

## Supporting Information

### **A Water Soluble Donor-Acceptor-Donor Conjugated Oligomer as a Photosensitizer for Mitochondria-Targeted Photodynamic Therapy**

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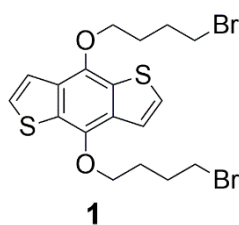
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## 1. General methods and materials

All the commercially available chemical reagents were used as received without further purification unless otherwise stated. Solvents used in Stille reactions were freshly distilled in appropriate methods. NMR spectra were recorded on a Bruker AMX 400 spectrophotometer with use of residual solvent or TMS as the internal reference. Low-resolution mass spectra were obtained with a Micromass GC-TOF mass spectrometer. High-resolution mass spectra (HRMS) were examined using an electrospray Agilent Q-TOF mass and reported as  $m/z$ . MALDI-TOF-MS was performed on a Bruker Daltonics autoflex speed analyzer with 2,5-dihydroxybenzoic acid (DHB) as the matrix. Ultraviolet-visible (UV-vis) spectra were taken on a Shimadzu UV-2600 spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 fluorimeter (PMT detector: 600 V, excitation and emission slit widths: 2.5 nm). The luminescence of  $^1\text{O}_2$  was determined on a FLS 980 time-resolved spectroscopy (Edinburgh, UK) equipped with a NIR detector.

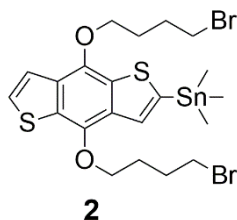
## 2. Synthesis and characterization



### *Synthesis of 4,8-bis(4-bromobutoxy)benzo[1,2-b;3,4-b]dithiophene (1)*

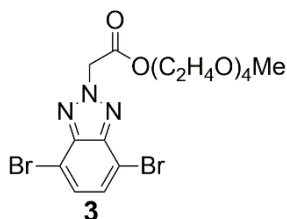
To a solution of 4,8-dihydrobenzo[1,2-b;3,4-b]dithiophen-4,8-dione (550 mg, 2.5 mmol) and zinc powder (487 mg, 7.5 mmol) in  $\text{H}_2\text{O}$  (20 mL) was added NaOH (1.2 g, 30 mmol). The reaction solution was stirred at reflux for 1 h under argon atmosphere. Then 1,4-dibromobutane (1.62 g, 7.5 mmol) and tetrabutylammonium bromide (TBAB) (0.25 mmol, 52 mg) were added and stirred for 3 h. The mixture was poured into cold water and extracted with  $\text{CH}_2\text{Cl}_2$  two times. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated to dryness in vacuo. Silica-gel column chromatography using petroleum ether/EtOAc (20:1) as eluent afforded **1** (824 mg,

67%) as a white solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.46 (d,  $J = 5.5$  Hz, 2H), 7.38 (d,  $J = 5.5$  Hz, 2H), 4.31 (m, 4H), 3.56 (m, 4H), 2.22 (m, 4H), 2.03 (m, 4H) ppm;  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  144.4, 131.5, 130.2, 126.4, 120.2, 72.8, 33.6, 29.6, 29.2 ppm. LRMS (EI): 492.0.



**Synthesis of 2-trimethyltin-4,8-bis(4-bromobutoxy)benzo[1,2-b;3,4-b]dithiophene (2)**

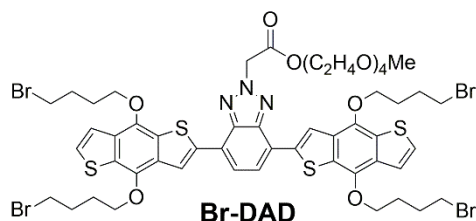
Compound **1** (492 mg, 1 mmol) and anhydrous THF (10 mL) were added into a flask under argon atmosphere. The reaction solution was cooled down to  $-78$  °C by a dry ice-acetone bath and *n*-butyllithium (0.55 mL, 1.1 mmol, 2 M in *n*-hexane) was added dropwise and stirred at  $-78$  °C for 1 h. Then trimethyltin chloride (1.5 mL, 1.5 mmol, 1.0 M in *n*-hexane) was added. The cooling bath was removed and the reaction mixture was allowed to warm to room temperature. After stirring for 4 h, the mixture was poured into cold water and extracted with *n*-hexane two times. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated to dryness in vacuo. Precipitation using isopropanol afforded **2** (537 mg, 82%) as a white solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.50 (s, 1H), 7.44 (d,  $J = 5.5$  Hz, 1H), 7.35 (d,  $J = 5.5$  Hz, 1H), 4.32 (m, 4H), 3.56 (m, 4H), 2.22 (m, 4H), 2.04 (m, 4H), 0.46 (s, 9H) ppm;  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  143.8, 143.5, 141.2, 134.3, 133.3, 131.1, 129.9, 127.6, 126.1, 120.3, 72.7, 72.5, 33.7, 33.6, 29.6, 29.2,  $-8.2$  ppm.



**Synthesis of 2,5,8,11-tetraoxatridecan-13-yl 2-(4,7-dibromo-2H-benzo[d][1,2,3]**

### *triazol-2-yl)acetate (3)*

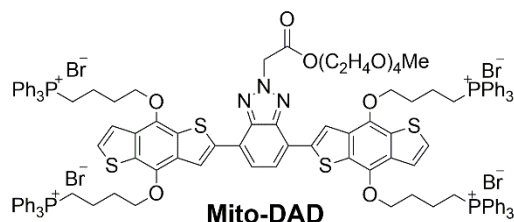
To a solution of 2-(4,7-dibromo-2*H*-benzo[d][1,2,3]triazol-2-yl)acetic acid<sup>[1]</sup> (333 mg, 1 mmol), dicyclohexylcarbodiimide (DCC) (227 mg, 1.1 mmol), and 4-dimethylaminopyridine (DMAP) (24 mg, 0.2 mmol) in dried CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added 3,6,9,12-Tetraoxatridecan-1-ol (250 mg, 1.2 mmol) under argon atmosphere. The reaction solution was stirred at 25 °C for 12 h and concentrated in vacuo. The residue was dissolved in EtOAc (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to dryness in vacuo. The residue was purified by silica column chromatography using petroleum ether/EtOAc (6:1) as eluent to afford **3** (410 mg, 78%) as a white solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.48 (s, 2H), 5.76 (s, 2H), 4.00 (m, 1H), 3.72 (m, 1H), 1.99–1.12 (m, 17H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 164.2, 152.8, 144.1, 130.1, 110.2, 59.4, 56.5, 50.4, 32.6, 30.8, 26.1, 25.3, 25.2, 24.7 ppm. LRMS (EI): 524.9.



### *Synthesis of Br-DAD*

To a solution of **2** (164 mg, 0.25 mmol) and **3** (53 mg, 0.1 mmol) in degassed THF (20 mL) were added Pd<sub>2</sub>(dba)<sub>3</sub> (20 mg) under argon atmosphere. The reaction solution was stirred at reflux for 16 h and concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with water (100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness in vacuo. Silica-gel column chromatography using petroleum ether/EtOAc (8:1) as eluent afforded **Br-DAD** (100 mg, 74%) as a deep yellow solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.58 (s, 2H), 7.79 (s, 2H), 7.48 (d, *J* = 5.5 Hz, 2H), 7.41 (d, *J* = 5.5 Hz, 2H), 5.91 (s, 2H), 4.39 (m, 8H), 4.13 (m, 1H), 3.72 (m, 1H), 3.62 (m, 8H), 2.30 (m, 8H), 2.09 (m, 8H), 1.98–1.14 (m, 17H) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 165.1, 153.0,

144.9, 144.0, 142.8, 138.9, 132.5, 130.4, 129.2, 126.6, 125.0, 124.6, 121.2, 120.4, 72.9, 72.7, 59.4, 56.6, 50.4, 34.0, 33.6, 32.7, 31.0, 29.6, 29.2, 29.1, 26.3, 25.3, 24.7 ppm. MALDI-TOF MS (+):  $m/z$  1348.0  $[M+H]^+$ .



