Supporting Information

A Rhodamine Based Turn-on Chemodosimeter for Monitoring Gold Ion Residues in Synthetic Products and Living Cells

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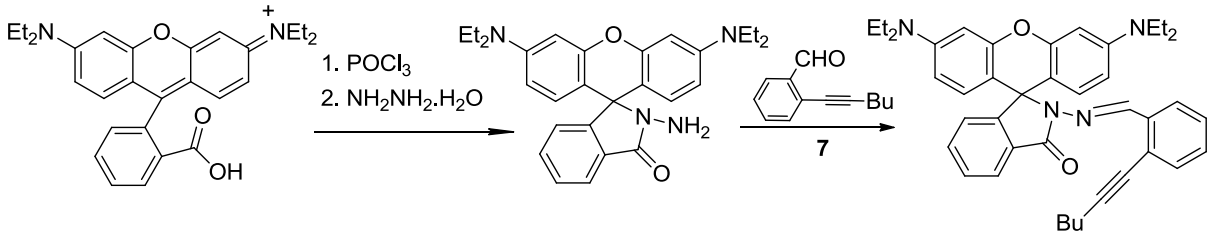
CONTENTS **PAGE**

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. Bruker MALDI-TOF-TOF Mass Spectrometer and Bruker Daltonics-microTOF-ESI-TOF were used for mass spectrometry analysis. UV absorption spectra were obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra were obtained using Varian Cary Eclipse Fluorescence spectrophotometer. Cell imagings were performed with Olympus CKX41 fluorescence microscope. Agilent 7500ce Octopole Reaction System (ORS) was used for inductively coupled plasma mass spectroscopy (ICP-MS) measurement. Samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 500 nm, the emission spectra were integrated over the range 525 nm to 750 nm. The slit width was 5 nm for both excitation and emission. The pH was recorded by HI-8014 instrument (HANNA). All measurements were conducted at least in triplicate.

2. Synthesis Section

a) Synthesis of 1 from Rhodamine B



Scheme S1: Synthesis of probe 1

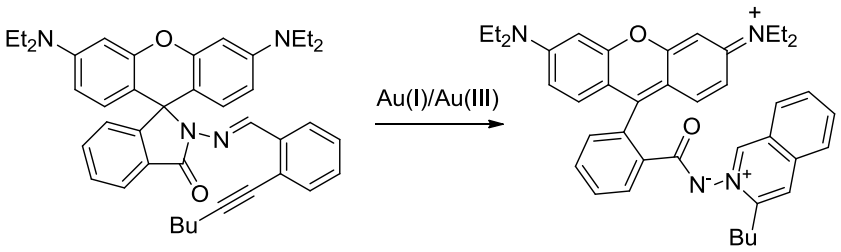
The rhodamine-B hydrazine **6** was prepared according to known procedure [1]

The 2-(hex-1-yn-1-yl)benzaldehyde **7** was prepared according to known procedure [2]

To a solution of rhodamine B hydrazide **6** (150mg, 0.33 mmol) in absolute ethanol (10 ml) was added 2-(hex-1-yn-1-yl)benzaldehyde **7** (123mg, 0.66 mmol). The solution was stirred overnight at room temperature. The reaction mixture was extracted with dichloromethane (3 x 10 mL). Then the collected organic layers were dried over anhydrous $MgSO_4$, concentrated under vacuum, and purified by column chromatography (hexane/EtOAc = 8/1) to give 140 mg of compound **1** (68%) as a pink solid. ^{1}H-NMR (400 MHz, $CDCl_3$) *δ:* 8.87 (s, 1H), 8.02 (dd, J=18.6 Hz, 7.2 Hz, 2H), 7.45 (quint, J=6.4 Hz, H), 7.26-7.08 (m, H), 6.57 (d, J= 8.0 Hz, 2H), 6.42 (s, H), 6.24 (dd, J=9.2 Hz, 2.4 Hz, 2H), 3.36-3.26 (m, 8H), 2.49 (t, J=7.2 Hz, 2H), 1.63 (quint, J=7.2 Hz, 2H), 1.51 (sextet, J=7.2 Hz, 2H), 1.15

(t, J=6.8 Hz, 12H), 0.99 (t, J=7.6 Hz, H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ:* 165.30, 152.78, 152.51, 148.86, 144.89, 136.17, 133.37, 132.19, 128.97, 127.62, 127.42, 125.12, 123.56, 123.45, 108.09, 105.85, 97.96, 95.79, 65.66, 44.29, 30.87, 22.23, 19.31, 13.77, 12.63. MS (MALDI-TOF): m/z: Calcd. for $C_{41}H_{44}N_4O_2$: 625.3464 $[M+H]^+$, Found: 625.291$[M+H]^+$. MS (ESI-TOF): m/z: Calcd. for $C_{41}H_{44}N_4O_2$: 625.3464 $[M+H]^+$, Found: 625.3544$[M+H]^+$.

b) Synthesis of 2 from compound 1

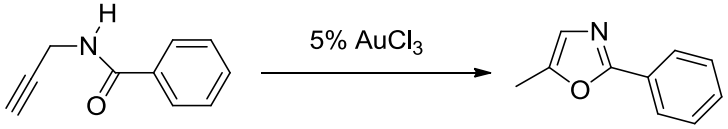


1 **2**

Scheme S2: Synthesis of compound 2

Compound **1** (38 mg, 0.06 mmol) was dissolved in CH_3CN/HEPES (1.6: 0.4 mL) and then $AuCl_3$ (18 mg, 0.06 mmol) was added. Subsequently, the reaction mixture was stirred at room temperature for 90 min. The reaction mixture was evaporated under reduced pressure, and it was filtered through celite. Then column chromatography was applied for the purification (CH_2Cl_2/MeOH 100:5) to obtain 15 mg of reddish liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.72 (d, *J*= 8.4 Hz, 1H), 8.24 (d, *J*=7.2 Hz, 1H), 7.91 (t, *J*=7.4 Hz, 1H), 7.79 (quint, *J*=7.6 Hz, 1H), 7.70 (s, 1H), 7.51-7.47 (m, 2H), 7.31-7.26 (m, 1H), 6.86 (d, J=8.8 Hz, 2H), 6.56 (dd, *J*=9.2 Hz, 2.4 Hz, 2H), 6.25 (s, 2H), 3.40-3.34 (m, H), 2.84 (t, *J*=8.0 Hz, 2H), 1.32-1.13 (m, H), 0.82 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.02, 155.80, 151.67, 149.02, 147.58, 141.09, 135.62, 133.21, 132.68, 130.61, 130.17, 129.16, 127.96, 126.68, 126.44, 125.79, 109.82, 106.62, 97.89, 45.03, 33.67, 29.68, 29.56, 22.88, 13.58, 12.45. MS (MALDI-TOF): m/z: Calcd. for $C_{41}H_{45}N_4O_2$: 625.3543 $[M^+]$, Found: 625.304 $[M^+]$. MS (ESI-TOF): m/z: Calcd. for $C_{41}H_{45}N_4O_2$ 625.3543 $[M^+]$, Found: 625.3557 $[M^+]$.

