

Synthesis and Evaluation of an Azo Dye for the Chromogenic Detection of Metal Cations †

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Abstract: A carboxylic acid azo dye, DabcyI, was synthesized and evaluated as a colorimetric chemosensor for metal cations with biological and environmental importance. The dye was prepared in high yield and characterized by ^1H and ^{13}C NMR and UV-vis absorption spectroscopies. A preliminary chemosensing study showed that DabcyI displayed a marked color change, from light yellow to pink, for Hg^{2+} , Fe^{2+} , Pd^{2+} , Sn^{2+} and Al^{3+} in acetonitrile solution. Consequently, spectrophotometric titrations were carried out for this dye with selected cations, which clearly indicated that DabcyI has potential application as a chromogenic probe for the cations under study with remarkable sensitivity and a marked color change.

Keywords: azo dyes; colorimetric chemosensor; DabcyI; metal cations; naked eye detection

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1. Introduction

Great efforts have been focused on developing selective and sensitive signaling probes for detecting metal ions because they are indispensable in most biological processes, but in abnormal levels these analytes may cause severe effects on human health and the environment [1–3].

Mercury is considered as one of the most dangerous metal cations in the ecosystem due to its well-known accumulative and toxic features, being listed as a persistent, bioaccumulative and toxic (PBT) substance [4,5]. Tin is an important vital trace mineral for human body, which is crucial for many tissues and organs. However, excess accumulation of tin may seriously interfere with respiratory, digestive and nervous system and in the environment may result from the degradation pathway of another PBT, tributyltin chloride [6,7]. Aluminium is extensively released in the environment owing to its wide application in human's daily life. An excess amount of Al^{3+} ions can damage the human nervous system and is closely related to many diseases such as Alzheimer's disease, Parkinson's disease, anemia, and gastrointestinal diseases [8,9]. Therefore, the development of chemosensors capable of accurately and efficiently detecting biologically and environmentally important metal ions is essential.

Among optical chemosensors, azo dyes are widely used as chromogenic probes due to their ease of synthesis, structural variability and tunability of color detection with naked eye [10,11]. A very popular azo compound is 4-[[4'-(*N,N*-dimethylamino)phenyl]diazanyl]benzoic acid, known as DabcyI, a push-pull azobenzene functionalized at the *para* positions of the phenyl rings [12]. Besides its biomolecular applications as a dark quencher in FRET-based probes, DabcyI has also been used as a precursor in colorimetric chemosensors for cation recognition [13,14].

Following the research group's interest on the synthesis and evaluation of optical chemosensors for various ions [15–18], we report herein the synthesis of DabcyI as well as

its evaluation as a potential colorimetric chemosensor for metal cations, through chemosensing studies in the presence of several cations in acetonitrile solutions.

2. Experimental Section

2.1. Instruments and Materials

Nuclear Magnetic Resonance (NMR) spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^1H and 100.6 MHz for ^{13}C using the solvent peak as an internal reference. The solvents are indicated in parentheses before the chemical shift values (δ relative to tetramethylsilane (TMS) and given in ppm). Assignments were supported by two-dimensional heteronuclear correlation techniques. Thin-layer chromatography (TLC) analyses were carried out on 0.25 mm-thick silica plates coated with fluorescent indicator F₂₅₄ (Merck KGaA, Darmstadt, Germany) and spots were visualized in a CN15 viewing cabinet under UV lamp at 365 nm (Vilber Lourmat, Marne-la-Vallée, France). UV/Vis absorption spectra were obtained using a Shimadzu UV/2501PC spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany). All reagents were purchased from Acros Organics (Geel, Belgium) and Sigma-Aldrich (St. Louis, MO, USA), and used as received.

