Luminescence and charge transfer. Part 4.[1] 'On–off' fluorescent PET (photoinduced electron transfer) sensors with pyridine receptors: 1,3-diaryl-5-pyridyl-4,5-dihydropyrazoles

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1,3-Diaryl-5-pyridyl-4,5-dihydropyrazoles, some of which have been previously synthesized for use as optical brighteners, satisfy the design criteria for fluorescent PET pH sensors by a proton-induced 'switching off' of fluorescence. In practice, they are the first family of 'on–off' fluorescent PET pH sensors whose signals cover most of the visible spectrum. They also show significant absorptiometric pH sensing action due to the coupling of the protonatable pyridine with the ICT (internal charge transfer) excited state of the 1,3-diaryl-4,5-dihydropyrazole. Experimentally identical pK_a values arise from the fluorimetric and absorptiometric pH dependences. The examination of 2-, 3- and 4-pyridyl regioisomers shows understandable differences in pK_a and maximum fluorescene quantum yield [φ_F(base)] values, but show no significant variations in fluorescence switching factors (FE).

One of our major aims in this series is to establish photoinduced electron transfer (PET)[2] as an important guiding principle for the design of fluorescent molecular sensors.[3] In previous parts of this series we have shown how the proton basicity of amines can be exploited for fluorescent pH sensor construction by appending aminoalkyl groups to various fluorophores in order to give rise to 'fluorophore–spacer–receptor' systems,[1,4] *e.g.* **1**. The power of a principle lies in the generality of its application. So we were encouraged by the recent report of luminescent PET pH sensors **2** based on phenolates within a calix[4]arene acting as proton receptors.[5] Protonation of amines or phenolates lead to the 'switching on' of fluorescence in these systems **1** or **2** due to the increased oxidation potential of the proton-bound receptor. Earlier work from our laboratory had also employed aromatic carboxylates as proton receptors in fluorescent PET sensing schemes.[6] In this instance, protonation leads to the 'switching off' of fluorescence of **3** due to the decreased reduction potential of the protonated receptor. With the amines, phenolates and carboxylates safely in the fold, we now look towards one of the remaining general classes of proton receptor: the pyridines.

The key to the incorporation of pyridine units as proton receptors within fluorescent PET sensors lies in the large decrease of the reduction potential that is induced upon protonation. The relevant values are −2.62 and −1.25 V (*vs.* SCE) for pyridine before and after protonation, respectively.[7] The electron deficiency of pyridinium derivatives lies at the heart of biological electron carriers such as NAD^+,[8] weedicides such as paraquat,[9] spectroscopically derived solvent polarity scales such as Z values[10] and self-assembly of supramolecular structures such as catenanes and rotaxanes.[11] A variety of fluorophores immediately suggest themselves as suitors on the simple theoretical basis of molecular orbital energy diagrams[3a,b] (see later). However, the convenience of synthesis must also be a criterion for the selection of the fluorophore as well as the intervening spacer module. This convenience factor can be maximized in the happy circumstance that a suitable target molecule has already been synthesized but for a different purpose.[12] This turned out to be the case when we examined the literature on one of our favourite fluorophores: the 4,5-dihydropyrazoles (Δ^2-pyrazolines).

1,3-Diaryl-4,5-dihydropyrazoles have found use as optical brighteners for many years.[13,14] More recent uses include fluorescent biological labels,[15] fluorescent sensors for solvent polarity[16] and fluorescent PET sensors for several cations.[6,17] Fields as diverse as singlet oxygen quenching,[18] hole transport in xerography.[19] aryl halide photochemistry[20] and electrogenerated chemiluminescence[21] have also received significant input from these heterocycles. 25 years ago, Tsukerman *et al.* showed that 3-(4-pyridyl)-4,5-dihydropyrazoles act as fluorescent pH indicators.[22,23] However, these molecules are integrated fluorophore–receptor systems where the protonation results in shifts of the emission wavelength arising from the internal charge transfer (ICT) excited state,[24,25] *i.e.* these are not fluorescent PET sensors with 'on–off' action. On the other hand, Szucs and co-workers examined the fluorescence of 4,5-dihydropyrazoles substituted with pyridyl units at 1-, 3- or 5-positions.[26] Of these, only the 5-pyridyl-4,5-dihydropyrazoles are exactly formatted to behave as fluorescent PET pH sensors[3] with a 1,3-diphenyl-4,5-dihydropyrazole fluorophore, a methine (sp^3 hybridized) carbon spacer and a pyridine receptor for protons. Though the work of Szucs was conducted primarily to develop optical brighteners, it gives us a springboard into a new series of 'on–off' fluorescent PET pH sensors **4**. The sensor series **4** encompasses a range of absorption and emission colours since 1,3-diaryl-4,5-dihydropyrazoles and other ICT fluorophores have substitutionally tunable electronic spectra.[13,14,24a]

