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Supporting Information for

A Fast-responsive Fluorescent Probe for Detection of Gold Ions in Water and Synthetic Products

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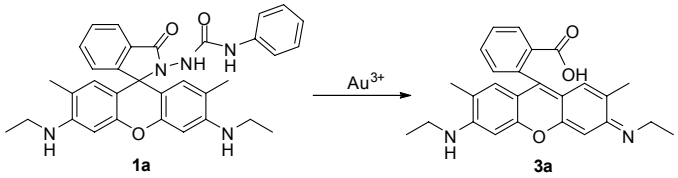
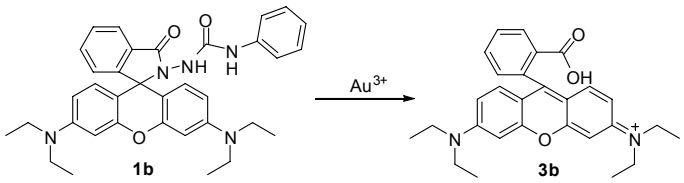
Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Melting points of compounds were measured on a Beijing Taike XT-4 microscopy melting point apparatus, all melting points were uncorrected; Low resolution mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP 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 spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; Cells imaging were performed with a Nikon eclipase TE300 inverted fluorescence microscopy; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; 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.

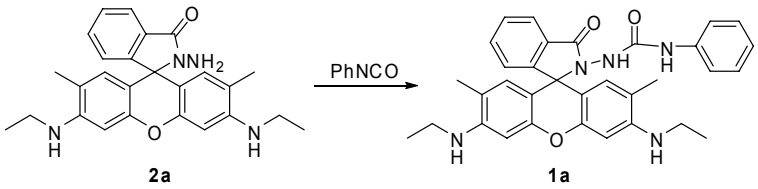


Synthesis of 3a from compound 1a: Compound **1a** (8.1 mg, 0.015 mmol) was dissolved in DMF/ pH 7.4 PBS (1: 1, 4 mL) and stirred at room temperature for 10 min, and then $HAuCl_4$ (5.1 mg, 0.015 mmol) in water (0. 1 mL) was added dropwise. Subsequently, the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was then poured onto a 1M KOH (10 mL) solution, and the crude product was extracted with CH_2Cl_2 (5 × 20 mL). The organic solvent was evaporated under reduced pressure, and the residue was purified by preparative thin layer chromatography (CH_2Cl_2: MeOH = 8: 1) to afford compound **3a** as a red powder (2.1 mg, isolated yield: 33.9%). mp 298-300 ^oC; ^1H NMR (400 MHz, CD_3OD) δ = 1.27-1.31 (t, J = 7.2 Hz, 6H, CH_3), 2.06 (s, 6H, Xanthene-CH_3), 3.42-3.47 (q, 4H, J = 7.2 Hz, CH_2), 6.81 (s, 2H, Xanthene-H), 6.94 (s, 2H, Xanthene-H), 7.18-7.20 (dd,

J = 6.8, 2.0 Hz , 1H, C_6H_4), 7.58-7.65 (m, 2H, C_6H_4), 8.08-8.10 (dd, *J* = 7.2, 1.6 Hz, 1H, C_6H_4); ^{13}C NMR (100 MHz, CF_3COOD) δ = 8.45, 37.73, 96.99, 114.81, 120.95, 123.42, 123.74, 124.23, 124.59, 126.01, 126.26, 128.01, 143.01, 149.17, 151.25, 163.56. ESI-MS m/z 415.3 $[M+H]^+$. HRMS (EI) *m/z* calcd for $C_{26}H_{26}O_3N_2$ (M): 414.1938. Found 414.1927; HRMS (EI) *m/z* calcd for $C_{26}H_{25}O_3N_2$ (M - H): 413.1860. Found 413.1856.

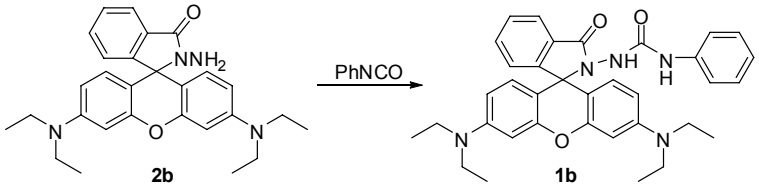


Synthesis of 3b from compound 1b: Compound **1b** (7.0 mg, 0.012 mmol) was dissolved in DMF/ pH 7.4 PBS (2: 1, 3 mL) and stirred at room temperature for 10 minutes, and then $HAuCl_4$ (4.0 mg, 0.012 mmol) in water (0.08 mL) was added dropwise. Subsequently, the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was evaporated under reduced pressure, and the residue was purified by preparative thin layer chromatography (CH_2Cl_2: EtOH = 20: 1) to afford compound **3b** as a red powder (2.3 mg, isolated yield: 40.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.27-1.30 (t, *J* = 6.0 Hz, 12H, CH_3), 3.54-3.55 (q, 8H, *J* = 6.4 Hz, CH_2), 6.67-6.72 (m, 4H, Xanthene-H), 7.02-7.04 (d, 2H, *J* = 8.8 Hz, Xanthene-H), 7.19-7.20 (d, *J* = 6.8, 1H, C_6H_4), 7.65-7.66 (d, *J* = 4.0, 2H, C_6H_4), 8.29-8.30 (d, *J* = 6.0, 1H, C_6H_4); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.63, 45.78, 96.37, 109.80, 112.88, 112.92, 128.34, 129.94, 131.42, 132.05, 154.49, 157.15, 166.80. ESI-MS m/z 443.2 $[M]^+$. HRMS (EI) *m/z* calcd for $C_{28}H_{30}O_3N_2$ (M - H): 442.2251. Found 442.2276.



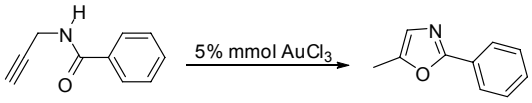
Synthesis of 1a: Rhodamine 6G hydrazide **2a** (37.0 mg, 0.086 mmol) was dissolved in DMF (5 mL) and heated to 50 □, and then PhNCO (16.0 mg, 0.13 mmol) in DMF (1 mL) was added dropwise.

