Electronic Supplementary Information (ESI)

A Merocyanine-based Colorimetric and Fluorescent Probe for Specifically Distinguishing Cysteine from Biothiols in Water and Imaging Application in Living Cells

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Experimental

1. Synthesis

*1.1 (E)-2-(4-hydroxystyryl)-1,3,3-trimethyl-3H-indolium iodide (compound **2**) was synthesized according to the literature procedure*[1].

^{1}H NMR (400 MHz, d_6-DMSO) δ = 1.784 (s, 6H), 4.102 (s, 3H), 6.967 (d, *J* = 8.8 Hz, 2H), 7.475 (d, *J* = 16.4 Hz, 1H), 7.561–7.638 (m, 2H), 7.843–7.865 (m, 2H), 8.150 (d, *J* = 8.4 Hz, 2H), 8.375 (d, *J* = 16 Hz, 1H), 10.815 (s, 1H). ^{13}C NMR (d_6-DMSO, 100 MHz) δ = 25.1, 33.6, 51.2, 108.8, 114.5, 115.9, 122.3, 125.5, 128.2, 128.3, 133.1, 141.3, 142.7, 153.2, 162.7, 180.9. ESI-MS *m/z* [$(M-I)^+$]: 278.2.

*1.2 Synthesis of (E)-2-(4-acryloyloxystyryl) -1,3,3-trimethyl-3H-indolium iodide (probe **3**).*

To a solution of **2** (132 mg, 0.325 mmol) in 2 mL of DMF, and then added 25 ml of CH_2Cl_2. After added anhydrous K_2CO_3 (45 mg, 325 mmol) and acryloyl chloride (0.2 ml, mixed with 5 mL of CH_2Cl_2) the reaction mixture was stirred at 0 ^{o}C and warmed to rt overnight. After removal of the insoluble materials by filtration, the filtrate was evaporated. The crude product was purified by chromatography (SiO_2, CH_2Cl_2/MeOH 20:1, v/v) to afford **3** as a orangered solid (106 mg, 75%). ^{1}H NMR (400 MHz, d_6-DMSO) δ = 1.809 (s, 6H), 4.181 (s, 3H), 6.224 (d, *J* = 10.4 Hz, 1H), 6.457 (dd, *J* = 17.2 Hz, *J* = 10.0 Hz, 1H), 6.598 (d, *J* = 17.2 Hz, 1H), 7.459 (d, *J* = 8.4 Hz, 2H), 7.561–7.638 (m, 2H), 7.711 (d, *J* = 16.4 Hz, 1H), 7.889–7.940 (m, 2H), 8.321 (d, *J* = 8.4 Hz, 1H), 8.842 (d, *J* = 16.4 Hz, 1H). ^{13}C NMR (d_6-DMSO, 100 MHz) δ = 24.7, 34.2, 51.8, 112.9, 114.8, 122.1, 122.2, 122.4, 126.8, 128.4, 129.0, 133.8, 141.3, 143.1, 151.2, 153.2, 163.2, 181.3. ESI-MS *m/z* [$(M-I)^+$]: 332.3.

2. Limit of detection in analysis

The limit of detection, expressed as the concentration c_L, or the quantity q_L, is derived from the smallest measure x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is given by the equation:

$$x_L = \bar{x}_{bi} + ks_{bi}$$

Where, $\bar{x}_{bi}$ is the mean of the blank measures, s_{bi} is the standard deviation of the blank measures, and *k* is a numerical factor chosen according to the confidence level desired.

$$c_L = (x_L - \bar{x}_B)/\mathrm{m}$$

Where, m is the slope of the linear regression equation. Combine two of the equations, Long and Winefordner defined c_L:

$$c_L = (k \cdot s_B)/\mathrm{m}$$

Generally, k =3, P < 0.01, we obtained c_L = 0.5 μM.

3. X-ray Diffraction Studies.

Single-crystal X-ray diffraction measurements were carried out on a Bruker APEX-II CCD diffractometer operating at 50 KV and 30 mA using Mo Kα radiation (λ=0.71073Å). Data collection and reduction were performed using SMART and SAINT software.[2] An empirical absorption correction was applied using the SADABS program.[3] The structure was solved by direct methods and refined by full-matrix least-squares on F^2 using the SHELXL-97 program package.[4]

Crystal data and details of the structure determination for compound **2** are summarized in Table S1. CCDC 970856 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

Formula	$C_{19}H_{20}INO$
Formula Weight	405.26
Crystal System	Orthorhombic
Space group	*Pnma*
a (Å)	20.3874 (8)

b (Å)	6.9055 (3)
c (Å)	12.3088 (5)
α (°)	90.00
β (°)	90.00
γ (°)	90.00
Z	4
Volume (Å^3)	1732.90 (12)
Density (calculated, Mg/m^3)	1.553
Absorption coefficient (mm^{-1})	1.47
F(000)	808
Crystal Size (mm^3)	0.21 × 0.21 × 0.20
Radiation (Å)	0.71073
Reflections collected	9013
Independent reflections	1755 (R_{int} = 0.023)
Date/restrains/parameters	1755 / 0 / 134
Final *R* indices [I>2.0σ (*I*)]	R_1 = 0.0311, wR_2 = 0.1247
R indices (all date)	R_1 = 0.0348, wR_2 = 0.1328
Goodness-of-fit on F^2	1.01

Table 1. Crystal Data and Details of the Structure Determination for compound **2.**

4. Distribution Coefficients of Probe 3 in Octanol/Water

The retention measurements by HPLC were made with a Waters 1525 (Breeze™ HPLC system) equipped with a C_{18} reversed phase HPLC column (75 mm×4.6 mm Symmetry® C_{18} 3.5 μm) and UV detector (Waters 2998 PDA) set at 384 nm. An injector with 5 μL sample loopwas used for the upper and the lower phases sample injections. The mobile phase was CH_3CN 50% (v/v) in distilled water and the flow rate was 0.5 mL/min.

Partition coefficients of the probe **3** in n-octanol/water system (log $P_{o/w}$) were determined by

a conventional shake-flask method. Briefly, probe **3** (10 mg) was dissolved in octanol saturated distilledwater (5 ml) and mixed with water saturated n-octanol (10 ml). The mixture was shaken on 3D shaking-table overnight. The absorbances of the upper and the lower phases showed on any other compound. In order to determine the concentration, the two phases were carefully separated. The Fig. S23-24 shows that the retention time of probe **3** in the water and octanol are appropriate and consistent with each other, peak shape is normal, shows that this method is applicable to the fat water distribution coefficient of determination of the sample. All values correspond to the mean of 3 measurements.

References

[1] J. Gu, U.R. Anumala, F. Lo Monte, T. Kramer, R. Heyny von Haussen, J. Holzer, V. Goetschy-Meyer, G. Mall, I.Hilger, C. Czech, B. Schmidt, 2-Styrylindolium based fluorescent probes visualize neurofibrillary tangles in Alzheimer's disease, Bioorg. Med. Chem. Lett., 22 (2012) 7667-7671.

[2] (a) *SMART*, 5.05 ed.; Bruker AXS, Inc.: Madison, WI, 1998. (b) *Bruker AdVanced X-ray Solutions SAINT*, version 6.45; Bruker AXS, Inc.:Madison, WI, 1997-2003.

[3] Sheldrick, G. M. *SADABS: Area-Detector Absorption Correction*; University of Göttingen: Göttingen, Germany, 1996.

[4] Heldrick, G. M. *SHELXL-97, Program for the Refinement of Crystal Structures*; University of Göttingen, Göttingen, Germany, 1997.

