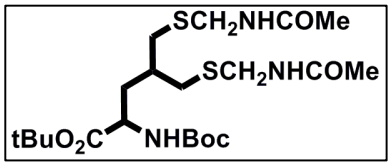
Abbreviations:

DMSO-Dimethyl Sulfoxide, TFA-Trifluoroacetic acid, TIPS-Triisopropylsilane, Fmoc-Osu-Fmoc-succinimide, TCEP: tris(2-carboxyethyl)phosphine, THF-Tetrahydrofuran, DMF: Dimethylformamide

Experimental Section:

All reactions were performed under argon to ensure an inert environment. All chemicals were purchased from Sigma-Aldrich, Alfa-Asar and Fisher scientific. All NMR spectra are recorded either in $CDCl_3$ or in CD_3OD unless otherwise mentioned in Varian 400MHz instrument. Triethylamine was distilled over KOH and stored over KOH under argon. All other anhydrous solvents were purchased from Sigma-Aldrich. Fmoc and side chain protected Amino acids were purchased from Novabiochem.

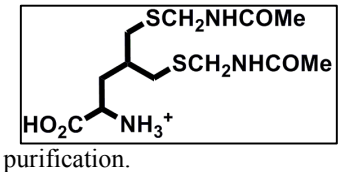
Synthesis of 1B: Compound **1A** [S1] (1g , 2.10 mmol) was dissolved in 6 mL dry DMSO in an oven dried round bottom flask. **Acetamidomethylthiol** [S2] (880 mg, 8.4 mmol) of was treated with 201mg (8.4 mmol) of NaH in 7.5 ml of dry DMSO. Resulting solution was stirred under argon for 15 min after which it was added to the previous solution in a drop wise fashion. The mixture was stirred until all the reactants were consumed and monitored by TLC (generally for 3 hrs). Following the completion of the reaction, 200 ml water was added to the reaction mixture and extracted with ethyl acetate. The organic layer was separated and washed with (5×100) ml of distilled water. Organic layer was dried over $MgSO_4$ and solvent was evaporated to dryness under reduced pressure. Resulting colorless oil was purified on a silica gel column using (1:1) CH_2Cl_2: Acetone. R_f=0.63(CH_2Cl_2:Acetone=1:1). Yield=700mg (67%), 1H NMR ($CDCl_3$) δ ppm 7.35 (b, 1H), 6.84(b, 1H), 5.25 (d,*J*=9.6 Hz, 2H), 4.37 (m, 2H), 4.21 (m,2H), 4.05 (m,1H), 2.84(m,2 H),2.46 (m, 2H), 1.97 (s,3H), 1.96 (s,3H),1.73(m,2H), 1.41(s,9H),1.37 (s,9H). 13C ($CDCl_3$) δ ppm 171.65, 170.30, 156.07, 82.40, 80.21, 51.86, 41.02, 39.92, 37.08, 34.69, 33.86, 33.01, 28.30, 27.96, 23.21, 23.16.



HRMS Calculated for $C_{21}H_{40}N_3O_6S_2$ (M^+) 494.2364, obtained 494.2359

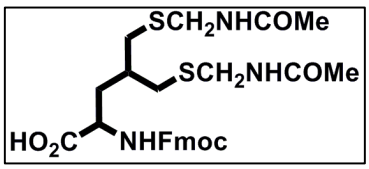
Synthesis of 1C:

Compound **1B (**150 mg, 0.304 mmol) was treated with 0.3 ml (3.9mmol) of TFA and 0.155 ml (0.76 mmol) of triisopropylsilane in a round botttom flask under strictly anaerobic condition. After 3 hours TFA was evaporated under reduced pressure and the resulting oily residue was triturated with ether to yield a white precipitate which was filtered and washed with another portion of ether. The compound was used for next step without any further purification.



Synthesis of 1D:

The solid precipitate of **1C** was transferred to a new round bottom flask and dissolved in 2 ml of 10% $NaHCO_3$. To this stirring solution under argon, 119 mg (0.353 mmol) of Fmoc-Osu dissolved in 0.70 ml of THF was added drop wise with vigorous stirring. The resulting solution was stirred overnight under argon. The organic layer was evaporated under reduced pressure and pH of the aqueous layer was adjusted to ~4 by the addition of 10% citric acid solution. The aqueous layer was then extracted with (5×100) ml of ethyl acetate. Combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Resulting solid was purified on a silica gel column using (1:1) CH_2Cl_2 : Acetone. R_f=0.33 (CH_2Cl_2: Acetone=1:1), Yiled=54mg (32% over two steps) 1H NMR (CD_3OD) δ ppm 7.67 (d, J=8Hz, 2H), 7.53 (d, J=8Hz, 2H), 7.27 (t, J=8Hz,2H), 7.19 (t,J=7.36,2H), 4.31 (m,1H), 4.20-4.0 (m,7H), 2.68-2.57(m,4H),2.0-1.92 (m,8H) 1.63 (m,1H).13C (CD3OD) δ ppm 171.92, 171.89, 156.99, 143.91, 143.70, 141.14, 127.37, 126.74, 124.75, 124.71, 119.53, 66.37, 53.79, 40.30, 40.10, 35.46, 34.25, 34.13, 33.19, 21.36. HRMS Calculated for $C_{27}H_{33}N_3O_6S_2$ ($M+Na^+$) *m/Z*=582.1735, obtained 582.1709, ($M+K^+$) = 598.1436, obtained 598.1448.

