Reactivity, Selectivity and Stability in Sulfenic Acid Detection:

A Comparative Study of Nucleophilic and Electrophilic Probes

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Contents:

Synthetic Methods and Materials.

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

Preparation of Cyclic sulfenamide (9)

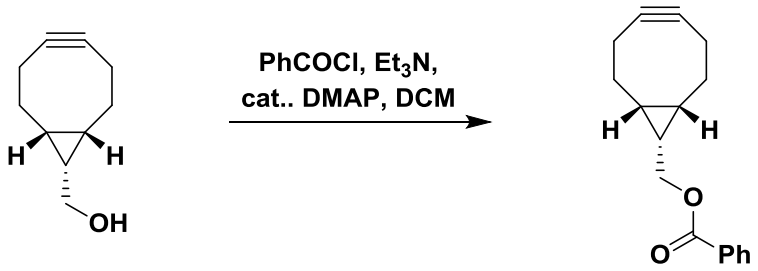
Methyl (S)-2-((R)-4-(((benzyloxy)carbonyl)amino)-3-oxoisothiazolidin-2-yl)-3-methylbutanoate **(9)** was prepared according to the literature procedure previously reported.[1]

Preparation of Nucleophiles and electrophiles

Cyclohexane-1,3-dione **(2e)**, 2-methylcyclohexane-1,3-dione **(25)**, methyl acetoacetate **(3a)**, 4-Chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole **(4)**, ((nitromethyl)sulfonyl)benzene **(13)** are commercially available and purchase from Sigma Aldrich. 2-(Phenylsulfonyl)acetonitrile **(14)** is commercially available and purchase from TCI. Diethyl malonate **(29)**, diethyl methylmalonate **(30)** are commercially available and purchased from Acros Organics. Diethyl fluoromalonate **(31)** is commercially available and purchased from Astatech.

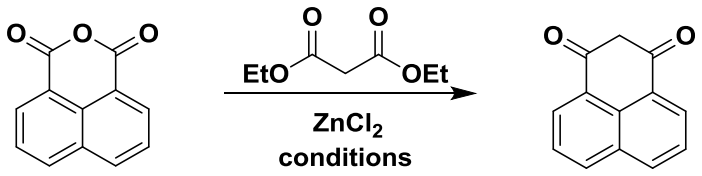
4-(ethylthio)cyclopentane-1,3-dione **(7a)** was prepared according to literature procedure.[2] 1-Benzylpiperidine-2,4-dione **(11)** and 1-benzyl-1H-benzo[*c*][1,2]thiazin-4(3*H*)-one 2,2-dioxide **(12)** were prepared according to literature procedure.[1]

Synthesis of ((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)methyl benzoate (6a)



Modified Procedure from literature[3]: To a solution of cyclooctyne (50 mg), triethylamine (0.14 ml) and DMAP (5 mg) in DCM (5 ml) was added benzoyl chloride (62 μl) at rt. Resulting reaction mixture was stirred at rt overnight. After the completion, a new product was formed (as seen by TLC). The reaction was quenched by the addition of saturated ammonium chloride. DCM layer was separated and aqueous layer was extracted with DCM (3 x 25 ml). Combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated to give a crude yellow solid. This crude product was purified by column chromatography using a gradient of 5% EtOAc/Hexanes to recover the pure product as white solid in 77% isolated yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05-8.09 (m, 2H), 7.57 (tt, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 7.42-7.49 (m, 2H), 4.43 (d, J = 8.3 Hz, 2H), 2.20-2.38 (m, 6H), 1.59 – 1.72 (m, 2H), 1.55 (p, J = 8.8 Hz, 1H), 0.99-1.07 (m, 2H); ^{13}C NMR (100 MHz, CDCl3): δ 166.7, 132.9, 130.5, 129.6, 128.4, 98.8, 63.0, 29.2, 21.4, 20.4, 17.5. LR-MS: $C_{17}H_{18}O_2$; Calculated: 254.33; Observed: 255.1 (M^++1).

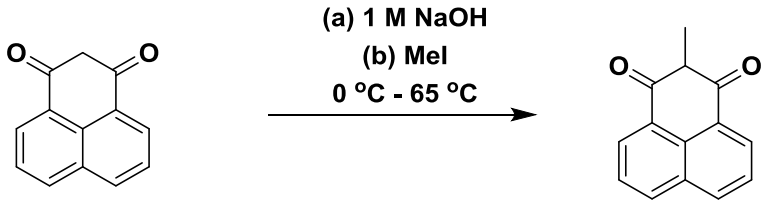
Synthesis of 1H-phenalene-1,3(2H)-dione (8a)



Modified Procedure from literature[4]: Naphthoic anhydride (2.5 g, 12.6 mmol) and diethyl malonate (10 ml, 62.4 mmol, 5 eq) were stirred together in a 100 ml round bottom flask at rt under N_2 pressure. To this free flowing slurry was added anhydrous zinc chloride (2.5 g, 18.3 mmol, 1.5 eq) and resulting slurry was heated and stirred at 175 ^{o}C for 7 h. The reaction mixture was cooled to RT and 50 ml hot water was added to it. Resulting slurry was filtered and residue was dissolved in ammonium hydroxide (75 ml). The solution was stirred for 15 minutes after which it was filtered. Filtrate was stirred with activated carbon for 30 minutes and filtered again.

Finally, the filtrate was acidified with acetic acid and allowed to stand at rt for 1 h to allow it to cool to rt. After filtration, the residue was washed with water and dried overnight. Yellow precipitates showed 90% purity without any purification. Crude yield was 88%. Small amount of it was purified by prep-HPLC and used in rate studies. 1H-NMR in DMSO-d6 (400 MHz): δ 11.77 (bs, 1H), 8.22 – 8.32 (m, 4H), 7.74 (dt, J_1 = 8.0 Hz, J_2 = 0.7 Hz), 6.03 (s, 1H); 13C-NMR in DMSO-d6 (100 MHz): 133.3, 131.6, 127.4, 126.7, 126.6, 105.5. LR-MS: $C_{13}H_8O_2$; Calculated: 196.20; Observed: 197.1 (M^++1).

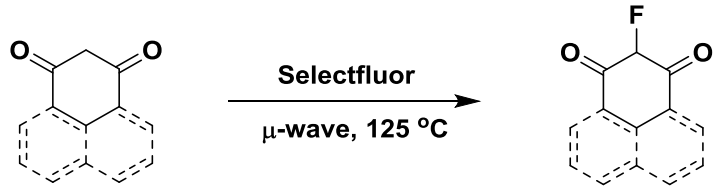
Methylation of C-2 following literature reported procedure[5]



Phenalene-1,3-dione **8** (0.89 g, 4.5 mmol) was dissolved in 1 M NaOH (4.5 ml, 4.5 mmol) in a scintillation vial (20 ml volume size) and cooled to 0 ^oC. Iodomethane (1.28 g, 9 mmol) was added to the resulting red/brown solution in one batch. Resulting reaction mixture was heated at 65 ^oC for 18 h with monitoring. After the completion, reaction mixture was cooled to rt, orange solid was filtered and washed with hexanes and cold water until a bright yellow solid is obtained. The product was then purified by prep-HPLC.

3-hydroxy-2-methyl-1*H*-phenalen-1-one (27): Yield 56%. ^{1}H-NMR in DMSO-d_6 (400 MHz): δ 8.33 (dd, J_1 = 7.3 Hz, J_2 = 1.1 Hz, 2H), 8.22 (dd, J_1 = 8.3 Hz, J_2 = 1.0 Hz), 7.74 (t, J = 8.0 Hz, 2H), 2.05 (s, 3H); ^{13}C-NMR in DMSO-d_6 (100 MHz): 132.6, 131.4, 127.3, 126.7, 126.6, 125.5, 113.2, 8.9; LR-MS: $C_{14}H_{10}O_2$; Calculated: 210.23; Observed: 211.1 (M^++1).

Fluorination of C-2 following literature reported procedure[6]



Procedure for μ-wave reaction: Into a septum-sealed microwave tube, the substrate (2 mmol), anhydrous acetonitrile (5 mL) and selectfluor (2 mmol, 1 eq) were charged. The resulting

mixture was irradiated in a microwave cavity at 125 ^{o}C (by modulation of power) for 6 h. The reaction mixture was cooled rapidly to room temperature by passing compressed air through the microwave cavity. Any insoluble material was filtered off. The solvent was removed from the filtrate *in vacuo*, and the crude products were analyzed by LC-MS and TLC. The product formed was purified by prep-HPLC and analyzed by ^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR.

3-hydroxy-2-fluoro-1*H*-phenalen-1-one (28): Yield: 45%. ^{1}H-NMR in DMSO-d_6 (400 MHz): δ 8.36 (dd, J_1 = 7.3 Hz, J_2 = 1.0 Hz, 2H), 8.31 (dd, J_1 = 8.2 Hz, J_2 = 0.9 Hz), 7.79 (t, J = 7.7 Hz, 2H); ^{13}C-NMR in DMSO-d_6 (100 MHz): 143.2, 140.9, 133.7, 131.4, 128.2 (d, J = 4.7 Hz), 126.9, 123.4; LR-MS: $C_{13}H_7FO_2$; Calculated: 214.20; Observed: 215.1 (M^++1).

Instrumentation for kinetics assay (Rate Studies)

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

KaleidaGraph (version 4.1.1) was used for graphing and further data analysis to obtain pseudo unimolecular 1st order rate constants, 2nd order rate constants and pH plots.

Following equations were used for the purpose of graphing:

Pseudo unimolecular 1st order rate constant (k_{obs}) for the product formation-

```
m1*(1-exp(-m2*m0));m1=max;m2=0.001;
```

Pseudo unimolecular 1st order rate constant (k_{obs}) for the product decomposition-

```
m1*(-exp(-m2*m0));m1=max;m2=0.001;
```

