Electronic Supporting Information

A Hemicyanine-based Fluorescent Probe for Hydrazine Detection in Aqueous Solution and Its Application in Real Time Bioimaging of Hydrazine as a Metabolite in Mice

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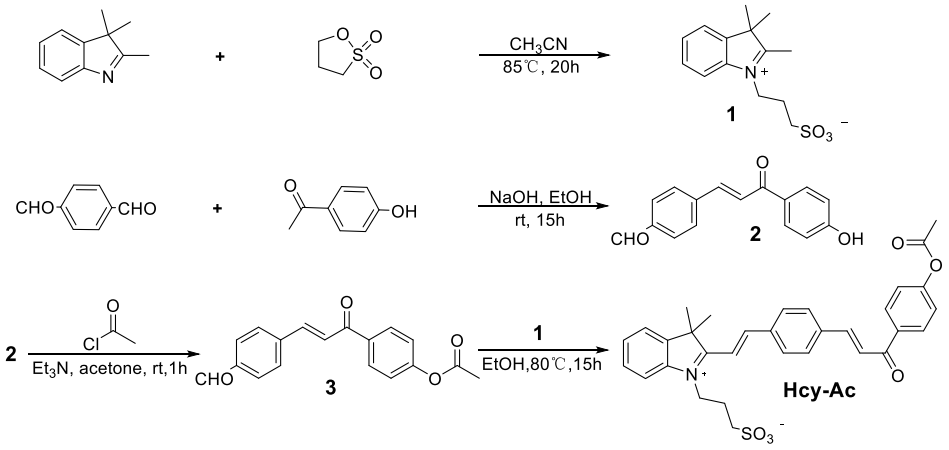
CONTENT

1. Materials and Instruments

All chemical reagents and solvents were obtained commercially and used as received without further purification unless otherwise stated. Ultrapure water was purified from Millipore. Silica gel P60 (Qingdao, mesh number 200-300) was used for column chromatography. NaCl, Na_2SO_4, $NaNO_2$, Na_2CO_3, KCl, $CaCl_2$, $FeCl_3$, $CuSO_4$, $MgSO_4$, NH_4Cl, *L*-Lys, *L*-Ile, *L*-Pro, *L*-Arg, *L*-Phe, *L*-His, ammonia, diethylamine, ethylenediamine, aniline, benzylamine, cyclohexylamine, ethanolamine, hydroxylamine, thiourea, isoniazid and acethydrazide were analytical grade products and their solutions in ultrapure water were used in selectivity study. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 or 600 M instrument and chemical shifts are given in ppm using the peak of residual proton signals of DMSO as the internal standard. Mass spectra (ESI) were carried out on a Finnigan LCQ^{DECA} spectrometer with ESI mode. UV−vis and fluorescence spectra were performed on a SHIMADZU UV-2450 spectrophotometer and a VARIAN Cary Eclipse FL1003 M013 spectrometer respectively. The pH value was measured using a digital pH meter (pHS-25, Century Ark, China). The fluorescence images of cells were recorded on an inverted fluorescence microscope (Olympus, Japan) and the fluorescence images of mice were taken using a small animal living imager (PerkinElmer IVIS Lumina Ⅲ). Absolute fluorescence quantum yield was measured with integrating sphere on HORIBA FL-3000.

2. Synthesis and Characterizations of Hcy-Ac



Scheme S1. Synthetic route of the probe Hcy-Ac

Synthesis of 3-(2, 3, 3-trimethyl-3*H*-indol-1-ium-1-yl) propane-1-sulfonate (1).

A solution of 2,3,3-trimethyl-3*H*-indole (1.00 g, 6.29 mmol) and propane sultone (1.16 g, 9.43 mmol) in acetonitrile (5 mL) was refluxed for 20 h. After cooling to room temperature, the purple solid precipitated was filtered, washed with acetone (2 mL) and dried, then compound **1** was obtained as a lavender powder (1.69 g, 95.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 7.4 Hz, 1H), 7.83 (d, J = 5.8 Hz, 1H), 7.61 (dd, J = 9.0, 5.3 Hz, 2H), 4.72 – 4.59 (m, 2H), 2.83 (s, 3H), 2.62 (t, J = 6.3 Hz, 2H), 2.24 – 2.06 (m, 2H), 1.53 (s, 6H). ^{13}C NMR (100MHz, DMSO-d_6) δ 197.0, 142.4, 141.7, 129.8, 129.4, 123.9, 115.9, 54.6, 47.9, 47.1, 24.2, 22.5, 14.3.

Synthesis of (*E*)-4-(3-(4-hydroxyphenyl)-3-oxoprop-1-en-1-yl) benzaldehyde (2).

A mixture of terephthalaldehyde (0.50 g, 3.73 mmol) and p-hydroxyacetophenone (0.25 g, 1.84 mmol) was dissolved in absolute ethanol (20 mL) and sodium hydroxide (0.22 g, 5.50 mmol) was added slowly with stirring. After the solution was stirred for 15 h at room temperature, dilute hydrochloric acid was added dropwise to a pH value of 7 and the bright yellow solid precipitated was filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (cyclohexane: ethyl acetate = 6:1, v/v) to afford intermediate **2** as a light yellow powder (0.19 g, 41.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 10.05 (s, 1H), 8.12 (t, J = 7.2 Hz, 2H), 8.09 (d, J = 2.9 Hz, 2H), 8.07 – 8.00 (m, 1H), 7.97 (d, J = 8.1 Hz, 2H), 7.73 (d, J = 15.6 Hz, 1H), 6.91 (d, J = 8.6 Hz, 2H). ^{13}C NMR (100

MHz, DMSO-d_6) δ 193.1, 187.4, 162.9, 141.5, 141.0, 137.3, 131.8, 130.3, 129.9, 129.7, 125.5, 115.9. MS (ESI, *m/z*): calcd. for $[C_{16}H_{12}O_3+Na]^+$, 275.0684, found 275.0676.

Synthesis of (*E*)-4-(3-(4-formylphenyl)acryloyl) phenyl acetate (3).

