Supporting Information

Cascade Reactions by Nitric Oxide and Hydrogen Radical for Anti-Hypoxia Photodynamic Therapy Using an Activatable Photosensitizer

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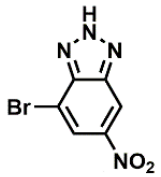
Methods

NMR spectra were recorded on a Bruker AMX 400 spectrophotometer. Low-resolution mass spectra were collected on a Micromass GC-TOF mass spectrometer (GCMS-QP2020). High-resolution mass spectra were obtained using an electrospray Agilent Q-TOF mass spectrometer system (Q-TOF-6540) and reported as *m/z.*

Ultraviolet−visible (UV−Vis) spectra were taken on a Shimadzu UV-2600 spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 fluorimeter. LED lamp (pE-4000, CoolLED Ltd., U.K.) was used as light source. A Waters ACQUITY H-Class UPLC equipped with PDA and fluorescence detectors was used to monitor the micro reaction process. Analytical LC was performed using a Water ACQUITY UPLC BEH C_{18} column (1.7 μm, 2.1×50 mm) eluting with a gradient of 10–100% acetonitrile for 4 min in 0.2% acetic acid at 40 °C. A total flow rate of 0.4 mL min^{-1} and an injection volume of 2 μL were applied. Absorption intensities were monitored at 370 nm, fluorescence emission monitored at 540 nm (λ_{ex} = 370 nm) unless otherwise stated. Statistical analysis was performed using SigmaPlot 12.5 software (USA). A confidence interval of 95% percent was set.

Synthesis and characterization

*Synthesis of 4-bromo-6-nitro-2H-benzo[d][1,2,3]triazole (**2**)*

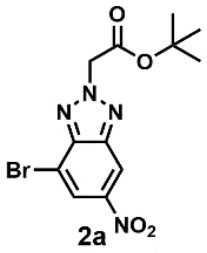


2

To a solution of 2-bromo-4,6-dinitroaniline (**1**) (2.60 g, 10 mmol) in ethanol (50 mL) and water (25 mL) was added $Na_2S•9H_2O$ (8.40 g, 35 mmol). The reaction was stirred at 25 °C for 20 min. The residue was poured into ethyl acetate (200 mL), washed with water (200 mL) and brine (200 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the crude product 3-bromo-5-nitrobenzene-1,2-diamine. To a solution of crude diamine product in 2:1 (v/v) mixture of acetic acid and DI water (30 mL) was added $NaNO_2$ (1.03 g, 15 mmol). The reaction was stirred

for 1 h at 25 °C. The mixture was poured into ice water (30 mL). The crude product was filtered and washed with water (200 mL). Silica-gel column chromatography using petroleum ether/ethyl acetate (5:1) as eluent afforded **2** (1.51 g, 62%) as a light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.94 (s, 1H), 8.48 (s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 145.3 140.7, 138.1, 122.7, 112.0, 107.4 ppm. HRMS (ESI-): *m/z* 240.9352 $[M–H]^–$, Calculated for $C_6H_2BrN_4O_2$: 240.9361; found 240.9352.

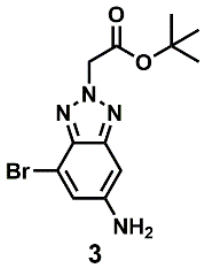
*Synthesis of tert-butyl 2-(4-bromo-6-nitro-2H-benzo[d][1,2,3]triazol-2-yl)acetate (**2a**)*



2a

To a solution of **2** (1.21 g, 5 mmol) in anhydrous DMF were added potassium *t*-butoxide (784 mg, 7 mmol) and *tert*-butyl bromoacetate (1.17 g, 6 mmol) under argon atmosphere. The reaction mixture was stirred at 25 °C for 12 h and then concentrated in vacuo. The residue was dissolved in ethyl acetate (100 mL), and washed with water (100 mL) and brine (100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Silica-gel column chromatography using petroleum ether/ethyl acetate (20:1) as eluent afforded **2a** (546 mg, 32%) as a light yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (s, 1H), 8.47 (s, 1H), 5.52 (s, 2H), 1.49 (9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.0, 146.9, 146.4, 142.9, 123.9, 115.6, 112.3, 84.7, 58.7, 28.0 ppm. LRMS (EI): 356.1.

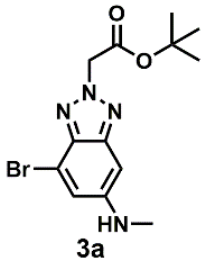
*Synthesis of tert-butyl 2-(4-bromo-6- amino -2H-benzo[d][1,2,3]triazol-2-yl)acetate (**3**)*



3

To a solution of **2a** (356 mg, 1 mmol) in acetic acid (20 mL) was added reduced iron powder (5.6 g, 100 mmol) under argon atmosphere. The reaction was stirred at 70 °C for 2 h. The mixture was diluted with ethyl acetate (100 mL). The obtained solutions were washed with water (100 mL) and brine (100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Silica-gel column chromatography using petroleum ether/ethyl acetate (3:1) as eluent afforded **3** (248 mg, 76%) as a light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.23 (s, 1H), 6.67 (s, 1H), 5.65 (s, 2H), 5.52 (s, 2H), 1.44 (s, 9H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.4, 149.0, 146.5, 138.5, 123.8, 109.9, 93.0, 83.0, 57.5, 28.1 ppm. HRMS (ESI+): *m/z* 327.0450 $[M+H]^+$, Calculated for $C_{12}H_{16}BrN_4O_2$: 327.0457; found 327.0450.

*Synthesis of tert-butyl 2-(4-bromo-6-(methylamino)-2H-benzo[d][1,2,3]triazol-2-yl)acetate (**3a**)*

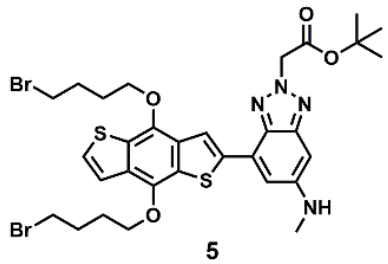


3a

To a solution of **3** (327 mg, 1 mmol) in anhydrous methanol (20 mL) were added dimethyl sulfate (252 mg, 2 mmol) and potassium carbonate (345 mg, 2.5 mmol) under argon atmosphere. The reaction mixture was stirred at 65 °C for 12 h and then quenched with ammonia (2 M). The mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate (50 mL), washed with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness in vacuo. Silica-gel column chromatography using petroleum ether/ethyl acetate (7:1) as

eluent afforded **3a** (220 mg, 65%) as a light yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (s, 1H), 7.61 (s, 1H), 5.33 (s, 2H), 2.87 (s, 3H), 1.48 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6, 144.3, 143.2, 141.6, 122.9, 112.4, 105.2, 84.2, 58.1, 31.1, 28.0 ppm. HRMS (ESI+): *m/z* 341.0608 $[M+H]^+$, Calculated for $C_{13}H_{18}BrN_4O_2$: 341.0613; found 341.0608.

Synthesis of ***5***



5

To a solution of **3a** (170 mg, 0.5 mmol) and **4** (392 mg, 0.6 mmol) in degassed THF (10 mL) was added $pd_2(dba)_3$ (23 mg, 0.025 mmol) under argon atmosphere. The reaction mixture was stirred at 65 °C for 24 h and concentrated in vacuo. The residue was dissolved in dichloromethane (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. Silica-gel column chromatography using petroleum ether/ethyl acetate (1:2) as eluent afforded **5** (285 mg, 76%) as a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (s, 1H), 7.48 (d, J = 4.48, 1H), 7.42 (d, J = 4.48, 1H), 7.16 (s, 1H), 6.75 (s, 1H), 5.44 (s, 2H), 4.39 (m, 4H), 3.62 (m, 4H), 2.97 (s, 3H), 2.30 (m, 4H), 2.10 (m, 4H), 1.54 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 147.8, 147.5, 144.8, 143.9, 138.9, 138.0, 132.5, 132.0, 130.4, 129.0, 126.4, 125.0, 121.4, 120.3, 118.7, 92.4, 83.5, 72.8, 72.7, 57.5, 33,7, 33.6, 31.0, 29.6, 29.2, 29.1, 28.1 ppm. HRMS (ESI+): *m/z* 753.0600 $[M+H]^+$, Calculated for $C_{31}H_{37}Br_2N_4O_4S_2$: 753.0603; found 753.0600.

Determination of fluorescence quantum yields

Quantum yields of DAPS and DANO (in methanol) were measured with coumarin 307 as a reference ($\Phi_{F(standard)}$ = 0.56 in ethanol).[1] The quantum yields, $\Phi_{F(sample)}$, were calculated according to Eq. 1 as following:

$$\Phi_{F(sample)} = \Phi_{F(standard)} \left(\frac{A_{standard}}{I_{standard}}\right) \left(\frac{I_{sample}}{A_{sample}}\right) \left(\frac{\eta_{sample}}{\eta_{standard}}\right)^2 \quad \text{Eq. 1}$$

where Φ_F is quantum yield, A the absorption intensity, I the integrated area under the emission spectra, and η the refractive index of the used solvent ($\eta_{ethanol}$ = 1.3614; $\eta_{methanol}$ = 1.3284).

