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Protonation of cyclen-based chelating agents containing fluorescent moieties

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The synthesis of three new polyamine receptors based on a common 1,4,8,11-tetraazacyclododecane (cyclen) platform with appended, *via* a methylene linker, a single quinoline (Q) or 8-hydroxy-quinoline (8-OH-Q) moiety (**L1** and H**L2**) or a Q and a 8-OH-Q unit (H**L3**) is reported. The proton binding features of these receptors, together with those of two similar receptors containing two equal Q moieties (**L4**) and two Q and two acetate groups (H_2**L5**), have been studied by means of potentiometric, ^{1}H NMR, UV-vis absorption an fluorescence emission measurements in aqueous solution at different pH values. While cyclen is a commonly used binding unit, Q and 8-OH-Q represent classical examples of signalling units in conjugated fluorescence chemosensors and their protonation features can strongly influence their ability in metal cation, and anion coordination and sensing. This study reveals that the structural features of these receptors are controlled by a subtle balance between hydrogen bonding and electrostatic repulsions occurring in their protonated species. At the same time, their emission properties are tuned by photoinduced electron and/or proton transfer processes taking place in the excited state.

Introduction

The conclusions section should come in this section at the end of the article, before the acknowledgements. Polyamines are among the most appealing and used platforms to build up chemical sensors, including luminescent reporters, to be used for detection of a variety of chemical species in aqueous media.[1-27] Water solubility, chemical stability, easily functionalizable structures and, overall, ability to bind both metal cations, in particular transition metals, and anions represent peculiar characteristics of this class of compounds, making them extremely versatile binding moieties for the constructions of chemosensors to be used in aqueous matrices. In the most common approach to the design of optical chemosensors, the binding site, the polyamine moiety, is covalently linked, through an appropriate spacer, to the signalling site, e.g. a fluorescent units.[28-30] The interaction of the target species with the receptor site induces electronic modulations in the signalling unit, thus resulting in detectable emission changes. The ability of polyamines to easily form charged polyammonium cations in water, even at neutral pH, is another striking property of these receptor units, which strongly affect their ability in both metal cation and anion coordination.[31-46] While protonation competes with the process of metal binding, thus inhibiting their coordination ability, in particular at acidic pH values, the formation of polyammonium cations favours anion binding, thanks to the formation of energetically stabilizing charge-charge interactions.[28,29] At the same time, protonation can also modulate the emission properties of luminescent signalling units. Photoinduced electron transfer (PET) processes, which induce emission quenching thanks to an electron transfer from the lone pairs of amine groups to the excited fluorophores, is normally observed in polyamine-based optical chemosensors and it is inhibited by protonation of the amine groups.[47,48] However, several signalling units are fluorogenic moieties containing acidic or basic groups, and, therefore, proton transfer processes can occur between the fluorophores and the polyamine binding unit. At the same time, polyammonium cations can also be involved in photoinduced proton transfer (PPT) processes with excited fluorophores possessing basic sites.[47,48] Therefore, the design of efficient fluorescent receptors based on polyamine subunits needs to consider not only the pure binding properties of the receptor site, but also its protonation characteristics.

With this in mind, in this paper we report on a series of potential chemosensors containing a 1,4,8,11-tetraazacyclododecane (cyclen) moiety,[7,49-52] one of the most used binding unit, with appended one or two equal or different signalling moieties (**L1**-H_2**L5** in Scheme 1). In this respect, we have chosen two 'classic' signalling moieties, e.g., quinoline (Q),[53-56] which contains a weakly basic heteroaromatic nitrogen and 8-hydroxyquinoline (8-OH-Q),[57-61] featuring a weakly acid hydroxyl group close to a protonable nitrogen. We have also

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introduced in this study receptor H_2**L5** containing two carboxylate groups appended to the polyamine framework. While **L1**-**L4** appear well suited to bind transition metals, the presence of two acetate groups would make this receptor capable to bind more hard metal cations, such as lanthanides. At the same time, however, the carboxylate groups can modulate the basicity properties of the polyamine macrocycle and/or influence the electron/proton transfer processes between the aliphatic nitrogens and the quinoline units.

We have then analyzed the pH dependence of the emission spectra of these molecules, in order to rationalize the effect of acid-base equilibria involving the binding unit and/or the fluorescent unit in the overall recognizing/signalling properties of the receptor.

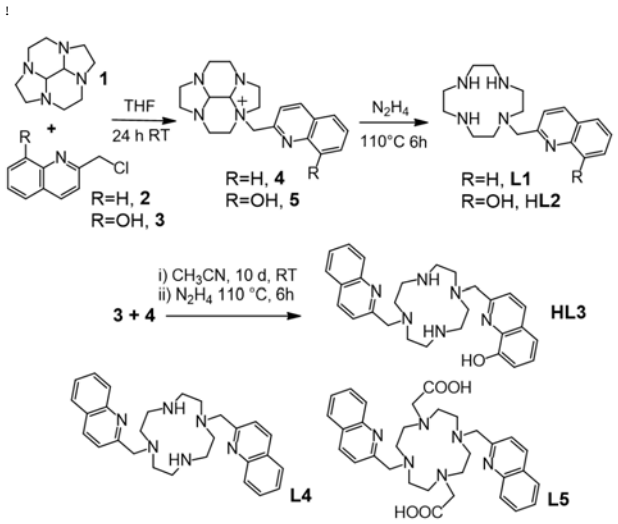
Results and discussion

Synthesis of the compounds

We have previously reported the synthesis of polyamines **L4** and H_2**L5**[62] (Scheme 1) by using, as starting material, the bis-aminal derivative of the tetraamine macrocycle 1,4,7,10-tetraazacyclododecane (cyclen), *i.e.* decahydro-2a,4a,6a,8a-tetraaza-cyclopenta[fg]acenaphthylene (**1** in Scheme 1).[63] The latter features two tertiary amine groups, in opposite positions within the cyclen framework, more reactive toward electrophilic agents. These structural characteristics can be exploited to synthesise the difunctionalized receptor **L4**, by simple reaction of **1** with two equivs. of 2-(chloromethyl)quinoline **2**. **L4** was subsequently used to obtain **L5** by reaction with chloroacetic acid. **1** can be also used to obtain mono-functionalized cyclen derivatives. In fact, the formation of the monofunctionalized derivatives of **1** makes slower the electrophilic attack on the second amine group. At the same time, in the present case, the monofunctionalized bis-aminals **4** and **5** are poorly soluble in THF, making easy their isolation. **4** and **5** resulted practically pure to be used in the following reaction without further purification. **4** and **5** can be deprotected by treatment with N_2H_4, affording the tetraamine macrocycles **L1** and H**L2**, which are finally purified as hydrobromide salts. However, **4** and **5** can be also exploited to insert a second functional group in the 7 position of the macrocyclic structure, taking advantage of the higher reactivity of the tertiary amine in 7 position with respect to the remaining nitrogens. Reaction of **4** with **3** in CH_3CN affords the bisaminal of cyclen functionalized with two different fluorescent units, Q and 8-OH-Q. The low solubility of the chloride salt of the diammonium cation allows for isolation of the bisaminal derivative, which can finally be deprotected with N_2H_4 to obtain the final cyclen derivative H**L3**

Acid-base properties

We first analysed the basicity properties of the five cyclen derivatives by means of potentiometric titrations, determining the species formed in solution and the corresponding protonation constants (Table 1).



Scheme 1. Synthetic routes to obtain ligand **L1**, H**L2** and H**L3** And drawings of **L4** and H_2**L5**.

