



# A new fluorogenic benzothiazolyl ionophore based upon calix[4]arene-crown-5 ether for calcium determination in aqueous media

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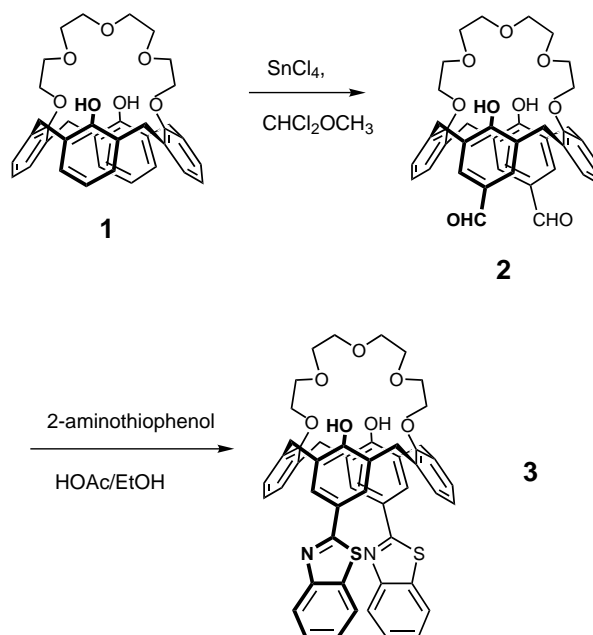
Received 20 December 2001; revised 3 April 2002; accepted 4 April 2002

**Abstract**—A new benzothiazolyl functionalized ionophore based upon the calix[4]arene-crown-5 ether has been prepared and its fluoroionophoric properties toward  $\text{Ca}^{2+}$  ions were investigated. The compound exhibited a pronouncedly selective fluoroionophoric behavior toward  $\text{Ca}^{2+}$  ions among the surveyed physiologically important metal ions of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{2+}$ . Detection limit for  $\text{Ca}^{2+}$  ions was found to be  $9 \times 10^{-5}$  M in dioxane–water (80:20, v/v) solution. © 2002 Elsevier Science Ltd. All rights reserved.

The development of specific chemosensors for the efficient detection of biologically relevant analytes is one of the most important areas in supramolecular chemistry.<sup>1,2</sup> In particular, the determination of  $\text{Ca}^{2+}$  in various biological systems has attracted much interest and many efficient systems are continually being developed.<sup>3</sup> For the recognition of  $\text{Ca}^{2+}$  ions, many of the specific ionophores that have been developed are based on the molecular platform of crown ethers, calixarenes, and a variety of EDTA type chelates.<sup>4,5</sup> With the versatile structural framework of calix[4]arenes,<sup>6</sup> various  $\text{Ca}^{2+}$ -selective ionophores having amide, carboxylic acid, and crown ether moieties have been developed.<sup>7–10</sup> Among them, calix[4]arene-crown ethers seem to have relatively well optimized structural properties<sup>10</sup> for the assay of  $\text{Ca}^{2+}$  ions at the millimolar level that is generally found in physiological systems. Meanwhile, thiazole functions are attractive and frequently employed as signaling devices for the construction of chromogenic as well as fluorogenic ionophores.<sup>11–14</sup> With this in mind, we postulated that appropriately designed ionophores combining well-established structural motifs of calix[4]arene-crown-5 ether and benzothiazole could function as both chromogenic and fluorogenic signaling molecular devices for  $\text{Ca}^{2+}$  ions. In this paper we report the synthesis and  $\text{Ca}^{2+}$ -selective chromogenic and/or fluorogenic ionophoric properties of a benzothiazolyl

ionophore based upon a calix[4]arene-crown-5 ether which can be utilized as a new sensing material for the determination of  $\text{Ca}^{2+}$  concentrations in physiological fluids.

Calix[4]arene-crown-thiazole **3** was prepared by the condensation of the dialdehyde derivative of *p*-H-



Scheme 1.

