



Doubly activated coumarin as a colorimetric and fluorescent chemodosimeter for cyanide

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ARTICLE INFO

Article history:

Received 23 September 2009

Revised 23 October 2009

Accepted 26 October 2009

Available online 30 October 2009

Keywords:

Chemodosimeter

Cyanide

Fluorescence

ABSTRACT

A new α,β -unsaturated nitro group and coumarin conjugate-based fluorescent chemodosimeter **1** was developed for the selective detection of cyanide anions. The chemodosimeter has shown a selective and sensitive response to cyanide anions over other various anions through a nucleophilic aromatic substitution reaction of the cyanide to **1**. When cyanide anions were added, the enhanced fluorescence intensity as well as the color changes of **1** was observed so that micromolar concentrations of cyanides were detectable by the naked eye.

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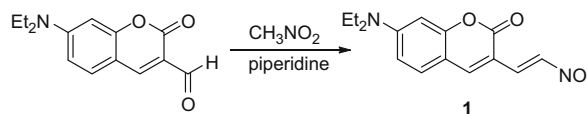
The cyanide ion is an extremely hazardous chemical that damages by absorption through the lungs, gastrointestinal tract, and skin, and can kill mammals upon binding to a heme unit.¹ The process of cellular respiration in mammals is inhibited by the cyanide anion, which interacts strongly with the heme unit at the active site of cytochrome a_3 .² Although cyanides have been found in many foods and plants, most environmental cyanides are released by industries involved in gold mining, electroplating, and metallurgy.³ Humans may be exposed to cyanides from dietary, industrial, environmental, and other sources. Consequently, there is great interest in the capability to detect the presence of the toxic cyanide anion by using chemosensors.⁴

Among the various chemical sensors, fluorescent chemosensors present many advantages such as high sensitivity, low cost, easy detection, and suitability as a diagnostic tool for biological concerns.⁵ There are, however, a few chemical probes that show both fluorescence turn-on and high selectivity upon the complexation of cyanide anions.^{4b,d,g}

Recently, Lee and co-workers reported a new type of cyanide sensor using a vinyl-substituted calix[4]pyrrole.⁴ⁱ The α,β -unsaturated vinyl malononitrile group in the sensor plays a role of the Michael acceptor for the cyanide anion. Motivated by this approach for a cyanide probe, we have developed and report herein a new Michael acceptor type of fluorescent chemodosimeter **1** for cyanide (Scheme 1).

Probe **1** has a coumarin group as a fluorescent signal unit and an unsaturated nitro group as a Michael acceptor unit toward the cyanide. The signaling unit is covalently linked to the reaction unit, and therefore the chemical reaction of **1** with a cyanide nucleophile will change the electronic structure of the probe enough to cause a significant photophysical alteration. The chemodosimeter **1** was prepared through the condensation reaction of nitromethane and the coumarinyl aldehyde, which was synthesized according to the literature procedure.⁶

In order to get an insight into the reaction, we investigated a thin layer chromatogram of **1** by adding cyanide and compared it with that of the probe itself. Upon the addition of the cyanide to **1**, a new brown spot on the TLC plate appeared and the reaction was almost complete within 1 h (Fig. S6). A ¹H NMR spectral analysis after column chromatographic purification of the new spot showed almost the same peaks as **1** except for the disappearance of an aromatic proton at 8.47 ppm (Fig. 1). The new spot turned out to be **2** by the analyses of the ¹H NMR and high resolution mass spectral data (Fig. S8). This unexpected result plausibly comes from the nucleophilic aromatic substitution (S_NAr^H) of the cyanide ion to **1**.



Scheme 1. The synthesis of **1**.

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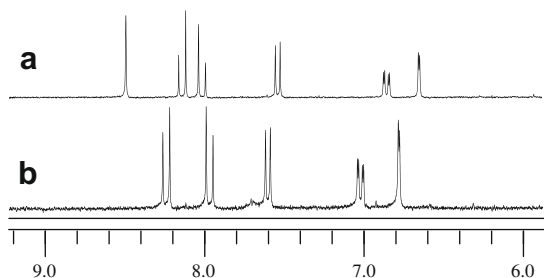


Figure 1. Partial ^1H NMR spectra of **1** in $\text{DMSO}-d_6$. (a) **1**; (b) **1** + 0.8 equiv of CN^- .

