About the use of an amide group as a linker in fluoroionophores: competition between linker and ionophore acting as chelating groups

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The photophysical and complexing properties of a series of aza-crown fluoroionophores based on coumarin 343 and on 3- and 6-methoxynaphthoic amides in acetonitrile are reported. The goal of the work was to probe the participation of the amide bridge linking the fluorophore and the ionophore in the metal chelation. The use of 3- and 6-methoxy substituents in the naphthoic amide fluorophores allowed us to maintain the charge transfer character of the system and to probe the participation of the methoxy group as ancillary ligand. The aza-crown unit is no longer complexing when the amide linker is included in a β-dicarbonyl sub-structure. The amide function itself is still able to form complexes, even if weaker, with the cations.

Introduction

Fluorescent molecular sensors for the selective detection of metal ions have attracted considerable attention due to their interest in analytical chemistry, biology, medicine (clinical diagnosis), environment, *etc.*[1,2] The design principles of such sensors, so-called fluoroionophores, have been reviewed.[3,4] In this frame, fluoroionophores based on coumarin 343 linked to monoaza-crown ether *via* a methylene group, were previously studied.[5–7] These probes contain an electron-withdrawing group, the carbonyl group of the lactone, conjugated to an electron-donating group, the nitrogen atom of the julolidyl ring. This gives to the absorption of light a charge transfer character. Since the carbonyl may interact with the cation chelated in the aza-crown site, marked photophysical changes are expected upon complexation, and were observed.[7] Bathochromic shifts and hyperchromicity were recorded in acetonitrile and ethanol principally in the cases of magnesium and calcium chelation.

Photosensing coumarins linked to the aza-crown by an amide group were reported in a subsequent work[8] (C343-crown shown hereunder), and in a further step the monoaza-crown receptor was replaced by a monoazabenzo-crown or a monoazadibenzo-crown in order to modify the selectivity of the fluorescent probe.[9,10] The consequences of these structural modifications were examined in terms of photophysical and complexing properties and indeed it was observed that the rigidification of the complexing cavity greatly improves the selectivity of the detection of alkaline-earth cations *versus* lithium cation. Whereas the complex of C343-crown with lithium in acetonitrile was nearly as stable as those with magnesium or calcium, the stability constant of C343-benzocrown with lithium is two orders of magnitude lower than those with alkaline-earth cations. In the case of C343-dibenzocrown, the interaction between the ligand and the lithium cation is so weak that the stability constant could not be determined.

Surprisingly, the stability constants for magnesium with all these C343-fluoroionophores were found to be very close, in acetonitrile (log K_S = 4.27, 4.59 and 4.24 for C343-crown, C343-benzocrown and C343-dibenzocrown respectively). Since the structural modification of the crown ether does not seem to influence the stability of the magnesium complex, this cation was supposed to be chelated by the two carbonyl groups and not by the crown ether heteroatoms. This kind of supra-molecular structure was already suggested by Alonso *et al.*[11]

It has also been shown that naphthalenic compounds bearing amide groups may be used as fluorescent chemo-sensors for alkali and alkaline-earth metal ions.[12,13]

Considering these results, we firstly decided to synthesize and study C343-diethylamide (C343-dea, see structure below), a fluoroionophore with a similar β-dicarbonyl sub-structure but without crown ether. In a second step, naphthalenic fluorophores linked to an aza-crown ether *via* an amide group or bearing a diethylamide function and substituted by a methoxy group were studied. These naphthalenic probes also contain an electron-donating group, the methoxy group, conjugated to an electron-withdrawing group, the carbonyl

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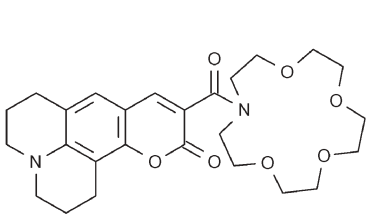
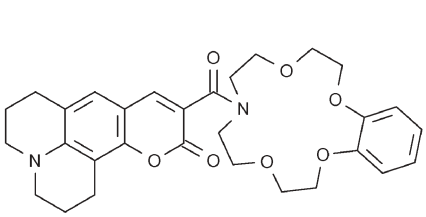


C343-crown



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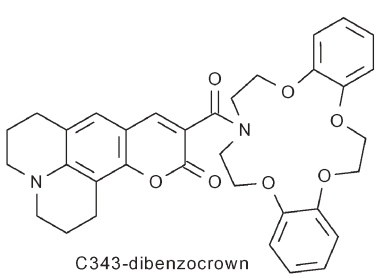
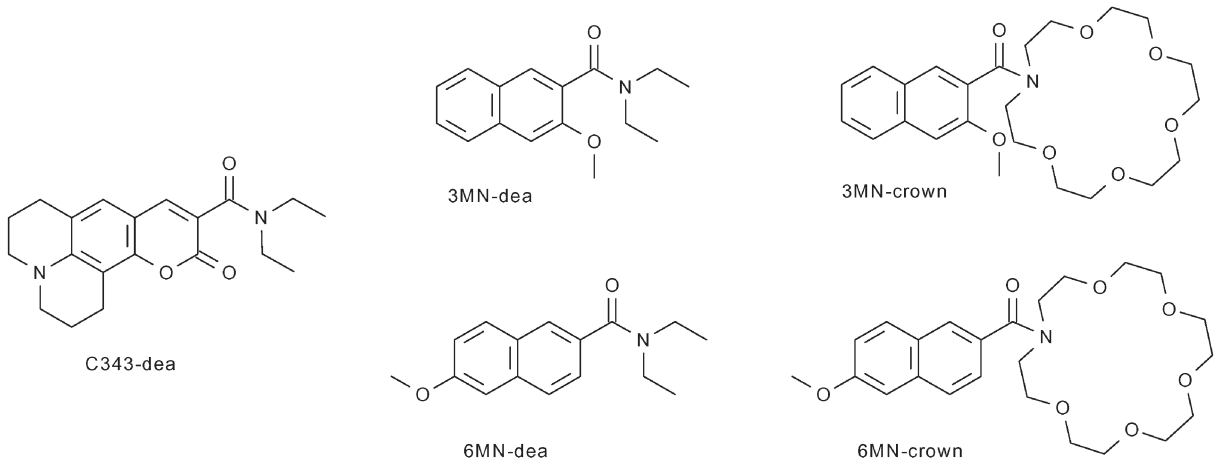


C343-dibenzocrown



amide group, and therefore are expected to behave as charge transfer sensors. The methoxy group was placed at a position where it is supposed to participate in the cation coordination as an ancillary complexing group while in other molecules the position of the methoxy substituent was changed in order to suppress the ancillary effect. Greater participation of the crown ether in the cation chelation should be expected in these cases, when compared to the coumarinic examples.

