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Electronic Supporting Information
for

Rhodol-based fluorescent probe for Au^{3+} detection and its application in bioimaging

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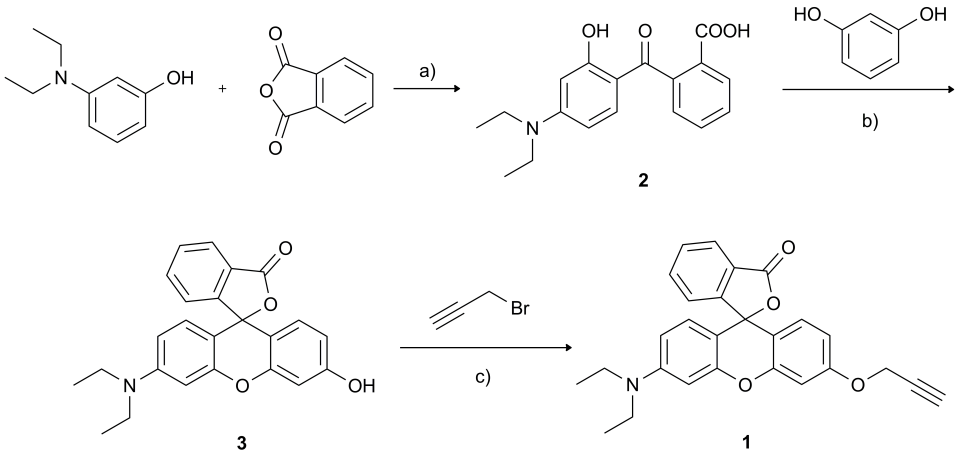
Materials and Instruments

All solvents and reagents of analytical grade were obtained commercially without further purification. Column chromatographic purification was performed on silica gel (70-230 mesh, Merck). The stock solutions of various metal ions such as metal chloride (Li^+, Na^+, K^+, Ni^{2+}), bromide (Cs^+), nitrate (Ag^+) and acetate (Ba^{2+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+}, Pd^{2+}, Pb^{2+}, Mg^{2+}, Zn^{2+}) were prepared in double–distilled water.

UV-vis absorption spectra were recorded on an Agilent 89090A spectrophotometer. Fluorescence emission measurements were performed on a Cary Eclipse fluorescence spectrophotometer with a 10-mm quartz cuvette. ^1H and ^{13}C NMR spectra were collected on a Bruker AV-400 (400 MHz) spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. HR-MS mass spectra were obtained in electrospray ionization (ESI) mode from a Bruker MicrOTOF mass spectrometer.

Synthesis



Scheme S1. Synthesis of probe **1**. Reagents and conditions: a) toluene, 130 °C, 20 h; b) resorcinol, TFA, 90 °C, 12 h; c) propargyl bromide, K_2CO_3, DMF, reflux, overnight.

Preparation of 2-(4-(*N*,*N*-diethylamino)-2-hydroxybenzoyl) benzoic acid (2)

Compound **2** was synthesized according to a previously reported procedure.[1] A suspension of 3-diethylaminophenol (1.46 g, 10.6 mmol) and *o*-phthalic anhydride (1.57 g, 10.6 mmol) in toluene was refluxed for 20 h. The reaction solvent was removed under vacuo and the crude product was resolved in CH_2Cl_2. The organic phase was washed with 1 N HCl and then dried over anhydrous Na_2SO_4. After the filtration and removal of solvent, the crude product was purified by silica gel column chromatography to give **2** as a white solid (2.08 g, 55% yield).

Preparation of rhodol (3)

Rhodol was prepared in excellent yield according to a reported literature procedure.[1] To a suspended solution of **2** (9.39 g, 0.03 mol) in trifluoroacetic acid (TFA, 25 mL) was added with resorcinol (3.40 g, 0.03 mol) and then the reaction mixture was stirred and refluxed for 24 h. After that the reaction mixture was cooled to room temperature and then evaporated under vacuo to provide the precipitated product. The crude product was further purified by recrystallization from EtOAc to obtain **3** as a white solid (11.92 g, 0.03 mol, 99 %yield).

