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A colorimetric and fluorescent turn-on chemosensor for Al^{3+} and its application in bioimaging

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ABSTRACT

The sensing properties of a boron dipyrromethene derivative **1** containing a *N*,*N*-(dimethylamino)styryl group at its α-position and an aniline moiety at *meso*-position were investigated by steady-state UV–vis absorption and fluorescence spectroscopy, which were found to exhibit wavelength ratiometric and large fluorescence enhancement in the presence of Al^{3+} with specific selectivity over other metal ions in aqueous media. Furthermore, confocal fluorescence microscopy experiments demonstrated that **1** could be used as a fluorescent probe for Al^{3+} in living cells.

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Chemosensors that offer easily detectable signals upon recognition metal ions with high sensitivity and selectivity have received considerable attention in the recent years.[1] Wavelength ratiometric and fluorescence turn-on probe combines the sensitivity of fluorescence with the convenience of a colorimetric assay.[2] In particular, ratiometric measurements have the important features of signal rationing, and thus increase the dynamic range and provide built-in correction for environmental effects.[3]

The development of chemosensors for the facile detection of Al^{3+} is of great importance because its toxicity not only hampers plant performance,[4] killing fish in acidified waters[5] but also damages the central nervous system to cause human illnesses like dementia and encephalopathy,[6] Parkinson's disease,[7] and Alzheimer's disease.[8] Recently a few Al^{3+}-responsive fluorescent sensors have been reported,[9] including a dual-channel fluorescence-enhanced Al^{3+} sensor,[9a] and a reversible photo-driven sensor based on photochromic spiropyran.[9b] However, sensing materials for Al^{3+} detection in aqueous media is still rare and there is no report on the detection of Al^{3+} in living cells. Therefore, the design of new probe for Al^{3+} which functions in aqueous media with a high selectivity remains highly desirable for environment and biological studies. Herein, we reported the sensing properties of a boradiaza-indacene (BDP) derivative, **1**[10] as a ratiometric and fluorescent turn-on sensor for Al^{3+} ion in aqueous media and in living cells. BDP derivatives have been extensively investigated as fluorescent switches and as probes for H^+,[11] Ca^{2+},[12] Cd^{2+},[13] Cu^+,[14] Cu^{2+},[15] Hg^{2+},[16] Mg^{2+},[17] and K^+[18] ions owing to their excellent photophysical properties.[19] It is found serendipitously that addition of Al^{3+} to our previously reported compound **1** resulted in a significant color change and concomitant fluorescent enhancement by the inhibition of both photoinduced electron transfer (PET) and internal charge transfer (ICT) quenching processes from the electron-donating dimethylamino substituents on **1** (Scheme 1).

To avoid the interference of H^+, a 6% HEPES buffer in MeCN (v/v, pH 7.0) was used for the spectroscopic investigations. The changes in absorption spectra during the Al^{3+} titration are shown in Figure 1. Compound **1** showed a typical BDP absorption band at 595 nm, which was about 100 nm red shifted compared to the

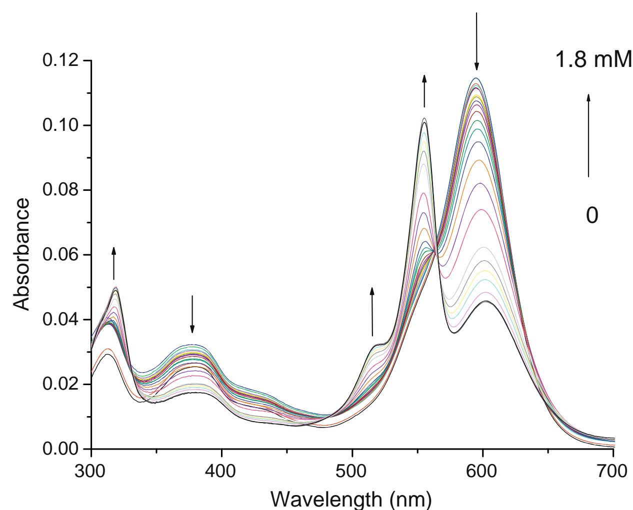

Figure 1. Absorbance titration spectra of **1** (2 μM) in HEPES buffer (6% HEPES in acetonitrile, v/v, pH 7.0) upon addition of increasing amounts of $Al(NO_3)_3$.

