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ORIGINAL PAPER

N-(2-(*N*’,*N*’-Diethylamino)ethyl)perylene-3,4-dicarboximide and its Quaternized Derivatives as Fluorescence Probes of Acid, Temperature, and Solvent Polarity

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Abstract In this manuscript, we report the fluorescence properties of *N*-(2-(*N*’,*N*’-diethylamino)ethyl)perylene-3,4-dicarboximide (**1**) and its quaternized derivative *N*-(2-(*N*’,*N*’-diethyl-*N*’-methylammonium)ethyl)perylene-3,4-dicarboximide tosylate (**2**) in organic solvents. The effects of carboxylic acids and amines on the fluorescence properties of these compounds were investigated. In addition, we studied the aggregation and fluorescence properties of (**2**) and its 9-bromo-substituted derivative (**3**) in aqueous solution. The fluorescent properties of these compounds change dramatically with the extent of aggregation, thus allowing these compounds to be used as fluorescent probes for changes in temperature and solvent polarity. For instance, the fluorescence emission intensity of **3** increases by about 28 times as the temperature of the solution increases from 10°C to 85°C. The fluorescent intensities of **2** and **3** in methanol are higher than that in water by about 8 and 25 times, respectively.

Keywords Perylene monoimide · Fluorescence probe · Photoinduced electron transfer · Aggregates

Introduction

Fluorescent compounds have important applications in clinical diagnostics, biomedical research, studies in life sciences, and as luminescent materials in optical devices [1]. Although there are reports of the photophysical properties of perylene-3,4-dicarboximides (or perylene monoimide) in organic solvents [2–4] and polymers [5–8] suggesting that these compounds have potential applications in bioassays [9–16], light harvesting systems, and photovoltaic devices [17–27], the structural effects on the fluorescence properties of perylene-3,4-dicarboximides, particularly in aqueous solution, remain largely unexplored. A deeper understanding of the structural effects on electronic transition and fluorescence properties of perylene-3,4-dicarboximides is important for developing applications and future design of fluorophores with desired properties.

We reported recently the direction-dependent electronic transition and fluorescence properties of *N*-(2-(*N*’,*N*’-diethylamino)ethyl)perylene-3,4-dicarboximide **1** and its quaternized derivative **2** in the crystal phase [28, 29]. In this manuscript, we report the fluorescence properties of these compounds and the 9-bromo-substituted derivative **3** in organic solvents and aqueous solution. The structures of compounds **1**–**3** are shown in Fig. 1.

We are interested in investigating the fluorescence properties of these compounds in solution for several reasons. Firstly, the flexible (*N,N*-diethylamino)ethyl group of compound **1** not only imparts solubility in common organic solvents but also present an electron rich amino group for quenching fluorescence emission of the perylene-3,4-dicarboximide ring by photoinduced electron transfer (Fig. 2). Protonation of the amino nitrogen lone-pair electrons will turn off this quenching mechanism, thereby allowing the use of **1** as fluorescence probe for detection of acids. Secondly, compounds that fluoresce in aqueous solution are generally useful in medical diagnostics, studies in life sciences, biomedical and biological research, and environmental analysis [30–35]. The quaternized derivative **2** has higher solubility than **1** in polar solvents and aqueous solution and is potentially useful in a broader range of applications. Water-soluble fluorophores with properties that vary with changes in environment are particularly important for sensing applications [36, 37]. In this

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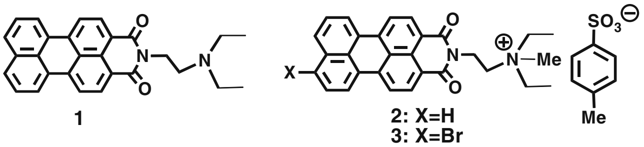

Fig. 1 Structures of perylene-3,4-dicarboximides **1**–**3**

paper, the effects of temperature and solvent polarity on the fluorescence properties of **2** in aqueous solution are presented. Thirdly, substituents on the perylene-3,4-dicarboximide ring may modulate the aggregation, and hence fluorescence properties of ionic perylene-3,4-dicarboximides in aqueous solution. We demonstrate such effect using the ionic 9-bromoperylene-3,4-dicarboximide derivative **3**.

Results and discussion

Effects of organic acids and bases on the UV-vis absorption and fluorescence properties of 1 and 2 in organic solvents

2-(*N,N*-Diethylamino)ethylperylene-3,4-dicarboximide (**1**) is soluble in organic solvents such as chloroform ($CHCl_3$) and dichloromethane (DCM), but less soluble in methanol (MeOH). The ionic derivative **2** has higher solubilities in MeOH than in $CHCl_3$. To allow comparison of the optical properties of **1** and **2** in organic solvents, UV-vis and fluorescence studies were performed in a mixture of DCM/MeOH. In this solvent mixture, compounds **1** and **2** have similar electronic transition properties which are not affected significantly by the addition of organic acids such as 5 vol% of formic acid (HCOOH) or acetic acid (CH_3COOH) (Fig. 3). In the presence of an organic base such as 5 vol% of piperidine, a small blue shift (about 10 nm) in the absorption band of **1** is resulted.

Both **1** and **2** exhibit fluorescence emission (λ_{max} of emission at about 560 nm) upon excitation at 491–495 nm; a Stokes shift of about 70 nm. As shown in Fig. 4, there is no significant change in fluorescence emission of **2** in the presence of as much as 5 vol % of CH_3COOH or HCOOH. At similar concentrations (1.0×10^{-7} M), the emission intensity of **1** is substantially lower than that of **2** and is greatly enhanced in the presence of CH_3COOH (Fig. 5). The enhancement can be observed in the presence of as little as 5 μM of CH_3COOH. Presumably, the fluorescence emission of **1** is quenched by a photoinduced electron transfer (PET) process [1, 30–35, 38, 39] in the presence of lone-pair electrons of the tertiary amino group of **1**. Removal of the lone-pair electrons of the amino group of **1** by protonation with acids prevents the quenching of fluorescence emission by PET. The results show that **1** serves as fluorescence probe of acids in organic solvents. Amino nitrogen lone-pair is not available in **2**, consequently, the emission of **2** is not quenched by intramolecular PET and does not vary with the acid concentration in solution. The pK_a values of the *N*-(2-(*N*',*N*'-diethylamino)ethyl) perylene-3,4-dicarboximide and perylene-3,4:9,10-bis (dicarboximide) in methanol or ethanol and water mixtures were found to be in the range of 9.5–9.8 [35], which are similar to the value of 9.9 determined for the pK_a of the monoprotonated species of 1-amino-2-diethylaminoethane and not significantly influenced by the aromatic rings. Base on these values, the pK_a value of **1** is estimated to be in the same range (9.5–9.8).

