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Preparation of unsymmetrical distyryl BODIPY derivatives and effects of the styryl substituents on their *in vitro* photodynamic properties†

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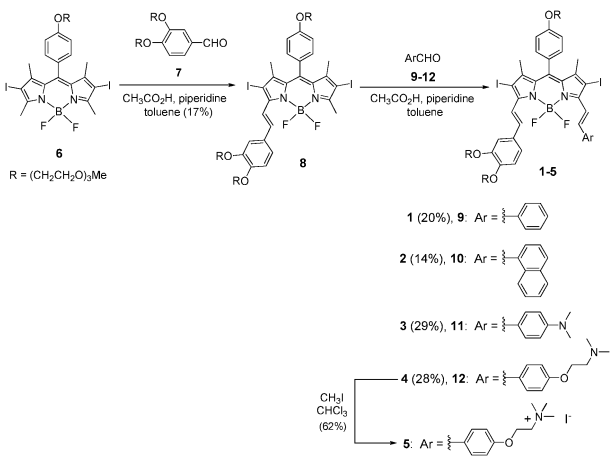
A series of unsymmetrical distyryl BODIPYs have been prepared which function as highly potent photosensitisers with *in vitro* IC_{50} values as low as 15 nM. Their cellular uptake, subcellular localisation and photocytotoxicity depend greatly on the styryl substituents.

Distyryl boron dipyrromethene (BODIPY) derivatives can be prepared readily by Knoevenagel condensation of 3,5-dimethyl BODIPYs with aryl aldehydes.[1] Having an extended π system, these compounds absorb strongly at *ca.* 620–660 nm and emit in the near-infrared region. These spectral properties can be tuned readily by chemical modification of the π skeleton. These characteristics, together with their high stability and solubility in many solvent systems, render them to be promising materials for various applications. In particular, these compounds serve as near-infrared fluorescent probes for various analytes,[2] building blocks for artificial photosynthetic models,[3] sensitisers for dye-sensitised solar cells[4] and fluorophores for one- and two-photon cell imaging.[5] By incorporating heavy halogen atoms onto the BODIPY core, these compounds behave as efficient photosensitisers which can generate singlet oxygen upon illumination.[6] This interesting property suggests that these compounds are potentially useful as photosensitisers for photodynamic therapy, which is a promising therapeutic modality for a variety of pre-malignant and malignant diseases.[7] However, this research field has been virtually unexplored. To our knowledge, only one example has been demonstrated to exhibit photodynamic activity at the cellular level so far.[8] We report herein a new series of unsymmetrical distyryl BODIPY derivatives (compounds **1–5**). By introducing two different styryl substituents, we hoped to impart a higher amphiphilicity to the compounds which may facilitate their cellular uptake[9] and control their subcellular localisation. The preparation and basic photophysical properties of this series of compounds, as well as the effects of the styryl substituents on their *in vitro* properties are described below.

Compounds **1–4** were prepared by sequential Knoevenagel condensation of diiodo BODIPY **6**[10] with two different aryl aldehydes (Scheme 1). Treatment with bis(triethylene glycol)-substituted benzaldehyde **7** first afforded the mono-styryl tris(triethylene glycol)-appended BODIPY **8**. The triethylene glycol chains were introduced to enhance the hydrophilicity, biocompatibility and cellular uptake of the dyes.[11] It was then followed by further condensation with aldehydes **9–12**, respectively, to give the corresponding distyryl BODIPYs **1–4**. Compounds **2** and **3** have a naphthyl or aminophenyl group attached directly to the π system, which can further shift the absorption to the red. Having a trialkylamino moiety, compound **4** could be *N*-methylated readily with iodomethane to give the cationic analogue **5** (Scheme 1). The experimental details and the characterising data are given in ESI.†

The electronic absorption spectra of compounds **1–5** were recorded in DMF (Fig. S1, ESI†) and the data are summarised in Table 1. It can be seen that the Q bands of **2–5** are red-shifted compared with that of **1**, particularly for compound **3**, which exhibits strong intramolecular charge transfer (ICT) due to the amino group.[1b] For all these compounds, the Q-band absorbance strictly followed Lambert–Beer's law [see the spectra of **1** (Fig. S2, ESI†) for exemplification], which suggested that they are essentially non-aggregated in DMF. Upon excitation at 610 nm, all the compounds (except **3**)



Scheme 1 Synthesis of unsymmetrical distyryl BODIPYs **1–5**.

