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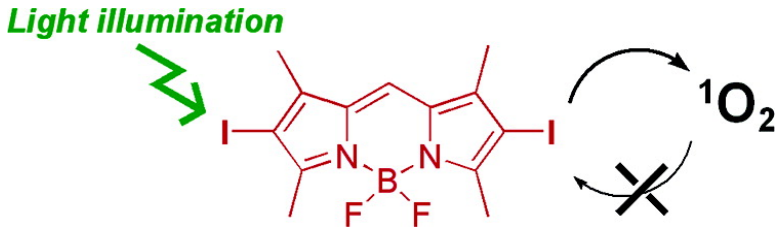

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Highly Efficient and Photostable Photosensitizer Based on BODIPY Chromophore

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Photosensitizers are chemical tools that produce reactive oxygen species (ROS) upon light illumination and are commonly used to cause physiological damage.[1] There are many reported or commercially available photosensitizers, but most have limitations, such as low photostability, structural instability, or a limited usable range of solvent conditions.[1−4] The development of novel photosensitizers, which are highly efficient, photostable, and widely applicable under various conditions, would be extremely useful.

Photosensitizers are frequently used to examine whether ROS affects a particular cellular molecule or signaling pathway, for example, enzyme activity,[5] apoptosis,[6] or gene expression.[7] Photodynamic therapy (PDT) for the treatment of cancer is another application.[8] In general, it is best to use a photosensitizer with a high absorption coefficient and high efficiency of ROS generation under a wide range of conditions. Biological targets of ROS (e.g., antioxidant substrate, enzyme, or protein of interest) exist in various environments, including the aqueous cytosolic environment and lipophilic membrane, so the availability of photosensitizers applicable over a wide range of solvent polarity should be advantageous. Moreover, good photostability upon repetitive excitation is highly desirable. Photobleaching is usually considered as a disadvantage of photosensitizers because the formation of photodegradation products complicates the interpretation of experimental results and lowers the efficiency of ROS generation.[9] Also, when a photosensitizer is used as a tumor-visualizing tool in PDT, high photostability is needed to allow monitoring for a sufficiently long period.

In this study, we report a novel photosensitizer, which is highly efficient, photostable, and usable in both lipophilic and aqueous environments. To develop this photosensitizer, we focused on the boron dipyrromethene (BODIPY) fluorophore since BODIPYs generally have high extinction coefficients (ϵ) and high quantum efficiencies of fluorescence (Φ_{fl}), which are relatively insensitive to environment[10] (i.e., solvent polarity or pH), and they are also resistant to photobleaching.[11] Thus, we hypothesized that the BODIPY fluorophore can be transformed into a general photosensitizing chromophore without loss of its unique characteristics by attaching heavy atoms directly onto the chromophore, making use of the so-called internal heavy-atom effect.[12,13]

To test this hypothesis, we synthesized 4,4-difluoro-2,6-diiodo-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (2I-BDP) by iodinating 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*- indacene (BDP) (Figure 1A). BDP has a high extinction coefficient (ϵ = 120 000 M^{-1} cm^{-1} at λ_{max} = 502 nm) and a high quantum efficiency of fluorescence (Φ_{fl} = 0.70) in MeOH. 2I-BDP also has a high extinction coefficient (ϵ = 110 000 M^{-1} cm^{-1} at λ_{max} =

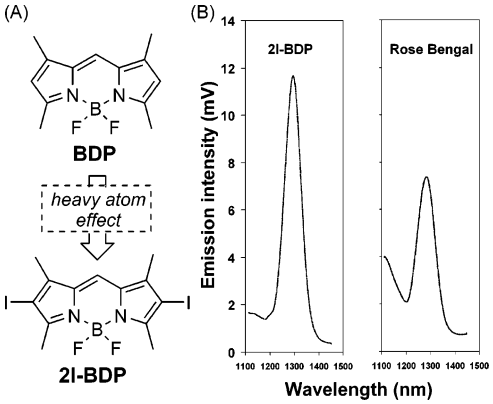

Figure 1. Structure and near-infrared singlet oxygen luminescence emission spectrum of 2I-BDP. (A) Structures of 2I-BDP and BDP. (B) Near-infrared singlet oxygen luminescence emission spectrum of 2I-BDP and Rose Bengal in MeOH (5×10^{-5} M) excited by an Ar laser light at 514 nm with 100 mW output power.

