Supporting information for

A Reversible Fluorescent Hg^{2+} Chemosensor based on a Receptor Composed of a Thiol Atom and an Alkene Moiety for Living Cell Fluorescence Imaging

Weiying Lin,* Xiaowei Cao, Yundi Ding, Lin Yuan, Quanxing Yu

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, P. R. China

E-mail: weiyinglin@hnu.cn

Page

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. 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 (0.40 g, 0.90 mmol) in EtOH (20 mL) was added prop-2-yn-1-amine (0.26 g, 4.5 mmol) and 5 drops of Et_3N. The solution was heated to reflux under N_2 atmosphere for 24h. Then the solution was concentrated under reduced pressure, and the resulting residue was purified by flash column chromatography (petrum/ CH_2Cl_2 = 2:1 to 1:1) to give 0.28 g (0.61 mmol, 68 %) of compound **2** as a white solid. M.p. = 198 - 200℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.92 (m, 1H), 7.46–7.43 (m, 2H), 7.06–7.04 (m, 1H), 6.34(s, 2H), 6.23(s, 2H), 5.33-5.23(m, 1H), 4.88-4.84 (q, 1H), 4.74-4.71(q, 1H), 3.74 (d, J = 3.4 Hz, 2H), 3.51 (s, 2H), 3.24–3.18 (q, 4H), 1.91 (s, 6H), 1.33 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 153.6, 151.7, 147.2, 133.4, 132.4, 131.2, 128.7, 128.0, 123.7, 122.8, 117.7, 116.5, 106.2, 96.4, 64.8, 43.0, 38.4, 16.7, 14.7; HRMS(EI) Calcd. for $C_{29}H_{31}N_3O_2$ 453.2416, found 453.2381 (M^+).

Synthesis of compound 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 resultign residue was purified by flash column chromatography with petroleum / CH_2Cl_2 = (4:1 to 1:1) as eluent to obtain compound **1** (0.13 g, 0.26 mmol, 60 %) as a light yellow solid. M.p. = 239 - 241℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.19–8.16 (m, 1H), 7.52–7.45 (m, 2H), 7.07–7.05 (m, 1H), 6.36(s, 2H), 6.12(s, 2H), 5.36-5.29 (m, 1H), 4.89-4.84(m, 1H), 4.74-4.70 (m, 1H), 4.14(d, *J* = 2.4 Hz, 2H), 3.55-3.54 (m, 2H), 3.26–3.18 (m, 4H), 1.89 (s, 6H), 1.33 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.4, 151.6, 151.1, 147.7, 147.6, 138.0, 132.4, 131.4, 128.5, 128.4, 125.0, 123.1, 118.0, 117.5, 104.6, 96.5, 73.3, 47.2, 38.4, 16.6, 14.6; HRMS(EI) Calcd. for $C_{29}H_{31}N_3OS$ 469.2188, found 469.2183 (M^+).

Synthesis of Compound 3: The synthesis of compound **3** was described previously.[1] M.p. = 268-271℃. ^{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, 3H), 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). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.7, 151.6, 151.3, 147.6, 138.2, 132.3, 128.6, 128.4, 124.8, 123.1, 118.1, 109.8, 96.6, 73.3, 39.6, 38.4, 16.7, 14.7, 12.3; MS(ESI) *m*/*z* 458.4 $(M + H)^+$.

Preparation of the test solution: The stock solution of probe **1** was prepared at 2×10^{-4} M in CH_3CN. 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. $PdCl_2$ solution was prepared in DMSO. The test solution of probe **1** (5 μM) in 3 mL neutral aqueous conditions (25 mM PBS buffer, pH 7.0, containing 2.5% CH_3CN as a co-solvent) was prepared by placing 0.075 mL of the probe stock

solution to 2.925 mL of 25 mM PBS buffer (pH = 7.0). The resulting solution was shaken well and allowed to stand for 30 min at room temperature before recording the spectra.

Cell culture and fluorescence imaging: Hela cells were grown in MEM (modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 ^oC. The cells were plated on 12-well plates and allowed to adhere for 24 h. Immediately before the experiments, the cells were washed with PBS buffer. The Hela cells were then incubated with sensor **1** (5 μM) in the culture medium for 30 min at 37°C. After washing with PBS three times to remove the remaining sensor, the cells were further incubated with $HgCl_2$ (10 μM) for 30 min at 37°C and imaged with a Nikon Eclipse TE300 equipped with a CCD camera. For a control experiment, the cells were incubated sequentially with the sensor (5 μM), Hg^{2+} (10 μM), and TPEN (1 mM) before taking the fluorescence images.

