Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

Fluorescent detection of biothiols based on a novel cascade reaction

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1. 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 SHIMADZU RF-5301PC fluorescence spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, 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 for thiol sensing

Deionized water was used throughout all experiments. A stock solution of **1** (1 mM) was prepared in CH_3CN. The stock solution of **1** was then diluted to 2 μM with the

solution of HEPES (10 mM, pH 7.4 contaning 5% CH_3CN). Spectra data were recorded in an indicated time after the addition of amino acids. Normally, excitation was at 465 nm. The excitation and emission slit width was 5 nm and 10 nm respectively.

Cell culture and fluorescence imaging: The HeLa cell line was provided by Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education (China). Cells were grown in RPMI 1640 medium supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO_2. Cells were plated on 6-well plate at 5×106 cells per well and allowed to adhere for 12 hours. We investigated the living cell bioimaging of Cys by using DMSO–PBS (1:500, v/v, pH 7.4) as a staining medium. HeLa cells were treated with 20 μM of probe **1** in the media for 30 min at 30 ^{o}C, and washed 3 times with PBS. For the control experiment, HeLa cells were pretreated with 500 μM NEM in the 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 the media for 30 min at 30 ^{o}C. Fluorescence imaging was performed with by a Olympus FV1000 Laser Scanning Confocal Microscope (Japan).

Quantum yield determination of probe 1 and methylfluorescein 2

Fluorescence quantum yields of **1** and methylfluorescein **2** were determined in HEPES buffer (pH 7.4, 10 mM, containing 5% CH_3CN) with fluorescein (Φ = 0.95, in 0.1 M NaOH) as a reference.[S3] Methylfluorescein **2** was obtained in the experiment by addition of 50 equiv of Cys to the solution of probe **1**. The quantum yields were calculated using Eq.1:

$$\Phi_u = [(A_s F A_u \eta^2)/ (A_u F A_s \eta_0^2)]\Phi s. \qquad \text{Eq.1}$$

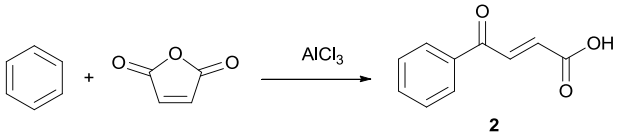
Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η_0 are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective

excitation wavelengths was controlled to be lower than 0.05.

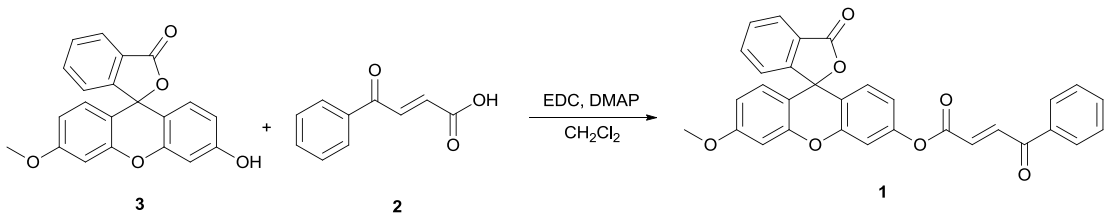
Quantum yield of **1**: Φ = 0.073

Quantum yield of methylfluorescein **2**: Φ = 0.265

Synthesis



Compound 2: 2 was synthesized according to the literature report without modification.[S1] To a stirred solution of maleic anhydride (2.45 g, 25 mmol) in 15 ml benzene was added $AlCl_3$ (7.2 g, 54 mmol) portionwise at room temperature. The mixture was stirred at 80 ^oC for 30 min. Then the content of the flask was poured onto 30 ml of ice-water and 7.5 ml of concentrated hydrochloric acid was added. The solution was extracted with 2×35 ml of EtOAc, dried with Na_2SO_4 and evaporated under reduced pressure to give 4.0 g of the product as a yellow solid. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.68 (br, 1H), 7.98–8.03 (m, 3H), 7.62–7.67 (m, 1H), 7.47–7.55 (m, 2H), 6.90 (d, J = 15.6 Hz, 1H).



