# Supporting Information

# A photosensitizer-drug conjugate for synergistic photodynamic therapy and photo-triggered camptothecin release

Bowen Xu,[a][b] Xuancheng Fu[a][b], Zhiwei Huang[a], Cijun Zhang[a][b] and Xiaoran Hu*[a][b]

[a] Department of Chemistry, Syracuse University, Syracuse, New York, 13244, United States
[b] BioInspired Institute, Syracuse University, Syracuse, New York, 13244, United States

*To whom all correspondence should be addressed: Prof. Dr. Xiaoran Hu, xhu156@syr.edu

## Contents

## 1. General Considerations

All reactions were conducted under standard air-free conditions under a nitrogen gas atmosphere with magnetic stirring unless otherwise mentioned. All reactants and solvents were purchased from commercial suppliers and used without further purification unless otherwise noted. Flash chromatography was performed on a Biotage isolera system with Yamazen corp. universal silica gel columns (Pore Size 60 angstroms, Particle Size 40-63 microns).

NMR spectra were acquired on a Bruker Avance III HD 400 MHz spectrometer. $^{1}H$ NMR spectra are reported relative to residual protonated solvent (7.26 ppm for $CDCl_3$, 2.50 ppm for DMSO-d6). $^{13}C$ NMR spectra are reported relative to residual protonated solvent (77.16 ppm for $CHCl_3$, 39.52 ppm for DMSO). Multiplicity abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ABq = AB quartet, m = multiplet, br = broad.

Mass spectra were acquired on a DART-SVP (Direct Analysis in Real Time) ion source (IonSense, Saugus, MA) coupled to an Exactive Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) at the Cornell Chemistry Mass Spectrometry Facility.

Light source and irradiation conditions
Irradiation was performed using a full-spectrum LED high-bay light (model QC-HB30F-2700K, Q QINGCHEN, Shenzhen, China; 300 W, 3000 K, 0-10 V dimmable, ETL-certified). A long-pass optical filter ($\lambda > 600$ nm) was placed in front of the light source to remove shorter-wavelength components.

Nanoparticle morphology was characterized by scanning electron microscopy (SEM) using a Zeiss Sigma 360 field-emission scanning electron microscope (FE-SEM). Samples were prepared by drop-casting 10 μL of nanoparticle suspension onto a glass substrate, followed by drying under ambient conditions overnight. The samples were then gently rinsed with ultrapure water three times to remove residual salts and dried again under ambient conditions. Prior to imaging, the dried samples were sputter-coated with a thin layer of gold (~15 nm) to improve conductivity. SEM images were acquired at an accelerating voltage of 5.0 kV, with a working distance of 5.2 mm, using a secondary electron detector (SE2) under high-vacuum conditions ($\sim 6.5 \times 10^{-6}$ mbar).

Materials
2-Mercaptoethanol (electrophoresis grade, ≥98%), acetone (certified ACS grade), trifluoroacetic acid (TFA), 4-dimethylaminopyridine (DMAP), and thiazolyl blue tetrazolium bromide (MTT, ≥98%) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Acetic acid (ACS grade) was obtained from VWR International (Radnor, PA, USA). (19S)-Camptothecin (CPT) was purchased from Arctom Biochem (China). 5-Mono(4-carboxyphenyl)-10,15,20-triphenylporphine was obtained from Aurum Pharmatech (USA). Triphosgene and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

(EDC·HCl, >99%) were purchased from AK Scientific (Union City, CA, USA). PLGA(10k)-mPEG(5k) was obtained from BroadPharm (USA). All reagents and solvents were used as received without further purification unless otherwise stated.

Corning™ RPMI-1640 medium (1×), fetal bovine serum (FBS), penicillin-streptomycin solution, Hoechst 33342 (trihydrochloride trihydrate), and LysoTracker™ Deep Red were purchased from Thermo Fisher Scientific (USA). Singlet Oxygen Sensor Green (SOSG) was obtained from Lumiprobe Corporation (USA). 2′,7′-Dichlorodihydrofluorescein diacetate ($H_2$DCFDA, ≥97% HPLC grade) was purchased from Sigma-Aldrich (USA). All cell culture reagents were used according to the manufacturers' instructions.

## 2. Supplementary Figures

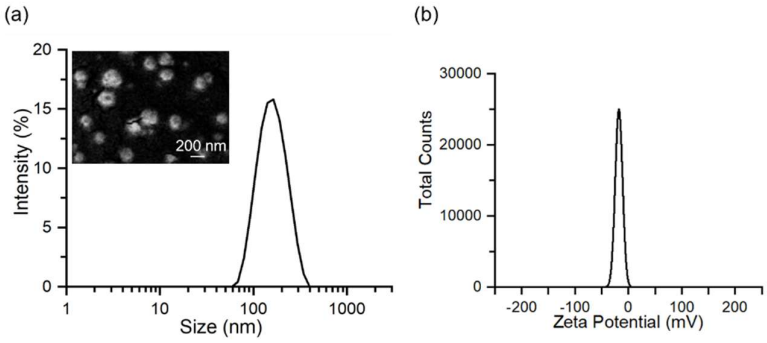



Figure S1. (a) Hydrodynamic diameter distribution of **ProCPTNPs** measured by dynamic light scattering (DLS), showing an average diameter of around 156.4 nm. Inset: SEM image showing spherical morphology and relatively uniform size distribution. Scale bar: 200 nm. (b) Zeta potential (−17.8 mV) of **ProCPTNPs**.

