Electronic Supplementary Material (ESI) for Chemical Communications

A fluorescein-based probe with high selectivity to cysteine over homocysteine and glutathione

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Experimental Section

General methods.

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Chromatography was carried out on silica gel 60 (230-400 mesh ASTM). ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 500. Mass spectra were obtained using a Waters Micromass Q-Tof micro mass spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer. UV absorption spectra were obtained on α-1860A UV/Vis Spectrometer.

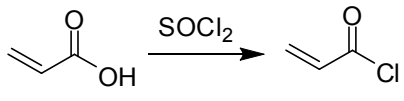
Preparation of amino acids solutions for fluorescent study

Stock solutions (10 mM) of amino acids and other anylates Cys, Hcy, GSH, Gly, Phe, Ser, Glu, Lys, Arg, His, Ala, Gln, Met and Tyr in distilled water were prepared. Stock solutions of probe **1** (1 mM) and probe **2** (1 mM) were also prepared in acetonitrile. In a typical experiment, test solutions were prepared by placing 15 μL of the probe stock solution into a test tube, diluting the solution to 3 mL with ethanol-phosphate buffer (20 mM, pH 7.4, 2:8 v/v), and adding an appropriate buffer of each analytes stock. Normally, excitation was at 478 nm. Both the excitation and emission slit widths were 1.5 nm/1.5 nm. Fluroscence spectra were measured after addition of analyte for 10 min. For low concentration titration of thiols, fluorescence spectra were measured after addition of thiols for 50 min, and both the excitation and emission slit widths were 3 nm.

Cell culture and fluorescence imaging

PC-12 cells were cultured in culture media (GIBCO RPMI 1640 supplemented with 10% FBS, 100 units/mL of penicillin, and 100 units /mL of streptomycin) at 37 ^{o}C in a humidified incubator, and culture media were replaced with fresh media every day. The cells were seeded at a density of 2×10^{6} cells per mL in culture media. After 24 h, the cells were treated without or with 500 μM NEM in culture media for 50 min at 37 ^{o}C. After washing with phosphate buffered saline (PBS) to remove the remaining NEM, the cells were further incubated with 50 μM of **1** in culture media for 30 min at 37 ^{o}C. The cells were imaged by confocal laser scanning microscopy (Olympus FV-1000).

Synthesis



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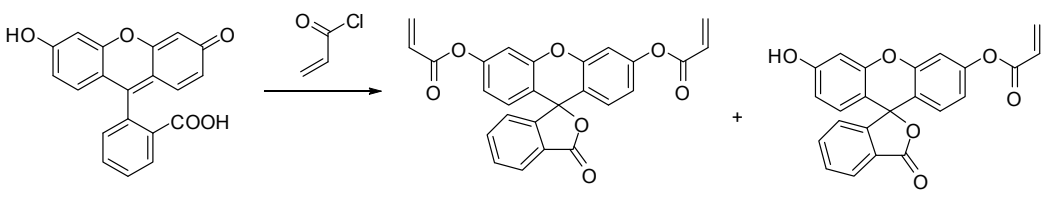

1 **2**

Compound 4

Acrylic acid (1g) was dissolved in 10 mL anhydrous dichloromethane. Then thionyl chloride(1 molar equivalent) was added to this solution while stirring under N_2 atm. The mixture was refluxed for 4 h. After the completion of reaction, the solvent was distilled out first at 39 ^oC and then acryloyl chloride at 73 ^oC to get pale yellow liquid in 86% yield.

Compound 1 and 2

Fluorescein(1g, 3.01mmol) was dissolved in 20 ml anhydrous CH_2Cl_2, acryloyl chloride(4 eq), Et_3N (4 eq) were added dropwise at 0 ^oC. After stirring at this temperature 90 min, the mixture was stirred at room temperature and stirred overnight. After evaporation of under reduced pressure, chromatography of the crude product on silica gel using CH_2Cl_2:CH_3OH(100:0.2) as eluent to afford 990 mg product (yield 75%).To get compound 2, fluorescein(100mg) was dissolved in 10 mL anhydrous CH_2Cl_2, acryloyl chloride(1.5 eq), Et_3N (2 eq) were added dropwise at 0 ^oC. After stirring at this temperature 90 min, the mixture was stirred at room temperature and stirred overnight. After evaporation of under reduced pressure, chromatography of the crude product on silica gel using CH_2Cl_2:CH_3OH (100:0.5) as eluent to afford 90.2 mg product (yield 78%).

