

# ChemMedChem

## Supporting Information

### **Significant Tumor Inhibition of Trimethyl-15<sup>2</sup>-[L-aspartyl]pheophorbide a in Tumor Photodynamic Therapy<sup>‡</sup>**

Anita Benić, Akmaral Kussayeva, Ivana Antol, Mario Vazdar, Zlatko Brkljača, Dan-Ye Chen, Yi-Jia Yan, Ying-Hua Gao,\* Zhi-Long Chen,\* and Davor Margetić\*

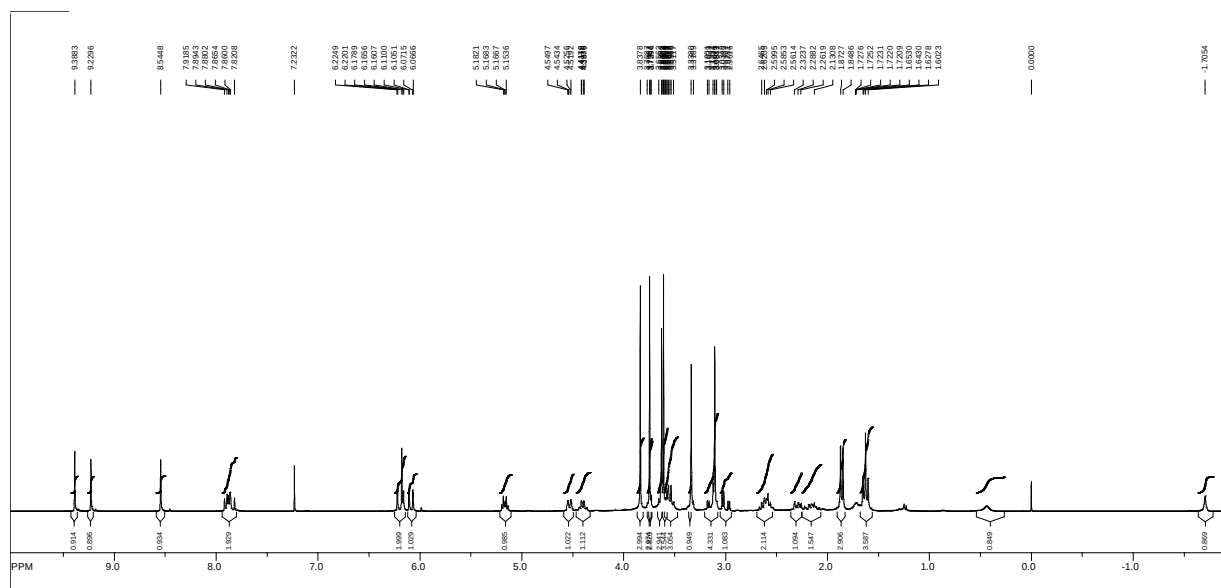
## Supporting Information

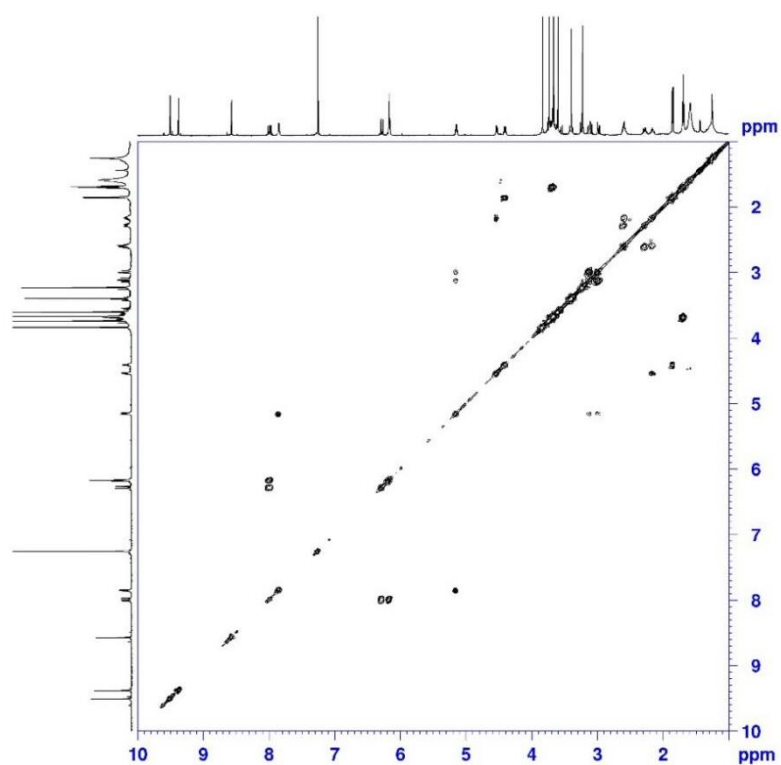
### Significant Tumor Inhibition of Trimethyl-15<sup>2</sup>-[L-aspartyl]pheophorbide a in Tumor Photodynamic Therapy

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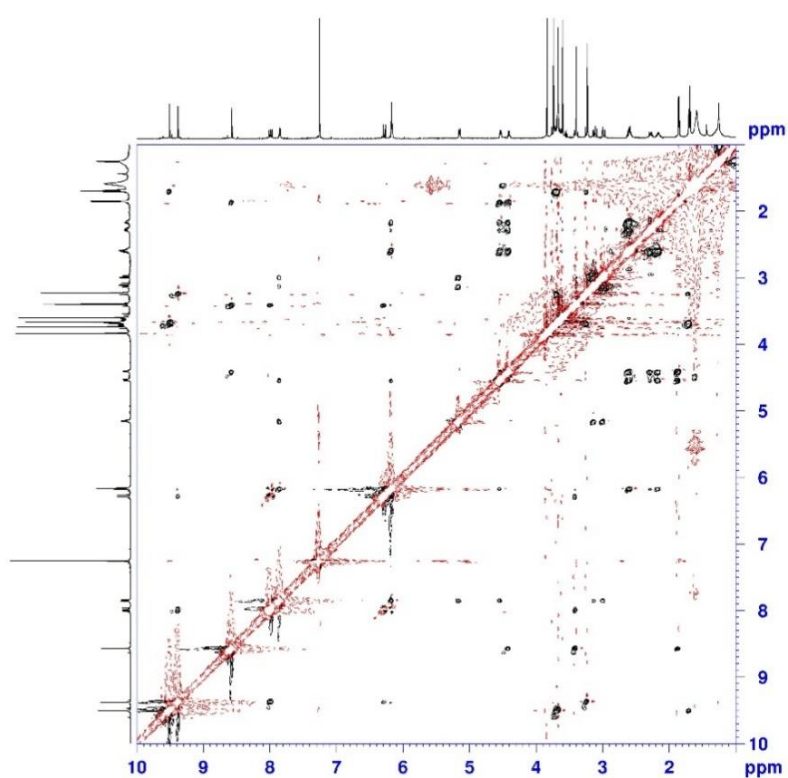
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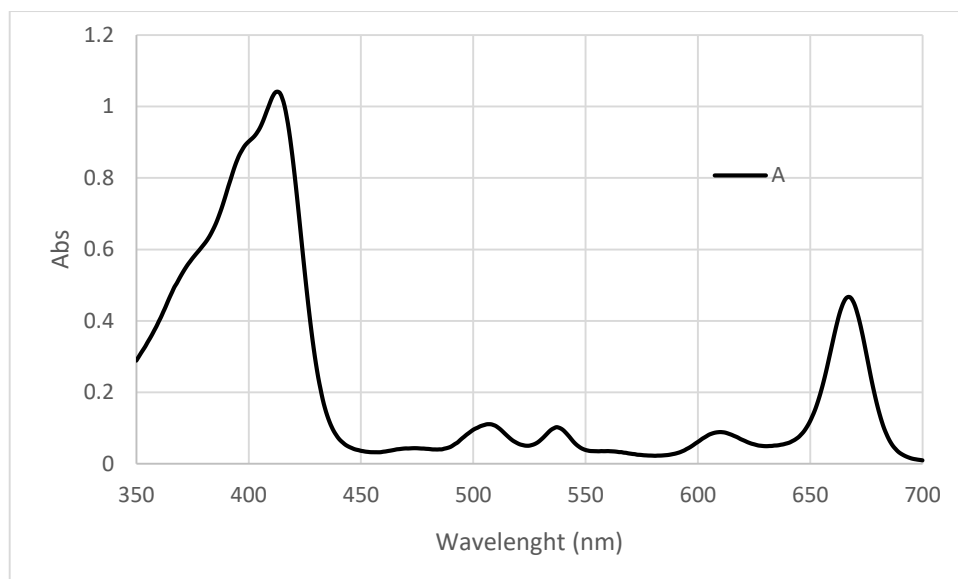




