# Electronic Supplementary Information (ESI)

## Ratiometric Fluorescence Detection of Cysteine and Homocysteine with a BODIPY Dye by Mimicking the Native Chemical Ligation

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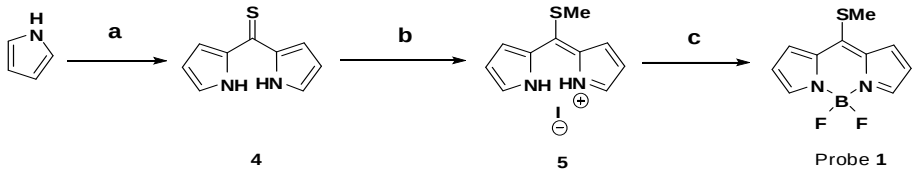
**General Methods**.

$^{1}$H and $^{13}$C NMR spectra were obtained using a Bruker DPX-300. UV absorption spectra were obtained using a HP8453 UV/Vis spectrophotometer. Fluorescence spectra were obtained using a Photon Technical International Fluorescence system. Commercially available reagents were used without further purification. Anhydrous solvents for synthesis were prepared using a solvent purification system. Thin-layer chromatography (TLC) was performed on precoated silica gel 60F-254 glass plates.

**Synthesis of probe 1.**




4 5 Probe **1**

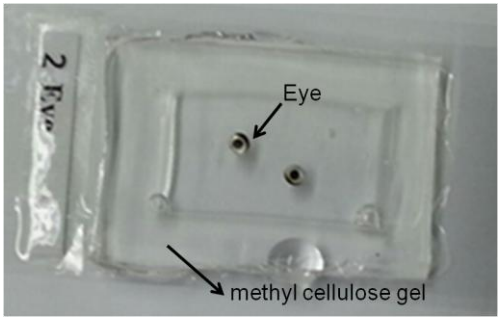
Reagents and conditions: (a) (i) $CSCl_2$ (0.52 equiv.), toluene, $Et_2O$, 25 ℃, 10 min. (ii) 10% MeOH in water, 25 ℃, 30 min. (b) $CH_3I$ (18 equiv.), $CH_2Cl_2$, 25 ℃, 15 h. (c) $Et_3N$ (6.0 equiv.), $BF_3{\cdot}Et_2O$ (9.0 equiv.), $CH_2Cl_2$, 25 ℃, 12 h.

**Synthesis of the probe 1**. It was prepared from pyrrole by following the literature procedure.[1] Only the characterization data are given here. The thiocarbonyl compound **4** was prepared as a crystalline dark-red solid (1.01 g, 76%) starting from pyrrole (1.02 g, 15.1 mmol): $^{1}$H NMR ($CDCl_3$, 300 MHz, 293 K): $\delta$ 6.40 (2H, m), 7.05 (2H, m), 7.20 (2H, m), 9.77 (2H, s), $^{13}$C NMR ($CDCl_3$, 300 MHz, 293 K): $\delta$ 112.5, 114.8, 127.7, 138.4, 193.2. Probe **1** was prepared starting from compound **4** (0.88 g, 5.0 mmol) via thiomethyl ether **5** as a crystalline dark red solid (0.84 g, 71% from two steps). $^{1}$H NMR ($CDCl_3$, 300 MHz, 293 K): $\delta$ 7.80 (2H, m), 7.26-7.42 (2H, m), 6.50-6.51 (2H, m), 2.91 (3H, s); $^{13}$C NMR ($CDCl_3$, 300 MHz, 293 K): $\delta$ 154.0, 140.8, 133.4, 127.3, 117.6, and 20.0.

**Preparation of the test solution**. A stock solution of the probe **1** (1.0 mM) was prepared in $CH_3CN$. For spectroscopic measurement, the test solution of the probe **1** (10 μM) in pH 7.4 HEPES buffer (10 mM, containing 1 % $CH_3CN$) was prepared in a cuvette (1 mL) and a required amount of the thiol stock solution was added to the probe **1** solution. The solutions of various sample species were prepared from Cys, Hcy, GSH, Val, Ser, Phe, Glucose, Thr, Glu, Leu, Ala, Try, His, Arg and $H_2O_2$. A fresh stock solution of each thiol (10 mM in DI water) was prepared every time before all the experiments. Each sample solution was shaken well and incubated for 20 min at room temperature before recording the spectra.

