Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

A novel BODIPY-based photosensitizer with pH-active singlet oxygen generation for photodynamic therapy in lysosome

Chengjun Wang, Ying Qian*

School of chemistry and chemical engineering, Southeast University, Nanjing, Jiangsu 211189, PR China

**Corresponding author: E-mail address: yingqian@seu.edu.cn*

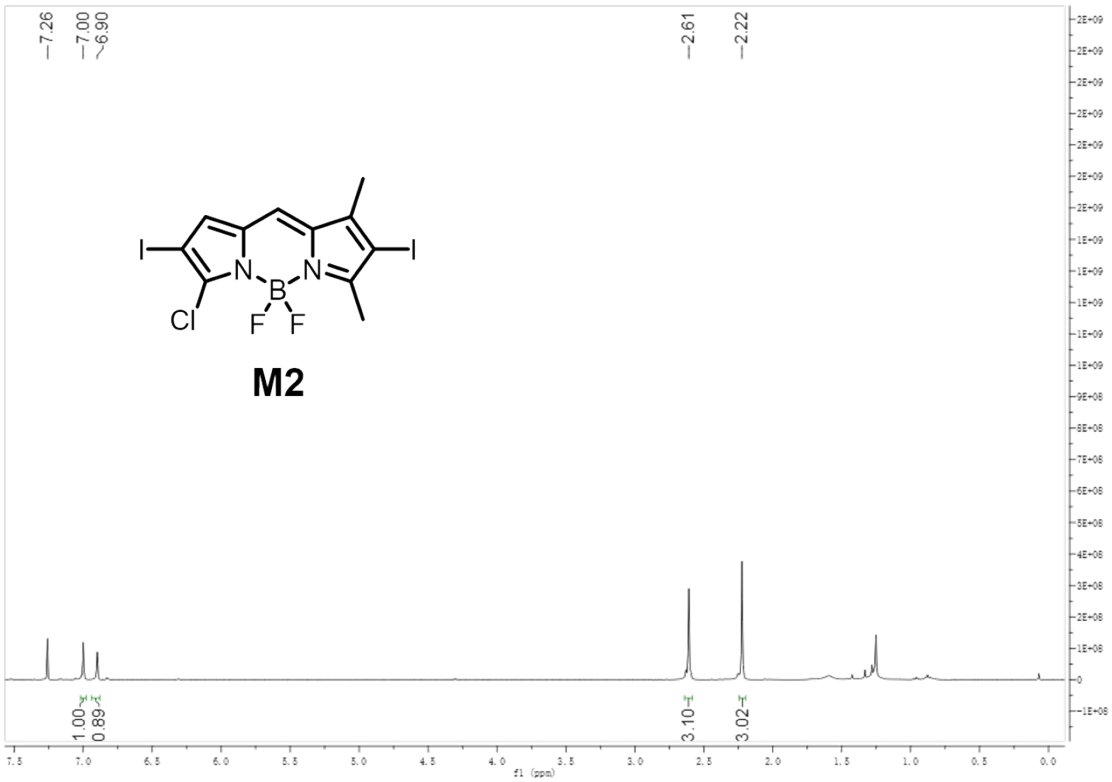

Figure S1. ^{1}H NMR of compound M2.

