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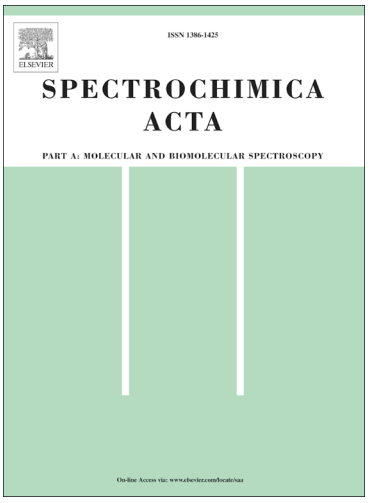

Highly Selective, Colorimetric Probes for Cyanide Ion Based on β –Formyl-BODIPY Dyes by an Unprecedented Nucleophilic Addition Reaction

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Highly Selective, Colorimetric Probes for Cyanide Ion Based on β–FormylBODIPY Dyes by an Unprecedented Nucleophilic Addition Reaction

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Highlights

An unprecedented nucleophilic addition between cyanide anion and α-position of BODIPY core was found.

Formyl groups on the BODIPY core enhanced the reactivity toward nucleophilic addition by cyanide anion

The probes showed visual, colorimetric and fluorescent sensing of cyanide anion.

Abstract

Two *β*-formylBODIPYs with strong daylight excitable fluorescence and highly selective visual and colorimetric response to cyanide anion (CN^-) have been prepared. NMR titration experiments have been performed to study the sensing mechanism for these two dyes. Surprisingly, cyanide anion is nucleophilic addition to the α-position of BODIPY core (the azafulvene framework) in aqueous system instead of the expected classical nucleophilic addition to the formyl moiety of the probes. This

nucleophilic addition of cyanide anion to the azafulvene framework causes the interruption of the π-conjugation of the BODIPY system, which brings a significant blue-shift (up to 104 nm) of the absorption maxima. A broadened and decreased absorption as well as ratiometrical fluorescence response (with maxima shifts from 523 to 670 nm) were observed with the titration of cyanide anion to probe **1b**.

Introduction

Cyanide anion has been widely used in industry, including the extracting gold from its ores, the electroplating and the manufacture of synthetic rubber and fibers [1-2]. In addition, the nitrogen metabolic pathways of many organisms, such as bacteria, fungi and algae also leads to the formation of cyanide anion [3]. On the other hand, cyanide anion itself is highly toxic. This may be attributed to the fact that it can rapidly interact with a heme unit in the active site of cytochrome a3, which inhibits the cellular respiration in mammals and leads to symptoms including vomiting, convulsion, loss of consciousness, and even death [4-7]. The World Health Organization (WHO) has regulated the allowable concentration of cyanide anion in drinking water to be less than 1.9 μM [8]. Therefore, the facile and sensitive detection of cyanide has attracted wide research attentions, especially the facile quantitative and qualitative detection of cyanide anion in the environmental and biological samples.

Although a set of sophisticated methods, including the mass spectrometry, electrochemistry as well as chromatography have been successfully used to detect cyanide [3, 9-15], however, the major limitations of these methods were that they need expensive instrumentation or tedious sample

pretreament. Considering the easy accessibility and operational simplicity of colorimetric and fluorescent detection of cyanide anion, which allows naked-eye detection of cyanide anion without resorting to some expensive spectroscopic instrumentation [16-19], extensive research efforts have been devoted to the development of reaction-based colorimetric and fluorescence probes by taking advantage of the nucleophilic reactivity of cyanide anion [20-21]. However, the low sensitivity issue remains a problem, which limits their applications in the real sample analysis.

BODIPY (boron dipyrromethene) dyes, have received increasing research interests in a wide research area, including sensors, imaging, labeling, photosensitizers due to their facile synthesis, easy functionalization, good stability, and tuneable photophysical properties, including large molar extinction coefficients [22-35]. Herein, we report the synthesis of two *β*–formylBODIPYs **1a** and **1b** (**Scheme 1**) and their highly selective visual and colorimetric response to cyanide anion over a series of common competition ions. NMR titration experiments indicate that this selective sensing of cyanide anion is based on an unprecedented nucleophilic addition of cyanide anion to the α–position of BODIPY core (the azafulvene framework, path b in **Scheme 1a**), which is in great contrast to those classical additions of cyanide anion to the *β*–formyl groups of the BODIPY core (path a in **Scheme 1a**).

Experimental Section

General materials, instruments, and methods were similar to our previous reports [26-28]. Detail information and UV–vis and fluorescence titration methods are available in the Supporting Information.

Synthesis and characterization: *meso*-MesitylBODIPY and BODIPYs **1a** and **1b** were synthesized using a slightly modified literature procedure [36-37].

