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Synthesis of BF_2 chelates of tetraarylazadipyrromethenes and evidence for their photodynamic therapeutic behaviour

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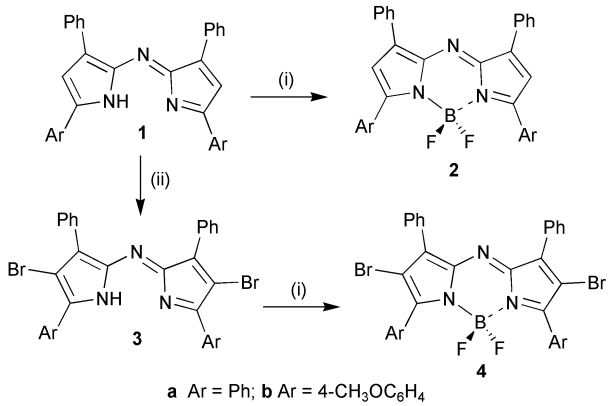
The synthesis, spectroscopic characteristics and *in vitro* cellular uptake properties of a new class of therapeutic window photosensitiser, namely the BF_2 chelates of 3,5-diaryl-1*H*-pyrrol-2-yl-3,5-diarylpyrrol-2-ylidene amines (tetraarylazadipyrromethenes), are described with the aim of developing a novel class of photodynamic therapeutic agents.

Photodynamic therapy (PDT) is a non-invasive technique for the treatment of a variety of solid tumour types by the combined use of visible or near-visible light with a photosensitising drug.[1] The therapeutic strategy is as follows: a photosensitiser of low dark toxicity is introduced into the body, which accumulates preferentially to some extent within the tumour. The tumour is then irradiated with low energy light of a wavelength that can pass through the body's therapeutic window (650–800 nm, *i.e.* beyond the absorbance of body tissue) resulting in excitation of the photosensitiser. The light-activated photosensitiser then transfers its excited state energy to surrounding biological tissue through singlet oxygen, resulting in oxidative cellular damage, leading to cell death *via* apoptosis and/or necrosis.[2] After light treatment, the photosensitiser is allowed to clear from the body. PDT can be considered as a highly selective form of cancer treatment, provided that the photosensitiser is non-toxic in the absence of light, only the irradiated areas are affected.

At the present time, Photofrin® is the most common clinically used PDT agent. Although it has been approved for use in the United States, Japan and Europe for the treatment of esophagael, lung, stomach and cervical cancers,[1b] it is widely recognised that it is far from being an ideal drug for use in PDT.[3] Despite its achievements to date, PDT is still in its developmental stages with a marked need to develop improved photosensitisers with better efficacy and side effect profiles. In order to further advance this novel form of treatment, it has become apparent that the development of new PDT compounds, together with a more thorough and integrated understanding of the multitude of targets/actions so far ascribed to PDT, is needed.

We now report the synthesis, photophysical properties and *in vitro* cellular uptake evaluation of a totally new class of potential PDT agent. Our photosensitisers are based on a BF_2 chelated azadipyrromethene core structure that has not been previously studied. The majority of PDT agents investigated to date are based on cyclic-tetrapyrrole macrocycles from which it can be synthetically difficult to generate an array of sequentially modified derivatives. However, our proposed class of non-porphyrin sensitisers[4] would be a good starting point as they are amenable to modification around the periphery of the chromophore which could allow for optimisation of all aspects of their photophysical and therapeutic properties.

The synthesis of tetraarylazadipyrromethenes **1** was first reported in the 1940s but since then this class of compound has remained unstudied.[5] We have repeated the synthesis of **1** using the reported three step literature procedure.[5a] In order to make **1** more structurally constrained and limit radiationless transitions, they were converted into the BF_2 chelates **2** by reaction with boron trifluoride diethyl etherate, diisopropylamine (DIEA) in CH_2Cl_2. As introduction of a heavy atom into a chromophore is known to enhance triplet state population (required for singlet oxygen generation) we brominated the free β-position of both pyrrole rings of **1**, giving **3** in high yields. Conversion of **3** into its BF_2 chelate **4** was readily achieved using the same conditions as for **1** (Scheme 1). Both **2** and **4** are metallic brown solids which have good solubility in organic solvents such as chloroform, toluene or THF and were characterised by ^1H, ^{13}C NMR, HRMS and CHN analysis.†



a Ar = Ph; **b** Ar = 4-$CH_3OC_6H_4$

Scheme 1 *Reagents and conditions*: (i) $BF_3.OEt_2$, DIEA, CH_2Cl_2, rt, 16 h; (ii) Br_2, benzene, rt, 2 h.