c) Synthesis of compound 5 by gold-catalyzed cyclization of propargylic amide



4 **5**

Scheme S3: Synthesis of compound 5

3. Cell imaging

HCT-116 cells were grown in Mc Coy's 5A medium supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5 % CO_2 at 37 ^{o}C. The cells were plated on 12mm cover glasses in 4-well plate and allowed to grow for 24h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated probe **1** (20 μM) for 30 min at 37 ^{o}C then washed with PBS three times. After incubating with Au^{3+} (10 μM, 20 μM and 40 μM) for 90 min at 37 ^{o}C, HCT-116 cells were rinsed with PBS three times, and Hoechst 34580 (2 μM) for 15 min at 37 ^{o}C then washed with PBS three times. Then the fluorescence images were acquired through an Olympus CKX41 fluorescence microscope.

References

1. V. Dujolsi, F. Ford and A. W. Czarnik, *J. Am. Chem. Soc.* 1997, **119**, 7386.

2. G. Dai and R. C. Larock, *Org. Lett*. 2001, **3**, 4035.

3. A. S. K. Hashmi, J. P. Weyrauch, W. Frey and J. W. Bats, *Org. Lett.* 2004**, 6**, 4391.

4. Absorption and Emission spectra of probe 1

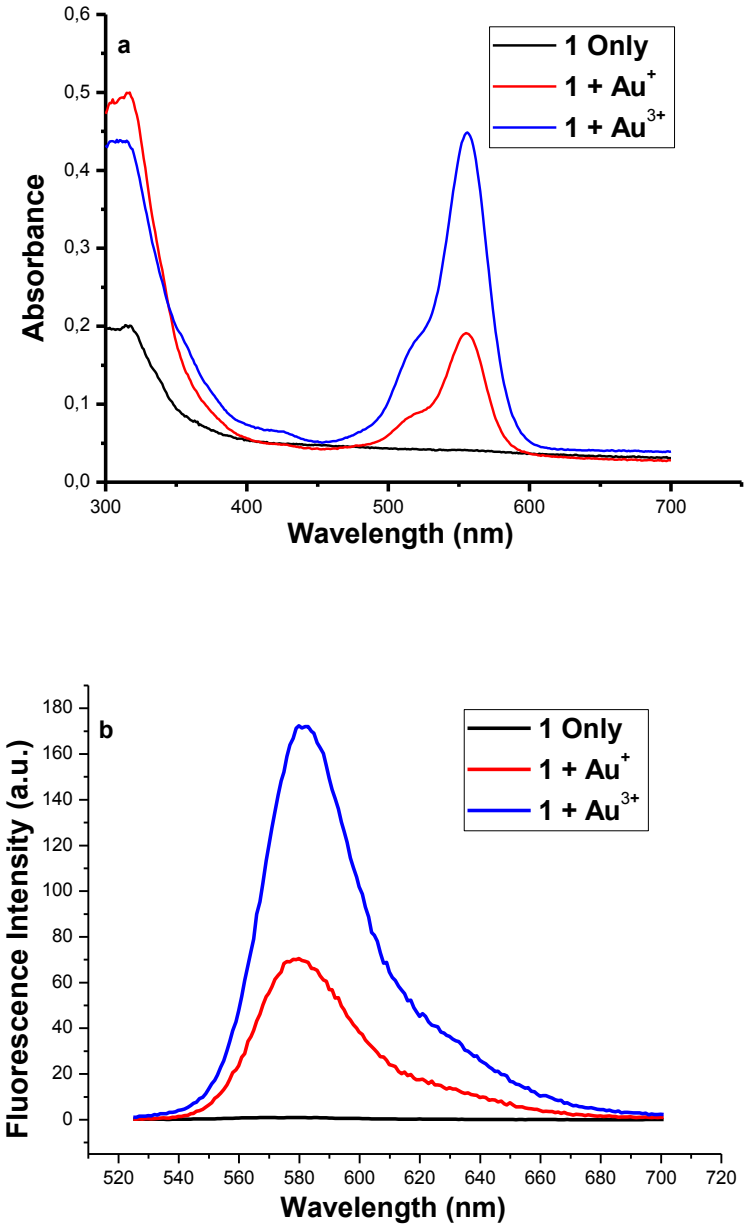


Figure S1 Absorption and Emission spectra of probe **1** (20 μM) and Au^{+} and Au^{3+} (60 μM) in 1:1 CH_3CN/HEPES buffer at pH = 7.0; (λ_{ex}: 500 nm, at 25 ^{o}C).

