Absorption and Fluorescence Properties of Acridinones, Thioacridinones, Aminoacridines and Related Crown Ethers

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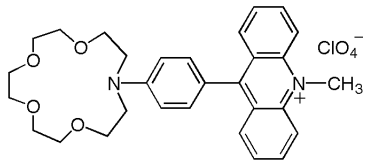
Received February 14, 2001

We report a study on the absorptive and emissive properties of 9-acridinones, 9-thioacridinones and 9-aminoacridines including six crown ether derivatives. The effect of solvents and of the addition of cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) on these properties has been studied. The absorption of the crown ether derivative of 9-thioacridinone is sensitive to solvents while the fluorescence of crown ethers derived from 9-aminoacridines shows some specificity towards cations. Empirical modeling was used to discuss the emission characteristics of these compounds.

J. Heterocyclic Chem., **38**, 1113 (2001).

Although the fluorescence of acridines and related compounds are well known properties that have been the subject of many fundamental studies and practical applications (mostly in molecular biology) [1-7]. The idea to obtain chromoionophoric and fluoroionophoric systems by associating a fluorescent probe to crown ethers is well documented in the literature [8]. However, there is only one publication dealing with the fluorescence of acridine-crown ethers [9] and it concerns compound **1**.



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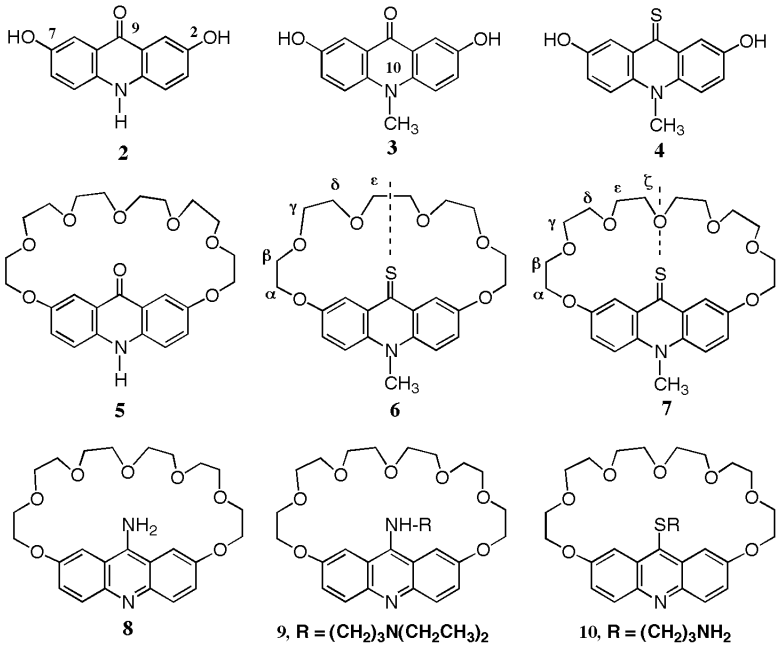
Therefore, we decided to study some acridine crown ethers and their precursors. In this paper, we will describe the absorptive and emissive properties of the following compounds: two 2,7-dihydroxy-9-acridinones **2** and **3**, one 9-thioacridinone **4**, and six crown ethers. These last compounds



2 **3** **4**

5 **6** **7**

8 **9**, R = $(CH_2)_3N(CH_2CH_3)_2$ **10**, R = $(CH_2)_3NH_2$

are linked to positions 2,7 of 9-acridinone **5**, 9-thioacridinone **6**, **7** and **10**, and 9-aminoacridines **8** and **9**. The effect of cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) on the absorption and fluorescence of some acridine-crowns has also been studied.

Results and Discussion.

Absorption Spectra.

Acridine derivatives intensively absorb UV and visible light. Multiple peaks are observed ranging between 245 nm and 510 nm. Table 1 reports the λ_{max} and ε_M obtained for compounds **5**, **7**, **8, 9** and **10**. As an example, the absorption spectra of derivatives **8** and **9** in DMSO and water + 1% DMSO are given in Figure 1.

