Acid–base strength and acido(fluoro)chromism of three push–pull derivatives of 2,6-distyrylpyridine

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Abstract

The acidochromism and acid–base properties of 2,6-distyrylpyridine (2,6-DStP) derivatives bearing on the sides push/pull substituents (namely two dimethylamino, one nitro, and one methoxy and two nitro groups in the case of 2,6-bis[(E)-2-(4-dimethylaminophenyl)ethenyl]pyridine, 2-[(E)-2-(4-nitrophenyl)ethenyl], 6-[(E)-2'-(4'-methoxyphenyl)ethenyl]pyridine and 2,6-bis[(E)-2-(4-nitrophenyl)ethenyl]pyridine, respectively) were investigated by stationary and time-resolved spectroscopies. The sensitivity of the absorption and emission spectrum to the medium acidity was found to enhance in the dimethylamino-derivative relative to the unsubstituted 2,6-DStP, also because of the second protonation by the $N(\text{CH}_3)_2$ group. Spectrophotometric titrations, also processed by a global fitting approach, gave pK_a values, for the protonation of the central pyridine, higher in the derivatives with electron-donor unities and lower in compounds bearing electron-acceptor groups. A fluorometric titration was performed in the case of the dimethylamino-derivative thanks to non-negligible emission efficiencies for both neutral and protonated species, unveiling an attractive naked-eye acido(fluoro)chromism from green to yellow upon pyridine protonation, and then to purple with the second protonation involving the lateral $N(\text{CH}_3)_2$ substituent. Due to the extremely short excited-state lifetimes, as resulted from femtosecond transient absorption experiments, the pK_a values for the excited state (pK_a^*) were estimated through the Förster cycle, revealing that the monoprotonated species of the dimethylamino-derivative would become upon excitation the only stable form in a wide range of pH.

Graphical abstract

Keywords Acid–base properties · Acido(fluoro)chromism · Push–pull systems · 2,6-Distyrylpyridine derivatives · Spectroscopic titrations, Förster cycle

Dedicated to Prof. Marija Šindler-Kulyk on the occasion of her 80th birthday.

Extended author information available on the last page of the article

1 Introduction

The excited-state behavior of aza-analogs of stilbenoid compounds, where one or two phenyl groups are substituted by pyridyl groups, has been extensively investigated in our [1–8] and other laboratories [9–13]. The acid–base equilibria and the protonation effect on the photobehavior of these aza-compounds in aqueous solutions have also attracted interest [14–22]. Inter alia, the 1,3-distyrylbenzene (1,3-DStB) analog where the central benzene ring was replaced by a pyridine, namely the 2,6-distyrylpyridine (2,6-DStP), has been studied to evidence the effect of the introduction of the heteroatom in the photophysical behavior and the rotamerism of the *EE* stereoisomer [23]. The comparison of 1,3-DStB and 2,6-DStP behavior in non-protic solvents has shown the noticeable effect of the central pyridine group in determining the structure of the most stable rotamer and the nature of its lowest excited states. Formation of intramolecular hydrogen bonds between the nitrogen and the next ethylene hydrogens in pyridine derivatives is known to stabilize the sterically favored longer-lived rotamer with the lowest emitting state of allowed character, thus leading to a different excited-state behavior from that of the parent hydrocarbon [24]. The conformational equilibrium in such pyridine-derivatives was found to be controlled by the proticity of the solvent [25]. In protic solvents strong intermolecular hydrogen bonds relax the intramolecular N–H interactions, that involve ethylenic hydrogens, destabilizing the most abundant and fluorescent species. Moreover, the presence of the central pyridine makes 2,6-DStP sensitive to the acidity of the medium.

