Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information for

A highly selective and sensitive fluorescence ratiometric probe for cyanide and its application for detection of cyanide in natural water sample and biological sample

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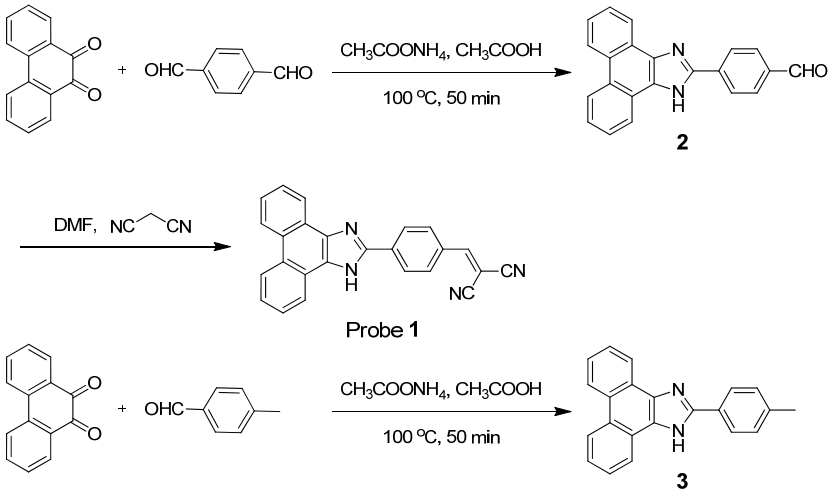
Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified and dried by standard methods prior to use. Twice-distilled water was used throughout all experiments. The solutions of various anions were prepared from their tetrabutylammonium salt. TLC analyses were 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. Melting points of compounds were measured on a Beijing Taike XT-4 microscopy melting point apparatus, and all melting points were uncorrected. Mass spectra were recorded on a LXQ Spectrometer (Thermo Scientific) operating on ESI. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz and 100 MHz respectively. Elemental (C, H, N) analysis were carried out using Flash EA 1112 analyzer. Electronic absorption spectra were obtained on a SHIMADZU UV-2450 spectrometer. Fluorescence spectra were measured on a Photon Technology International (PTI) Quantamaster fluorometer with 3 nm excitation and emission slit widths. The pH measurements were performed with a pH-3c digital pH-meter (Shanghai ShengCi Device Works, Shanghai, China) with a combined glass-calomel electrode.



Scheme S1. Synthesis of the probe **1** and reference compound **3**.

Synthesis of 4-(1*H*-phenanthro[9,10-*d*]imidazol-2-yl)benzaldehyde (2):

Terephthalaldehyde (402 mg, 3 mmol), 9,10-phenanthroquinone (208 mg, 1 mmol), and ammonium acetate (1.54 g, 20 mmol) were added in glacial AcOH (10 mL), and then the mixture was heated to 100 °C for 50 min. The hot solution was cooled to room temperature, and the resulting precipitate was collected by filtration and washed with acetate acid, dilute sodium hydrogen carbonate solution, and water. After dried under reduced vacuum, the precipitate was further purified by column chromatography on silica gel (acetone) to afford the compound **2** as yellow solid (245.9 mg, 76.3%). Mp: > 300°C. ^{1}H NMR (DMSO-d_6, 400MHz), δ (ppm): 7.67 (m, 2H), 7.77 (m, 2H), 8.14 (d, J = 8.4 Hz, 2H), 8.53 (d, J = 8.4 Hz, 2H), 8.57 (d, J = 7.6 Hz, 1H), 8.63 (d, J = 7.6 Hz, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.90 (d, J = 8.4 Hz, 1H), 10.10 (s, 1H). MS (ESI) m/z 323.1 $[M+H]^+$.

Synthesis of 2-(4-(1*H*-phenanthro[9,10-*d*]imidazol-2-yl)benzylidene) malononitrile (1):

A mixture of compound **2** (150 mg, 0.46 mmol), malononitrile (0.5 mL, 7.94 mmol), dry DMF (10 mL) was stirred overnight under N_2 atmosphere at room temperature. After the solvent was removed under vacuum, the resulting mixture was purified by column chromatography on silica gel (CH_2Cl_2) to afford the compound **1** as orange solid (138.4 mg, 81.2%). Mp: > 300°C. ^{1}H NMR (DMSO-d_6, 400MHz), δ (ppm): 7.68 (m, 2H), 7.77 (m, 2H), 8.10 (d, J = 8.4 Hz, 2H), 8.47 (d, J = 8.4 Hz, 2H), 8.49 (s, 1H), 8.57 (d, J = 7.6 Hz, 1H), 8.60 (d, J = 7.6 Hz, 1H), 8.85 (d, J = 8.4 Hz, 1H), 8.89 (d, J = 8.4 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 81.5, 113.8, 114.8, 122.6, 124.5, 126.3, 126.9, 127.8, 128.5, 131.7, 131.9, 135.5, 147.8, 160.7. MS (ESI) m/z 369.68 $[M-H]^-$. Elemental analysis calcd (%) for $C_{25}H_{14}N_4$: C 81.06, H 3.81, N 15.13; found C 80.89, H 3.83, N 15.08.

