Supporting information

A specific nucleophilic ring-opening reaction of aziridines as a unique platform for the construction of hydrogen polysulfides sensors

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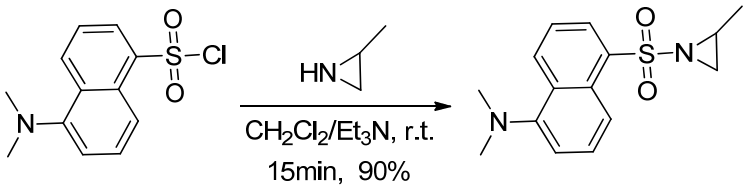
Materials and Methods: All solvents were reagent grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-13 NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported relative to chloroform (δ 7.26) for ^{1}H NMR and chloroform (δ 77.0) for ^{13}C NMR. Absorption spectra were recorded on a Thermo 300 UV/VIS spectophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on Cary Eclipse fluorescence spectrophotometer. The two-photon excitation and fluorescence spectra were measured by Zeiss multiphoton confocal microscope. The excitation source is a tunable Ti:Sapphire femtosecond pulsed laser (Coherent Chameleon). It produces 140 fs pulses at 80 MHz repetition rate. The fluorescence from the samples

is dispersed by a prism and detected by the 32-channel photomultiplier tube array inside the microscope (Zeiss QUASAR detection unit)

Na_2S_2 used in this study was prepared and characterized following a known protocol (Takata et al, *Inorg. Chem*., 2003, *42*, 3712-3714； *Chem. Lett*., 2002, 454-455). This salt can now be purchased from Dojindo molecular technologies, INC (http://www.dojindo.com/store/p/947-SulfoBiotics-Sodium-polysulfides.html).

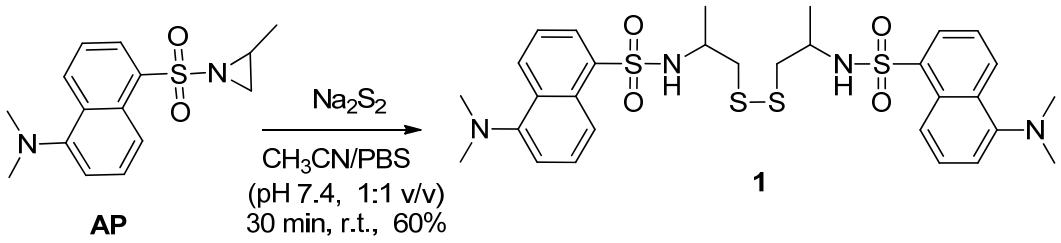
Synthesis of Probe AP



AP

To a mixture of 2-methylaziridine (1.0 mmol) and Et_3N (1.0 mmol) in CH_2Cl_2 (10 mL) was added dansyl chloride (0.85 mmol) at room temperature. The mixture was stirred for 15 min. Then solvent was evaporated under reduced pressure and resulted residue was subjected to falsh column chromatography for purification with hexane/ethyl acetate (1:1, v/v). AP was obtained as oil in 90% yield (222 mg).^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (m, 2 H), 8.20 (d, J = 6.0 Hz, 1 H), 7.56 (m, 2 H), 7.20 (d, J = 9.0 Hz, 1 H), 2.98 (m, 1 H), 2.89 (s, 6 H), 2.72 (d, J = 6.0 Hz, 1 H), 2.05 (d, J = 6.0 Hz, 1 H), 1.25 (d, J = 6.0 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.8, 134.2, 131.4, 130.7, 130.1, 129.5, 128.5, 123.3, 120.5, 115.6, 45.7, 36.5, 35.6, 17.2; HRMS m/z 291.1155 $[M+H]^+$; calcd for $C_{15}H_{19}N_2O_2S$: 291.1167.