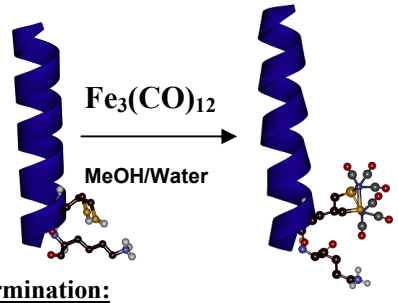


Synthesis of the Peptides: All peptides were synthesized in a CEM Liberty SPS automated peptide synthesizer on a PAL-ChemMatrix resin using standard Fmoc-based SPPS with a slight variation for **Peptide 1** in 0.1 mmol scale. All of the amino acids were double coupled at all positions. For **Peptide 1**, first three amino acids were coupled using the synthesizer after which the resin was removed from the synthesizer and manually deprotected using 20% piperdine in DMF. Then it was coupled with 2.5 fold

excess of **1D**, using standard coupling conditions for 12 hours. Completion of the reaction was monitored by cleaving a small portion of resin and then characterizing the mass of the system. The resin was then put back in the synthesizer and rest of the synthesis was carried out as usual. All the peptides were manually deprotected and then acetylated in the N-terminus. For K16Dt, the Acm protecting group on the side chain was cleaved using I_2 (15 equivalent excess) in DMF for 2 hours on the resin. For **Peptide 2**, the peptide was cleaved from the resin using 95% TFA, 2.5% water and 2.5% TIPS. **Peptide 1** was cleaved from the resin using 94% TFA, 2.5% water, 2.5% BME, 1% TIPS. Following the cleavage the resin was filtered off and collected TFA was evaporated using a stream of nitrogen through the solution. Ice cold ether was added to the residue which resulted in a white precipitate that was centrifuged and the resulting pallet was collected after decanting the ether. The solid precipitate was dissolved in water and lyophilized to get a white fluffy powder. Peptides were purified on a Vydac-C18 semiprep column using A (0.1% TFA in water) and B (95% acetonitrile, 5% water and 0.1% TFA) solvent system. For **Peptide 1**, after lyopholization it was dissolved in water and a large excess of TCEP was added to the solution to avoid oxidation of the dithiol unit. Appropriate fractions from HPLC purification were collected and lyophilized. Mass of the peptides was confirmed in MALDI and purity of the peptides was checked by re-injecting them on a C-18 analytical peptide. All the peptides used for further experiments were at least 99% pure (Fig.1-3). Cluster incorporated peptide was also purified in a similar manner except no reducing agent was added to minimize cluster degradation.

Cluster Incorporation Reaction:

2.4 mg (1.2mmol) of **Peptide 1** was dissolved in 0.5ml of degassed water in a round bottom flask fitted with a water condenser. To this solution, was added 4.44 mg (8.7 mmol) of $Fe_3(CO)_{12}$ dissolved in 1.7 ml of methanol. The reaction mixture was refluxed under argon for two hours. In the course of the reaction a change in color was observed from green to red. The solvent was evaporated under reduced pressure. Resulting brown solid was dissolved in 10 ml of water. 0.1 ml of this solution was centrifuged and the supernatant was injected on a Vydac C-18 analytical column to monitor the progress of the reaction. UV-Vis absorbance was monitored at 220 nm and 335 nm simultaneously to follow cluster incorporated peptide. Cluster incorporated peptide was purified on a Vydac semipreparative C-18 column using 5ml/min flow rate. Appropriate fractions were collected and lyopholyzed. Identity of the peptides was confirmed using MALDI-MS for apo peptides and ESI-MS for holo-peptide. Purity of the peptide was confirmed by reinjecting the purified peptides on a C-18 analytical column.



Protein Concentration determination:

For **peptide 1** and **2**, peptide concentration was determined from absorbance of at 276 nm (ε=1455). **Peptide1-$[Fe_2(CO)_6]$** has an absorption peak at 327 nm that overlaps with the absorption peak at 276 nm, so absorbance at 276 nm is not a correct reflection of peptide concentration. To determine the concentration of **Peptide1-$[Fe_2(CO)_6]$** two different techniques were employed. Firstly, a fluorimetric standardization curve was constructed using **peptide 1** as standard. Serial dilutions of stock **peptide 1** in 10 mM TRIS of 7.5 pH were made. Samples were excited at 276 nm and emission spectra were monitored in 280-400 nm range. A standard curve was constructed by plotting maximum emission intensity vs. concentration. From this standard curve, concentration of unknown **peptide 1-$[Fe_2(CO)_6]$** was determined. Peptide concentration of the same unknown sample was also determined using Amino Acid Analysis technique (UC-Davis proteomics facility). Concentrations determined from two different techniques were within 4% of each other.

FTIR:

FTIR spectra were recorded on a Bruker Vertex 70 series instrument by depositing a thin film on a CaF_2 window and drying it under vacuum. The optical chamber was flushed with Nitrogen for 15 minutes before each scan.

UV-Vis:

UV-Vis spectra were recorded using a Cary-50 UV–vis spectrometer in water.

CD Spectroscopy:

CD spectra were recorded in a JASCO J-815 spectropolarimeter. All the CD spectra were recorded in 10mM TRIS pH=7.5 buffer. For each peptide, CD spectra were recorded at three different concentrations (30uM, 50uM and 100uM). Each spectra is an average of 6 accumulations. For **Peptide 1**, the peptide was reduced with TCEP to exclude any kind of intermolecular or intramolecular covalent bond formation. For **Peptide 1-[$Fe_2(CO)_6$]** the sample was dissolved in a degassed buffer solution to avoid aerial degradation of cluster. CD spectra were recorded for two different temperatures, 4°C and 20°C respectively. Fraction helix (f_{helix}) was determined using the following equation.

