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A Zn^{2+} Specific Fluorescent Molecular Probe for the Selective Detection of Endogenous Cyanide in Biorelevant Samples

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Contents

Synthetic details, spectral changes of **3** and **4**.

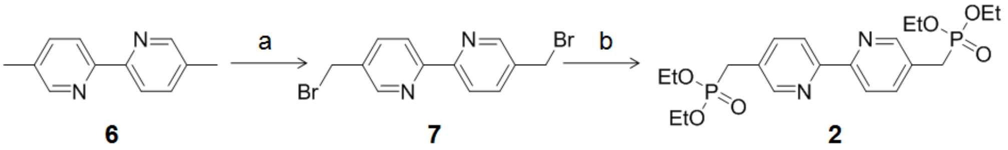
Experimental Section

General

Solvents and reagents were purified and dried by usual methods. All starting materials were obtained from commercial suppliers and used as received. All melting points were determined with Mel-Temp-II melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR were measured on a 500 MHz Bruker Avance DPX Spectrometer. High Resolution Mass Spectra were recorded with a JEOL JMS600. FT-IR spectra were recorded using a Shimadzu IR Prestige-21 Fourier Transform Infrared spectrophotometer. Electronic absorption spectra were recorded on a Shimadzu UV-3101 PC NIR scanning spectrophotometer and the emission spectra were measured on a SPEX-Fluorolog F112X spectrofluorimeter.

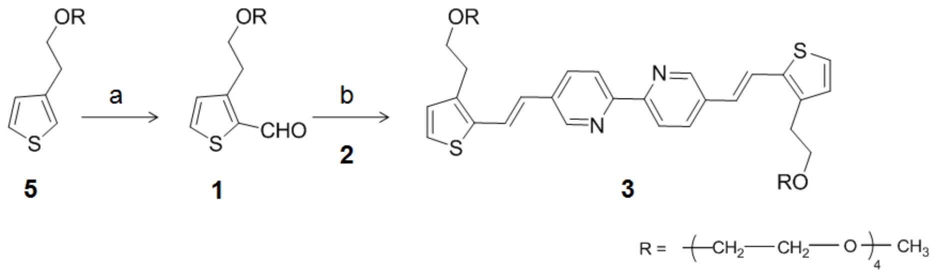
Synthesis and characterization

Scheme for the synthesis of 2



Scheme S1. Reagents and conditions: a) NBS, CCl_4, 18h; b) $P(OEt)_3$, 100 °C, 12 h.

Scheme for the synthesis of 4



Scheme S2. Reagents and conditions: a) $POCl_3$, DMF b) NaH, THF, 70 °C, 10 h.

Synthesis of 1

To a two-neck RB flask under argon atmosphere, $POCl_3$ (0.320 mL, 3.65 mmole) were taken and allowed to stirr in an ice cold condition. Dimethyl formamide (DMF), (1.1 mL, 14.6 mmol) were added slowly into it. The reaction mixture was allowed to stir for 1 h. After the formation of the ylide (pale yellow color), glycolated thiophene **5** (1 gm, 3.65 mmol) was added slowly into the reaction mixture. After 12 h, the reaction mixture was poured into ice water in a beaker. A fresh solution of 0.5 N NaOH was added and heated up to 80 °C. The solution was then extracted with dichloromethane and purified using silica gel column chromatography (100-200 mesh, 30% ethylacetate-hexane as eluent). A pale yellow liquid was obtained. Yield 45%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 10.01 (s, -CHO),7.05 (d, 1H, aromatic), 7.60 (d, 1H, aromatic), 3.6 (t, 2H), 3.5 (m, 12H), 3.38 (s, $-OCH_3$); ^{13} C NMR $CDCl_3$, (125 MHz) δ 29.30, 59.01, 70.44, 96.27, 131.10, 133.73, 182.18; FAB-MS: $[M]^+$ Calcd for $C_{14}H_{22}O_5S$, 302.9; found 303.