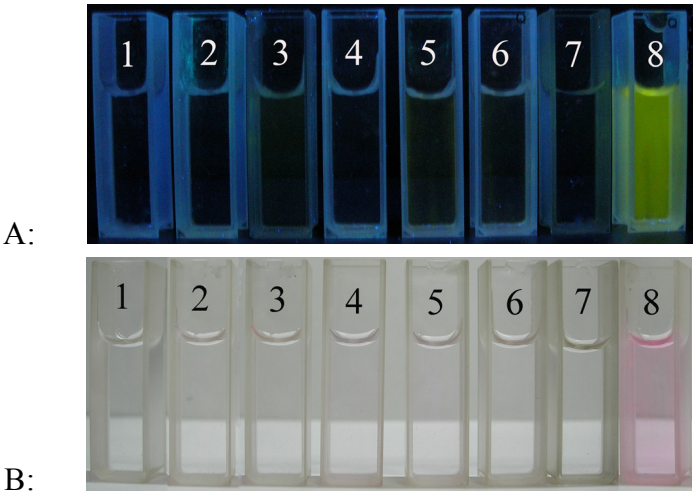

Figure S1. Color changes of the sensor **1** solution (5 μM) with 2 equiv. of different metal ions in PBS buffer solution (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent): 1. free sensor **1**; 2. sensor **1** + Cd^{2+}; 3. sensor **1** + Au^{3+}; 4. sensor **1** + Fe^{2+}; 5. sensor **1** + Cu^{2+}; 6. sensor **1** + Ag^{+}; 7. sensor **1** + Pd^{2+}; 8. sensor **1** + Hg^{2+}. A) visual fluorescence color on excitation at 365 nm using a handheld UV lamp; B) visible color.

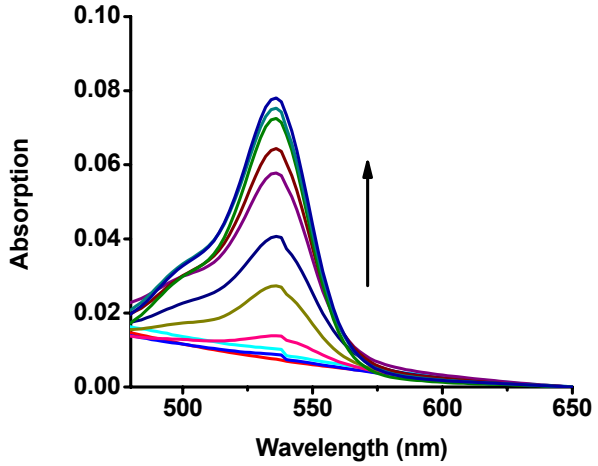

Figure S2. Absorption spectra of sensor **1** (5 μM) in PBS buffer solution (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent) in the presence of different concentraions of Hg^{2+} (0 – 2.0 equiv.).

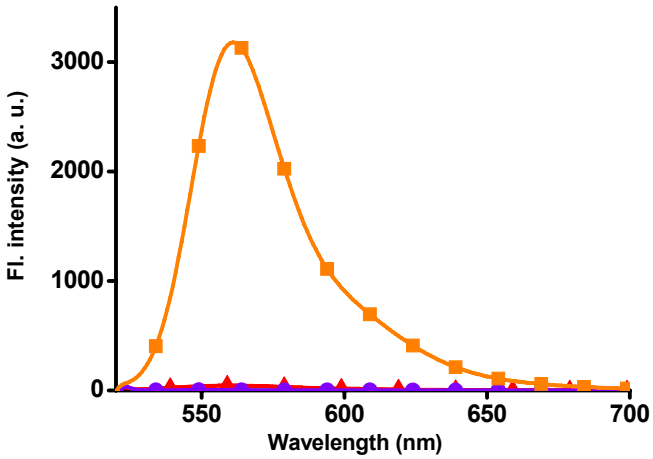

Figure S3. Fluorescence spectra of sensor **1** only (●) in PBS buffer solution (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent), and with the successive addition of 2 equiv. of Hg^{2+} (■), and excess of EDTA (▲). Excitation at 500 nm.

