Supporting Information

for

Iminocoumarin-Cu(II) ensemble-based chemodosimeter toward thiols

Hyo Sung Jung,[†] Ji Hye Han,[‡] Yoichi Habata,[‡] Chulhun Kang[§] and Jong Seung Kim *[†]*

[†]Department of Chemistry, Korea University, Seoul, 136-704, Korea;
[‡]Department of Chemistry, Toho University, Chiba, 274-8510, Japan;
[§]The School of East-West Medical Science, Kyung Hee University, Yongin 446-701, Korea.

E-mails: kangch@khu.ac.kr; jongskim@korea.ac.kr

- **Methods**

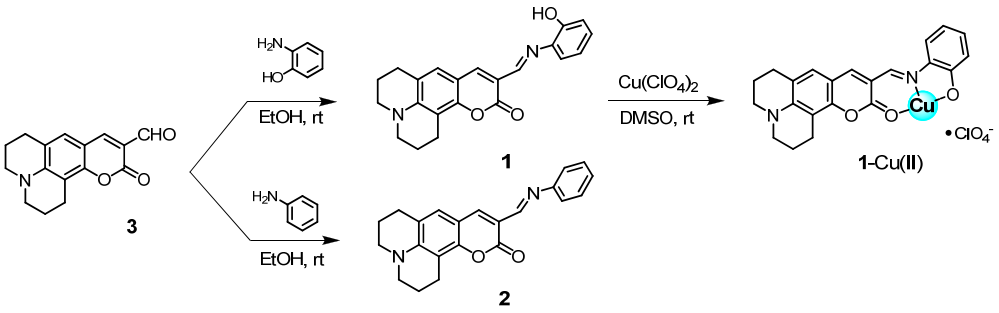
General information and materials. All fluorescence and UV/Vis absorption spectra were recorded with Shimadzu RF-5301PC and Shinco S-3100 spectrophotometers, respectively. All respective NMR and mass spectra were recorded with a Varian instrument (400 MHz) and JMS-700 MStation mass spectrometer. All analytes were purchased from Aldrich and used as received. All solvents were analytical reagents from Duksan Pure Chemical Co., Ltd. All DMSO for spectra detection was HPLC reagent grade, without fluorescent impurity; H_2O was deionized.

Spectroscopic Data. Stock solutions (1.0 mM) of the biological relevant analytes [amino acids (Cys, Hcy, GSH, Ala, Arg, Asn, Asp, Gln, Gluc, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Tau, Thr, Trp, Tyr, Val) were prepared in twice-distilled water. Stock solutions of **1**-Cu(II) (0.5 mM) were prepared in DMSO by mixing **1** and $Cu(ClO_4)_2$ with the ratio of 1:1.2. Stock solutions of compound **2** and **3** (0.5 mM) were also prepared in DMSO. Typically, test solutions were prepared by placing 40 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each analyte stocks, and diluting the solution to 4 mL with PBS buffer solution (10 mM, pH 7.4). For all measurements of fluorescence spectra, excitation was at 479 nm with all excitation slit widths is 1.5 nm, that of emission is 1.5 nm. UV/Vis and fluorescence titration experiments were performed using 5 μM and 2 μM of **1**, **1**-Cu(II), **2** and **3** in 1.0 % DMSO aqueous solution (pH 7.4, 10 mM PBS buffer) with varying concentrations of the metal perchlorate salts and amino acids at room temperature.

Cell culture, NEM and compound 1 treatment. A human hepatoma cell line, HepG2, was grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, 1% penicillin, and 10,000 Unit/mL of streptomycin at 37 °C under humidified air containing 5% CO_2. Cells (1.0×10^5) were located and stabilized in a single well of a 24-well plate. When 80% confluence was reached, the cells were washed with 1.0 mL of phosphate buffered saline (PBS) twice and finally incubated with 1.0 mL of PBS containing **1** (final concentration of 2.0 μM) for the following confocal experiment. For the *N*-ethyl maleimide (NEM) treated samples, before the media were finally replaced with PBS containing compound **1** (the concentration of its stock solution was 1mM in DMSO), the cells were incubated with the media containing NEM at variable concentrations for 1 h at 37 °C. The fluorescence images in confocal experiments were obtained at 1 min after the cells were treated with compound **1**.

Fluorescence imaging. Fluorescence images of the cells treated with compound **1** were undertaken by a confocal microscope from Carl Zeiss (LSM 510 META model). After compound **1** was added to the cells, the images were obtained in 2 min. The excitation source was a 488 nm argon laser and an emission image was acquired using a longpath filter (> 505 nm). All images were taken under the same experimental parameters to minimize possible variations in fluorescence intensity.

- **Synthesis**



Scheme S1. Synthetic pathways to compounds **1**, **2** and **1**-Cu(II).

Synthesis of compound 1.

A portion of **3**[S1] (269 mg, 1 mmol) and 2-aminophenol (153 mg, 1.4 mmol) were combined in absolute ethanol (10 mL) to yield a scarlet precipitate for a moment. The solution was stirred under reflux conditions for 4 hours, and the precipitate was filtrated, washed with absolute ethanol three times, then recrystallized with C_2H_5OH to get red crystal **1** (312.3 mg) in 87% yield. m.p. 170 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ 8.68 (s, 1H; CH), 8.51 (s, 1H; CH), 7.35 (d, J = 8.37 Hz, 1H; CH), 7.16 (t, J = 7.70 Hz, 1H; CH), 7.03 (s, 1H; CH), 6.99 (d, J = 8.22 Hz, 1H; CH), 6.98 (t, J = 7.62 Hz, 1H; CH), 3.33 (q, J = 5.62 Hz, 4H; CH_2), 2.91 (t, J = 6.42 Hz, 2H; CH_2), 2.78 (t, J = 4.00 Hz, 2H; CH_2), 1.99 (m, 4H). ^{13}C NMR (100 MHz, CDCl3): 163.0, 154.8, 152.9, 149.2, 147.8, 142.1, 134.5, 127.3, 126.6, 125.4, 124.3, 119.5, 118.0, 114.2, 109.0, 106.5, 50.5, 50.1, 27.7, 21.5, 20.6, 15.0. FAB-MS calc. for $C_{22}H_{20}N_2O_3$ $[M+H]^+$ 361.2, found 361.3.