$$f_{helix} = \frac{[\theta]_{222}}{40000(1 - 2.5/N)}$$

Where [θ] is mean residue molar ellipticity at 222nm and N is the number of backbone amide bonds. Error in f_{helix} is reported as standard deviation of the mean. It is worthy to mention that our fraction helix deviate significantly from the reported value of equivalent peptides mainly because of the difference in conditions used to record the CD spectra. Most of the reported CD spectra for equivalent peptides are reported in 1 M NaCl, 1 mM sodium phosphate, 1 mM sodium citrate, and 1 mM sodium borate (pH 7) at 0 °C, where as all our spectra are recorded in 10mM TRIS, pH=7.5 buffer at 4°C and 20°C to get a better (S/N) below 200nm. We monitored molar ellipticity at 222 nm as a function of temperature to evaluate the stability of the peptides to thermal denaturation. Qualitatively, we were able to observe enhanced stability of peptide **1-[$Fe_2(CO)_6$]**; the lack of a folded baseline, however, prevented a rigorous analysis of thermodynamic parameters.

Photocatalytic Hydrogen Production:

In a typical photocatalytic hydrogen evolution experiment 9.33 uM **Peptide 1-[$Fe_2(CO)_6$]** , 150uM $Ru(bipyridine)_3,6H_2O$ and 50mM ascorbate in 50mM of citrate buffer of pH= 4.5 in total 1mL were taken in an airtight cuvette with 1 cm path length. All the solutions were degassed extensively to exclude any atmospheric oxygen. The cuvette was then irradiated with a 450W xenon lamp using a pyrex cutoff filter excluding wavelength <410nm and an IR cut off filter to exclude light > 700nm. The light intensity at the sample was maintained 1100 W/m^2 for all the experiments. For the control experiments, all the components of the reaction mixture were added in the same ratio except the photosensitizer i.e. $Ru(bpy)_3,6H_2O$. The Hydrogen evolved as a function of time was monitored by taking 100uL of sample overhead gas with a gas tight syringe and injecting and analyzing on a GC (SRI instruments, Model no. 310C) using a 5Å molecular sieve column, a thermal conductivity detector and Ar carrier gas. The GC instrument was calibrated using a gas standard (1% H_2 and bulk N_2).

Electrochemistry:

All electrochemical studies were performed on CH-Instruments model 420B electrochemical workstation using a SCE reference electrode, a Pt-mesh counter electrode and a glassy carbon working electrode (3mm diameter). 100 mM citrate and 25mM NaCl buffer of desired pH (3.6, 4.0, 4.5, 5.0 and 5.5) was utilized to record the cyclic voltammogram. All scans were performed at 200mV/s scan rate. Catalyst concentration was 42 μM. A chemical degradation of the catalyst was observed after 1st scan that deposited on the electrodes. Continuous cleaning of the electrode was necessary to successfully repeat the electrochemical scans. An increase of current around -1.1V was observed as the pH of the solution was lowered. No current increase was observed with substoichiometric ratio of proton to catalyst. (Fig. 8)

Figure Descriptions:

Figure 1: Analytical HPLC trace of K16L.

Figure 2: Analytical HPLC trace of K16Dt.

Figure 3: Analytical HPLC trace of K16Dt_$Fe_2(CO)_6$

Figure 4: Analytical HPLC trace of K16Dt before and after cluster incorporation

Figure 5:CD spectra of K16L,K16Dt, K16Dt_$Fe_2(CO)_6$

Figure 6: ESI-MS of **Peptide 1_[2Fe-2S].**

Figure 7:pH dependance of peak current

Figure 8: peak current difference vs. equivalence of proton plot.

Figure 9: 1H NMR spectrum of **1B**.

Figure 10: 13C NMR spectrum of **1B**.
Figure 11: 1H NMR specturm of **1D.**
Figure 12:13C NMR spectrum of **1D.**

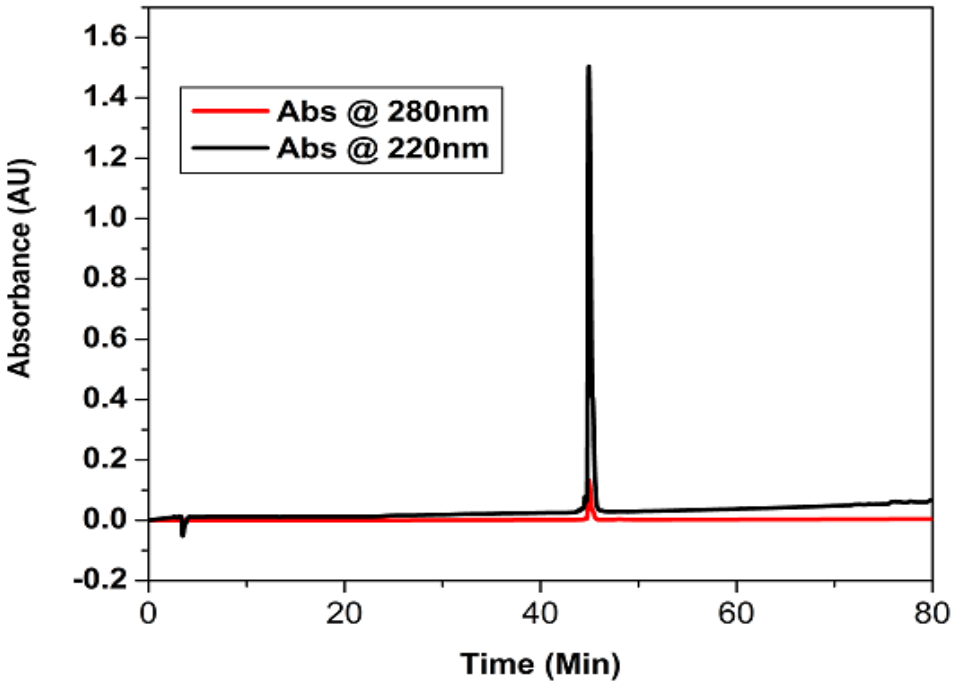


Figure 1: Analytical HPLC of pure K16L. 1%/ minute gradient from solvent A to solvent B was used.