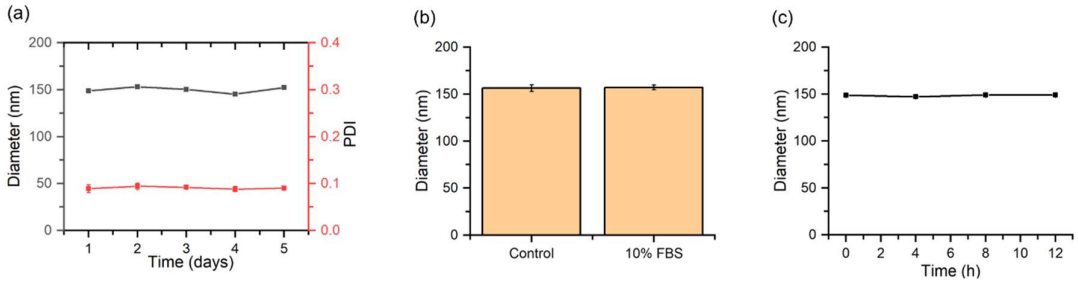



Figure S2. Stability test of **ProCPTNPs** monitored by DLS. (a) Hydrodynamic diameter distribution of **ProCPTNPs** after incubation in PBS (pH 7.4) for 1–5 days; (b) Hydrodynamic diameter distribution of **ProCPTNPs** following incubation in PBS buffer (control) or PBS buffer containing 10% FBS at 37 °C for 1 day. (c) Hydrodynamic diameter distribution of **ProCPTNPs** after incubation in RPMI-1640 cell culture medium at 37 °C for 0, 4, 8 and 12 h. Data are shown as mean ± SD (n = 3).

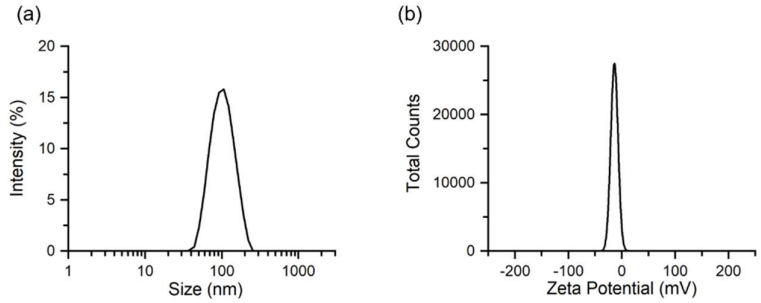



Figure S3. (a) Hydrodynamic diameter distribution of **TPPNPs** measured by DLS, showing an average diameter of around 105.4 nm. (b) Zeta potential (-14.7 mV) of **TPPNPs**.

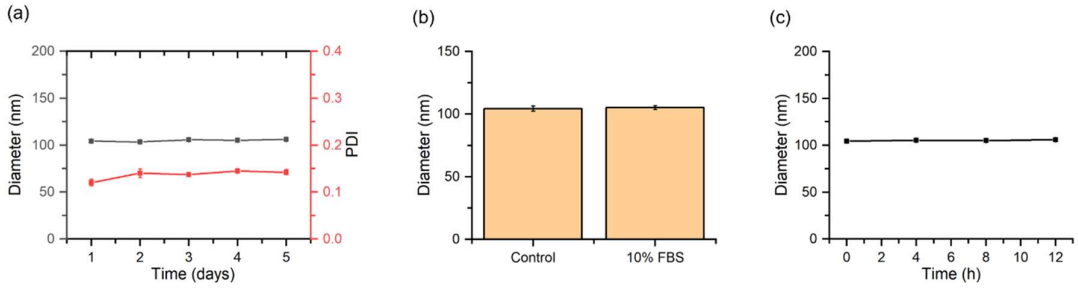



Figure S4. Stability test of **TPPNPs** monitored by DLS. (a) Hydrodynamic diameter distribution of **TPPNPs** after incubation in PBS (pH 7.4) for 1–5 days; (b) Hydrodynamic diameter distribution of **TPPNPs** following incubation in PBS buffer (control) or PBS buffer with 10% FBS at 37 °C for 1 day. (c) Hydrodynamic diameter distribution of **TPPNPs** after incubation in RPMI-1640 cell culture medium at 37 °C for 0, 4, 8 and 12 h. Data are shown as mean ± SD (n = 3).

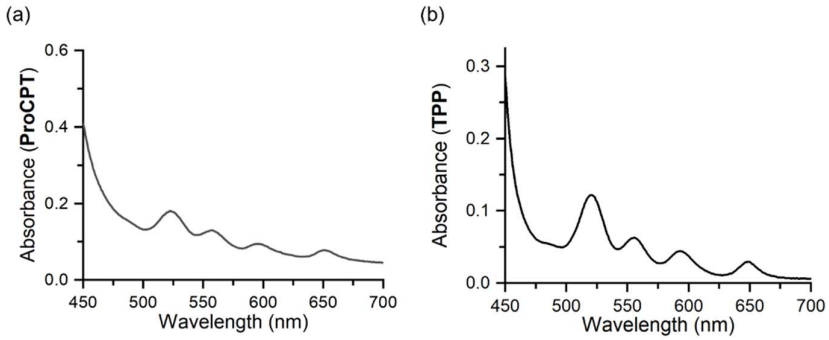



Figure S5. (a) Absorption spectra of **ProCPT** in PBS buffer. (b) Absorption spectra of TPP in PBS buffer.