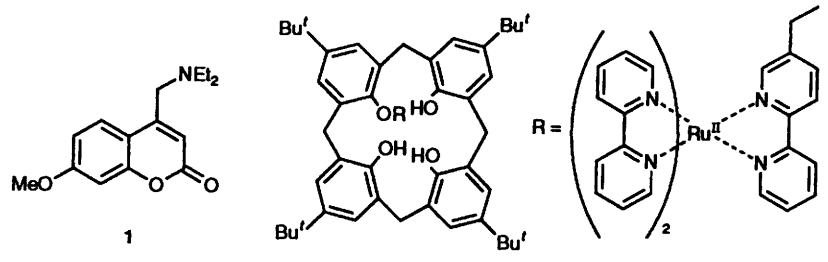
Results and discussion

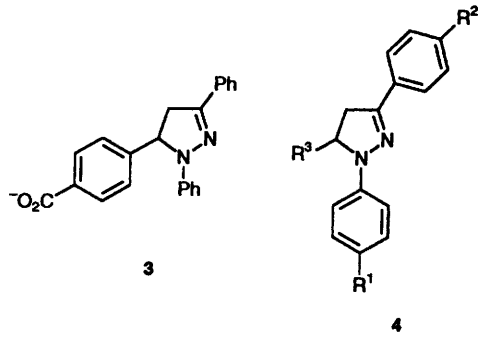
Fig. 1 is a schematic representation of the energies of the relevant frontier molecular orbitals of **4a–c**. Electron transfer from the photoexcited 1,3-diphenyl-4,5-dihydropyrazole fluorophore is significantly more exergonic when the acceptor is protonated pyridine rather than when it is unprotonated. Therefore, the competitive deexcitation channel of fluorescence is strongly quenched only when the medium is sufficiently acidic to protonate the pyridine receptor. The information in Fig. 1 and its congeners can be summarized in terms of eqns. (1)[27] and

$$\Delta G_{PET} = -E_{S.fluorophore} + E_{ox.fluorophore} - E_{red.receptor} + \Delta G_{ion\ pair} \quad (1)$$

(2)[3a] where ΔG_{PET} is the thermodynamic driving force for PET,

$$E_{S.fluorophore} = E_{ox.fluorophore} - E_{red.fluorophore} \quad (2)$$





4a R^1 = H, R^2 = H, R^3 = 2-Pyridyl
4b R^1 = H, R^2 = H, R^3 = 3-Pyridyl
4c R^1 = H, R^2 = H, R^3 = 4-Pyridyl
4d R^1 = H, R^2 = H, R^3 = Ph
4e R^1 = H, R^2 = CN, R^3 = 2-Pyridyl
4f R^1 = H, R^2 = CN, R^3 = 3-Pyridyl
4g R^1 = H, R^2 = CN, R^3 = 4-Pyridyl
4h R^1 = H, R^2 = CN, R^3 = Ph
4i R^1 = OMe, R^2 = CN, R^3 = 2-Pyridyl
4j R^1 = OMe, R^2 = CN, R^3 = 3-Pyridyl
4k R^1 = OMe, R^2 = CN, R^3 = 4-Pyridyl
4l R^1 = OMe, R^2 = CN, R^3 = Ph