To the solution of **2** (0.19 g, 0.75 mmol) in anhydrous acetone (3 mL) was added acetyl chloride (0.18 g, 2.30 mmol) dropwise at room temperature, followed by a solution of triethylamine (0.12 g, 1.19 mmol) in acetone (1 mL). After stirring for 1h, the white solid precipitated was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in dichloromethane (4 mL) and the organic phase was washed with 10 mL distilled water and saturated brine, dried over anhydrous sodium sulfate and evaporated, then, pure compound **3** was obtain as a yellow powder (0.19 g, 85.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.29 – 8.23 (m, 2H), 8.16 – 8.08 (m, 3H), 7.98 (d, *J* = 8.2 Hz, 2H), 7.81 (d, *J* = 15.7 Hz, 1H), 7.39 – 7.33 (m, 2H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.1, 188.4, 169.3, 154.8, 142.8, 140.7, 137.5, 135.3, 130.8, 130.3, 129.9, 125.2, 122.8, 21.4. MS (ESI, *m/z*): calcd. for $[C_{18}H_{14}O_4+Na]^+$, 317.0790, found 317.0789.

Synthesis of Hcy-Ac.

A mixture of **1** (0.27 g, 0.96 mmol) and **3** (0.19 g, 0.65 mmol) in absolute ethanol (5 mL) was heated to reflux for 15 h, then the reactant was concentrated under reduced pressure and the residue obtained was purified by column chromatography (CH_2Cl_2: MeOH = 40:1, v/v) to give pure **Hcy-Ac** as an orange powder (0.08 g, 22.2%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.49 (d, *J* = 16.3 Hz, 1H), 8.41 (d, *J* = 8.1 Hz, 2H), 8.30 (d, *J* = 8.5 Hz, 2H), 8.18 (d, *J* = 15.6 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 2H), 8.05 (d, *J* = 16.1 Hz, 2H), 7.92 – 7.89 (m, 1H), 7.83 (d, *J* = 15.6 Hz, 1H), 7.67 – 7.62 (m, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 4.97 – 4.86 (m, 2H), 2.72 – 2.64 (m, 2H), 2.33 (s, 3H), 2.22 (s, 2H), 1.83 (s, 6H). ^{13}C NMR (100MHz, DMSO-d_6) δ 188.4, 182.3, 169.4, 154.8, 153.2, 144.6, 143.1, 141.3, 139.5, 136.9, 135.4, 131.8, 130.9, 130.1, 129.6, 124.6, 123.6, 122.8, 115.9, 114.4, 63.3, 52.8, 47.6, 25.9, 25.3, 21.4. MS (ESI, *m/z*): calcd. for $[C_{32}H_{31}NO_6S+Na]^+$, 580.1872, found 580.1856.

3. Experimental Procedures for Spectroscopic Analysis and Bioimaging

Spectroscopic Analysis and Methods

2 μmol of probe **Hcy-Ac** was dissolved in 100 μL of DMSO and then diluted to 200 mL with PBS buffer (pH=7.4, 10 mM). 2 mL of sample solutions for absorption and fluorescence measurements were shaken well at 25℃ for 25 min before the spectra were recorded.

MTT assay of the probe

A standard MTT assay was conducted against HeLa cells to evaluate the cytotoxicity of **Hcy-Ac** and the results were shown in Fig. S1.

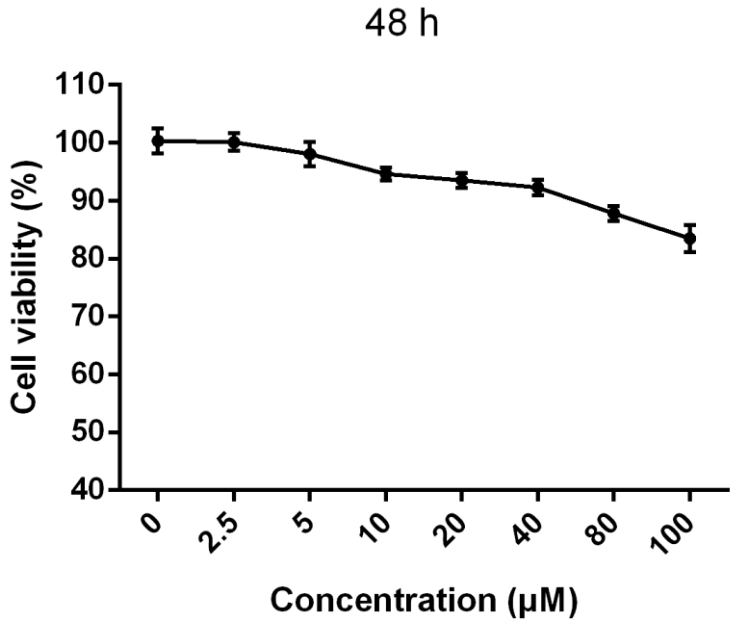


Fig. S1 Viability of HeLa cells estimated by MTT assay at different concentrations of **Hcy-Ac** for 48h.

Confocal Microscopy Imaging

Hela cells were purchased from the American type culture collection (ATCC) and stored in liquid nitrogen. After the cells were taken out and placed in water bath at 37℃ rapidly, the supernatant was discarded after centrifuged at 1500 rpm for 3 minutes. The bottom cell pellet was suspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with heat-inactivated 10% (v/v) fetal bovine serum and cultured in a cell

culture incubator at 37℃ under 5% CO_2 environment. When the cell density reached to 80%-90%, the adherent cells were digested with trypsin, collected by centrifugation and subcultured. Owning to the inherent toxicity of hydrazine, a large number of cells died when the concentration of hydrazine was higher than 50 μM. Thus, the concentrations of **Hcy-Ac** and hydrazine adopted for living cell bioimaging were selected to be 10 μM and 50 μM respectively.

Hela cells were seeded in a 12-well plate at a density of 5×10^3 cells per well and cultured until the cells were adherent. Then, the growth medium was removed and replaced with PBS buffer (pH=7.4, 10 mM, 1 mL) or probe **Hcy-Ac** (10 μM, 1 mL) and incubated at 37°C for 12 h. Half of the **Hcy-Ac**-treated HeLa cells were further washed three times with PBS buffer (pH=7.4, 10 mM) and then treated with hydrazine (50 μM, 1 mL) at 37°C for another 0.5 h. After all the cells were washed three times with PBS buffer (pH=7.4, 10 mM), confocal microscopy imaging was performed upon excitation at 430nm and images with λ_{em} = 480-590 nm were recorded.

