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Water-Soluble Green Perylenediimide (PDI) Dyes as Potential Sensitizers for Photodynamic Therapy

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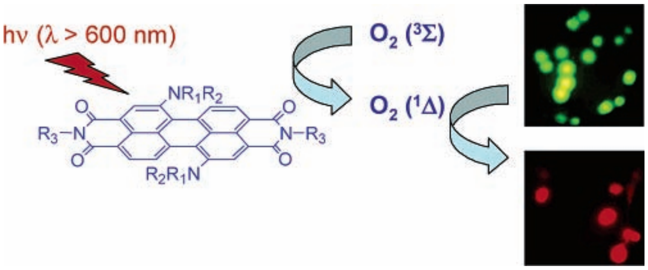
ABSTRACT



A series of water-soluble green perylenediimide (PDI) dyes have been synthesized. On red light excitation, these dyes were shown to be efficient generators of singlet oxygen, and in cell culture media, they were shown to display significant light-induced cytotoxic effects on the human erythroleukemia cell line (K-562). It appears that highly versatile PDI dyes are likely to find new applications in photodynamic therapy.

Photodynamic therapy (PDT) is an innovative, noninvasive approach in the treatment of several types of cancers and macular degeneration.[1] There are additional reported applications of PDT in the arterial wall plaque removal, infectious diseases, precancerous skin lesions, and in the treatment of multidrug-resistant tumors.[2] Mainly for historical reasons, most of the photodynamic sensitizing agents are porphyrin derived, although it is widely acknowledged that porphyrins are not optimal reagents for PDT. An ideal photodynamic reagent should possess the following qualities: high extinction coefficient beyond 630 nm, good singlet oxygen yield, low dark toxicity, constant composition (no isomeric mixtures), straightforward synthesis, and appropriate solution behavior. The development of second-generation photosensitizers is, therefore, a very active research field, and many non-porphyrin reagents are proposed. Among these, phthalocyanines and naphthocyanines,[3] texaphyrins,[4] squaraines,[5] and azadipyrromethenes[6] should be mentioned.

Perylenediimides (PDIs), on the other hand, are highly versatile dyes and pigments which find applications in diverse fields, such as organic light-emitting diodes,[7] molecular switches and wires,[8] light-harvesting arrays,[9] photoreactive

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(1) (a) Bonnet, R. *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science: Amsterdam, 2000. (b) Bonnet, R.; Martinez, G. *Tetrahedron* **2001**, *57*, 9513−9547 and references therein.

(2) Capella, M. A. M.; Capella, L. S. *J. Biomed. Sci.* **2003**, *10*, 361−366.

(3) (a) Ali, H.; van Lier, J. E. *Chem. Rev.* **1999**, *99*, 2379−2450. (b) Lukyanets, E. A. *J. Porphyrins Phthalocyanines* **1999**, *3*, 424−432. (c) Allen, C. M.; Sharman, W. M.; van Lier, J. E. *J. Porphyrins Phthalocyanines* **2001**, *5*, 161−169. (d) Tedesco, A. C.; Rotta, J. G. C.; Lunardi, C. N. *Curr. Org. Chem.* **2003**, *7*, 187−196. (e) Lo, P.-C.; Huang, J.-D.; Cheng, D. Y. Y.; Chan, E. Y. M.; Fong, W.-P.; Ko, W.-H.; Ng, D. K. P. *Chem.*−*Eur. J.* **2004**, *10*, 4831−4838.

(4) (a) Sessler, J. L.; Miller, R. A. *Biochem. Pharmacol.* **2000**, *59*, 733−739. (b) Mody, T. D.; Sessler, J. L. *J. Porphyrins Phthalocyanines* **2001**, *5*, 134−142.

(5) (a) Ramaiah, D.; Eckert, I.; Arun, K. T.; Weidenfeller, L.; Epe, B. *Photochem. Photobiol.* **2002**, *76*, 672−677. (b) Ramaiah, D.; Eckert, I.; Arun, K. T.; Weidenfeller, L.; Epe, B. *Photochem. Photobiol.* **2004**, *79*, 99−104.

(6) (a) Killoran, J.; Allen, L.; Gallagher, J. F.; Gallagher, W. M.; O'Shea, D. F. *Chem. Commun.* **2002**, 1862. (b) Gorman, A.; Killoran, J.; O'Shea, C.; Gallagher, W. M.; O'Shea, D. F. *J. Am. Chem. Soc.* **2004**, *126*, 10619−10631.

