Fluorescent Chemosensors Containing Polyamine Receptors

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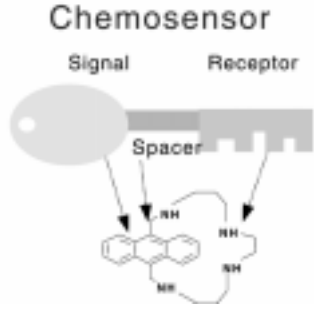
Chemosensors have attracted interest in many different scientific fields, such as environmental chemistry, medicine, and the processing and storage of information. These molecular-scale devices have the advantage of working on the same spatial scale as the chemical structures that are responsible for macroscopic behaviour observed in the environment or those associated with health problems. Moreover, they allow the construction of molecular-scale devices for information storage. In this review, we describe a family of chemosensors based on a polyamine receptor and a fluorescent signalling unit. Polyamine receptors are water-soluble ambidentate receptors; they are able to coordinate either metal ions, when sufficient deprotonated amino groups are available, or anionic species, when there are sufficient protonated amino groups. On the other hand, the use of fluorescent signalling units confers the advantage of an immediate visual response/signal.

Introduction

A standard chemosensor can be defined as a molecule containing three basic units, each one performing a precise function: (i) a receptor unit, (ii) a signalling unit, and (iii) a spacer (Scheme 1).[1,2] The first of these, the receptor unit, plays the role of recognizing and reversibly binding a given target substrate. The signalling unit, in turn, must be capable of either producing a signal or significantly altering its signal intensity in response to the binding of the substrate,



Scheme 1. Schematic representation of a chemosensor

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MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

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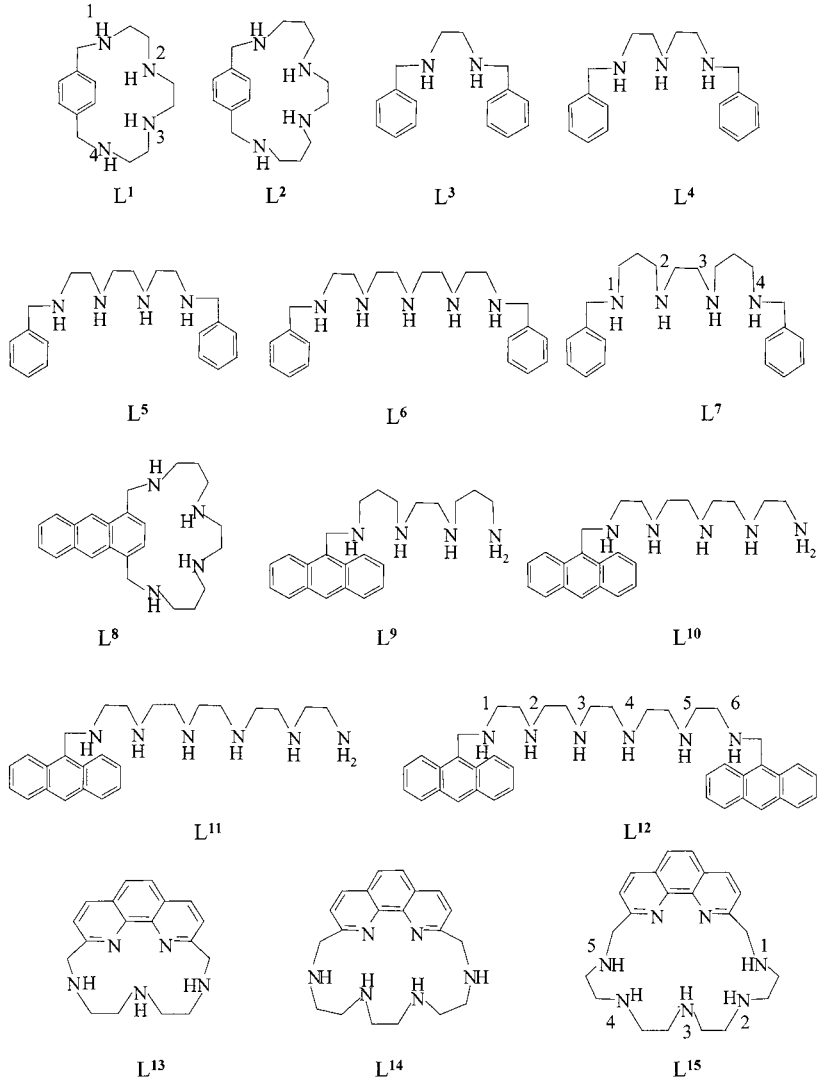
preferably without changing the other photochemical properties. With fluorescent chemosensors, chelation enhancement of the fluorescence (CHEF) or chelation enhancement of the quenching (CHEQ) are commonly encountered responses. Finally, the third unit, the spacer, links the binding and signalling units and controls their mutual separation and geometrical arrangement. These parameters play a crucial role in determining the performance of the device.

A particularly interesting goal is the development of chemosensors capable of functioning in aqueous solution, as highlighted by Czarnick.[2c] Many chemosensors that function efficiently in less polar solvents do not give adequate responses in water either due to low solubility limits or owing to weak complexation of the target species.[1,2] This is a severe drawback because water is the solvent in which most chemical processes occur. In order to achieve sufficiently strong complexation of anions or metal ions in water, a rational approach is the use of polyamine fragments as receptor units. Polyamine compounds are well known as ambidentate receptors capable of coordinating metal ions when sufficient deprotonated amino groups are available, or anionic species when there are sufficient protonated amino groups. Therefore, their binding capacities can be readily tuned by adjusting the pH value.[3] This is a further reason why these compounds have been synthesized and their properties explored.

In this paper, we have attempted to review the effects of the binding of protons, cations, and anions on families of fluorescent chemosensors containing receptor units based on open-chain or cyclic polyamine fragments (Scheme 2). Excellent reviews dealing with earlier work in the field of fluorescent sensors have been published by Czarnick[2c] and by de Silva et al.[1b]

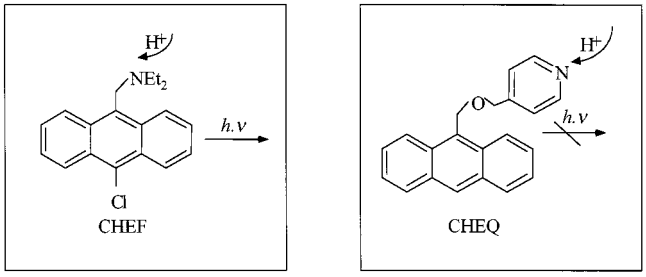
Sensing Protons

CHEQ or CHEF effects on the fluorescence emission resulting from the binding of protons are rather common. Systems of this type have been used to construct ON/OFF switches, which is a very important goal in the growing field of semiochemistry.[4,5] The two effects can be illustrated by



Scheme 2. Chemical structures of the fluorescent chemosensors studied

the following examples based on the anthracene chromophore (Scheme 3).[6,7]



Scheme 3. Examples of systems that function as ON/OFF switches

In the first case, a quenching process involving photoinduced electron transfer (PET) from the free lone pair of the amine pendant arm to the excited fluorophore occurs. This process can be inhibited when the amine is protonated (pK_a = 7.7), resulting in the appearance of a fluorescence emission.[6,7] In the second case, the species containing the pyridinium fragment quenches fluorescence, while this is not the case with unprotonated pyridine. Therefore, protonation has the opposite effect and thus the intensity of the fluorescence emission decreases on increasing the mole fraction of the protonated form (pK_a = 5.0). In this case, the quenching effect has been ascribed to an electron-transfer process from the photo-excited fluorophore to the pyridinium fragment.[7]

Benzene Fluorophore

Polyazacyclophane Macrocyclic Receptors

The azacyclophanes 2,5,8,11-tetraaza[12]paracyclophane (L^1) and 2,6,9,13-tetraaza[14]paracyclophane (L^2) are cyclic chemosensors constructed by linking a polyamine chain and a 1,4-dimethylbenzene fragment (Scheme 2).

According to crystallographic, NMR, and molecular mechanics studies, in solution these compounds preferentially adopt conformations in which the polyamine chain is arched above the aromatic ring. Indeed, this feature could also be observed in a structure reported by Altava et al.[8] for the analogous compound 13,14,16,17-tetramethyl-2,6,10-triaza[11]paracyclophane, which contains a triamine chain as the receptor unit and a durene moiety as the fluorophore. On the other hand, the structure shows that the three acidic protons located on the aliphatic nitrogen atoms are in an *exo* disposition and that the macrocycle assumes an overall open conformation. This arrangement minimizes the electrostatic repulsion between the charged polyammonium groups. It is important to note that the benzylic nitrogen atoms are located ca. 1.45 Å above the plane defined by the aromatic ring. On the other hand, the distance between the benzylic nitrogen atoms is remarkably large, amounting to 6.85 Å.

