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Journal of Photochemistry and Photobiology A: Chemistry 135 (2000) 163–169

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.nl/locate/jphotochem

Ion-responsive fluorescent compounds
VI. Coumarin 153 linked to rigid crowns for improvement of selectivity

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Received 14 February 2000; accepted 4 April 2000

Abstract

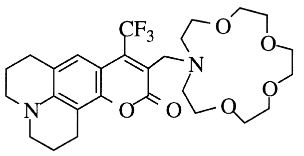
The photophysical and complexing properties of fluoroionophores consisting of the coumarin C153 linked to dibenzo-16-crown-5 (C153-DBC) and tribenzo-19-crown-6 (C153-TBC) are described in acetonitrile and ethanol. The carbonyl group of the coumarin moiety is in direct interaction with a bound cation. The selectivity of these compounds for a given alkali or alkaline earth metal ion was found to be better than that of previously reported crowned coumarins owing to the rigidity of dibenzo- and tribenzocrowns. In acetonitrile, a very high selectivity for Ca^{2+} versus Mg^{2+} has been found. In ethanol, C153-DBC is selective for Na^+ and C153-TBC is selective for K^+, as expected from the relative size of the crown cavity.

Keywords: Fluorescence; Supramolecular photochemistry; Sensors; Alkali; Alkaline earth metal ion

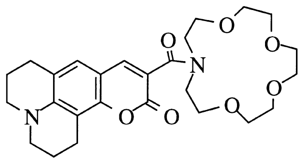
1. Introduction

The development of fluorescent molecular sensors for metal ions, especially for cations of biological interest, has been the object of considerable effort [1–4]. The design principles of such sensors, so-called fluoroionophores consisting of a fluorophore linked to a complexing unit, have been reviewed [5,6]. The changes in the photophysical properties in the presence of a cation possibly involve various photoinduced processes such as electron transfer, charge transfer, excimer formation and energy transfer.

We have previously reported the photophysical and complexing properties of fluoroionophores composed of a coumarin linked to azacrowns via a methylene bridge or an amide bridge [7–10]. The carbonyl group of the coumarin moiety interacts with the bound cation. The photophysical changes upon complexation with a metal ion can be described in terms of enhancement of the intramolecular charge transfer in the coumarin owing to direct interaction between the bound cation and the carbonyl group. The selectivity was found to be poor in C153-crown(O4) because of the flexibility of the crown as well as the spacer between the dye and the crown. In order to improve the selectivity and to decrease the pH sensitivity, the methylene bridge was replaced by an amide bridge (C343-crown). An improvement was observed in the selectivity towards alkaline earth cations with respect to alkali cations.



C153-crown(O4)



C343-crown

Further improvement in selectivity is expected from the use of a more rigid crown containing phenyl groups, as demonstrated previously [11]. Dibenzo-16-crown-5 (DBC) was found to be selective for Na^+ [12]; a high K^+ selectivity is expected when using tribenzo-19-crown-6 (TBC) [13]. Therefore, in order to improve the selectivity towards a given cation, we have replaced the azacrown ether by a dibenzocrown ether and a tribenzocrown ether. The synthesis, and the photophysical and complexing properties of these new fluoroionophores that are called C153-DBC and C153-TBC are reported in this paper.

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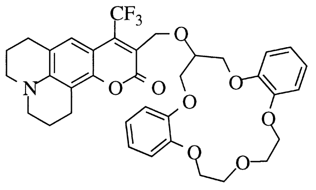
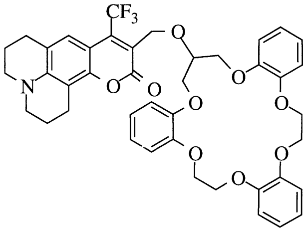


C153-DBC



C153-TBC

2. Materials and methods

2.1. Synthesis of the crown

Sym-(hydroxy)dibenzo-16-crown-5 was prepared according to Cram et al. [14] and Markowitz et al. [15]. *Sym*-(hydroxy)tribenzo-19-crown-6 was prepared according to Bartsch et al. [13]. The chloromethyl derivative of C153 (C153-CH_2Cl) was synthesised as described previously [8] and used as a crude product.

