Supporting Information for:

Redox-based Selective Fluorometric Detection of Homocysteine

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1. General Information

Solvents and reagents were purchased from VWR International, Oakwood Product Inc., or Sigma-Aldrich Co. and used without purification unless specified otherwise. When necessary, solid reagents were dried under high vacuum. Reactions with compounds sensitive to air or moisture were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Thin layer chromatography (TLC) was run on Sorbtech W/UV254 plates (0.25 mm thick), and visualized under UV-light or by a Ce-Mo staining solution (phosphomolybdate, 25 g; $Ce(SO_4)_2.4H_2O$, 10 g; conc. H_2SO_4, 60 mL; H_2O, 940 mL) with heating. Flash chromatography was performed using Fluka silica gel 60 (mesh size: 0.040-0.063 mm) using a weight ratio of ca. 30:1 for silica gel over crude compound. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer (400 and 100MHz, respectively) in deuterated chloroform ($CDCl_3$), methanol-d_4 (CD_3OD), and DMSO-d_6 with either tetramethylsilane (TMS) (0.00 ppm) or the NMR solvent as the internal reference. UV-Vis absorption spectra were recorded on a Shimadzu PharmaSpec UV-1700 UV-Visible spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5310PC spectrofluorophotometer. 96-Well plates were read and recorded on a PerkinElmer 1420 multi-label counter.

2. Synthesis and Characterization

5-(2-Aminoethylamino)naphthalene-1-sulfonic acid 4 and 5-(*N*-(2-(dimethylamino)ethyl)-N-methylamino)naphthalene-1-sulfonic acid 5 was performed following literature reported procedures. [1]

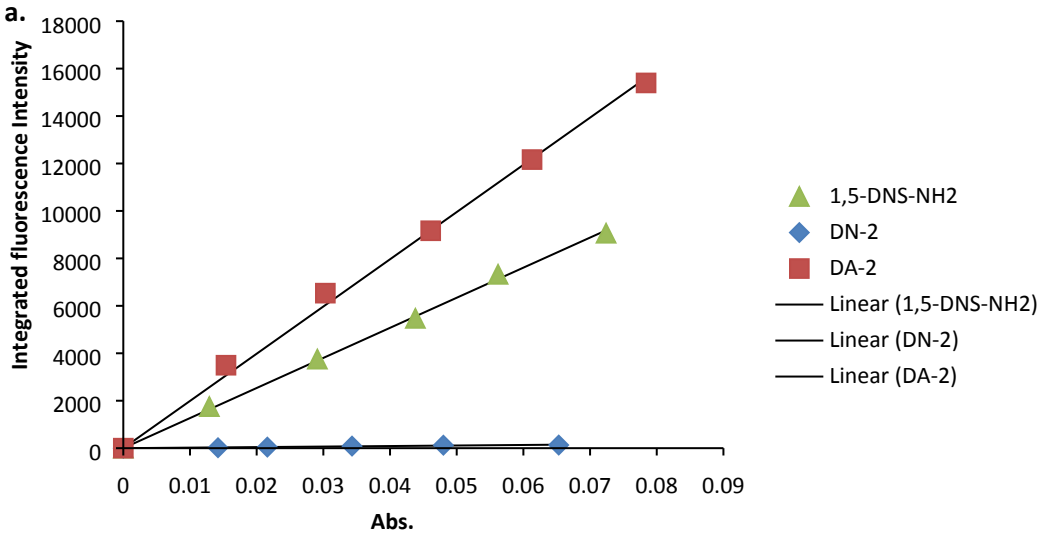
5-(*N*-(2-(Dimethylamino)ethyl)-*N*-methylamino)naphthalene-1-sulfonyl azide 1: To a Ar protected solution of **5** (110 mg, 0.36 mmol) in $POCl_3$ (570 µL, 6.2 mmol) was added PCl5 in 2 portions (430 mg, 2.1 mmol). The reaction was stirred on ice bath for 1 h, then warmed to room temperature, and stirred for additional 2 h. The reaction mixture was poured into 10 g ice. EtOAc extraction and evaporation gave crude intermediate 5-(*N*-(2-(dimethylamino)ethyl)-*N*-methylamino)naphthalene-1-sulfonyl chloride, which was dissolved in MeOH (2 mL) and added