### Synthesis of Mito-DAD

To a solution of Br-DAD (27 mg, 0.02 mmol) in degassed  $CH_3CN$  (10 mL) was added  $PPh_3$  (52 mg, 0.2 mmol) under argon atmosphere. The reaction solution was stirred at reflux for 48 h and concentrated in vacuo. Silica-gel column chromatography using DCM/MeOH (10:1) as eluent afforded Mito-DAD (41 mg, 86%) as a deep yellow solid;  $^1H$  NMR (400 MHz,  $CD_3CN$ ):  $\delta$  8.45 (s, 2H), 7.84–7.61 (m, 60H), 7.47 (s, 2H), 7.33 (d,  $J = 5.5$  Hz, 2H), 7.13 (d,  $J = 5.5$  Hz, 2H), 6.18 (s, 2H), 4.27 (m, 8H), 3.99 (m, 1H), 3.72 (m, 8H), 3.41 (m, 1H), 2.15 (m, 8H), 1.87 (m, 8H), 1.81–0.86 (m, 17H) ppm;  $^{13}C$  NMR (100 MHz,  $CD_3CN$ ):  $\delta$  162.7, 152.3, 143.9, 142.9, 141.8, 138.0, 134.7, 133.5, 133.4, 133.3, 131.2, 131.1, 129.9, 129.8, 129.6, 128.1, 126.7, 124.0, 123.4, 120.8, 119.8, 118.6, 118.5, 117.7, 117.6, 72.6, 72.1, 63.2, 59.3, 54.0, 50.4, 31.4, 30.6, 30.5, 30.4, 30.3, 25.2, 24.9, 24.7, 24.5, 21.4, 20.9, 19.0 ppm. HRMS (ESI+):  $m/z$  523.4415  $[M+H_2O]^{4+}$ , Calculated for  $C_{125}H_{123}N_3O_{11}P_4S_4^{4+}$ : 523.4242.

### 3. Measurement of fluorescence quantum yields

Fluorescence quantum yields of Br-DAD (in PhMe or MeOH) and Mito-DAD (in MeOH) were determined at room temperature (20 °C) using  $[Ru(bpy)_3]Cl_2$  as standard ( $\Phi = 0.028$  in  $H_2O$ ),<sup>[2]</sup> within an absorbance range of 0.01–0.05. The quantum yields were calculated based on Eq. 1:

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

where  $\Phi$  is quantum yield,  $A$  is absorption,  $I$  is luminescence intensity, and  $\eta$  is

refractive index of solvent.

#### 4. Measurement of singlet oxygen quantum yield

Singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) of Mito-DAD (in MeOH) was determined at room temperature (20 °C) using 1,3-diphenylisobenzofuran (DPBF) as  $^1\text{O}_2$  trapping agent and  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  as standard ( $\Phi_{\Delta} = 0.87$  in MeOH). Briefly, Mito-DAD or  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  was dissolved in MeOH containing 20  $\mu\text{M}$  DPBF. Each prepared solution was irradiated with a LED Lamp (460 nm, 1.5  $\text{mW}/\text{cm}^2$ ) in an interval of 5 s. The oxidation of DPBF was monitored by the change of absorption spectra. With Mito-DAD and  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  in test solution sharing the same OD in 460 nm, Singlet oxygen quantum yields could be calculated based on Eq. 2:

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

where  $K$  represents the degradation rate constants of DPBF at 410 nm.

#### 5. Measurement of intracellular ROS

The probe 2,7-Dichlorofluorescein diacetate (DCFH-DA) was used to measure the generation of intracellular ROS. 100  $\mu\text{L}$  of  $1.7 \times 10^4/\text{mL}$  HeLa cell suspension was seeded in each well of the 96 well plate and allowed to grow in 5%  $\text{CO}_2$  at 37 °C for 24 h. After rinse with PBS, the cells were incubated with Mito-DAD (5  $\mu\text{M}$ ) for 4 h and DCFH-DA (10  $\mu\text{M}$ ) for 20 min at 37 °C. The cells were washed with PBS and exposed to light irradiation for 2 min ( $\lambda_{\text{ex}}$  470 nm, 16  $\text{mW}/\text{cm}^2$ ) with a LED lamp. After irradiation, the green fluorescence intensity of cells was immediately measured by the inverted fluorescent microscope. The control experiment was carried out without irradiation treatment using the same procedure.

#### 6. Cell viability assay under normal condition

HeLa cell was seeded in the 96 well plate and allowed to grow for 24 h. Then the cells were washed with PBS and incubated in the fresh growth medium containing Mito-DAD at varying concentrations (0, 0.25, 0.5, 1, 2.5, and 5  $\mu\text{M}$ ). After incubation for 4 h, the cells were exposed to light irradiation for 3 min with a LED lamp. As

control, no light irradiation was applied. After incubation for 24 h in dark, the cells were washed twice with PBS, and then dispersed using Trypsin/Versene. After resuspended in fresh growth medium, the cells were treated with the same volume of 0.4% trypan blue in PBS for 5 min. Cell viability was determined using a hemocytometer chamber.

## **7. Cell viability assay under hypoxia condition**

HeLa cell was seeded in the 96 well plate and allowed to grow for 24 h. Then the cells were washed with PBS and incubated in the fresh growth medium containing Mito-DAD (0 or 5  $\mu\text{M}$ ) under 5%  $\text{O}_2$  (Anaerobic bag). After incubation for 4 h, the cells were exposed to light irradiation for 1-5 min with a LED lamp ( $\lambda_{\text{ex}}$  470 nm, 16  $\text{mW}/\text{cm}^2$ ). After incubation for 2 h in dark (5%  $\text{O}_2$ ), the cells were washed twice with PBS, and then dispersed using Trypsin/Versene. After resuspended in fresh growth medium, the cells were treated with the same volume of 0.4% trypan blue in PBS for 5 min. Cell viability was determined using a hemocytometer chamber.

## **8. Fluorescence imaging**

HeLa cells were seeded in 4-well glass-bottomed plates ( $\Phi = 24$  mm) at a density of  $8 \times 10^4$  cells per well. After incubation for 24 h, the cells were incubated in fresh growth medium containing 5  $\mu\text{M}$  Mito-DAD for 2 h or 4 h. Then the cells were washed twice with PBS, followed by incubation with 100 nM Mito Tracker (Invitrogen) for 25 min. Confocal fluorescence imaging experiments were performed with a Zeiss laser scanning microscope 710 with a 63 $\times$  oil objective lens, using Zen 2008 software (Carl Zeiss). Mito-DAD was excited by a 458 nm diode laser, with emission collected between 580 and 750 nm. Mito Tracker was excited by a 488 nm diode laser, with emission collected between 500 and 540 nm.

## **9. JC-1 staining**

HeLa cells were treated with 5  $\mu\text{M}$  Mito-DAD for 4 h in dark. Then the cells were washed twice with PBS. After irradiation by a LED lamp (0 or 2 min,  $\lambda_{\text{ex}}$  470 nm; 16  $\text{mW}/\text{cm}^2$ ), the cells were cultured for 30 min. Then the cells were washed twice with PBS, followed by addition of JC-1 (5  $\mu\text{g}/\text{mL}$ ) in 1 $\times$  incubation buffer. The



cells were incubated for 20 min. After multiple rinse with PBS, the cells were imaged by an inverted fluorescent microscope. As control, HeLa cells were treated with CCCP (10  $\mu$ M) for 30 min. Then the cells were stained with JC-1 and imaged using the same procedure.

## **10. Live/dead staining**

HeLa cells were treated with 5  $\mu$ M Mito-DAD for 4 h in dark. Then the cells were washed twice with PBS. After irradiation by a LED lamp ( $\lambda_{\text{ex}}$  470 nm, 16 mW/cm<sup>2</sup>), the cells were cultured for 2 h or 24 h. Then the cells were washed twice with PBS, followed by addition of calcein-AM (1.6  $\mu$ g/ml) and PI (1.6  $\mu$ g/ml) in PBS. The cells were incubated for 20 min. After multiple rinse with PBS, the cells were imaged by an inverted fluorescent microscope.