2.1.1. C153-DBC synthesis

An amount of 24 mg (1.5 eq) of NaH (60% dispersion in mineral oil) was introduced in a round-bottom flask under argon and washed three times with dry pentane. Pentane was replaced by 10 ml of dry THF and 230 mg (1 eq) of *Sym*-(hydroxy)dibenzo-16-crown-5 were added. The mixture was stirred at room temperature for 1 h and 240 mg of the crude C153-CH_2Cl dissolved in 30 ml of THF were added slowly. The reaction mixture was left stirring overnight at room temperature. After evaporation of the solvent under reduced pressure, the mixture was dissolved in 30 ml of methylene chloride and washed three times with 10 ml of water. After solvent evaporation under reduced pressure, the product was purified by flash chromatography on silica gel (eluent CH_2Cl_2:MeOH (95:5)) affording 220 mg (50% yield) of the desired product which can be further recrystallised in ethyl acetate/hexane. The purity of the product was checked by HPLC: a single peak with 100% peak purity analysis was observed on the C18 reversed phase with MeOH:H_2O (90:10) eluent.

C153-TBC was similarly synthesised with 65% yield.

2.1.1.1. Analysis of C153-DBC. ^1H NMR ($CDCl_3$): δ (ppm) 1.95 (m, 4H); 2.75 (t, 2H); 2.85 (t, 2H); 3.28 (m, 4H); 3.88–4.00 (m, 4H); 4.12–4.20 (m, 6H); 4.24 (p, 1H); 4.33–4.37 (m, 2H); 4.96 (s, 2H); 6.81–6.88 (m, 4H); 6.90–6.95 (m, 2H); 7.04–7.06 (m, 2H); 7.17 (s, 1H).

^{13}C NMR ($CDCl_3$): δ (ppm) 20.24, 20.38, 21.21, 27.78, 49.36, 49.83, 64.06, 67.62, 69.62, 71.27, 77.35, 103.58, 106.24, 113.27, 117.02, 118.52, 118.63, 121.24, 122.58, 123.19, 123.22, 139.60, 146.11, 148.44, 150.32, 150.76, 162.17.

2.1.1.2. Analysis of C153-TBC. ^1H NMR ($CDCl_3$): δ (ppm) 1.95 (m, 4H); 2.74 (t, 2H); 2.86 (t, 2H); 3.30 (m, 4H); 4–4.40 (m, 13H); 4.85 (s, 2H); 6.80–7.10 (m, 12H); 7.15 (s, 1H).

^{13}C NMR ($CDCl_3$): δ (ppm) 20.97, 21.08, 22.02, 28.51, 50.10, 50.58, 64.12, 68.40, 68.81, 71.07, 77.45, 104.29, 106.95, 115.23, 116.32, 117.9, 119.23, 119.42, 122.46, 122.59, 123.19, 123.92, 124.01, 140.20, 146.91, 149.82, 149.87, 150.54, 151.80, 162.05.

2.2. Solvent and salts

Acetonitrile from Aldrich (spectrometric grade) and absolute ethanol from SDS (spectrometric grade) were used as solvents for absorption and fluorescence measurements. The alkali and alkaline earth perchlorates and potassium thiocyanate used were from Alfa and of the highest quality available, and were vacuum-dried over P_2O_5 prior to use.

2.3. Apparatus

UV–VIS absorption spectra were recorded on a Varian Cary 5E spectrophotometer. Corrected emission spectra were obtained on an SLM-Aminco 8000C and 48000S. The fluorescence quantum yield was determined using C153 as a reference (Φ=0.38 in ethanol) [16]. All experiments were performed at 20°C.

The stability constants were determined using the SPECFIT software version 211 (Spectrum Software Associates).