Preparation of probe 1:

Rhodol (193.7 mg, 0.5 mmol) and potassium carbonate (K_2CO_3, 103.2 mg, 0.75 mmol, 1.5 equiv.) were dissolved in DMF. A reaction mixture was added with propargyl bromide (65 μL, 0.75 mmol, 1.5 equiv.) and the reaction mixture was refluxed overnight. After cooling to room temperature, the reaction mixture was evaporated under vacuo. The crude product was resolved in CH_2Cl_2 and then extracted with saturated sodium bicarbonate and

brine solution (2 times). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuo. The crude product was further purified by silica gel column chromatography to provide **1** as an orange solid (170 mg, 0.4 mmol, 79.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.00 (d, *J* = 7.5 Hz, 1H), 7.56-7.68 (m, 2H), 7.19 (d, *J* = 7.5 Hz, 1H), 6.85 (d, *J* = 7.5 Hz, 1H), 6.60-6.72 (m, 2H), 6.56 (dd, *J* = 2.4, 6.4 Hz, 1H), 6.45 (d, *J* = 3.0 Hz, 1H), 6.35 (dd, *J* = 2.3, 17.9 Hz, 1H), 4.70 (d, *J* = 2.4 Hz, 2H), 3.25 (q, *J* = 7.1 Hz, 4H), 2.55 (t, *J* = 2.3 Hz, 1H), 1.18 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.69, 159.97, 154.06, 153.85, 153.76, 150.50, 135.59, 130.02, 129.68, 128.08, 125.61, 124.83, 113.41, 112.21, 109.08, 105.80, 102.68, 98.18, 84.79, 78.48, 76.53, 56.34, 44.76, 12.60. HRMS (ESI) calcd for $C_{27}H_{23}NO_4$ $[M + H]^+$: 426.1700, found: 426.1702.

Product identification 1e

A suspension of probe **1** (10 mg, 0.0235 mmol) in 3 mL of CH_2Cl_2 was added $HAuCl_4$ (10.2 mg, 0.0259 mmol, 1.1 equiv.) at room temperature. After 12 h, the solvent was removed under vacuo and the residue product was dissolved in CH_2Cl_2, followed by filtrated through Celite. The crude product was purified by silica gel column chromatography to provide **1e** (3.6 mg, 36% yield). ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.86 (d, *J* = 7.1 Hz, 1H), 7.67 (td, *J* = 7.5, 1.1 Hz, 1H), 7.60 (td, *J* = 7.5, 0.8 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 6.65 (d, *J* = 2.1 Hz, 1H), 6.55-6.44 (m, 3H), 6.40 (d, *J* = 6.9 Hz, 2H), 3.34 (q, *J* = 7.1 Hz, 4H), 1.24-1.08 (m, 3H), 1.05 (t, *J* = 7.1 Hz, 6H).

Cell culture and fluorescence imaging

HeLa cells were grown in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS). The cells were plated in a 12-well plate containing the treated cover glass at a density of 5×10^5 cells per well in culture media. After 24 h, the cells were washed with Dulbecco's

phosphate-buffered saline (DPBS) buffer (pH 7.4) (1 mL×3 times) and fixed with (1:1 v/v) MeOH in DPBS buffer at -20 °C, followed by MeOH at -20 °C. Before the experiments, the cells were washed with DPBS buffer and incubated with 100 μM $HAuCl_4$ for 1 h. Subsequently, the cells were washed with DPBS buffer and incubated with 30 μM of probe **1** in DPBS buffer for 2 h. Finally, the cells were washed with DPBS buffer and mounted with a DAPI staining solution. The cell imaging was performed by a confocal laser-scanning microscope Olympus FV10i-DOC.

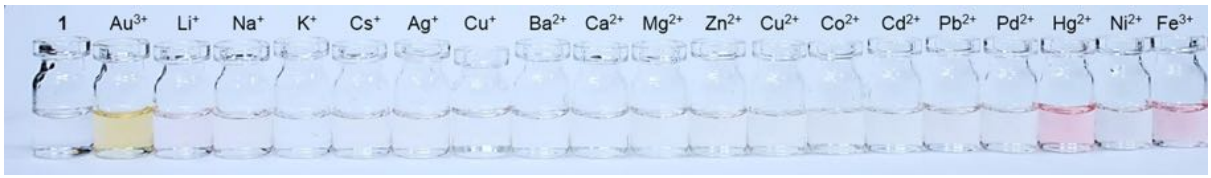


Figure S1. Color changes of probe **1** (20 μM) in the presence of Au^{3+} and other metal ions (10 equiv.) in 60% v/v DMSO/H_2O.

