PHOTOPHYSICAL CHARACTERISATION, METAL ION BINDING AND ENANTIOMERIC RECOGNITION OF CHIRAL LIGANDS CONTAINING PHENAZINE FLUOROPHORE

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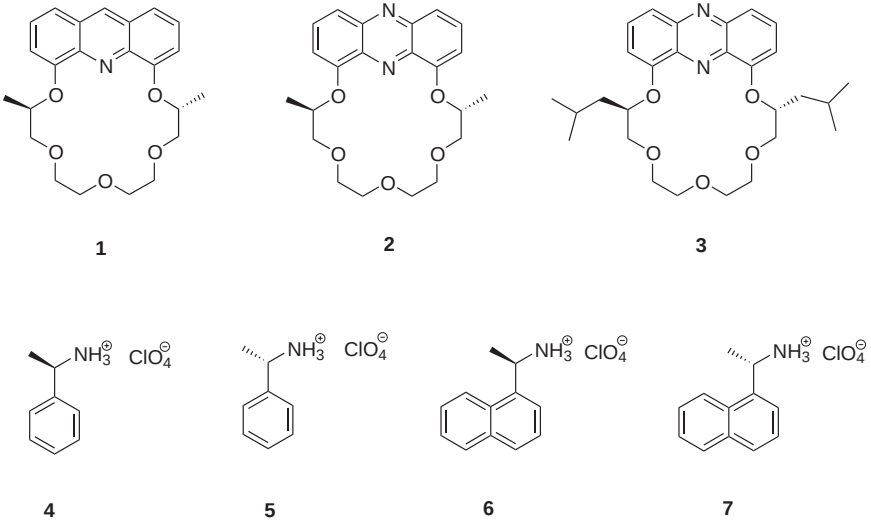
Enantiomerically pure dimethyl- and diisobutyl-substituted phenazino-18-crown-6 ligands bind metal and ammonium ions and also primary aralkylammonium perchlorates in acetonitrile with high affinity, causing pronounced changes in their luminescence properties. In addition, they show enantioselectivity towards chiral primary aralkylammonium perchlorates. The possibility to monitor the binding process by photoluminescence spectroscopy can gain ground for the design of very efficient enantioselective chemosensors for chiral species. The observed changes in the photophysical properties are also an important tool for understanding the interactions present in the adduct.

Keywords: Chiral crown compounds; Fluorescence spectroscopy; Enantiomeric recognition; Chemosensors; Supramolecular chemistry; Macrocycles; Crown ethers; Phenazines.

Enantiomeric recognition, as a special case of molecular recognition, is a very important and frequently occurring phenomenon in nature. Examples include the metabolism of single enantiomeric forms of amino acids and saccharides in biosynthetic pathways. This phenomenon, which involves the discrimination between the enantiomers of a chiral molecule (guest) by an enantiomerically pure chiral receptor (host), can be performed using relatively simple synthetic molecules such as crown ethers. Since the pioneering studies of Cram and his co-workers on optically pure bis(1,1-dinaphthalenyl)-22-crown-6 ligands in the early 1970s[1], a great variety of enantiomerically pure crown ether-type host molecules have been prepared with

the aim of enhancing their selectivity to the enantiomers of chiral guest molecules[2]. Among them a number of enantiomerically pure crown ethers have been prepared, containing pyridine[3], pyrimidine[4], phenanthroline[5], acridine[6], and phenazine[7] units. These hosts and their selectivity towards the enantiomers of organic ammonium salts have been extensively studied[8].

In a recent work[9], we proposed the use of the changes of luminescence properties of the acridine fluorophore attached at the chiral 18-crown-6 crown ether framework (see ligand **1** in Scheme 1) to monitor the binding event with chiral organic ammonium salts. Generally speaking, this possibility makes the receptor species very interesting as a suitable fluorescent chemosensor for enantiomeric recognition, a phenomenon rapidly gaining attention in biological, pharmaceutical, analytical and separation sciences, and in food technology[10–17].



