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Selective Fluorometric Sensing of Calcium Cation by C-Pivot Lariat Monoaza-crown Ether with Two Pyrene Moieties

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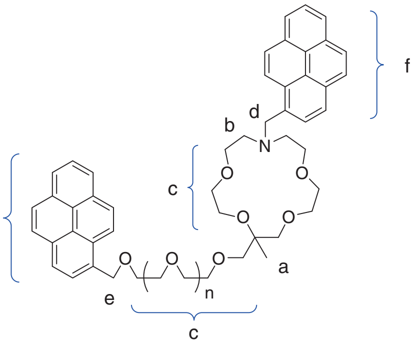
A new type of C-pivot lariat monoaza-crown ether **1b** containing a 15-crown-5 ring and two pyrene moieties on the sidearms was found to be a highly sensitive and selective fluorescent chemosensor toward Ca^{2+} in the presence of Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+}.

Much attention has been focused on the development of sensing techniques for specific cations in a variety of research fields of chemistry, biology, and medicine.[1] Selective and sensitive chemosensors for Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} are strongly desired for detection of these cations in living organisms.[2] Supramolecular chemosensors used for cation recognition are composed of a binding part for guest cations and a sensing part to read information about the complexation.[3] Thus, a number of chemosensors have been developed by an elaborate combination of both binding and sensing parts.[3] Recently, we found that C-pivot lariat crown ethers[4] with two pyrenylmethyl groups on the electron-donating sidearms are potentially useful as selective chemosensors for alkaline earth metal cations.[5] In this case, effective coordination of the electron-donating sidearm to metal cations resulted in changing the spatial distance of the two pyrene rings. The ratiometry of monomer and excimer emissions of the pyrene moieties[6] was successfully used for the selective detection of metal cations. A combination of this sensing mechanism with photoinduced electron transfer (PET), another effective method for detecting metal cations,[3,7] should afford an interesting strategy to develop new fluorescent chemosensors. From this point of view, we describe the design and synthesis of novel chemosensors derived from C-pivot lariat monoaza-crown ethers containing a monoaza-15-crown-5 ring and two pyrene moieties and their fluorescent properties toward Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} cations.

Monoaza-15-crown-5 was selected as the main binding site of new fluorescent chemosensors suitable for Ca^{2+}, since effective collaboration of the 15-crown-5 ring with an electron-donating sidearm upon complexation with Ca^{2+} was expected based on our previous studies on lariat ethers.[8] An electron-donating sidearm was introduced to the C-pivot position of the monoaza-crown ether by the reaction of a key intermediate, 5-bromomethyl-5-methyl-1,4,7,10-tetraoxa-13-azacyclopentadecane,[9] with ethylene glycol or diethylene glycol under basic conditions to give the corresponding C-pivot lariat monoaza-crown ethers, respectively (Scheme S1[13]). The reaction of these compounds with 1-bromomethylpyrene was carried out in THF in the presence of NaH at reflux for 36 and 60 h to afford **1a** and **1b** in 46% and 32% yield, respectively. The structures (Figure 1) were confirmed by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis.[10]



1a n = 0
1b n = 1

Figure 1. Structures of **1a** and **1b**; labels a–f denote the corresponding hydrogen atoms.