Subsequently, the reaction mixture was heated to 80-100 □ and further stirred for 0.5 hour. The hot solution was cooled to room temperature, and the solvent was removed under reduced pressure. The resulting residue was purified on a silica gel column (CH_2Cl_2 / petroleum = 1 : 2) to afford compound **1a** as a yellow powder (19.1 mg, isolated yield: 42.1%). mp 236-238 °C; ^{1}H NMR (400 Hz, $CDCl_3$): δ = 1.19-1.23 (t, 6H, C*H*$_3$), 1.83 (s, 6H, Xanthene-C*H*$_3$), 3.08-3.13 (q, 4H, C*H*$_2$), 5.73 (s, 1H, CON*H*Ph), 6.19 (s, 2H, Xanthene-*H*), 6.30 (s, 2H, Xanthene-*H*), 6.40 (s, 1H, N*H*CONHPh), 6.86-6.88 (d, *J* = 7.6 Hz, 3H, Ar-*H*), 7.04-7.07 (t, *J* = 7.8 Hz, 2H, Ar-*H*), 7.19 (s, 1H, Ar-*H*), 7.51-7.59 (m, 2H, Ar-*H*), 7.96-7.98 (d, *J* = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 Hz, $CDCl_3$):14.62, 16.78, 29.67, 38.29, 67.14, 97.23, 104.73, 118.37, 119.56, 123.08, 123.75, 124.72, 127.22, 128.43, 128.92, 129.31, 134.12, 137.61, 147.89, 150.39, 152.73, 155.17, 167.56; ESI-MS m/z 548.3 $[M+H]^+$; HRMS (EI) *m/z* calcd for $C_{33}H_{33}N_5O_3$ (M): 547.2578. Found 547.2594.



2b **1b**

Synthesis of 1b: Rhodamine B hydrazide **2b** (100.0 mg, 0.22 mmol) in toluene (60 mL) was heated to 80 □ for 30 min, and then PhNCO (30.0 mg, 0.22 mmol) in toluene (3 mL) was added dropwise. Subsequently, the reaction mixture was heated to 110 □ and further stirred for 0.5 hour. The hot solution was cooled to room temperature, and the solvent was removed under reduced pressure. The resulting residue was purified on a silica gel column (CH_2Cl_2 / petroleum = 1 : 2) to afford compound **1b** as a yellow powder (38.1 mg, isolated yield: 32.1%). ^{1}H NMR (400 Hz, $CDCl_3$): δ = 1.06-1.09 (t, *J* = 6.8, 12H, CH_3), 3.23-3.28 (q, *J* = 6.8, 8H, C*H*$_2$), 6.15 (s, 1H, CON*H*Ph), 6.21 (dd, *J* = 8.8, 2.4 Hz, 2H, Xanthene-*H*), 6.35 (d, *J* = 2.0 Hz, 2H, Xanthene-*H*), 6.71-6.73 (d, *J* = 8.8 Hz, 2H, Xanthene-*H*), 6.97-7.00 (t, *J* = 7.2 Hz, 1H, Ar-*H*), 7.04-7.06 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 7.18-7.20 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 7.36-7.38 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 7.40-7.42 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 7.43-7.47 (d, *J* = 7.8 Hz, 1H, Ar-*H*), 8.19-8.21 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 11.05 (s, 1H, N-*H*); ^{13}C NMR (100 Hz, $CDCl_3$): 11.59, 43.33, 57.74, 97.16, 106.16, 107.06, 119.12, 122.98, 126.56, 126.80, 127.06, 127.48, 127.89, 129.00, 133.08, 136.46, 145.27, 147.83, 149.15, 151.59, 164.01; ESI-MS m/z 576.1 $[M+H]^+$; HRMS (EI) *m/z* calcd for $C_{35}H_{37}N_5O_3$ (M): 575.2891. Found 575.2893.



4 **5**

Synthesis of compound 5 by gold-catalyzed cyclization of propargylic amide (*Org. Lett.* **2004**, *6*, 4391-4394.): Amide **4** (100 mg) was dissolved in approximately 500 µl of dichloromethane, and 5 mol-% $AuCl_3$ was added. Subsequently, the reaction mixture was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure, and the resulting crude product was purified by column chromatography on silica with petrol ether and dichloromethane as eluent (3: 2, V/V) or neutral/basic Al_2O_3 with petrol ether as eluent to afford compound **5** as a primrose (after silica column purification) or colorless liquid (after neutral/basic Al_2O_3 column purification). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.40-2.41 (d, J = 1.2 Hz, 3H, CH_3), 6.85-6.86 (d, J = 1.2 Hz, 1H, oxazole-*H*), 7.43-7.46 (m, 3H, C_6H_5), 8.01-8.03 (dd, J = 8.0, 1.8 Hz, 2H, C_6H_5). Compound known: A. Hassner, B. Fischer, *Tetrahedron* **1989**, 45 (19), 6249-6262; C. Kashima, H. Arao, *Synthesis* **1989**, 873-874.