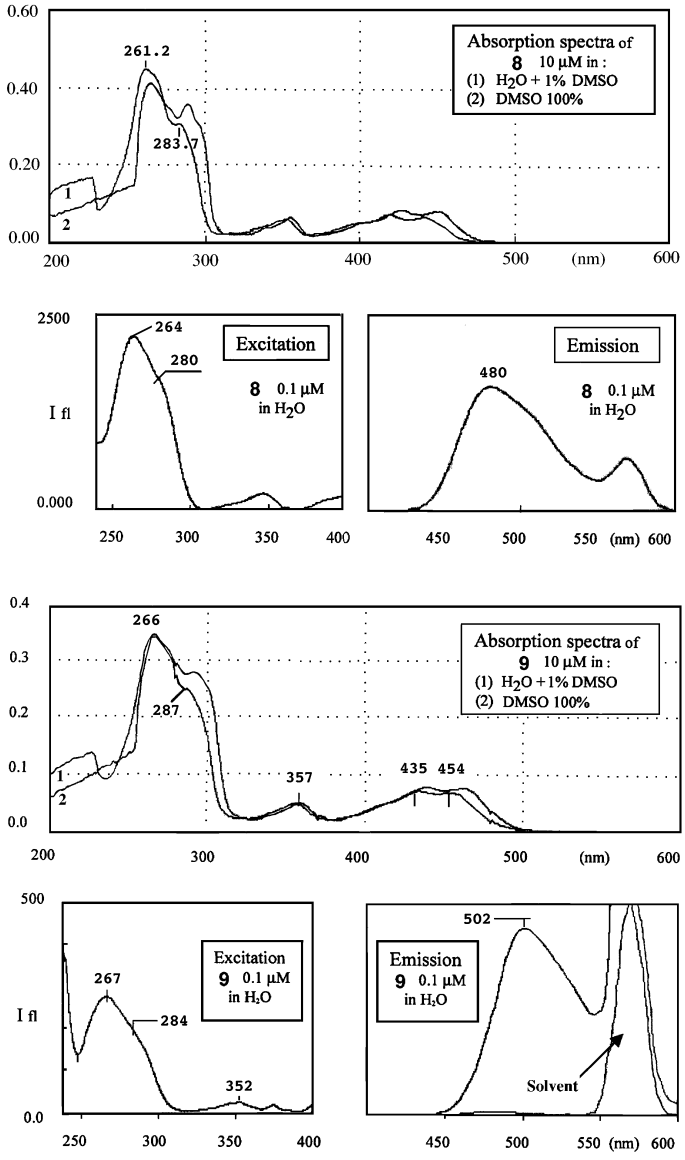


Figure 1. Absorption, excitation and emission spectra of **8** and **9**.

Absorption is very intense in the UV for compound **10** and less for the other compounds which rank in the following order: **5** > **8** > **7** > **9**. Thus, the oxygen atom on the acridine ring is more effective for increasing absorption than the nitrogen atom of an amine or the sulfur atom. Substitution of a hydrogen atom of the amine group by a bulky group decreases the absorption while addition of a bulky group on the sulfur atom increases considerably the absorption.

Effect of Solvents.

The effect of solvents on the absorption spectra of crown ethers was studied using water and DMSO as solvents. Except in the case of **10**, where only a slight hypsochromic effect was observed around 280 nm, increasing polarity of solvent produces a large change of the absorption spectra of **7** (hypsochromic and hyperchromic effects in the UV and visible range). The same effect, but less important, was observed for compounds **8**, **9** (Figure 1) and **5**. Therefore, it appears that thioacridinone derivative **7**, and not the other crown ethers, could be a useful indicator for the change of polarity of the environment [10].

Effect of Ions on the Absorption Spectra.

The absorption spectra of compounds **5**, **7**, and **10** in water are not sensitive to the addition of 140 m*M* NaCl or KCl. In contrast, compounds **8** and **9** are more sensitive to these salts and display a slight hyperchromic effect over all the spectra. So, for compound **8**, the effect of the concentration of K^+ and Na^+ ions on the absorption at 265 nm (the peak of the spectrum) was investigated. The results shown in Figure 2 indicate first an increase of absorption, which can attain 11%, followed by a decrease in the zone of high ionic strength. Moreover, some selectivity for the cation is observed. Compound **9** interacts more specifically with K^+ than with Na^+.