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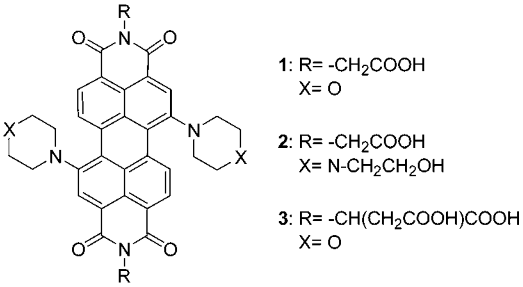
thin films,[10] solar cells,[11] and dye lasers,[12] among others. One important problem to be addressed in many applications is solubility. Substitution at the bay region (positions 1, 6, 7, and 12) is known to increase solubility in organic solvents.[13] Water solubility is a more challenging problem[14] as these dyes have a strong tendency to aggregate even at very low concentrations in aqueous solutions. Yet, for potential biological applications, it is highly desirable to obtain water-soluble PDIs, especially with long wavelength absorption and emission. Simple substitutions at the imide groups do not change the spectral characteristics significantly, and extending the perylene core to form higher rylenes[15] reduces the chances of water solubility even further. The breakthrough came in 1999, when Wasielewski showed[16] that amine substitutions at the bay region resulted in remarkable green chromophores with absorptions in the desired region of the spectrum. Emission characteristics were also reported recently.[17]

Considering the requirements for a successful sensitizing agent for PDT, and the likely benefits of amino acid modification in cell permeation, we targeted perylenediimide derivatives (**1**−**3**). The synthesis is straightforward, starting from 1,7-dibromoperylenetetracarboxylic acid dianhydride.[18] The required dibromosubstituted perylenediimides were synthesized in analogy to the general practice of PDI synthesis.[8−12] The amines used were either *tert*-butyl ester of glycine and L-aspartic acid (diester). The intermediate dyes were purified by silica gel column chromatography. Then, following Wasielewski procedures, these compounds were reacted either in neat morpholine or in dry NMP with *N*-(2-hydroxyethyl)piperazine. Following chromatographic purification,[19] the *tert*-butyl groups were removed in DCM/TFA to yield water-soluble derivatives. Both the novel *tert*-butyl ester precursors and the deprotected dyes were characterized by various spectroscopic methods (Supporting Information). In pH 7.4 buffer solutions, the absorption spectra of compounds **1**−**3** show broad and red-shifted charge-transfer peaks centered at 676, 649, and 642 nm for compounds **1**, **2**, and **3**, respectively. The extinction coefficients in the same buffer solutions were near 20 000 M^{-1} cm^{-1}, which is considerably higher than that of porphyrins in the same spectral region. Fluorescence emission (near 750 nm), as expected, was barely detectable in aqueous solutions.



1: R= $-CH_2COOH$
X= O

2: R= $-CH_2COOH$
X= $N-CH_2CH_2OH$

3: R= $-CH(CH_2COOH)COOH$
X= O

Singlet oxygen generation capacities of these novel water-soluble PDI dyes were studied in 2-propanol. The experiments were performed in such a way that the requirements of both the red light and PDI sensitizers were unequivocally established. Red light from 240 W tungsten lamps, filtered to remove light with $\lambda < 600$ nm was used to excite PDI dyes. The fluence rate was determined to be 11 mW/cm^2. Thus, Figure 1A shows the change in the absorbance of the selective[20] singlet oxygen trap 1,3-diphenyl-*iso*-benzofuran (50.0 μM) in the presence of 5.0 μM of PDI dyes (**1**−**3**).

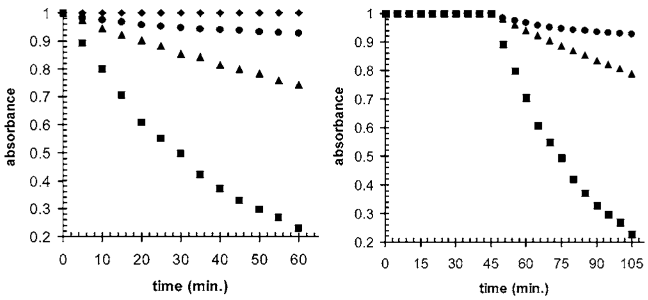


Figure 1. (A) (left) The change in the absorbance (at 415 nm) of 1,3-diphenylisobenzofuran (50 μM) in 2-propanol under red-light illumination in the presence of 5.0 μM dyes (circles, **1**; triangles, **2**; squares, **3**; and diamonds, no added dye. (B) (right) Same concentration of dyes (circles, **1**; triangles, **2**; squares, **3**) and DPBF; illumination starts after 45 min dark treatment.