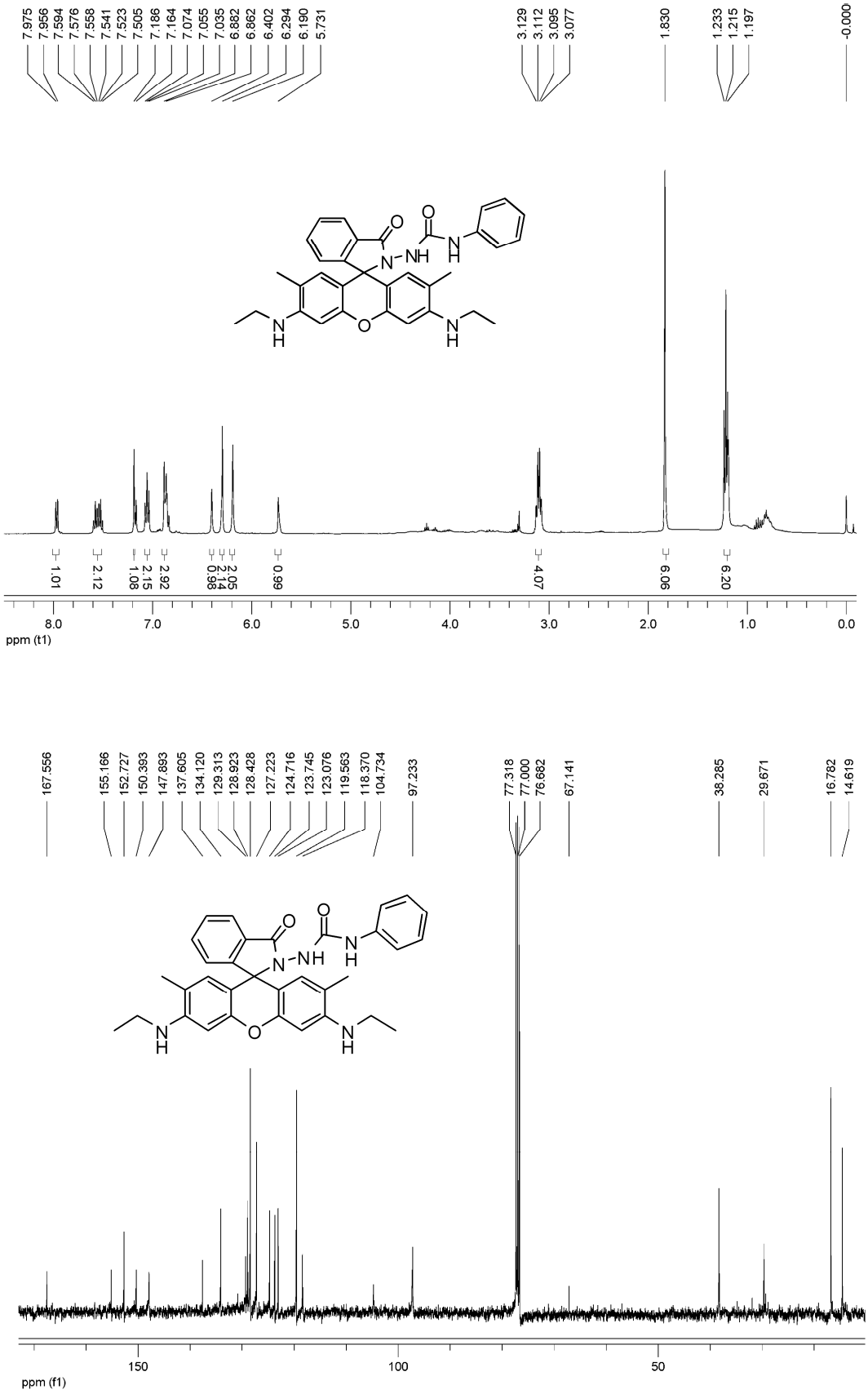


Figure S1. ^{1}H and ^{13}C NMR spectra of compound **1a** in $CDCl_3$.

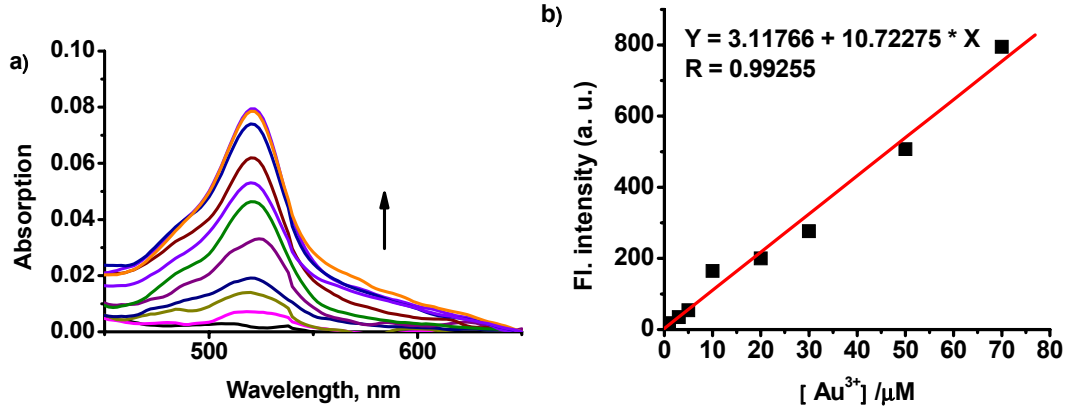


Figure S2. a) Absorption spectra of probe **1a** (10 μM) in the presence of Au^{3+} (0-120 μM) in PBS (pH 7.4, containing 0.3% DMF as a cosolvent). b) The plot of fluorescence intensity at 549 nm vs. Au^{3+} concentration (0.5, 1, 3, 5, 7, 10, 30, 50, and 70 μM). [**1a**] = 10 μM

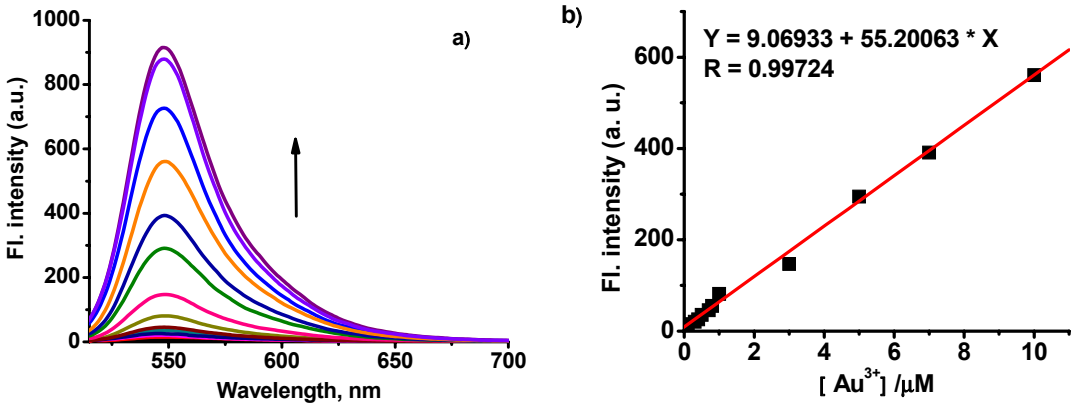


Figure S3. a) Fluorescence spectra of probe **1a** (10 μM) in the presence of Au^{3+} (0-20 μM) in PBS/ MeOH solvent (pH 7.4, 9: 1, v/v) with excitation at 500 nm. b) Fluorescence intensity at 549 nm vs. Au^{3+} concentration. [**1a**] = 10 μM, Au^{3+} = 0.1, 0.3, 0.4, 0.5, 0.7, 0.8, 1, 3, 5, 7, and 10 μM, respectively.

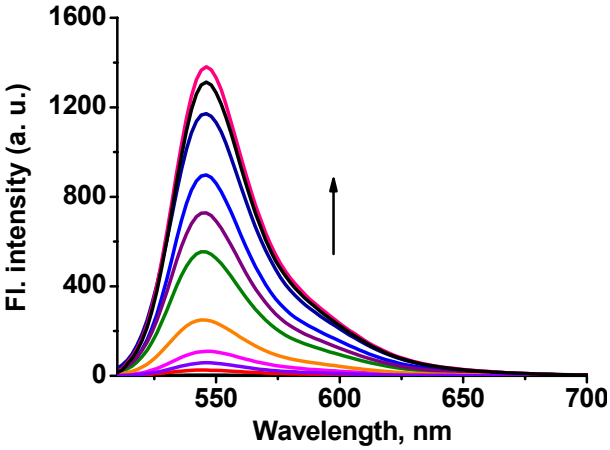


Figure S4. Fluorescence spectra of probe **1a** (10 μM) in the presence of increasing concentrations of Au^{+} (0-120 μM) in PBS (pH 7.4, containing 0.3% DMF as a cosolvent) with excitation at 500 nm.

