Supporting information

"Highly efficient and photostable photosensitizer based on BODIPY chromophore"

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Materials and General Information. General chemicals were of the best grade available, supplied by Wako Pure Chemical, and used without further purification. Special chemicals were 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (BODIPY® 505/515, Molecular Probes), tetrabutylammonium perchlorate (TBAP, electrochemical grade, Fluka), *N*,*N*-dimethylformamide (DMF, fluorometric grade, Dojindo), acetonitrile (fluorometric grade, Dojindo), methyl alcohol (fluorometric grade, Dojindo), acetone (fluorometric grade, Wako Pure Chemical), methylene chloride (fluorometric grade, Dojindo), and chloroform (fluorometric grade, Dojindo). Ethanol was used after appropriate distillation. NMR spectra were recorded on a JNM-LA300 (JEOL) instrument at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR. δ

values are given in ppm relative to tetramethylsilane. Mass spectrum (MS) was measured with a JEOL JMS-T100LC AccuTOF (ESI).

Synthesis of 4,4-difluoro-2,6-diiodo-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene; 2I-BDP. (Scheme S1) Iodic acid (2.0 eq.) dissolved in a minimum amount of water was added dropwise over 20 min to a solution of 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (1.0 eq.) and iodine (2.5 eq.) in EtOH. This mixture was then warmed for 20 min at 60 °C. After cooling, the mixture was evaporated under vacuum. The crude product was purified by silica gel chromatography and recrystallized from chloroform and *n*-hexane to afford 2I-BDP as bright red needles (yield 83 %). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.22 (s, 6H); 2.54 (s, 6H); 7.10 (s, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 13.75, 15.68, 82.00, 120.22, 132.81, 144.35, 157.70. HRMS (ESI-): calcd for $[M - 1]^{-1}$; Found. 498.9186. Anal. Calcd for $C_{13}H_{13}BF_2I_2N_2$:N, 5.60; C, 31.24; H, 2.62. Found: N, 5.42; C, 31.39; H, 2.76.

Measurement of Photochemical Properties of BDP and 2I-BDP. (Figure S1, Table S1) Steady-state fluorescence spectroscopic studies were performed on an F 4500 (Hitachi). UV-vis spectra were obtained on a UV-1650PC (Shimazu). The solution contained 0.1% (v/v) DMF as a cosolvent in the case of 2I-BDP. For determination of the quantum efficiency of fluorescence (Φ_{fl}), fluorescein in 0.1 M NaOH was used as a fluorescence standard. The quantum efficiencies of fluorescence were obtained with the following equation (*F*

denotes fluorescence intensity at each wavelength and Σ[*F*] was calculated by summation of fluorescence intensity)

$$\Phi_{fl}^{sample} = \Phi_{fl}^{standard} Abs^{standard} \Sigma [F^{sample}]/Abs^{sample} \Sigma [F^{standard}]$$

Figure S1 shows absorption and fluorescence spectra of BDP and 2I-BDP in MeOH. Table S1 summarizes the photochemical properties of BDP and 2I-BDP.

Singlet Oxygen Detection by Near-Infrared Spectroscopy. (Figure 1, Table S2) According to the method of Arakane et al. [SR1], singlet oxygen was detected by using a near-infrared emission spectrometer (Ultra-sensitive Ge detector, model E1-S, Edinburgh Instruments Ltd., UK), cooled with liquid nitrogen, to obtain luminescence emission spectra from a test solution circulated in a quartz flow cell excited with argon laser light (Ar laser equipment, Innova 70-4, Coherent Inc., USA) at 514.5 nm with 100 mW output power. The argon laser beam was chopped at 800 Hz by an acoustooptic modulator (A-160, Hoya Co., Japan) with a driver (110-DS, HOYA Co., Japan). The output signal from the Ge detector was led through a preamplifier (model 116, E. G. & G. Princeton Applied Research, USA) to a lock-in amplifier (model 124A, E. G. & G. Princeton Applied Research, USA), synchronized with an internal standard signal, and recorded on an XY recorder. Table S2 compares the emission intensity of 2I-BDP and RB in MeOH. The values were calibrated using the molar extinction coefficient (ε_{514}) and then normalized to the value of RB. The efficiency (I/ε_{514}) of $^1O_2$ generation of 2I-BDP was 1.34 times greater than that of RB.

