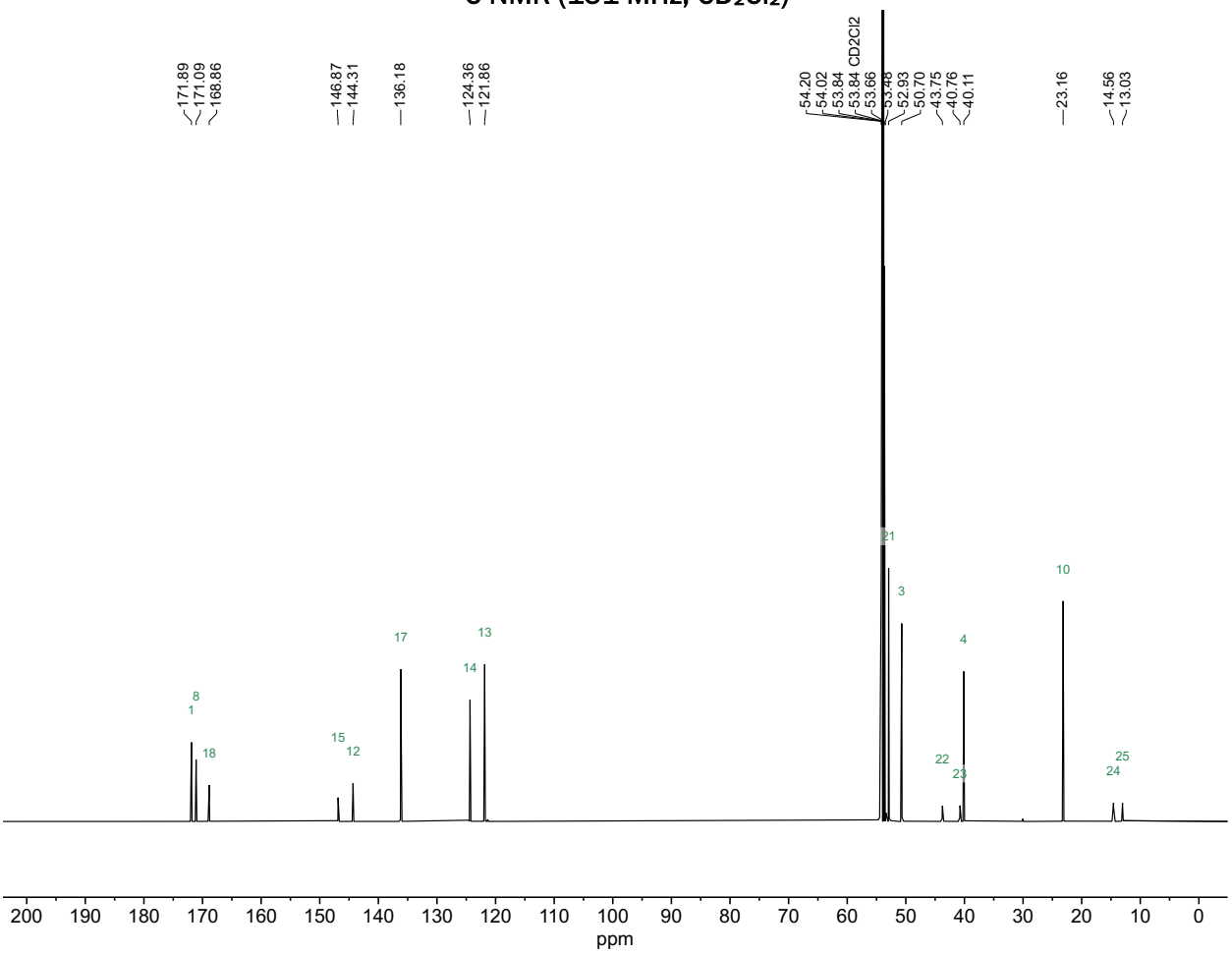
Numbering scheme for cysteine N-transfer product and corresponding NMRs:



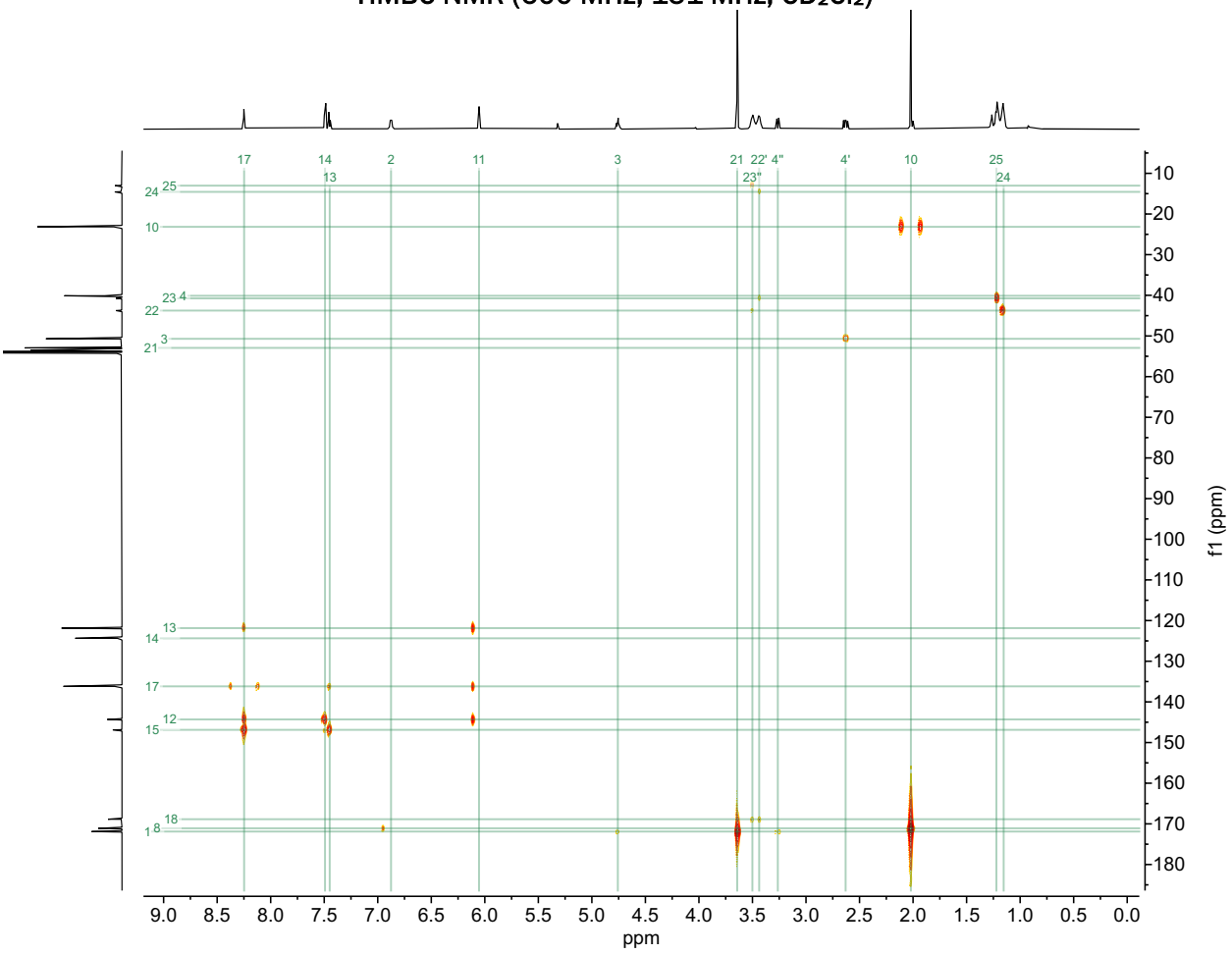
^{1}H NMR (600 MHz, CD_2Cl_2)



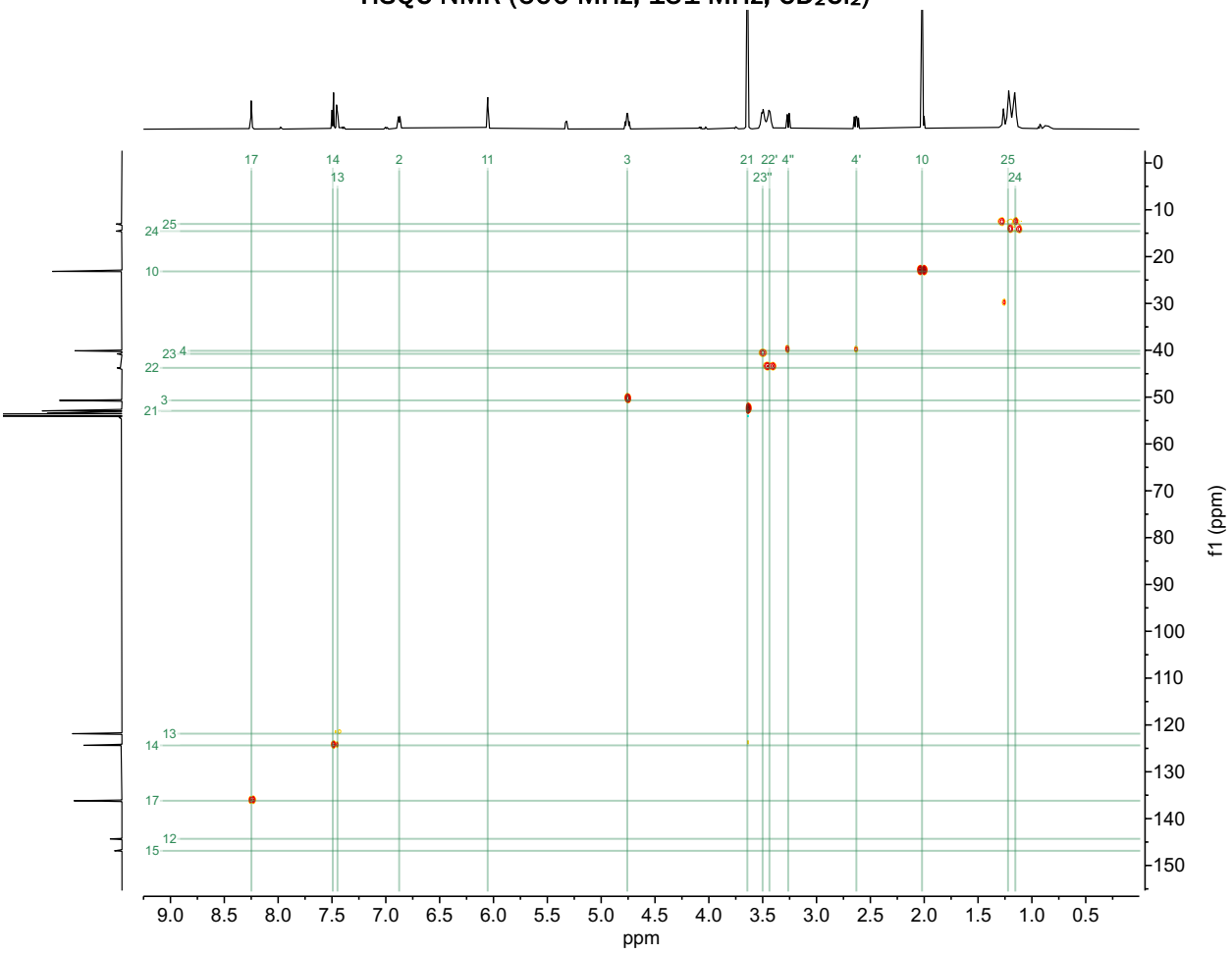
^{13}C NMR (151 MHz, CD_2Cl_2)



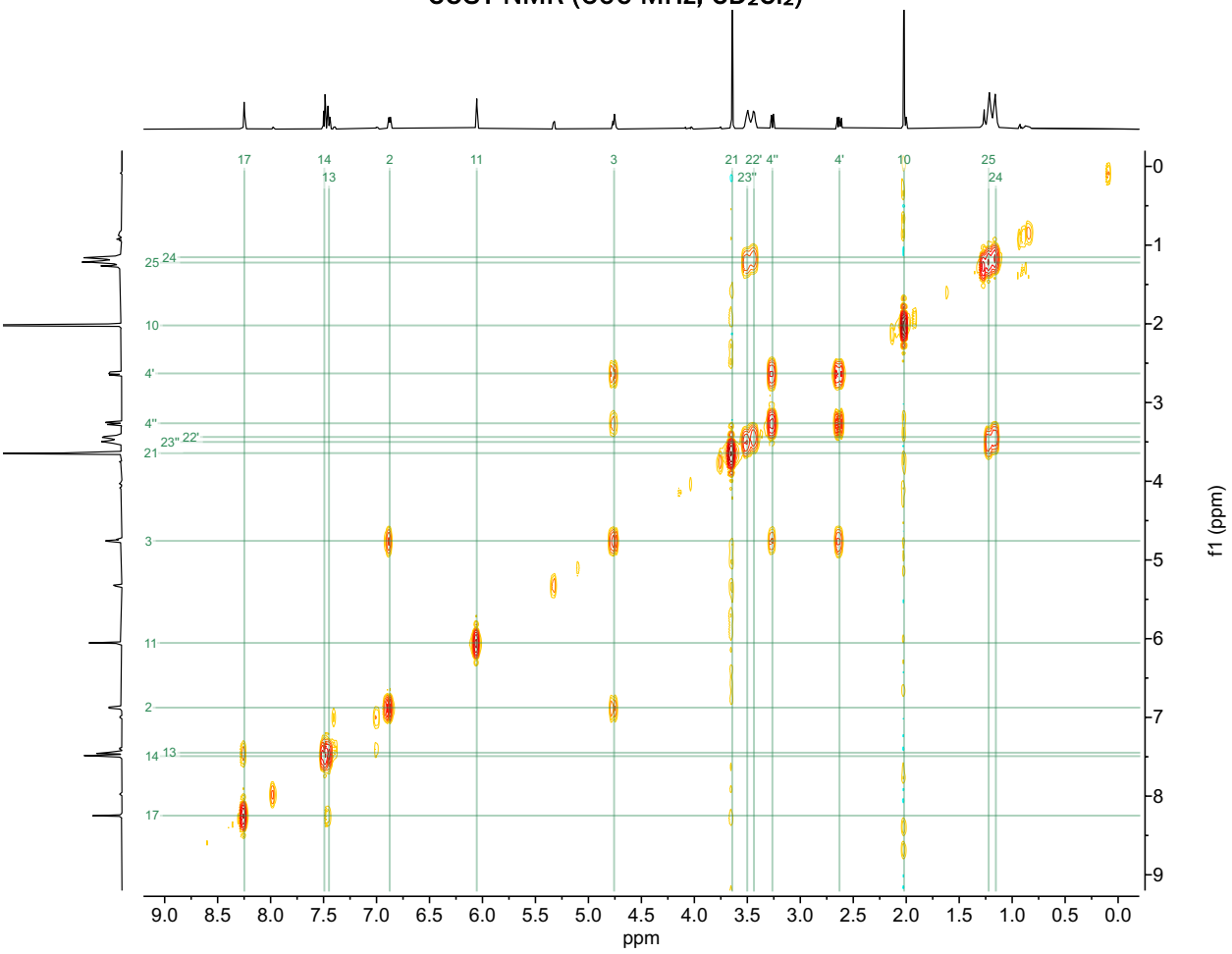
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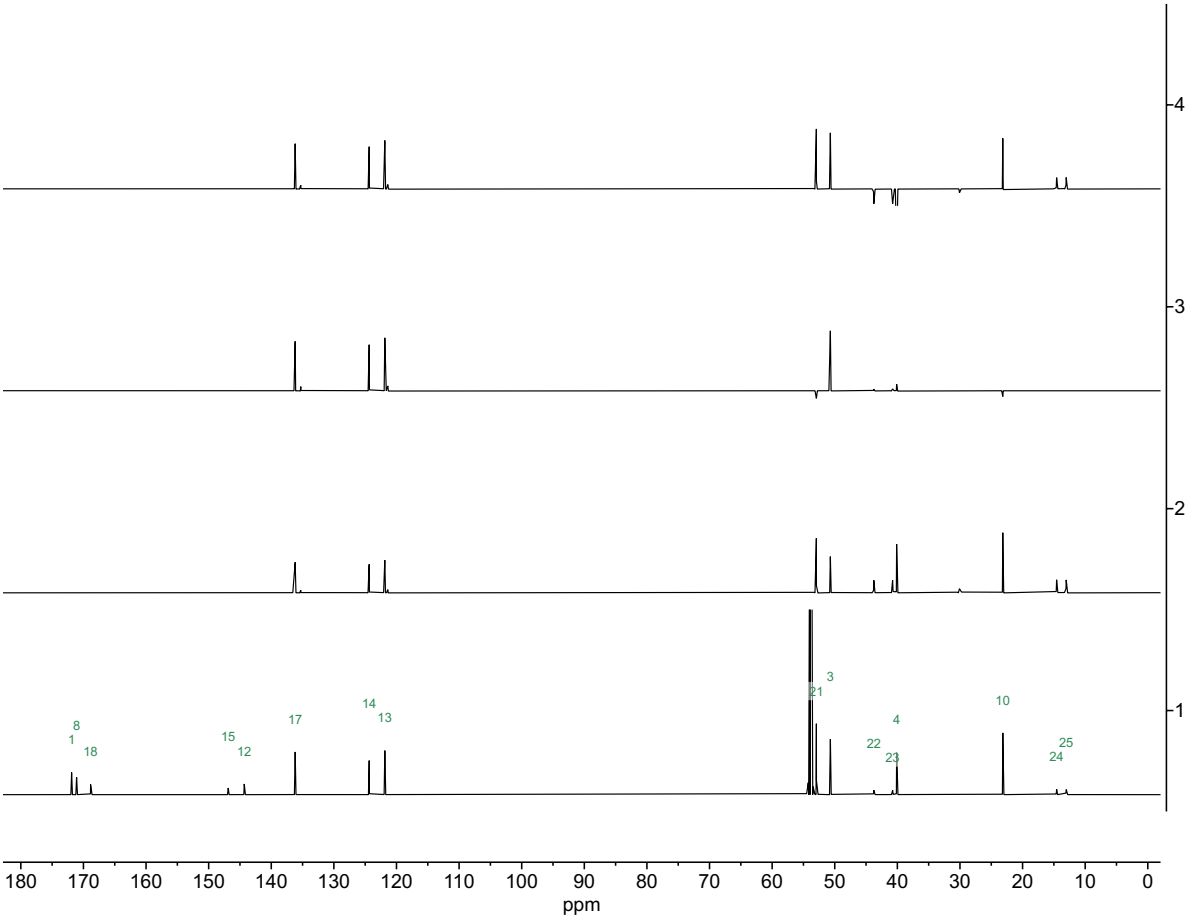
HSQC NMR (600 MHz, 151 MHz, CD_2Cl_2)



COSY NMR (600 MHz, CD_2Cl_2)



DEPT-135, DEPT-90, DEPT-45, ^{13}C NMR (151 MHz, CD_2Cl_2)



Rate of oxaziridine WW reaction with Trp

Reactions mixtures were created at the given concentrations using a stock solution of Trp (2 mM in 5% ACN in water) and a stock solution of oxaziridine WW (4.0 mM nitrone WW in ACN irradiated for 10 min at 370 nm), the final reaction mixture was 2.0 mL of 10 mM sodium phosphate buffer, pH 7.0. The irradiated nitrone stock was added to reaction mixture approximately 10–15 s before data collection began. Fluorescence intensity of Trp was averaged over 1 s starting every 2 s between measurements using a Varian Cary Eclipse fluorescence spectrophotometer.

First set of data was collected by measuring fluorescence with an excitation wavelength of 280 nm (10 nm slit) and emission wavelength of 360 nm (20 nm slit). A pseudo-first order reaction kinetics analysis was performed to estimate the second order rate constant (Figure S32). Given the nearly complete reaction by the first timepoint (10–15 s), we believe that the calculated rate constant (76 M^{-1} s^{-1}) should only be viewed as a lower bound on the actual second order rate constant.

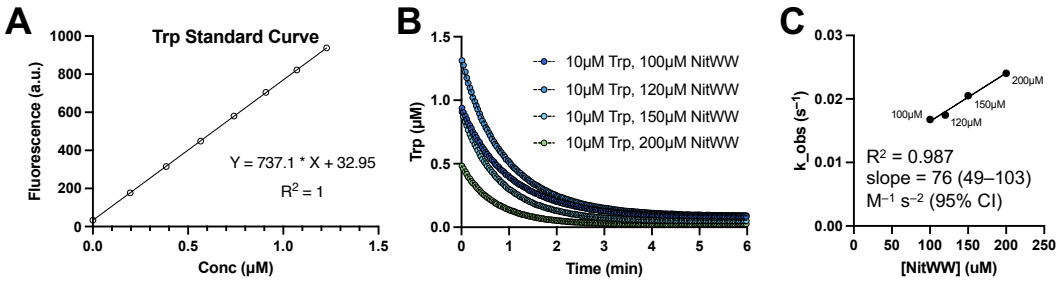


Figure S32. Trp consumption by oxaziridine WW at various ratios over time. (**A**) Standard curve of tryptophan fluorescence, (**B**) concentration of Trp over time following the addition of oxaziridine WW, (**C**) linear fit of the rate constant plotted over the starting concentration of oxaziridine WW in solution gives a second order rate constant of 76 M^{-1} s^{-1}.

Second set of data was collected by measuring fluorescence with an excitation wavelength of 280 nm (10 nm slit) and emission wavelength of 360 nm (10 nm slit). Bayesian modeling was used to establish most likely values for the second order rate constant (Figure S33). Non-informative uniform priors were used for the analysis ($0.01<\sigma<2.00$ μM, $10<k_1<10{,}000$ M^{-1} s^{-1}, $0.01\times10^{-3}<k_2<100\times10^{-3}$ s^{-1}), the data was assumed to follow a normal distribution with standard deviation of σ, and initial reagent concentrations were constrained to the values given. Obtained MAP values of parameters: k_1 = 511.3 M^{-1} s^{-1}, k_2 = 0.00518 s^{-1}, σ = 0.192 μM. The resulting MAP k_2 value (5.18×10^{-3} s^{-1}) is relatively close to the rate constant obtained through a different method under similar conditions described earlier (0.89×10^{-3} s^{-1}, Figure S14).

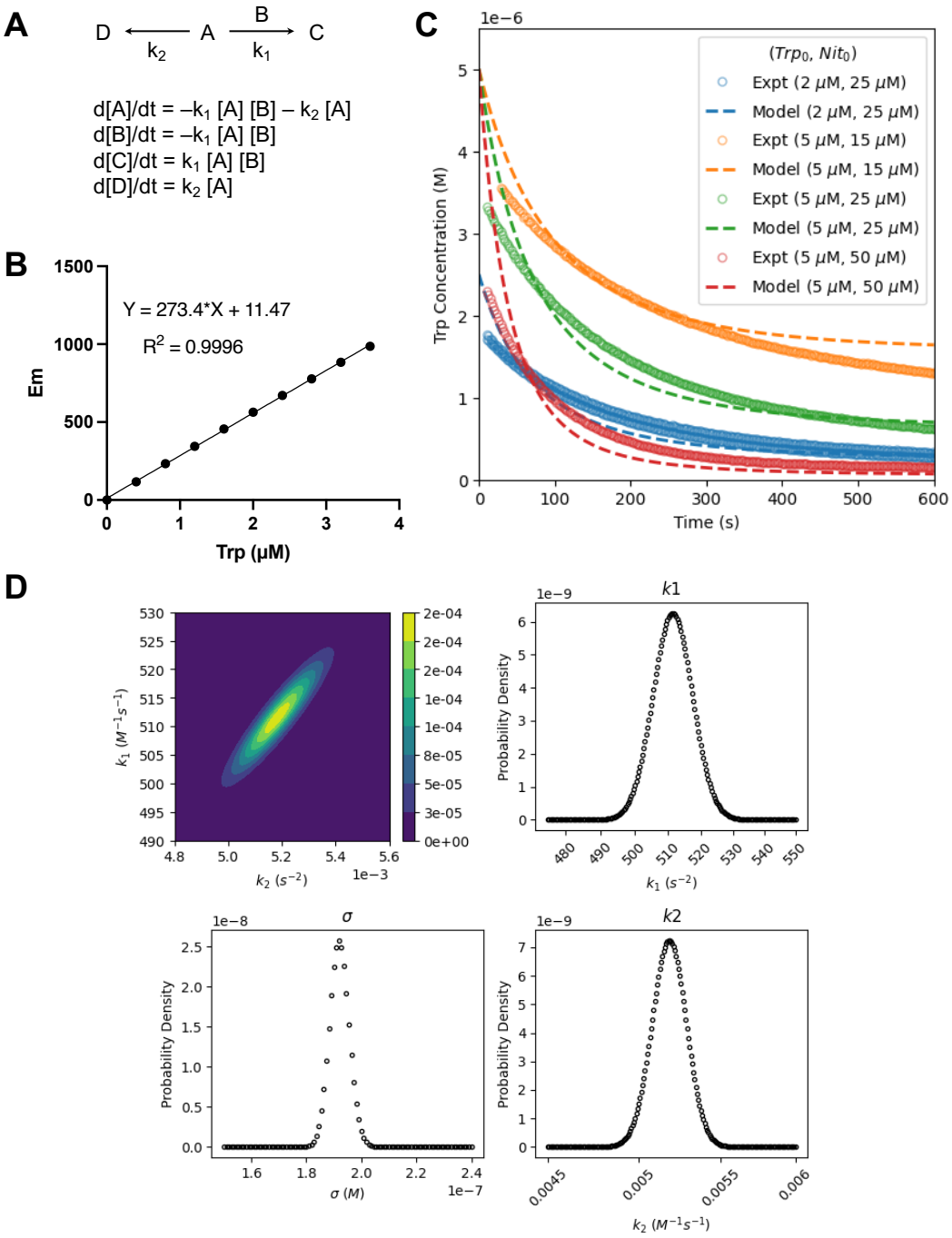


Figure S33. (**A**) Reaction scheme used for modeling and rate equations, A: oxaziridine, B: Trp, C: conjugation product, D: oxaziridine decomposition product. (**B**) Standard curve of Trp fluorescence. (**C**) Experimental data (open circles) and model fits (dashed lines, using MAP values for k_1 and k_2) for 4 unique sets of starting conditions (initial Trp and irradiated nitrone concentrations in each reaction) (two replicates of one condition are included, blue). (**D**) A posterior distribution of k1 and k2 (σ set to MAP value of 0.192 μM) (top left graph), and marginal distributions of each model parameter. MAP values of parameters: k_1 = 511.3 $M^{-1}s^{-1}$, k_2 = 0.00518 s^{-1}, σ = 0.192 μM.

Influence of solvent on methionine versus tryptophan selectivity

N-Ac *C*-OMe tryptophan and methionine were combined in equimolar ratios (100 μM each) in reaction solution with phosphate buffer (10 mM, pH 7.0) using indicated solvent ratios. Nitrone **WW** was irradiated in a solution (10 mM) of acetonitrile and added to the reaction mixture for a final concentration of 100 μM (1 equiv) in the reaction mixture. Each reaction mixture contained approximately 1%$_{v/v}$ acetonitrile in addition to the shown solvent ratios.

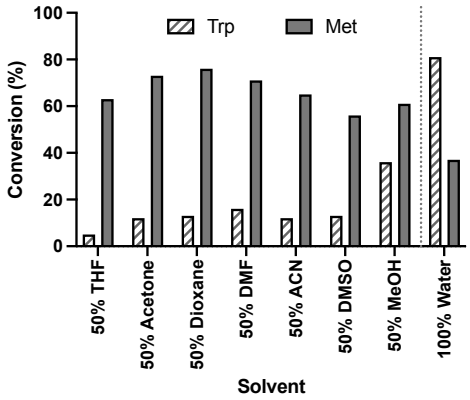


Figure S34. Impact of solvent type on tryptophan versus methionine selectivity of oxaziridine **WW**.

Protein conjugation

Antibody fragments

Nitrone solutions (4mM) in acetonitrile were irradiated for 5 min at 370 nm, then 100 equiv were added (10 μL for 200 μM in reaction) to a solution of Fab (2 μM) in phosphate buffered saline (0.1x) at pH 7. After 45 min at rt the reactions were quenched with 1,000 equiv of 2-(methylthio)ethan-1-ol (10 μL of 40 mM stock in water for a final concentration of 2 mM in reaction). The reactions were analyzed by LC-MS (Figure S36,Figure S37).

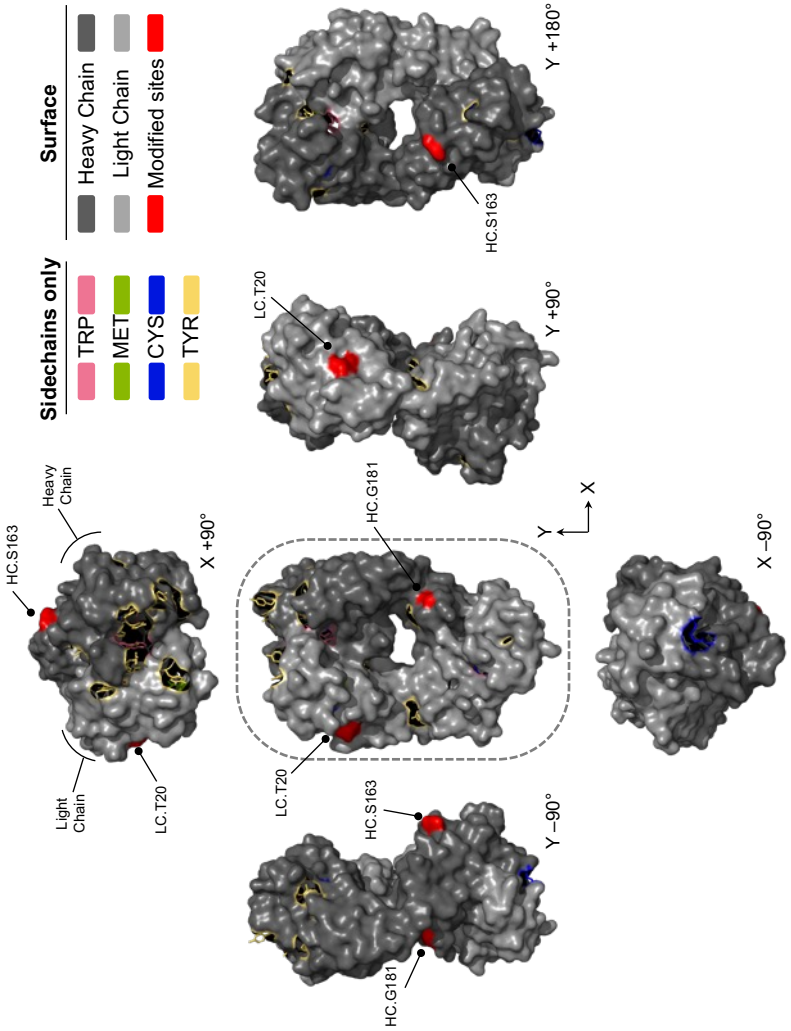


Figure S35. Structure of Fab mutants showing modified residues (red surface) and other possible sites of reaction (Trp, Met, Cys, and Tyr). Author numbering system from the crystal structure was used.

