New family of polyamine macrocycles containing 2,5-diphenyl[1,3,4]oxadiazole as a signaling unit. Synthesis, acid–base and spectrophotometric properties†

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Synthesis and acid–base properties for three fluorescent polyamine macrocycles 9,12,15,24,25-pentaaza-26-oxatetracyclo[21.2.1.0^{2,7}.0^{17,22}]hexaicosa-2,4,6,17,19,21,23,25^{1}-octaene (**L1**), 9,12,15,18,27,28-hexaaza-29-oxatetracyclo[24.2.1.0^{2,7}.0^{20,25}]enneicosa-2,4,6,20,22,24,26,28^{1}-octaene (**L2**) and 9,12,15,18,21,30,31-heptaaza-32-oxatetracyclo[27.2.1.0^{2,7}.0^{23,28}]diatriconta-2,4,6,23,25,27,29,31^{1}-octaene (**L3**) are reported. Each ligand contains the 2,5-diphenyl[1,3,4]oxadiazole (PPD) unit incorporated in the polyamine macrocycle. The protonation constants of **L1–L3** were determined by means of potentiometric measurements in 0.15 mol dm^{-3} NaCl aqueous solution at 298.1 K. All the ligands are highly fluorescent in aqueous solution under acidic conditions (pH < 2) and their emission drastically decreases when the pH is increased. At pH > 8, a total quenching of fluorescence is observable in all the ligands. The fluorescence is given by the PPD unit, while the behavior as a function of pH can be rationalized on the basis of photoinduced intramolecular electron transfer (PET) from the HOMO of the donor macrocycle nitrogen atoms to the excited fluorophore unit. The insertion of PPD in a polyamine skeleton strongly improves the fluorescence quantum yield of this class of ligands with respect to those already known.

Introduction

In recent years, efforts have been devoted to the synthesis and characterization of ligands able to selectively bind to a specific metal ion undergoing a concomitant spectroscopic response, such as color change, variation in fluorescence both in emission wavelength and intensity, which are important in the host–guest chemistry. An important class of ligands is the macrocyclic molecules, which are more selective than open-chain ones and usually form more stable complexes.[1] The presence of a photosensitive group, such as a chromophore or a fluorophore, makes such compounds suitable for use as simple optical sensors.[2–4]

Polyamine macrocycles can be excellent building blocks for use in molecular recognition studies of several kinds of differently charged substrates.[5] For this reason, one of the synthetic strategies has been the insertion of one or more photoactive groups in the polyamine skeleton with the aim to exploit the photochemical properties of the inserted group preserving those of the polyamines. In this way, several aza-macrocycles incorporating phenolic,[6] biphenolic,[7] and various N- and O-based heteroaromatic functions,[8] have been synthesized in our group and studied as potential sensors.

In this paper, we report the synthesis and a complete study on the acid–base behavior of a new family of three fluorescent polyaza-macrocycles suitable for use as photoactive chemosensors: 9,12,15,24,25-pentaaza-26-oxatetracyclo[21.2.1.0^{2,7}.0^{17,22}]hexaicosa-2,4,6,17,19,21,23,25^{1}-octaene (**L1**), 9,12,15,18,27,28-hexaaza-29-oxatetracyclo[24.2.1.0^{2,7}.0^{20,25}]enneicosa-2,4,6,20,22,24,26,28^{1}-octaene (**L2**) and 9,12,15,18,21,30,31-heptaaza-32-oxatetracyclo[27.2.1.0^{2,7}.0^{23,28}]diatriconta-2,4,6,23,25,27,29,31^{1}-octaene (**L3**) (Chart 1). Each ligand shows the 2,5-diphenyl[1,3,4]oxadiazole (PPD) group as a photoactive moiety, which is inserted in a polyamine macrocyclic skeleton. The choice of PPD was based on its photochemical properties, as it is a well known fluorophore belonging to 1,3,4-oxadiazole derivatives, one of the most widely studied classes of electron-injection/hole-blocking materials due to their electron deficiency, high photoluminescence quantum yield, and good thermal and chemical stabilities.[9] Most fluorescent ligands reported in the literature with an aromatic group inserted in a polyamine skeleton exhibit medium or low fluorescence quantum yield; the introduction of PPD improves the photochemical performances

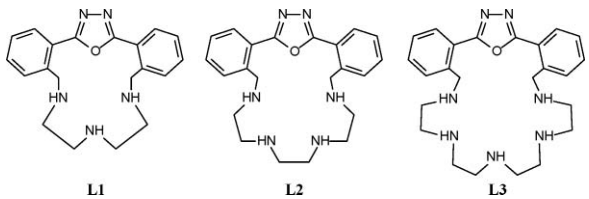

Chart 1 The ligands

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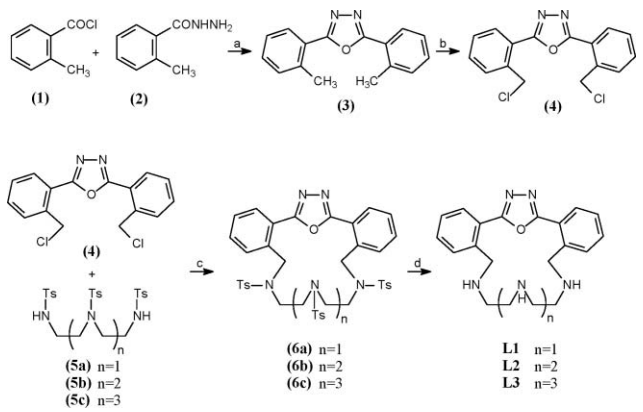
of this class of ligands making them also suitable for use under high-dilution conditions.