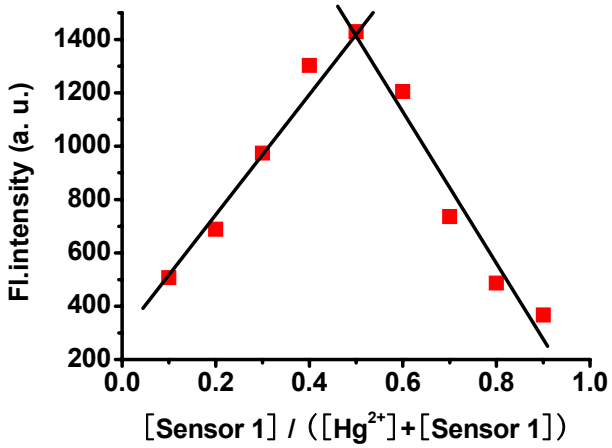

Figure S4. Job's plot of sensor **1** with Hg^{2+} according to the method of continuous variations. The fluorescent intensity was recorded at 561 nm. The total concentrations of sensor **1** and Hg^{2+} were kept constant at 10 μM.

Determination of dissociation constant: According to the titration experiment, the apparent dissociation constant (K_d) was determined using the following equation: [2] $F - F_{min} = \triangle F = [Hg^{2+}](F_{max} - F_{min})/(K_d + [Hg^{2+}])$, where F is the observed fluorescence, F_{max} is the saturated fluorescence for the Hg^{2+}-**1** complex, and F_{min} is the fluorescence for the free chemosensor **1**. When plot the reciprocal of $\triangle F$ against the reciprocal of concentration of Hg^{2+}, as shown in Figure S4, a linear relation equation was obtained: $Y = A + BX$. K_d was calculated from B/A.

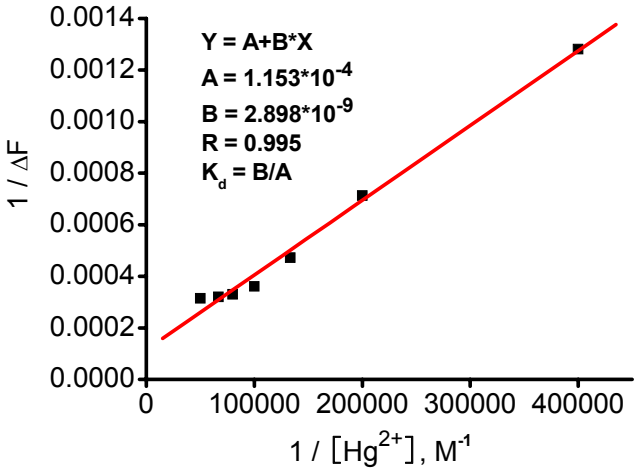

Figure S5. Benesi–Hildebrand plot for Hg^{2+}-bound **1**. Note: the Y axis denotes the reciprocal of $\triangle F$, and the X axis denotes the reciprocal of the concentration of Hg^{2+}.

Detection limit: The detection limit was determined from the fluorescence titration data based on a reported method.[3] According to the result of titration experiment, the fluorescent intensity data at 561 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 S6), and the point at which this line crossed the axis was considered as the detection limit (2.75×10^{-8} M).

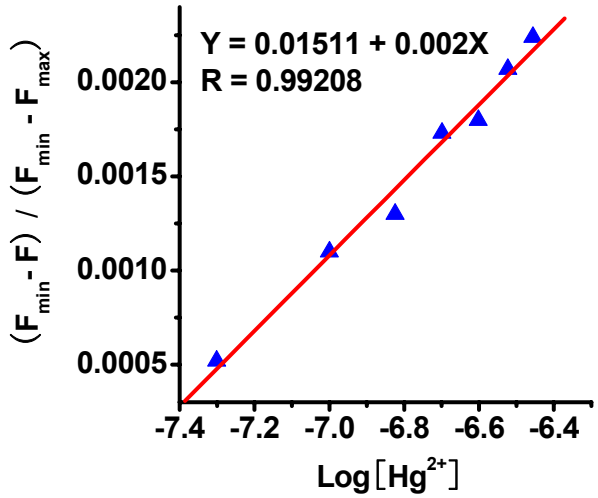

Figure S6. Normlized response of fluorescence signal to changing Hg^{2+} concentrations. (Ex. 500 nm)

