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Teaching a Known Molecule New Tricks: Optical Cyanide Recognition by 2-[(9-Ethyl-9*H*-carbazol-3-yl)methylene]propanedinitrile in Aqueous Solution

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The colorimetric and fluorescent cyanide recognition properties of 2-[(9-ethyl-9*H*-carbazol-3-yl)methylene]-propanedinitrile (**1**) in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0) solution were evaluated. The optical recognition process of probe **1** exhibited high sensitivity and selectivity to cyanide ion with the detection limit of 2.04×10^{-6} M and barely interfered by other coexisting anions. The sensing mechanism of probe **1** is speculated to undergo a nucleophilic addition of cyanide to dicyanovinyl group present in compound **1**. The colorimetric and fluorescent dual-modal response to cyanide makes probe **1** has a potential utility in cyanide detection.

Key Words : Cyanide, Recognition, Colorimetric, Fluorescence, Carbazole

Introduction

During the past two decades, considerable efforts have been paid to the development of abiotic receptors for biologically important anions due to their fundamental roles in a wide range of chemical, environmental and biological processes.[1-6] Among them, cyanide anion recognition has attracted more attention due to its high toxicity which eventually lead to a variety of diseases. Cyanide occurs naturally both in geologic and biologic world and it binds heme cofactors to inhibit the process of cellular respiration in mammals.[7] Although cyanides have been found in many foods and plants, most environmental cyanides are released by industries involved in gold mining, electroplating and metallurgy.[8] Consequently, considerable efforts have been devoted to the detection of cyanide by using colorimetric and fluorescent probes.[9] Currently, there are several methodologies and detecting principles that have been applied to cyanide ion recognition, which includes metal ion displacement,[10,11] demetalation of preassembled complexed sensor,[12-16] utilizing nucleophilic attack of CN^- on activated carbonyl groups[17-30] or Michael acceptor type of activated C=C double bond.[31-35] Nevertheless, the innate nucleophiphilic nature of cyanide ion can minimize or avoid the interference by other anions such as fluoride and acetate[29] during the detection and hence, it is widely used.

Even though many cyanide probes, including some more efficient sensing systems, have been well documented, development of probes for the detection of cyanide ions in polar or aqueous solution[36-40] is still desired. Moreover, in most cases tetrabutyl ammonium (TBA) salt of cyanide was used for cyanide recognition which has several drawbacks. Therefore, for practical applications, the detection of cyanide ion by using KCN is more meaningful.

Herein, we report the colorimetric and fluorescent recognition of cyanide ion by using a simple probe, 2-[(9-ethyl-9*H*-carbazol-3-yl)methylene]propanedinitrile (**1**) in aqueous media.

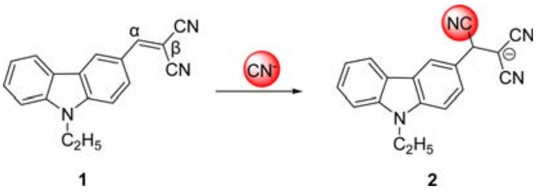
Experimental

General Methods and Materials. All the solvents were of analytical grade, obtained from commercial sources and used without further purification. UV-vis absorption spectra were measured on a SP-1900 spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., Chian). Fluorescence measurements were performed on a 970 CRT spectrofluorometer (Shanghai Sanco, China). HRMS was carried out on a Micromass UPLC Q/Tof mass spectrometer.

Results and Discussion

Compound **1**, 2-[(9-ethyl-9*H*-carbazol-3-yl)methylene]-propanedinitrile, was facilely synthesized by the condensation of 9-ethylcarbazole-3-carbaldehyde and malononitrile.[41] It has already been used for the examination of the influence of molecular conformation on the absorption spectra,[42] study on photoluminescence of donor-acceptor carbazole chromophores[43] and as a material for optical applications.[44] Based on the nucleophilic nature of the cyanide ion, it is reasonable to expect that the cyanide ion could attack the dicyanovinyl group in compound **1** to generate the stabilized anionic species **2** (Scheme 1), which reduces the extent of conjugation and a possible change in the spectral properties could be observed. Indeed, this speculation played a role in the cyanide recognition process.

