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Colorimetric Detection of Cyanide with a Chromogenic Oxazine

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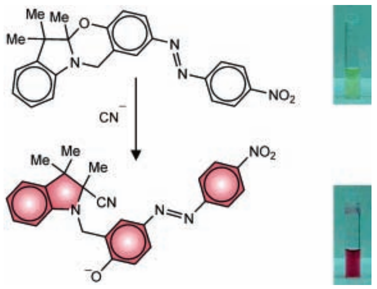
ABSTRACT



We have designed a chromogenic oxazine for the colorimetric detection of cyanide. Indeed, the [1,3]oxazine ring of our compound opens to form a 4-nitrophenylazophenolate chromophore in response to cyanide. This quantitative chromogenic transformation permits the detection of micromolar concentrations of cyanide in water. Furthermore, our chromogenic oxazine is insensitive to the presence of large concentrations of fluoride, chloride, bromide, or iodide anions, which are generally the principal interferents in the colorimetric detection of cyanide.

The ingestion of cyanide has deleterious consequences on cell metabolism.[1] This particular anion has a strong affinity for transition metals and binds efficiently the active site of cytochrome oxidase. As a result, cyanide inhibits the electron-transport chain in mitochondria, and relatively small amounts (0.5−3.5 mg/kg of body weight) of this anion are lethal to humans.[2] Similarly, cyanide concentrations greater than 1.9 μM in drinking water can have long-term or even acute effects.[3] Unfortunately, the reluctance of cyanide to decompose in the environment,[4] coupled to its widespread use in a diversity of industrial processes,[5] can be a serious threat to human health. Indeed, the accidental spillage of cyanide and its intentional release as a fishing agent or even as a warfare agent can ultimately contaminate drinking waters.

Several analytical protocols have been developed for the efficient determination of micromolar amounts of cyanide in water.[6] Some of them are extremely sensitive, as well as specific, but require either multiple experimental steps with tedious sample pretreatments or sophisticated instrumentation. The design of chromogenic chemosensors[7−9] for anion recognition[10−19] would, instead, permit the qualitative, or

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(1) Baskin, S. I.; Brewer, T. G. In *Medical Aspects of Chemical and Biological Warfare*; Sidell, F. R., Takafuji, E. T., Franz, D. R., Eds.; TMM Publications: Washington, 1997; pp 271−286.

(2) Muir, G. D. *Hazards in the Chemical Laboratory*; The Royal Chemical Society: London, 1977.

(3) *Guidelines for Drinking-Water Quality*; World Health Organization: Geneva, 1996.

(4) Baird, C.; Cann, M. *Environmental Chemistry*; Freeman: New York, 2005.

(5) Young, C.; Tidwell, L.; Anderson, C. *Cyanide: Social, Industrial, and Economic Aspects*; Minerals, Metals, and Materials Society: Warrendale, 2001.

(6) Ikebukuro, K.; Nakamusa, H.; Karude, I. In *Handbook of Water Analysis*; Nollet, L. M., Ed.; Marcel Dekker: New York, 2000: Vol. 102, pp 367−385.

(7) Desvergne, J.-P., Czarnik, A. W., Eds.; *Chemosensors of Ion and Molecular Recognition*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1997.

(8) Ricco, A. J., Crooks, R. M. *Acc. Chem. Res.* **1998**, *31*, 199−324.

(9) Ellis, A. B., Walt, D. R. *Chem. Rev.* **2000**, *100*, 2477−2738.

(10) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302−308.

(11) Scheerder, J.; Engbersen, J. F. J.; Reinhoudt, D. N. *Recl. Trav. Chim. Pays. Bas.* **1996**, *115*, 307−320.

(12) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609−1646.

(13) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.

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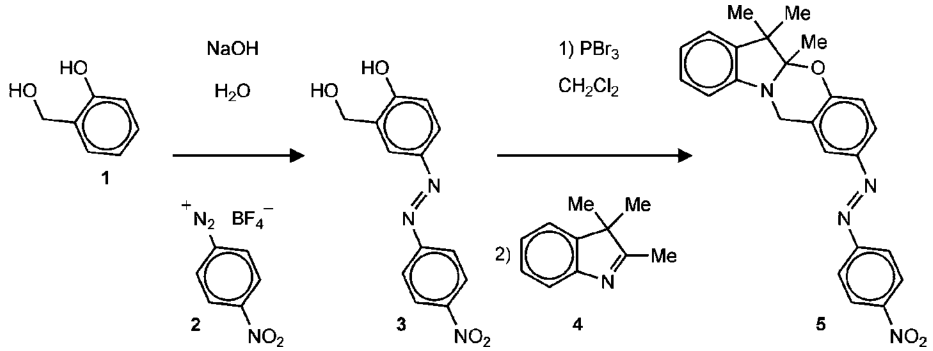