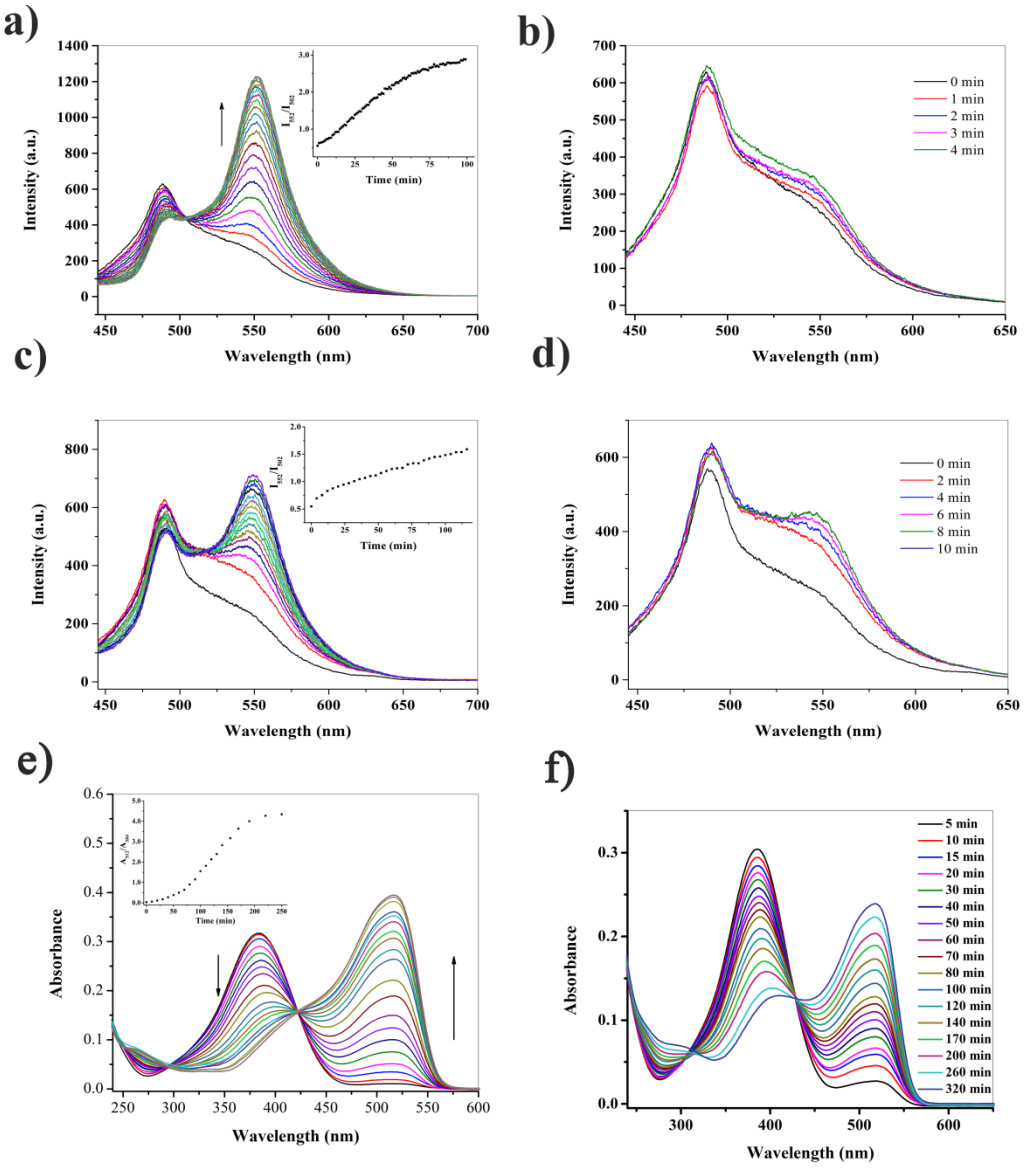


Fig. S1. Time-dependent fluorescence spectral and UV-vis spectral changes of probe **3** (10 μM) with Hcy (10 equiv) or GSH (10 equiv) in phosphate buffer (10 mM, pH 7.40). **(a)** Fluorescence spectral of Hcy (λex = 418 nm). Inset: Fluorescence intensity ratios R (I_{552}/I_{502}) with addition of 100 μM Hcy depending on time. **(b)** Fluorescence spectral of Hcy within 4 min, λex = 418 nm. **(c)** Fluorescence spectral of GSH (λex = 418 nm). Inset: Fluorescence intensity ratios R (I_{552}/I_{502}) with addition of 100 μM GSH depending on time. **(d)** Fluorescence spectral of GSH within 10 min, λex = 418 nm. **(e)** UV-vis spectral of Hcy. Inset: Absorbance ratio (A_{512}/A_{384}) with addition of 100 μM Hcy depending on time. **(f)** UV-vis spectral of GSH. Inset: Absorbance ratio (A_{512}/A_{384}) with addition of 100 μM GSH depending on time.

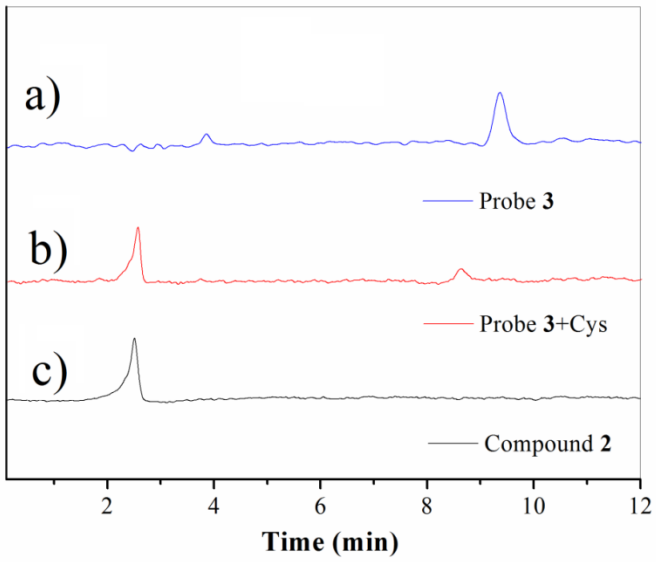


Fig. S2. HPLC chromatograms (from highest to lowest) of probe **3** and Cys (a) Before addition of Cys. (b) Reaction mixture after incubation for 15 min at room temperature. (c) Compound **2** prepared as the authentic sample. The reaction was carried out in water. The HPLC gradient was as follows: solvent A (water), solvent B (CH_3CN); 0-12 min, 80% B. The peaks were monitored by UV absorbance at 490 nm.

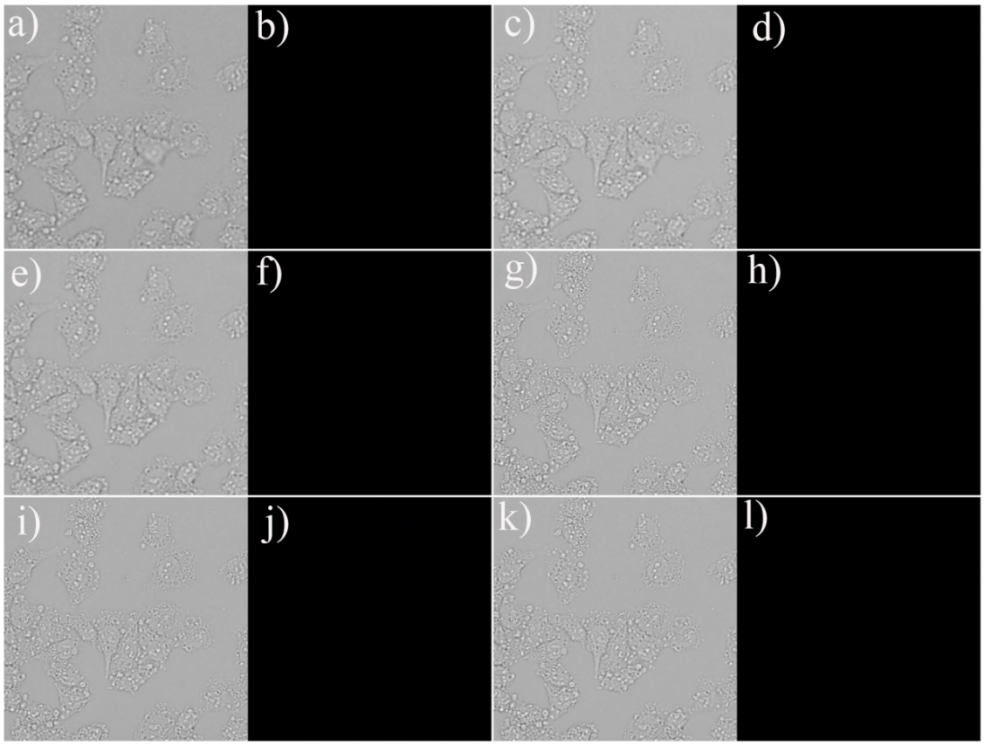


Fig. S3. Fluorescence images of living *Hela* cells incubated at different time. The cells were pretreated with 100 μM N-ethylmaleimide for 1.0 h, and then incubated with 50 μM probe **3**. (**a, b**) 40 min; (**c, d**) 40 min; (**e, f**) 50 min; **(g, h)** 60 min; (**g, h**) 70 min; (**i, j**) 80 min; (**k, l**) 90 min. Scale bar is 20 mm. Brightfield images (**a, c, e, g, I, k**), fluorescence images (**b, d, f, h, j, l**).

