Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A Guanidinium modified Rhodamine-based fluorescent probe for in-vitro/vivo imaging of gold ions

Erman Karakuş[a], Gülçin Çakan Akdoğan[b] and Mustafa Emrullahoğlu,*[a]

[a]Department of Chemistry, Faculty of Science, İzmir Institute of Technology, Urla, 35430, İzmir, Turkey

[b]Dokuz Eylül University, İzmir Biomedicine and Genome Center, İzmir, Turkey

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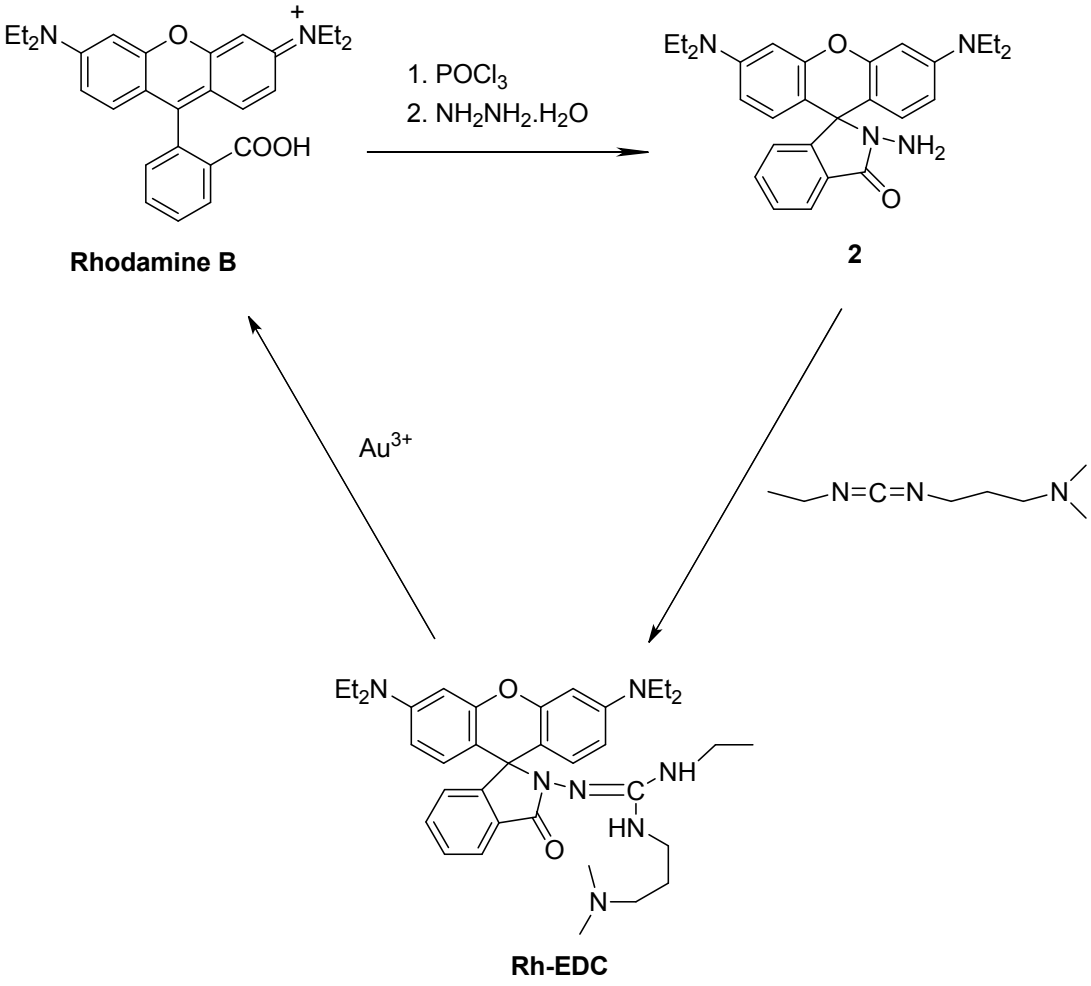
1. General methods: All reagents were purchased from commercial suppliers (Aldrich and Merck) and used without further purification. ^{1}H NMR and ^{13}C NMR were measured on a Varian VNMRJ 400 Nuclear Magnetic Resonance Spectrometer. UV absorption spectra were obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra were obtained using Varian Cary Eclipse Fluorescence spectrophotometer. HRMS data were acquired on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS. Cell imaging was performed with Olympus CKX41 fluorescence microscope. Samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 525 nm, the emission spectra were integrated over the range 535 nm to 700 nm (slit width: 5/5). pH was recorded by HI-8014 instrument (HANNA). All measurements were conducted at least in triplicate.