Figure 2 shows the fluorescence spectra of **1a** and **1b** at a concentration of 1.0×10^{-6} M in CH_3CN in the absence and presence of metal cations. These compounds possess two types of pyrenylmethyl groups, one at the terminal of the electron-donating sidearm of the C-pivot position and the other at the N-pivot position of the monoaza-crown ring. It should be noted that the fluorescence of both **1a** and **1b** based on the pyrene ring was effectively quenched due to the PET from the amino group, in spite of the presence of two pyrene rings for the single amino group. This result showed that both pyrene rings followed the PET mechanism.[7b] Upon complexation with a metal cation, the nitrogen lone pair no longer participates in the PET process, causing recovery of the fluorescence. A remarkable increase of monomer emission at 375 nm was observed for **1a** upon the addition of Mg^{2+} (16 times) and Ca^{2+} (18 times), without a significant increase of excimer emission at 475 nm (Figure 3). On the other hand, **1b** with its longer electron-donating sidearm had an increased excimer emission for Ca^{2+} (12 times) and monomer emission for Mg^{2+} (8 times). Since the increase of excimer emission is attributed to the intramolecular π–π stacking of the two pyrene rings, this finding strongly suggests that the length of the oxyethylene chain on the sidearm plays a crucial role in bringing the two pyrene rings into close proximity. It should be noted that the increase of excimer emission upon the addition of Ca^{2+} observed in fluorophore **1b** was completely in contrast to the decrease of excimer emission in the case of C-pivot lariat crown ethers previously reported.[5]

The stability constants (K) of the complex were evaluated from plots of monomer or excimer emission intensities vs. [metal]/[ligand] by means of a nonlinear least-square curve-fitting method.[11] The curve showed that both ligands formed 1:1 complexes. The log K values of **1a** and **1b** toward metal cations

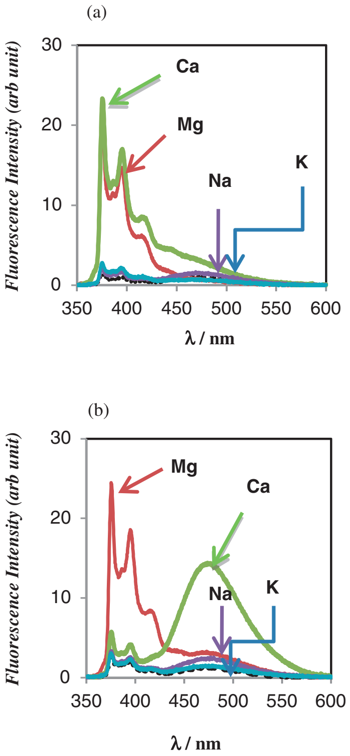


Figure 2. (a) Fluorescence spectra of **1a** (1×10^{-6} M) and (b) **1b** (1×10^{-6} M) in CH_3CN in the absence (black dotted line) and presence of metal cations (3.2×10^{-6} M). Excitation wavelength: 340 nm.

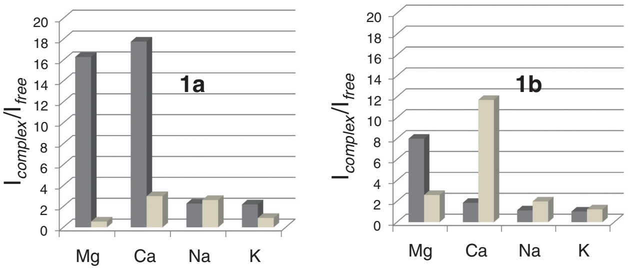


Figure 3. Change of fluorescence intensity of **1a** or **1b** (1.0×10^{-6} M) upon addition of metal ions (3.2×10^{-6} M) in CH_3CN; dark gray column: monomer emission at 375 nm; pale gray column: excimer emission at 475 nm. Excitation wavelength: 340 nm.

are summarized in Table 1. Both **1a** and **1b** showed a good affinity toward Ca^{2+}. This result is reasonably explained by considering the fitness of the cavity size of the ligand and the cation size and the difference in the charge density of alkaline earth metal cation and alkali metal cation.