Animal Models and In Vivo Imaging

The 6-week-old Kunming mice were provided by Animal Experimental Center of Sichuan University. All animal experiments were performed in compliance with the relevant laws and guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The animal studies were approved by the Ethical Committee on Animal Care and Use of West China School of Pharmacy, Sichuan University. Generally, three groups of mice were used for *in vivo* bioimaging. In the first group, the mouse was only given a tail-vein injection of **Hcy-Ac** (25 μL, 50 μM) and then imaged (Figure S-1a). In the second group, the mouse was injected with **Hcy-Ac** (25 μL, 50 μM) followed by hydrazine (25 μL, 500 μM) by tail-vein (Figure S-1b). In the last group, the mouse was given a tail-vein injection of **Hcy-Ac** (25 μL, 50 μM) followed by an intragastric administration of isoniazid (5.4 mg) in 0.6 mL PBS buffer (pH=7.4, 10mM) (Figure S-1c). Fluorescence images were taken at different time points (0, 5, 15, 30, 60, 90, 120 min) to evaluate the fluorescence intensity at 520 nm using IVIS spectrum imaging system upon excitation at 440 nm.

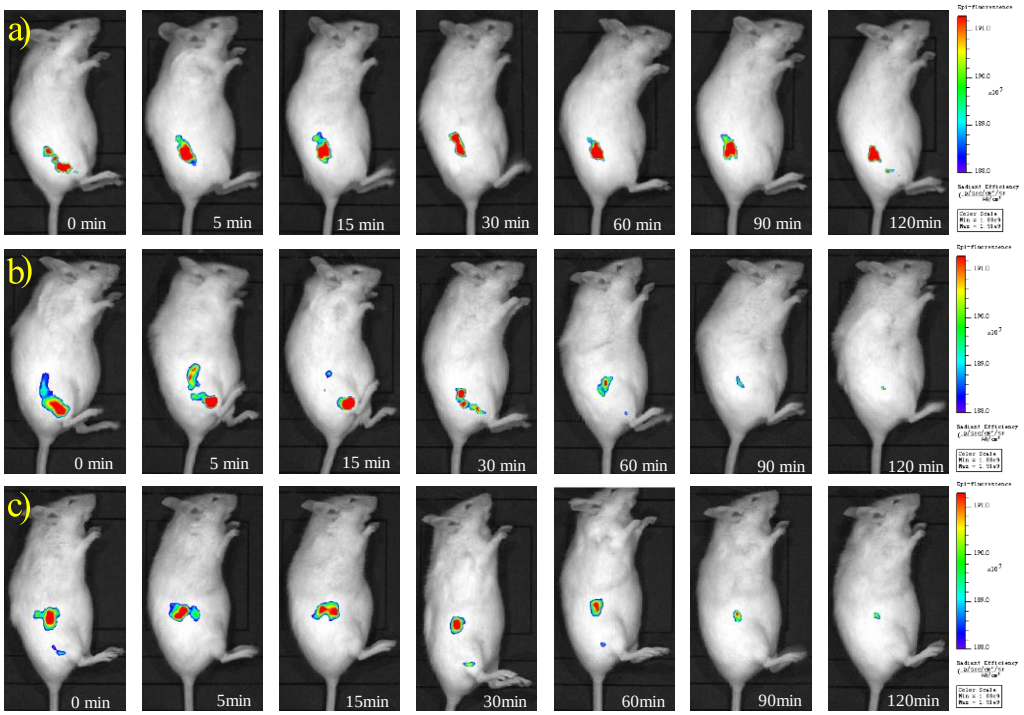


Fig. S2 Fluorescence images (pseudocolor) of a Kunming mouse. a: the mouse was given a tail-vein injection of **Hcy-Ac** (25 μL, 50 μM). b: the mouse was given a tail-vein injection of **Hcy-Ac** (25 μL, 50 μM) followed by hydrazine (25 μL, 500 μM). c: the mouse was given a tail-vein injection of **Hcy-Ac** (25 μL, 50 μM) followed by an intragastric administration of INH (5.4 mg) in 0.6 mL PBS buffer (pH = 7.4, 10 mM). The mice were imaged with an excitation filter of 440 nm and an emission filter of 520 nm. Images were taken at 0, 5, 15, 30, 60, 90 and 120 min.