2. Synthesis Section



Scheme S1: Synthesis and hydrolysis of **Rh-EDC**

The rhodamine-B hydrazide **2** was prepared according to known procedure [1]

Hydrolysis of Rh-EDC with Au^{3+}

Rh-EDC (30 mg, 0.046 mmol) was dissolved in EtOH/Phosphate buffer (1:1 mL) and then $AuCl_3$ (13 mg, 0.046 mmol) was added. Subsequently, the reaction mixture was stirred at room temperature for 5 min. The reaction mixture was evaporated under reduced pressure, and it was filtered through celite and $MgSO_4$. Rhodamine B was obtained as 20 mg without applying further purification method. ^{1}H-NMR (400 MHz, D_2O) δ: 8.07-8.05 (m, 1H), 7.62 (t, J=3.6 Hz, 2H), 7.11-7.09 (m, 1H), 6.81 (d, J=9.6 hZ, 2H), 6.58 (d, J=9.6 Hz, 2H), 6.41 (s, 2H), 3.33-3.30 (m, 8H), 1.00 (t, J=7.2 Hz, 12H). ^{13}C NMR (100 MHz, D_2O) δ: 168.9, 159.0, 155.0, 132.8, 132.6, 131.0, 130.7, 130.4, 130.0, 113.9, 112.5, 95.8, 45.6, 11.9.

3. Kinetic Study

The reaction of **Rh-EDC** (10 μM) with $AuCl_3$ (1 equiv) in phosphate buffer was monitored by the fluorescence intensity at 580 nm at 25 °C. The pseudo-first-order rate constant for the reaction was determined by the following equation:

$$\ln [(Fmax - Ft)/Fmax] = -k' t$$

where Ft and Fmax are the fluorescence intensities at 580 nm at time t and the maximum value obtained after the reaction was complete, respectively, and k′ is the pseudo-first-order rate constant. The plot for the reaction between the **Rh-EDC** and $AuCl_3$ (1 equiv) is shown in Figure S3. The negative slope of the line represents the pseudo-first-order rate constant.

Reference

1. V. Dujolsi, F. Ford and A. W. Czarnik *J. Am. Chem. Soc.* 1997, **119**, 7387.

4. Absorption and emission spectra of Rh-EDC with Au^{3+}

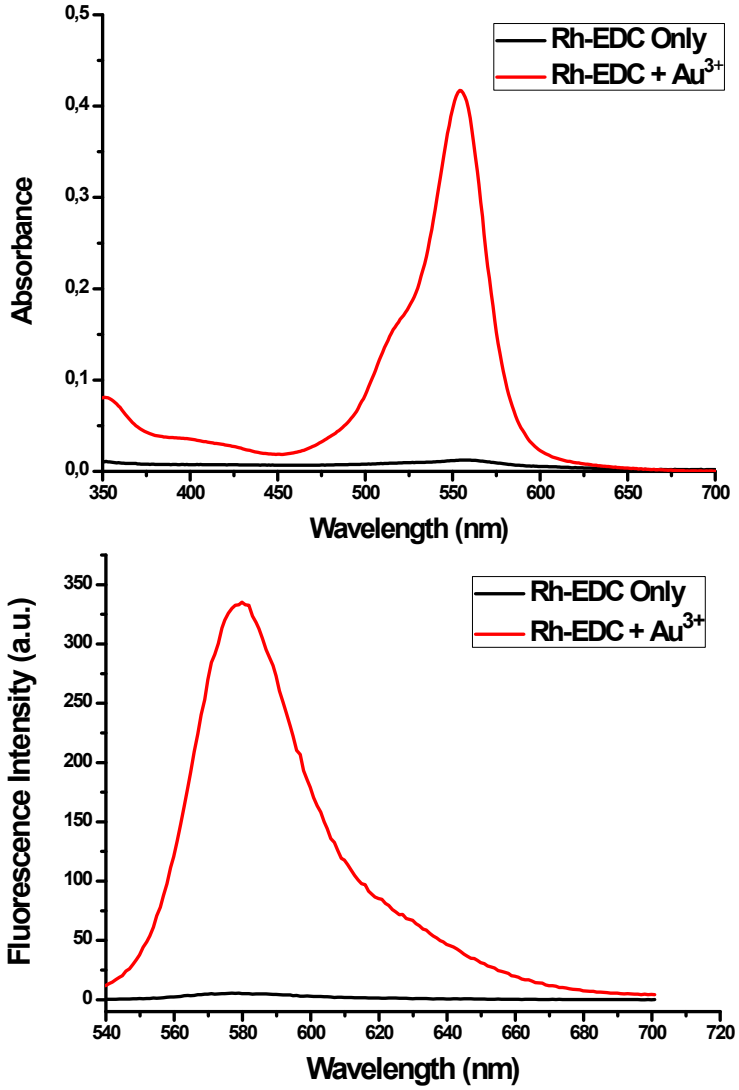


Figure S1 Absorption and Emission spectra of **Rh-EDC** (10 μM) and Au^{3+} (5.0 equiv.) in phosphate buffer at pH = 7.0; (λ_{ex}: 525 nm, at 25 °C).

