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Novel Ca^{2+}-selective merocyanine-type chromoionophore derived from calix[4]arene-diamide

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Abstract—The synthesis and calcium-selective chromogenic ion binding properties of benzothiazolium derivative of calix[4]arene are described. The merocyanine-type ionophore derived from calix[4]arene-diamide showed selective chromogenic properties toward Ca^{2+} ions in aqueous methanol. The compound showed significant Ca^{2+} ion dependent changes in UV–vis spectral properties in the presence of other physiologically important metal ions (Na^+, K^+, and Mg^{2+}). The pH sensitive chromogenic behavior also suggests that the compound can be used as a sensitive pH indicator around pH 6.5. © 2003 Elsevier Science Ltd. All rights reserved.

The development of selective sensors for the chemically and biologically important metal ions is one of the most interesting areas in the field of supramolecular chemistry.[1] Calixarenes have received considerable research efforts as the basis for the numerous designs of genuine functional ionophores.[2] The selective ionophoric properties of calix[4]arene–diamide framework for the recognition of Ca^{2+} ions are well investigated.[3] Although there are a wide variety of Ca^{2+}-sensitive sensors for the sensing of calcium ions in chemical and biological systems,[4] there still needs to be more sensors to meet the varying types of samples in terms of wavelength and concentration ranges. Particularly, the sensitive and convenient determination of calcium ions, such as naked eye detection,[5] in aqueous solution is essentially important for the convenient monitoring of the ions in many chemical and biological systems. Benzothiazole functions have widely used as a major constituent for the design of merocyanine[6] and hemicyanine[7] type optical materials[8] and also have a good spectroscopic characteristic for the design of chromogenic[9] and fluorogenic[10] signaling of binding processes. We report here the synthesis of a new chromogenic ionophore derived from calix[4]arene–diamide framework having benzothiazolium ion moieties. The prepared ionophore revealed a pronounced Ca^{2+}-selective chromogenic behavior over other physiologically important metal ions of Na^+, K^+, and Mg^{2+}. Furthermore, the ionophore exhibited pH-sensitive spectral changes, which might be utilized as a new pH-probe around pH 6.5.

Selective formylation of calix[4]arene-diamide **1** with $CHCl_2OCH_3/SnCl_4$ afforded dialdehyde **2** in good yield (93%).[11] Aldehyde **2** was reacted with 3-ethyl-2-methylbenzothiazolium iodide in the presence of pyridine in EtOH[12] to yield desired benzothiazolium derivative **3** (97%).[13] The reaction was hastened with molecular sieve by using Soxhlet extraction apparatus to remove the water formed efficiently. The prepared compound showed a typical AB quartet for the bridging methylene protons at δ 4.50 and 3.53 characteristic of a cone type conformation of calix[4]arene framework. Alkenic protons on the styryl functions of merocyanine moiety revealed a large vicinal coupling of alkene ($J = 15.6$ Hz) indicating a *trans* configuration of the olefinic structure in the ionophore. For comparison purpose, control compound **4** having no calix[4]arene moiety in the structure was prepared similarly from 3,5-dimethyl-4-hydroxybenzaldehyde (Scheme 1).[13]

We first investigated the preliminary chromogenic responses of compound **3** toward the physiologically important metal ions (Na^+, K^+, Mg^{2+}, and Ca^{2+} ions) in common organic solvents. In methanol, the ionophore **3** (1.25×10^{-5} M) showed a strong absorption band around 450 nm and weak bands at 530 and 590 nm resulting in light orange colored solution. Upon interaction with 100 equiv. of Ca^{2+} ions in perchlorate, the absorption band around 530 and 590 nm increased significantly while the band at 450 nm decreased. The

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Keywords: chromogenic ionophore; calix[4]arene diamide; merocyanine; Ca^{2+}-selectivity; pH indicator.

