Supporting Information for

Fluorescence turn-on detection of Cu^{2+} in water samples and living cells based on the unprecedented copper-mediated dihydrorosamine oxidation reaction

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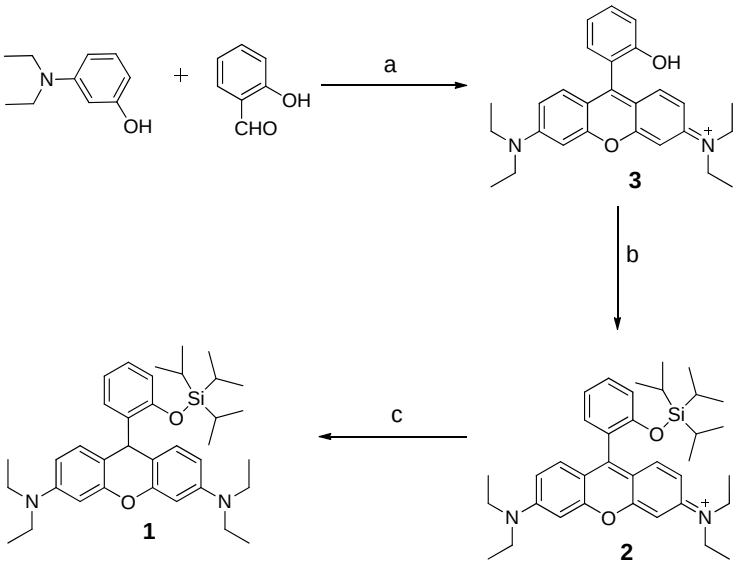
Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Melting points of compounds were measured on a Beijing Taike XT-4 microscopy melting point apparatus, and all melting points were uncorrected. Low resolution mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. High resolution mass spectrometric (HRMS) analysis was measured on a Finnigan MAT 95 XP spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. X-ray diffraction analysis was performed by using a Bruker Smart-1000 X-ray diffractometer. Electronic absorption spectra were obtained on a SHIMADZU UV-2450 spectrometer. Photoluminescent spectra were recorded with a HITACHI F4500 fluorescence spectrophotometer with the excitation and emission slit widths at 2.5 nm. Cell imaging was performed with a Nikon Eclipse TE2000-S inverted microscope. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.



Scheme S1. Synthesis of compound **1**. a): p-TsOH, propionic acid, 65°C, overnight; chloranil, 2h, 22.4%. b) TIPSCl, Et_3N, DMF, 75°C, 2h, 72%. c) NaBH4, THF, 82%.

Synthesis

Compound **3**: A solution of salicylaldehyde (365 mg, 3 mmol), 3-diethylaminophenol (990 mg, 6 mmol), and *p*-TsOH (76 mg, 0.40 mmol) in propionic acid (6 mL) were stirred at 65°C overnight. Then the solvent was removed under reduced pressure, and the resulting solid was dissolved in a mixture of MeOH (20 mL) and CH_2Cl_2 (20 mL), to which chloranil (370 mg, 1.5 mmol) was subsequently added. The mixture was vigorously stirred for 2 h, and then concentrated under reduced pressure. The residue was purified by flash chromatography (acetone: MeOH = 9:1) on silical gel to give a dark purple solid (280 mg, 22.4 %). M.p.258 - 260°C; ^{1}H NMR (400 MHz, CD_3OD), δ: 7.48 (t, J = 7.2 Hz, 1H), 7.37 (d, J = 10 Hz, 2H), 7.19 (d, J = 8 Hz, 1H), 7.06 (m, 4H), 6.93 (d, J = 2.4, 2H), 3.66 (q, J = 7.2 Hz, 8H), 1.29 (t, J = 7.2 Hz, 12H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ: 159.5, 157.8, 157.1, 156.1, 133.3, 132.9, 131.8, 120.7, 120.4, 117.2, 115.2, 114.9, 97.1, 46.8, 12.8 ppm; MS m/z (ESI): 415.2 (M^+); UV-vis (CH_3CN), λ_{max} (logε) = 556 nm (4.96); fluorescence (CH_3CN), λ_{em} = 575 nm; Φ_f = 0.23.

Compound **2**: To a solution of compound **3** (268 mg, 0.645 mmol) in dry DMF (2 ml) were added chlorotriisopropylsilane (TIPSCl) (0.7 ml, 3.87 mmol) and Et_3N (1 mL, 7.2 mmol). The reaction mixture was kept in dark and stirred for 2 h at 75°C, and then the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (acetone: petroleum ether = 1:2) to afford compound **2** as a red solid (265 mg, 72%). ^{1}H NMR (400 MHz, *d*-acetone), δ: 7.62 (t, J = 8, 1H), 7.40 (m, 3H), 7.28 (t, J = 7.2, 1H), 7.22 (m, 3H), 7.02 (s, 2H), 3.80 (q, J = 7.2, 8H), 1.34 (t, J = 7.2,

12H), 1.12 (m, 3H), 0.8 (m, 18H) ppm; ^{13}C NMR (100 MHz, *d*-acetone) δ: 158.8, 156.7, 153.9, 132.9, 132.7, 131.6, 123.9, 122.0, 119.7, 115.2, 114.5, 96.8, 46.5, 17.9, 17.5, 13.6, 13.3, 12.8 ppm; MS m/z (ESI): 571.3 (M^+); UV-vis (CH_3CN), λ_{max} (logε) = 558 nm (4.96); fluorescence, λ_{em} = 578 nm; Φ_f = 0.22.

Compound **1**: A pink solution of compound **2** (200 mg, 0.35 mmol) in THF (25 ml) with stirring was added $NaBH_4$ (15 mg, 0.396 mmol) in portion slowly at room temperature. After the solution became colorless, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography on basic alumina (petroleum ether as eluent) to afford compound **1** as a colorless solid (164 mg, 82%). ^{1}H NMR (400 MHz, *d*-acetone) δ: 7.02 (m, 1H), 6.91 (d, *J* = 8.4, 1H), 6.86 (m, 3H), 6.77 (t, *J* = 7.2, 1H), 6.35 (m, 4H), 5.73 (d, 1H), 3.36 (q, *J* = 7.2, 8H), 1.46(m, 3H), 1.17 (m, 30H) ppm; ^{13}C NMR (100 MHz, *d*-acetone) δ: 153.1, 148.4, 131.6, 130.8, 130.7, 127.5, 122.1, 118.7, 118.6, 112.9, 108.3, 99.3, 44.8, 42.8, 42.2, 18.9, 18.6, 18.27, 18.20, 18.1, 17.8, 17.7, 15.1, 14.6, 14.0, 13.9, 13.7, 12.9, 12.8 ppm; HRMS (EI) calcd for $C_{36}H_{52}N_2O_2Si_1$ (M^+): 572.3793, found: 572.3790.