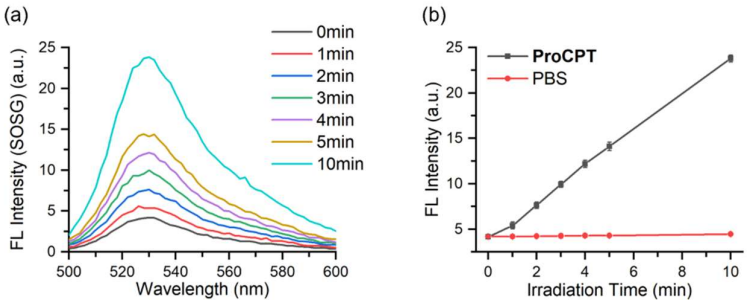



Figure S6. (a) Generation of singlet oxygen determined by the SOSG probe for **ProCPT**. (b) The changes in the fluorescence intensity of SOSG (emission peak = 530 nm) with or without **ProCPT** under red light irradiation (> 600 nm, 40 mW $cm^{-2}$). Data are shown as mean ± SD (n = 3).

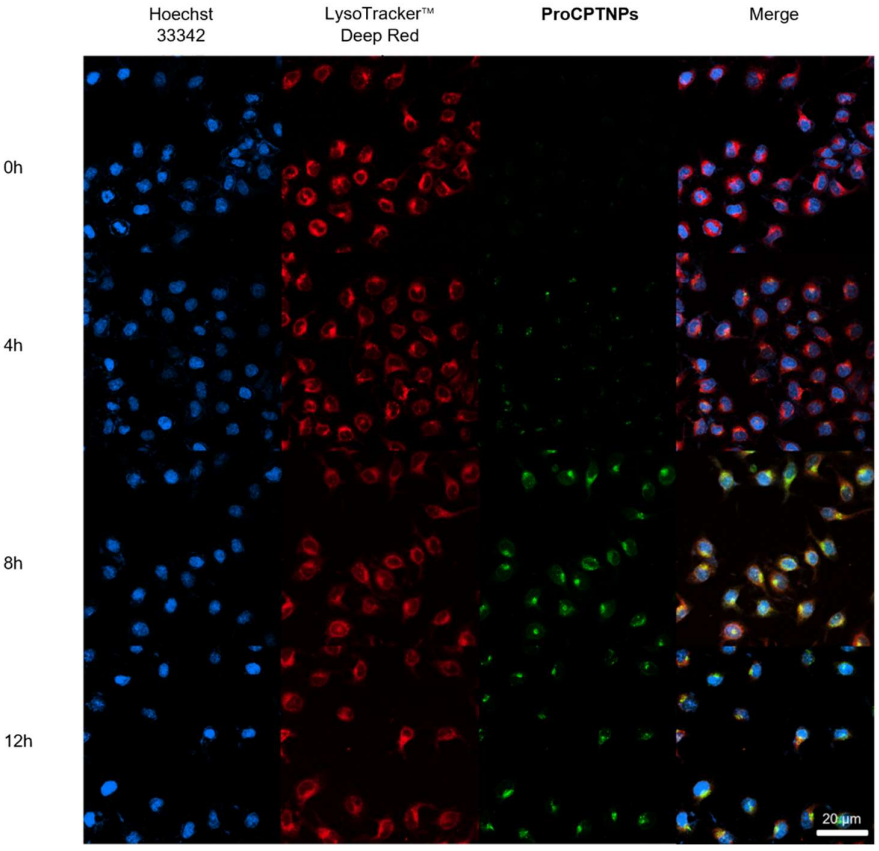



Figure S7. Confocal fluorescence images of HeLa cells treated with **ProCPTNPs** for 0, 4, 8 and 12 h. Arbitrary colors are shown in the figure for visual clarity only and do not represent the actual fluorescence emission colors: blue, Hoechst 33342 (nucleus); red, LysoTracker™ (lysosomes); green, **ProCPTNPs.** Scale bar: 20 μm.

## 3. Calculation of the CI-Fa curve

### 3.1. Determination of effective molar concentrations of CPT and TPP in nanoparticle formulations

To enable accurate comparison in combination index (CI) analysis, nanoparticle mass concentrations (μg $mL^{-1}$) were converted into effective molar concentrations of the active components (CPT or TPP) based on their respective loading contents and molecular weights.

Drug loading content
The loading content was calculated according to the formulation ratios:
**ProCPTNPs:**
1 mg **ProCPT** + 10 mg PLGA-PEG
CPT weight fraction = 1 / 11 = 9.1 wt%
**TPPNPs**:
0.5 mg TPP + 10 mg PLGA-PEG
TPP weight fraction = 0.5 / 10.5 = 4.76 wt%
Conversion formula
The effective molar concentration of each active component was calculated as:

$$C_{drug}(\mu M) = \frac{C_{NP}(\mu g\, mL^{-1}) \times wt\%}{MW(g\, mol^{-1})}$$

where:
$C_{NP}$ = nanoparticle mass concentration
wt% = weight fraction of active component
MW = molecular weight of active component

Example calculation (**ProCPTNPs**, 1.25 μg $mL^{-1}$)
1.25 μg $mL^{-1}$ × 9.1% = 0.1136 μg $mL^{-1}$
0.1136 μg $mL^{-1}$ / 1211 g $mol^{-1}$ = 0.0937 μM
(MW of **ProCPT** = 1211 g $mol^{-1}$)
Example Calculation (**TPPNPs**, 1.25 μg $mL^{-1}$)
1.25 μg $mL^{-1}$ × 4.76% = 0.0595 μg $mL^{-1}$
0.0595 μg $mL^{-1}$ / 658.76 g $mol^{-1}$ = 0.090 μM
(MW of TPP = 658.76 g $mol^{-1}$)

