Supporting Information

On-Resin Macrocyclization of Peptides Using Vinyl Sulfonamides as a Thiol-Michael "Click" Acceptor

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Abbreviations

MeOH; Methanol
TFA; Trifluoroacetic acid
TES; Triethylsilane
DMF; N,N-Dimethylformamide
DIC; N.N-Diisopropylcarbodiimide
DCM; Dichloromethane

TES; Triethylamine
Mmt; Monomethoxytrityl
RP-HPLC; Reversed-phase high-performance liquid chromatography
RBF; Round bottom flask
DBU; Diazabicyclo[5.4.0]undec-7-ene
TLC; Thin -layer chromatography
NMR; Nuclear magnetic resonance spectroscopy
VSA; Vinylsulfonamide
FTIR; Fourier-transform infrared spectroscopy
DMSO; Dimethylsulfoxide
ESI-UPLC; Electrospray ionization – Ultra performance liquid chromatography
HSQC; Heteronuclear single quantum coherence
HMBC; Heteronuclear multiple bond correlation
COSY; Homonuclear correlation spectroscopy
NOESY; Nuclear Overhauser effect spectroscopy
TOCSY; Total correlation spectroscopy

1. **Materials**

All reagents unless otherwise stated were purchased from Millipore Sigma. Fmoc-protected Amino acids and RAM (0.43 meq/g) resin for peptide synthesis were purchased from Chempep. TFA, TES, t-butyl sarcosine, and t-butyl glycine were purchased from ChemImpex. 2-chloroethanesulfonyl chloride was purchased from TCI America. All deuterated solvents were purchased from Fischer Scientific. RediSep Rf Gold columns (Teledyne Isco) were used for small molecule purification.

2. Instruments

Kinetic experiments were performed using Fourier-transform infrared spectroscopy (FTIR; IS-50, Thermofisher). Peptides were synthesized using an automated microwave peptide synthesizer (Liberty Blue^{TM}; CEM Corporation). Peptides and model systems were characterized using NMR spectroscopy (AV400 and AV600 MHz, Bruker) and electrospray ionization-ultrahigh performance liquid chromatography (ESI-UPLC; Xevo G2-S QTof, Waters). Peptides were purified using reverse-phase high performance liquid chromatography (RP-HPLC; XBridge BEH C18 prep column, Waters). Synthesized model compounds for kinetics studies were purified using flash chromatography (CombiFlash Rf, Teledyne Isco).

3. Synthetic Procedures

3.1. Unfunctionalized Peptide Synthesis

The peptide sequence SarRGDSdFC was synthesized using standard Fmoc solid-phase peptide synthesis (SPPS) on a Liberty Blue^{TM} microwave peptide synthesizer (Sar stands for sarcosine and lower case ‘d’ denotes a D-isomer amino acid). Rink amide resin (0.43 meq/g) was used at a 0.25 mmol scale (581 mg). Each amino acid was dissolved in DMF at a concentration of 0.2 M. Activator solutions of N,N’-Diisopropylcarbodiimide (DIC) and Oxyma Pure were made at 0.5 M and 1 M in DMF, respectively. After placing the resin in the microwave synthesizer vessel, the resin was preswelled for 5 min in DMF

and agitated. The amino acids were then coupled to the resin in the presence of 4 eq of Fmoc-protected amino acid (0.2 mM, 5 mL), DIC (0.5 mM, 2 mL), and Oxyma Pure (1 mM, 1 mL) for 2 min under microwave irradiation (90 °C). All amino acids were double coupled. Following coupling, the resin was washed 4 times with DMF (7 mL). Deprotection steps consisted of 10 mL of 20% piperdine in DMF with microwave irradiation (90 °C) for 0.5 min. Upon final deprotection of the end-group sarcosine, the resin was removed from the reaction vessel, transferred to a manual glass peptide synthesis vessel, and washed (3x) with DMF before further modification of the peptide.

3.2. Peptide Vinyl Sulfonamide Functionalization and Cyclization

The peptide was first swollen in 5 mL of DCM and mixed for 15 min. Following swelling, the resin was washed (3x, 5 mL) with DCM. The peptide vessel was then purged with nitrogen for 1 h. After purging, 5 mL of anhydrous DCM was added to the resin and mixed under inert atmosphere. While mixing, the vessel was charged with anhydrous TEA (289 µL, 202 mg, 2 mmol, 8 eq) and mixed for 5 min. Next, 2-chloroethanesulfonyl chloride (104 µL, 163 mg, 1 mmol, 4eq) was added dropwise to the peptide vessel. When addition was finished, the vessel was left to stir for 2 h. The peptide was rinsed with DCM (3x, 5 mL), MeOH (1x, 5 mL), and DCM (3x, 5 mL). Functionalization of the end group sarcosine was performed twice under the same conditions. A microcleavage was performed on the resin and run through an ESI-UPLC to verify complete functionalization of the end-group sarcosine with the vinyl sulfonamide. Based on the ESI-UPLC of the microcleaved resin, the functionalization step was repeated until complete consumption of starting peptide on the resin had occurred.

Selective deprotection of the thiol was performed using dilute TFA. The resin was swollen in 5 mL of DCM and mixed for 15 min. The resin was then washed with DCM (3x, 5 mL) and subjected to 3 % trifluoroacetic acid (TFA) and 5 % Triethylsilane (TES) in DCM (5 mL, 30 s, 15 x). Next, a few beads were collected and subjected to a qualitative Ellman's test to determine if free thiols were present. The solution turned yellow suggesting that thiols had been generated. Following this test, the resin was subjected to 5 mL of 5 % TEA in DCM and was then washed with DCM (5 mL, 3x), MeOH (5 mL, 1x), and DMF (5 mL, 3x).

Following deprotection of Mmt protected cysteine, the peptide was split into thirds. One-third of the resin was subjected to complete cleavage from the resin to produce the linear peptide. To cleave the peptide, 2.5 mL of a cleavage cocktail consisting of 95 % TFA, 2.5 % TES, 2.5 % H_2O, and 50 mg/mL phenol was added to the resin. The resin was mixed for 2 h before it was filtered and the cleavage solution was collected. The resin was then subjected to 5 mL of TFA and mixed for an additional 30 min. Filtering was again performed and the eluent was collected. Both TFA solutions were combined and slowly evaporated with N_2 to concentrate the sample. Once reduced to < 5 mL, the peptide was added dropwise to ice cold ether (40 mL). White precipitated peptide was centrifuged followed by decanting of the diethyl ether. Again, the peptide was washed with 40 mL of ice cold ether and centrifuged before decanting the diethyl ether. The peptide was then dried under vacuum overnight.

The other two-thirds resin was subjected to the cyclization procedure. 5 ml of DMF was added to the resin and allowed to swell for 15 min while mixing. The resin was then washed with DMF (5 mL, 3x). A solution of 2 mL of DMF and 10 mol% DBU (2.6 µL)

was added to the resin. For 1 h the resin was mixed before being filtered and washed with DMF (5 mL, 3x), MeOH (5 mL, 1x), and DCM (5 mL, 3x). After washing, the resin was subjected to the same cleavage cocktail as described above (for the first 1/3 of the resin). 5 mL of a cleavage cocktail, consisting of 95 % TFA, 2.5 % TIPS, 2.5 % H_2O, and 50 mg/mL phenol, was added to the resin. The resin was mixed for two hours followed by filtration of the TFA solution, which was collected and set aside. Again, the resin was further mixed with 5 ml of TFA for 30 min, which was collected and combined with the initial cleavage cocktail eluent. The combined filtrate was evaporated using a stream of N_2 until roughly 5 mL of volume remained, which was added dropwise to ice cold ether (40 mL). White precipitates formed and were centrifuged before decanting of the diethyl ether. The peptide was again washed with 40 mL of cold diethyl ether and centrifuged before the diethyl ether was decanted. The resin was finally dried overnight on vacuum.

3.3. Peptide Purification

Both linear and cyclized peptides were purified using reverse-phase high performance liquid chromatography (RP-HPLC). Peptides were dissolved at 18 mg/mL in 95:5 Millipore water to acetonitrile with 0.2% TFA and sonicated for 1 h. After sonication, the peptide solution was filtered through a 0.45 micron filter to remove any insoluble material. After which, the peptide was injected into the column undergoing a water to acetonitrile solvent gradient from 85:15 to 78:22 over 20 min. Peptide fractions were collected and concentrated with a stream of N_2 to a volume of about 20 mL and was re-diluted to 40 mL using DI water. The solution was frozen and lyophilized to afford the desired peptide products.

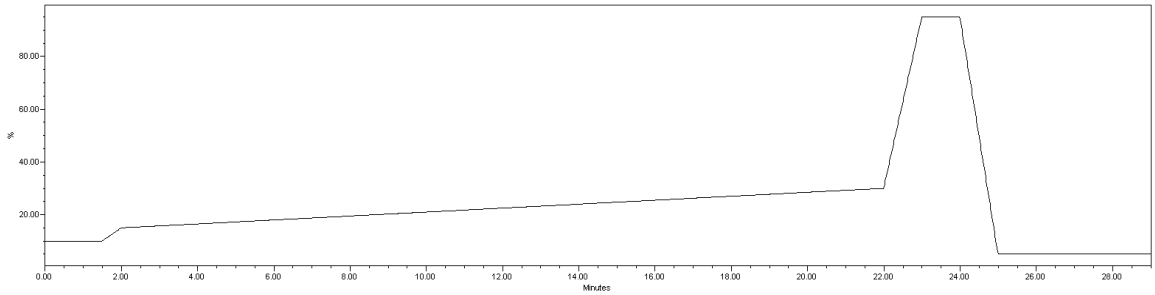


Figure S1. RF-HPLC acetonitrile to water gradient method used to purify both linear and cyclized RGDS peptides.

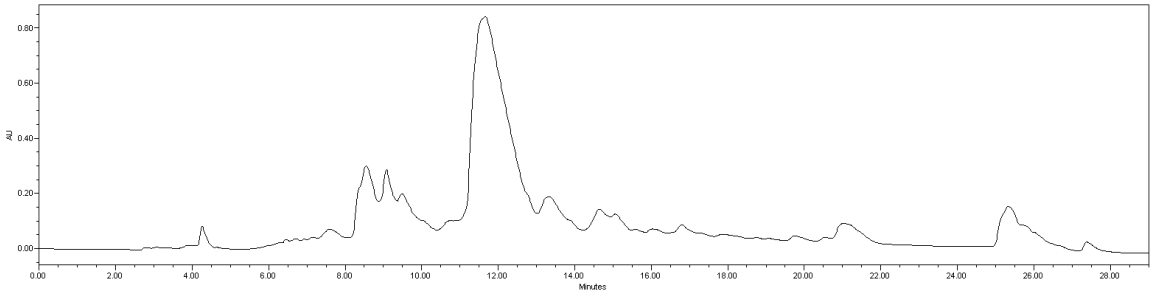


Figure S2. RF-HPLC prep scale purification of linear vinylsulfonamide functionalized RGDS peptide. Max peak eluted at 11.63 min.

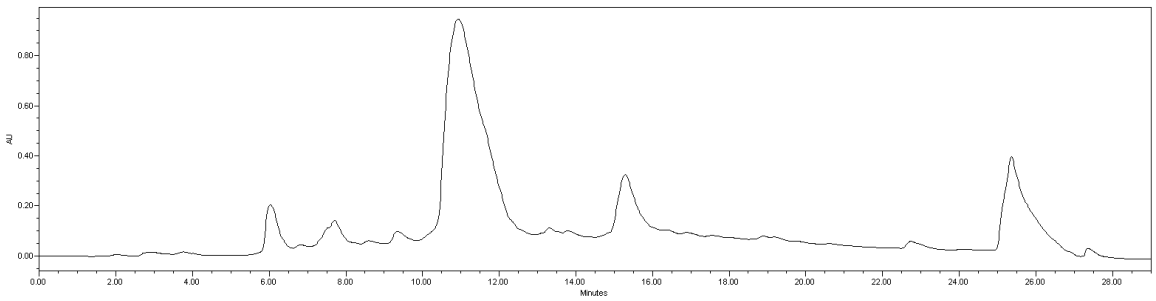
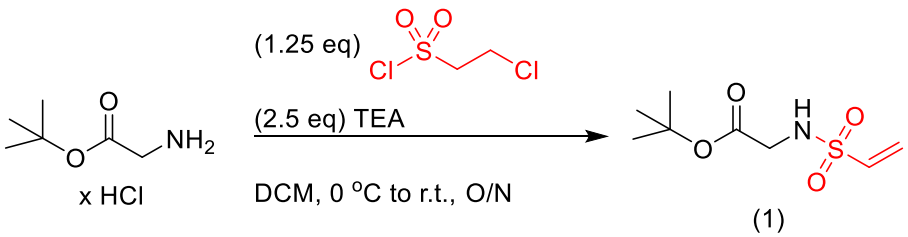


Figure S3. RF-HPLC prep scale purification of cyclized vinylsulfonamide functionalized RGDS peptide. Max peak eluted at 10.92 min.

3.4. Microcleavage of RAM Resin

To perform microcleavages of the linear and cyclized peptides, 10 to 20 beads of resin were subjected to 1 mL of TFA and mixed for 2 h. The TFA solution was evaporated using a steady stream of N_2. Once completely dry, 500 µL of Millipore water was added and mixed thoroughly. Then, the peptide was filtered through a 0.45 µm filter. The sample was then subjected to UPLC-ESI. An ACQUITY UPLC Protein BEH C4 column was used for the purification. A gradient of 100:0 to 5:95 water:acetonitrile was used to elute the crude peptide.

3.5. Model Compounds



Compound (1, E5): To an oven dried N_2 purged 250 mL RBF was added 2.5 g (15 mmol, 1 eq) of t-butyl glycine hydrochloride. Then, 100 mL of anhydrous DCM was added to the RBF. After, 5.22 mL of TEA (37.5 mmol, 2.5 eq) was slowly added to the solution. Upon complete addition of TEA, the RBF was cooled in an ice bath and allowed to stir for 15 min. To the cooled solution, 1.96 mL (3.056 g, 18.75 mmol, 1.25 eq) 2-chloroetanesulfonyl chloride was added dropwise over 15 min. The reaction was allowed to warm to room temperature and was stirred overnight. The next day, saturated $NaHCO_3$ (100 mL) was added to the solution and the organic phase was extracted with H_2O (2x 50 mL) and brine (1x 50 mL). The organic phase was then dried over Na_2SO_4, filtered, and evaporated. The residue was subjected to column purification using a gradient of hexanes and ethyl acetate (0% to 50%). Fractions were collected and evaporated to produce (1, E5) as a white solid (1.83 g, 55.1%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94-7.65 (t, 1H), 6.83-6.53 (dd, 1H), 6.14-5.85 (dd, 2H), 3.72-3.51 (d, 2H), 1.62-1.20 (s, 9H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 168.96, 137.76, 125.45, 81.68, 44.67, 28.11.

Mass: $C_8H_{15}NO_4S$ $[M+Na]^+$ Calculated: 244.06, Found: 243.95

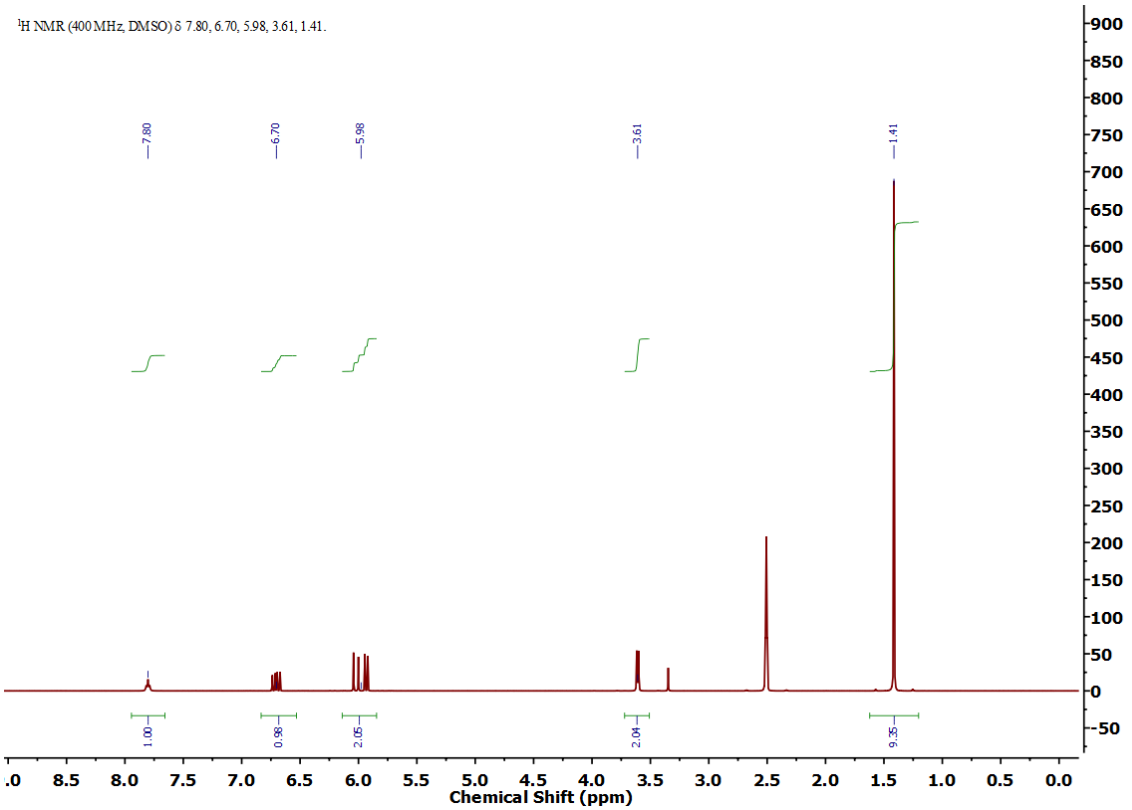


Figure S4. ^{1}H NMR of compound (1).

