Supplementary Information (SI) for ChemComm.

Supporting Information

A Spiropyran-Based Colorimetric Probe for the Highly Selective and Sensitive Detection of Copper(II) Ions

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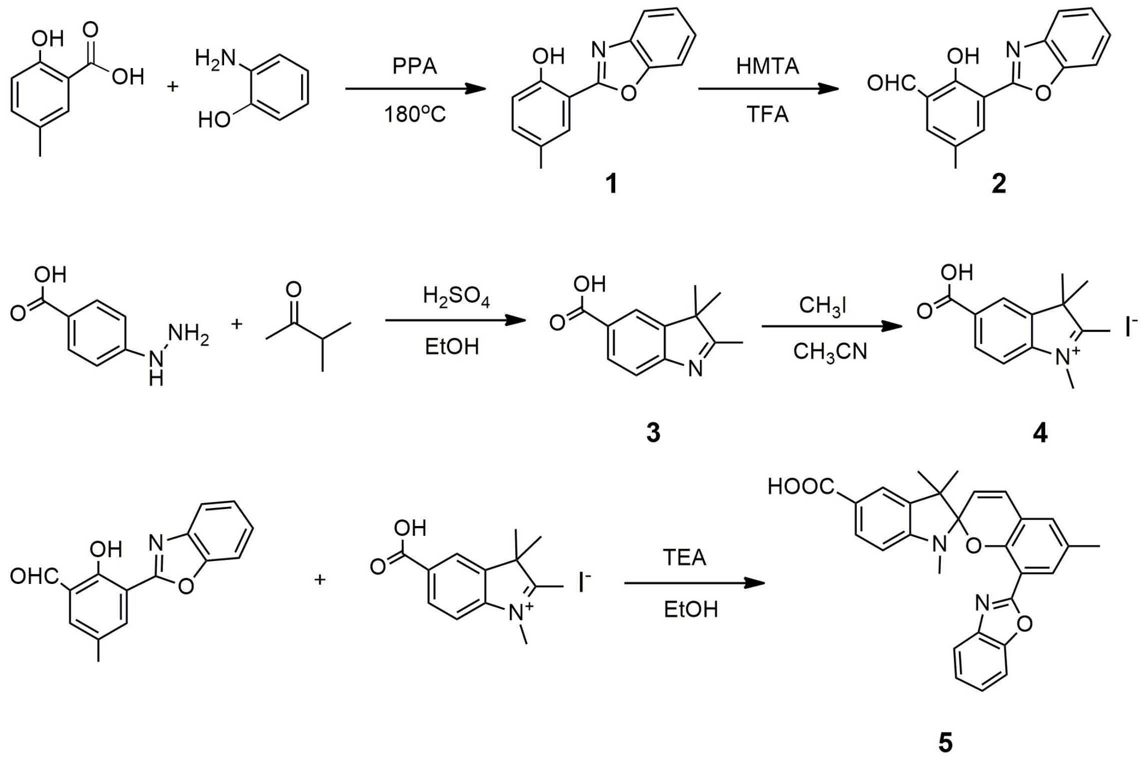
Experimental Section/Methods

Materials.

All starting materials were obtained from commercial suppliers and used as received. Solvents were dried and purified using standard methods prior to use. The synthetic route of benzoxazole-spiropyran was shown in Scheme S1.



Scheme S1. Synthetic route of the benzoxazole-spiropyran (SP) derivative.

Synthesis and Characterization.

Synthesis of 2-(benzo[d]oxazol--2-yl)-4-methylphenol (**1**)[1]. 6.5 g of 5-methylsalicylic acid (47 mmol), 5.0 g of 2-aminophenol (47 mmol) were dissolved in 100 mL of polyphosphoric acid (PPA). The mixture was heated at 180°C in oil bath for 7 h under N_2 atmosphere. After reaction, the mixture was cooled to room temperature and poured into ice. The precipitate was collected by filtration, washed thoroughly with water, and dried under vacuum. The crude solid was then purified by silica gel column chromatography (CH_2Cl_2) to afford 6.3 g of pure colorless product **1** (yield: 59%). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 2.40$ (s, 3H), 7.05 (d, *J*=8.4, 1H), 7.40 (m, 2H), 7.63 (m, 1H), 7.75 (m, 1H), 7.86 (m, 1H), 11.30 (s, 1H). Anal. calcd. for $C_{14}H_{11}NO_2$: C 74.65, H 4.92, N 6.22; found: C 74.52, H 4.85, N 6.13.

Synthesis of 3-(benzo[d]oxazol-2-yl)-2-hydroxy-5-methylbenzaldehyde (**2**)[2]. 2.41 g of above compound **1** (10 mmol) and 7 g of hexamethylenetetramine (50 mmol) were dissolved in 50 mL of trifluoroacetic acid. The mixture was refluxed for 24 h. After cooling to room temperature, the solution was poured into 500 mL of water. The orange precipitate was collected by filtration, and washed with water three times. After drying under vacuum, 2.69 g of pure compound **2** was obtained (yield: 95%). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 2.44$ (d, *J*=0.7, 3H), 7.45 (m, 2H), 7.67 (m, 1H), 7.81 (m, 2H), 8.13 (m, 1H), 10.62 (s, 1H). Anal. calcd. for $C_{15}H_{11}NO_3$: C 71.14, H 4.38, N 5.53; found: C 71.06, H 4.37, N 5.44.

Synthesis of 2,3,3-trimethyl-3H-indole-5-carboxylic acid (**3**)[3]. 2.0 mL of concentrated sulfuric acid was added dropwise to an absolute ethanol solution (250 mL) containing 10.0 mL of 3-Methyl-2-butanone (92 mmol) and 10.0 g of 4-hydrazinobenzoic acid (66 mmol). The mixture was refluxed for 10 h. After cooling to ambient temperature, the reaction mixture was filtered. The filtrate was treated with 100 mL of saturated aqueous $NaHCO_3$ solution, then washed with CH_2Cl_2 (50 mL×3). The aqueous phase was adjusted to pH 4~5 using aqueous HCl solution and extracted with CH_2Cl_2 (50 mL×3). The combined organic phases were extracted with 200 mL of brine and then dried over anhydrous Na_2SO_4. After evaporation to dryness, 12.1 g of brownish product **3** was obtained (yield: 90%). ^1H NMR (400 MHz, $CDCl_3$) δ = 1.39 (s, 6H), 2.42 (s, 3H), 7.73 (d, *J*=8.1, 1H), 8.09 (d, *J*=1.6, 1H), 8.18 (dd, *J*=1.7, 8.1, 1H), 10.88 (s, 1H). Anal. calcd. for $C_{12}H_{13}NO_2$: C 70.92, H 6.45, N 6.89; found: C 71.02, H 6.33, N 6.75.

