

Highly Sensitive and Selective “Turn-On” Fluorescent Chemodosimeter for Cu²⁺ in Water via Cu²⁺-promoted Hydrolysis of Lactone Moiety in Coumarin

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Materials and Methods

Reagents and Apparatus

Absolute DMSO of analytical grade and deionized water (distilled) were used throughout the experiment. All the materials for synthesis were purchased from commercial suppliers and used without further purification. The solutions of metal ions were prepared from their nitrate salts of analytical grade. HEPES (purchased from Alfa) buffer solutions were prepared using 10 mM HEPES and proper amount of NaOH or HCl under adjustment by a pH meter. Absorption spectra were determined on a JASCO V-550 UV-VIS spectrophotometer equipped with an ETC-505 peltier thermostatted single cell holder. Fluorescence spectra measurements were performed on a JASCO FP-6500 spectrofluorimeter equipped with an ETC-273T peltier thermostatted single cell holder. All pH measurements were made with a Model PHS-3C pH meter (Shanghai, China). NMR spectra were recorded using a JOEL JNM-ECA300 spectrometer operated at 300 MHz. ESI MS were obtained on an API3000 LCMS spectrometer. Elemental analysis was carried out on a CE-440 elemental analyzer.

Procedures of metal ion sensing by **1**.

A 1.0×10^{-3} M stock solution of **1** was prepared in absolute DMSO. To 5 mL glass tubes with 3.96 mL buffer (10 mM HEPES of desire pH) containing different amount of metal ions, 40 μ M of the stock solution was added to each tube by a pipette to obtain solutions of 10 μ M **1**. After well mixed, the solutions were allowed to stand at 50 °C for 15 min, then cooling to room temperature, absorption or fluorescence spectra were recorded at 25 °C. If the concentration of **1** needs to be 2.0 μ M, then a 2.0×10^{-4} M stock solution in DMSO of **1** was prepared following a similar way. In low pH (< 8.0) environment, the response time was rather long. The time for standing was 60 min or more.

Determination of kinetic parameters

The solution was prepared using a similar procedure for metal ion sensing. After well mixed, the solutions were allowed to stand at 50 °C and the fluorescence enhancement rate was recorded at the same time.

Studying the sensing mechanism of **1** for Cu^{2+}

A 100 mL solution (DMSO- d_6 /phosphate buffer (pH 8.0) = 1 : 9, v/v) containing 50 μ M **1** and 50 μ M Cu^{2+} was heated at 50 °C for 30 min. After the reaction, the buffer was neutralized by HCl to pH 7.0 and extracted by CH_2Cl_2 to remove 2-aminophenol. The aqueous phase was collected and further acidified by HCl to pH 5.0. After extraction by CH_2Cl_2 , the organic layer was collected and evaporated in vacuo. The resulting slurry containing product and DMSO- d_6 was then dissolved in DMSO- d_6 for NMR characterization and in CH_2Cl_2 for ESI MS analysis, respectively.

Synthesis of compound **1**, **2** and **3**

Synthesis of 7-diethylaminocoumarin-3-aldehyde (**2**)

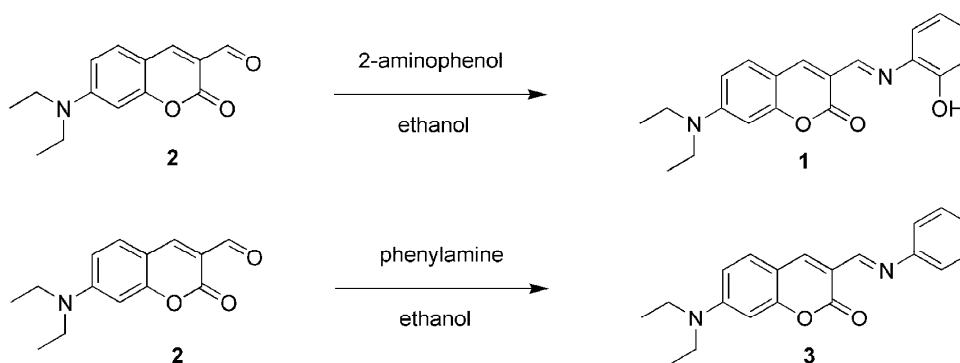
7-diethylaminocoumarin-3-aldehyde (**2**) was prepared through a reported procedure.¹ $^1\text{H-NMR}$ (CDCl_3) δ (ppm): 10.11 (s, 1H), 8.23 (s, 1H), 7.42-7.39 (d, 1H), 6.66-6.62 (dd, 1H), 6.48-6.47 (d, 1H), 3.51-3.44 (q, 4H), 1.29-1.23 (t, 6H). $^{13}\text{C-NMR}$ (CDCl_3) δ (ppm): 187.9, 161.9, 159.0, 153.5, 145.4, 132.6, 114.2, 110.3, 108.3, 97.2, 45.4 (2C), 12.5 (2C). ESI mass spectrometry: m/z 246.2 (27% $[\text{M} + \text{H}]^+$), 368.1 (100% $[\text{M} + \text{Na}]^+$); M^+ calculated 336.2. Anal. Calcd for $\text{C}_{14}\text{H}_{15}\text{NO}_3$: C 68.56%, H 6.16%, N 5.71%, O 19.57%. Found: C 68.34%, H 6.16%, N 5.48%.

Synthesis of compound 1

A 245 mg aliquot of **2** (1.0 mmol) was dissolved in 30 mL hot absolute ethanol. A 164 mg aliquot of 2-aminophenol (1.5 mmol) that was dissolved in 15 mL absolute ethanol was added. The mixture was refluxed for 2 h to yield a scarlet precipitate. The precipitate was filtrated, washed with hot absolute ethanol three times, After drying under reduced pressure, 258 mg **1** was obtained as a scarlet solid (77%). ¹H-NMR (DMSO-d₆) δ (ppm): 8.98 (s, 1H), 8.83 (s, 1H), 8.66 (s, 1H), 7.62-7.59 (d, 1H), 7.18-7.16 (d, 1H), 7.10-7.04 (t, 1H), 6.90-6.79 (m, 3H), 6.62 (s, 1H), 3.53-3.46 (q, 4H), 1.18-1.13 (t, 6H). ¹³C-NMR (pyridine-d₅) δ (ppm): 162.0, 157.9, 153.2, 153.0, 152.0, 141.1, 138.9, 131.0, 128.1, 120.0, 118.7, 116.7, 115.59, 109.9, 108.8, 97.1, 44.7 (2C), 12.3 (2C). ESI mass spectrometry: m/z 337.3 (100% [M + H]⁺), 359.2 (28% [M + Na]⁺); M⁺calculated 336.2. Anal. Calcd for C₂₀H₂₀N₂O₃: C 71.41%, H 5.99%, N 8.33%, O 14.27%. Found: C 71.32%, H 6.01%, N 8.34%.