The recognition of anions with probe **1** was investigated in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0) solution. Solution **1** (20 μM) displays a yellow color and exhibits three distinct absorption bands at 289, 323 and 409 nm. The two shorter wavelength bands are attributed to the π-π* and n-π* transitions and the longer one is for an intramolecular



Scheme 1. Reaction of **1** with cyanide ion for the formation of **2**.

charge transfer (ICT) transition. Upon addition of 20 equiv of CN^- (potassium salt), the yellow color bleached gradually to colorless. The three absorption intensities decreased significantly, in which the absorbance at 409 nm decreased dramatically to near zero. Concomitantly, a new absorption band centered at 263 nm was developed (Figure 1). Due to the observable gradual color bleaching, a time-dependent absorption changes was consequently examined and the results were depicted in Figure 2. The results reveal that the interaction between **1** and CN^- can complete within 2 hours. Thus, all the spectra were recorded after 2 hours of anion addition to probe **1**.

Furthermore, the effects of other anions such as F^-, Cl^-, Br^-, I^-, AcO^-, HSO_4^-, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, SCN^- and NO_3^- (all used as sodium salt, 20 equiv for each) on the absorption change were also conducted (Figure 1). As shown in Figure 1, only CN^- induced the dramatic absorption decrease accompanying a naked eye detectable color change from yellow to colorless. However, other anions did not cause any noticeable color and absorption spectral changes. These results demonstrate the high selectivity of probe **1** to CN^-.

As shown in Figure 3, solution **1** behaves strong fluorescence with a maximum emission at 539 nm. Upon addition of 20 equiv of CN^- to solution **1**, the fluorescence intensity was completely quenched. However, addition of other anions caused no or slight emission changes. These results further indicate the high selectivity of **1** toward CN^-.

The CN^- sensing property of **1** was further investigated by absorption and fluorescence titration experiments and the results were depicted in Figures 4 and 5, respectively. Figure

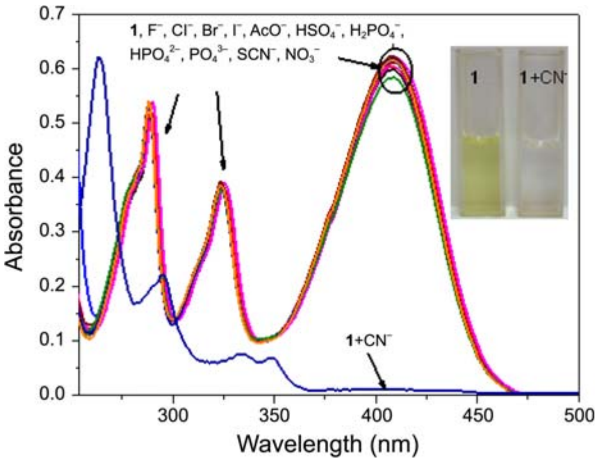


Figure 1. Absorption spectra changes of solution **1** (2.0×10^{-5} M) in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0) upon addition of 20 equiv of various anions.

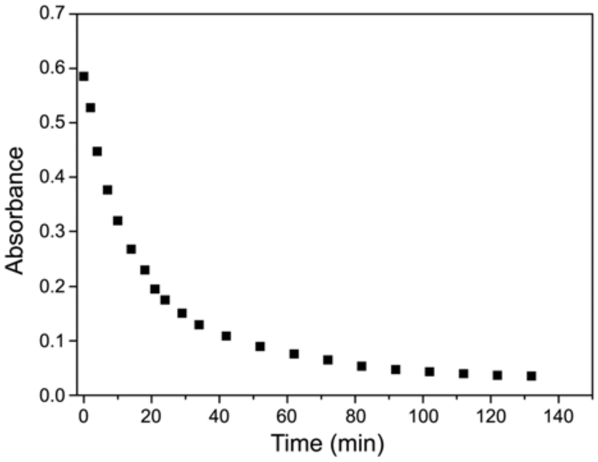


Figure 2. Absorption intensity changes of solution **1** (2.0×10^{-5} M) at 409 nm in the presence of 20 equiv of CN^- as a function of time in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0).

