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Coumarin–indanedione conjugate as a doubly activated Michael addition type probe for the colorimetric and ratiometric fluorescent detection of cyanide

Xianshu Zhou [a], Xin Lv [b], Junsheng Hao [b], Diansheng Liu [a],*, Wei Guo [b],*

[a] *Institute of Applied Chemistry, Shanxi University, Taiyuan 030006, China*
[b] *School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, China*

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ABSTRACT

A coumarin–indanedione conjugate as an activated Michael addition type probe was prepared for cyanide ion detection. The colorimetric and ratiometric fluorescent response of the probe to cyanide ion is due to the Michael addition of cyanide to the doubly activated Michael receptor of the probe which blocks an intramolecular charge transfer process. The probe displays a fast response to cyanide ion at room temperature, and a maximal ratiometric fluorescent signal is achieved in the presence of only 2 equivalents of cyanide ion. Moreover, competitive species did not induce any significant changes both in color and emission intensity ratio (I_{485}/I_{609}), demonstrating the high selectivity of the probe to cyanide.

1. Introduction

Anion recognition is an area of growing interest in supramolecular chemistry due to its important role in a wide range of environmental, clinical, chemical, and biological applications [1]. Among various anions, cyanide is one of the most concerned. Cyanide is a basic component in the preparation of a wide variety of products ranging from plastic, fibers, gold, dyes, chelating agents for water treatment, to pharmaceuticals [2]. However, even very small amounts of the cyanide anion are extremely toxic to living creatures. Cyanide is a potent inhibitor of some metallo-enzymes and non-metallo-enzymes. This leads to diseases of the vascular, cardiac, visual, endocrine, central nervous and metabolic systems [3]. Thus, the development of cyanide-selective colorimetric or fluorescent probes is in high demand. A variety of cyanide-selective colorimetric or fluorescent probes has been developed over the past ten years by making use of the coordination ability and the nucleophilic reactivity of cyanide [4]; the operation of such probes involves addition with metal complexes [5–7], displacement method [8–12], as well as bond-forming reaction between the cyanide and either an electrophilic carbon [13,14] or a boron center [15,16].

The Michael addition or Michael reaction, addition of stabilized anions (e.g., enolate, cyanide) to α,β-unsaturated carbonyl and related compounds, is a prototype for the conjugate addition reactions of various nucleophiles that find wide utility in organic syntheses [17]. In recent years, this powerful reaction has been successfully used in the design of the colorimetric and fluorescent probes for cyanide as well as other species [18–35]. Generally, Michael addition type probes can be constructed by covalently linking the Michael acceptor with signaling group. The reaction of nucleophiles with the Michael acceptor will cause a change in color or fluorescence of the signaling group.

Compared with the general Michael acceptors, the doubly activated acceptors, in which two electron-withdrawing groups are attached to the C=C group [29–31], are more reactive [32–35], and allow the Michael addition reaction to occur under mild condition. However, it was noted that the probes with the doubly activated acceptors usually exhibit low florescence quantum yield in solution due to the rapid non-radioactive deactivation of the singlet exited state through torsional relaxation around the single bond that connects the Michael acceptor with fluorophore [36,37] (Fig. 1). Thus, most of this type probes only show fluorescence OFF–ON effect upon interaction with analytes [20,31,34,35]. As the change in fluorescence intensity is the only detection signal, factors such as

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* Corresponding authors. Tel./fax: +86 351 7011600.
E-mail address: guow@sxu.edu.cn (W. Guo).

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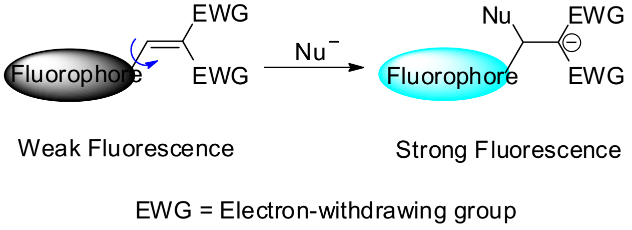


Fig. 1. Schematic representation of Michael acceptor type probes.

variabilities in excitation and emission efficiency, sample environments, and probe distribution can interfere with the signal output. By contrast, ratiometric fluorescent probes can eliminate most or all ambiguities by self-calibration of two emission bands, and seem to be more favorable for sensing target ions in comparison with fluorescence intensity-based probes [38–40]. However, up to now, only a limited number of Michael addition type probes for the ratiometric fluorescent detection of cyanide have been reported in literature [30,41].

Herein, we report a coumarin–indanedione conjugate (**1**) as doubly activated Michael addition type probe for the colorimetric and ratiometric fluorescent detection of cyanide. The probe displays high selectivity and sensitivity for CN^- over other anions with a fast response. In addition, as a control, a coumarin–indanedione conjugate (**2**) with a nitro group in the indanedione moiety was also synthesized, and its capability to sense cyanide was tested.