Using fluorescein (quantum yield of 1.0 in MeOH with 0.01 M KOH) as reference standard [40], the fluorescence quantum yield of **2** in MeOH was determined to be 0.50. The quantum yield of **1** in DCM is only 0.06 presumably due to quenching by intramolecular PET. The quantum yield of **1** in MeOH/DCM (v:v=50:50) is 0.12, slightly higher than that in DCM, presumably because of the presence of some protonated form in MeOH/DCM (v:v=50:50). Fluorescence emission of the protonated form is not quenched by intramolecular PET.

Results of the time-resolved fluorescence lifetime studies of the effect of acids are consistent with the results of the steady state experiments. The lifetime of a 1.0×10^{-6} M solution of **2** in MeOH/DCM (v:v=50:50) is 5.3 ns and it does not change in the presence CH_3COOH (1–5 vol %) in MeOH/DCM. In contrast, the lifetime of **1** changes in the presence of acid. Lifetime studies of **1** in MeOH/DCM (v:v=50:50), indicated the presence of a species (20%) with a lifetime of 5.0 ns, and a short-lived species (80%) with a lifetime of about 1.0 ns. Presumably, **1** has a short lifetime of about 1 ns because of quenching by the nitrogen lone-pair of the amino side chain, and some protonated form of **1** is present in MeOH/DCM resulting in the longer lifetime of 5.0 ns observed. After the addition of CH_3COOH (1–5 vol %), the amino group of **1** is protonated, resulting in a species of lifetime of 5.3 ns only.

Although the fluorescence emission of **2** is not quenched by intramolecular PET process, it can be quenched by intermolecular PET. Quenching of **2** by intermolecular PET was studied using piperidine (an alicyclic amine) and *N*-

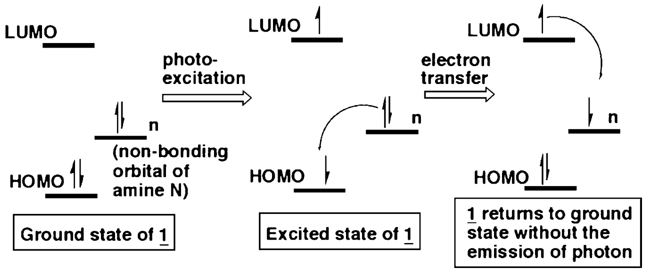

Fig. 2 Diagram illustrating the quenching of fluorescence emission of **1** by photoinduced electron transfer (PET) from the non-bonding orbital of amine nitrogen

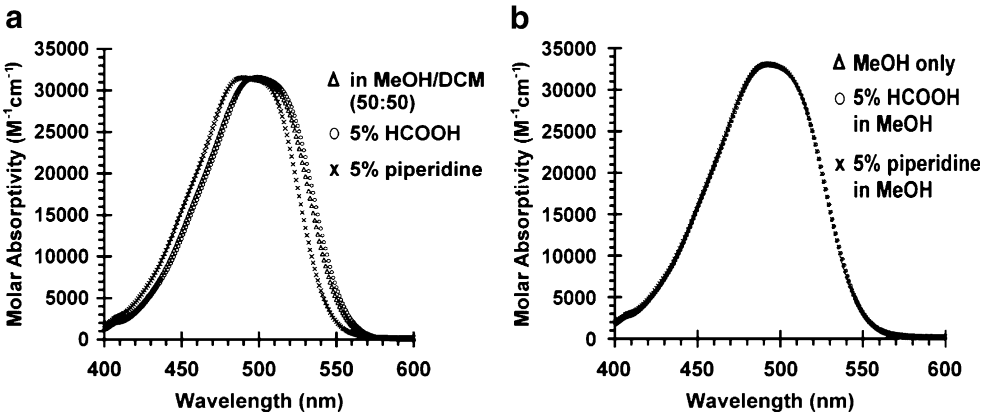

Fig. 3 (**a**) Absorption spectra of **1** in (Δ) MeOH/DCM (v/v=50/50) and (o) MeOH/DCM/HCOOH (v/v/v=47.5/47.5/5), and (x) MeOH/DCM/piperidine (v/v/v=47.5/47.5/5). (**b**) Absorption spectra of **2** in (Δ) MeOH/DCM (v/v=50/50) and (o) MeOH/DCM/HCOOH (v/v/v=47.5/47.5/5), and (x) MeOH/DCM/piperidine (v/v/v=47.5/47.5/5)

ethylaniline (an aromatic amine). As shown in Fig. 6, the addition of piperidine (e.g., 1 vol%, ~0.1 M) to a solution of **2** (e.g., 1×10^{-7} M) in methanol has no significant effect on the wavelengths of emission, however, it lowers the fluorescence intensity of **2**. The quenching effect is weak and requires a high molar ratio of piperidine to cause a significant decrease in fluorescence intensity. The quenching constants (K_D) determined from the slopes of the linear Stern-Volmer plots (Fig. 7) are 1.3, 1.8, and 2.3 M^{-1} at 25°C, 35°C, and 45°C, respectively. The increase in quenching constant with temperature suggests that the decrease in fluorescence is caused by collisional quenching effect [1] of piperidine. Diffusion rate, and thus, quenching by collision increase as temperature increases.

