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Synthesis and fluorescence studies of novel bis(azacrown ether) type chemosensors containing an acridinone unit

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ABSTRACT

Three new bis(azacrown ether)s containing an acridinone fluorescent signalling unit were prepared by reacting 9-chloro-4,5-bis(chloromethyl)acridine with monoazacrown ethers with different cavity sizes followed by a hydrolysis step. Their fluorescent characteristics and the complexation properties of the one containing two monoaza-18-crown-6 ether receptors towards selected metal ions were studied. The operation of the latter ligand is based on the photoinduced electron transfer (PET) process, thus it shows fluorescence enhancement in the presence of metal ions. Since the sensor molecule contains two receptor moieties, it is able to form two types of complex with 1:1 and 1:2 ligand to metal ion ratios, which have different emission spectra. The reason for this spectral difference may be that the 1:1 complex is a sandwich type one in which the acridinone unit can undergo structural changes.

1. Introduction

Fluorescent chemosensors for metal ion analysis are of great importance due to their potential applications in a wide range of areas such as cell biology, biochemical analysis, medical diagnosis and monitoring of contamination by heavy metals.[1] Fluorescence spectroscopy is an attractive tool owing to its sensitivity, selectivity, versatility and relatively simple handling.[2,3] Furthermore, the concept of a photoinduced electron transfer (PET) system for designing sensor molecules based on a fluorophore–spacer–receptor structure is quite useful due to its capability of signalling the recognition event very sensitively.[4–8] PET type fluorescence behaviour means that the free sensor molecule exhibits poor fluorescence due to an efficient intramolecular quenching process (PET) in the excited state, but shows large fluorescence enhancement in the presence of cations without spectral shifts.[4–8] Incorporating an acridinone moiety as a signalling unit into a sensor molecule is very advantageous because of its strong fluorescence[9–11] and great photostability.[12] Recently, we synthesized several achiral and chiral mono(azacrown ether) type PET sensor molecules containing an acridinone or an *N*-methylacridinone fluorescent unit and studied their complexation properties towards various cationic guests.[13] Bis(azacrown ether) type ligands, which have two receptor moieties within a molecule, can form complexes of different structures, thus they exhibit particular complexing abilities compared to the mono(azacrown ether) type ones.[14] Among the complex formation modes, the intramolecular sandwich complex can be emphasized from our point of view, the formation of which depends on the flexibility of the sensor molecule and the proximity of the crown ether rings.[14] This complex form can cause an enhanced selectivity towards large metal ions.[14] Several reported sensor molecules match the bis(azacrown ether) and the PET system concepts based on a fluorophore–short alkylene spacer–azacrown receptor structure using different signalling units and their complexation properties were examined towards metal ions and α,ω-alkanediammonium ions.[15–21]

Herein we report the synthesis of three new fluorescent bis(azacrown ether) type sensor molecules in which an acridinone signalling unit was attached to two monoazacrown ethers through methylene bridges. Their fluorescent characteristics and the complexation properties of the one having two monoaza-18-crown-6 ether receptors towards selected metal ions (Li^+, Na^+, K^+, Ag^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}) were studied in detail.

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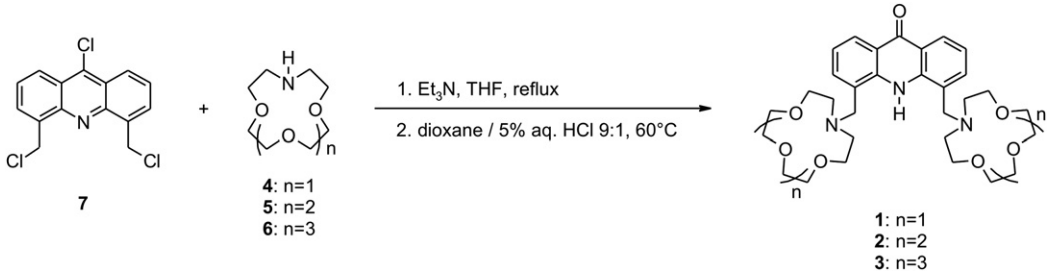
2. Results and discussion

2.1. Synthesis

Bis(azacrown ether) chemosensors **1**–**3** containing an acridinone fluorescent signalling unit were synthesized by alkylation of monoazacrown ethers **4**–**6** with bis(chloromethyl)-substituted acridine derivative **7** followed by a hydrolysis step (Scheme 1). The alkylation reactions were performed in dry THF in the presence of triethylamine as a base and the obtained crude products were hydrolized in a mixture of dioxane/5% aqueous hydrochloric acid (9:1) to give the desired bis(azacrown ether)s (**1**–**3**) containing the acridinone unit. The overall yields for these two steps are rather low (10% for **1** and **2**, 9% for **3**), probably, because the electrophilic carbon atom having a chlorine atom at position 9 of the trichloro derivative **7** can also react with the monoazacrown ethers **4**–**6**, and also, because during the hydrolysis the benzylic carbon atom of the protonated benzyl amine type intermediates can be attacked by the nucleophilic water molecules, too.

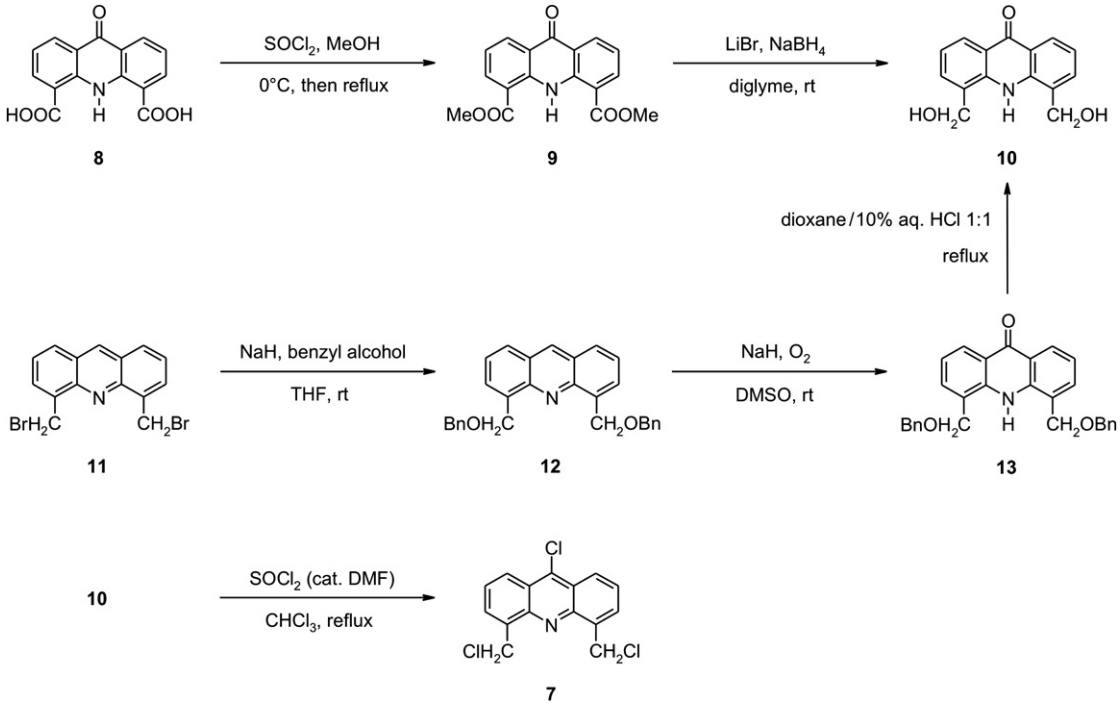
Bis(hydroxymethyl)acridinone **10** was prepared in two different ways (Scheme 2). On the one hand the synthesis was started from oxoacridine dicarboxylic acid **8**,[22] which was treated with thionyl chloride in dry methanol to obtain its dimethyl ester **9**. The latter (**9**) was reduced by sodium tetrahydridoborate and lithium bromide in dry diethylene gylcol dimethyl ether with a modification of a reported procedure[23] to give bis(hydroxymethyl) derivative **10**. On the other hand, bis(bromomethyl)acridine **11**[24,25] was reacted with the sodium salt of benzyl alcohol in dry THF to yield bis(benzyloxymethyl)acridine **12**, which was oxidized to acridinone derivative **13** under an oxygen atmosphere in dry DMSO in the presence of a strong base sodium hydride as reported[26] for similar acridine derivatives. To obtain bis(hydroxymethyl) derivative **10**, the benzyl protecting groups were removed by boiling bis(benzyloxymethyl)acridinone **13** in a mixture of dioxane/10% aqueous hydrochloric acid (1:1).



