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Synthesis and binding properties of calix[4]biscrown-based fluorescent molecular sensors for caesium or potassium ions

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The synthesis of two new fluorescent molecular sensors based on calix[4]biscrowns is reported. A dioxycoumarin fluorophore was incorporated into one of the crowns or into both crowns, leading to Calix-COU1 and Calix-COU2, respectively. The stability constants of the 1 : 1 and 1 : 2 complexes with potassium and caesium ions in ethanol or acetonitrile were measured. An anticooperative effect was observed when binding a second cation to the 1 : 1 complex owing to electrostatic repulsion. The selectivity of Calix-COU1 in ethanol expressed as the ratio of the stability constants of the 1 : 1 complexes was found to be about 4 × 10^4 for Cs^+ *versus* Na^+ and greater than 500 for K^+ *versus* Na^+. The origin of the cation-induced changes in the photophysical properties is discussed.

Introduction

In the design of fluorescent molecular sensors for cation recognition,[1] much attention should be paid to the selectivity towards a given cation and against possible interfering cations. In this regard, outstanding selectivities can be obtained by using calixarene-based ligands,[2] and several fluorescent molecular sensors involving such ligands have been reported.[3–9]

The photophysical processes perturbed by the presence of a bound cation can be photoinduced electron transfer, photoinduced charge transfer, excimer formation or energy transfer.[10]

Among calixarene-based ligands, calix[4]biscrown ethers deserve particular attention. In particular, calix[4]biscrown-6 ethers exhibit a high selectivity towards caesium ion against sodium ion,[11–13] so that their use for the removal of caesium from nuclear waste was suggested. It is therefore of interest to incorporate a fluorophore into one of the crowns or both crowns in order to signal the recognition of a cation. After the first example with naphthalene reported by two of us,[11] we decided to incorporate a coumarin derivative. Larger cation-induced photophysical changes are indeed expected; the two other reasons are (i) the very good photochemical stability of coumarin dyes, (ii) the commercial availability of dihydroxymethylcoumarin which can be easily inserted into a crown ether by the same method as that used for naphthalene.[14] Independently, Brown and coworkers[9] reported a calix[4]biscrown-6 ether in which an anthracene fluorophore is inserted into both crowns. Such a fluorescent sensor is based on cation-control of photoinduced electron transfer (PET-type fluorescent sensor) involving anthracene which has a poor photochemical stability. In contrast, our sensor is based on cation-control of photoinduced charge transfer (PCT-type fluorescent sensor) and the dioxycoumarin fluorophore has a good photochemical stability.

In the present paper, we report the synthesis and binding properties of fluorescent molecular sensors (Fig. 1) which are well suited for the recognition of caesium ion. But it is also the aim of this paper to examine the suitability of these compounds for recognition of potassium ion. In fact, the data reported on cation binding showed that the selectivity of calix[4]biscrown-6 ethers towards potassium ion against sodium ion was in some cases almost as good as the selectivity for caesium ion. Molecular sensors of potassium are indeed of great interest in analytical biochemistry. One of the challenges in this field is indeed the rapid and reasonably-priced detection of this ion in blood plasma and urine. Moreover, continuous monitoring is desirable in the situation of open-heart surgery. The difficulty arises from the presence of sodium ion in those media at a concentration much higher than that of potassium ion. High selectivity is therefore required.