Synthesis of compound 1



To the solution of **AP** (0.24 mmol) in CH_3CN (2.0 mL) and PBS buffer (2.0 mL, 50 mM, pH 7.4, containing 0.02 mmol CTAB) was added Na_2S_2 (0.272 mmol). The mixture was stirred for 30 min at r.t. and then diluted with ethyl acetate (50 mL). The organic layer was seperated and dried by $MgSO_4$, and concentrated. Purification by flash column chromatography with hexane/ethyl acetate (1:1, v/v) afforded compound **1** as a mixture of diastereoisomers (60 % yield, 93 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, *J* = 9.0 Hz, 2 H), 8.25 (m, 4 H), 7.53 (m, 4 H), 7.17 (m, 2 H), 4.99 (d, *J* = 9.0 Hz, 1 H), 4.87 (d, *J* = 9.0 Hz, 1 H), 3.46 (m, 2 H), 2.89 (s, 12 H), 2.54 (m, 4 H), 1.01 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.2, 135.4, 135.2, 130.8, 130.1, 130.0, 129.9, 129.8, 128.7, 123.4, 119.0, 115.5, 49.5, 49.2, 45.9, 45.7, 45.1, 20.6, 20.4; HRMS m/z 647.1852 $[M+H]^+$; calcd for $C_{30}H_{39}N_4O_4S_4$: 647.1854.

Preparation of the solutions and fluorescence measurments

The stock solution of **AP** (1 mM) was prepared in DMSO. The stock solution of cetrimonium bromide (CTAB, 5 mM) was prepared in EtOH.

Unless otherwise noted, all the measurements were carried out for 30 min at room temperature in 50 mM PBS buffer (pH 7.4) with 50 μM CTAB according to the following procedure: in a test tube, 3 mL of 50 mM PBS buffer (pH 7.4) and 40 μL of the stock solution of CTAB (final concentration: 50 μM) were mixed, followed by addition of a requisite volume of testing species sample solution. The final volume of the reaction solution was adjusted to 3.96 mL with 50 mM PBS buffer (pH 7.4). To this solution was then added 40 μL of the stock solution of **AP** (final concentration: 10 μM). After mixing and standing for 30 min at room temperature, a 3.5-mL portion of the reaction solution was transferred into a 1-cm quartz cell to measure fluorescence

with λ_{ex} = 350 nm. In the meantime, a blank solution containing no testing species sample was prepared and measured under the same conditions for comparison.

Preparation of ROS solutions

The concentration of H_2O_2 was determined using the absorption at 240 nm (ε= 43.6 M^{-1} cm^{-1}). The concentration of ClO^- was determined using the absorption at 292 nm (ε= 360 M^{-1} cm^{-1}). Superoxide solution (O_2^-) was prepared by adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min.

Hydroxy radical (•OH) was generated *in situ* by addition of $FeSO_4$ stock solution into a solution containing excess H_2O_2 through Fenton chemistry. Final concentration of H_2O_2 was 250 μM. Final concentration of ferrous ion was 25 μM.

Singlet oxygen ($^1O_2$) was generated in situ by addition of the hypochlorite stock solution into a solution containing excess of H_2O_2. Final concentration of H_2O_2 was 250 μM. Final concentration of ClO^- was 25 μM.

Quantum Yields

The quantum yield of the compounds in PBS buffer was calculated according to the equation:[1]

$$\Phi_{sample} = \Phi_{standard} \times (I_{sample}/ I_{standard}) \times (A_{standard}/ A_{sample}) \times (n_{sample}/ n_{standard})^2$$

Φ denotes the quantum yield; *I* denotes the area under the fluorescence band; *A* denotes the absorbance at the excitation wavelength; n denotes the refractive index of the solvent.

For quantum yields of **AP** and **1**, they were determined using 7-hydroxycoumarin as a standard by comparing the area under the corrected emission spectrum of the test sample with that of a solution of 7-hydroxycoumarin excited at 330 nm in sodium phosphate buffer (0.1 M; pH 7.4), which has a quantum efficiency of 0.76 according

[1] H. Sunahara, Y. Urano, H. Kojima, T. Nagano, *J. Am. Chem. Soc.*, **2007**, *129*, 5597-5604.

to the literature.[2]

For solid samples, an integrating sphere was used to calculate quantum yield.[3]

Measurement of Two-Photon Cross Section

The relative two-photon absorption cross-section of **1** was calculated by using the following formula:[4] $\delta = \delta_r(S_s\Phi_r\varphi_r c_r)/(S_r\Phi_s\varphi_s c_s)$, where the subscripts s and r denote the sample and reference molecules. The intensity of the signal collected by a CCD detector was denoted as S. Φ is the fluorescence quantum yield. φ is the overall fluorescence collection efficiency of the experimental apparatus. The number density of the molecules in solution was denoted as c. δ_r is the TPA cross section of the reference molecule. The two-photon induced fluorescence intensity was measured at 700−840nm by using fluorescein (1μM, pH = 13) as the reference.[5]

H_2S_n detection in bovine plasma

The stock solution of **AP** (1 mM) was prepared in DMSO. The stock solution of Na_2S_2 (0.4 mM) was prepared in PBS buffer. 1 mL of commercially available bovine plasma was deproteined by adding 2 mL cold pure ethanol (-20 ℃) and then centrifuging for 30 min at 2 ℃. The separated supernatant was collected to use for analysis of deproteinized bovine plasma.