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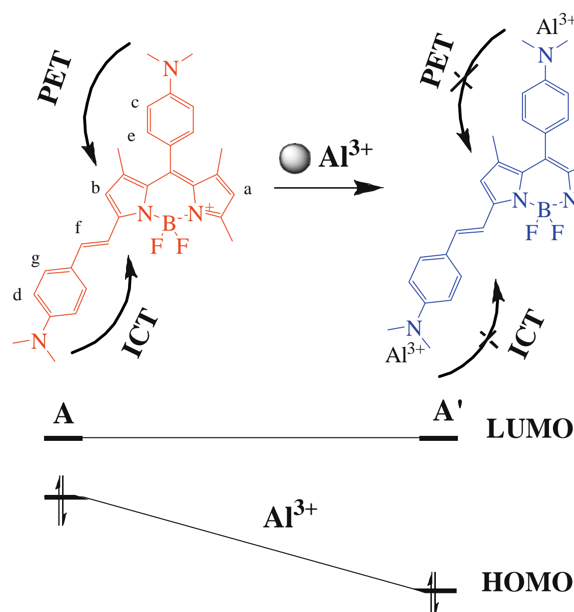
classic BDP fluorophore due to the styryl extension at α-position. Upon addition of Al^{3+}, the intensity of the absorption maximum gradually decreased following the formation of a new band centered at 555 nm with an isosbestic point at 563 nm. The spectrofluorimeter titration of **1** upon addition of Al^{3+} is shown in Figure 2. Compound **1** displayed a weak and broad emission band centered at 700 nm with the quantum yield of 0.016, which resulted from the efficient PET and ICT quenching of the excited state of the BODIPY chromophore from the electron-donating dimethylamino moieties at its *meso*- and α-position. As the concentration of Al^{3+} ion increased, the emission intensity at 700 nm decreased; meanwhile, a significant enhancement at 566 nm was observed. As shown in Scheme 1, the binding of Al^{3+} to **1** reduced the electron-donating ability of the nitrogen atoms of *N*,*N*-(dimethylamino)styryl group which is in conjugation to the BDP core, thus suppressing the ICT process and causing the blue shift in its absorption and emission spectra. While the coordination of Al^{3+} to the nitrogen atom of aniline group at *meso*-position of the BDP core suppressed the PET quenching process, thus leading to an enhancement of the emission. For an excellent chemosensor, high selectivity is a matter of necessity. In the present work, its Al^{3+} response was not interfered in the background containing appropriate metal ions. Figure 3 shows the color change and the emission response of **1** in the presence of various cations. Among the metal ions studied, the addition of Al^{3+} and Cu^{2+} changed the blue solution of **1** into a faint pink color, whereas the solution showed a strong green emission in the presence of Al^{3+} and a weak brown luminescence containing Hg^{2+}. In contrast to Al^{3+}, the addition of Hg^{2+} only slightly red shifted the absorbance of **1** (Fig. 4); this might be due to the minor conformation change in **1**. Studies of the selectivity of **1** by means of fluorescence spectroscopy were then carried out on the related heavy, transition, and main group metal ions. Only the addition of Al^{3+} resulted in a prominent fluorescent enhancement, whereas very weak variations of fluorescent spectra of **1** were observed upon the addition of excesses of other metal ions such as Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Ca^{2+}, and Mg^{2+} (Fig. 5). From the curve of fluorescence intensity changes at 566 nm upon addition of increasing amount of Al^{3+} ions to **1** (Fig. 6), the binding constant pK_d was determined to be 2.9 and the detection limit was 8×10^{-5} M in aqueous solution. These facts suggested that **1** could selectively recognize Al^{3+} ion with a high selectivity under physiological condition.



Scheme 1. Proposed interaction of compound **1** with Al^{3+}.

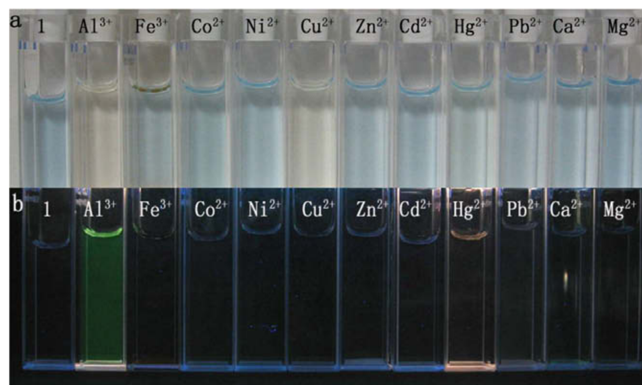


Figure 3. The color change (a, under ambient light) and fluorescent responses (b, irradiated with 365 nm by using a UV lamp) of **1** (2 μM) in HEPES buffer (6% HEPES in acetonitrile, v/v, pH 7.0) upon addition of 2 mM different metal ions.