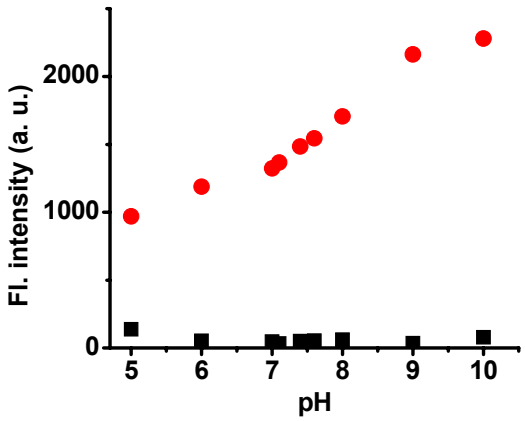


Figure S5. The fluorescence intensity of probe **1a** (10 μM) at various pH condition in the absence (■) or presence (●) of Au^{3+} (10 equiv.) in water (containing 0.3 % DMF as a cosolvent).

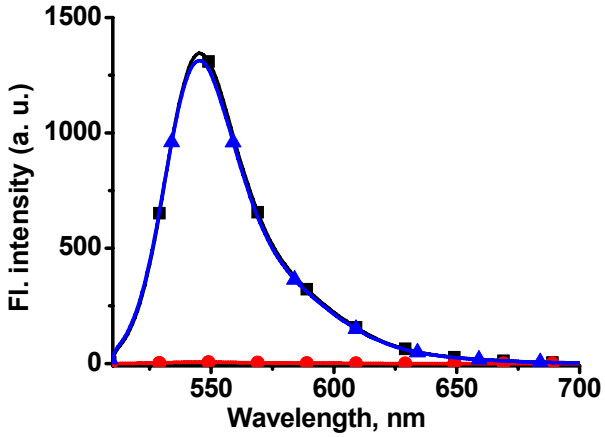


Figure S6. Emission spectra of probe **1a** (●), **1a** + 10 equiv. Au^{3+} (■), and **1a** + 10 equiv. Au^{3+} + 100 equiv. tetrabutylammonium cyanide (▲). Excitation at 500 nm.

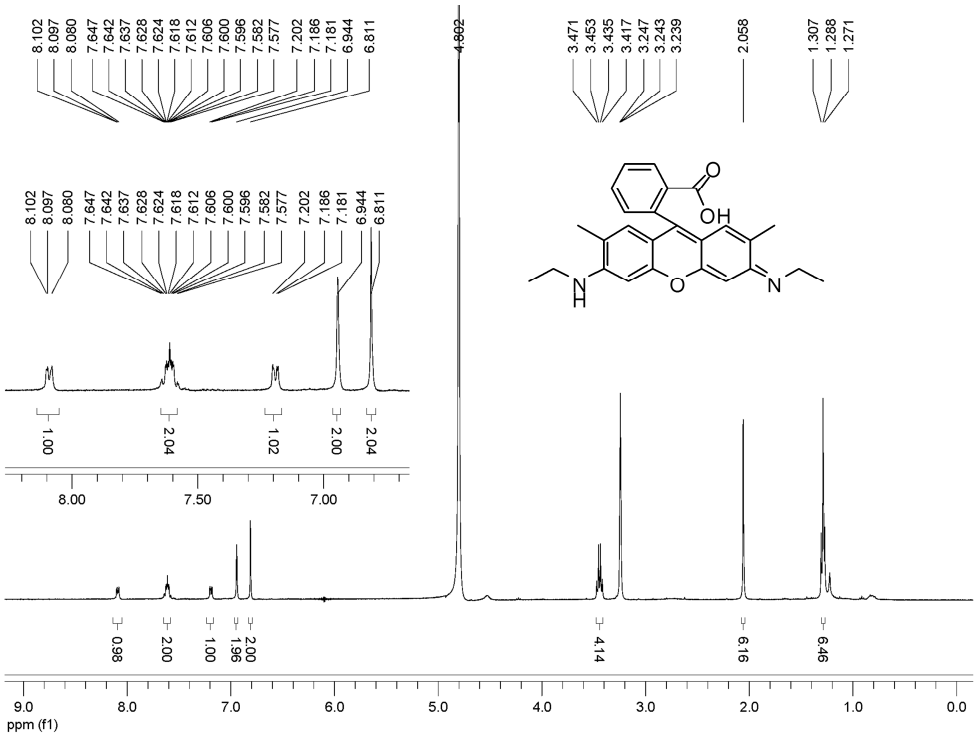


Figure S7. ^{1}H NMR spectrum of compound **3a** in CD_3OD.

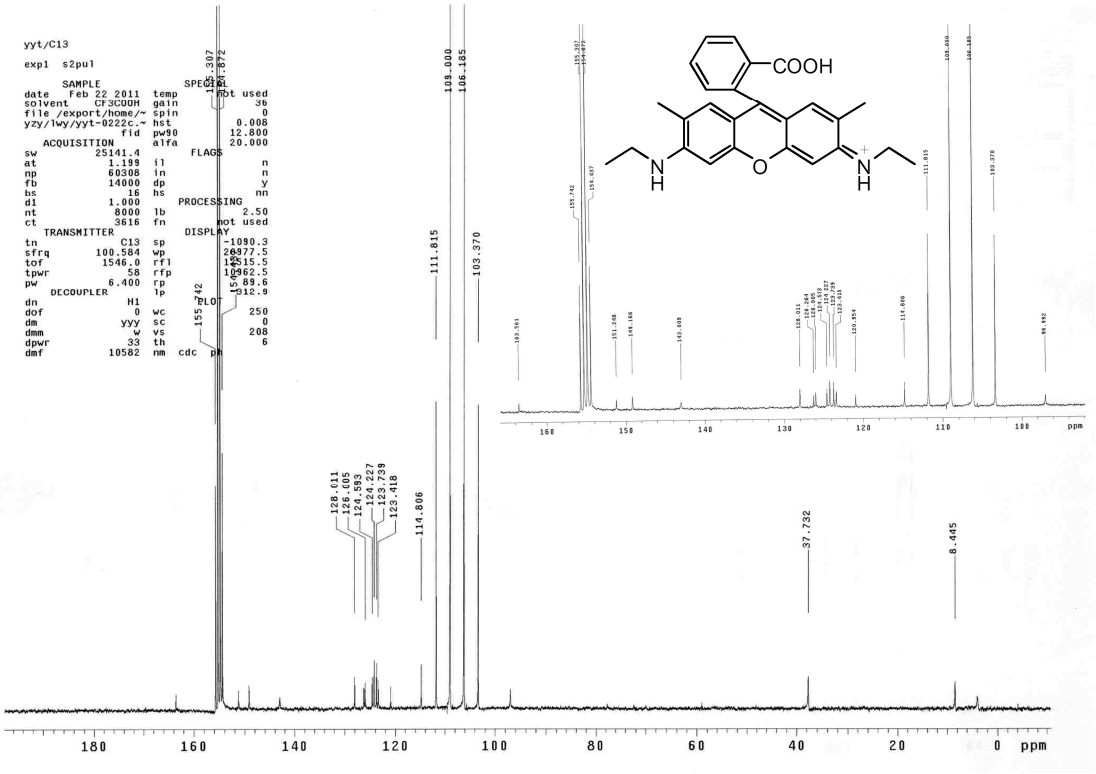


Figure S8. ^{13}C NMR spectrum of compound **3a** in CF_3COOD.

