Supporting Information for：

Nitroolefin-based coumarin as a colorimetric and fluorescent dual probe for biothiols

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Experimental Section

General information and methods. All reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel 60 (200–300 mesh). Absorption spectra were taken on an Agilent 8453 spectrophotometer. Fluorescence spectra were taken on HITACHI F-4500 fluorescence spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 and 150MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer.

Procedures of thiols sensing

Deionized water was used throughout all experiments. A stock solution of **1** (5 mM) was prepared in DMF. The stock solution of **1** was then diluted to the corresponding concentration (20 μM, 5 μM) with the solution of CH_3CN/HEPES (1:1, v/v, 0.1 M, pH 7.4). Spectra data were recorded in an indicated time after the addition of amino acids. Normally, excitation was at 410 nm. The excitation and emission slit width was 5 nm and 10 nm respectively.

Procedure for preparation of human blood samples

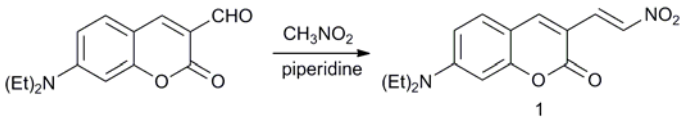
The human blood samples were treated according the reported literature procedure.[S1] 500 μL of plasma were vigorously mixed with 40 μL of hydrochloric acid 0.2 M and with 20 μL of PPh_3 400 mM (in water-acetonitrile 20:80 v/v and 2.0 M hydrochloric acid) and incubated for 15 minutes at room temperature, in order to hydrolyze the disulfide bonds. 500 μL of this hydrolysed plasma were gently mixed with 500 μL of acetonitrile in order to precipitate plasma proteins followed by centrifugation at 3000 g for 20 minutes. Then supernatant deproteinized plasma was collected filtered through a nylon filter (0.22 μm).

Fluorescence Imaging

The Tetrahymena thermophila cells was provided by Key Laboratory of Chemical Biology

and Molecular Engineering of Ministry of Education (China). Cells were grown in 1×SPP medium (1% proteose peptone, 0.2% glucose, 0.1% yeast extract, 0.003% EDTA ferric sodium salt) at 30 ^{o}C. The Tetrahymena thermophila cells were treated with 20 μM of probe **1** in culture media for 30 min at 30 ^{o}C and washed 3 times with PBS. For the control experiment, the cells were treated with 20 μM N-ethylmaleimide (NEM) in culture media for 30 min at 30 ^{o}C. After washing with phosphate buffered saline (PBS) to remove the remaining NEM, the cells were further incubated with 20 μM of **1** in culture media for 30 min at 30 ^{o}C.

Synthesis of probe 1.



Scheme S1. Synthesis of probe **1**.

Probe **1**[S2] was synthesized according to the literature report without modification. 7-Diethylamino-3-formylcoumarin (245.2 mg, 1 mmol),[S3] nitromethane (64.4 μL, 1.2 mmol), piperidine (118.5 μL, 1.2 mmol) were dissolved in 5 mL EtOH in a flask. The reaction mixture was stirred overnight at 40 ^{o}C and then the solvent was removed under the reduced pressure and the residue was purified by column chromatography using CH_2Cl_2/n-hexane (v/v 2:1, R_f= 0.50) afforded the desired probe **1** as a red solid (123.4mg, yield 43 %).

^{1}H NMR (DMSO-*d*6, 600 MHz): δ 8.46 (s, 1H), 8.10 (d, *J*= 13.2 Hz, 1H), 7.98 (d, *J*= 13.2 Hz, 1H), 7.50 (d, *J*= 9 Hz, 1H), 6.82 (d, *J*= 9 Hz, 1H), 6.62 (s, 1H), 3.50 (q, *J*= 7.2 Hz, 4H), 1.14 (t, *J*= 7.2 Hz, 6H). ^{13}C NMR (DMSO-*d*6, 150 MHz): δ 159.66, 157.48, 153.47, 150.44, 136.36, 136.23, 131.89, 110.93, 109.16, 109.03, 96.76, 44.99, 12.84 (13 carbon peaks). ESI-MS: calcd for $(M+Na)^+$ 311.10, found 311.10 $(M+Na)^+$.