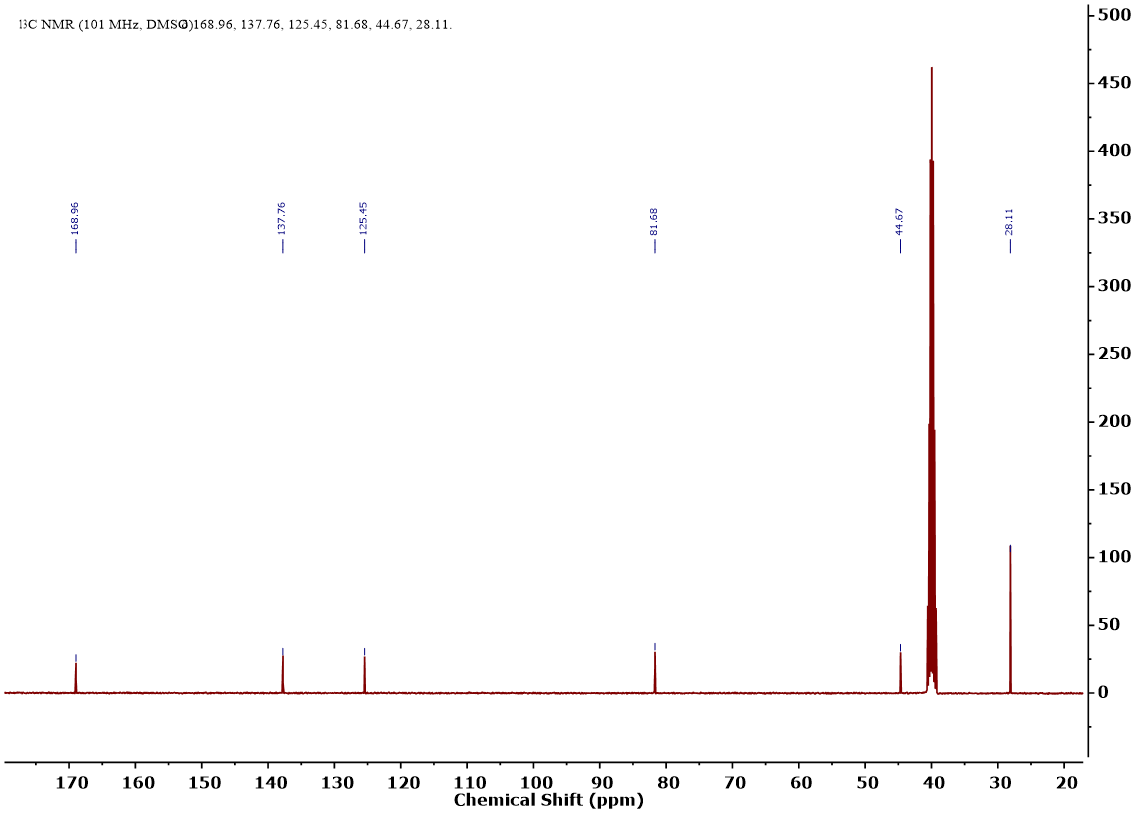
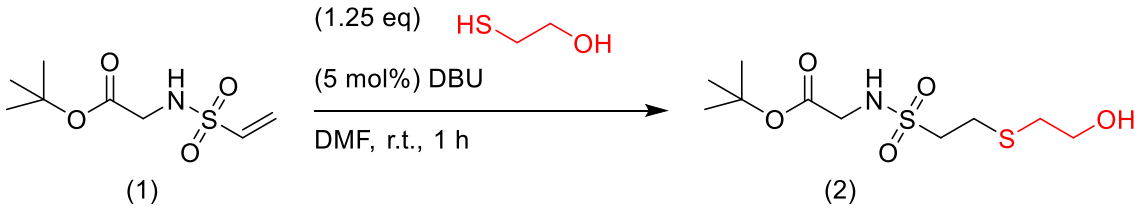


Figure S5. ^{13}C NMR of compound (1).



(1) (2)

Compound (2): 750 mg (3.19 mmol, 1 eq) of (1) and 500 µL of DMF was added to a 1 dram vial and mixed. 281 µL (312.5 mg, 4 mmol, 1.25 eq) of 2-mercaptoethanol was then added to the solution. Once thoroughly mixed, 24 µL (24.4 mg, 0.16 mmol, 0.05 eq) of DBU was added and the reaction was allowed to mix for 1 h. Once the reaction had finished (as determined by TLC), the solution was diluted with 50 mL of DCM and was extracted with water (2x 50 mL) and dried over Na_2SO_4. The organic solution was evaporated and subjected to column purification using a gradient of hexanes and ethyl acetate (0% to 50%). Fractions of the product were collected and dried to produce (2) as a white solid (912 mg, 91.3%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77-7.642 (t, 1H), 4.93-4.74 (t, 1H), 3.78-3.67 (d, 2H), 3.59-3.48 (dt, 2H), 3.30-3.21 (m, 2H), 2.89-2.79 (m, 2H), 2.66-2.57 (t, 2H), 1.58-1.31 (s, 2H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 81.69, 61.18, 53.43, 44.74, 34.30, 28.13, 25.23.

Mass: $C_{10}H_{22}NO_5S_2$ $[M+H]^+$ Calculated: 300.09, Found: 300.90

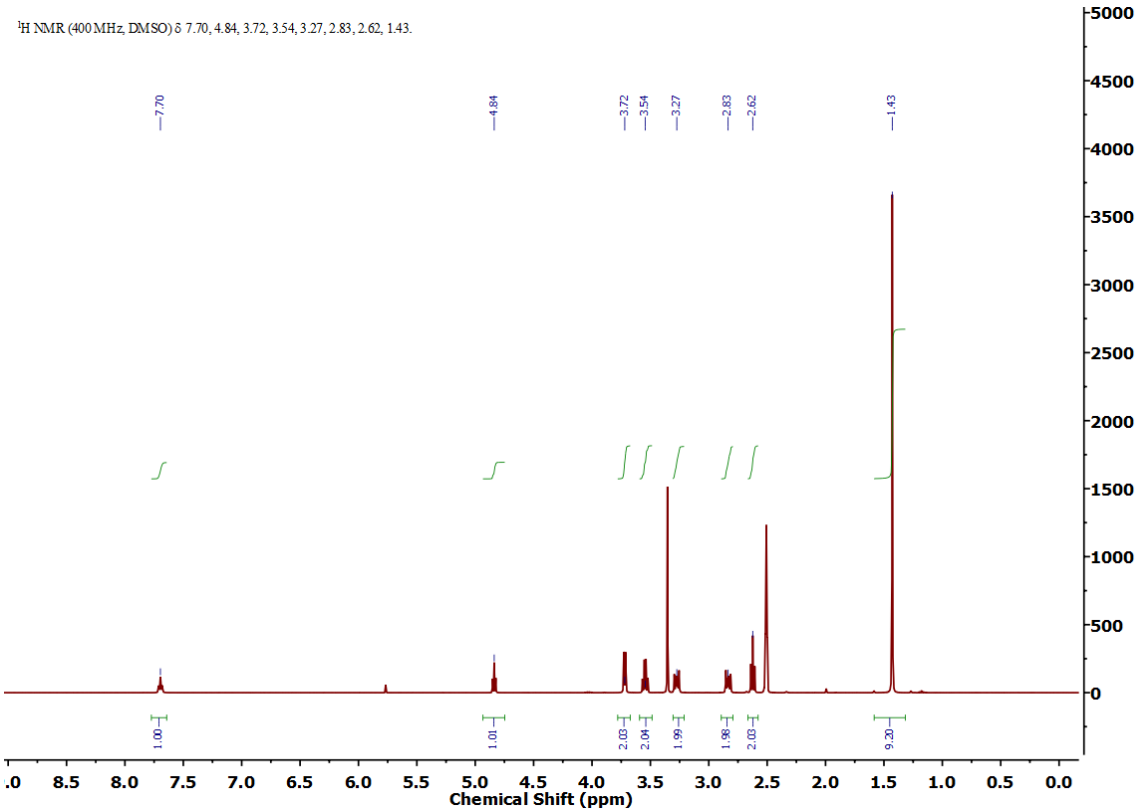


Figure S6. ^{1}H NMR of compound (2).

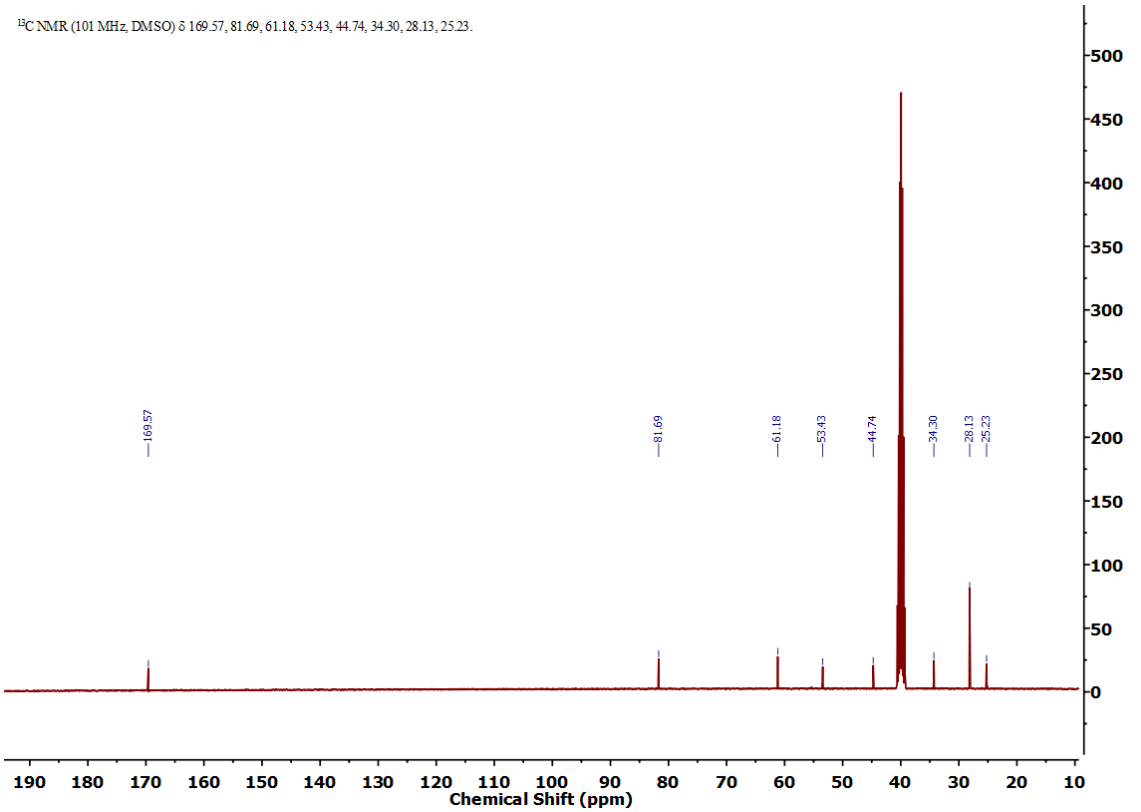
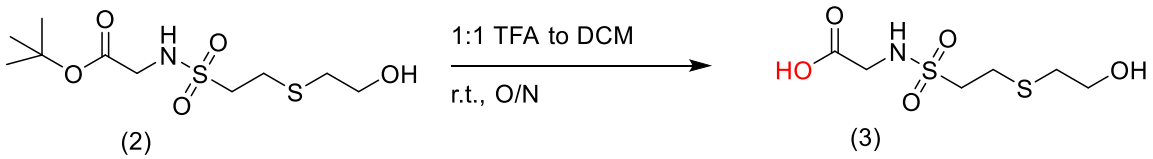


Figure S7. ^{13}C NMR of compound (2).



(2) (3)

Compound (3): 912 mg (3.05 mmol, 1 eq) of (2) and 5 mL of DCM was added to a 20 ml vial and stirred until all solid had dissolved. 5 mL of TFA was then added and the mixture was stirred continuously overnight. The reaction was then concentrated in vacuo and the product was subjected to column purification using a gradient of 0% to 5% MeOH in DCM. Fractions with the desired product were collected to produce a white solid (285 mg, 38.5%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71-7.62 (t, 1H), 4.65-4.45 (t, 2H), 3.77-3.72 (d, 2H), 3.33-2.85 (m, 6H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 171.91, 67.28, 53.05, 44.08, 29.24, 24.94.

Mass: $C_6H_{14}NO_5S_2$ $[M+Na]^+$ Calculated: 266.01, Found: 266.01

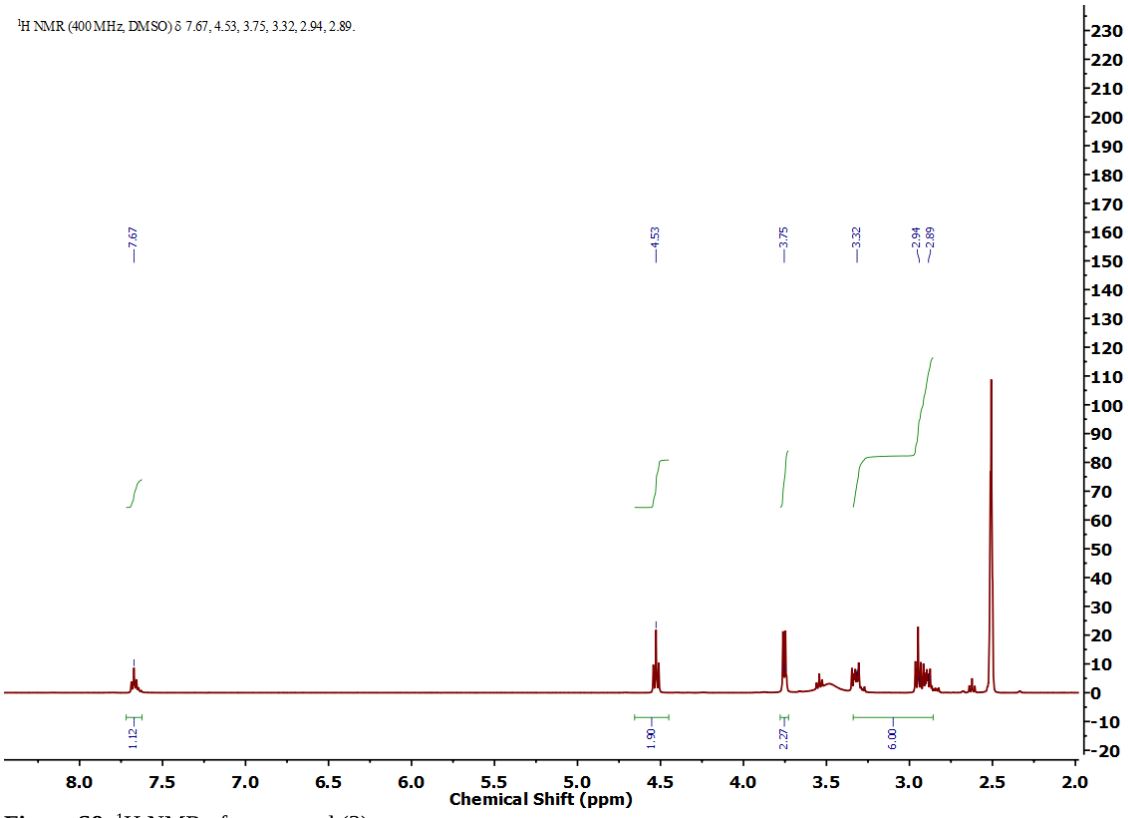


Figure S8. [1]H NMR of compound (3).