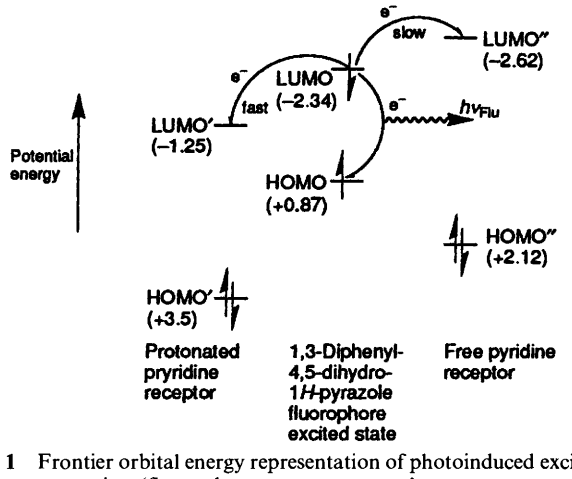


Fig. 1 Frontier orbital energy representation of photoinduced excited state processes in a 'fluorophore–spacer–receptor' system composed of a 1,3-diphenyl-4,5-dihydropyrazole fluorophore excited state and a pyridine receptor for protons *e.g.* **4a–c** when proton-free and when protonated. Redox potentials (V *vs.* SCE), from refs. 7 and 31, are given in parentheses as a measure of MO energies. The energies are not to scale. The oxidation potential of the pyridinium ion is estimated by subtracting the modulus of the reduction potential from the singlet energy, approximated by the value for benzene.[50] It is understood that only one of the three electron transfer acts shown can take place for any one molecule.

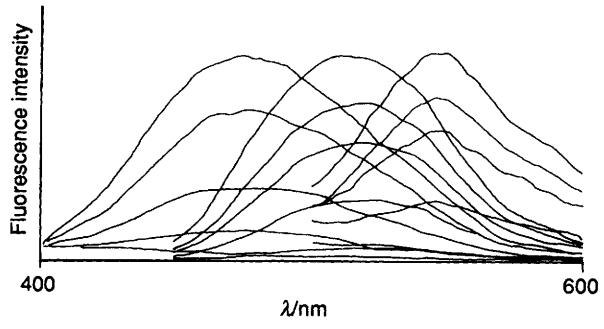


Fig. 2 pH Dependence of fluorescence emission spectra of 10^{-5} mol dm^{-3} **4b** (left set of spectra), **4f** (centre set) and **4k** (right set) in MeOH–H_2O (1 : 4, v/v). The detector gain for each set has been scaled to give similar maximum intensities at limiting high pH values. The pH values are, in order of decreasing intensity, 7.2, 4.6, 3.8, 3.2 and 1.8 for **4b**, 7.6, 4.7, 4.3, 3.8 and 3.0 for **4f** and 6.4, 5.0, 4.7, 4.0 and 2.2 for **4k**. The excitation wavelengths for each set of spectra are 342, 380 and 384 nm, respectively.

E_{ox} and E_{red} are the appropriate oxidation and reduction potentials, E_S is the singlet energy and $\Delta G_{ion\,pair}$ is the attractive energy between a contact radical ion pair, taken as −0.1 eV,[28] when a charge separation results from the PET process. This would apply to a PET between electrically neutral components such as 1,3-diphenyl-4,5-dihydropyrazole and unprotonated pyridine. On the other hand, PET from 1,3-diphenyl-4,5-dihydropyrazole to protonated pyridine would simply result in a charge translocation and the $\Delta G_{ion\,pair}$ term is irrelevant. Such charge shift electron transfers can differ from charge separating versions with regard to the relevance of the Marcus inverted region[29] to their kinetics, according to detailed numerical calculations.[30] Extensive electrochemical investigations by Pragst[31] gives $E_{red.fluorophore}$ as −2.34 V (*vs.* SCE) for **4a–d** and allows us to estimate the corresponding value for **4e–h** as −1.2 V,[6] from linear free energy relations.[32] While the corresponding value for **4i–l** is not available, it most likely lies in between the potentials noted above. Application of eqns. (1) and (2) now allows the estimation of ΔG_{PET} values for **4a–c** as −1.1 and +0.2 eV in acidic and basic solution, respectively. The corresponding quantities for **4e–g** are +0.1 and +1.3 eV.