For product formation and subsequent decomposition

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m1*(exp(-m2*m0)-exp(-m3*m0));m1=1000;m2=1;m3=0.1
```

Sample Preparation and General LC-MS Assay

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

Assay - For each rate study, to a 2 mL solution of the nucleophile, was added 1 mL solution of cyclic sulfenamide. Effective concentrations were: Cyclic sulfenamide – 10, 25 or 50 or 100 μM; Nucleophile – 50, 125 or 250 μM, 1.0 mM or higher. Resulting reaction mixture was quenched after regular intervals by taking out a measured aliquot (300 μL) and adding it to a LC-MS vial containing formic acid (100 μL) and analyzing it by LC-MS. Area under the curve for the product formation at each time point was obtained from LC and plotted against time to get k_{obs}.

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

Assay approximation - In the cases of kinetically faster nucleophiles (where < 1 mM nucleophile concentrations were used), the k_{obs} was adjusted to [Nu] = 1 mM by multiplying the observed rate values with appropriate factor for comparison purposes. The correction presupposes that the same rate law applies throughout the entire concentration range, which may or may not hold true.

Standard deviation for k_{obs} reported are $\leq$ 7.5% and representative plot for each nucleophile is shown here.

Electrospray ionization mass spectrometry (ESI-MS). The molecular mass of Gpx3-S-Nucleophile adduct was measured on a LTQ XL linear Ion trap mass spectrometer (Thermo Scientific) connected to a liquid chromatography (LC) system. Each Gpx3-S-nucleophile adduct (3 μM, 8 μL) was separated and desalted onto a C8 column and subsequently analyzed in the mass spectrometer. Spectra were acquired in the positive ion mode. The deconvolution program MagTran was used to obtain the mass spectra.

Fig. S1. Sample LC plot - For the reaction of dipeptide-SOH **(10)** with 1,3-cyclohexanedione **(2e)** showing the thioether adduct formation.

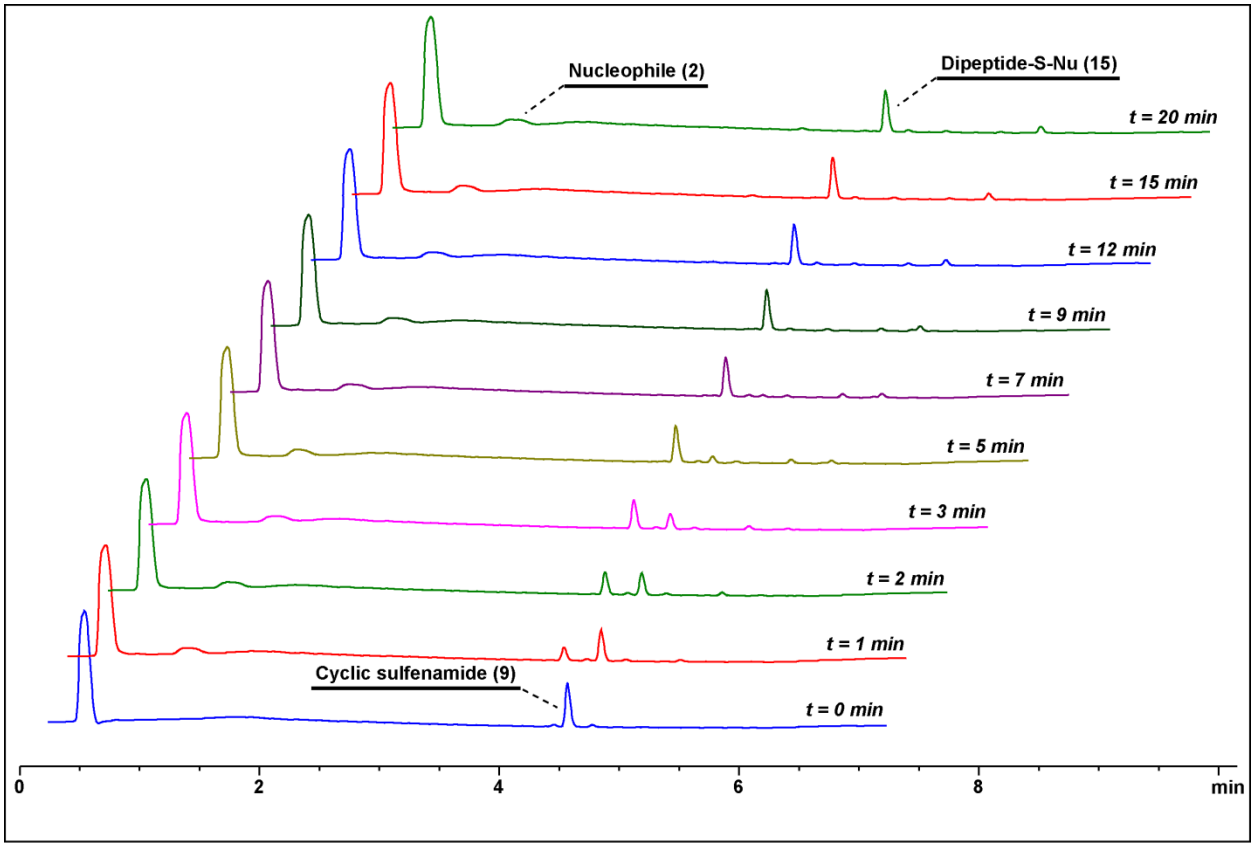


Fig. S2 Sample MS plot – For the reaction of dipeptide-SOH **(10)** with 1,3-cyclohexanedione **(2e)** showing the thioether adduct formation (*m/z* 479.2)

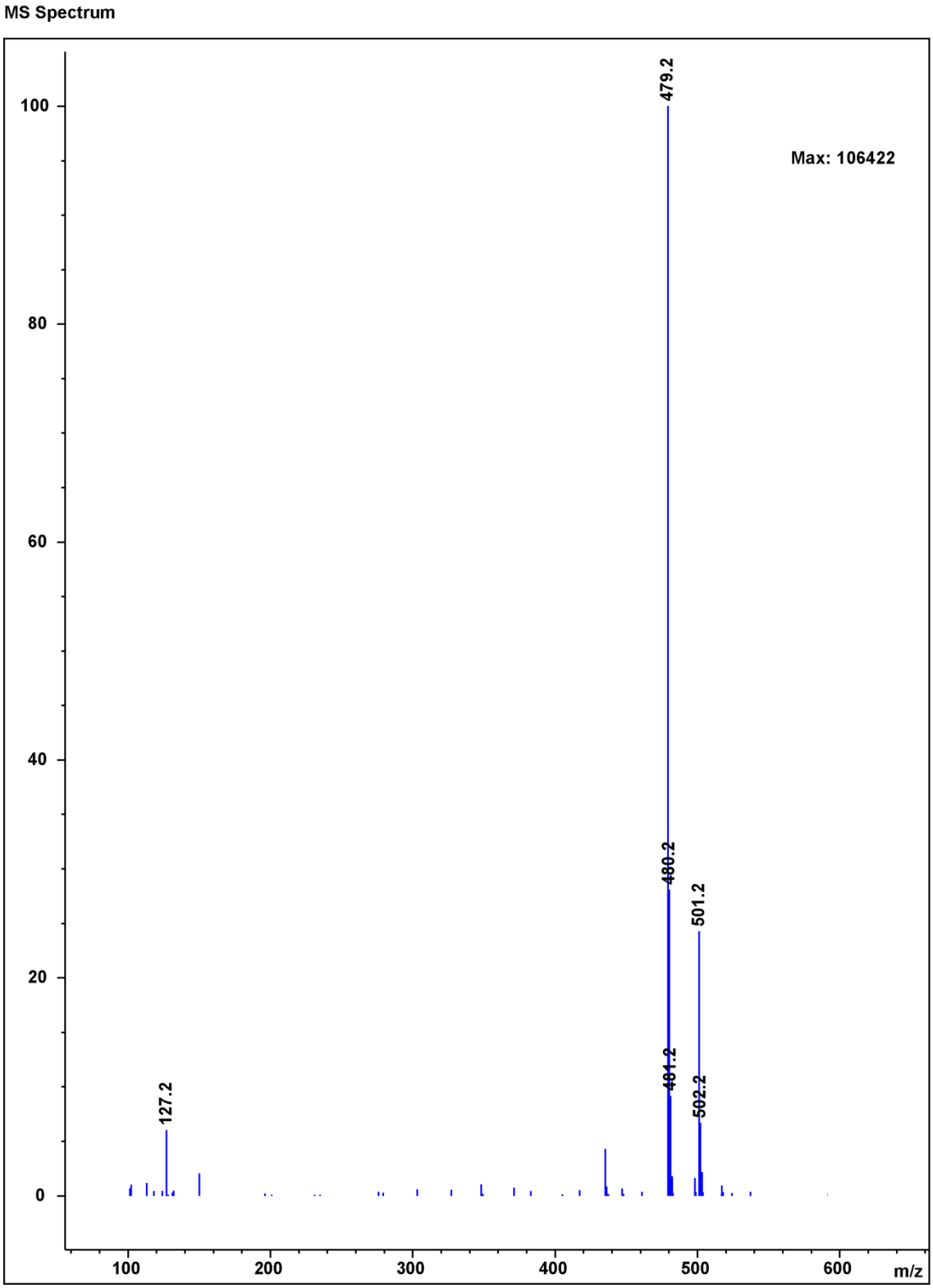


Fig. S3 Sample rate plot