5. Time-dependent fluorescence change of Rh-EDC with Au^{3+}

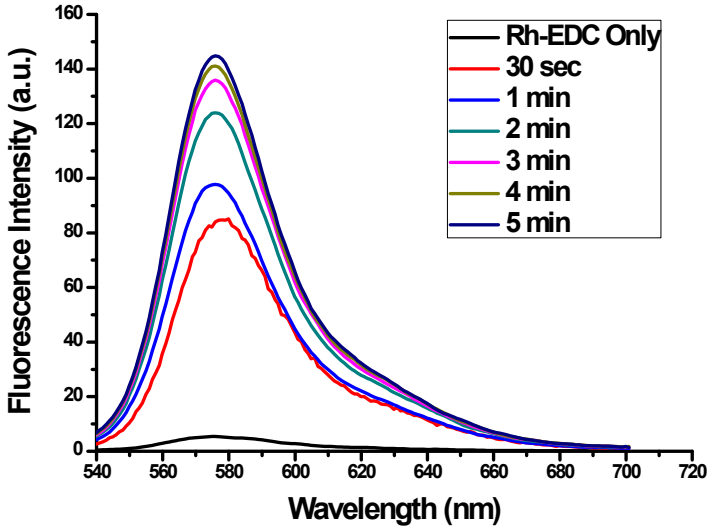


Figure S2 Time-dependent fluorescence change of **Rh-EDC** (10 μM) in the presence of an 1.0 equivalent of $AuCl_3$ measured in phosphate buffer at pH = 7.0

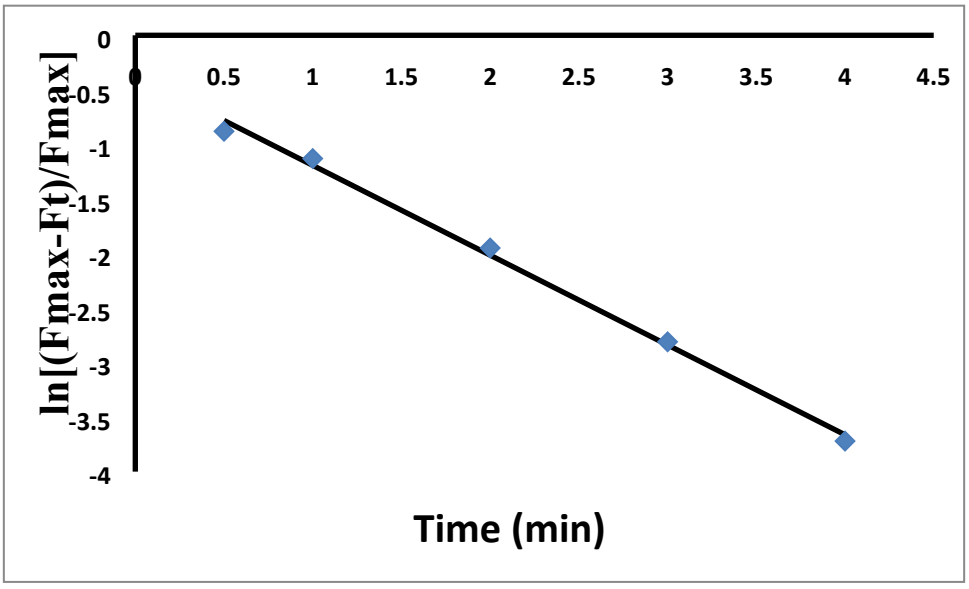


Figure S3 A Pseudo-first-order kinetic plot of the reaction between **Rh-EDC** (10 μM) in the presence of an 1.0 equivalent of $AuCl_3$ measured in phosphate buffer at pH = 7.0. k_{obs}= 1.37 x 10^{-2} sec^{-1}

6. Fluorescence titration of Rh-EDC with Au^{3+}

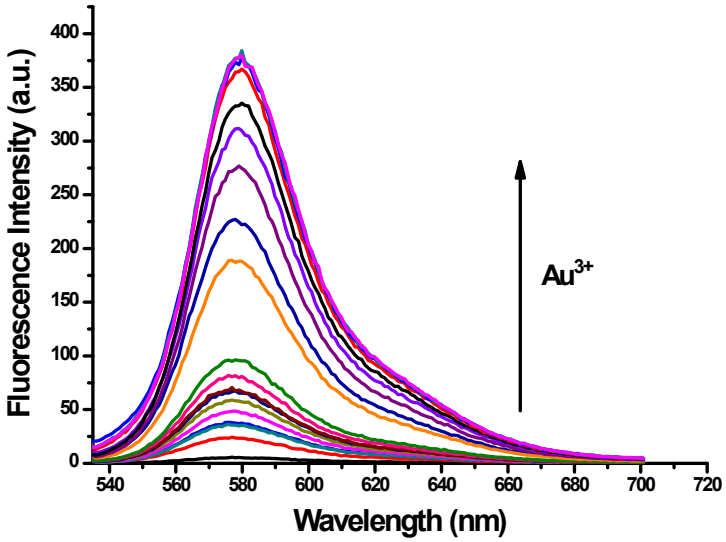


Figure S4 Fluorescence spectra of **Rh-EDC** (10 μM) in phosphate buffer at pH = 7.0 in the presence of Au^{3+} (mole equivalents = 0. 1 - 10.0)