Additionally, the introduction of side electron-donor (D) and/or electron-acceptor (A) units such as dimethyl-amino, methoxy, and nitro groups gives to the systems push–pull character [26–29] making these molecules interesting as candidate components in devices in the fields of optoelectronics, medicine, and communications [30–34]. The push–pull character of such systems implies marked solvatochromic behavior coupled with non-negligible non-linear optical (NLO) properties [35–39]. In this type of systems, such features can also be coupled with acidochromic behavior, which becomes particularly appealing when the pK_a values fall within mildly acidic or nearly neutral pH ranges. The possibility of detecting pH in biological fluids and mapping its values within living organisms in a non-invasive manner is indeed highly desirable, and acidochromic probes are undoubtedly up to the task [40, 41]. In particular, water-soluble acidochromic compounds may allow healthy tissues and tumor cells to be recognized based on the dysregulated pH within the interstitial space of solid tumors (5.0) relative to blood plasma (7.4) [42].

Scheme 1 Molecular structures of *E,E*-4,4'-pyridine-2,6-diylbis(ethene-2,1-diyl))bis(*N,N*-dimethylaniline) (DAQP), *E,E*-2-(*p*-nitrostyryl)-5-(*p*-methoxystyryl)pyridine (MIX) and *E,E*-2,6-bis[2-(4-nitrophenyl)ethenyl]pyridine (QP)

In the present work, we aim at investigating the effects of pH on the spectral and photophysical properties of the three push–pull compounds shown in Scheme 1, namely a dipolar-type molecule with a donor–bridge–acceptor (D– π –A) motif, *E,E*-2-(*p*-nitrostyryl)-5-(*p*-methoxystyryl)pyridine (MIX), and two quadrupolar-type molecules with a D– π –A– π –D and A– π –D– π –A motives, *E,E*-4,4'-pyridine-2,6-diylbis(ethene-2,1-diyl))bis(*N,N*-dimethylaniline) (DAQP) and *E,E*-2,6-bis[2-(4-nitrophenyl)ethenyl]pyridine (QP), respectively. The comparison with the protonation effects already studied in the case of the unsubstituted 2,6-distyrylpyridine will allow some generalizations.

2 Experimental

2.1 Chemicals

The investigated compounds are shown in Scheme 1. The compounds were investigated in aqueous solutions (deionized water, PURELAB option, ELGA). The addition of some percentage of acetonitrile (MeCN, VWR Chemicals) was necessary due to solubility issues. The different pHs were obtained by use of commercial buffer solutions (Pan-Reac AppliChem by ITW Reagents) in the range 4–8, while HClO₄ acid was added to reach pH < 4, down to *H*₀ = –1.08.

2.2 Synthesis and characterization of the 2,6-distyrylpyridine products

MIX and QP were synthesized for previous papers (see Refs. [35, 36] and Sect. 1 in the dedicated Supporting Information section). The newly synthesized DAQP was prepared according to the procedure described below.

Preparation of *E,E*-4,4'-pyridine-2,6-diylbis(ethene-2,1-diyl))bis(*N,N*-dimethylaniline) (DAQP): To a stirred

solution of 2,6-dimethylpyridine in tetrachloromethane, *N*-bromosuccinimide (2 eq) and azobisisobutyronitrile (0.1 eq) were added. The reaction mixture was heated on reflux and irradiated with a halogen lamp (75 W) overnight. After cooling down to RT, the reaction mixture was filtrated to remove succinimide and evaporated. After the removal of the solvent, extraction with dichloromethane and water was carried out. The extract was dried and concentrated. The crude product was dissolved in benzene and triphenylphosphine (PPh₃) in benzene was added. After stirring overnight at RT, the precipitate was filtered off and used in the next step after drying. To a stirred solution of obtained phosphonium salt (0.45 mmol) and *p*-dimethylaminobenzaldehyde (0.45 mmol) in ethanol sodium ethoxide was dropwise added (10.3 mg, 0.45 mmol Na dissolved in 5-mL ethanol). Stirring was continued for 4 h at RT. After removal of the solvent, the residue was worked up with water and toluene and dried with MgSO₄. The crude reaction product was chromatographed and the mixtures of isomers were obtained on silica gel column using petroleum ether/diethylether mixture as eluent (5%). The mixture of isomers was dissolved in toluene and a catalytic amount of I₂ was added. After 4 h on reflux, TLC was checked and it could be concluded that only one isomer was present in the reaction. After removal of the solvent, the crude reaction product was chromatographed and the pure *E,E*-isomer was obtained on silica gel column using petroleum ether/diethylether mixture as eluent (5%).