3. Results and discussion

3.1. Cation-induced photophysical changes

The absorption and corrected emission spectra of C153-DBC with its complexes and C153-TBC with its complexes are shown in Figs. 1 and 2, respectively. The spectral characteristics of the free ligands and the various complexes are given in Table 1 for C153-DBC and in Table 2 for C153-TBC. The spectral shifts under complexation may be explained, as reported previously, by the direct interaction of the bound cation with the carbonyl group of the coumarin. It should be noted that the spectral shifts are larger with C153-DBC and C153-TBC as compared to those observed with C153-crown and C343-crown. These different systems differ in the bridging group separating the coumarin and the crown. In the case of C153-DBC and C153-TBC, the bridging arm contains one more atom than the systems C153-crown and C343-crown. One may indeed expect C153-DBC and C153-TBC to adopt a conformation allowing the cation to move closer to the carbonyl group upon excitation as a result of increasing electron density in the excited state (Scheme 1). In contrast, the shifts of the absorption spectra appear to be less dependent on the fluoroionophore structure.

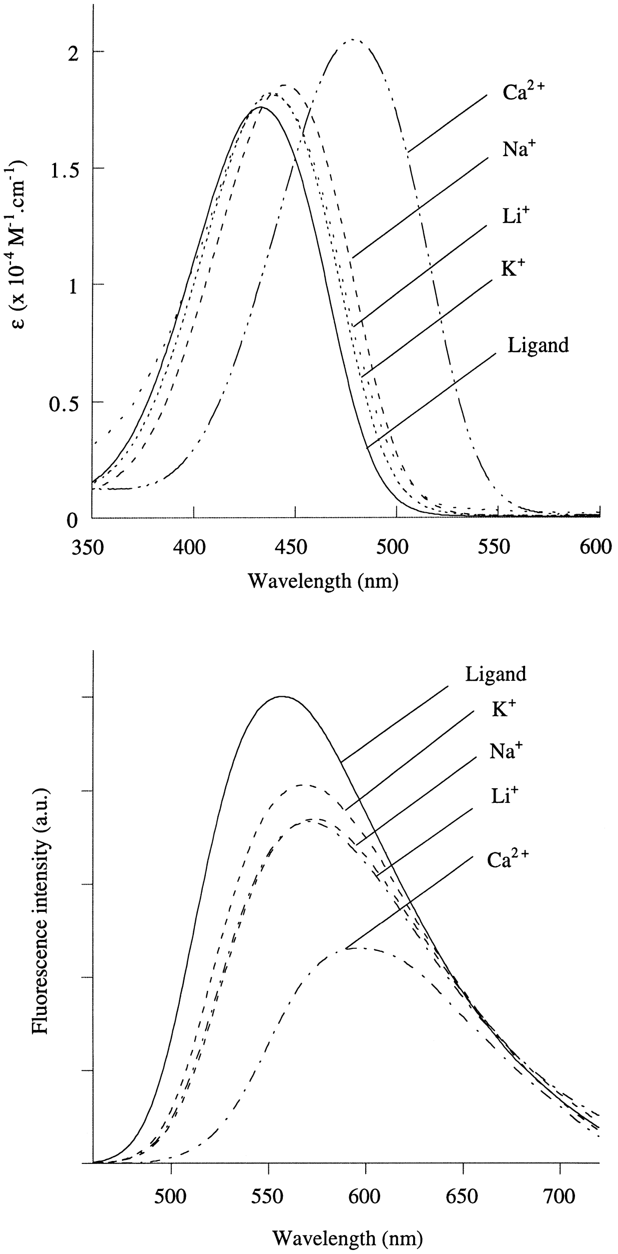


Fig. 1. Absorption and corrected emission spectra of C153-DBC in acetonitrile and its complexes with perchlorate salts.