Singlet Oxygen Detection by Using 1,3-diphenylisobenzofuran (DPBF). (Table 1) A solution of photosensitizer (1×10^{-6} M) and DPBF (2×10^{-5} M) was irradiated with a 500 W Xe light source (SM-3, Bunkoh-keiki Co., Ltd.), which was filtered to around 530 nm (in the case of 2I-BDP) or around 556 nm (in the case of Rose Bengal). The solution contained up to 0.2% (v/v) DMF as a cosolvent. Reaction of DPBF with $^1O_2$ was monitored by measuring the reduction in the intensity of the absorption band at 410 nm. UV-vis spectra were obtained on an Agilent 8453 UV-vis spectrometer. The rate of DPBF consumption at the initial stage (i.e., the slope) was calibrated in terms of relative number of absorbed photons (i.e., absorbance at 530 nm in the case of 2I-BDP, and 556 nm in the case of Rose Bengal), which corresponds to the relative efficiency of $^1O_2$ generation by photosensitizer in each solvent.

Photobleaching Experiments. (Figure 2) An aerobic methanol solution of 2I-BDP (1×10^{-6} M, containing 0.1% (v/v) DMF as a cosolvent) or Rose Bengal (1×10^{-6} M) was repetitively illuminated by a laser beam at 546 nm, at which the extinction coefficients are about the same. Laser illumination (546 nm, 0.1 W, emission interval 0.1 sec) was repeated from 0 cycle to 8 cycle (1 cycle = 128 pulses × 100), and UV-vis spectra were obtained after each illumination cycle. Laser illumination were conducted with a SURELITE OPO (Continuum). UV-vis spectra were obtained on an Agilent 8453 UV-vis spectrometer.

Cyclic Voltammetry. Cyclic voltammetry was performed on a 600A electrochemical analyzer (ALS). A

three-electrode arrangement in a single cell was used for the measurement: a Pt wire as the auxiliary electrode, GC electrode as the working electrode, and an Ag/Ag+ electrode as the reference electrode. The sample solution contained 0.1 M tetrabutylammonium perchlorate as a supporting electrolyte in MeOH.

Cell Photosensitization Assay. (Figure 3, Figure S2)

Cell Culture. HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Corp., Carlsbad, CA), supplemented with 10% fetal bovine serum (Invitrogen Corp.), 1% penicillin, and 1% streptomycin (Invitrogen Corp.) at 37 °C in a 5% CO_2/95% air incubator. The cells were grown on an uncoated 35-mm-diameter glass-bottomed dishes (MatTek, Ashland, MA).

Microscopy. The imaging system comprised an inverted microscope (IX71; Olympus) and a cooled CCD camera (Cool Snap HQ; Roper Scientific, Tucson, AZ). The microscope was equipped with a xenon lamp (AH2-RX; Olympus), a 40× objective lens (Uapo/340, N.A. 1.35; Olympus), a dichroic mirror (DM505; Olympus, DM570; Olympus, Q565lp; Chroma Tech.Corp.), an excitation filter (BP470-490; Olympus, BP530-550; Olympus, HQ535/50; Chroma Tech.Corp.), and an emission filter (BA510-550; Olympus, BA590; Olympus). The whole system was controlled using MetaFluor 6.1 software (Universal Imaging, Media, PA).

Protocol of Cell Photosensitization Assay.

HeLa cells attached to glass-bottomed dishes (1-5 × 10^5 cells/ml) were washed twice with Hanks' balanced salt solution (HBSS) buffer (Invitrogen Corp.). The extracellular solution was replaced with 1 μM 2I-BDP

(containing 0.1% DMSO) in HBSS or vehicle (0.1% DMSO in HBSS) and incubated for 30 min at r.t.. Then, the cells were washed twice with HBSS and illuminated with a xenon lamp (AH2-RX), which was filtered to around 535 ± 25 by an excitation filter (HQ535/50; Chroma Tech.Corp.) through the objective lens under the fluorescence microscope. The light power at the focal plane was about 5 mW/cm^2 at 530 nm. After illumination, the extracellular solution was replaced with 2 μM Calcein/AM and 4 μM ethidium homodimer-1 (EthD-1) (LIVE/DEAD®VIability/Cytotoxicity kit, Molecular Probes)[SR2] in HBSS and incubated for 30 min at r.t.. Then, cells were washed with HBSS and the two-color fluorescence cell viability test was conducted under a fluorescence microscope. The fluorescence image was acquired by using a BP470-490 excitation filter and a BA510-550 emission filter for Calcein imaging (living cells), and a BP530-550 excitation filter and a BA590 emission filter for EthD-1 imaging (dead cells). In the control experiment, vehicle (0.1% DMSO in HBSS)-loaded cells did not show any cytotoxic effect, whether or not light illumination was conducted, showing that light illumination at 530 nm did not affect for cell viability under this condition (Figure S2).

