# New Charge-transfer Probe for Solvent Polarity: Fluorescent Hydrogen-bonding Switch

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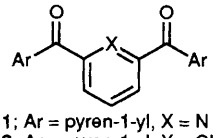
A new fluorescent probe highly sensitive to solvent polarity, hydrogen bonding and with a large deuterium isotope effect on its fluorescence quenching, is reported.

Fluorescent probes with a high sensitivity for solvent polarity are valuable in the study of heterogeneous media, organized media and biological media.[1] A new chromophore whose fluorescence maximum shifts to longer wavelengths with solvent polarity, featuring a specific site for hydrogen bonding with the solvent or a quencher, is reported here. The presence of a single nitrogen atom in the chromophore seems to be responsible for the sensitivity of the probe-fluorescence to hydrogen bonding solvents. Apparently, hydrogen bonding at this site controls the rate of the excited state decay, functioning as a fluorescent hydrogen bonding switch. This sensitivity towards hydrogen bonding solvents and quenchers is accompanied by a large deuterium isotope effect on the fluorescence quenching. These inferences are based on a comparison of the fluorescence quenching of the diketones **1** and **2** (Scheme 1) by quenchers such as water, phenol, aniline and anisole. It is important to note that the two probes **1** and **2** differ by a single nitrogen atom in the spacer connecting the two pyrene chromophores. Current results are in contrast to optical sensors that generate intense colour in the presence of polar




**1**; Ar = pyren-1-yl, X = N
**2**; Ar = pyren-1-yl, X = CH

**Scheme 1**

solvent molecules.[2] The observed solvent induced shifts in the fluorescence spectra and the large deuterium isotope effect on the fluorescence quenching of **1** and **2** can be explained by an intramolecular charge transfer excited state which is very likely to be twisted in geometry.[3]

The diketone, 2,6-bis(pyren-1-oyl)pyridine (DPP, **1**) (Scheme 1) and the corresponding carbon-analogue, 1,3-bis(pyren-1-oyl)benzene (DPB, **2**), were prepared in nearly 60% yield, by reacting pyrene with the corresponding aromatic diacid chloride in the presence of anhydrous aluminum chloride, in an inert solvent such as chloroform. After about 3 h the reaction was quenched with ice–water and the resulting product was purified by column chromatography on alumina to remove the unreacted pyrene. The final product was purified by recrystallization from a 1:1 mixture of hexane–chloroform. $^1$H NMR, $^{13}$C NMR, IR and mass spectra and elemental analysis of **1** and **2** support the structures shown in Scheme 1.

The electronic absorption spectra of **1** and **2** show very broad peaks in the 350 to 400 nm region, in addition to the absorption bands of the pyrene chromophore [Fig. 1(*A*)]. The absorption spectra are independent of solvent polarity or the hydrogen bonding ability of the solvent. In contrast, excitation into the long wavelength absorption bands of **1** or **2** results in a broad fluorescence spectrum that is distinct from the typical pyrene fluorescence spectrum [Fig. 1(*B*)] and is highly sensitive to the solvent polarity. Typically, the emission maximum of **1** shifted from 472 nm in a nonpolar solvent such as cyclohexane to 570 nm in an aprotic solvent such as acetonitrile. Similar red shifts in the fluorescence spectra were observed for **2** with maxima at 430 and 513 nm in cyclohexane and acetonitrile, respectively. The relative fluorescence intensities and the maxima for **1** in various solvents are as follows: (solvent, fluorescence $\lambda_{max}$/nm, relative yield with respect to the yield in benzene): benzene, 507 (1.0); chloroform, 546 (0.62); pyridine 545 (0.52); acetone 552 (0.32); dimethylformamide 558; dimethyl sulfoxide 565 (0.23) and acetonitrile 570 (0.15). The emission maxima for **2** are (in nm): cyclohexane 431; benzene 440; chloroform 473; acetone 488; dimethylformamide 497; dimethyl sulfoxide 513 and acetonitrile 512.

The insensitivity of the absorption spectra to the solvent polarity and the large red shifts in the emission maxima in polar solvents indicate large Stoke's shifts in highly polar solvents. The solvent induced relaxation of the excited state which is very likely to be a twisted intramolecular charge transfer state is consistent with these observations.[3,4] The emission spectra of both **1** and **2** are independent of the concentration of the fluorophore indicating that the emission is not due to excimer formation between two different molecules. Preliminary studies of fluorescence lifetimes of these new probes indicate that the excited states are very short-lived (<1 ns) on nanosecond timescales. These observations are consistent with emission from an intramolecular charge transfer excited state rather than from an excimer. The absence of distinct vibrational bands, a large red shift of fluorescence with increased solvent polarity and a decrease in the emission yield with increased solvent polarity, are consistent with this assignment.