N-ethylaniline is a more effective quencher than piperidine. Quenching of emission of **2** (1×10^{-7} M) at 35°C occurs in the presence of *N*-ethylaniline at concentrations as low as 0.5 mM, and only about 0.01 M of *N*-ethylaniline is required for 50% quenching. An upward curvature is observed for the Stern-Volmer plot (Fig. 8, left). The dependence of F_0/F on the concentration of *N*-ethylaniline is second order in the concentration of *N*-ethylaniline. This suggests that the quenching of **2** occurs by both collisional quenching and a static mechanism [1]. The values of collision quenching constant (K_D) and static quenching constant (K_S) were determined from the plot of $((F_o\text{-}F)/F[N\text{-ethylaniline}])$ versus [*N*-ethylaniline]. According to Eq. 1, the slope of the linear plot equals K_DK_S and the intercept equals (K_D+K_S) [1]. From the slope and intercept of the plot (Fig. 8, right), the values of K_D and K_S at 35°C were calculated to be 4.5 M^{-1} and 97 M^{-1}, respectively.

$$(F_o - F)/F[N - \text{ethylaniline}] = K_DK_S[N - \text{ethylaniline}] + (K_D + K_S) \tag{1}$$

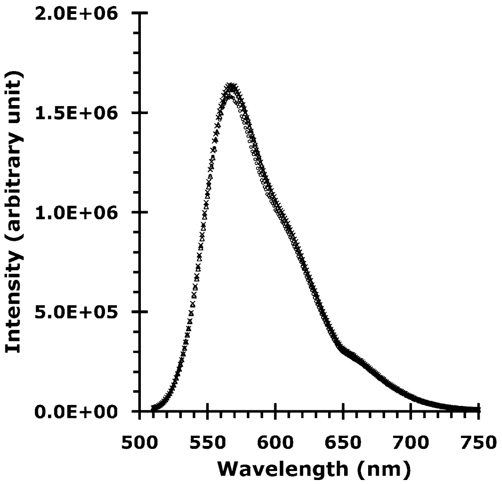

Fig. 4 Fluorescence spectra of **2** (1.0×10^{-7} M) (o) in MeOH/DCM (v/v=50/50), (x) in MeOH/DCM/CH_3COOH (v/v/v=47.5/47.5/5), and (Δ) in MeOH/DCM/HCOOH (v/v/v=47.5/47.5/5). The spectra in the presence of CH_3COOH or HCOOH overlapped with the spectra in the absence of acids

Effects of solvent polarity and temperature on UV-vis absorption and fluorescence properties of 2 and 3 in MeOH and aqueous solution

The ionic derivatives **2** and **3** have higher solubilities in MeOH than in $CHCl_3$. They can also be dissolved in water to give dilute solutions at room temperature. Compounds **2** (1.0×10^{-5} M) and **3** (1.0×10^{-5} M) in MeOH have similar λ_{max} of absorption. At similar concentrations, the λ_{max} values of these compounds in water are blue-shifted compared to that in MeOH. The molar absorptivities of these compounds in water are lower than those in MeOH (see λ_{max} values and molar absorptivities provided in the experimental section.)

The aggregation of **2** and **3** in water was investigated using UV-vis spectroscopy. Upon varying the concentration of **2** over the range of 1.0×10^{-7} M to 1.0×10^{-3} M, a blue shift in λ_{max} from 504 nm to 482 nm in the visible spectra was observed due to aggregation of **2** (Fig. 9a). Similar shift was observed for **3** (Fig. 9b). At a low concentration (1.0×10^{-7} M), the λ_{max} of **3** is at 487 nm. It shifts gradually

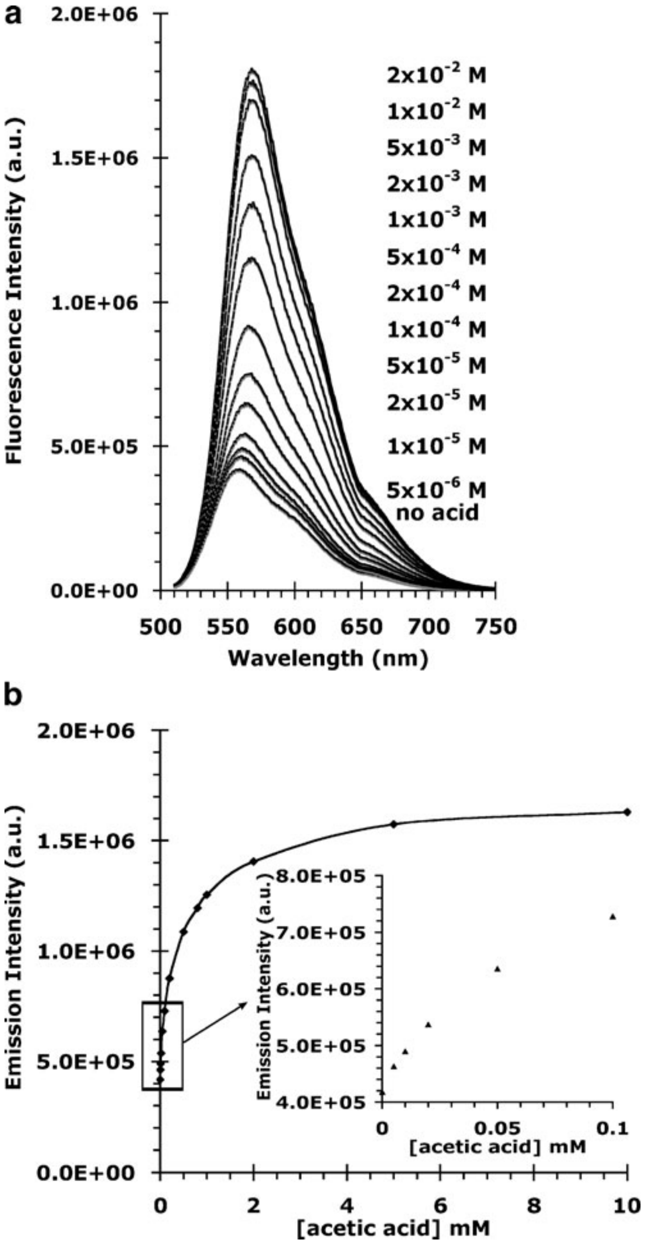

Fig. 5 Effect of CH_3COOH on fluorescence emission of **1** (1.0×10^{-7} M) in MeOH/DCM (v/v=50/50)

