Supporting Online Material for

Light-triggered, Self-immolative Nucleic Acid-drug Nanostructures

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Materials and Methods Phosphoramidites and supplies for DNA synthesis were purchased from Glen Research Co. All other materials were purchased from Sigma-Aldrich Co., VWR International LLC., or Fisher Scientific Inc., and used without further purification unless otherwise indicated. ^{1}H-and ^{13}C-NMR spectra were recorded on a Varian 400 MHz spectrometer (Varian Inc., CA, USA). Chemical shifts (δ) are reported in ppm. MALDI-ToF MS measurements were taken on a Bruker Microflex LT mass spectrometer (Bruker Daltonics Inc., MA, USA). Ultraviolet-visible spectroscopy (UV-Vis) data were obtained on a Cary 4000 UV-Vis spectrophotometer (Varian Inc., CA, USA). Gel images were acquired on a Fluorochem Q imager (ProteinSimple Co., CA, USA). High performance liquid chromatography was performed using a Waters HPLC system (Waters Co., MA, USA, Waters 2998 Photodiode Array Detector, Waters 1525 Binary HPLC Pump, and Waters Symmetry® C18 3.5 μm, 4.6×75 mm reverse phase column). Dynamic Light Scattering (DLS) data were recorded on a Zetasizer Nano-ZSP (Malvern Instruments Ltd., UK). Infrared (IR) measurements were carried out on a Bruker Tensor FT-IR spectrometer. Fluorescence data were taken on a Cary Eclipse fluorescence spectrophotometer (Varian Inc., CA, USA).

Synthesis of compound 2 Compound **1** (Scheme S1, 1.015 g, 6 mmol) was dissolved in anhydrous DMSO (10 mL). The solution was used to dissolve K_2CO_3 (0.911 g, 6.6 mmol) and 18-crown-6 (0.087 g, 0.33 mmol). The mixture was stirred at 70 ^{o}C for 30 min, before propargyl bromide (500 μL, 6.6 mmol, 1.1 eq.) was added. The reaction was monitored by TLC (EtOAc:Hex=1:4). After 4 hours, the mixture was quenched with 13.2 mL 1 M HCl, and 7 mL Nanopure™ water was added. The product was then extracted three times (3 × 20 mL) with ethyl acetate. The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:Hex=1:4 to 1:2) to give **2** as white solid (1.039 g, 81.6%). ^{1}H-NMR (400 MHz, d-acetone): δ 8.16-8.18 (d, 1H), 7.55-7.56 (d, 1H), 7.08-7.11 (dd, 1H), 5.02-5.03 (d,

2H), 4.97-4.98 (d, 2H), 4.70-4.71 (m, 1H), 3.17-3.19 (t, 1H). ^{13}C-NMR (400 MHz, d-acetone): δ 162.23, 142.42, 140.65, 127.47, 114.03, 113.24, 78.09, 77.27, 61.18, 56.27.

Synthesis of compound 3 Compound **2** (0.215 g, 1.04 mmol) and triethylamine (160 μL, 1.14 mmol) were dissolved in 4 mL dichloromethane (DCM). Triphosgene (0.112 g, 1.13 mmol) was then added to the reaction mixture, which was allowed to stir at room temperature for 30 min. The completion of the reaction was confirmed by TLC (EtOAc:Hex=1:4). The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:Hex=1:7) to give **3** as pale yellow oil (0.200 g, 85.5%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.12-8.14 (d, 1H), 7.25-7.26 (d, 1H), 6.99-7.02 (dd, 1H), 4.98 (s, 2H), 4.79-4.80 (d, 2H), 2.59-2.60 (t, 1H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 161.60, 141.46, 135.60, 128.30, 117.79, 114.70, 77.66, 77.02, 56.61, 43.80.

Synthesis of compound 4 The self-immolative precursor **4** was synthesized based on a modified literature method.[1] NMR spectroscopy confirmed the successful synthesis. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.06 (s, 2H), 4.84 (s, 4H), 4.63 (s, 2H), 0.92-0.93 (s, 27H), and 0.08-0.12 (s, 18H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 152.37, 132.29, 125.99, 124.08, 65.28, 63.29, 26.27, 26.12, 18.71, 18.54, -4.92, -5.18.