In spite of the low fluorescence emission quantum yield of the benzene fluorophore, which precludes any practical application, the properties shown by chemosensors of this type are very much representative of the general behaviour exhibited by chemosensors based on a polyamine receptor. Owing to the presence of the spacer between the signalling and the receptor units, these compounds show absorption and fluorescence emission spectra that largely resemble those of the benzene chromophore. This behaviour is more or less general, also being observed when the benzene ring is substituted by other signalling units (vide infra).

The protonation sequence of this family of receptors has been studied in detail by ^{13}C and ^{1}H NMR.[9,10] According to these studies, the protons on the two central nitrogen atoms are the most easily removed. In Figure 1, the fluorescence emission titration curves of L^1 and L^2 are presented, showing the mole fraction distributions of the various protonated species as determined by potentiometry.

The deprotonation sequence of the chemosensors is also included for discussion purposes. By inspection of the figure it can clearly be seen that the fully protonated species gives rise to the highest emission intensity and that removal of the first proton leads to a large decrease in the fluorescence emission intensity. However, total extinction of the emission is only observed following loss of the second proton from the other central nitrogen atom.

The quenching effect of the fluorescence emission can easily be explained in terms of an exergonic intramolecular electron-transfer process from the lone pair of the amines to the excited fluorophore.[9] However, protonation of the amines increases their oxidation potential by ca. 2.1 eV,[11] changing the process from an exoergonic to an endergonic one. Therefore, the fully protonated forms of these chemosensors exhibit the highest fluorescence emission intensities. Another aspect to take into account is the relative position of the lone pairs with respect to the π system. The probability of electron transfer depends on the distance between the two partners, as well as on the overlap between the donor and the acceptor orbitals. Removal of the central protons leads to a decrease in the overall electrostatic repulsion and hence the nitrogen atoms labelled N2 and N3 can become closer to the π system favouring the quenching process (Figure 1).

Finally, the weak fluorescence emission observed for the triprotonated species $[H_3L^1]^{3+}$ can be ascribed to the fact that the two central nitrogen atoms are equivalent and hence share the proton; this would still provide a certain degree of protection for the lone pairs.

The fluorescence emission titration curve of L^2 is similar to that of L^1 (Figure 1), suggesting that it may be similarly interpreted. However, the triprotonated form of L^2 exhibits a less efficient quenching effect than in the case of L^1. The two compounds differ in the dimensions of their chains, i.e. 12 atoms in L^1 compared with 14 atoms in L^2. For this reason, the central nitrogen atoms in L^2 are further away from the π system than in L^1, and consequently the electron-transfer process is less efficient.

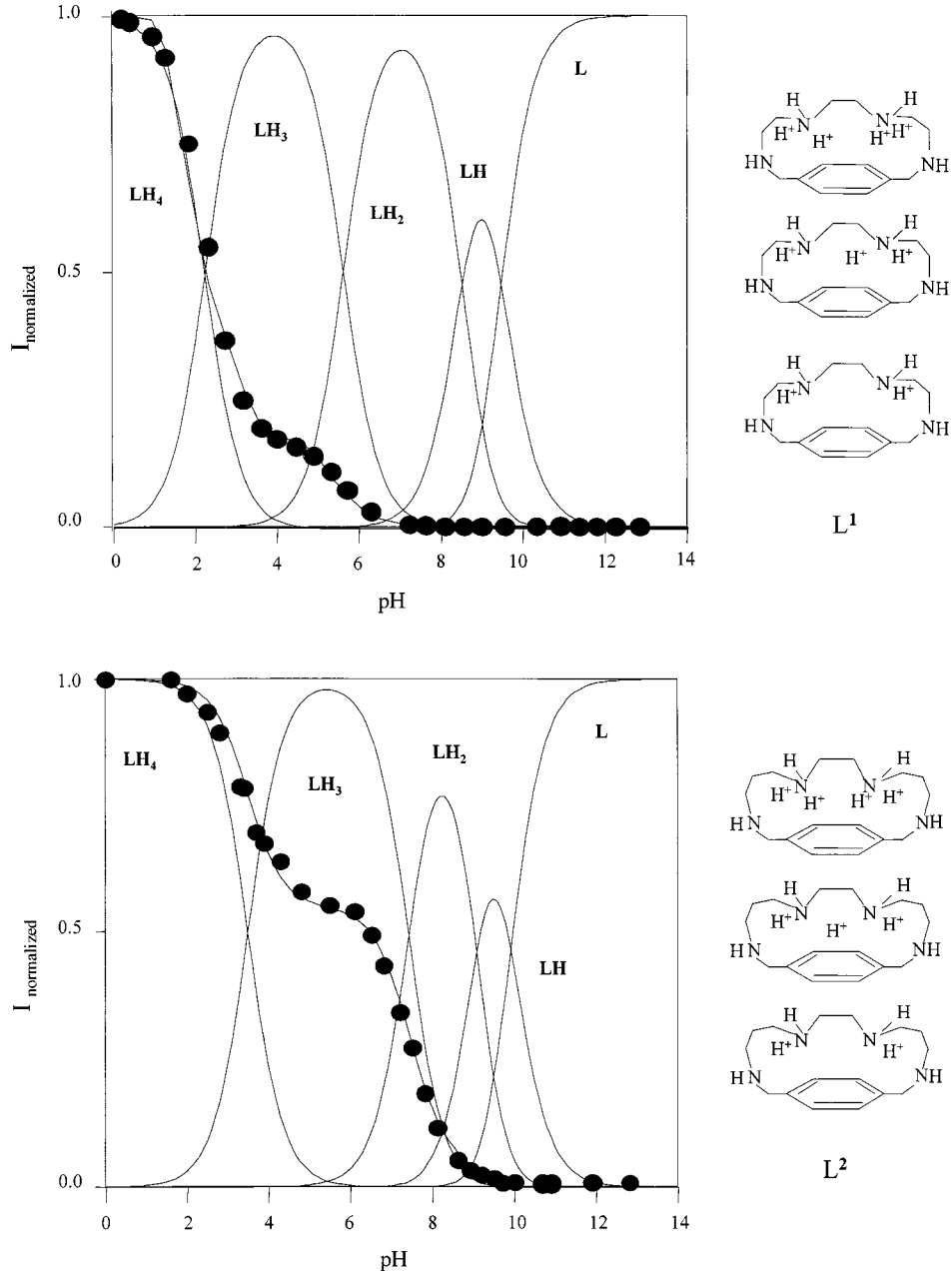


Figure 1. Fluorescence emission titration curves, along with the mole fraction distribution and deprotonation sequence of the chemosensors L^1 and L^2

N,N′-Dibenzylated Polyamine Receptors

In this class of compounds the signalling unit is made up of two terminal benzene rings, separated by a spacer from a polyamine open-chain receptor of variable length, L^3 to L^7 (Scheme 2).

The fluorescence emission titration curves (Figure 2) were obtained using the same methodology as mentioned above. The intensity of the fluorescence emission spectrum of the smallest member of this family, L^3, is practically unaffected by the loss of the first proton. This result suggests that, in contrast to the findings of Thomas and Davidson[12,13] regarding the naphthalene fluorophore, the benzylic nitrogen atoms in these compounds are not very efficient in inducing the quenching process. This becomes apparent when compound L^4 is used, for which a significant quenching effect is observed upon removal of the first proton from a nitrogen atom in the centre of the chain.