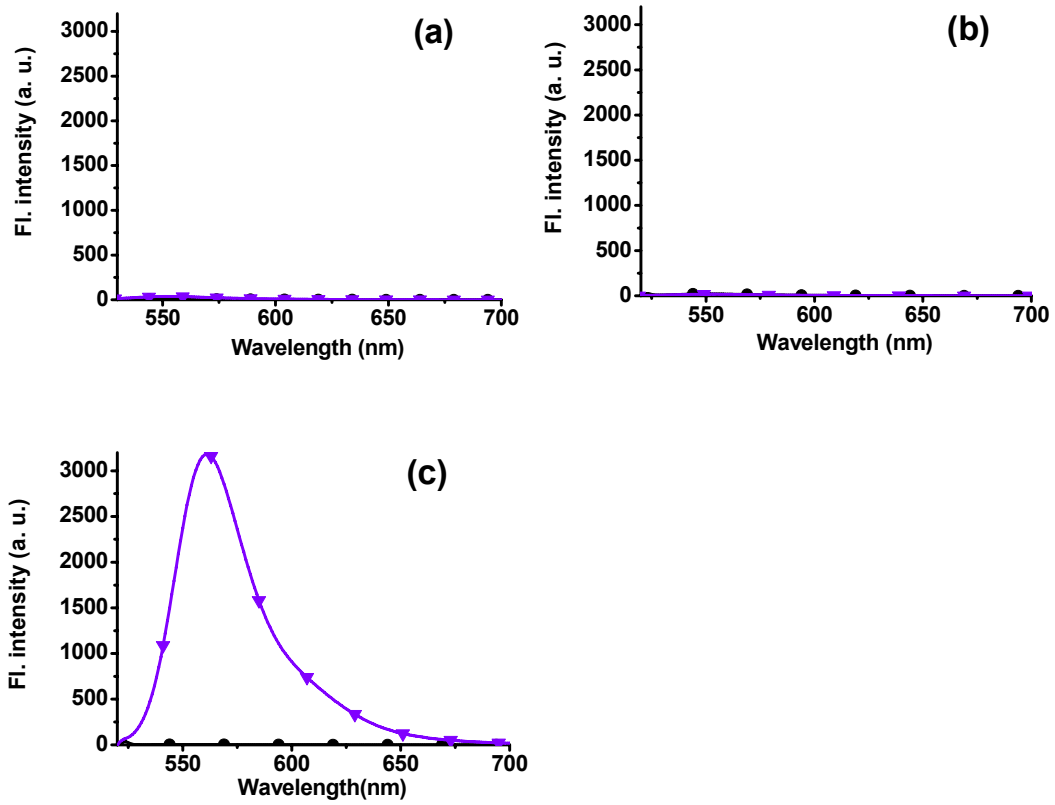

Figure S7. (a) Fluorencence spectra of the model compound **2** (●), the model compound **2** + Hg^{2+} (2 equiv.) (▼). (b) Fluorencence spectra of the model compound **3** (●), and the model compound **3** + Hg^{2+} (2 equiv.) (▼) in PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent). (c) For comparison, fluorencence spectra of sensor **1** (●), sensor **1** + Hg^{2+} (2 equiv.) (▼) was also showed. The concentration of the model compound **2**, the model compound **3**, and sensor **1** was 5 μM.