Comparing the binding affinity for acidic protons of **L1**-H_2**L5**, Table 1 shows that **L1** and **L4** display similar basicity properties. Both receptors show a remarkably high value of the first protonation constant (Log K > 11). A second protonation step is then observed in the alkaline pH region (Log K = 8.90 and 8.72 for **L1** and **L4**, respectively). Finally, one or two further protonation steps are observed below pH 3. This behaviour partially parallels the protonation characteristics of the macrocyclic parent cyclen, which displays two remarkably high protonation constants (log K_1 = 11.02, log K_2 = 9.98).[64,65] In fact, in the $[H(cyclen)]^+$ and $[H_2(cyclen)]^{2+}$ species the two acidic protons are localized on two amine groups separated by a not-protonated nitrogen and form a stabilizing hydrogen bonding network involving all nitrogens within the macrocyclic cavity. However, cyclen shows a very low tendency to give species with protonation degree higher than 2, due to the increase of the electrostatic repulsions between ammonium groups within the macrocyclic framework.

Localization of the ammonium groups within the cyclic framework can influence the photophysical properties of fluorescent polyamine. Therefore, we performed ^{1}H NMR experiments at different pH values to determine the protonation sites in the different species formed by **L1** and **L4** in aqueous solutions. Figure 1 reports the ^{1}H chemical shifts of **L4** in D_2O solution at different pH values. The receptor presents a D_{2h} time-averaged symmetry all over the pH range investigated, making the spectra easily interpretable. Formation of the $[H$**L4**$]^+$ and $[H_2$**L4**$]^{2+}$ species below pH 11 induces a downfield shift for all signals of aliphatic protons, more marked for the methylene bridge 1_{AL} and for the signals of the ethylene chain (Figure 1). Moreover, the resonances of the Q moieties are also affected by the first two protonation steps, suggesting that the aromatic nitrogens are involved in proton binding. Despite the higher basicity displayed by secondary amine groups with respect to tertiary ones in aqueous solution,[64] it seems likely that the H^+ ions of $[H$**L4**$]^+$ and $[H_2$**L4**$]^{2+}$ are partially localized on the tertiary nitrogens A and A', enabling the formation of stabilizing

Table 1. Protonation constants (log K) of the receptors (NMe_4NO_3 0.1 M, 298 K).

Equilibrium	**L1**	**L4**
	Log K	
$\mathbf{L} + H^+ = [H\mathbf{L}]^+$	10.71(5)	10.54(4)
$[H\mathbf{L}]^+ + H^+ = [H_2\mathbf{L}]^{2+}$	8.90(7)	8.72(6)
$[H_2\mathbf{L}]^{2+} + H^+ = [H_3\mathbf{L}]^{3+}$	2.3(1)	2.74(8)
$[H_3\mathbf{L}]^{3+} + H^+ = [H_4\mathbf{L}]^{4+}$		2.0(1)

Equilibrium	H**L2**	H**L3**
	Log K	
$\mathbf{L}^- + H^+ = [H\mathbf{L}]$	11.06(6)	11.08(2)
$[H\mathbf{L}] + H^+ = [H\mathbf{L}]^+$	10.54(5)	9.85(4)
$[H_2\mathbf{L}]^+ + H^+ = [H_3\mathbf{L}]^{2+}$	8.85(5)	8.79(6)
$[H_3\mathbf{L}]^{2+} + H^+ = [H_4\mathbf{L}]^{3+}$	2.88(7)	2.3 (1)

Equilibrium	H_2**L5**
	Log K
$\mathbf{L}^{2-} + 2H^+ = [H_2\mathbf{L}]$	21.04(6)
$[H_2\mathbf{L}] + H^+ = [H_3\mathbf{L}]^+$	6.70(7)
$[H_3\mathbf{L}]^+ + H^+ = [H_4\mathbf{L}]^{2+}$	6.41(7)
$[H_4\mathbf{L}]^{2+} + H^+ = [H_5\mathbf{L}]^{3+}$	4.21(8)
$[H_5\mathbf{L}]^{3+} + H^+ = [H_6\mathbf{L}]^{4+}$	3.48(8)

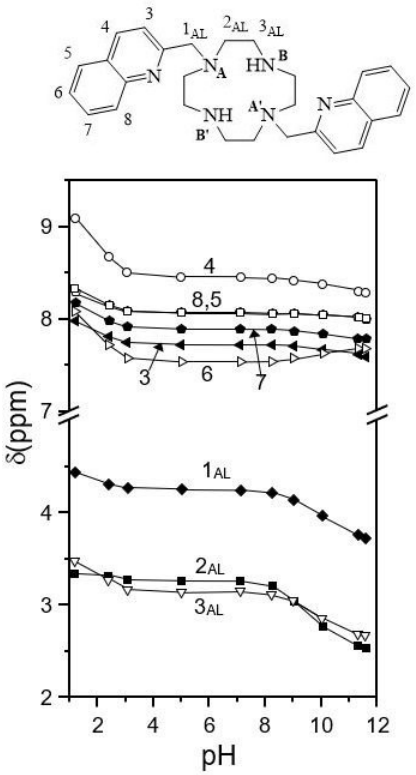


Fig.1. Plot of the ^{1}H NMR chemical shifts of **L4** as a function of pH.

hydrogen bonds with the heteroaromatic nitrogens of quinoline, as sketched in Scheme 2. This would explain the large downfield shift observed for the 1_{AL} signal. In this hypothesis, the signal of the ethylenic chain, which experiences the larger shift, can be attributed to the 2_{AL} methylene group.

The formation of the $[H_3\mathbf{L4}]^{3+}$ and $[H_4\mathbf{L4}]^{4+}$ species below pH 4 is accompanied by a marked downfield shift of the resonances of the Q units and of 3_{AL} of the ethylenic chains. Conversely, the 2_{AL} resonance remains almost unchanged. Considering that the protonation constant of Q is 4.94 log units,[66] while the third protonation step of cyclen is often not detected in aqueous solution,[65] the shifts observed in ^{1}H NMR spectra can be rationalized hypothesizing that a reorganization of the acidic proton distribution occurs in the $[H_3\mathbf{L4}]^{3+}$ and $[H_4\mathbf{L4}]^{4+}$ species. In the latter, the two Q moieties would be protonated, while the remaining two H^+ ions would be localized on the N_B and $N_{B'}$ amine groups, far from the protonated heteroaromatic units. This localization of the ammonium groups is strongly supported by the crystal structure of the $[H_4\mathbf{L4}]^{4+}$ cation (Figure 2), where the acidic protons have been localized, by means of Fourier density map, on the amine groups N_B and $N_{B'}$ and the heteroaromatic nitrogens during the refinement of the solid state structures by X-ray single crystal data. The whole molecule assumes a rather flat structure, ensuring an optimal minimization of the electrostatic repulsion between the positive charges gathered on the polyammonium cation (a full description of the crystal structure the $[H_4\mathbf{L4}]^{4+}$ cation is given within the supporting information). Similar results are also obtained in the case of **L1**, although in this case the presence of four different resonances for the methylene groups makes difficult a correct attribution of the methylene groups of the cyclen unit. However, the analysis of the pH dependence of ^{1}H NMR resonances shows an upfield shift of all aliphatic signals, indicating that the first two protons are bound to the amine groups of cyclen. The resonance of the methylene linker between cyclen and Q experiences the larger shift (see Supporting Information, Figure S19). This fact, together with the shifts observed for the aromatic protons at alkaline pH values would suggest that one of two first protonation steps occurs on the tertiary nitrogen of cyclen, probably shared *via* hydrogen bonding with the Q nitrogen. Finally, at acidic pH values, the third protonation step occurs on the heteroaromatic nitrogen, as testified by the large shift observed for the signals of the Q protons.