Results and discussion

Synthesis

The synthetic pathway used to obtain the ligands **L1–L3** is depicted in Scheme 1. The heteroaromatic scaffold 2,5-bis[2-(chloromethyl)phenyl][1,3,4]oxadiazole (**4**) was synthesized by chlorination with SO_2Cl_2 of its precursor 2,5-bis(2-methylphenyl)[1,3,4]oxadiazole (**3**), obtained following the procedure reported by Mashraqui and co-workers.[10] The reaction used to obtain the tosylated macrocycles **6a**, **6b** and **6c**, a modification of the Richman–Atkins method,[11] involves cyclization of the polytosylated polyamines **5a**, **5b** and **5c**, respectively, with 1 equivalent of 2,5-bis[2-(chloromethyl)phenyl][1,3,4]oxadiazole (**4**), in the presence of an alkaline carbonate base. The final compounds **6a**, **6b** and **6c** were obtained using a 1 + 1 cyclization scheme and purified from the crude products by flash chromatography. Finally, the desired ligands **L1–L3** were obtained from the cleavage of the tosyl groups by using hydrobromic acid in acetic acid. All the compounds were further purified as hydrobromide salts by recrystallization from cold water–48% aqueous HBr mixture.



Scheme 1 Synthesis of the ligands. Conditions: (**a**) i. dioxane, 100 °C, 2 h, ii. $POCl_3$, 100 °C, 3 h; (**b**) SO_2Cl_2, benzoyl peroxide, chlorobenzene, 90 °C, 2 h; (**c**) K_2CO_3, DMF, 90 °C, 2 h; (**d**) 33% HBr–CH_3COOH, 90 °C, 24 h.

Acid–base behavior

Potentiometric studies. Table 1 summarizes the basicity constants of **L1–L3** potentiometrically determined in 0.15 mol dm^{-3} NaCl aqueous solution, at 298.1 K, monitoring the 2–12 pH-range. Under these experimental conditions, all the ligands can be fully protonated, reaching the cationic species with H_kL^{k+} stoichiometry, where k is the number of secondary amine groups present in the macrocycle. Examining the stepwise protonation constants, it appears evident that the number of constants shown by each ligand coincides with the numbers of secondary amine functions, thus suggesting that the protonation probably takes place at these functions. In particular, **L1** shows one relatively high basicity constant (log $K_1 = 9.56$), one of intermediate and one of much lower value (log $K_2 = 7.10$, log $K_3 = 2.90$), **L2** shows two relatively high and similar basicity constants (log $K_1 = 9.19$, log $K_2 = 8.96$), one of intermediate and one of much lower value (log $K_3 = 6.11$, log $K_4 = 2.48$), while **L3** shows two relatively high basicity constants (log $K_1 = 9.30$, log $K_2 = 9.18$), one of intermediate (log $K_3 = 7.23$) and two of lower values for the last two protonation steps (log $K_4 = 3.37$, log $K_5 = 2.15$) (see Table 1). In all the ligands, the trend of the stepwise basicity constants follows the general criterion of the minimum electrostatic repulsion between charges of the same type; this is generally observed in the protonation of polyamines containing only ethylene chains, and these protonation constants are in agreement with the corresponding ones for the polyaza-macrocycles of the polyaza-cyclophane series with analogous sizes[12a] also containing heteroaromatic groups, such as phenantroline,[12b] bipyridyl[12c] and terpyridyl,[12d] suggesting that the PPD aromatic moiety should not be directly involved in protonation in the pH range investigated.

Table 1 Protonation constants (log K) of ligands **L1**, **L2** and **L3** potentiometrically determined in 0.15 mol dm^{-3} NaCl aqueous solution at 298.1 K

	log K		
Reaction	**L1**	**L2**	**L3**
$L + H^+ = HL^+$	9.56(2)[a]	9.19(1)	9.30(1)
$HL^+ + H^+ = H_2L^{2+}$	7.10(4)	8.96(2)	9.18(2)
$H_2L^{2+} + H^+ = H_3L^{3+}$	2.90(4)	6.11(4)	7.23(2)
$H_3L^{3+} + H^+ = H_4L^{4+}$	—	2.48(4)	3.37(3)
$H_4L^{4+} + H^+ = H_5L^{5+}$	—	—	2.15(4)

[a] Values in parentheses are the standard deviations on the last significant figure.

UV-Vis and fluorescence studies. UV-Vis absorption and fluorescence electronic spectra were performed at different pH values to obtain further information about the role of PPD in the acid–base behavior of the ligands. At pH = 12, all ligands exhibit an absorption band with λ_{max} at 267 nm ($\varepsilon = 12\,300$ $cm^{-1}mol^{-1}dm^3$) for **L1**, and 275 nm for **L2** ($\varepsilon = 14\,000$ $cm^{-1}mol^{-1}dm^3$) and **L3** ($\varepsilon = 13\,000$ $cm^{-1}mol^{-1}dm^3$); by lowering the pH, a red shift of λ_{max} can be observed together with an increase in absorptivity. At pH = 2 the λ_{max} is 283, 280 and 278 nm for **L1**, **L2** and **L3**, respectively (see Fig. 1a, 2a and 3a), and, as observable, the shift is more marked for **L1** than for **L2** and **L3**. Taking into account that the absorption and fluorescence spectra of free PPD do not change in the range of pH (2–12) examined ($\lambda_{max} = 282$ nm, $\lambda_{em} = 341$ nm), and that the PPD undergoes protonation only under strongly acid conditions,[13] we can suppose that the λ_{max} change observed for the ligands was not due to the protonation of PPD but most likely to an interaction *via* H-bonding between the PPD group and the protonated secondary amines of the macrocycles, as shown for other similar ligands containing aza-heteroaromatic moieties inserted in the polyaza skeleton, reported in the literature;[12,14] this interaction depends on the degree of protonation as well as on the size of the macrocycle.