5. Time-dependent fluorescence change of probe 1

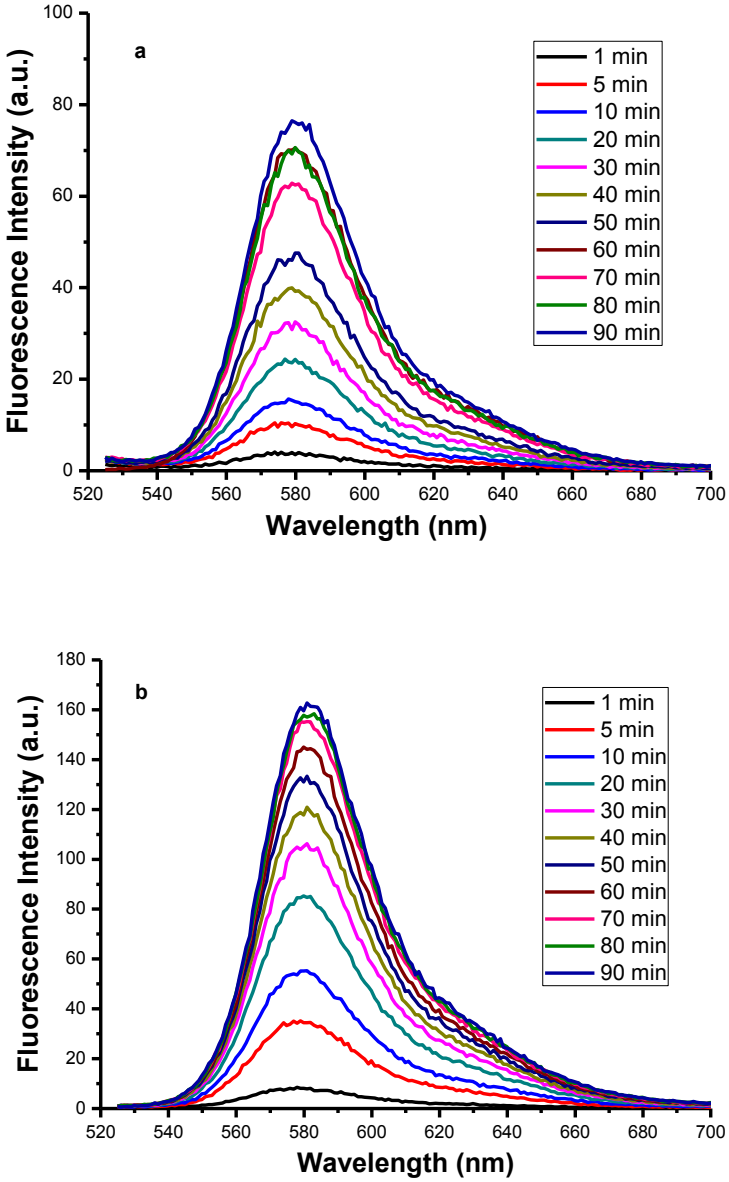


Figure S2 Time-dependent fluorescence change of probe **1** (20 μM) in the presence of an 60 μM of (*a*) AuCl and (*b*) $AuCl_3$ measured in 1:1 CH_3CN/HEPES buffer at pH = 7.0

6. Fluorescence titration of 1 with Au^{3+}

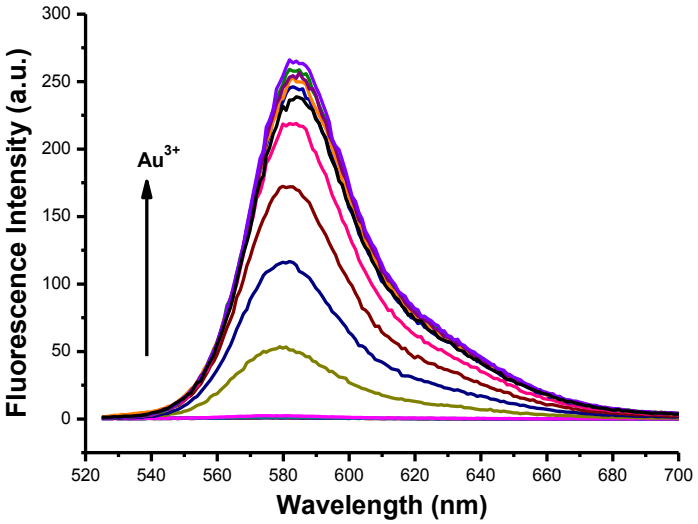


Figure S3 Fluorescence spectra of 1 (20 μM) in 1:1 CH_3CN/HEPES buffer at pH = 7.0 in the presence of Au^{3+} (2 to 200 μM, 0.1 to 10.0 equiv.)

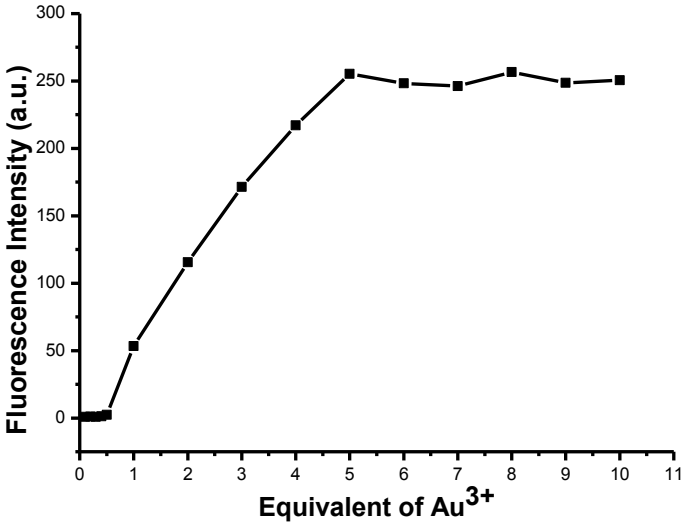
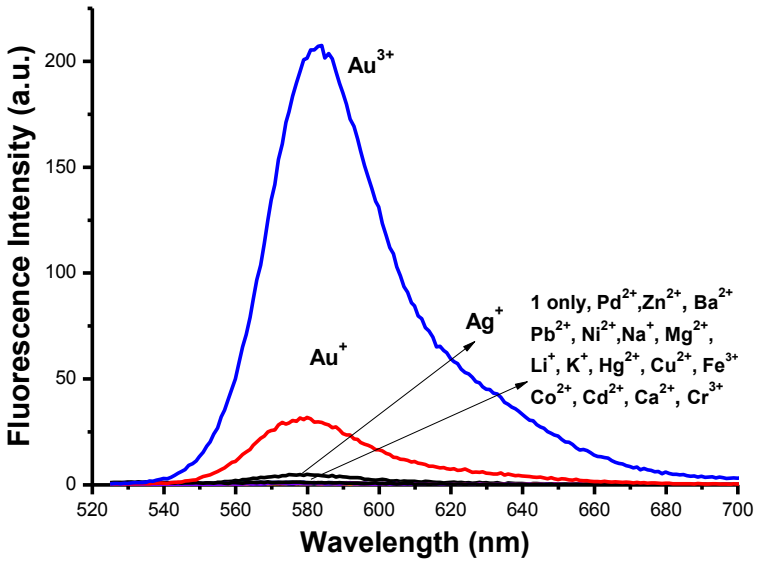


Figure S4 Fluorescence intensity changes of probe **1** (λ_{max}: 580 nm) vs equivalents of Au^{3+}