Compared to **L1** and **L4**, the most significant difference in the protonation behaviour of H**L2** and H**L3** is the formation of deprotonated anionic forms of the receptors ($\mathbf{L}^-$, see Table 1) at strongly alkaline pH values, which can be related to deprotonation of the 8-OH-Q hydroxyl group. In fact, 8-OH-Q

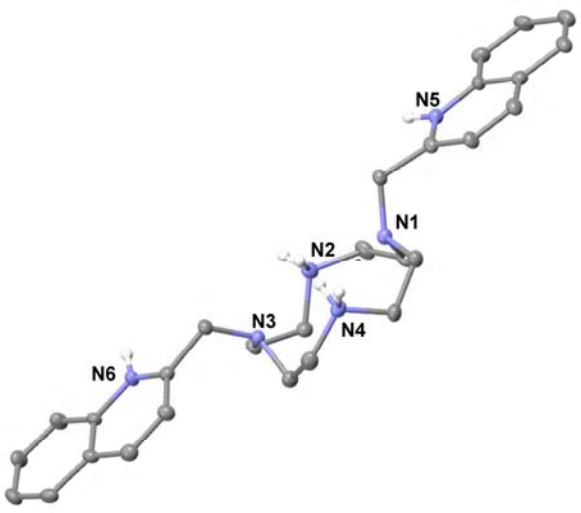


Fig.2. ORTEP-3 view of the H_4**L4**$^{4+}$ cation. Ellipsoids are drawn at the 20% probability. Hydrogen atoms bonded to carbon atoms have been omitted for the sake of clarity.

deprotonates at alkaline pH values (Log K_a = 9.9) and, similarly to quinoline, is protonated in acidic medium (pK_a = 5.1).[67] Interestingly enough, the protonation constant of 8-OH-Q is similar to the first one of cyclen, and, therefore, the formation of zwitterionic forms at alkaline pH values cannot be ruled out. Considering the ^{1}H NMR spectra recorded at different pH values, the most striking difference between the spectra recorded at pH 12.0 and 11.1, where the formation of the H**L2** occurs in solution, is the marked downfield shift of the singlet of the methylene bridge 1_{AL} (Figure 3). Minor shifts affect the other aliphatic signals, while only the resonance of the aromatic proton in 3 position is affected by the formation of the species. These results suggest that the first H^+ ion is bound by the macrocyclic moiety, although the quinoline nitrogen is probably also involved in proton binding, as observed in the case of **L1** and **L4**. As a result, H**L2** is in zwitterionic form, the hydroxyl group being deprotonated. Further pH decrease, up to pH 9.0 induces marked changes in the chemical shift of all the aromatic protons, to indicate that the formation of the H_2**L2**$^+$ species implies protonation of the phenolate group of 8-OH-Q. Binding of the third H^+ ion at neutral pH gives rise to an upfield shift of the aliphatic signals of the cyclen moiety, accompanied by line broadening. Minor spectral changes are observed in the aromatic sub-spectrum. As in the case of **L4**, in H_3**L2**$^{2+}$ two H^+ are likely to be shared between the aliphatic amine groups of cyclen. More interestingly the spectra recorded below pH 3 show a remarkable upfield shift of the 1_{AL} signal of the methylene bridge, a sharpening of the other aliphatic resonances and an upfield shift of the aromatic signals. As already suggested for the highly charged species of **L1** and **L4**, in H_4**L2**$^{3+}$ the quinoline nitrogen is protonated, while the remaining couple of H^+ ions are localized on the secondary amine groups, thus minimizing the electrostatic repulsion between the positive charge and 'freezing' the structure of the polyammonium cation.

H**L3** suffers of low solubility in the concentrations used for the NMR experiments in D_2O ($5x10^{-3}$-$1x10^{-2}$ M), from neutral to alkaline pH range, limiting the use of this technique in this study. However, an upfield shift of the resonances of the methylene groups linking the fluorophores to the cyclen units is clearly observed from pH 5 to 2, accompanied by shifts of the signals of the heteroaromatic units. Most likely, the last protonation step occurs on the two nitrogen of the quinoline side arms, analogously to that observed in the case of **L4**.

Differently from the other receptors, H_2**L5** contains two types of functionalities, appended to the cyclen unit, with remarkably different chemical properties. Furthermore, it can form a doubly negatively charged species (**L5**$^{2-}$). In this case, the two first protonation equilibria are not singularly detected in our potentiometric titrations and only the overall constant relative to the addition of two H^+ ions to the **L5**$^{2-}$ anions can be calculated, as normally found in the case of two protonation equilibria occurring in very similar pH ranges. The ^{1}H and, at a lower extent, the ^{13}C NMR spectra are affected by fluxionality in the aliphatic region, making the signal attribution difficult (Figure 4). Despite the broadness affecting the signals, upfield shifts of the resonances in the aliphatic region of the ^{1}H NMR spectra are observed by decreasing pH from 11.5 to 9.5, while the aromatic signals are almost not sensitive to ligand protonation. Therefore, the two first protonation steps likely occur on the aliphatic macrocyclic ring and originate a H_2**L5** zwitterion, while the Q moieties seem to be not involved in H^+ binding. Further decrease of pH does not change both the ^{1}H and ^{13}C NMR spectral feature of the molecule up to slight acidic values, where the H_3**L5**$^+$ and H_4**L5**$^{2+}$ species are formed in

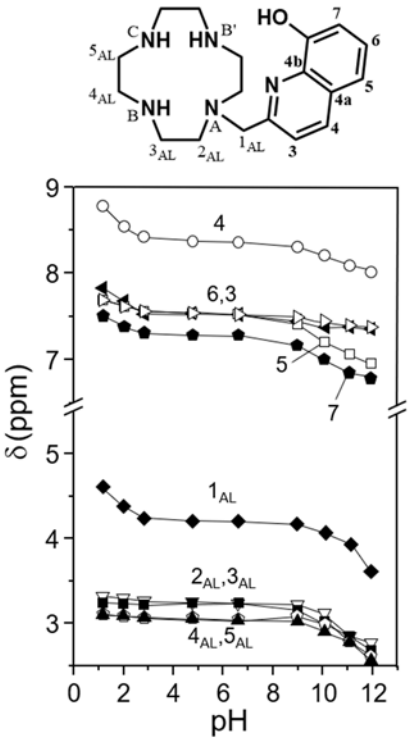


Fig.3. Plot of the ^{1}H NMR chemical shifts of H**L2** as a function of pH.

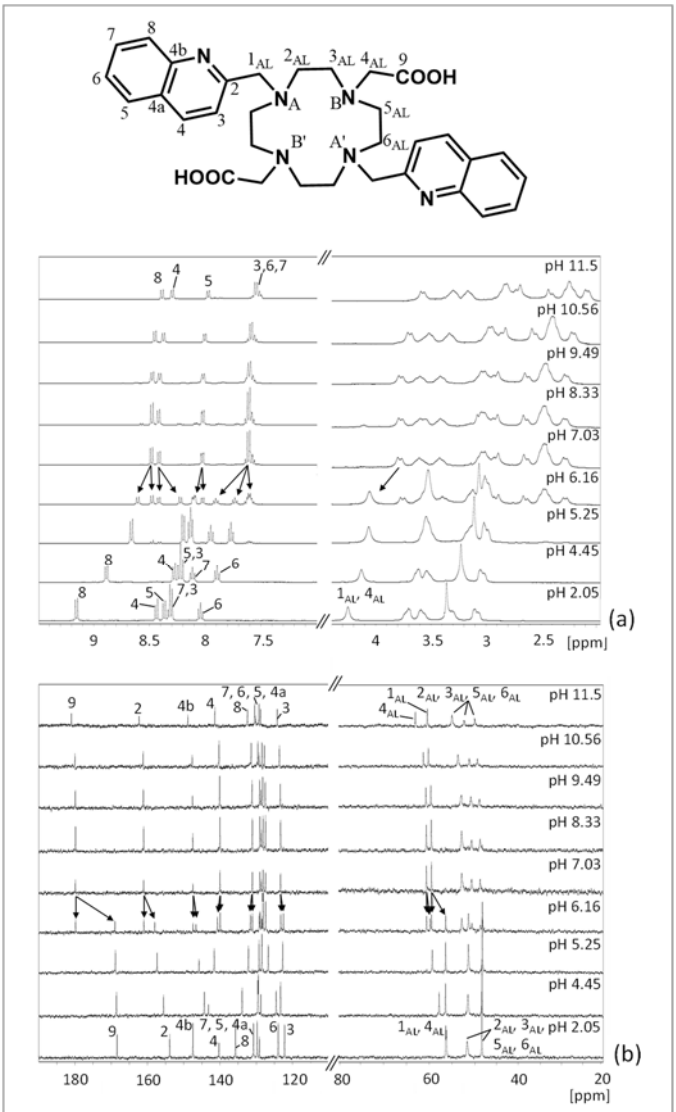


Fig. 4. ^{1}H (a) and ^{13}C (b) NMR spectra of H_2**L5** at different pH values.