Fig. 2 exemplifies the clear 'switching off' of fluorescence upon protonation for the cases of **4b**, **4f** and **4k**. Note that the shape and wavelength position of each case are independent of pH, since the major contributor by far to the fluorescence is the base form of **4b**, **f** and **k**. Such simple 'on–off' fluorescence switching action is particularly remarkable since it occurs with uniformly high efficiency across the sensor series with FE values of 25–100 (Table 1) while traversing a large fraction of the visible spectrum. While the maximum emission wavelengths of **4a–k** range from 476 to 543 nm, their broad emission bands allows proton-controlled reversible optical switching at wavelengths from 450 to 600 nm. This large spectral range becomes possible because the photoexcited 1,3-diphenyl-4,5-dihydropyrazole system develops significant positive charge density on

Table 1 Electronic absorption and fluorescence parameters for **4**[a]

Parameter	4a	4b	4c	4e	4f	4g	4i	4j	4k
λ_{abs}(acid)/nm	331	342	340	370	378	372	368	390	382
log ε(acid)	4.21	4.17	4.16	4.30	4.32	4.31	4.26	4.29	4.31
λ_{abs}(base)/nm	355	352	356	391	388	390	393	399	398
log ε(base)	4.25	4.21	4.21	4.34	4.36	4.36	4.30	4.33	4.34
λ_{isos}/nm	336	342	348	372	380	376	368	380	384
$E_{acid} - E_{base}$/kJ mol^{-1} [b]	24.4	10.1	16.0	17.6	8.4	14.7	19.6	7.6	12.6
pK_a [c]	3.5	4.5	4.7	3.2	4.2	4.4	3.4	4.2	4.4
λ_{Flu}(base)/nm [d]	476	476	475	517	511	515	543	540	545
$10^3\varphi_F$(acid) [e]	3.3	1.2	0.6	1.5	1.3	1.6	0.2	0.2	0.3
$10^3\varphi_F$(base) [e]	130	87	30	150	140	148	7.4	7.3	6.7
FE [f]	40	70	50	100	110	90	40	30	25
pK_a' [g]	3.5	4.3	4.5	3.2	4.2	4.5	3.5	4.3	4.5
ΔpK_a [h]	2.5	1.3	1.4	2.8	1.5	1.6	2.6	1.5	1.6

[a] Sensors and model compounds (10^{-5} mol dm^{-3}) in aerated MeOH–H_2O (1:4, v/v) except where noted otherwise. pH Dependent fluorescence measurements were carried out with excitation at isosbestic wavelengths. The molecules **4d**, **h** and **l** act as model fluorophores for the sensor sets **4a–c**, **e–g** and **i–k**, respectively. These model compounds have the following spectral characteristics in 100% MeOH. **4d**; λ_{abs} = 353 nm, log ε = 4.30 (ε in all cases is in units of dm^3 mol^{-1} cm^{-1}), λ_{Flu} = 463 nm (corrected), φ_F = 0.46. **4h**; λ_{abs} = 393 nm, log ε = 4.40, λ_{Flu} = 502 nm (corrected), φ_F = 0.56. **4l**; λ_{abs} = 405 nm, log ε = 4.33, λ_{Flu} = 638 nm (corrected), φ_F = 0.050. [b] $E = hc/\lambda_{abs}$. [c] These values are obtained by analysing the pH dependence of absorbance (A) values at appropriate wavelengths according to the equation log $[(A_{max} - A)/(A - A_{min})] = \mp$pH $\pm$ pK_a.[47] Either set of signs on the right hand side of this equation can be valid, depending on the analytical wavelength chosen. Isosbestic points (λ_{isos}) are observed in all cases. The fit of experimental points to the equation is good [average least squares correlation coefficient (r) = 0.992, average number of points (n) = 7]. The average experimental gradient = 1.00, standard deviation = 0.07 for the nine cases. [d] Obtained from fluorescence spectra which have been corrected for wavelength dependence of response of the photon detection system using quinine bisulfate.[48] The λ_{Flu}(acid) values are indeterminable due to the weakness of the emission. [e] **4h** (φ_F = 0.56 in MeOH[14]) is used as a secondary standard. [f] Fluorescence enhancement factor = φ_F(base)/φ_F(acid). [g] These values are obtained by analysing the pH dependence of fluorescence quantum yield (φ_F) values according to the equation log $[(\varphi_{Fmax} - \varphi_F)/(\varphi_F - \varphi_{Fmin})]$ = pH − pK_a'.[3a] The use of this equation is facilitated by the proportionality of φ_F to fluorescence intensities[49] due to the pH invariance of spectral shape and position in all cases. The fit of experimental points to this equation is good (r = 0.997, n = 7). The average experimental gradient = 1.00, standard deviation = 0.04 for the nine cases. [h] The value p$K_{a.model}$ − [(pK_a + pK_a')/2] where the model for the receptor in **4a**, **e** and **i** is 2-methylpyridine (pK_a = 6.0).[40] The corresponding models for the receptor in **4b**, **g** and **k**, and **c**, **g** and **k** are 3-methylpyridine (pK_a = 5.7)[40] and 4-methylpyridine (pK_a = 6.0).[40] These p$K_{a.model}$ values are for aqueous solutions.