Compound 1: ^{1}H NMR ($CDCl_3$, 500MHz) δ (ppm): 8.04(1H, d, J=7.6Hz), 7.70-7.62(2H, m), 7.20(1H, d, J=7.6Hz), 7.15(2H, s), 6.88-6.84(4H, m), 6.62(2H, d, J=17.3Hz), 6.34-6.29(2H, m), 6.05(2H, d, J=10.5Hz) ^{13}C NMR ($CDCl_3$, 500MHz) δ (ppm):163.07, 163.83, 152.85, 151.93, 151.53, 135.25, 133.26, 130.01, 128.90, 127.43, 125.17, 124.02, 117.65, 116.47, 110.31, 81.61. TOF MS *m/z* = 441.0977 $[M + H^+]^+$, calc. for $C_{26}H_{17}O_7$ = 441.0974.

Compound 2: ^{1}H NMR ($CDCl_3$, 500MHz) δ (ppm):8.02(1H, d, J=7.5Hz), 7.68-7.60(2H, m), 7.13-7.12(2H, m), 6.84-6.79(2H, m), 6.71(1H, s), 6.65-6.62(2H, m), 6.54(1H, d, J=8.4Hz), 6.35-6.30(1H, m), 6.06(1H, d, J=10.5Hz) ^{13}C NMR ($CDCl_3$, 500MHz) δ (ppm):169.69, 164.22, 158.09, 153.01, 152.18, 151.89, 151.17, 135.21, 133.43, 129.87, 129.22, 129.08, 127.47, 126.43, 125.10, 124.05, 117.29, 112,59, 110.71, 110.33, 103.13. TOF MS *m/z* = 387.0871 $[M + H^+]^+$, calc. for $C_{23}H_{15}O_6$ = 387.0869.

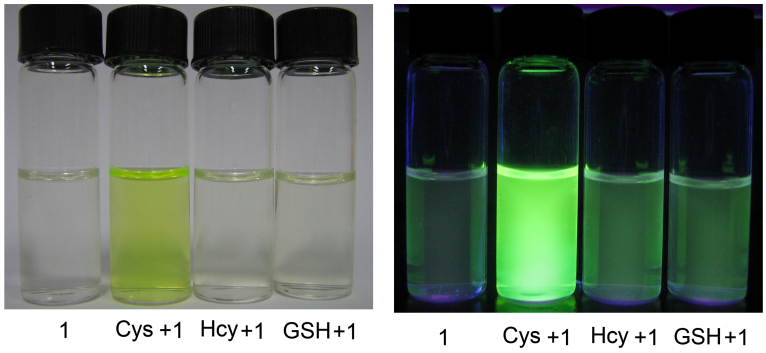

Fig.S1. Color and fluorescence changes of **1** (20 μM) in ethanol-phosphate buffer (20 mM, pH 7.4, 2:8 v/v) in the presence of 200 μM Cys, Hcy and GSH.