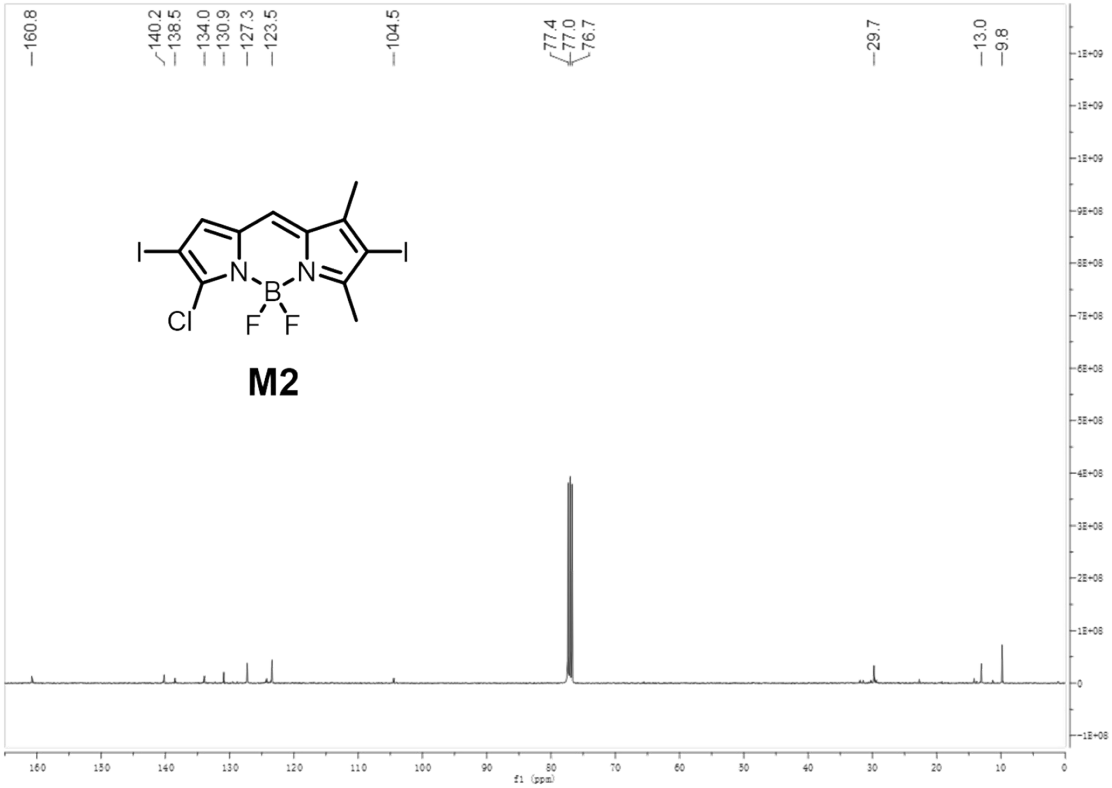

Figure S2. ^{13}C NMR of compound M2.

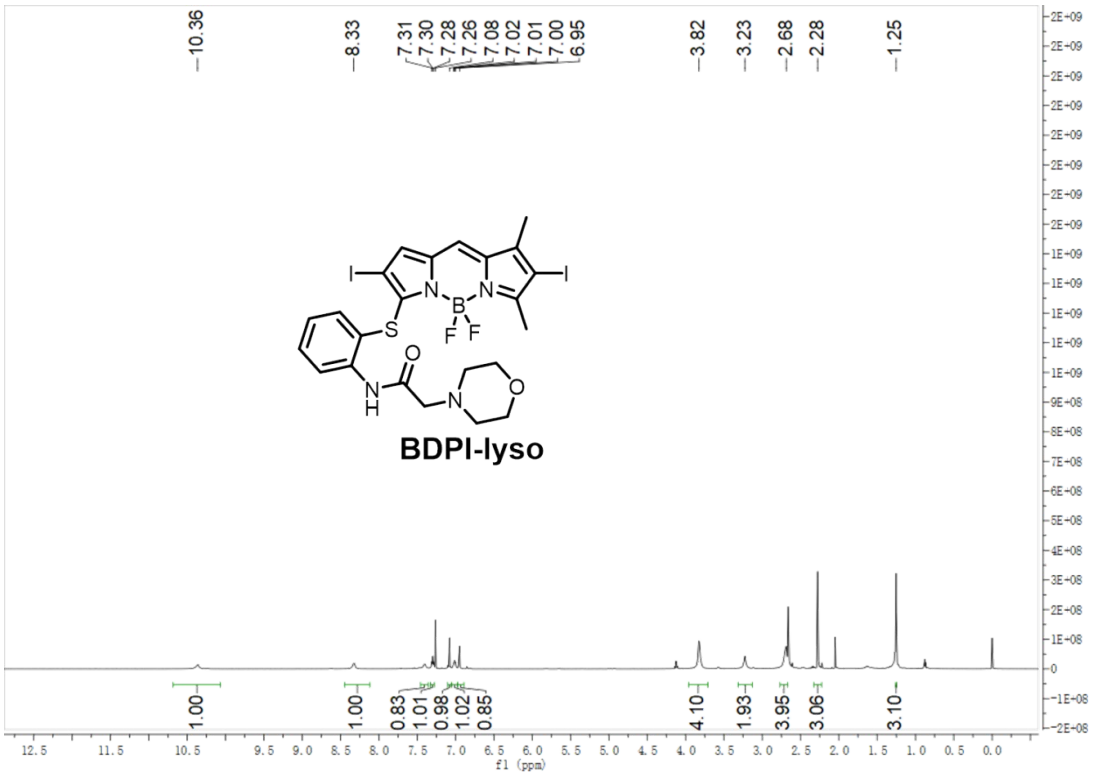

Figure S3. ^{1}H NMR of photosensitizer BDPI-lyso.