DAQP: orange solid; mp 119–120 °C; R_f =0.4 (petroleum ether); ¹H NMR (CDCl₃, 600 MHz) δ /ppm: 7.53–7.50 (m, 4H), 7.51 (d, 2H, J =16.2 Hz), 7.21 (d, 1H, J =7.5 Hz), 7.00 (d, 2H, J =16.2 Hz), 6.95 (d, 2H, J =7.5 Hz), 6.72 (d, 4H, J =8.8 Hz), 3.00 (s, 12H); ¹³C NMR (CDCl₃, 150 MHz) δ /ppm: 155.5 (2s), 149.9 (2s), 136.0 (2d), 127.8 (4d), 124.6 (2s), 120.2 (2d), 117.5 (2d), 117.3 (d), 111.7 (4d), 39.8 (4q); HRMS (TOF ES⁺) m/z calculated for C₂₅H₂₇N₃ 369.2265; found 369.2262.

Characterization: The ¹H and ¹³C NMR spectra were recorded on a spectrometer at 300 and 600 MHz. All NMR spectra were measured in CDCl₃ using tetramethylsilane as reference. High-resolution mass spectra (HRMS) were obtained on a matrix-assisted laser desorption/ionization time-of-flight MALDI-TOF/TOF mass spectrometer (4800 Plus MALDI-TOF/TOF analyzer, Applied Biosystems Inc., Foster City, CA, USA) equipped with Nd:YAG laser operating at 355 nm with firing rate 200 Hz in the positive ion reflector mode. 1600 shots per spectrum were taken with mass range 100–1000 Da, focus mass 500 Da and delay time 100 ns. Nicotinamide and azithromycin were used for external mass calibration in positive ion mode. Each spectrum was internally calibrated, providing measured mass accuracy within 5 ppm of theoretical mass. Melting points were obtained using an Original Kofler Mikroheitzisch apparatus (Reichert, Wien) and are uncorrected. Silica gel (Merck

0.063–0.2 mm) and aluminum oxide (Merck 0.063–0.2 mm) were used for chromatographic purifications. Solvents were purified by distillation. The boiling range of petroleum ether, used for chromatographic separation, was 40–70 °C. 6-Methyl-2-pyridinecarboxaldehyde, 2,6-dimethylpyridine, *p*-dimethylaminobenzaldehyde, 6-methyl-2-pyridinecarboxaldehyde, *p*-nitrobenzaldehyde, and 2,6-pyridinedicarboxaldehyde were obtained from a commercial source.

2.3 Stationary spectroscopic techniques

Steady-state absorption measurements were carried out by a Cary 4E (Varian) spectrophotometer. A Spex Fluorolog-2 F112AI spectrofluorometer was instead used for excitation and emission fluorescence spectra at room temperature. Air-equilibrated and de-aerated dilute solutions (absorbance < 0.1 at the excitation wavelength) were used for the fluorometric measurements. The fluorescence quantum yields (ϕ_F , experimental error $\pm 10\%$ and $\pm 20\%$ when $\phi_F \leq 10^{-4}$) were obtained by employing tetracene (ϕ_F =0.17 in air-equilibrated cyclohexane) [43] and 9,10-diphenylanthracene (ϕ_F =0.73 in air-equilibrated cyclohexane) [44] as reference compounds, taking also into account the different refractive indexes of the used solvents. To determine the pK_a of the acid–base equilibria, as well as the concentration profiles and the spectra of intermediate species, the global fitting of multivariate spectrophotometric data was carried out by the ReactLab Equilibria software (Jplus Consulting). The parameters sum-of-squares (ssq) and deviation standard for the residuals (σ) were used to evaluate the goodness of the fits.