SCHEME 1

The preparation and characterisation of new chemosensors is the first step in the development and fabrication of efficient devices for the real-time determination of chemical species[17]. Of various kinds of chemosensors, the luminescent ones present many advantages since luminescence measurements are usually very sensitive, cheap, easily performed, and versatile[18].

For detection of the target analyte, two different processes are needed: molecular recognition and signal transduction, i.e., the mechanism by which the complexation of the sensor with the analyte causes a change in

the properties of the sensor itself. This means that chemosensors have to be built by components able to perform all these functions. Chiral ligands **2** and **3** (see Scheme 1) can perform both processes: the crown ether skeleton is able to bind cationic species, and the photophysical properties of the inserted phenazine fluorophore change upon the association event. As a result, we demonstrate here that photoluminescence spectroscopy can be a very sensitive tool for monitoring the chiral recognition of the enantiomerically pure crown ethers **2** and **3** towards the enantiomers of chiral organic ammonium salts.

EXPERIMENTAL

Materials

(3*R*,13*R*)-3,13-Dimethyl-2,5,8,11,14-pentaoxa-20,26-diazatetracyclo[13.9.3.$0^{19,27}$.$0^{21,25}$]heptacosa-1(25),15,17,19,21,23,26-heptaene (**2**)[7], (3*R*,13*R*)-3,13-diisobutyl-2,5,8,11,14-pentaoxa-20,26-diazatetracyclo[13.9.3.$0^{19,27}$.$0^{21,25}$]heptacosa-1(25),15,17,19,21,23,26-heptaene (**3**)[7], (*R*)-(1-phenylethyl)ammonium perchlorate [(*R*)-PEA] (**4**)[8f], (*S*)-1-phenylethylammonium perchlorate [(*S*)-PEA] (**5**)[8f], (*R*)-1-(1-naphthyl)ethylammonium perchlorate [(*R*)-NEA] (**6**)[8f], (*S*)-1-(1-naphthyl)ethylammonium perchlorate [(*S*)-NEA] (**7**)[8f] were prepared as reported earlier. The solvent used for photophysical measurements was acetonitrile from Merck (UVASOL). All other reagents were purchased from Fluka or Aldrich and used without purification.

Photophysical Measurements

UV-VIS absorption spectra were taken with a Perkin Elmer Lambda 16 spectrophotometer. Uncorrected emission and corrected excitation spectra were obtained with a Perkin Elmer LS 50 spectrofluorimeter. The fluorescence lifetimes (uncertainty ±5%) were obtained with an Edinburgh single-photon counting apparatus, in which the flash lamp was filled with $^2H_2$. Luminescence quantum yields (uncertainty ±15%) were determined using quinine sulfate in aqueous 5×10^{-1} M H_2SO_4 ($\Phi = 0.546$)[19]. In order to allow comparison of emission intensities, corrections for instrumental response, inner filter effects, and phototube sensitivity were performed[18a].

Spectrophotometric and fluorimetric titrations were carried out as described earlier[20]. All cations were added as perchlorate salts.

RESULTS AND DISCUSSION

The absorption spectra of ligands **2** and **3** in acetonitrile (Fig. 1) are, as expected, very similar, showing, in the 320–400 nm region, the typical absorption band of the phenazine chromophore, although slightly red-shifted, and a new, broad and unstructured band in the 400–480 nm region.

As far as the luminescence spectra concerns, ligands **2** and **3** (Fig. 2) show an intense fluorescence band in the 440–700 nm region, with a maximum

at 520 nm, a lifetime of 7.0 ns and a fluorescence quantum yield of 0.07. This behaviour is very different from that of the parent phenazine itself, since its fluorescence band has been reported to be at a higher energy (ca. 400 nm), with a shorter lifetime (20 ps) and a lower quantum yield (0.0015)[21]. The different photophysical behaviour of phenazine and ligands **2** and **3** has to be related to the electron donating effect of the alkoxy substituents at the 1 and 9 positions of the phenazine ring system in the latter two compounds. This effect introduces a new transition that is responsible