**Detection limit.** The detection limit was estimated based on the fluorescence titration.[2] The probe **1** was used at 10 μM and the slit was adjusted to 2 nm. To determine the S/N ratio, the emission intensity of the probe **1** without addition of Cys/Hcy was measured for five times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the Cys/Hcy concentration was obtained. The detection limit, $3\sigma_{bi}/m$, was then calculated, where $\sigma_{bi}$ is the standard deviation of blank measurements, m is the slope between intensity versus sample concentration.

**Dissection of zebrafish.** An adult zebrafish (4-month-old with identifiable organs) was exposed to 10 μM of the probe **1** in E3 media (15 mM NaCl, 0.5 mM KCl, 1.0 mM $MgSO_4$, 1.0 mM $CaCl_2$, 0.15 mM $KH_2PO_4$, 0.05 mM $Na_2HPO_4$, 0.7 mM $NaHCO_3$, 5% methylene blue; pH 7.4) for 30 min at 27 ℃. After being washed with E3 media to remove the remaining probe, the zebrafish was dissected to isolate the tissues and organs for imaging by fluorescence microscopy and by a dissecting microscope. For the control experiment B, the zebrafish was pretreated with 1.0 mM of *N*-ethylmaleimide (NEM) for 30 min. After being washed with E3 media to remove the remaining NEM, the zebrafish was further incubated with 10 μM of the probe in E3 media for 30 min at 27 ℃. For the control experiment C, the zebrafish was pretreated with 500 μM NAC (*N*-acetylcysteine) for 30 min. After being washed with E3 media to remove the remaining NAC, the zebrafish was further incubated with 10 μM of the probe in E3 media for 30 min at 27 ℃. After being washed with E3 media to remove the remaining probe, the zebrafish was anesthetized in 0.2% Tricaine (ethyl 3-aminobenzoate, an anesthetic) solution. The zebrafish was dissected into a piece of eye, gill, liver, heart, fin, and air bladder. The dissected tissues were kept in plates containing DPBS (Dulbecco's phosphate buffered saline; pH 7.4) solution. For imaging, each of the tissues was immobilized onto a methyl cellulose gel (7% wt/vol) that was placed in the center of a glass-bottomed dish, and then it was capped with a cover glass (see the below photo for the eye sampling).



**Fluorescence microscopy.** Confocal fluorescence imaging experiments were performed on Leica TCS SP5 II Adv. System. The microscope was equipped with multiple visible laser lines (405, 458, 476, 488, 496, 514, 561, 594, 633 nm) for confocal imaging. Fluorescence signals were obtained through Hyd PMT(Hybrid detector PhotoMultiplier Tube; Leica) with CH1 (423–490 nm) and CH2 (500–550 nm). UV Lens wheel: Lens 10X.

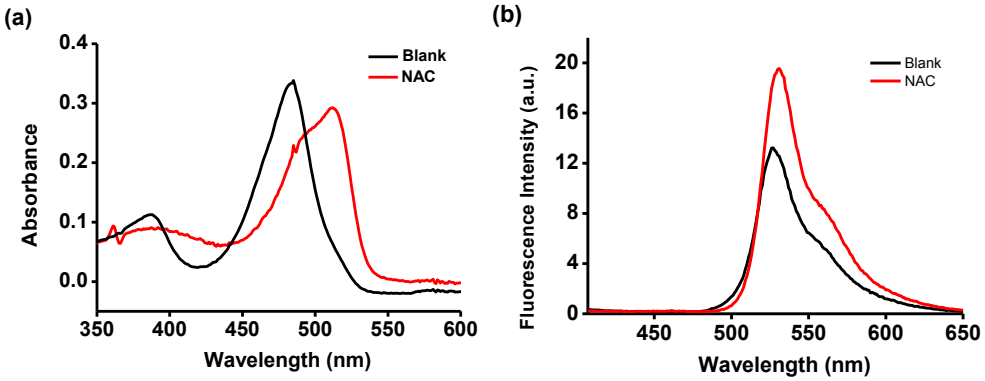



**Fig. S1** (a) Absorption and (b) emission spectrum for the reaction product between *N*-acetyl-cysteine (1.0 mM) and the probe **1** (10 μM) in HEPES buffer (pH 7.4, containing 1 % $CH_3CN$).