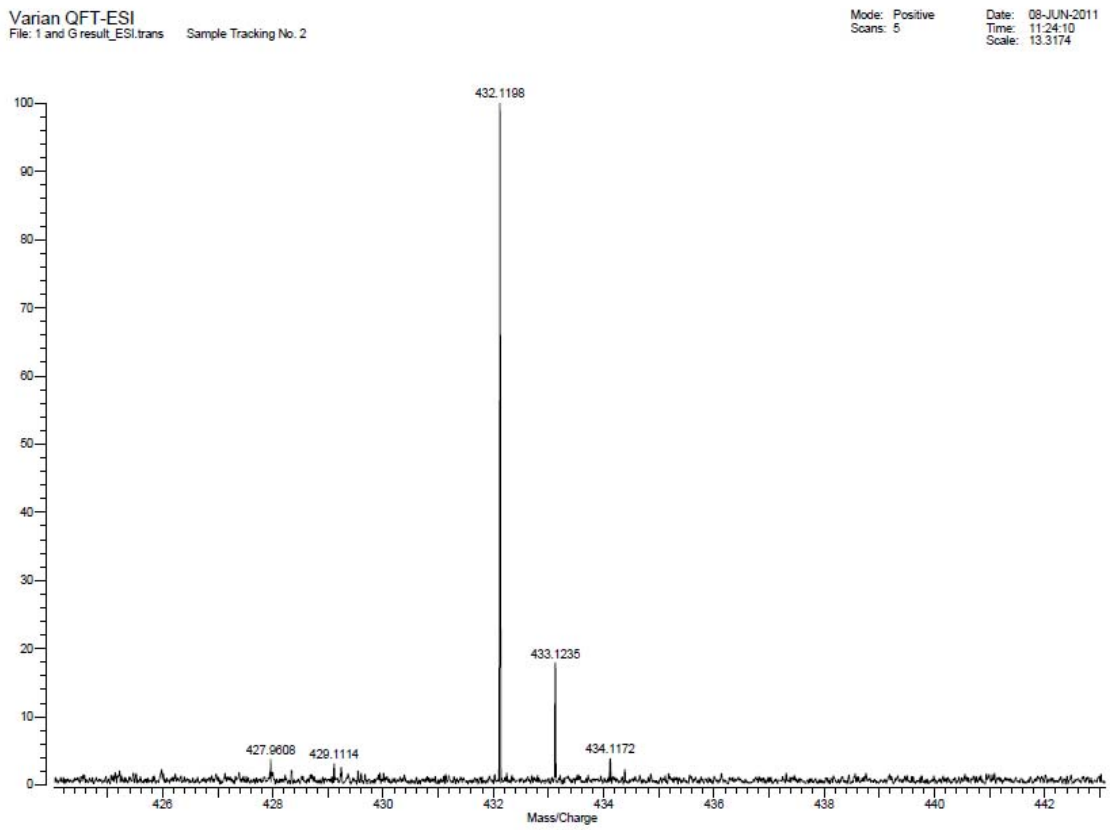


Fig. S1 ESI-MS of the probe **1** titrated with Cys.

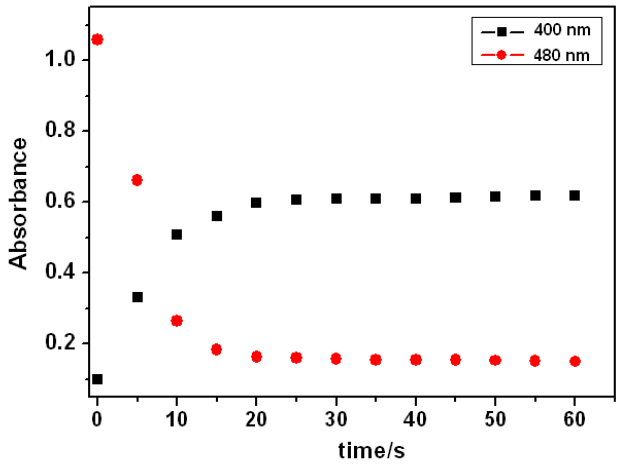


Fig. S2 The kinetic study of the response of the probe **1** to Cys at 25 ^{o}C in CH_3CN-HEPES buffer (0.1 M, pH = 7.4, 1: 1, v/v). Condition: [**1**] = 20 μM, [Cys] = 2 mM.

