Rhodamine-sugar based turn-on fluorescent probe for the detection of cysteine and homocysteine in water

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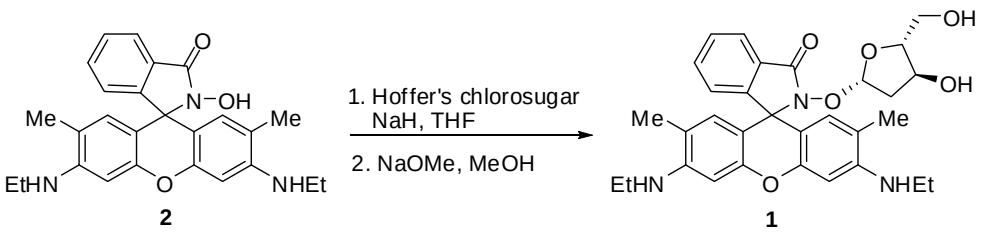
1. Reagents and general methods

General synthetic materials and methods: Silica gel 60 (230-400 mesh, Merck) was used for column chromatography. Analytical thin layer chromatography was performed using Merck 60 F_{254} silica gel (precoated sheets, 0.25 mm thick). All reagents and solvents for reactions were used as received with the following exceptions. Tetrahydrofuran (THF) was distilled from sodium (Na) and benzophenone. Ethyl acetate (EtOAc) and hexanes were distilled. All other chemicals used were purchased from Sigma-Aldrich and were used as received.

Spectroscopic materials and methods: Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ unless otherwise stated, with tetramethylsilane (TMS) as internal reference at ambient temperature, mainly on a Bruker Avance II-400 Fourier Transform Spectrometer operating at 400 MHz for ^1H and at 100.6 MHz for ^{13}C. *J* values were given in Hz. Mass spectra were recorded on a ZQ-4000 LC-MS and QUATTRO LC Triple Quadrupole Tandem mass spectrometer for both low resolution and high resolution mass spectra. Melting points were measured on a Z289078 (Sigma-Aldrich) microscope and were uncorrected. Infrared absorption spectra were recorded as a solution in $CHCl_3$ on a Avatar 360 FT-IR spectrophotometer. Fluorescence emission spectra were obtained using a Hitachi F-4500 spectrofluorimeter linked to a Pentium PC running SpectraCalc software package. The slit width was 5.0 nm for both excitation and emission. The photon multiplier voltage was 400 V. Samples were contained in 10.0 nm path length quartz cuvettes (3.5 mL volume). Upon excitation at 500 nm, the emission spectra were integrated over the range 510-650 nm. The stock solution of 0.001 M (**1**) was prepared by dissolving **1** in MeOH and without light irradiation. The work solutions of 1×10^{-5} M (**1**) was obtained by diluting the MeOH stock solution with H_2O. Stock solutions of 0.01 M amino acids were prepared by dissolving the appropriate amount of each compound in distilled water. Working solutions were prepared by successive diluting the stock solutions with distilled water. All measurements were conducted at least in triplicate and room temperature (25 ^oC).

2. Synthesis of 1 from rhodamine hydroxamic acid (2)



2 **1**

The rhodamine-hydroxamic acid was prepared according to the known procedure.[1]
To a solution of rhodamine-hydroxamic acid (42 mg, 0.098 mmol) in THF (3.0 mL) was added dropwise into a suspension of sodium hydride (4 mg, 0.167 mmol) in an equal amount of THF (1.0 mL) at 0 ^{o}C. After stirring for 30 min at 0 ^{o}C, a solution of Hoffer's chlorosugar[2] (50 mg, 0.128 mmol) in an equal amount of THF (1.0 mL) was added dropwise. The solution was allowed to warm to room temperature and stirring for 2 h. The reaction was quenched with water (10 mL) and the reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the collected organic layers were dried over anhydrous $MgSO_4$. The mixture was concentrated under vacuum and the crude product was purified by column chromatography (hexanes/EtOAc = 2:1 to 1:1) to give 73 mg (0.093 mmol, 95%) of sugar-protection compound. The isolation compound (50 mg, 0.064 mmol) was dissolved in MeOH (3.0 mL), and then 25 wt% of NaOMe (0.2 mL) was added into the flask via syringe under an atmosphere of nitrogen gas. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure. The crude products were purified by silica gel chromatography (EtOAc/hexanes = 3:1 to 3:1 + MeOH 10%) to give 34 mg (0.062 mmol, 97%) of compound **1** as a white solid.

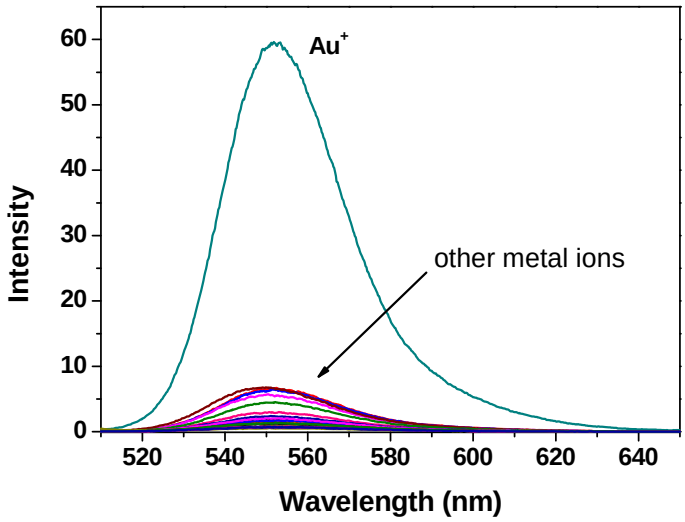
Compound 1: R_f= 0.2 (hexanes/EtOAc/MeOH = 2:1:0.2); mp 228-230 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.94–7.92 (m, 1H), 7.53–7.50 (m, 2H), 7.08–7.0 (m, 1H), 6.52 (s, 1H), 6.36 (d, *J* = 5.2 Hz, 2H), 6.07 (s, 1H), 5.76 (dd, *J* = 4.4, 9.6 Hz, 1H), 4.75 (d, *J* = 4.8 Hz, 1H), 4.57 (s, 1H), 3.92 (s, 1H), 3.85–3.84 (m, 1H), 3.60-3.56 (m, 3H), 3.24-3.18 (m, 4H), 2.15-2.09 (m, 2H), 1.95 (s, 3H), 1.88 (s, 3H), 1.36-1.30 (m, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 166.1, 152.5, 149.9, 148.0, 147.6, 133.5, 129.1, 129.0, 128.7, 128.0, 124.0, 123.5, 118.3, 118.0, 108.6, 105.6, 105.5, 96.7, 89.1, 69.9, 66.7, 61.2, 40.3, 38.5, 17.0, 14.8; IR (film, cm^{-1}) 3385, 2971, 2932, 2863, 1670, 1636, 1621, 1518, 1463, 1449, 1422, 1348, 1271, 1202, 1160, 1095, 1014, 849, 752; HRMS (FAB) *m/z* calcd. for $C_{31}H_{36}N_3O_6$ [$(M + H)^+$] 546.2604; found 546.2604.