4. NMR and MS Spectra

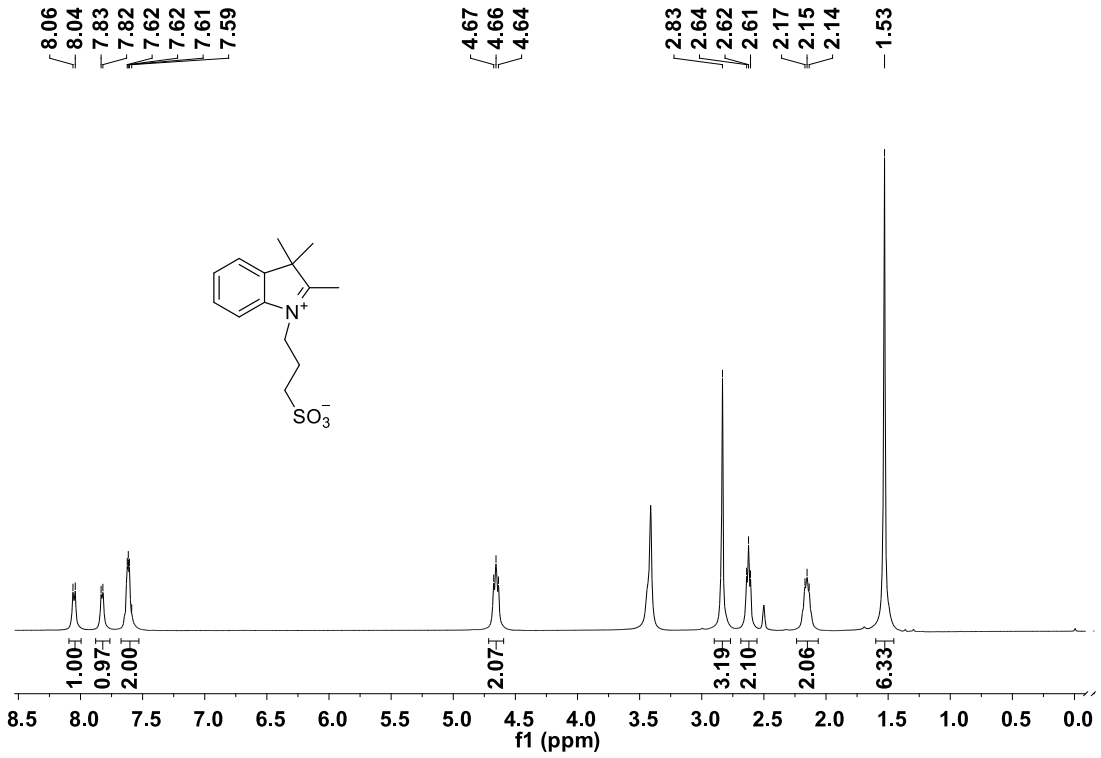


Fig. S3 ^{1}H NMR of **1** in DMSO-d_6

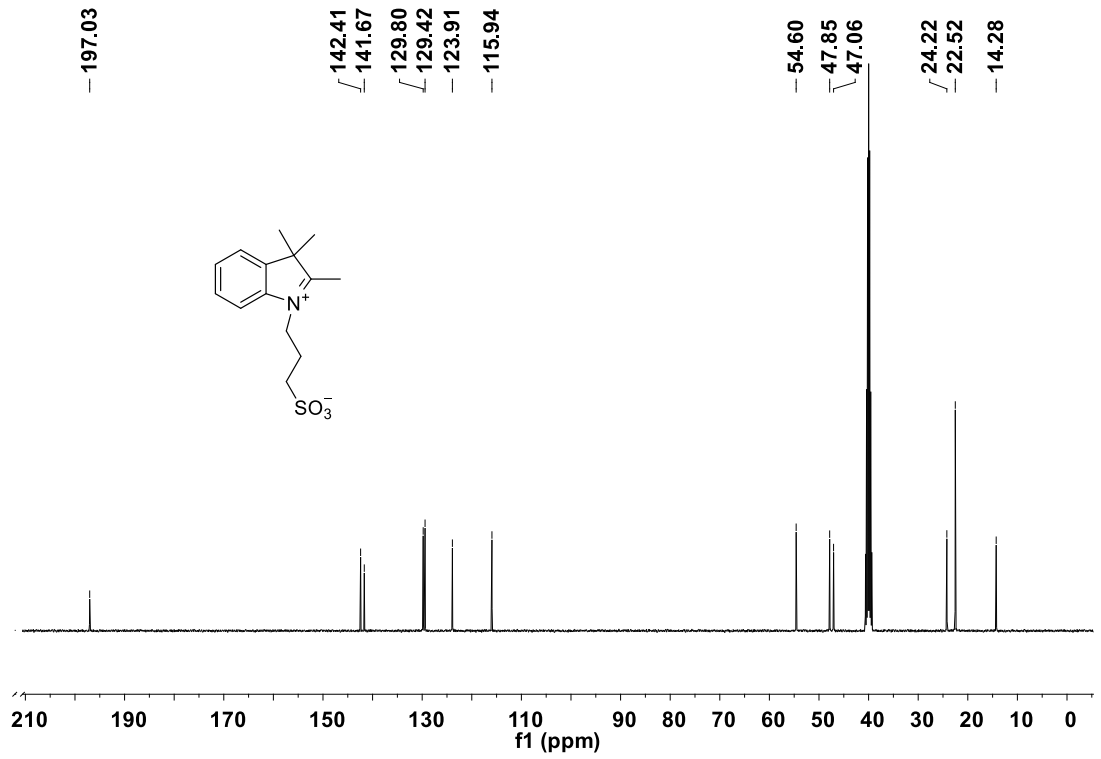


Fig. S4 ^{13}C NMR of **1** in DMSO-d_6

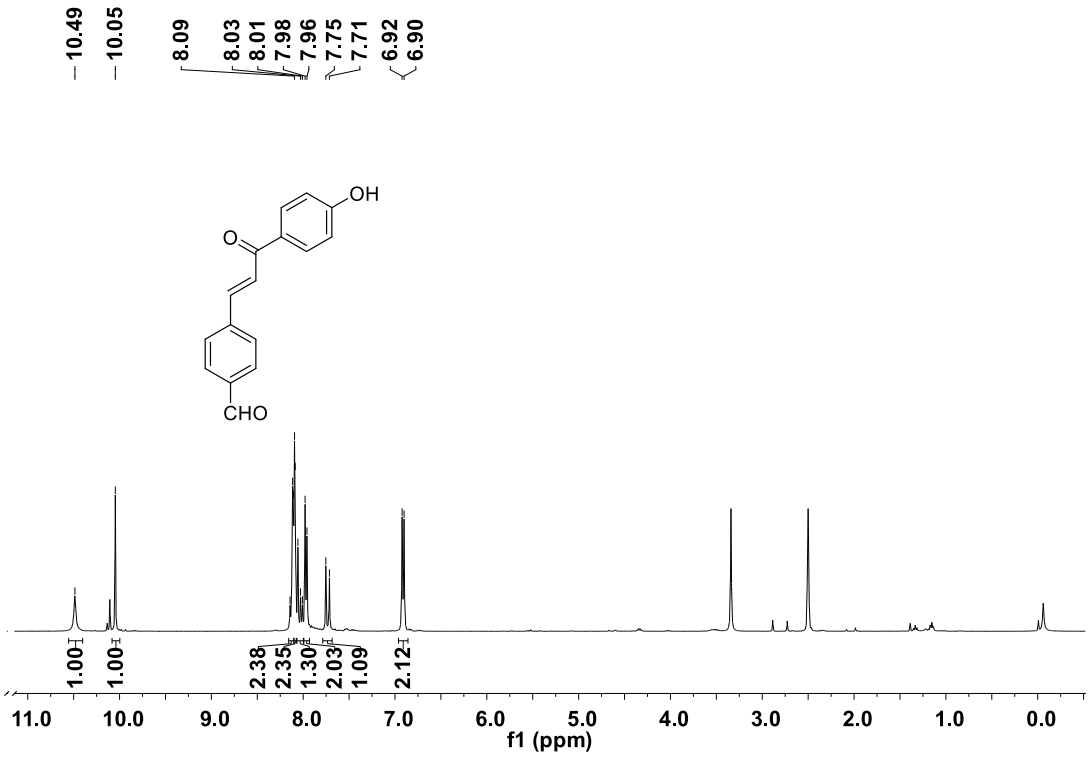