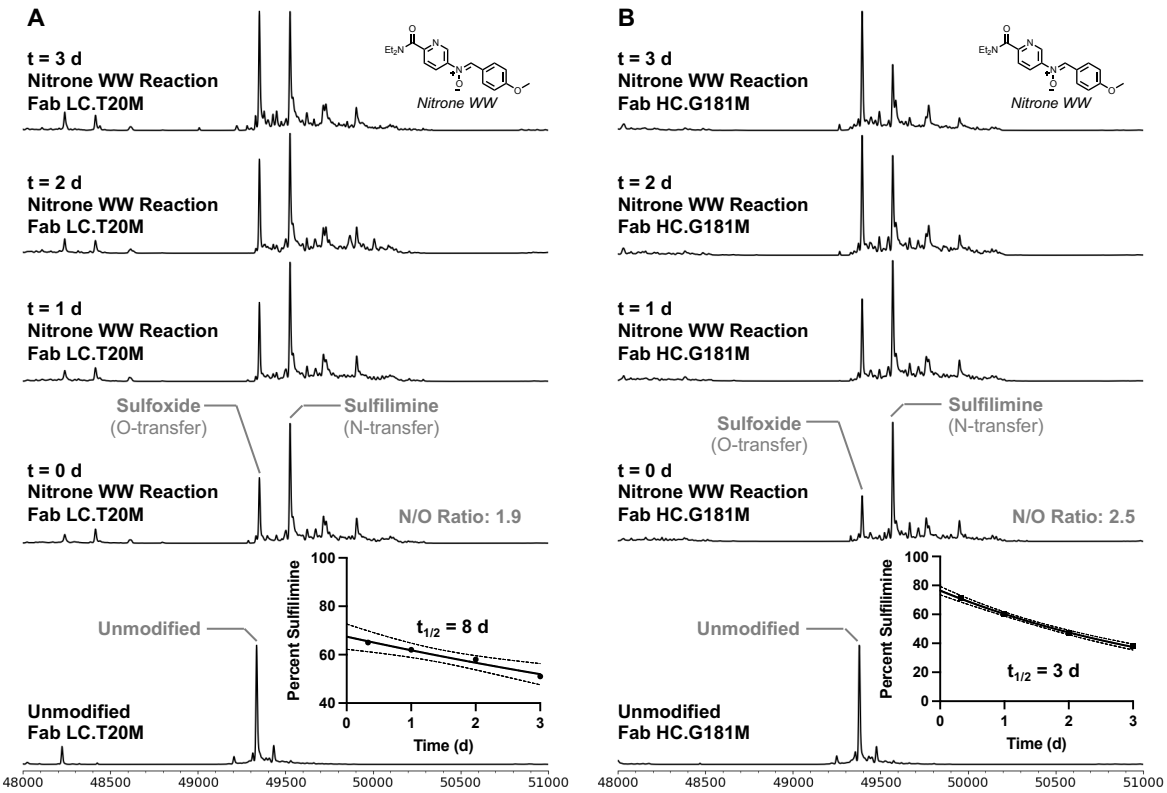


Figure S36. Nitrone modification of HER-2 selective antibody fragments with a single introduced methionine modification at (**A**) light chain T20M and (**B**) heavy chain G181M. Sulfilimine conjugation product was monitored over time to assess hydrolysis rates and fit to first order decay function with plateau constrained at zero, $Y = Y_0*\exp(-k*t)$ (insets).

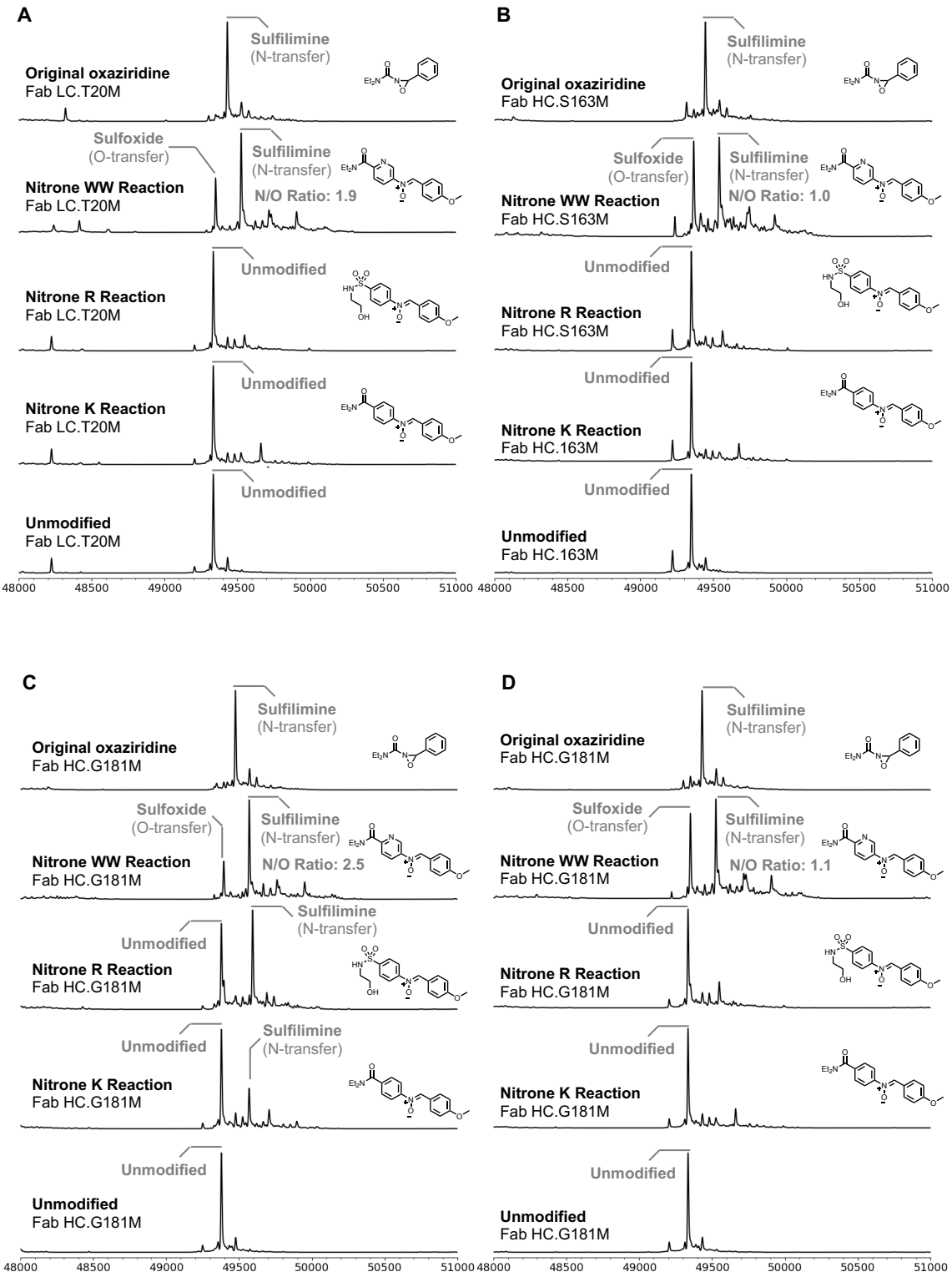


Figure S37.Comparison of labeling of a HER-2 selective Fab antibody fragment by three nitrones and the original oxaziridine at methionine sites. The Fabs used for labeling had methionine residues incorporated at the (**A**) light chain T20M, (**B**) heavy chain S163M, (**C**) heavy chain G181M, (**D**) heavy chain G181M.

α-Chymotrypsin and Thioredoxin

Solutions of protein (10 μM) and nitrones (20 equiv, 200 μM) in PBS at pH 7.0 were irradiated for 5 min at 370 nm, then oxaziridines were added and the reactions analyzed by LC-MS within 2 h. Reactions were allowed to proceed overnight at rt and were analyzed again after 16 h by LC-MS (Figure S23).

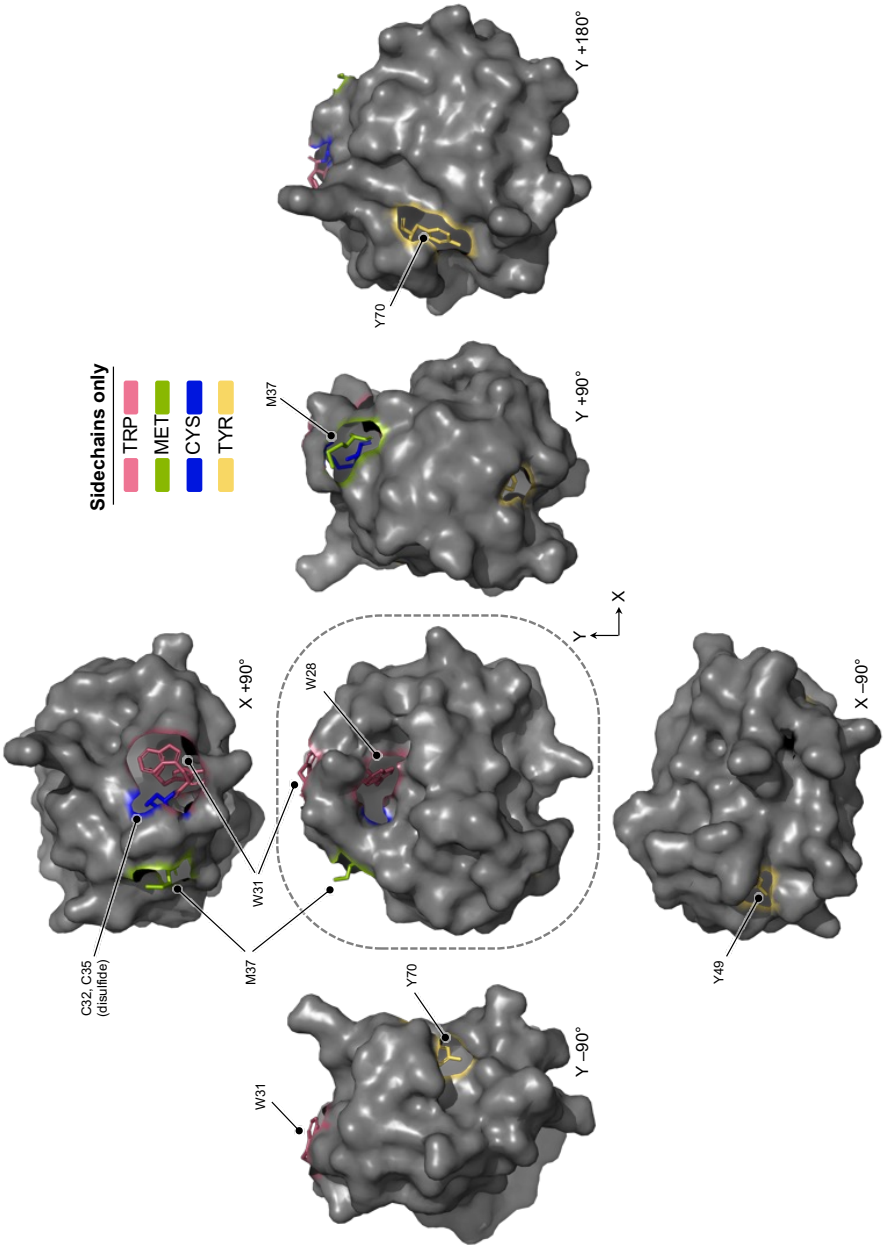


Figure S38. Structure of Thioredoxin (PDB 2TRX) showing possible reactive residues (Trp, Met, Cys, Tyr). Likely sites of modification are W28, W31, and M37. Author numbering system from the crystal structure was used.

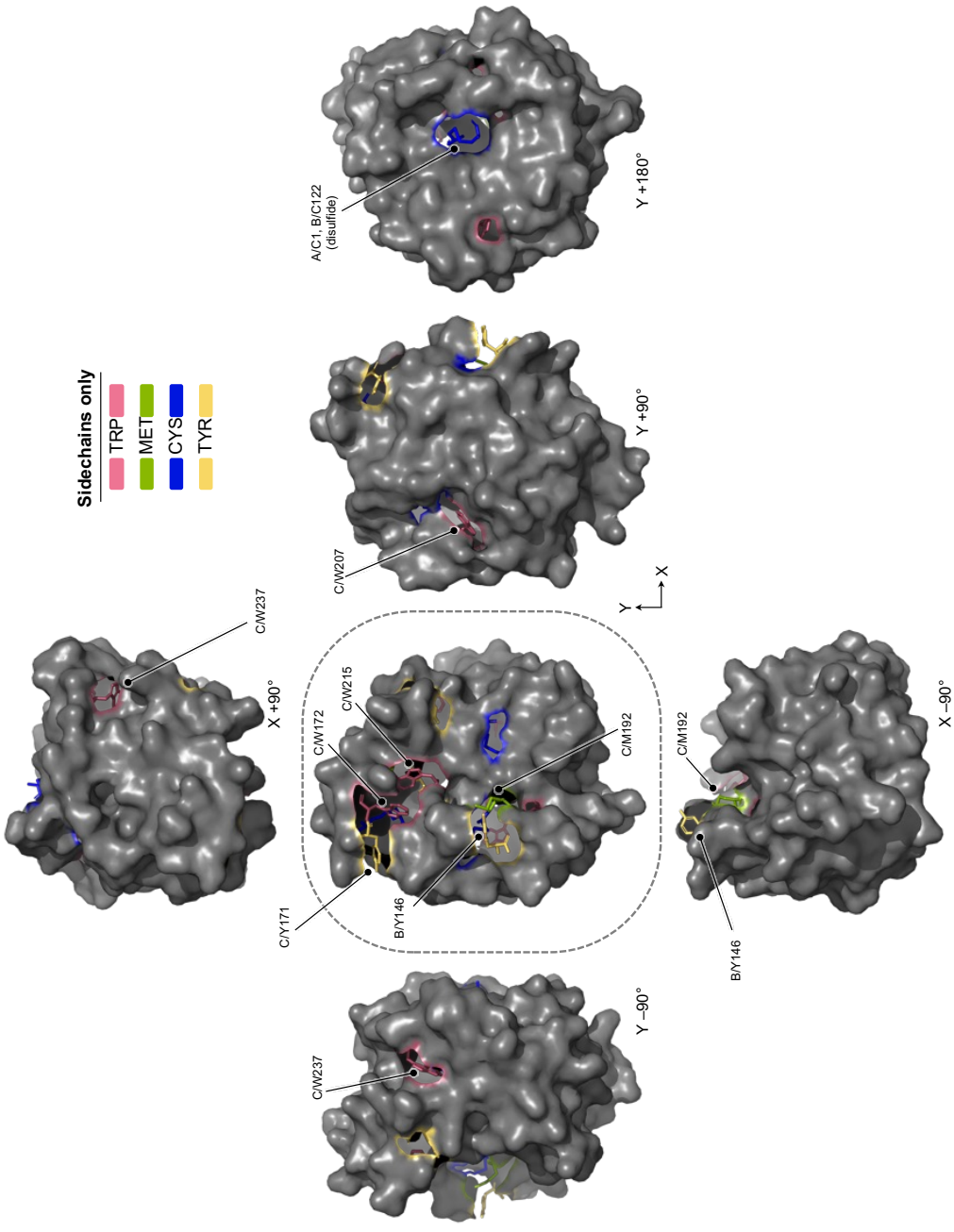


Figure S39. Structure of bovine α-chymotrypsin (PDB 1YPH) showing possible reactive residues (Trp, Met, Cys, Tyr). Likely sites of modification are C/M192, C/M192, C/W215, and C/W235. Author numbering system from the crystal structure was used.

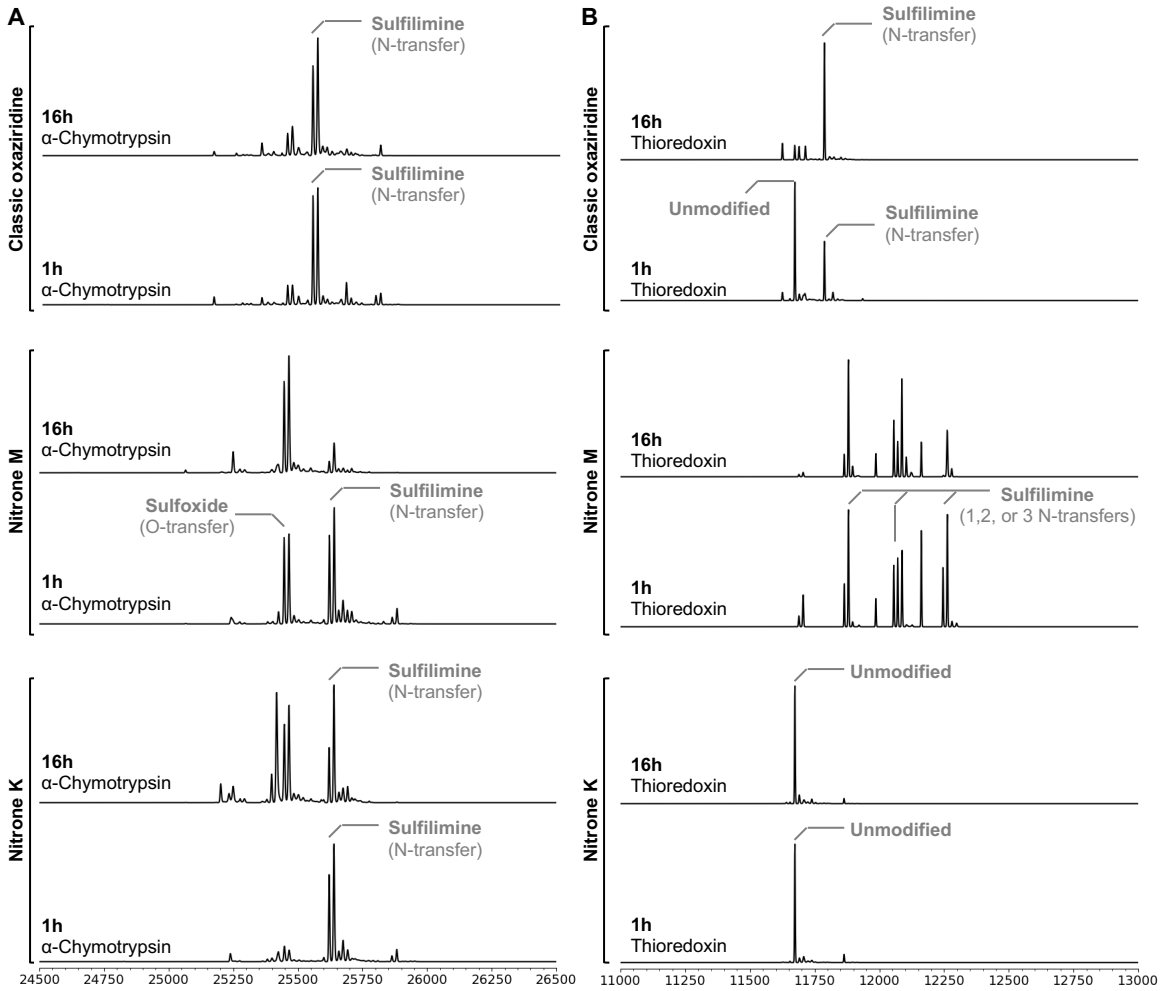


Figure S40. Modification of (**A**) α-Chymotrypsin or (**B**) thioredoxin with nitrones **K** and **M** or the N-carbamoyl *N′*,*N′*-diethyl oxaziridine.

Proteins (15 μM) and nitrones (13 equiv, 200 μM) in PBS at pH 7 were irradiated for 5 min at 370 nm. A size exclusion G-25 resin (NAP) column was used to remove excess nitrone reagent and the purified samples were incubated at rt and analyzed by LC-MS over time (Figure S24).

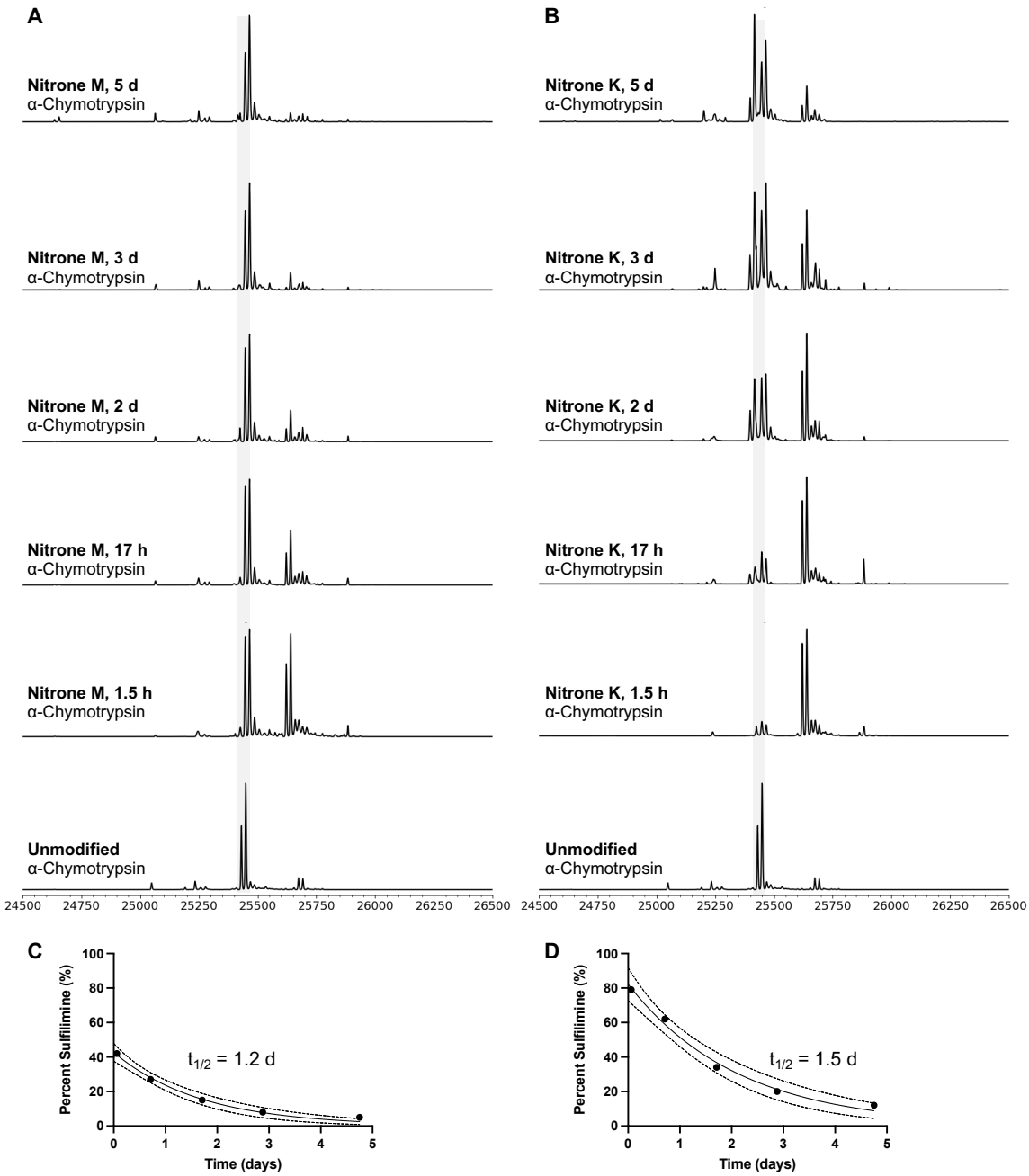


Figure S41. Measurement of hydrolytic stability of sulfilimine conjugates with α-Chymotrypsin after reaction with (**A**) nitrone **M** and (**B**) nitrone **K**, (**C**,**D**) measurements across time were fit to first order decay functions with plateau constrained at zero, $Y = Y_0*\exp(-k*t)$.

In situ versus separate irradiation

Nitrones were either irradiated for 5 min at 370 nm in acetonitrile (20X stock) prior to addition to proteins or *in situ* with proteins (10 μM proteins in 1X PBS at pH 7.0) at ratios indicated. The reactions were incubated at rt for 30 min, then analyzed by LC-MS (Figure S25).

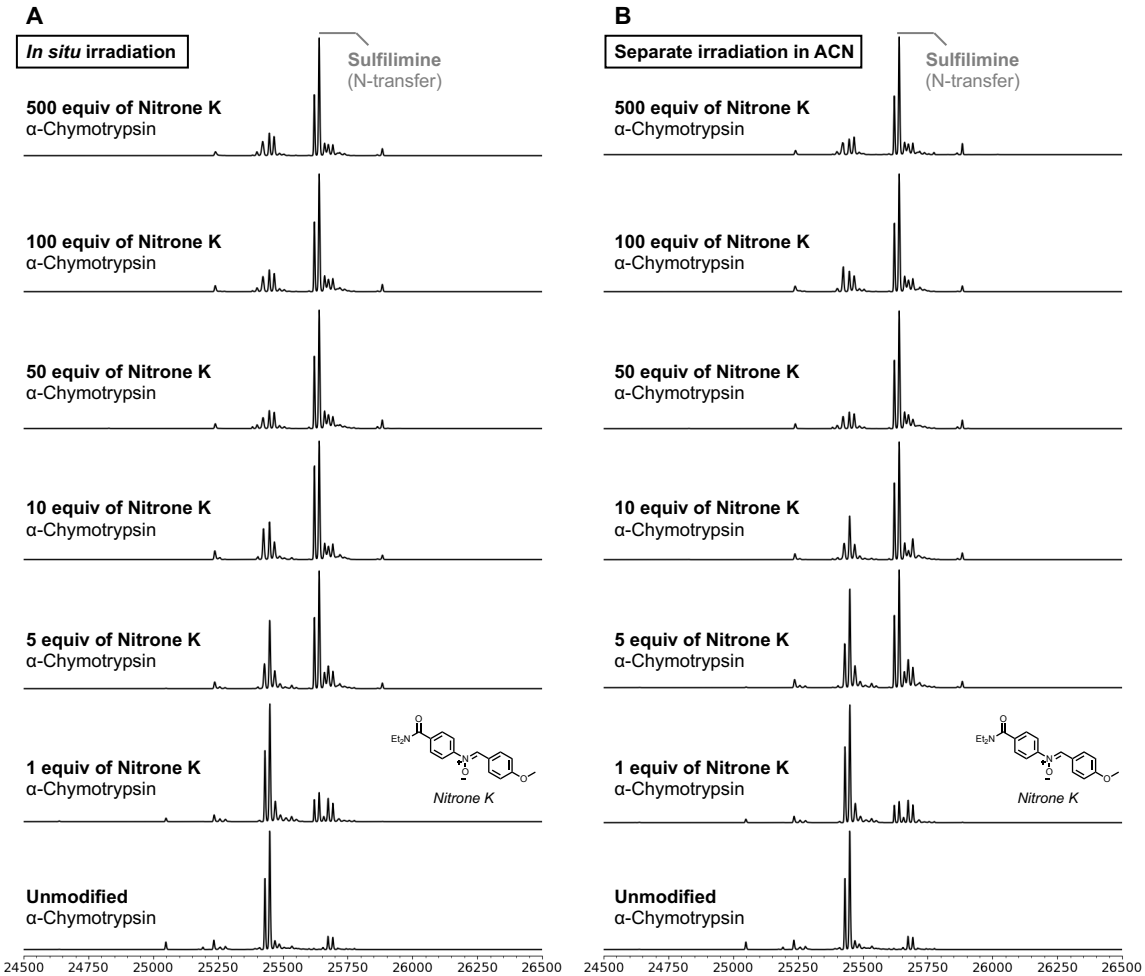


Figure S42. α-Chymotrypsin labeling with nitrone **K** at a range of equivalents by irradiation (**A**) *in situ* or (**B**) prior to addition to protein solution.

Bromodomain 1 of BRD4

The sequence of Bromodomain 1 (BD1) of BRD4 used in these studies is shown below and includes residues 42–168 (underlined) of the canonical Uniprot sequence (O60885) with an N-terminal extension.

GPLGS STNPPPPET SNPNKPKRQT NQLQYLLRVV LKTLWKHQFA WPFQQPVDAV KLNLPDYYKI IKTPMDMGTI KKRLENNYYW NAQECIQDFN TMFTNCYIYN KPGDDIVLMA EALEKLFLQK INELPTEE.

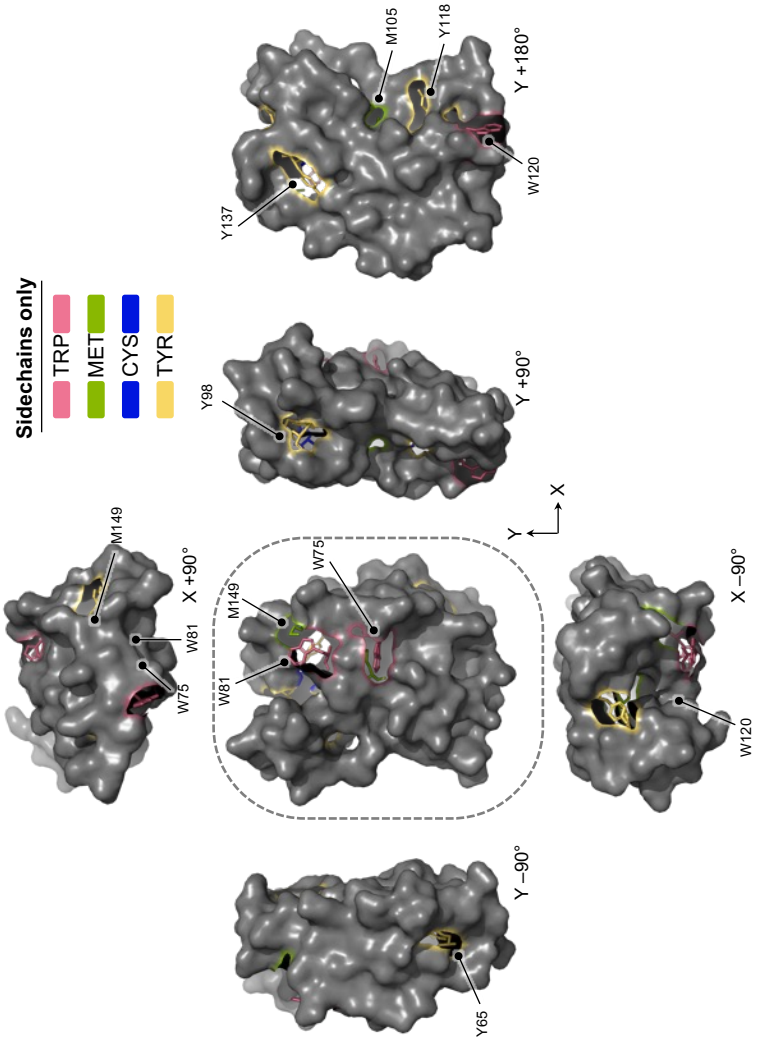


Figure S43. Structure of BRD4 BD1 (PDB 4Z1A) showing possible reactive residues (Trp, Met, Cys, Tyr). Likely sites of modification are W75, W81, W120, and M149. Author numbering system from the crystal structure was used.

Lysate and live cell proteome profiling by gel-based ABPP

Lysate generation. HEK cells (ATCC) were cultured in DMEM (Gibco) containing 10% BSA and 1% Penn/Strep in a humidified incubator (37 °C, 5% CO_2). HEK cells were harvested and prepped for native lysate generation using a native lysis buffer (50 mM HEPES pH 7.5 ,150 mM NaCl, 1.5 mM $MgCl_2$, 0.8% NP-40, 5% Glycerol, 1 mM DTT, 1x HALT protease and phosphatase inhibitors) and sonication at 20% amplitude (2 seconds ON, 5 seconds OFF, 30 seconds; 3x's). Cellular debris was removed by centrifugation at 20,000xg for 10 minutes at 4 °C. The clarified supernatant was collected, and protein concentration was determined by BCA (Peirce). On the day of the experiment, the lysates

were diluted down to 5 mg/mL in 50 mM HEPES pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 0.4% NP-40, 1 mM DTT.

Each nitrone reaction was made by adding HEK293 cell lysate (40 µL) diluted in PBS to 250 µL total volume. Nitrone stocks were made in DMSO at 20 mM concentration and 5 µL of stock was added to each reaction for a 100 µM nitrone reaction concentration. All reaction were irradiated at 370 nm for 5 min. The reactions were further incubated at rt for 10 min. Then BCA assay was performed to normalize the protein concentration across reactions. The BCA assay was performed according to manufacturer's protocol. A premade mix of rhodamine-azide (1.25 µL of 5 mM stock in DMSO), $CuSO_4$ (10 µL of 50 mM, 12.48 mg/mL stock in water), TCEP (10 µL of 50 mM, 12.51 mg/mL stock in water), and TBTA (10 µL of 5 mM, 2.65 mg/mL stock in 1:4 DMSO/*tert*-butanol) was added to each reaction mixture and incubated on ice for 45 min. Reaction analyzed by SDS-PAGE gel and imaged using rhodamine fluorescence and silver stain.

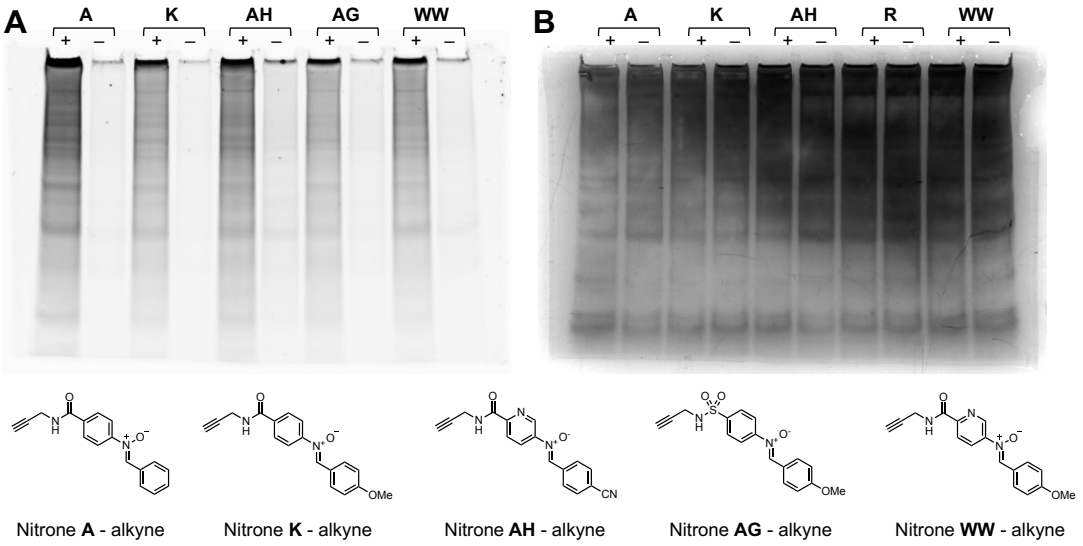


Figure S44. Labeling HEK cell lysate with various nitrone alkynes. Non-reducing PAGE gel visualized by (**A**) rhodamine fluorescence and (**B**) silver stain. Additional image in Figure S35.