2.2. Synthesis of Dabcyl 2

A mixture of 4-aminobenzoic acid (0.500 g, 1 equiv.), HCl 1 M (7.5 mL) and HCl 6 M (0.42 mL) was cooled to 0–5 °C. Aqueous NaNO₂ (0.251 g, 1 equiv., in 1 mL of water) was added and the reaction mixture was stirred for 30 min. The diazonium salt solution previously prepared was added drop wise to a solution of *N,N*-dimethylaniline (0.46 mL, 1 equiv.) in AcOH (1.25 mL) and H₂O (0.83 mL). The pH was adjusted to 4 with a saturated aqueous solution of sodium acetate, whereupon precipitation occurred. The precipitated dye was filtered, washed with water and diethyl ether, and dried at 40 °C. Compound **2** (Figure 1) was obtained as a dark red solid without additional purification (0.892 g, 91%).

^1H NMR (400 MHz, DMSO-*d*₆): δ = 3.06 (6H, s, 2xCH₃), 6.83 (2H, dd, J = 2 and 7.2 Hz, H-3' and H-5'), 7.81 (4H, dd, J = 2.8 Hz and 9.2 Hz, H-3, H-5, H-2' and H-6'), 8.05 (2H, dd, J = 2.0 Hz and 7.2 Hz, H-2 and H-6) ppm.

^{13}C NMR (100.6 MHz, DMSO-*d*₆): δ = 39.96 (2xCH₃), 111.74 (C3' + C5'), 121.85 (C3 + C5), 125.42 (C2' + C6'), 130.65 (C2 + C6), 131.13 (C1), 142.78 (C1'), 153.15 (C4'), 155.15 (C4), 167.12 (C=O) ppm.

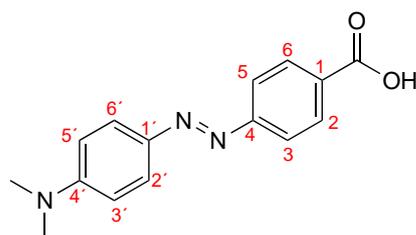


Figure 1. Structure of Dabcyl 2.

2.3. Preliminary Chemosensing Studies and Spectrophotometric Titrations of Dabcyl 2

Evaluation of Dabcyl **2** as a colorimetric chemosensor was performed in the presence of several cations (Ag^+ , K^+ , Li^+ , Na^+ , Cu^+ , TBT^+ , Hg^{2+} , Ca^{2+} , Co^{2+} , Pb^{2+} , Mn^{2+} , Fe^{2+} , Zn^{2+} , Ni^{2+} , Cd^{2+} , Cu^{2+} , Pd^{2+} , Cs^{2+} , Sn^{2+} , Fe^{3+} and Al^{3+}). Solutions of compound **2** and solutions of these cations were prepared in UV-grade acetonitrile.

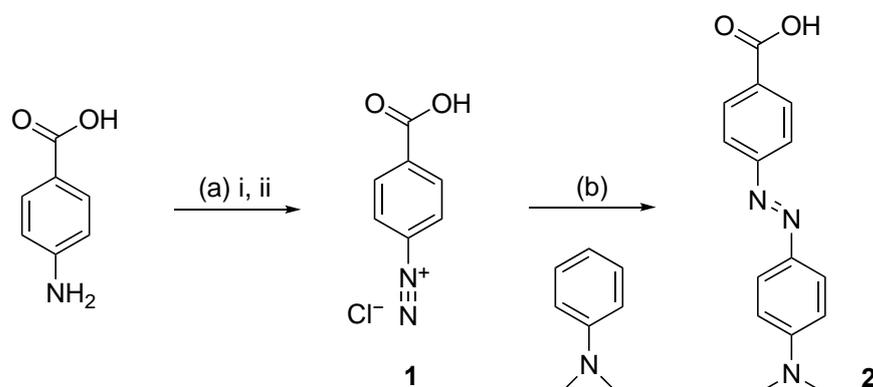
A preliminary study was carried out by addition of 20 equivalents of each cation (1×10^{-2} M) to the solution (1 mL, 1×10^{-5} M) of dye **2** and the assessment of the color was evaluated by naked eye. Given this preliminary result, spectrophotometric titrations of Dabcyl **2** with selected cations were performed by the sequential addition of each cation

(5×10^{-3} M) to the solution (3 mL, 1×10^{-5} M) of dye **2**, and the absorption spectra were collected until the absorbance plateau was reached.