N(1) and the 1-phenyl ring which can be stabilized by a 4-methoxy group on the 1-phenyl ring. Similarly, the resulting negative charge density on N(2), C(3) and the 3-phenyl ring can be stabilized by a 4-cyano group (**4e–l**).[13,14,24a,33]

Table 1 also collects data on the pH dependence of the absorption spectra of (**4a–c**, **e–g** and **i–k**). The large proton-induced hypsochromism seen here is larger in magnitude than the proton induced shifts seen in **1** and relatives whose maximum value was 18 kJ mol^{-1}. While the presence of an electrically chargeable group near an ICT chromophore appears to give rise generally to pH dependent absorption spectra,[1] the lack of such an effect in 1,3-diaryl-4,5-dihydropyrazoles with benzoate groups appended at the 5-position [6] makes the present results particularly interesting. One of the relevant differences between the benzoate–benzoic acid and the pyridine–pyridinium systems is the extent of delocalization of the charge onto the aromatic ring carbon atoms, especially the one adjacent to C(5) of the pyrazole heterocycle. The weakness of this charge delocalization in the benzoate–benzoic acid system is an important reason why substituent effects do not involve any large resonance contribution and why this system is the cornerstone of the historical development of linear free energy relationships.[34] On the other hand, resonance is a strong contributor to substituent effects in the pyridine–pyridinium systems regarding 2- and 4-positions.[35] Any charge transmitted in this manner to the aromatic ring carbon adjacent to the pyrazole C(5) spacer will interact electrostatically with the positive terminal of the pyrazole excited state dipole near N(1) and the 1-aryl ring. This largely rationalizes the proton-induced hypsochromism observed here as well as the ranking of its magnitude ($E_{acid} - E_{base}$) among the three pyridyl isomers, *i.e.* 2-pyridyl, *e.g.* **4a** > 4-pyridyl, *e.g.* **4c** > 3-pyridyl, *e.g.* **4b**. Additional through space interactions between the various atoms of the pyridinium unit and the pyrazoline ICT excited state dipole, especially at its positive terminal, strengthens this rationalization further.

Guest-induced changes in electronic absorption spectra are frequently interpreted as arising from ground state complexation. It must be emphasised that, in the present instances, the extrafluorophoric pyridyl unit is essential for the observation of proton-induced hypsochromism. Experiments with corresponding model molecules **4d**, **h** and **l**, as far as solubility constraints allow, do not show this effect. Thus, it is the ground state complexation of the pyridyl unit with the proton that indirectly leads to the effect in the manner discussed in the previous paragraph. Also, it must be noted that electronic absorption spectroscopic observations necessarily deal with Franck–Condon excited states of the chromophore under examination. We also remained alert to the possibility of charge transfer complexation [36] between the pyrazole fluorophore and the protonated pyridyl unit nearby, in spite of the nearly orthogonal planes [37] occupied by these two moieties. Dramatic absorption spectral evidence for charge transfer between non-parallel (non-sandwich) donor–acceptor pairs is available in several bridged systems.[38] In the event, the protonated sensors **4a–c**, **e–g** and **i–k** did not show any low-energy absorption bands attributable to this cause. According to some elegant studies conducted recently by Saeva,[39] pyridinium units which are conformationally constrained to remain quasi-parallel with an anthracene moiety display clear charge transfer absorption, whereas related structures devoid of this restraint do so only weakly. Nevertheless, fluorescence quenching is uniformly strong in both series.