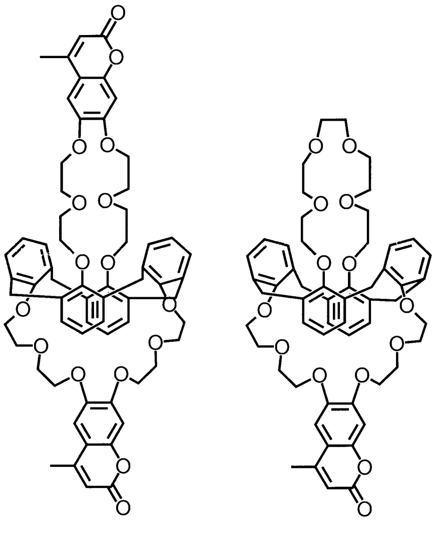
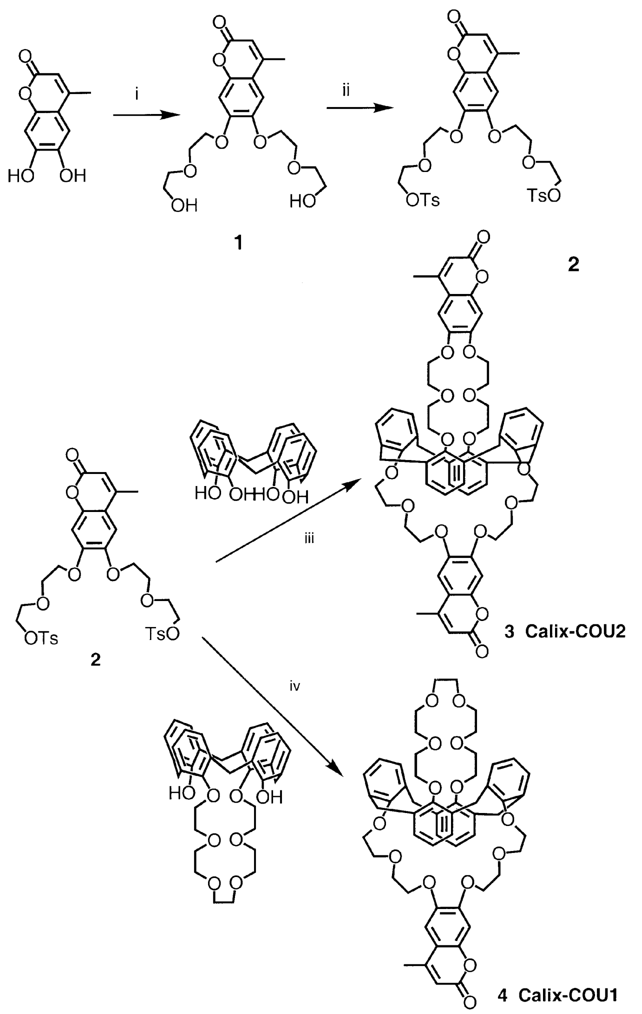


Fig. 1 Structure of Calix-COU1 and Calix-COU2.

Results and discussion

Synthesis

The synthesis of Calix-COU2 and Calix-COU1 is presented in Scheme 1. Compound **1** is obtained by reacting 2-(2-chloro-



Scheme 1 *Reagents and conditions*: i) $ClCH_2CH_2OCH_2CH_2OH$, K_2CO_3, CH_3CN, ii) TsCl, NEt_3, CH_2Cl_2; iii) calix[4]arene, K_2CO_3, CH_3CN; iv) calix[4]arene monocrown-6, K_2CO_3, CH_3CN.

ethoxy)ethanol with commercially available 6,7-dihydroxy-4-methylcoumarin. Ditosylate **2** is synthesized by reaction of **1** with toluene-*p*-sulfonyl chloride in the presence of triethylamine in CH_2Cl_2 in 76% yield.

Calix-COU2 was prepared in 43% yield by condensing calix[4]arene with 2 equiv. of ditosylate **2** and 10 equiv. of K_2CO_3 in refluxing acetonitrile for 2 weeks. The synthesis of Calix-COU1 was done starting from calix[4]crown-6[15] with 1 equiv. of ditosylate **2** and 10 equiv. of K_2CO_3 in refluxing acetonitrile for 6 days.

Calix-COU1 and Calix-COU2 had ^1H NMR spectra characteristic of the 1,3-alternate conformation.

Cation binding properties of Calix-COU1 and Calix-COU2

Addition of sodium perchlorate, or potassium thiocyanate, or caesium acetate to solutions of Calix-COU1 and Calix-COU2 induced only slight changes in the absorption spectra in ethanol (Fig. 2) and acetonitrile which precludes spectrophotometric titrations.

Fluorimetric titrations of Calix-COU1 and Calix-COU2 with Cs^+, K^+ and Na^+ were carried out in ethanol and acetonitrile. Let us consider first the complexation of Calix-COU2 with K^+ in ethanol. The ratio $R = [M^+]/[\text{ligand}]$ was varied from 0 to 2100. Evolution of the emission spectra of Calix-COU2 on addition of K^+ is displayed in Fig. 3A and an example of the titration curve at 410 nm is shown in Fig. 3B. Addition of K^+ causes fluorescence quenching. Two regimes can be distinguished: in the first regime (up to $R \approx 10$), the fluorescence intensity decreases moderately but steeply, and the fluorescence spectrum is blue shifted by 3 nm; in the second regime ($R > 10$), the fluorescence intensity decreases more gradually, but quenching is more efficient. The fluorescence quantum yield decreases from 0.29 (free ligand) to 0.05 at full complexation.