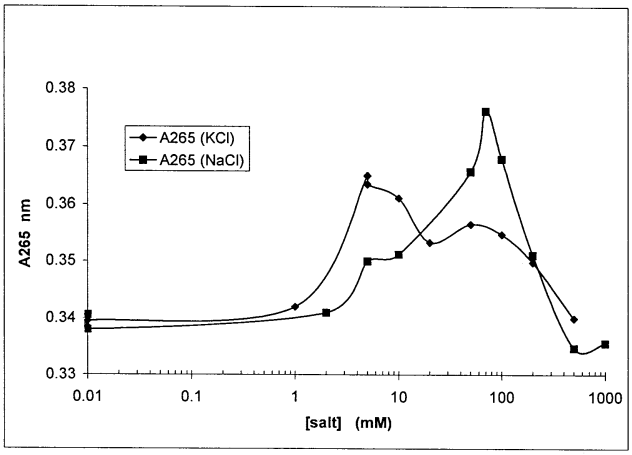


Figure 2. Effect of the concentration of NaCl and KCl on the absorption at 265 nm of compound **9** (10 µ*M* in water and 1% DMSO). The temperature was maintained at 25 °C. Some points were measured 3 times at any moment and the values obtained were the same ± 3‰.

Fluorescence.

Since fluorescence is a more sensitive method than UV absorption, we have used this method to investigate the effect of solvent and cations with compounds **2** to **10**. Figure 3 shows an example of the spectra of excitation and emission of fluorescence obtained with **8** and **9**. As expected, excitation spectra reflected perfectly the absorption spectra of these compounds. An intense band of emission of fluorescence is observed in the visible wavelength around 480 nm (**8**) and 500 nm (**9**). Table 1 gathers the results obtained with all the compounds in H_2O and Table 2 resumes those obtained in THF and THF plus 15% H_2O.

Table 1
Spectral Characteristics (Absorption, Excitation and Emission of Fluorescence) of Some Crown Ethers of Acridinones, Thioacridinones, and Aminoacridines [a]

Compound	Absorption λ_{max} (nm)	ε_M ($M^{-1}.cm^{-1}$)	Excitation (λ_{em} = 480 nm) λ_{max} (nm)	Emission (λ_{exc} = 280 nm) λ_{max} (nm)
5	253.7	56000	254.4	
	277.7	48800		
	284.6	58100	273.8	470.8
	334.8	3350	331.6	484 (s)
	389.7	5100	390 (s)	
	409.1	8800		
	429.7	8800		
7	245.7	36000	255.5	
	281.1	34500	276.1	481.4
	300 (s)	13500		
	308.6	9200		
	372.6	7600		
	424.0	4900		
	445.7	6400		
	480.9	8200		
	507.4	10300		

Table 1 (continued)

Compound	Absorption λ_{max} (nm)	ε_M ($M^{-1}.cm^{-1}$)	Excitation (λ_{em} = 480 nm) λ_{max} (nm)	Emission (λ_{exc} = 280 nm) λ_{max} (nm)
8	261.2	45600	264	
	283.4	31700	280 (s)	480
	352.9	6200	345	502 (s)
	402.3	5040		
	420.6	7600		
	454.8	6900		
9	266.3	34100	267	
	273.1 (s)	31800		
	286.8	24400	284 (s)	502
	356.6	4600	352	
	414.8 (s)	5040		
	435.4	7600		
10	265.6	109400		
	280.0	15700	448	
	371.4	12900		
	384.0	18500		
	412.6 (s)	8500		

[a] Absorption spectra were obtained at 25°C with 10 μM of compound in water + 1% DMSO. (s) means a shoulder. Fluorescence spectra (excitation and emission) were obtained in the same solvent with 0.1 μM of compounds.

Luminescence can be measured in two ways: either from the intensity of the emission band at maximum wavelength or from the area of the emission band, both in arbitrary units (AUF). Assuming that the emission bands have similar shapes, it is expected that these two are related measures. This is actually the case, the intensity being roughly proportional to the area, for instance

Table 2
Fluorescence Results (λ in nm)