## **11. In vitro Two-photon photodynamic therapy**

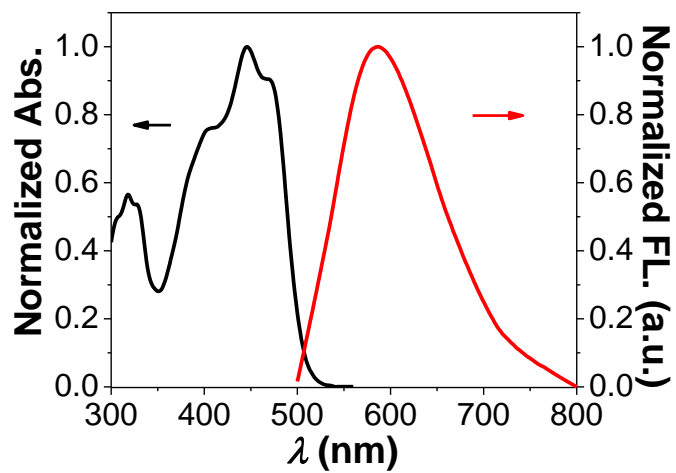
HeLa cells were seeded in 4-well glass-bottomed plates ( $\Phi = 24$  mm) at a density of  $8 \times 10^4$  cells per well. After incubation for 24 h, the cells were incubated in fresh growth medium containing 5  $\mu$ M Mito-DAD for 4 h. Then the cells were washed twice with PBS, followed by addition of fresh growth medium. Two-photon (TP) imaging experiments were performed with an Olympus FV1000MPE laser scanning microscope with a 40 $\times$  objective lens, using FV10-ASW 3.1 Viewer software. Mito-DAD was excited by an 800 nm femtosecond Ti-Sapphire laser ( $\sim 4$  W at 800 nm;  $\sim 1\%$  average power in the focal plane), with emission collected between 580 and 750 nm.

## 12. Supplementary table and figures

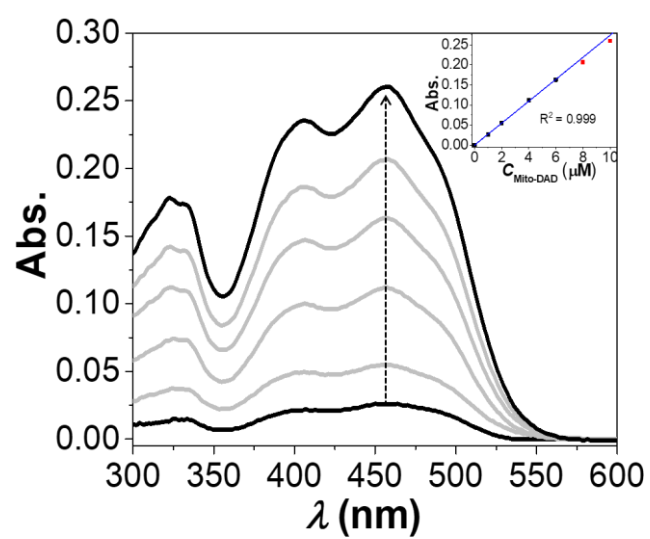
**Table S1.** Brief comparison of the present work with presentative published approaches on PDT activated by photosensitizers absorbing visible light.

Presentative work	PS	$\lambda_{\text{abs}}/\lambda_{\text{em}} (\lambda_{\text{ex}}(\text{TP}))$	$\Phi_{\Delta}$	Targeted Organelle	OP-PDT	TP-PDT	Hypoxia-PDT
Chao et al. <i>Angew. Chem. Int. Ed.</i> <b>2015</b> , <i>54</i> , 14049	Ru1-Ru3	450 nm/600 nm (800 nm)	0.92-0.99 (Methanol)	Lysosome	(2 $\mu\text{M}$ ) 450 nm, 10 J/cm <sup>2</sup> ; IC <sub>50</sub> 1.5 $\mu\text{M}$	—	—
Huang et al. <i>Angew. Chem. Int. Ed.</i> <b>2016</b> , <i>55</i> , 9947	Ir-P(ph)3	455 nm/590 nm	0.17 (DMF)	Mitochondria	(5 $\mu\text{M}$ ) 470 nm, 22 mW/cm <sup>2</sup> , 30 min; 2.3% cell viability (39.6 J/cm <sup>2</sup> )	—	5% O <sub>2</sub> (5 $\mu\text{M}$ ) 470 nm, 22 mW/cm <sup>2</sup> , 30 min; 3.3% cell viability
	Ir-alkyl	455 nm/600 nm	0.21 (DMF)	Lysosome	(5 $\mu\text{M}$ ) 470 nm, 22 mW/cm <sup>2</sup> , 30 min; ~60% cell viability (39.6 J/cm <sup>2</sup> )	—	5% O <sub>2</sub> (5 $\mu\text{M}$ ) 470 nm, 22 mW/cm <sup>2</sup> , 30 min; ~80% cell viability
Liu et al. <i>Chem. Sci.</i> <b>2015</b> , <i>6</i> , 4580	TPECM-2TPP	405 nm/610 nm	—	Mitochondria	(20 $\mu\text{M}$ ) white light, 100 mW/cm <sup>2</sup> , 8 min; ~25% cell viability (48 J/cm <sup>2</sup> )	—	—
Weil et al. <i>J. Am. Chem. Soc.</i> <b>2017</b> , <i>139</i> , 2512	cHSA-PEO-TPP-Ru	460 nm/617 nm (810 nm)	—	Mitochondria	(~1 $\mu\text{M}$ ) 470 nm, 20 mW/cm <sup>2</sup> , 5 min; ~1% cell viability (6 J/cm <sup>2</sup> )	—	—
Dong et al. <i>Chem. Sci.</i> <b>2018</b> , <i>9</i> , 2188	DPPBDPI (D- $\pi$ -A- $\pi$ -D)	540 nm/570 nm	0.8 (DCM)	Lysosome	(0.15 $\mu\text{M}$ ) Xenon lamp, 40 mW/cm <sup>2</sup> , 8 min; ~15% cell viability (19.2 J/cm <sup>2</sup> )	—	—
Tang et al. <i>chem. Sci.</i> <b>2018</b> , <i>9</i> , 5165	Azabenzan-throne derivative	486 nm/618 nm (aggr)	0.129 (aggr)	Lysosome	(10 $\mu\text{M}$ ) 525 nm, 1.67 mW/cm <sup>2</sup> , 20 min; ~1% cell viability (2 J/cm <sup>2</sup> )	—	—
This work	Mito-DAD	455 nm/652 nm (800 nm)	0.64 (21% O <sub>2</sub> , Methanol); 0.52 (5% O <sub>2</sub> , Methanol)	Mitochondria	(5 $\mu\text{M}$ ) 470 nm, 16 mW/cm <sup>2</sup> , 3 min; 5.2% cell viability (2.9 J/cm <sup>2</sup> )	4 W at 800 nm, 5 min	5% O <sub>2</sub> (5 $\mu\text{M}$ ) 470 nm, 16 mW/cm <sup>2</sup> , 5 min; 6.6% cell viability (4.8 J/cm <sup>2</sup> )

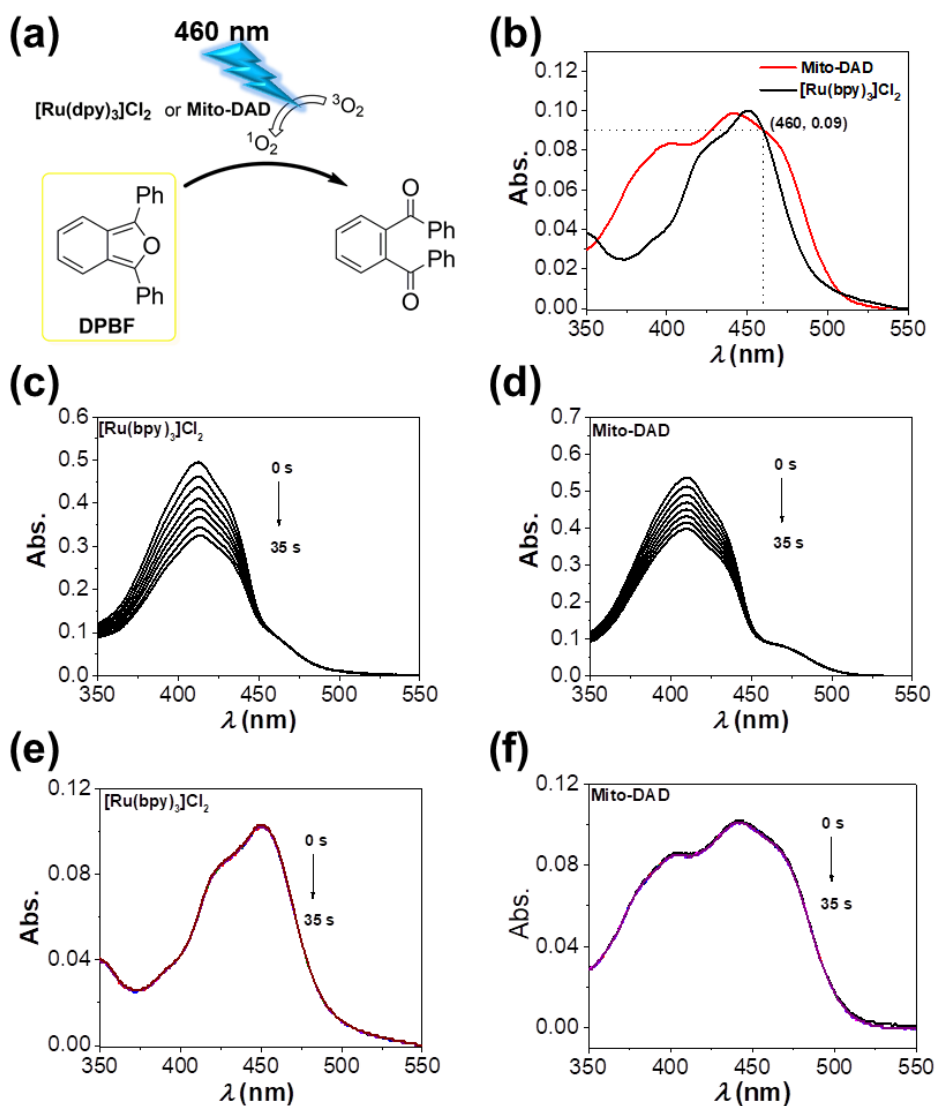
“—” means “unavailable”.



