Supporting Information for

Development of a Reversible Fluorescent Gold Sensor with High Selectivity

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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 and dried by standard methods prior to use. Twice-distilled water was used throughout all experiments. The solutions of metal ions were prepared from $ZnCl_2$, $CoCl_2$, $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, $MgSO_4 \cdot 7H_2O$, KCl, NaCl, $CaCl_2$, $HgCl_2$, $CdCl_2 \cdot 2.5H_2O$, $MnSO_4 \cdot H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$, $AgNO_3$, and $FeCl_2$, respectively. Melting points were determined with a Beijing taike XT-4 microscopy and were uncorrected. ESI-MS analyses were performed using a Waters Micromass ZQ-4000 spectrometer. High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer. Electronic absorption spectra were obtained on a LabTech UV Power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; Cells imaging were performed with a Nikon eclipase TE300 inverted fluorescence microscopy; ^1H spectra were measured on an INOVA-400 spectrometer using TMS as an internal standard. TLC analyses were 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.

Experimental Section

Synthesis of compound 3: Rhodamine 6G hydrazide (compound **2)** was synthesized according to a reported method for the preparation of Rhodamine B hydrazide. [3a] AcOH (two drops) was added to a solution of Rhodamine 6G hydrazide (compound **2)** (37.0 mg, 0.086 mmol) and 8-hydroxyquinoline-2-carbaldehyde (10.0 mg, 0.057 mmol) in EtOH (3 mL). Then the reaction mixture was stirred at 50℃ for 1 hour. The solvent of the mixture was removed under reduced pressure, and the resulting residue was purified on a silica gel column (petroleum ether / acetone = 3: 1) to afford compound **3** as a yellow powder (30.4 mg, isolated yield: 90.2%). mp 236-238 ; ^1H NMR (400 MHz, $CDCl_3$), δ = 8.45 (s, 1H), 8.13-8.11 (d, *J* = 9.2 Hz, 1H), 8.07-8.05 (dd, *J* = 8.0 Hz, 1H), 8.02-8.00 (d, *J* = 8.4 Hz, 1H), 7.52-7.49 (m, *J* = 3.2 Hz, 2H), 7.38-7.35(t, *J* = 8.0Hz, 1H), 7.24-7.22 (dd, *J* = 6.8Hz, 1H), 7.10-7.08 (m, *J* = 1.2Hz, 2H), 6.46(s, 2H), 6.37 (s, 2H), 3.25-3.19 (q, *J* = 7.2 Hz, 4H) 1.87 (s, 6H) 1.32-1.29 (t, *J* = 5.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 165.5, 152.5, 152.3, 152.1, 151.2, 147.7, 145.2, 137.4, 135.8, 133.9, 128.4, 128.1, 127.9, 127.4, 123.8, 123.7, 118.8, 118.1, 117.7, 110.0, 105.7, 96.9, 77.3, 77.0, 76.7, 65.9, 38.3, 16.7, 14.7. ESI-MS m/z = 584.4 [M+H]+, calc. for $C_{36}H_{33}N_5O_3$ =583.3; HRMS (EI): m/z calcd for $[C_{36}H_{33}N_5O_3]$:583.2578, Found: 583.2569.

Synthesis of compound 1: Zinc powder (11.1 mg, 1.7 mmol) and two drops of acetic acid were added to compound **3** (50 mg, 0.086 mmol) in dichloromethane (4 mL). The reaction mixture was stirred at 0℃ for 0.5 h. The solvent was removed under reduced pressure, and the resulting crude product was purified by column chromatography on silica with dichloromethane and methanol as eluent (50: 1, V/V) to afford compound **1** in 60% yield as a pink solid. ^1HNMR (400 MHz, d6-DMSO), δ = 9.57(s, 1H), 8.04-8.02 (d, *J* = 7.6.0Hz, 1H), 7.88-7.86 (q, *J* = 2.4Hz, 1H), 7.59-7.53 (m, *J* = 3.2Hz, 2H),7.43-7.41 (t, *J* = 8.0Hz, 1H), 7.33-7.31 (d, *J* = 8.0Hz, 1H), 7.12-7.06 (q, *J* = 8.0Hz 2H), 7.02-6.99 (q, *J* = 2.8Hz, 1H), 6.27 (s, 2H), 6.16(s, 1H), 6.11 (s, 2H), 4.92 (s, 2H), 4.15 (s, 2H), 3.18-3.15 (t, *J* = 6.0Hz, 4H) 1.75 (s, 6H) 1.29-1.24 (t, *J* = 7.2Hz, 6H); ^{13}C NMR (100 MHz, d6-DMSO), 165.7, 156.6, 151.8, 151.2, 147.1, 132.6, 129.7, 128.1, 127.5, 127.3, 126.6,