Scheme 1. Preparation of bis(azacrown ether) type fluorescent chemosensors **1**–**3**.

Acridine derivative **7** was prepared by treating bis(hydroxymethyl)acridinone **10** with thionyl chloride in dry chloroform in the presence of a catalytic amount of DMF (Scheme 2). Acridine derivative **7** was expected to be easily convertable to the appropriate acridinone derivative [4,5-bis(chloromethyl)acridin-9(10*H*)-one] according to our previous observations.[13] However, applying the same experimental conditions as used for the hydrolysis of the crude 9-chloro-4-chloromethylacridine [a mixture of dioxane/water (19:1) at rt],[13] the reaction did not take place. Furthermore, acridine derivative **7** withstood the conditions of purification by column chromatography, so it was suitable for the alkylation reactions.

Comparing the efficiency of the two synthetic routes for bis(hydroxymethyl) derivative **10**, it should be mentioned that the two precursors (**8** and **11**) were prepared from commercially available starting materials, 2-chloro-1,3-dimethylbenzene and acridine in three steps and one step, respectively. Although the overall yield of bis(hydroxymethyl) derivative **10** following the pathway started from 2-chloro-1,3-dimethylbenzene was twice as



Scheme 2. Preparation of acridine derivative 7 and acridinone derivative **10**.

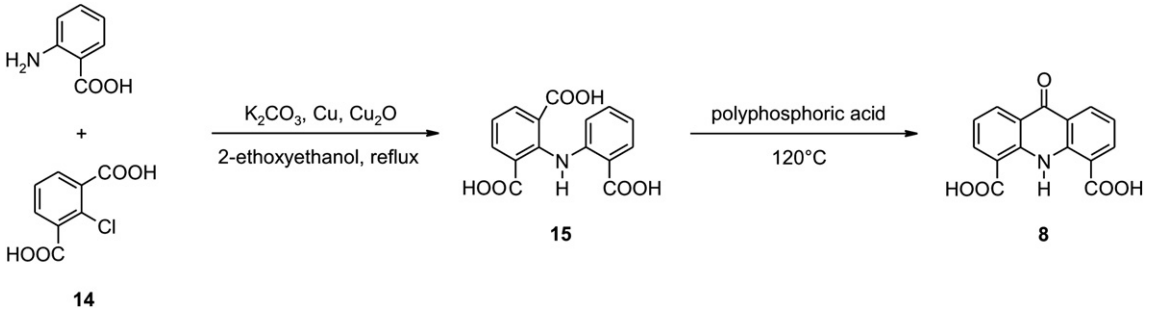
much (10%) as that of **10** applying the route started from acridine (5%), the former pathway consisted of six steps and the latter route only four steps. The synthesis of acridinone dicarboxylic acid **8** was started from 2-chloro-1,3-dimethylbenzene, which was oxidized with potassium permanganate in water to obtain dicarboxylic acid **14**.[27] The latter (**14**) was reacted with anthranilic acid in the presence of potassium carbonate, copper powder and copper(I) oxide in dry 2-ethoxyethanol using a modification of the reported procedure of an analogous compound[26] to give tricarboxylic acid **15**,[22] which was then heated in polyphosphoric acid to yield oxoacridine dicarboxylic acid **8**[22] (Scheme 3). Bis(bromomethyl)acridine **11** was prepared by treating acridine with bromomethyl methyl ether in sulfuric acid as reported.[24,25]

values are larger (2.6–5.7 times) in dichloromethane than in acetonitrile and methanol, but less significantly than in the case of chemosensors **16**–**18**[13] (Table 1).

Table 1
Fluorescence quantum yields of **1**–**3** and **16**–**18** in different solvents

Compound	Φ_f (CH_2Cl_2)	Φ_f (MeCN)	Φ_f (MeOH)
1	0.12	0.021	0.046
2	0.10	0.019	0.022
3	0.10	0.043	0.027
16	0.60[a]	—	0.15[a]
17	0.59[a]	—	0.070[a]
18	0.55[a]	—	0.038[a]

[a] These values were taken from literature.[13]



Scheme 3. Modified preparation of oxoacridine dicarboxylic acid **8**.

2.2. Fluorescence characterization

The fluorescence spectra of ligands **1**–**3** in dichloromethane (Fig. 1) are very similar to those of their mono(azacrown ether) analogues **16**–**18** (Fig. 2) both in dichloromethane and methanol.[13] However, in acetonitrile and methanol new bands appeared in the emission spectra of ligands **1**–**3** in the wavelength region above 450 nm (Fig. 1). The fluorescence quantum yields of chemosensors **1**–**3** were determined in these solvents and it can be stated that the

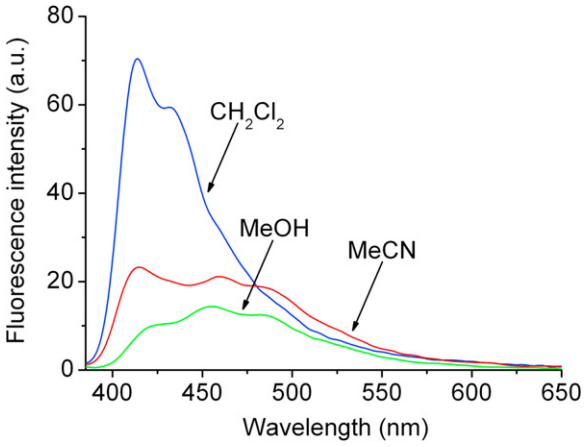


Figure 1. Fluorescence emission spectra of **3** (20 μM) in MeOH, MeCN and CH_2Cl_2, λ_{ex}=370 nm.