Measurement of singlet oxygen generation efficiency

The relative singlet oxygen generation efficiency (Φ_Δ) was determined at 20 ºC using 1,3-diphenylisobenzofuran (DPBF) as a $^1O_2$ indicator and $[Ru(bpy)_3]Cl_2$ as a reference (Φ_Δ = 0.87 in MeOH).[2] DANO, DAPS or $[Ru(bpy)_3]Cl_2$ was dissolved in methanol containing 20 μM DPBF. Each of the prepared solutions was irradiated with an LED lamp (λ 435 nm, 1.5 mW cm^{-2}) at 10 s intervals. The oxidation of DPBF was monitored by the change of absorption spectra. As DANO, DAPS, and $[Ru(bpy)_3]Cl_2$ were tested with the same optical density (OD) at 435 nm, the Φ_Δs were calculated by Eq. 2:

$$\Phi_{\Delta,sample} = \Phi_{\Delta,standard} \left(\frac{K_{sample}}{K_{standard}}\right) \quad \text{Eq. 2}$$

where K represents the decomposition rates of DPBF at 410 nm.

Measurement of Two-Photon Cross Section

The two-photon cross section of sample ($\boldsymbol{\delta}_s$) in methanol was measured by the two-photon induced fluorescence method and a femto-second laser (AVESTA, TiF-100) was used as the excitation source. Rhodamine 6G was selected as the reference ($\boldsymbol{\delta}$ = 65 GM, at 800 nm in methanol).[3] The $\boldsymbol{\delta}$ value of DAPS was calculated according to Eq. 3:

$$\delta_s = \delta_r \left(\frac{S_s \Phi_r \phi_r C_r}{S_r \Phi_s \phi_s C_s} \right) \quad \text{Eq. 3}$$

where S represents the signal intensity of CCD detector, Φ represents fluorescence quantum yield, ϕ represents the overall fluorescence collection efficiency of the experimental apparatus, C represents the number density of the molecules in solution, and r represents the reference molecule (rhodamine 6G, $\Phi = 0.94$ in methanol[4]).

Cell culture

HeLa cells (American Type Culture Collection) were cultured in RPMI 1640 medium supplemented containing 10% fetal bovine serum (FBS, Biological Industries), ampicillin (50 U mL^{-1}) and streptomycin (50 U mL^{-1}) at 37 °C in a humidified incubator with 5% CO_2. Mitsubishi™ AnaeroPack-Anaero (Japan) gas generator was used for cultivation of cells under hypoxic environment. Oxygen indicator (SMART, China) was used to detect the oxygen levels in the chamber.

Confocal fluorescence imaging

HeLa cells were seeded in 4-well glass-bottomed plates (Φ = 24 mm) at a density of 8×10^4 cells per well and cultured for 24 h. After washing with PBS, DANO (5 μM) in fresh medium was added and incubated at 37 °C for 2 h. Then the cells were cultured with 100 nM Mito Tracker Red (Invitrogen) for 30 min, followed by rinse with PBS for three times. After that, the cells were irradiated for 1 min or 2 min with LED lamp (λ 405 nm, 20 mW cm^{-2}) before imaging by CLSM. Confocal fluorescence images of cells were obtained using a Zeiss laser scanning microscope 710 equipped with a 63× oil objective lens. The green fluorescence of products was excited at 405 nm, and collected at 470−540 nm. The red fluorescence of Mito Tracker Red was excited at 543 nm, and collected at 570−650 nm. Zen 2008 software (Carl Zeiss) was used for image acquisition and statistic analysis.

JC-1 assay

HeLa cells were seeded in 4-well glass-bottomed plates (Φ = 24 mm) at a density of 8×10^4 cells per well and cultured for 24 h. After washing with PBS, the cells were incubated with fresh medium containing DANO (0 or 5 μM) for 2 h. Then, the cells cultured with JC-1 dye (5 μg mL^{-1}, Mitochondrial Membrane Potential Assay Kit) for 30 min, followed by washed by PBS for three times. After that, cells were irradiated with LED light for 0 or 1 min (λ 405 nm, 20 mW cm^{-2}) before confocal fluorescence imaging. The green fluorescence of JC-1 monomer was excited at 488 nm, and collected at 510−540 nm. The red fluorescence of J-aggregates was excited at 543 nm, and collected at 580−620 nm.

Detection of intracellular superoxide radical

HeLa cells were seeded in 96 well plates at a density of 1×10^5 cells per well. The cells were incubated with DANO (0 or 5 μM) for 2 h followed by incubation with DHE (10 μM, in PBS) for another 30 min. After that, the cells were irradiated with LED light for 1 min (λ 405 nm, 20 mW cm^{-2}). The red fluorescence signal of cells was collected by an inverted fluorescent microscope.

Detection of intracellular nitric oxide

The detection of $^{\bullet}$NO was performed using the similar procedure described for the detection of superoxide radical except that DAF-FM DA (5 μM, in PBS) was used as the $^{\bullet}$NO-specific probe. The green fluorescence signal of cells was collected by an inverted fluorescent microscope.

Detection of intracellular singlet oxygen

The detection of $^1O_2$ was performed by using the similar procedure described for the detection of superoxide radical except that SOSG (5 μM) was used as $^1O_2$-specific probe. Standard maintenance medium (SMM) was applied to enhance cell permeability of SOSG.[5] The green fluorescence signal of cells was collected by an inverted fluorescent microscope.

Detection of intracellular peroxynitrite

The detection of $ONOO^-$ was performed using the similar procedure described for the detection of superoxide radical except that TPNIR-FP (2 μM) was used as the $ONOO^-$-specific probe. The red fluorescence of cells was collected by an inverted fluorescent microscope.

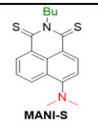
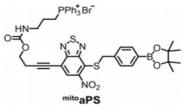
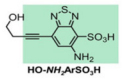
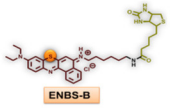
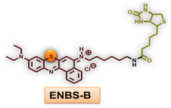
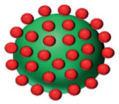
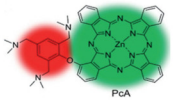
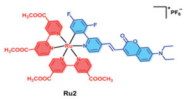
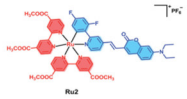
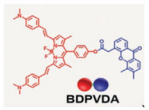
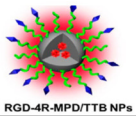
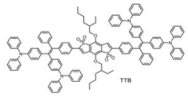
Selectivity assay

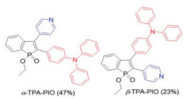
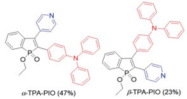
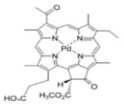
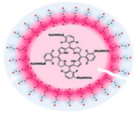
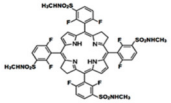
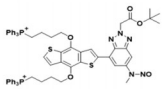
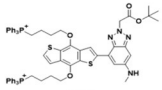
Selectivity assay was performed by recording fluorescence spectra of reaction mixture containing DANO (5 μM) and various analytes (200 μM) including NO_2^-, NO_3^-, SO_3^{2-}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, H_2O_2, ClO^-, glycine (Gly), glutamate (Glu), Arg (arginine), NADH, L-cysteine (Cys) and GSH after 2 min of LED light irradiation (λ 405 nm, 20 mW cm^{-2}). Fe^{3+}, Fe^{2+}, Zn^{2+}, and Cu^{2+} stock solutions (in HEPES) were prepared using the corresponding chlorine salt. The reaction mixture was prepared by mixing 20 μL of analyte solution (20 mM) and 1.98 mL of DANO (5 μM) solution in 10% acetonitrile–PBS or HEPES (pH 7.4).

Detection of hydrogen radical by 4-vinylbenzoic acid (PVBA)

Hydrogen radical was detected by hydrogenation reaction using 4-vinybenzoic acid (PVBA) as the substrate. The mixture containing DANO (1 mM), GSH (1 mM), and PVBA (10 mM) were irradiated with LED light for 0 or 1 min (λ 405 nm, 20 mW cm^{-2}), and subjected to HRMS measurement without further purification.

Table S1. Comparison of recently reported type I and dual type photosensitizers for PDT.[a]

References	Therapeutic agent	Activation	Photosensitizer	Reaction mechanism	$\lambda_{ex}/\lambda_{em}$ ($\lambda_{ex(TP)}$)	Φ_{Δ}	Targeted Organelle	OP-PDT	TP-PDT	Hypoxia-PDT
J. Yoon et al. *J. Am. Chem. Soc*. **2019**, 141,16243	 NanoMANI-S	Protein	 MANI-S	Type I & Type II	560 nm / NA (NA)	~1.0	–	0.5 μM, 560 nm (0.1 W cm^{-2}), 10 min, 11% cell viability (60 J cm^{-2})	–	0.5 μM, 560 nm (0.1 W cm^{-2}), 10 min, 13% cell viability (1% O_2, 60 J cm^{-2})
F. Feng et al. *Angew. Chem. Int. Ed.* **2020**, 59,12122	 ^{mito}aPS	GSH-H_2O_2	 HO-NH_2ArSO$_3$H	Type I & Type II	405 nm / 515 nm (800 nm)	0.59	Mitochondria	10 μM, 405 nm (30 mW cm^{-2}), 5 min, <10 % cell viability (9 J cm^{-2})	10 μM, 800 nm (~100 mW cm^{-2}), 120 s	10 μM, 405 nm (30 mW cm^{-2}), 6 min, <10% cell viability (1% O_2, 10.8 J cm^{-2})
X. Peng et al. *J. Am. Chem. Soc.* **2018**, 140,14851	 ENBS-B	–	 ENBS-B	Type I	660 nm / 690 nm (NA)	not detectable	Lysosome	0.63 μM, 660 nm, 12 J cm^{-2}, <10 % cell viability	–	0.63 μM, 660 nm, <10 % cell viability (2% O_2, 12 J cm^{-2})
J. Yoon et al. *Angew. Chem. Int. Ed.* **2018**, 57, 9885	 NanoPcA	–	 PcA	Type I	655 nm / NA (NA)	not detectable	–	50 nM, 655 nm (0.4 W cm^{-2}), 10 min, ~100% antibacterial effects (240 J cm^{-2})	–	–
W. Huang et al. *Chem. Sci.*, **2018**,9, 502	 Ru2	–	 Ru2	Type I & Type II	460 nm / 750 nm (NA)	0.16	Mitochondria	10 μM, white light (30 mW cm^{-2}), 10 min, <10 % cell viability (18 J cm^{-2})	–	–
X. Dong et al. *Small* **2020**, 16, 2001059	 PBV NPs	pH	 BDPVDA	Type I & Type II	730 nm / NA (NA)	NA	Lysosome	20 μg/mL, 730 nm (0.1 W cm^{-2}), 8 min, <10 % cell viability (48 J cm^{-2})	–	20 μg/mL, 730 nm (0.1 W cm^{-2}), 8 min, <10 % cell viability (2% O_2, 48 J cm^{-2})
B. Tang et al. *ACS Nano,* **2020**, 14, 854	 RGD-4R-MPD/TTB NPs	–	 TTB	Type I & Type II	510 nm / 696 nm (NA)	NA	–	10 μg/mL, white light (0.2 W cm^{-2}), 10 min, < 20 % cell viability (120 J cm^{-2})	–	–