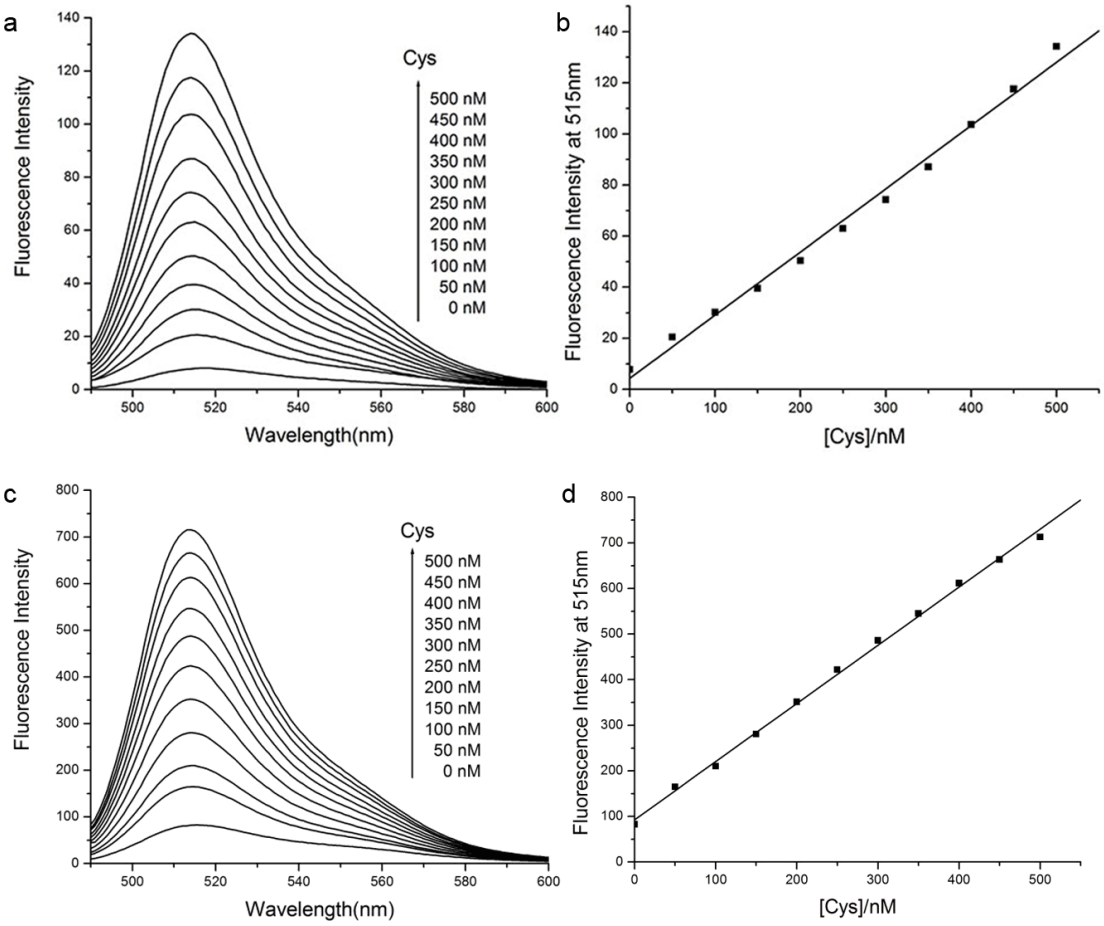

Fig.S2. a: Fluorescence of **1** (1 μM) in the presence of various concentrations of Cys (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 nM) in ethanol-phosphate buffer (20 mM, pH 7.4, 2:8 v/v) (λ_{ex} = 478 nm, slit: 3 nm/3 nm). b: The change in the fluorescence intensity of **1** (1 μM) at 515 nm against varied concentrations of Cys. c: Fluorescence of **2** (1 μM) in the presence of various concentrations of Cys (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 nM) in ethanol-phosphate buffer (20 mM, pH 7.4, 2:8 v/v) (λ_{ex} = 478 nm, slit: 3 nm/3 nm). d: The change in the fluorescence intensity of **2** (1 μM) at 515 nm against varied concentrations of Cys.