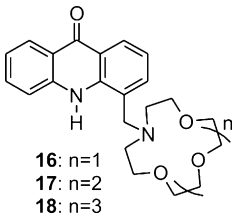


Figure 2. Reported mono(azacrown ether) analogues **16**–**18**.

2.3. Complexation studies on chemosensor 3

Since chemosensor **3** has a modular (fluorophore–methylene spacer–azacrown receptor) structure, a PET type fluorescence response was expected upon complexation with cationic guests similar to our previous experiences in the case of its mono(azacrown ether) analogue **18**.[13] We started our complexation studies in methanol as in the case of ligand **18**.[13] In general, ligand **3** showed fluorescence enhancement upon addition of various metal ions as expected, but in most cases the positions of the emission spectra of metal ion complexes were different from those of ligand **18** (Fig. 3). Six of the ten metal ions studied (K^+, Ag^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}) caused similar type emission spectral changes, namely a fluorescence increase upon complexation mainly in the wavelength region above 450 nm (Fig. 4). However, in the case of Cu^{2+}, Pb^{2+} and Na^+, after addition of an excess of the metal ion in question, a second spectrum form appeared on the titration series of spectra (Fig. 5), the position of which was similar to the emission spectra of ligand **18**

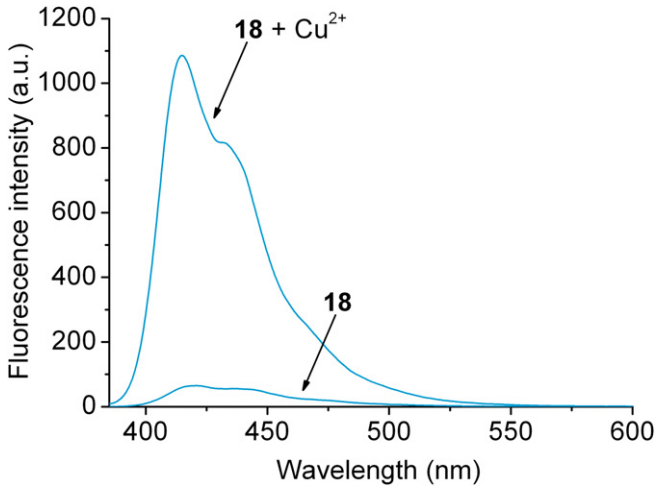


Figure 3. Fluorescence emission spectra of free ligand **18** (20 μM) and its complex with Cu^{2+} (200 equiv) in MeOH, λ_{ex}=370 nm.

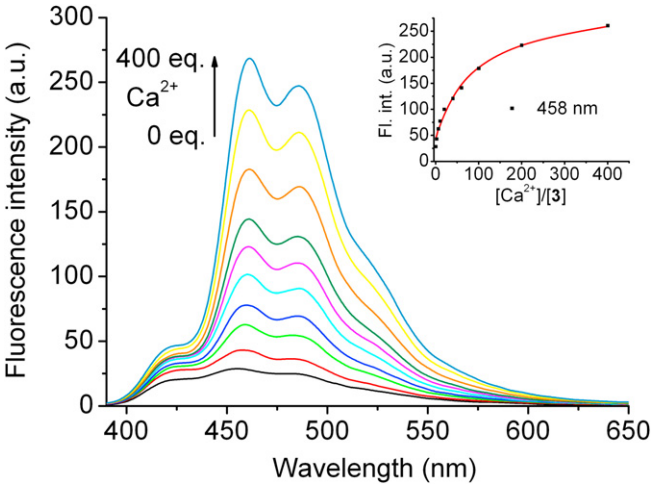


Figure 4. Fluorescence emission series of spectra of **3** (20 μM) on increasing addition of Ca^{2+} (0–400 equiv) in MeOH, λ_{ex}=370 nm. Inset: titration curve (0–400 equiv) at 458 nm, solid line: fitted curve.

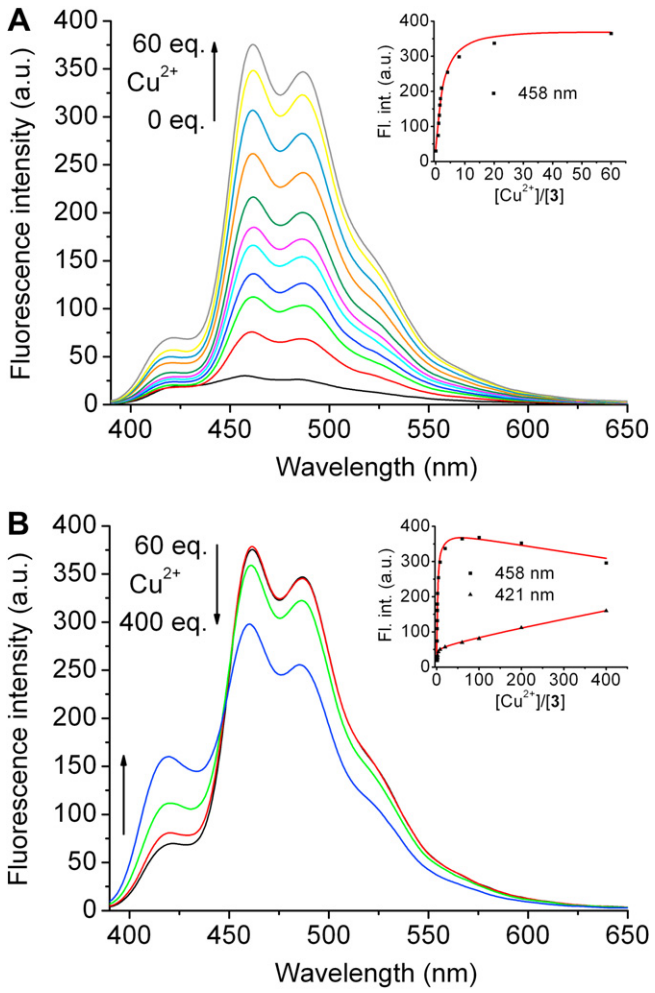


Figure 5. Fluorescence emission series of spectra of **3** (20 μM) on increasing addition of Cu^{2+} (A: 0–60 equiv, B: 60–400 equiv) in MeOH, λ_{ex}=370 nm. Insets: titration curves (A: 0–60 equiv at 458 nm, B: 0–400 equiv at 421 and 458 nm), solid lines: fitted curves.

and its metal ion complexes (Fig. 3). The absorption spectrum remained essentially unchanged upon titration of ligand **3** with metal ions in almost all cases, which is typical for PET type sensor molecules, the only exception was the effect of Ag^{+} (Fig. 6).

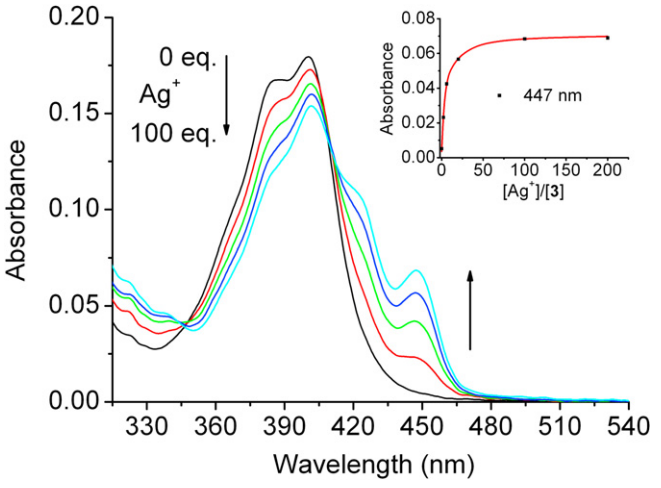


Figure 6. UV–vis titration series of spectra of **3** (20 μM) on increasing addition of Ag^{+} (0–100 equiv) in MeOH. Inset: titration curve (0–200 equiv) at 447 nm, solid line: fitted curve.