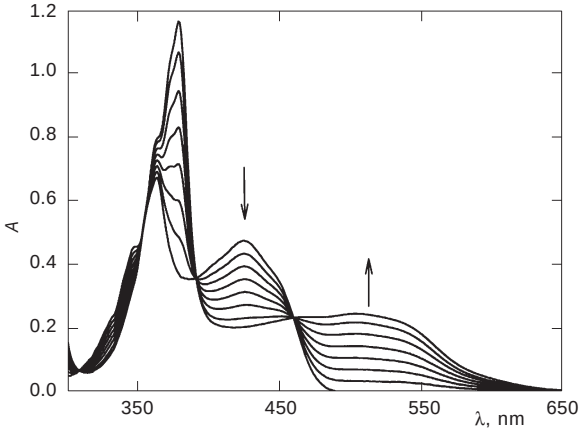


FIG. 1
Electronic absorption spectra of **3** in acetonitrile recorded upon addition of increasing amounts of trifluoromethanesulfonic acid

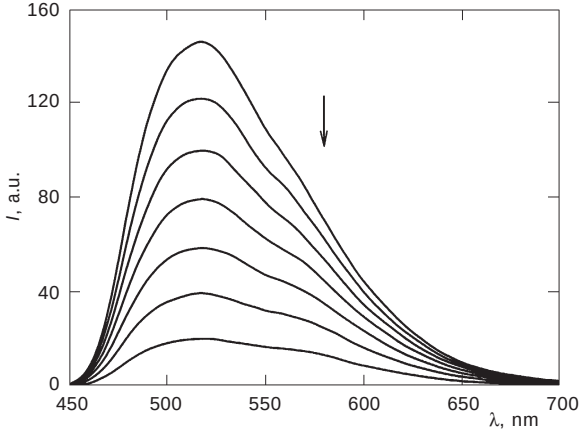


FIG. 2
Fluorescence spectra (λ_{exc} = 420 nm) of **3** in acetonitrile recorded upon addition of increasing amounts of trifluoromethanesulfonic acid

for the low-energy absorption band and for the fluorescence feature characteristic for this kind of crown compounds[7,22].

Addition of trifluoromethanesulfonic (triflic) acid to an acetonitrile solution of ligands **2** and **3** leads to the appearance of a new absorption band in the 460–600 nm region (Fig. 1) and complete quenching of the fluorescence band, typical for the unprotonated species (Fig. 2). The observed changes can be attributed to the acid/base process leading to the protonation of the nitrogen atom of the phenazine inside the crown ether cavity. No evidence for an additional protonation could be obtained even upon addition of a ten-fold excess of the triflic acid. A similar drastic change was also observed for the acridine crown ether[9] **1**.

Small changes in the absorption spectrum of ligand **2** are induced by addition of Na^+, K^+, NH_4^+, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Zn^{2+} ions. On the contrary, a noticeable increase in the fluorescence intensity (see Fig. 3 for NH_4^+ ions) has been observed in the presence of these ions, with the only exception of Zn^{2+} that does not have any influence on the fluorescence spectrum. With the exception of Zn^{2+}, it was possible to determine, from the changes observed, the association constants of **2** with all these ions. The data are gathered in Table I; they refer to association processes with a 1:1 stoichiometry. Processes with a different stoichiometry were negligible under the experimental conditions used. The intensity increase suggests the possibility of monitoring, with a good sensitivity, the presence of these metal ions in solution. However, the observed selectivity is modest, since the association

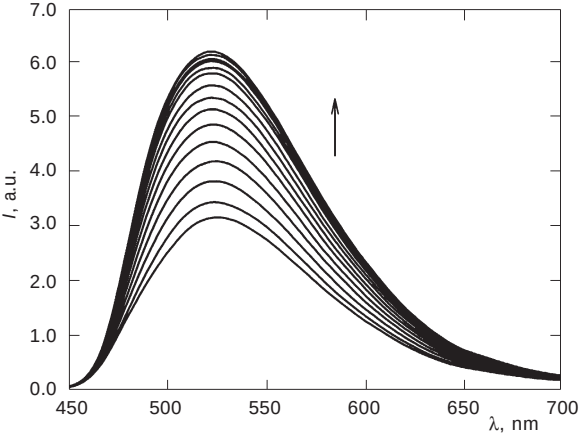


FIG. 3
Fluorescence spectra (λ_{exc} = 420 nm) of **3** in acetonitrile recorded upon addition of increasing amounts of NH_4^+ ions

constant clearly depends on the ion charge, but the observed differences are relatively small for ions having the same charge, with the only exception of calcium ions.