Synthesis of compound 2.

Synthetic procedures are similar to that of **1** to give 275.5 mg, 0.80 mmol in 80% yield. m.p. 168 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1H), 8.42 (s, 1H), 7.37 (t, J = 7.94 Hz, 2H), 7.24 (m, 3H), 6.98 (s, 1H), 3.33 (q, J = 5.62 Hz, 4H), 2.91 (t, J = 6.42 Hz, 2H), 2.78 (t, J = 4.00 Hz, 2H), 1.99 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$): 162.8, 155.2, 152.7, 151.9, 147.7, 141.3, 129.2, 126.9, 125.9, 121.2, 119.3, 114.0, 108.6, 106.2, 50.2, 49.8, 29.7, 27.5, 21.2, 20.3, 20.2; FAB-MS calc. for $C_{22}H_{20}N_2O_2$ $[M+H]^+$ 345.2, found 345.3.

Ref. S1 N. C. Lim, J. V. Schuster, M. C. Porto, M. A. Tanudra, L. Yao, H. C. Freake and C. Brückner, *Inorg. Chem.*, 2005, **44**, 2018–2030.

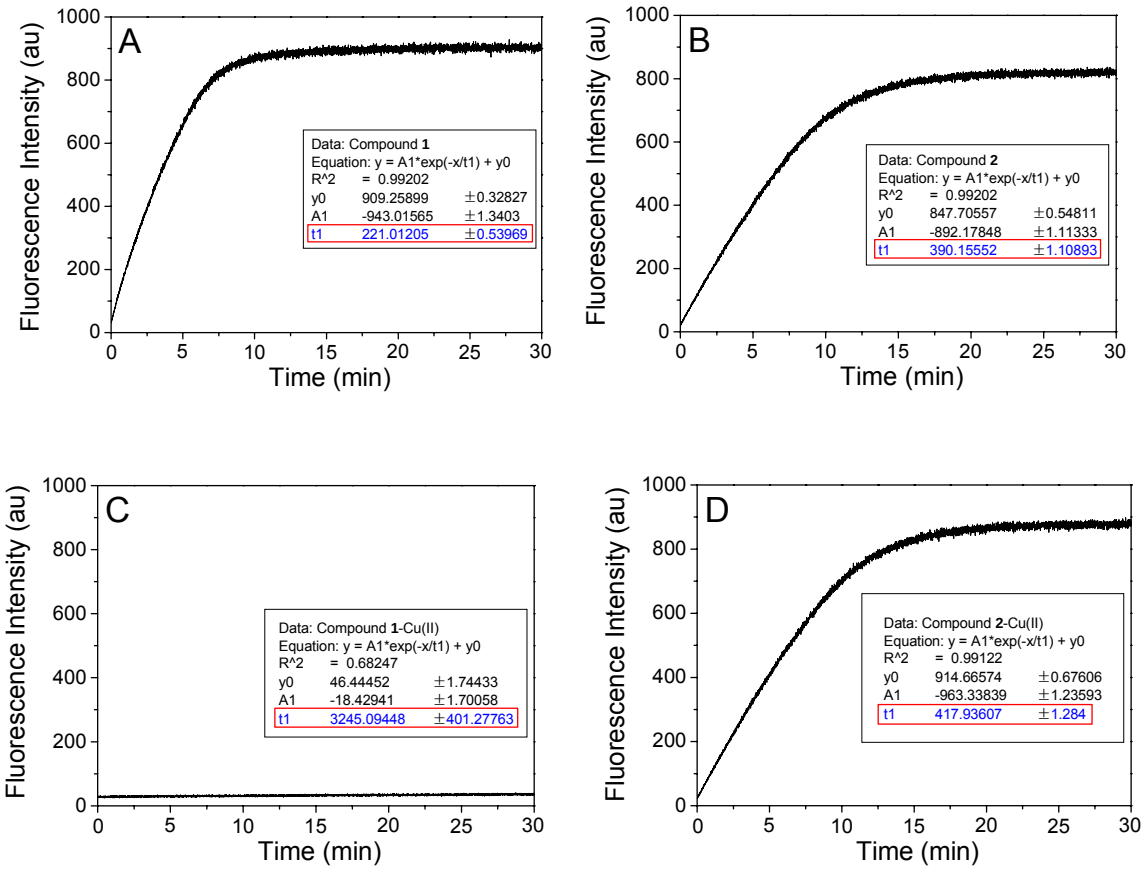


Figure S1. Time course of the fluorescence response of (a) **1**, (b) **2**, (c) **1**-Cu(II), and (d) **2**-Cu(II) (2 μM, respectively) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0 % DMSO). The excitation and emission wavelength were 479 and 514 nm, respectively. The boxes indicate kinetic analysis results based on first order decay model.

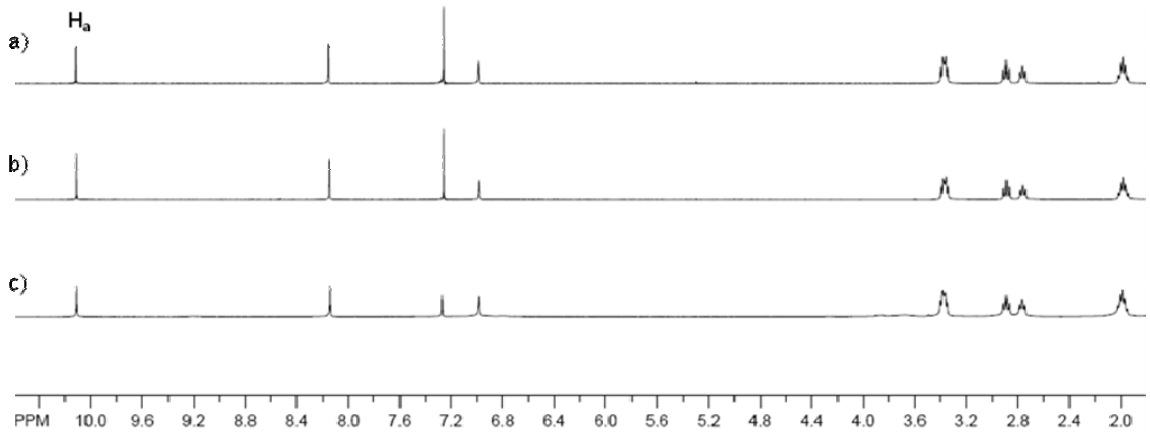


Figure S2. ^{1}H NMR spectra of coumarinaldehyde in $CDCl_3$; a) synthesized **3**; b) isolated from **1** and c) the mixture of **1**-Cu(II) in the presence of GSH in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% CH_3CN).

