Supplementary Material (ESI) for Chemical Communications

Supporting information for

A highly selective and sensitive fluorescent probe for Hg^{2+} imaging in live cells based on a rhodamine-thioamide-alkyne scaffold

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Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Melting points of compounds were measured on a Beijing Taike XT-4 microscopy melting point apparatus, and all melting points were uncorrected. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. Cell imaging was performed with a Nikon Eclipse TE300 inverted microscope using excitation filters (510-560 nm). TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Synthesis of compound 2. To a solution of rhodamine 6G (0.96 g, 2.0 mmol) in EtOH (20 mL) was added prop-2-yn-1-amine (0.22 g, 4.0 mmol). The solution was heated to reflux under N_2 atmosphere for 24h. Then the solution was concentrated under vacuum and purified by flash column chromatography (petroleum / CH_2Cl_2 = 2:1 to 1:1) to give 0.36 g (0.80 mmol, 40%) of compound **2** as a white solid. mp = 259-261℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.95–7.93 (m, 1H), 7.45–7.41 (m, 2H), 7.06–7.03 (m, 1H), 6.36 (s, 2H), 6.29 (s, 2H), 3.93 (d, J = 2.8 Hz, 2H), 3.51 (bs, 2H), 3.24–3.19 (m, 4H), 1.90 (s, 6H), 1.76 (t, J = 2.4 Hz, 1H), 1.32 (t, J = 7.2 Hz, 6H);^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.6, 154.0, 151.8, 147.4, 132.7, 130.2, 128.8, 128.0, 123.7, 123.0, 117.7, 105.4, 96.6, 78.3, 70.0, 65.0, 38.4, 28.6, 16.6, 14.7; MS (ESI): m/z = 452.2 $[M+H]^+$.

Synthesis of compound 3. Preparation of compound **3** was conducted by the same synthetic procedure for compound **2**. mp = 239-242℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93–7.91 (m, 1H), 7.46–7.40 (m, 2H), 7.04–7.02 (m, 1H), 6.36 (s, 2H), 6.26(s, 2H), 3.52 (d, *J* = 2.8 Hz, 2H), 3.24-3.15 (m, 6H), 1.91 (s, 6H), 1.33 (t, *J* = 7.2 Hz, 6H), 0.78 (t, *J* = 6.8 Hz, 3H); MS (ESI): m/z = 442.5 $[M+H]^+$.

Synthesis of probe 1. Compound **2** (0.20 g, 0.44 mmol) and Lawesson's reagent (0.14 g, 0.35 mmol) were dissolved in dry benzene (20 mL), and the reaction mixture was refluxed for 30 min under N_2 atmosphere. After removal of benzene under reduced pressure, the resulting residue was purified by flash column chromatography with petroleum / CH_2Cl_2 = (2:1 to 1:1) as eluent to obtain compound **1** (96 mg, 0.21 mmol, 46%) as a red solid. mp = 192-195℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.19–8.17 (m, 1H), 7.48–7.46 (m, 2H), 7.06–7.04 (m, 1H), 6.38 (s, 2H), 6.18 (s, 2H), 4.32 (d, *J* = 2.0 Hz, 2H), 3.55 (bs, 2H), 3.25–3.19 (m, 4H), 1.89 (s, 6H), 1.79 (t, *J* = 2.4, 1H), 1.33 (t, *J* = 4.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 191.4, 151.7, 151.6, 147.8, 137.0, 132.7, 128.6, 128.4, 125.1, 123.2, 118.0, 96.6, 73.3, 70.3, 38.4, 33.1, 16.6, 14.7. HRMS (EI) m/z calcd for $C_{29}H_{29}N_3OS$ $[M]^+$: 467.2026; Found: 467.2027. Elemental analysis: calcd (%) for $C_{29}H_{29}N_3OS$: C, 74.48; H, 6.25; N, 8.99; Found C, 74.63; H, 6.42; N, 8.74.

Compound **4**: Preparation of compound **4** was conducted by the same synthetic procedure for compound **1**. mp = 269-271℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.18–8.16 (m, 1H), 7.51–7.44 (m, 2H), 7.06–7.04 (m, 1H), 6.39 (s, 2H), 6.15 (s, 2H), 3.55 (q, 2H), 3.25–3.20 (m, 4H), 1.90 (s, 6H), 1.33 (t, *J* = 6.8 Hz, 6H), 0.82 (t, *J* = 6.8 Hz, 3H). MS (ESI): m/z = 458.4 $[M+H]^+$.

Synthesis of compound 7. The solution of $HgCl_2$ (0.017g, 0.064 mmol) dissolved in twice distilled water (10 mL) was added to the solution of compound **1** (0.030g, 0.064 mmol) dissolved in DMF (10 mL) and incubated for 3 hours. The solvents

were then removed under reduced pressure, and the resulting residue was purified by flash column chromatography with CH_2Cl_2 / CH_3CH_2OH= (50:1 to 20:1) as eluent to obtain compound **7** (24 mg, 0.053 mmol, 83%) as a red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.92–7.89 (m, 1H), 7.74–7.70 (m, 2H), 7.29–7.27 (m, 1H), 6.89 (s, 2H), 6.70 (s, 2H), 6.21 (s, 2H), 5.10 (m, 2H), 4.67 (t, *J* = 3 Hz, 2H), 3.53 (m, 4H), 2.21 (s, , 6H), 1.45 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 157.1, 156.0, 132.4, 131.1, 130.4, 128.9, 125.7, 109.8, 104.3, 94.0, 38.7, 29.7, 17.4, 13.8. HRMS (EI) m/z calcd for $C_{29}H_{29}N_3OS$ $[M-H]^+$: 467.2026; Found: 467.2012.

Preparation of the test solution.

The stock solution of probe **1** was prepared at 1×10^{-4} M in DMF. The solutions of various testing species were prepared from $AgNO_3$, $HAuCl_4$, $CaCl_2$, $MgCl_2$ $CdCl_2 \cdot 1/2H_2O$, $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $FeCl_2$, $FeCl_3$, $HgCl_2$, $MnSO_4 \cdot H_2O$, $NiCl_2 \cdot 6H_2O$, $Pb(NO_3)_2$, $ZnCl_2$ in the twice-distilled water, and $PdCl_2$ test solution was prepared in DMSO. The test solution of probe **1** (5 μM) in 3 mL aqueous solution (25 mM PBS buffer, pH 7.2, containing 20% DMF as a co-solvent) was prepared by placing 0.15 mL of the probe stock solution (in DMF) , 0.45 mL DMF, and 2.4 mL of 25 mM PBS buffer (pH = 7.2). The resulting solution was shaken well and allowed to stand for 3 min at room temperature before recording the spectra.