The fluorescence emission titration curve of L^5 raises another interesting question. In this case, a moderate quenching effect is seen following the first deprotonation. By comparing L^4 with L^5, it can be concluded that while in the former receptor electron transfer from the lone pair of the central deprotonated nitrogen atom to either benzene ring should be equally probable, in L^5 only one of the benzene rings is suitably orientated. The other ring is too distant from the free lone pair to permit an efficient electron trans-

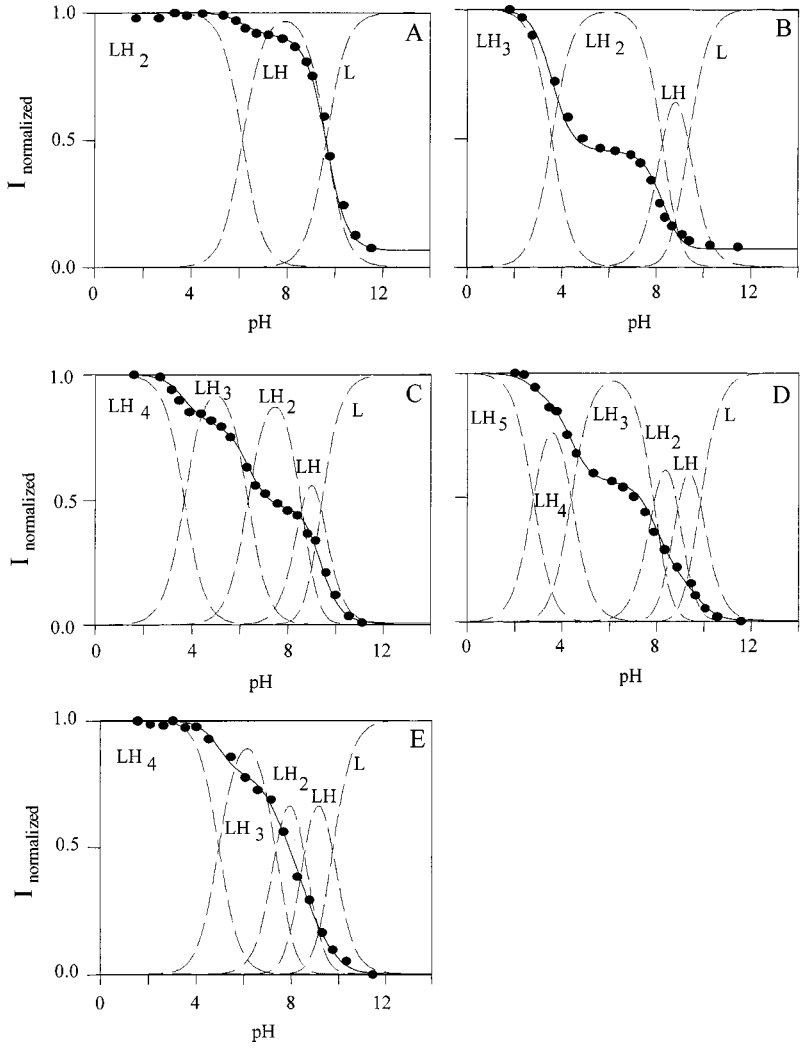


Figure 2. Fluorescence emission titration curves of A-L^3, B-L^4, C-L^5, D-L^6, and E-L^7, along with the respective mole fraction distribution curves

fer. On this basis, L^5 can be predicted to show ca. one-half of the efficiency of L^4, which is indeed borne out by experiment (see Figure 2). Removal of a second proton from L^5 (which is known to occur from the central atoms as well) should double the electron-transfer probability, as is also observed experimentally.

The largest member of this family, L^6, has a chain bearing five nitrogen atoms. The first two deprotonation steps occur at the nitrogen atoms labelled N2 and N4. At this stage, the situation can be compared with that resulting from the first deprotonation of L^3 or with that following the second deprotonation of L^5.

The fluorescence emission titration curves of compound L^7 were also determined. In this compound, nitrogen atoms N1 and N2 as well as N3 and N4 are separated from the benzene fragments by three carbon atoms, rather than two in the case of L^5. This structural feature is reflected in the titration curve of L^7 in that the quenching observed upon removing two protons is less efficient than in the case of L^5.

Anthracene Fluorophore

A great improvement in the efficiency of polyamine-based chemosensors can be achieved by substituting benzene, which has a low fluorescence quantum yield, φ_f = 0.06, by a more intrinsically luminescent unit, such as naphthalene, φ_f = 0.21, or anthracene, φ_f = 0.3. In this section, we report a family of chemosensors incorporating anthracene fluorophore.[14,15] In Figure 3, the titration curves of the chemosensors L^8 and L^9 are presented. As for the benzene analogues, the fluorescence emission is almost totally quenched when the two central nitrogen atoms are deprotonated.

An identical pattern is apparent in the fluorescence emission titration curves of the linear analogues L^{10} and L^{11}, also shown in Figure 3. The quenching effect is seen when nitrogen atoms N2 and N3 are completely deprotonated. In effect, according to CPK models, these two nitrogen atoms are the only ones that can overlap efficiently with the π system of the anthracene.

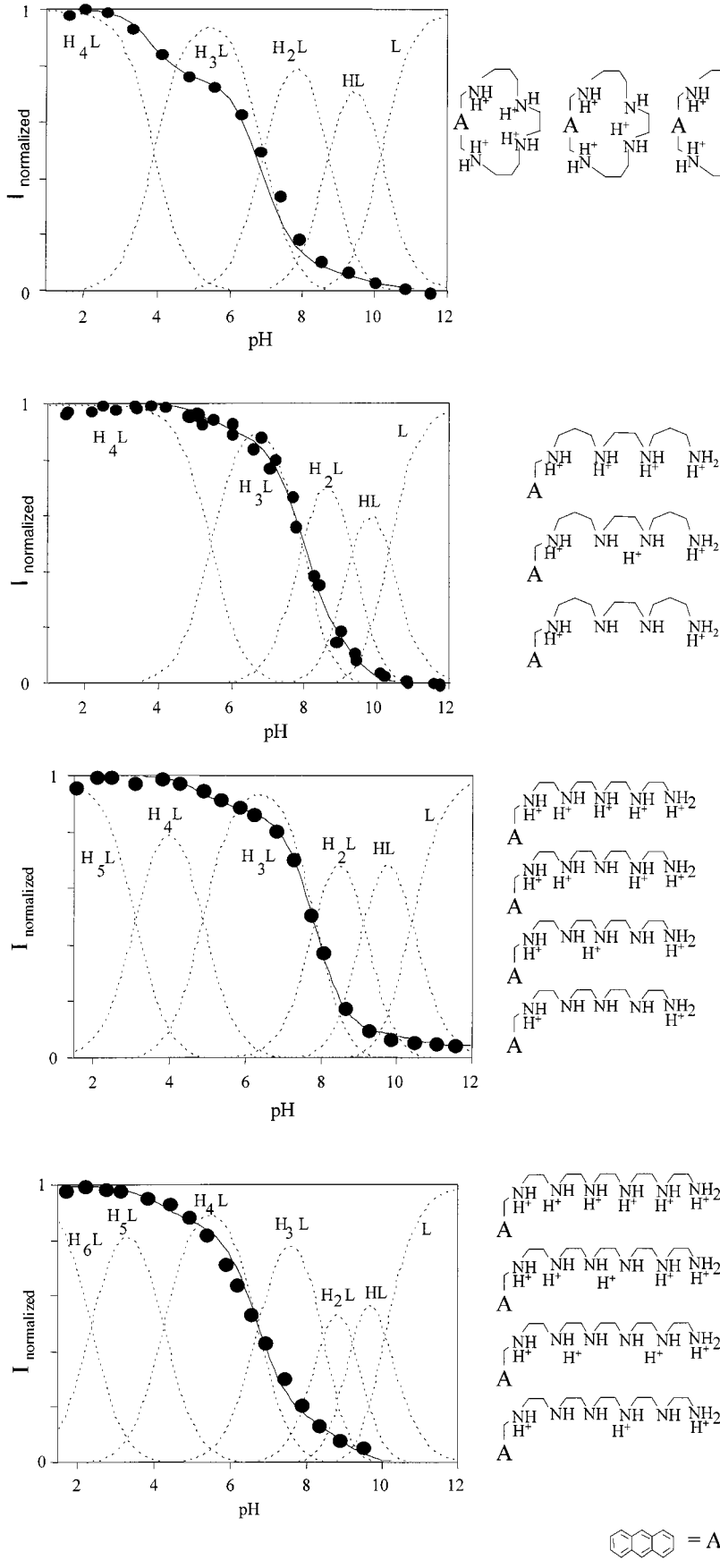


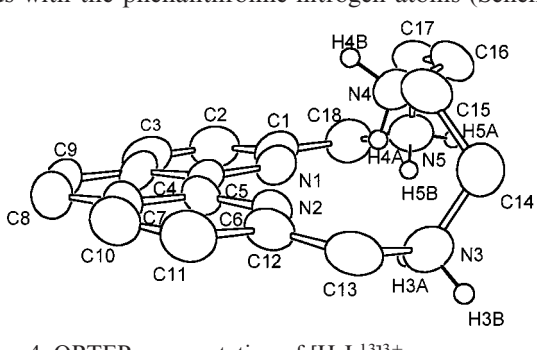
Figure 3. Fluorescence emission titration curves for L^8, L^9, L^{10}, and L^{11}, along with the respective mole fraction distribution curves and deprotonation sequence of the chemosensors

Phenanthroline Fluorophore

The first generation of chemosensors were based on aromatic heterocycles, e.g. those containing nitrogen or oxygen atoms, and their functions (binding and signalling) stemmed from the same part of the molecule. For this reason, they were called *intrinsic chemosensors*. Among these, phenanthroline derivatives have received much attention in recent years by virtue of their intense fluorescence and due to the presence of two aromatic nitrogen atoms that can act cooperatively in binding cations.[16] In the second generation of chemosensors, known as *conjugate chemosensors*, the binding and signalling moieties of the molecule are separated by a spacer and the two functions can be ascribed to different parts of the molecule, as shown in Scheme 2.[1]

In this context, a new series of polyamine macrocycles containing a phenanthroline unit as an integral part of the cyclic framework has recently been reported.[17,18] The simplest member of the series, 2,5,8-triaza[9]-10,23-phenanthrolinophane, L^{13}, contains a triamine chain linking the 2,9-phenanthroline positions. Therefore, this system can be considered as being both an intrinsic and a conjugate chemosensor: While the polyamine unit is used exclusively for the function of binding, the phenanthroline unit can be used not only for signalling but for binding as well.