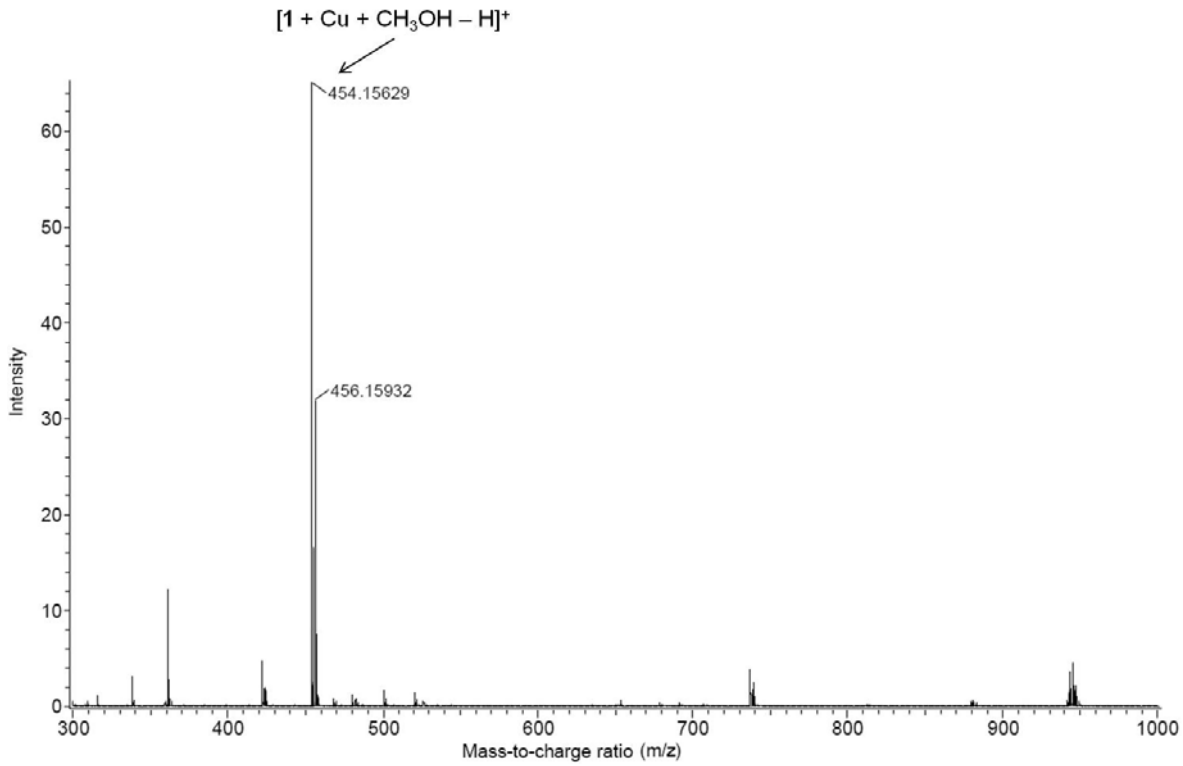


Figure S3. ESI-MS of **1**-Cu(II).

Table S1. Photophysical data of reaction mixtures [a]

The added Compounds	Absorbance max (nm)	Emission max (nm)	Relative quantum yield (Φ_f)
1	464	514	0.67
2	464	514	0.65
3	464	514	0.66
1-Cu(II)	522	514	0.02
1-Cu(II) + Thiol (10 eq)	464	514	0.66

[a] Φ_f: Relative Fluorescence Quantum Yield (fluorescein in 0.1N NaOH as a reference, Φ_f = 0.85).[S1] After compounds (2.0 μM) were added to the buffered solution, the photophysical data of the reaction mixture were obtained after 30 min at room temperature.

Ref. S2 J.-S. Wu, W.-M. Liu, X.-Q. Zhuang, F. Wang, P.-F. Wang, S.-L. Tao, X.-H. Zhang, S.-K. Wu and S.-T. Lee, *Org. Lett.* 2007, **9**, 33.

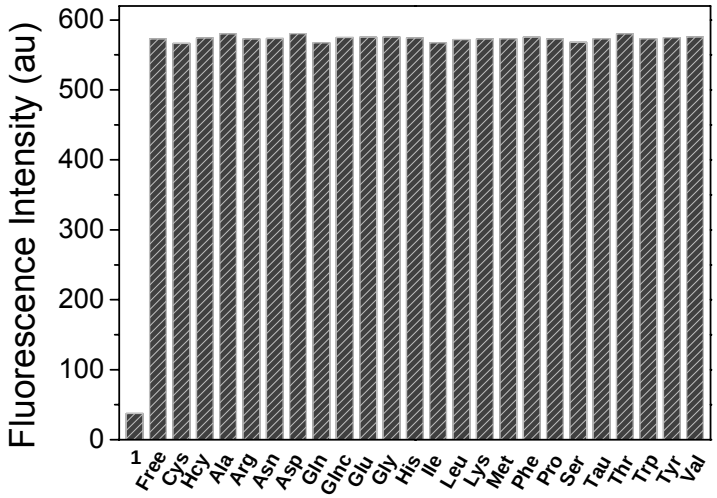


Figure S4. Fluorescence emission histogram of probe **1**-Cu(II) (2.0 μM) toward GSH (10 equiv) in the presence of different competing amino acids (10 equiv) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO). The wavelengths for excitation and emission were 479 nm and 514 nm, respectively.