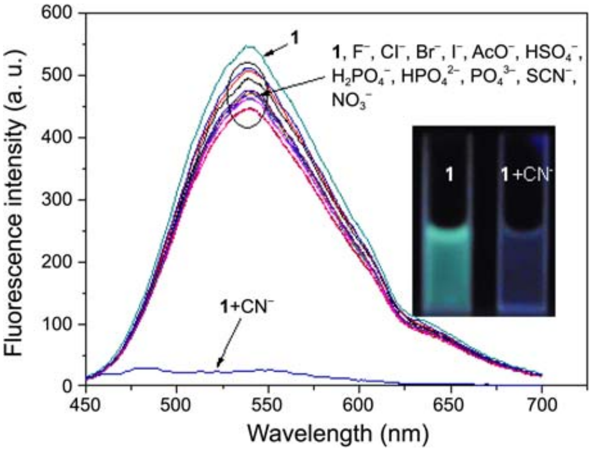


Figure 3. Fluorescence spectra changes of solution **1** (2.0×10^{-5} M) in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0) upon addition of 20 equiv. of various anions. λ_{ex} = 409 nm, λ_{em} = 539 nm.

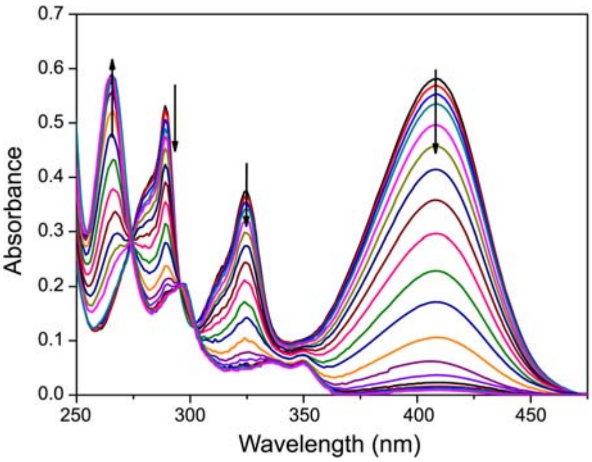


Figure 4. Absorption spectra changes of solution **1** (2.0×10^{-5} M) in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0) upon addition of different amounts of CN^- (0 to 20 equiv).

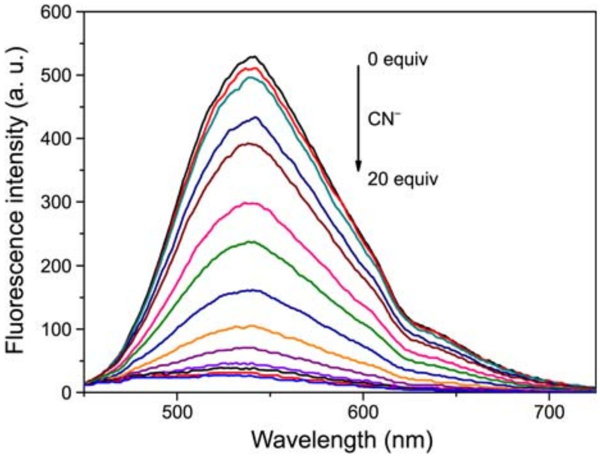


Figure 5. Fluorescence spectra changes of solution **1** (2.0×10^{-5} M) in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0) upon addition of different amounts of CN^- (0 to 20 equiv). λ_{ex} = 409 nm, λ_{em} = 539 nm.

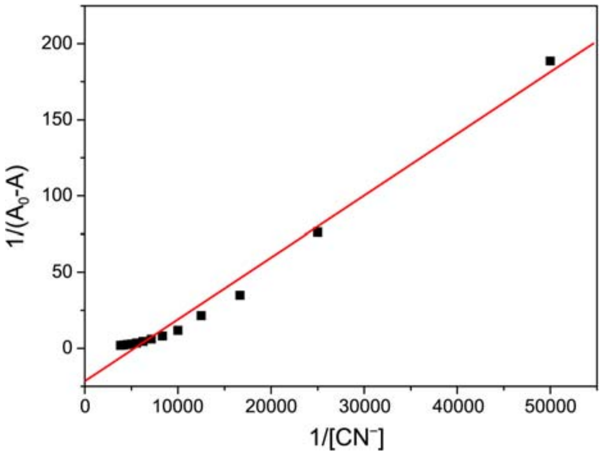


Figure 6. Benesi-Hildebrand plot (absorbance at 409 nm) of **1** assuming 1:1 binding stoichiometry with CN^-.