In the crystal structure of the fully protonated form of L^{13}, containing three bromide anions as counterions, the macrocycle is seen to adopt a bent configuration. The phenanthroline unit and the benzylic nitrogen atoms N3 and N5 are almost coplanar, and this plane makes a dihedral angle of 104.10°, with the plane formed by the N3, N4, and N5 amino groups. The aliphatic amino groups (N4, N5, and N6) are in an *endo* conformation, enabling H-bond interactions inside the macrocyclic cavity. Specifically, the hydrogen atom linked to the central amine group points inside the macrocyclic cavity, giving rise to intramolecular H bonds with the phenanthroline nitrogen atoms. The nitrogen atoms N3 and N5 are also partially enclosed in the ligand cavity, and thus they can also form intramolecular H bonds with the phenanthroline nitrogen atoms (Scheme 4).



Scheme 4. ORTEP representation of $[H_3L^{13}]^{3+}$

As for the benzene and anthracene analogues, the absorption spectra of the whole molecules resemble that of the phenanthroline chromophore and protonation of the chain has only a small influence on their shape and intensity. However, the fluorescence emission spectra are completely quenched at basic pH values. When considering a fluorophore such as phenanthroline, containing lone pairs, the possibility of inversion between the strongly emissive π-π* state and the poorly emissive n-π* state has to be taken into account. In particular, protonation can stabilize the n-π* state relative to the π-π* state, leading to inversion of the states and thus to a decrease in the fluorescence emission intensity. This is the most probable explanation for the quenching effect observed for the fully protonated form. As discussed above, in this form, the nitrogen atoms of the phenanthroline unit are involved in H bonds with the protons of the chain. On removing the first proton, which is known to occur from the central nitrogen atom, the chemosensor becomes more emissive. At this point, two opposite effects come into operation. On the one hand, the intramolecular hydrogen bond becomes weaker and thus the fluorescence emission tends to increase. On the other hand, deprotonation of the central atom leaves the respective nitrogen lone-pair free to participate in the quenching process by electron transfer, which is thermodynamically favourable. However, a general feature of this class of compounds is that only when a benzylic nitrogen atom is deprotonated is the emission extinguished, and hence the diprotonated form is still emissive. This behaviour is exactly opposite to that seen with polyazacyclophane and *N,N′*-dibenzylated polyamine chemosensors (vide infra). This difference can be attributed to the fact that in the latter compounds the benzylic amino groups are in an *exo* conformation, whereas in the phenanthroline derivatives an *endo* conformation is adopted.

By increasing the length of the polyamine moiety, i.e. by replacing the diethylenetriamine chain by a triethylenetetraamine unit, L^{14}, or a tetraethylenepentaamine unit, L^{15}, larger macrocycles are obtained. The fluorescence emission titration curves show essentially the same features, i.e. a total quenching at basic pH and a partial quenching at very acidic pH values (Figure 4).

As a matter of fact, the marked decrease in the fluorescence emission intensity in very acidic media corresponds to the formation of the fully protonated forms of these compounds. This quenching effect increases in the order $L^{13} < L^{14} < L^{15}$, exactly mirroring the increasing involvement of the nitrogen atoms in stabilizing the protonated forms through hydrogen bonding. The observed decrease in the fluorescence emission can thus be rationalized in terms of possible inversion of the energy between the n-π* state and the π-π* state upon protonation (even partial) of the phenanthroline nitrogen atoms.

As observed for L^{13}, the larger macrocycles show a dramatic quenching effect at more basic pH values. In the case of L^{14}, the emission disappears with the loss of the second proton, while for L^{15} this is only seen on removing the third proton. In both cases, the resulting forms correspond to deprotonation at the benzylic nitrogen atoms. Once again, this behaviour seems to indicate a quenching process involving electron transfer from the lone pairs of the deprotonated amines to the excited phenanthroline. Because

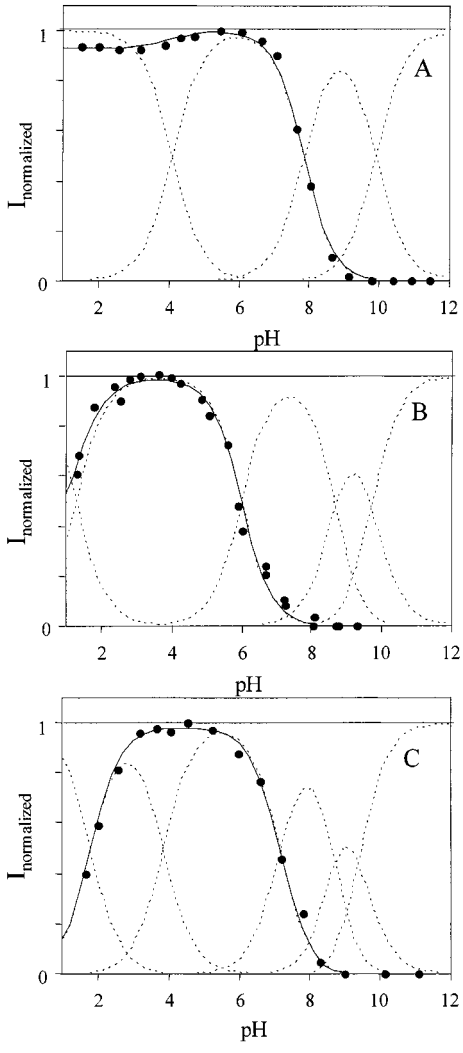


Figure 4. Fluorescence emission titration curves of A-L[13], B-L[14], and C-L[15], along with the respective mole fraction distribution curves

phenanthroline is only ca. 0.1 eV more difficult to reduce than anthracene, the process is also thermodynamically favourable.

Sensing Metal Cations

Photoelectron transfer from the amines to the excited fluorophore can also be suppressed by the binding of a cation at the receptor unit because, like the proton, the positively charged metal ion can increase the oxidation potential of the amine. However, coordination to metals such as copper(II) or nickel(II) can give rise to an enhancement of the quenching as the presence of such metal ions can offer alternative, non-radiative pathways, e.g. through the formation of non-radiative charge-transfer states. This situation can generally be detected through the appearance of new, intense absorption bands in the UV/Vis spectrum. The metal ion can also participate in the quenching by energy transfer or electron transfer, or quench through the heavy-atom effect. Some of these quenching processes are, however, precluded when the electronic configuration of the metal ion is d^{10}. This is the case with the zinc(II) and cadmium(II) cations, complexes of which can exhibit CHEF effects.

Sensing Zinc(II)

Complexes involving zinc(II) have attracted much attention, not only because of the large CHEF effect exhibited by this metal ion upon coordination, but also in view of the key roles played by such complexes in the active sites of zinc-containing enzymes such as carbonic anhydrase.[19−21]

The zinc(II) complexes of polyazacyclophanes and *N,N′*-dibenzylated polyamine receptors have been studied in detail. The absorption spectra of the chemosensors are not substantially altered in the presence of this metal ion, as reported by Czarnik in the case of a series of anthrylazamacrocycles.[2]

As for proton binding, plotting the fluorescence emission titration curves in conjunction with the mol fraction distributions of the various species present in solution, as determined by potentiometry, proved very helpful in understanding the emissive behaviour of these systems. This procedure led to good fitting and allowed us to dissect the relative contribution of each species present to the overall emission.