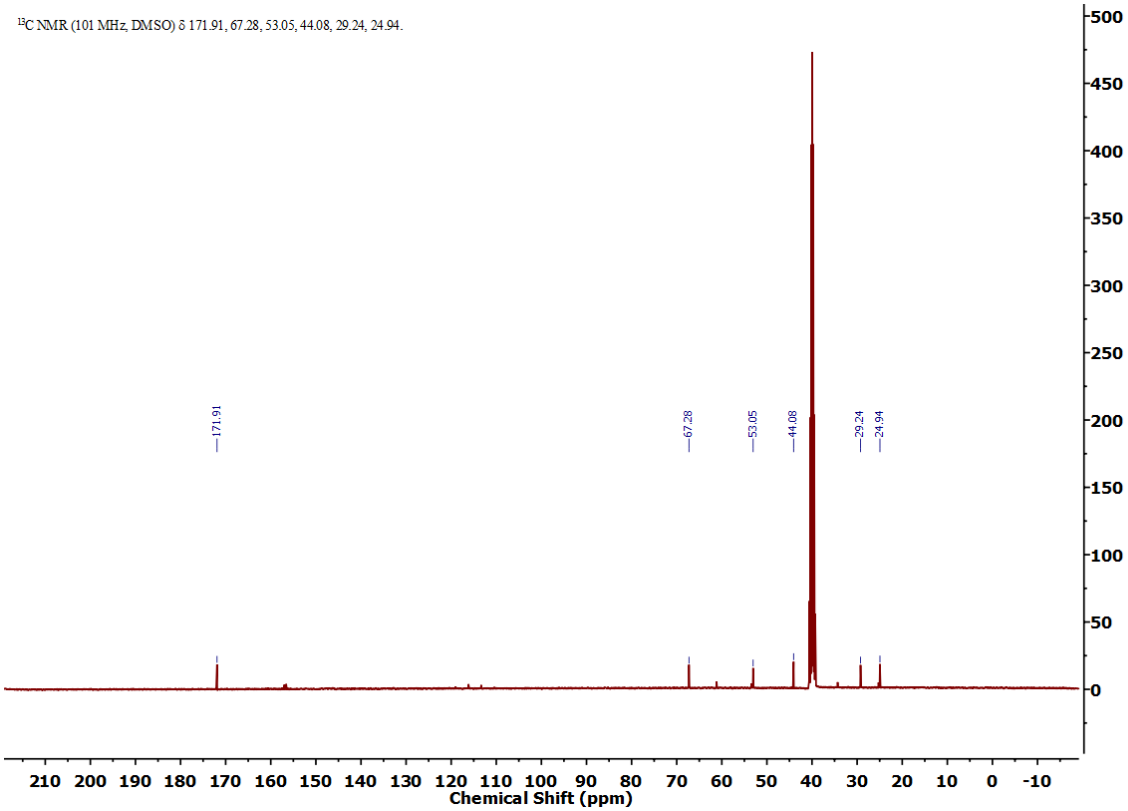
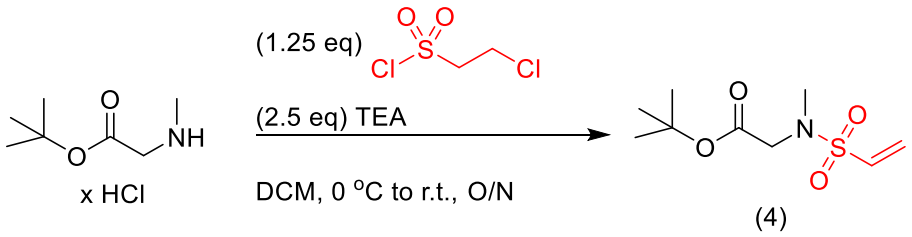


Figure S9. [13]C NMR of compound (3).



Compound (4, E6): To an oven dried N_2 purged 250 mL RBF was added 2.5 g (13.8 mmol, 1 eq) of t-butyl methyl glycine hydrochloride and 100 mL of DCM. 4.8 mL of TEA (3.49 g, 34.5 mmol, 2.5 eq) was added to the solution. The RBF was then cooled in an ice bath and allowed to stir for 15 min. Finally, 1.804 mL (2.813 g, 17.263 mmol, 1.25 eq) of 2-chloroethanesulfonyl chloride was added dropwise to the reaction over 15 min. The reaction was allowed to warm to room temperature overnight. The reaction was quenched with 100 mL of $NaHCO_3$ and the organic phase was extracted with H_2O (2x 50 mL) and brine (1x 50 mL). The organic phase was then dried over Na_2SO_4 and filtered. Once evaporated, the residue was subjected to column purification using a gradient of hexanes and ethyl acetate (0% to 40%). After the fractions were collected and evaporated, the clear oil product was collected, which crystallized upon standing to form (4, E6) as a white solid (2.1 g, 64.6%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.96-6.56 (dd, 1H), 6.23-5.95 (dd, 2H), 4.07-3.73 (s, 2H), 2.90-2.66 (s, 3H), 1.66-1.18 (s, 9H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 168.46, 134.84, 127.83, 82.05, 51.45, 35.47, 28.14.

Mass: $C_9H_{18}NO_4S$ $[M+H]^+$ Calculated: 236.09, Found: 236.18

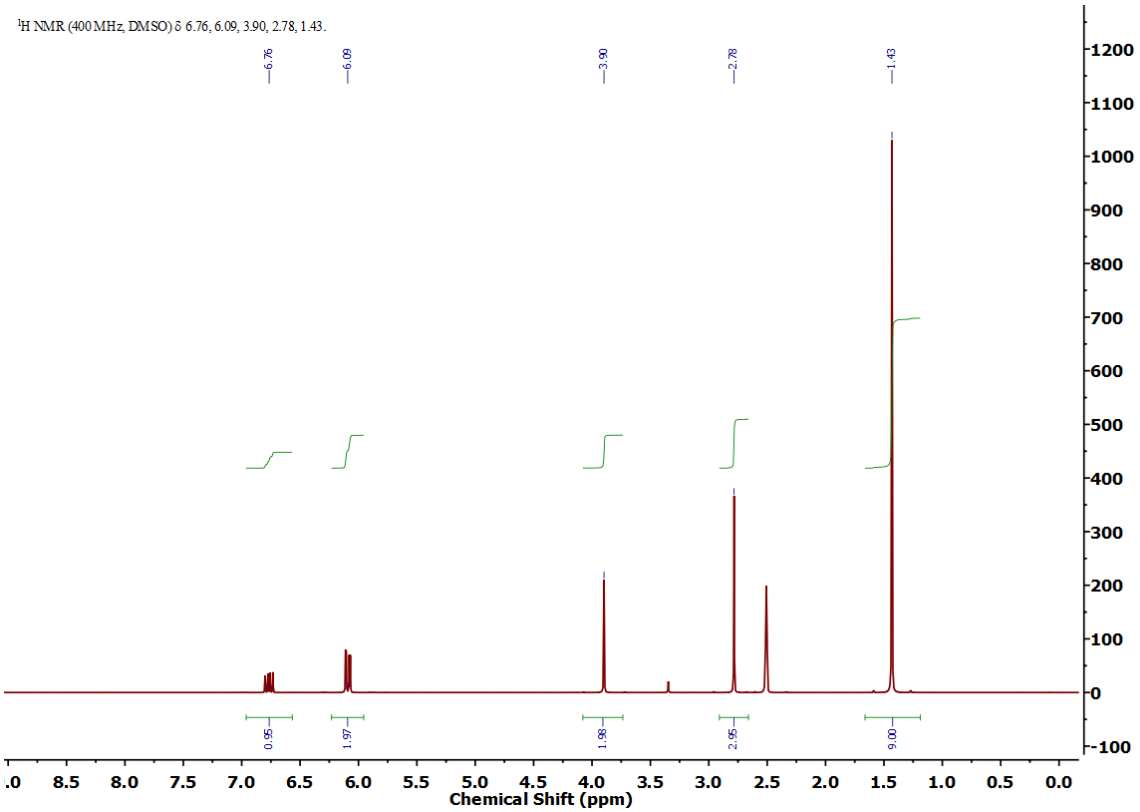


Figure S10. ^{1}H NMR of compound (4).

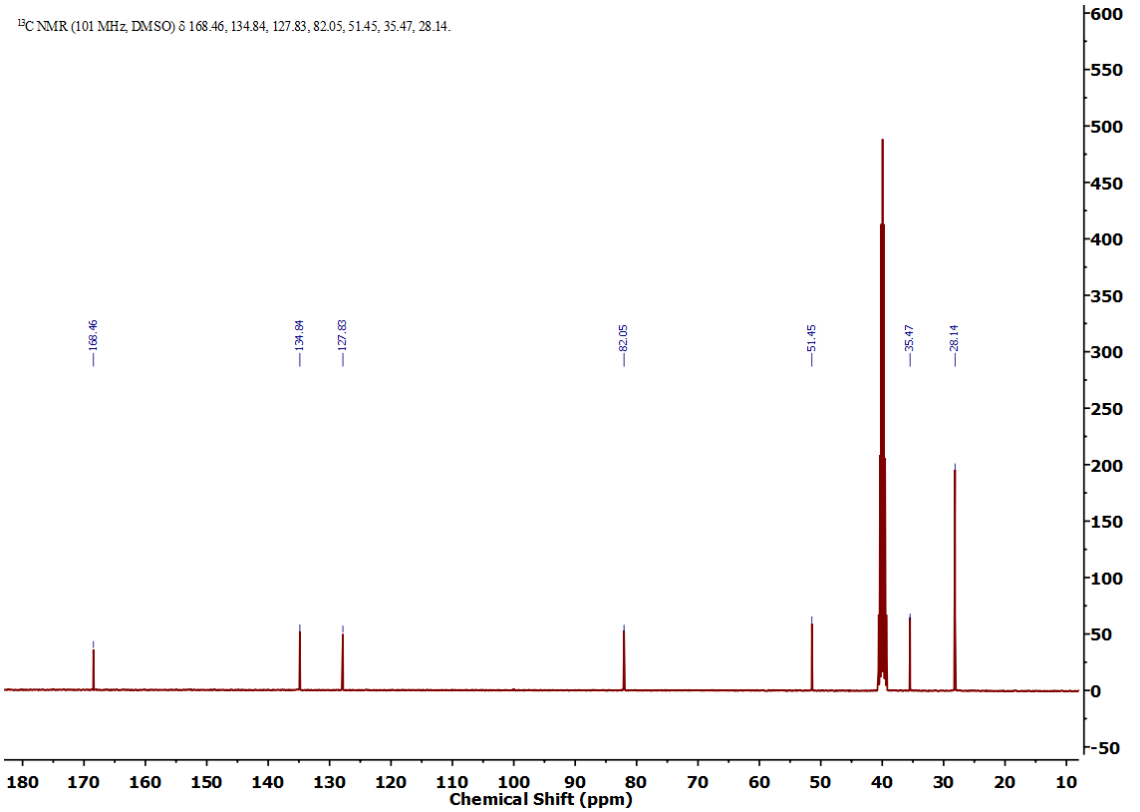
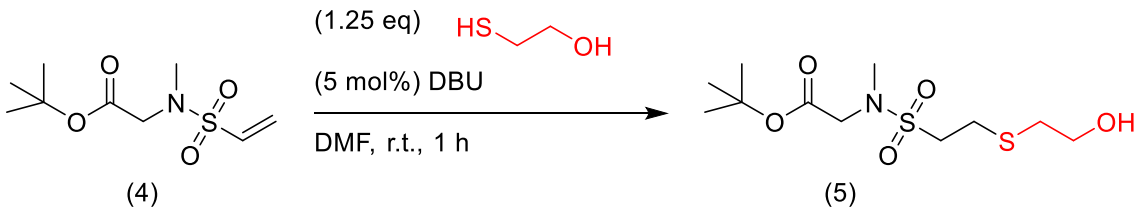


Figure S11. ^{13}C NMR of compound (4).



(4) (5)

Compound (5): 750 mg (3.4 mmol, 1 eq) of (4) and 500 µL of DMF was added to a 1 dram vial, followed by the addition of 331 µL (4.24 mmol, 1.25 eq) of 2-mercaptoethanol. After complete dissolution of the reaction mixture, 24.1 µL (24.5 mg, 0.16 mmol, 0.05 eq) of DBU was added. The solution was mixed thoroughly for 1 hour before being diluted with 50 mL of DCM. The organic phase was washed with water (2x 50 mL) and dried over Na_2SO_4. Upon drying, the residue was subjected to column purification using hexanes and ethyl acetate (0% to 50%). The fractions containing the product were collected and dried to produce (5) as a clear oil that solidified upon standing to form a white solid (0.962 mg, 96.3%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 4.93-4.74 (t, 1H), 4.01-3.84 (s, 2H), 3.59-3.5 (dt, 2H), 3.41-3.58 (m, 2H), 3.57-3.32 (s, 3H), 2.85-2.76 (m, 2H), 2.66-2.59 (t, 2H), 1.50-1.37 (s, 9H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 168.81, 81.99, 61.25, 51.58, 51.46, 35.71, 34.33, 28.15, 24.99.

Mass: $C_{11}H_{24}NO_5S_2$ $[M+Na]^+$ Calculated: 336.43, Found: 336.03

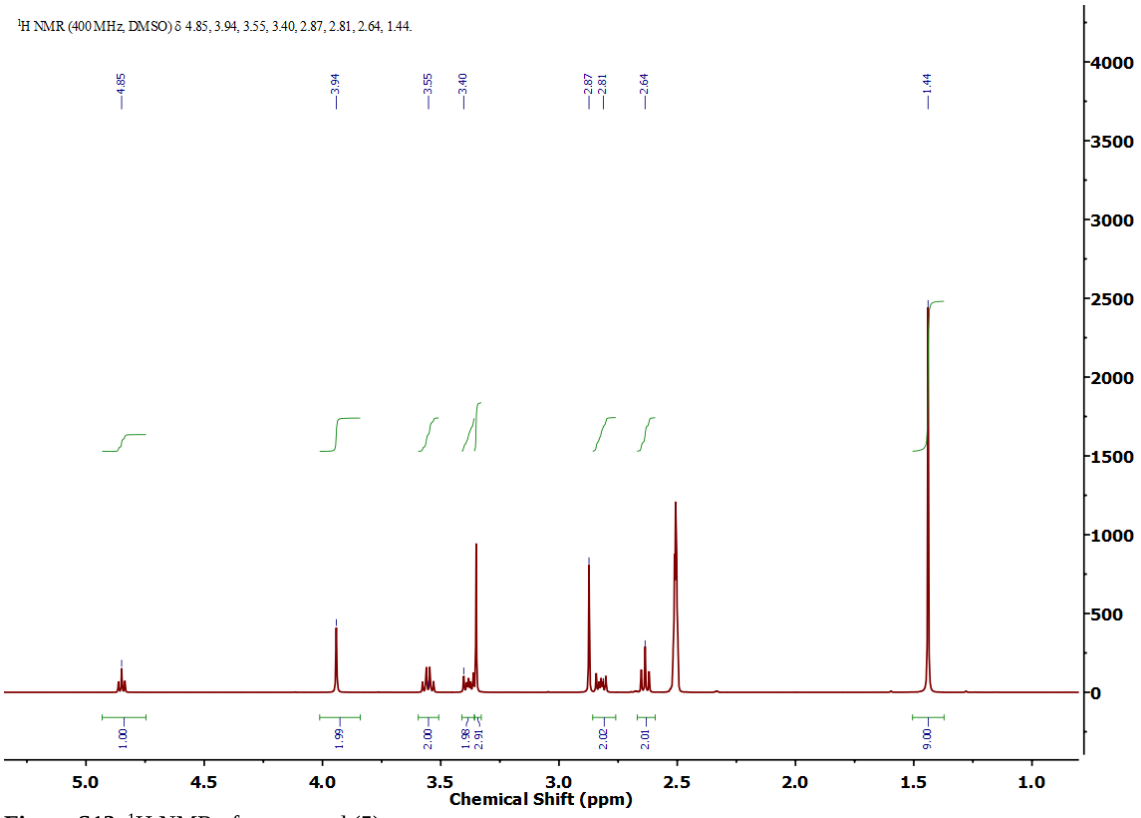


Figure S12. ^{1}H NMR of compound (5).