Synthesis of 2-(*p*-tolyl)-1*H*-phenanthro[9,10-*d*]imidazole (3):

p-Tolualdehyde (120 mg, 1 mmol), 9,10-phenanthroquinone (208 mg, 1 mmol), and ammonium acetate (1.54 g, 20 mmol) were added in glacial AcOH (10 mL), and

then the mixture was heated to 100 ^{o}C for 50 min. After cooling to room temperature, the solution was poured into water (50mL), and the white precipitate was collected by filtration. The crude product was purified by column chromatography on silica gel (dichloromethane: petroleum ether = 4:1, v/v) to afford the compound **3** as white solid (181.9 mg, 59%). Mp: 290-292 ^{o}C. ^{1}H NMR (Acetone-d_6, 400MHz), δ (ppm): 2.42 (s, 3 H), 7.38 (d, J = 8.0 Hz, 2H), 7.62-7.74 (m, 4 H), 8.25 (d, J = 8.0 Hz, 2H), 8.49 (d, J = 7.6 Hz, 1H), 8.75 (d, J = 8.0 Hz, 1H), 8.84 (d, J = 8.0 Hz, 1H), 8.88 (d, J = 8.4 Hz, 1H). MS (ESI) m/z 309.1 $[M+H]^{+}$.

Measurement procedures: A stock solution of probe **1** was prepared at 2.5×10^{-4} M in CH_3CN, and a stock solution of various anions (1×10^{-3} M) was prepared by dissolving an appropriate amount of anions in water. Test solutions in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4) solution (5 mL) were prepared by placing 0.2 mL of probe **1** stock solution, 3.8 mL CH_3CN, and an appropriate aliquot of each testing species stock into a 5 mL volumetric flask, and then diluting the solution to 5 mL with 20 mM potassium phosphate buffer (pH 7.4). The resulting solution was shaken well and incubated at room temperature for 10 min before recording the spectra.

Determination of fluorescence quantum yield: Fluorescence quantum yield was determined using the solutions of quinine sulfate (Φ_F = 0.546 in 1N H_2SO_4)[1] as a standard. The quantum yield was calculated using the following equation: [2-4]

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

Determination of the detection limit: The detection limit was determined from fluorescence titration data based on a reported method.[5-9] According to the result of titration experiment, the graph of $(I_{min}-I) / (I_{min}-I_{max})$ versus log $[CN^-]$ was plotted, where the I is the fluorescence intensity at 458 nm, I_{min} and I_{max} are the minimum and maximum fluorescence intensity at 458 nm respectively. A linear regression curve was then fitted (Fig. S4), and the intercept of the line at x-axis was taken as detection limit.

Detection of cyanide in natural water sample: The crude water samples from Yangtzi River, pond water and tap water were filtered through microfiltration membrane before use. For determination of CN^- in water samples, the pH value of the water samples was firstly adjusted to 7.4 by addition of aliquot aqueous sodium hydroxide or hydrochloric acid, and then diluting 4 mL of probe **1** (12.5 μM) in CH_3CN with the water samples to 5 mL in a 5 mL volumetric flask. The resulting

solution was shaken well and incubated for 10 min at room temperature. After that, the emission ratio (I_{458}/I_{618}) was recorded. The CN^- in these water samples was not detected. Next, the water samples were spiked with standard CN^- solutions at different concentration levels and then analyzed with probe **1**, the results are shown in Table S1.

Detection of endogenous biological cyanide in cassava: The extraction of cyanide from the bitter cassava root was according to a reported procedure.[10] Fresh peeled cassava roots were well crushed and stored in a sealed tube for 60 min at room temperature to release the cyanide. Then, 2 g of finely crushed material was diluted with 10 mL of water and centrifuged at 10000 r/min for 10 min. The supernatant as cassava extract were used for further analysis.