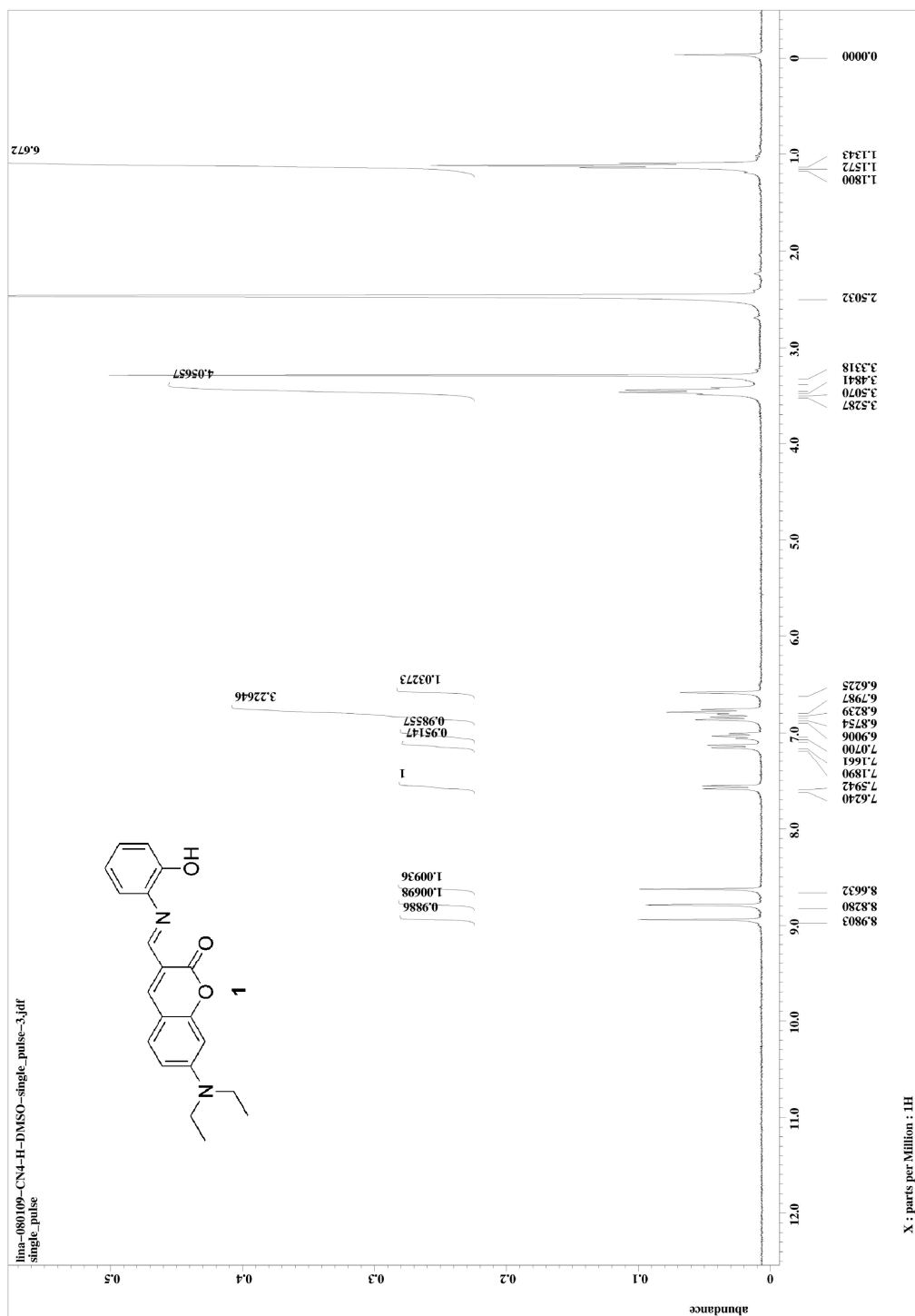
Synthesis of compound 3

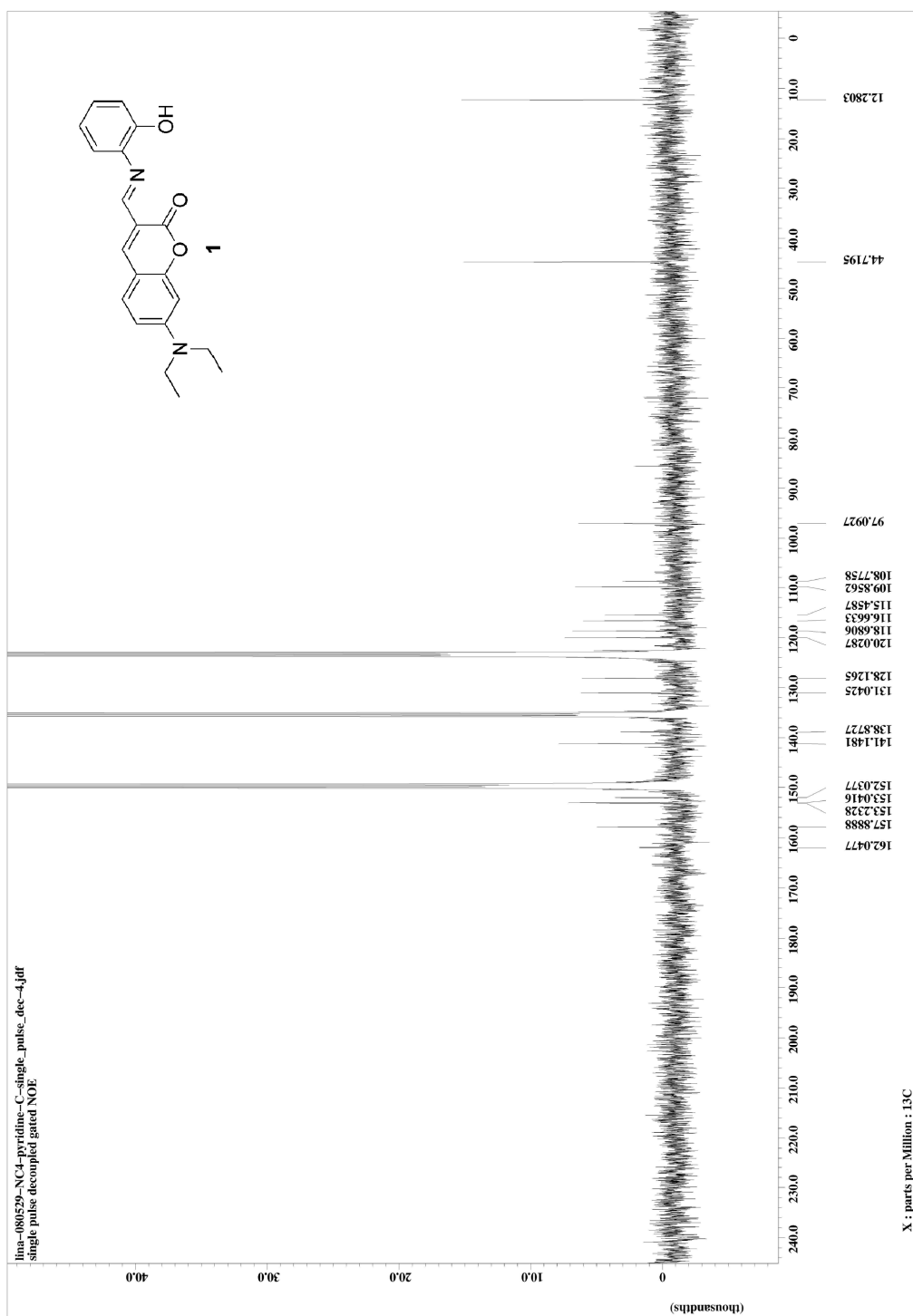
A 245 mg aliquot of **2** (1.0 mmol) was dissolved in 30 mL hot absolute ethanol. A 0.456 mL of phenylamine (466 mg, 5.0 mmol) was added. The mixture was refluxed for 4 h. After cooling to room temperature, the reaction mixture was allowed to stand at 4 °C overnight. The precipitate formed was filtrated, washed with cold absolute ethanol three times. After drying under reduced pressure, 210 mg **1** was obtained as a yellow solid (66%). ¹H-NMR (DMSO-d₆) δ (ppm): 8.58 (s, 1H), 8.52 (s, 1H), 7.69-7.65 (d, 1H), 7.44-7.38 (m, 2H), 7.26-7.20 (m, 3H), 6.81-6.78 (dd, 1H), 6.60-6.59 (d, 1H), 3.52-3.45 (q, 4H), 1.17-1.12 (t, 6H). ¹³C-NMR (DMSO-d₆) δ (ppm): 161.2, 157.2, 154.4, 152.0, 151.7, 141.4, 131.6, 129.3 (2C), 125.9, 120.9 (2C), 113.7, 109.9, 108.0, 96.4, 44.1 (2C), 12.4 (2C). ESI mass spectrometry: m/z 321.2 (100% [M + H]⁺), 343.2 (50% [M + Na]⁺); M⁺calculated 336.2. Anal. Calcd for C₂₀H₂₀N₂O₂: C 74.98%, H 6.29%, N 8.74%, O 9.99%. Found: C 74.87%, H 6.31%, N 8.50%.

