Acid–Base Sensors Based on Novel Quinone-Type Dyes

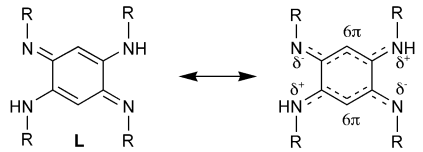
Mourad Elhabiri,[a] Olivier Siri,[b] Alejandra Sornosa-Tent,[a] Anne-Marie Albrecht-Gary,*[a] and Pierre Braunstein*[b]

Abstract: We present a detailed study on the acid–base behaviour of a family of "potentially antiaromatic" *p*-benzoquinonediimine ligands. These 12π electron molecules can be considered as constituted of two chemically connected but electronically not conjugated 6π-electron subunits. Upon successive protonation, "mono" and "double" cyanine-type chromophores are generated in solution and allow a precise and sensitive spectrophotometric detection. These molecules represent a new class of tunable quinones whose electronic and structural properties can be triggered by proton input, as established by a complete physicochemical study involving a combination of potentiometric and spectrophotometric methods (absorption and emission).

Keywords: absorption · benzoquinonediimine · fluorescence spectroscopy · protonation · sensors

Introduction

Spectrophotometric chemosensors constitute a large class of "reporter" molecules able to bind to other molecules or ions and signal their presence.[1] In particular, acid–base sensors capable of indicating the presence or absence of protons are of great importance in for example life sciences, detectors responding to environmental changes, and memories or logic gates in nanotechnology.[2,3] Fluorescent sensors are especially interesting due to the high sensitivity of this method and numerous fluorescent systems displaying bimodal response "on" and "off" at near-neutral pH 4.5–8 have been reported and have led to practical applications for intracellular pH measurements.[4] Cyanine dyes, which constitute a subgroup of the polymethine dyes, have been used as sensor molecules for the fluorescent detection of analytes in diagnostics.[5,6] Despite their success, only few examples of fluorescent cyanine biosensors sensitive to proton concentration have been reported,[6] mainly due to difficulties associated with photodecomposition, self-aggregation or hydrolysis in aqueous media. Therefore, synthetic efforts are currently in progress in order to overcome these limitations and to produce new and stable spectrophotometric sensors based on cyanine-type dyes. Other interesting candidates as spectrophotometric chemosensors are molecules exhibiting a quinonoid structure, which have attracted the interest of a large community for decades[7] owing to their chemical and physical properties. More specifically, research on the relation between colour and structure has, since a long time, involved benzoquinonediimines such as **L**, which are used as colorants for various applications. These 12π electron molecules should actually be better considered as constituted of two 6π subsystems,[8] chemically connected by C–C single bonds but electronically not conjugated (Scheme 1).[9,10]



Scheme 1. Resonance forms in 2,5-diamino-1,4-benzoquinonediimines.

We have previously shown that *N*-neopentyl *p*-benzoquinonediimine[9] $\mathbf{L^1}$ undergoes significant spectrophotometric changes as a function of pH.[10] Upon successive protonation of $\mathbf{L^1}$, "mono" and "double" cyanine-type chromophores are generated in solution.

An X-ray crystal structure analysis of the monoprotonated derivative $(\mathbf{L^1})H^+$ established that protonation occurred at one of the imine functions and generated an iminium ion stabilised by intramolecular delocalisation, so that the posi-

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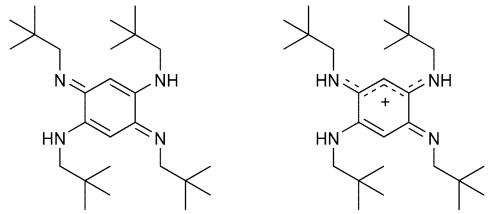
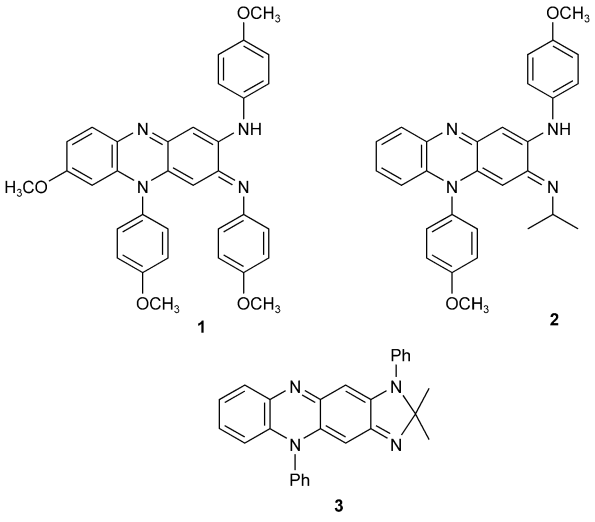
tive charge is delocalised over the N=C=C=C=N subunit.[10] Therefore $(\mathbf{L^1})H^+$ contains in the solid state two different conjugated π-systems, with and without delocalisation. The X-ray structure analysis of the diprotonated species $(\mathbf{L^1})H_2^{2+}$ similarly showed that each positive charge is delocalised, leading to a double cyanine-type chromophore for



$\mathbf{L^1}$ $(\mathbf{L^1})H^+$

which the 6π-subsystems are connected to each other by two C–C single bonds.[10] Since $\mathbf{L^1}$ appeared to be the first molecule of its kind with two separated, conjugated and localised 6π electron systems that can be tuned by protonation to become delocalised, this prompted us to evaluate such systems as spectrophotometric sensors for proton detection. The electronic situation in our molecules is novel with respect to previously reported cyanine or cyanine-type dyes.[7,11] In related systems **1**–**3** involving a quinoxalino-phenazine backbone, the two "face-to-face" π-systems are not fully independent owing to a "peripheral" C=C connection between the endocyclic protonations sites which allows conjugation between the two 6π electron systems.[12–18]