In a test tube, 3 mL of 50 mM PBS buffer (pH 7.4) and 500 μL deproteinized bovine plasma were mixed. To this solution was added 40 μL of 1 mM the stock solution of **AP** (final concentration:10 μM) with 40 μL CTAB (final concentration: 50 μM), followed by addition of a requisite volume of Na_2S_2 solution. The final volume of the reaction solution was adjusted to 4 mL with 50 mM PBS buffer (pH 7.4). After mixing and standing for 30 min, a 3.5 mL portion of the reaction solution was

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[2] K. Setsukinai, Y. Urano, K. Kikuchi, T. Higuchi, T. Nagano, *J. Chem. Soc*., *Perkin Trans. 2*, **2000**, 2453.

[3] (a)L.-O. Pålsson, A. P. Monkman, *Adv. Mater.*, **2002**, 14, 757-758.(b) Y. Li, Z. Li, Y. Wang, A. Compaan, T. Ren and W. Dong, *Energy Environ. Sci.*, **2013**, 6, 2907.

[4] G. Mao, T. Wei, X. Wang, S. Huan, D. Lu, J. Zhang, X. Zhang, W. Tan, G. Shen, R. Yu, *Anal. Chem.*, **2013**, 85, 7875−7881.

[5] M. A. Albota, C. Xu, W. W. Webb, *Appl. Opt*., **1998**, *37*, 7352-7356.

transferred into a 1-cm quartz cell to measure fluorescence with λ_{ex} = 350 nm, PMT detector voltage = 600V. In the meantime, a blank solution containing no Na_2S_2 solution was prepared and measured under the same conditions for comparison.

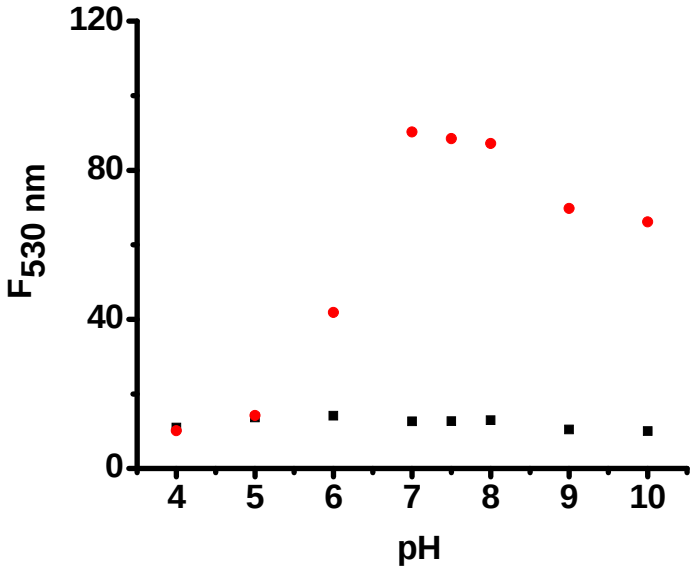


Fig. S1 Fluorescence intensities of **AP** (10 μM) under different pH values in the absence (■) or presence (●) of Na_2S_2 (25 μM). The reactions were carried out for 30 min at room temperature in different pH values solution with 50 μM CTAB. Data were acquired at 530 nm and excited at 350 nm.

