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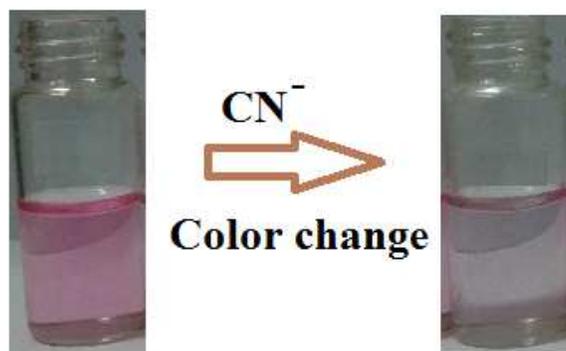
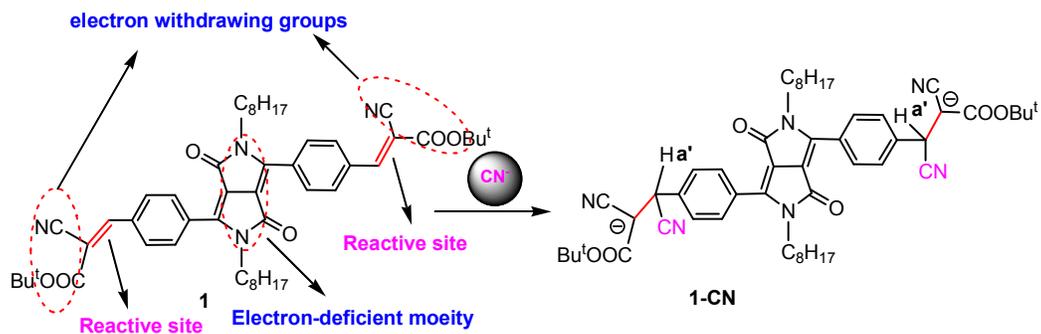
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A new colorimetric probe **1** comprising diketopyrrolopyrrole-based Michael receptor, which recognized cyanide anion with high selectivity was designed and synthesized.

**A colorimetric probe based on diketopyrrolopyrrole and *tert*-butyl
cyanoacetate for cyanide detection**

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Abstract

A new colorimetric probe **1** comprising diketopyrrolopyrrole-based Michael receptor, which recognized cyanide anion with high selectivity was designed and synthesized. Addition of CN^- aqueous solution to **1** in THF resulted in a rapid color change from pink to light yellow together with a large blue shift from 530 to 487 nm, while other anions did not induce any significant color change. Furthermore, the Michael addition of cyanide to **1** elicited 94% fluorescence quenching at 640 nm accompanying with appearance of a weak new emission at 560 nm, which constituted the fluorescence signature for cyanide detection. The detection limits were $0.75 \mu\text{M}$ using the fluorescence spectra changes, which was far lower than the WHO guideline of $1.9 \mu\text{M}$. Moreover, **1**-based test strips could successfully detect CN^- in water solutions.

Keywords, Chemosensors; Cyanide anion; Colorimetric; Diketopyrrolopyrrole;

1. Introduction

Cyanide is well known as one of the most toxic species and is extremely harmful to mammals. Any accidental release of cyanide to the environment causes serious problems.¹ Therefore, the maximum permissible level of cyanide in drinking water is set at 1.9 μM by the World Health Organization (WHO). Nevertheless, cyanide salts are still widely used as industrial materials in gold mining, electroplating, plastics production and other fields. Therefore, it is highly desirable to develop sensitive, selective and quick detection methods for toxic cyanide anions.

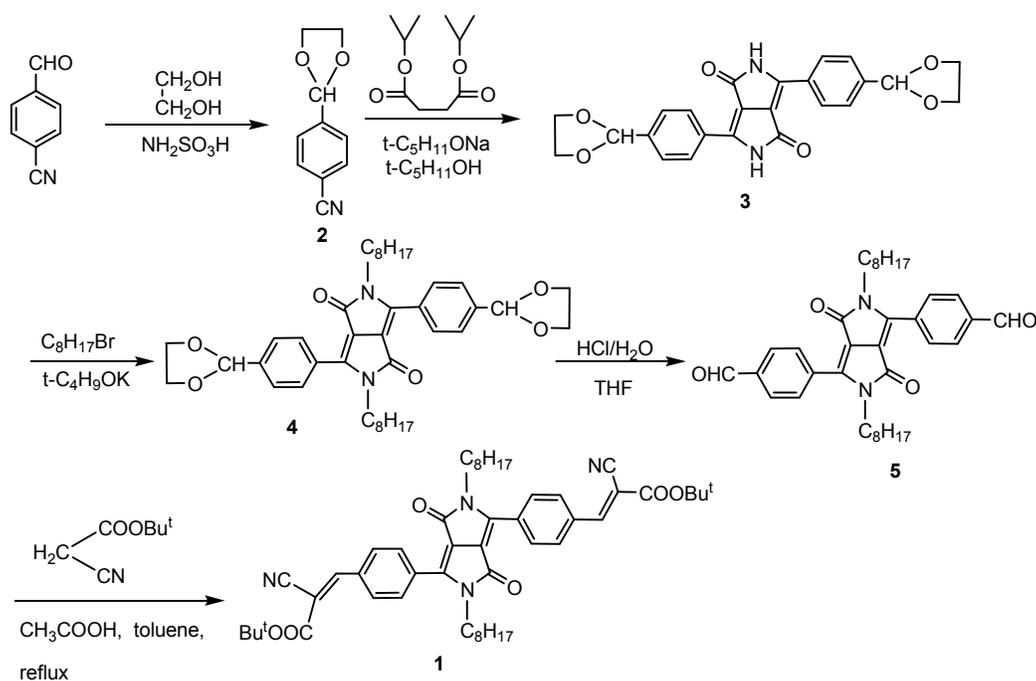
Traditionally, hydrogen bonding or supramolecular interactions have been used in the detection of low concentrations of cyanide in solution.²⁻³ These approaches, however, usually result in poor selectivity over other common anions.⁴ The burgeoning field of reaction-based indicators has made progress in this area because of the unique reactivity of CN^- toward a variety of organic functional groups including $\text{C}=\text{O}$, $\text{C}=\text{N}$, $\text{C}=\text{C}$, and so on.⁵ The irreversible formation of chemical bonds can provide chemodosimetric information and develop ratiometric fluorescent probes. Unfortunately, none of these is ideal. For instance, the cationic borane receptor was very promising in that reaction with cyanide took place in water, but failed to produce a color change, a shortcoming that restricted its utility.^{5k} Likewise, the oxazine-based indicators required specific biphasic conditions,^{5q, 5r} while the acridinium salts required an elevated reaction temperature.^{5s} There is an ever-present need to develop new reaction-based CN^- sensors, as these can help overcome lingering obstacles in its detection, such as selectivity, sensitivity, response times, sensor stability, reaction