solution. Surprisingly, the ^{13}C NMR spectra recorded at slightly acidic pH values (pH 6.23 in Figure 4) show the appearance of a second set of signals, indicating the presence in solution of two species/conformers slowing exchanging on the NMR time scale. Interestingly enough, while the ^{13}C NMR spectra recorded at alkaline pH values display the typical peak of the carboxylate group at 180 (add exact value) ppm, the new sub-spectrum arising at pH 6.23 features the typical signal of the carboxylic acid carbon at 170 ppm, indicating that the formation of the H_3**L5**$^{+}$ and H_4**L5**$^{2+}$ species in slightly acidic aqueous solution implies protonation of the carboxylate groups. Further decrease of pH leads to complete disappearance of the ^{1}H and ^{13}C resonances attributable to the H_2**L5** form (see the spectra recorded at pH 5.25). Below pH 5.25, remarkable shifts affect the aromatic ^{13}C and ^{1}H spectral zones and the signal of the methylene group linking the heteroaromatic units to cyclen, in accord with protonation of the Q nitrogens with the formation of the [H_5**L5**]$^{3+}$ and [H_6**L5**]$^{4+}$.

pH dependence of the UV-vis absorption and fluorescence emission spectra

The presence of the cyclen moiety and the ability of its amine groups to interact with hydrogen bonding acceptor sites strongly affect the photophysical properties of both Q and 8-OH-Q fluorophores.

The absorption spectrum of Q in aqueous solution presents a structured band at 313 and a broad band at 277 nm.[68,69] The formation of the quinolinium cation changes these spectral features with the disappearance of the 277 nm absorption and a marked increase of the absorbance at 313 nm. The absorption spectra of **L1** and **L4** parallel these spectral features. In fact, both ligands display the typical structured band of quinoline featuring two peaks at 316 and 303 nm and a band at lower energy centred at 275 nm. As shown in Figure 5a for **L4** (see Supporting Information for **L1**), a slight decrease of both absorption bands is observed between pH 12 and 7, where protonation of the cyclen units occur in solution, probably due to the formation of hydrogen bonds with the ammonium groups of cyclen, as previously hypothesized on the basis of the ^{1}H NMR measurements, and/or to conformational changes upon cyclen protonation. Conversely, marked changes in the spectra occur below pH 4, with a marked increase of the absorbance at 316 nm and the disappearance of the band at 275 nm, as a consequence of protonation of Q with the formation of the [H_4**L4**]$^{4+}$species (Figure 5b for **L4**, Figure S20 for **L1**), in good agreement with the result obtained from the ^{1}H NMR titration. Considering the fluorescence emission, Q features a band at 332 nm, while the quinolinium ion (QH^{+}) presents an emission band red-shifted at 410 nm.[68] Quinoline is also a known photo-base, featuring an increased basicity in the excited state. **L1** and **L4** are almost non emissive at strongly alkaline pH values (Figure 5c,d), where the polyamine ring is not protonated and, therefore, the emission is likely to be inhibited by a PET effect from the aliphatic amines, in particular the tertiary amine groups adjacent to the Q unit, to the excited fluorophore. Below pH 9.5, the system presents a clear increase of the emission at 391 nm up to pH 4. A shoulder at ca 340 nm, which tends to disappear as the pH decreases, can be also noted. The emission at 391 nm strongly resembles that of the quinolinium (QH^{+}), occurring at far lower energy than that of Q. Conversely the shoulder at 330 nm is likely to be due to quinoline, in equilibrium in solution with its protonated form and, therefore, tends to disappear below pH 7. The observed emission at 391 nm, occurs at somewhat lower wavelength than that reported for the QH^{+} ion, suggesting that, in the present case, the protonated heteroaromatic nitrogen shares the H^{+} ion *via* hydrogen bonding with the adjacent tertiary amines of the macrocycle. This would also inhibit PET effect from the tertiary amine to the fluorophore, justifying the observed emission intensity increase at pH < 9.5. However, while in the fundamental state the acidic protons in the less protonated forms of the receptor are mainly localized on the aliphatic amines, a PPT process would occur in the excited state, translating the H^{+} ion on the quinoline nitrogens, as schematically sketched in Scheme 2. Further decrease of pH

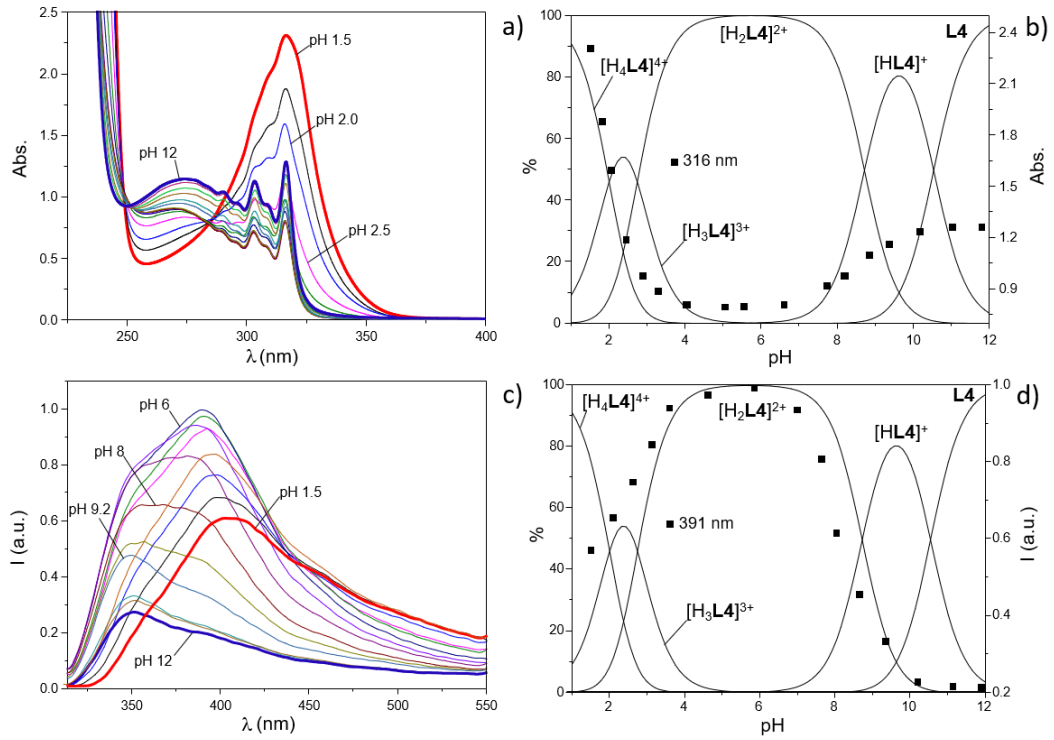
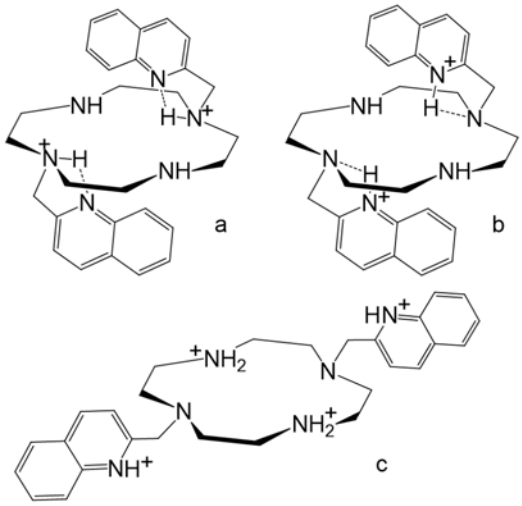