Aliquots of the cassava extract (0 μL, 20 μL, 40 μL, 60 μL,80 μL, or 100 μL) were added to 4 mL probe **1** solution (12.5 μM, in CH_3CN) in a 5 mL volumetric flask, and then diluting the solution to 5 mL with 20 mM potassium phosphate buffer (pH 7.4). After the resulting solution incubated at room temperature for 10 min, the emsision ratios (I_{458} / I_{618}) (λ_{ex}= 370 nm) were recorded.

The unknown content of cyanide in cassava sample was estimated by using the standard addition method with tetrabutylammonium cyanide (5×10^{-3} M stock) as the standard. Different volumes of the CN^- stock (0, 1, 2, 3, or 4 μL) was added directly to a mixture of 4 mL probe **1** solution (12.5 μM, in CH_3CN) and 10 μL cassava extract in a 5 mL volumetric flask, and then diluting the mixture to 5 mL with 20 mM potassium phosphate buffer (pH 7.4). The resulting solution was shaken well. After 10 min, the emsision ratios (I_{458} / I_{618}) (λ_{ex}= 370 nm) were recorded (Fig. S15). The content of cyanide in the cassava root was analyzed to be 123.4 mg/kg.

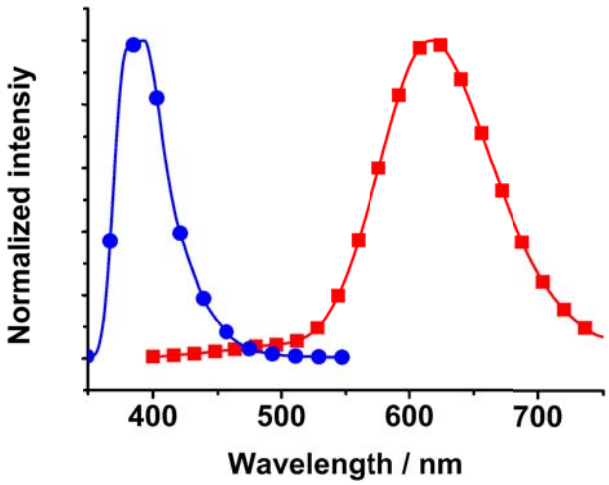


Figure S1. Normalized fluorescence emission spectra of probe **1** (■) and reference compound **3** (●) in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4), the excitation wavelength was at 370 and 326 nm respectively.

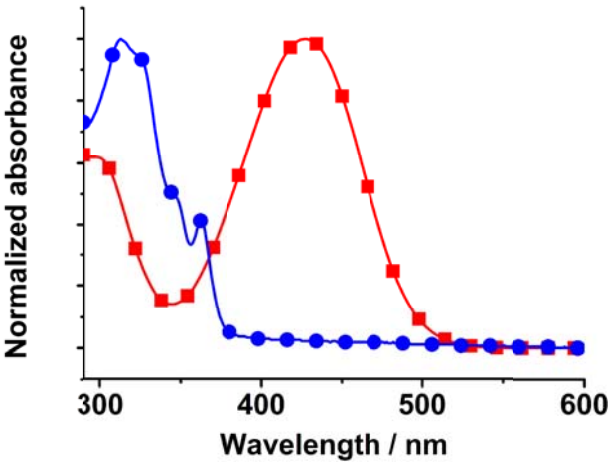


Figure S2. Normalized absorption spectra of probe **1** (■) and reference compound **3** (●) in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4).

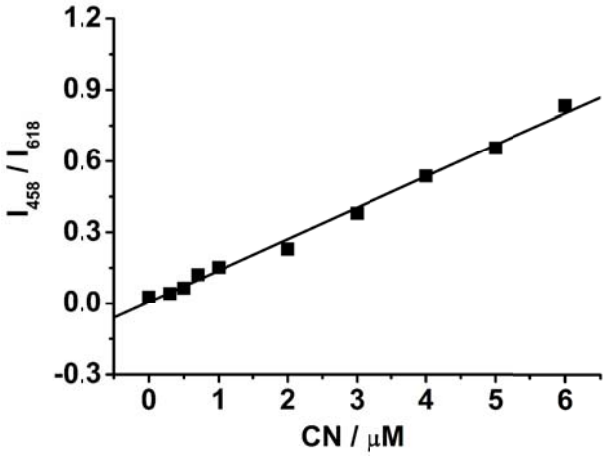


Figure S3. The emission ratio (I_{458} /I_{618}) of probe **1** (10 μM) to various amount of CN^- (0 to 6 μM). The data were acquired after incubation of probe **1** with CN^- for 10 min in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4) at room temperature. Excitation was provided at 370 nm.