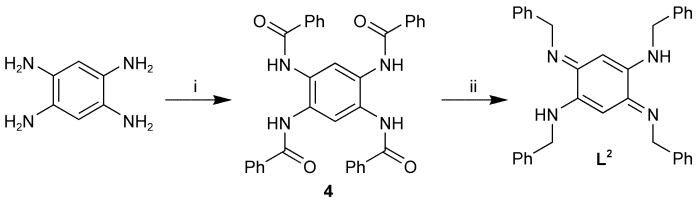


1 **2** **3**

Herein, we wish to report the synthesis and full characterisation of the new N-substituted *p*-benzoquinonediimine $\mathbf{L^2}$ which contains benzyl groups as the light emitting components and four nitrogen atoms as the protonation sites. By analogy with aryl-containing polyamines receptors,[19,20] the use of benzyl groups as N-substituents is expected to lead to fluorescent sensors for which photophysical properties could be triggered by proton input, thus allowing "on/off" switching of the luminescence.[6,21] The stability of $\mathbf{L^2}$ and of its neopentyl analogue $\mathbf{L^1}$ in aqueous media allowed their physico-chemical and electronic properties to be determined in methanol/water (80/20 *w*/*w*). Their protonated forms have been fully characterised by a combination of spectrophotometric methods (absorption and/or emission) and potentiometry.

Results and Discussion

Synthesis and characterisation: The *N*,*N*′,*N*″,*N*‴-tetrabenzyl-2,5-diamino-1,4-benzoquinonediimine base, noted $\mathbf{L^2}$, was prepared in two steps similarly to the neopentyl analogue $\mathbf{L^1}$ (Scheme 2).[9,10] Tetraaminobenzene was first reacted with benzoyl chloride in acetonitrile and NEt_3, to afford the tetrabenzamidobenzene (**4**) which was fully characterised. This intermediate was then reduced with $LiAlH_4$ in THF yielding $\mathbf{L^2}$ as an orange solid after aerobic workup.

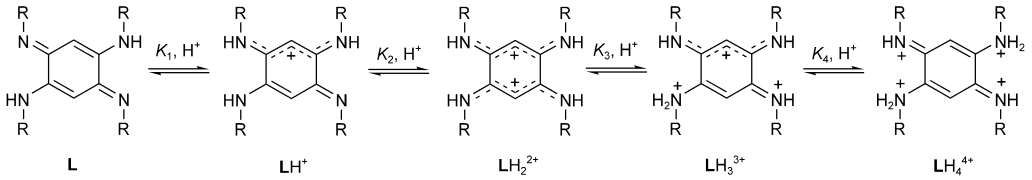


4 $\mathbf{L^2}$

Scheme 2. i) $PhC(O)Cl/NEt_3/CH_3CN/Ar$; ii) $LiAlH_4$/Ar, THF/aerobic workup.

The successive protonations of **L** (**L** = $\mathbf{L^1}$ or $\mathbf{L^2}$) led to the formation of "mono" and "double" cyanine-type systems $\mathbf{L}H^+$ and $\mathbf{L}H_2^{2+}$, respectively (Scheme 3). Compounds $\mathbf{L^2}$, $(\mathbf{L^2})H^+$ and $(\mathbf{L^2})H_2^{2+}$ were fully characterised by ^1H NMR spectroscopy and elemental analyses. Further protonation leads to $\mathbf{L}H_3^{3+}$ and $\mathbf{L}H_4^{4+}$ which will be characterised by spectrophotometric methods.

UV/Vis absorption: Compounds $\mathbf{L^1}$ and $\mathbf{L^2}$ are soluble in dichloromethane, tetrahydrofuran or acetonitrile, sparingly soluble in methanol and insoluble in water whereas their protonated forms are more soluble in methanol and soluble



L $\mathbf{L}H^+$ $\mathbf{L}H_2^{2+}$ $\mathbf{L}H_3^{3+}$ $\mathbf{L}H_4^{4+}$

Scheme 3. Protonation of *N*,*N*′,*N*″,*N*‴-tetrasubstituted-2,5-diamino-1,4-benzoquinonediimine-type ligands ($\mathbf{L^1}$: R = benzyl and $\mathbf{L^2}$: R = neopentyl).

in water (ca. 10^{-4} M). $\mathbf{L^1}$ is much more soluble in organic solvents than $\mathbf{L^2}$, which facilitates purification by crystallisation of its protonated forms. The acido–basic properties were thus examined in a mixture methanol/water (80/20 *w*/*w*). *p*-Benzoquinonemonoimine and *p*-benzoquinonediimine derivatives are known to undergo rapid hydrolysis in water and fast mixing techniques (e.g. stopped-flow) are generally required to determine their protonation constants.[22,23] Interestingly, $\mathbf{L^1}$ and $\mathbf{L^2}$ were found to be stable under our experimental conditions and not altered by reversible variations of pH. The UV/Vis absorption spectra of $\mathbf{L^1}$ and $\mathbf{L^2}$ were recorded in a large span of pH values (Figure 1).