Compound	Solvent	Concentration	$\lambda_{excitation}$	$\lambda_{max(emission)}$	Intensity	Surface
2	THF	10^{-6} M	281	444.0	417	19,000
3	THF	10^{-6} M	283	452.0	407	18,500
4	THF	10^{-6} M	283	452.5	125	5,500
5	THF	10^{-6} M	282	441.0	318	17,200
	THF	10^{-5} M	282	------	>1000	------
	THF+15% H_2O	10^{-6} M	282	452.5	248	15,600
	THF+15% H_2O	10^{-5} M	282	------	>1000	------
6	THF	10^{-6} M	285	442.5	112	5,300
	THF	10^{-5} M	285	442.0	687	30,900
	THF+15% H_2O	10^{-6} M	285	456.0	156	9,900
	THF+15% H_2O	10^{-5} M	285	456.0	815	47,800
7	THF	10^{-6} M	285	449.5	122	6,700
	THF	10^{-5} M	285	448.5	570	28,600
	THF+15% H_2O	10^{-6} M	285	463.5	105	7,100
	THF+15% H_2O	10^{-5} M	285	464.0	521	32,600
8	THF	10^{-6} M	281	475.0	140	9,800
	THF	10^{-5} M	281	477.5	306	22,700
	THF+15% H_2O	10^{-6} M	281	478.5	346	23,000
	THF+15% H_2O	10^{-5} M	281	------	>1000	------
9	THF	10^{-6} M	281	496.0	20	1,300
	THF	10^{-5} M	281	496.5	98	6,600
	THF+15% H_2O	10^{-6} M	281	501.0	25	3,100
	THF+15% H_2O	10^{-5} M	281	500.0	243	18,200

for data obtained at 1 μ*M* solutions in THF or in THF plus 15% water, linear regression **(1)** is found:

Intensity = (0.018±0.001) area with n = 13 and R^2 = 0.94(1)

Also, there is a linear relationship between the intensity of emission and the concentration of crown ethers (Table 3). The sensitivity of the detection of these compounds is less than 0.1 μ*M* (it is 0.01 μ*M* and less for compounds **5** and **8**).

Table 3

Fluorescence Emission of Crown Ether Derivatives in Water +1% DMSO as a Function of Concentration. These Values Were Obtained on a Spectrofluorimeter (Hitachi) with Excitation and Emission width of 10 nm

Compound	$\lambda_{excitation}$ (nm)	$\lambda_{emission}$ (nm)	Fluorescence intensity (AUF) 0.1 μ*M*	0.3 μ*M*	0.5 μ*M*	1 μ*M*
5	280	465	2327			
7	280	480	341		1768	3295
8	280	480	1650	4780		
9	280	500	414	1315	2100	4417

As with the absorption method, the polarity of the solvent significantly changes the emission spectra. We decided to try to express the position (in nm) of the maximum of the emission band as a linear combination of different factors. It is possible to build up an empirical model using the data obtained with 1 μ*M* solutions in THF and in THF + 5% H_2O of Table 2 and the following descriptors:

Position 9:	O = 0, NH_2 = 0, S = 1, NHR = 2.5
Position 10:	H = 0, N lone pair = 0.5, CH_3 = 1
Crown:	absence = 0, seven oxygen atoms = 1, six oxygen atoms = 2
Solvent:	THF = 0, THF + 5% H_2O = 1
Solv/Pos. 9:	all = 0 except NH_2/THF-H_2O = 1.5, NH_2/THF = 2, NHR/THF-H_2O = 2.5

The descriptors were empirically built after several trials. The following two multiple regression equations are found:

$\lambda_{max(emission)}$ (nm) = (444.9±0.9) + (2.9±0.8) Position 9 + (6.2±1.2) Position 10 - (5.2±0.7) Crown + (12.9±0.8) Solvent + (15.2±0.6) Solvent/Position 9, n = 13, R^2 = 0.997(2)

Intensity (AUF) = (350±39) - (124±26) Position 9 - (52±34) Crown, n =13, R^2 = 0.76(3)

The position of the emission band in nm depends on all the factors, particularly on the addition of water to THF (a bathochromic effect of 13 nm on average) but this effect is more important for 9-amino derivatives than for the other compounds. The *N*-methylation also produces a bathochromic shift of 6.2 nm on average while the effect of substituting the oxygen by a sulfur atom at position 9 is weak (+2.9 nm).

The intensity of the band is only dependent on the substituents at position 9 and on the presence of a crown ether in the structure, but the dependence is only roughly followed as shown by the low correlation coefficient of equation (3). Note that the substitution of an oxygen (factor = 0) by a sulfur (factor = 1) produces an important hypochromic effect (-124 AUF's).

