

Single-Atom Replacement as a General Approach Towards Visible-Light/Near-Infrared Heavy-Atom-Free Photosensitizers for Photodynamic Therapy

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1. General experimental information

All solvents and chemicals for synthesis were purchased from Alfa Aesar and Chem-Impex and used as received without further purification unless otherwise specified. Live/Dead dual staining kit was bought from Thermo Fisher Scientific. CCK-8 kit was purchased from Dojindo Molecular Technologies, Inc. CellTiter-Glo 3D Cell Viability kit was from Promega. 3D multicellular tumor spheroids (MCTS) were seeded in Corning 96-well Spheroid Microplates (Cat. No. 4442).

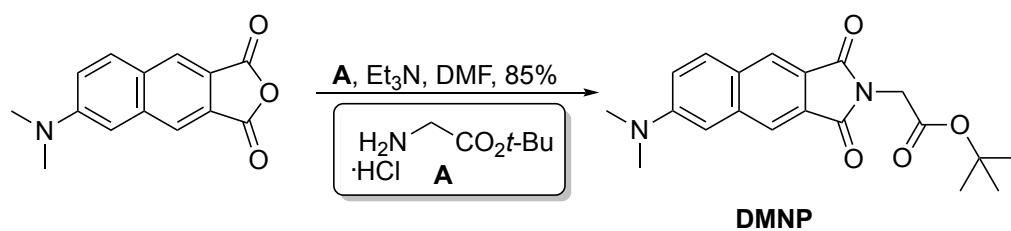
The ^1H NMR spectroscopic measurements were carried out using a Bruker-600 NMR at 600 MHz with tetramethylsilane (TMS) as internal reference. Electrospray ionization (ESI) mass spectra were performed on a Bruker MicroToF ESI LC-MS System in positive-ion mode. The steady-state absorption spectra were obtained with a ThermoFisher Evolution 220 UV-Vis spectrophotometer in 1 cm path length quartz cells. Fluorescence spectra were recorded using spectrophotometer (SPEX FluoroLog-3). Quantum yield in DMSO was measured relative to the fluorescence of Rhodamine B ($\Phi = 0.65$) in ethanol, quinine sulfate ($\Phi = 0.55$) in 0.5 M H_2SO_4 or fluorescein in 0.1 M NaOH. FT-IR spectrum was taken on a PerkinElmer Spectrum 100 FT-IR Spectrometers. Confocal fluorescent images of living cells were performed using Nikon A1R-si Laser Scanning Confocal Microscope (Japan), equipped with lasers of 405/488/561/638 nm. Plate reader was from Infinite® 200 PRO (TECAN). Light sources are Prior Lumen200 with 463/20, 585/40 or 615/30 filters and 730 LED (675-750 nm) (Mouser Electronics). Universal optical power meter was from MELLES GRIOT.

2. Synthesis and characterization

General preparation of thiocarbonyl compounds

To a mixture of carbonyl compounds ($0.04 \text{ M}\cdot\text{L}^{-1}$) and Lawesson's reagent ($0.024 \text{ M}\cdot\text{L}^{-1}$) in Schlenk flask, 5 mL dry toluene was added. The mixture was heated 120°C for 1 h and then cooled to room temperature. For the double-thionation compounds, a mixture of carbonyl compounds ($0.04 \text{ M}\cdot\text{L}^{-1}$) and Lawesson's reagent ($0.048 \text{ M}\cdot\text{L}^{-1}$) was refluxed for 24 h. Solvent was evaporated under reduced pressure. The residue was purified by flash silica gel column chromatography to afford the desired compound as solids in yields of 20-80%.

The synthesis and characterization including ^1H NMR, ^{13}C NMR, electrospray ionization mass (ESI-MS), FT-IR, UV-vis and fluorescence spectra of SACD, SCou, SDMAP, SDMAP-Halo, and SNile Red can be found in the reference literature.¹



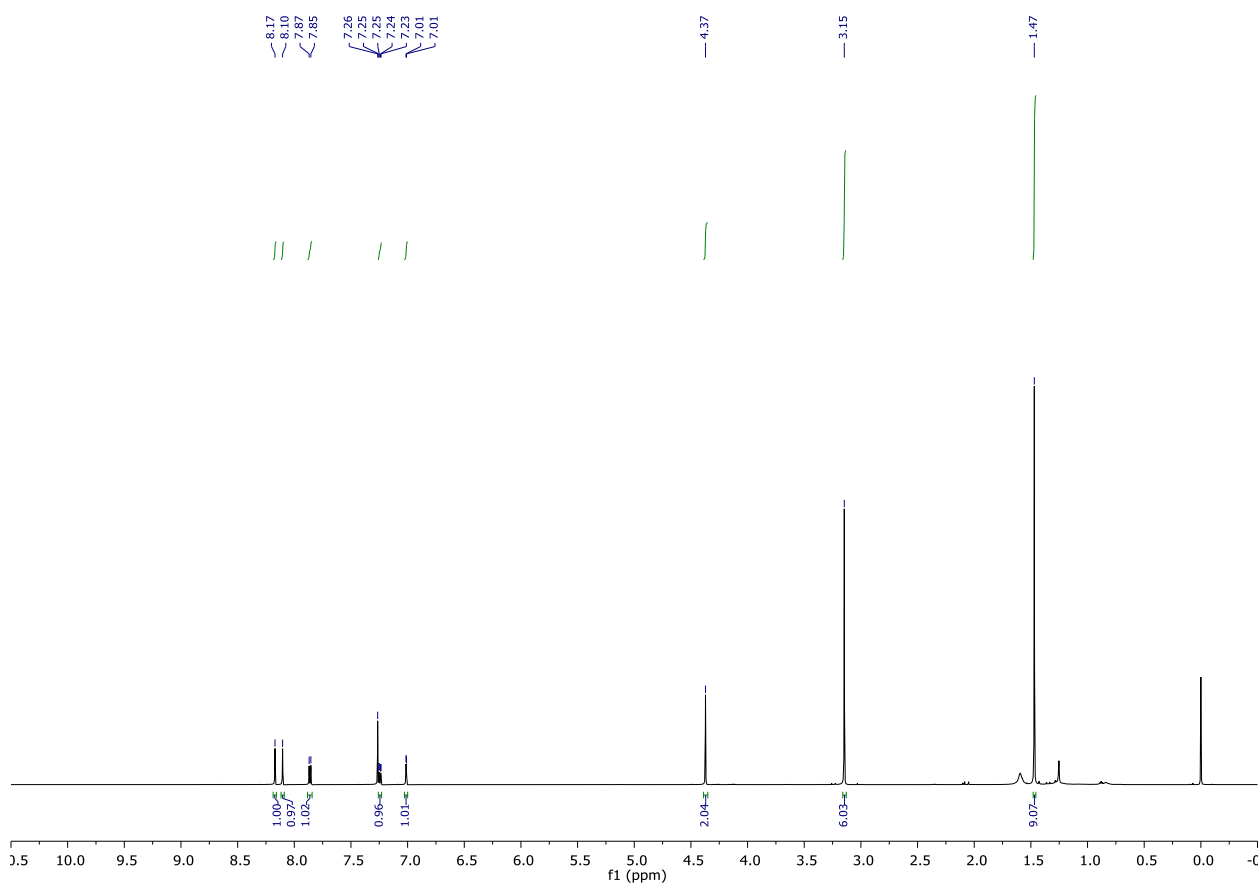
To a solution of anhydride (90 mg, 0.37 mmol) and glycine *tert*-butyl ester hydrochloride (125 mg, 0.75 mmol, 2.0 eq) in DMF (5 mL), Et₃N (154 μL, 1.11 mmol, 3.0 eq) was added, and the reaction was stirred at 80 °C for 12 h. Then diluted with water and extracted with EtOAc (50 mL x 3). The combined organic phase was washed with water and brine, dried over Na₂SO₄, filtered, and evaporated. Flash chromatography on silica gel (20% EtOAc/hexanes) yielded 111mg (85%) of **DMNP** as a yellow solid.

R_f = 0.4 (ethyl acetate: hexane = 1: 2, v/v)

HRMS (ESI): calcd. for C₂₀H₂₃N₂O₄⁺ [M+H]⁺ 355.1652, found 355.1600.

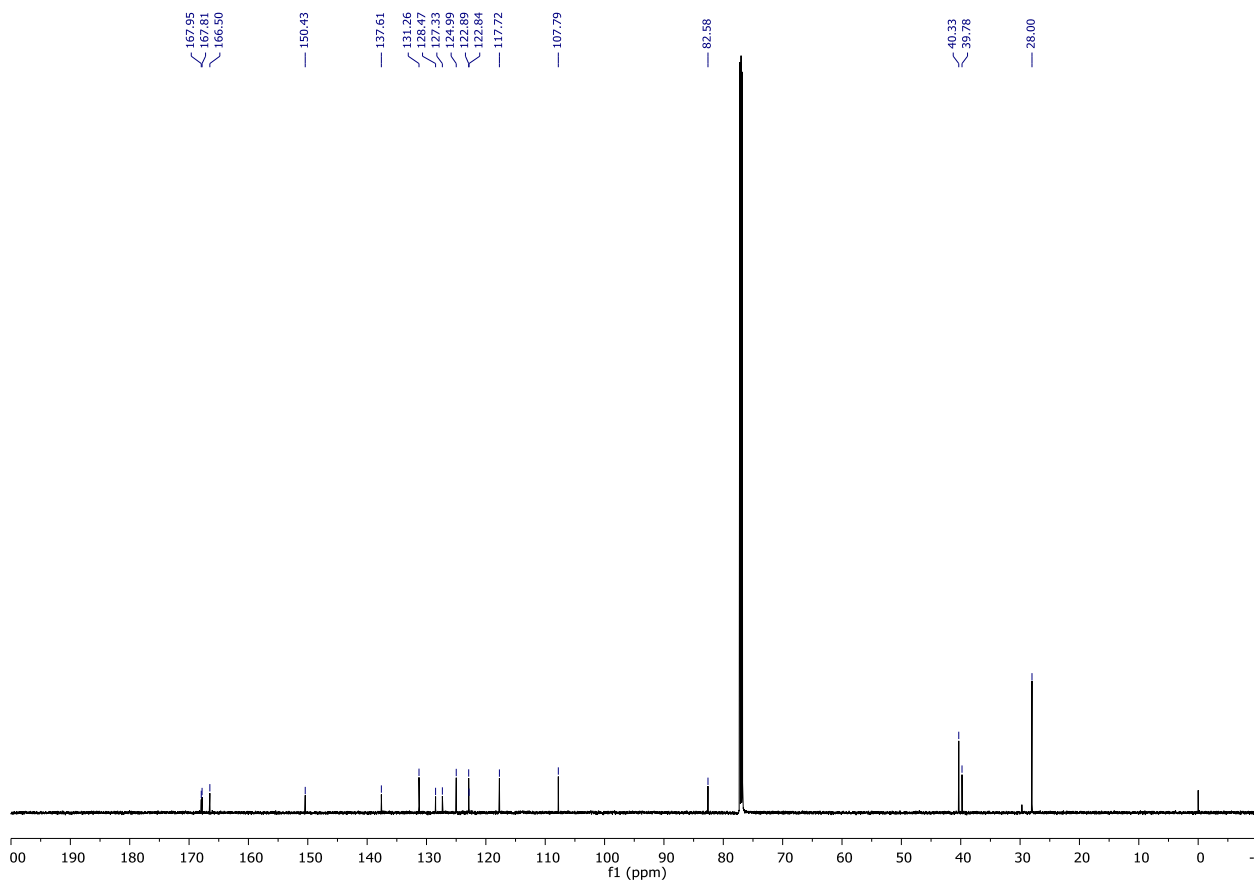
¹H NMR (600 MHz, CDCl₃) δ 8.17 (s, 1H), 8.10 (s, 1H), 7.86 (d, *J* = 9.1 Hz, 1H), 7.24 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.01 (d, *J* = 2.6 Hz, 1H), 4.37 (s, 2H), 3.15 (s, 6H), 1.47 (s, 9H).