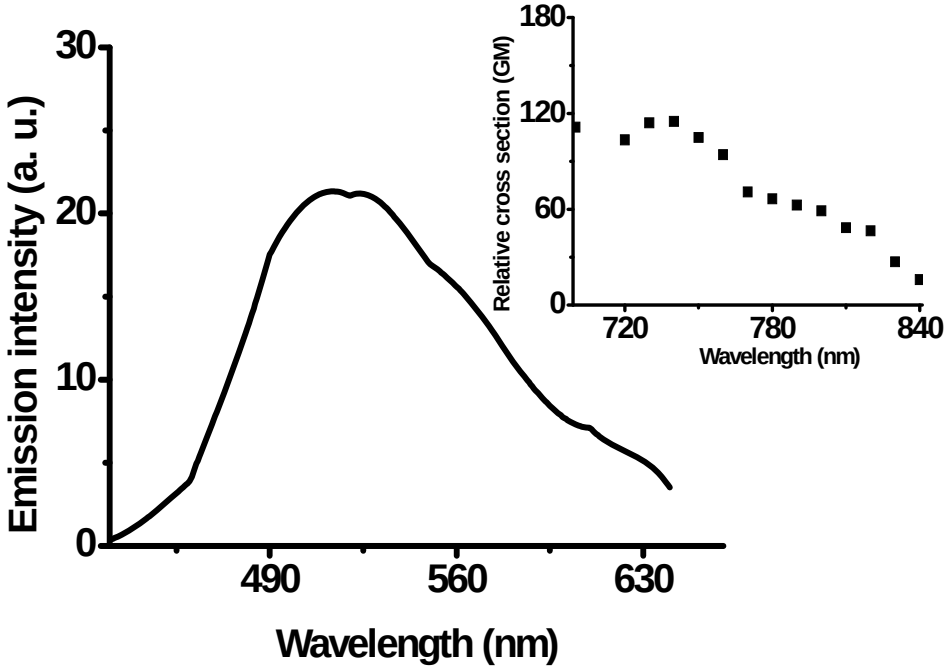


Fig. S2 The two-photon induced excitation spectrum and emission spectrum (λ_{em} = 530 nm, λ_{ex} = 740 nm) of **1** in PBS buffers.

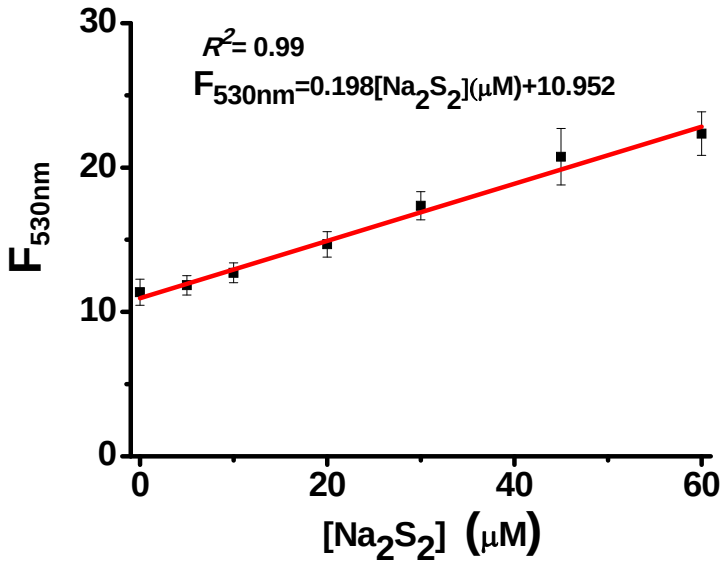
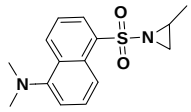
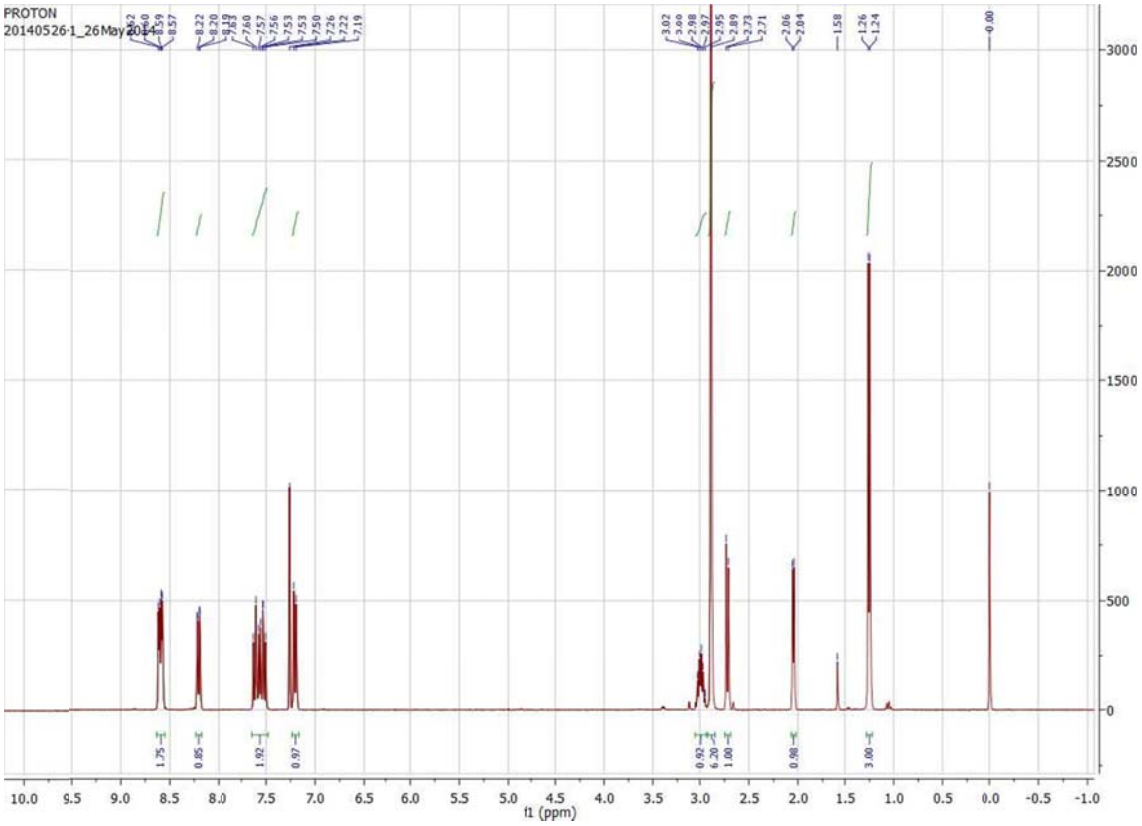


