Supporting Information

Selective Modification of Sulfamidate-Containing Peptides

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Content

1. HPLC chromatograms of selective peptide backbone modification reactions

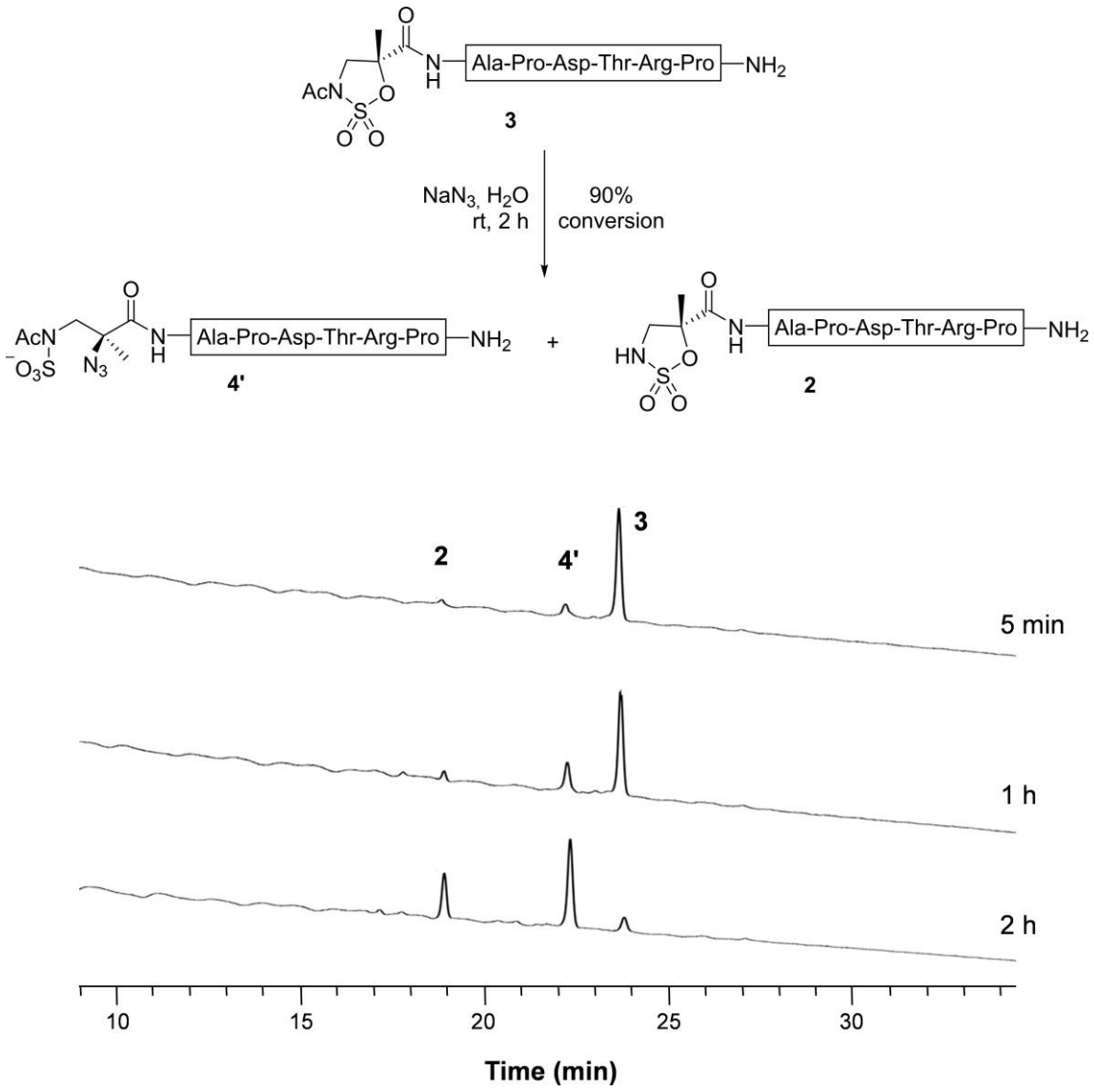


Fig. S1. Monitoring of reaction of peptide **3** with sodium azide in water. HPLC analyses were performed using linear gradient of 1 % to 31% B solvent in 37 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

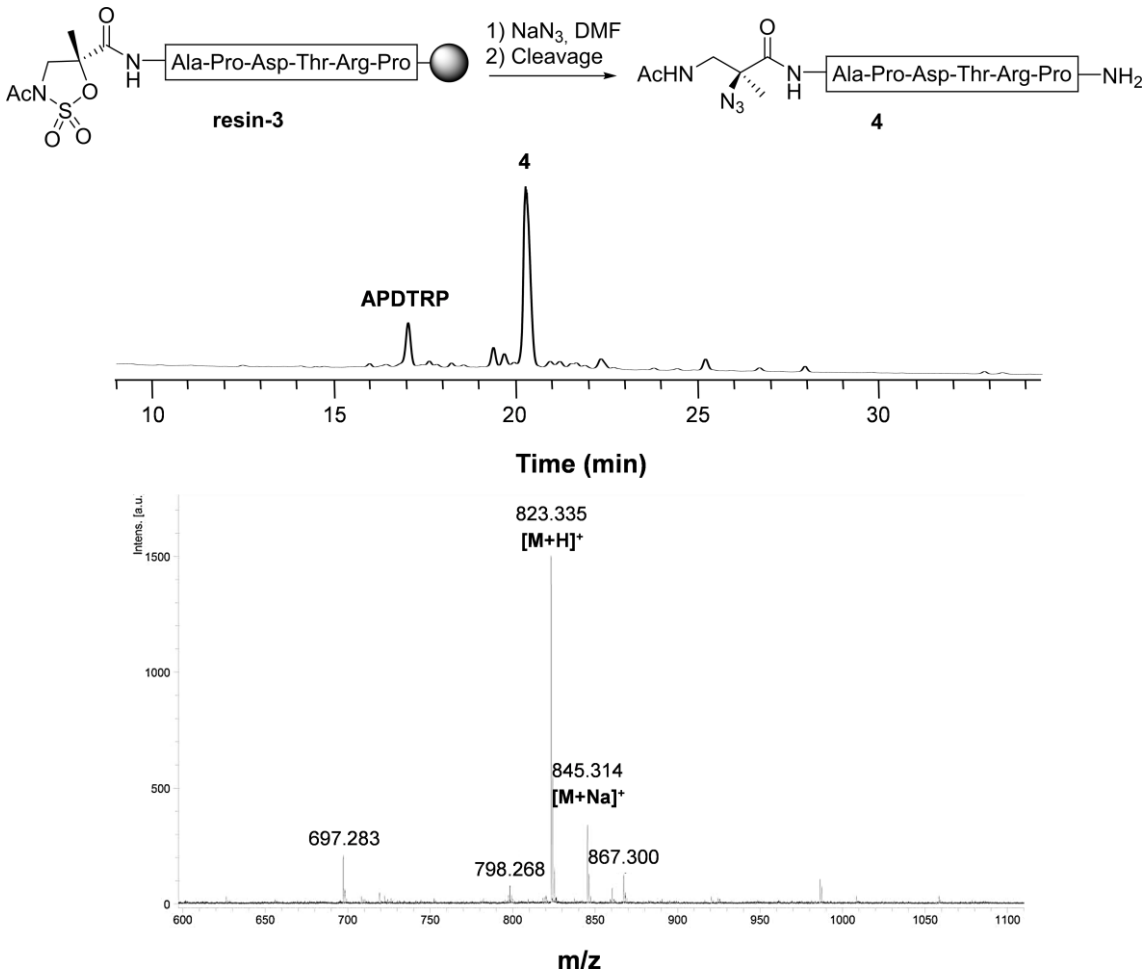


Fig. S2. Analytical RP-HPLC chromatogram and MS chromatogram for the crude mixture of on-resin synthesis of peptide **4** (t_R = 20.25 min). Linear gradient 1-31% B over 37 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile. N-deacetylated peptide **2** (t_R: 18.8 min; m/z = 818.881 for $[M+H]^+$) was not observed. Unmodified peptide Ac-Ala-Pro-Asp-Thr-Arg-Pro-NH_2 (**APDTRP**, tr: 17.0 min; m/z = 697.283 for $[M+H]^+$) was obtained as a by-product originating from the incomplete solid-phase coupling of sulfamidate **1** (ca. 70% conversion) and subsequent *N*-acetylation prior to ring-opening with NaN_3.

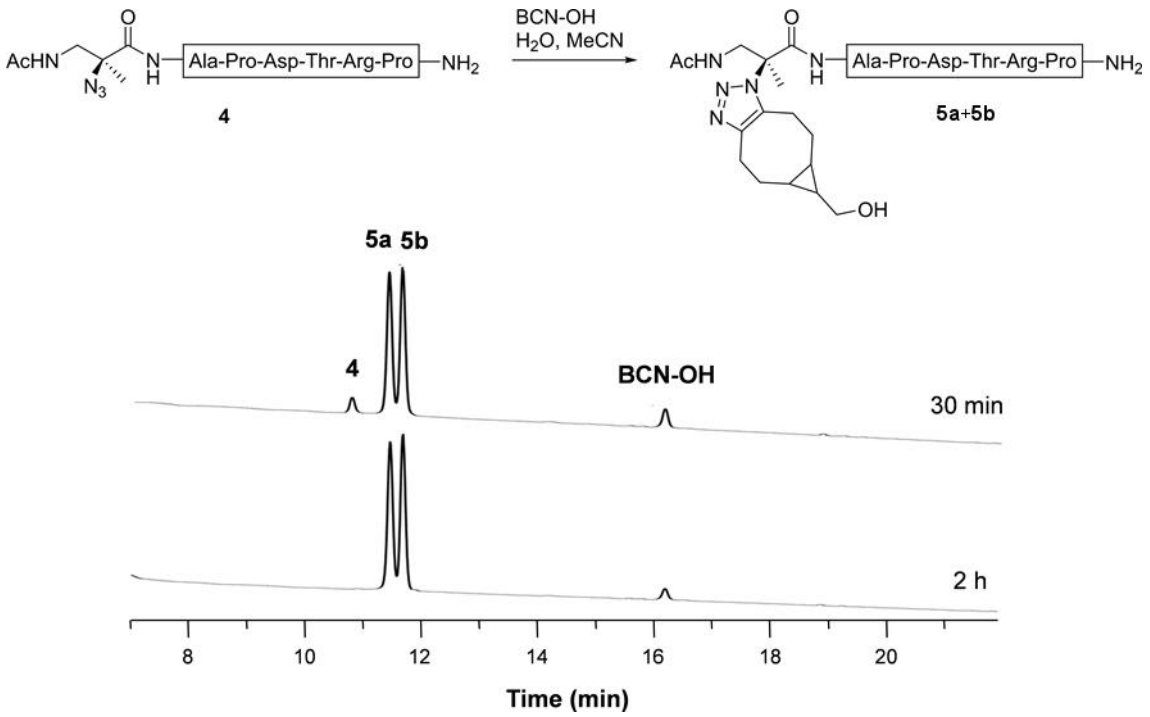


Fig. S3. Monitoring of reaction of peptide **4** with BCN-OH in a water/acetonitrile mixture. HPLC analyses were performed using linear gradient of 5 % to 65% B solvent in 30 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

2. Optical study of fluorsecent peptide 7

The ultraviolet-visible absorption spectrum of compound **7** (10^{-5} M solution in DMSO) has been registered, observing an absorption band with maximum at 331 nm. Then, the emission of the compound was studied and a maximum emission wavelength was observed at 399 nm.

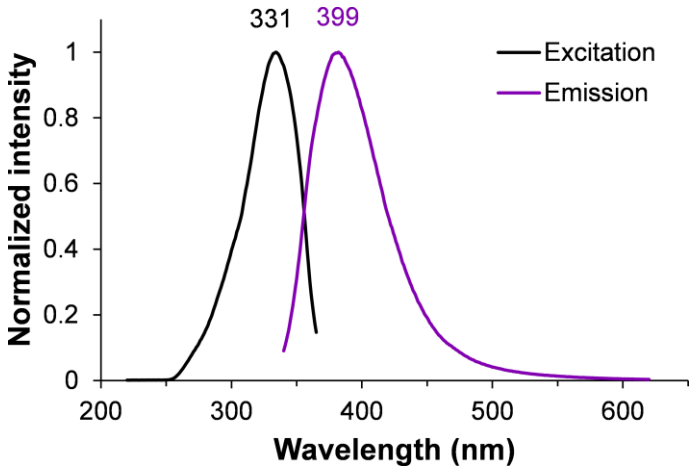


Fig. S4. Excitation and emission spectra for peptide **7** in DMSO.

3. HPLC chromatograms for pH-controlled release reactions

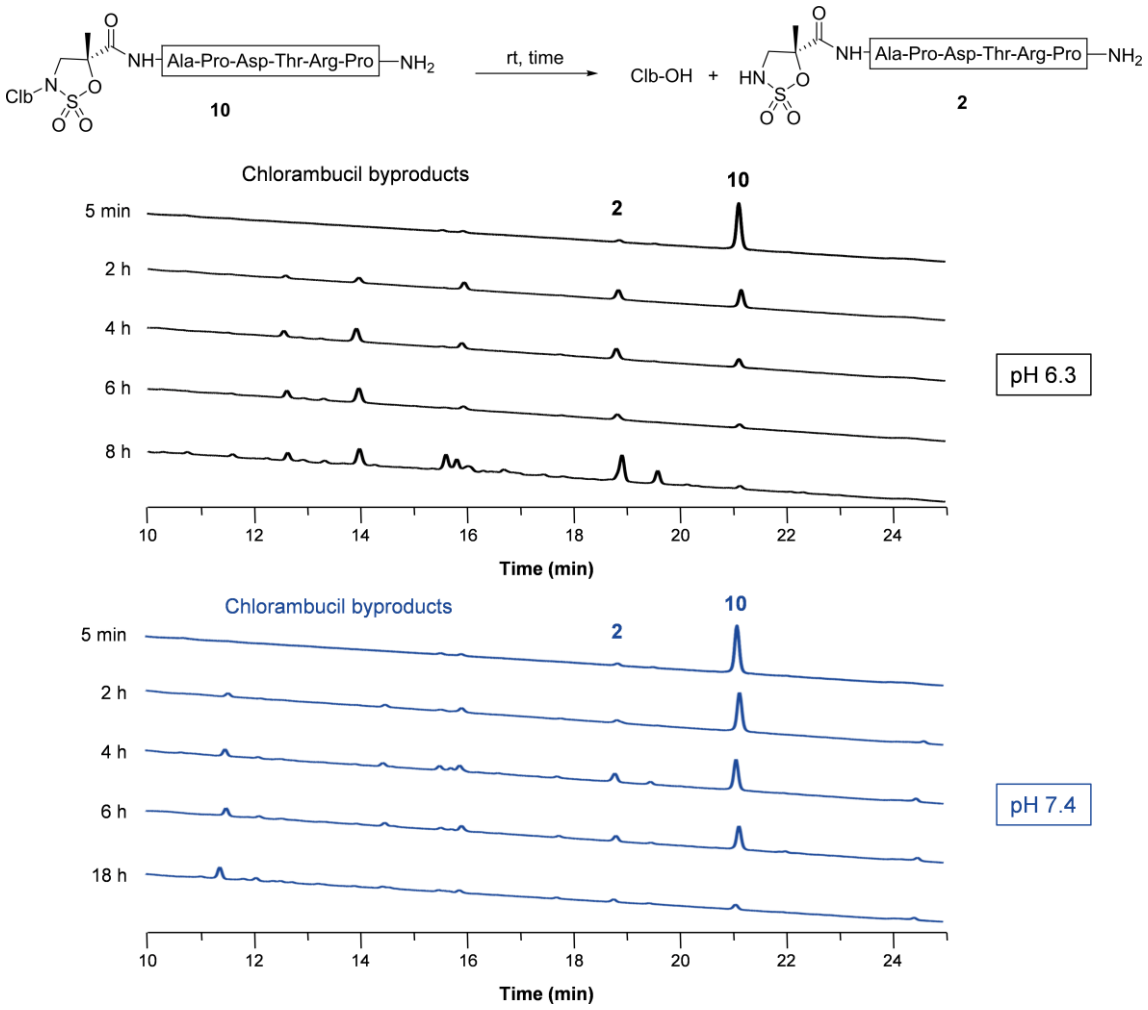


Fig. S5. Monitoring of the pH-controlled release of chlorambucil (Clb-OH) from peptide **10** in a PBS (pH 7.4 or 6.3) solution. HPLC analyses were performed using linear gradient of 5 % to 95% B solvent in 30 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

4. NMR spectra for intramolecular N→X acyl shift reactions

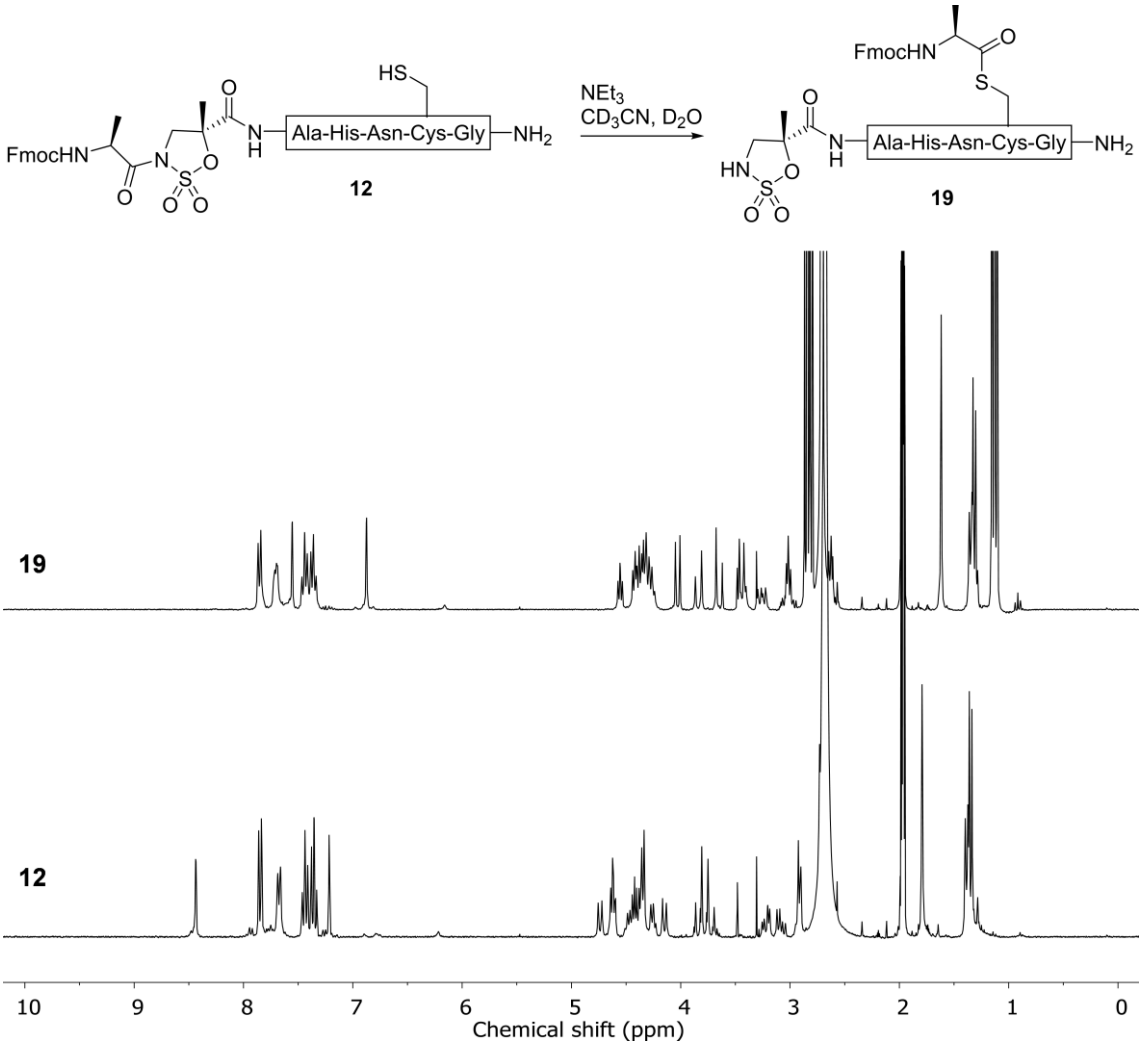


Fig. S6. ^{1}H NMR spectra ((300 MHz), 24:1 CD_3CN/D_2O) for intein-like sulfamidate-containing peptide **12** and the corresponding transacylated peptide **19** upon treatment with triethylamine.

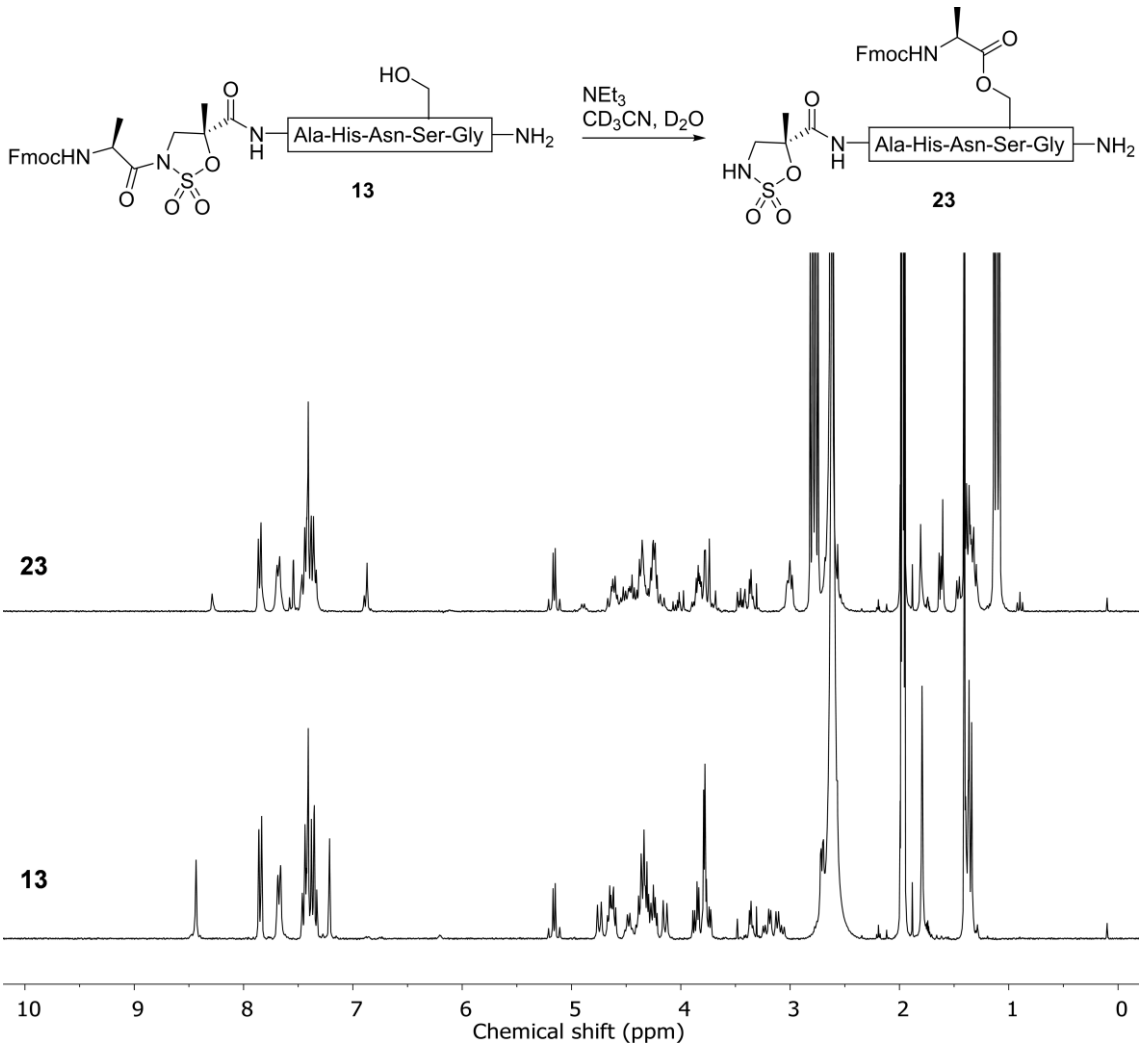


Fig. S7. ^{1}H NMR spectra ((300 MHz), 24:1 CD_3CN/D_2O) for intein-like sulfamidate-containing peptide **13** and the corresponding transacylated peptide **23** upon treatment with triethylamine.

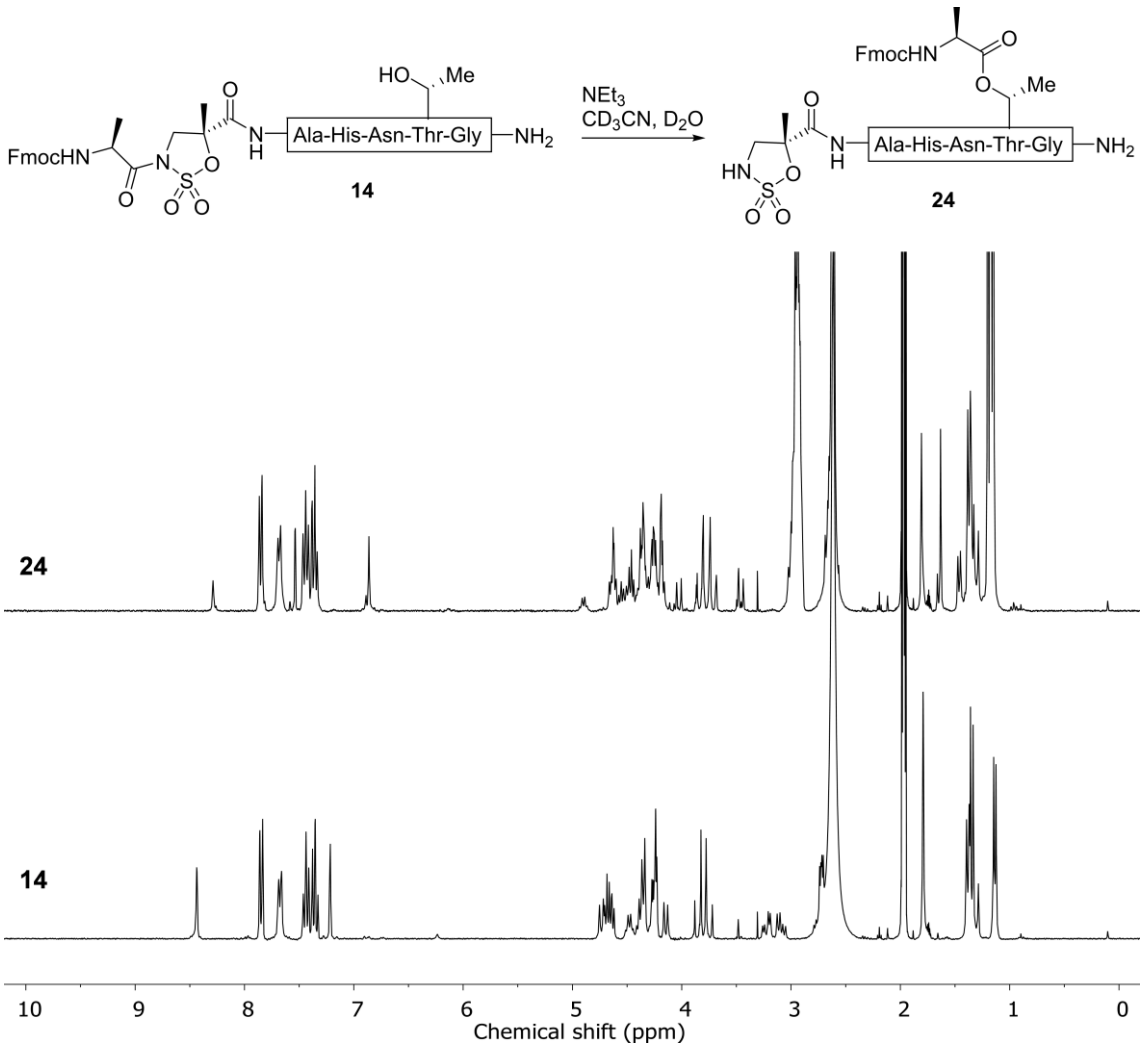


Fig. S8. ^{1}H NMR spectra ((300 MHz), 24:1 CD_3CN/D_2O) for intein-like sulfamidate-containing peptide **14** and the corresponding transacylated peptide **24** upon treatment with triethylamine.