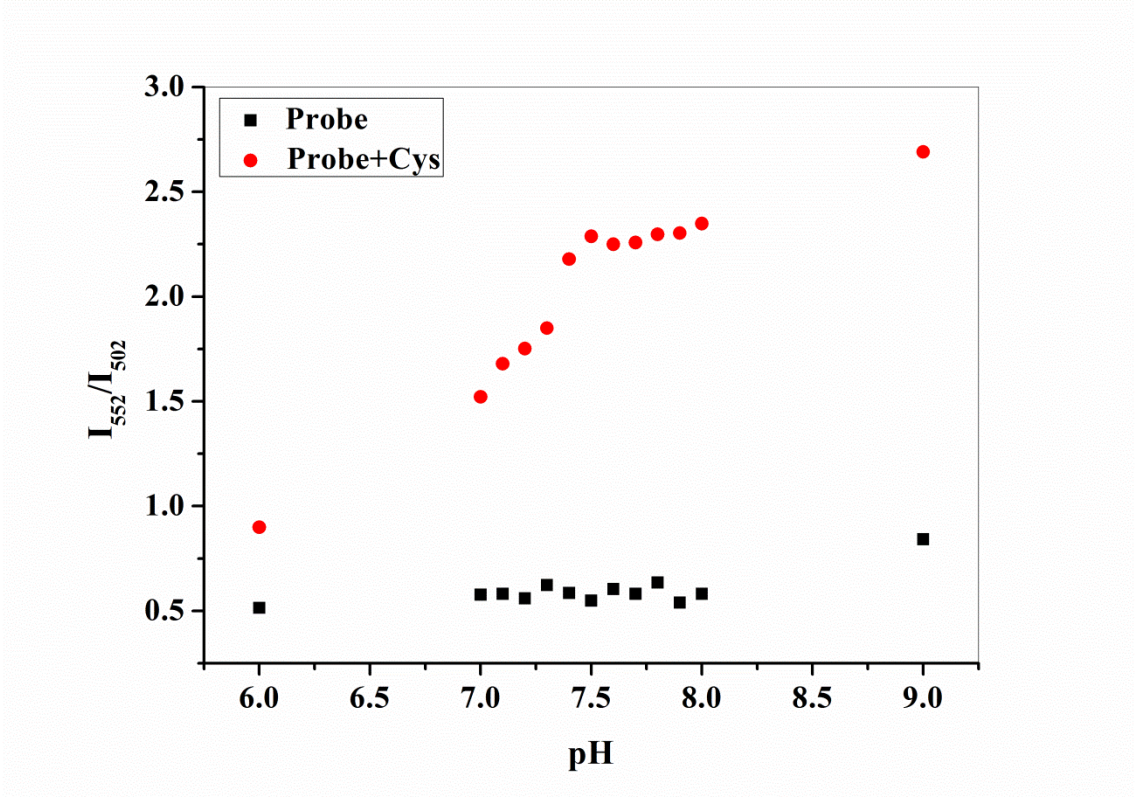
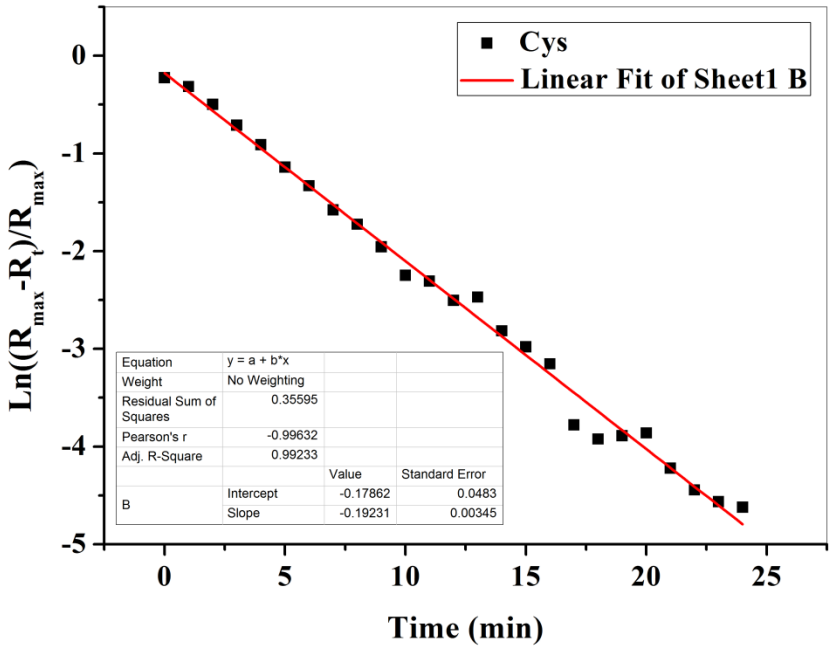


Fig. S4. Fluorescence intensity ratio R (I_{552}/I_{502}) of probe **3** (■) and probe **3** (10 μM) with Cys (0.1 mM) (●) at various pH values. Effect of pH on the Fluorescence intensity ratio R (I_{552}/I_{502}) of probe **3** (■) and probe **3** (10 μM) with Cys (0.1 mM) after 10 min (●), respectively. The excitation wavelength was 418 nm.

a)



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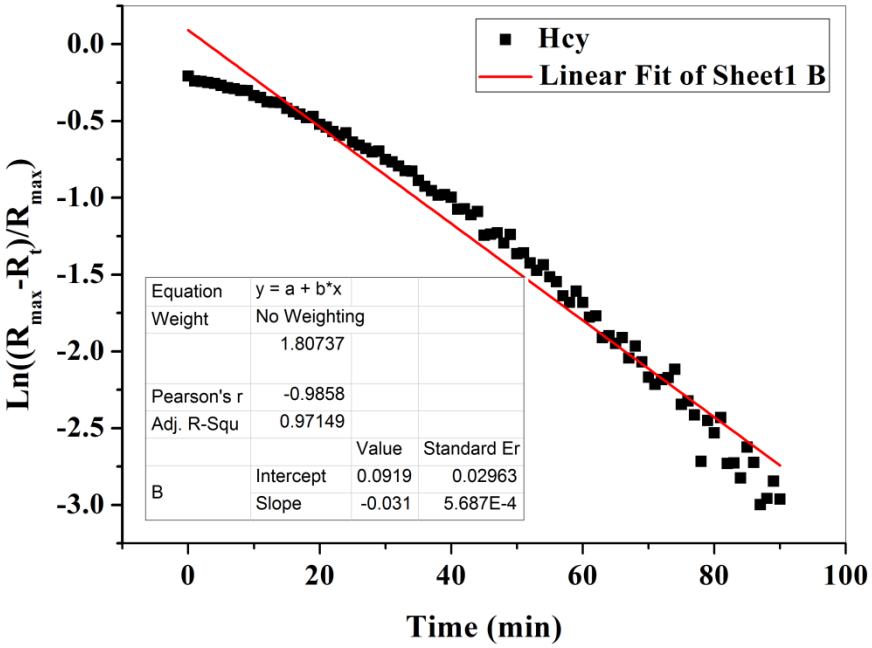

c)

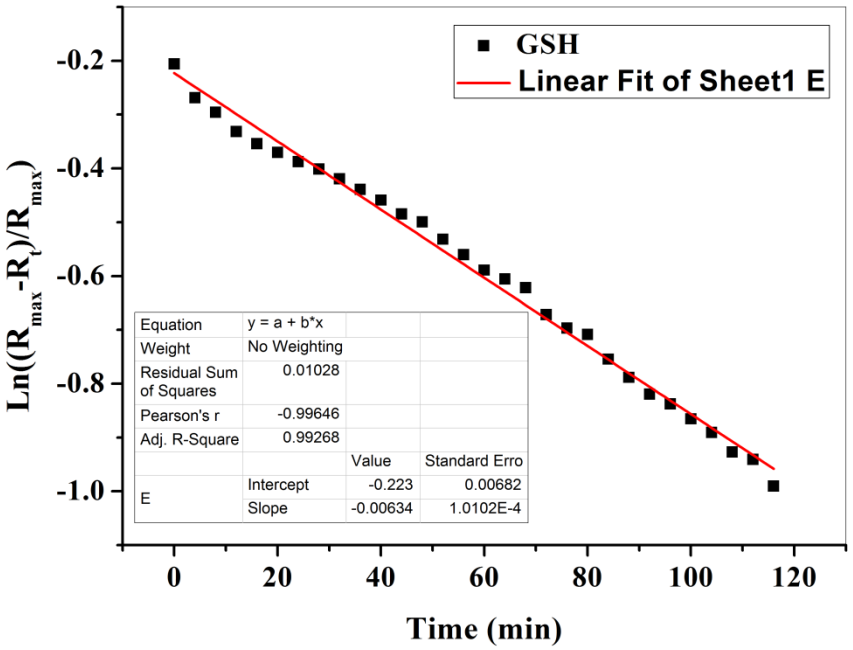

Fig. S5. **(a)** Pseudo first-order kinetic plot of reaction of probe **3** (10^{-5} M) with Cys (10^{-4}M), slope = -0.192 so k' = -0.192 min^{-1} and $k = 3.2\times10^{3}$ $M^{-1}s^{-1}$. **(b)** Pseudo first-order kinetic plot of reaction of probe **3** (10^{-5} M) with Hcy (10^{-4}M), slope = -0.031 so k' = -0.031 min^{-1} and $k = 5.2\times10^{2}$ $M^{-1}s^{-1}$. **(c)** Pseudo first-order kinetic plot of reaction of probe **3** (10^{-5} M) with GSH (10^{-4}M), slope = -0.006 so k' = -0.006 min^{-1} and $k = 1.3\times10^{2}$ $M^{-1}s^{-1}$.

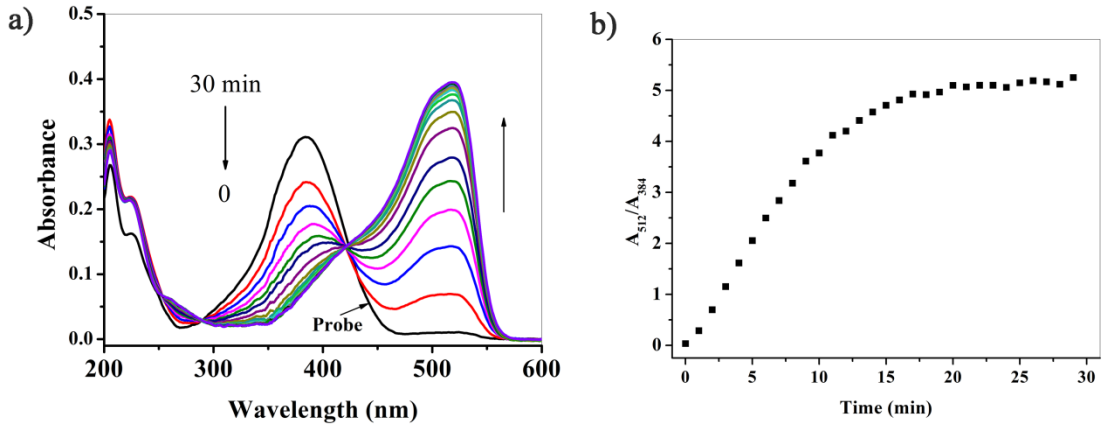

Fig. S6. (a) Time-dependent UV-vis spectral changes of probe **3** (10 μM) with Cys (10 equiv) inphosphate buffer (10 mM, pH 7.4). **(b)** Absorption spectra of 490 nm with addition of 100 μM Cys depending on time.