Compound 1: 3 was synthesized according to the literature report.[S2] To a mixture of compounds **2** (0.264 g, 1.5 mmol), **3** (0.173 g, 0.5 mmol), EDC (0.288 g, 1.5 mmol), and DMAP (18.3 mg, 0.15 mmol) was added CH_2Cl_2 (20 ml) at room temperature. The mixture was stirred for 12 hours. Then solvent was evaporated under reduced pressure and resulted residue was subjected to silica gel chromatography with CH_2Cl_2/EtOAc (30:1), giving 0.14 g of probe **1** (55.6 %) as a light yellow solid. ^1H NMR ($CDCl_3$, 300 MHz): δ8.02–8.30 (m, 4H), 7.70–7.77 (m, 3H), 7.59 (t, J = 7.5 Hz, 2H), 7.34–7.40 (m, 2H), 6.89–7.07 (m, 4H), 6.69–6.77 (m, 2H), 3.83 (s, 3H); ^{13}C NMR (DMSO, 75 MHz): 188.6, 168.0, 162.8, 160.7, 151.8, 151.0, 150.5, 138.2,

135.3, 133.8, 129.9, 128.5, 125.1, 124.4, 123.5, 117.6, 116.3, 111.9, 109.8, 100.3, 81.1, 55.2; HRMS: calcd for 505.1282 $(M+H)^+$, found 505.1278; calcd for 527.1107 $(M+Na)^+$, found 527.1096.

References

S1 A. Tuncer, S. Filiz, O. A. Evren, Y. Aycil, R. Nurten, WO 2004/000874.

S2 L. Mugherli, O. N. Burchak, F. Chatelain, M. Y. Balakirev, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4488–4491.

S3 (a) A. T. R. Williams, S. A. Winfield, J. N. Miller, *Analyst* **1983**, *108*, 1067–1071; (b) H. Li, J. Fan, M. Hu, G. Cheng, D. Zhou, T. Wu, F. Song, S. Sun, C. Duan, X. Peng, Chem. Eur. J., **2012**, *18*, 12242-12250.