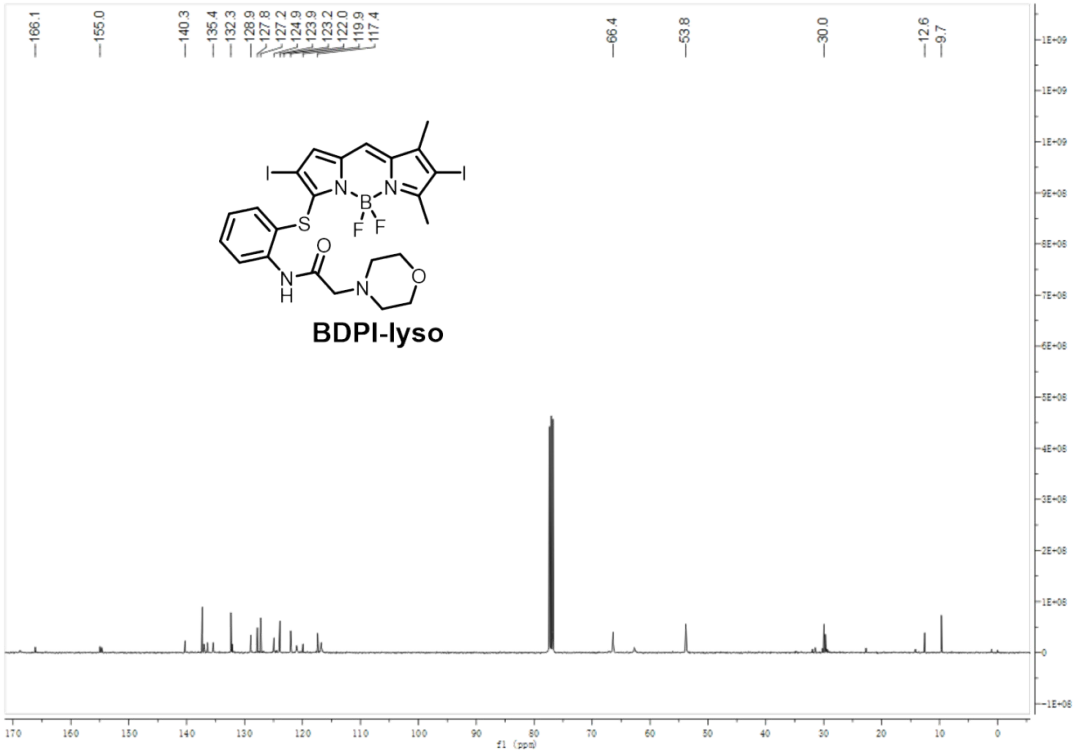

Figure S4. ^{13}C NMR of photosensitizer BDPI-lyso.

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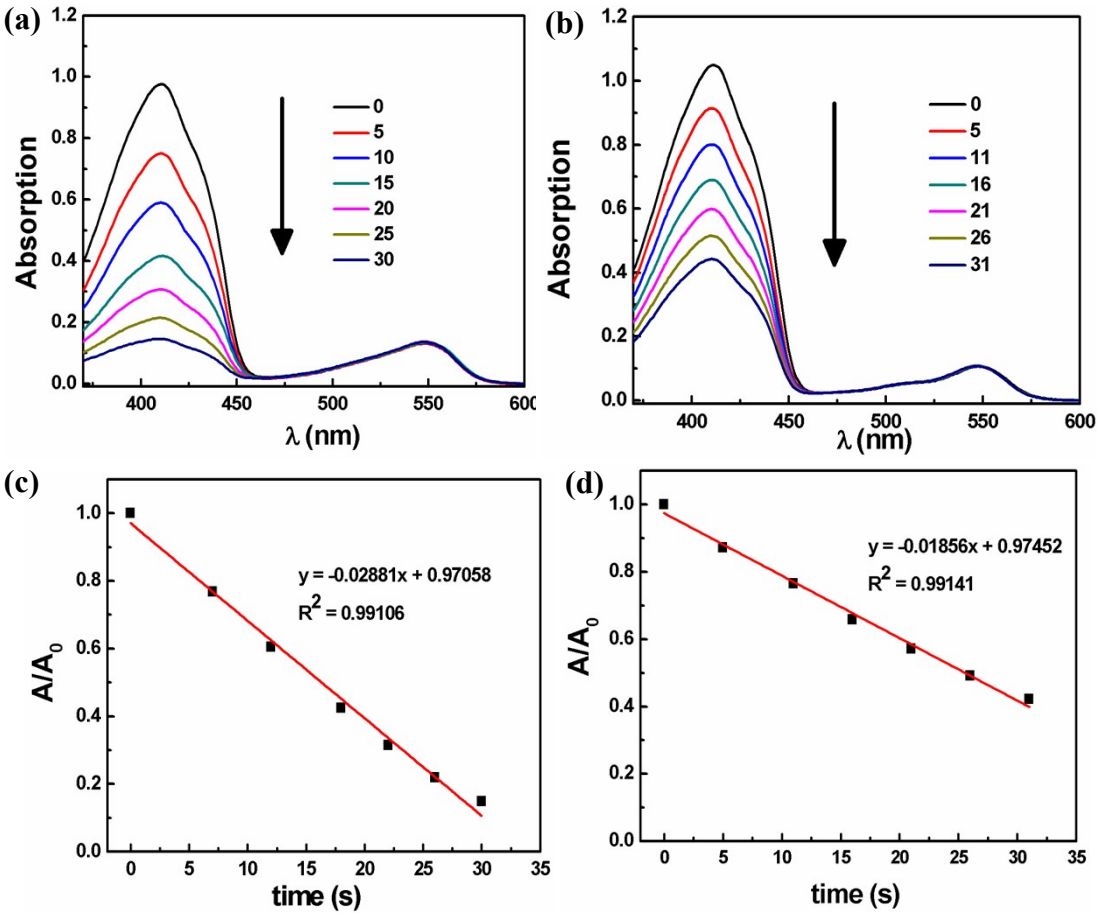