The aim of this work was to study the photophysical and complexing properties of the ligands and those of their complexes with alkaline (Li^+, Na^+, K^+) and alkaline-earth (Mg^{2+}, Ca^{2+}) cations in acetonitrile. From these results, it should be possible to outline the function of the crown ether and/or the amide bridge in the cation chelation and we could determine if the methoxy group acts as an auxiliary complexing group when it is placed in position 3 in the naphthalenic compounds.

Experimental

Solvents and reagents

Coumarin 343, 3- and 6-methoxy-2-naphthoic acids were purchased from Aldrich and 1-aza-18-crown-6 was from Fluka. PyBOP and diisopropylethylamine were ACROS products. All reagents were used as received. Absorption and fluorescence spectra were recorded in acetonitrile from Fisher Scientific (HPLC grade). Lithium, sodium, alkaline-earth perchlorates and potassium thiocyanate were purchased from Alpha (highest quality available) and were vacuum-dried over P_2O_5 prior to use. Deuterated chloroform and acetonitrile for NMR measurements were Aldrich products.

Apparatus and methods

UV-Vis absorption spectra were obtained on a Varian Cary 50 spectrophotometer. Emission spectra were obtained on a SPEX Fluorolog 1681 spectrofluorometer and on a Varian Cary Eclipse spectrofluorometer. Fluorescent quantum yields were determined using naphthalene in cyclohexane as reference ($\Phi_F = 0.23 \pm 0.02$, $\lambda_{exc} = 267$ nm[14]) or coumarin 314 in ethanol ($\Phi_F = 0.68$, $\lambda_{exc} = 408$ nm[15]).

The titrations were realized by progressive additions of small aliquots of a ligand solution highly concentrated in cations salts to 2.5 ml of a ligand solution of the same concentration (concentration range: 1–4 $\times$ 10^{-5} M) in order to avoid dilution effects. For C343-dea, the absorption spectrum was recorded after each addition. For the naphthalenic probes, titrations were done by fluorescence and in that case the emission spectra were recorded after each cation addition and were corrected for small variations of absorption if necessary. The stability constants of complexes were determined from absorption or fluorescence intensity evolution upon metal addition using equations reported in ref. 10 and processed by Origin© software.

The apparatus and method for fluorescence decay times measurements were described in a previous paper.[10]

NMR spectra were recorded on a BRUKER Avance 500 spectrometer. NMR spectra of complexes were recorded using a 10 mm probe. The concentrations of solutions in deuterated acetonitrile were 10^{-3} M for ^1H spectra and 10^{-2} M for ^{13}C spectra.

Infrared spectra were obtained on a Biorad FTS 135 spectrophotometer, using KBr pellets or $CDCl_3$ solutions. Mass spectra were obtained on a LCQ apparatus with an APCI source (Thermo FINNIGAN, San Jose, CA, USA). Melting points were determined with a BÜCHI apparatus. HPLC analyses were performed on a SpectraPhysics SP 8800, equipped with an Econosil normal phase or a C18 Biorad reversed phase column.

HRMS and ESI-MS analyses of the complexes were performed in the laboratory of bioorganic mass spectrometry in the University Louis Pasteur of Strasbourg (Prof. A. Van Dorsselaer). Spectra of the complexes were obtained on a triple quadrupole apparatus Quattro II (Micromass, Altringham, UK). The ESI source was warmed to 70 °C and a potential of 10 V was applied on the entry cone in order to transfer the supramolecular species to the gas phase without fragmentation. Every scan was recorded from 200 to 2000 *m*/*z* and several scans were added to obtain the final spectrum. Every spectrum was reproduced three times. Ligand concentrations were 10^{-4} M and 10^{-3} M for 3MN-crown and 6MN-dea respectively. These concentrations are higher than those used for UV-Vis spectroscopy experiments. Significant formation of complexes is then allowed without using a high cation concentration that is unfavourable for the ESI process.

Synthesis

C343-dea, 3- and 6MN-dea were obtained from coumarin 343, 3- or 6-methoxy-2-naphthoic acids and diethylamine using the coupling agent PyBOP.[16] The same method was tested to synthesize 3- and 6MN-crown but purification difficulties were encountered due to by-products formed during the reaction. Similar problems were encountered with DCC as coupling agent. The amides 3- and 6MN-crown were finally synthesized by the reaction of the corresponding naphthoic acid with 1-aza-18-crown-6 *via* the formation of the acyl chloride.

Synthesis of C343-dea. A mixture of 498 mg (1 eq.) of coumarin 343, 184 mg (1.4 eq.) of diethylamine, 966 mg (1.8 eq.) of PyBOP and 890 mg (4 eq.) of diisopropylethylamine was stirred overnight at room temperature in 70 ml of a 1 : 1 mixture of CH_2Cl_2 and CH_3CN. After solvent evaporation, the crude product was purified by column chromatography on silica ($CHCl_3$–iPrOH 9 : 1) resulting in yellow thick oil that slowly crystallized. Yield: 89%.

Mp: 148 °C. ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 7.73 (s, 1H), 6.87 (s, 1H), 3.53 (m, 2H), 3.30 (m, 6H), 2.86 (t, 2H), 2.75 (t, 2H), 1.96 (m, 4H), 1.20 (m, 6H). ^{13}C NMR ($CDCl_3$, 500 MHz), δ (ppm): 166.21, 159.64, 151.98, 146.74, 143.16, 125.61, 118.90, 117.02, 107.58, 106.23, 50.10, 49.70, 43.37, 39.55, 27.53, 21.36, 20.44, 20.25, 14.29, 12.97. MS (APCI): 341 (MH^+), 300, 268. HPLC (normal phase, $CHCl_3$–EtOH 95 : 5), purity: 99.1%. HRMS calculated for $[M + Na]^+$: 363.1685; found: 363.1692.

Synthesis of 3MN-dea. A mixture of 296 mg (1 eq.) of 3-methoxy-2-naphthoic acid, 220 µl (1.46 eq.) of diethylamine, 830 mg (1.10 eq.) of PyBOP and 2 ml (7.84 eq.) of diisopropylethylamine was stirred overnight at room temperature. After solvent evaporation, the crude product was purified by column chromatography on silica ($CHCl_3$–iPrOH 9 : 1) and was recrystallized in ethyl acetate to give white crystals. Yield: 96%.

Mp: 111 °C. ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 7.75 (m, 2H), 7.67 (s, 1H), 7.46 (m, 1H), 7.36 (m, 1H), 7.16 (s, 1H), 3.93 (s, 3H), 3.62 (m, 2H), 3.16 (m, 2H), 1.29 (t, 3H), 1.03 (t, 3H). ^{13}C NMR ($CDCl_3$, 500 MHz), δ (ppm): 168.40, 153.73, 134.45, 128.75, 128.42, 127.87, 126.88, 126.88, 126.59, 124.19, 105.72, 55.57, 42.85, 38.91, 13.92, 12.92. MS (APCI): 258 (MH^+), 185. FTIR (KBr pellet): $\nu_{C=O}$: 1624 cm^{-1}. HPLC (normal phase, $CHCl_3$–EtOH 97.5 : 2.5), purity: 99.4%. HRMS calculated for $[M + Na]^+$: 280.1313; found: 280.1309.