2. Supplementary Spectra

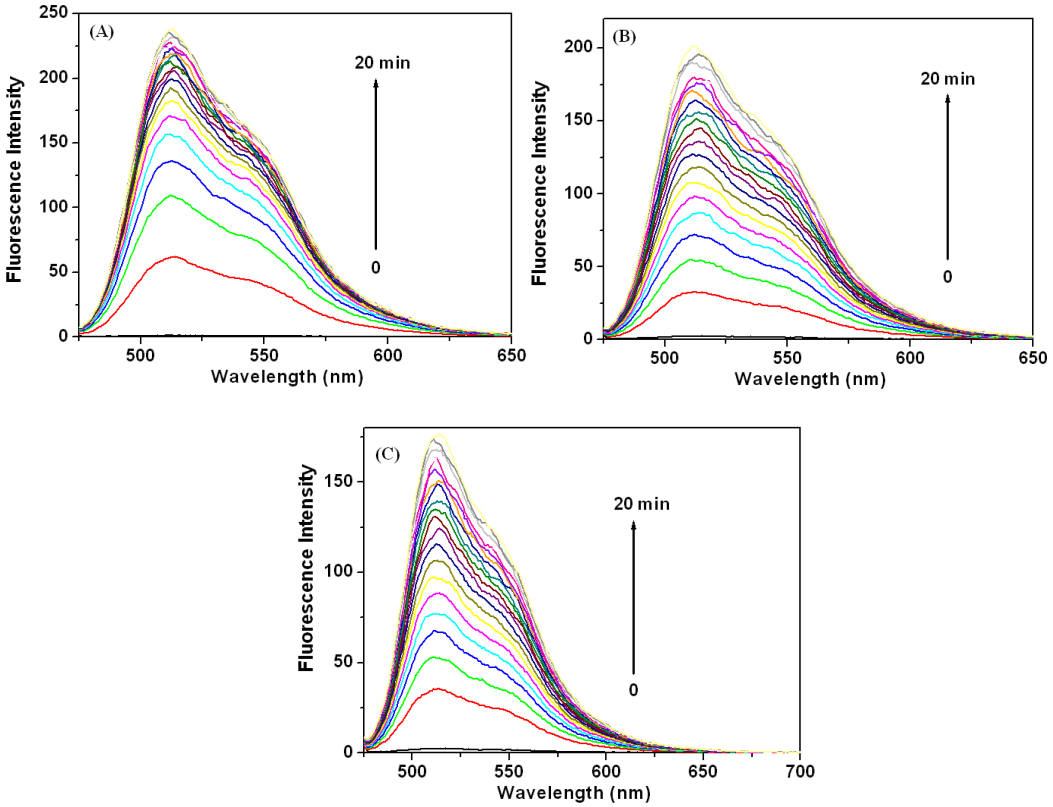


Fig. S1. Time-dependent fluorescence spectra of the probe **1** (2 μM) to several sulfydryl-containing compounds (100 μM) in HEPES buffer (pH 7.4, 10 mM, containing 5% CH_3CN) at 20 ^oC. (A) *N*-acetyl-protected Cys (NAC); (B) thiophenol; (C) mercaptoacetic acid. λ_{ex} = 465 nm, λ_{em}= 512 nm. Slit: 5 nm/5 nm.

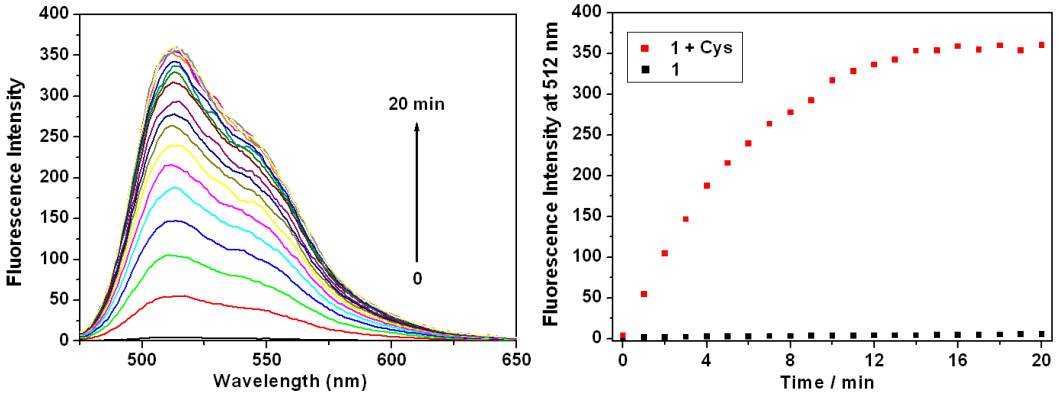


Fig. S2. Time-dependent fluorescence spectra of the probe **1** (2 μM) to Cys (10 μM) in HEPES buffer (pH 7.4, 10 mM, containing 5% CH_3CN) at 20 ^oC.

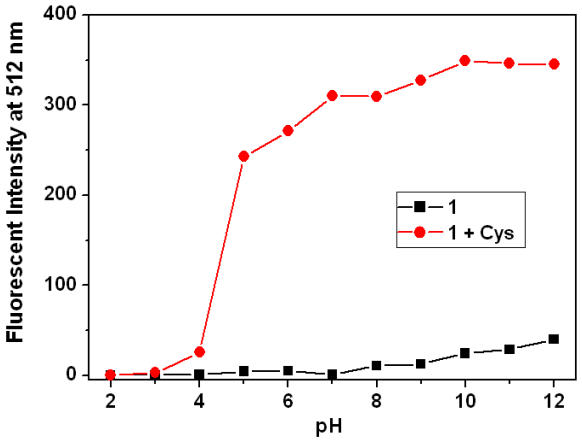


Fig. S3 The fluorescent intensities at 512 nm for probe **1** (2 μM) in the absence or presence of Cys (10 μM) at varied pH values.