Cell culture and fluorescence imaging: Human Tca-8113 cells were seeded in a 12-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum for 24 h. Human Tca-8113 cells were then incubated with probe **1** (1 μM) in the culture medium for 30 min at 37^{o}C. After washing with PBS three times to remove the remaining probe **1**, the cells were further incubated with $HgCl_2$ (2 μM) for 30 min at 37^{o}C. After washing with PBS three times to remove the remaining Hg^{2+}, the fluorescence images were acquired with a Nikon Eclipse TE300 equipped with a CCD camera.

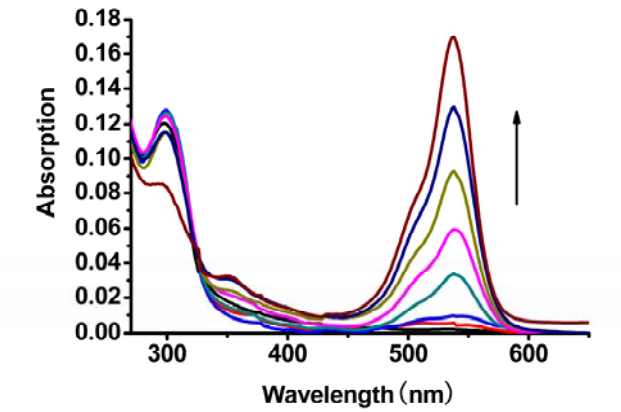

Figure S1. Absorption spectra of **1** (5 μM) in the presence of Hg^{2+} (0.0 to 2.0 equiv.) in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent).

Detection limit: The detection limit was determined from the fluorescence titration data based on a reported method.[1] According to the result of titration experiment, the fluorescent intensity data at 566 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to these normalized fluorescent intensity data (Figure S2), and the point at which this line crossed the axis was considered as the detection limit (3.9×10^{-8} M).

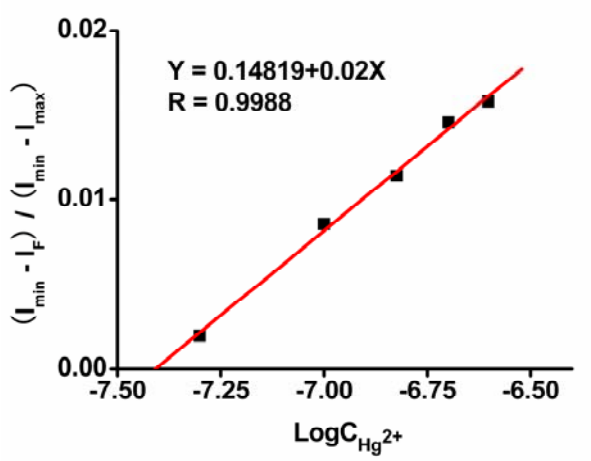

Figure S2. Normalized response of fluorescence signal to changing Hg^{2+} concentrations in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent). (Ex. 500 nm; Em. 566 nm).

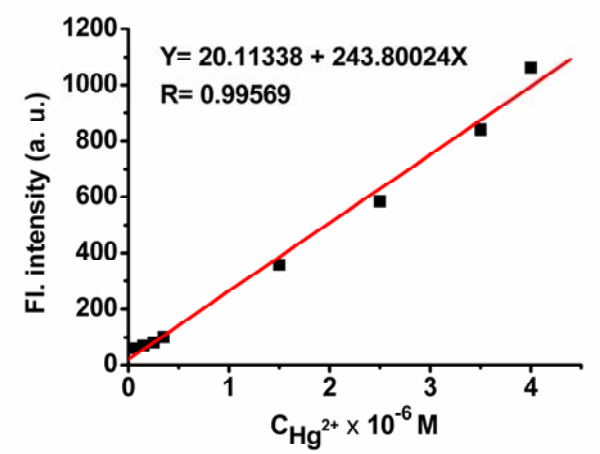

Figure S3. Plot of the fluorescent intensity at 566 nm as a function of the Hg^{2+} concentration in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent).

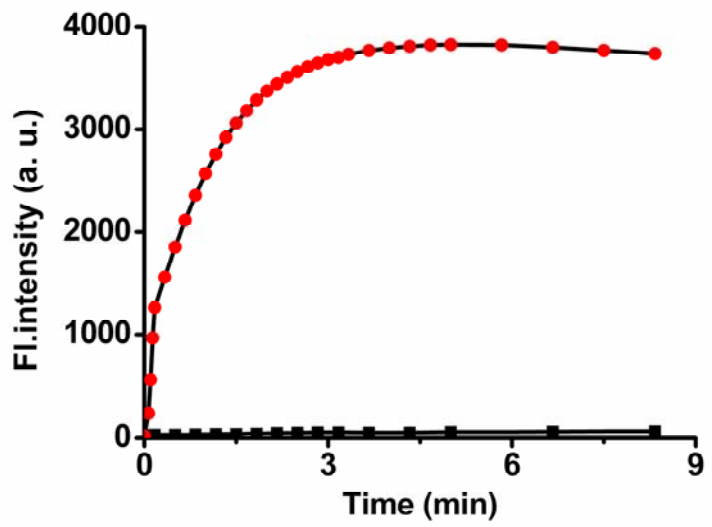

Figure S4. Time-dependent fluorescence intensity changes of probe **1** (5 μM) in the absence（■) or presence（●) of Hg^{2+} (2 equiv.) in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent). (Ex. 500 nm; Em. 566 nm).

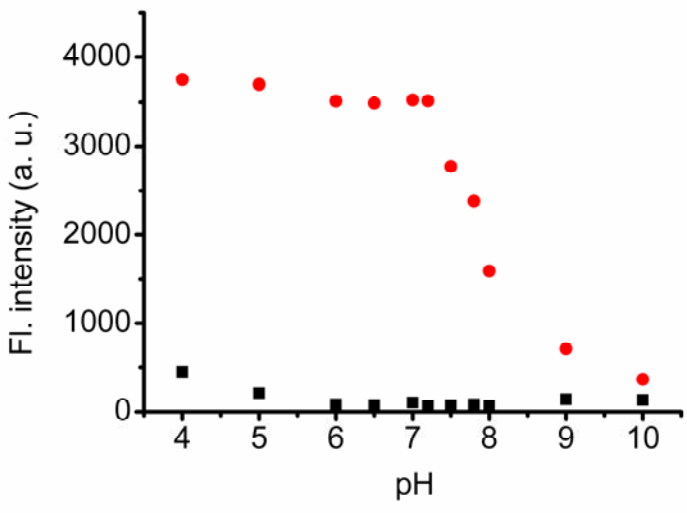

Figure S5. Fluorescence intensity of probe **1** (5 μM) without (■) or with (●) the addition of Hg^{2+} (2.0 equiv.) at various pH values. (Ex. 500 nm; Em. 566 nm).