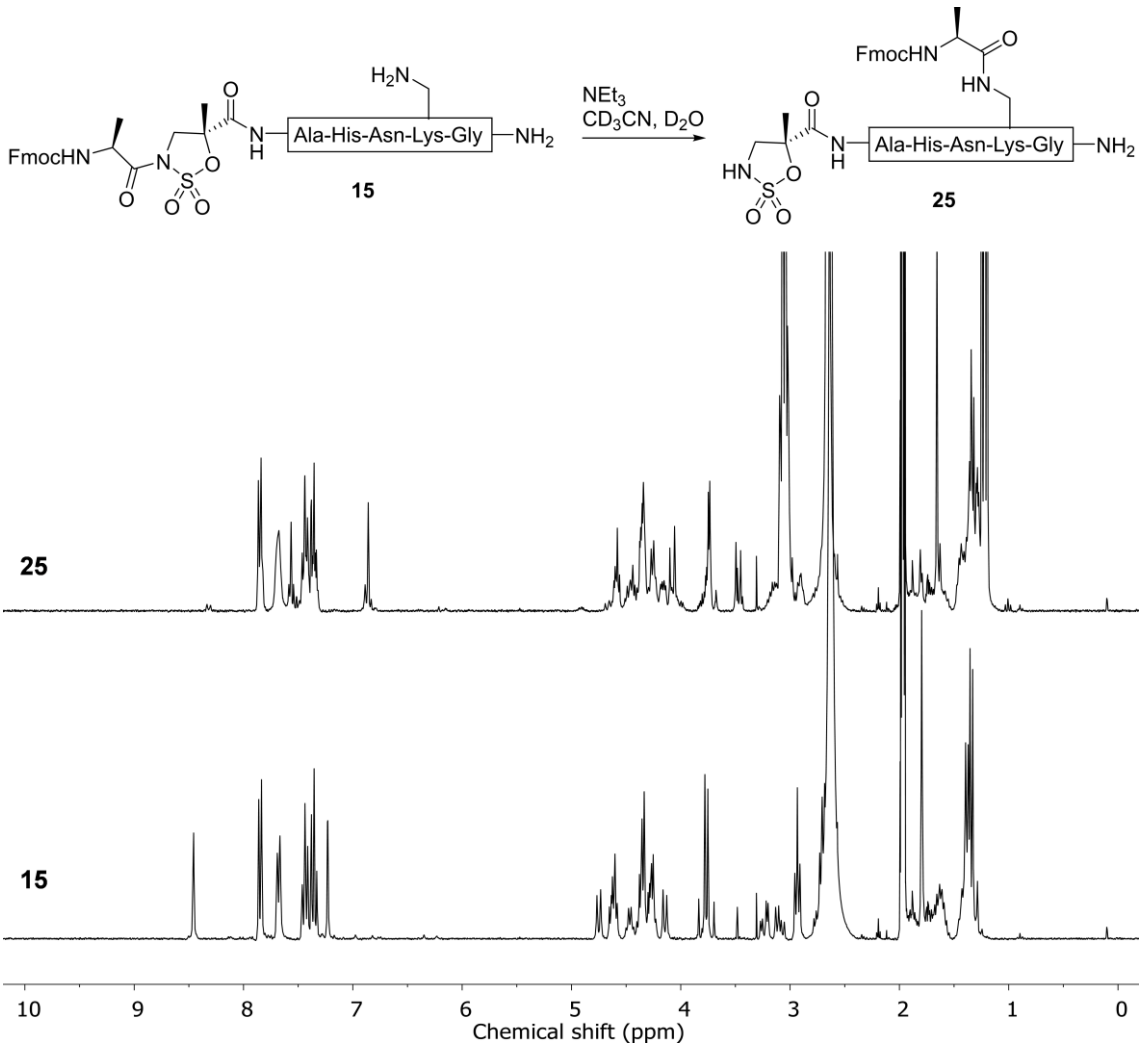


Fig. S9. ^{1}H NMR spectra ((300 MHz), 24:1 CD_3CN/D_2O) for intein-like sulfamidate-containing peptide **15** and the corresponding transacylated peptide **25** upon treatment with triethylamine.

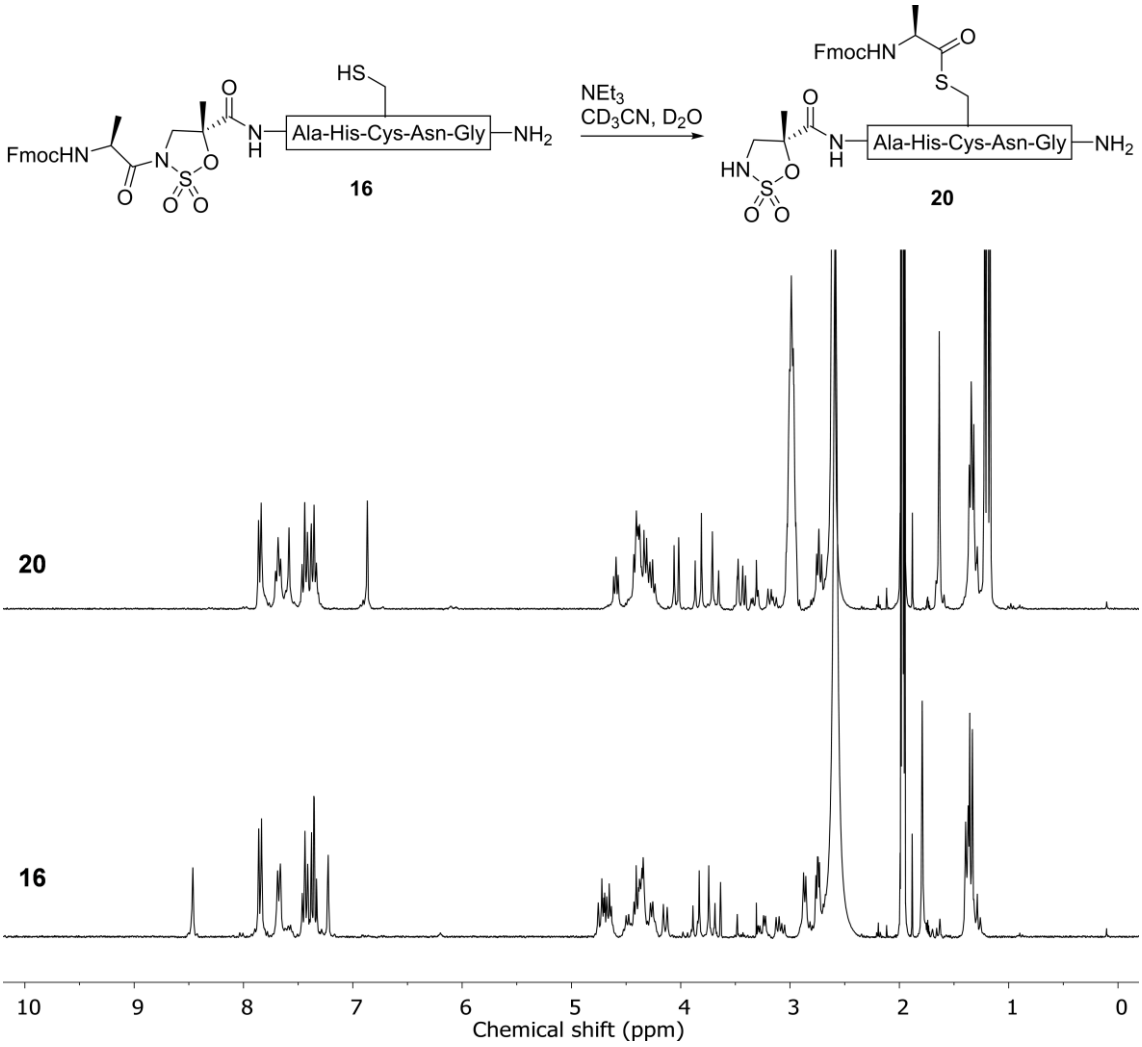


Fig. S10. ^{1}H NMR spectra ((300 MHz), 24:1 CD_3CN/D_2O) for intein-like sulfamidate-containing peptide **16** and the corresponding transacylated peptide **20** upon treatment with triethylamine.

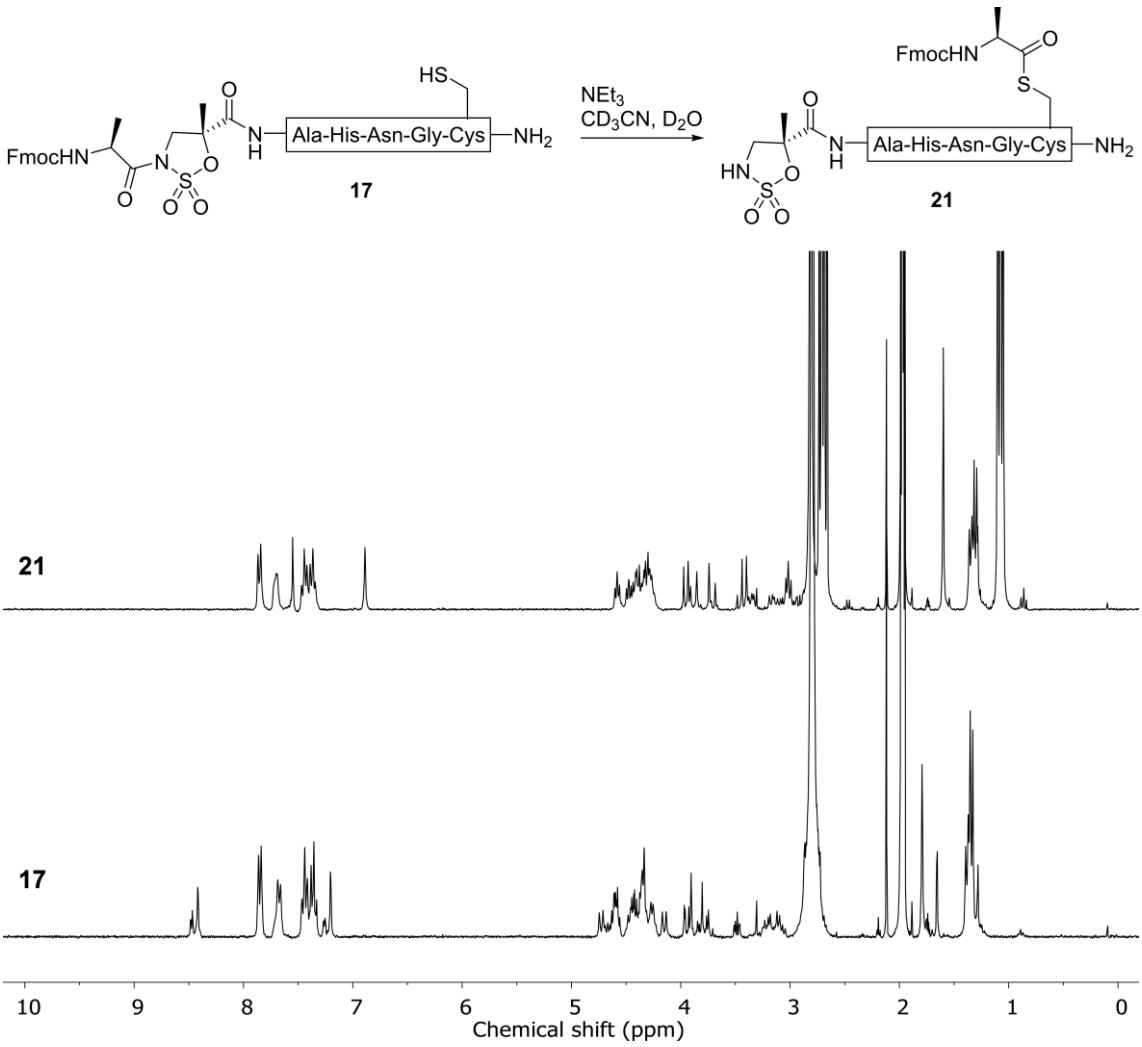


Fig. S11. ^{1}H NMR spectra ((300 MHz), 24:1 CD_3CN/D_2O) for intein-like sulfamidate-containing peptide **17** and the corresponding transacylated peptide **21** upon treatment with triethylamine.

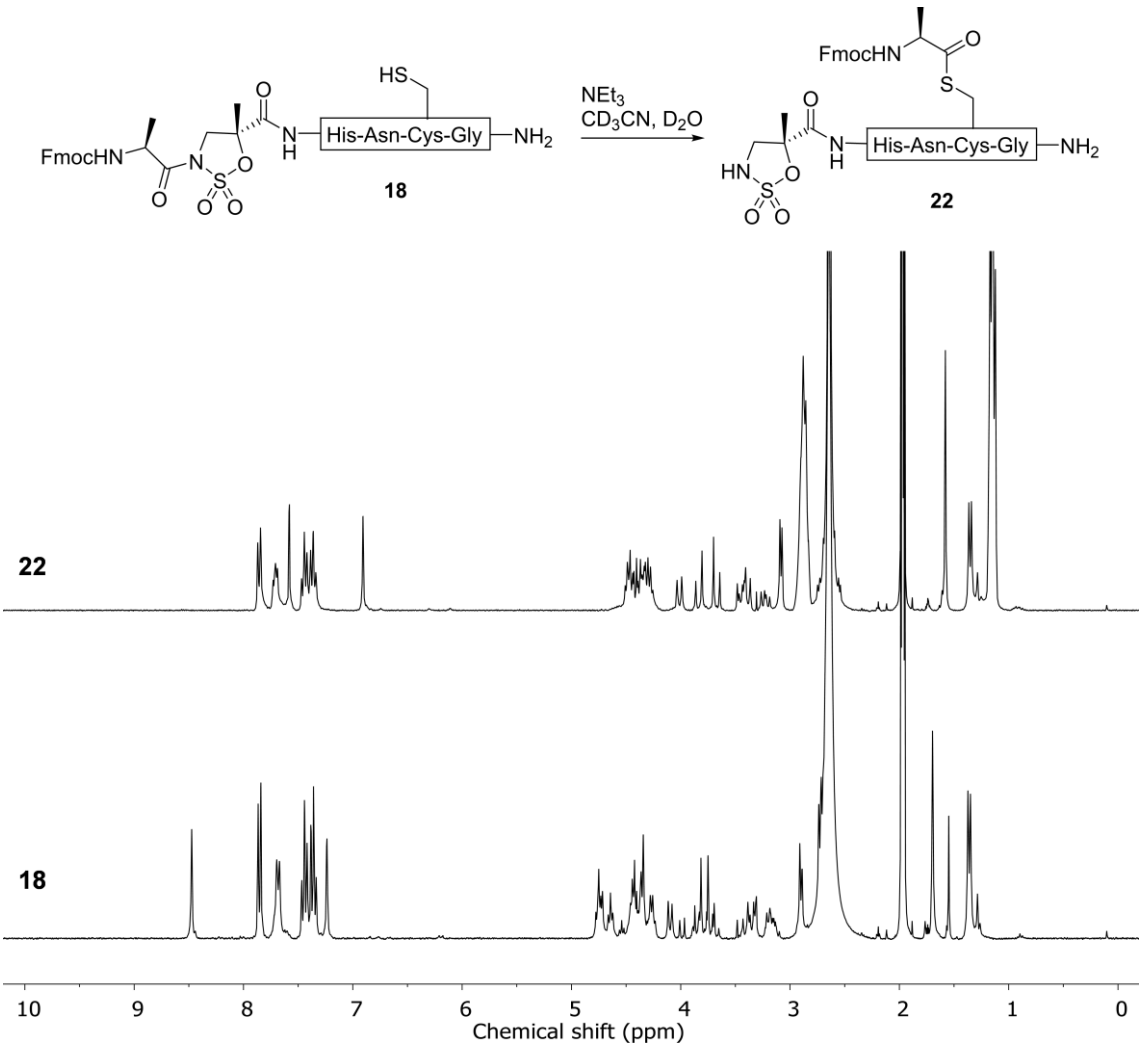


Fig. S12. ^{1}H NMR spectra ((300 MHz), 24:1 CD_3CN/D_2O) for intein-like sulfamidate-containing peptide **18** and the corresponding transacylated peptide **22** upon treatment with triethylamine.

5. HPLC chromatograms for intramolecular N→X acyl shift reactions

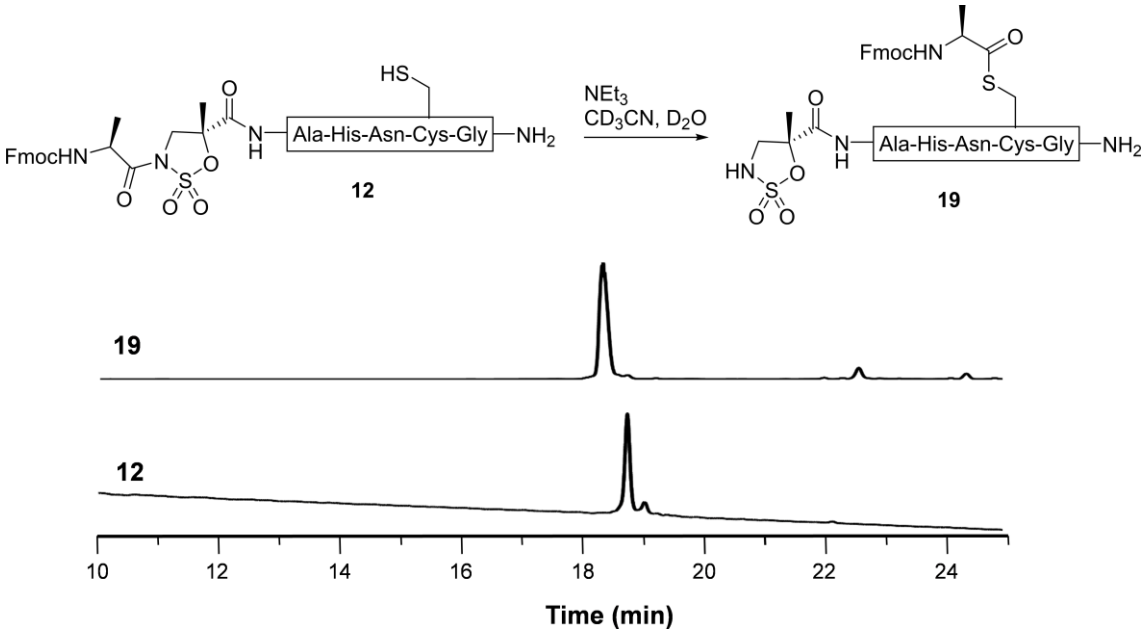


Fig. S13. Analytical HPLC chromatograms for intein-like sulfamidate-containing peptide **12** and the corresponding transacylated peptide **19** upon treatment with triethylamine. HPLC analyses were performed using linear gradient of 1% to 71% B solvent in 35 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

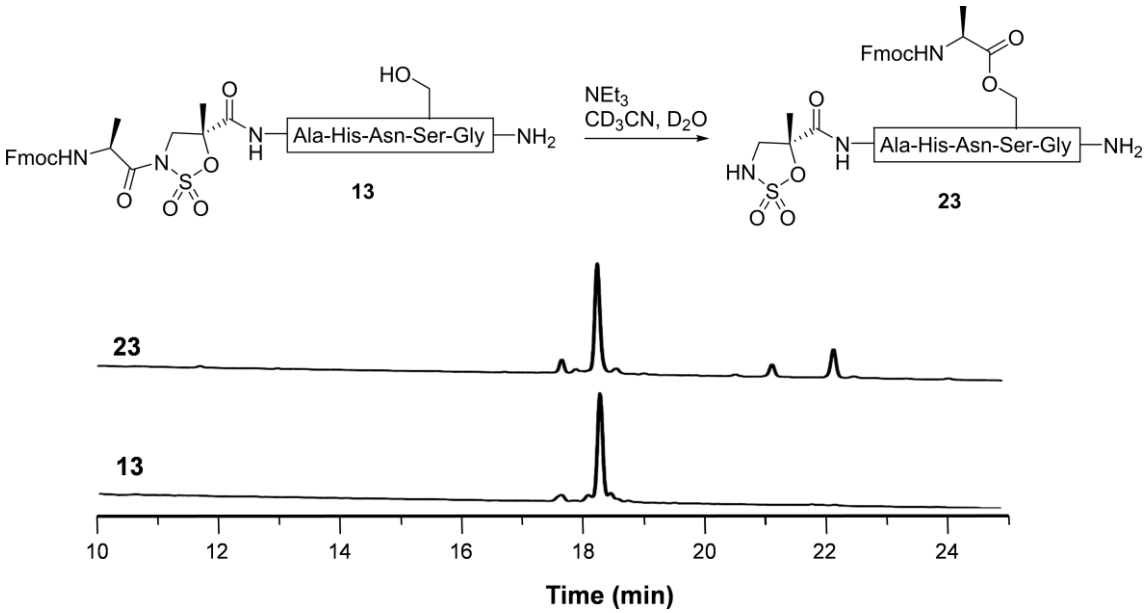


Fig. S14. Analytical HPLC chromatograms for intein-like sulfamidate-containing peptide **13** and the corresponding transacylated peptide **23** upon treatment with triethylamine. HPLC analyses were performed using linear gradient of 1% to 71% B solvent in 35 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

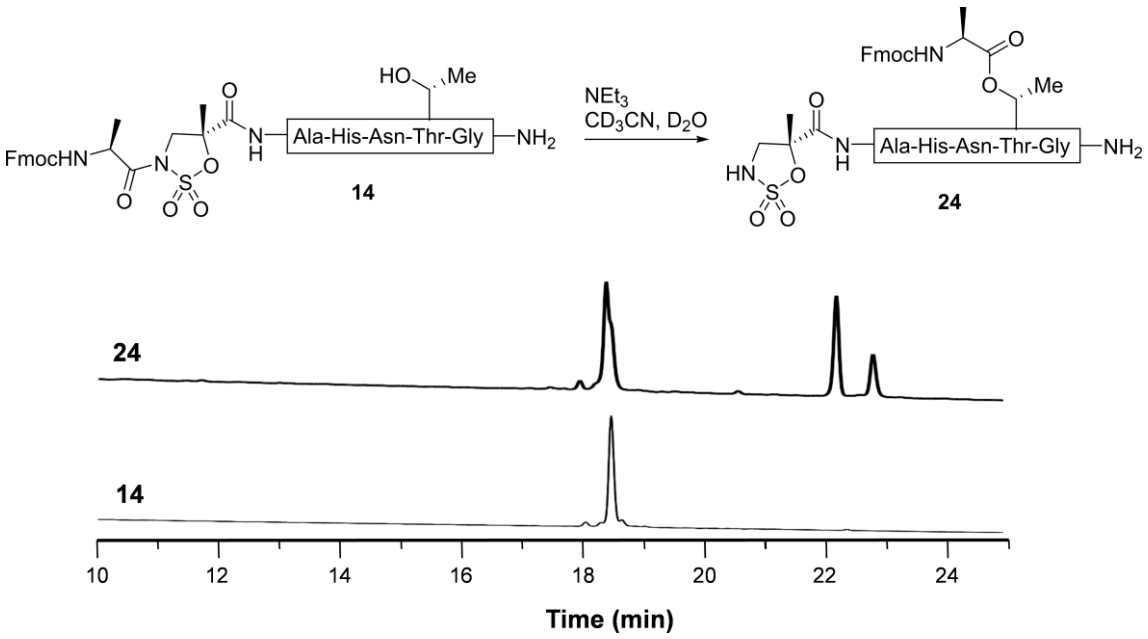


Fig. S15. Analytical HPLC chromatograms for intein-like sulfamidate-containing peptide **14** and the corresponding transacylated peptide **24** upon treatment with triethylamine. HPLC analyses were performed using linear gradient of 1% to 71% B solvent in 35 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

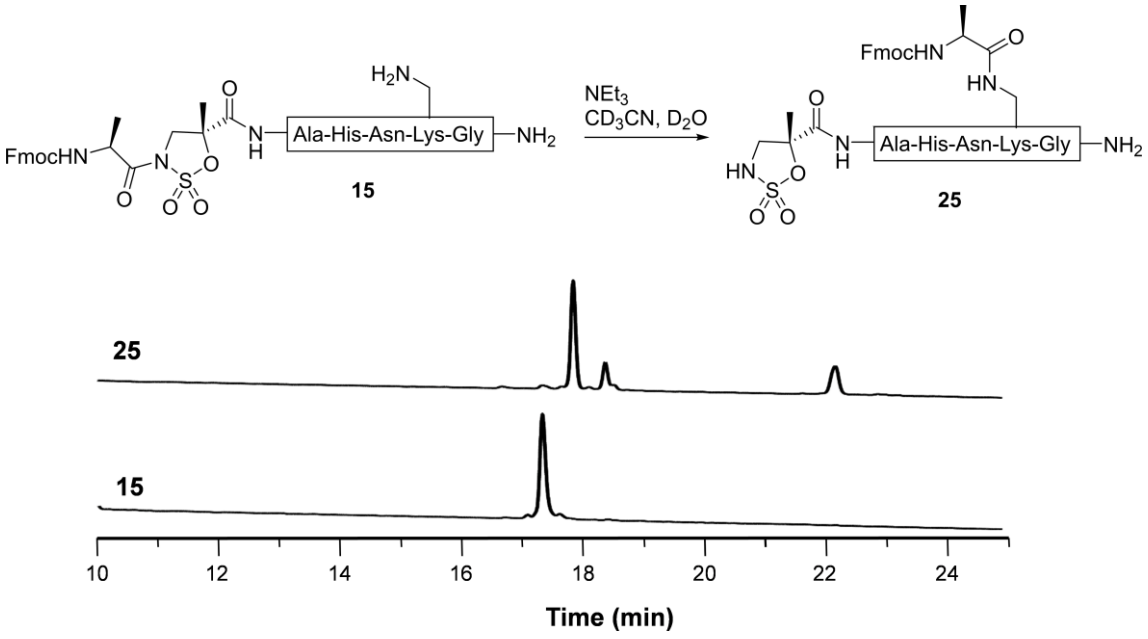


Fig. S16. Analytical HPLC chromatograms for intein-like sulfamidate-containing peptide **15** and the corresponding transacylated peptide **25** upon treatment with triethylamine. HPLC analyses were performed using linear gradient of 1% to 71% B solvent in 35 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

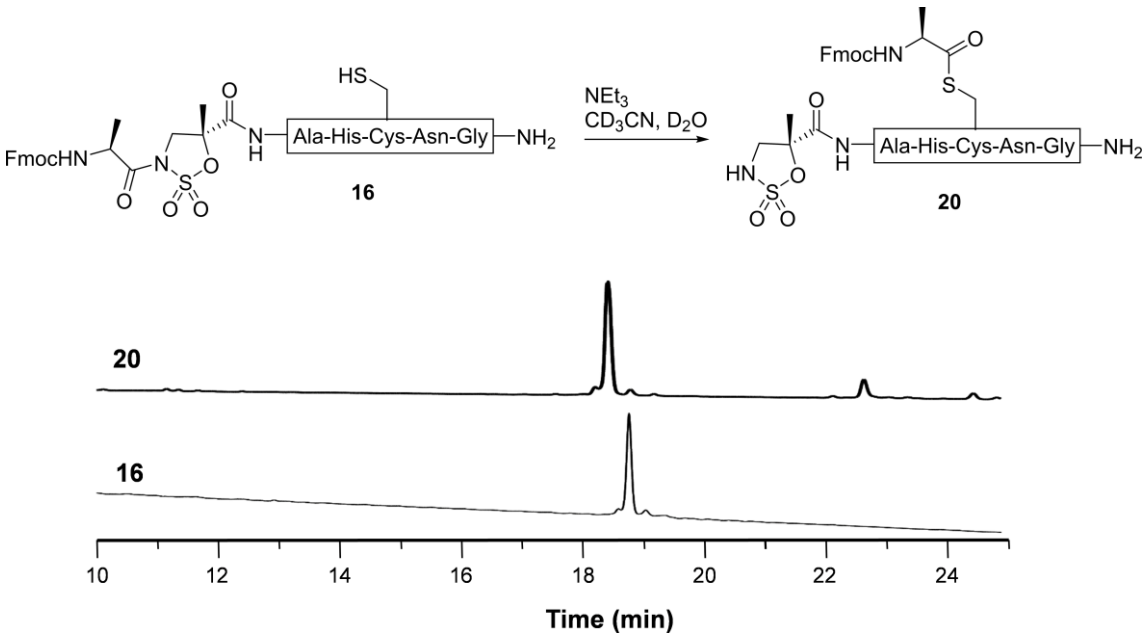


Fig. S17. Analytical HPLC chromatograms for intein-like sulfamidate-containing peptide **16** and the corresponding transacylated peptide **20** upon treatment with triethylamine. HPLC analyses were performed using linear gradient of 1% to 71% B solvent in 35 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