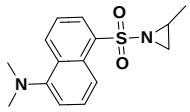
Fig. S3 The plot of fluorescence intensities of **AP** (10 μM) with varied concentrations of Na_2S_2 (0, 5, 10, 20, 30, 45, 60 μM) in diluted deproteinized bovine plasma. The reactions were carried out for 30 min at room temperature. The data represents the average of five independent experiments.



AP

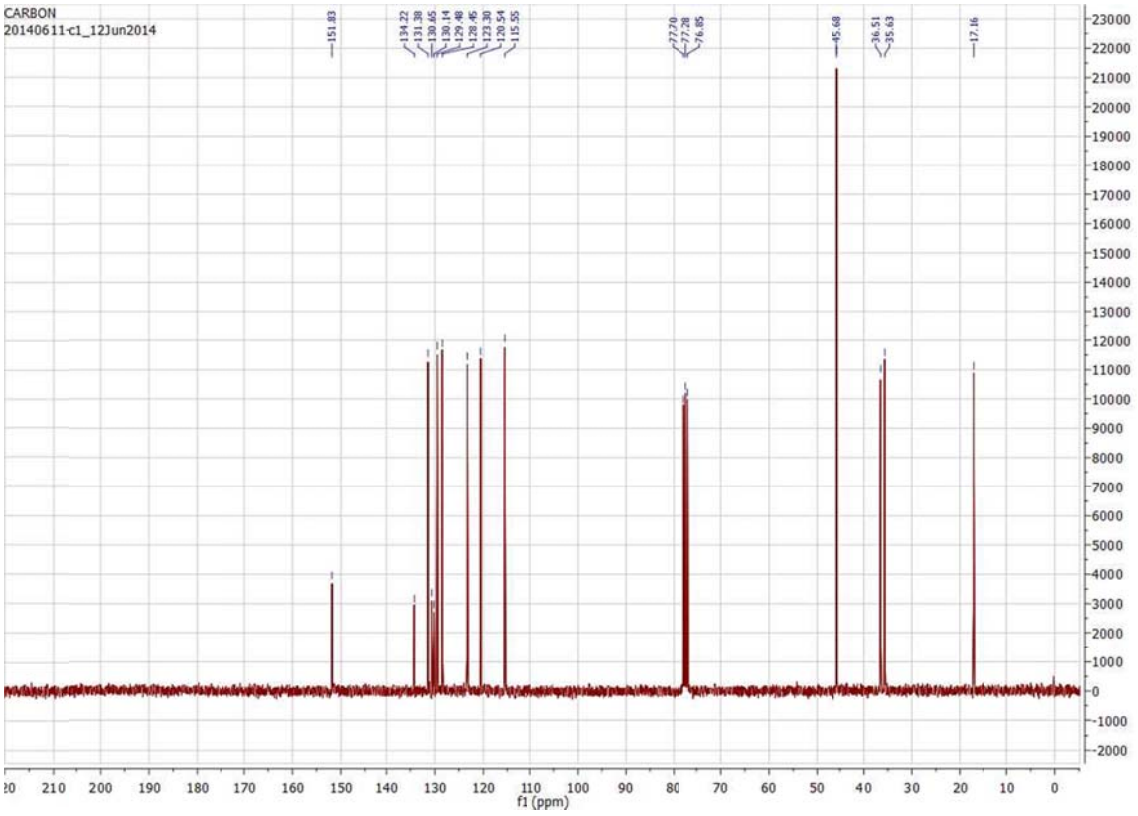
^{1}H NMR (300 MHz, $CDCl_3$)