Figure 1. Synthesis of the chromogenic [1,3]oxazine **5**.

even quantitative, determination of this particular analyte with fast and simple colorimetric assays. Indeed, few compounds able to change their ability to absorb and/or emit light in the presence of cyanide have been identified.[20−25] These chemosensors are not particularly sensitive, but their fast chromogenic response can be detected by the naked eye. As a result, they might permit the rapid and preliminary screening of potentially contaminated aqueous solutions. Unfortunately, most of the chromogenic compounds developed so far suffer the interference of other anions. Specifically, the discrimination of cyanide from halides is rather problematic.[20,22b,23]

In the wake of our research program on photochromic switches,[26] we have designed the chromogenic oxazine **5** (Figure 1) for the colorimetric detection of cyanide. We have synthesized this compound in two steps starting from commercial precursors. The first step involves the coupling of 2-hydroxymethylphenol (**1**) with 4-nitrobenzenediazonium tetrafluoroborate (**2**) in alkaline conditions. The second step requires the bromination of the resulting azophenol (**3**), followed by the reaction with 2,3,3′-trimethyl-3*H*-indole (**4**) in situ. The overall yield of the target oxazine **5** is 39%.

In acetonitrile, the treatment of **5** with Bu_4NCN results in the opening of the central [1,3]oxazine ring with the

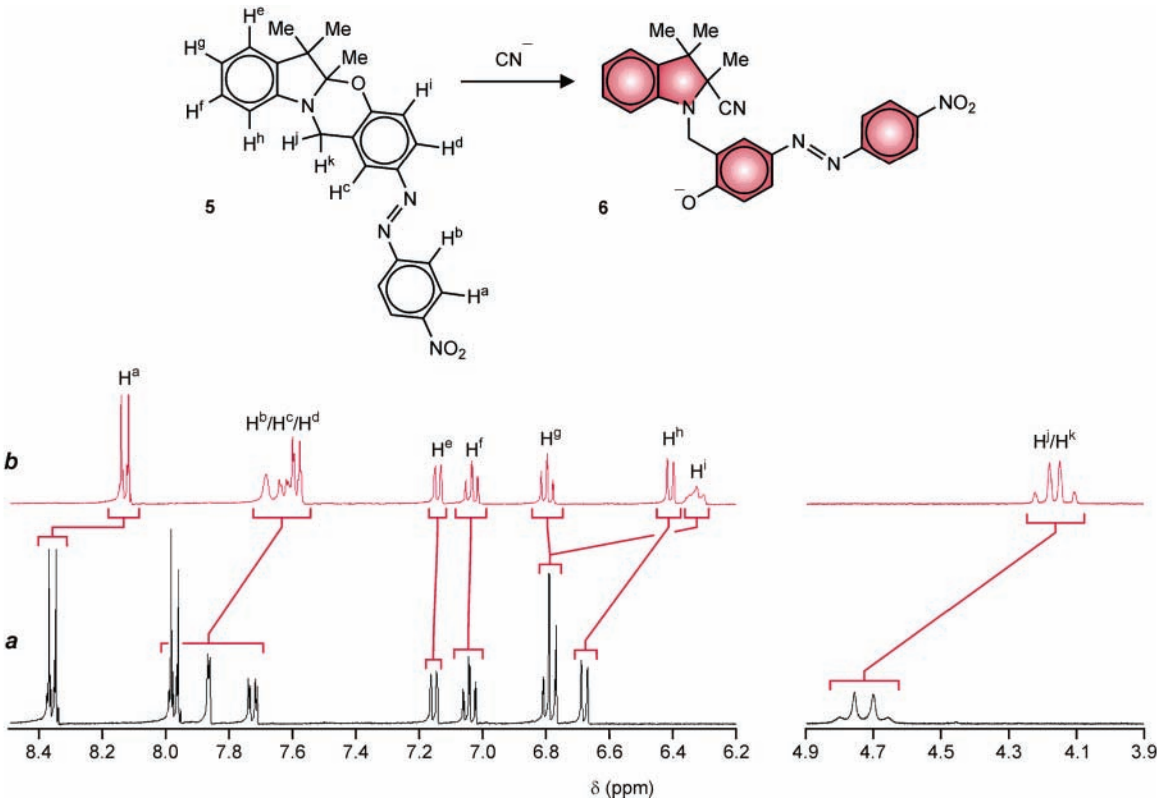

Figure 2. Partial ^1H NMR spectra (400 MHz, 10 mM, acetonitrile-d_3, 20 °C) of **5** before (*a*) and after (*b*) the addition of Bu_4NCN (10 equiv).