BODIPY **1a** was prepared by reacting *meso*-mesitylBODIPY (50 mg, 0.16 mmol) in 10 mL 1,2-dichloroethane with the reaction mixture of DMF (3.0 mL) and $POCl_3$ (3.0 mL) for 10 h at 80 °C. After workup, the crude product was collected and purified using column chromatography (silica gel, hexane/ CH_2Cl_2 = 3/1, v/v) to give **1a** in 81% yield (44 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 (s, 1H), 8.25 (s, 1H), 8.16 (s, 1H), 7.03- 6.91 (m, 4H), 6.65 (s, 1H), 2.37 (s, 3H), 2.09 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.9, 150.4, 150.0, 143.0, 139.7, 137.9, 136.2, 135.1, 133.7, 131.9, 128.8, 128.6, 127.4, 121.8, 21.3, 20.1, HRMS (EI): calcd. for $C_{19}H_{17}BF_2N_2O$, $[M]^+$ 338.1402; found 338.1408.

BODIPY **1b** was prepared in 75% isolated yield (29 mg) using a similar procedure described above for **1a**, from the reaction of **1a** (85 mg, 0.25 mmol) with the mixture of DMF (6.0 mL) and $POCl_3$ (6.0 mL). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.87 (s, 2H), 8.46 (s, 2H), 7.28 (s, 2H), 7.02 (s, 2H), 2.39 (s, 3H), 2.10 (s, 6H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 185.3, 154.3, 147.0, 136.4, 134.5, 132.5, 128.9, 128.8, 20.7, 19.4. HRMS (EI): calcd. for $C_{20}H_{17}BF_2N_2O_2$, $[M]^+$ 366.1351; found 366.1356.

Results and Discussion

First, *β*–formylBODIPY **1a** was synthesized by Vilsmeier-Haack reaction of *meso*-mesitylBODIPY in 81% yield following our previously reported procedure. Further Vilsmeier-Haack reaction of **1a** gave *β,β'*–diformylBODIPY **1b** in 75% yield. The symmetrical substituted pattern of **1b** was confirmed by NMR spectroscopy, in which a singlet signal at 9.87 ppm for the two protons of aldehyde group, and two sets of singlet signals at 8.46 and 7.02 ppm for the α and γ pyrrolic protons on the BODIPY core were observed. The solvent-dependent photophysical properties of **1a** and **1b** at various common organic solvents with different polarities have been studied as summarized in **Figures S1-2** and **Table S1** in Supporting Information. The *β*–formylBODIPYs **1a** and **1b** showed absorption maxima centered at 501 and 509 nm in toluene, respectively. There is a small red-shift of the absorption bands with respect to that of the starting *meso*-mesitylBODIPY. The increase of the polarity of solvents (from toluene to methanol) leads to a slightly blue-shift in absorption bands for both **1a** and **1b** (**Table**

S1). No obvious solvent-dependent fluorescence emission was observed for BODIPYs **1a** and **1b**, except a slightly increase of the fluorescence quantum yields for **1b** with increase of the polarity of solvents (0.66 in toluene, 0.72 in chloroform, 0.63 in THF and 0.12 in acetonitrile, **Table S1**).

Next, the sensing behavior of BODIPYs **1a** and **1b** to cyanide anion was studied in a CH_3CN-HEPES buffer solution (v/v = 9:1, pH = 7.4). In this buffer solution, BODIPY **1a** (1×10^{-5} M) showed an absorption peak at 492 nm (**Figure 1a**), which gradually disappeared with the increase of the concentration of cyanide anion. Meanwhile a new absorption band centered at 388 nm gradually increases with an isosbestic point at 425 nm. This blue-shift of the absorption spectra is in good consistence with an apparent visible color change from light green to yellow under ambient light irradiation condition that can be visualized by naked eyes (inserted picture in **Figure 1a)**. As shown in **Figure 1b**, the increase of the concentration of cyanide anion leads to the gradual decrease of the fluorescence intensity of **1a** at 517 nm. There is a good linear relationship between the fluorescence intensity at 517 nm and the CN^- concentration in the range of 0-450 μM ($R^2 = 0.992$, **Figure S3**) with a detection limit at 2.5×10^{-5} M (S/N = 3).