¹H spectrum

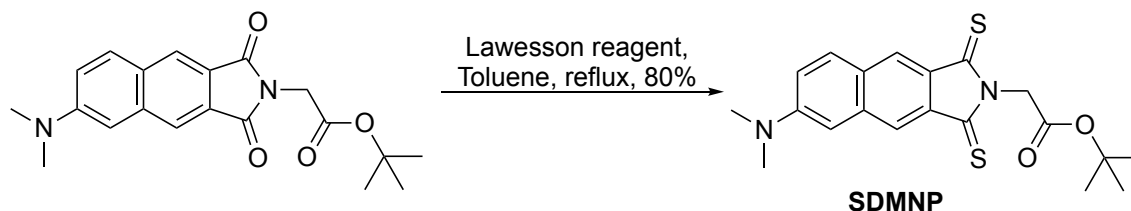
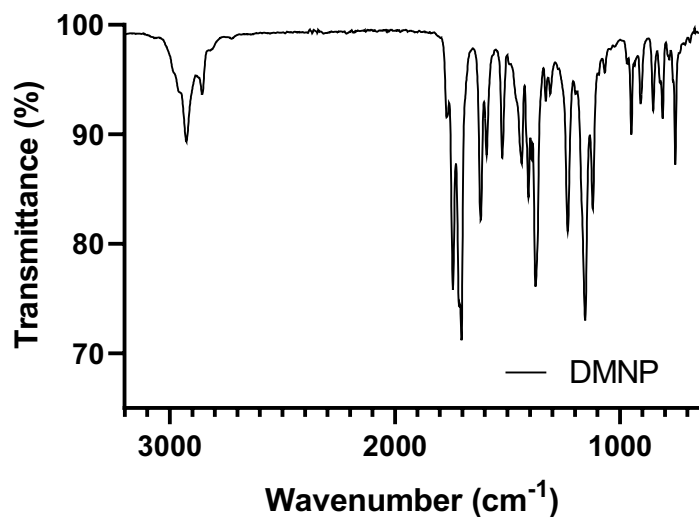


^{13}C NMR (150 MHz, CDCl_3) δ 167.95, 167.81, 166.50, 150.43, 137.61, 131.26, 128.47, 127.33, 124.99, 122.89, 122.84, 117.72, 107.79, 82.58, 40.33, 39.78, 28.00.

^{13}C spectrum



IR for DMNP



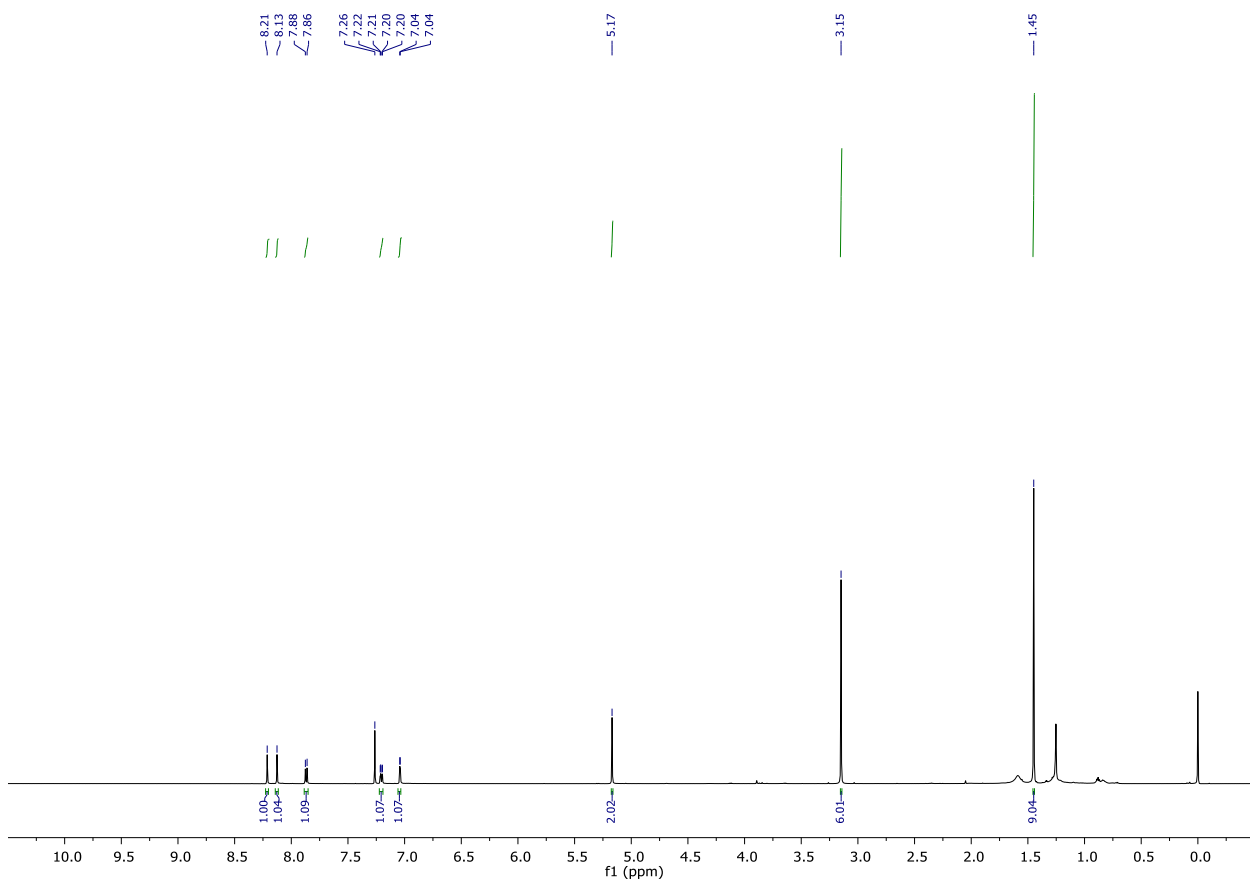
To a mixture of carbonyl compounds (30 mg, 85 μmol) and Lawesson's reagent (68 mg, 170 μmol) in a Schlenk tube, 5 mL dry toluene was added. The mixture was heated 130 $^{\circ}\text{C}$ for 12 h and then cooled to room temperature. Solvent was evaporated under reduced pressure. The residue was purified by flash silica gel column chromatography (20% EtOAc/hexanes) yielded 26 mg (80%) of **SDMNP** as a purple solid.

$R_f = 0.6$ (ethyl acetate: hexane = 1: 2, v/v)

HRMS (ESI): calcd. for $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_2\text{S}_2^+$ $[\text{M}+\text{H}]^+$ 387.1195, found 387.1125.

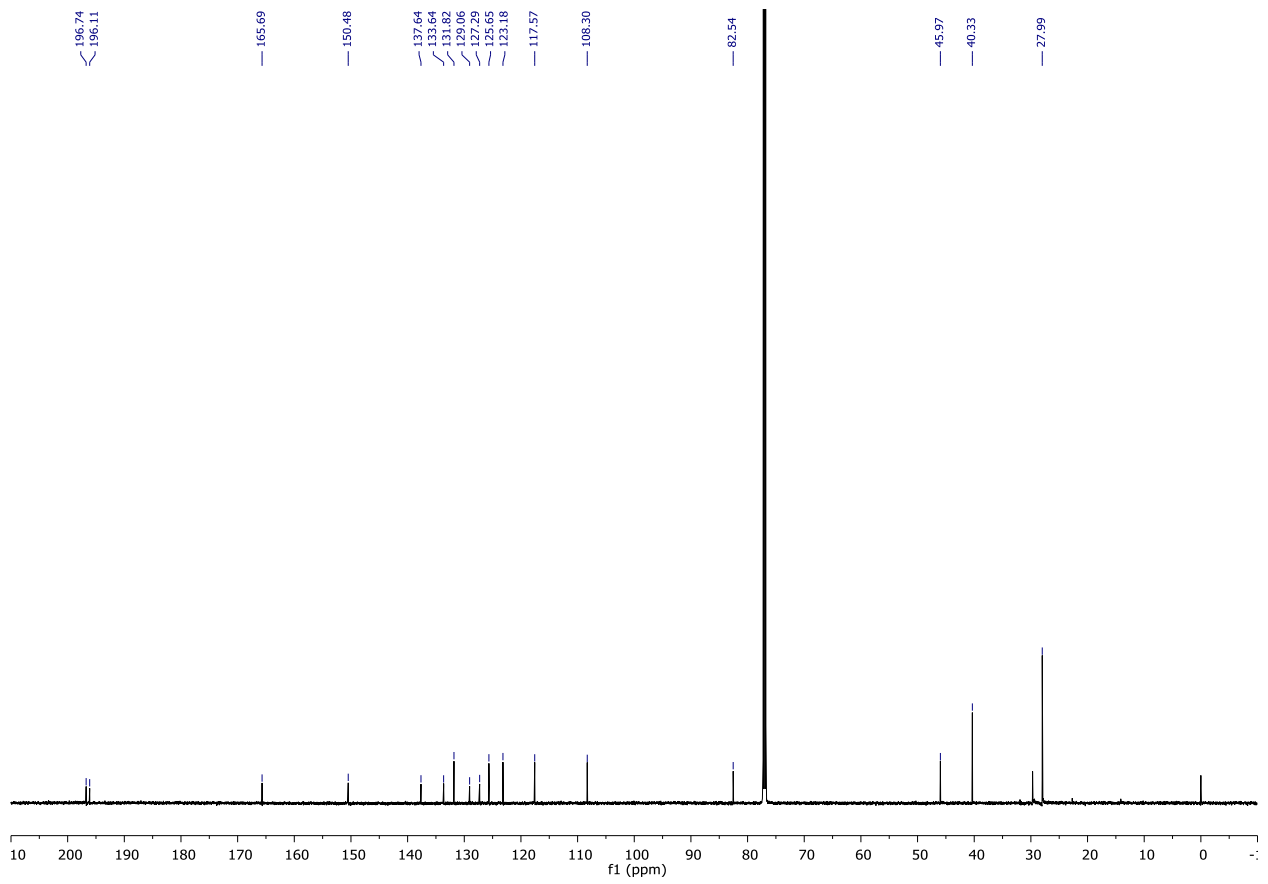
^1H NMR (600 MHz, CDCl_3) δ 8.21 (s, 1H), 8.13 (s, 1H), 7.87 (d, $J = 9.1$ Hz, 1H), 7.21 (dd, $J = 9.1, 2.6$ Hz, 1H), 7.04 (d, $J = 2.6$ Hz, 1H), 5.17 (s, 2H), 3.15 (s, 6H), 1.45 (s, 9H).

^1H spectrum

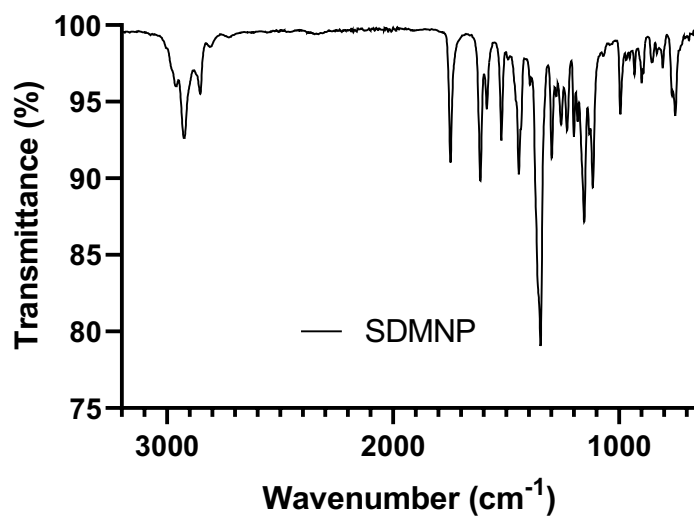


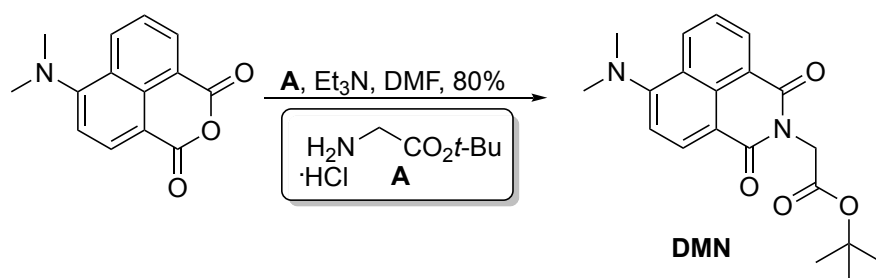
^{13}C NMR (150 MHz, CDCl_3) δ 196.74, 196.11, 165.69, 150.48, 137.64, 133.64, 131.82, 129.06, 127.29, 125.65, 123.18, 117.57, 108.30, 82.54, 45.97, 40.33, 27.99.