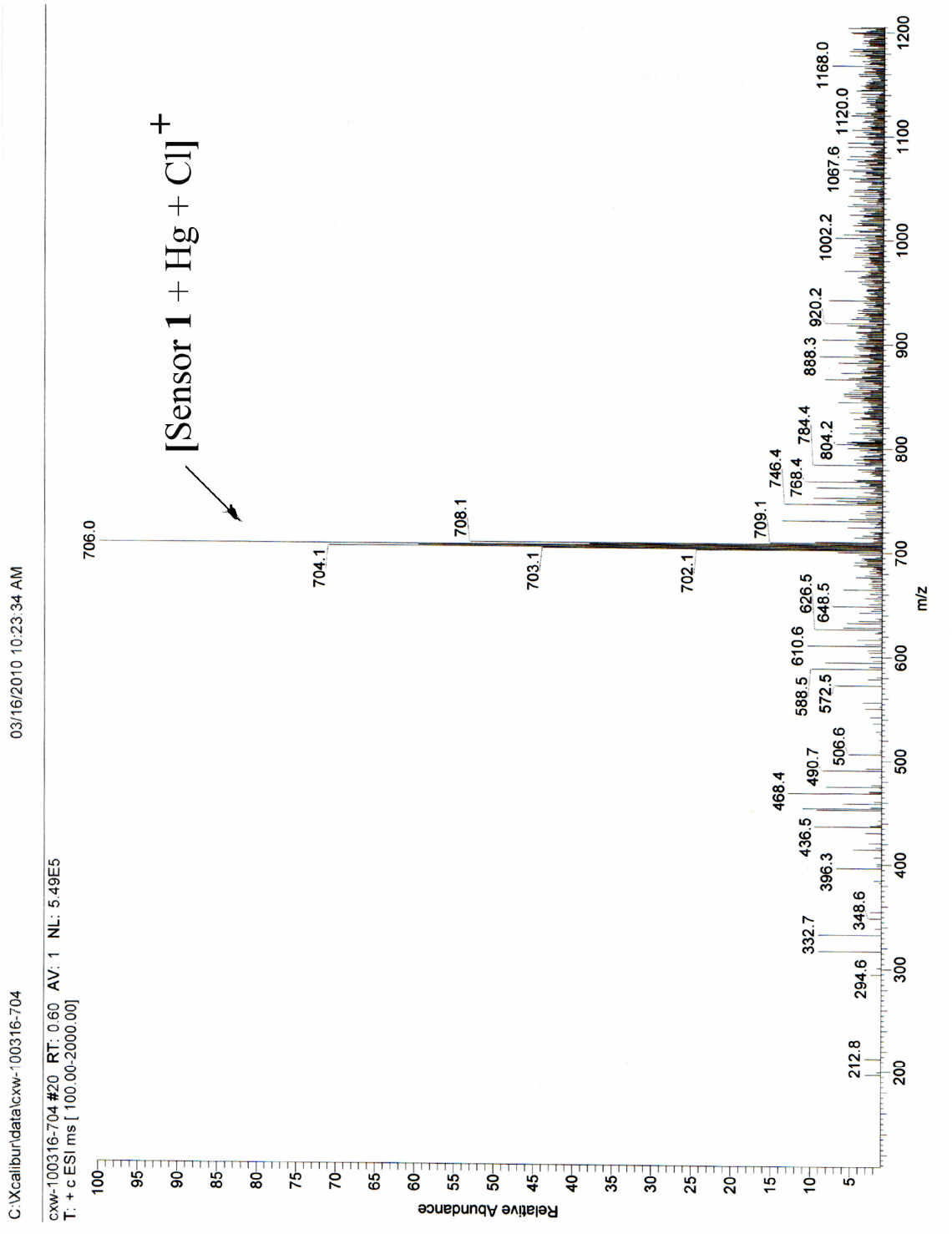

Figure S8. Mass spectrum of chemosensor **1** (20 μM) with 2 equiv. of Hg^{2+} in the PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent).