4 showed the gradual decrease of absorption intensities at 289, 323 and 409 nm upon incremental addition of CN^- into solution **1**. The titration reached saturation when 20 equiv of CN^- was added to solution **1** and the yellow color changed to colorless. Fluorescence titration results (Figure 5) showed that the original strong emission of solution **1** was gradually quenched on increasing the amount of CN^- ion and reached saturation when 20 equiv of CN^- was added. Both UV-vis and fluorescence titration results demonstrate the good sensitivity of **1** to CN^-. Linear fitting of the absorption titration profiles using Benesi-Hildebrand plot based on a 1:1 binding mode results in a good linearity (correlation coefficient is over 0.99, Figure 6), which strongly support the 1:1 binding stoichiometry of **1** and CN^-.

The peak at *m*/*z* 297.1148 (negative mode) form the Q-TOF mass data indicates the formation of anionic species **2**, *i.e.* **1**-CN^- adduct species, which strongly support the 1:1 addition reaction. The absorption spectrum of **2** looks quite similar to that of 9-ethylcarbazole which doesn't have the dicyanovinyl acceptor site. So, the nucleophilic cyanide ion attacked the α-position of dicyanovinyl group[37] and interdicted the π-conjugation of the dicyanovinyl group with carbazole skeleton. Therefore, the inhibited electron-accepting ability leads to a hypsochromic shift of absorption and emission quench.[33]

Subsequently, the UV-vis and fluorescence competition experiments were carried out to evaluate the anti-interference ability of probe **1**. UV-vis studies reveal that the coexistence of equal amount of other anion does not cause any noticeable interference on the CN^- recognition (Figure 7). The fluorescence intensity of probe **1** on the cyanide recognition was also completely quenched in the presence of other competitive anion, except $H_2PO_4^-$ which could induce

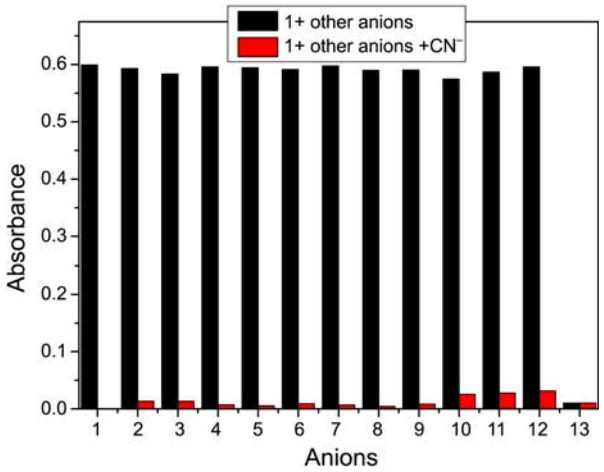


Figure 7. The absorbance of solution **1** (2.0×10^{-5} M) at 409 nm to various anions. The black bars represent the absorbance of **1** in the presence of 20 equiv of miscellaneous anions, the red bars represent the absorbance of the above solution upon addition of 20 equiv of CN^-. 1. **l**, 2. $H_2PO_4^-$, 3. I^-, 4. Cl^-, 5. Br^-, 6. F^-, 7. AcO^-, 8. HSO_4^-, 9. HPO_4^{2-}, 10. PO_4^{3-} 11. SCN^-, 12. NO_3^-, 13. CN^-.

