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Proceeding Paper

Chromo-Fluorogenic Detection of Cyanide Ion with a Cyanine Probe [†]

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Abstract: Due to the extreme toxicity of cyanide in biological systems and its widespread presence in the environment, great efforts have been focused on developing sensitive and selective methods for cyanide detection. In this regard, a sulfo-cyanine dye **1** was evaluated as a colorimetric and fluorimetric probe for various anions. Chemosensing studies revealed that this compound exhibited remarkable selectivity and sensitivity for CN^- in acetonitrile solution, with a marked colour change from cyan to colourless and the fluorescence emission was switched off. The detection limit was estimated to be as low as 0.45 μM, and the binding mode was studied using NMR techniques.

Keywords: colorimetric chemosensor; cyanide; cyanine dye; fluorescent probe; naked eye

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1. Introduction

The adverse health effects caused by specific anions underscore the importance of easy and straightforward anion recognition. Excessive levels of anions such as cyanide or nitrate can disturb physiological processes, leading to health problems. For instance, cyanide ions (CN^-) are highly toxic because of their ability to bind to the ferric form of cytochrome-c in the mitochondrial electron transport chain. This binding disrupts the production of adenosine triphosphate (ATP) and thereby inhibits the cellular respiration. However, cyanide is widely used in a variety of industrial processes, including in paper, textiles, and plastics manufacturing methods, as well as in metallurgy for electroplating and gold and silver extraction [1,2]. Therefore, the selective and sensitive detection of cyanide ions is essential for human well-being, environmental preservation, and the advancement of scientific and medical knowledge in the context of cyanide exposure.

Nowadays, optical chemosensors designed for anion detection are highly attractive for biological and environmental applications because of their simplicity, cost-effectiveness, real-time response, and ease of detection through optical changes (colour and/or fluorescence), without requiring advanced equipment [2–4]. This provides reliable quantitative and qualitative analyses for a variety of analytes. Over the past years, various promising optical chemosensors have been developed for sensing cyanide ions [2,4–8]. Among these probes, cyanine dyes offer numerous advantages, including high sensitivity, tunable optical properties, and biocompatibility, making them valuable tools in analytical chemistry and biosensing applications [9].

Following our previous work focused on the synthesis of a sulfo-cyanine dye and its evaluation as an optical chemosensor for cations [10,11], we now report its chromo-fluorogenic ability for anion detection in acetonitrile solution. Therefore, the interaction of this compound with several anions was evaluated by preliminary chemosensing studies, followed by UV/Vis, fluorescence and NMR titrations.

2. Experimental Section

2.1. Instruments and Materials

UV/Vis absorption spectra were obtained using a Shimadzu UV/2501PC spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany) and fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer (HORIBA Europe GmbH, Darmstadt, Germany) in standard quartz cuvettes with 1 cm optical path. NMR spectra were recorded on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C, using the solvent peak as the internal reference at 25 °C; the chemical shift values (δ relative to TMS) are given in ppm. All commercially available reagents were used as received. The synthesis and structural characterization of dye **1** in MeOH-d_4 has been reported by us elsewhere [10]. In this report, NMR spectra were obtained in DMSO-d_6.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.30–1.38 (2H, m, CH_2-c), 1.45–1.53 (2H, m, CH_2-b), 1.65–1.70 (14H, m, CH_2-d and 4xCH_3), 1.92 (2H, t, *J* = 7.2 Hz, CH_2-e), 3.58 (1H, s, NCH_3), 4.06 (2H, t, *J* = 7.2 Hz, CH_2-a); 6.25 (1H, d, *J* = 14.4 Hz, H-α or H-α'), 6.29 (1H, d, *J* = 14.4 Hz, H-α or H-α'), 6.57 (1H, t, *J* = 12.4 Hz, H-γ), 7.29 (2H, d, *J* = 8.0 Hz, H-7 and H-7′), 7.62 (2H, dd, *J* = 1.6 and 8.0 Hz, H-6 and H-6′), 7.79 (2H, d, *J* = 1.2 Hz, H-4 and H-4′), 8.34 (2H, t, *J* = 13.2 Hz, H-β and H-β') ppm.