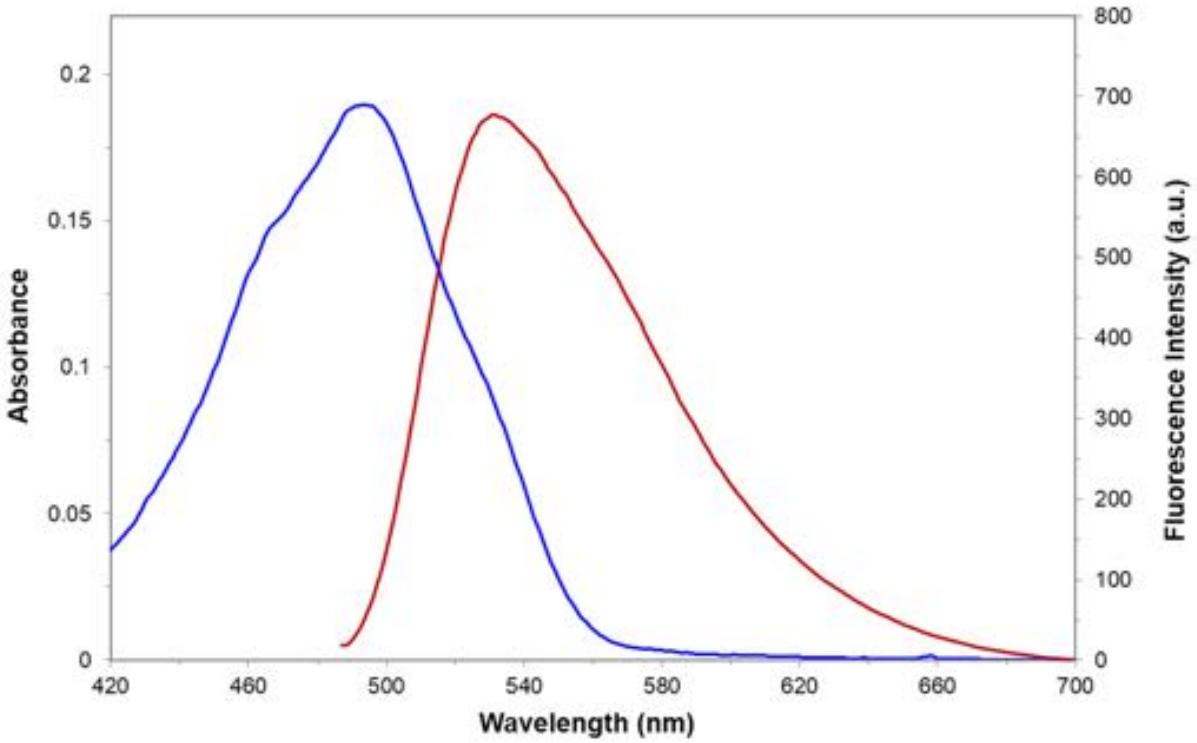


Figure S2. Absorbance (blue line) and fluorescence (red line) spectra of probe **1** (20 μM) in the presence of Au^{3+} (10 equiv.) in 60% v/v DMSO/H_2O.

