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A ratiometric near-infrared pH-responsive fluorescent dye based on distyryl BODIPY†

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A novel distyryl boron dipyrromethene substituted with two 4-(dimethylamino)phenylethynyl groups at the 2- and 6-positions has been synthesised and characterised. It exhibits remarkable and reversible pH-responsive changes in the absorption and fluorescence emission spectra, both in organic and in aqueous media.

There has been considerable interest in organic materials that absorb and emit in the near-infrared region.[1] Owing to the increased optical transparency and lower tissue auto-fluorescence in this region, this class of materials serves as promising fluorescent probes for *in vivo* imaging.[2] Fabrication of near-infrared absorbing dyes in solar cells can extend the absorption region to cover most of the solar spectrum, which can improve the power conversion efficiency.[3] The use of these materials in other technological sectors, such as laser printing, information storage, telecommunication, and displays has also been well documented.[1] These widespread and important applications have stimulated extensive studies in the development of new and superior near-infrared materials.

Distyryl boron dipyrromethenes (BODIPYs) are versatile near-infrared dyes of which the absorption and emission properties can be easily tuned by chemical modification of the π systems.[4] Some of the desirable characteristics of these dyes include strong absorption in the near-infrared region, high fluorescence quantum yield, reasonably long excited singlet state lifetime, and good solubility and stability in many solvent systems. As a result, they have been widely studied as fluorescent probes for metal ions,[5] building blocks for artificial photosynthetic models,[6] and photosensitisers for photodynamic therapy[7] and dye-sensitised solar cells.[8] Recently, we have been interested in the construction of photoinduced electron and energy transfer systems based on BODIPYs and their styryl analogues.[9] As an extension of this work, we report herein a novel distyryl derivative which is connected to a triethylene glycol chain at the meso phenyl substituent and two 4-(dimethylamino)phenylethynyl groups at the 2- and 6-positions. Like in other pegylated distyryl BODIPYs,[5a,7a,10] the triethylene glycol chain can enhance the hydrophilicity of the molecule. The introduction of two 4-(dimethylamino)phenylethynyl substituents not only shifts the absorption and emission positions further to the red, but also imparts a pH-responsive property to the dye. Acidic environments are known to be associated with a variety of diseases such as cancer, cystic fibrosis and immune dysfunction.[11] Some intracellular compartments such as endosomes, lysosomes and phagosomes are also acidic in nature. Near-infrared dyes that are sensitive to pH changes are therefore of particular interest for application in bio-imaging.[12]

The synthetic route used to prepare this compound is shown in Scheme 1. The triethylene glycol monomethyl ether substituted BODIPY **2** was first prepared by treating benzaldehyde **1** with 2,4-dimethylpyrrole *via* sequential condensation, oxidation and complexation reactions. The 2- and 6-positions of the BODIPY skeleton of **2** were iodinated by using a mixture of iodine and iodic acid to give the diiodo BODIPY **3**. This compound then underwent Knoevenagel condensation with 4-methoxybenzaldehyde in the presence of piperidine to give the distyryl BODIPY **4**. The target compound **5** was obtained in moderate yield by palladium-catalysed Sonogashira coupling reaction of **4** with 4-(dimethylamino)phenylethyne. While compounds **2**, **3** and **5** could be purified readily by chromatography on silica gel columns, the purification of compound **4** required an additional step of size-exclusion chromatography with Bio-beads S-X1 beads. The experimental details and characterisation data are given in the ESI.†

The electronic absorption spectrum of **5** was first recorded in chloroform (Fig. S1, ESI†). It showed a Soret band peaking at 401 nm with a shoulder at 365 nm, an intense Q band at 708 nm, together with a weak absorption at 510 nm. The Q band strictly followed the Lambert–Beer law suggesting that the compound is not significantly aggregated in chloroform. It was red-shifted by *ca.* 50–60 nm compared with that of **4** (662 nm) and some other typical distyryl BODIPYs.[4] The large bathochromic shift could be attributed to the extended conjugation due to the arylethynyl moieties and the intramolecular charge transfer (ICT) process induced by the amino groups.[5] The extent of shift was comparable with that observed for the 2,6-bis(arylethynyl)BODIPY analogues.[13]