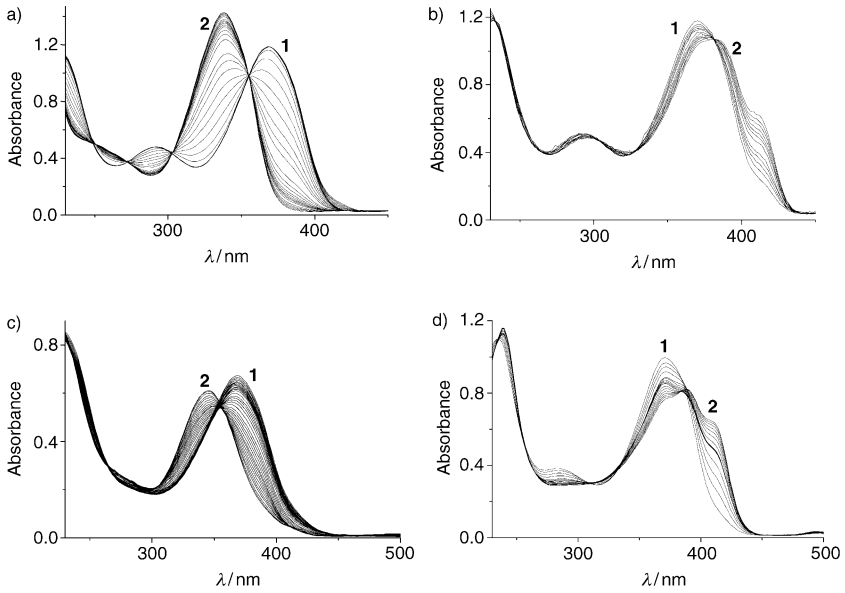


Figure 1. Absorption spectra of $\mathbf{L^1}$ and $\mathbf{L^2}$ as a function of pH. Solvent: methanol/water (80/20 *w*/*w*); $T = 25.0 \pm 0.2$ °C; $I = 0.5$ M ($N(n\text{Bu})_4ClO_4$); $l = 1.0$ cm; a) $[\mathbf{L^1}]_{tot} = 5.76 \times 10^{-5}$ M, 1) pH 2.66, 2) pH 10.19; b) $[\mathbf{L^1}]_{tot} = 5.23 \times 10^{-5}$ M, 1) pH 2.20, 2) pH 1.31; c) $[\mathbf{L^2}]_{tot} = 4.23 \times 10^{-5}$ M), 1) pH 2.86, 2) pH 8.03; d) $[\mathbf{L^2}]_{tot} = 4.35 \times 10^{-5}$ M, 1) pH 2.52, 2) pH 1.30.

The processing[24,25] of the spectrophotometric and potentiometric data led to the respective protonation constants of $\mathbf{L^1}$ and $\mathbf{L^2}$ (Table 1).

Table 1. Protonation constants of ligands $\mathbf{L^1}$ and $\mathbf{L^2}$.[a]

Equilibrium	$\mathbf{L^1}$ $\log\beta_n \pm 3\sigma$	$\mathbf{L^2}$ $\log\beta_n \pm 3\sigma$
$\mathbf{L} + H^+ \rightleftarrows \mathbf{LH}^+$ (β_1)	8.28 ± 0.01[b]	7.96 ± 0.05[b]
	8.09 ± 0.09[c]	7.5 ± 0.2[c]
$\mathbf{L} + 2H^+ \rightleftarrows \mathbf{LH_2}^{2+}$ (β_2)	14.03 ± 0.09[b]	13.76 ± 0.09[b]
	13.7 ± 0.2[c]	12.9 ± 0.2[c]
$\mathbf{L} + 3H^+ \rightleftarrows \mathbf{LH_3}^{3+}$ (β_3)	–	14.6 ± 0.2[c]
$\mathbf{L} + 4H^+ \rightleftarrows \mathbf{LH_4}^{4+}$ (β_4)	16.5 ± 0.2[c]	–

[a] methanol/water (80/20 *w*/*w*); $T = 25.0 \pm 0.2$ °C. [b] $I = 0.1$ M (NEt_4ClO_4). [c] $I = 0.5$ M ($N(n\text{Bu})_4ClO_4$). [d] $\mathbf{L} + NH^+ \overset{\beta_n}{\rightleftarrows} \mathbf{LH}_n^{n+}$ $1 \le n \le 4$ and $\mathbf{L} = \mathbf{L^1}$ or $\mathbf{L^2}$.

β_1 and β_2 were attributed to the protonation of the imine functions, in agreement with literature data available for closely related compounds.[22,26,27] A comparison between 1,4-benzoquinonemonoimine ($\log\beta_1 = 3.72 \pm 0.06$),[22] 1,4-benzoquinonediimine ($\log\beta_1 = 5.75$; $\log\beta_2 < 7.25$),[22] $\mathbf{L^1}$ and $\mathbf{L^2}$ clearly shows a drastic increase of the respective protonation constants mainly due to inductive and mesomeric effects. In a statistical model, a difference between two successive protonation constants of two identical and independent sites of a molecule is expected to be $\Delta\log K = 0.6$.[28] However, a much larger $\Delta\log K$ value (≈ 2.2 for $\mathbf{L^1}$ and ≈ 2.5 for $\mathbf{L^2}$) was observed between K_1 and K_2, that could be explained by strong electrostatic repulsions between the two independent conjugated subunits in $(\mathbf{L^1})H_2^{2+}$ and $(\mathbf{L^2})H_2^{2+}$ (Scheme 3 and Table 1).[28] Under very acidic conditions, low constants could be determined for further protonation of $\mathbf{L^1}$ and $\mathbf{L^2}$ (Table 1). For $\mathbf{L^2}$, β_3 was easily attributed to the protonation of one of the four equivalent nitrogen sites of $(\mathbf{L^2})H_2^{2+}$ (Scheme 3). Its low value ($\log\beta_3 = 14.6 \pm 0.2$) could be explained by the resulting loss of the optimal delocalisation and symmetry occurring in $(\mathbf{L^2})H_2^{2+}$.[10,29] Owing to the decrease of conjugation, protonation of $(\mathbf{L^2})H_3^{3+}$ could be expected to follow a mechanism with less interactions and then closer to a statistical process.[28] The influence in the chemical structure of the N-substituents, neopentyl in $\mathbf{L^1}$ and benzyl in $\mathbf{L^2}$, respectively, does not significantly affect the protonation constants owing to the presence of a common methylenic spacer in $\mathbf{L^1}$ and $\mathbf{L^2}$, which is a poor electronic relay.

As an example, the corresponding distribution diagram[30] of the species resulting from protonation of $\mathbf{L^1}$ versus pH is given in Figure 2. It shows that under suitable conditions of acidity, $\mathbf{L^1}$, $(\mathbf{L^1})H^+$ and $(\mathbf{L^1})H_2^{2+}$ can be isolated almost quantitatively as pure species.[10]

The electronic spectra of the protonated species of $\mathbf{L^1}$ and $\mathbf{L^2}$ are given in Figure 3.