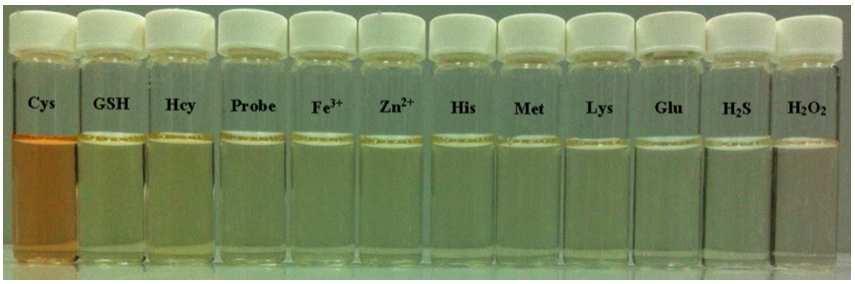

Fig. S7. The color change of probe **3** in pH 7.40 of phosphate buffer solution in the presence of 10 equiv. of certain amino acids, important metal ions or redox species at 10 min.

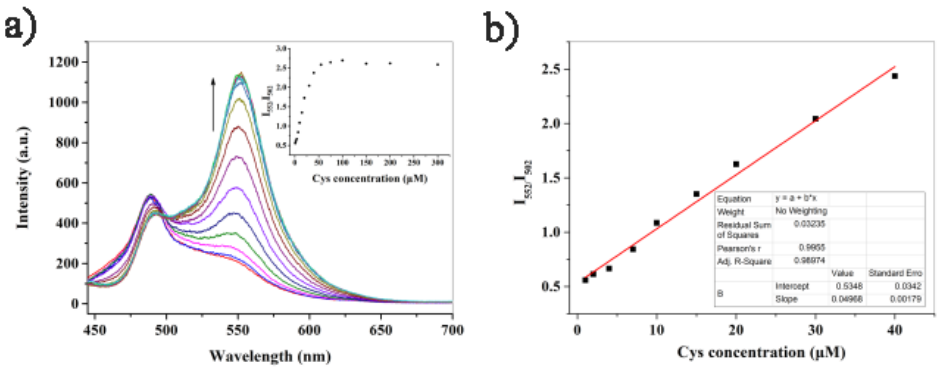

Fig. S8 (a) Fluorescence spectra of probe **3** (10 μM) with the titration of Cys (1, 2, 4, 7, 10, 15, 20, 30, 40, 55, 75, 100, 150, 200,300 μM) (λ_{ex}=418 nm) in phosphate buffer (10 mM, pH 7.4). (**b**) Fluorescence intensity ratio R (I_{552}/I_{502}) changes of probe **3** as a function of concentration Cys (0-40μM). Each spectrum was recorded at 10 min after the addition of Cys to probe **3**.

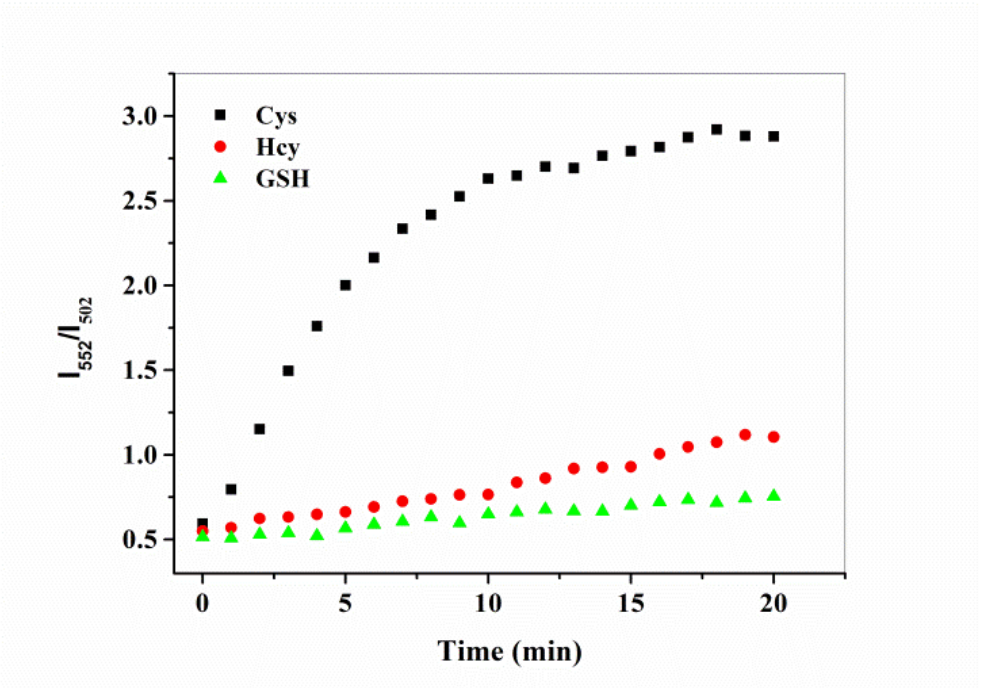

Fig. S9. Fluorescence intensity ratio R (I_{552}/I_{502}) changes of probe **3** (10 μM) with addition of 100 μM Cys, Hcy and GSH depending on time in phosphate buffer (10 mM, pH 7.4). λ_{ex} = 418 nm. The spectrum was recorded at 10 min.

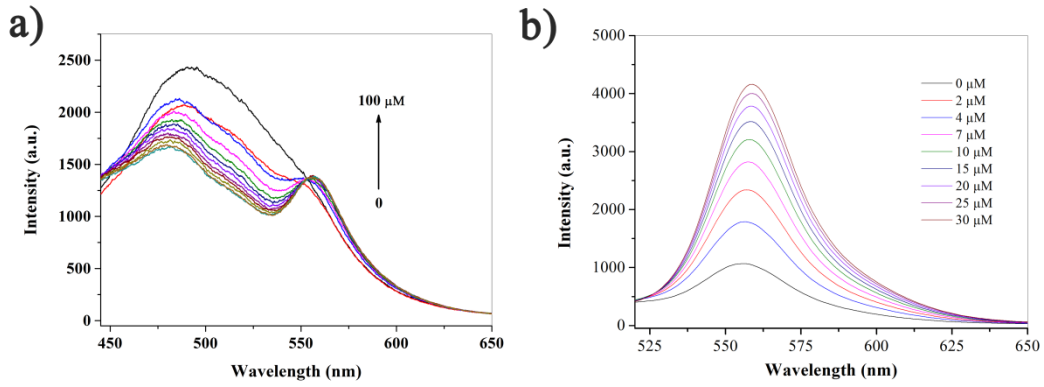

Fig. S10. Fluorescence spectra of probe **3** (10 μM) and Cys (0–40 μM) in diluted deproteinized human plasma. The plasma was diluted with phosphate buffer (10 mM, pH 7.4) and the reaction monitored at 10 min. **(a)** λ_{ex} = 418 nm. **(b)** λ_{ex} = 490 nm.

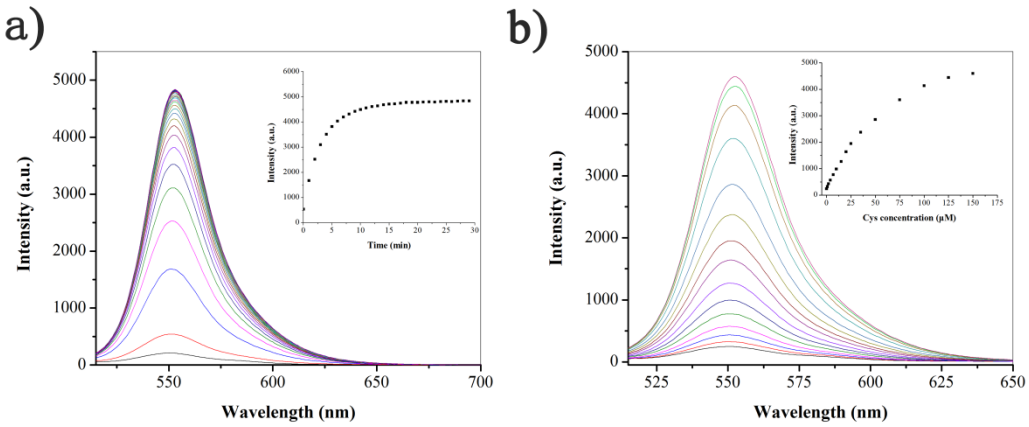

Fig. S11. Fluorescence spectra study upon excitation at 490 nm. **(a)** Time-dependent fluorescence spectral changes of probe **3** (10 μM) with Cys (10 equiv) in phosphate buffer (10 mM, pH 7.40). Inset: Fluorescence intensity at 552 nm *vs* time; (**b**) Fluorescence spectra of probe **3** (10 μM) with the titration of Cys (1, 2, 4, 7, 10, 15, 20, 35, 50, 75, 100, 125, 150 μM) (λ_{ex}=490 nm) in phosphate buffer (10 mM, pH 7.4). Each spectrum was recorded at 10 min after the addition of Cys to probe **3**.