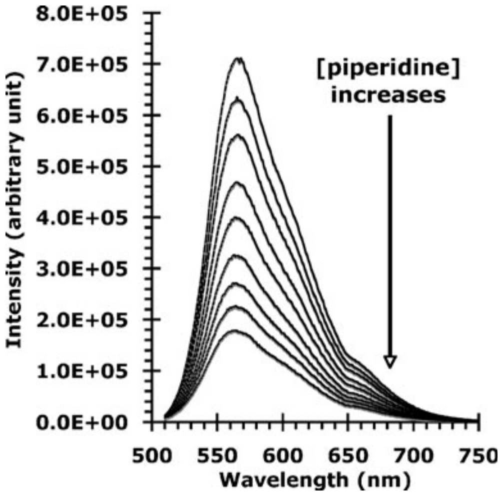

Fig. 6 Effect of piperidine on the fluorescence properties of **2** (1.0×10^{-7} M) in MeOH at 25°C. The intensities of emission spectra decreased gradually as the volume % of piperidine was increased from 0 to 1, 2, 4, 6, 9, 12, 15, and 20 vol%

to 475 nm as the concentration increases to 1.0×10^{-4} M. Spectral changes in the visible region of **2** and **3** suggest the formation of H-aggregates [41, 42] of these compounds in water. Studies reported for other ionic aromatic dyes in aqueous solution suggested that there is no optimum aggregation size and no critical concentration for the formation of aggregates [43–45].

Further evidence for the formation of H-aggregates is provided by the temperature-dependent absorption spectra of these compounds. As shown in Fig. 10, the λ_{max} of absorption of **2** (1×10^{-5} M) in water is red-shifted from 485 nm at 10°C to 495 nm at 80°C. The red shift is accompanied by an increase in molar absorptivity. A red shift from 480 nm at 10°C to 490 nm at 80°C was observed for **3** (1×10^{-5} M). Such changes are indicative of the formation of H-aggregation of these compounds in water at low temperature. The extent of aggregation decreases as temperature increases resulting in an increase in λ_{max} and molar absorptivity.

Compounds **2** and **3** fluoresce in water and show an excitation band with a maximum at 491 nm and an emission band at about 590 nm; a very large Stokes shift of almost 100 nm. The effect of temperature on the fluorescent properties of these compounds is dramatic. As temperature was raised from 10°C to 80°C, about 14-fold increase in emission intensity was observed for a 1×10^{-5} M solution of **2**

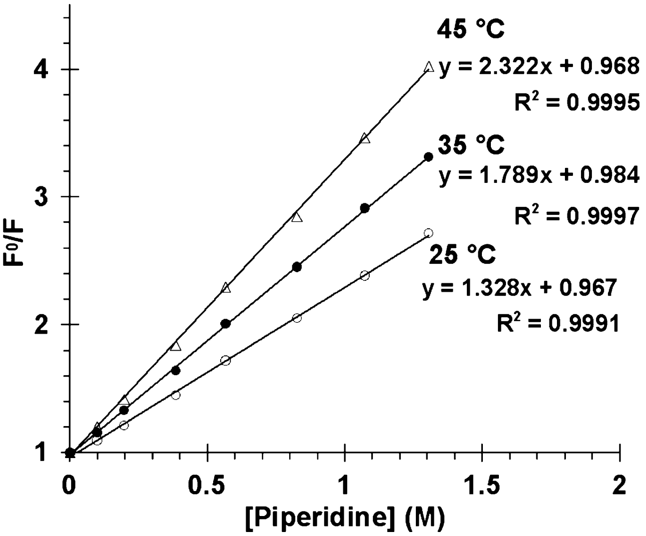

Fig. 7 Stern-Volmer plots of quenching of 1.0×10^{-7} M of **2** in MeOH by piperidine at 25°C (Δ), 35°C (•), and 45°C (o)

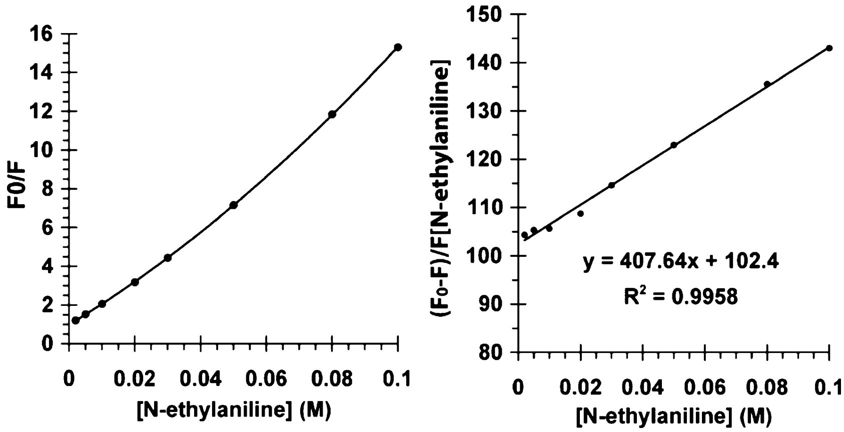

Fig. 8 (Left) Stern-Volmer plots of quenching of 1.0×10^{-7} M of **2** in MeOH by *N*-ethylaniline at 35°C. (Right) A plot of the apparent quenching constant ((F$_o$-F)/F[*N*-ethylaniline]) versus [*N*-ethylaniline]

(Figs. 11a and 12). At a low temperature, the aggregation of **2** in water results in significant self-quenching. The extent of aggregation decreases at a higher temperature, consequently reducing the extent of self-quenching.