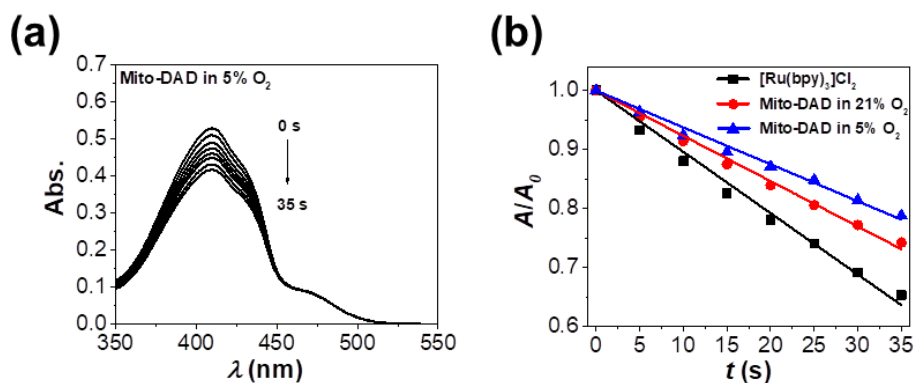
**Fig. S1** Normalized UV-vis absorption and emission spectral of Br-DAD in toluene.



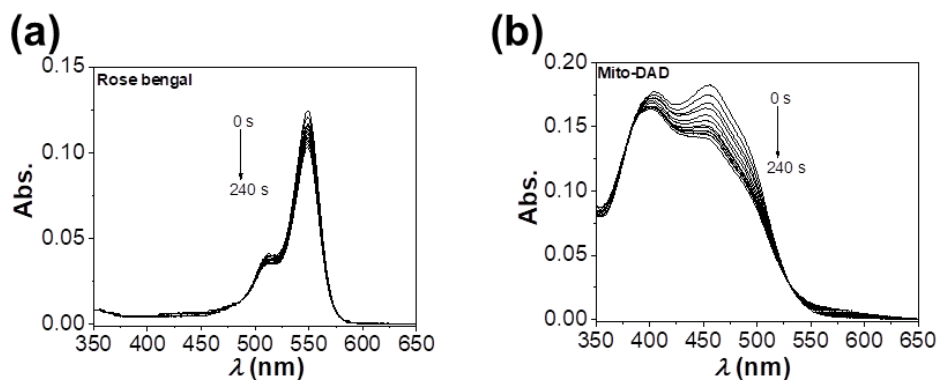
**Fig. S2** UV-vis spectra of Mito-DAD (1.0~10  $\mu\text{M}$ ) in PBS. Insert: plot of absorption intensity at 455 nm as a function of Mito-DAD concentration.



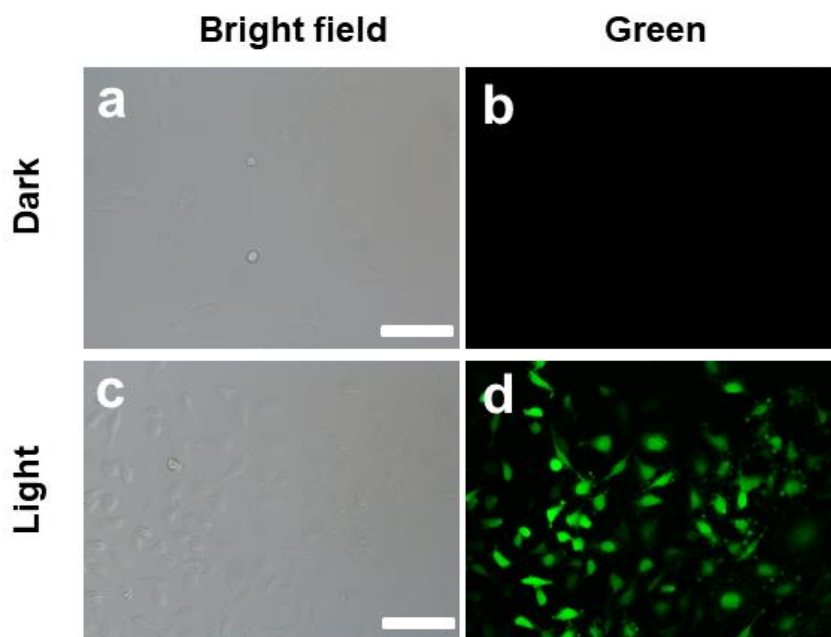
**Fig. S3** (a) Mechanism of  $\Phi_{\Delta}$  examination using DPBF as a  $^1\text{O}_2$  trapping agent. (b) UV-vis absorption spectra of solutions containing  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  or Mito-DAD in MeOH in the absence of DPBF. Change of UV-vis absorption spectra of DPBF (20  $\mu\text{M}$ ) in the presence of (c)  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  or (d) Mito-DAD in MeOH upon irradiation with LED light ( $\lambda_{\text{ex}}$  460 nm, 1.5  $\text{mW}/\text{cm}^2$ ) at 5 s intervals. UV-vis absorption spectra of (e)  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  and (f) Mito-DAD in MeOH were monitored after various periods of LED light irradiation ( $\lambda_{\text{ex}}$  460 nm, 1.5  $\text{mW}/\text{cm}^2$ ) at 5 s intervals.



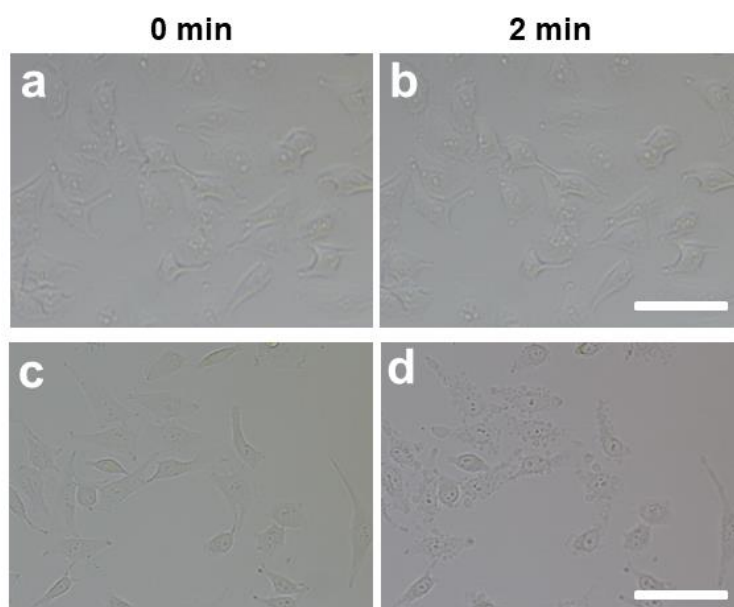
**Fig. S4** (a) Change of UV-vis absorption spectra of DPBF (20  $\mu\text{M}$ ) in the presence of Mito-DAD in MeOH upon irradiation with LED light ( $\lambda_{\text{ex}}$  460 nm, 1.5  $\text{mW}/\text{cm}^2$ ) at 5 s intervals under 5%  $\text{O}_2$ . (b) Plot of  $A/A_0$  as a function of irradiation time.  $A_0$  and  $A$  denote the optical intensity of DPBF in the beginning and under varying time of light exposure ( $\lambda_{\text{ex}}$  460 nm, 1.5  $\text{mW}/\text{cm}^2$ ), respectively, in the presence of Mito-DAD in 5%  $\text{O}_2$ -equilibrated methanol.  $\text{Ru}(\text{bpy})_2\text{Cl}_2$  in methanol and Mito-DAD in 21%  $\text{O}_2$ -equilibrated methanol were shown for comparison.