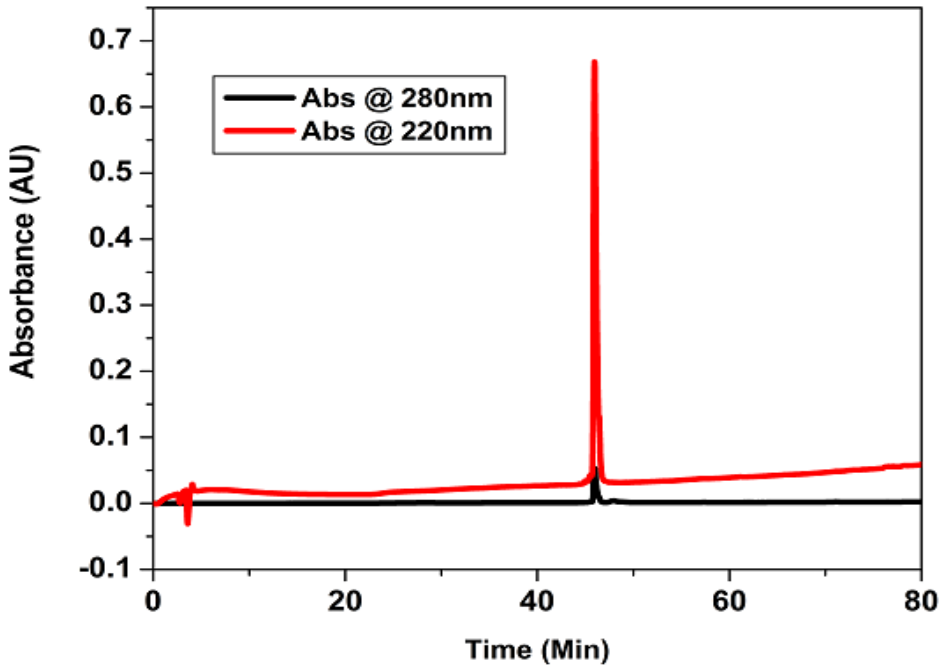


Figure 2: Analytical HPLC of pure K16Dt with 1%/minute gradient.

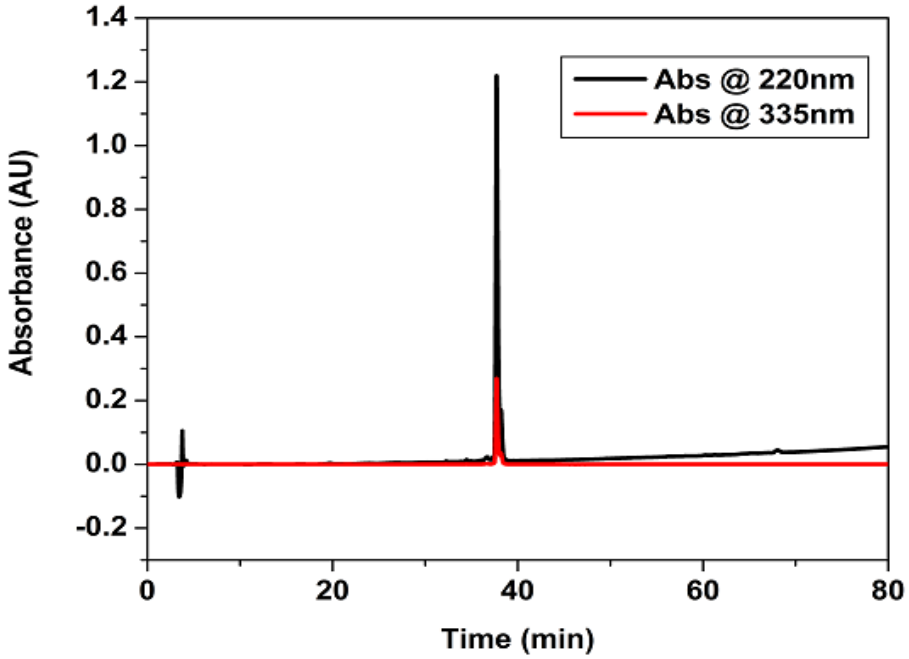


Figure 3: Analytical HPLC of pure K16Dt_$Fe_2(CO)_6$. 1%/minute gradient from solvent A to solvent B was used.