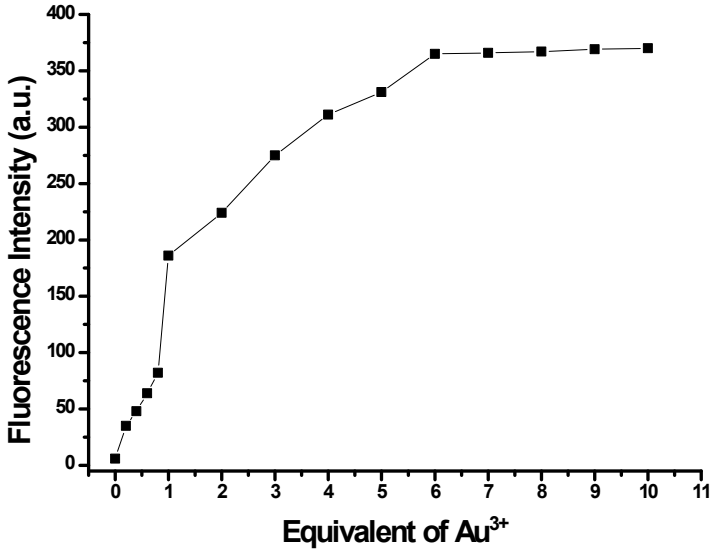


Figure S5 Fluorescence intensity changes of **Rh-EDC** (λ_{max}: 580 nm) vs equivalents of Au^{3+}

7. The fluorescence responses of Rh-EDC with Au^{3+} and other metals

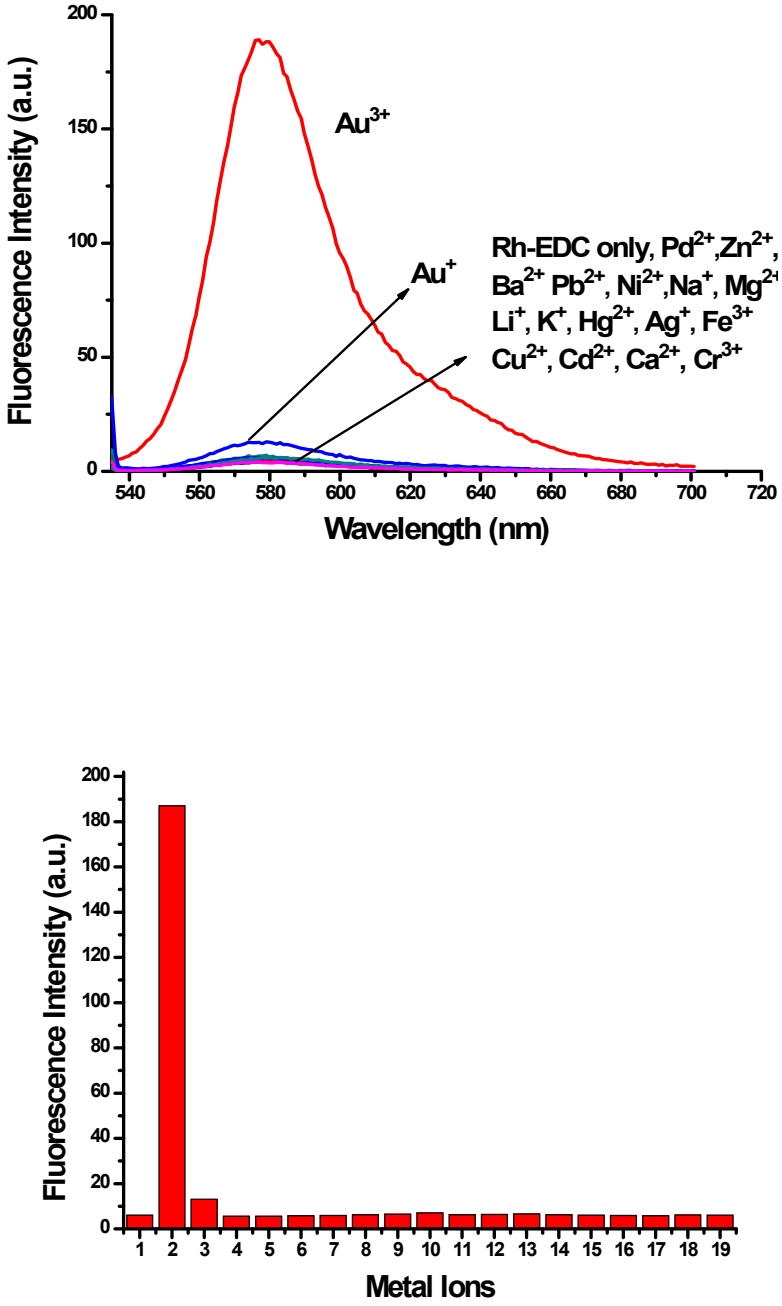


Figure S6 Fluorescence intensities of **Rh-EDC** (10 μM) in phosphate buffer at pH = 7.0 at λ_{max}: 580 nm in the presence of 10.0 equivalent of the cations interest: 1, **Rh-EDC** only; 2, Au^{3+} (1.0 equiv.); 3, Au^{+}; 4, Ag^{+}; 5, Ba^{2+}; 6, Ca^{2+}, 7, Cd^{2+}; 8, Cr^{3+}; 9, Cu^{2+}; 10, Fe^{3+}; 11, Hg^{2+}, 12, K^{+}; 13, Li^{+}; 14, Mg^{2+}; 15, Na^{+}; 16, Ni^{2+}; 17, Pb^{2+}; 18, Pd^{2+}; 19, Zn^{2+}