| Nanoparticle μg $mL^{-1}$ | 1.25 | 2.5 | 5 | 10 | 20 | 40 | 80 | 160 |
|---|---|---|---|---|---|---|---|---|
| **ProCPT** μM | 0.0937 | 0.1874 | 0.3748 | 0.7496 | 1.4992 | 2.9984 | 5.9968 | 11.9936 |
| Viability % | 84.93862 | 78.88722 | 69.80843 | 57.39061 | 46.18167 | 23.25351 | 13.69526 | 9.08224 |
| **TPP** μM | 0.0904 | 0.1808 | 0.3616 | 0.7232 | 1.4464 | 2.8928 | 5.7856 | 11.5712 |
| Viability % | 88.29718 | 83.28772 | 78.69568 | 70.37167 | 66.46667 | 60.16079 | 48.26801 | 30.03704 |
| **CPT** μM | 0.016866 | 0.033732 | 0.067464 | 0.134928 | 0.269856 | 0.539712 | 1.079424 | 2.158848 |
| Viability % | 89.76767 | 85.16855 | 77.43245 | 73.15231 | 60.21523 | 50.36933 | 40.40234 | 27.77604 |

Table S1. Conversion of nanoparticle mass concentrations to effective molar concentrations of active components. Nanoparticle formulations were tested at mass concentrations ranging from 1.25 to 160 μg $mL^{-1}$. The corresponding molar concentrations of **ProCPT**, CPT (equivalent),

and TPP were calculated based on their loading fractions and molecular weights. Cell viability (%) values obtained from MTT assays are included to enable calculation of fraction affected (Fa) for subsequent median-effect and CI analysis.

### 3.2. Determination of median-effect parameters

Cell viability data were converted to the fraction affected (Fa) using:

$$Fa = 1 - \frac{\text{viability (\%)}}{100}$$

The median-effect equation was applied:

$$\frac{Fa}{1-Fa} = \left(\frac{D}{Dm}\right)^m$$

Linearization gives:

$$\log_{10}\left(\frac{Fa}{1-Fa}\right) = m\log_{10}(D) - m\log_{10}(Dm)$$

Linear regression of

$$Y = \log_{10}(Fa/(1-Fa))$$

Versus

$$X = \log_{10}(D)$$

provided the slope (m) and intercept.

Chemotherapy (CPT)
$m_A = 0.6323$
$Dm_A$ ($IC_{50}$) = 0.5376 μM
$R^2 = 0.996$
Fitted equation:

$$\log_{10}\left(\frac{Fa}{1-Fa}\right) = 0.6323\log_{10}(D) + 0.1704$$

Photodynamic therapy (TPP)
$m_B = 0.5377$
$Dm_B$ ($IC_{50}$) = 4.1073 μM
$R^2 = 0.971$
Fitted equation:

$$\log_{10}\left(\frac{Fa}{1-Fa}\right) = 0.5377\log_{10}(D) - 0.3299$$

Both single-drug fits showed strong linear correlation ($R^2 \geq 0.97$), indicating reliable median-effect modeling.

### 3.3. Calculation of equivalent single-agent dose (Dx)

For a given effect level $Fa_k$ obtained from combination treatment, the equivalent single-drug dose required to produce the same effect was calculated as:

$$Dx_A = Dm_A \left( \frac{Fa_k}{1 - Fa_k} \right)^{1/m_A}$$

$$Dx_B = Dm_B \left( \frac{Fa_k}{1 - Fa_k} \right)^{1/m_B}$$

where:
$Dm_A$ = 0.5376 μM
$m_A$ = 0.6323
$Dm_B$ = 4.1073 μM
$m_B$ = 0.5377

### 3.4. Combination index calculation

Because chemotherapy (CPT) and photodynamic therapy (TPP) act through independent mechanisms, the mutually nonexclusive model was applied:

$$CI = \frac{D_A}{Dx_A} + \frac{D_B}{Dx_B} + \frac{D_A D_B}{Dx_A Dx_B}$$

where:
$D_A$ = concentration of CPT in combination
$D_B$ = concentration of TPP in combination
$Dx_A$ and $Dx_B$ are the calculated equivalent single-drug doses at the same Fa level
CI values were interpreted as:
CI < 1: synergism, CI = 1: additive, CI > 1: antagonism

| Fa | $D_A$ (μM) | $D_B$ (μM) | $Dx_A$ (μM) | $Dx_B$ (μM) | CI |
|---|---|---|---|---|---|
| 0.151 | 0.0169 | 0.0937 | 0.0349 | 0.165 | 1.33 |
| 0.211 | 0.0337 | 0.187 | 0.0686 | 0.354 | 1.3 |
| 0.302 | 0.0675 | 0.375 | 0.143 | 0.864 | 1.11 |
| 0.426 | 0.135 | 0.75 | 0.335 | 2.36 | 0.85 |
| 0.538 | 0.27 | 1.499 | 0.685 | 5.46 | 0.78 |
| 0.767 | 0.54 | 2.998 | 3.55 | 37.8 | 0.24 |
| 0.863 | 1.079 | 5.997 | 9.88 | 126.0 | 0.16 |
| 0.909 | 2.159 | 11.57 | 20.5 | 298.0 | 0.15 |

Table S2. Calculation of Combination Index (CI) values at different fraction affected (Fa) levels.