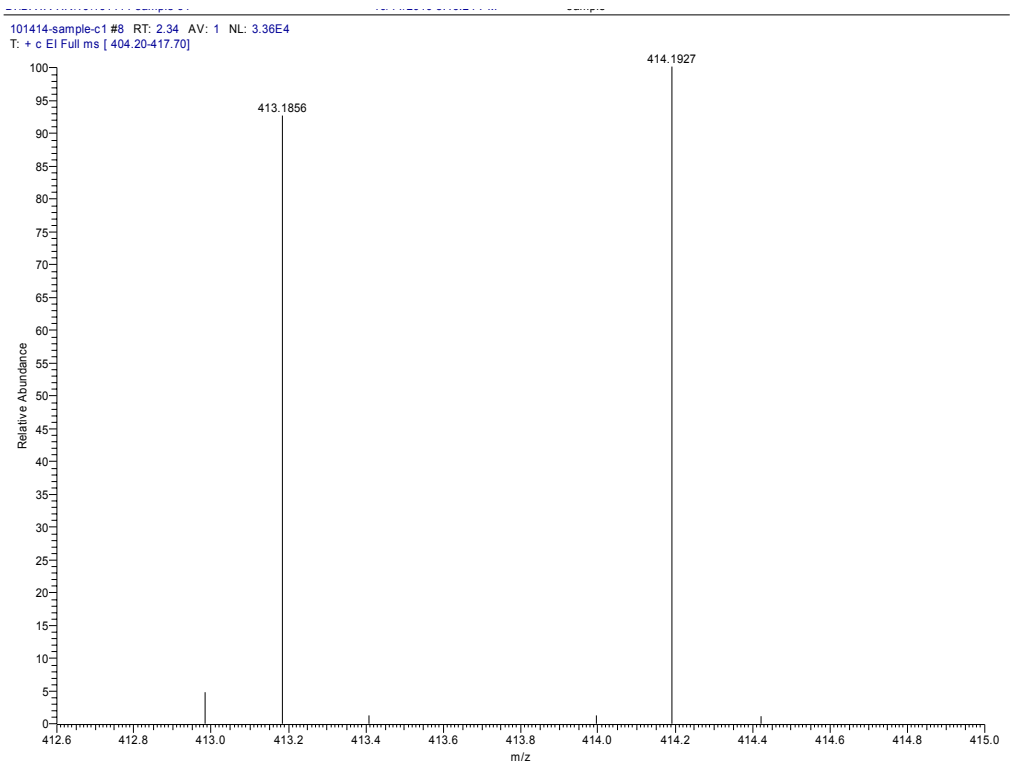


Figure S9. HRMS (EI) spectrum of compound **3a**. HRMS (EI) *m/z* calcd for $C_{26}H_{26}O_3N_2$ (M): 414.1938. Found 414.1927; HRMS (EI) *m/z* calcd for $C_{26}H_{25}O_3N_2$ (M - H): 413.1860. Found 413.1856.

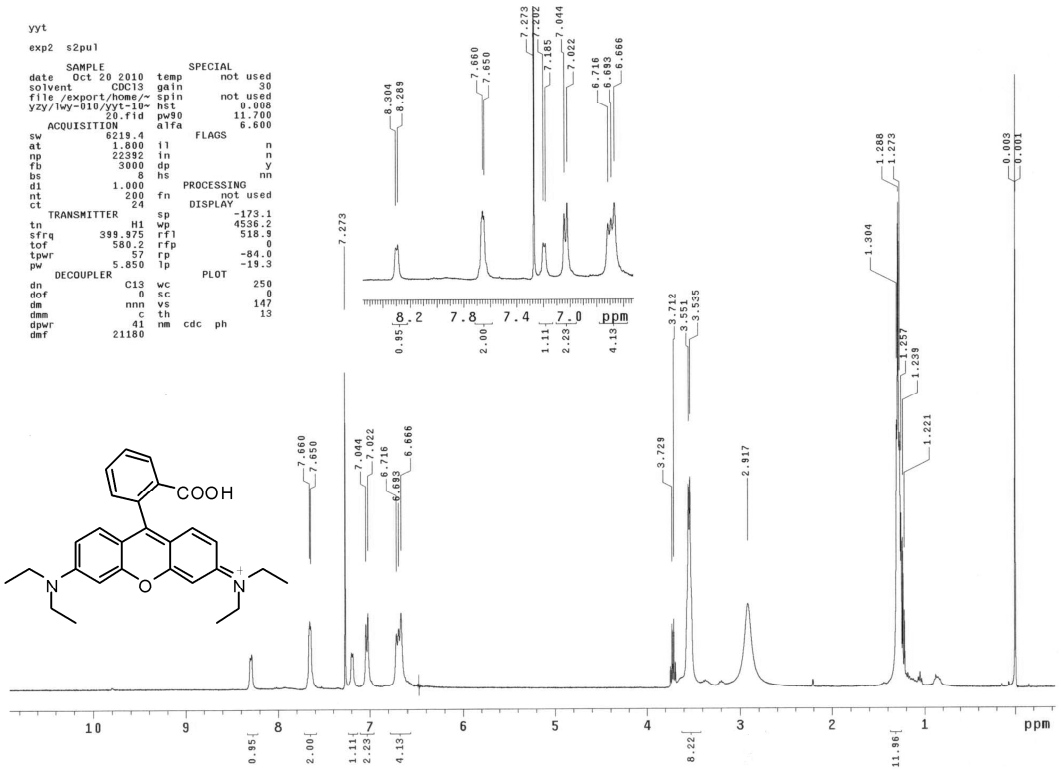


Figure S10. ^{1}H NMR spectrum of compound **3b** in $CDCl_3$.

