

Fluorescent Monitoring of Complexation of Phthalimide-Fused Crown Ethers with Alkali Metal Ions

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Phthalimide-fused mono(crown ether)s (**1** and **2**) and bis(crown ether)s (**3** and **4**) were prepared. These imide derivatives strongly fluoresce in methanol, and the fluorescence intensity is affected by alkali metal ions due to crown–ion interactions. While fluorescence increases upon complexation involving a single macrocycle, it decreases when the fluorophores take a face-to-face arrangement. This property was used to monitor the process of ion complexation and concomitant conformation changes. With the incremental addition of cesium ions (M) to the bis(crown ether)s (**L**), a folded sandwich complex (LM) initially formed, which subsequently transformed into a more extended LM_2-type complex. This behavior, which was independently confirmed by ^{1}H NMR spectroscopy, is clearly manifested in the fluorescence intensity.

Fluorescent ionophores, in which a fluorophore unit is combined with a receptor site for ions, are widely used to monitor ion-recognition events.[1,2] There are several approaches connecting a fluorophore and a receptor. One of them is the spaced fluorophore–receptor system, in which a fluorophore and a receptor are connected through a spacer module, but effectively separated in terms of structure and electronic interactions. Another is the integrated fluorophore–receptor system in which a fluorophore and a receptor share part of the molecular framework. The former system is widely exploited in photo-induced electron-transfer sensors, since it can give rise to a large on/off ratio, depending on the presence or absence of metal ions, and is thus useful as sensors and switches. On the other hand, fluorescence from the latter type of fluorescent ionophores may reflect the recognition event in a more subtle fashion. Whereas this is disadvantageous for the purpose of sensing and switching, the integrated fluoro-ionophores are more sensitive to their microenvironment, and can thus be more useful for shedding light on the recognition process.[3]

It is well known that a metal ion too large to be accommodated in a single macrocycle associates with two macrocycles to form a sandwich complex.[4] Bis(crown ether)s, in which two crown ethers are connected via a spacer, are particularly effective in this respect due to entropic gain in the process of bringing two macrocycles together around the captured ion. This sandwich complexation is reflected in enhanced affinity and altered selectivity toward a given metal ion.[4] Furthermore, it can be used to control the conformation of a molecule, aiming to effect biomimetic allosterism or other molecular-scale functions.[5] Although the importance of, and interest in, sandwich complexation are well recognized, examples in which the sandwich complexation is monitored by means of fluorescence are rare,[6,7] probably because an appropriate fluorophore system that can differentiate a 2:1 sandwich complex from a usual 1:1 complex is hard to implement in a host compound. We have prepared bis(crown ether)s **3** and **4** for the purpose of monitoring the process of sandwich complexation by fluorescence. The dialkoxyphthalimide group and crown-ether ring are integrated structurally and electronically in these host compounds. The phthalimide moieties are desinged to be forced to stack with one another when the host forms a sandwich complex with a metal ion and takes a folded conformation. It was hoped that the stacking is reflected in the fluorescence properties of the fluorophore, as is indeed the case, as described below. Before describing the behaviors of the bis(crown ether)s, the fluorescence properties of mono(crown ether)s **1** and **2** are described as model compounds, which may also be of interest in their own right (Chart 1).

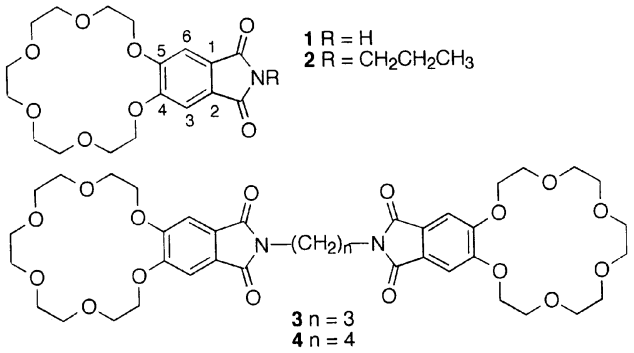

Chart 1.