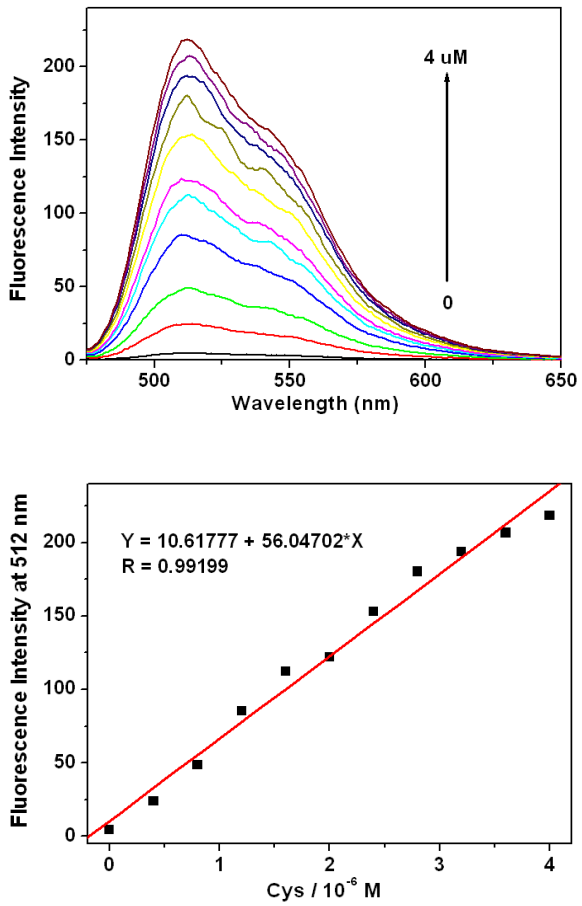


Fig. S4 (A) Fluorescence spectral changes of **1** (2 μM) upon addition of Cys (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0 μM) (λ_{ex} = 465 nm, λ_{em}= 512 nm; slit, 5 nm/5 nm). (B) The fluorescence intensity of **1** at 512 nm as a function of the concentration of Cys. Each spectrum was recorded after 15 min upon addition Cys in HEPES buffer (pH 7.4, 10 mM, containing 5% CH_3CN) at 20 ^oC.

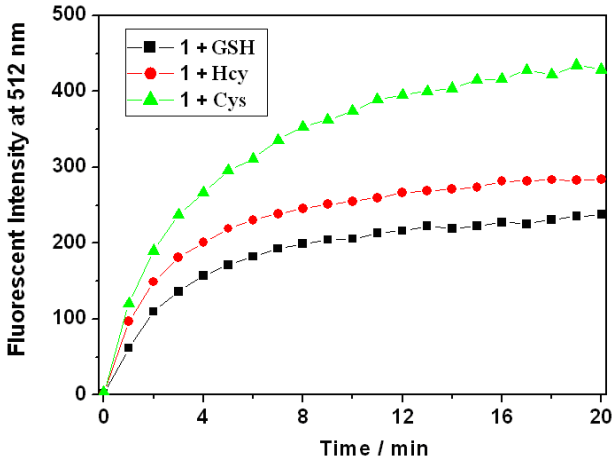


Fig. S5 The kinetic study of the response of the probe **1** (2 μM) to 100 μM of Cys, Hcy and GSH, respectively, in HEPES buffer (pH 7.4, 10 mM, containing 5% CH_3CN) at 20 ^oC.

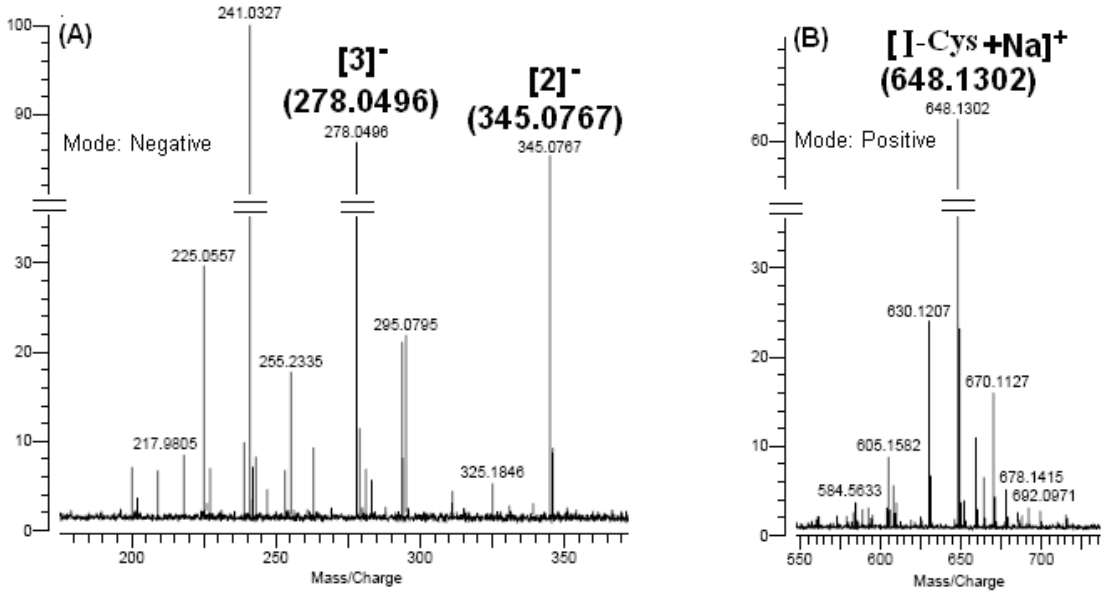


Fig. S6 HRMS charts of probe **1** upon addition of Cys obtained after 0.5 h (A) and within 1 min (B).