Synthesis of 5-carboxy-1,2,3,3-tetramethyl-3H-indol-1-ium iodid (**4**). 2.03 g of product **3** A (10 mmol) and 1 mL of methyl iodide (16 mmol) were dissolved 30 mL of acetonitrile. The mixture was refluxed for 14 h. After reaction, the mixture was cooled to 0 °C. The resulting precipitate was filtered, washed with diethyl ether (20 mL×3) and dried under vacuum. 1.9 g of pure white product **4** was obtained (yield: 55%). ^1H NMR (400 MHz, D_2O) δ = 1.43-1.48 (s, 6H), 3.17-3.22 (d, *J*=0.9, 2H), 3.88-3.92 (s, 3H), 7.66-7.72 (d, *J*=8.4, 1H), 8.05-8.13 (dt, *J*=1.3, 8.5, 1H), 8.16-8.21 (d, *J*=1.6, 1H). Anal. calcd. for $C_{13}H_{16}INO_2$: C 45.23, H 4.67, N 4.06; found: C 45.03, H 4.55, N 4.11.

Synthesis of 8-(benzo[d]oxazol-2-yl)-1',3',3',6-tetramethylspiro[chromene-2,2'-indoline]-5'-carboxylic acid (*benzoxazole-spiropyran,* **5**). 345 mg of 4 (1 mmol) and 253 mg of 2 (1 mmol) were dissolved in 30 mL of methanol. 150 μL of piperidine (1.5 mmol) was added. The mixture was refluxed for 6 h. After cooling to ambient temperature, the reaction solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography (CH_3OH: CH_2Cl_2 = 3:100, v/v) to afford 325 mg of pure off-white product **5** (yield: 65%). ^1H NMR (400 MHz, DMSO-d_6) δ = 1.17-1.24 (s, 4H), 1.28-1.34 (s, 3H), 2.29-2.33 (s, 3H), 2.72-2.76 (s, 3H), 5.95-6.03 (d, *J*=10.3, 1H), 6.66-6.73 (d, *J*=8.2, 1H), 6.84-6.91 (d, *J*=7.9, 1H), 7.11-7.18 (d, *J*=10.3, 1H), 7.20-7.28 (td, *J*=1.4, 7.7, 1H), 7.25-7.34 (m, 2H), 7.60-7.67 (m, 1H), 7.69-7.78 (dd, *J*=2.0, 15.9, 2H), 7.84-7.91 (dd, *J*=1.8, 8.2, 1H), 12.37-12.42 (s, 1H). Anal. calcd. for $C_{28}H_{24}N_2O_4$: C 74.32, H 5.35, N 6.19; found: C 71.24, H 5.33, N 6.05.

General.

^1H NMR spectra were recorded on a Bruker AV400 spectrometer. Elemental analysis was performed on a Carlo–Erba-1106 instrument. UV-vis spectra were carried out on JASCO UV-550 spectrophotometer. Atomic absorption spectra were measured on HITACHI Z-2000 atomic absorption spectrometer.

0.1 mM benzoxazole-spiropyran (SP) methanol solution was employed to study photochromic behavior. The light source used for the UV-induced ring-opened process (SP→MC) was UV lamp (λ = 365 nm, 40 W) and the distance of UV source–sample was 10 cm with a light intensity of 280 $mW \cdot cm^{-2}$ in the dark. Natural condition (ambient temperature and visible light conditions) was employed for the recovery of the original colorless solution. All experiments were conducted at controlled low temperatures.

To prepare methanolic SP solutions at different pH values, a representative procedure follows: 20 μL of methanolic HCl (1 M), 20 μL of methanolic SP solution (0.1 mM), and 160 μL methanol were combined in a seal-capped vial. After shaking, a methanolic SP solution at pH=1 was obtained. Solutions at other pH values were prepared similarly.

1 mM SP methanol solution and 0.2 mM nitrate or trifluoromethanesulfonate salts of several cations' aqueous solution (Pb^{2+}, Zn^{2+}, Co^{2+}, Ag^+, $Fe^{2+/3+}$, Hg^{2+}, Na^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}) were used as stock solutions for detection test. All tests

maintained 0.1 mM SP. For the detection to 10^{-4} M cations, 20 μL of SP stock solutions, 100 μL of cationic stock solutions and 80 μL of methanol/H_2O solution (1:1, v/v) were combined in a seal-capped vial. The resulting solution was shaken well. As for the detection to 10^{-5} M cations, 20 μL of SP stock solutions, 10 μL of cationic stock solutions and 170 μL of methanol/H_2O solution (1:1, v/v) were mixed. The immediate color changes were observed visually.

Test strips were prepared by immersion of the filter paper in 0.02 mM SP/THF solution for 3 mins and then air-dried under ambient conditions. The obtained strips were then briefly immersed in aqueous cation solutions at room temperature, with immediate visual color changes observed.

Instruments.

^{1}H NMR spectra were recorded on a Bruker AV400 spectrometer. UV-vis spectra were performed on JASCO UV-550 spectrophotometers. FT-IR spectra were measured on a Bruker Tensor 27 FT-IR spectrometer. High-resolution mass spectrometry (HRMS) experiments were conducted on a Bruker Solari X 9.4T Spectrometer. Elemental analysis was performed on a Carlo–Erba-1106 instrument.