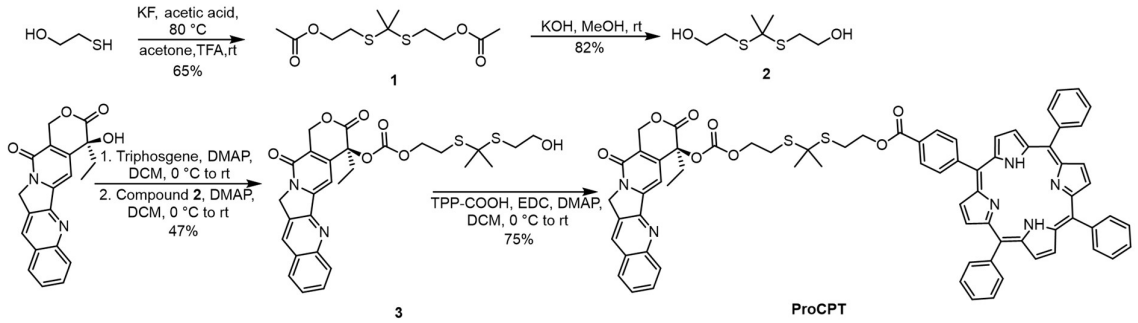
### 3.5. Representative result

At Fa ≈ 0.50 (corresponding to the $IC_{50}$ level of combination treatment):

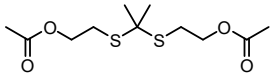
$$CI(IC_{50}) \approx 0.80$$

indicating synergistic interaction between chemotherapy and PDT.
Confidence intervals for CI values were estimated by bootstrap resampling (n = 5000).
The CI-Fa plot demonstrated a progressive decrease in CI with increasing Fa, further confirming enhanced synergism at higher effect levels.

## 4. Synthetic details



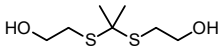

Scheme S1. Synthesis of $^1O_2$-responsive prodrug **ProCPT**.



1

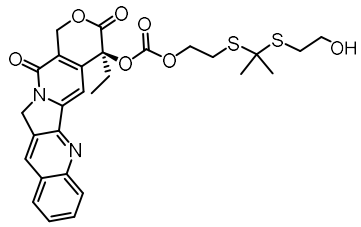
**Compound 1.** 2-Mercaptoethanol (5.00 g, 63.99 mmol, 1.0 equiv) and KF (4.60 g, 79.14 mmol, 1.2 equiv) were dissolved in glacial acetic acid (100 mL) in a 250 mL round-bottom flask. The reaction mixture was heated to 80 °C and stirred for 18 h. After completion, the mixture was cooled to room temperature, diluted with water (200 mL), and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed sequentially with saturated $NaHCO_3$ and brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude material was passed through a short silica gel column to afford 2-mercaptoethyl acetate as a crude product, which was used in the next step without further purification.

To a solution of the crude 2-mercaptoethyl acetate (4.00 g, 33.33 mmol, 2.2 equiv) in acetone (ca. 20 mL) was added trifluoroacetic acid (TFA, 140 μL, catalytic). The reaction was stirred at room temperature for 24 h. Upon completion, the reaction mixture was concentrated and purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give compound 1 as a colorless liquid (6.11 g, 65 % overall yield after two steps). $^1$H NMR (400 MHz, Chloroform-d): δ 4.23 (t, *J* = 8.0 Hz, 4H), 2.87 (t, *J* = 8.0 Hz, 4H), 2.07 (s, 6H), 1.62 (s, 6H).



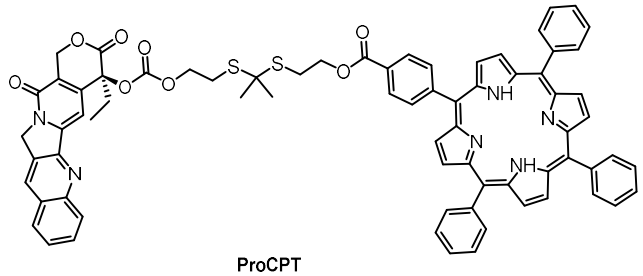
2

**Compound 2.** Compound **1** (2.00 g, 7.14 mmol, 1.0 equiv) and potassium hydroxide (1.80 g, 32.13 mmol, 4.0 equiv) were suspended in methanol (20 mL) in a 50 mL round-bottom flask. The reaction mixture was stirred at room temperature for 16 h. After completion (monitored by TLC), the solvent was removed under reduced pressure. The residue was dissolved in water (≈ 30 mL) and the aqueous phase was carefully acidified to pH around 6-7 with 1 M HCl. The aqueous mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure to afford compound 2 as a liquid (1.15 g, 82 % yield). $^1$H NMR (400 MHz, Chloroform-d): δ 3.80 (t, *J* = 6.0 Hz, 4H), 2.89 (t, *J* = 6.0 Hz, 4H), 2.10 (t, *J* = 6.0 Hz, 2H), 1.64 (s, 6H). The $^1$H NMR spectrum matches with that reported in literature.[1]



3

**Compound 3.** Triphosgene (85.2 mg, 0.287 mmol, 1/3 equiv) was dissolved in anhydrous dichloromethane (DCM, 2.0 mL) in a dry, $N_2$-flushed 50 mL round-bottom flask, and the solution was cooled to 0 °C. Camptothecin (300 mg, 0.861 mmol, 1.0 equiv) was dissolved in anhydrous DCM (1.0 mL) and added to the triphosgene solution at 0 °C. DMAP (525.9 mg, 4.308 mmol, 5.0 equiv) dissolved in anhydrous DCM (1.0 mL) was added dropwise to the reaction mixture. The reaction mixture was allowed to warm to room temperature and was stirred for 1 h. Without purification, the reaction mixture was cooled again to 0 °C, and compound **2** (198.7 mg, 1.033 mmol, 1.2 equiv) in anhydrous DCM (1.0 mL) was added. The reaction was then stirred at room temperature for 16 h. The reaction mixture was concentrated and purified by silica gel column chromatography (hexane/acetone = 1:2) to give compound 3 as a white solid (233.3 mg, 47 % overall yield). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.72 (s, 1H), 8.17 (dd, $J$ = 12.5, 8.4 Hz, 2H), 7.89 (m, 1H), 7.73 (m, 1H), 7.09 (s, 1H), 5.53 (s, 2H), 5.33 (s, 2H), 4.77 (t, $J$ = 5.5 Hz, 1H), 4.25 (t, $J$ = 6.5 Hz, 2H), 3.46 (q, $J$ = 6.9 Hz, 2H), 2.85 (t, $J$ = 6.5 Hz, 2H), 2.68 – 2.55 (m, 2H), 2.18 (m, 2H), 1.47 (d, $J$ = 14.1 Hz, 6H), 0.93 (t, $J$ = 7.4 Hz, 3H). The $^1$H NMR spectrum matches with that reported in literature.[2]