Synthesis of 6MN-dea. A mixture of 305 mg (1 eq.) of 6-methoxy-2-naphthoic acid, 220 µl (1.41 eq.) of diethylamine, 867 mg (1.14 eq.) of PyBOP and 1 ml (3.92 eq.) of diisopropylethylamine was stirred for 30 h at room temperature. After solvent evaporation, the crude product was chromatographed twice on a silica column ($CHCl_3$–iPrOH 9 : 1 and Et_2O–$CHCl_3$ 1 : 1), yielding the product as a colourless oil. Yield: 82%.

^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 7.80 (s, 1H), 7.75 (d, 1H), 7.75 (d, 1H), 7.44 (dd, 1H), 7.18 (dd, 1H), 7.14 (m, 1H), 3.93 (s, 3H), 3.2–3.7 (broad, 4H), 1.1–1.3 (broad, 6H). ^{13}C NMR ($CDCl_3$, 500 MHz), δ (ppm): 171.56, 158.43, 134.84, 132.43, 129.89, 128.25, 127.02, 125.85, 124.66, 119.57, 105.75, 55.42, 43.23, 39.45, 14.39, 13.07. MS (APCI): 258 (MH^+), 185. FTIR ($CDCl_3$): $\nu_{C=O}$: 1613 cm^{-1}. HPLC (normal phase, CH_2Cl_2–iPrOH 95 : 5), purity: 99.5%. HRMS calculated for $[M + H]^+$: 258.1494; found: 258.1494.

Synthesis of 3MN-crown. Thionyl chloride (120 µl, 1.05 eq.) dissolved in 20 ml of dry CH_2Cl_2, was added dropwise to a solution of 315 mg (1 eq.) of 3-methoxy-2-naphthoic acid dissolved in 20 ml of CH_2Cl_2 containing one drop of dimethylformamide. The reaction mixture is refluxed for 45 min. The solvent (40 ml) was eliminated by normal pressure distillation during which time 20 ml of fresh CH_2Cl_2 were added. After cooling, 0.8 ml (3.88 eq.) of Et_3N were added to the mixture, followed by dropwise introduction of 488 mg (1.19 eq.) of 1-aza-18-crown-6 dissolved in 10 ml of CH_2Cl_2. The mixture was refluxed for 30 min and left stirring overnight at room temperature. After being washed twice with 20 ml of aqueous ammonia (pH 9) and three times with 20 ml of deionised water, the organic phase was evaporated under reduced pressure. Finally, the crude product is chromatographed on silica gel (CH_2Cl_2–iPrOH 9 : 1) affording a colourless oil. Yield: 85%.

^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 7.75 (m, 2H), 7.69 (s, 1H), 7.46 (m, 1H), 7.36 (m, 1H), 7.16 (s, 1H), 3.94 (s, 3H), 3.4–4.0 (broad, 24H). ^{13}C NMR ($CDCl_3$, 500 MHz), δ (ppm): 169.56, 153.63, 134.64, 128.49, 128.28, 128.02, 127.48, 127.11, 126.74, 124.37, 105.93, 70.92–69.75, 55.72, 49.55, 46.16. MS (APCI): 448 (MH^+), 185. FTIR ($CDCl_3$): $\nu_{C=O}$: 1627 cm^{-1}. HPLC (normal phase, $CHCl_3$–EtOH 97.5 : 2.5), purity: 99.9%. HRMS calculated for $[M + H]^+$: 448.2335; found: 448.2339.

Synthesis of 6MN-crown. The fluoroionophore 6MN-crown was synthesised following the method used for 3MN-crown from 125 µl (1.14 eq.) of thionyl chloride, 315 mg (1 eq.) of 6-methoxy-2-naphthoic acid, 0.8 ml (3.88 eq.) of Et_3N and 488 mg (1.19 eq.) of 1-aza-18-crown-6. A colourless oil was obtained. Yield: 66%.

^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 7.85 (s, 1H), 7.75 (d, 1H), 7.75 (d, 1H), 7.48 (m, 1H), 7.17 (m, 1H), 7.13 (m, 1H), 3.94 (s, 3H), 3.5–3.9 (broad, 24H). ^{13}C NMR ($CDCl_3$, 500 MHz), δ (ppm): 173.54, 158.54, 134.96, 132.02, 130.02, 128.27, 127.08, 126.53, 125.08, 119.61, 105.80, 70.83–69.82, 55.49, 50.32, 46.34. MS (APCI): 448 (MH^+), 185. FTIR ($CDCl_3$): $\nu_{C=O}$: 1623 cm^{-1}. HPLC (normal phase, $CHCl_3$–EtOH 97.5 : 2.5), purity: 99.8%. HRMS calculated for $[M + Na]^+$: 470.2155; found: 470.2167.

Results and discussion

A) C343-dea

Absorption. The absorption spectra of C343-dea as free ligand and as complexes with various alkaline and alkaline-earth cations are reported in Fig. 1 and detailed in Table 1.

Complexation by a cation induces a bathochromic shift as expected and a hyperchromicity as already observed for C343-crown.[8] Among the tested cations (Li^+, Na^+, K^+, Mg^{2+} and

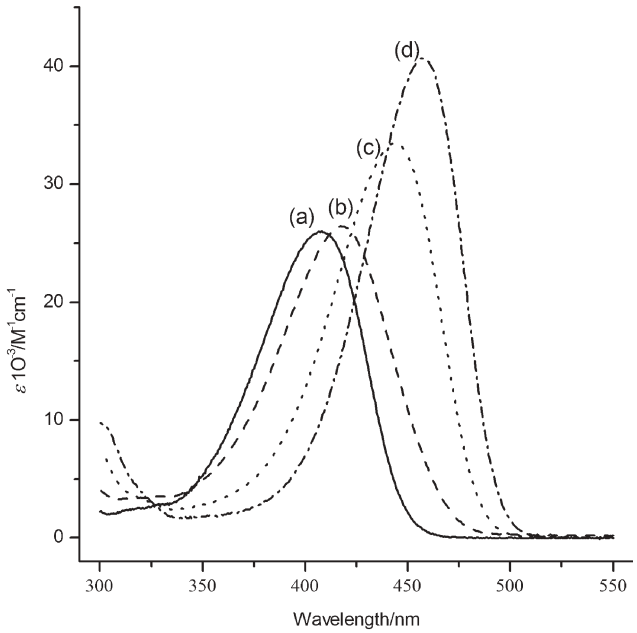


Fig. 1 Absorption spectra of C343-dea: (a) free ligand; (b) its complex with Li^+; (c) its complex with Ca^{2+}; (d) its complex with Mg^{2+}.