Fig. 5. (a) Absorption spectra of aqueous solutions of **L4** at different pH values and (b) absorbance values at 316 nm (black squares) superimposed to the distribution diagram of the species present in solution ([**L4**] = 5 x 10^{-4} M). (c) Fluorescence spectra of the ligand at different pH values and (d) plot of the fluorescence emission at 391 nm (black squares) superimposed to the distribution diagram of the species present in solution ([**L4**] = 2.5 x 10^{-5} M, λ_{exc} = 295 nm).

leads to a shift of the fluorescence emission up to 410 nm, the typical emission band of QH^+ and simultaneous emission quenching. On the basis of this experimental observation, we propose that the further protonation steps would occur on the secondary amine groups of the macrocycle. Analogously to the fundamental state, the receptor would assume an overall 'open' conformation to minimize the electrostatic repulsions between positive charges, in which tertiary aliphatic amine will be no more involved in H^+ binding and, therefore, available to quench the emission of the quinolinium ion. A similar behaviour has also been observed for **L1**. In this case, however, the increase of the emission at *ca* 400 nm occurs from neutral to slightly acidic pH values (see Figure S26).

The addition of two acetate moieties as appended functional groups on the macrocyclic ring strongly modifies the emission properties of the receptor. The pH dependence of UV-vis spectrum of H_2**L5** strongly resembles that of **L1** and **L4**, with a marked increase of the absorbance at 313 nm below pH 4, accounting for protonation of the quinoline nitrogens with the formation of the $[H_5\mathbf{L5}]^{3+}$ and $[H_6\mathbf{L5}]^{4+}$ species (Figures 6a,b). Differently from **L1** and **L4**, the fluorescence emission spectra at alkaline pH values feature a structured band at 350 nm, attributable to not protonated quinoline, while the red shifted band of quinolinium appears only in the acidic region (Figure 6c). Therefore, in this case the first protonation steps in the excited state seem to occur on the polyamine chain rather than on the Q fluorophores. The different behaviour from **L1** and **L4** can be related to the presence of two acetate groups, which can favour protonation of the cyclen moiety thanks to the formation of stabilizing hydrogen bond network involving protonated amines and anionic carboxylate functions, as often observed in polyamino-polycarboxylate receptors.[64,70] As a consequence, the PPT process observed in the first protonation steps of **L1** and **L4** would be inhibited in the case of H_2**L5** by the presence of the two anionic functionalities. By decreasing pH, the quinoline



Scheme 2. Sketch of the distribution of the H^+ ions in the fundamental (a) and excited (b) state of H_2**L4** and in H_4**L4** (c, both fundamental and excited state).

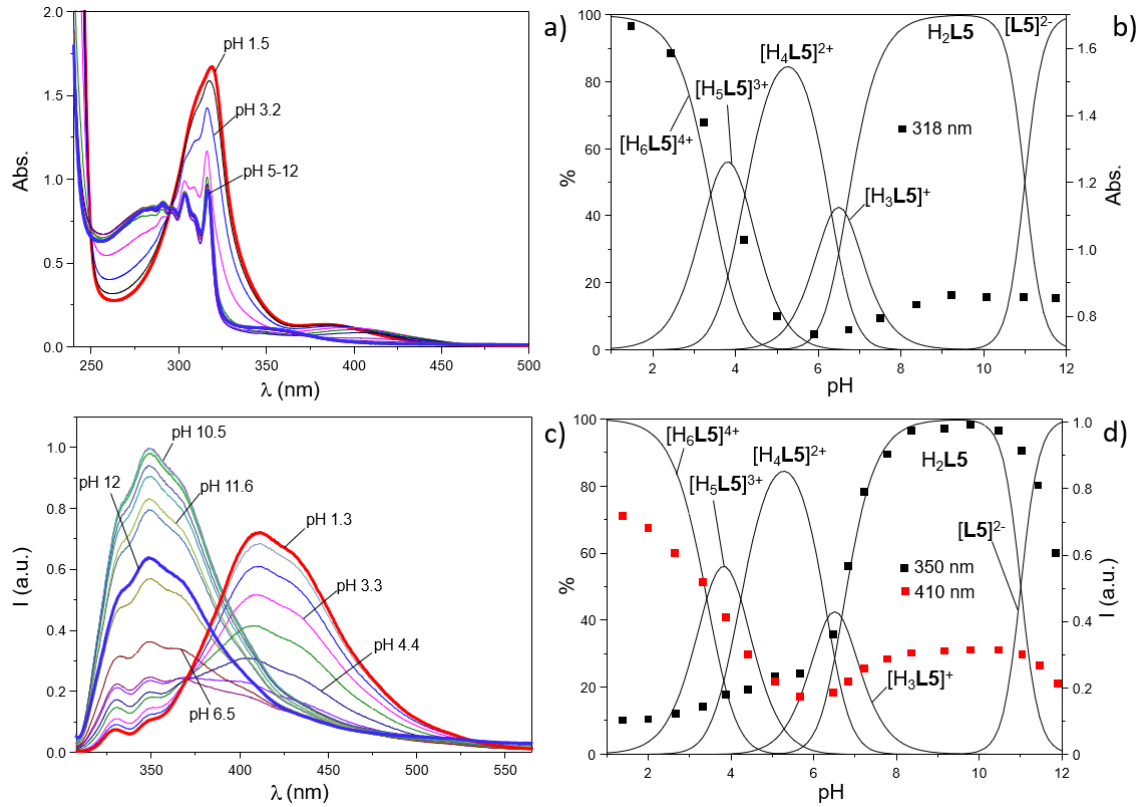


Fig. 6. (a) Absorption spectra of aqueous solutions of H_2**L5** at different pH values and (b) absorbance values at 316 nm (black squares) superimposed to the distribution diagram of the species present in solution ([H_2**L5**] = 5.5 x 10^{-4} M). (c) Fluorescence spectra of the ligand at different pH values and (d) plot of the fluorescence emission at 350 nm (black squares) and 410 nm (red squares) superimposed to the distribution diagram of the species present in solution ([H_2**L5**] = 2.5 x 10^{-5} M, λ_{exc} = 295 nm).

emission band disappears and, below pH 5, a band at 410 nm, attributable to quinolinium appears in the spectra, accounting for protonation of the Q moieties, which occurs in the case of H_2**L5** only at acidic pH values. Compared to **L1** and **L4**, the photobasicity of Q is therefore strongly reduced, probably due to the electrostatic repulsion between the QH^+ and the H^+ ions gathered on the macrocyclic framework.

H**L2** and H**L3** contains an 8-hydroxyquinoline (8-OH-Q) unit, together with a simple quinoline in the case of H**L3**. The photophysical properties of 8-OH-Q are more complex than those of Q. 8-OH-Q can be protonated at acidic pH values (pK_a = 5.1), but also contains a weakly basic hydroxyl function (pK_a = 9.9), which deprotonates in alkaline media.[61,67,71] Furthermore, it can generate a zwitterionic form, $8\text{-}O^-\text{-}QH^+$. 8-OH-Q is mainly in its non-zwitterionic form in water at neutral pH, which features a strong absorption band at 303 nm at neutral pH in aqueous solution. A minor amount of its tautomeric form give rise to an extremely weak band at 440 nm. Protonation of 8-OH-Q induces the appearance of a new band at 358 nm, while deprotonation of 8-OH-Q leads to the disappearance of the 440 nm absorption band and to the formation of a broad band with maximum at 354 nm. 8-OH-Q is poorly luminescent at neutral pH, due to an intermolecular PPT process between the hydroxyl group (which is a relatively strong photoacid) and the nearby quinoline nitrogen (which is, in turn, a strong photobase).[61,67,71,72] In addition, in protic media, intermolecular PPT processes involving solvent molecules can also occur, further decreasing the fluorescence quantum yield of this kind of fluorophore. However, a broad band centered at 432 nm can be observed and it has been attributed to the presence in solution of minor percentages of the non-zwitterionic form in solution.[67] The 8-OH-Q emission is red-shifted upon protonation (ca 60 nm) or deprotonation (more than 100 nm). However, the presence of a polyamine moiety linked to heteroaromatic nucleus can modify both the acid-base behaviour of 8-OH-Q and its photophysical properties as well.