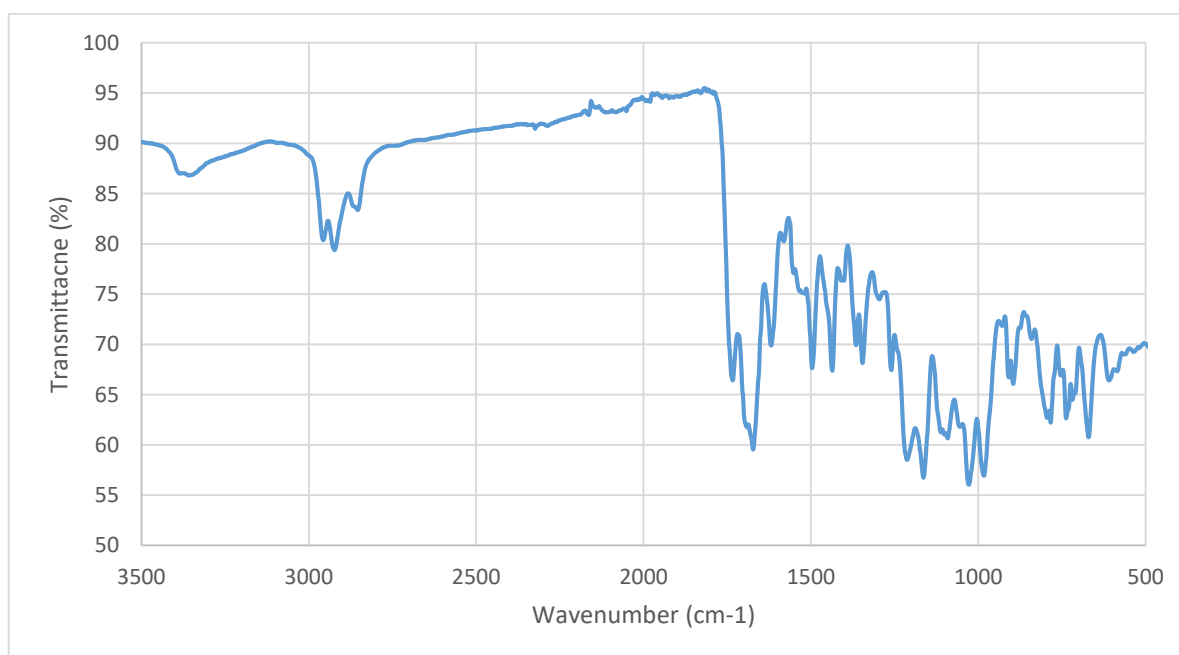
**Figure S3.** COSY NMR spectrum of trimethyl 15<sup>2</sup>-[L-aspartyl] pheophorbide a (**PS5**) in CDCl<sub>3</sub> (600 MHz)



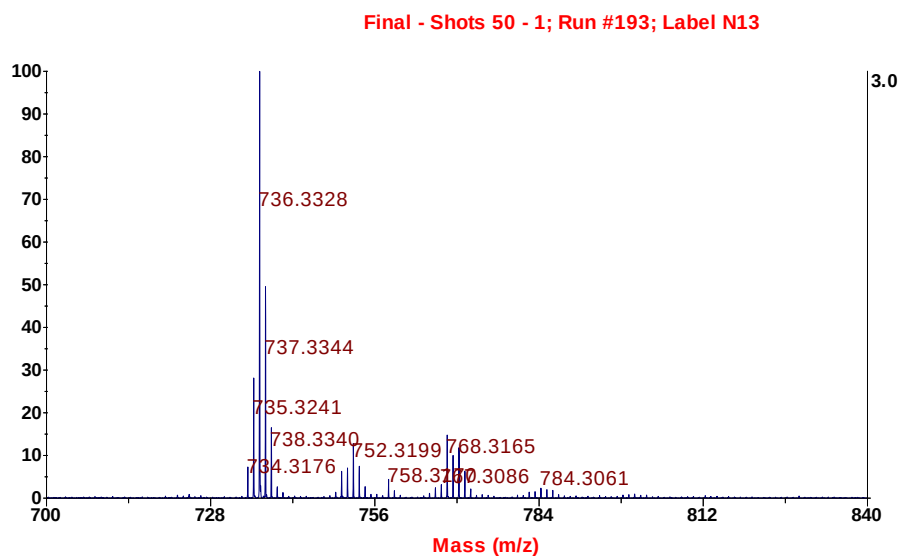
**Figure S4.** NOESY NMR spectrum of trimethyl 15<sup>2</sup>-[L-aspartyl] pheophorbide a (**PS5**) in CDCl<sub>3</sub> (600 MHz)



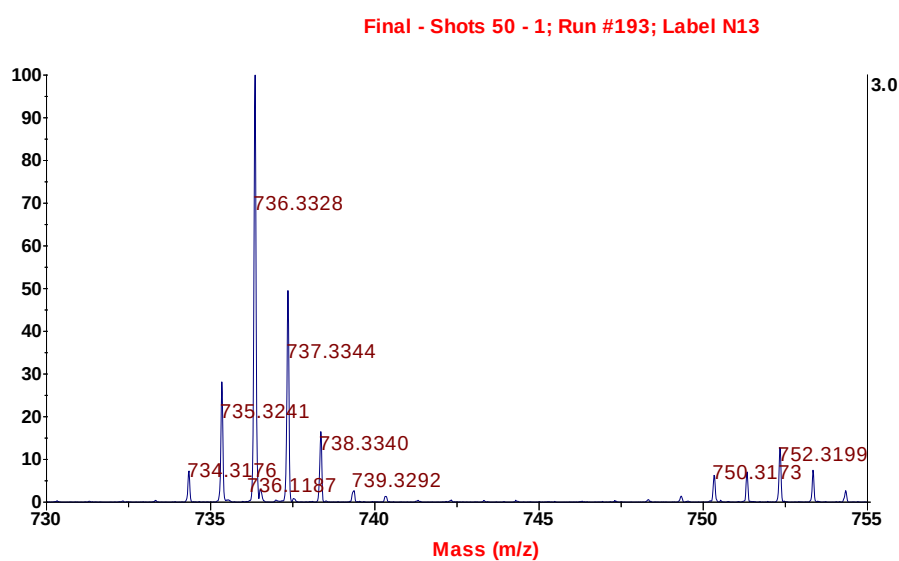
**Figure S5.** UV-Vis spectrum of trimethyl 15<sup>2</sup>-[L-aspartyl] pheophorbide a (PS5) in CHCl<sub>3</sub>



**Figure S6.** IR spectrum of trimethyl 15<sup>2</sup>-[L-aspartyl] pheophorbide a (PS5)

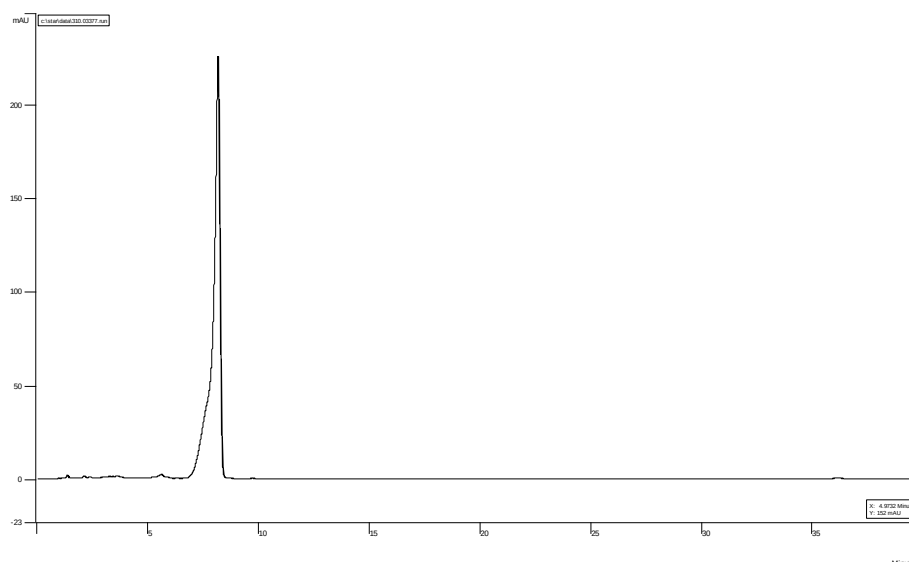


**Figure S7.** Mass spectrum of trimethyl 15<sup>2</sup>-[L-aspartyl] pheophorbide a (PS5) in the range  $m/z$  700-840



**Figure S8.** Mass spectrum of trimethyl 15<sup>2</sup>-[L-aspartyl] pheophorbide a (PS5) in the range  $m/z$  730-756

Analytical HPLC method: Varian ProStar 230 HPLC instrument with Microsorb-MV 100-5 column (C18, 150 mm  $\times$  4.6 mm  $\times$  1/4 inch, 5  $\mu$ m); solvent system: A) MeOH : NH<sub>4</sub>OAc (0.5 N in water); B) acetone : MeOH = 80 : 20. Program: 1) 0 - 27 min (80% - 5 % A), 2) 27-34 min (5 % A) 3) 34-36 min (5 % - 80 % A). Flow rate 1.5 mL/min; wavelength 414 nm; injection volume 10  $\mu$ L.