2.4 Förster cycle for determining pK_a*

From an experimental point of view, the direct measurement of the constant for the acid–base equilibrium in the singlet excited state (pK_a*) was performed by fluorometric titration in the presence of fast partial or complete acid–base re-equilibration in the excited state, the latter being operative if it is fast enough to efficiently compete with the deactivation processes of the basic and acidic forms in S₁. In systems where no equilibration in the excited state is observed or for systems scarcely fluorescent, an (approximate) estimation of pK_a* could be carried out by the indirect method proposed by Förster [45, 46] and Weller [47–50]. pK_a* can be estimated according to Eq. (1) knowing pK_a in the ground state and measuring the energy difference between the 0,0 transitions of the protonated and neutral forms ($\Delta\nu$). The latter was estimated from the intersection point of both the normalized absorption and fluorescence spectra of the two species (see below, Fig. 5).

$$\Delta pK = pK_a^* - pK_a = 0.625 \Delta \nu (\text{cm}^{-1}) / T. \quad (1)$$

2.5 Transient absorption measurements

The experimental setup for ultrafast transient absorption experiments has previously been reported [29, 51–53]. The 400 nm excitation pulses (ca. 60 fs) are generated by an amplified Ti: Sapphire laser system (Spectra Physics). The Helios transient absorption spectrometer (Ultrafast Systems) is characterized by a temporal resolution of about 150 fs and a spectral resolution of 1.5 nm. A small portion of the 800-nm light passes through an optical delay line (time window of 3200 ps) and is focused onto a Sapphire crystal (2 mm thick) to generate a white-light in the 450–800 nm spectral range (probe pulse). All measurements were carried out under the magic angle in a 2-mm cell at an absorbance of about 0.5 at 400 nm (concentration $\approx 2 \times 10^{-4}$ M). The solution was stirred during the experiments to avoid photoproduct interferences. Photodegradation was checked recording the absorption spectra before and after the time-resolved measurement where no significant change was observed. Transient absorption data were analyzed using the Surface Explorer PRO (Ultrafast Systems) and GloTarAn software. The former performs Singular Value Deconvolution of the 3D matrix to extract principal components (spectra and kinetics), and successively Global Analysis to obtain the Decay Associated Spectra, DAS. The latter was used to perform Target Analysis assuming successive steps to describe the evolution of transients providing the Species Associated Spectra (SAS) [54, 55].

2.6 Computational details

Quantum–mechanical calculations were carried out using the Gaussian 16 package [56]. Density functional theory (DFT) based on the B3LYP method [57] was used to optimize the geometry and to obtain the properties of the substrates in the ground state, whereas the lowest excited singlet states were characterized by time-dependent (TD) DFT WB97XD excited-state calculations [58]. In both cases, a 6–31+G(d) basis set was employed. Water solvation effects were included in the calculations by means of the conductor-like polarizable continuum model (CPCM) [59].

3 Results and discussion

3.1 Spectrophotometric and fluorometric titrations

The investigated compounds exhibited a different response to light absorption depending on the acidity of the medium. The titrations were carried out in aqueous buffers adding

different percentages of MeCN (% v/v) for solubility issues based on the system considered. The spectrophotometric titrations and their fitting results are shown in Figs. 1, 2, S1 and S2. In the case of DAQP, to get an insight into the effect exerted on the pKa values by the different amounts of MeCN, two spectrophotometric titrations were performed at the lowest (20% v/v MeCN, Fig. 1) and highest (60% v/v MeCN, Fig. S2) percentages of organic solvent required by these molecular systems. Starting from a neutral pH (7/8), the increase of the solution acidity causes a red-shift of the absorption spectrum of all the studied compounds. In the case of the dimethylamino-derivative, a further decrease of the medium pH leads to the drop of the bathochromic band and the parallel appearance of new absorption in the UV region, at higher energy if compared with the spectrum recorded in neutral solutions, pointing to the presence of two acid–base equilibria in the large pH range investigated. The spectrophotometric titrations followed at suitable monitoring wavelengths (430, 393, and 376 nm for DAQP, MIX, and QP, respectively) provided the pKa values reported in Table 1. The protonation of the central pyridine gives rise to a red-shift of the absorption spectrum, as expected [14–16, 21] and confirmed by the quantum–mechanical calculations (see below). Therefore, the first site of protonation is supposed to be the nitrogen of the central pyridine unit also for DAQP, while the second site of protonation in the latter compound is the nitrogen of the dimethylamino group, in agreement with the significant blue-shift of the absorption spectrum [60, 61]. When a higher percentage of MeCN was used (Fig. S2), analogous spectral modifications were recorded and the same acid–base equilibria could be recognized for DAQP, although a reduction is peculiar to both pKa values, with the second acid–base equilibrium being more affected by the increased amount of MeCN ($pK_{a1} = 5.4$ vs. 5.6 and $pK_{a2} = 1.6$ vs. 2.8 at 60% v/v and 20% v/v, respectively). As is the case for the first acid–base constant, these differences are however small and do not prevent the comparison between the pKa of the three molecules for the protonation of the central pyridine (see below).

