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New ON–OFF type Ca^{2+}-selective fluoroionophore having boron–dipyrromethene fluorophores

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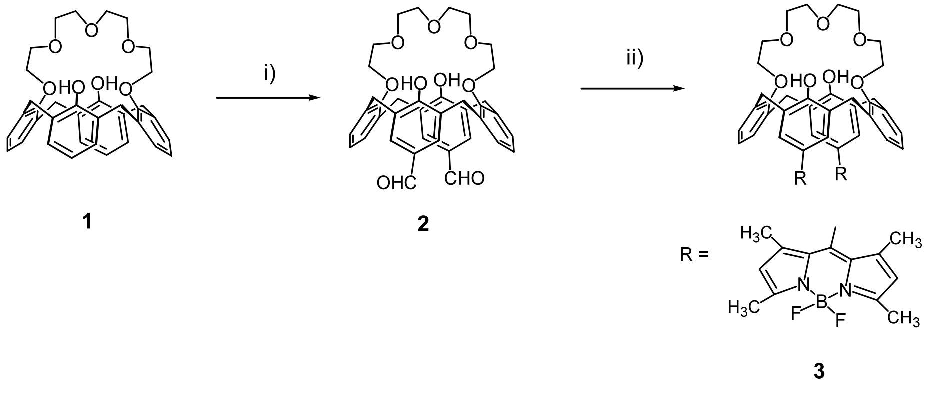
Abstract—A new fluorogenic ionophore has been prepared by conjugating calix[4]-crown-5 ether with boron–dipyrromethene fluorophore. The ionophore exhibited a pronounced selective ON–OFF type response toward Ca^{2+} ions over other physiologically important metal ions of Na^+, K^+, and Mg^{2+}. The interaction with Ca^{2+} ions resulted in very efficient quenching of fluorescence at 507 nm excited by relatively long wavelength irradiation at 480 nm. The selectivity ratios toward Ca^{2+} ions over other tested physiologically important metal ions were larger than 180 in aqueous 95% MeOH solution.

The design of efficient and selective functional ionophores for the detection of ionic guests is one of the most attractive topics in supramolecular chemistry. They are generally devised by conjugating a selective molecular recognition unit with efficient signaling handles that can induce significant changes in electrochemical or spectroscopical behavior.[1] Calixarenes have received much interest as basic molecular scaffold for the construction of many ionophore systems for the sensing of chemically and biologically important metal ions.[2] Among them, calix[4]arene-crown ethers are particularly attractive for their unique binding characteristics toward alkali, alkaline earth, and ammonium ions. Many interesting compounds having chromogenic and/or fluorogenic responses toward a variety of important metal ions have been developed based on the versatile molecular framework of calix[4]arene-crown ethers.[3,4] Recently boron–dipyrromethene (BDP) fluorophore has been attracted wide interest in the construction of selective and efficient fluorescent chemical sensors.[5–10] They are very attractive signaling handles because they have high absorption coefficients and high quantum yield, as well as can be excited by relatively longer wavelength (~500 nm) irradiations.[11] In this paper we report the



Scheme 1. *Reagents and conditions*: (i) $CHCl_2OCH_3$, $SnCl_4$, $CHCl_3$; (ii) 2,4-dimethylpyrrole, TFA, CH_2Cl_2, then *p*-chloranil, Et_3N, BF_3–Et_2O.

Keywords: calix[4]arene-crown ether; fluoroionophore; boron–dipyrromethene; Ca^{2+}-selectivity.

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synthesis and fluoroionophoric properties of a new BDP derivative based on the well-known molecular recognition framework of calix[4]arene-crown-5 ether for the signaling of biologically important Ca^{2+} ions by visible light excitation. In fact, visible light–excitable Ca^{2+} sensors are known to offer several advantages over UV light–excitable probes.[12] The prepared ionophore showed a very selective and efficient ON–OFF type signal transduction toward Ca^{2+} ions in aqueous MeOH solution over other physiologically important metal ions.