B. Tang et al. *Chem. Sci.*, **2020**, 11, 3405		–		Type I & Type II	580 nm / 630 nm (NA)	NA	ER	10 μM, white light (20 mW cm^{-2}),30 min, <10 % cell viability (36 J cm^{-2})	–	–
A. Scherz et al. *J. Am. Chem. Soc* . **2005**, 127,6487	–	–		Type I & Type II	532 nm / NA (NA)	~1.0	–	–	–	–
J. M. Dąbrowski et al. *ACS Appl. Mater. Interfaces* **2016**, 8, 22039		–		Type I & Type II	740 nm / 750 nm (NA)	NA	Lysosome	5 μM, 735 nm LED , 0.32 J cm^{-2},5 min, <20 % cell viability	–	–
this work		GSH-Light		Type I & Type II	405 nm / 550 nm (800 nm)	0.42	Mitochondria	5 μM, 405 nm (20 mW cm^{-2}), 4 min, <10 % cell viability (1% O_2, 4.8 J cm^{-2})	5 μM, 800 nm (~100 mW cm^{-2}), 120 s	5 μM, 405 nm (20 mW cm^{-2}), 6 min, <10 % cell viability (1% O_2, 7.2 J cm^{-2})

[a] "NA" means "not available". "OP-PDT" and "two-photon PDT" denote "one-photon PDT" and "two-photon PDT", respectively.

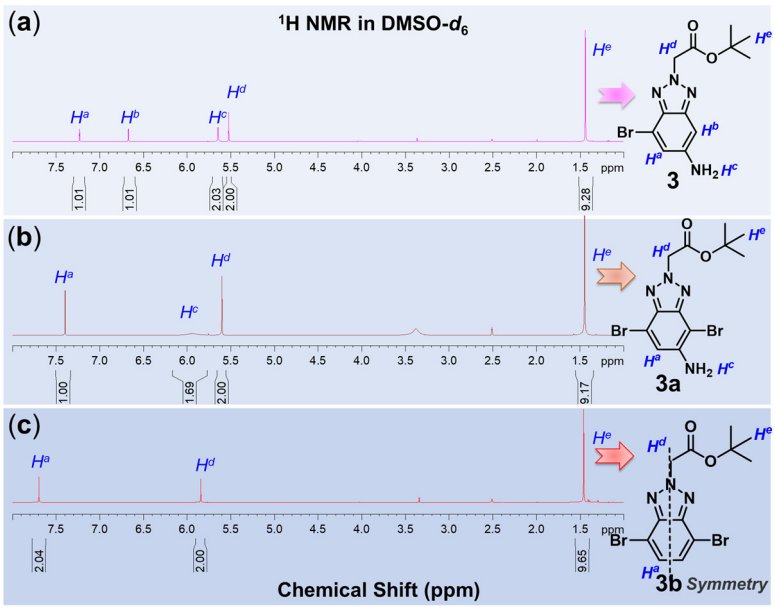


Figure S1. ^{1}H NMR spectra of **(a) 3**, **(b) 3a**, and **(c) 3b** in DMSO-d_6.

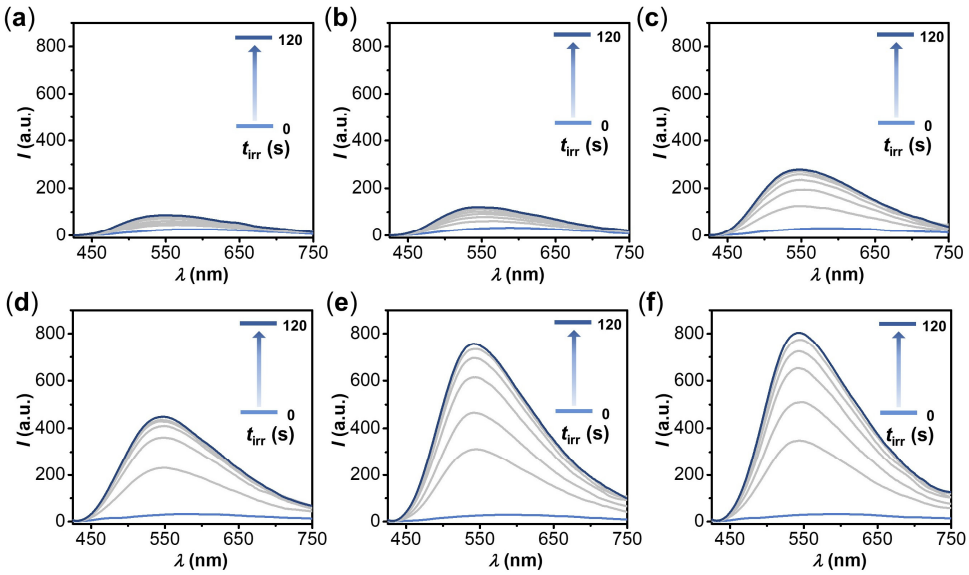


Figure S2. The t_{irr}–dependent fluorescence spectra of 10% acetonitrile-PBS solutions containing DANO (5 μM) in the presence of GSH. The concentrations of GSH were **(a)** 0 μM, **(b)** 5 μM, **(c)** 50 μM, **(d)** 100 μM, **(e)** 200 μM and **(f)** 500 μM. The solutions were irradiated by LED light (λ 405 nm, and 20 mW cm^{-2}) for 0−120 s.

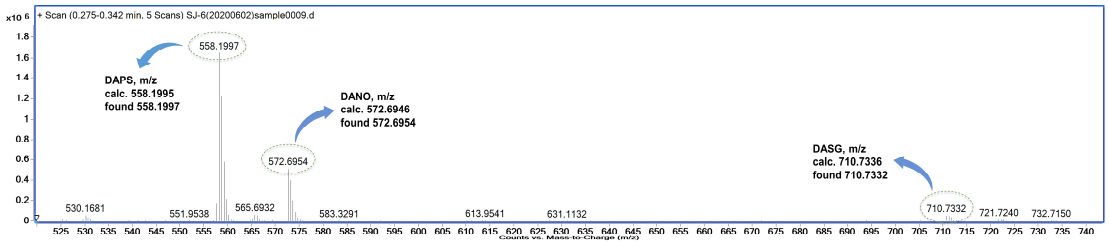


Figure S3. HRMS analysis of the reaction mixture containing DANO (5 μM) and GSH (200 μM) after LED light irradiation (λ 405 nm, 20 mW cm^{-2}, t_{irr} = 2 min).

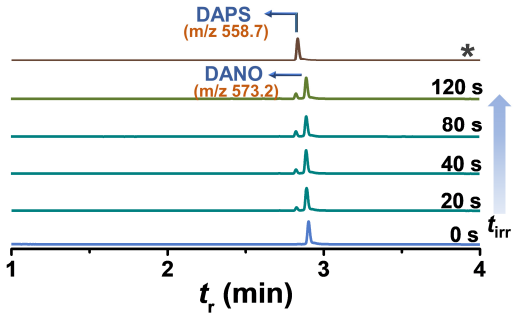


Figure S4. The UPLC spectra of solutions containing DANO (5 μM) in 10% acetonitrile-PBS buffer (pH 7.4) upon LED light irradiation (λ 405 nm, and 20 mW cm^{-2}, t_{irr} = 0−120 s). Reaction was monitored in the 370 nm channel by a PDA detector.

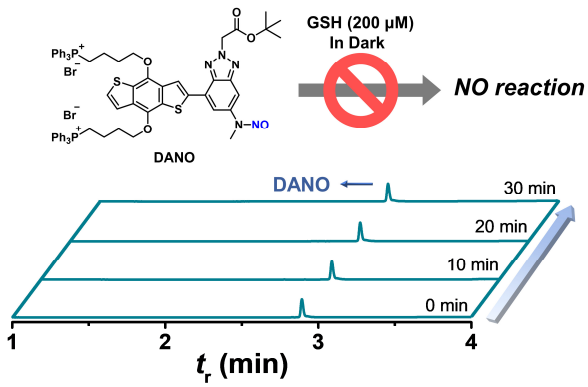


Figure S5. The UPLC spectra of reaction mixture containing DANO (5 μM) and GSH (200 μM) in 10% acetonitrile-PBS buffer (pH 7.4) in dark. The reaction was monitored over a period of 0−30 min, detected in the 370 nm channel by a PDA detector.