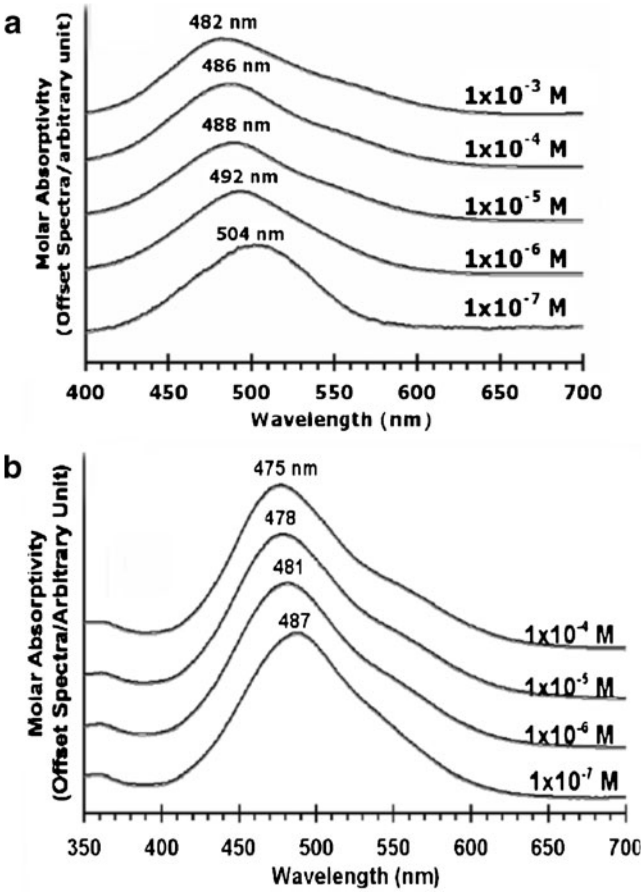

Fig. 9 Series of offset visible spectra of (**a**) **2** of concentrations from 1.0×10^{-3} M (top spectrum) to 1.0×10^{-7} M (bottom spectrum) and (**b**) **3** of concentrations from 1.0×10^{-4} M (top spectrum) to 1.0×10^{-7} M (bottom spectrum) in water

The substitution of hydrogen at the 9-position of the perylene-3,4-dicarboximide ring by bromine resulted in very significant difference in the temperature-dependent aggregation and fluorescence properties of **3**. At 10°C, the emission intensity of **3** (1.0×10^{-5} M) is significantly lower than that of **2** at similar concentrations (Fig. 11b). This is attributed to the bromine substitution that (1) causes quenching of fluorescence by heavy atom effect and (2) modulates the solubility and stacking of aromatic rings, thereby, increases aggregation and self-quenching of **3**. More importantly, the fluorescence emission intensity increases dramatically by about 28-fold as the temperature of the solution increases from 10°C to 85°C (Figs. 11b and 12).

In addition to temperature, the fluorescence emissions of **2** and **3** in aqueous solution can be varied by the addition of a less polar solvent, such as methanol. As shown in Fig. 13, the λ_{max} of emission of **2** and **3** in methanol are slightly blue-shifted (about 20 nm) compared to that in water. The relaxation of the excited state dipole of fluorophores by solvation of solvent dipoles will result in lower energy of the excited state [38, 39]. Since stabilization of the excited state dipole in methanol is less effective than in water, emission in methanol is at higher energy or shorter wavelength. In addition to different λ_{max} values of emission, the fluorescent intensities of **2** and **3** in methanol are higher than that in water by about 8 and 25 times, respectively. Several factors contribute to the lower fluorescent emission intensities in water compared to methanol. The molar absorptivities of **2** and **3** in water are lower than those in methanol. Furthermore, the rate constants of non-radiative decays of fluorophores are not the same in different solvents. Most significantly, the aggregation of these fluorophores in water results in reabsorption and self-quenching of fluorescent emission.

Using fluorescein (quantum yield of 1.0 in methanol with 0.01 M KOH) as reference standard, the fluores-

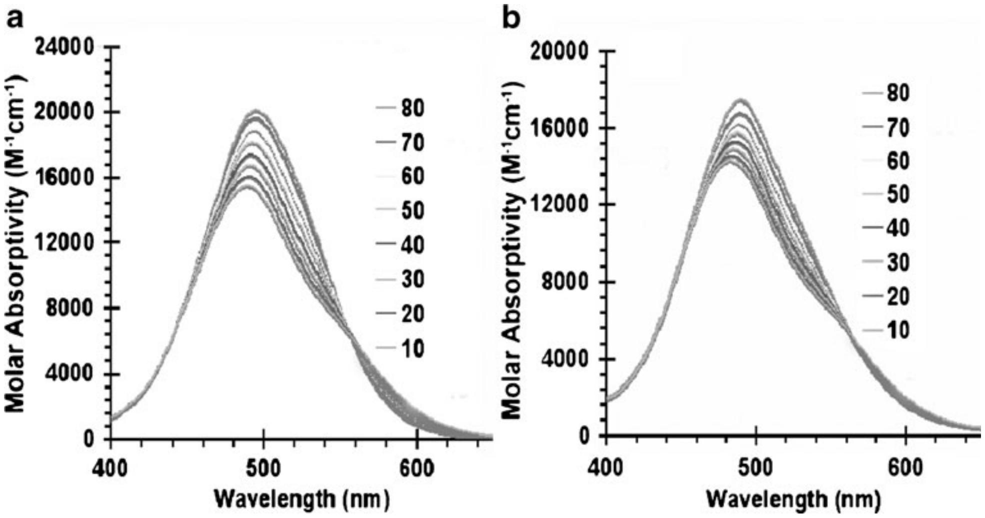

Fig. 10 Spectra showing the effect of temperature on the uv-vis absorption properties of (**a**) **2** (1×10^{-5} M) and (**b**) **3** (1×10^{-5} M) in water. Spectra were taken at increments of 10°C in temperature

cence quantum yields of **2** and **3** in MeOH were determined to be 0.50 and 0.41, respectively (Table 1). The lower quantum yield observed for **3** is probably caused by the presence of bromine which increases fluorescence quenching by heavy atom effect. The average quantum yields of the aggregates in water were also determined. In consistent with the lower fluorescent emission intensities in the steady state spectra observed for these compounds in water, the average quantum yields of the aggregates of **2** and **3** in water were found to be lower than the quantum yields in methanol. Furthermore, the quantum yields decrease as self-quenching increase with aggregation at higher concentrations.