Synthesis of photocleavable linker 5 Compound **3** (0.120 g, 0.53 mmol) and precursor **4** (0.150 g, 0.28 mmol) were dissolved in 2 mL DMSO, followed by the addition of K_2CO_3 (80 mg, 0.58 mmol) and 18-crown-6 (10 mg, 0.038 mmol). The reaction mixture was stirred at 100 ^{o}C for 2 h. The reaction was monitored by TLC (EtOAc:Hex =1:19 and 1:7). Thereafter, 2 mL of water was added to the reaction mixture, and 3×5 mL diethyl ether was used to extract the product. The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was then dissolved in 5 mL methanol and excess Amberlyst-15 resin was added. The solution was stirred overnight and the resin was removed by filtration. The solvent was removed under reduced pressure and the crude product was purified by silica gel

column chromatography (EtOAc:Hex=2:1) to give the photocleavable linker **5** as white solid (26.0 mg, 24.4%). ^{1}H-NMR (400 MHz, d-acetone): δ 8.25-8.27 (d, 1H), 7.80-7.81 (d, 1H), 7.43 (s, 2H), 7.18-7.22 (dd, 1H), 5.42 (s, 2H), 5.04-5.05 (d, 2H), 4.70-4.71 (m, 4H), 4.62-4.64 (m, 2H), 3.22-3.23 (t, 1H); hydroxyl protons (3H): 4.12-4.18 (m) and 2.80-2.84 (m). ^{13}C-NMR (400 MHz, d-acetone): δ 162.33, 153.08, 140.31, 138.78, 138.21, 135.10, 127.62, 126.71, 113.99, 113.87, 78.04, 77.45, 72.82, 63.88, 63.75, 59.38, 59.25, 56.42. IR spectrum see Figure S2.

Synthesis of linker-CPT$_3$, 6 (Figure S2) Camptothecin (CPT, 7.6 mg, 21.8 μmol), 4-dimethylaminopyridine (DMAP, 11.4 mg, 93.3 μmol), and triphosgene (3.7mg, 12.5 μmol) were dissolved in 1.5 mL DCM, and the solution was stirred for 15 minutes. Thereafter, linker **5** (2.6 mg, 7.0 μmol) was added to the reaction mixture and the solution was shaken at room temperature on a Thermomixer (Eppendorf Thermo mixer C) overnight. The solution was dried *in vacuo* and the solids were suspended in acetonitrile/H_2O (1:1 vol:vol). Centrifugation (12000 rpm, 5 min) was used to remove insoluble components. The supernatant containing **6** was filtered and subjected to HPLC separation to give **6** (*ca*. 30% yield). Of note, compound **6** (and subsequent conjugates) can be degraded by the light inside the HPLC detector, and therefore must be collected with the detector switched off.

General protocol for the modification of amine-DNA with azido-dPEG®$_4$-NHS ester

DNA (0.28 μmol) was dissolved in 500 μL 0.1 M sodium bicarbonate solution. Azido-dPEG®$_4$-NHS ester (Quanta Biodesign Co., 1.4 mg, 3.6 μmol) was dissolved in 36 μL acetonitrile and was added to the DNA solution. The mixture was shaken on a Thermomixer at room temperature for 2 h, and excess NHS ester was removed by HPLC.

General protocol for "click" coupling reactions Stock solutions for click chemistry were prepared: 1 mg CuBr was dissolved in 70 μL DMSO, 54 mg tris(benzyltriazolylmethyl)amine (TBTA) was dissolved in 1 mL DMSO, and 20 mg sodium ascorbate was dissolved in 1 mL H_2O. CuBr and TBTA stock solutions were then pre-mixed in 1:2 (vol:vol) ratio. Compound **6** (0.2 mg,

0.13 μmol) was dissolved in 90 μL DMSO, to which an aqueous solution containing azide-modified DNA (50 μL, 5-25 OD) was added. After brief stirring, 10 μL of the pre-mixed ligand-Cu(I) solution was added to the reaction mixture, followed by 50 μL of the sodium ascorbate stock solution. The mixture was shaken on a Thermomixer at 40 ^{o}C for 1 h. Thereafter, the solvent was removed under reduced pressure. The crude product was dissolved in water and was purified by HPLC.