The most important observation of this paper concerns the effect on the fluorescence properties of connecting the 2,7-dihydroxy groups with the crown ether. The coefficients of equations (2) and (3) show that the crown ether produces both an hypsochromic effect (5.2 nm for the larger ring and 10.4 nm for the small one) and a weak and not very significant hypochromic effect. Concerning 9-aminoacridine crown ethers **8** and **9**, the first one is more fluorescent than the second one.

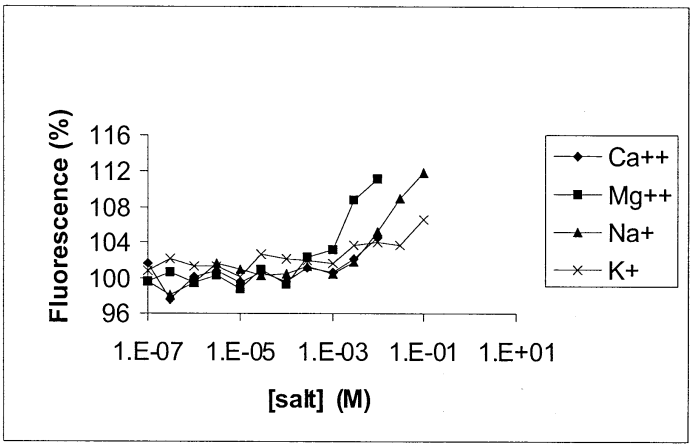


Figure 3. Effect of Na^+, K^+, Ca^{2+} and Mg^{2+} on the emission fluorescence of **8**. Fluorescence is expressed in percent of the value observed at 0 salt. All the salts are in the chloride form. Each point is the mean of 3 determinations.

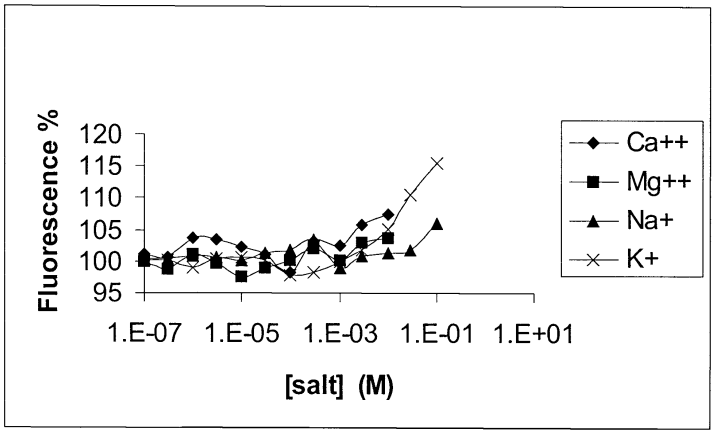


Figure 4. Effect of Na^+, K^+, Ca^{2+} and Mg^{2+} on the emission fluorescence of **9**. Fluorescence is expressed in percent of the value observed at 0 salt. All the salts are in the chloride form. Each point is the mean of 3 determinations.

The results obtained by the fluorescence technique are well related to those obtained by the absorption method. So it was interesting to see the effect of cations of biological importance (Na^+, K^+, Ca^{2+} and Mg^{2+}) on the fluorescence emission. Figures 3 and 4 show the effect of the concentration of cations on the fluorescence of **8** at 0.1 μ*M* and **9** at 0.5 μ*M* in TRIS-Cl 1 m*M*, pH 7.4. The effect is observed only when the concentration is > 10^{-2} *M*.

As observed by the absorption method, cations increase the intensity of fluorescence of compounds **8** and **9**. K^+ is more selective than Na^+ with **9**, while with **8** Na^+ is slightly more selective than K^+. Moreover, among the divalent cations used, the interaction of Mg^{2+} is more selective with **8** than with **9** and better recognized than the other cations. Fluorescence of **5** and **7** is not sensitive to the addition of 10 m*M* or 100 m*M* KCl (results not shown).

The selectivity towards monovalent and divalent cations in the case of **8** and **9**, suggest that the interaction of cations with crown ethers derived from 9-aminoacridine, could be increased probably by increasing the cavity of the crown able to receive the cation like in the well-known gramicidin [11]. In this case, a larger modification of the fluorescence is expected, which could be easily used in the studies of many biological systems.