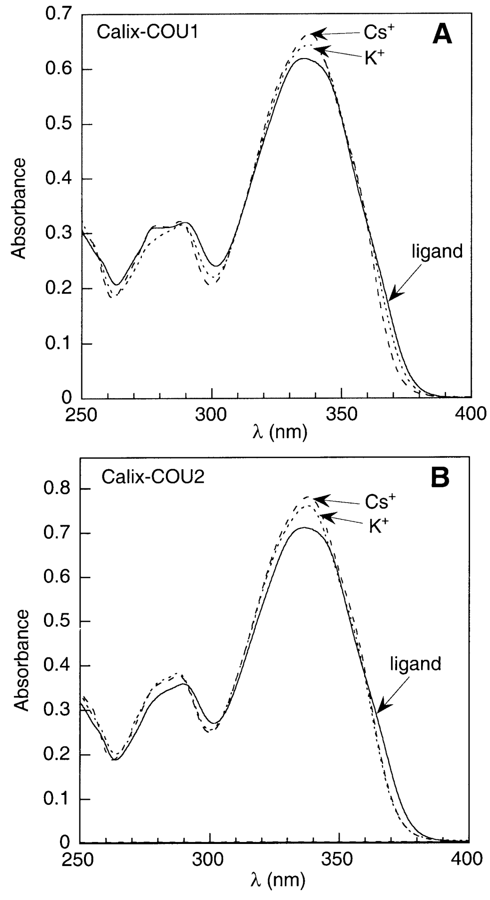


Fig. 2 Absorption spectra in ethanol of Calix-COU1 (5.4×10^{-5} mol l^{-1}) (A) and Calix-COU2 (3.24×10^{-5} mol l^{-1}) (B) in the absence of cation and in the presence of potassium thiocyanate and caesium acetate at a concentration of 0.001 M.

Under the same experimental conditions, the evolution of the emission spectra of Calix-COU2 on addition of Cs^+ is displayed in Fig. 4A and an example of the titration curve at 410 nm is shown in Fig. 4B. The final level of fluorescence intensity is higher than in the case of potassium complexation. The cation-induced changes in fluorescence intensity will be discussed in the next section.

Similar trends were observed for complexation of K^+ and Cs^+ with Calix-COU1 in ethanol, and with Calix-COU1 and Calix-COU2 in acetonitrile.

Analysis of the evolution of the whole emission spectra by means of the SPECFIT programme (using global analysis with 250 wavelengths) showed that the titration curves were consistent with the formation of two complexes, 1 : 1 and 2 : 1 (metal : ligand), with K^+ and Cs^+. The ability of calix[4]-biscrowns to form such complexes was previously observed by mass spectroscopy[16] and by UV–visible spectroscopy.[12] The constants K_{11} and K_{21} of the successive equilibria are defined as follows:

$$\mathrm{M + L \rightleftharpoons ML} \qquad K_{11} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]} \qquad (1)$$

$$\mathrm{M + ML \rightleftharpoons M_2L} \qquad K_{21} = \frac{[\mathrm{M_2L}]}{[\mathrm{M}][\mathrm{ML}]} \qquad (2)$$

Table 1 Stability constants of the complexes of Calix-COU1 and Calix-COU2 with alkali metal ions in ethanol and acetonitrile

Ligand	Solvent	Cation	log K_{11}	log K_{21}	log β_{21}
Calix-COU1	EtOH	Cs^+	6.9 ± 0.1$_5$	3.91 ± 0.15	10.8 ± 0.1$_5$
		K^+	5.03 ± 0.09	2.47 ± 0.09	7.5 ± 0.04
		Na^+	2.3 ± 0.05		
	CH_3CN	Cs^+	5.77 ± 0.03	3.36 ± 0.07	9.1 ± 0.1
		K^+	5.03 ± 0.03	2.3 ± 0.03	7.33 ± 0.04
		Na^+	2.57 ± 0.12		
Calix-COU2	EtOH	Cs^+	6.68 ± 0.09	3.81 ± 0.1	10.0 ± 0.1
		K^+	4.81 ± 0.07	2.46 ± 0.06	7.29 ± 0.06
		Na^+	2.48 ± 0.06		
	CH_3CN	Cs^+	6.0 ± 0.1	4.3 ± 0.2	10.3 ± 0.2
		K^+	4.25 ± 0.02	2.19 ± 0.03	6.44 ± 0.03
		Na^+	2.38 ± 0.06		