2. Experiment

2.1. Materials and general methods

All reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel 60 (200–300 mesh). Absorption spectra were taken on a Varian 50 Bio spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer. The ^1H NMR and ^{13}C NMR spectra were recorded using Bruker Avance III 600 at 600 and 150 MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. 7-Diethylaminocoumarin-3-carboxaldehyde **3** [42] and compound **1** [43] were synthesized according to the literature reported procedures (Scheme 1).

*2.2. Synthesis of compounds **1** and **2***

*2.2.1. Compound **1***

To a solution of coumarin aldehyde **3** (245 mg, 1.00 mmol) and 1,3-indanedione **2** (175 mg, 1.20 mmol) in EtOH (5 mL) was added 2 drops of piperidine. The reaction mixture was stirred overnight at room temperature, and then the solvent was removed under the reduced pressure. The residue was purified by column chromatography using CH_2Cl_2/EA (v/v 5:0.05, Rf = 0.22) to give the desired probe as a dark green solid (310 mg, yield 83%). Mp: 221–222 °C. ^1H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.99 (s, 1H), 7.90–7.94 (m, 4H), 7.63 (d, $J = 9.12$, 1H), 6.87–6.89 (m, 1H), 6.66 (d, $J = 2.28$, 1H), 3.55 (q, $J = 7.02$, 4H), 1.16–1.19 (t, $J = 7.02$, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 189.4, 161.0, 158.3, 153.8, 148.9, 141.4, 139.1, 135.5, 132.9, 125.8, 122.6, 111.0, 108.6, 96.7, 44.7, 30.6, 12.4 (17 carbon peaks). HRMS [ESI]: *m*/*z*, calcd for $[M + H]^+$ 374.1387; Found 374.1384.

*2.2.2. Compound **2***

The compound can be synthesized according to a similar procedure of **1**. In fact, it was obtained as *cis* and *trans* isomer as suggested by ^1H NMR spectra. Presently, it is difficult for us to separate them due to the almost same TLC performance. However, as control compound, the isomer is usable for studying the substituent effect. Yield: 48%. ^1H NMR (600 MHz, DMSO-d_6) δ 9.94 and 9.81 (2s, 1H), 8.27 and 8.23 (2d, $J_1 = 8.4$, $J_2 = 7.8$, 1H), 8.09 (m, 1H), 8.07 and 8.02 (2s, 1H), 7.70 and 7.67 (2d, $J_1 = 9.0$, $J_2 = 9.0$, 1H), 6.92 (m, 1H), 6.70 (d, $J = 3.6$, 1H), 3.58 (q, $J = 7.2$, 4H), 1.18 (t, 6H). HRMS [ESI]: *m*/*z*, calcd for $[M + Na]^+$ 441.1063; Found 441.1061.

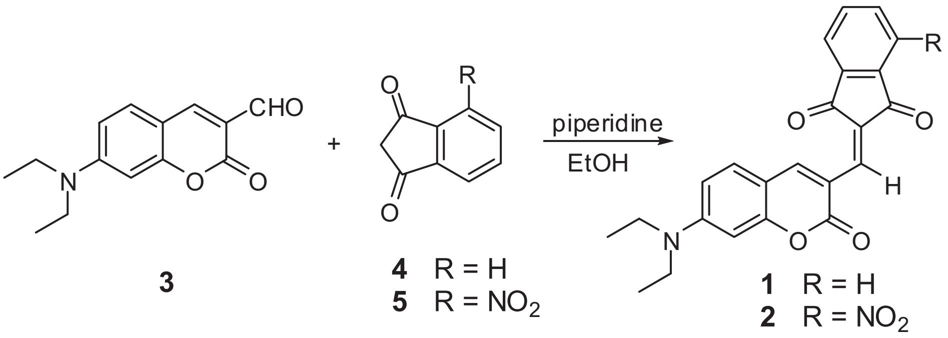
2.3. Procedures of anions sensing

Deionized water was used throughout all experiments. Cyanide anion and the other anions were prepared from *n*-Bu_4N salts. A stock solution of **1** (10 mM) was prepared in DMF. The stock solution of **1** was then diluted to the corresponding concentration (10 μM) with the solution of CH_3CN. The tetrabutylammonium cyanide stock solution of 1.0×10^{-1} M was diluted to 1.0×10^{-2} M and 1.0×10^{-3} M with deionized water for spectra titration studies. Spectral data were recorded in an indicated time after the addition.

3. Results and discussion

*3.1. Design and synthesis of probe **1***

Probe **1** could be conveniently synthesized via the condensation of diethylaminocoumarin-aldehyde with 1,3-indanedione in ethanol (see: Experimental section). Structural identification of the compound was confirmed by ^1H NMR, ^{13}C NMR, and HRMS



Scheme 1. Synthesis of compounds 1 and 2.