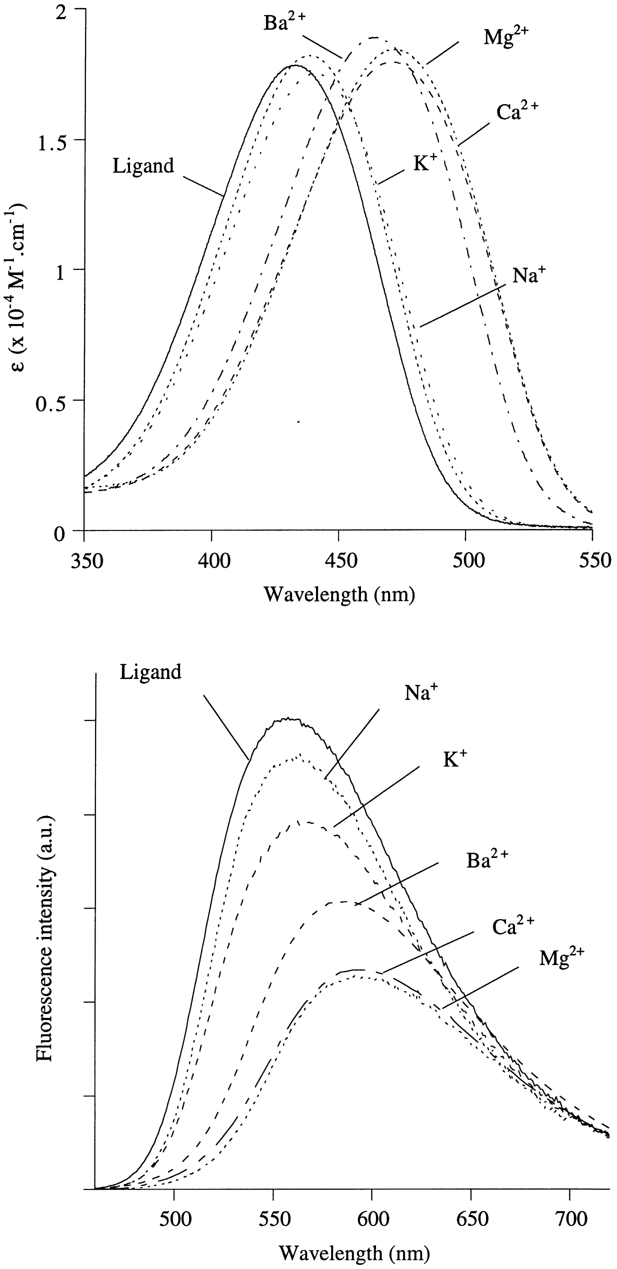


Fig. 2. Absorption and corrected emission spectra of C153-TBC in acetonitrile and its complexes with perchlorate salts.

A decrease was observed in the fluorescent quantum yield upon complexation: the larger the charge density of the bound cation, the lower the quantum yield of the complex. The fluorescence decays of C153-DBC and its complexes were measured in acetonitrile. They were found to be monoexponential. The lifetime was 3.34 ns for the free ligand, and the values for the complexes follow those of the fluorescence quantum yields. By calculating the radiative and nonradiative rate constants from the values of the fluorescence quantum yield and the excited-state lifetime, it turns out that the radiative rate constant is only slightly affected by a bound cation, whereas the nonradiative rate constant is increased significantly and seems to be correlated to the charge density of the cation.

In ethanol, red shifts of the absorption and emission spectra were also observed upon addition of cation (Figs. 3 and 4). For cations with a high charge density (Mg^{2+}, Ca^{2+}), the stability constants are too low to attain full complexation upon addition of a reasonable amount of salt. Therefore, no reliable values of the photophysical characteristics of the complex can be obtained in these cases.

Table 1
Absorption and fluorescence properties of C153-DBC and C153-TBC and its complexes with alkali and alkaline earth metal ions in acetonitrile at room temperature[a]