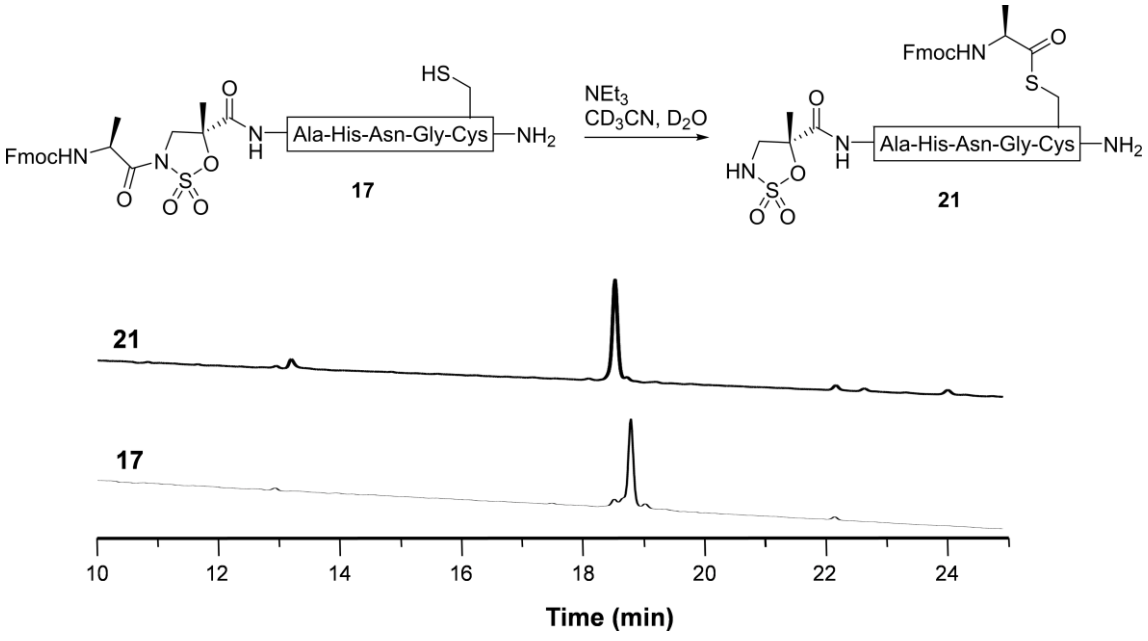


Fig. S18. Analytical HPLC chromatograms for intein-like sulfamidate-containing peptide **17** and the corresponding transacylated peptide **21** upon treatment with triethylamine. HPLC analyses were performed using linear gradient of 1% to 71% B solvent in 35 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

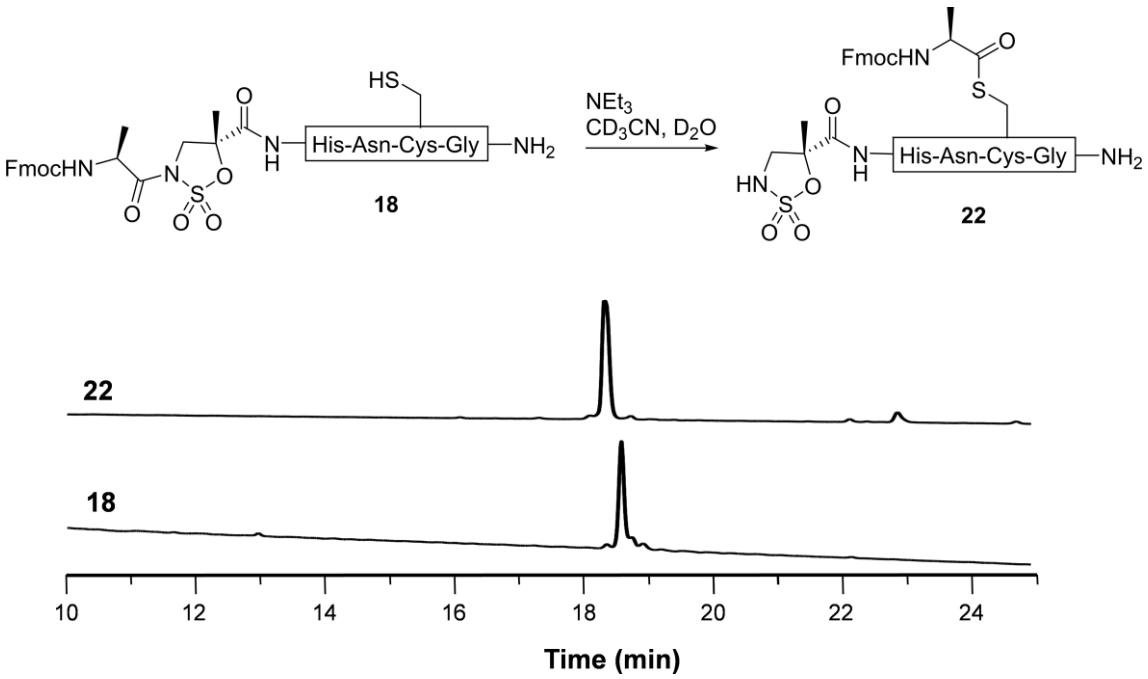


Fig. S19. Analytical HPLC chromatograms for intein-like sulfamidate-containing peptide **18** and the corresponding transacylated peptide **22** upon treatment with triethylamine. HPLC analyses were performed using linear gradient of 1% to 71% B solvent in 35 min (1 mL/min). Solvent A: H_2O containing 0.1% TFA (v/v); solvent B: acetonitrile.

6. NEM assay on peptides 19 and 26

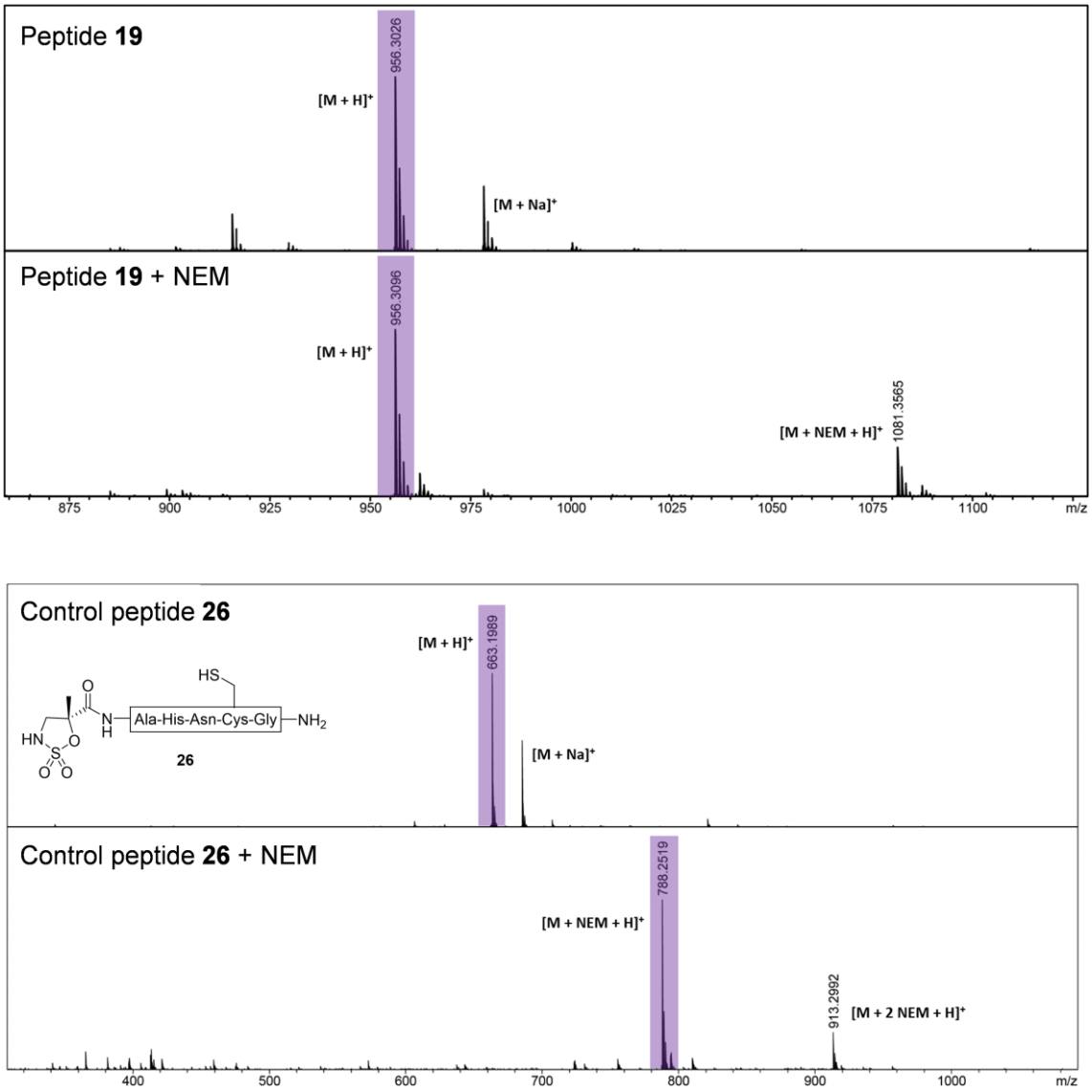
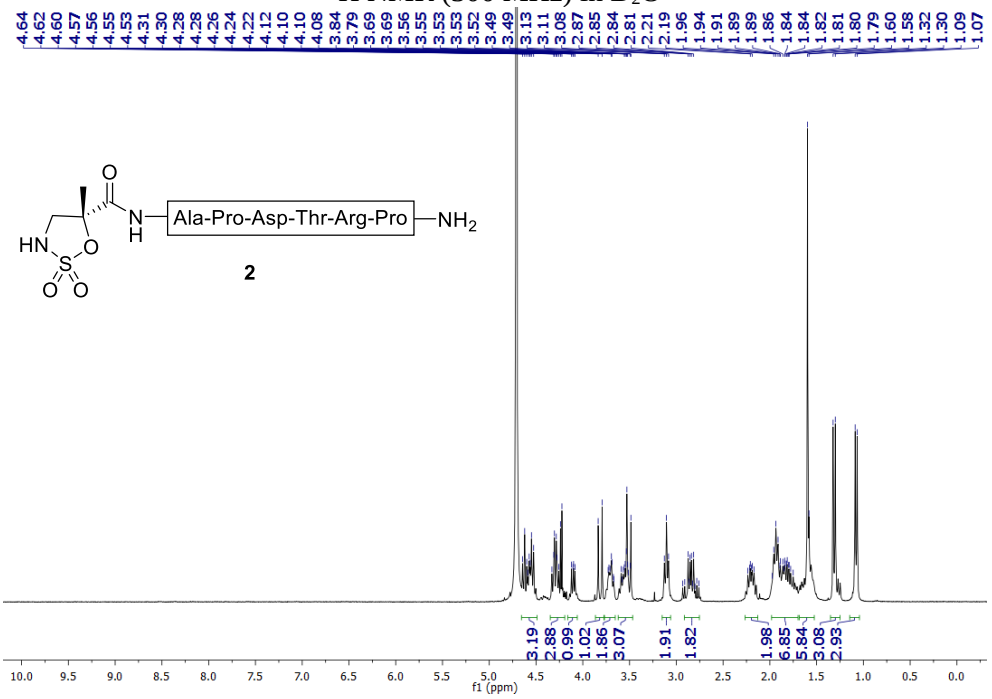


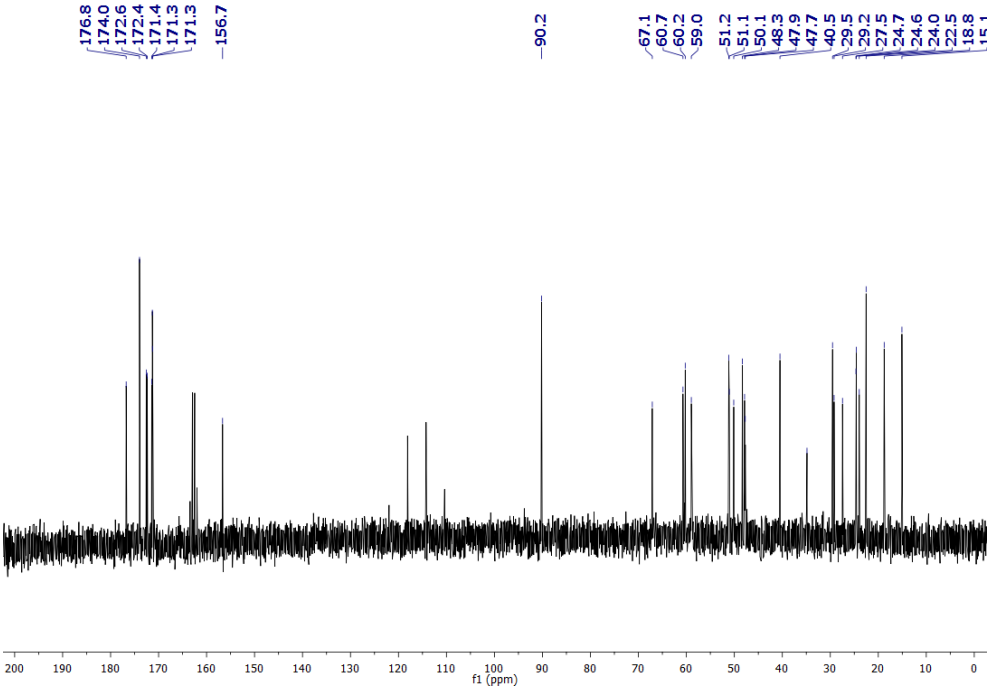
Fig. S20. MALDI spectra of peptides **19** and **26** as well as the corresponding products obtained upon treatment with an excess of *N*-ethylmaleimide (NEM). Peptide **19** did not react with NEM, suggesting that the cysteine residue was already blocked. The minor peak corresponds to the addition of NEM to the histidine residue. On the other hand, peptide **26** which bears a free cysteine residue reacted completely with NEM. The minor peak correspond to the addition of a second equivalent of NEM to the histidine residue.