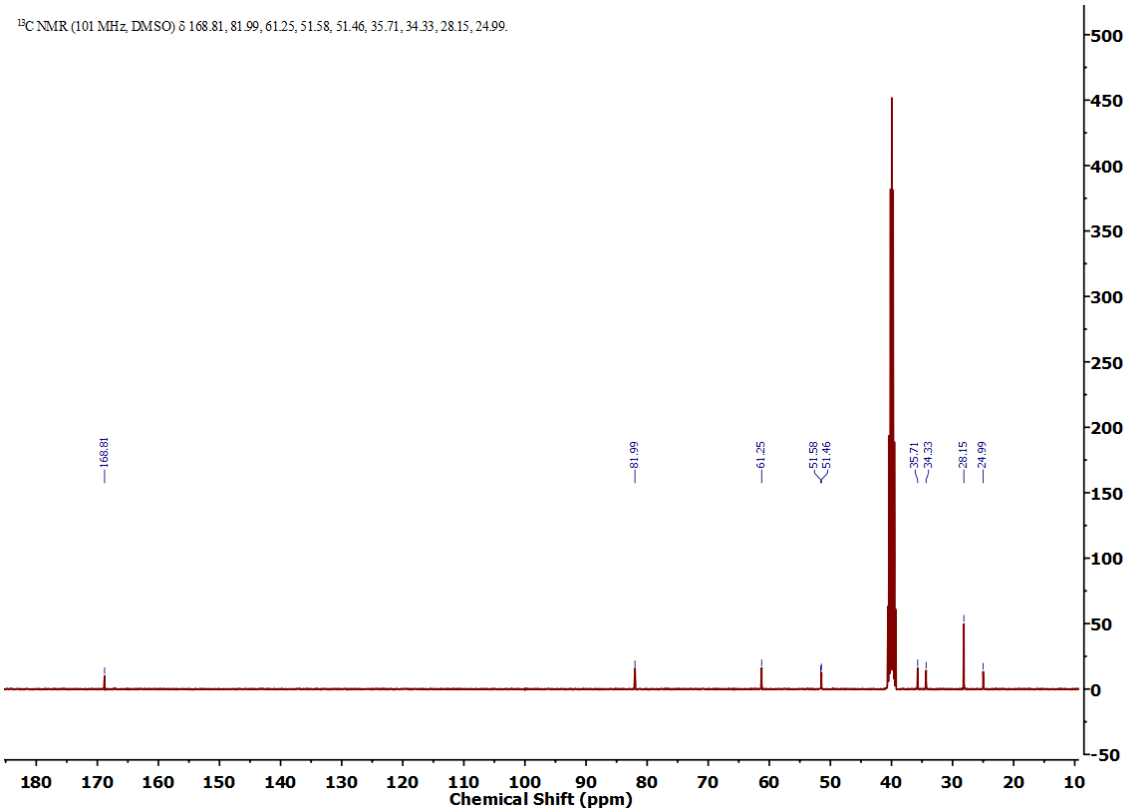
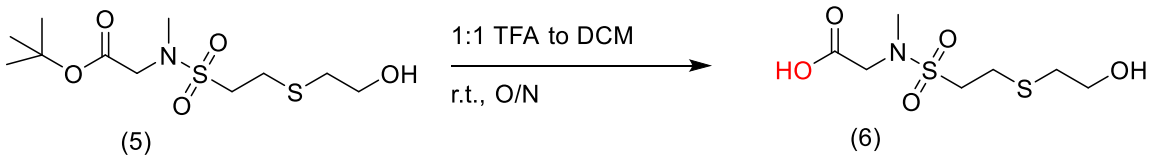


Figure S13. ^{13}C NMR of compound (5).



(5) (6)

Compound (6): 0.962 (0.307 mmol, 1 eq) of (5) and 5 mL of DCM was added to a 20 ml vial and stirred until all solid residue had dissolved. Next, 5 mL of TFA was added and stirred overnight. The reaction was concentrated in vacuo and subjected to column purification with a gradient of 0% to 5% MeOH to DCM. The desired fractions were collected, and the solvent was evaporated to produce a clear oil that crystallized upon standing (434 mg, 54.9%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 4.60-4.44 (t, 2H), 4.04-3.89 (s, 2H), 3.43-3.40 (m, 2H), 2.98-2.85 (m, 7H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 171.13, 67.46, 51.33, 50.74, 35.70, 29.34, 24.76.

Mass: $C_7H_{16}NO_5S_2$ $[M+Na]^+$ Calculated: 280.31, Found: 280.00

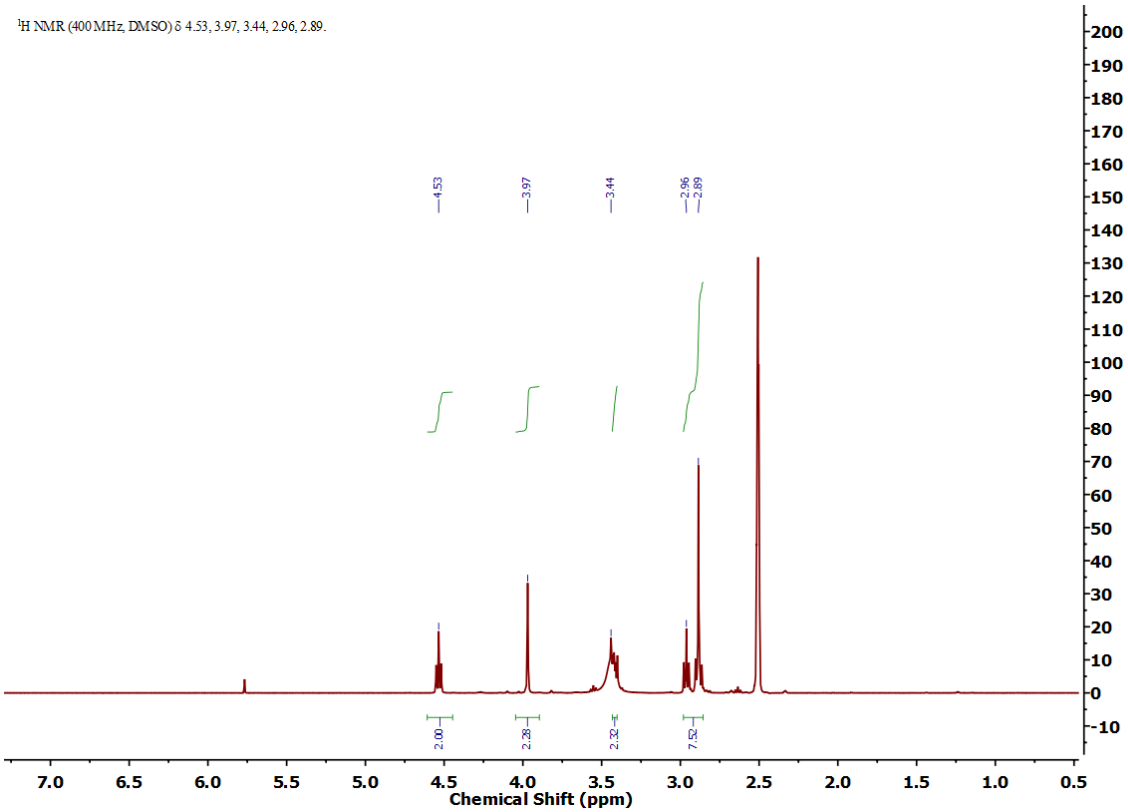


Figure S14. ^{1}H NMR of compound (6).

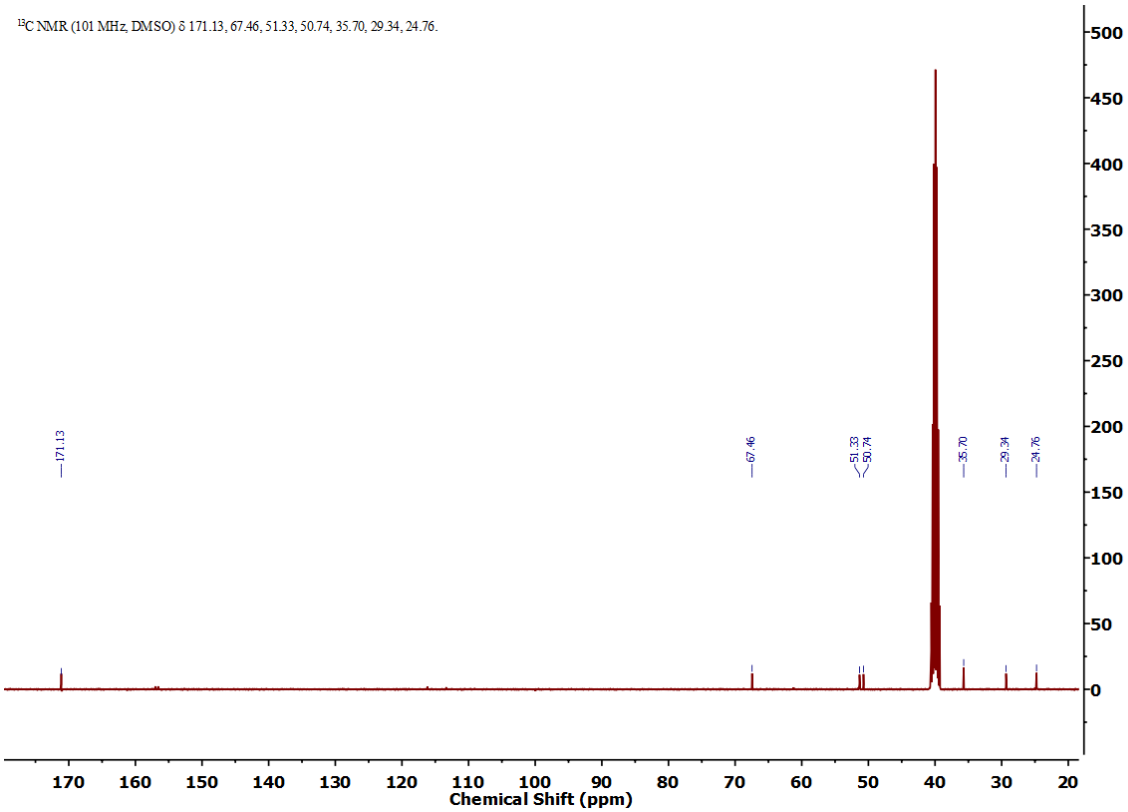


Figure S15. ^{13}C NMR of compound (6).

3.6. Degradation Study

Degradation of model compounds were performed using an AV600 MHz NMR. A model thiol-acrylate product, (methyl 3-[(2-hydroxyethyl)thio]propionate), was synthesized *in situ* by adding methyl acrylate (50 mg, 0.58 mmol, 1 eq) and mercaptoethanol (45.4 mg, 0.58 mmol, 1 eq) together with a catalytic amount of TEA (0.59 mg, 0.006 mmol, 0.01 eq). The neat reaction was vortexed for 15 minutes after which 300 µL of D_2O was added and mixed. A total of 100 µL of the solution was diluted with 900 µL of 25 mM pH 4 sodium acetate buffer and another 100 µL was diluted with 900 µL of 25 mM pH 10 borate buffer. The samples were subsequently mixed and the NMR spectra taken at various time intervals. For products (3) and (6), 5 mg of each sample were dissolved in 100 µL of D_2O and diluted with either 900 µL of 25 mM pH 4 sodium acetate buffer or pH 10 borate buffer at room temperature. NMR measurements were taken over an 8-day period.

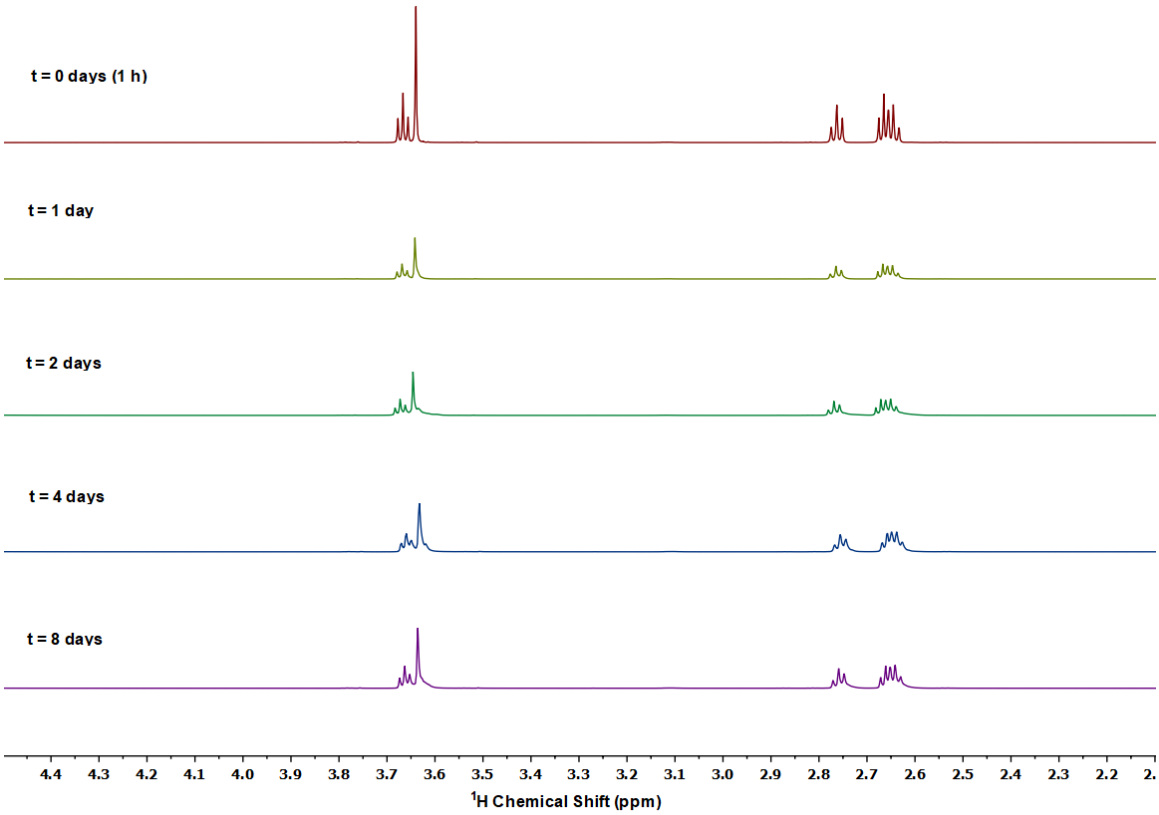


Figure S16. ^{1}H NMR of methyl 3-[(2-hydroxyethyl)thio]propionate in 9:1 25 mM **pH 4** sodium acetate buffer to D_2O. Reaction was run over an 8-day period with periodic NMR measurements. No degradation of the ester bond was seen under these conditions over an 8-day period.