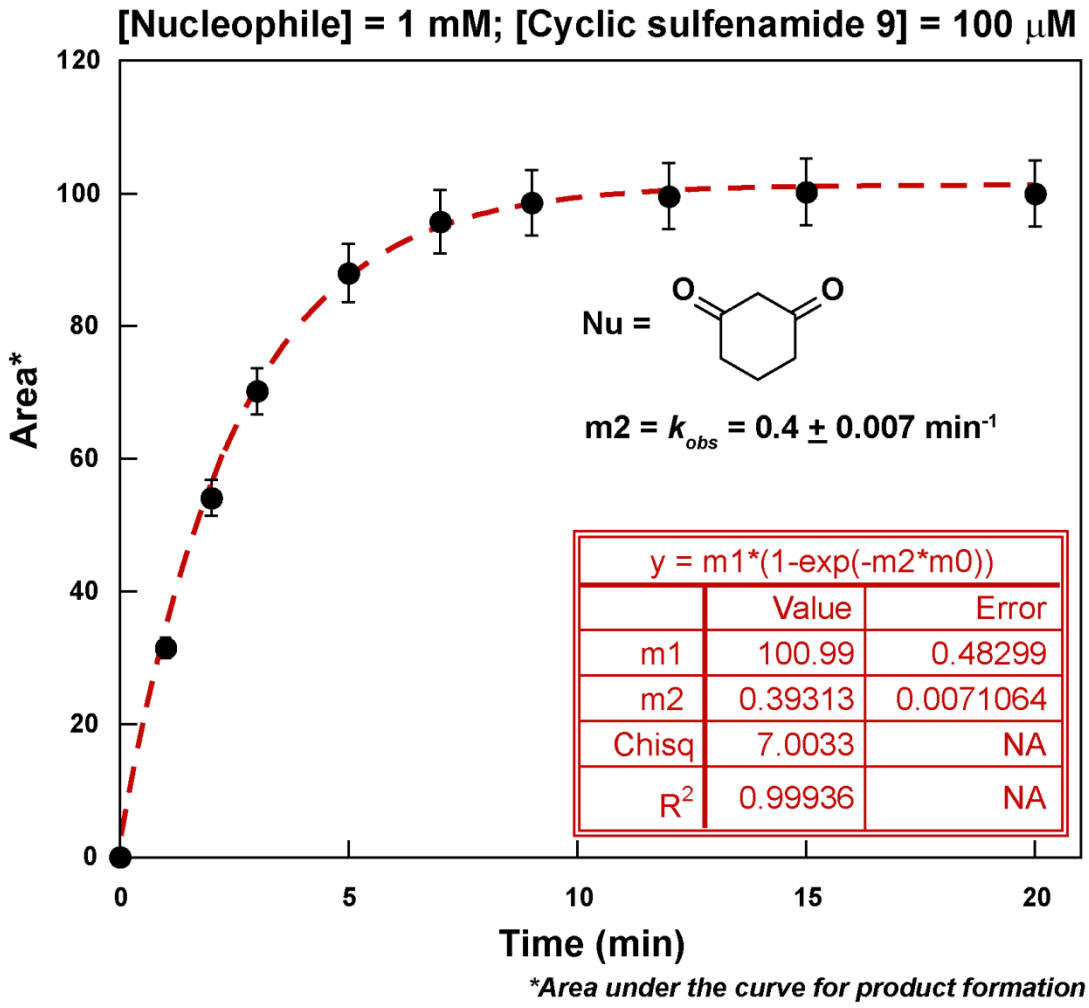
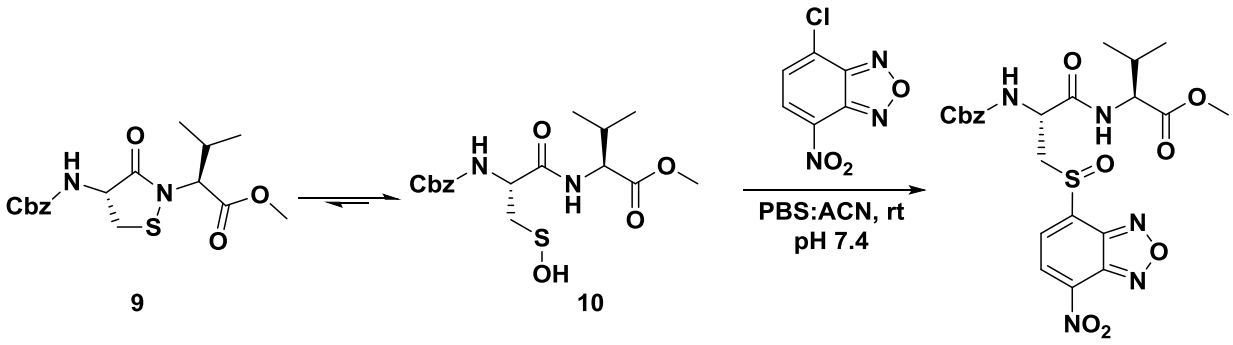


Fig. S4 Reaction of dipeptide-SOH **10** (1 mM) with NBD-Cl **4** (1 mM) under PBS:ACN (10 mM, 2:1, pH 7.4, rt) conditions



LC traces over 2 days

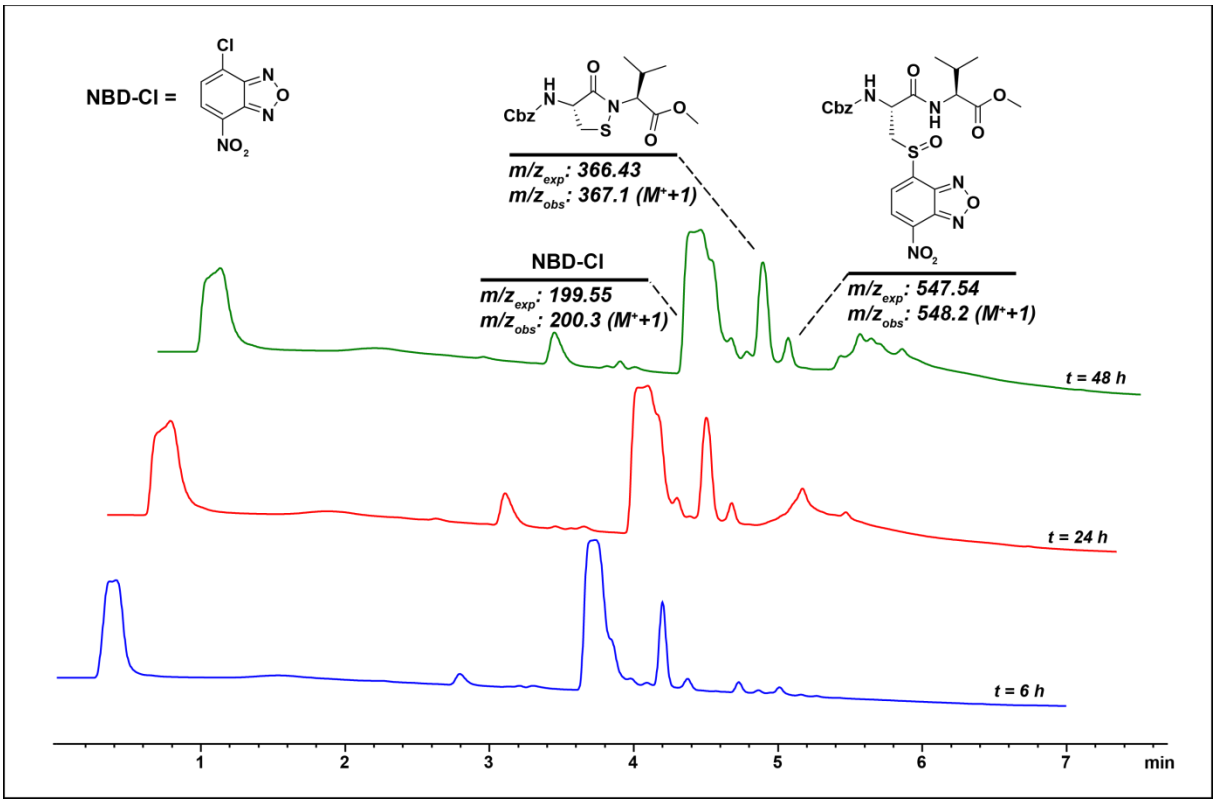
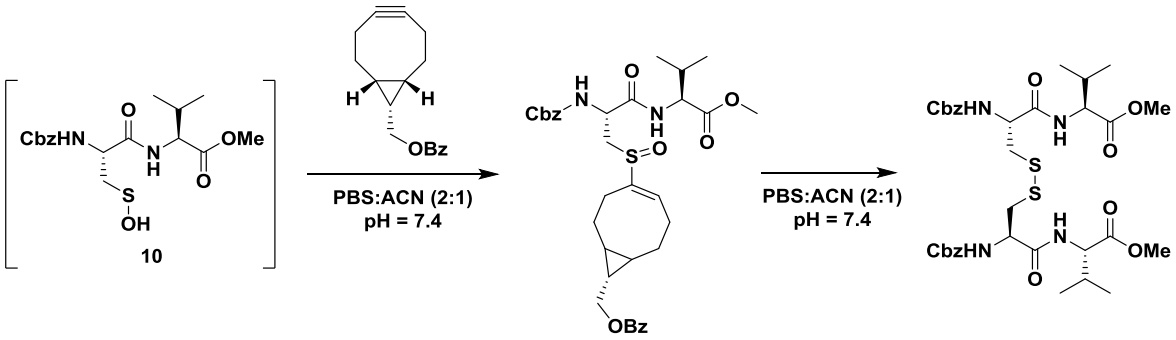
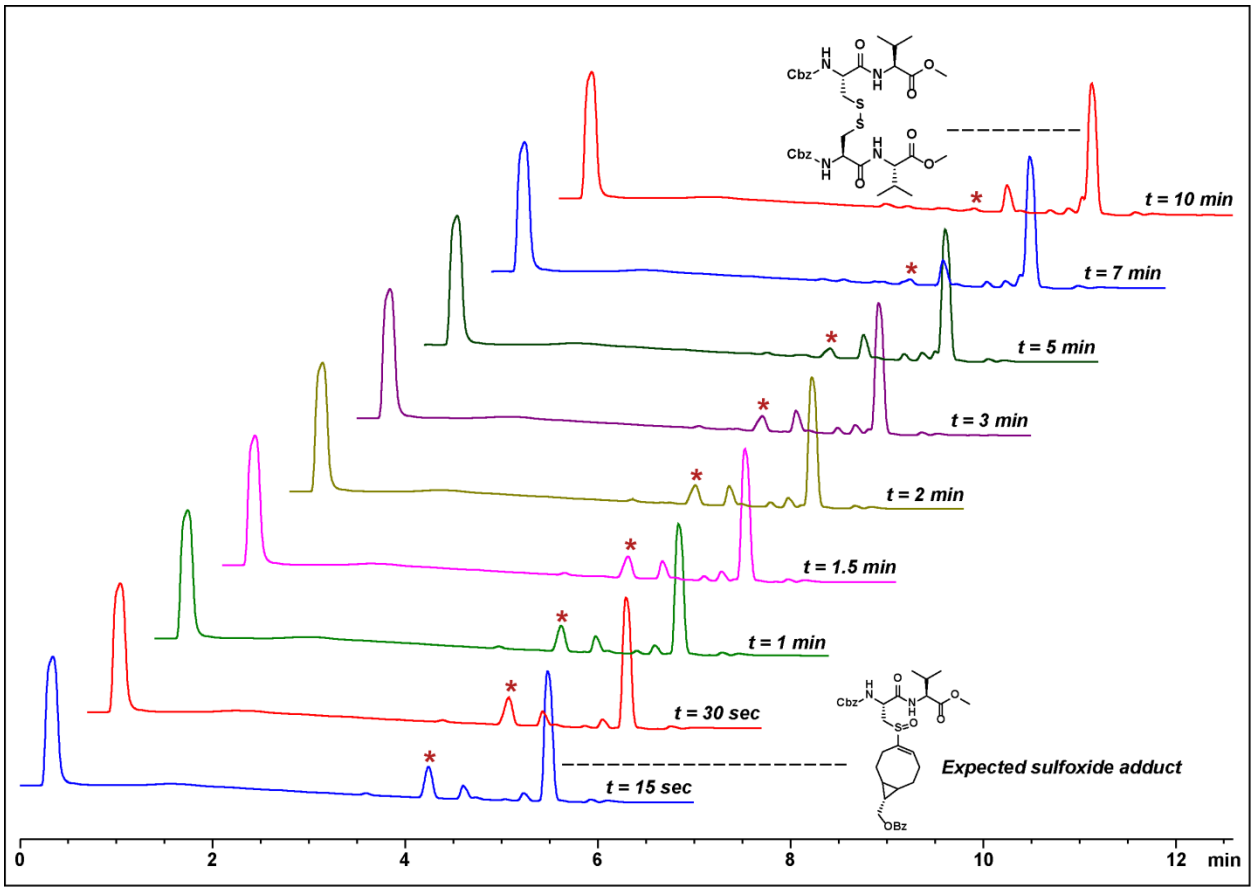
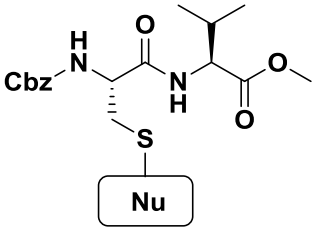


Fig. S5 Reaction of the strained cycloalkyne ((1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-yl)methyl benzoate **(6a)** with dipeptide-SOH **10.**





Stability of Dipeptide-S-Nu adducts under reductive conditions.



(1) Synthesize and prep-HPLC purify all analogs
(2) Confirm the formation by LC-MS, ^{1}H-NMR and ^{13}C-NMR

(a) Stability over 12 h at pH 7.4
(b) DTT (5 mM)
(c) GSH (5 mM)
(d) TCEP (5 mM)

Procedure: A 25 mM solution of dipeptide-S-Nu adduct was prepared in DMSO. Solutions of DTT (500 mM), GSH (500 mM) and TCEP (500 mM) were prepared in 50 mM HEPES, 100 mM NaCl (pH 7.4). 10 μL of dipeptide-S-Nu solution and 10 μL of each reducing agent solution was added to 980 μL of buffer (50 mM HEPES, 100 mM NaCl, pH 7.4, RT). Effective concentrations were as follow:

(1) Dipeptide-S-Nu = 250 μM

(2) Reducting agent = 5 mM

(3) Buffer = 50 mM HEPES, 100 mM NaCl, pH 7.4

Each reaction mixture was analyzed by LC-MS time course.

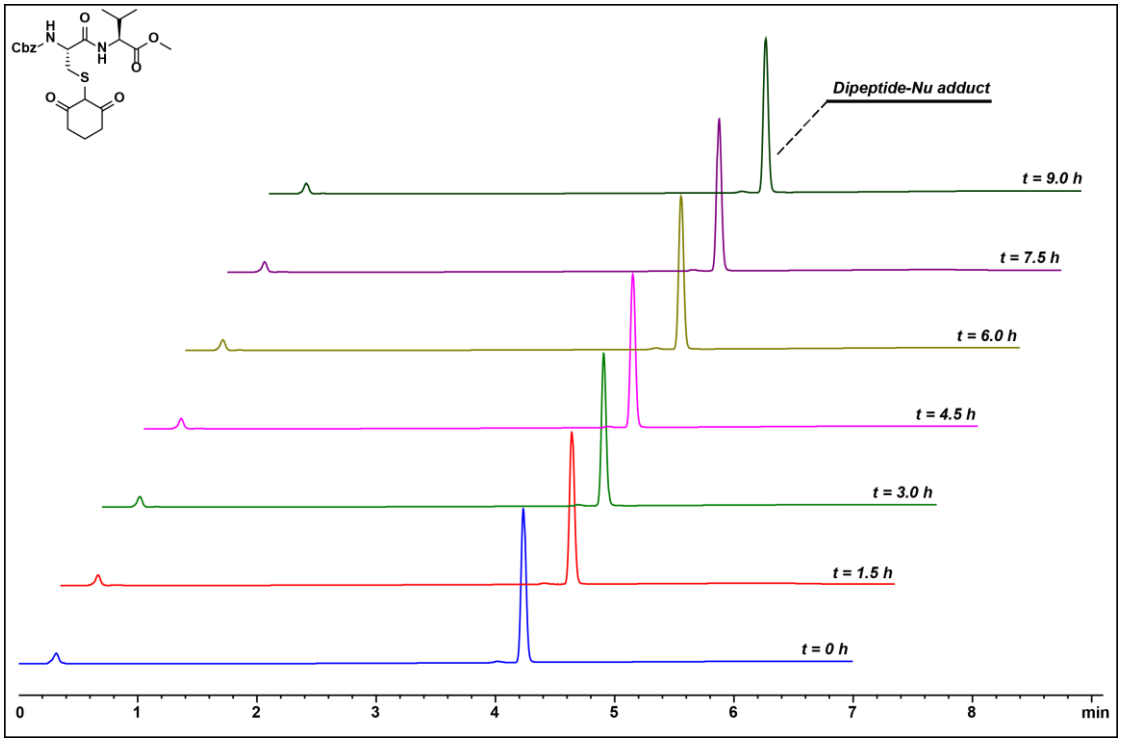


Fig. S6 Stability of dipeptide-Nu adduct **15** (250 μM) in buffer over a course of 9 h.

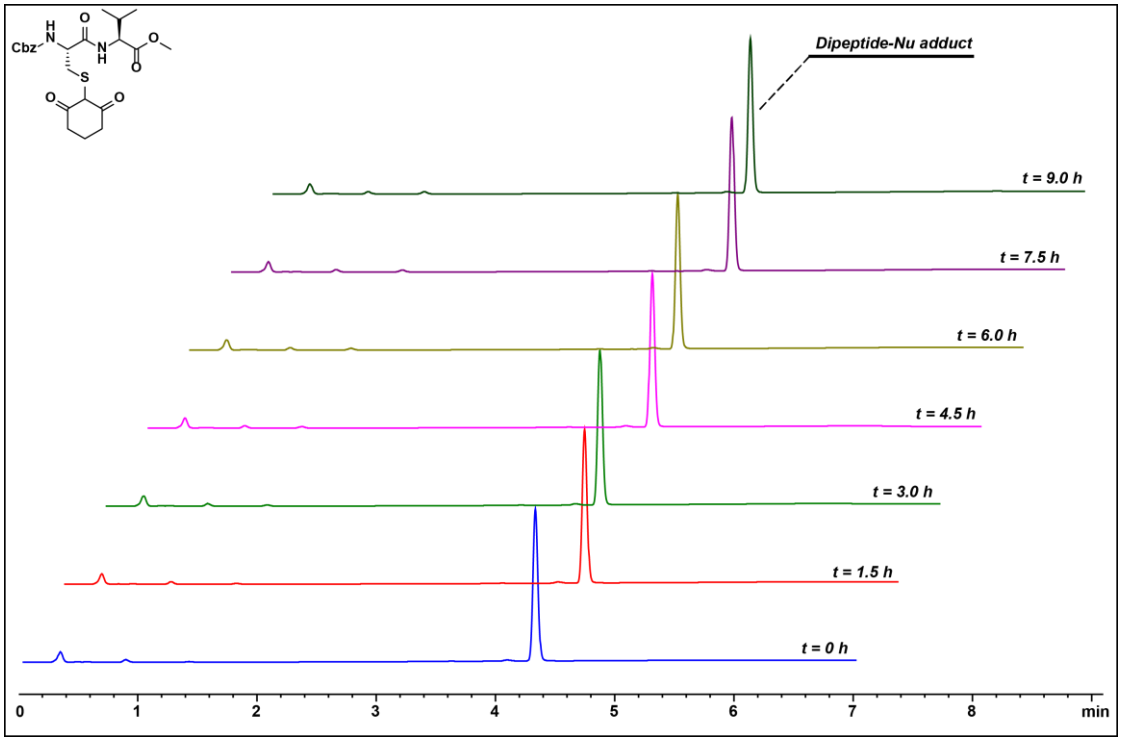


Fig. S7 Stability of dipeptide-Nu adduct **15** (250 μM) in presence of DTT (5 mM) in buffer over a course of 9 h.

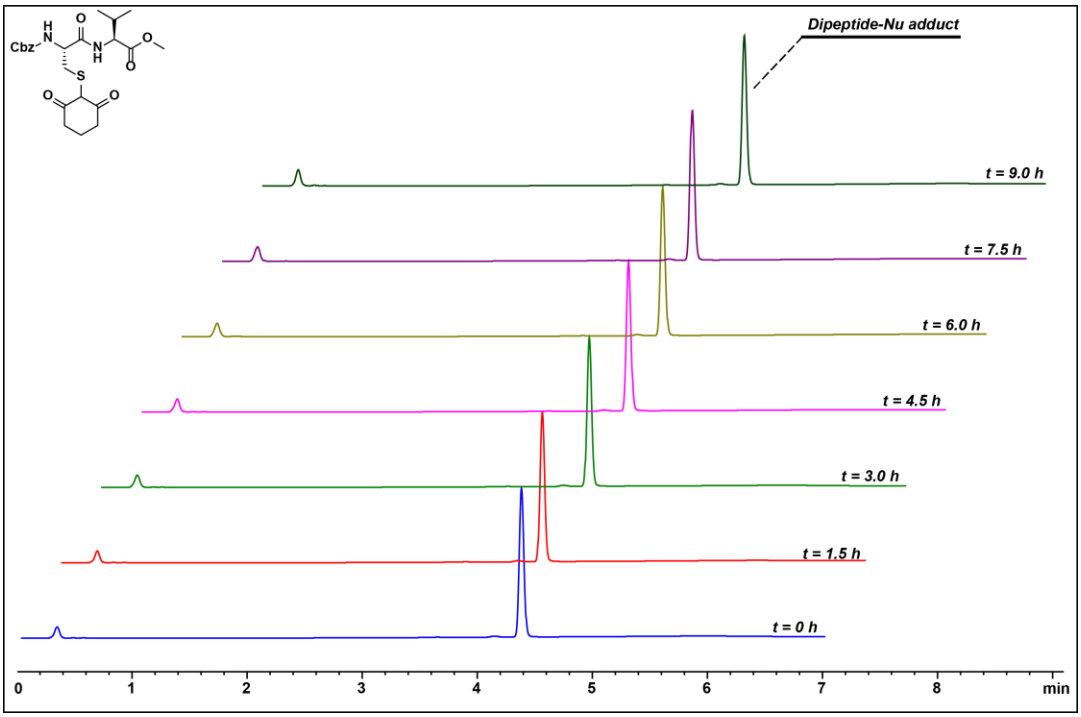


Fig. S8 Stability of dipeptide-Nu adduct **15** (250 μM) in presence of GSH (5 mM) in buffer over a course of 9 h.

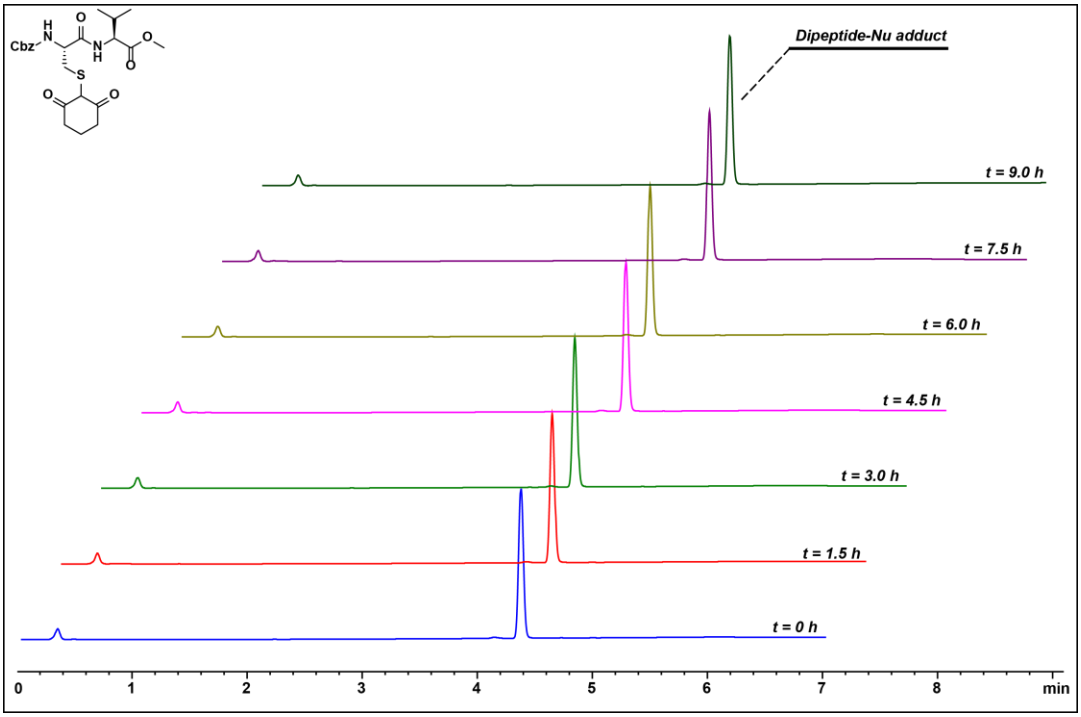


Fig. S9 Stability of dipeptide-Nu adduct **15** (250 μM) in presence of TCEP (5 mM) in buffer over a course of 9 h.

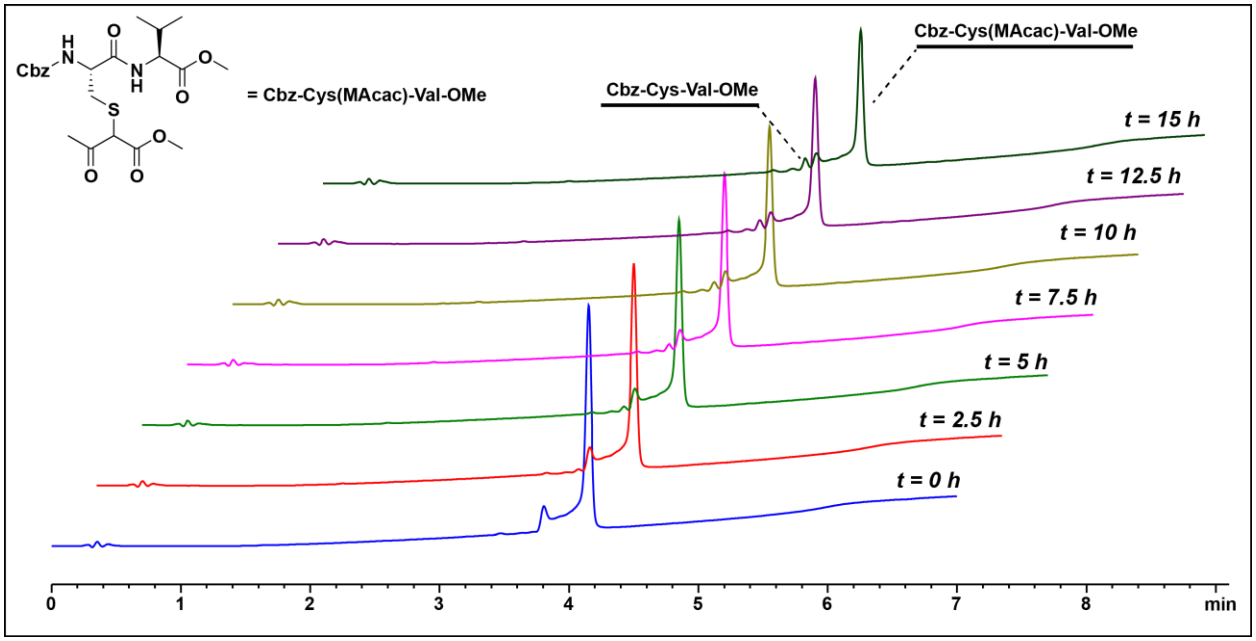


Fig. S10 Stability of Cbz-Cys(Macac)-Val-OMe **16** (250 μM) in buffer over a course of 12 h.

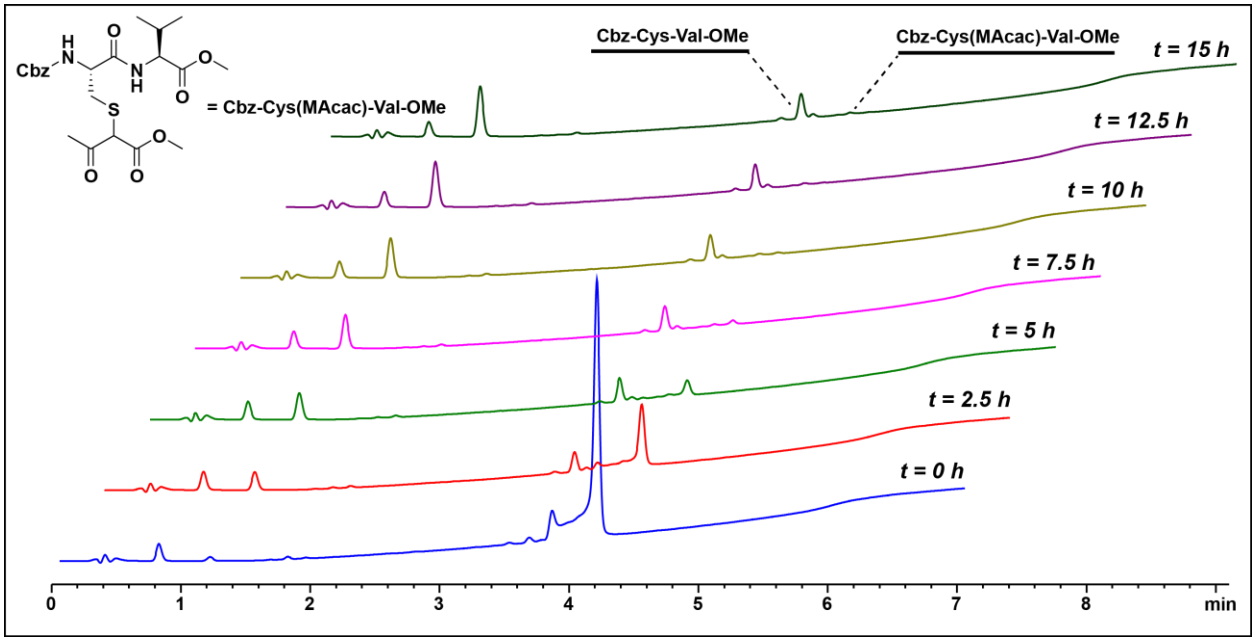


Fig. S11 Stability of Cbz-Cys(Macac)-Val-OMe **16** (250 μM) in presence of DTT (5 mM) in buffer over a course of 12 h.

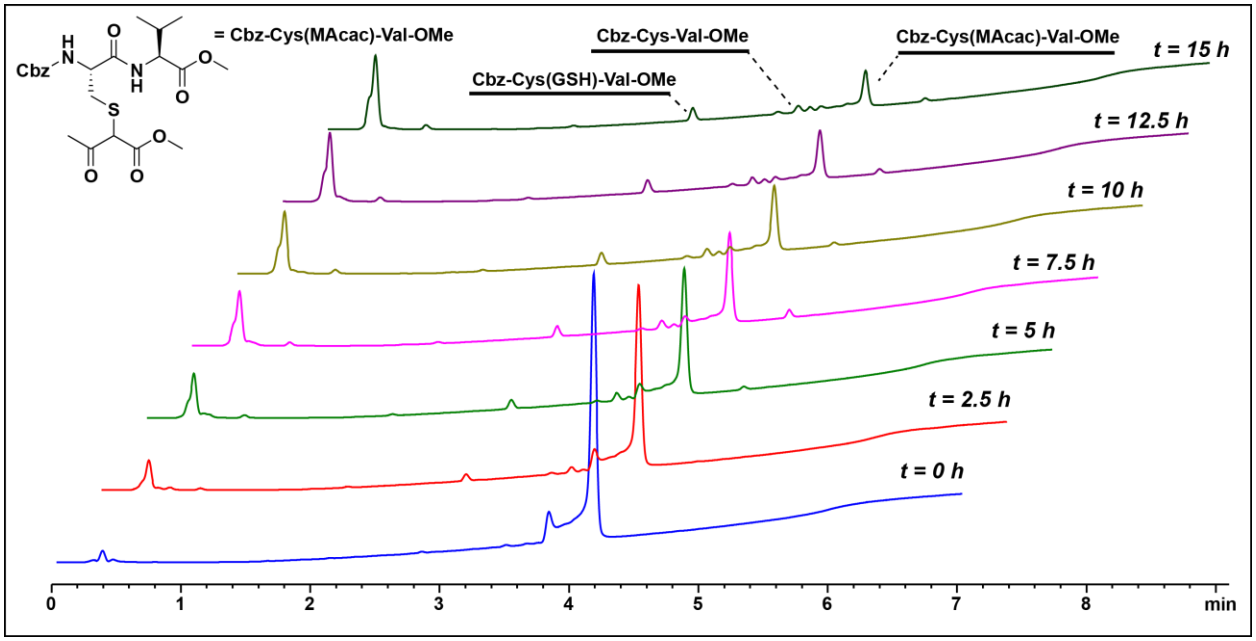