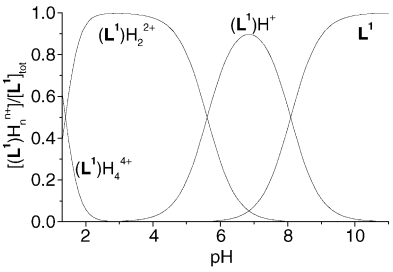


Figure 2. Distribution diagram of the species resulting from protonation of $\mathbf{L^1}$. Solvent: methanol/water (80/20 *w*/*w*); $T = 25.0 \pm 0.2$ °C; $I = 0.5$ M ($N(n\text{Bu})_4ClO_4$). $[\mathbf{L^1}]_{tot} = 5.00 \times 10^{-5}$ M.

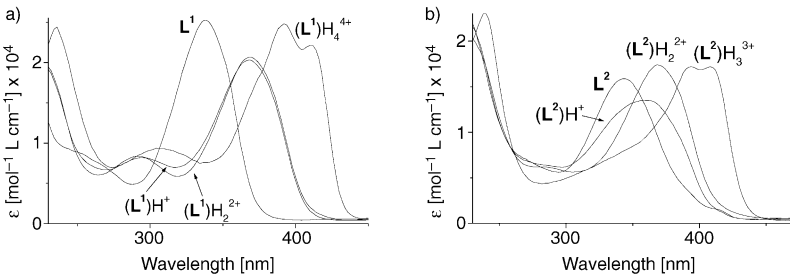


Figure 3. Electronic spectra of the different species resulting from protonation of a) $\mathbf{L^1}$ and b) $\mathbf{L^2}$; solvent: methanol/water (80/20 *w*/*w*); $T = 25.0 \pm 0.2$ °C; $I = 0.5$ M ($N(n\text{Bu})_4ClO_4$).

A comparison between the spectrophotometric data for 1,4-benzoquinonemonoimine (**7**) ($\lambda_{max} = 262$ nm)[22], 1,4-benzoquinonediimine (**12**) ($\lambda_{max} = 265.5$ nm)[22], $\mathbf{L^1}$ ($\lambda_{max} = 338$ nm) and $\mathbf{L^2}$ ($\lambda_{max} = 345$ nm) shows that substitution of positions 2 and 5 by amine functions leads to a bathochromic shift of the intraquinone transitions of about 80 nm (Table 2). N-Substitution of the imine functions of **7** and **12** by aryl groups leads to large bathochromic shifts (> 200 nm) of the maximum of the intraquinone $\pi \rightarrow \pi^*$ absorption band[22,26,31,32] as shown in indoaniline (**9**, **10** and **11**), *N*-phenyl benzoquinonediimine (**13**, **14**) or phenazine (**15**) type compounds. This indicates the presence of more extended π-systems most likely due to significant delocalisation through the "peripheral" N-substituents (Table 2). Furthermore, N-substitution of the amine and imine nitrogen atoms by aryl groups (**5**, **6**) similarly results in red shifts of the intraquinone $\pi \rightarrow \pi^*$ absorption band compared to that of neopentyl or benzyl derivatives $\mathbf{L^1}$ and $\mathbf{L^2}$. The presence of methylenic spacers in the N-substituents of the latter two molecules interrupts electronic communication and results in confined systems with shorter wavelength absorptions. The larger red shift for **6**[12] compared with azophenine (**5**)[33] is due to a "peripheral" C=C connection between the endocyclic protonations sites which allows conjugation between the two 6π electron systems. The intraquinone $\pi \rightarrow \pi^*$ transitions for $(\mathbf{L^1})H^+$ and $(\mathbf{L^1})H_2^{2+}$ are similar and experience bathochromic shifts of about 25–30 nm compared to $\mathbf{L^1}$ (Figure 3 and Table 2). This is in agreement with recent DFT calculations on $\mathbf{L^1}$ to be published elsewhere[10] as well as with data obtained for **5** (Table 2). Compared to $\mathbf{L^2}$, the intraquinone $\pi \rightarrow \pi^*$ transitions for $(\mathbf{L^2})H^+$ and $(\mathbf{L^2})H_2^{2+}$ are shifted to longer wavelengths by 7 and 24 nm, respectively (Figure 3 and Table 2).[33]

Fluorescence: When the *p*-benzoquinonediimine system was N-substituted by an alkyl (R = neopentyl, $\mathbf{L^1}$) or an aryl (R = phenyl, **5**) group, no significant emission signal was observed. In contrast, compound $\mathbf{L^2}$ with benzylic moieties as N-substituents revealed interesting emission properties and the effect of pH on the fluorescence spectra of $\mathbf{L^2}$ was investigated. The absorbance at the chosen excitation wavelength ($\lambda_{exc} = 358$ nm) was kept lower than 0.1 to minimise resorption processes and remained constant throughout the fluorescence titration (Figure 4). Upon excitation into the transitions at 358 nm, a maximum of emission arising from the $^1\pi\pi^*$ state was observed at 480 nm at pH 10.53 (Figure 4). Upon successive protonation of the imine functions, the ligand-centred fluorescence significantly decreased to almost zero, with a concomitant shift to longer wavelengths ($\lambda_{em} = 529$ nm), when both imine receptors are protonated (Figure 4).

The processing of the spectrofluorimetric data led to protonation constants close to those obtained in the ground-state by absorption spectrophotometry (Table 3).