Conclusions

Overall, perylene-3,4-dicarboximide is an excellent scaffold for constructing fluorophores with desired properties for sensing applications. The solubility, aggregation, and fluorescence properties can be modulated by the structure of the group attached to the imide nitrogen and substituent on the aromatic ring. Compounds **1–3** can be excited with visible light at wavelengths of commercially available lasers and fluorescence emission can be observed without the need to remove oxygen from the solution. The large Stokes shift permits easy filtering of emission light from excitation light in studies by fluorescence microscopy and spectroscopy techniques.

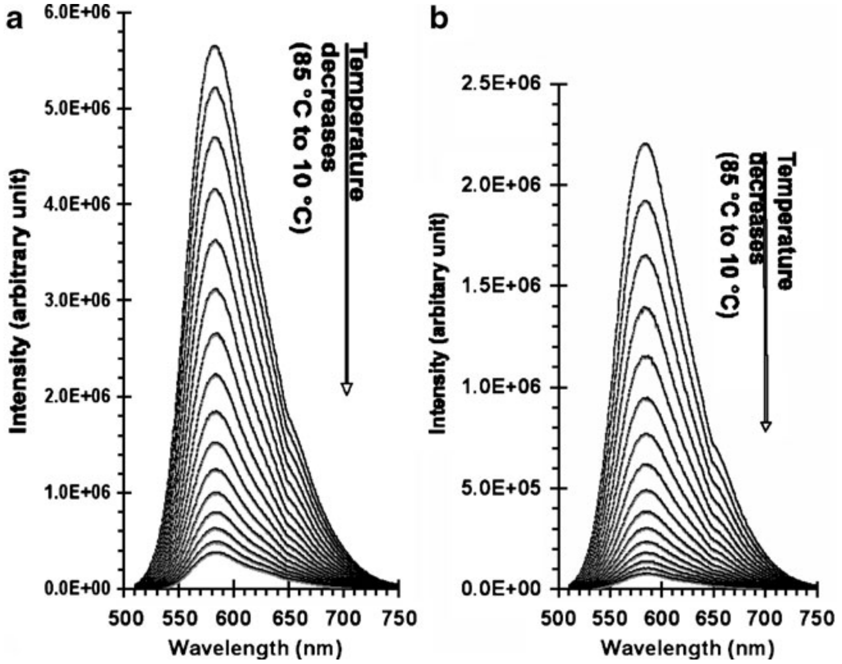

Fig. 11 Temperature-dependent fluorescence excitation and emission spectra of (**a**) **2** (1.0×10^{-5} M) and (**b**) **3** (1.0×10^{-5} M) in water. Spectra were taken at increments of 5°C in temperature. Excitation spectra were acquired at emission wavelength of 586 nm and the emission spectra were acquired at excitation wavelength of 491 nm

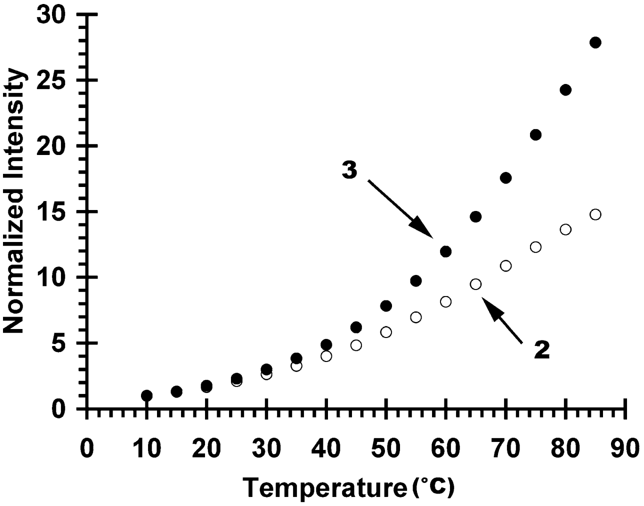

Fig. 12 Plot showing the effect of temperature on the fluorescent emission intensity at λ_{max} of aqueous solutions (1.0×10^{-5} M) of **2** and **3**. For comparison of the relative increase in intensity, the emission intensities are normalized with respect to the intensity at 10°C

This study shows that the fluorescence emission of *N*-(2-(*N*',*N*'-diethylamino)ethyl)perylene-3,4-dicarboximide **1** is partially quenched by photoinduced electron transfer from the lone-pair electrons of its amino side chain. Fluorescence emission is enhanced by protonation of the amino side chain suggesting that **1** may serve as fluorescent probe for acidic environment. Our studies show that **1** can detect carboxylic acids in the μM concentration range. Quaternization of the amino side chain of **1** resulted in **2** with higher fluorescent quantum yield and longer lifetime than **1**. The fluorescence emission of **2** is not affected by carboxylic acids. The results of this research demonstrated that the fluorescence emission of perylene-3,4-dicarboximide could be switched on or off by controlling the availability of lone-pair electrons of an amino group.

The ionic compounds **2** and **3** are soluble in water and have potential applications as fluorescence probes of temperature and solvent polarity. The fluorescence emission intensity of **3** increases dramatically by about 28-fold as the temperature of the solution increases from 10°C to 85°C. In addition, the fluorescent intensities of **2** and **3** in methanol are higher than that in water by about 8 and 25 times, respectively. The 9-bromo substituent of **3** has substantially change the extent of aggregation of the ionic perylene-3,4-dicarboximides in water and resulted in a much larger increase in fluorescence emission with temperature and solvent polarity than **2**.