Fig. S5 ^{1}H NMR of **2** in DMSO-d_6

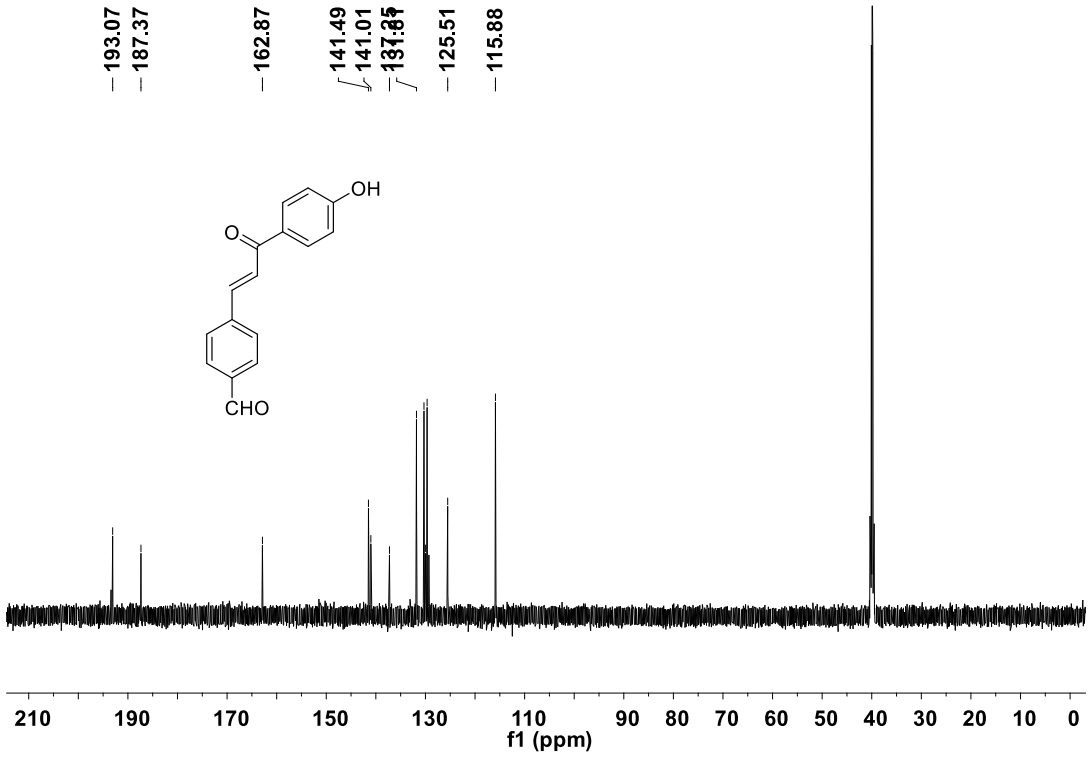


Fig. S6 ^{13}C NMR of **2** in DMSO-d_6

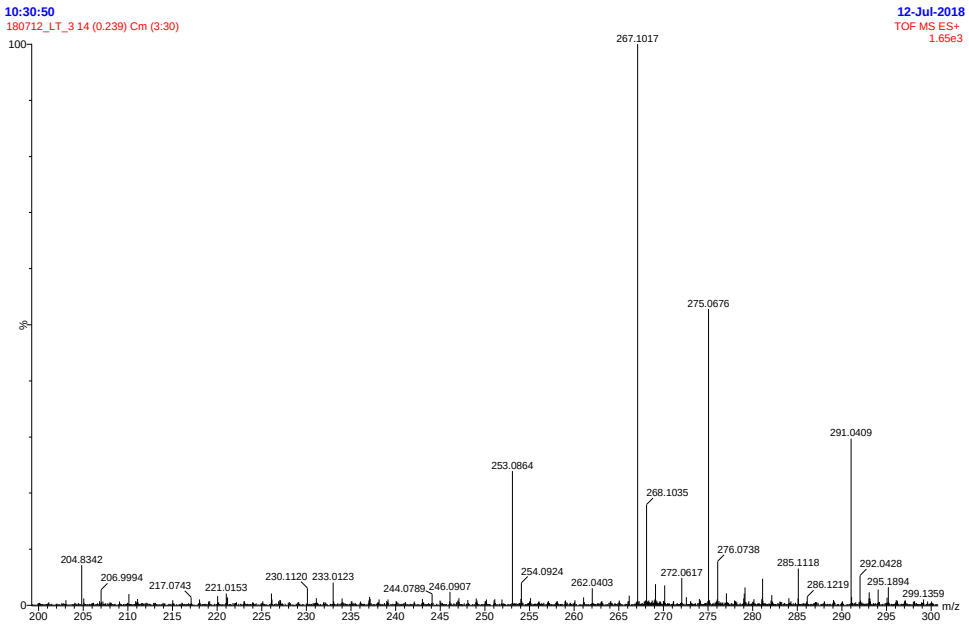


Fig. S7 Mass spectrum of **2**