7. The fluorescence responses of probe 1 with Au^{3+} and other metals.



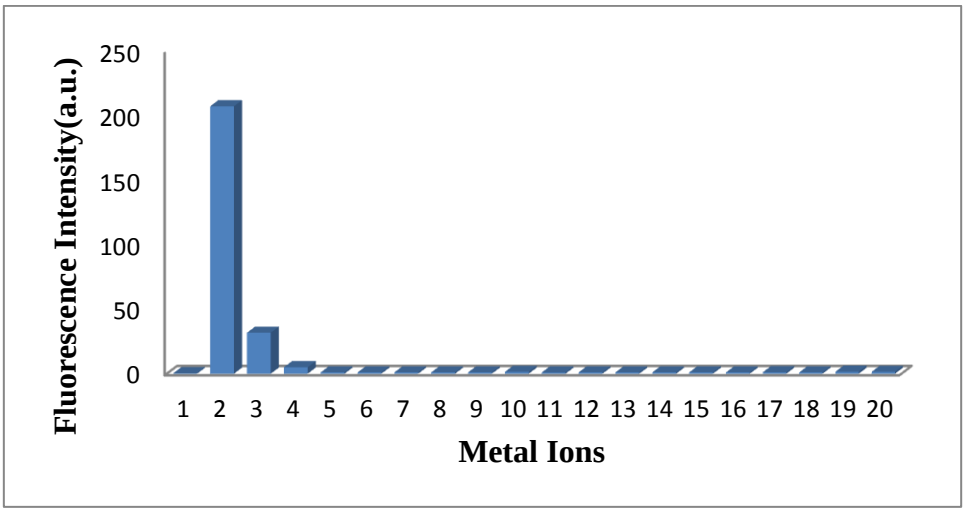


Figure S5 Fluorescence intensities of probe **1** (20 μM) in 1:1 CH_3CN/HEPES buffer at pH = 7.0 at λ_{max}: 580 nm in the presence of the cation (100 μM): 1, probe **1** only; 2, Au^{3+}; 3, Au^{+}; 4, Ag^{+}; 5, Zn^{2+}; 6, Pb^{2+}, 7, Ni^{2+}; 8, Na^{+}; 9, Mg^{2+}; 10, Li^{+}; 11, K^{+}, 12, Hg^{2+}; 13, Cu^{2+}; 14, Co^{2+}; 15, Cd^{2+}; 16, Ca^{2+}; 17, Ba^{2+}; 18, Pd^{2+}; 19, Fe^{3+}; 20, Cr^{3+}

8. The fluorescence responses of probe 1 in the presence of Au^{3+} and other metal ions.

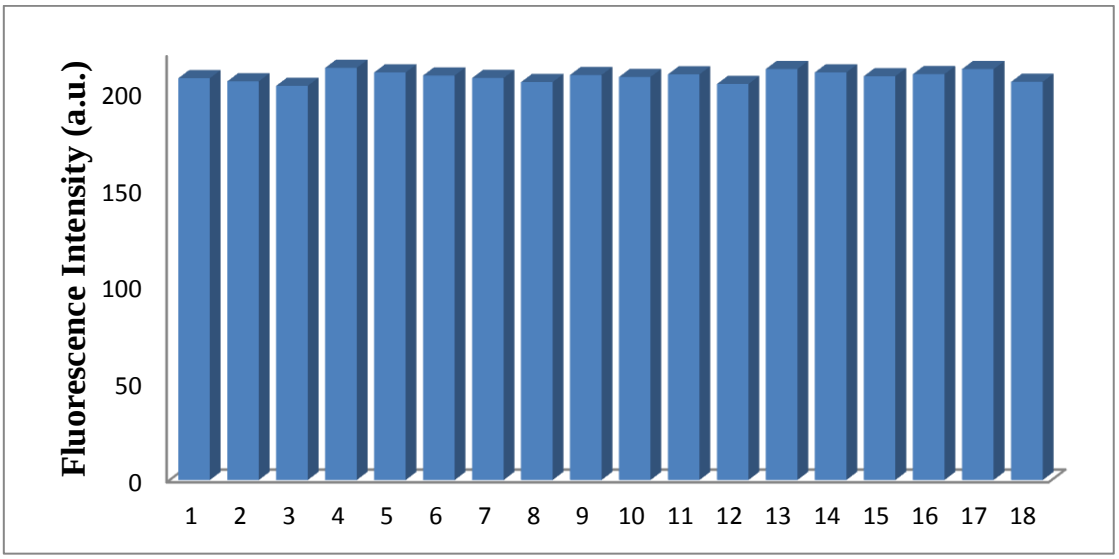
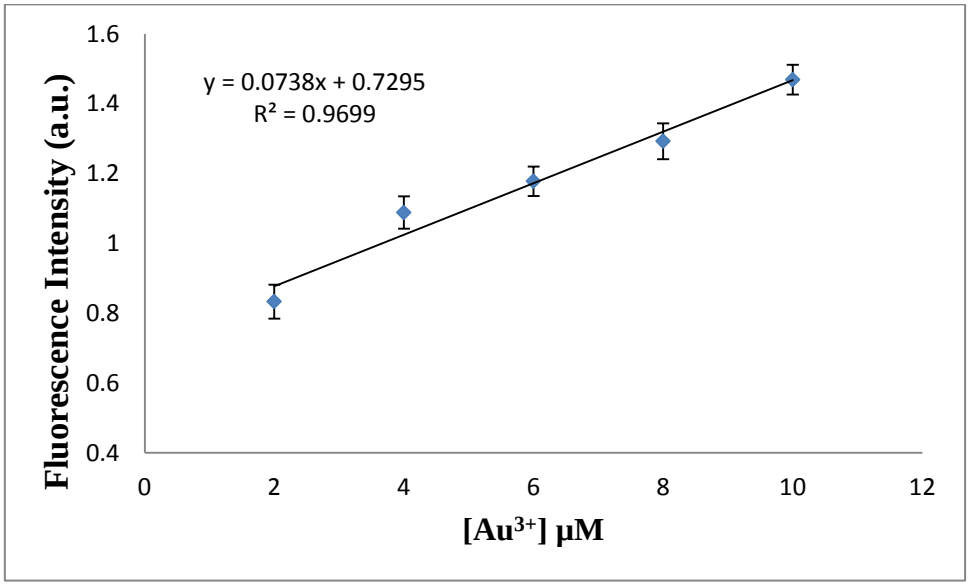