Experimental

Compounds **1** and **2** were synthesized according to procedures described previously [28, 29]. Compound **3** was synthesized by reaction of 9-bromo-(*N*-(2-(*N*',*N*'-diethylamino)ethyl)perylene-3,4-dicarboximide (**4**) with methyl *p*-toluenesulfonate as described below.

N-(2-(N',N'-diethyl-N'-methylammonium)ethyl)-9-bromo-perylene-3,4-dicarboximide tosylate (3) Methyl *p*-toluenesulfonate (2.50 g, 13.5 mmol) was dissolved into 20 mL of toluene. Then 9-bromo-2-(*N,N*-diethylamino)ethylperylene-3,4-dicarboximide **4** (0.50 g, 1.0 mmol) was added and the reaction mixture was refluxed overnight. After the reaction mixture was cooled to room temperature, the solid was collected by suction filtration and washed with toluene and ether. The solid was dried under vacuum overnight and then suspended into 50 mL of $CHCl_3$. The suspension was refluxed overnight. Then the resulting solid was collected by suction filtration and dried over vacuum overnight to give **3** (0.59 g, 86%). ^{1}H NMR (400 MHz, CD_3OD): δ=7.67 (d, 2H, ^3J (H,H)=8.4 Hz, Ar-H), 7.63 (d, 1H, ^3J (H,H)=9.6 Hz,

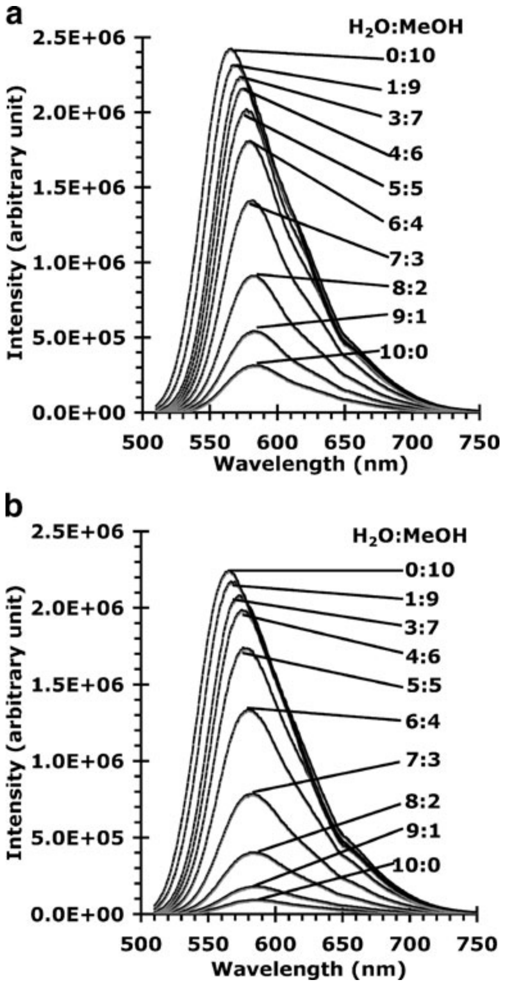

Fig. 13 Effect of solvent on the fluorescence emission spectra of (**a**) **2** (1×10^{-6} M) and (**b**) **3** (1×10^{-6} M). The emission spectra were acquired at an excitation wavelength of 491 nm

Table 1 Quantum yields (Φ) of **2** and **3** in MeOH and water at 25°C

Entries	Solution	Φ of **2**	Φ of **3**
1	In methanol	0.50±0.02	0.42±0.008
2	2×10^{-7} M in water	0.17±0.004	0.079±0.006
3	5×10^{-7} M in water	0.13±0.006	0.051±0.003
4	1×10^{-6} M in water 1×10^{-5} M in water	0.11±0.006	0.035±0.002
5a	at 80°C	0.13±0.003	0.072±0.001
5b	at 25°C	0.021±0.0002	0.0061±0.00009
5c	at 10°C	0.010±0.00009	0.0026±0.00004

Ar-H), 7.61 (d, 1H, ^3J (H,H)=8.8 Hz, Ar-H), 7.60 (d, 1H, ^3J (H,H)=7.6 Hz, Ar-H), 7.52 (d, 1H, ^3J (H,H)=7.6 Hz, Ar-H), 7.38 (d, 1H, ^3J (H,H)=8.0 Hz, Ar-H), 7.28 (d, 1H, ^3J (H,H)= 9.2 Hz, Ar-H), 7.27 (t, 1H, ^3J (H,H)=8.4 Hz, Ar-H), 7.21 (d, 1H, ^3J (H,H)=8.0 Hz, Ar-H), 7.18 (d, 2H, ^3J (H,H)=8.0 Hz, Ar-H), 7.14 (d, 1H, ^3J (H,H)=7.6 Hz, Ar-H), 4.30 (t, 2H, ^3J (H,H)=7.6 Hz, -CH2), 3.54 (m, 4H, -N-$(CH_2)_2$-), 3.46 (t, 2H, -CH_2-N-$(CH_2)_2$), 3.16 (s, 3H, -N-CH_3), 2.30 (s, 3H, CH_3 of *p*-toluenesulfonate), 1.47 (t, 6H, ^3J (H,H)=7.2 Hz, -CH_2-CH_3) ppm; ^{13}C NMR (100 MHz, CF_3COOD): δ=168.3, 167.7, 146.4, 140.7, 140.5, 138.6, 135.2, 135.2, 134.0, 133.9, 133.2, 132.9, 131.5, 131.2, 130.3, 130.0 129.4, 129.2, 127.9, 127.2, 127.0, 122.8, 122.4, 120.0, 119.9, 60.1, 58.3, 49.7, 36.4, 21.7, 8.9 ppm; UV (MeOH, 1.0×10^{-5} M)) λ_{max}=262 (ε=29,700), 338 (ε=2,800), 354 (ε=3,520), λ_{max}=493 (ε= 35,500) nm, (water, 1.0×10^{-5} M)) λ_{max}=360 (ε=2,820), 481 (ε=16,500) nm; MS (ESI+, *m/z*) Calcd for $[C_{29}H_{26}BrN_2O_2]^+$: 513.12; Found: 513.28.