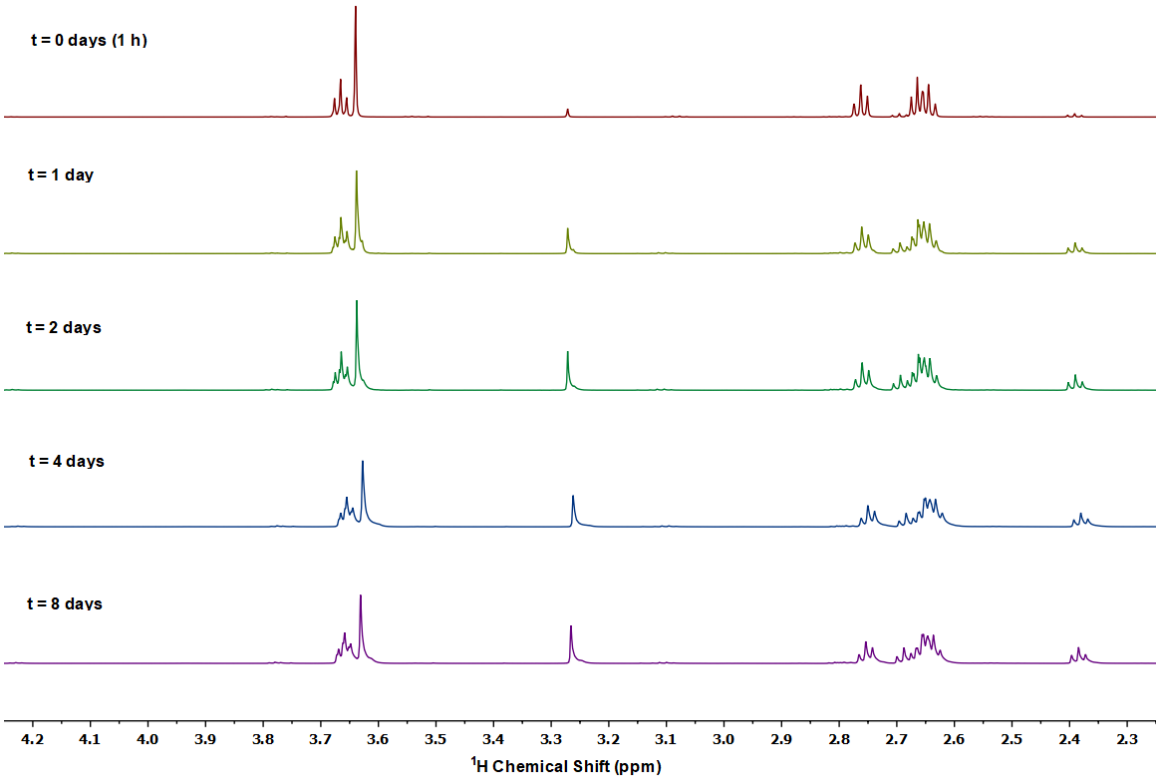


Figure S17. ^{1}H NMR of Methyl 3-[(2-hydroxyethyl)thio]propionate in 9:1 25 mM **pH 10** borate buffer to D_2O. Reaction was run over an 8-day period with periodic NMR measurements. Over the 8-day period, the generation of MeOH (δ = 3.26 ppm) was observed due to degradation, while additional chemical shifts corresponding to the acid product (δ = 2.38 and 2.78 ppm) were observed.

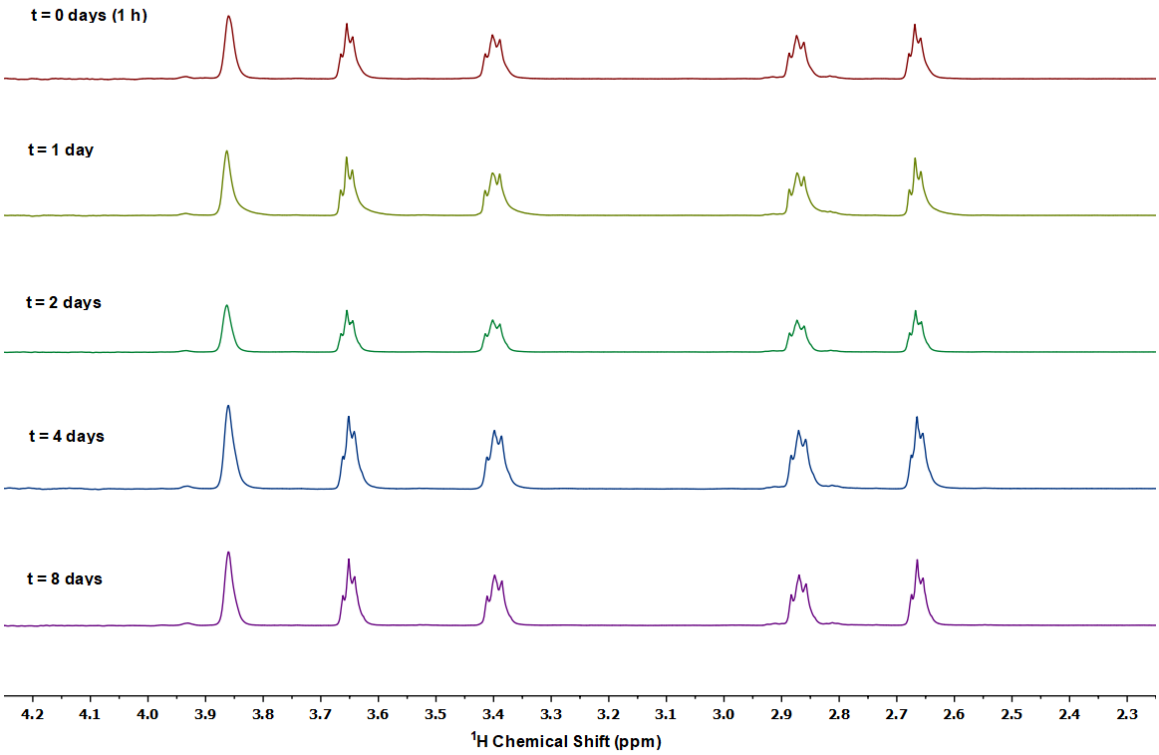


Figure S18. ^{1}H NMR of compound (3) in 9:1 25 mM **pH 4** sodium acetate buffer to D_2O. Reaction was run over an 8-day period with periodic NMR measurements. No observable degradation was seen over the 8-day period demonstrating the stability of the sulfonamide bond.

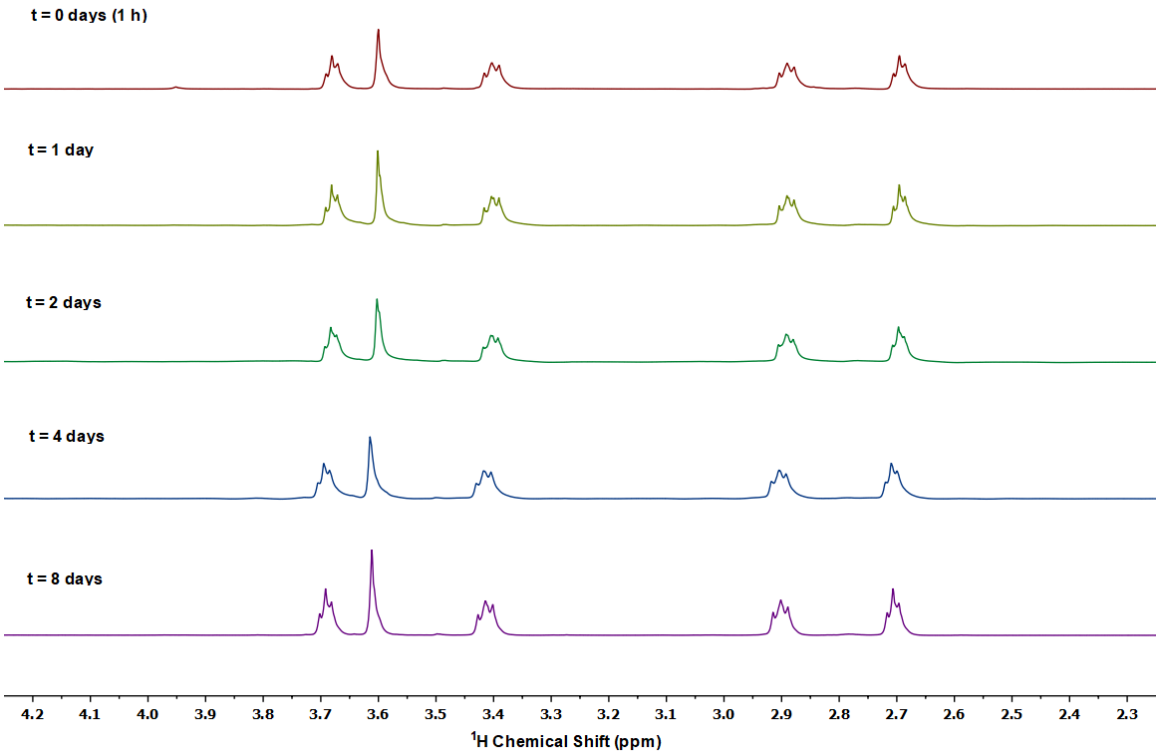


Figure S19. ^{1}H NMR of compound (3) in 9:1 25 mM **pH 10** borate buffer to D_2O. Reaction was run over a 8-day period with periodic NMR measurements. No observable degradation was seen over the 8-day period demonstrating the stability of the sulfonamide bond.

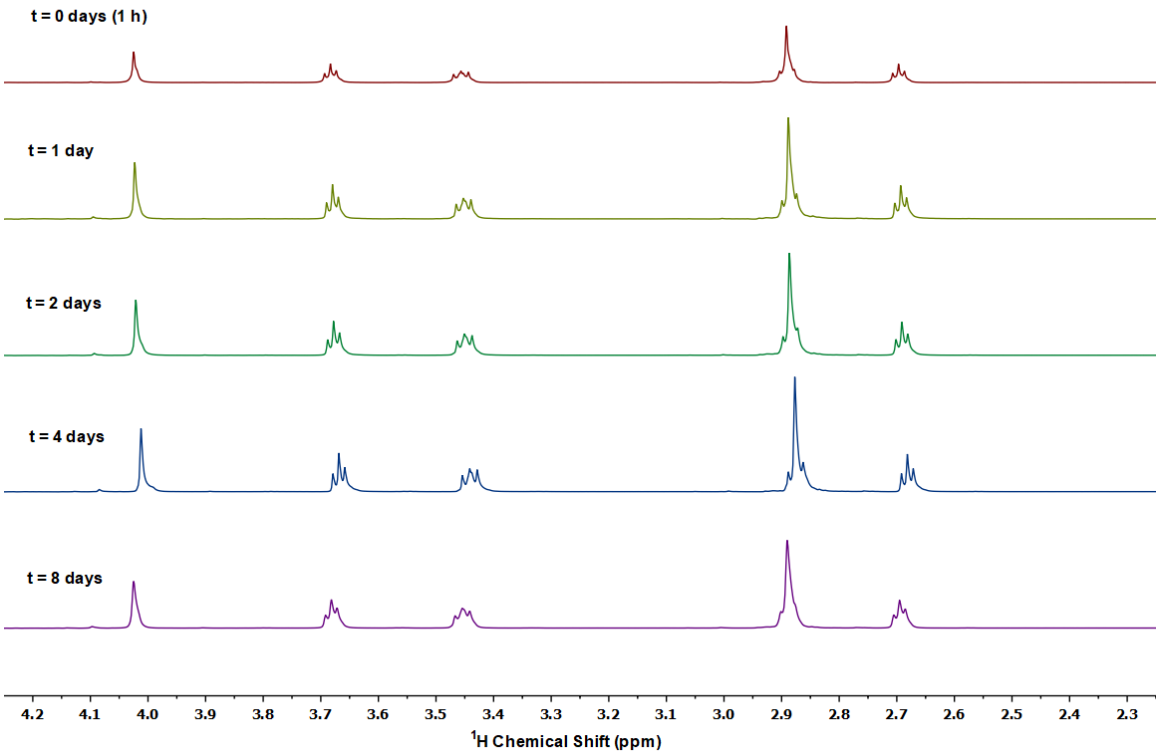


Figure S20. ^{1}H NMR of compound (6) in 9:1 25 mM **pH 4** sodium acetate buffer to D_2O. Reaction was run over an 8-day period with periodic NMR measurements. No observable degradation was seen over the 8-day period demonstrating the stability of the sulfonamide bond.

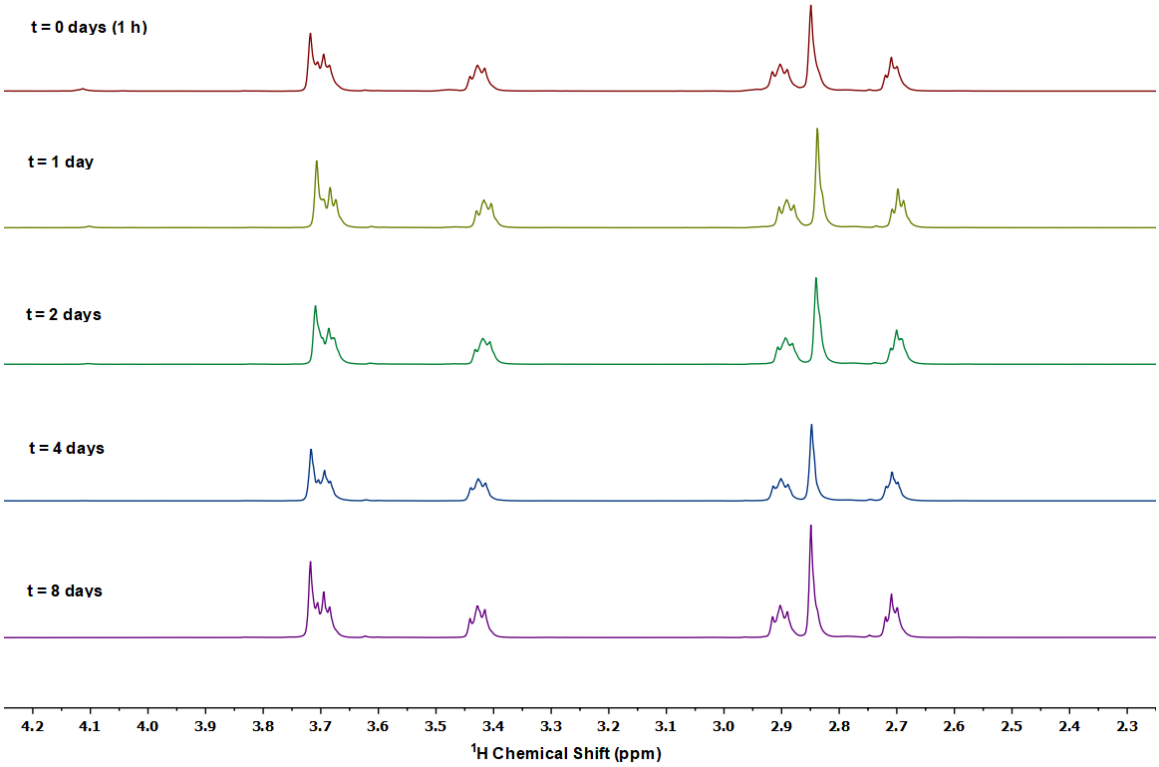


Figure S21. ^{1}H NMR of compound (6) in 9:1 25 mM **pH 10** borate buffer to D_2O. Reaction was run over a 8-day period with periodic NMR measurements. No observable degradation was seen over the 8-day period demonstrating the stability of the sulfonamide bond.

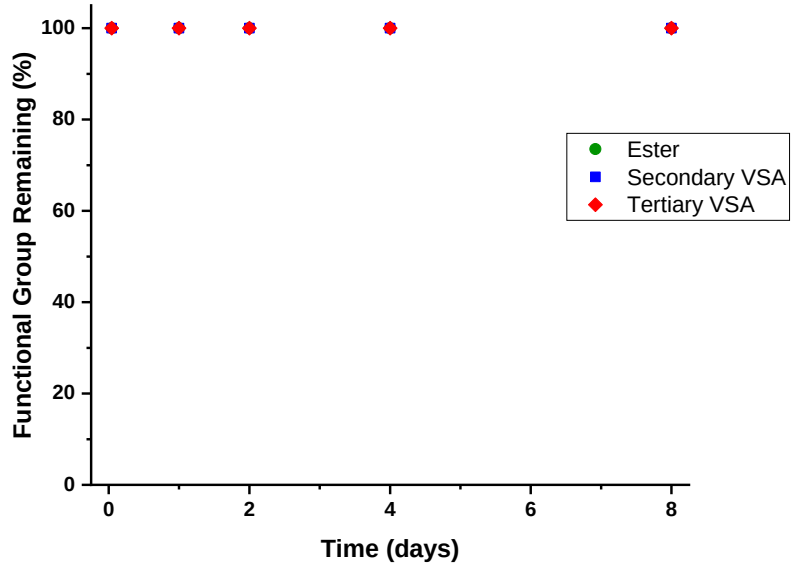


Figure S22. Degradation profile of the model ester, secondary vinyl sulfonamide, and tertiary vinyl sulfonamide in pH 4 25 mM acetate buffer over an 8-day period based on the above NMR integrations. No appreciable degradation was witnessed across each of the three functional groups.