Previous experimental and theoretical studies highlighted that the dipole moment of the PPD moiety increases almost two-fold on excitation, suggesting that the excited state is twisted intramolecular charge transfer (TICT).[15] Upon excitation, the electronic density increases on the oxygen and nitrogen atoms of the [1,3,4]oxadiazole ring, thus raising its proton affinity with

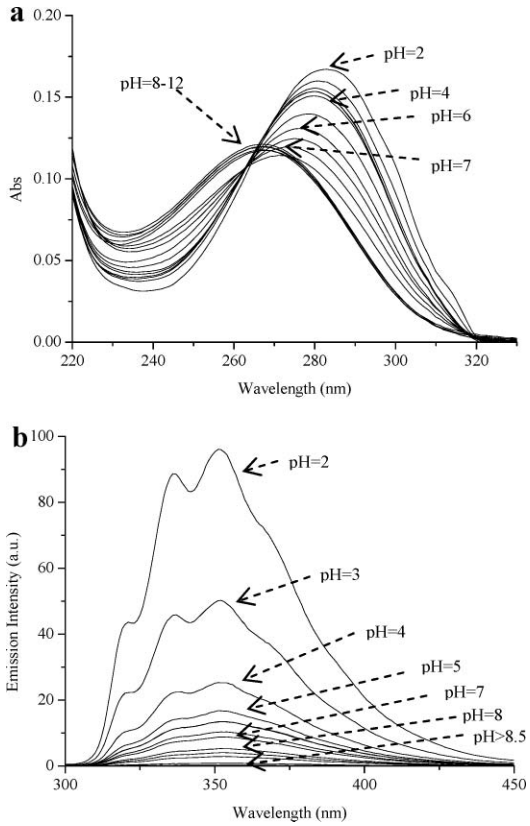


Fig. 1 (**a**) UV-Vis absorption and (**b**) fluorescence emission ($\lambda_{ex} = 265$ nm) spectra of **L1** registered at various pH values. Experimental conditions: [**L1**] $= 1 \times 10^{-5}$ M, $I = 0.15$ M aqueous NaCl, $T = 298.1$ K.

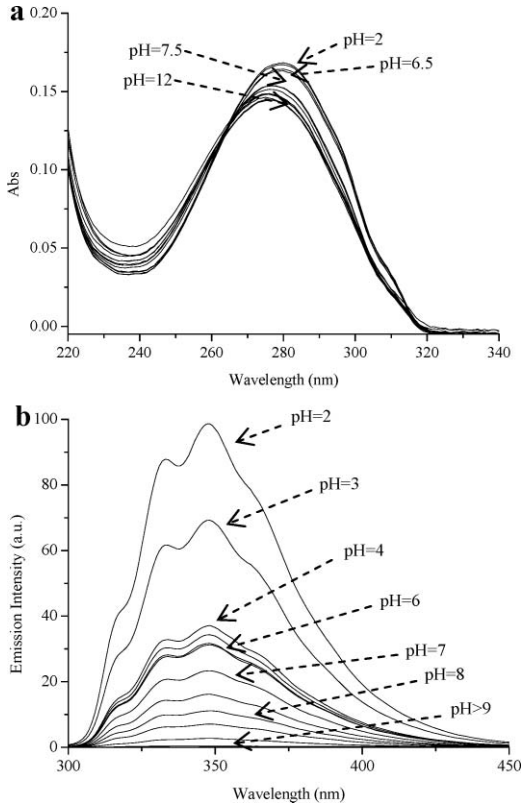


Fig. 2 (**a**) UV-Vis absorption and (**b**) fluorescence emission ($\lambda_{ex} = 265$ nm) spectra of **L2** registered at various pH values. Experimental conditions: [**L2**] $= 1 \times 10^{-5}$ M, $I = 0.15$ M aqueous NaCl, $T = 298.1$ K.

respect to the fundamental state, as demonstrated by determining the pK_a value for the protonated HPPD$^+$ in water–sulfuric acid mixtures, which were found to be p$K_a = -2.29$ for the singlet fundamental state S_0 and p$K_a = 2.63$ for the singlet excited state S_1.[13] In ligands **L1–L3** the PPD electronic levels are perturbed by the presence of the polyamine base connected to its phenyl rings. Taking into account that the excited state S_1 is more basic than the fundamental one S_0, it can be reasonably supposed that the presence of protons in the macrocycle better stabilizes the S_1 state than S_0 *via* hydrogen bonding, as well as that the N lone-pairs in the deprotonated species perturb the electronic state of the PPD, making the S_1 state more unstable than the S_0 state. This produces a considerable red shift of the absorption band going from basic to acid pH values,[16] as schematically depicted in Fig. 4. This effect is particularly marked for **L1** probably because, due to its smaller size and its greater rigidity, the acidic protons located on the polyamine moiety are held closer to the oxadiazole ring with respect to the other ligands; in fact, the effect decreases when the macrocyclic ring size is increased. However, conformational changes in the aromatic rings (angles between the planes), occurring in the several protonated species and affected by both the size and the protonation degree of the macrocycle, could also explain these data.

Fluorescence emission spectra were recorded for each ligand upon excitation at the isosbestic point ($\lambda_{ex} = 265$ nm for **L1** and **L2**; $\lambda_{ex} = 255$ nm for **L3**). All the ligands were found to be highly fluorescent in the acid pH range, showing a "four finger"-shaped emission band due to vibration structure, with the maximum emission at $\lambda_{em} = 350$ nm and remarkable emission quantum yields at pH = 2 ($\Phi_f = 0.57$, 0.65 and 0.72 for **L1**, **L2** and **L3** respectively). The high quantum yield exhibited by this class of ligands is an upgrade compared to the previously synthesized polyaza-cyclophanes.[12,14] At higher pH the fluorescence emission decreases and is totally quenched at pH around 8, for all the ligands. This behavior can be rationalized in terms of quenching of the excited state due to photoinduced intramolecular electron transfer (PET) from the lone pairs of the polyamine nitrogen atoms to the excited fluorophore moiety;[3a] the closer the amine function is to PPD the higher the effect is. Total quenching of the fluorescence takes place when at least one amine function sufficiently close to PPD is completely deprotonated, *i.e.* neither bearing protons nor being involved in any H-bonding. In the most common case of fluorescent amino macrocycle, the nitrogen atoms prone to give PET to fluorophore are the benzyl ones, but when the macrocycle size is small non-benzyl nitrogens can also do it because the electron transfer is dependent on the distance between the donor atom and the fluorophore. The trend of the emission intensity at 350 nm together with the distribution diagram of the species as a function of pH is reported in Fig. 5.