Fig. S12 Stability of Cbz-Cys(Macac)-Val-OMe **16** (250 μM) in presence of GSH (5 mM) in buffer over a course of 12 h.

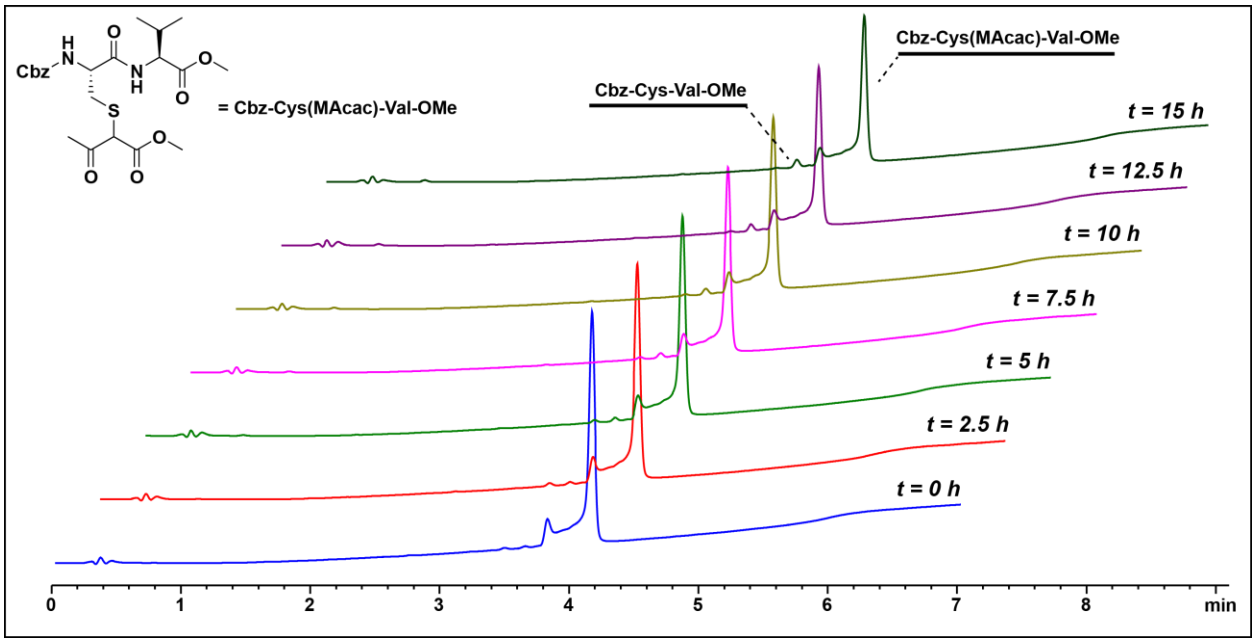


Fig. S13 Stability of Cbz-Cys(Macac)-Val-OMe **16** (250 μM) in presence of TCEP (5 mM) in buffer over a course of 12 h.

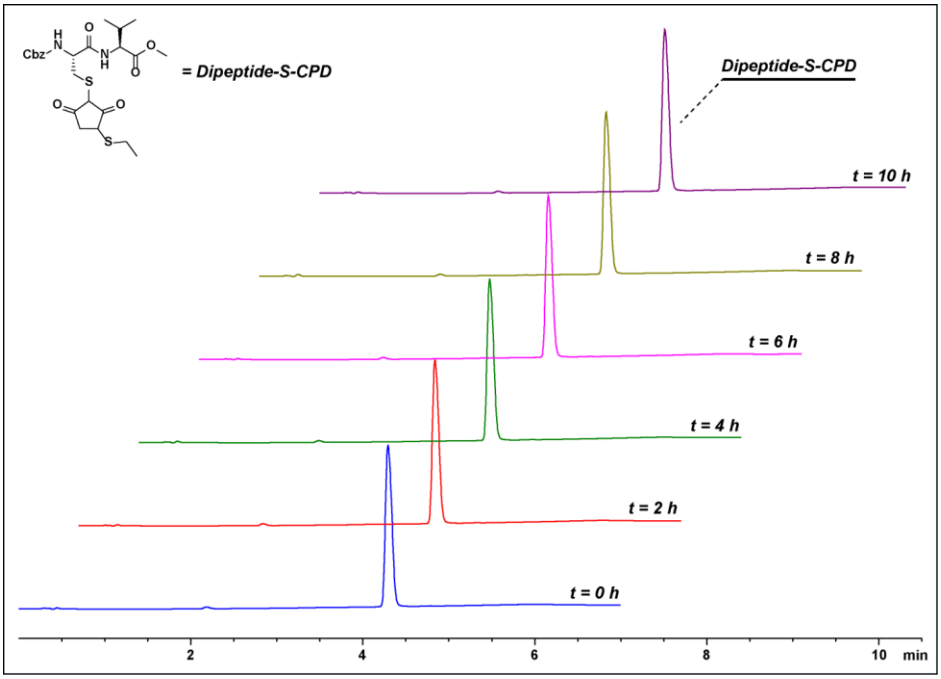


Fig. S14 Stability of Cbz-Cys(CPD)-Val-OMe **17** (250 μM) in buffer over a course of 10 h.

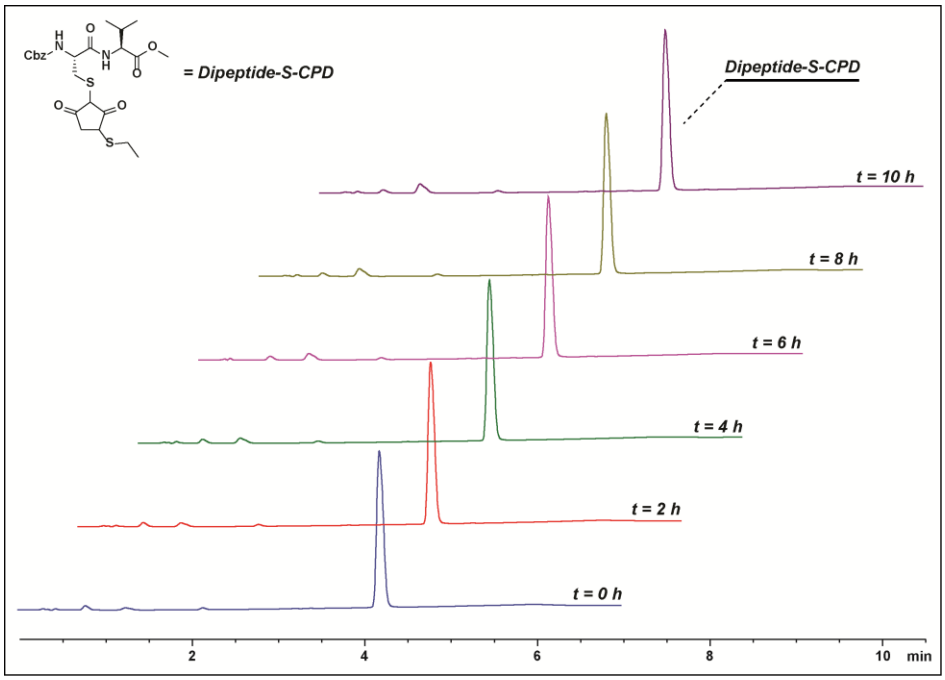


Fig. S15 Stability of Cbz-Cys(CPD)-Val-OMe **17** (250 μM) in presence of DTT (5 mM) in buffer over a course of 10 h.

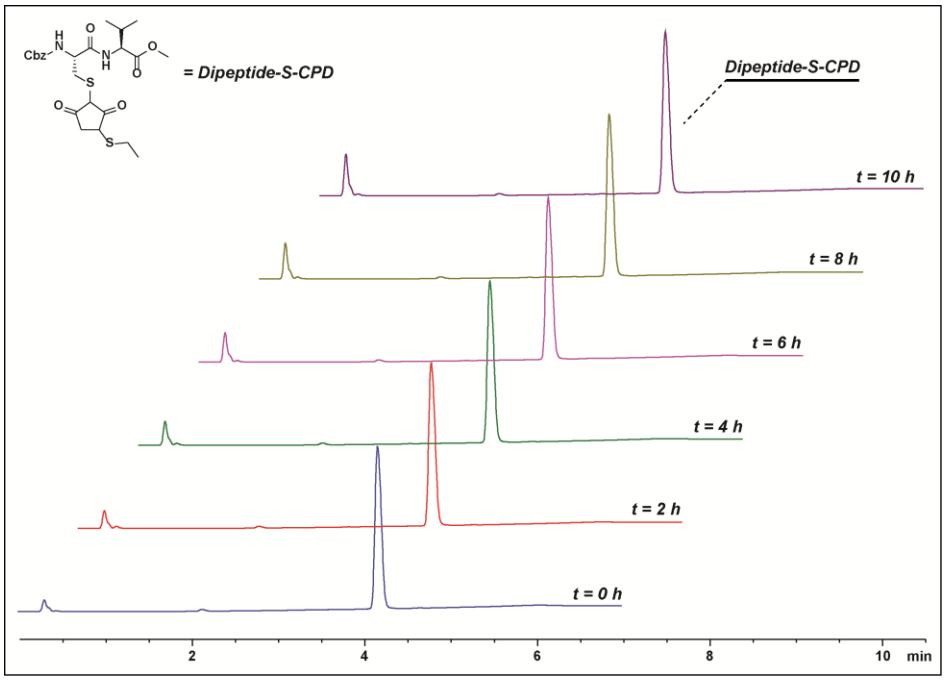


Fig. S16 Stability of Cbz-Cys(CPD)-Val-OMe **17** (250 μM) in presence of GSH (5 mM) in buffer over a course of 10 h.

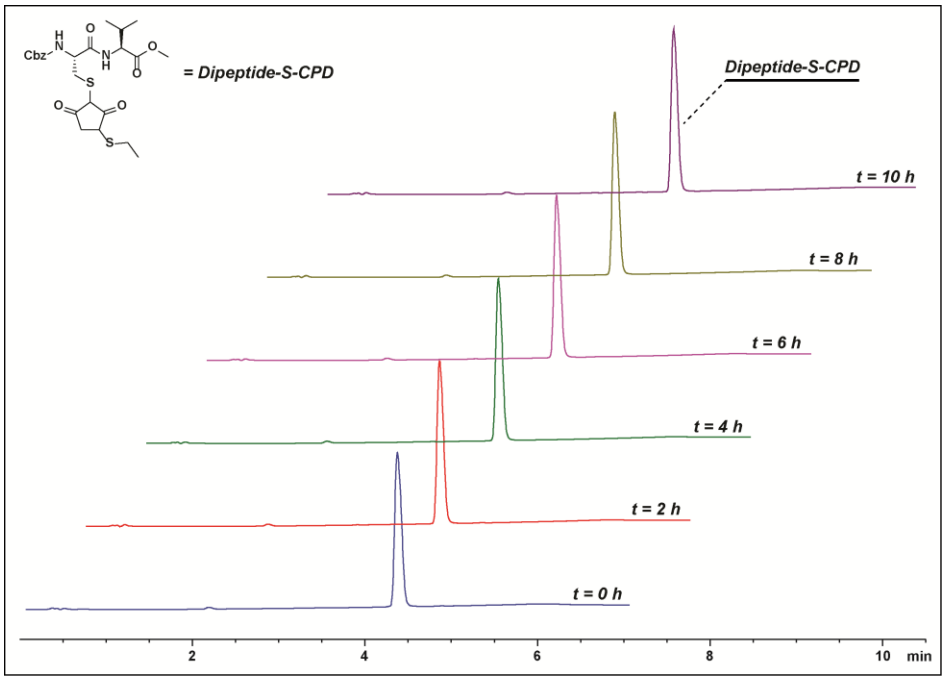


Fig. S17 Stability of Cbz-Cys(CPD)-Val-OMe **17** (250 μM) in presence of TCEP (5 mM) in buffer over a course of 10 h.

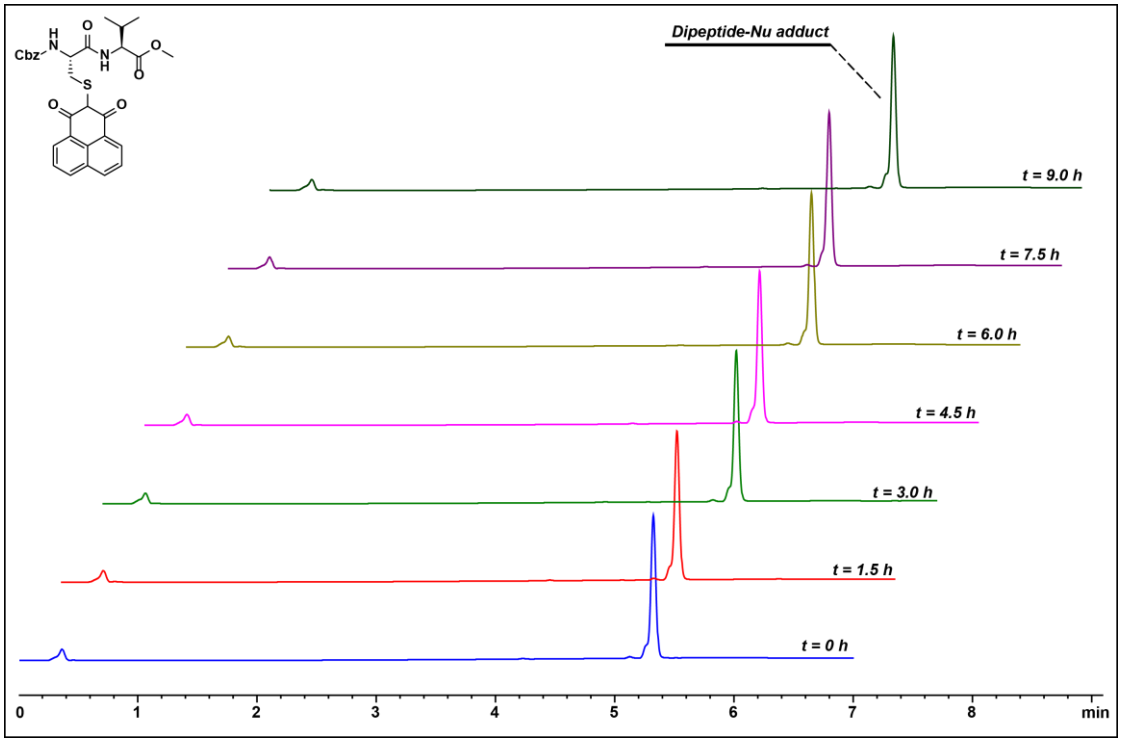


Fig. S18. LC trace of dipeptide-Nu adduct **18** (250 μM) in buffer (pH 7.4) over 9 h.

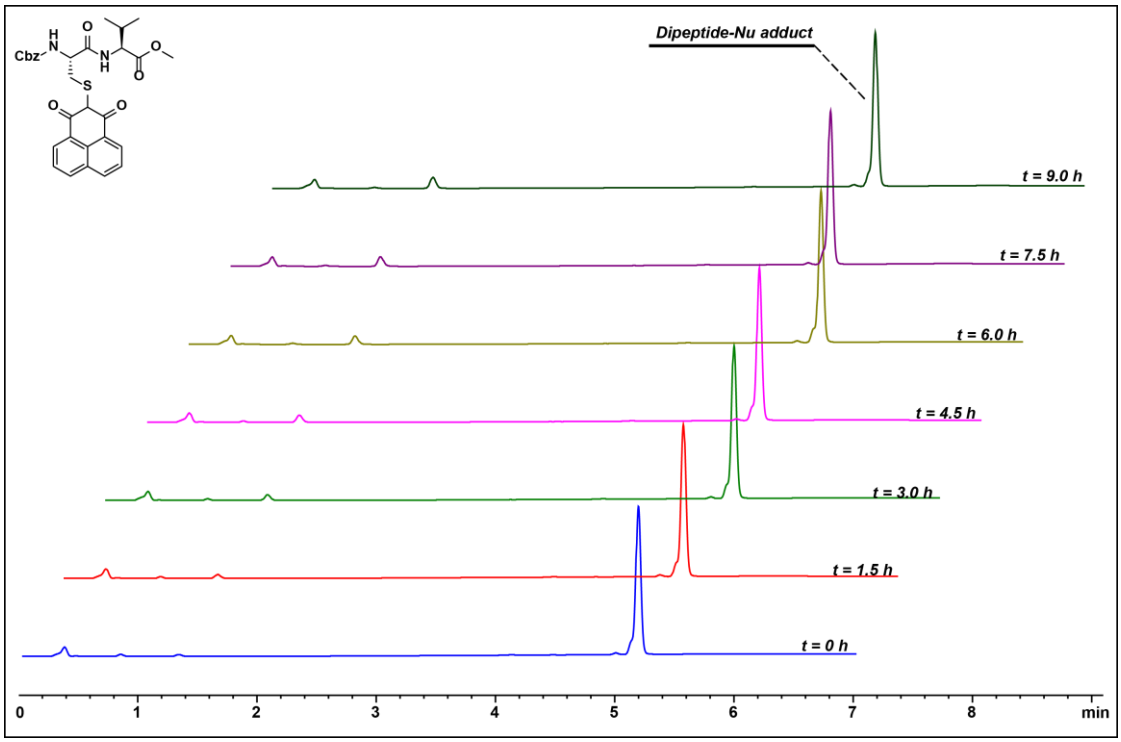


Fig. S19 LC trace of the reaction of dipeptide-Nu adduct **18** (250 μM) with DTT (5 mM) over 9 h.

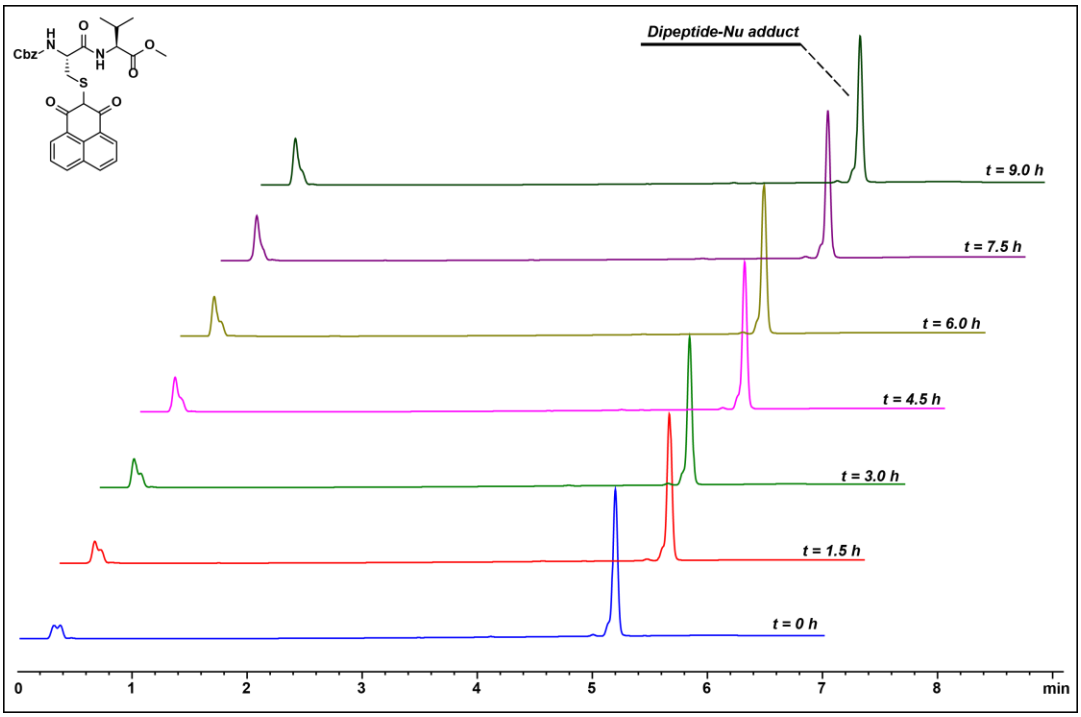


Fig. S20 LC trace of the reaction of dipeptide-Nu adduct **18** (250 μM) with GSH (5 mM) over 9 h.

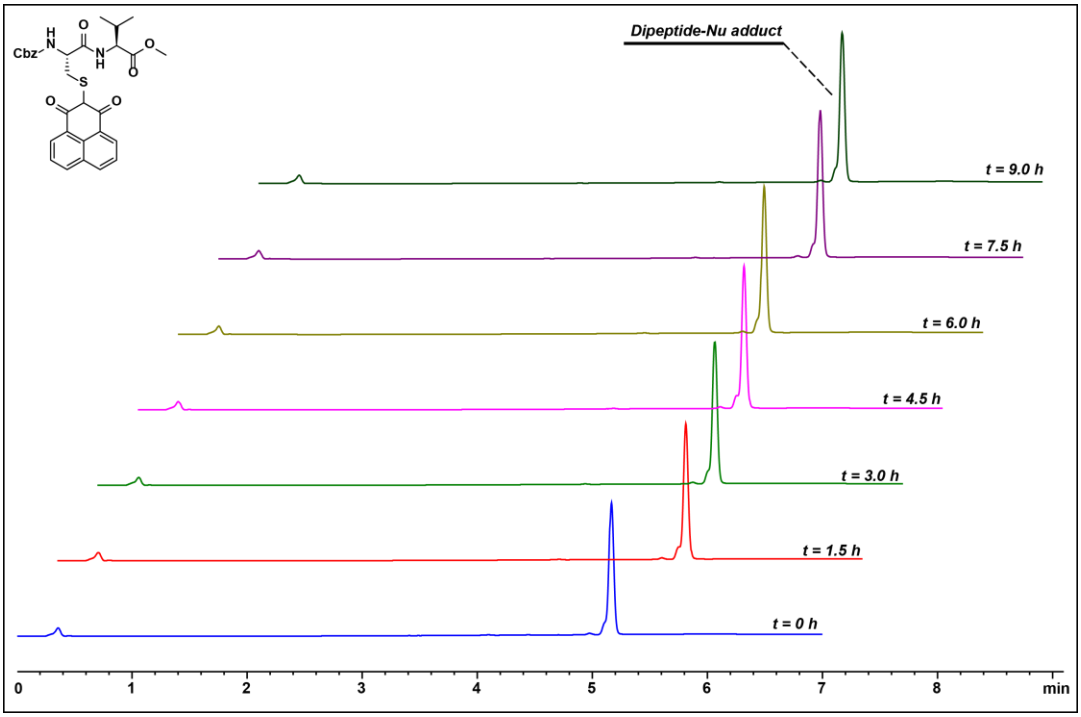


Fig. S21 LC trace of the reaction of dipeptide-Nu adduct **18** (250 μM) with TCEP (5 mM) over 9 h.

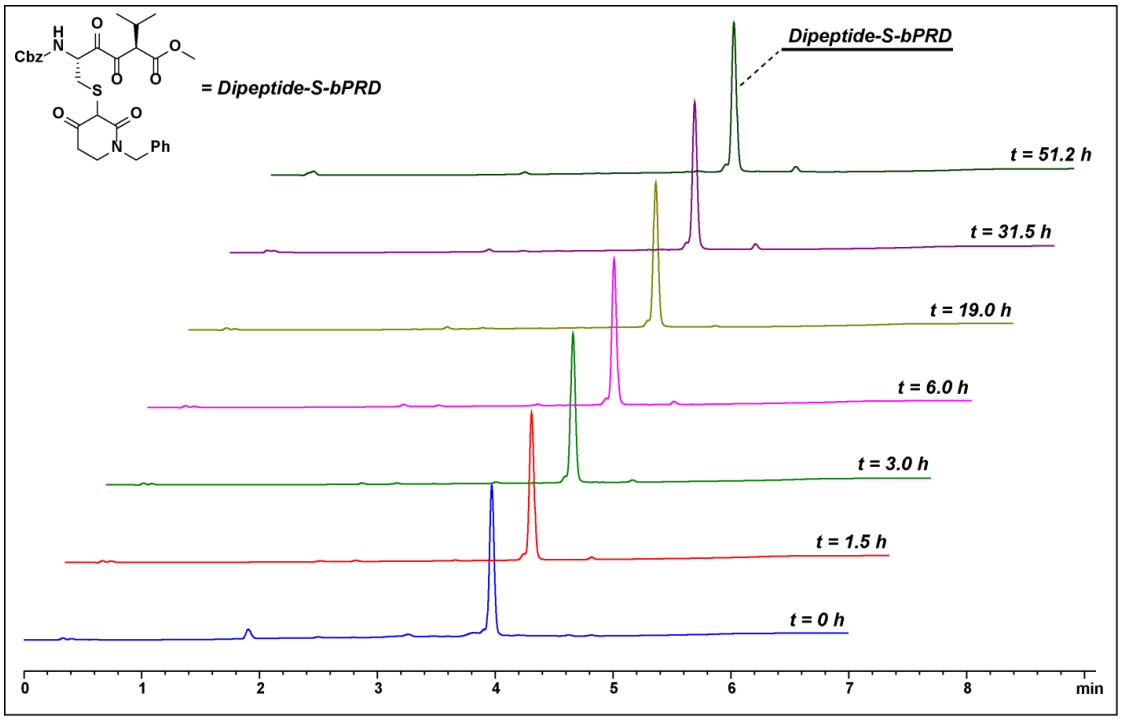


Fig. S22 Stability of Cbz-Cys(bPRD)-Val-OMe **19** (250 μM) in buffer over a course of 52 h.

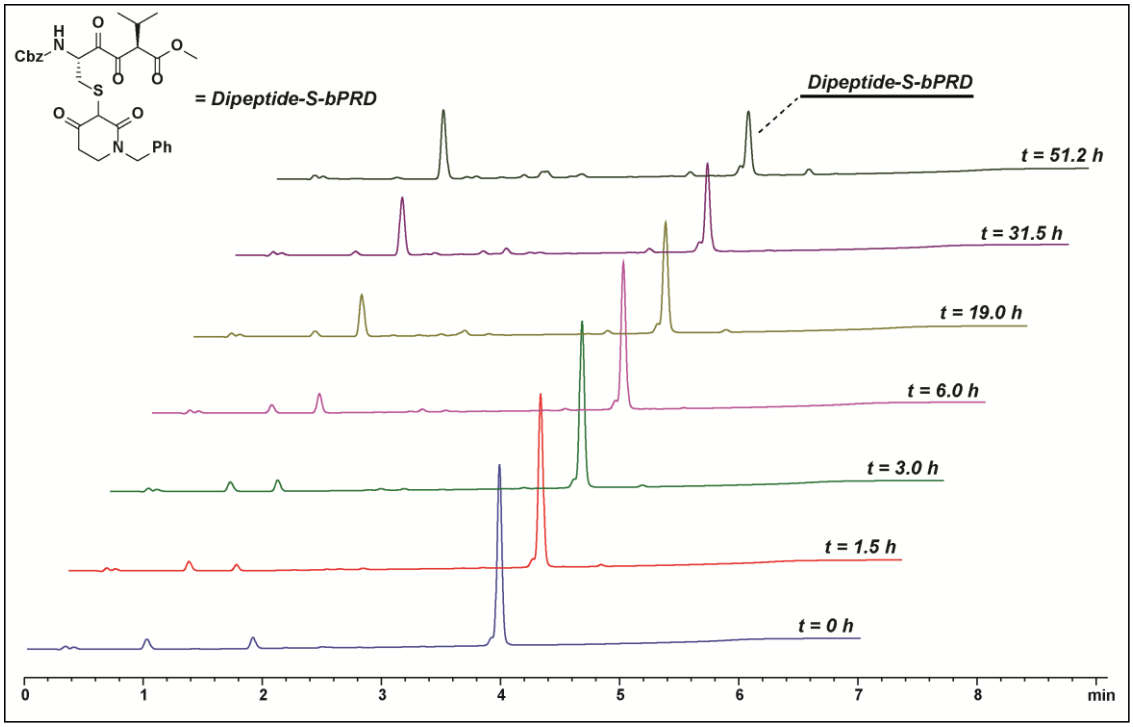


Fig. S23 Stability of Cbz-Cys(bPRD)-Val-OMe **19** (250 μM) in presence of DTT (5 mM) in buffer over a course of 52 h.

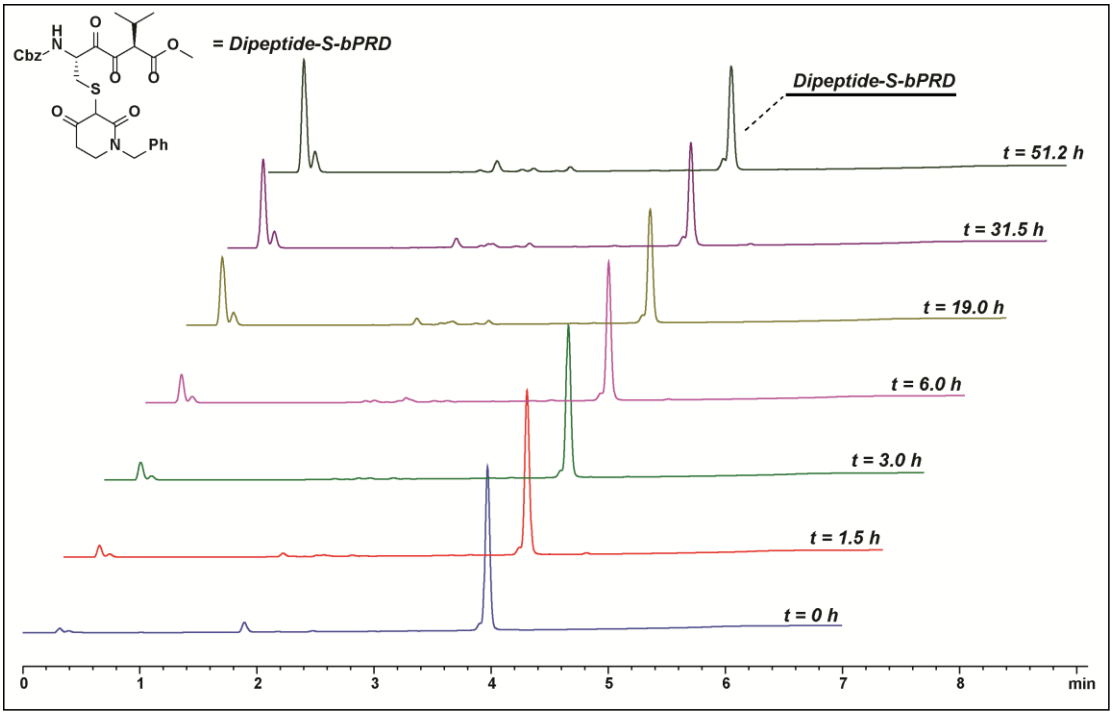


Fig. S24 Stability of Cbz-Cys(bPRD)-Val-OMe **19** (250 μM) in presence of GSH (5 mM) in buffer over a course of 52 h.

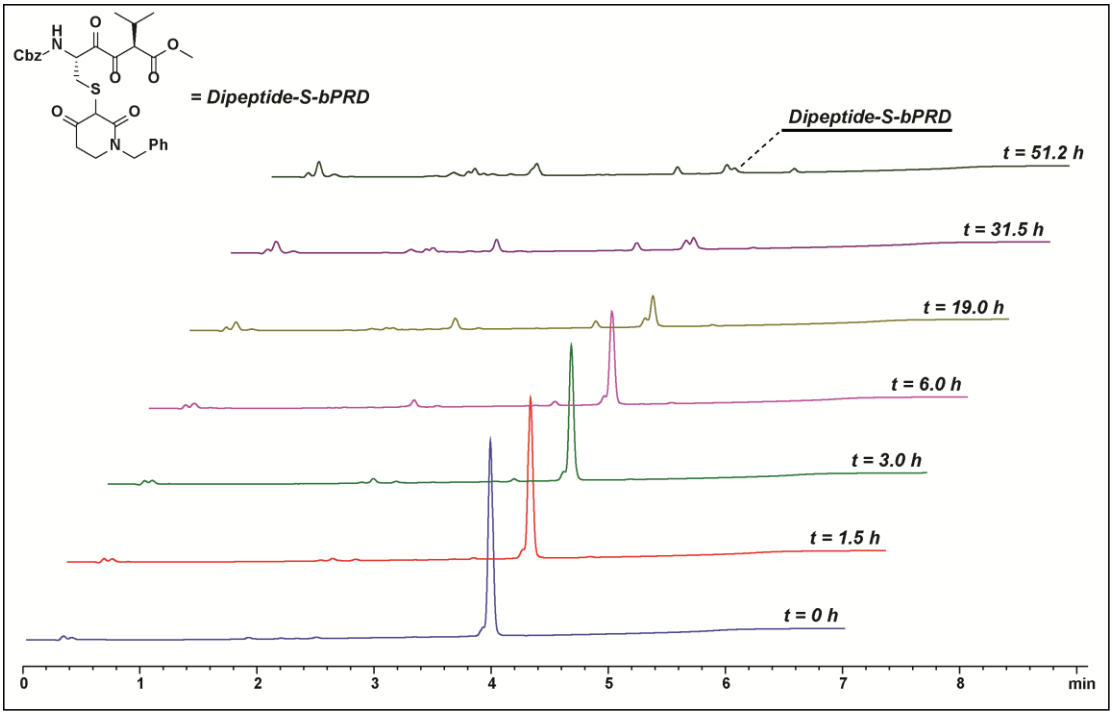


Fig. S25 Stability of Cbz-Cys(bPRD)-Val-OMe **19** (250 μM) in presence of TCEP (5 mM) in buffer over a course of 52 h.

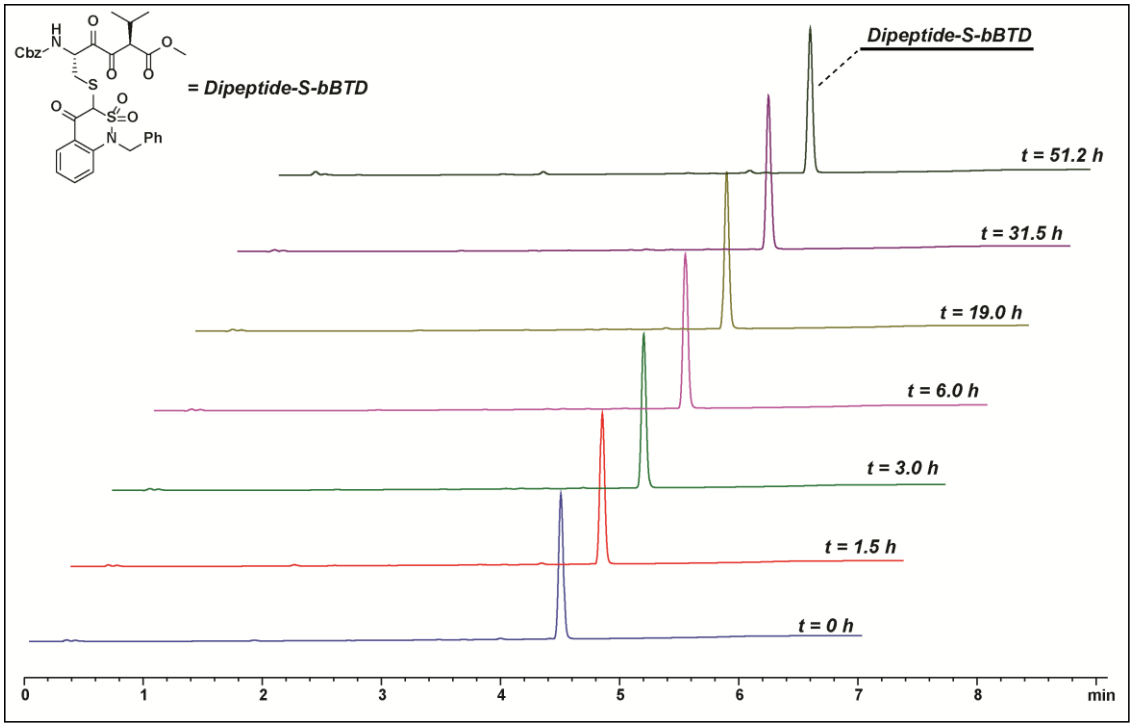


Fig. S26 Stability of Cbz-Cys(bBTD)-Val-OMe **20** (250 μM) in buffer over a course of 52 h.

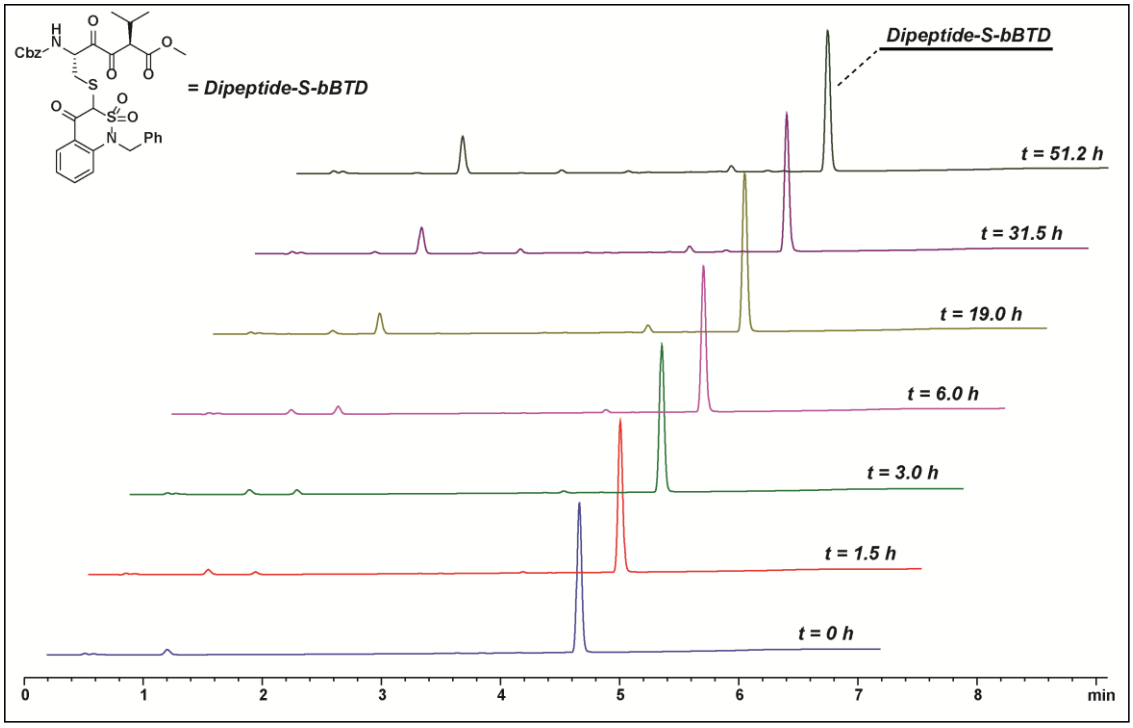