Further insight into the nature of the excited state was provided by the quantitative evaluation of the fluorescence energy with respect to the solvent polarity parameter, $E_T(30)$.[5] The emission intensities of both **1** and **2** strongly vary with solvent polarity, decreasing with increased solvent polarity index. A plot of the fluorescence energy, corresponding to the emission maxima, as a function of $E_T(30)$ gave a biphasic plot for **1** and a linear plot for **2** (Fig. 2).[5] In the low polarity region of the plot, the slopes of both **1** and **2** are nearly the same (−0.84 for **1** and −0.76 for **2**), and provide strong evidence for the charge transfer nature of the excited state.[6] In the high polarity region of the plot, the slope for **1** decreased to −0.33 suggesting that the nature of the excited state altered considerably.[7] A similar biphasic behaviour was not observed for **2**. Clearly, the presence of a nitrogen atom in the six-membered ring of the spacer is responsible for this difference between **1** and **2**. The role of the hetero atom in the excited state dynamics was further examined by quenching experiments.

If the nitrogen atom is involved in the excited state dynamics then the fluorescence yields should be sensitive to protic solvents. Indeed, the charge transfer emission from **1** is dramatically quenched by water with a large deuterium isotope effect whereas the emission from the carbon-analogue **2** shows only a poor sensitivity to water (Fig. 3). The fluorescence quenching constant was estimated from the Stern–Volmer equation, $I_o/I = 1 + K_{SV}[Q]$ where $I$ and $I_o$ are the intensities in the presence and absence of water, [Q] the concentration of water and $K_{SV}$ the quenching constant. Fluorescence from **1** is quenched by water with a quenching constant of 2.2 ± 0.03 dm$^3$ mol$^{-1}$. The $K_{SV}$ value decreases to

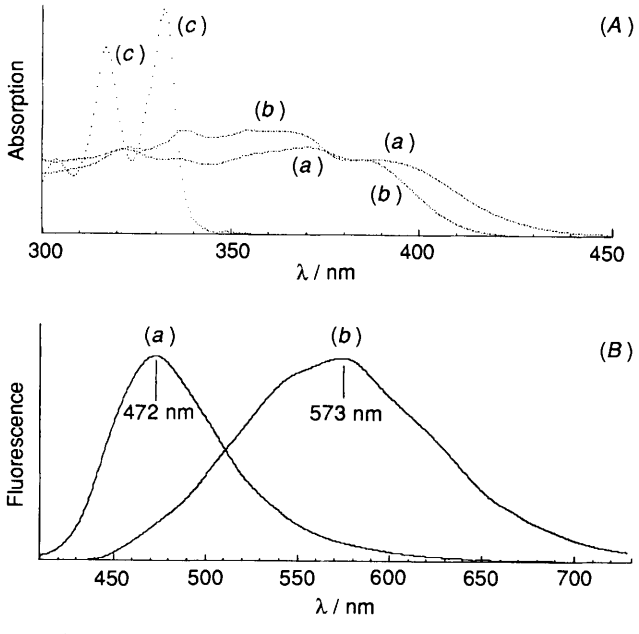



**Fig. 1** (*A*) Absorption spectra of DPP (*a*), DPB (*b*) and pyrene (*c*) in cyclohexane. (*B*) Fluorescence spectra of DPP in (*a*) cyclohexane ($\lambda_{max}$ 472 nm) and (*b*) dimethyl sulfoxide ($\lambda_{max}$ 573 nm). The excitation wavelength was 396 nm.

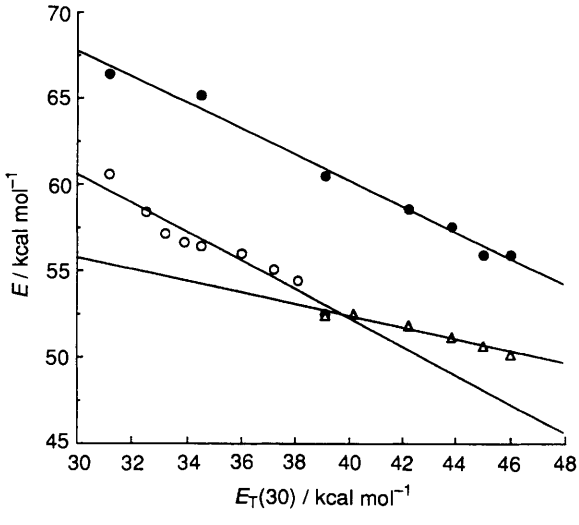



**Fig. 2** A plot of emission energy in (kcal mol$^{-1}$) *vs.* the solvent polarity parameter $E_T(30)$ for DPB (●); DPP at low solvent polarity (○) and DPP at high solvent polarity (△). The biphasic nature of the plot is evident for DPP and its initial slope is similar to that of DPB.