The pH dependence of the absorption spectra, as well as the fluorescence spectra, endows the molecules **4a–c**, **e–g** and **i–k** with two sensory channels. Thus, the additional absorptiometric sensing channel can confirm the results of proton density obtained *via* fluorescence, if their pH dependencies are identical. Table 1 collects the pK_a and pK_a' values that can be extracted from a detailed analysis of these pH dependences. The equality, within experimental error, of pK_a and pK_a' values for a given sensor confirms that the ground state protonation equilibrium

is maintained during the short lifetime of the singlet excited state. Related 4,5-dihydropyrazoles have fluorescence lifetimes around 2 ns even in non-polar media.[33] The equality of proton binding constants obtained *via* absorptiometric and fluorimetric channels for PET sensors with ICT fluorophores, which has been discussed recently for aminoalkyl heterocycles,[1] is now found to be true for pyridyl systems as well.

As can be seen in Table 1, the pK_a and pK_a' values are subject to regiocontrol, with the 2-pyridyl isomers **4a**, **e** and **i** yielding consistently lower numbers. The ΔpK_a values, where the corresponding methylpyridine is taken as a model, are essentially identical for a given regioisomer with all three fluorophores and show a larger number for the 2-pyridyl cases. Previously, we have encountered situations where an otherwise satisfactory model receptor unit is found to be inadequate for quantitative comparison of proton binding ability.[3a,4] Usually, steric inhibition of solvation of the protonated receptor by the bulky fluorophore is responsible, even though the presence of the intervening spacer unit must reduce the magnitude of this effect. This factor appears to be operating in the present instances since 2-pyridyl regioisomers would indeed have considerable congestion at the protonation site, more so than their 3- and 4-pyridyl counterparts. The pK_a values of *tert*-butylpyridines show a 0.2 unit reduction for the 2-isomer only when compared with the methylpyridines.[40] The larger pK_a differences seen in **4a**, **e** and **i** must have an additional contributory factor. These 2-pyridyl isomers would have their protonated nitrogen centre relatively close to the electron withdrawing C(5)–N(1) system of the dihydropyrazole ring. Hydroxymethylpyridines, which have a rather similar atom array, show ΔpK_a values (relative to the corresponding methylpyridines) of 1.1, 0.8 and 0.7 for the 2-, 3- and 4-isomers, respectively.[40] So the pK_a values of sensors **4a–c**, **e–g** and **i–k** appear to be displaced from those of the parent proton receptors due to a combination of polar and steric effects of the 4,5-dihydropyrazol-5-yl system.

The fluorescence quantum yield data in Table 1 deserve special discussion because they form the core of fluorescent PET sensor action. In ideal situations, the maximum quantum yields [φ_F(base) in the present instance] should approach the quantum yield of the parent fluorophore.[3a] Solubility limitations of the parent fluorophores **4d**, **h** and **l** necessitated the use of 100% MeOH as solvent for their study. The sensors themselves were examined in the more practical medium of 20% MeOH–80% H_2O (v/v) which also exerts a stronger hydrogen bond acidity. We have previously demonstrated that solvent hydrogen bond acidity strongly contributes to the quenching of 4,5-dihydropyrazole fluorescence[16] according to a linear solvation energy relationship.[41] Therefore, it is no surprise that the φ_F(base) values of all the sensors significantly undershoot the parent quantum yields. Similarly, the λ_{Flu}(base) values are significantly longer than the parent fluorescence wavelengths due to the higher polarity of the sensor solutions with their high water content. Further analysis of φ_F(base) values is possible according to the previous insight that the quenching is due to the strengthening of a hydrogen bond from the solvent to the N(2) lone electron pair of the 4,5-dihydropyrazole in its ICT excited state.[16] The electron density on N(2) will be reduced in the excited state of the sensors **4e–g** due to the electron withdrawal of the 3-(4-cyanophenyl) group. Hence, the fluorescence quenching is reduced accordingly. The opposite effect is seen with sensors **4i–k** due to the electron releasing 1-(4-methoxyphenyl) moiety in their ICT excited states. Thus, the φ_F(base) values follow the ranking; **4e–g** > **a–c** > **i–k**. However, there is another contributor to this ranking. As noted earlier (and pictured in Fig. 1), a PET quenching channel is possible in the base form of the sensors **4a–c**, though it would be relatively slow owing to the small endergonicity of +0.2 eV. Nevertheless, this would cause a significant fluorescence loss at ambient temperature. Such a PET quenching is much less likely in the cyano substituted sensors **4e–g** due to their much lower fluorophore LUMO energy. The endergonicity of +1.3 eV in these cases is essentially insurmountable thermally under the experimental conditions. Such a PET step in the base form of the sensors can also rationalize the φ_F(base) order of **4a** > **b** > **c** because the solvational reorganization of the resulting radical ion pair is subject to steric hindrance. The solvation of the negative charge density in the pyridine radical anion would be expected to concentrate on the nitrogen centre. The exposure to solvent molecules would be in the order **4c** > **b** > **a**. Szucs also observed the fluorescence intensity order **4d** > **a** > **b** > **c** in ethanol[26] which can now be understood as above.