Quantification of DNA_{20}-CPT The concentration for as-synthesized DNA-CPT conjugates is unknown. Because free CPT and DNA-conjugated CPT have slightly different extinction coefficients, direct measurement of CPT absorbance at 350 nm should suffer from inherent errors. Therefore, an assay was developed to establish a quantitative relationship between the optical density (OD) at 260 nm and the amount of DNA_{20}-CPT in solution. The OD of a DNA_{20}-CPT solution at 260 nm was measured by a Thermo Scientific NanoDrop 2000 spectrophotometer. Separately, the solution was treated with prolonged UV light, which allows the conjugate to fully decompose and release free CPT. The solution containing free CPT was analyzed by HPLC, and the peak correlating with the CPT was quantified using a standard curve established using free CPT. It is determined that 9.3 nmol CPT and 3.1 nmol DNA are present in 1 OD (260 nm) of DNA_{20}-CPT.

Determination of critical micelle concentration (CMC) A series of concentrations of DNA-CPT conjugates were created by adding aliquots of concentrated DNA-CPT to the appropriate solutions (PBS with 5 mM Mg^{2+} for DNA_{20}-CPT, and PBS for DNA_5-CPT). The mixtures were placed in a Zetasizer Nano ZSP instrument, and the mean intensity of scattered light at backward (173.0 degrees) scattering angle was obtained as time-averaged count rate (in kcps). The count rates were plotted against conjugate concentration. Count rate increased with regard to conjugate concentration at a lower rate before micelle formation, and at a higher rate

thereafter. Each stage appeared to have a linear relationship (Figure S7). The inflection point denoting the CMC can be identified from the plot as the onset of the second stage.

Gel electrophoresis analysis of light-induced cleavage DNA_{20}-CPT and Cy3-DNA_{20} (31 nmol/mL) were electrophoresed in an agarose gel (1%, 0.5x Tris-Borate-EDTA buffer) for 30 min. Thereafter, the gel was imaged by a multiplex gel imager (Fluorochem Q, Protein Simple Co.), with two channels collected: excitation: trans-UV, emission: Cy2 (CPT), and excitation: Cy3, emission: Cy3 (Cy3-DNA_{20}). Next, the gel was exposed to UV light (365 nm, 10 mJ/s•cm^2) for 5 min, and was further electrophoresed for 30 min, before a second gel image was acquired.

Drug release kinetics Five vials of DNA_{20}-CPT solution in PBS buffer (10 μL, 31 nmol/mL) were each exposed to UV light (365 nm, 10 mJ/s•cm^2) for 0, 1, 2, 4, and 8 min, respectively. Thereafter, the samples were diluted by adding an additional 90 μL PBS buffer, and were analyzed by HPLC. The area under the curve for the peak correlating with the conjugate (measured at 350 nm) without UV treatment was regarded as 100% non-degraded. The decomposition was determined by measuring the reduction of the conjugate peak for samples treated with UV light. The experiment was repeated 3x and the values are averaged.

Gel electrophoresis analysis on the effect of Mg^{2+} ion on self-assembly Agarose gel (5%) was made using 0.05x PBS buffer instead of TBE buffer due to EDTA chelation with magnesium ions. Conjugates and their corresponding free dye-labeled DNA strands (~1 OD/mL) were electrophoresed in 0.05x PBS buffer (100 V, 30 min). Intermittent cooling was required to avoid heating and melting of the gel. To observe the effect of magnesium ions, the experiment was repeated in the presence of 2 mM $MgCl_2$ and with a 60 min electrophoresis time, and multiplex gel images were acquired (CPT excitation: trans-UV, emission: Cy2; DNA excitation: Cy2, emission: Cy2).

Transmission electron microscopy Samples (5 OD/mL, 4 μL) were deposited on the copper side of the carbon-coated copper grids. After 1.5 min, the sample was wicked away, and the grid was stained by depositing 4 μL of 2% uranyl acetate onto the grid. The stain was allowed to stay for 1 min before being wicked away. All TEM samples were imaged on a JEOL JEM 1010 electron microscope utilizing an accelerating voltage of 80 kV.

DNA nuclease stability assay Excess (1.5 equiv) complementary dabcyl-DNA_{20} was added to solutions of pre-formed DNA_{20}-CPT nanostructures (containing an equivalent of 1000 nM free DNA) or free Cy3-DNA_{20} (1000 nM) in DNase I buffer (10 mM tris, 2.5 mM $MgCl_2$, and 0.5 mM $CaCl_2$, pH=7.5). To anneal the duplexes, the solutions were heated to 60 ^oC for 1 h and cooled down to room temperature slowly over 30 min. The solutions were gently shaken overnight. Thereafter, aliquots of the samples were withdrawn and were diluted into 1 mL 100 nM solutions using DNase I buffer. Next, DNase I (0.1 unit, Sigma Aldrich) was added to the solution, and Cy3 fluorescence was monitored every 3 sec for 1 h (excitation: 512 nm, emission: 570 nm). The measurements were repeated 3x.

