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The Benzil−Cyanide Reaction and Its Application to the Development of a Selective Cyanide Anion Indicator

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Abstract: The benzil−cyanide reaction is a cyanide-specific reaction that has been exploited to produce a colorimetric indicator for this toxic anion. This was done by producing a π-extended analogue of benzil, **7**, which is soluble in a 70:30 (v/v) mixture of methanol−water. In this medium, dilute solutions of **7** are yellow but produce colorless products when exposed to low concentrations of cyanide anion ($\geq 1.7\ \mu$M; added as an aqueous NaCN solution), but no other common anions (e.g., OH^-, F^-, N_3^-, benzoate$^-$, and $H_2PO_4^-$). On the basis of these observations and supporting mechanistic analyses, it is concluded that the modified benzil system **7** is a promising cyanide anion indicator that is attractive in terms of its selectivity, ease-of-use, water compatibility, and the low, naked-eye discernible cyanide detection limit it provides.

Introduction

Cyanide binds to the ferric form of cytochrome-*c* and inhibits the mitochondrial electron-transport chain.[1] It is thus highly toxic to living creatures. Nonetheless, the use of cyanide salts remains widespread, particularly in gold mining, electroplating, and metallurgy.[2] Despite safeguards and increasing levels of monitoring and control, accidental releases of cyanide into the environment do occur. This can lead to disastrous consequences, as underscored by the large cyanide spill that took place in Romania in 2000, an event that was considered to be the worst environmental disaster in Europe since Chernobyl.[3] There is thus a well-appreciated need for cyanide-selective receptors, sensors, and indicators. Indeed, considerable effort within the anion recognition community has been devoted to preparing such species.[4] However, many of the cyanide anion receptors reported to date have relied on hydrogen-bonding motifs and, as a consequence, have generally displayed poor selectivities relative to other anions.[4] To overcome this limitation, reaction-based receptors, rationally designed cyanide anion indicators, have been developed recently; these include oxazines, such as **1**,[5] cationic borane derivatives, for example, **2**,[6] and acridinium salts such as **3**.[7] Unfortunately, none of these is ideal. In many cases, the mechanistic basis for reaction is not fully established. More importantly, there are practical limitations to their use. For instance, the cationic borane receptor **2**, while very promising in that reaction with cyanide takes place in water, fails to produce a color change, a shortcoming that restricts its utility. Likewise, the oxazine-based indicators required specific biphasic conditions, while the acridinium salts required an elevated reaction temperature. There thus remains a need for yet-improved reaction-based cyanide indicator systems. Recently, we carried out a mechanistic study of the benzil rearrangement reaction in organic solvents and used this analysis to produce a benzil-based indicator, **4**, which could be used to detect the presence of cyanide anion in the absence of water.[8] Further analysis of the underlying chemistry has now led us to produce a modified benzil indicator, **7**, which undergoes a different cyanide-driven reaction, the so-called benzil−cyanide reaction, in aqueous media. As detailed below, this new system can be used to detect the presence of cyanide via a naked-eye discernible color change in 70:30 (v/v) methanol−water at a limit of detection below the maximum permissible level for drinking water set by the World Health Organization (1.9 μM).[9]

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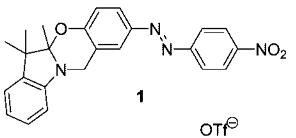

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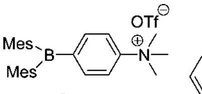

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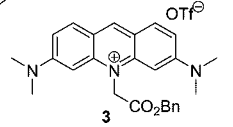

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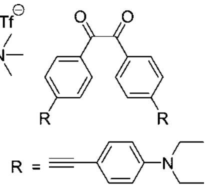

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Results and Discussion

Our recent studies of **4** confirmed that in the absence of water and in the presence of an organic-soluble cyanide anion source, specifically tetrabutylammonium cyanide (TBACN), a benzil rearrangement took place; this produced cyanobenzoyl benzoate in decent yields in aprotic solvents via intermediate **C** (Schemes 1 and 2).[8] This same intermediate is implicated in the venerable benzil−cyanide reaction,[10] kinetic studies of which were reported in 1958.[10a] Cleavage of benzil derivatives according to this latter reaction produces the corresponding benzaldehyde derivatives and benzoate esters in MeOH. Only a catalytic amount of cyanide is required to trigger this reaction, and this can be provided by the use of simple CN$^-$ salts that have been rendered miscible in MeOH via the addition of water. The benzil−cyanide reaction also leads to bond cleavage and thus an obvious change in the π-conjugation pathway of the starting benzil derivative. We therefore considered that the benzil−cyanide reaction would prove superior to the benzyl rearrangement-based process we used previously for cyanide anion detection.[8] In particular, we thought it might allow detection of cyanide in the presence of water, provided a benzil-like system could be produced that would give rise to a detectible color change following cyanide-mediated bond cleavage.