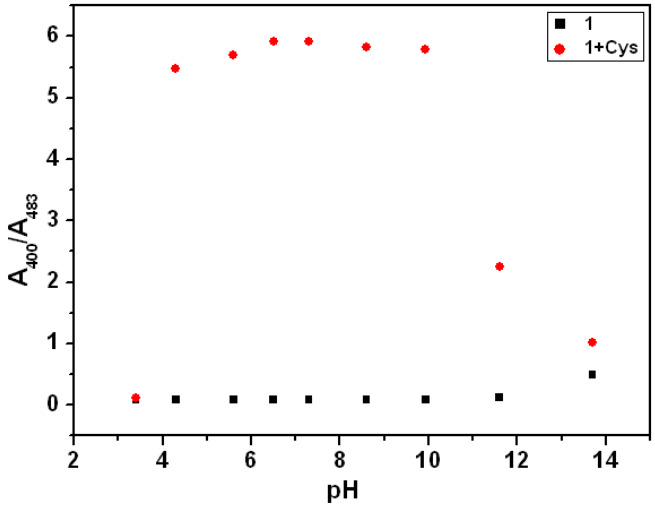


Fig. S3 The absorption intensities ratios at 400 nm and 480 nm for prove **1** (20 μM) in the absence or presence of Cys (100 equiv) at varied pH values.

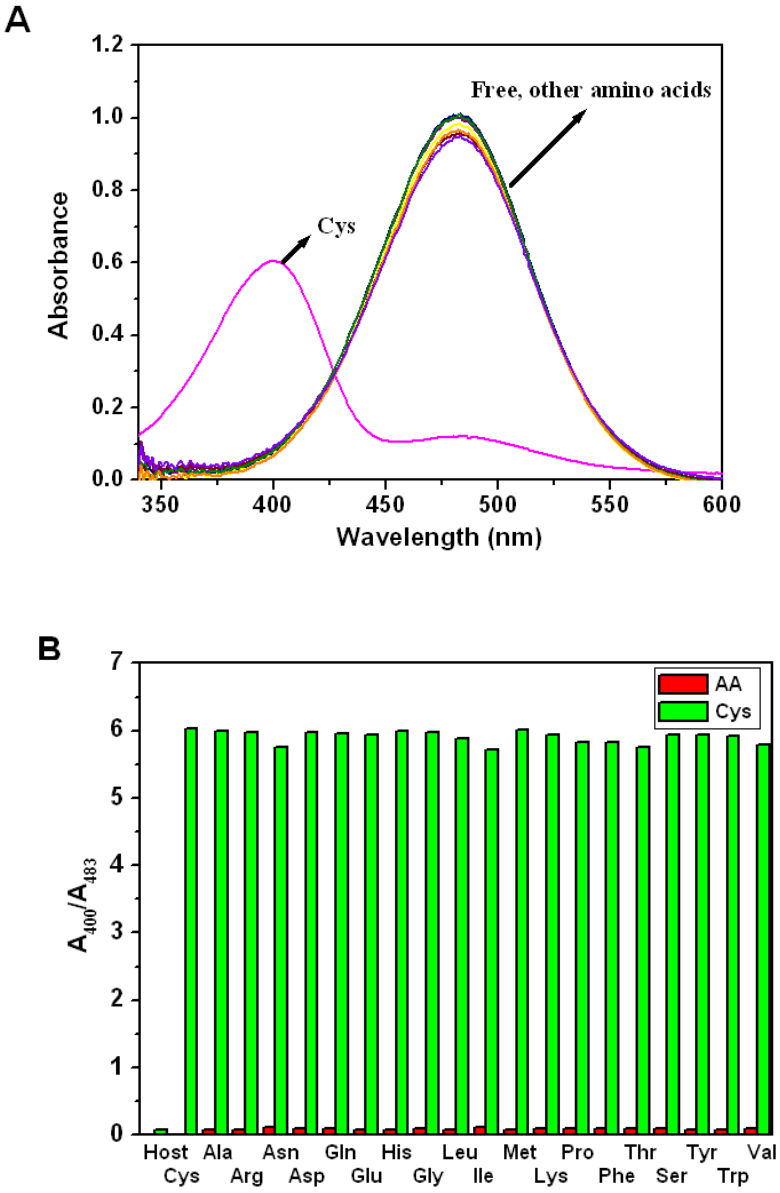


Fig. S4 (A) UV-vis absorption spectra of the probe **1** (20 μM) in CH_3CN-HEPES buffer solution (0.1 M, pH = 7.4, 1: 1, v/v) in the absence or presence of 2 mM amino acids (AA) including Cys, Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val. (B) Its competition graph with Cys.

