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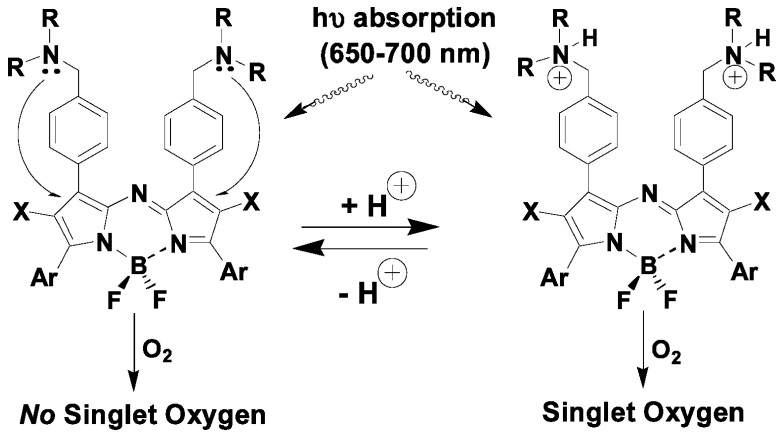
J. Am. Chem. Soc., **2005**, 127 (47), 16360-16361• DOI: 10.1021/ja0553497 • Publication Date (Web): 04 November 2005



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Supramolecular Photonic Therapeutic Agents

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Photodynamic therapy (PDT) is a noninvasive technique for the treatment of a variety of cancer tumors by the combined use of low energy light and a photosensitizing drug.[1] In clinical use a photosensitizer of minimal dark toxicity is introduced into the body, which accumulates within the tumor by passive diffusion. The tumor is then irradiated with low energy light at a wavelength that can pass through the body's therapeutic window (650−900 nm) resulting in excitation of the photosensitizer from S_0 to S_1. Following absorption, the photophysical pathway involves an intersystem crossing (ISC) from photosensitizer S_1 to T_1 and energy transfer from T_1 to ground-state oxygen, which produces singlet oxygen (Figure 1). Singlet oxygen is the key cytotoxic agent in the therapeutic process, which can induce cell death by an apoptotic and/or necrotic mechanism. This combination of light, photosensitizer, and oxygen as a means of inducing cell death is unique as a mode of disease treatment.

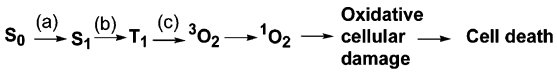

Figure 1. Photophysical pathway for the PDT process: (a) absorbance, (b) intersystem crossing, (c) energy transfer.

We have recently disclosed a new photosensitizer structural platform, the BF_2-chelated azadipyrromethenes **1**, from which a sequentially modified array of PDT agents has been developed (Figure 2).[2a,b] This has led to identification of lead compounds based upon this core structure which display in vitro efficacy from the micro- to nanomolar range for a diverse set of human cancer cell types.[2c] Optimal in vitro efficacy was obtained by exerting molecular control of the excited-state populations through exploitation of the heavy atom effect (bromine substitution).[2b]

Despite its promising results to date, the selectivity of PDT leaves much to be desired as it often gives rise to clinical side effects such as prolonged light sensitivity. Typical approaches taken to improve selectivity are the encapsulation of the photosensitizer in ligand-modified liposomes or the use of sensitizer-antibody conjugates.[3] Herein, we outline a new approach to achieving PDT selectivity based upon the *reversible* off/on switching of the key therapeutic property (singlet oxygen generation) of a supramolecular photonic therapeutic agent (SPTA). This could be accomplished by covalently attaching a substrate-specific receptor to our PDT agents. In the absence of the substrate, the unbound receptor would effect a rapid quench of the photosensitizer excited state by a photoinduced electron transfer (PET) mechanism, thereby shutting down singlet oxygen production (Figure 3, pathway A). In contrast, singlet oxygen generation would occur if the substrate were available to bind with the receptor, as the PET pathway would be switched off (Figure 3, pathway B). A related photophysical mechanism has been successfully exploited in supramolecular fluorescent and phosphorescent sensors for the detection of various substrates.[4]

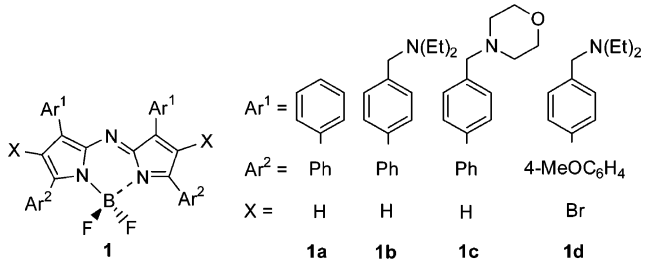

Figure 2. BF_2-chelated azadipyrromethenes.