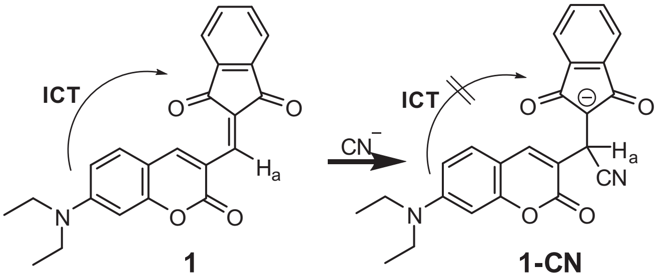


Fig. 2. Sensing mechanism of **1** toward cyanide.

spectroscopy. Note that the chemical shift of vinylic proton was found in 9.92 ppm, probably due to the existence of an intramolecular C–H···O hydrogen-bonding interaction [44,45] between the coumarin carbonyl O atom and the vinylic proton.

Probe **1** is composed of a diethylaminocoumarin fluorophore and a 1,3-indanedione-containing doubly activated Michael acceptor for cyanide anion (Fig. 2). Such a molecular design makes probe **1** possess the expanded π-conjugation as well as the strong ICT from the diethylaminocoumarin to the conjugated 1,3-indanedione moiety, which will lead to the red absorption and emission. It is expected that cyanide anion can add to the β-position of the doubly activated Michael acceptor to generate the stabilized anionic species **1**–**CN** [29]. Importantly, the addition reaction will block the intramolecular charge transfer (ICT) due to interruption of the conjugation, by which a hypsochromic shift of absorption and emission of diethylaminocoumarin should be induced. As a result, a ratiometric fluorescence response as well as an obvious color change could be expected. These could serve as the basis for the cyanide sensing of probe **1**.

3.2. Sensing mechanism of probe 1 toward cyanide

The binding pattern between **1** and CN^- was first examined by a ^1H NMR titration experiment (Fig. 3). Upon addition of *n*-Bu_4NCN to a DMSO-d_6 solution of **1**, the signals attributed to **1** disappeared with the appearance of a set of new signals. Moreover, the signal of the vinylic proton (H_a) at around 9.92 ppm was shifted to 4.71 ppm upon the addition of cyanide, suggesting the formation of the stabilized anionic species **1**–**CN** [29]. Furthermore, this formation of **1**–**CN** adduct was also characterized by mass spectrometry analysis, in which the peaks at m/z 399.1337 (calcd = 399.1350) corresponding to $[\mathbf{1} + CN]^-$ were clearly observed.

3.3. Colorimetric response of probe 1 to cyanide

As shown in Fig. 4, free **1** shows a main absorption band at 548 nm in CH_3CN, which can be assigned to the ICT bands. Upon addition of CN^-, the absorbance at 548 nm was gradually decreased with a new peak appearing at 390 nm. 2 Equiv. of cyanide is sufficient to drive the reaction to completion. Concomitantly, an obvious color change from purple to colorless was clearly observed, suggesting the ICT is turned off due to the Michael addition of cyanide to **1**. The fact that only 2 equiv. of cyanide used in the assay is impressive as many reported cyanide probes require high equivalents of cyanide to reach a maximal spectra signal [5–16].

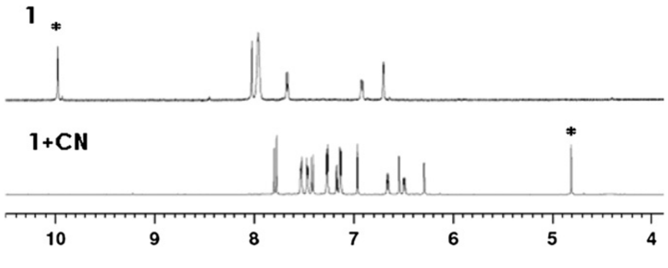


Fig. 3. ^1H NMR spectral changes upon the addition of cyanide anion (2 equiv., as its tetrabutylammonium salts) to **1** (10 mM) in DMSO-d_6.

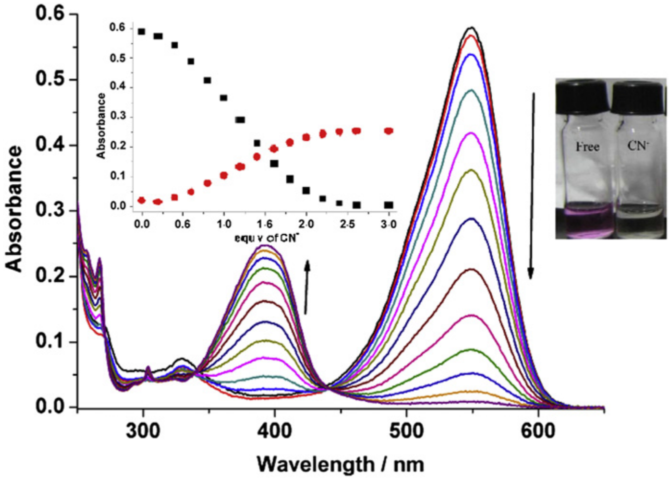


Fig. 4. Concentration-dependent changes in absorption spectra of probe **1** (10 μM) upon gradual addition of CN^- (0–3 equiv.) in CH_3CN. Inset: Changes of absorption intensity of probe **1** at 548 nm (■) and 390 nm (● in red) in the presence of different equiv. of CN^- (0–3 equiv.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Ratiometric fluorescence response of probe 1 toward cyanide