into a stirred solution of NaN_3 (243 mg, 3.75 mmol) in a 1:1 mixed solvent of MeOH/H_2O (4 mL). The reaction mixture was stirred at room temperature for 2 h. MeOH was evaporated and the product was extracted with EtOAc. The organic phase was washed with water and brine, and dried over Na_2SO_4. Solvent evaporation followed by flash chromatography (CH_2Cl_2:Hex:EtOAc, 1:2:0.2) gave a light yellow solid (90 mg, 75% yield). ^{1}H NMR ($CDCl_3$): 8.64 (d, J = 8.4 Hz, 1 H), 8.34 (d, J = 7.2 Hz, 1 H), 8.64 (d, J = 8.8 Hz, 1 H), 7.74-7.64 (m, 2 H), 7.39 (d, J = 7.6 Hz, 1 H), 3.59 (t, J = 7.0 Hz, 2 H), 3.12 (t, J = 7.0 Hz, 2 H), 2.91 (s, 3 H), 2.91 (s, 6 H); ^{13}C NMR ($CDCl_3$): 149.8, 134.1, 131.7, 131.0, 130.4, 129.7, 129.2, 124.0, 120.7, 118.3, 60.6, 51.1, 50.4, 45.1; IR 2916.9, 2421.7, 2130.7, 1571.2, 1465.2, 1166.6, 792.6, 743.8; MS (ES+) 333.9 $(M+1)^+$.

5-(*N*-(2-(Dimethylamino)ethyl)-*N*-methylamino)naphthalene-1-sulfonyl amide 2: ^{1}H NMR ($CDCl_3$): 8.51-8.46 (m, 2 H), 8.26 (d, J = 7.2 Hz, 1 H), 7.65-7.57 (m, 2 H), 7.40-7.38 (m, 1 H), 3.52 (t, J = 6.0 Hz, 2 H), 3.13 (t, J = 6.0 Hz, 2 H), 2.84 (s, 3 H), 2.66 (s, 6 H); ^{13}C NMR ($CDCl_3$): 149.8, 139.1, 130.6, 129.5, 128.7, 127.5, 127.0, 123.7, 120.8, 117.3, 60.1, 50.2, 49.9, 43.5; MS (ES+) 308.0 $(M+1)^+$.

3. Relative quantum yield determination of DN-2 (**1**) and DA-2 (**2**)

1,5-DNS-NH_2 was used as the reference for relative quantum yield determination. Absorption and emission (λ_{Ex}= 325 nm) spectra were recorded for a series of concentrations (16 µM, 12 µM, 8 µM, 4 µM and 0 µM in acetonitrile or deionized water) of 1,5-DNS-NH_2, DN-2 (**1**) and DA-2 (**2**). Integrated fluorescence intensity was plotted against the absorption values at each concentration (Figures S5 and S6)[2]. Relative quantum yield values can be calculated using slopes of each compound.



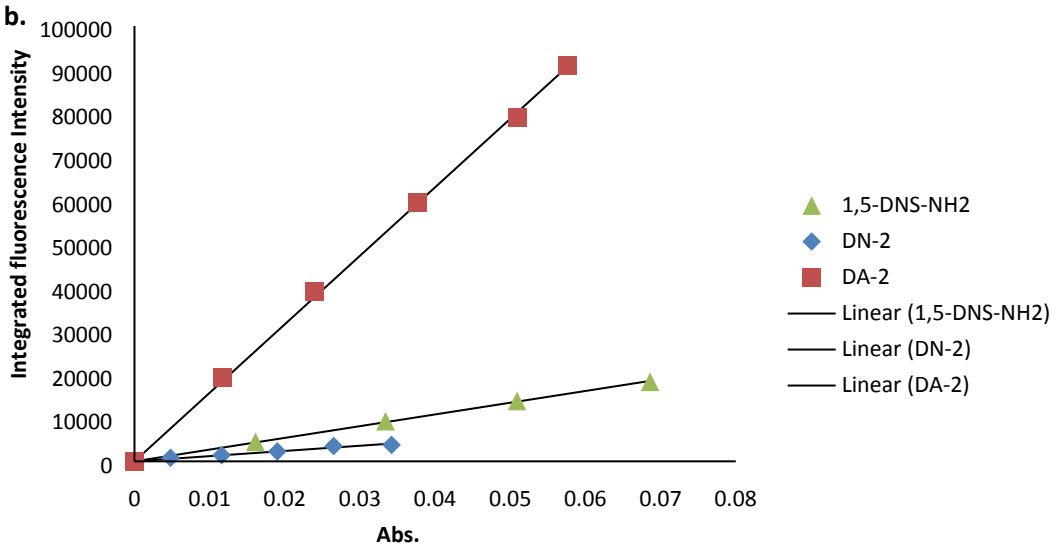


Figure S1. a. Quantum yield determination of DN-2 (**1**) and DA-2 (**2**) in acetonitrile; b. quantum yield determination of DN-2 (**1**) and DA-2 (**2**) in water.