conditions, *etc.*

On the other hand, diketopyrrolopyrrole (DPP) and its derivatives represent a class of brilliant red dyes and exhibit large extinction coefficients, high fluorescence quantum and exceptional high stability upon exposure to light, weather, and heat, which makes them to be good candidates for chemosensor.⁶ Deng *et al.* used DPP as fluorophore and malononitrile moiety as reactive site for thiol, which simultaneously displayed the colorimetric and ratiometric signal transduction.^{6f} Hua's group reported a colorimetric and ratiometric fluorescent chemosensor for fluoride ion based on DPP due to deprotonation of the amide moiety by fluoride ion.^{6g} To date, only limited DPP-derived probes for reaction-based cyanide detection have been reported. Jang *et al* reported a DPP-based colorimetric and fluorescent probe for cyanide detection, in which cyanide attacked carbonyl carbon atom of DPP ring.⁷ In this regard, we have recently reported a DPP-indandione conjugate for cyanide detection, which exhibited high sensitivity and selectivity to cyanide through the exciplex process.⁸ However, an improvement in the DPP-indandione conjugate system is required because the emission band located at 572 nm, which largely hinder their application *in vivo* imaging.

Considering our objectives, we designed probe **1** (Scheme 1) keeping in mind the following. (1) The signal reporting of probe derives from more reactive vinyl moiety activated by electron withdrawing cyano and ester groups, which could selectively react and enhance the sensitivity of the nucleophilic addition reaction between the vinyl group and CN^- . (2) Electron-deficient (DPP) is selected as chromophore

considering its many advantageous features including its enhanced vinyl moiety reactivity with CN^- and strong red fluorescence as well as its distinct rigid ring structure. (3) The DPP-*tert*-butyl cyanoacetate conjugate (**1**) will have longer emission band at 640 nm. Herein, we report **1** as doubly activated Michael addition type probe for the colorimetric fluorescent detection of cyanide. The probe displays high selectivity and sensitivity for CN^- over other anions with a fast response.



Scheme 1. Synthesis of Probe **1**.

2. Experimental

2.1. Chemicals and instruments

Nuclear magnetic resonance spectra were recorded on Bruker Avance III 400 MHz and chemical shifts are expressed in ppm using TMS as an internal standard. The UV-vis absorption spectra were recorded using a Helios Alpha UV-Vis scanning

spectrophotometer. Fluorescence spectra were obtained with a Hitachi F-4500 FL spectrophotometer with quartz cuvette (path length = 1 cm). KHSO_4 , NaF, NaCl, KBr, KI, Na_2CO_3 , NaHCO_3 , Na_2SO_3 , NaHSO_3 , Na_2SO_4 , Na_2S , CH_3COONa , Na_3PO_4 , KNO_3 , Na_2HPO_4 and NaH_2PO_4 were purchased from Guangzhou Chemical Reagent Company.

N-Methyl-2-pyrrolidone (NMP) was dried with CaH_2 and distilled under nitrogen atmosphere. Other solvents were obtained from commercially available resources without further purification. 2,5-Dioctyl-3,6-bis(4'-formylphenyl)pyrrolo[3,4-c]pyrrole-1,4-dione (compound **5**) was synthesized according to our published literature.

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The recognition between **1** and different anions was investigated by UV-Vis and fluorescence spectroscopy in THF solution at room temperature. The stock solution of **1** and anions was at a concentration of 10.0 mM. After the **1** and anions with desired concentrations were mixed, they were measured by UV-Vis and fluorescence spectroscopy.

2.2. Synthesis of compound **1**

A mixture of **5** (56.8 mg, 0.10 mmol), *tert*-butyl cyanoacetate (56.4 mg, 0.40 mmol), ammonium acetate (48 mg, 0.63 mmol), and acetic acid (1 mL) in toluene (20 mL) was refluxed under argon atmosphere for 5 h. After cooling to room temperature, the reaction was quenched by water and the mixture was extracted with CH_2Cl_2 . The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by

rotary evaporation. The crude product was further purified by column chromatography to give **1** as a dark red solid in 85% yield. m.p. 160-161 °C.