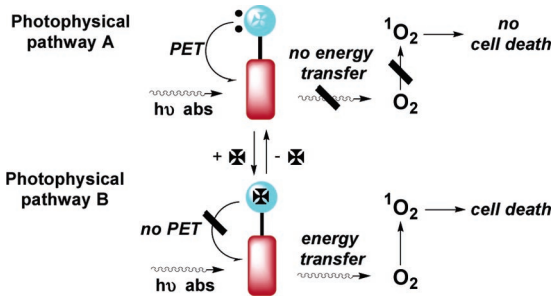

Figure 3. Design and function of an SPTA. Blue circle, substrate-specific receptor. Red rectangle, photosensitizer. Black cross, substrate.

As proof-of-principle, a series of SPTA analogues **1b**−**d** with pH-responsive amine receptors was synthesized following previously reported methods, and their properties were compared to those of the tetra-phenyl derivative **1a**. The substrate required to activate the SPTA would be a proton source of sufficient strength to protonate the amine receptor. A potential clinical application would be the targeting of the interstitial fluid surrounding a tumor mass which is known to have a lower pH profile (6.5−6.8) than normal tissue.[5] Direct PDT action on the tumor vascular system has been previously demonstrated as a means of causing tumor destruction.[6] The amine receptors have been chosen such that their protonated apparent pK_a values vary from just below physiological pH at 6.8 for **1b** to 4.8 for **1d** when measured in aqueous formulated solutions of Cremophor EL (CrEL). It should be noted that pK_a values of amines in a micellar microenvironment are often lower than might be expected.[7] The hypotheses to be tested were whether (i) singlet oxygen generation could be controlled by solution pH and (ii) cells could activate the SPTA and as a consequence cause their own death.

The ability of **1a**−**d** to modulate singlet oxygen generation in response to an acidic environmental stimulus was tested by trapping with 1,3-diphenylisobenzofuran (DPBF).[2b] Photosensitizer **1a**, which

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10.1021/ja0553497 CCC: $30.25

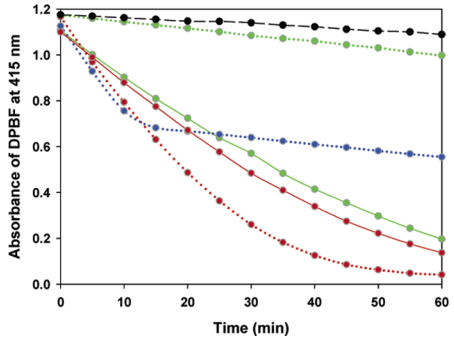

Figure 4. Comparative photooxidation of 1,3-diphenylisobenzofuran (DPBF) (5×10^{-5} M) in DMF with no sensitizer (black dashed line), **1a** in DMF (green solid line), **1a** in DMF/0.05 M HCl (red solid line), **1b** in DMF (green dotted line), **1b** in DMF/0.05 M HCl (red dotted line), and **1b** in DMF/0.05 M HCl for 10 min then treated with 0.05 M $NaHCO_3$ (blue dotted line). **1a** and **1b** at 5×10^{-6} M. Light > 590 nm used.

does not contain an amine receptor group, showed no significant variance in singlet oxygen generation when analyzed in DMF or DMF with an aliquot of 0.05 M HCl (Figure 4, Table 1, entry 1). In comparison, singlet oxygen generation by **1b** displayed a marked reliance upon the presence or absence of a proton source. In DMF alone, the generation of singlet oxygen was very low being almost equivalent to the control experiment, which contained no photosensitizer. However, following addition of an aliquot of acid, the singlet oxygen generation profile was superior to that observed for **1a** (Figure 4). Comparing singlet oxygen generation rates of **1b** in the presence and absence of acid gave a relative rate enhancement of 8.5 (Table 1, entry 2). Similarly, **1c** and **1d** each gave acid enhanced rate increases when compared to a nonacidic solution (Table 1, entries 3, 4). The ability to reversibly switch the singlet oxygen generation on and off was shown by the addition (after 10 min) of a neutralizing equivalent of base into an acidic solution of **1b** during irradiation. The profile of the plot in Figure 4 (blue line) clearly shows how singlet oxygen generation has been virtually switched off in response to removal of the acid substrate. A corresponding off/on switching of the fluorescence intensity was also recorded for **1b**−**d**; for example a 56-fold fluorescence enhancement was observed for **1b** upon amine protonation (Supporting Information). This would substantiate a PET mechanism being the predominate mode of singlet oxygen control.[8]