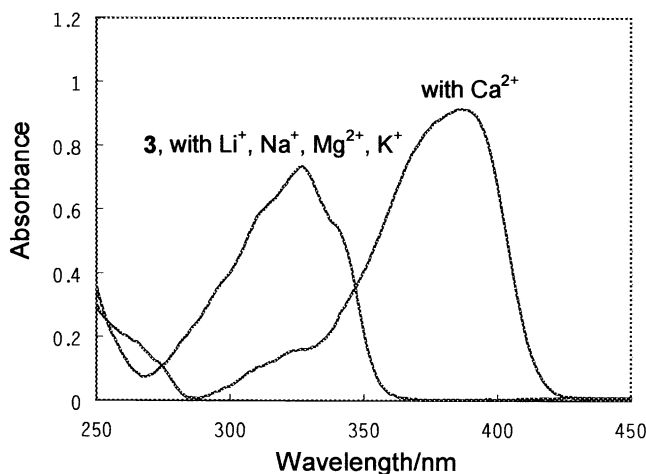
**Keywords:** fluoroionophore; calix[4]-crown ether; benzothiazole;  $\text{Ca}^{2+}$ -selectivity; aqueous media.

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calix[4]arene-crown ether **2** with 2-aminothiophenol, as shown in Scheme 1. Dialdehyde **2** was prepared by the selective 1,3-diformylation of calix-crown ether **1**<sup>15</sup> with  $\text{CHCl}_2\text{OCH}_3/\text{SnCl}_4$  following a slightly modified procedure for the preparation of diether-dialdehydes.<sup>16</sup> Reaction of dialdehyde **2** with 2-aminothiophenol in  $\text{AcOH}/\text{EtOH}$ <sup>12</sup> afforded the bis(benzothiazole) derivative **3** in moderate yield (59%).<sup>17</sup>

Preliminary ionophoric properties of the thiazole derivative **3** were investigated by UV spectroscopy. The UV spectrum of **3** ( $1.0 \times 10^{-5}$  M) was measured in a dioxane– $\text{H}_2\text{O}$  (80:20, v/v) mixture with 200 equiv. of triethylamine in the presence and absence of the surveyed metal ions. An aqueous dioxane solution was used as solvent, as **3** has only limited solubility in pure water. Guest metal ions tested were representative alkali and alkaline earth metal ions of physiological interest ( $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$  ions in perchlorate). Upon interaction with  $\text{Ca}^{2+}$  ions, the colorless solution of **3** was transformed into a fluorescent pale purple one, which can be seen easily even with the naked eye. For example, as shown in Fig. 1, with 100 equiv. of  $\text{Ca}^{2+}$  ions, the absorbance of **3** at  $\lambda_{\text{max}} = 330$  nm decreased with a concomitant increase in a new absorption band at  $\lambda_{\text{max}} = 380$  nm. Other tested metal ions induced almost no change in the UV spectral behavior of **3**. In this case, the presence of triethylamine was found to be essential for the chromogenic behavior of the benzothiazole ionophore **3** toward  $\text{Ca}^{2+}$  ions.<sup>12</sup>

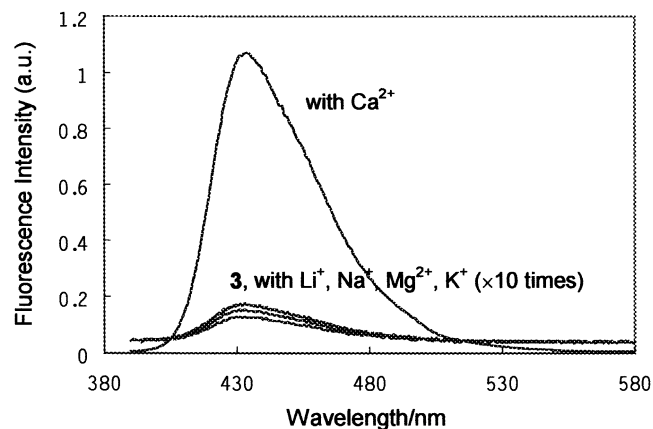
Based on these preliminary observations for the selective chromogenic behavior of **3** toward  $\text{Ca}^{2+}$  ions, we have investigated the fluoroionophoric properties of **3**. In the same dioxane– $\text{H}_2\text{O}$  solvent system, free ionophore **3** ( $5.0 \times 10^{-6}$  M) in the presence of 200 equiv. of triethylamine showed a broad fluorescence spectrum around 380 nm ( $\lambda_{\text{ex}} = 330$  nm). Upon interaction with  $\text{Ca}^{2+}$  ions (100 equiv.), the fluorescence behavior of **3** changes significantly: a large decrease in fluorescence intensity at 380 nm with concomitant increase in fluorescence intensity at 430 nm was observed. This