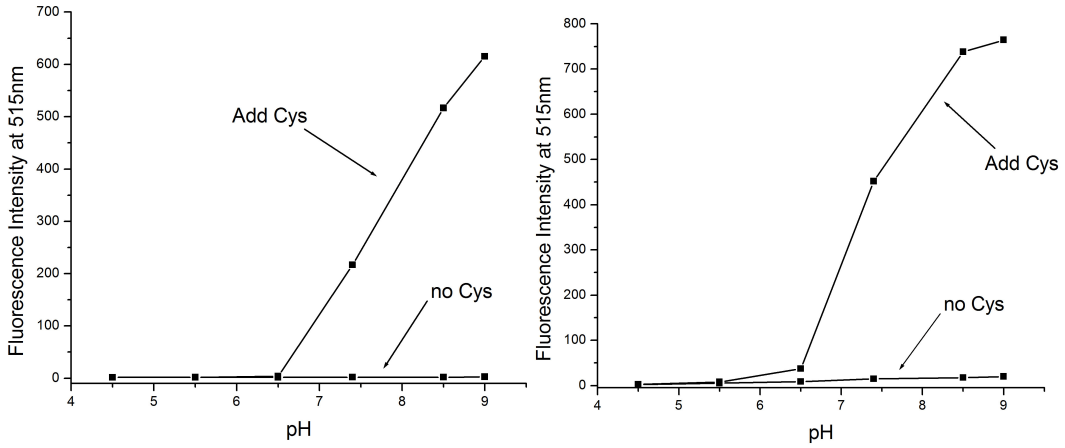

Fig.S3. Left: The fluorescence intensity of **1** at 515 nm in the presence and absence of Cys under different pH (5 μM sensor **1** in CH_3CN; λ_{ex} = 478 nm; Slit: 1.5 nm/1.5 nm). Right: The fluorescence intensity of **2** at 515 nm in the presence and absence of Cys under different pH (5 μM sensor **2** in CH_3CN; λ_{ex} = 478 nm; Slit: 1.5 nm/1.5 nm). (Phosphate-ethanol buffer (20 mM, 8:2 v/v) was used for pH 4.5, 5.5 and 7.4, MOPS-ethanol buffer (20 mM, 8:2 v/v) was used for pH 6.5, Tris-HCl-ethanol buffer (20 mM, 8:2 v/v) was used for pH 8.5, CHES-ethanol buffer (20 mM, 8:2 v/v) was used for pH 9.0).

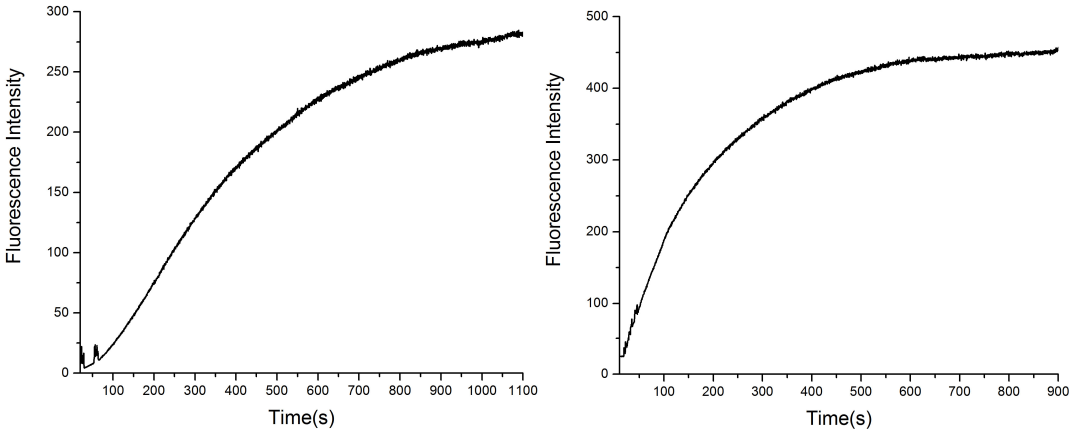

Fig. S4. Left: Time-dependent fluorescence intensity of **1**(5 μM) at 515 nm in the presence of 50 μM Cys. Right: Time-dependent fluorescence intensity of **2**(5 μM) at 515 nm in the presence of 50 μM Cys.