^{13}C spectrum



IR for SDMNP





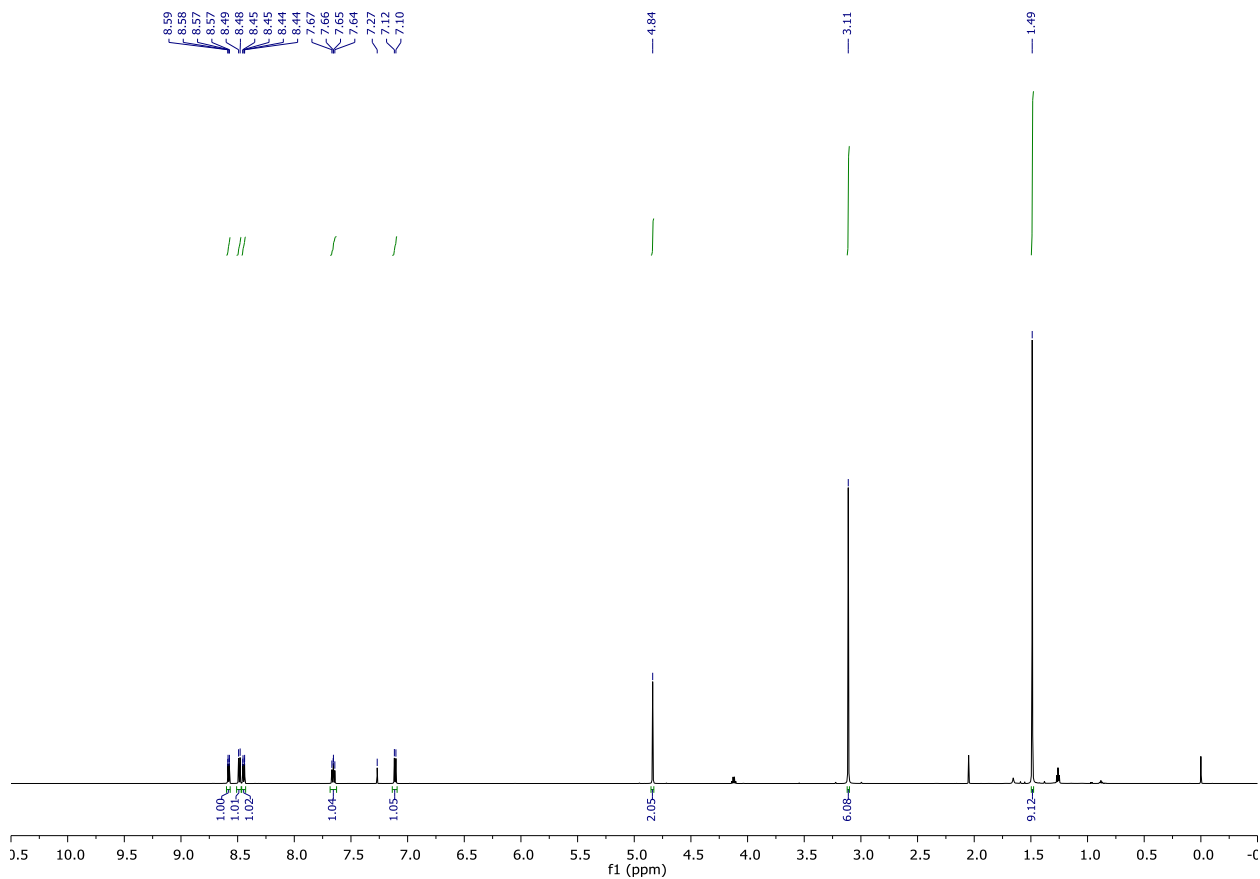
To a solution of anhydride (150 mg, 0.62 mmol) and glycine tert-butyl ester hydrochloride (209 mg, 1.24 mmol, 2.0 eq) in DMF (5 mL), Et₃N (261 μ L, 1.86 mmol, 3.0 eq) was added, and the reaction was stirred at 80 °C for 12 h. Then diluted with water and extracted with EtOAc (50 mL x 3). The combined organic phase was washed with water and brine, dried over Na₂SO₄, filtered, and evaporated. Flash chromatography on silica gel (20% EtOAc/hexanes) yielded 176 mg (80%) of **DMN** as a yellow solid.

R_f = 0.5 (ethyl acetate: hexane = 1: 2, v/v)

HRMS (ESI): calcd. for C₂₀H₂₃N₂O₄⁺ [M+H]⁺ 355.1652, found 355.1603.

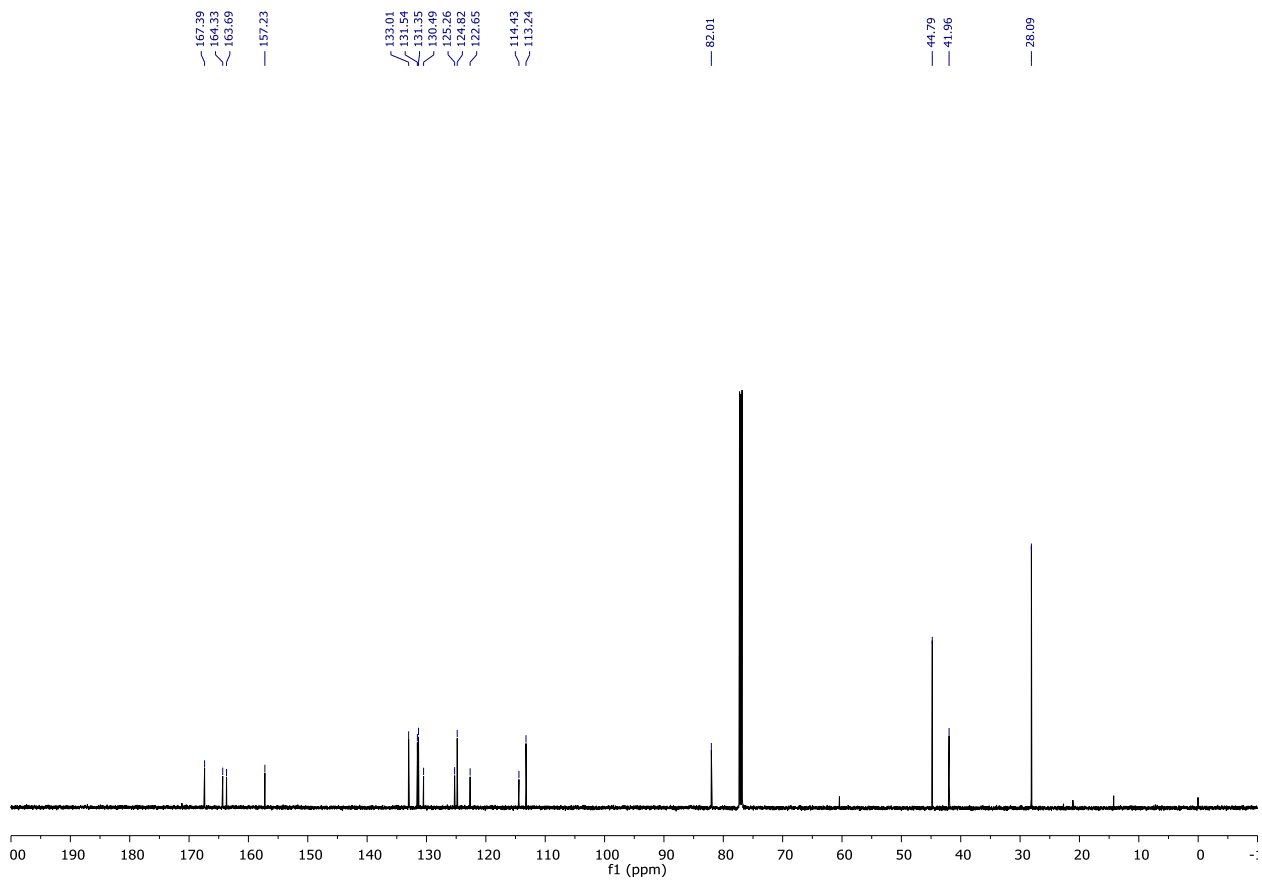
¹H NMR (600 MHz, CDCl₃) δ 8.58 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.48 (d, *J* = 8.1 Hz, 1H), 8.45 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.66 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.11 (d, *J* = 8.2 Hz, 1H), 4.84 (s, 2H), 3.11 (s, 6H), 1.49 (s, 9H).

¹H spectrum

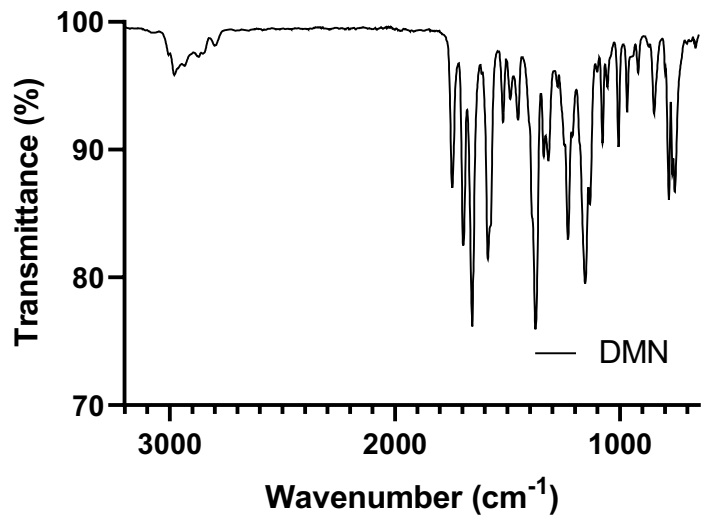


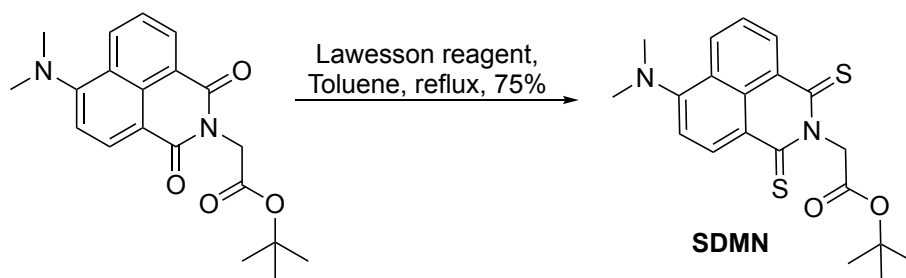
^{13}C NMR (150 MHz, CDCl_3) δ 167.39, 164.33, 163.69, 157.23, 133.01, 131.54, 131.35, 130.49, 125.26, 124.82, 122.65, 114.43, 113.24, 82.01, 44.79, 41.96, 28.09.