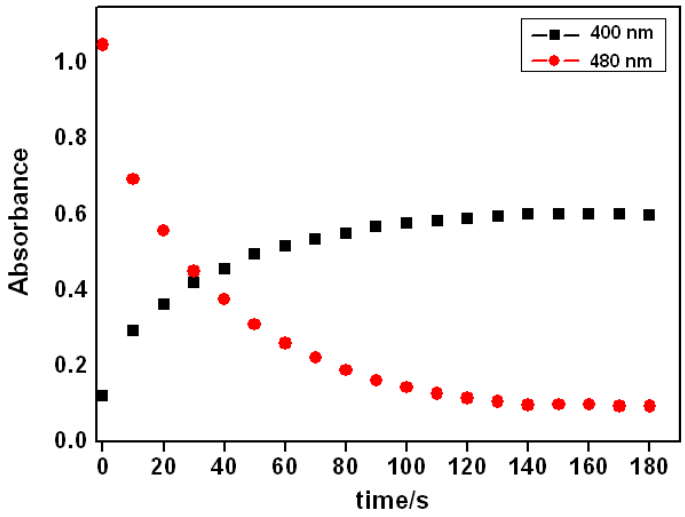


Fig. S5 The kinetic study of the response of the probe **1** to Hcy at 25 °C in CH_3CN-HEPES buffer (0.1 M, pH = 7.4, 1: 1, v/v). Condition: [**1**] = 20 μM, [Hcy] = 2 mM.

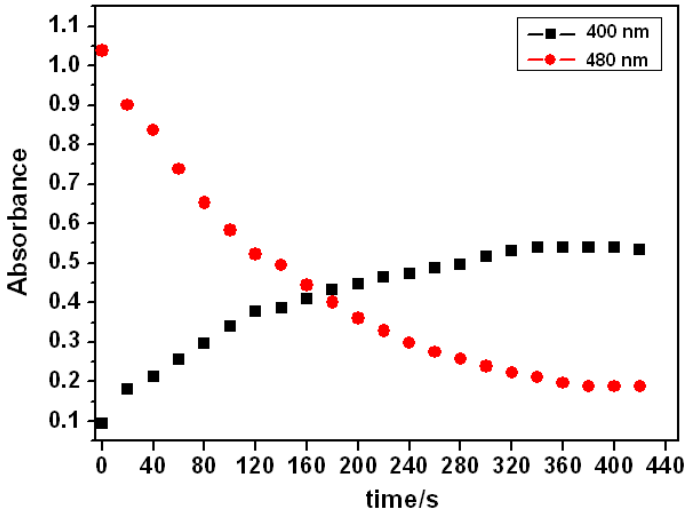


Fig. S6 The kinetic study of the response of the probe **1** to GSH at 25 °C in CH_3CN-HEPES buffer (0.1 M, pH = 7.4, 1: 1, v/v). Condition: [**1**] = 20 μM, [GSH] = 2 mM.

Detection limit

The detection limit was calculated based on the fluorescence titration.[S4] To improve the sensitivity, **1** was employed at 1 μM and the slit was adjusted to 10 nm/20 nm. To determine the S/N ratio, the emission intensity of **1** without Cys was measured by 10 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 concentration could be obtained in the 0 – 60 μM (R = 0.9994), as shown in Fig. S7. The detection limit is then calculated with the equation: detection limit = $3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements, m is the slope between intensity versus sample concentration. The detection limit was measured to be 8.6×10^{-7} M at S/N = 3 (signal-to-noise ratio of 3:1).