Figure S5. (a) The degradation of the absorption of DPBF in the presence of BDPI-lyso in EtOH and light illumination (0.8 mW/cm2, 525 nm) for various time (s); (b) the degradation of the absorption of DPBF in the presence of Rose Bengal in EtOH and light illumination (0.8 mW/cm2, 525 nm) for various time (s); (c) linear fitting of the normalized DPBF absorption (A/A_0) at 410 nm in EtOH against various light (0.8 mW/cm^2, 525 nm) illumination time in the presence of BDPI-lyso; (d) linear fitting of the normalized DPBF absorption (A/A_0) at 410 nm in EtOH against various light (0.8 mW/cm^2, 525 nm) illumination time in the presence of Rose Bengal.

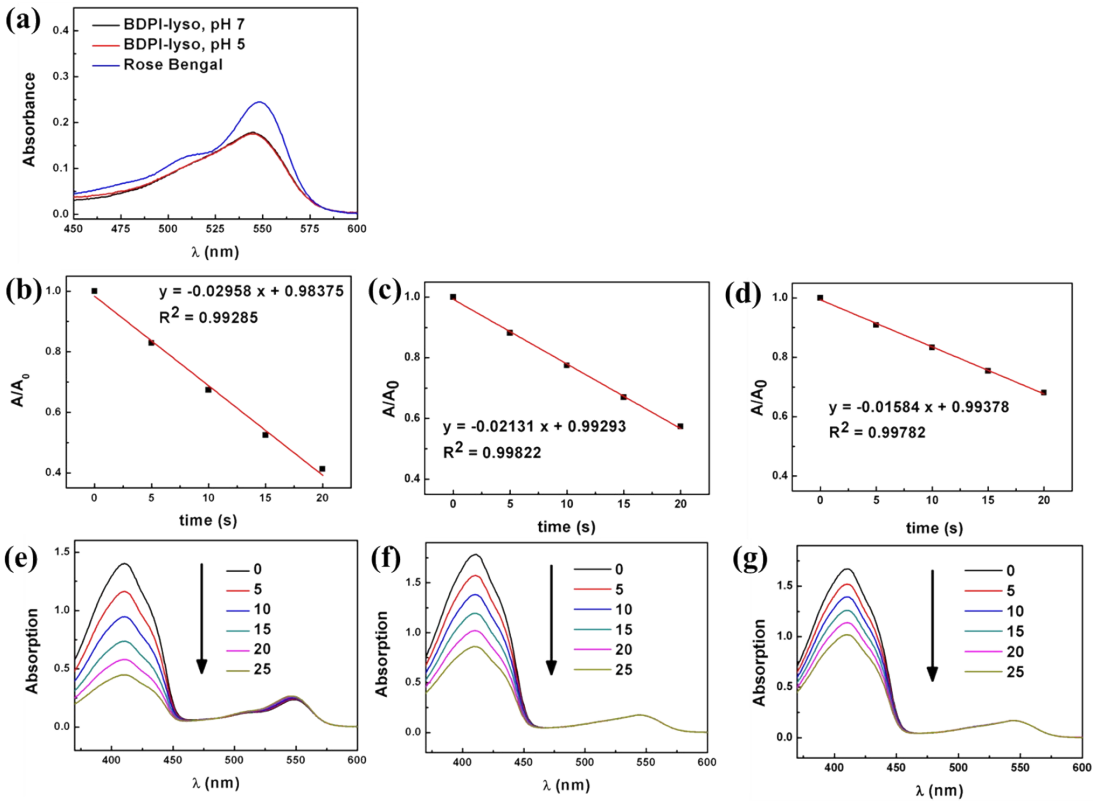

Figure S6. (a) The absorption of BDPI-lyso in different pH conditions and Rose Bengal in EtOH before addition of DPBF; (b) linear fitting of the normalized DPBF absorption (A/A_0) at 410 nm in EtOH against various light (0.8 mW/cm^2, 525 nm) illumination time in the presence of Rose Bengal; (c) linear fitting of the normalized DPBF absorption (A/A_0) at 410 nm in acid solution (EtOH/PBS = 1:1, pH = 