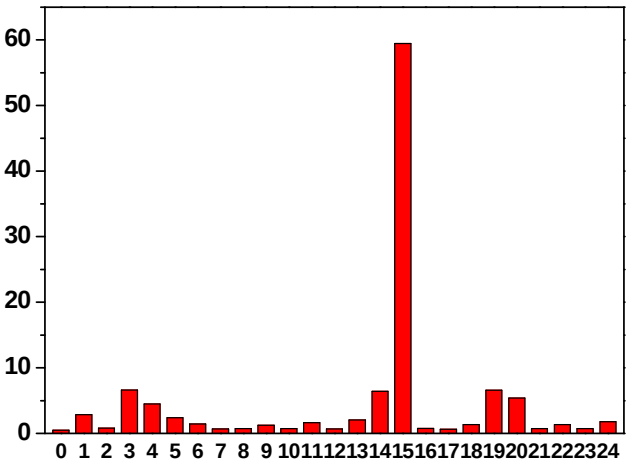
3. Reference

1. Y.-K. Yang, H. J. Cho, J. Lee, I. Shin and J. Tae, *Org. Lett.*, 2009, **11**, 859.

2. a) M. Hoffer, *Chem. Ber.*, 1960, **93**, 2777; b) V. Rolland, M. Kotera and J. Lhomme, *Synth. Commun.*, 1997, **27**, 3505; c) M. Ogino, Y. Yoshimura, A. Nakazawa, I. Saito and K. Fujimoto, *Org. Lett.*, 2005, **7**, 2853.

4. Fluorescence intensity changes of 1 with metal ions

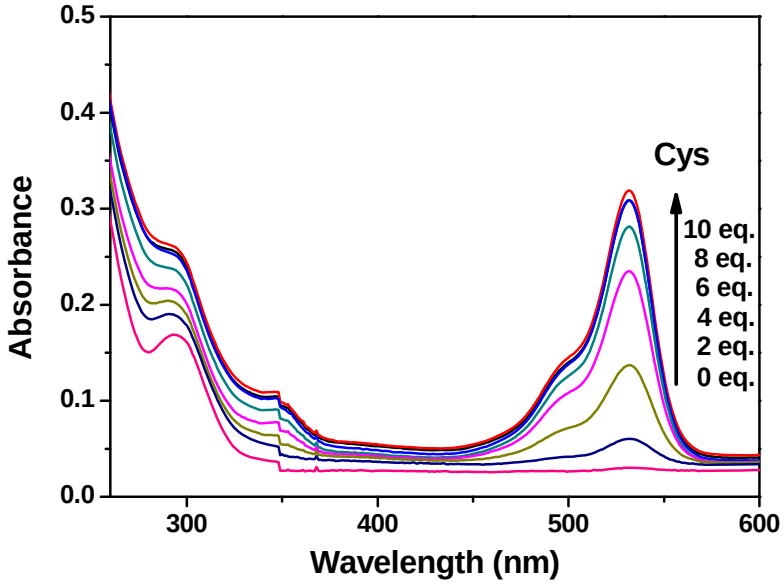




Metals ions: 0. Only; 1. Fe^{3+}; 2. Fe^{2+}; 3. Au^{3+}; 4. Hg^{2+}; 5. Zn^{2+}; 6. Pb^{2+}; 7. Ca^{2+}; 8. Co^{2+}; 9. Mn^{2+}; 10. Mg^{2+}; 11. Cu^{2+}; 12. Cd^{2+}; 13. Al^{3+}; 14. Cr^{3+}; **15. Au^{+}**; 16. Ag^{+}; 17. Na^{+}; 18. Li^{+}; 19. Pd^{2+}; 20. Pt^{2+}; 21. Rh^{2+}; 22. Ni^{2+}; 23. K^{+}; 24. Ba^{2+}. Fluorescence spectra (λ_{ex} = 500 nm, λ_{em} = 553 nm) of probe **1** (10 μM) in H_2O (1% MeOH + 0.1% PBS buffer) at 25 ^{o}C in the presence of 3.0 equiv of metal ions, respectively.

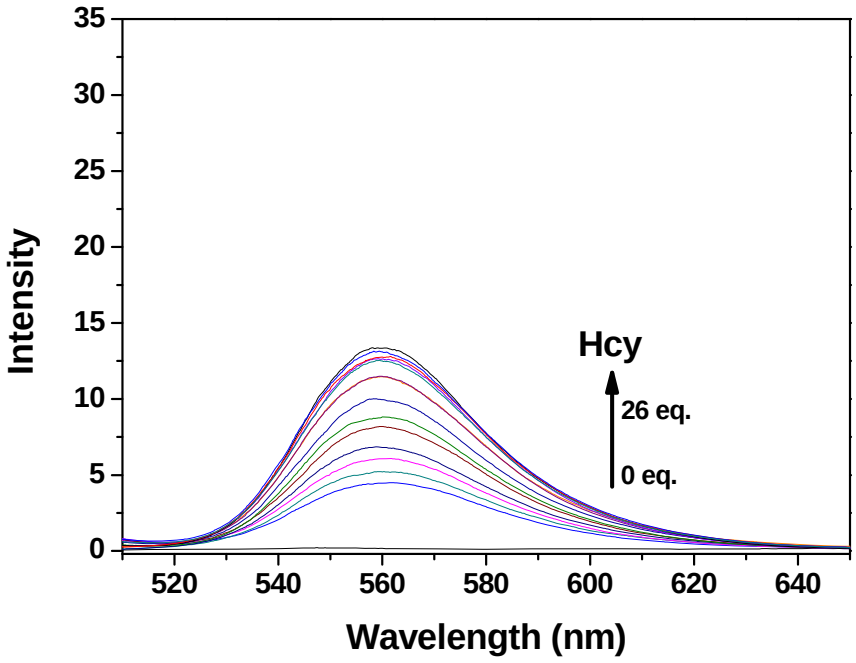
5. UV-vis absorption changes of 1–Au^{+} with Cys

UV-vis absorption is changes of **1**–Au^{+} upon addition of Cys in H_2O (MeOH 1%) at 25 ^{o}C. The arrows indicate the signal changes as increasing in the concentration of Cys (0, 2, 4, 6, 8 and 10 equiv), respectively. [**1**] = 1.0×10^{-5} M, [Au^{+}] = 1 equiv. Each spectrum is acquired after 1 min of each Cys addition.



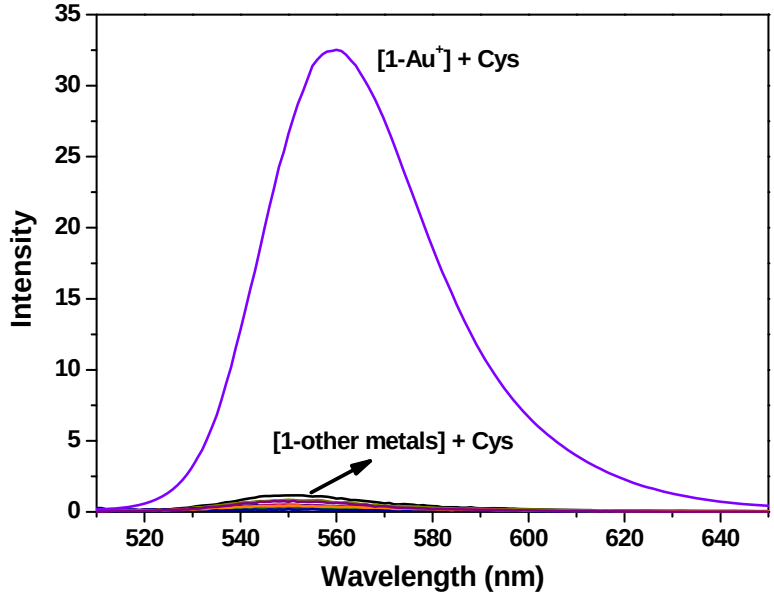
6. Fluorescence titration of 1–Au^{+} with Hcy