X-Ray crystal structure determination of **2b** demonstrated the conjugated nature of the chromophore with comparable bond lengths for the bridging nitrogen N1 to both pyrrole rings (C1, C2) and for both pyrrole nitrogen to boron bonds (Fig. 1).

A study of the spectroscopic properties of **2a** and **4a** in chloroform demonstrated that they have a sharp absorption band at 650 nm with a full width at half maximum (fwhm) of 49 and 47 nm and extinction coefficients of 79,000 (Fig. 2, Table 1).

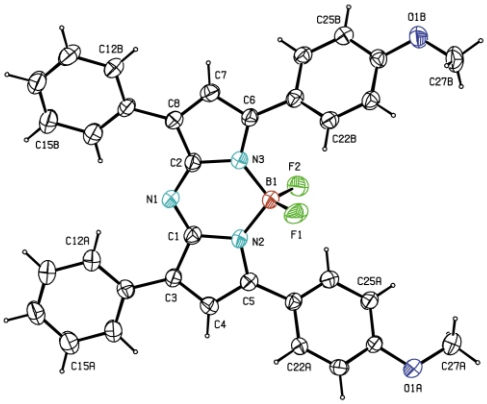


Fig. 1 X-Ray crystal structure of **2b** (grown from toluene–methanol bilayer, co-crystallised with a molecule of toluene, not shown for clarity). Selected bond lengths N(1)-C(1) 1.317(4), N(1)-C(2) 1.326(4), N(2)-B(1) 1.562(5), N(3)-B(1) 1.561(5).‡

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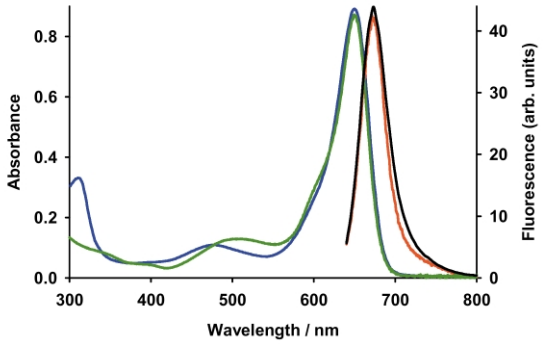

Fig. 2 Absorption (blue) and emission spectra (red) of **2a**; absorption (green) and emission spectra (black) of **4a** in $CHCl_3$.

Table 1 Spectroscopic absorbance properties of **2** and **4**[a]

Compound	λ_{max}[b]/ nm	fwhm[b]/ nm	ε[b]/ M^{-1} cm^{-1}	λ_{max}[c]/ nm	fwhm[c]/ nm
2a	650	49	79,000	658	53
2b	688	55	85,000	696	57
4a	650	47	79,000	651	57
4b	679	57	75,000	685	86

[a] Room temperature. [b] $CHCl_3$. [c] Water/cremophor.

Introduction of an electron donating methoxy group on the phenyl rings at C5 and C6 resulted in an increase in extinction coefficient for **2b** and a significant bathochromic shift of absorption bands for **2b** and **4b** to 688 and 679 nm respectively. In order to achieve water solubility of the photosensitisers the micelle forming reagent cremophor was utilised with solutions of **2** and **4** in water–cremophor giving rise to a small absorbance bathochromic shift and slight broadening of the bands (Table 1).

Excitation of **2a** and **4a** in chloroform at 630 nm gave a fluorescence band at 672 and 673 nm, respectively, (Fig. 2). The fluorescence quantum yield of **2a** was 0.34 but, as would be expected, is significantly reduced for **4a** at 0.012 (Table 2). Similarly **2b** had a fluorescence quantum yield of 0.36 (λ_{em} 715 nm) and **4b** was lower at 0.10 (λ_{em} 714 nm). In general, substitution of bromine into the chromophore has minimal effect on absorption and fluorescence wavelength or band shape but considerably reduces the fluorescence quantum yield.