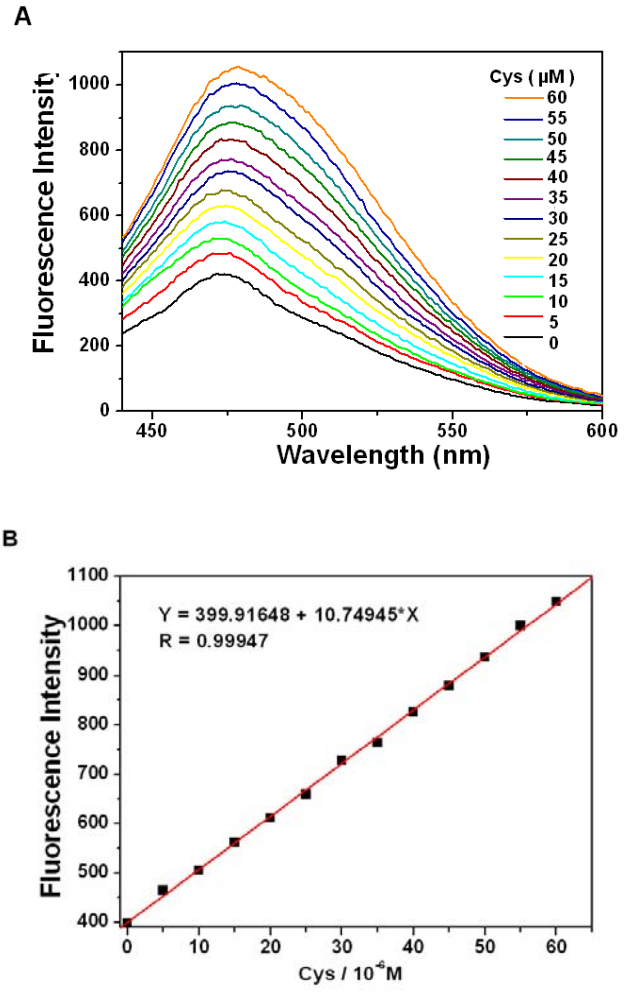


Fig. S7 (A) Fluorescence response of **1** (1 μM) to Cys ($\lambda_{ex}/\lambda_{em}$ = 410 /480 nm. Slit: 10 nm/20 nm) in CH_3CN–HEPES buffer (0.1 M, pH = 7.4, 1:1, v/v). (B) Fluorescence intensity at 480 nm of **1** (1 μM) upon addition of Cys (0–60 μM).

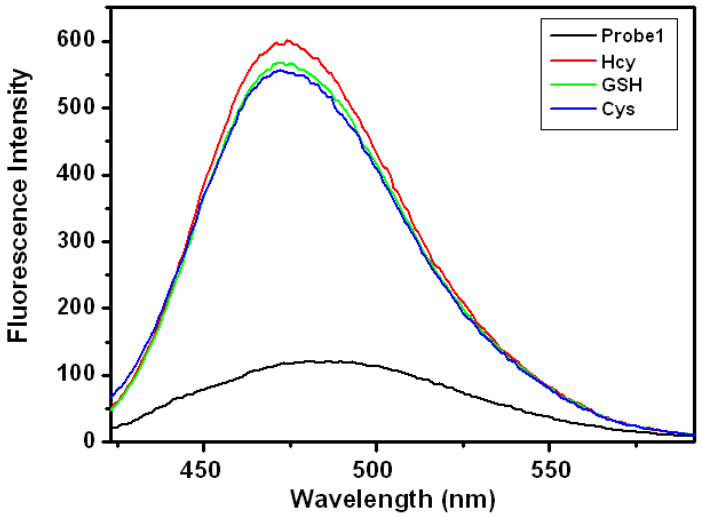


Fig. S8 Fluorescence spectra (5 μM) of **1** in CH_3CN-HEPES buffer (0.1 M, pH = 7.4, 1: 1, v/v) in the presence of thiols (100 eq, respectively) with an excitation at 410 nm after 3 min (slit = 5/10).

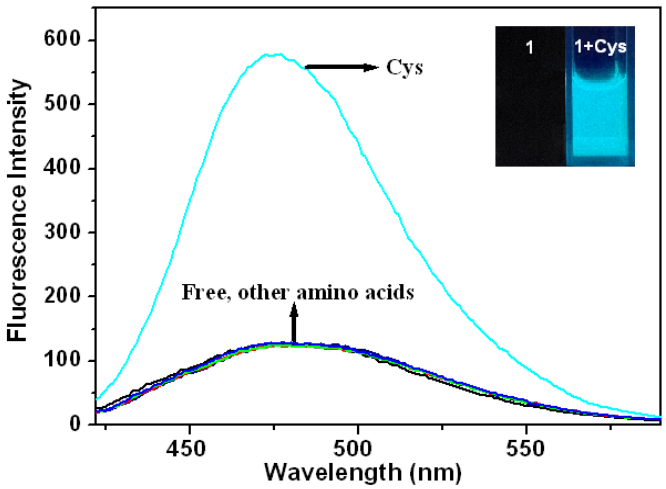


Fig. S9 Fluorescence spectra for probe **1** (5 μM) in CH_3CN–HEPES buffer (0.1 M, pH = 7.4, 1:1, v/v) in the presence of various amino acids at an excitation wavelength of 410 nm after 1 min. Inset: fluorescence emission color changes of **1** (10 μM) in the absence or presence of Cys (100 equiv).