7. Copies of the NMR spectra for all new compounds

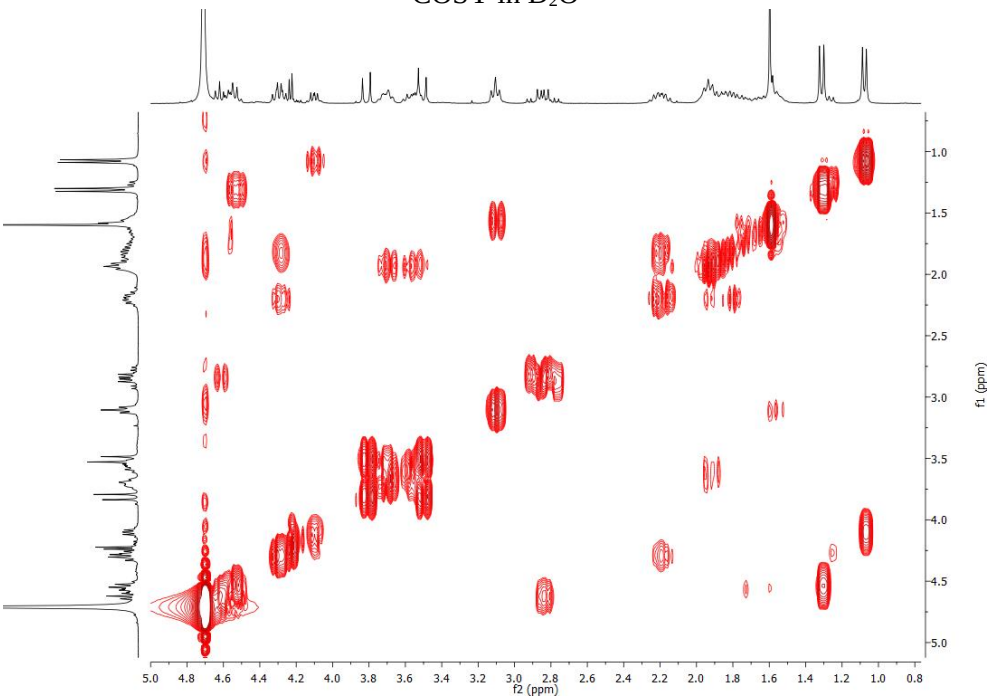
^{1}H NMR (300 MHz) in D_2O



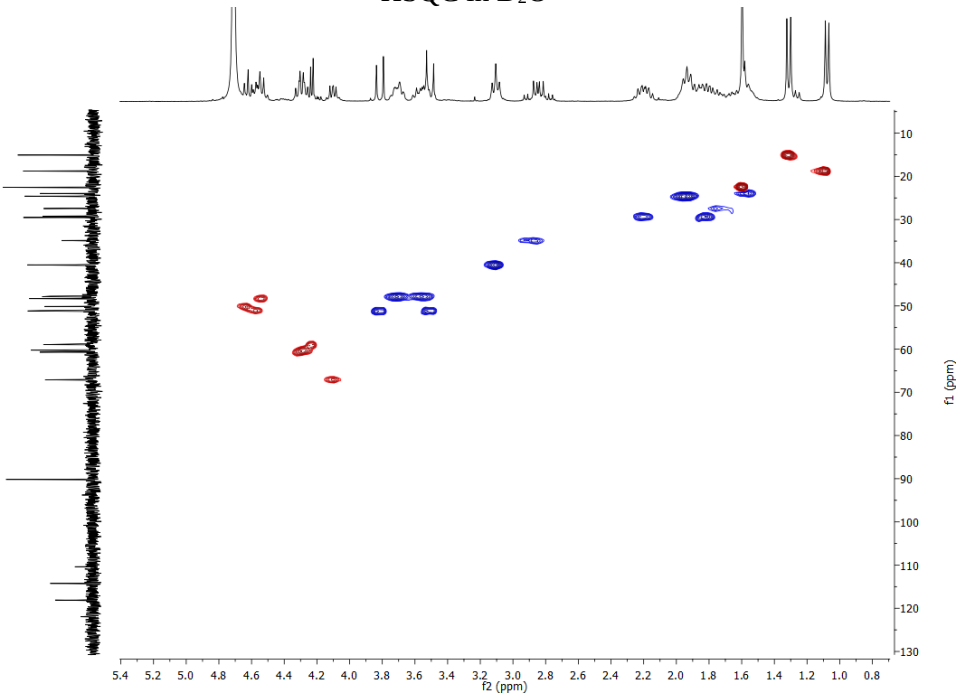
^{13}C NMR (75 MHz) in D_2O



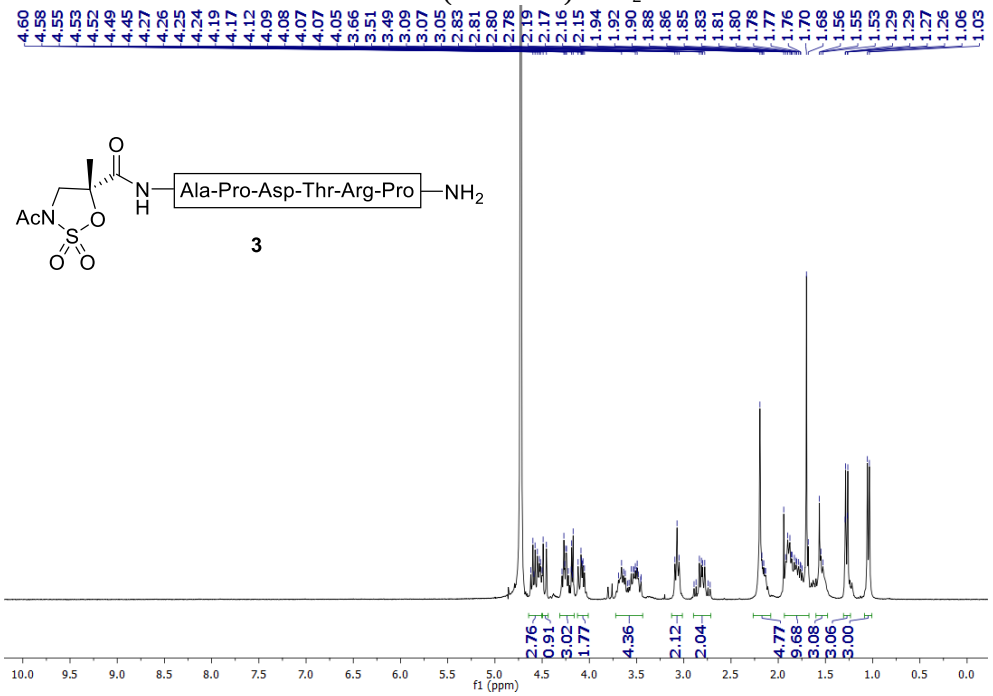
COSY in D_2O



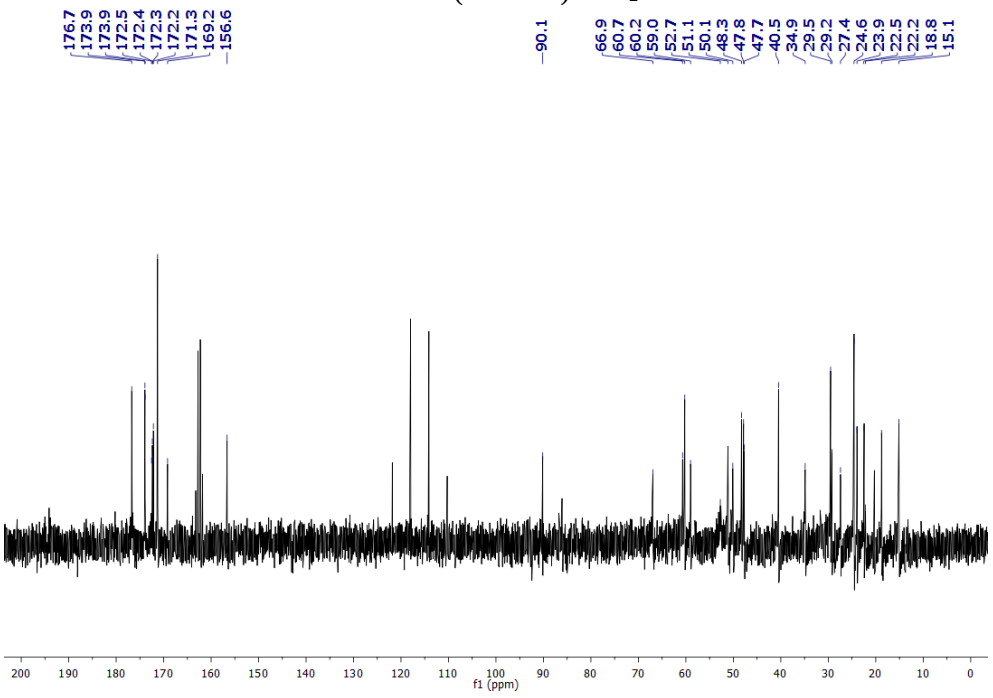
HSQC in D_2O



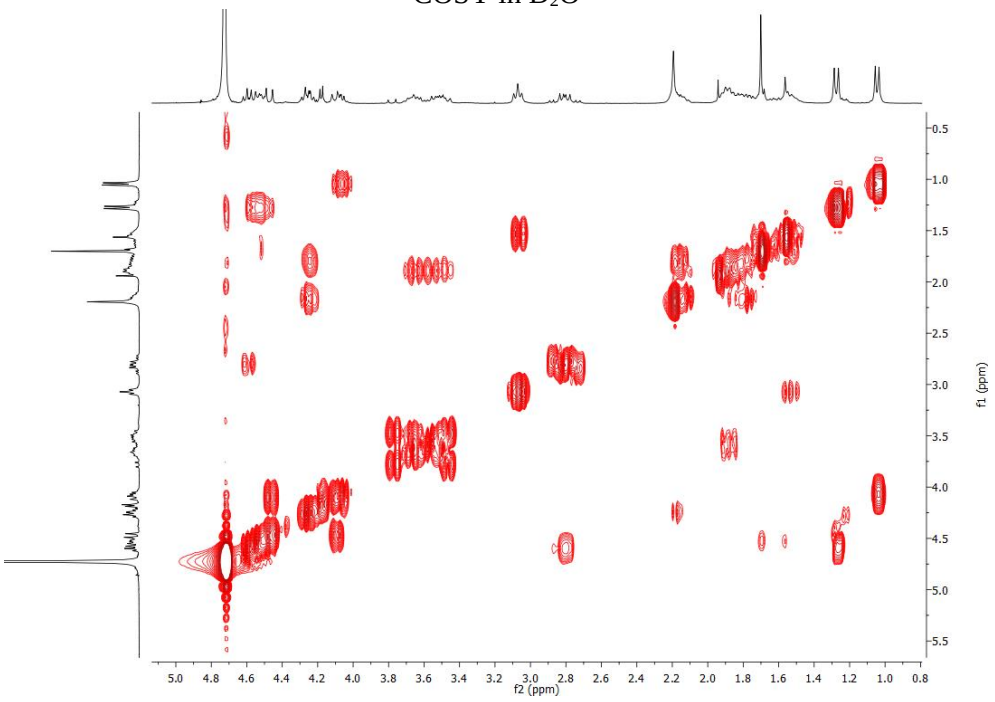
^{1}H NMR (300 MHz) in D_2O



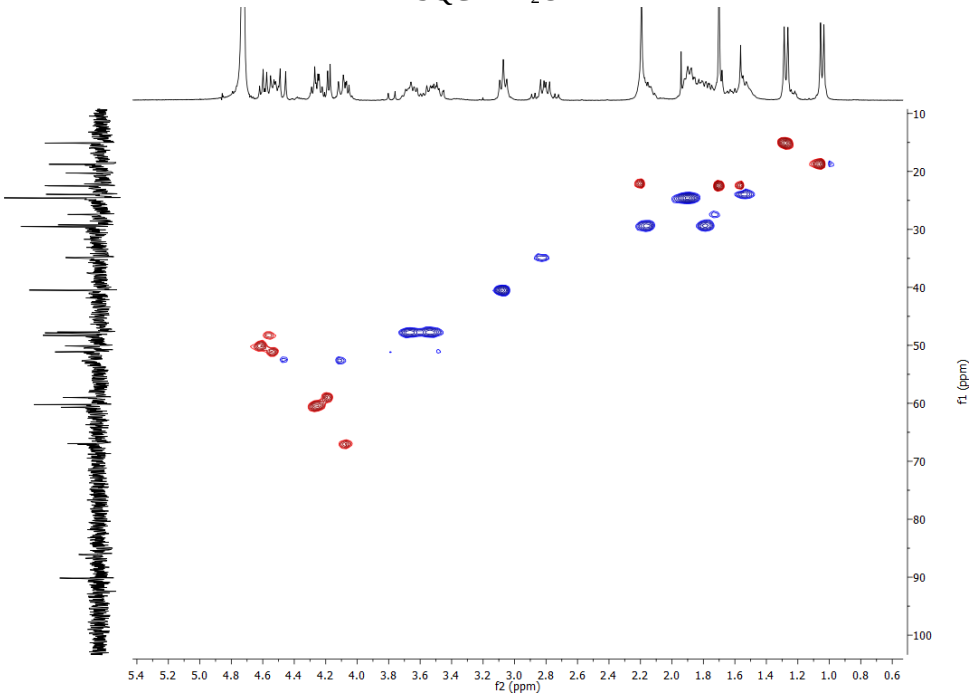
^{13}C NMR (75 MHz) in D_2O



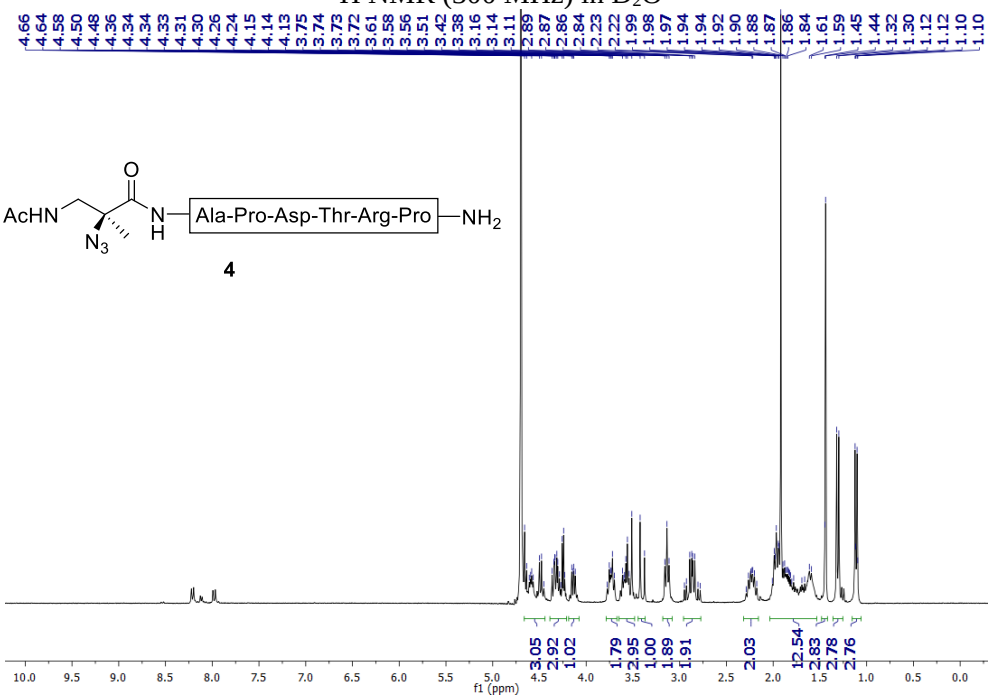
COSY in D_2O



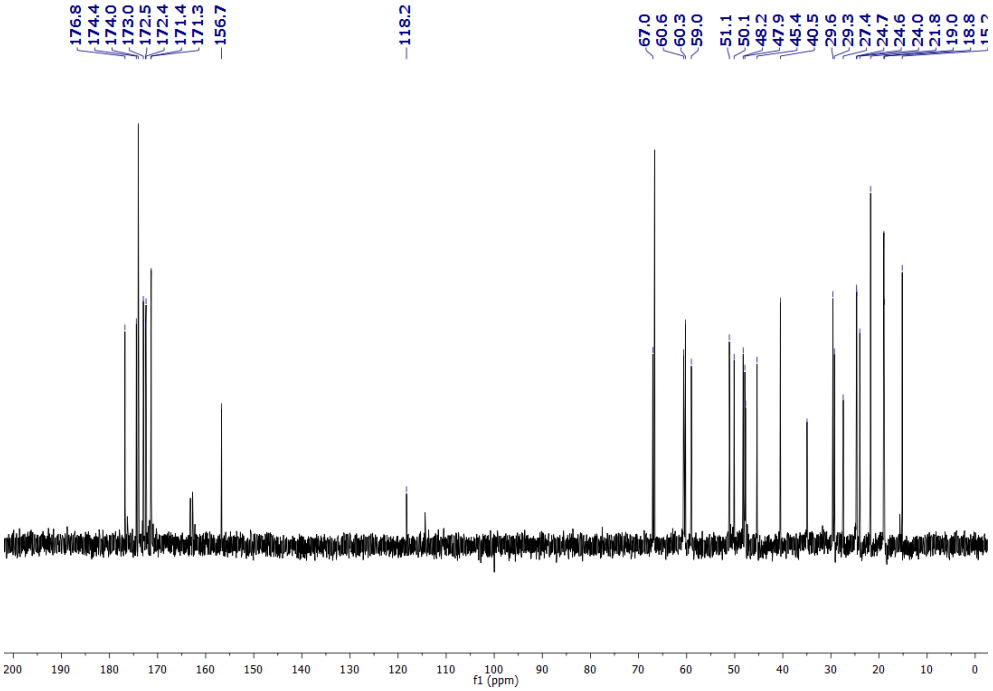
HSQC in D_2O



^{1}H NMR (300 MHz) in D_2O



^{13}C NMR (75 MHz) in D_2O



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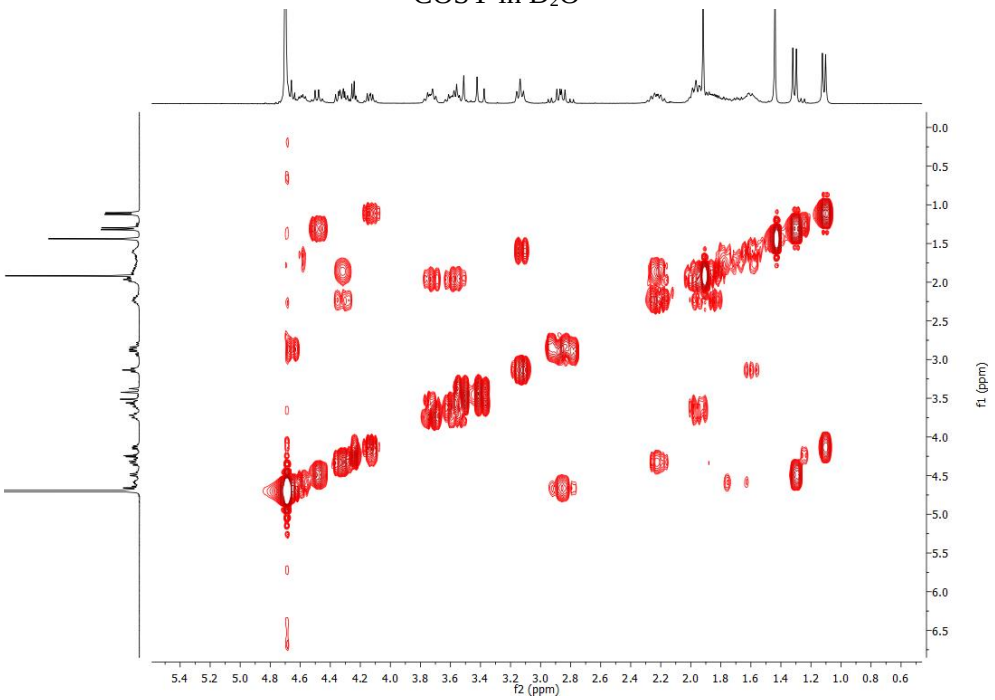
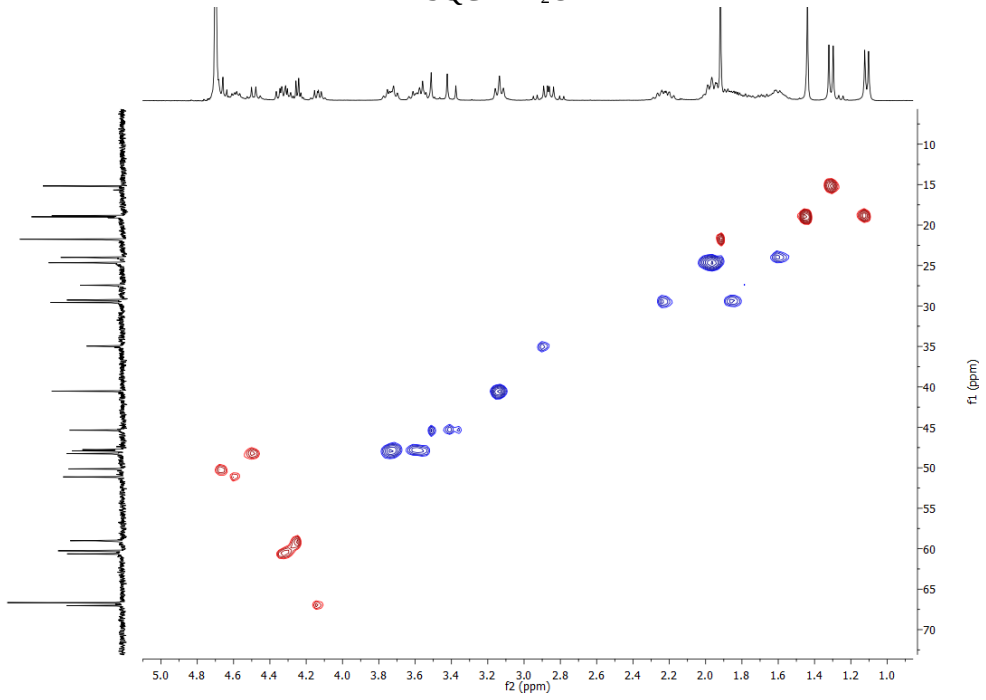
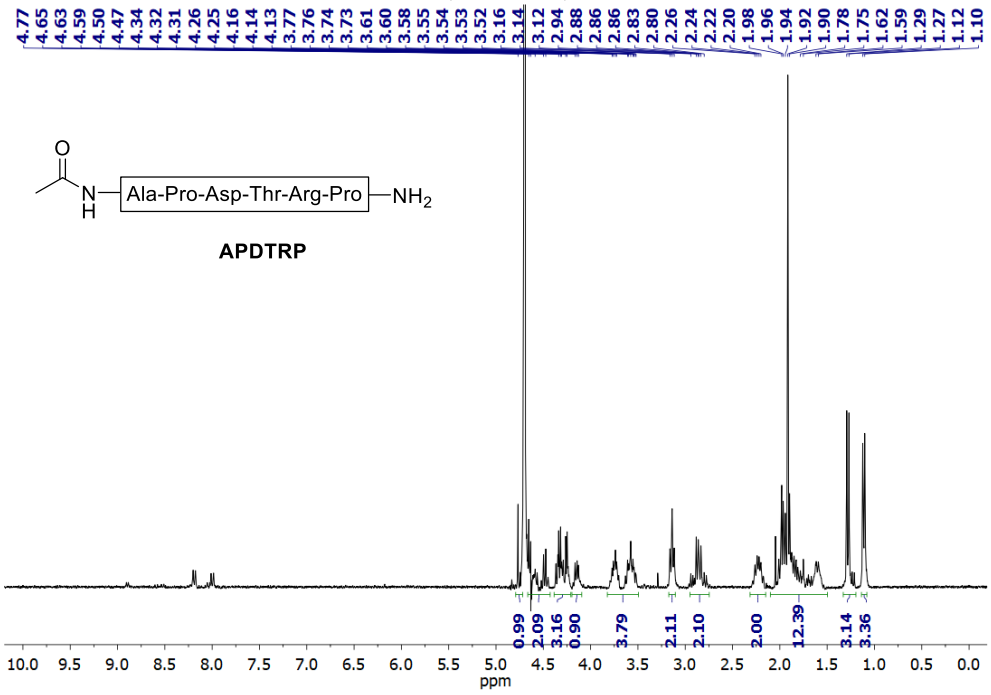