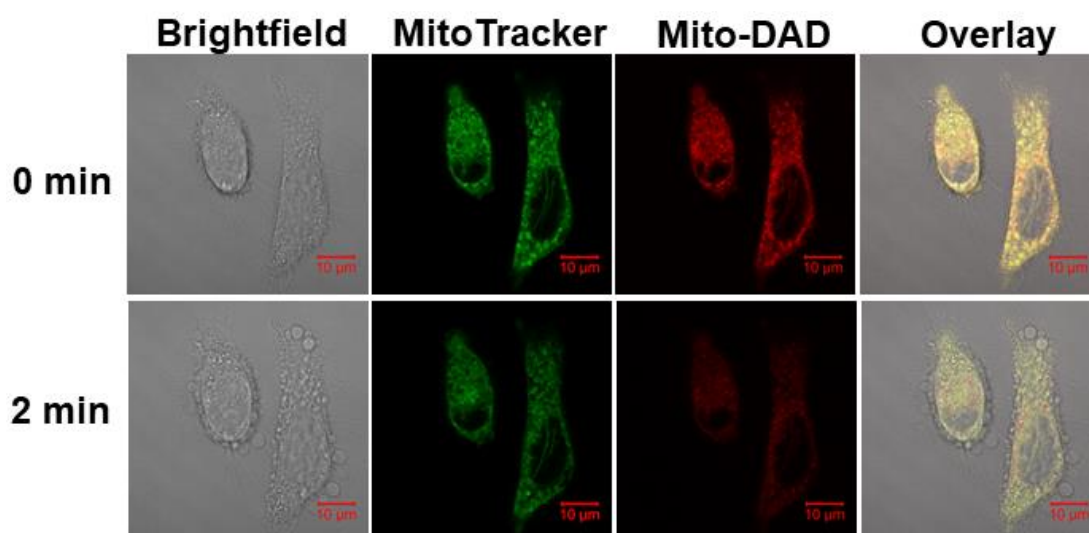
**Fig. S5** Changes of UV-vis absorption spectra of (a) Rose bengal or (b) Mito-DAD in PBS (10 mM) after various periods of LED light irradiation (16  $\text{mW}/\text{cm}^2$ ,  $\lambda_{\text{ex}}$  550 nm and 470 nm for Rose Bengal and Mito-DAD, respectively) at 20 s intervals.



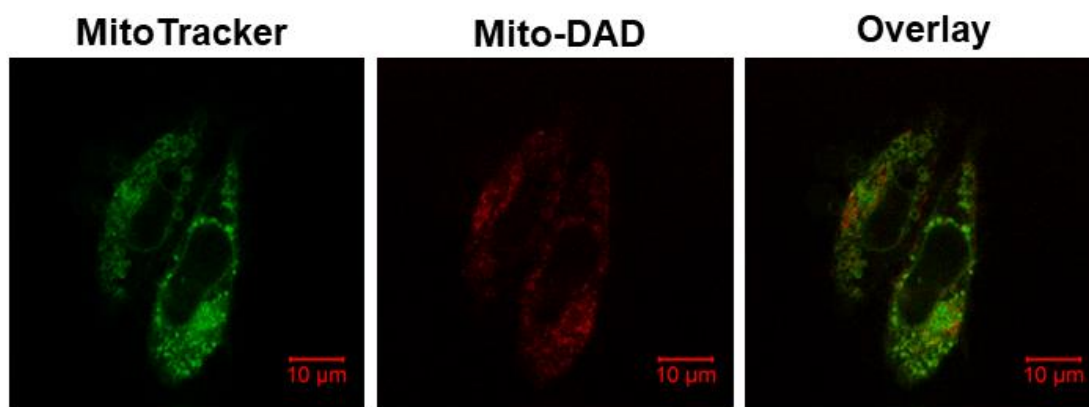
**Fig. S6** Intracellular ROS generation in Mito-DAD (5  $\mu$ M) treated HeLa cells without light irradiation (**a-b**) or with light irradiation (2 min, 16 mW/cm<sup>2</sup>;  $\lambda_{\text{ex}}$  470 nm) (**c-d**), detected by DCFH-DA in green channel under fluorescence microscopy. Scale bars indicate 50  $\mu$ m.



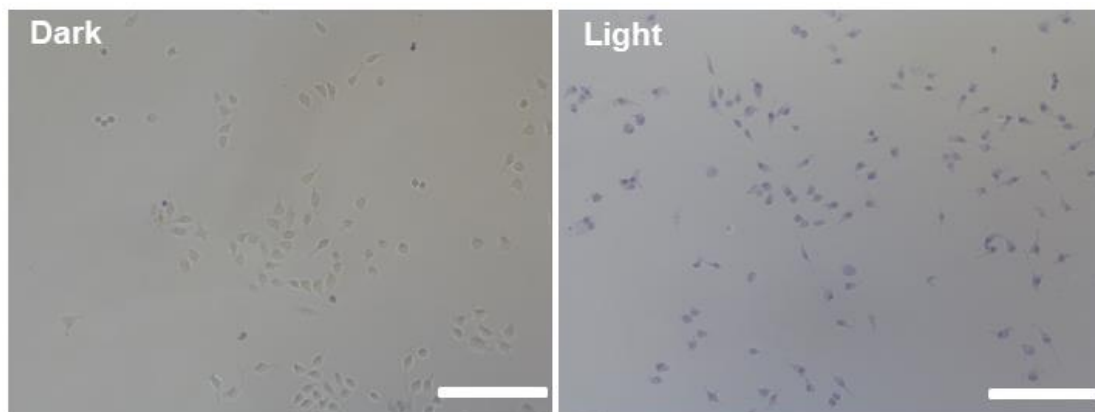
**Fig. S7** HeLa cells before and after light irradiation (2 min, 16 mW/cm<sup>2</sup>;  $\lambda_{\text{ex}}$  470 nm) (**a-b**) in the absence and (**c-d**) presence of 5  $\mu$ M Mito-DAD. Scale bars indicate 50  $\mu$ m.



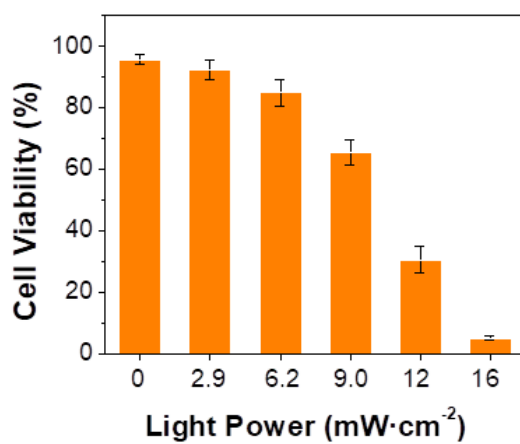
**Fig. S8** CLSM images of Mito-DAD and Mito Tracker Green co-treated HeLa cells before and after light irradiation ( $\lambda_{\text{ex}}$  470 nm, 16 mW/cm<sup>2</sup>, 2 min).



**Fig. S9** CLSM images of Mito-DAD (5  $\mu$ M) treated HeLa cells. The HeLa cells were stained by Mito Tracker Green, a membrane potential indicator, after light irradiation with light irradiation ( $\lambda_{\text{ex}}$  470 nm, 16 mW/cm<sup>2</sup>, 2 min).