A new calix[4]arene-crown-5 ether derivative having BDP fluorophore at the upper rim of phenol ring was prepared from the aldehyde derivative **2**. Aldehyde **2** was prepared by the selective formylation of calix[4]arene-crown-5 ether with $SnCl_4/CHCl_2OCH_3$ in chloroform following the reported procedures.[4,13] The dialdehyde **2** was condensed with 2,4-dimethylpyrrole in the presence of TFA, which was subsequently oxidized (*p*-chloranil), neutralized (Et_3N), and treated with $BF_3 \cdot Et_2O$ to afford the desired BDP derivative **3** (18%) (Scheme 1).[14]

The prepared BDP derivative showed a characteristic AB-type splitting pattern in its ^1H NMR spectrum for the bridging methylene protons of calix[4]arene revealing a cone shaped conformation in chloroform. Interestingly, there are two ^1H resonances (δ 6.01 and 5.92) for the pyrrole protons of BDP moiety, that is due to the orthogonal orientation of BDP functions with respect to the phenyl ring of calix[4]arene. With this orthogonal structure the two protons of pyrrole moieties will take *exo* and *endo* positions with respect to the calixarene backbone and are no longer equivalent. Similar behavior was also observed for the methyl protons of pyrrole rings (δ 2.56 and 2.55 for the distal and δ 1.59 and 0.89 for the proximal methyl protons, respectively).

The ionophoric properties of BDP derivative **3** were investigated by fluorescence measurements. Earlier we have reported the Ca^{2+} selective OFF–ON type ionophoric behaviors of calix[4]arene-crown-5 ether having benzothiazole functions as signaling unit that was excited by the irradiation in UV region of 330 nm.[4] Ionophore **3** showed an intense green fluorescence at $\lambda_{max} = 507$ nm, which is excited by visible region irradiation of 480 nm having much lower energy, with a small shoulder around 545 nm in MeOH. The selective fluorogenic responses of **3** toward physiologically important metal ions (Na^+, K^+, Mg^{2+}, and Ca^{2+}) were tested by the treatment of the ionophore (2.5×10^{-6} M) with 50 equiv. of metal ions in MeOH. However, addition of metal ions resulted in no significant changes in fluorescence spectra of **3** in spite of the possible interactions of this ionophore with some of the tested metal ions.

This insensitive behavior is due to the well-known fact that the spectral response of BDP appended phenol moiety depends on the pH of the medium.[9] So we tried to find the optimum pH condition for the selective signaling of the binding process by the addition of aliquots of LiOH solution to the mixture of ionophore and metal ions in MeOH. Separate measurements confirmed that the Li^+ ions are irrelevant to the fluorescent behavior of the calix[4]arene-BDP **3**. With the addition of increasing amount of LiOH solution to the **3**-M^{n+} solution ([**3**] $= 2.5\times10^{-6}$ M and $[M^{n+}] = 1.25\times10^{-4}$ M), the fluorescence intensity starts to decrease significantly especially for Ca^{2+} ions. For example, upon addition of 100 μL of 0.1 M LiOH solution into 5 mL of MeOH solution of **3**, the solution responds exclusively to the Ca^{2+} ions and the greenish fluorescent solution changed into a light orange colored one, which can be detected even with naked eye. The changes in fluorescence spectrum in the presence of metal ions are shown in Figure 1. In fact, the Ca^{2+} ions induced almost complete quenching of fluorescence up to baseline level: that is 390-fold decrease in fluorescence intensity at 507 nm. On the other hand, the other metal ions showed almost no changes in fluorescence spectrum in both intensity and position. The selectivity toward Ca^{2+} ions over other surveyed metal ions of Na^+, K^+, and Mg^{2+} ions, estimated from the ratio of the changes in fluorescence intensity at 507 nm was larger than 380. In the present system, the efficient quenching of the fluorescence emission is due to the PET (photoinduced electron transfer)-like electron transfer from the phenolate group to the BDP fluorophore.[6,9]

The use of aqueous solutions as analytical medium is frequently prerequisite for the developments of sensors for the metal ion analysis in biological systems. So, we next tested the responses of **3** toward metal ions in aqueous MeOH solution having various compositions of water. The results are summarized in Figure 2.