In the case of the most fluorescent DAQP, it was possible to carry out a fluorometric titration (Figs. 3, S3). This was performed exciting at two excitation wavelengths (355 and 384 nm, left and right panels of Fig. 3, respectively) that represent the isosbestic points between the three different species observed (neutral, DAQP, mono-protonated, DAQPH^+ , and bis-protonated, DAQPH_2^{2+}). A bell-shaped emission spectrum centered at 503 nm was recorded at pH 7/8. The increase of the medium acidity induces the disappearance of this band and the simultaneous growth of a new band centered at 596 nm. A further pH reduction implies a new acid–base equilibrium of fluorescent species to be observed, the 596-nm band decreases in intensity until it completely disappears at

Fig. 1 (Top panel) Acidochromism of DAQP in Water/MeCN mixture: pH = 1 (left), 4 (middle) and 7 (right). (Bottom panels) Spectrophotometric titration (left) of compound DAQP (20% v/v MeCN) in a large pH range and relative pK_a fitting at 430 nm (right)

Fig. 2 Spectrophotometric titration of compound MIX (30% v/v MeCN, left graph) and QP (60% v/v MeCN, right graph) in a large pH range

pH ≤ 0 to the advantage of a hypsochromic band centered at 395 nm. The best fitting of the fluorometric titration followed at suitable wavelengths by acid–base equations furnished the pK_a values also reported in Table 1.

3.2 pK_a* determination by the Förster cycle

The very low fluorescent quantum yields of the investigated compounds (see below Table 2) ruled out the possibility

Table 1 Acid–base properties of DAQP, MIX, and QP

Compound	$\lambda_{\text{abs}}/\text{nm}$	$\lambda_{\text{em}}/\text{nm}$	pK _a ^a	pK _a (fluo) ^b	pK _a (ReactLab) ^c	pK _a ^{*d}
2,6-DStP	289, 335	408	4.3			10.1
2,6-DStPH ⁺	272 ^{sh} , 300, 372	460				
DAQP	356	503				
DAQPH ⁺	430	596	5.6 ± 0.1	5.9 ± 0.1	5.67	13.9
DAQPH ₂ ²⁺	328	395	2.8 ± 0.1	3.1 ± 0.2	2.63	−16.1
MIX	327, 370 ^{sh}	420	3.4 ± 0.1		3.45	9.2
MIXH ⁺	329 ^{sh} , 394	450				
QP	277 ^{sh} , 328 ^{sh} , 366	405	2.0 ± 0.1		2.05	4.0
QPH ⁺	297 ^{sh} , 376	430				

Absorption (λ_{abs}) and emission (λ_{em}) maxima, acid–base equilibrium constant in the ground (pK_a) and excited (pK_a^{*}) states. The properties of 2,6-DStP are also reported for comparison purposes [21]

^aDetermined by spectrophotometric titrations analyzed by best fitting at suitable absorption wavelengths

^bDetermined by fluorometric titrations analyzed by best fitting at suitable emission wavelengths

^cDetermined by spectrophotometric titrations elaborated by the global analysis performed by ReactLab software

^dCalculated using the Förster cycle, Eq. (1)