Determination of the fluorescence quantum yield: [1-3] Fluorescence quantum yield was determined in CH_3CN using optically matching solutions of rhodamine 6G (fluorescence quantum yield = 0.9 in ethanol [4]) as standard and the quantum yield was calculated using the following equation:

$$\Phi_f = \Phi_r (A_r F_s / A_s F_r) (n_s / n_r)^2$$

where, A_s and A_r are the absorbance of the sample and the reference, respectively, at the same excitation wavelength, F_s and F_r are the corresponding relative integrated fluorescence intensities, and n is the refractive index of the solvent.

Preparation of the test solution: The stock solution of probe **1** was prepared at 4×10^{-4} M in CH_3CN. The solutions of various testing species were prepared from $AgNO_3$, $AlCl_3 \cdot 6H_2O$, $CaCl_2$, $CdCl_2 \cdot 1/2H_2O$, $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $FeCl_2$, $FeCl_3$, $HgCl_2$, $MnSO_4 \cdot H_2O$, $NaNO_2$, $NiCl_2 \cdot 6H_2O$, $Pb(NO_3)_2$, $ZnCl_2$, NaF, 30% H_2O_2, respectively. •OH was prepared according to a literature method.[5] The test solution of probe **1** (10 μM) in 2 mL neutral aqueous conditions (20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent) was prepared by placing 0.05 mL of the probe stock solution and 0.75 mL CH_3CN in 1.2 mL of 33.3 mM HEPES buffer (pH = 7.4). The resulting solution was shaken well and incubated for 30 min at room temperature before recording the spectra. The reaction of probe **1** (2 mL, 10 μM) with H_2O_2 catalyzed by HRP was performed by adding HRP (final concentration 1U/mL) and H_2O_2 (final concentration 50 μM) to the probe **1** solution, and the resulting solution was incubated at room temperature before recording the spectra.

Determination of Cu^{2+} concentrations in the water samples: The spring water samples were obtained from YueLu Mountain, and the suspension was filtered before use. The water samples were spiked with different concentrations of Cu^{2+} and were then treated with the buffer (20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent) containing probe **1** (10 μM). After 30 min incubation, the fluorescence spectra were recorded, and the concentrations of copper were calculated based on the calibration curve.

Visual emission detection of Cu^{2+} in the new born calf serum: The commercial available new born calf serum samples were spiked with different concentrations of Cu^{2+} and were then treated with the buffer (20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent) containing probe **1**(10 μM). After 30 min incubation, the fluorescence spectra were recorded and the emission photographs were taken under a handheld 365 nm UV lamp.

Cell culture and fluorescence imaging: Nasopharyngeal carcinoma cells were seeded in a 12-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum for 24 h. Nasopharyngeal carcinoma cells were then incubated with $CuCl_2$ (0 or 5 equiv.) in the culture medium for 30 min at 37°C. After washing with PBS three times to remove the remaining copper ions, the cells were further incubated with probe **1** (10 μM) for 30 min at 37°C. Subsequently, the fluorescence images were acquired with a Nikon Eclipse TE2000-S equipped with a CCD camera. A G-2E/C 528-553 nm excitation filter and a G-2E/C 590-650 nm emission filter were employed.

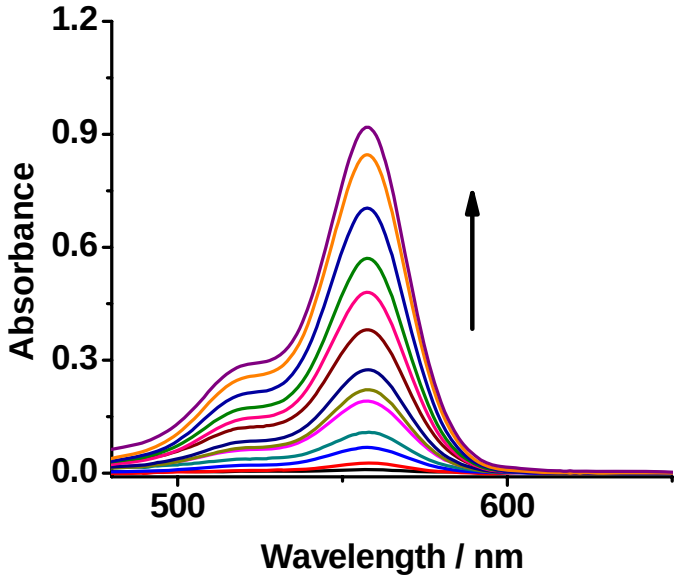


Figure S1. Absorption spectral changes of probe **1** (10 μM) upon addition of Cu^{2+} (0-5 equiv.) in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent. The spectra were recorded after mixing copper ions with probe **1** for 30 minutes at room temperature.

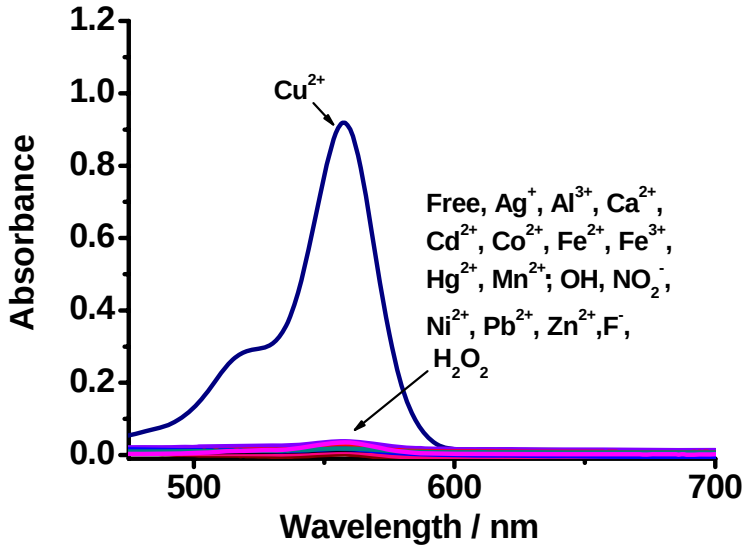


Figure S2. The absorption spectra of probe **1** (10 μM) in the absence and presence of different species (5 equiv.) in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent.