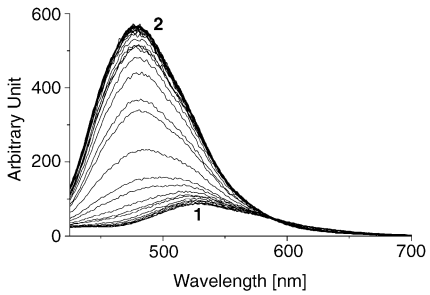
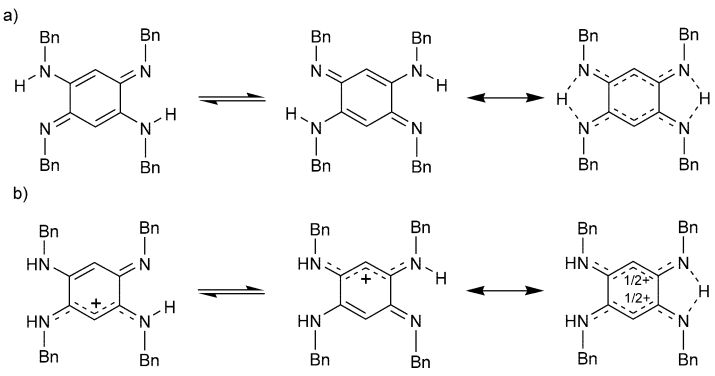


Figure 4. Fluorescence spectra of $\mathbf{L^2}$ (7.58×10^{-6} M) as a function of pH. Solvent: methanol/water (80/20 *w*/*w*); $T = 25.0 \pm 0.2$ °C; $I = 0.1$ M ($N(C_2H_5)_4ClO_4$); $l = 1$ cm, $\lambda_{exc} = 358$ nm. Spectra: 1) pH 3.34; 2) pH 10.53. Excitation and emission slits: 3 nm.

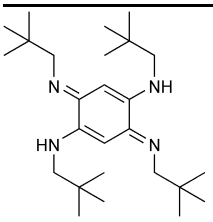
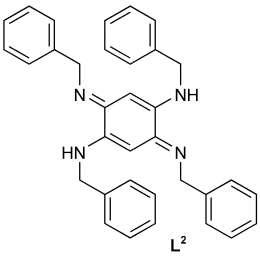
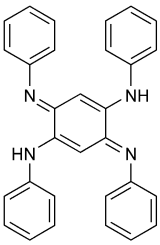
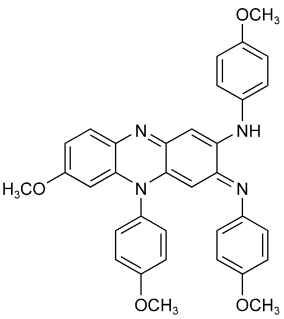
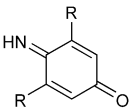
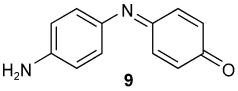
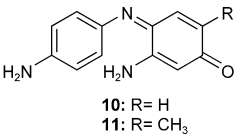
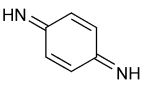
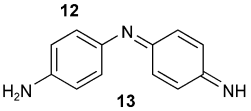
The absolute quantum yield scarcely varies between pH 9.8 and 6.6 (~0.18), whereas it significantly decreases below pH 6.6 and amounts to 0.03 at pH 2.8 (Figure 5a), in agreement with the relative quantum yield calculated from the emission fluorescence titration (Figure 5b).[34]

^{1}H NMR spectroscopy of $\mathbf{L^2}$ clearly showed that a fast intramolecular double proton transfer, which involves two tautomers, occurs in solution and leads to a structure of higher symmetry (Scheme 4a). Upon the first protonation, a decrease of one quarter of the total fluorescence intensity could be expected and this is consistent with our experimental observations (Figure 5b, Scheme 4a and Table 3). The



Scheme 4. Tautomeric equilibria of a) $\mathbf{L^2}$ and b) $(\mathbf{L^2})H^+$ in solution.

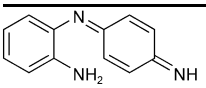
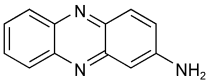
Table 2. Spectrophotometric data ($\pi \rightarrow \pi^*$ transition) for benzoquinone mono- and diimine derivatives.[a]

Ligand	**L** λ_{max} ($\log\varepsilon^{\lambda max}$) [nm]	$\mathbf{LH^+}$ λ_{max} ($\log\varepsilon^{\lambda max}$) [nm]	$\mathbf{LH_2^{2+}}$ λ_{max} ($\log\varepsilon^{\lambda max}$) [nm]	Ref.
 **L¹**	338 (4.40)	368 (4.30)	368 (4.32)	[a]
 **L²**	345 (4.30) 344 (4.26)	352 (4.23)	369 (4.29)	[a] [b]
 **5**	389 (4.33)			[b], [33]
 **6**	505 (4.10)	530 (4.18)	–	[12]
 **7**: R= H **8**: R= CH_3	262 (**7**) 275 (**8**)	–		[23]
 **9**	554 (4.05)	–		[31]
 **10**: R= H **11**: R= CH_3	511 (3.98) (**10**) 492 (3.96) (**11**)	– 428 (3.55) (**11**)	– –	[26]
 **12**	257 (4.43) 265.5 (4.42)	–	–	[22]
 **13**	484 (3.91)	672 (4.6)	–	[32]

^{1}H NMR spectrum of $(\mathbf{L^2})H^+$ revealed a C_2 symmetry in solution which could be explained similarly by a fast proton transfer involving two tautomers (Scheme 4b). Further protonation leads to a more symmetrical $(\mathbf{L^2})H_2{}^{2+}$ species, for which the four equivalent light-emitting benzylic groups are switched off (Scheme 3, Figures 5 and 6).