A PAGE-based comparison of labeling using nitrone alkynes (WW, R, K, A, AH, and D), N-carbazoyl oxaziridine alkyne, and N-sulfonyl oxaziridine alkyne (Ox-W18 as reported in *Nature* **2024**, 627, 680–687) was designed to eliminate cysteine labeling.. Lysate (approx. protein concentration of 0.2 mg/mL) was treated with iodoacetamide (2.0 mM, 30 min at rt), then nitrone or oxaziridine reagent (50 µM, 60 min at rt). Then rhodamine azide was attached via CuCAAC (50 µM Rh-N3, 2 mM TCEP, 200 µM THPTA, 2.5 mM CuSO4 in reaction for 1 h at rt.) Then the reactions were reduced with DTT (10 mM, 30 min at rt) and denatured with LDS buffer (5 min at 95 °C) and assayed by PAGE gel. PAGE was imaged by rhodamine fluorescence, then stained with Coomassie as loading control.

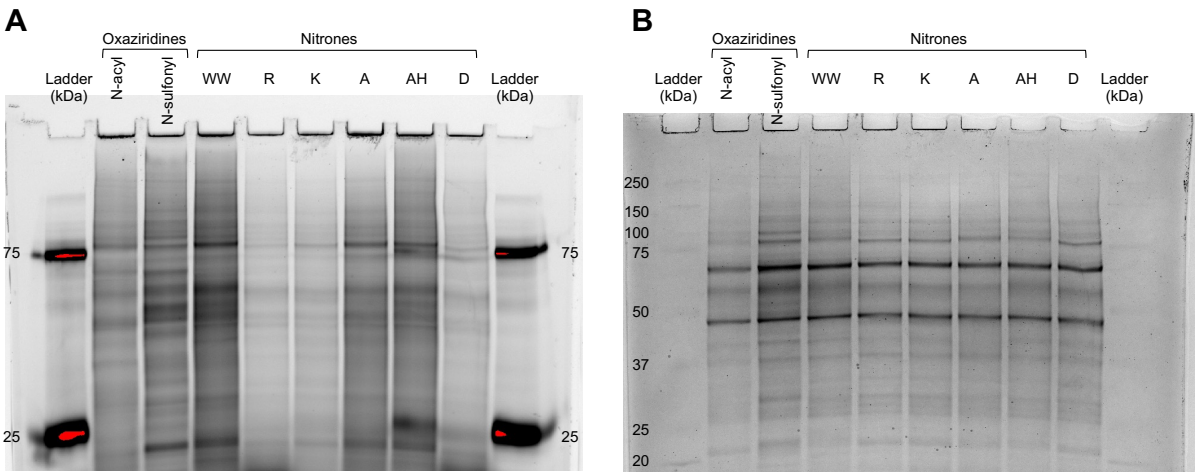


Figure S45. PAGE gel showing cell lysate labeling using N-acyl and N-sulfonyl oxaziridines and nitrones imaged by (A) rhodamine fluorescence and (B) Coomassie stain. Full gel image in Figure S35.

HeLa cells plated at 0.2×10^6 cells/well in 12-well plates. All media replaced with optiMEM just prior to treatment. Incubated with 100 µM nitrone-alkyne for indicated time at the indicated temperature. All wells were washed with PBS, then nitrone was added to 0 h timepoint and all wells were irradiated at 370 nm for 5 min. Media were immediately aspirated and cells lysed using 300 µL of RIPA buffer with protease inhibitor and 2 mM 2-(methylthio)ethan-1-ol quench. Lysate was labeled with Rh-azide through CuAAC and run on SDS-PAGE as described previously without specifically excluding cys labeling.

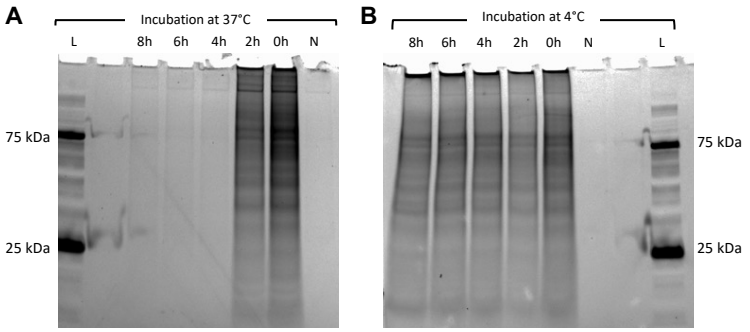


Figure S46. Cellular stability of nitrone **WW** during proteomic profiling in live HeLa cells. Cells were treated with nitrones and incubated for 0, 2, 4, 6, or 8 h at (**A**) 37 °C or (**B**) 4 °C. Labeling disappears with incubation longer than 2 h at 37 °C but is retained across all incubation times at 4 °C. L = ladder, N = medium only, no treatment. Additional image in Figure S35.

Cellular toxicity

Cytotoxicity of nitrone **WW**-alkyne compared to the original *N*-carbamoyl *N'*-(2-propynyl) oxaziridine reagent were measured. HeLa cells were plated in two 96-well plates at a density of 1×10^5 cells/well, some wells were left empty as 0% viability controls. After 24 h the medium was removed, the cells washed with DPBS and 140 µL of optiMEM serum- and indicator-free medium was added to each well. The nitrone and oxaziridine sample dilutions were prepared in 10/90 DMSO/water solutions and added to the appropriate wells in both plates. A 10/90 DMSO/water solution without

any solute was used as the 100% viability control. Approximately 10 min following the addition of treatment one plate was irradiated for 5 min with 370 nm light source. Both plates were incubated at 37 °C with 5% CO_2 for 24 h prior to the MTS assay. The MTS assay was carried out according to manufacturer's protocol (Promega, G111A). A comparison of untreated wells in the irradiated and non-irradiated samples shows cells are 88±7% viable after irradiation (mean±sd).

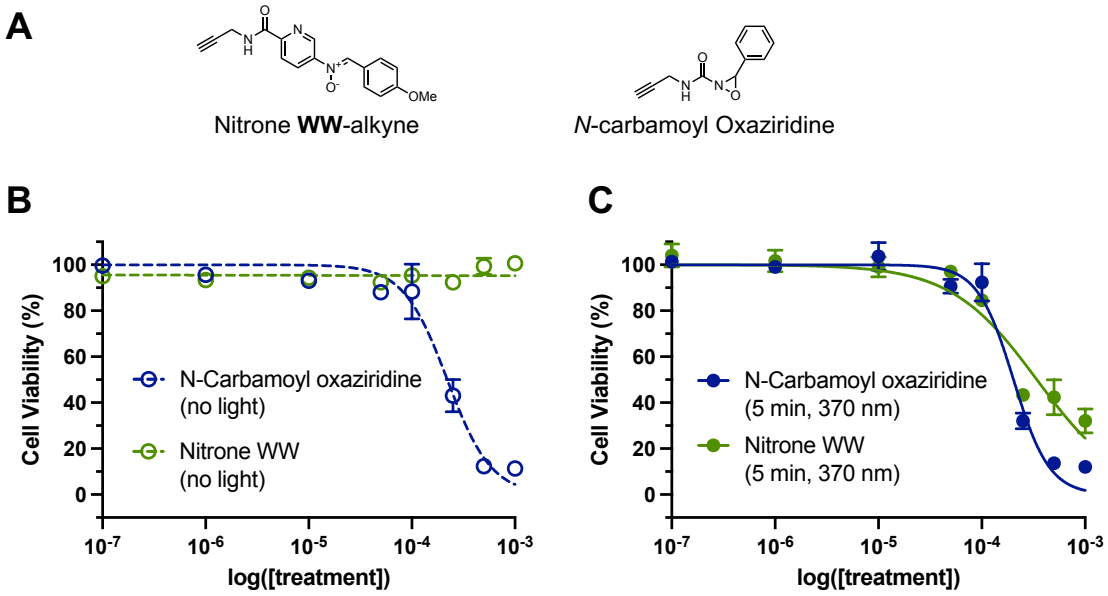


Figure S47. Cytotoxicity of *N*-carbamoyl *N'*-(2-propynyl) oxaziridine and nitrone **WW**-alkyne. (**A**) Chemical structures of the treatments. (**B**) Cell viability without irradiation. (**C**) Cell viability with irradiation. Average and standard deviation of three experimental replicates shown, curves show least-squares fit of sigmoidal function with variable IC_{50} and Hill slope parameters: $Y=100/(1+(IC_{50}/X)^{Hill_Slope})$.

Chemoproteomic profiling

Nitrone profiling using label-free quantitation.

Native HEK lysate (1 mg, 200 µL of 5 mg/mL lysate) was treated with 100 µM nitrone probes (50 mM DMSO stock) and incubated for 45 minutes at 4 °C. Following pre-incubation, the native lysates were irradiated with 365 nm light for 5 minutes at 4 °C. Following photoirradiation, the samples were prepped to introduce an acid-cleavable DADPS biotin azide for enrichment (Vector lab, CCT-1330, 100 µM final concentration, 4 µL of 5 mM stock) via copper catalyzed alkyne-azide cycloaddition with $CuSO_4$ (4 µL of 50 mM stock in water, final concentration is 1 mM), TBTA (12 µL of 1.7 mM in 4:1 tBuOH:DMSO, 100 µM final concentration), TCEP (4 µL of 50 mM stock in water, final concentration is 1 mM) and allowed to mix for 1 hour at room temperature. Proteins were precipitated with the addition of acetone (1 mL) and stored at -20 °C overnight. Protein precipitates were collected with centrifugation (3000xg, 10 minutes) and pellets were washed twice with 1 mL acetone with sonication (10% amplitude, 2 seconds ON, 2 seconds OFF, 10 seconds, 1x) and subsequent centrifugation. Pellets were resuspended with 200 µL 50 mM HEPES pH 8.0 containing 10 mM DTT (Pierce) and 0.2% RapiGest (Waters) and sonicated (10% amplitude, 2 seconds ON, 2 seconds OFF, 10 seconds, 3x's) to re-solubilize the protein pellet. Reduction of disulfides was allowed to proceed for 30 minutes at 55 °C with mixing followed by alkylation with 25 mM iodoacetamide (Pierce) for 30 minutes with mixing at room temperature in the dark. Following alkylation, excess iodoacetamide was quenched with the addition of 10 mM DTT and allowed to react for 10 minutes at room temperature. The samples were

next treated with 10 µg Trypsin/LysC (Promega) and allowed to digest overnight at 37 °C with mixing at 750 rpm on thermomixer (Eppendorf). The following day, the digest was diluted up to 1 mL in 50 mM HEPES pH 7.3 containing 0.2% SDS and treated with 100 µL of UltraLink Streptavidin resin (Thermo, 53116) to enrich for probe-modified peptides (4 hours at room temperature). Following enrichment, the beads were washed 5x's with 50 mM HEPES pH 7.3, 0.2% SDS; 5x's with 50 mM HEPES pH 7.3; and 5x's with water. Afterwards the enriched modified peptides were eluted with treatment with 150 µL 10% formic acid (2x's). The combined eluants were concentrated to dryness in Teflon plates and resuspended in mobile phase A (2% acetonitrile, 0.1% formic acid) for MS acquisition.

Mass spectrometry acquisition: Samples were loaded directly onto an 25cm Aurora Ultimate analytical column (IonOpticks) using an EasynLC 1200 (Thermo Fisher Scientific). Peptides were separated over a 120-minute gradient starting from 5% mobile phase A (2% acetonitrile, 0.1% formic acid) up to 50% mobile phase B (70% acetonitrile, 0.1% formic acid) at 300 nL/min with column heating to 45 °C (Sonation). Peptides were analyzed by label-free quantitation on a Thermo Orbitrap Fusion Lumos (Thermo Fisher Scientific) mass spectrometer in data dependent acquisition (DDA) mode with top 10 ions being submitted for MSMS. Full MS scans were collected in a scan range of 375-1500 m/z at a resolution of 60,000 on the orbitrap and a maximum injection time of 118 ms. The most intense peaks were selected for CID at 32% collision energy and detected on the ion trap.

Data analysis with FragPipe: To survey the landscape of mass shifts observed across all peptides and analyze the amino acid selectivity of the nitrone probes an OpenSearch and OffsetSearch was performed using MSFragger as described previously using FragPipe v21.1 with MSFragger 4.0 and philosopher 5.1.0.[5] To quantify specific amino acids the samples were searched against the human Uniprot database (20,649 entries) alongside an integrated contaminant database using FragPipe v 21.1 using the default settings for label-free quantification with matched between runs (LFQ-MBR). Cleavage site was set to Trypsin with cleavage at lysine and arginine but not before proline. Oxidation (+16 and +32) of cysteine and methionine were set as variable modifications alongside carbamidomethylation of cysteine. Furthermore, nitrone modifications (Nitrone AH and WW = 316.1648 Da; Nitrone K and A = 315.1696; Nitrone D and R = 351.1365) were set to tryptophan, methionine, cysteine, and tyrosine as variable modifications. Precursor and fragment mass tolerance were set to 20 ppm. False discovery rate for PSMs, peptides, and proteins was set to 1% using a target-decoy approach.

Hyperreactivity profiling by isoDTB-ABPP.

HEK293 cell lysate was harvested and prepared using sonication, and proteome concentration was normalized to 2.0 mg/mL. For each biological replicate, two 1 mL aliquots of 2.0 mg/mL were used. Prior to tag labeling, each aliquot was treated with 5 µL of nitrone **WW**-alkyne solution (40 or 4 mM stock in DMSO) for a final concentration of 200 or 20 µM. The samples were then irradiated at 370 nm for 5 min at rt. Subsequently, each sample set was treated with 120 µL of either heavy (20 µM nitrone) or light (200 µM nitrone) isoDTB tags containing master mix for 2 h at rt. Master mix contains 510 µL of TBTA (0.9 mg/mL in 1:4 in DMSO/tBuOH), 165 µL of $CuSO_4$ (12.5 mg/mL in H_2O), 165 µL of TCEP (14.0 mg/mL in H_2O), and 160 µL of isoDTB tag (4 mg of either heavy or light tag in DMSO, Click chemistry Tools, 1565). After the reaction, one heavy tagged and one light tagged labeled sample were combined and precipitated in acetone at –20 °C overnight.

After the precipitation, the samples were centrifuged at 7,000 g for 10 min at 4 °C. The supernatant was removed, and the precipitated protein samples were resuspended in cold methanol. Protein pellets were washed with cold methanol (500 µL, 3 times) and dissolved in 600 µL of 8 M urea in 0.1 M tetraethylammonium bromide (TEAB) buffer. Pellets were completely dissolved in the urea solution by sonication, and the urea concentration was adjusted to 2 M urea by adding 1800 µL of 0.1 M TEAB. Two replicates were then combined into a single container, and the combined sample sets were further diluted with 2400 µL of PBS containing 0.2% NP40 (w/v). Proteome was then

incubated with high-capacity streptavidin agarose beads (200 μL per sample, ThermoFisher, 20357) for overnight at 4 °C on hula mixer.

After incubation, the beads were centrifuged for 1 min at 1000 g and the supernatant were removed. The remaining beads were then washed in following order: 3 x PBS with 0.1% NP40, 3 x PBS, and 3 x water. Next, the protein-bound beads were resuspended in 600 μL of 8 M urea in 0.1 M of TEAB buffer, and treated with 30 μL DTT (31 mg/mL, 200 mM in water) for 45 min in 37 °C, then 30 μL iodoacetamide (74 mg/mL, 400 mM in water) for 30 min at 23 °C, subsequently, 30 μL DTT stock for 30 min at 23 °C. After incubation, the beads were centrifuged for 1 min and the supernatants were removed. The protein-bound beads were resuspended in 400 μL of 2 M urea in 0.1 M TEAB. 8 μL of 0.5 mg/mL sequencing grade trypsin (Promega, V5111) was then added, and the bead bound proteins were digested overnight at 37 °C. The resulting samples were then diluted with 800 μL of 0.1% NP40 in PBS, and the beads were washed with the same buffer three times and three more times with PBS and water. Bead-bound peptides were then eluted with 0.1% formic acid in 50% acetonitrile (v/v). Eluted samples were dried using a vacufuge and re-dissolved in 300 μL of water with 0.1% TFA. Suspended samples were then fractionated using high pH reversed-phase peptide fractionation kits (ThermoFisher, 84688).

To survey the landscape of mass shifts observed across all peptides and analyze the amino acid selectivity of the nitrone probes an OpenSearch and OffsetSearch was performed using MSFragger as described previously using FragPipe v21.1 with MSFragger 4.0 and philosopher 5.1.0.[5] Modifications were determined as an increase in mass by 654.3350 Da for the "light" and 660.3425 Da for the "heavy" modification across tryptophan, tyrosine, and methionine. A closed search was performed using FragPipe using the isoDTB-ABPP workflow default settings with minor adjustments. The cleavage site was set to trypsin. Variable modifications for oxidation (+16 and +32) were set for cysteine and methionine alongside carbamidometylation of cysteine. Furthermore, heavy and light isoDTB nitrone modifications were set to tryptophan, methionine, cysteine, and tyrosine as variable modifications. Precursor and fragment mass tolerance were set at 20ppm. False discovery rate for PSMs, peptides, and proteins was set to 1% using a target-decoy approach. High confident modified sties were found at least two times across 4 replicates in the same experiment.

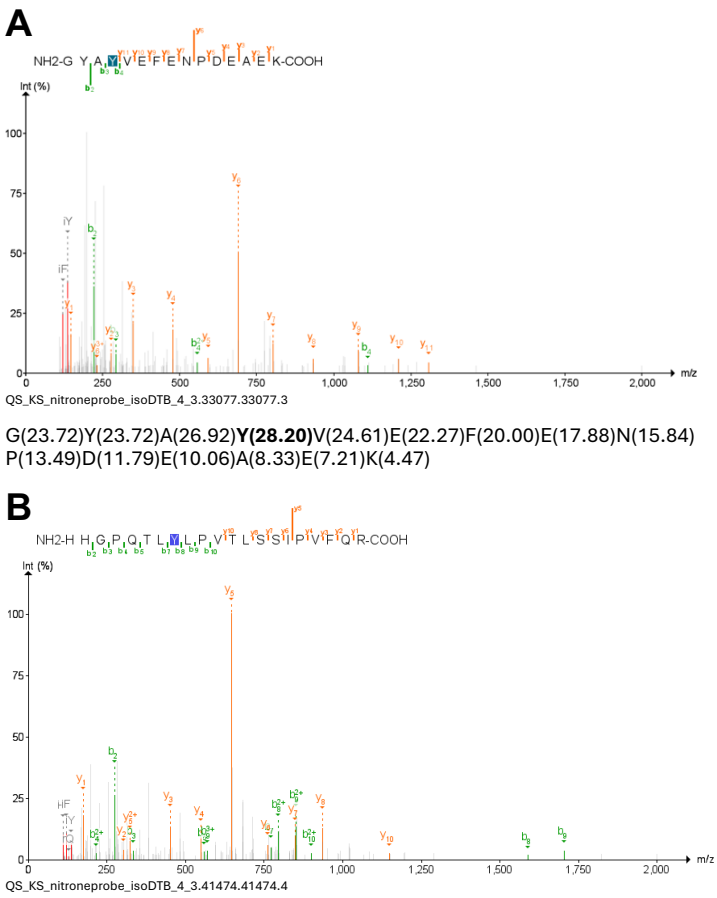


Figure S48. **(A)** MS/MS spectrum matched to peptide GYAyVEFENPDEAEK with a mass shift +654. Positional scores computed by MSFragger (hyperscores computed by placing the mass shift on each amino acid) show the most likely localization to be on Y4, even though there is only one shifted (modification containing fragment) matched, b4. Placing the mass shift on other sites results in lower hyperscores. For example, placing it on V5 leads to the loss of an unshifted fragment ion y11. Similarly, placing it on A3 leads to the loss of an unshifted fragment b3. **(B)** Same as in (A), MS/MS spectrum matched to peptide. HHGPQTLyLPVTLSSIPVFQR. Y8 site has the highest MSFragger positional score. Moving the mass shift to the left (to L7) results in a loss of unshifted fragment b7, whereas moving it to the right results is a loss of shifted fragment b8.

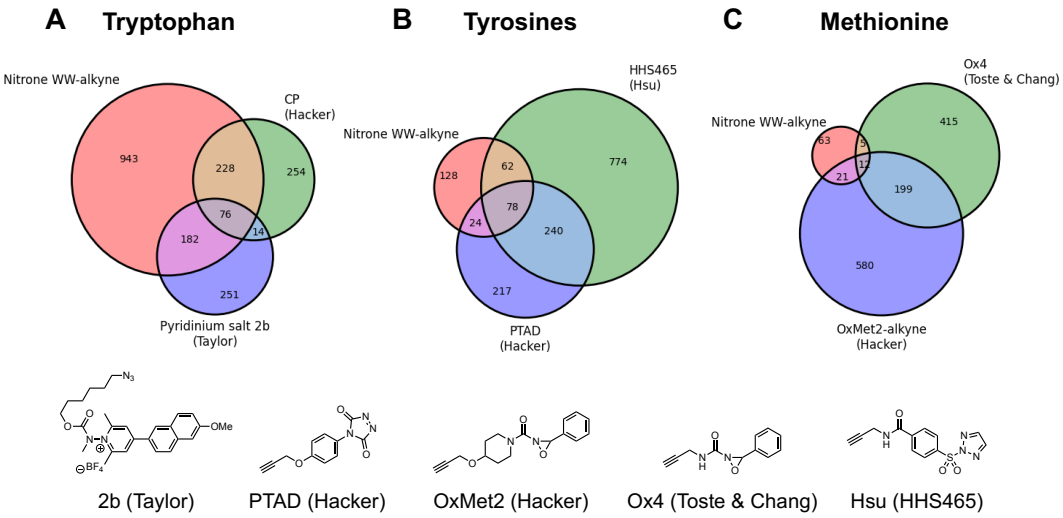


Figure S49. Overlap between unique *proteins* labeled by nitrones (from isoDTB-ABPP hyperreactivity profiling) and other reagents reported by the Hacker,[5] with reagents from the Hacker, Taylor,[6] and Hsu[7] labs (reagent names from the original publications are given in parentheses) at (**A**) tryptophan, (**B**) methionine, or (**C**) tyrosine residues.

Table S3. Overlap between modified tryptophan *residues*.

		Nitrones						
		K	**A**	**AH**	**WW**	**D**	**R**	**N-sulfonyl oxaziridine[1]**
Nitrones	**K**	70	64	56	58	11	41	11
	A	64	115	80	86	11	51	18
	AH	56	80	362	287	14	67	57
	WW	58	86	287	367	14	70	55
	D	11	11	14	14	18	14	10
	R	41	51	67	70	14	83	17
N-sulfonyl oxaziridine[1]		11	18	57	55	10	17	341

Considering only the Trp residues that appeared modified in all replicates (2 replicates for nitrones from label-free profiling and 3 replicates for N-sulfonyl oxaziridine from publication). Diagonal values indicate the number of sites that appeared in all replicates for that reagent, off diagonal values indicate overlap of those sets between reagents.[1] N-Sulfonyl oxaziridine labeled sites are from the overlap of individual replicates in the supplementary data of "Ox-W18 desthiobiotin probe" as reported in *Nature* **2024**, 627, 680–687.

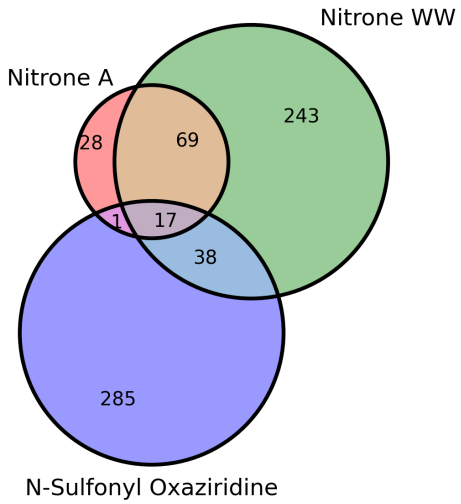


Figure S50. Overlap of modified *Trp sites* between nitrones A and WW alkynes (intersection of two replicates from label-free profiling), and an *N*-sulfonyl oxaziridine (data for Ox-W18 from *Nature* **2024**, 627, 680–687).

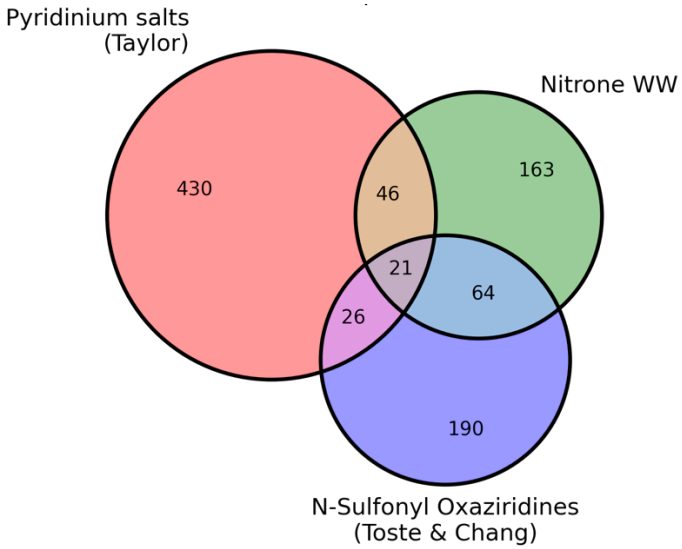


Figure S51. Overlap between *proteins* enriched using Nitrone WW alkyne (intersection of two replicates from label-free profiling), *N*-sulfonyl oxaziridine alkyne (reagent Ox-W18, intersection of 3 replicates as reported in *Nature* **2024**, 627, 680–687), and *N*-carbamoyl pyridinium salt (reagent 2b as reported in *J. Am. Chem. Soc.* 2022, 144, 14, 6227–6236, replicate data not provided).

Additional gel Images

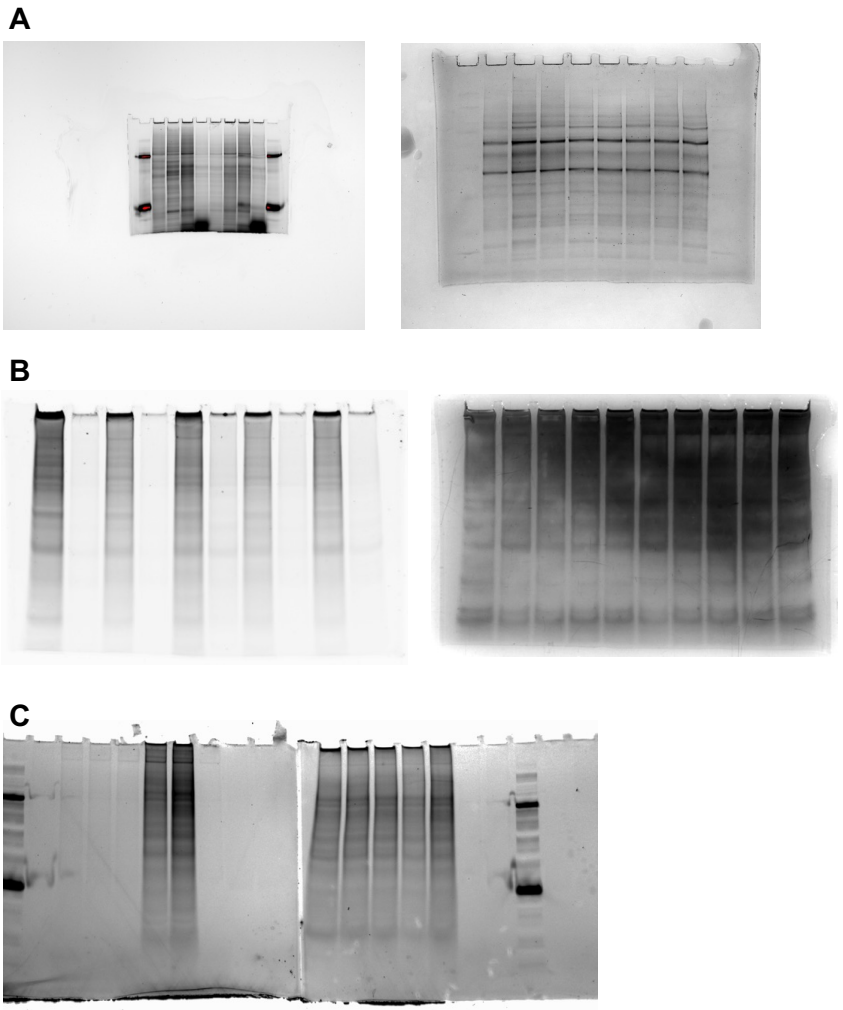
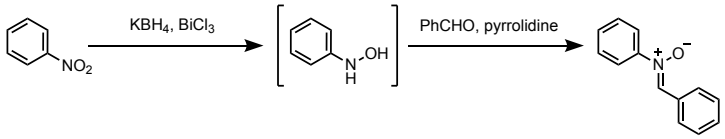


Figure S52. Uncropped images for (**A**) Figure 4 and Figure S29, (**B**) Figure S28, and (**C**) Figure S30.

Nitrone synthesis

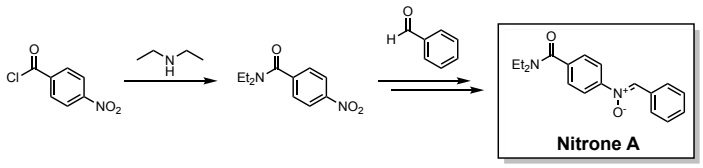
General procedure



Nitro compound (1.0 equiv) and bismuth chloride (0.2 equiv) were added to a solution of 10% water, 90% ethanol at approximately 400 mM concentration, resulting in a white suspension. The reaction mixture was purged with nitrogen and cooled to 0 °C. Potassium borohydride (1.4 equiv) was added in batches while stirring, the reaction mixture turned black and gas evolved. The reaction mixture was maintained under a nitrogen atmosphere and sonicated for approximately 0.5 h or until starting material was entirely consumed, as judged by TLC (10/90 MeOH/DCM, UV visualization). The

black suspension was filtered through a layered pad of celite and silica, then washed with copious amounts of methanol, stopping prior to the elution of black-colored material off the silica. The solution was concentrated under reduced pressure using a rotary evaporator. The resulting hydroxylamine was immediately used without further purification. The reduction product was entirely solubilized in a suitable solvent (usually DCM, but otherwise ACN, THF, MeOH or mixtures thereof) at a concentration of approximately 1 M. Aldehyde (2–5 equiv) and either: (A) pyrrolidine (0.2 equiv) or (B) methanesulfonic acid (0.2 equiv) and 3 Å molecular sieves were added and the mixture was allowed to stir overnight at rt, covered in aluminum foil. If copious amounts of precipitate formed, it was filtered off and often found to be the pure nitrone. Otherwise, the solution was purified without work-up using silica chromatography with an eluent that provided optimal resolution (usually 1-5% methanol in either dichloromethane or 1:1 mixture of dichloromethane/ethyl acetate).