Figure S3. Color changes of probe **1** (10 μM) in the presence of 5 equiv. of various species in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent. 1: free, 2: Fe^{3+}, 3: H_2O_2, 4: Cu^{2+}, 5: F^-, 6: Co^{2+} : (a) visible color and (b) visual fluorescence color on excitation at 365 nm using a handheld UV lamp.

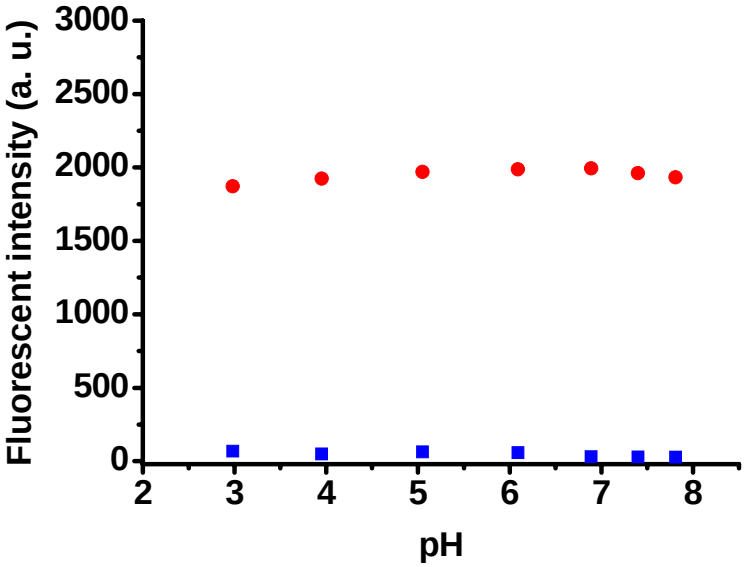


Figure S4. The variations of fluorescence intensity at 580 nm of probe **1** (10 μM) in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent in the absence (■) or presence (●) of Cu^{2+} (5 equiv.) as a function of pH. Excitation wavelength was 554 nm.

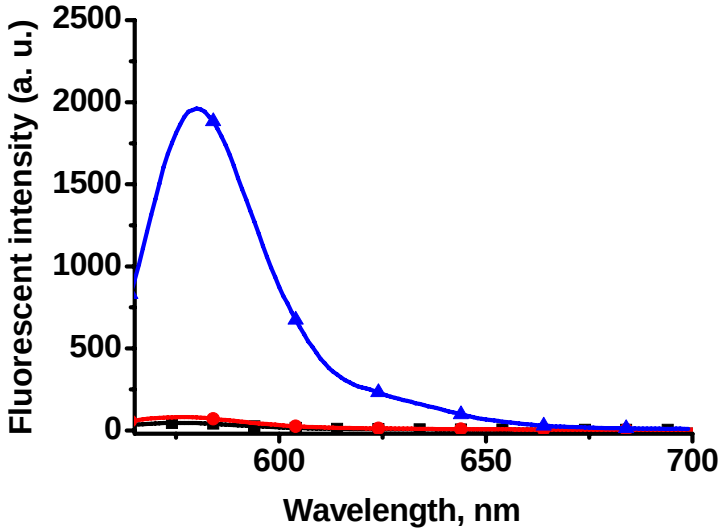


Figure S5. The fluorescence spectra of probe **1** (10 μM) in the absence of Cu^{2+} at room temperature for 30 minutes (■) or three days (●) in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent. For comparison, the fluorescence spectrum of Probe **1** + 5 equiv. Cu^{2+} (▲) at room temperature for 30 minutes was also showed.

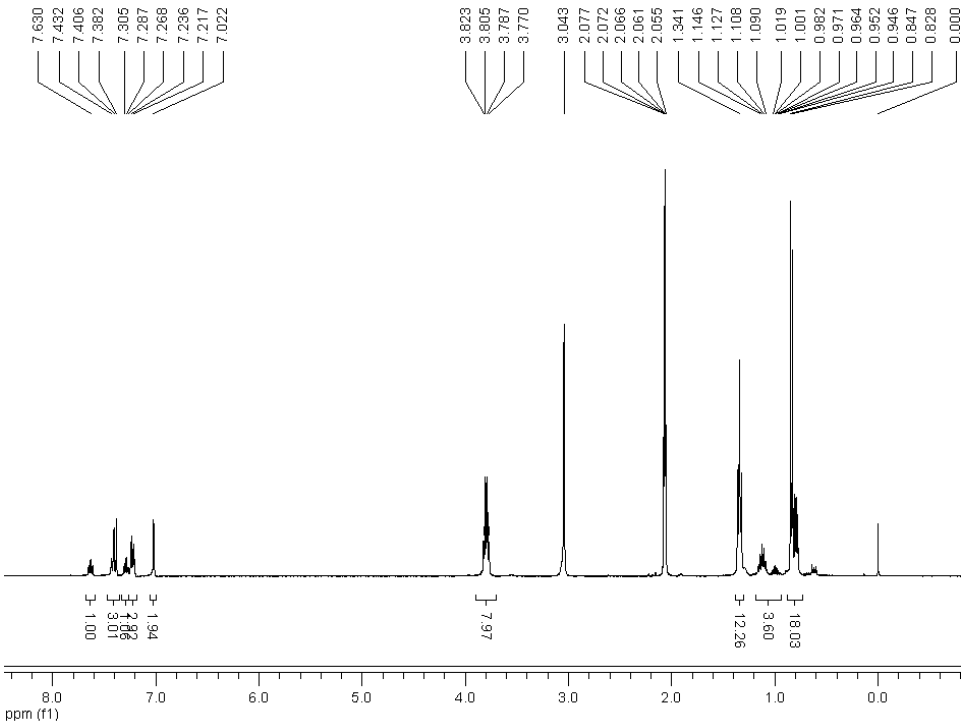


Figure S6. ^{1}H NMR spectrum of the isolated product of probe **1** + Cu^{2+}.