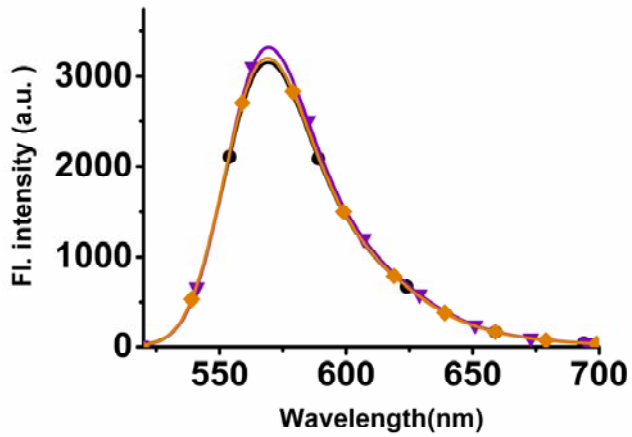

Figure S6. Fluorescence spectra of (▼) probe **1** + $HgCl_2$ (2 equiv), (◆) probe **1** + $Hg(ClO_4)_2$ (2 equiv), (●) probe **1** + $Hg(NO_3)_2$ (2 equiv) in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent). Excitation at 500 nm. The concentration of probe **1** was 5 μM.

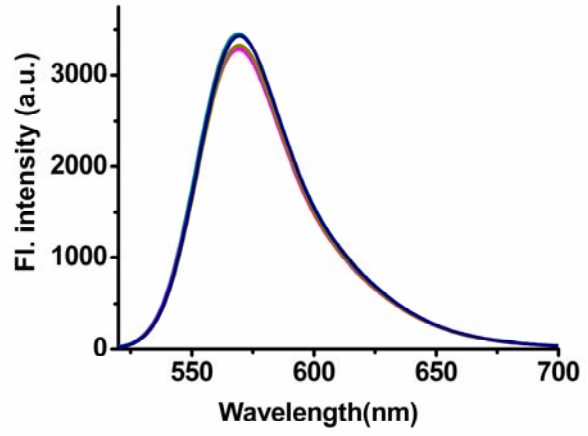

Figure S7. The fluorescence spectra of probe **1** (5 μM) + Hg^{2+} (2 equiv.) in the presence of different concentrations of chloride (10, 20, 50, 100, or 200 equiv.).

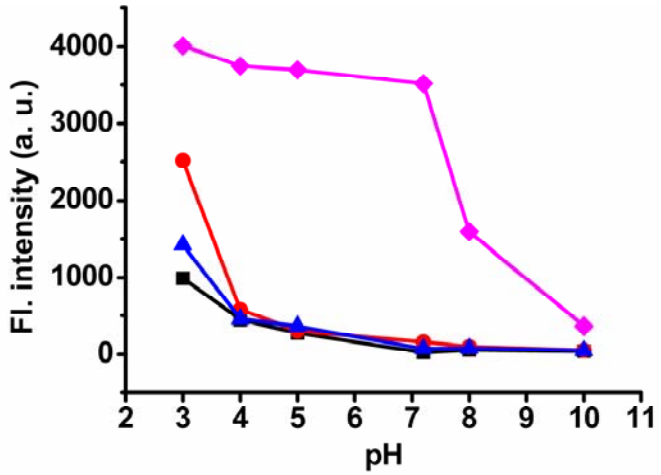

Figure S8. Fluorescence intensities of free probe **1** (5 μM) (■), probe **1** (5 μM) + Au^{3+} 2.0 (equiv) (●), or probe **1** (5 μM) + Pd^{2+} 2.0 (equiv) (▲) at various pH values. For comparison, fluorescence intensities of probe **1** (5 μM) + Hg^{2+} 2.0 (equiv) (◆) at various pH values were also showed. (Ex. 500 nm; Em. 566 nm).

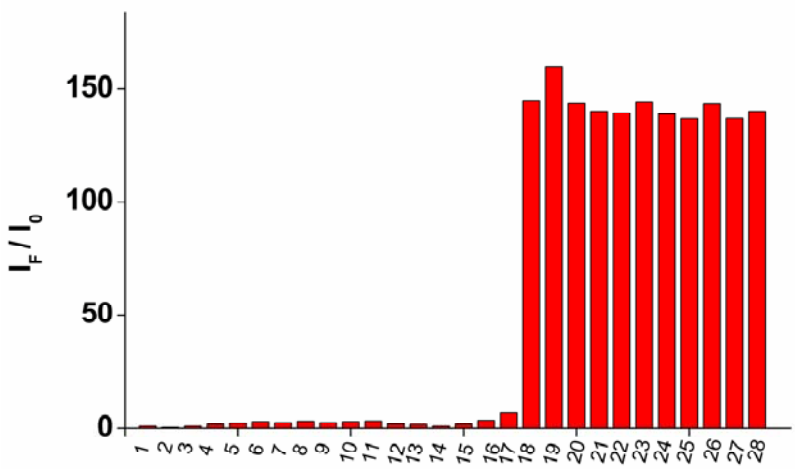

Figure S9. Fluorescence response of probe **1** (5 μM) to the various species (200 equiv. for Na^+, K^+, Ca^{2+}, Mg^{2+}, 2 equiv. for other tested metal ions) in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent). 1: free probe **1**; 2: Ag^+; 3: K^+; 4: Co^{2+}; 5: Cu^{2+};6: Fe^{2+}; 7: Fe^{3+}; 8: Ni^{2+}; 9: Mg^{2+}; 10: Pb^{2+}; 11: Zn^{2+};12: Pd^{2+}; 13: Ca^{2+}; 14: Na^+; 15: Mn^{2+}; 16: Cd^{2+}; 17: Au^{3+}; 18: Hg^{2+}; 19: Au^{3+} + Hg^{2+}; 20: Ca^{2+} + Hg^{2+}; 21: Cd^{2+} + Hg^{2+}; 22: Cu^{2+} + Hg^{2+}; 23: Fe^{3+} + Hg^{2+}; 24: Mn^{2+} + Hg^{2+}; 25: Na^+ + Hg^{2+}; 26: Pb^{2+} + Hg^{2+}; 27: Pd^{2+} + Hg^{2+}; 28: K^+ + Hg^{2+}.