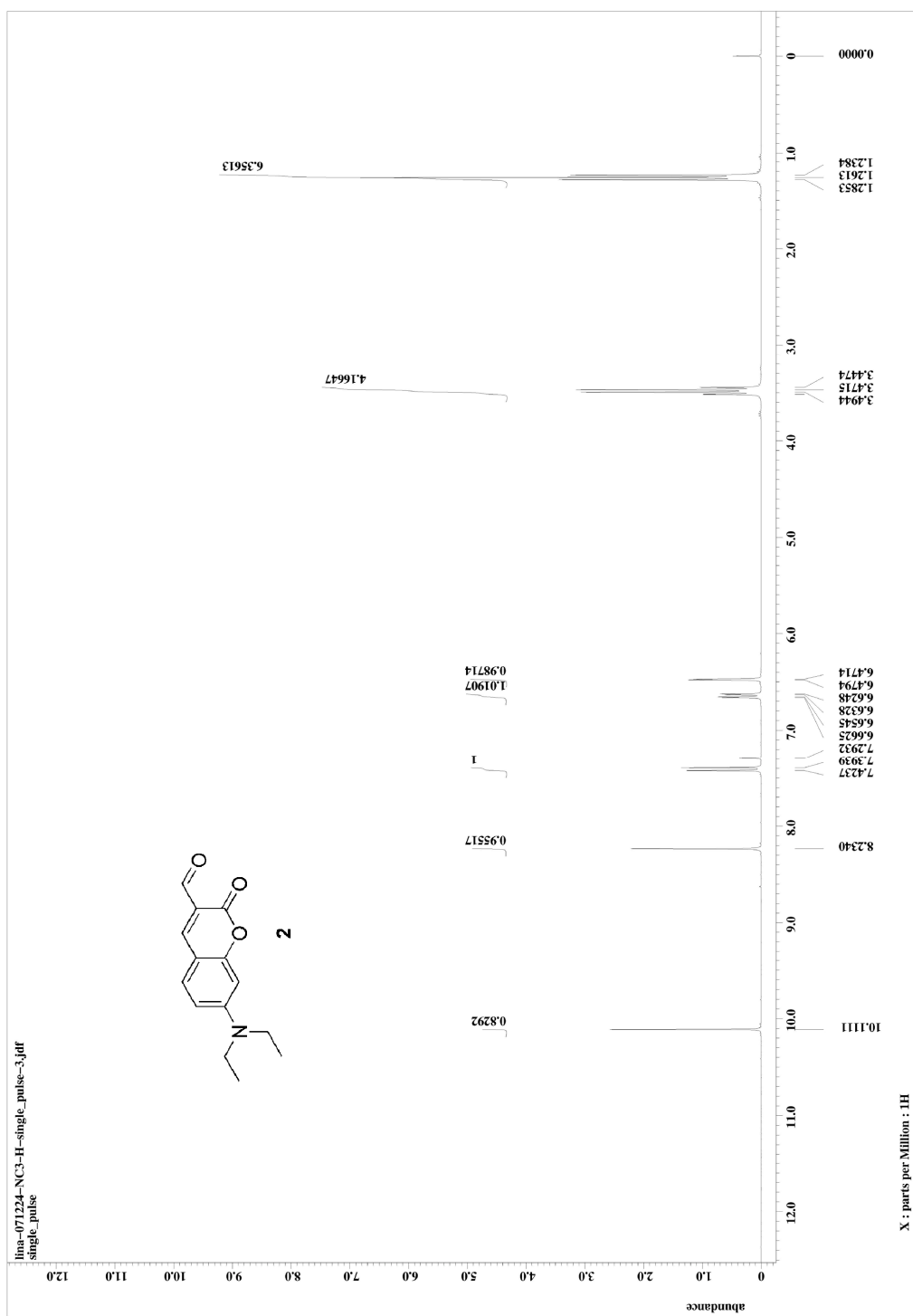


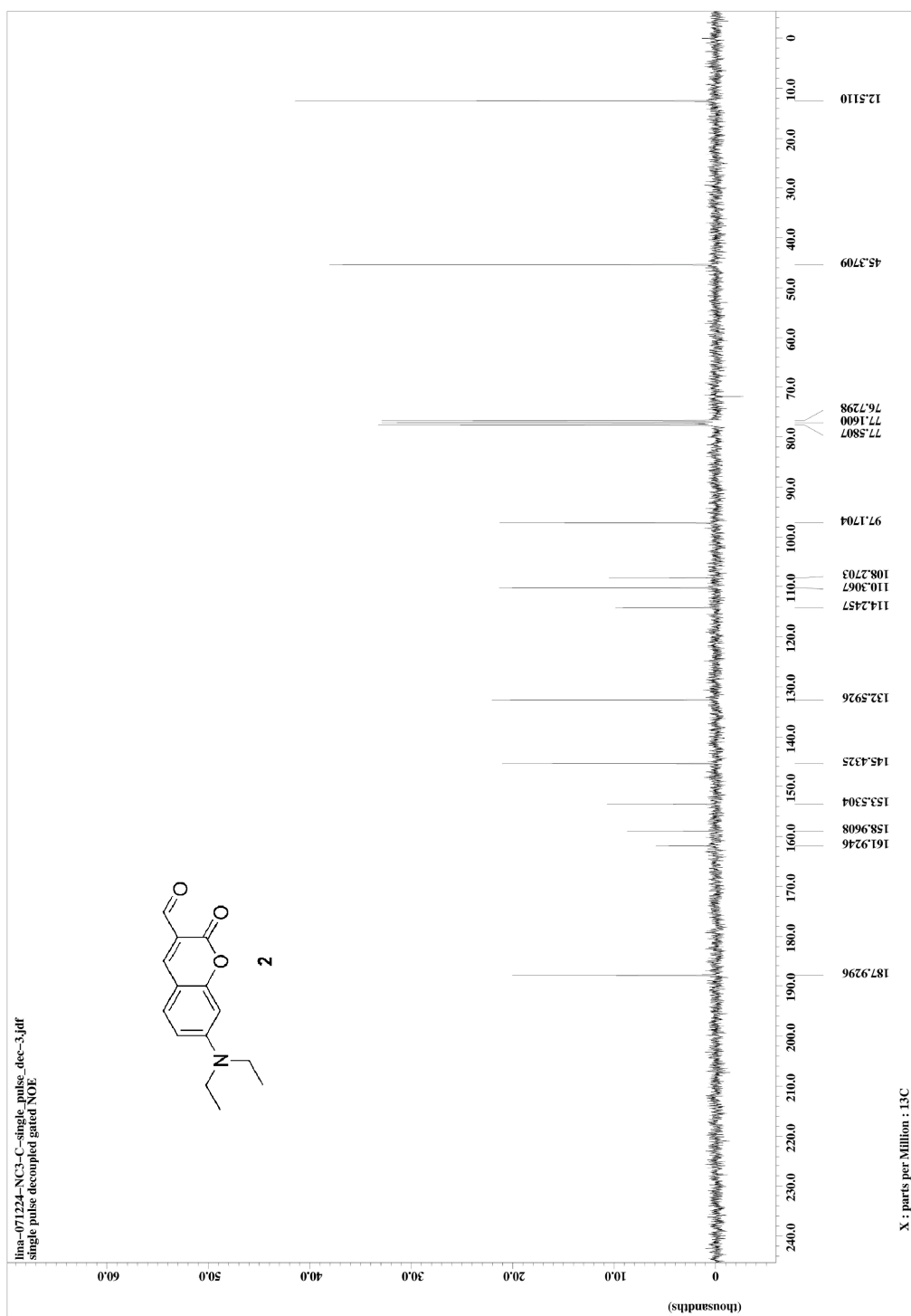
Scheme S1. Synthesis of compounds **1** and **3**.

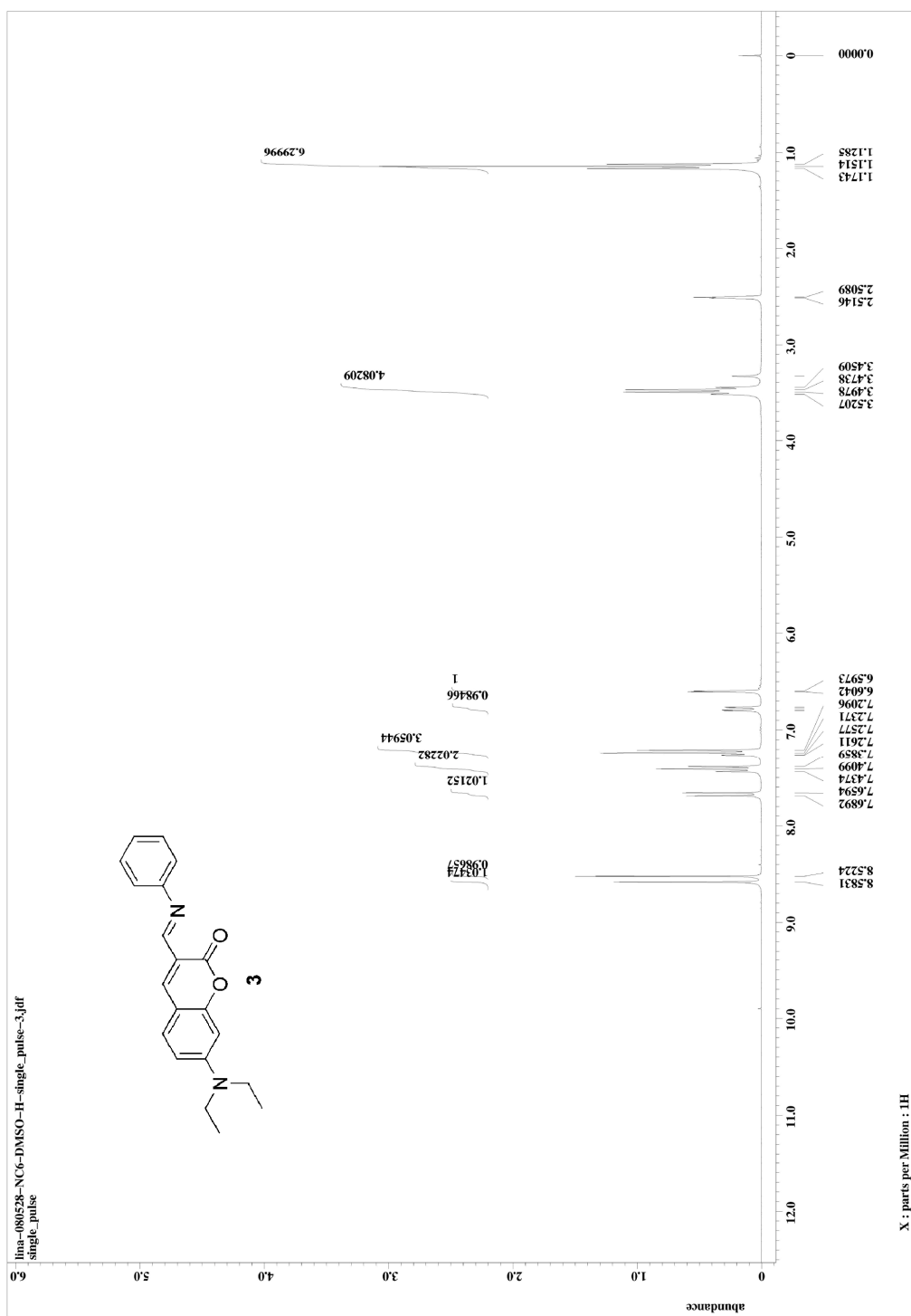
^1H - and ^{13}C -NMR Spectra of compound 1, 2 and 3