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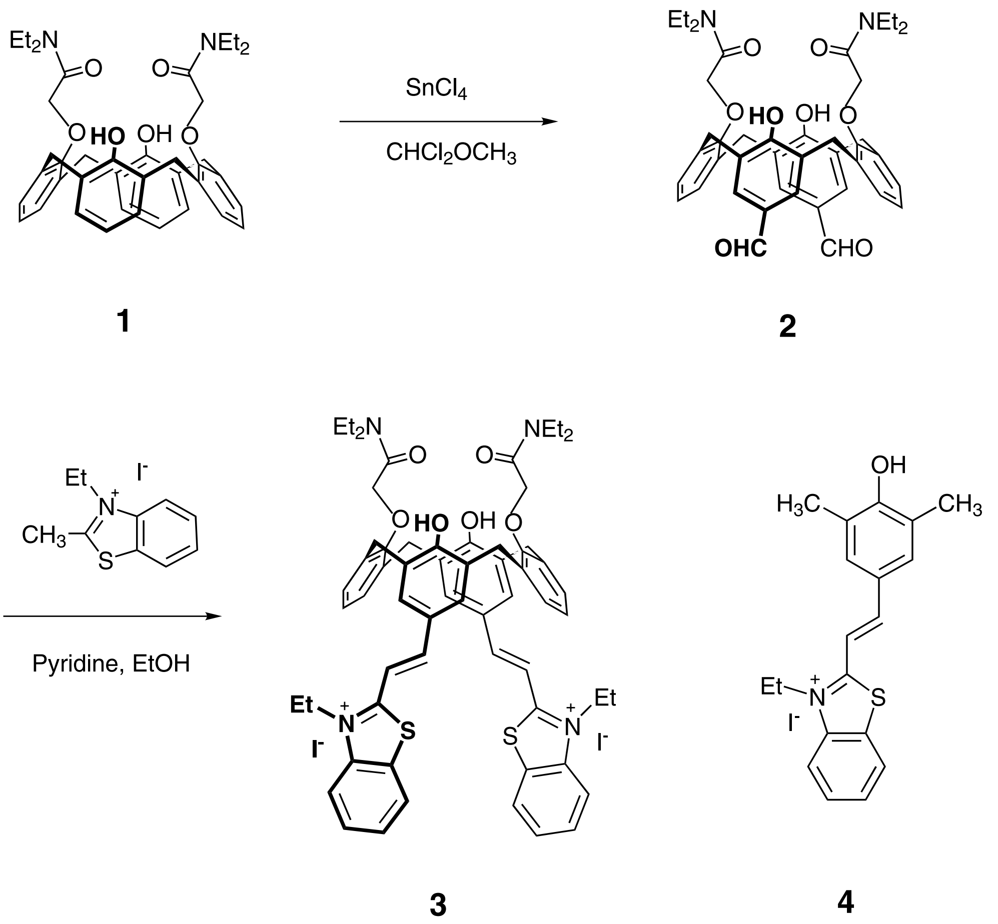

Scheme 1.

changes in absorption bands resulted in the color change from light orange to purple. Other metal ions (Na^+, K^+, and Mg^{2+}) also exhibited similar but significantly reduced spectral changes compared with Ca^{2+} ions.

The preliminary results of Ca^{2+}-selective response of **3** obtained in methanol solution was not so sufficient for the selective detection of Ca^{2+} ions in many chemical systems. So, we tried to optimize the Ca^{2+}-selectivity by testing various solvent systems focusing mainly on aqueous medium. Among them, aqueous MeOH (H_2O: MeOH = 1:1, v/v) solution gave a considerably enhanced selectivity toward Ca^{2+} ions (Fig. 1). In this solvent system, the spectral characteristic of **3** is somewhat different with that of 100% MeOH solution due to the increased polarity of the medium. Absorption bands at 450 nm and 590 nm were blue-shifted to 434 nm and 580 nm, respectively, while the 530 nm band was not affected. In addition to this, the absorption intensity of free **3** at 434 nm increased markedly and the band at 580 nm decreased significantly. The addition of 100 equiv. of Ca^{2+} ions to the ionophore solution again resulted in a large enhancement of the band at 580 nm with concomitant decrease in 434 nm band. On the other hand, the other metal ions showed relatively insignificant changes in absorption spectra when compared with the results of 100% MeOH solution that makes the enhanced selectivity of **3** toward Ca^{2+} ions. The selective chromogenic response toward Ca^{2+} ions from orange to purple can be readily detected with naked eye (Fig. 1).