Ca^{2+}) only Na^+ and K^+ do not induce spectral modifications. The observed properties are very close to that registered for C343-crown.[8,10]

Emission. The emission properties of C343-dea and its complexes in acetonitrile are reported in Table 2 and illustrated in Fig. 2.

Emission maxima of C343-dea and its complexes are close to the maxima of C343-crown but the fluorescence quantum yields are somewhat lower (0.56 for C343-dea and 0.65 for C343-crown[10]). This might be due to a greater flexibility of the diethylamine group compared to the aza-crown ether.

The fluorescence decay time of free C343-dea is monoexponential, but in the cases of complexes a biexponential decay is observed consisting of a longer (4.18 ns) and a shorter decay time (1.3 ns for the magnesium complex and 0.6 ns for the calcium complex). The longer decay time that represents more than 90% of the decay may be tentatively assigned to a simple 1 : 1 complex in which the metal chelation induces a rigidification of the structure. The short component might reveal the presence of a second kind of complex with another stoichiometry for which the close proximity of the two coumarinic rings leads to some fluorescence quenching.

Table 1 Absorption properties of free and complexed C343-dea in acetonitrile

	Radius/Å	Charge density/q Å^{-1}	λ_{max}/nm	λ_{iso}/nm	$\varepsilon \times 10^{-3}$/M^{-1} cm^{-1}
Ligand			**407.5**		**26.0**
Li^+	0.90	1.11	418.5	410.5	26.5
Mg^{2+}	0.86	2.32	457.0	424.0	40.7
Ca^{2+}	1.14	1.75	443.0	418.5	33.7

[a] λ_{max}: maximum absorption wavelength; λ_{iso}: isosbestic point, ε: extinction coefficient.

Table 2 Emission properties of free and complexed C343-dea in acetonitrile

	Radius/Å	Charge density/q Å^{-1}	λ_{em}/nm	Φ_F	τ_F/ns (f_i)
Ligand			**472**	**0.56**	**3.70**
Li^+	0.90	1.11	491	0.50	
Mg^{2+}	0.86	2.32	501	0.52	4.18 (0.92)
					1.34 (0.08)
Ca^{2+}	1.14	1.75	497	0.51	4.18 (0.96)
					0.61 (0.04)

[a] λ_{em}: emission maximum; Φ_F: fluorescence quantum yield; τ: fluorescence lifetime; f_i: fractional contribution to the steady-state intensity (satisfactory values—below 1.2—of the reduced χ^2 were obtained in all cases).

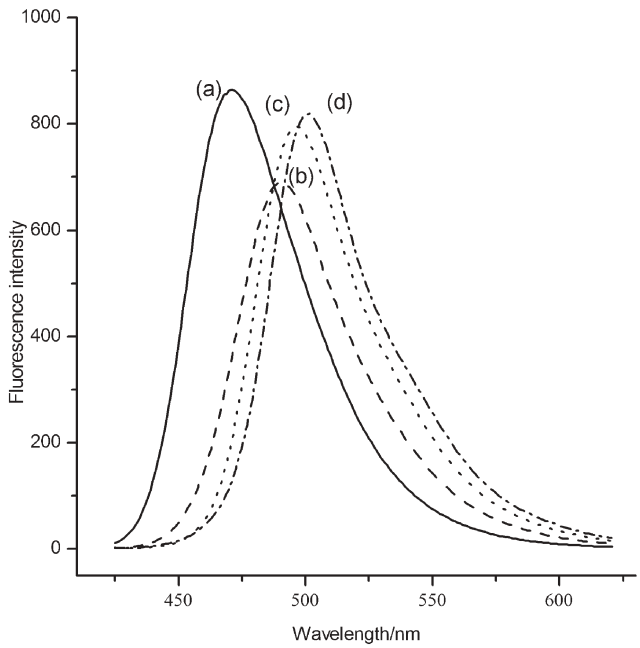


Fig. 2 Emission spectra of C343-dea: (a) free ligand; (b) its complex with Li^+; (c) its complex with Ca^{2+}; (d) its complex with Mg^{2+}.

B) Naphthalenic fluoroionophores

Absorption. The absorption spectra of the four naphthalenic ligands show that the replacement of the diethylamine by the aza-crown ether does not significantly modify the ligand absorption spectra. The modifications of absorption spectra upon addition of the tested cations are very small and will not be detailed here.

Emission. The emission characteristics of the four ligands and their complexes are presented in Table 3. Quantum yields and fluorescence lifetimes were only determined when full complexation could be reached. This is not always possible due to the weak stability of some complexes. In the other cases, the observed trends in the photophysical changes induced by the cations are indicated.

As for the absorption spectra, the replacement of the diethylamine by the crown ether does not influence the emission properties.

Table 3 Emission properties of naphthalenic ligands and their complexes in acetonitrile

	Cations		3MN-dea			6MN-dea		
	Radius/Å	Charge density/q Å^{-1}	λ_{em}/nm	Φ_F	τ_F/ns (f_i)	λ_{em}/nm	Φ_F	τ_F/ns (f_i)
Ligand			**367**	**0.003**	**0.11 (0.30)** **0.28 (0.70)**	**352**	**0.022**	**0.07 (0.01)** **1.40 (0.99)**
Li^+	0.90	1.11	367	↑	—	353	↓	—
Na^+	1.16	0.86	367	0.003	—	353	0.022	—
K^+	1.52	0.66	367	0.003	—	353	0.022	—
Mg^{2+}	0.86	2.32	393	↑ ↑ ↑	—	354	↓ ↓ ↓	—
Ca^{2+}	1.14	1.75	389	↑ ↑	—	352	↓ ↓	—

			3MN-crown			6MN-crown		
			λ_{em}/nm	Φ_F	τ_F/ns (f_i)	λ_{em}/nm	Φ_F	τ_F/ns (f_i)
Ligand			**367**	**0.007**	**0.15 (0.18)** **0.60 (0.82)**	**353**	**0.019**	**0.23 (0.02)** **1.42 (0.99)**
Li^+	0.90	1.11	368	↑ ↑	—	353	↑	—
Na^+	1.16	0.86	370	0.016	0.28 (0.16) 1.66 (0.84)	353	0.020	0.99 (0.19) 1.90 (0.81)
K^+	1.52	0.66	369	↑	—	353	0.019	—
Mg^{2+}	0.86	2.32	416	0.055	0.63 (0.04) 4.03 (0.34) 9.40 (0.62)	400	0.024	0.10 (0.21) 0.28 (0.34) 0.84 (0.45)
Ca^{2+}	1.14	1.75	376	0.028	0.20 (0.02) 0.78 (0.16) 2.19 (0.82)	354	0.025	0.17 (0.02) 1.23 (0.37) 3.34 (0.61)

[a] λ_{em}: emission maximum; Φ_F: fluorescence quantum yield; ↑, ↓: increase, decrease of the fluorescence intensity (when full complexation cannot be reached); τ: fluorescence lifetime; f_i: fractional contribution to the steady-state intensity (satisfactory values—below 1.2—of the reduced χ^2 were obtained in all cases).