Nitrone A

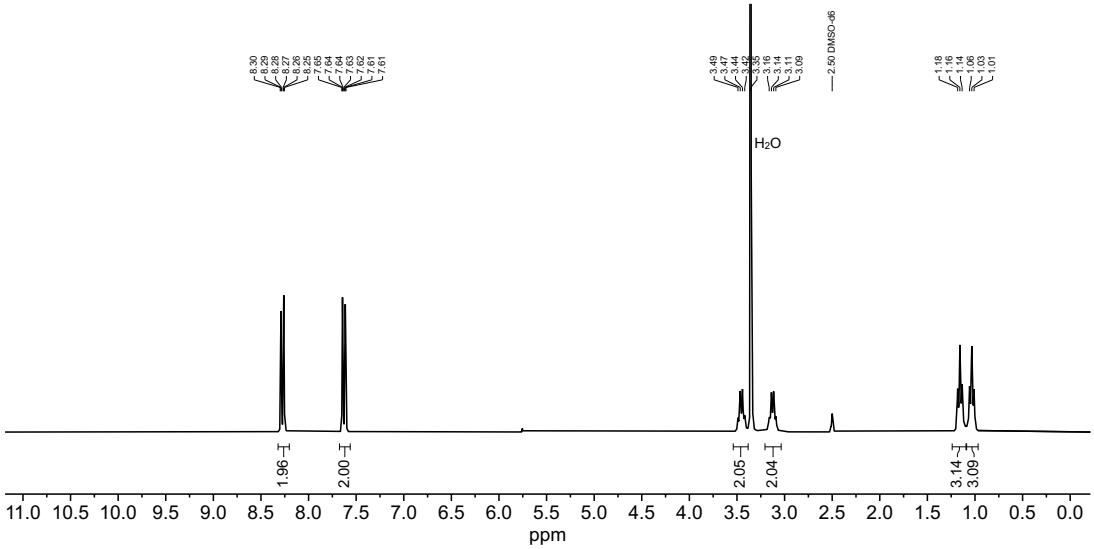


N,*N*-diethyl-4-nitrobenzamide

4-Nitrobenzoyl chloride (5.0 g, 27 mmol, 1.0 equiv) was dissolved in 24 mL of dichloromethane (DCM) under nitrogen atmosphere and cooled to 0 °C. Diethylamine (5.6 mL, 67 mmol, 2.5 equiv) was added dropwise. The reaction was allowed to warm to rt while stirring over 2 h. Solvent was removed using a rotary evaporator under reduced pressure, the crude was reconstituted in ethyl acetate and extracted with 0.1 M NaH2PO4 (pH 4) until the aqueous layer remained colorless. Organic layer was washed with brine, dried with Na_2SO_4, and concentrated using a rotary evaporator under reduced pressure. The resulting red oil, which crystallized upon cooling, was used without further purification. Yield 75%.

^{1}H NMR (300 MHz, DMSO) δ 8.33 – 8.22 (m, 1H), 7.68 – 7.58 (m, 1H), 3.45 (q, *J* = 7.1 Hz, 1H), 3.13 (q, *J* = 7.0 Hz, 1H), 1.16 (t, *J* = 7.0 Hz, 2H), 1.03 (t, *J* = 7.1 Hz, 2H).

^{1}H NMR

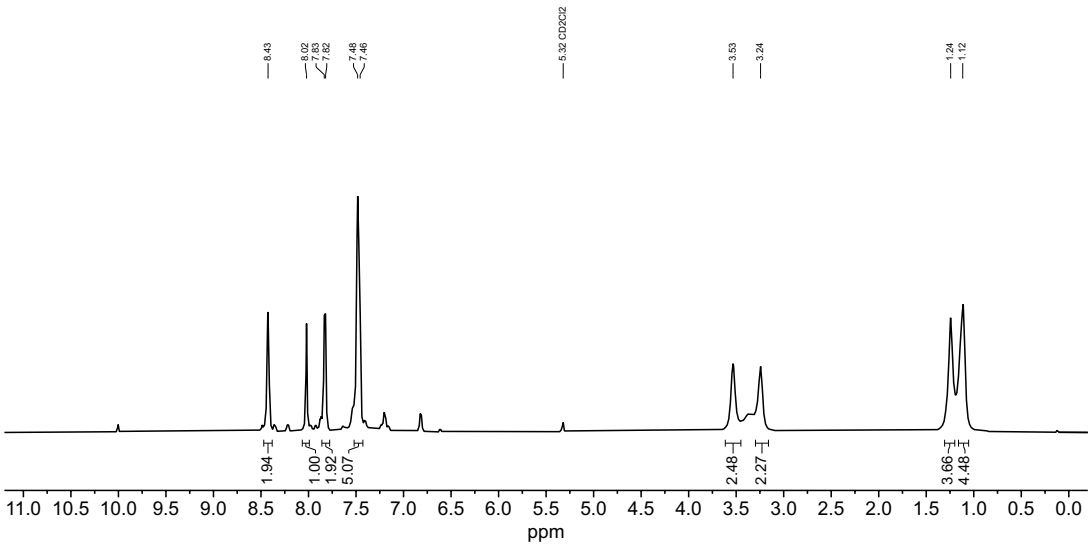


(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-1-phenylmethanimine oxide (Nitrone A)

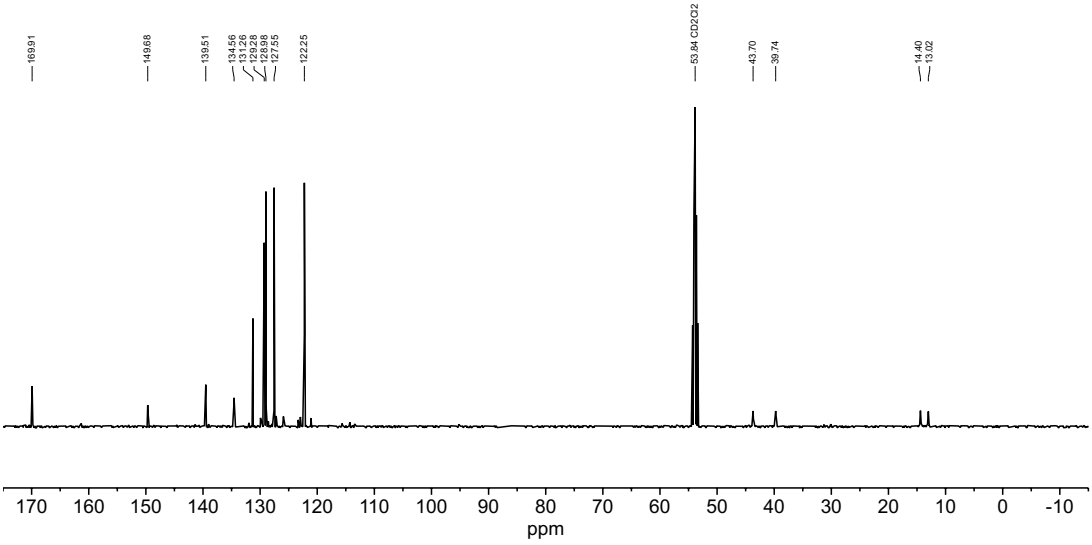
Prepared according to the general procedure for synthesis of nitrones. Yield: 48% of a buff colored solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.43 (s, 2H), 8.02 (s, 1H), 7.83 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 15.8 Hz, 5H), 3.53 (br s, 2H), 3.24 (br s, 2H), 1.24 (br s, 3H), 1.12 (br s, 3H).
^{13}C NMR (126 MHz, CD_2Cl_2) δ 169.91, 149.68, 139.51, 134.56, 131.26, 129.28, 128.98, 127.55, 122.25, 43.70, 39.74, 14.40, 13.02.
HRMS (ESI) m/z for $C_{18}H_{20}N_2O_2$, $[2M+K]^+$ calcd: 883.5498, found: 883.5487; $[2M+Na]^+$ calcd: 867.5758, found: 867.5754; $[M+Na]^+$ calcd: 445.2825, found: 445.2857.

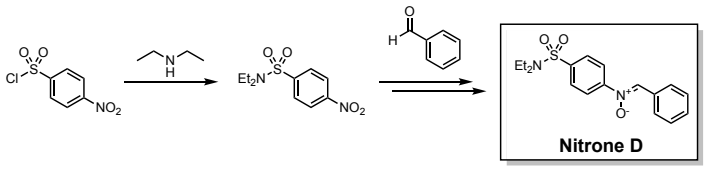
^{1}H NMR



13C NMR



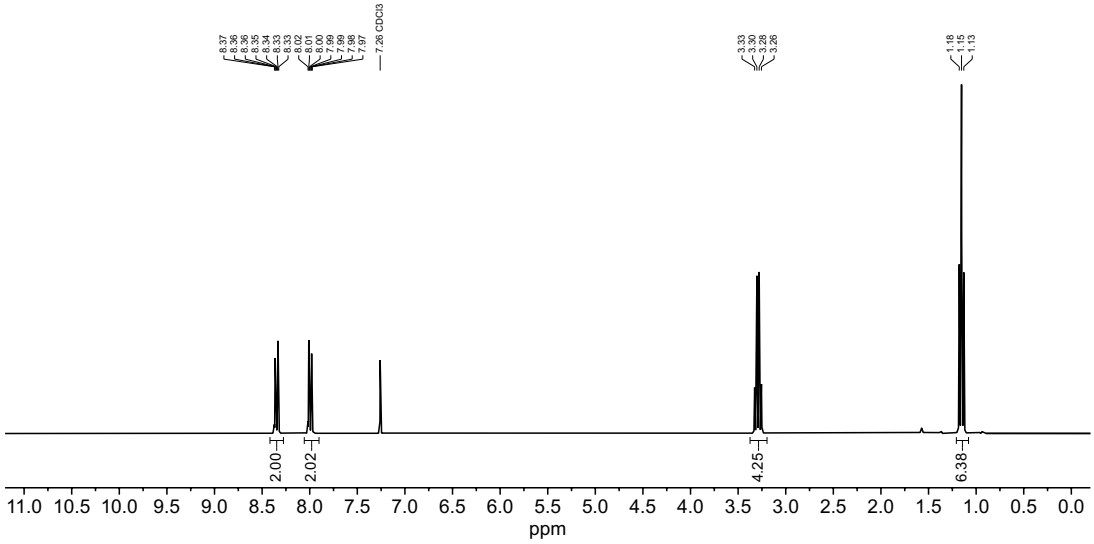
Nitrone D



***N*,*N*-diethyl-4-nitrobenzenesulfonamide**
4-Nitrobenzenesulfonyl chloride (4.0 g, 18 mmol, 1.0 equiv) was dissolved in 18 mL of tetrahydrofuran (THF) and cooled to 0 °C. Diethylamine (4.7 mL, 45 mmol, 2.5 equiv) was added dropwise, then the reaction was allowed to warm to rt and stir overnight. The solvent was removed using a rotary evaporator under reduced pressure, the residue was reconstituted in ethyl acetate, washed with 0.1 M NaH2PO4 until the aqueous layer was colorless. Washed the organic layer with brine and dried with Na_2SO_4, then concentrated using a rotary evaporator under reduced pressure to give a tan solid. Yield: 92%

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 – 8.31 (m, 2H), 8.03 – 7.94 (m, 2H), 3.29 (q, *J* = 7.1 Hz, 4H), 1.15 (t, *J* = 7.1 Hz, 6H).

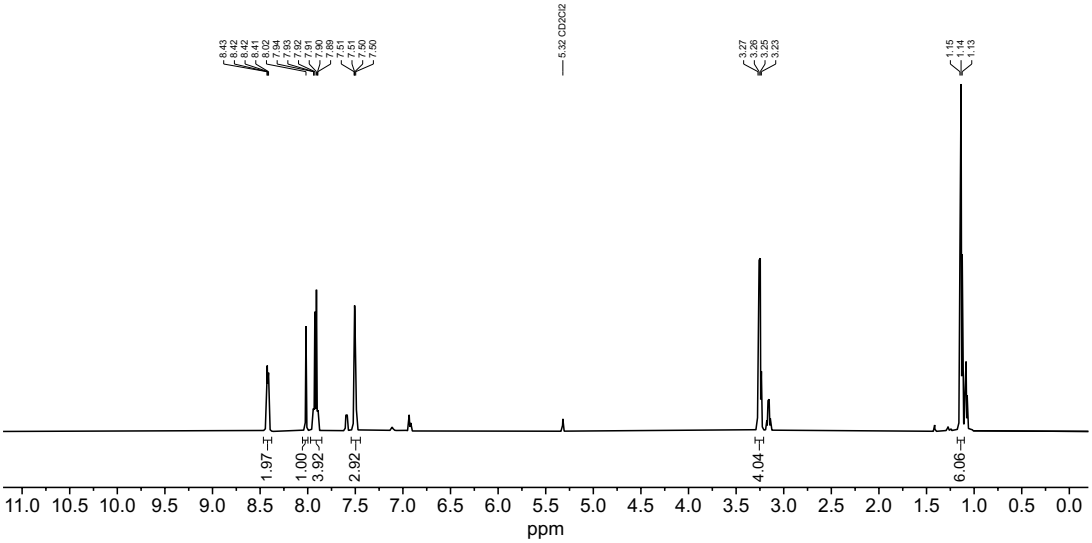
^{1}H NMR



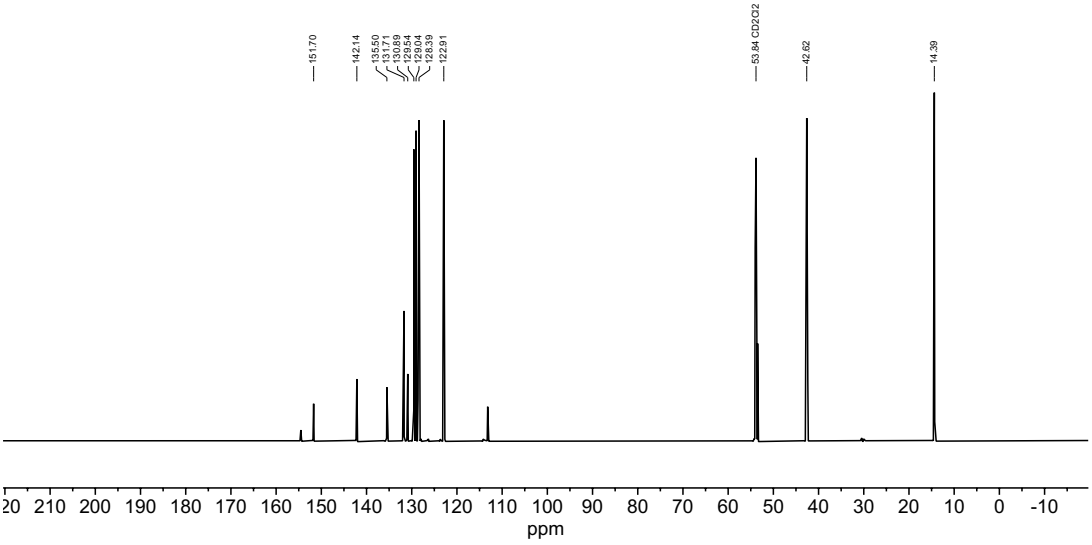
(*Z*)-*N*-(4-(*N*,*N*-diethylsulfamoyl)phenyl)-1-phenylmethanimine oxide (Nitrone D)
Prepared according to the general procedure for synthesis of nitrones. Yield: 54% of a tan solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.42 (dd, *J* = 7.4, 2.4 Hz, 2H), 8.02 (s, 1H), 7.92 (q, *J* = 8.8 Hz, 4H), 7.52 – 7.49 (m, 3H), 3.25 (q, *J* = 7.2 Hz, 4H), 1.14 (t, *J* = 7.2 Hz, 6H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 151.70, 142.14, 135.50, 131.71, 130.89, 129.54, 129.04, 128.39, 122.91, 42.62, 14.39.
HRMS (ESI) m/z for $C_{17}H_{20}N_2O_3S$ $[M+H]^+$ calcd: 333.12729, found: 333.12795.

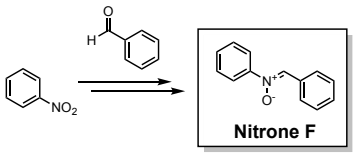
^{1}H NMR



13C NMR



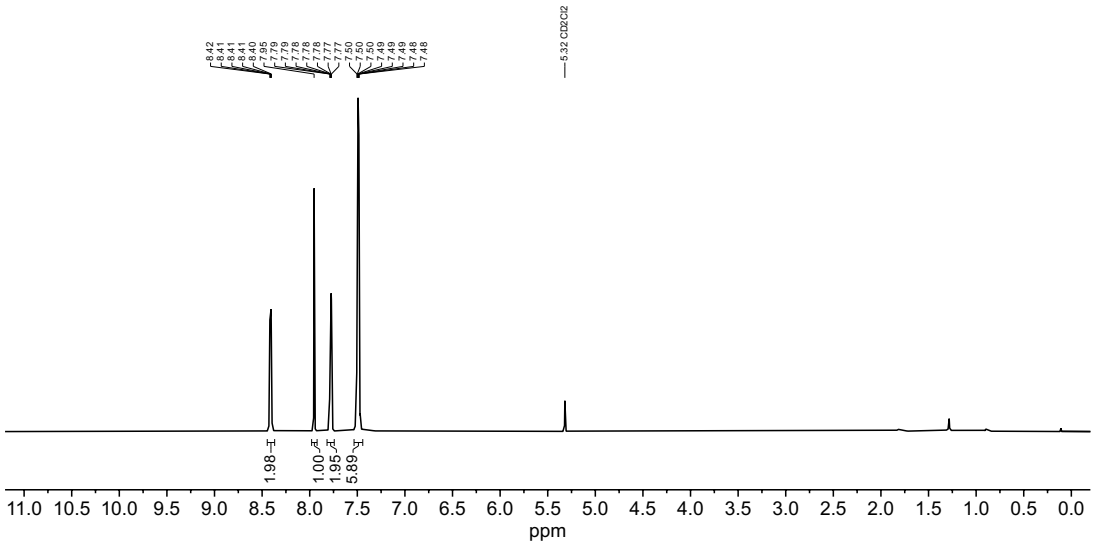
Nitrone F



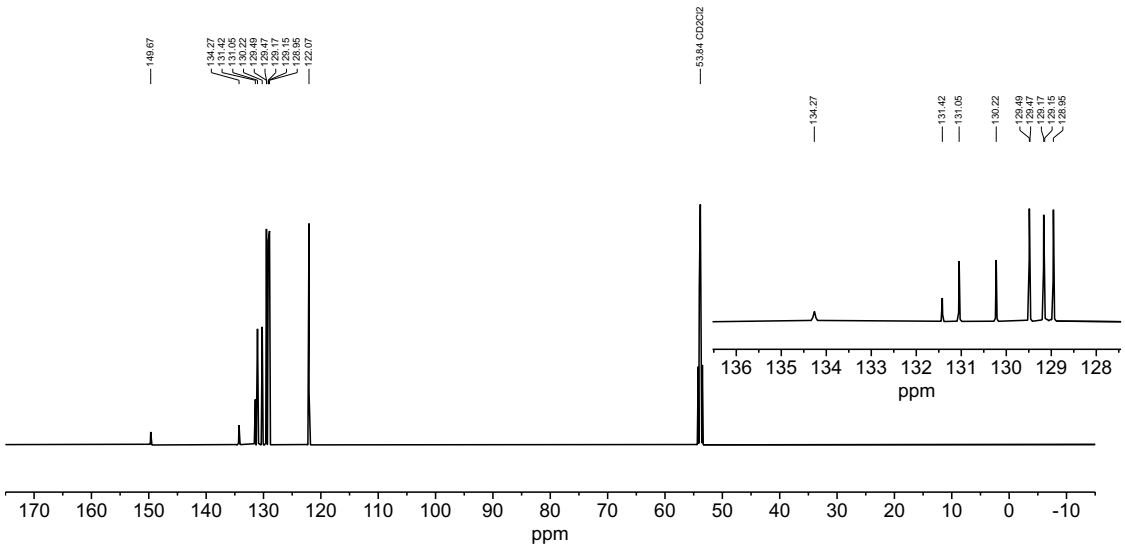
(*Z*)-*N*,1-diphenylmethanimine oxide (Nitrone F)
Prepared according to the general procedure for synthesis of nitrones. Yield: 14% of a buff colored solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.43 – 8.39 (m, 2H), 7.95 (s, 1H), 7.80 – 7.76 (m, 2H), 7.52 – 7.47 (m, 6H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 149.67, 134.27, 131.42, 131.05, 130.22, 129.49, 129.47, 129.17, 129.15, 128.95, 122.07.
HRMS (ESI) m/z for $C_{13}H_{11}NO$ $[M+H]^+$ calcd: 198.09189, found: 198.09255

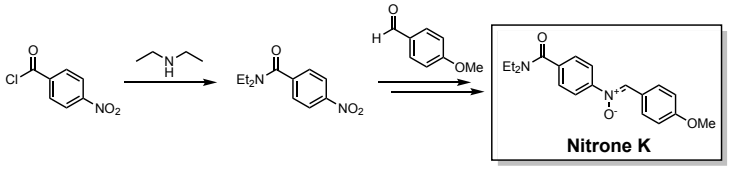
^{1}H NMR



13C NMR


149.67
134.27
131.42
131.05
130.22
129.49
129.47
129.17
129.15
128.95
122.07
53.84 CD2Cl2
134.27
131.42
131.05
130.22
129.49
129.47
129.17
129.15
128.95
136 135 134 133 132 131 130 129 128
ppm
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

Nitrone K

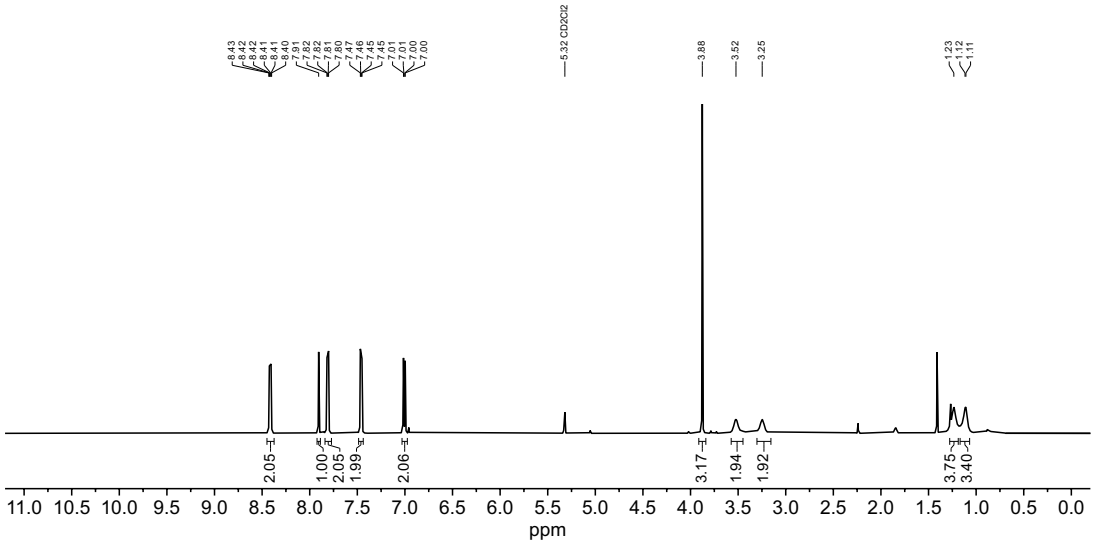


N,*N*-diethyl-4-nitrobenzamide
Prepared according to procedure outline in Nitrone A.

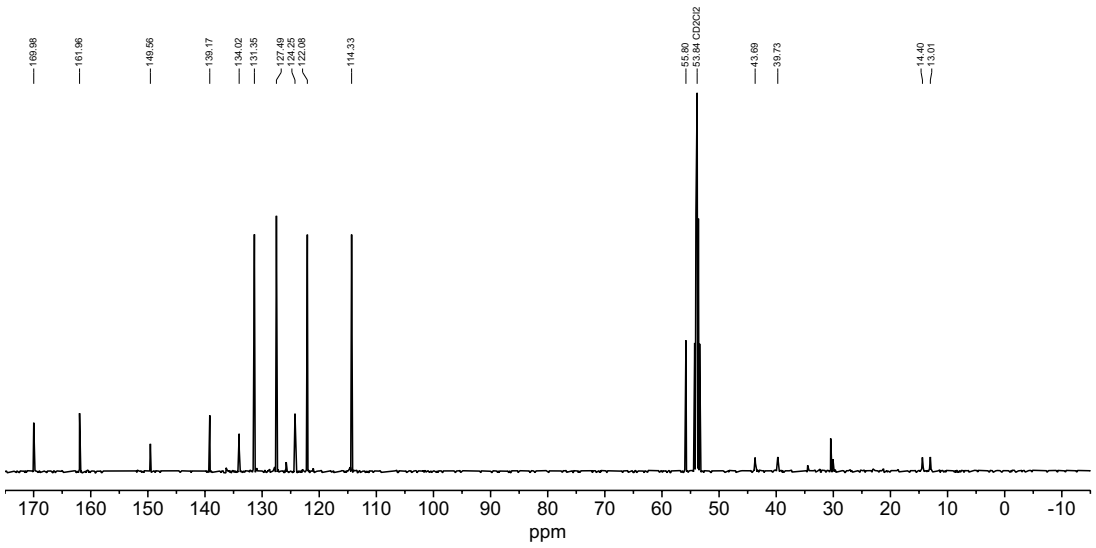
(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-1-(4-methoxyphenyl)methanimine oxide (Nitrone K)
Prepared according to the general procedure for synthesis of nitrones. Yield: 47% of a buff colored solid.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.44 – 8.39 (m, 2H), 7.91 (s, 1H), 7.83 – 7.79 (m, 2H), 7.48 – 7.44 (m, 2H), 7.03 – 6.99 (m, 2H), 3.88 (s, 3H), 3.52 (br s, 2H), 3.25 (br s, 2H), 1.23 (br s, 3H), 1.11 (br d, *J* = 4.8 Hz, 3H).
^{13}C NMR (126 MHz, CD_2Cl_2) δ 169.98, 161.96, 149.56, 139.17, 134.02, 131.35, 127.49, 124.25, 122.08, 114.33, 55.80, 43.69, 39.73, 14.40, 13.01.
HRMS (ESI) m/z for $C_{19}H_{22}N_2O_3$ $[M+H]^+$ calcd: 327.17087, found: 327.17166.

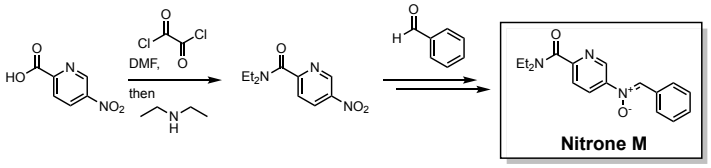
^{1}H NMR



13C NMR


169.98
161.96
149.56
138.17
134.02
131.35
127.49
124.45
122.08
114.33
55.80
53.84 CD2Cl2
43.69
39.73
14.40
13.01
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

Nitrone M



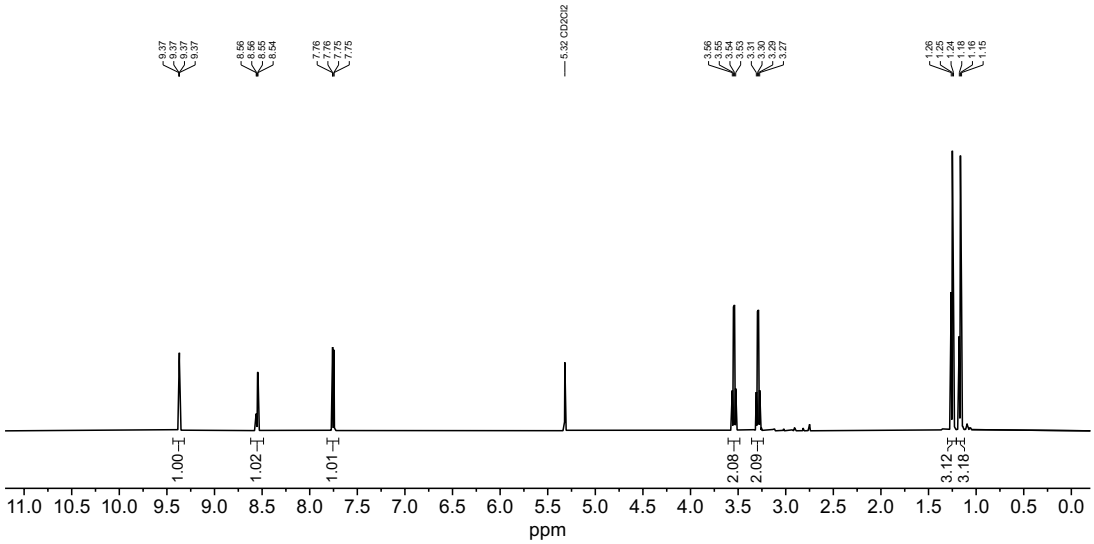
N,*N*-diethyl-5-nitropicolinamide

Anhydrous DMF (2.0 mL, 26 mmol, 0.43 equiv) was added to 250 mL of anhydrous DCM and the solution cooled to 0°C. Oxalyl chloride (16 mL, 23 g, 178 mmol, 3.0 equiv) was added slowly, gas evolved. Immediately upon finishing addition of oxalyl chloride, the 5-nitropicolinic acid (10 g, 60 mmol, 1.0 equiv) was added in batches over the course of approximately 30 min. The solution mixture was allowed to warm to rt slowly and stirred for 5 h. The solution was then dried under reduced pressure using a rotary evaporator in a fume hood with saturated sodium bicarbonate in the solvent collection trap. The resulting pale-yellow solid was used without further purification.

A part of the crude product (6.0 g, 32 mmol, 1.0 equiv) was reconstituted in ~ 250 mL of anhydrous DCM and cooled to 0°C. Diethylamine (10. mL, 7.1 g, 96 mmol, 3.0 equiv) was added slowly, the reaction color became darker red. The reaction mixture was allowed to warm to rt and stir overnight. The crude reaction mixture was washed with 0.2 M Na_2HPO_4 and then 1 M HCl until the aqueous phase was nearly color-free, then brine, finally dried with sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was used without further purification. Yield: 64% of a tan solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 9.37 (dd, *J* = 2.6, 0.7 Hz, 1H), 8.55 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.75 (dd, *J* = 8.5, 0.8 Hz, 1H), 3.54 (q, *J* = 7.1 Hz, 2H), 3.29 (q, *J* = 7.1 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 3H).

^{1}H NMR

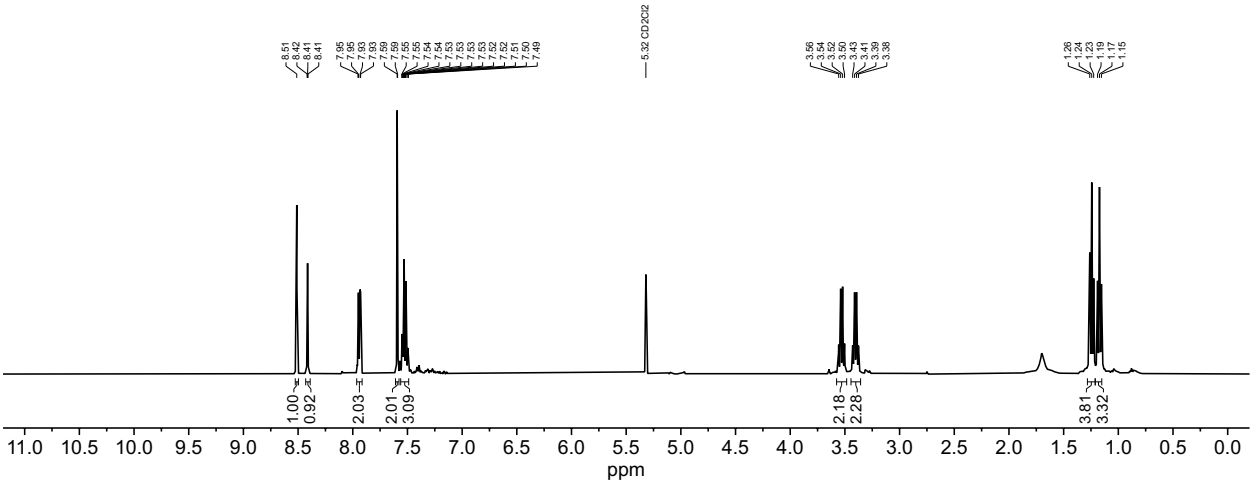


(*Z*)-*N*-(6-(diethylcarbamoyl)pyridin-3-yl)-1-phenylmethanimine oxide (Nitrone M)

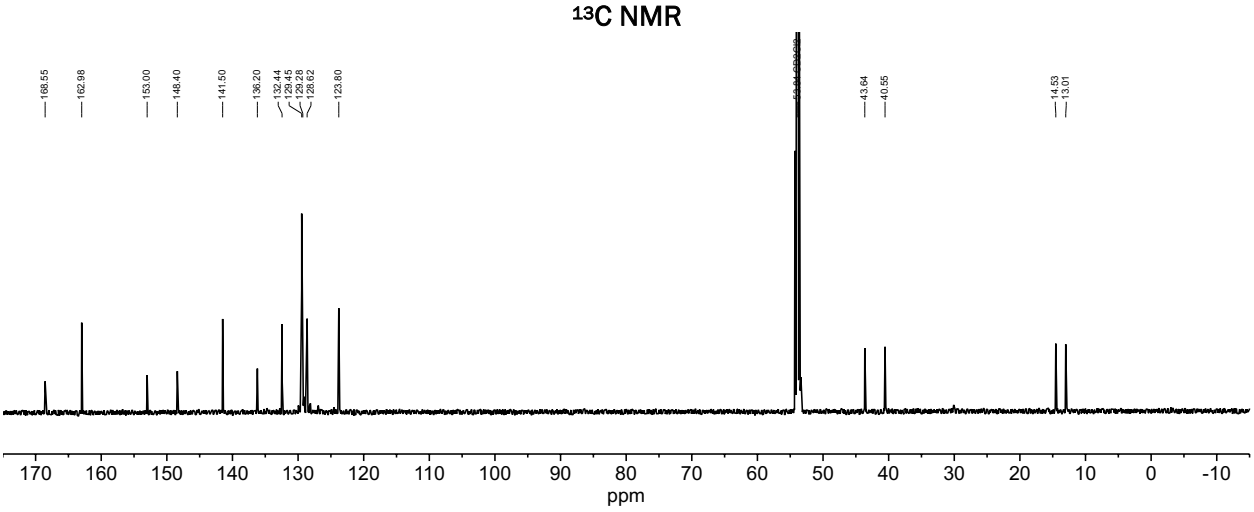
Prepared according to the general procedure for synthesis of nitrones. Yield: 15% of an amber colored oil.