**Figure 1.** UV–vis absorption spectra of **3** in dioxane– $\text{H}_2\text{O}$  (80:20, v/v) in the presence and absence of metal ions.  $[\mathbf{3}] = 1.0 \times 10^{-5}$  M.  $[\text{M}^{n+}] = 1.0 \times 10^{-3}$  M.  $[\text{NEt}_3] = 2.0 \times 10^{-3}$  M.

fluorescence behavior was again unique for  $\text{Ca}^{2+}$  ions among the surveyed metal ions and other metal ions showed almost negligible fluorescence changes at both 380 and 430 nm. This type of fluorescence signaling is mainly attributed to the deprotonation of the phenol moiety assisted by the interaction with the guest metal ions, as reported earlier.<sup>12</sup> The selective fluorogenic behavior of **3** toward  $\text{Ca}^{2+}$  ions was more evident with the fluorescence spectra obtained at another excitation wavelength ( $\lambda_{\text{ex}} = 380$  nm) (Fig. 2). In this case, the fluorescence intensity at 430 nm was much more dramatically enhanced for  $\text{Ca}^{2+}$  ions compared to the other surveyed metal ions.

The selective fluorogenic behavior of **3** toward given metal ions was semi-quantitatively compared using the ratio of the fluorescence intensities of **3** in the presence ( $I$ ) and absence ( $I_0$ ) of given guest metal ions at 430 nm ( $\lambda_{\text{ex}} = 380$  nm). The ionophore **3** showed a high selectivity ratio toward  $\text{Ca}^{2+}$  ions over other metal ions with  $I/I_0$  values greater than 85 in dioxane– $\text{H}_2\text{O}$  (80:20) (Table 1). The remaining metal ions revealed  $I/I_0$  values less than 2. In fact, during the survey for an appropriate solvent system for **3**, we tried to optimize the  $\text{Ca}^{2+}$  selectivity by varying the composition of the dioxane– $\text{H}_2\text{O}$  solvent system. As the amount of water in the mixed solvent system increased, the fluorescence intensity  $I$  for the **3**– $\text{Ca}^{2+}$  system was found to decrease significantly, resulting in a reduction in the selectivity ratios. For example, with a 50:50 mixture of dioxane–



**Figure 2.** Fluorescence spectra of **3** in the presence and absence of varying metal ions ( $\lambda_{\text{ex}} = 380$  nm) in dioxane– $\text{H}_2\text{O}$  (80:20, v/v).  $[\mathbf{3}] = 5.0 \times 10^{-6}$  M.  $[\text{M}^{n+}] = 5.0 \times 10^{-4}$  M.  $[\text{NEt}_3] = 1.0 \times 10^{-3}$  M.

**Table 1.** Fluorescence intensity ratios ( $I/I_0$ ) of **3** for metal ions at 430 nm in various solvent systems<sup>a</sup>

% $\text{H}_2\text{O}$ in dioxane	$\text{Li}^+$	$\text{Na}^+$	$\text{K}^+$	$\text{Mg}^{2+}$	$\text{Ca}^{2+}$
1	0.8	1.8	1.0	0.8	175
20	1.4	1.2	1.4	1.0	85.5
50	1.0	1.2	1.2	0.8	2.3

<sup>a</sup>  $I$  and  $I_0$  denote fluorescence intensity of **3** in the presence of 100 equiv. of metal ions and in the absence of metal ions, respectively, at 430 nm ( $\lambda_{\text{ex}} = 380$  nm).  $[\mathbf{3}] = 5.0 \times 10^{-6}$  M.  $[\text{M}^{n+}] = 5.0 \times 10^{-4}$  M.  $[\text{NEt}_3] = 1.0 \times 10^{-3}$  M.