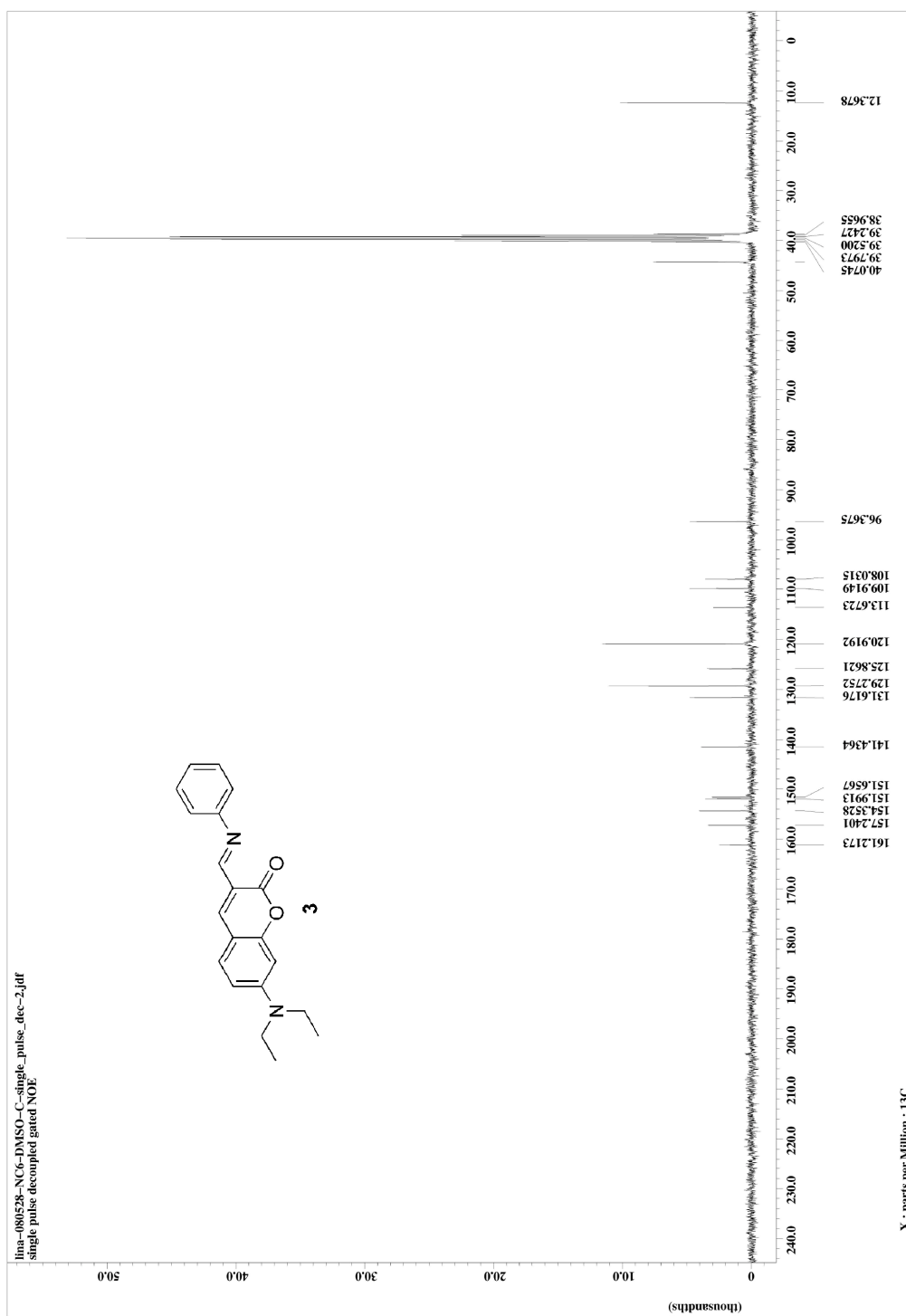












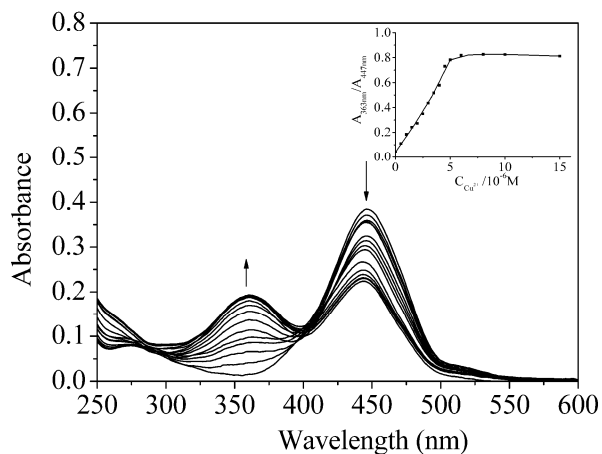


Figure S1. Absorption spectra of **1** (10 μM) in the absence and presence of Cu^{2+} in 1% (v/v) DMSO/water buffered by 10 mM HEPES at pH 8.0. $[\text{Cu}^{2+}]$ changes from 0 to 15 μM as indicated by arrows. Inset shows ratio of absorbance at 363 and at 447 nm as a function of Cu^{2+} concentration. Spectra were measured at 25 $^{\circ}\text{C}$ after 15 min heating at 50 $^{\circ}\text{C}$.

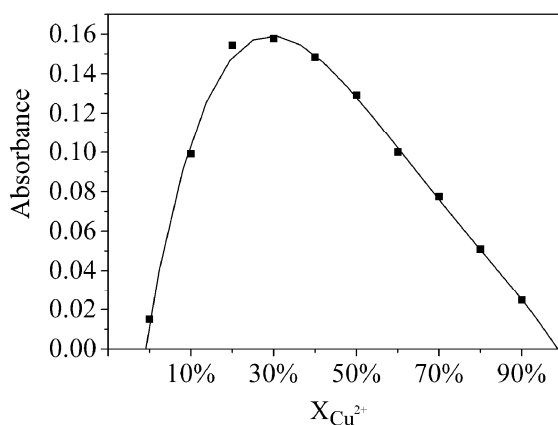


Figure S2. Job's plot data for evaluating the stoichiometry of **1**- Cu^{2+} complex. The total concentration of **1** and Cu^{2+} was 10 μM . The wavelength of absorbance is 363 nm. Spectra were measured at 25 $^{\circ}\text{C}$ after 15 min heating at 50 $^{\circ}\text{C}$.