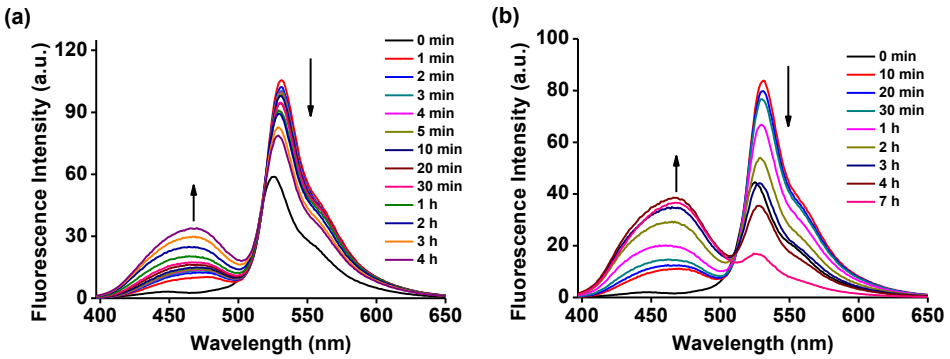



**Fig. S2** Time-dependent fluorescence spectra of the probe **1** (10 μM) with 1.0 mM of GSH, measured at (a) 25 °C and (b) 37 °C. Each spectrums were acquired in HEPES buffer (pH 7.4, containing 1 % $CH_3CN$). Excitation wavelength is 400 nm.

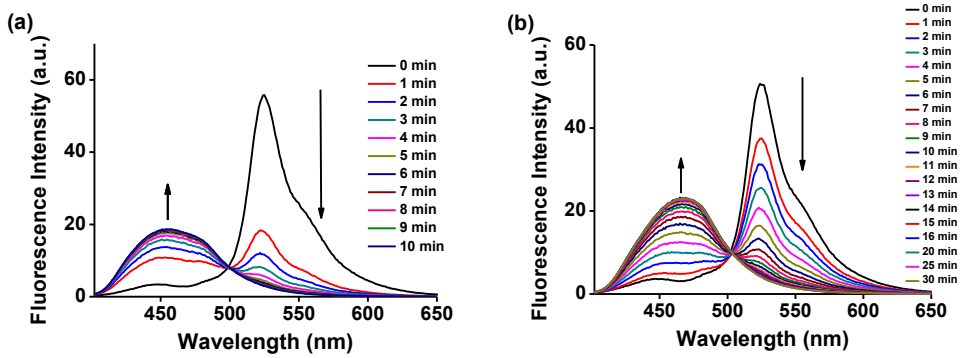



**Fig. S3** Time-dependent fluorescence spectra of the probe **1** (10 μM) with 0.1 mM of (a) Cys and (b) Hcy. Each spectrum was acquired in HEPES buffer (pH 7.4, containing 1 % $CH_3CN$) at 25 °C. Excitation wavelength is 400 nm.