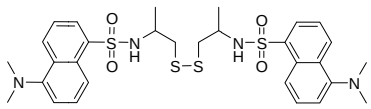




AP

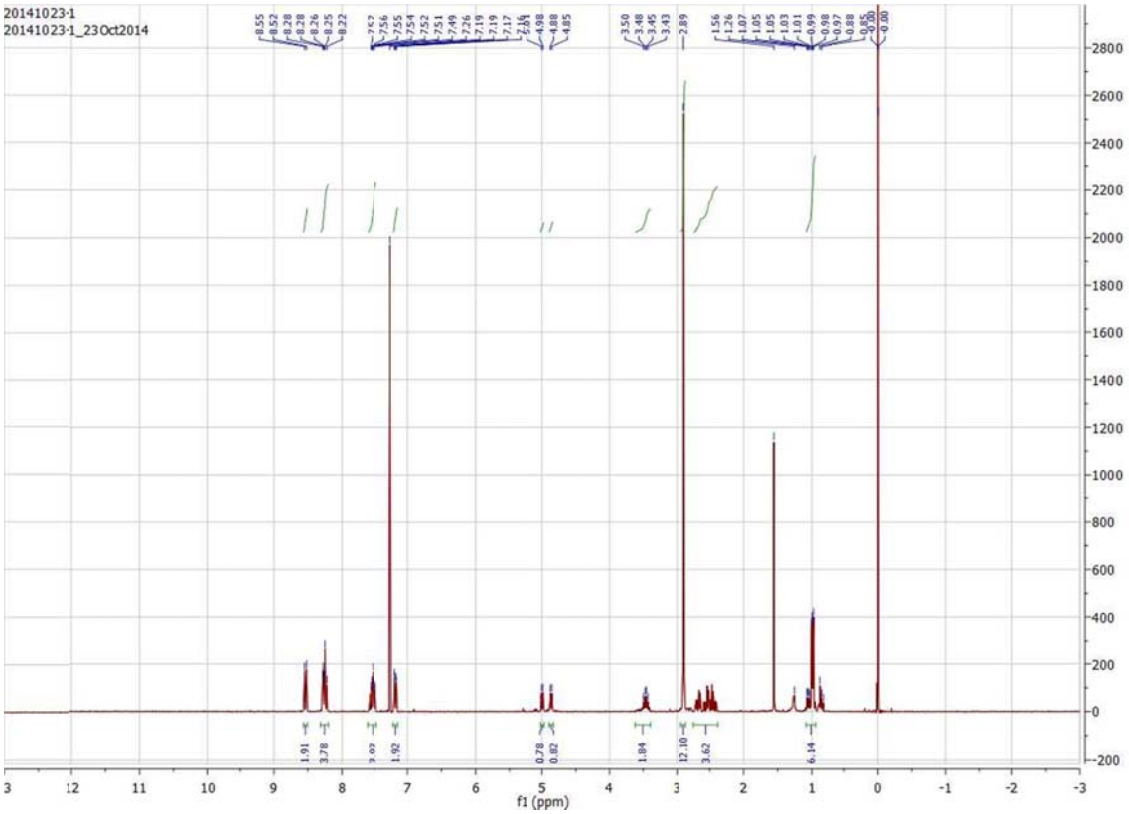
^{13}C NMR (75 MHz, $CDCl_3$)

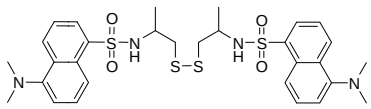




1

^{1}H NMR (300 MHz, $CDCl_3$)





1

^{13}C NMR (75 MHz, CD**C**l$_3$)