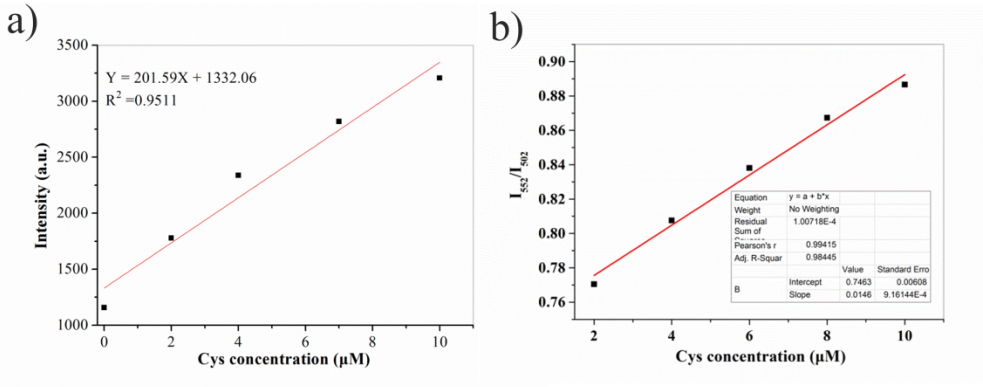

Fig. S12. Plot of fluorescence intensity/ intensity ratio as a function of Cys concentration in diluted deproteinized human plasma. Phosphate buffer (10 mM, pH 7.4) wa used to dilute the plasma sample and the reaction was monitored at 10 min, λ_{ex} = 490 nm.

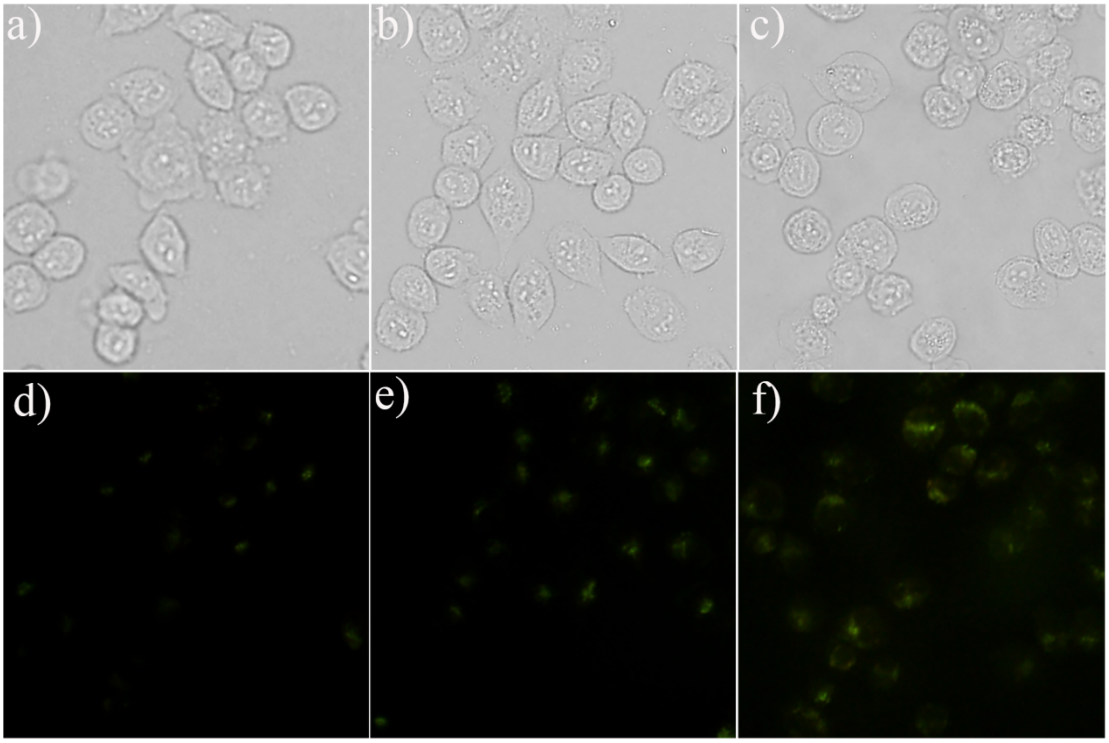

Fig. S13. Fluorescence images of living Hela cells incubated with various concentrations of probe **3**. Hela cells loaded with probe **3** for 30 min. (**a, d**) 10 μM, (**b, e**) 30 μM, (**c, f**) 50 μM. Scale bar is 20 mm. Brightfield images (**a, b, c**), fluorescence images (**d, e, f**).

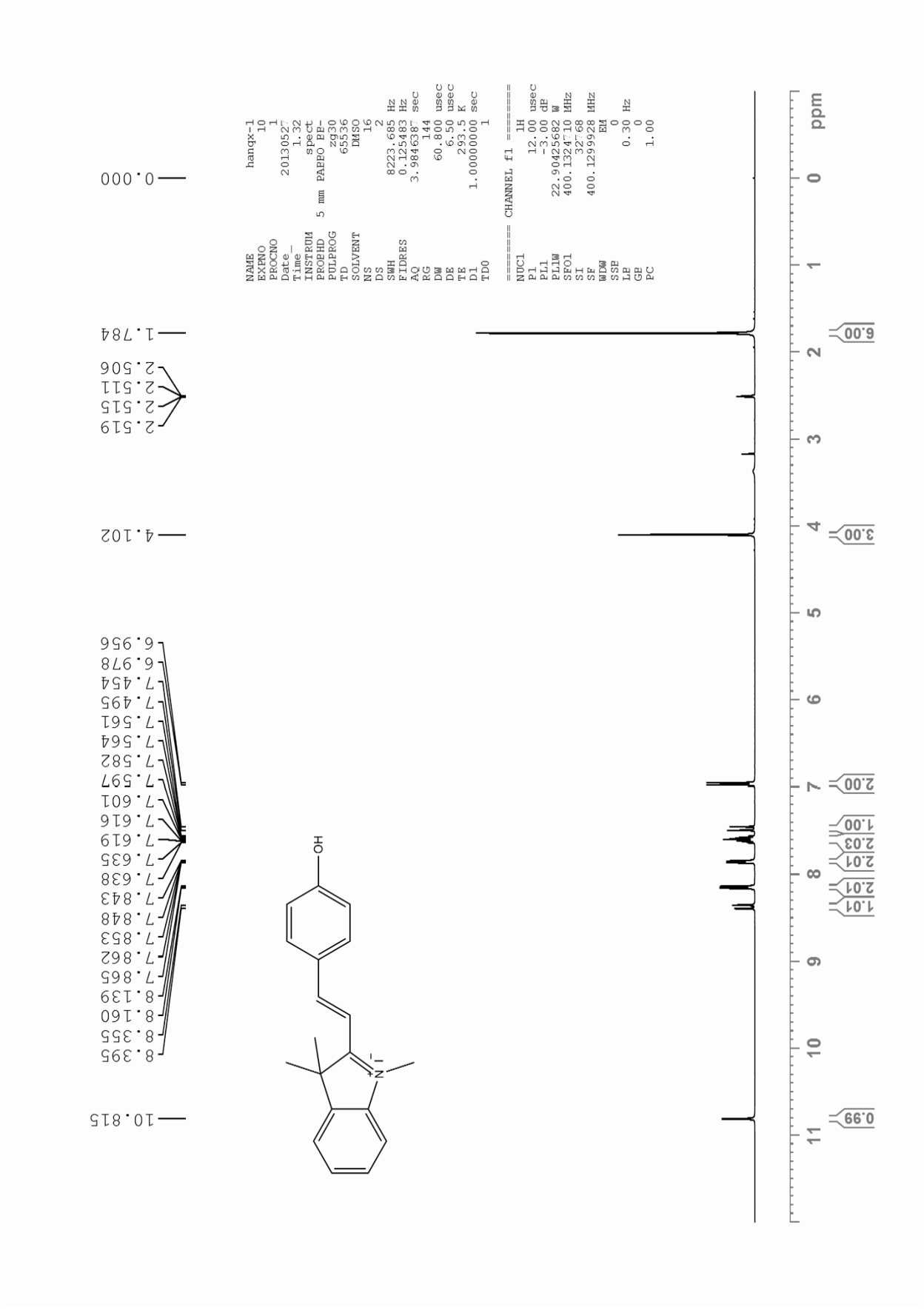

Fig. S14. ^{1}H MNR (400 MHz) spectrum of compound **2** in d_6-DMSO.