The molecules bearing a diethylamide function are able to interact only with cations of high charge density. A variation of the fluorescence intensity is induced by these cations with the effect being in opposite directions for 3MN-dea (increase of intensity) and 6MN-dea (decreasing intensity). This opposite effect will be discussed later. A red shift of 26 and 22 nm is observed for 3MN-dea when the solution is saturated with magnesium and calcium cations respectively.

The emission spectra of probes bearing an aza-crown ether are modified by almost all the tested cations and full complexation can be reached for both probes with Na^+, Mg^{2+}, Ca^{2+} (Fig. 3 and Fig. 4). In all these cases, the metal chelation

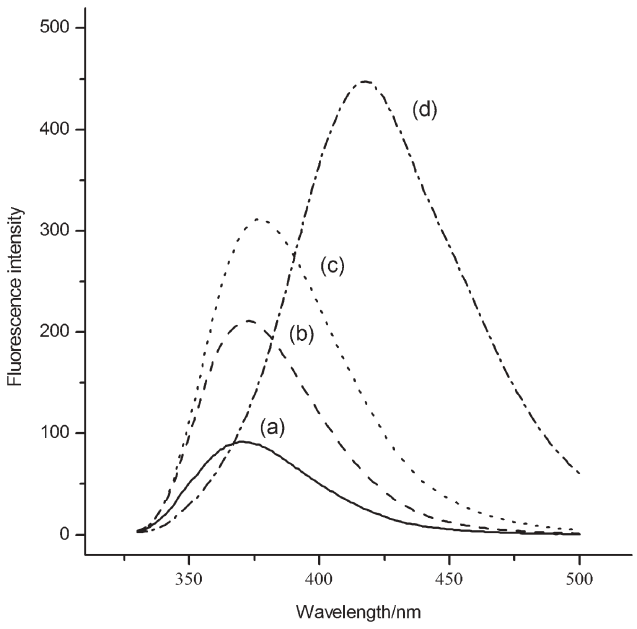


Fig. 3 Emission spectra of (a) 3MN-crown ($c = 5.9 \times 10^{-5}$ M) and its complexes (at full complexation) with (b) Na^+; (c) Ca^{2+}; (d) Mg^{2+} in acetonitrile; λ_{exc}: 315 nm.

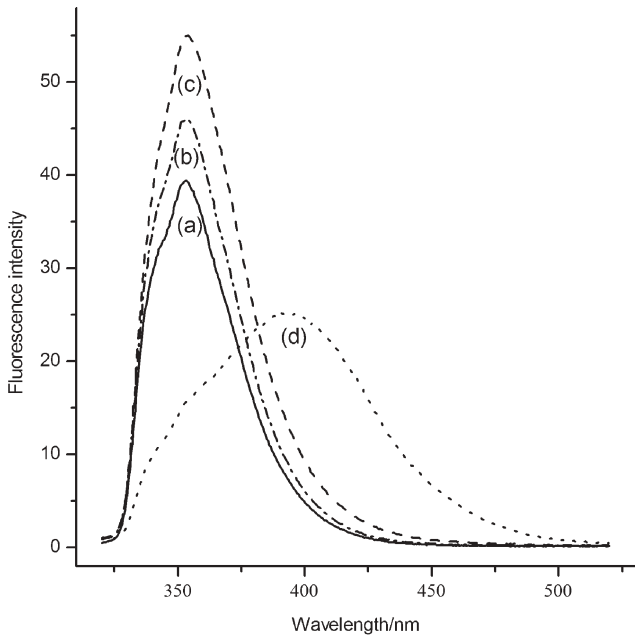


Fig. 4 Emission spectra of (a) 6MN-crown ($c = 5.8 \times 10^{-5}$ M) and its complexes (at full complexation) with (b) Na^+; (c) Ca^{2+}; (d) Mg^{2+} in acetonitrile; λ_{exc}: 267 nm

induces an increase of the fluorescence intensity. Large red shifts of 49 and 47 nm are observed for magnesium complexes with 3- and 6MN-crown respectively.

The observed bathochromic shifts increase significantly with the charge density of the cation. This is coherent with the stronger interaction between the ligand carbonyl group and the cation and the greater resulting stabilisation of the excited charge transfer state. The variation of fluorescence intensity is also related to the cation charge density. For most of the complexes, cation binding leads to an increase of the fluorescence quantum yield. It is accepted that naphthalenic or aromatic compounds having amide groups are strongly quenched by intersystem crossing to a triplet sate and/or by the rotational relaxation linked to excited state rotations around the three naphthalene–CO, CO–NH and N–alkyl bonds. This may explain why, when complexed by cations, these rotors are less available for relaxation.[13] An increase of the rigidity of the system by cation complexation may therefore be an explanation for the fluorescence enhancement. The case of 6MN-dea will be discussed later.

Two exponentials were needed in all cases to fit the experimental decay curves. In the case of the free ligands, both contributions are significant for 3MN derivatives while the shorter component may be considered negligible for the 6MN derivatives. This means that the methoxy group, when close to the amide group, is probably responsible for an excited state conformational equilibrium leading to two different emitting conformers. This seems coherent with the observed increase of Φ_F when these rotations are slowed down in the presence of the cation. In the case of the complexes, the situation is still more complicated which might be the sign of the presence of different complexes with various structures or stoichiometries.

C) Stoichiometry and stability constants of the complexes in acetonitrile

The stability constants were obtained from the evolution of the absorption or emission spectra upon cation addition (*cf.* Apparatus and methods section). An example of the evolution of 3MN-crown emission spectrum upon $NaClO_4$ addition and the corresponding titration curve are presented in Fig. 5 and 6. The ML stoichiometry was firstly tested in every case and found to be adapted to most of the complexes. The stability constants calculated for that stoichiometry are given in Table 4 and defined according to eqn. (1):

$$M + L \rightleftharpoons ML \quad K_s = \frac{[ML]}{[M] \times [L]} \qquad (1)$$

The stability constant of the C343-dea magnesium complex is very close to the values obtained for the C343-crown derivatives. This clearly demonstrated that the β-dicarbonyl sub-structure of our coumarinic compounds is responsible for the chelation of magnesium cation. For the calcium complex, it is not possible to simply compare this new result with the previous ones obtained for the crowned fluoroionophores since complexes with a stoichiometry differing from 1 : 1 were observed for C343-crown and C343-benzocrown. Lithium cation weakly binds C343-dea. For lithium, a drastic effect is