	Diameter (Å)	Charge density q (Å^{-1})	λ_{abs} (nm)	$\varepsilon\times10^{-3}$ (l mol^{-1} cm^{-1})	λ_{iso} (nm)	λ_{em} (nm)	Φ_F
C153-DBC							
Ligand			434	17.6		558	0.21
Li^+	1.36	1.47	438	18.2	430	571	0.16
Na^+	1.94	1.03	444	19	431	569	0.16
K^+	2.66	0.75	438	19	426	568	0.17
Mg^{2+}	1.32	3.03	[b]	[b]	448	[b]	[b]
Ca^{2+}	1.98	2.02	479	20.5	450	598	0.092
C153-TBC							
Ligand			432.5	17.8		558	0.23
Li^+	1.36	1.47	[b]	[b]	[b]	[b]	
Na^+	1.94	1.03	440	17.5	438.5	563	0.21
K^+	2.66	0.75	438	18.2	431	567	0.19
Mg^{2+}	1.32	3.03	442	18.7	449.5	591.5	0.11
Ca^{2+}	1.98	2.02	472	18.0	450	593	0.12
Ba^{2+}	2.68	1.49	464.5	18.8	445	584	0.15

[a] λ_{abs}: absorption maximum; ε: molar absorption coefficient; λ_{iso}: wavelength of the isosbestic point; Φ_F: fluorescence quantum yield.
[b] The stability constants are too small to yield a reliable value characteristic of the complex.

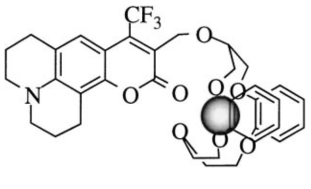
Table 2
Absorption and fluorescence properties of C153-DBC and C153-TBC and its complexes with alkali and alkaline earth metal ions in ethanol at room temperature[a]

	Diameter (Å)	Charge density q (Å^{-1})	λ_{abs} (nm)	$\varepsilon\times10^{-3}$ (l mol^{-1} cm^{-1})	λ_{iso} (nm)	λ_{em} (nm)	Φ_F
C153-DBC							
Ligand			431.3	14.96		558	0.2
Li^+	1.36	1.47	[b]	[b]	[b]	[b]	[b]
Na^+	1.94	1.03	440	15.83	428	566	0.16
K^+	2.66	0.75	442	15.81	430	565	0.15
Mg^{2+}	1.32	3.03	[b]	[b]	430	[b]	[b]
Ca^{2+}	1.98	2.02	[b]	[b]	447	[b]	[b]
C153-TBC							
Ligand			431.5	14.83		558	0.22
Li^+	1.36	1.47	[b]	[b]	[b]	[b]	
Na^+	1.94	1.03	441	15.13	434	561	0.19
K^+	2.66	0.75	439	15.68	426	567	0.20
Mg^{2+}	1.32	3.03	[b]	[b]	437	[b]	[b]
Ca^{2+}	1.98	2.02	[b]	[b]	445	[b]	[b]
Ba^{2+}	2.68	1.49	459	16.1	442	576	0.17

[a] λ_{abs}: absorption maximum; ε: molar absorption coefficient; λ_{iso}: wavelength of the isosbestic point; Φ_F: fluorescence quantum yield.
[b] The stability constants are too small to yield a reliable value characteristic of the complex.

3.2. *Stoichiometry and stability constant of the complexes*

The stoichiometry and the association constant were determined by using the variation in either absorbance or fluorescence intensity upon addition of metal ion. An example of the spectral evolution upon addition of salt is given in Fig. 5.



Scheme 1.

The stability constants obtained for the complexes in acetonitrile and in ethanol are reported in Table 3. Satisfactory fits were found for the 1:1 complex for C153-DBC with alkali and alkaline earth cations. In the case of the complexation of C153-TBC with Ba^{2+} and Ca^{2+} in acetonitrile and with Ba^{2+} in ethanol, the titration curves are consistent with the formation of two complexes viz. 1:1 and 2:1 (ligand:metal). The following equilibria are to be considered:

$$M + L \rightleftharpoons ML, \quad K_1 = \frac{[ML]}{[M][L]}$$

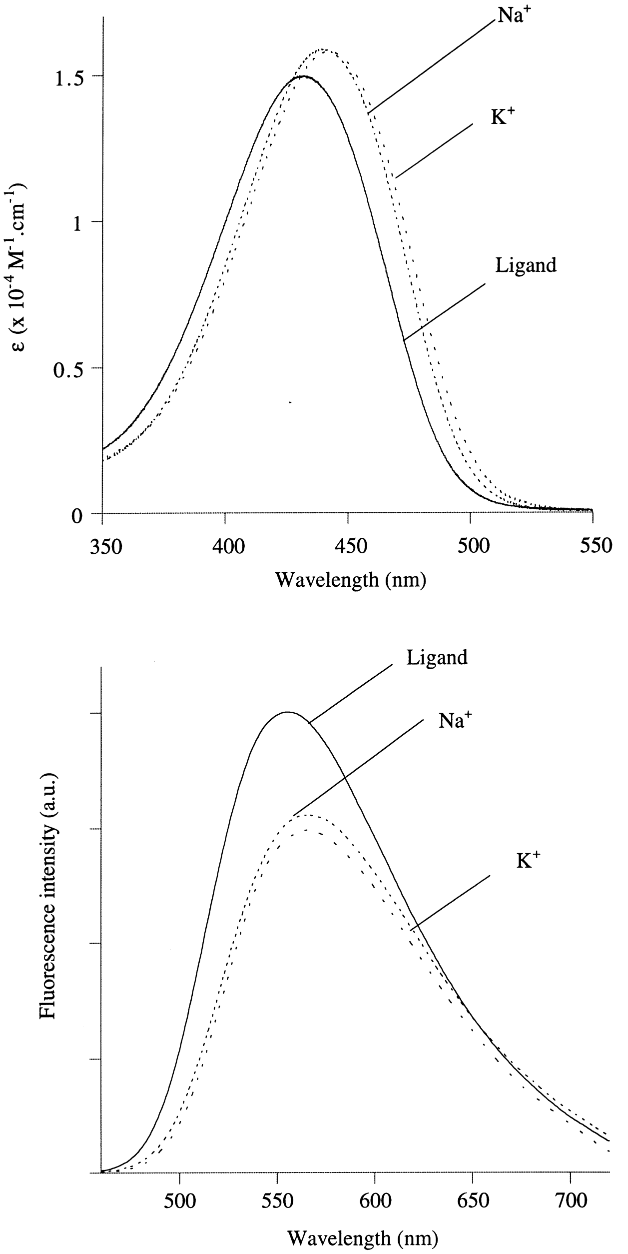


Fig. 3. Absorption and corrected emission spectra of C153-DBC in ethanol and its complexes with perchlorate salts.

$$M + 2L \rightleftharpoons ML_2, \quad K_2 = \frac{[ML_2]}{[M][L]^2}$$

Similar complexes have already been reported in the literature for the binding of benzocrowns linked to pyrene [17] or bistyryl benzene [18]. In the present work, the sandwich complexes observed were found only in the case of the complexation of C153-TBC with Ca^{2+} and Ba^{2+}. A steric hindrance may account for this observation.

The values of the stability constants obtained in acetonitrile clearly show that the use of a more rigid crown by incorporating phenyl groups leads to an improvement in the selectivity (Table 3). The selectivity for Ca^{2+}/Mg^{2+},