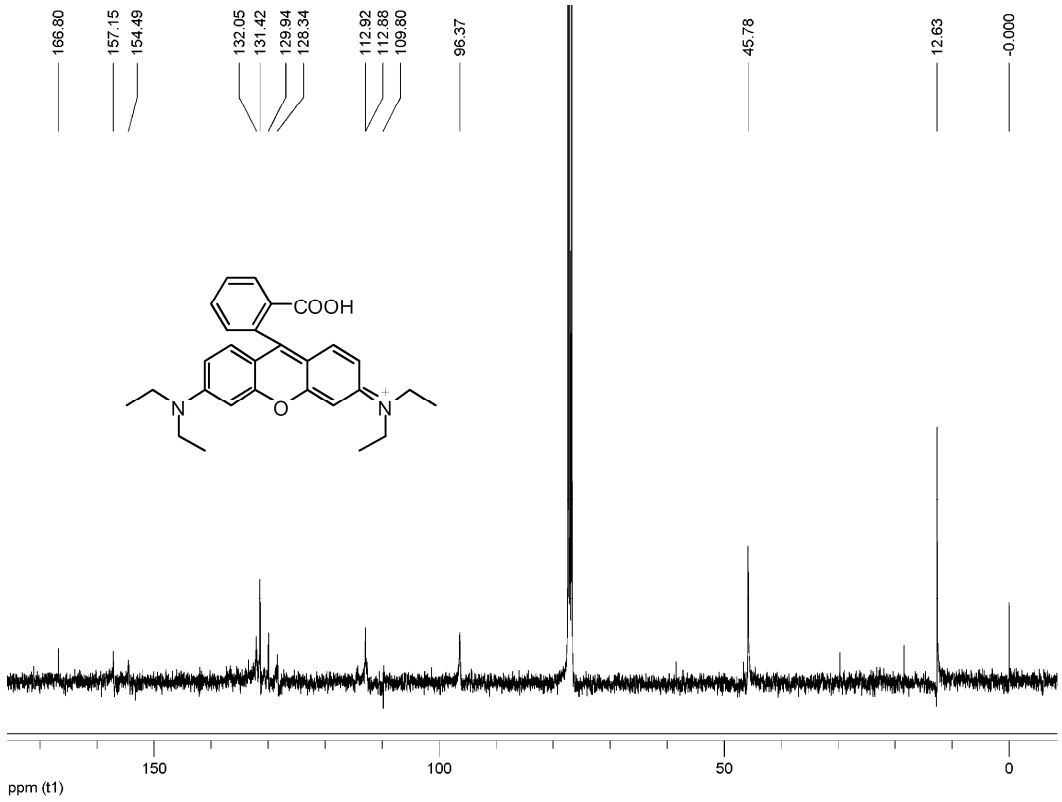


Figure S11. ^{13}C NMR spectrum of compound **3b** in $CDCl_3$.

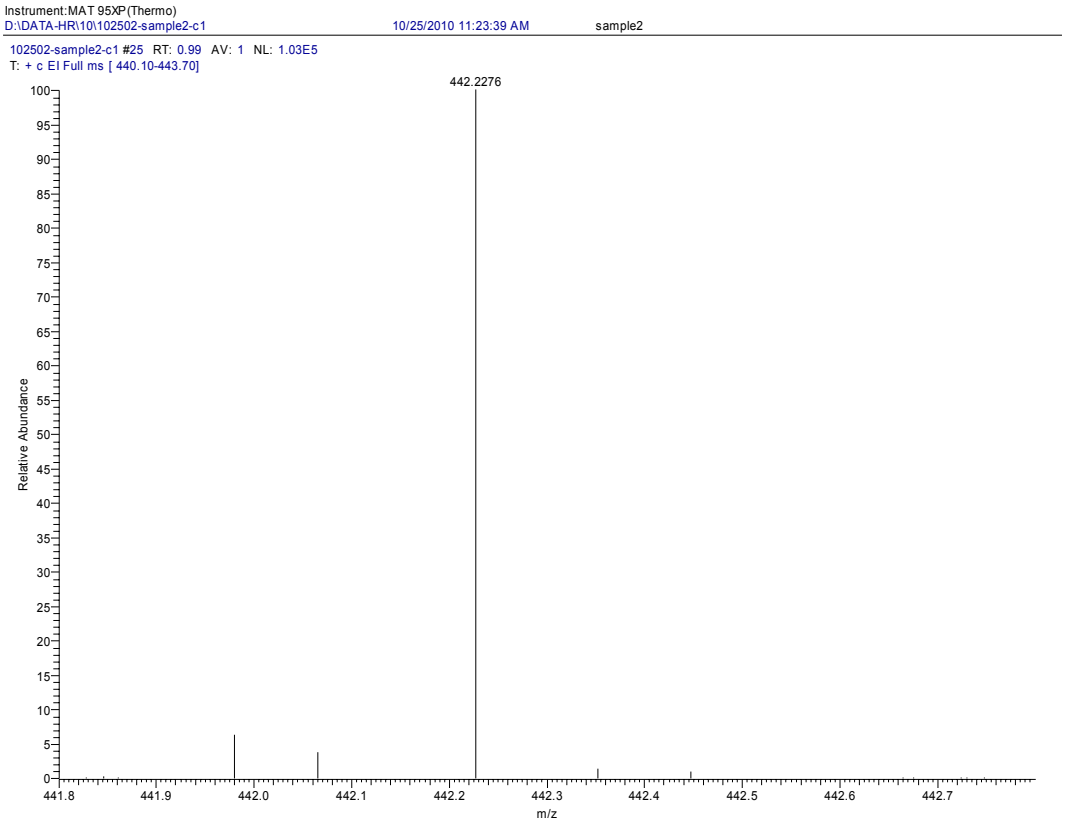
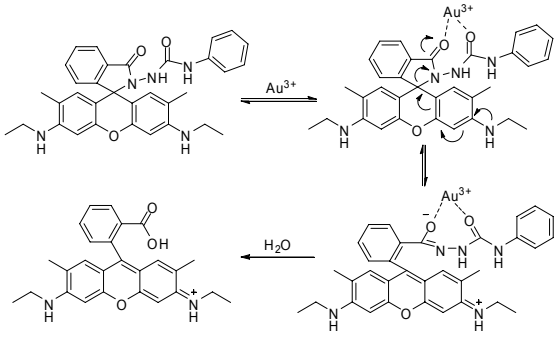


Figure S12. HRMS (EI) spectrum of compound **3b**. HRMS (EI) *m/z* calcd for $C_{28}H_{30}O_3N_2$ (M - H): 442.2251. Found 442.2276.



Scheme S1. A proposed reaction mechanism for Au^{3+}-mediated hydrolysis of acylsemicarbazides to carboxylic acids.

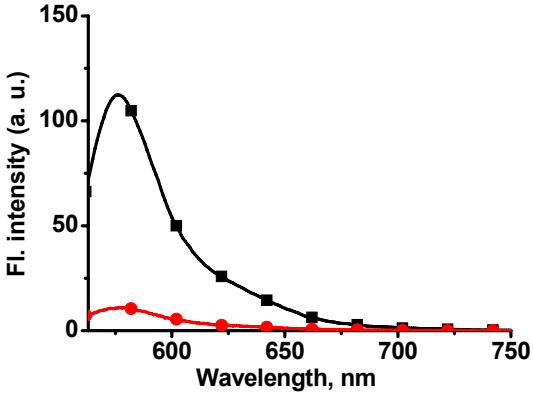


Figure S13. Fluorescence spectra of compound **1b** in the absence (●) or presence (■) of Au^{3+} (10 equiv.) in PBS (pH 7.4, containing 0.3% DMF as a cosolvent) with excitation at 550 nm.