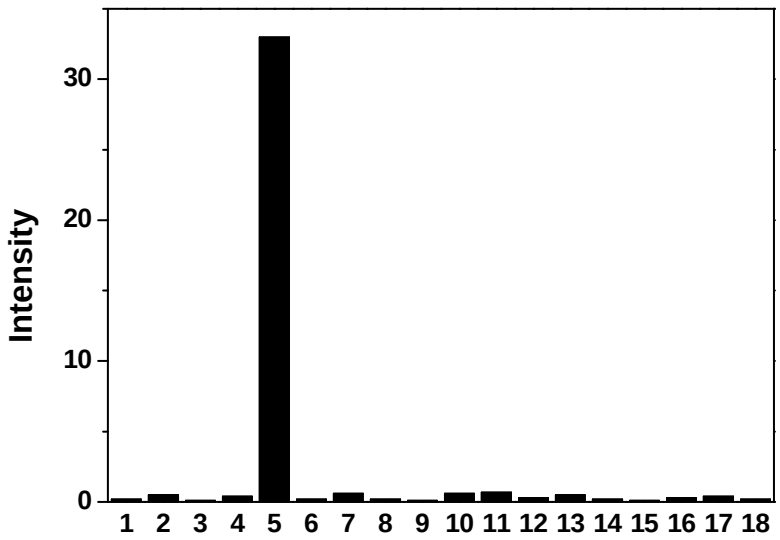
Fluorescence spectra (λ_{ex} = 500 nm) of **1**–Au^{+} in H_2O (MeOH 1%) in the presence of Hcy at 25 ^{o}C. The arrows indicate the signal changes as increasing in the additions of Hcy (0, 2, 5, 7, 10, 12, 15, 20, 23, and 26 equiv), respectively. [**1**] = 1.0 × 10^{-5} M, [Au^{+}] = 1 equiv. Each spectrum is acquired after 2 min of each Hcy addition.



7. Effects of metal ions with Cys

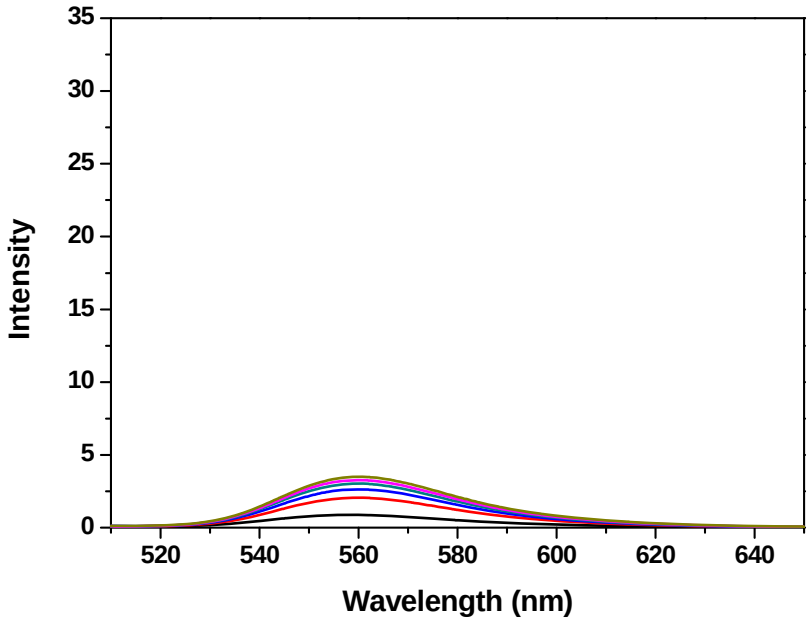
Effects of metal ions on the fluorescence intensity (λ_{ex} = 500 nm, λ_{em} = 560 nm) changes of probe **1** in H_2O (MeOH 1%) at 25 °C. [**1**-other metal ions such as 1. Fe^{3+} + Cys; 2. Fe^{2+} + Cys; 3. Hg^{2+} + Cys; 4. Zn^{2+} + Cys; 5. Au^{+} + Cys; 6. Pb^{2+} + Cys; 7. Ca^{2+} + Cys; 8. Co^{2+} + Cys; 9. Mn^{2+} + Cys; 10. Mg^{2+} + Cys; 11. Cu^{2+} + Cys; 12. Cd^{2+} + Cys; 13. Al^{3+} + Cys; 14. Cr^{3+} + Cys; 15. Ag^{+} + Cys; 16. Na^{+} + Cys; 17. Li^{+} + Cys; 18. Ni^{2+} + Cys]; [**1**] = 1.0 × 10^{-5} M; [metal ions] = 1 equiv; [Cys] = 20 equiv, respectively.





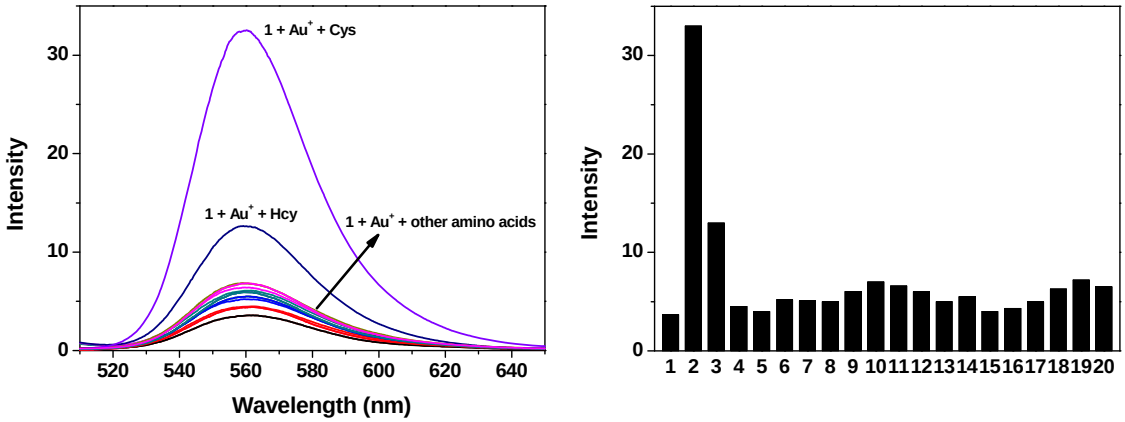
8. Effects of 1–Au^{3+} ions with Cys

Effects of metal ions on the fluorescence intensity (λ_{ex} = 500 nm) changes of probe **1** in H_2O (MeOH 1%) at 25 ^oC. The fluorescence is weak enhancement as increasing in the additions of Cys (0, 1, 5, 10, 15 and 20 equiv), respectively. [**1**] = 1.0 × 10^{-5} M, [Au^{3+}] = 1 equiv. Each spectrum is acquired after 2 min of each Cys addition.