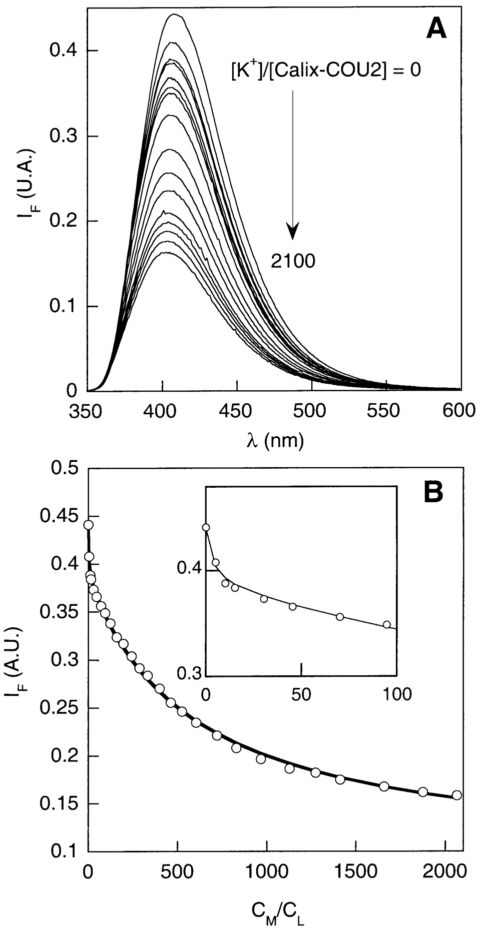


Fig. 3 A: Evolution of the emission spectrum of Calix-COU2 (1.9 × 10^{-5} mol l^{-1}) upon addition of potassium thiocyanate in ethanol. λ_{exc} = 310 nm. B: Titration curve at 410 nm.

The global equilibrium for the formation of the complex M_2L is

$$2\,M + L \rightleftharpoons M_2L \qquad \beta_{21} = \frac{[M_2L]}{[M]^2[L]} \qquad (3)$$

The values of K_{11}, K_{21} and β_{21} obtained from the titration data analyzed by SPECFIT are given in Table 1. K_{21} turned out to be much smaller than K_{11}, which means that the complexation of a second cation is made more difficult by the presence of a bound cation. The system can be considered as anticooperative because the ratio K_{21}/K_{11} is in all cases much smaller than the statistical value of 1/4 that would be observed if the two binding sites were identical and independent.[17] Such an anticooperative effect is most likely due to electrostatic repulsion between the two cations; an unfavorable conformational change induced in the free crown by the bound cation in the other crown can also be invoked.

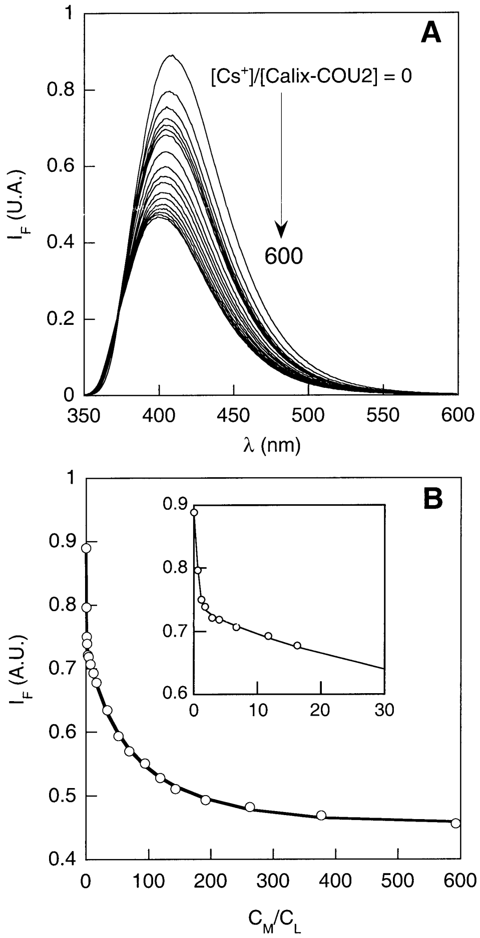


Fig. 4 A: Evolution of the emission spectrum of Calix-COU2 (1.9 × 10^{-5} mol l^{-1}) upon addition of caesium acetate in ethanol. λ_{exc} = 310 nm. B: Titration curve at 410 nm.

Regarding the complexation with Na^+, satisfactory fits of the titration curves were found with a single 1 : 1 complex in the investigated range of concentration because a much larger excess of sodium salt would be required to observe a significant effect due to the formation of the 2 : 1 complex. The stability constants of the 1 : 1 complexes of Calix-COU1 and Calix-COU2 with Na^+ are much lower than those of the complexes with Cs^+ or K^+. This is consistent with a poorer fit of Na^+ to the size of the crown cavity as compared to Cs^+, and to a lesser extent, K^+.