Furthermore, the spectrum changes of BODIPY **1b** after reaction with CN^- were also investigated by absorption and fluorescence emission spectroscopy. Surprisingly, the increasing addition of CN^- leads to the gradual decrease of the absorption band of **1b** centred at 498 nm and an appearance of a broader new band centred at 513 nm with a distinct isosbestic point at 455 nm (**Figure 2a**). This clearly indicates a chemical reaction between probe **1b** and CN^-. In addition, there is a gradual decrease of the fluorescence emission of **1b** centered at 523 nm with an appearance of a gradual appearance of a new band centered at 670 nm with a distinct isosbestic point at 615 nm with the increase of the concentration of CN^- (**Figure 2b**). This can be visualized by naked eyes since there is a significant visible color

change from light orange to pink was observed under day light irradiation condition. This indicates that probe **1b** could be used as a ratiometrical fluorescence probe for cyanine ion (**Figure S4**).

To demonstrate the high specificity of these two reaction-based probes toward CN^-, some common competing anions were comparatively studied under the same conditions. As shown in Figures **3** and **4**, probes **1a** and **1b** are highly selective toward CN^- over common competing anions. The addition of other common competing anions including AcO^-, Br^-, Cl^-, ClO_4^-, F^-, $H_2PO_4^{2-}$, HSO_3^-, HSO_4^-, NO_3^-, SCN^-, SO_4^{2-} leads to negligible variations in both the absorption and fluorescence spectra for both probes **1a** and **1b**. It is worth noticed that although the addition of F^- leads to the decrease of fluorescence emission for **1b,** no spectra shift was observed. This is in great contrast to CN^- which brings not only the decrease of the fluorescence emission but also a red-shift of the spectra..

To demonstrate the high selectivity of probe **1a** and **1b** toward to CN^-, the competition experiments were performed (**Figures S5** and **S6**). The fluorescence competition experiments indicated that the presence of other competing anions only leads to a negligible to slight variation of the fluorescence response induced by CN^-. Therefore, probes **1a** and **1b** may be used for the selective detection of cyanine anion in the presence of other common competing species.

In addition, the visible light (daylight) excitation and emission (green light) properties of probes **1a** and **1b** renders the naked-eye detection of CN^- (**Figure 5)**. Under daylight irradiation condition, the addition of CN^- to probes **1a** and **1b** leads to a dramatic colour change from light green (visible emission by excitation of daylight) to yellow (for **1a**), and from yellow to pink (for **1b**) respectively. By contrast, the addition of other competition anions causes negligible variations of the colour.

The significantly blue shifts of absorption maxima indicated that the BODIPY core conjugation was broken, especially in the reaction of BODIPY **1a** and cyanine ion. Since the direct addition of formyl group on BODIPY **1a** with cyanine ion (**Scheme 1a**, path a) can not affect the conjugation of the BODIPY core, thus we speculate that the cyanide ion may attack azafulvene ring of BODIPY core as shown in **Figure 6**. In fact, our group have recently disclosed that BODIPY derivative with electron withdrawn group at β–position shows enhanced reactivity toward the direct oxidative nucleophilic substitution and can efficiently react with various amines regioselectively at the α-position of BODIPY core at room temperature [38].

To further probe the proposed reaction mechanism, ^{1}H NMR titration experiments were carried out. The variation of the ^{1}H NMR spectra for probes **1a** and **1b** with the addition of CN^- were recorded in $CDCl_3$ at room temperature (**Figures 6** and **S7**), respectively. The addition of CN^-, brings a significant up-field shift of the proton signals corresponding to the aldehyde proton (H_a) and aromatic protons (H_b-H_f) in comparison with those in probes **1a** and **1b**, respectively. As shown in **Figure 6**, the addition of 1 equiv CN^- leads to the disappearance of the chemical shift of the aldehyde proton (H_a) at 9.82 ppm and the appearance of a new signal at 9.62 ppm ($H_{a'}$). This confirms that the formyl group in **1a** remains after the reaction CN^-. Significantly, a upfield shift of the azafulvene proton (H_c) from 8.16 ppm to 5.51 ppm ($H_{c'}$) was observed with the addition of CN^-. This may be attributed to the formation of sp^3 hybridized carbon anion due to the nucleophilic addition to the C=N double bond of the azafulvene framework. This result is in good agreement with the blue shifted absorption bands observed with the addition of CN^-. Probe **1b** shows a similar ^{1}H NMR titration behaviour to that of probe **1a** (**Figure S5**), in which the aldehyde groups remain untouched with the reaction with CN^-. Our initial attempts to prepare and isolate the proposed possible α-CN substituted BODIPYs *via* the atmospheric oxidation of **1a-CN** and **1b-CN** (**Scheme 1**) by exposure the reaction mixture to the air were failed. Fortunately,

the addition of a strong oxidant $Pb(OAc)_4$ to the reaction mixture of **1b** and CN^- smoothly promotes the oxidation reaction to afford the corresponding α-CN substituted BODIPY as stable product in 75% yield (**Scheme S1**). The formation of this oxidation product as was confirmed by NMR spectra and HRMS.