**Figure S9.** HPLC spectrum of compound **PS5** ( $t_R$  = 8.16 min).

## PHOTOPHYSICAL PROPERTIES

### Photophysical properties

UV-vis absorption spectrum was measured on an Ultraviolet-visible Spectrophotometer (Model V-530, Japan). Fluorescence measurements were carried out with a Fluorescence Spectrometer (FluoroMax-4, France). Compound **PS5** was dissolved in DMSO to get the 10  $\mu$ M concentration solutions. Slits were kept narrow to 1 nm in excitation and 1 nm in emission. All the measurements were carried out at room temperature.

### Evaluation of singlet oxygen generation

In vitro photo-bleaching was conducted by observing the UV-vis spectra of compound **PS5** at 10  $\mu$ M concentrations dissolved in DMSO. The solution of compound **PS5** in the cuvette was then irradiated with a 650 nm laser (therapeutic light) at various time points for 90 min. The UV-vis spectra were obtained every 10 min in 90 min.

The  $^1\text{O}_2$  generation was evaluated with DPBF as a singlet oxygen ( $^1\text{O}_2$ ) scavenger.<sup>1</sup> A mixed solution of compound (2  $\mu$ M) and DPBF (60  $\mu$ M) was irradiated with a 650 nm laser and the



absorption value at 417 nm was monitored using a UV-vis spectrophotometer every 10 s. The observed rate constants ( $k$ ) were calculated from  $\ln ([DPBF]_t / [DPBF]_0) = -kt$ , where  $[DPBF]_t$  and  $[DPBF]_0$  represent the ultraviolet-visible absorbance at time  $t$  and time 0, respectively.<sup>2</sup> Comparison of the slopes for the samples with the reference yielded the singlet oxygen quantum yield straightforwardly as given in the equation below.

$$\Phi_{\Delta}^S = \frac{K_S}{K_R} \times \Phi_{\Delta}^R$$

The superscripts/subscripts S and R stand for the sample and the reference, respectively.

## BIOLOGICAL EVALUATION

### Chemicals and reagents

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), the Hoechst 33342, Mito-Tracker Green (MTG), Lyso-Tracker Blue and ER-Green [DiOC6(3)] were purchased from KeyGen BioTech, China. 1,3-diphenylisobenzofuran (DPBF), dimethyl sulfoxide (DMSO), and *N, N*-dimethylformamide (DMF) were obtained from Sinopharm Chemical Reagent Co., Ltd. All the chemicals and reagents were of analytical grade and used without any purification.

### Cell culture

The lung cancer cells (A549) were purchased from the Type Culture Collection, the Chinese Academy of Sciences. The cell line was cultured in F-12K medium added with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin, and 1 mM glutamine. Cells were incubated at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere.

### MTT assay

The cells ( $4 \times 10^5$  cells/well) were seeded in 96-well micro-plates in a complete medium for dark and light-dependent cytotoxicity measurement. After 24 h, the cells were washed once and incubated for another 24 h with a complete medium containing various concentrations of compound (0, 0.1, 0.3, 0.5, 0.7, and 0.9 µM), respectively. All samples were handled in the dark. Cellular survival was measured using a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide (MTT) assay. Briefly, at the end of the incubation period, 20 µl MTT (5 mg/mL) reagent was added to each well and incubated for 4 h at 37°C. Then, the medium was removed, and the formazan complex was solubilized with 150 µL DMSO. The absorbance of the complex was measured with a microplate reader at a wavelength of 570 nm.

### Subcellular localisation assay

The cells were grown in 35 mm Petri dish and incubated at 37 °C with 5  $\mu$ M **PS5** for 4 h in the dark, then rinsed in the medium and incubated with Mito-Tracker-Green (200 nM), Lyso-Tracker Blue (5  $\mu$ M), or ER-Tracker Green (500 nM), respectively. After washed with PBS, the cells were fixed for 10 min at 4 °C with 4% paraformaldehyde and then examined by fluorescence with confocal microscopy (Carl Zeiss LSM 700, Jena, Germany). Photosensitizer **PS5** was excited at 415 nm and monitored at 600-700 nm.

### **ROS generation**

A549 cells were seeded in a 35 mm cell culture dish at a density of  $1 \times 10^4$  cells/well. After 24 h, the cells were treated with **PS5**. After 4 h, the cells were treated with PDT (650 nm) and stained with DCFH-DA according to the manufacturer's instructions.

### **Caspase 3 activity**

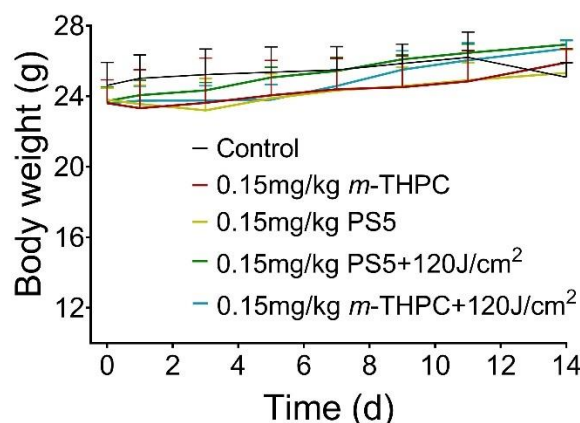
Caspase activity was determined by a colorimetric assay based on the ability of caspase-3 to change acetyl-Asp-Glu-Val-Asp *p*-nitroanilide (Ac-DEVD-pNA) into a yellow formazan product (*p*-nitroaniline (pNA)). An increase in absorbance at 405 nm was used to quantify the activation of caspase activity.

### **Animal models**

Balb/c nude mice (5 weeks old), were used to establish a lung cancer mouse model. Tumors were allowed to grow to about 150-170 mm<sup>3</sup> in volume before being used for PDT.

### ***In vivo* therapeutic efficacy**

The A549 tumor-bearing Balb/c nude mice were randomly divided into 5 groups: (1) PBS as a control, (2) 0.15 mg/kg *m*-THPC without irradiation, (3) 0.15 mg/kg **PS5** without irradiation, (4) 0.15 mg/kg *m*-THPC with irradiation, and (5) 0.15 mg/kg **PS5** with irradiation. Therapy was continued through tail vein injection for 14 days. The mice were locally irradiated with a 650 nm laser (200 mW/cm<sup>2</sup>, 120 J/cm<sup>2</sup>) after injection for PDT treatment. The body weight and the tumor size of each mouse were recorded to evaluate the therapy efficiency and toxicity: the tumor volume (V) was calculated:  $V = a \times b^2/2$  (a, the length of the tumor; b, the width of the tumor). Finally, organs of the mice, including the heart, livers, spleen, lung, kidney, and tumors, were harvested and collected immediately for H&E and TUNEL histology analysis.



**Figure S10.** The body weight of A549 xenograft tumor mice with different treatments.

### Statistical analysis

Graphics were done by Origin Pro 8.0 (Graph Software, USA). All results are presented as mean  $\pm$  SD. Statistical significance was determined by unpaired two-tailed t-tests or two-way analysis of variance. \*  $P < 0.05$  and \*\*  $P < 0.01$  were considered statistically significant.

### Intracellular localization

The subcellular localization of photosensitizer **PS5** was evaluated by a confocal laser scanning microscope (CLSM). The experiments were conducted using the Mito-Track Green (MTG), Lyso-Track Green (LTG), and ER-Tracker Green (ETG).

### Plasma concentration of chlorophyll derivatives

#### 1. Drug dissolution

The compounds (**PS5**, *m*-THPC, 7.5 mg) were dissolved in dichloromethane (DCM, 3 mL) and then polyoxyethylene castor oil (200  $\mu$ L) was added. After mixing, the organic solvent was evaporated by a rotary evaporator. Finally, the samples with PBS were mixed to the final concentration. We found that compound **PS5** precipitates quickly when dissolved in PBS. *m*-THPC was dissolved in THF by the same method.