4. Effect of Fe(II), Cu(I), and ascorbic acid on DN-2 fluorescence

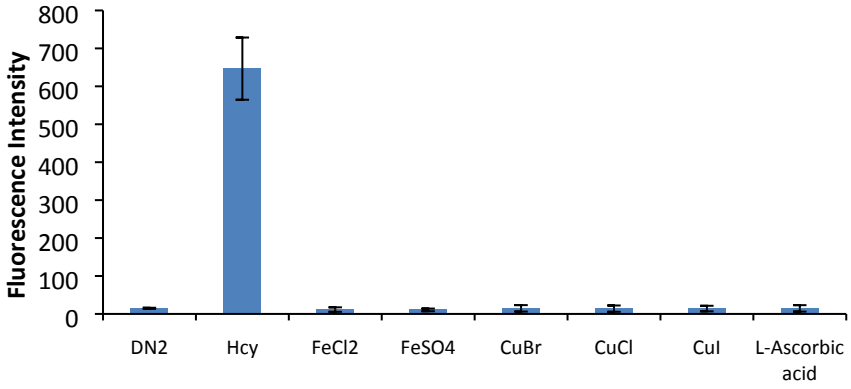


Figure S2. Fluorescence response of DN-2 to $FeCl_2$, $FeSO_4$, CuBr, CuCl, CuI, and ascorbic acid in comparison with negative control (DN-2 alone) and positive control (Hcy addition to DN-2 solution). (DN-2 120 μM, analytes 100 μM in 100 mM sodium phosphate buffer at pH 7.4 with 10% ethanol, fluorescence intensities were recorded 1 h after the addition of reducing species. Data represents the average of three independent experiments.)

5. Effect of cysteine fluctuation on homocysteine quantitation

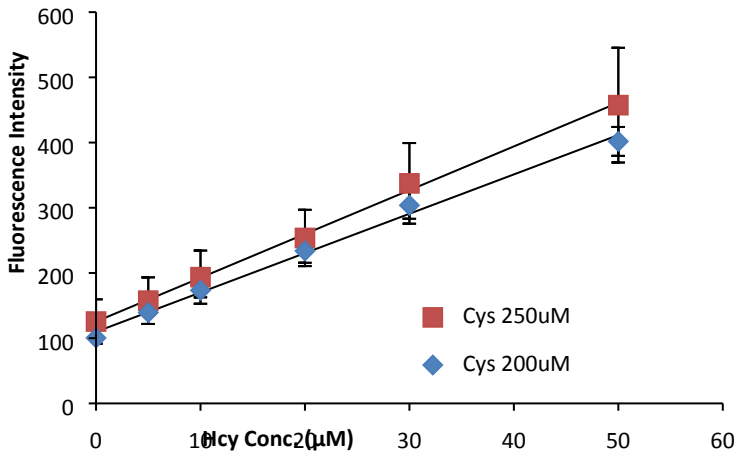


Figure S3. Calibration curve of homocysteine in the presence of 200 and 250 μM of cysteine. (DN-2 120 μM, Hcy 0-50 μM in 100 mM sodium phosphate buffer at pH 7.4, with 10% ethanol; fluorescence intensities at 517 nm were recorded 60 min after the addition of Hcy. Data represents the average of three independent experiments.)

6. Influence of Zn^{2+} on fluorescence response of DN-2 to Hcy

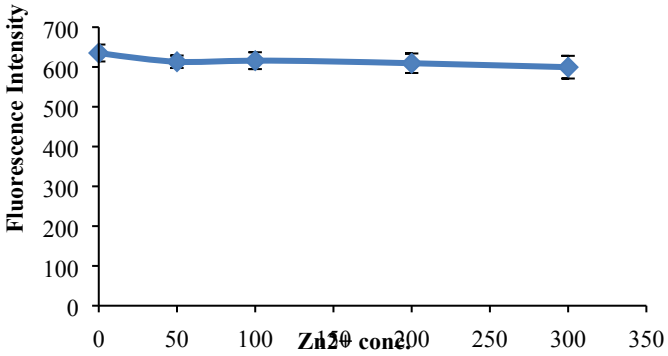


Figure S4. Fluorescence response of DN-2 to Hcy in the presence of different concentrations of Zn^{2+}. DN-2 120 μM, Hcy 100 μM in 100 mM phosphate buffer with 10% ethanol at pH 7.4. Fluorescence was recorded on a fluorometer after reaction for 120 min in the presence of 0, 50, 100, 200 and 300 μM of $ZnCl_2$, respectively. Data represents the average of three independent experiments.