Fig. S27 Stability of Cbz-Cys(bBTD)-Val-OMe **20** (250 μM) in presence of DTT (5 mM) in buffer over a course of 52 h.

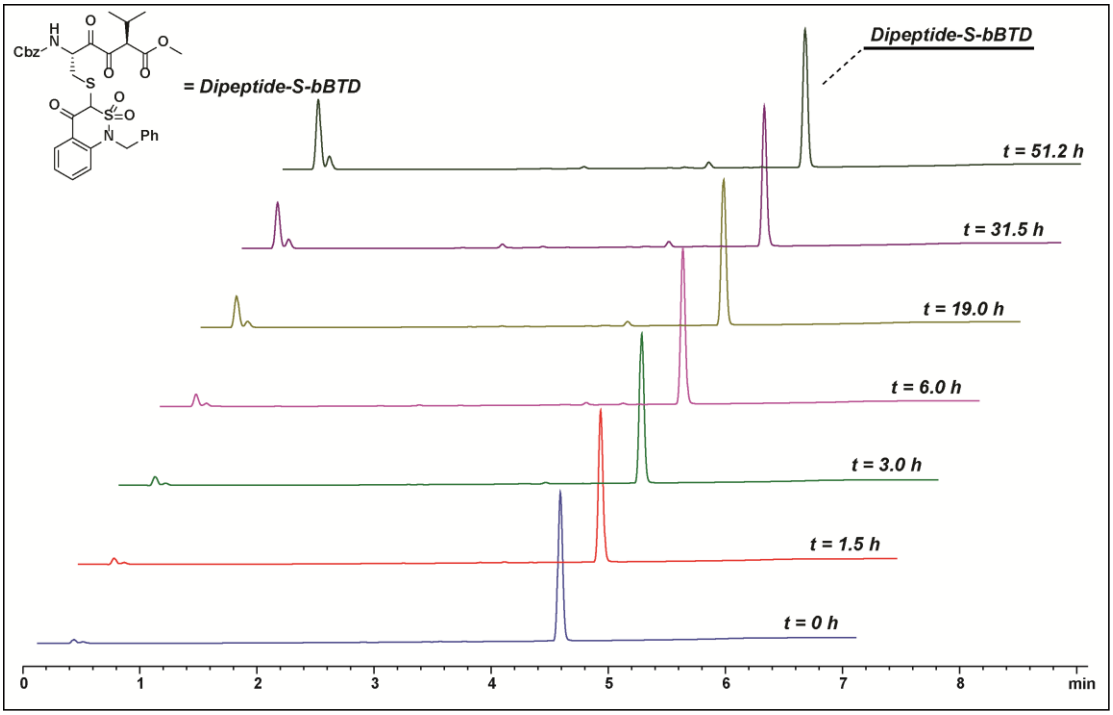


Fig. S28 Stability of Cbz-Cys(bBTD)-Val-OMe **20** (250 μM) in presence of GSH (5 mM) in buffer over a course of 52 h.

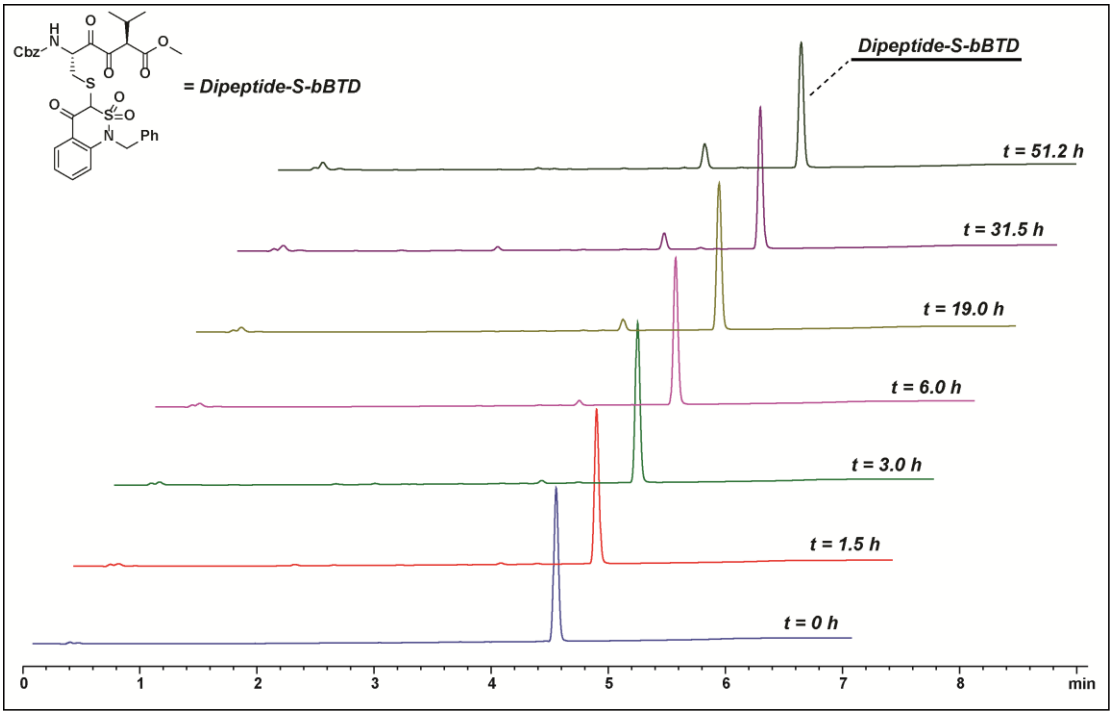


Fig. S29 Stability of Cbz-Cys(bBTD)-Val-OMe **20** (250 μM) in presence of TCEP (5 mM) in buffer over a course of 52 h.

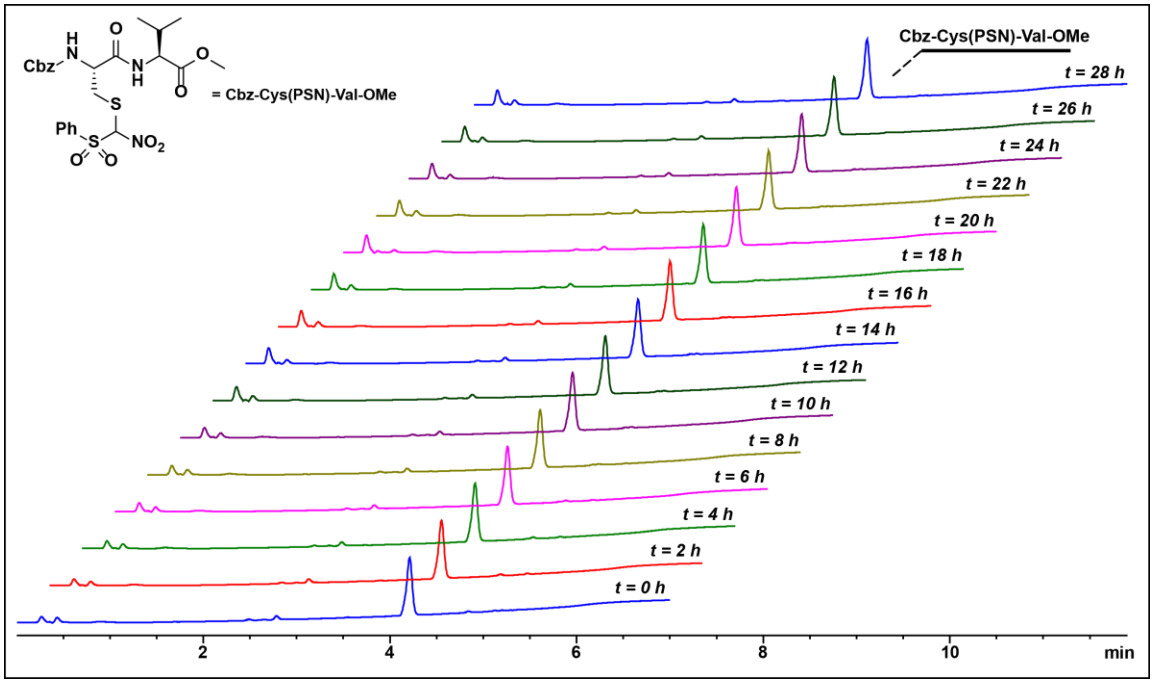


Fig. S30 LC trace of Cbz-Cys(PSN)-Val-OMe **21** (250 μM) in buffer (pH 7.4) over 28 h.

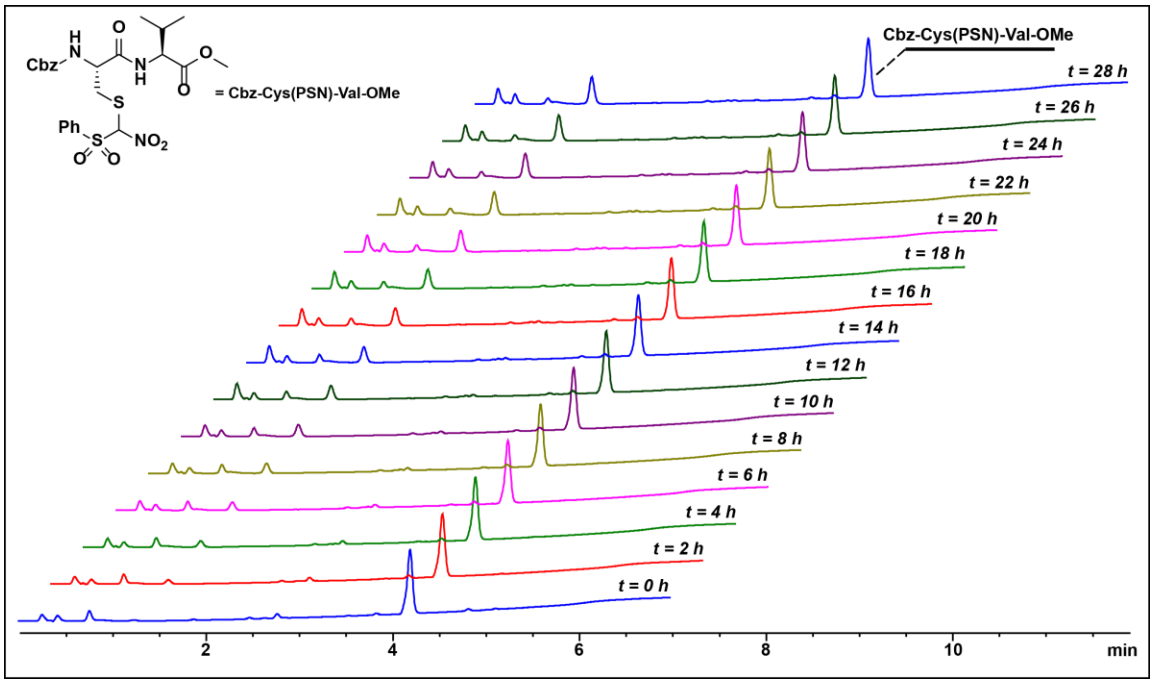


Fig. S31 LC trace of the reaction of Cbz-Cys(PSN)-Val-OMe **21** (250 μM) with DTT (5 mM) over 28 h.

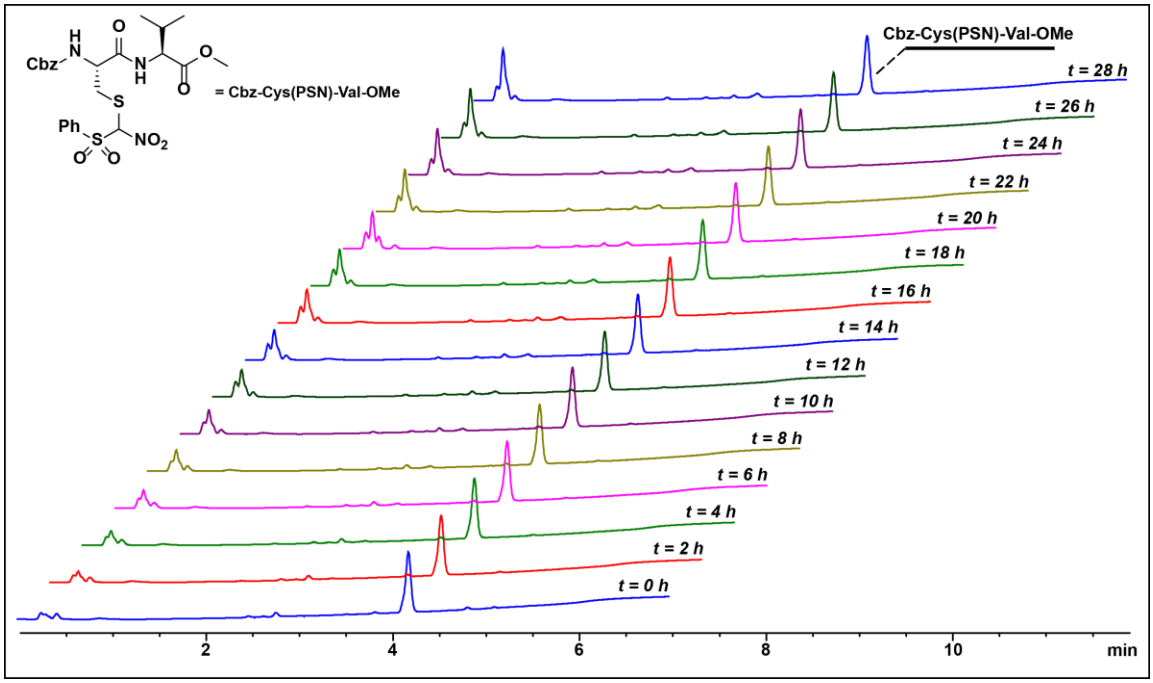


Fig. S32 LC trace of the reaction of Cbz-Cys(PSN)-Val-OMe **21** (250 μM) with GSH (5 mM) over 28 h.

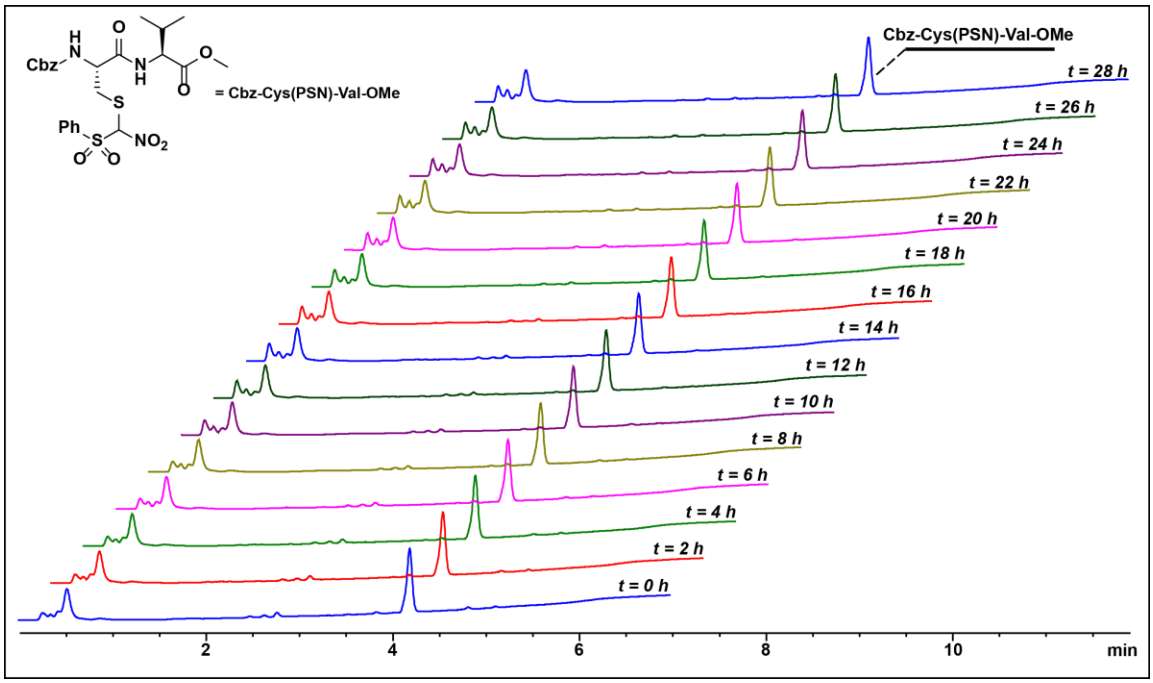


Fig. S33 LC trace of the reaction of Cbz-Cys(PSN)-Val-OMe **21** (250 μM) with TCEP (5 mM) over 28 h.

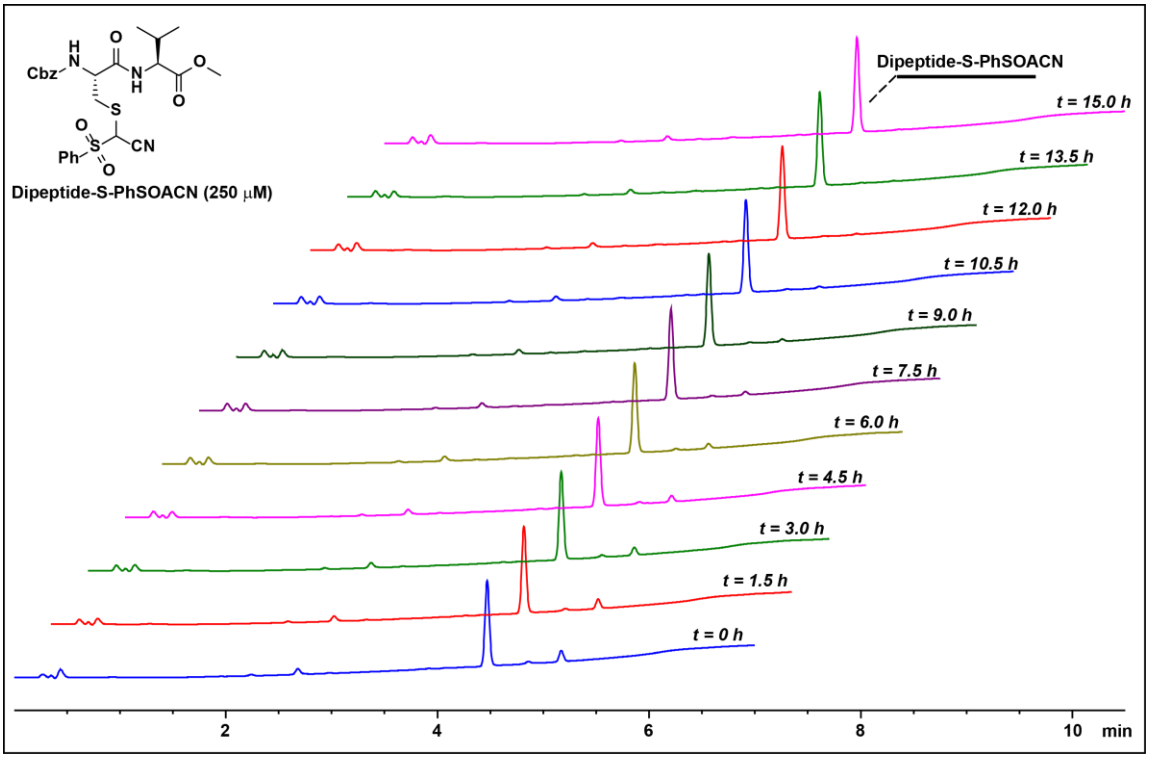


Fig. S34 Stability of Cbz-Cys(PSA)-Val-OMe **22** (250 μM) in buffer over a course of 12 h.

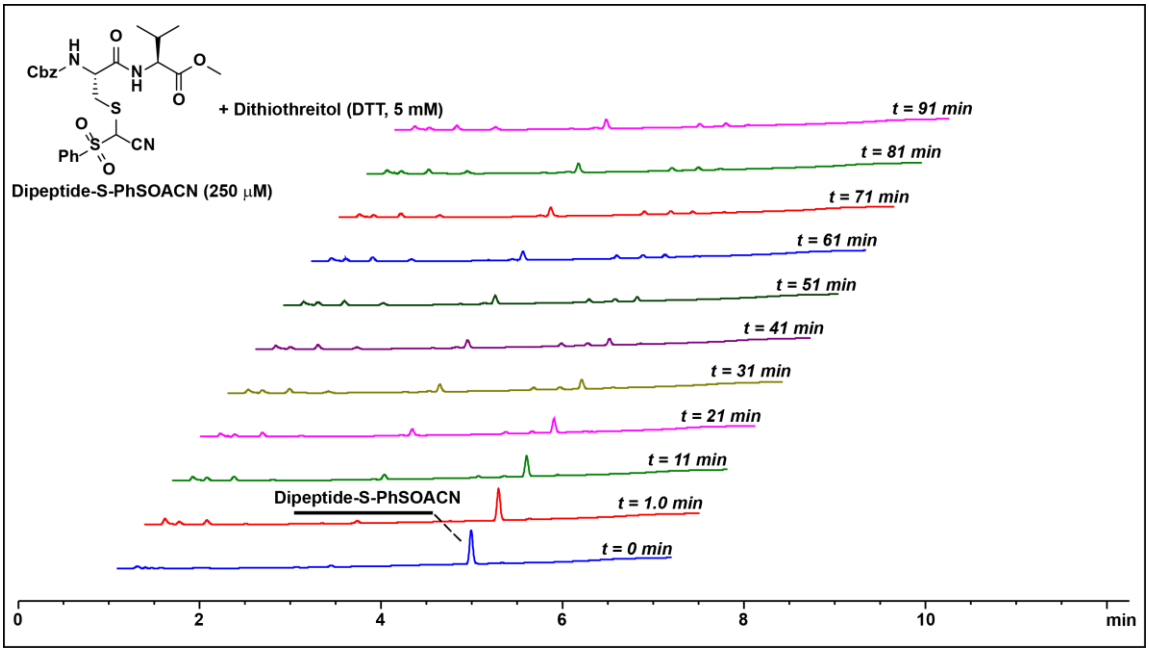


Fig. S35 Stability of Cbz-Cys(PSA)-Val-OMe **22** (250 μM) in presence of DTT (5 mM) in buffer over a course of 12 h.

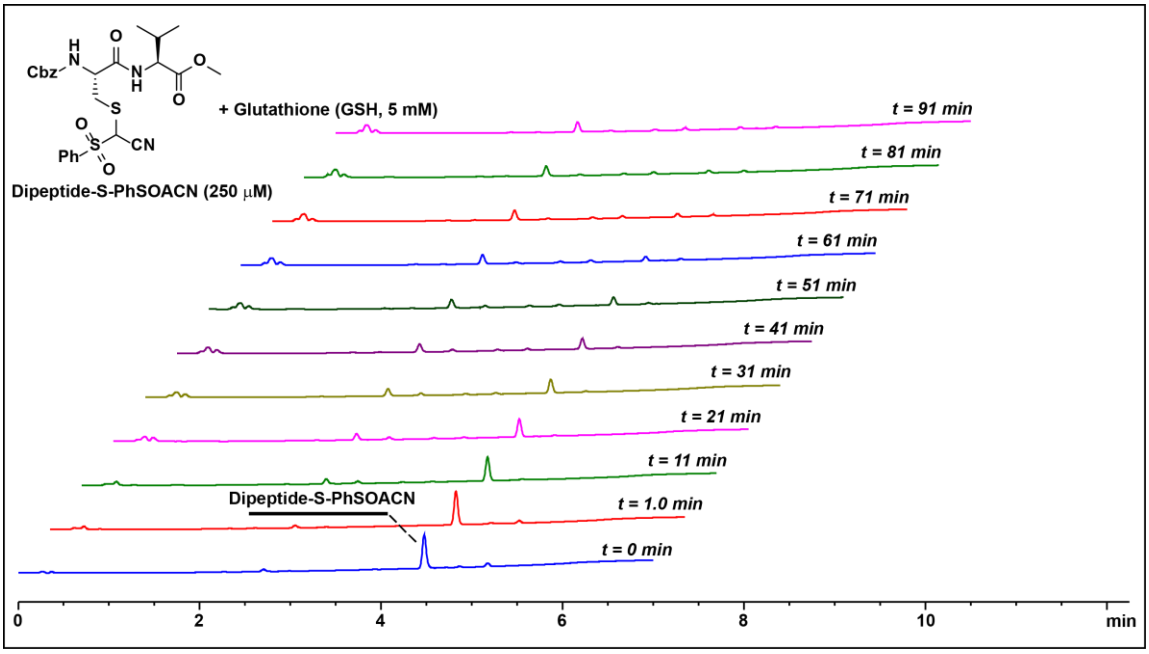


Fig. S36 Stability of Cbz-Cys(PSA)-Val-OMe **22** (250 μM) in presence of GSH (5 mM) in buffer over a course of 12 h.

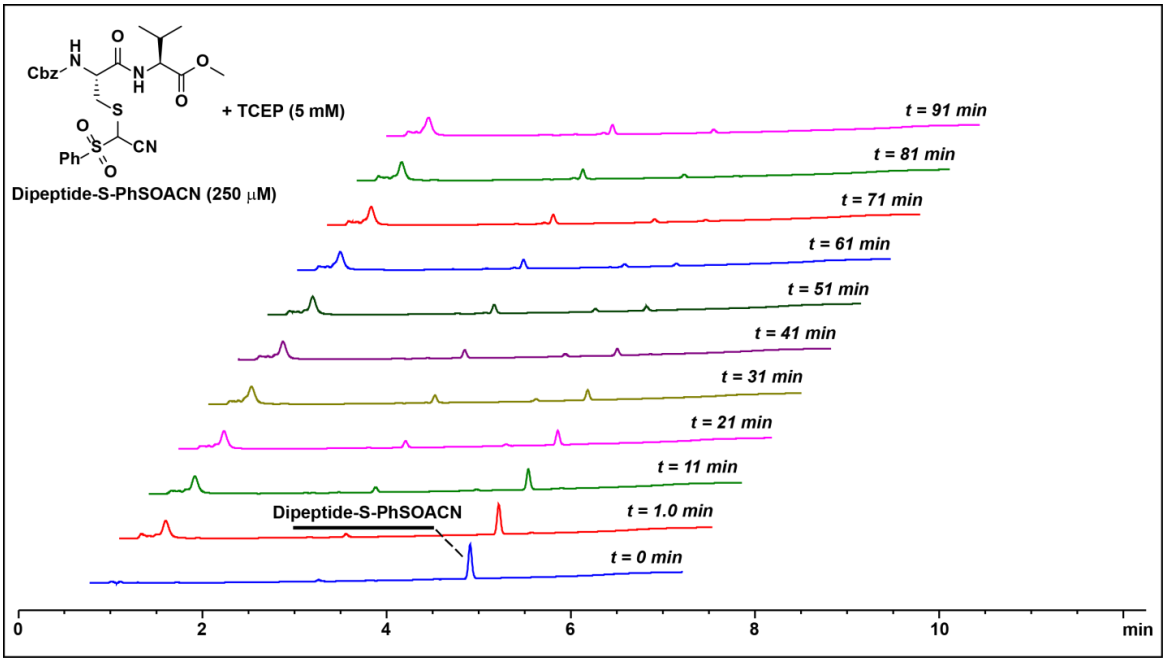


Fig. S37 Stability of Cbz-Cys(PSA)-Val-OMe **22** (250 μM) in presence of TCEP (5 mM) in buffer over a course of 12 h.

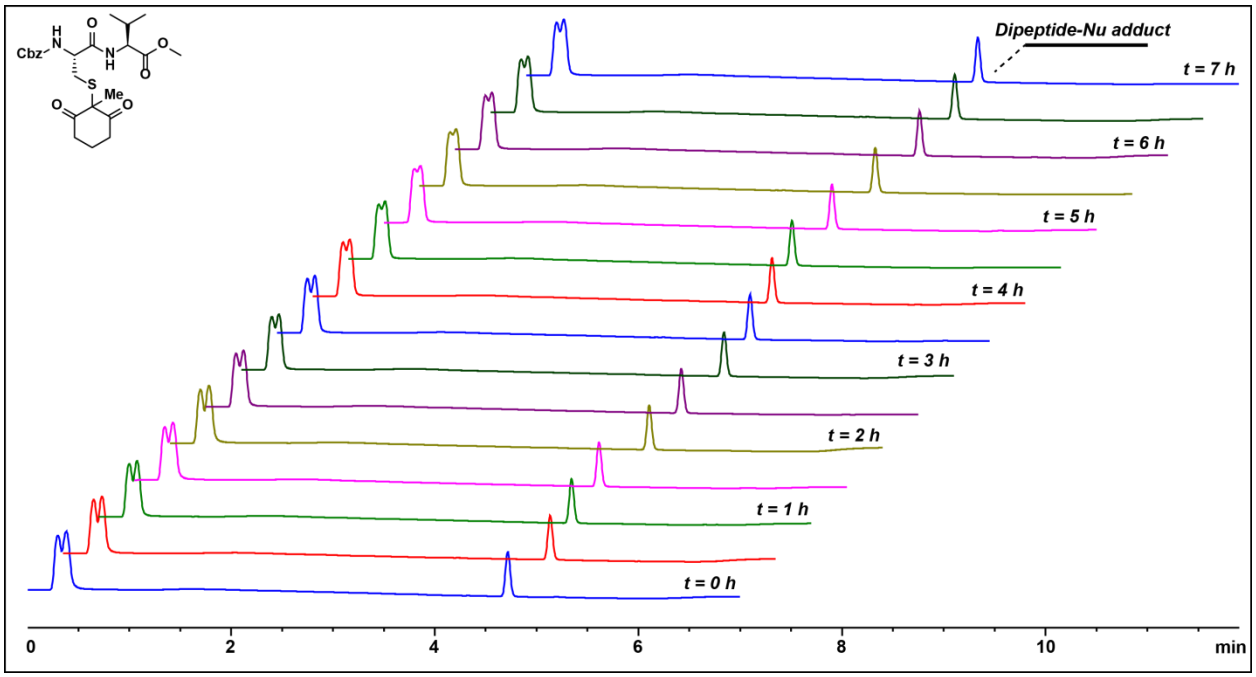


Fig. S38 Stability of dipeptide-Nu adduct (250 μM) in buffer over a course of 7 h.

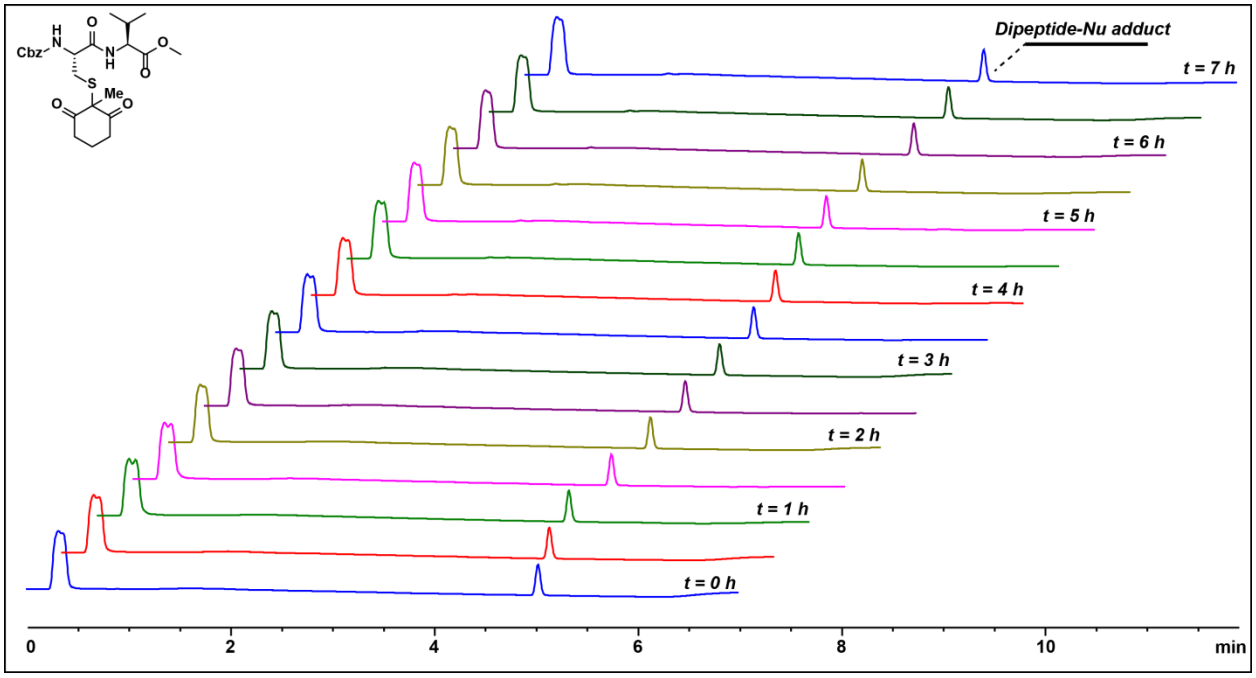


Fig. S39 Stability of dipeptide-Nu adduct (250 μM) in presence of DTT (5 mM) in buffer over a course of 7 h.

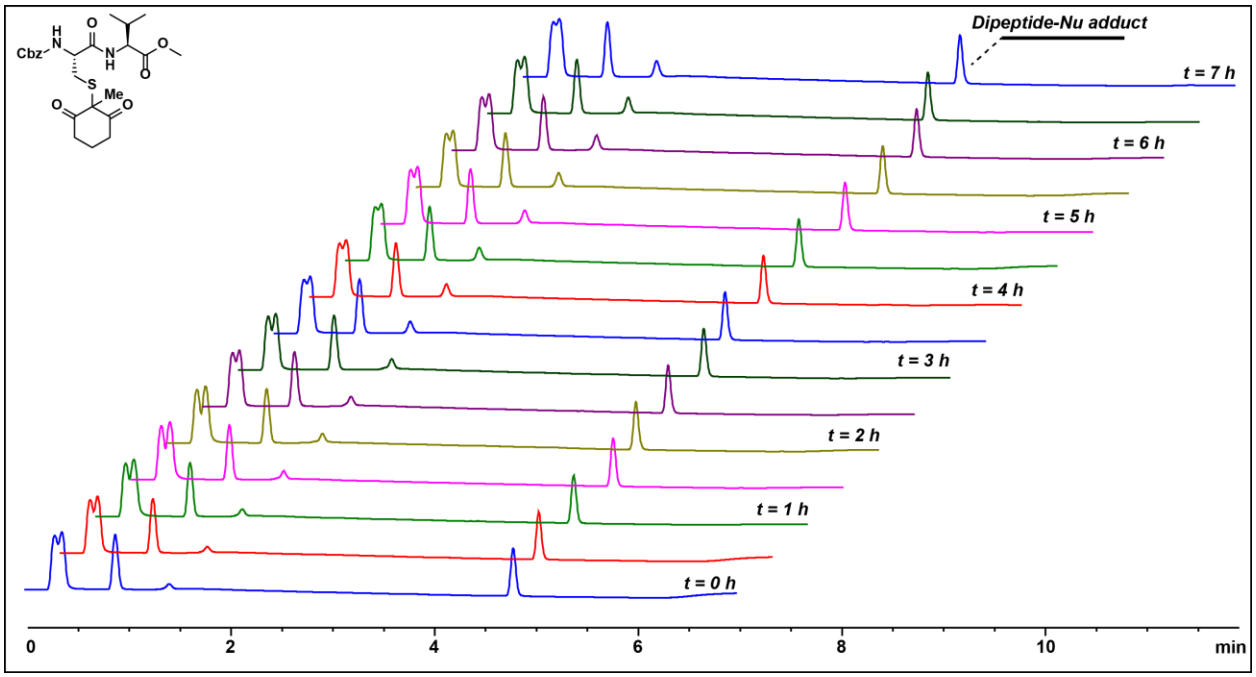


Fig. S40 Stability of dipeptide-Nu adduct (250 μM) in presence of GSH (5 mM) in buffer over a course of 7 h.

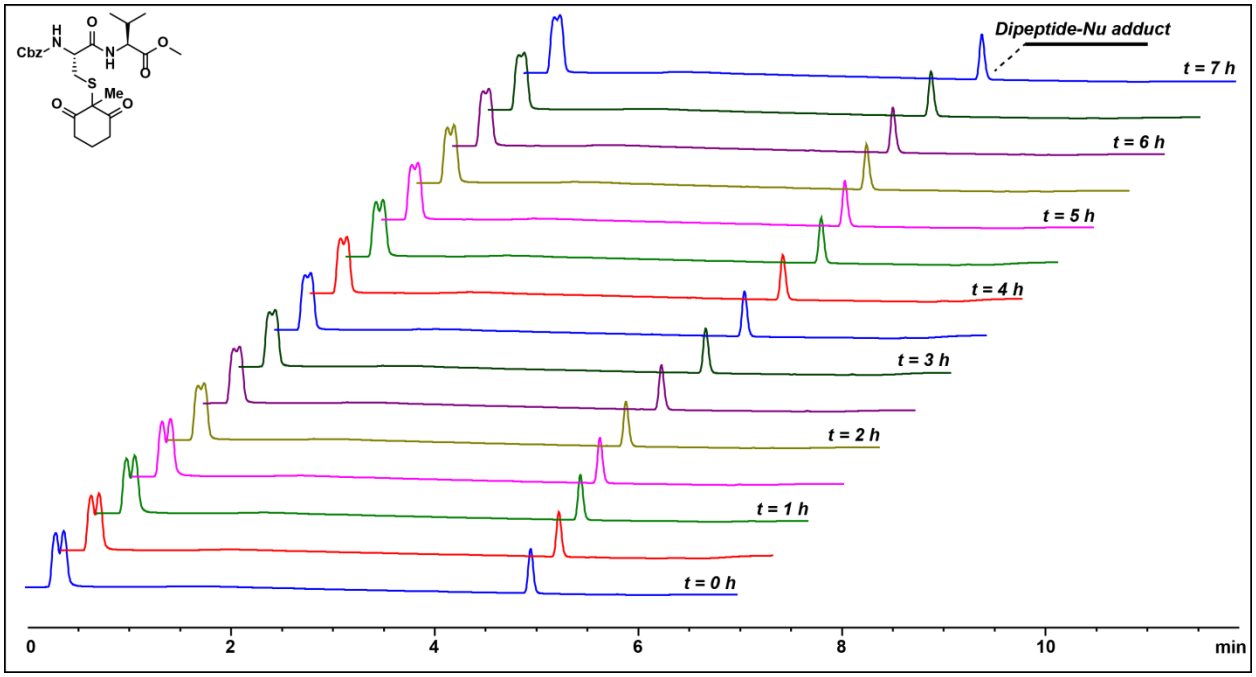


Fig. S41 Stability of dipeptide-Nu adduct (250 μM) in presence of TCEP (5 mM) in buffer over a course of 7 h.

Fig. S42 Rate plot for the reaction of Dipeptide-S-Nu **(15)** with GSH (5 mM)