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† Electronic supplementary information (ESI) available: Experimental details and characterising data, absorption and fluorescence spectra of **1–5** in DMF and DMEM; absorption spectra of **1** at various concentrations in DMF, cytotoxic effects of **1**, **4** and **5**, results of subcellular localisation studies of **4** and **5**, and ^{1}H and $^{13}C\{^{1}H\}$ NMR and ESI mass spectra of **1–5** and **8**. See DOI: 10.1039/c1cc10727e

Table 1 Electronic absorption and fluorescence emission data for compounds **1–5** in DMF

Compound	λ_{max} (nm) (log ε)	λ_{em} (nm)[a]	Φ_F[b]
1	340 (4.55), 450 (4.12), 653 (4.88)	677	0.19
2	340 (4.44), 390 (4.54), 465 (4.19), 659 (4.88)	688	0.15
3	339 (4.53), 410 (4.53), 517 (4.35), 709 (4.81)	—	—
4	327 (4.43), 382 (4.58), 453 (4.21), 665 (4.91)	695	0.18
5	327 (4.40), 377 (4.57), 450 (4.19), 661 (4.89)	686	0.15

[a] Excited at 610 nm. [b] Relative to ZnPc in DMF as the reference [fluorescence quantum yield (Φ_F) = 0.28].

showed a fluorescence emission at 677–695 nm (Fig. S3, ESI†) with a fluorescence quantum yield (Φ_F) of 0.15–0.19 relative to unsubstituted zinc(II) phthalocyanine (ZnPc) (Φ_F = 0.28) (Table 1).[12] The Stokes shifts were in the range of 24–30 nm. Compound **3** was essentially non-fluorescent as a result of ICT in the excited state.[1b]

The efficiency of these compounds in generating singlet oxygen, as reflected by the rate of decay of the singlet-oxygen quencher 1,3-diphenylisobenzofuran (DPBF) in DMF was also compared. As shown in Fig. 1, all these distyryl BODIPYs can induce photo-degradation of DPBF with a comparable efficiency, which is also similar to that of ZnPc. The only exception is compound **3**, which is clearly a less efficient singlet-oxygen generator. Presumably, the strong ICT process shortens the singlet-state lifetime and disfavors the population of triplet state to sensitise the formation of singlet oxygen.

The *in vitro* photodynamic activities of **1–5** in Tween 80 emulsions were then investigated against HT29 human colorectal carcinoma cells. Fig. 2 shows their dose-dependent survival curves. It can be seen that all of them are essentially non-cytotoxic in the absence of light. However, upon illumination with red light ($\lambda > 610$ nm), they exhibit different degrees of cytotoxicity. The IC_{50} and IC_{90} values, defined as the dye concentrations required to kill 50% and 90% of the cells, respectively, are summarised in Table 2. The photocytotoxicity follows the order **4** ≈ **5** > **1** > **3** > **2**. Compound **4** and its *N*-methylated derivative **5** are particularly potent with IC_{50} values of 15–17 nM, which are much lower than those of the classical photosensitiser porfimer sodium (IC_{50} = 4.6 μg mL^{-1} under the same experimental conditions *vs.* 22 ng mL^{-1} for **4** and **5**) and pheophorbide *a* (IC_{50} = 0.5 μM),[13] and are comparable with those of the most potent phthalocyanine-based photosensitisers reported by us so far.[14] Although the

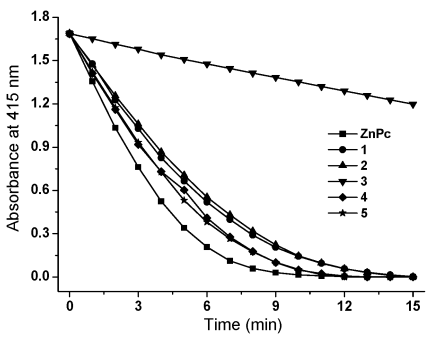

Fig. 1 Comparison of the rates of decay of DPBF (initial concentration = 75 μM) in DMF, as monitored spectroscopically at 415 nm, using **1–5** as the photosensitisers (4 μM) and ZnPc (4 μM) as the reference.

naphthyl group of **2** can induce a bathochromic shift on the absorption and emission positions, the photocytotoxicity of this compound is the lowest in this series. Compound **3** is also a less efficient photosensitiser.

To account for their different photocytotoxicity, their aggregation behavior in the Dulbecco's modified Eagle's medium (DMEM) was first examined by absorption and fluorescence spectroscopic methods (Fig. S5, ESI†). For all the compounds, the Q band remained relatively sharp and an intense emission band was observed (except **3**, which was essentially non-fluorescent due to ICT). These observations suggested that these compounds remained relatively non-aggregated in the culture medium.

The cellular uptake of these compounds was then examined by fluorescence microscopy. HT29 cells were incubated respectively with all these compounds (2 μM) for 2 h. Upon excitation at 633 nm, the bright field and fluorescence (650–720 nm) images of the cells were then captured (Fig. 3a), and the intracellular fluorescence intensities were determined (Fig. 3b). Compounds **4** and **5** showed much stronger intracellular fluorescence throughout the cytoplasm. The intensity, which is an indicator of the cellular uptake, followed the order: **4** ≈ **5** > **1** > **2** ≈ **3**. Generally, it is in good agreement with the trend observed for the photocytotoxicity. It is expected that **3** has a very different fluorescence emission efficiency compared with the other compounds. Hence its intracellular fluorescence intensity may not directly reflect the cellular uptake. However, in light of its low photocytotoxicity, we did not pursue further the exact uptake of this compound, and put our focus on the highly photocytotoxic **4** and **5**.