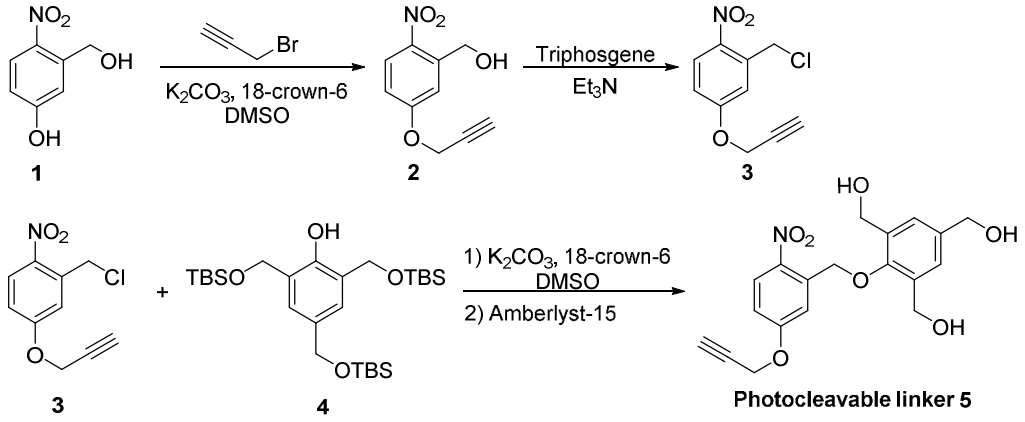
Confocal laser scanning microscopy SK-BR-3 cells were seeded into 35 mm glass bottom cell culture dishes (1×10^5 cells/well) and were cultured overnight in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 ^oC in a 5% CO_2 atmosphere. Then, the cells were washed with PBS buffer 3x and were incubated with pre-formed DNA_{20}-CPT nanostructures (1 μM equivalent DNA) in fresh FBS-free RPMI-1640 medium. After incubation for 6 h, the cells were washed with PBS buffer 4x. Afterwards, the cells were observed by a confocal microscope system (Olympus fluoview FV1000) immediately.

Cytotoxicity SK-BR-3 cells were cultured in RPMI 1640 media (Life Technologies 11875-093) supplemented with 10% fetal bovine serum (Gemini Bio-Products 100-106) and 1% Antibiotic-Antimycotic (Life Technologies 15240-062). To determine whether UV exposure

affects cell viability, SK-BR-3 cells were seeded at 5000 cells/well in 96-well plates and were irradiated with UV light (365 nm, 10 mJ/s•cm^2) the following day for up to 270 s. Cell viability was tested by CellTiter-Glo 2.0 at 24 h post-irradiation. Figure S7 shows that UV irradiation does not have a substantial effect on cell viability at 24 h post-irradiation. To assess the efficacy of DNA_{20}-CPT nanostructures, SK-BR-3 cells were seeded in 96-well plates at 5000 cells/well. Free CPT or DNA_{20}-CPT were added the following day and 6 h after drug addition, cells were irradiated at 365 nm for 4 min with approximate intensity 10 mJ/s•cm^2. Cell viability was tested by CellTiter-Glo 2.0 (Promega G9242) according to manufacturer's protocol at 3 days post-irradiation.

Table S1. Oligonucleotide sequences used in this study. Amine-modified DNA strands have been modified with azido-dPEG®4-NHS ester prior to all experiments.