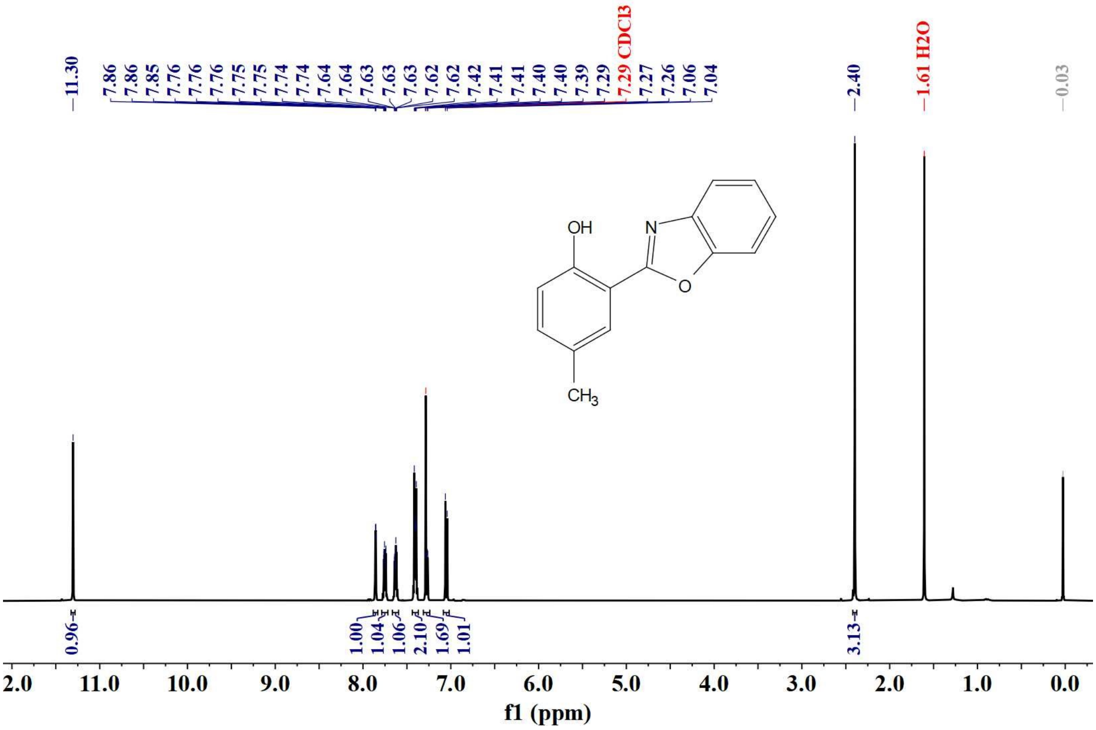


Fig. S1. ^{1}H NMR spectra of 2-(benzo[d]oxazol--2-yl)-4-methylphenol (1).

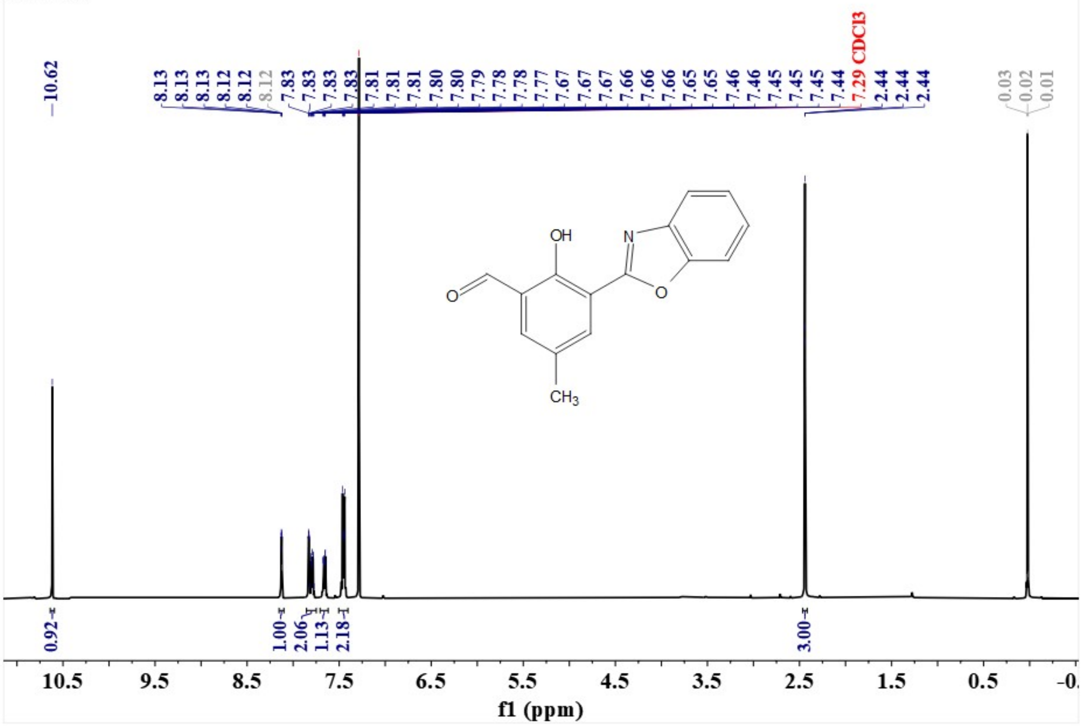


Fig. S2. ^{1}H NMR spectra of 3-(benzo[d]oxazol-2-yl)-2-hydroxy-5-methylbenzaldehyde (2).