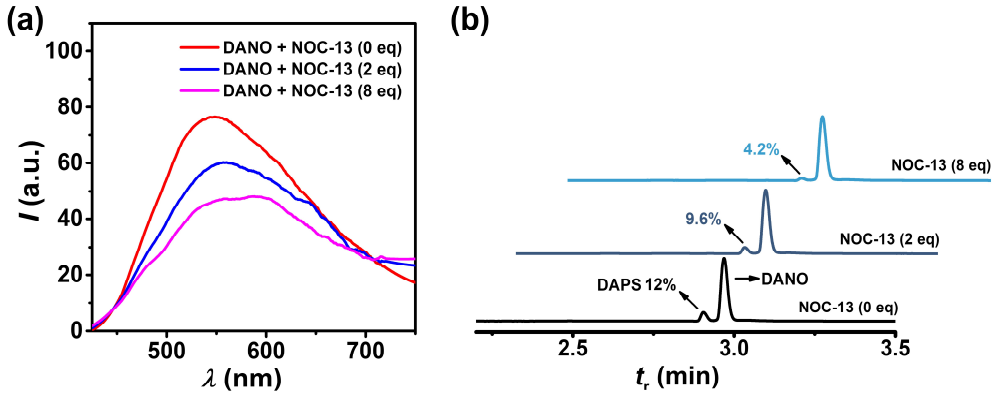


Figure S6. (a) Fluorescence spectra and (b) UPLC analysis of solutions containing DANO (5 μM) and NOC-13 (0, 2, or 8 eq.) upon LED light irradiation (1 min, λ 405 nm, 20 mW cm^{-2}).

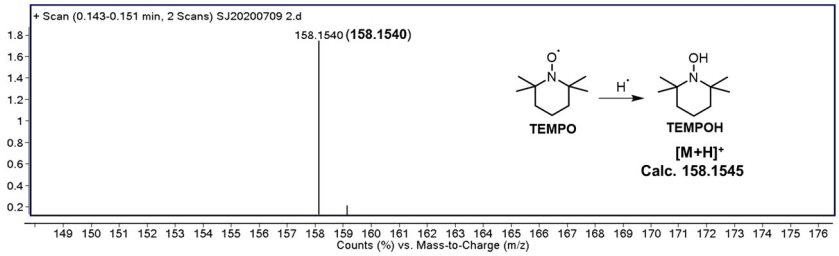


Figure S7. HRMS analysis of the reaction mixture containing TEMPO, DANO, and GSH (1 mM) after LED light irradiation (λ 405 nm, 20 mW cm^{-2}, t_{irr} = 1 min).

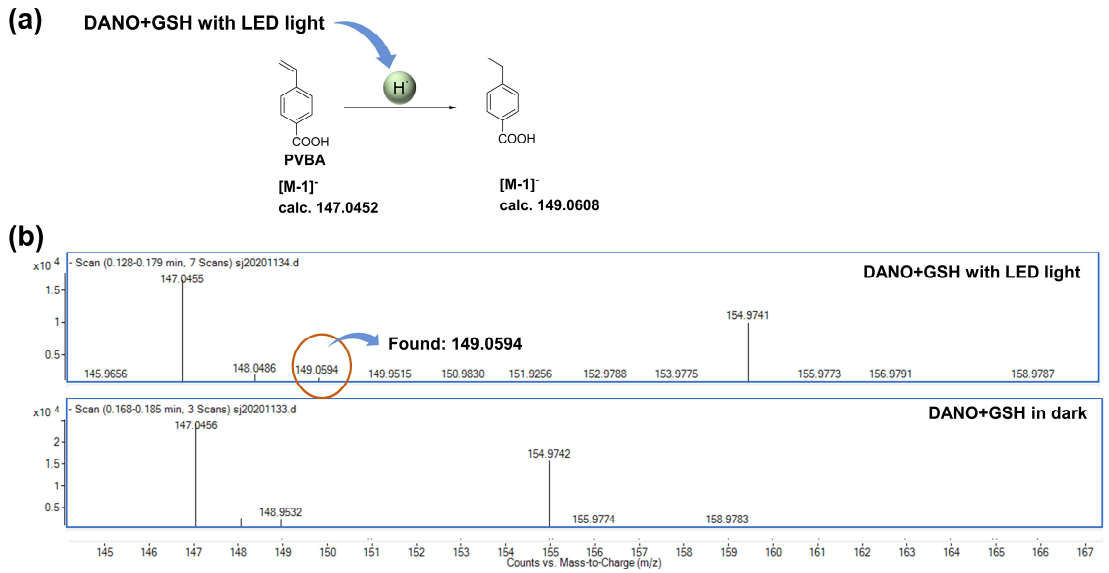


Figure S8. (a) Schematic illustration of H• detection using PVBA as trapper. (b) HRMS analysis of the reaction mixture containing DANO (1 mM), GSH (1 mM), and PVBA (10 mM) with or without 1 min LED irradiation (λ 405 nm, 20 mW cm^{-2}). The test solution for HRMS was diluted for 1000-fold.

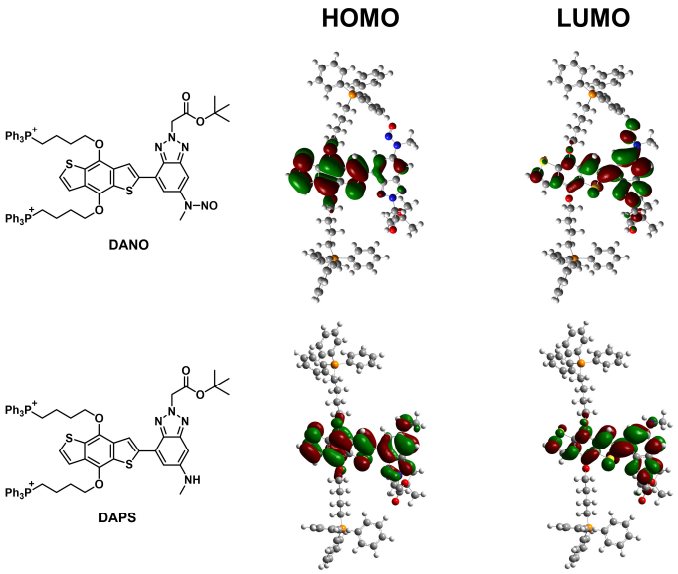


Figure S9. The frontier molecular orbitals of DANO and DAPS. Calculations were performed with the DFT method [B3LYP/6-31G (d)] using Gaussian 09 program.

Table S2. Selective parameters for the vertical excitation (UV-Vis absorptions) of DANO and DAPS on a basis of the optimized ground state geometries at a TDDFT/ B3LYP/6-31G (d) level.

Compound	States	ET	Energy, eV/λ nm	*f*	Composition	CI
DANO	Singlet State	$S_0 \rightarrow S_1$	2.87/433	0.0008	H→L	0.7019
		$S_0 \rightarrow S_2$	3.90/428	0.2383	H-3→L	0.5940
	Triplet State	$S_0 \rightarrow T_1$	2.06/603	0.0000	H→L	0.5527
		$S_0 \rightarrow T_3$	2.52/492	0.0000	H-2→L	0.2769
DAPS	Singlet State	$S_0 \rightarrow S_1$	2.98/415	0.5243	H→L	0.7024
		$S_0 \rightarrow S_2$	3.16/392	0.0044	H-1→L	0.6976
	Triplet State	$S_0 \rightarrow T_1$	1.99/623	0.0000	H→L	0.6512
		$S_0 \rightarrow T_3$	2.89/429	0.0000	H-1→L+7	0.1845

ET: Elecreonic transition
CI: Coefficient of the wavefunction for each excitations.

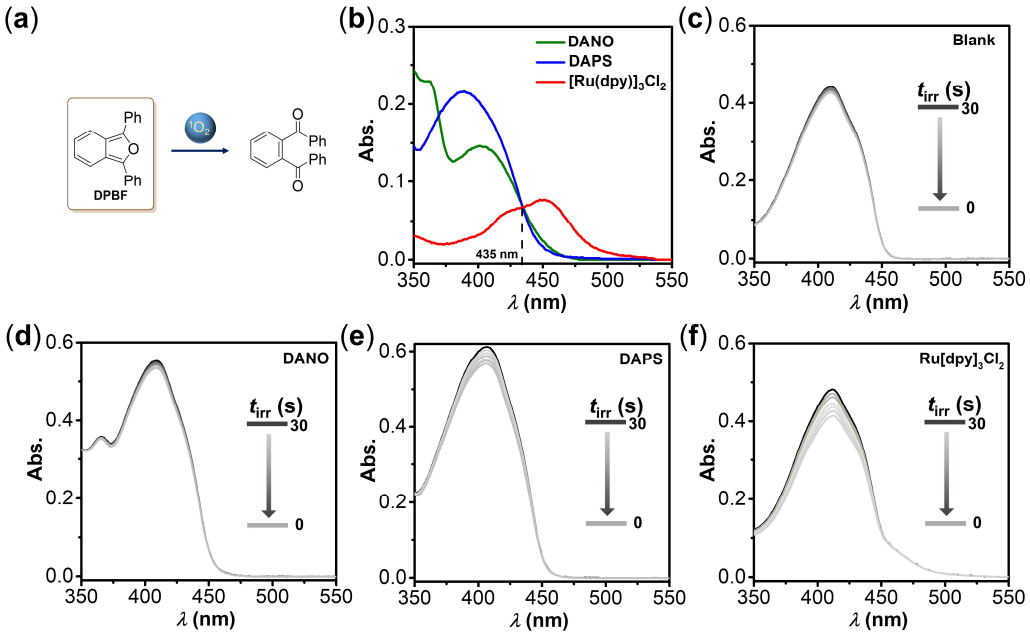


Figure S10. **(a)** Schematic illustration of mechanism of Φ_Δ examination using DPBF as a $^1O_2$ trapping agent. **(b)** UV−Vis absorption spectra of solutions containing DANO, DAPS, or $[Ru(bpy)_3]Cl_2$ in methanol, with the same OD value at 435 nm. Change of UV−Vis absorption spectra of methanol solutions containing DPBF (20 μM) and **(c)** blank, **(d)** DANO (20 μM), **€** DAPS (20 μM) and **(f)** $[Ru(bpy)_3]Cl_2$ (20 μM), upon LED light irradiation (λ 405 nm, and 1.5 mW cm^{-2}, t_{irr} = 0−30 s at 5 s intervals).