5.10) against various light (0.8 mW/cm^2, 525 nm) illumination time in the presence of BDPI-lyso; (d) linear fitting of the normalized DPBF absorption (A/A_0) at 410 nm in neutral solution (EtOH/PBS = 1:1, pH = 7.24) against various light (0.8 mW/cm^2, 525 nm) illumination time in the presence of BDPI-lyso; (e) the degradation of the absorption of DPBF in the presence of Rose Bengal in EtOH and light (0.8 mW/cm^2, 525 nm) illumination for various time (s); (f) the degradation of the absorption of DPBF in the presence of BDPI-lyso in acid solution (EtOH/PBS = 1:1, pH = 5.10) and light (0.8 mW/cm^2, 525 nm) illumination for various time (s); (g) the degradation of the absorption of DPBF in the presence of BDPI-lyso in neutral solution (EtOH/PBS = 1:1, pH = 7.24) and light illumination (0.8 mW/cm^2, 525 nm) for various time (s).

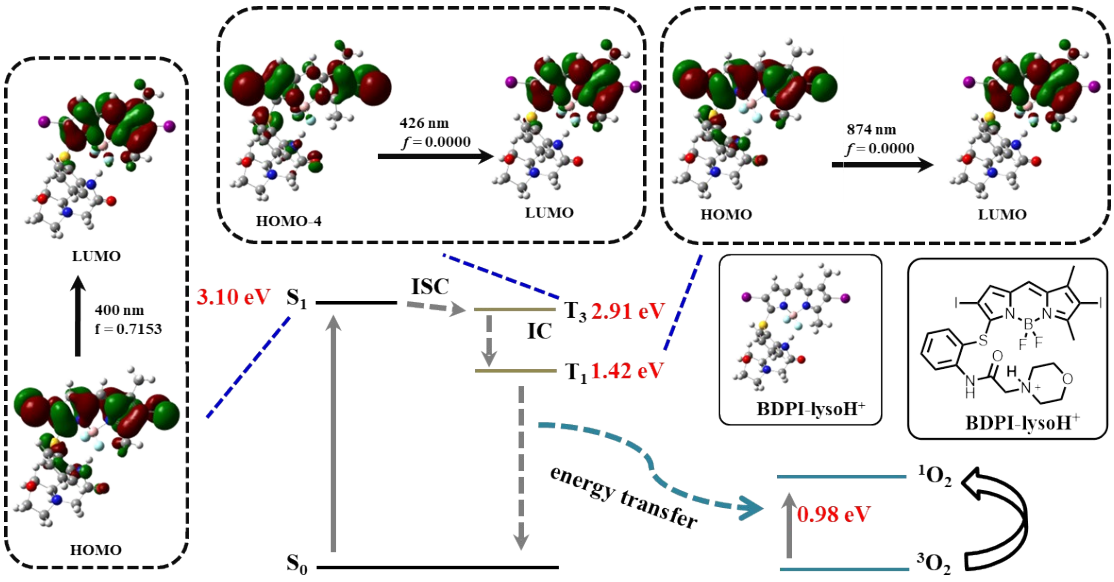

Figure S7. The energies and frontier molecular orbital diagrams of BDPI-lysoH$^+$ were calculated using TD-DFT//cam-B3LYP/6-31G(d)/LANL2DZ level in EtOH based on the geometry optimized by DFT//cam-B3LYP/6-31G(d)/LANL2DZ basis set.