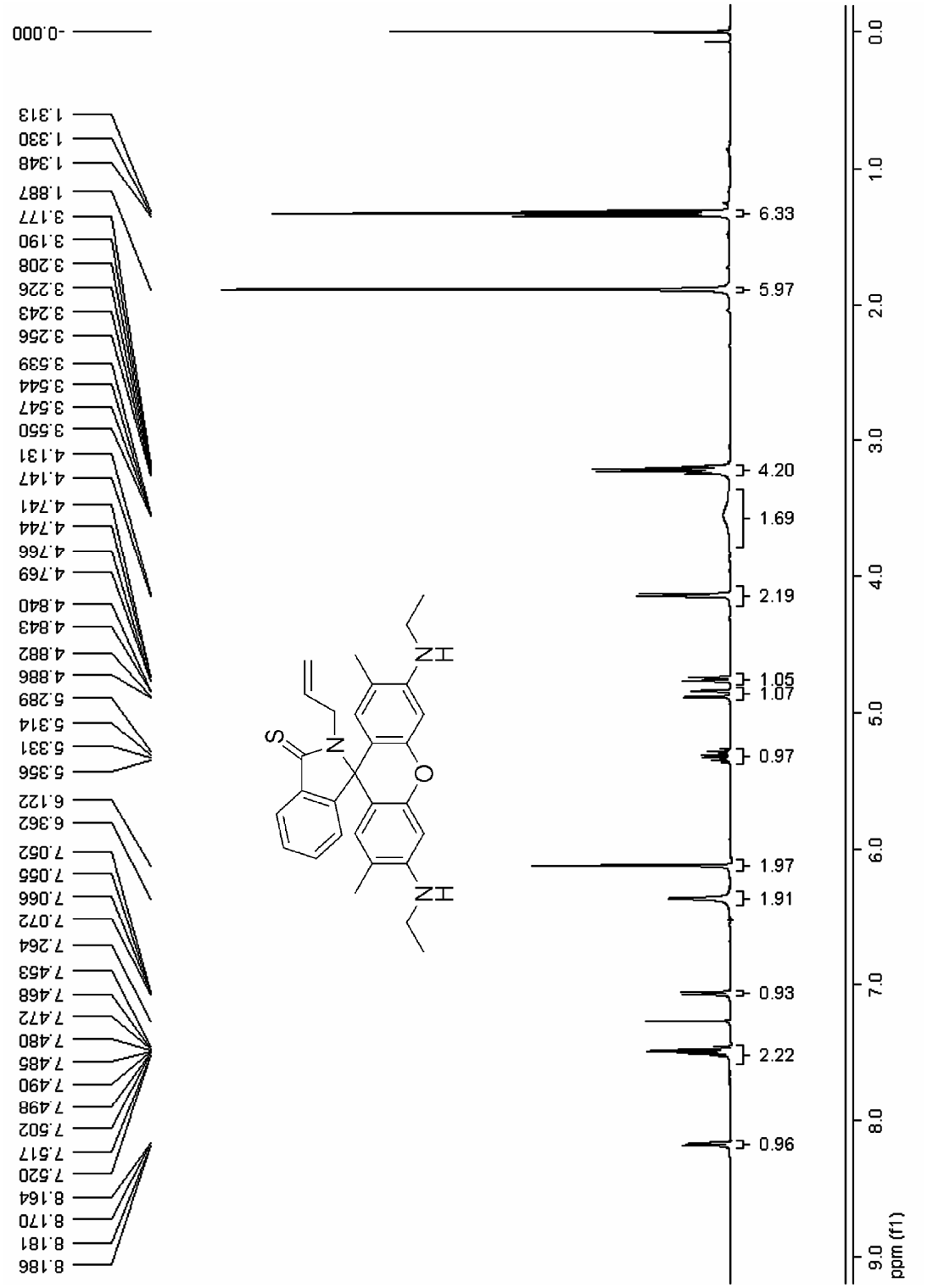

Figure S9. ^{1}H NMR spectrum of sensor **1**.

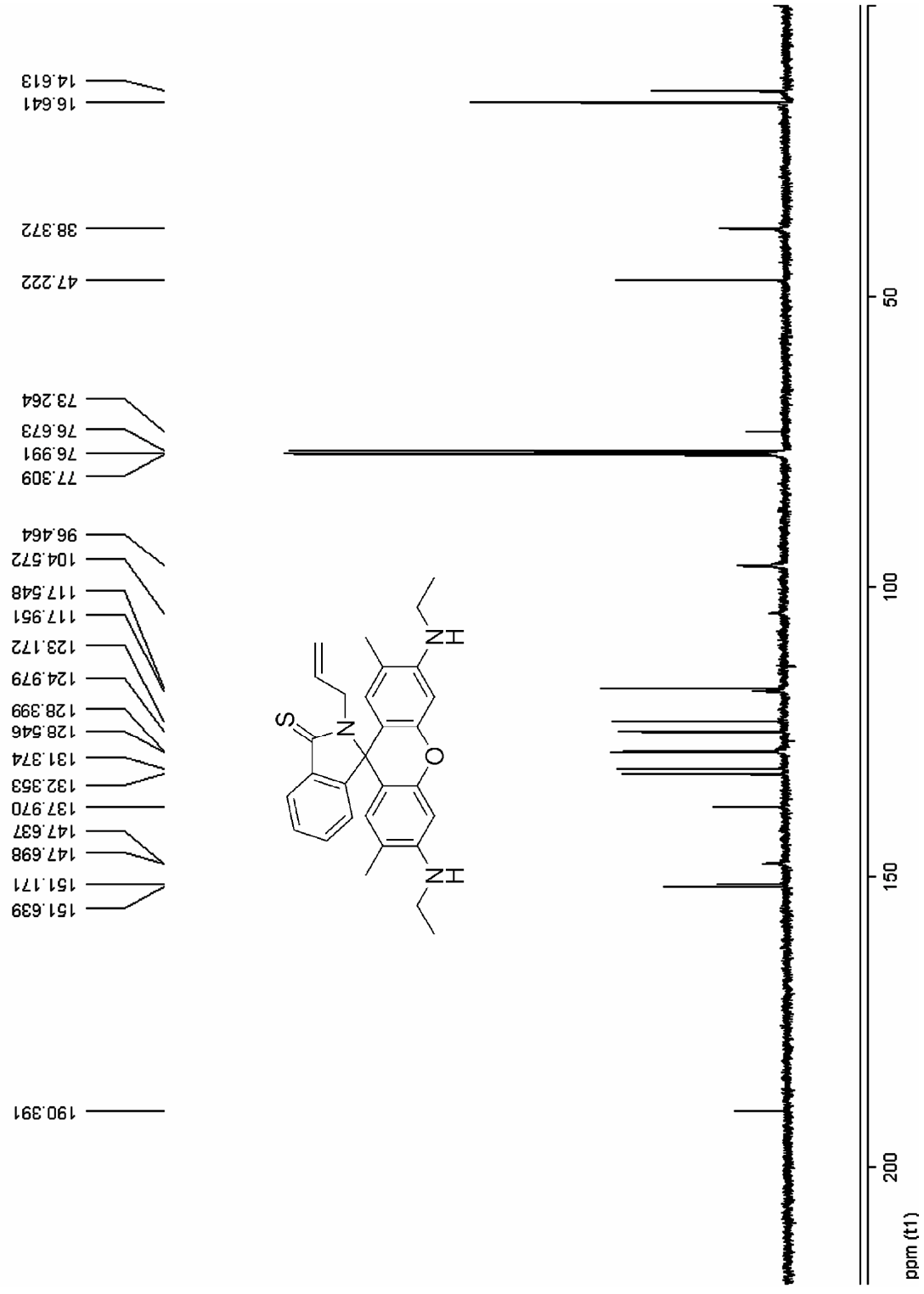

Figure S10. ^{13}C NMR spectrum of sensor **1**.