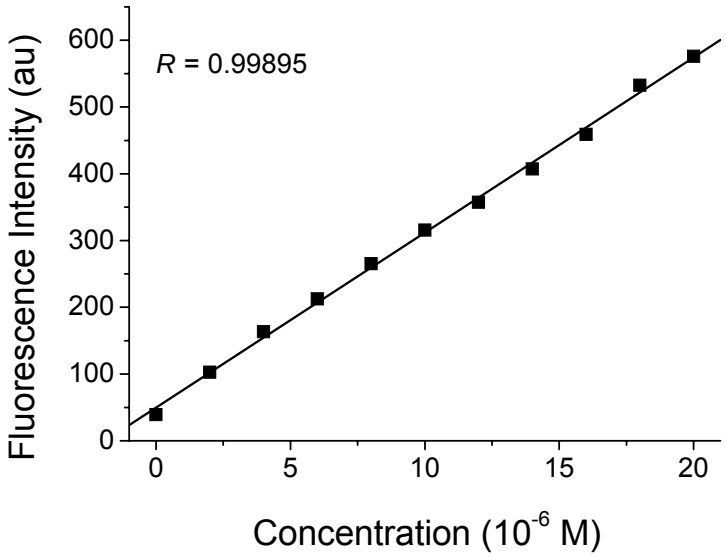


Figure S5. Fluorescence responses of **1**-Cu(II) (2.0 μM) in the presence of sub-micromolar level of GSH. The emission intensity at 514 nm was measured at 5 min after the various amount of GSH were added. The excitation wavelength was 479 nm.

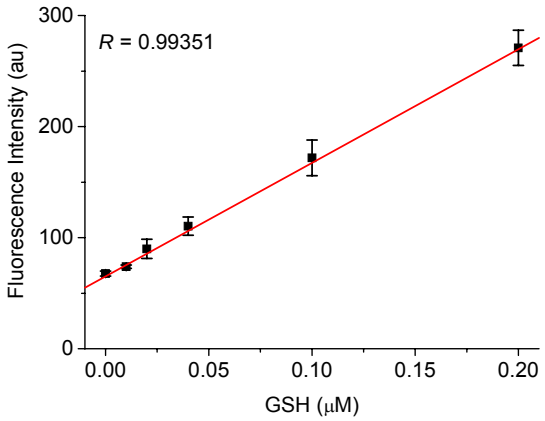


Figure S6. Fluorescence responses of **1**-Cu(II) (2.0 μM) in the presence of sub-nanomolar level of GSH. The emission intensity at 514 nm was measured at 5 min after the various amount of GSH were added. The excitation wavelength was 479 nm. The error bars indicate the standard deviation (n=3).

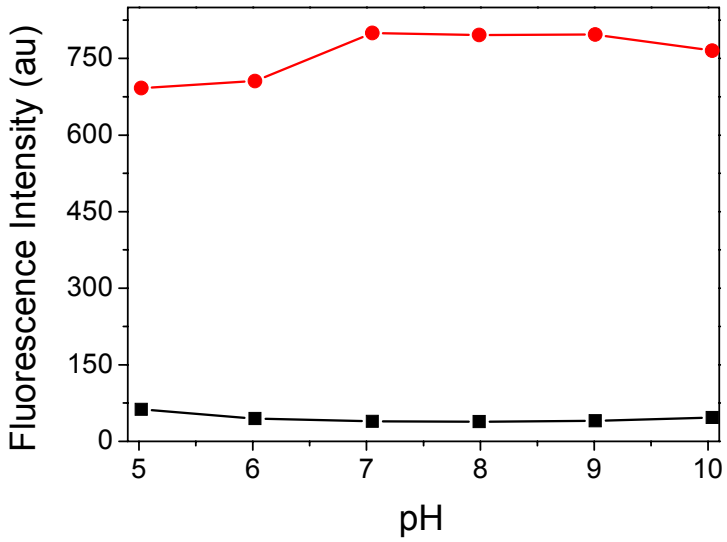


Figure S7. pH-dependent fluorescence profiles of **1**-Cu(II) (2.0 μM) and the corresponding GSH (10 eq) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO). Excitation at 479 nm after 10 min. (slit = 1.5/1.5).

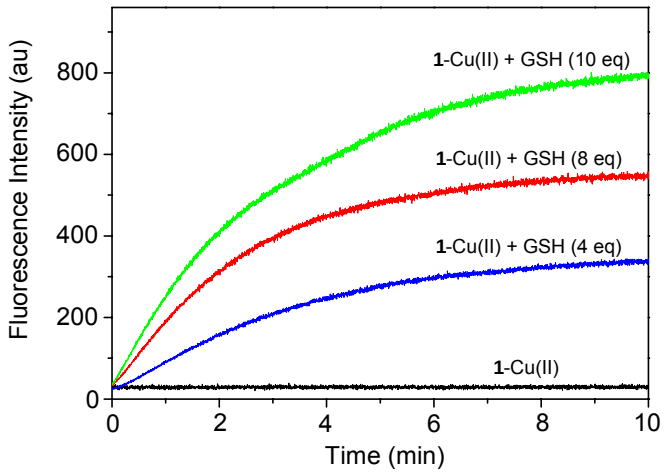


Figure S8. Time course of the fluorescence response of **1**-Cu(II) (2.0 μM, respectively) in the presence of various concentration of GSH in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0 % DMSO). The excitation and emission wavelength were 479 and 514 nm, respectively.

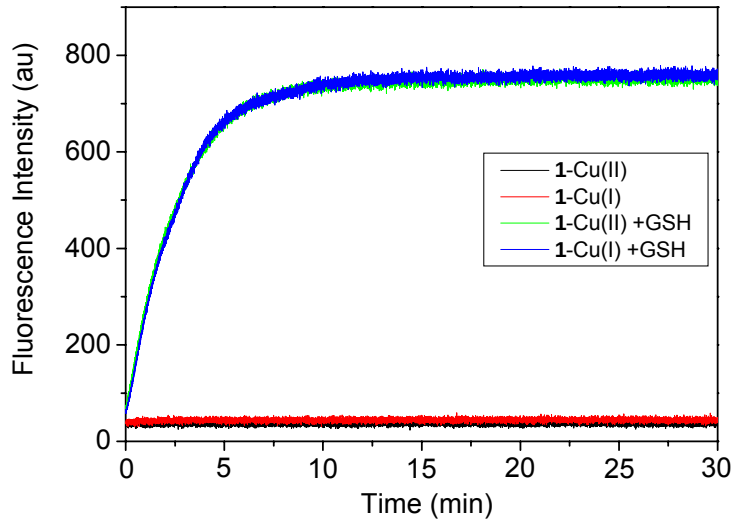


Figure S9. Time course of the fluorescence response of **1**-Cu(II) and **1**-Cu(I) (2.0 μM, respectively) in the absence or presence of GSH (10 eq) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0 % DMSO). The excitation and emission wavelength were 479 and 514 nm, respectively.