Upon addition of trifluoroacetic acid (TFA), the Q band at 708 nm and the shoulder at *ca.* 650 nm shifted gradually to give

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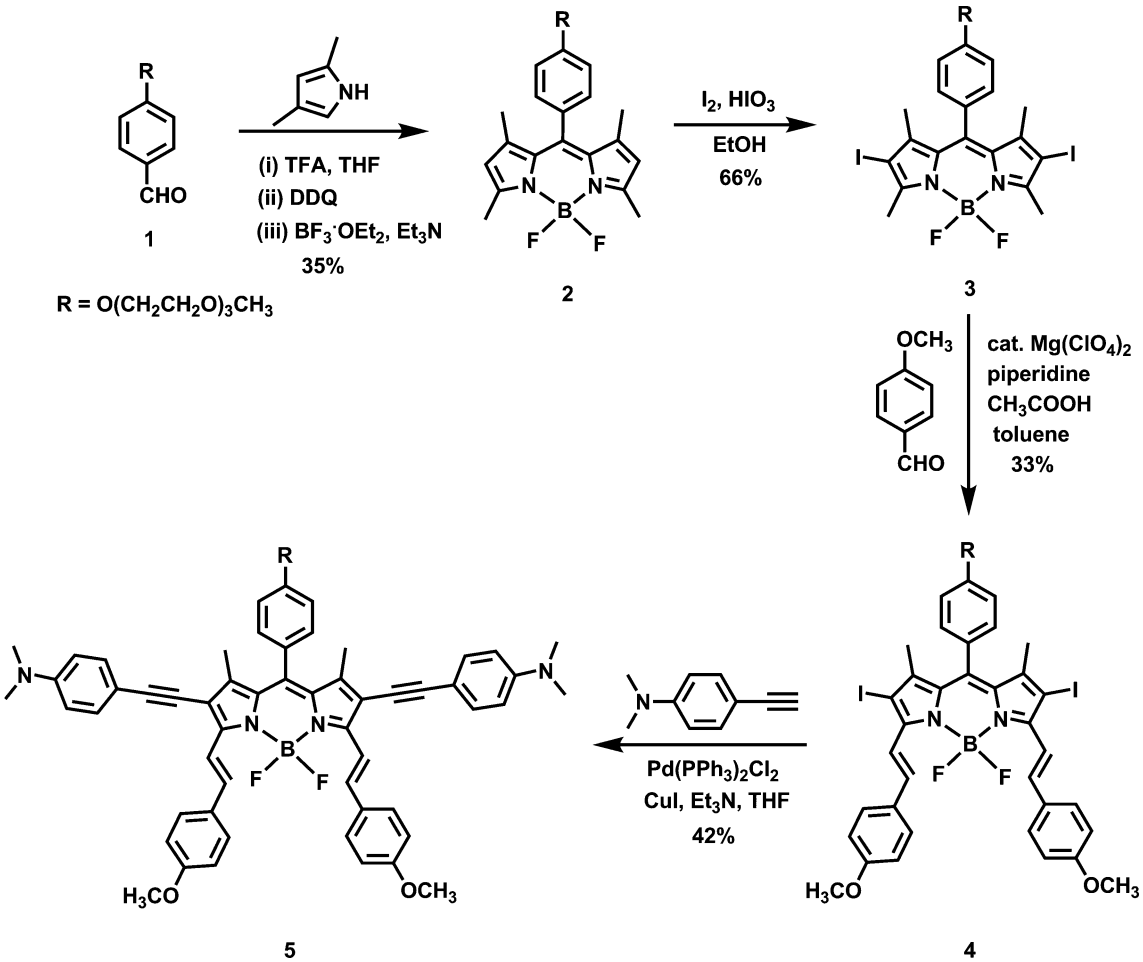
† Electronic supplementary information (ESI) available: Experimental procedure, characterisation data, and ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra for **2**–**5**; electronic absorption and fluorescence spectra of **5** in different solvents; change in fluorescence spectrum of **5** in chloroform upon addition of a large excess of TFA; change in color and fluorescence of **5** in water with 0.05% (v/v) Tween 80 as the pH changes from 5.95 to 0.38. See DOI: 10.1039/c0ob01252a