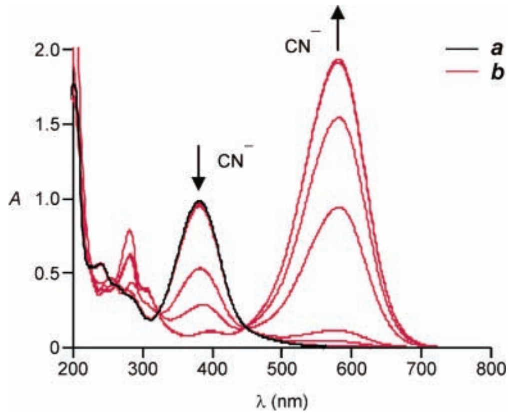

Figure 3. Absorption spectra of a solution of **5** (0.1 mM, MeCN, 20 °C) recorded before (*a*) and after (*b*) the addition of increasing amounts of Bu_4NCN (1−15 000 μM).

quantitative formation of **6** (Figure 2). The ^1H nuclear magnetic resonance (NMR) spectra recorded before (*a* in Figure 2) and after (*b*) the addition of Bu_4NCN confirm the transformation of **5** into **6**. The chemical shifts of most aromatic protons decrease with this process. The largest change (−0.45 ppm) is observed for the set of resonances associated with the proton (H^i) in the *ortho* position relative to the phenolate oxygen atom of **6**. Furthermore, the AB system for the diastereotopic methylene protons (H^j and H^k) is maintained with the conversion of **5** into **6**, confirming that both compounds have a chiral center. However, this particular set of resonances shifts by −0.57 ppm with the formation of **6**. In addition, the fast atom bombardment mass spectrum recorded after the addition of Bu_4NCN shows a peak at *m*/*z* 442 for **6**.

The absorption spectrum of **5** (*a* in Figure 3) shows a band centered at 381 nm. After the addition of increasing amounts of Bu_4NCN, this band fades with the concomitant appearance of a new absorption at 581 nm (*b* in Figure 3), corresponding to the 4-nitrophenylazophenolate chromophore of **6**. In fact, the large molar extinction coefficient of this fragment (ca. 50 mM^{-1} cm^{-1}) coupled to the efficiency of the transformation of **5** into **6** offers the opportunity to detect micromolar amounts of cyanide. Indeed, a Bu_4NCN concentration of 1 μM translates into an absorbance at 581 nm of 0.01 (*b* in Figure 3).

The colorimetric detection of cyanide in water is generally complicated by the interference of halide anions.[20,22b,23] As a result, we have tested the spectroscopic response of acetonitrile solutions of **5** to the addition of aqueous solutions of NaF, NaCl, NaBr, NaI, and NaCN. The absorption spectrum (*a*−*e* in Figure 4) is essentially unaffected by the presence of halide anions, even when their concentration is relatively high (10 mM). The characteristic absorption of **6** can only be observed in the presence of cyanide (*f* in Figure 4). Thus, our chromogenic oxazine responds selectively to cyanide and is virtually insensitive to fluoride, chloride, bromide, and iodide. Furthermore, the transformation of **5** into **6** causes the pronounced changes in color evident in the photographs of Figure 4.

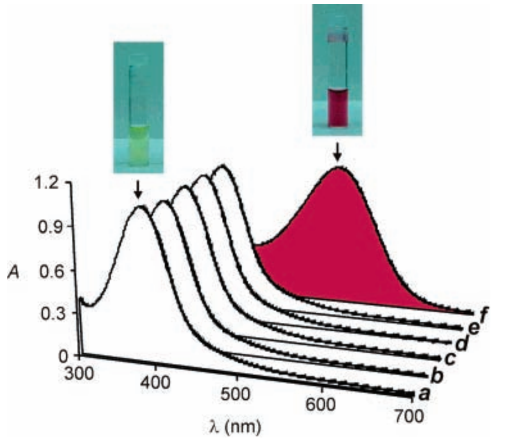

Figure 4. Absorption spectra of a solution of **5** (0.1 mM, MeCN, 550 μL, 20 °C) recorded after the addition of aqueous sodium phosphate buffer (pH = 7.6, 100 μL) without (*a*) and with 10 mM of NaF (*b*), NaCl (*c*), NaBr (*d*), NaI (*e*), or NaCN (*f*). Photographs of the solutions corresponding to spectra *a* and *f*.