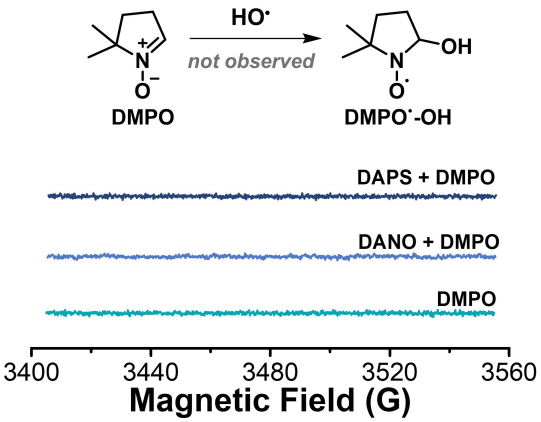


Figure S11. ESR spectra for the detection of •OH using DMPO (in 20% acetonitrile−water) as a spin trapper. The reaction mixture containing only DMPO (100 mM), DMPO (100 mM)/DANO (1 mM), or DMPO (100 mM)/DAPS (1 mM) upon LED light irradiation (λ 405 nm, 20 mW cm^{-2}, t_{irr} = 1 min).

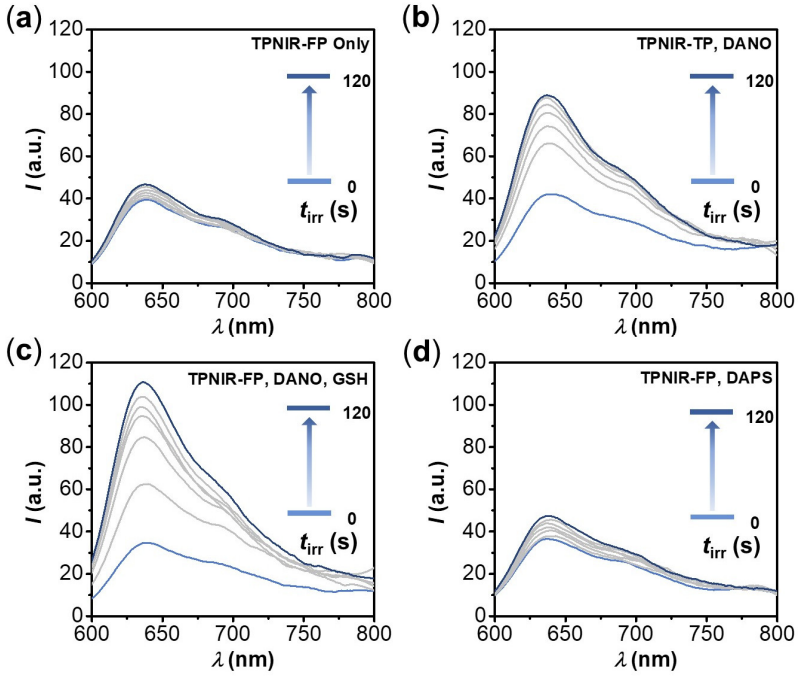


Figure S12. Fluorescence spectra of reaction mixture containing TPNIR-FP ($ONOO^-$ probe) and different substrates **(a)** blank, **(b)** DANO (5 μM), **(c)** DANO (5 μM) and GSH (200 μM), **(d)** DAPS (5 μM), after LED light irradiation (λ 405 nm, 20 mW cm^{-2}, t_{irr} = 0−120 s).

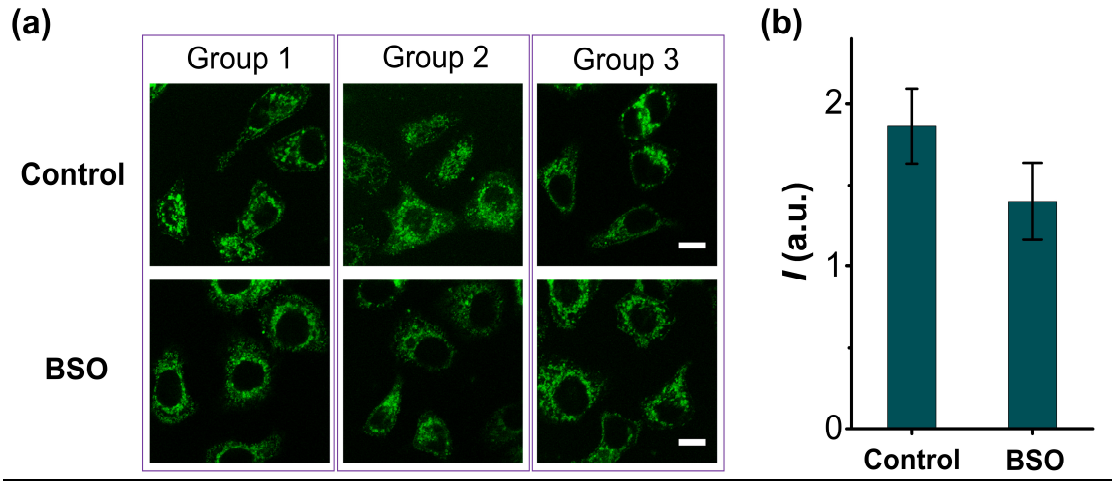


Figure S13. (a) CLSM images of HeLa cells treated by DANO (5 μM) after 1 min LED light irradiation (λ_{ex} 405 nm, 20 mW cm^{-2}). The cells were pretreated with PBS (control) or L-buthionine -(S,R)-sulfoximine (BSO, 10 μM) for 1 h. (b) the median fluorescence intensities derived from images in (a). Scale bars, 10 μm.

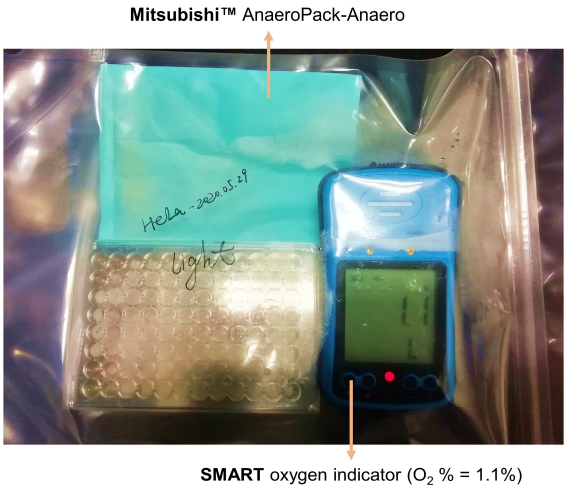


Figure S14. The photograph of Mitsubishi^{TM} device for mimicking hypoxic environment and measuring the content of O_2. For experiments under hypoxia, the content of O_2 was maintained at approximately 1%.

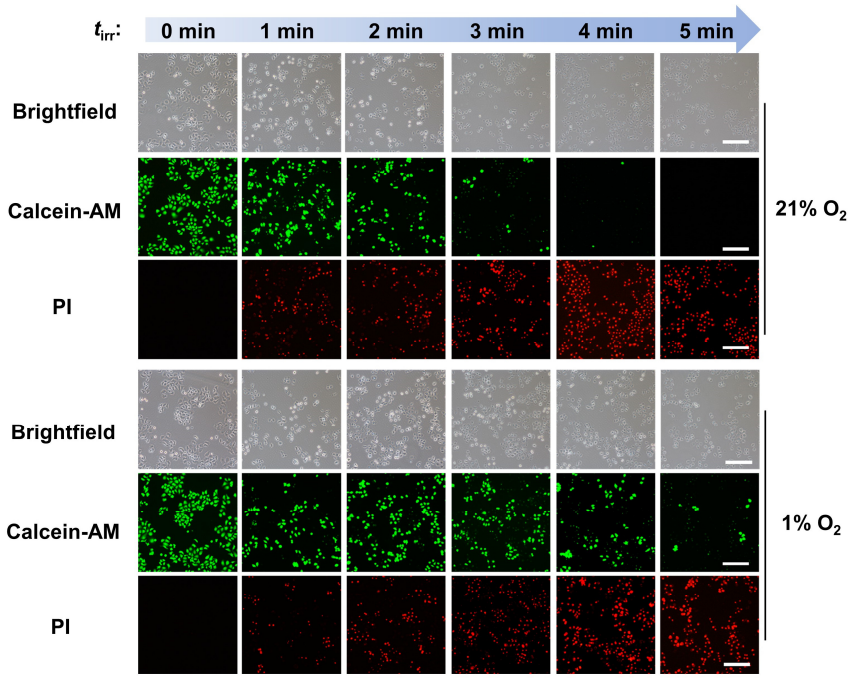


Figure S15. Brightfield and fluorescence images of DANO (5 μM)-treated HeLa cells after LED light irradiation (λ 405 nm, 20 mW cm^{-2}, t_{irr} = 0–5 min). The cells were stained with Calcein-AM/PI. Scale bars: 200 μm.