For **4** and **5**, their subcellular localisation was also investigated. The cells were first incubated with either **4** or **5** in the medium for 2 h, then stained with LysoTracker DND 26, MitoTracker Green FM or ER-Tracker Green (for 10–30 min), which are specific fluorescent dyes for lysosomes, mitochondria and endoplasmic reticulum, respectively. As shown in Fig. 4, both compounds show substantial intracellular fluorescence, but compound **4** is distributed throughout the cytoplasm, while compound **5** tends to localise in the cell membrane. To take a closer examination, the fluorescence caused by **4** (excited at 633 nm, monitored at 675–720 nm) could superimpose with the fluorescence caused by the LysoTracker (excited at 488 nm, monitored at 510–560 nm) and partially overlap with the fluorescence caused by the MitoTracker (excited at 488 nm, monitored at 510–560 nm), but not with that caused by the ER-Tracker (Fig. S6, ESI†). The results suggested that compound **4** has high affinity to the lysosomes and can also bind to the mitochondria. For the cationic *N*-methylated derivative **5**, it is not exclusively localised in any organelles,

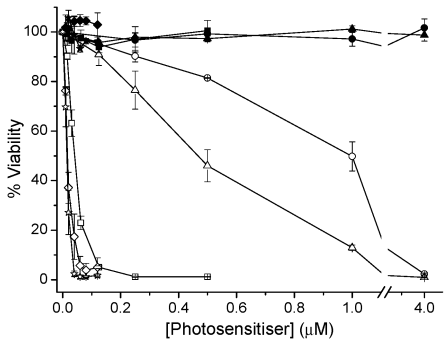

Fig. 2 Comparison of the cytotoxic effects of **1** (squares), **2** (circles), **3** (triangles), **4** (rhombuses) and **5** (stars) on HT29 cells in the absence (closed symbols) and presence (open symbols) of light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}). Fig. S4 (ESI†) shows the enlarged region for **1**, **4** and **5**. Data are expressed as mean value ± standard error of the mean value (SEM) of three independent experiments, each performed in quadruplicate.

Table 2 IC_{50} and IC_{90} values of compounds **1–5** against HT29 cells

Compound	IC_{50} (nM)	IC_{90} (nM)
1	42	108
2	1000	2600
3	470	1270
4	17	53
5	15	34

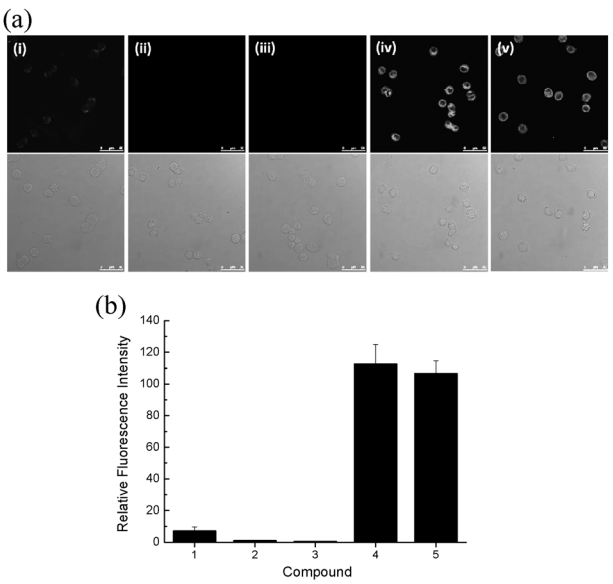

Fig. 3 (a) Bright field (lower row) and intracellular fluorescence (upper row) images of HT29 cells after incubation with (i) **1**, (ii) **2**, (iii) **3**, (iv) **4** and (v) **5** (all at 2 μM) for 2 h. (b) Comparison of the intracellular fluorescence intensities of compounds **1–5**. Data are expressed as mean value ± standard deviation (number of cells = 50).

but shows bright fluorescence in the cell membrane (Fig. S7, ESI†). It is likely that due to the cationic nature of this compound, it is preferentially retained in the membrane's lipid bilayer.

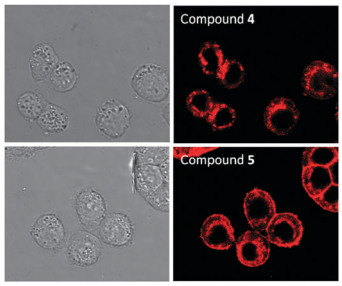

Fig. 4 Bright field (left column) and fluorescence (right column) images of HT29 after incubation with **4** or **5** in the medium for 2 h.

In conclusion, we have prepared a new series of unsymmetrical distyryl BODIPYs and evaluated their *in vitro* photodynamic activities. It has been found that these properties depend greatly on the styryl substituents. The amino derivative **4** and its *N*-methylated analogue are highly photocytotoxic with IC_{50} values down to 15 nM. The high potency could be attributed to their low aggregation tendency, high efficiency in generating singlet oxygen and high cellular uptake. These compounds, however, show a remarkably different subcellular localisation property as a result of minor modification of the substituent.

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