#### 2. Standard curve

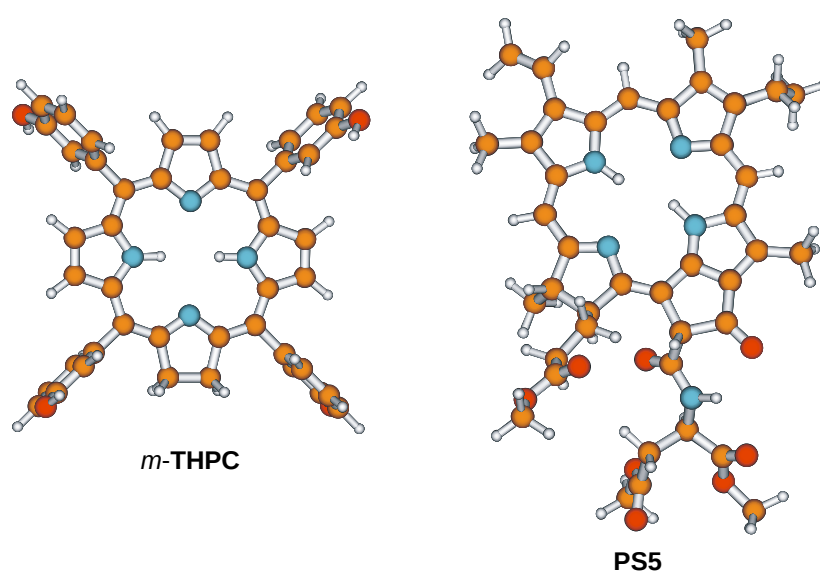
36 KM mice were randomly divided into groups ( $n=18$ ): *m*-THPC, **PS5**. The mice were sacrificed after taking a blood sample. The photosensitizers were diluted in different concentrations (0, 0.00125, 0.0025, 0.005, 0.01, 0.015 mg/mL) with plasma. The samples were separated by centrifugation at 12000 rpm for 10 min at 4  $^{\circ}$ C. The supernatant (250  $\mu$ L) was mixed with DMSO (2.75 mL) and the fluorescence intensity of the solution was measured by a fluorescence spectrophotometer.

#### 3. Experiment Method

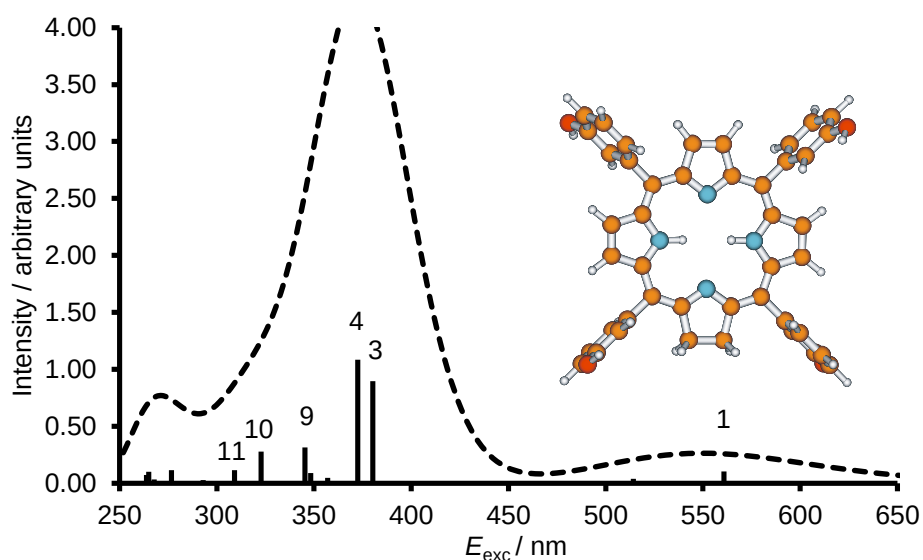
60 KM mice (28 - 30 g, males) were fed in the environment under constant temperature (25-26  $^{\circ}$ C) and humidity (50-60%), with a 12 h light/dark cycle. The mice were randomly divided into groups ( $n=30$ ): *m*-THPC, **PS5**. The mice were sacrificed at different times after a tail vein

intravenous injection of compounds (5 mg/kg). Blood samples were collected into the polypropylene microfuge tubes containing heparin sodium. Plasma was separated by centrifugation at 12000 rpm for 10 min at 4 °C. Plasma (250  $\mu$ L) was mixed with DMSO (2.75 mL) and the fluorescence intensity of the solution was measured by a fluorescence spectrophotometer.

## COMPUTATION OF PHOTOPHYSICAL PROPERTIES



**Figure S11.** B3LYP/6-311+G(d) optimized structures of *m*-THPC, and PS5.

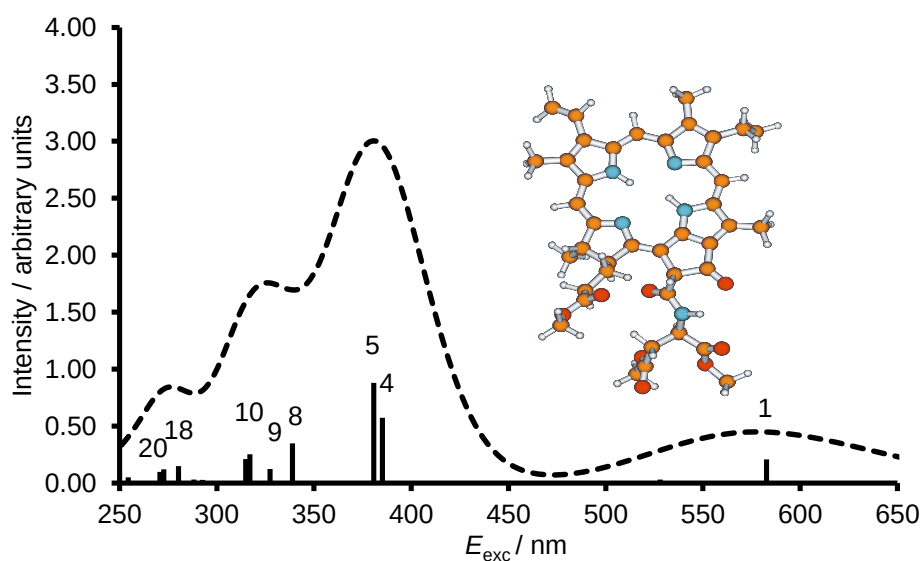


**Figure S12.** Simulated UV-Vis spectra of *m*-THPC calculated at TD DFT level of theory using PBE0 density functional and Pople type 6-311+G(2d,p) basis set.

**Table ST1.** TD-DFT calculated vertical excitation energies ( $E_{\text{exc}}$ ), oscillator strengths ( $f$ ) and leading configurations for *m*-THPC at the PBE0/6-311+G(2d,p) level of theory.