¹H-NMR (THF-*d*₈, 400 MHz, δ), 8.15 (s, 2H), 8.08 (d, 2H), 7.99 (d, 2H), 3.75 (t, 4H), 1.62-0.75 (m, 48H). ¹³C-NMR (THF-*d*₈, 100 MHz, δ), 161.20, 160.24, 151.32, 146.36, 133.61, 131.61, 130.46, 128.94, 114.55, 110.26, 105.70, 82.69, 41.00, 28.77, 28.55, 26.14, 22.12, 13.04. HRMS (ESI, *m/z*), [M + H]⁺ calcd. for (C₅₀H₆₂N₄NaO₆), 837.4562, found, 837.4571.

3. Results and discussions

3.1. Synthesis and structural characterization

Compared with the general Michael acceptors, the doubly activated acceptors, in which two electron-withdrawing groups are attached to the C=C group,¹⁰⁻¹¹ are more reactive,¹²⁻¹⁵ and allow the Michael addition reaction to occur under mild condition. Clearly, to improve the sensitivity, it is necessary to enhance the reactivity of the probe to cyanide. We reasoned that this could be accomplished by increasing the electrophilicity of the β-carbon. With this in mind, we decided to introduce electron withdrawing cyano and ester groups, as well as electron-deficient DPP moiety to afford probe **1**. Thus, it is anticipated that probe **1** should be highly reactive to CN⁻.

2,5-Dioctyl-3,6-bis(4'-formylphenyl)pyrrolo[3,4-c] pyrrole-1,4-dione (compound **5**) was prepared from *p*-cyanobenzaldehyde as a starting material, in four steps following Scheme 1. Probe **1** was obtained by a straightforward aldol-type condensation reaction of compound **5** with *tert*-butyl cyanoacetate in 85% yield as a dark red solid.

The ^1H NMR spectrum of probe **1** showed the distinctive signals corresponding to the resonance of vinyl proton at 8.15 ppm. The ^1H , ^{13}C NMR and HRMS spectra of **1** were shown in Figs. S1-S3 (supporting information).

3.2. Spectral characteristics of **1** with CN^-

The photophysical properties of **1** were studied in THF. **1** showed two major absorption peaks at 355 and 530 nm. To exploit its sensing behavior, we first examined the time dependent changes in the absorption spectra of **1** (10 μM) upon reaction with CN^- (3 equiv.) at room temperature. Generally, reaction-based chemosensors suffer from a long response time. In our case, the response of **1** to CN^- was found to be very fast. The addition of CN^- aqueous solution elicited not only a significant absorption increase around 487 nm but also dramatic absorption decreases around 355 and 530 nm (Fig. 1). After 8 minutes, the UV-Vis spectra of **1** were unchangeable, indicating nucleophilic addition reaction between the vinyl group and CN^- was completed. It is well known that the nucleophilicity of anions is greatly decreased in water due to hydrogen bond formation between the anions and water molecules. For instance, Guo's group reported that the reaction of anthracene-indanedione Michael receptor and cyanide could be completed within 2 minutes at an elevated temperature of 50 $^\circ\text{C}$ in 9/1 CH_3CN -water, and 10 equiv. of cyanide was required to reach the spectral saturation.¹⁶ Herein, in the presence of 3 equiv of cyanide, the nucleophilic CN^- addition to **1** occurred very rapidly (within 8 minutes), indicating high reactivity was the unique feature of **1**, which was impressive

as many reported cyanide probes require high equivalents of cyanide and long reaction time to reach a maximal spectral signal.¹⁷⁻²⁷

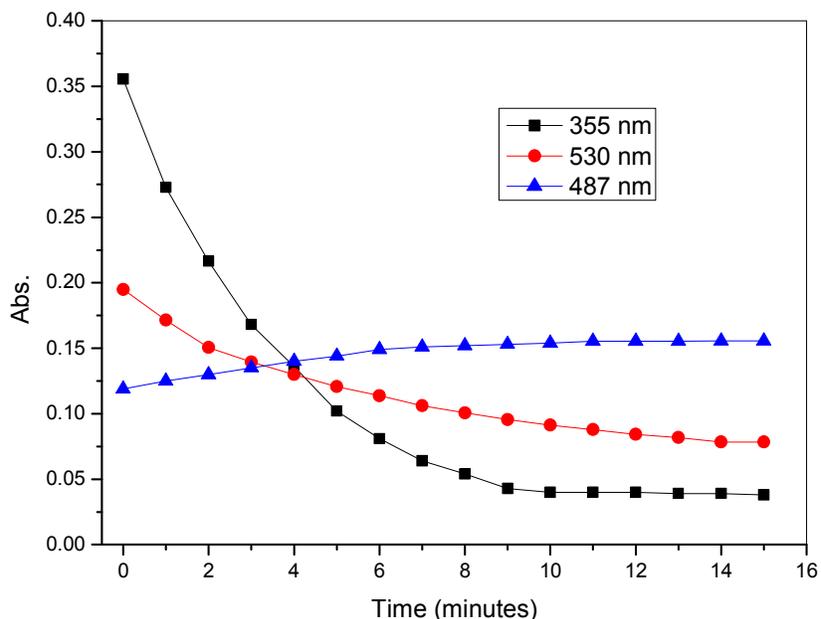


Fig. 1. Time-dependent absorption intensity of probe **1** (10 μM) in THF at 355 (■ in black), 530 (● in red) and 487 nm (▲ in blue) in the presence of CN^- (3 equiv) at room temperature.