^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.51 (s, 1H), 8.41 (t, *J* = 1.7 Hz, 1H), 7.94 (dd, *J* = 7.9, 1.7 Hz, 2H), 7.59 (d, *J* = 1.6 Hz, 2H), 7.56 – 7.49 (m, 3H), 3.53 (q, *J* = 7.1 Hz, 2H), 3.40 (q, *J* = 7.1 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 4H), 1.17 (t, *J* = 7.0 Hz, 3H).
^{13}C NMR (126 MHz, CD_2Cl_2) δ 168.55, 162.98, 153.00, 148.40, 141.50, 136.20, 132.44, 129.45, 129.28, 128.62, 123.80, 43.64, 40.55, 14.53, 13.01.
HRMS (ESI) m/z for $C_{17}H_{20}N_3O_2$ $[M+H]^+$ calcd: 298.1550, found: 298.15575.

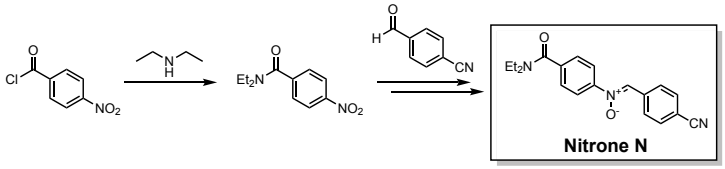
^{1}H NMR



^{13}C NMR



Nitrone N



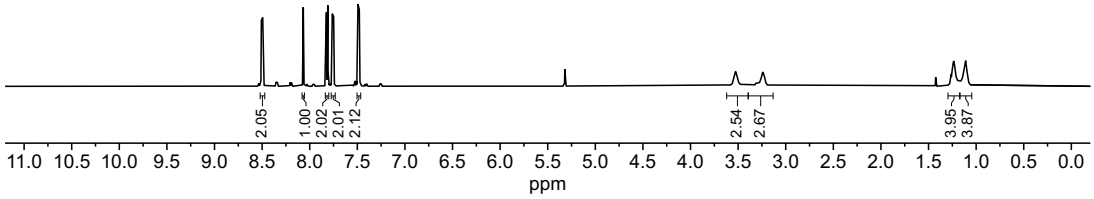
N,*N*-diethyl-4-nitrobenzamide
Prepared according to procedure outline in Nitrone A.

(*Z*)-1-(4-cyanophenyl)-*N*-(4-(diethylcarbamoyl)phenyl)methanimine oxide (Nitrone N)
Prepared according to the general procedure for synthesis of nitrones. Yield: 43% of a buff colored solid.

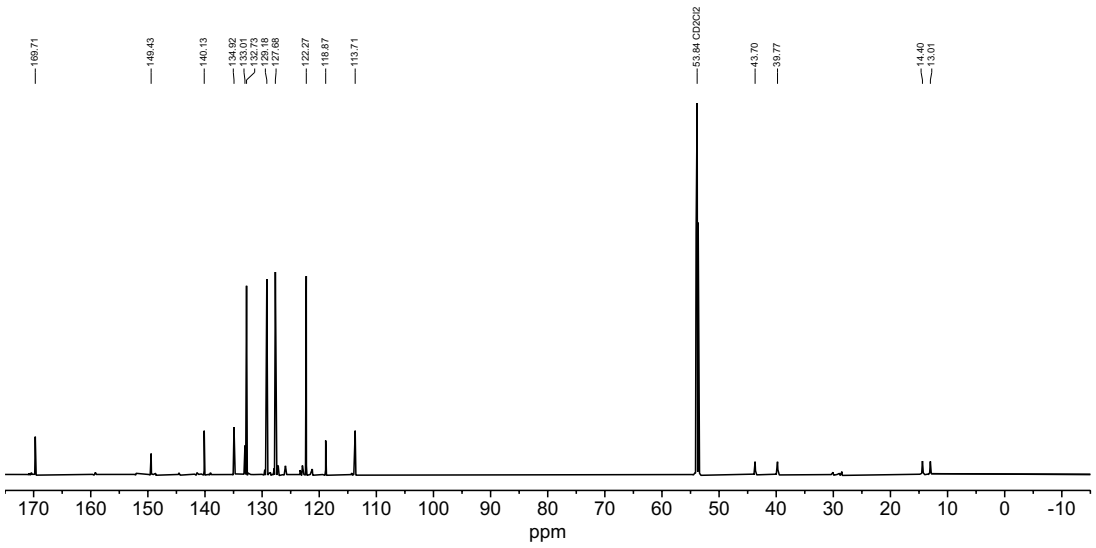
^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.52 – 8.48 (m, 2H), 8.07 (s, 1H), 7.84 – 7.80 (m, 2H), 7.77 – 7.74 (m, 2H), 7.51 – 7.47 (m, 2H), 3.52 (br s, 2H), 3.24 (br s, 2H), 1.24 (br s, 3H), 1.11 (br s, 3H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 169.71, 149.43, 140.13, 134.92, 133.01, 132.73, 129.18, 127.68, 122.27, 118.87, 113.71, 43.70, 39.77, 14.40, 13.01.
HRMS (ESI) m/z for $C_{19}H_{19}N_3O_2$ $[M+H]^+$ calcd: 322.15555, found: 322.15614.

^{1}H NMR

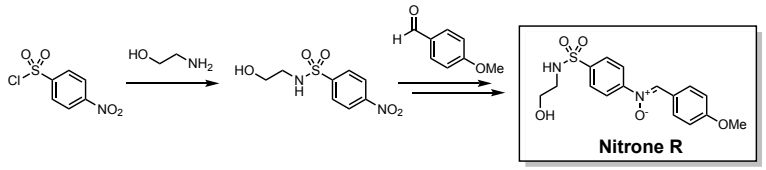




13C NMR


169.71
148.43
140.13
134.92
133.01
132.73
129.18
127.68
122.27
118.87
113.71
53.84 CD2Cl2
43.70
39.77
14.40
13.01
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

Nitrone R

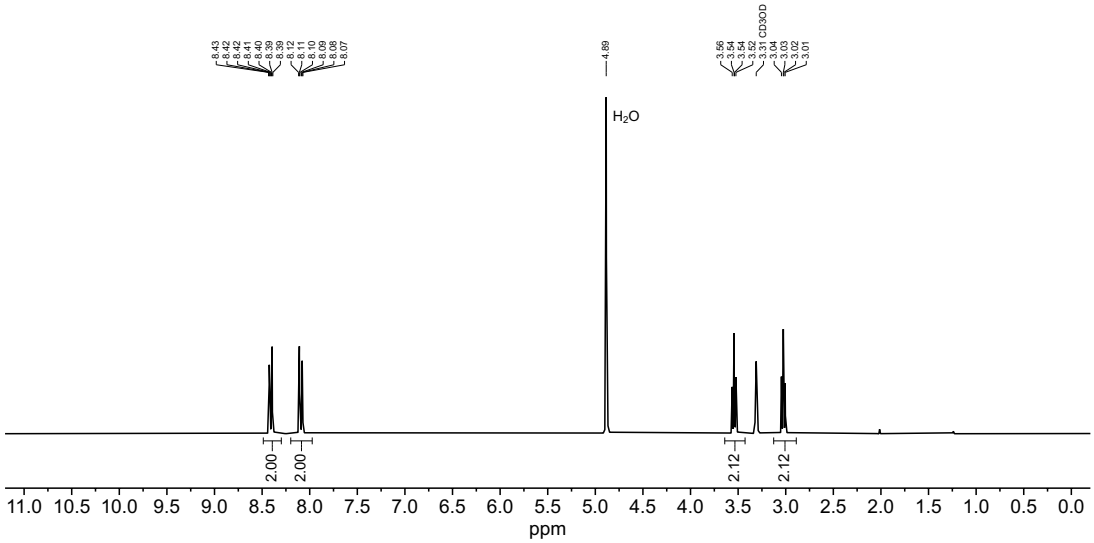


N-(2-hydroxyethyl)-4-nitrobenzenesulfonamide

Ethanolamine (2.1 g, 2.0 mL, 34 mmol, 2.5 equiv) was dissolved in 13 mL of anhydrous THF and cooled to 0 °C under nitrogen. A solution of 4-nitrobenzenesulfonyl chloride (3.0 g, 14 mmol, 1.0 equiv) in 5.0 mL of anhydrous THF was added to the reaction dropwise. The reaction was allowed to stir for 1 h at 0 °C prior to warming to rt and stirring an additional 1 h. The solvent was removed under reduced pressure, the reaction was reconstituted in ethyl acetate, washed with saturated sodium bicarbonate solution, 1 M HCl, and brine. The organic layer was dried with sodium sulfate, filtered, and concentrated under reduced pressure. The product was used without further purification. Yield: 88% of slightly yellow powder.

^{1}H NMR (300 MHz, MeOD) δ 8.46 – 8.36 (m, 2H), 8.15 – 8.04 (m, 2H), 3.54 (t, *J* = 5.8 Hz, 2H), 3.03 (t, *J* = 5.8 Hz, 2H).

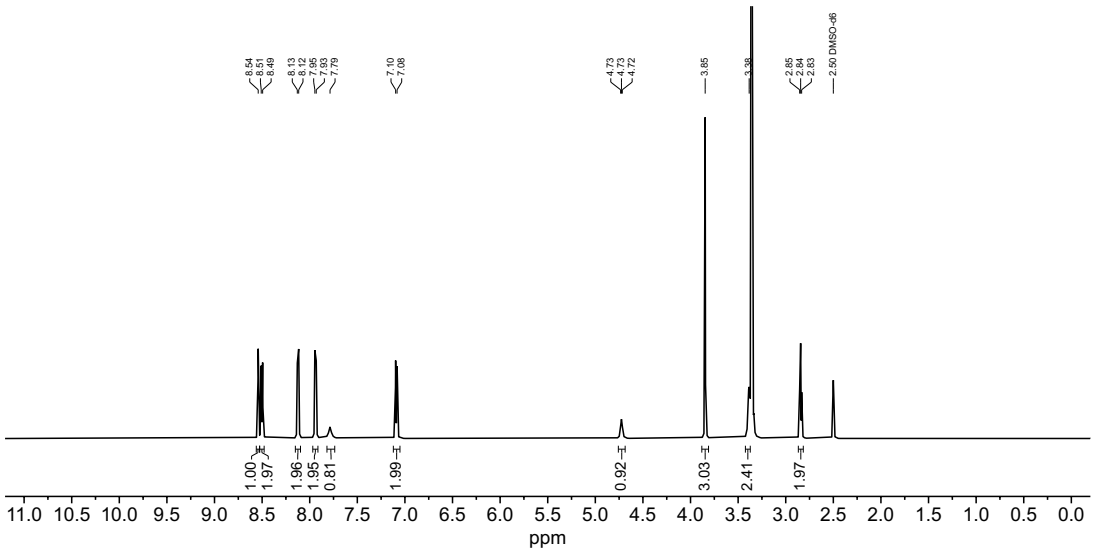
^{1}H NMR



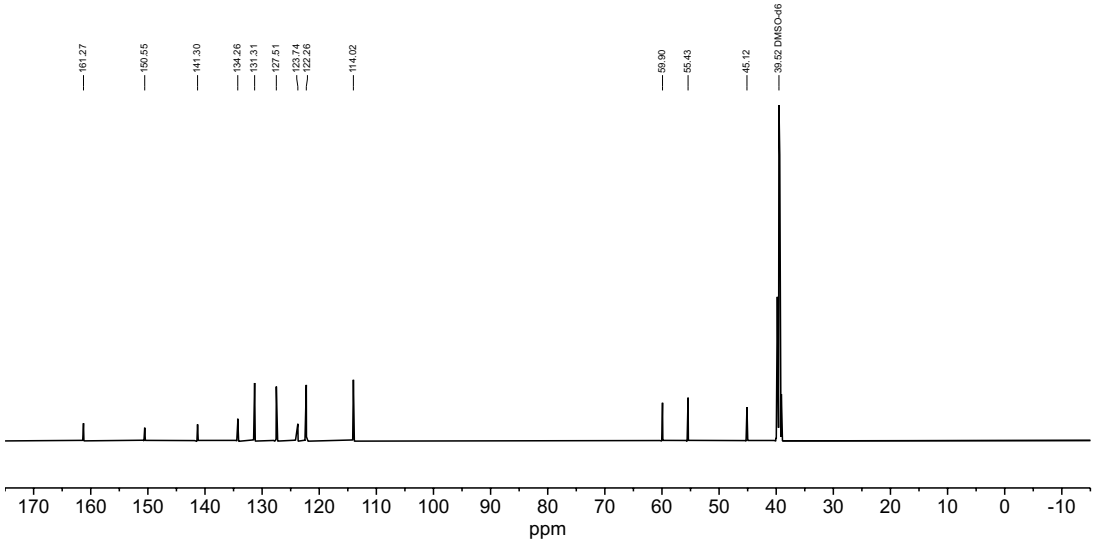
(*Z*)-*N*-(4-(*N*-(2-hydroxyethyl)sulfamoyl)phenyl)-1-(4-methoxyphenyl)methanimine oxide (Nitrone R)
Prepared according to the general procedure for synthesis of nitrones. Yield: 35% of a yellow solid.

^{1}H NMR (600 MHz, DMSO) δ 8.54 (s, 1H), 8.50 (d, *J* = 9.0 Hz, 2H), 8.12 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.7 Hz, 2H), 7.79 (s, 1H), 7.09 (d, *J* = 9.0 Hz, 2H), 4.73 (t, *J* = 5.4 Hz, 1H), 3.85 (s, 3H), 3.39 – 3.38 (m, 2H), 2.84 (t, *J* = 6.3 Hz, 2H).
^{13}C NMR (151 MHz, DMSO) δ 161.27, 150.55, 141.30, 134.26, 131.31, 127.51, 123.74, 122.26, 114.02, 59.90, 55.43, 45.12.
HRMS (ESI) m/z for $C_{16}H_{18}N_2O_5S$ $[M+H]^+$ calcd: 351.10147, found: 351.10199.

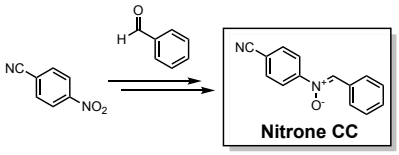
^{1}H NMR



13-C NMR

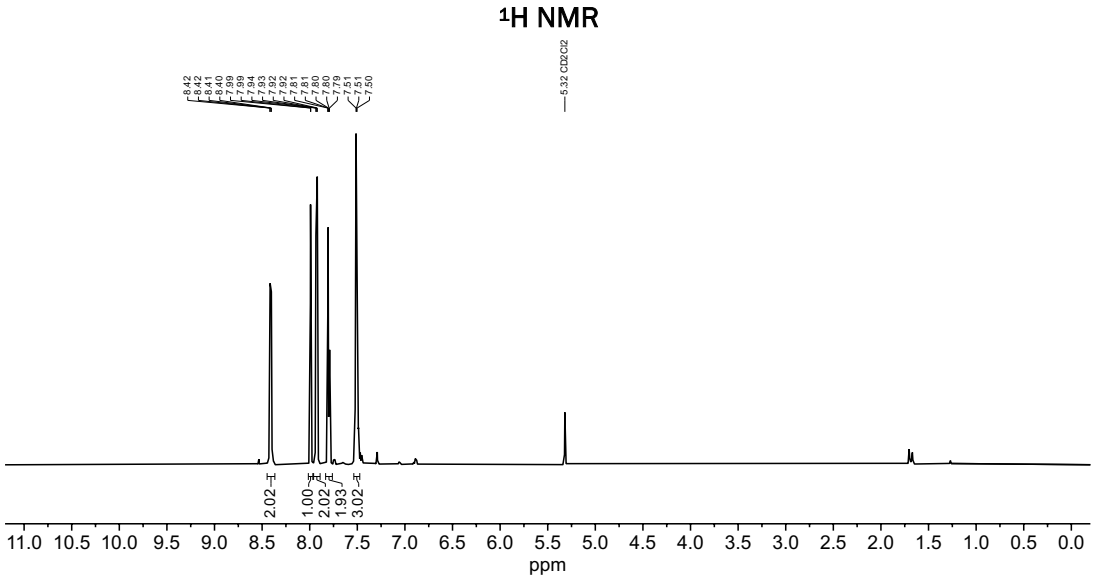


Nitrone CC

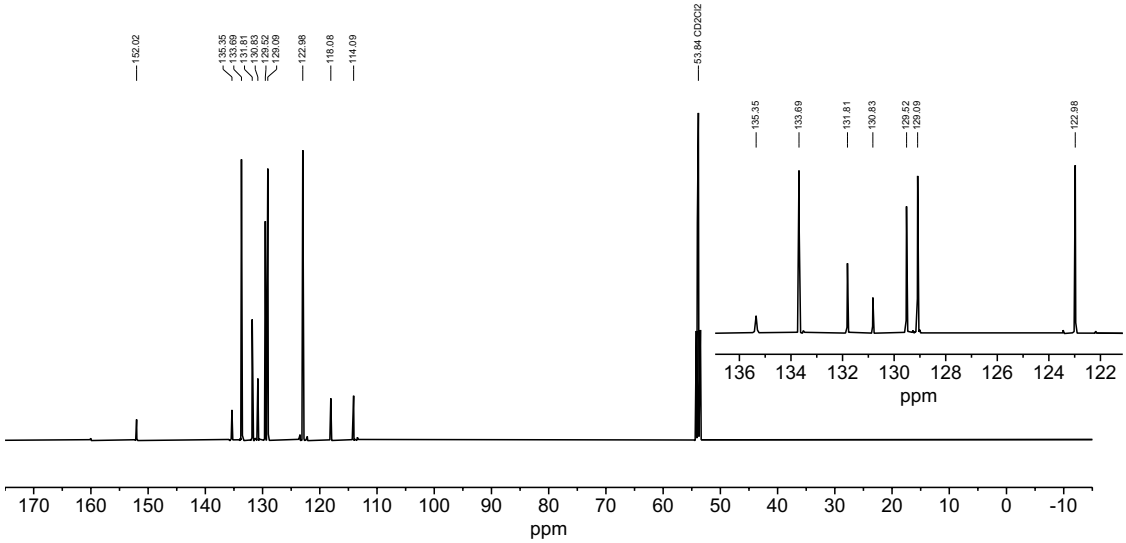


(*Z*)-*N*-(4-cyanophenyl)-1-phenylmethanimine oxide (Nitrone CC)
Prepared according to the general procedure for synthesis of nitrones. Yield: 7% of a yellow solid.

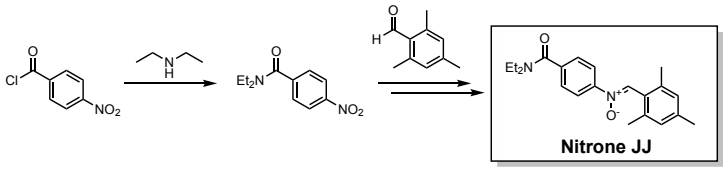
^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.41 (dd, *J* = 7.6, 2.2 Hz, 2H), 8.01 – 7.98 (m, 1H), 7.95 – 7.91 (m, 2H), 7.80 (dd, *J* = 8.8, 2.2 Hz, 2H), 7.52 – 7.49 (m, 3H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 152.02, 135.35, 133.69, 131.81, 130.83, 129.52, 129.09, 122.98, 118.08, 114.09.
HRMS (ESI) m/z for $C_{14}H_{10}N_2O$ $[M+H]^+$ calcd: 223.08714, found: 223.08681.



13C NMR


152.02
135.35
133.69
131.81
130.83
129.52
129.09
122.98
118.08
114.09
53.84 CD2Cl2
135.35
133.69
131.81
130.83
129.52
129.09
122.98
136 134 132 130 128 126 124 122
ppm
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

Nitrone JJ

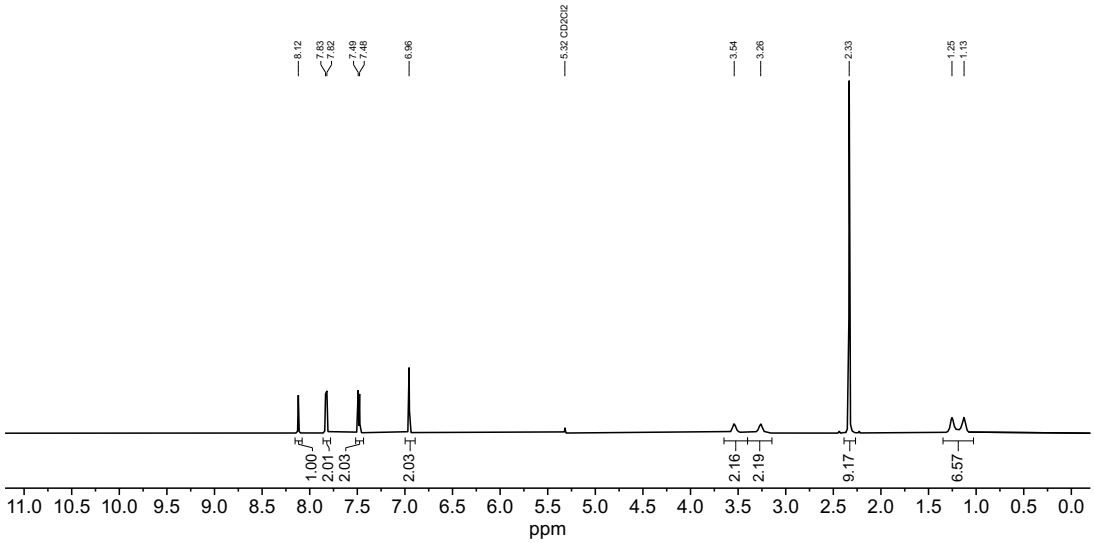


***N*,*N*-diethyl-4-nitrobenzamide**
Prepared according to procedure outline in Nitrone A.

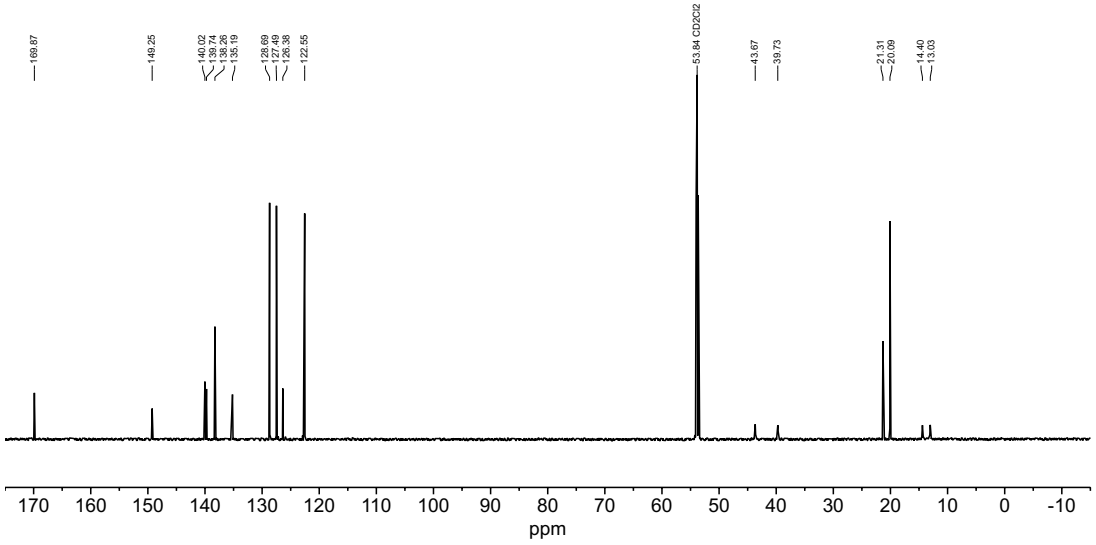
(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-1-mesitylmethanimine oxide (Nitrone JJ)
Prepared according to the general procedure for synthesis of nitrones. Yield: 28% of a tan solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.12 (s, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.48 (d, *J* = 8.5 Hz, 2H), 6.96 (s, 2H), 3.54 (br s, 2H), 3.26 (br s, 2H), 2.33 (s, 9H), 1.25 (br s, 3H), 1.13 (br s, 3H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 169.87, 149.25, 140.02, 139.74, 138.26, 135.19, 128.69, 127.49, 126.38, 122.55, 43.67, 39.73, 21.31, 20.09, 14.40, 13.03.
HRMS (ESI) m/z for $C_{21}H_{26}N_2O_2$, [2M+Na]$^+$ calcd: 699.3880, found: 699.3907, [M+Na]$^+$ calcd: 361.1886, found: 361.1908, [2M+H]$^+$ calcd: 677.4061, found: 677.4049.

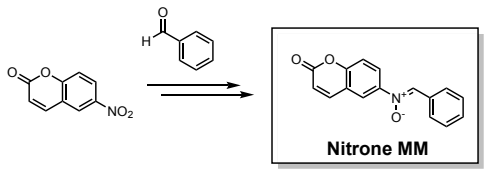
^{1}H NMR



^{13}C NMR


169.87
149.25
140.02
138.74
136.20
135.19
128.69
127.49
126.38
122.55
53.84 CD2Cl2
43.67
39.73
21.31
20.09
14.40
13.03
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

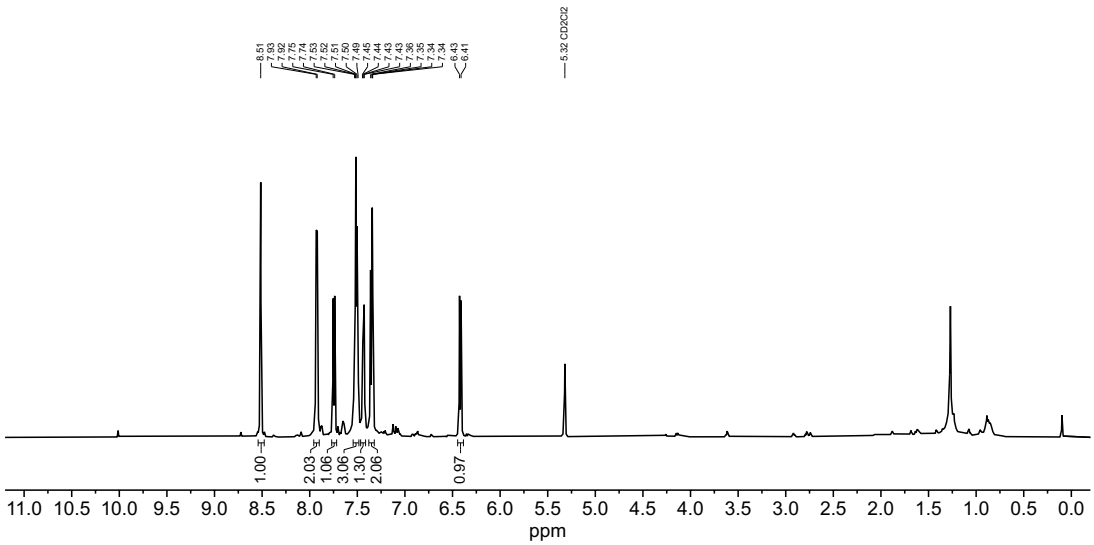
Nitrone MM



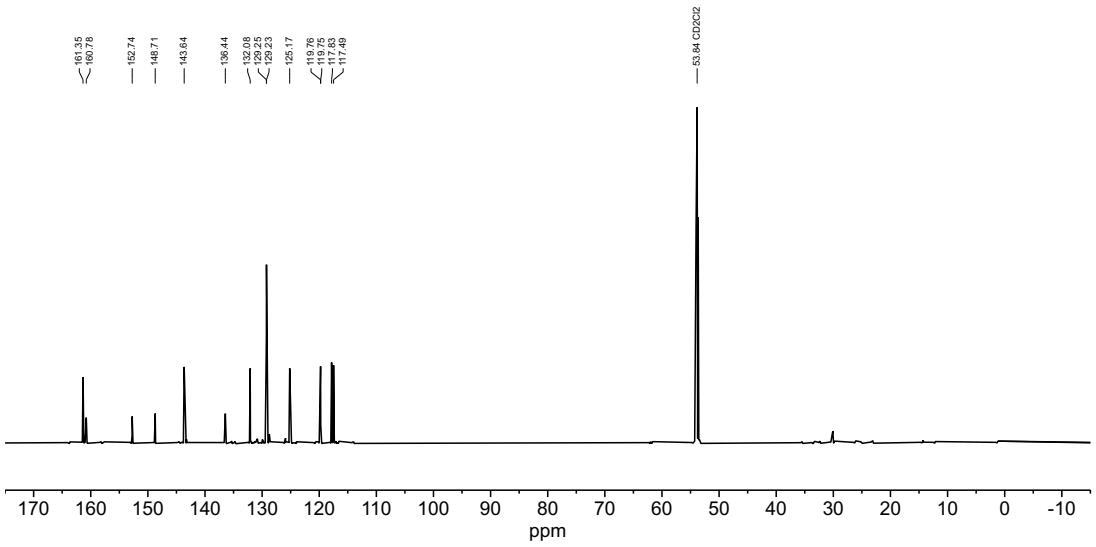
(*Z*)-*N*-(2-oxo-2*H*-chromen-6-yl)-1-phenylmethanimine oxide (Nitrone MM)
Prepared according to the general procedure for synthesis of nitrones. Yield: 20% of a buff colored solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.51 (s, 1H), 7.93 (d, *J* = 6.3 Hz, 2H), 7.75 (d, *J* = 9.6 Hz, 1H), 7.54 – 7.49 (m, 3H), 7.44 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.37 – 7.33 (m, 2H), 6.42 (d, *J* = 9.5 Hz, 1H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 161.35, 160.78, 152.74, 148.71, 143.64, 136.44, 132.08, 129.25, 129.23, 125.17, 119.76, 119.75, 117.83, 117.49.