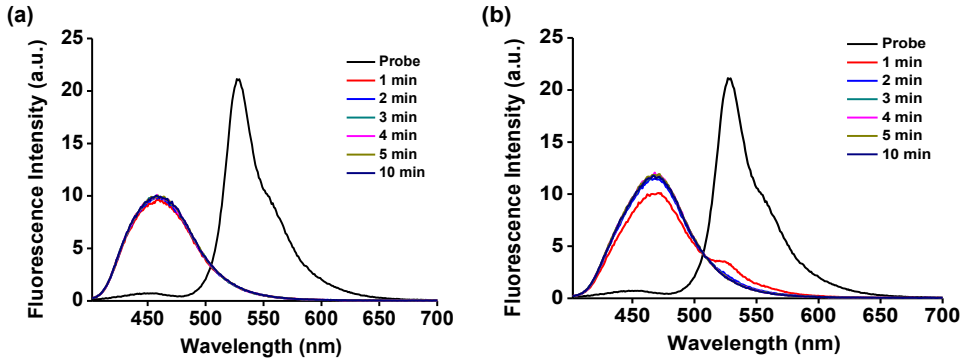



**Fig. S4** Time-dependent fluorescence spectra of the probe **1** (10 μM) with 1.0 mM of (a) Cys and (b) Hcy. Each spectrum was acquired in HEPES buffer (pH 7.4, containing 1 % $CH_3CN$) at 25 $^oC$. Excitation wavelength is 400 nm.

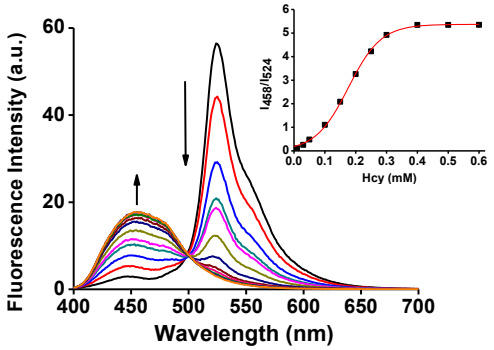



**Fig. S5** Fluorescence spectra of the probe **1** (10 μM) upon addition of increasing concentration of Hcy. Each spectrums were acquired in HEPES buffer (pH 7.4, containing 1 % $CH_3CN$) at 25 $^oC$. Excitation wavelength is 400 nm.

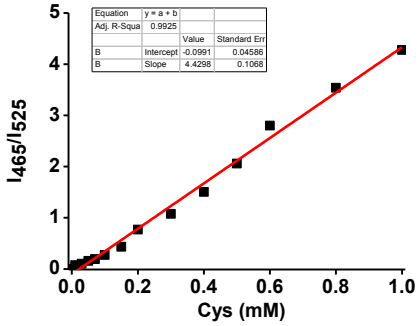



**Fig. S6** The emission ratio of the probe **1** (10 μM) to various concentrations of Cys. The data was acquired after incubation of the probe **1** with Cys for 20 min in pH 7.4 HEPES buffer (containing 1% $CH_3CN$) at 25 $^oC$. Excitation wavelength is 400 nm.

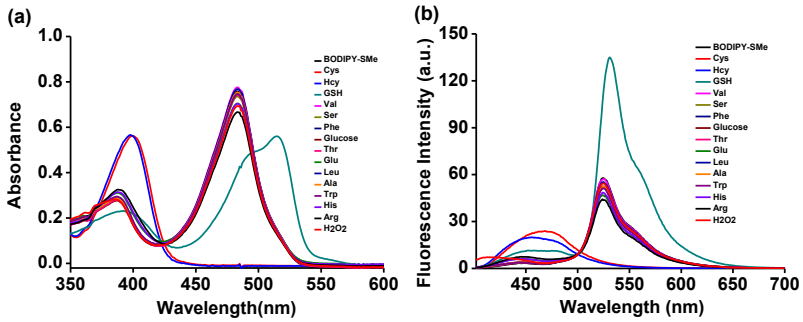



**Fig. S7** (a) Absorption and (b) emission spectra of the probe **1** (10 μM) upon addition of various amino acids in HEPES buffer (pH 7.4, containing 1 % $CH_3CN$) at 25 $^oC$. Excitation wavelength is 400 nm.