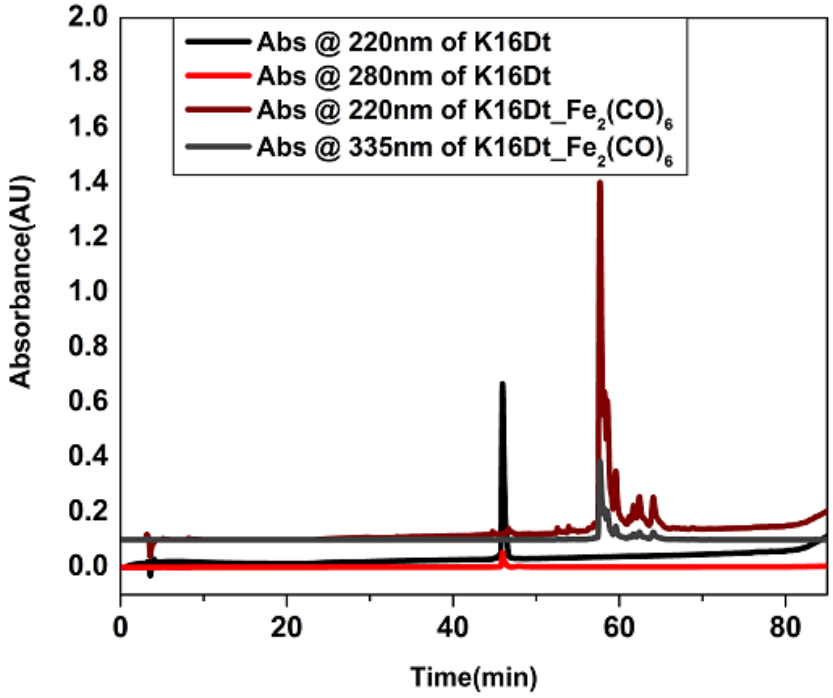


Figure 4: HPLC traces of K16Dt before and after metallocluster incorporation showing~100% conversion. The trace for K16Dt_$Fe_2(CO)_6$ is upscaled a little bit for clarity.

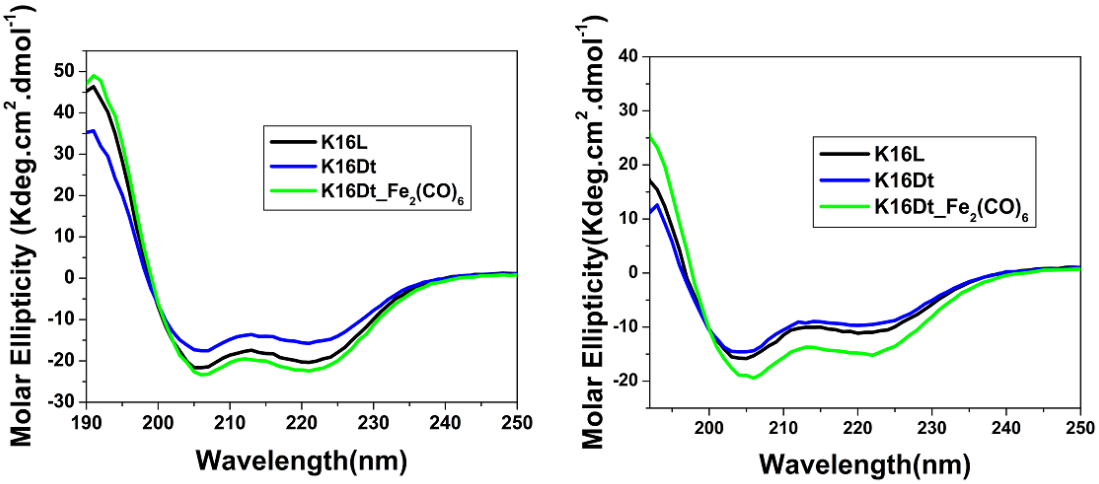


Figure 5: CD spectra at 4°C (left panel) and 20°C (right Panel)