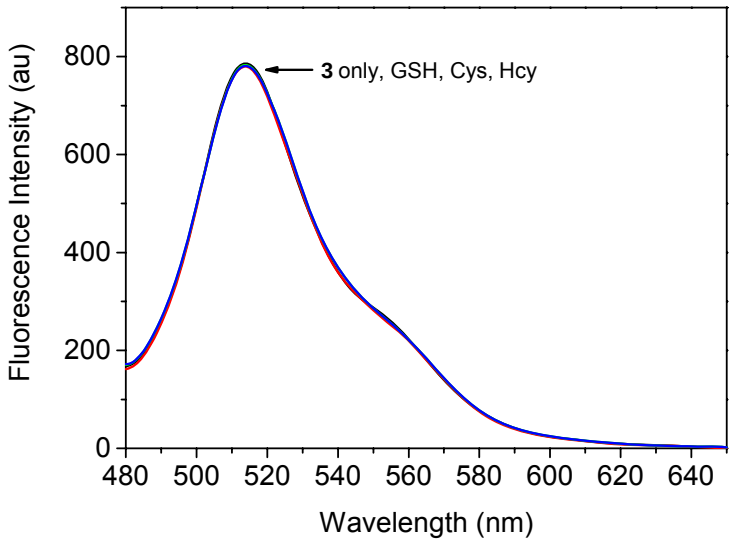


Figure S10. Fluorescence spectra (2.0 μM) of **3** in aqueous solution (10 mM PBS buffer, pH 7.4, 10 % DMSO) in the presence of thiols (10 eq, respectively) with an excitation at 479 nm after 30 min (slit=1.5/1.5).

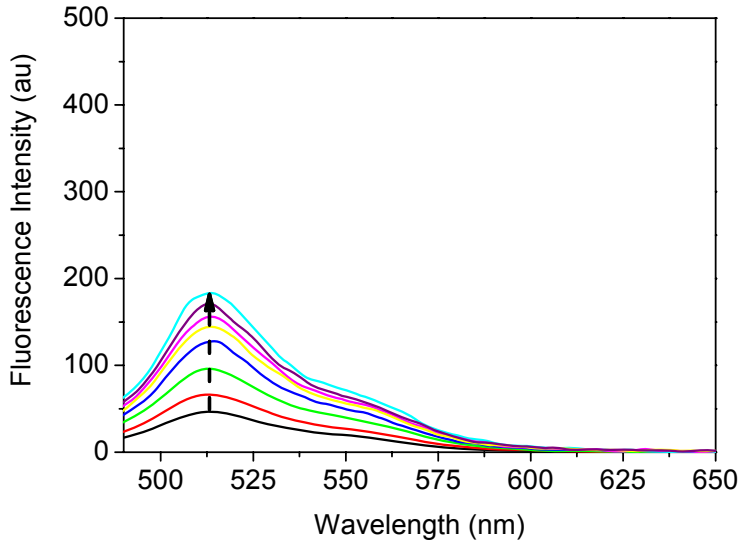


Figure S11. Fluorescence spectra (2.0 μM) of **1**-Cu(II) in aqueous solution (10 mM PBS buffer, pH 7.4, 1% DMSO) upon addition of various concentrations of Cys (0 – 14 eq to [**1**-Cu(II)]) after 5 min. Excitation at 479 nm (slit = 1.5/1.5).

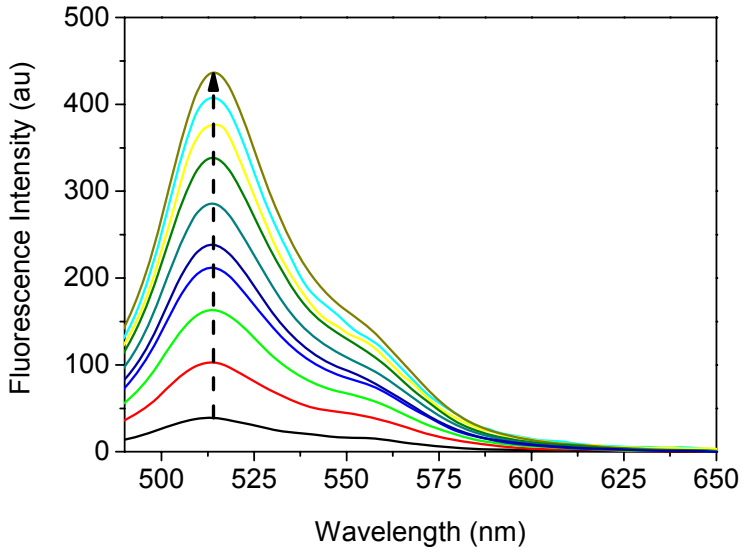


Figure S12. Fluorescence spectra (2.0 μM) of **1**-Cu(II) in aqueous solution (10 mM PBS buffer, pH 7.4, 1% DMSO) upon addition of various concentrations of Hcy (0 – 14 eq to [**1**-Cu(II)]) after 5 min. Excitation at 479 nm (slit = 1.5/1.5).

- **FAB-MS data**

Mass spectrometric data were obtained on a JMS-700 MStation mass spectrometer in Korea University (Korea).