All of the fluorescence spectral changes were evaluated using global nonlinear regression analysis by means of the SPECFIT/32™ program and the stability constants of metal ion–ligand complexes were determined (Table 2). In the latter cases (Cu^{2+}, Pb^{2+} and Na^{+}), the best fits were obtained when we assumed two complex forms with 1:1 and 1:2 ligand to metal ion ratios, whereas in the other cases the spectra could be fitted satisfactorily using only one species with 1:1 stoichiometry. It is noteworthy to mention that the log β_1 value of complex **3**–Ag^{+} determined from the UV–vis titration series of spectra (4.08) was in good agreement with that obtained from the emission changes (3.85, Table 2).

Table 2
Stability constants for metal ion complexes of **3** in MeOH and MeCN[a]

	MeOH		MeCN	
	log β_1	log β_2	log β_1	log β_2
Li^{+}	—[b]	—[b]	3.86	5.79
Na^{+}	4.77	7.02	4.97	8.28
K^{+}	4.14	—	5.06	7.35
Mg^{2+}	2.88	—	4.71	7.99
Ca^{2+}	2.66	—	6.78	13.12
Zn^{2+}	3.13	—	7.78	11.70
Cd^{2+}	2.94	—	5.31	7.75
Cu^{2+}	4.36	5.77	—[c]	—[c]
Ag^{+}	3.85	—	4.15	6.53
Pb^{2+}	5.21	7.79	7.12	13.46

[a] The values β_1 and β_2 denote the overall equilibrium constants of the 1:1 and 1:2 stoichiometric complexes, respectively.
[b] No complexation could be observed.
[c] Complexation took place, but the emission spectral changes could not be fitted satisfactorily.

The presence of both 1:1 and 1:2 complex forms during the titration experiments with metal ions was more pronounced in acetonitrile than in methanol. In acetonitrile, the two spectrum forms mentioned above appeared on the emission series of spectra in all cases (Fig. 7). Thus, we had to consider both 1:1 and 1:2 stoichiometric complexes to obtain the best fit of the fluorescence spectra in order to determine the complex stability constants (Table 2). The absorption spectrum in acetonitrile was also unchanged upon complexation. The difference between the complexation behaviour of ligand **3** in the presence of metal ions in the two solvents, methanol and acetonitrile, is demonstrated in the case of Ca^{2+} (Figs. 4 and 7).

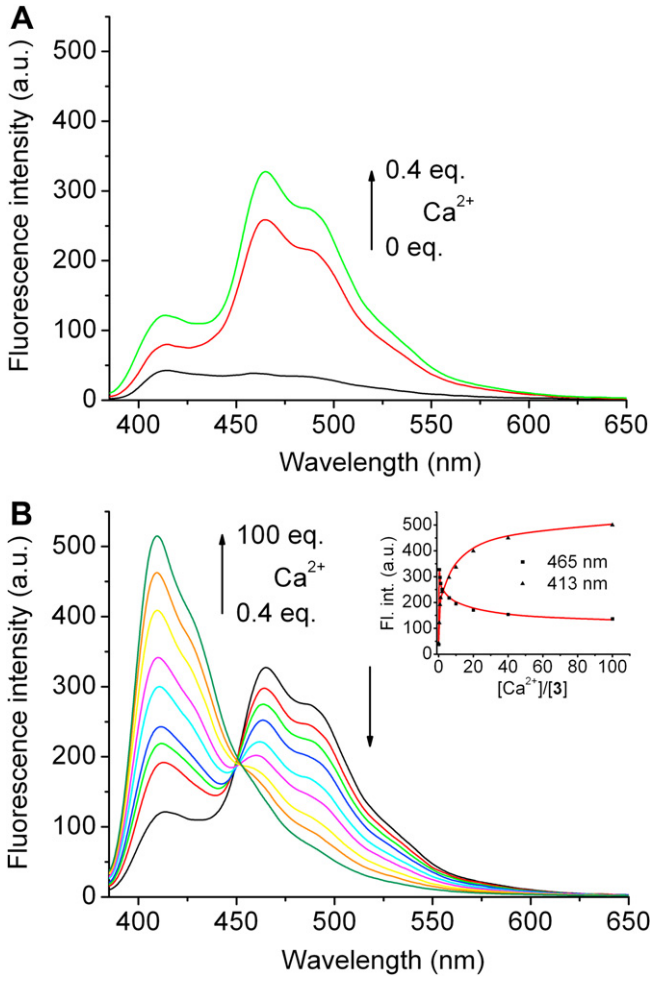


Figure 7. Fluorescence emission series of spectra of **3** (20 μM) on increasing addition of Ca^{2+} (A: 0–0.4 equiv, B: 0.4–100 equiv) in MeCN, λ_{ex}=370 nm. Inset: titration curves (0–100 equiv) at 413 and 465 nm, solid lines: fitted curves.

The fluorescence behaviour of chemosensor **3** upon complexation with metal ions significantly differs from its mono(azacrown ether) analogue **18**.[13] While ligand **18** showed only emission increase upon complexation without spectral shifts,[13] in the case of ligand **3**, spectral shifts were also observed in the presence of cations besides the fluorescence enhancement, which is untypical in the case of PET sensor molecules. Based on our observations mentioned above, we can explain this behaviour as follows. The spectrum shown in the presence of a larger excess of metal ion (e.g., at 100 equiv of Ca^{2+} in acetonitrile, Fig. 7B) belongs to the 1:2 stoichiometric complex in which the two crown ethers bind two metal ions independently from each other. This can be confirmed by that the position and shape of the spectrum were similar to those of the spectrum of ligand **18** in its complexed form (Fig. 3). The other spectrum, which arose at the beginning of a titration process (e.g., at 0–60 equiv of Cu^{2+} in methanol, Fig. 5A) can be attributed to the presence of the 1:1 stoichiometric complex. We assume that it is a sandwich type complex in which the two crown ethers bind one metal ion. The presence of a simple 1:1 complex in which one crown ether ring binds one cation and the other crown cavity remains unbound can be excluded, since the position and shape of the spectrum were different from those of the 1:2 complex. The difference in the spectra of 1:1 and 1:2 complexes of ligand **3** can be explained by that the acridinone unit undergoes structural changes in the sandwich complex. The latter changes can be attributed to the acridinone–9-hydroxyacridine tautomerism[28] or the deprotonation of the acridinone NH.[29] Since the absorption spectrum was unchanged upon complexation (with the only exception of Ag^{+}), these structural changes could take place only in the excited singlet state. A confirmation of the deprotonation of the acridinone NH in the assumed sandwich complex may be provided by the fact that the emission spectrum of the latter was very similar to that of the deprotonated form of ligand **3** in the presence of a large excess of Me_4NOH (Fig. 8). Either the tautomerism or the deprotonation of the acridinone unit could promote the participation of the nitrogen atom of the latter in the complexation process with a metal ion or vice versa.