To get insight into the binding of CN^- with **1**, the absorption spectra of **1** in THF upon titration with CN^- aqueous solution were recorded. As shown in Fig. 2, the absorption peaks of **1** at 355 and 530 nm gradually decreased following the formation of a new band centered at 487 nm. Meanwhile, two isosbestic points at 416 and 472 nm were observed, which implied new species with less conjugation were formed. Notably, the ratios of A_{497}/A_{530} and A_{497}/A_{355} increased over 2.15 and 20.43-fold in presence of 20 equiv. CN^- , respectively.

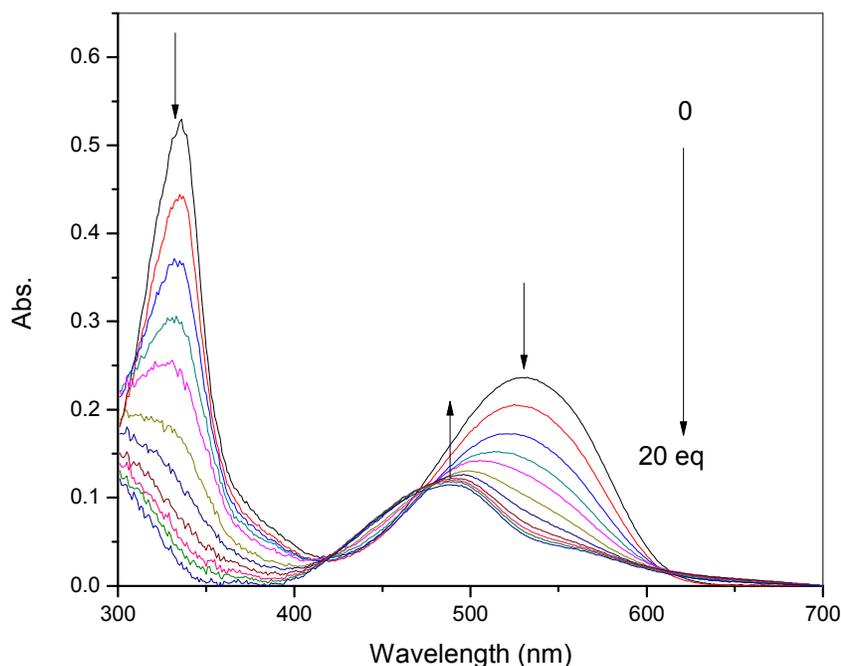


Fig. 2. UV-Vis spectral changes of **1** in THF (10 μM) with the increasing concentrations of cyanide anion aqueous solution.

The CN⁻ sensing property was further examined through fluorescent titration studies in detail. As shown in Fig. 3, **1** showed a major emission peak at 640 nm. Upon addition of CN⁻ aqueous solution to **1**, the PL intensity at 640 nm was decreased gradually and a new emission peak at 560 nm appeared. A plateau with the addition of 80 equiv. of CN⁻ with 94% fluorescence quenching at 640 nm was achieved. The inset of Fig. 3 showed the emission color photographs of **L** in the presence of CN⁻ under excitation at 365 nm, where strong red emission was absent.

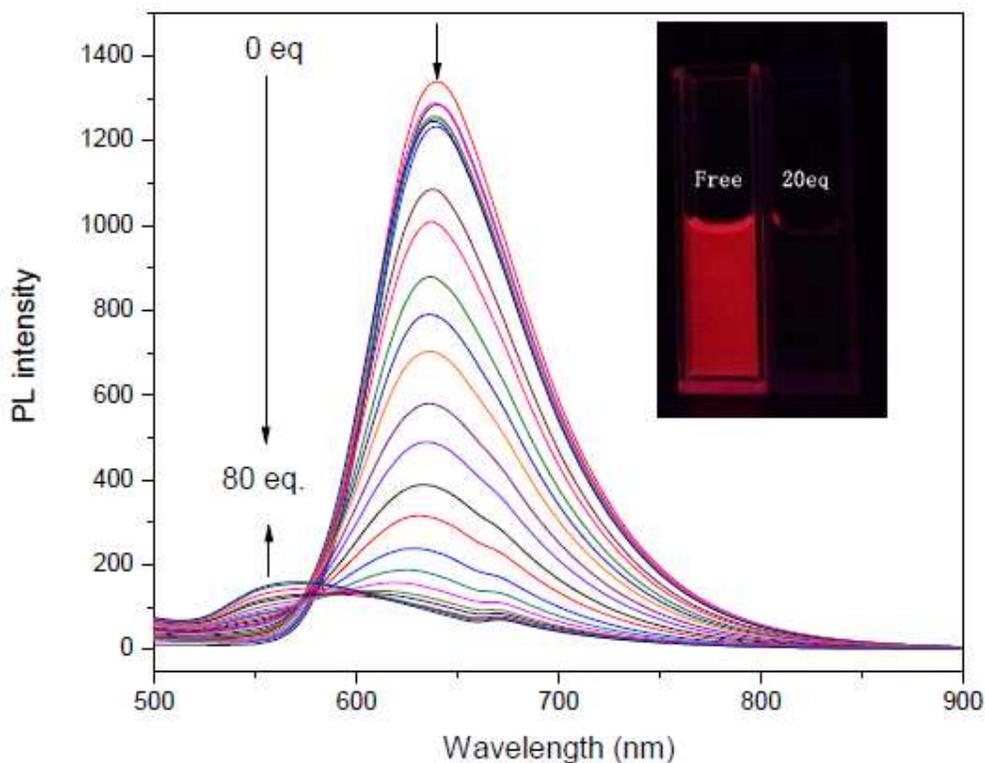


Fig. 3. Fluorescence spectral changes of **1** (10 μM) in THF with the increasing concentrations of CN^- in THF.