123.6, 122.0, 120.0, 117.5, 117.2, 104.9, 95.4, 64.8, 54.8, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 38.7, 37.3, 16.7, 14.0; ESI-MS m/z = 586.1 [M+H]+, calc. for $C_{36}H_{35}N_5O_3$ =585.3; HRMS (MALDI): m/z calcd for $[C_{36}H_{36}N_5O_3]^+$:586.2813, Found: 586.2794.

Cell culture and fluorescence imaging: The 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 ℃. The cells were plated on 12-well plates and allowed to adhere for 24 h. Immediately before the experiments, HeLa cells were pre-treated with sensor **1** (5 μM) in the growth medium for 30 min and washed three times with PBS. Then the cells were incubated with Au^{3+} (10 eq) for 30 min. Subsequently, the cells were washed three times with PBS to remove the remaining Au^{3+} ions. Inverted microscope fluorescence imaging was performed with a Nikon Eclipse TE2000-S equipped with a CCD camera.

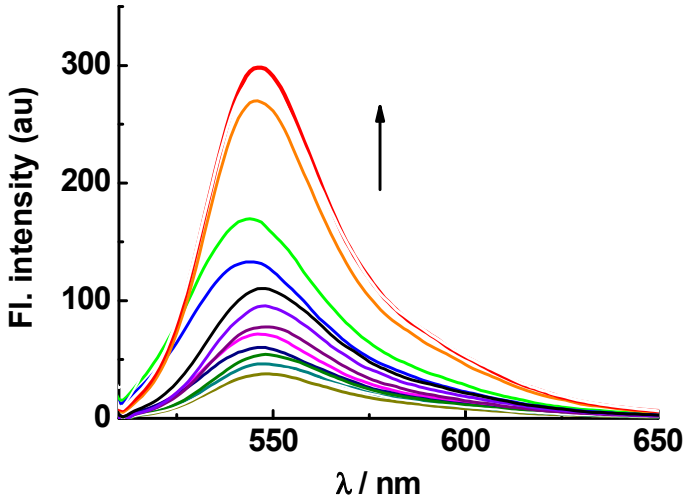


Figure S1. Fluorescence spectra (excitation at 500 nm) changes of compound **2** (5 μM) upon addition of increasing concentrations (0 –10 equiv.) of Cu^{2+} in H_2O/EtOH, 7:3, v/v.

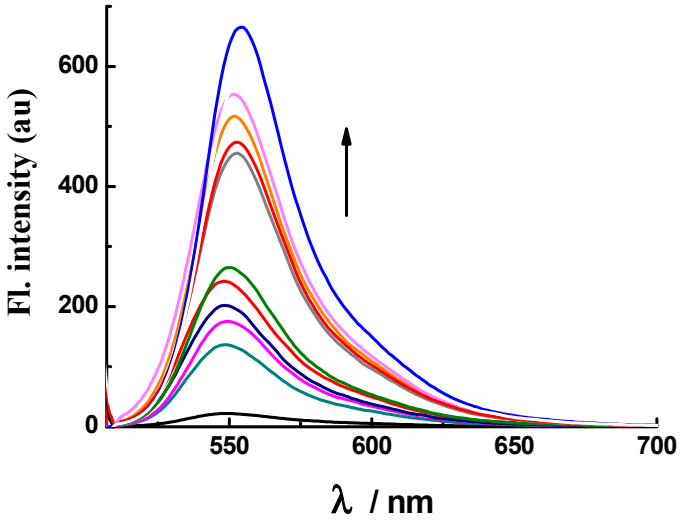


Figure S2. Fluorescence spectra (excitation at 500 nm) changes of compound **2** (5 μM) upon addition of increasing concentrations (0 – 20 equiv.) of Au^{3+} in H_2O/EtOH, 7:3, v/v.