Scheme 1 Synthesis of distyryl BODIPY **5**.

two new bands at 678 and 622 nm with three isobestic points at 554, 475 and 409 nm. The colour of the solution turned from grass green to blue green (Fig. 1). The changes could be explained by protonation of the amino groups of **5** by TFA, which inhibits the ICT process resulting in a blue shift.[14] The changes were reversible upon addition of triethylamine, which suggests that compound **5** is stable under these acidic and basic environments.

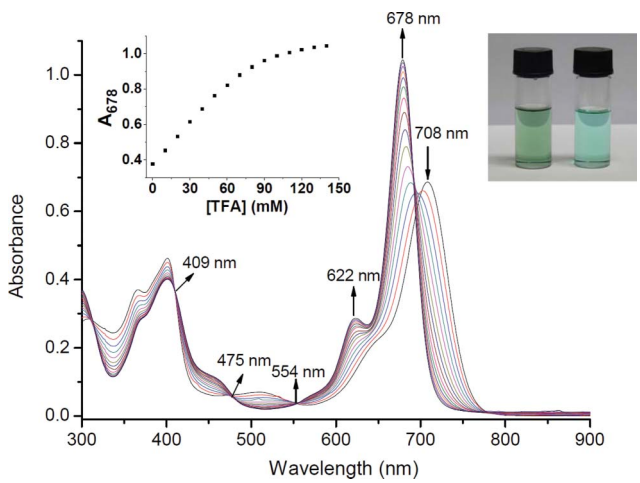


Fig. 1 Change in electronic absorption spectrum of **5** in chloroform (5 μM) upon addition of TFA (up to 140 mM) at ambient temperature. The left inset plots the Q-band absorbance at 678 nm *vs.* the concentration of TFA. The right inset shows that the colour of the solution changed from grass green (left) to blue green (right) upon addition of 140 mM of TFA.

In the absence of TFA, compound **5** in chloroform (1 μM) gave a weak fluorescence at 736 nm with a fluorescence quantum yield (Φ_F) of 0.005 relative to unsubstituted zinc(II) phthalocyanine (ZnPc) in DMF (Φ_F = 0.28).[15] Upon addition of TFA (up to 34 mM), the emission band shifted to 694 nm and increased in intensity gradually resulting in a very large blue shift of 42 nm (Fig. 2), again as a result of inhibition of the ICT process.[14] Further addition of TFA (up to 140 mM) further increased the fluorescence intensity at 694 nm (Fig. S2, ESI†). The fluorescence quantum yield reached the value of 0.46 after addition of 140 mM of TFA, and the fluorescence emission turned to bright red (left inset of Fig. 2). The absorption and fluorescence data under different acidic conditions are compiled in Table 1.

In contrast to the electronic absorption spectrum recorded in chloroform, the spectrum of **5** in water exhibited a very broad Q band indicating that the compound is highly aggregated in water. In fact, distyryl BODIPY even with a polysulfonated moiety at the meso phenyl substituent has also been found to be aggregated in aqueous solution, as shown by the blue-shifted H-dimer band.[16] Upon addition of 0.05% (v/v) Tween 80, which is a common nonionic surfactant, the Q band was sharpened showing that the

Table 1 Electronic absorption and fluorescence data for **5** under different conditions

Solvent	Acidic condition	λ_{max}/nm (log ε)	λ_{em}/nm[a]	Φ_F[b]
Chloroform	In the absence of TFA	365 (4.88), 401 (4.98), 510 (4.17), 708 (5.15)	736	0.005
	[TFA] = 140 mM	366 (4.73), 400 (4.90), 462 (4.33), 622 (4.76), 678 (5.32)	694	0.46
Water[c]	pH = 5.95	367 (4.77), 404 (4.83), 516 (4.07), 717 (4.95)	—	—
	pH = 0.38	368 (4.60), 401 (4.76), 465 (4.20), 628 (4.64), 682 (5.09)	640, 697	0.15

[a] Excited at 409 (in chloroform) or 423 (in water) nm. [b] Using ZnPc in DMF as the reference ($\Phi_F = 0.28$). [c] With 0.05% (v/v) Tween 80.