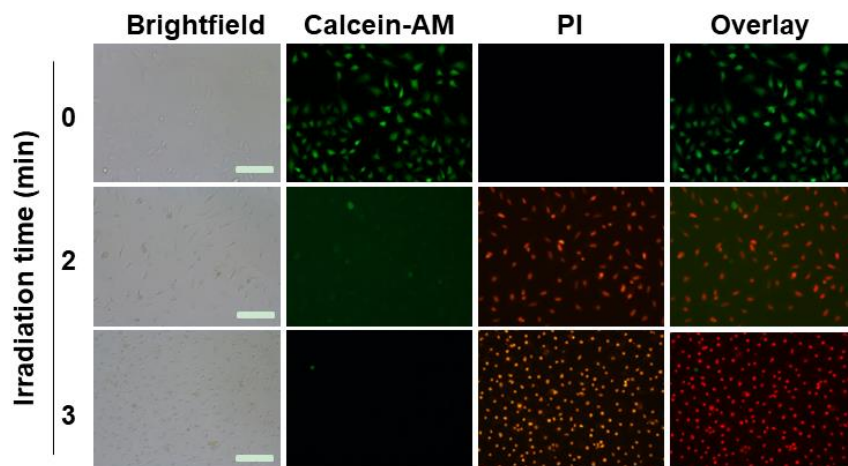


**Fig. S10** Trypan blue stained HeLa cells. The Mito-DAD (5  $\mu\text{M}$ ) treated cells were exposed to light irradiation ( $\lambda_{\text{ex}}$  470 nm, 16  $\text{mW}/\text{cm}^2$ , 3 min) and incubated for 24 h before Trypan blue staining. As control, no light irradiation was applied. Scale bars indicate 100  $\mu\text{m}$ .

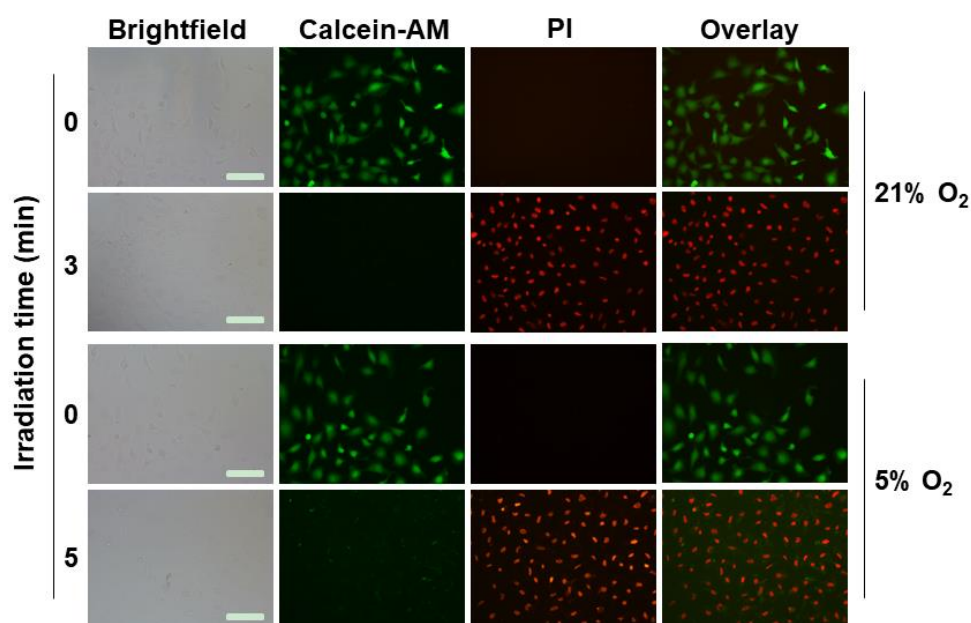


**Fig. S11** Light power-dependent viabilities of HeLa cells incubated with 5  $\mu\text{M}$  Mito-DAD.



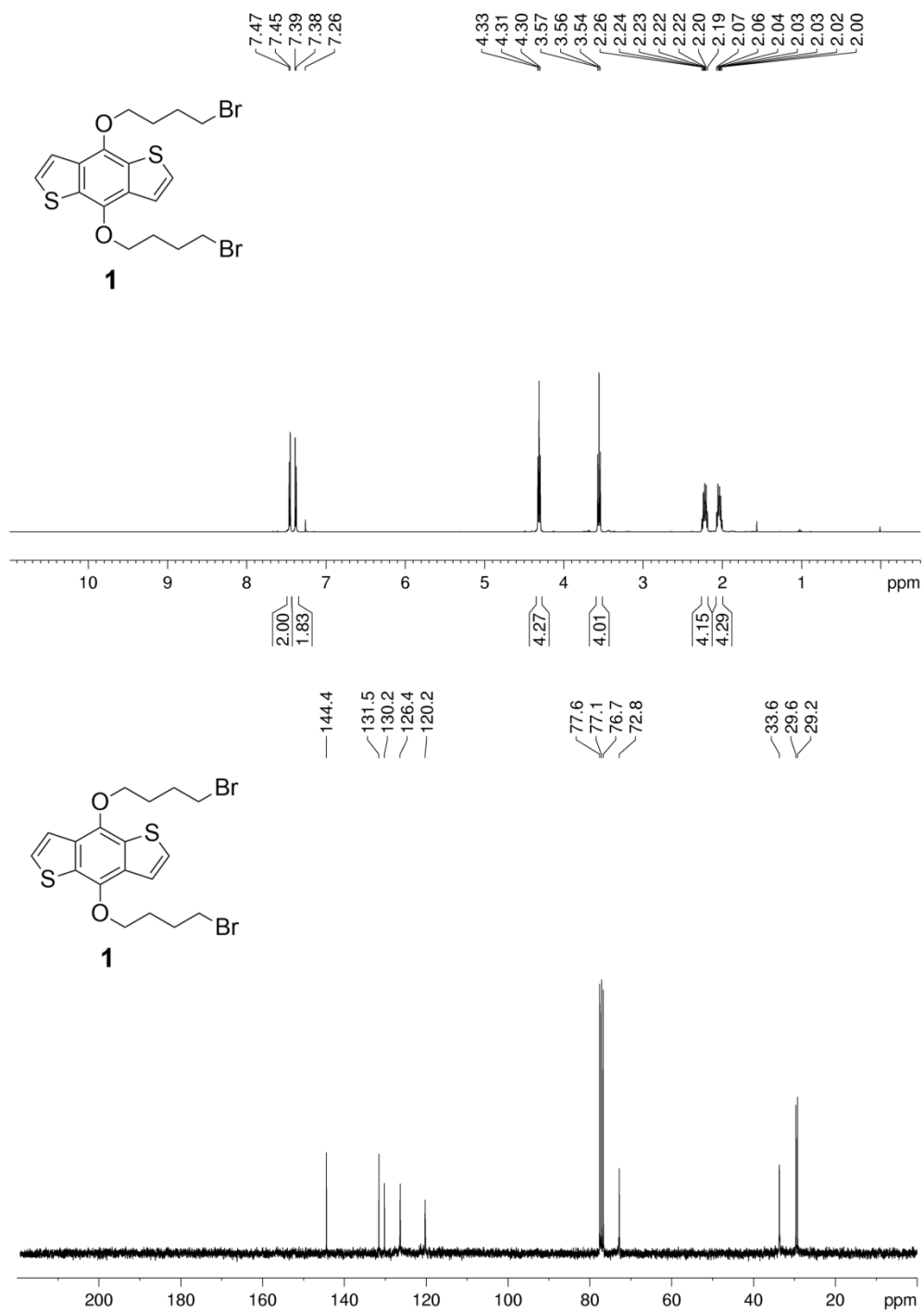


**Fig. S12** Live/dead staining of Mito-DAD (5  $\mu$ M) treated HeLa cells with varied light irradiation times. After further incubated for 24 h, the live and dead cells were stained by Calcein-AM and PI, respectively. The scale bars indicate 100  $\mu$ m.