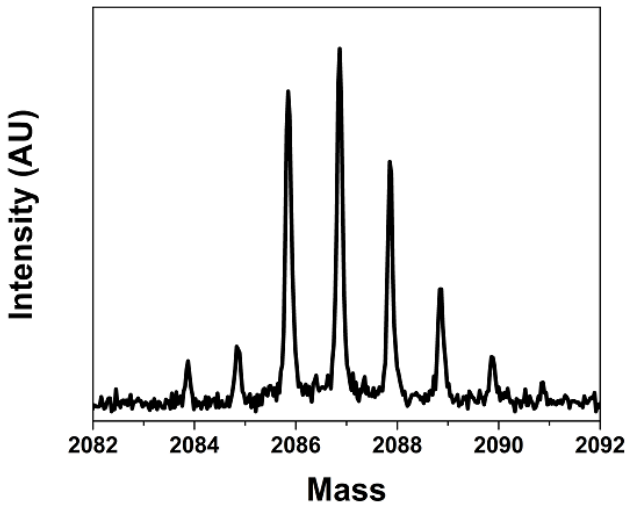


Figure 6: ESI-MS of **Peptide 1_[2Fe-2S]**

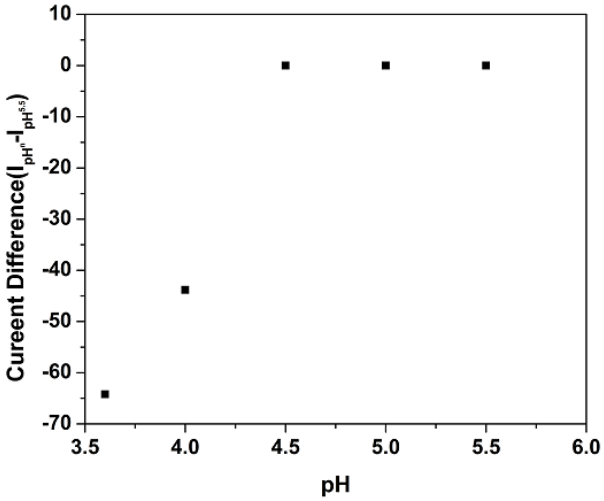


Figure 7: pH dependance of peak Current

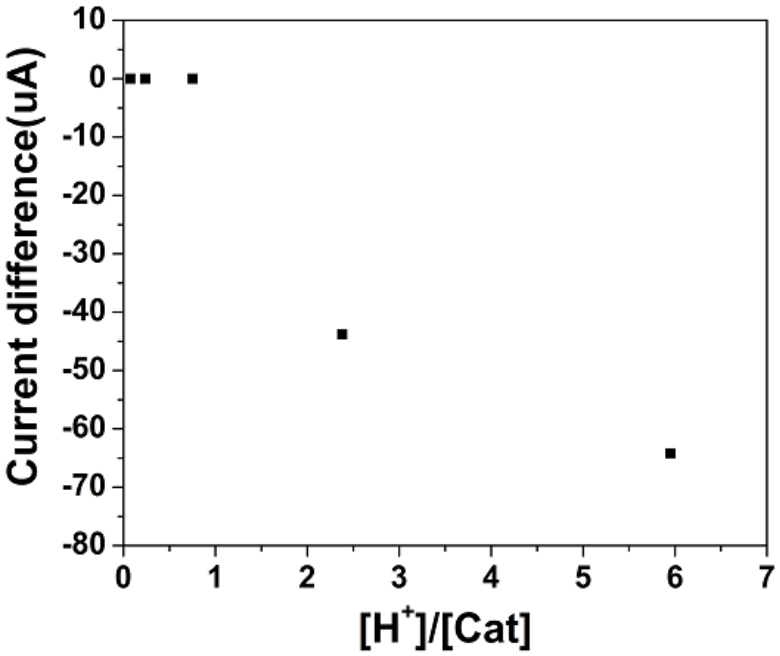


Figure 8: Peak Current diffence vs equivalence of proton Plot