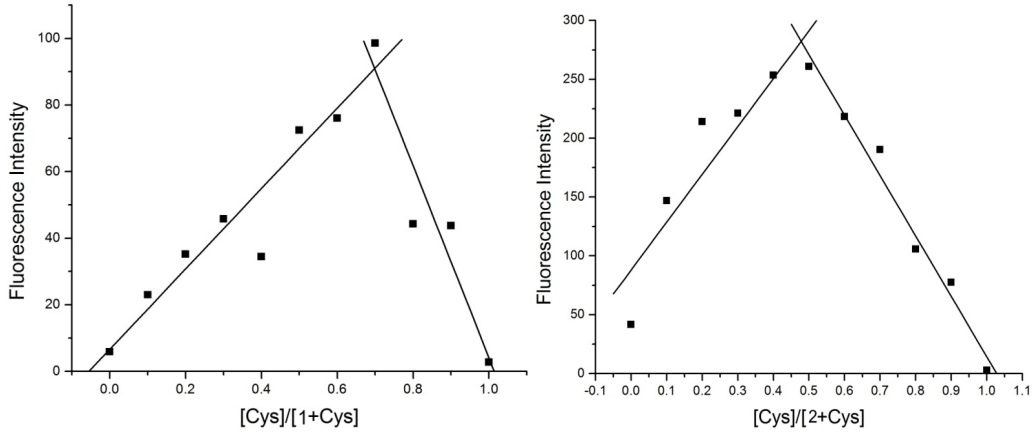

Fig.S5. Left: Job's plot of the reaction between **1** and Cys in ethanol-phosphate buffer (20 mM, pH 7.4, 2:8 v/v). Total concentration of **1** and Cys was kept constant at 10.0 μM. Right: Job's plot of the reaction between **2** and Cys in ethanol-phosphate buffer (20 mM, pH 7.4, 2:8 v/v). Total concentration of **2** and Cys was kept constant at 10.0 μM.

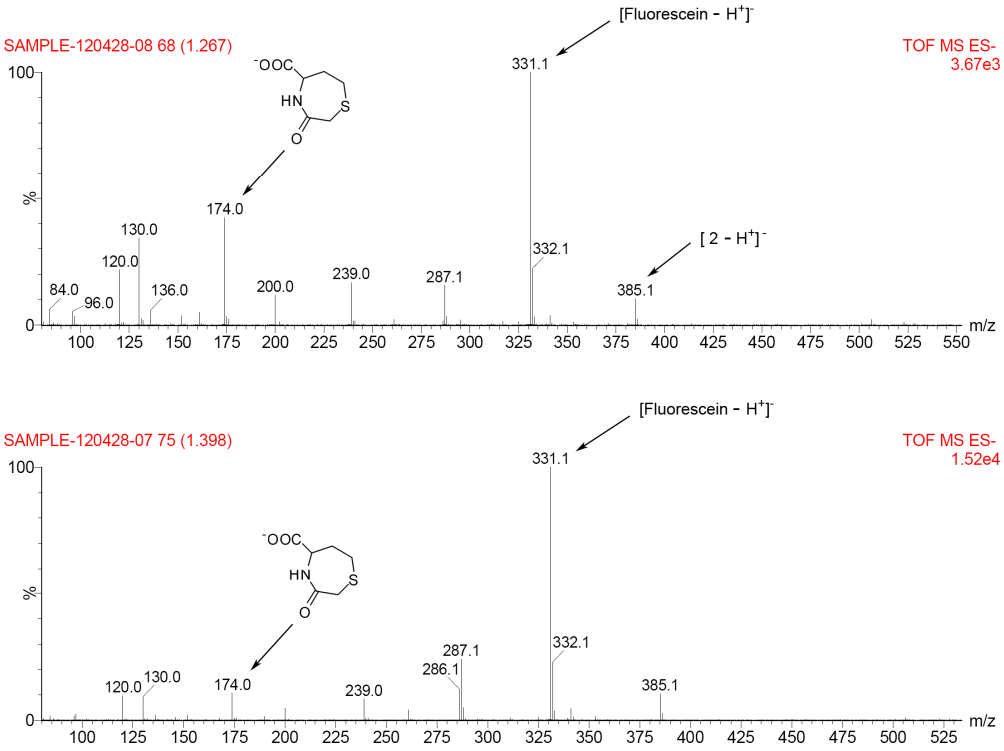

Fig.S6. Top: The ESI mass of product obtained by mixing sensor **1** and Cys; Bottom, The ESI mass of product obtained by mixing sensor **2** and Cys.