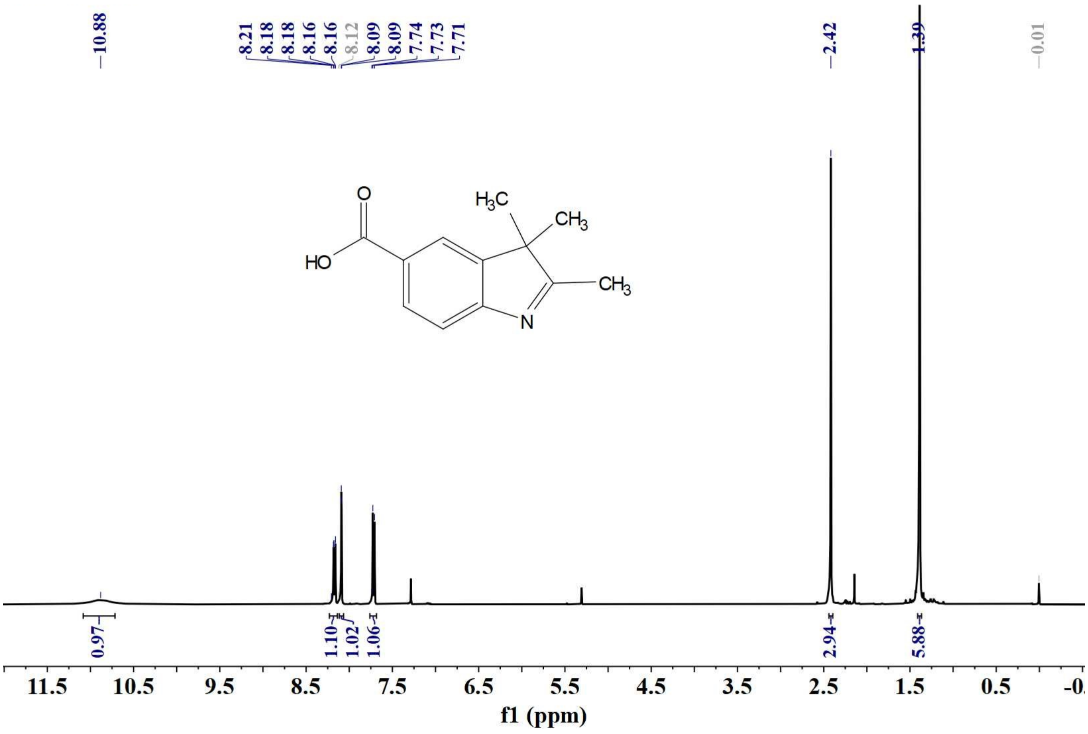


Fig. S3. ^{1}H NMR spectra of 2,3,3-trimethyl-3H-indole-5-carboxylic acid (3).

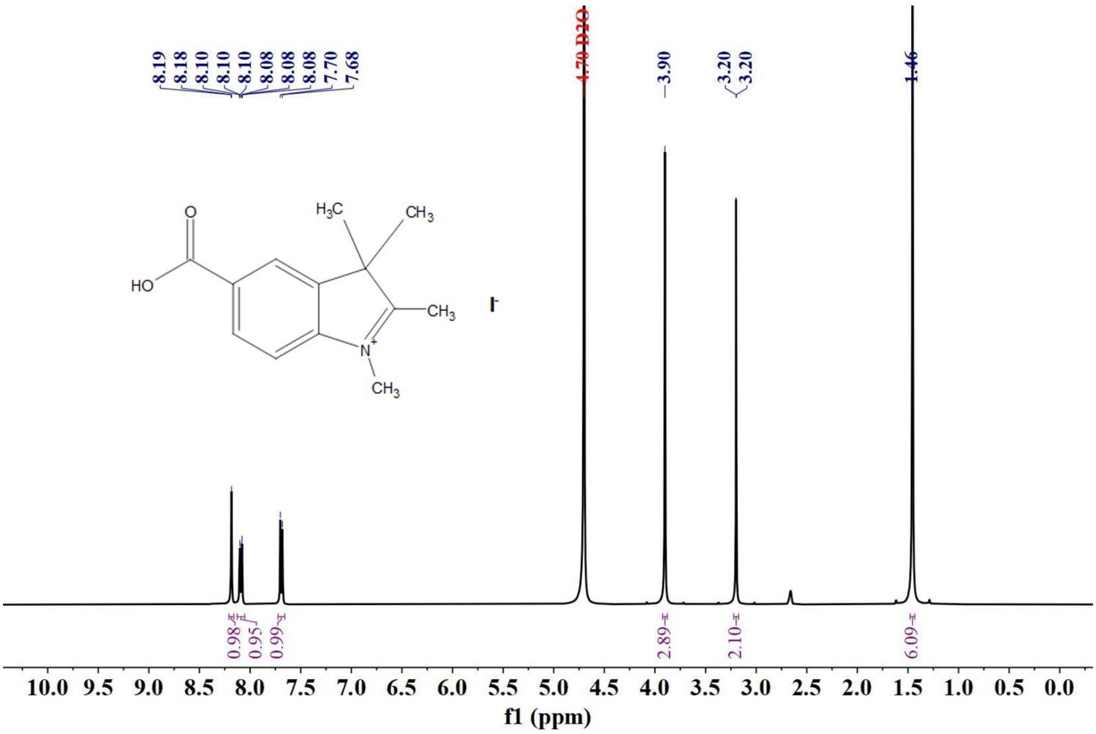


Fig. S4. ^{1}H NMR spectra of 5-carboxy-1,2,3,3-tetramethyl-3H-indol-1-ium iodid (4).

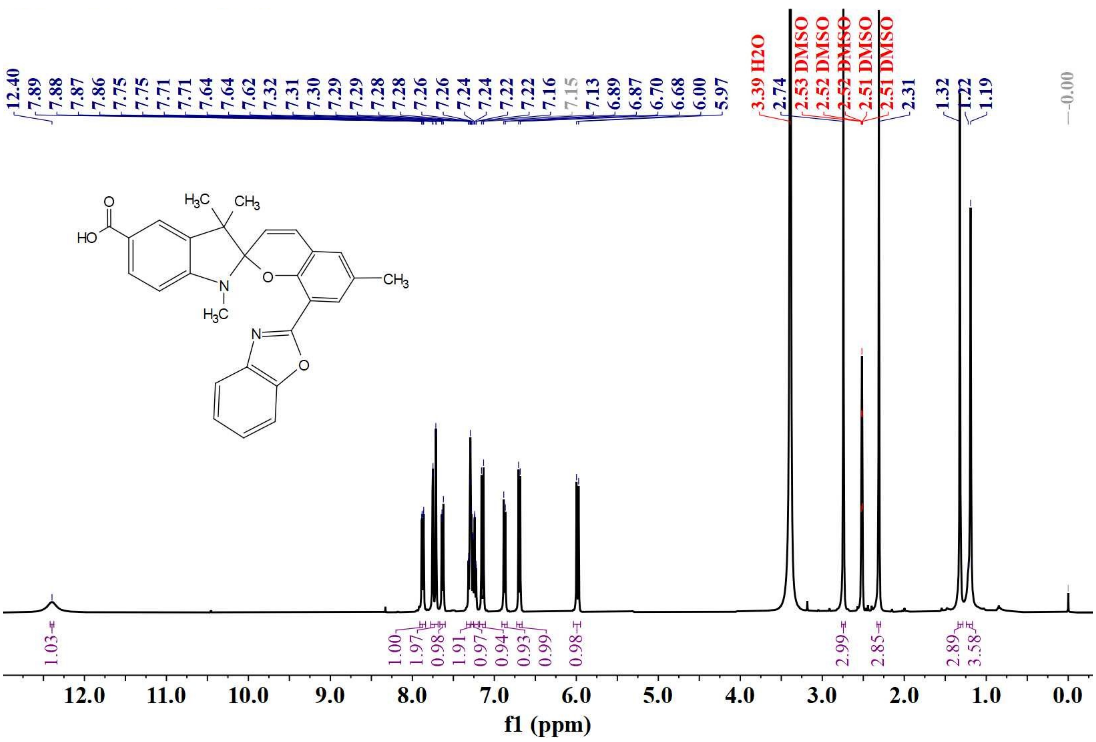


Fig. S5. ^{1}H NMR spectra of benzoxazole-spiropyran (5, SP).