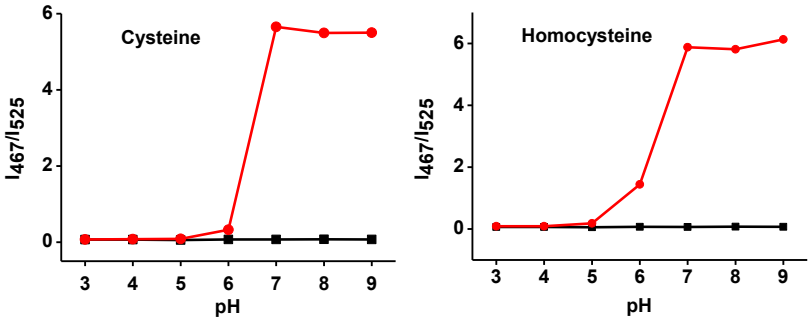



**Fig. S8** The fluorescent ratio of the probe **1** (10 μM) at various pH value in the absence (■) or presence (●) of Cys, Hcy (0.1 mM). The spectra were recorded after incubation of the probe **1** with Cys/Hcy for 20 min at 25 $^oC$. Excitation wavelength is 400 nm.

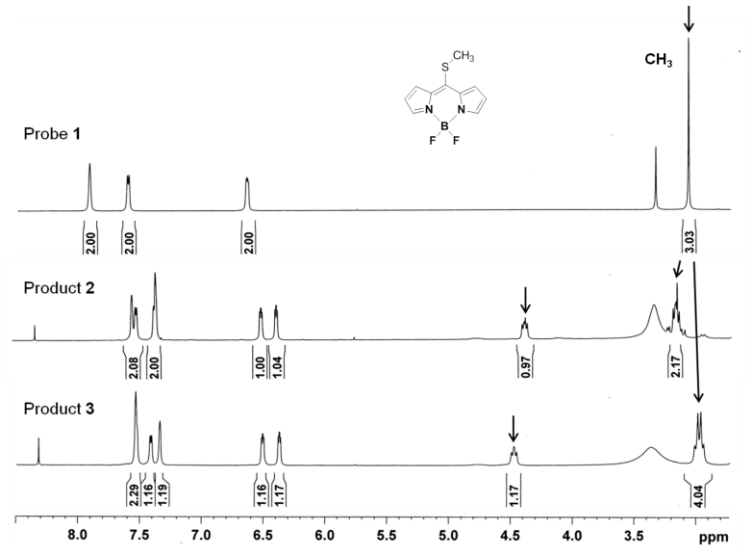



**Fig. S9** $^1H$ NMR spectra of the probe **1** and its reaction product with Cys (compound **2**) and Hcy (compound **3**) dissolved in DMSO-$d_6$.

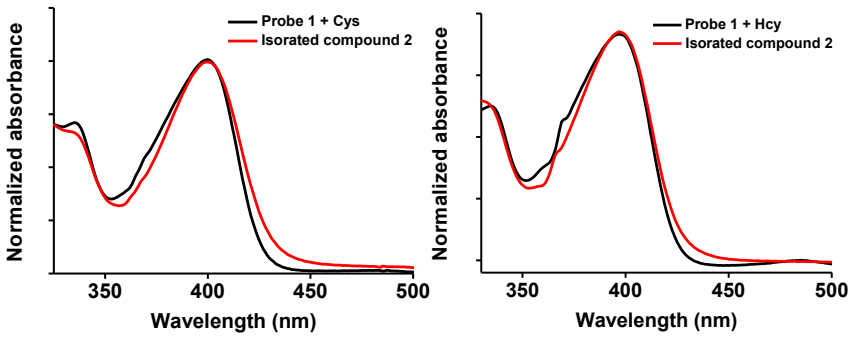



**Fig. S10** The normalized absorption spectra of the probe **1** titrated with Cys/Hcy and the isolated compound **2** and **3**.