NMR studies. In order to monitor the relation between the trend of the fluorescence with the acidic proton distribution in the species, pH-dependent ^{1}H NMR spectra were recorded over the pH range of the potentiometric measurements. ^{1}H–^{1}H and ^{1}H–^{13}C NMR 2D correlation experiments were performed to assign all the signals. The pattern of resonances found in the ^{1}H and ^{13}C NMR

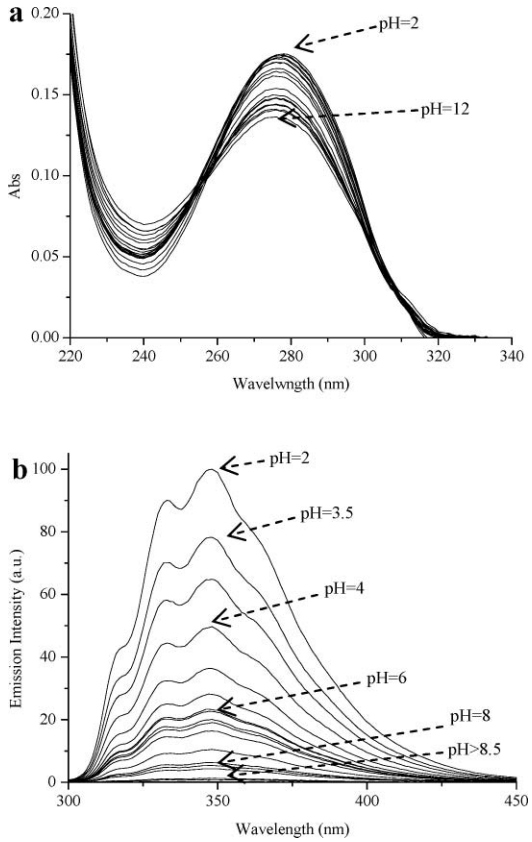


Fig. 3 (**a**) UV-Vis absorption and (**b**) fluorescence emission ($\lambda_{ex} = 255$ nm) spectra of **L3** registered at various pH values. Experimental conditions: [**L3**] $= 1 \times 10^{-5}$ M, $I = 0.15$ M aqueous NaCl, $T = 298.1$ K.

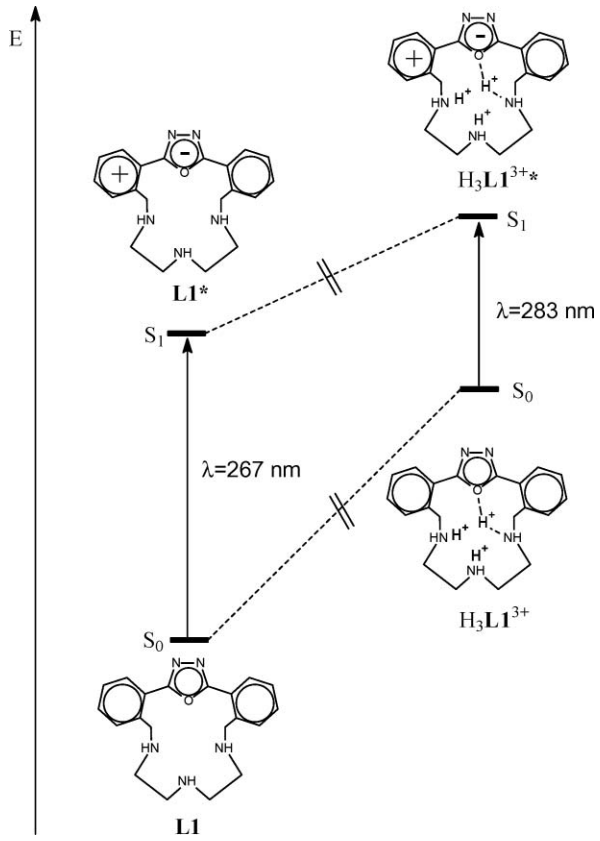


Fig. 4 Proposed model for singlet in fundamental (S_0) and excited (S_1) state of the neutral **L1** and fully protonated H_3**L1**$^{3+}$ species.

spectra (see experimental part) indicates a C_{2v} symmetry on the NMR time scale for all the ligands, which is preserved throughout the pH range investigated. A proposed proton disposition in all the species of the ligands is depicted in Fig. 6.

Fig. 7 reports the chemical shifts in the most significant resonances of **L2**, reported as a function of pH. The ^{1}H NMR spectrum recorded at pH = 12, where the neutral **L2** species is prevalent in solution, shows three aliphatic and four aromatic resonances: a singlet at $\delta = 2.73$ ppm integrating four protons (4H) attributed to the resonance of H8 (H8, 4H), a multiplet at $\delta = 2.80$ ppm (H7 and H6, 8H), a singlet at $\delta = 3.67$ ppm (H5, 4H), a multiplet at $\delta = 7.39$ ppm (H2, 2H), a doublet of doublets at $\delta = 7.48$ ppm (H4, 2H), a multiplet at $\delta = 7.60$ ppm (H3, 2H) and a doublet of doublets at $\delta = 7.69$ ppm (H1, 2H).

Examining Fig. 7, starting from pH = 12 and going towards pH = 9.3, where the H**L2**$^+$ species is prevalent in solution, all the aliphatic resonances shift downfield. In particular, the signals of H5 and H6 exhibit a marked shift, thus suggesting that the first protonation step mainly involves the benzyl nitrogen atoms, which are, however, shared and stabilized *via* H-bond with the closer amine function. By lowering the pH to 7.8, at which H_2**L2**$^{2+}$ is prevalent in solution, the resonances of H6 and H5 further shift downfield, highlighting that the second acidic proton is again mainly located on the other benzyl amine function (see Fig. 6). However, the protons, although mainly located in those positions, are shared and/or stabilized *via* H-bonding with all the other amine functions, as highlighted by the downfield shift in all aliphatic resonances. This situation affects the fluorescence, and in fact the ligand becomes weakly fluorescent (Fig. 5b). The third and fourth protonation steps take place on the two non-benzyl amine functions, as highlighted by the downfield shift observed for the signals of H8 and H7 from pH = 7.8 to pH = 4.6 (corresponding to the H_2**L2**$^{2+} \rightarrow H_3$**L2**$^{3+}$ step), as well as from pH = 4.6 to pH = 2.0, where the fully protonated H_4**L2**$^{4+}$ species is prevalent in solution (Fig. 5b). Taking into account that the first two protonation steps take place, above all, on the benzylic positions, we can suppose that in **L2**, PET starts mainly from the benzyl nitrogen atoms. However, the sharing of the acidic protons on all the amine functions, as well as the presence of close lone pairs, do not allow full emission of the PPD in the H_2**L2**$^{2+}$ species. The main fluorescent species is the fully protonated one, in which the benzyl N atoms are fully protonated, while the H_3**L2**$^{3+}$ species, in which one of the two amine function are not fully protonated (see Fig. 6), is less fluorescent. Analogous remarks can be made for **L3** (data not reported, see Fig. 6).