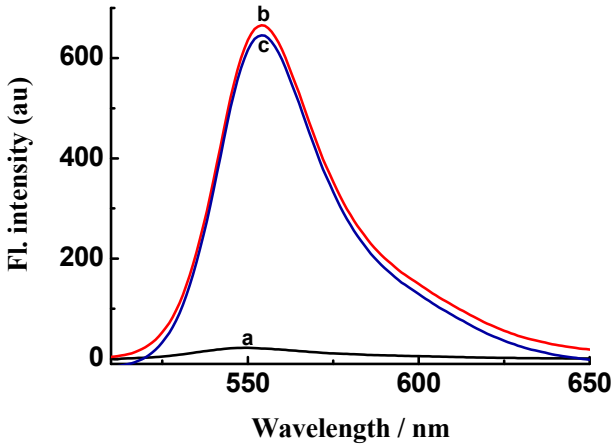


Figure S3. Fluorescence intensity changes of the solutions. a: compound **2** (5 μM); b: compound **2** (5 μM) + Au^{3+} (20 eq); c: compound **2** (5 μM) + Au^{3+} (20 eq) + CN^- (excess amount).

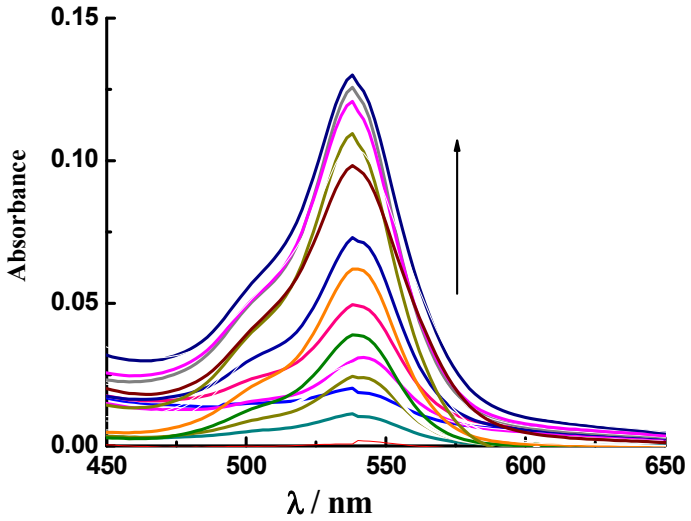


Figure S4. Absorption spectra changes of sensor **1** (5 μM) upon addition of increasing concentrations (0 – 20 equiv.) of Au^{3+} in H_2O/EtOH, 7:3, v/v.

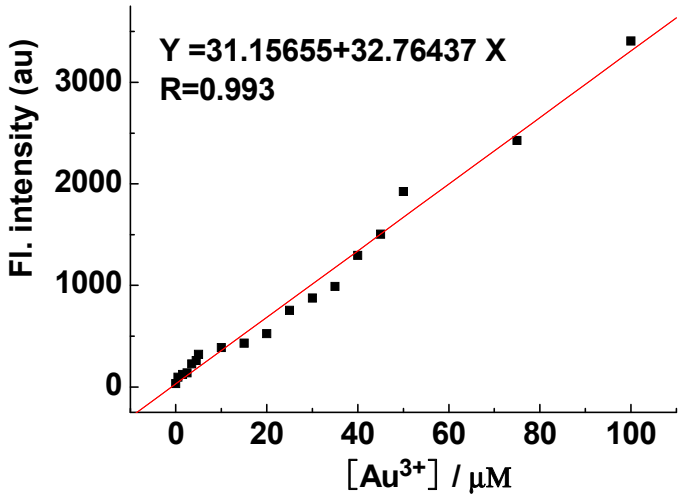


Figure S5. The correlation between the fluorescence intensity at 556 nm and the concentrations of Au^{3+}.