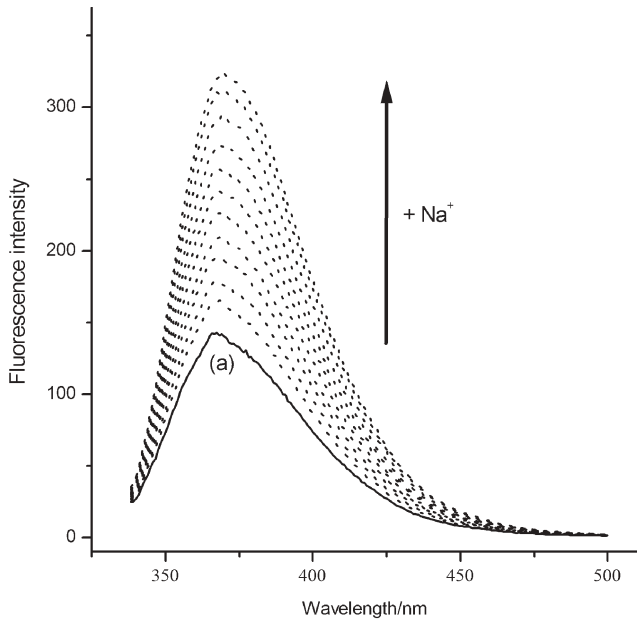


Fig. 5 Evolution of the emission spectrum of (a) 3MN-crown (c = 1.8×10^{-5} M) upon $NaClO_4$ (c = 0; 1.2×10^{-5}; 2.3×10^{-5}; 3.5×10^{-5}; 4.6×10^{-5}; 6.9×10^{-5}; 9.8×10^{-5}; 1.4×10^{-4}; 2.2×10^{-4}; 4.0×10^{-4}; 9.5×10^{-4}; 2.8×10^{-3} M) addition, λ_{exc} = 330 nm.

observed since the stability constant of the complex drops from log K_s = 3.26 for C343-crown to 1.7 for C343-dea.

Calcium and magnesium cations are complexed by 3- and 6MN-dea with stability constants of the same order of magnitude. Since the position of the methoxy group does not modify the stability of these complexes, it seems that no ancillary chelation occurs with this substituent. Although satisfactory fits are obtained using 1 : 1 (metal : ligand) stoichiometry for 6MN-dea complexes, these complexes

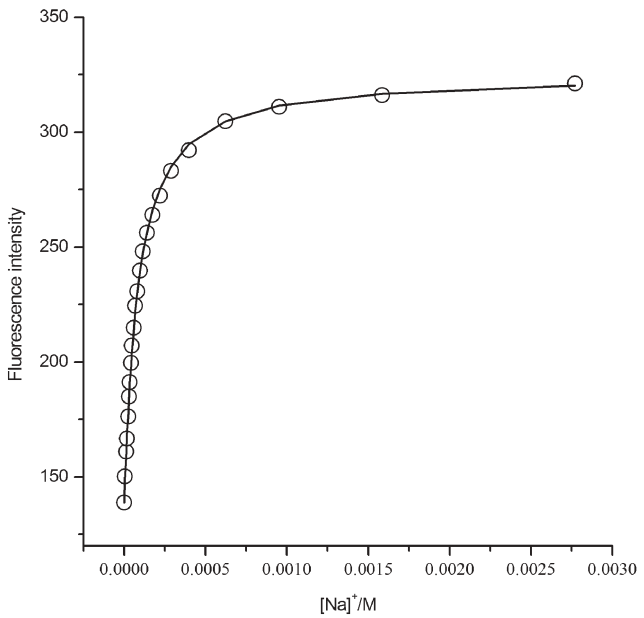


Fig. 6 Titration curve of 3MN-crown (c = 1.8×10^{-5} M) by $NaClO_4$ at 371 nm and fit to 1 : 1 stoichiometry.

Table 4 Stability constants of complexes according to the hypothesis of 1 : 1 stoichiometry

	Log K_s				
	C343-dea	3MN-dea	6MN-dea	3MN-crown	6MN-crown
Li^+	1.70 ± 0.01	[a]	[a]	2.61 ± 0.02	[a]
Na^+	[a]	[a]	[a]	4.13 ± 0.01	3.95 ± 0.02
K^+	[a]	[a]	[a]	2.62 ± 0.02	[a]
Mg^{2+}	4.46 ± 0.01	2.55 ± 0.02	2.98 ± 0.02	(3.78 ± 0.02)[b]	(4.34 ± 0.01)[b]
Ca^{2+}	3.81 ± 0.01	2.63 ± 0.02	2.92 ± 0.02	5.26 ± 0.01	5.71 ± 0.04

[a] Cation-induced spectroscopic changes too small to determine a constant. [b] Stoichiometry 1 : 1 used for calculation but found inadequate by visual inspection of the fitted curve.

showed a surprising decrease of fluorescence intensity we have not explained yet. A proposed hypothesis is the formation of complexes of stoichiometry 2 : 2 and not 1 : 1. Indeed, in this case, a cation would be complexed by the methoxy group of 6MN-dea and at the same time by the carbonyl group of another ligand molecule. This would bring the aromatic rings close together and could lead to the formation of less fluorescent excimeric complexes.

Stability constants are of the same order of magnitude for 3- and 6MN-crown with calcium cations. As mentioned for 3- and 6MN-dea, the methoxy group does not seem to participate in the complexation. Normally the 18-crown-6 ether cavity is adapted for cations of the size of potassium cations. But it appears that smaller cations, like sodium cation, are more strongly bound by this aza-crown cavity than potassium cation. We suppose that the oxygen atoms of the crown ether are coordinating the cation. The nitrogen atom, whose electron-donating character is diminished by its conjugation in the amide link, is probably not involved in the cation complexation, leaving a smaller chelating cavity. Such a sodium complex of 18-membered ring crown amide was studied in ref. 17 by X-ray crystallography. In this solid state structure, the sodium cation is coordinated in the macroring and the non-coordinating amide nitrogen atom is puckered outward. Furthermore this X-ray study reveals that this sodium complex is in a dimeric form with the carbonyl group of another ligand molecule used to fulfil the coordination sphere of the sodium cation.

The 1 : 1 stoichiometry is not adapted for magnesium complexes of 3- and 6MN-crown. Better results were obtained considering M_2L and M_3L_2 stoichiometries. Stability constants for these stoichiometries are defined as follows:

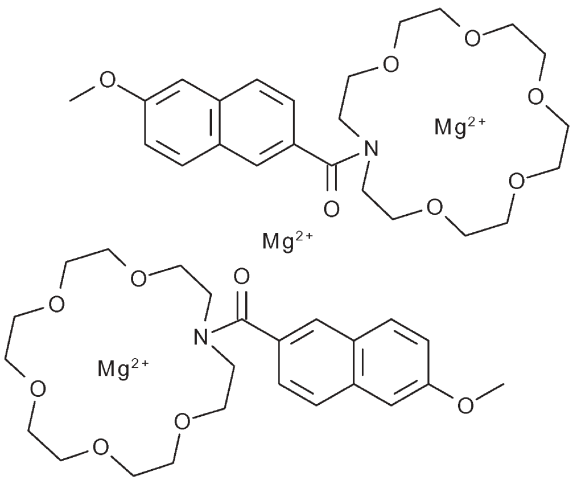
$$M + L \rightleftharpoons ML \quad K_1 = \frac{[ML]}{[M] \times [L]} \tag{2}$$

$$2ML + M \rightleftharpoons M_3L_2 \quad K_2 = \frac{[M_3L_2]}{[ML]^2 \times [M]} \tag{3}$$

or

$$ML + M \rightleftharpoons M_2L \quad K_2 = \frac{[M_2L]}{[ML] \times [M]} \tag{4}$$