It should be noted that the stability constants of the complexes with Calix-COU1 and Calix-COU2 (Table 1) are in good agreement with those previously reported for calix[4]arene-biscrown ethers.[12]

An important point concerns the selectivity for Cs^+ or K^+

Table 2 Fluorescence characteristics of Calix-COU1 and Calix-COU2 and their complexes with alkali and alkaline-earth metal ions in ethanol: wavelengths of the emission maxima (λ_{em}) and fluorescence quantum yields (Φ_F)

Ligand	Cation	Charge density/Q Å^{-1}	Stoichiometry	λ_{em}/nm	Φ_F[a]
Calix-COU2				408	0.29
	Na^+	1.03	1 : 1	408	0.26
	K^+	0.75	1 : 1	405	0.26
	K^+	0.75	2 : 1	397	0.05
	Cs^+	0.60	1 : 1	406	0.24
	Cs^+	0.60	2 : 1	399	0.12
Calix-COU1				408	0.28
	Na^+	1.03	1 : 1	408	0.26
	K^+	0.75	1 : 1	406	0.25
	K^+	0.75	2 : 1	395	0.06
	Cs^+	0.60	1 : 1	406	0.24
	Cs^+	0.60	2 : 1	400	0.12

[a] Error: 5–10%.

versus Na^+, expressed as the ratio of the stability constants. Taking the stability constants of the 1 : 1 complexes in ethanol, the selectivity for Cs^+ *versus* Na^+ was found to be 4.0×10^4 with Calix-COU1 and 1.6×10^4 with Calix-COU2. The selectivity for K^+ *versus* Na^+ was found to be 540 with Calix-COU1 and 220 with Calix-COU2.

Spectral characteristics and fluorescence quantum yields of Calix-COU1, Calix-COU2 and their complexes with alkali metal ions

The photophysical data are collected in Table 2. The molar absorption coefficient of Calix-COU2 is twice as large as that of Calix-COU1, and the fluorescence quantum yields are identical. Therefore, it can be concluded that there is no interaction in the ground and excited state between the two coumarin moieties in Calix-COU2, as expected.

The data of Table 2 show that complexation with alkali ions induces a more or less efficient quenching of fluorescence. For understanding this effect, it should be recalled that coumarin itself (*i.e.* without any substituent on the phenyl ring) is non-fluorescent and that introduction of an electron-donating group at the 7-position (*e.g.* amino or methoxy groups) leads to intense fluorescence accompanied by a red shift of the fluorescence spectrum. This can be interpreted in terms of intramolecular charge transfer from the donor group at the 7-position to the lactone carbonyl group (acceptor). The case of methoxy substituents at the 7- and 6-positions has been the object of particular attention.[18]

In our compounds a bound cation that is in interaction with the oxygen atoms at the 7- and 6-positions decreases the electron-donating character of these atoms, which reduces the intramolecular charge transfer; therefore, in addition to a blue shift of the fluorescence spectrum, the fluorescence quantum yield is decreased and ultimately tends to be that of coumarin without substituents. It can be thus expected that the quenching efficiency is larger when the distance between the cation and those oxygen atoms is shorter, and/or when the charge density of the cation is larger.

Table 2 shows that for a given cation, the fluorescence quantum yields of the 1 : 1 complexes of Calix-COU1 and Calix-COU2 are identical within experimental error (5–10%). This is consistent with the fact that the affinity of the crowns for a cation does not significantly depend on whether it contains a coumarin moiety or not. For these 1 : 1 complexes, the quenching efficiency increases in the order $Na^+ < K^+ < Cs^+$, *i.e.* in the order of the size of the cations. In fact, the size of Cs^+ fits well the cavity size of the crown whereas the size of K^+—and *a fortiori* Na^+—is smaller, which results in looser complexes, *i.e.* with a larger average distance between the cation and the oxygen atoms.[19] Similar trends were previously reported for complexes of Na^+ and K^+ with another fluoroionophore containing the same dioxycoumarin[20] included in a crown-ether.

The fluorescence spectra of the 1 : 1 and 2 : 1 complexes of Calix-COU2 with K^+ or Cs^+ are provided by data analysis using the SPECFIT programme. These spectra are shown in Fig. 5 together with the spectrum of the free ligand. According to Scheme 2 showing the various complexes, and taking into account the fact that the two binding sites of Calix-COU2 have the same affinity for a cation, the fluorescence spectrum of the 1 : 1 complex was expected to be the half sum of the spectrum of the free ligand and that of the 2 : 1 complex. In contrast to this expectation, the fluorescence spectrum of the 1 : 1 complex appears to be closer to that of the free ligand than it should be. Moreover, the cation-induced blue shift of the fluorescence spectrum is much less marked for the 1 : 1 complex than for the 2 : 1 complex.