Experimental

Fluorometric-grade methanol was purchased from Kanto

Chemical and used as received. Acetates were used as alkali metal salts. The electronic absorption spectra were recorded on a Shimadzu UV-2400PC. The fluorescence spectra were taken on a Shimadzu RF-5300PC. The temperature of the sample solutions was kept at 25 °C by circulating thermostatically controlled water. The sample concentrations were 10 μM and 5 μM for the mono(crown ether)s and bis(crown ether)s, respectively, in the fluorescence measurements so that the concentration of the imide unit would be constant (1 M = mol dm^{-3}). The fluorescence quantum yields were determined using 2-aminopyridine as a standard. The melting points were measured on a Yanako MP500D apparatus. ^{1}H NMR spectra were taken on a JEOL JNM-GX400 spectrometer, using TMS as an internal standard. HRMS spectra were taken on JEOL-JMS600H mass spectrometer.[8] ZINDO calculations were carried out using a Sony Techtronics CAChe package on the geometry optimized structure with INDO/1 parameters. The association constants were determined by a home-made least-squares curve fitting program using either the fluorescence intensity or the ^{1}H NMR chemical shift changes. For definitions of the association constants (K, K_1, and K_2), see footnotes b–d in Table 1. The conditional standard deviations were less than 10% of the K values.

After 2,5,8,11,14,17-hexaoxabicyclo[16.4.0]dodeca-1(18),19,-21-triene-20,21-dicarboxylic acid or the corresponding anhydride[5] was dissolved in methanol, the solution was mixed and heated with urea, 1-propylamine, 1,3-diaminopropane, and 1,4-diaminobutane, giving the desired imides, **1**, **2**, **3**, and **4**, respectively, which were purified as described below.

The imide **1** was crystallized from methanol (30%), mp 183.5–185.5 °C; Found: C, 56.25; H, 5.94; N, 3.66%; HRMS *m/z* 381.1427. Calcd for $C_{18}H_{23}NO_8$: C, 56.69; H, 6.08; N, 3.67%; 381.1423; ^{1}H NMR ($CDCl_3$) δ 3.69 (4H, s, OCH_2); 3.73 (4H, t, J = 4 Hz, OCH_2); 3.79 (4H, t, J = 4 Hz, OCH_2); 3.96 (4H, t, J = 4 Hz, OCH_2); 4.26 (4H, t, J = 4 Hz, OCH_2); 7.27 (2H, s, ArH); 7.92 (1H, s, NH). The *N*-propylimide **2** was crystallized from methanol (30%), mp 167.5–169 °C; Found: C, 59.33; H, 6.77; N, 3.29%; HRMS: *m/z* 423.1894. Calcd for $C_{21}H_{29}NO_8$: C, 59.56; H, 6.96; N, 3.31%; 423.1893; ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.5 Hz, CH_3); 1.71 (2H, sextet, J = 7.5 Hz, $MeCH_2$), 3.59 (2H, t, J = 7.5 Hz, NCH_2), 3.7–3.85 (12H, OCH_2), 3.96 (4H, t, J = 4.5 Hz, OCH_2), 4.24 (4H, t, J = 4.5 Hz, OCH_2), 7.27 (2H, s, ArH). The trimethylene-linked **3** was purified by silica-gel PLC using chloroform/methanol (= 7/3) as eluent and crystallization from methanol (49%), mp 172.5–174.5 °C; Found: *m/z* 802.3221. Calcd for $C_{39}H_{50}N_2O_{16}$: 802.3159;[9] ^{1}H NMR ($CDCl_3$) δ 2.05 (2H, quintet, J = 7.2 Hz, $CH_2CH_2CH_2$); 3.7–3.8 (28 H, OCH_2, NCH_2); 3.96 (8H, t, J = 4 Hz, OCH_2); 4.24 (8H, t, J = 4 Hz, OCH_2); 7.25 (4H, s). The tetramethylene-linked **4** was purified by silica-gel colunm chromatography and crystallization from chloroform/methanol (44%), mp 188.4–189.3 °C; Found: C, 58.38; H, 6.27; 3.42%; HRMS *m/z* 817.3384. Calcd for $C_{40}H_{52}N_2O_{16}$: C, 58.82; H, 6.42; N, 3.43%; HRMS *m/z* 817.3395 (MH); ^{1}H NMR ($CDCl_3$) δ 1.6–1.7 (4H, $CH_2CH_2CH_2CH_2$); 3.6–3.8 (28H, OCH_2, NCH_2); 3.95 (8H, t, J = 4 Hz, OCH_2); 4.24 (8H, t, J = 4 Hz, OCH_2), 7.24 (4H, s, ArH).