The behavior of **L1** is slightly different. Fig. 8 reports the chemical shifts in the most significant resonances of **L1**, reported as a function of pH. From pH = 12 to pH = 8, when the H**L1**$^+$ species is prevalent in solution, all the aliphatic resonances shift downfield due to the increase of positive charge density on the macrocycle, showing that the first H$^+$ is fully shared between all the three amine functions. By lowering the pH to 5, when the di-protonated H_2**L1**$^{2+}$ species is prevalent (see Fig. 5a), a marked downfield shift of H6 and H5 resonances was observed, while the signal of H7 did not shift. This suggests that the second protonation fixes the two acidic protons on the benzylic functions

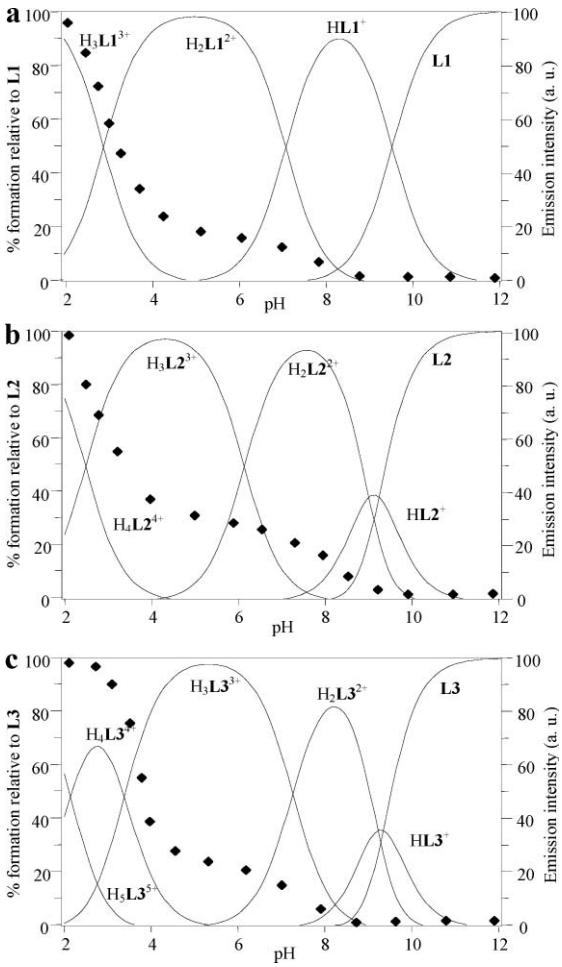


Fig. 5 Distribution curves of the species (—) and emission intensity at $\lambda_{em} = 350$ nm (◆) of **L1** (a), **L2** (b) and **L3** (c) in aqueous solution as a function of pH; [L] = 1×10^{-5} M, $I = 0.15$ M NaCl, $T = 298.1$ K, $\lambda_{ex} =$ 265 nm (**L1**), 265 nm (**L2**), 255 nm (**L3**).

due to the minimization of electrostatic repulsion. This species is very poorly emitting, showing that PET takes place mainly from the central non-benzylic nitrogen, which, due to the small size of the macrocycle, is close to the PPD moiety. Moving to pH = 2, where the fully protonated species is prevalent in solution, the signal of H7 undergoes the greater downfield shift while those of H5 did not substantially shift, reaching the highest emitting H_3**L1**$^{3+}$ species.

Fig. 9 reports the spectra of the aromatic part of all ligands recorded at pH = 12 and 2; all the resonances shift downfield moving from the free to the fully protonated species. The shift, as previously reported, cannot be attributed to a direct protonation of the PPD unit, but to an increase of the positive charge density on the ligand and its participation in the stabilization of the fully protonated species *via* H-bonding, as previously suggested by the UV-Vis absorption experiments. It is possible to observe that all the three ligands show a similar pattern of resonances when present in the fully protonated species. Under these conditions, the overall molecules are stiffened and, for this reason, the PPD units are probably arranged in the same way in all the ligands; the resulting spectra show a multiplet integrating six protons (H2, H3 and H4, see Fig. 7 and 8) and a signal (dd) at lower field, which accounts for two protons (H1). In the neutral species, all the resonances shift upfield in agreement with the reduced positive charge density on the ligands. **L2** and **L3** show a similar pattern of signals, while **L1** slightly differs, exhibiting a pattern of signals similar to the protonated species. Once again, this could be ascribed to the small size of **L1**; this makes **L1** stiff also in the neutral species, affecting the PPD conformation in a similar way in all the species.

Concluding remarks

The new polyamine macrocyclic ligands **L1**, **L2** and **L3** synthesized and characterized contain the photoactive heterocycle PPD (2,5-diphenyl[1,3,4]oxadiazole) inserted in polyaza-macrocycle fragments of different size and with different numbers of secondary amine functions. In aqueous solution, all the ligands show the typical acid–base behavior of a polyaza-cyclophane of comparable size, where the aromatic part is not involved in the acid–base processes. This suggests that the PPD group is not directly involved in the protonation steps. The fluorescence emission depends on the protonation state of the ligands and it can be rationalized on the basis of quenching of the excited state due to photoinduced intramolecular electron transfer (PET) from the lone-pair of the macrocycle nitrogen atoms to the excited state of the fluorophore moiety; in fact, going from acidic pH, at which the fully protonated species are prevalent in solutions, to alkaline solutions, a gradual quenching of fluorescence was observed for all the ligands. It is to highlight the high emission quantum yield of the fully protonated ligands, ranging from 0.57 to 0.75, making this class of ligands more sensitive with respect to the other polyaza-cyclophanes previously reported. The study of the fluorescence emission trend highlighted the on–off fluorescence behavior modulated by pH exhibited by the entire family, thus making these systems appealing for multiple applications.