a

b

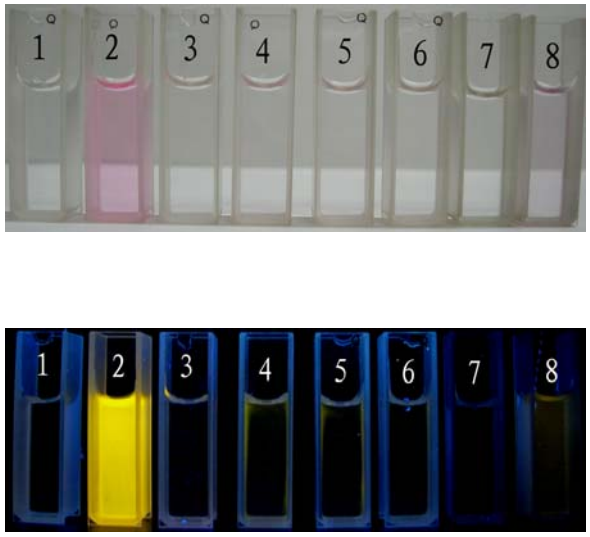

Figure S10. Color changes of the probe **1** solution (5 μM) with 2 equiv. of different metal ions in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent): 1. free probe **1**; 2. probe **1** + Hg^{2+}; 3. probe **1** + Cd^{2+}; 4. probe **1** + Cu^{2+}; 5. probe **1** + Pd^{2+}; 6. probe **1** + Ag^+; 7. probe **1** + Fe^{2+}; 8. probe **1** + Au^{3+}. a) Visible color; b) Visual fluorescence color on excitation at 365 nm using a handheld UV lamp.

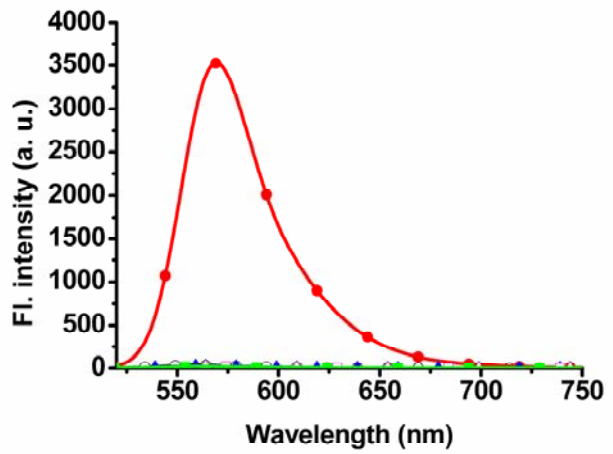

Figure S11. Fluorescence spectra of control compound **2** (△), control compound **2** + Hg^{2+} (2 equiv) (▲), control compound **4** (□), and control compound **4** + Hg^{2+} (2 equiv) (■) in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent). For comparison, the fluorescence spectra of probe **1** (○) and probe **1** + Hg^{2+} (2 equiv) (●) were also showed. Excitation at 500 nm. The concentration of control compound **2**, control compound **4,** and probe **1** was 5 μM.

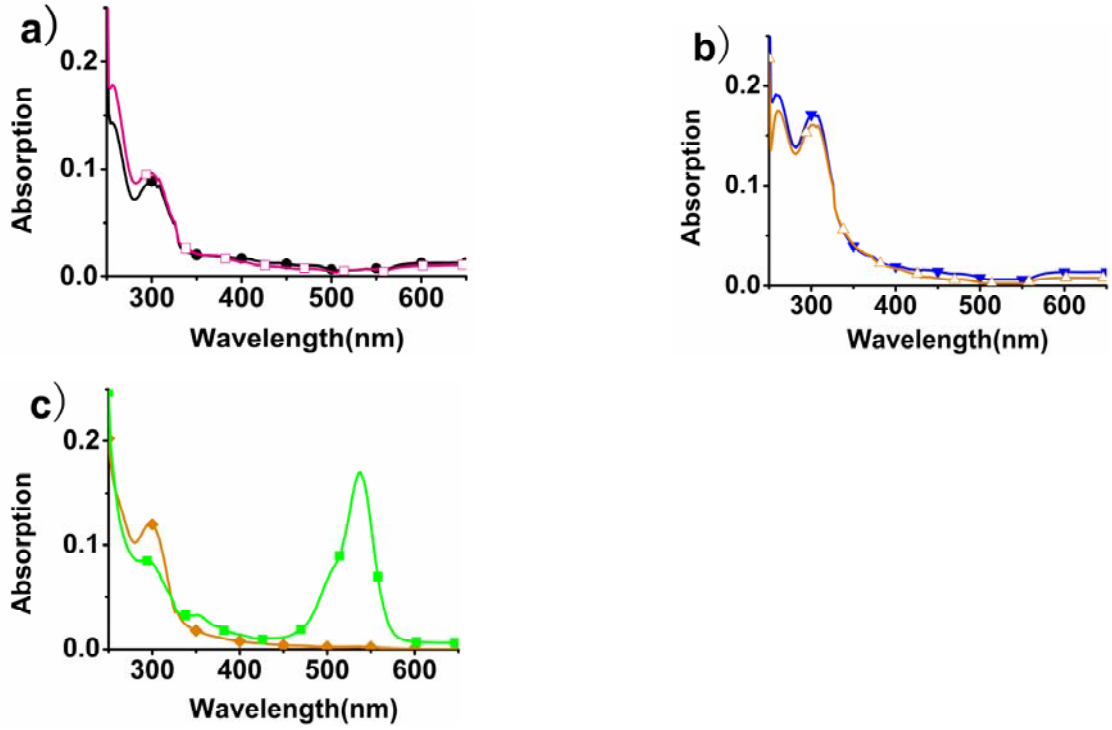

Figure S12. a) Absorption spectra of control compound **2** (●), and control compound **2** + Hg^{2+} (2 equiv.) (□). b) Absorption spectra of control compound **4** (▼), and control compound **4** + Hg^{2+} (2 equiv) (△) in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent). For comparison, c) the absorption spectra of probe **1** (◆) and probe **1** + Hg^{2+} (2 equiv) (■) were also showed. The concentration of control compound **2**, control compound **4**, and probe **1** was 5 μM.

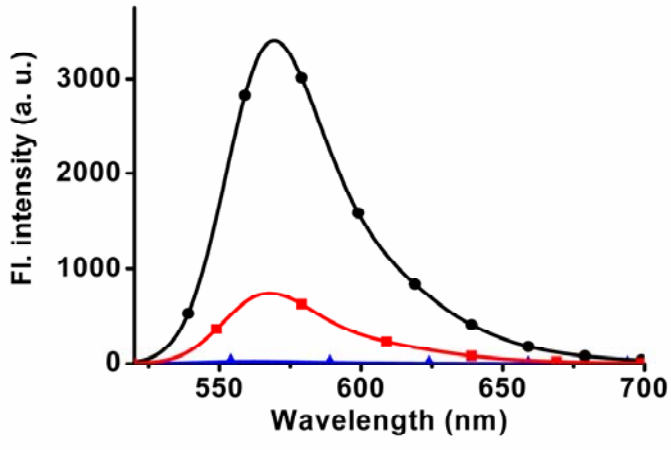

Figure S13. Fluorescence spectra of free probe **1** (▲), and probe **1** with the successive addition of 2 equiv of Hg^{2+} (●) and 8 equiv of I^- (■). Excitation at 500 nm. The spectra were recorded in the PBS buffer (25 mM, pH 7.2, containing 20% DMF as a co-solvent).