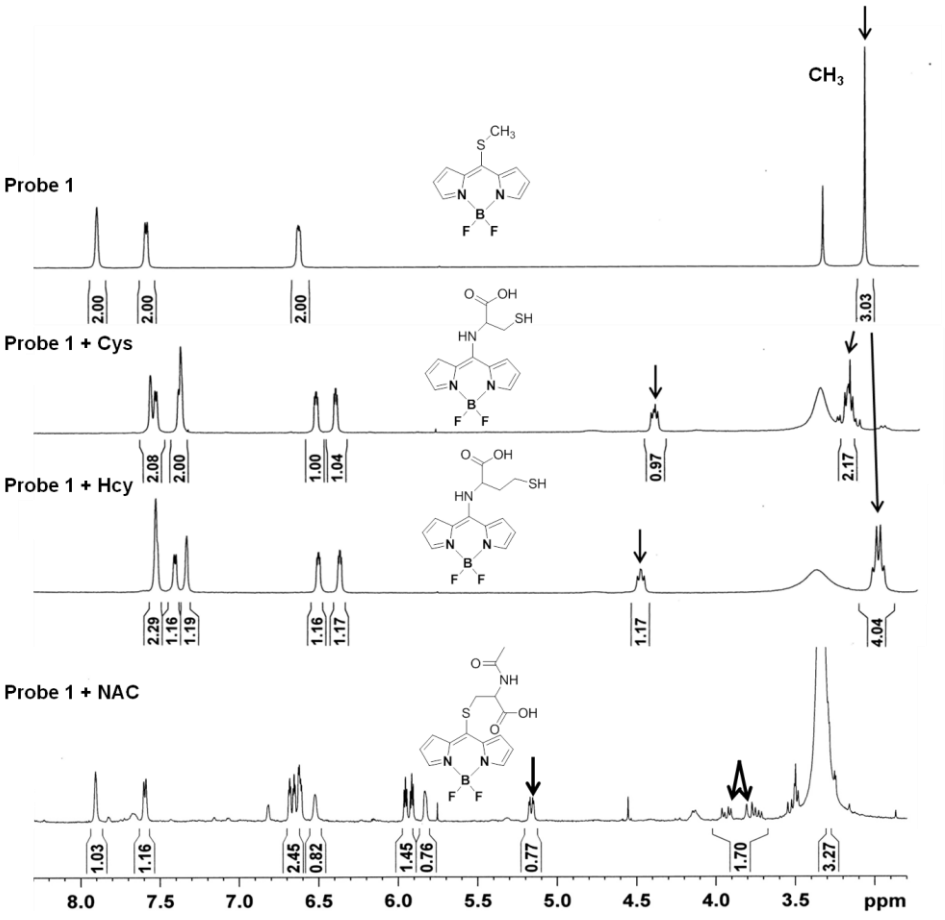



**Fig. S11** $^{1}$H NMR spectrum of the reaction product between *N*-acetyl-cysteine and the probe **1** in DMSO-$d_6$.

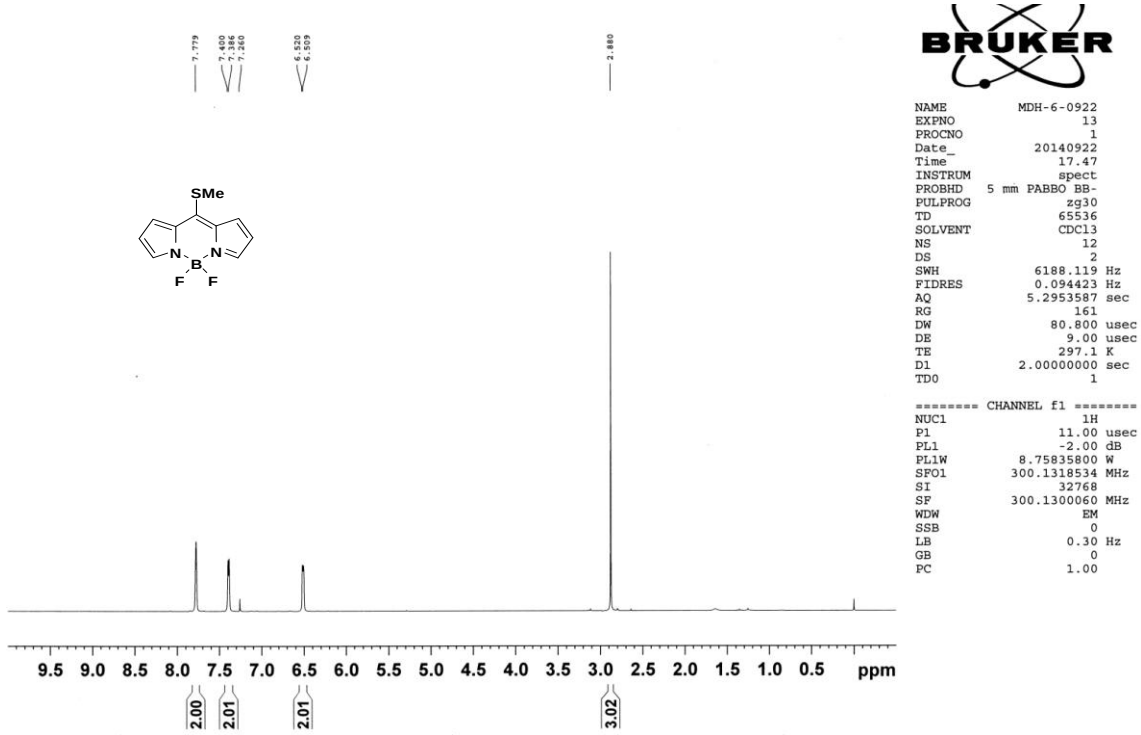



**Fig. S12** [1]H NMR spectra of probe **1.