The addition of NaCN in neutral buffer to an acetonitrile solution of **5** causes the appearance of the characteristic band of **6** in the absorption spectrum (*f* in Figure 4). However, the cyanide concentration necessary to impose a detectable change on the absorption spectrum is significantly greater than that sufficient to observe similar effects in the absence of water (Figure 3). Presumably, the partial protonation of the phenolate chromophore of **6** is responsible for this behavior. This limitation can be overcome by operating with a two-phase system, a phase transfer catalyst, and higher pH. Specifically, the chromogenic oxazine **5** can be co-dissolved

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(14) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 487−516.

(15) Bianchi, A., Bowman-James, K., Garcia-España, E., Eds.; *Supramolecular Chemistry of Anions*; Wiley-VCH: New York, 1997.

(16) Gale, P. A.; Anzenbacher, P., Jr.; Sessler, J. L. *Coord. Chem. Rev.* **2001**, *222*, 57−102.

(17) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3119−3130.

(18) Martínez-Máñez, R.; Sancerón, F. *Chem. Rev.* **2003**, *103*, 4419−4476.

(19) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192−202.

(20) Miyaji, H.; Sessler, J. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 154−157.

(21) Kim, Y.-H.; Hong, J.-I. *Chem. Commun.* **2002**, 512−513.

(22) (a) Ros-Lis, J. V.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2002**, 2248−2249. (b) Jiménez, D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J. *Tetrahedron Lett.* **2002**, *43*, 2823−2825.

(23) (a) Anzenbacher, P., Jr.; Tyson, D. S.; Jursíková, K.; Castellano, F. N. *J. Am Chem. Soc.* **2002**, *124*, 6232−6233. (b) Anzenbacher, P., Jr.; Jursíková, K.; Aldakov, D.; Marquez, M.; Pohl, R. *Tetrahedron* **2004**, *60*, 11163−11168.

(24) Chow, C.-F.; Lam, M. H. W.; Wong, W.-Y. *Inorg. Chem.* **2004**, *43*, 8387−8393.

(25) (a) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Anal. Chim. Acta* **2004**, *522*, 9−17. (b) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Dyes Pigm.* **2005**, *64*, 49−55. (c) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *J. Am Chem. Soc.* **2005**, *127*, 3635−3641.

(26) Tomasulo, M.; Sortino, S.; Raymo, F. M. *Org. Lett.* **2005**, *7*, 1109−1112.

with Bu_4NCl in dichloroethane. The treatment of the resulting solution with NaCN in sodium phosphate buffer (pH = 9.0) results in the coloration of the organic phase. Indeed, the absorption spectra (*a* and *b* in Figure 5) of the organic phase show the characteristic absorbance of the phenolate chromophore of **6**, even when the cyanide concentration is only 1 μM. In fact, the detection limit of our chromogenic oxazine under these experimental conditions is comparable to those of the best fluorescence chemosensors for cyanide developed so far.[24,25] Furthermore, it is compatible with the maximum acceptable level of cyanide in drinking water, which is 1.9 μM.[3]

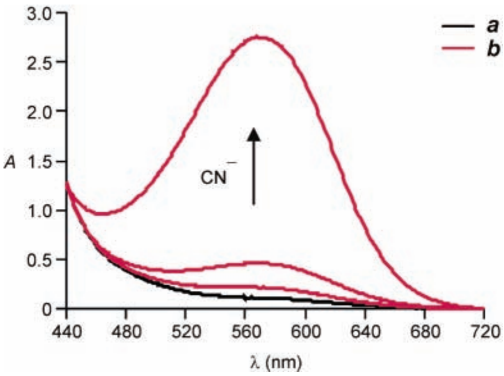

Figure 5. Absorption spectra of a solution of **5** (1 mM, 200 μL, dichloroethane, 20 °C) and Bu_4NCl (0.1 M) after treatment with sodium phosphate buffer (100 μL, pH = 9.0) without (*a*) or with (*b*) increasing amounts of NaCN (1−100 μM) and dilution with dichloroethane (120 μL).

In summary, we have designed a chromogenic oxazine for the detection of micromolar concentrations of cyanide in water. Our compound responds to this particular analyte with the opening of its [1,3]oxazine ring and the formation of a 4-nitrophenylazophenolate chromophore. In fact, this transformation causes drastic changes in color, which can easily be detected by the naked eye. Furthermore, our compound is not affected by the common interferents that usually plague the colorimetric detection of cyanide.

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Supporting Information Available: Experimental procedures for the synthesis of **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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