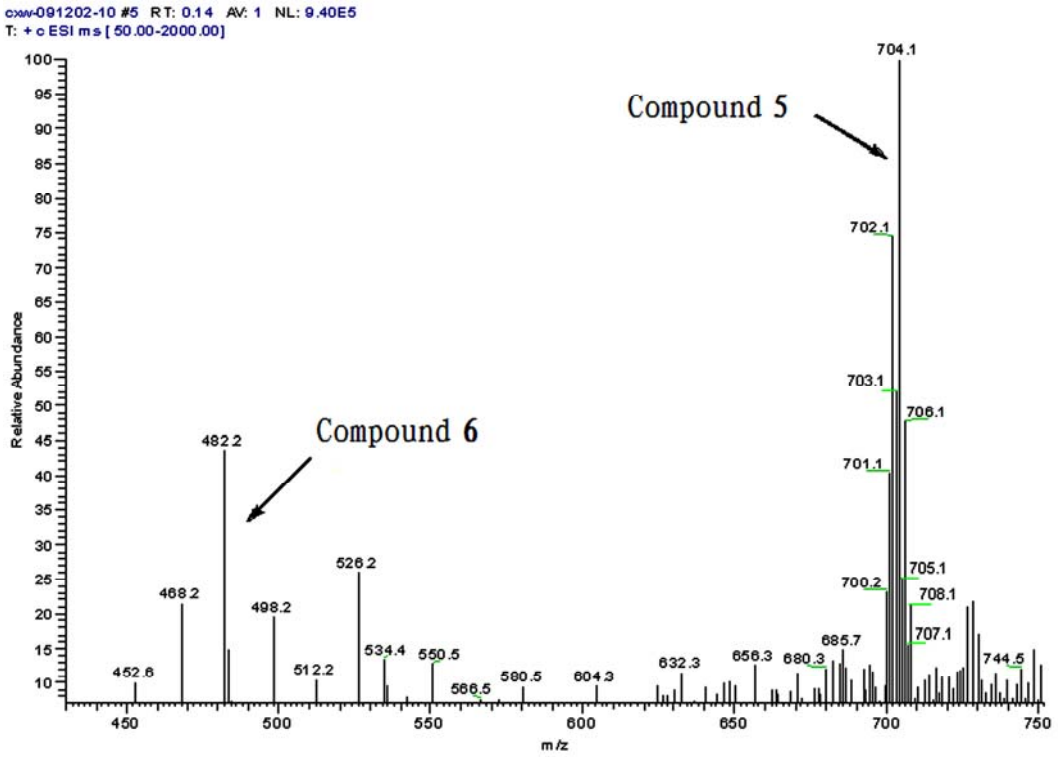

Figure S14. Mass spectrum of probe **1** solution (20 μM) with 2 equiv. of Hg^{2+}.

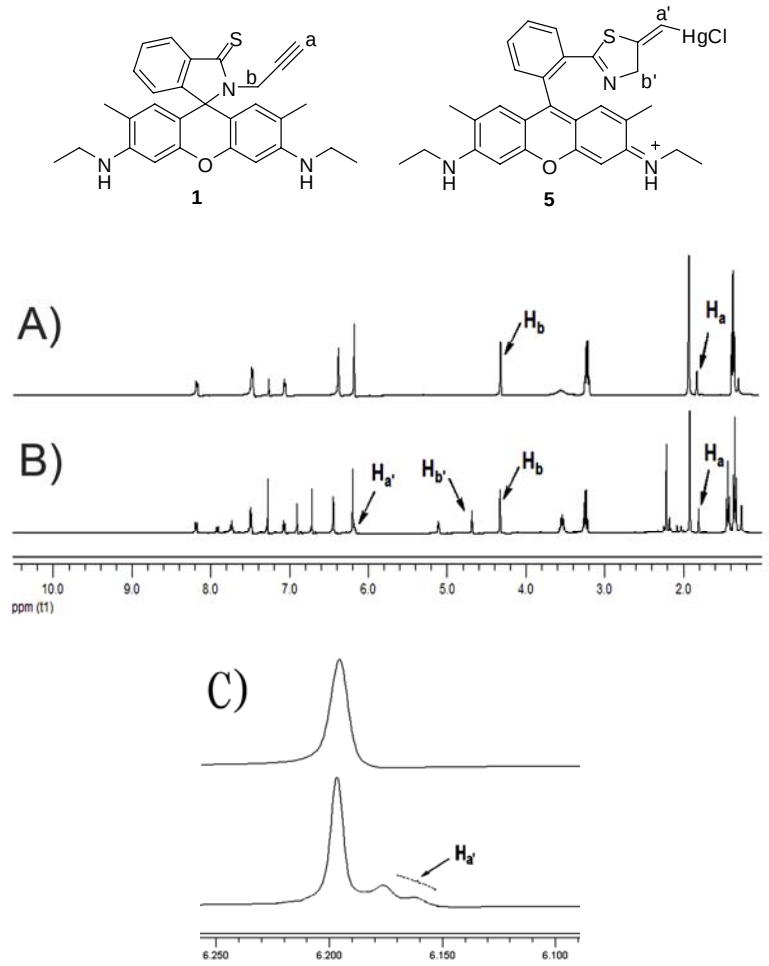

Figure S15. ^{1}H NMR spectra of (A) free probe **1** and (B) probe **1** + Hg^{2+} (1 equiv.) in $CDCl_3$. C) Amplified ^{1}H NMR spectra of the above spectra ranged from δ = 6.10 to 6.25.