As the amount of water in MeOH–H_2O mixture increases, the efficiency of fluorescence quenching with Ca^{2+} ions decreases significantly without affecting the response of other metal ions. That resulted in the reduced selectivity toward target metal ions of Ca^{2+} over other metal ions. For example, the ratio of I_o/I (I_o and I represent the fluorescence intensity in the absence and presence of metal ions, respectively) decreased from

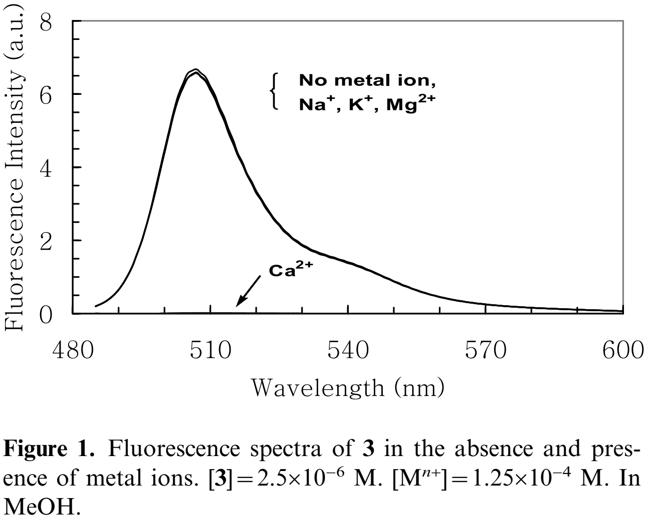

Figure 1. Fluorescence spectra of **3** in the absence and presence of metal ions. [**3**] $= 2.5\times10^{-6}$ M. $[M^{n+}] = 1.25\times10^{-4}$ M. In MeOH.

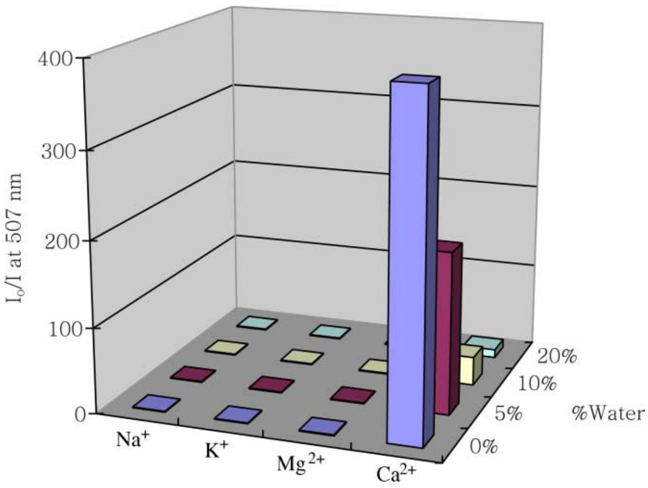

Figure 2. Changes in fluorescence intensity of **3** as a function of water composition in aqueous MeOH. [**3**]$=2.5\times10^{-6}$ M. [M^{n+}]$=1.25\times10^{-4}$ M. $\lambda_{ex}=480$ nm.

391 to 9.8 by changing the composition of water from 0 to 20%. However, the selectivity toward Ca^{2+} ions remains sufficiently high up to 5% of water ($I_o/I=188$) and the selective response can be clearly observable with naked eye (Fig. 3). The aqueous 95% MeOH solution seems to be a suitable medium for the practical application of this ionophore compromising the Ca^{2+}-selectivity and necessity of aqueous solution. The selectivity toward Ca^{2+} ions was further ascertained by the fluorescence measurements under the competitive conditions. For example, the fluorescence intensity profile of the ionophore **3** with 50 equiv. of Ca^{2+} ions was not affected by the presence of 500 equiv. of other background metal ions of Na$^+$, K$^+$, and Mg^{2+} ions.