Name of the compound	DNA sequence
DNA_5-CPT	5'-NH_2-TTT TT-3'
DNA_{4F}	5'-NH_2-TTT T-Fluorescein-3'
DNA_9-CPT	5'-NH_2-TTT TTT TTT-3'
DNA_{8F}	5'-NH_2-TTT TTT TT-Fluorescein-3'
DNA_{13}-CPT	5'-NH_2-TTT TTT TTT TTT T-3'
DNA_{12F}	5'-NH_2-TTT TTT TTT TTT-Fluorescein-3'
DNA_{17}-CPT	5'-NH_2-TTT TTT TTT TTT TTT TT-3'
DNA_{16F}	5'-NH_2-TTT TTT TTT TTT TTT T-Fluorescein-3'
Cy3-DNA_{20}	5'-NH_2-TTT TTC TCC ATG GTG CTC AC-Cy3-3'
Dabcyl-DNA_{20}	5'-dabcyl-GTG AGC ACC ATG GAG AAA AA-3'



Scheme S1. Synthetic route of the photocleavable linker, **5**.

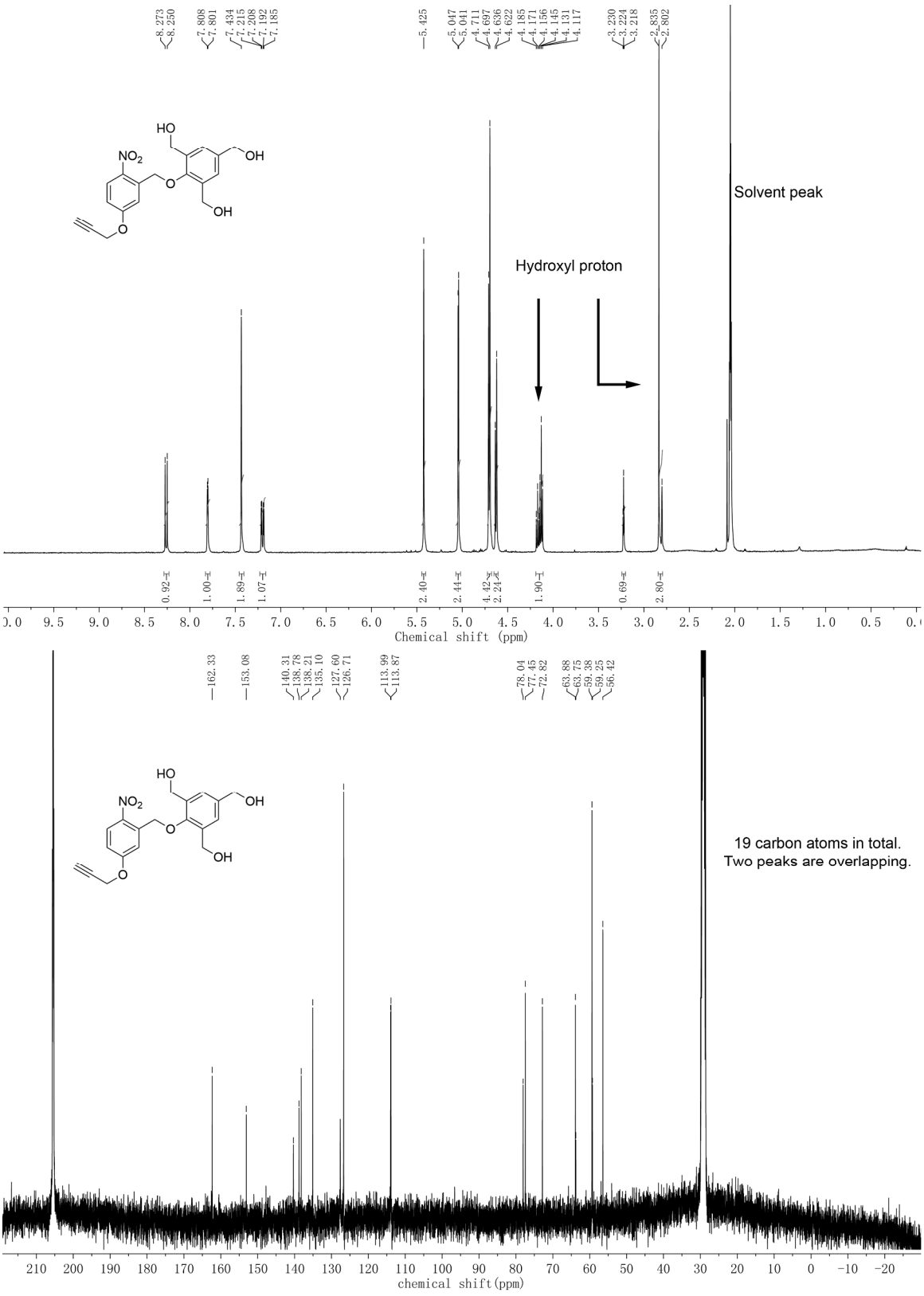


Figure S1. ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of **5** in d-acetone.