534 nm), but a much lower quantum efficiency of fluorescence (Φ_{fl} = 0.02) in MeOH than BDP (Figure S1, Table S1), suggesting that the intersystem crossing efficiency (Φ_{isc}) from the lowest singlet excited state to the triplet state has been enhanced by the internal heavy-atom effect. We then examined the ability of 2I-BDP to generate singlet oxygen ($^1O_2$). Figure 1B shows the near-infrared emission spectrum[14] of 2I-BDP in MeOH excited with an Ar laser at 514 nm. The near-infrared emission spectrum of 2I-BDP showed a narrow peak at 1268 nm, which is characteristic of $^1O_2$ generation and is stronger than that of Rose Bengal (RB), a commonly used photosensitizer for studies on oxidative stress in biological systems.[6] The efficiency of $^1O_2$ generation of 2I-BDP was 1.34 times greater than that of RB (Table S2), showing that 2I-BDP provides a highly efficient scaffold for developing novel $^1O_2$ generators.

We then examined the ability of 2I-BDP to generate $^1O_2$ in various solvents. This was achieved experimentally by following the disappearance of the 410 nm absorbance band of 1,3-diphenylisobenzofuran (DPBF),[1] a known $^1O_2$ scavenger, at the initial concentration of 2×10^{-5} M in each solvent in the presence of 1×10^{-6} M photosensitizer under light illumination. Table 1 shows the rate of DPBF consumption at the initial stage (i.e., the slope), which corresponds to the efficiency of $^1O_2$ generation. 2I-BDP generated $^1O_2$ in all of the solvents in Table 1 almost equally. The lower efficiency of $^1O_2$ generation in MeOH and aqueous solution probably reflects the shorter lifetime of $^1O_2$ in them as compared with other solvents.[15,16] On the other hand, RB could not be used in nonpolar solvents, including CH_2Cl_2 and $CHCl_3$, because of its low solubility (data not shown). These results demonstrate that 2I-BDP can generate $^1O_2$ in various environments, suggesting that it would offer considerable flexibility as an experimental tool.

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Table 1. Singlet Oxygen Generation by 2I-BDP in Various Solvents

	2I-BDP						RB
	aqueous solution[a]	CH_3CN	CH_3OH	acetone	CH_2Cl_2	$CHCl_3$	CH_3OH
slope ($\times 10^4$ s^{-1})	45	128	44	130	129	142	29
relative number of absorbed photons	0.09	0.11	0.12	0.12	0.096	0.086	0.13
relative efficiency of $^1O_2$ generation	0.048	0.11	0.037	0.11	0.14	0.17	0.022

[a] With 0.1 M sodium phosphate buffer, pH 7.4 containing 50% MeOH.

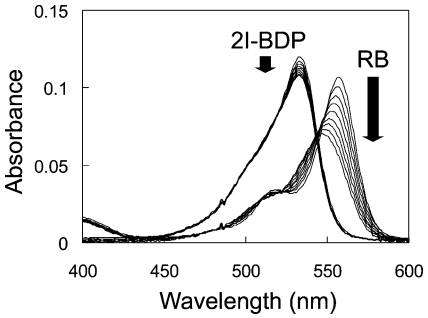

Figure 2. Comparison of photostability of 2I-BDP and Rose Bengal. The change of the absorption spectra of 2I-BDP or Rose Bengal in aerobic MeOH (1×10^{-6} M) upon repetitive laser illumination (546 nm, 0.1 W, emission interval 0.1 s) is shown from 0 cycle to 8 cycles (1 cycle = 128 pulses $\times$ 100).