According to the thermodynamic Förster cycle method,[35] the frequency difference between absorption and emission could nevertheless be used to approximate the K^* values for $\mathbf{L^2}$.[36] $\log K_1^*$ and $\log K_2^*$ values amount to 9.0 ± 0.2 and 8.8 ± 0.3, respectively. These values are consistent with earlier results which showed that the nitrogens of the imine functions become more basic in the excited state S1.[37] Moreover, these results suggest a decrease of the repulsive interactions between the two positively charged halves of the $(\mathbf{L^2})H_2{}^{2+}$ species in the excited state (Scheme 4). For aryl-containing polyamine receptors, it has been demonstrated that unprotonated amine and/or imine groups are in most cases efficient intramolecular electron transfer quenchers of the aromatic excited state (photoinduced electron transfer, PET).[19,20,38,39] Our system stands in interesting contrast, which may be explained by an intramolecular charge transfer state (ICT)[40] from the amino to the imino nitrogen functions in $\mathbf{L^2}$ (Scheme 4). Theoretical calculations were performed on a model of **L** for which the N-substituent is a hydrogen. Its π molecular orbitals can be deduced from an interaction diagram between π orbitals of $[HN\text{-}(CH)_3\text{-}NH_2]$ and/or $[H_2N\text{-}(CH)_3\text{-}NH_2]^+$ fragments (Figure 6).[10] A comparison between the π orbitals of the protonated fragment $[H_2N\text{-}(CH)_3\text{-}NH_2]^+$ and the neutral [HN-

Table 2. (Continued)

Ligand	**L** $\lambda_{max}(\log\varepsilon^{\lambda^{max}})$ [nm]	$\mathbf{LH^+}$ $\lambda_{max}(\log\varepsilon^{\lambda^{max}})$ [nm]	$\mathbf{LH_2^{2+}}$ $\lambda_{max}(\log\varepsilon^{\lambda^{max}})$ [nm]	Ref.
 **14**	480 (3.51)	520 (3.93)	–	[27]
 **15**	448 (3.80)	517 (3.99)	–	[27]

[a] This work; solvent: CH_3OH/H_2O (80/20 *w*/*w*); $T=25.0\pm0.2$ °C; $I=0.5$ M. [b] This work; solvent: CH_2Cl_2; $T=25.0\pm0.2$ °C. Ref. [12]: solvent: CH_3CN/water (70/30 *v*/*v*); $T=25$ °C. Ref. [22]: solvent: water; $T=25$ °C; kinetic determinations. Ref. [23]: solvent: water; $T=20$ °C; kinetic determinations. Ref. [26]: solvent: water; $T=25$ °C; kinetic determinations. Ref. [27]: solvent: water; $T=25$ °C; kinetic determinations. Ref. [31]: solvent: water; $T=25$ °C; kinetic determinations. Ref. [32]: solvent: water; $T=25$ °C; kinetic determinations. Solvent: CH_2Cl_2; $T=25.0\pm0.2$ °C. Ref. [33]: solvent: CH_2Cl_2; $T=25.0$ °C.

Table 3. Protonation constants and emission data of ligand $\mathbf{L^2}$.[a]

Equilibrium	$\log\beta_n\pm3\sigma$	λ_{em}^{max} [nm][b]	$Q_{LH_n^{n+}}^{rel}/Q_L^{rel}$[c]
$\mathbf{L^2}$	–	480	1.00
$\mathbf{L^2} + H^+ \overset{\beta_1}{\rightleftharpoons} (\mathbf{L^2})H^+$	7.9 ± 0.3	477	0.91
$\mathbf{L^2} + H^+ \overset{\beta_2}{\rightleftharpoons} (\mathbf{L^2})H_2^{2+}$	13.9 ± 0.4	530	0.22

[a] Solvent: methanol/water (80/20 *w*/*w*); $T=25.0\pm0.2$ °C; $I=0.1$ M (NEt_4ClO_4). [b] $\lambda_{exc}=358$ nm; excitation and emission slits: 3 nm. [c] Determined from recalculated, normalised fluorescence spectra. The errors are estimated to 1 nm for the wavelengths and to 10% for the quantum yields.

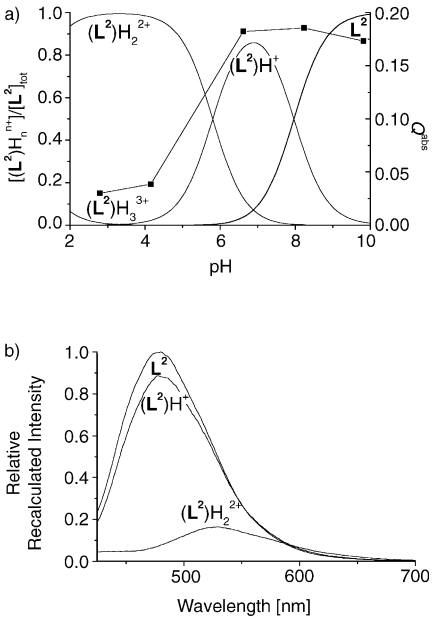


Figure 5. Ligand-centred emission quantum yields (a) and recalculated, normalised fluorescence spectra of $\mathbf{L^2}$, $(\mathbf{L^2})H^+$ and $(\mathbf{L^2})H_2^{2+}$ (b) Solvent: methanol/water (80/20 *w*/*w*); $T=25.0\pm0.2$ °C; $I=0.1$ M (NEt_4ClO_4). a) Protonation constants of Table 1; $[\mathbf{L^2}]_{tot}=7.58\times10^{-6}$ M; standard: quinine sulfate in 0.05 M H_2SO_4, $Q_{abs}=0.546$.

$(CH)_3$-NH_2] moiety shows a lowering of the symmetry from C_{2v} to C_i, respectively with a concomitant increase of the π-orbital coefficient of the imine group in **L**.

Therefore, electronic excitation leads to an intramolecular charge transfer from the donor (amino group) to the acceptor end (imino group), in agreement with the enhancement of the emission signal observed until pH ≈6.5. To explain the unexpected fluorescence quenching with decreasing pH, we suggest that the PET quenching mechanism occurs by protonation of the imine units in the excited state with the loss of the ICT character as shown in the interaction diagram between the π levels of two $[H_2N\text{-}(CH)_3\text{-}NH_2]^+$ fragment.[10] The small difference between the K_1* and K_2* values suggests a decrease of the π-electron delocalisation. The fluorescence quenching observed under acidic conditions is therefore mainly due to a possible PET deactivation mechanism.