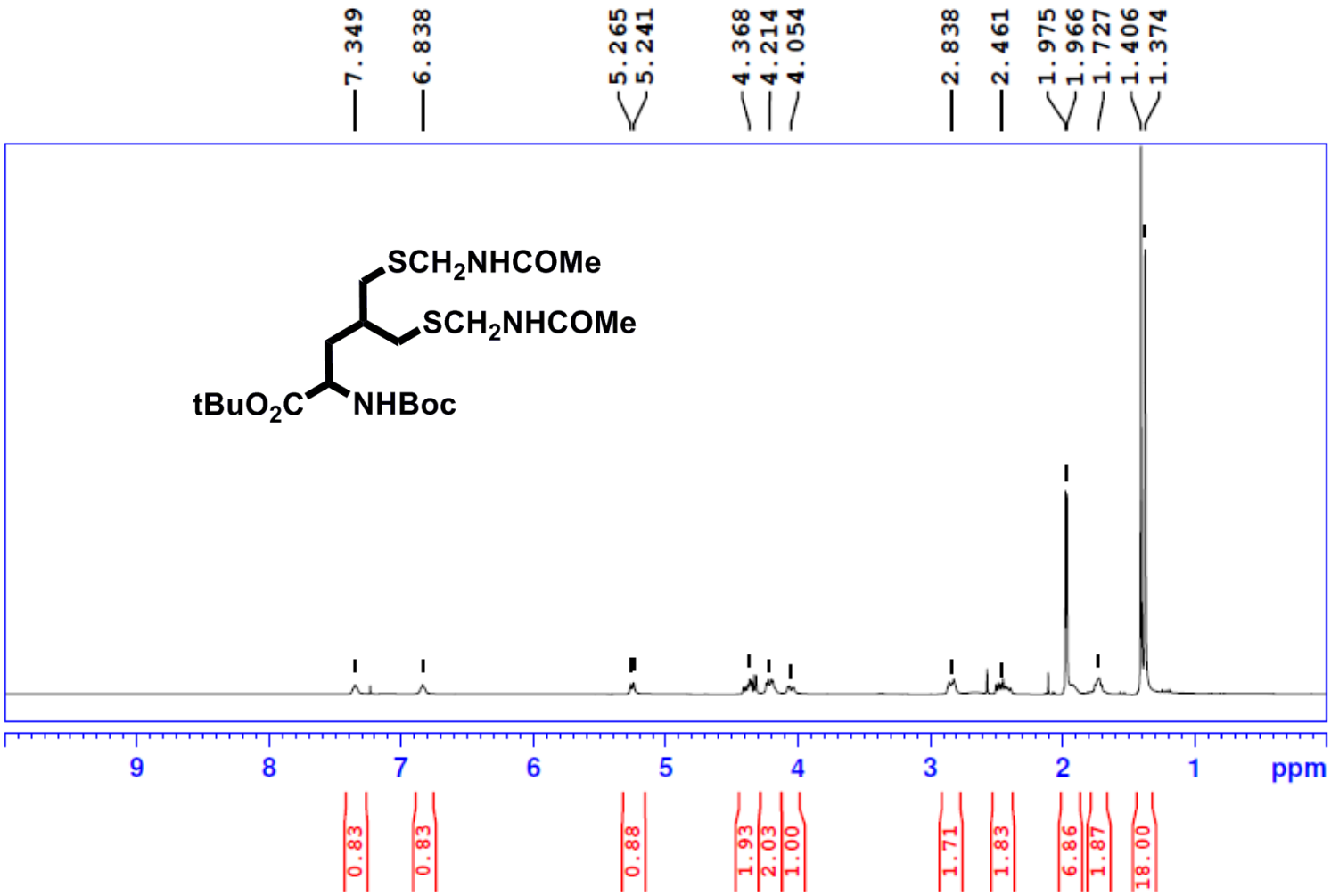


Figure 9: 1H NMR spectrum for compound **1B**

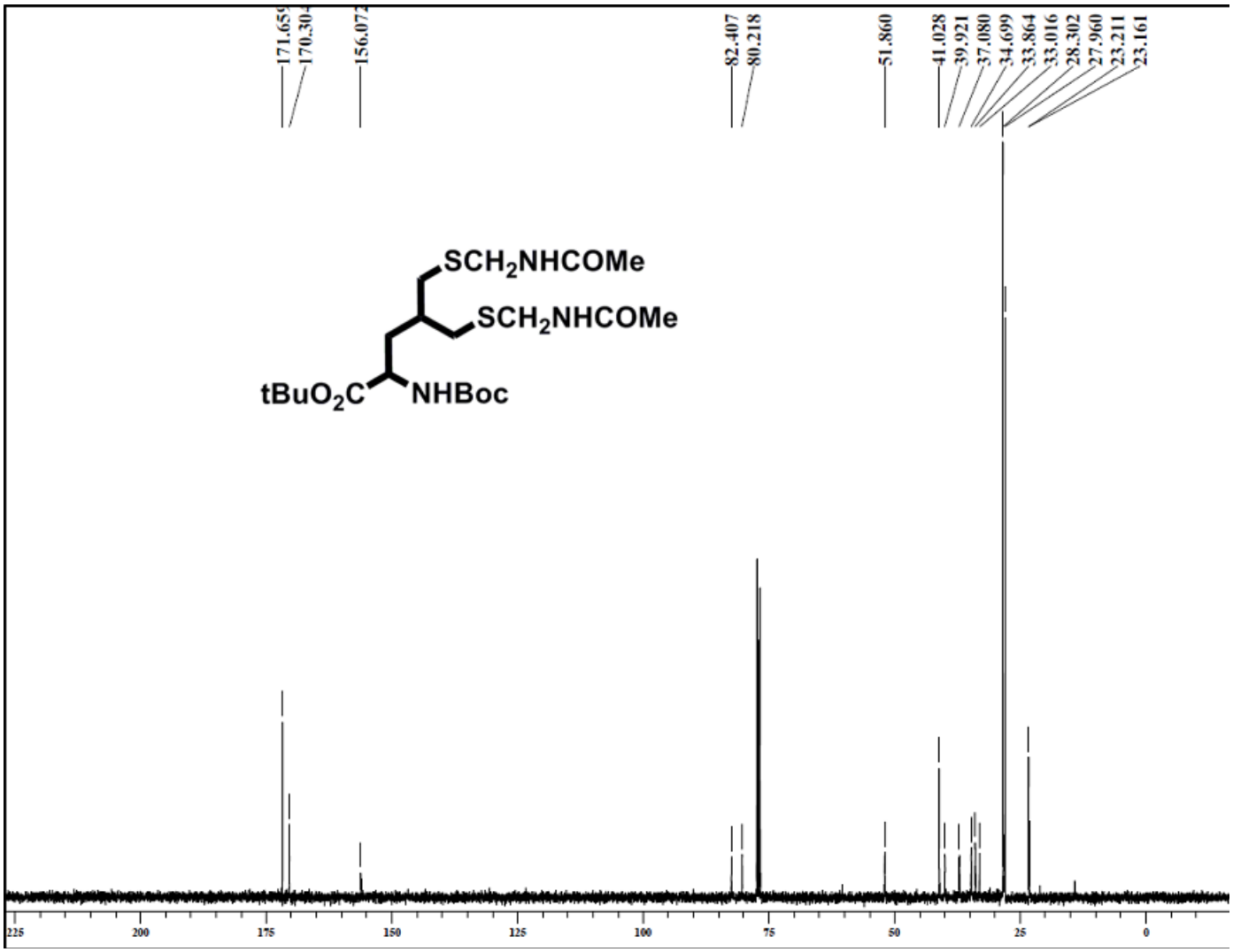


Figure 10: 13C NMR spectrum for compound **1B**

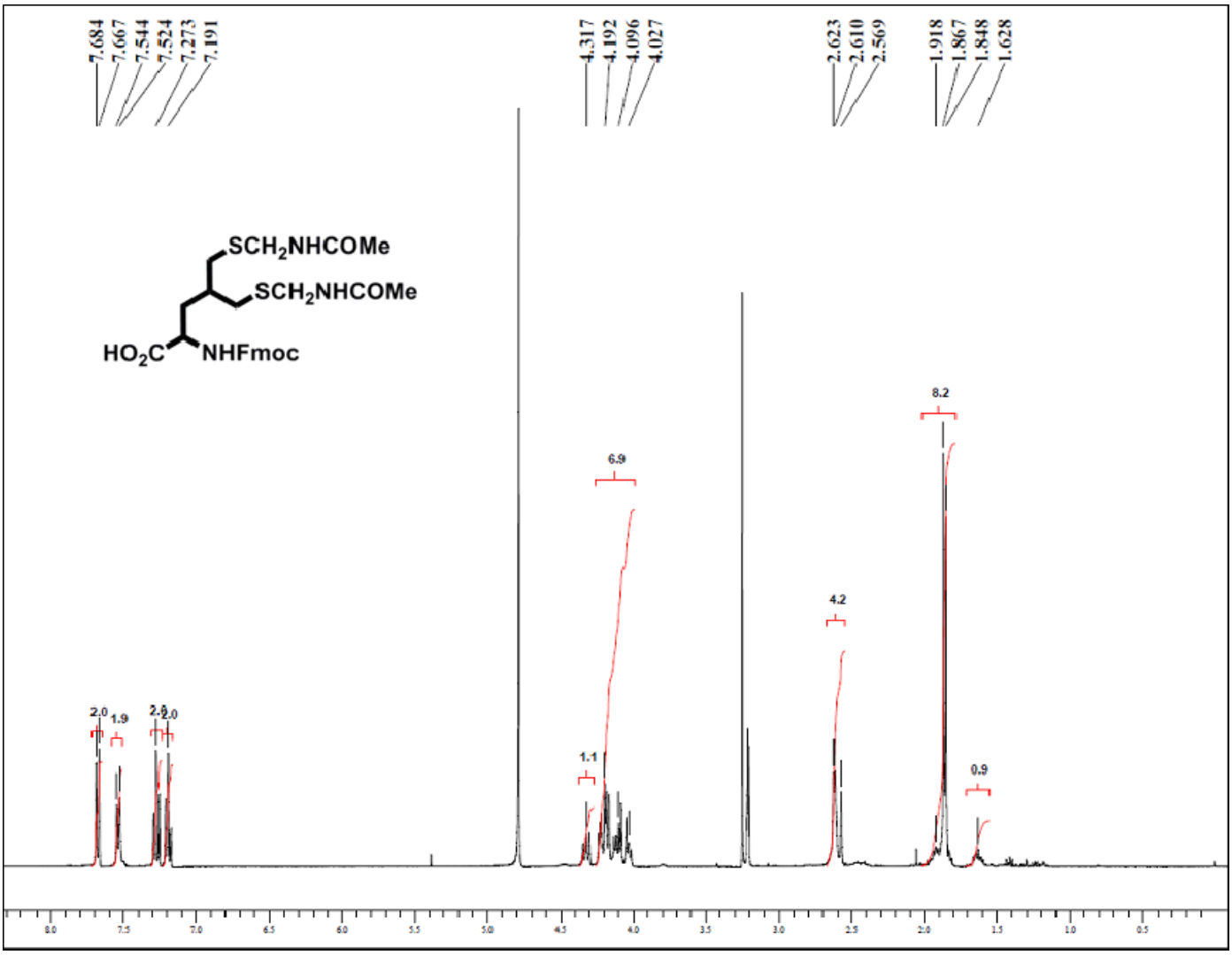


Figure 11: 1H NMR spectra of **1D.**