Efficient cellular uptake and cytoplasmic localization of formulated **1b** were observed with confocal laser scanning microscopy (Figure 5, left). Compounds **1b**−**d** were assayed in the MRC5-SV40 transformed fibroblast cell line. The analogues **1b, c** showed no dark toxicity (0 J cm^{-2}) in the tested concentration range, whereas, for **1d**, dark toxicity was observed at high concentrations with an EC_{50} value of 4.5 μM (Table 1). Comparison of the light-induced (16 J cm^{-2}) toxicity results for **1a**−**c** was very encouraging. **1a** has no receptor, and we have previously reported it to be an efficient agent for inducing cell death with an EC_{50} of 0.50 μM.[2c] Gratifyingly, **1b** had a marginally better efficacy at 0.30 μM, and **1c** had a marginally lower activity at 1.0 μM (Table 1, Figure 5). The dibrominated analogue **1d**, which combines excited-state molecular control by the heavy atom effect to promote ISC and supramolecular photochemical control with amine receptors, has an EC_{50} value of 5.8 nM, which is indicative of clinical potential (Table 1, Figure 5). Due to the complexity of cellular microenvironments the exact mode of intracellular switching is still under investigation.

Table 1. pK_a in H_2O/CrEL,[a] Acid-Enhanced Singlet Oxygen Generation[b] and in Vitro MRC5 Cell Line EC_{50} Assay Data[c] for **1a**−**d**

entry		pK_a	$^1O_2$ acidic rate increase	$EC_{50} \times 10^{-6}$ (M) MRC5 cell	
				0 J cm^{-2}	16 J cm^{-2}
1	**1a**		1.2	>100	0.50 (±0.1)[d]
2	**1b**	6.8	8.5	>100	0.30 (±0.1)
3	**1c**	4.8	2.9	>100	1.0 (±0.2)
4	**1d**	6.6	10.6	4.5 (±0.7)	0.0058 (±0.003)

[a] pK_a by fluorescence titration in H_2O/CrEL solutions. [b] Rate of singlet oxygen generation in acidic DMF/rate in DMF. [c] Light dose of either 0 or 16 J cm^{-2} with standard deviation in brackets. [d] Reference 2c.

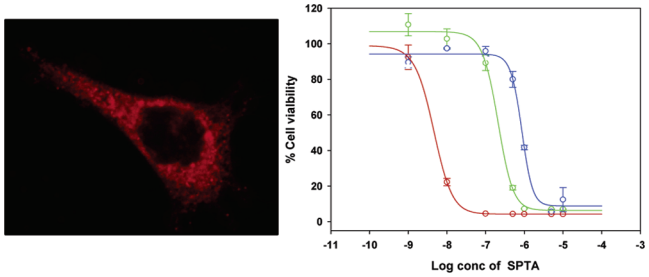

Figure 5. Left: CLSM image showing cytoplasmic localization (red color) of **1b** (dark area is the cell nucleus). Right: In vitro efficacy of **1b** (green), **1c** (blue), and **1d** (red) in MRC5 cell line.

We have outlined a new strategy to therapeutic selectivity in which a supramolecular therapeutic agent could manufacture a cytotoxic agent (singlet oxygen) in response to one exogenous stimulus (light) and one endogenous stimulus (microenvironment pH). The absence of the specific endogenous stimulus would switch off the therapeutic function of the SPTA thereby providing selectivity. As the photophysical mechanism which controls this selectivity is general, it may be applied to other substrates by employing different receptors. More advanced biological assaying is underway to determine the full potential of this concept.

Acknowledgment. This work was supported by Science Foundation Ireland. S.O.McD. thanks IRCSET for funding.

Supporting Information Available: Analytical data for **1b**−**d**. ^1H, ^{13}C NMR, UV−vis spectra, pK_a, off/on fluorescence switching, singlet oxygen measurements, dark toxicity for **1b**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0553497