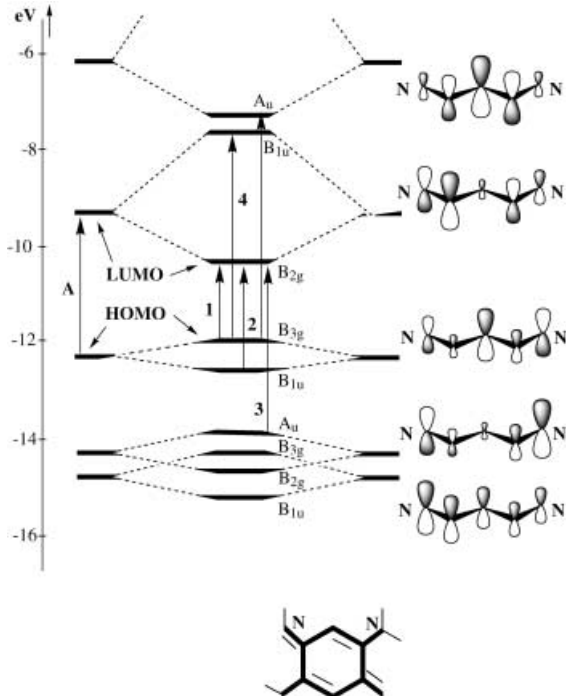


Figure 6. Orbital interaction diagram obtained for a model of **L** with R = H (Scheme 1) by means of EHT calculations. Imino nitrogen (left) and amino nitrogen (right).

Conclusion

Absorption and/or fluorescence spectrophotometric titrations of *p*-benzoquinonediimine derivatives (ligands $\mathbf{L^1}$ and

L^2), over a wide pH range, have enabled the determination of their acid–base properties in the ground and excited states. The structural changes observed in the solid state upon protonation are retained in solution as demonstrated by the complete physico-chemical studies presented here. The resonance effects in the protonated forms of L^1 and L^2 are triggered by protonation and especially lead to a fluorescent "on/off" switch L^2 modulated by proton input. Fluorescent cyanine-type dyes can be used as markers for different bio-applications,[6,21] and the detection of protons is of ongoing interest for monitoring the acidity regulation in biological media.[41] Valuable pH sensitive fluorescent dyes for biological applications require acid–base properties within the critical intracellular pH range (pH 5–8).[4] Molecule L^2 which was found to be sensitive in the interesting pH range between 4 and 7, gave rise to an unexpected large fluorescence decrease under acidic conditions (~80%). Therefore, L^2 can be regarded as a sensitive H^+ chemosensor with large Stokes shift (>120 nm) by comparison with the conventional fluorophores, such as fluorescein (25 nm at pH 9).[42] In conclusion, 2,5-diamino-1,4-benzoquinonediimines bearing methylene groups could constitute stable and tunable spectrophotometric probes with predetermined physico-chemical properties in aqueous solution.

Experimental Section

General: Analytical-grade reagents were obtained from commercial suppliers and were used as received. Azophenine (**5**) is a long known 2,5-diamino-1,4-benzoquinonediimine which can be obtained in various ways[43] from aniline. Solvents were distilled under argon prior to use and dried by standard methods. Distilled water was further purified by passing through a mixed bed of ion-exchanger (Bioblock Scientific R3-83002, M3-83006) and activated carbon (Bioblock Scientific ORC-83005) columns and was boiled for two hours and saturated with argon before use. Spectrophotometric grade methanol (Merck, SeccoSolv, p.a.) was also saturated with free-CO_2 and O_2 argon prior to use (Sigma Oxiclear cartridge).

CAUTION: *Perchlorate salts combined with organic ligands are potentially explosive and should be handled in small quantity and with the necessary precautions.*[44]

^{1}H NMR spectra were recorded in $CDCl_3$ and [D_6]DMSO on a AC300 Bruker spectrometer, operating at 300 MHz for ^{1}H NMR spectra. Chemical shifts are reported in δ units, in parts per million (ppm) relative to the singlet at $\delta = 7.26$ for $CDCl_3$. Splitting patterns are designed as s, singlet; m, multiplet; br, broad. In the ^{1}H NMR spectra of the protonated forms of L^2, the NH resonance could not be identified. This could result from rapid proton exchange with the other protonated forms present in small amount. Elemental analyses were performed by the "Service de microanalyses de L'Institut de Chimie", Strasbourg. EI mass spectral analyses were recorded on a Finnigan TSQ 700. The EHT calculations have been carried out as detailed in ref. [10].

1,2,4,5-Tetrabenzamidobenzene (4): Similarly to the procedure described for the synthesis of analogues,[10] tetraaminobenzene tetrahydrochloride (500 mg, 1.76 mmol) was reacted with benzoyl chloride (990 mg, 7.04 mmol) to afford **4** as a white solid (720 mg, 74%). ^{1}H NMR ([D_6]DMSO): δ = 7.5 (m, 12H, H_{arom}), 7.95 (m, 8H, H_{arom}), 8.01 (s, 2H, H_{arom}), 10.15 ppm (s, 4H, NH); MS (40 eV, EI): m/z: 554 [M^+]: elemental analysis calcd (%) for $C_{34}H_{26}N_4O_4$: C 73.63, H 4.73, N 10.10; found: C 72.53, H 4.67, N 10.03.