7. Determination of the pKa values of the sulfhydryl groups on cysteine and homocysteine

Automatic pH titration on a (Accumet@ Research, AR10 pH meter) was used to determine the pKa values of the sulfhydryl groups on cysteine and homocysteine. NaOH solution (99.35 mM) was standardized with potassium hydrogen phthalate (KHP) and used for titration of 5 mM cysteine hydrochloride and 5 mM homocysteine hydrochloride. Titration curve was created and analyzed using TitriSoft 2.51. Experiment was triplicated to obtain the average pKa values (8.25 for cysteine and 8.9 for homocysteine).

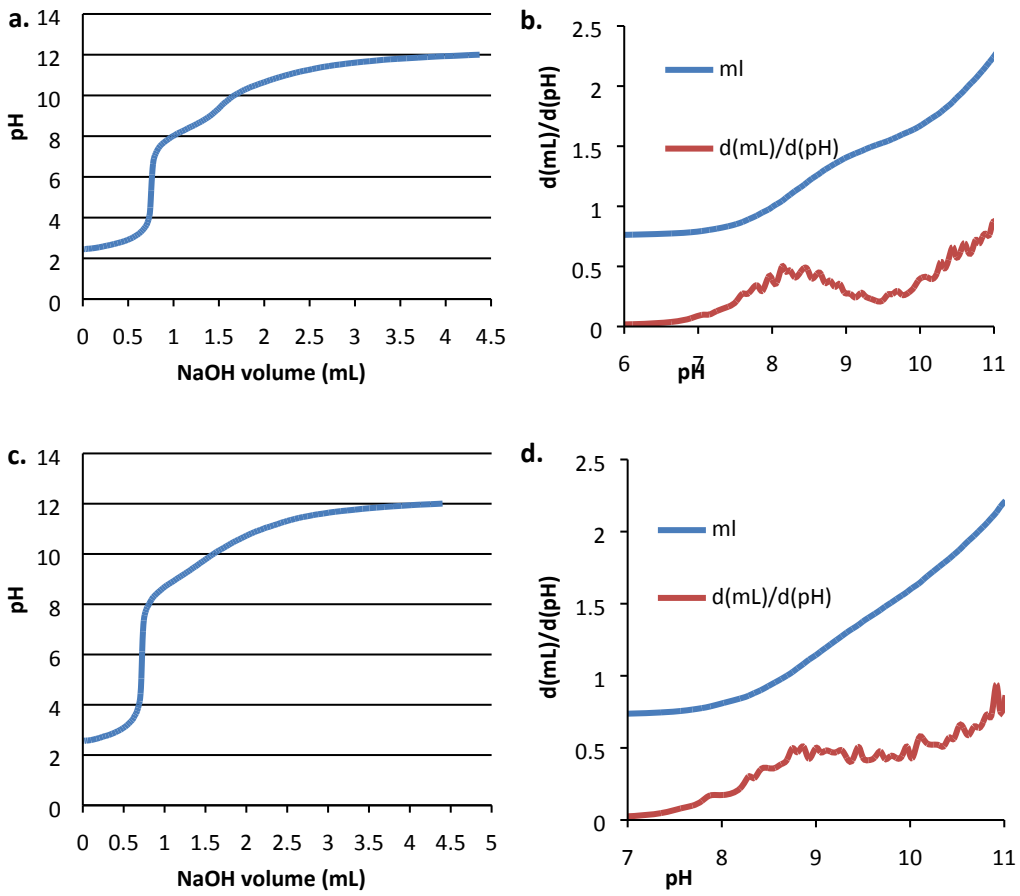


Figure S5. a. Titration curve of 5 mM cysteine hydrochloride; b. Calculation of the sulfhydryl group pKa of cysteine; c. Titration curve of 5 mM homocysteine hydrochloride; d. Calculation of the sulfhydryl group pKa of homocysteine.