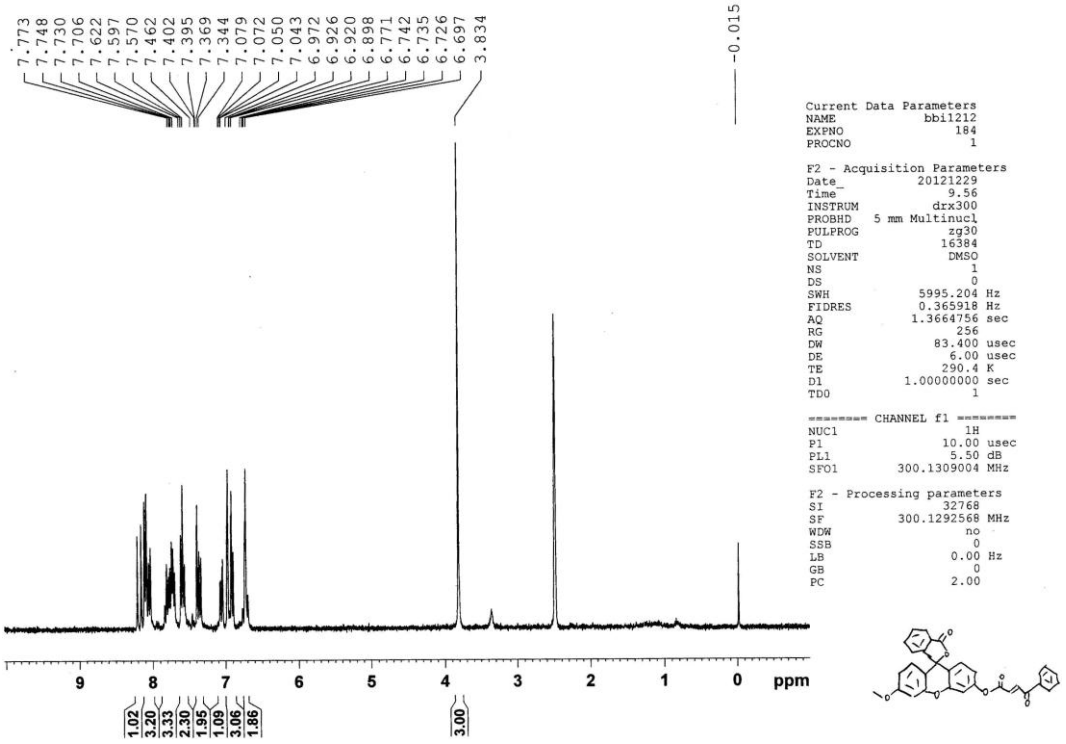


Fig. S7 ^{1}H NMR chart of **1** (DMSO-d_6) (300 MHz)**.**

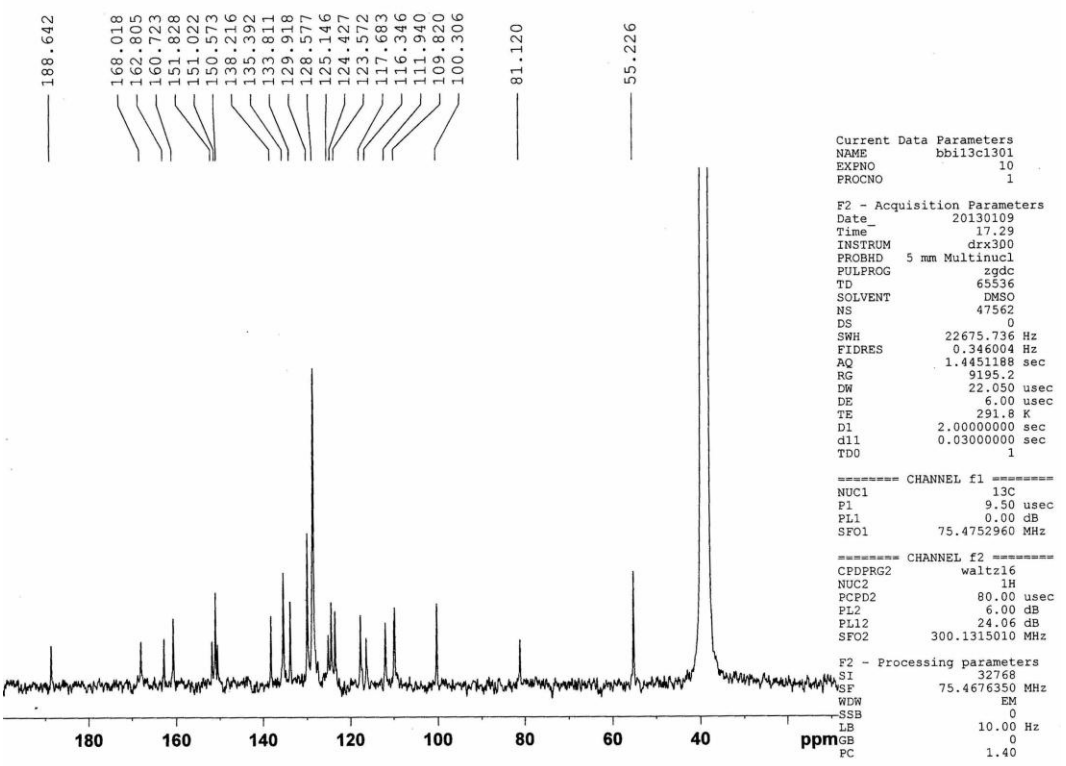