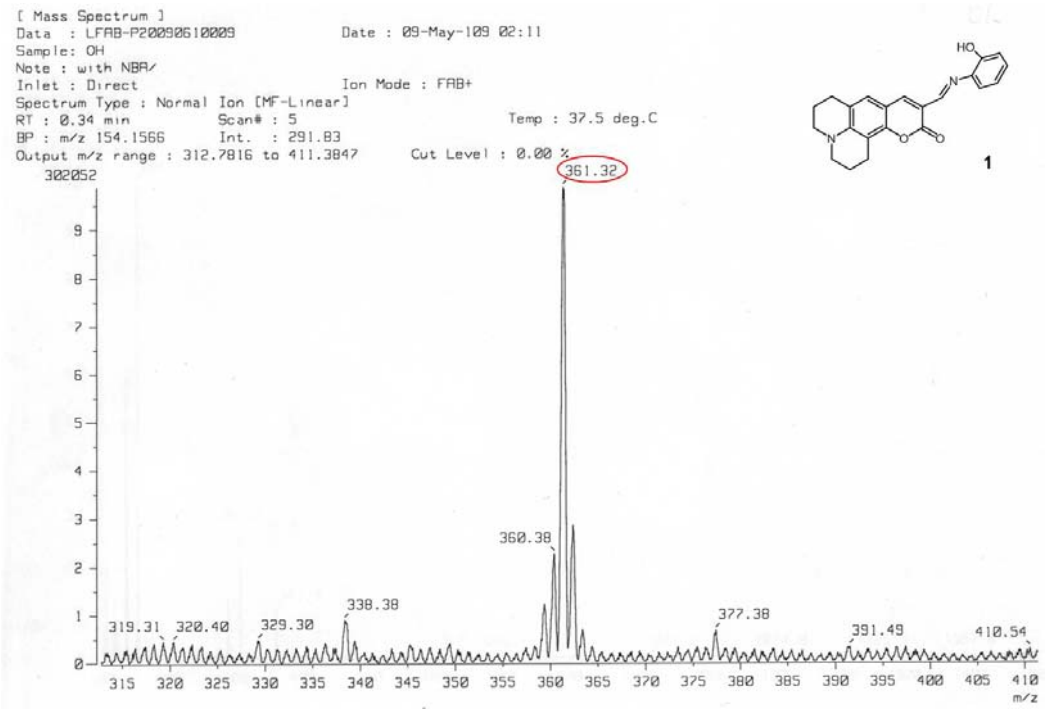


Figure S13. FAB-MS of **1**.

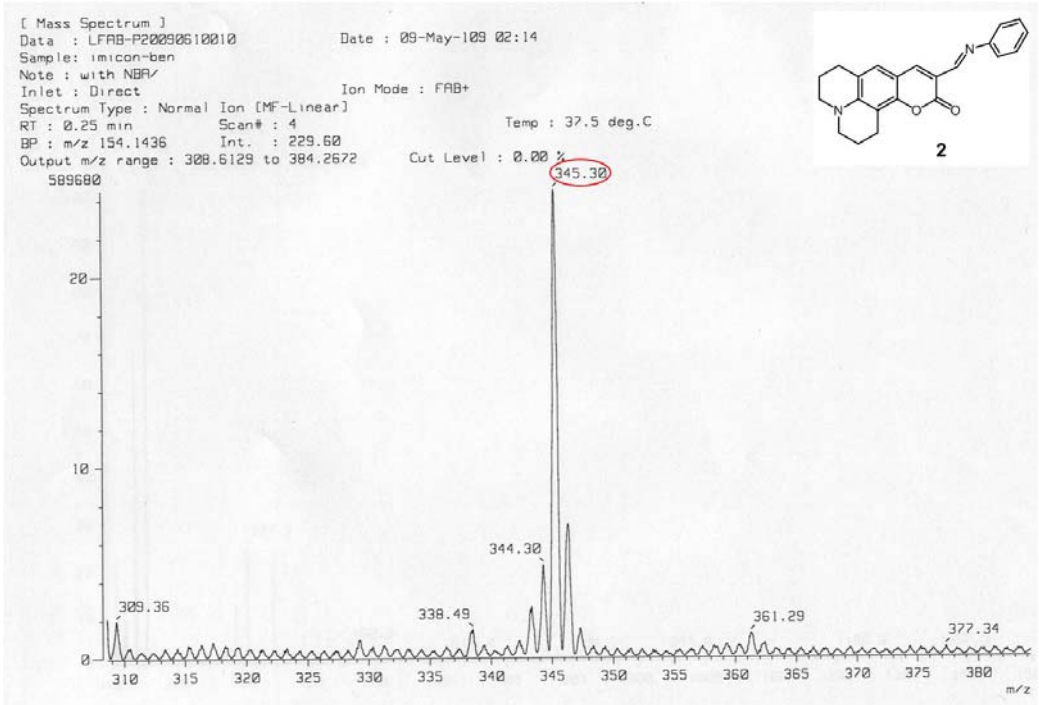


Figure S14. FAB-MS of **2**.

- **^{1}H NMR and ^{13}C NMR copies**

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a VARIAN (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) spectrometer with chemical shifts reported as ppm (in $CDCl_3$, TMS as internal standard).