3. Results and Discussion

3.1. Synthesis and Characterization of Dabcyl **2**

The dye was prepared by an azo coupling reaction between an aryl diazonium salt and an activated electron-rich aromatic component (Scheme 1). Firstly, the diazotization of 4-aminobenzoic acid using sodium nitrite as nitrosating agent resulted in the diazonium cation **1**, which was not isolated. Then, the SEAr-type reaction of the diazonium salt **1** with *N,N*-dimethylaniline gave Dabcyl **2** with an excellent yield (91%), without additional purification. The synthesized compound was characterized by ^1H and ^{13}C NMR spectroscopy, and the obtained data was in agreement with the expected structure.



Scheme 1. Synthesis of Dabcyl **2**: (a) (i) HCl 1 M, HCl 6 M, r.t.; (ii) NaNO_2 , H_2O , 0°C , 30 min (b) AcOH, H_2O , r.t.

The photophysical properties of Dabcyl **2** were analyzed in acetonitrile solution (1×10^{-5} M). The compound exhibited a large UV-vis absorbance band with a high molar absorption coefficient ($\log \epsilon = 4.49$) at 439 nm. This is a typical feature of azobenzene derivatives related to their significant number of vibronic states in each energy levels S_n [19]. Furthermore, because of the isomerization of their azo bridge, either through a rotation mechanism around the $-\text{N}=\text{N}-$ double bond or an inversion mechanism upon UV-vis irradiation, these dyes are usually weakly or non-fluorescent compounds. Hence, the fluorescence properties of Dabcyl have not been studied in this work [19,20].

3.2. Preliminary Chemosensing Studies of Dabcyl **2**

A preliminary evaluation of Dabcyl **2** as a colorimetric chemosensor was performed in acetonitrile solution in the presence of various cations. The study was performed by addition of 20 equivalents of each cation to the compound's solution (Figure 2). The chromogenic response of the compound **2** was remarkably visible to the naked eye, with a color change from light yellow to pink in the presence of Hg^{2+} , Fe^{2+} , Pd^{2+} , Sn^{2+} and Al^{3+} .



Figure 2. Color changes observed for Dabcyl **2** in acetonitrile solution after addition of 20 equivalents of each cation.

3.3. Spectrophotometric Titrations of Dabcyll 2

Considering the preliminary results and the effects above-mentioned of Hg^{2+} , Sn^{2+} and Al^{3+} on human health and the environment, the sensing properties of Dabcyll 2 towards these cations have been examined in more detail using UV–Vis spectroscopy in acetonitrile solution. The increase in the ratio of these cations led to the gradual disappearance of the absorption band at 439 nm and the appearance of a new peak at 510 nm, with an isosbestic point at 465 nm (Figure 3). The significant red shift in the UV–vis spectra ($\Delta\lambda = 71$ nm) resulted in a color change from light yellow to pink, which can be easily detected by the naked eye. Furthermore, the sensitivity of Dabcyll 2 towards these cations was evident, since the addition of only 1.5 equivalents of Al^{3+} was necessary to reach the absorbance plateau, while the interaction with Hg^{2+} and Sn^{2+} required a higher number of equivalents to achieve the plateau, 6 and 7 equivalents, respectively. Therefore, Dabcyll 2 can be used to detect these cations with remarkable sensitivity.