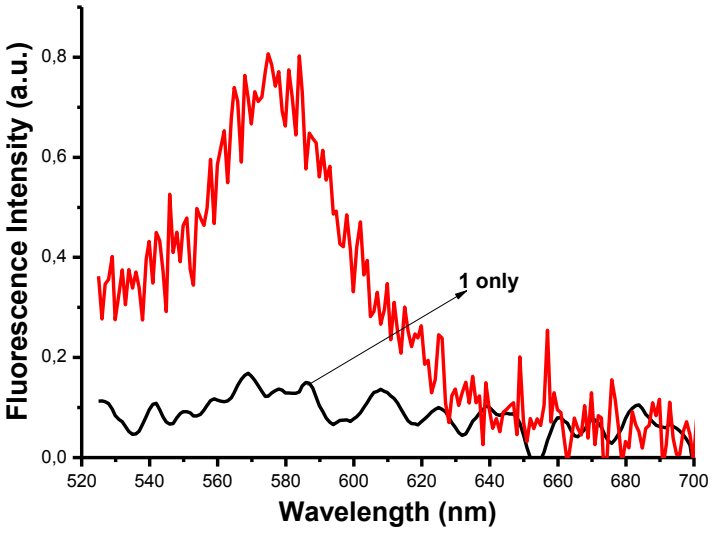
Figure S6 Fluorescence intensities of probe **1** (20 μM) in 1:1 CH_3CN/HEPES buffer at pH = 7.0 at λ_{max}: 580 nm in the presence Au^{3+} (100 μM) and of the following metal ions (200 μM): 1, none; 2, Ag^{+}; 3, Ba^{2+}; 4, Pd^{2+}; 5, Zn^{2+}; 6, Pb^{2+}, 7, Ni^{2+}; 8, Na^{+}; 9, Mg^{2+}; 10, Li^{+}; 11, K^{+}, 12, Hg^{2+}; 13, Cu^{2+}; 14, Co^{2+}; 15, Cd^{2+}; 16, Ca^{2+}; 17, Cr^{3+}; 18, Fe^{3+}

9. Determination of detection limit

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of probe **1** (20 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 – 10 μM (R = 0.9699). 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 2.0 μM at S/N = 3.



A



B

Figure S7 A) Fluorescence change of probe **1** (20 μM) upon addition of $AuCl_3$ (2.0 to 10.0 μM, 0.1 to 0.5 equiv.) in 1:1 CH_3CN/HEPES buffer at pH = 7.0. **B)** Fluorescence spectrum of probe **1** (20 μM) upon addition of $AuCl_3$ (2.0 μM / 0.6 ppm) in 1:1 CH_3CN/HEPES buffer at pH = 7.0

10. Effect of water content

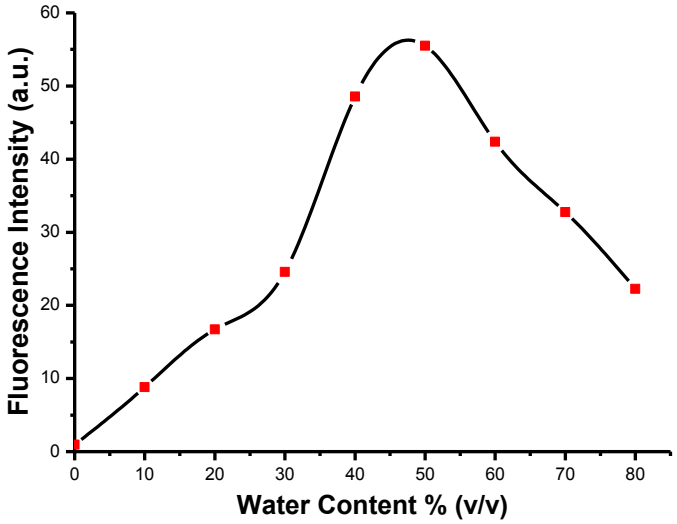


Figure S8 Effect of water content on the fluorescence intensity of probe **1** (20 μM) in the presence of Au^{3+} (20 μM) at pH= 7.0

11. Effect of pH

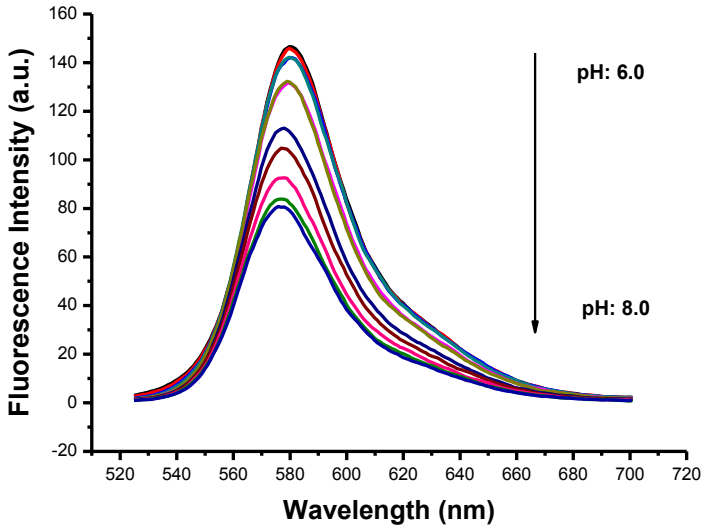
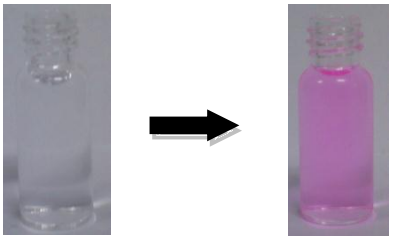


Figure S9 Effect of different pH values (pH: 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8 and 8.0) on the fluorescence intensity of probe **1** (20 μM) in the presence of Au^{3+} (60 μM)

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

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

The crude compound **5** was loaded into silica gel column and eluted with Hexane: EtOAc (4:1 v/v). For qualitative analysis 20 µM probe **1** solution was added into one of the collected fraction for proving that the fraction contained Au^{3+} by changing color from colorless to pink as demonstrated figure below.