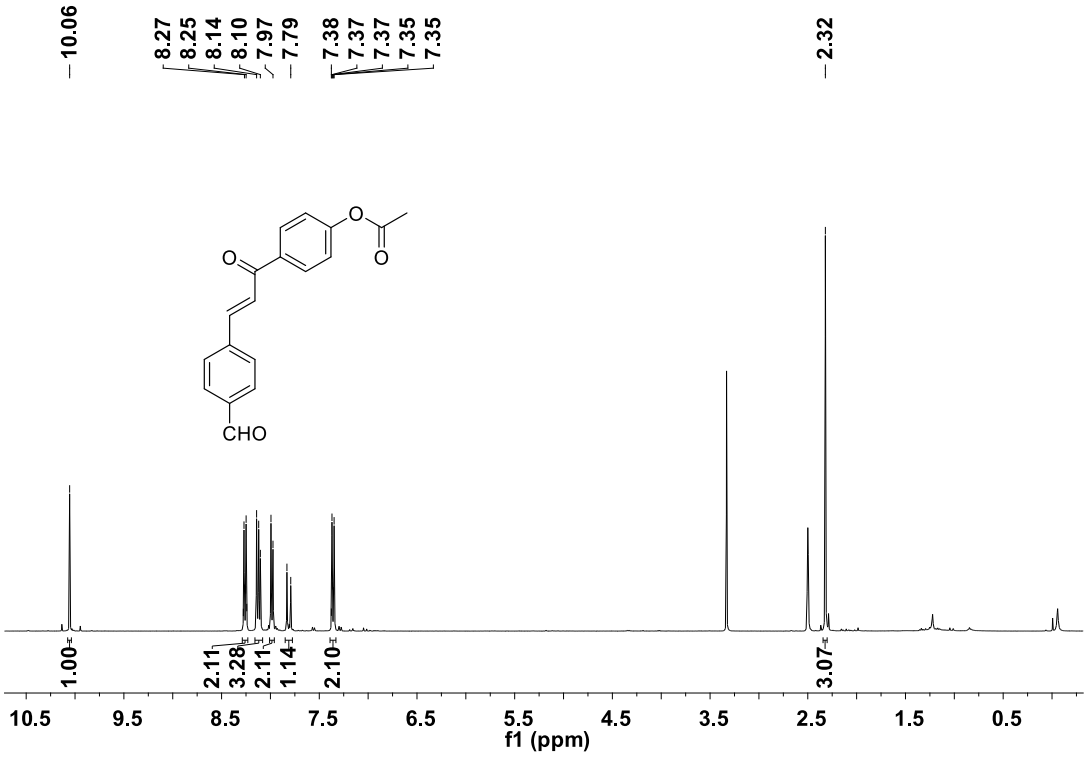


Fig. S8 ^{1}H NMR of **3** in DMSO-d_6

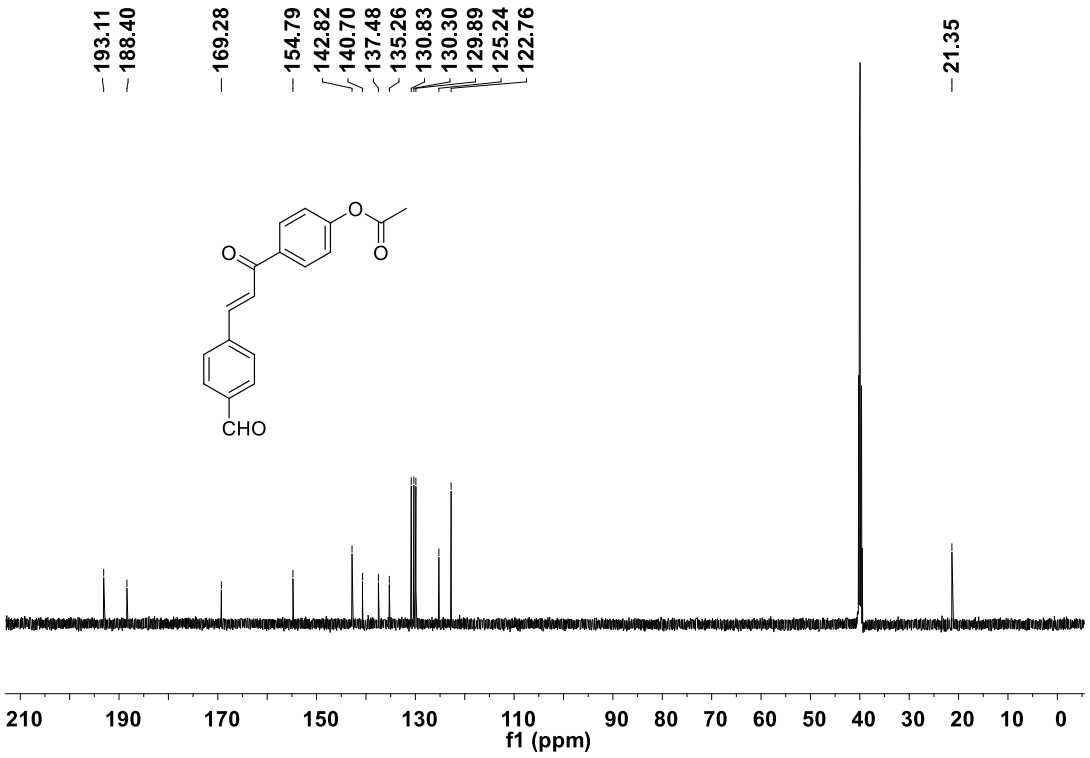


Fig. S9 ^{13}C NMR of **3** in DMSO-d_6

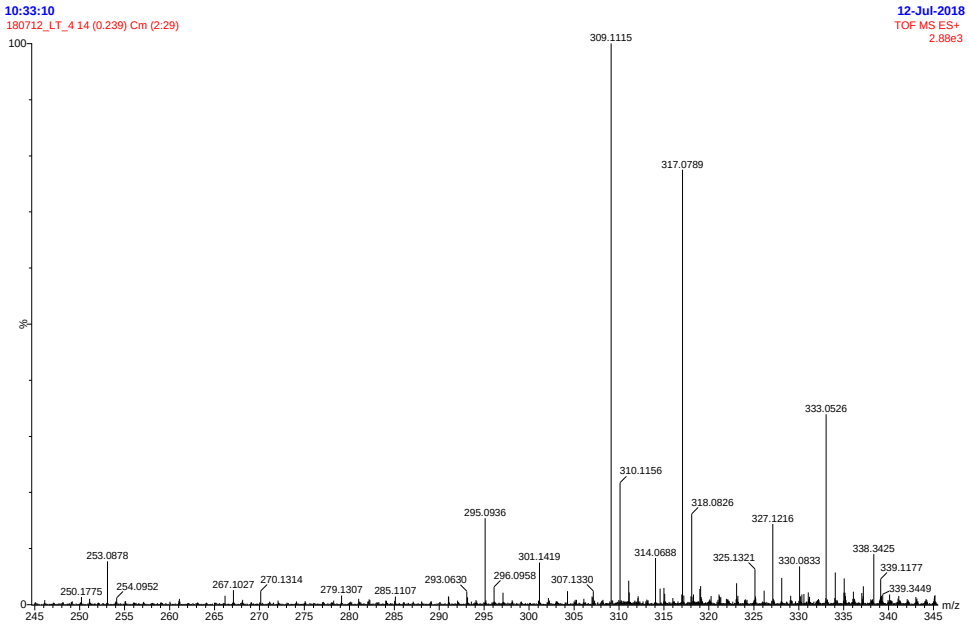


Fig. S10 Mass spectrum of **3**