Although the increase in the fluorescence intensity is, as stressed above, noticeable, the complexation with Na^+, K^+, NH_4^+, Ca^{2+}, Sr^{2+}, and Ba^{2+} leads to changes in photophysical properties that are different from those observed upon protonation, since in the latter case a complete quenching proceeded. In particular, this is true for ammonium ions. This finding clearly indicates that no proton transfer occurs from the guest to the phenazine moiety, in agreement with what has already been observed for the acridine crown ether[9] **1**. In general, the involvement of the nitrogen lone pair in the complexation is much less pronounced than that observed upon protonation.

When enantiomerically pure chiral organic ammonium ions **4**–**7** were added to the acetonitrile solutions of **2** and **3**, red shift and decreased intensity of the band lying in the 400–480 nm region were observed in the absorption spectra (see Fig. 4 for the adduct of **2** and **6**). The observed changes are qualitatively similar to those following complexation of metal and NH_4^+ ions; again, an acid/base process can be ruled out. In these cases, however, π–π interactions are possible between the phenazine chromophore and the aromatic units of the chiral organic ammonium ions. This can explain the greater changes observed upon the addition of **4**–**7**. As expected, the effects are greater when the aromatic system of the guest is more extended[7,22].

TABLE I
Association constants and fluorescence quantum yields of the complexes of **2** with inorganic cations

Complex	Φ	K_{assoc}, l mol^{-1}
2	0.07	–
2·Sr^{2+}	0.18	1.1×10^7
2·Ba^{2+}	0.23	2.1×10^7
2·Ca^{2+}	0.10	5.5×10^5
2·Na^+	0.21	5.1×10^5
2·K^+	0.19	3.4×10^5
2·NH_4^+	0.13	6.2×10^5

Concerning the fluorescence spectra, addition of ammonium ions **4** or **5** causes, upon excitation at 420 nm, an increase in the luminescence intensity, as already observed upon addition of alkali and alkaline earth metal ions. This result is in perfect agreement with the changes caused by addition of inorganic cations.

A different behaviour is instead observed upon addition of **6** and **7**. In these cases, the luminescence of the phenazine chromophore is quenched, as shown in Fig. 5. However, it is worth stressing that the adducts of **2** and

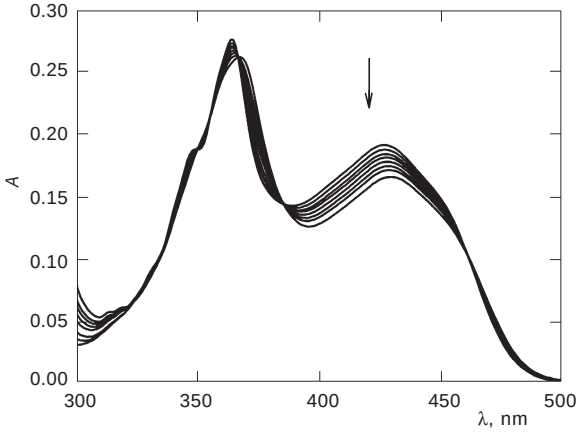


FIG. 4
Electronic absorption spectra of **2** in acetonitrile recorded upon addition of increasing amounts of **6**

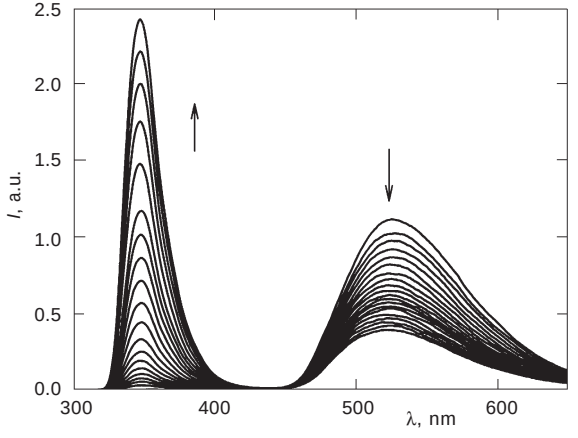


FIG. 5
Fluorescence spectra (λ_{exc} = 420 nm) of **2** in acetonitrile recorded upon addition of increasing amounts of **6**