Experimental section

General methods

UV absorption spectra were recorded at 298 K on a Varian Cary-100 spectrophotometer equipped with a temperature control unit. Fluorescence emission spectra were recorded at 298 K on a Varian Cary-Eclypse spectrofluorimeter and the spectra are uncorrected. The fluorescence quantum yields of fully protonated ligands (Φ_f) were determined by comparing the integrated fluorescence spectra of the sample in aqueous HCl at pH = 2 with 2,2′-biphenol in acetonitrile ($\Phi_f = 0.29$).[17] ESI-MS spectra were recorded on a Thermo Quest LCQ Duo LC/MS/MS spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 298.1 K on a Bruker Avance instrument, operating at 200.13 and 50.33 MHz, respectively. For the spectra recorded in D_2O, the peak positions are reported with respect to HOD (4.75 ppm) for ^{1}H NMR spectra, while dioxane was used as reference standard in ^{13}C NMR spectra ($\delta =$ 67.4 ppm). For the spectra recorded in $CDCl_3$ the peak positions are reported with respect to TMS. All reagents and solvents used were of analytical grade.

EMF measurements

Equilibrium constants for protonation and complexation reactions of the ligands were determined by pH-metric measurements in 0.15 mol dm^{-3} NaCl at 298.1 ± 0.1 K, using the fully automatic

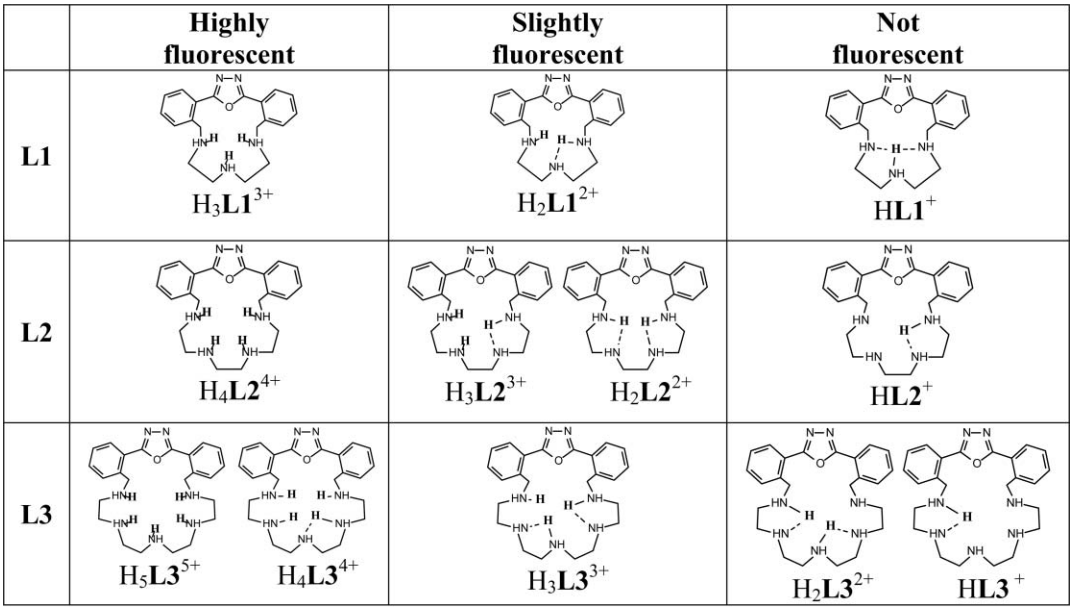


Fig. 6 Proposed disposition of the H^+ in the protonated forms of the ligands.

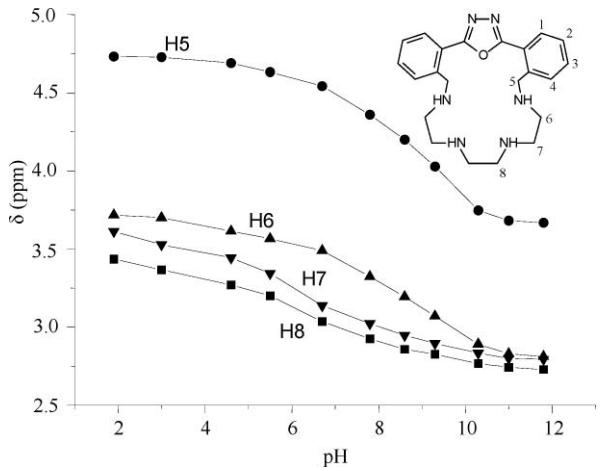


Fig. 7 Trend of the ^{1}H NMR signals for the **L2** aliphatic protons as a function of pH. (■ = H8, ▼ = H7, ▲ = H6, ● = H5).

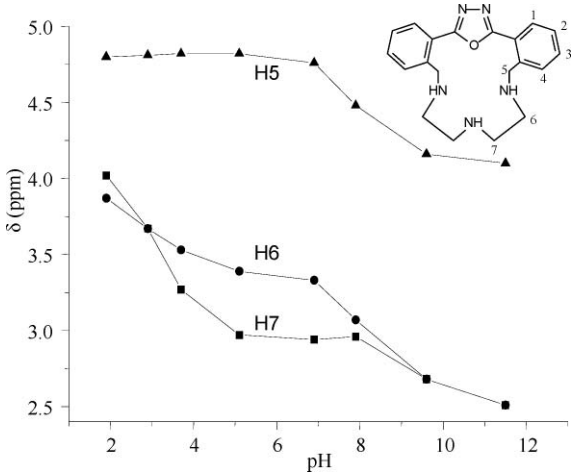


Fig. 8 Trend of the ^{1}H NMR signals for the **L1** aliphatic protons as a function of pH. (■ = H7, ● = H6, ▲ = H5).