In order to elucidate the function of the electron-donating sidearm, ^{1}H NMR spectral changes of **1a** and **1b** were examined upon addition of $Ca(ClO_4)_2$ in 9:1 v/v $CD_3CN/CDCl_3$ (Figure S3[13]). Pyrene protons (f) of **1a** and **1b** were observed at 7.95–8.62 ppm in the absence of Ca^{2+}. Upon addition of an equimolar amount of Ca^{2+}, the pyrene protons were shifted upfield to 7.63–8.50 ppm for **1a** and 7.42–8.32 ppm for **1b**. A larger upfield shift of the pyrene protons of **1b** compared to those of **1a** provided evidence for the proximity of the two pyrene rings of **1b**. The singlet peak of the methylene protons (e) of **1a** at 5.22 ppm was separated into a pair of doublet and shifted downfield to 5.4 ppm upon addition of Ca^{2+}, but the corresponding peak (e) of **1b** at 5.22 ppm was only shifted downfield to 5.3 ppm and was not split. This result may suggest that the pyrenylmethyl group on the electron-donating sidearm of **1b** moves freely and can easily access another pyrene ring. In the case of the **1a**$\cdot Ca^{2+}$ complex, all oxygen atoms of the electron-donating sidearm of **1a** coordinate to Ca^{2+}, and the movement of the pyrenylmethyl group is highly restricted. In addition, the length of the electron-donating sidearm of **1a** may be too short to promote π–π interaction of the two pyrene rings in the complex with Ca^{2+}.

Table 1. The log K values of **1a** and **1b** for metal cations in CH_3CN

Compound	Mg^{2+}	Ca^{2+}	Na^+	K^+
1a	6.7	7.9	5.0	nd
1b	6.3	7.1	5.1	nd

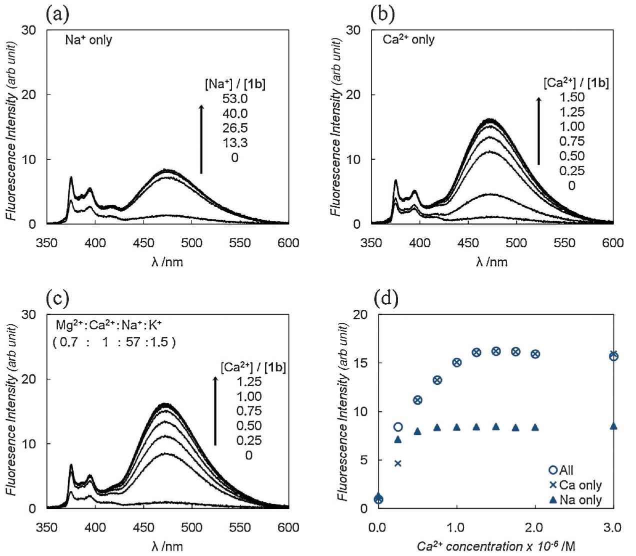


Figure 4. Fluorescence spectral changes of **1b** (1.0×10^{-6} M) with different concentrations of alkali metal and alkaline earth metal cations: (a) Na^+ only, (b) Ca^{2+} only, (c) Mg^{2+}, Ca^{2+}, Na^+, K^+, and (d) fluorescence intensity changes of **1b** at 475 nm (○ Mg^{2+}, Ca^{2+}, Na^+, K^+; × Ca^{2+} only; and ▲ Na^+ only) in CH_3CN. Excitation wavelength: 340 nm.