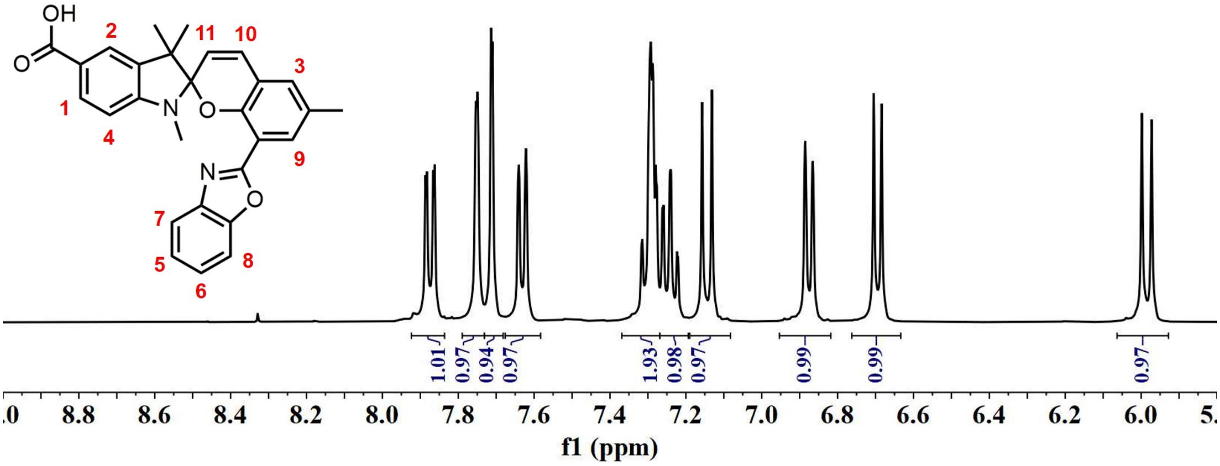


Fig. S6. ^{1}H NMR spectra of benzoxazole-spiropyran in the low-field of 5.8-9.0 ppm.

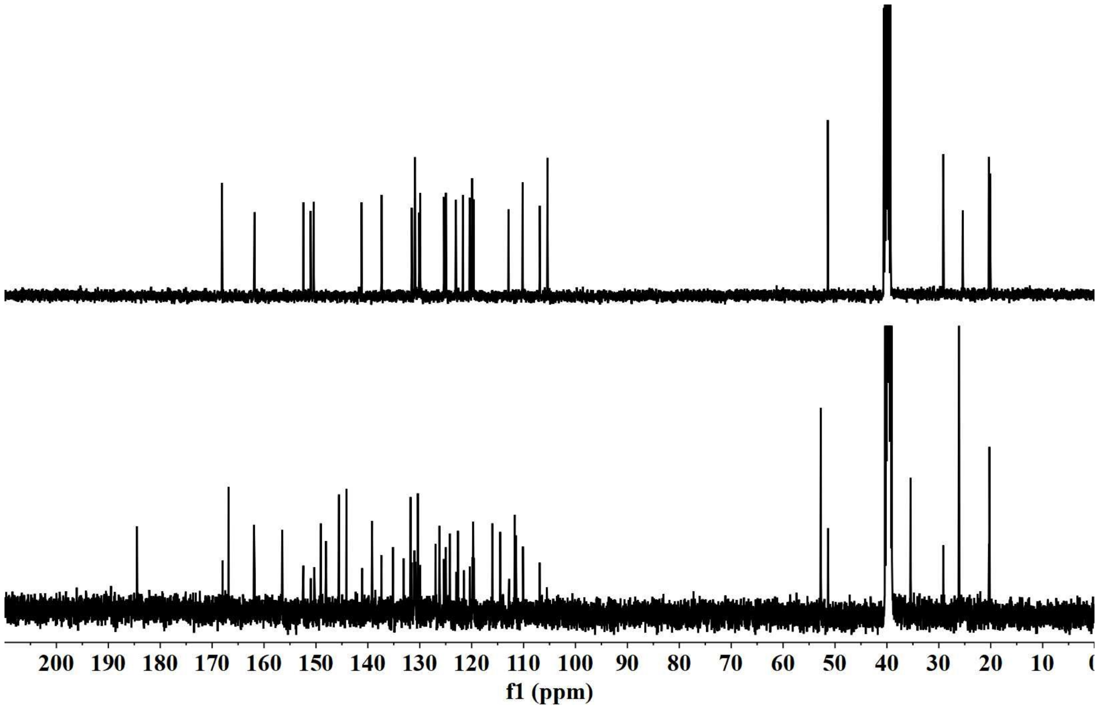


Fig. S7. ^{13}C NMR spectra of SP in DMSO-*d*6 without (top) and with (bottom) DCl.