H<sub>2</sub>O, the  $I/I_0$  for Ca<sup>2+</sup> ions was drastically reduced to 2.3, an almost total loss of selectivity toward Ca<sup>2+</sup> ions. On the other hand, however, with a dioxane–H<sub>2</sub>O ratio of 99:1, the fluorescence intensity ratio for Ca<sup>2+</sup> ions increased significantly, to 175—that is, twice as large as that of the dioxane–H<sub>2</sub>O (80:20) system. Although employing the 99:1 mixed solvent is desirable in terms of the enhanced selectivity toward Ca<sup>2+</sup> ions, we feel that a 99% aqueous dioxane solution might cause some inconvenience and be unfavorable for the possible practical applications of **3** in the determination of Ca<sup>2+</sup> levels in physiological fluids.

To gain an insight into the possibility of using **3** in the analysis of Ca<sup>2+</sup> ions in physiological fluids, the fluorescence response to Ca<sup>2+</sup> ions over the concentration range of physiological environments was investigated in a dioxane–water (80:20) solution. That is, the fluorescence intensity at 430 nm ( $\lambda_{\text{ex}} = 380$  nm) was monitored as a function of Ca<sup>2+</sup> ion concentration between 0.1 and 10 mM, in the presence of physiological background metal ions ([Na<sup>+</sup>] = 138 mM, [K<sup>+</sup>] = 4 mM, and [Mg<sup>2+</sup>] = 3 mM).<sup>18</sup> The fluorescence intensity increases in a sigmoidal pattern with increasing [Ca<sup>2+</sup>] over the surveyed concentration range, that is almost superimposable with the result obtained for the system without any background metal ions. From this plot, the detection limit<sup>19</sup> of **3** for the Ca<sup>2+</sup> ions in the presence of background metal ions was estimated to be  $9 \times 10^{-5}$  M. Other metal ions showed very little fluorescence changes in similar titrations and the addition of interfering ions up to 5 mM (1000-fold excess) results in almost no change in the fluorescence intensity of **3** with Ca<sup>2+</sup> ions. Although the requirement of a relatively large quantity of triethylamine for the signaling is not so beneficial for some practical applications, the results obtained imply a possibility of the present benzothiazolyl derivative for the analysis of Ca<sup>2+</sup> ions in physiological fluid systems after suitable optimization.<sup>20</sup>

The binding constant for the **3**–Ca<sup>2+</sup> complex in dioxane–H<sub>2</sub>O (80:20) was estimated to be  $3.2 \times 10^4$  M<sup>-1</sup> by a Scatchard plot<sup>21</sup> of the changes in fluorescence intensity as a function of Ca<sup>2+</sup> ion concentration. The Ca<sup>2+</sup>-selectivity of the present ionophore system might be due to the high affinity of the calix[4]arene-crown-5 structure<sup>10,22</sup> toward some of alkaline earth metal ions, especially in the presence of basic triethylamine. Upon metal ion assisted deprotonation of the phenolic moiety, the interaction with phenolate moiety of ionophore will be much favorable for the doubly charged alkaline earth metal ions compared with the singly charged alkali metal ions. Within the alkaline earth metal ions, **3** showed decreasing fluorescence response in the following order: Ca<sup>2+</sup> > Sr<sup>2+</sup> > Ba<sup>2+</sup> >> Mg<sup>2+</sup>. Obviously the possible interferences of Ba<sup>2+</sup> and Sr<sup>2+</sup> ions are insignificant in physiological systems. The Ca<sup>2+</sup> selectivity of **3** can be further confirmed by the results of FAB mass spectrometric analysis. The mass spectrum of **3** measured in the presence of 10 equiv. each of competing metal ions Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> ions in perchlorate in *m*-NBA matrix showed very high Ca<sup>2+</sup> selectivity by exhibiting an intense peak at  $m/z = 877.25$  ascribable to

the [3-H+Ca]<sup>+</sup> complex. Other peaks for plausible species arising from the other tested metal ions were not observed.

In summary, we have designed a new Ca<sup>2+</sup>-selective chromogenic and/or fluorogenic ionophore that has a high selectivity and sensitivity for Ca<sup>2+</sup> over other physiologically important alkali and alkaline earth metal ions. We believe that the prepared compound can be used as a new probe for the fluorogenic sensing of biologically important Ca<sup>2+</sup> ions in aqueous environments.

### Acknowledgements

This work was supported by a grant from Korea Research Foundation (2000-DP0278), which is gratefully acknowledged.