Calculation of Association Constant[1]

The association constant was determined from the fluorescence titration data according to a reported method [1] for a 1:1 metal-ligand binding mode. If a 1:1 metal-ligand complex is formed between a metal ion and a ligand, one can describe the equilibrium as follows:

$$M + Ligand \underset{}{\overset{K}{\rightleftharpoons}} M(Ligand) \quad \text{.....(1)}$$

Where M and M(Ligand) denote a metal ion and its complex, respectively. The corresponding association constant, *K*, can be expressed as follows:

$$K = \frac{[M(Ligand)]}{[Ligand][M]} \quad \cdots\cdots\ \cdots\cdots\ \cdots\cdots\ \cdots\cdots\ (2)$$

A response function for M is given below following the mass law:

$$\frac{\alpha}{1-\alpha} = \frac{1}{KC_T[M]} \quad \cdots\cdots\ \cdots\cdots\ \cdots\cdots\cdots\cdots(3)$$

where C_T denotes the total concentration of the ligand in the system, α defined as the ratio between the free ligand concentration ([C]) and the total concentration of ligand C_T:

$$\alpha = \frac{[C]}{C_T} \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots(4)$$

α can be determined from the emission changes in the presence of different concentrations of M:

$$\alpha = \frac{F - F_{max}}{F_{min} - F_{max}} \cdots\cdots\cdots\ \cdots\cdots\cdots\ \cdots\cdots\cdots\cdots\cdots\cdots(5)$$

where F_{max} and F_{min} are the limiting emission values for α = 1 (in the absence of M) and α = 0 (the ligand is completely complexed with M), respectively.

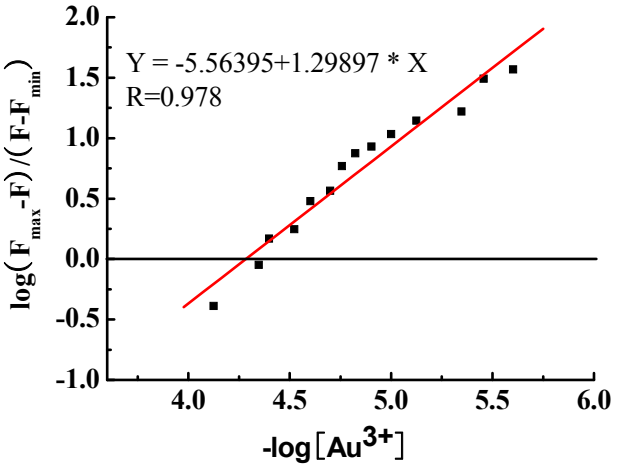


Figure S6. The binding constant of sensor **1** and Au^{3+} in H_2O/EtOH, 7:3, v/v.
$K = 3.7 \times 10^5$. LogK=5.56