Zinc(II) Complexes with Polyazacyclophane Macrocyclic Receptors

In the case of polyazacyclophane macrocyclic receptors, the presence of a *p*-phenylene spacer prevents the simultaneous coordination of both benzylic nitrogen atoms to the same metal ion. This feature leads to unsaturated coordination sites, often of low symmetry, that are of interest with regard to the promotion of particular reactivities such as the stabilization of low-valent metal complexes or the activation of hydrolytic pathways.[22−25]

The structural characteristics of these compounds are reflected in the fluorescence emission properties of their various forms. In all the $[ML]^{2+}$ complexes, one of the benzylic nitrogen atoms is neither protonated nor coordinated and can thus take part in a PET quenching process, thereby accounting for the lack of an emission (Figure 5).

Curiously, in these cases, the two titration curves (in the presence and absence of the metal) are identical; only H_4L^{4+} and H_3L^{3+} are emissive and these species do not interact appreciably with zinc(II), hence their mol fraction distributions as a function of the pH value are not significantly affected.

At this point, the question arises as to why the benzylic nitrogen atoms should become efficient at quenching when involved in the metal complex, even though they are seemingly poor quenchers in the free chemosensor. A likely explanation for this behaviour is that these benzylic nitrogen atoms would be transformed from an *exo* conformation in the uncoordinated macrocycle to an essentially *endo* conformation in the coordinated complex.

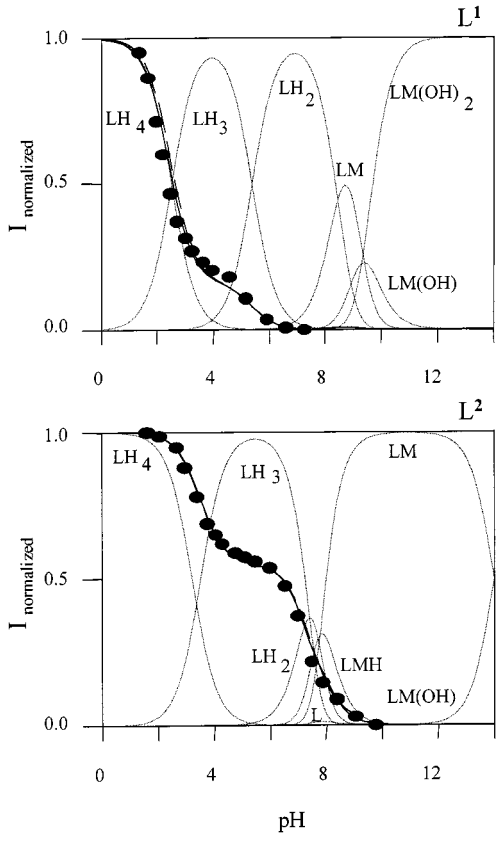


Figure 5. Steady-state fluorescence emission titration curves of (a) L^1, (b) L^2, in the absence of zinc(II) (traced line) and in the presence of an equimolar amount of zinc(II) (full line); mole fraction distributions in the presence of zinc(II) are also represented (full line)

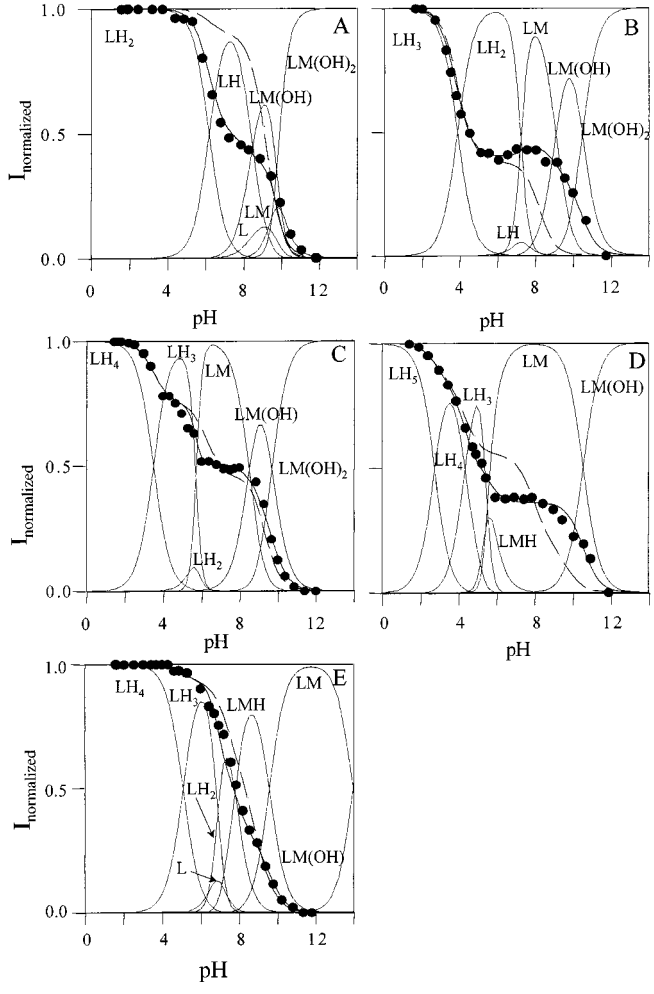


Figure 6. Steady-state fluorescence emission titration curves of A-L^3, B-L^4, C-L^5, D-L^6, and E-L^7 in the absence of zinc(II) (traced line) and in the presence of an equimolar amount of zinc(II) (full line); mole fraction distributions in the presence of zinc(II) are also represented (full line)

Zinc(II) Complexes with *N*,*N*′-Dibenzylated Receptors

The fluorescence emission titration curves for the zinc(II) complexes with ligands L^3 to L^7 are presented in Figure 6.[26]

On comparing the fluorescence emission properties of these complexes with those of the polyazacyclophane analogues, it can be seen that for these ligands the $[ZnL]^{2+}$ complexes and the protonated analogues are emissive, except in the case of the largest member of this family.

In the case of the ligands L^4 and L^5, three complexes $[ZnL]^{2+}$, $[ZnL(OH)]^{+}$, and $[ZnL(OH)_2]$, could be detected by potentiometry, showing significant, weak, and no emission, respectively. In all the complexes, the nitrogen atoms can be expected to be fully involved in the coordination to the metal center, except in the case of the dihydroxy complexes, where the lack of an emission may be rationalized in terms of partial decomplexation. The zinc(II) complexes display emissions with quantum yields ca. half of that of the emissions detected for the fully protonated forms of the free ligands. This implies that coordination to the zinc(II) ion, like protonation, prevents the PET between the amines and the fluorophore, albeit less efficiently. When the water coordinated to the zinc(II) cation loses a proton, the protective effect of the metal ion decreases and is lost completely following removal of a second proton from another water ligand. In the case of L^6, potentiometric titrations predict the existence of the species $[ZnHL]^{3+}$, $[ZnL]^{2+}$, and $[ZnL(OH)]^{+}$. The lack of fluorescence emission for the form $[ZnL]^{2+}$ can be explained by the fact that in this species only four of the five nitrogen atoms are involved in the binding, allowing the free nitrogen atom to carry out the quenching process.

Zinc(II) Complexes with Anthracene Fluorophores

The fluorescence emission titration curves for the zinc(II) complexes of the chemosensors L^9 to L^{11} are presented in Figure 7.[14]

This class of compounds shows some remarkable advantages in comparison with the chemosensors incorporating a benzene unit: (i) substantially higher fluorescence quantum yields, (ii) much greater differences in emission between the free and the complexed forms.