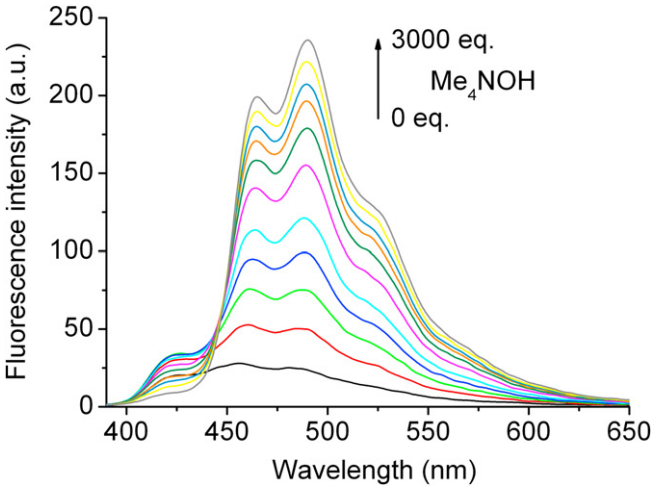


Figure 8. Fluorescence emission series of spectra of **3** (20 μM) on increasing addition of Me_4NOH (0–3000 equiv) in MeOH, λ_{ex}=370 nm.

In general, it can be stated about the complexation ability of ligand **3** that it formed stronger complexes in acetonitrile than in methanol (Table 2) and the presence of the 1:2 stoichiometric complex during the titration experiments was much more pronounced in the former solvent as discussed earlier (Figs. 4 and 7, Table 2). Comparing the effects of various metal ions in methanol, in the presence of 4 equiv of them, the largest fluorescence enhancements were caused by Cu^{2+}, Pb^{2+} and Ag^{+} (Fig. 9), but in larger excesses the other metal ions (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}) also induced significant changes. In the case of ligand **18**, only Cu^{2+}, Pb^{2+} and K^{+} caused considerable fluorescence enhancement

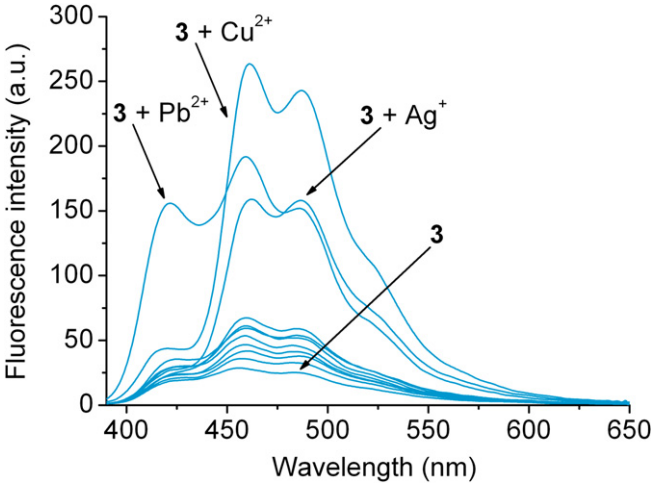


Figure 9. Fluorescence enhancement of **3** (20 μM) in the presence of 4 equiv of various metal ions (Cu^{2+}, Pb^{2+}, Ag^{+} and the others in order of increasing fluorescence intensity: Li^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, K^{+}, Cd^{2+}, Zn^{2+}) in MeOH, λ_{ex}=370 nm.

in 400-fold excess.[13] This means that ligand **3** formed stronger complexes with the metal ions studied as compared to its mono-(azacrown ether) analogue **18**, probably due to the presence of two receptor moieties in the same molecule, but no sufficient selectivity between the metal ions examined could be observed. Furthermore, the spectral shifts and simultaneous presence of the two complex forms upon complexation made it difficult to compare these fluorescence changes with each other, thereby it cannot be susceptible for sensing a metal ion in a wider concentration range.

3. Conclusion

The synthesis and characterization of three new bis(azacrown ether)s containing an acridinone fluorescent signalling unit and their unreported precursors have been performed. The key intermediate of the fluorescent unit [bis(hydroxymethyl) derivative **10**] was prepared in two different ways. Comparing the efficiency of them, the overall yield of bis(hydroxymethyl) derivative **10** following the pathway started from 2-chloro-1,3-dimethylbenzene was twice as much (10%) as that of **10** applying the route started from acridine (5%), although the former pathway consisted of six steps and the latter route only four steps. The complexation properties of the chemosensor containing two monoaza-18-crown-6 ether receptors (**3**) towards selected metal ions (Li^+, Na^+, K^+, Ag^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}) were studied in detail. Based on its structure, it was expected to show PET type fluorescence response upon complexation with metal ions, namely a fluorescence increase without spectral shifts. However, besides the fluorescence enhancement in the presence of cationic guests, spectral shifts were also observed due to the formation of two types of complex with 1:1 and 1:2 ligand to metal ion ratios, which have different emission spectra. The reason for this spectral difference can be that the 1:1 complex is proposed to be a sandwich type one in which the acridinone unit undergoes structural changes. These structural changes could be either the deprotonation or tautomerism of the acridinone unit. The spectra of the complexes of ligand **3** with 1:2 stoichiometry were similar to those of the complexes of mono-(azacrown ether) analogue **18**. Because of the ability of ligand **3** to form two types of complex having different emission spectra and stability constants, it is complicated to compare the fluorescence intensity changes upon complexation with metal ions and to define selectivity, thereby it would be difficult to use it as a selective sensor molecule. However, these observations allowed us to get a deeper insight into the complexation process, which can contribute to the future design of more selective sensor molecules suitable for practical applications.

4. Experimental

4.1. General

Infrared spectra were recorded on a Zeiss Specord IR 75 spectrometer. NMR spectra were recorded either on a Bruker DRX-500 Avance spectrometer (at 500 MHz for ^1H and 125.8 MHz for ^{13}C spectra) or on a Bruker 300 Avance spectrometer (at 300 MHz for ^1H and 75.5 MHz for ^{13}C spectra) and it is indicated in each individual case. Mass spectra were recorded on a ZQ2000 MS instrument (Waters Corp.) using ESI method. Elemental analyses were performed in the Microanalytical Laboratory of the Department of Organic Chemistry, Institute of Chemistry, L. Eötvös University, Budapest, Hungary. Melting points were taken on a Boetius micro-melting point apparatus and were uncorrected. Starting materials were purchased from Aldrich Chemical Company unless otherwise noted. Silica gel 60 F_{254} (Merck) plates were used for TLC. Silica gel 60 (70–230 mesh, Merck) was used for column chromatography. Romil Ltd (Cambridge, UK) SuperPurity Solvent grade THF stored under argon was used as purchased. All other solvents were dried and purified according to well-established methods.[30] Evaporations were carried out under reduced pressure. Ratios of solvents are given in volumes (v/v).