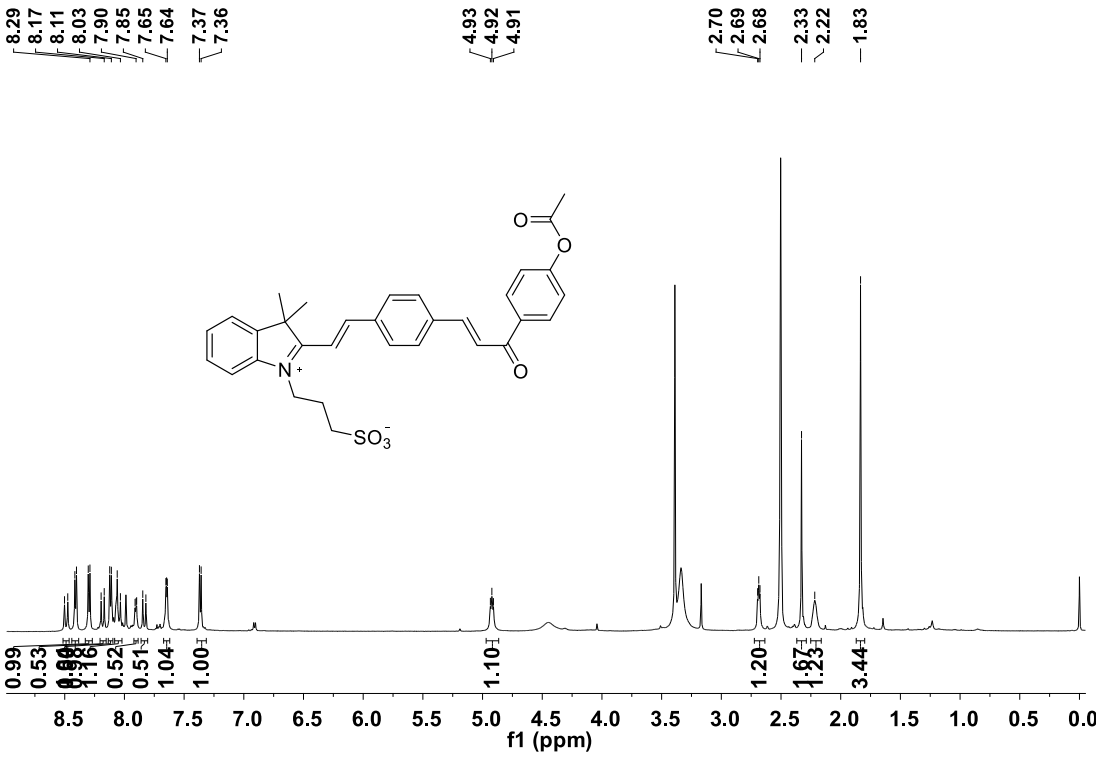


Fig. S11 ^{1}H NMR of **Hcy-Ac** in DMSO-d_6

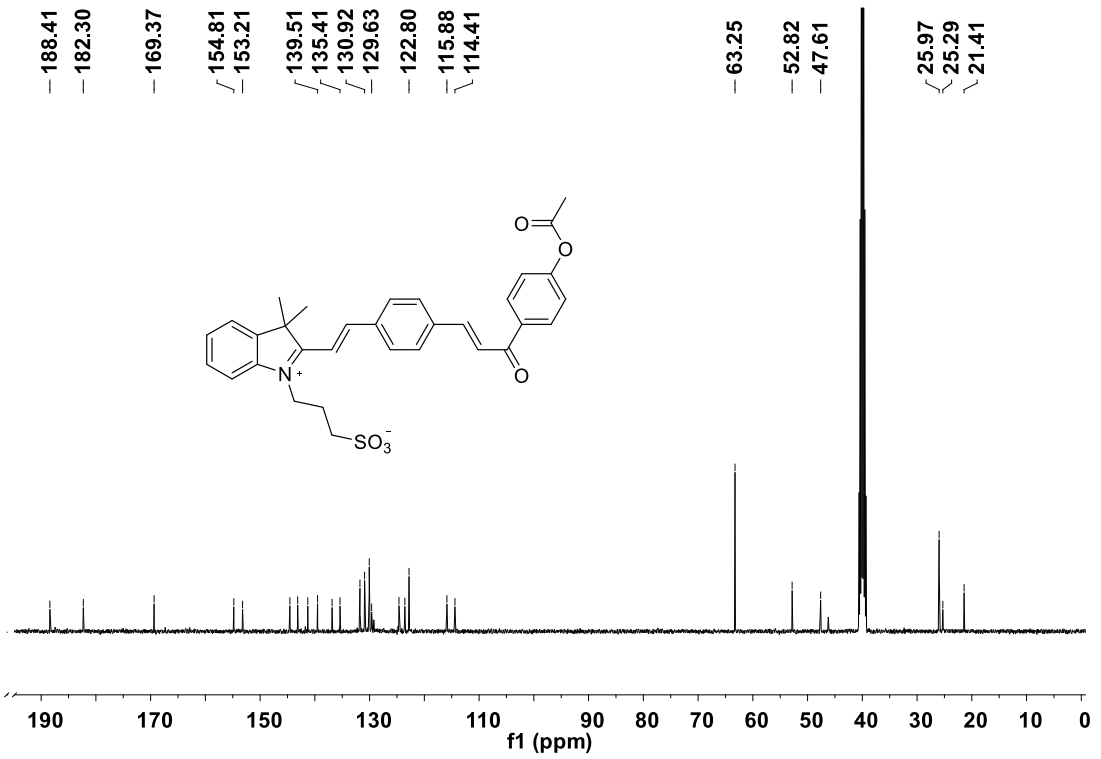


Fig. S12 ^{13}C NMR of **Hcy-Ac** in DMSO-d_6

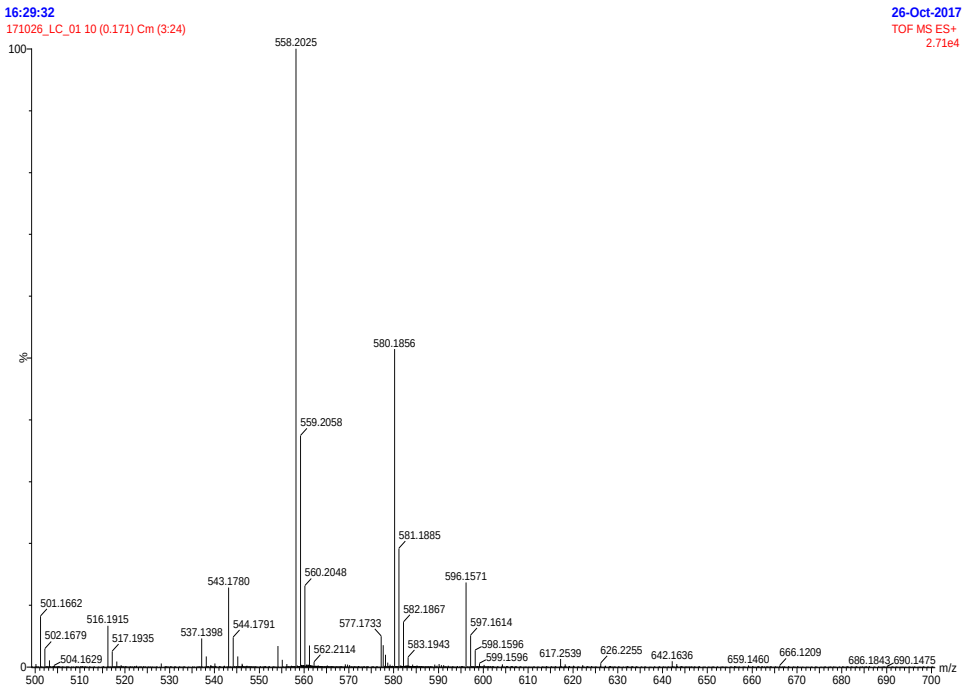