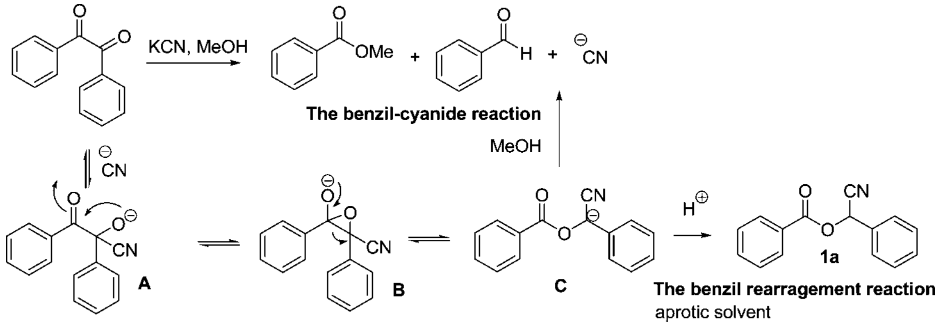
To exploit the benzil−cyanide reaction for the purposes of cyanide indicator development, it is necessary to produce a π-extended benzil derivative that is soluble in MeOH. Unfortunately, the previous indicator (**4**) was designed to be soluble in organic media and proved, as expected, to be insoluble in MeOH. We therefore focused on synthesizing a benzil derivative, the putative reaction-based indicator **7**, which was expected soluble in MeOH and, perhaps, MeOH−water mixtures. It was prepared in 38% yield by subjecting bis(4-ethynylphenyl)ethanedione **5**[11] and 4-iodo-*N*,*N*-bis(2-methoxyethyl)benzenamine **6**[12] to Sonogashira coupling. The unexpected low yield is thought to reflect the fact that intermediate **5** is consumed more rapidly than **6** during the reaction. Consistent with this latter supposition was the finding that the optimized yield of 38% (based on **6**) was only obtained when 1 extra equiv of **5** was used.

The ability of **7** to act as a reaction-based cyanide anion sensor was monitored by UV−vis spectroscopy, as shown in Figures 1 and 2. In the absence of an added anion, the absorption maximum of **7** (2.30×10^{-5} M in 80:20 (v/v) MeOH−water) appears at 410 nm. After the addition of 1 equiv of sodium cyanide and base (10 μM, 1 N aqueous NaOH) to 2 mL of this aqueous methanolic solution of **7**, a large bathochromic shift was observed ($\Delta\lambda_{max} = 43$ nm), with all spectral changes being complete within 10 min with occasional shaking (Figure 1a). The blue shift in the absorption maximum produced as the result of this treatment is reflected in a change in the color of the solution from yellow to colorless (Figure 1b), a feature that allows for facile qualitative and semiquantitative analysis via simple naked-eye inspection.[13]

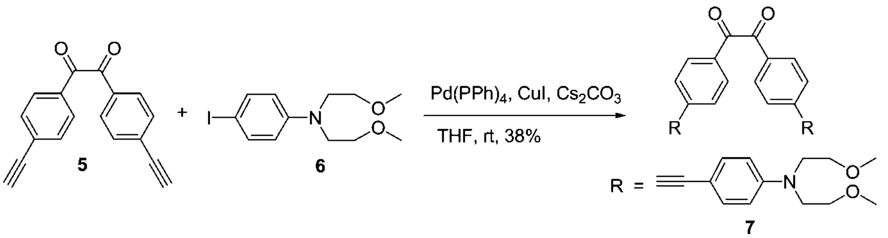
In the absence of added base, the color changes (and presumably the underlying benzil−cyanide reaction) were not complete within 1 h under what were otherwise identical conditions. This observation is consistent with the intuitively reasonable conclusion that intermediate **C** is subject to more rapid solvolysis in a basic medium. However, it is important to appreciate that even in the absence of base, the reaction produces the same final products albeit more slowly. The specific nature of these products will be discussed in context of Figures 3 and 4 below.