Fig. 3 Spectrofluorimetric titrations of compound DAQP (20% v/v MeCN) in a large pH range by exciting the sample at 355 nm (left) and 384 nm (right)

to experimentally determine the constant for the acid–base equilibrium in the singlet excited state (pK_a^{*}). Moreover, in the case of the most fluorescent DAQP, no clear sign of re-equilibration in S₁ was observed, in agreement with the very short lifetimes of the first singlet excited state measured using transient absorption spectroscopy with femtosecond resolution in time (see below Table 2). This finding suggests that proton transfer in S₁ fails to compete with fast non-radiative deactivation paths, as trans→cis photoisomerization and, in the case of QP, triplet production through intersystem crossing (see below paragraph 3.3, Table S6). Therefore, pK_a^{*} values were estimated through the Förster cycle by applying Eq. (1). The obtained results are shown in Table 1. All the three compounds become stronger bases

upon photoexcitation, as reported in the literature in the case of the analogous 2-styrylpyridine and 2,6-DStP [14–16, 21]. On the contrary, the bis-protonated DAQP becomes a much stronger acid in the excited state in agreement with the calculated negative pK_a^{*} (Table 1).

A comparison with 2,6-DStP shows the role of different substituents on the acid–base properties of the studied compounds (see Table 1). The presence of nitro, dimethylamino, and methoxy groups always produces a red-shift of the absorption and emission spectra relative to the unsubstituted 2,6-DStP, due to a partial charge transfer character of the absorption transitions, as pointed out by the calculations (see below and Sect. 2 of Supp. Inf.). Accordingly, the largest bathochromic shift, both in absorption and emission, was

Table 2 Emission properties (fluorescence quantum yield in air equilibrated solutions, ϕ_{air} , S_1 lifetimes, τ_F , and relative kinetic constant, k_F) of compounds DAQP, MIX, and QP in water/MeCN mixtures at different pHs

	pH	$\lambda_{\text{exc}}/\text{nm}$	ϕ_{air}	τ_F/ps	$k_F/10^8 \text{ s}^{-1}$
DAQP	1	330	1.4×10^{-3}	23	1.9
	2	329	2.1×10^{-3}		
	3	329	2.8×10^{-3}		
	4	431	4.4×10^{-3}		
	5	431	6.3×10^{-3}		
	6	363	1.82×10^{-2}		
	7	357	2.46×10^{-2}		
	8	357	1.80×10^{-2}		
MIX	1	395	1×10^{-4}	0.49	2.0
	5	329	1.3×10^{-3}		
	6	329	7×10^{-4}		
	8	327	7×10^{-4}		
QP	1	377	1.3×10^{-3}	16	0.81
	4	367	7×10^{-4}		
	6	327	1.1×10^{-3}		
	8	327	1.5×10^{-3}		

Kinetic constants were obtained by considering S_1 lifetimes provided by femtosecond transient absorption experiments with $\lambda_{\text{pump}} = 400 \text{ nm}$ reported in Table S6

observed for the monoprotonated DAQPH⁺ with the central charged pyridinium unit and the side strong electron-donor dimethylamino, while negligible red-shift and blue-shift, in the absorption and emission spectrum respectively, were recorded in the case of the monoprotonated QPH⁺, bearing the electron-withdrawing nitro-groups.

An electron-donor group, such as the dimethylamino in the case of DAQP, makes the pKa and pKa* values higher than those shown by the reference molecule. Thus, the effect of the strong electron-donor group is to facilitate the protonation of the central pyridine, making this unit a stronger base. On the contrary, when an electron-withdrawing group, such as the nitro-group, is present both the pKa and pKa* decrease by 1–2 units (respectively for MIX and QP) if compared to the unsubstituted 2,6-DStP. The effect is stronger for QP where there are two strong electron-acceptor groups. An interesting result of the application of the Förster cycle is that the photoexcitation not only makes the monoprotonated form of DAQP stable in a wide pH range, but this range is much wider if compared to that related to the ground state. If the acid–base equilibrium was kinetically possible, the monoprotonated species in the excited state would always prevail.