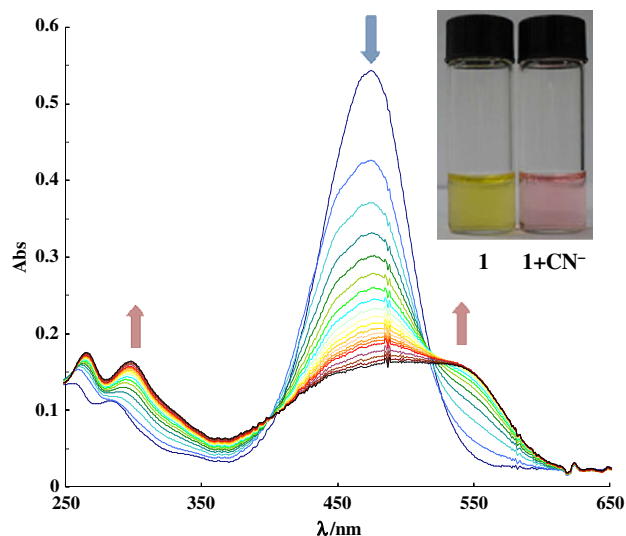


Figure 2. Time-dependant UV-vis spectral change in the presence of 1 equiv of CN^- to **1** ($10\ \mu\text{M}$, CH_3CN). Inset: color change observed by the naked eye.

The chemical reaction was also confirmed by UV-vis spectroscopy. Upon the addition of cyanide (1 equiv) to **1** ($10\ \mu\text{M}$ in CH_3CN), the absorption maximum at $\lambda\ 468\ \text{nm}$ decreased while new maxima appeared around $\lambda\ 300\ \text{nm}$ and $550\ \text{nm}$ with a pseudo-isosbestic point at $\lambda\ 400\ \text{nm}$ (Fig. 2). These spectral changes could be observed by the naked eye. The light orange color of **1** in CH_3CN changed into pink as soon as cyanide ions were added.

The sensing phenomena were also monitored by fluorescence spectroscopy. As cyanide ions were added to **1** ($10\ \mu\text{M}$ in CH_3CN), the fluorescence emission intensity of the dosimeter increased more than fivefold and was saturated at 1 equiv of cyanide. However, other anions such as F^- , Cl^- , Br^- , I^- , N_3^- , H_2PO_4^- , HSO_4^- , and AcO^- did not cause any significant changes in their fluorescence emission intensities, even in the presence of 10 equiv of guests (Fig. 3). The fluorescence profiles at $468\ \text{nm}$ showed a higher selectivity for cyanide over other various anions (Fig. 4). Competitive fluorescence experiments also corroborated the selectivity of **1** toward cyanide anions (Fig. S4).

A large fluorescence increase can be applied to the detection of cyanide anions by the naked eye. When the chemodosimeter was excited at $365\ \text{nm}$ in acetonitrile, a bright blue fluorescence was observed only in the solution of **1** with cyanide anions (Fig. 5).

To evaluate the detection limit of cyanide in solution, we have measured the fluorescence changes by increasing the amount of cyanide. The fluorescence intensity of **1** increased almost threefold relative to **1** even in the presence of $3.0\ \mu\text{M}$ of cyanide, indicating the concentration limit of detection (LOD) for cyanide to be less than $3.0\ \mu\text{M}$ (Fig. S5).⁷ This means that our fluorescent chemodos-

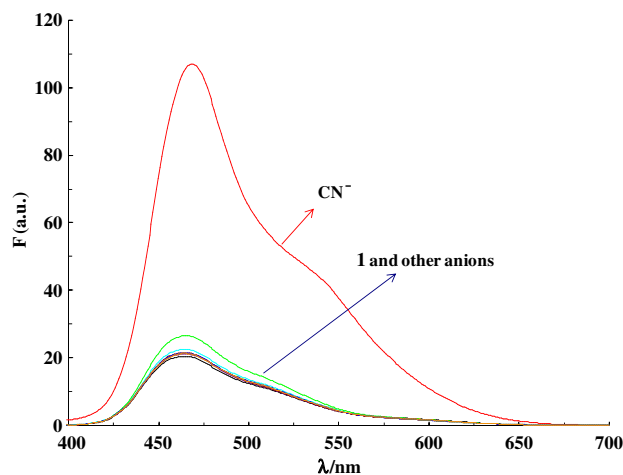


Figure 3. Fluorescence spectra of **1** ($10\ \mu\text{M}$) in CH_3CN after 4 h upon the addition of 10 equiv of anions (CN^- , F^- , Cl^- , Br^- , I^- , N_3^- , H_2PO_4^- , HSO_4^- , AcO^- as *n*-Bu₄N salt).