8. The fluorescence responses of Rh-EDC in the presence of Au^{3+} and other metal ions

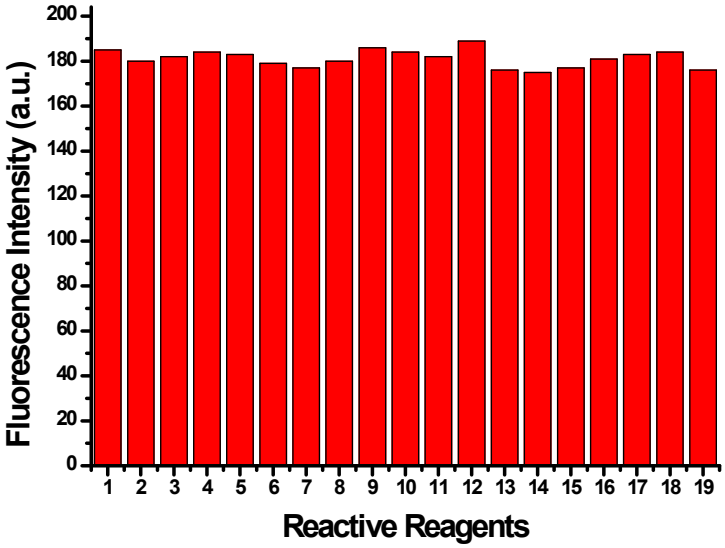


Figure S7 Fluorescence intensities of **Rh-EDC** (10 μM) in phosphate buffer at pH = 7.0 at λ_{max}: 580 nm in the presence Au^{3+} (1.0 equiv.) and 10.0 equivalent of the cations interest: 1, **Rh-EDC** only; 2, Au^{+}; 3, Ag^{+}; 4, Ba^{2+}; 5, Ca^{2+}, 6, Cd^{2+}; 7, Cr^{3+}; 8, Cu^{2+}; 9, Fe^{3+}; 10, Hg^{2+}, 11, K^{+}; 12, Li^{+}; 13, Mg^{2+}; 14, Na^{+}; 15, Ni^{2+}; 16, Pb^{2+}; 17, Pd^{2+}; 18, Zn^{2+}; 19, Cysteine.

9. Effect of pH

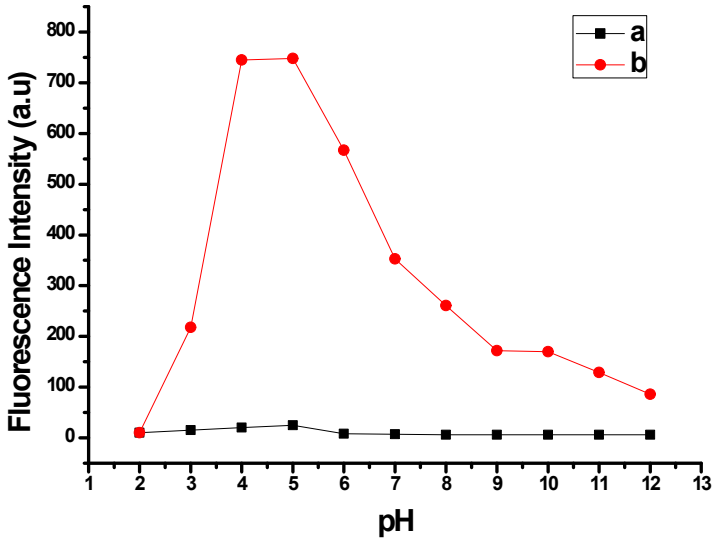


Figure S8 Effect of pH on the fluorescence intensity of **Rh-EDC** (10 μM) in phosphate buffer in the absence (a) and presence (b) of Au^{3+} (5.0 equiv.)

10. Determination of Detection Limit of Au^{3+}

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of **Rh-EDC** (10 µM) without Au^{3+} was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and Au^{3+} concentration could be obtained in the 0 – 0,3 µM (R = 0.996). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 2 nM at S/N = 3.