^{13}C spectrum



IR for DMN





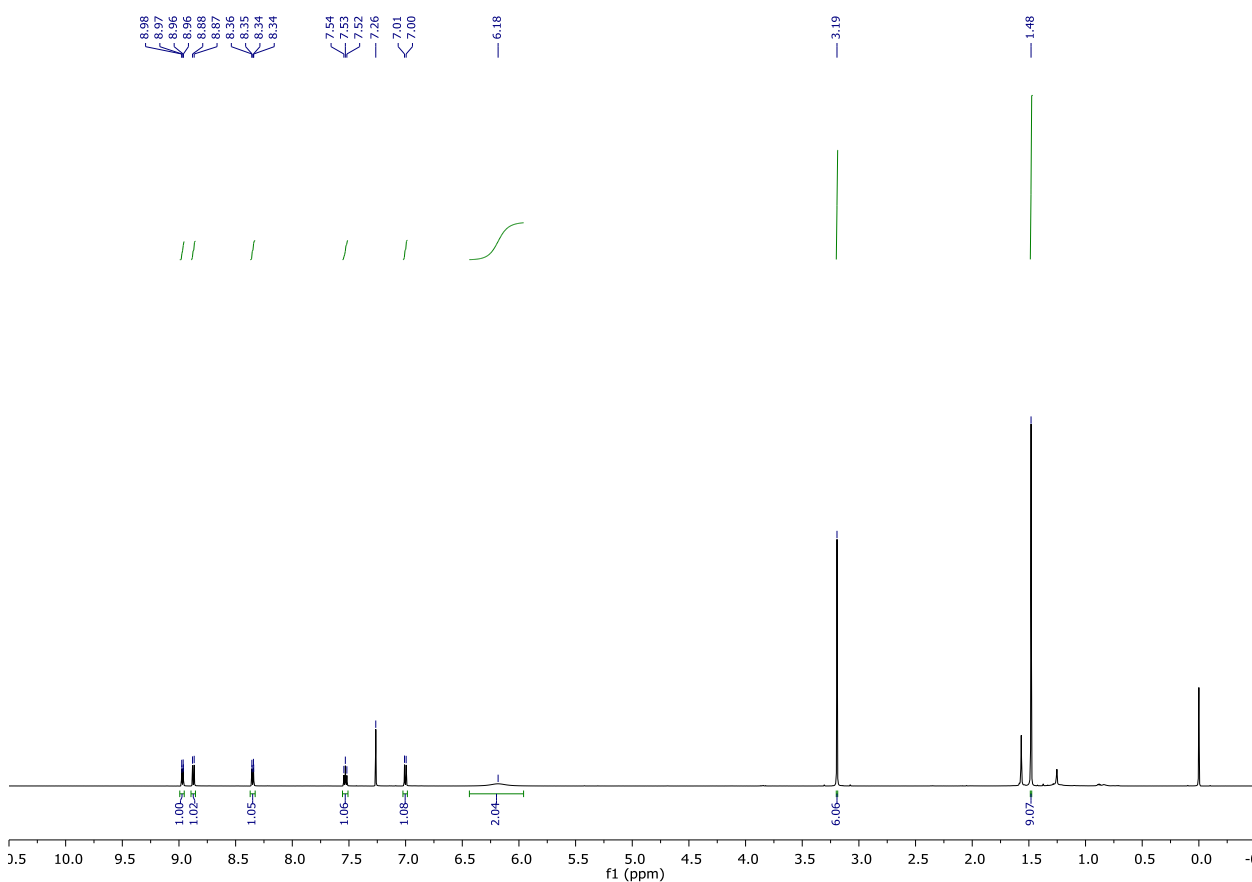
To a mixture of carbonyl compound **DMN** (30 mg, 85 μmol) and Lawesson's reagent (68 mg, 170 μmol) in Schlenk tube, 5 mL dry toluene was added. The mixture was heated 130 $^{\circ}\text{C}$ for 36 h and then cooled to room temperature. Solvent was evaporated under reduced pressure. The residue was purified by flash silica gel column chromatography (20% EtOAc/hexanes) yielded 25 mg (75%) of **SDMN** as a purple solid.

$R_f = 0.5$ (ethyl acetate: hexane = 1: 2, v/v)

HRMS (ESI): calcd. for $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_2\text{S}_2^+ [\text{M}+\text{H}]^+$ 387.1195, found 387.1127.

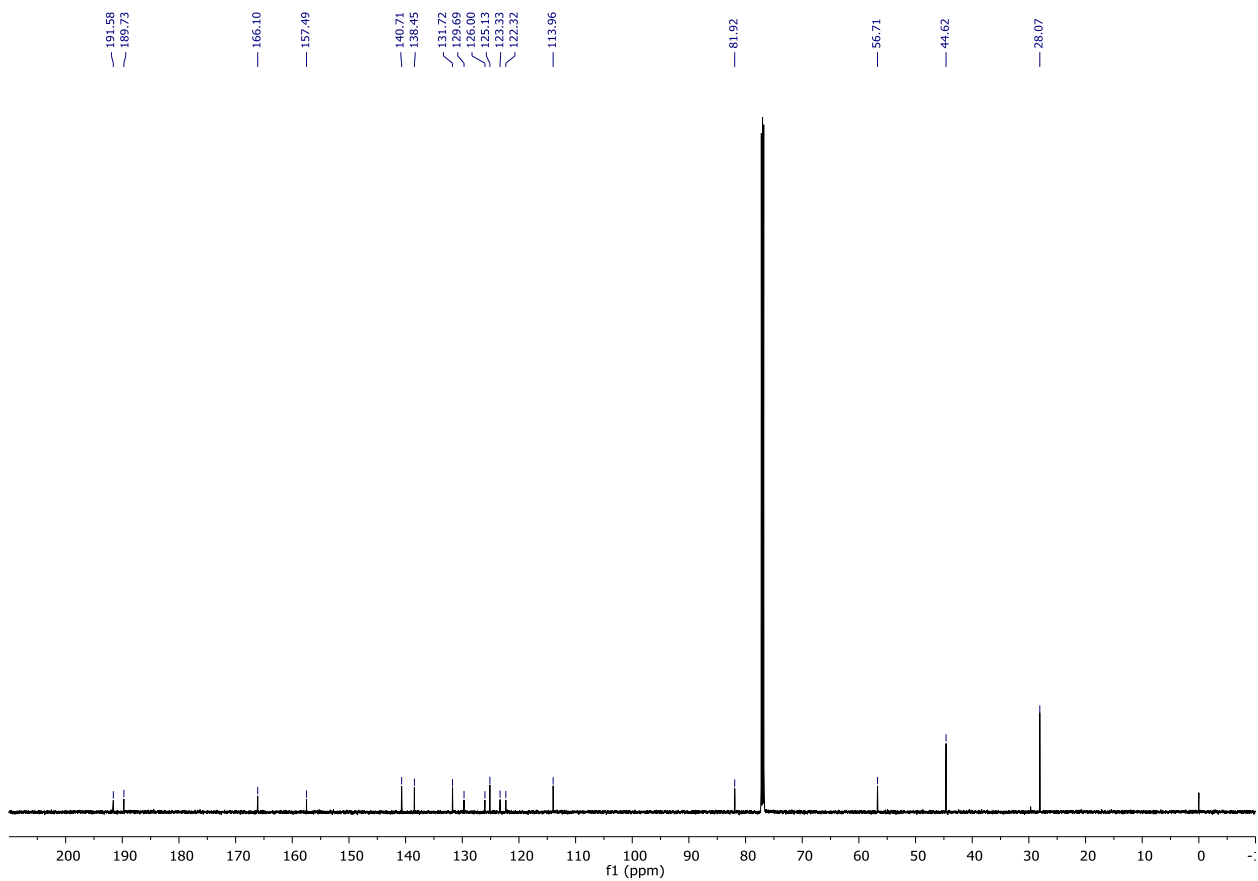
^1H NMR (600 MHz, CDCl_3) δ 8.97 (dd, $J = 7.6, 1.2$ Hz, 1H), 8.87 (d, $J = 8.7$ Hz, 1H), 8.35 (dd, $J = 8.3, 1.3$ Hz, 1H), 7.53 (t, $J = 8.0$ Hz, 1H), 7.00 (d, $J = 8.7$ Hz, 1H), 6.18 (s, 2H), 3.19 (s, 6H), 1.48 (s, 9H).

^1H spectrum

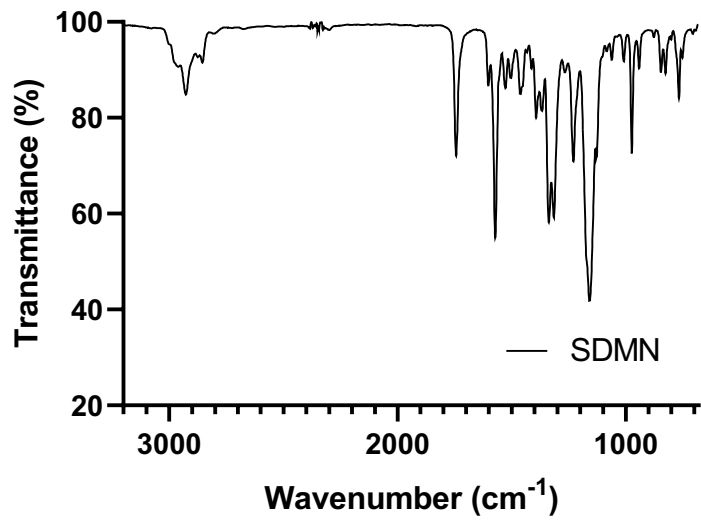


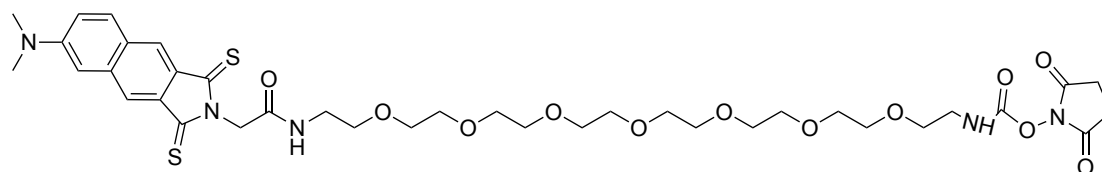
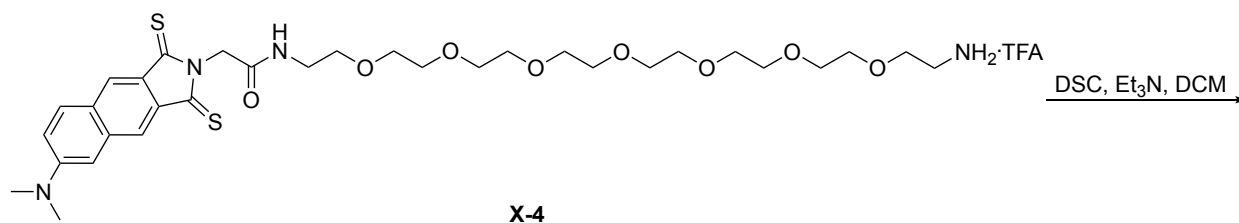
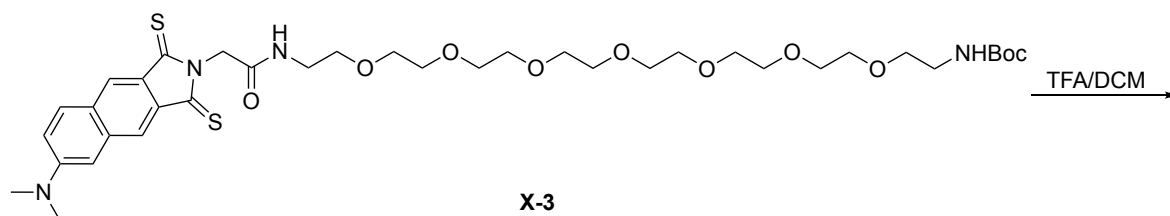
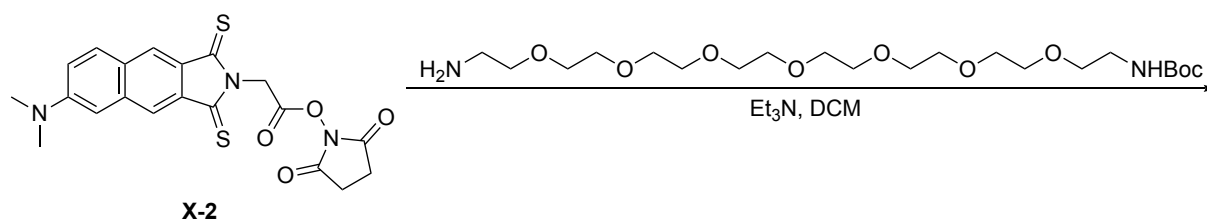
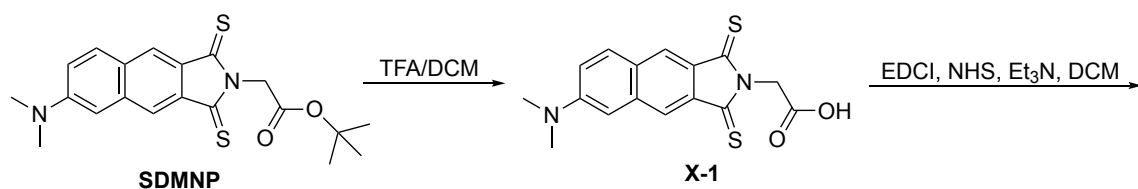
^{13}C NMR (150 MHz, CDCl_3) δ 191.58, 189.73, 166.10, 157.49, 140.71, 138.45, 131.72, 129.69, 126.00, 125.13, 123.33, 122.32, 113.96, 81.92, 56.71, 44.62, 28.07.