Table S1 Determination of thiols in human plasma.[a]

Sample	Added Cys/μM	Determined thiols/μM*	Recovery%
Human plasma	0	312 ± 4[b]	–
	100	417 ± 7[c]	101.2
	200	524 ± 9[c]	102.3

*Mean ± standard deviation. Relative standard deviations were calculated on the basis of three measurements.

[a] Thiols concentrations were determined using a standard addition method[S1,S5] by measuring the increase of the intensity of the band of **1** at 480 nm. The standard curve in Fig S7 was used to extrapolate the unknown amount of thiols. [b] Aliquots of the deproteinized plasma after reduction were added directly to the CH_3CN–HEPES buffer (2 mL; 0.1 M, pH = 7.4, 1:1, v/v) containing probe **1** (1 μM), and the emission intensity at 480 nm was recorded, whereby the unknown concentrations of thiols in human blood samples were determined to be 312 μM. [c] For recovery studies, know concentrations of cysteine were added to plasma samples and the total thiol concentration was determined following the method outlined above.

References

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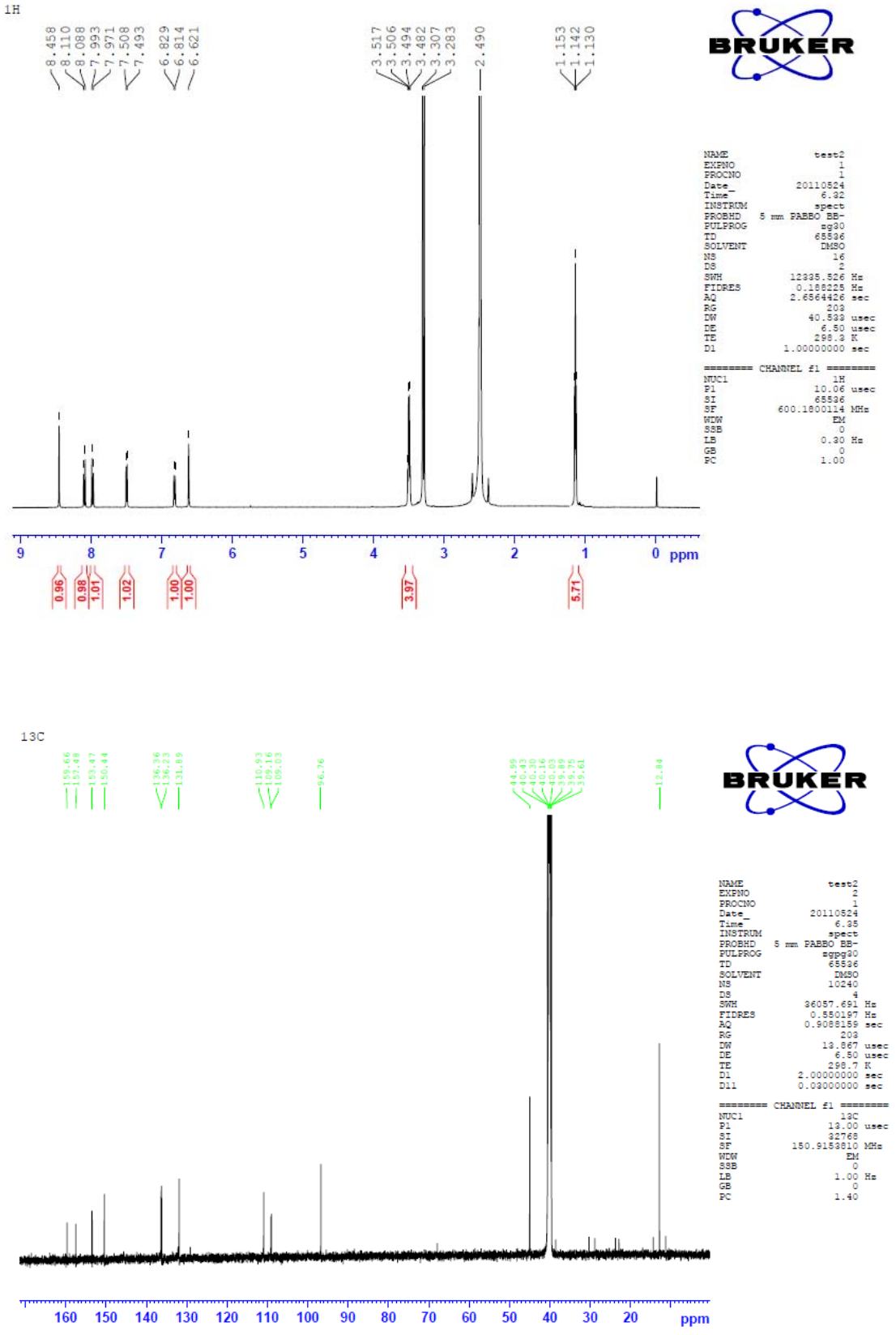