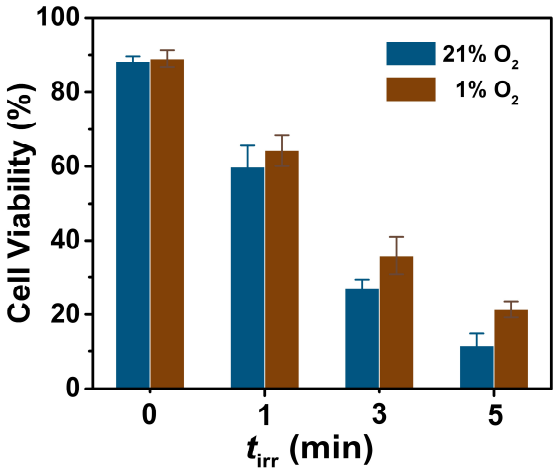


Figure S16. Viabilities of HeLa cells treated with DAPS (5 μM) under 21% or 1% O_2 condition, determined 24 h post LED light irradiation (λ 405 nm, 0, 1, 3, or 5 min, 20 mW cm^{-2}).

Structural characterization

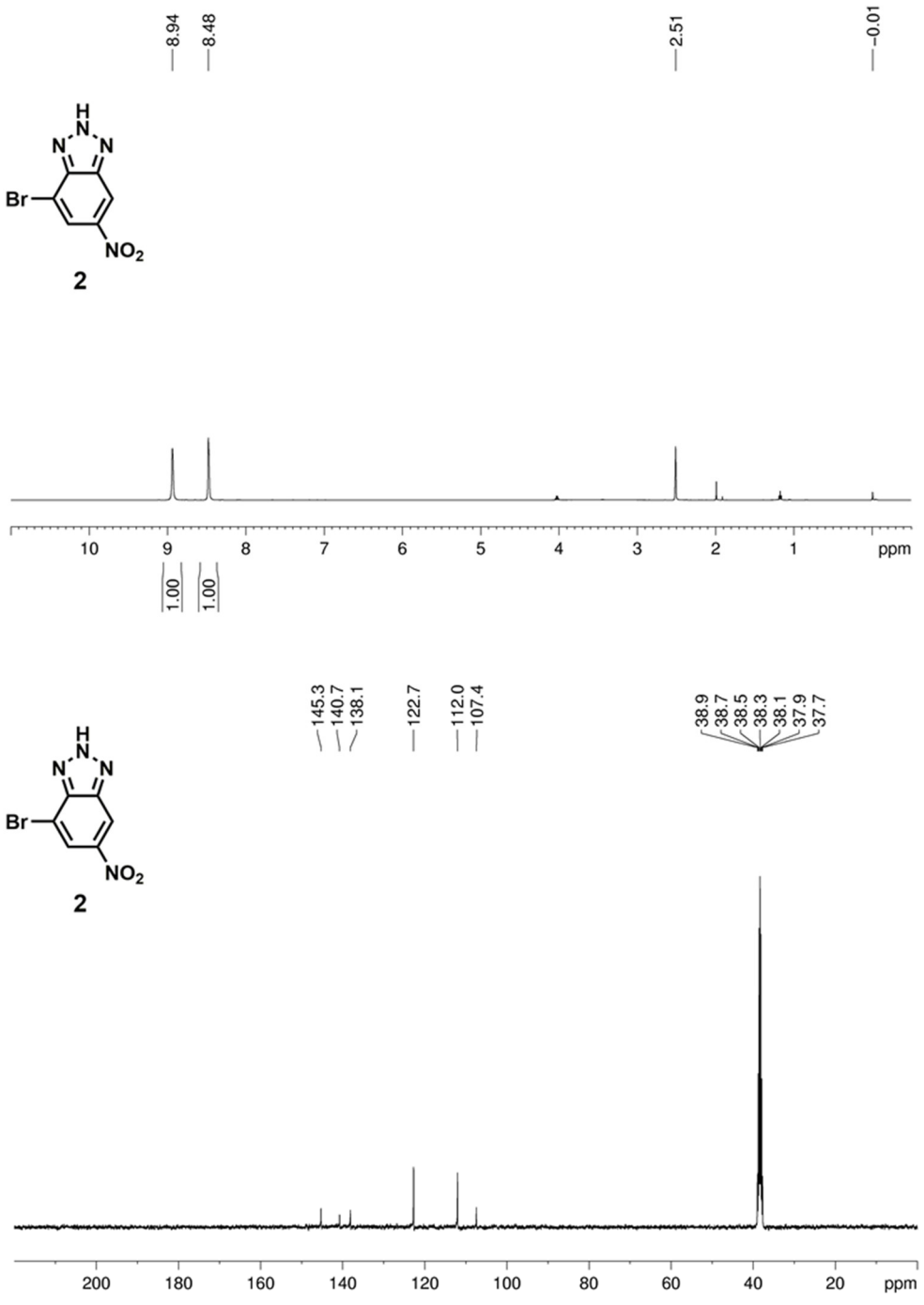


Figure S17. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **2** in DMSO-*d*6.

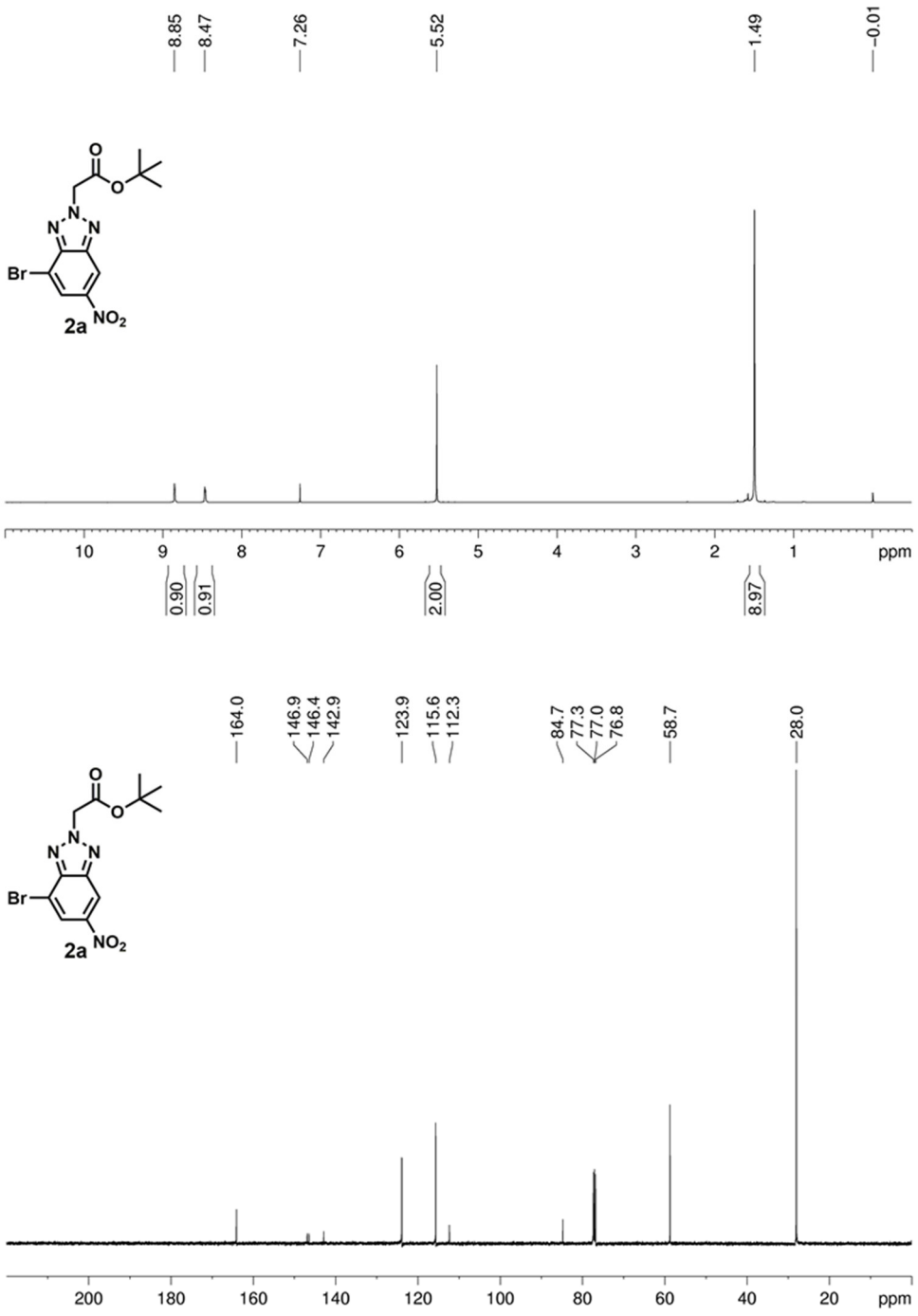


Figure S18. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **2a** in $CDCl_3$.