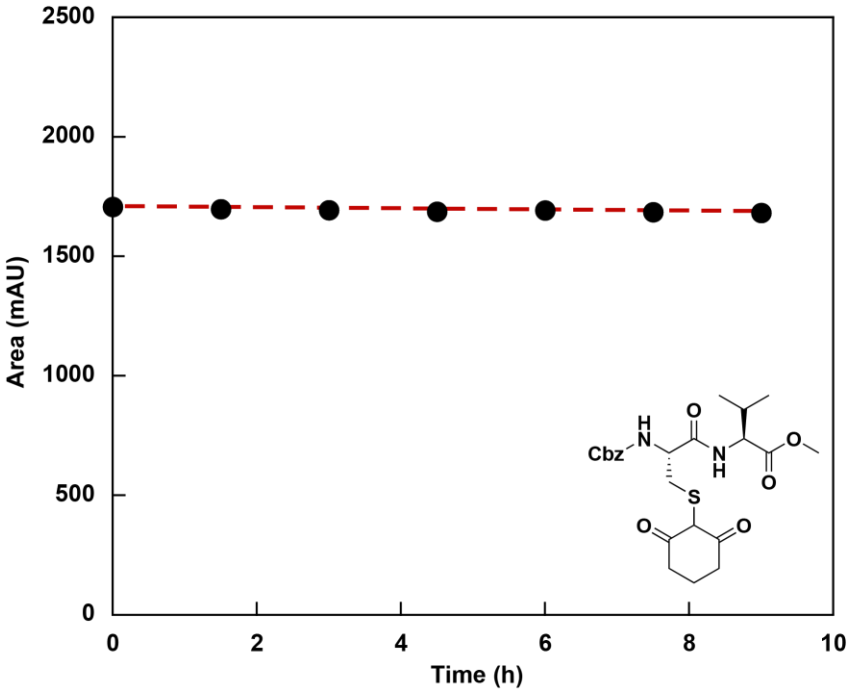


Fig. S43 Rate plot for the reaction of Dipeptide-S-Nu **(16)** with GSH (5 mM)

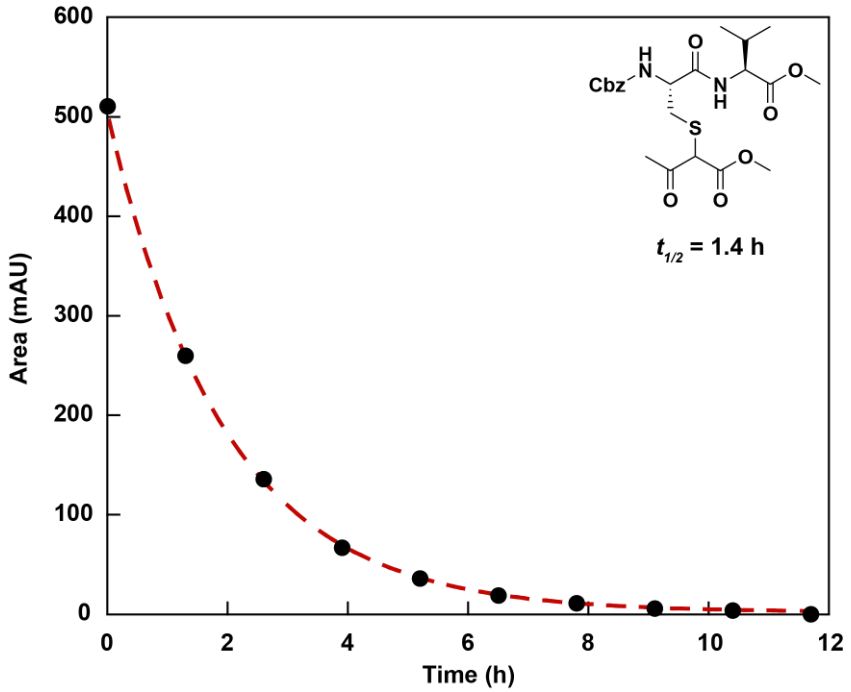


Fig. S44 Rate plot for the reaction of Dipeptide-S-Nu **(17)** with GSH (5 mM)

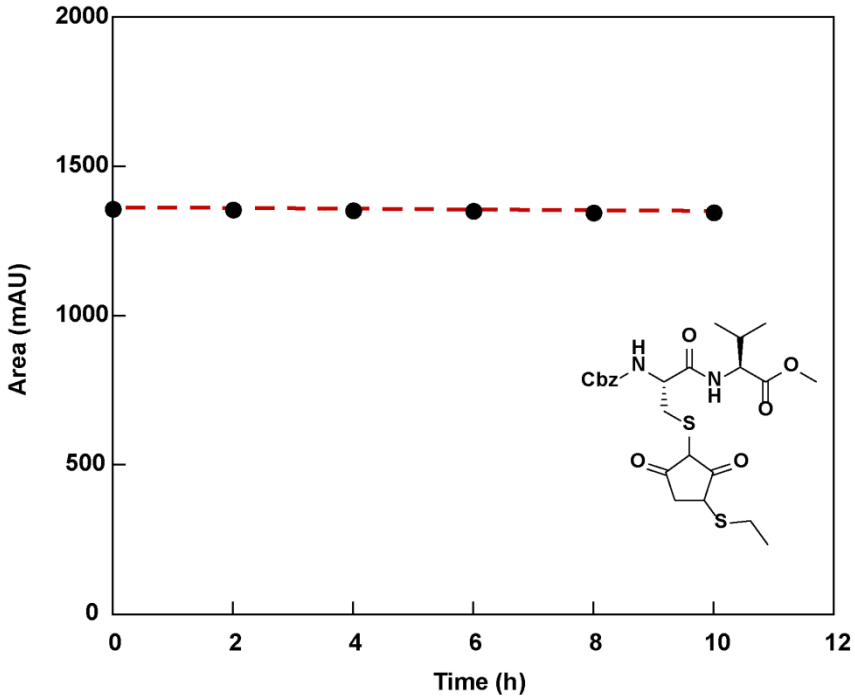


Fig. S45 Rate plot for the reaction of Dipeptide-S-Nu **(18)** with GSH (5 mM)

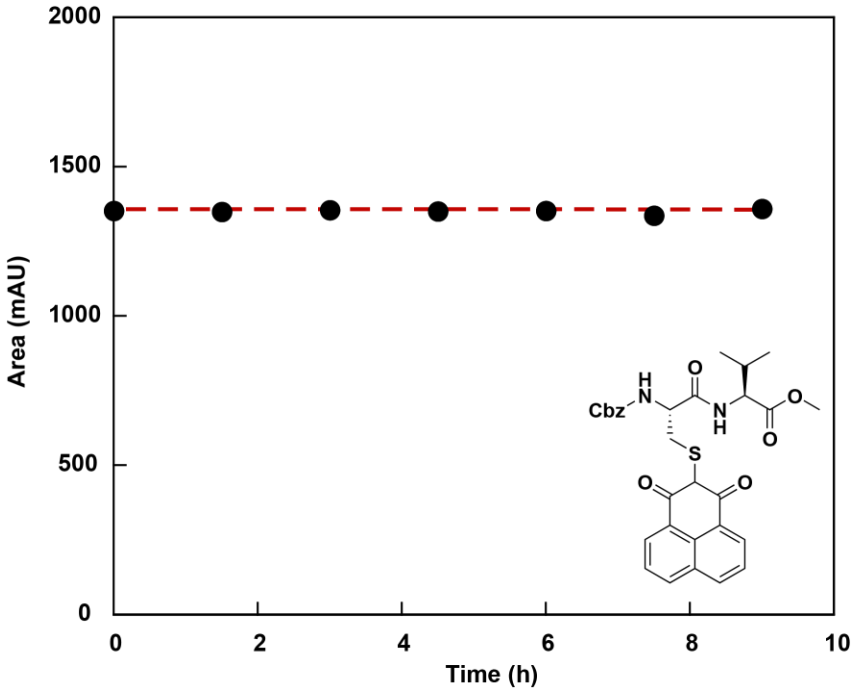


Fig. S46 Rate plot for the reaction of Dipeptide-S-Nu **(19)** with GSH (5 mM)

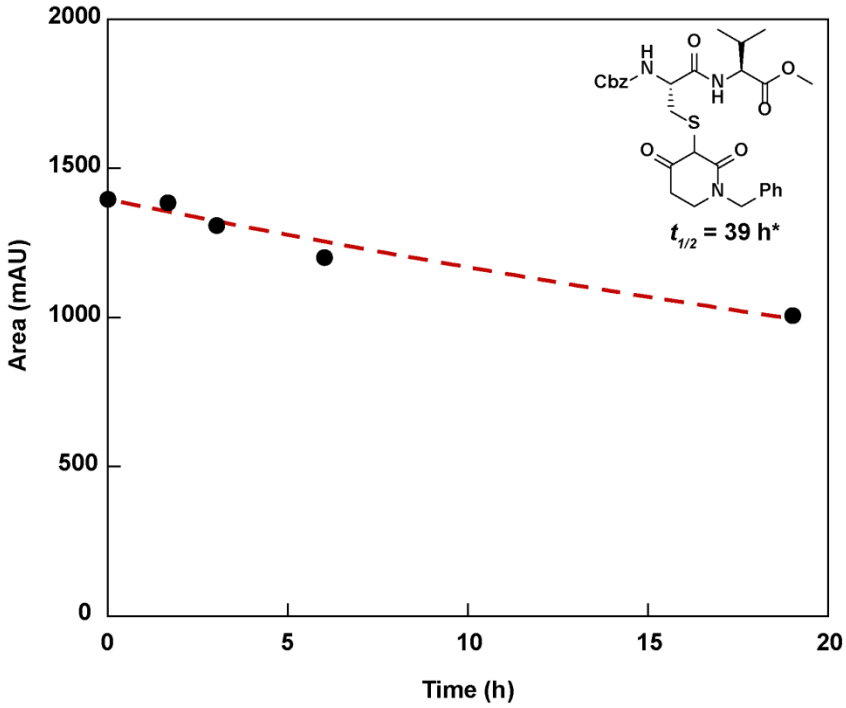


Fig. S47 Rate plot for the reaction of Dipeptide-S-Nu **(20)** with GSH (5 mM)

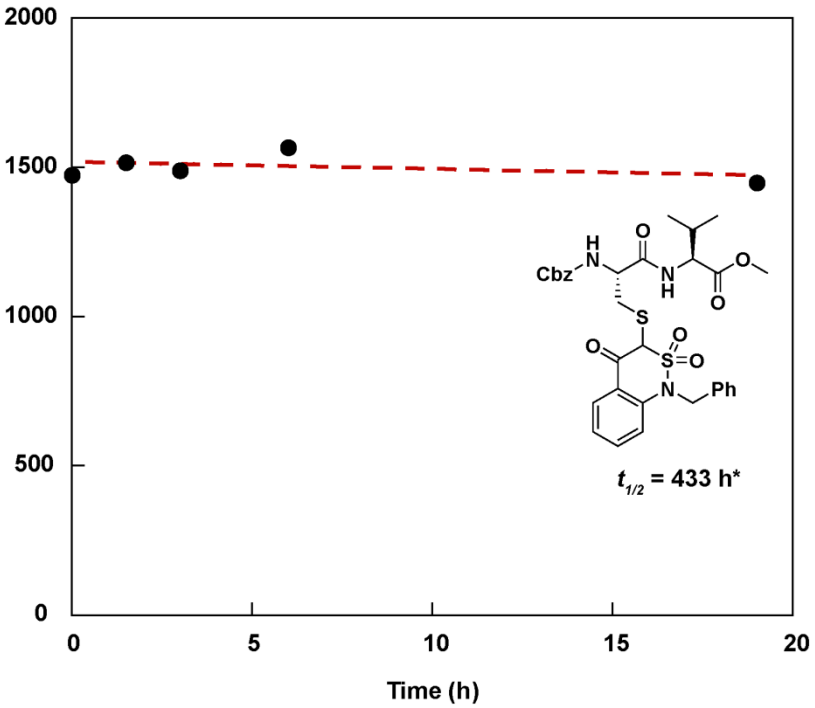


Fig. S48 Rate plot for the reaction of Dipeptide-S-Nu **(21)** with GSH (5 mM)

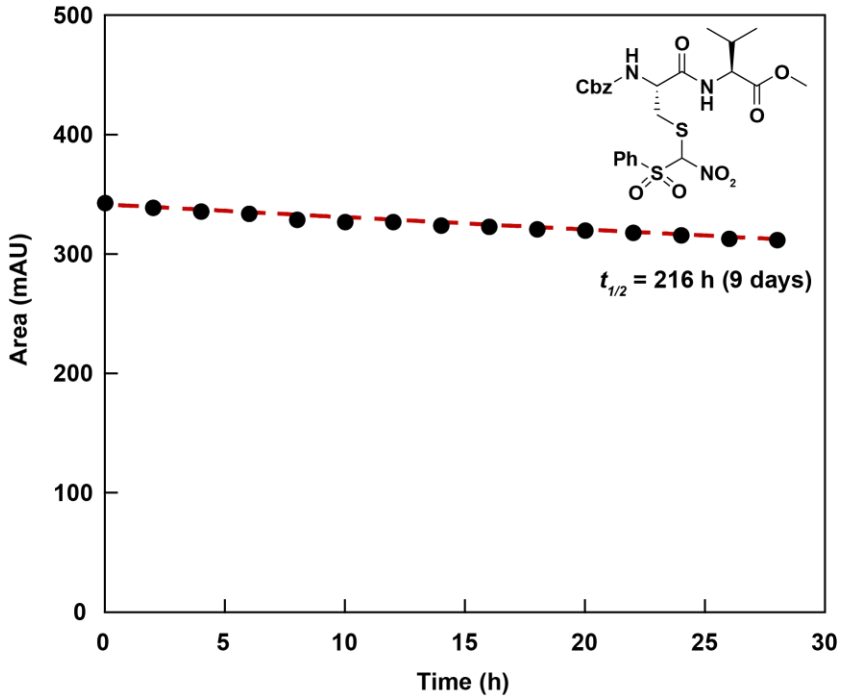
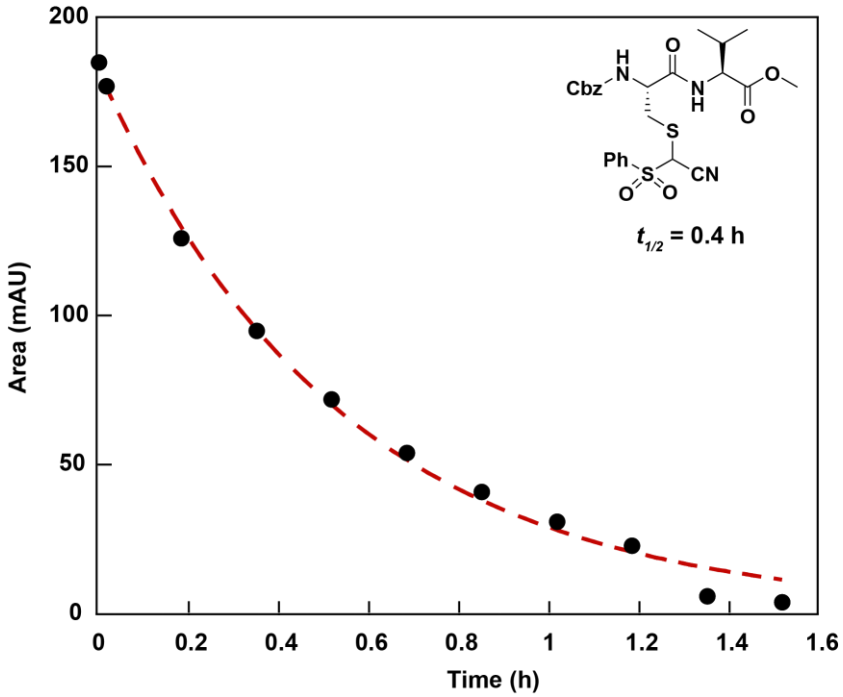
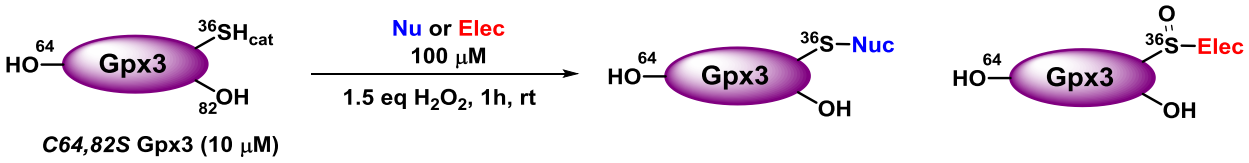


Fig. S49 Rate plot for the reaction of Dipeptide-S-Nu **(22)** with GSH (5 mM)



Scheme S1. Labeling of recombinant C64,82S Gpx3-SOH with various nucleophiles

Reaction:



(1) 100 mM stock solutions of nucleophiles/electrophiles were prepared in DMSO – If not soluble at room temperature, the eppendorf tubes were heated in 100 ^{o}C water bath for 2 minutes. 100 mM stock solutions were then diluted to 2 mM solution in 70% Gpx3 labeling buffer (50 mM HEPES, 100 mM NaCl, pH = 7.4), 30% DMSO.

(2) C64, 82S Gpx3 was thawed at 0 ^{o}C and reduced with 50 mM (2M, 5 µL) DTT for 20 minutes on ice.

A 2 M solution of DTT in water was prepared (31 mg of DTT in 100 µl of water). 5 µl of this DTT solution was added to the protein solution (200 µl) giving an effective DTT concentration of 50 mM.

(3) C64, 82S Gpx3-SH was buffer exchanged to labeling buffer (50 mM HEPES, 100 mM NaCl, pH = 7.4) using pre-equilibrated Nap-5 column. Nap-5 column was equilibrated by passing 10 ml of Gpx3 labeling buffer.

(4) Determine the concentration of C64, 82S Gpx3 by using A_{280} (ε = 24410 $M^{-1}cm^{-1}$).

(5) Label 10 µM of C64, 82S Gpx3 with 1.5 eq of H_2O_2 and 100 µM probe keeping DMSO $\leq$ 5%. Incubate for 1 h [REACTION VOLUME = 100 µl].

Order of addition was as follow:

(a) Appropriate amounts of Gpx3 labeling buffer were added to the eppy tubes.

(b) C64, 82S Gpx3 was added to the tube and the contents were mixed gently.