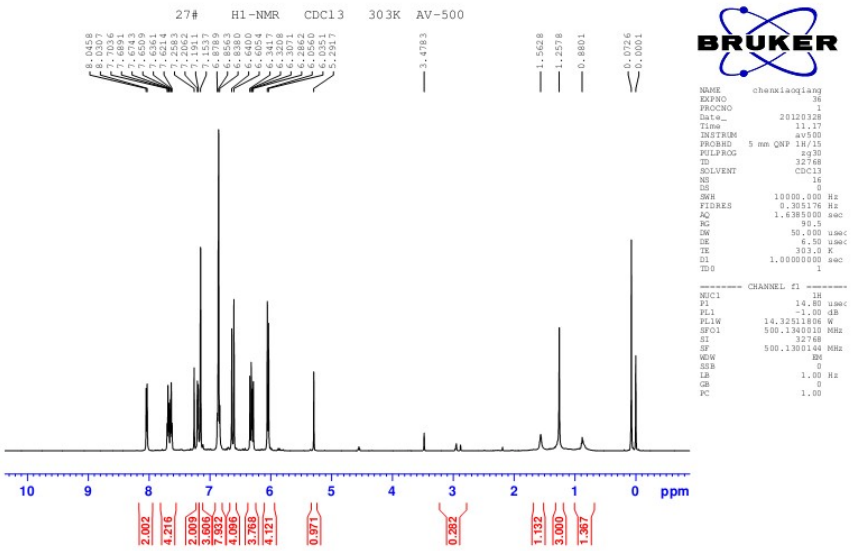

Fig.S7. ^{1}H NMR spectrum of **1** in $CDCl_3$.

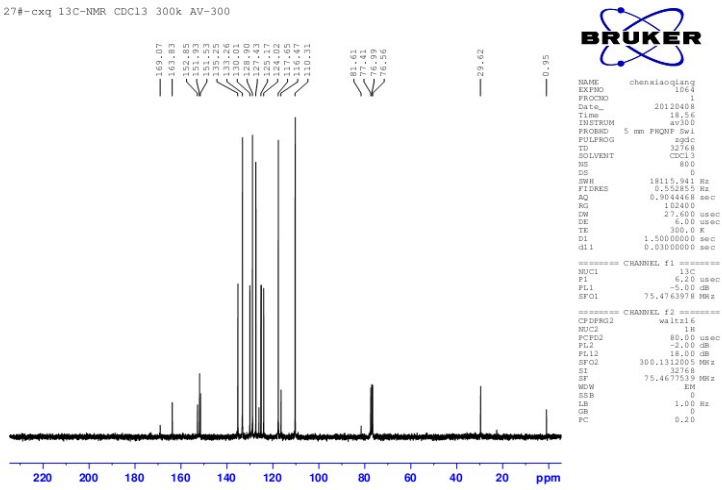

Fig.S8. ^{13}C NMR spectrum of **1** in $CDCl_3$.

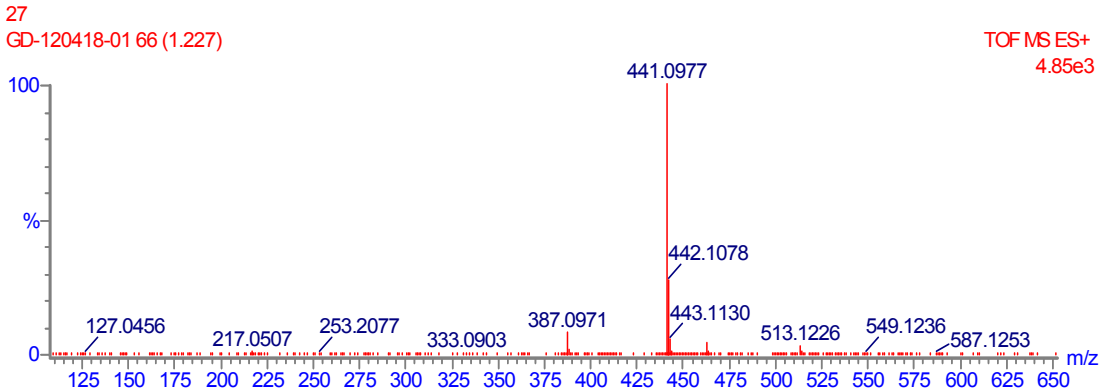

Fig.S9. ESI mass spectrum of **1**.