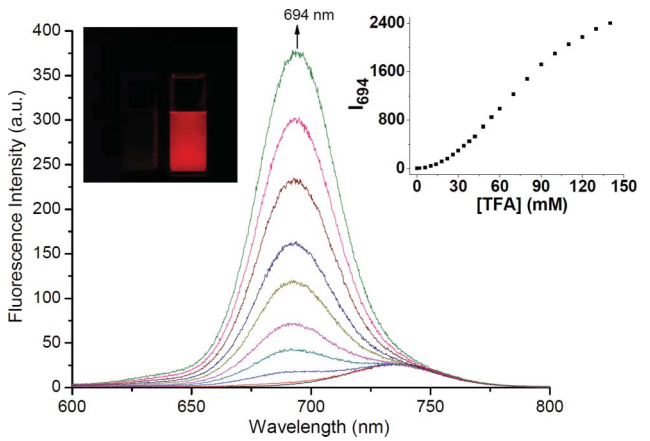


Fig. 2 Change in fluorescence emission spectrum of **5** in chloroform (1 μM) upon addition of TFA (up to 34 mM) (excited at 409 nm) at ambient temperature. The right inset plots the fluorescence intensity at 694 nm *vs.* the concentration of TFA. The left inset shows that the fluorescence emission turned to bright red after addition of 140 mM of TFA.

aggregation of **5** was significantly reduced (Fig. S3, ESI†, which also shows the spectra in chloroform and DMF for comparison). In fact, the Q band at 717 nm also followed the Lambert–Beer law (Fig. S4, ESI†). It is worth noting that in more polar solvents, such as DMF and water [with 0.05% (v/v) Tween 80], compound **5** was nearly non-fluorescent (Fig. S5, ESI†), which could be explained by the stronger ICT effect in polar media resulting in a weaker fluorescence.[14]

Since compound **5** is essentially non-aggregated in water with 0.05% (v/v) Tween 80, its pH-dependent effects were studied in this medium. Fig. 3 shows the change in absorption spectrum of **5** at different pH. With decreasing pH value from 5.95 to 0.38, the Q band at 717 nm shifted gradually to give a sharp and intense Q band at 682 nm and a vibronic band at 628 nm. Three isobestic points at 570, 481 and 423 nm were also observed. The colour of the solution also changed from grass green to blue green as observed in chloroform (Fig. S6, ESI†). A sigmoidal plot of the absorbance at 682 nm *vs.* the pH afforded an apparent pK_a value of 0.95 ± 0.07 (the inset of Fig. 3), which is comparable with that of the bis[4-(dimethylamino)phenyl] aza-BODIPY analogue reported by O'Shea and coworkers.[17]

As shown in Fig. 4, with decreasing the pH value from 5.95 to 0.38, the fluorescence emissions at 697 and 640 nm gradually enhance, and the fluorescence quantum yield finally reaches a value of 0.15 (Table 1). The fluorescence of the solution also turned to bright red as shown in Fig. S7 (ESI†). A sigmoidal plot of the fluorescence intensity at 697 nm *vs.* the pH gave an apparent pK_a value of 0.83 ± 0.02 (the inset of Fig. 4), which is very close to that derived from the absorption data.

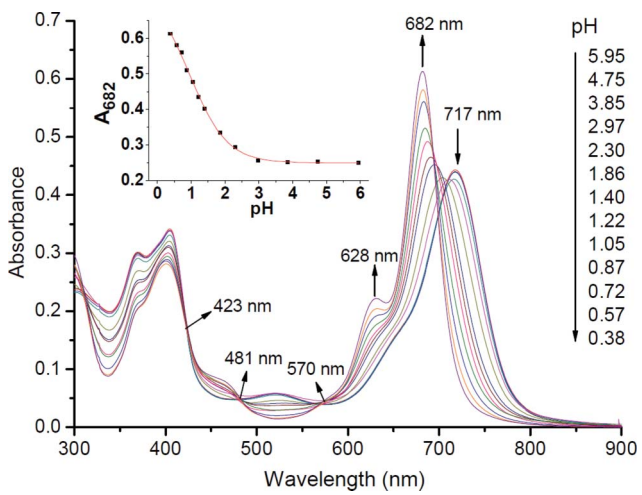


Fig. 3 Electronic absorption spectra of **5** in water with 0.05% (v/v) Tween 80 (5 μM) at different pH at ambient temperature. The inset plots the Q-band absorbance at 682 nm *vs.* the pH value.