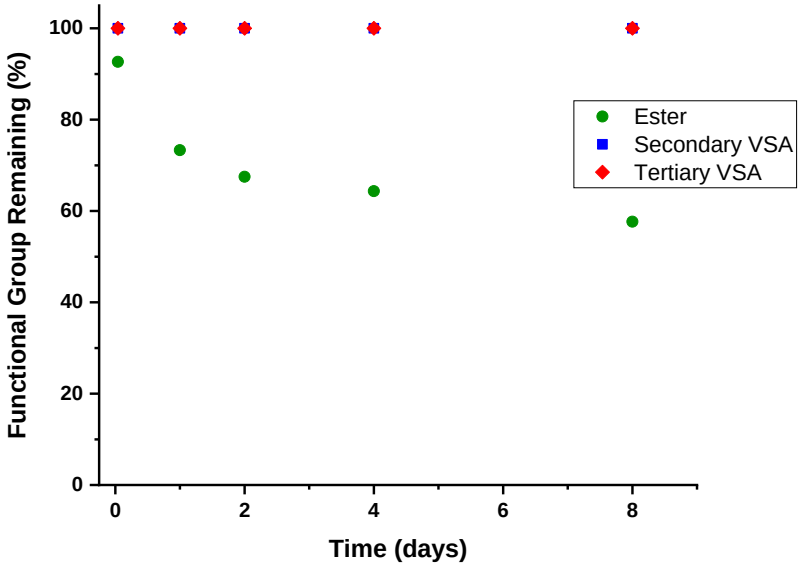


Figure S23. Degradation profile of the model ester, secondary vinyl sulfonamide, and tertiary vinyl sulfonamide in pH 10 25 mM borate buffer over an 8-day period based on the above NMR integrations. The sulfonamides showed improved stability in basic media demonstrating their ability to resist hydrolytic degradation. However, under the same basic conditions, 42% of the model ester had degraded as tracked by the MeOH peak (δ = 3.26 ppm).

4. Real-time FTIR Experiments

Solutions containing 0.5 M thiol and 0.5 M ene were prepared in DMSO. 1.4 mM of DBU was then added to the reaction mixture. The reaction kinetics were measured via real-time Fourier Transform Infrared (FTIR) spectroscopy using an IS-50 spectrometer (Thermofisher). Aliquots of the reaction mixture were then injected into an SL-2 liquid cell equipped with polished calcium fluoride windows (International Crystal Laboratories) using a 0.2 mm thick spacer. Each sample was placed into the FTIR after 30 seconds from adding and mixing the DBU catalyst. Using a Beer's Law type analysis, the reaction kinetics were monitored by tracking the area under the peak corresponding to the S-H between 2484 and 2545 cm^{-1} as shown in **Figure S24.** Real-time FTIR spectra tracking the disappearance of the thiol peak between 2484 and 2545 cm-1.The area under the peak at these concentrations and at the constant path length is directly proportional to the concentration of the thiol. Conversion was then calculated by measuring the ratio of the peak area at a specific time relative to the initial peak area. Relative rates were calculated at 2 min.

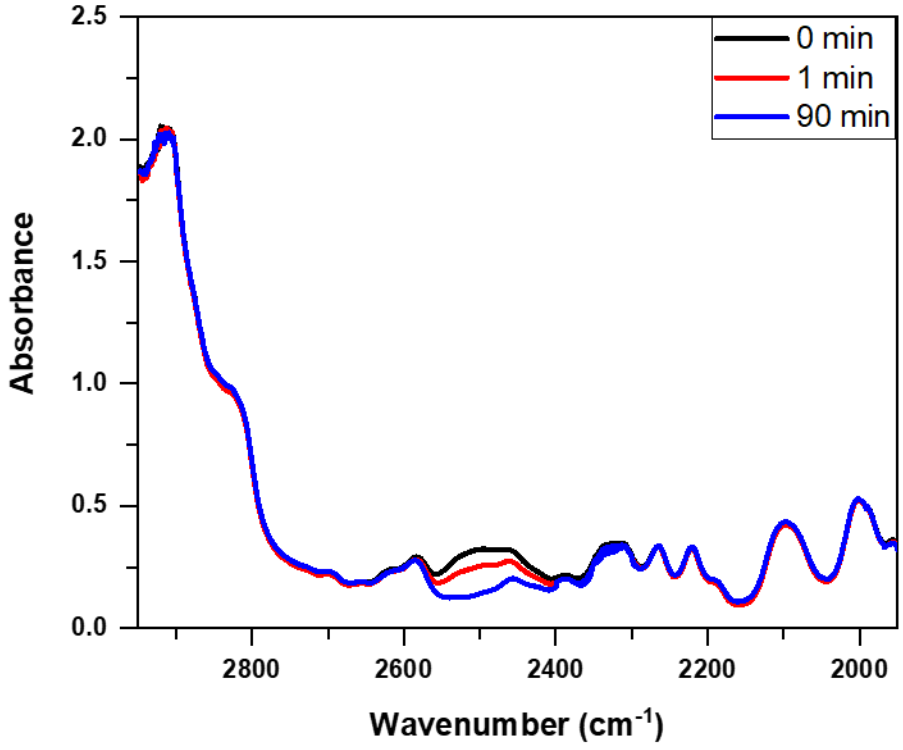


Figure S24. Real-time FTIR spectra tracking the disappearance of the thiol peak between 2484 and 2545 cm^{-1}.

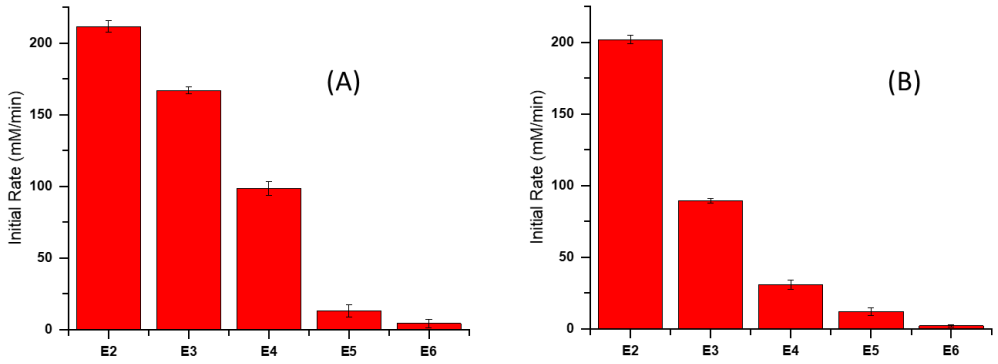


Figure S25. Initial rates calculated at 2 min of (A) T1 and (B) T2 reacting with alkenes E2, E3, E4, E5, and E6.

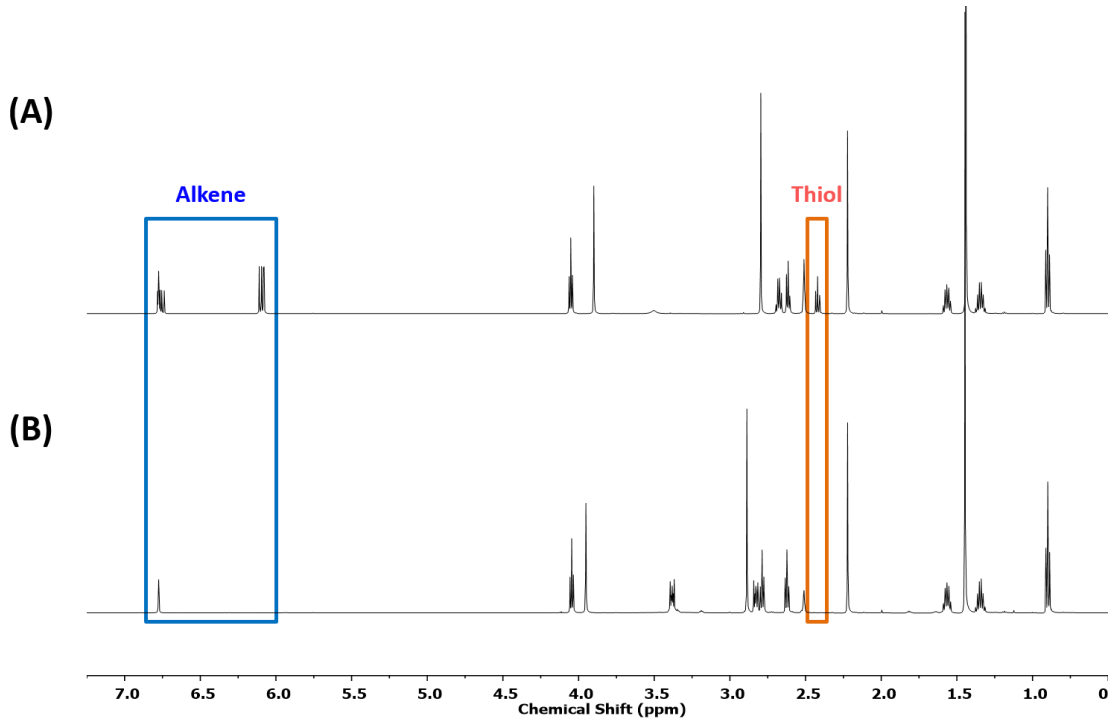


Figure S26. NMR of T1 and E5 (A) without DBU and (B) with DBU after 15 min. The reaction was run at a 0.3 M concentration of total functional groups with 10 mol% of DBU as a catalyst. NMRs show the thiol and alkene peaks disapear proportionally. Peaks were compared to a mesitylene internal standard.

5. ESI of Linear and Cyclized Peptides

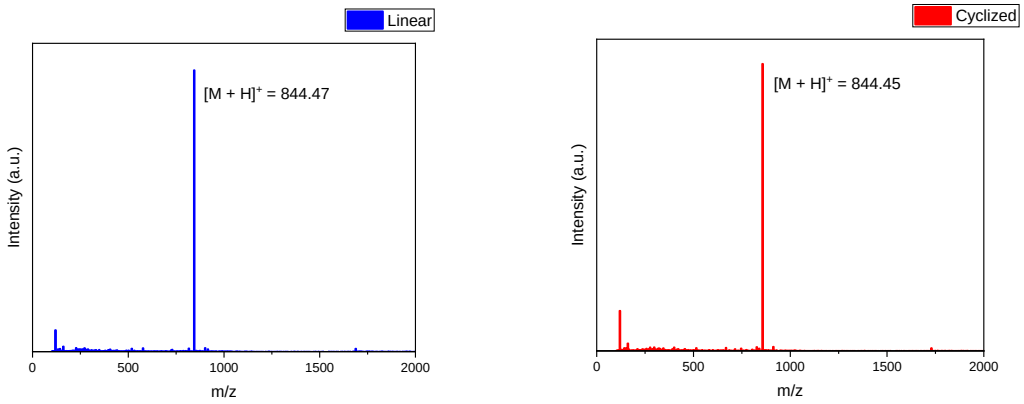


Figure S27. ESI spectra of the linear and cyclized peptide demonstrating expected molecular weights.

6. NMR Spectra of Linear and Cyclized Peptides

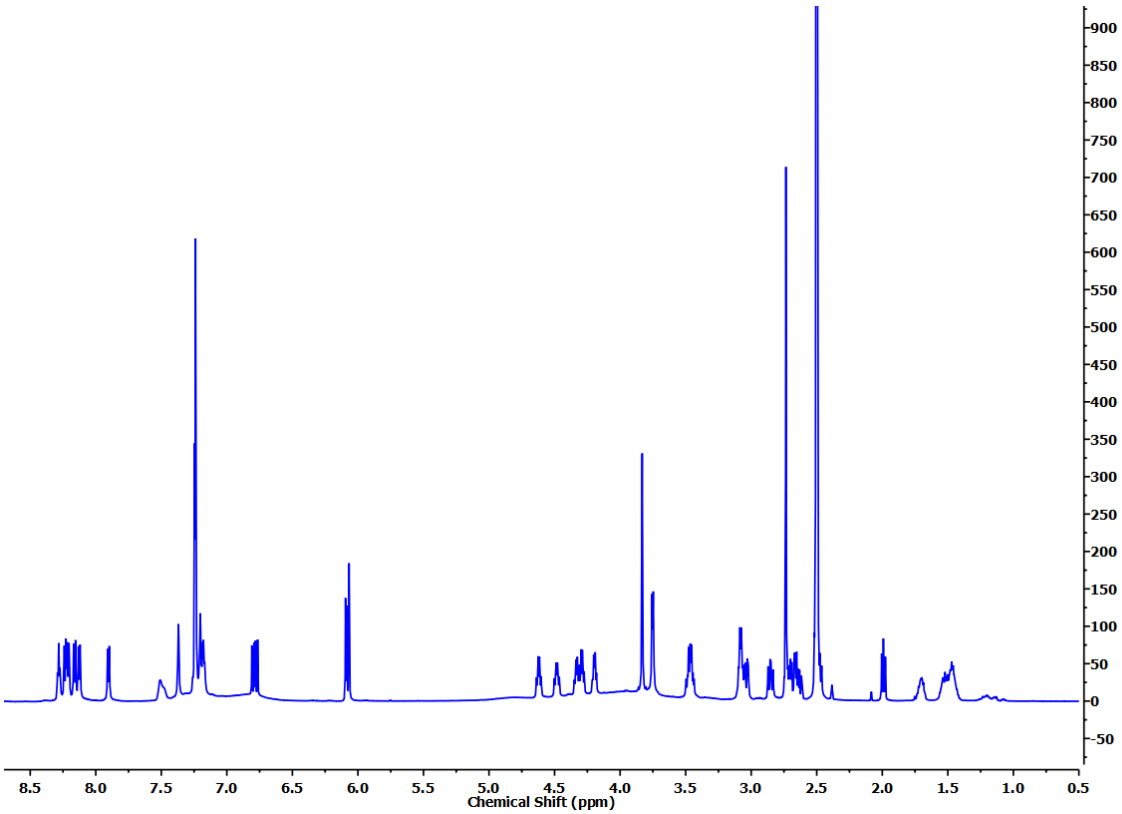


Figure S28. Proton NMR of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

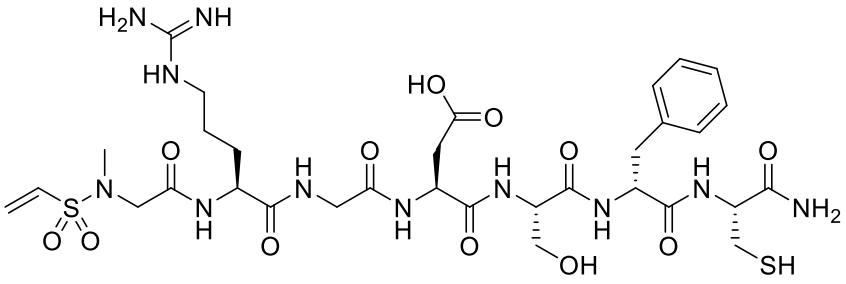


Figure S29. Structure of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

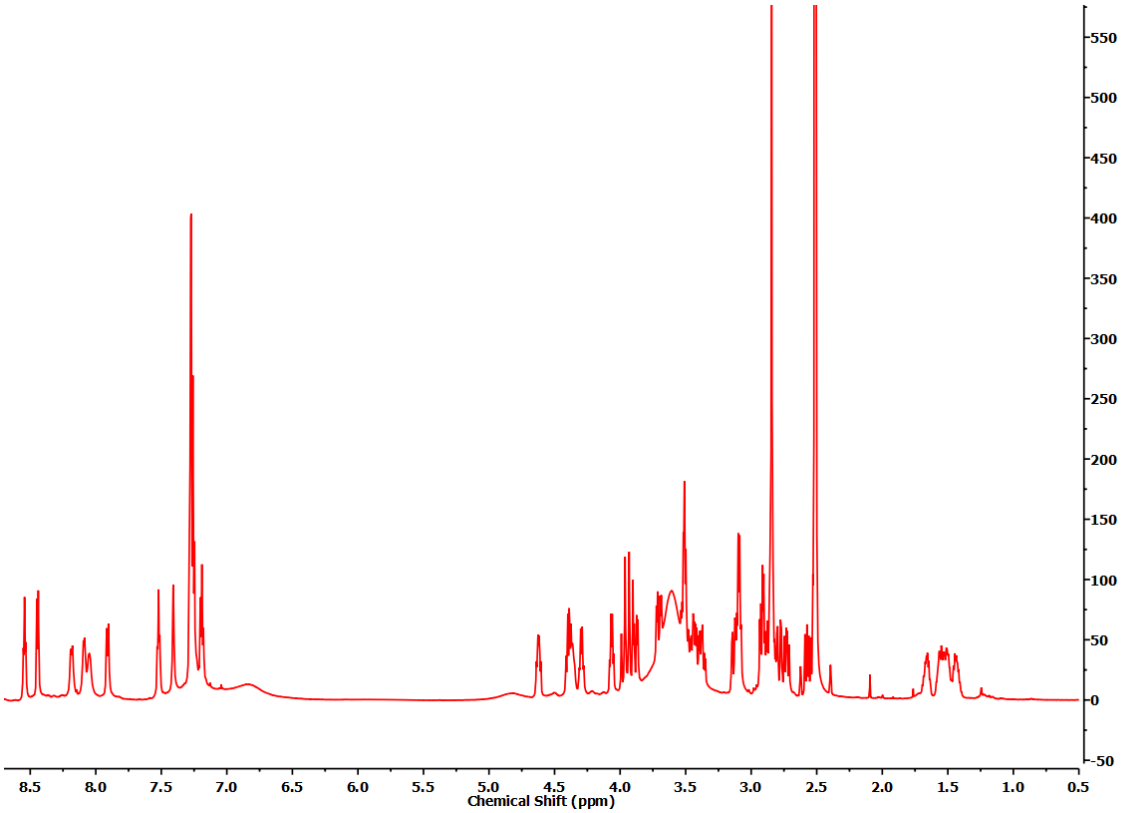


Figure S30. Proton NMR of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH_2.