Further studies revealed that, in the case of **7**, the rate of the cyanide-promoted reaction is independent of water concentration up to a limit of 30% water v/v, with the solution becoming turbid once a limit of 50% water in MeOH is reached. Using a dilute solution of **7** (7.20×10^{-6} M) in 70:30 (v/v) MeOH−water allows a limit of detection (LoD) of less than 1.7 μM to be

Scheme 1. Proposed Mechanisms of the Benzil−Cyanide Reaction



Scheme 2. Synthetic Scheme



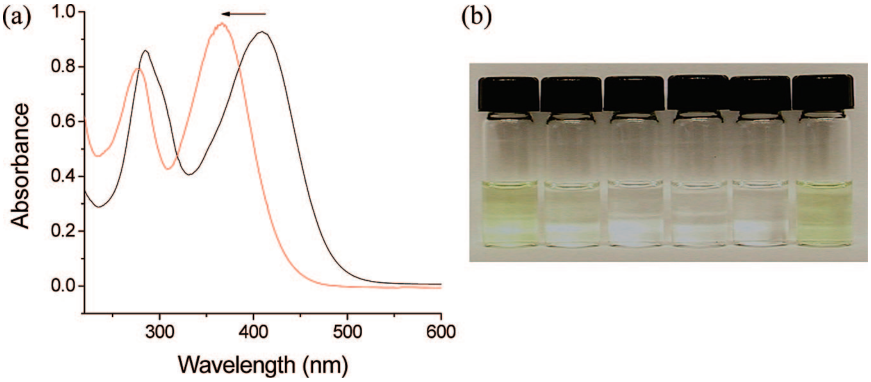


Figure 1. (a) UV–vis spectra of indicator **7** (2.30×10^{-5} in MeOH/water (80:20, v/v)) recorded before and 10 min after the addition of sodium cyanide (NaCN, 1 equiv and 10 μL of 1 N NaOH in water) to the cuvette containing 2 mL of the solution of **7**. (b) Color changes observed 1 h after the addition of varying quantities of NaCN (as 6.95×10^{-4} M stock solution in water) and 1 N NaOH (10 μL in water) to solutions of indicator **7** (7.20×10^{-6} M in MeOH/water (70:30, v/v); initial volume of **7** = 2 mL). From left to right: 0, 1.7, 3.4, 6.8, 13.6, and 0 μM of added [CN$^-$], respectively.

Figure 2. Color changes observed upon the addition of various anions to solutions of indicator **7** (2.30×10^{-5} in MeOH/water (80:20, v/v)) within 10 min. From left to right: **7** only, **7** + NaCN (1 equiv) + NaOH (10 μL of 1 N aqueous NaOH), **7** + NaOH (10 μL of 1 N aqueous NaOH), **7** + TBA·F$^-$ (10 equiv), **7** + TBA·N$_3^-$ (10 equiv), **7** + TBA·BzO$^-$ (10 equiv), **7** + TBA·H$_2$PO$_4^-$ (10 equiv). The initial solution volume of **7** was 2 mL.

attained via simple naked-eye analysis in MeOH/water within 1 h (Figure 1b).

To evaluate the selectivity of indicator **7**, various potentially competing anions, including OH$^-$ (10 μL of 1 N aqueous NaOH), F$^-$ (as the trihydrate), N$_3^-$, BzO$^-$, and H$_2$PO$_4^-$ as TBA salts (10 equiv of each anion was added from a stock solutions of appropriate concentrations made up in MeOH) were added to solutions of **7** (2.30×10^{-5} in MeOH/water (70:20, v/v); initial volume = 2 mL). In no case was a change in color observed. In contrast, cyanide caused a complete loss in color. Such absolute yes/no selectivity is generally not seen in hydrogen-bonding-based receptors and is often precluded in reaction-based indicator systems due to competing reactions (e.g., nucleophilic addition of anions other than cyanide).[14] More importantly, direct anion interference experiments were conducted under the same conditions (i.e., **7**, 2.30×10^{-5} in MeOH/water (70:20, v/v); initial volume = 2 mL) in the presence of other anions, including OH$^-$ (10 μL of 1 N aqueous NaOH), F$^-$ (as the trihydrate), N$_3^-$, BzO$^-$, Cl$^-$, HS$_2$O$_4^-$, and H$_2$PO$_4^-$ as TBA salts (1 equiv of each anion) with or without sodium cyanide (1 equiv). A discernible color change was only seen in the presence of cyanide. Furthermore, this color change was observed without any significant change in the reaction rate. It is particularly noteworthy that the addition of aqueous NaOH did not change the color of the solution even after 12 h, thus ruling out simple deprotonation effects, which are the basis of a number of promising noncyanide anion indicators.[15]