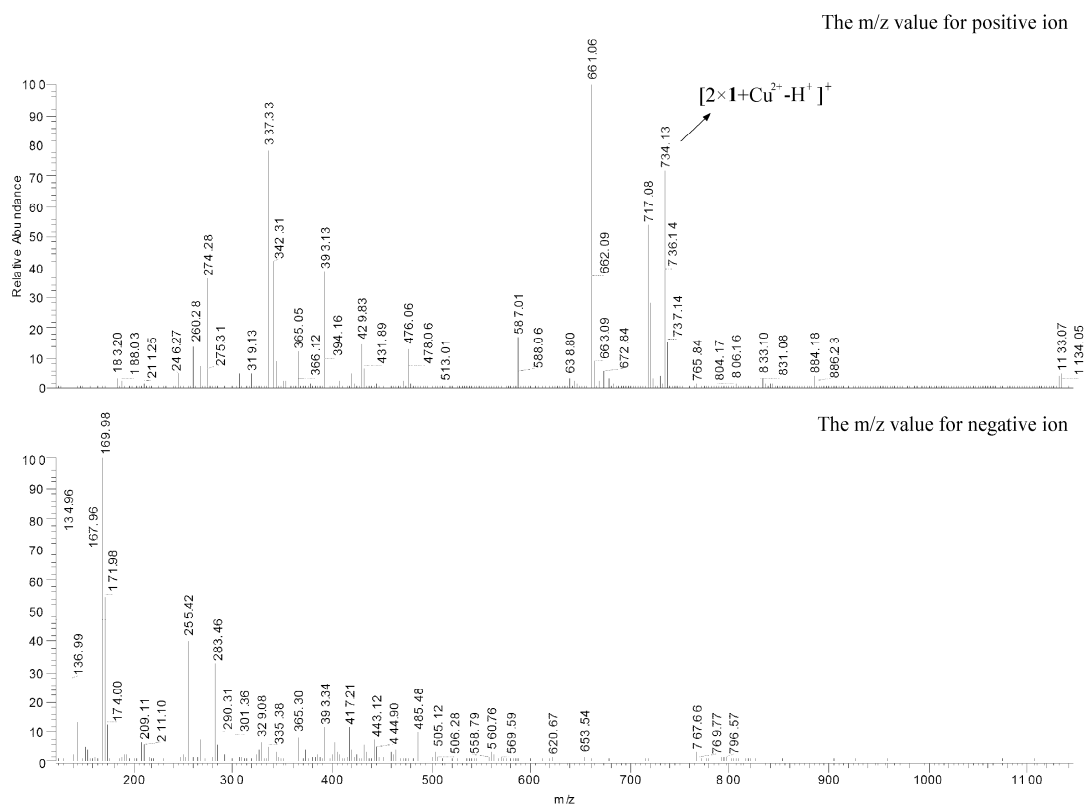


Figure S3. ESI mass spectra of **1** in the presence of 1.2 equivalent Cu^{2+} in absolute ethanol.

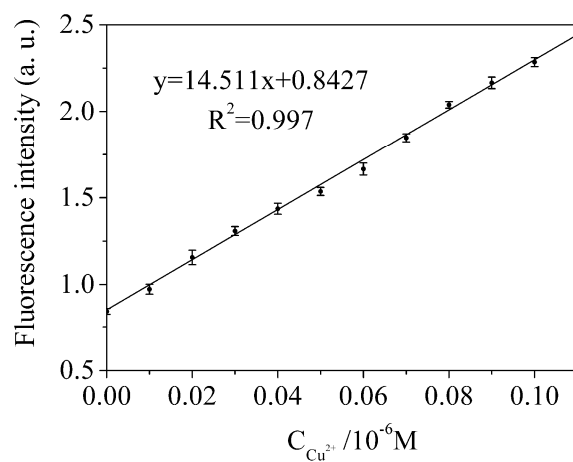


Figure S4. Fluorescence intensity at 453 nm of **1** (2 μM) as a function of Cu^{2+} concentration. Spectra were measured at 25 $^{\circ}\text{C}$ after 15 min heating at 50 $^{\circ}\text{C}$ in 1% (v/v) DMSO/water buffered by 10 mM HEPES at pH 8.0.

Table S1. Comparison of the performance of compound **1** and selected analogous compounds

	Sensing mechanism	Signal to noise ratio(fluorescence)	LOD	Selectivity to other metal ions	Reference
1	Hydrolysis of lactone	50	20 nM	>100 times	This work
2	Hydrolysis of lactam	20	10 nM	Interference from Hg ²⁺	4(a)
3	Hydrolysis of normal ester	Fluorescence quenching	NR	A kinetic discrimination of different metal ions	4(b)
4	Hydrolysis of normal ester	<16	300 fmol	>100 times	4(c)
5	Hydrolysis of normal ester	>50	10 nM	>100 times	4(d)
6	Hydrolysis of hydrazone	18	<100 nM	>100 times	4(h)
7	Heck reaction	NR	30 nM	Interference from Co ²⁺ , Ni ²⁺ and Cd ²⁺	4(j)

LOD: limits of detection

NR: not reported

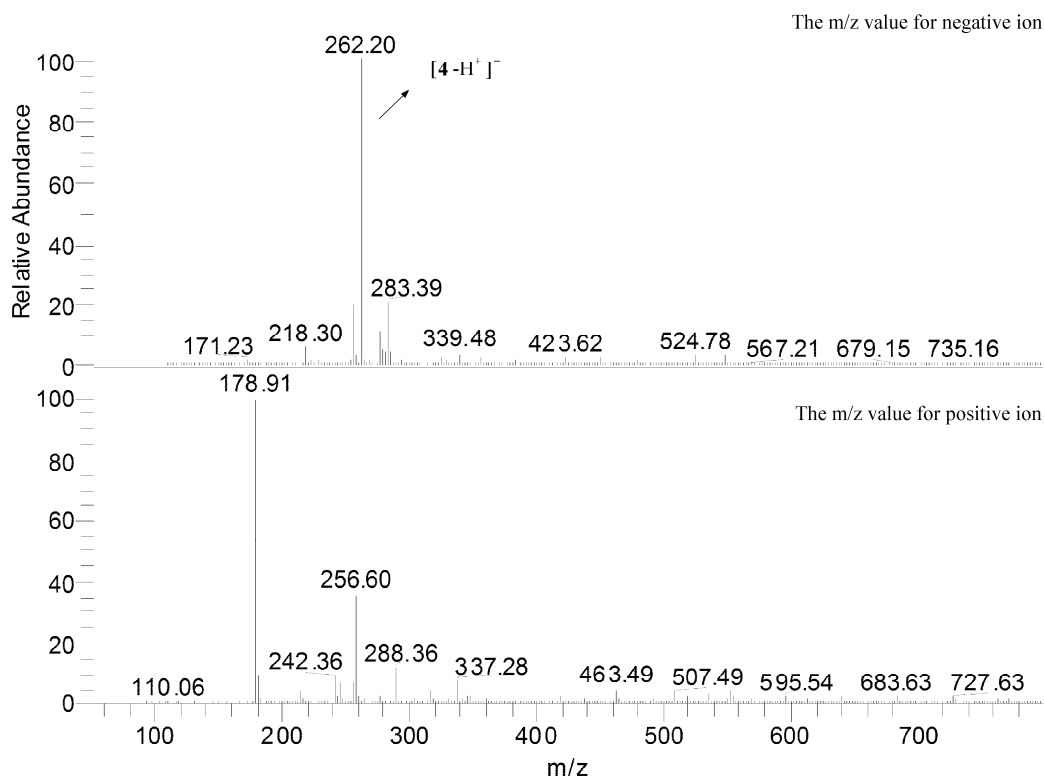


Figure S5. ESI mass spectra of the isolated fluorescent product of **1**-Cu²⁺ after 30 min heating at 50 °C