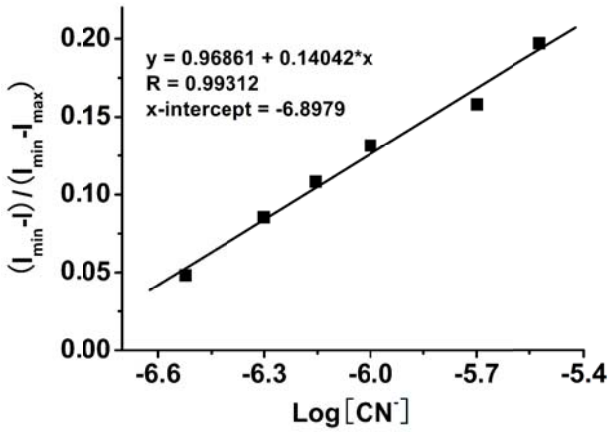


Figure S4. Plot of (I_{min}- I) / (I_{min}-I_{max}) versus log [CN^-] for probe **1**. Calculated detection limit = 0.126 μM.

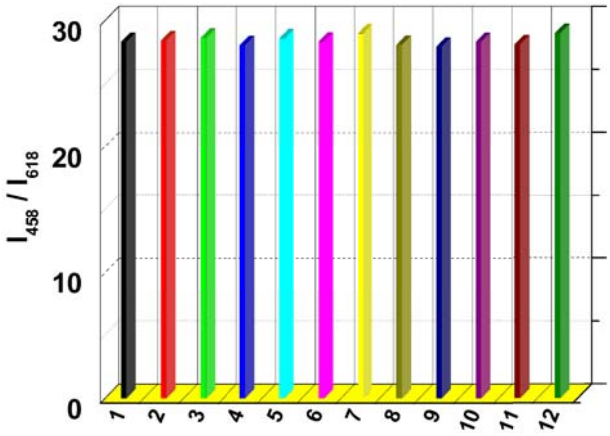


Figure S5. Fluorescence ratiometric response of probe **1** (10 μM) to 20 μM of CN^- in the presence of 20 μM of other anions. 1) blank; 2) F^-; 3) Cl^-; 4) Br^-; 5) I^-; 6) HSO_3^-; 7) CH_3COO^-; 8) ClO_4^-; 9)$H_2PO_4^-$; 10) HCO_3^-; 11) NO_3^-; 12) SCN^-. The excitation wavelength was 370 nm.

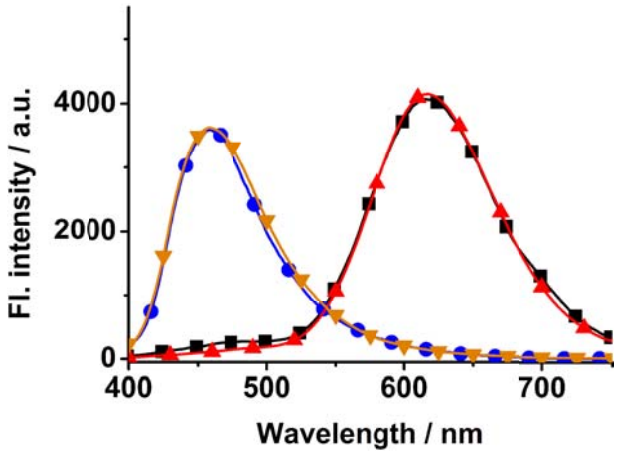


Figure S6. The fluorescence emission spectra of probe **1** (10μM) (▲), probe **1** (10μM) + 2-mercaptoethanol (20μM) (■), probe **1** (10μM) + CN^- (20 μM) (▼), and probe **1** (10μM) + 2-mercaptoethanol (20μM) + CN^- (20 μM) (●). The spectra were performed in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4). The excitation wavelength was 370 nm.

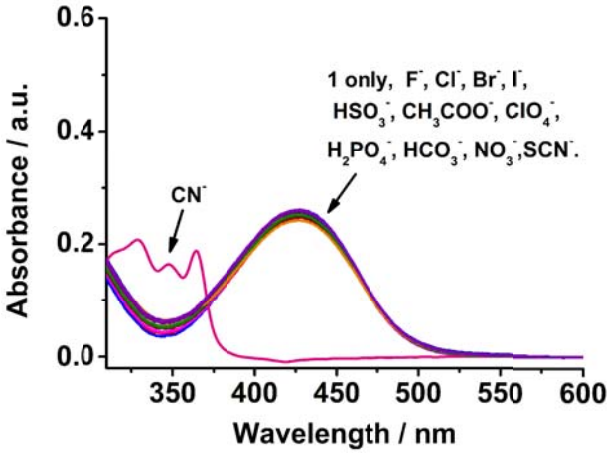


Figure S7. Absorption spectra of probe **1** (10 μM) in the presence of various anions (20μM) in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4) at room temperature.