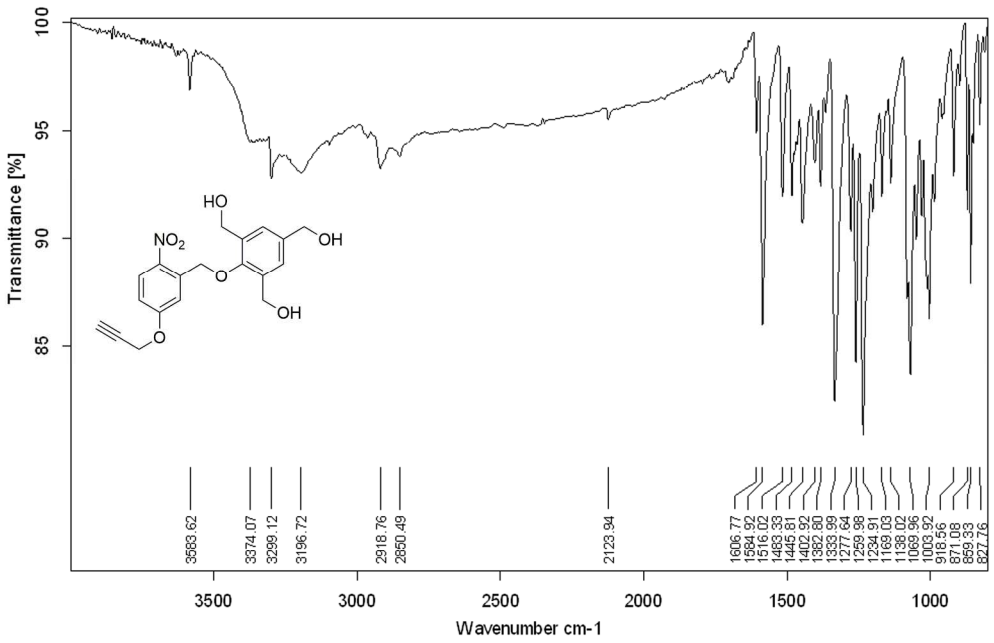
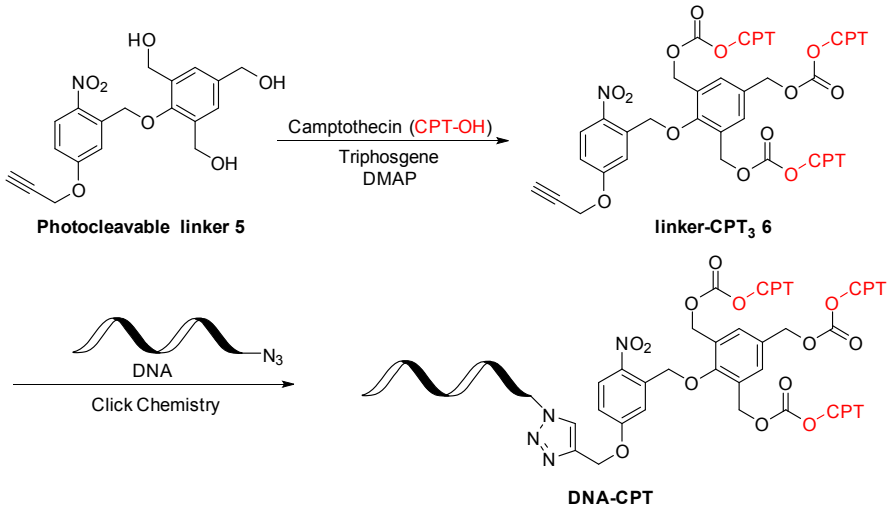