Synthesis of 5,5'-bis(bromomethyl)-2,2'-bipyridine (7)

To a solution of 5,5'-dimethyl-2,2'-bipyridine (**6**) (10 mmol) in 50 mL of dry CCl_4 was added *N*-bromosuccinimide (20.5 mmol) and AIBN. The reaction mixture was refluxed for 18-20 h, cooled, filtered and the solvent was removed under reduced pressure to give the crude product which was further precipitated using dichloromethane. Yield 80-90%; mp. 188 °C; ^{1}H NMR (500 M Hz, $CDCl_3$, TMS) δ 4.53 (s, 4H, CH_2Br), 7.79 (m, 2H, aromatic), 8.34 (m, 2H, aromatic), 8.61 (m, 2H, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.43, 121.25, 133.28, 137.70, 149.27, 155.19.

Synthesis of the 5,5'-Bis-(diethyl phosphonomethyl)-2,2'-bipyridine (2)

The phosphonate ester, **2** was prepared by the reaction of the corresponding 5,5’-bis(bromomethyl)-2,2’-bipyridine derivatives (**7**) (2 mmol) with 3 mL of triethyl phosphite at 80-85 °C for 10-12 h followed by the removal of the unreacted triethyl phosphite under vacuum. Yield 90-95%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.12 (m, 12H, CH_3), 3.21 (s, 4H, CH_2P), 4.14 (m, 8H, OCH_2), 7.34 (m, 2H, aromatic), 8.01 (m, 2H, aromatic), 8.30 (m, 2H, aromatic).

Synthesis of 3

A suspension of sodium hydride (12 mmol) in dry THF was added slowly to a solution of the bisphosphonate (**2**) (2 mmol) and the aldehyde **1** (4 mmol) in THF. After refluxing for 12 h, the reaction mixture was cooled followed by the removal of the THF under reduced pressure to give a pasty residue. The residue was suspended in water and extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give the crude product, which was further purified by column chromatography over silica gel using petroleum ether as eluent. Yield 37%; yellow pasty solid; ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 8.7 (s, 1H, aromatic), 8.3 (m, 1H, aromatic), 7.8 (m, 1H, aromatic), 7.39 (1H, vinylic, J = 16.03), 7.15 (1H, vinylic, J = 16.08), 7.0 (s, 1H aromatic), 6.8 (s, 1H, aromatic), 3.6 (m, 12H), 3.5 (m, 2H), 3.3 (s, 3H, -OCH_3); ^{13}C NMR $CDCl_3$, (125 MHz) δ 30.70, 58.90, 70.20, 71.80, 120.83, 122.19, 128.56, 132.80, 137.12, 141.93, 147.87, 154.39; FAB-MS: $[M]^+$ Calcd for $C_{40}H_{52}N_2O_8S_2$, 752.32; found 753.0.

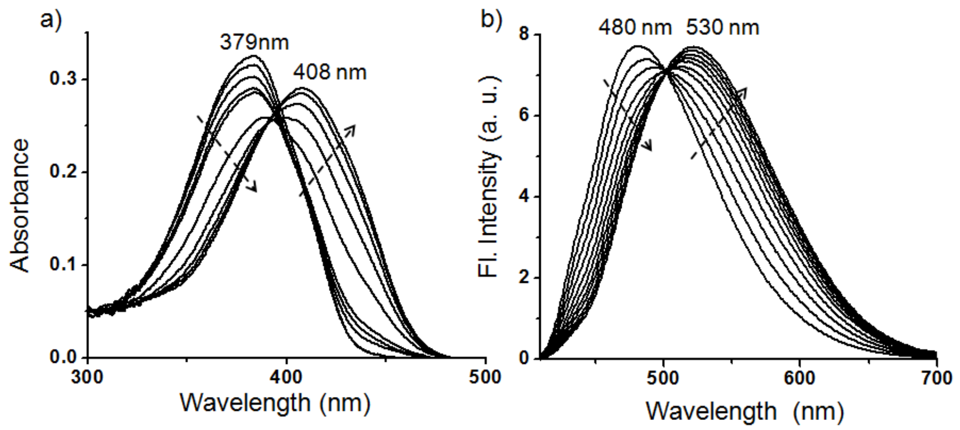


Figure S1. a) Changes in the a) absorption and b) emission spectra of **3** [6 × 10^{-6} M] with the addition of one equivalent of zinc chloride in 4:1 acetonitrile/water (HEPES buffer 0.01 M, pH 7.2).