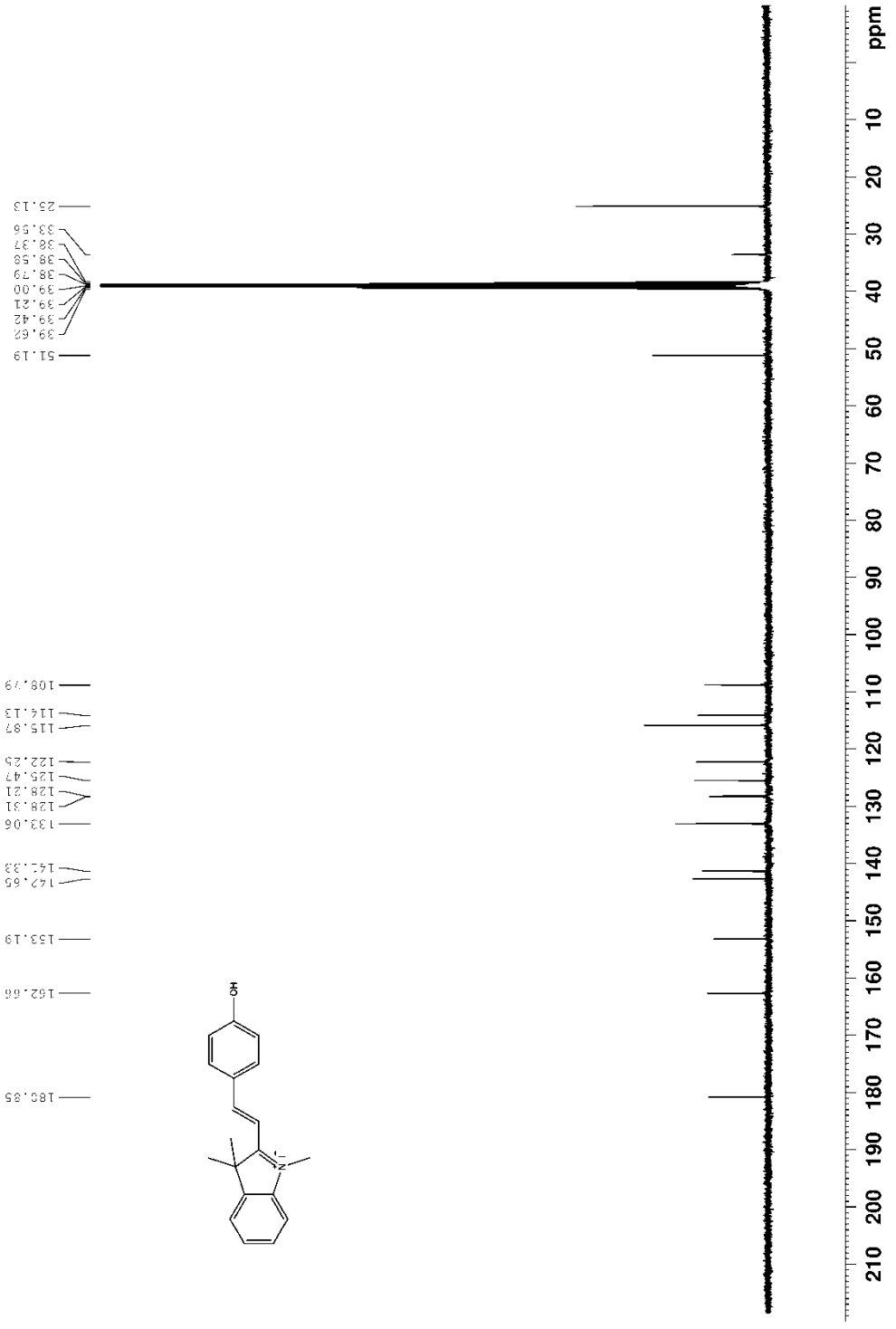

Fig. S15. ^{13}C MNR (100 MHz) spectrum of compound **2** in d_6-DMSO.

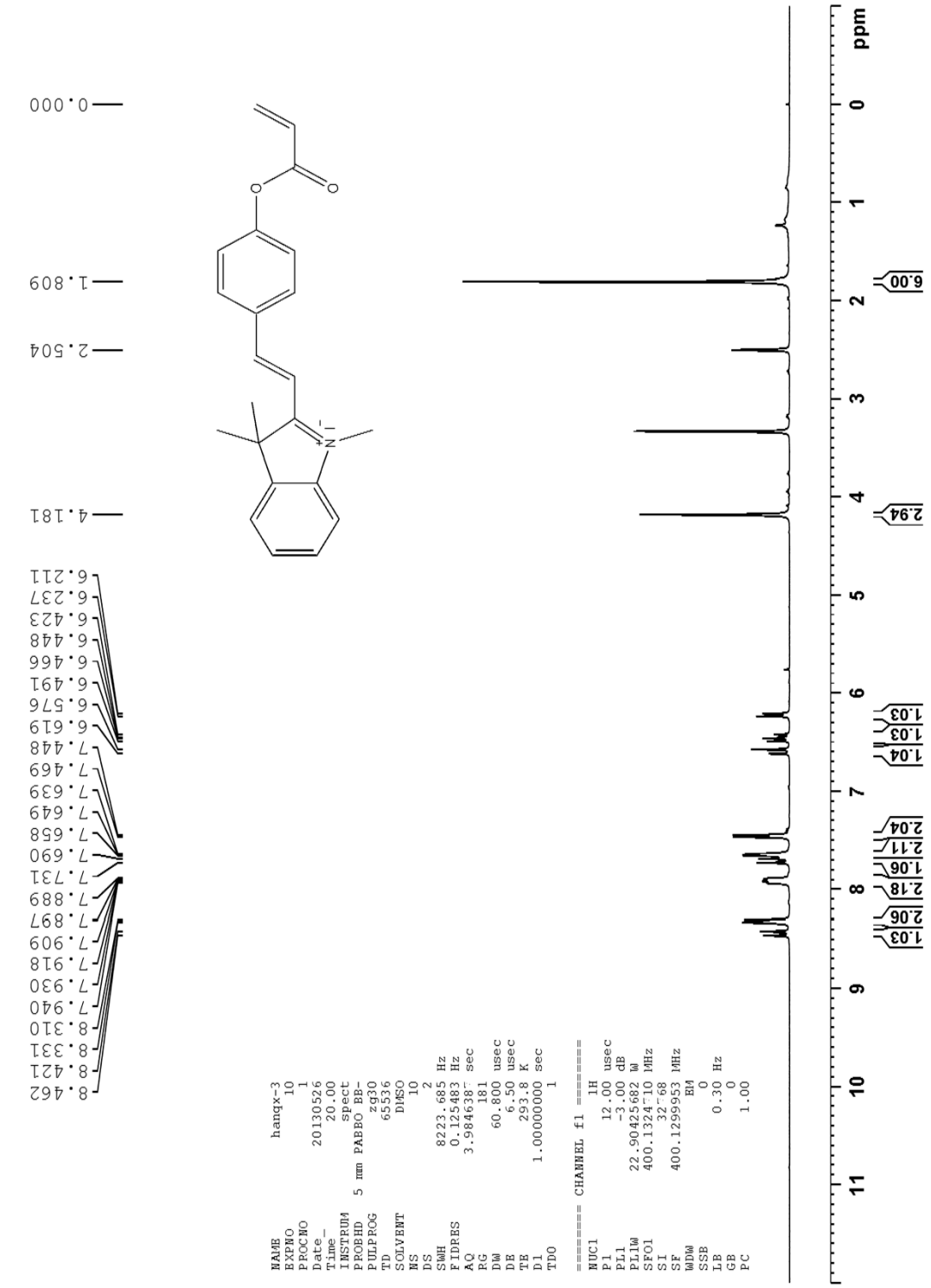

Fig. S16. [1]H MNR (400 MHz) spectrum of probe **3** in d_6-DMSO.

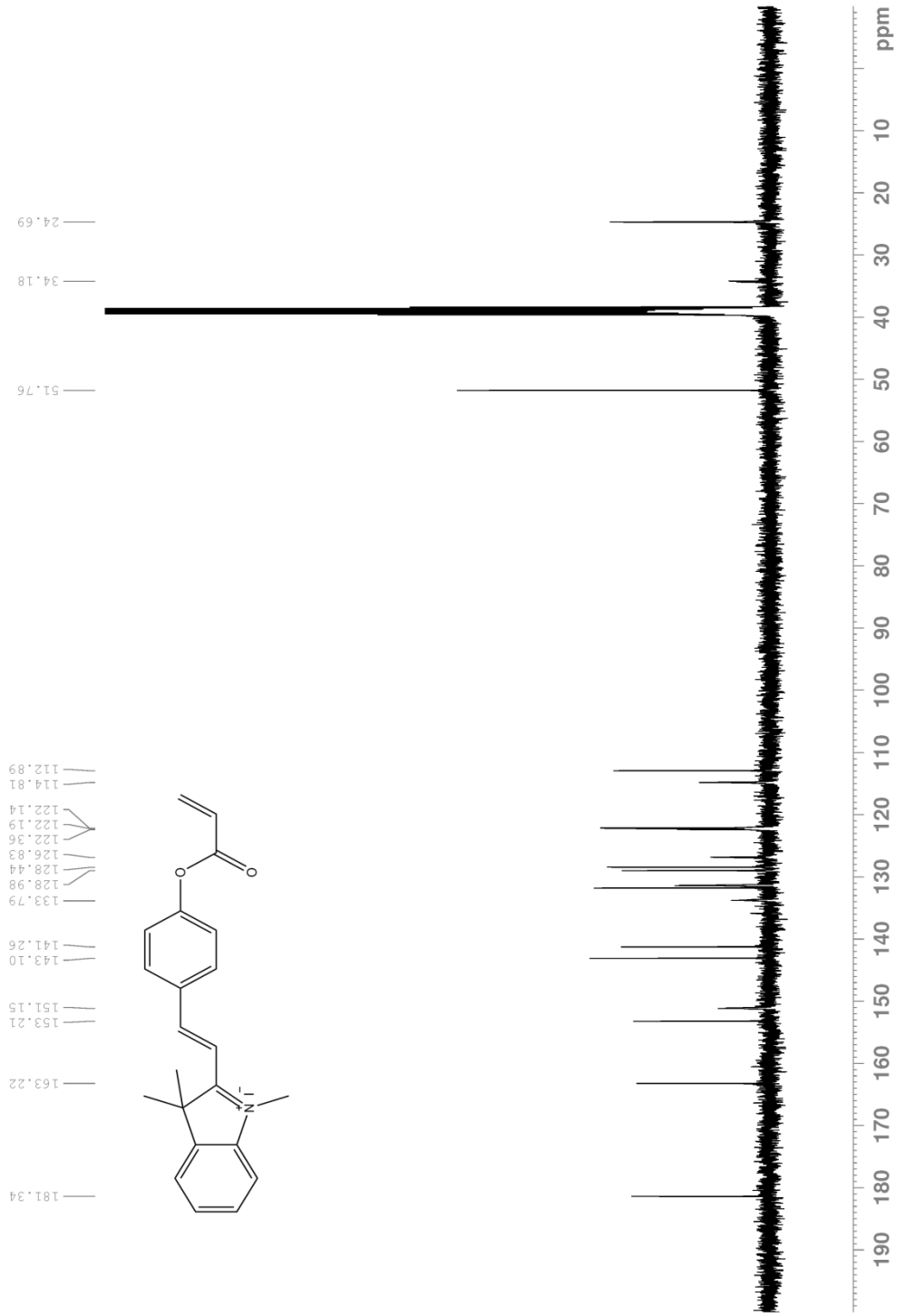

Fig. S17. ^{13}C MNR (100 MHz) spectrum of probe **3** in d_6-DMSO.