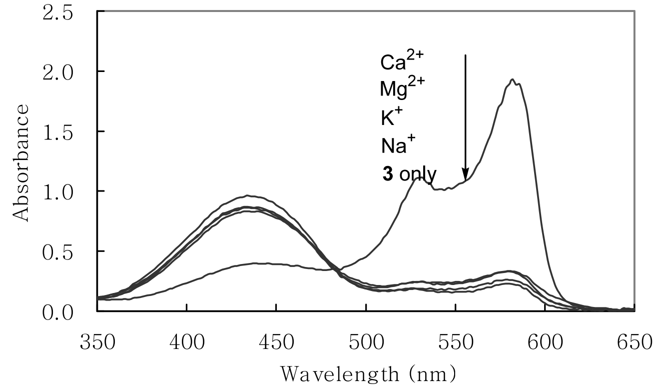

Figure 1. Absorption spectra of **3** in aqueous MeOH solution. [**3**] = 1.25×10^{-5} M. [M^{n+}] = 1.25×10^{-3} M in MeOH–H_2O (1:1, v/v).

The Ca^{2+}-selective ionophoric properties of **3** could be confirmed by the NMR and mass spectrometric observations. In its ^1H NMR spectrum, the compound **3** showed significant changes in the resonances for the

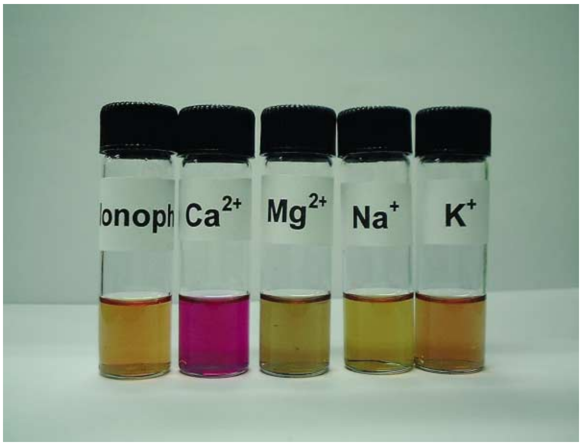

Figure 2. Color changes of **3** in the presence of metal ions. [**3**] = 1.25×10^{-5} M. [M^{n+}] = 1.25×10^{-3} M in MeOH–H_2O (1:1, v/v).

benzothiazolium moiety and bridging methylene protons in the presence of Ca^{2+} ions in DMSO-d_6 solution. In contrast to this, other metal ions of Na^+ and K^+ exhibited almost no changes in the spectra. The ionophoric properties toward Ca^{2+} ions were further evidenced by the observation of a strong peak for $[M+Ca+ClO_4]^+$ at $m/z = 1163.9$ in FAB (*m*-NBA) and MALDI-TOF spectra. The role of the calix[4]arene moiety in this ionophore is confirmed by the control experiments with the model compound of benzothiazolium **4** which devoid of the calix[4]arene binding site. Compound **4** has no chromogenic resposes toward Ca^{2+} ions as well as other tested metal ions under the same experimental conditions (Fig. 2).

We next determined the detection limit of ionophore **3** from the Ca^{2+} ion concentration dependent chromogenic responses (Fig. 3). From the plot of the changes in absorption intensity as a function of Ca^{2+} ion concentration, the detection limit[14] was estimated to be 1.2×10^{-6} M that is sufficient for the analysis of micromolar concentration range of calcium ions in many chemical and biological systems. The binding stoichiometry for the **3**–Ca^{2+} system was found to be 1:1 and the association constant determined by the nonlinear curve fitting procedure[15] was found to be 2.4×10^{6} M^{-1}.

With the observation of Ca^{2+} selectivity, the possibility of ionophore **3** for the chromogenic determination of calcium ion concentration in biological samples was investigated. The changes in absorption spectra were monitored as a function of [Ca^{2+}] in the presence of physiologically important metal ions ([Na^+] = 145 mM, [K^+] = 5 mM, and [Mg^{2+}] = 2 mM)[16] as background. The ionophore **3** showed almost identical Ca^{2+}-dependent spectral responses in the presence of competing background metal ions in MeOH–H_2O (1:1, v/v) solvent system with somewhat increased residual chromogenic behavior due to the presence of high concentration of the background metal ions. The plot of the absorption intensity ratio at 530 and 434 nm (Abs_{530}/Abs_{434}) versus [Ca^{2+}] showed a well-defined dependency on calcium ion concentration which suggests the practical applicability of compound **3** as a probe for the calcium ions in the common concentration range of 2.5–5 mM in physiological samples.