N-(2-N',N'-diethylamino)ethyl)-9-bromoperylene-3,4-dicarboximide (4) 2-(*N,N*-diethylamino)ethylperylene-3,4-dicarboximide (**1**) (3.01 g, 7.15 mmol) and 150 mL of concentrated sulfuric acid were mixed in a 500-mL round-bottom flask. The mixture was cooled to −10°C, then 0.48 mL of Br_2 was added slowly with a 1-mL syringe. After the reaction mixture was stirred at −10 to −5°C for 4 h, it was poured into 750 mL of cold distilled water in a 2,000-mL flask in an ice bath. About 400 mL of 30% $NH_4OH_{(aq)}$ was added dropwise until the pH was 8–9. A bright red solid formed was filtered and washed with 300 mL of 5% $NH_4OH_{(aq)}$ and then distilled water. The solid was dried in an vacuum oven at 110°C. The bright red solid was purified by recrystallization in 100 mL of 1% triethylamine in DMF solution. The filtered solid was washed with ethyl ether and dried under vacuum to yield **4** (2.96 g) in 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ=8.30 (d, 1H, ^3J (H,H)) 8.0 Hz, Ar-H), 8.27 (d, 1H, ^3J (H,H)) 8.5 Hz, Ar-H), 8.09 (d, 2H, ^3J (H,H)) 8.5 Hz, Ar-H), 8.01 (d, 1H, ^3J (H,H)) 8.0 Hz, Ar-H), 7.93 (d, 1H, ^3J (H,H)) 8.0 Hz, Ar-H), 7.80 (d, 1H, ^3J (H,H)) 8.0 Hz, Ar-H), 7.66 (d, 1H, ^3J (H,H)) 8.5 Hz, Ar-H), 7.51 (d, 1H, ^3J (H,H)) 8 Hz, Ar-H), 4.27 (t, 2H, ^3J (H,H)) 7.5 Hz, α-CH_2), 2.82 (t, 2H, ^3J (H,H)) 7.5 Hz, β-CH_2), 2.72 (q, 4H, ^3J (H,H)) 7.0 Hz, -NCH_2CH_3), 1.14 (t, 6H, ^3J (H,H)) 8.0 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ=163.7, 163.7, 136.1, 136.0, 132.7, 131.3, 1,312, 131.1, 130.0, 129.3, 129.3, 128.7, 128.0, 126.2, 126.0, 124.1, 123.4, 121.2, 120.5, 120.2, 50.0, 47.9, 38.2, 12.5 ppm; UV ($CHCl_3$, 1.0×10^{-5} M) λ_{max}= 475 (ε=33,150), 497 (ε=34,100) nm; HRMS (FAB, *m/z*) Calcd 499.1021, found 499.1004; mp 246°C.

Deuterated trifluoroacetic acid (TFA-d, D-99.5%), deuterated chloroform ($CDCl_3$, D-99.8%), and deuterated methanol (CD_3OD, D-99.8%) were purchased from Cambridge Isotopes. Formic acid (HCOOH(aq) conc. +88%) and glacial acetic acid (100.0%) were purchased from ACROS Organics and Fisher Scientific, respectively.

^{1}H and ^{13}C NMR spectra were acquired on either a Varian MR 400 MHz or a GE QE 300 MHz spectrometer. Infrared spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. Spectra of powdered samples were obtained in the form of KBr pellets prepared with dried KBr using a mini-press from SpectraTech, Inc. Mass spectra were recorded with Waters Micromass XQ detector using ESI^+ and ESI^-. Electronic spectra were obtained with a dual-beam Perkin Elmer Lambda 950 and software UV-WIN Lab version 5.1.5.

Fluorescence spectra were acquired using a Jobin-Yvon Horiba Fluorolog 3–222 spectrophotometer and software FluorEssence. Lifetime measurement were performed at 25°C using the Fluorolog 3 equipped with a NanoLED-492, FluoroHub TCSPC, a single photon detection cooled photocathode TBX-05, and Datastation DAS6 Foundation Software. The lifetime of fluorescein in MeOH was determined under the same conditions for comparison and was found to be 4.1 ns. All studies were preformed without purging the solutions with inert gas to remove oxygen.

Quantum yields were determined according to the method reported by Williams and his coauthors [46]. Solutions of three concentrations (2.0×10^{-7} M, 5.0×10^{-7} M, and 1.0×10^{-6} M) were prepared for fluorescein in MeOH (0.01 N KOH) and perylene-3,4-dicarboximide **1**–**3** in the solvents to be studied. The UV-vis absorption spectra of solutions of both fluorescein and **1**–**3** were acquired using a 10 or 1-cm path length cuvette. Electronic spectra were obtained with a dual-beam Perkin Elmer Lambda 950 and software UV-WIN Lab version 5.1.5. Fluorescence spectra were acquired using a Jobin-Yvon Horiba Fluorolog 3–222 spectrophotometer and software FluorEssence. Emission in range from 460 nm to 750 nm was acquired when the sample in a 1-cm path length fluorescence cuvette was excited at 450 nm. The integrated fluorescence intensity (that is, the area of the fluorescence spectrum from 490 nm to 750 nm) was calculated and note down from all fluorescence spectrum. A graph of integrated fluorescence integration vs absorbance (at 450 nm) was plotted for each compound. The slopes of the graphs are proportional to the quantum yield of the different samples. Absolute values were calculated according to the following equation [46]:

$$\Phi_X = \Phi_{ST}(\mathrm{Grad}_X/\mathrm{Grad}_{ST})\left(\eta_X^2/\eta_{ST}^2\right)$$

Where Φ is the fluorescence quantum yield, ST and X denote standard and test respectively, η is the refractive index of the solvent, and *Grad* is the slope from the plot of integrated fluorescence intensity *vs* absorbance.

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