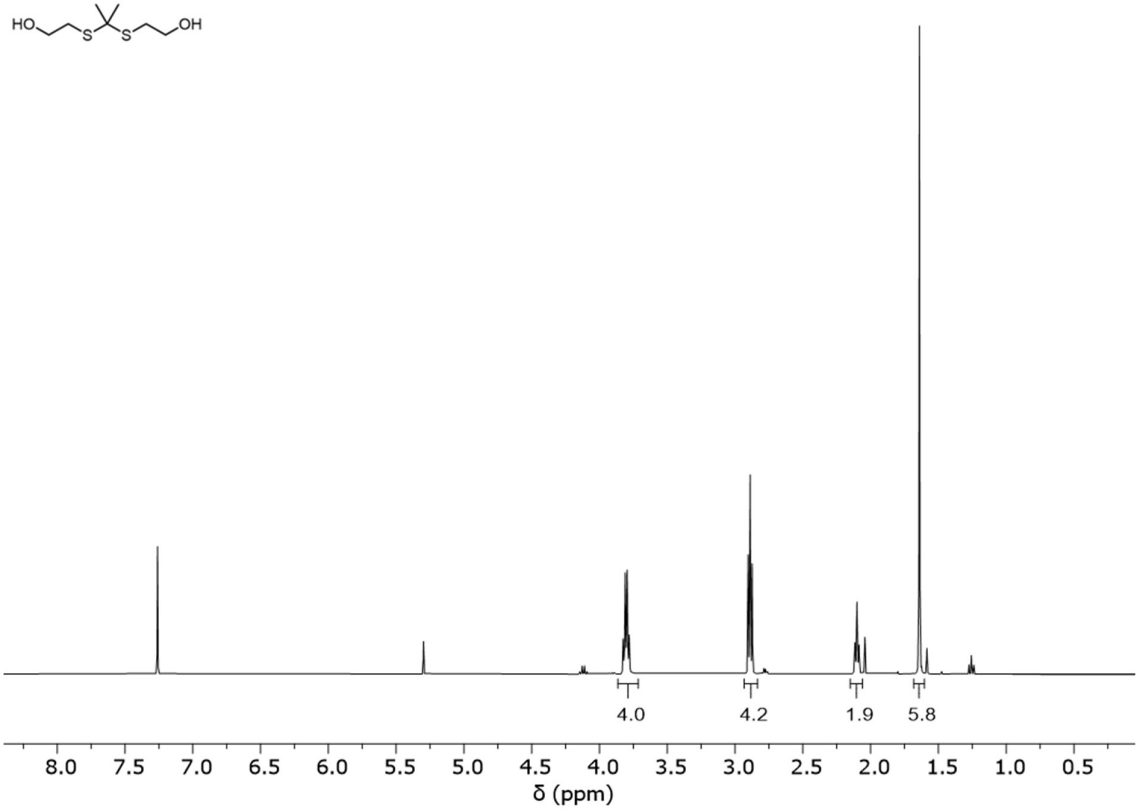


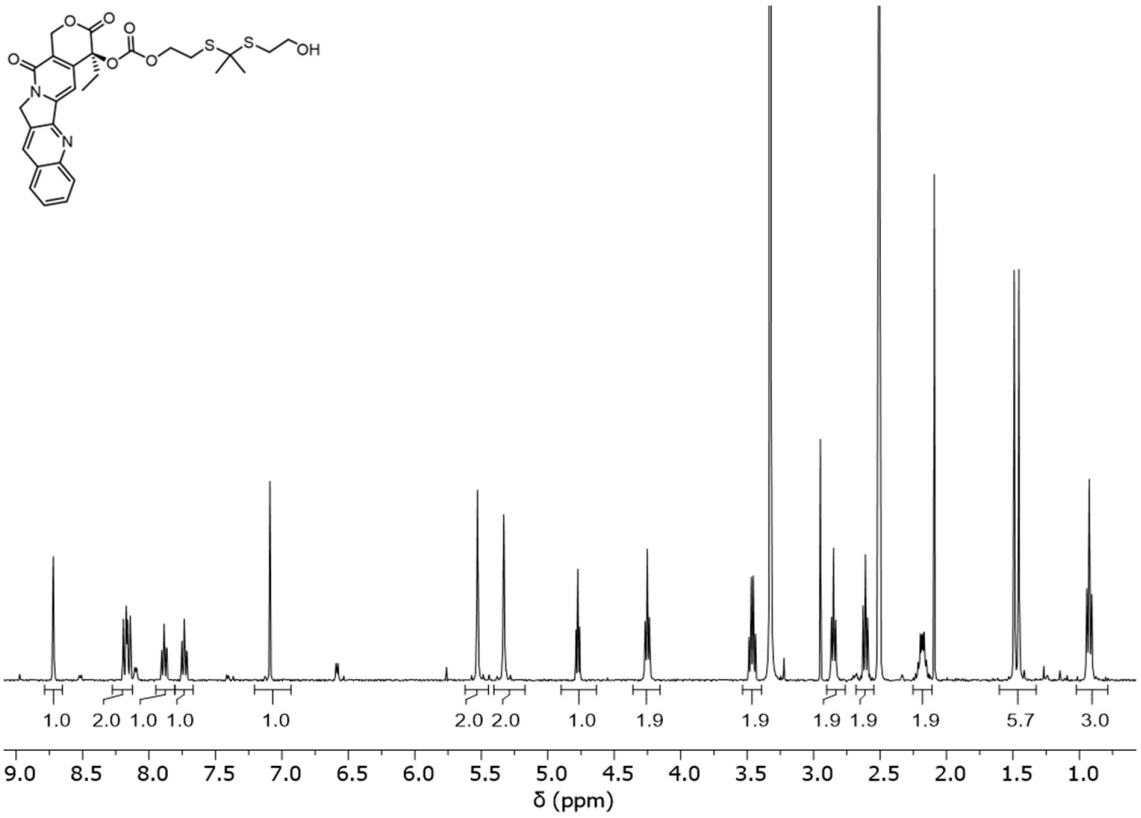
ProCPT

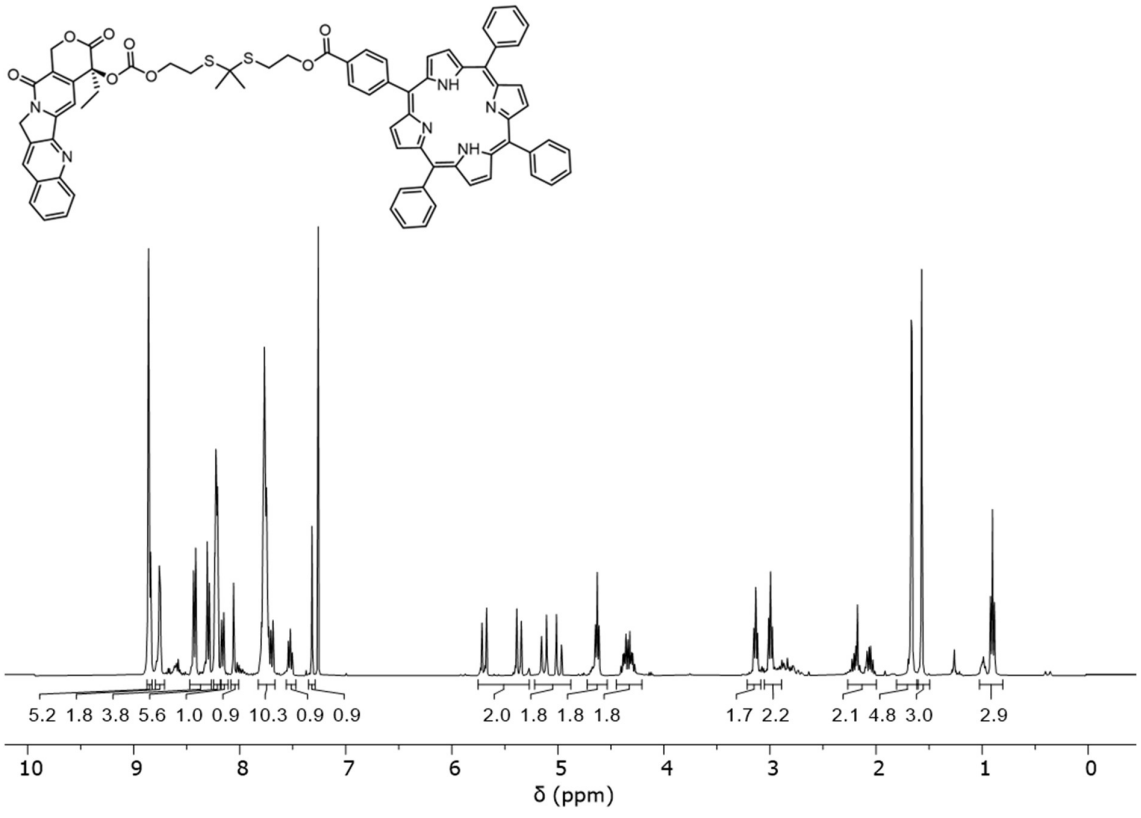
Synthesis of **ProCPT**. Compound **3** (88.7 mg, 0.070 mmol, 1.0 equiv) and 5-Mono(4-carboxyphenyl)-10,15,20-triphenylporphyrin (TPP-COOH, 50.0 mg, 0.070 mmol, 1.0 equiv) were dissolved in anhydrous DCM (1.0 mL) under nitrogen and cooled to 0 °C. A solution of DMAP (13.9 mg, 0.114 mmol, 1.5 equiv) in anhydrous DCM (0.5 mL) was added, followed by dropwise addition of a solution of EDC HCl (17.6 mg, 0.114 mmol, 1.5 equiv) in anhydrous DCM (1.0 mL). The reaction mixture was then allowed to warm to room temperature and stirred for 16 h. After completion (monitored by TLC), the solvent was removed under reduced pressure, and the crude residue was purified by silica gel column chromatography (dichloromethane/ethyl acetate = 10:1) to afford **ProCPT** as a dark red solid (60.15 mg, 75 % yield). $^1$H NMR (400 MHz, Chloroform-d) δ 8.85 (d, $J$ = 8.0 Hz, 6H), 8.75 (d, $J$ = 4.8 Hz, 2H), 8.47 – 8.27 (m, 4H), 8.22 (m, 6H), 8.16 (d, $J$ = 8.6 Hz, 1H), 8.06 (s, 1H), 7.82 – 7.67 (m, 10H), 7.52 (dd, $J$ = 8.1, 6.8 Hz, 1H), 7.32 (s, 1H), 5.75 – 5.27 (m, 2H), 5.22 – 4.88 (m, 2H), 4.63 (t, $J$ = 6.8 Hz, 2H), 4.34 (m, 2H), 3.13 (t, $J$ = 6.9 Hz, 2H), 3.00 (t, $J$ = 7.1 Hz, 2H), 2.27 – 2.00 (m,