Conclusion

In summary, we have prepared two new colorimetric and ratiometric probes **1a** and **1b** for the selective "naked-eye" detection of cyanide anion over other common competition anions based on an unprecedented nucleophilic addition of cyanide ion to the azafulvene framework of the BODIPY chromophore instead of the expected formyl moieties. The addition of cyanide ion brings a remarkably dual change: a blue-shift in absorption (from 492 to 388 nm) and an efficient quenching in the fluorescence for probe **1a**, and a ratiometrical red-shift of the fluorescence emission band for probe **1b** (from 523 to 670 nm). Possible mechanism of these reaction based cyanide probes were studied. Both the ^1H NMR titration and the oxidative nucleophilic addition products indicates that probes **1a** and **1b** underwent an unprecedented nucleophilic addition of cyanide anion to the α-position of BODIPY core (the azafulvene framework) instead of classical β-formyl addition products.

Acknowledgements

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Figure 1. (a) Absorption titration and (b) fluorescence titration spectra of **1a** (10 μM) with the addition of CN^- from 0 to 500 μM in CH_3CN-HEPES buffer (v/v = 9:1, pH = 7.4). Data was recorded 10 min after the addition of CN^-. Inserted pictures: color changes of probe **1a** (10 μM) in the presence of 50 equiv of cyanide anion under daylight (left) and UV irradiation (right) conditions.

Figure 2. (a) Absorption titration and (b) fluorescence titration spectra of **1b** (10 μM) with the addition of CN^- from 0 to 300 μM in CH_3CN-HEPES buffer (v/v = 9:1 , pH = 7.4). Each spectrum was collected

10 min after addition of CN^-. Inserted pictures: color changes of probe **1b** (10 μM) in the presence of 30 equiv of cyanide anion under daylight (left) or UV irradiation (right) conditions.

Figure 3. (a) Overlaid absorption and (b) fluorescence emission spectra (right, λ_{ex} = 460 nm) of **1a** (10 μM) in the presence of 50 equiv CN^- and some common competing anions (100 equiv) in CH_3CN-HEPES buffer (v/v = 9:1, pH = 7.4). [0: Blank, 1: AcO^-, 2: Br^-, 3: Cl^-, 4: ClO_4^-, 5: F^-, 6: $H_2PO_4^-$, 7: HSO_3^-, 8: HSO_4^-, 9: NO_3^-, 10: SCN^-, 11: SO_4^{2-}, 12:CN^-].

Figure 4. (a) Overlaid absorption and (b) fluorescence emission spectra (right, λ_{ex} = 470 nm) of **1b** (10 μM) in the presence of 30 equiv CN^- and some common competing anions (100 equiv) in CH_3CN-HEPES buffer (v/v = 9:1, pH = 7.4). [0: Blank, 1: AcO^-, 2: Br^-, 3: Cl^-, 4: ClO_4^-, 5: F^-, 6: $H_2PO_4^-$, 7: HSO_3^-, 8: HSO_4^-, 9: NO_3^-, 10: SCN^-, 11: SO_4^{2-}, 12:CN^-].

Figure 5. Images of **1a** (a) and **1b** (b) (10 μM) in the presence of CN^- (50 equiv for **1a** and 30 equiv for **1b**) and 100 equiv of various competing anions in CH_3CN-HEPES buffer (v/v = 9:1, pH = 7.4) under daylight (top) and handhold UV-irradiation (bottom) conditions. [0: Blank, 1: AcO^-, 2: Br^-, 3: Cl^-, 4: ClO_4^-, 5: F^-, 6: $H_2PO_4^-$, 7: HSO_3^-, 8: HSO_4^-, 9: NO_3^-, 10: SCN^-, 11: SO_4^{2-}, 12: CN^-]

Figure 6. Proposed mechanism and ^1H NMR titration spectra of BODIPY **1a** (8 mM) with and without the addition of cyanide anion (0.5 and 1.0 equiv, respectively) in $CDCl_3$ solution at 25°C. ^1H NMR spectra was immediately recorded upon addition of TBACN.

Scheme 1. (a) Chemical structures of probes **1a** and **1b** and the proposed nucleophilic addition sensing mechanisms. (b, c) Overlaid absorption (black line) and emission (red line) spectra of probes **1a-CN**

(b, 10 μM **1a** in the presence of 50 equiv CN^-) and **1b-CN** (c, 10 μM **1b** in the presence of 30 equiv CN^-) in CH_3CN-HEPES solution (v/v = 9:1, pH = 7.4), respectively. Inserted pictures: the corresponding photos of probes **1a-CN** (b) and **1b-CN** (c) under daylight (left) or 360 nm light irradiation (right) conditions.