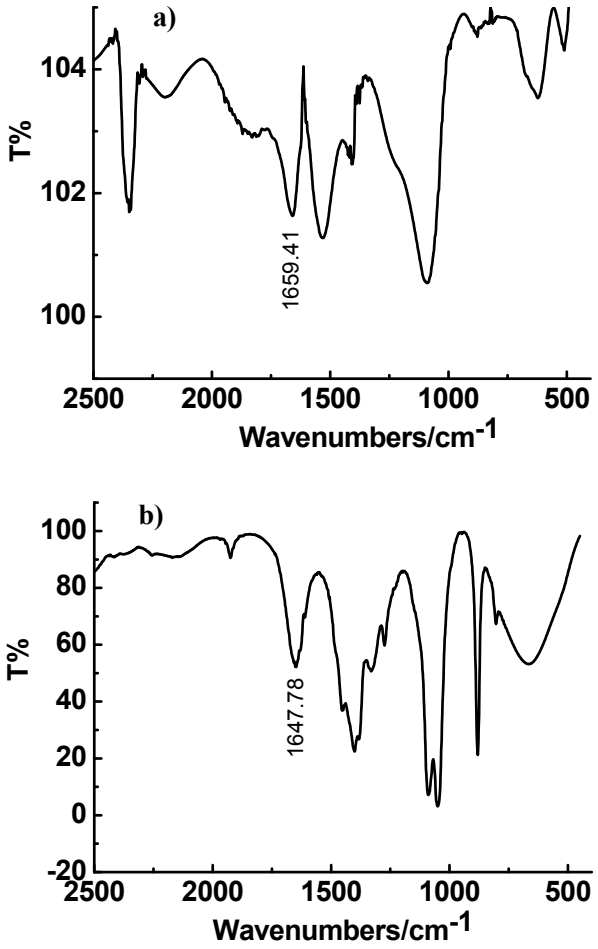
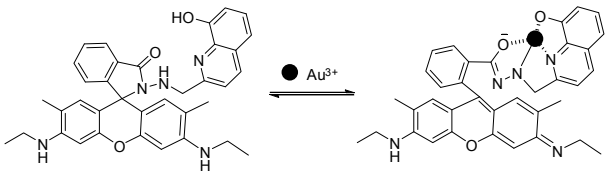


Figure S7. Infrared spectra of sensor **1** (a), and sensor **1** with Au^{3+} (b).



Scheme S1. Proposed mechanism for the fluorescent changes of sensor **1** upon the addition of Au^{3+}.

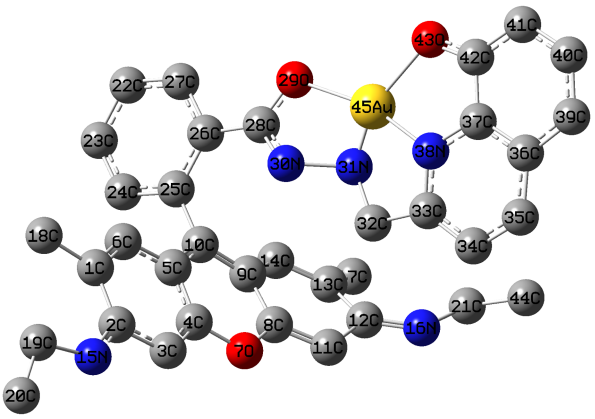


Figure S8. Calculated structure of the sensor **1**-Au^{3+} complex. Gray, red, blue, and yellow atoms denote C, O, N, and Au atoms, respectively. H atoms are omitted for clarity.

Table S1. Selected B3LYP/GEN (pop = nbo) optimized geometries of the ground state of the sensor **1**-Au^{3+} complex.

Bond	Bond length (Å)	Bond	Bond angle (°)
Au-O29	2.279	O29-Au-N38	167.71
Au-O43	2.133	N31-Au-O43	161.80
Au-N31	1.452	N31-Au-N38	88.72
Au-N29	2.041	N38-Au-O43	74.66
		O29-Au-N31	80.32
		O29-Au-O43	116.88

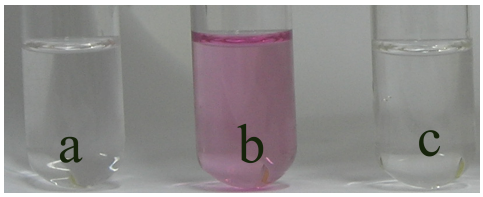


Figure S9. The visible color changes of the solutions. a: sensor **1** (5 μM); b: sensor **1** (5 μM) + Au^{3+} (20 eq); c: sensor **1** (5 μM) + Au^{3+} (20 eq) + CN^- (excess amount).

Calculation of detection limit: The detection limit was determined from the fluorescence titration data based on a reported and broadly used method [2]: According to the result of titrating experiment, the fluorescent intensity data at 556 nm were normalized between the minimum intensity and the maximum intensity and are displayed in Figure S8. A linear regression curve was then fitted to these normalized fluorescent intensity data, and the point at which this line crossed the ordinate axis was considered as the detection limit (4.8×10^{-8} M).