Free probe **1** in CH_3CN exhibits two main emission bands centering at 609 and 485 nm (Fig. 5), which can be assigned to the ICT and the local bands, respectively [41]. With the addition of CN^-, the emission at 609 nm decreased sharply, followed by the increases of emission of the diethylaminocoumarin moiety at

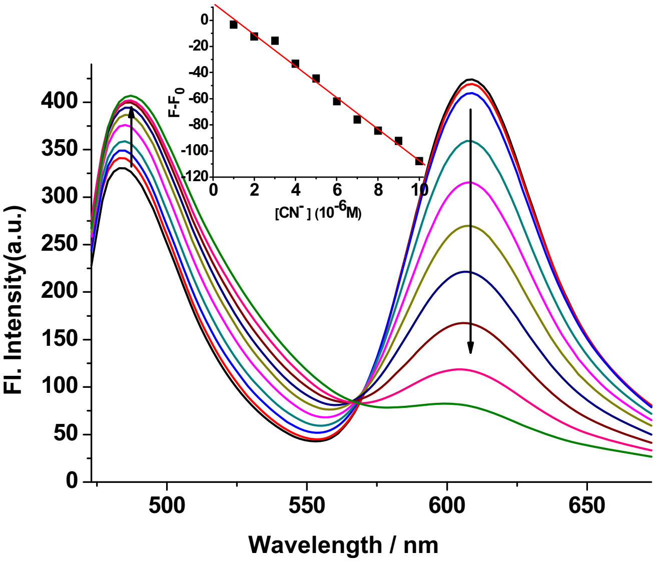


Fig. 5. Fluorescence titration spectra of probe **1** (10 μM) in CH_3CN upon addition of CN^- (0–2 equiv.). $\lambda_{ex} = 440$ nm.

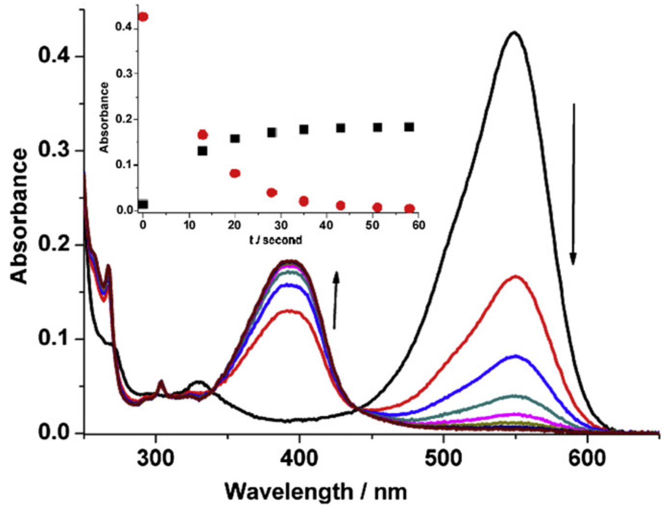


Fig. 6. Time-dependent UV–vis spectra of **1** upon the addition of CN^- (2 equiv.) in CH_3CN. Inset: Time-dependent absorption intensity of probe **1** at 548 nm (● in red) and 390 nm (■) in the presence of CN^- (2 equiv.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

485 nm, indicating that the Michael addition reaction interrupted the π-conjugation and blocked the ICT process, after which the fluorescence of diethylaminocoumarin recovered. This is in good agreement with the aforementioned design concept. In addition, the emission changes also resulted in a well-defined isoemissive point at 520 nm. In the presence of 2 equiv. of cyanide, the changes of the emission intensities nearly became constant [46], and a ca. 6.5-fold enhancement in ratiometric value of I_{485}/I_{609} (from 0.77 to 5.04) is achieved with respect to the cyanide-free solution. It is noteworthy that the difference in the two emission wavelengths is very large (emission shift: $\Delta F = 124$ nm), which contributes to the accurate measurement of the intensities of the two emission peaks. The detection limit for cyanide was determined as 0.18 μM based on $S/N = 3$ (Fig. 5, inset), meaning that **1** is operable well below the WHO cyanide standard in drinking water (1.9 μM) [47].