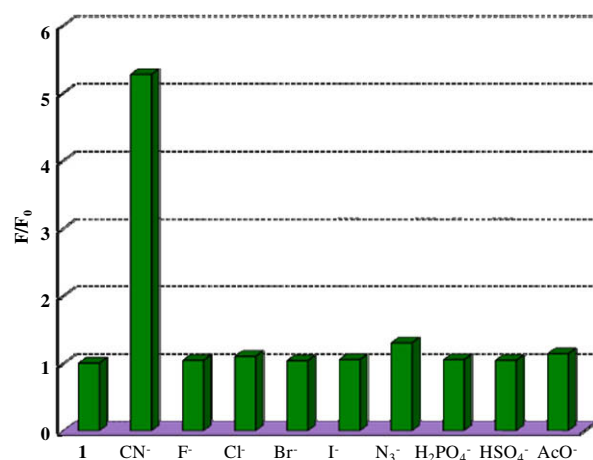


Figure 4. Relative fluorescence intensities of **1** ($10\ \mu\text{M}$ in CH_3CN) in the presence of various anions (4 h, 10 equiv except for 1 equiv of CN^-), where F_0 is the fluorescence intensity exerted by **1** only. $\lambda_{\text{ex}}\ 390\ \text{nm}$, $\lambda_{\text{em}}\ 468\ \text{nm}$.



Figure 5. Fluorescence responses of **1** ($10\ \mu\text{M}$) in the presence of 10 equiv of different anions. (a) only **1**, (b) CN^- , (c) F^- , (d) Cl^- , (e) Br^- , (f) I^- , (g) N_3^- , (h) H_2PO_4^- , (i) HSO_4^- (tetrabutylammonium salt).

imeter is operating well at almost the same levels as the World Health Organization (WHO) cyanide detection standard ($1.9\ \mu\text{M}$)⁸ and is therefore useful to determine the lethal level (ca. $20\ \mu\text{M}$) of cyanide in fire victims.⁹

From the spectroscopic and chromatographic evidence, we propose a plausible mechanism for the Michael acceptor **1** (Fig. 6). Because the unsaturated nitro group of **1** is one of the good Michael acceptors, cyanide can be added to the β - or δ -position of the unsaturated nitro group, where the δ -position is doubly activated

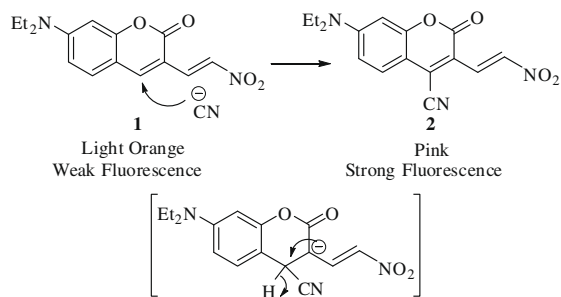


Figure 6. Proposed reaction mechanism of **1** toward CN^- .

by both the nitro and the coumarinyl carbonyl group. The Michael addition to the δ -position of the Michael acceptor and the unexpected hydride removal¹⁰ via $\text{S}_{\text{N}}\text{Ar}^{\text{H}}$ will retrieve the aromatic stability of **1** and lead to a fluorescence change of the fluorophore.

In summary, a chemodosimeter having an α,β -unsaturated nitro group as a Michael acceptor activating unit and a coumarin skeleton as a signaling unit was synthesized to evaluate its fluorescence properties in the presence of anions. The chemodosimeter displayed both colorimetric and fluorometric changes selectively for the cyanide anion over other anions in acetonitrile. The enhanced fluorescence intensity is observed by the nucleophilic aromatic substitution of the cyanide anion toward the doubly activated δ -position of the unsaturated nitro group of **1** and the unexpected hydride removal.

Acknowledgment

We thank the National Research Foundation of Korea (KRF-2008-314-C00206) for financial support.

Supplementary data

Supplementary data (experimental procedures) associated with this paper can be found, in the online version, at [doi:10.1016/j.tetlet.2009.10.113](https://doi.org/10.1016/j.tetlet.2009.10.113).

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- At this time, the mechanism of the hydride removal is not fully understood. Further mechanistic study is still under investigation.