Results and Discussion

Mono(crown ether)s, 1 and 2. The absorption spectrum for **1** has peaks at 296 and 337 nm (Table 1 and Fig. 1a). The ZINDO calculations show that the lowest energy absorption bands correspond to a $\pi\pi^*$ transition with a considerable charge-transfer character. The HOMO includes p_z-orbitals of alkoxy oxygens and carbons-1, -2, -4, and -5 of the benzene ring, while the LUMO lies on the carbonyls and carbons-1 and -2 of the benzene ring.[10] Hence, it is expected from a simple electrostatic consideration that a cation bound to the macrocycle lowers the HOMO level, but exerts less effect on the LUMO. This argument has been corroborated by the observation of a blue-shift in λ_{max} by 8 nm with isosbestic points upon the addition of potassium acetate, as shown in Fig. 1a.

Upon excitation into these absorption bands, compound **1** emits strong fluorescence at 480 nm. Figure 1b shows the effect of the addition of potassium acetate on the fluorescence spectrum of **1** excited at isosbestic 330 nm in methanol. A blue-shift by 16 nm and an increase in the intensity are observed. The fluorescence increases monotonously with increasing concentration of the added ions. Similar moderate increases in the fluorescence intensity upon the addition of alkali metal ions are observed for related fluorescent crown ethers.[11–13] It was shown that rigidifying the molecular framework of the host compounds induced by ion complexation suppresses the rate of internal quenching, leading to a fluorescent enhancement.[13]

Bcause the changes in the fluorescence intensity fit well to the calculated curves based on the 1:1 complexation, the association constants (K) and limited relative intensities (I_∞/I_0) at

Table 1. Absorption and Fluorescence Properties of **1**–**4** in Methanol at 25 °C

	Absorption λ_{max}/nm ($\log \varepsilon$)	Fluorescence λ_{max}/nm (ϕ)	$\log (K/M^{-1})$ (I_∞/I_0[a)])			
			Na^+	K^+	Rb^+	Cs^+
1	296 (3.3) 337 (3.3)	480 (0.22)	3.9 (1.44)	4.4 (1.57)	3.9 (1.34)	3.3 (1.26)
2	296 (3.3) 346 (3.2)	488 (0.13)	3.8 (1.52)	4.5 (1.70)	3.8 (1.46)	3.4 (1.27)
3	296 (3.6) 348 (3.5)	490 (0.05)	3.2 (4.4)[b)]	3.9 (4.0)[b)]	3.1 (3.3)[b)]	4.7 (0.24)[c)], 1.7 (3.2)[d)]
4	296 (3.5) 347 (3.5)	490 (0.06)	3.4 (3.9)[b)]	4.1 (4.3)[b)]	3.2 (3.0)[b)]	4.6 (0.39)[c)], 2.0 (2.6)[d)]

a) Relative fluorescence intensity at the λ_{max} of the complex. The limited value I_∞ at [M] $\rightarrow \infty$ was estimated by the curve-fitting procedure. b) Apparent association constants with respect to a single crown unit, C ([C] = [L] (L = **1**, **2**; [C] = 2[L] (L = **3**, **4**)), based on the assumption that each macrocycle in the bis(crown ether)s behaves independently: K = [CM]/[C][M]. See text. c) For the first ion binding: K_1 = [LM]/[L][M]. d) For the second ion binding, K_2 = [LM_2]/[LM][M].