With the hypothesis of M_2L stoichiometry, the magnesium complex stability constants obtained were K_1 = 3.77 ± 0.03 and K_2 = 3.96 ± 0.02 for 3MN-crown, K_1 = 4.35 ± 0.04 and K_2 = 4.45 ± 0.01 for 6MN-crown. In the case of M_3L_2 stoichiometry, the results were K_1 = 3.69 ± 0.02 and K_2 = 9.14 ± 0.02, K_1 = 3.63 ± 0.02 and K_2 = 7.90 ± 0.08 for the same complexes, respectively. Although these stoichiometries (proposed structure of M_3L_2 showed below) fit satisfactorily with experimental data, they cannot be considered as established on the basis of these fittings.



D) NMR experiments on the complexation of 3MN-crown and 6MN-dea

NMR spectroscopy can be used to determine if a particular group of a ligand participates in cation complexation or not.[18] Two complexes were studied by NMR: 3MN-crown with Na^+ and 6MN-dea with Mg^{2+}. The first one was chosen because of its high stability constant and its unexpected complexation selectivity. The second one was studied because of its particular photophysical behaviour since a decrease of fluorescence intensity is observed upon metal chelation while an increase was observed in all other studied cases. A possible explanation for this different behaviour would be the formation of a 2 : 2 complex. NMR experiments were done to probe this proposal.

^{1}H NMR spectra of 3MN-crown and its complex with sodium were recorded. The protons of the crown ether part of the ligand appeared as a set of multiplets from 3.30 ppm to 3.78 ppm and are shifted, upon sodium chelation, from 3.50 to 4.08 ppm (Fig. 7). A singlet corresponding to the methoxy group appeared at 3.92 ppm and was not affected by the presence of sodium cation. No shift was observed for aromatic signals. This confirms our view of this complex: the sodium cation is well fitted in the crown ether cavity and the methoxy group is not involved in the cation chelation.

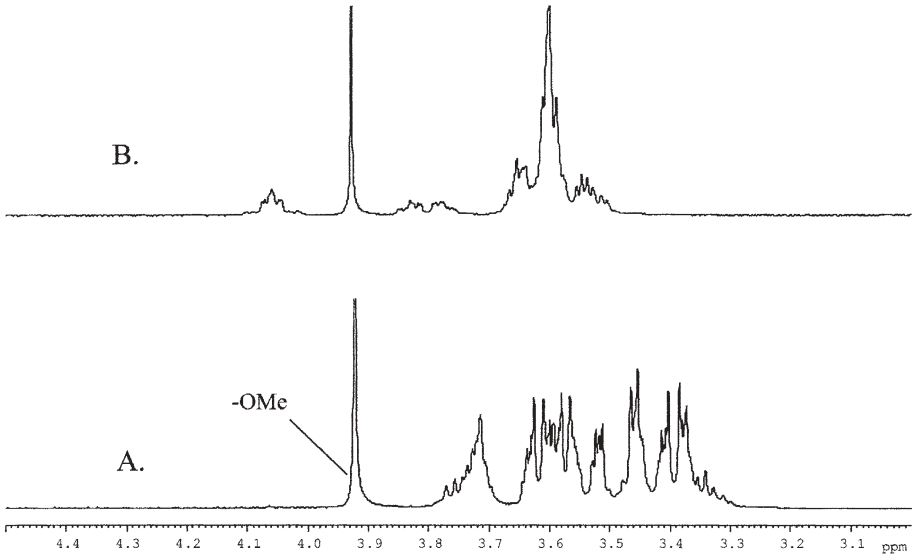
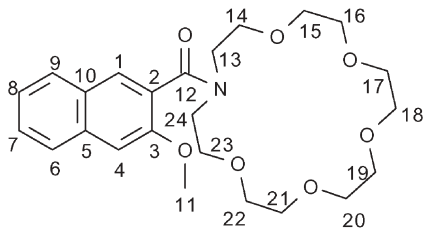


Fig. 7 Part of ^{1}H NMR spectra of A: 3MN-crown ($1.41\ 10^{-3}$ M) and B: its complex with Na^{+}.

Table 5 Chemical shift differences between ^{13}C peaks of 3MN-crown ($c = 9.74 \times 10^{-3}$ M) and its complex with Na^{+}



^{13}C	$\Delta\delta$ (ppm)
12	−0.13
3	−0.12
5	0.12
1, 2, 6, 7, 9, 10	−0.34 to +0.05
8	0.12
4	0.15
14 to 23	−0.16 to +2.61
11	0.02
13, 24	−0.24
	−1.44

^{13}C NMR spectra of the same complex showed significant shifts for ^{13}C of the crown ether but not for the methoxy group nor for the carbonyl group (Table 5). Therefore, it is concluded that the cation does not interact with the carbonyl group in the ground state. But, since red shifts of the emission spectrum and fluorescence enhancement are observed upon metal chelation by 3MN-crown, we supposed that the cations interact with the carbonyl group in the excited state. So, after light excitation, the ligand undergoes intramolecular charge transfer and the electronic density on the carbonyl group is increased. This may yield the formation of a tighter complex with the cation located closer to the carbonyl group in the excited state. This effect would be in the opposite direction compared to the cation photoejection reported by Martin *et al.*[19] In this case, the fluorescent sensor was built in a reversed way compared to our probes: their fluorophore presents also a light induced charge transfer character but the cation interacts with the electron-donating part of the sensor. Moreover, Van den Bergh *et al.*[20] reported that, for calcium complexation by a fluoroionophore, excited and ground state stability constants may be strongly different.

^{1}H and ^{13}C NMR spectra of 6MN-dea and its complex with magnesium are shown in Fig. 8 and Table 6.

The formation of a 2 : 2 complex (as previously supposed) is expected to lead to peak shifts for the carbonyl and methoxy groups. The peaks of the carbonyl group and atoms nearby are shifted, showing that the cation is complexed by this group. However, the positions of the ^{1}H and ^{13}C peaks of the methoxy group are not influenced by the presence of the cation and the hypothesis of a 2 : 2 complex is therefore to be rejected. Furthermore, the rotation of the C–N amide link is shown by the NMR spectra to be difficult due to the double bond character of this link and to be completely hindered when the cation is coordinated to the carbonyl group: two well defined quadruplets of two non-equivalent ethyl groups are apparent in the complex while they are clearly less discriminated in the free ligand. This is an observation that, on one hand, confirms the rigidification of such a system by complexation but, on the other hand, does not explain the fluorescence decrease observed in the case of the 6MN-dea magnesium complex.