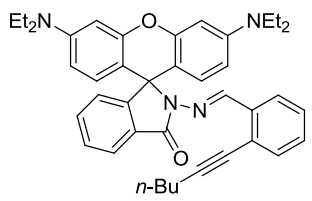


Then, the rest of the other fractions combined and solvents were removed to afford the product in a liquid form. A sample of compound **5** (2.0 mg) was weighed and dissolved in 2.0 mL of CH_3CN: HEPES (1:1 v/v) at pH= 7.0 Stock solution of probe **1** solution was prepared (2.5 x 10^{-3} M) and 100 µL added into the solution containing compound **5**. The resulting solution was shaken at room temperature before recording the fluorescence spectra. Based on the standard calibration curve, the content of residual gold ions in compound **5** was determined as $5.90x10^{-7}$ mole/mg.

b) Detection of residual Au^{3+} content in compound 5 using inductively-coupled plasma mass spectroscope (ICP-MS)

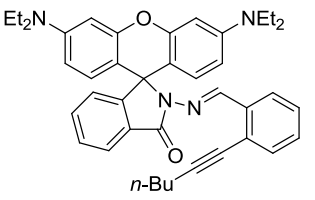
A sample of compound **5** (5.0 mg) prepared in 2.0 mL aqua regia (HNO_3: HCl, 1:3 v/v) and incubated 30 min. Then, degradation process was applied with Cem Mars X microwave instrument. The solution was completed to 50.0 mL with deionized water. The resulting solution was subjected to ICP-MS analysis. A standard calibration curve was acquired with the known concentration of Au^{3+} solutions. The measurement was conducted in triplet. The residual gold ions in compound **5** were measured as $6.27x10^{-7}$mole/mg.