3.5. Response time

Time-dependent UV–vis spectra of **1** were monitored in the presence of 2 equiv. cyanide (Fig. 6). The result revealed that the Michael addition reaction can be finished within 60 s, indicating that **1** has rapid detection ability for cyanide.

3.6. Selectivity investigation

For the purpose of evaluating selectivity of **1** to cyanide, color and fluorescence spectral changes upon addition of 5 equiv. of various anion including F^-, Cl^-, Br^-, I^-, AcO^-, SCN^-, N_3^-, NO_3^-, SO_4^{2-}, $H_2PO_4^-$, OH^- and S^{2-} as n-Bu_4N salts in CH_3CN were studied. These competitive species did not induce any significant changes both in color and emission intensity ratio (I_{485}/I_{609}) at all (Figs. 7 and 8), indicating the high selectivity of **1** to cyanide. Interestingly, although some probes for S^{2-} have been reported by taking advantage of its nucleophilicity and coordination ability [48,49], in our conditions, the Michael addition of this species with **1** did not occur. Moreover, to the best of our knowledge, the probe for S^{2-} based on Michael addition reaction has not been previously reported.

3.7. Substituent effect

To further improve the sensitivity, it is necessary to enhance the reactivity of the Michael receptor in this type of probes. Thus, a coumarin–indanedione conjugate (**2**) with a strongly electron-withdrawing nitro group in the indanedione moiety was synthesized, and its capability to sense cyanide was tested. As suggested

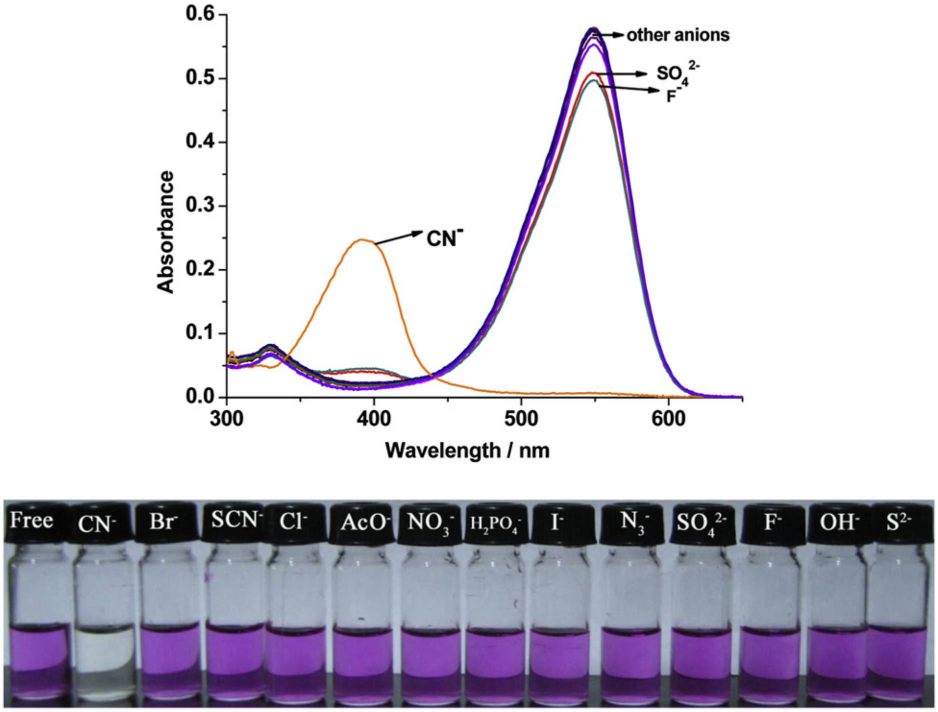


Fig. 7. Absorption spectra of probe **1** (10 μM) in the presence of 5 equiv. of various anion species (CN^-, F^-, Cl^-, Br^-, I^-, SCN^-, N_3^-, NO_3^-, AcO^-, SO_4^{2-}, and $H_2PO_4^-$ as n-Bu_4N salts in CH_3CN) (Top). Color change photograph of probe **1** in the absence or presence of various species (5 equiv.) as n-Bu_4N salts in CH_3CN (Bottom).

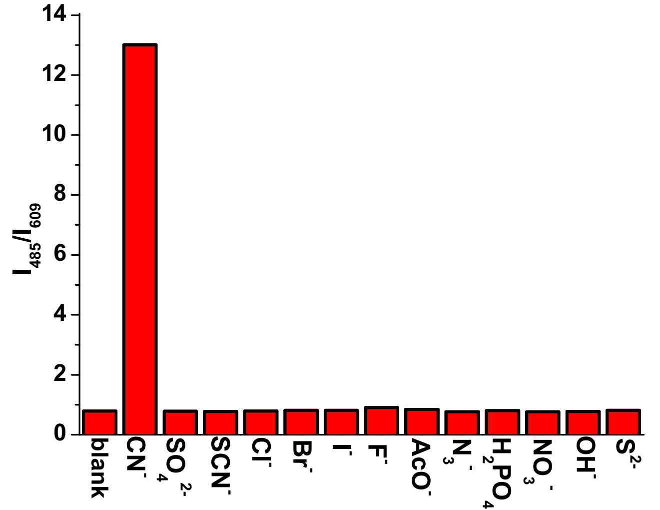


Fig. 8. Emission ratio (I_{485}/I_{609}) of probe **1** in the absence or presence of various species (5 equiv.) as n-Bu$_4$N salts in CH$_3$CN with excitation at 440 nm.