8. Selectivity of other dansyl azide analogues for homocysteine

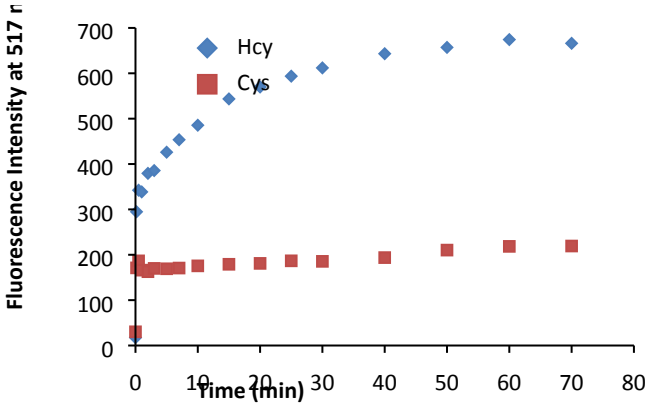


Figure S6. Time dependent fluorescence emission (517 nm) of 1,5-DNS-Az in the presence of Hcy and Cys (DNS-Az 120 μM, amino thiols 100 μM in 100 mM sodium phosphate buffer at pH 7.4 with 10% ethanol).

9. Fluorescence response of DN-2 to thiols at different pH:

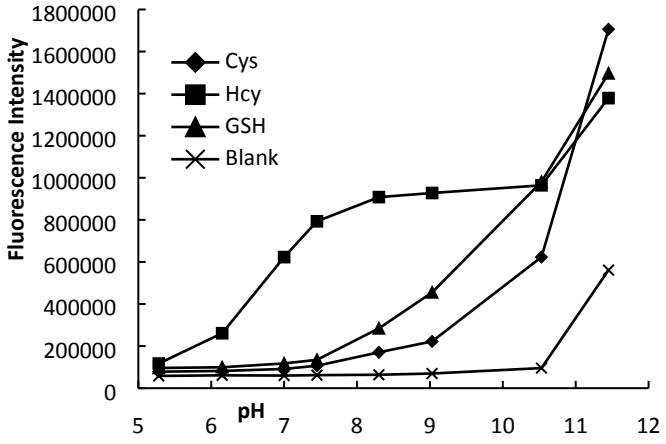


Figure S7. pH dependent fluorescence response of DN-2 to thiols. Fluorescence intensity was read on a microplate reader (Excitation filter 340 nm, Emission filter 535 nm). DN-2 100 μM, thiols 100 μM in 100 mM sodium phosphate buffer at pH values of 5.3, 6.2, 7.0, 7.5, 8.3, 9.0, 10.5, and 11.5.