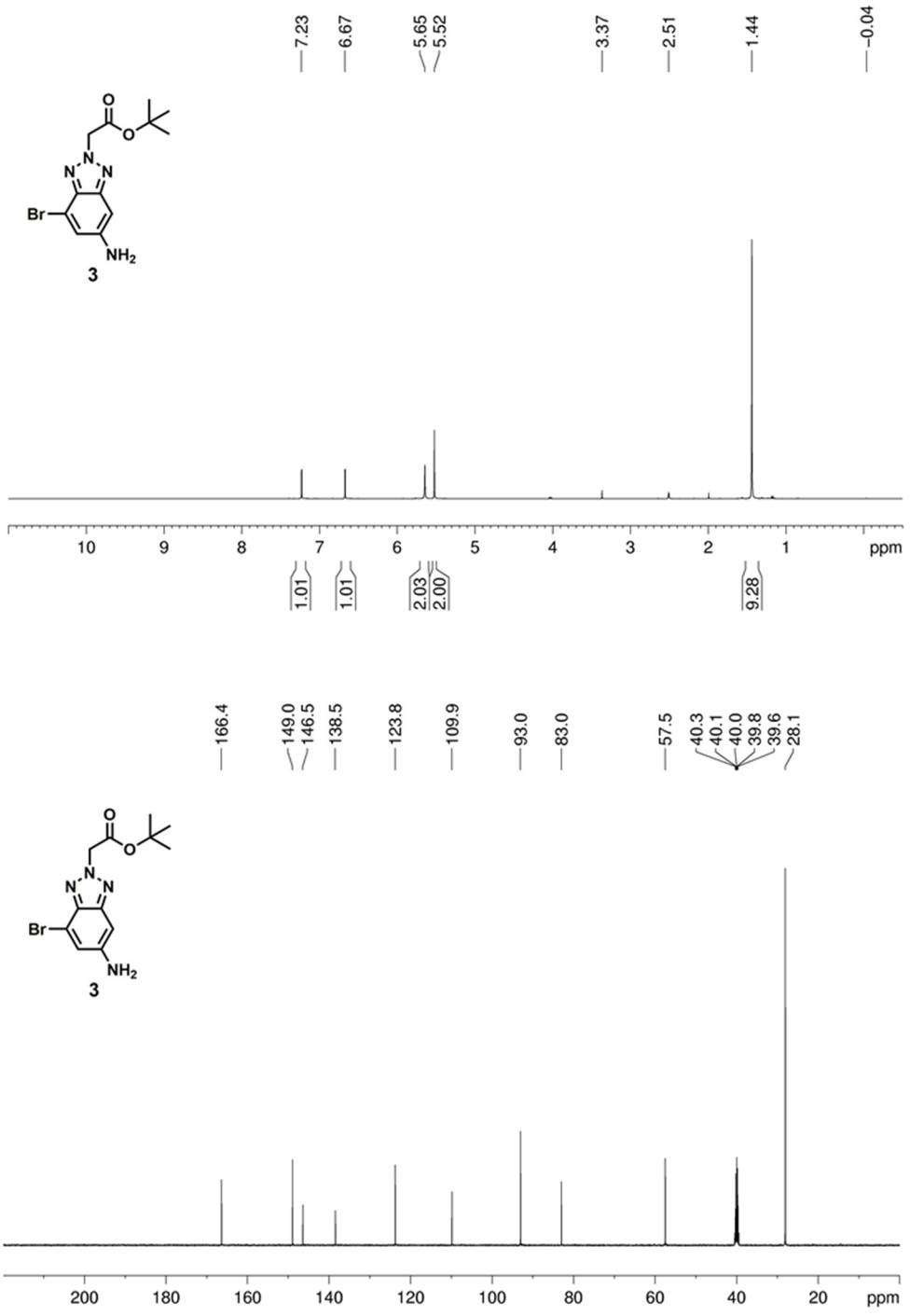


Figure S19. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **3** in DMSO-*d*6.

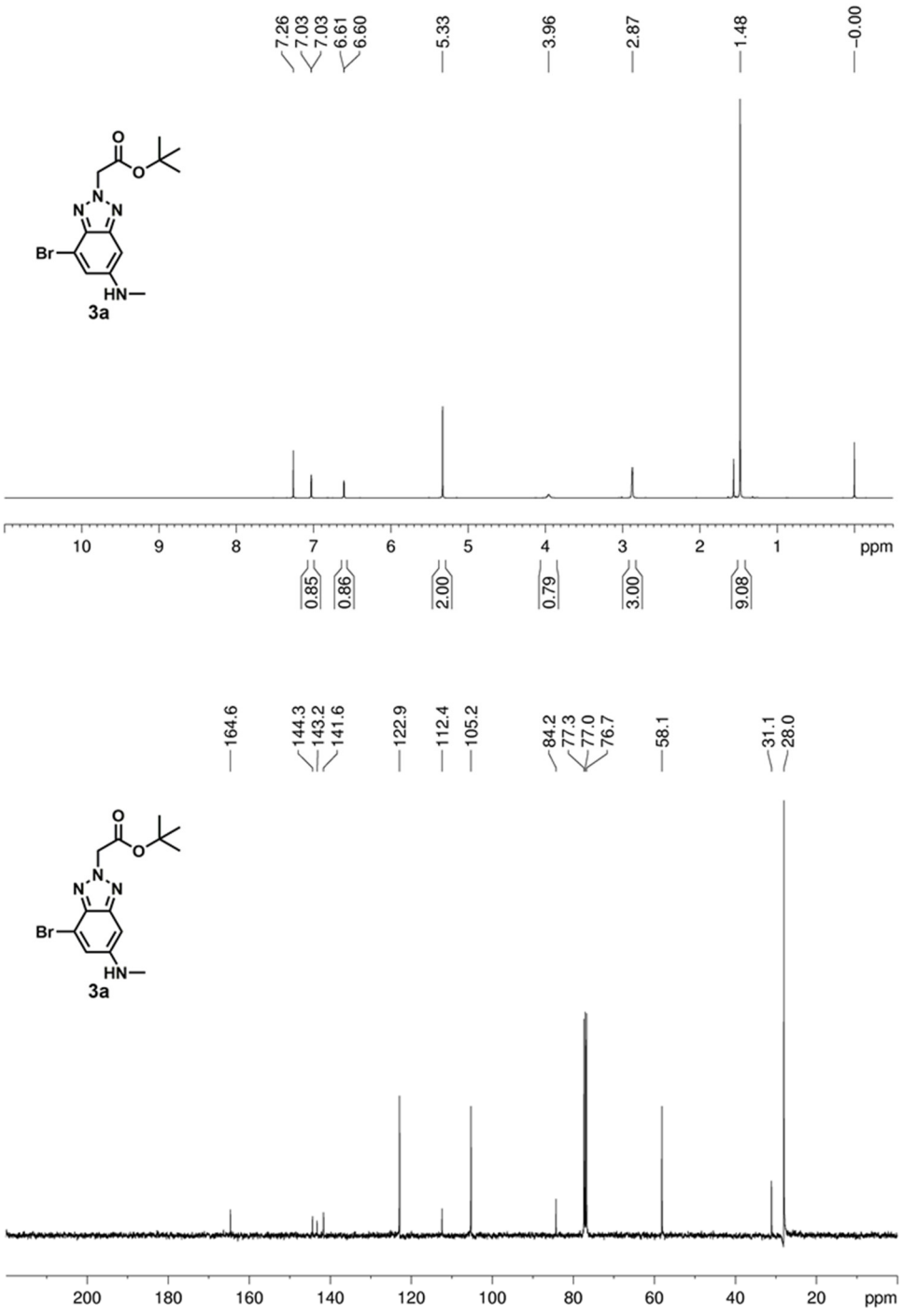


Figure S20. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **3a** in $CDCl_3$.

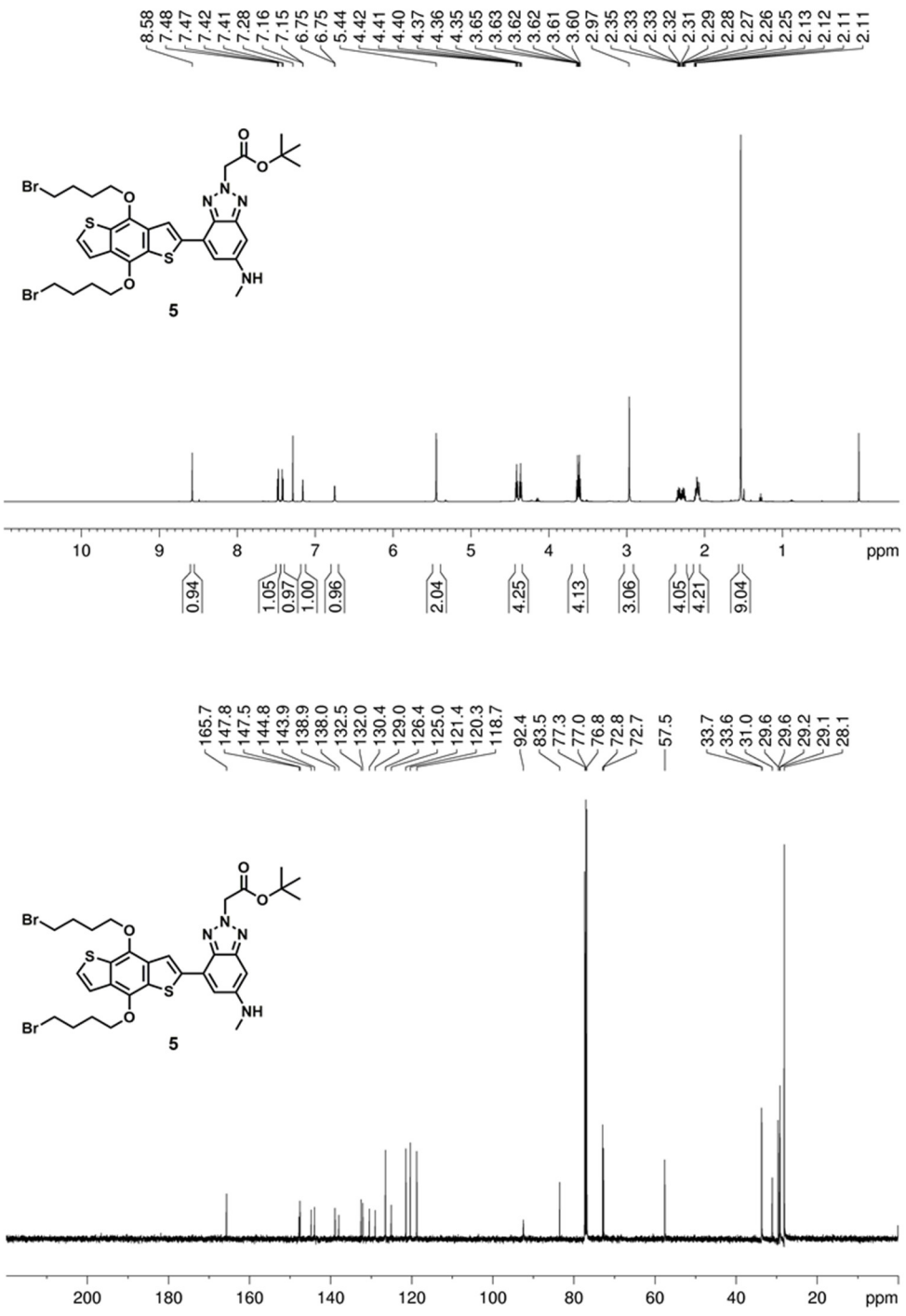


Figure S21. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **5** in $CDCl_3$.