The determinants of the cyanide-mediated cleavage reaction were studied in detail using liquid chromatography–mass spectrometry (LC–MS). The addition of cyanide and an additive (10 μL of aqueous 1 N NaOH) to a 2 mL sample of sensor **7** gives rise to fragments in the LC–MS that are consistent with the formation of benzaldehyde **7a** and benzoate **7b** (Figure 3).

The identity of these peaks was independently confirmed from a small-scale benzil–cyanide reaction carried out in MeOH/CH$_2$Cl$_2$ (2:1, v/v). These solvent conditions were chosen to ensure a homogeneous reaction. The two major compounds were isolated by preparative thin layer chromatography in 97% and 71% yield, respectively (Scheme 3). Standard ^{1}H NMR spectroscopic and mass spectrometric analyses allowed these species to be assigned as **7a** and **7b**, respectively.[16]

With authentic samples of **7a** and **7b** in hand, co-injection experiments, involving of samples obtained under sensing conditions, were carried out. As can be seen from an inspection of Figure 4, LC–MS analysis revealed that the intensity of the left-most peak (only) is increased upon the addition of authentic **7a**, whereas the intensity of the right peak only increases upon

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(16) The fact that compounds **7a** and **7b** were isolated in different relative ratios is ascribed to difficulties in separation, rather than any intrinsic differences in yield (the R_f values of the two products **7a** and **7b** are too close to allow facile separation, even on a preparative silica gel plate).

(17) The extent of the intensity increase was not the same in these two experiments; this was not unexpected because these experiments were only carried out in a qualitative manner without the concentrations of the species involved being rigorously controlled.

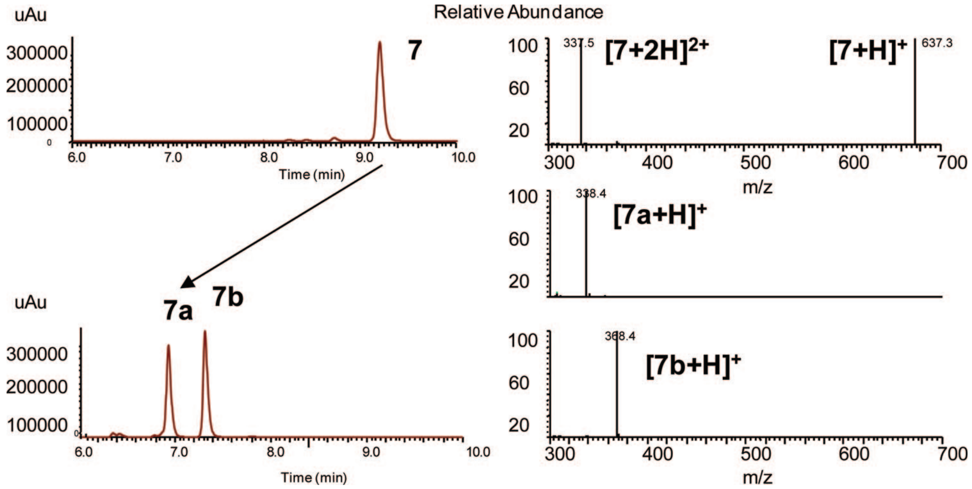


Figure 3. Peaks in the liquid chromatograms that undergo change upon the addition of cyanide anion (1 equiv) and 10 μL of 1 N aqueous NaOH to 2 mL of a solution of indicator **7** (2.30×10^{-5} M in MeOH−water (80:20, v/v)) (left) and their corresponding mass spectra as determined by LC−mass spectrometry (right).