equipment that has already been described;[18a] EMF data were acquired with the PASAT computer program.[18b] The combined glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by Gran's method,[18c,d] which gives the standard potential E° and the ionic product of water (pK_w = 13.83(1) at 298.1 K in 0.15 mol dm^{-3} NaCl, $K_w = [H^+][OH^-]$). At least three potentiometric titrations were performed for each system in the pH range 2–12, and all titrations were treated either as single sets or as separate entities, for each system; no significant variations were found in the values of the determined constants. The HYPERQUAD computer program was used to process the potentiometric data.[18e]

Synthesis

All chemicals were purchased from Aldrich, Fluka and Lancaster in the highest quality commercially available. 2,5-Bis(2-methylphenyl)[1,3,4]oxadiazole (**3**) was prepared from 2-methylbenzoyl chloride (**1**) (Aldrich) and 2-methylbenzhydrazide (**2**) as reported.[10] 2,5-Bis[2-(chloromethyl)phenyl]-[1,3,4]oxadiazole (**4**) was prepared as reported.[19] 1,4,7-Tris(4-methylbenzensulfonyl)-1,4,7-triazaheptane (**5a**), 1,4,7,10-tetrakis(4-methylbenzensulfonyl)-1,4,7,10-tetraazadecane[20a] (**5b**) and 1,4,7,10,13-pentakis(4-methylbenzensulfonyl)-1,4,7,10,13-pentaazatridecane[20b] (**5c**) were prepared as reported.

9,12,15-Tris(4-methylbenzensulfonyl)-9,12,15,24,25-pentaaza-26-oxatetracyclo[21.2.1.0^{2,7}.0^{17,22}]hexaicosa-2,4,6,17,19,21,23,25^1-octaene (6a)

Over a period of 4 h, a solution of **5a** (1.8 g, 3.1 mmol) in 100 cm^3 of anhydrous DMF was added to a suspension of **4** (1.0 g, 3.1 mmol) and K_2CO_3 (4.3 g, 31.0 mmol) in 200 cm^3 of anhydrous DMF, at 90 °C under nitrogen. The reaction mixture was maintained at

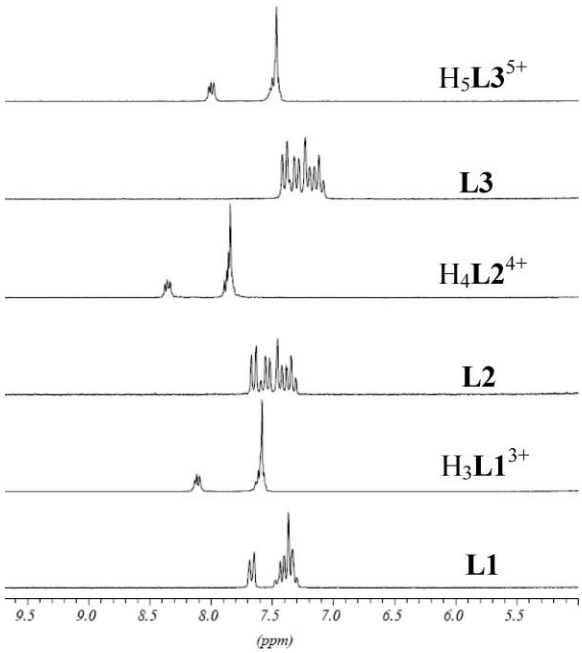


Fig. 9 ^{1}H NMR spectra of the aromatic resonances recorded in D_2O solution at pH = 2 and 12.

90 °C for further 2 h. Subsequently, the mixture was cooled to room temperature then concentrated under reduced pressure to one third of the initial volume, then poured under stirring into cold water (1 dm^3). The resulting white precipitate was filtered off, washed with cold water, dried under vacuum and purified by flash chromatography (silica gel, chloroform) obtaining **6a** as a white solid (1.8 g, 72%).

MS *m*/*z* (ESI): 812.2 (M + H$^+$); ^{1}H NMR ($CDCl_3$) δ = 7.92 (2H, dd), 7.85 (6H, d) 7.72 (2H, d), 7.53 (4H, m), 7.36 (4H, d), 7.26 (2H, d), 5.08 (4H, s), 3.04 (8H, m), 2.46 (6H, s), 2.39 (3H, s); ^{13}C NMR ($CDCl_3$): δ = 164.5, 143.7, 143.6, 137.6, 136.0, 135.6, 133.5, 132.4, 129.9, 129.8, 128.9, 128.8, 127.7, 127.6, 122.3, 49.1, 47.8, 47.5, 21.6 ppm; Anal. for $C_{41}H_{41}N_5O_7S_3$ (812.0): Calcd (%) C 60.65, H 5.09, N 8.62; Found (%) C 60.5, H 5.2, N 8.5.

9,12,15,18-Tetrakis(4-methylbenzensulfonyl)-9,12,15,18,27,28-hexaaza-29-oxatetracyclo[24.2.1.0^{2,7}.0^{20,25}]enneaicosa-2,4,6,20,22,24,26,28^1-octaene (6b)

This compound was synthesized from **5b** (1.6 g, 2.2 mmol), **4** (0.7 g, 2.2 mmol) and K_2CO_3 (3.0 g, 22.0 mmol) following the same procedure reported for **6a** obtaining **6b** as a white solid (1.2 g, 54%).

MS *m*/*z* (ESI): 1009.3 (M + H$^+$); ^{1}H NMR ($CDCl_3$) δ = 7.91 (2H, d), 7.80 (6H, d) 7.67 (4H, d), 7.51 (4H, m), 7.30 (8H, m), 4.99 (4H, s), 3.38 (4H, m), 3.20 (4H, m), 3.13 (4H, s), 2.44 (6H, s), 2.42 (6H, s); ^{13}C NMR ($CDCl_3$): δ = 164.0, 143.6, 143.5, 137.2, 136.8, 134.8, 132.6, 132.2, 129.9, 129.8, 129.1, 128.6, 127.7, 127.5, 122.3, 50.2, 49.2, 48.9, 47.4, 21.6 ppm; Anal. for $C_{50}H_{52}N_6O_9S_4$ (1009.2): Calcd (%) C 59.51, H 5.19, N 8.33; Found (%) C 59.4, H 5.2, N 8.2.