Inspection of Figure 7 shows that the metal complexes however $[ZnL]^{2+}$ as well as their protonated forms are strongly emissive, with the sole exception of the $[ZnL^{11}]^{2+}$ species. In contrast, the hydroxo complexes are not emissive. A puzzling matter that needs to be addressed is the contrast

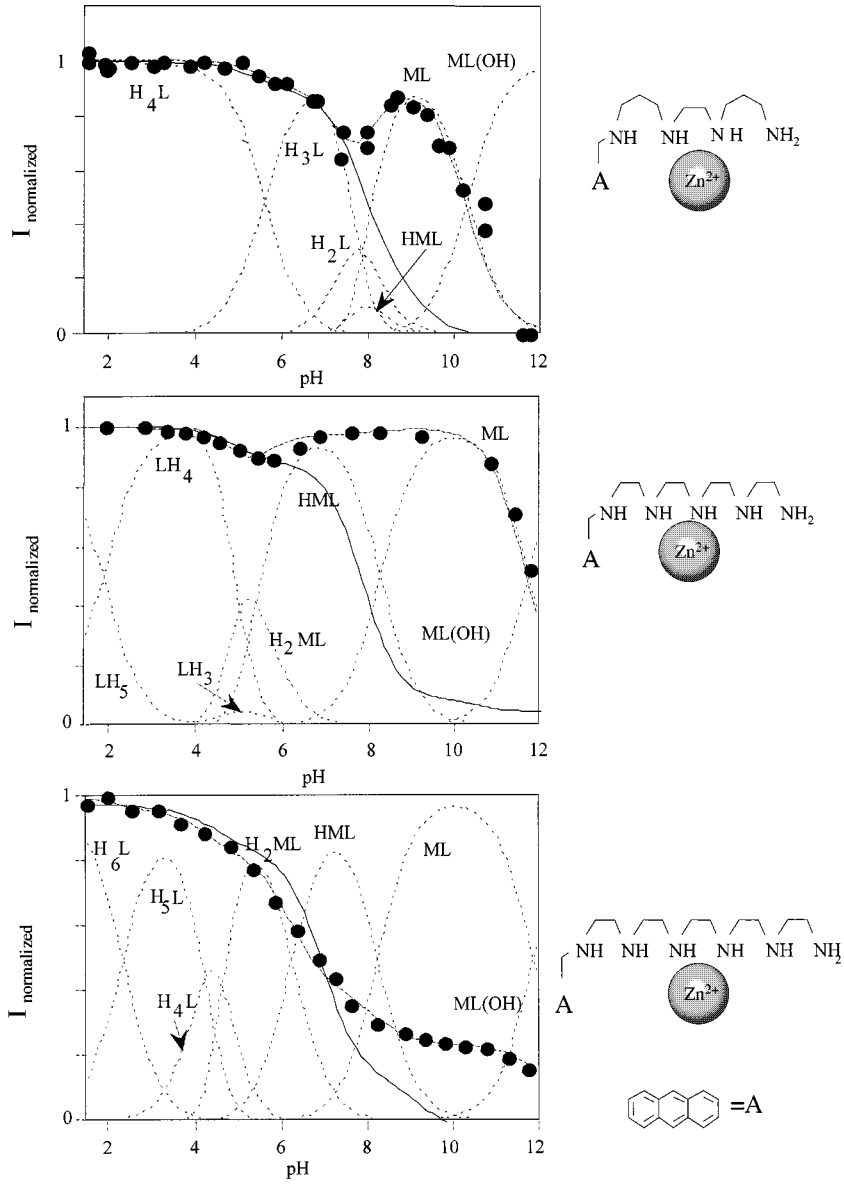


Figure 7. Steady-state fluorescence emission titration curves of L^9 to L^{11} in the absence of zinc(II) (full line) and in the presence of an equimolar amount of zinc(II) (traced dotted line); mole fraction distributions in the presence of zinc(II) are also represented (dotted line)

between the intense fluorescence emission of the species $[ZnL^{10}]^{2+}$ and the weak emission of the complex $[ZnL^{11}]^{2+}$.

Assuming that in these compounds the best coordination is achieved when four nitrogen atoms are simultaneously bound to the metal center, the $[ZnL^{11}]^{2+}$ species would possess a free or only weakly coordinated nitrogen atom, which could thus be responsible for quenching.

An interesting feature that emerges from Figure 7 is the crucial importance of the receptor length. The best performance was observed with the chemosensor of intermediate length.

In general, zinc(II) complexation by the conjugate chemosensors gives rise to two different effects: (i) the mol fraction distributions of the highly protonated and emissive forms of the free ligand are decreased and shifted to lower pH values, and these are replaced by less emissive metal complexes, thereby explaining the CHEQ effect; and (ii) it allows the existence of emissive metal complexes under conditions of moderately basic pH values where, in the absence of the metal ion, no emissive species of the free ligand are present, thereby leading to a CHEF effect. These two effects are thus the result of the existence of differentiated domains for each type of emissive species.

Zinc(II) Complexes with Phenanthroline Chromophores[17,18]

The crystal structure of the complex $[ZnL^{13}(H_2O)](ClO_4)_2$ indicates that the coordination geometry around the metal atom can be described as a distorted tetrahedron, the vertices of which are occupied by the phenanthroline nitrogen atoms N1 and N2, the central aliphatic nitrogen atom N4, and a water molecule. The zinc(II) cation forms two long bonds to the benzylic nitrogen atoms (N3−Zn 2.460 Å, N5−Zn 2.512 Å) so as to achieve a pseudo-prismatic geometry, the bases of the prism being defined by N2,

N3, N4 and N1, O1, N5, respectively (Figure 8A). According to this structure, both benzylic nitrogen atoms are weakly involved in metal coordination. A similar effect has been described for polyazamacrocyclic receptors containing a benzene unit as an integral part of the ring.[26] Furthermore, the phenanthroline unit displays electron-withdrawing properties, which reduce the basicity and the donor ability of the benzylic amino groups adjacent to the heteroaromatic moiety. These characteristics disfavour coordination of the metal center by the benzylic amino groups, leading to an unsaturated coordination environment for the zinc(II) ion. As a consequence, water molecules can be bound at the free binding sites of the metal center, as has indeed been shown by a crystal-structure analysis of the complex. Facile deprotonation of the coordinated water gives the hydroxo complex in aqueous solution at slightly alkaline pH values, as well as a dihydroxo species in strongly alkaline solutions. The aforementioned structural characteristics strongly affect the fluorescence emission features of this zinc(II) complex. Figure 8B shows the fluorescence emission titration curve of L^{13} in the presence of equimolar amounts of zinc(II) ions, from which an efficient quenching effect is apparent.

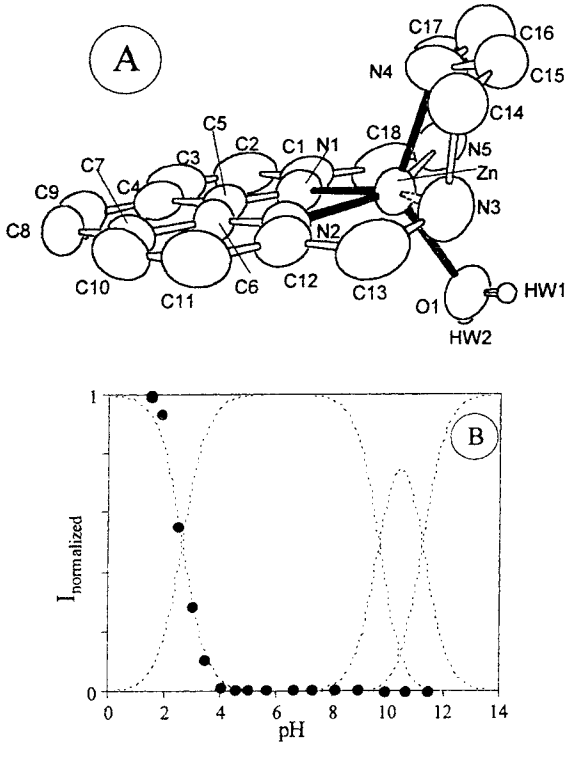


Figure 8. **A**: ORTEP representation of the $[ZnL^{13}(H_2O)]^{2+}$ cation; **B**: fluorescence emission titration curve of L^{13} in the presence of an equimolar amount of zinc(II)

This unexpected behaviour can be rationalized by considering the particular characteristics of the ligand L^{13} and of its zinc(II) complexes. According to the structure of the complex cation $[ZnL^{13}(H_2O)]^{2+}$, both benzylic amino groups are weakly involved in the metal coordination. These nitrogen atoms are close to the fluorophore and, as shown previously, they are able to efficiently quench the fluorescence. Moreover, no protonated forms of the zinc(II) complex could be detected by potentiometry. Therefore, in this complex the lone pairs on the benzylic nitrogen atoms are available for the electron-transfer process, accounting for the lack of fluorescence emission.

Sensing Cadmium(II)

Figure 9 shows the fluorescence emission titration curves obtained for the chemosensors L^9 to L^{11} in the presence of equimolar quantities of cadmium(II). The basic trend is similar to that observed for the complexes with zinc(II). However, due to the lower propensity of the cadmium(II) complexes to form hydroxo species when compared with zinc(II), the fluorescence emission extends to more basic pH values. For example, strong fluorescence emissions can still be detected at pH = 12.