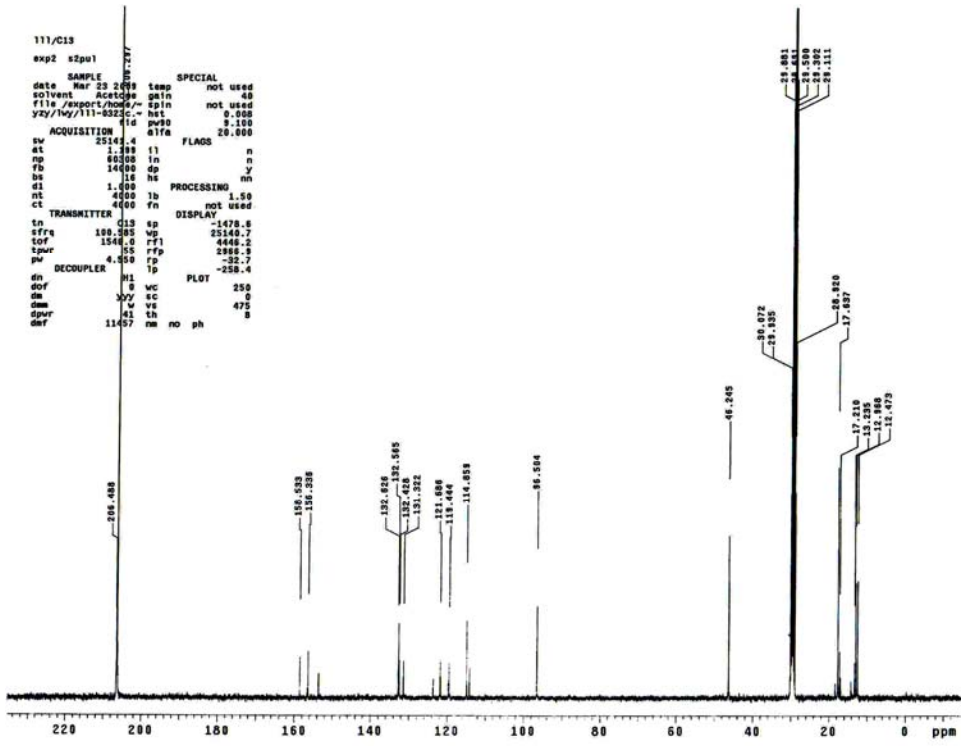


Figure S7. ^{13}C NMR spectrum of the isolated product of probe **1** + Cu^{2+}.

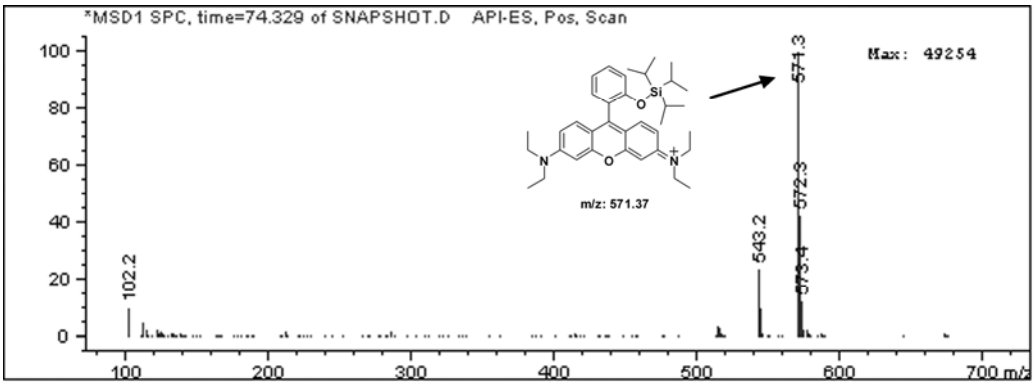


Figure S8. The mass spectrum of the isolated product of probe **1** + Cu^{2+}.

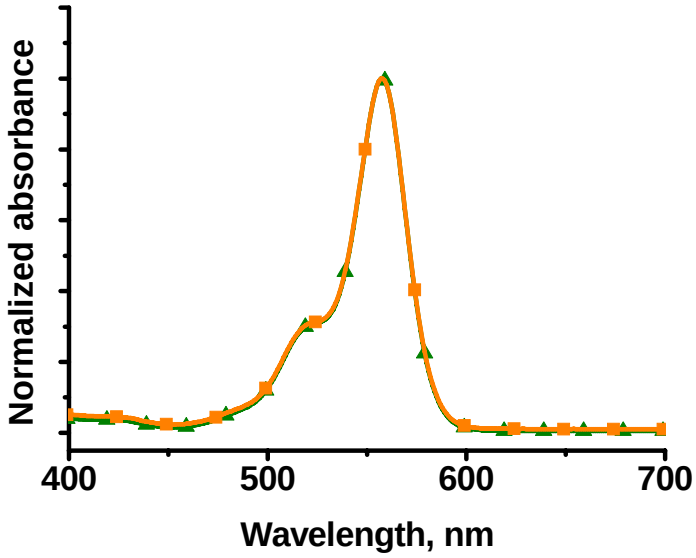


Figure S9. The normalized absorption spectra of the isolated product of probe **1** + Cu^{2+} (■) and the standard compound **2** (▲).

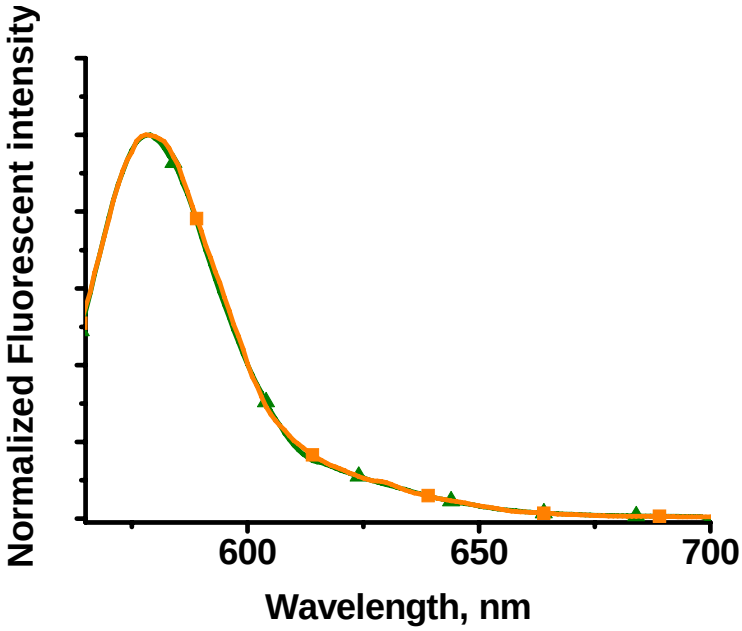


Figure S10. The normalized emission spectra of the isolated product of probe **1** + Cu^{2+} (■) and the standard compound **2** (▲). Excited at 554 nm.