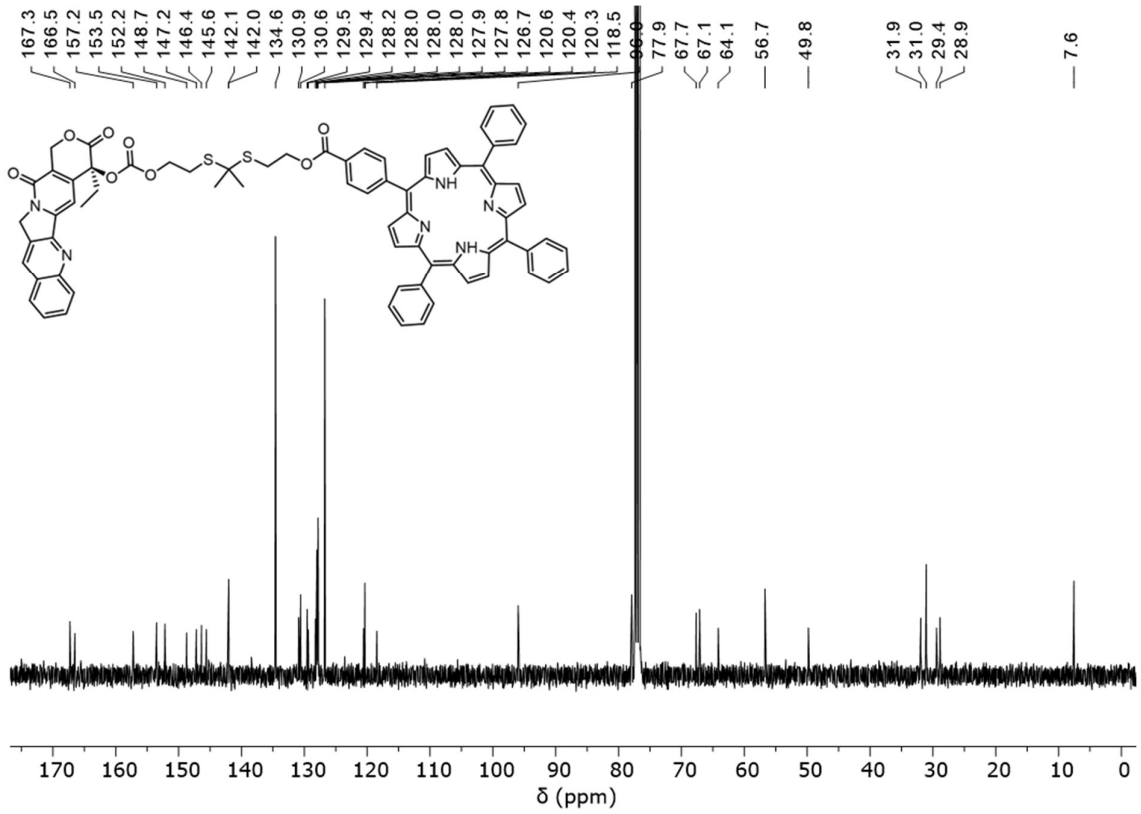
2H), 1.66 (d, $J$ = 2.7 Hz, 5H), 1.57 (s, 3H), 1.02 – 0.81 (m, 3H). $^{13}$C NMR (100 MHz, Chloroform-d) δ 167.29, 166.51, 157.22, 153.51, 153.27, 152.18, 148.73, 147.18, 146.37, 145.58, 142.07, 142.02, 134.57, 130.91, 130.58, 129.54, 129.37, 128.24, 128.04, 128.01, 127.96, 127.91, 127.80, 126.73, 120.57, 120.37, 120.30, 118.46, 95.97, 77.91, 67.65, 67.10, 64.14, 56.71, 49.80, 31.93, 31.05, 29.42, 28.87, 7.56. HRMS (ESI$^+$) m/z: calcd for $C_{73}H_{59}N_6O_8S_2$ $[M + H]^+$ 1212.3831; found 1212.3830.

## 5. NMR Spectra




1.0 2.0 1.0 1.0 1.0 2.0 2.0 1.0 1.9 1.9 1.9 1.9 1.9 5.7 3.0
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
δ (ppm)


5.2 1.8 3.8 5.6 1.0 0.9 10.3 0.9 0.9 2.0 1.8 1.8 1.8 1.7 2.2 2.1 4.8 3.0 2.9
10 9 8 7 6 5 4 3 2 1 0
δ (ppm)




Reference

[1] W. Wang, C. Zhu, B. Zhang, Y. Feng, Y. Zhang and J. Li, *J. Am. Chem. Soc.*, 2023, **145**, 16642–16649. https://doi.org/10.1021/jacs.3c04109

[2] W. Zhou, Y. Liu, G. Liu, Y. Zhang, G. Feng and G. Xing, *Angew. Chem., Int. Ed.*, 2024, **63**, e202413350. https://doi.org/10.1002/anie.202413350