UV–vis spectra were taken on an UNICAM UV4-100 spectrophotometer controlled by VISION 3.4 software (ATI UNICAM, Cambridge, UK). Fluorescence spectra were recorded on a Perkin–Elmer LS 50B luminescent spectrometer supplied with an FL WinLab 3.0™ software (Perkin–Elmer Corp., USA). Both the emission and excitation spectra were corrected by the spectrometer software. Quartz cuvettes with path length of 1 cm were used. Fluorescence quantum yields were determined relative to quinine sulfate (Φ_f=0.546 in 0.5 M H_2SO_4).[2] Spectrophotometric titrations were carried out according to the literature.[31,32] Perchlorate salts of the metal cations were used in general with the exception of $AgNO_3$ and KSCN. All of metal ion salts were of analytical grade. The stability constants were determined by global nonlinear regression analysis using SPECFIT/32™ program.

4.2. General procedure for the synthesis of crown ethers 1–3

A solution of monoazacrown ether **4** (543 mg, 3.1 mmol) or **5** (680 mg, 3.1 mmol) or **6** (816 mg, 3.1 mmol), acridine derivative **7** (404 mg, 1.3 mmol) and triethylamine (1.1 mL, 7.8 mmol) in dry THF (6 mL) was stirred under Ar at reflux for 2 days. The volatile compounds were evaporated and the residue was stirred in a mixture of freshly distilled dioxane/5% aqueous HCl solution (9:1, 20 mL) at 60 °C for a day. The volatile materials were removed and the residue was dissolved in a mixture of CH_2Cl_2 (24 mL) and water (24 mL). The pH of the aqueous phase was adjusted to 9 by addition of 5% aqueous Me_4NOH solution and the phases were mixed well and separated. The aqueous phase was extracted with CH_2Cl_2 (3×12 mL). The combined organic phase was dried over $MgSO_4$, filtered and the solvent was removed. The crude products were purified by column chromatography on silica gel using 3:1:0.04 acetone/hexane/triethylamine as an eluent to give bis(azacrown ether)s **1**–**3** as described below for each compound.

*4.2.1. 4,5-Bis(1,4,7-trioxa-10-azacyclododecan-10-ylmethyl)acridin-9(10H)-one (**1**).* Yield: 74 mg (10%). Yellow oil; R_f=0.72 (silica gel TLC, 1:0.5:20 MeOH/triethylamine/acetone); IR (neat) ν_{max} 3481, 3080, 2854, 1621, 1614, 1594, 1523, 1435, 1358, 1256, 1093, 758 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.17 (br s, 0.5 mol of complexed H_2O, 1H), 2.92 (t, J=5 Hz, 8H), 3.51–3.64 (m, 24H), 4.08 (s, 4H), 7.19 (t, J=8 Hz, 2H), 7.69 (d, J=8 Hz, 2H), 8.41 (d, J=8 Hz, 2H), 11.00 (s, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 54.87, 59.15, 69.93, 70.68, 71.36, 121.07, 121.68, 125.56, 126.48, 133.94, 139.86, 178.82; MS: 570 $(M+1)^+$. Anal. Calcd for $C_{31}H_{43}N_3O_7\cdot 0.5H_2O$: C, 64.34; H, 7.66; N, 7.26. Found: C, 64.29; H, 7.47; N, 7.03.

*4.2.2. 4,5-Bis(1,4,7,10-tetraoxa-13-azacyclopentadecan-13-ylmethyl)-acridin-9(10H)-one (**2**).* Yield: 86 mg (10%). Yellow oil; R_f=0.61 (silica gel TLC, 1:0.5:20 MeOH/triethylamine/acetone); IR (neat) ν_{max} 3484, 3080, 2864, 1616, 1608, 1600, 1528, 1440, 1352, 1256, 1128, 760 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.13 (br s, 0.5 mol of complexed H_2O, 1H), 2.87 (t, J=6 Hz, 8H), 3.50–3.66 (m, 32H), 3.99 (s, 4H), 7.12 (t, J=8 Hz, 2H), 7.60 (d, J=8 Hz, 2H), 8.34 (d, J=8 Hz, 2H), 11.27 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 54.12, 58.54, 69.39, 70.36, 70.56, 71.14, 121.02, 121.72, 125.22, 126.44, 133.75, 139.99, 178.87; MS: 658 $(M+1)^+$. Anal. Calcd for $C_{35}H_{51}N_3O_9\cdot 0.5H_2O$: C, 63.04; H, 7.86; N, 6.30. Found: C, 62.98; H, 7.60; N, 6.27.

*4.2.3. 4,5-Bis(1,4,7,10,13-pentaoxa-16-azacyclooctadecan-16-ylmethyl)acridin-9(10H)-one (**3**).* Yield: 87 mg (9%). Yellow oil; R_f=0.43 (silica gel TLC, 1:0.5:20 MeOH/triethylamine/acetone); IR (neat) ν_{max} 3478, 3080, 2864, 1616, 1608, 1596, 1528, 1440, 1352,

1256, 1116, 760 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.69 (br s, 1 mol of complexed H_2O, 2H), 2.90 (t, J=6 Hz, 8H), 3.47–3.66 (m, 40H), 4.02 (s, 4H), 7.13 (t, J=8 Hz, 2H), 7.63 (d, J=8 Hz, 2H), 8.34 (d, J=8 Hz, 2H), 11.54 (s, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 53.43, 57.76, 69.30, 70.27, 70.72 (very high, probably two carbon 13 signals together), 70.93, 120.80, 121.50, 125.27, 126.10, 133.38, 139.86, 178.73; MS: 746 $(M+1)^+$. Anal. Calcd for $C_{39}H_{59}N_3O_{11}\cdot H_2O$: C, 61.32; H, 8.05; N, 5.50. Found: C, 61.04; H, 7.93; N, 5.29.

4.3. 9-Chloro-4,5-bis(chloromethyl)acridine (7)

A mixture of bis(hydroxymethyl)acridinone **10** (1.63 g, 6.4 mmol), thionyl chloride (14 mL, 0.19 mol), dry DMF (0.2 mL) and dry $CHCl_3$ (70 mL) was stirred at reflux until the TLC analysis showed that the reaction was completed (approximately 6 h). The volatile compounds were evaporated at 40 °C and the residue was dissolved in a mixture of CH_2Cl_2 (80 mL) and ice-cold 5% aqueous NaOH solution (40 mL). The phases were mixed well and separated. The organic phase was dried over $MgSO_4$, filtered and the solvent was removed. The residue was purified by column chromatography on silica gel using 1:20 $CHCl_3$/hexane as an eluent to give **7** (1.15 g, 58%) as yellow crystals. Mp: 151–153 °C; R_f=0.94 (silica gel TLC, 1:1:10 EtOH/AcOH/toluene); IR (KBr) ν_{max} 3024, 2920, 1664, 1624, 1564, 1536, 1436, 1396, 1328, 1260, 1096, 1048, 824, 808, 760, 752, 736, 616, 592 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.57 (br s, 0.5 mol of complexed H_2O, 1H), 5.47 (s, 4H), 7.62–7.66 (m, 2H), 7.97 (d, J=8 Hz, 2H), 8.40 (d, J=8 Hz, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 43.07, 124.44, 125.45, 127.06, 130.92, 136.46, 141.96, 145.92. Anal. Calcd for $C_{15}H_{10}Cl_3N\cdot 0.5H_2O$: C, 56.37; H, 3.47; Cl, 33.28; N, 4.38. Found: C, 56.43; H, 3.48; Cl, 33.07; N, 4.19.