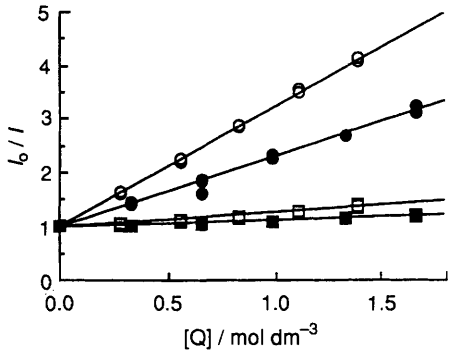



**Fig. 3** Quenching of the DPP emission (○, ●) and DPB emission (□, ■) by $H_2O$ (○, □) and $D_2O$ (●, ■). Triplicate measurements were made at each concentration.

1.29 ± 0.03 dm$^3$ mol$^{-1}$ when deuterium oxide was used as the quencher instead of water. The observed large kinetic isotope effect with $H_2O$ and $D_2O$ ($K_{SV}H_2O/K_{SV}D_2O$ = 1.7) clearly establishes the sensitivity of the fluorescence intensity to hydrogen bonding and the role of hydrogen bonding in the deactivation of the excited state.[8]

In contrast, the carbon analogue is much less sensitive to the presence of water (almost by an order of magnitude) with a $K_{SV}$ = 0.27 ± 0.02 dm$^3$ mol$^{-1}$ and a deuterium isotope effect ($K_{SV}H_2O/K_{SV}D_2O$) of ~2.1. Efficient quenching of emission from **1** and the poor quenching of the emission from **2** by water with the observed isotope effects indicate that hydrogen bonding to the nitrogen in the six-membered ring of **1** is perhaps very efficient in the deactivation of the excited state. Although hydrogen bonding to the carbonyl oxygens of both **1** and **2** may be expected to be important in the quenching mechanism, its role seems to be minor as indicated by the poor quenching observed with **2** by water. However, the large deuterium isotope effect observed with **2** confirms the hydrogen bonding ability of the carbonyl oxygens in the excited state even though its role in the deactivation is quite weak. From these various observations, we conclude that the nitrogen atom in **1** plays a significant role in the charge separation as well as in the excited state deactivation through hydrogen bonding. The single hetero atom in the spacer of **1** provides a favourable site for hydrogen bonding and serves as a fluorescent hydrogen bonding switch.[1]

The role of hydrogen bonding in the fluorescence quenching and the recognition of the hetero atom by small hydrogen bonding molecules was further established in quenching experiments with phenol, aniline and anisole, in a non-polar solvent such as benzene. The corresponding $K_{SV}$ values with **1** are 18.4 ± 0.73, 18.16 ± 0.68 and 1.24 ± 0.31 dm$^3$ mol$^{-1}$, respectively. The quenching constants for phenol and aniline are within experimental error and indicate that the quenching is perhaps due to the hydrogen bonding ability of the quencher. Consistent with this observation, the methylated analogue of phenol, anisole shows only a poor quenching constant. From these results one predicts that the quenching of fluorescence from **2** by these quenchers should be very weak. Indeed, the quenching constants with the carbon analogue for phenol, aniline and anisole are 3.31 ± 0.36, 5.67 ± 0.6 and 0.96 ± 0.28 dm$^3$ mol$^{-1}$, respectively. The slightly larger value with aniline when compared to phenol or anisole could be due to electron transfer mechanism for the quenching rather than hydrogen bonding. Thus, the contrasting behaviour between **1** and **2** as well as the hydrogen bonding and non-hydrogen bonding quenchers further lend support for the charge transfer excited state and its quenching by hydrogen bonding molecules. Since quenching constants decrease dramatically when the hetero atom is absent, it serves as a key element in the recognition by the hydrogen bonding quenchers. By increasing the length of the spacer one could provide larger cavities for hydrogen bonding molecules. Such probes may be useful in the development of fluorescent chemical sensors and extend the realm of optical sensors developed so far.[2]

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