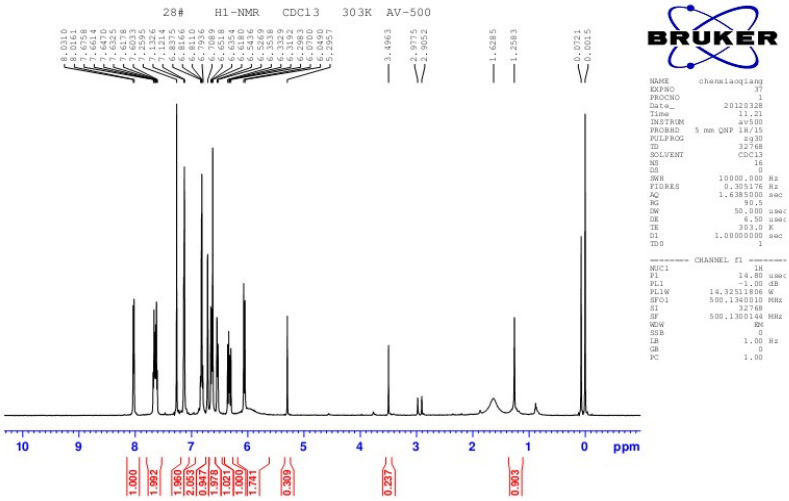

Fig.S10. ^{1}H NMR spectrum of **2** in $CDCl_3$.

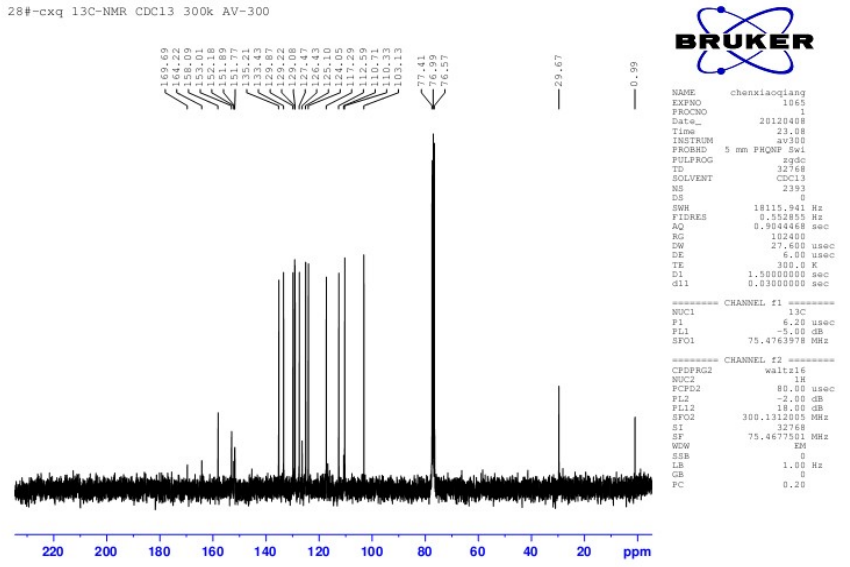

Fig.S11. ^{13}C NMR spectrum of **2** in $CDCl_3$.

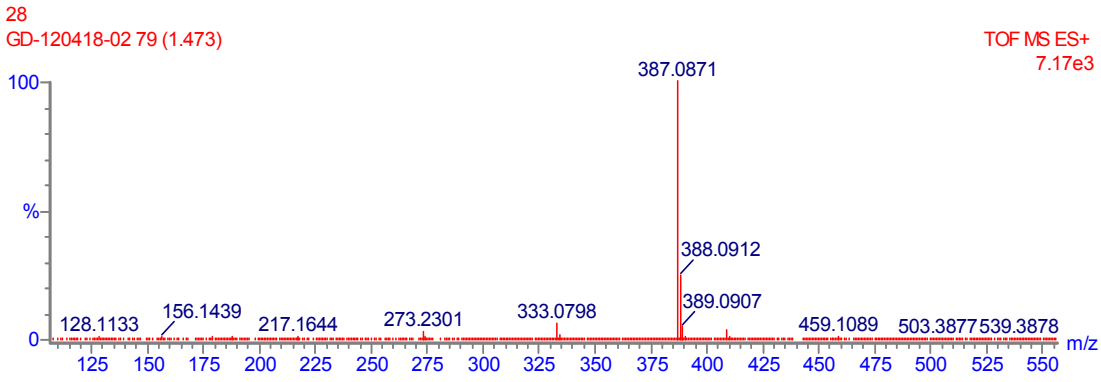

Fig.S12. ESI mass spectrum of 2.