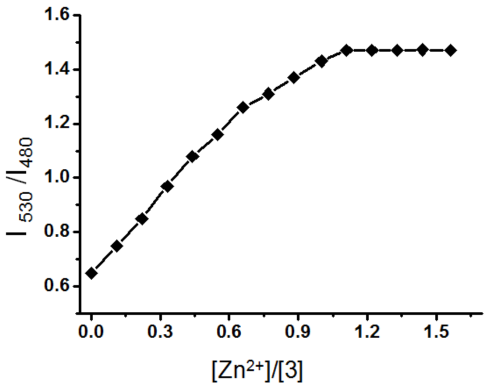


Figure S2. Plot of the fluorescence intensity ratio as the function of added Zn^{2+} concentration.

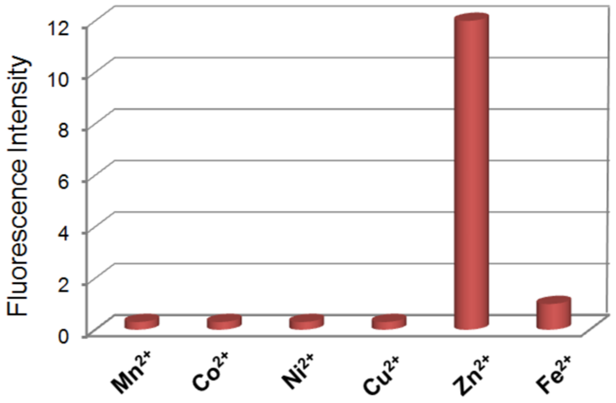


Figure S3. Fluorescence responses of **3** (6 µM) monitored at 530 nm with various transition metal ions.

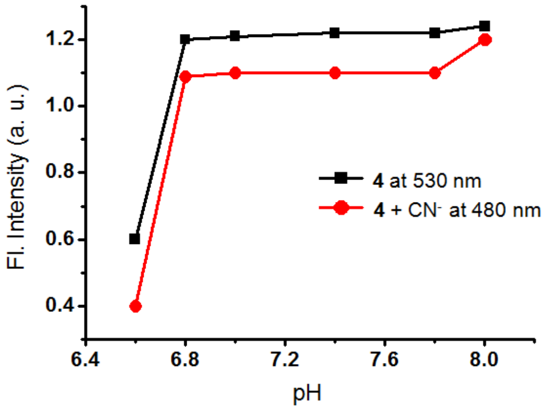


Figure S4. Emission intensity variation of **4** at emission maximum against different pH (HEPES buffer) 4:1 acetonitrile/water in the absence (black line) and presence (red line) of cyanide. Within the biological pH window of 6.8-7.4, **4** did not show considerable emission intensity variation though fluorescence decrease is observed below pH 6.8.

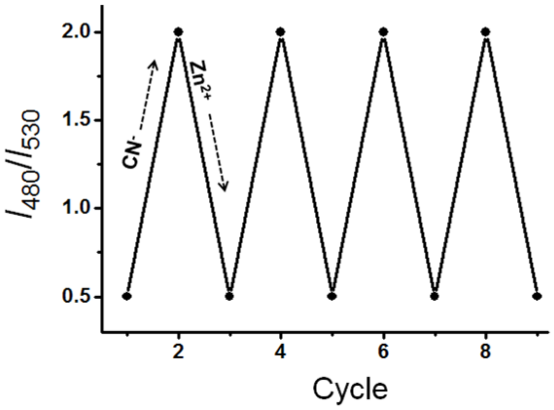


Figure S5. Ratiometric fluorescence responses of **4** during eight continuous cycles of addition of CN^- and $ZnCl_2$.

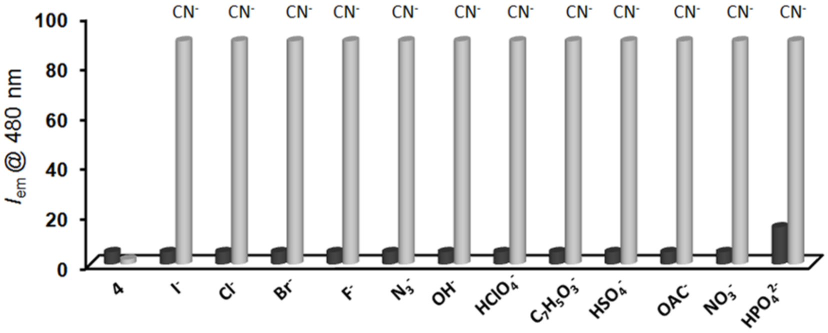


Figure S6. Plot showing the response of **4** [6 × 10^{-6} M] to different anions. The emission intensity was monitored at 480 nm in acetonitrile-water (4:1) (HEPES buffer 0.01M, pH 7.2). Black bars represent response of **4** with different anions. Gray bars shows the enhancement in emission of **4**

with CN^- in the presence of excess (20 times) of different anions. All emission intensities were recorded in Bio-Tek Cell reader using 96-well plate, 200 µL per well.