As shown in Figure 7a for H**L2**, the absorption spectra recorded from pH 12 to pH 10 present a single broad band centered at ca 350 nm, which is attributed to the deprotonated 8-OH-Q moiety. By decreasing pH, this band progressively disappears, while the formation of a single band at ca 300 nm, attributed to the neutral (non-zwitterionic) form of 8-OH-Q, can be observed. These spectral changes, due to protonation of the hydroxyl group of 8-OH-Q occur with the formation of the $[H_2\mathbf{L2}]^+$ and $[H_3\mathbf{L2}]^{2+}$ protonated species, in good agreement with the results obtained from the ^{1}H NMR titration. The spectra do not undergo further changes up to pH 3, when the absorbance at 300 nm starts increasing and red-shifting, and, overall, the formation of a shoulder at ca 360 nm can be observed in the spectra, in agreement with protonation of the 8-OH-Q nitrogen at strongly acidic pH values, in good agreement, once again, with the results obtained from ^{1}H NMR measurements. Interestingly

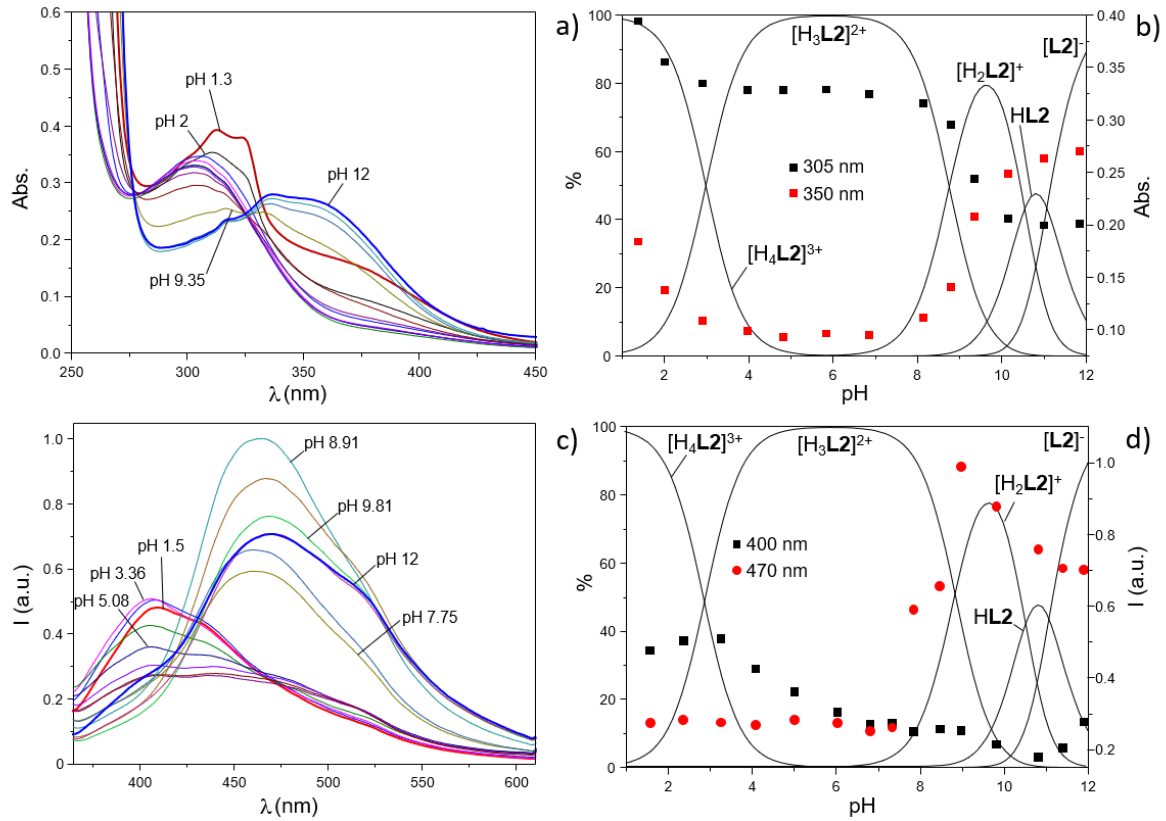
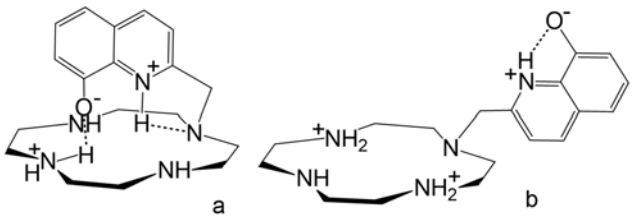


Fig. 7. (a) Absorption spectra of aqueous solutions of H**L2** at different pH values and (b) absorbance values at 305 nm (black squares) and 350 nm (red squares) superimposed to the distribution diagram of the species present in solution ([H**L2**] = 1.12 x 10^{-4} M). (c) Fluorescence spectra of the ligand at different pH values and (d) plot of the fluorescence emission at 400 nm (black squares) and 470 nm (red circles) superimposed to the distribution diagram of the species present in solution ([H**L2**] = 2.5 x 10^{-5} M, λ_{exc} = 320 nm).

enough, the heteroaromatic nitrogen seems to display lower affinity for acidic protons than that observed for the quinoline nitrogens of **L1** and **L2**. This fact can be related to the possible formation of an hydrogen bonding interaction between the heteroaromatic nitrogen and the OH group, as normally proposed for 8-OH-Q alone, making the nitrogen of 8-OH-Q less available for proton binding.

The system displays a different protonation behaviour in the excited state. At pH 12 H**L2** presents an emission band with a maximum at 470 nm with a shoulder at ca 530 nm (Figure 7c). By decreasing pH from 12 to 8.91 the emission at 470 nm increases, while the shoulder disappears. While the shoulder observed at the most higher pH values can be attributed to the presence in solution of minor amounts of the anionic form of 8-OH-Q, the band at 470 nm strongly resembles that observed for its cationic form. This appears in contrast with the basicity properties of 8-OH-Q alone in the excited state. In this case, in fact, the cationic form is observed only at strongly acidic pH values, where protonation of the phenolate oxygen occurs in solution. On the other hand, in **L2** 8-OH-Q is in close proximity with the cyclen moiety, which is, in turn, a rather strong base and can gather on its macrocyclic framework up two ammonium groups at pH *ca* 10. We can suggest that the anionic phenolate group of 8-OH-Q can interact via hydrogen bonding with the ammonium function(s) of the macrocycle. Simultaneously, an H^+ ion can be shared between the 8-OH-Q nitrogen and the adjacent tertiary nitrogen of the macrocycle. This proton distribution would explain the band at ca 470 nm observed at alkaline pH values.

Interestingly enough, pH decrease from 8.91 to 7 leads to quenching of this emission band and, below pH 7, to the appearance of a new band at 400 nm, which is the typical emission of neutral 8-OH-Q. As already observed for **L1** and **L4**, gathering of two or more protons on the macrocyclic framework can induce a proton redistribution among the nitrogen atoms to minimize the electrostatic repulsion between positive charges. This, together with an overall stiffening of the molecule, may lead to a more 'opened' structure in which the



Scheme 3. Sketches of the proposed structures of the $[H_2L2]^+$ and $[H_3L2]^{2+}$ species formed in the excited state.