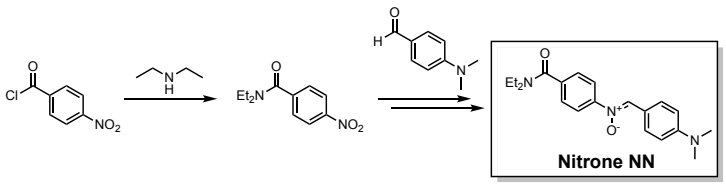
^{1}H NMR



^{13}C NMR


161.35
160.78
152.74
148.71
143.64
136.44
132.08
129.25
129.23
125.17
119.76
119.35
117.63
117.49
53.84 CD2Cl2
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

Nitrone NN

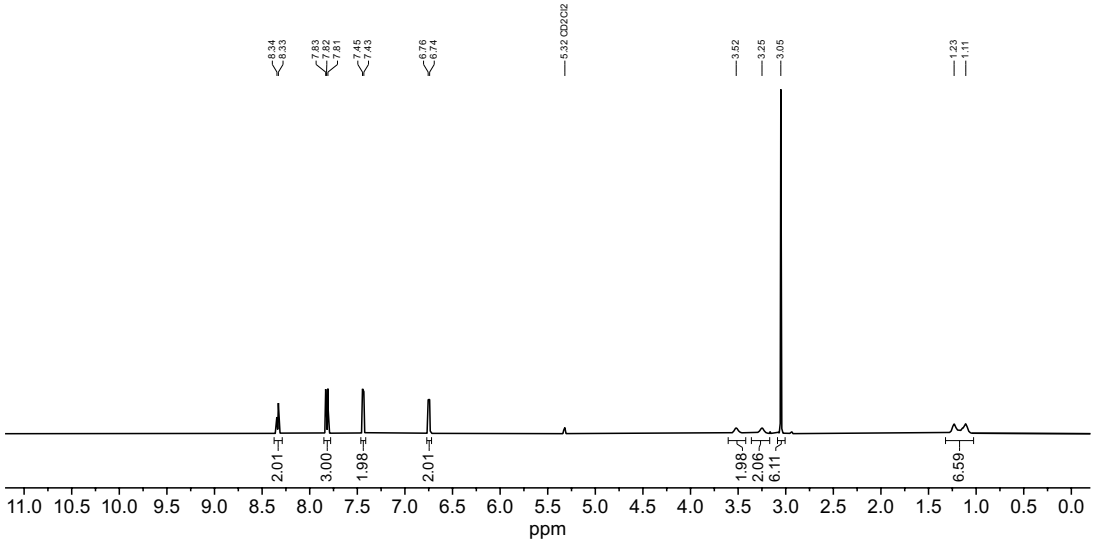


***N*,*N*-diethyl-4-nitrobenzamide**
Followed procedure outlined in Nitrone A synthesis.

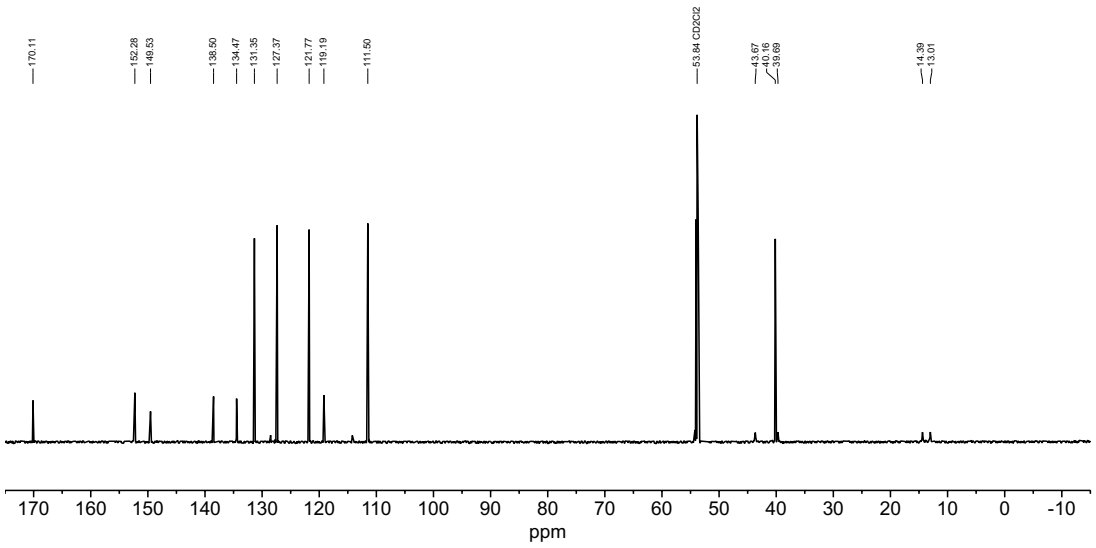
(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-1-(4-(dimethylamino)phenyl)methanimine oxide (Nitrone NN)
Prepared according to the general procedure for synthesis of nitrones. Yield: 67% of red solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.34 (d, *J* = 9.1 Hz, 2H), 7.83 (s, 1H), 7.82 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.5 Hz, 2H), 6.75 (d, *J* = 9.2 Hz, 2H), 3.52 (br s, 2H), 3.25 (br s, 2H), 3.05 (s, 6H), 1.23 (br s, 3H), 1.11 (br s, 3H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 170.11, 152.28, 149.53, 138.50, 134.47, 131.35, 127.37, 121.77, 119.19, 111.50, 43.67, 40.16, 39.69, 14.39, 13.01.
HRMS (ESI) m/z for $C_{20}H_{25}N_3O_2$, [2M+H]+ calcd: 679.3967, found: 679.3987; [M+H]+ calcd: 340.2020, found: 340.2045; [M+Na]+ calcd: 362.1839, found: 362.1857.

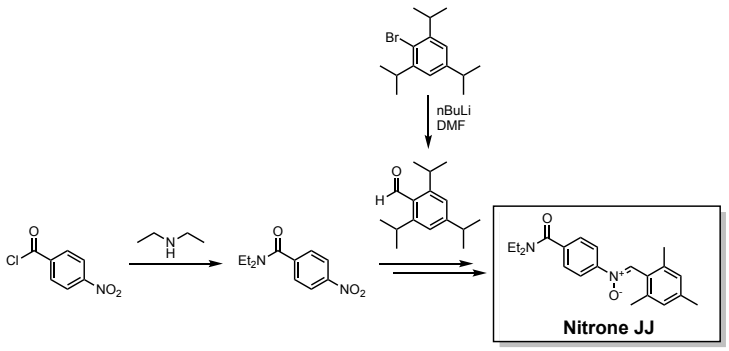
^{1}H NMR



^{13}C NMR


170.11
152.28
149.53
138.50
134.47
131.35
127.37
121.77
119.19
111.50
53.84 CD2Cl2
43.67
40.16
39.69
14.39
13.01
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

Nitrone OO



N,*N*-diethyl-4-nitrobenzamide

Followed procedure outlined in Nitrone A synthesis.

2,4,6-triisopropylbenzaldehyde

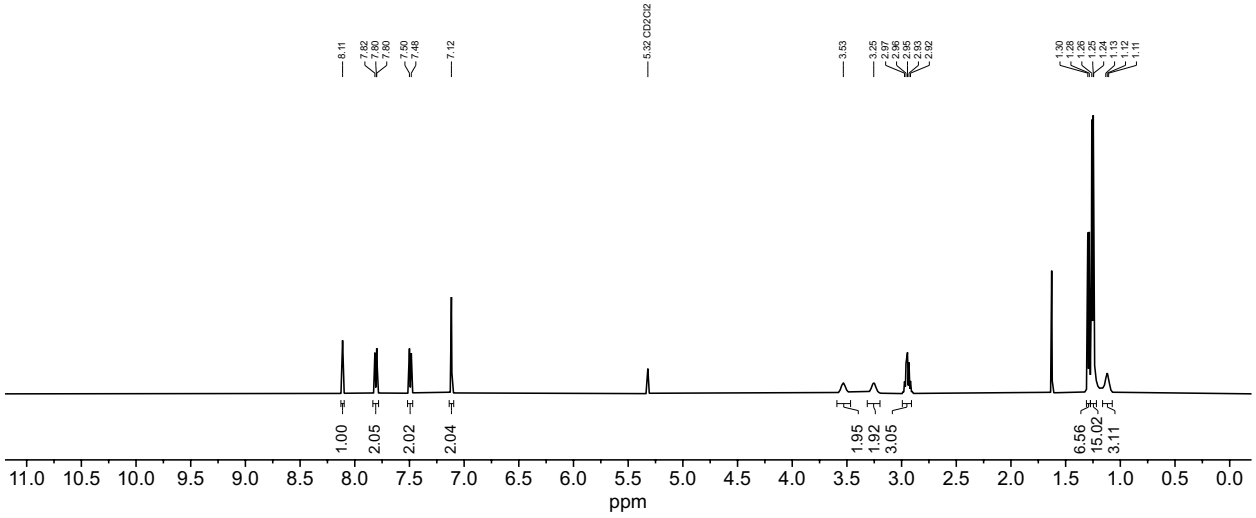
2-bromo-1,3,5-triisopropylbenzene (2.0 g, 7.06 mmol, 1 equiv) was dissolved in 20 mL of dry THF and cooled to –78 °C. *n*-Butyllithium (3.88 mL of 2 M solution, 7.77 mmol, 1.1 equiv) was added dropwise, the solution allowed to stir for 20 min at –78 °C and became milky white. Dry DMF (0.60 mL, 7.77 mmol, 1.1 equiv) was added dropwise and the solution became clear. Allowed to stir for 30 min at –78 °C prior to warming to rt and stirring for an additional 2 h. The reaction was then poured into ~50 mL of water and extracted with diethyl ether. The organic layer was washed with water, brine, dried with sodium sulfate, and concentrated under reduced pressure. This crude product contained starting material and desired product (yield of aldehyde ~60%) and was used without further purification.

(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-1-(2,4,6-triisopropylphenyl)methanimine oxide (Nitrone OO)

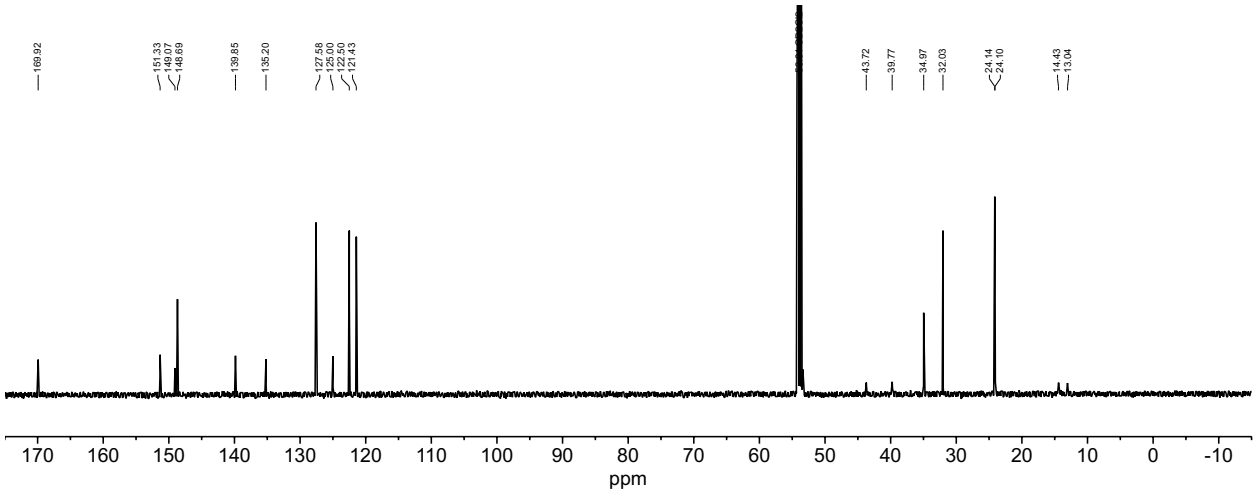
Prepared according to the general procedure for synthesis of nitrones. Yield: 46% of a tan solid.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.11 (s, 1H), 7.83 – 7.79 (m, 2H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.12 (s, 2H), 3.53 (br s, 2H), 3.25 (br s, 2H), 2.95 (p, *J* = 6.9 Hz, 3H), 1.29 (d, *J* = 6.9 Hz, 6H), 1.25 (d, *J* = 6.9 Hz, 12H), 1.24 (br t, 3H), 1.12 (br t, *J* = 6.6 Hz, 3H).
^{13}C NMR (126 MHz, CD_2Cl_2) δ 169.92, 151.33, 149.07, 148.69, 139.85, 135.20, 127.58, 125.00, 122.50, 121.43, 43.72, 39.77, 34.97, 32.03, 24.14, 24.10, 14.43, 13.04.
HRMS (ESI) m/z for $C_{27}H_{38}N_2O_2$, $[M+H]^+$ calcd: 423.30115, found: 423.30281.

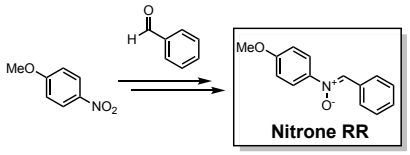
^{1}H NMR



^{13}C NMR

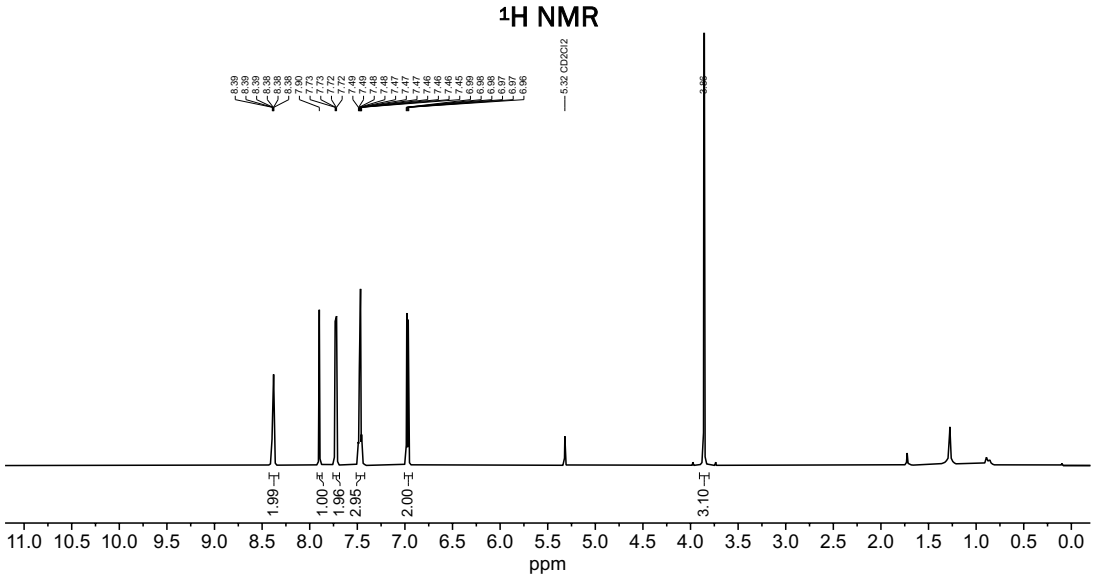


Nitrone RR

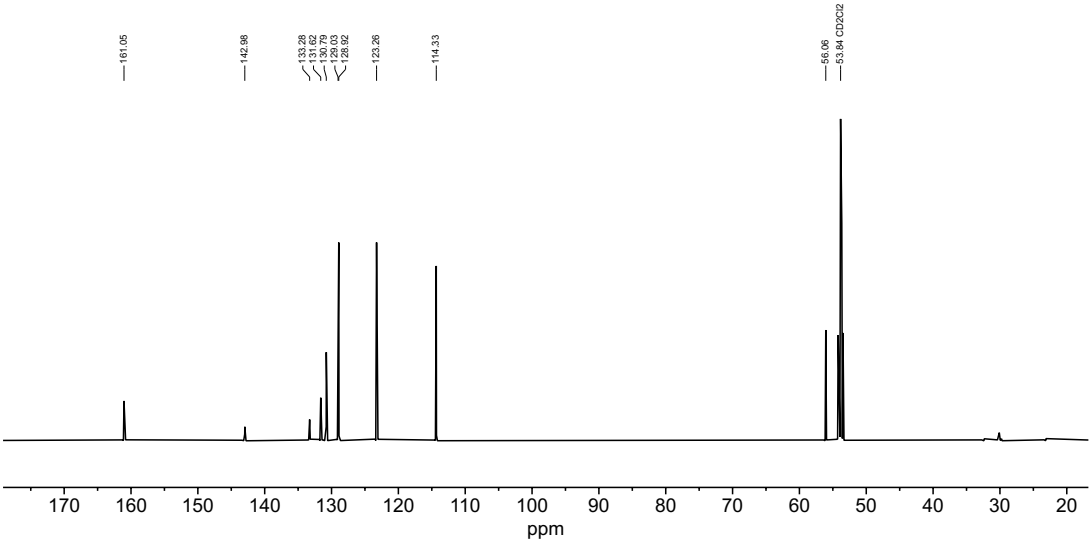


(*Z*)-*N*-(4-methoxyphenyl)-1-phenylmethanimine oxide (Nitrone RR)
Prepared according to the general procedure for synthesis of nitrones. Yield: 18% of a tan colored solid.

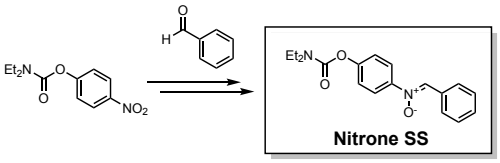
^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.41 – 8.35 (m, 2H), 7.90 (s, 1H), 7.74 – 7.70 (m, 2H), 7.50 – 7.44 (m, 3H), 7.00 – 6.94 (m, 2H), 3.86 (s, 3H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 161.05, 142.98, 133.28, 131.62, 130.79, 129.03, 128.92, 123.26, 114.33, 56.06.
HRMS (ESI) m/z for $C_{14}H_{13}NO_2$ $[M+H]^+$ calcd: 228.10245, found: 228.10295.



13C NMR


161.05
142.98
133.28
131.62
130.79
129.03
128.92
123.26
114.33
56.06
53.84 CD2Cl2
170
160
150
140
130
120
110
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60
50
40
30
20
ppm

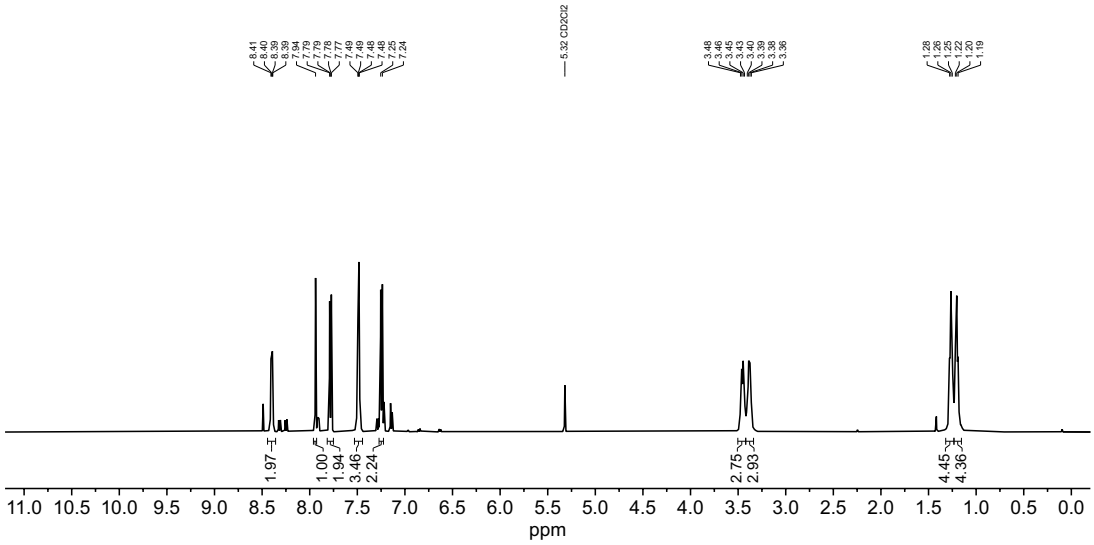
Nitrone SS



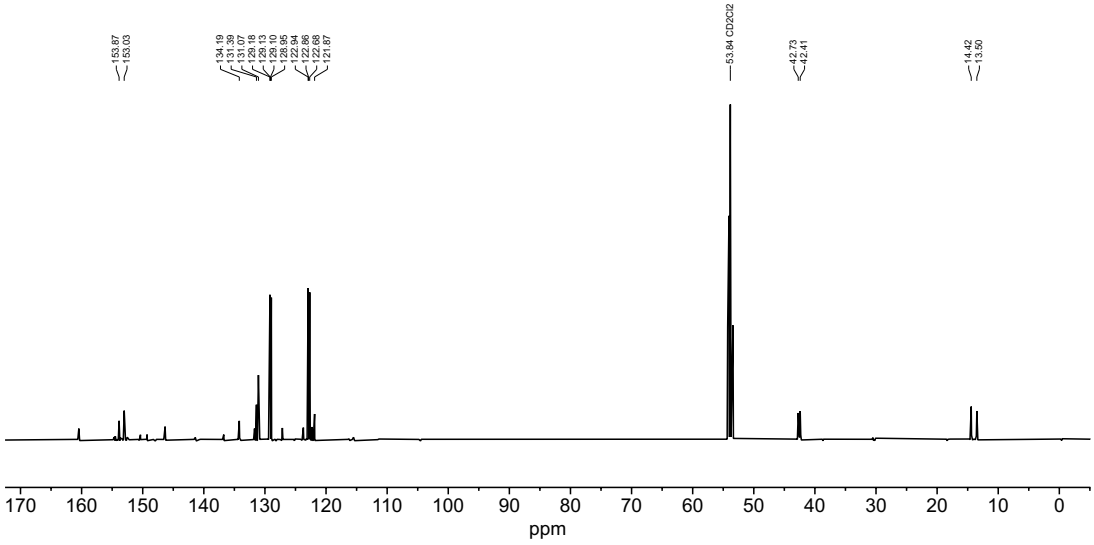
(*Z*)-*N*-(4-((diethylcarbamoyl)oxy)phenyl)-1-phenylmethanimine oxide (Nitrone SS)
Prepared according to the general procedure for synthesis of nitrones. Yield: 68% of a yellow solid.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.40 (dd, *J* = 6.9, 2.9 Hz, 2H), 7.94 (s, 1H), 7.80 – 7.76 (m, 2H), 7.51 – 7.47 (m, 3H), 7.27 – 7.23 (m, 2H), 3.45 (q, *J* = 7.1 Hz, 2H), 3.38 (q, *J* = 7.1 Hz, 2H), 1.26 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (126 MHz, CD_2Cl_2) δ 153.87, 153.03, 134.19, 131.39, 131.07, 129.18, 129.13, 129.10, 128.95, 122.94, 122.86, 122.68, 121.87, 42.73, 42.41, 14.42, 13.50.
HRMS (ESI) m/z for $C_{18}H_{20}N_2O_3$ $[M+H]^+$ calcd: 313.15522, found: 313.15618.

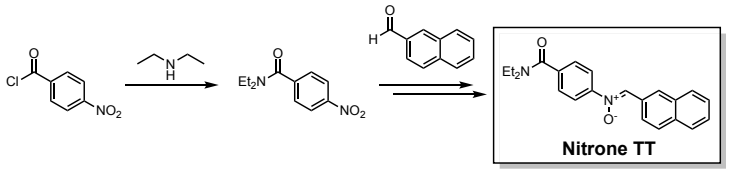
^{1}H NMR



13C NMR


153.87
153.03
134.19
131.39
131.07
129.18
129.13
129.10
128.95
122.94
122.86
122.68
121.87
53.84 CD2Cl2
42.73
42.41
14.42
13.90
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

Nitrone TT

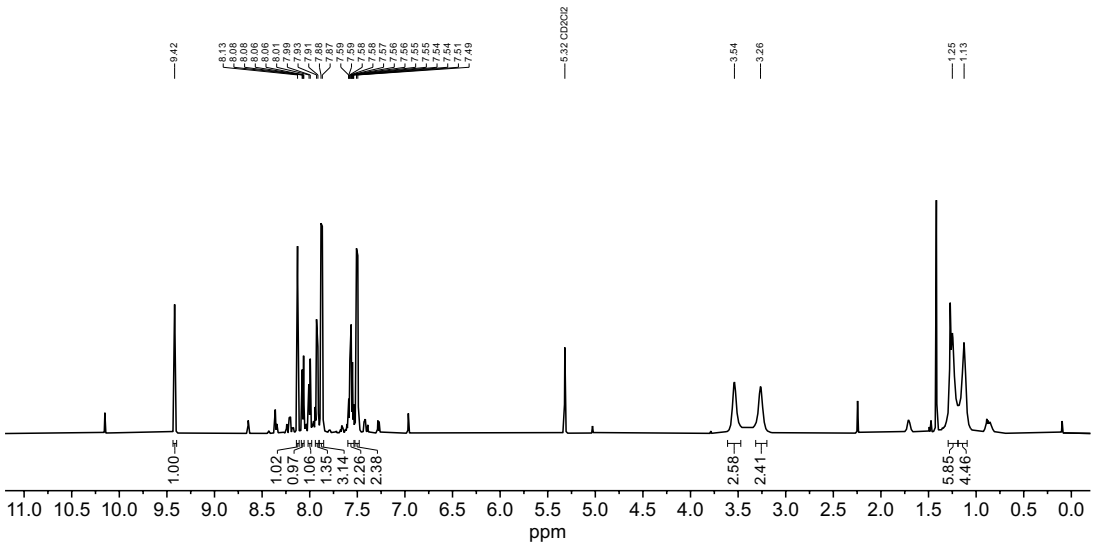


N,*N*-diethyl-4-nitrobenzamide
Prepared according to procedure outline in Nitrone A.

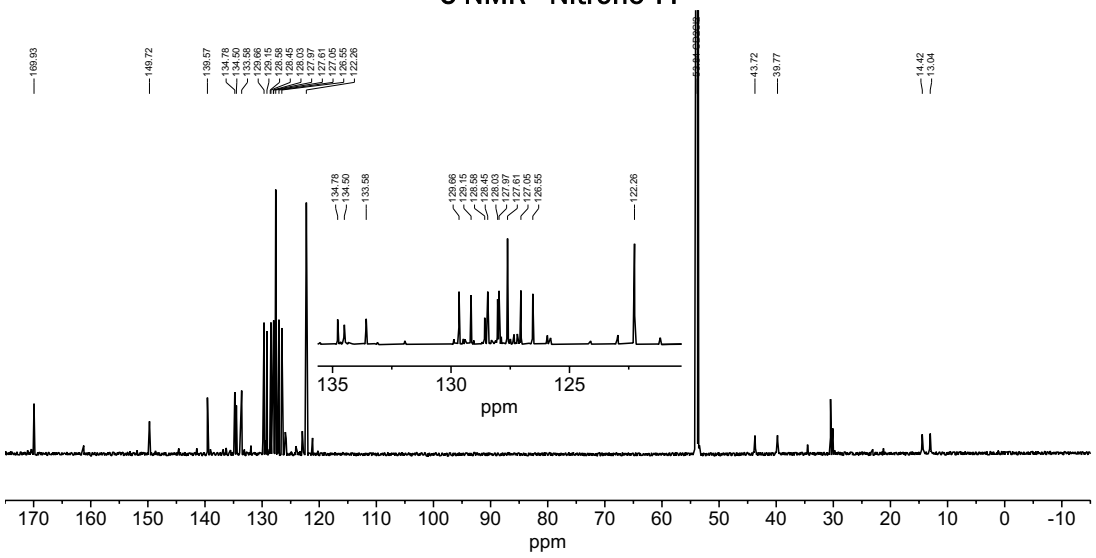
(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-1-(naphthalen-2-yl)methanimine oxide (Nitrone TT)
Prepared according to the general procedure for synthesis of nitrones. Yield: 14% of a tan solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 9.42 (s, 1H), 8.13 (s, 1H), 8.07 (d, *J* = 8.6, 1.7 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.88 – 7.85 (m, 3H), 7.62 – 7.53 (m, 2H), 7.50 (d, *J* = 8.3 Hz, 2H), 3.54 (br s, 2H), 3.26 (br s, 2H), 1.25 (br s, 3H), 1.12 (br s, 3H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 169.93, 149.72, 139.57, 134.78, 134.50, 133.58, 129.66, 129.15, 128.58, 128.45, 128.03, 127.97, 127.61, 127.05, 126.55, 122.26, 43.72, 39.77, 14.42, 13.04.
HRMS (ESI) m/z calcd for $C_{22}H_{22}N_2O_2$ $[M+H]^+$: 347.17595, found: 347.17549.

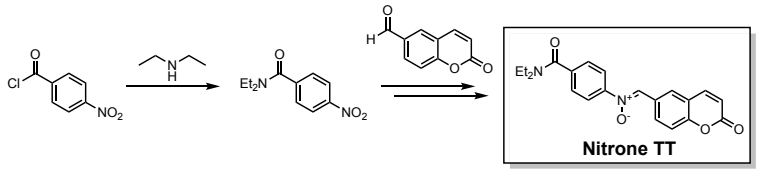
^{1}H NMR - Nitrone TT



^{12}C NMR - Nitrone TT



Nitrone UU

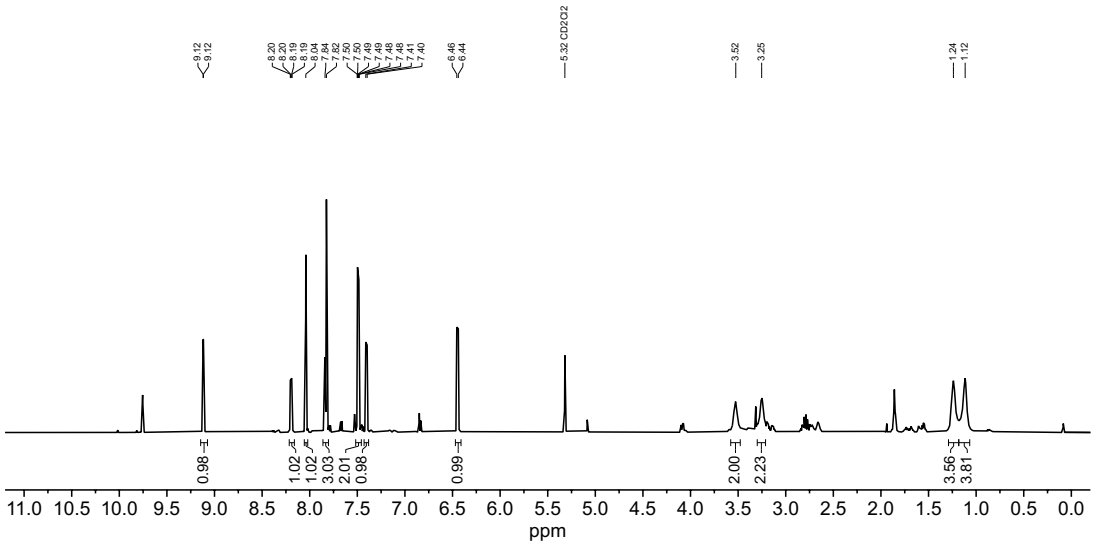


***N*,*N*-diethyl-4-nitrobenzamide**
Prepared according to procedure outlined for Nitrone A.

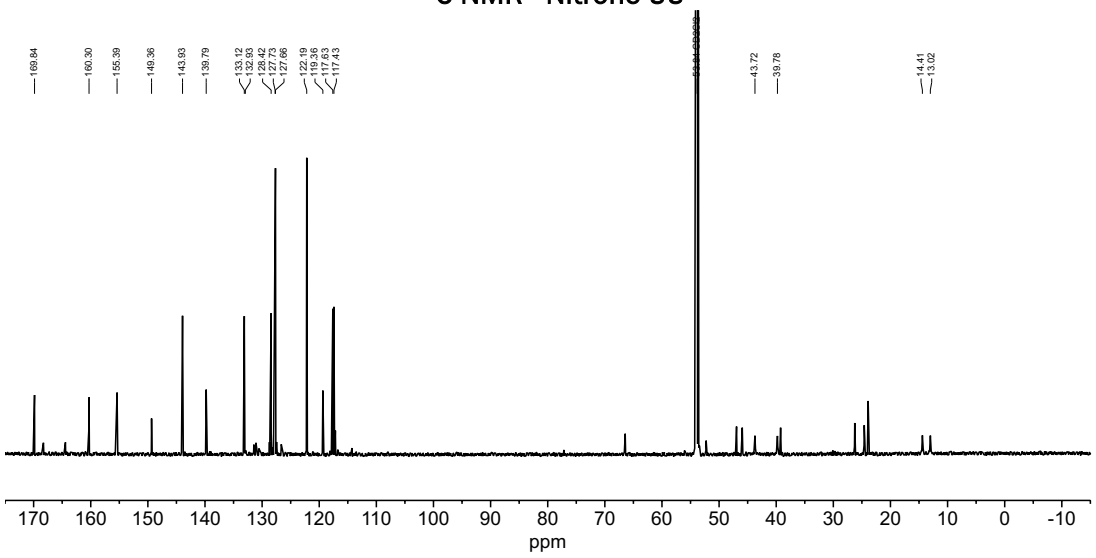
(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-1-(2-oxo-2*H*-chromen-6-yl)methanimine oxide (Nitrone UU)
Prepared according to the general procedure for synthesis of nitrones. Yield: 75% of a buff colored solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 9.12 (d, *J* = 2.0 Hz, 1H), 8.19 (dd, *J* = 8.8, 2.1 Hz, 1H), 8.04 (s, 1H), 7.83 (d, *J* = 8.8 Hz, 3H), 7.51 – 7.48 (m, 2H), 7.40 (d, *J* = 8.7 Hz, 1H), 6.45 (d, *J* = 9.6 Hz, 1H), 3.52 (br s, 2H), 3.25 (br s, 2H), 1.24 (br s, 3H), 1.12 (br s, 3H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 169.84, 160.30, 155.39, 149.36, 143.93, 139.79, 133.12, 132.93, 128.42, 127.73, 127.66, 122.19, 119.36, 117.63, 117.43, 43.72, 39.78, 14.41, 13.02.
HRMS (ESI) m/z for $C_{21}H_{20}N_2O_4$ $[M+H]^+$ calcd: 365.15013, found: 365.15079.