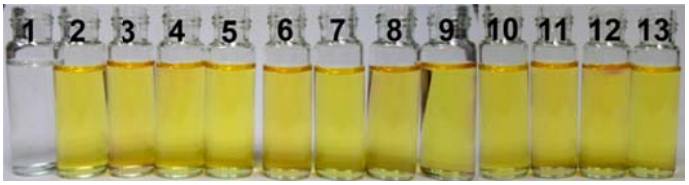


Figure S8. Visible color changes of probe **1** (10 μM) in the presence of various anions (20μM). 1) CN^-; 2) blank; 3) F^-; 4) Cl^-; 5) Br^-; 6) I^-; 7) HSO_3^-; 8) CH_3COO^-; 9) ClO_4^-; 10)$H_2PO_4^-$; 11) HCO_3^-; 12) NO_3^-; 13) SCN^-.

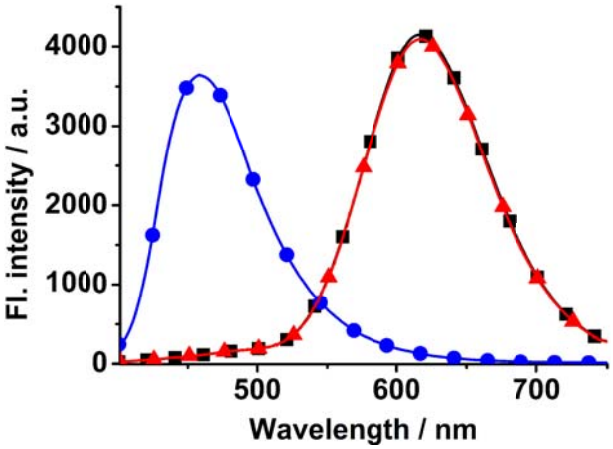


Figure S9. The fluorescence spectra (λ_{ex} = 370 nm) of probe **1** (10 μM) in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4) kept for 0.5 hour (■) and for 1 day (▲). For comparison, the fluorescence spectrum of probe **1** with addition of CN^- (20 μM) for 10 min was also showed (●).

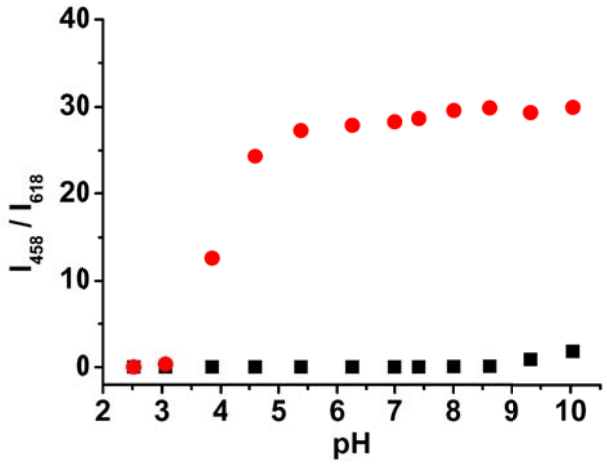


Figure S10. The variations of emission ratio of probe **1** (10 μM) in the absence (■) or presence (●) of CN^- (20 μM) as a function of pH. Excitation wavelength was 370 nm.

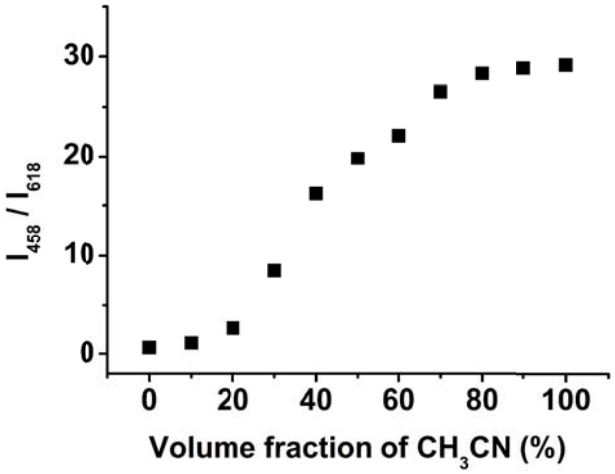


Figure S11. The effect of CH_3CN volume fraction on ratiometric response of probe **1** (10 μM) to CN^- (20 μM). The data were acquired after incubation of probe **1** with CN^- for 10 min at room temperature. Excitation was provided at 370 nm, and the ratio of emission intensities at 458 and 618 nm were measured.