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(7) (a) Angadi, M. A.; Gosztola, D.; Wasielewski, M. R. *Mater. Sci. Eng. B* **1999**, *63*, 191−194. (b) Ranke, P.; Bleyl, I.; Simmerer, J.; Haarer, D.; Bacher, A.; Schmidt, H. W. *Appl. Phys. Lett.* **1997**, *71*, 1332−1334.

(8) (a) Hayes, R. T.; Wasielewski, M. R.; Gosztola, D. *J. Am. Chem. Soc.* **2000**, *122*, 5563−5567. (b) Davis, W. B.; Svec, W. A.; Ratner, M. A.; Wasielewski, M. R. *Nature* **1998**, *396*, 60−63.

(9) Tornizaki, K.; Loewe, R. S.; Kirmaier, C.; Schwartz, J. K.; Retsek, J. L.; Bocian, D. F.; Holten, D.; Lindsey, J. S. *J. Org. Chem.* **2002**, *67*, 6519−6534.

(10) (a) Fuller, M. J.; Wasielewski, M. R. *J. Phys. Chem. B* **2001**, *105*, 7216−7219. (b) Fuller, M. J.; Walsh, C. J.; Zhao, Y.; Wasielewski, M. R. *Chem. Mater.* **2002**, *14*, 952−953.

(11) (a) Gregg, B. A.; Cormier, R. A. *J. Am. Chem. Soc.* **2001**, *123*, 7959−7960. (b) Breeze, A. J.; Salomon, A.; Ginley, D. S.; Gregg, B. A.; Tillmann, H.; Horhold, H. H. *Appl. Phys. Lett.* **2002**, *81*, 3085−3087.

(12) (a) Sadrai, M.; Hadel, L.; Sauers, R. R.; Husain, S.; Krogh-Jespersen, K.; Westbrook, J. D.; Bird, F. R. *J. Phys. Chem.* **1992**, *96*, 7988−7996. (b) Gvishi, R.; Reisfeld, R.; Brushtein, Z. *Chem. Phys. Lett.* **1993**, *213*, 338−344.

(13) (a) Dotcheva, D.; Klapper, M.; Mullen, K. *Macromol. Chem. Phys.* **1994**, *195*, 1905−1911. (b) Wurthner, F.; Thalacker, C.; Sautter, A. *Adv. Mater.* **1999**, *11*, 754−758.

(14) (a) Qu, J.; Kohl, C.; Pottek, M.; Mullen, K. *Angew. Chem., Int. Ed.* **2004**, *43*, 1528−1531. (b) Margineanu, A. et al. *J. Phys. Chem. B* **2004**, *108*, 12242−12251.

(15) (a) Langhals, H.; Schonmann, G.; Feiler, L. *Tetrahedron Lett.* **1995**, *36*, 6423−6424. (b) Holtrup, F. O.; Muller, G. R. J.; Quante, H.; De Feyter, S.; De Schryver, F. C.; Mullen, K. *Chem.*−*Eur. J.* **1997**, *3*, 219−225. (c) Kohl, C.; Becker, S.; Mullen, K. *Chem. Commun.* **2002**, 2778−2779.

(16) Zhao, Y.; Wasielewski, M. R. *Tetrahedron Lett.* **1999**, *40*, 7047−7050.

(17) Sugiyasu, K.; Fujita, N.; Shinkai, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 1229−1233.

(18) (a) Rohr, U.; Schlichting, P.; Bohm, A.; Gross, M.; Meerholz, K.; Brauchle, C.; Mullen, K. *Angew. Chem., Int. Ed.* **1998**, *37*, 1434−1437. (b) Bohm, A.; Arms, H.; Henning, G.; Blaschka, P. U.S. Patent 6,143,905, 2000.

(19) Wurthner, F.; Stepanenko, V.; Chen, Z.; Saha-Moller, C. R.; Kocher, N.; Stalke, D. *J. Org. Chem.* **2004**, *69*, 7933−7939. This recent report cautions about the presence of 1,6-isomers carried from the anhydride bromination step. Fortunately, in all three derivatives that we report, 1,6-isomers can be chromatographically removed as minor products following amine substitution in the bay region.

(20) Guiraud, H. J.; Foote, C. S. *J. Am. Chem. Soc.* **1976**, *98*, 1984−1986.