^{13}C spectrum



IR for SDMN





SDMNP-NHS

To a stirred solution of **SDMNP** (10 mg, 26 μ mol) in CH_2Cl_2 (2 mL) was added TFA (4 mL) at 0 $^\circ\text{C}$. The reaction mixture was gradually warmed to room temperature and stirred for 4 h. Solvents and volatiles were removed in vacuo, the residue was dissolved in CH_2Cl_2 (5 mL) and concentrated again to afford the desired amine as the corresponding TFA salt, which was used directly in the next step without further purification.

To a solution of acid **X-1** in DMF (3 mL), Et₃N (52 μL, 0.3 mmol) was added, followed by the addition of EDCI (18 mg, 0.12 mmol) and NHS (14 mg, 0.12 mmol) in one portion at 0 °C. The mixture was stirred at room temperature for 10 h, and then diluted with water and extracted with EtOAc (30 mL x 3). The combined organic phase was washed with 1 N aqueous hydrochloric acid solution (20 mL x 2), saturated sodium bicarbonate solution (20 mL x 2) and brine, dried over Na₂SO₄, filtered, and evaporated. The resulting NHS ester **X-2** used directly in the next step without further purification.

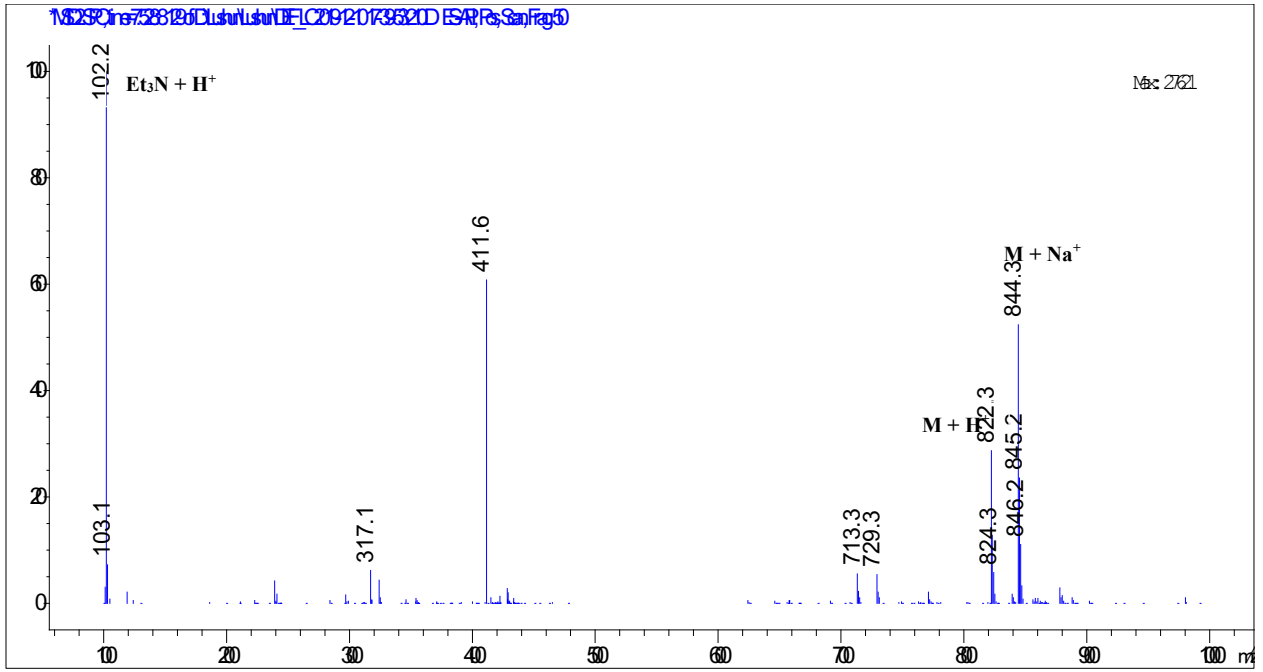
X-2 LC-MS (ESI): calcd. for C₂₀H₁₈N₃O₄S₂⁺ [M+H]⁺ 428.1, found 428.0.

To a solution of NHS ester **X-2** and *t*-Boc-*N*-amido-PEG7-amine (11 mg, 24 μmol) in CH₂Cl₂ (3 mL) was added Et₃N (52 μL, 0.3 mmol) at 0 °C. The mixture was stirred at room temperature for 10 h, and then diluted with water and extracted with EtOAc (30 mL x 3). The combined organic phase was washed with 1 N aqueous hydrochloric acid solution (20 mL x 2), saturated sodium bicarbonate solution (20 mL x 2) and brine, dried over Na₂SO₄, filtered, and evaporated. The resulting compound **X-3** used directly in the next step without further purification.

To a stirred solution of compound **X-3** in CH₂Cl₂ (2 mL) was added TFA (2 mL) at 0 °C. The reaction mixture was gradually warmed to room temperature and stirred for 1 h. Solvents and volatiles were removed in vacuo, the residue was dissolved in CH₂Cl₂ (5 mL) and concentrated again to afford the desired amine as the corresponding TFA salt, which was used directly in the next step without further purification.

To a solution of acid **X-4** in CH₂Cl₂ (3 mL) was added Et₃N (52 μL, 0.3 mmol), followed by addition of DSC (10 mg, 0.04 mmol) in one portion at 0 °C. The mixture was stirred at room temperature for 11 h, and then diluted with water and extracted with EtOAc (30 mL x 3). The combined organic phase was washed with 1 N aqueous hydrochloric acid solution (20 mL x 2), saturated sodium bicarbonate solution (20 mL x 2) and brine, dried over Na₂SO₄, filtered, and evaporated. The resulting NHS ester **SDMNP-NHS** was used directly in antibody conjugation without further purification.

SDMNP-NHS LC-MS (ESI): calcd. for C₃₇H₅₂N₅O₁₂S₂⁺ [M+H]⁺ 822.3, found 822.3.



3. Quantum yield determination

Quantum yields were measured with Rhodamine B as a reference ($\Phi=0.65$), quinine sulfate ($\Phi = 0.55$) in 0.5 M H₂SO₄ or fluorescein in 0.1 M NaOH. Fluorescence measurements were performed in 1 cm quartz cells with 5 μ M samples in DMSO on spectrophotometer (SPEX FluoroLog-3) (slits 5 \times 5). The fluorescence quantum yield, Φ_f (sample), were calculated according to equation as following:

$$\frac{\Phi_{f,sample}}{\Phi_{f,ref}} = \frac{OD_{ref} \cdot I_{sample} \cdot d_{sample}^2}{OD_{sample} \cdot I_{ref} \cdot d_{ref}^2}$$

Φ_f : quantum yield of fluorescence;
 I: integrated emission intensity;
 OD: optical density at the excitation wavelength;
 d: refractive index of solvents, $d_{DMSO}=1.478$; $d_{ethanol}=1.36$; $d_{water}=1.33$.

4. Singlet oxygen quantum yield determination

Singlet oxygen quantum yields were calculated according to the literature. The initial absorbance of DPBF was adjusted to about 1.0 in DCM. The photosensitizer was added to the cuvette and photosensitizer's absorbance was adjusted to under 0.2 to minimize the possibility of singlet oxygen quenching by the dyes. The photooxidation of DPBF was monitored by UV-vis spectra over 100 s. The quantum yield of singlet oxygen generation was calculated by a relative method comparing to Ru(bpy)₃²⁺ for SCou and SACD ($\Phi_{\Delta} = 0.73$ in MeOH) and Methylene Blue for SDMAP, SDMNP, SDMN and SNile Red ($\Phi_{\Delta} = 0.57$ in DCM). Light source: 453-473 nm for SACD and SCou; 600-630 nm for SDMAP, SDMNP, SDMN, SNile Red; 565-605 nm for Nile Red; 405 nm for the rest. Φ_{Δ} were calculated according to the following equation:

$$\Phi_{\Delta,sample} = \Phi_{\Delta,standard} \cdot \frac{m_{sample} F_{standard}}{m_{standard} F_{sample}}$$

The m is the slope of the plot ΔA of DPBF (at 410 nm) vs. irradiation time, and F is the absorption correction factor, which is given by

$$F = \int (1 - 10^{-OD}) d\lambda$$

OD at the irradiation wavelength.

5. Computational details

In this work, we performed geometry optimizations for ground (S_0) and excited states (S_1 and T_1) followed by harmonic vibrational frequencies calculation with hybrid density functional, B3LYP,²⁻³ using the program package Gaussian 09 (Revision E.01)⁴. The 6-311G(d) basis set⁵⁻⁶ was used for all atoms. Frequency calculations were performed on the optimized structures to ensure that they were minimum energy structures by the absence of imaginary frequency. The spin-orbit coupling (SOC) integrals between

the spin state S_1 and T_1 were estimated at the optimized S_1 structures using effective atomic charge (Zeff) method by PySOC.⁷⁻⁸

6. Cell culture

HeLa, SK-BR-3 and MDA-MB-468 cells were incubated in complete medium (Dulbecco's modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin) at 37°C in atmosphere containing 5% CO₂.

7. CCK-8 assay

Cells were seeded in flat-bottomed 96-well plates, 1×10^4 cells per well, with 200 μ L complete culture media for 24 h. After washed with PBS three times, the cells were incubated with different concentrations of photosensitizers for 2 h. All stock solutions were prepared in DMSO (2 mM) and diluted with complete medium. Then the cells were washed with PBS (pH 7.4) three times, incubated in the phenol red-free culture media and then get irradiated with white light (Prior Lumen200, $0.4 \mu\text{W} \cdot \text{cm}^{-2}$) or red light (Prior Lumen200, filter 615/30, $0.4 \mu\text{W} \cdot \text{cm}^{-2}$) or 730 LED (675-750 nm) for different irradiation time. Then the cell culture media is changed back by complete culture media. After cultured for 24 h, 10 μ L Cell Counting Kit-8 (CCK-8) solution and 90 μ l PBS (pH 7.4) were added per well simultaneously. After 1-2 hours, the absorbance at 450 nm was read by 96-well plates reader. The viability of Hela cells was calculated by the following equation:

$$\text{CV} = \frac{(\text{As}-\text{Ab})}{(\text{Ac}-\text{Ab})} \times 100\%$$

CV stands for the viability of cells, As, Ac and Ab stand for the absorbance of cells containing photosensitizer, cell control (0 μ M photosensitizer) and blank control (wells containing neither cells nor photosensitizer).