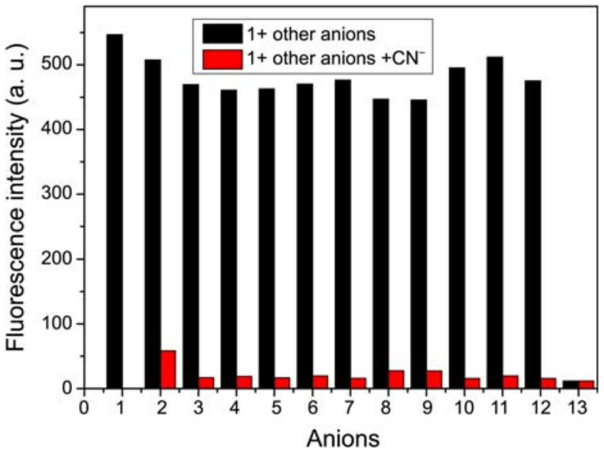


Figure 8. The fluorescence intensity of solution **1** (2.0×10^{-5} M) at 539 nm to various anions. The black bars represent the emission intensity of **1** in the presence of 20 equiv of miscellaneous anions, the red bars represent the emission intensity of the above solution upon addition of 20 equiv of CN^-. 1. **l**, 2. $H_2PO_4^-$, 3. I^-, 4. Cl^-, 5. Br^-, 6. F^-, 7. AcO^-, 8. HSO_4^-, 9. HPO_4^{2-}, 10. PO_4^{3-} 11. SCN^-, 12. NO_3^-, 13. CN^-.

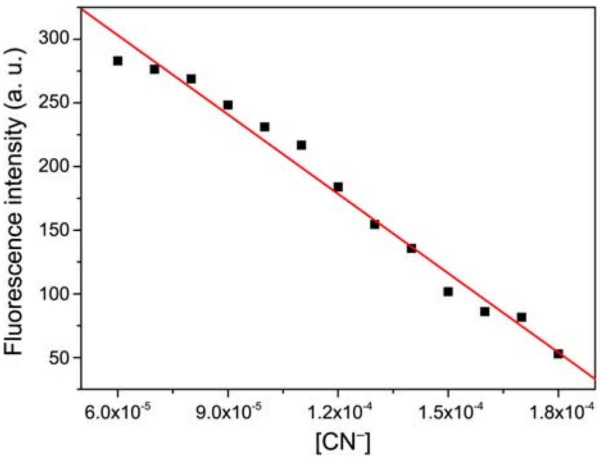


Figure 9. Fluorescence intensity of solution **1** (1.0×10^{-5} M) *versus* the concentration of CN^- at low concentration range (6.0×10^{-5} to 1.8×10^{-4} M).

a slight perturbation leading to a weak emission (Figure 8). Results of these competition experiments state that the CN^- recognition by probe **1** is barely interfered by coexisting anions.

In addition, to check its practical applicability, the fluorescent detection limit of **1** for CN^- was evaluated. Under the present conditions, a good linear relationship between the fluorescence intensity and CN^- concentration was obtained and found to be in the range of 6.0×10^{-5} to 1.8×10^{-4} M (R = 0.9913) (Figure 9). The detection limit was calculated to be 2.04×10^{-6} M with the equation: detection limit = $3S/\rho$,[45] where S is the standard deviation of blank measurements, ρ is the slope between intensity *versus* sample concentration. According to the World Health Organization (WHO), the maximum contaminant level of cyanide has been set as 2.7×10^{-6} M in drinking water.[46] Compared with the CN^- detection limits of some reported probes (ranging from 7.4×10^{-8} M[39] to 2.3×10^{-6} M[34]), the detection limit of probe **1** to CN^- is under the upper limit of cyanide concentration in drinking water. This fact indicates that probe **1** has a potential utility in CN^- concentration detection in aqueous media.

Conclusion

New application of 2-[(9-ethyl-9*H*-carbazol-3-yl)methylene]propanedinitrile (**1**) as a cyanide probe in CH_3CN-H_2O (2/1, v/v, HEPES 10 mM, pH = 7.0) solution was studied. Probe **1** exhibits high selectivity and sensitivity toward CN^- and the recognition process is hardly interfered by other coexisting anions. Probe **1** interacts with CN^- *via* 1:1 binding stoichiometry with a detection limit of 2.04×10^{-6} M. The colorimetric and fluorescent dual-modal response makes probe **1** has a potential applicability for environmental cyanide detection.

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