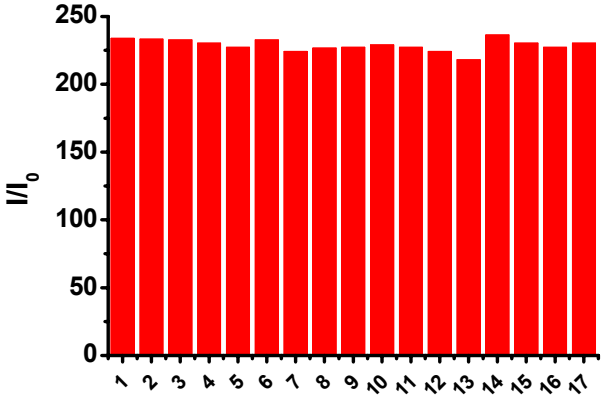


Figure S14. Fluorescence intensity changes (I/I_0) of probe **1a** (10 μM) in response to Au^{3+} (10 equiv.) in the presence of various metal species (10 equiv.) in PBS (pH 7.4, containing 0.3 % DMF as a cosolvent). 1. Au^{3+}; 2. Au^{3+} + Ag^{+}; 3. Au^{3+} + Ca^{2+}; 4. Au^{3+} + Cd^{2+}; 5. Au^{3+} + Co^{2+}; 6. Au^{3+} + Cr^{3+}; 7. Au^{3+} + Cu^{2+}; 8. Au^{3+} + Zn^{2+}; 9. Au^{3+} + Fe^{2+}; 10. Au^{3+} + Fe^{3+}; 11. Au^{3+} + Hg^{2+}; 12. Au^{3+} + Mg^{2+}; 13. Au^{3+} + Mn^{2+}; 14. Au^{3+} + Ni^{2+}; 15. Au^{3+} + Pb^{2+}; 16. Au^{3+} + Na^{+}, 17. Au^{3+} + K^{+}. Excitation at 500 nm; emission at 549 nm.

HeLa cell incubation and imaging using Probe 1a: 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 ^{o}C. The cells were plated on 6-well plates and allowed to adhere for 24 h. Immediately before the experiments, the cells were washed with PBS buffer, and then the cells were incubated with probe **1a** (5 μM) and Hoechst 33258 (4.5 μM) for 30 min at 37 ºC in PBS buffer (containing 0.3% DMF as a cosolvent), and then washed with PBS three times. After incubating with Au^{3+} (10 μM) for another 30 min at 37 ºC, the HeLa cells were rinsed with PBS three times, and the fluorescence images were acquired through a Nikon eclipase TE300 inverted fluorescence microscopy equipped with a cooled CCD camera (Figure 3 and Figure S14).

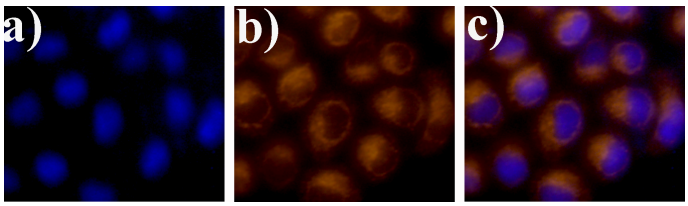


Figure S15. HeLa cells were co-incubated with probe **1a** (5 μM) and Hoechst 33258 (4.5 μM) for 30 min, washed with PBS for three times, then further incubated with Au^{3+} (10 μM) for 30 min. (a) emission from the blue channel (nuclear staining); (b) emission from the red channel; and (c) overlay of the blue and red channels.

Cytotoxicity assays: HeLa cells were grown in the modified Eagle's medium (MEM) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 ^{o}C. Immediately before the experiments, the cells were placed in a 96-well plate, followed by addition of increasing concentrations of probe **1a** (99% MEM and 1% DMSO). The final concentrations of the probe were kept from 5 to 200 μM (n = 3). The cells were then incubated at 37 $^{\circ}$C in an atmosphere of 5% CO_2 and 95% air at 37 ^{o}C for 24, 48, and 72h, respectively, followed by MTT assays. Untreated assay with MEM (n = 3) was also conducted under the same conditions.

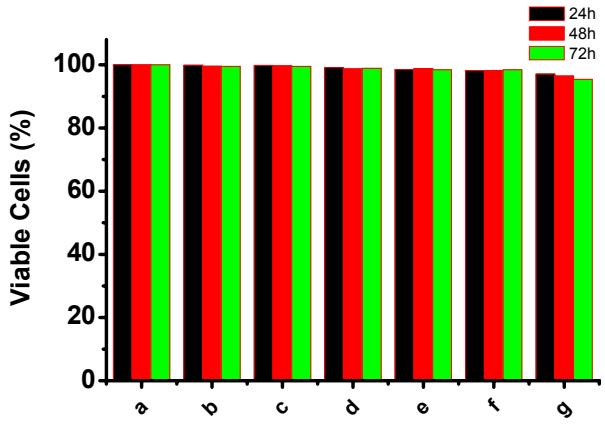
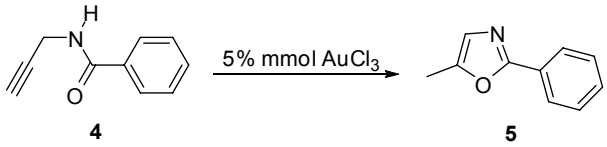


Figure S16. Cytotoxicity assay of probe **1a** at different concentration (a: 0 μM; b: 5 μM; c: 10 μM; d: 20 μM; e: 50 μM; f: 100 μM; g: 200 μM;) for HeLa cells.



Scheme S2. $AuCl_3$-catalyzed cycloisomerization of propargylic amides.

Quantitative detection of residual Au^{3+} content in compound 5 purified by silica gel chromatography