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 25.66 (CH_2-b), 26.26 (CH_2-c), 26.83 (CH_2-d), 26.93 (2xCH_3), 27.09 (2xCH_3), 31.19 (NCH_3), 37.07 (CH_2-e), 43.52 (CH_2-a), 48.81 (C-3 or C-3′), 48.85 (C-3 or C-3′), 103.36 (C-α or C-α'), 103.59 (C-α or C-α'),110.00 (C-7 or C-7′), 110.11 (C-7 or C-7′), 119.81 (C-4 or C-4′), 119.90 (C-4 or C-4′), 125.68 (C-γ), 125.96 (C-6 or C-6′), 126.07 (C-6 or C-6′), 140.37 (C-3a or C-3a′), 140.48 (C-3a or C-3a′),142.00 (C-7a′), 142.72 (C-7a), 145.16 (C-5 or C-5′), 145.25 (C-5 or C-5′), 154.13 (C-β or C-β'), 154.17 (C-β or C-β'), 172.86 (C-2′), 173.57 (C-2), 174.51 (C=O) ppm.

2.2. Stock Solutions

Evaluation of dye **1** as a colorimetric and fluorimetric chemosensor was carried out in the presence of various anions (F^-, I^-, Br^-, CN^-, ClO_4^-, HSO_4^-, NO_3^-, BzO^-, $H_2PO_4^-$, CH_3COO^-) in the form of hydrated tetrabutylammonium salts. Solutions of dye **1** (*ca.* 1.0×10^{-5} to 5.0×10^{-6} M) and the selected anions (1.0×10^{-2}) were prepared in UV-grade acetonitrile.

2.3. Preliminary Chemosensing Studies and Titrations

Preliminary chemosensing studies were performed by the addition of 20 equivalents of each ion to the solution of compound **1**. Spectrophotometric and spectrofluorimetric titrations were carried out by the sequential addition of the ion to the solution of **1** (3 mL), and the absorption and fluorescence spectra were measured until reaching the maximum optical change. Emission scans were recorded by excitation at 590 nm, with a 3 nm slit. NMR titrations were carried out by a similar gradual addition of the ion (6.0×10^{-1} M) to the solution of **1** (2.0×10^{-2} M) in DMSO-d_6 at 25 °C.

2.4. Determination of the Detection Limit (DL)

The detection limit (DL) was calculated using the equation $DL = 3\sigma/S$, where σ is the standard deviation of the fluorescent intensity of the analyte free solution and S is the slope of the linear plot of concentration-dependent fluorescence response [12].

3. Results and Discussion

3.1. Preliminary Chemosensing Studies

In our previous work, we reported the synthesis and photophysical characterization of sulfo-cyanine **1** (Figure 1), as well as its evaluation as an efficient colorimetric probe for cations, especially for Cu^{2+} and Fe^{3+} [10,11]. Here, we study the sensing ability of this compound for different anions with environmental and medicinal relevance.

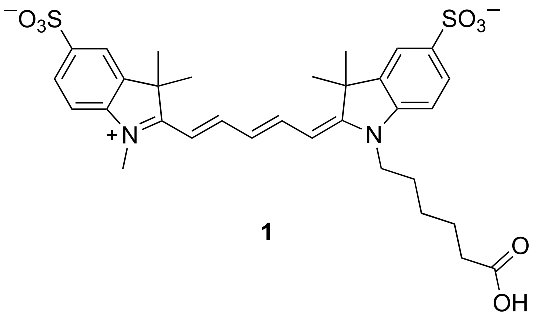

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Figure 1. Structure of probe **1**.

Firstly, the selectivity of probe **1** towards the selected anions was evaluated by naked-eye perceivable colour changes, through the addition of 20 equivalents of each anion to the solution of **1**. This compound displayed a selective response in the presence of CN^-, showing a remarkable colour change from cyan to colourless (Figure 2). No obvious colorimetric change was observed upon addition of the other ions to the solution.