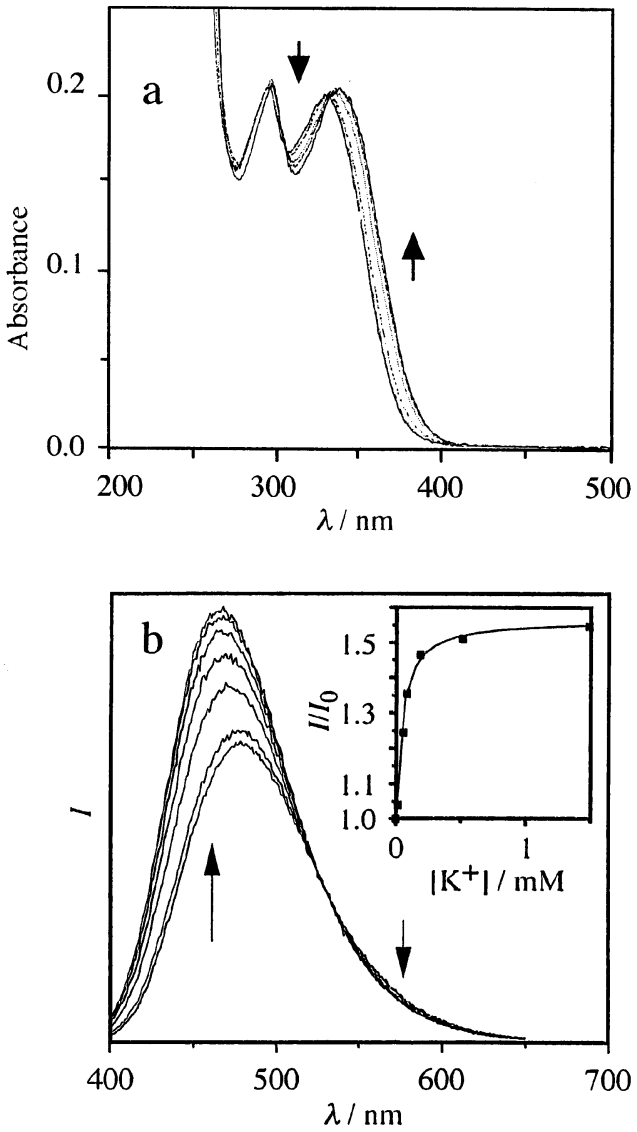

Fig. 1. Changes in the spectra of **1** upon the addition of potassium acetate in methanol at 25 °C. (a) The absorption changes of **1** (100 μM). (b) The fluorescence changes of **1** (10 μM). The inset shows the relative fluorescence intensity at 464 nm. The calculated curve is superimposed on observed values shown as squares.

the λ_{max} of the complex were obtained, and are summarized in Table 1. The ion selectivity is in the order $K^+ > Na^+ \approx Rb^+ > Cs^+ >> Li^+$, which is in accord with the known selectivity of 18-crown-6, although the *K* values are smaller than those reported for benzo-18-crown-6[4] because of a decreased electron density on the phenolic oxygens due to the electron-withdrawing imide group. The fluorescence and its ion-responsive behavior of *N*-propyl derivative **2** are also given in Table 1. Although the quantum yield is diminished nearly by half by *N*-substitution, the values of *K* as well as that of I_∞/I_0 are similar to those of unsubstituted **1**.