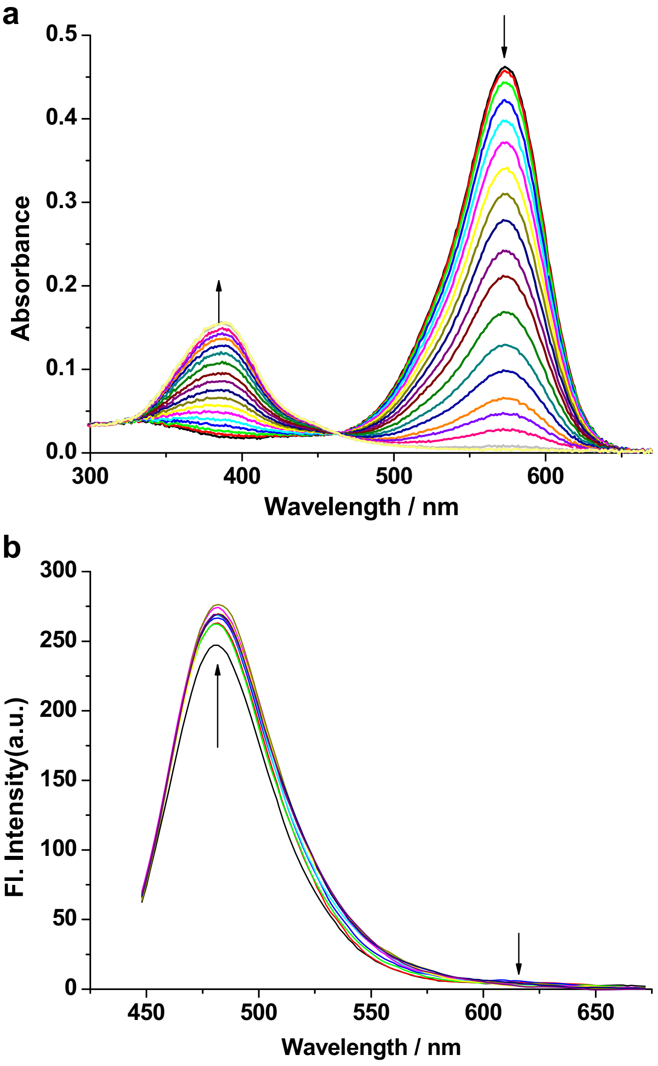


Fig. 9. Concentration-dependent changes in absorption (a) and fluorescence (b) spectra of probe **2** (10 μM) upon gradual addition of CN$^-$ (0–1 equiv.) in CH$_3$CN.

by absorption titration (Fig. 9a), 1 equiv. of cyanide is sufficient to drive the reaction to completion. Moreover, the reaction is very fast relative to that of **1** with cyanide, and completed within 20 s. Thus, compound **2** displays higher reactivity than compound **1**. However, signals acquired from absorption spectra are not as efficient as those evaluated from fluorescence spectra. In fact, the compound displayed very weak fluorescence at long wavelength (625 nm) possibly due to the oxidative electron transfer from excited **2** to the LUMO of the nitro groups upon photoexcitation. As a result, upon addition of cyanide, only very small ratiometric changes were observed (Fig. 9b).

4. Conclusions

In conclusion, we have developed a new colorimetric and ratiometric fluorescent probe **1** for CN$^-$ based on a coumarin–indanedione conjugate. The probe displays remarkable dual changes in absorption and emission spectrum for cyanide over other anions in CH$_3$CN with a fast response (less than 1 min).