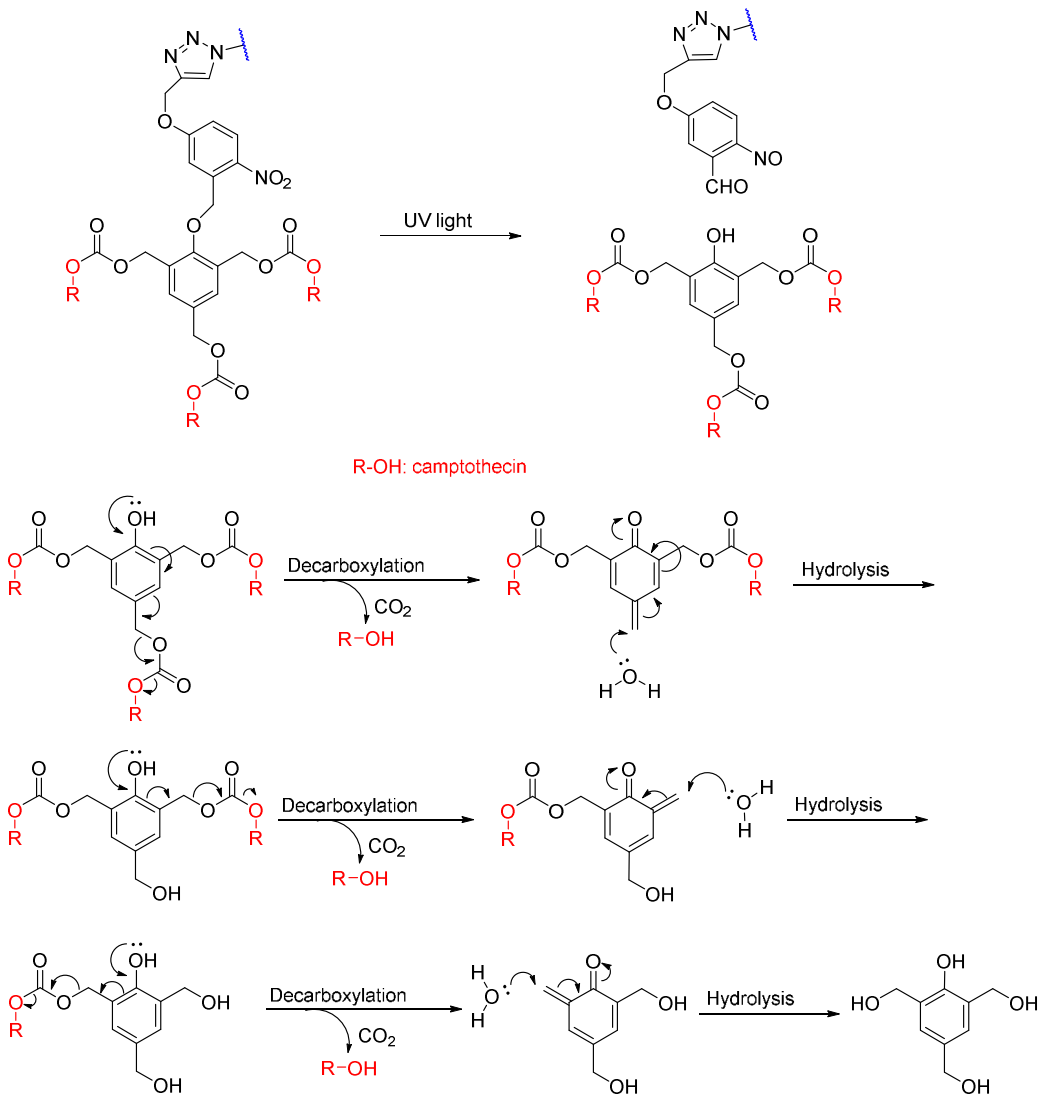