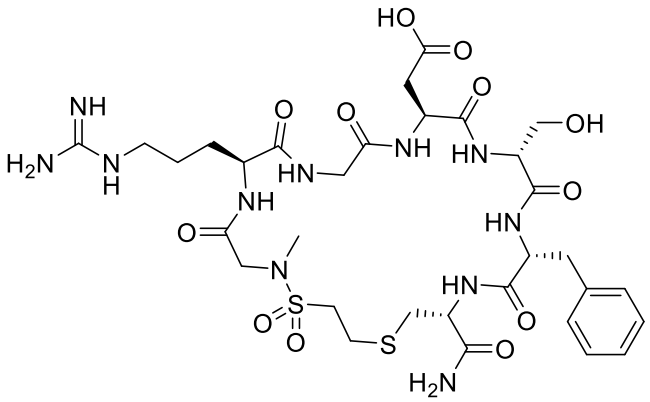


Figure S31. Structure of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH2.

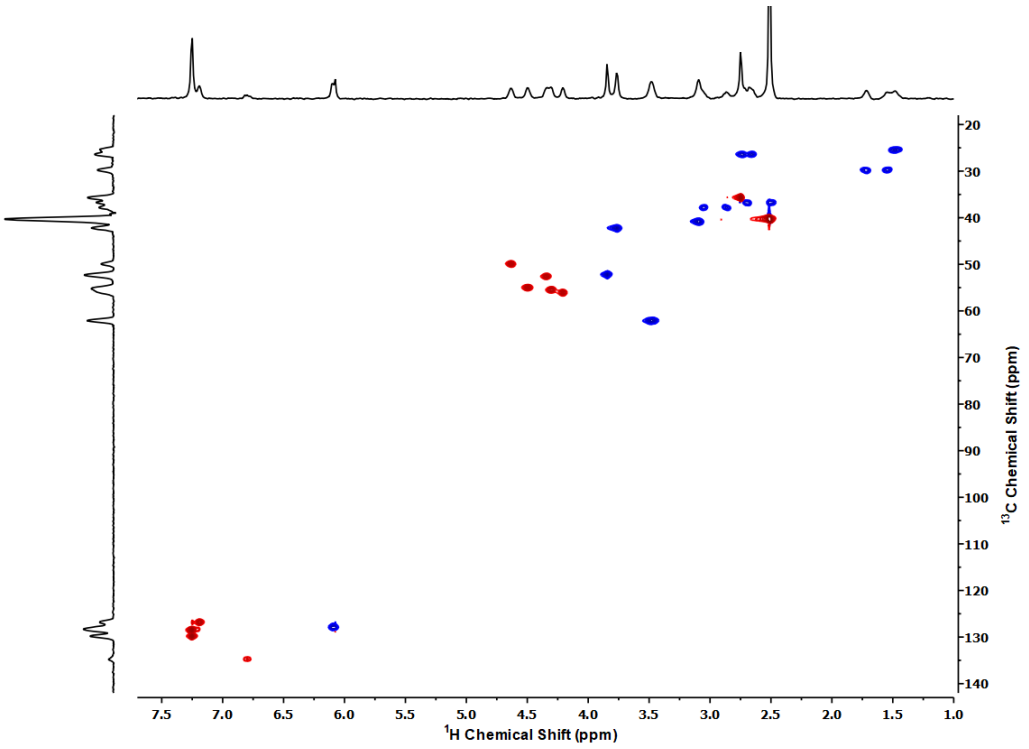


Figure S32. ^{1}H-^{13}C HSQC of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

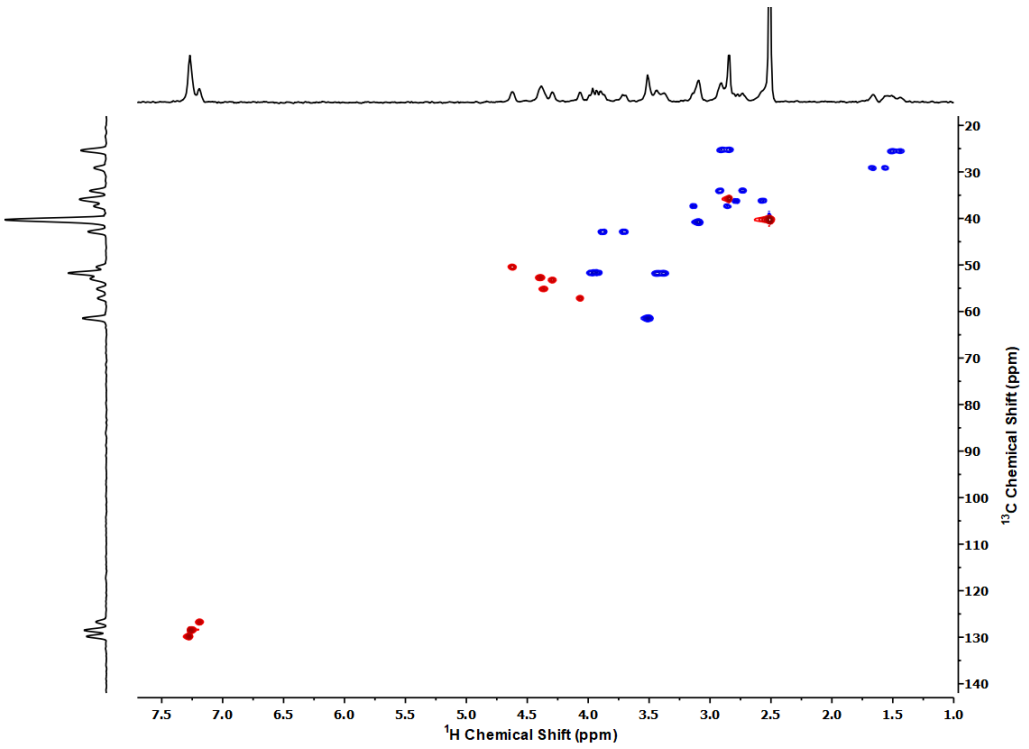


Figure S33. ^{1}H-^{13}C HSQC of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH_2.

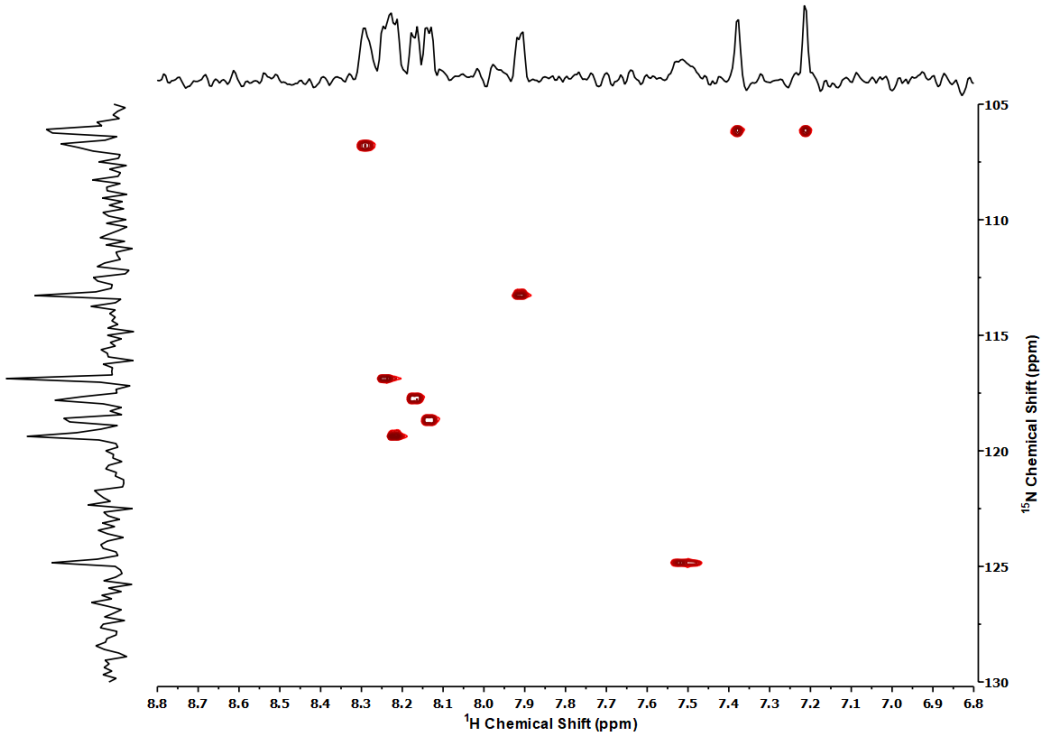


Figure S34. ^{1}H-^{15}N HSQC of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

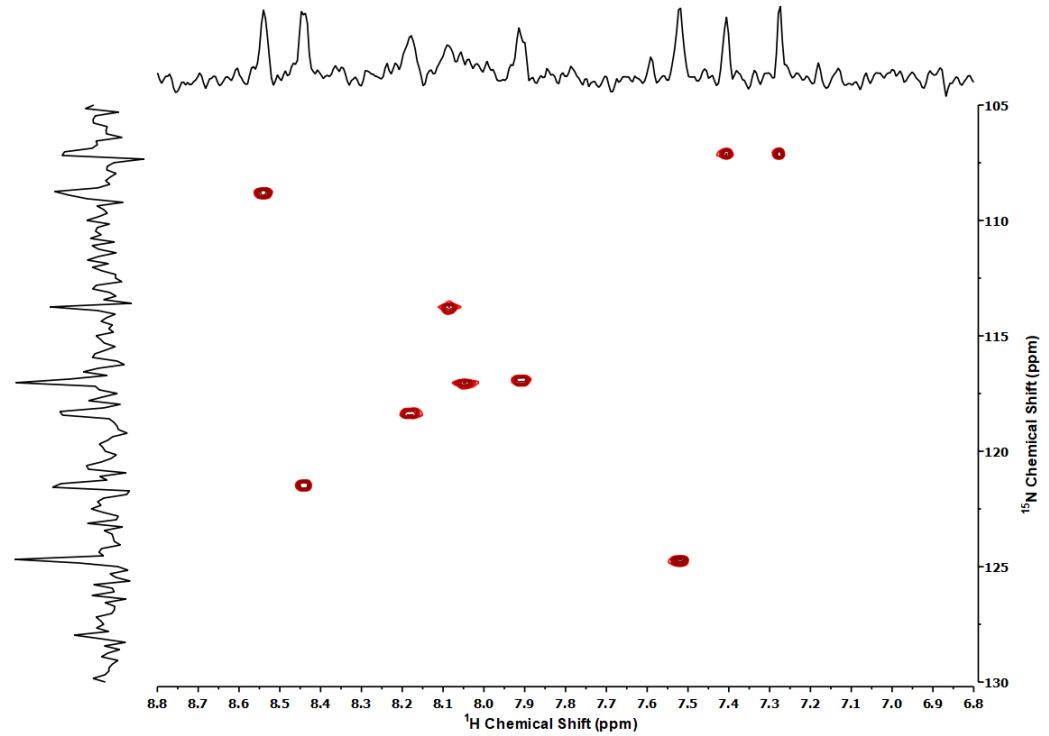


Figure S35. ^{1}H-^{15}N HSQC of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH_2.

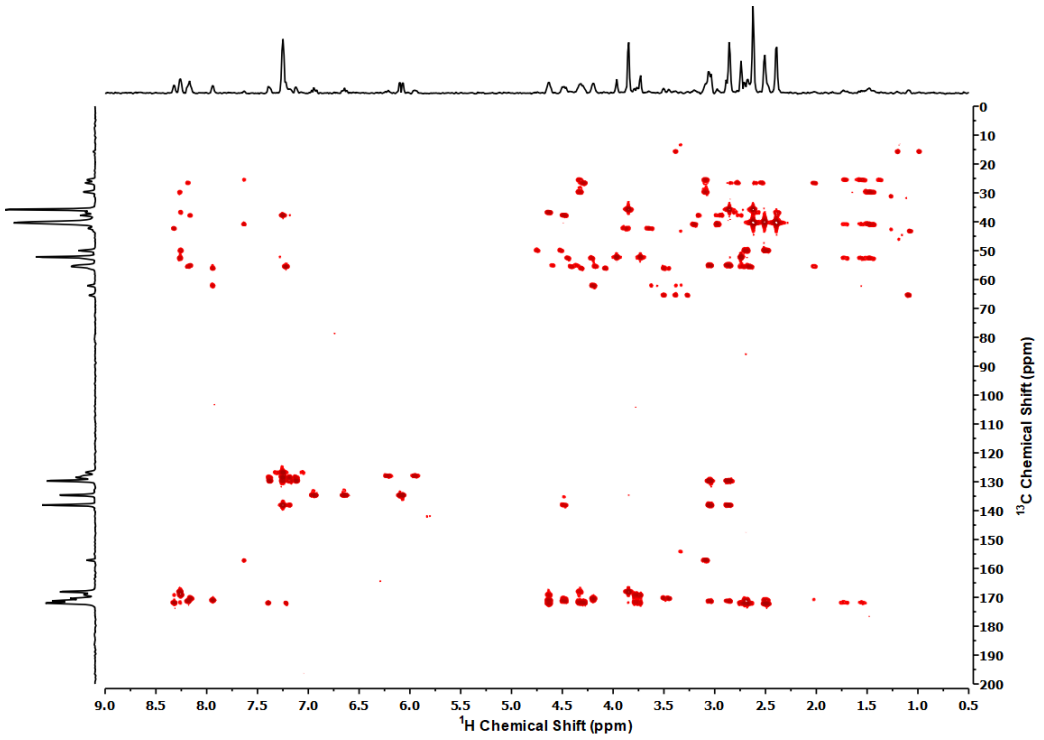


Figure S36. HMBC of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

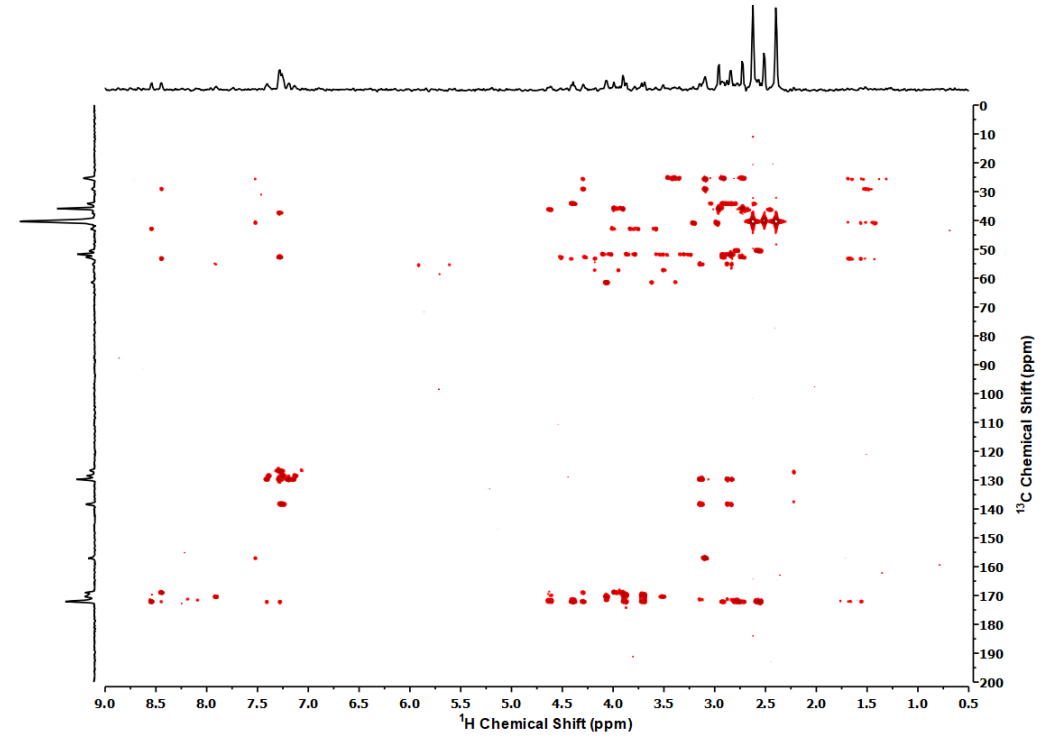


Figure S37. HMBC of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH_2.