L^2: Similarly to the procedure described for the synthesis of analogues,[10] compound **4** (720 mg, 1.30 mmol) was reduced with $LiAlH_4$ and L^2 was obtained as a deep orange solid (458 mg, 71%). ^{1}H NMR ($CDCl_3$): δ = 4.44 (s, 8H, CH_2), 5.40 (s, 2H, $H_{olefinic}$), 6.90 (br s, 2H, NH), 7.29 ppm (m, 20H, H_{arom}); MS (40 eV, EI): m/z: 496 [M^+]; elemental analysis calcd (%) for $C_{34}H_{32}N_4$: C 82.21, H 6.50, N 11.29; found: C 80.96, H 6.49, N 11.23.

(L^2)H^+: Similarly to the procedure described for the synthesis of analogues[10] but using acetone as solvent instead of Et_2O during the work-up, a few drops of diluted HCl were added to a solution of L^2 dissolved in THF until the colour changed from yellow to deep red. (L^2)H^+ was obtained as a red solid (145 mg, 56%). ^{1}H NMR ($CDCl_3$): δ = 4.51 (br s, 8H, CH_2), 5.33 (br s, 2H, $H_{olefinic}$), 7.20–7.26 (m, not integrated owing the $CDCl_3$ resonance, H_{arom}), 7.30–7.35 ppm (m, 12H, H_{arom}); elemental analysis calcd (%) for $C_{34}H_{33}ClN_4{\cdot}H_2O$: C 74.10, H 6.40, N 10.17; found: C 72.93, H 6.05, N 10.06.

(L^2)H_2^{2+}: Similarly to the procedure described for the synthesis of analogues,[10] a large excess of HCl was added to a solution of L^2 dissolved in THF. (L^2)H_2^{2+} was obtained as a green solid (118 mg, 67%). ^{1}H NMR ($CDCl_3$): δ = 4.63 (s, 8H, CH_2), 5.33 (br s, 2H, $H_{olefinic}$), 7.19–7.24 (m, 8H, H_{arom}), 7.30–7.35 ppm (m, 12H, H_{arom}); elemental analysis calcd (%) for $C_{34}H_{34}Cl_2N_4$: C 71.70, H 6.02, N 9.84; found: C 70.71, H 6.09, N 9.68.

Potentiometric and UV/Vis titrations: All solutions were prepared using an AG 245 Mettler Toledo analytical balance (precision 0.01 mg). The ionic strength was adjusted to 0.1 M or 0.5 M with tetraethylammonium perchlorate (Fluka, purum) and tetrabutylammonium perchlorate (Fluka, purum), respectively. Solutions of L^1 (5.23×10^{-5} M and 5.76×10^{-5} M) and L^2 (4.23×10^{-5} M and 4.35×10^{-5} M) were prepared by quantitative dissolution of a solid sample in methanol/water (80/20 *w*/*w*). An aliquot of 40 mL was introduced into a jacketed cell (Metrohm) maintained at 25.0±0.2 °C by the flow of a Haake FJ thermostat. The free hydrogen ion concentrations were measured with a combined glass electrode (Metrohm 6.0234.500, Long Life). The Ag/AgCl reference electrode was filled with 0.1 M NaCl (Fluka, p.a.) in MeOH/H_2O (80/20 *w*/*w*). Potential differences were given by a Tacussel Isis 20.000 millivoltmeter. Standardisation of the millivoltmeter and verification of the linearity ($2.00 < pH < 13.60$) of the electrode were performed using buffers according to classical methods.[45] The titration of L^1 ($2.66 < pH < 10.19$, [L^1] = 5.76×10^{-5} M) and L^2 ($2.86 < pH < 8.03$, [L^2] = 4.23×10^{-5} M) were carried out by addition of known volumes of 8.27×10^{-3} M tetrabutylammonium hydroxide solution. The titration of L^1 ($1.31 < pH < 2.20$, [L^1] = 5.23×10^{-5} M) and L^2 ($1.30 < pH < 2.52$, [L^2] = 4.35×10^{-5} M) were carried out by addition of known volumes of a 9.98×10^{-1} M perchloric acid solution. Special care was taken to ensure that complete equilibration was attained. Simultaneous pH and UV/Vis measurements (230–600 nm) were recorded. Absorption spectra were recorded using a Varian CARY 50 (Varian) probe UV/Vis spectrophotometer fitted with Hellma optical fibres (Hellma, 041.002-UV) and immersion probe made of quartz suprazil (Hellma, 661.500-QX).

Potentiometric and fluorescence titrations: A stock solution of L^2 (7.58×10^{-6} M, I = 0.1 M NEt_4ClO_4, Fluka, puriss) was prepared by dilution of a mother solution (3.76×10^{-6} M) and an aliquot of 40 mL was introduced into a jacketed cell (Metrohm) maintained at 25.0±0.2 °C by the flow of a Haake FJ thermostat. The initial pH was adjusted at 3.34 with perchloric acid (1.15 M), and the titration of L^2 was carried out by addition of known volumes of a 4.05×10^{-2} M tetrabutylammonium hydroxide solution. An aliquot (2 mL) was taken after each addition of base, and fluorescence emission spectra (350–700 nm) were recorded versus pH with 1 cm quartz optical cell (Hellma, 110-QS) on a Perkin–Elmer LS-50B maintained at 25.0±0.2 °C by the flow of a Haake FJ thermostat. The excitation wavelength was 358±1 nm and the slit widths were set at 3 nm for both excitation and emission. The light source was a pulsed xenon flash lamp with a pulse width at half peak height < 10 μs and power equivalent to 20 kW. Absorption spectra were also measured along the fluorescence titration by using a Varian CARY 300 spectrophotometer.

Refinement of the data: The spectrophotometric data were processed with both the Specfit[24] and Letagrop-Spefo[25] programs, which adjust the stability constants and the corresponding extinction coefficients of the species formed at equilibrium. Letagrop-Spefo[25] uses the Newton–Raphson algorithm to solve mass balance equations and a pit-mapping method to minimise the errors and determine the best values of the parameters. Specfit[24] uses factor analysis to reduce the absorbance matrix and to extract the eigenvalues prior to the multiwavelength fit of the reduced data set according to the Marquardt algorithm.[46]

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