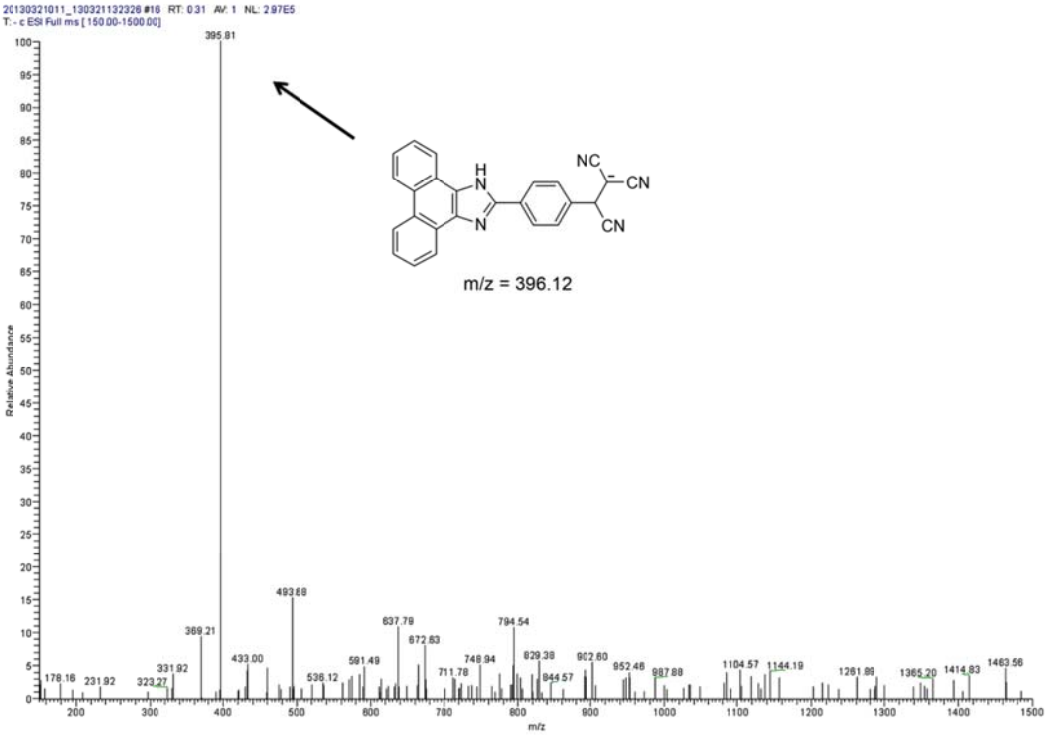


Figure S12. The ESI-Ms spectra of the probe **1** + CN^-.

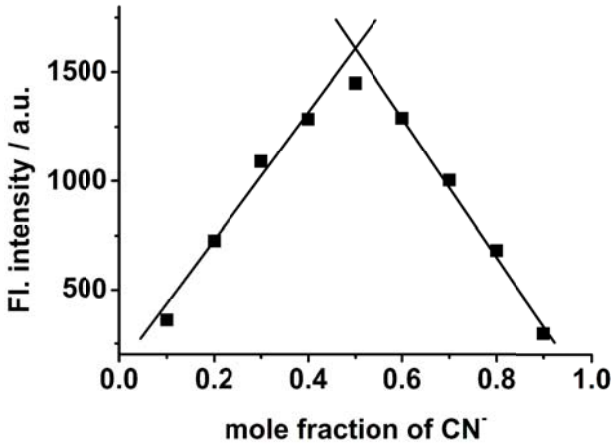


Figure S13. Job plot of the reaction between **1** and CN^- in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4). The variation of the emission at 458 nm was plotted as a function of the mole fraction of CN^-.

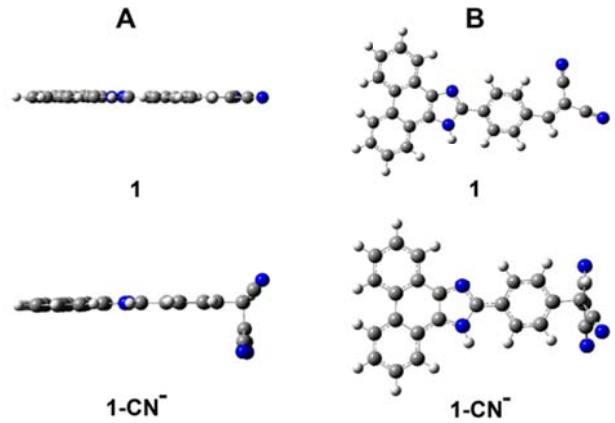


Figure S14. Optimized ground-state geometries of the probe **1** and **1**-CN^-. A) The side-view; B) The top-view.