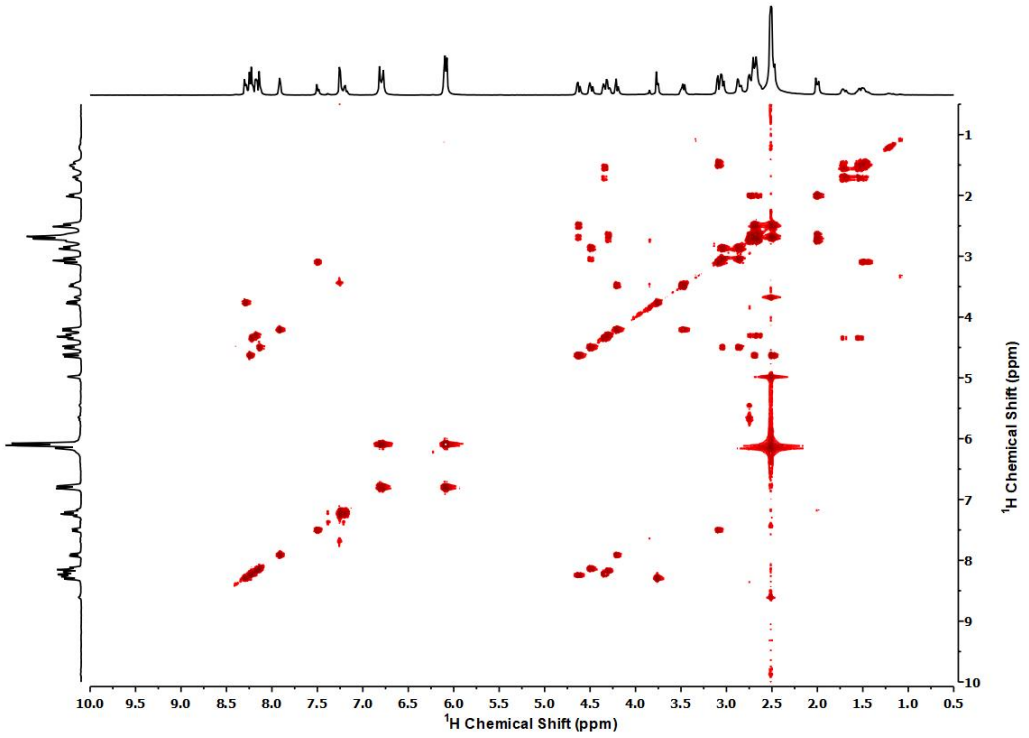


Figure S38. COSY of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

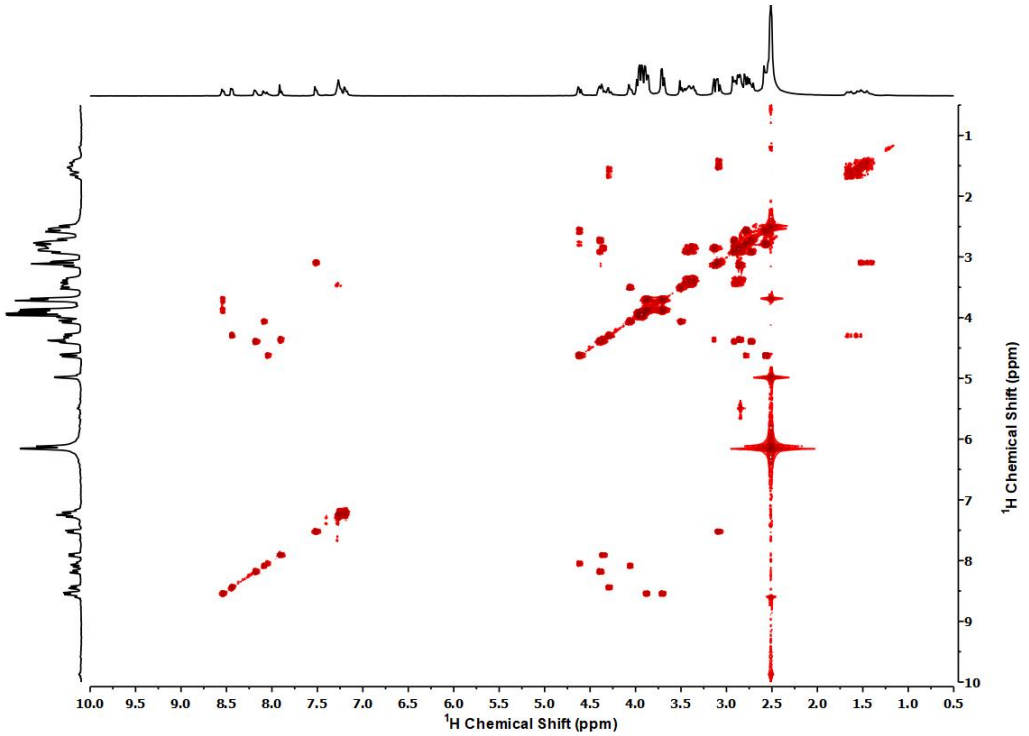


Figure S39. COSY of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH_2.

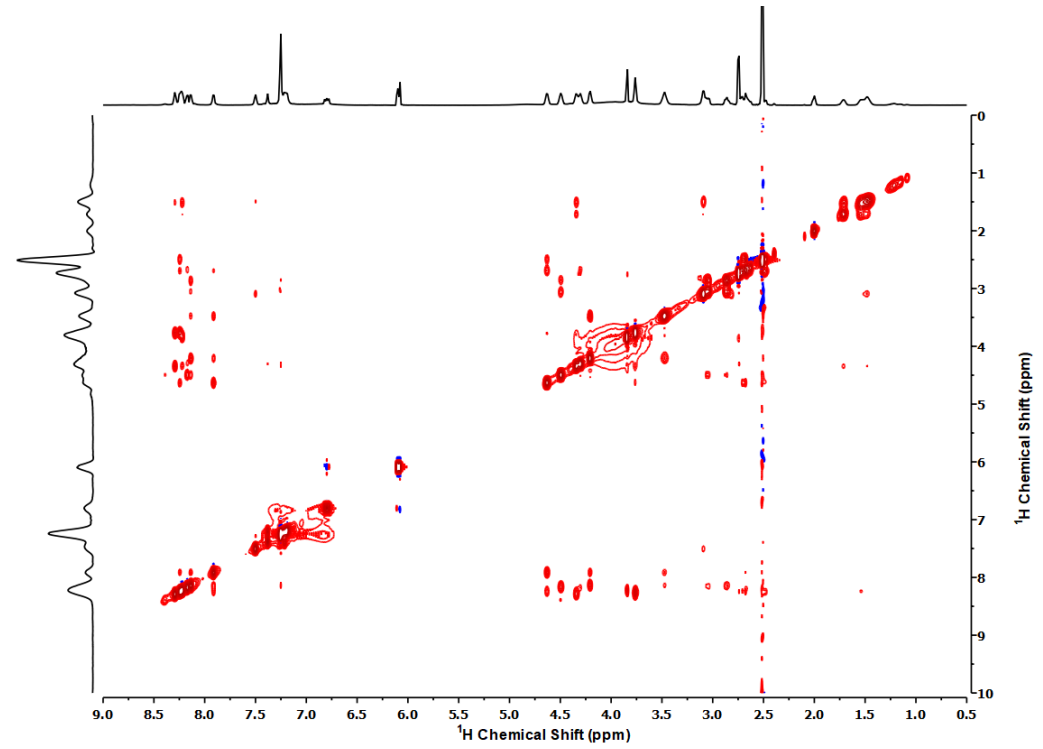


Figure S40. NOESY of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

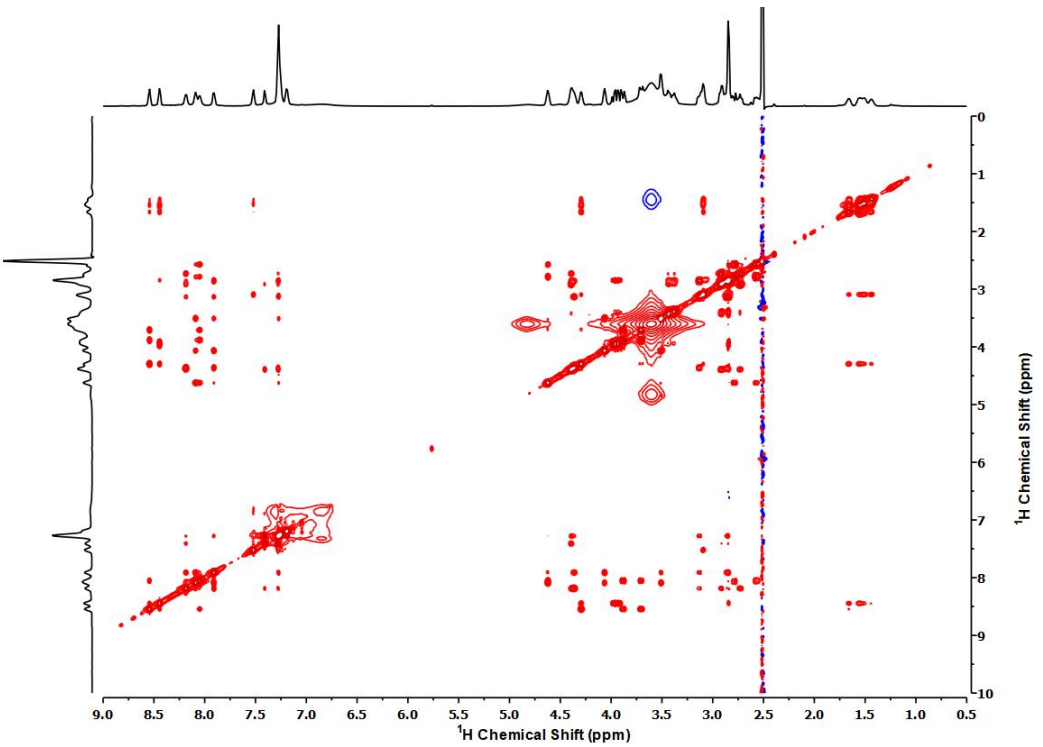


Figure S41. NOESY of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH_2.

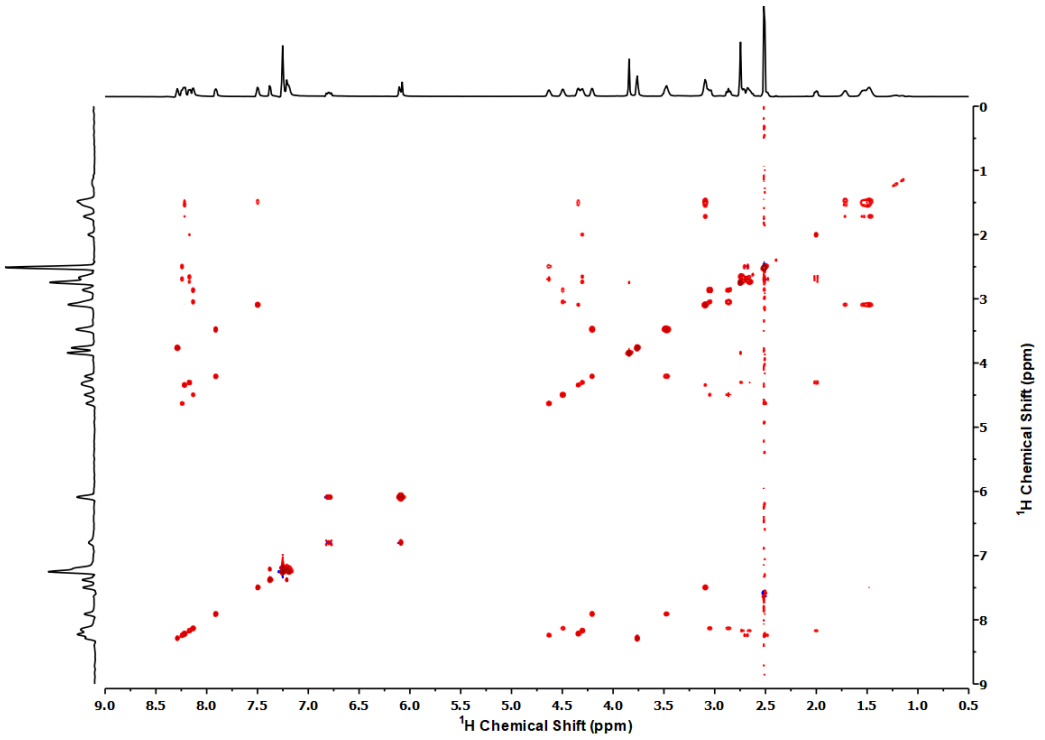


Figure S42. TOCSY of the linear peptide with the sequence (VS)SarRGDSdFC-NH_2.

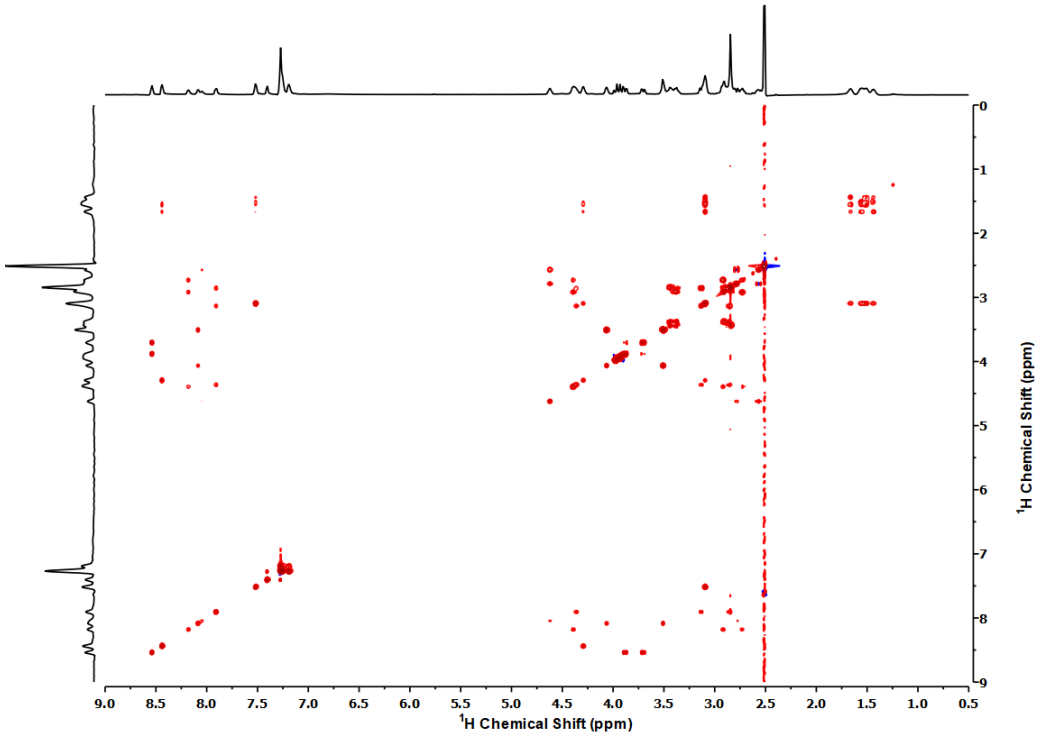


Figure S43. TOCSY of the cyclized peptide with the sequence (VS)SarRGDSdFC-NH_2.