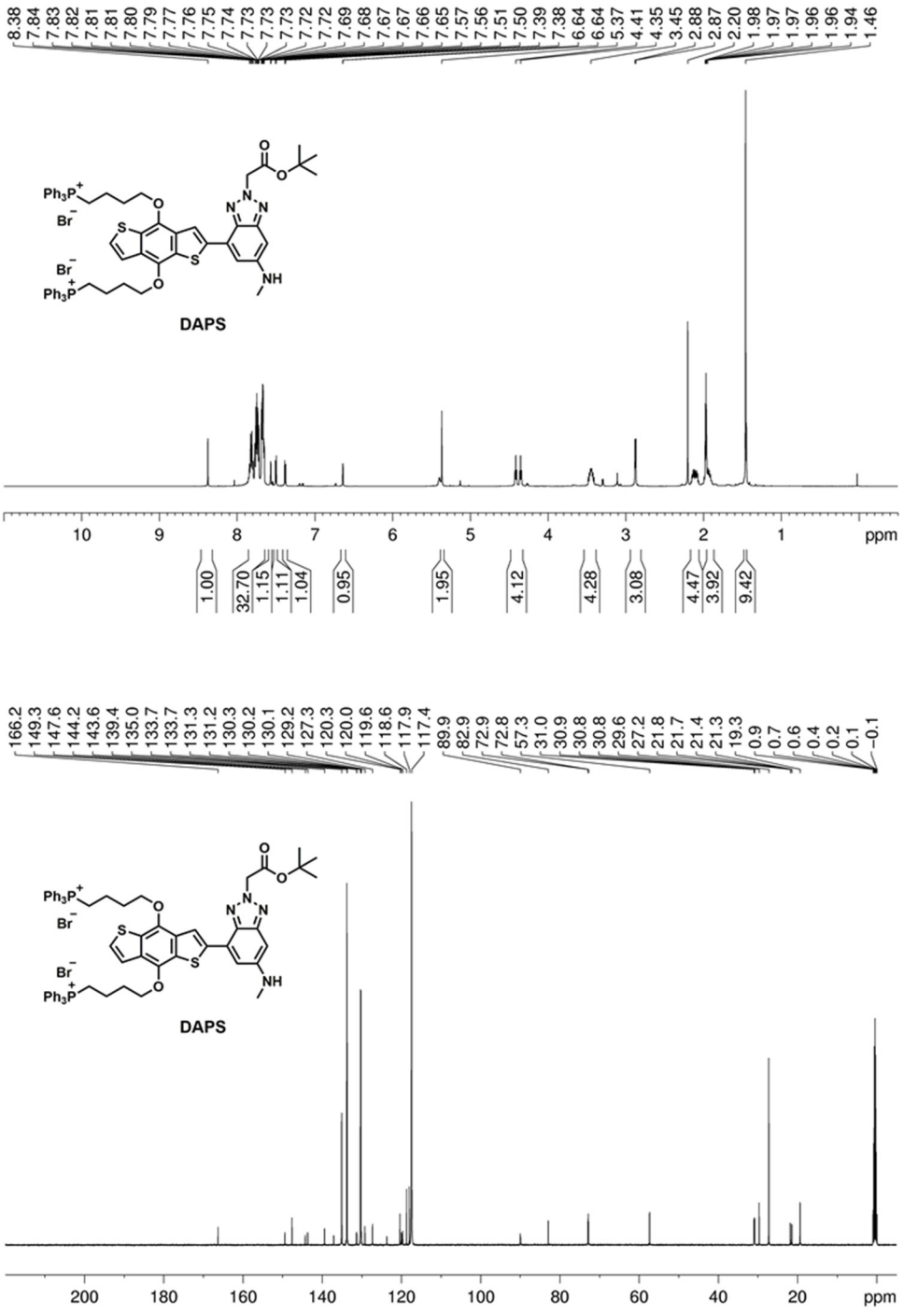


Figure S22. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of DAPS in CD_3CN.

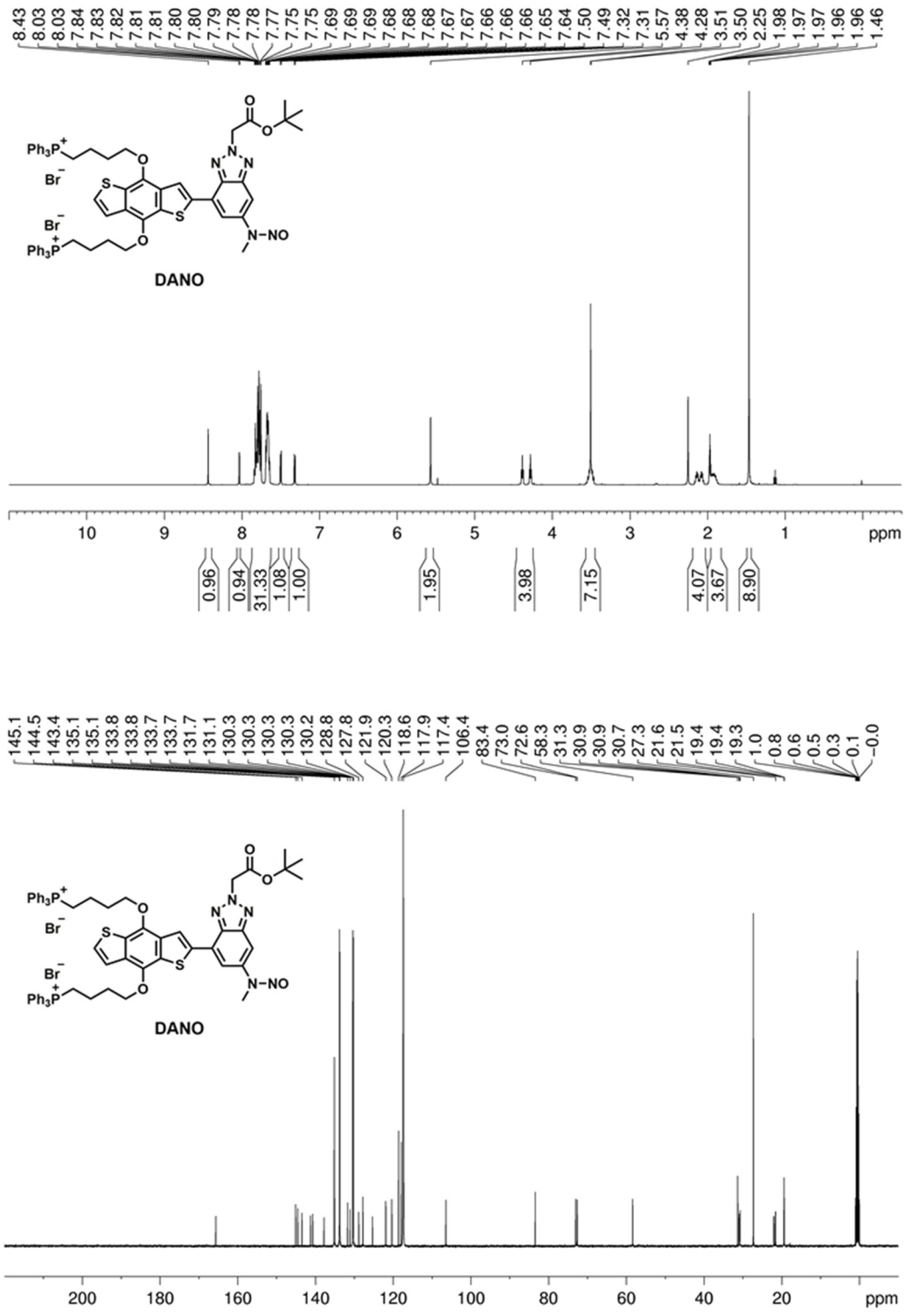


Figure S23. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of DANO in CD_3CN.

References

(1) Reynolds, G. A.; Drexhage, K. H. New coumarin dyes with rigidized structure for flashlamp-pumped dye lasers. *Opt. Commun.* **1975**, *13*, 222–225.

(2) Tanielian, C.; Wolff, C.; Esch, M. Singlet Oxygen Production in Water: Aggregation and Charge-Transfer Effects. *J. Phys. Chem.* **1996**, *100*, 6555–6560.

(3) Makarov, N. S.; Drobizhev, M.; Rebane, A. Two-photon absorption standards in the 550–1600 nm excitation wavelength range. *Opt. Express* **2008**, *16*, 4029–4047.

(4) Penzkofer, A. Phosphorescence quantum yield determination with time-gated fluorimeter and Tb(III)-acetylacetonate as luminescence reference. *Chem. Phys.* **2013**, *415*, 173–178.

(5) Hatz, S.; Lambert, J. D. C.; Ogilby, P. R. Measuring the lifetime of singlet oxygen in a single cell: addressing the issue of cell viability. *Photochem. Photobiol. Sci.* **2007**, *6*, 1106–1116.