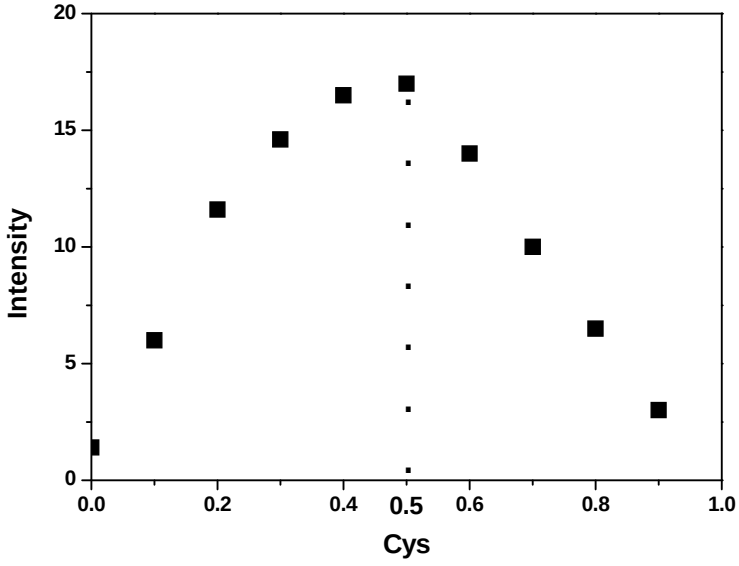


9. Effects of 1–Au^+ with amino acids

Effects of amino acids on the fluorescence intensity (λ_{ex} = 500 nm, λ_{em} = 560 nm) changes of probe **1** in H_2O (MeOH 1%) at 25 °C. [**1**] = 1.0 × 10^{-5} M; [Au^+] = 1 equiv; [amino acid] = 20 equiv, respectively; 1. **1** + Au^+; 2. **1** + Au^+ + Cys; 3. **1** + Au^+ + Hcy; 4. **1** + Au^+ + Ala; 5. **1** + Au^+ + Val; 6. **1** + Au^+ + Leu; 7. **1** + Au^+ + Iso; 8. **1** + Au^+ + Pro; 9. **1** + Au^+ + Met; 10. **1** + Au^+ + Thr; 11. **1** + Au^+ + Try; 12. **1** + Au^+ + Gly; 13. **1** + Au^+ + Ser; 14. **1** + Au^+ + Asp; 15. **1** + Au^+ + Phe; 16. **1** + Au^+ + Glu; 17. **1** + Au^+ + Tyr; 18. **1** + Au^+ + Lys; 19. **1** + Au^+ + His; 20. **1** + Au^+ + Arg.

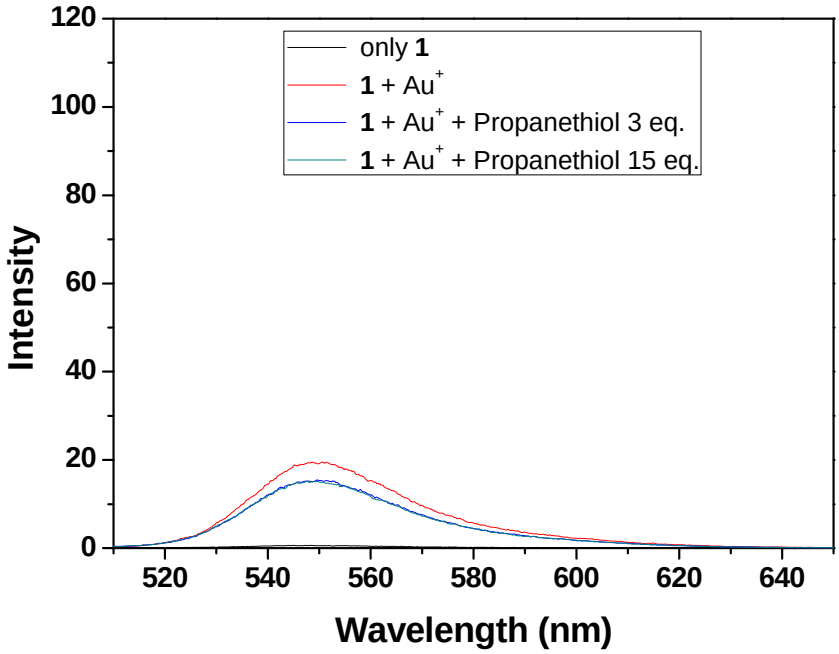


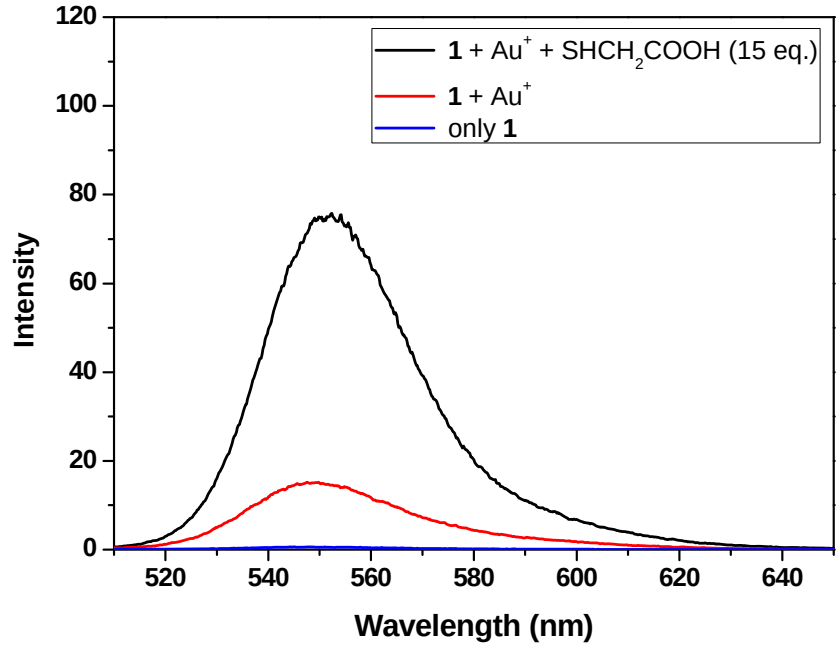
Job's plot according to the method of continuous variations, indicating the 1:1 binding stoichiometry of **1**–Au^+/Cys.