HSQC in D_2O



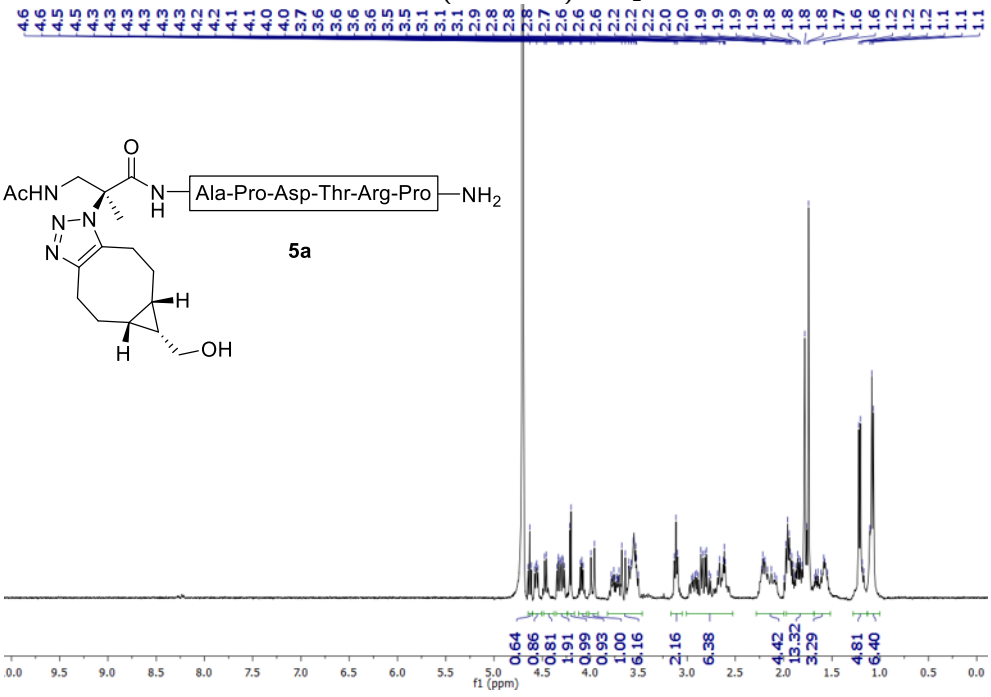
Compound **APDTRP**

^{1}H NMR (300 MHz) in D_2O



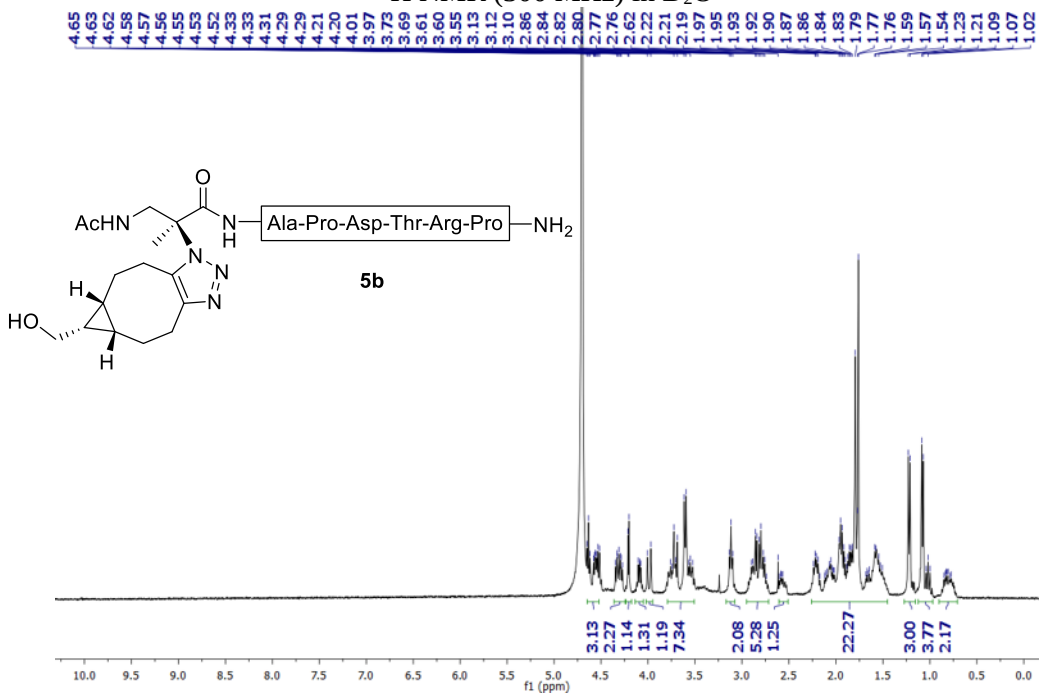
Compound **5a**

^{1}H NMR (300 MHz) in D_2O



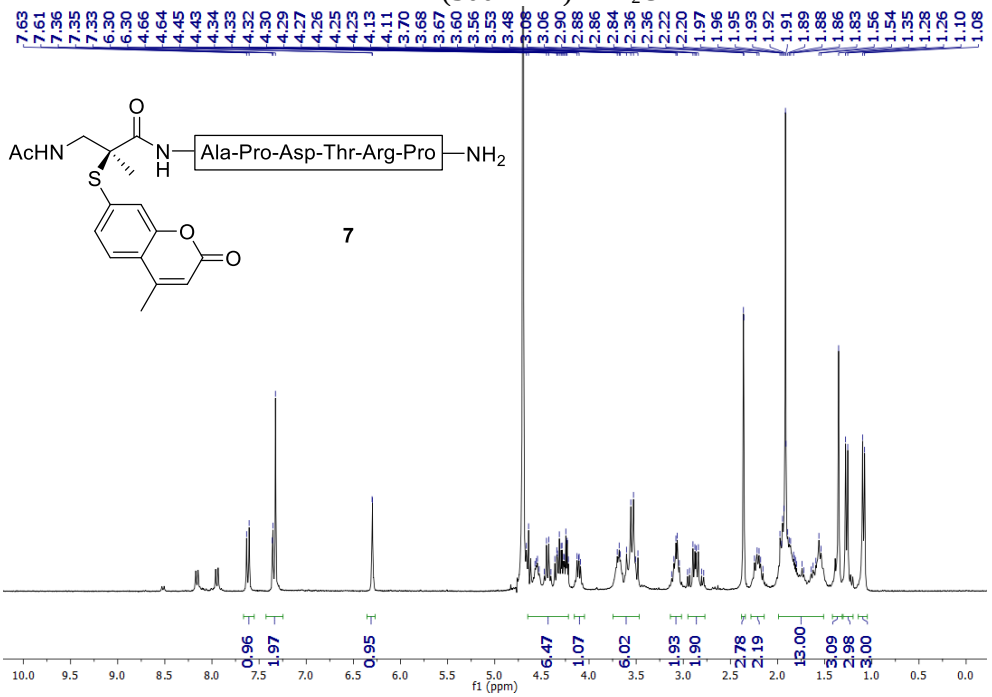
Compound **5b**

^{1}H NMR (300 MHz) in D_2O

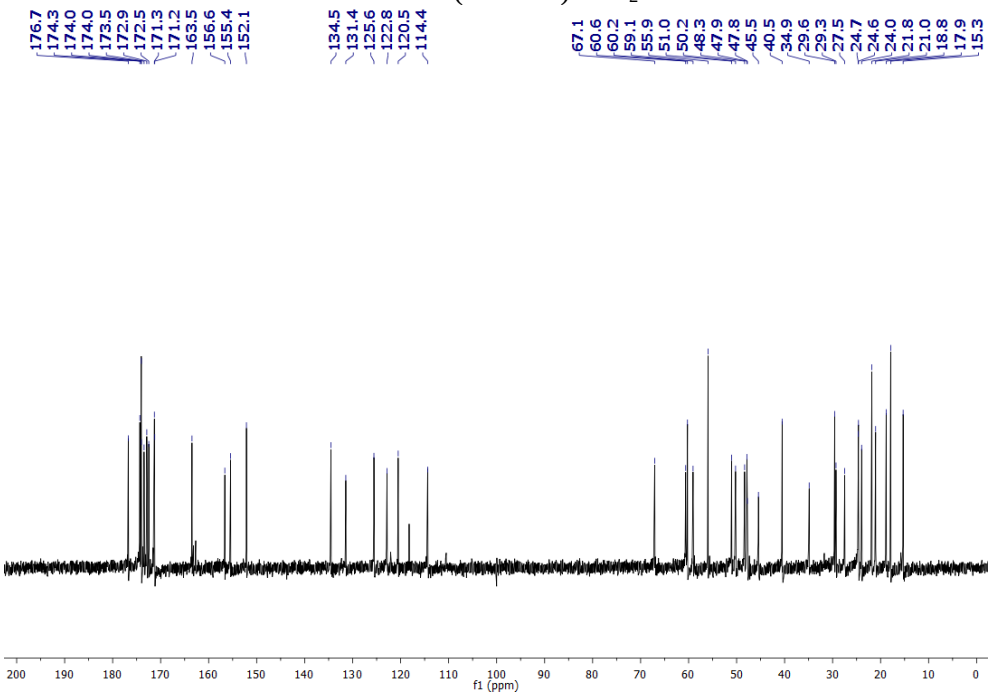


Compound **7**

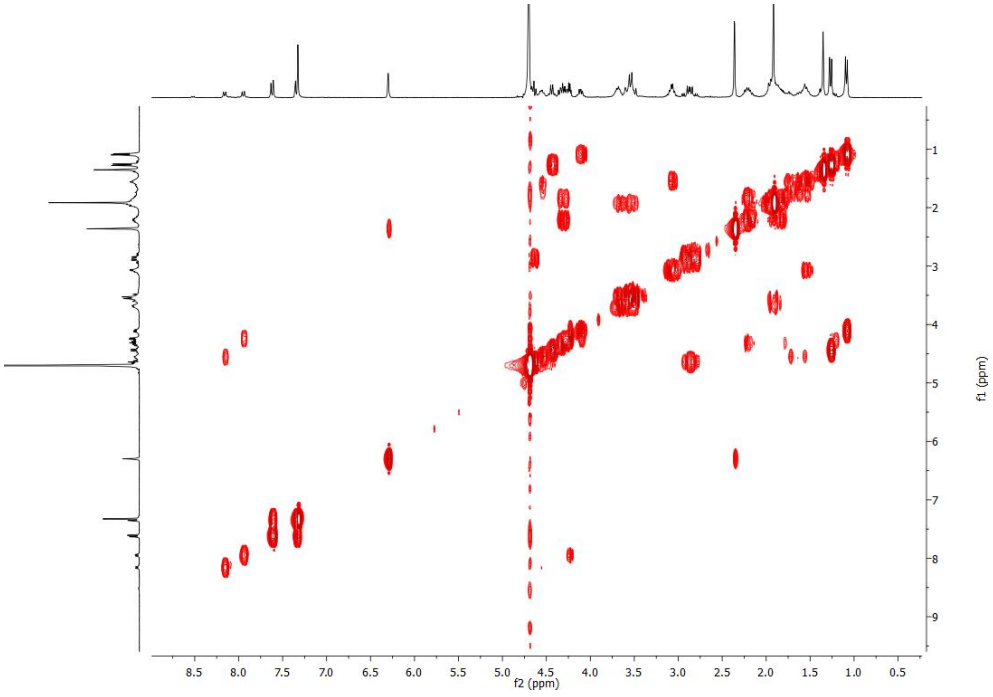
^{1}H NMR (300 MHz) in D_2O



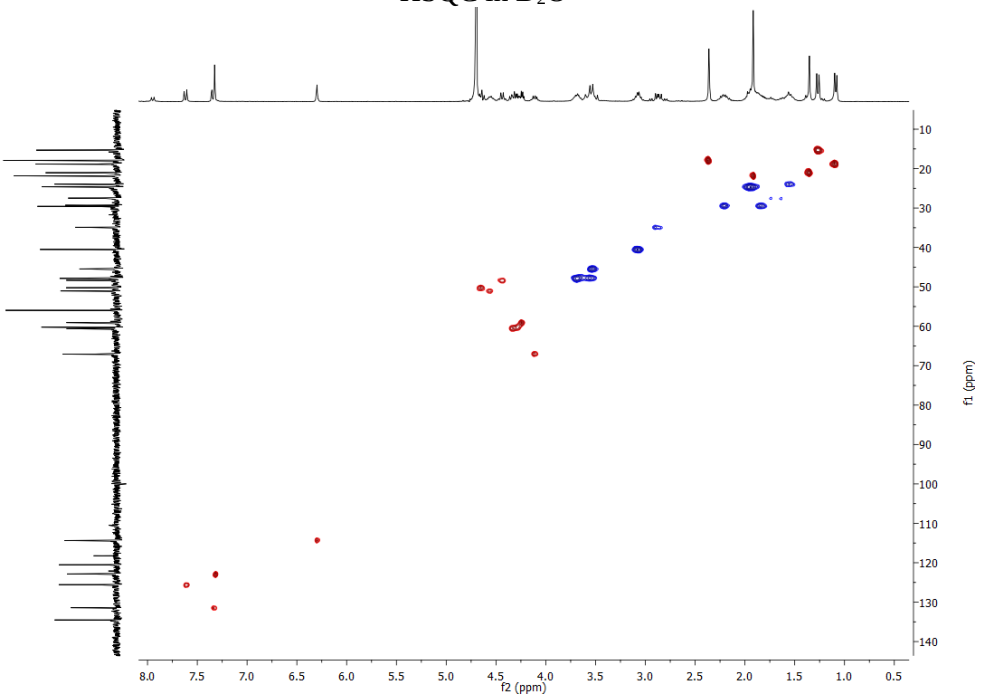
^{13}C NMR (75 MHz) in D_2O



COSY in D_2O

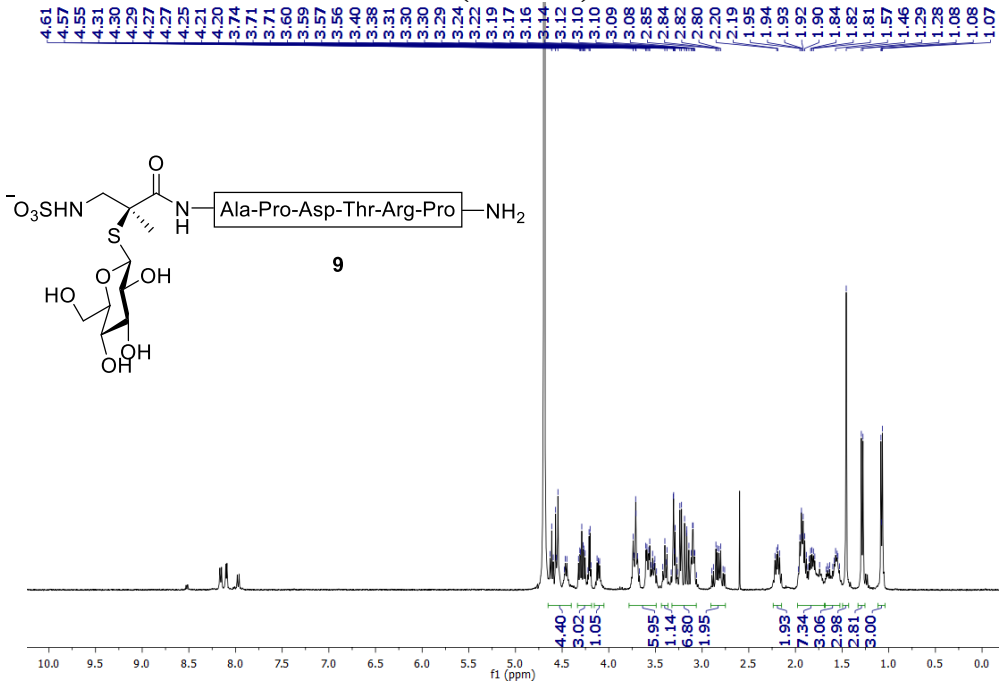


HSQC in D_2O

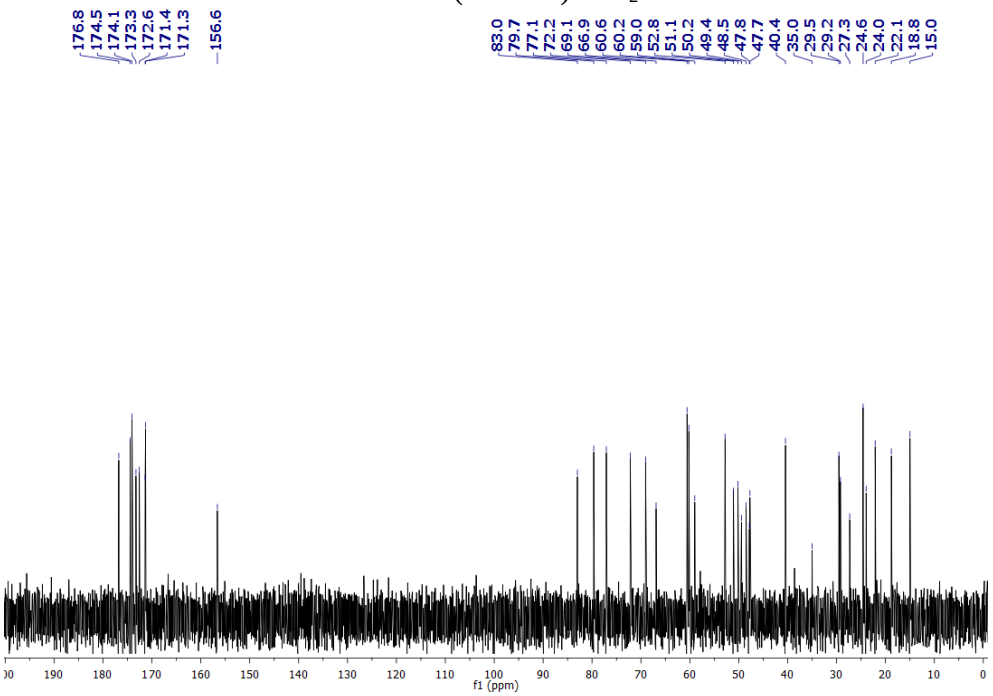


Compound **9**

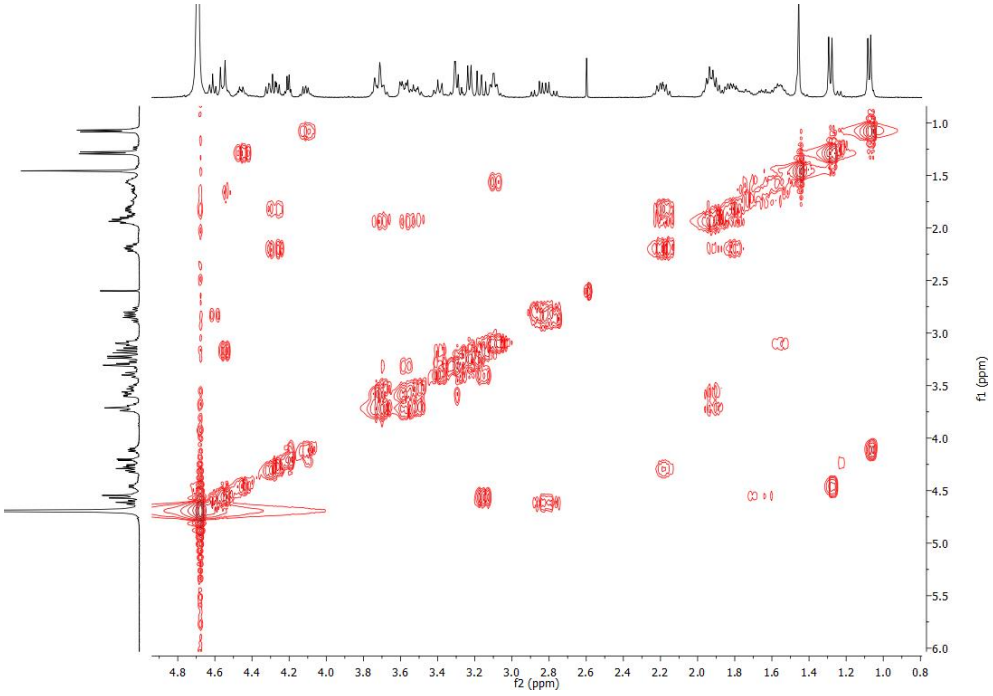
^1H NMR (400 MHz) in D_2O



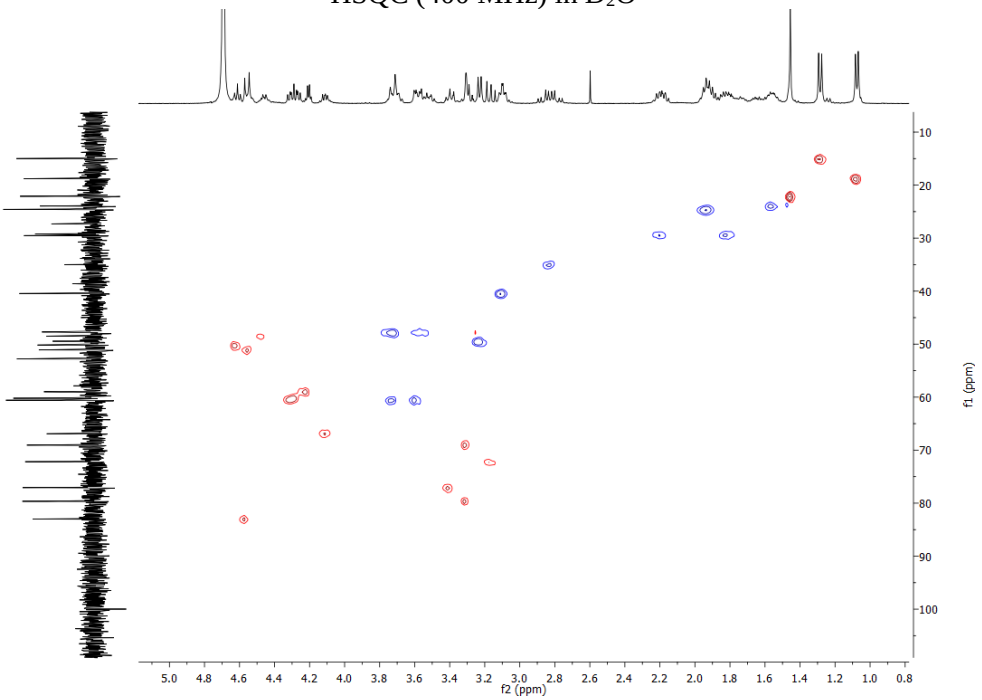
^{13}C NMR (75 MHz) in D_2O



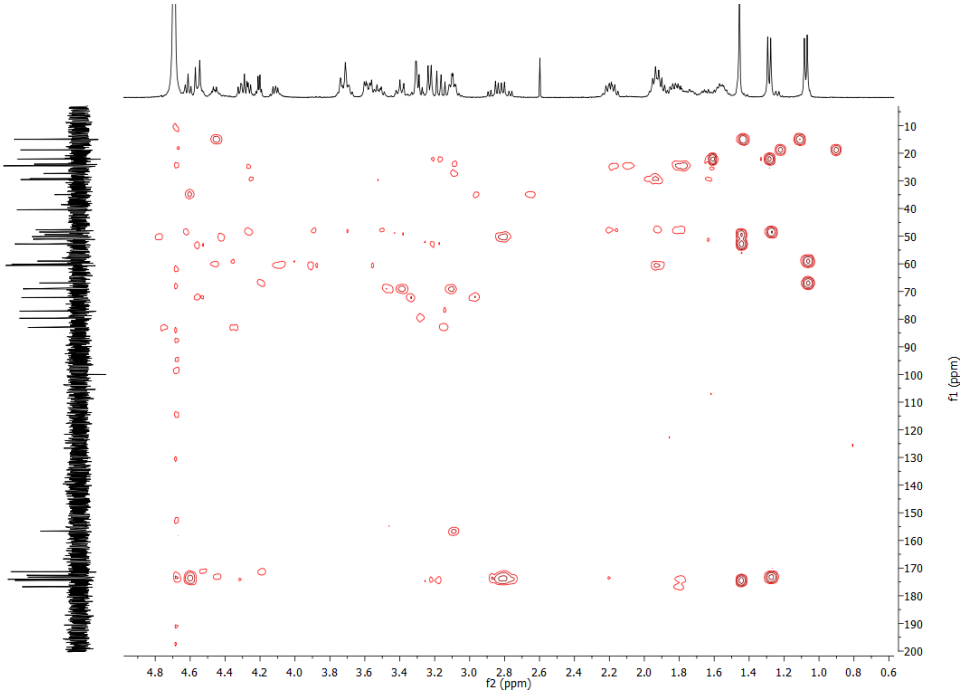
COSY in D_2O



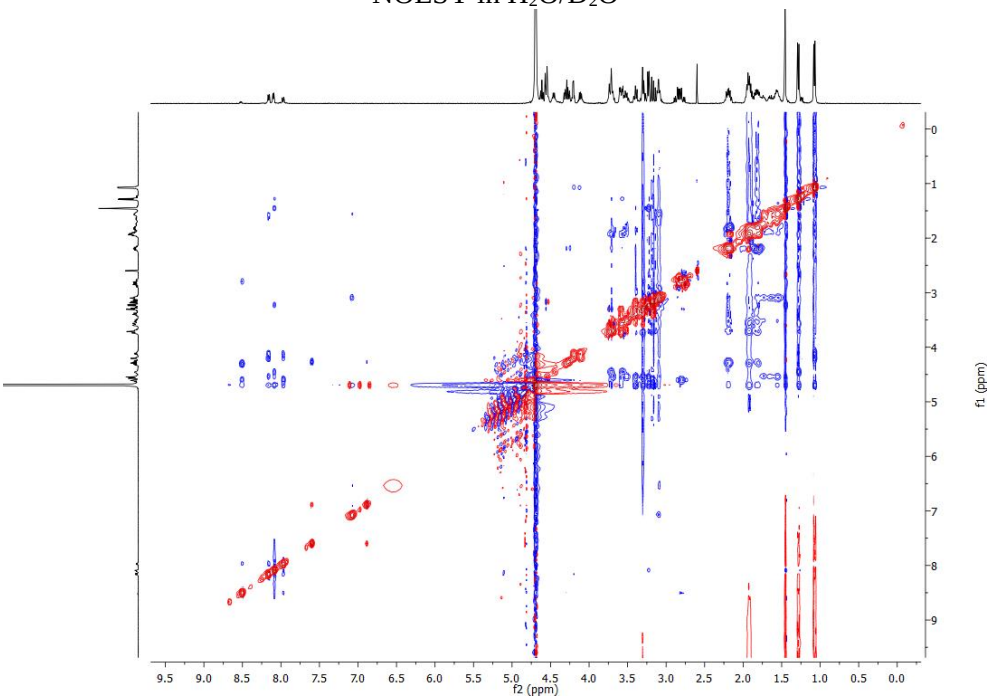
HSQC (400 MHz) in D_2O



HMBC in D_2O

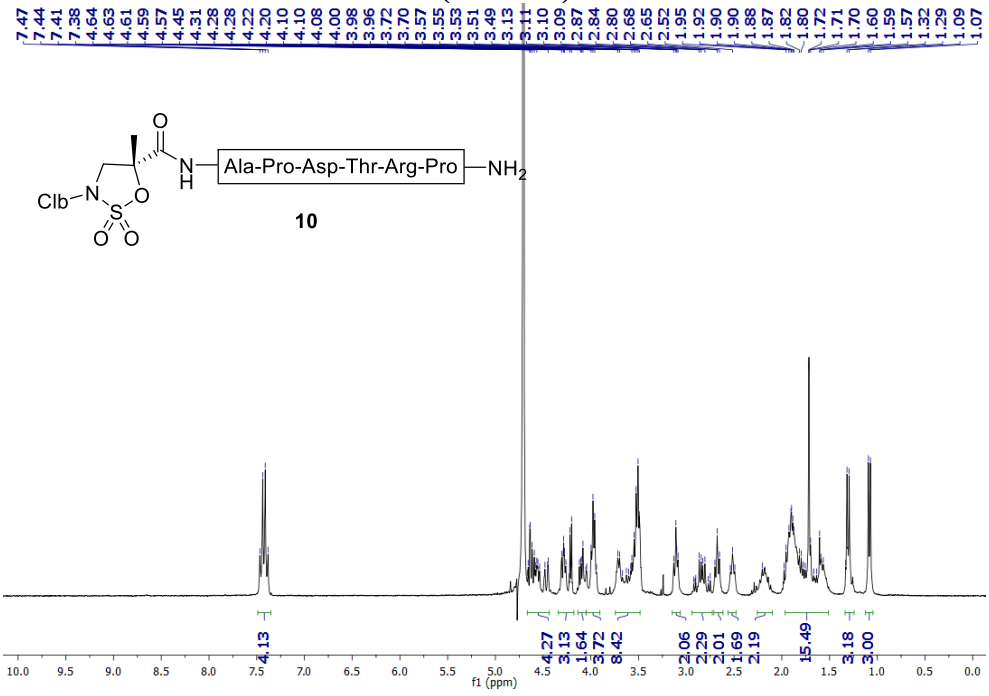


NOESY in H_2O/D_2O

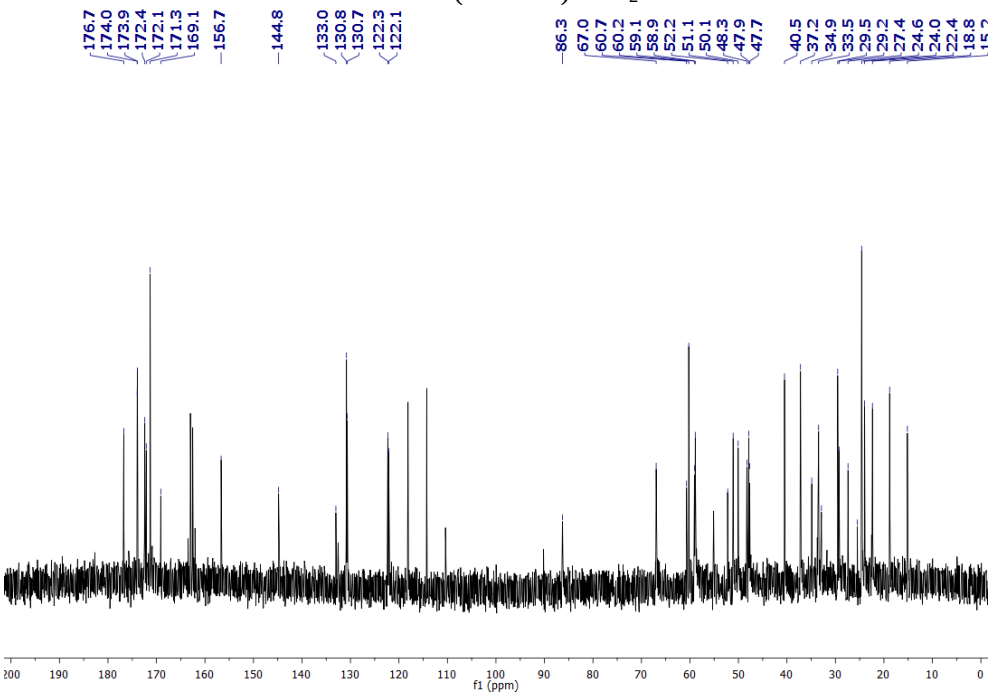


Compound **10**

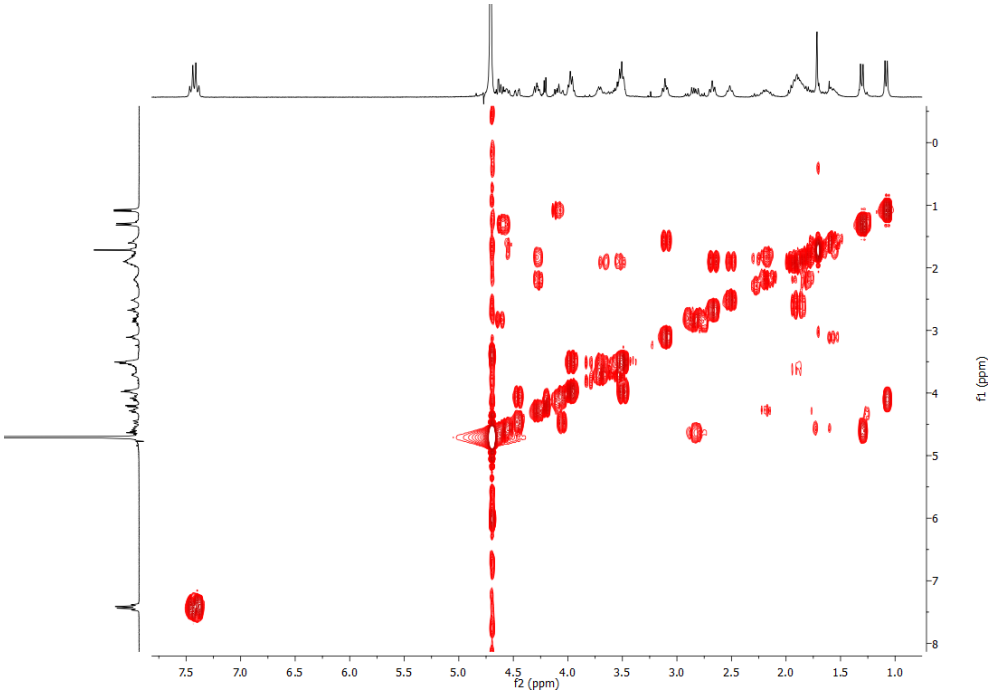
^1H NMR (300 MHz) in D_2O



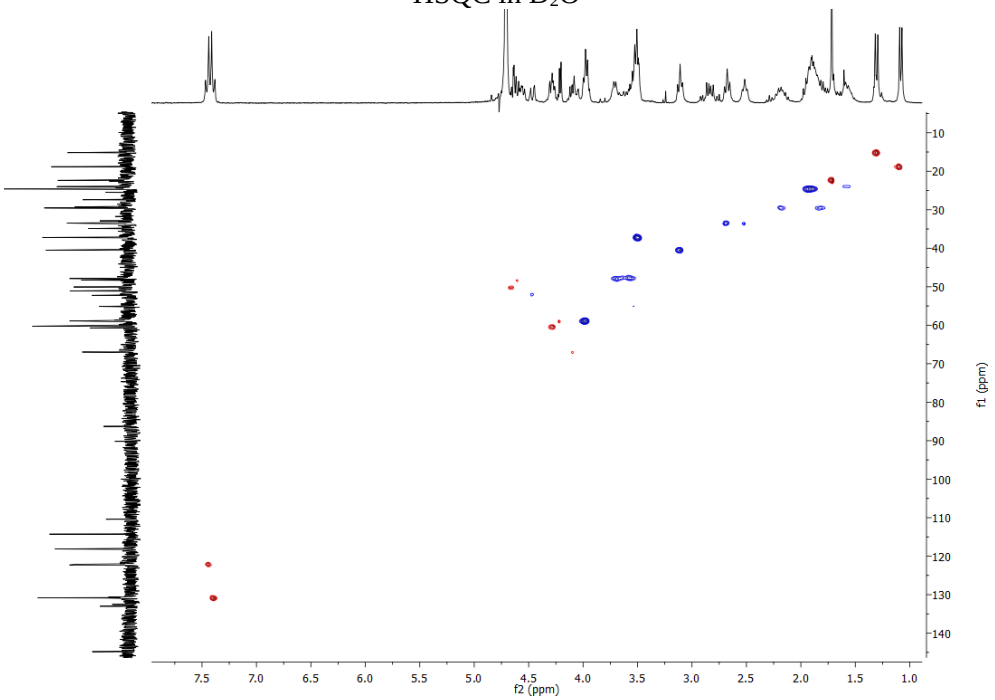
^{13}C NMR (75 MHz) in D_2O



COSY in D_2O

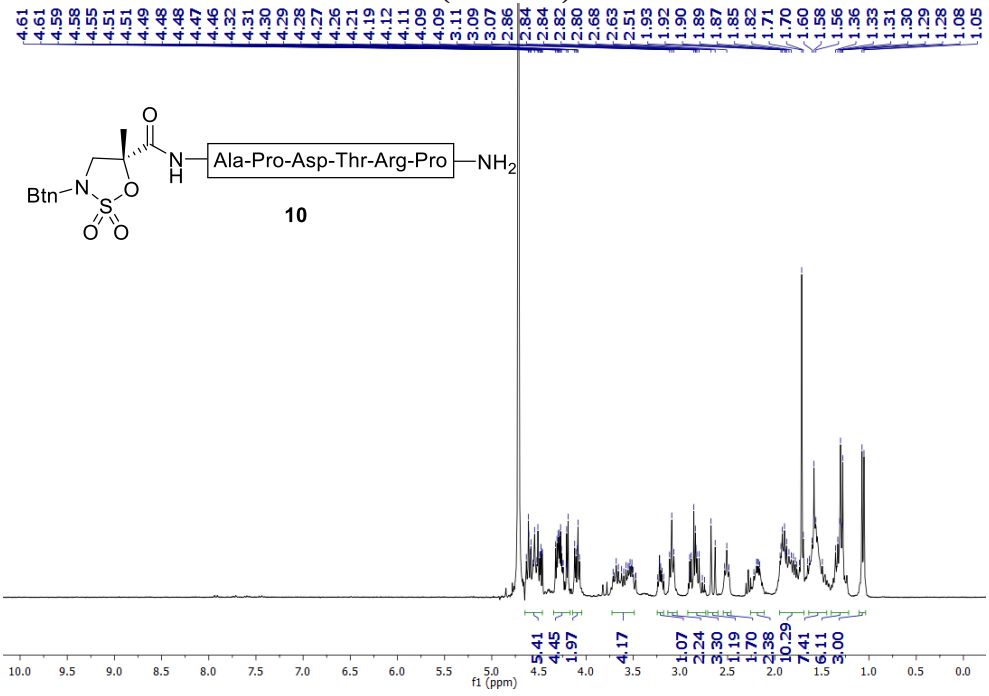


HSQC in D_2O

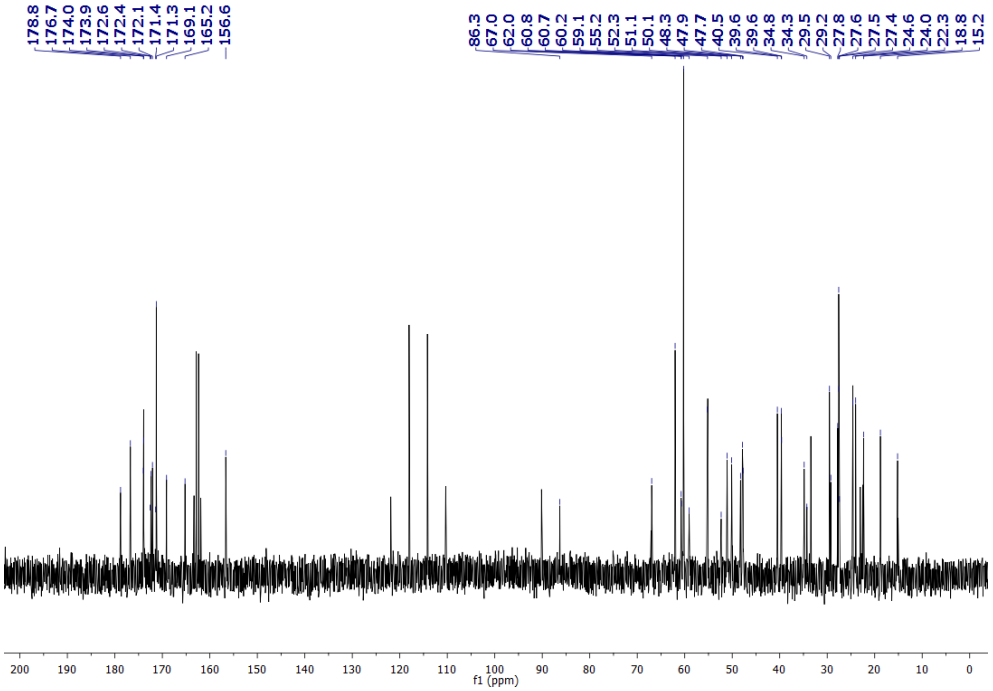