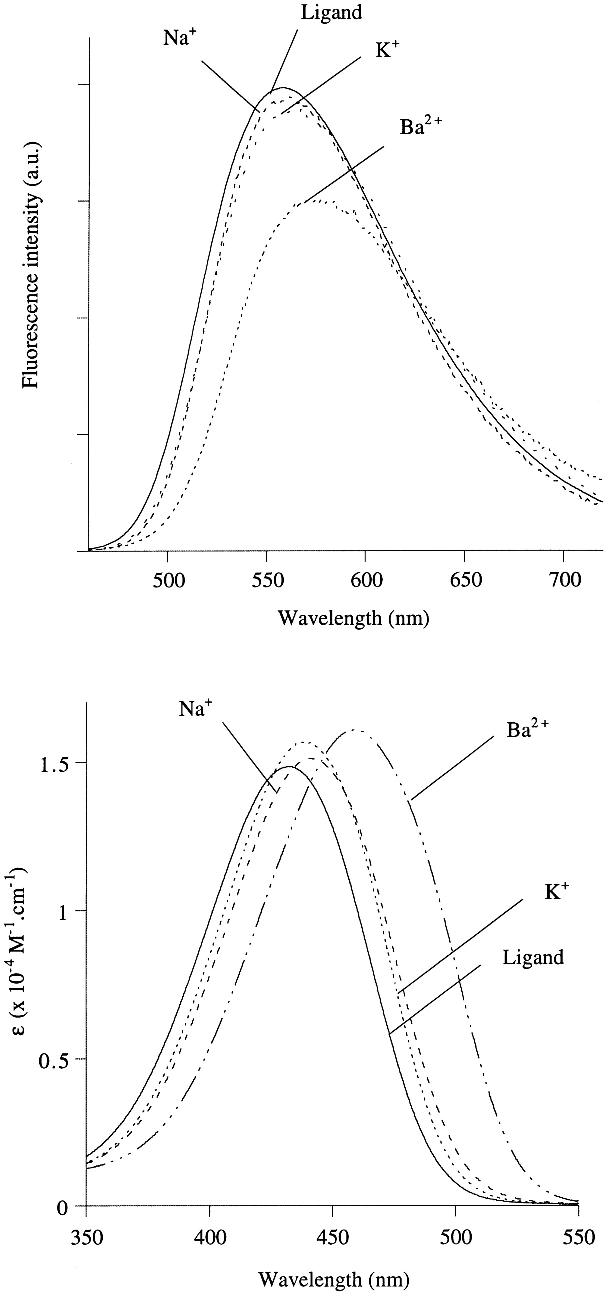


Fig. 4. Absorption and corrected emission spectra of C153-TBC in ethanol and its complexes with perchlorate salts.

expressed as the ratio of the stability constants, was found to be 12 500 for C153-DBC and 58 000 for C153-TBC. Mg^{2+} is too small to fit in the crown. As compared to the above, the selectivity was only 3.5 in the case of C343-crown. Regarding the distinction between Na^+ and K^+, the selectivity of C153-DBC for Na^+ versus K^+ is $Na^+/K^+=16$.

In ethanol, the stability constants are reported in Table 3. It is worth noting that the stability constants are much higher than those obtained with the same DBC [19] thanks to the participation of the carbonyl group in the complexation. The stability constants of the complexes of C153-DBC and

Table 3
Stability constants of the complexes of C153-DBC and C153-TBC in acetonitrile[a]

Solvent	Compound	Li^+ (1.36 Å)	Na^+ (1.94 Å)	K^+ (2.66 Å)	Mg^+ (1.32 Å)	Ca^+ (1.98 Å)	Ba^+ (2.68 Å)
Acetonitrile	C153-DBC	1.96±0.03	4.2±0.05	2.98±0.03	1.48±0.03	5.58±0.02	
	C153-TBC	1.30±0.02	3.81±0.02	4.08±0.02	2.73±0.02	$\log K_1$=7.5±0.3 $\log K_2$=13.7±0.4	$\log K_1$=8.6±0.3 $\log K_2$=15±0.3
Ethanol	C153-DBC	<1	3.5±0.02	3.1±0.02	1.4±0.02	1.3±0.03	
	C153-TBC	<1	3.03±0.02	4.34±0.02	<1	1.1	$\log K_1$=8.59±0.3 $\log K_2$=15±0.3

[a] The values are expressed as $\log K_S$. The ionic diameter is indicated in brackets.

C153-TBC with Na^+ and K^+ are much higher than with Li^+, Mg^{2+} and Ca^{2+} because the latter cations have a higher charge density and are thus more solvated than complexed in contrast to the observation in acetonitrile. The selectivity Na^+/K^+ was found to be 3 for C153-DBC. The selectivity of this compound with respect to the other alkali and alkaline earth cations is in good agreement with that of a similar pendent lariat ether (dibenzo-16-crown-5 oxyacetate) [20]. The K^+/Na^+ selectivity of C153-TBC was found to be 20 (instead of 2 in acetonitrile).