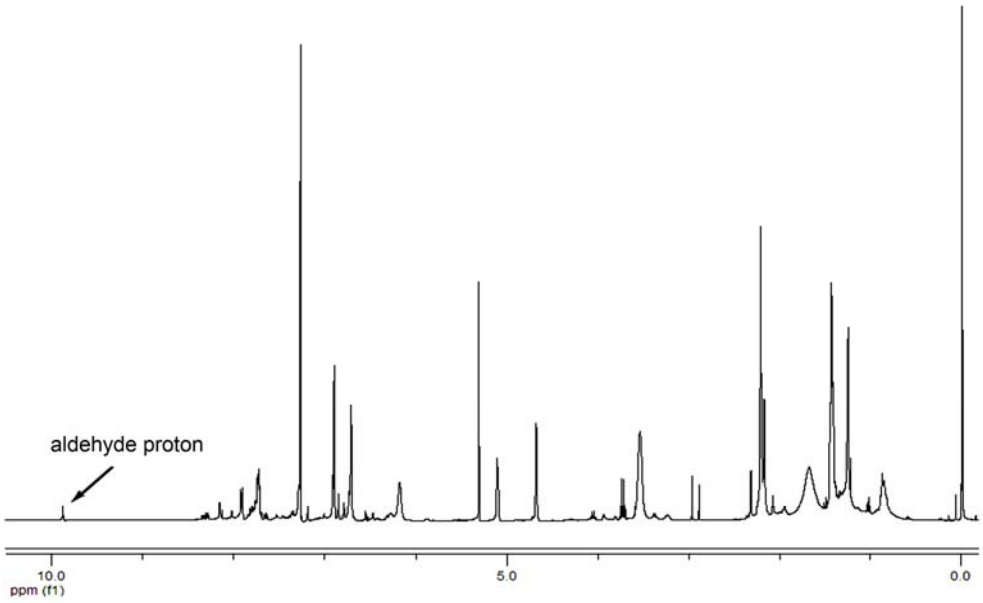

Figure S16. ^{1}H NMR spectrum of the reaction mixture of probe **1** with Hg^{2+}: The mixture of equimolar probe **1** and Hg^{2+} were incubated in DMF:H_2O (v/v 1:1) for 2 hours, and the solvents were then removed in vacuum. The resulting residual was further treated with $CDCl_3$ for ^{1}H NMR recording.

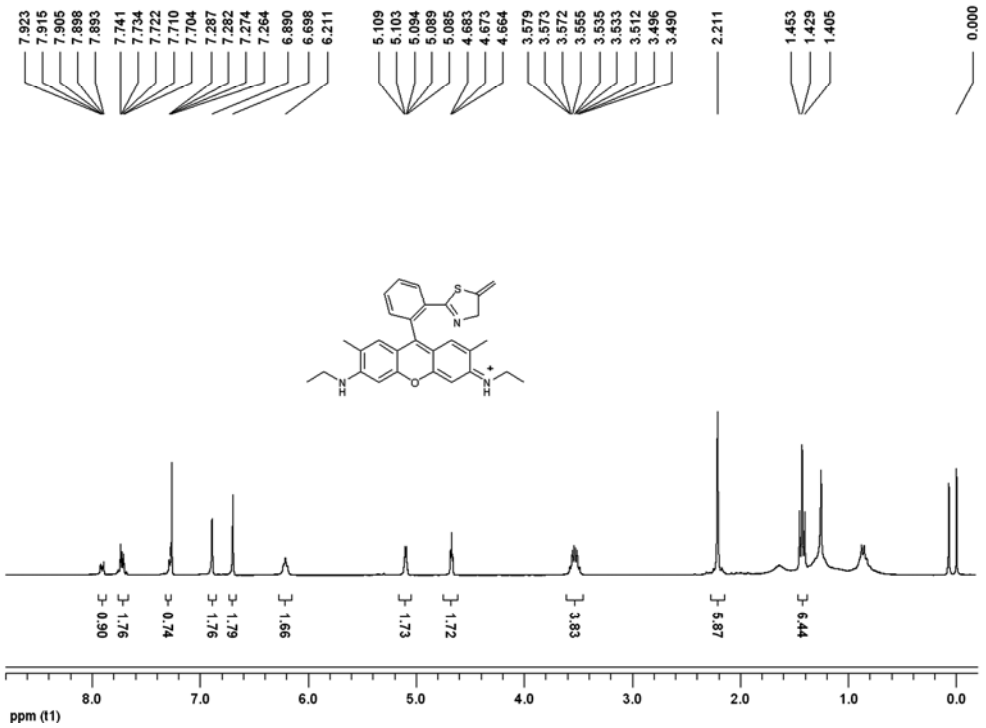

Figure S17. ^{1}H NMR spectrum of compound **7**.

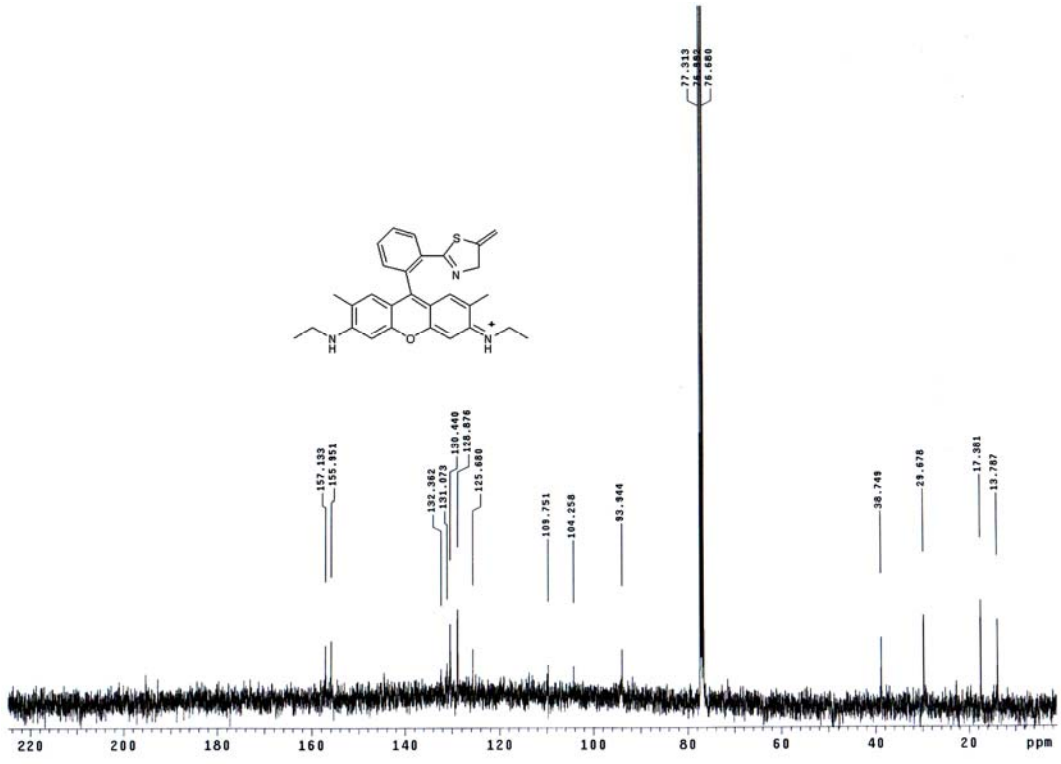

Figure S18. ^{13}C NMR spectrum of compound **7**.