Compound **11**

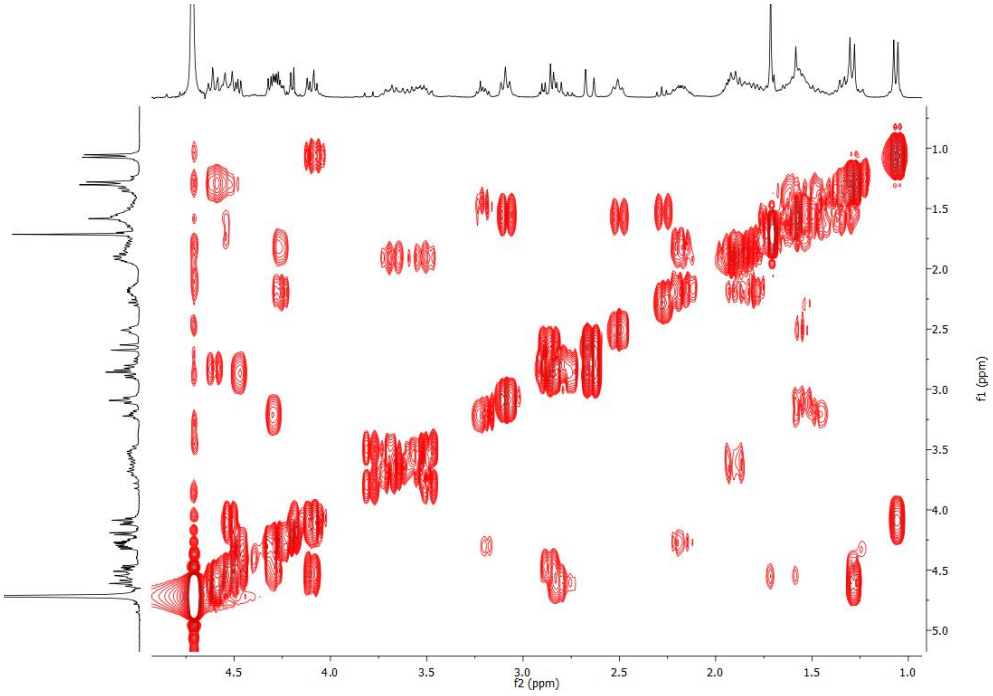
^1H NMR (300 MHz) in D_2O



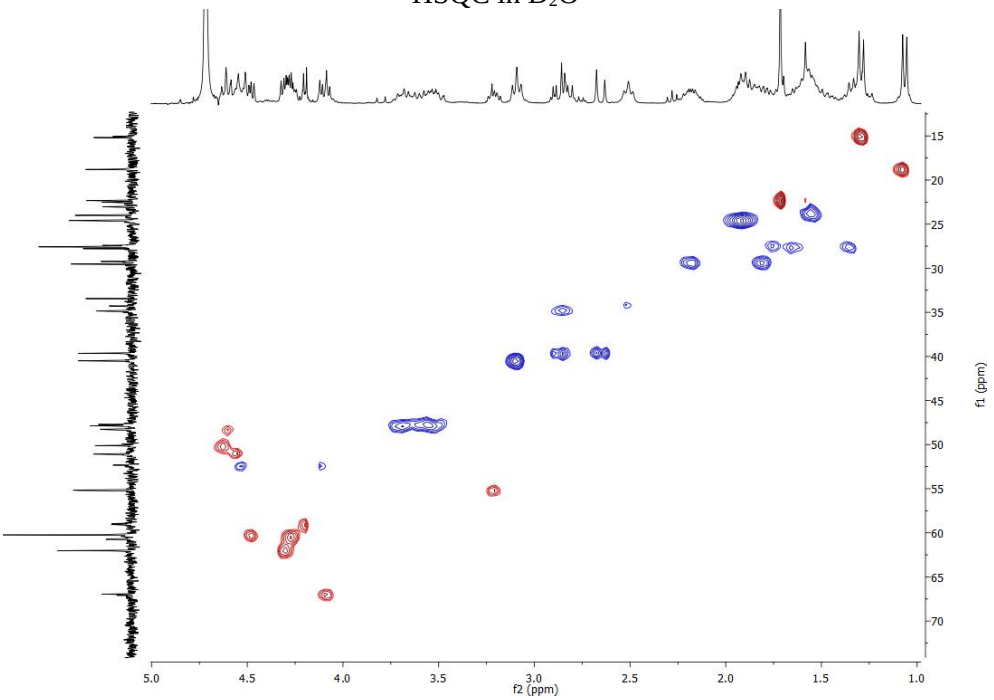
^{13}C NMR (75 MHz) in D_2O



COSY in D_2O

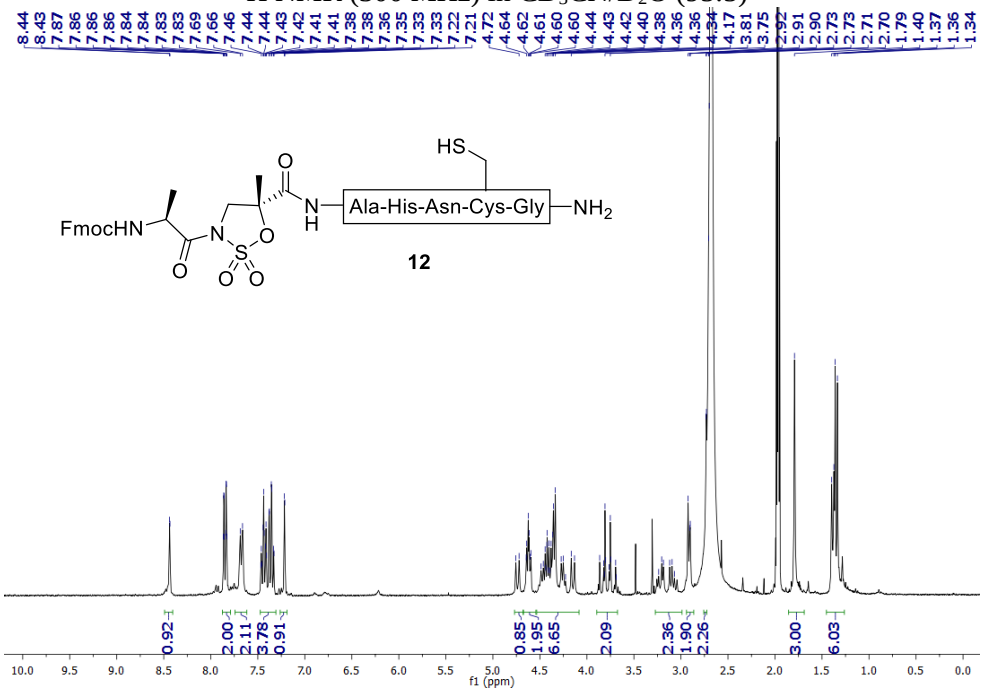


HSQC in D_2O

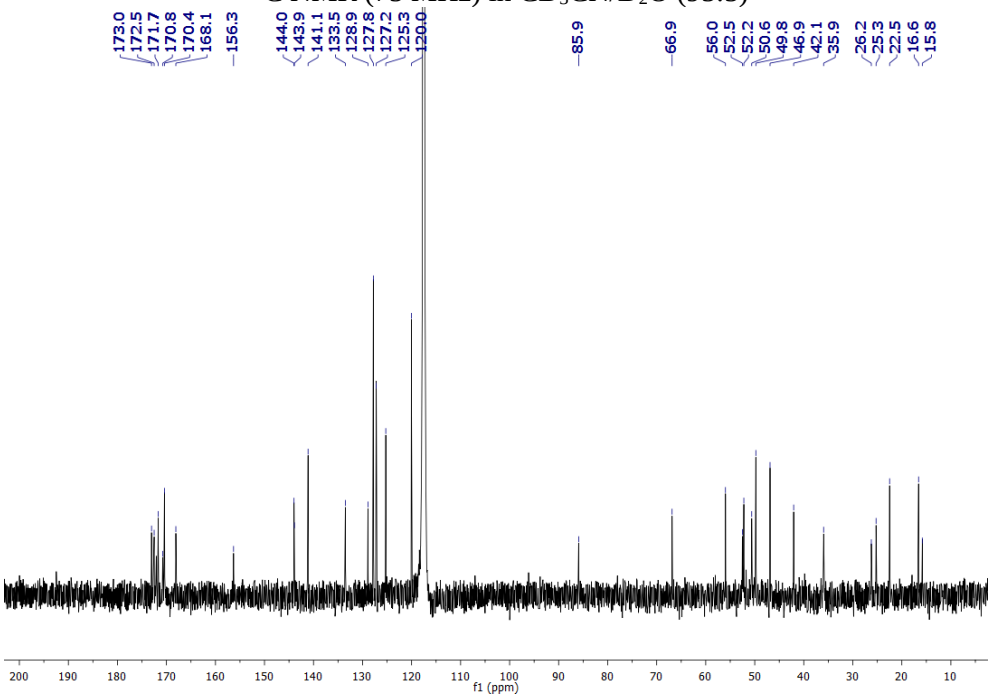


Compound **12**

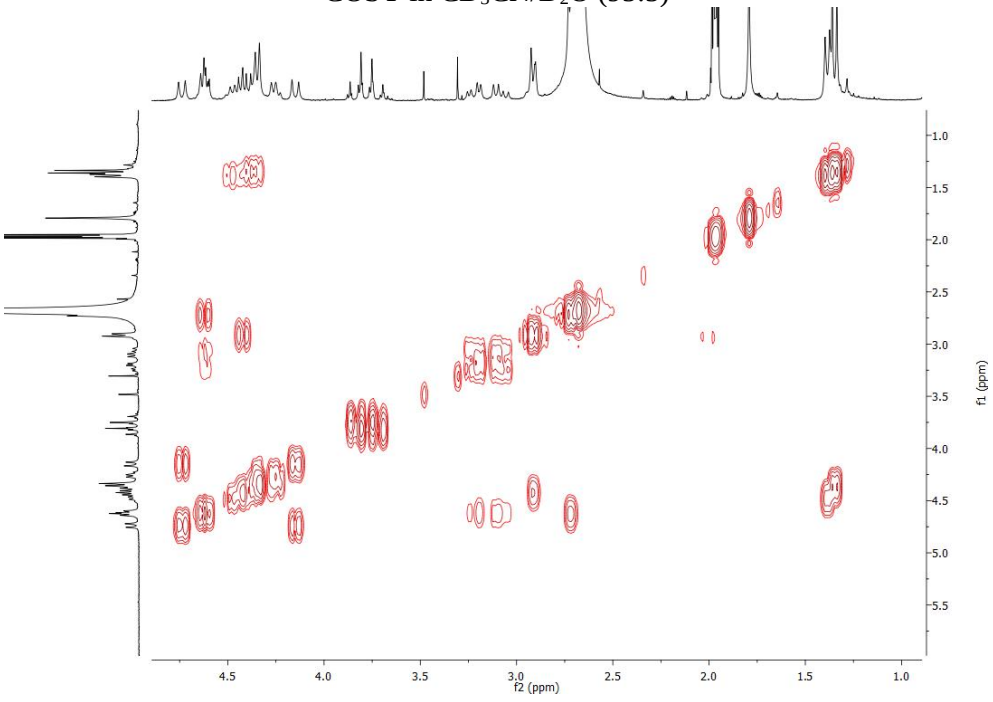
^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



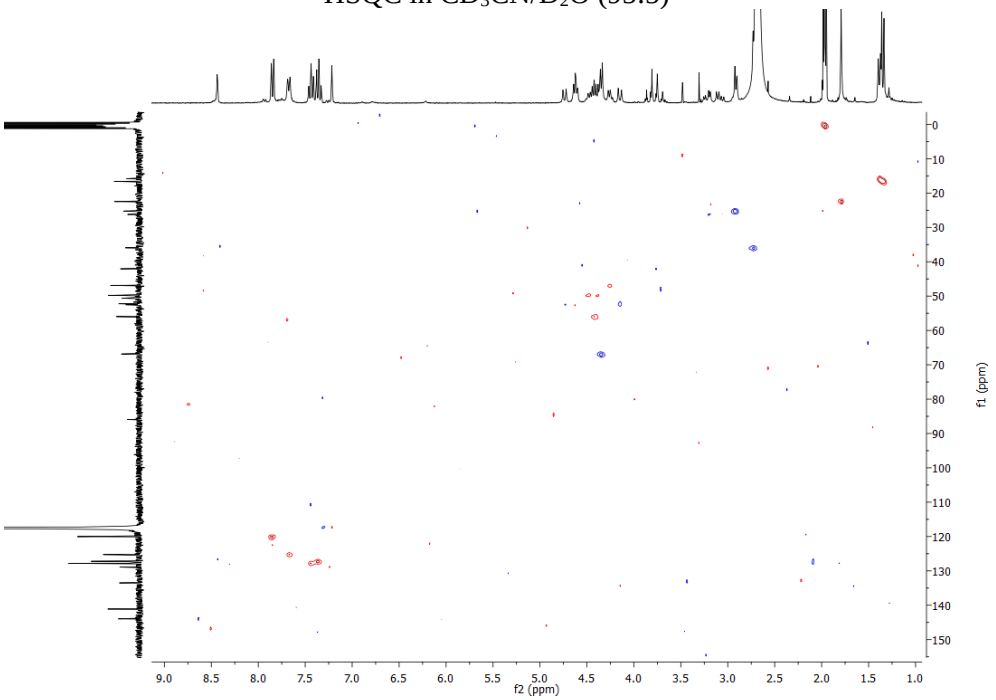
^{13}C NMR (75 MHz) in CD_3CN/D_2O (95:5)



COSY in CD_3CN/D_2O (95:5)

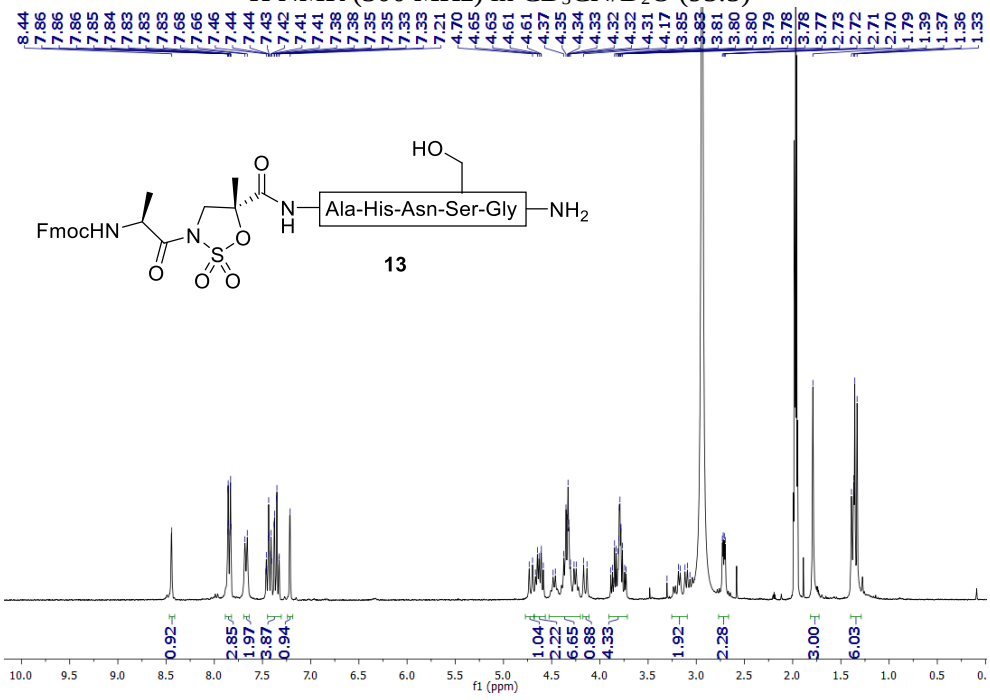


HSQC in CD_3CN/D_2O (95:5)

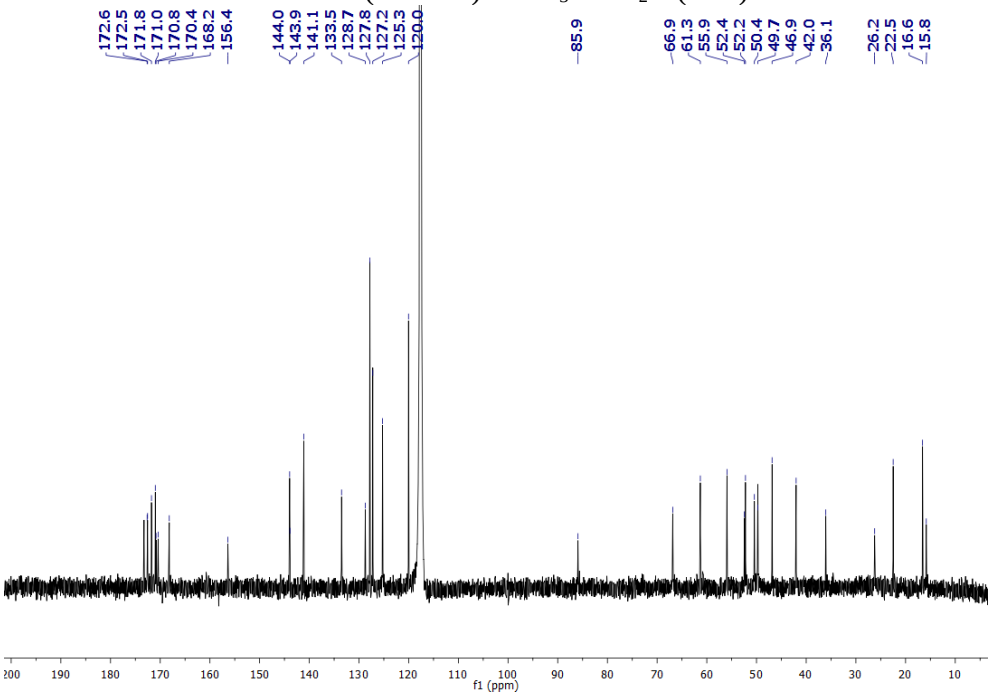


Compound **13**

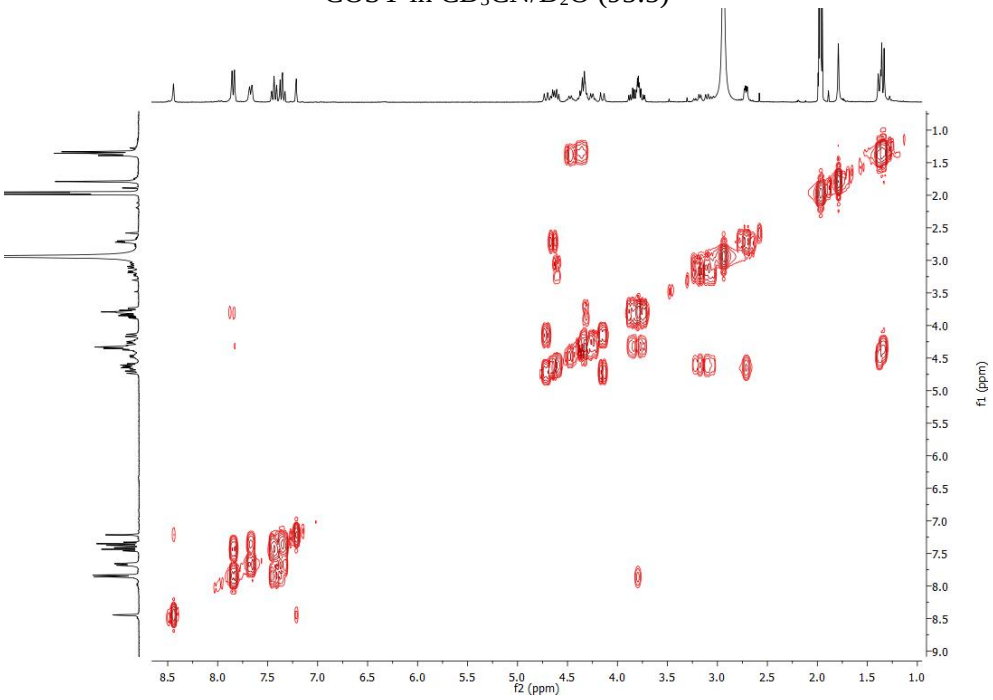
^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



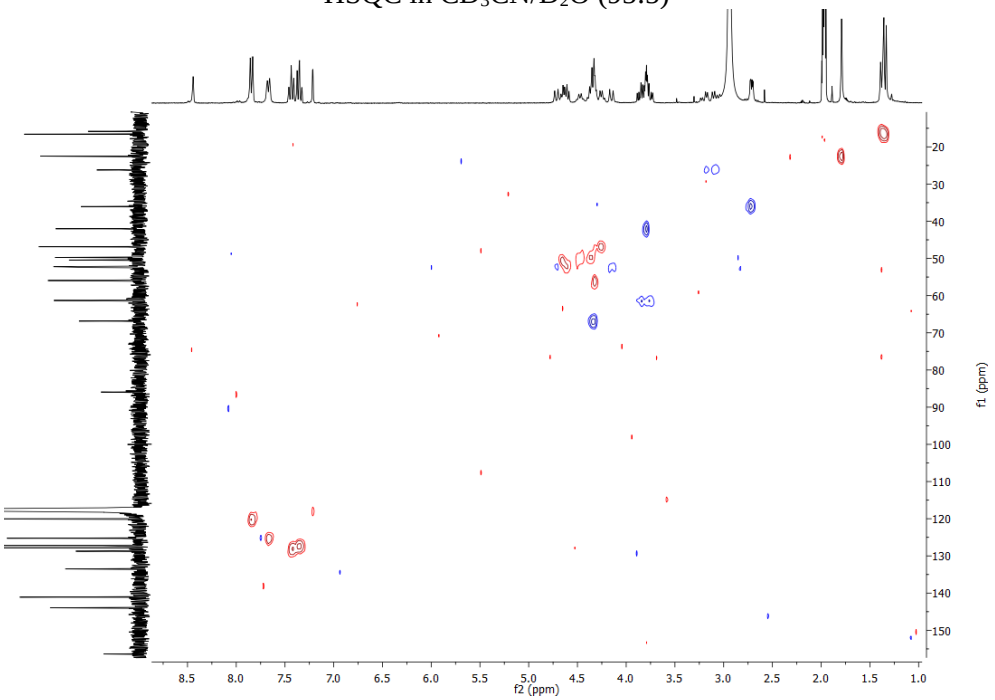
^{13}C NMR (75 MHz) in CD_3CN/D_2O (95:5)



COSY in CD_3CN/D_2O (95:5)

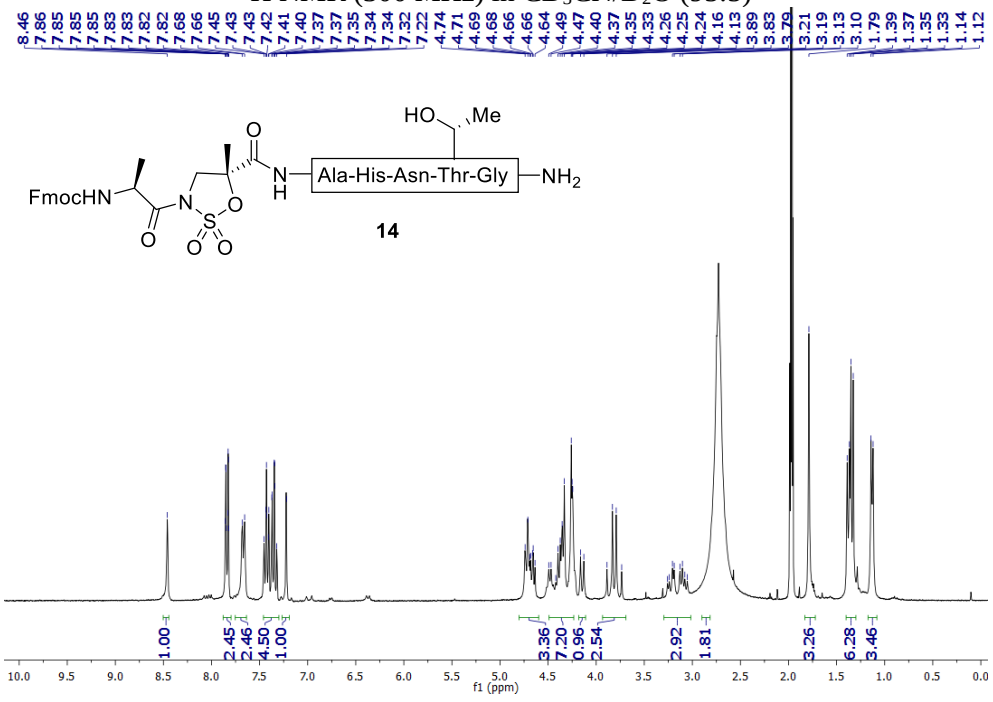


HSQC in CD_3CN/D_2O (95:5)

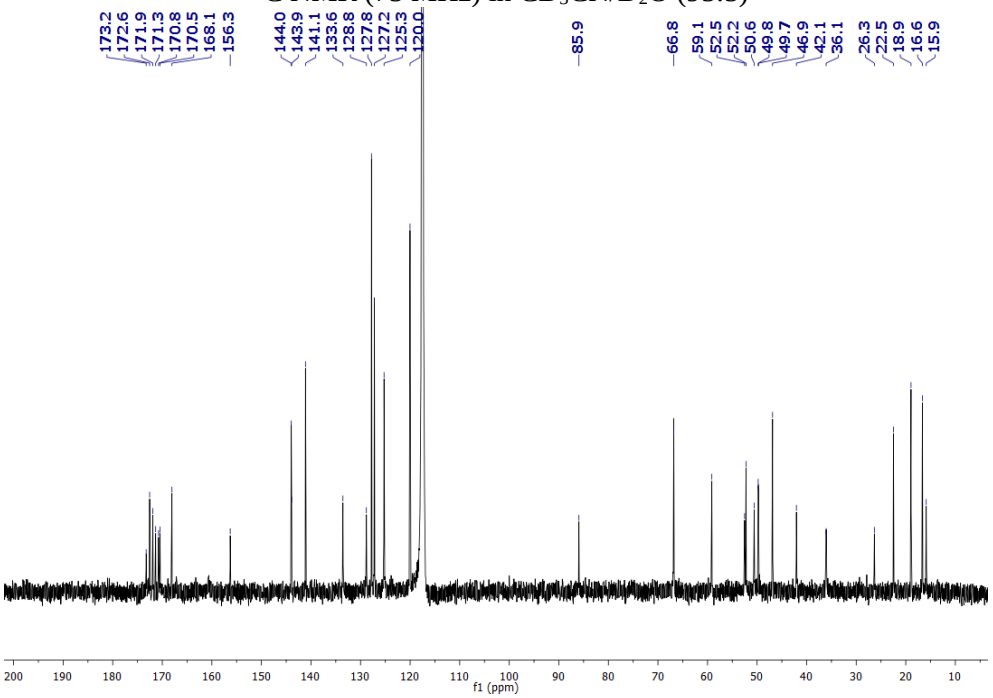


Compound **14**

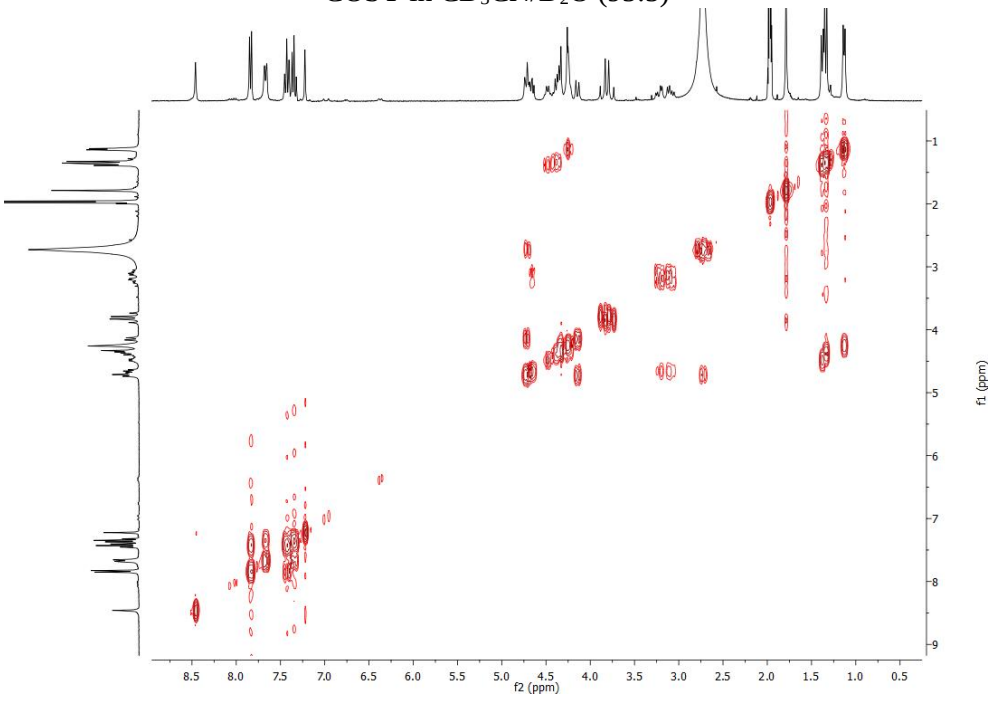
^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



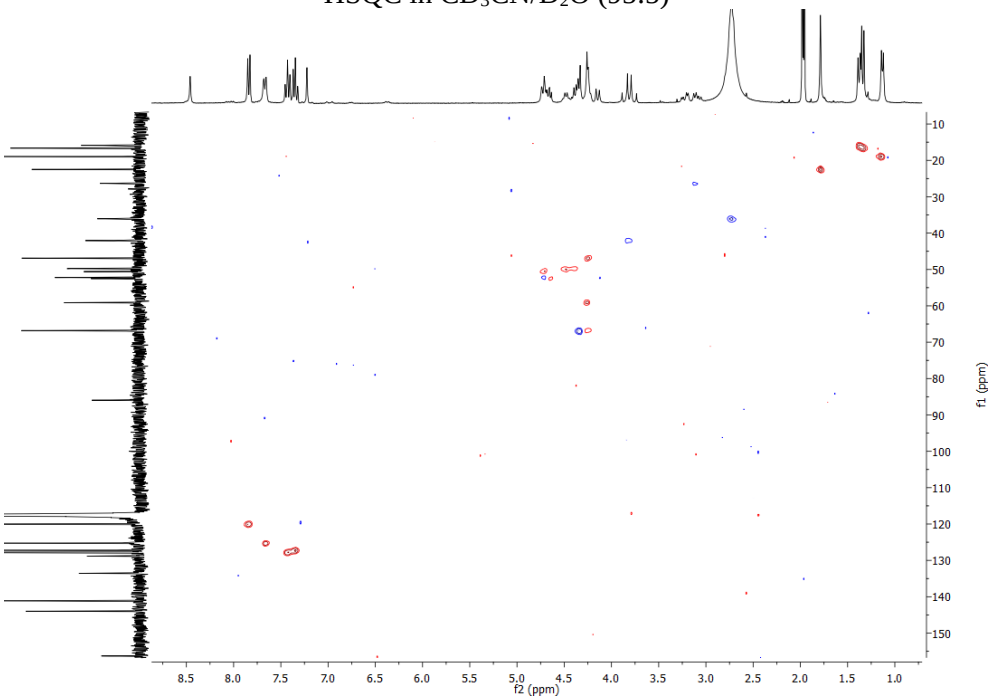
^{13}C NMR (75 MHz) in CD_3CN/D_2O (95:5)



COSY in CD_3CN/D_2O (95:5)

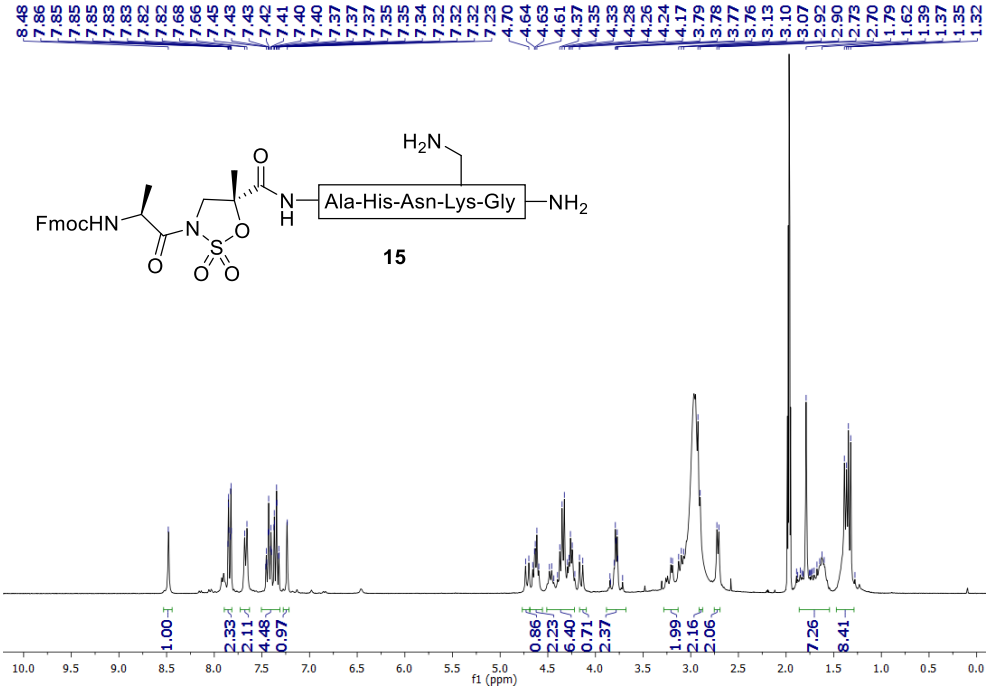


HSQC in CD_3CN/D_2O (95:5)