3 with **6** and **7** contain two different luminescent moieties, i.e., a naphthalene derivative of the ammonium ion and the phenazine unit incorporated in the crown ether framework. The association of such adducts can, consequently, be monitored through the analysis of two different luminescence signals if excitation is properly performed. In particular, if excitation is performed at 290 nm, where the light is mainly absorbed by the naphthalene chromophore, increasing the amount of **6** and **7** causes quenching of the corrected intensity of the band typical of the phenazine chromophore (Fig. 5). At the same time, a band shows up with maximum at 330 nm, typical of the naphthalene derivatives. The increase in its intensity is very modest up to the addition of one equivalent of the ammonium ion, while it is much more evident afterwards (Figs 5 and 6).

The analysis of the corrected fluorescence intensities at different wavelengths as a function of the concentration of the added organic ammonium ions afforded the possibility to determine the association constants of the different adducts, which are gathered in Table II, together with their fluorescence quantum yields and excited state lifetimes.

The intensity changes for **6** and **7** are different from those observed for the inorganic cations and organic ammonium ions **4** and **5**. As previously discussed, a decrease in the intensity of the fluorescence band can be found, typical of the phenazine chromophore, upon excitation both at 420 nm, where only the phenazine unit absorbs, and at 290 nm, where a relevant percentage of the light is absorbed by the naphthalene moiety. This finding clearly indicates that the complexation with **6** and **7** intro-

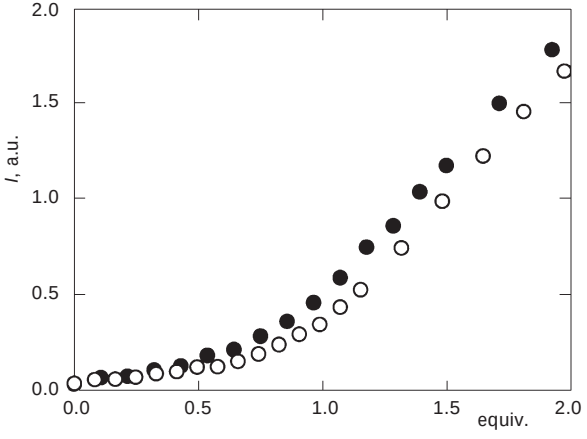


FIG. 6

Fluorescence intensity (λ_{exc} = 290 nm, λ_{em} = 335 nm) recorded after addition of increasing amounts of **6** (○) and **7** (●) to a solution of **2** in acetonitrile

duces a new non-radiative decay path for the luminescence of the phenazine chromophore. This phenomenon is also supported by the decrease in the excited state lifetime of phenazine in the adduct. In the adduct, also the fluorescence of the naphthalene unit is drastically quenched, and the intensity recorded in the 300–400 nm region can be attributed only to the free, not complexed, organic ammonium ion. As can be easily seen in Fig. 7, the only thermodynamically allowed mechanism that can be invoked for the quenching of both units is a photoinduced electron transfer. As a matter of fact, the singlet state of the phenazine chromophore is lower in energy than the singlet and triplet excited states of naphthalene, so that the energy transfer from phenazine to naphthalene is not possible. In addition, a photoinduced proton transfer to the phenazine can also be ruled out, since this mechanism should work also for the adducts with NH_4^+, **4**, and **5**, where increased fluorescence intensity has been observed instead. The fluorescence quenching of phenazine is not complete, probably because the process is only slightly exoergonic, as was found for the adducts with the same ammonium ions and the acridine host **1**. It should also be noted that a decrease in the luminescence intensity (Table II) is higher in the adduct of **2** and **7** compared with that observed for the adduct of **2** and **6**. This finding is in agreement with a stronger interaction in the former system, as previously reported[22].