| No.                    | $E_{\text{exc}}/\text{eV}$ | $f$           | Leading configurations         | Coefficients   | $E_{\text{exc}}/\text{nm}$ |
|------------------------|----------------------------|---------------|--------------------------------|----------------|----------------------------|
| Singlet excited states |                            |               |                                |                |                            |
| <b>1</b>               | <b>2.211</b>               | <b>0.1046</b> | <b>Singlet-A 178 -&gt; 179</b> | <b>0.60665</b> | <b>561</b>                 |
| 2                      | 2.411                      | 0.0399        | Singlet-A 177 -> 179           | 0.5720         | 514                        |
| <b>3</b>               | <b>3.261</b>               | <b>0.8954</b> | <b>Singlet-A 177 -&gt; 180</b> | <b>0.57159</b> | <b>380</b>                 |
| <b>4</b>               | <b>3.328</b>               | <b>1.0843</b> | <b>Singlet-A 178 -&gt; 180</b> | <b>0.52174</b> | <b>373</b>                 |
| 5                      | 3.380                      | 0.0001        | Singlet-A 176 -> 179           | 0.66986        | 367                        |
| 6                      | 3.473                      | 0.0481        | Singlet-A 175 -> 179           | 0.68646        | 357                        |
| 7                      | 3.559                      | 0.089         | Singlet-A 173 -> 179           | 0.61905        | 348                        |
| 8                      | 3.563                      | 0.0018        | Singlet-A 174 -> 179           | 0.68234        | 348                        |
| <b>9</b>               | <b>3.590</b>               | <b>0.3157</b> | <b>Singlet-A 172 -&gt; 179</b> | <b>0.58721</b> | <b>345</b>                 |
| <b>10</b>              | <b>3.842</b>               | <b>0.2774</b> | <b>Singlet-A 176 -&gt; 180</b> | <b>0.55369</b> | <b>323</b>                 |
| <b>11</b>              | <b>4.010</b>               | <b>0.1156</b> | <b>Singlet-A 171 -&gt; 179</b> | <b>0.44768</b> | <b>309</b>                 |
| 12                     | 4.020                      | 0.0109        | Singlet-A 175 -> 180           | 0.68968        | 308                        |
| 13                     | 4.046                      | 0.0044        | Singlet-A 171 -> 179           | 0.43172        | 306                        |
| 14                     | 4.099                      | 0.0006        | Singlet-A 164 -> 179           | 0.49894        | 302                        |
| 15                     | 4.129                      | 0.0132        | Singlet-A 170 -> 179           | 0.56644        | 300                        |
| 16                     | 4.134                      | 0.0117        | Singlet-A 178 -> 181           | 0.56078        | 300                        |
| 17                     | 4.143                      | 0.0036        | Singlet-A 169 -> 179           | 0.41068        | 299                        |
| 18                     | 4.160                      | 0.0001        | Singlet-A 174 -> 180           | 0.69596        | 298                        |
| 19                     | 4.163                      | 0             | Singlet-A 178 -> 182           | 0.58282        | 298                        |
| 20                     | 4.169                      | 0.0013        | Singlet-A 173 -> 180           | 0.65997        | 297                        |
| 21                     | 4.231                      | 0.0272        | Singlet-A 168 -> 179           | 0.55084        | 293                        |
| 22                     | 4.275                      | 0.0026        | Singlet-A 178 -> 183           | 0.64847        | 290                        |
| 23                     | 4.279                      | 0.0013        | Singlet-A 167 -> 179           | 0.45539        | 290                        |
| 24                     | 4.292                      | 0.017         | Singlet-A 166 -> 179           | 0.55482        | 289                        |
| 25                     | 4.295                      | 0.0041        | Singlet-A 178 -> 184           | 0.57367        | 289                        |
| 26                     | 4.318                      | 0.02          | Singlet-A 177 -> 181           | 0.48866        | 287                        |
| 27                     | 4.336                      | 0.0018        | Singlet-A 163 -> 179           | 0.63248        | 286                        |
| 28                     | 4.342                      | 0.01          | Singlet-A 177 -> 182           | 0.65418        | 286                        |
| 29                     | 4.436                      | 0.0004        | Singlet-A 177 -> 183           | 0.66111        | 279                        |
| 30                     | 4.466                      | 0.0104        | Singlet-A 177 -> 184           | 0.69067        | 278                        |
| 31                     | 4.481                      | 0.1153        | Singlet-A 165 -> 179           | 0.44625        | 277                        |
| 32                     | 4.531                      | 0.0034        | Singlet-A 164 -> 180           | 0.4038         | 274                        |
| 33                     | 4.547                      | 0.0157        | Singlet-A 178 -> 185           | 0.46831        | 273                        |
| 34                     | 4.631                      | 0.0333        | Singlet-A 171 -> 180           | 0.493          | 268                        |
| 35                     | 4.636                      | 0.0069        | Singlet-A 178 -> 187           | 0.56692        | 267                        |
| 36                     | 4.646                      | 0.0198        | Singlet-A 178 -> 186           | 0.59212        | 267                        |
| 37                     | 4.678                      | 0.0998        | Singlet-A 170 -> 180           | 0.43204        | 265                        |
| 38                     | 4.699                      | 0.0716        | Singlet-A 170 -> 180           | 0.49694        | 264                        |
| 39                     | 4.715                      | 0.0191        | Singlet-A 178 -> 188           | 0.55358        | 263                        |
| 40                     | 4.777                      | 0.0002        | Singlet-A 178 -> 190           | 0.5761         | 260                        |

| Triplet excited states |       |   |           |            |         |     |
|------------------------|-------|---|-----------|------------|---------|-----|
| 1                      | 1.298 | 0 | Triplet-A | 177 -> 179 | 0.6727  | 955 |
| 2                      | 1.525 | 0 | Triplet-A | 178 -> 179 | 0.6991  | 813 |
| 3                      | 2.018 | 0 | Triplet-A | 178 -> 180 | 0.65763 | 614 |
| 4                      | 2.257 | 0 | Triplet-A | 177 -> 180 | 0.68731 | 549 |
| 5                      | 2.990 | 0 | Triplet-A | 172 -> 179 | 0.56951 | 415 |
| 6                      | 3.070 | 0 | Triplet-A | 172 -> 180 | 0.56173 | 404 |
| 7                      | 3.326 | 0 | Triplet-A | 176 -> 179 | 0.52608 | 373 |
| 8                      | 3.349 | 0 | Triplet-A | 175 -> 179 | 0.56416 | 370 |
| 9                      | 3.438 | 0 | Triplet-A | 174 -> 179 | 0.42763 | 361 |
| 10                     | 3.451 | 0 | Triplet-A | 173 -> 179 | 0.47878 | 359 |
| 11                     | 3.474 | 0 | Triplet-A | 171 -> 179 | 0.32909 | 357 |
| 12                     | 3.536 | 0 | Triplet-A | 166 -> 179 | 0.4144  | 351 |
| 13                     | 3.551 | 0 | Triplet-A | 167 -> 179 | 0.34712 | 349 |
| 14                     | 3.603 | 0 | Triplet-A | 170 -> 184 | 0.25371 | 344 |
| 15                     | 3.621 | 0 | Triplet-A | 174 -> 179 | 0.26605 | 342 |
| 16                     | 3.624 | 0 | Triplet-A | 173 -> 179 | 0.38138 | 342 |
| 17                     | 3.639 | 0 | Triplet-A | 174 -> 179 | 0.37212 | 341 |
| 18                     | 3.680 | 0 | Triplet-A | 178 -> 185 | 0.32865 | 337 |
| 19                     | 3.871 | 0 | Triplet-A | 164 -> 179 | 0.49282 | 320 |
| 20                     | 3.892 | 0 | Triplet-A | 176 -> 180 | 0.51008 | 319 |
| 21                     | 3.904 | 0 | Triplet-A | 175 -> 180 | 0.41853 | 318 |
| 22                     | 3.921 | 0 | Triplet-A | 175 -> 180 | 0.40989 | 316 |
| 23                     | 3.994 | 0 | Triplet-A | 163 -> 179 | 0.64203 | 310 |
| 24                     | 4.074 | 0 | Triplet-A | 178 -> 181 | 0.4842  | 304 |
| 25                     | 4.079 | 0 | Triplet-A | 178 -> 182 | 0.30295 | 304 |
| 26                     | 4.109 | 0 | Triplet-A | 177 -> 185 | 0.29375 | 302 |
| 27                     | 4.113 | 0 | Triplet-A | 174 -> 180 | 0.3895  | 301 |
| 28                     | 4.124 | 0 | Triplet-A | 178 -> 182 | 0.26029 | 301 |
| 29                     | 4.127 | 0 | Triplet-A | 170 -> 179 | 0.57903 | 300 |
| 30                     | 4.146 | 0 | Triplet-A | 178 -> 183 | 0.34384 | 299 |
| 31                     | 4.151 | 0 | Triplet-A | 173 -> 180 | 0.39615 | 299 |
| 32                     | 4.194 | 0 | Triplet-A | 174 -> 180 | 0.36166 | 296 |
| 33                     | 4.200 | 0 | Triplet-A | 177 -> 181 | 0.28567 | 295 |
| 34                     | 4.212 | 0 | Triplet-A | 169 -> 179 | 0.31903 | 294 |
| 35                     | 4.245 | 0 | Triplet-A | 168 -> 179 | 0.61044 | 292 |
| 36                     | 4.268 | 0 | Triplet-A | 169 -> 179 | 0.47623 | 291 |
| 37                     | 4.299 | 0 | Triplet-A | 177 -> 181 | 0.50334 | 288 |
| 38                     | 4.302 | 0 | Triplet-A | 178 -> 183 | 0.42318 | 288 |
| 39                     | 4.330 | 0 | Triplet-A | 177 -> 182 | 0.44712 | 286 |
| 40                     | 4.339 | 0 | Triplet-A | 177 -> 182 | 0.35431 | 286 |



**Figure S13.** Simulated UV-Vis spectra of protonated guanidine derivative **PS5** calculated at TD DFT level of theory using PBE0 density functional and Pople type 6-311+G(2d,p) basis set.