Acknowledgments

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References

[1] Amendola V, Esteban-Gómez D, Fabbrizzi L, Licchelli M. What anions do to N–H-containing receptors. Acc Chem Res 2006;39:343–53.
[2] Miller GC, Pritsos CA. Cyanide: Soc., Ind. Econ. Aspects. In: Proc. Symp. Annu. Meet. TMS, 2001, p. 73–81.
[3] Baird C, Cann M. Environmental chemistry. New York: Freeman; 2005.
[4] Xu Z, Chen X, Kim HN, Yoon J. Sensors for the optical detection of cyanide ion. Chem Soc Rev 2010;39:127–37.
[5] Kim YH, Hong JI. Ion pair recognition by Zn-porphyrin/crown ether conjugates: visible sensing of sodium cyanide. Chem Commun 2002;5:512–3.
[6] Chow CF, Lam MHW, Wong WY. A heterobimetallic ruthenium(II)–copper(II) donor–acceptor complex as a chemodosimetric ensemble for selective cyanide detection. Inorg Chem 2004;43:8387–93.
[7] Lee JH, Jeong AR, Shin IS, Kim HJ, Hong JI. Fluorescence turn-on sensor for cyanide based on a cobalt(II)–coumarinylsalen complex. Org Lett 2010;12: 764–7.
[8] Kim HN, Guo Z, Zhu W, Yoon J, Tian H. Recent progress on polymer-based fluorescent and colorimetric chemosensors. Chem Soc Rev 2011;40:79–93.
[9] Lou X, Zhang L, Qin J, Li Z. An alternative approach to develop a highly sensitive and selective chemosensor for the colorimetric sensing of cyanide in water. Chem Commun 2008;44:5848–50.
[10] Lou X, Qin J, Li Z. Colorimetric cyanide detection using an azobenzene acid in aqueous solutions. Analyst 2009;134:2071–5.
[11] Zeng Q, Cai P, Li Z, Qin J, Tang BZ. An imidazole-functionalized polyacetylene: convenient synthesis and selective chemosensor for metal ions and cyanide. Chem Commun 2008;9:1094–6.
[12] Lou X, Qiang L, Qin J, Li Z. A new rhodamine-based colorimetric cyanide chemosensor: convenient detecting procedure and high sensitivity and selectivity. ACS Appl Mater Interfaces 2009;1:2529–35.
[13] Yu H, Zhao Q, Jiang Z, Qin J, Li Z. A ratiometric fluorescent probe for cyanide: convenient synthesis and the proposed mechanism. Sensors Actuators B: Chem 2010;148:110–6.
[14] Ren J, Zhu W, Tian H. A highly sensitive and selective chemosensor for cyanide. Talanta 2008;75:760–4.
[15] Jamkratoke M, Ruangpornvisuti V, Tumcharern G, Tuntulani T, Tomapatanaget B. A-D-A sensors based on naphthoimidazoledione and boronic acid as turn-on cyanide probes in water. J Org Chem 2009;74: 3919–22.
[16] Ros-Lis JV, Martinez-Manez R, Soto J. Subphthalocyanines as fluorochromogenic probes for anions and their application to the highly selective and sensitive cyanide detection. Chem Commun 2005;42:5260–2.
[17] Tokoroyama T. Discovery of the Michael reaction. Eur J Org Chem 2010;10: 2009–16.
[18] Kim G-J, Kim H-J. Coumarinyl aldehyde as a Michael acceptor type of colorimetric and fluorescent probe for cyanide in water. Tetrahedron Lett 2010;51: 2914–6.
[19] Isaad J, Achari AE. Biosourced 3-formyl chromenyl-azo dye as Michael acceptor type of chemodosimeter for cyanide in aqueous environment. Tetrahedron 2011;67:5678–85.

[20] Kim G-J, Kim H-J. Doubly activated coumarin as a colorimetric and fluorescent chemodosimeter for cyanide. Tetrahedron Lett 2010;51:185–7.

[21] Park S, Kim H-J. Highly selective chemodosimeter for cyanide based on a doubly activated Michael acceptor type of coumarin thiazole fluorophore. Sensors Actuators B: Chem; 2011. doi:10.1016/j.snb.2011.10.038.

[22] Park S, Kim H-J. Highly activated Michael acceptor by an intramolecular hydrogen bond as a fluorescence turn-on probe for cyanide. Chem Commun 2010;46:9197–9.

[23] Chen X, Zhou Y, Peng X, Yoon J. Fluorescent and colorimetric probes for detection of thiols. Chem Soc Rev 2010;39:2120–35.

[24] Kim HJ, Lee H, Lee JH, Choi DH, Jung JH, Kim JS. Bisindole anchored mesoporous silica nanoparticles for cyanide sensing in aqueous media. Chem Commun 2011;47:10918–20.

[25] Huo F-J, Su J, Sun Y-Q, Yin C-X, Chao J-B. A new ring-opening chromene molecule: colorimetric detection of cyanide anion. Chem Lett 2010;39:738–40.

[26] Dong Y-M, Peng Y, Dong M, Wang Y-W. A selective, sensitive, and chromogenic chemodosimeter for cyanide based on the 1,1′-binaphthyl scaffold. J Org Chem 2011;76:6962–6.

[27] Lou B, Chen Z-Q, Bian Z-Q, Huang C-H. Multisignaling detection of cyanide anions based on an iridium(iii) complex: remarkable enhancement of sensitivity by coordination effect. New J Chem 2010;34:132–6.