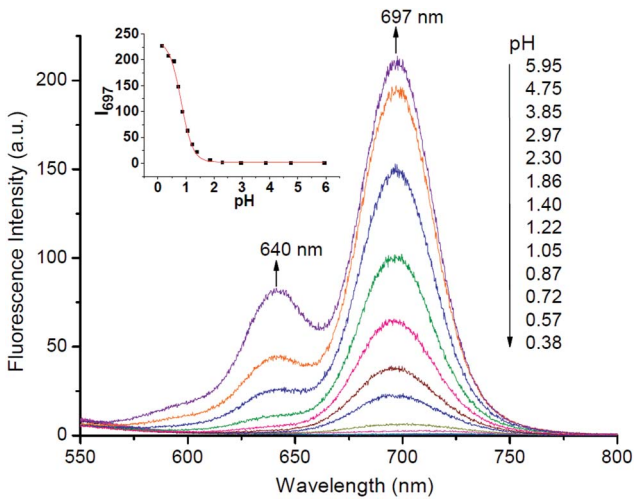


Fig. 4 Fluorescence emission spectra of **5** in water with 0.05% (v/v) Tween 80 (1 μM) at different pH (excited at 423 nm) at ambient temperature. The inset plots the fluorescence emission intensity at 697 nm *vs.* the pH value.

To examine whether these pH-dependent changes are reversible, aqueous sodium hydroxide (12 M) and concentrated HCl were

used to change the pH back and forth between 0.38 and 5.95. The ratios of the absorbance at 682 and 717 nm were measured and the results are shown in Fig. 5. It is clear that this process has a high reversibility showing that compound **5** has a high stability under these conditions.

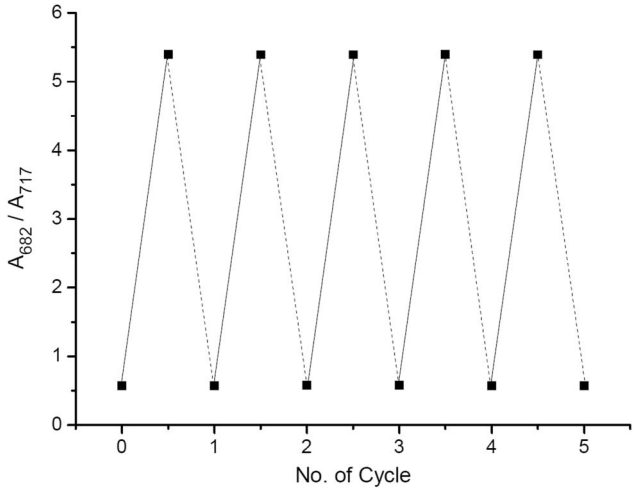


Fig. 5 Change in the ratio of the absorbance at 682 and 717 nm of **5** in water with 0.05% (v/v) Tween 80 (5 μM). The pH value was switched back and forth between 0.38 and 5.95 using aqueous sodium hydroxide (12 M) and concentrated HCl. The solid and dashed lines represent addition of HCl and NaOH, respectively.

In summary, we have prepared and characterised a novel near-infrared fluorescent dye based on a distyryl BODIPY scaffold. It exhibits remarkable pH-responsive changes in absorption and emission properties with a high reversibility. For biomedical applications, further structural modification of this dye is deemed necessary, for example, by incorporating more water-solubilising groups to enhance the solubility and reduce the aggregation tendency in aqueous media,[18] and other acid-sensitive groups so that the dyes can respond in different pH regions. This work is currently under investigation.

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