Furthermore, the fluorimetric behaviour of probe **1** was also studied by recording the fluorescence intensity of **1** before and after the addition of the selected anions. It was found that probe **1** exhibited a complete quenching of fluorescence upon interaction with CN^-, while other ions did not induce relevant changes (Figure 3).

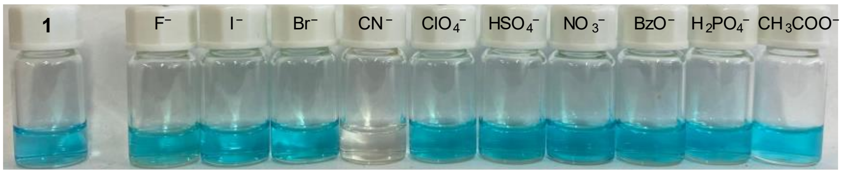

Figure 2. Colorimetric responses of probe **1** in acetonitrile solution (1.0×10^{-5} M) before and after the addition of 20 equiv. of various anions.

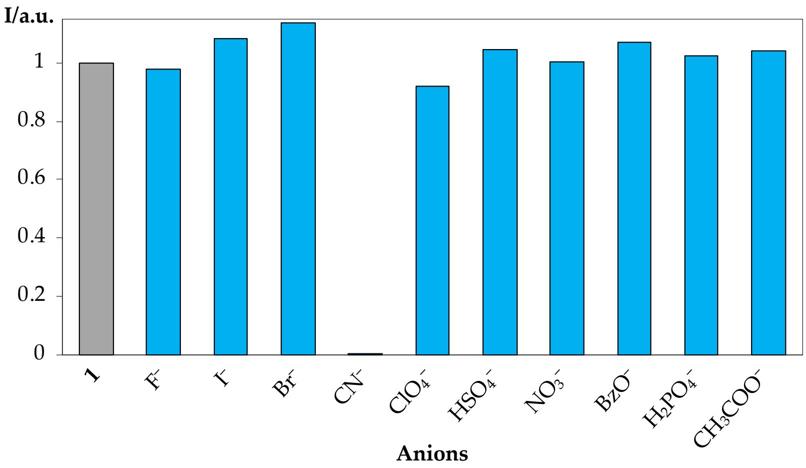

Figure 3. Normalized fluorescence responses of probe **1** at 672 nm (λ_{exc} = 590 nm) in acetonitrile solution (5.0×10^{-6} M) before and after the addition of 20 equiv. of various anions.

3.2. Spectrophotometric and Spectrofluorimetric Titrations

Considering the most promising result in both colorimetric and fluorescence preliminary chemosensing studies, the interaction of probe **1** with CN^- was evaluated by spectrophotometric and spectrofluorimetric titrations in acetonitrile solution. The ion-free solution showed an intense absorption band at 651 nm (ε_{max} = 13 6304 M^{-1} cm^{-1}) and a strong fluorescence intensity at 672 nm (Figure 4).