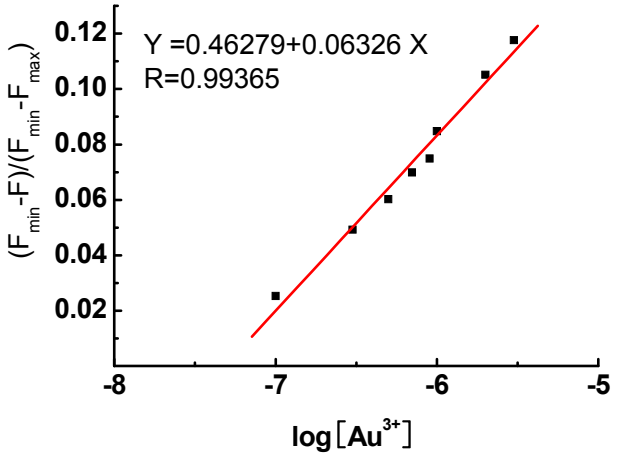


Figure S10. The plot of fluorescence intensity at 556 nm vs. Au^{3+} concentrations.

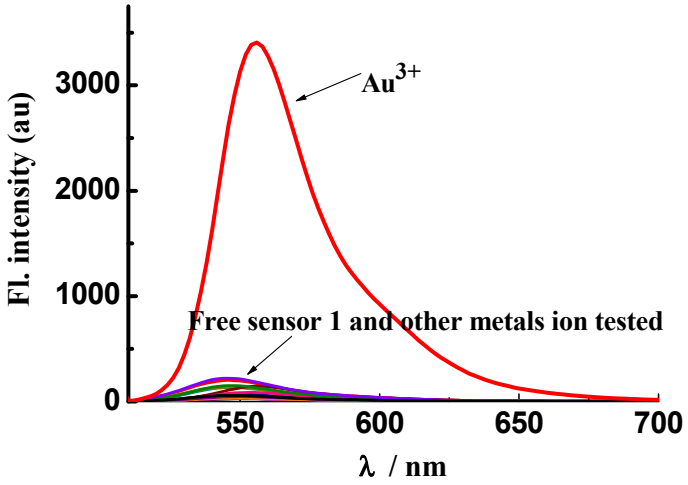


Figure S11. Fluorescence spectra of sensor **1** (5 μM) with metal ions (20 equiv.) in H_2O/EtOH, 7:3, v/v. The metal species include Au^{3+}, Au^{+} Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, and Zn^{2+}.

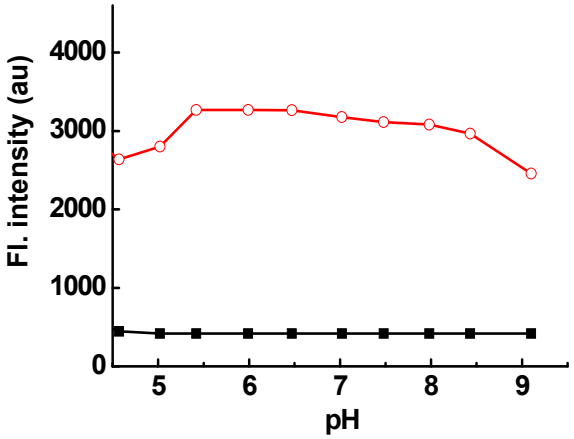


Figure S12. The fluorescence responses (at 556 nm) of free sensor **1** (5 μM) (■) and sensor **1** (5 μM) + 20 equiv Au^{3+} (○) in H_2O/EtOH, 7: 3, v/v as a function of different pH values.

References

[1] R. Yang, K. Li, K. Wang, F. Zhao, N. Li, F. Liu, *Anal. Chem.* **2003**, *75*, 612.

[2] a) H. Kim, K.-S. Moon, S. Shim, and J. Tae, *Chem. Asian J.,* **2011**, DOI: 10.1002/asia.201100126. (b) M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, *Anal. Chem.* **1996**, *68*, 1414. (c) A. Caballero, R. Martinez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tárraga, P. Molina, J. Veciana, *J. Am. Chem. Soc.* **2005**, *127*, 15666.