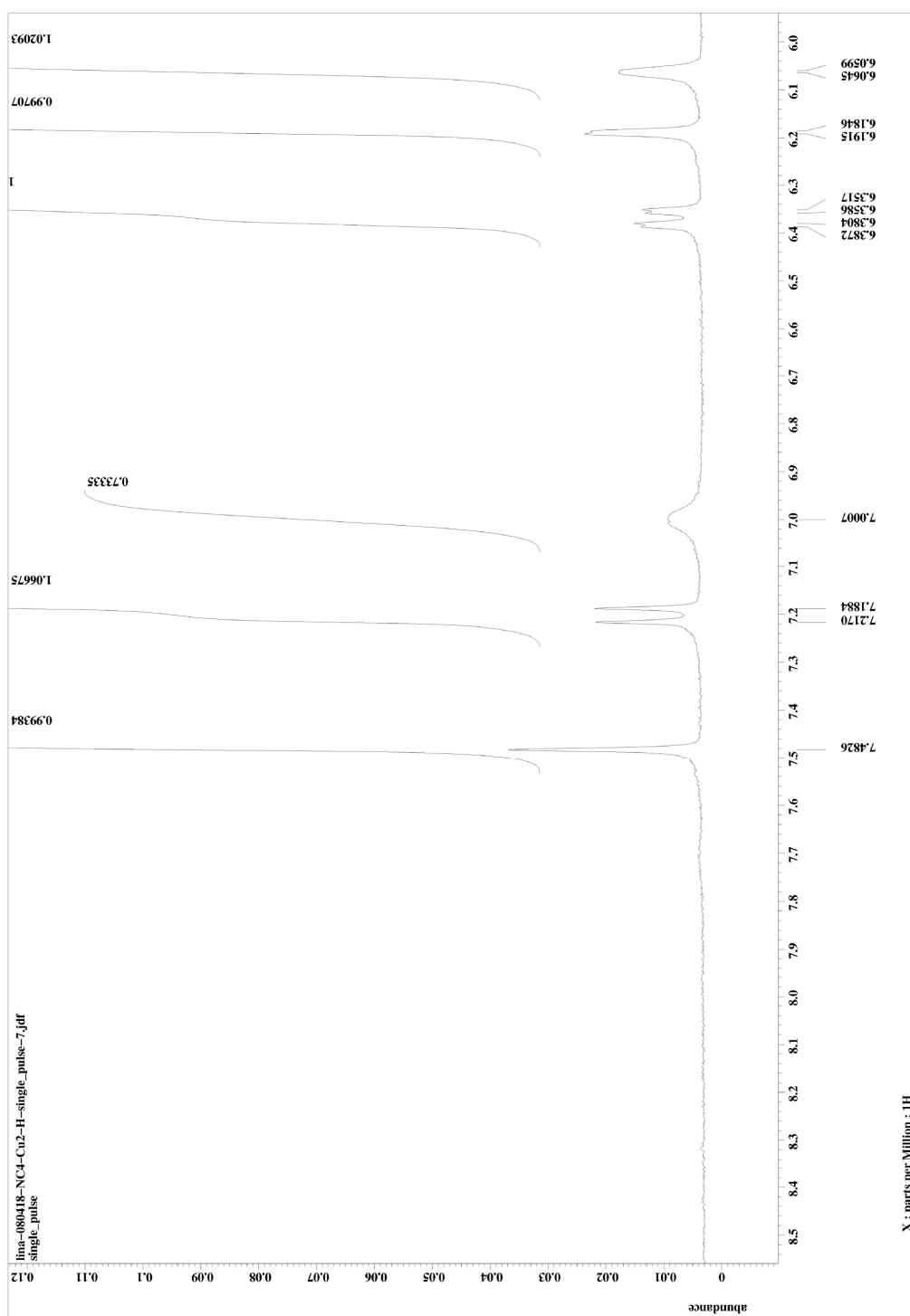


Figure S6. ^1H -NMR spectra of the isolated fluorescent product of 1-Cu^{2+} after 30 min heating at $50\text{ }^\circ\text{C}$.

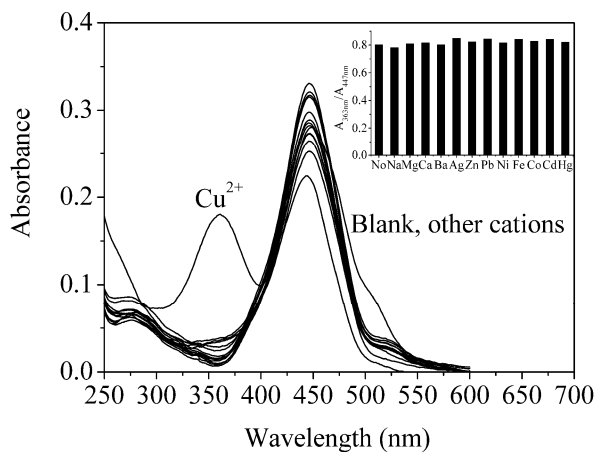


Figure S7. Absorption spectra of **1** (10 μM) in the absence and presence of different metal ions (10 μM) in 1% (v/v) DMSO/water buffered by 10 mM HEPES at pH 8.0. Ions: Na⁺, Mg²⁺, Ca²⁺, Ba²⁺, Ag⁺, Zn²⁺, Pb²⁺, Ni²⁺, Fe³⁺, Co²⁺, Cd²⁺, Hg²⁺. Spectra were measured at 25 °C after 15 min heating at 50 °C. Inset: Ratio of absorbance at 363 and at 447 nm of **1** (10 μM) with Cu²⁺ (5 μM) in the absence and presence of different metal ions (10 μM).