10. Fluorescence spectra of 1–Au^+ with propanethiol and thioglycolic acid

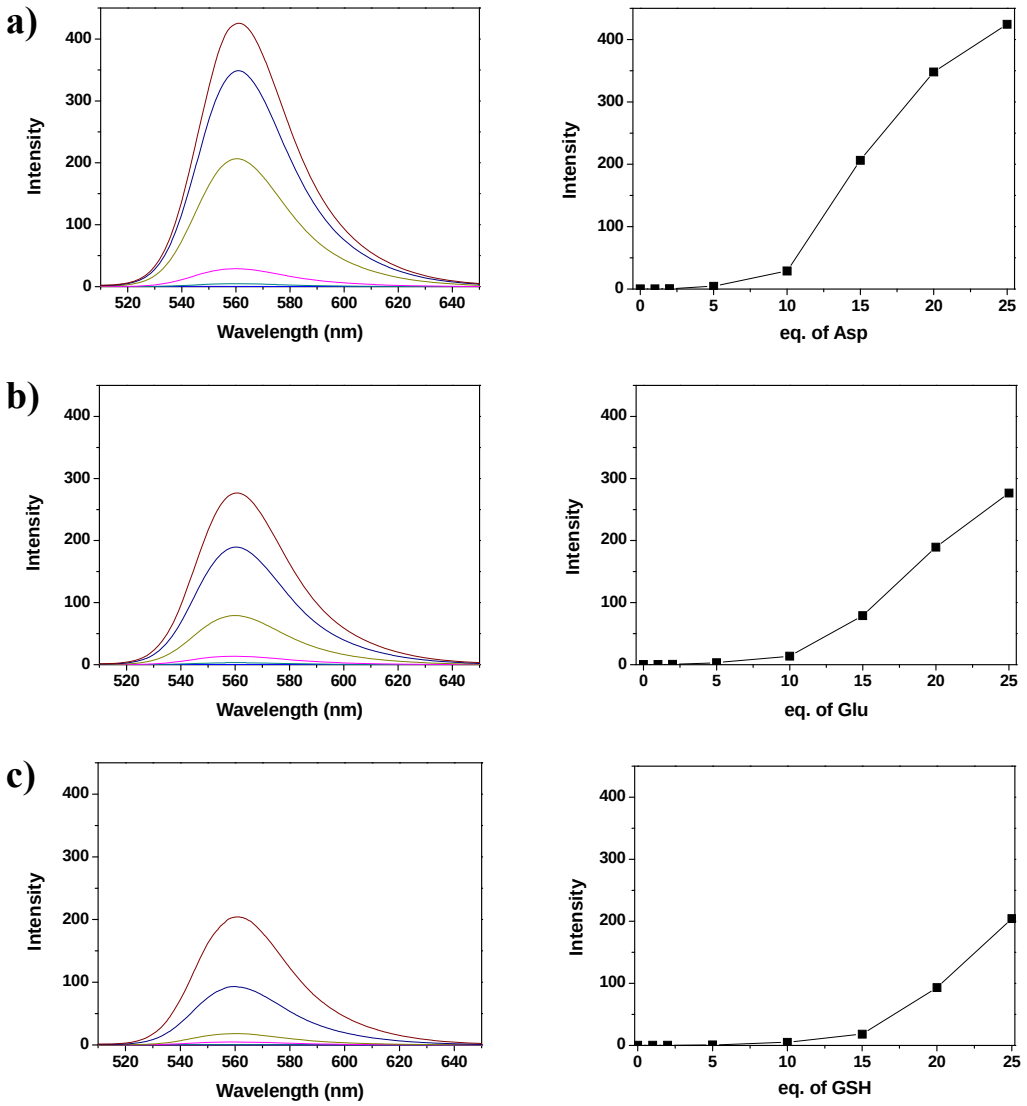
Fluorescence response (λ_{ex} = 500 nm) of **1**-Au^+ upon addition of propanethiol and thioglycolic acid in H_2O (MeOH 1%) at 25 °C. [**1**] = 1.0×10^{-5} M; [Au^+] = 1 equiv; [Propanethiol] = 3 equiv and 15 equiv; [Thioglycolic acid] = 15 equiv.





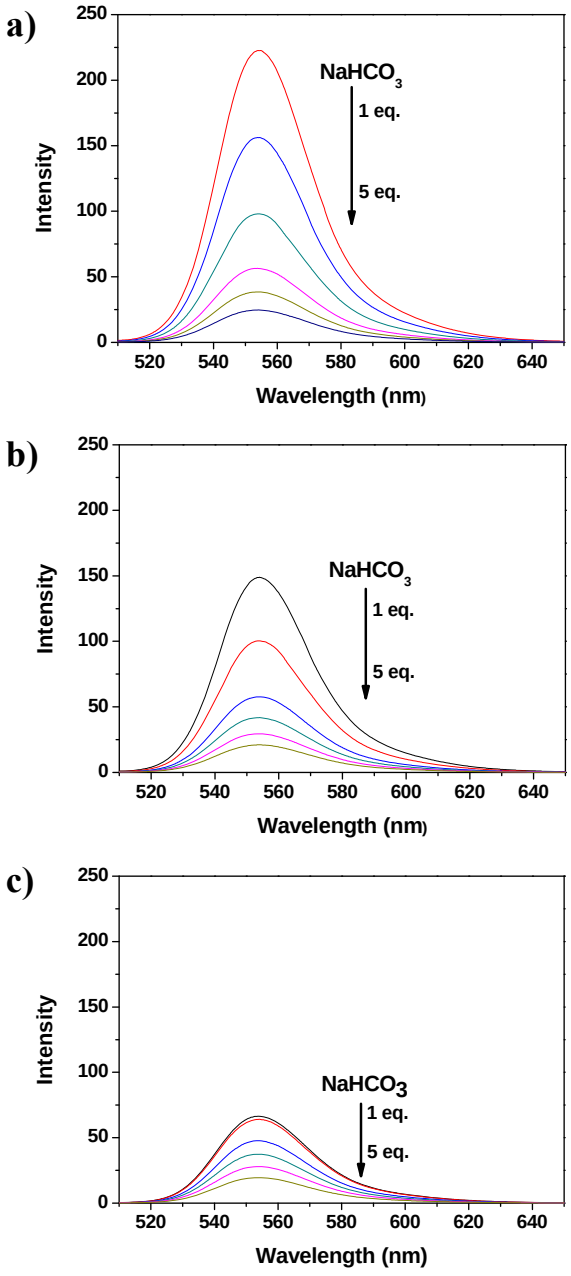
11. Fluorescence spectra of 1 with amino acids (Asp, Glu, GSH)

Fluorescence response (λ_{ex} = 500 nm, λ_{em} = 560 nm) of **1** (1.0×10^{-5} M) upon addition of Asp, Glu and GSH in H_2O (MeOH 1%) at 25 ^{o}C.



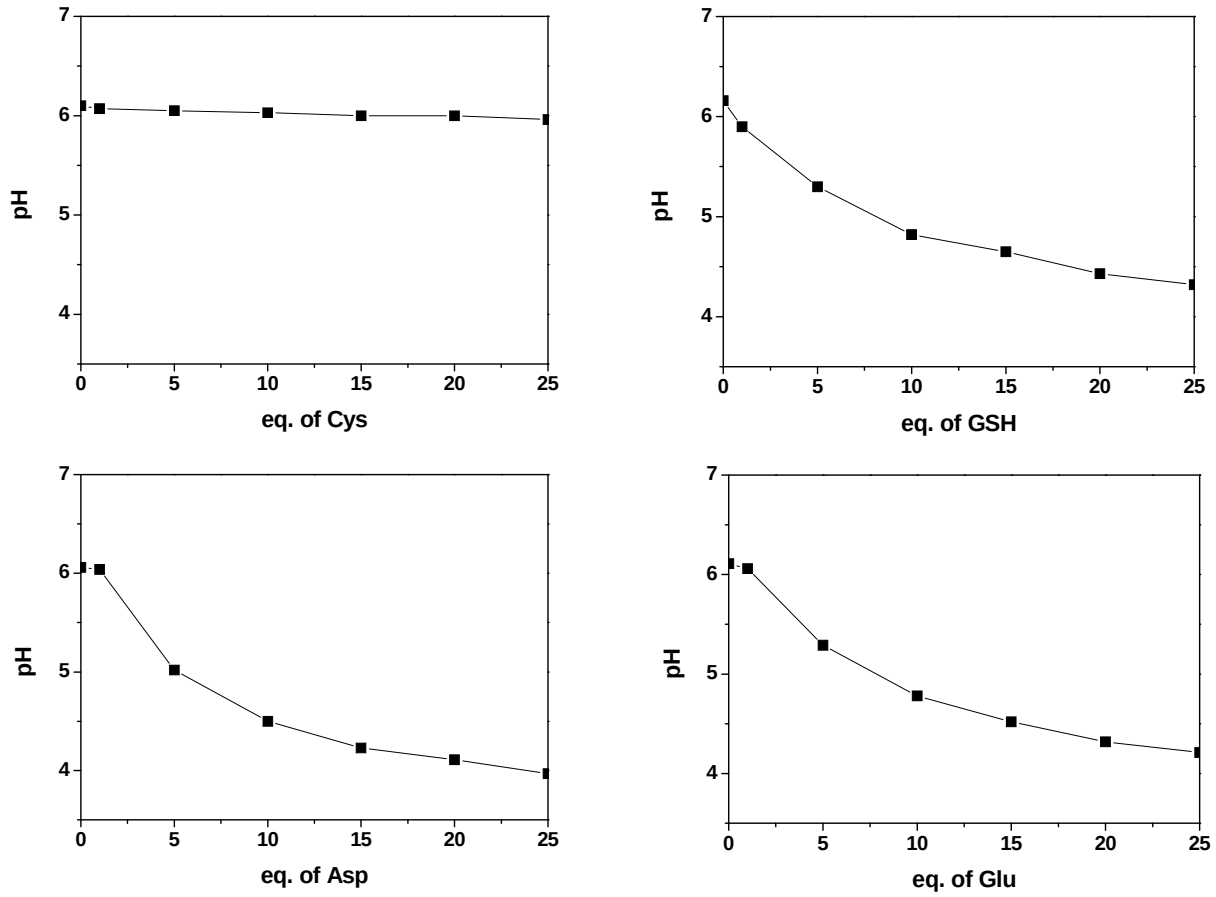
12. Fluorescence spectra of 1–amino acids with $NaHCO_3$