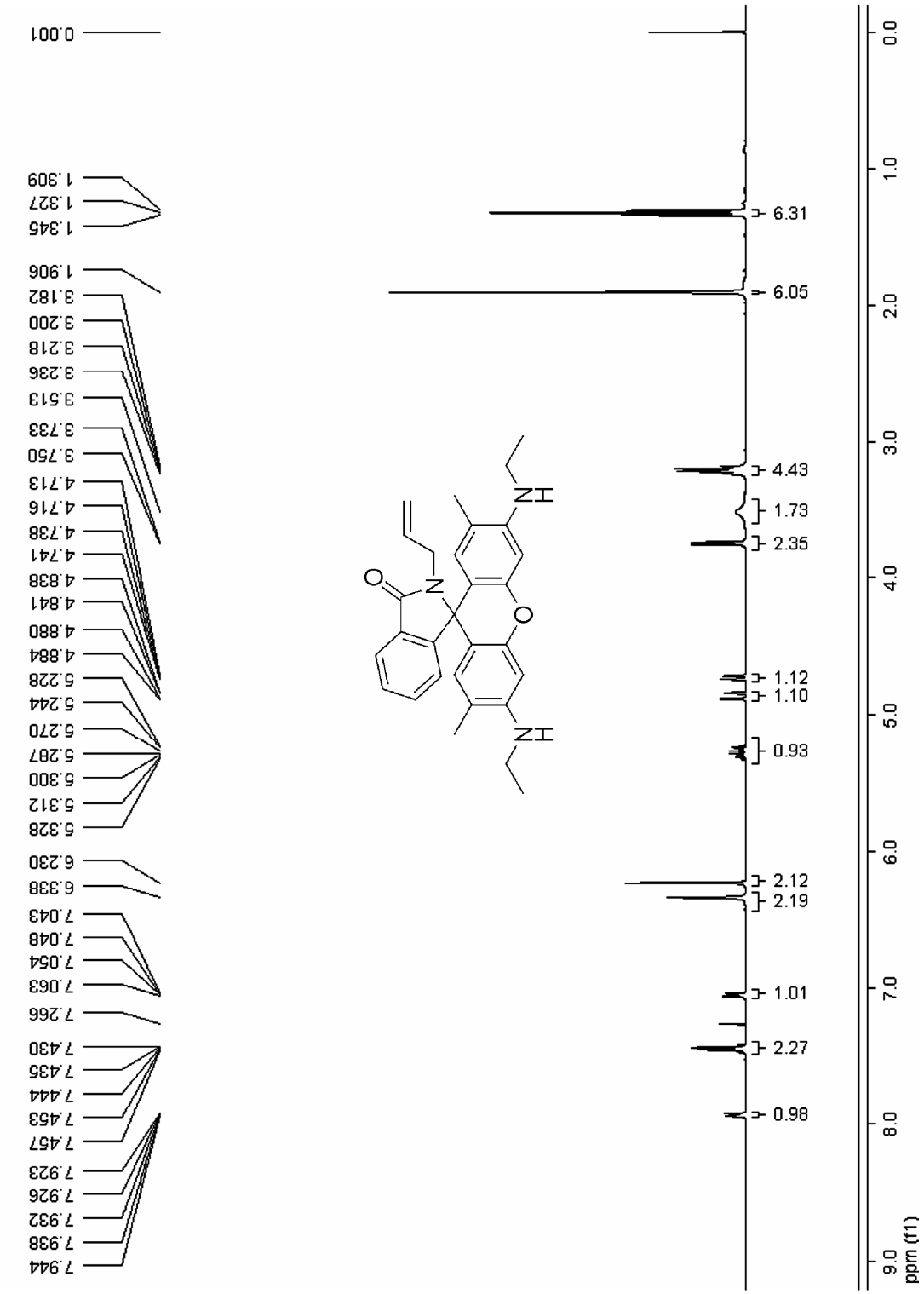

Figure S11. ^{1}H NMR spectrum of compound **2**.