(c) Subsequently, probe solution was added to each of the tubes. For the probe, 5 µL of a 2 mM solution was used. This gave 100 µM effective probe concentration for each reaction and kept the DMSO concentration at < 5%.

(e) Hydrogen peroxide solution was added last. A 1 mM solution of hydrogen peroxide was prepared in water by serial dilution starting with commercially available 8.8 M solution. 1.5 µl of this 1 mM peroxide solution was used in each case to give an effective concentration of 15 µM.

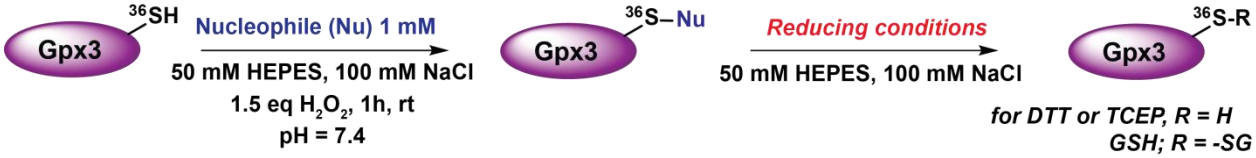
(f) Reaction mixtures were incubated for 1 h at rt.

(6) The reaction mixtures were quenched by the filtration through pre-equilibrated P30 columns.

(7) During the filtration through P30 columns, each sample was buffer exchanged to 25 mM ammonium bicarbonate (pH 8.0).

(8) Each sample was then analyzed by LTQ-MS by injecting 3 µM concentration in 0.1% formic acid in water.

Scheme S2. Stability of Gpx3-Nu adduct under reducing conditions



Procedure: The labeling of Gpx3-SOH was performed exactly as described in Scheme S1. For each nucleophile, in addition to the reduced & oxidized Gpx3 and nucleophile labeling controls the same labeling reaction was conducted in 4 different eppendorf tubes.

1st tube was incubated as is to test the stability of thioether adduct at 1 h.

2nd tube was incubated with DTT (5 mM) and stability was evaluated at 1h.

3rd tube was incubated with TCEP (5 mM) and stability was evaluated at 1h.

4th tube was incubated with GSH (5 mM) and stability was evaluated at 1h.

The reaction was quenched by removing the reducing agents by filteration through P30 column. Subsequently, the samples were analyzed by LTQ-MS and results are summarized below.

Fig. S50 Reversibility of Gpx3-S-Nu adduct under reducing conditions.

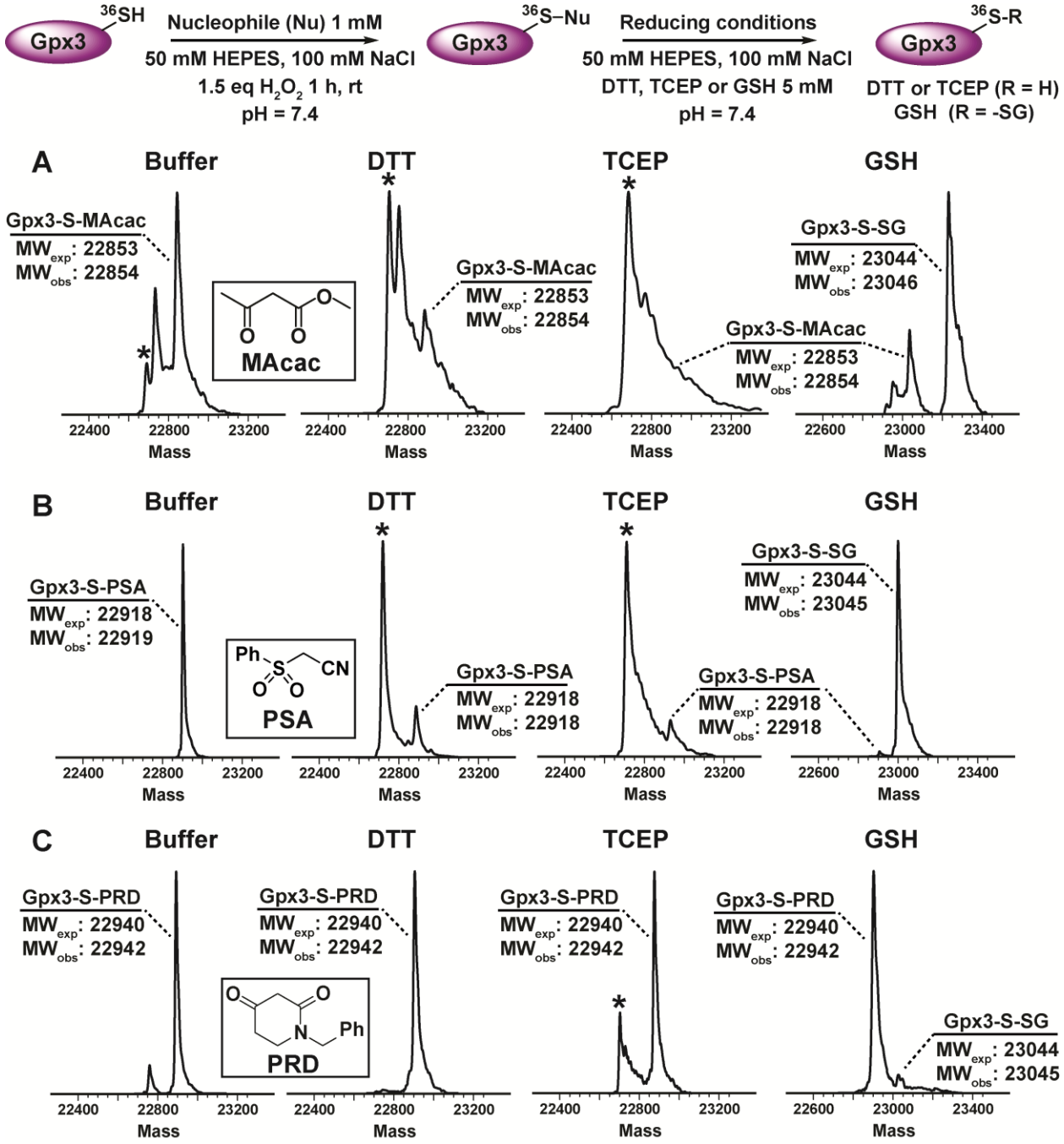
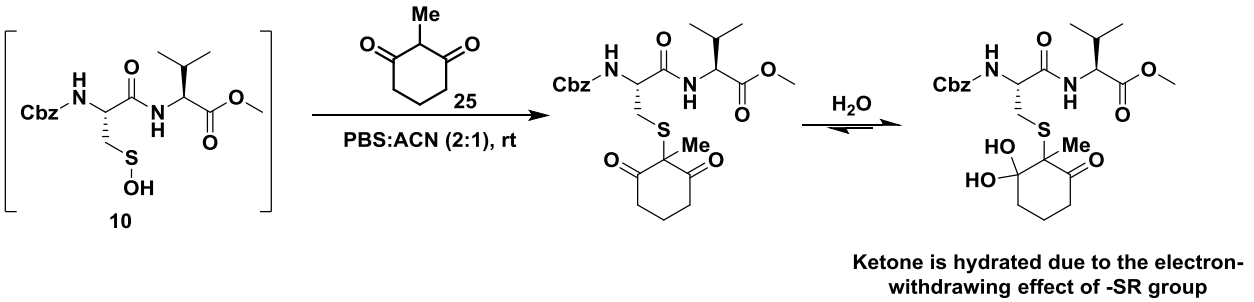


Fig. S51 LC-trace for the reaction of 2-methylcyclohexane-1,3-dione **(25)** with dipeptide-SOH **10** resulting in the formation of dipeptide-S-Nu adduct and its subsequent hydration.



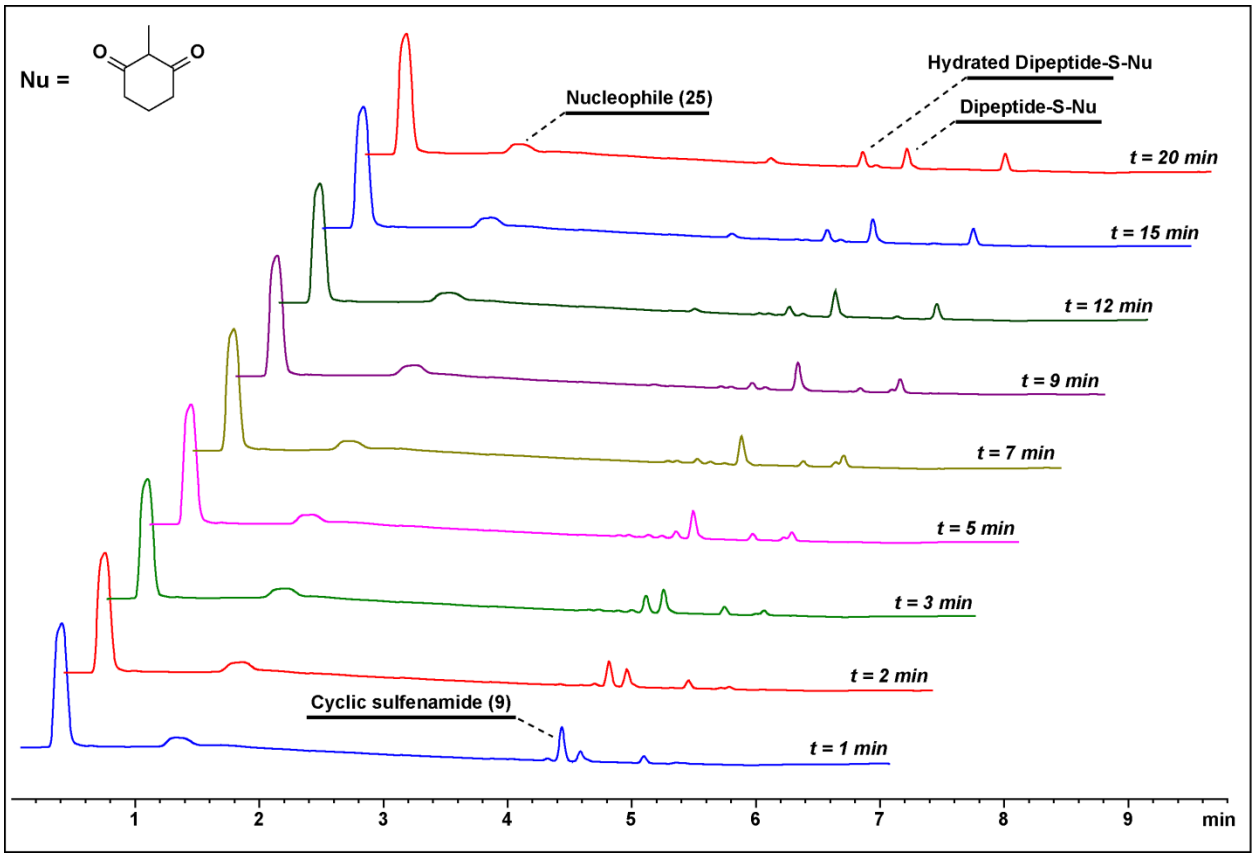


Fig. S52 Rate plot for the reaction of 2-methylcyclohexane-1,3-dione **(25)** with dipeptide-SOH **10** resulting in the formation of dipeptide-S-Nu adduct and its subsequent hydration.