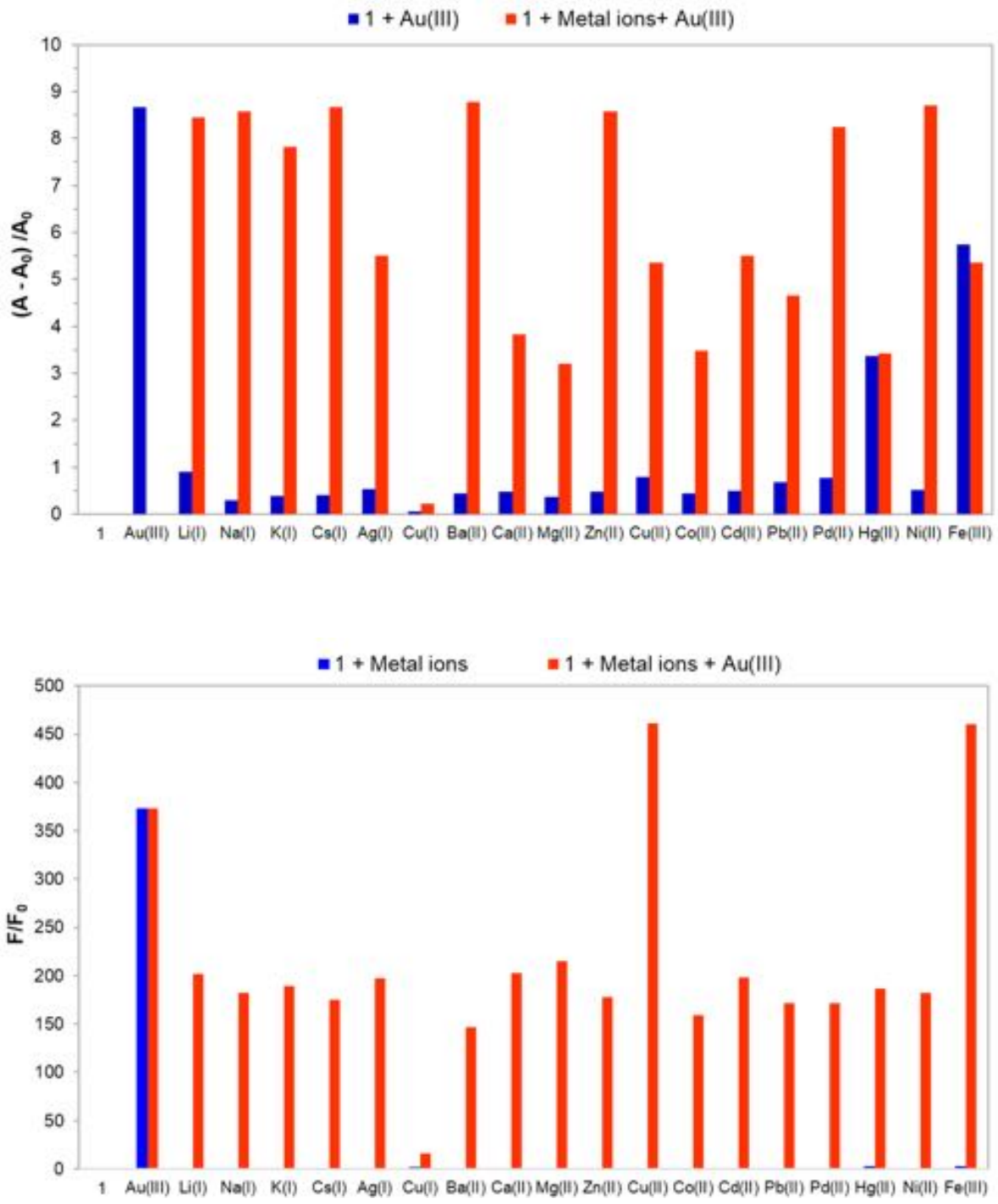


Figure S3. Absorbance and fluorescence responses of probe **1** in the presence of Au^{3+} and other metal ions (10 equiv.) in 60% v/v DMSO/H_2O. Excitation at 480 nm and maximum emission at 526 nm.