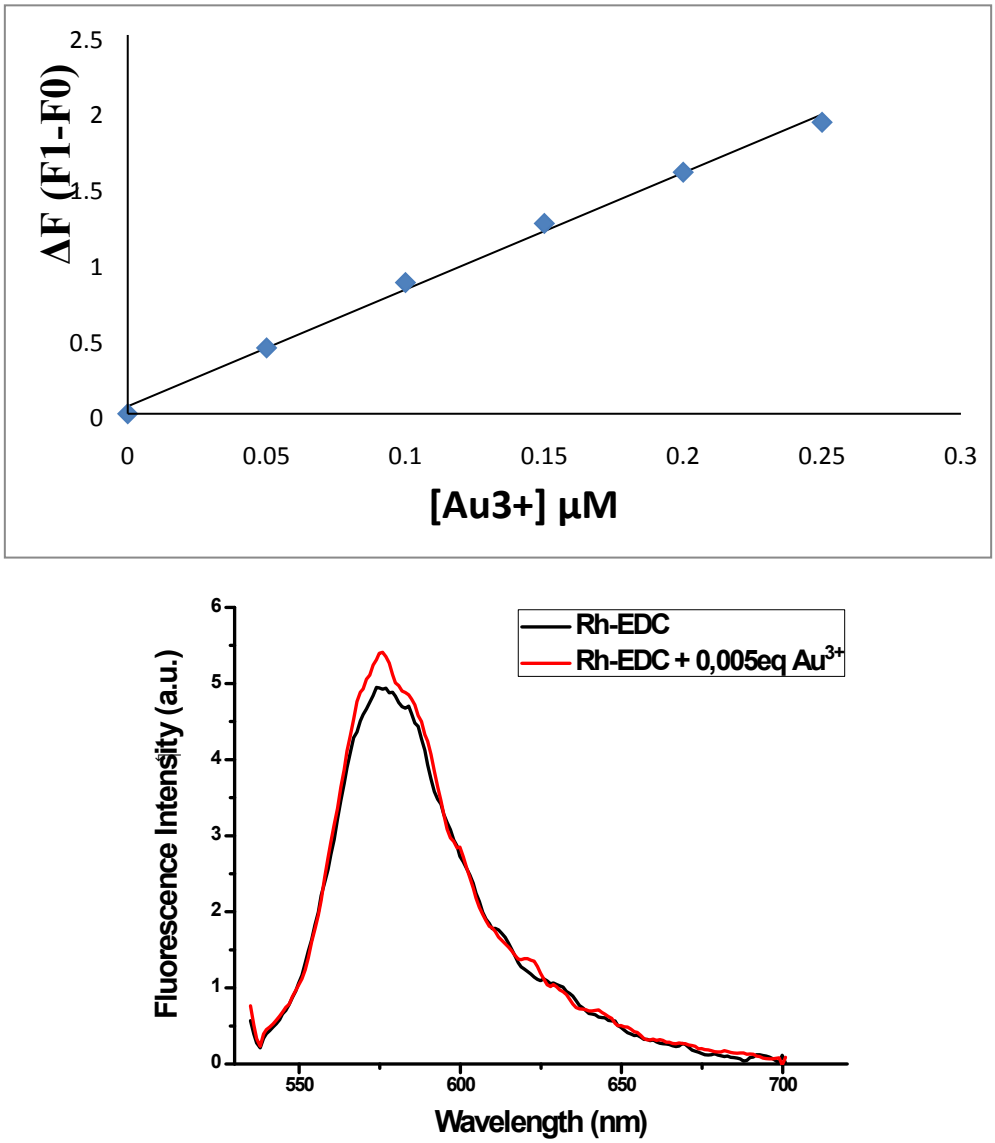
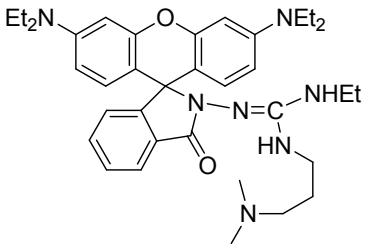
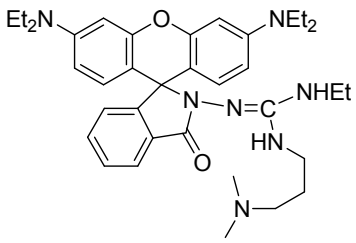


Figure S9 (a) Fluorescence changes of **Rh-EDC** (10 µM) upon addition of Au^{3+} (0.05 to 0.3 µM, 0.005 to 0.025 equiv.) **(b)** Fluorescence spectra of **Rh-EDC** (10 µM) in the presence of Au^{3+} (0.05 µM, 0.005 equiv.) in phosphate buffer at pH = 7.0