10. Fluorescence response of DN-2 to Hcy in phosphate buffer at 37 ºC:

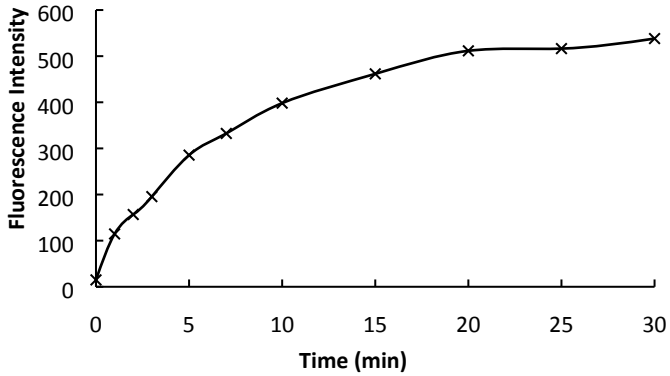
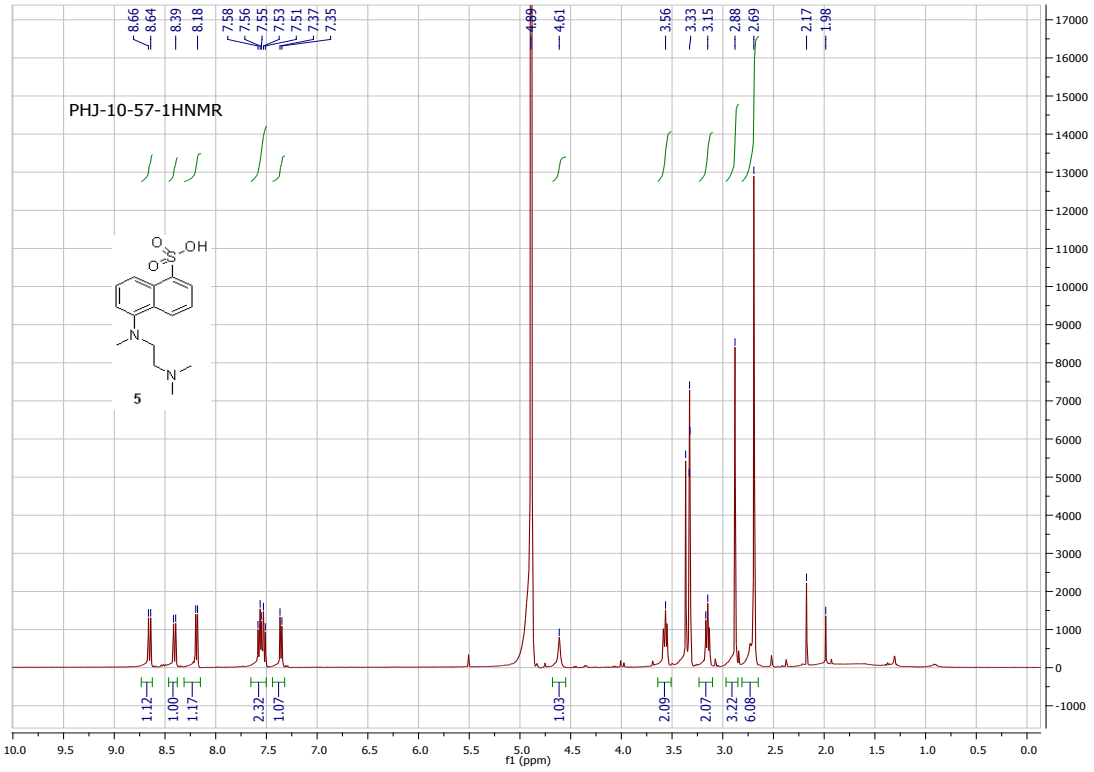
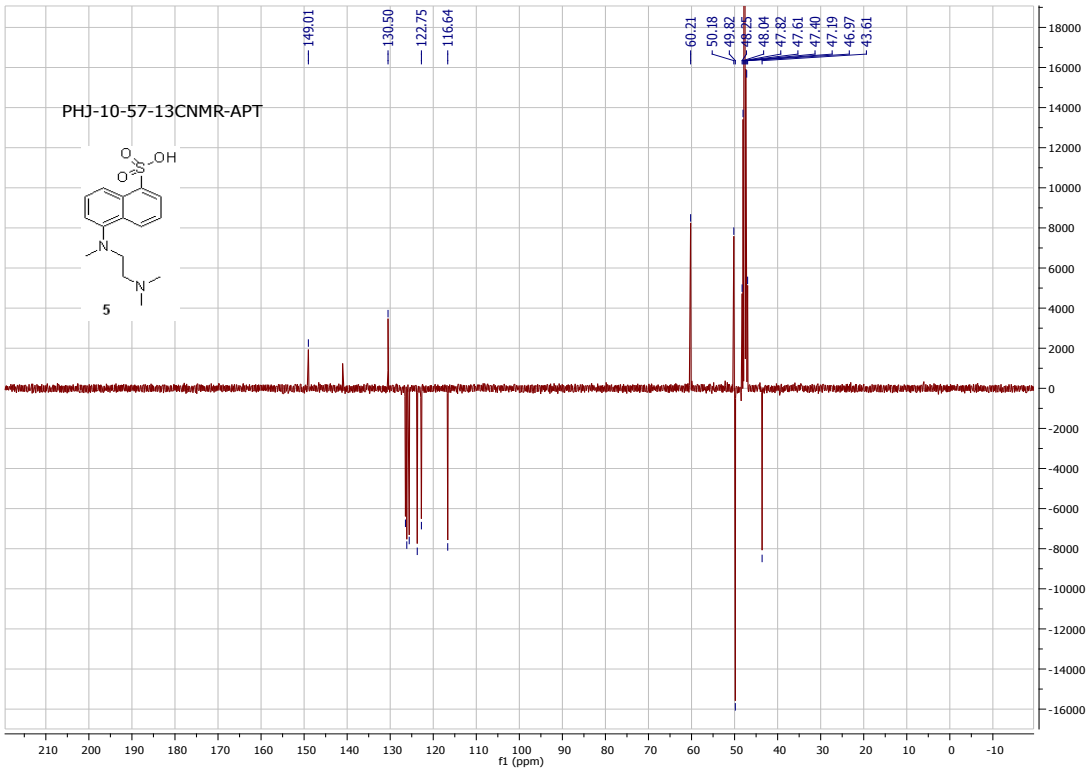