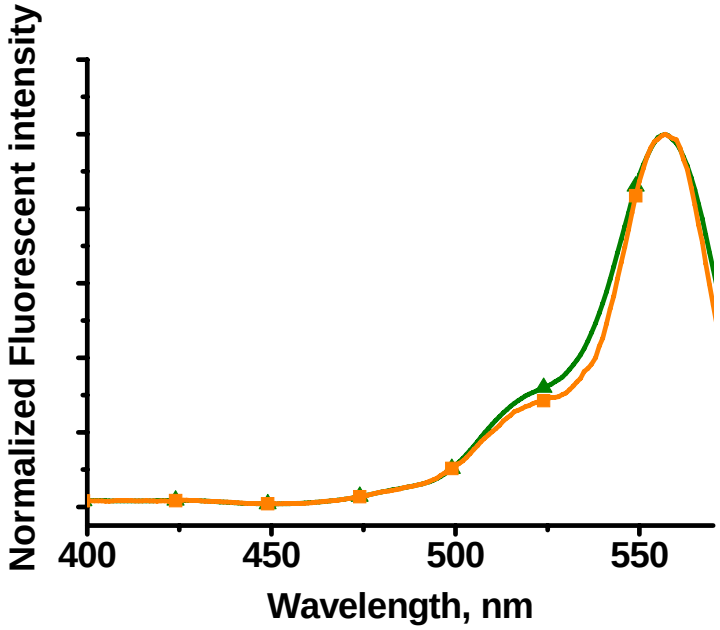


Figure S11. The normalized excitation spectra of the isolated product of probe **1** + Cu^{2+} (■) and the standard compound **2** (▲). Recorded at 580 nm.

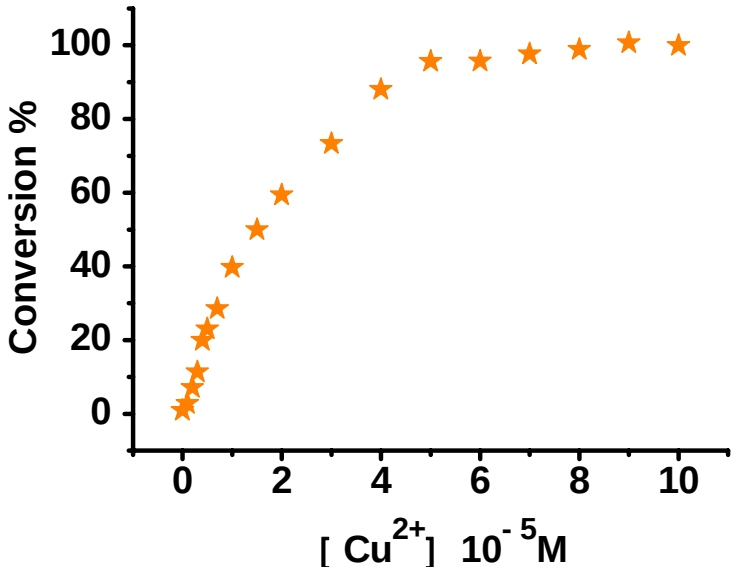


Figure S12. The conversion of probe **1** (10 μM) to compound **2** in the presence of various concentration of Cu^{2+} at room temperature. The conversion was obtained by the comparison of the absorbance (at 558 nm) of probe **1** + Cu^{2+} with that of the standard compound **2**. The Cu^{2+}-mediated oxidation was conducted at room temperature for 30 minutes in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent.

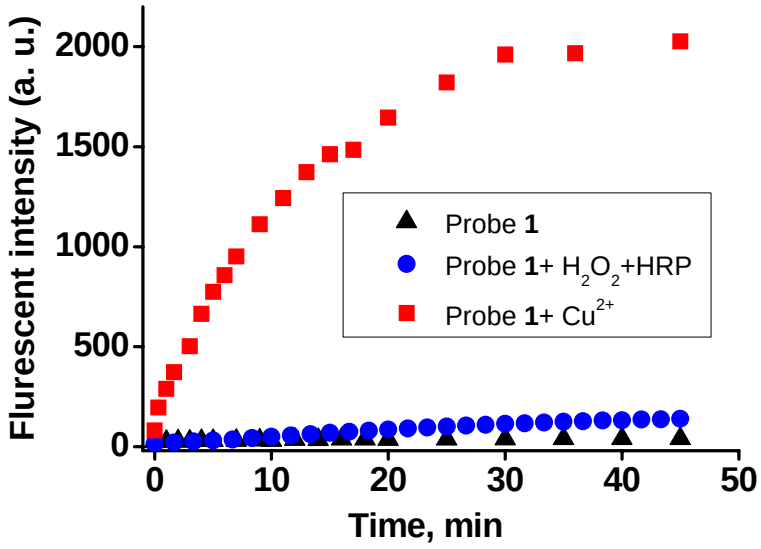


Figure S13. Time course of the fluorescent intensity changes of probe **1** (10 μM) in the absence and presence of various species (5 equiv.) (λ_{ex} = 554 nm) in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent. (HRP = horseradish peroxidase).

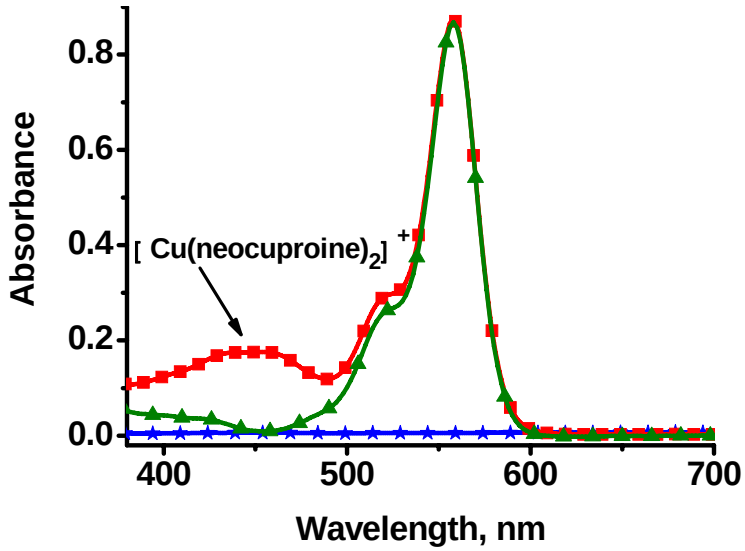


Figure S14. The absorption spectra of probe **1** (10 μM) + 5 equiv. Cu^{2+} in the absence (▲) or presence (■) of 5 equiv. neocuproine in 20 mM HEPES buffer, pH 7.4, containing 40% CH_3CN as a co-solvent. For comparison, the absorption spectrum of 5 equiv. neocuproine (★) was also displayed.