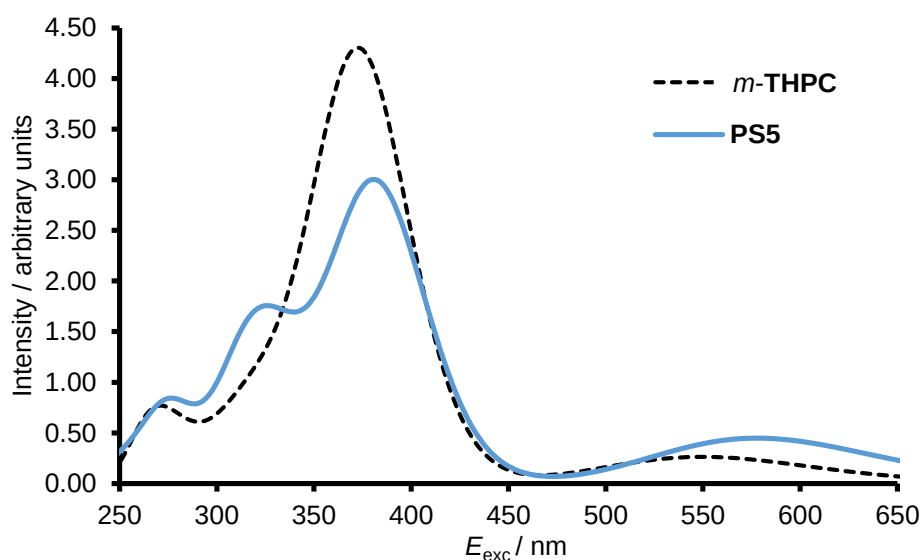
**Table ST2.** TD-DFT calculated vertical excitation energies ( $E_{\text{exc}}$ ), oscillator strengths ( $f$ ) and leading configurations for **PS5** at the PBE0/6-311+G(2d,p) level of theory.

| No.                    | $E_{\text{exc}}/\text{eV}$ | $f$           | Leading configurations         | Coefficients   | $E_{\text{exc}}/\text{nm}$ |
|------------------------|----------------------------|---------------|--------------------------------|----------------|----------------------------|
| Singlet excited states |                            |               |                                |                |                            |
| <b>1</b>               | <b>2.128</b>               | <b>0.2073</b> | <b>Singlet-A 195 -&gt; 196</b> | <b>0.63629</b> | <b>583</b>                 |
| 2                      | 2.348                      | 0.0306        | Singlet-A 194 -> 196           | 0.59856        | 528                        |
| 3                      | 3.041                      | 0.0090        | Singlet-A 193 -> 196           | 0.68026        | 408                        |
| <b>4</b>               | <b>3.219</b>               | <b>0.5730</b> | <b>Singlet-A 195 -&gt; 197</b> | <b>0.44638</b> | <b>385</b>                 |
| <b>5</b>               | <b>3.257</b>               | <b>0.8805</b> | <b>Singlet-A 194 -&gt; 197</b> | <b>0.43976</b> | <b>381</b>                 |
| 6                      | 3.469                      | 0.0088        | Singlet-A 192 -> 196           | 0.69027        | 357                        |
| 7                      | 3.487                      | 0.0017        | Singlet-A 191 -> 196           | 0.60809        | 356                        |
| <b>8</b>               | <b>3.659</b>               | <b>0.3486</b> | <b>Singlet-A 193 -&gt; 197</b> | <b>0.60047</b> | <b>339</b>                 |
| <b>9</b>               | <b>3.787</b>               | <b>0.1245</b> | <b>Singlet-A 195 -&gt; 198</b> | <b>0.62899</b> | <b>327</b>                 |
| <b>10</b>              | <b>3.912</b>               | <b>0.2532</b> | <b>Singlet-A 194 -&gt; 198</b> | <b>0.48578</b> | <b>317</b>                 |
| 11                     | 3.930                      | 0.0728        | Singlet-A 187 -> 196           | 0.57799        | 315                        |
| <b>12</b>              | <b>3.937</b>               | <b>0.2096</b> | <b>Singlet-A 190 -&gt; 196</b> | <b>0.44452</b> | <b>315</b>                 |
| 13                     | 4.012                      | 0.0011        | Singlet-A 185 -> 196           | 0.53352        | 309                        |
| 14                     | 4.102                      | 0.0106        | Singlet-A 189 -> 196           | 0.62897        | 302                        |
| 15                     | 4.183                      | 0.0067        | Singlet-A 188 -> 196           | 0.63360        | 296                        |
| 16                     | 4.237                      | 0.0292        | Singlet-A 192 -> 197           | 0.61083        | 293                        |
| 17                     | 4.301                      | 0.0312        | Singlet-A 186 -> 196           | 0.47027        | 288                        |
| <b>18</b>              | <b>4.423</b>               | <b>0.1481</b> | <b>Singlet-A 195 -&gt; 199</b> | <b>0.58789</b> | <b>280</b>                 |
| 19                     | 4.506                      | 0.0046        | Singlet-A 187 -> 197           | 0.45428        | 275                        |
| <b>20</b>              | <b>4.545</b>               | <b>0.1211</b> | <b>Singlet-A 194 -&gt; 199</b> | <b>0.63704</b> | <b>273</b>                 |
| 21                     | 4.552                      | 0.0031        | Singlet-A 191 -> 197           | 0.51934        | 272                        |
| <b>22</b>              | <b>4.578</b>               | <b>0.0970</b> | <b>Singlet-A 184 -&gt; 196</b> | <b>0.58886</b> | <b>271</b>                 |

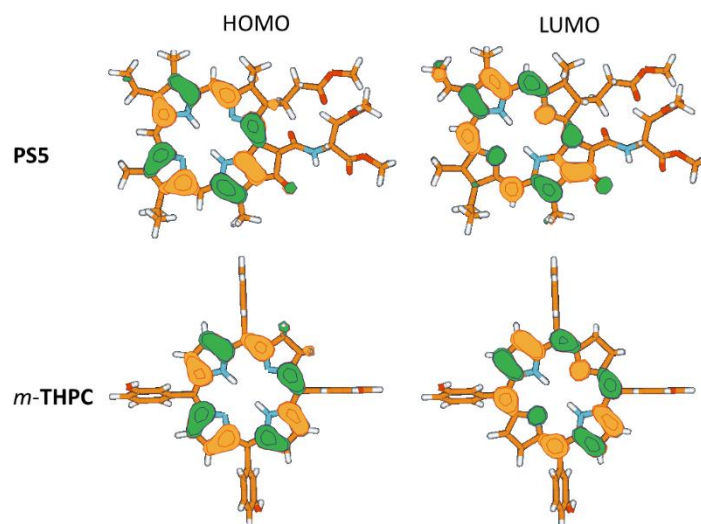
|                        |       |        |           |            |         |      |
|------------------------|-------|--------|-----------|------------|---------|------|
| 23                     | 4.682 | 0.0020 | Singlet-A | 195 -> 200 | 0.63885 | 265  |
| 24                     | 4.710 | 0.0059 | Singlet-A | 186 -> 197 | 0.38891 | 263  |
| 25                     | 4.756 | 0.0069 | Singlet-A | 190 -> 197 | 0.47194 | 261  |
| 26                     | 4.781 | 0.0009 | Singlet-A | 195 -> 201 | 0.55332 | 259  |
| 27                     | 4.794 | 0.0120 | Singlet-A | 191 -> 198 | 0.42314 | 259  |
| 28                     | 4.838 | 0.0003 | Singlet-A | 183 -> 196 | 0.67386 | 256  |
| 29                     | 4.854 | 0.0061 | Singlet-A | 189 -> 197 | 0.42606 | 255  |
| 30                     | 4.873 | 0.0515 | Singlet-A | 193 -> 198 | 0.59732 | 254  |
| 31                     | 4.880 | 0.0074 | Singlet-A | 195 -> 202 | 0.57646 | 254  |
| 32                     | 4.928 | 0.0046 | Singlet-A | 194 -> 200 | 0.59400 | 252  |
| 33                     | 4.937 | 0.0012 | Singlet-A | 195 -> 203 | 0.49136 | 251  |
| 34                     | 4.988 | 0.0004 | Singlet-A | 188 -> 197 | 0.63489 | 249  |
| 35                     | 5.004 | 0.0004 | Singlet-A | 182 -> 196 | 0.68422 | 248  |
| 36                     | 5.010 | 0.0046 | Singlet-A | 194 -> 201 | 0.48775 | 247  |
| 37                     | 5.020 | 0.0280 | Singlet-A | 194 -> 201 | 0.37354 | 247  |
| 38                     | 5.112 | 0.0019 | Singlet-A | 186 -> 197 | 0.46472 | 243  |
| 39                     | 5.119 | 0.0096 | Singlet-A | 194 -> 202 | 0.46279 | 242  |
| 40                     | 5.128 | 0.0004 | Singlet-A | 195 -> 205 | 0.34539 | 242  |
| Triplet excited states |       |        |           |            |         |      |
| 1                      | 1.203 | 0      | Triplet-A | 194 -> 196 | 0.6257  | 1031 |
| 2                      | 1.420 | 0      | Triplet-A | 195 -> 196 | 0.6594  | 873  |
| 3                      | 2.039 | 0      | Triplet-A | 195 -> 197 | 0.6538  | 608  |
| 4                      | 2.342 | 0      | Triplet-A | 194 -> 197 | 0.6780  | 529  |
| 5                      | 2.626 | 0      | Triplet-A | 193 -> 196 | 0.6113  | 472  |
| 6                      | 2.673 | 0      | Triplet-A | 192 -> 196 | 0.5723  | 464  |
| 7                      | 2.916 | 0      | Triplet-A | 193 -> 197 | 0.5825  | 425  |
| 8                      | 3.104 | 0      | Triplet-A | 195 -> 198 | 0.5939  | 399  |
| 9                      | 3.179 | 0      | Triplet-A | 191 -> 196 | 0.4225  | 390  |
| 10                     | 3.197 | 0      | Triplet-A | 189 -> 196 | 0.3760  | 388  |
| 11                     | 3.413 | 0      | Triplet-A | 190 -> 196 | 0.4023  | 363  |
| 12                     | 3.671 | 0      | Triplet-A | 195 -> 199 | 0.2845  | 338  |
| 13                     | 3.694 | 0      | Triplet-A | 187 -> 196 | 0.4046  | 336  |
| 14                     | 3.712 | 0      | Triplet-A | 187 -> 196 | 0.4191  | 334  |
| 15                     | 3.773 | 0      | Triplet-A | 185 -> 196 | 0.4906  | 329  |
| 16                     | 3.784 | 0      | Triplet-A | 184 -> 196 | 0.4597  | 328  |
| 17                     | 3.959 | 0      | Triplet-A | 195 -> 199 | 0.3981  | 313  |
| 18                     | 4.090 | 0      | Triplet-A | 186 -> 196 | 0.2991  | 303  |
| 19                     | 4.104 | 0      | Triplet-A | 192 -> 197 | 0.3848  | 302  |
| 20                     | 4.157 | 0      | Triplet-A | 188 -> 196 | 0.6018  | 298  |
| 21                     | 4.188 | 0      | Triplet-A | 195 -> 199 | 0.3543  | 296  |
| 22                     | 4.314 | 0      | Triplet-A | 194 -> 199 | 0.4199  | 287  |
| 23                     | 4.386 | 0      | Triplet-A | 189 -> 197 | 0.4556  | 283  |
| 24                     | 4.427 | 0      | Triplet-A | 187 -> 197 | 0.5394  | 280  |
| 25                     | 4.493 | 0      | Triplet-A | 191 -> 197 | 0.4563  | 276  |
| 26                     | 4.547 | 0      | Triplet-A | 191 -> 197 | 0.3650  | 273  |
| 27                     | 4.576 | 0      | Triplet-A | 191 -> 198 | 0.4096  | 271  |