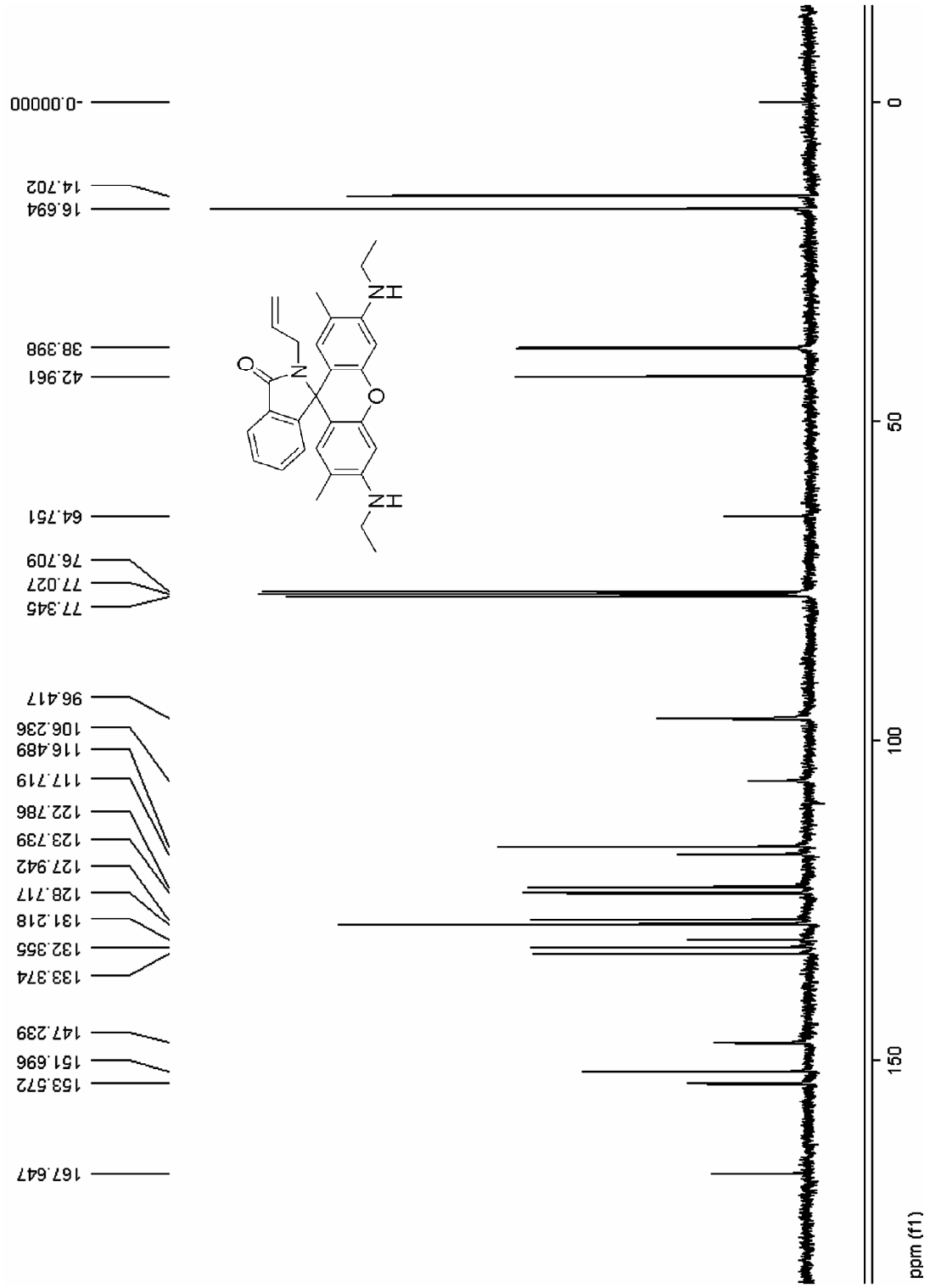

Figure S12. ^{13}C NMR spectrum of compound **2**.

References

1. Lin, W.; Cao, X.; Ding, Y.; Yuan, L.; Long, L. *Chem. Commun.* **2010**, DOI: 10.1039/b927373e.
2. (a) Rosenthal, J.; Lippard, S.J. *J. Am. Chem. Soc.* 2010, 10.1021/ja909148v. (b) Mizukami, S.; Okada, S.; Kimura, S.; Kikuchi, K. *Inorg. Chem.* **2009,** *48,* 7630–7638. (c) Xu, Z.; Baek, K. –H.; Kim, H. N.; Cui, J.; Qian, X.; Spring, D. R.; Shin, I.; Yoon, J. *J. Am. Chem. Soc.* **2010,** *132,* 601–610.
3. (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.