Table S1. Determination of CN^- concentrations in natural water samples.

Sample	CN^- spiked (mol L^{-1})	CN^- recovered (mol L^{-1}) [a]	Recovery (%)
Yangtzi River 1	0	Not detected	-
Yangtzi River 2	2.00×10^{-6}	$(2.06 \pm 0.05) \times 10^{-6}$	103.0
Yangtzi River 3	2.00×10^{-5}	$(2.01 \pm 0.03) \times 10^{-5}$	100.5
Pond water 1	0	Not detected	-
Pond water 2	2.00×10^{-6}	$(1.98 \pm 0.02) \times 10^{-6}$	99.0
Pond water 3	2.00×10^{-5}	$(2.03 \pm 0.03) \times 10^{-5}$	101.5
Tap water 1	0	Not detected	-
Tap water 2	2.00×10^{-6}	$(2.04 \pm 0.03) \times 10^{-6}$	102.0
Tap water 3	2.00×10^{-5}	$(1.95 \pm 0.04) \times 10^{-5}$	97.5

[a] Relative standard deviations were calculated based on three times of measurement.

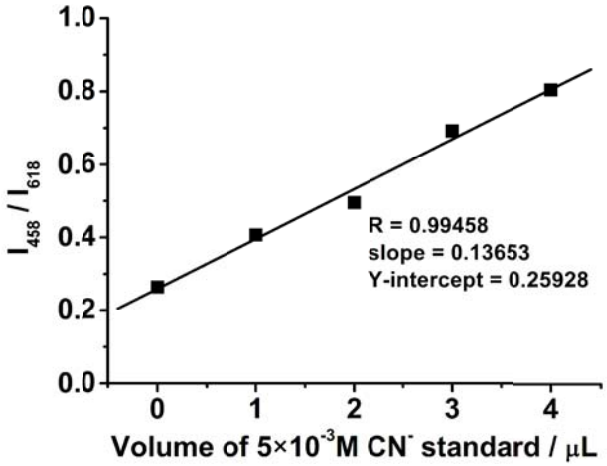


Figure S15. The ratiometric response (I_{458}/I_{618}) of probe **1** (10 μM) to 10 μL cassava extract including different volumes (0, 1, 2, 3, or 4 μL) of standard CN^- stock (5×10^{-3} M). Excitation was provided at 370 nm.

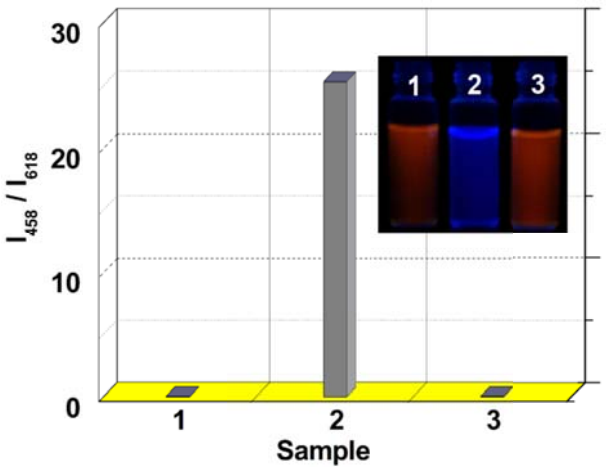


Figure S16. Ratiometric response of 5 mL probe **1** (10μM, in 20 mM potassium phosphate buffer/CH_3CN (v/v 1: 4, pH 7.4)) in the presence of various samples. Sample 1: 0.1 mL potassium phosphate buffer buffer; sample 2: 0.1 mL cassava extract; sample 3: 0.1 mL heat-treated cassava extract. Inset: visual fluorescence color changes of probe **1** (10 μM) in the presence of above mentioned samples.