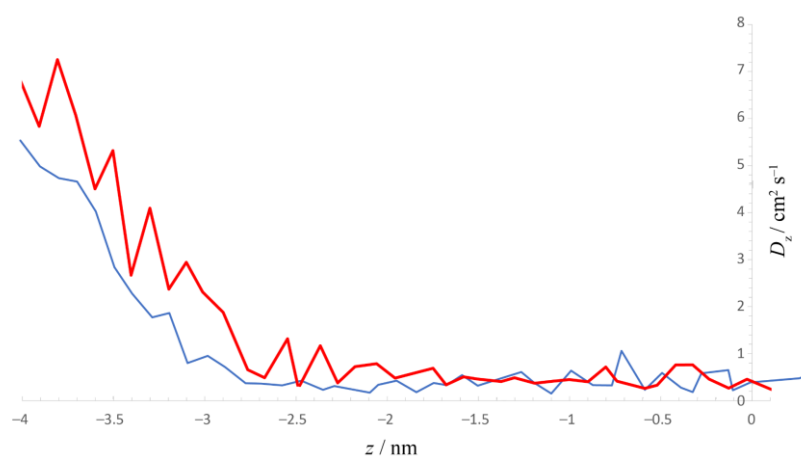
|    |       |   |           |            |        |     |
|----|-------|---|-----------|------------|--------|-----|
| 28 | 4.608 | 0 | Triplet-A | 191 -> 198 | 0.1996 | 269 |
| 29 | 4.658 | 0 | Triplet-A | 195 -> 200 | 0.3864 | 266 |
| 30 | 4.663 | 0 | Triplet-A | 185 -> 197 | 0.3282 | 266 |
| 31 | 4.748 | 0 | Triplet-A | 195 -> 200 | 0.3960 | 261 |
| 32 | 4.756 | 0 | Triplet-A | 193 -> 198 | 0.4874 | 261 |
| 33 | 4.816 | 0 | Triplet-A | 195 -> 201 | 0.2702 | 257 |
| 34 | 4.835 | 0 | Triplet-A | 183 -> 196 | 0.6679 | 256 |
| 35 | 4.853 | 0 | Triplet-A | 179 -> 196 | 0.2883 | 255 |
| 36 | 4.871 | 0 | Triplet-A | 194 -> 200 | 0.3746 | 255 |
| 37 | 4.886 | 0 | Triplet-A | 189 -> 198 | 0.2590 | 254 |
| 38 | 4.912 | 0 | Triplet-A | 189 -> 198 | 0.2735 | 252 |
| 39 | 4.950 | 0 | Triplet-A | 188 -> 197 | 0.3398 | 250 |
| 40 | 4.953 | 0 | Triplet-A | 195 -> 205 | 0.3061 | 250 |



**Figure S14.** Comparison of the simulated UV/Vis spectra of *m*-THPC and investigated molecules PS5. The simulation is based on the BPE0/6-311+G(2d,p) calculations of excitation energies and oscillator strengths.



**Figure S15.** HOMO and LUMO Kohn-Sham orbitals of *m*-THPC and **PS5** at the PBE0/6-311+G(2d,p) level of theory.



**Figure S16.** Position-specific diffusion coefficients for *m*-THPC (blue) and **PS5** (red) calculated via Hummer's positional autocorrelation extension of Wolf-Roux estimator.

**Cartesian coordinates** for optimized structures calculated at the B3LYP/6-311+G(2d,p) level of theory

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***m*-THPC**

|   |           |           |           |
|---|-----------|-----------|-----------|
| C | 4.358835  | -3.552010 | -1.455448 |
| C | 3.825105  | -3.153621 | -0.230476 |
| C | 4.364197  | -3.673253 | 0.946767  |
| C | 5.424750  | -4.575353 | 0.901651  |
| C | 5.958963  | -4.968203 | -0.322915 |
| C | 5.419973  | -4.453224 | -1.493667 |
| C | 2.686242  | -2.180551 | -0.167945 |
| C | 3.003684  | -0.827875 | -0.095069 |
| N | 2.125944  | 0.195422  | -0.022925 |
| C | 2.799342  | 1.364419  | 0.029389  |
| C | 4.307543  | 1.167520  | -0.019757 |
| C | 4.449613  | -0.353384 | -0.084313 |
| C | 2.237535  | 2.634501  | 0.113639  |
| C | 3.176485  | 3.802763  | 0.149606  |
| C | 3.663246  | 4.291991  | 1.361154  |
| C | 4.538720  | 5.375043  | 1.374526  |
| C | 4.937041  | 5.982947  | 0.192083  |
| C | 4.448787  | 5.499081  | -1.019019 |
| C | 3.573804  | 4.415288  | -1.039305 |
| C | 0.867800  | 2.945452  | 0.162790  |
| N | -0.182929 | 2.071733  | 0.152212  |
| C | -1.384292 | 2.752809  | 0.196490  |
| C | -1.056016 | 4.142051  | 0.240258  |
| C | 0.306139  | 4.260144  | 0.223483  |
| C | -2.655543 | 2.187380  | 0.176930  |
| C | -3.808286 | 3.138409  | 0.241155  |
| C | -4.136179 | 3.754663  | 1.449178  |
| C | -5.206132 | 4.643408  | 1.520140  |
| C | -5.957393 | 4.928725  | 0.383194  |
| C | -5.628549 | 4.315082  | -0.817892 |
| C | -4.563051 | 3.422726  | -0.896735 |
| C | -2.941032 | 0.809628  | 0.098623  |
| N | -2.019225 | -0.194185 | 0.028038  |
| C | -2.739638 | -1.352056 | -0.023764 |
| C | -4.163020 | -1.074366 | 0.011567  |
| C | -4.288395 | 0.272031  | 0.094414  |
| C | -2.204657 | -2.652872 | -0.111145 |
| C | -0.851126 | -2.971706 | -0.161976 |
| N | 0.203030  | -2.078608 | -0.146078 |
| C | 1.397588  | -2.741310 | -0.183699 |
| C | 1.089444  | -4.137779 | -0.232270 |
| C | -0.270848 | -4.275626 | -0.215481 |
| C | -3.161272 | -3.802089 | -0.149272 |
| C | -3.826211 | -4.215523 | 1.004970  |
| C | -4.708283 | -5.291045 | 0.950453  |