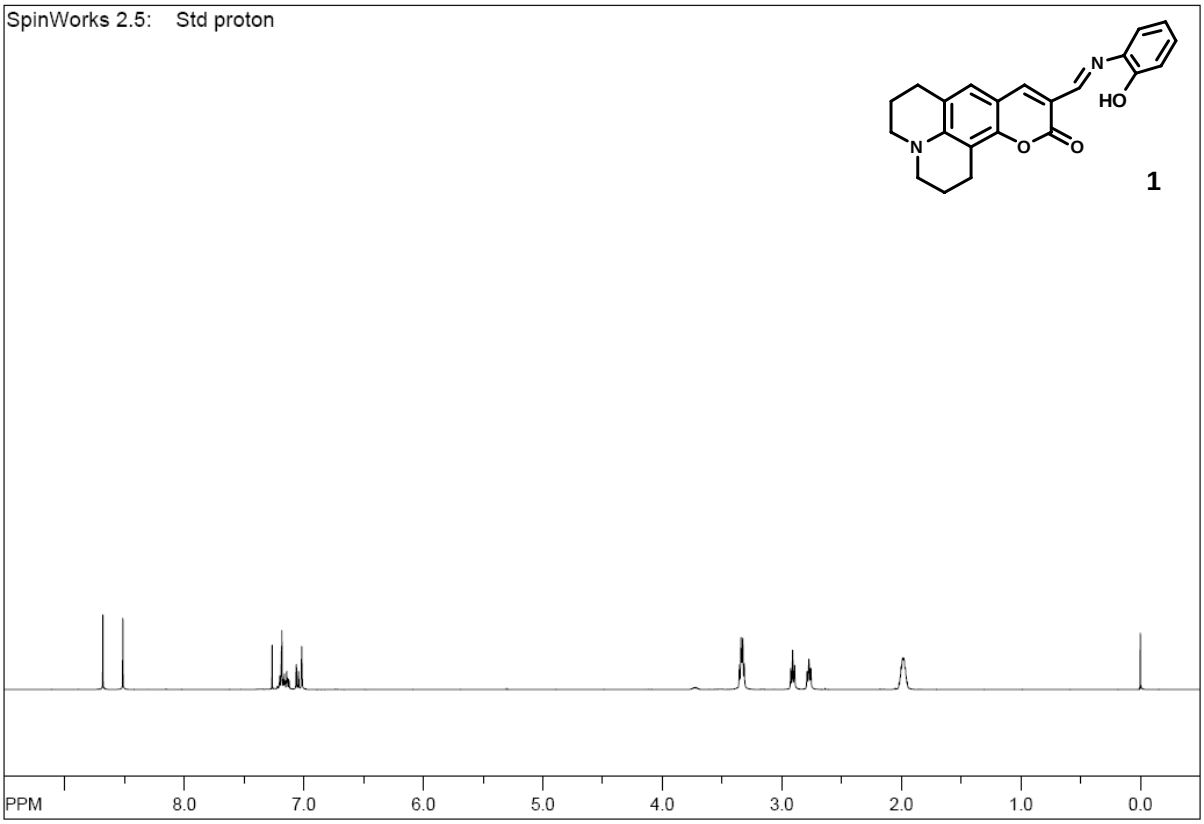


Figure S15. ^{1}H NMR spectra (400 MHz) of **1** (10 mM) in $CDCl_3$.

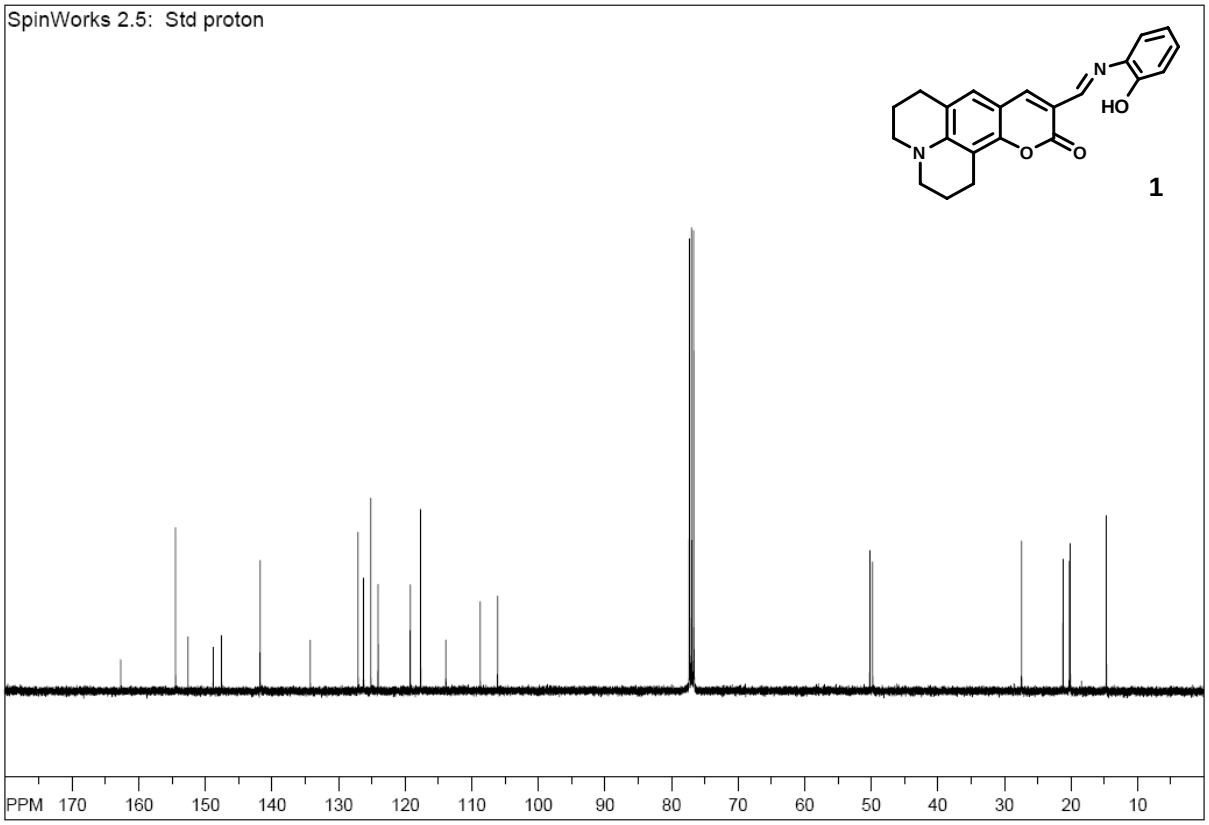


Figure S16. ^{13}C NMR spectra (100 MHz) of **1** in $CDCl_3$.