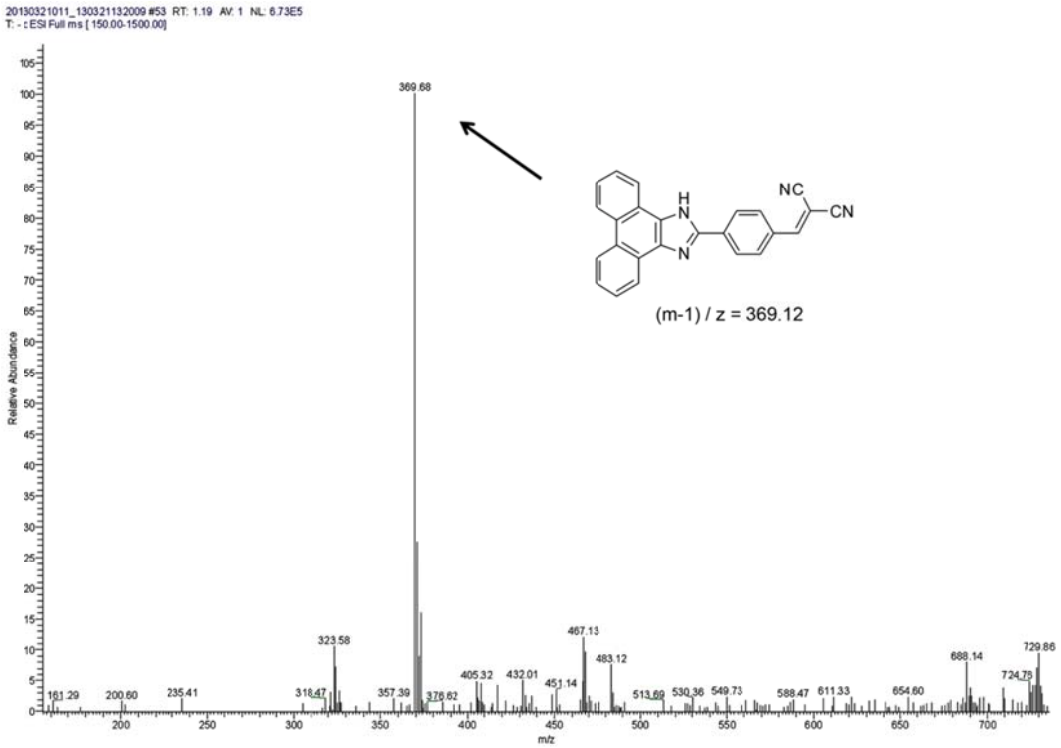


Figure S17. ESI-Ms spectra of probe **1**.

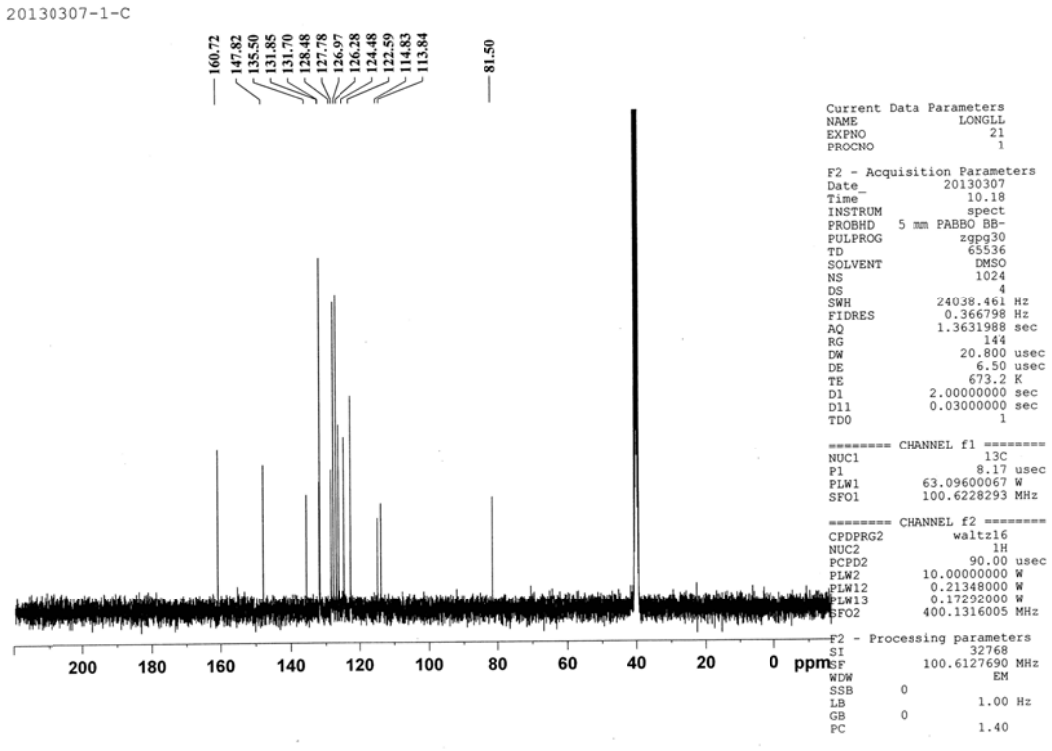


Figure S18. ^{13}C NMR spectra of probe **1**.

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