1. ***Detection of residual* Au^{3+} *content in compound 5 using probe 1a*.**

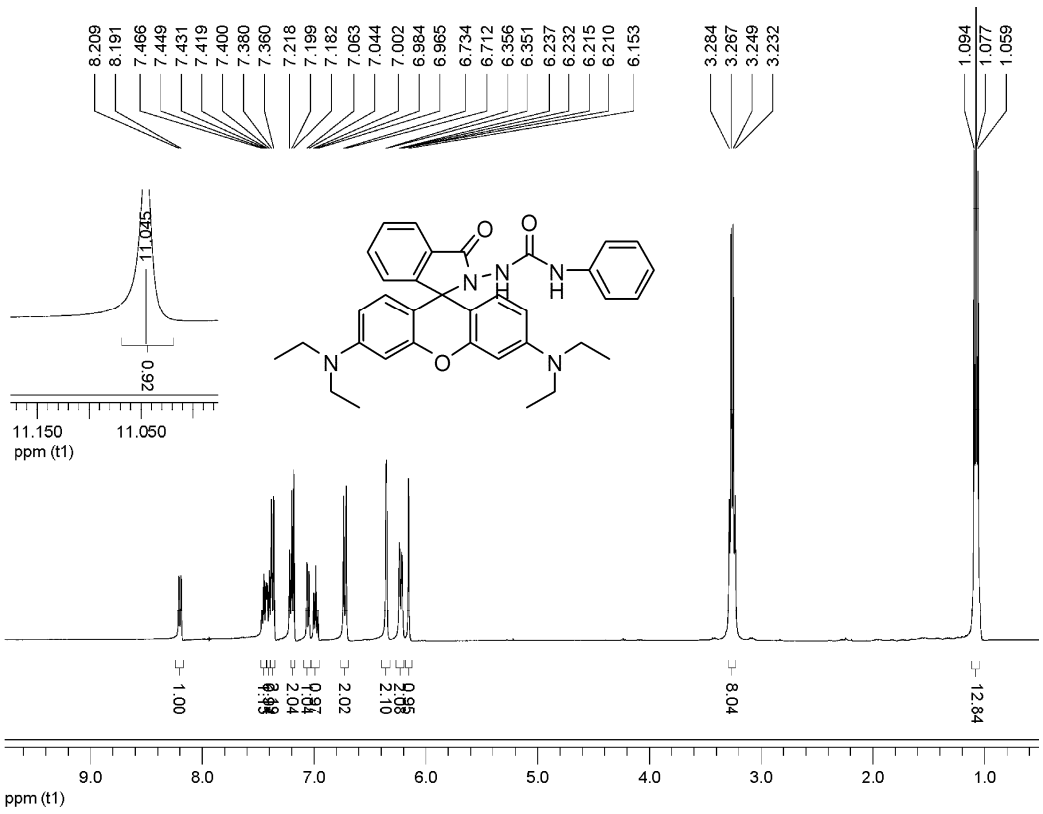
Crude oxazole **5** was prepared from propargylic amide **4** based on a reported Au^{3+}-catalyzed transformation (Scheme S2) (*Org. Lett.* **2004**, *6*, 4391-4394.). See page S6 in the Supporting Information for the synthetic detail. The crude compound **5** was loaded into a silica gel column and eluted with CH_2Cl_2: petroleum ether (2: 3, V/V). The final few fractions of compound **5** which contained Au^{3+} residue as demonstrated by the qualitative analysis in Figure 4B were combined and then the solvents were removed to afford the product in a liquid form.

A sample of compound **5** (2.5 mg) prepared as above described was accurately weighted and then dissolved in MeOH (5.0 mL) to give the sample test solution. The probe **1a** test solution (10 μM) was prepared by placing 0.01 mL probe **1a** stock solution (3 mM) and 2.7 mL PBS (25 mM, pH 7.4) into a test tube. Subsequently, 0.3 mL of the sample test solution was added to the probe **1a** test solution. The resulting solution was shaken and incubated at room temperature before recording the fluorescence

spectra. Every experiment was carried out in triplet. Based on the standard calibration curve as shown in Figure S3b and the measured data of fluorescence intensity, the content of the residual gold ions in compound **5** was determined as (3.21 ±0.25) × 10^{-7} mole/mg.

2. Detection of residual* Au^{3+} *content in compound 5 using inductively-coupled plasma optical emission spectroscope (ICP-OES).

A sample of compound **5** (14.5 mg) prepared as above described was accurately weighted into a beaker (note: the beaker was washed with a neutral detergent, HNO_3, and deionized water before use). The sample was then heated to near dryness. After cooling to room temperature, 1.0 mL regia aqua (HNO_3: HCl, v: v = 1: 3) was added and incubated for 10 min. The dissolved solution was transferred to 5.0 mL volumetric flask, diluted to total 5.0 mL with deionized water. The resulting solution was then subjected to ICP-OES analysis. A standard calibration curve was acquired with the known concentrations of Au^{3+} solutions. The measurement was conducted in triplet. The content of the residual gold ions in compound **5** was measured as (3.31 ±0.21) × 10^{-7} mole/mg.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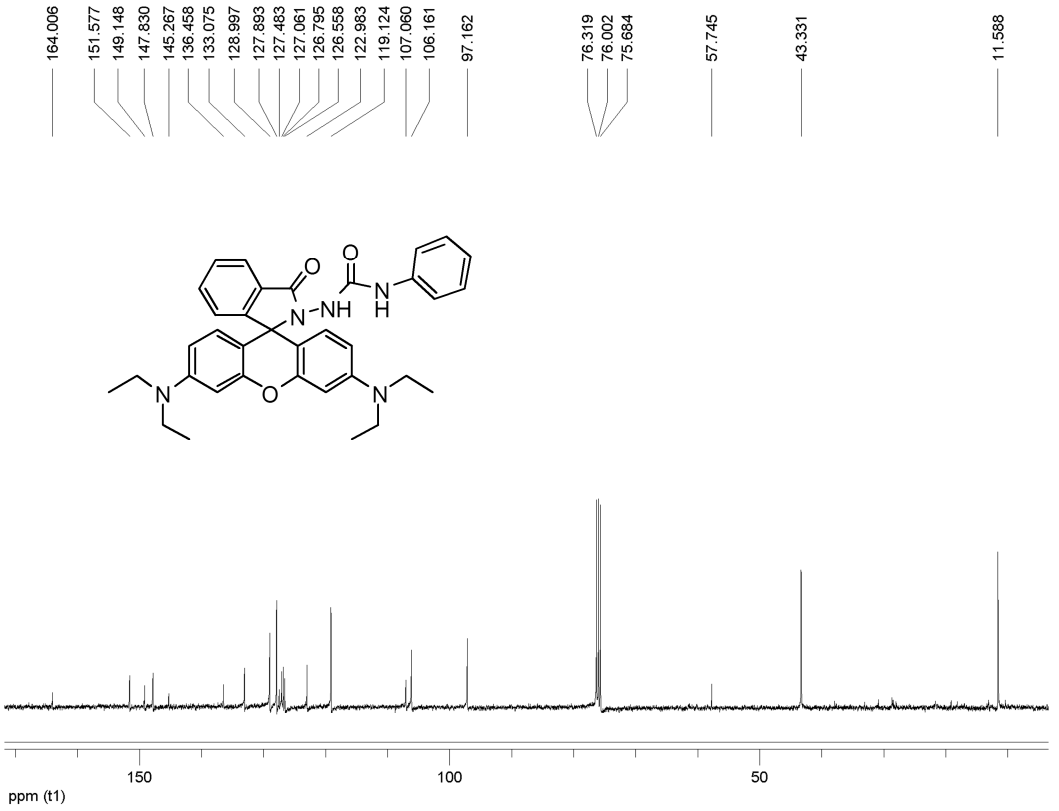
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