3.3 Selectivity of **1** with CN^-

For the purpose of evaluating selectivity of **1** to cyanide, the absorption spectral change of **1** upon addition of other anions and biothiols was also investigated. Dramatic change of the absorption spectrum induced by CN^- was observed, while almost no changes could be found in presence of other anions (F^- , Cl^- , Br^- , I^- , CO_3^{2-} , HCO_3^- , SO_3^{2-} , HSO_3^- , SO_4^{2-} , S^{2-} , SCN^- , CH_3COO^- , NO_3^- , PO_4^{3-} , HPO_4^{2-} and H_2PO_4^-) and biothiols (cysteine (Cys), homocysteine (Hcy) and glutathione (GSH)) (Fig. 4a). More importantly, the color change from pink to light yellow can be clearly observed by the naked eye in presence of CN^- , while other anions and biothiols did not induce

any significant color change, which suggested that naked-eye selective detection of CN^- became possible (Fig. 4b).

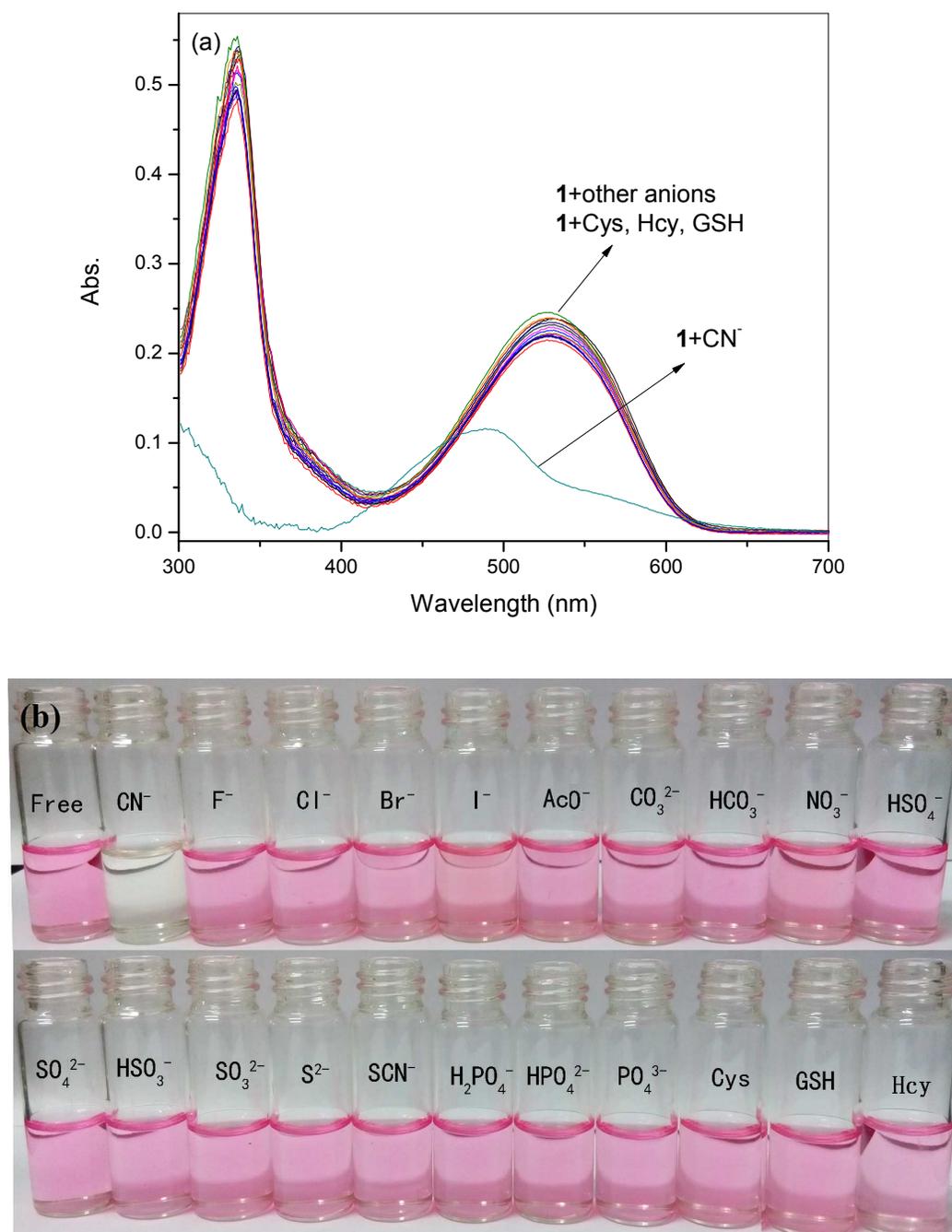
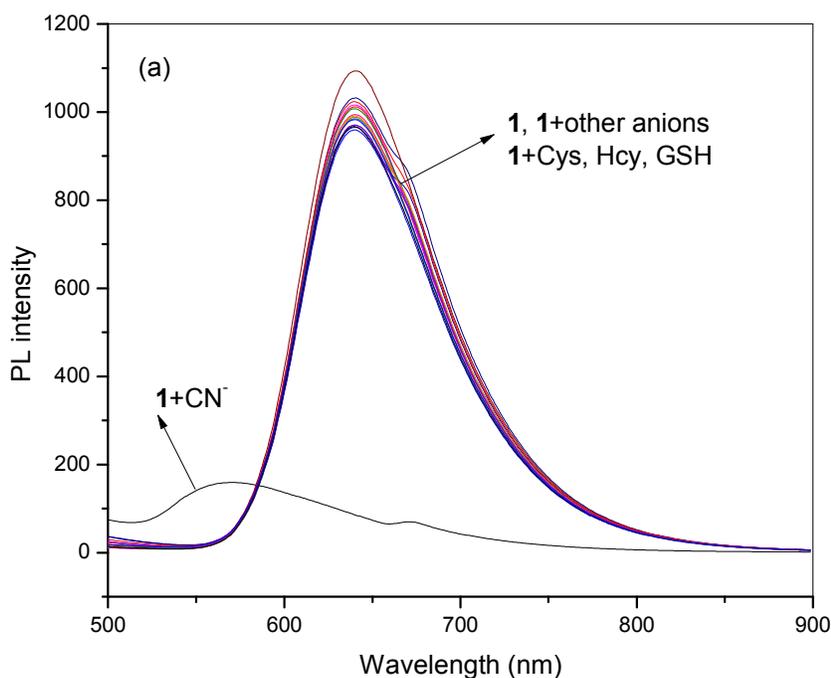