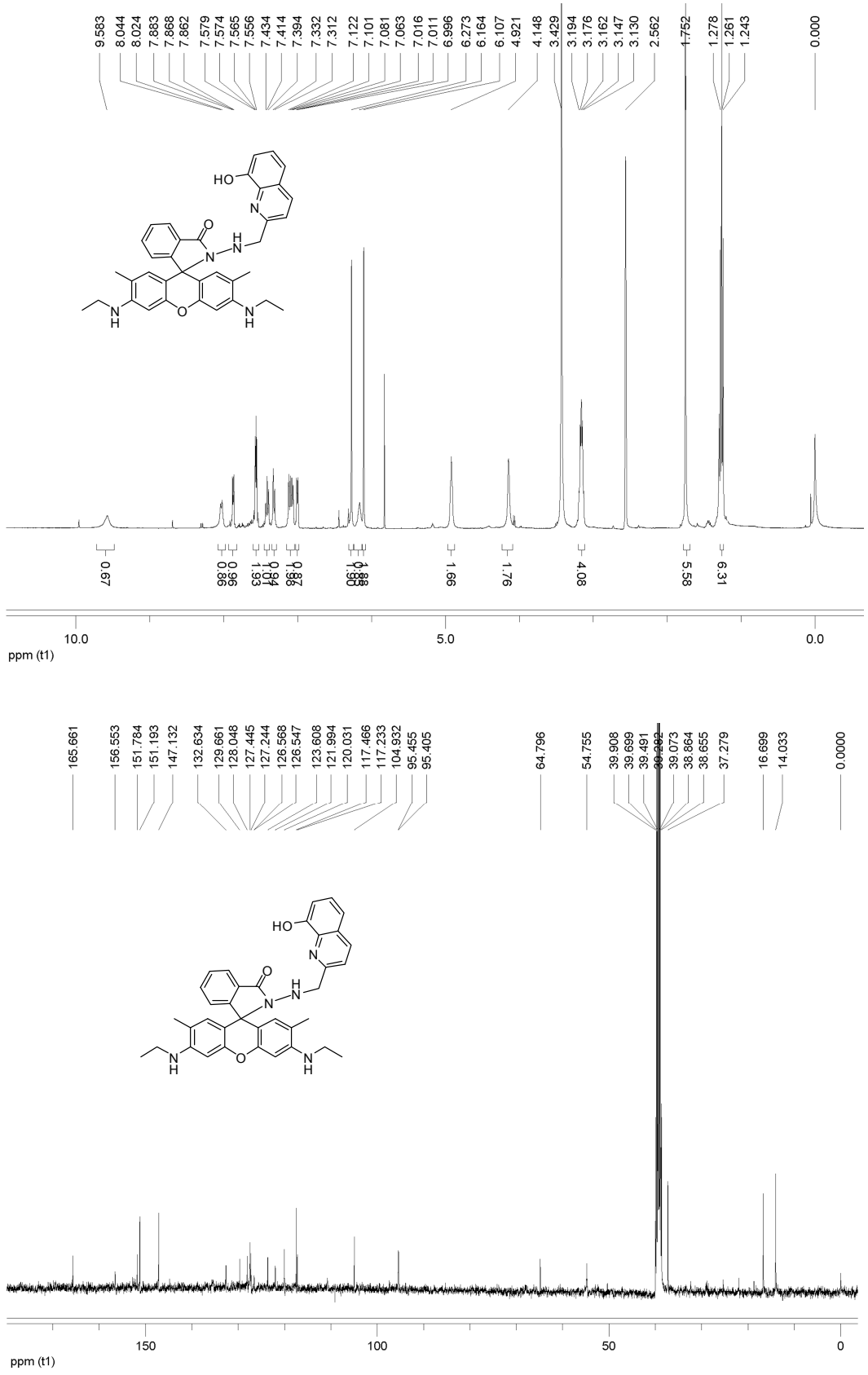


Figure S13. ^{1}H NMR and ^{13}C NMR spectra of sensor **1** in d6-DMSO.