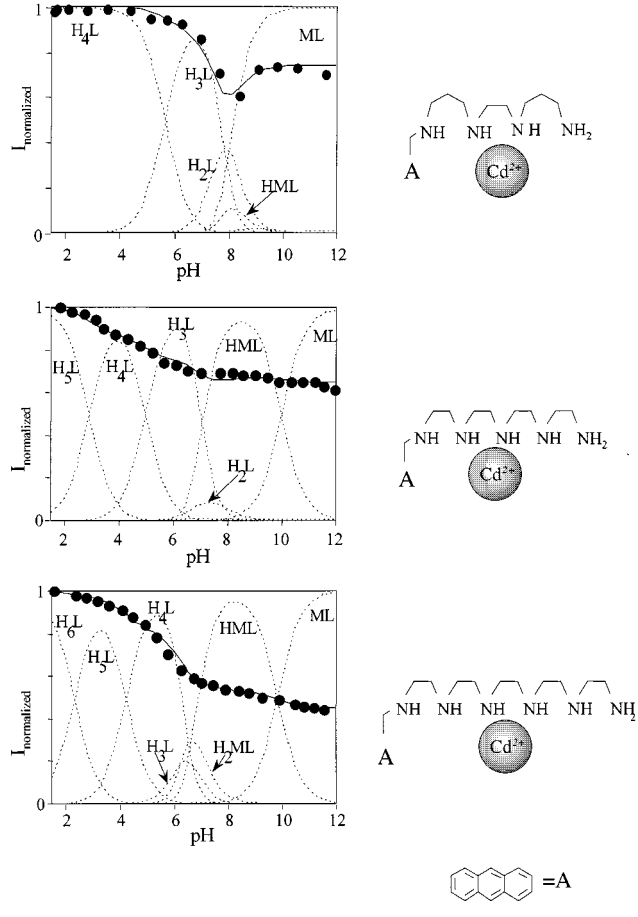


Figure 9. Fluorescence emission titration curves obtained for the chemosensors L^9 to L^{11} in the presence of equimolar quantities of cadmium(II)

Sensing Copper(II)

In contrast to the situation with zinc(II) ions, complexation of copper(II) ions by L^1 to L^7 leads to large increases in the molar absorption coefficients as well as to blue shifts in the UV/Vis absorption maxima. These new

and very intense bands are characteristic of charge-transfer processes and are indicative of significant interactions between the molecular orbitals of the ligand and those of the metal center (Figure 10A). As a consequence, the excited states are no longer centred on the ligand but display metal character. No fluorescence emission was observed for these complexes, the recorded fluorescence being purely due to protonated forms of the free ligands (Figure 10B). According to potentiometric studies, several species with all the nitrogen atoms involved in coordination to the metal ion and/or protonated are present in solution. In spite of this, no emission was observed. A possible explanation for the absence of fluorescence emission in the copper(II) complexes can be found in the charge-transfer characteristics of the excited state, which can offer a channel for the deactivation of the excited state involving the metal center.

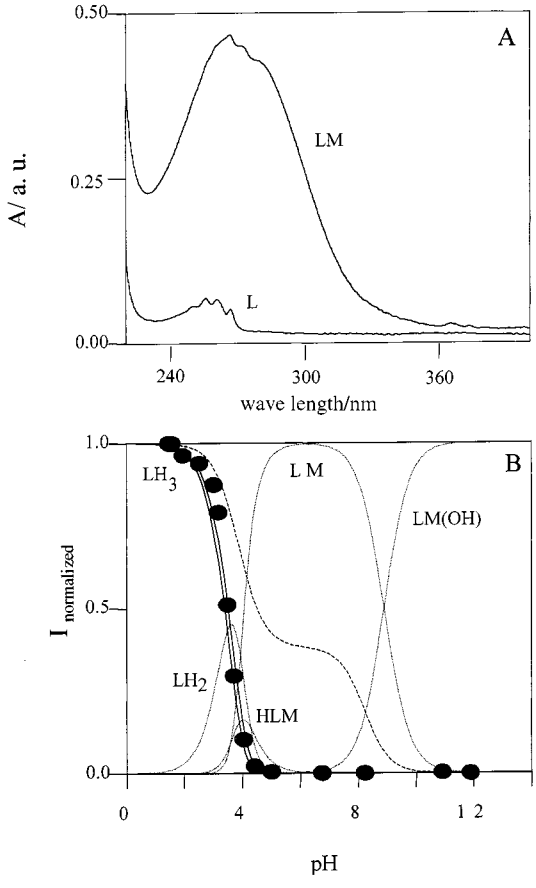


Figure 10. **A**: Absorption spectra of L^4 at pH = 6, in the absence and in the presence of an equimolar amount of copper(II); **B**: fluorescence emission titration curves obtained for L^4 in the presence of an equimolar amount of copper(II)

Sensing Anions

The chemosensors described throughout this work can also be used to bind anions. One of the obvious strategies for achieving this goal is to work at pH values at which the polyamine receptor is fully or partially protonated, thereby using electrostatic attraction as a driving force for anion binding. In another strategy, complexes of the polyamine ligands with strong Lewis acids such as divalent metal ions, in particular zinc(II), are used as receptors. Adducts of this type show strong affinities for anions as the metal center offers a coordination site at which the substrate can be bound. This latter strategy has been brilliantly developed by Kimura and co-workers. These authors have designed molecules based on the well-known [12]aneN_4 and [12]aneN_3 macrocyclic receptors. Coordination of substrates such as benzoic acid,[27] phthalic acid,[27] sulfonamides,[28] or carboxamides[29] is favoured by strong interactions between the zinc(II) ion and the respective anions. In this regard, macrocyclic receptors bearing pendant arms incorporating additional donor atoms are particularly interesting. This is exemplified by the receptor cyclen, which has pendant aromatic sulfonamide moieties that can bind to the metal center through their sulfonamide *N*-anions.[31] Pendant arms containing phenolate,[30] dimethylaminopropyl,[31] pyridine,[31] uracil,[32] and alcohol[33] moieties have also been described.

Sensing Nucleotides

Nucleotide sensing and quantification by means of luminescent probes represents an important target in supramolecular chemistry due to their many biological and biomedical implications. Lehn and co-workers have prepared two very interesting multi-site binding receptors based on the well-known macrocyclic structure of 1,13-dioxa-4,7,10,16,19,22-hexaazatetracosane (bisdien); to which one or two acridine luminescent groups, respectively, have been covalently bonded to the central nitrogen atoms of these polyamine fragments through aminopropyl side-arms. The second of these receptors seems to be particularly well suited for nucleotide recognition since, apart from displaying large variations in its emission properties upon coordination to nucleotides, it does not induce hydrolytic ATP cleavage.[34] Other types of receptors developed by Lehn and Zinic were based on bis(phenanthridinium) fragments; their emissions were subject to large quenching effects as a result of π-stacking interactions with the adenine moieties of nucleotides.[35] These interactions gave constants as high as ca. 5.8 logarithmic units and were independent of the polyphosphate chains of the nucleotides.

Recently, it was shown that, in spite of their apparent simplicity, the chemosensors L^9, L^{10}, and L^{11}, but not L^8, strongly interact with nucleotides exhibiting high degrees of selectivity for ATP. The presence of the anthracene unit allows the chemical inputs to be readily transformed into light signals.[36]

pH-Metric titrations of aqueous solutions of L^9 and ATP in a 1:1 molar ratio over the pH range 2.5−11 show the formation of ATP−L^9 adducts with degrees of protonation varying from 6 to 1.7. The distribution diagram is depicted in Figure 11. The conditional stability constants vary from 6.5 to 2 in the pH range explored. Fluorimetric titrations on the ATP−L^9 system show a bell-shaped curve; thus, light emission is strongly quenched at pH values lower than 4,

the emission reappears at pH ≈ 7, and then decreases once more, eventually disappearing at pH > 10. A similar result has been obtained by Czarnick et al.[37] in a study of the complexation of various anions, such as ATP, by two anthrylpolyamines.