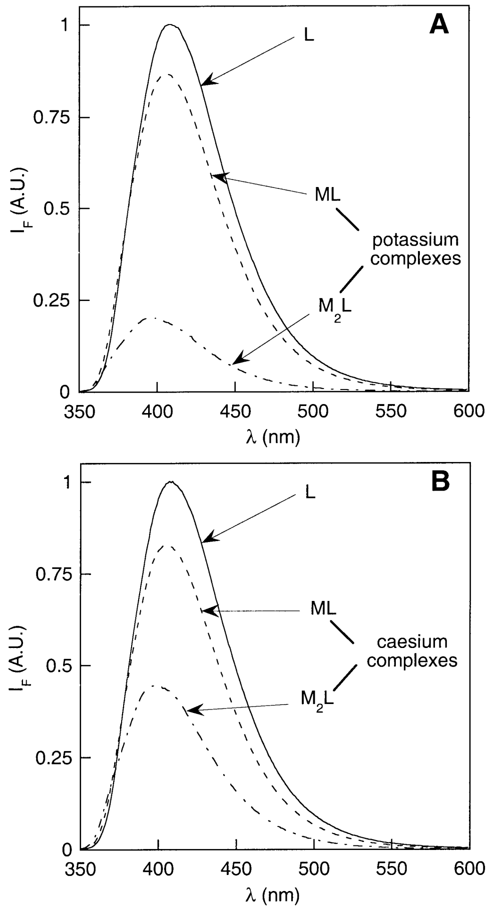
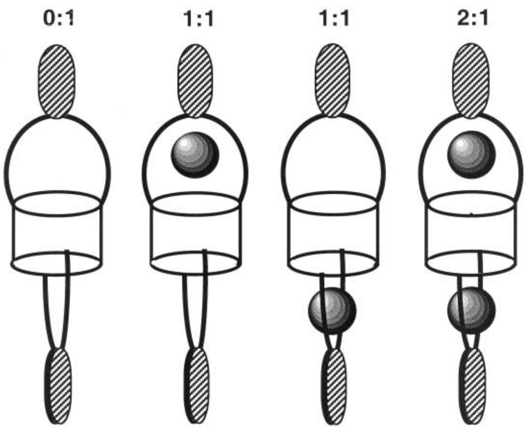
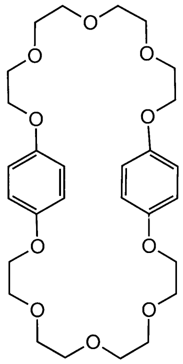


Fig. 5 Fluorescence spectra of the 1 : 1 and 2 : 1 complexes of Calix-COU2 in ethanol calculated from titration data analysis using the SPECFIT programme. (A) potassium; (B) caesium.

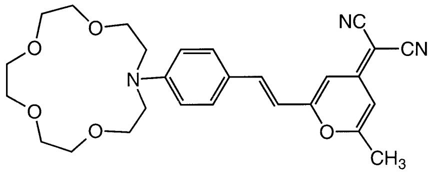


Scheme 2

These observations led us to conclude that the interaction between a bound cation and an excited coumarin fluorophore is weaker in the 1 : 1 complex than in the 2 : 1 complex. The first explanation that comes to mind is the closer distance between cation and coumarin in the 2 : 1 complex because the electrostatic repulsion between the two bound cations forces them to be closer to the coumarin moiety as compared to the 1 : 1 complex. However, another cause arises from the photoinduced charge transfer that occurs from the two oxygen atoms linked to the phenyl moiety of the coumarin to the electron-withdrawing carbonyl group of the coumarin. In fact, such a charge transfer upon excitation reduces the electron density on those oxygen atoms which may even become positively polarized, and results in a decrease of the coordination strength between the bound cation and those oxygen atoms, as already observed in benzenocoronands.[21,22] Similar effects have been reported in complexes of a “crowned” laser dye, DCM-crown,[23] in which the bound cation is in interaction with the azacrown nitrogen atom conjugated with an electron-withdrawing group. Photoinduced disruption of this interaction was demonstrated.[24]



benzenocoronand
(BBO_5O_5)



DCM-crown

Regarding the 2 : 2 complexes, the fluorescence quantum yield of the caesium complex was found to be larger than that of the potassium complex. The smaller charge density of Cs^+ can account for this difference.