Concerning the association constants, they all are very large, indicating that the receptors **2** and **3** have a good affinity to the organic ammonium ions studied in this work. Comparing the data related to the two receptors,

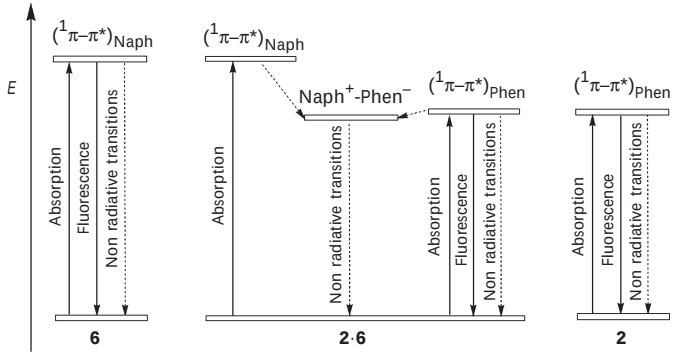


FIG. 7
Schematic representation of the energy levels of **2**, **6**, and their adduct

it can be noted that the association constants with **4** and **5** are larger for **2** than for **3**, as expected because of the larger steric hindrance in **3**. In addition, the association constants with the ions **6** and **7** are smaller for **2** and larger for **3** compared to the values for **4** and **5**. These findings are due to the interplay of two different effects, viz., the steric hindrance and the π–π interactions between the phenazine chromophore and the aromatic ring of the ammonium ion, as already shown[22]. Furthermore, again in agreement with the previously reported results[7,22], the heterochiral adducts (i.e., crown ether complexes with the ammonium ions **5** and **7**) are more stable than the homochiral ones (crown ether complexes with **4** and **6**).

It is noteworthy to emphasize that the degree of enantiomeric recognition is lower for **2** and **3** than for the acridine crown **1**. This result can be due to a different ability to form hydrogen bonding with the ammonium ions and to give rise to π–π interactions with the aromatic moiety of the guest. In any case, the enantiomeric recognition ability shown by **2** and **3** is sufficiently high to make them interesting systems for practical applications. Moreover, **2** and **3** offer the advantage, compared to **1**, of a stronger fluorescence intensity change upon complexation, which is particularly important for the design of sensory devices[17].

TABLE II
Association constants, excited state lifetimes, and fluorescence quantum yields of the complexes of **2** and **3** with primary organic cations **4**–**7**

Complex	Φ	τ, ns	K_{assoc}, l mol^{-1}	K_s/K_r [a]
2	0.07	7	–	–
3	0.07	7	–	–
2–**4**	0.17	21	5.6×10^5	–
2–**5**	0.17	23	9.9×10^5	1.8
2–**6**	0.014	1.1	3.3×10^5	–
2–**7**	0.010	1.3	5.0×10^5	1.5
3–**4**	0.14	22.3	2.0×10^5	–
3–**5**	0.14	23.2	2.5×10^5	1.3
3–**6**	0.016	0.7	2.1×10^5	–
3–**7**	0.016	1.0	3.4×10^5	1.6

[a] K_s/K_r is the ratio between the association constant of the (*S*)-primary organic cation with the (*R*,*R*)-crown ether complex and that of the (*R*)-primary organic cation with the (*R*,*R*)-crown ether complex; it is proportional to the enantioselectivity.

CONCLUSIONS

The association process between the enantiomerically pure phenazino-18-crown-6 ligands **2** and **3** and the enantiomers of organic ammonium ions causes large changes in the fluorescence intensity of single components. This allows the process to be monitored by luminescence spectroscopy with a very high sensitivity. The changes in the fluorescence intensity for **2** and **3** are even bigger than those previously observed for the enantiopure acridino-18-crown-6 ligand **1**, but, on the other hand, the enantioselectivity is lower. The results reported in this work give further insight into the design of efficient chemosensors for enantiomeric discrimination, a field gaining growing interest in practical applications.

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