(Supporting references)

(SR1) Arakane, K.; Ryu, A.; Takarada, K.; Masunaga, T.; Shinmoto, K.; Kobayashi, R.; Mashiko, S.; Nagano, T.; Hirobe, M. *Chem. Pharm. Bull.* **1996**, *44*, 1-4.

(SR2) Papadopoulos, N. G.; Dedoussis, G. V. Z.; Spanakos, G.; Gritzapis, A. D.; Baxevanis, C. N.; Papamichail, M. *J. Immunol. Methods* **1994**, *177*, 101-111.

Table S1. Photochemical properties of BODIPY Derivatives[a]

	absorbance max [nm]	excitation coefficient (ε) [$\times 10^4$ M^{-1} cm^{-1}]	emission max [nm][b]	ralative quautum yield (Φ_{fl})[c]
BDP	502	12	508	0.70
2I-BDP	534	11	548	0.02

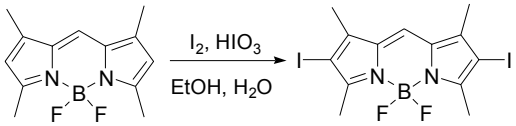
[a]Measured in MeOH. [b]Excited at 490 nm. [c]Quantum yield of fluorescence was determined using that of fluorescein (0.85) in 0.1 M NaOH aq. as a standard.

Table S2. Comparison of relative emission intensities at 1268 nm in solutions of 2I-BDP and Rose Bengal.

	A_{514}	ε_{514}	$^1O_2$ emission (I) [mV]	I/ε_{514}	I/ε_{514} (rel.)
2I-BDP	2.03	4.06×10^4	11.6	2.86×10^{-4}	1.34
Rose Bengal	1.66	3.32×10^4	7.1	2.14×10^{-4}	1

The emission intensity at 1268 nm was measured in methanol solution of a dye at the concentration of 5×10^{-5} M, excited by Ar laser light at 514 nm with 100 mW output power. A_{514}: absorbance at 514 nm in each solution, ε_{514}: molar extinction coefficient at 514 nm.

Scheme S1. Synthetic scheme of 2I-BDP.



BDP **2I-BDP**

Figure S1. Absorption and fluorescence spectra of BODIPY derivatives. (A) Absorption spectra of BDP and 2I-BDP in MeOH. (B) Fluorescence spectra of BDP and 2I-BDP in MeOH.

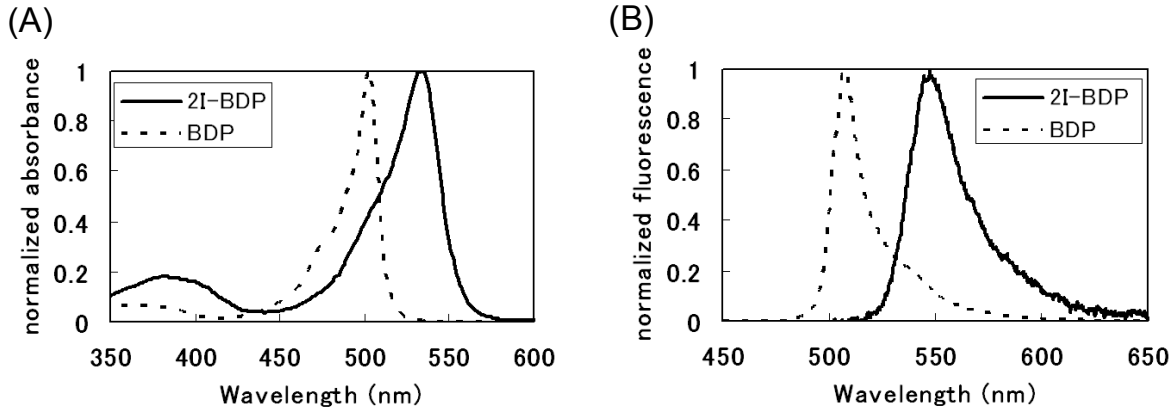


Figure S2. Light phototoxicity to HeLa cells. (A-C) Differential interference contrast (DIC) and fluorescence images of HeLa cells loaded with calcein AM (live cell marker) and EthD-1 (dead cell marker) after vehicle (0.1% DMSO in HBSS) loading and light illumination (535 ± 25, 5mW, 1 min). (D-F) Loading with vehicle (0.1% DMSO in HBSS) alone had no toxic effect in this assay. Scale bar indicates 5 μm.