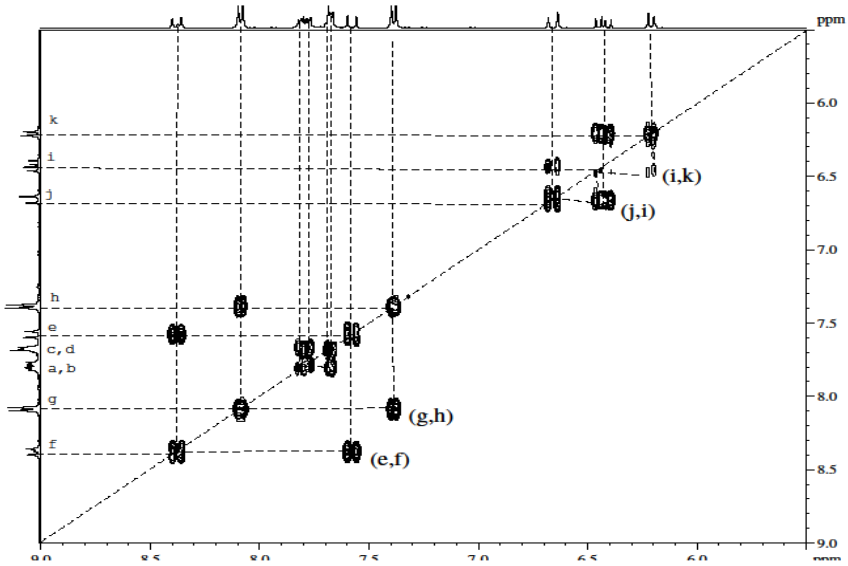

Fig. S18. $^{1}H - {}^{1}H$ COSY NMR spectrum of probe **3** in D_2O/CD_3OD (1:1, V/V).