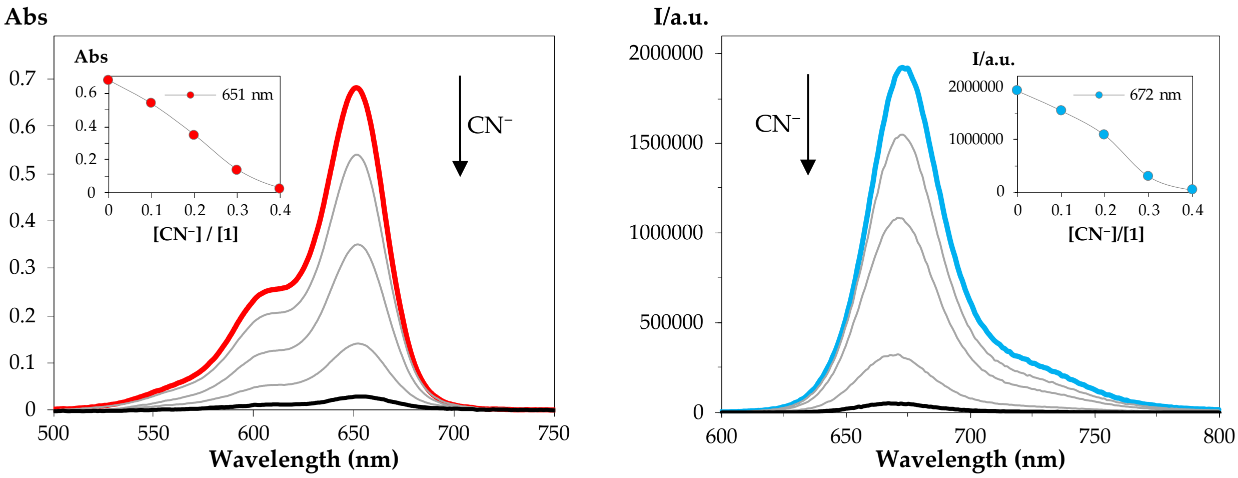

Figure 4. Spectrophotometric and spectrofluorimetric of probe **1** with CN^- in acetonitrile solution: [**1**] = 5.0×10^{-6} M. Inset: absorption at 651 nm and emission at 672 nm as a function of added anion equivalents.

It was found that probe **1** exhibited a decrease in absorbance and fluorescence intensity in response to increasing amounts of cyanide. Notably, the sensitivity of **1** towards CN^- was evident, as the addition of a low number of equivalents (0.4 equiv.) was enough to cancel the absorbance and for the complete quenching of fluorescence. The molar extinction coefficient for the complex **1**-CN^- (651 nm) was found to be $\varepsilon_{max} = 1544\ M^{-1}\ cm^{-1}$).

Based on the fluorimetric titration, the detection limit (DL) of **1** for CN^- was calculated by DL = $3\sigma/S$ method [12]. The DL for CN^- was found to be 0.45 μM, which is lower than the WHO limit for cyanide in drinking water (1.9 μM) [13]. Overall, the results suggest that compound **1** could be used as an efficient chromofluorescent probe for CN^- with high sensitivity in acetonitrile solution.

3.3. NMR Studies

The mode of complexation of probe **1** with CN^- was further investigated by ^1H NMR titrations. Upon addition of cyanide ions, the aromatic and vinyl protons attributed to probe **1** disappeared, while a set of new signals appeared (Figure 5a). This result is close to those described by Qiu et al. [7], suggesting that the complex Cy5-CN is likely a product of a nucleophilic addition. In this reaction, CN^- acted as a nucleophile in the nucleophilic addition at one of the iminium carbons of the indolium salts of Cy5, as strongly indicated by the shifts observed for protons adjacent to the nitrogen atoms. Indeed, comparison of ^{13}C NMR spectrum of probe **1** with ^{13}C NMR and DEPT 135 spectra of probe **1** with 0.5 equiv. of CN^- confirms the presence of new quaternary carbon signals at 80.04 and 117.62 ppm which could be ascribed to the cyano group carbon and C2, respectively (Figure 5b). These results suggest the formation of a new species, such as **2** (Figure 6).