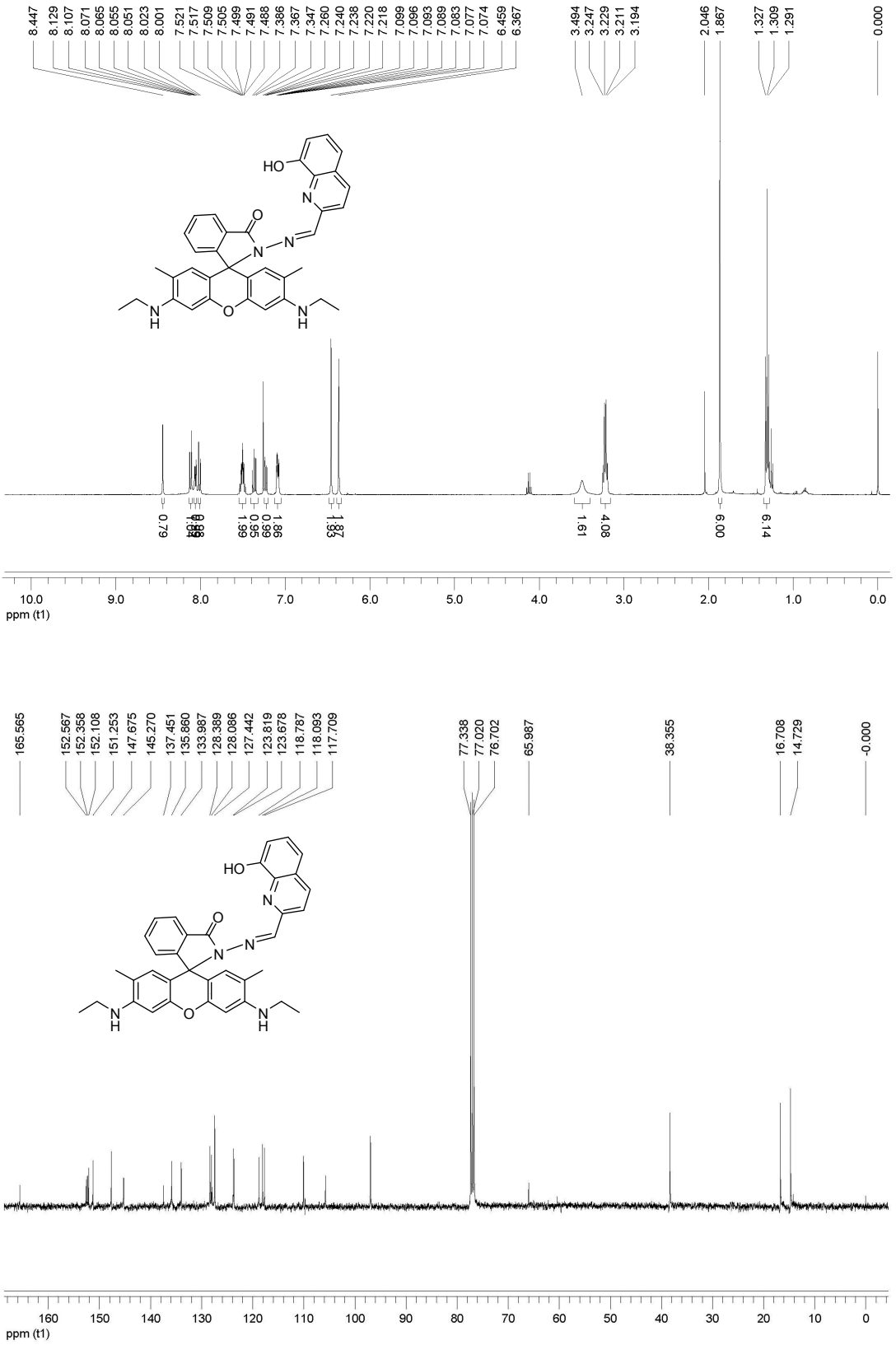


Figure S14. ^{1}H NMR and ^{13}C NMR spectra of compound **3** in $CDCl_3$.