4. Conclusion

Replacement of the azacrown in the previously reported fluoroionophores (C153-crown and C343-crown) by a dibenzocrown or a tribenzocrown leads to the expected improvement in selectivity. It should be emphasised that the selectivity of these compounds is strongly solvent-dependent as a result of the interplay between solvation and complexation. In acetonitrile, a very high selectivity towards calcium versus magnesium has been observed. In ethanol, the complexes of C153-DBC and C153-TBC with Na^+ and K^+ were found to be much more stable than those with the other alkali and alkaline earth cations. Regarding the distinction between Na^+ and K^+, it was found that C153-DBC is selective for Na^+ and C153-TBC is selective for K^+, as expected from the relative size of the crown cavity. The K^+/Na^+ selectivity of C153-TBC is promising when the selective detection of K^+, which is of major interest in human serum where Na^+ is present in a larger concentration than Na^+ ($[Na^+]/[K^+]\sim 30$), is the aim.

The present investigation on crowned coumarins in line with the previous ones shows that the most important parameters responsible for selectivity in such compounds are (i) the rigidity of the link between the fluorophore and the crown, (ii) the rigidity of the crown itself and (iii) the size of the crown.

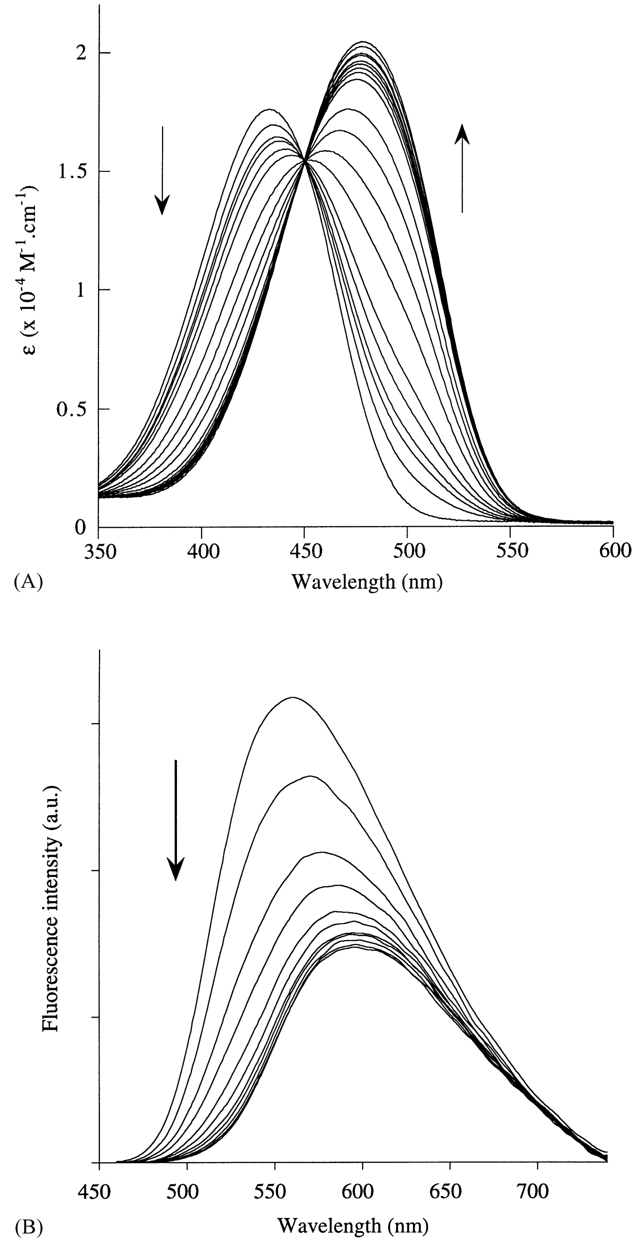


Fig. 5. Evolution of the absorption (A) and corrected emission spectra (B) of C153-DBC in acetonitrile on addition of calcium perchlorate.

Acknowledgements

Mr. Jean-Pierre Lefèvre (CNAM, ENS-Cachan) is gratefully acknowledged for his assistance with lifetime measurements. The Belgian FNRS and French CNRS institutions are thanked for providing financial support.

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