EXPERIMENTAL

Fluorescence measurements were carried out with two instruments, a Hitachi spectrofluorimeter model D2000 and a Fluorescence Spectrometer Perkin Elmer LS 50. Nmr spectra were recorded in deuteriochloroform or DMSO-d_6 with TMS as internal standard on a Bruker-AMX 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Melting points were measured with a Mettler FP61 apparatus. Commercially available reagents and compounds were purchased from Aldrich and were used without further purification.

Compounds **2** (2,7-dihydroxy-9-acridinone), **3** (2,7-dihydroxy-10-methyl-9-acridinone), **5** [2,7-(epoxyethanoxyethanoxyethanoxyethanoxyethanoxyethanoxy)acridin-9(10*H*)-one], **8** [2,7-(epoxyethanoxyethanoxyethanoxyethanoxyethanoxyethanoxy)acridin-9-amine] and **9** [*N*,*N*-dimethyl-1,3-propanediamine, *N'*-2,7-(epoxyethanoxyethanoxyethanoxy-ethanoxy-ethanoxyethanoxy)acridin-9-yl] have been described by us [12].

2,7-Dihydroxy-10-methyl-9-thioacridinone (**4**).

First, 2,7-dimethoxy-10-methyl-9-thioacridinone was prepared by treatment of 2,7-dimethoxy-10-methyl-9-acridinone [10] with phosphorous pentasulfide (P_4S_{10}) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)pyrimidinone as solvent. The compound, mp 204 °C, was obtained in 94% yield.

Anal. Calcd. for $C_{16}H_{15}NO_2S$: C, 67.37; H, 5.26; N, 4.91. Found: C, 67.46; H, 5.31; N, 4.78.

After heating under reflux for 24 hours a mixture of 2,7-dimethoxy-10-methyl-9-thioacridinone (2 g, 7 mmoles), 50 ml of 48% hydrobromic acid and 50 ml of acetic anydride, the solution was poured into water, then neutralized with diluted ammonium hydroxide. A dark violet solid precipitates, which was washed with cold and hot water and then dried. Yield 1.65 g (92%), mp 175 °C; ^{1}H-nmr (DMSO-d_6): δ 4.08 (s, 3H, CH_3), 7.41 (dd, 2H, H-3 and H-6), 7.88 (dd, 2H, H-4 and H-5, J = 9.4), 8.44 (d, 2H, H-1 and H-8, J = 3.0); ^{13}C-nmr (DMSO-d_6): δ 108.9 (C-1 and C-8), 153.0 (C-2 and C-7), 124.8 (C-3 and C-6), 118.4 (C-4 and C-5), 192.1 (C-9), 130.8 and 131.1 (C-8a, C-9a, C-4a and C-10a), 34.9 (CH_3).

Anal. Calcd. for $C_{14}H_{11}NO_2S$: C, 65.37; H, 4.28; N, 5.45. Found: C, 65.61; H, 4.48; N, 5.50.

Reaction Between 2,7-Dihydroxy-10-methyl-9-thioacridinone (**4**) and Penta(ethyleneglycol) ditosylate.

Compound **4** (1 g, 3.9 mmoles) was dissolved into 400 ml of anhydrous dimethylformamide heated at 100 °C under nitrogen atmosphere; then, cesium fluoride was added (3.1 g, 20.35 mmoles) and the mixture stirred for 2 hours. Afterwards, a solution of penta(ethyleneglycol) ditosylate (2.4 g, 4.4 mmoles) in 50 ml of dimethylformamide was added dropwise. The mixture was stirred for 4 days at 100 °C under nitrogen atmosphere. Dimethylformamide was eliminated under reduced pressure, the residue extracted with 400 ml of hot acetonitrile and then filtered off. The solution was evaporated yielding an oily residue that was extracted with acetone. The solid part, mostly cesium fluoride, was filtered off and eliminated. The acetone solution was evaporated, treated with ethanol diethyl ether 1:1, and the obtained garnet-coloured crystals were filtered and dried. Compound **6** [10-methyl-2,7-(epoxyethanoxyethanoxyethanoxyethanoxyethanoxy)acridine-9(10*H*)-thione], yield 150 mg (8%), mp 161 °C; ^{1}H-nmr (deuteriochloroform): δ 3.35 (m, 4H, CH_2-ε), 3.40 (m, 4H, CH_2-δ), 3.51 (m, 4H, CH_2-γ), 3.83 (t, 4H, CH_2-β), 3.97 (s, 3H, CH_3), 4.51 (t, 4H, CH_2-α), 7.35 (dd, 2H, H-3 and H-6), 7.49 (d, 2H, H-1 and H-8, J = 3.0), 8.92 (d, 2H, H-4 and H-5, J = 9.4); ^{13}C-nmr: (deuteriochloroform): δ 112.7 (C-1 and C-8), 154.9 (C-2 and C-7), 125.9 (C-3 and C-6), 116.4 (C-4 and C-5), 196.0 (C-9), 131.6 and 132.1 (C-8a, C-9a, C-4a and C-10a), 34.9 (CH_3), 67.5 (C-α), 71.6 (C-β), 70.9 (C-γ), 71.1 (C-δ) and 70.4 (C-ε). The X-ray strucure of this compound has already been published [13].