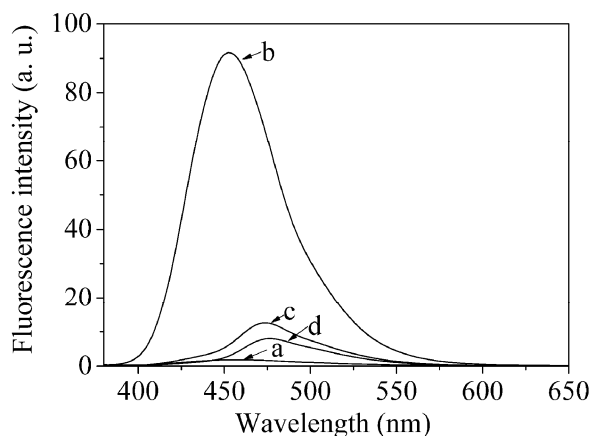


Figure S8. Fluorescence spectra of **1** in the absence and presence of aminophenol in 1% (v/v) DMSO/water buffered by 10 mM HEPES at pH 8.0. a: **1** only, [**1**] = 10 μM. b: [**1**] = 10 μM, [Cu²⁺] = 10 μM. c: [**1**] = 10 μM, [Cu²⁺] = 10 μM, the concentration of aminophenol was 20 μM. d: [**1**] = 10 μM, [Cu²⁺] = 10 μM, the concentration of aminophenol was 50 μM. Excitation was performed at 363 nm. Spectra were measured at 25 °C after 15 min heating at 50 °C.

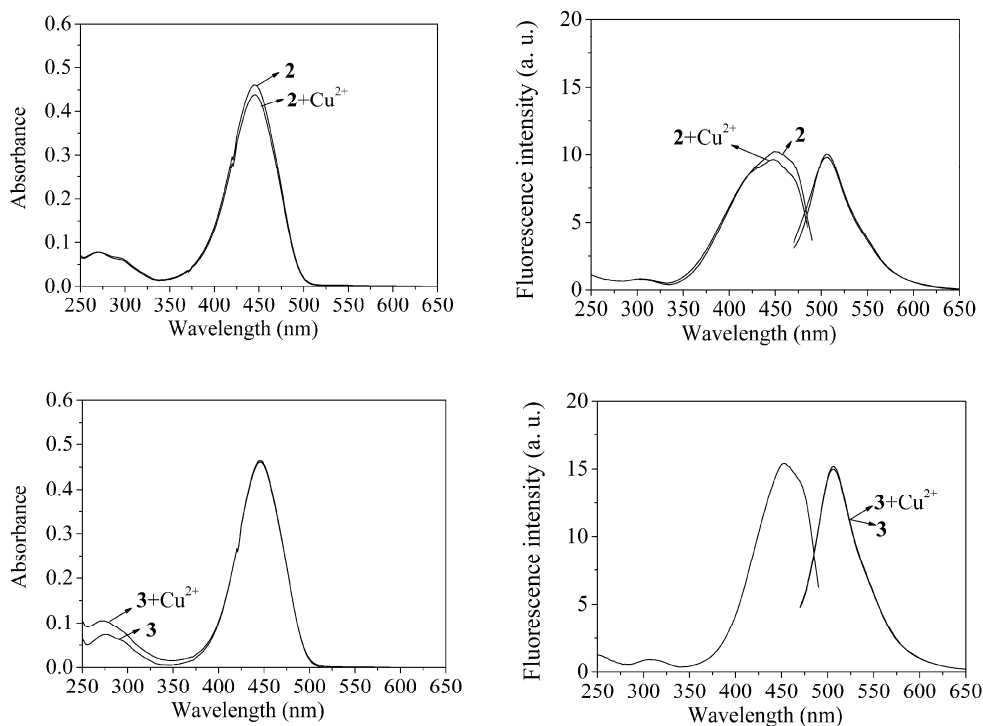


Figure S9. Absorption and fluorescence spectra of compounds **2** (10 μM) and **3** (10 μM) in the absence and presence of Cu^{2+} in 1% (v/v) DMSO/water buffered by 10 mM HEPES at pH 8.0. Spectra were measured at 25 $^{\circ}\text{C}$ after 15 min heating at 50 $^{\circ}\text{C}$.

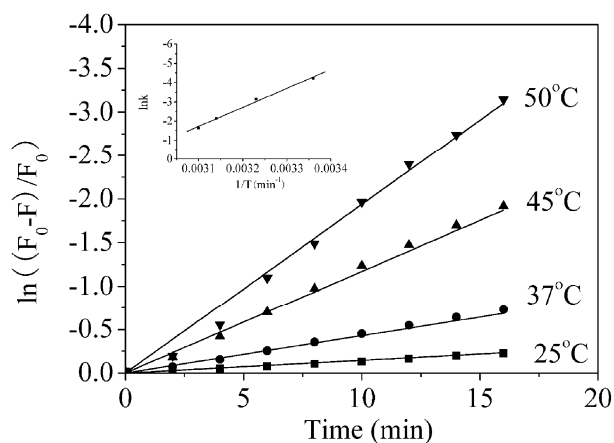


Figure S10. Curves fitted with one-order reaction dynamics ($\ln((F-F_0)/F_0) = -kt$) and speed constant (k) was calculated: $k = 0.015 \text{ min}^{-1}$ ($T = 25 \text{ }^{\circ}\text{C}$), $k = 0.043 \text{ min}^{-1}$ ($T = 37 \text{ }^{\circ}\text{C}$), $k = 0.12 \text{ min}^{-1}$ ($T = 45 \text{ }^{\circ}\text{C}$), $k = 0.19 \text{ min}^{-1}$ ($T = 50 \text{ }^{\circ}\text{C}$). Conditions: $[\mathbf{1}] = 10 \mu\text{M}$, $[\text{Cu}^{2+}] = 5 \mu\text{M}$, ex/em = 363/453 nm. Buffer: HEPES (10 mM pH = 8.0), 1% (v/v) DMSO/water. Inset: Curves fitted with Arrhenius expressions ($\ln k = -E_a/RT + \ln A$) and activation energy was calculated: $E_a = 83.2 \text{ KJ/mol}$.

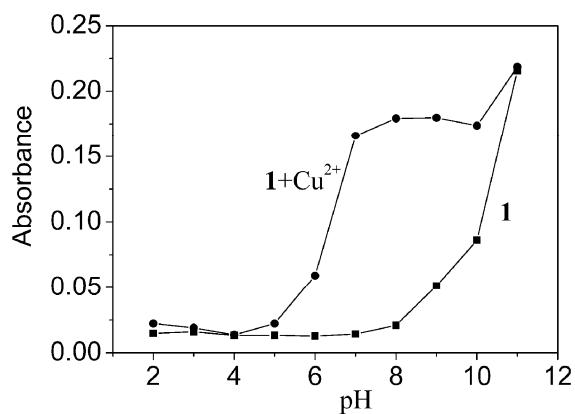


Figure S11. Absorbance at 363 nm of **1** (10 μM) in the absence and presence of 10 μM Cu^{2+} in 1% (v/v) DMSO/water buffered by 10 mM HEPES at different pH. Spectra were measured at 25 $^{\circ}\text{C}$ after 60 min heating at 50 $^{\circ}\text{C}$.

References

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