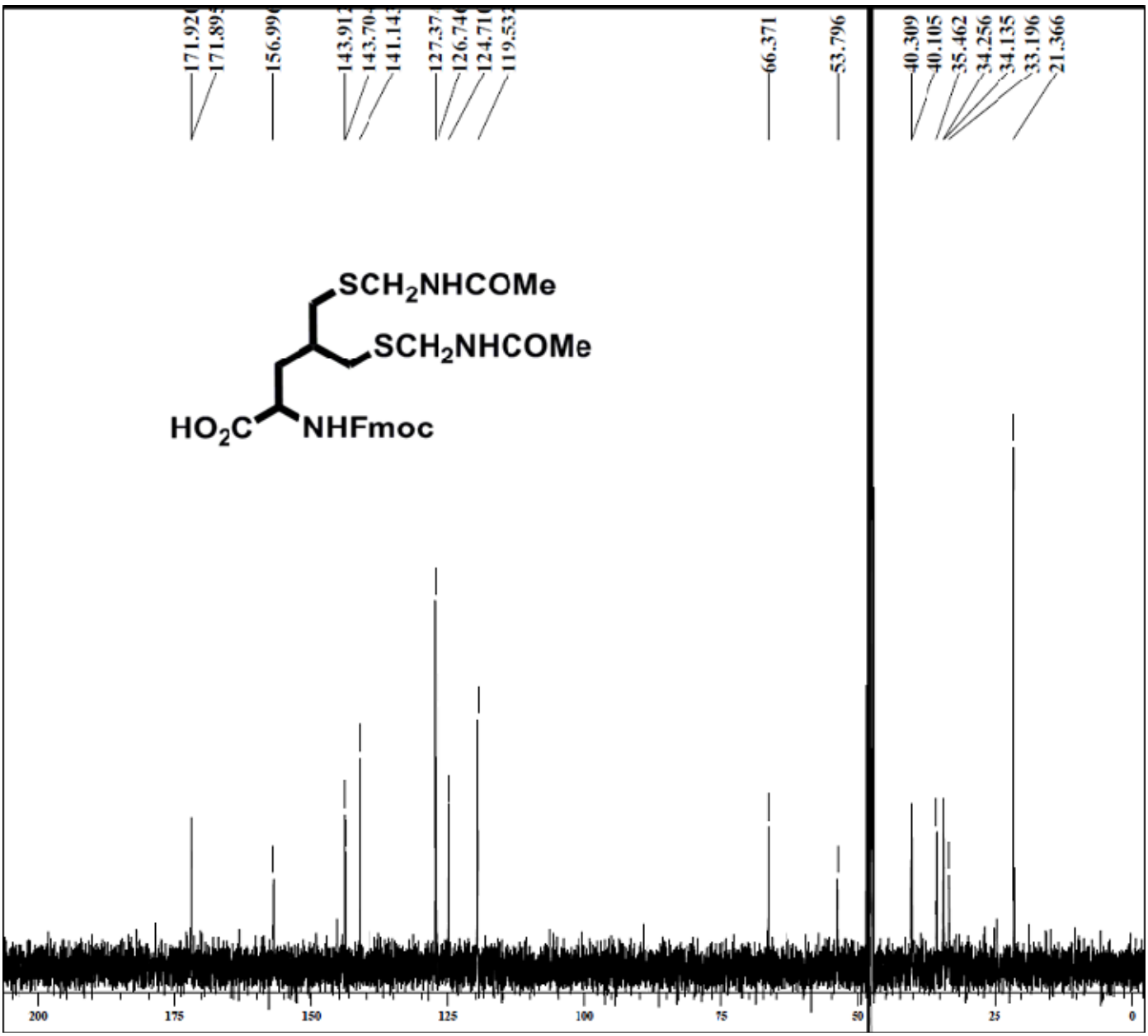


Figure 12: 13C NMR spectra of **1D**

References:

S1: E. Morera, F.Pinnen, G. Lucente , *Org. Lett.*, 2002, **4**, 1139-1142.

S2:R. Hartung, G. Rainer, S. Schlaf, G. Silvennoinen, K. Polborn, P. Mayer, H. Pfaendler, *Synthesis,* 2009, 3, 495-501