For the dark cytotoxicity, every step is exactly the same as above only without irradiation.

For the cytotoxicity in presence of sodium azide, we add sodium azide during the irradiation step.

IC₅₀ values were calculated by Prism 6 using "log(inhibitor) vs. response -- Variable slope (four parameters)".

8. Generation and analysis of 3D multicellular tumor spheroids (MCTS)

The preparation of MCTS was with the aid of Corning 96-well Spheroid Microplates following their guidelines. A density of 10000 cells per well in 250 μ L of media. The MCTS were cultivated and maintained for four days. The formation, integrity, and diameter of the MCTS was monitored by z-stacking imaging from confocal laser scanning microscope (CLSM).

9. Cell viability of MCTS after PDT

The cytotoxicity of MCTS was assessed by measurement of the ATP concentration using CellTiter-Glo 3D Cell Viability kit (Promega). After cultured for 4 days, MCTS were treated with different concentrations of SDMNP by replacing 50% of the media with drug supplemented media and incubation for 2 h. After this

time, the MCTS were divided into two identical groups. The first group was strictly kept in the dark. The second group was exposed to white light (Prior Lumen200, 400-700 nm, $0.4 \mu\text{W cm}^{-2}$). After the irradiation, all groups were incubated an additional 24 h. The ATP concentration was measured using a CellTiter-Glo 3D Cell Viability kit (Promega) by measuring the generated chemiluminescence with an infinite 200 PRO (Tecan) plate reader. The obtained data were analyzed with the GraphPad Prism software.

10. Live/dead cell dual staining of MCTS

After cultured for 4 days, MCTS were treated with $2 \mu\text{M}$ SDMNP by replacing 50% of the media with drug supplemented media in the dark for 2 h. After this time, the MCTS were exposed to a white light (Prior Lumen200, 400-700 nm, $0.4 \mu\text{W cm}^{-2}$). 24 h after the irradiation the MCTS viability was tested using a Live/Dead dual staining kit for mammalian cells (Thermo Fisher). MCTS were incubated with $2 \mu\text{M}$ calcein AM and $4 \mu\text{M}$ propidium iodide for 30 min. Then the MCTS were washed with PBS gently once and turned to confocal laser scanning microscope. Images were taken under conditions as follows: $20\times$ lens with a resolution of 1024×1024 and a speed of 0.5 frames per second, 561 nm excitation wavelength and 552 to 617 nm detector slit, and 488 nm excitation wavelength and 500 to 530 nm detector slit. Differential interference contrast (DIC) and fluorescent images were processed and analyzed using ImageJ.

11. Live/dead cell dual staining of monolayer HeLa cells

HeLa cells were placed onto 0.1 mM poly-D-lysine coated glasses in complete media for 24 h. The cells were incubated with $2 \mu\text{M}$ SDMNP for 2 h. After washed with PBS for three times, cells were cultured in phenol red-free media and get irradiated with white light (Prior Lumen200, $0.4 \mu\text{W}\cdot\text{cm}^{-2}$) for 20 min. Then the cell culture media is changed by complete culture media containing $2 \mu\text{M}$ calcein AM and $4 \mu\text{M}$ propidium iodide and the cells were cultured for another 30 min. Then the cells were washed with PBS gently once and turned to confocal laser scanning microscope. Images were taken under conditions as follows: $60\times$ immersion lens with a resolution of 1024×1024 and a speed of 0.5 frames per second, 561 nm excitation wavelength, and 552 to 617 nm detector slit, and 488 nm excitation wavelength and 500 to 530 nm detector slit. Differential interference contrast (DIC) and fluorescent images were processed and analyzed using ImageJ.

12. Confocal imaging of intracellular ROS level

ROS detection reagent (H2DCFDA) was purchased from Invitrogen (Life Technologies). HeLa cells were placed on poly-D-lysine-coated sterile glass coverslips in cell culture dishes containing complete medium (2 mL) for 24 h. $2 \mu\text{M}$ (2 mM dissolved in DMSO) was added to complete culture medium and cultured for another 2 h. After washing with PBS three times, culture medium with $10 \mu\text{M}$ H2DCFDA was added to the dishes, and the cells were incubated for 30 min. After washing three times with PBS, they were immersed in phenol red-free culture media (1 mL) and irradiated for 20 min with white light (Prior Lumen200, $0.4 \mu\text{W}\cdot\text{cm}^{-2}$). Images were taken under conditions as follows: $60\times$ immersion lens with a

resolution of 1024×1024 and a speed of 0.5 frames per second, excitation wavelength 488 nm, 515/30 detector.

13. Preparation of Trastuzumab-SDMNP (Tras-SDMNP) conjugate.

Trastuzumab (10 μM, in saturated benzoic acid water solution) was reacted with 10 equivalents of SDMNP-NHS (100 μM, in saturated benzoic acid water solution) at 37°C for 12 hours. The resulting Tras-SDMNP was first purified by Corning® Spin-X® UF 6 mL Centrifugal Concentrator (10,000 MWCO Membrane) and followed by Thermo Scientific™ Zeba™ Spin Desalting Columns (40K MWCO) following the manufactures' instructions to concentrate and to remove the excess SDNMP-NHS. The isolated protein concentration was quantified by SDS-PAGE analysis followed by Coomassie staining compared to the wild type Trastuzumab.

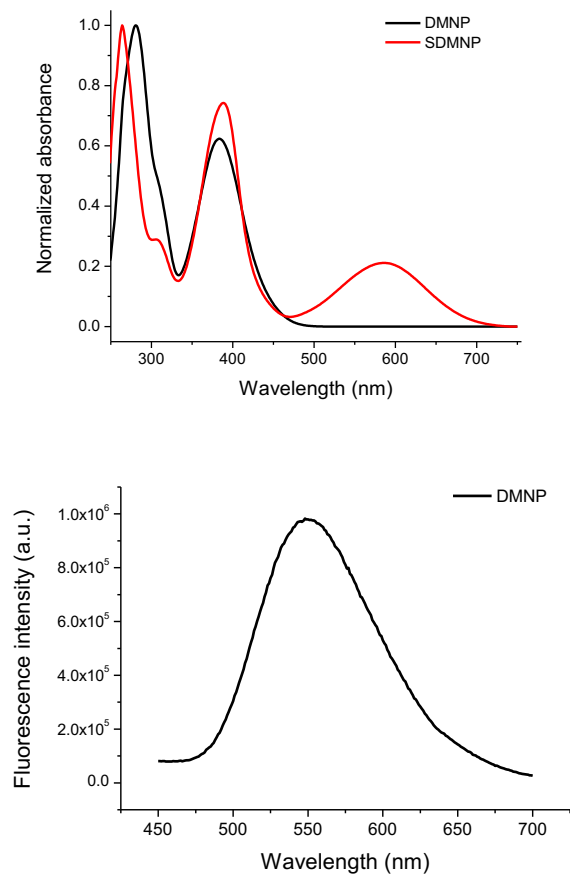


Fig. S1 UV-vis absorption (top) and fluorescence spectra (bottom) of DMNP and SDMNP.

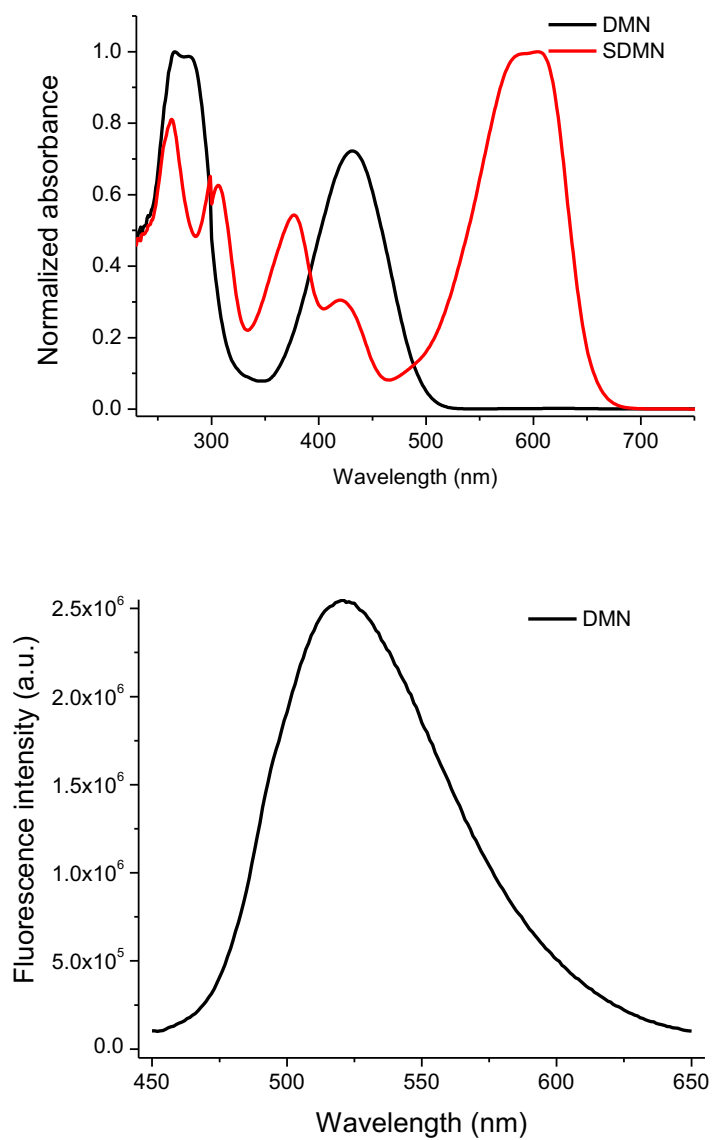


Fig. S2 UV-vis absorption (top) and fluorescence spectra (bottom) of DMN and SDM.