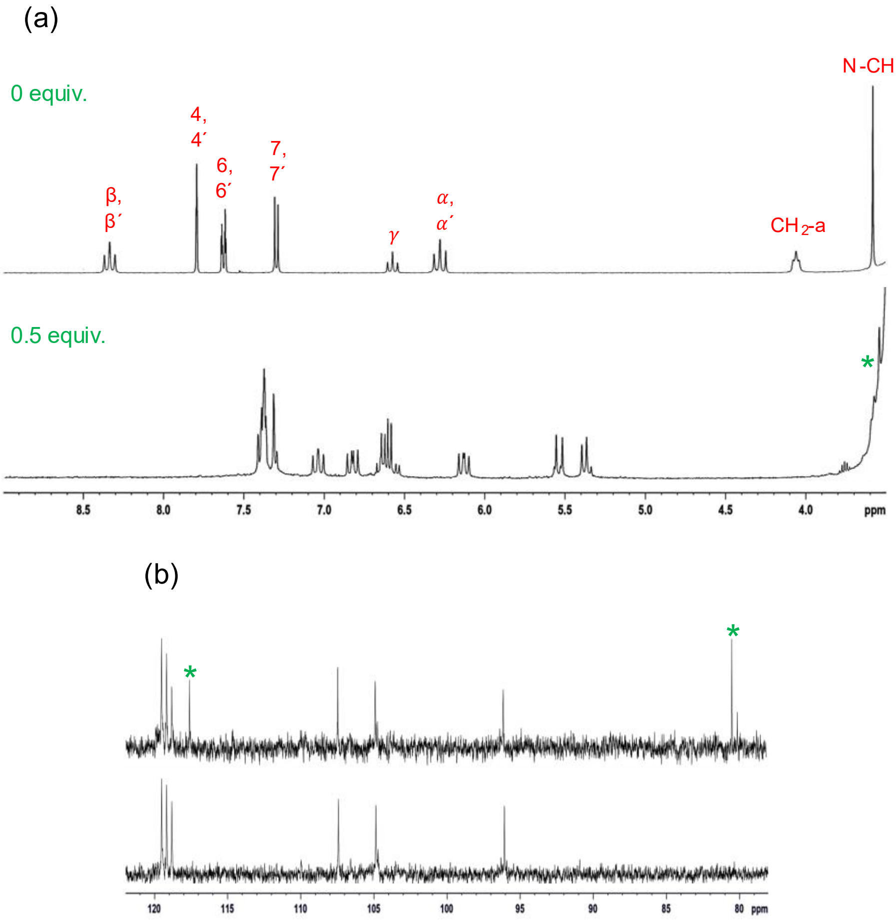

Figure 5. NMR studies: (**a**) partial ^1H NMR spectra of **1** before and after the addition of 0.5 equiv. of CN^- (* overlap of water signal in the DMSO-d_6 solution); (**b**) partial ^{13}C NMR (top) and DEPT 135 (bottom) of **1** with CN^- (0.5 equiv.) in DMSO-d_6 (* indicates new cyano group carbon and C2).

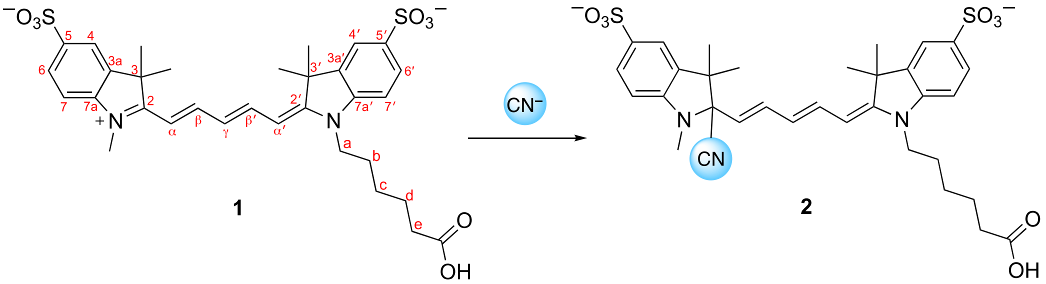

Figure 6. Suggested structure for species **2**.

4. Conclusions

In summary, we have reported a cyanine dye-based chromofluorescent chemosensor, which exhibited various advantageous properties, including a high molar absorption coefficient at 651 nm (ε_{max} = 136,304 M^{-1} cm^{-1}); a selective and sensitive response towards CN^-, showing a remarkable colour change from cyan to colourless and a complete quenching of fluorescence with a low detection limit (0.45 µM). NMR studies suggest a mechanism involving nucleophilic addition of cyanide to the iminium carbon. Therefore, probe **1** could be used to detect cyanide ions qualitatively (naked eye) and quantitatively (UV-Vis and fluorescence spectroscopy) with high sensitivity in acetonitrile solution.

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