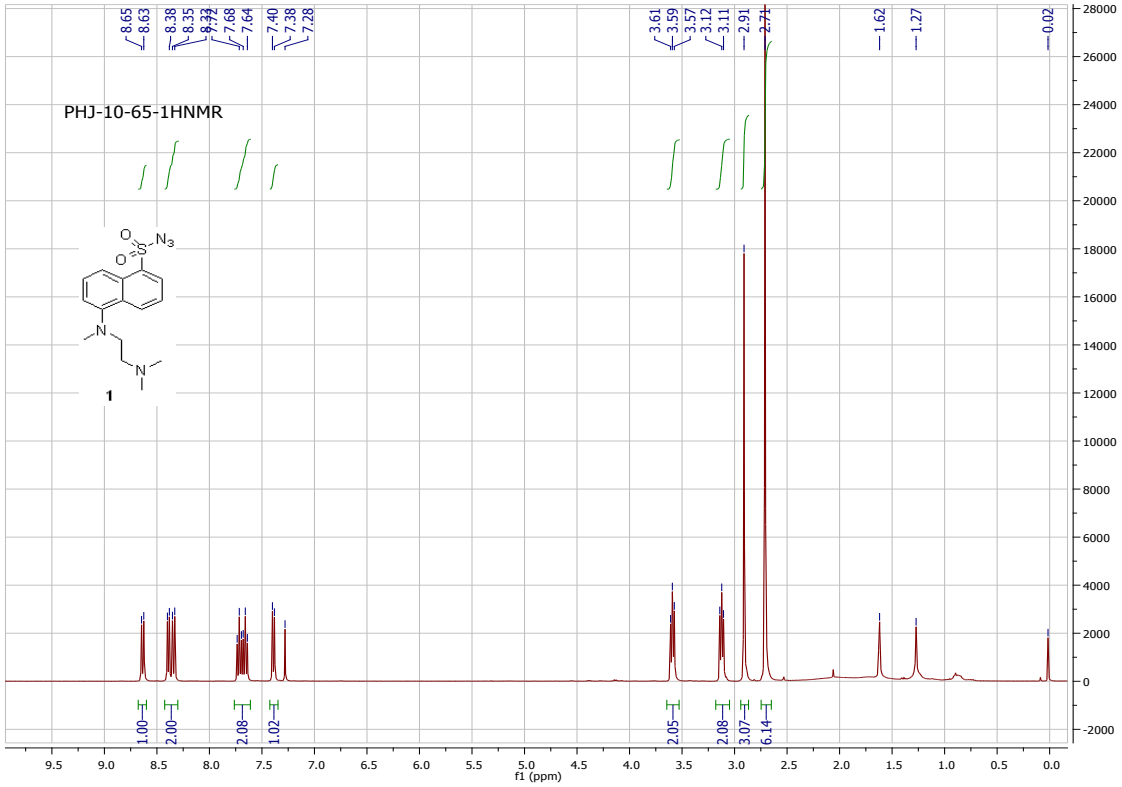
Figure S8. Reaction time profile at 37 °C, (DN-2 120 μM, amino thiols 100 μM in 100 mM sodium phosphate buffer at pH 7.5, 37 °C, fluorescence intensities at 517 nm were recorded at each time point)