Table S1. Determination of Cu^{2+} concentrations in the water samples.

Sample	Cu^{2+} spiked (mol L^{-1})	Cu^{2+} recovered (mol L^{-1})[a]	Recovery (%)
YueLu spring 1	0	Not detected	—
YueLu spring 2	1.00×10^{-6}	$(0.96 \pm 0.11) \times 10^{-6}$	96.0
YueLu spring 3	5.00×10^{-6}	$(5.03 \pm 0.08) \times 10^{-6}$	100.6
YueLu spring 4	15.00×10^{-6}	$(14.89 \pm 0.12) \times 10^{-6}$	99.3
YueLu spring 5	20.00×10^{-6}	$(19.99 \pm 0.10) \times 10^{-6}$	99.9

[a] Relative standard deviations were calculated on the basis of three measurements.

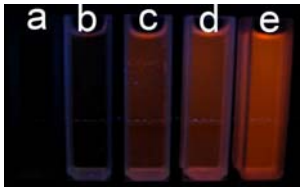


Figure S15. Visual fluorescence color change of probe **1** (10 μM) treated with the new born calf serum pre-spiked with increasing concentration of Cu^{2+} (a, 0 μM; b, 5 μM; c, 20 μM; d, 30 μM; e, 50 μM).

Table S2. Crystallographic parameters for probe **1**

Compound	Probe **1**
Chemical formula	$C_{36}H_{52}N_2O_2$ Si
formula weight	572.89
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	P2(1)/c
T (K)	293(2)
a (Å)	19.8209(19)
b (Å)	13.1032(12)
c (Å)	13.9853(13)
α(°)	90.00
β(°)	108.316(2)
γ(°)	90.00
V(Å^3)	3448.2(6)
Z	4
D (mg/m^{-3})	1.104
F(000)	1248
μ(Mo *K*a)(mm^{-1})	0.100
θ range (º)	1.08 ~ 25.50
Goodness of fit on F^2	0.859
R_1, wR_2 [$I > 2\sigma(I)$]	0.0769, 0.1948
Reflections collected / unique	17917 / 6406 [R(int) = 0.1102]
R indices (all data)	0.1507, 0.2303

$$R = \Sigma||F_0| - |F_c||/\Sigma|F_0|,\ wR_2 = \{\Sigma[w(F_0^2 - F_c^2)^2]/\Sigma[w(F_0^2)^2]\}^{1/2}$$

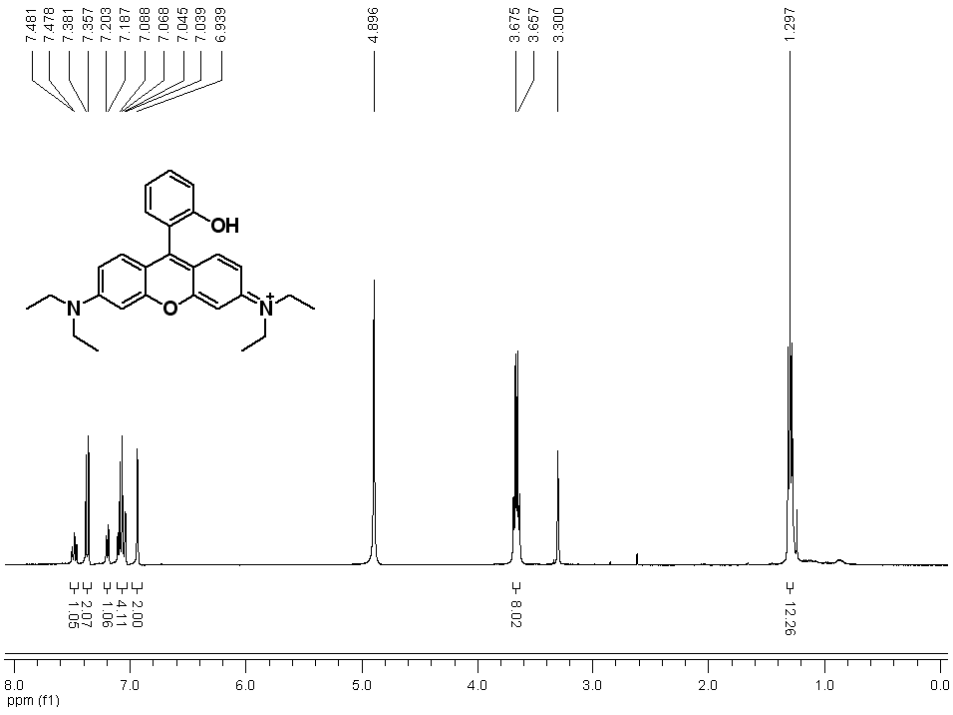


Figure S16. ^{1}H NMR spectrum of compound **3**.

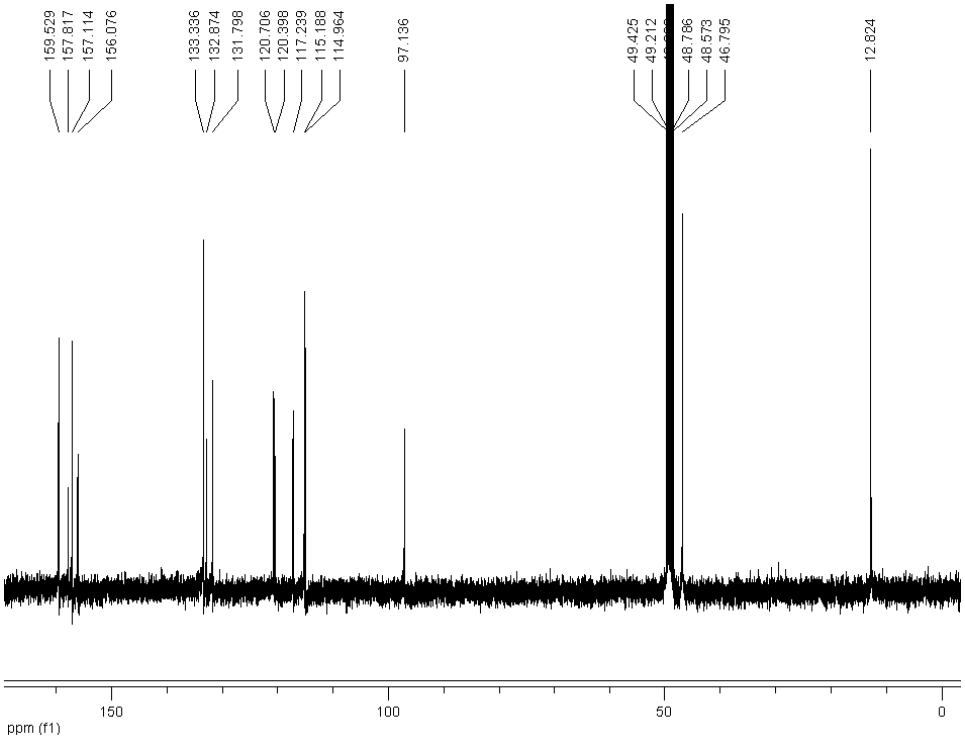


Figure S17. ^{13}C NMR spectrum of compound **3**.