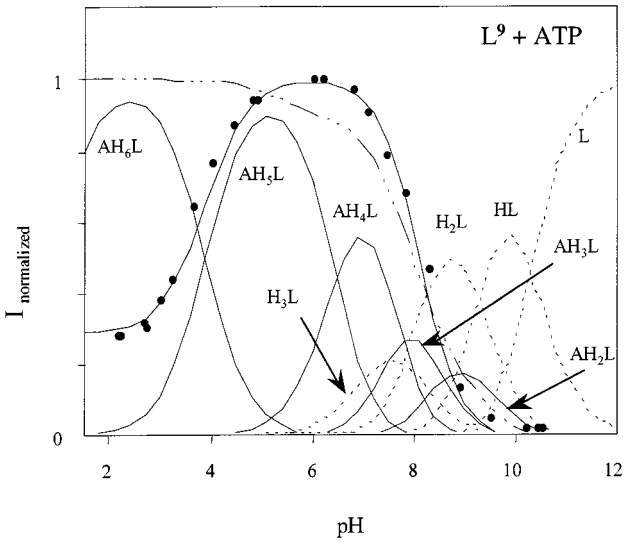


Figure 11. Fluorescence emission titration curves of L^9 in presence of equimolar amounts of ATP (•); fitting (——) was achieved as a linear combination of the mole fraction distribution of the various adducts as determined by potentiometry; the titration curve in the absence of ATP (—···—) is also included for comparison purposes

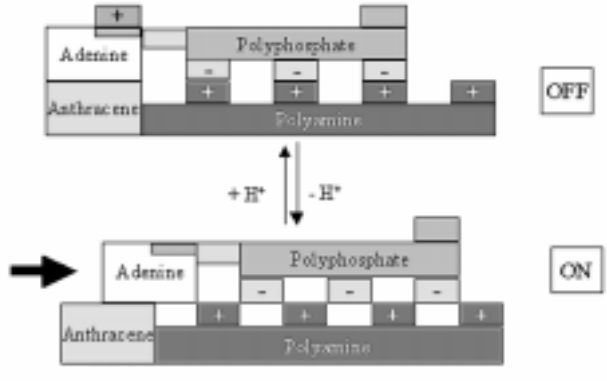
We suggest that this behaviour may be interpreted on the basis of the various species formed in solution, and considering the luminescent behaviour of the free receptor.

As mentioned above, from spectrofluorimetric titrations of the free receptor it could be inferred that H_4L^{4+} and H_3L^{3+} were the only emissive species, with a relative fluorescence emission quantum yield of $\Phi(H_4L^{4+})/\Phi(H_3L^{3+}) = 0.85$. In the presence of ATP, these species continue to emit, irrespective of whether they are complexed to ATP or free, except for the H_6LA^{6+} species formed by the association of the fully protonated receptor L^9 and the diprotonated form of ATP, which gives rise to a quenching effect. Comparing the fluorescence emission titration curves of the free and complexed receptor (Figure 11), the small increase in the fluorescence emission (CHEF) observed for the ATP−L^9 system at a neutral pH value can be attributed to the increase in apparent basicity of the receptor that occurs upon coordination to ATP. Perhaps the most striking feature of this system is the dramatic CHEQ effect (almost 60%) observed at pH < 4 (see Figure 11). A plausible interpretation of such quenching could be a π-stacking interaction between the anthracene unit of the receptor and the adenine fragment of the guest species, resulting by the charge state of both of them at these pH values. Evaluation of the stability constants at pH = 2.0 provides a value of 5.8 logarithmic units. Interestingly, the monoprotonated form of ATP does not give rise to such a quenching effect. It seems likely that the matching between the triphosphate chain of $HATP^{3-}$ and the polyammonium fragment of L^9 alters the relative disposition of the aromatic moieties in the two molecules, thereby preventing efficient π-stacking. On examining the behaviour of ADP, AMP, adenine, and tripolyphosphate, it was observed that only the first of these produced a CHEQ effect, however with a much lower constant (log K = 3.7 at pH = 2.2) than that found for ATP.

Therefore, it seems that the molecular topology of the receptor is a key factor in facilitating the π-stacking pattern. Indeed, experiments carried out with the anthracenophane 2,6,9,13-tetraaza[14](9,10)-anthracenophane (L^8) did not reveal any significant effect. On the other hand, analogous ligands containing the same fluorophore but with different polyamine chains (L^{10} and L^{11}) give rise to similar CHEQ effects with very similar association constants (L^{10}: log K = 5.6; L^{11}: log K = 5.4; both at pH = 2.2). It is noteworthy that the constants obtained are not affected by the chain length or the number of nitrogen atoms in the chain, but only by the molecular arrangement of the receptor. The values of these stability constants are, on the other hand, rather similar to those obtained by Lehn and Zinic for the interaction of ATP and other nucleotides with their bis-(phenanthridinium)-based receptors.[35]

A related receptor can be constructed by appending two methylanthracene fragments at both ends of a linear pentamethylenehexaamine chain, L^{12}. Interaction of L^{12} with ATP also leads to a very significant quenching of the emission at low pH values, from which an association constant of log K = 5.7 at pH = 2.2 can be calculated.

The similar values of the constants obtained for ATP binding with all the receptors at acidic pH values, together with the lack of notable effects for AMP and adenine, suggest that the requirements for observing significant CHEQ effects are: (i) a certain degree of anchorage of the polyphosphate chain of ATP by the polyammonium fragments of the receptor, and (ii) topological complementarity of the partners (see Scheme 5).



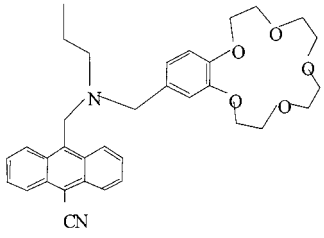
Scheme 5. Schematic representation of ATP "anchorage"

These results highlight the potential use of these simple receptors as efficient luminescent ATP chemosensors. Further work is currently in progress with the aim of obtaining related receptors with improved binding characteristics.[37]

Molecular Devices and Machines

Logic Gates at a Molecular-Level

Chemosensors can be regarded as devices that behave essentially as logic gates since in these systems chemical binding (the "input") results in a change in fluorescence intensity (the "output") of the receptor, which can be translated as a simple logic operation. Among other authors,[37,39,40] de Silva and co-workers were the first to contribute to this growing field of chemistry.[3] For example, an AND logic gate operating with two input channels was described.[41] In this system, the fluorescence signal depends on whether hydrogen ions, sodium ions, or both are bound to the receptor molecule (Scheme 6).



Scheme 6. Chemical structure of de Silva's ligand that operates as a logic device[41]

Recently, we described a NAND logic gate based on the system ATP−L^9 (Figure 11).[36] The initial state is that of the free chemosensor in the pH range 5−8. The inputs are obtained by: (i) addition of protons in order to reach a final pH of ca. 2, and (ii) addition of a slight excess of ATP at pH = 6. We consider there to be a final output of 1 when the intensity of the fluorescence emission exceeds 0.8 relative units, and of 0 when the intensity of the emission is below 0.4 relative units. In the absence of any input, the system exhibits fluorescence emission and thus the output signal is 1. The same output is obtained if only ATP or protons are added to the solution. When both inputs are present, a quenching effect occurs and the output becomes 0 (see Table 1).

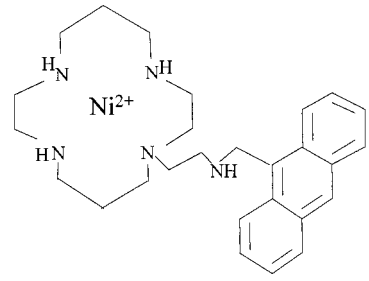
Table 1. Truth table of the logic operation NAND

$[H^+]$	[ATP]	Em
0	0	1
0	1	1
1	0	1
1	1	0

Molecular Machines

Metal Scorpionate Complexes

One interesting system, based on the presence of a pendant arm, has been reported by Fabbrizzi and coworkers.[42] This system uses the Ni^{II} complex of a cyclam ring separated from an anthracene chromophore by a methylene spacer (Scheme 7).



Scheme 7. Chemical structure of Fabbrizzi's molecular machine[42]

The fully protonated form, corresponding to a structure where the protonated alkylamine side chain is forced away from the ring through electrostatic repulsion, exhibits the most intense emission. A fluorimetric titration in acetonitrile/water (4:1) showed that on removing the first proton (from the alkylamine side chain) the emission was reduced to 60% of the original. In this last species the metal center is hexacoordinated by the cyclam nitrogen donors at the equatorial positions and by the alkylamine group and water molecule at the axial positions. Complete quenching occurs upon removal of a second proton from the water molecule linked to the metal center. All these processes are reversible and thus the system can be considered as an elementary molecular machine driven by the pH value. The pendant arm is forced to oscillate between the metal center and a more distant point by modulation of an external parameter, i.e. the pH value.

Conclusions

The study of supramolecular species capable of existing in different states that may be interconverted by external stimuli is a topic of great interest and has led to predictable applications in the field of nanotechnology. Fluorescent chemosensors containing polyamine receptors are versatile systems capable of detecting cations or anions and, depending on their incorporated units, function as molecular devices or machines. The systems reported in this review are capable of detecting substrates at low concentrations and exhibit high sensitivity in water. However, their selectivities still need to be improved if they are to find application in real situations.

Acknowledgments

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