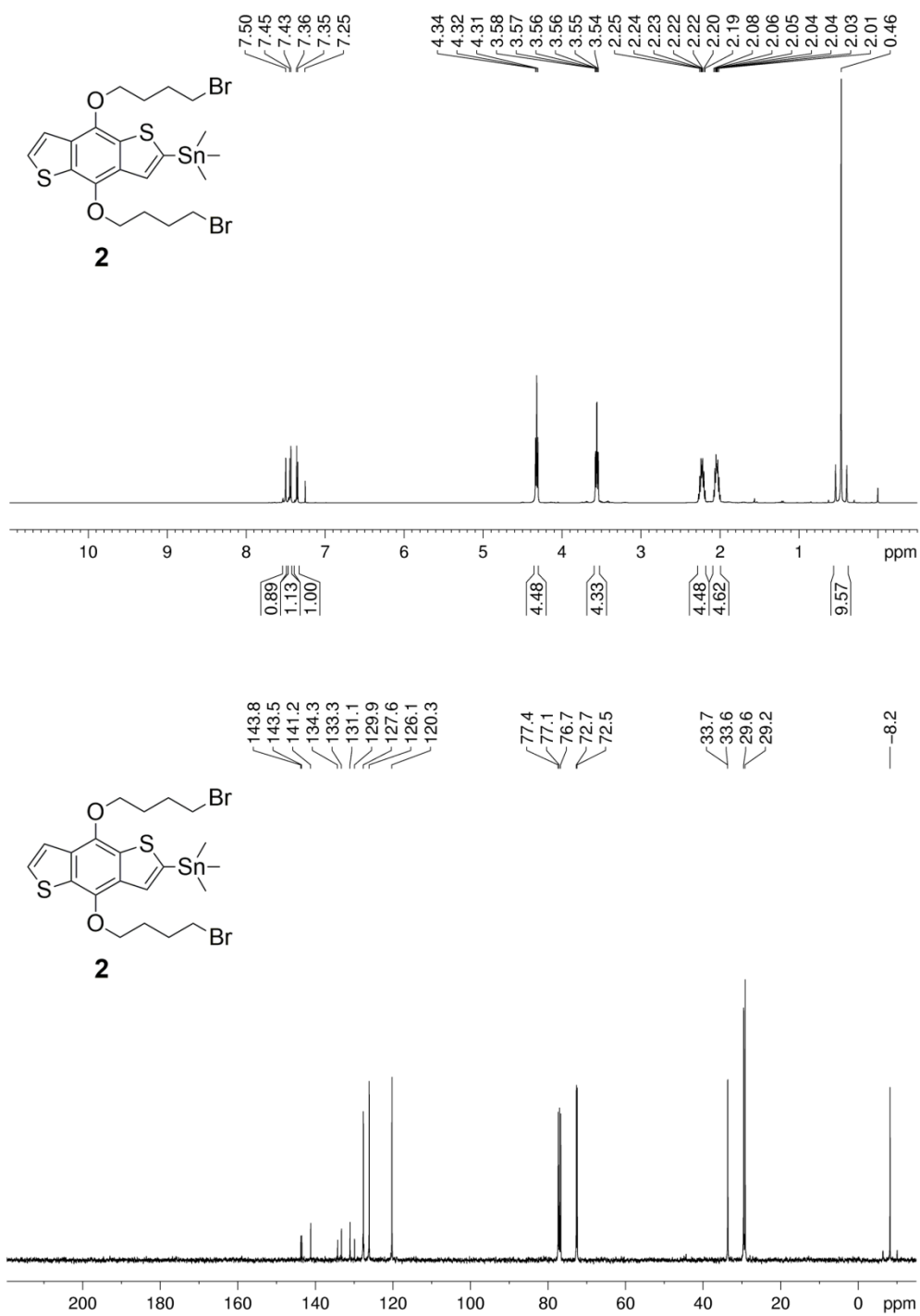


**Fig. S13** Live/dead staining of Mito-DAD (5  $\mu$ M) treated HeLa cells with varied light irradiation times under 21% and 5% O<sub>2</sub>. The live and dead cells were stained by Calcein-AM and PI, respectively, 2 h post irradiation treatment. The scale bars indicate 100  $\mu$ m.