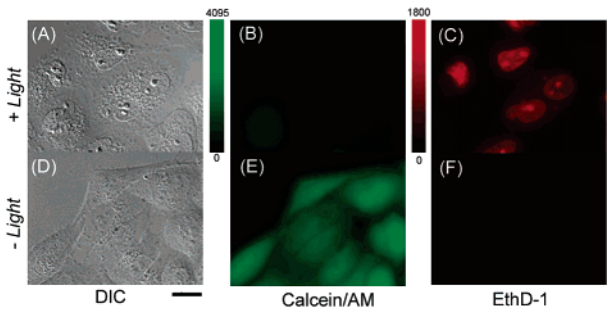

Figure 3. Cell photosensitization by 2I-BDP. (A−C) Differential interference contrast (DIC) and fluorescence images of HeLa cells loaded with calcein AM (living cell marker) and EthD-1 (dead cell marker) after photosensitization with 2I-BDP. (D−F) Loading with 2I-BDP alone had no toxic effect in this assay. Scale bar indicates 5 μm.

The photostability of 2I-BDP was then compared with that of RB. Figure 2 shows the changes of absorption spectra of 2I-BDP or RB upon repetitive laser illumination. These results indicate that 2I-BDP was much more resistant to photobleaching than RB. To elucidate the reason for this, bearing in mind that the efficiency of $^1O_2$ generation by 2I-BDP was greater than that by RB, we measured the oxidation potential of the photosensitizers by cyclic voltammetry because degradation of photosensitizer (i.e., photobleaching) is partly due to oxidation of photosensitizers by $^1O_2$.[1] The oxidation potentials of 2I-BDP and RB in MeOH were 1.13 (V vs SCE) and 0.89 (V vs SCE), respectively, showing that 2I-BDP has a more positive oxidation potential than RB and, thus, is more resistant to oxidation by $^1O_2$. Thus, a suitable design strategy for developing photosensitizers with greater photostability could be to appropriately control the oxidation potential.

Finally, we examined whether 2I-BDP can be used as a tool for cell photosensitization. HeLa cells loaded with 1 μM 2I-BDP for 30 min were illuminated with green light (535 $\pm$ 25 nm, 5 mW/cm^2) for 1 min under a fluorescence microscope, and then cell viability was assayed through the use of the esterase and nucleic acid intercalating dyes, calcein AM, and ethidium homodimer-1 (EthD-1). The combination of 2I-BDP and light illumination resulted in cellular toxicity (Figure 3), suggesting that 2I-BDP is a potentially useful reagent for cell photosensitization, studies on oxidative stress, or PDT.

In conclusion, a novel photosensitizer, 2I-BDP, was developed without loss of the unique characteristics of the BODIPY fluorophore (i.e., high ϵ, high photostability, and insensitivity to solvent environment) simply by changing the high Φ_{fl} to high Φ_{isc}, resulting in high efficiency of $^1O_2$ generation from the triplet excited state. We demonstrated that 2I-BDP has a much higher efficiency of $^1O_2$ generation than a conventional photosensitizer. It also has high photostability owing to the positive oxidation potential of the chromophore. Furthermore, 2I-BDP should be usable in a wide range of environments. Thus, 2I-BDP meets most of the key criteria for photosensitizers, and we propose it as a candidate for various applications. We have already shown that BODIPY can be easily modified chemically for the preparation of various derivatives and is available for the development of potentially useful bioimaging fluorescence probes.[17] We now plan to develop 2I-BDP derivatives with functional groups that recognize specific proteins as an approach toward a highly efficient photoinactivation technique.

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Supporting Information Available: Synthesis, experimental details, and characterization of 2I-BDP. This material is available free of charge via the Internet at http://pubs.acs.org.

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