[28] Ros-Lis JV, Martinez-Manez R, Soto J. A selective chromogenic reagent for cyanide determination. Chem Commun 2002;19:2248–9.

[29] Kim S-H, Hong S-J, Yoo J, Kim SK, Sessler JL, Lee C-H. Strapped calix[4]pyrroles bearing a 1,3-indanedione at a β-pyrrolic position: chemodosimeters for the cyanide anion. Org Lett 2009;11:3626–9.

[30] Liu Z, Wang X, Yang Z, He W. Rational design of a dual chemosensor for cyanide anion sensing based on dicyanovinyl-substituted benzofurazan. J Org Chem 2011;76:10286–90.

[31] Jung HS, Ko KC, Kim GH, Lee AR, Na YC, Kang C, et al. Coumarin-based thiol chemosensor: synthesis, turn-on mechanism, and its biological application. Org Lett 2011;13:1498–501.

[32] Hong SJ, Yoo J, Kim S-H, Kim JS, Yoon J, Lee C-H. beta-Vinyl substituted calix 4 pyrrole as a selective ratiometric sensor for cyanide anion. Chem Commun; 2009:189–91.

[33] Jiao L, Liu M, Zhang M, Yu C, Wang Z, Hao E. Visual and colorimetric detection of cyanide anion based on a “turn-off” daylight fluorescent molecule. Chem Lett 2011;40:623–5.

[34] Vallejos S, Estevez P, Garcia FC, Serna F, de la Pena JL, Garcia JM. Putting to work organic sensing molecules in aqueous media: fluorene derivative-containing polymers as sensory materials for the colorimetric sensing of cyanide in water. Chem Commun 2010;46:7951–3.

[35] Wu X, Xu B, Tong H, Wang L. Highly selective and sensitive detection of cyanide by a reaction-based conjugated polymer chemosensor. Macromolecules 2011;44:4241–8.

[36] Kim T-I, Jeong MS, Chung SJ, Kim Y. An iminocoumarin-based fluorescent probe for the selective detection of dual-specific protein tyrosine phosphatases. Chem Eur J 2010;16:5297–300.

[37] Kim T-I, Kim H, Choi Y, Kim Y. A fluorescent turn-on probe for the detection of alkaline phosphatase activity in living cells. Chem Commun 2011;47:9825–7.

[38] Kikuchi K. Design, synthesis and biological application of chemical probes for bio-imaging. Chem Soc Rev 2010;39:2048–53.

[39] Liu B, Tian H. A ratiometric fluorescent chemosensor for fluoride ions based on a proton transfer signaling mechanism. J Mater Chem 2005;15:2681–6.

[40] Liu B, Tian H. A selective fluorescent ratiometric chemodosimeter for mercury ion. Chem Commun 2005;25:3156–8.

[41] Yuan L, Lin W, Yang Y, Song J, Wang J. Rational design of a highly reactive ratiometric fluorescent probe for cyanide. Org Lett 2011;13:3730–3.

[42] Wu J-S, Liu W-M, Zhuang X-Q, Wang F, Wang P-F, Tao S-L, et al. Fluorescence turn on of coumarin derivatives by metal cations: a new signaling mechanism based on C=N isomerization. Org Lett 2006;9:33–6.

[43] Griffiths J, Millar V, Bahra GS. The influence of chain length and electron acceptor residues in 3-substituted 7-*N*,*N*-diethylaminocoumarin dyes. Dyes Pigm 1995;28:327–39.

[44] Corey EJ, Lee TW. The formayl C–H···O hydrogen bond as a critical factor in enantioselective Lewis acid-catalyzed reactions of aldehydes. Chem Commun; 2001:1321.

[45] Yoshitake Y, Nakagawa H, Eto M, Harano K. Effect of C=O···H–Ar interaction on endo/exo selectivity in the Diels-Alder reaction of phenyl-substituted cyclopentadienones. Tetrahedron Lett 2000;41:4395.

[46] The use of two equivalents of cyanide anions does not mean that the probe can react with two cyanide anions. In fact, the 1:1 binding pattern has been demonstrated by Job’s plot. Although cyanide could sometimes add to C-4 of the coumarin unit (ref. 20), in our conditions, only one cyanide anion was found to add to β position of the Michael receptor, as suggested by ^{1}H NMR titration experiment and mass spectrometry analysis.

[47] Guidelines for dinking-water qunlity. Geneva: World Health Organization; 1996.

[48] Yang XF, Wang L, Xu H, Zhao M. A fluorescein-based fluorogenic and chromogenic chemodosimeter for the sensitive detection of sulfide anion in aqueous solution. Anal Chim Acta 2009;631:91.

[49] Choi MG, Cha S, Lee H, Jeon HL, Chang SK. Sulfide-selective chemosignaling by a Cu^{2+} complex of dipicolylamine appended fluorescein. Chem Commun; 2009:7390.