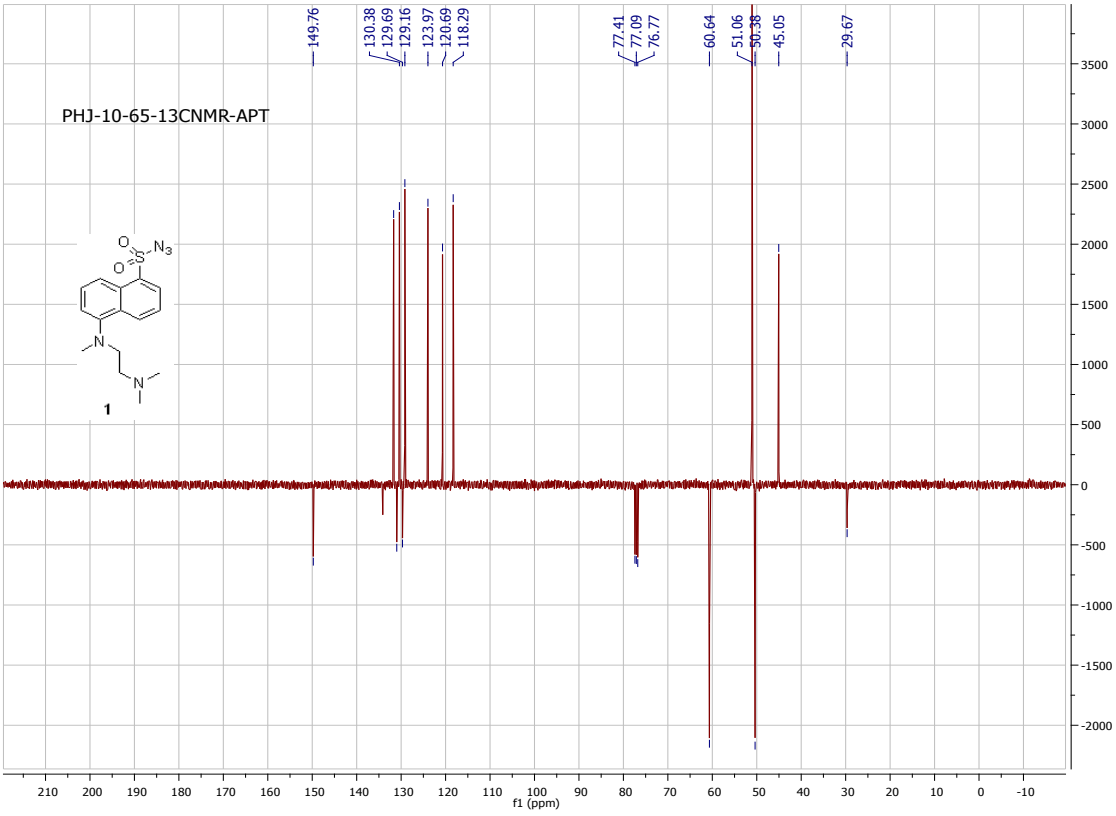
11. Spectroscopic Data:
    Spectra of Compound **5**:

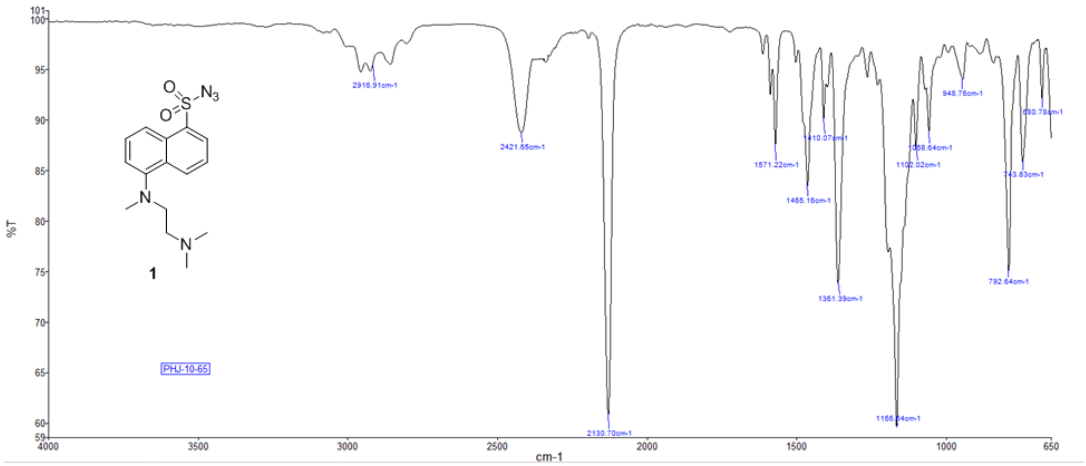




Spectra of Compound **1**:







Reference

1. N. A. O'Connor, S. T. Sakata, H. Zhu and K. J. Shea, *Org Lett*, 2006, **8**, 1581-1584.
2. A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst*, 1983, **108**, 1067-1071.
3. J. G. Ma, G. Stoter, J. Verweij and J. H. M. Schellens, *Cancer Chemoth Pharm*, 1996, **38**, 391-394.