Fig. 4. (a) UV-vis absorption spectra and (b) color changes of **1** in THF (10 μM) upon addition of 10 equiv of each anion aqueous solution.

Meanwhile, only CN^- rendered a remarkable “Turn-Off” fluorescence response, whereas all other anions revealed a negligible change in the fluorescent spectra of **L** (Fig. 5a). In the presence of 80 equivalents of CN^- , an emission peak at 640 nm with 94% fluorescence quenching was observed. Fig. 5b showed the photographs of **L** in the presence of different anions and biothiols under excitation at 365 nm using a portable UV lamp. The disappearance of intense red color of the solution upon interaction of **L** with CN^- was present. However, other anions and biothiols did not induce any significant emission color change.



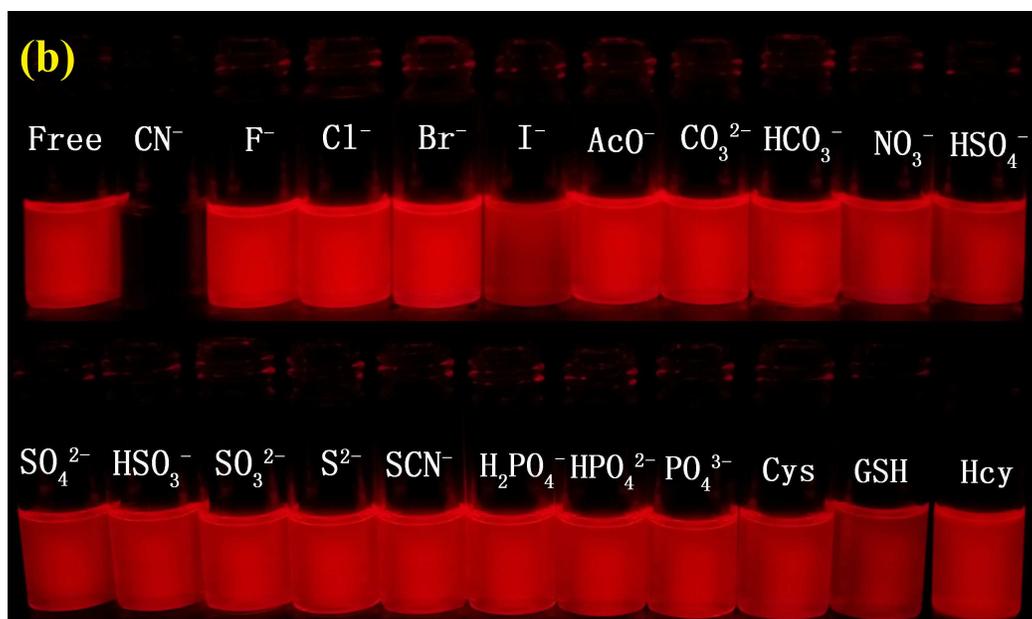


Fig. 5. Fluorescence and (b) emission color changes of **1** in THF (10 μM) upon addition of 10 equiv of each anion aqueous solution.

Another important feature of **1** is its high selectivity toward the CN^- in presence of other competitive anions. Changes of fluorescence spectra of **1** (10 μM) caused by CN^- (80 equiv) and miscellaneous competing species (80 equiv) were recorded in Fig. 6. As can be seen, these competitive species did not lead to any significant interference. In the presence of these anions and biothiols, the CN^- still produced similar optical spectral changes. These results showed that the selectivity of sensor **1** toward CN^- was not affected by the presence of other competitive species.