Fluorescence response (λ_{ex} = 500 nm) of **1** with Asp, Glu and GSH upon additions of $NaHCO_3$ (1, 2, 3, 4, and 5 equiv) in H_2O (MeOH 1%) at 25 ^oC. [**1**] = 1.0×10^{-5} M; a) Asp 10 equiv; b) Glu 10 equiv; c) GSH 10 equiv.



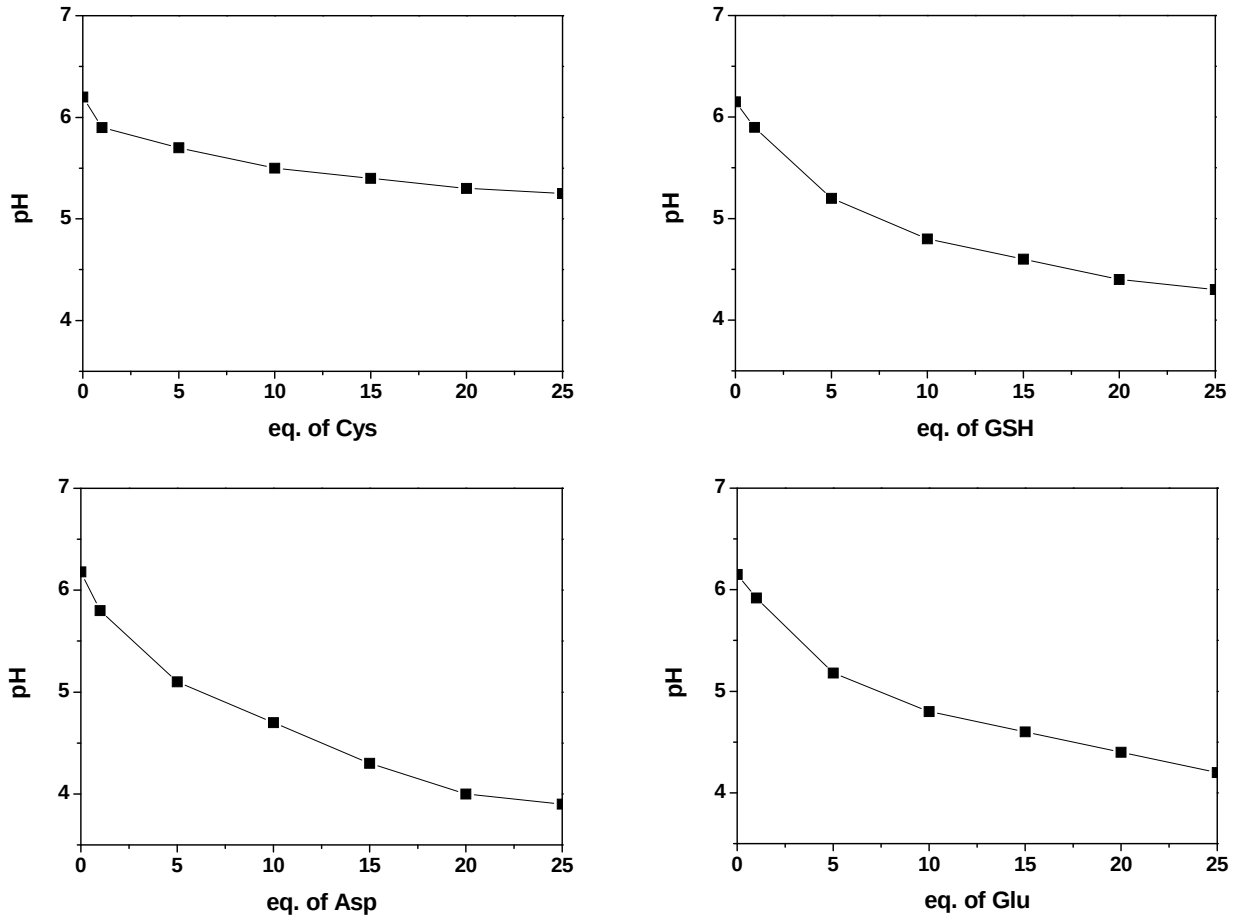
13. The pH value changes depending on acidic amino acid

Figure shows the pH value changes depending on Cys, GSH, Asp and Glu (1, 5, 10, 15, 20, 25; respectively) with Au^{+} (1 equiv) in H_2O (MeOH 1%) at 25 ^{o}C.



14. The pH value changes depending on acidic amino acid with probe 1

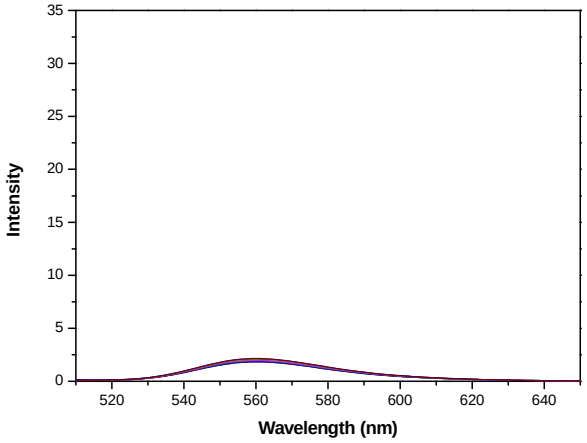
Figure shows the pH value changes depending on Cys, GSH, Asp and Glu (1, 5, 10, 15, 20, 25; respectively) with **1**–Au^{+} (1 equiv) in H_2O (MeOH 1%) at 25 ^{o}C.