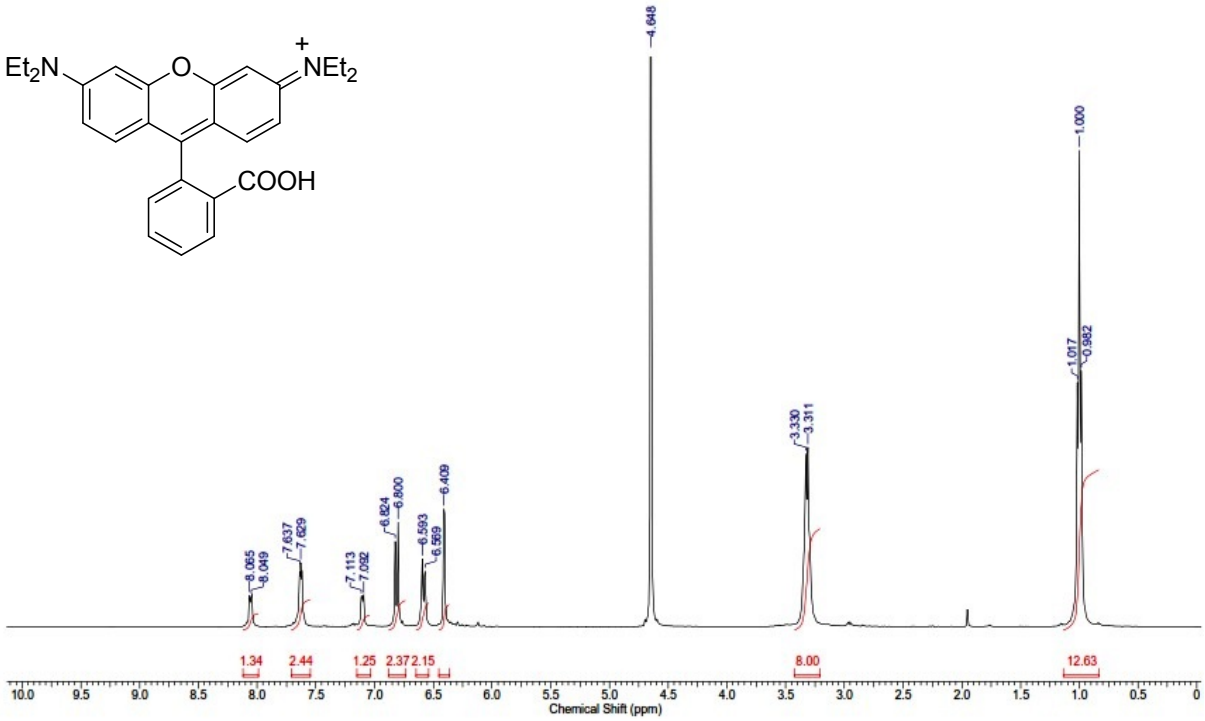


^{1}H NMR of Rh-EDC

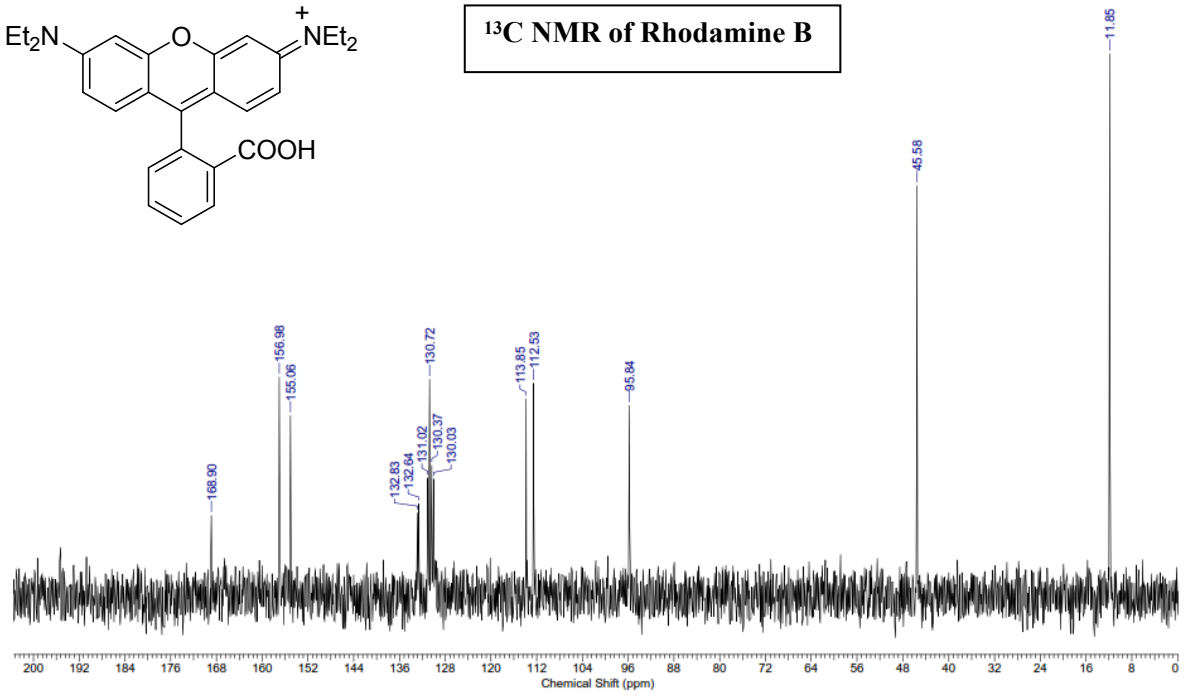


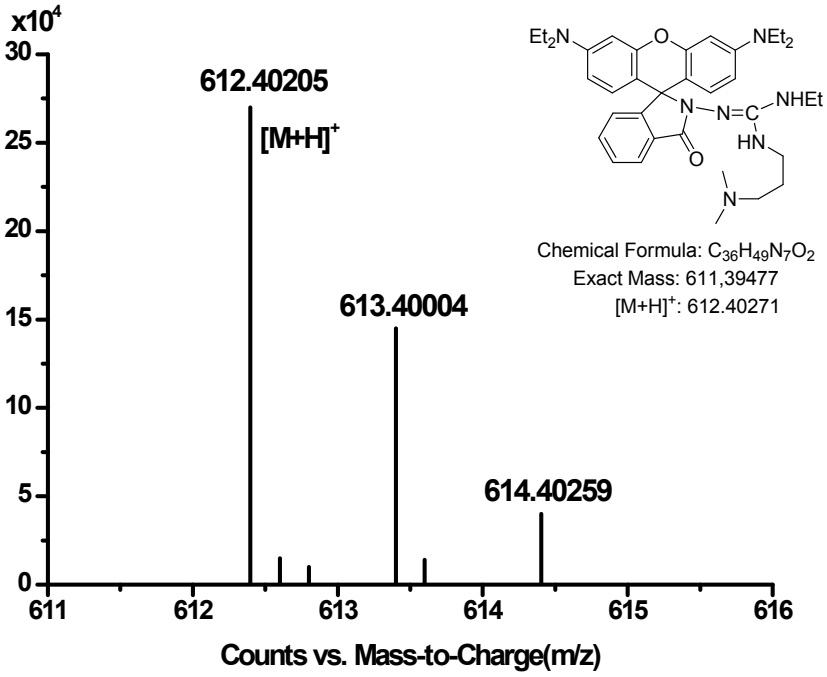
^{13}C NMR of Rh-EDC

^{1}H NMR of Rhodamine B

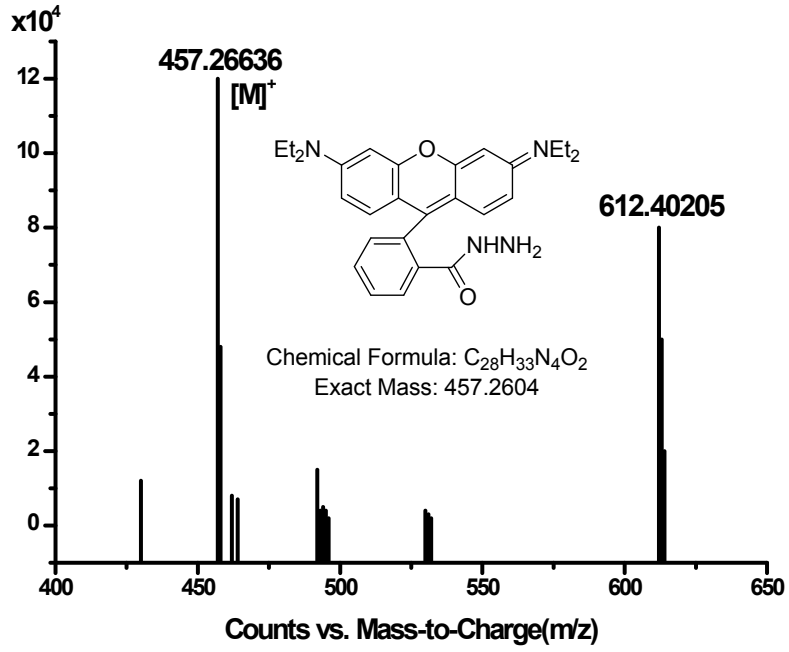


^{13}C NMR of Rhodamine B



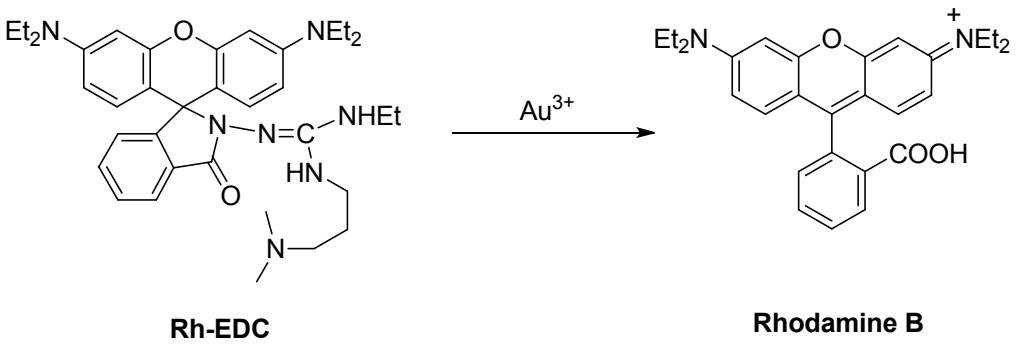