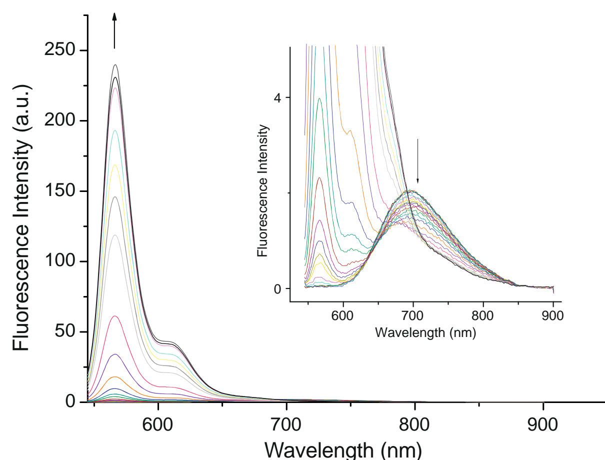


Figure 2. Fluorescence response (λ_{ex} = 543 nm) for **1** (2 μM) in HEPES buffer (6% HEPES in acetonitrile, v/v, pH 7.0) in the presence of increasing amount of $Al(NO_3)_3$. (Inset spectra: amplified the small band at 700 nm.)

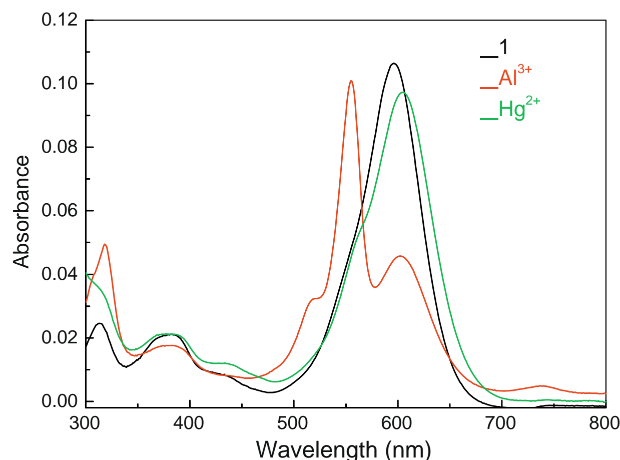


Figure 4. Absorption spectra of **1** (2 μM) in HEPES buffer (6% HEPES in acetonitrile, v/v, pH 7.0) upon addition of 2 mM different metal ions (Al^{3+} red, Hg^{2+} green).

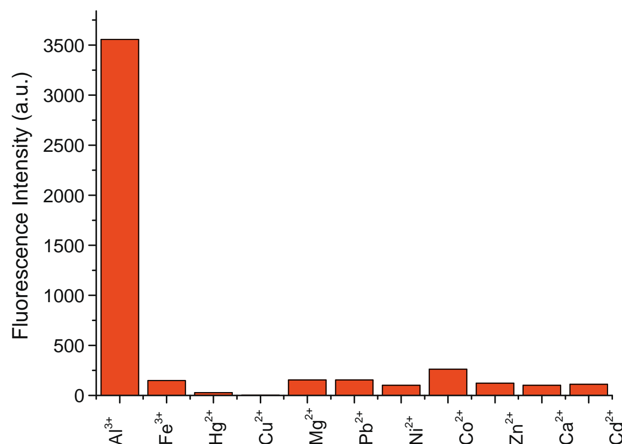


Figure 5. Fluorescent enhancement responses (λ_{ex} = 543 nm) of **1** (2 μM) upon the addition of 2 mM different metal ions in HEPES buffer (6% HEPES in acetonitrile, v/v, pH 7.0).

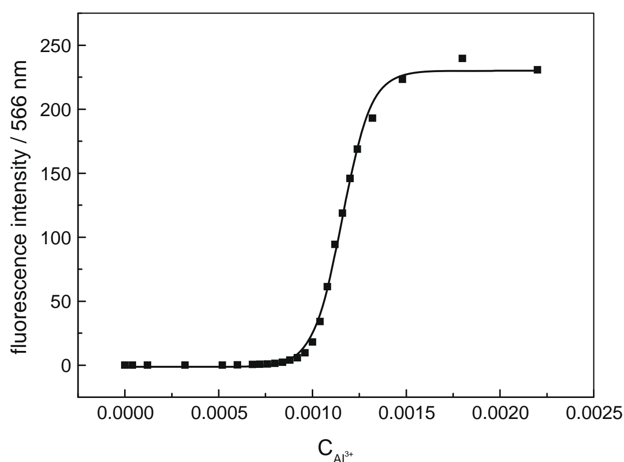


Figure 6. Curve of fluorescence intensity at 566 nm of **1** versus increasing concentration of Al^{3+} in HEPES buffer (6% HEPES in acetonitrile, v/v, pH 7.0). The concentration of **1** was 2 μM. The dissociation constant K_d is deduced to be 1.6×10^{-3} (pK_d = 2.9).