Bis(crown ether)s, 3 and 4. The molar-absorption coefficients for the bis(crown ether)s, **3** and **4**, are as expected from the values for **2**; the coefficient values per chromophore for **3** and **4** are about the same as that found for **2** as shown in Table 1. It was confirmed that the coefficient values are independent of the concentration, ranging from 5 to 50 μM. However, the fluorescence quantum yields for the bis(crown ether)s are less than half that for the mono-counterpart **2**. These results in the absorption and fluorescence spectra imply some intramolecular electronic interactions between the two chromophores, not in the ground state but also in the excited state. In the ^1H NMR spectra in CD_3OD, the protons of the benzene rings in **2** (1 mM), **3** (0.5 mM), and **4** (0.5 mM) exhibit resonances at 7.40, 7.25, and 7.21 ppm, respectively. These upfield shifts in the bis-compounds, which are most reasonably explicable by a ring-current effect, suggest that the aromatic rings take a face-to-face arrangement to some extent as a time-averaged conformation. The polarity of the solvent appears to play a role in facilitating the fluorophore-fluorophore interactions, since the resonances of the same protons in **2**, **3**, and **4** appear at as closely as 7.27, 7.25, and 7.24 ppm, respectively, in less polar $CDCl_3$.

Clear isosbestic points in the absorption spectra and a monotonous increase in the fluorescence emission similar to the cases of **1** and **2** are observed upon the addition of Na^+ or K^+ to **3** or **4**. It is different, though, in that the relative limiting fluorescence intensities in these cases are much larger than that for **2**: around 4-fold enhancement is achieved. This is related to the fact that the fluorescence of these bis(crown ether)s is weaker than that of **2** in the absence of ions due to intramolecular fluorophore interactions, as described above. The ion complexation and conformational unfolding (see below), which both result in a fluorescence increase, are expected to add up to the augmented effect for the bis(crown ether)s.

The apparent association constants of **3** and **4** with Na^+ and K^+ displayed in Table 1 are calculated based on the assumption that the two macrocycles behave independently. These values are consistently smaller than those of **2**, and **1** for that matter, by factors of 4–5. The process of ion (M) complexation with the bis(crown ether)s (**L**) consists of two steps, i.e. the first ion binding to form LM and the second ion binding to form LM_2. Although the affinity of the first ion binding may be comparable to that for **2**, the second ion binding must be disfavored due to electrostatic repulsion. In other words, the apparent diminished affinity toward ions indicates the presence of communication between the two ions captured by the same bis(crown ether) molecule. Because the fluorescence cannot distingush between the first and second ion binding, the thus-obtained association constants involve contributions from both processes. The *K* values for **4** are larger than those for **3** by small, but consistent, margins. The longer spacer in **4** may alleviate more effectively the electrostatic repulsion in the second ion binding. A rare opportunity to distingush these processes clearly is provided by using Cs^+ as described below.

A two-stage change in the absorption spectra takes place upon an incremental addition of Rb^+ or Cs^+ to **3** or **4**, although the change is small. We focus our discussion on the fluorescence, since the change is much more distinctive. The fluores-