^{1}H NMR of probe 1



1

^{13}C NMR of probe 1



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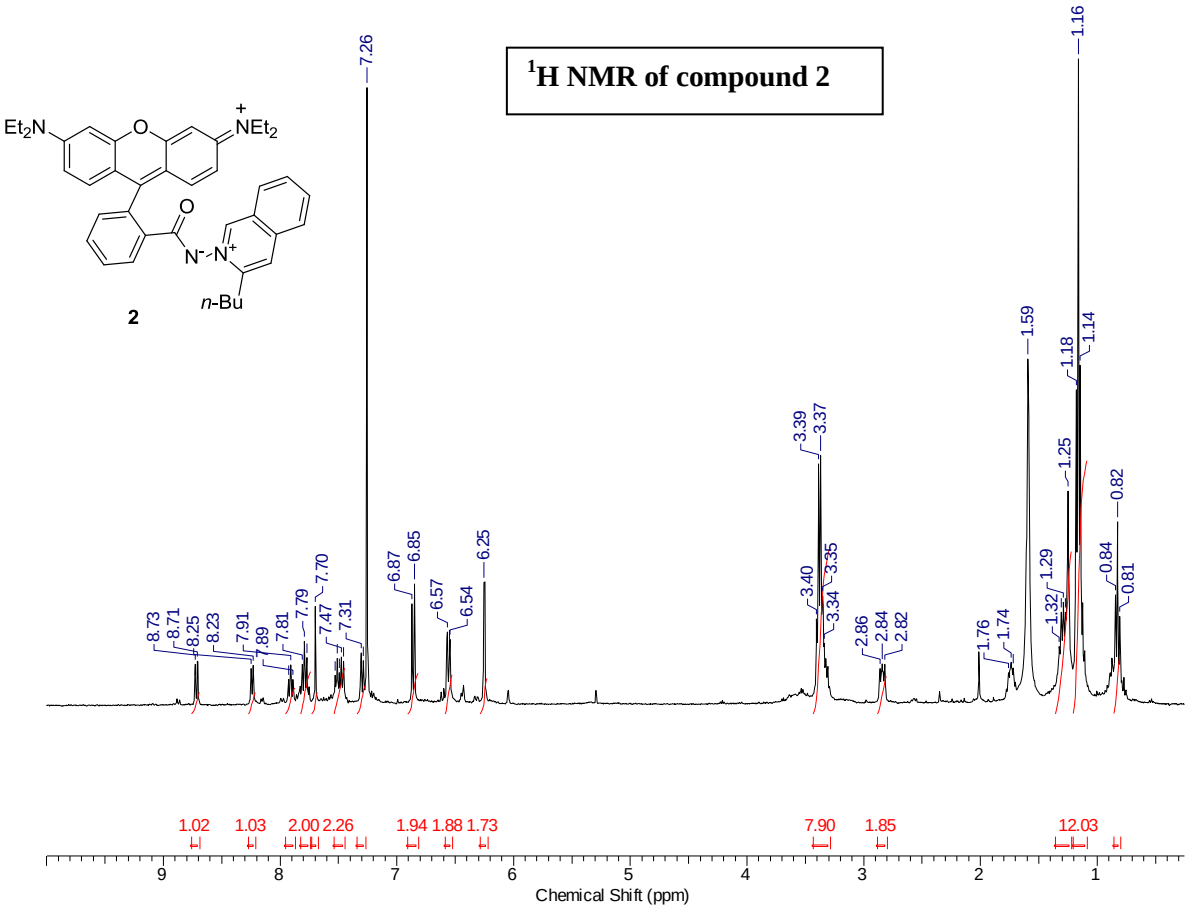
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1.18
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3.37
1.25
0.82
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7.79
6.25
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8.73
8.71
8.25
8.23
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7.81
7.47
7.31
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6.54
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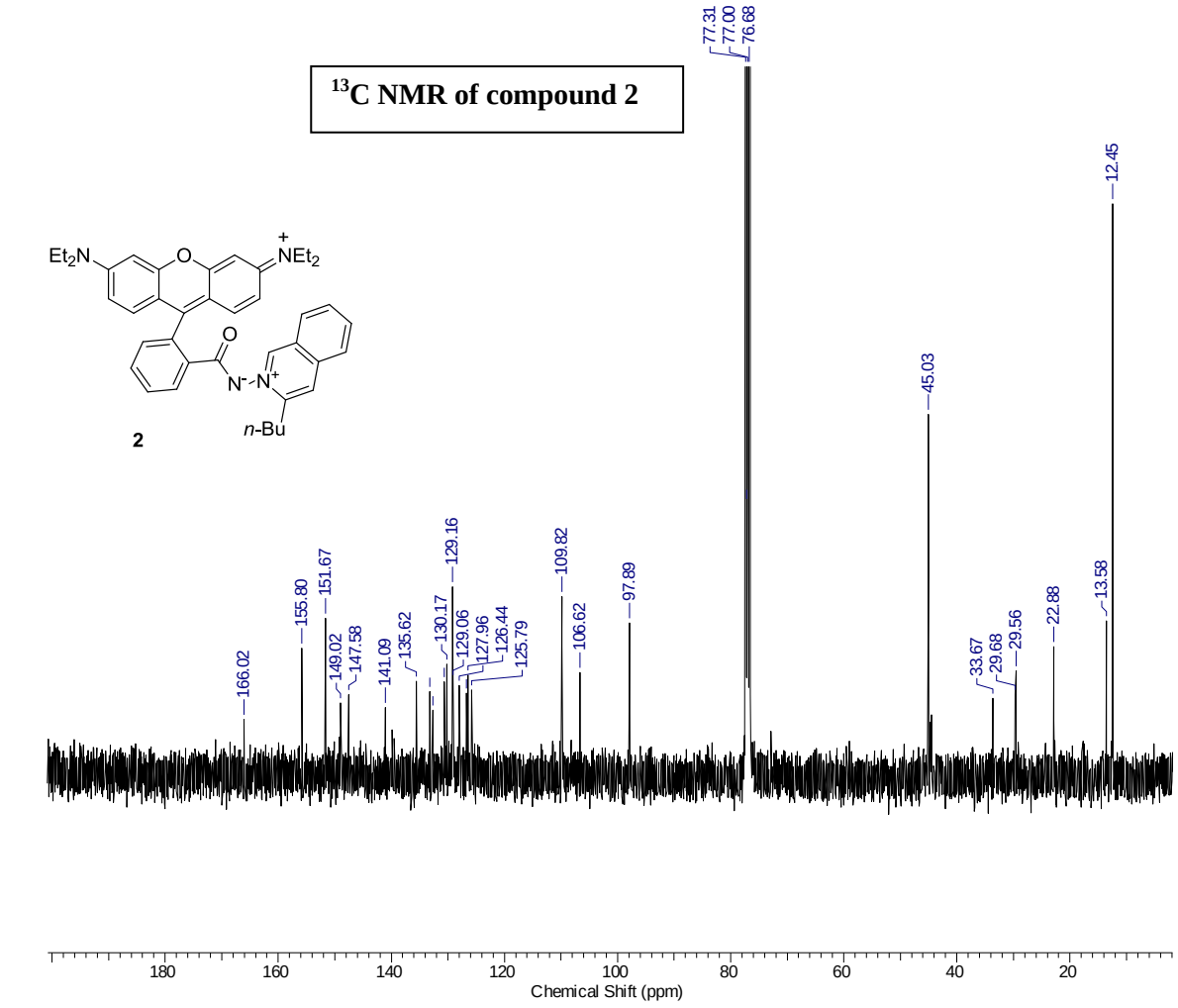
Chemical Shift (ppm)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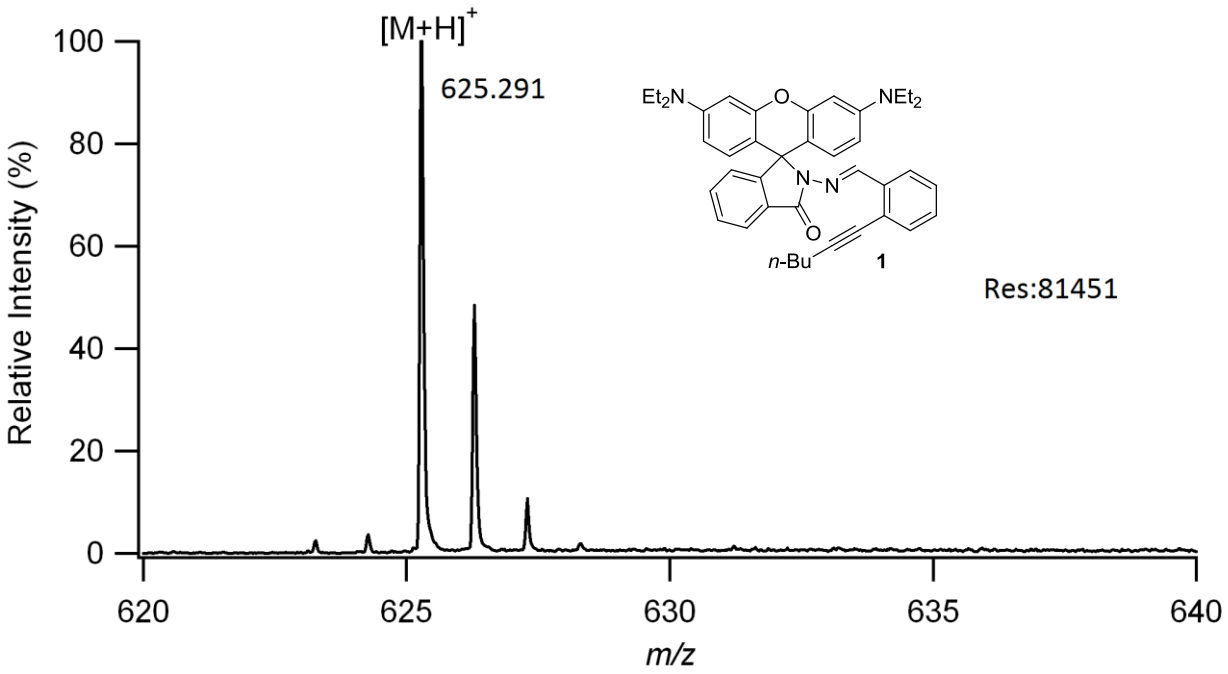
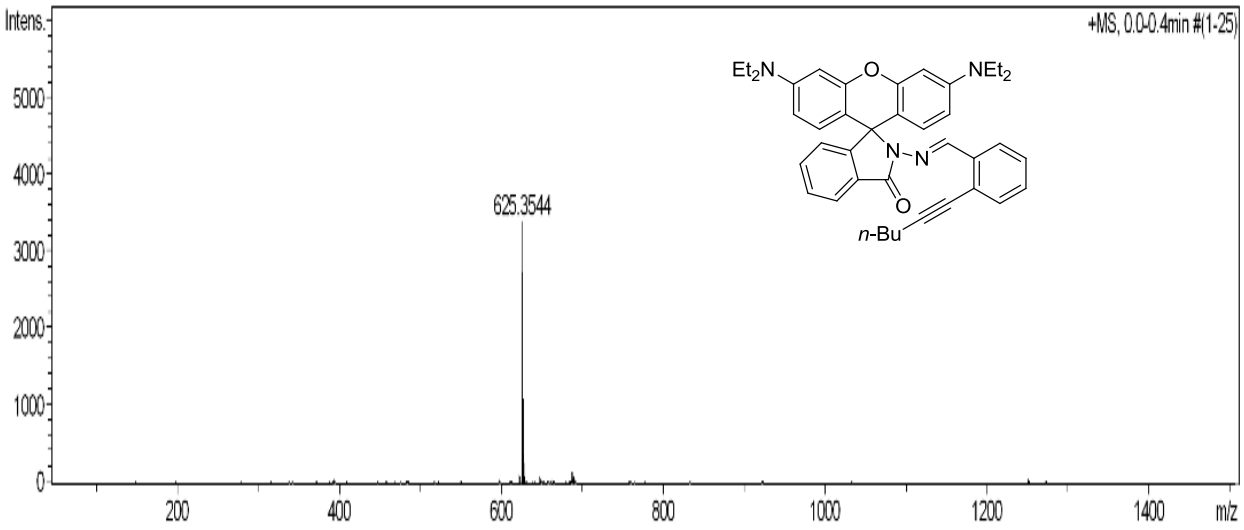
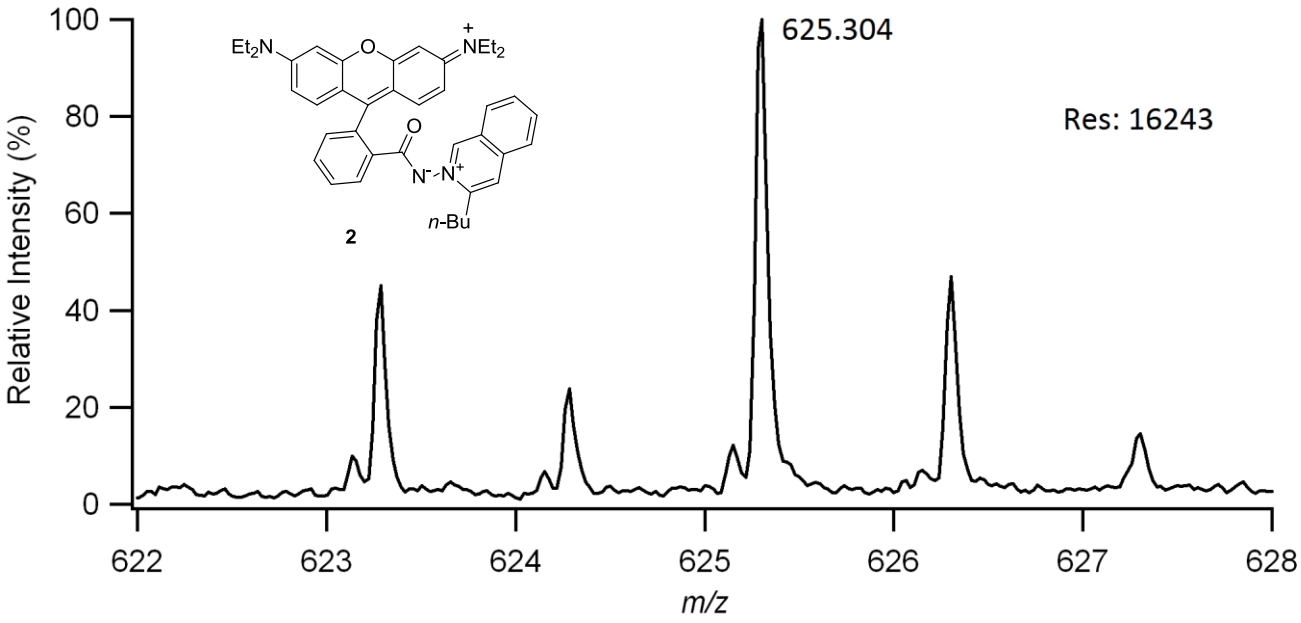