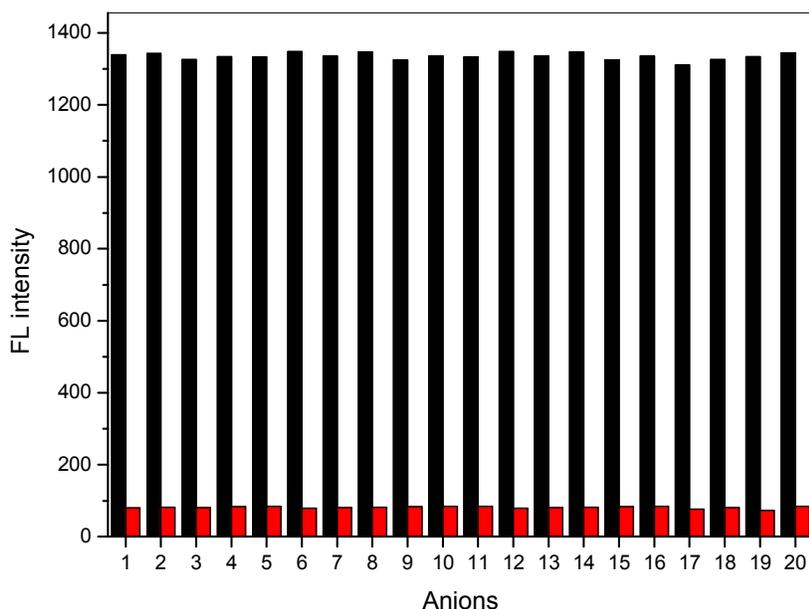


Fig. 6. Selectivity of **1**. The black bars represent fluorescence intensity at 640 nm of **1** (10 μM) in THF in the presence of other competitive species (800 μM). The red bars represent the fluorescence intensity that occurs upon the subsequent addition of 800 μM of CN^- to the above solution. From 1 to 20, control, F^- , Cl^- , Br^- , I^- , CO_3^{2-} , HCO_3^- , SO_3^{2-} , HSO_3^- , SO_4^{2-} , S^{2-} , SCN^- , CH_3COO^- , NO_3^- , PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- , Cys, GSH and Hcy.

The detection limit of **1** for CN^- was calculated based on the fluorescence titration data according to a reported method.²⁸ Under optimal conditions, calibration graphs for the determination of CN^- were constructed. The decreased fluorescence intensity of the system showed a good linear relationship with the concentration of CN^- in the range of 1~ 9 μM ($R^2 = 0.998$), as shown in Fig. 7. The detection limit for CN^- was determined as 0.75 μM based on $S/N = 3$, which was far lower than the WHO

guideline of 1.9 μM cyanide.

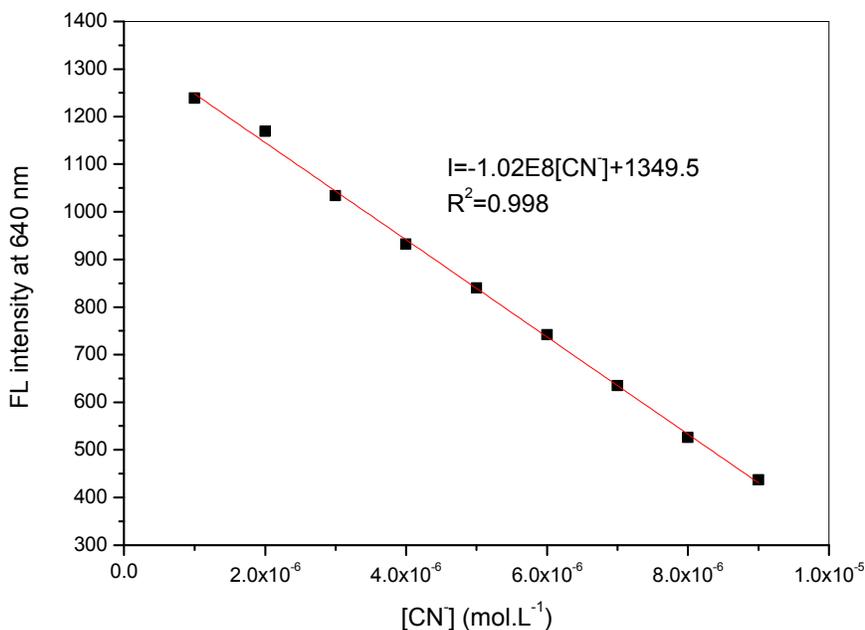


Fig. 7. The linear relation for concentration of CN^- in the range of 1–10 μM .

To gain further insight into the nature of **1**-cyanide interactions, the ^1H , ^{13}C NMR and MALDI-TOF-MS spectra were monitored (Figs. S4-S6, supporting information). As shown in Fig. 8 for partial ^1H NMR spectrum of $[\mathbf{1}\text{-CN}]^-$, it was obvious that the resonance signal corresponding to the vinyl proton at 8.16 ppm disappeared completely, whereas a new signal grew at 5.25 ppm corresponding to the α -proton (H_a). Meanwhile, the benzyl protons displayed upfield shift compared to those of **1** due to the breaking of the conjugation. ^{13}C NMR spectrum of $[\mathbf{1}\text{-CN}]^-$ also confirmed the reaction between of **1** and CN^- (Fig. S5, supporting information). In Fig. S6 (supporting information), a MALDI-TOF-MS spectrum revealed its cyanide adduct (found for $[\mathbf{1}\text{-CN}]^-$, 867.899). All these observations clearly indicated that the cyanide

anion was added to the vinyl group, and the anionic species was formed. The possible reactive mechanism between **1** and CN^- ions was shown in Scheme 2.