Fig. S8 ^{13}C NMR chart of **1** (DMSO-d_6) (75 MHz)**.**

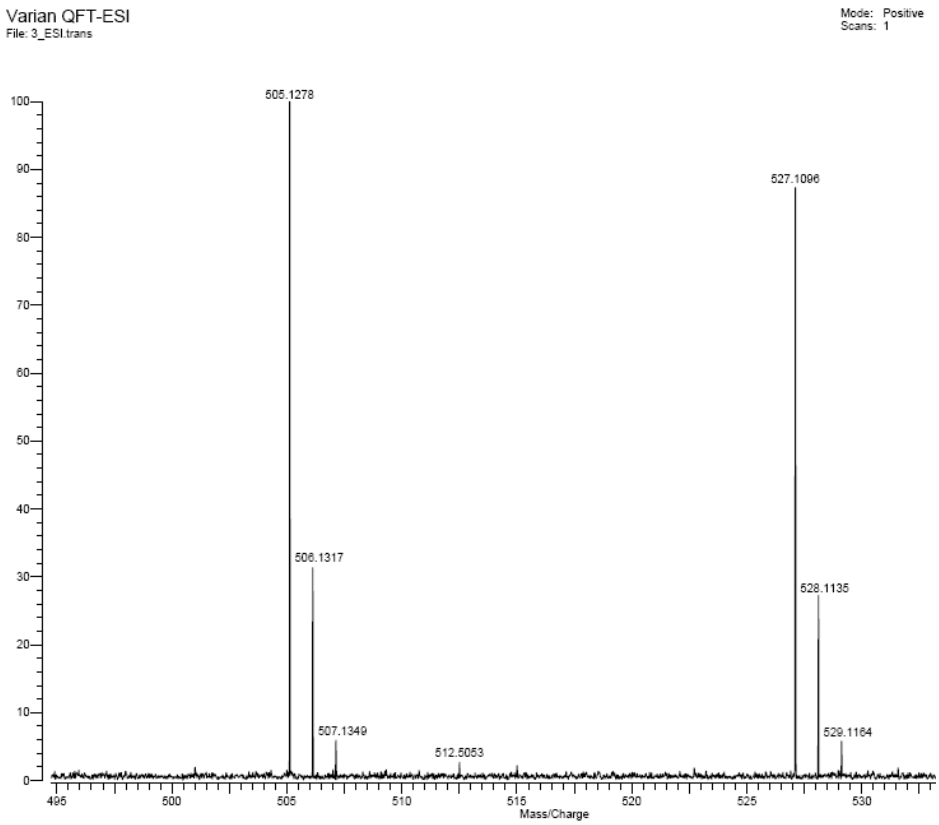


Fig. S9 HRMS chart of **1**.