4.4. Dimethyl 9-oxo-9,10-dihydroacridine-4,5-dicarboxylate (9)

To a suspension of oxoacridine dicarboxylic acid **8**[22] (2.5 g, 8.8 mmol) in dry MeOH (600 mL) was added dropwise thionyl chloride (25 mL, 0.34 mol) under Ar at 0 °C. The reaction mixture was stirred at this temperature for 1 h then it was allowed to warm to rt, stirred at rt for 30 min and finally refluxed for a day. For completion of the reaction, this procedure was repeated two more times. The precipitate was filtered off and dried. The crude product was recrystallized from DMF to give **9** (1.64 g, 60%) as yellow crystals. Mp: 231–233 °C; R_f=0.48 (silica gel TLC, 1:5 AcOH/toluene); IR (KBr) ν_{max} 3160, 1708, 1648, 1612, 1520, 1464, 1436, 1400, 1296, 1280, 1192, 1140, 752, 680, 496 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, T=360 K) δ 4.03 (s, 6H), 7.41 (t, J=8 Hz, 2H), 8.46 (d, J=8 Hz, 2H), 8.54 (d, J=8 Hz, 2H), 13.42 (s, 1H). Anal. Calcd for $C_{17}H_{13}NO_5$: C, 65.59; H, 4.21; N, 4.50. Found: C, 65.51; H, 3.98; N, 4.46.

4.5. 4,5-Bis(hydroxymethyl)acridin-9(10*H*)-one (10)

*4.5.1. Starting from oxoacridine dicarboxylic acid dimethyl ester **9**.* A suspension of lithium bromide (9.55 g, 0.11 mol) and sodium tetrahydridoborate (4.54 g, 0.12 mol) in dry diglyme (18 mL) was stirred under Ar at rt for 1 h. Dimethyl ester **9** (2.0 g, 6.4 mmol) was added in one portion and the reaction mixture was stirred at rt for 3 days. The mixture was poured into a stirred ice-cold 5% aqueous HCl solution (160 mL) and the precipitate was filtered off. It was washed with water (3×50 mL) and dried to give **10** (1.16 g, 71%) as a yellow solid. The crude product was pure enough to use it in the next step without purification. A small amount of **10** was recrystallized from DMF to give an analytical sample as yellow crystals. Mp: 227–231 °C; R_f=0.19 (silica gel TLC, 1:1:10 EtOH/AcOH/toluene); IR (KBr) ν_{max} 3600–2400, 1628, 1608, 1600, 1576, 1532, 1448, 1416, 1360, 1272, 1256, 1024, 1004, 752, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.50 (br s, 1 mol of complexed H_2O, 2H), 4.91 (s, 4H), 5.86 (br s, 2H), 7.23 (t, J=8 Hz, 2H), 7.66 (d, J=8 Hz, 2H), 8.19 (d, J=8 Hz, 2H), 10.93 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 61.79, 120.75, 120.84, 125.24, 128.39, 131.92, 139.29, 177.01. Anal. Calcd for $C_{15}H_{13}NO_3\cdot H_2O$: C, 65.93; H, 5.53; N, 5.13. Found: C, 65.81; H, 5.62; N, 5.09.

*4.5.2. Starting from bis(benzyloxymethyl)acridinone **13**.* A solution of acridinone derivative **13** (1.0 g, 2.3 mmol) in a mixture of freshly distilled dioxane/10% aqueous HCl solution (1:1, 100 mL) was stirred at reflux for 2 days. The volatile compounds were evaporated at 40 °C and the residue was triturated with MeOH (10 mL). It was kept at rt for overnight then in the freezer for a day. The precipitate was filtered off and dried to give **10** (0.31 g, 53%) as a yellow solid. The crude product was pure enough to use it in the next step without purification. A small amount of **10** was recrystallized from DMF to give an analytical sample as yellow crystals. Compound **10** obtained this way was identical in every aspect to that prepared by the previous procedure.

4.6. 4,5-Bis(benzyloxymethyl)acridine (12)

A solution of benzyl alcohol (1.78 g, 16.5 mmol) in dry THF (25 mL) was added dropwise under Ar at rt to a well stirred suspension of NaH (0.66 g, 16.5 mmol, 60% dispersion in mineral oil) in dry THF (5 mL). After addition of benzyl alcohol the mixture was stirred at reflux for 1 h. The reaction mixture was cooled down to rt and bis(bromomethyl)acridine **11**[24,25] (2.0 g, 5.5 mmol) was added to it in one portion. Stirring was continued at rt for 3 days. The volatile compounds were removed and the residue was dissolved in a mixture of CH_2Cl_2 (100 mL) and water (100 mL). The phases were mixed well and separated. The aqueous phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic phase was dried over $MgSO_4$, filtered and the solvent was removed. The crude product was purified first by flash chromatography on silica gel using 1:1:200 *i*-PrOH/triethylamine/hexane as an eluent then by recrystallization from diisopropyl ether to give **12** (1.18 g, 51%) as pale yellow crystals. Mp: 67–69 °C; R_f=0.62 (silica gel TLC, 1:30 EtOH/toluene); IR (KBr) ν_{max} 3088, 3027, 2869, 1619, 1604, 1600, 1537, 1497, 1455, 1394, 1354, 1304, 1131, 1118, 890, 754, 727, 694 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.83 (s, 4H), 5.46 (s, 4H), 7.30–7.52 (m, 10H), 7.57 (t, J=8 Hz, 2H), 7.93 (d, J=8 Hz, 2H), 7.98 (d, J=8 Hz, 2H), 8.75 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 68.90, 73.41, 125.86, 126.42, 127.53, 127.81, 127.96, 128.10, 128.64, 136.48, 137.00, 138.92, 146.15. Anal. Calcd for $C_{29}H_{25}NO_2$: C, 83.03; H, 6.01; N, 3.34. Found: C, 82.94; H, 5.98; N, 3.08.

4.7. 4,5-Bis(benzyloxymethyl)acridin-9(10*H*)-one (13)

A mixture of bis(benzyloxymethyl)acridine **12** (1.97 g, 4.7 mmol), NaH (0.71 g, 28 mmol, dry, 95%) and dry DMSO (40 mL) was stirred under O_2 at rt for 4 days. The solvent was evaporated at 45 °C and the residue was triturated with water (100 mL) then its pH was adjusted to 7 by addition of AcOH. EtOAc (100 mL) was added to the mixture and the phases were mixed well and separated. The aqueous phase was extracted with EtOAc (3×50 mL). The combined organic phase was dried over $MgSO_4$, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel using 1:5 EtOAc/hexane as an eluent to give **13** as a yellow solid (0.57 g, 28%). A small amount of **13** was recrystallized from EtOH to give an analytical sample as yellow crystals. Mp: 101–102 °C; R_f=0.43 (silica gel TLC, 1:2 EtOAc/hexane); IR (KBr) ν_{max} 3483, 3080, 2888, 2858, 1621, 1607, 1589, 1522, 1437, 1357, 1256, 1097, 751 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.71 (br s, 1 mol of complexed H_2O, 2H), 4.41 (s, 4H), 4.74 (s, 4H), 7.13–7.34 (m, 12H), 7.44 (d, J=8 Hz, 2H), 8.46 (d, J=8 Hz, 2H), 10.54 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 70.82, 72.05, 121.06, 122.12, 124.13, 127.77, 128.24, 128.44, 128.69, 133.78, 137.47, 140.16, 178.65.