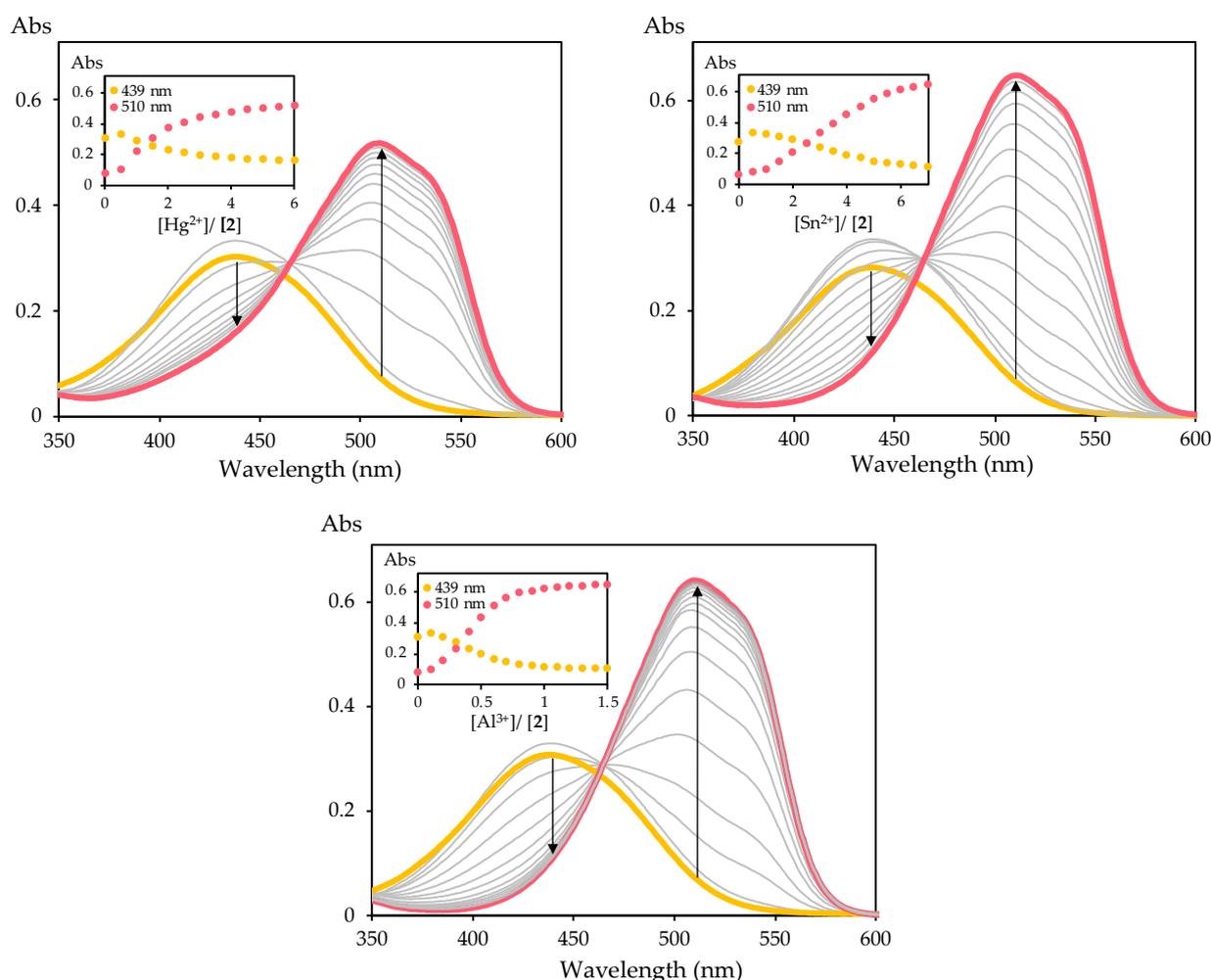


Figure 3. Spectrophotometric titration of Dabcyll 2 with addition of increasing amounts of Hg^{2+} , Sn^{2+} and Al^{3+} in acetonitrile. The inset represents the normalized absorption ($[\text{2}] = 1 \times 10^{-5}$ M).

4. Conclusions

This work reports the synthesis and evaluation of Dabcyll acid 2 as a colorimetric chemosensor for metal cations. This compound had a colorimetric response for different cations in acetonitrile with a marked color change, from light yellow to pink, in the presence of Hg^{2+} , Fe^{2+} , Pd^{2+} , Sn^{2+} and Al^{3+} . Moreover, the sensing properties of Dabcyll 2 with selected cations were analyzed by UV–Vis spectroscopy in acetonitrile solution, confirming the sensitivity of this dye towards Hg^{2+} , Sn^{2+} and Al^{3+} . Therefore, Dabcyll 2 could be a

sensitive potential colorimetric probe for the detection of these analytes. In the near future, further studies will be performed in water solutions to determine the potential application of Dabcyl as a chromogenic probe for cations in environmental and biological samples.

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