9,12,15,18,21-Pentakis(4-methylbenzensulfonyl)-9,12,15,18,21,30,31-heptaaza-32-oxatetracyclo[27.2.1.0^{2,7}.0^{23,28}]-diatriconta-2,4,6,23,25,27,29,31^1-octaene (6c)

This compound was synthesized from **5c** (7.0 g, 7.1 mmol), **4** (2.3 g, 7.1 mmol) and K_2CO_3 (9.7 g, 70.0 mmol) following the same procedure reported for **6a** obtaining **6c** as a white solid (4.6 g, 54%).

MS *m*/*z* (ESI): 1206.3 (M + H$^+$); ^{1}H NMR ($CDCl_3$) δ = 7.93 (2H, d), 7.83 (2H, d), 7.78 (4H, d), 7.63 (4H, d) 7.57 (2H, t), 7.44 (2H, t), 7.34 (2H, d), 7.32 (4H, d), 7.29 (4H, d), 7.23 (2H, d), 5.07 (4H, s), 3.53 (4H, m), 3.07 (12H, m), 2.47 (6H, s), 2.44 (6H, s), 2.41 (3H, s); ^{13}C NMR ($CDCl_3$): δ = 164.0, 143.8, 143.6, 143.3, 137.2, 136.8, 134.6, 133.8, 131.8, 131.7, 129.8, 129.6, 128.2, 127.6, 127.5, 127.4, 122.8, 51.1, 50.5, 49.9, 48.8, 47.1, 21.6, 21.6, 21.5 ppm; Anal. for $C_{59}H_{63}N_7O_{11}S_5$ (1206.5): Calcd (%) C 58.74, H 5.26, N 8.13; Found (%) C 58.8, H 5.2, N 8.1.

9,12,15,24,25-Pentaaza-26-oxatetracyclo[21.2.1.0^{2,7}.0^{17,22}]-hexaicosa-2,4,6,17,19,21,23,25^1-octaene (L1)

Macrocycle **6a** (1.8 g, 2.2 mmol) and phenol (5.0 g, 53.2 mmol) were dissolved in HBr–CH_3COOH (33%, 40 ml). The solution was stirred at 90 °C for 24 h. The resulting suspension was filtered and washed with CH_2Cl_2 several times. The solid obtained was recrystallized from cold water–48% aqueous HBr mixture to give **L1**·3HBr as a white solid (0.9 g, 70%).

MS *m*/*z* (ESI): 350.2 (M + H$^+$); ^{1}H NMR (DMSO-d_6) δ = 9.09 (6H, br), 8.34 (2H, m), 7.79 (6H, m), 4.93 (4H, br), 3.82 (4H, br), 3.58 (4H, br) ppm; ^{13}C NMR (DMSO-d_6): δ = 164.3, 134.7, 133.3, 131.4, 131.1, 130.0, 122.8, 49.7, 44.1, 43.6 ppm; Anal. for $C_{20}H_{26}Br_3N_5O$ (592.2): Calcd (%) C 40.57, H 4.43, N 11.83; Found (%) C 40.7, H 4.6, N 11.7; UV-Vis ($NaOH_{aq}$, pH = 12) λ_{max} = 267 nm, ε = 12300 mol^{-1} cm^{-1} dm^3.

9,12,15,18,27,28-Hexaaza-29-oxatetracyclo[24.2.1.0^{2,7}.0^{20,25}]-enneaicosa-2,4,6,20,22,24,26,28^1-octaene (L2)

This compound was synthesized from **6b** (2.2 g, 2.2 mmol), phenol (6.6 g, 70.2 mmol) and HBr–CH_3COOH (33%, 55 ml) following the same procedure reported for **L1**, obtaining **L2**·4HBr as a white solid (0.8 g, 54%).

MS *m*/*z* (ESI): 393.2 (M + H$^+$); ^{1}H NMR (D_2O, pH = 3) δ = 8.12 (2H, m), 7.63 (6H, m), 4.54 (4H, s), 3.56 (4H, m), 3.51 (4H, m), 3.36 (4H, s); ^{13}C NMR (D_2O, pH = 3): δ = 163.9, 133.2, 133.1, 131.1, 129.9, 129.2, 122.7, 50.4, 42.5, 42.4, 41.7 ppm; Anal. for $C_{22}H_{32}Br_4N_6O$ (716.2): Calcd (%) C 36.90, H 4.50, N 11.73; Found (%) C 37.0, H 4.6, N 11.7; UV-Vis ($NaOH_{aq}$, pH = 12) λ_{max} = 275 nm, ε = 14000 mol^{-1} cm^{-1} dm^3.

9,12,15,18,21,30,31-Heptaaza-32-oxatetracyclo[27.2.1.0^{2,7}.0^{23,28}]-diatriconta-2,4,6,23,25,27,29,31^1-octaene (L3)

This compound was synthesized from **6c** (2.0 g, 1.7 mmol), phenol (6.4 g, 68.0 mmol) and HBr–CH_3COOH (33%, 50 ml) following the same procedure reported for **L1**, obtaining **L3**·5HBr as a white solid (1.0 g, 71%).

MS *m*/*z* (ESI): 436.3 (M + H$^+$); ^{1}H NMR (D_2O, pH=3) δ = 8.17 (2H, m), 7.62 (6H, m), 4.59 (4H, s), 3.72 (4H, t), 3.51 (4H, t), 3.29 (4H, t,br), 3.11 (4H, t,br); ^{13}C NMR (D_2O, pH = 3): ^{13}C NMR

(D_2O, pH=3): δ = 163.8, 133.2, 132.7, 131.3, 129.8, 129.3, 122.7, 51.3, 44.6, 44.2, 43.9, 41.8 ppm; Anal. for $C_{24}H_{38}Br_5N_7O$ (840.1): Calcd (%) C 34.31, H 4.56, N 11.67; Found (%) C 34.3, H 4.6, N 11.6; UV-Vis ($NaOH_{aq}$, pH=12) λ_{max} = 275 nm, ε = 13000 mol^{-1} cm^{-1} dm^3.

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