3.3 ReactLab simulations and quantum–mechanical calculations

A global fitting procedure of all the spectral data matrix using the React-Lab™ Equilibria Program gave the molar absorption coefficients and the concentration profiles as a function of pH of three different species in the case of DAQP (shown in Fig. 4, upper panel) and two species for MIX and QP in Fig. 4, middle and lower panels, respectively. The more reliable pKa values calculated through the global fitting are reported in the sixth column of Table 1 and are in fair agreement with those values determined by spectrophotometric and fluorometric titrations analyzed at suitable wavelengths.

It has to be recalled that these flexible molecules can exist in fluid solutions at room temperature as a dynamic equilibrium among different conformers [60] (elongated and compressed) originating by rotation of the vinyl groups around the quasi-single bonds between the central pyridine and the double bonds (Scheme 2). In the case of 2,6-DStP [21] and even more for MIX [36] and QP [35], the prevalence of the compressed rotamers is due to the presence of intramolecular H-type N⋯H bonds involving the nearby ethene hydrogens. However, in protic solvents, the establishment of strong intermolecular bonds weakens the N⋯H intramolecular interactions causing the conformational equilibrium to shift towards the elongated species [23–25]. Moreover, in the protonated 2,6-DStP and its derivatives the conformational equilibrium was found to be largely shifted towards the elongated geometries owing to steric hindrance in the compressed conformations among the proton and the ethene hydrogens [21]. For these reasons, in the present paper the elongated rotamers of the investigated compounds were assumed to be largely prevalent in solution and the quantum mechanical calculations were performed on the elongated species only.

The optimized structures in the ground state, the calculated electronic spectra together with the frontier molecular orbitals involved in the first absorption transitions for the protonated species of the investigated compounds and the neutral DAQP are reported in Figs. S4, S5, Tables S1, S2, S3, S4 and S5 in the dedicated Supporting Information section and compared with those previously reported for the neutral QP [35] and MIX [38]. The calculated spectra are in fair agreement with the experimental ones and are also shown as vertical bars in Fig. 4. The only data which disagree with the experimental findings are those relating to the absorption spectrum of the bis-protonated DAQPH₂²⁺ for which the calculations resulted to be unable to reproduce the experimental strong blue-shift observed. In fact, the absorption maximum experimentally detected for the second protonation of DAQP was found to move from 430 to 328 nm,

Fig. 4 Molar absorption coefficients (left panels) and the concentration profiles as a function of pH (right panels) of three different species in the case of DAQP (upper panel) and two species for MIX (middle panel) and QP (lower panel). The calculated energy of the

absorption transitions with their oscillator strength are also reported as red bars for the monoprotonated species and black bars for the neutral species. In the case of the neutral species of QP and MIX, the calculated data are retrieved from ref. 35 and 38, respectively

Scheme 2 Conformational equilibrium of 2,6-DStP derivatives

Fig. 5 Normalized absorption and emission spectra of the neutral and protonated species of DAQP in water/MeCN mixtures at different pHs. The intersection points between the normalized absorption and emission spectra of the neutral and protonated species are also reported

while a much smaller shift from 460 to 430 nm is predicted by calculations (see Table S5).

3.4 Spectral and fluorescence properties of neutral and protonated species

The absorption and emission spectra peculiar to the different neutral and protonated species for the investigated compounds were collected and normalized in Fig. 5 for the case of DAQP and in Fig. S16 for the remaining compounds. The intersection points between the absorption and relative fluorescence for the different species used to estimate the 0,0 transition energies to be manipulated in the Förster cycle equation are also reported in Figs. 5 and S15.

All the compounds proved weakly emitting in all the investigated pH ranges. DAQP is the most fluorescent with $\phi_F \cong 2 \times 10^{-2}$ for the base. Protonation generally causes a decrease of the fluorescence quantum yield, roughly of one order of magnitude in the case of DAQP and MIX (Table 2).