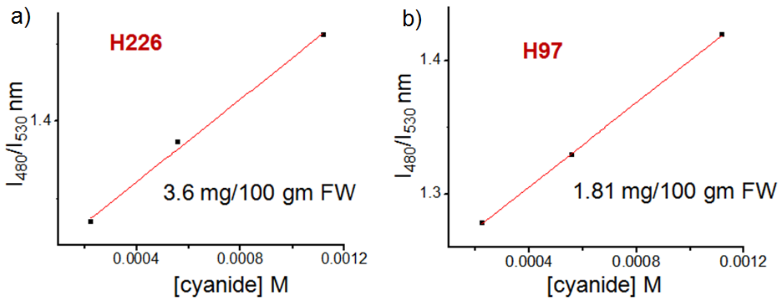


Figure S7. Standard addition plot for the determination of cyanide anion content in fresh latex of high yield cassava varieties: a) from cassava variety H226; b) from H97. **4** (6×10^{-6} M) was taken in 4:1 (v/v) acetonitrile/water mixture buffered at a pH 7.2. The ratio of emission intensity at 480 nm and 530 nm were plotted against known concentration of tetrabutyl ammonium cyanide. Concentration of unknown cyanide ions in a specific volume of the leaf juice (300 µL) was calculated from the slope of the straight line.

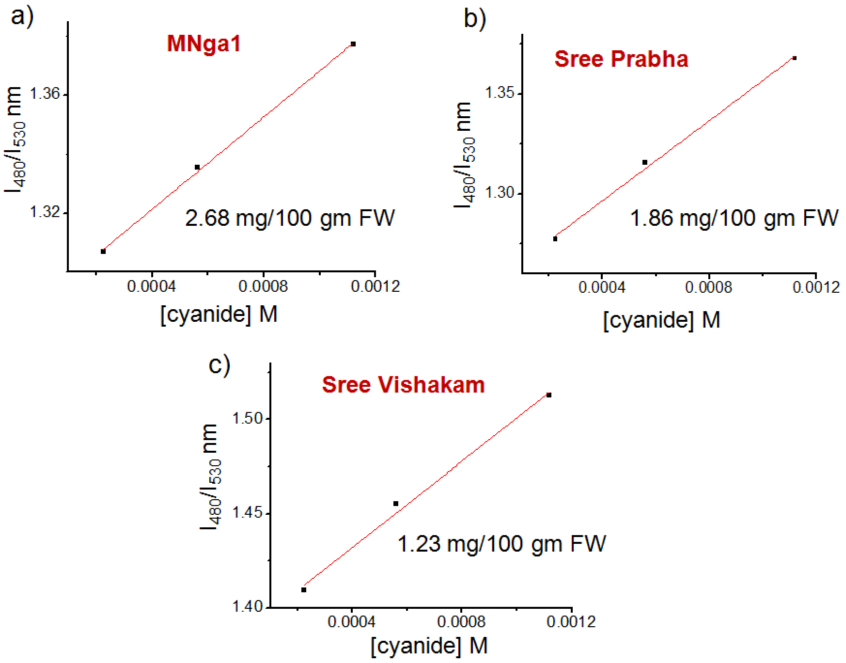


Figure S8. Standard addition plot for the determination of cyanide anion content in fresh latex of high yield cassava varieties a) from cassava variety MNga1 b) from Sree Prabha and c) Sree Vishakam. **4** (6×10^{-6} M)was taken in 4:1 (v/v) acetonitrile/water mixture buffered at a pH 7.2. The ratio of emission intensity at 480 nm and 530 nm were plotted against known concentration of tetrabutyl ammonium cyanide. Concentration of unknown cyanide ions in a specific volume of the leaf juice (300 µL) was calculated from the slope of the straight line.