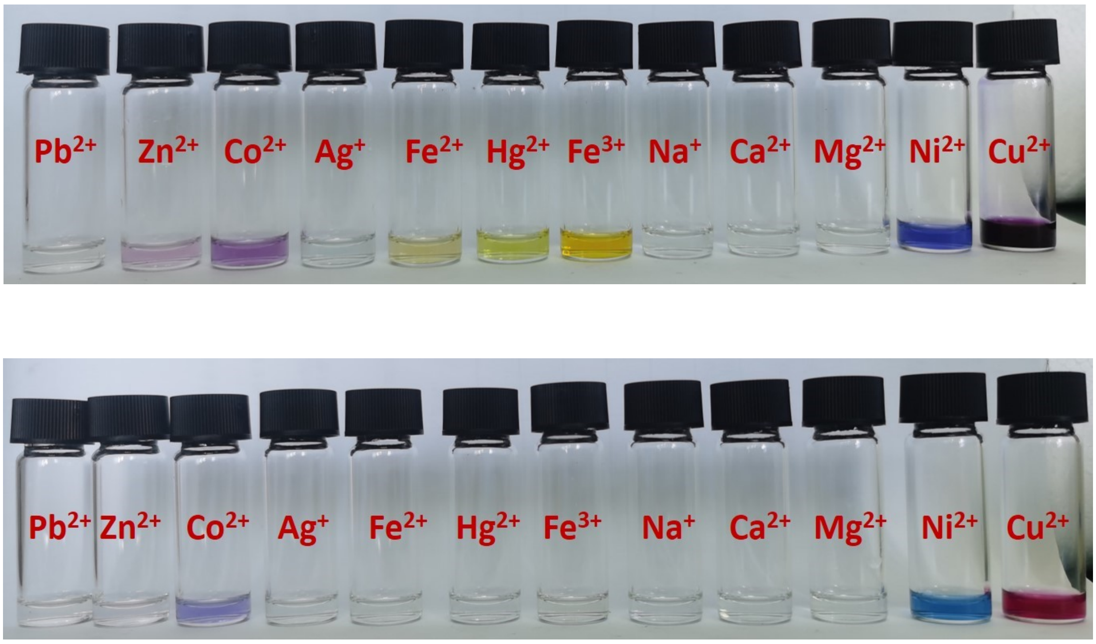


Fig. S8. Photographic images of SP (0.1 mM) with various metal ions in CH_3OH/H_2O (1:1, v/v). The concentration of metal ions 10^{-4} (top) and 10^{-5} M (bottom), respectively.

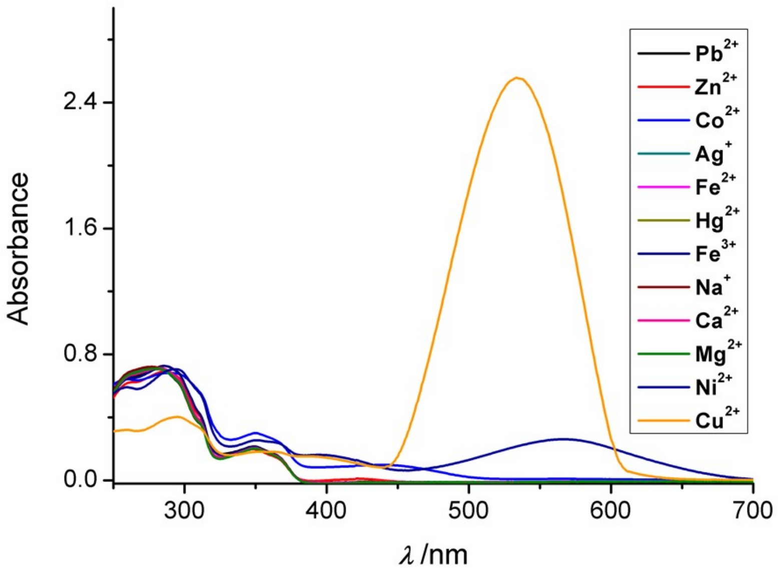


Fig. S9. UV-vis spectra of SP upon addition of various cations (0.1 equiv.). The final concentrations of SP and cations are 10^{-4} and 10^{-5} M, respectively.

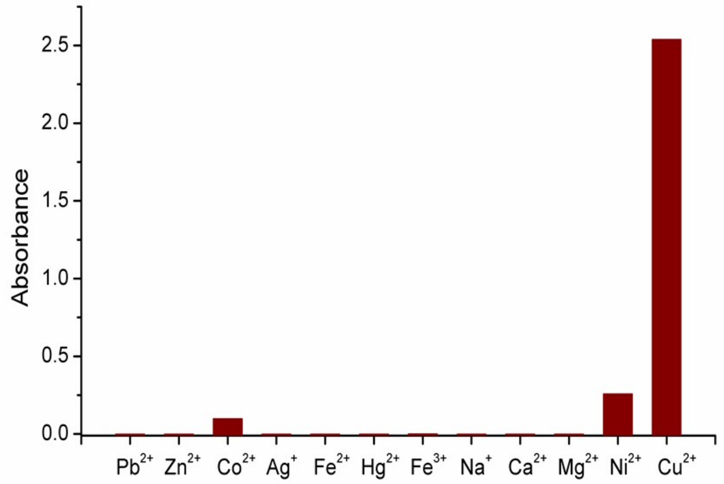


Fig. S10. Absorbance of SP upon addition of various cations (0.1 equiv.) at the λ_{max} = 535 nm. The final concentrations of SP and cations are 10^{-4} and 10^{-5} M, respectively.