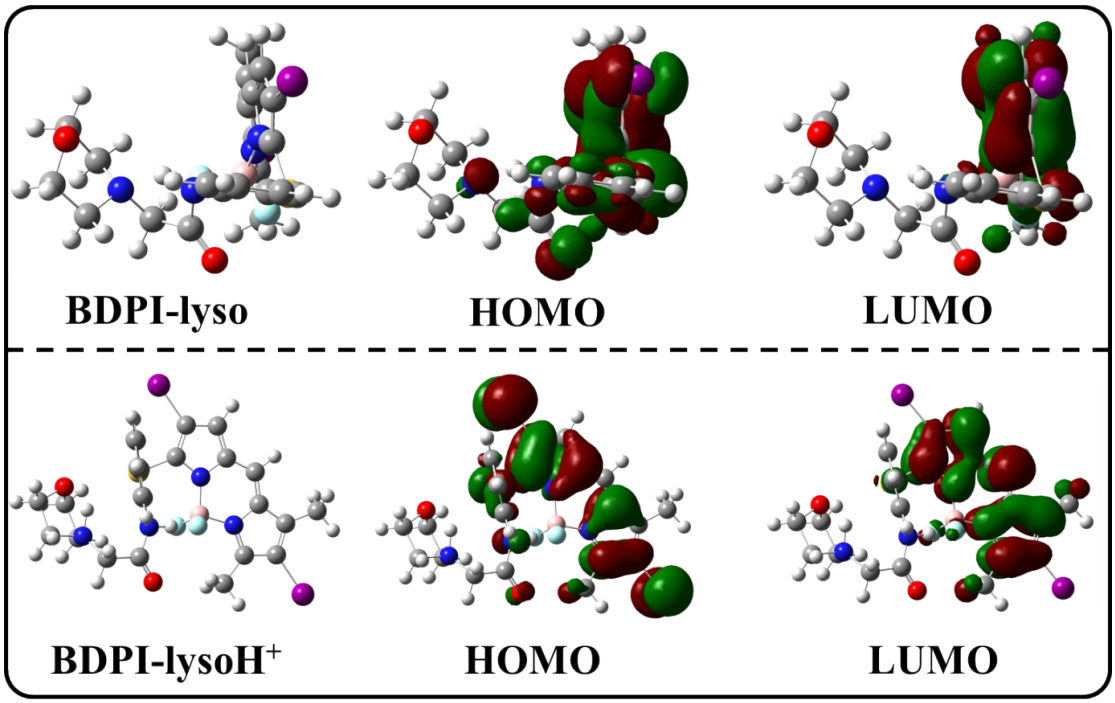

Figure S8. The optimized structures and Frontier Molecular Orbital diagrams of BDPI-lyso and BDPI-lysoH$^+$. TD-DFT calculations were performed at the EtOH-cam-B3LYP/6-31G (d) /LANL2DZ level based on DFT-optimized structures at cam-B3LYP/6-31G (d) /LANL2DZ level.

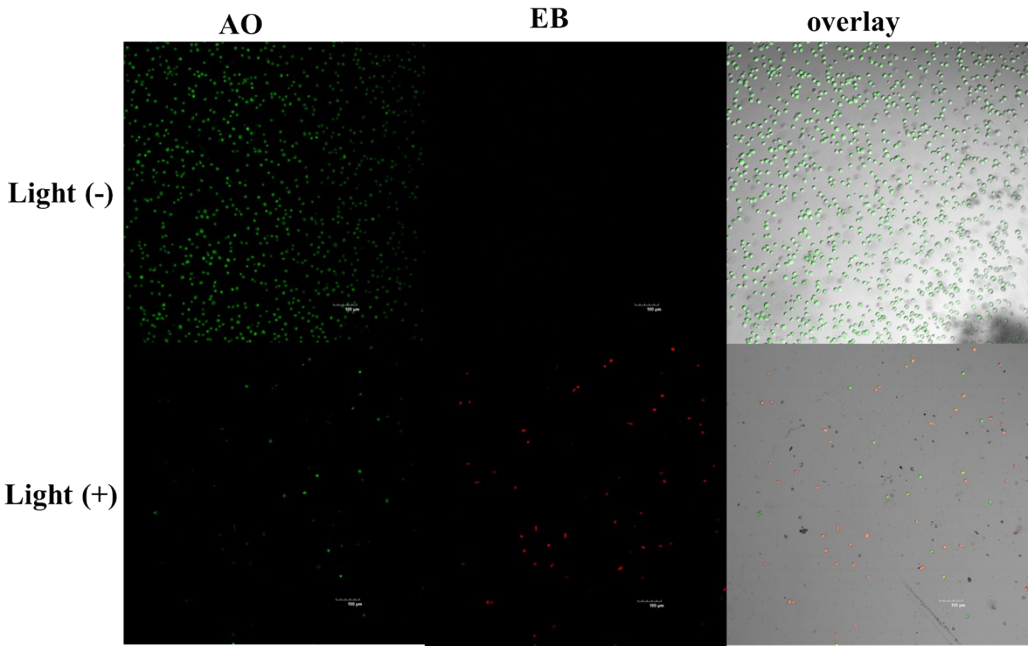

Figure S9. Live/dead staining of BDPI-lyso (1 μM) treated Bel-7402 cells in the absence and presence of light illumination (4 mW/cm^2, 525 nm, 30 min).