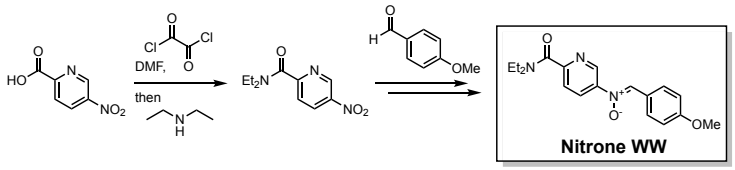
^{1}H NMR - Nitrone UU



^{13}C NMR - Nitrone UU



Nitrone WW

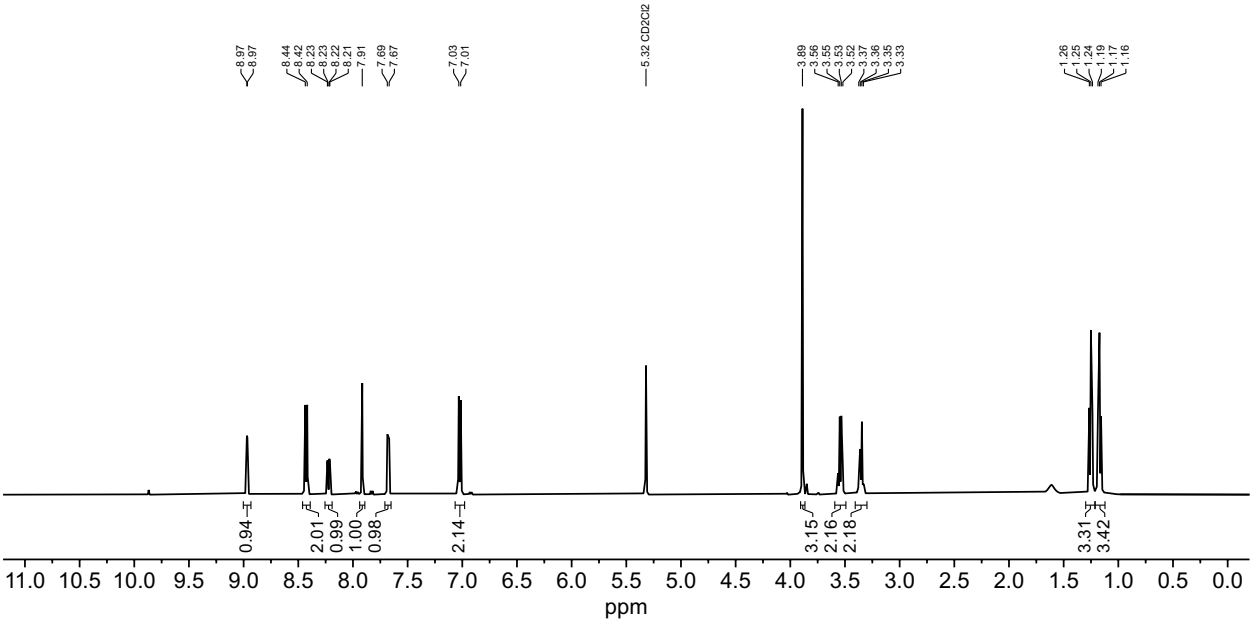


***N*,*N*-diethyl-5-nitropicolinamide**
Prepared according to procedure outlined for Nitrone M.

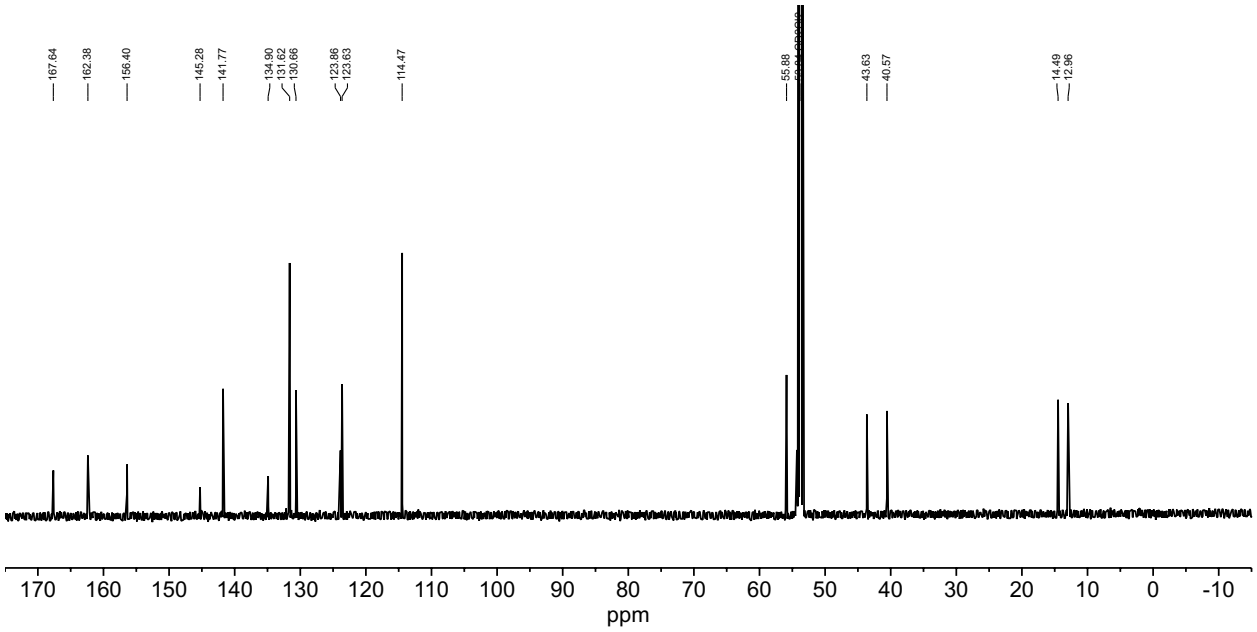
(*Z*)-*N*-(6-(diethylcarbamoyl)pyridin-3-yl)-1-(4-methoxyphenyl)methanimine oxide (Nitrone WW)
Prepared according to the general procedure for synthesis of nitrones. Yield: 65% of a buff colored solid.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.97 (d, *J* = 2.5 Hz, 1H), 8.43 (d, *J* = 8.7 Hz, 2H), 8.22 (dd, *J* = 8.4, 2.6 Hz, 1H), 7.91 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 8.6 Hz, 2H), 3.89 (s, 3H), 3.54 (q, *J* = 7.1 Hz, 2H), 3.35 (q, *J* = 7.0 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.17 (t, *J* = 7.0 Hz, 3H).
^{13}C NMR (126 MHz, CD_2Cl_2) δ 167.64, 162.38, 156.40, 145.28, 141.77, 134.90, 131.62, 130.66, 123.86, 123.63, 114.47, 55.88, 43.63, 40.57, 14.49, 12.96.
HRMS (ESI) m/z for $C_{18}H_{21}N_3O_3$, $[M+K]^+$ calcd: 366.1215, found: 366.1230; $[M+Na]^+$ calcd: 350.1475, found: 350.1483; $[2M+Na]^+$ calcd: 677.3058, found: 677.3078.

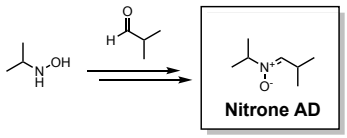
^{1}H NMR



13-C NMR


167.64
162.38
156.40
145.28
141.77
134.90
131.62
130.66
123.86
123.63
114.47
55.88
43.63
40.57
14.49
12.96
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
ppm

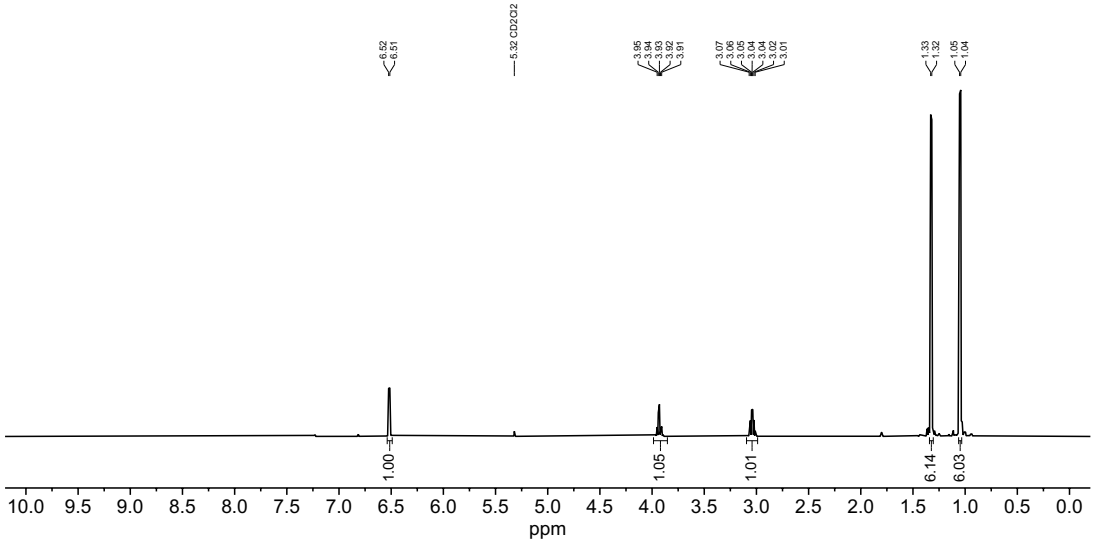
Nitrone AD



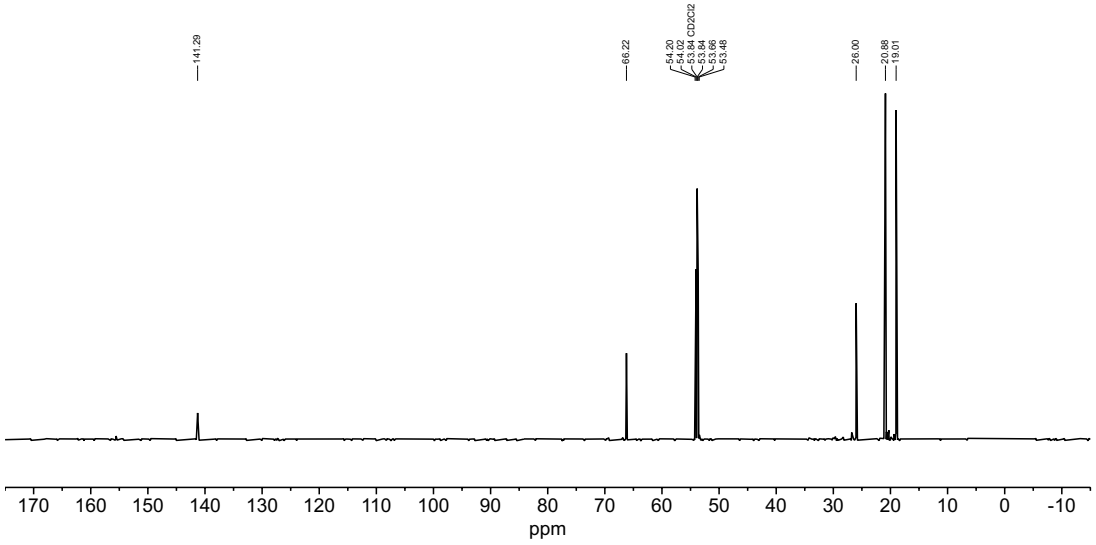
(*Z*)-*N*-isopropyl-2-methylpropan-1-imine oxide (Nitrone AD)
Prepared according to the general procedure for synthesis of nitrones. Yield: 72% of a tan solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 6.52 (d, *J* = 7.0 Hz, 1H), 3.93 (p, *J* = 6.5 Hz, 1H), 3.04 (dq, *J* = 13.8, 6.9 Hz, 1H), 1.33 (d, *J* = 6.6 Hz, 6H), 1.05 (d, *J* = 6.9 Hz, 6H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 141.29, 66.22, 54.20, 54.02, 53.84, 53.66, 53.48, 26.00, 20.88, 19.01.
HRMS (ESI) *m/z* for $C_7H_{15}NO$ [M+H]+ calcd: 130.12319, found: 130.12323.

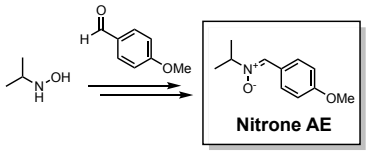
^{1}H NMR - Nitrone AD



^{13}C NMR - Nitrone AD



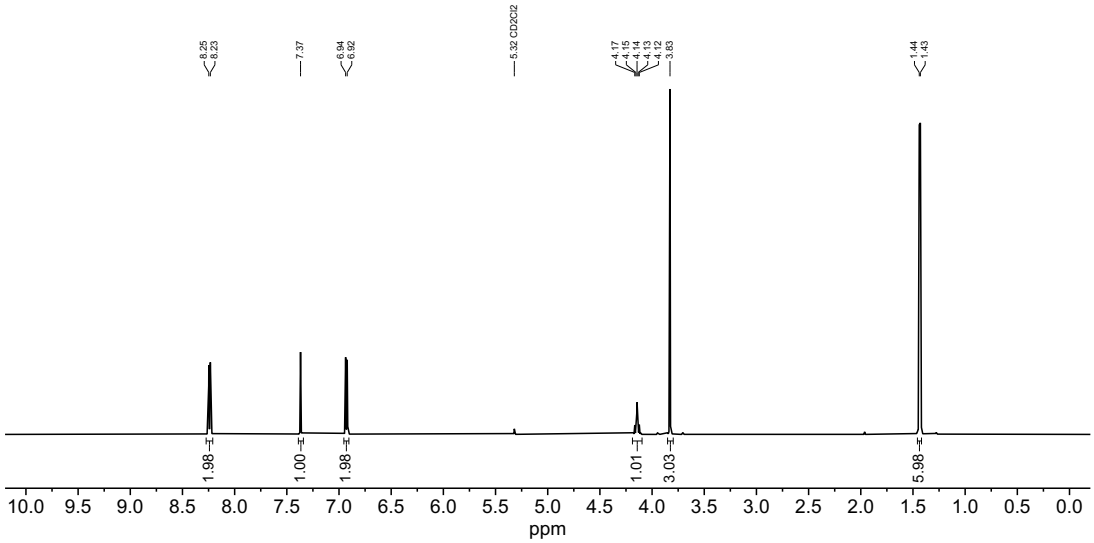
Nitrone AE



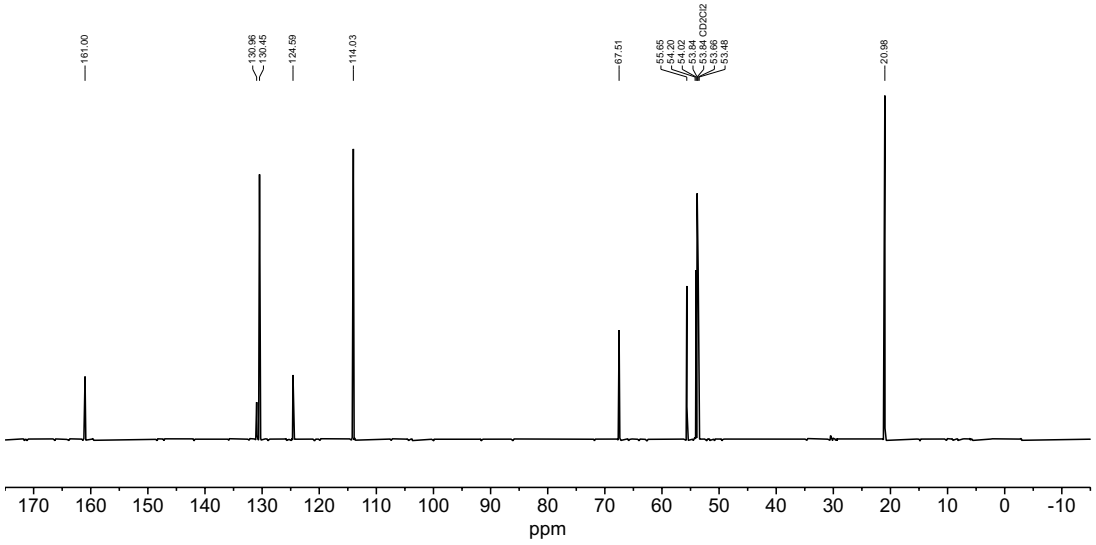
(*Z*)-*N*-isopropyl-1-(4-methoxyphenyl)methanimine oxide (Nitrone AE)
Prepared according to the general procedure for synthesis of nitrones. Yield: 17% of a buff colored solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.24 (d, *J* = 8.9 Hz, 2H), 7.37 (s, 1H), 6.93 (d, *J* = 9.0 Hz, 2H), 4.14 (p, *J* = 6.5 Hz, 1H), 3.83 (s, 3H), 1.43 (d, *J* = 6.5 Hz, 6H).
^{13}C NMR (151 MHz, CD_2Cl_2) δ 161.00, 130.96, 130.45, 124.59, 114.03, 67.51, 55.65, 54.20, 54.02, 53.84, 53.66, 53.48, 20.98.
HRMS (ESI) m/z for $C_{11}H_{15}NO_2$, $[M+H]^+$ calcd: 194.1181, found: 194.11843.

^{1}H NMR - Nitrone AE



^{13}C NMR - Nitrone AE



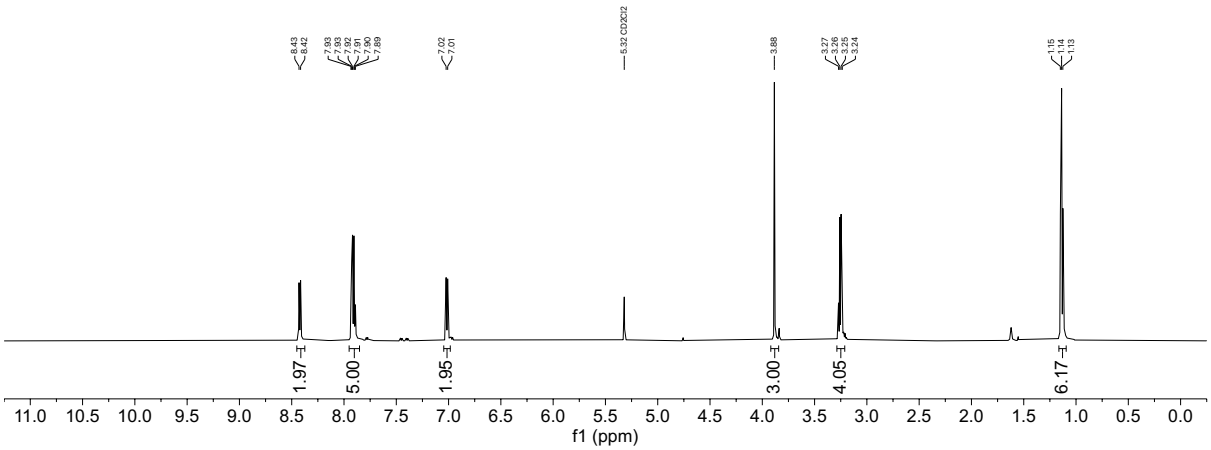
Nitrone AG

(*Z*)-*N*-(4-(*N*,*N*-diethylsulfamoyl)phenyl)-1-(4-methoxyphenyl)methanimine oxide (Nitrone AG)

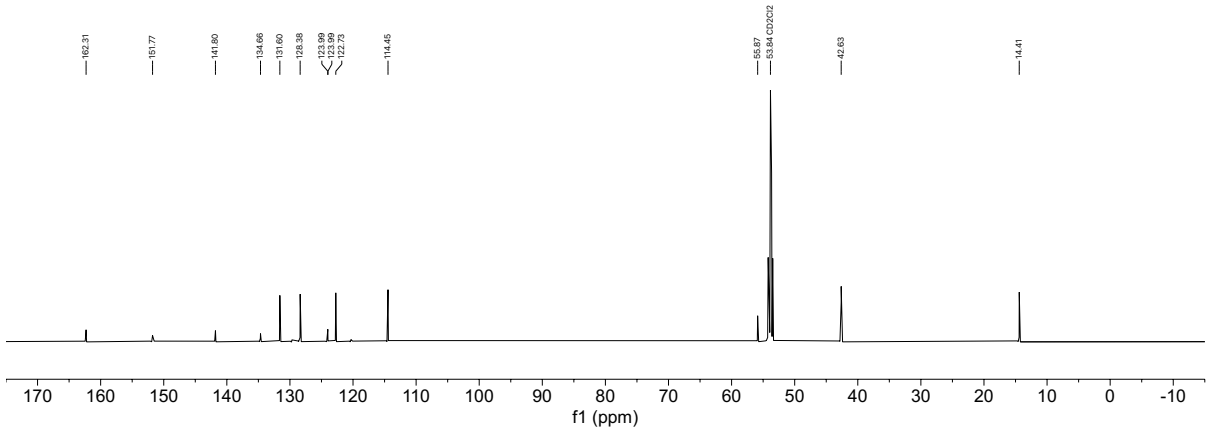
Prepared according to the general procedure for synthesis of nitrones. Yield: 57% of cream colored solid.

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.43, 8.42, 7.93, 7.93, 7.92, 7.91, 7.90, 7.89, 7.02, 7.01, 3.88, 3.27, 3.26, 3.25, 3.24, 1.15, 1.14, 1.13.
^{13}C NMR (151 MHz, CD_2Cl_2) δ 162.31, 151.77, 141.80, 134.66, 131.60, 128.38, 123.99, 123.99, 122.73, 114.45, 55.87, 42.63, 14.41.
HRMS (ESI) m/z for $C_{18}H_{23}N_2O_4S$ $[M+H]^+$ calcd: 363.13730, found: 363.13850.

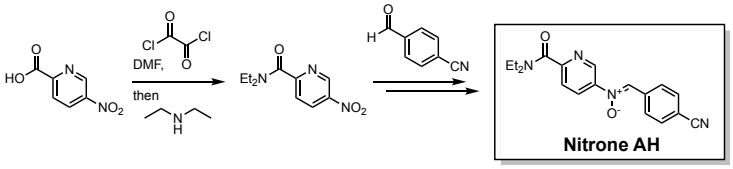
^{1}H NMR - Nitrone AG



^{13}C NMR – Nitrone AG



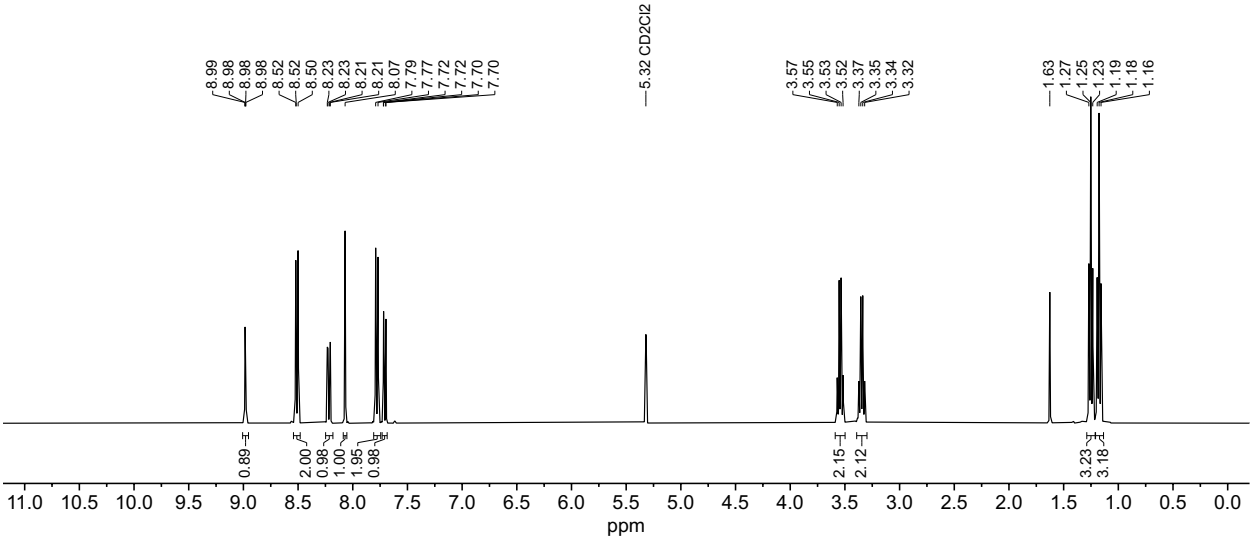
Nitrone AH



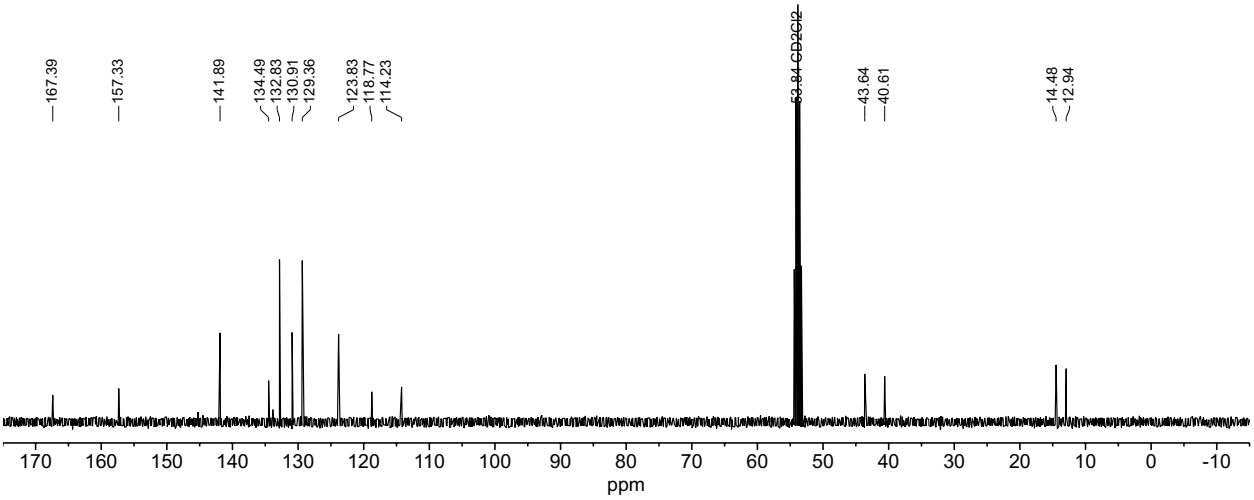
(*Z*)-1-(4-cyanophenyl)-*N*-(6-(diethylcarbamoyl)pyridin-3-yl)methanimine oxide (Nitrone AH)
Prepared according to the general procedure for synthesis of nitrones. Yield: 59% of a cream colored solid.

^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.98 (dd, *J* = 2.6, 0.8 Hz, 1H), 8.51 (d, *J* = 8.6 Hz, 2H), 8.22 (dd, *J* = 8.4, 2.6 Hz, 1H), 8.07 (s, 1H), 7.78 (d, *J* = 8.6 Hz, 2H), 7.71 (dd, *J* = 8.4, 0.8 Hz, 1H), 3.54 (q, *J* = 7.1 Hz, 2H), 3.35 (q, *J* = 7.1 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.18 (t, *J* = 7.1 Hz, 4H).
^{13}C NMR (101 MHz, CD_2Cl_2) δ 167.39, 157.33, 141.89, 134.49, 132.83, 130.91, 129.36, 123.83, 118.77, 114.23, 43.64, 40.61, 14.48, 12.94.
HRMS (ESI) m/z for $C_{18}H_{19}N_4O_2$ $[M+H]^+$ calcd: 323.15048, found: 323.15131.

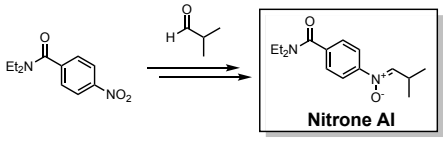
^{1}H NMR - Nitrone AH



^{13}C NMR - Nitrone AH

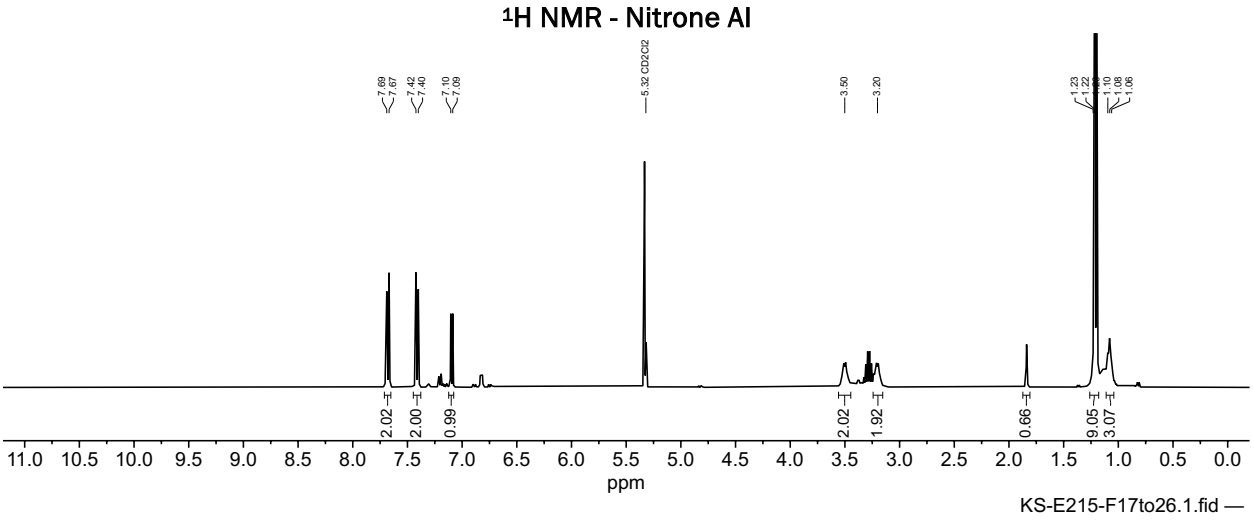


Nitrone AI

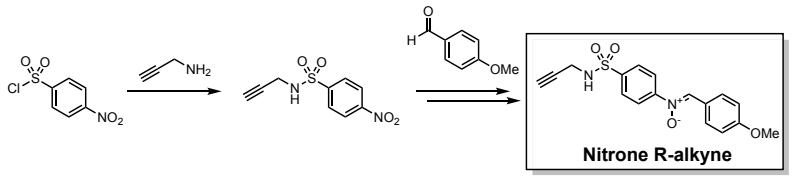


(*Z*)-*N*-(4-(diethylcarbamoyl)phenyl)-2-methylpropan-1-imine oxide (Nitrone AI)
Prepared according to the general procedure for synthesis of nitrones.

^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.68 (d, *J* = 8.6 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.09 (d, *J* = 7.2 Hz, 1H), 3.55 – 3.45 (m, 2H), 3.23 – 3.15 (m, 0H), 1.25 – 1.22 (m, 3H), 1.21 (d, *J* = 6.9 Hz, 6H), 1.12 – 1.04 (m, 3H), 3.23 – 3.16 (m, 2H).
HRMS (ESI) m/z for $C_{15}H_{23}N_2O_2$ $[M+H]^+$ calcd: 263.17540, found: 263.17624.



Nitrone R-alkyne

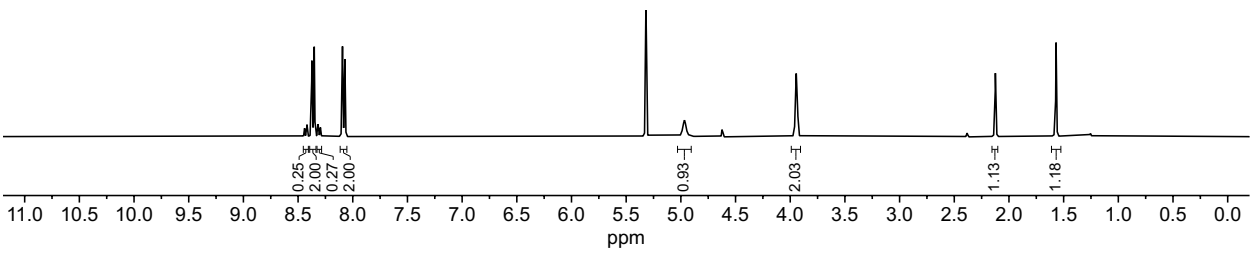


4-nitro-*N*-(prop-2-yn-1-yl)benzenesulfonamide

4-Nitrobenzenesulfonyl chloride (5.0 g, 23 mmol, 1.0 equiv) was dissolved in anhydrous tetrahydrofuran (30 mL) and cooled to 0 °C in an ice bath. Triethylamine (4.7 mL, 3.4 g, 34 mmol, 1.5 equiv) and propargylamine (1.6 mL, 1.4 g, 25 mmol, 1.1 equiv) were added to solution slowly. The reaction solution was allowed warm to rt and stir overnight. The solvent was removed using a rotary evaporator under reduced pressure. Added approximately 60 mL of 0.1 M NaH_2PO_4 solution, and extracted 4 times with dichloromethane. The organic layers were combined and washed with saturated $NaHCO_3$ and brine. The solution was dried with sodium sulfate and concentrated. Purified with a gradient of acetone/hexanes or ethyl acetate/hexanes. Isolated 3.54 g, 65% yield.