E) Mass spectrometry analysis of 3MN-crown and 6MN-dea in the presence of Mg^{2+}

Mass spectrometry measurements were performed on complexes of 3MN-crown and 6MN-dea with magnesium, trying to get further information about stoichiometries: fluorimetric titration of 3MN-crown by Mg^{2+} could not be fitted as a 1 : 1 complex and a 2 : 2 stoichiometry was considered (and disproved by NMR measurements) in the case of the magnesium complex of 6MN-dea. Mass spectrometry is used in supramolecular chemistry thanks to electrospray ionisation (ESI). This technique is a soft ionisation technique and allows the supramolecular species to be transferred from the solution to the gas phase.[21–23]

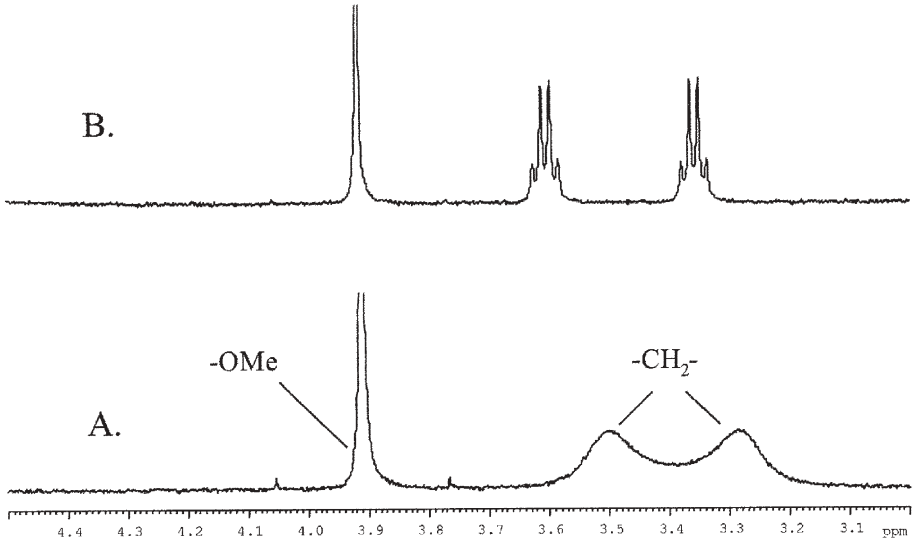
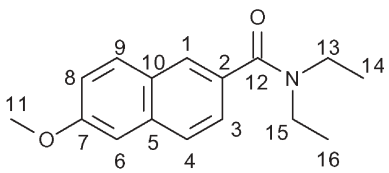


Fig. 8 Part of ^{1}H NMR spectra of A: 6MN-dea ($c = 1.28 \times 10^{-3}$ M) and B: its complex with magnesium.

Table 6 Chemical shift differences between ^{13}C peaks of 6MN-dea ($c = 1.01 \times 10^{-2}$ M) and its complex with magnesium



^{13}C	$\Delta\delta$ (ppm)	^{13}C	$\Delta\delta$ (ppm)
12	1.35	3	−0.44
7	0.64	8	0.59
5	0.50	6	0.21
2	−3.54	11	0.14
9	0.18	15 + 13	1.21
10	−0.30		1.74
4	0.20	16 + 14	−0.35

In the case of the magnesium complex of 3MN-crown, this preliminary study tends to show that the stoichiometry 1 : 1 is in the majority as indicated by signals at *m/z* 256.3 ($[LMgCH_3CN]^{2+}$) and 570.0 ($[LMgClO_4]^{+}$). M_3L_2 and M_2L were not found and this leads us to reject our hypothesis. In the same way, and confirming the NMR results, the mass spectra of magnesium complex of 6MN-dea never showed a 2 : 2 complex but the 1 : 1 complex is once again the major species as recorded by the signals at *m/z* 222.6 ($[LMg(CH_3CN)_4]^{2+}$) and 462.2 ($[LMg(CH_3CN)_2ClO_4]^{+}$). Minority species like ML_2 or ML_3 (for instance, $[(6MN\text{-}dea)_2Mg(CH_3CN)_2]^{2+}$ at *m/z* 310.2 or $[(6MN\text{-}dea)_3MgCH_3CN]^{2+}$ at *m/z* 418.4) are present in both cases and in a larger proportion for 6MN-dea than for 3MN-crown complex because this first ligand does not have a well defined ionophore.

It is also important to note that the observed complexes of 3MN-crown with magnesium are as follows: $[LMgCH_3CN]^{2+}$ and $[LMgClO_4]^{+}$. It seems that the coordination sphere of magnesium cannot be completed by the chelating atoms of 3MN-crown and the cation needs an extra ligand that can be the solvent or the anion. The major observed complex (stoichiometry 1 : 1) in the case of 6MN-dea with magnesium contains four molecules of solvents. More extra ligands are present in this case as can be expected since 6MN-dea offers fewer coordination sites than the 3MN-crown ligand.

The role of these co-ligands is very often neglected in this type of studies and this may well be of importance for explaining why stoichiometries are not always easy to determine: small spectral differences may be expected between two 1 : 1 complexes according to the nature of the co-ligands (acetonitrile, residual water molecule or anion) and the type of 1 : 1 complex that is formed may change during the titration. Association with solvent molecules is expected to dominate at the beginning but anions may be more important at the end of the titration if a large excess of salt is necessary to reach full complexation, which is often the case especially in absorption or emission titrations. Of course in ESI-MS experiments, the evaporation step may influence the nature of the co-ligands according to their relative volatility.

The ESI-MS experiments reveal the presence of complexes of different stoichiometries and the possible change of co-ligands. Both may explain the deviations observed in fittings (see for instance note *b* of Table 4).

Conclusion

The close similarity between the complexation constants found for C343-dea and in preceding works for C343-crown, C343-benzocrown and C343-dibenzocrown clearly demonstrates that the β-dicarbonyl substructure common to these molecules is responsible for the magnesium complexation and that the aza-crown cavity does not seem to play a role in the alkaline-earth cation chelation. Things may be less clear-cut for calcium complexes where competitive complexation seems possible. With the naphthalenic molecules it is clearly demonstrated that amide functions, when alone, are still able to complex calcium and magnesium but with weaker constants, while alkali metal ions do not affect the photophysics of these molecules.

Even if this group was shown to help complexation in other cases,[24] no ancillary chelating effect of the methoxy substituent was demonstrated in the 3MN-fluoroionophores.

In almost all cases a 1 : 1 stoichiometry has been demonstrated. NMR and mass spectrometry measurements, for the cases studied, give arguments favouring a major 1 : 1 complex even when the fluorimetric titration does not fit for this stoichiometry. The presence of minor complexes of different stoichiometries and the possibility of changes in the co-ligands are possible reasons for the deviations observed in certain cases.

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