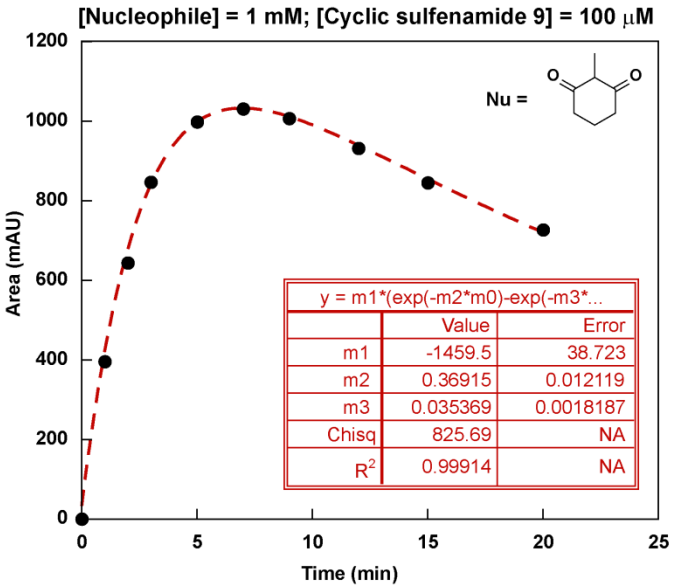


Fig. S53 Rate plot for the reaction of 2-methyl-1*H*-phenalene-1,3(2*H*)-dione **(27)** with dipeptide-SOH **10** resulting in the formation of dipeptide-S-Nu adduct and its subsequent hydration.

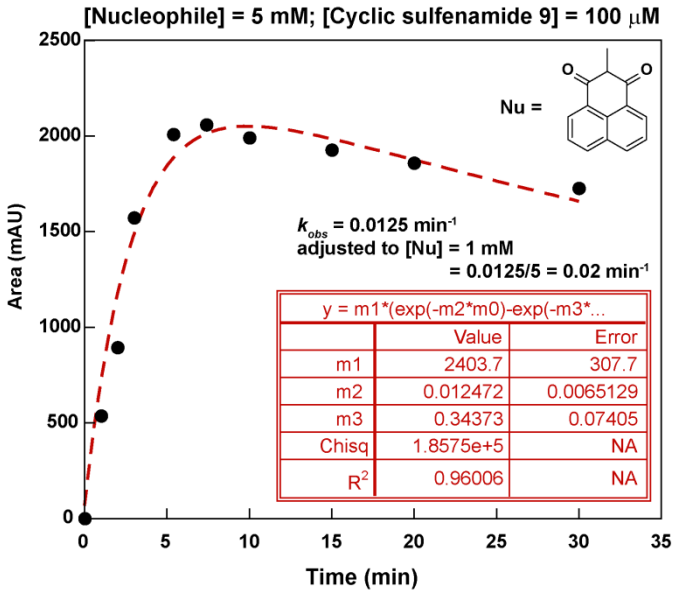
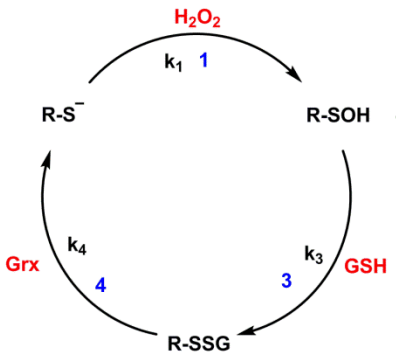


Fig. S54 Steady-state model to calculate the amount of protein-SOH captured by nucleophiles in the presence of GSH.



$$\frac{d[RSOH]}{dt} = k_2[RSOH][Nu] \quad (1)$$

$$\frac{d[RSOH]}{dt} = k_3[RSOH][GSH] \quad (2)$$

$$\textit{Proportion of R-SOH reacting with Nucleophile (Nu)} = \frac{k_2[Nu]}{k_2[Nu] + k_3[GSH]} \quad (3)$$

For HSA

[Nu] = 100 μM [GSH] = 2 mM

k_3 = 2.9 $M^{-1}s^{-1}$

For oxMKP3

[Nu] = 100 μM [GSH] = 2 mM

k_3 = 2 $M^{-1}s^{-1}$

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[1] Gupta, V., and Carroll, K. S. (2016) Profiling the Reactivity of Cyclic C-Nucleophiles towards Electrophilic Sulfur in Cysteine Sulfenic Acid, *Chem. Sci. 7*, 400-415.

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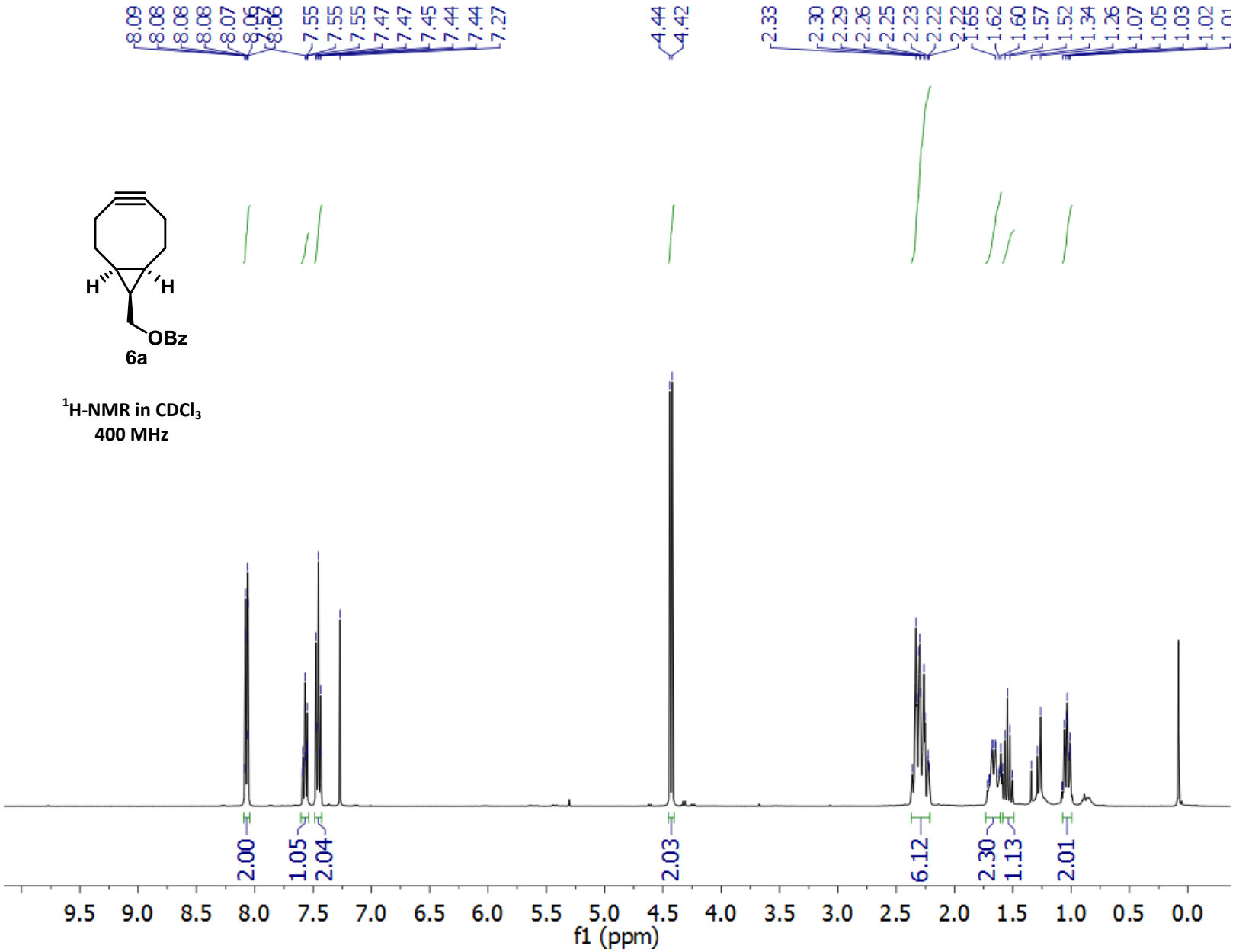
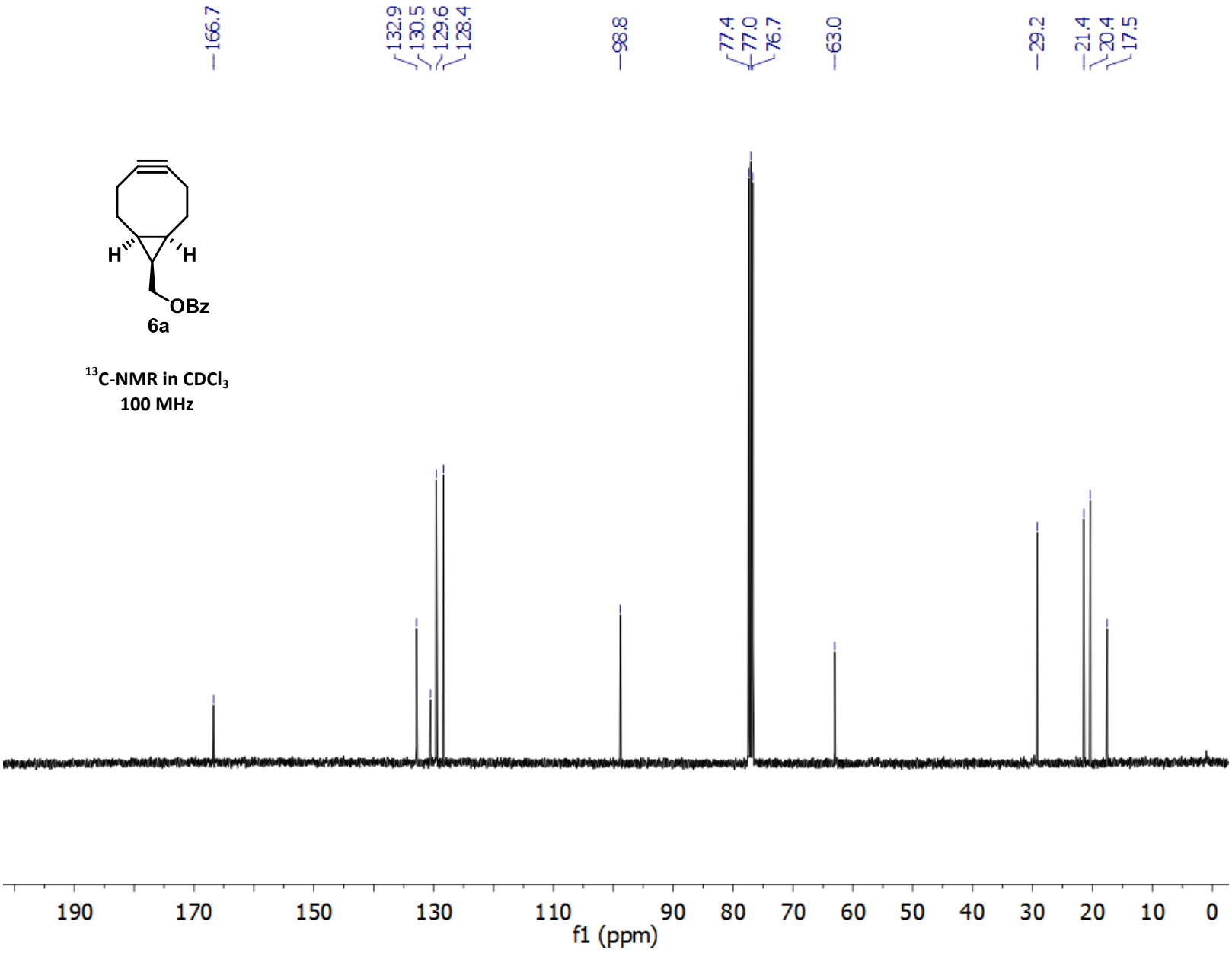
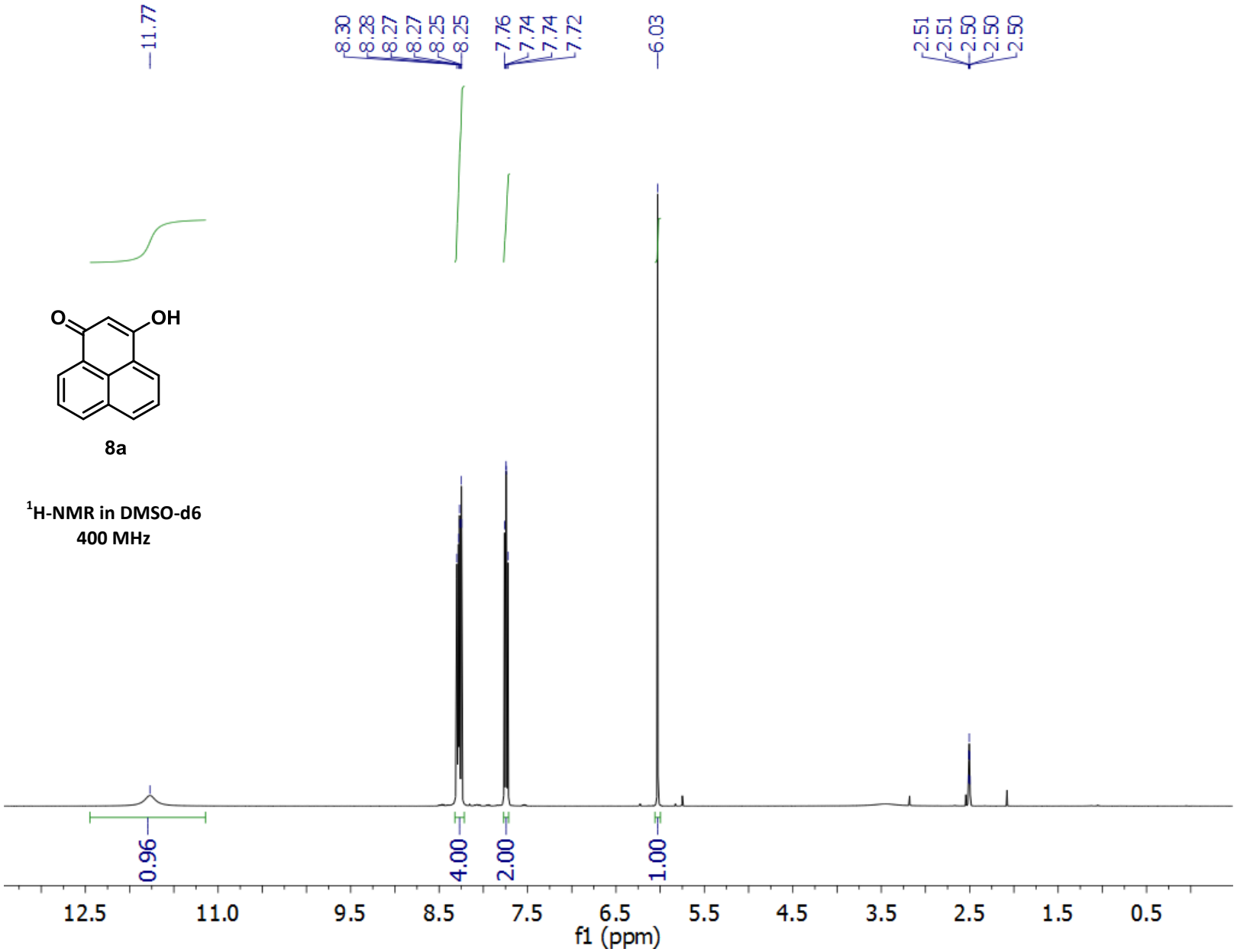
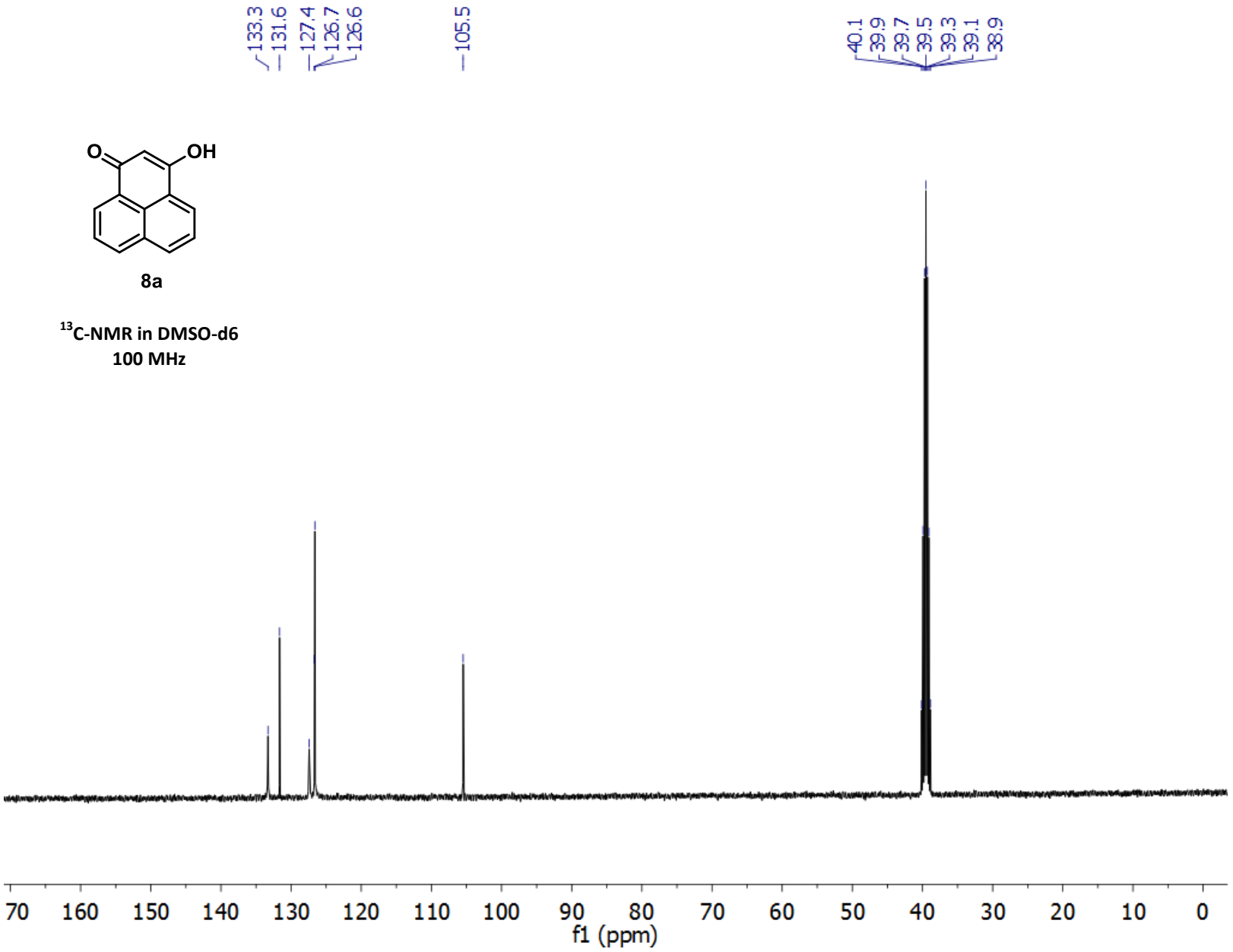
NMR Spectra



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

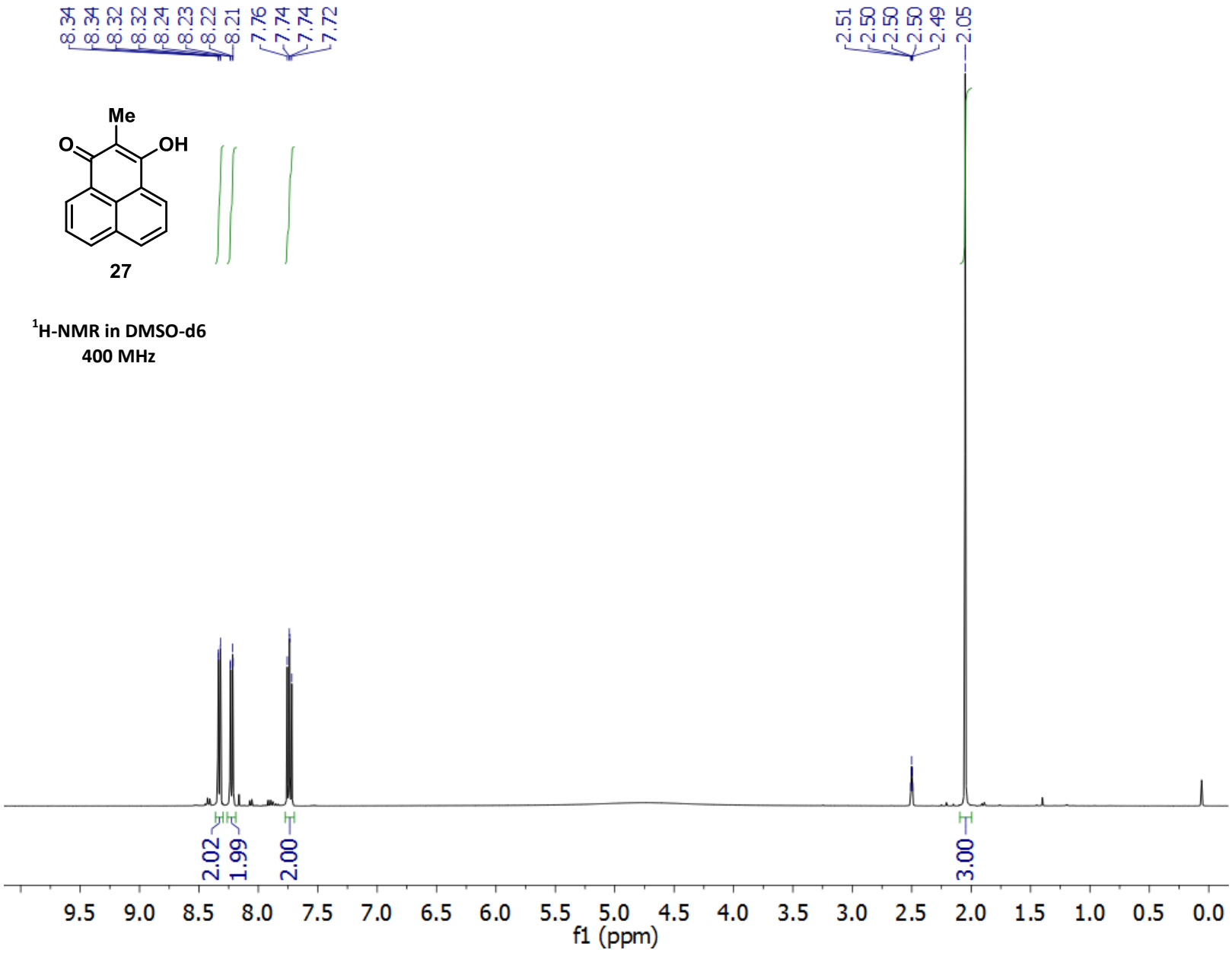

166.7
132.9
130.5
129.6
128.4
98.8
77.4
77.0
76.7
63.0
29.2
21.4
20.4
17.5
H
H
OBz
6a
13C-NMR in CDCl3
100 MHz
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


11.77
8.30
8.28
8.27
8.27
8.25
8.25
7.76
7.74
7.74
7.72
6.03
2.51
2.51
2.50
2.50
2.50
O
OH
8a
1H-NMR in DMSO-d6
400 MHz
0.96
4.00
2.00
1.00
12.5
11.0
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)



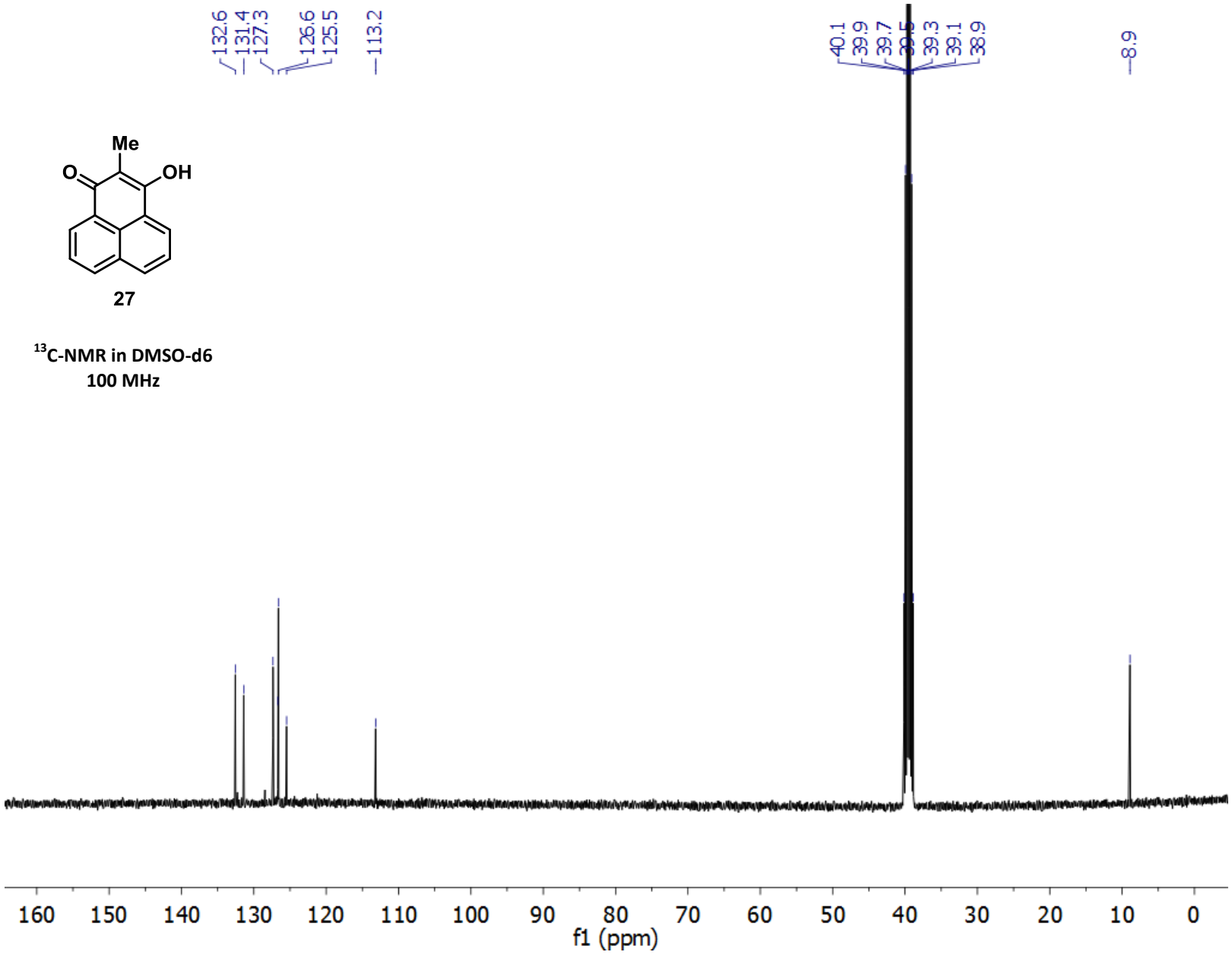
8a

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



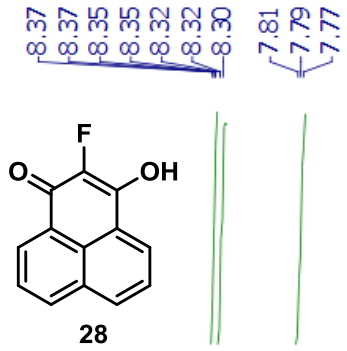
27

^{1}H-NMR in DMSO-d6
400 MHz



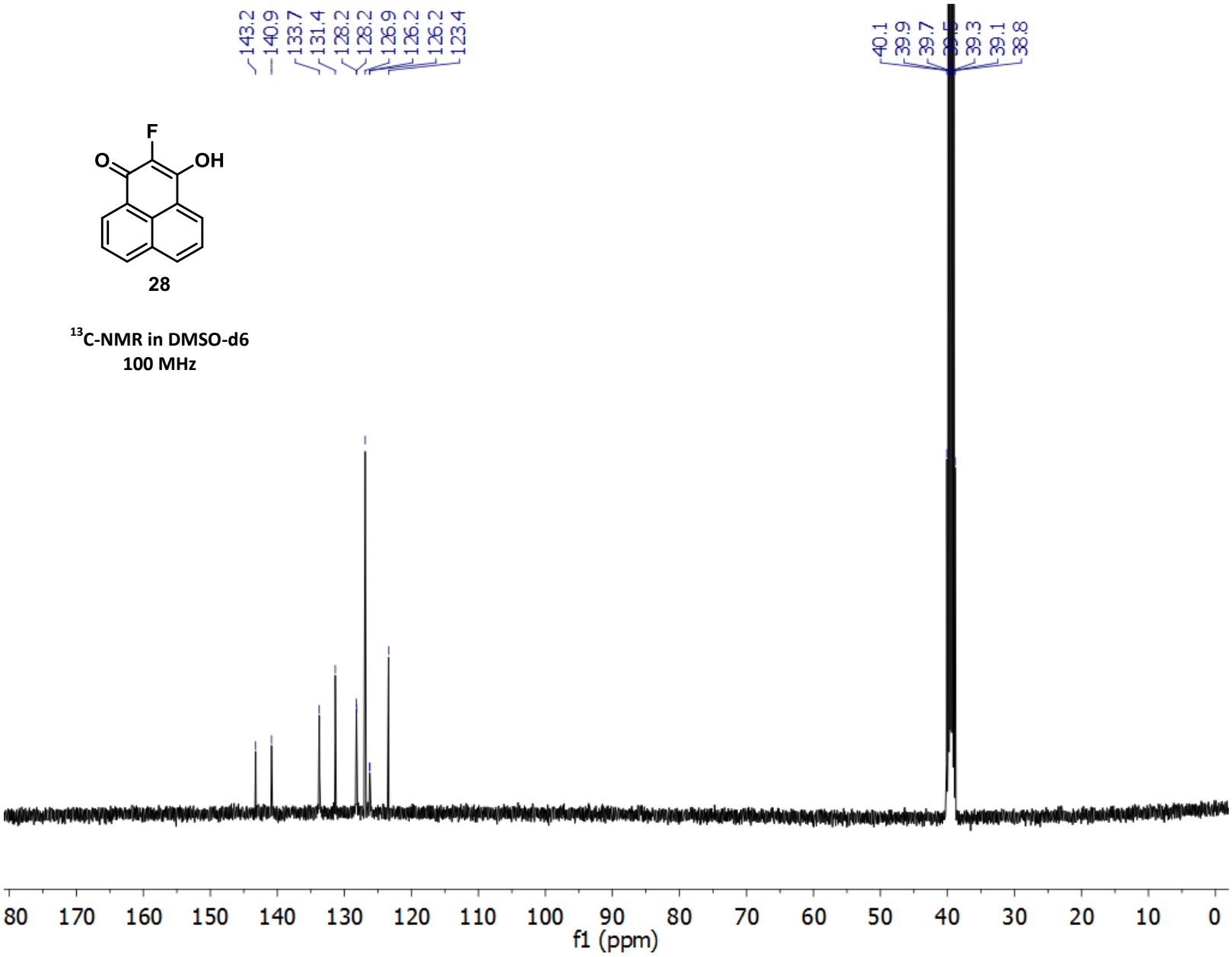
27

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



28

^{1}H-NMR in DMSO-d6
400 MHz


143.2
140.9
133.7
131.4
128.2
128.2
126.9
126.2
126.2
123.4
40.1
39.9
39.7
39.5
39.3
39.1
38.8
F
O
OH
28
13C-NMR in DMSO-d6
100 MHz
80
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

28

^{19}F-NMR in DMSO-d6
400 MHz