^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.36 (d, *J* = 9.0 Hz, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 4.97 (s, 1H), 3.95 (s, 1H), 2.12 (td, *J* = 2.5, 1.0 Hz, 1H).

^{1}H NMR

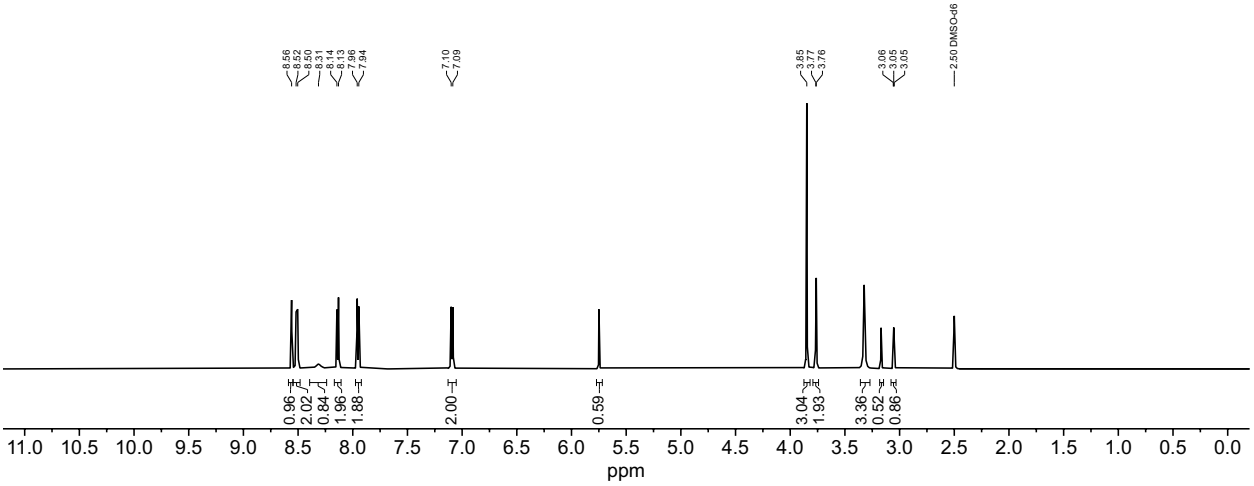


(*Z*)-1-(4-methoxyphenyl)-*N*-(4-(*N*-(prop-2-yn-1-yl)sulfamoyl)phenyl)methanimine oxide (Nitrone R-alkyne)

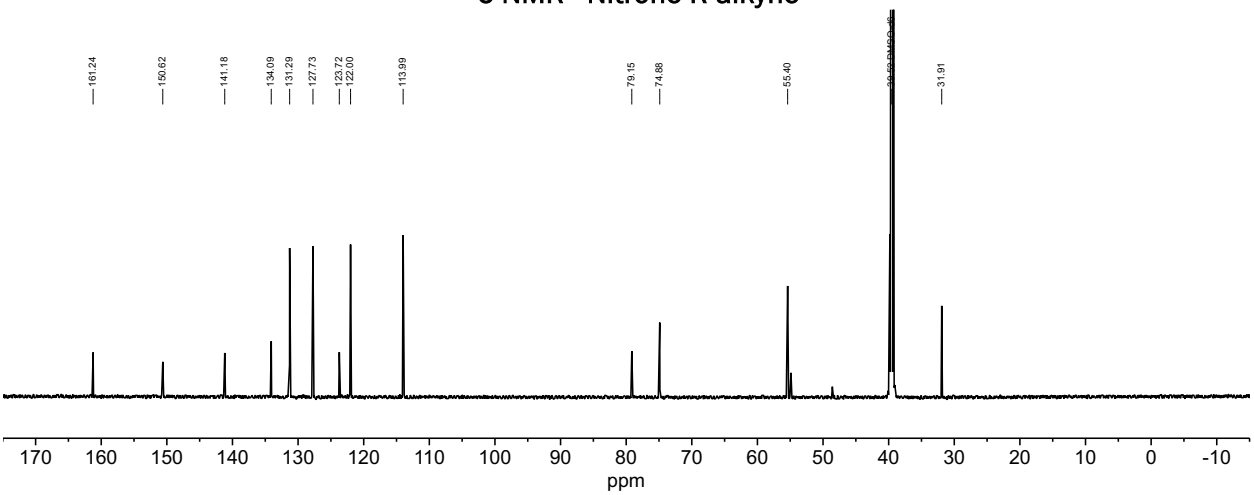
Prepared according to the general procedure for synthesis of nitrones but solvent was methanol instead of dichloromethane. Yield: 80% of a tan solid.

^{1}H NMR (600 MHz, DMSO) δ 8.56 (s, 1H), 8.51 (d, *J* = 8.9 Hz, 2H), 8.31 (br s, 1H), 8.14 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.7 Hz, 2H), 7.09 (d, *J* = 8.9 Hz, 2H), 3.85 (s, 3H), 3.76 (d, *J* = 2.6 Hz, 2H), 3.05 (t, *J* = 2.5 Hz, 1H).
^{13}C NMR (151 MHz, DMSO) δ 161.24, 150.62, 141.18, 134.09, 131.29, 127.73, 123.72, 122.00, 113.99, 79.15, 74.88, 55.40, 31.91.
HRMS (ESI) m/z for $C_{17}H_{17}N_2O_4S$ $[M+H]^+$ calcd: 345.09035, found: 345.09132.

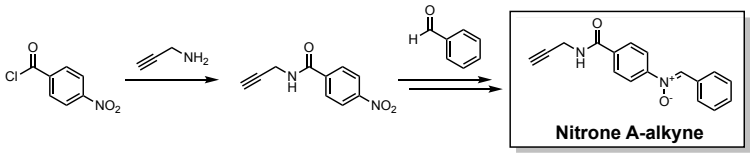
^{1}H NMR - Nitrone R-alkyne



^{13}C NMR - Nitrone R-alkyne



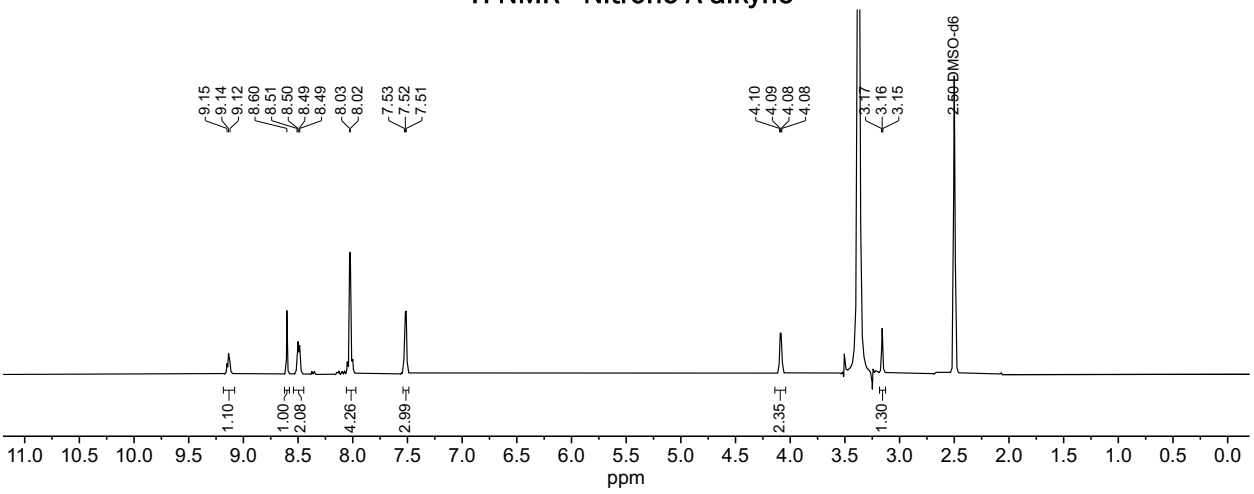
Nitrone A-alkyne



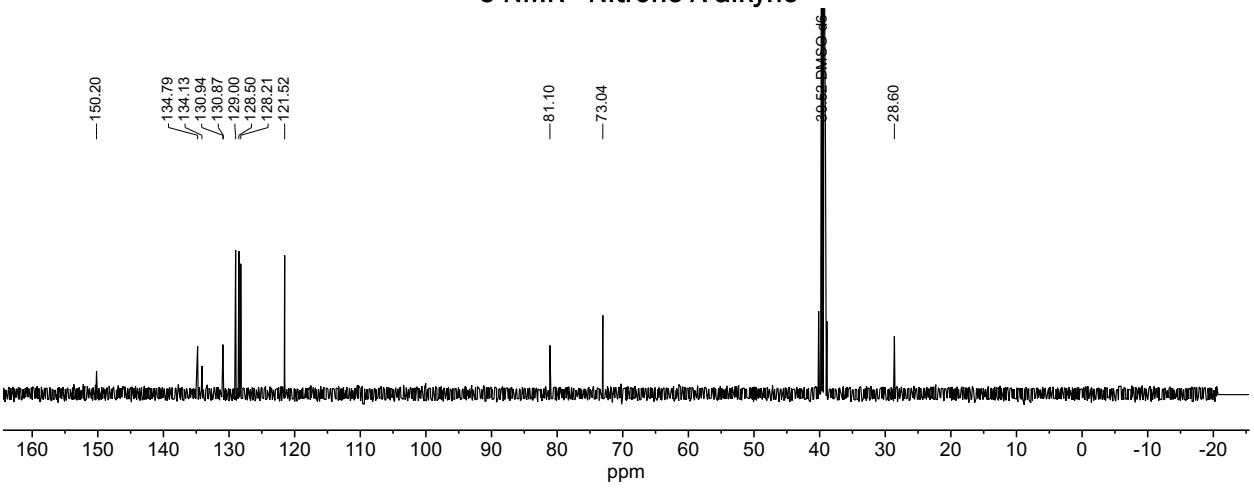
(*Z*)-1-phenyl-*N*-(4-(prop-2-yn-1-ylcarbamoyl)phenyl)methanimine oxide (Nitrone A-alkyne)
Prepared according to the general procedure for synthesis of nitrones. Yield: 70% of a tan colored solid.

^{1}H NMR (400 MHz, DMSO) δ 9.14 (t, *J* = 5.5 Hz, 1H), 8.60 (s, 1H), 8.50 (dd, *J* = 6.7, 3.0 Hz, 2H), 8.03 (d, *J* = 2.4 Hz, 4H), 7.54 – 7.49 (m, 3H), 4.09 (dd, *J* = 5.5, 2.5 Hz, 2H), 3.16 (t, *J* = 2.5 Hz, 1H).
^{13}C NMR (101 MHz, DMSO-D_6) δ 164.81, 150.20, 134.79, 134.13, 130.94, 130.87, 129.00, 128.50, 128.21, 121.52, 81.10, 73.04, 28.60.
HRMS (ESI) m/z for $C_{17}H_{15}N_2O_2$ $[M+H]^+$ calcd: 279.11280, found: 279.11308.

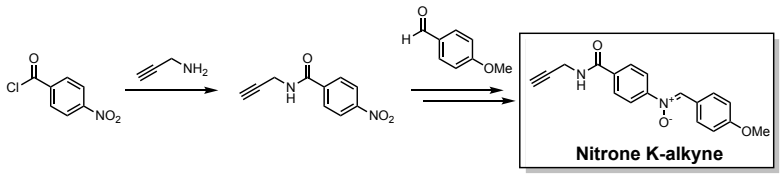
^{1}H NMR - Nitrone A-alkyne



^{13}C NMR - Nitrone A-alkyne



Nitrone K-alkyne

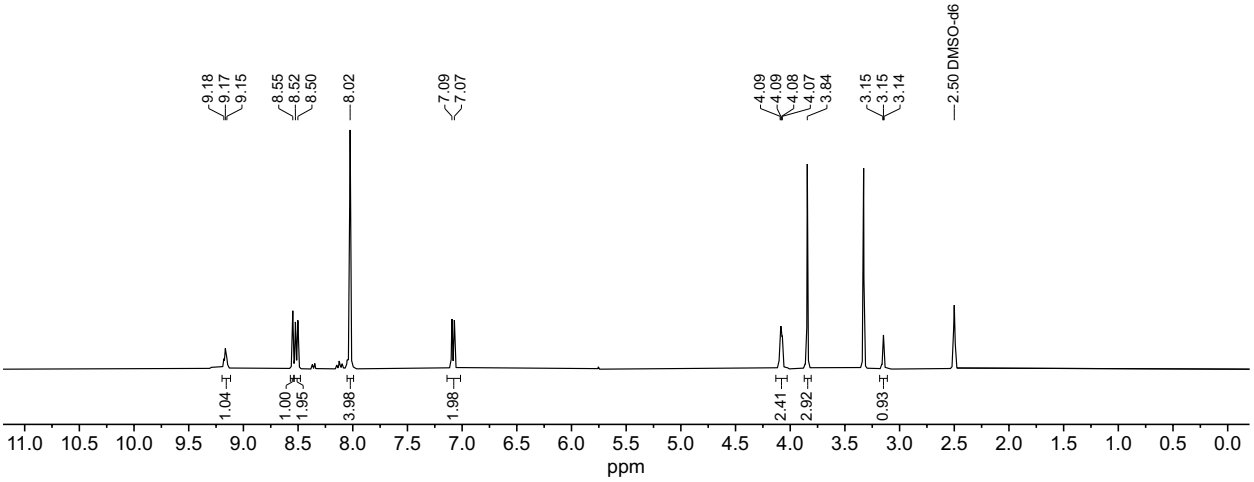


(*Z*)-1-(4-methoxyphenyl)-*N*-(4-(prop-2-yn-1-ylcarbamoyl)phenyl)methanimine oxide (Nitrone K-alkyne)

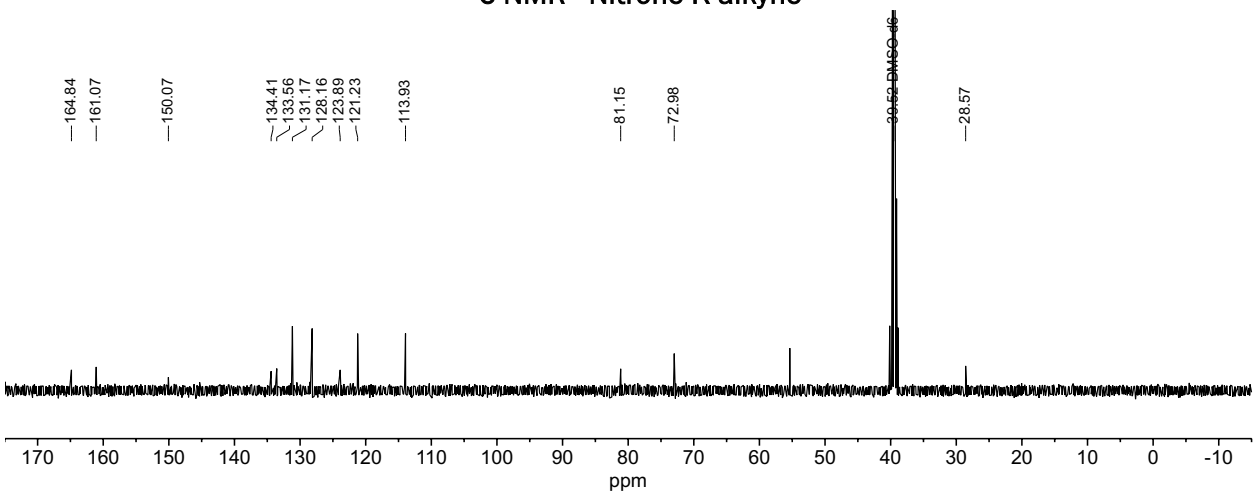
Prepared according to the general procedure for synthesis of nitrones. Yield: 23% of a white solid.

^{1}H NMR (400 MHz, DMSO-D_6) δ 9.17 (t, *J* = 5.6 Hz, 1H), 8.55 (s, 2H), 8.51 (d, *J* = 9.0 Hz, 1H), 8.02 (s, 4H), 7.08 (d, *J* = 9.0 Hz, 2H), 4.08 (dd, *J* = 5.5, 2.5 Hz, 2H), 3.84 (s, 3H), 3.15 (t, *J* = 2.5 Hz, 1H).
^{13}C NMR (101 MHz, DMSO-D_6) δ 164.84, 161.07, 150.07, 134.41, 133.56, 131.17, 128.16, 123.89, 121.23, 113.93, 81.15, 72.98, 28.57.
HRMS (ESI) m/z for $C_{18}H_{17}N_2O_3$ $[M+H]^+$ calcd: 309.12337, found: 309.12394.

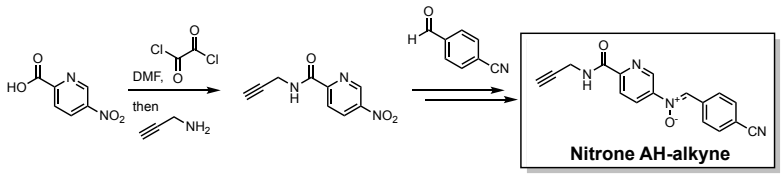
^{1}H NMR - Nitrone K-alkyne



^{13}C NMR - Nitrone K-alkyne



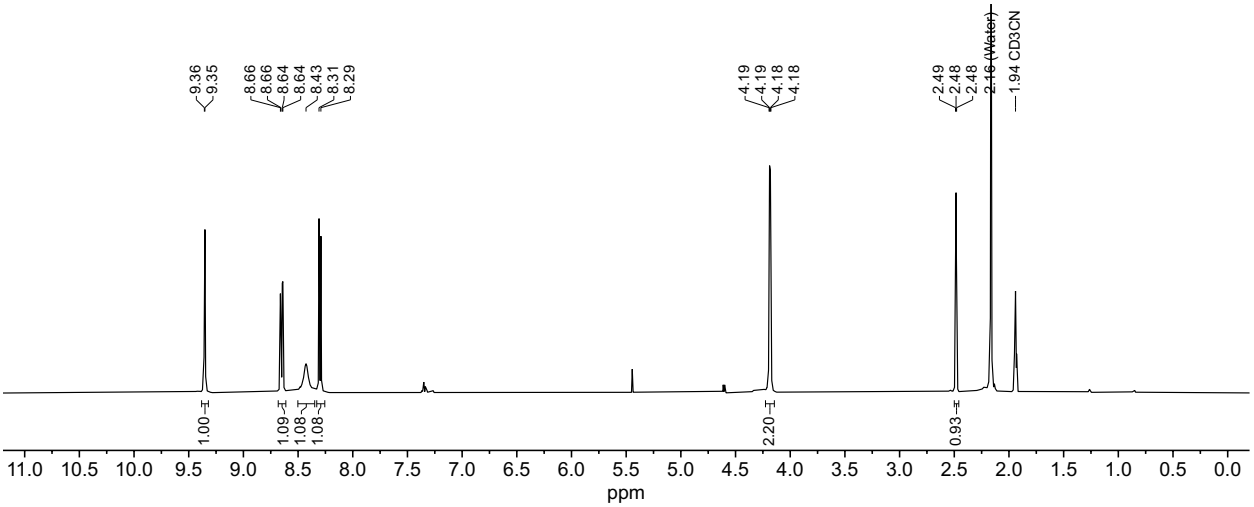
Nitrone AH-alkyne



5-nitro-*N*-(prop-2-yn-1-yl)picolinamide

5-nitropicolinic acid (5.0 g, 30.0 mmol, 1.0 equiv) and *N*,*N*-dimethylformamide (0.46 mL, 0.44 mg, 6.0 mmol, 0.2 equiv) were combined in ~100 mL of anhydrous dichloromethane and cooled to 0°C in an ice bath. 5-Nitropicolinic acid is sparingly soluble in dichloromethane. Oxalyl chloride (7.7 mL, 11.3 g, 89 mmol, 3.0 equiv) was added by syringe. The reaction was allowed to warm to rt and stirred until all starting materials dissolved, usually ~3 h. The solution was concentrated using a rotary evaporator and under vacuum, then reconstituted in ~150 mL of anhydrous dichloromethane, cooled to 0°C in an ice bath, and triethylamine (12.4 mL, 9.0 g, 89.2 mmol, 3 equiv) and prop-2-yn-1-amine (2.9 mL, 2.5 g, 44.6 mmol, 1.5 equiv) were added. The reaction mixture was allowed to warm to rt and stirred overnight. The product was purified by silica gel chromatography using hexane to ethyl acetate gradient. Yield: 70% of tan flakes.

^{1}H NMR



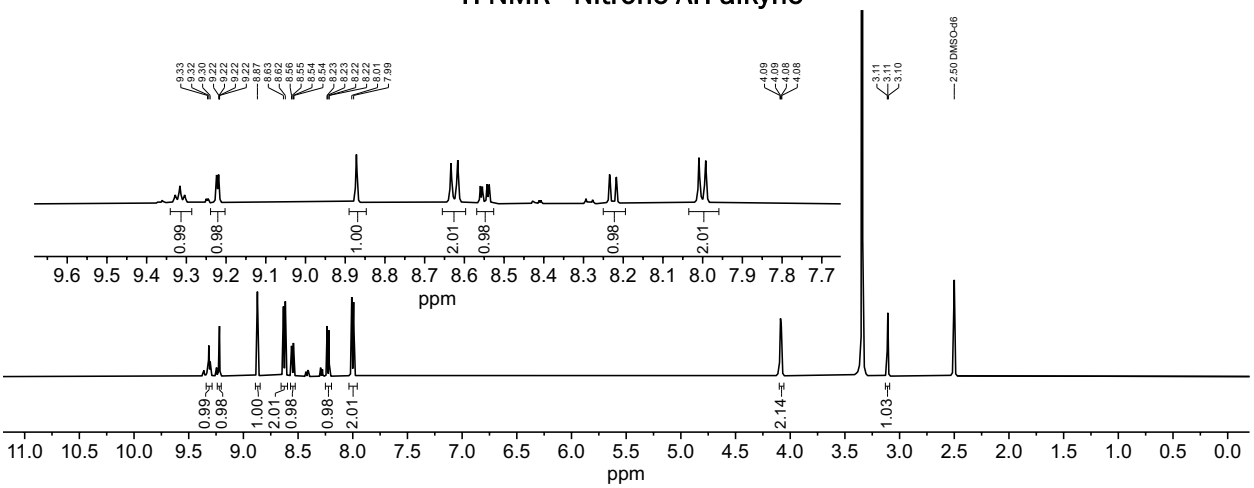
^{1}H NMR (500 MHz, CD_3CN) δ 9.35 (d, *J* = 2.6 Hz, 1H), 8.65 (dd, *J* = 8.6, 2.6 Hz, 1H), 8.43 (s, 1H), 8.30 (d, *J* = 8.6 Hz, 1H), 4.19 (dd, *J* = 6.1, 2.5 Hz, 2H), 2.48 (t, *J* = 2.5 Hz, 1H).

(*Z*)-1-(4-cyanophenyl)-*N*-(6-(prop-2-yn-1-ylcarbamoyl)pyridin-3-yl)methanimine oxide (Nitrone AH-alkyne)

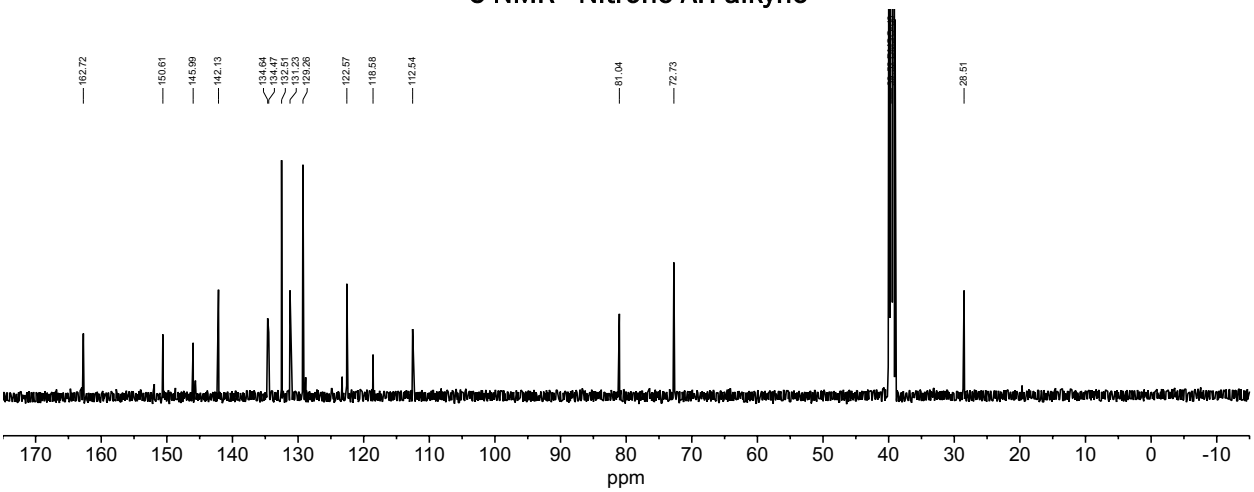
Prepared according to the general procedure for synthesis of nitrones. Yield: 68% of a tan colored solid.

^{1}H NMR (500 MHz, DMSO) δ 9.32 (t, *J* = 6.0 Hz, 1H), 9.22 (dd, *J* = 2.6, 0.8 Hz, 1H), 8.87 (s, 1H), 8.63 (d, *J* = 8.6 Hz, 2H), 8.55 (dd, *J* = 8.5, 2.6 Hz, 1H), 8.23 (dd, *J* = 8.5, 0.7 Hz, 1H), 8.00 (d, *J* = 8.6 Hz, 2H), 4.09 (dd, *J* = 6.0, 2.5 Hz, 2H), 3.11 (t, *J* = 2.4 Hz, 1H).
^{13}C NMR (126 MHz, DMSO) δ 162.72, 150.61, 145.99, 142.13, 134.64, 134.47, 132.51, 131.23, 129.26, 122.57, 118.58, 112.54, 81.04, 72.73, 28.51.
HRMS (ESI) m/z for $C_{17}H_{13}N_4O_2$ $[M+H]^+$ calcd: 305.10330, found: 305.10354.

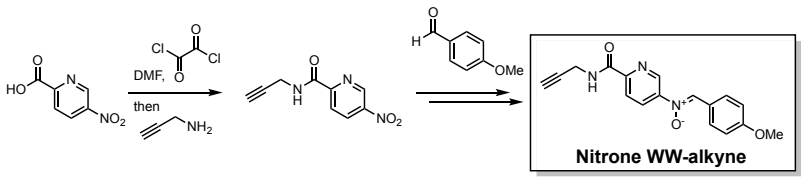
^{1}H NMR - Nitrone AH-alkyne



^{13}C NMR - Nitrone AH-alkyne



Nitrone WW-alkyne



5-nitro-*N*-(prop-2-yn-1-yl)picolinamide

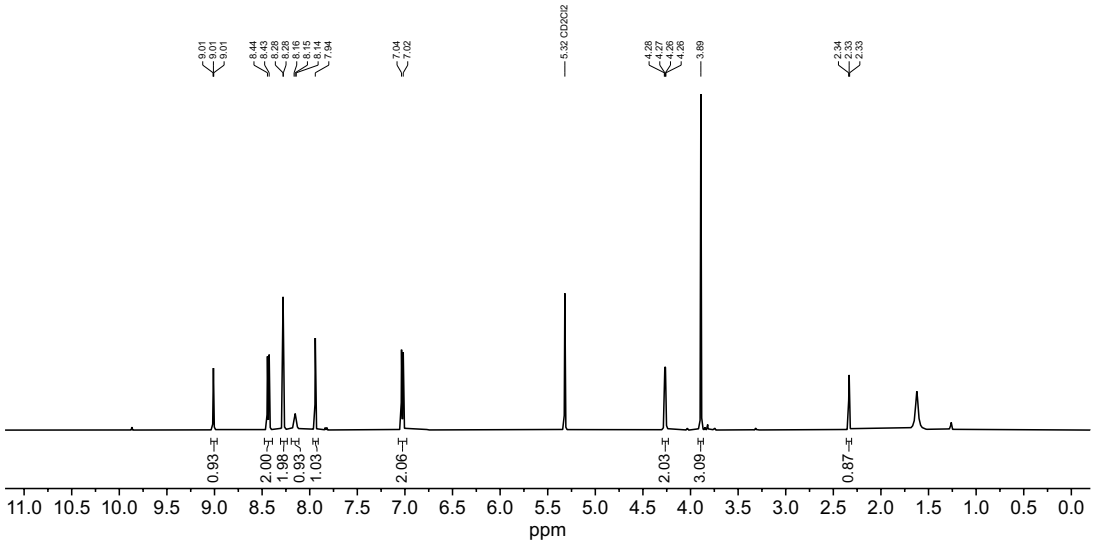
Followed synthesis method outlined for nitrone AH-alkyne.

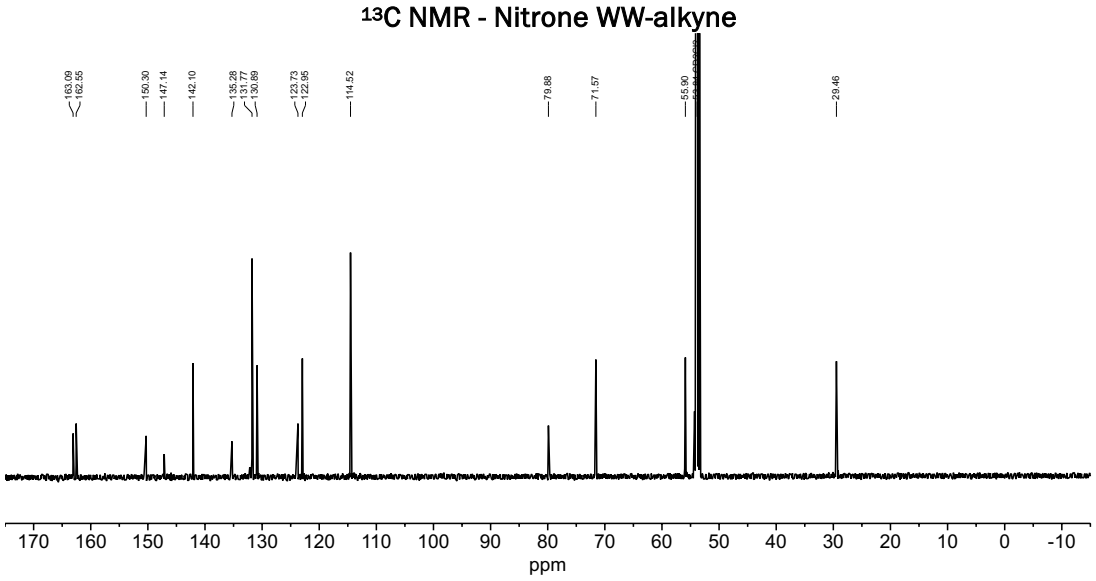
(*Z*)-1-(4-methoxyphenyl)-*N*-(6-(prop-2-yn-1-ylcarbamoyl)pyridin-3-yl)methanimine oxide (Nitrone WW-alkyne)

Prepared according to the general procedure for synthesis of nitrones. Yield: 78% of a tan colored solid.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 9.01 (t, *J* = 1.7 Hz, 1H), 8.43 (d, *J* = 9.0 Hz, 2H), 8.28 (d, *J* = 1.6 Hz, 2H), 8.16 (t, *J* = 5.7 Hz, 1H), 7.94 (s, 1H), 7.03 (d, *J* = 9.0 Hz, 2H), 4.27 (dd, *J* = 5.8, 2.6 Hz, 2H), 3.89 (s, 3H), 2.33 (t, *J* = 2.6 Hz, 1H).
^{13}C NMR (126 MHz, CD_2Cl_2) δ 163.09, 162.55, 150.30, 147.14, 142.10, 135.28, 131.77, 130.89, 123.73, 122.95, 114.52, 79.88, 71.57, 55.90, 29.46.

^{1}H NMR - Nitrone WW-alkyne





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