ESI(P),ZYM-0702-B,20250708

Analysis Info

Acquisition Date 7/7/2025 3:27:56 PM

Analysis Name D:\Data\ESI\LiuMingHua\2025\2025-07\ZYM-0702-B_000001.d

Sample Name ZYM-0702-B

Instrument solariX

Acquisition Parameter

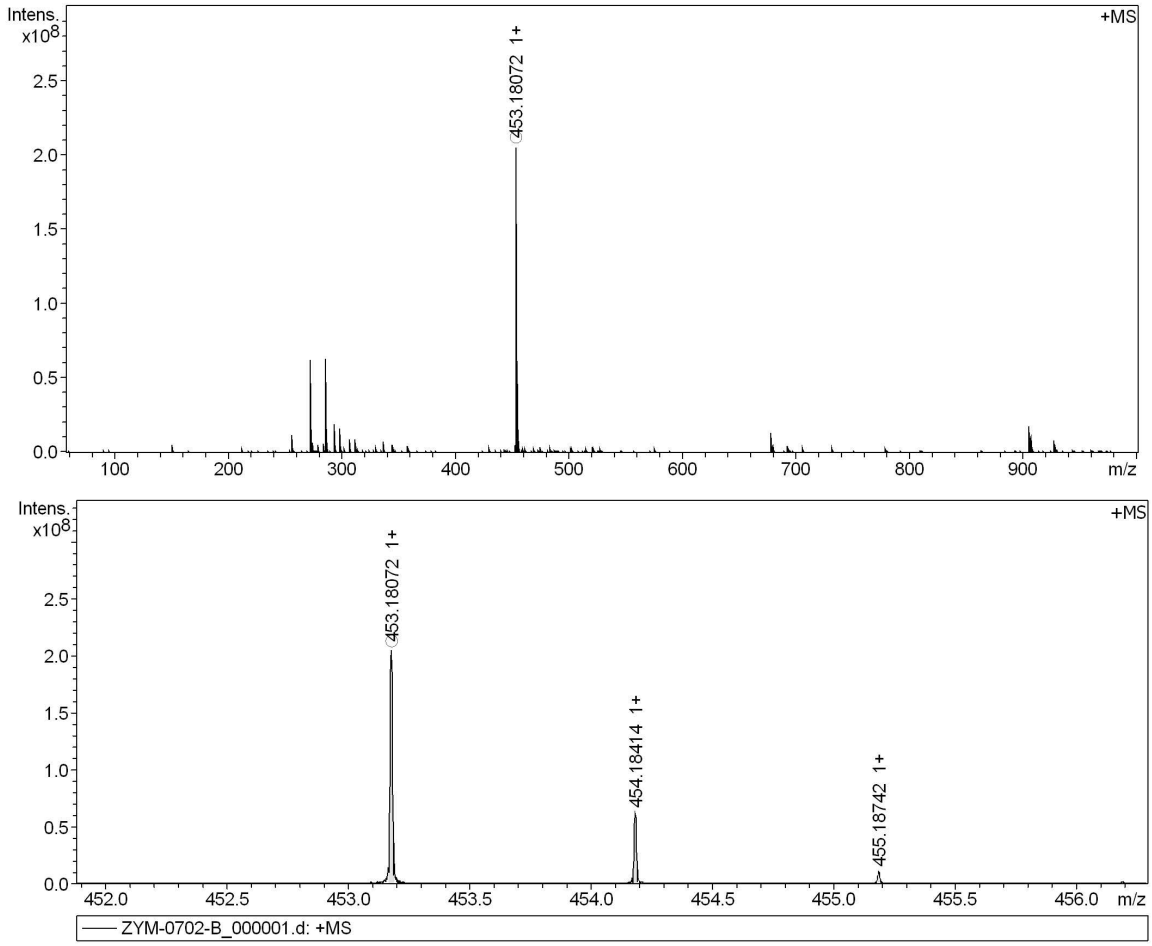
Acquisition Mode Single MS

Polarity Positive

Broadband Low Mass 57.7 m/z

Acquired Scans 7

Broadband High Mass 1000.0 m/z



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e^- Conf	N-Rule
453.180723	1	C28H25N2O4	100.00	453.180884	-0.4	0.2	8.4	17.5	even	ok

Fig. S11. The original high-resolution mass spectrum (HRMS) of SP in methanol.

ESI(P),ZYM-0702-C,20250708

Analysis Info

Acquisition Date 7/7/2025 3:34:27 PM

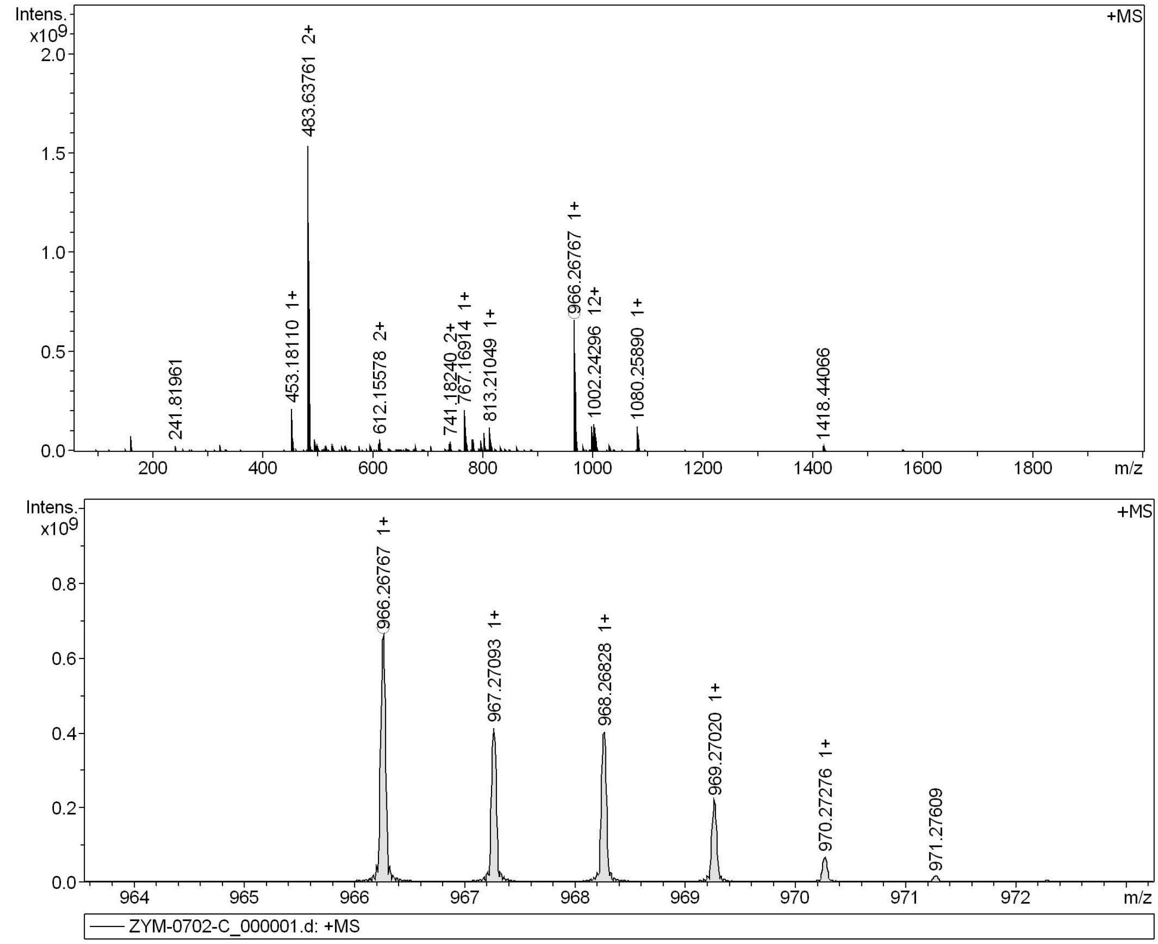
Analysis Name D:\Data\ESI\LiuMingHua\2025\2025-07\ZYM-0702-C_000001.d

Sample Name ZYM-0702-C

Instrument solariX

Acquisition Parameter

Acquisition Mode	Single MS	Acquired Scans	10
Polarity	Positive		
Broadband Low Mass	57.7 m/z	Broadband High Mass	2000.0 m/z



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
966.267665	1	C56H47CuN4O8	100.00	966.268438	0.8	0.8	18.0	35.0	odd	ok

Fig. S12. The original high-resolution mass spectrum (HRMS) of SP upon addition of 0.5 equivalent Cu^{2+} in methanol/ H_2O (1:1, v/v).