Based on these observations we have determined the binding constant for **3**-Ca^{2+} system and the detection limit for the analysis of Ca^{2+} ions. As the concentration of calcium ions increased the fluorescence intensity decreased while the position and shape of the fluorescence spectra were not affected, that is typical of PET type mechanism.[15] The changes in fluorescence intensity were monitored as a function of added Ca^{2+} ion concentration in MeOH–H$_2$O (95:5, v/v) (Fig. 4). The almost complete quenching of the fluorescence down to the base line was observed with 10 equiv. of Ca^{2+} ions. From this titration result, binding constant for **3**-Ca^{2+} system was obtained by the nonlinear curve fitting procedure[16] and was found to be 5.4×10^5 M^{-1}. The detection limit[17] for the analysis of Ca^{2+} ions was estimated to be 2×10^{-6} M in same medium.

Figure 3. Fluorescence of **3** in the absence and presence of metal ions. From left to right: no metal ion, in the presence of Ca^{2+}, Mg^{2+}, and K$^+$ ions. [**3**]$=2.5\times10^{-6}$ M and [M^{n+}]$=1.25\times10^{-4}$ M in MeOH–H$_2$O (95:5, v/v).

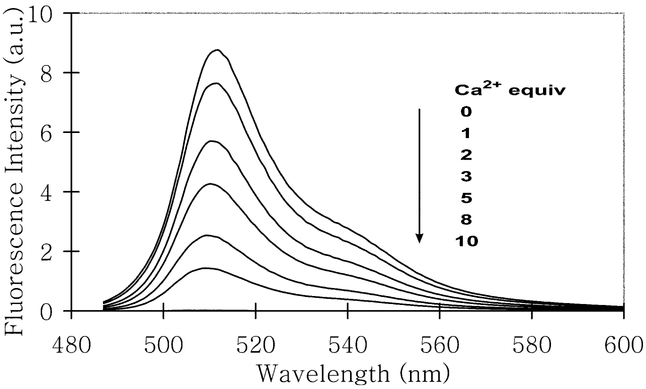

Figure 4. Changes in fluorescence intensity of **3** as a function of [Ca^{2+}]. [**3**]$=2.5\times10^{-6}$ M. In MeOH–H$_2$O (95:5, v/v).

In conclusion, a new fluorogenic ionophore based on the well-known structural motif of calix[4]arene-crown-5 ether was prepared by appending BDP moiety on the upper rim of the calix[4]arene. The compound exhibited a high selectivity toward Ca^{2+} ions in the presence of other physiologically important metal ions. The prominent ON–OFF type quenching effect can also be utilized as a useful handle for the design of other functional supramolecular switching systems.

Acknowledgements

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14. Selected data for **3**. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.94 (s, 2H, ArOH), 7.00 (s, 4H, ArH), 6.81 (d, $J=7.5$ Hz, 4H, ArH), 6.65 (t, $J=7.5$ Hz, 2H, ArH), 6.01 and 5.92 (s, 2H each, pyrrole-H), 4.49 (d, $J=12.9$ Hz, 4H, $ArCH_2Ar$), 4.13 (s, 8H, OCH_2), 3.99 (br t, $J=4.5$ Hz, 4H, OCH_2), 3.90 (br t, $J=5.1$ Hz, 4H, OCH_2), 3.38 (4H, $J=13.5$ Hz, 4H, $ArCH_2Ar$), 2.56 and 2.55 (s, 6H each, pyrrole-CH_3), 1.59 and 0.89 (s, 6H each, pyrrole-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.6, 155.0, 154.1, 152.2, 143.7, 143.4, 142.7, 141.1, 133.4, 132.3, 132.1, 129.6, 129.1, 127.9, 125.5, 125.0, 121.3, 121.1, 101.4, 71.1, 70.3, 30.9, 14.9, 13.5. HRMS (FAB, *m*-NBA) calcd for $C_{62}H_{64}B_2F_4N_4O_7$ 1074.490, found 1074.496.
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