Table S1. Selected parameters[a] for the calculated singlet state energy level and triplet state energy level of BDPI-lyso and BDPI-lysoH$^+$.

Compound	States	Elecreonic transition	Energy, eV/λ, nm	*f*[b]	Composition[c]	CI[d]
BDPI-lyso	Singlet state	$S_0 \rightarrow S_1$	2.96/420	0.9130	H→L	0.6849
		$S_0 \rightarrow S_2$	3.17/391	0.0242	H-1→L	0.6360
		$S_0 \rightarrow S_3$	3.54/350	0.0954	H-4→L	0.6207
	Triplet state	$S_0 \rightarrow T_1$	1.33/934	0.0000	H→L	0.6550
		$S_0 \rightarrow T_2$	2.75/452	0.0000	H-4→L	0.4948
		$S_0 \rightarrow T_3$	2.89/429	0.0000	H-5→L	0.5053
BDPI-lysoH$^+$	Singlet state	$S_0 \rightarrow S_1$	3.10/400	0.7153	H→L	0.6850
		$S_0 \rightarrow S_2$	3.44/360	0.0020	H-1→L	0.5992
		$S_0 \rightarrow S_3$	3.49/355	0.0952	H-3→L	0.4043
	Triplet state	$S_0 \rightarrow T_1$	1.42/874	0.0000	H→L	0.6454
		$S_0 \rightarrow T_2$	2.57/482	0.0000	H-5→L	0.4056
		$S_0 \rightarrow T_3$	2.91/426	0.0000	H-4→L	0.4269

[a] Parameters were calculated by TDDFT/cam-B3LYP/6-31G(d)/LANL2DZ (EtOH was used as solvent), based on the optimized ground-state geometries using the method DFT/cam-B3LYP/6-31G(d)/LANL2DZ. [b]Oscillator strength. [c]H, HOMO (highest occupied molecular orbital) and L, LUMO (lowest unoccupied molecular orbital). [d]Coefficient of the wavefunction for each excitations.

Table S2. Comparison of photosensitizer BDPI-lyso in our article and other lysosome-targeted photosensitizers in other articles about the singlet quantum yields in acid and neutral conditions.

Reference	Φ_Δ (acid), Φ_Δ (neutral)
Our article	0.38 (pH 7.24), 0.51 (pH 5.10)
Dyes and Pigments, 2017, **147**, 99	Not given
J. Mater. Chem. B, 2016, **4**, 1862	0.25 (pH 7), 0.35 (pH 5)
Chem. Commun., 2016, **52**, 148	0.227 (pH 7.4), 0.284 (pH 4)
Chem. Eur. J., 2018, **24**, 10999	Not given in neutral condition, 0.71 (pH 4–5.5)
Dyes and Pigments, 2017, **147**, 476	Not given

Methods

1. Measurement of fluorescence quantum yield of BDPI-lyso

Fluorescence quantum yields of BDPI-lyso (in EtOH) was determined using Rhodamine B as standard (Φ_r = 0.65 in EtOH)[1], within an absorption around 0.05. The quantum yield was calculated based on Eq. 1:

$$\Phi_s = \Phi_r \times \frac{A_r}{A_s} \times \frac{F_s}{F_r} \times \frac{{n_s}^2}{{n_r}^2} \qquad \text{Eq. 1}$$

Where Φ is the fluorescence quantum yield, A is the absorption at the excitation wavelength (A<0.05), F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts s and r refer to the unknown and the standard, respectively.

2. Measurement of singlet oxygen quantum yield of BDPI-lyso

Singlet oxygen quantum yield (Φ_Δ) of BDPI-lyso was determined using DPBF as singlet oxygen trapping agent and Rose Bengal (RB) as standard (Φ_Δ = 0.68 in EtOH)[2]. The quantum yields were calculated based on Eq. 2:

$$\Phi_{\Delta,\ s} = \Phi_{\Delta,\ RB} \times \frac{k_s}{k_r} \times \frac{F_r}{F_s} \qquad \text{Eq. 2}$$

Where $\Phi_{\Delta,\ s}$ is the singlet oxygen quantum yield of BDPI-lyso, *k* is the bleach rate

of normalized DPBF absorption (410 nm) against the illumination time, F is the absorption correction factor at the excitation wavelength (525 nm), $\Phi_{\Delta, RB}$ was the reported singlet oxygen quantum yield of Rose Bengal.