8-OH-Q moiety cannot interact *via* hydrogen bonding with the macrocycle (Scheme 3), in which the 8-OH-Q moiety is in its zwitterionic form normally observed in this pH region for this heterocycle.

H**L3** is the most intriguing polyamine, due to the simultaneous presence of appended Q and 8-OH-Q units. The UV-vis spectrum recorded at pH 12 shows a band at ca 340 nm, attributable to the deprotonated 8-OH-Q and a more intense structure band at 320 nm, due to the superimposed absorption from both the heteroaromatic units (Figure S27). pH decrease up to pH 8 induces disappearance of the band at 340 nm, as expected considering the formation of the 8-OH-Q neutral form, and a decrease of the absorption at 320. Further pH decrease does not change the spectra up to pH 4. At lower pH values, the increase of the 320 nm absorbance indicates protonation of the Q moiety. Considering the fluorescence emission, This receptor is basically not emissive all over the pH range investigated. This behaviour can be tentatively ascribed to energy and/or proton transfer process between the two heteroaromatic units, which can enable non-radiative decay processes.

Experimental

L4 and H_2**L5** were synthesized as previously reported.[62] **1** was obtained by using the procedure described in ref. 63.

Synthesis of L1, HL2 and HL3

*4a-methylen-2-quinolyn-dodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene monochloride salt (**4**·Cl).* A solution of 2-(chloromethyl)quinoline (**2**, 1.6 g, 9 mmol) in THF (10 mL) was added dropwise to a stirred solution of **1** (1.6 g, 8.24 mmol) in THF (10 mL). The solution was then stirred at room temperature for six hours. The yellowish precipitate formed was filtered and washed with THF, obtaining **4**·Cl, which was used without further purification**.**

(Yield: 2.53 g, 6.80 mmol) Anal. calcd. for $C_{20}H_{26}N_5Cl$: C 64.59, N 18.83, H 7.05; found C 64.64, N 18.89, H 7.10; ^{1}H-NMR (400 MHz, MeOD): δ(ppm) 8.52 (d, 1H, J=8.44 Hz, H8), 8.16 (d, 1H, J=8.48 Hz, H4), 8.05 (d, 1H, J=8 Hz, H5), 7.88 (t, 1H, J=8.04 Hz, H7), 7.75-7.70 (m, 2H, H6, H3), 5.32 (d, 1H, J=14.2 Hz), 5.06 (d, 1H, J=14.2 Hz), 4.63-4.57 (m, 2H, 1_{AL}), 4.07-4.01 (m, 1H), 3.97-3.90 (m, 2H), 3.79-3.75 (m, 1H), 3.57-3.51 (m, 1H), 3.41-3.36 (m, 1H), 3.32-3.23 (m, 2H), 3.23-3.11 (m, 2H), 3.08-2.95 (m, 2H), 2.89-2.77 (m, 2H), 2.62-2.49 (m, 2H). ESI MS (m/z) 336.2186 (**4**$^+$)

*1-methylen-2-quinolyl-1,4,7,10-tetraazacyclododecane three-hydrobromide salt (**L1**·**3**HBr).* 1 g of **4**·Cl (2.69 mmol) is dissolved in 25 ml of N_2H_4 and 5 ml of EtOH and stirred at 110 ° C for 6 h. To the resulting mixture, cooled at room temperature, were added 15 ml of 5 M NaOH. The solution was extracted with chloroform (5x50 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduce pressure. The crude product was dissolved in ethanol. Concentrated (48%) HBr was added dropwise to obtain **L1**·3HBr as a yellowish solid.

Yield: 1.17 g, 2.1 mmol. Anal. calcd. for $C_{18}H_{30}N_5Br_3$: C 38.87, N 12.59, H 5.44; found C 38.98, N 12.61, H 5.49; ^{1}H NMR (MeOD, 400 MHz): δ(ppm) 9.09 (d, 1H, H8, J=8.5 MHz), 8.26 (d, 1H, H4, J=8.6 MHz), 8.23 (d, 1H, H5, J=9.1 MHz), 8.12 (t, 1H, H7, J=7.9 MHz), 8.08 (d, 1H, H3, J=8.6 MHz), 7.92 (t, 1H, H6, J=7.3 MHz), 4.38 (s, 2H, 1_{AL}), 3.33-3.31 (m, 4H), 3.17-3.16 (m, 4H), 3.03-2.98 (m, 8H). ^{13}C NMR (D_2O, pD < 2, 400 MHz): δ(ppm) 154.34, 148.38, 139.19, 136.12, 130.74, 129.81, 129.12, 123.36, 120.82, 55.60, 48.85, 45.09, 42.50, 42.12314.236. ESI MS (m/z) 314.236 (z = 1, [**L1** + H]$^+$); 157.620 (z = 2, [**L1** + 2H]$^{2+}$).

*4a-methylen-2-(8-hyrdoxyquinolyn)-dodecahydro-2a,4a,6a,8ª-tetraazacyclopenta[fg]acenaphthylene monochloride salt (**5**·Cl).* This product was obtained from 2-(chloromethyl)quinolin-8-ol (**3**, 850 mg, 4.39 mmol) and **1** (750 mg, 3.86 mmol) in THF (10mL) by using the same procedure reported for **4**.

Yield: 1.02 g, 2.63 mmol. Anal. calcd. for $C_{20}H_{26}N_5ClO$: C 61.93, N 18.05, H 6.76; found C 62.02, N 18.10, H 6.81; ^{1}H NMR (MeOD, 400 MHz): δ(ppm) 8.48 (d, 1H, J=8.44 Hz, H4), 7.77 (d, 1H, J=8.40 Hz, H3), 7.60 (t, 1H, J=8.16 Hz, H6), 7.51 (d, 1H, J=8 Hz, H5), 7.26 (d, 1H, J=7.9 Hz, H7), 5.27 (d, 1H, J=13.64 Hz), 5.01 (d, 1H, J=13.64 Hz), 4.81-4.71 (m, 2H, 1_{AL}), 3.94-3.74 (m, 2H), 3.57-3.48 (m, 1H), 3.43-3.35 (m, 1H), 3.32-3.16 (m, 4H), 3.08-2.95 (m, 2H), 2.88-2.76 (m, 2H), 2.65-2.51 (m, 2H).

*1-methylen-2-(8-hydroxyquinolyl-1,4,7,10-tetraazacyclododecane three-hydrobromide salt (**L2**·**3**HBr).* This compound was obtained by treatment of **5**·Cl (0.82 g, 2.11 mmol) with N_2H_4 as reported for **L1**·3HBr**.**

Yield: 0.89 g, 1.56 mmol. Anal. calcd. for $C_{18}H_{30}Br_3N_5O$: C 37.78, N 12.24, H 5.29; found C 37.82, N 12.28, H 5.31; ^{1}H NMR (D_2O, pD < 2, 400 MHz): δ(ppm) 8.99 (d, 1H, H4, J=8.7 MHz), 8.05 (d, 1H, H3, J=8.6 MHz), 7.71-7.69 (m, 2H, H5, H6), 7.48-7.42 (d, 1H, H7), 4.43 (s, 2H, 1_{AL}), 3.36-3.28 (m, 4H), 3.24-3.17 (m, 4H), 3.08-2.99 (m, 8H). ^{13}C NMR (D_2O, pD < 2, 400 MHz): δ(ppm) 153.94, 147.90, 147.80, 131.30, 130.30, 130.16, 123.62, 120.18, 117.72, 55.30, 49.06, 45.04, 42.61, 42.23; ESI MS (m/z) 330.227 (z = 1, [H**L2** + H]$^+$).