Table 2 Spectroscopic fluorescence properties of **2** and **4**[a]

Compound	λ_{em}/nm[b]	Φ_f[c]	λ_{em}/nm[d]
2a	672	0.34	683
2b	715	0.36	727
4a	673	0.012	679
4b	714	0.10	719

[a] Room temperature. [b] $CHCl_3$. [c] Relative to magnesium tetra-*tert*-butylphthalocyanine in $CHCl_3$ (Φ_f = 0.84).[7] [d] Water/cremophor.

The ability of **2** and **4** to produce singlet oxygen would be a prerequisite for type II PDT behaviour. Qualitative comparison of singlet oxygen production of **2a** and **4a** by trapping with 1,3-diphenylisobenzofuran, a known efficient quencher of singlet oxygen,[6] demonstrated that both were capable of generating singlet oxygen. In accord with the reduced fluorescence quantum yields, **4a** (5×10^{-8} M) was more effective in generating singlet oxygen even at a one hundred fold lower concentration than **2a**. The facile introduction of the heavy atom bromine allows us to incorporate control over this essential photophysical parameter of our PDT agents (Fig. 3). Cellular uptake of a photosensitiser is required for it to act as a PDT agent. Water–cremophor solutions of **2a** (5×10^{-6} M) were added to HeLa tumour cells and incubated for periods of 1, 5, 15, 30 and 60 minutes followed by measurement of an averaged fluorescence intensity per fixed area of individual cells. We observed an efficient, time dependent, uptake of **2a** with intracellular maximum reached after 60 min (Fig. 4, left image). Staining of the nucleus of the cells with 4′,6-diamidino-2-phenylindole (DAPI) prior to treatment with **2a** gave good contrast imaging and confirmed localisation of the sensitiser to the cytoplasm (Fig. 4, right image). Light induced toxicity assays are currently underway and will be reported elsewhere.

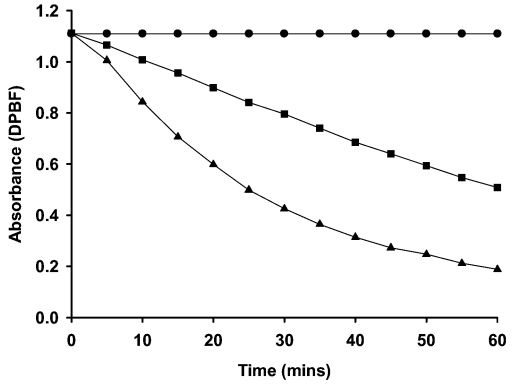

Fig. 3 Comparative photooxidation of 1,3-diphenylisobenzofuran (DPBF) (5×10^{-5} M) in isopropyl alcohol with no photosensitiser (●), **2a** (5×10^{-6} M) (■) and **4a** (5×10^{-8} M) (▲). Filtered light < 590 nm used.

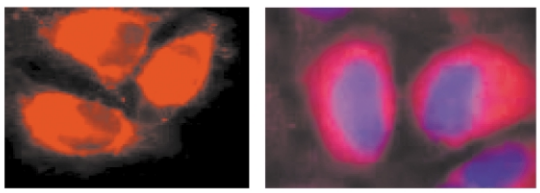

Fig. 4 Left image; cellular uptake of **2a** (red colour) in HeLa cancer cells visualised with a fluorescence microscope (rhodamine filter) (dark area is the cell nucleus). Right image; cellular localisation of **2a**; nucleus is co-stained with DAPI (blue) and cytoplasmic localisation of **2a** (red).

In summary we have developed a new class of therapeutic window photosensitiser with spectroscopic evidence to substantiate their ability to function as PDT agents. We now aim to generate an array of sequentially modified water soluble derivatives that incorporate targeting systems for enhanced tumour selectivity. The highly fluorescent dyes are also being studied as *in vivo* sensors and fluorescent switches.

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Notes and references

† Synthetic procedures will be reported elsewhere.

‡ *Crystal data* for **2b** ($C_{34}H_{26}BF_2N_3O_2{\cdot}0.5(C_7H_8)$): M = 603.46, monoclinic, $P2_1/c$, a = 18.124(2), b = 7.4096(7), c = 23.634(3) Å, β = 110.188(7)°, V = 2978.8(6) Å^3, T = 294 K, Z = 4, ρ_{calc} = 1.346 Mg m^{-3}, 9803 reflections measured, 5871 unique (R_{int} = 0.033), wR_2 = 0.175 (all data). CCDC 184817. See http://www.rsc.org/suppdata/cc/b2/b204317c/ for crystallographic data in CIF or other electronic format.

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