|   |           |           |           |
|---|-----------|-----------|-----------|
| C | -4.941912 | -5.961970 | -0.242273 |
| C | -4.280565 | -5.548274 | -1.395487 |
| C | -3.393540 | -4.475560 | -1.348926 |
| H | 1.827407  | -4.921358 | -0.269445 |
| H | -0.835627 | -5.192699 | -0.229698 |
| H | 3.946648  | -3.373145 | 1.902989  |
| O | 5.981815  | -5.110994 | 2.031702  |
| H | 6.782366  | -5.670695 | -0.337668 |
| H | 4.787170  | 1.608088  | 0.855742  |
| H | -1.781338 | 4.937752  | 0.271335  |
| H | -4.949851 | -1.810254 | -0.021712 |
| H | 4.980042  | -0.699119 | -0.973011 |
| H | 0.885841  | 5.167584  | 0.245373  |
| H | -5.197441 | 0.848828  | 0.146513  |
| H | 3.939354  | -3.158492 | -2.373055 |
| H | -3.552888 | 3.533120  | 2.337124  |
| H | -4.312659 | 2.945850  | -1.835637 |
| H | 3.352135  | 3.825928  | 2.287882  |
| H | 3.190951  | 4.043878  | -1.984950 |
| H | -3.649584 | -3.695103 | 1.937494  |
| H | -2.880165 | -4.154426 | -2.249522 |
| H | 5.830653  | -4.760944 | -2.448071 |
| O | -5.565471 | 5.266589  | 2.685367  |
| H | -6.210367 | 4.536418  | -1.704784 |
| H | 4.913323  | 5.752685  | 2.318608  |
| O | 4.862290  | 6.130547  | -2.161166 |
| H | -5.220163 | -5.611828 | 1.849984  |
| O | -4.541587 | -6.234031 | -2.551710 |
| H | -6.784674 | 5.622735  | 0.458438  |
| H | 5.615354  | 6.826495  | 0.187592  |
| H | -5.626882 | -6.798238 | -0.298624 |
| H | 0.086290  | -1.076210 | -0.098175 |
| H | -0.112007 | 1.065052  | 0.104402  |
| H | 4.734668  | 1.672744  | -0.888219 |
| H | 4.985843  | -0.768009 | 0.771509  |
| H | 5.523132  | -4.777821 | 2.811267  |
| H | -4.984199 | 4.982104  | 3.399701  |
| H | 4.450448  | 5.718798  | -2.929257 |
| H | -4.038436 | -5.850300 | -3.278808 |

---

**PS5**

|   |          |           |           |
|---|----------|-----------|-----------|
| C | 5.708033 | 1.344126  | -0.576614 |
| C | 6.179675 | 2.666822  | -0.927465 |
| C | 5.082803 | 3.505677  | -0.974890 |
| C | 3.916453 | 2.717710  | -0.671242 |
| C | 2.600776 | 3.167697  | -0.632639 |
| C | 6.463976 | 0.198751  | -0.428912 |
| C | 6.030529 | -1.095049 | -0.095320 |
| C | 6.930441 | -2.240551 | 0.036380  |

|   |           |           |           |
|---|-----------|-----------|-----------|
| C | 6.145458  | -3.301943 | 0.374063  |
| C | 4.779816  | -2.787562 | 0.437513  |
| C | 1.438735  | 2.451180  | -0.348874 |
| C | 0.100818  | 0.793009  | 0.220333  |
| C | 3.656464  | -3.544187 | 0.757111  |
| C | -0.214238 | -0.504680 | 0.576266  |
| C | 0.707070  | -1.559179 | 0.691063  |
| C | 0.135039  | -2.800404 | 1.060444  |
| C | 1.143314  | -3.756302 | 1.145290  |
| C | 2.349125  | -3.067344 | 0.819072  |
| H | 2.466263  | 4.215417  | -0.865514 |
| H | 7.525632  | 0.315402  | -0.600483 |
| H | 3.797966  | -4.593797 | 0.980473  |
| N | 4.749517  | -1.448153 | 0.146112  |
| N | 1.389289  | 1.158683  | -0.013510 |
| C | -1.564304 | -1.096282 | 0.972294  |
| H | -1.894305 | -0.687413 | 1.933429  |
| C | -1.910819 | 2.221733  | 1.037314  |
| H | -2.434739 | 1.313290  | 1.322917  |
| H | -1.417172 | 2.580939  | 1.944690  |
| C | -2.959041 | 3.230074  | 0.566368  |
| H | -2.581594 | 4.256147  | 0.567796  |
| H | -3.246020 | 3.026050  | -0.470140 |
| C | -1.289440 | -2.616271 | 1.254405  |
| O | -2.146691 | -3.407014 | 1.593896  |
| C | -2.723260 | -0.900142 | -0.005963 |
| O | -2.597425 | -0.361784 | -1.098110 |
| C | -4.234042 | 3.192686  | 1.379790  |
| O | -4.438400 | 2.479001  | 2.331792  |
| O | -5.146452 | 4.041479  | 0.872976  |
| C | -5.147505 | -1.078885 | -0.256502 |
| H | -5.030488 | -1.351012 | -1.306969 |
| C | -6.446167 | 4.030325  | 1.505696  |
| H | -6.344615 | 4.151003  | 2.583514  |
| H | -6.983900 | 4.868942  | 1.070772  |
| H | -6.959870 | 3.096094  | 1.279213  |
| C | -0.253866 | 3.805924  | -1.706562 |
| H | 0.437123  | 4.626383  | -1.910561 |
| H | -0.189698 | 3.096771  | -2.534899 |
| H | -1.264891 | 4.218343  | -1.692360 |
| C | 1.011338  | -5.201932 | 1.492259  |
| H | 0.000721  | -5.417785 | 1.836812  |
| H | 1.215197  | -5.835076 | 0.623367  |
| H | 1.715843  | -5.489339 | 2.276943  |
| C | 8.410423  | -2.202791 | -0.176602 |
| H | 8.893445  | -1.479137 | 0.486949  |
| H | 8.865603  | -3.176000 | 0.009780  |
| H | 8.659267  | -1.915523 | -1.202853 |
| C | 6.547119  | -4.729018 | 0.590236  |
| H | 7.598225  | -4.773153 | 0.886679  |

|   |           |           |           |
|---|-----------|-----------|-----------|
| H | 5.983668  | -5.149268 | 1.429127  |
| C | 6.333894  | -5.605039 | -0.655996 |
| H | 5.286737  | -5.594879 | -0.965883 |
| H | 6.928027  | -5.233623 | -1.493822 |
| H | 6.623780  | -6.640942 | -0.462735 |
| C | 5.063074  | 4.976184  | -1.239860 |
| H | 4.921125  | 5.196130  | -2.302947 |
| H | 4.256671  | 5.467888  | -0.693729 |
| H | 6.004866  | 5.434373  | -0.934318 |
| C | 8.073948  | 3.925382  | -1.954177 |
| H | 7.438528  | 4.541808  | -2.576642 |
| H | 9.141611  | 4.086614  | -2.034750 |
| C | 7.585201  | 2.983911  | -1.143011 |
| H | 8.294188  | 2.377234  | -0.586658 |
| N | 2.028364  | -1.738082 | 0.551535  |
| H | 2.744567  | -1.065034 | 0.307462  |
| N | 4.344279  | 1.440260  | -0.438409 |
| H | 3.738787  | 0.666762  | -0.209579 |
| C | 0.065633  | 3.109910  | -0.379127 |
| H | 0.024437  | 3.854819  | 0.425167  |
| C | -0.874802 | 1.928328  | -0.052762 |
| H | -1.424346 | 1.635060  | -0.951750 |
| N | -3.919786 | -1.317330 | 0.470218  |
| H | -3.963756 | -1.903594 | 1.295636  |
| C | -5.518762 | 0.424256  | -0.173949 |
| H | -5.654517 | 0.716241  | 0.865270  |
| H | -4.665809 | 0.967304  | -0.585042 |
| C | -6.243581 | -1.934422 | 0.359616  |
| O | -6.149591 | -2.525473 | 1.405254  |
| O | -7.346462 | -1.911506 | -0.401706 |
| C | -6.742816 | 0.831249  | -0.953195 |
| O | -7.695407 | 1.410280  | -0.487894 |
| O | -6.631252 | 0.500426  | -2.251431 |
| C | -8.494722 | -2.596256 | 0.140966  |
| H | -9.282061 | -2.468406 | -0.596721 |
| H | -8.268106 | -3.651535 | 0.289340  |
| H | -8.781159 | -2.148910 | 1.092302  |
| C | -7.765966 | 0.817784  | -3.076307 |
| H | -7.955417 | 1.891195  | -3.067315 |
| H | -7.503351 | 0.481013  | -4.075781 |
| H | -8.651216 | 0.295059  | -2.713210 |

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