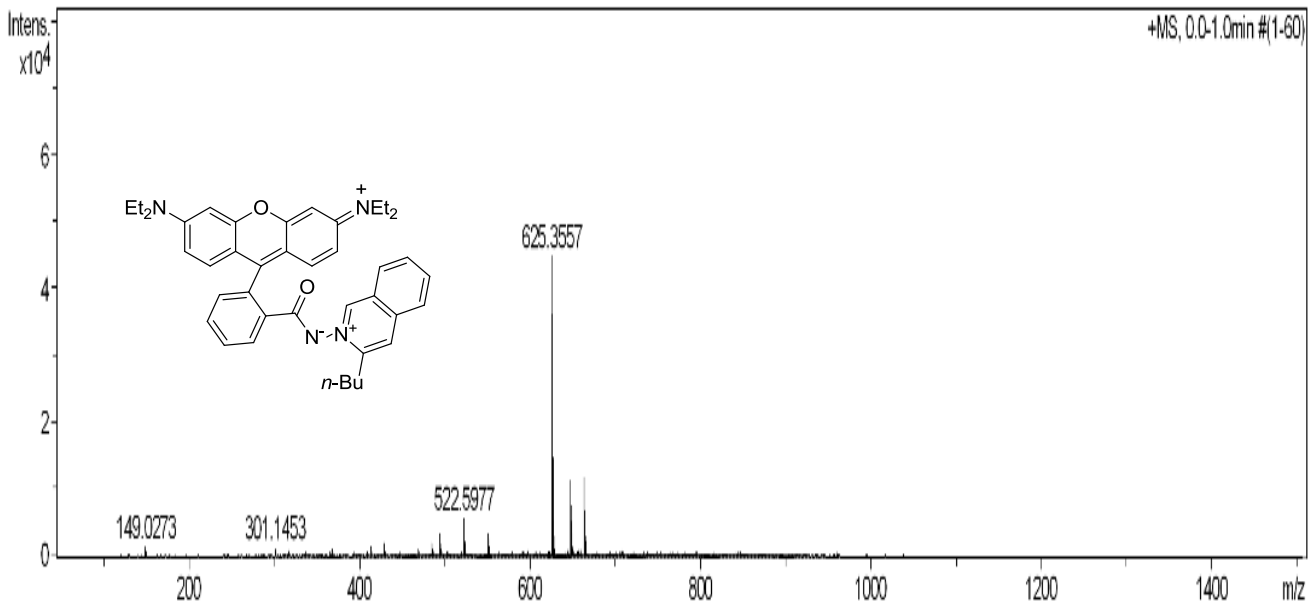
MALDI-TOF result of compound **2**



ESI-TOF MS result of compound **2**



MALDI-TOF result of probe **1**



ESI-TOF MS result of probe **1**