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17. **Preparation of dialdehyde 2:** Calix[4]arene-crown-5 **1** (0.116 g, 0.2 mmol) was dissolved in chloroform (15 mL) and the solution was cooled to  $-10^{\circ}\text{C}$ . To this solution 1,1-dichlorodimethyl ether (0.06 g, 0.5 mmol) and  $\text{SnCl}_4$  (0.52 g, 2 mmol) were rapidly added. The reaction mixture was stirred at room temperature for 2 h and quenched with a 0.1 M aqueous HCl solution (10 mL). The organic layer was washed three times with water and evaporated under reduced pressure. The residue was purified by column chromatography ( $\text{SiO}_2$ , ethyl acetate:hexane=1:1) to afford **2** (100 mg, 78%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 9.82 (s, 2H), 8.77 (s, 2H), 7.66 (s, 4H), 6.92 (d,  $J=7.5$  Hz, 4H), 6.77 (t,  $J=7.5$  Hz, 2H), 4.44 (d,  $J=13.2$  Hz, 4H), 4.14 (m, 4H), 4.09 (m, 4H), 3.92 (t,  $J=5.1$  Hz, 4H), 3.85 (t,  $J=5.1$  Hz, 4H), 3.49 (d,  $J=13.2$  Hz, 4H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 191.3, 159.9, 152.2, 132.5, 131.2, 129.7, 128.9, 128.7, 125.9, 71.3, 70.2, 31.0; MS (FAB, *m*-NBA): found:  $m/z$  639.7. Calcd for  $\text{C}_{38}\text{H}_{39}\text{O}_9$ : ( $\text{M}+\text{H}$ ) $^+$  639.7. **Preparation of bis(benzothiazole) 3:** To a solution of dialdehyde **2** (0.10 g, 0.16 mmol) in a mixture of ethanol (10 mL) and acetic acid (0.5 mL) was added 2-aminothiophenol (50 mg, 0.38 mmol). The yellow solution was refluxed overnight under  $\text{N}_2$  and then solvents were removed under reduced pressure. The dark residue was taken up into chloroform and washed with water three times. The solvent was evaporated under reduced pressure and the product was purified by column chromatography ( $\text{SiO}_2$ , ethyl acetate:hexane=1:1) to yield the desired thiazole **3** (80 mg, 59%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 8.29 (s, 2H), 8.03 (d,  $J=8.1$  Hz, 2H), 7.87 (m, 2H), 7.86 (s, 4H), 7.46 (t,  $J=7.5$  Hz, 2H), 7.33 (t,  $J=7.6$  Hz, 2H), 6.98 (d,  $J=7.8$  Hz, 4H), 6.77 (t,  $J=7.4$  Hz, 2H), 4.49 (d,  $J=13.2$  Hz, 4H), 4.15 (m, 4H), 4.11 (m, 4H), 3.95 (t,  $J=5.1$  Hz, 4H), 3.87 (t,  $J=5.1$  Hz, 4H), 3.53 (d,  $J=13.2$  Hz, 4H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 168.9, 156.9, 154.6, 152.4, 135.0, 132.7, 129.7, 129.0, 128.3, 126.4, 125.8, 124.8, 122.8, 121.7, 71.3, 70.4, 31.1; MS (FAB, *m*-NBA): found:  $m/z$  849.3. Calcd for  $\text{C}_{50}\text{H}_{45}\text{N}_2\text{O}_7\text{S}_2$ : ( $\text{M}+\text{H}$ ) $^+$  849.3. Anal. calcd for  $\text{C}_{50}\text{H}_{44}\text{N}_2\text{O}_7\text{S}_2\cdot\text{H}_2\text{O}$ : C, 69.26; H, 5.35; N, 3.23. Found: C, 69.43; H, 5.67; N, 3.30%.
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20. With the results obtained in the present study, a plausible procedure for the analysis of  $\text{Ca}^{2+}$  ions in physiological fluids might be suggested as following. The treatment of the aliquots of physiological sample with the ionophore solution (containing 200 equiv. of triethylamine in dioxane) and then adjust the composition of the solution as dioxane– $\text{H}_2\text{O}$  (80:20, v/v) by adding required amount of pure dioxane. However, as previously mentioned more optimization of the experimental condition to enhance the response and selectivity of **3** toward  $\text{Ca}^{2+}$  ions along with the prevention of possible precipitation of the sample component is still necessary before practical applications.
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