Fig. S13 Mass spectrum of **Hcy-Ac**

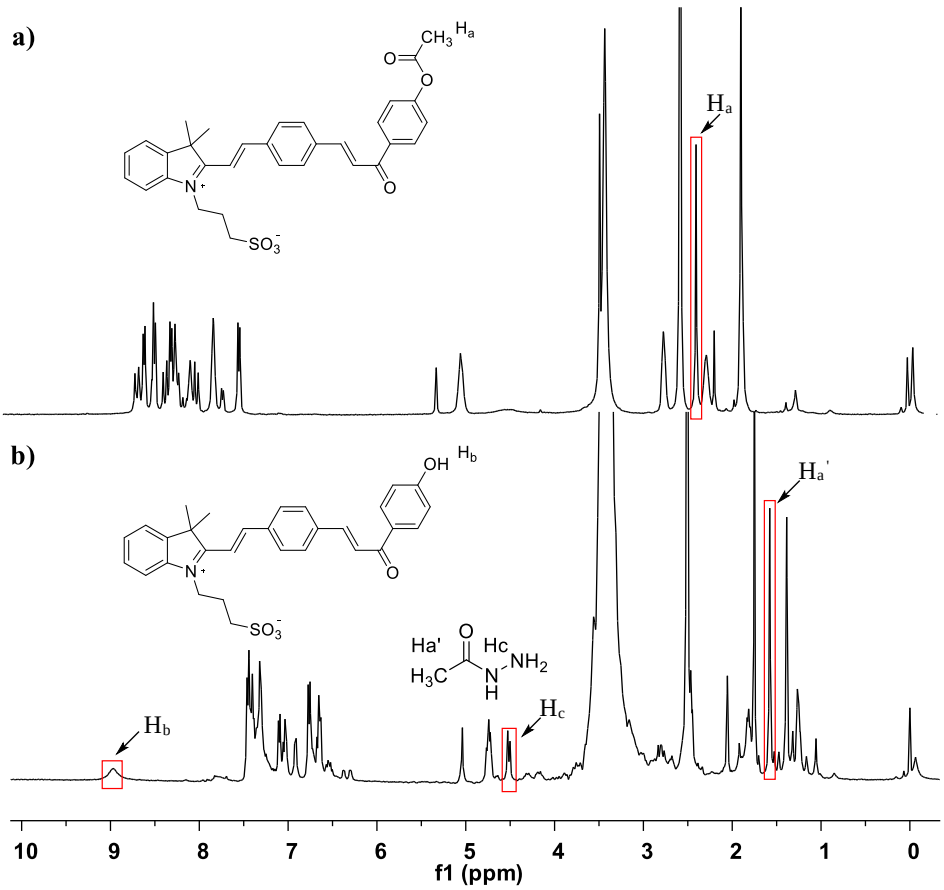


Fig. S14 ^{1}H NMR (400MHz, DMSO-d_6) spectra of (a) pure **Hcy-Ac** (16 mM) and (b) **Hcy-Ac** (16 mM) with the addition of 20 equiv of hydrazine