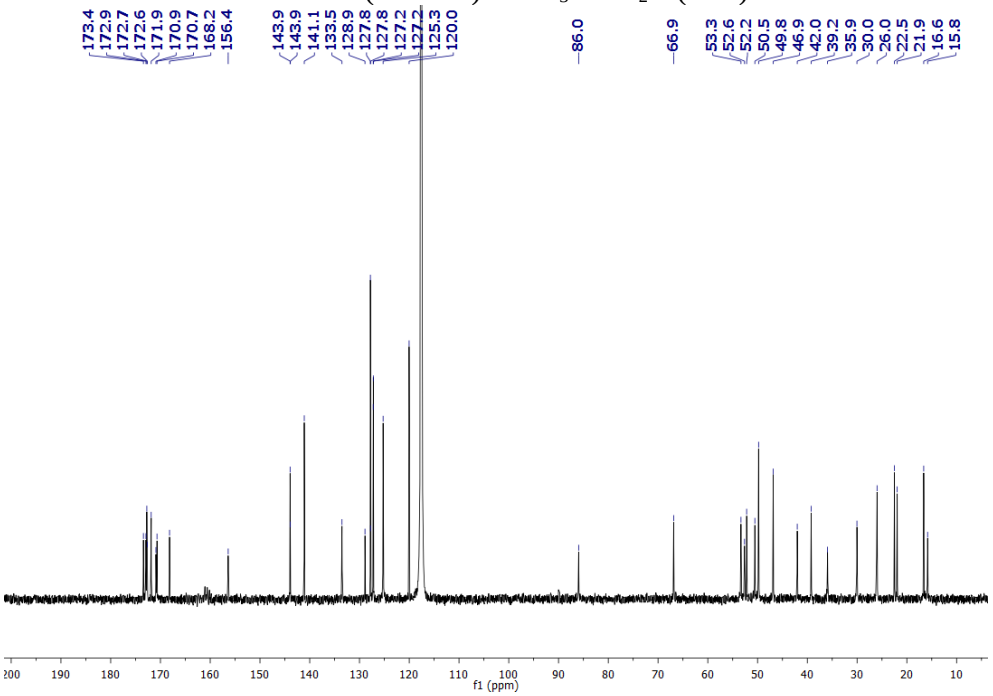


Compound **15**

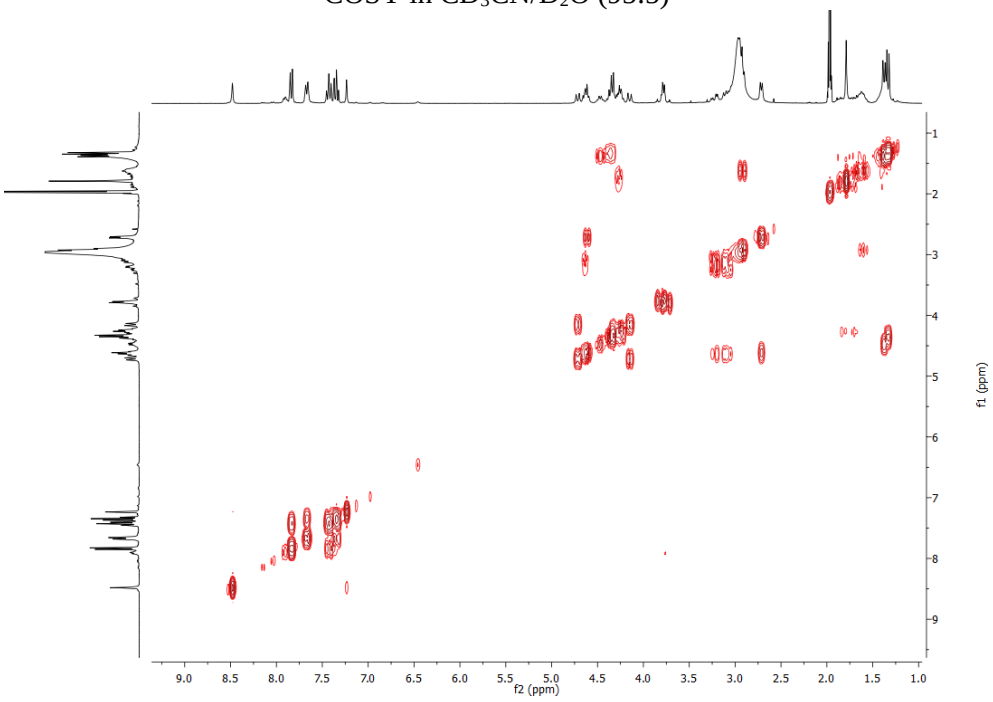
^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



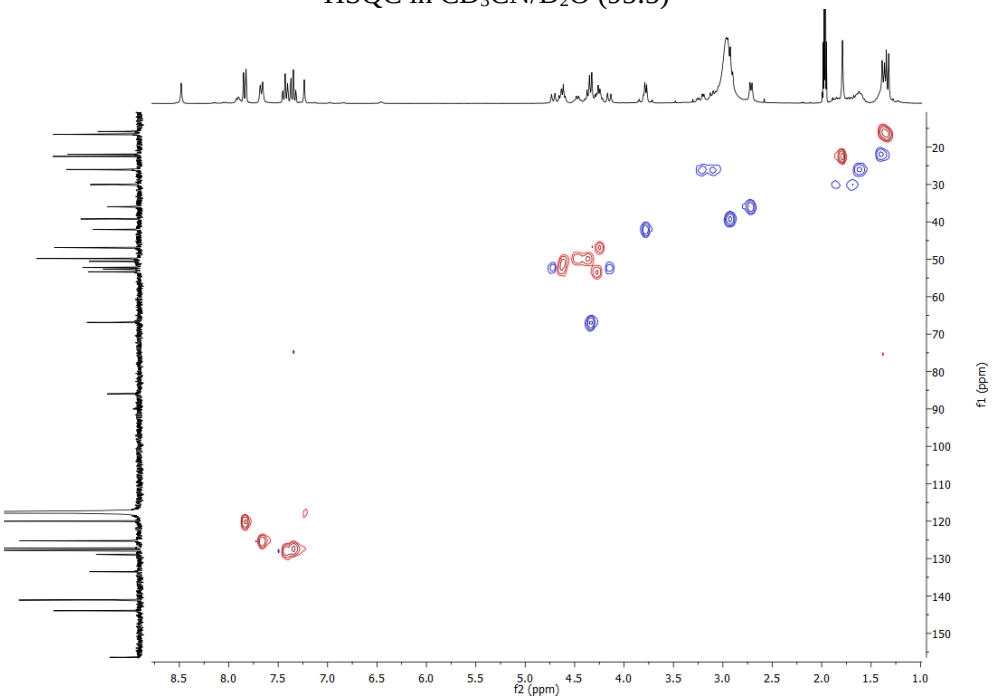
^{13}C NMR (75 MHz) in CD_3CN/D_2O (95:5)



COSY in CD_3CN/D_2O (95:5)

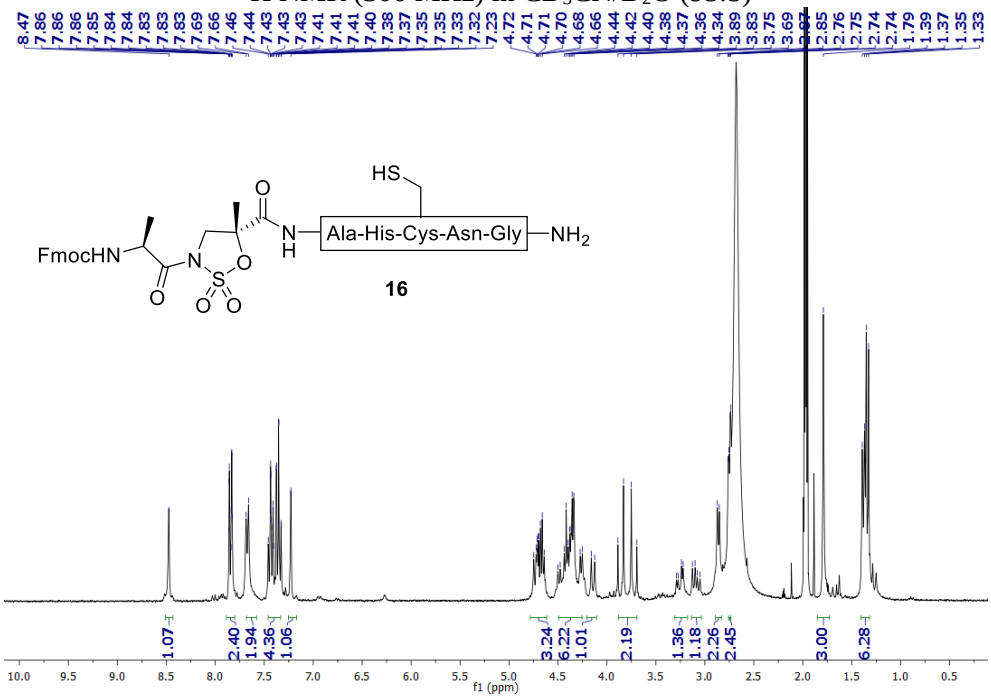


HSQC in CD_3CN/D_2O (95:5)

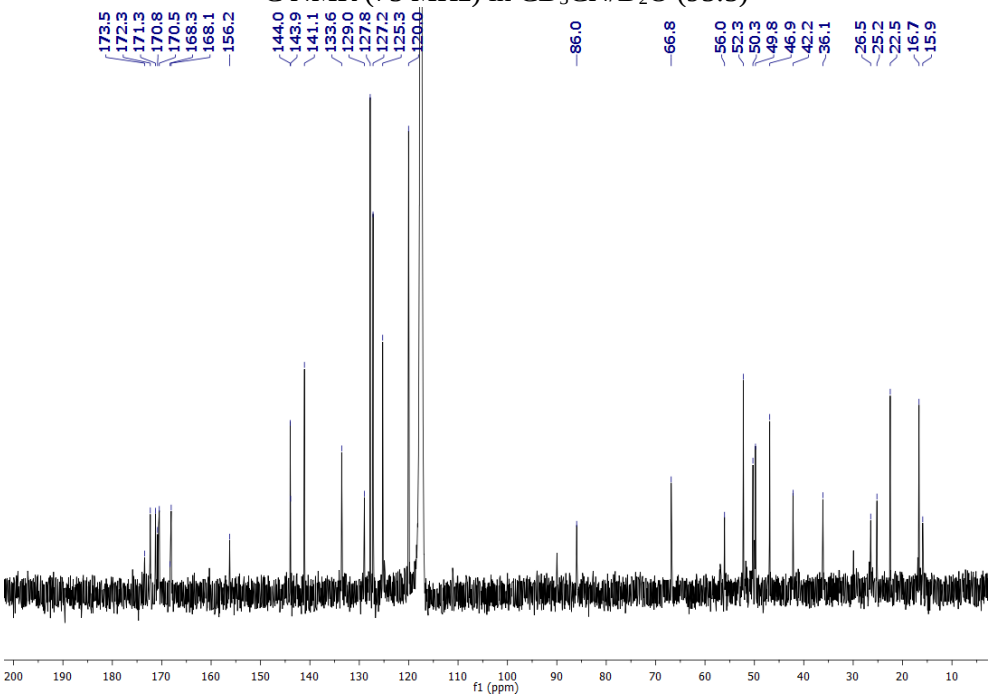


Compound **16**

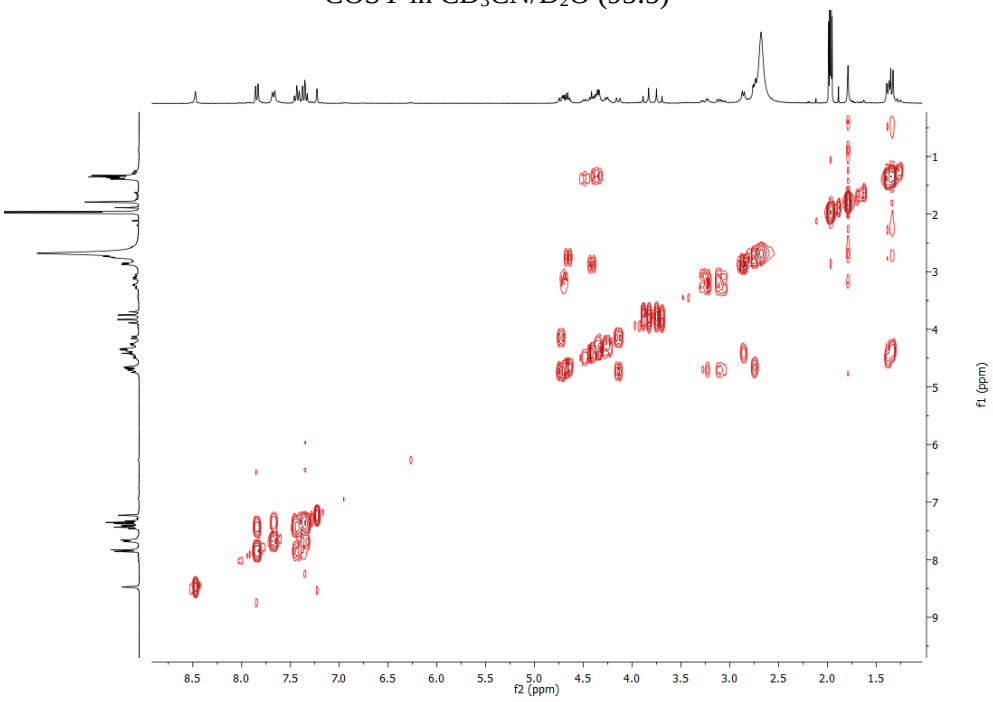
^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



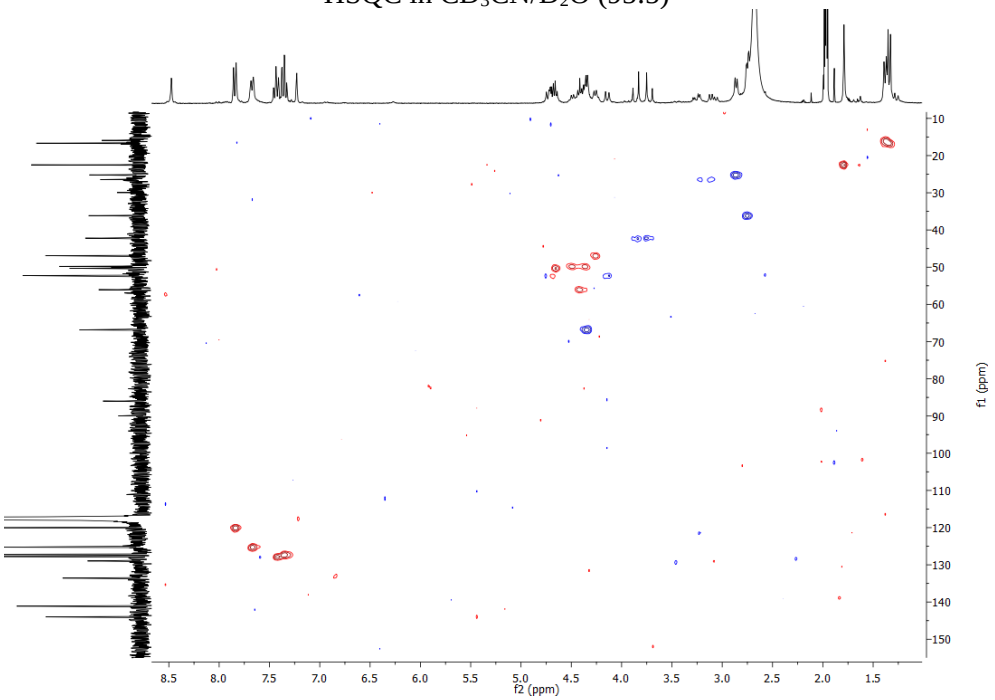
^{13}C NMR (75 MHz) in CD_3CN/D_2O (95:5)



COSY in CD_3CN/D_2O (95:5)

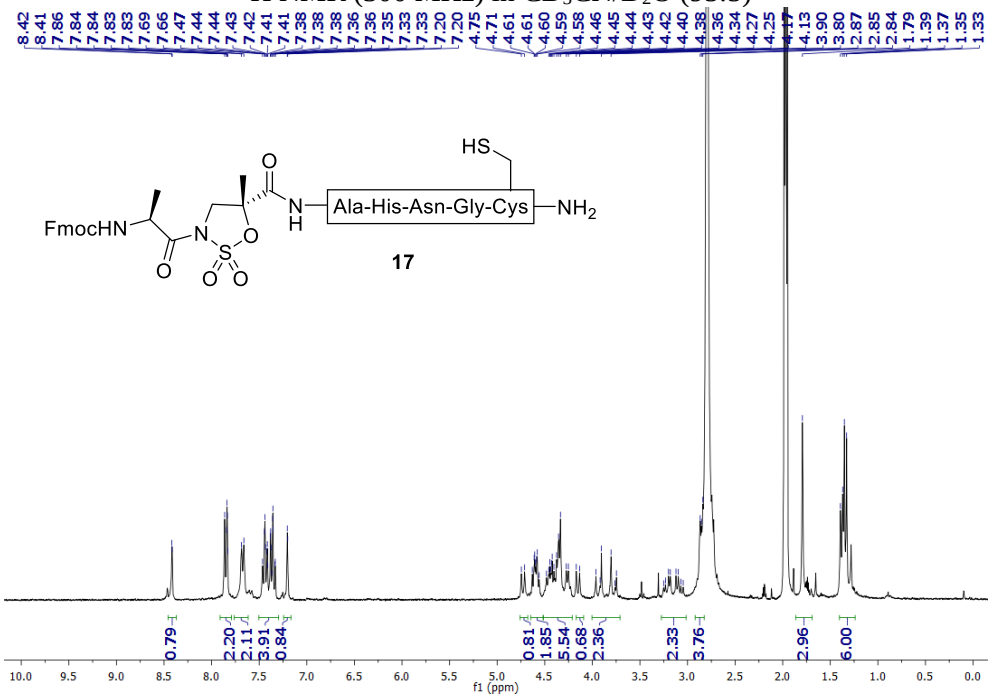


HSQC in CD_3CN/D_2O (95:5)

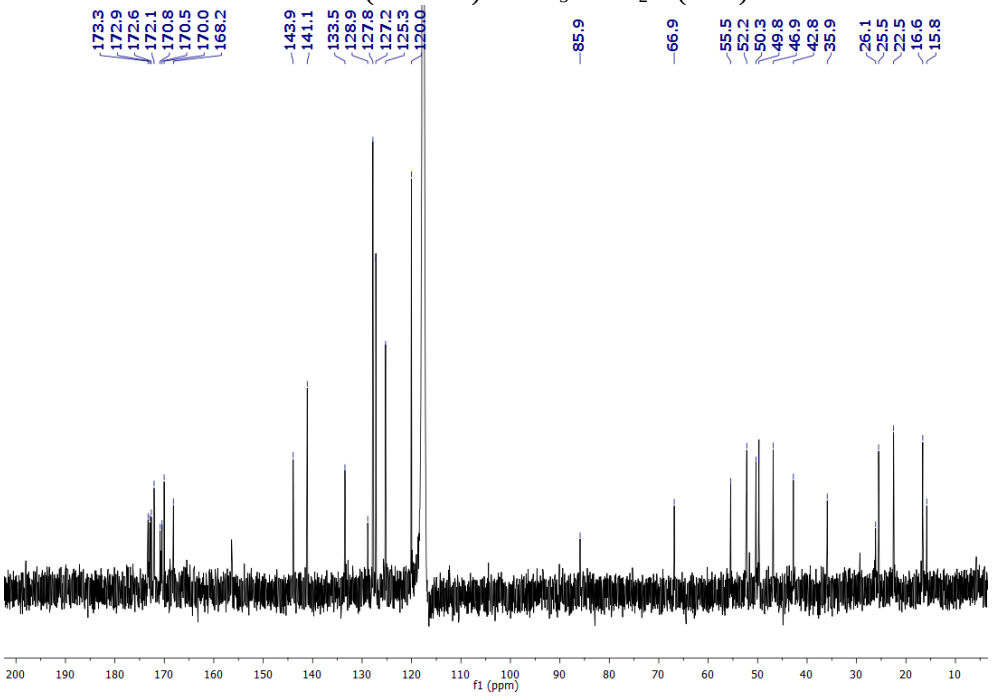


Compound **17**

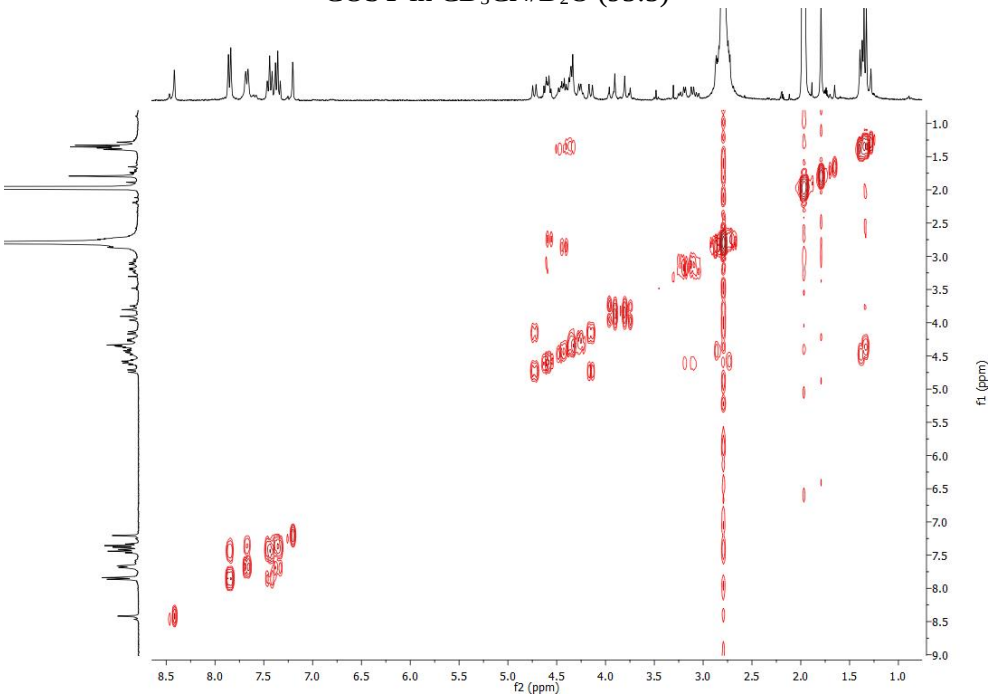
^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



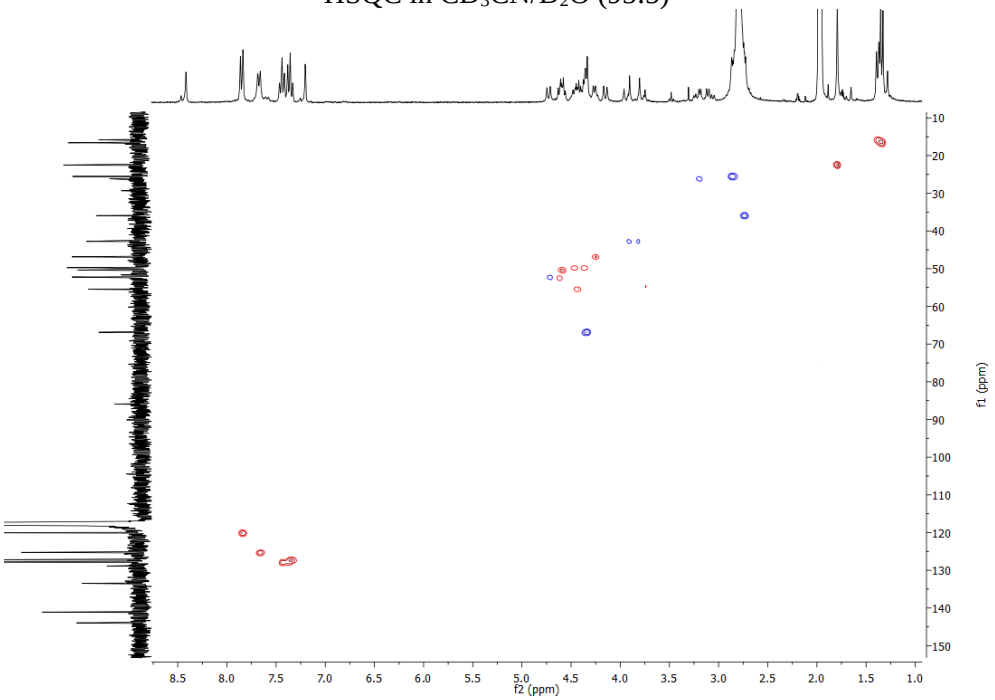
^{13}C NMR (75 MHz) in CD_3CN/D_2O (95:5)



COSY in CD_3CN/D_2O (95:5)

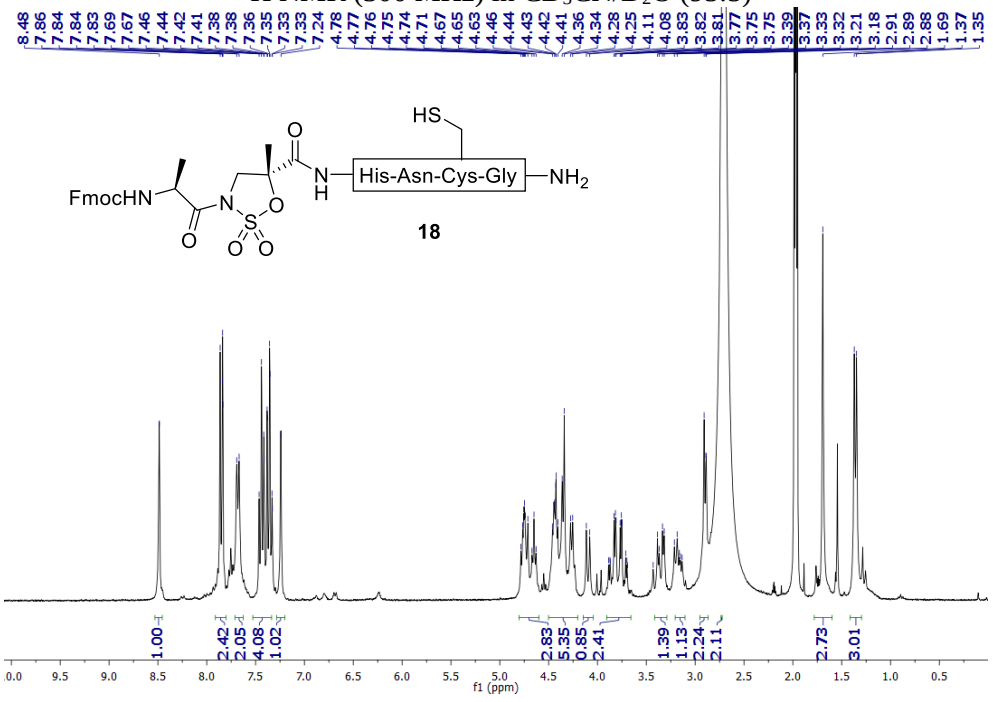


HSQC in CD_3CN/D_2O (95:5)