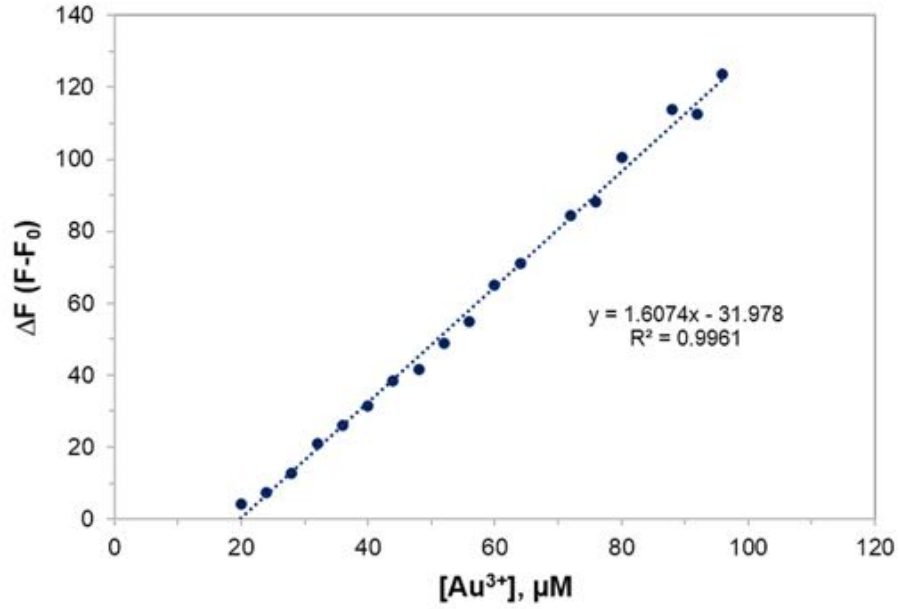


Figure S4. The calibration curve of probe **1** in the presence of Au^{3+} (20-96 μM) in 60% v/v DMSO/H_2O. Excitation at 480 nm and maximum emission at 526 nm. The detection limit (LOD) of Au^{3+} was calculated from the following equation: LOD = 3σ/slope; σ is the standard derivation of the blank solution; *slope* is the slope of the calibration curve.

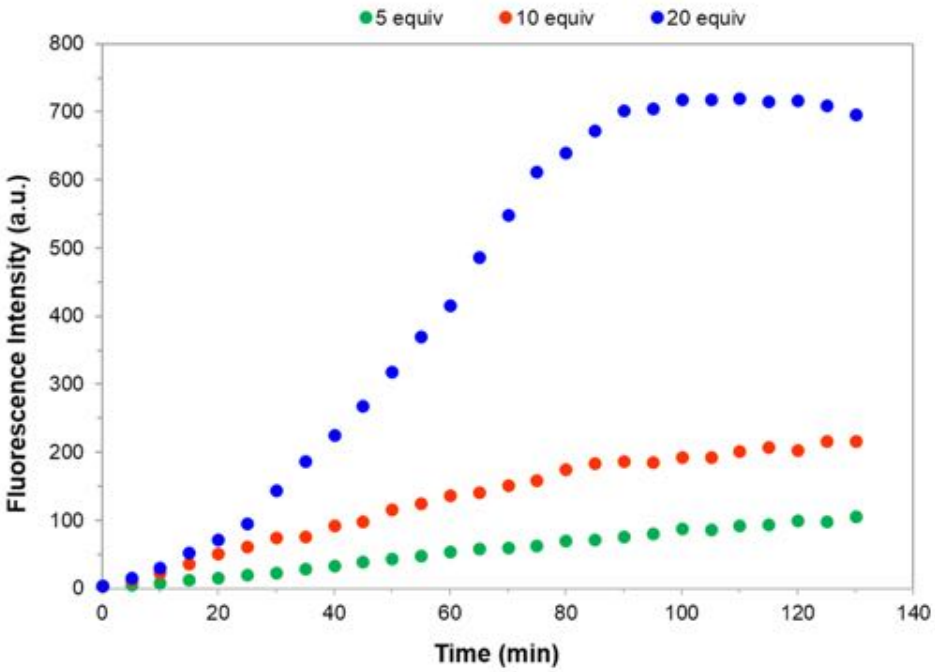


Figure S5. Time-dependent fluorescence intensity of probe **1** (20 μM) upon the addition of Au^{3+} (5, 10 and 20 equiv.) in 60% v/v DMSO/H_2O. Excitation at 480 nm and maximum emission at 526 nm.