Next, the selective fluorescent detection of Ca^{2+} using **1b** was examined in the presence of metal cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}) relevant to living organisms. The concentrations of these metal chlorides were prepared by considering the human serum constitution[12] to be 142×10^{-3} (Na^+), 4.14×10^{-3} (K^+), 1.69×10^{-3} (Mg^{2+}), and 2.50×10^{-3} M (Ca^{2+}) in water, and then the solutions were diluted ten times with CH_3CN to generate the stock solutions (9:1 = CH_3CN:H_2O). The fluorescent spectral changes of **1b** (1.0×10^{-6} M) with different concentrations of metal cations are shown in Figure 4. The addition of Na^+ or Ca^{2+} increased the excimer emission of pyrene rings at 475 nm (Figures 4a and 4b). However, the maximum fluorescence intensity with Ca^{2+} was much higher than that with Na^+, and a large excess of metal cations was needed to achieve the maximum fluorescence with Na^+. The fluorescence behavior of **1b** in the presence of a mixture of Na^+, K^+, Mg^{2+}, and Ca^{2+} was found to be very similar to that of **1b** in the presence of only Ca^{2+} (Figure 4c). Thus, the fluorescence intensities at 475 nm were plotted as the functions of Ca^{2+} concentration (Figure 4d). After exceeding 0.5×10^{-6} M of Ca^{2+} concentration, the plots of the fluorescence intensities for a mixture of metal cations coincided with those for only Ca^{2+} at the same concentrations. This result demonstrates that **1b** can selectively detect Ca^{2+} in spite of the disturbance of other metal cations. Based on the graph of Figure 4d, a standard curve prepared by plotting Ca^{2+} concentrations on the x axis and the differences of fluorescence intensities of **1b** between the mixed system and the Ca^{2+} single system on the y axis (Figure S4[13]) yielded a good linear relationship. As a result, the concentrations of Ca^{2+} were successfully determined at $0.25\text{–}1.25 \times 10^{-6}$ M in the presence of a mixture of Na^+, K^+, Mg^{2+}, and Ca^{2+}.

In summary, two kinds of C-pivot lariat monoaza-crown ethers containing a 15-crown-5 ring and two pyrene moieties on the sidearms (**1a** and **1b**) were prepared and their fluorescent properties toward Na^+, K^+, Mg^{2+}, and Ca^{2+} were examined. These chemosensors can discriminate among various metal cations by using both ratiometry of the pyrene excimer and monomer emissions and the PET mechanism. Compound **1b** was found to be a highly sensitive and selective chemosensor for Ca^{2+}. Continuing molecular design of this type of fluorophore is underway in our laboratory.

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10 **1a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (d, $J = 9.3$ Hz, 1H), 8.33 (d, $J = 9.3$ Hz, 1H), 8.19–7.82 (m, 16H), 5.22 (s, 2H), 4.28 (s, 2H), 3.82–3.23 (m, 20H), 2.99–2.74 (m, 4H), 1.19 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 133.2, 131.4, 131.2, 131.2, 131.1, 130.8, 130.7, 130.6, 129.8, 129.3, 128.0, 127.6, 127.4, 127.3, 127.3, 126.9, 126.9, 126.9, 125.8, 125.7, 125.1, 124.9, 124.8, 124.8, 124.7, 124.6, 124.4, 124.3, 124.2, 123.5, 76.8, 74.5, 73.8, 71.7, 71.2, 71.1, 70.4, 70.1, 70.0, 69.5, 69.4, 62.2, 59.1, 54.6, 54.4, 18.4. MS(ESI) m/z: 758 $[M + Na^+]$. Anal. Calcd for $C_{48}H_{49}NO_6$: C, 78.34; H, 6.71; N, 1.90%. Found: C, 78.09; H, 6.65; N, 1.84%. **1b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (d, $J = 9.3$ Hz, 1H), 8.34 (d, $J = 9.0$ Hz, 1H), 8.17–7.90 (m, 16H), 5.22 (s, 2H), 4.27 (s, 2H), 3.72–3.34 (m, 24H), 2.91–2.78 (m, 4H), 1.15 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 133.2, 131.3, 131.2, 131.2, 131.1, 130.8, 130.7, 130.6, 129.7, 129.3, 128.0, 127.6, 127.4, 127.3, 127.3, 126.9, 126.9, 126.9, 125.8, 125.7, 125.1, 124.9, 124.8, 124.8, 124.7, 124.6, 124.4, 124.3, 124.2, 123.4, 76.7, 74.4, 73.7, 71.8, 71.1, 71.0, 70.7, 70.5, 70.3, 70.1, 70.0, 69.5, 69.4, 62.2, 59.1, 54.5, 54.4, 18.3. MS(ESI) m/z: 802 $[M + Na^+]$. Anal. Calcd for $C_{50}H_{53}NO_7$: C, 77.00; H, 6.85; N, 1.80%. Found: C, 76.97; H, 6.81; N, 1.67%.

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