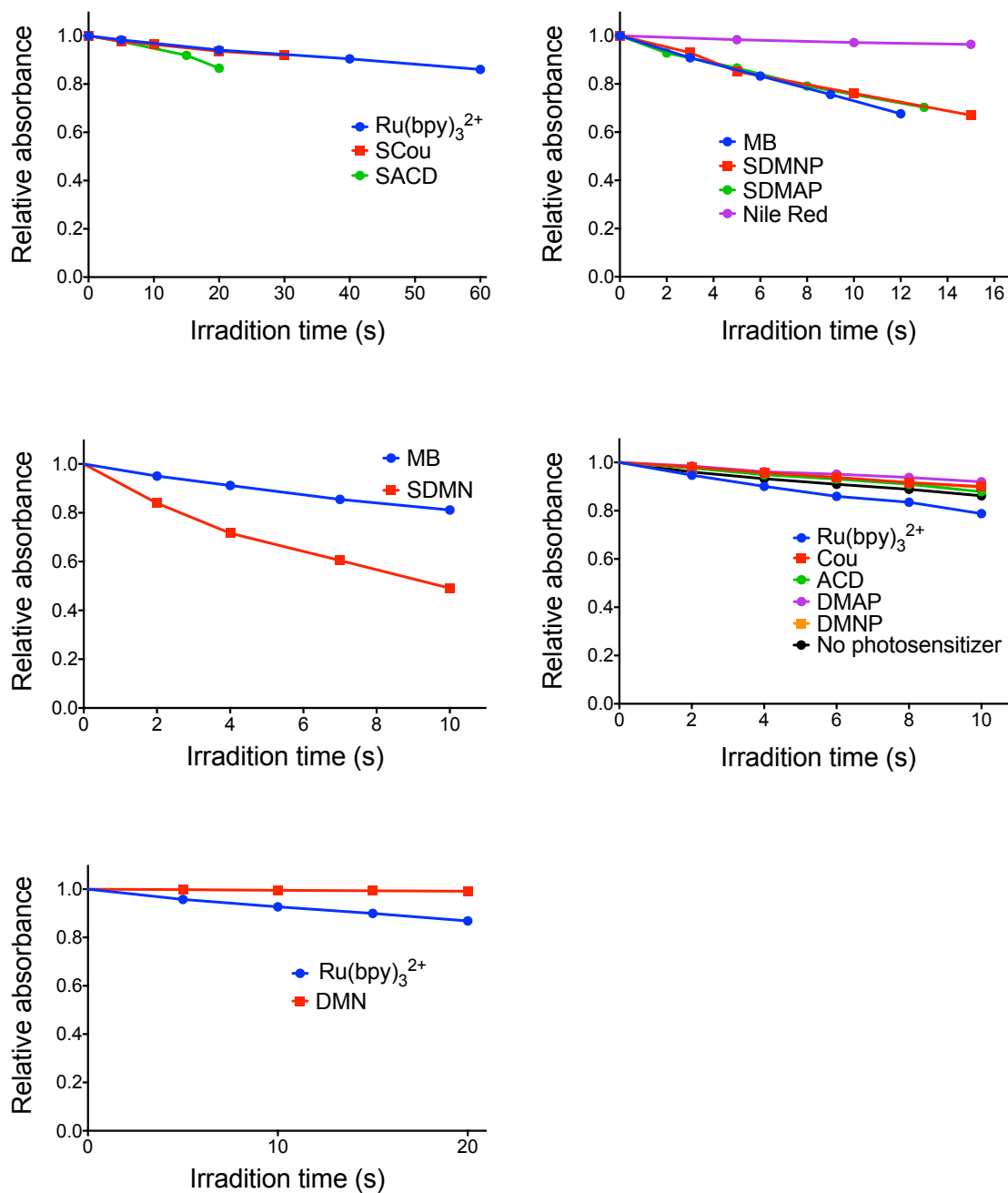


Fig. S3 Plots of changes in absorbance by DPBF at 410 nm against irradiation time in the presence of different compounds. Ru(bpy)₃²⁺ as standard for SCou and SADC ($\Phi_{\Delta} = 0.73$ in MeOH) and Methylene Blue as standard for SDMAP, SDMNP, SDMNP, and Nile Red ($\Phi_{\Delta} = 0.57$ in DCM). Light source: 453-473 nm for SADC and SCou; 600-630 nm for SDMAP, SDMNP, SDMNP, Nile Red; 565-605 nm for Nile Red; 405 nm for the rest.

Table S1. Calculated spin-orbit coupling (SOC) constants between S1 state and T1 state of DMNP and SDMNP.

Head 1	$ \langle S1 H_{SO} T1\rangle $	$ \langle S1 H_{SO} T1\rangle_x $	$ \langle S1 H_{SO} T1\rangle_y $	$ \langle S1 H_{SO} T1\rangle_z $
DMNP	0.18892 cm ⁻¹	0.30669 cm ⁻¹	0.18892 cm ⁻¹	0.15059 cm ⁻¹
SDMNP	71.81998 cm ⁻¹	101.60090 cm ⁻¹	71.81998 cm ⁻¹	2.55381 cm ⁻¹

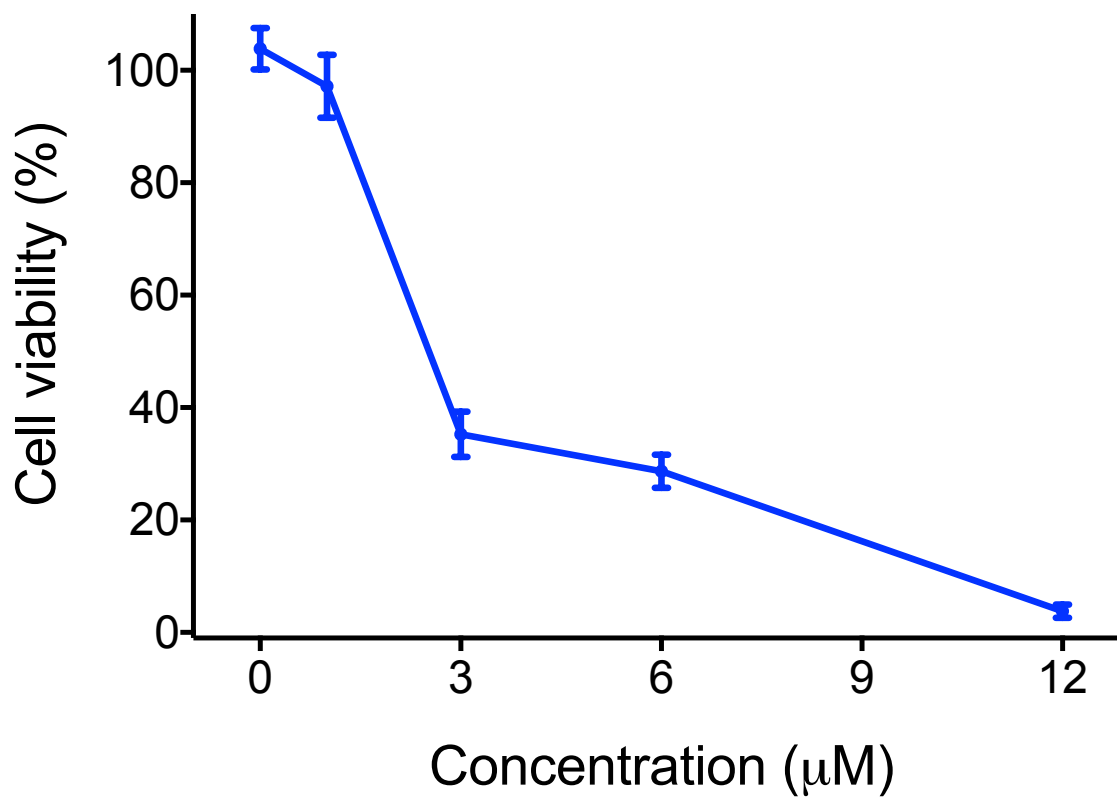


Fig. S4 Cell viability of SNile Red under 730 nm light.

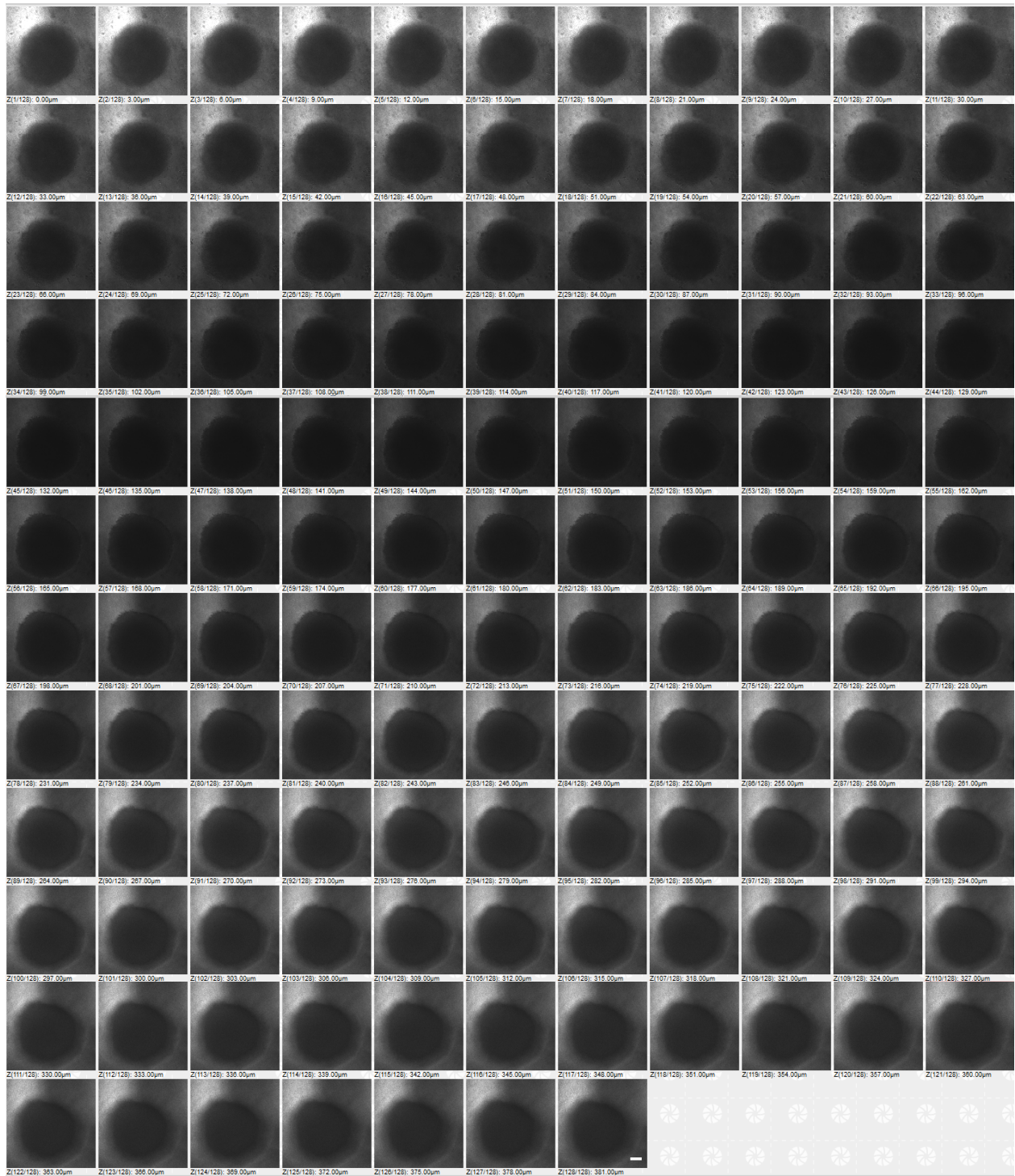


Fig. S5 z-stacking confocal images of MCTS. Scale bat: 100 µm.

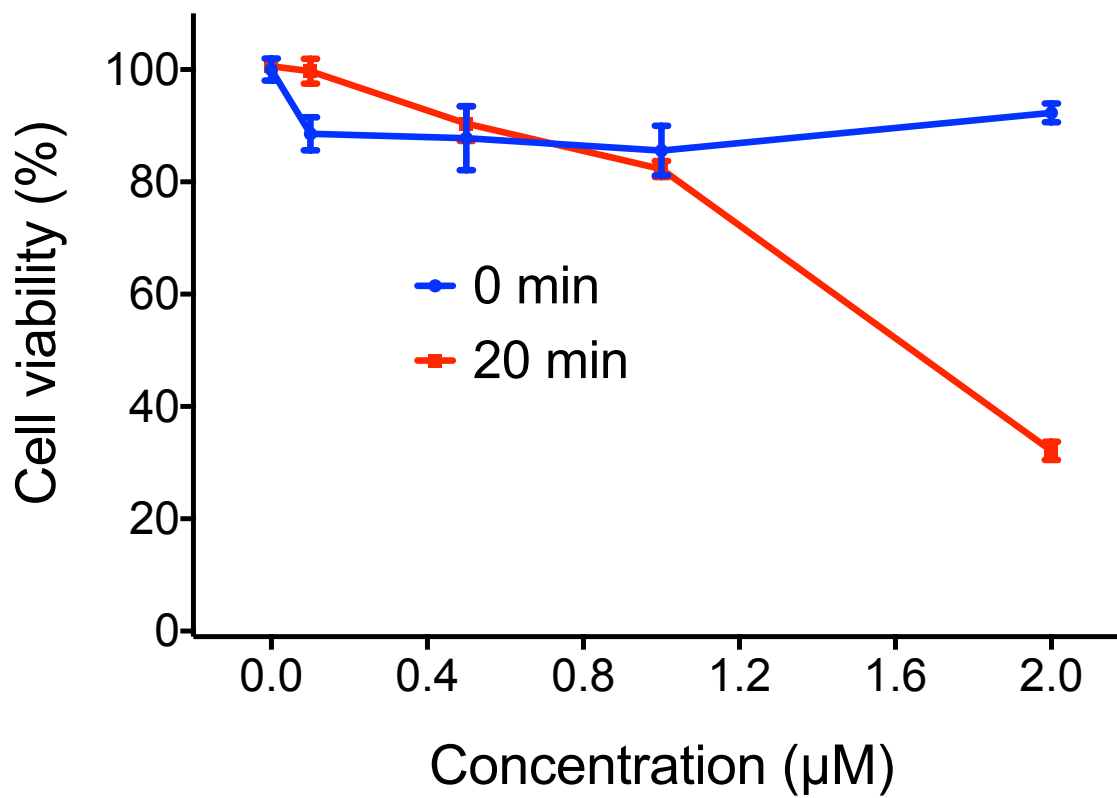


Fig. S6 Cell viability of MCTS using CellTiter-Glo 3D Cell Viability kit (Promega).