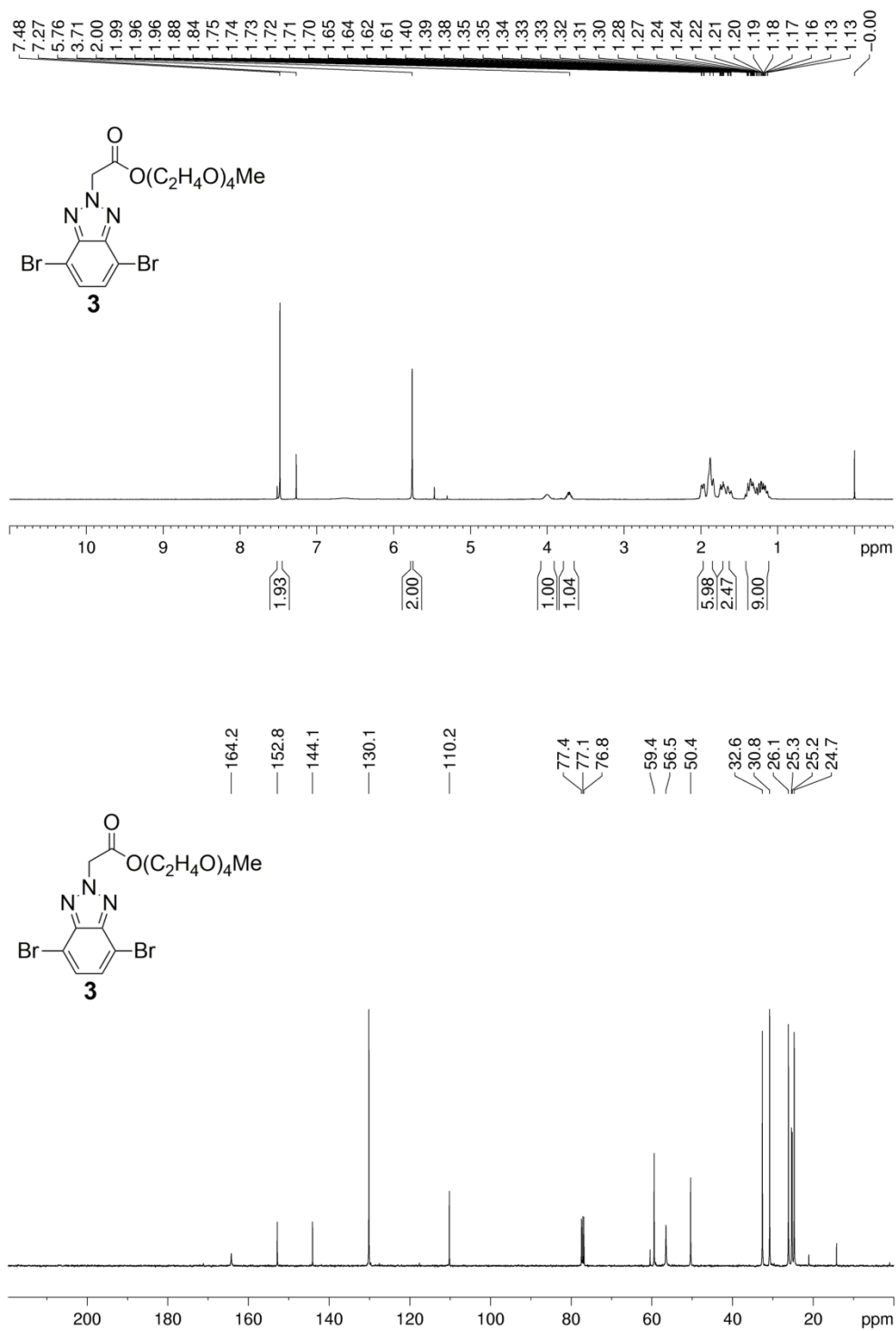
### 13. Structural characterization



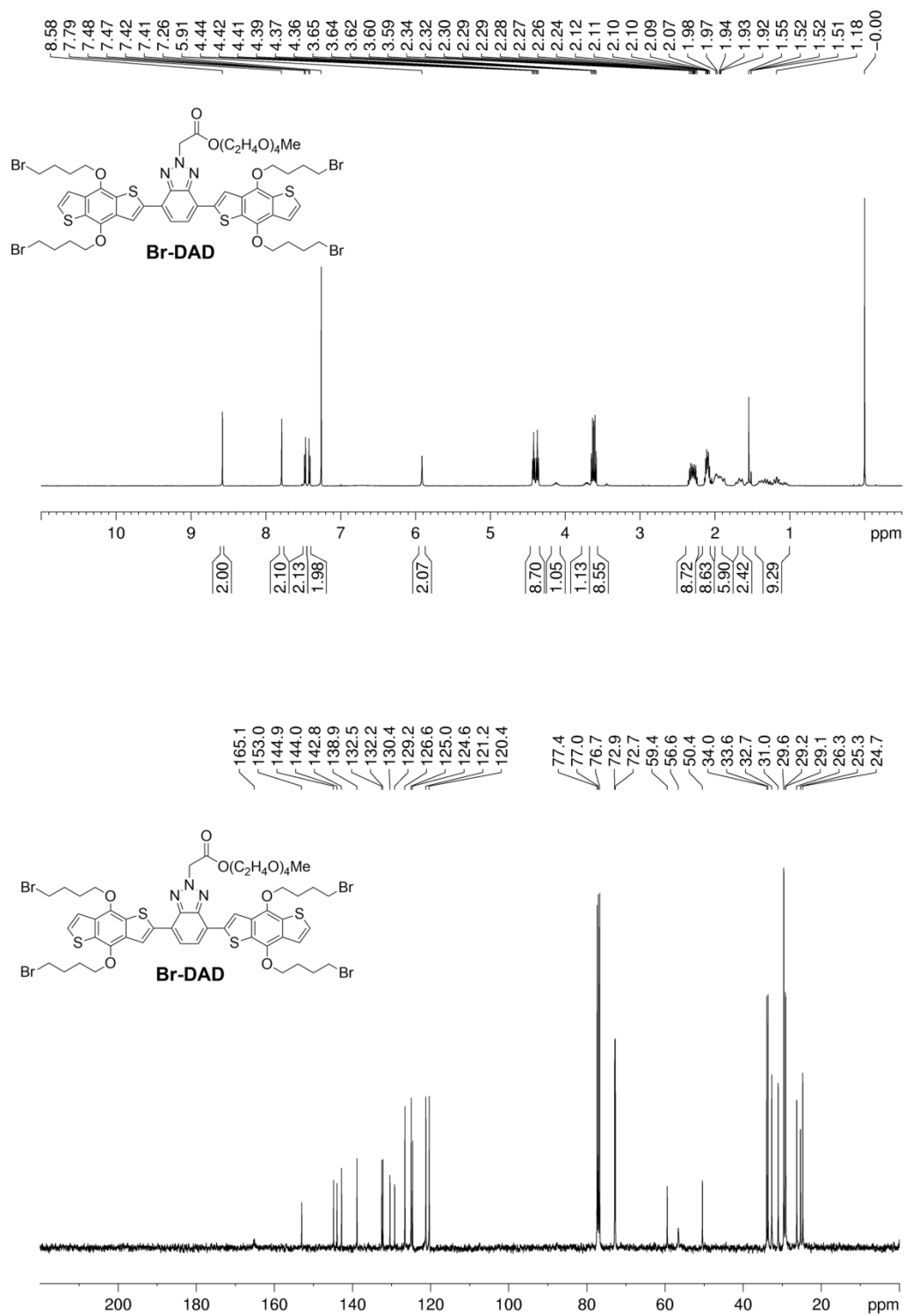
**Fig. S14** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of **1** in CDCl<sub>3</sub>.



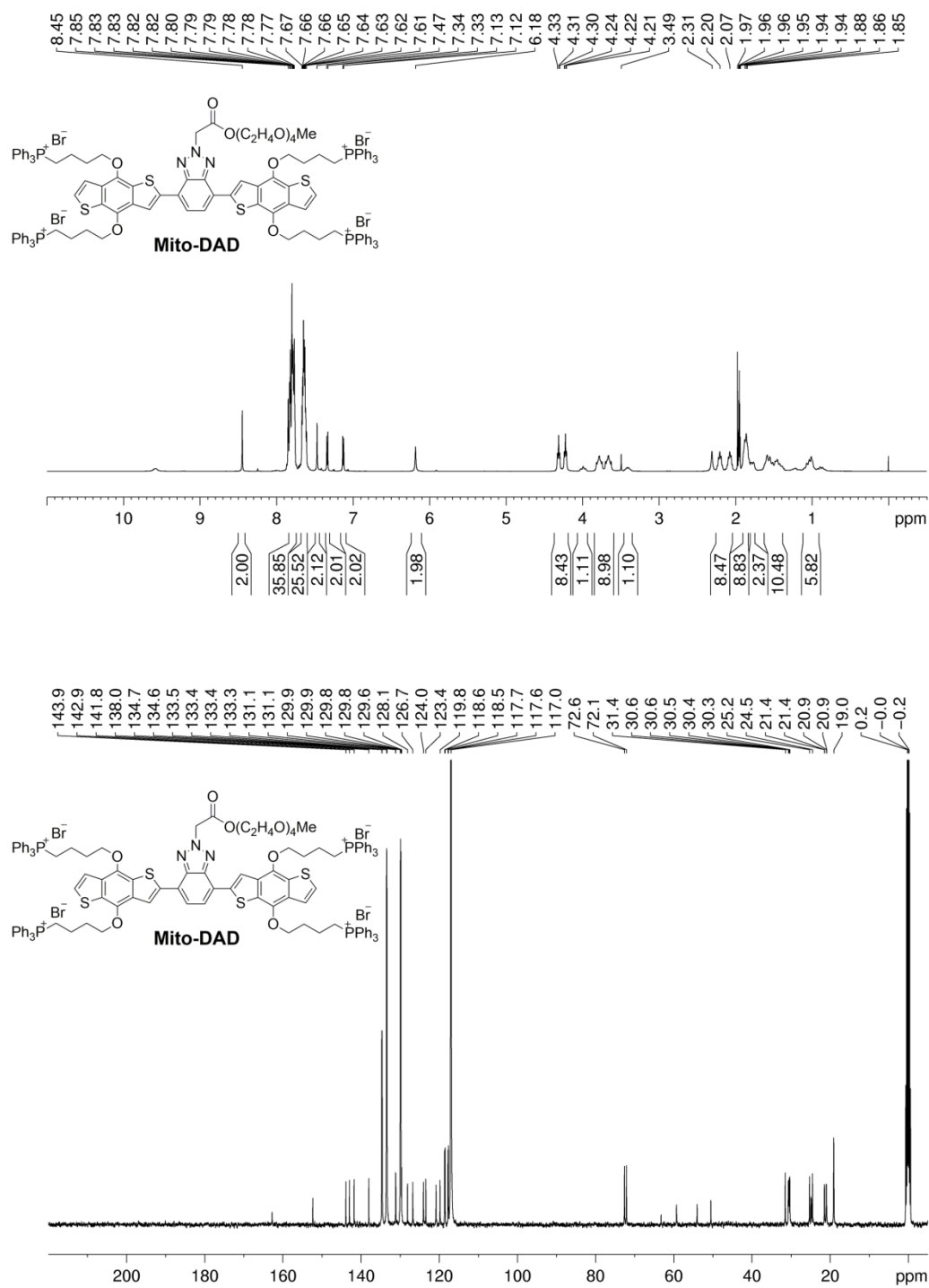
**Fig. S15**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of **2** in  $\text{CDCl}_3$ .



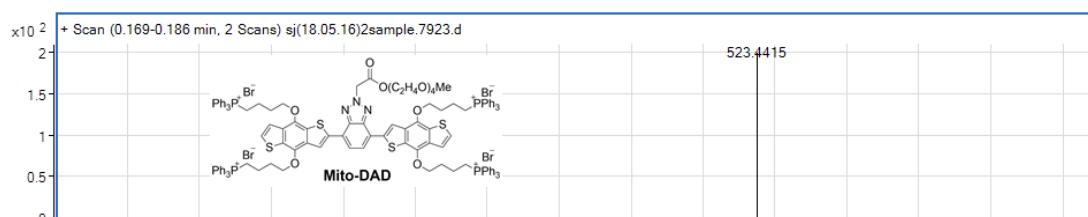
**Fig. S16** <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectra of **3** in CDCl<sub>3</sub>.



**Fig. S17** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Br-DAD in CDCl<sub>3</sub>.



**Fig. S18**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of Mito-DAD in  $\text{CD}_3\text{CN}$ .



**Fig. S19** HRMS spectrum of Mito-DAD.

#### 14. References

- [1] F. C. Teixeira, C. M. Rangel, A. P. S. Teixeira, *Heteroatom Chem.*, **2015**, 26, 236.
- [2] C. Latouche, P. H. Lanoë, J. A. G. Williams, et al, *New J. Chem.*, **2011**, 35, 2196.