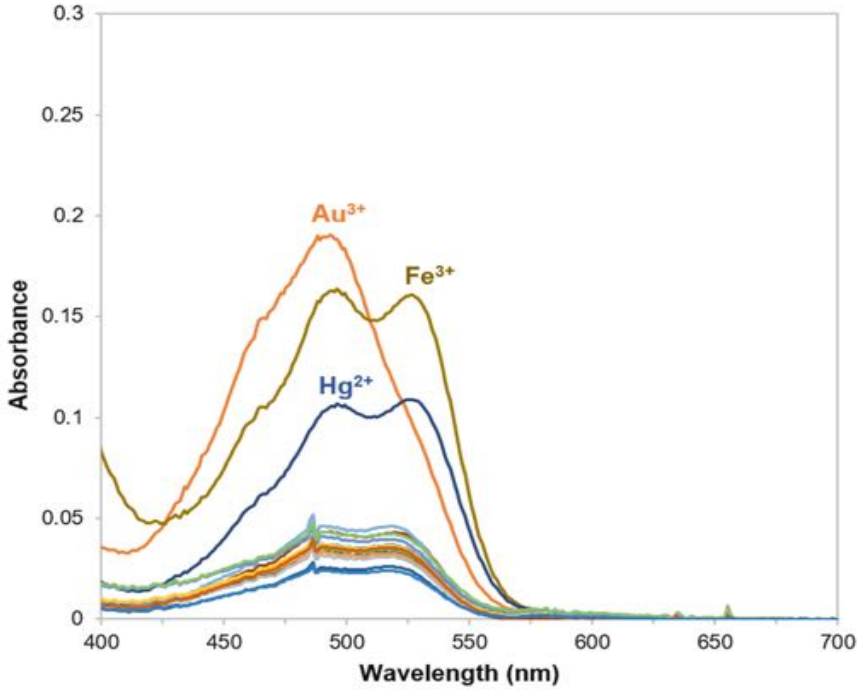


Figure S6. Absorbance spectra of probe **1** (20 μM) in the presence of Au^{3+} and other metal ions (10 equiv.) in 60% v/v DMSO/H_2O.

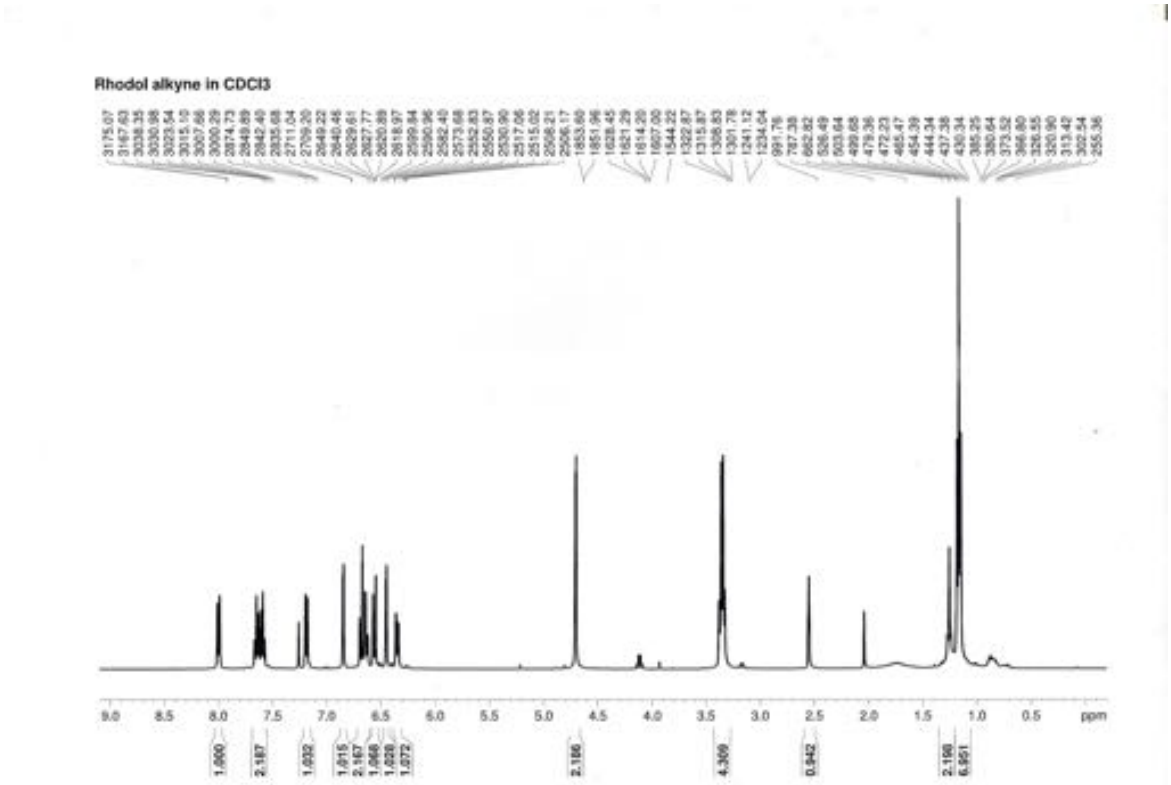


Figure S7. ^{1}H NMR spectra of probe **1** in $CDCl_3$.