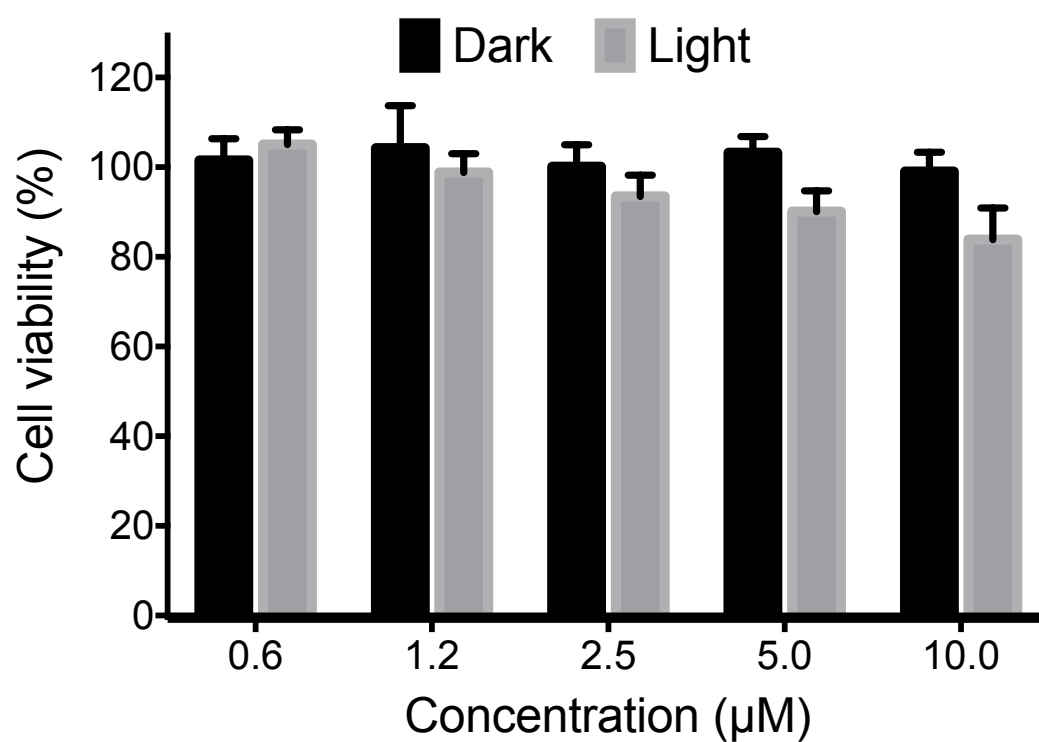
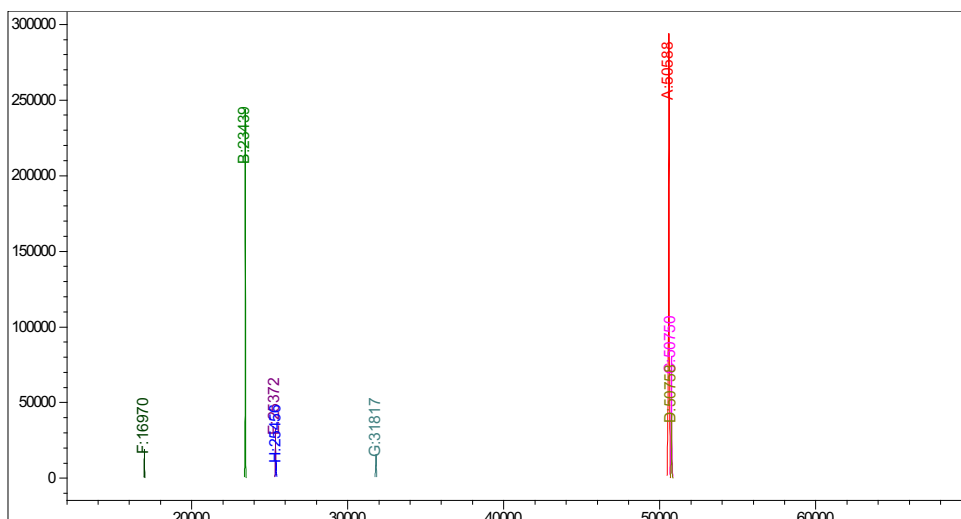


Fig. S7 Cell viability of methylene blue under dark or white light (400-700 nm).



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Fig. S8 ESI-MS of Tras-SDMNP, expected mass: $[M+Na]^+$ 50587 Da.

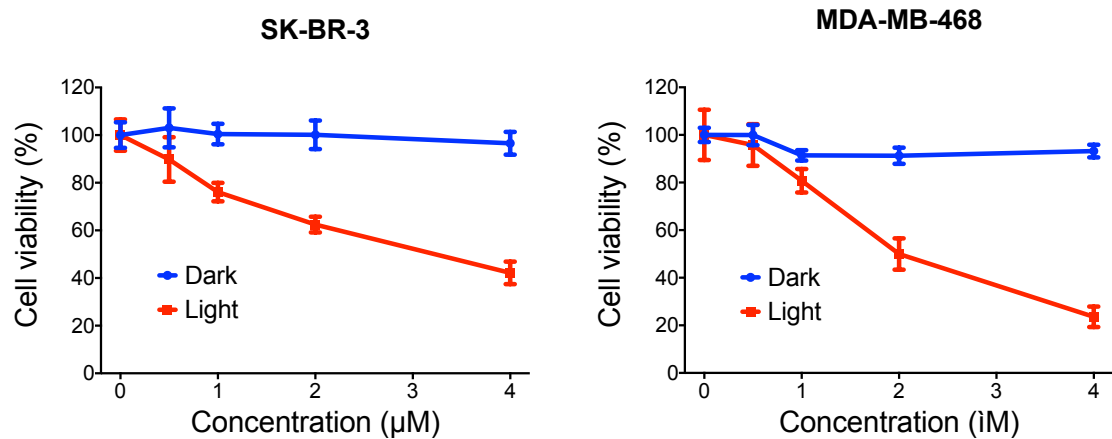


Fig. S9 Cell viability of SK-BR-3 and MDA-MB-468 cells with 24 h-incubation of 0.5 μM Tras-SDMNP after different irradiation times.

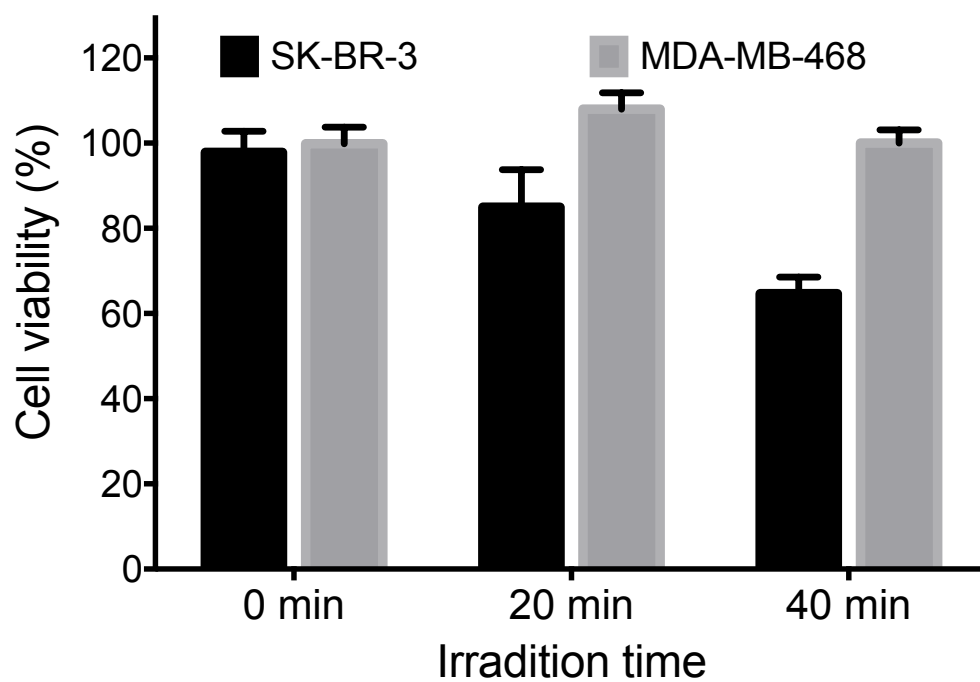


Fig. S10 Cell viability of SK-BR-3 and MDA-MB-468 cells with 24 h-incubation of 0.5 μM Tras-SDMNP after different irradiation times.

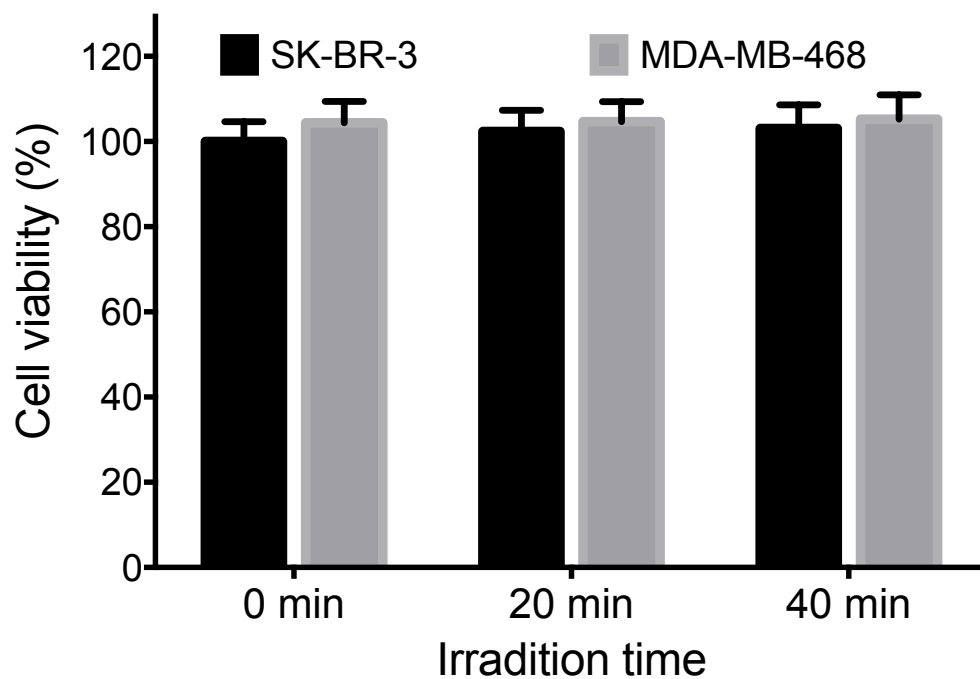


Fig. S11 Cell viability of SK-BR-3 and MDA-MB-468 cells after co-incubated with 0.5 μM Tras-SDMNP and 0.5 μM wt-Tras in presence of different irradiation times.

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