15. Fluorescence spectra of 1–Au^{+} with amino acids (GSH, Asp, Glu) at pH 7.0

Fluorescence response (λ_{ex} = 500 nm) of **1** (10 μM) with Au^{+} (1 equiv) upon addition of GSH (a), Asp (b) and Glu (c) (0, 1, 5, 10, 15, 20, 25 equiv) in PBS-buffer (pH 7.0, MeOH 1%) at 25 ^{o}C, respectively.

a)



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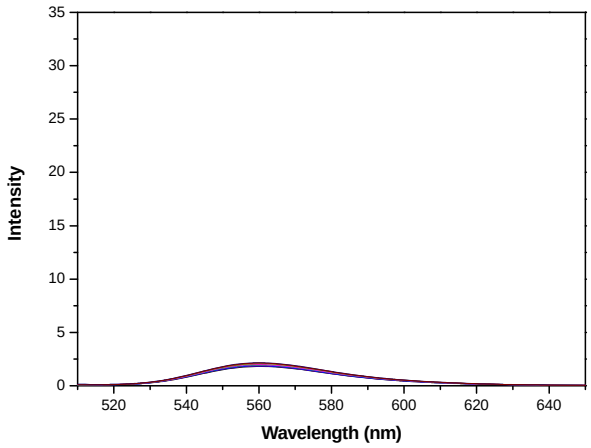
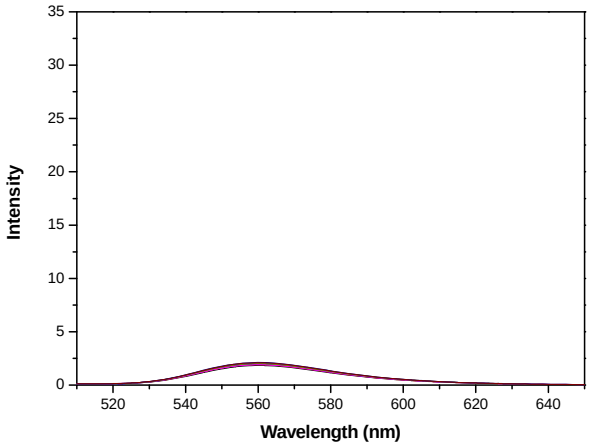
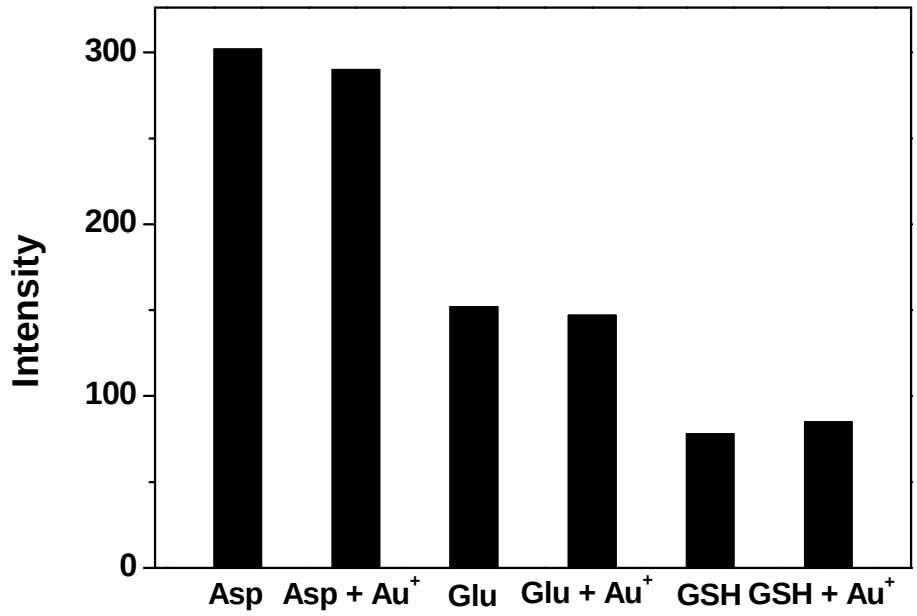


c)



16. Fluorescence spectra of 1 in the absence and presence of Au^+ with acidic amino acids

Fluorescence response (λ_{ex} = 500 nm, λ_{em} = 560 nm) of **1** (10 μM) with Au^+ (1 equiv) upon addition of Asp (20 equiv) Glu (20 equiv) and GSH (20 equiv) in H_2O (MeOH 1%) at 25 ^oC, respectively.



17. Color changes of 1–Au^+ in the presence of different amino acids

Color change of the H_2O (MeOH 1%) solution of **1**–Au^+ in the presence of different amino acids; 1. **1**–Au^+; 2. **1**–Au^+ + His; 3. **1**–Au^+ + Gly; 4. **1**–Au^+ + Cys; 5. **1**–Au^+ + Leu; 6. **1**–Au^+ + Ser; 7. **1**–Au^+ + Asn; 8. **1**–Au^+ + Hcy; 9. **1**–Au^+ + Ala; 10. **1**–Au^+ + Val; 11. **1**–Au^+ + Arg; 12. **1**–Au^+ + Iso; 13. **1**–Au^+ + Pro; 14. **1**–Au^+ + Met; 15. **1**–Au^+ + Try; 16. **1**–Au^+ + Thr; 17. **1**–Au^+ + Gln; 18. **1**–Au^+ + Phe; 19. **1**–Au^+ + Tyr; 20. **1**–Au^+ + Lys.

[**1**] = 2.0×10^{-5} M, [Au^+] = 1 equiv, [amino acid] = 10 equiv.