SPECTRUM - MS
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Full ms [465.700 - 481.700] - Range: 465.700 - 481.700
Scan No. 10 of 22
Scan #: 10
RT: 2.80
Data points: 1

Mass	Relative Intensity	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
467.2012	100.0	467.2026	-2.9	-1.4	17.0	C_{29} H_{29} O_1 N_3 S_1

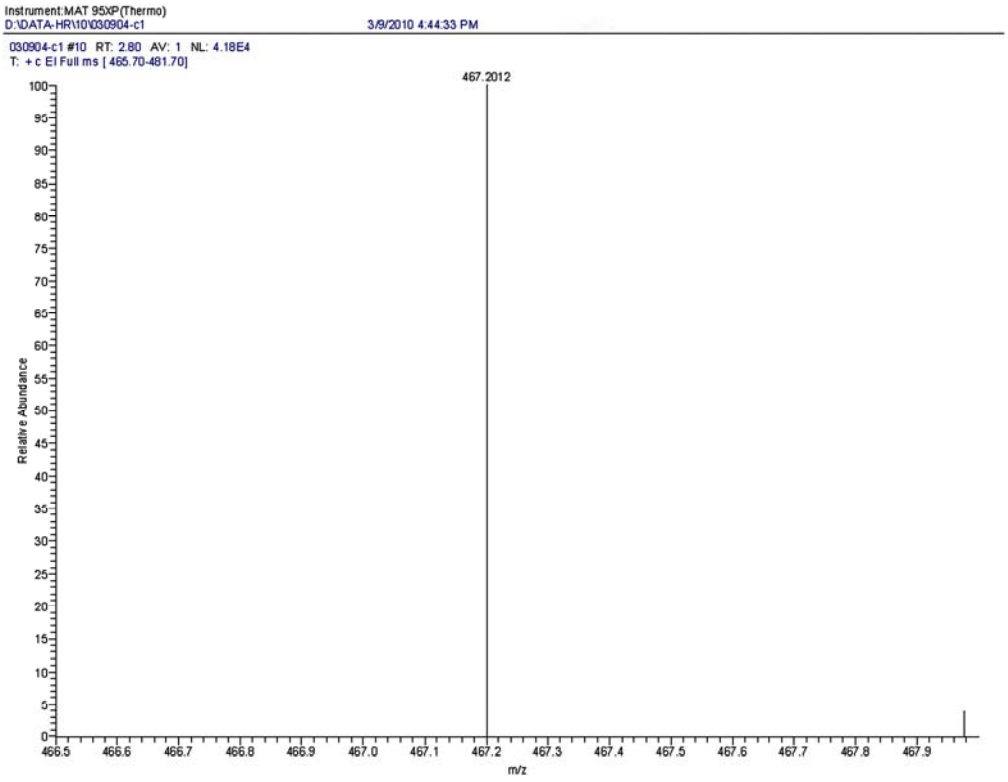

Figure S19. HRMS (EI) spectrum of compound **7.**

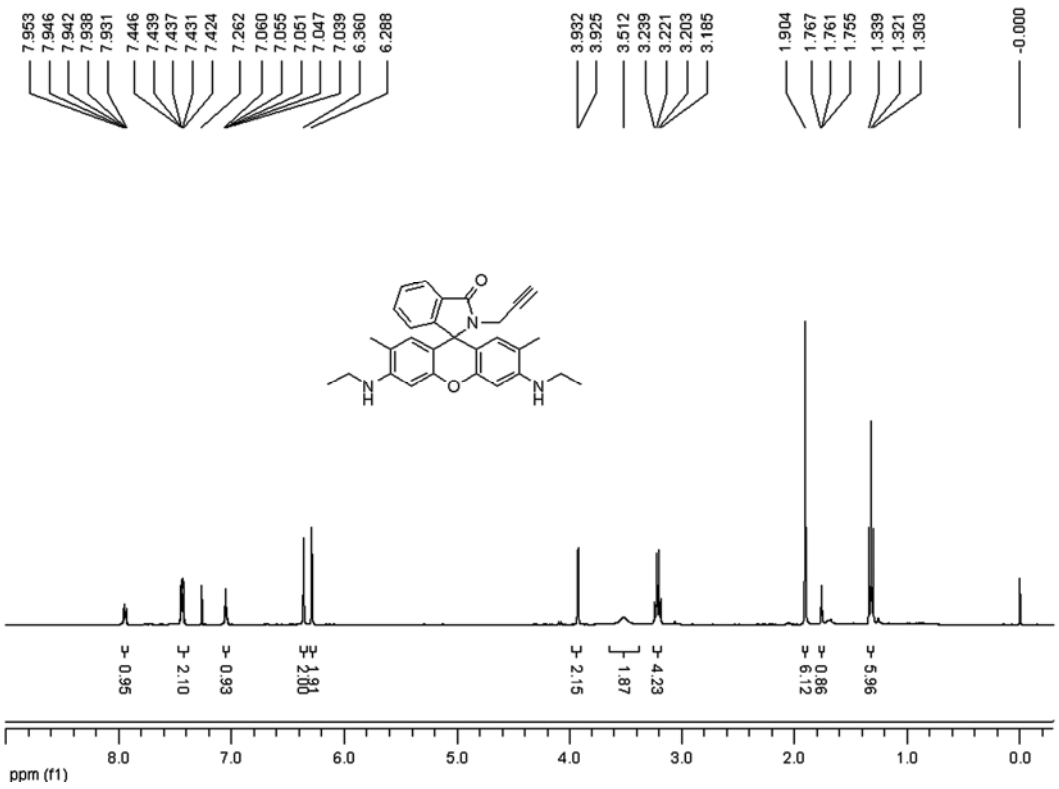

Figure S20. ^{1}H NMR spectrum of compound **2**.