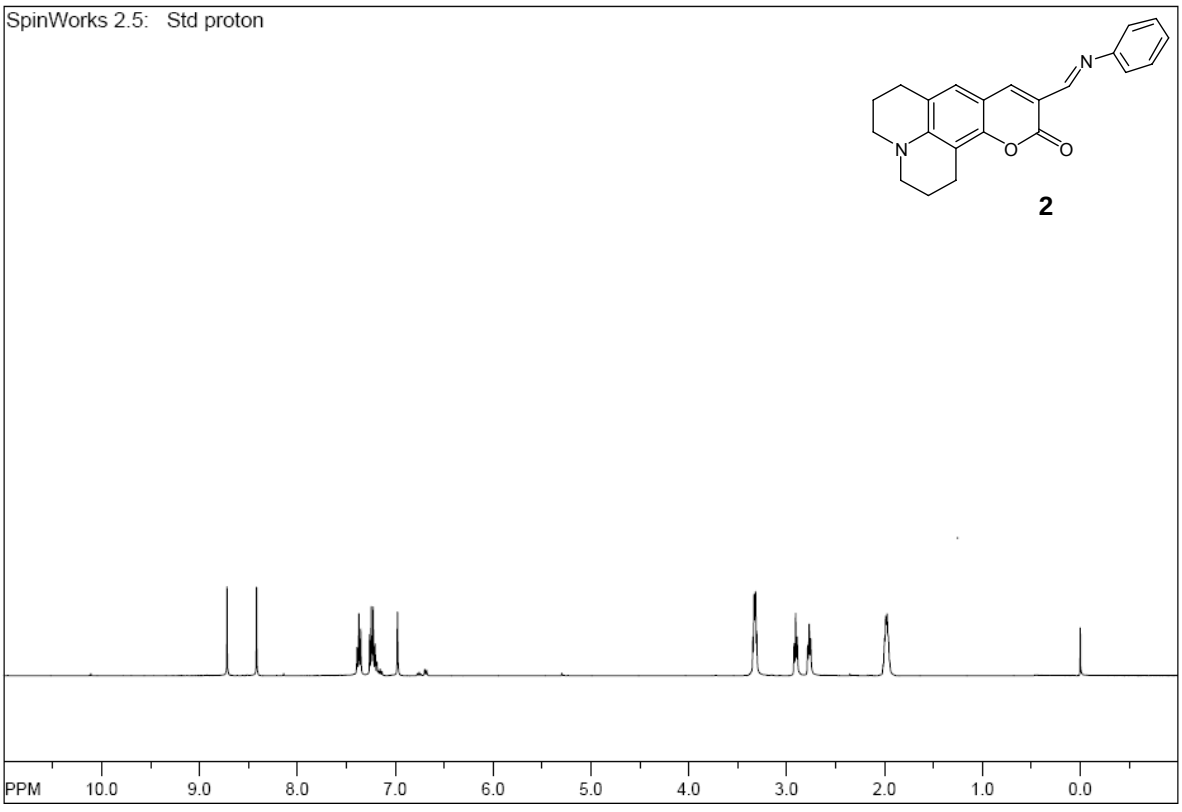


Figure S17. ^{1}H NMR spectra (400 MHz) of **2** (10 mM) in $CDCl_3$.

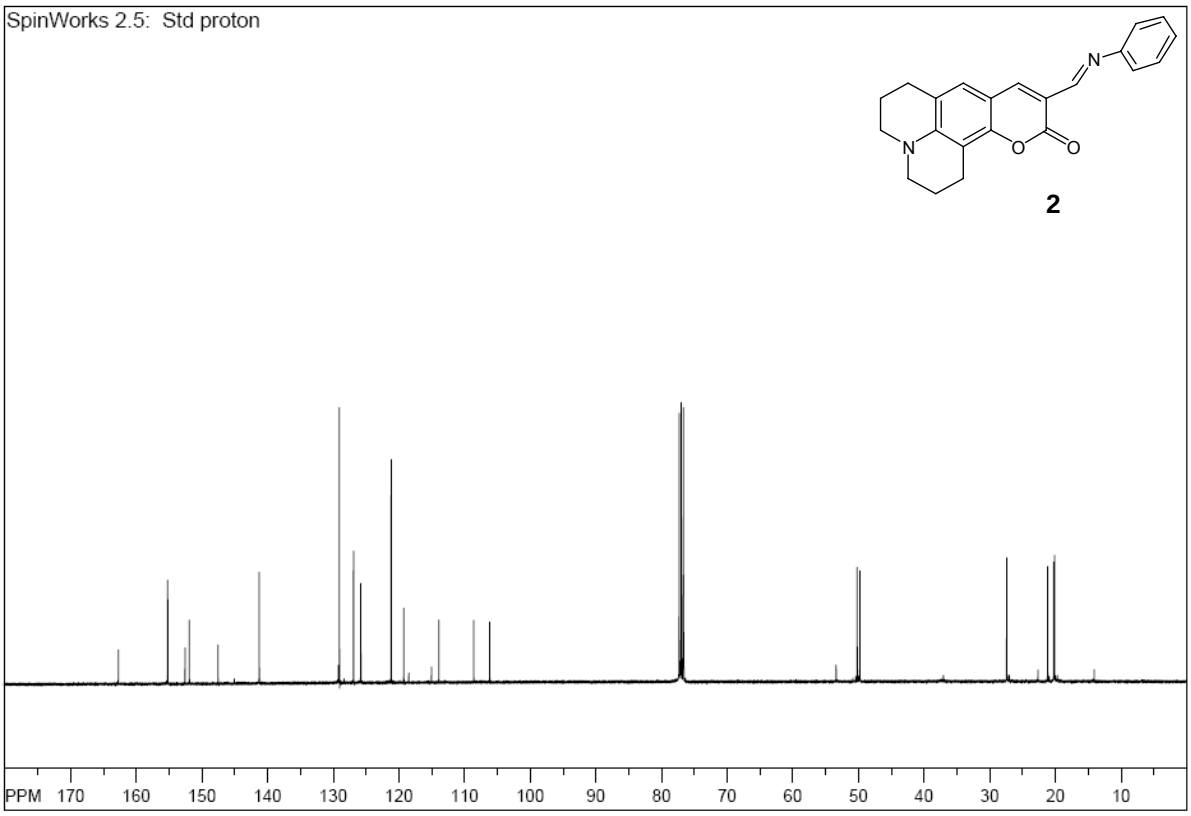


Figure S18. ^{13}C NMR spectra (100 MHz) of **2** in $CDCl_3$.

- **Crystal data for the X-ray diffraction studies**.

Table S2. Crystal data and structure refinement for **1**-Cu(II).

Empirical formula	C49 H49 Cl11 Cu2 N4 O16
Formula weight	1466.95
Temperature	120 K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 8.2013(16) Å b = 9.0211(17) Å c = 19.626(4) Å
Volume	1419.7(5) $Å^3$
Z	1
Density (calculated)	1.716 Mg/m^3
Absorption coefficient	1.339 mm^{-1}
F(000)	744
Crystal size	0.39 x 0.10 x 0.02 mm^3
Theta range for data collection	1.06 to 28.42°.
Index ranges	-10<=h<=10, -12<=k<=11, -14<=l<=26
Reflections collected	10273
Independent reflections	6949 [R(int) = 0.0289]
Completeness to theta = 28.42°	97.7 %
Absorption correction	Empirical
Max. and min. transmission	0.9699 and 0.6253
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6949 / 3 / 469
Goodness-of-fit on F^2	1.039
Final R indices [I>2sigma(I)]	R1 = 0.0571, wR2 = 0.1375
R indices (all data)	R1 = 0.0870, wR2 = 0.1566
Largest diff. peak and hole	1.352 and -0.918 $e.Å^{-3}$