Anal. Calcd for $C_{29}H_{25}NO_3 \cdot H_2O$: C, 76.80; H, 6.00; N, 3.09. Found: C, 76.69; H, 6.07; N, 2.93.

4.8. 2-[(2-Carboxyphenyl)amino]isophthalic acid (15)

A mixture of 2-chloroisophthalic acid **14** (10.43 g, 52 mmol), anthranilic acid (7.13 g, 52 mmol), finely powdered anhydrous K_2CO_3 (14.37 g, 104 mmol), copper powder (110 mg), copper(I) oxide (110 mg) and 2-ethoxyethanol (40 mL) was stirred vigorously under Ar at reflux for a day. The solvent was removed at 50 °C and the residue was dissolved in 2% aqueous KOH solution (250 mL). Charcoal (1.0 g) was added to the aqueous solution, boiled for 10 min, filtered then cooled to 0 °C in an ice-water bath. The pH of the aqueous solution was adjusted to 2 with concentrated aqueous HCl solution. The precipitate was filtered off, washed with ice-cold water (3×50 mL) and dried. The crude product was recrystallized from water to give **15** (8.30 g, 53%) as yellow crystals. Mp: 253–255 °C [lit.[22] mp: 258–259 °C (aqueous MeOH)]; R_f=0.44 (silica gel TLC, 1:3 AcOH/toluene); IR (KBr) ν_{max} 3600–2300, 1692, 1616, 1580, 1504, 1472, 1460, 1448, 1408, 1396, 1224, 1176, 1160, 1096, 828, 752, 676, 648 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 6.75–6.82 (m, 2H), 7.18 (t, J=8 Hz, 1H), 7.27 (t, J=8 Hz, 1H), 7.84 (d, J=8 Hz, 1H), 7.93 (d, J=8 Hz, 2H), 10.67 (br s, 1H), 12.95 (br s, 3H).

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Supplementary data

^1H and ^{13}C NMR spectra of chemosensors **1**–**3**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.02.076.

References and notes

1. *Chemosensors of Ion and Molecule Recognition*; Desvergne, J. P., Czarnik, A. W., Eds.; NATO ASI Series C; Kluwer: Dordrecht, The Netherlands, 1997; Vol. 492.
2. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer: New York, NY, 1999.
3. Valeur, B. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH: Weinheim, Germany, 2002.
4. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
5. Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3–40.
6. de Silva, A. P.; McClean, G. D.; Moody, T. S.; Weir, S. M. In *Handbook of Photochemistry and Photobiology*; Nalwa, H. S., Ed.; American Scientific: Stevenson Ranch, CA, 2003; Vol. 3, Chapter 5.
7. Montalti, M.; Prodi, L.; Zaccheroni, N. In *Handbook of Photochemistry and Photobiology*; Nalwa, H. S., Ed.; American Scientific: Stevenson Ranch, CA, 2003; Vol. 3, Chapter 6.
8. Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588.
9. Albert, A. *The Acridines*, 2nd ed.; Edward Arnold: London, UK, 1966.
10. Acheson, R. M. In *The Chemistry of Heterocyclic Compounds*, 2nd ed.; Weissberger, A., Taylor, E. C., Eds.; Wiley: New York, NY, 1973; Vol. 9.
11. Siegmund, M.; Bendig, J. *Ber. Bunsenges. Phys. Chem.* **1978**, *82*, 1061–1068.
12. Rothman, J. H.; Still, W. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 509–512.
13. Móczár, I.; Huszthy, P.; Mezei, A.; Kádár, M.; Nyitrai, J.; Tóth, K. *Tetrahedron* **2010**, *66*, 350–358.
14. Fery-Forgues, S.; Al-Ali, F. *J. Photochem. Photobiol., C* **2004**, *5*, 139–153.
15. de Silva, A. P.; Sandanayake, K. R. A. S. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1173–1175.
16. Kim, S. K.; Bang, M. Y.; Lee, S.-H.; Nakamura, K.; Cho, S.-W.; Yoon, J. *J. Inclusion Phenom. Macrocycl. Chem.* **2002**, *43*, 71–75.
17. Geue, J. P.; Head, N. J.; Ward, A. D.; Lincoln, S. F. *Dalton Trans.* **2003**, 521–526.
18. Hua, J.; Wang, Y.-G. *Chem. Lett.* **2005**, *34*, 98–99.
19. Kondo, S.; Kinjo, T.; Yano, Y. *Tetrahedron Lett.* **2005**, *46*, 3183–3186.
20. Mashraqui, S. H.; Sundaram, S.; Bhasikuttan, A. C.; Kapoor, S.; Sapre, A. V. *Sens. Actuators, B* **2007**, *122*, 347–350.
21. Mashraqui, S. H.; Sundaram, S.; Khan, T.; Bhasikuttan, A. C. *Tetrahedron* **2007**, *63*, 11093–11100.
22. Rewcastle, G. W.; Denny, W. A. *Synthesis* **1985**, 217–220.
23. Kavadias, G. *Chim. Chron., New Ser.* **1994**, *23*, 79–95.
24. Chiron, J.; Galy, J.-P. *Synlett* **2003**, 2349–2350.
25. Di Giorgio, C.; De Méo, M.; Chiron, J.; Delmas, F.; Nikoyan, A.; Jean, S.; Dumenil, G.; Timon-David, P.; Galy, J.-P. *Bioorg. Med. Chem.* **2005**, *13*, 5560–5568.
26. Huszthy, P.; Köntös, Z.; Vermes, B.; Pintér, Á *Tetrahedron* **2001**, *57*, 4967–4975.
27. Stapleton, G.; White, A. I. *J. Am. Pharm. Assoc.* **1954**, *43*, 193–200; (*Chem. Abstr.* **1955**, *49*, 8968).
28. Szalay, L.; Farkas, V.; Vass, E.; Hollósi, M.; Móczár, I.; Pintér, Á.; Huszthy, P. *Tetrahedron: Asymmetry* **2004**, *15*, 1487–1493.
29. Lin, C.; Simov, V.; Drueckhammer, D. G. *J. Org. Chem.* **2007**, *72*, 1742–1746.
30. Riddick, J. A.; Bunger, W. B.; Sakano, T. K. In *Techniques of Chemistry*, 4th ed.; Weissberger, A., Ed.; Wiley-Interscience: New York, NY, 1986; Vol. 2.
31. Kádár, M.; Biró, A.; Tóth, K.; Vermes, B.; Huszthy, P. *Spectrochim. Acta, Part A* **2005**, *62*, 1032–1038.
32. Móczár, I.; Huszthy, P.; Maidics, Z.; Kádár, M.; Tóth, K. *Tetrahedron* **2009**, *65*, 8250–8258.