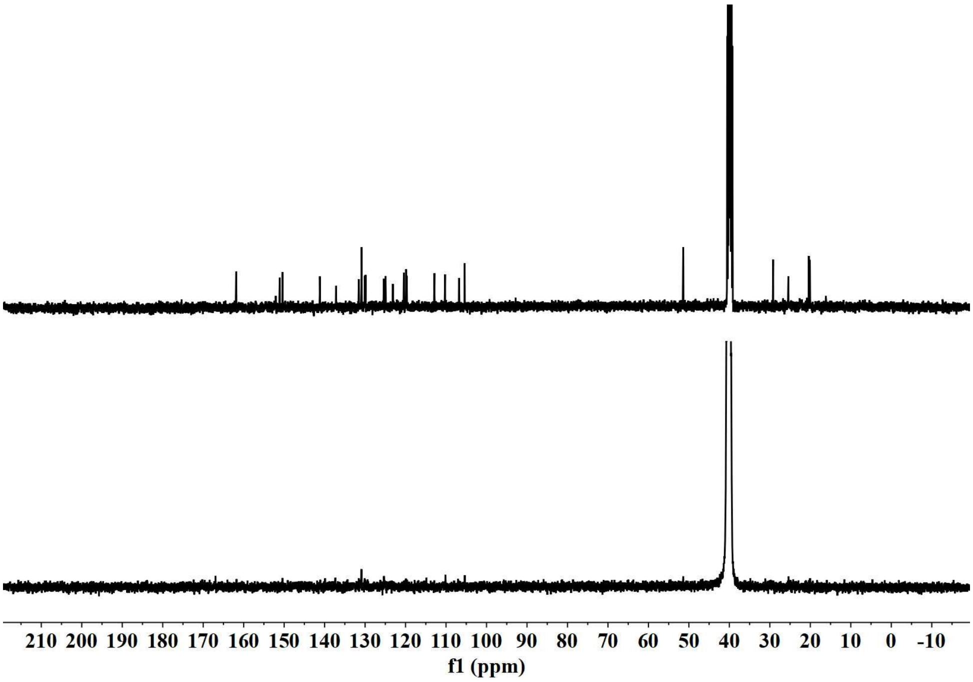
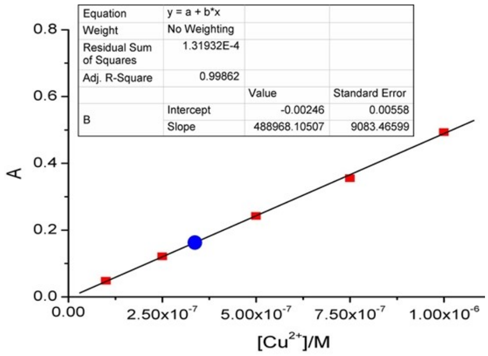


Fig. S13. ^{13}C NMR spectra of SP in DMSO-*d*6 upon addition of different equivalents $Cu(NO_3)_2$ in D_2O at 25°C. the top and bottom were 0.125 and 0.5 equiv., respectively.



Equation	y = a + b*x		
Weight	No Weighting		
Residual Sum of Squares	1.31932E-4		
Adj. R-Square	0.99862		
		Value	Standard Error
B	Intercept	-0.00246	0.00558
	Slope	488968.10507	9083.46599

Fig. S14. The calibration curve of Cu^{2+} ions with the benzoxazole-spiropyran probe. The blue is absorbance of the nearshore seawater of Jinsha Bay, Zhanjiang with the probe. λ = 535 nm.

Fig. S15. The naked-eye detection of the nearshore seawater of Jinsha Bay, Zhanjiang with the probe.

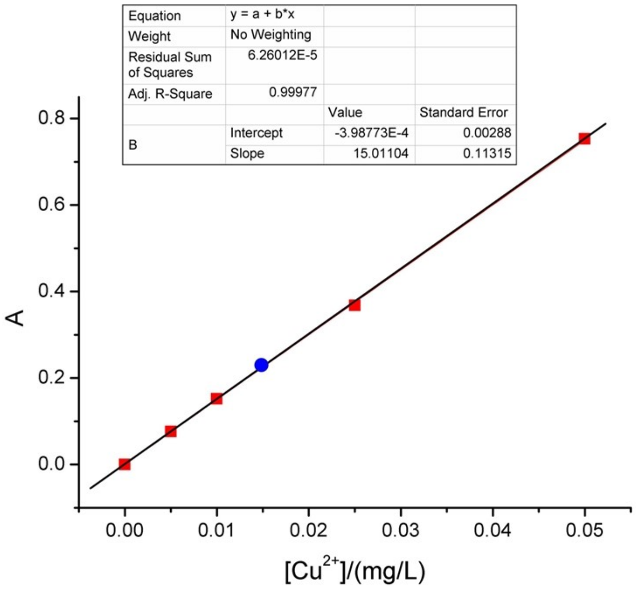


Fig. S16. The calibration curve of Cu^{2+} ions by atomic absorption spectroscopy (AAS). The blue is absorbance of the same seawater sample of Jinsha Bay, Zhanjiang. λ = 324.75 nm.

Supplementary Reference

1. D. Back, G. Oliveira, M. Ballin, V. Corbellini, *Inorg. Chim. Acta*, 2010, **363**, 807-812.
2. W. Chen, Y. Xing, Y. Pang, *Org. Lett.*, 2011, **13**, 1362-1365.
3. M. Tomasulo, S. Kaanumal, S. Sortino, F. Raymo, *J. Org. Chem.*, 2007, **72**, 595-605.