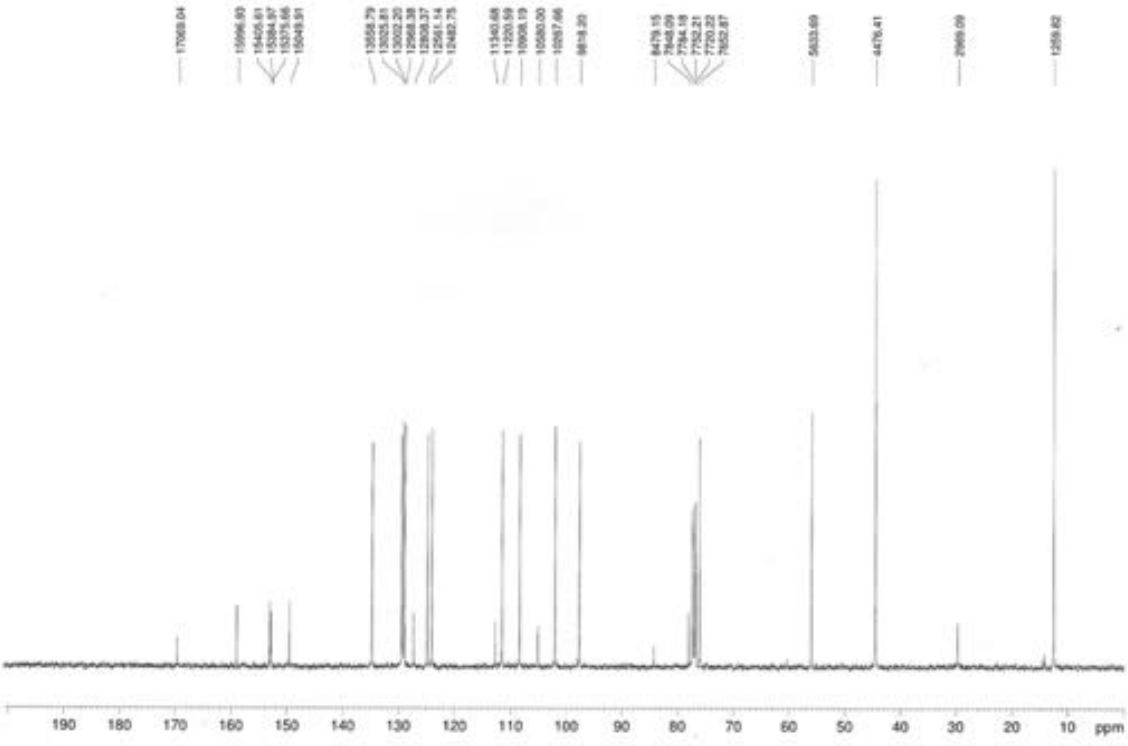


Figure S8. ^{13}C NMR spectra of probe **1** in $CDCl_3$.

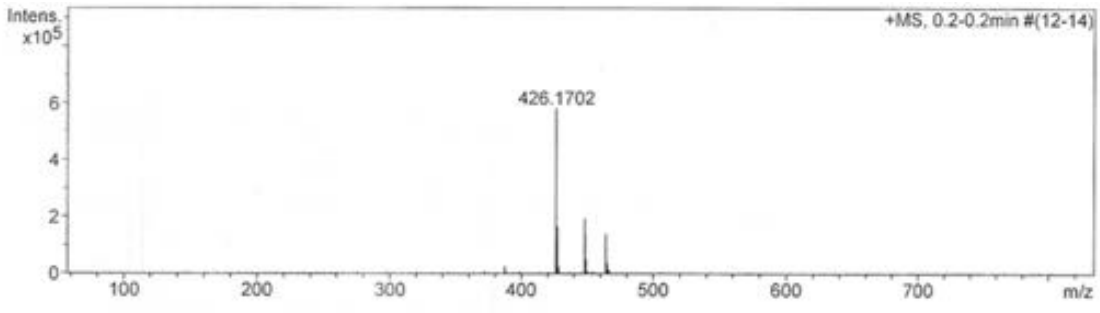


Figure S9. HR-ESI-MS of probe **1**.