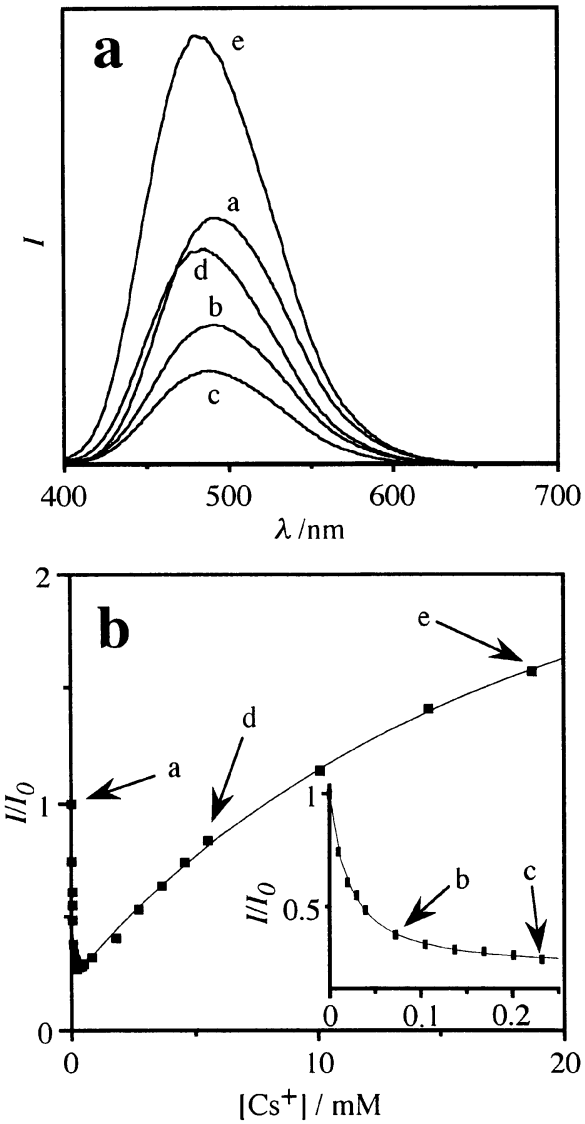

Fig. 2. Fluorescence of **3** (5 μM) upon the addition of cesium acetate in methanol at 25 °C. (a) Spectral change. (b) Relative fluorescence intensity at 482 nm. The low concentration range is shown magnified in the inset. The calculated curve is superimposed on observed values shown as squares.

cence decreases by adding these ions in a low concentration range; this effect is especially pronounced for the larger Cs^+, as shown in Fig. 2. This behavior is readily explained by assuming the formation of a sandwich complex.[4,5] The intramolecular sandwich-complex formation forces the two imide fluorophores to take a face-to-face stacking conformation, which should be the reason for the diminished fluorescence. Apparently, the ππ interactions of the imide moieties are signigicantly enhanced by the sandwich-complex formation in the bis(crown ether)s, which take a face-to-face conformation to some extent, even in the absence of ions, as described previously. Similar fluorescence quenching due to stacking interactions is known for other π-systems and is ascribed to concentration quenching.[7] With the further addition of these ions, a molecule of **3** begins to accommodate two ions (one ion in each macrocycle) and the behavior turns to a "normal" one, that is, an increase in the fluorescence intensity.

The association constants for the sandwich-complex formation between the bis(crown ether)s and Cs^+ are the largest among the entries given in Table 1, which underscores the effectiveness of sandwich complexation. The second ion binding to the sandwich complex is nearly three orders of magnitude less favored. This is also much more unfavorable than the 1:1 complex formation between **2** and Cs^+, due to the electrostatic repulsion between the first and second ions. In the case of Rb^+, only a small initial decrease in the fluorescence intensity was observed; it was not possible to determine the association constant for the first ion binding. Apparently, the association constant for the first ion binding is not so large that the second ion binding begins well before the sandwich-complex formation is completed.

The fluorescence behavior is fully consistent with the ^{1}H NMR spectra. For example, the resonance at 7.25 ppm (0.5 mM in CD_3OD) of the aromatic protons of **3** is first shifted upfield to 7.11 ppm upon the addition of up to 1 mM Cs^+ (92% of **3** forms the sandwich complex based on the association constant), then down-field to 7.38 ppm with further addition of 100 mM Cs^+ (90% of **3** forms LM_2-type complex), as shown in Fig. 3. The downfield shift is indicative of unfolding of the host conformation in the process of the LM_2 complex formation. Although the first association constant could not be determined accurately due to experimental uncertainties, the second association constant derived from NMR ($\ln K_2 = 1.6$) agreed with the value determined from the fluorescence titration. These processes are shown schematically in Fig. 4.