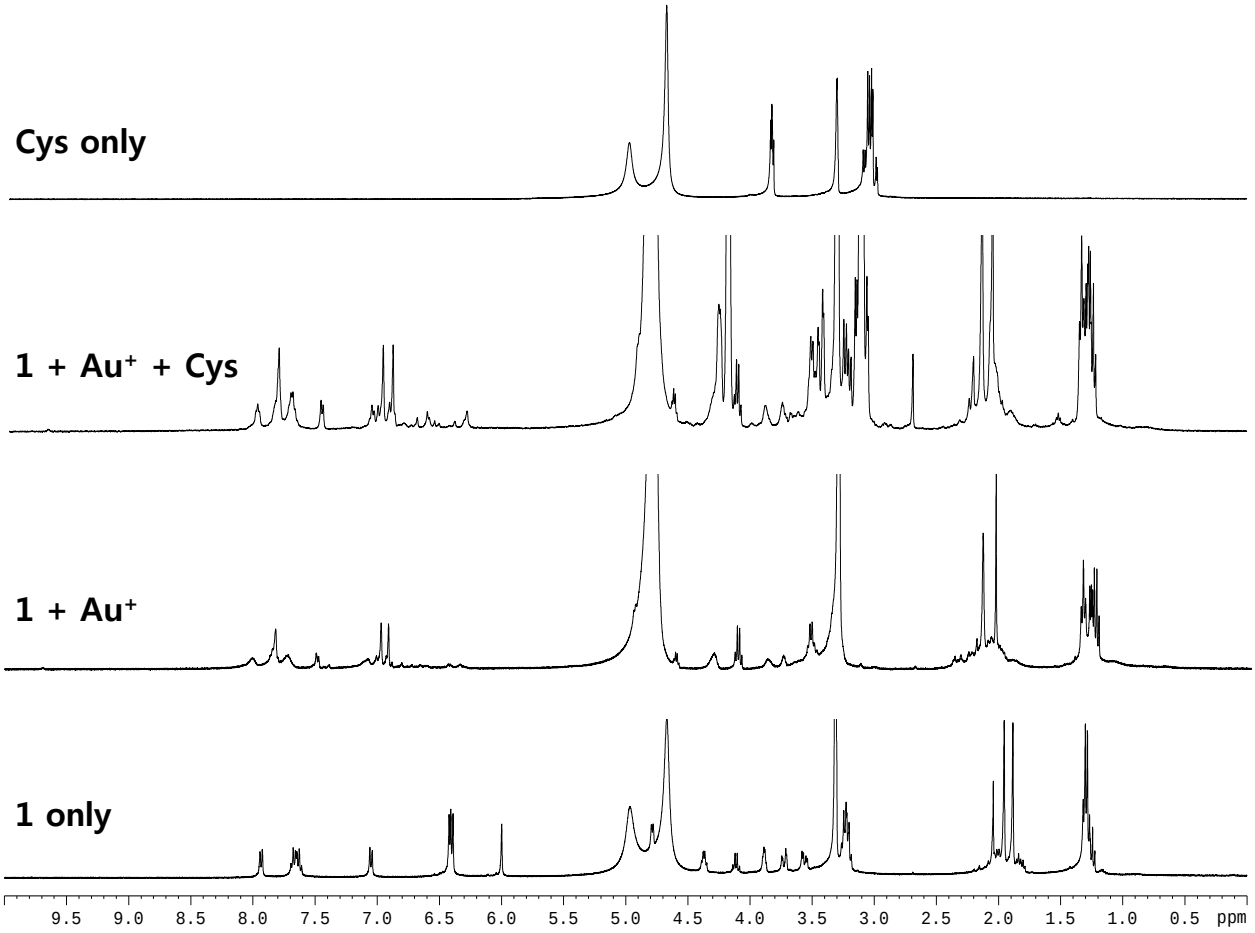
Color Changes

1 2 3 4 5 6 7 8 9 10

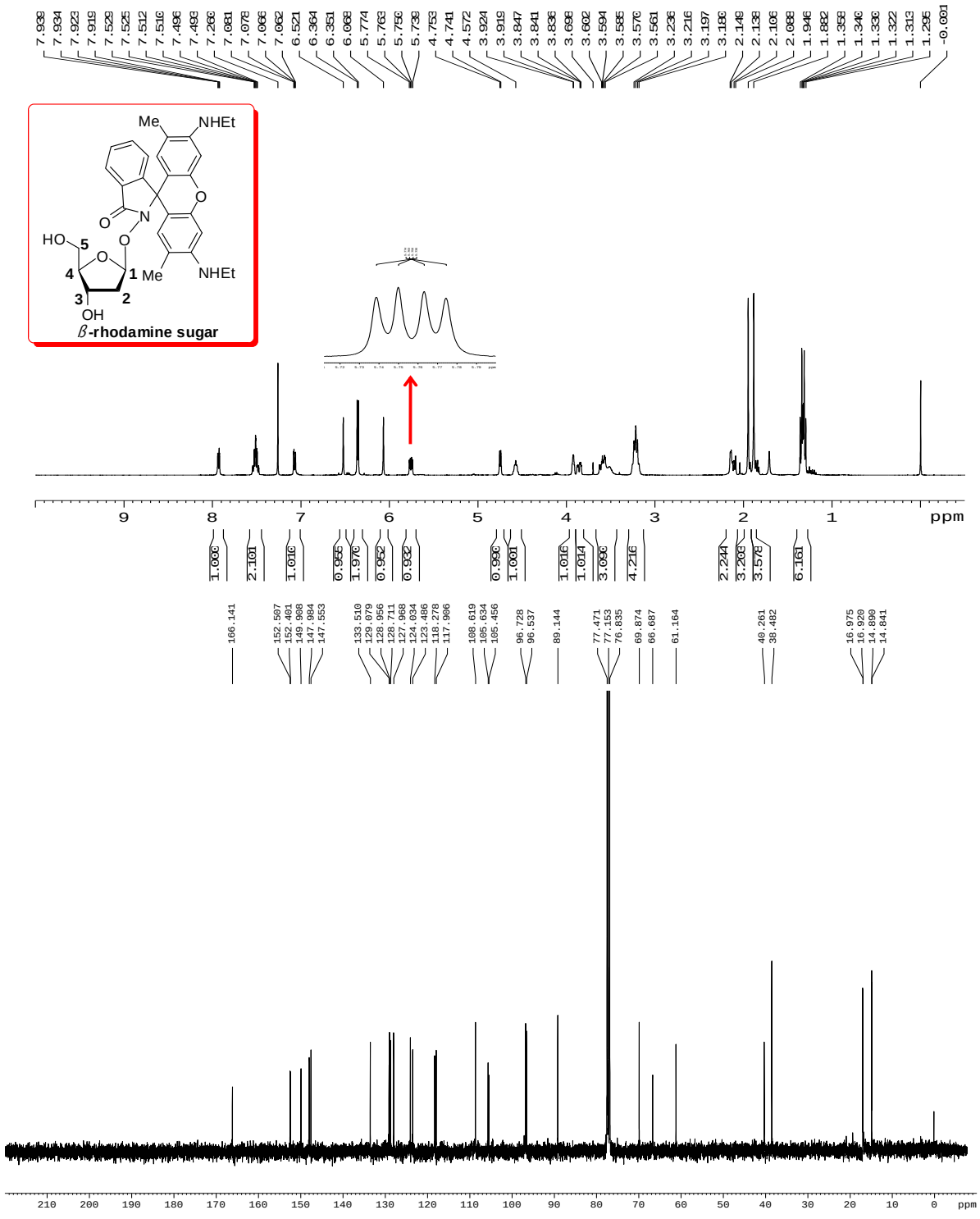
11 12 13 14 15 16 17 18 19 20

18. ^{1}H NMR spectra of 1, 1–Au^+ and 1–Au^+ + Cys in CD_3OD:D_2O at 25 ^{o}C

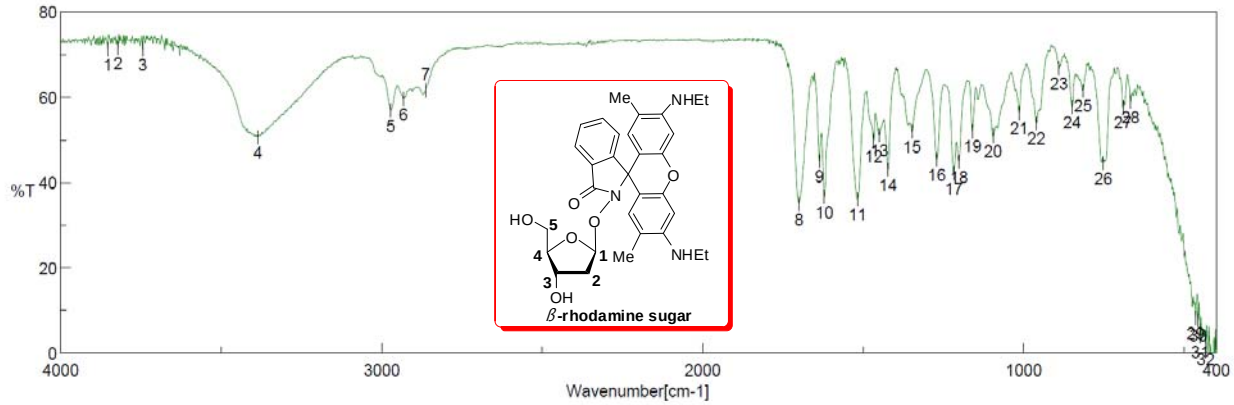
^{1}H NMR spectra (400 MHz) were performed with a CD_3OD:D_2O (2:1 v/v) solution of **1** (0.0046 M) and a CD_3OD:D_2O (2:1 v/v) solution of AuCl (0.046 M) and Cys (0.46 M). The AuCl solution was introduced in portions (60 μL corresponds to 1.5 equiv) and, after addition of Cys (40 μL corresponds to 10 equiv) the solution was maintained for 5 min at 25 ^{o}C.



19. ^{1}H NMR and ^{13}C NMR spectra of 1



20. IR spectra of 1



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Operator			
File Name	Memory#1		
Sample Name	Au-6		
Comment			

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9: 1636.3, 45	10: 1620.88, 36.6329	11: 1517.7, 36.0597	12: 1467.08, 49.8143
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25: 815.742, 61.6041	26: 752.102, 44.5815	27: 690.873, 57.6619	28: 666.767, 58.9339
29: 464.278, 8.26476	30: 456.565, 7.70675	31: 450.297, 3.83117	32: 433.423, 2.13147
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