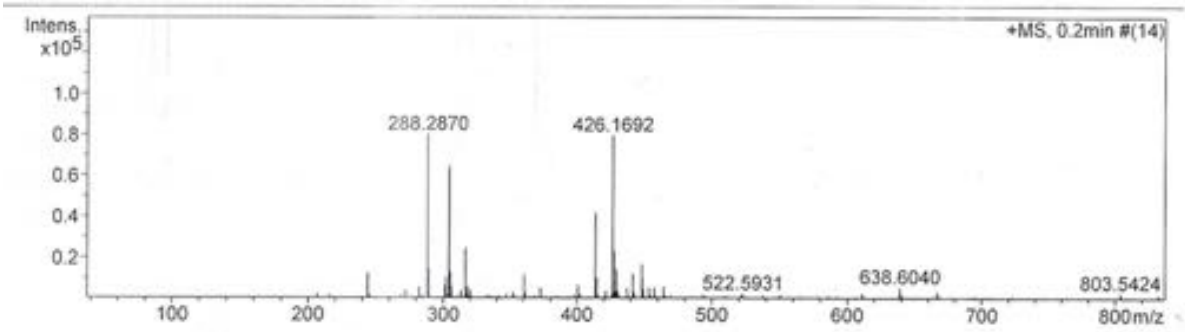


Figure S10. HR-ESI-MS of probe **1** upon the addition of Au^{3+} in (60% v/v) $MeCN/H_2O$.

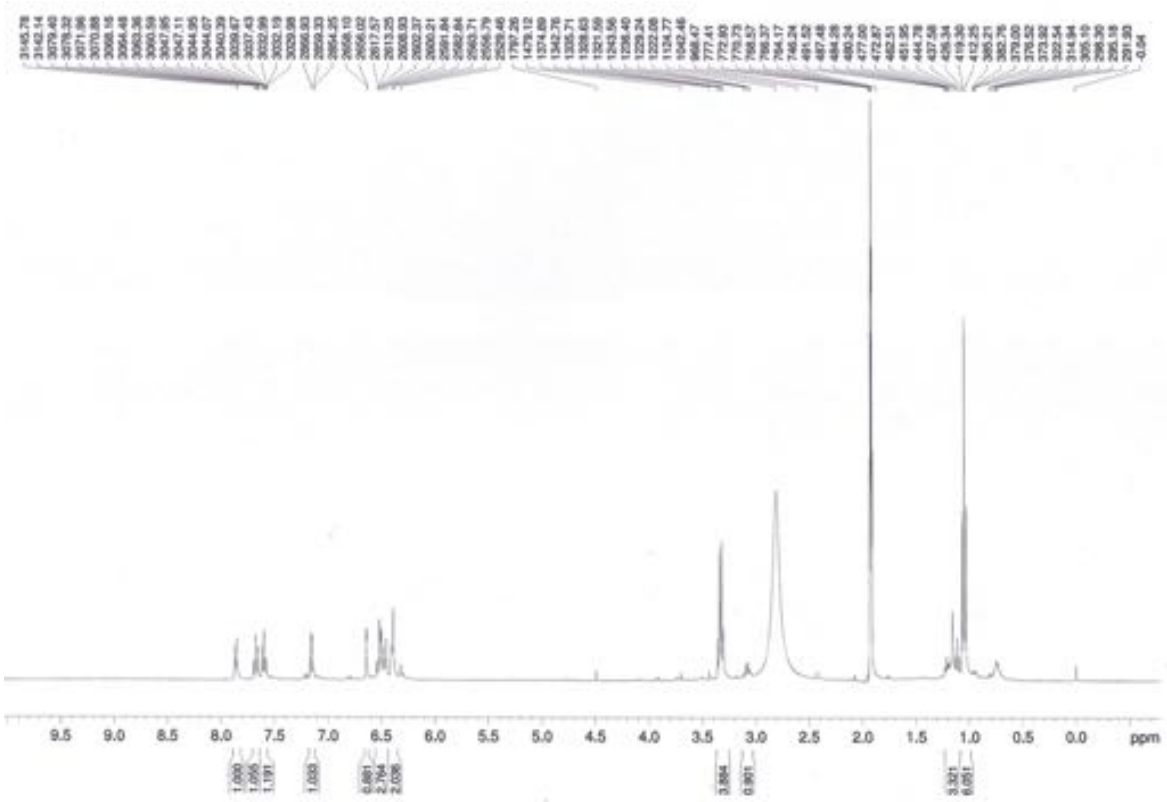


Figure S11. ^{1}H NMR spectra of the product **1e** in acetone-d_6.

References

1. H. G. Im, H. Y. Kim, S.-K. Chang, *Sens. Actuators B-Chem.* 2014, **191**, 854.