For all the monoprotonated species of the investigated compounds, very short transient lifetimes (from fractions to tens of picoseconds, Fig. 6) were obtained by femtosecond transient absorption measurements, in accordance with the very low fluorescence yields measured with steady-state techniques and with the presence of intramolecular charge transfer processes under photoexcitation as highlighted by the calculated HOMO–LUMO orbitals (see Fig. 7 for the case of MIXH⁺ and related Figs. S6, S7, S8, S9, S10, S11, S12, S13, S14 and S15 in Sect. 3 of the Supplementary Information for the other compounds). In the case of DAQP,

Fig. 6 Kinetics (open black circles) obtained from the femtosecond transient absorption experiments of DAQPH⁺, MIXH⁺ and QPH⁺ ($\lambda_{exc}=400$ nm) at significant wavelengths, and their fittings (solid red line). The negative signal recorded for DAQPH⁺ is due to stimulated emission, while the positive amplitude in MIXH⁺ and QPH⁺ kinetics is ascribable to excited-state absorption

where the dimethylamino groups act as electron-donating groups, in the LUMO configuration, the electronic charge is located primarily on the central pyridine (Fig. S10). The charge movement under excitation is predicted to be more important in DAQPH⁺ owing to the positively charged electron-acceptor pyridinium unit (Fig. S12). For the bis-protonated DAQPH₂²⁺, a complete localization of the excitation in the arm bearing the non-protonated dimethylamino group is predicted, in agreement with the loss of conjugation accompanied by the strong blue shift of the absorption and emission spectra (Fig. S14). In QP the two nitro-electron-withdrawing groups attract the charge density from the central pyridine following the transition. Finally, for the asymmetric compound, MIX, there is an evident charge transfer from one arm of the molecule to the other, specifically from the methoxy group to the nitro group at the opposite edge of the structure (Fig. 7). The short singlet lifetimes provided by femtosecond transient absorption data were used to evaluate the kinetic constant values of the radiative process which were found to be fully allowed, even though not efficient,

Fig. 7 Frontier molecular orbitals of MIXH⁺

for all the protonated species of the three compounds under study (see the last column in Table 2).

4 Conclusions

The acid–base properties of three derivatives of the 2,6-distyrylpyridine bearing electron-donor and electron-acceptor groups were studied in buffered water with adding some percentage of acetonitrile for solubility problems. Their absorption and emission spectra were found to shift towards the red when protonating the nitrogen of the central pyridine ring, while the protonation of the nitrogen of the dimethylamino group of DAQP causes a marked blue shift. This behavior gives rise to an eye-catching acidochromism for the DAQP fluorescence, where the green emission of the neutral species turns yellow under protonation of the pyridine and changes to violet when the second protonation has also occurred. The pK_a values were provided by spectrophotometric titrations applying the global fitting of multivariate data by the ReactLab Equilibria software. The pK_a in the excited state (pK_a^{*}), on the other hand, was estimated through the Förster cycle. The protonation of the nitrogen of the central pyridine proved easier relative to the unsubstituted 2,6-DStP, both in the ground and excited states, when strong electron-donor groups are present, as observed in the dimethylamino-derivative DAQP. Conversely, strong electron-withdrawing units at the ends of 2,6-DStP cause the depletion of the electron

density on the pyridine nitrogen making the protonation harder, as experienced for MIX, where the effect of the nitro group overcomes that of the mild electron-donor methoxy substituent, and even more for QP since it bears two nitro groups. In the case of DAQP, a second protonation was observed in the explored pH range due to the protonation of the nitrogen of one dimethylamino group. Interestingly, the monoprotonated species was found to be the only thermodynamically favored form in the excited state, as suggested by the application of the Förster cycle, although any possible excited-state re-equilibration is prevented by the extremely short S₁ lifetime (tens of picoseconds) due to the intramolecular charge transfer occurring upon photoexcitation, as proved by femtosecond transient absorption experiments and TD-DFT calculations. However, the marked acido(fluoro)chromic behavior exhibited by DAQP together with a pK_a value of about 5.6 are all attractive features in light of possible applications as fluorescent probes of pH modulation in living cells, for recognition of healthy tissues from tumor cells. Hence, the present results, pointing out the chromism of the investigated systems as a function of pH and the relation between pK_a/pK_a^{*} and molecular structure, are of interest for driving the synthesis of new and improved compounds with acid–base properties suitable for applications as environment-sensitive probes and/or as photoacids and photobases.

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Declarations

Conflict of interest The authors have no conflict of interest.

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