The fluorescence enhancement (FE) factor between the 'switched on' and the 'switched off' states are uniformly high (>25) for all the sensors studied. While this result is very pleasing from a practical end-user's viewpoint, it raises questions for the sensor designers. As noted above, sensor sets **4a–c** and **e–g** differ by more than 1 eV in their ΔG_{PET} values whether with or without protonation. Interestingly, such large differences in thermodynamic driving force are not reflected in significant differences in FE values. Previously, these two quantities have been found to be related by others[42] and ourselves[1] in the case of several aminoalkyl substituted fluorophores. Nevertheless, close structural relatives based on protonated **3** show significant fluorescence quenching even when $\Delta G_{PET} = +0.9$ eV.[6] Thus, PET rates arising from these 5-substituted 4,5-dihydropyrazoles remain rather fast (when compared with their fluorescence rates) even at relatively high endergonicities.

Another question concerns regiochemistry. At the design stage, sensor sets such as **4a–c** were particularly interesting because the 'fluorophore–spacer' assembly could be connected to the receptor at the 2-, 3- or 4-position of the pyridine moiety. Since the LUMO of the pyridinium ion would have significant differences in electron density at these three positions,[43] some variation of PET kinetics and subsequent fluorescence quenching was anticipated. This was not found. In contrast, we have recently found strong regiocontrol of FE values in systems where the 'receptor–spacer' assembly could be connected to an ICT fluorophore in different ways.[44] Nevertheless, the 4,5-dihydropyrazoles still hold several secrets regarding the subtleties of their PET behaviour with electron deficient 5-aryl substituents. It is therefore important to note Sahyun's laser flash photolysis experiments on 5-(4-nitrophenyl)-1,3-diphenyl-4,5-dihydropyrazole[37,45] which give credence to the long-suspected PET process[31,20] as the cause of fluorescence quenching in this[23,31,46] and related cases.[20]

In summary, variously substituted 1,3-diaryl-4,5-dihydropyrazoles can be coupled at the 5-position to pyridine *via* its 2-, 3- or 4-position to yield fluorescent PET pH sensors which show strong 'switching off' of fluorescence with protons across most of the visible spectrum. These systems also show supporting absorptiometric pH sensory behaviour due to a proton-induced hypsochromism.

Experimental

Electronic absorption and fluorescence spectra were obtained with Perkin-Elmer lambda-9 and LS-5B spectrometers, respectively. The synthesis of the various sensors and model molecule **4l** were carried out according to the method of Szucs[26] who has reported the syntheses of **4a–c**. Model molecules **4d**[14] and **h**[14] were also prepared according to literature methods. The characterization of the new compounds are detailed below.

3-(4-Cyanophenyl)-1-phenyl-5-(2-pyridyl)-4,5-dihydropyrazole (4e)

Bright yellow crystals (50% yield) obtained from methanol, mp 178–179 °C (Found: C, 77.7; H, 5.1; N, 17.5. $C_{21}H_{16}N_4$ requires C, 77.8; H, 5.0; N, 17.3%); $\delta_H(CDCl_3)$ 8.63 (d, 1 H, 6-PyH), 7.78–6.87 (m, 12 H, ArH), 5.57 (dd, 1 H, PyCHN), 3.91 (dd, 1 H, CH_2C=N) and 3.26 (dd, 1 H, CH_2C=N); *m/z* 324 (M^+, 43%), 247 (18), 246 (100), 219 (22), 218 (66), 96 (18), 79 (29) and 77 (17).