Fig. S10 The ^{1}H NMR and ^{13}C NMR spectra of the **1** in DMSO-*d*6.

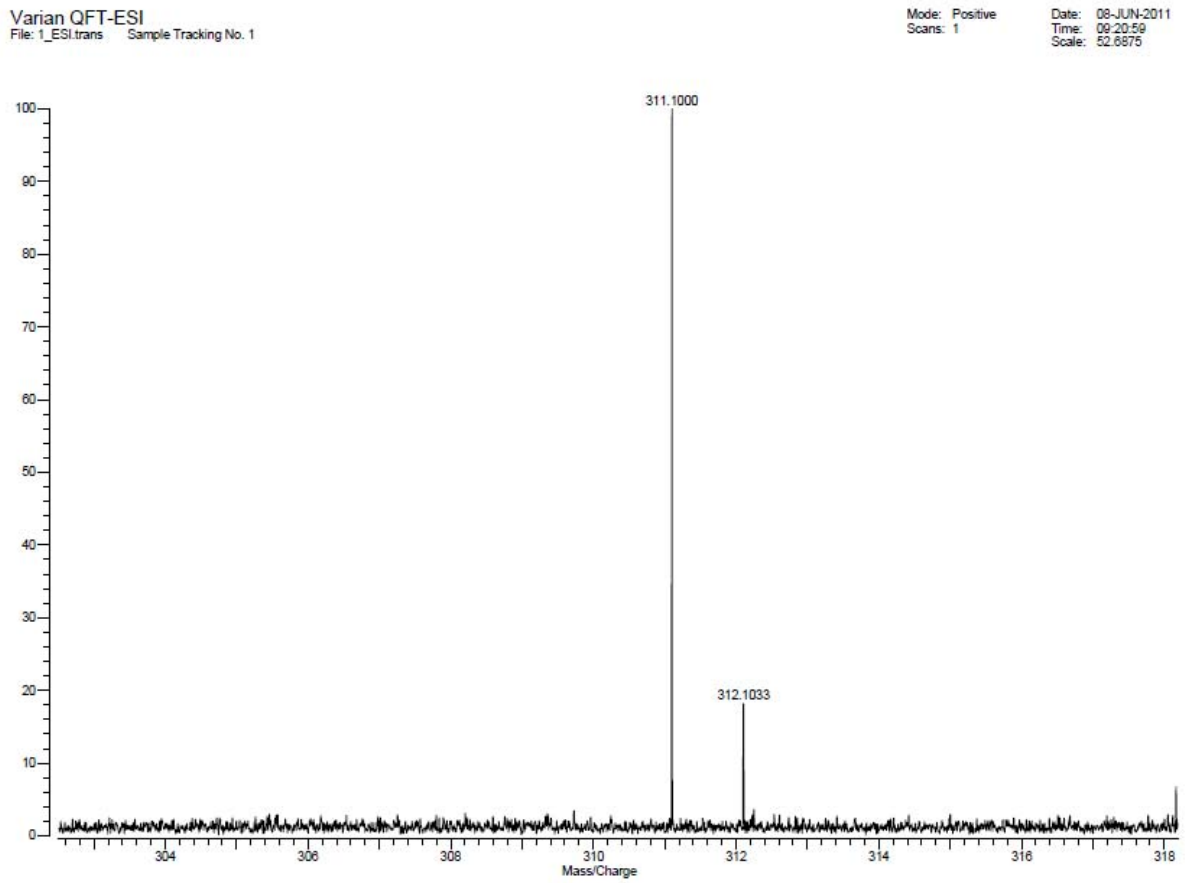

Fig. S11 ESI-MS of the probe **1.**