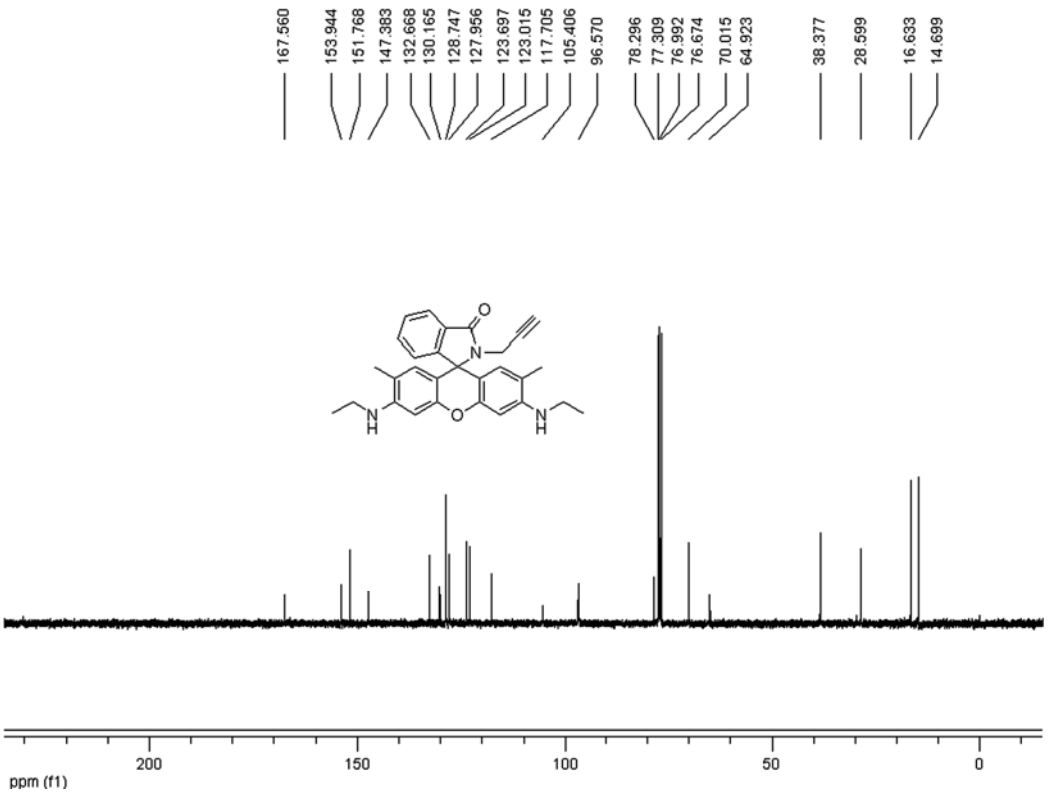

Figure S21. ^{13}C NMR spectrum of compound **2**.

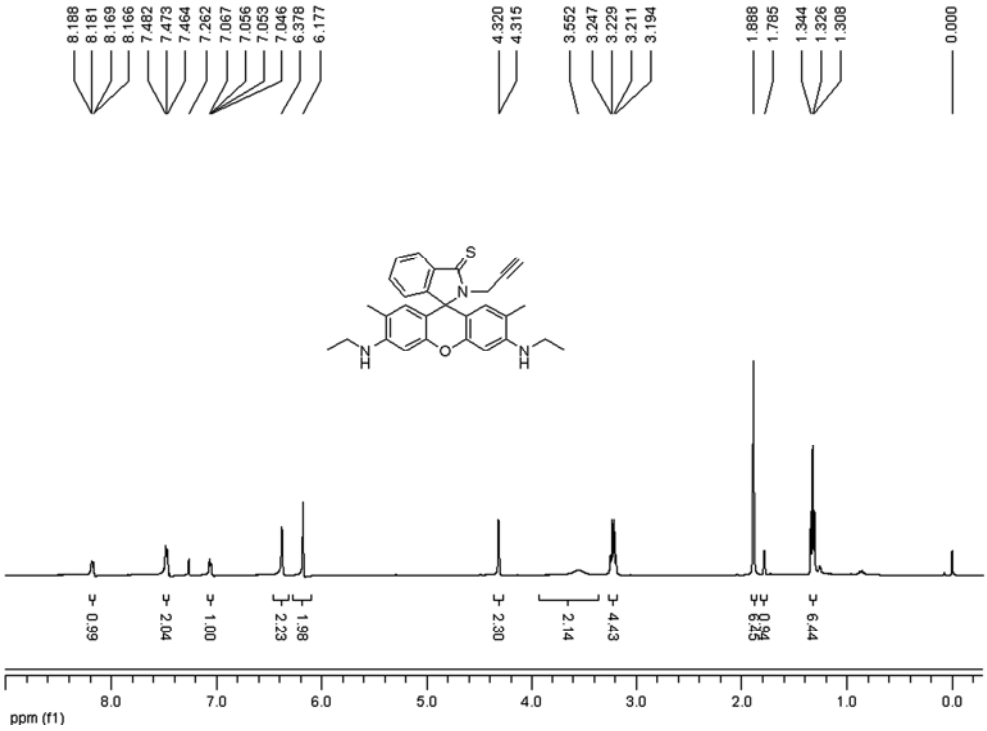

Figure S22. ^{1}H NMR spectrum of probe **1**.

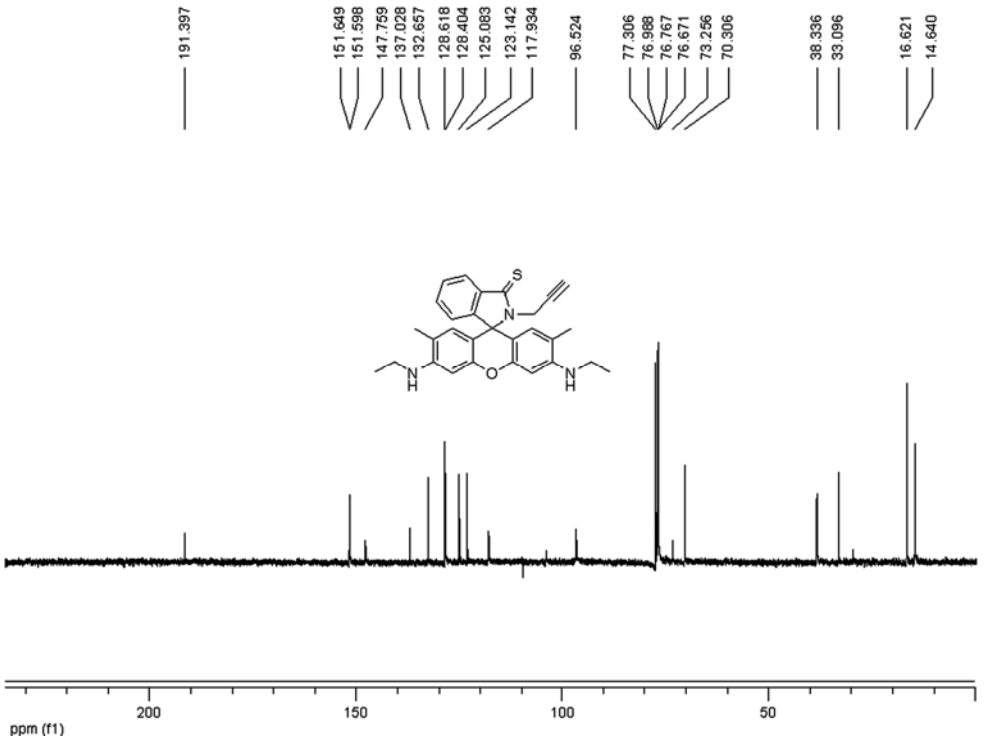

Figure S23. ^{13}C NMR spectrum of probe **1**.

References:

1 (a) M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, *Anal. Chem.* 1996, **68**, 1414; (b) A. Caballero, R. Martınez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tarraga, P. Molina, J. Veciana, *J. Am. Chem. Soc.* 2005, **127**, 15666. (c) W. Lin, L. Yuan, Z. Cao, Y. Feng, L. Long, *Chem. Eur. J.* 2009, **15**, 5096.