3-(4-Cyanophenyl)-1-phenyl-5-(3-pyridyl)-4,5-dihydropyrazole (4f)

Bright yellow crystals (55% yield) obtained from methanol, mp 143–144 °C (Found: C, 77.7; H, 5.1; N, 17.4); $\delta_H(CDCl_3)$ 8.65 (s, 1 H, 2-PyH), 8.48 (d, 1 H, 6-PyH), 7.78–6.83 (m, 11 H, ArH), 5.39 (dd, 1 H, PyCHN), 3.89 (dd, 1 H, CH_2C=N) and 3.13 (dd, 1 H, CH_2C=N); *m/z* 324 (M^+, 100%), 246 (46), 96 (81), 95 (28), 91 (46), 79 (15) and 77 (18).

3-(4-Cyanophenyl)-1-phenyl-5-(4-pyridyl)-4,5-dihydropyrazole (4g)

Bright yellow crystals (50% yield) obtained from methanol, mp 209–210 °C (Found: C, 77.3; H, 5.1; N, 17.2); $\delta_H(CDCl_3)$ 8.63 (d, 2 H, 2-, 6-PyH), 7.78–6.87 (m, 11 H, ArH), 5.39 (dd, 1 H, PyCHN), 3.93 (dd, 1 H, CH_2C=N) and 3.13 (dd, 1 H, CH_2C=N); *m/z* 324 (M^+, 100%), 246 (65), 96 (22), 91 (34) and 77 (20).

3-(4-Cyanophenyl)-1-(4-methoxyphenyl)-5-(2-pyridyl)-4,5-dihydropyrazole (4i)

Bright yellow crystals from ethanol, mp 218–219 °C (Found: C, 74.2; H, 5.3; N, 15.7. $C_{22}H_{18}N_4O$ requires C, 74.6; H, 5.1; N, 15.8%); $\delta_H(CDCl_3)$ 8.64 (d, 1 H, 6-PyH), 7.93–6.82 (m, 11 H, ArH), 5.50 (dd, 1 H, PyCHN), 4.01 (m, 1 H, CH_2C=N), 3.41 (s, 3 H, OCH_3) and 3.27 (m, 1 H, CH_2C=N); *m/z* 354 (M^+, 100%), 352 (12), 122 (13) and 121 (20).

3-(4-Cyanophenyl)-1-(4-methoxyphenyl)-5-(3-pyridyl)-4,5-dihydropyrazole (4j)

Bright yellow crystals from ethanol, mp 150–151 °C (Found: C, 74.7; H, 5.1; N, 15.3); $\delta_H(CDCl_3)$ 8.68 (s, 1 H, 2-PyH), 8.57 (d, 1 H, 6-PyH), 7.82–6.77 (m, 10 H, ArH), 5.41 (dd, 1 H, PyCHN), 3.82 (dd, 1 H, CH_2C=N), 3.73 (s, 3 H, OCH_3) and 3.14 (dd, 1 H, CH_2C=N); *m/z* 354 (M^+, 100%), 329 (12), 276 (15), 122 (17), 121 (29), 106 (12) and 80 (14).

3-(4-Cyanophenyl)-1-(4-methoxyphenyl)-5-(4-pyridyl)-4,5-dihydropyrazole (4k)

Bright yellow crystals from ethanol, mp 148–149 °C (Found: C, 74.0; H, 5.1; N, 15.9); $\delta_H(CDCl_3)$ 8.61 (d, 2 H, 2-, 6-PyH), 7.74–6.78 (m, 10 H, ArH), 5.30 (dd, 1 H, PyCHN), 3.85 (dd, 1 H, CH_2C=N), 3.74 (s, 3 H, OCH_3) and 3.09 (dd, 1 H, CH_2C=N); *m/z* 354 (M^+, 100%), 339 (16), 276 (26), 122 (11), 121 (23), 106 (12) and 80 (15).

3-(4-Cyanophenyl)-1-(4-methoxyphenyl)-5-phenyl-4,5-dihydropyrazole (4l)

Yellow–orange crystals from ethanol, mp 145–146 °C (Found: C, 77.5; H, 5.4; N, 11.9. $C_{23}H_{19}N_3O$ requires C, 78.2; H, 5.4; N, 11.9%); $\delta_H(CDCl_3)$ 7.74–6.74 (m, 13 H, ArH), 5.30 (dd, 1 H, PyCHN), 3.80 (dd, 1 H, CH_2C=N), 3.74 (s, 3 H, OCH_3) and 3.11 (dd, 1 H, CH_2C=N); *m/z* 353 (M^+, 100%), 338 (11), 122 (13), 121 (22) and 80 (11).

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