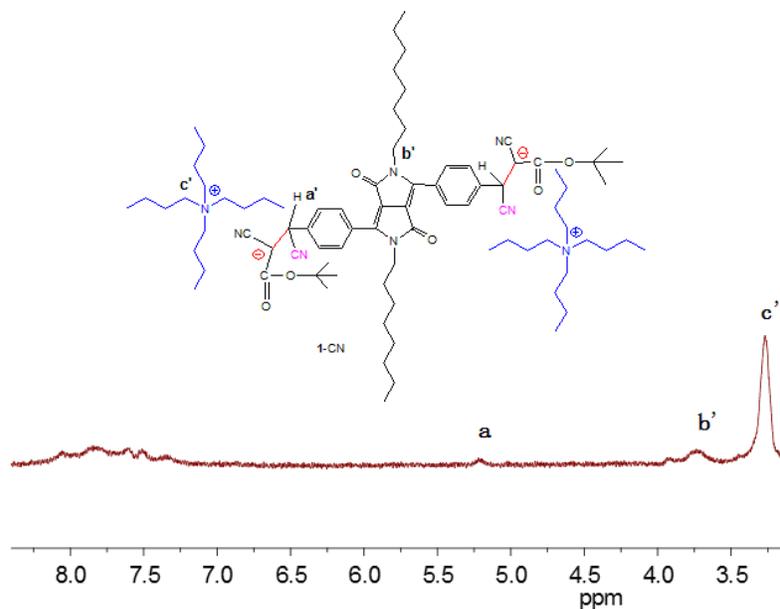
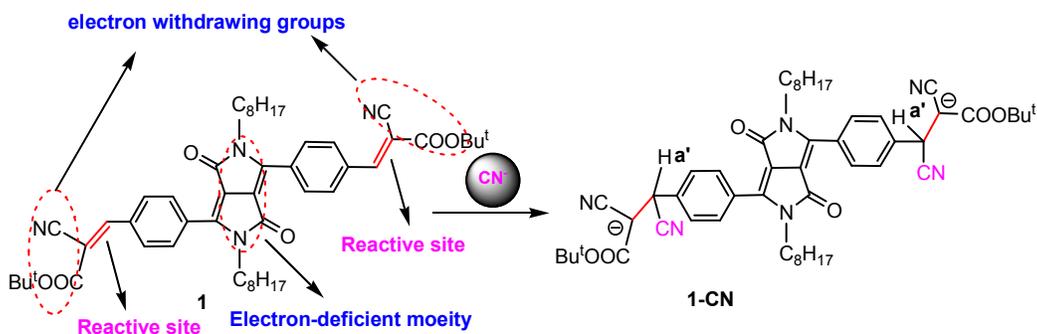


Fig. 8. Partial ^1H NMR spectrum of **1-CN⁻** in CD_3COCD_3 .



Scheme 2. Schematic illustration of nucleophilic addition reaction of CN^- with $\text{C}=\text{C}$ bond of **1**.

3.5 Practical application

Active materials-based test strips represent a group of convenient probing substrate for practical utilization. **1**-based test strip was thus fabricated by immersing filter

paper into the THF solution of **1** (1.0×10^{-3} M) and drying in air, which was energy- and cost-effective. The corresponding probing experiments were carried out subsequently. The results indicated that this protocol really took effect. The obvious color change from pink to yellow was observed by immersing this test strips in aqueous solutions of CN^- , exhibiting colorimetric changes differentiable to naked eyes (Fig. 9a). As shown in Fig. 9b, when the **1**-exposed test strip was immersed into aqueous solutions of CN^- (0.01 M), strong red fluorescence disappeared and easily distinguished. Since the color change was rapidly and clearly detected, the test strips could conveniently detect CN^- in water solutions.

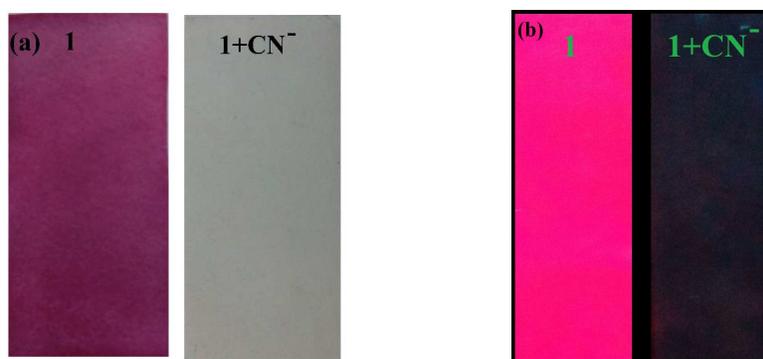


Fig. 9. (a) Color and (b) emission changes of **1**-based test strips before and after addition of CN^- .

4. Conclusions

A colorimetric fluorescent CN^- probe (**1**) was designed with diketopyrrolopyrrole (DPP) as the fluorophore, which shows absorption at 355 and 530 nm as well as a red emission at 640 nm with large Stokes shift (110 nm). In the presence of CN^- , the absorption peak shows 43 nm blue-shift and 94% fluorescence quenching at 640 nm.

Correspondingly, the color of the probe solution changed from pink to light yellow, and the fluorescence was quenched upon interaction with CN^- . Test strips for detection CN^- in practical applications are successfully realized.

Acknowledgements

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