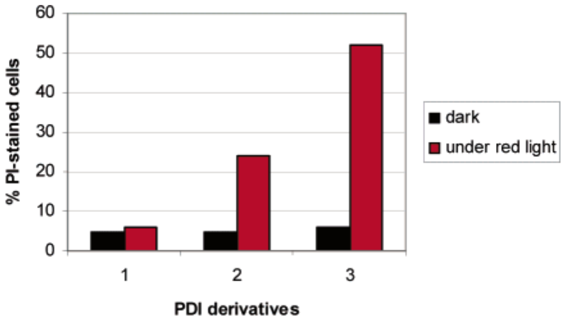

Figure 2. Percentage of propidium iodide stained K-562 cell line as assessed by flow cytometry. Black bars represent percent cell death following 4 h of incubation in dark, and red bars indicate cell death following illumination under red light for the same period, both in the presence of green PDI dyes **1**−**3** (1.5 mM). Cells were cultured in full medium. The percent cell death values represent the average of three runs.

The solutions were aerated for 5 min before the irradiation started. Under these experimental conditions, the absorbance at the peak absorbance wavelength (415 nm) of 1,3-diphenyl-*iso*-benzofuran (no added dye) did not change, whereas the solutions containing PDI dyes showed significant decreases in absorbance. To eliminate any potential contribution to the absorbance changes from dark reactions, we also recorded absorbance under the same conditions for 45 min in dark, followed by a 60 min period of irradiation with red light (Figure 1B). In dark, none of the compounds studied resulted in any absorbance changes in the reaction mixture. Only when the light was turned on did the degradation of the singlet oxygen trap start. Relative singlet oxygen generation efficiencies were determined in comparison to hematoporphyrin IX, which has a singlet oxygen quantum yield of 0.65 in methanol. Relative singlet oxygen generation rates under the conditions employed (which is a more relevant parameter[6b]) are 0.04, 0.22, and 1.2 for the sensitizers **1**, **2**, and **3**, respectively (hematoporphyrin IX = 1).

In vitro cytotoxic effects of the PDI dyes were studied using human erythroleukemia cell line (K-562). Cells were seeded at a density of 2 × 10^6/dish in RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 μg/mL penicillin, and 100 U/ml streptomycin (full medium). Varying concentrations of dyes (1.2−4.3 mM) were added on cells which were kept either in dark (control) or under filtered red light (λ > 600 nm) for a period of 4 h at room temperature at a fluence rate of 200 mW/cm^2. The percentage of cell death was evaluated by flow cytometry. Irradiated cells exhibited significantly higher percentage of cell death compared to the cells kept in dark (Figure 2). The PDI dye **3** was again the most active, in keeping with the singlet oxygen generation efficiencies. The efficiency photodynamic cytotoxicity was further demonstrated in a double staining experiment under a fluorescence microscope (Figure 3). In negative control experiments with irradiation at the same fluence rate, but with no added dye, no changes in viability of the cells were detected either in flow cytometry or under a fluorescence microscope. Thus, we successfully demonstrated that long wavelength absorbing water-soluble green PDI dyes are efficient generators of singlet oxygen and potential sensitizers for PDT when excited in the therapeutic window. High photo- and chemical stability, strong absorption in red, and availability via straightforward syntheses could make these novel PDI dyes viable alternatives to current PDT reagents.

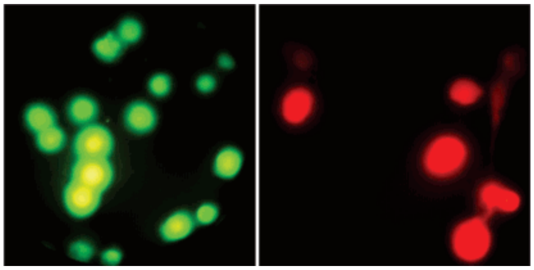

Figure 3. Fluorescence microscope images (×100) of acridine orange (AO) and propidium iodide (PI) double-stained K-562 cells, following 4 h of incubation in dark (left panel) and following irradiation with red light (λ > 600 nm) (right panel) for the same duration. Cells in full medium were incubated with 1.5 mM green PDI dye **3**. Live cells are preferentially stained with AO (green), whereas dead cells are preferentially stained with the charged dye PI (red) due to increased cellular permeability.

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Supporting Information Available: Experimental procedures and complete ref 14b. This material is available free of charge via the Internet at http://pubs.acs.org.

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