Conclusion

The calix[4]biscrown-based fluorescent molecular sensors described in this paper exhibit an excellent selectivity towards caesium ion *versus* sodium ion. The selectivity towards potassium ion *versus* sodium ion is less good but still quite promising for practical applications. The poor solubility of these new sensors in water is not a drawback because our aim is to design an optical device in which the molecular sensor will be immobilized in a polymer or a sol-gel film. Insolubility in water is then preferable to minimize leaching.

Regarding the cation-induced photophysical changes, further experiments are necessary to further characterize the photodisruption of the cation–coumarin interaction, and to see whether the cation moves towards the tube-shaped cavity composed of four phenyl rings where it can be stabilized by cation–π interaction.[25] Time-resolved fluorescence and pump-probe experiments are in progress with this aim.

Experimental

Starting materials for synthesis

All commercial solvents and all starting materials and basic reagents were used without further purification.

Analytical procedures

Melting points were measured in sealed capillaries under nitrogen with a Büchi 500 apparatus. Chromatography columns were prepared from Merck Kieselgel N°. 11567. ^1H NMR spectra were recorded at room temperature on a Bruker SY 200 spectrometer. FAB-MS spectra were obtained on a VG-Analytical ZAB-HF apparatus. Elemental analyses were performed at the Service de Microanalyse de l’Institut de Chimie de Strasbourg.

Synthesis

***6,7-Bis[2-(2-hydroxyethoxy)ethoxy]-4-methylchromen-2-one* (1).** 6,7-Dihydroxy-4-methylcoumarin (14.41 g, 75.0 mmol) and K_2CO_3 (31.10 g, 225.0 mmol) were stirred at room temperature in acetonitrile (500 ml) for 2 h. Then, the mixture was heated to reflux and 2-(2-chloroethoxy)ethanol (22.42 g, 180.0 mmol) was added. After 3 days of reflux, additional K_2CO_3 (15.55 g, 112.5 mmol) and 2-(2-chloroethoxy)ethanol (11.21 g, 90.0 mmol) were added. After refluxing for 3 days, the solvents were evaporated. The residue was neutralized with 1 M HCl and the aqueous phase was extracted with dichloromethane. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. Chromatography on a silica column with an 80 : 20 dichloromethane : acetone mixture as eluent gave pure diol **1** (13.60 g; 37%) as a white solid, mp = 72–73 °C. ^1H NMR (200 MHz; $CDCl_3$) δ in ppm from TMS: 2.28 (s, 3H; CH_3), 2.77 (s, 2H; OH), 3.67–3.80 (m, 8H; OCH_2), 3.92–3.98 (m, 4H; OCH_2), 4.19–4.22 (m, 4H; OCH_2), 6.17 (s, 1H; ArH), 6.85 (s, 1H; ArH), 7.03 (s, 1H; ArH). Elemental analysis calcd for $C_{18}H_{24}O_8$: C, 58.69; H 6.57; found: C, 58.73; H, 6.53%.

***Ditosylate of 6,7-bis[2-(2-hydroxyethoxy)ethoxy]-4-methylchromen-2-one* (2).** A mixture of **1** (5.90 g, 16.0 mmol) and toluene-*p*-sulfonyl chloride (12.41 g, 64.0 mmol) in dichloromethane (150 ml) was cooled at 0 °C. Then, triethylamine (12.91 g, 128.0 mmol) was added dropwise. After 24 h, the reaction mixture was neutralized to pH ~ 1 by addition of 1 M HCl. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was chromatographed on a silica column with a 95 : 5 mixture of dichloromethane : acetone as eluent to give pure ditosylate **2** (8.35 g; 76%) as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$): δ in ppm from TMS: 2.37 (s, 3H, CH_3), 2.41 (s, 6H, CH_3), 3.72–3.81 (m, 8H, OCH_2), 4.12–4.18 (m, 8H, OCH_2), 6.16 (s, 1H, ArH), 6.80 (s, 1H, ArH), 7.09 (s, 1H, ArH), 7.30 (d, $J = 8.2$ Hz, 4H, ArH of tosyl), 7.78 (d, J = 8.2 Hz, 4H of tosyl). Elemental analysis calcd for $C_{32}H_{36}O_{12}S_2$: C, 56.79; H, 5.36; found: C, 56.88; H, 5.41%.