3. MTT assay to evaluate the dark toxity and phototoxity of BDPI-lyso

Preparation BDPI-lyso pretreated Bel-7402 cells: cells were seeded in 96-well plates at 100 μL/well (density of 5000 cells/well) and incubated for 24 h. Various concentrations of BDPI-lyso (0, 0.25, 0.5, 1, 2, 4 μM) were added into each well and incubated for 12 h.

Dark cytoctoxity: After the removal of BDPI-lyso-containing medium, the cells were rinsed twice with PBS and the fresh medium containing MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) was added and incubated for 4 h. After removal of the MTT-containing medium, DMSO (100 μL/well) was added and the absorption at 490 nm was recorded. The cell viability was calculated utilizing the following equation:

$$\text{Cell Viability} = OD_{490}\ (\text{sample})/OD_{490}\ (\text{control})$$

Phototoxity: After the removal of BDPI-lyso-containing medium, the cells were rinsed twice with PBS and the fresh medium was added. Then the cells were illuminated by the LED lamp (525 nm) at a power density of 4 mW/cm^2 for 10 min and 30 min with an ice bag disposed below the 96-well plates to eliminate the influence of temperature. Then the cells were subject to continuous incubation for 12 h. After removal of the medium, the fresh medium containing MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) was added and incubated for 4 h. After removal of the MTT-containing medium, DMSO (100 μL/well) was added and the absorption at 490 nm was recorded. The cell viability was calculated utilizing the above-mentioned equation

4. Confocal Fluorescence Microscopy

Cell images: Bel-7402 cells were seeded in glass-bottomed plates (Φ = 24 mm) at a density of 1×10^5 cells per well. After incubation for 24 h, the medium was removed and washed with PBS twice. Fresh medium containing BDPI-lyso (1 μM) was added and incubated for 30 min. After removal of the medium and PBS washing for three times and addition of fresh medium, cell image was conducted before and after light illumination (525 nm, 4 mW/cm^2) for various time using the cofocal laser scanning microscopy (CLSM).

AO/EB dual staining

Bel-7402 cells were seeded in two plates (Φ = 30 mm) at a density of 1×10^5 cells. After incubation for 24 h, the medium was removed. Fresh medium containing BDPI-lyso (1 μM) was added and incubated for 12 h. After removal of the medium and PBS washing for three times and addition of fresh medium, the cells in one plate were illuminated with LED light (525 nm, 4 mW/cm^2, 30 min) and incubated for another 12 h. Another plate of cells was subjected the subsequent operation directly. Cells of all the above-mentioned plates were washed with PBS twice and were subject to the trypsin enzymic digestion (without EDTA). The suspension of cells was collected and centrifugal separated. Cells were washed with PBS twice and added with an appropriate amount of acridine orange/ethidium bromide (AO/EB) working solution (10 μL, in 1× biding buffer). The suspension was incubated for 10 min and dropped on a glass slice. Cofocal fluorescence imaging experiments in green and red channel were performed.

Measurement of ROS: The probe 2,7-Dichlorofluorescein diacetate (DCFH-DA) was used to measure the generation of intracellular ROS. Bel-7402 cells were seeded in glass-bottomed plates (Φ = 24 mm) at a density of 1×10^5 cells per well. After incubation for 24 h, the medium was removed and washed with PBS twice. Medium without FBS containing DCFH-DA (3 μM) was added and incubated for 20 min. The

medium was removed and washed with PBS twice. Fresh medium was added and cell image in red and green channel was conducted before and after light illumination (525 nm, 4 mW/cm^2, 5 min) using the cofocal laser scanning microscopy (CLSM).

Lyso-Tracker Green colocalization: Bel-7402 cells were seeded in glass-bottomed plates (Φ = 24 mm) at a density of 1×10^5 cells per well. After incubation for 24 h, the medium was removed and washed with PBS twice. Fresh medium containing BDPI-lyso (1 μM) was added and incubated for 30 min. The medium was removed and fresh medium containing appropriate amount of Lyso-Tracker Green was added and incubated for 30 min. The medium was removed and washed with PBS twice. Fresh medium was added and cell image in red and green channel was conducted before and after light illumination (525 nm, 4 mW/cm^2, 15 min) using the cofocal laser scanning microscopy (CLSM).

Reference

[1] T. Karstens, K. Kobs, *J Phys. Chem.*, 1980, **84**, 1871.

[2]R.W. Redmond, J.N. *Gamlin, Photochem. Photobiol.*, 1999, **70**, 391.