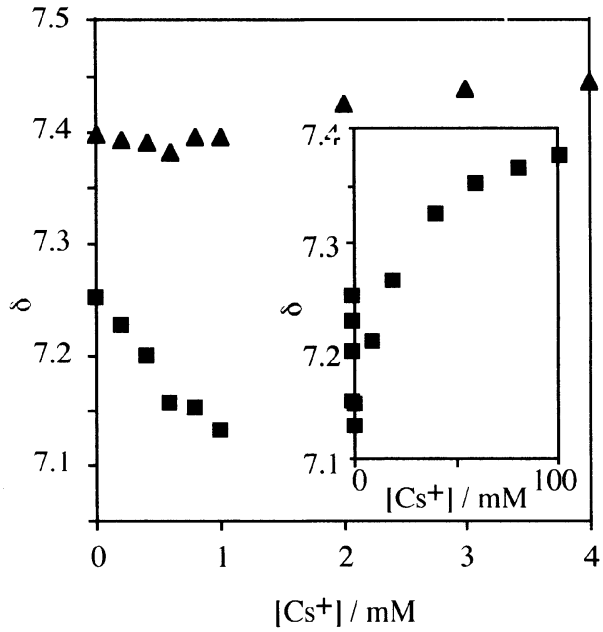

Fig. 3. Cesium ion induced chemical shift changes of the aromatic protons in **2** (1 mM; triangle) and **3** (0.5 mM; square) in CD_3OD at 25 °C. A wider concentration range (0–100 mM) in the case of **3** is shown in the inset.

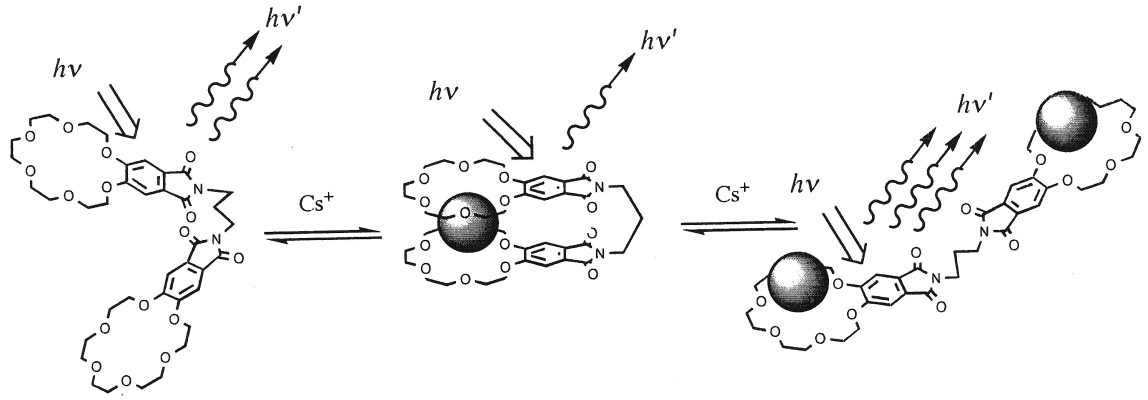

Fig. 4. Schematic representation of the first ion binding to form a sandwich complex and the second ion binding to form a LM_2 complex.

A similar ^{1}H NMR titration was conducted for mono(crown ether) **2** and the results are also included in Fig. 3. The chemical shift of aromatic protons of **2** undergoes a small upfield shift and then turns to a downfield shift. The fact that the initial upfield shift for **2** is much smaller than that for **3** suggests that although the *inter*molecular sandwich-complex formation actually occurs, it is much more disfavored than *intra*molecular sandwich complexation of **3**. Note that the NMR experiments were carried out for two-orders-of-magnitude concentrated samples (~mM) that favor intermolecular processes compared to those for fluorescence measurements (~10 μM). Indeed, the fluorescence behavior of mono(crown ether)s did not show any indication of intermolecular complex formation.

We have thus demonstrated here that the fluorophore used in this study, i.e. phthalimide fused with a crown ether macrocycle, can be used to monitor ion complexation and associated conformational changes. It has recently been shown that 4,5-dimethoxyphthalimide is useful for the chromophoric derivatization of the amino group for circular dichroism studies directed towards the determination of the absolute configuration of chiral amines.[14] It can be envisaged that these properties of the phthalimide unit may find use as optical probes for a microenvironment under wider circumstances, some of which are being explored in our laboratories.

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8 We are grateful to I. Yoshikawa for the measurements of MS spectra.

9 Compound **3** was not crystaline, and it was not possible to obtain satisfactory microanalysis. Its purity was confirmed by HPLC.

10 See Chart 1 for numbering.

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