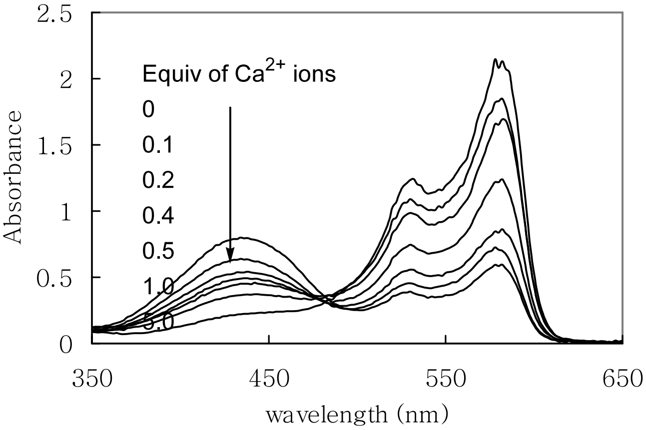

Figure 3. Absorption spectra of **3** as a function of [Ca^{2+}]. [**3**] = 1.25×10^{-5} M in MeOH–H_2O (1:1, v/v).

The prepared compound has structural characteristics comprising of phenolic function of calix[4]arene framework conjugated with benzothiazolium moiety which might revealed a sensitive change in its chromogenic behavior as a function of the pH of the medium. Therefore, we measured the pH dependent absorption behavior of **3** by varying the pH of the solution with 0.1 M HCl or 0.1 M LiOH. The color of aqueous methanolic solution of **3** is orange in acidic region around pH 4 having 430 nm band as major absorption while in basic region around pH 8 the color transformed into a bluish purple with 590 nm band as major response. This type of response is frequently observed for other merocyanine type dyes of related structures.[6] As the pH of the medium increases from 4 to 8, the absorption spectra transformed progressively from the acidic form into basic form (Fig. 4). This pH-dependent spectral behavior of **3** could be used as a new pH-probe around pH 6.5. We also have investigated the pH-

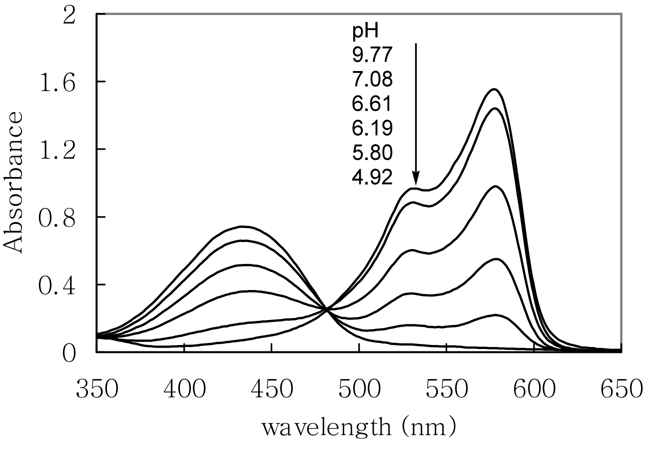

Figure 4. Effect of pH on the absorption spectra of **3** in MeOH–H_2O (1:1, v/v).

dependency of the Ca^{2+}-selective chromogenic behavior of the ionophore **3** and found that the compound showed a distinct color change from yellow around pH 6 to red around pH 7 in the presence of 100 equiv. of Ca^{2+} ions. The color transition has occurred at similar pH range compared with the results of ionophore itself in the absence of any metal ions. So the practical pH range for the selective chromogenic detection of Ca^{2+} ions with compound **3** seems to be in the region below pH 6.

In conclusion, a new chromogenic ionophore derived from calix[4]arene–diamide derivative having appended benzothiazolium moiety showed a selective and sensitive chromogenic behavior toward Ca^{2+} ions in aqueous MeOH solution. The response was retained in the presence of competing metal ions of physiologically important Na^+, K^+, and Mg^{2+} ions. The ionophore also revealed a sensitive variation in its absorption behavior as a function of the pH, which can be used as a new pH-probe in the region of pH 6.5.

Acknowledgements

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