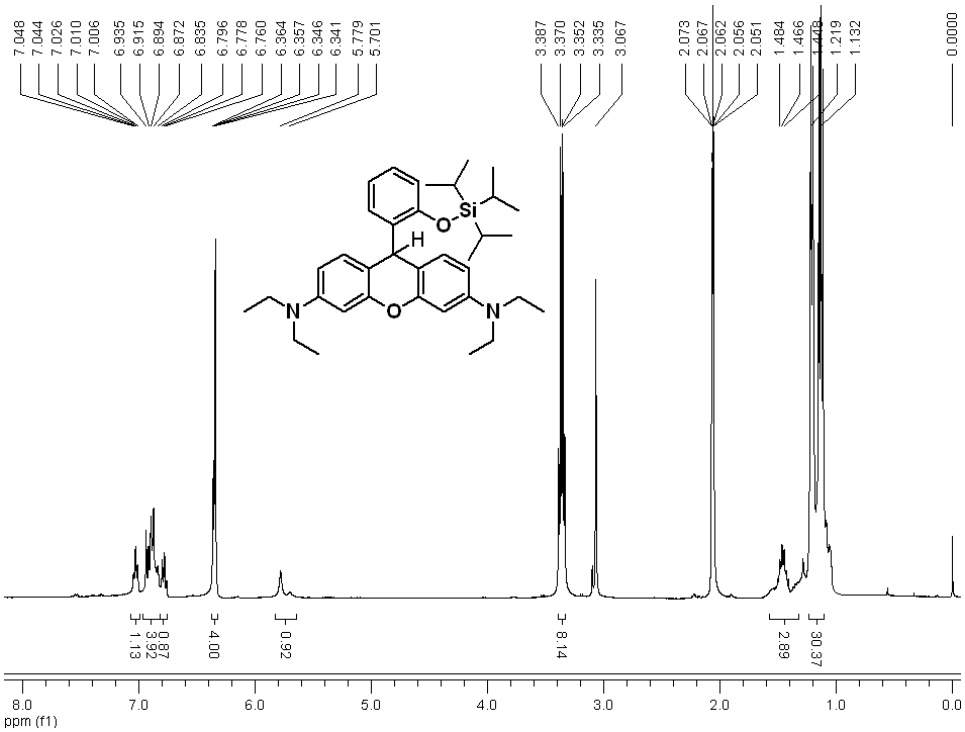


Figure S18. ^{1}H NMR spectrum of compound **1**.

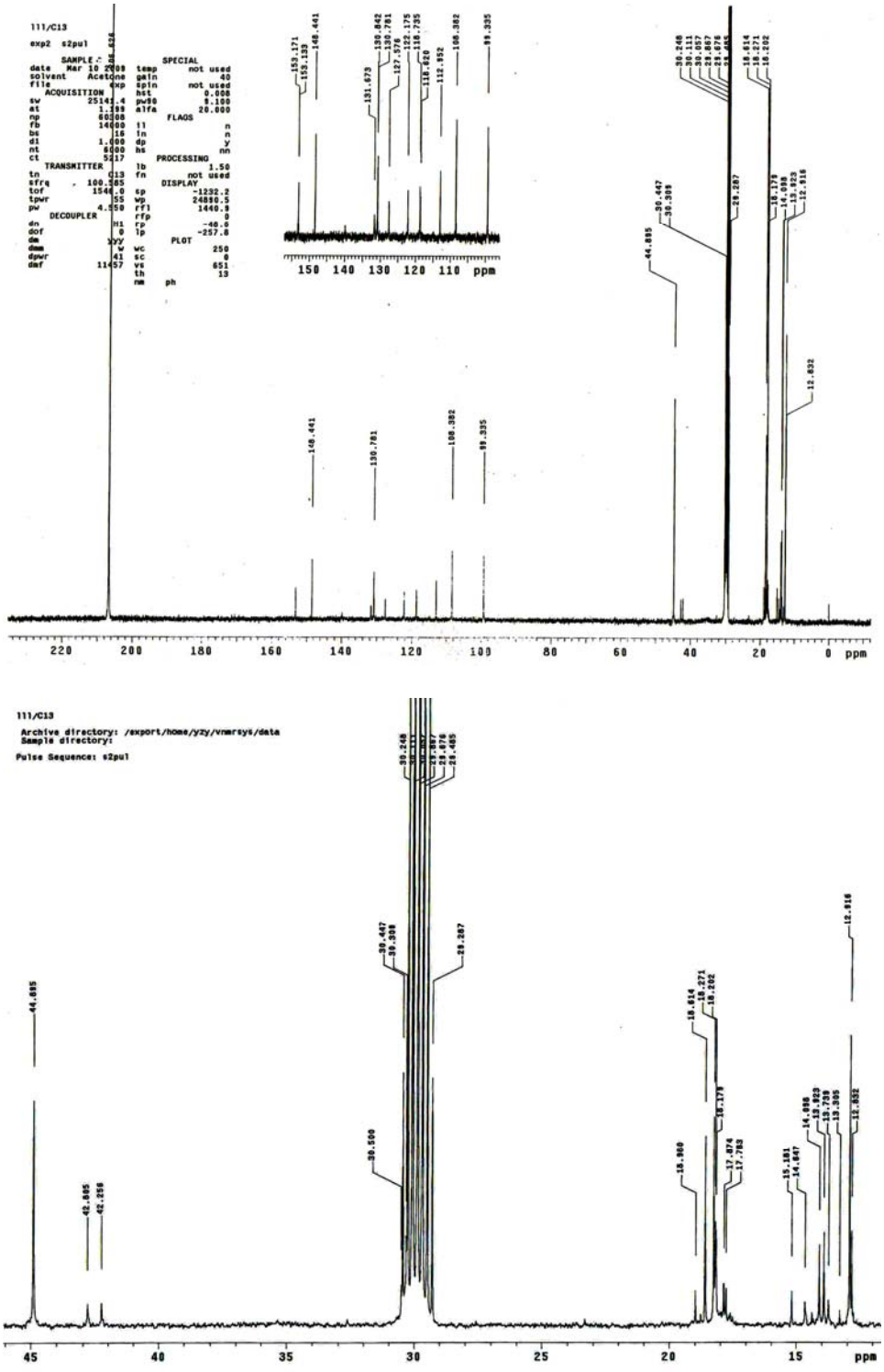


Figure S19. ^{13}C NMR spectrum of compound **1** (the expansion of chemical shifts in the region from 10 ppm to 45 ppm is shown in the bottom).