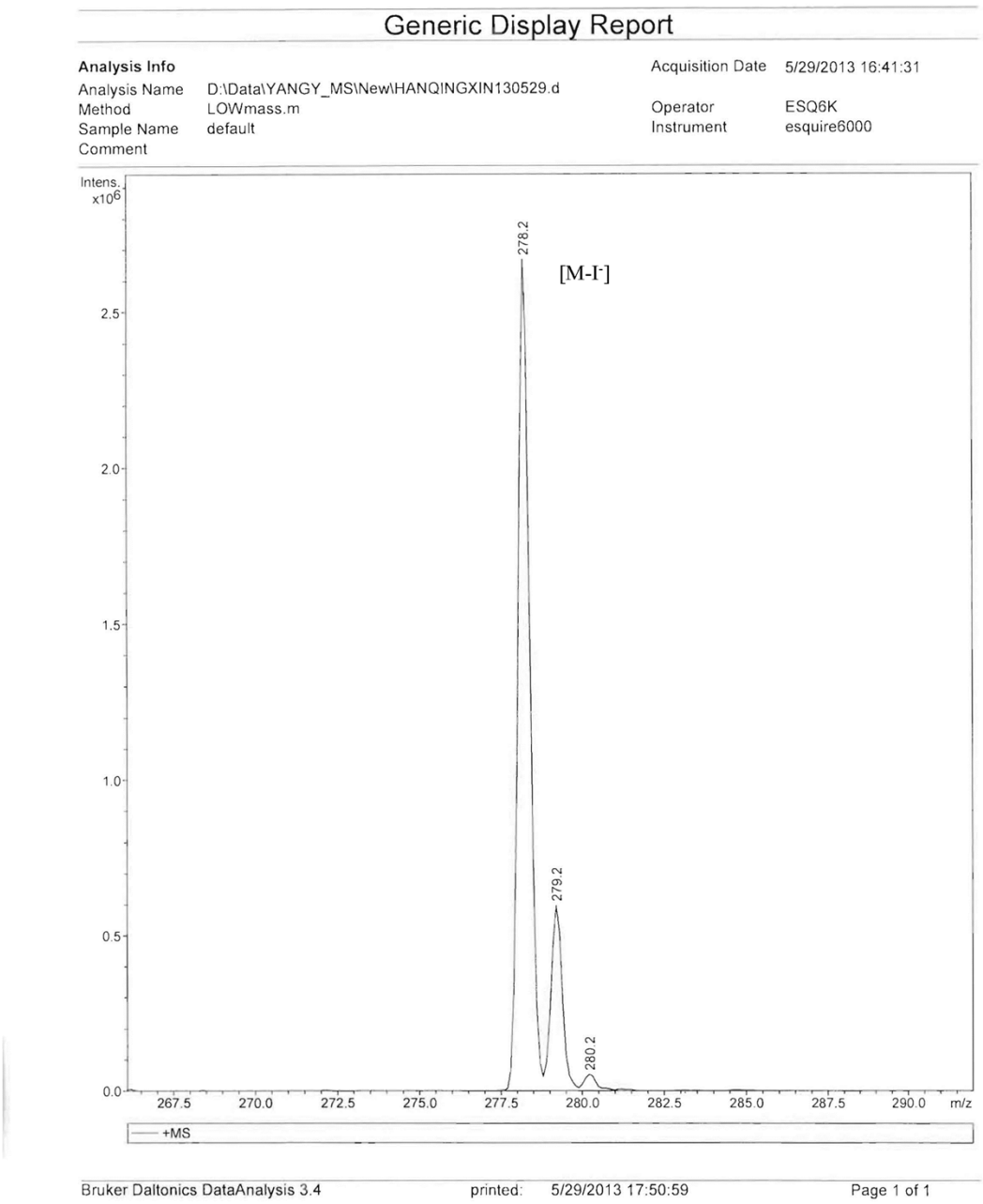

Fig. S19. Mass spectrum (ESI) of compound **2.**

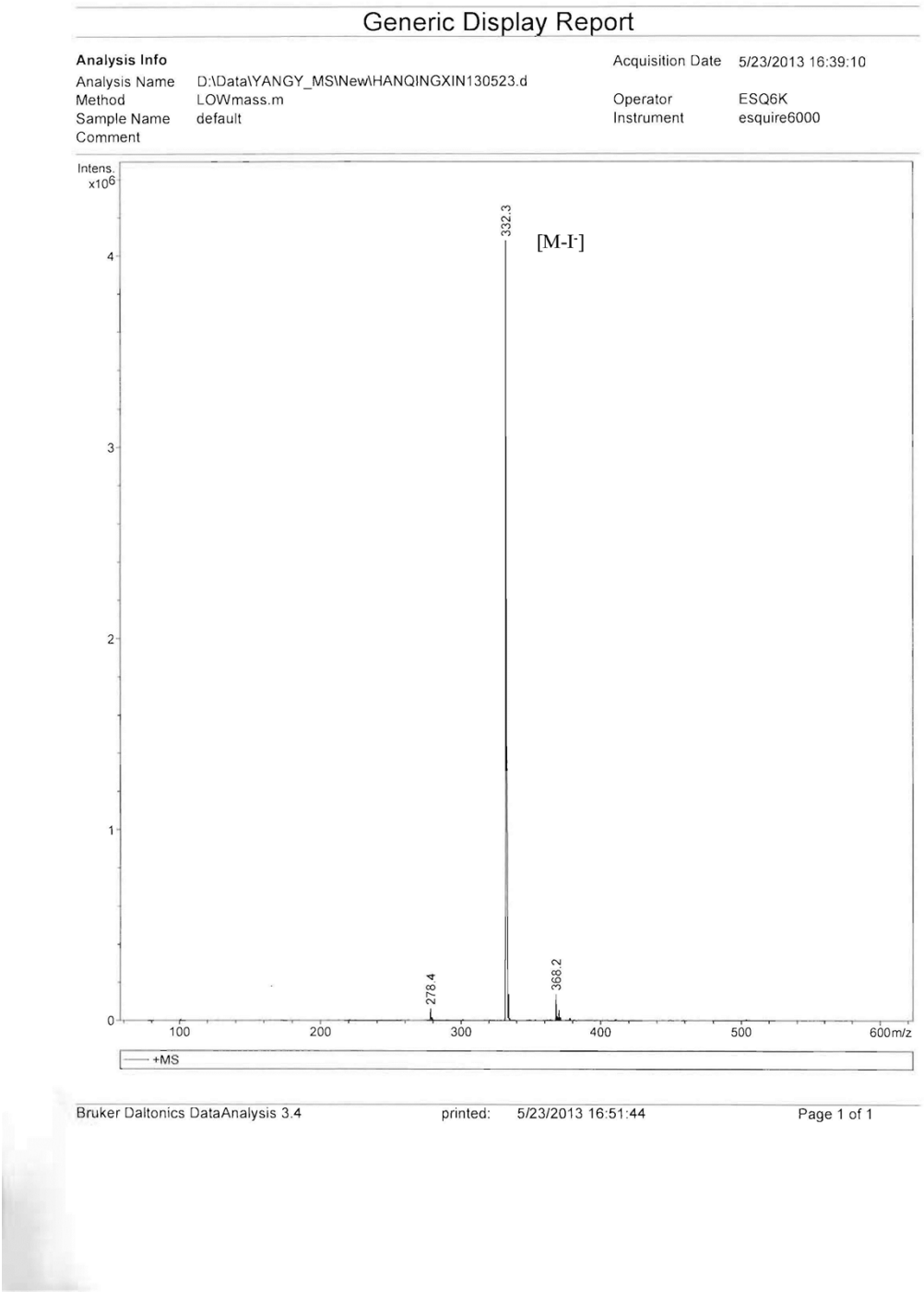

Fig. S20. Mass spectrum (ESI) of probe **3.**

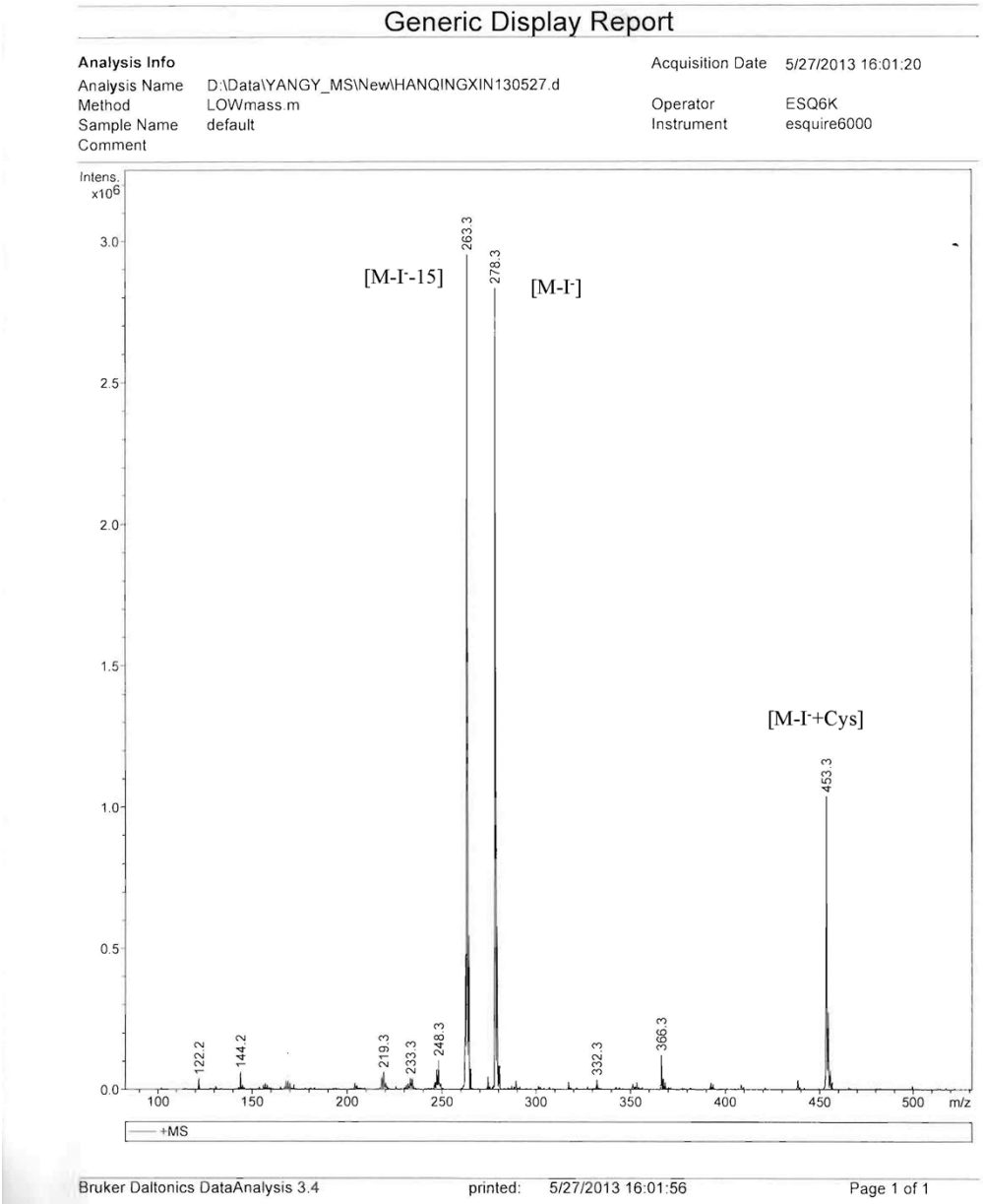

Fig. S21. Mass spectrum (ESI) obtained from the reaction of **4** with Cys**.**

LZDX

项目名称: 2014

用户名称: Breeze 用户 (Breeze)

Breeze 2 HPLC System

样品信息

样品名称:	W-N-3	采集者:	Breeze
样品类型:	未知	样品组名称:	
瓶号:	1	采集方法组:	hanqx08
进样次数:	12	处理方法:	W 3
进样体积:	5.00 ul	通道名称:	384.0 纳米
运行时间:	10.0 Minutes	处理通道注释:	PDA 384.0 纳米
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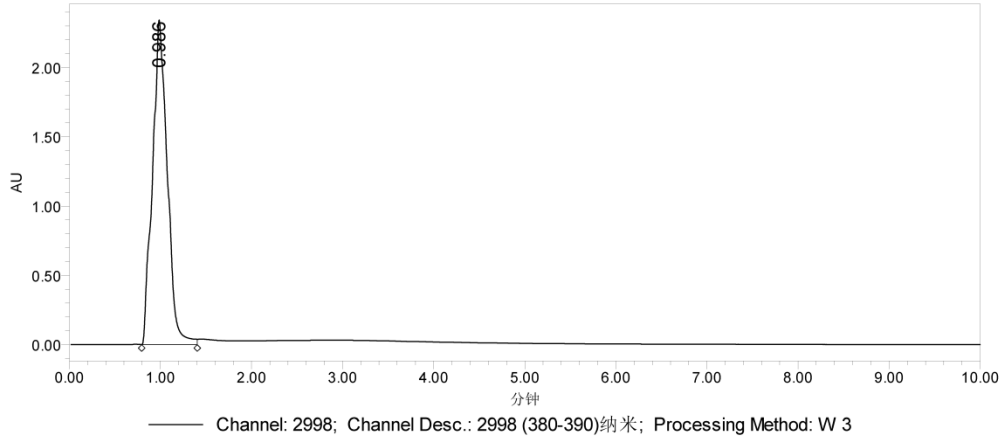

名称:

	通道说明	保留时间 (分钟)	面积 (微伏*秒)	% 面积	高度 (微伏)
1	2998 (380-390)纳米	0.986	26394307	100.00	2342097

Fig. S22. The retention measurements of probe **3** in water phase.

LZDX

项目名称: 2014

用户名称: Breeze 用户 (Breeze)

Breeze 2 HPLC System

样品信息

样品名称:	O-3	采集者:	Breeze
样品类型:	未知	样品组名称:	
瓶号:	1	采集方法组:	hanqx08
进样次数:	6	处理方法:	W 3
进样体积:	5.00 ul	通道名称:	384.0 纳米
运行时间:	10.0 Minutes	处理通道注释:	PDA 384.0 纳米
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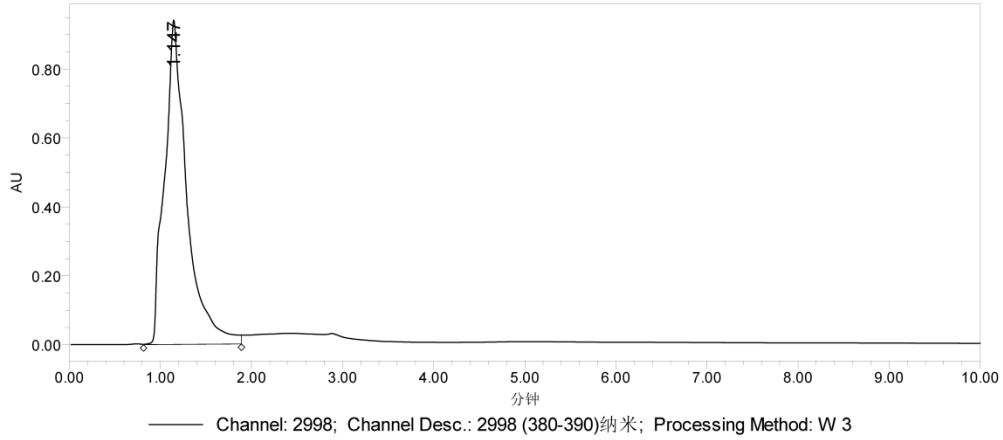

名称:

	通道说明	保留时间 (分钟)	面积 (微伏*秒)	% 面积	高度 (微伏)
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Fig. S23. The retention measurements of probe **3** in octanol phase.