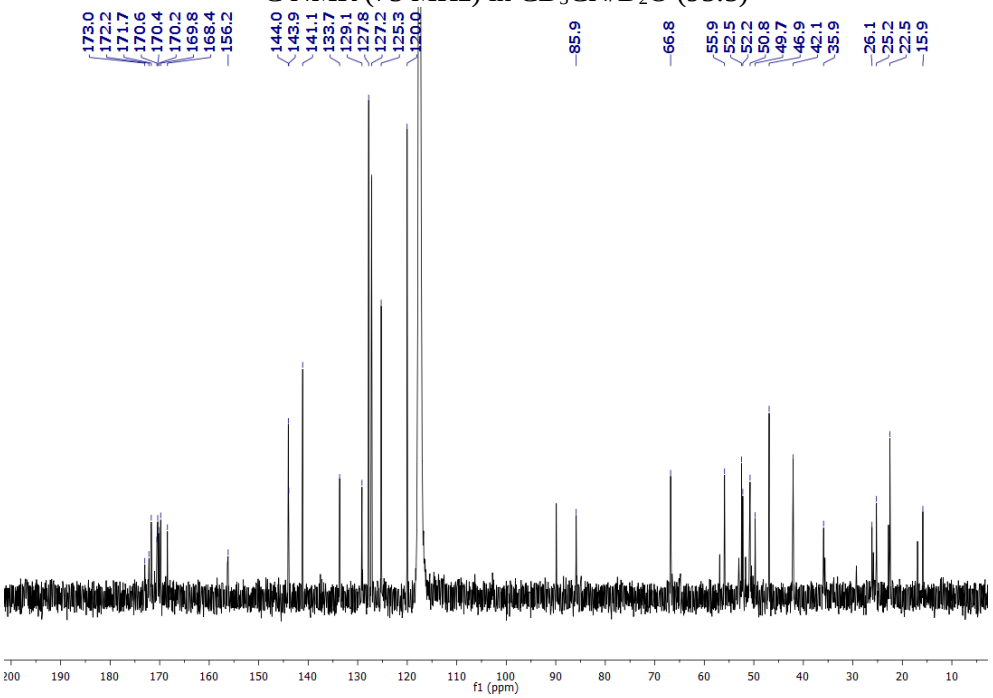


Compound **18**

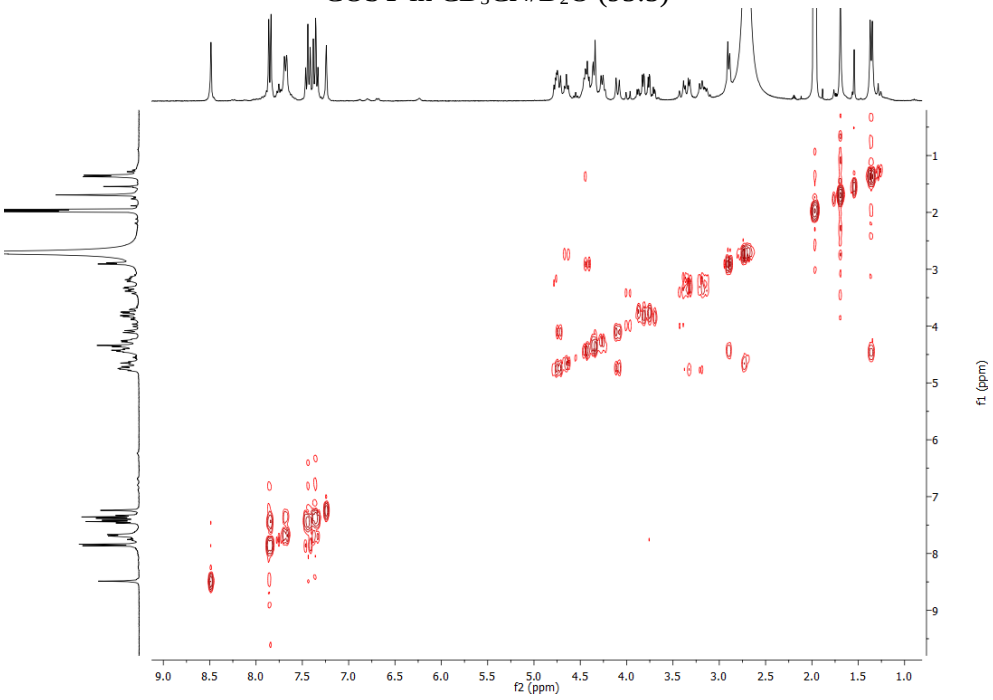
^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



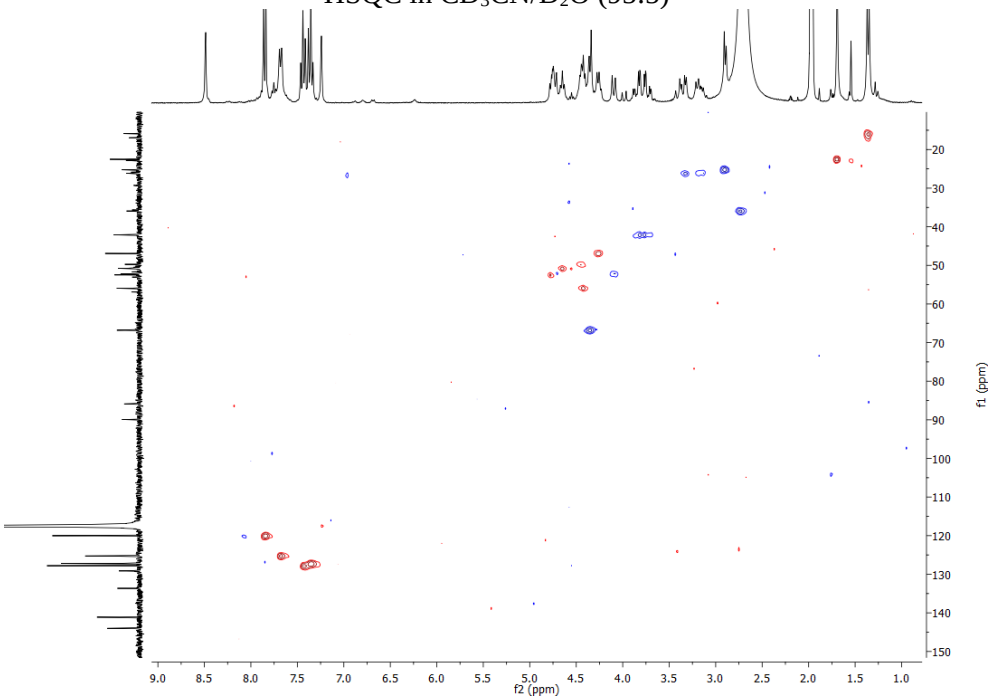
^{13}C NMR (75 MHz) in CD_3CN/D_2O (95:5)



COSY in CD_3CN/D_2O (95:5)

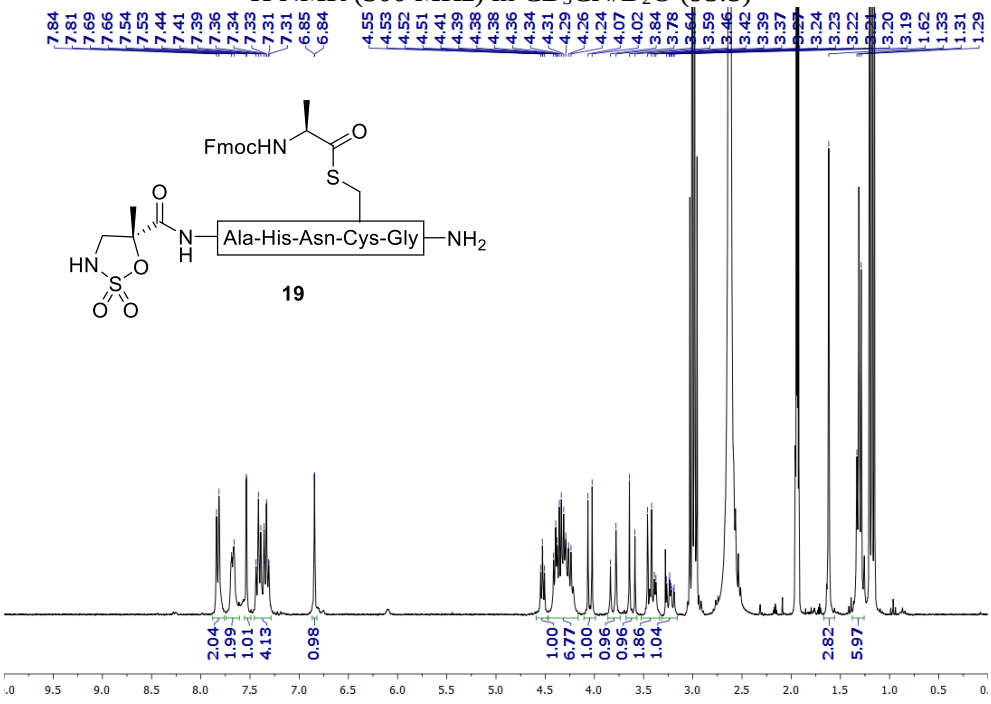


HSQC in CD_3CN/D_2O (95:5)



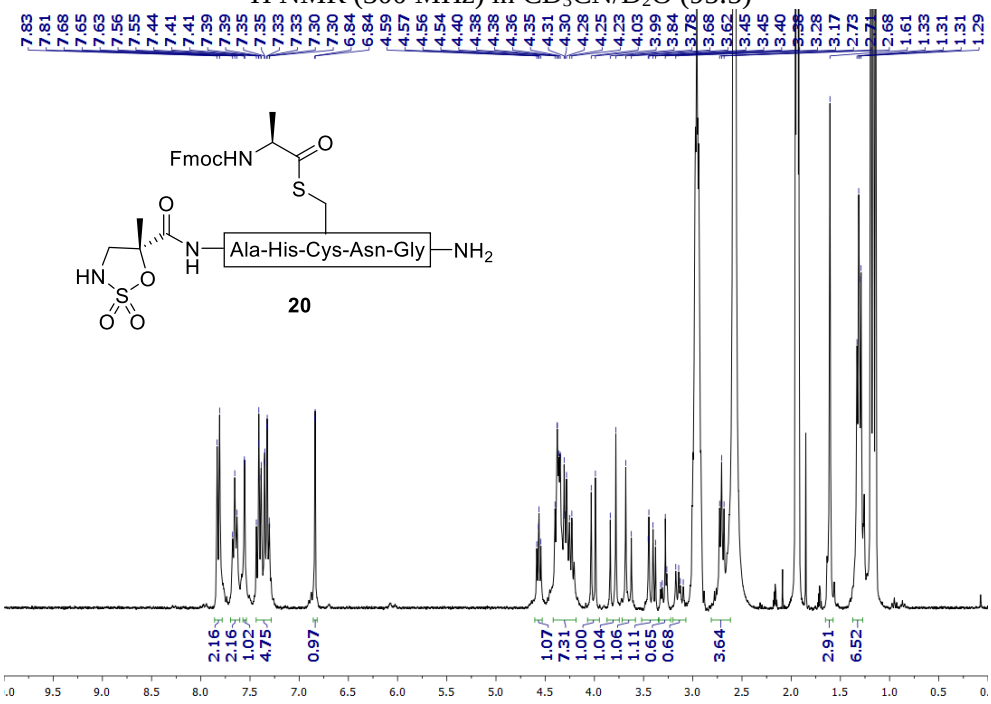
Compound **19**

^1H NMR (300 MHz) in CD_3CN/D_2O (95:5)



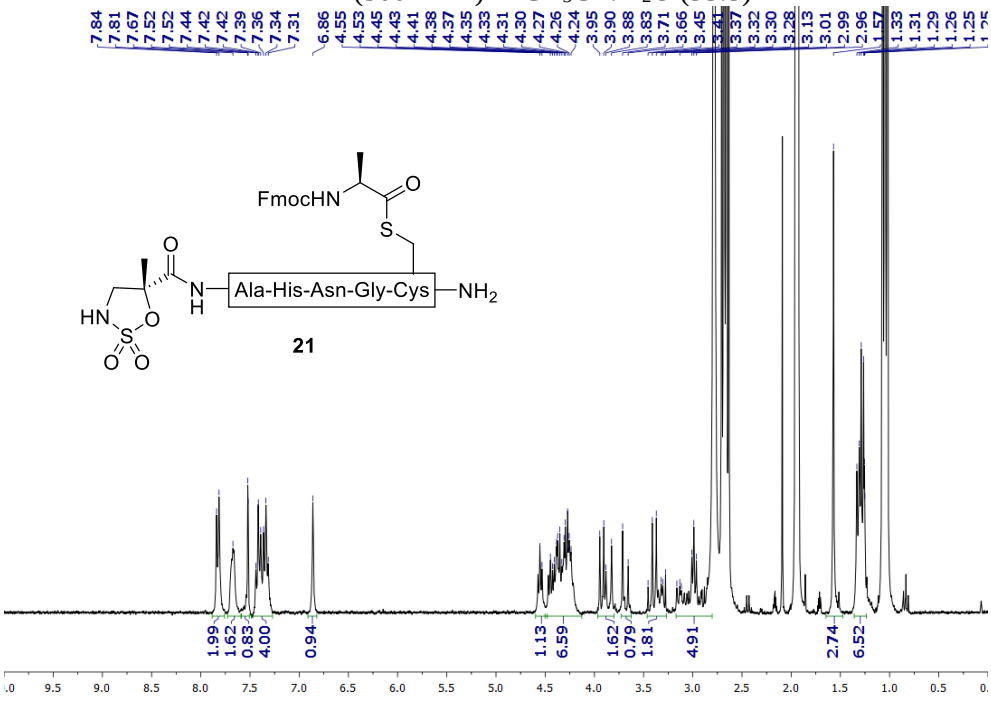
Compound **20**

^{1}H NMR (300 MHz) in CD_3CN/D_2O (95:5)



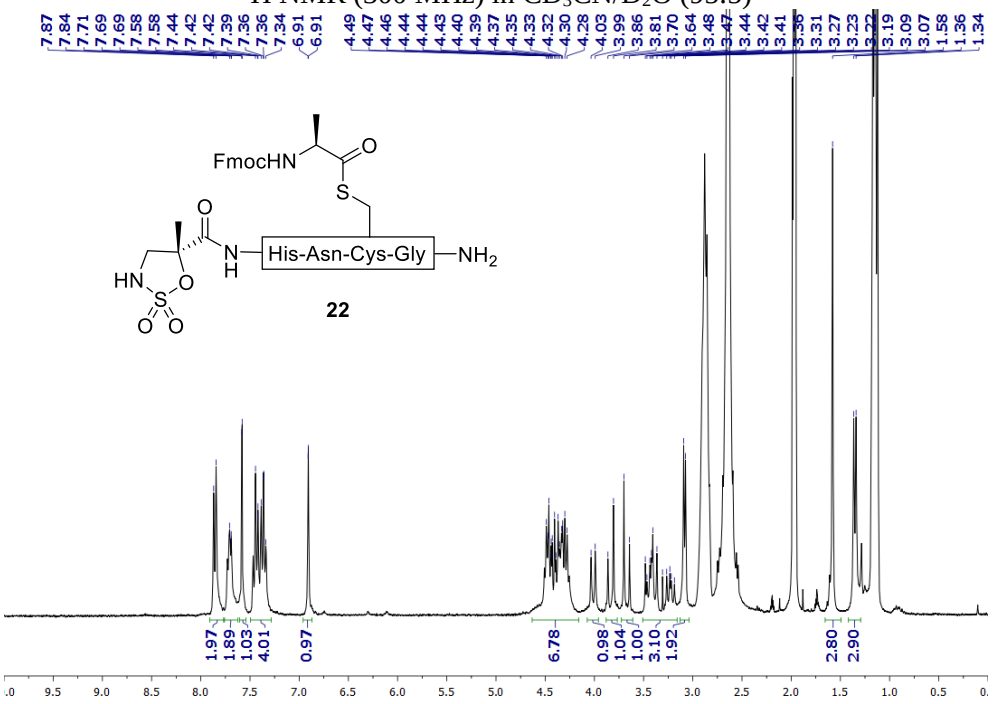
Compound **21**

^{1}H NMR (300 MHz) in CD_3CN/D_2O (95:5)



Compound **22**

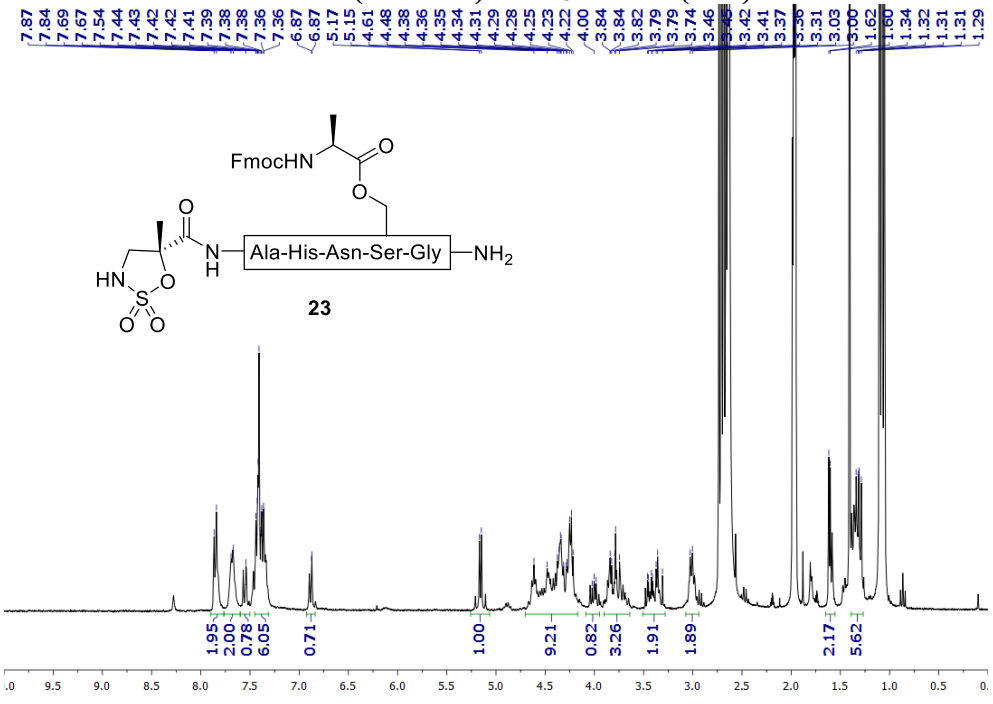
^{1}H NMR (300 MHz) in CD_3CN/D_2O (95:5)



22

Compound **23**

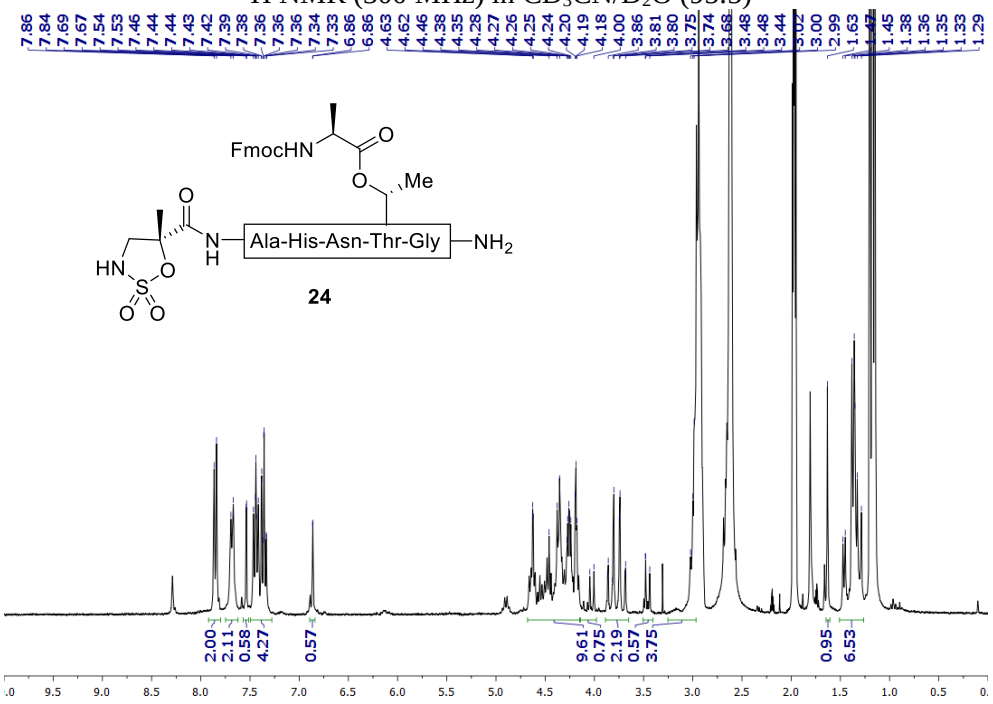
^{1}H NMR (300 MHz) in CD_3CN/D_2O (95:5)



23

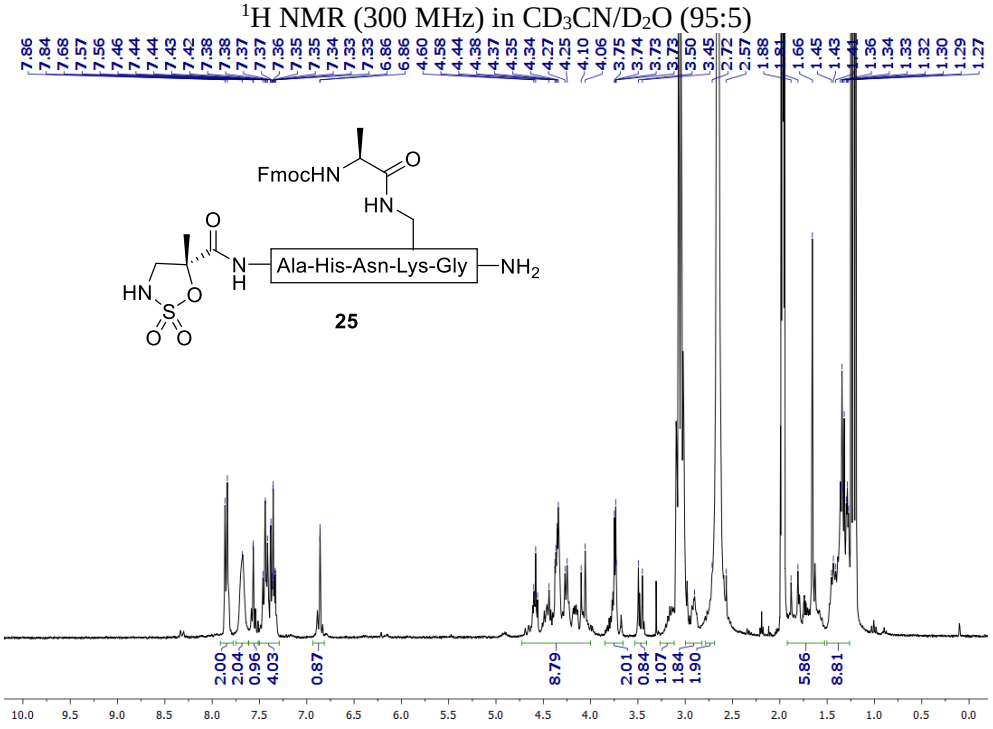
Compound **24**

^{1}H NMR (300 MHz) in CD_3CN/D_2O (95:5)



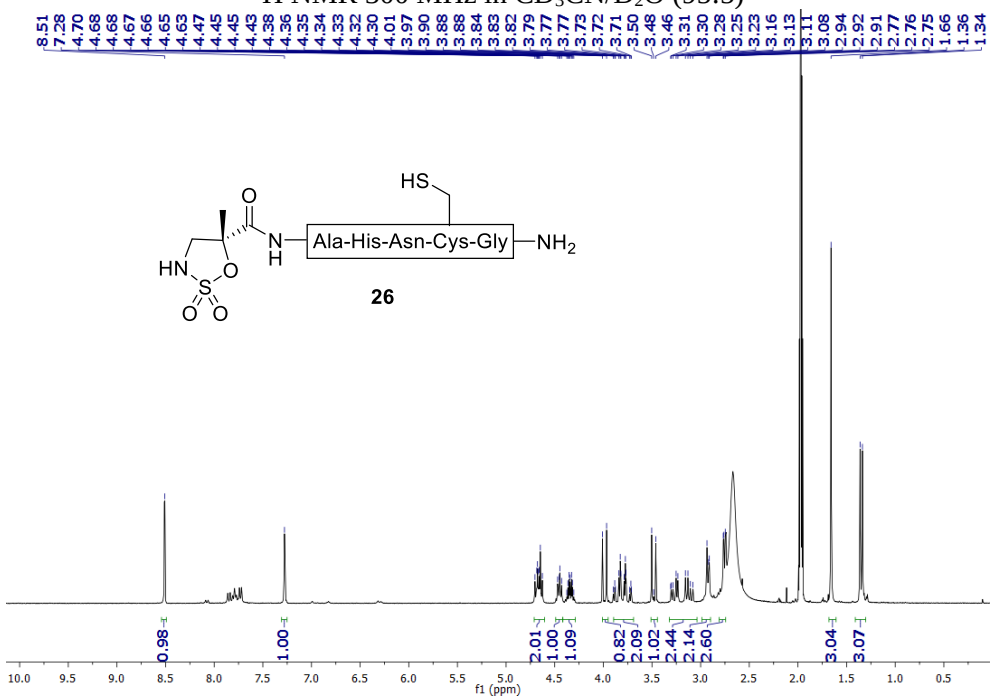
Compound **25**

^{1}H NMR (300 MHz) in CD_3CN/D_2O (95:5)

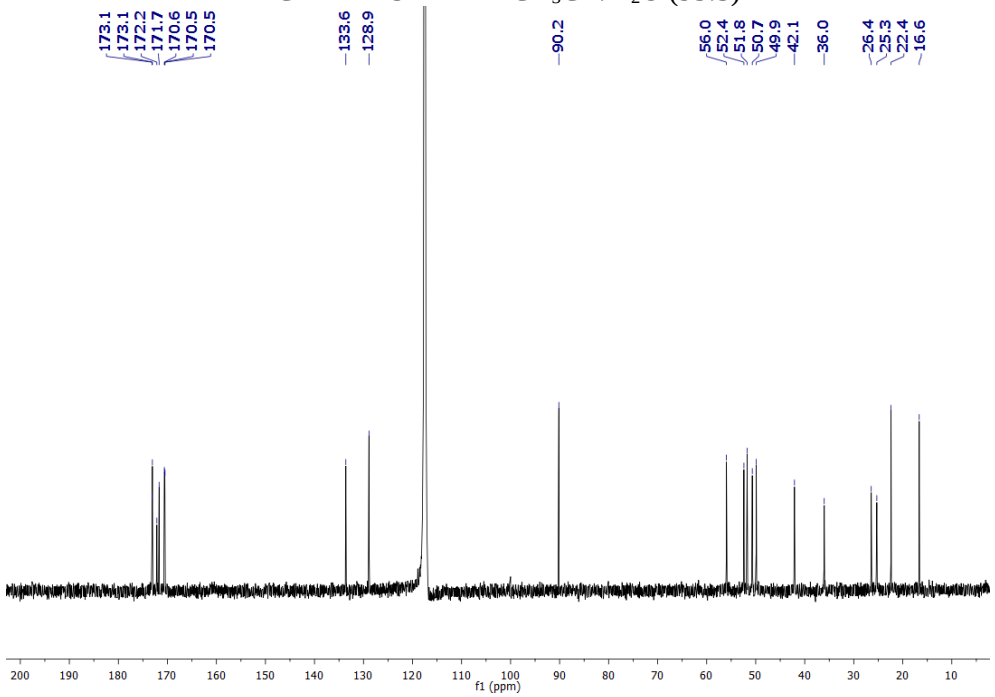


Compound **26**

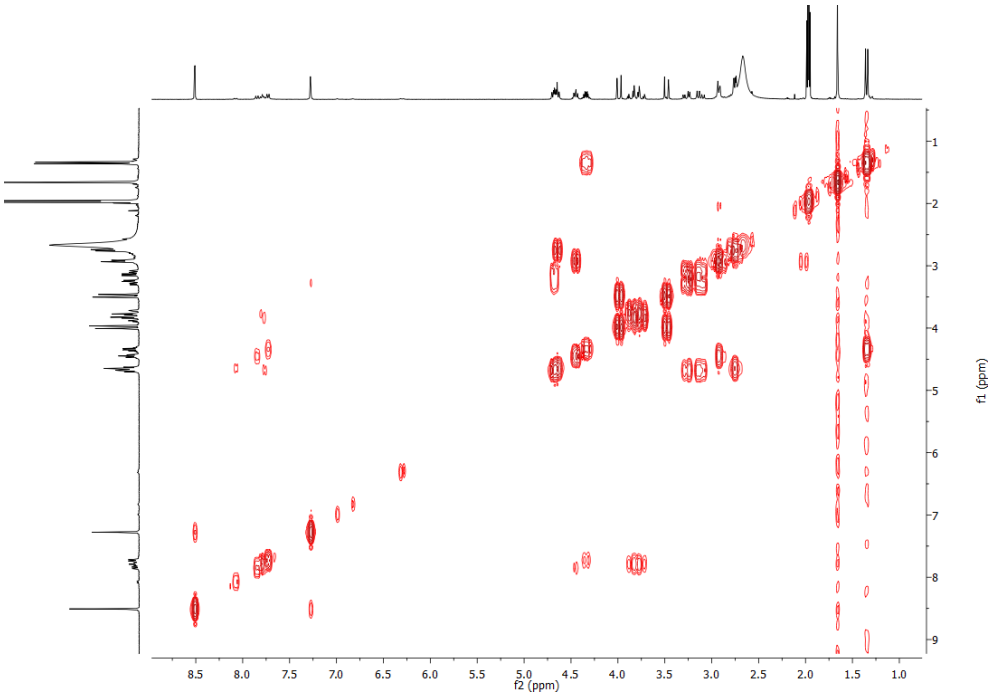
^{1}H NMR 300 MHz in CD_3CN/D_2O (95:5)



^{13}C NMR 75 MHz in CD_3CN/D_2O (95:5)



COSY 300 MHz in CD_3CN/D_2O (95:5)



HSQC 300 MHz in CD_3CN/D_2O (95:5)