***25,27:26,28-Bis{4-methyl-2-oxochromene-6,7-diylbis[2-(2-oxyethoxy)ethoxy]}calix[4]arene* (3).** Calix[4]arene (2.12 g; 5.0 mmol) and K_2CO_3 (13.38 g; 100.0 mmol) were stirred in acetonitrile (300 ml) at room temperature for 1 h. Then, ditosylate **2** (6.76 g; 10.0 mmol dissolved in 10 ml of acetonitrile) was added and the reaction mixture was refluxed for 10 days. After cooling to room temperature, the solvents were removed under reduced pressure. The residue was dissolved in dichloromethane and water and neutralised to pH ~ 1 with 1 M HCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. Chromatography on a silica column with an 80 : 20 mixture of dichloromethane : acetone as eluent followed by precipitation with methanol gave product **3** (2.39 g; 43%) as a white solid, mp = 164–165 °C. ^1H NMR (200 MHz, $CDCl_3$): δ in ppm from TMS: 2.41 (s, 6H; CH_3), 3.59–3.62 (m, 20H; OCH_2), 3.78 (s, 8H; $ArCH_2Ar$), 3.89–3.91 (m, 4H; OCH_2), 4.13–4.16 (m, 8H; OCH_2), 6.19 (s, 2H; ArH), 6.70 (t, J = 7.4 Hz, 4H; ArHcalix-*para*), 6.90 (s, 2H; ArH), 7.08 (d, J = 7.4 Hz, 8H; ArHcalix-*meta*), 7.16 (s, 2H; ArH). FAB positive, m/z = 1089.2. Elemental analysis calcd for $C_{64}H_{64}O_{16}$: C, 70.57; H 5.92; found: C, 70.64; H, 5.88%.

***25,27-{4-Methyl-2-oxochromene-6,7-diylbis[2-(2-oxyethoxy)ethoxy]}-26,28-{ethylenebis[2-(2-oxyethoxy)ethoxy]}calix-[4]arene* (4).** Calix[4]arene monocrown-6 (1.25 g; 2.0 mmol) and K_2CO_3 (2.76 g; 20.0 mmol) were stirred at room temperature for 2 h in acetonitrile (120 ml). Then ditosylate **2** (1.35 g; 2.0 mmol dissolved in 10 ml of acetonitrile) was added. After 7 days of reflux, the mixture was cooled to room temperature and the solvents were removed under reduced pressure. The residue was dissolved in dichloromethane and water and acidified to pH ~ 1 with 1 M HCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. Chromatography on a silica column with a mixture of 90 : 10 dichloromethane : acetone followed by precipitation with methanol gave product **4** (1.36 g; 71%) as a white solid, mp = 132–133 °C.

^1H NMR (200 MHz, $CDCl_3$): δ in ppm from TMS: 2.42 (s, 3H; CH_3), 3. 38 (t, J = 6.2 Hz, 4H; OCH_2), 3.47–3.67 (m, 20H; OCH_2), 3.71 (s, 8H; $ArCH_2Ar$), 3.80 (s, 4H; OCH_2), 3.86 (t, J = 6.2 Hz, 4H; OCH_2), 4.10 (t, J = 6.2 Hz, 2H; OCH_2), 4.19 (t, J = 6.1 Hz, 2H; OCH_2), 6.19 (d, J = 1.2 Hz, 1H; ArH), 6.70 (t, J = 7.4 Hz, 2H; ArHcalix-*para*), 6.87 (t, J = 7.4 Hz, 2H; ArHcalix-*para*), 6.89 (s, 1H; ArH), 7.06 (s, 1H; ArH), 7.08 (d, J = 7.4 Hz, 4H; ArHcalix-*meta*), 7.15 (d, J = 7.4 Hz, 4H; ArHcalix-*meta*). FAB positive, m/z = 959.40. Elemental analysis calcd for $C_{56}H_{62}O_{14}$: C, 70.13; H 6.52; found: C, 70.14; H, 6.58%.

Solvent and salts

Acetonitrile from Aldrich (spectrometric grade) and absolute ethanol from SDS (spectrometric grade) were used as solvents for absorption and fluorescence measurements. Sodium perchlorate and potassium thiocyanate from Alfa were used; they were of the highest quality available and vacuum dried over P_2O_5 prior to use.

Spectroscopic measurements and calculations

UV–visible absorption spectra were recorded on a Varian Cary 5E spectrophotometer. Corrected emission spectra were obtained on an SLM-Aminco 8000C spectrofluorometer. The fluorescence quantum yields were determined using quinine sulfate dihydrate in H_2SO_4 (0.05 M) as a reference ($\Phi_F = 0.546$). All solvents used were of spectroscopic grade.

Global analysis of the evolution of the whole fluorescence spectra was performed with Specfit Global Analysis System V3.0 for 32-bit Window System. This programme uses singular value decomposition and non linear regression modelling by the Levenberg–Marquardt method.[26]

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