HRMS result of **Rh-EDC**



HRMS result of **Rhodamine Hydrazide**

TLC Image of the Hydrolysis Reaction of Rh-EDC Mediated with Au (III) Ion



Rh-EDC **Rhodamine B**

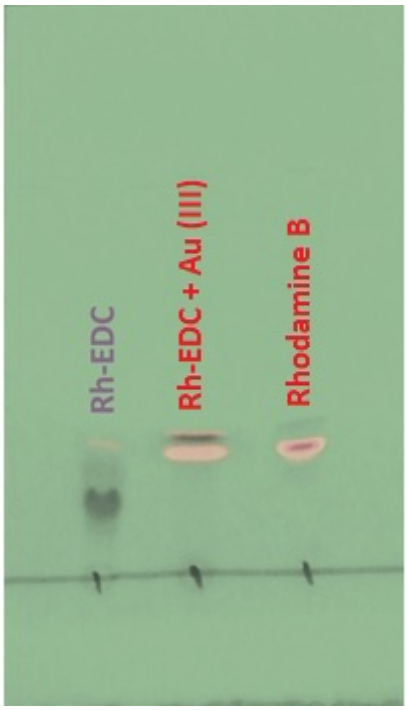


Figure S10 TLC image of the hydrolysis reaction of **Rh-EDC** mediated with Au (III) ion