*4a-methylen-2-quinolyn-8a-methylen-2'(8-hydroxyquinolyn)-dodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene dichloride salt (**6**·2Cl).* A solution of 8-(hydroxy)-2-(chloromethyl)-quinoline (**3**, 0.6 g, 3.1 mmol) in CH_3CN (10 mL) was added dropwise to a stirred solution of **4**·Cl (818 mg, 2.2 mmol) in CH_3CN (10 mL). The yellowish precipitate formed was filtered off, affording the bis-derivatized bisaminal, which was used without further purification.

Yield 0.81 g, 1.43 mmol. Anal. calcd. for $C_{30}H_{34}Cl_2N_6O$: C 63.71, N 14.86, H 6.06; found C 63.75, N 14.92, H 6.11; ^{1}H NMR (MeOD, 400 MHz): δ(ppm) 8.53 (m, 2H, J=9.6 Hz, H8(Q),H7(Q)), 8.17 (d, 1H, J=8.52 Hz, H4(HQ)), 8.06 (d, 1H, J=8.28 Hz, H4(Q)), 7.88 (d, 1H, J=7.12 Hz, H5(Q)), 7.84 (d, 1H, J=8.44 Hz, H3(HQ)), 7.78 (d, 1H, J=8.36 Hz, H3(Q)), 7.74 (t, 1H, J=7.32 Hz, H6(HQ)), 7.60 (t, 1H, J=8.04 Hz, H6(Q)), 7.53 (d, 1H, J=7.4 Hz, H5 (HQ)), 7.26 (d, 1H, J=7.4 Hz, H7(HQ)), 5.37 (d, 1H, J=14.16 Hz), 5.34 (m, 3H), 5.15 (s, 2H), 4.64-4.55 (m, 2H), 4.19-3.96 (m, 6H), 3.71-3.59 (m, 2H), 3.55-3.41 (m, 4H), 3.23-3.08 (m, 2H).

*1-methylen-2-(8-hydroxyquinolyl-7-methylen-2-quinoline-1,4,7,10-tetraazacyclododecane three-hydrobromide salt (**L3**·**3**HBr).* This compound was obtained by treatment of **6**·2Cl (0.70 g, 1.24 mmol) with N_2H_4 as reported for **L1**·3HBr**.** Yield 0.68 g, 0.95 mmol. Anal. calcd. for $C_{28}H_{39}Br_3N_6O$: C 47.01, N 11.75, H 5.50; found C 46.96, N 11.70, H 5.56; ^{1}H NMR (D_2O, pD < 2, 400 MHz): δ(ppm) 8.27 (d, 1H, J=8.6 Hz, H8(Q)), 7.89 (m, 3H, H7(Q), H4(HQ), H4(Q)), 7.77 (d, 1H, J=8.3 Hz, H3(HQ)), 7.68 (m, 2H, J=8.6 Hz, H6(HQ), H5(Q)), 7.42 (d, 1H, J=8.4 Hz, H5(HQ)), 7.37 (d, 1H, J=8.1 Hz, H3(Q)), 7.14 (t, 1H, J=7.90 Hz, H6(Q)), 6.70 (d, 1H, J=7.56 Hz, H7(HQ)), 4.36 (s, 2H), 4.28 (s, 2H), 3.50-3.26 (m 8H), 3.20-2.88 (m, 8H). ^{13}C NMR (D_2O, pD < 2, 400 MHz): δ(ppm) 157.62, 156.31, 151.35, 145.47, 139.70, 138.50, 137.45, 134.98, 130.40, 129.77, 129.32, 128.18, 127.59, 122.40, 121.89, 121.31, 120.98, 120.14, 113.17, 56.83, 55.98, 49.69, 49.49, 43.25, 43.11, ESI MS (m/z) 471.286 (z = 1, [H**L3** + H]$^+$); 236.1484 (z = 2, [H**L3** + 2H]$^{2+}$).

NMR measurements

NMR experiments were carried out by using on a Bruker Avance 400 MHz instrument. The pH was calculated from the measured pD value by using the relationship pH=pD–0.40.[75]

ESI-Mass spectrometry

Stock solutions 10^{-5} M of the compounds were prepared dissolving the samples in H_2O or MeOH. The ESI mass study was performed using a TripleTOF® 5600+ high-resolution mass spectrometer (Sciex, Framingham, MA, U.S.A.), equipped with a DuoSpray® interface operating with an ESI probe. Respective ESI mass spectra were acquired through direct infusion at 7 µL/min flow rate.

Electronic absorption and fluorescence measurements

Absorption and fluorescence spectra were registered on a Perkin-Elmer Lambda 6 spectrophotometer and on a Perkin-Elmer LS55 spectrofluorimeter, respectively. An excitation wavelength of 295 or 320 nm was used to record fluorescence spectra of ligands. All measurements were performed at 298.0 ± 0.1 K.

Potentiometric measurements

All pH measurements (pH = −log [H$^+$]) employed for the determination of ligand protonation constants were carried out in 0.10 M NMe_4Cl aqueous solution at 298.1 ± 0.1 K by conventional titration experiments under inert atmosphere. The equipment and procedure used were as previously described.[73] The standard potential $E°$ and the ionic product of water (pK_w = 13.83(1) at 298.1 ± 0.1 K in 0.10 M NMe_4Cl) were determined by Gran's method.[74] At least three measurements (with about 100 data points for each) were performed for each system in the pH ranges 2−10.5. In all experiments, ligand concentration [L] was about 0.5×10^{-3} M. The computer program HYPERQUAD[75] was used to calculate both protonation and stability constants from potentiometric data. Distribution diagrams were plotted by using the Hyss program.[76]

Concluding remarks

The photophysical properties of receptors **L1** and **L4** are strongly influenced by the changes in molecular conformation upon successive proton binding. Basically, in **L1**-**L4** tertiary nitrogen of cyclen and the adjacent heteroaromatic nitrogen represent a rather strongly basic site, thanks to the formation of a stabilizing hydrogen bond between the protonated nitrogen and the adjacent basic site. This leads to an overall 'closed' molecular conformation, at least in the less protonated forms of the receptors, which can favour PPT processes from aliphatic ammonium groups to the basic sites of the fluorophores. This greatly affects the emission properties, while minor changes are observed in the absorption spectra. In the case of **L1**-**L4**, the system is basically quenched or poorly emissive at strongly alkaline pH values. Conversely, at slightly alkaline pH values, where the first protonation steps occur in solution, the spectra of **L1**-**L4** feature emission bands that resemble those of the protonated forms of Q and 8-OH-Q, likely due to the formation of stabilizing hydrogen bonding interactions between the protonated heteroaromatic nitrogen and the tertiary amine of cyclen. Partial localization of positive charge on the tertiary nitrogens also inhibits possible PET processes from the tertiary amine to the fluorophore, justifying the observed emission. In the case of H**L2**, hydrogen bonding interactions between the protonated macrocycle and the phenolate group can also favour a PPT process from an aliphatic NH^+ group and the phenolate oxygen. In both cases, these hydrogen bonding interaction seem to be lost at acidic pH values, where further protonation of the receptors leads to a more 'open' molecular conformation, in which the acidic protons are localized on the heteroaromatic nitrogen and the secondary amine groups to minimize electrostatic repulsions between protonated nitrogens. Therefore, **L1** and **L4** display the typical emission of the QH^+ ion, while **L2** shows the typical band of neutral 8-OH-Q, which are, in turn, progressively quenched at strongly acidic pH values by a PET process from the not-protonated tertiary amine(s) to the excited fluorophore. The NH^+---N interactions are also lost in H_2**L5**. In this case, however, this occurs at alkaline pH values, where the ability of the carboxylate groups to act as hydrogen bonding acceptor sites make the adjacent tertiary nitrogen the preferred protonation site in the less protonated forms of the receptor. Therefore, at alkaline pH values, H_2**L5** presents the typical emission band of Q, while and the emission of the QH^+ ion is observed only at acidic pH values, as a consequence of protonation of the Q nitrogen.

Conflicts of interest

There are no conflicts of interest to declare.

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