**

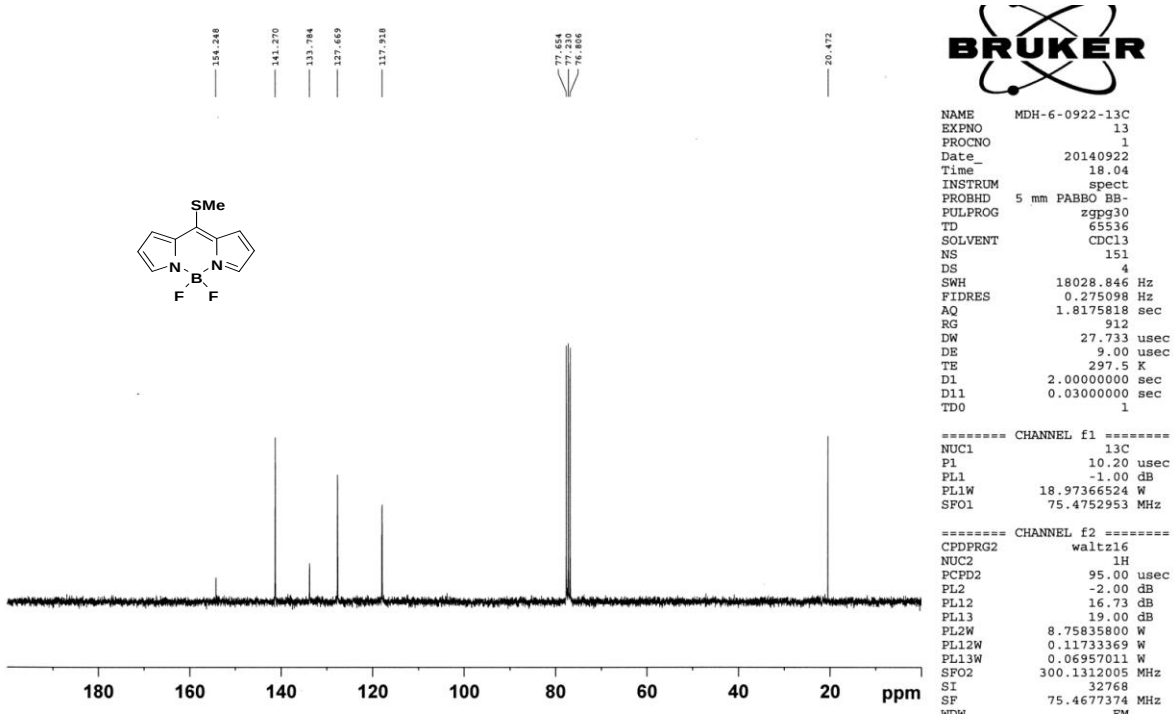



**Fig. S13** [13]C NMR spectra of probe **1.**

1 T. V. Goud, A. Tutary, J. F. Biellmann, *Tetrahedron*. 2006, **62**, 5084–5091.
2 B. P. Joshi, J. Park, W. I. Lee, K. Lee, *Talanta*. 2009, **78**, 903–909.