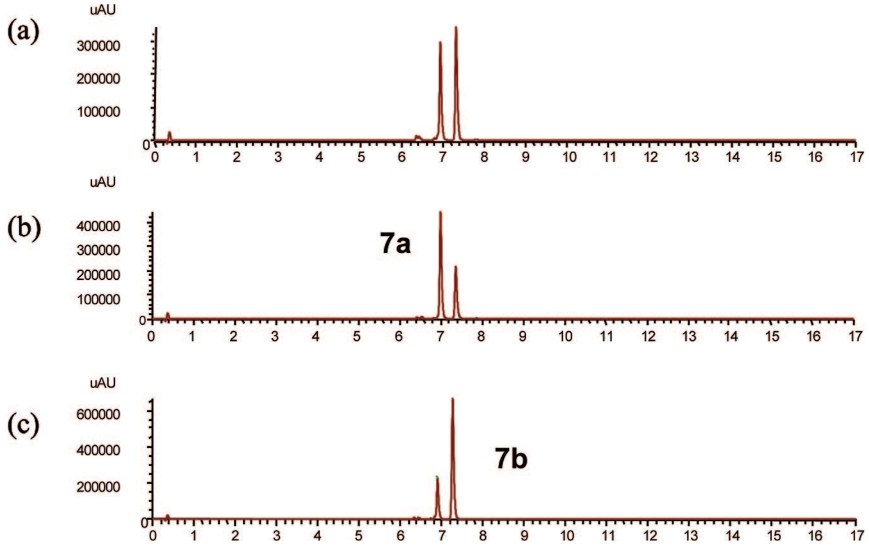
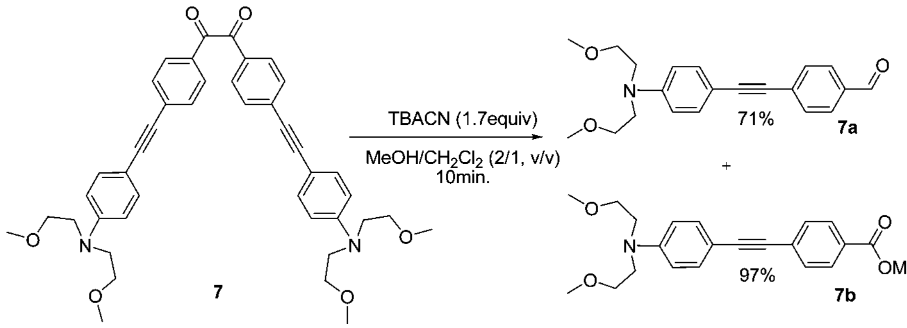


Figure 4. (a) LC profiles obtained from the sample used to produce Figure 3. (b) The sample of experiment (a) after being co-injected with a sample of **7a** produced from an independent synthesis and purification protocol. (c) The sample of experiment (a) after being co-injected with a sample of **7b** produced from an independent synthesis and purification protocol.

Scheme 3. Synthetic Scheme



co-injection of authentic **7b**.[17] Taken in concert, these results are fully consistent with the proposal that **7** undergoes a benzil−cyanide reaction to produce **7a** and **7b** when exposed to small quantities of cyanide anion in aqueous methanol. The rupture of the π-framework that occurs as a result thus provides a mechanistic rationale for the proposed signal-producing process, that initial chromophore-containing solution (pale yellow in color) is transformed into one that lacks any appreciable visible absorption bands (and thus appears clear).

Conclusion

The solubilized benzil derivative **7** was found to act as a highly selective reaction-based colorimetric indicator for cyanide in aqueous methanolic media. The high selectivity for cyanide is thought to reflect the fundamental nature of the benzil—cyanide reaction. Under the reaction conditions used to establish the selectivity and limits of detection, the transformations produced by adding cyanide to aqueous methanolic solutions of **7** do indeed follow the pathway expected for a benzil—cyanide reaction, as established by a combination of LC—mass spectrometric analysis and independent synthesis. Presumably as a consequence, the modified benzil indicator **7** is a highly promising cyanide anion indicator, in terms of ease-of-use, selectivity, tolerance for aqueous environments, as well as visual detection limits. It is expected that appropriate modifications may allow this system to be optimized further, and work along these lines is currently in progress.

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Supporting Information Available: Synthetic experimental and characterization data for compounds **7**, **7a**, and **7b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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