In line with the proposed mechanism, the weak interaction between Al^{3+} and the nitrogen atoms of *N,N*-(dimethylamino) moieties was further supported by the signal changes in the ^{1}H NMR spectra of **1** titrated with Al^{3+} (Fig. 7). Upon the addition of Al^{3+}, the resonance signals corresponding to the protons on the phenyl rings (H^c, H^d, H^e, and H^g) shifted significantly to the downfield. This could be due to the decreasing of the ring currents on the phenyl

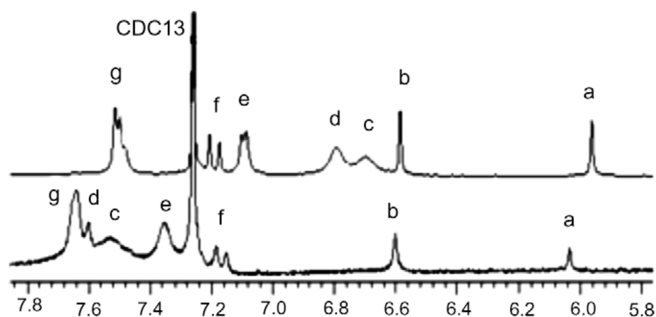


Figure 7. Partial ^{1}H NMR (500 MHz) spectrum of **1** in $CDCl_3$: **1** in $CDCl_3$ (top) and **1**+Al^{3+} (2 equiv) (below).

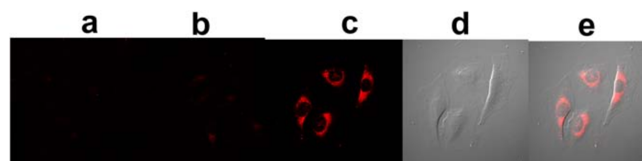


Figure 8. Confocal fluorescence and bright-field images of HeLa cells. (a) Cells supplemented with 1 mM $Al(NO_3)_3$ in the growth media for 24 h at 37 °C; (b) cells stained with 10 μM **1** for 10 min at 25 °C; (c) $Al(NO_3)_3$ supplemented cells loaded with 10 μM **1** for 10 min at 25 °C; (d) bright-field image of cells shown in panel c; and (e) the overlay image of (c) and (d) (λ_{ex} = 543 nm).

rings after Al^{3+} binding to the nitrogen atoms of the dimethylamino groups.

Owing to its favorable spectroscopic properties, **1** might be ideally suitable for fluorescence imaging in living cells to monitor Al^{3+}. Confocal fluorescence microscopy measurement was carried out. The HeLa cells were grown in MEM (modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) at 37 °C and 5% CO_2. Cells (5×10^8/L) were plated on 14 mm glass cover slips and allowed to adhere for 24 h. After being supplemented with 1 mM $Al(NO_3)_3$ in the growth media for 24 h at 37 °C, there was no intracellular fluorescence (Fig. 8a). Cells stained with 10 μM **1** for 10 min at 25 °C led to a weak intracellular fluorescence (Fig. 8b). The cells were then supplemented with 1 mm $Al(NO_3)_3$ in the growth medium for 24 h at 37 °C and loaded with **1** under the same condition, whereupon a significant increase in the fluorescence from the intracellular area was observed (Fig. 8c). Bright-field measurements after treatment with Al^{3+} and **1** confirmed that the cells were viable throughout the imaging experiments (Fig. 8d). As depicted in Figure 8e, the overlay of fluorescence and bright-field images revealed that the fluorescence signals were localized in the perinuclear area of the cytosol, indicating a subcellular distribution of Al^{3+}. These results demonstrated that **1** might be used for detecting Al^{3+} within biological samples.

In summary, we have presented a highly selective chemosensor for Al^{3+} based on BODIPY with two (dimethylamino) moieties. The inhibition of the ICT from the α-(dimethylamino)styryl moiety and PET from the *meso*-(dimethylamino) group upon binding Al^{3+} in this probe caused wavelength ratiometric and fluorescence enhancement. Confocal fluorescence microscopy experiments have shown that **1** can be used to monitor Al^{3+} in living cells and map its subcellular distraction. The results provide a useful design strategy for the synthesis and application of new fluorescent sensors for other transition metal ions in living cells.

Acknowledgments

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