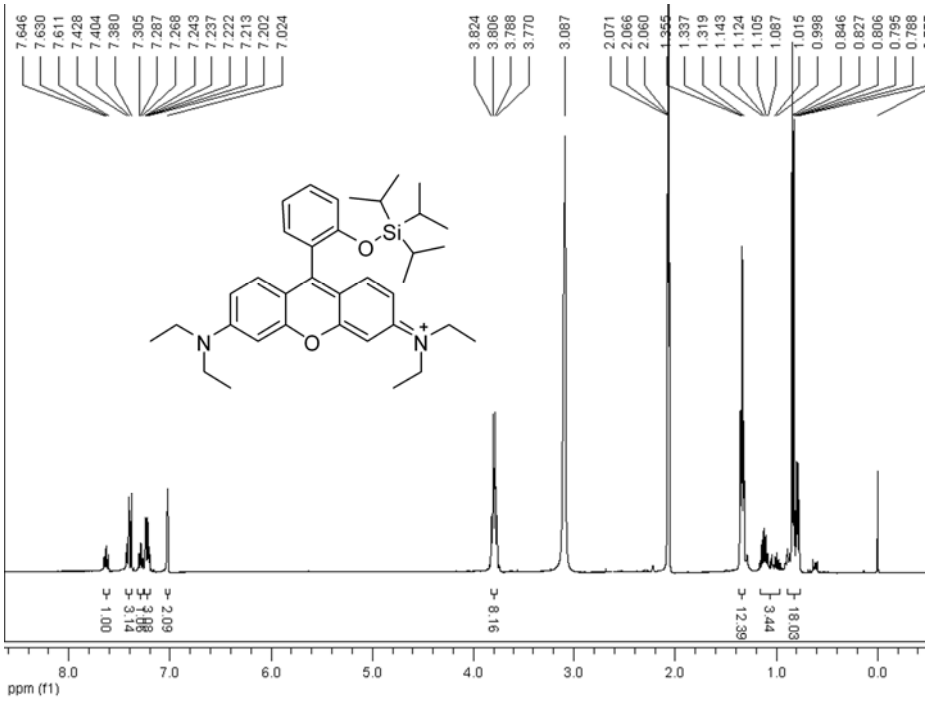


Figure S20. [1]HNMR spectrum of the conjugated rosamine compound **2**.

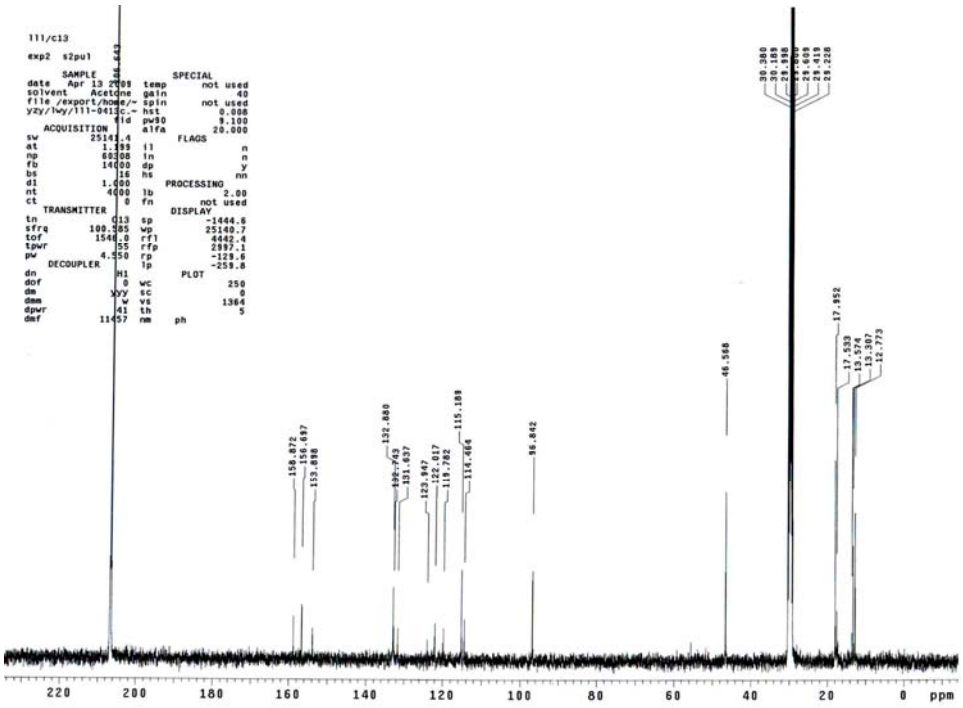


Figure S21. [13]C NMR spectrum of the conjugated rosamine compound **2**.

References:

1 (a) C. A. Parker, W. T. Rees, *Analyst*, 1960, **85**, 587; (b) S. F. Forgues, D. Lavabre, *J. Chem. Educ.*, 1999, **76**, 1260.

2 (a) I. B. Berlman, *Handbook of fluorescence spectra of aromatic molecules,* Academic Press, New York, **1971**. (b) A. Ajayaghosh, P. Carol, S. Sreejith, *J. Am. Chem. Soc.*, 2005, **127**, 14962.

3 W. Lin, L. Long, L. Yuan, Z. Cao, B. Chen, W. Tan, *Org. Lett.*, 2008, **10**, 5577.

4 V. K. Praveen, S. J. George, R. Varghese, C. Vijayakumar, A. Ajayaghosh, *J. Am. Chem. Soc.*, 2006, **128**, 7542.

5 Z. Sun, F. Liu, Y. Chen, P. K. H. Tam, D. Yang, *Org. Lett.*, 2008, **10**, 2171.