Anal. Calcd. for $C_{24}H_{29}NO_6S$: C, 62.74; H, 6.32; N, 3.05. Found: C, 62.95; H, 6.49; N, 3.17.

Reaction Between 2,7-Dihydroxy-10-methyl-9-thioacridinone (**4**) and Hexa(ethyleneglycol) ditosylate.

Compound **4** (1 g, 3.9 mmoles) was dissolved into 400 ml of anhydrous dimethylformamide heated at 100 °C under nitrogen atmosphere; then, cesium fluoride was added (3.1 g, 20.35 mmoles) and the mixture stirred for 1 hour. Afterwards, a solution of hexa(ethyleneglycol) ditosylate (2.6 g, 4.4 mmoles) in 50 ml of dimethylformamide was added dropwise. The mixture was stirred during 4 days at 100 °C under nitrogen atmosphere. Dimethylformamide was eliminated under reduced pressure, the residue extracted with 400 ml of hot acetonitrile and then filtered off. The solution was evaporated yielding an oily residue that was extracted with acetone. The solid part, mostly cesium fluoride, was filtered off and eliminated. The acetone solution was evaporated, treated with ethanol diethyl ether 1:1, and the obtained brick-red residue filtered and dried. Compound **7** [10-methyl-2,7-(epoxyethanoxyethanoxyethanoxyethanoxyethanoxyethanoxy)acridine-9(10*H*)-thione] yield 250 mg (13%), mp 134 °C; ^{1}H-nmr (deuteriochloroform): δ 3.26 (m, 4H, CH_2-ζ), 3.37 (m, 4H, CH_2-ε), 3.52 (m, 4H, CH_2-δ), 3.67 (m, 4H, CH_2-γ), 3.92 (t, 4H, CH_2-β), 4.02 (s,

3H, CH_3), 4.51 (t, 4H, CH_2-α), 7.42 (dd, 2H, H-3 and H-6), 7.56 (d, 2H, H-1 and H-8, J = 2.9), 8.83 (d, 2H, H-4 and H-5, J = 9.4); ^{13}C-nmr (deuteriochloroform): δ 111.8 (C-1 and C-8), 154.6 (C-2 and C-7), 125.6 (C-3 and C-6), 116.8 (C-4 and C-5), 194.5 (C-9), 131.1 and 131.8 (C-8a, C-9a, C-4a and C-10a), 34.8 (CH_3), 67.6 (C-α), 71.0, 70.9, 70.3, 70.0 and 69.9 (C-β, C-γ, C-δ, C-ε and C-ζ).

Anal. Calcd. for $C_{26}H_{33}NO_7S$: C, 62.03; H, 6.56; N, 2.78. Found: C, 62.41; H, 6.85; N, 2.65.

Fluorescence Experiments.

The position of the excitation line used was between 281 and 285 nm. In all cases, a series of experiments were carried out in order to determine the optimum concentration, *i.e.*, the concentration which results in the maximum of emission. These concentrations, in the range 10^{-5} to 10^{-6} *M*, are those reported on Table 1. More concentrated solutions show concentration quenching [14].

The effect of metals on the fluorescence of compounds **8** and **9** were determined in dimethyl-sulfoxide. A 10^{-7} *M* solution of the acridine crown ethers was prepared, then increasing quantities of the sodium, potassium, calcium and magnesium chlorides dissolved in water were added. The cells were maintained at 25 °C and the solutions stirred during the recording.

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