Figure S2. Infrared spectrum of **5**.



Scheme S2. Synthetic route of DNA-CPT conjugates.



Scheme S3. Camptothecin release pathway after UV irradiation.

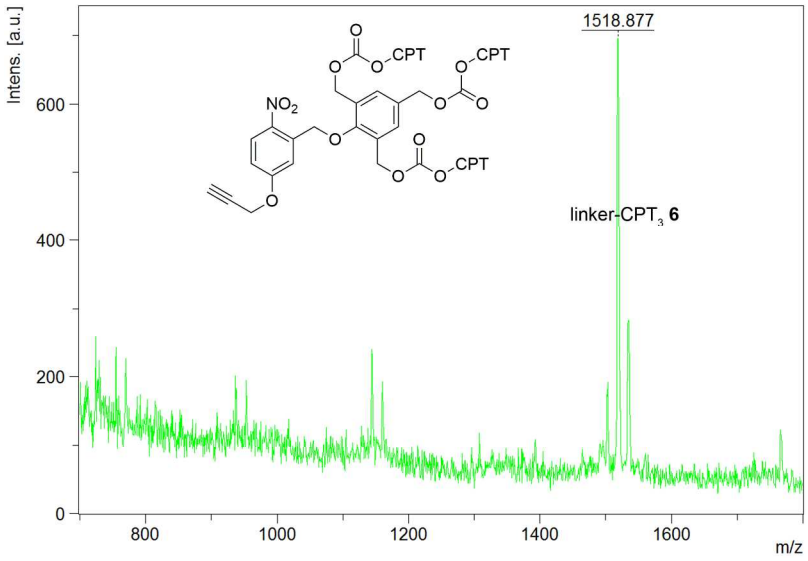


Figure S3. MALDI-ToF MS of **6**.

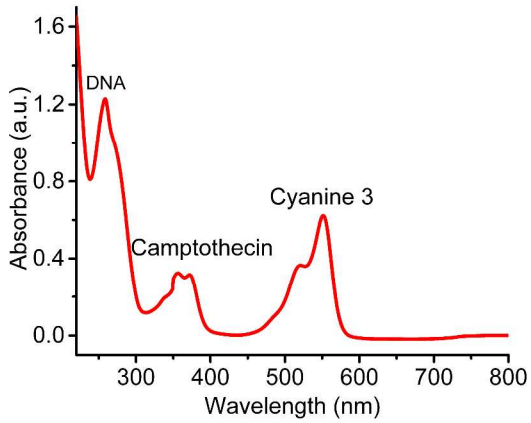


Figure S4. UV-Vis spectrum of the DNA_{20}-CPT conjugate.

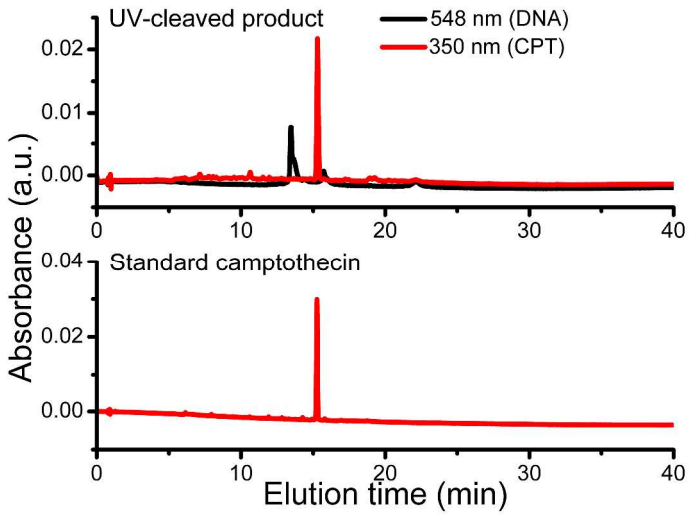


Figure S5. HPLC chromatograms of released products from UV-treated DNA-drug conjugates (top) and free, as-purchased CPT (bottom), showing agreement in retention times.

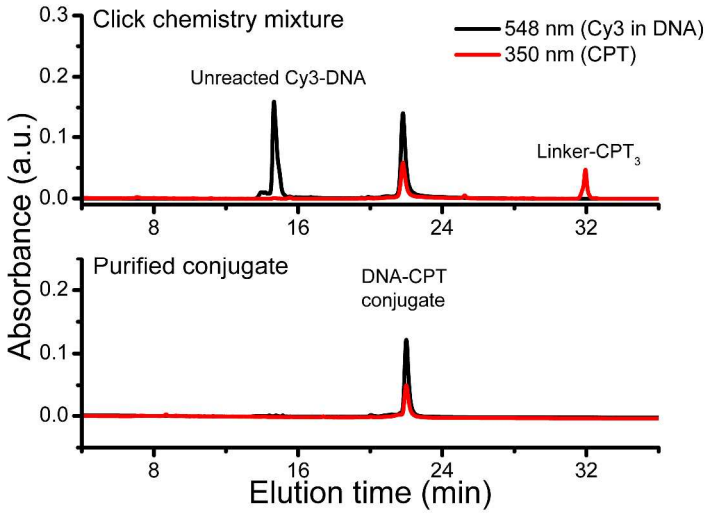


Figure S6. HPLC chromatograms of the reaction mixture for the coupling of Cy3-DNA and CPT trimer by click chemistry (top) and purified DNA-CPT conjugate (bottom).

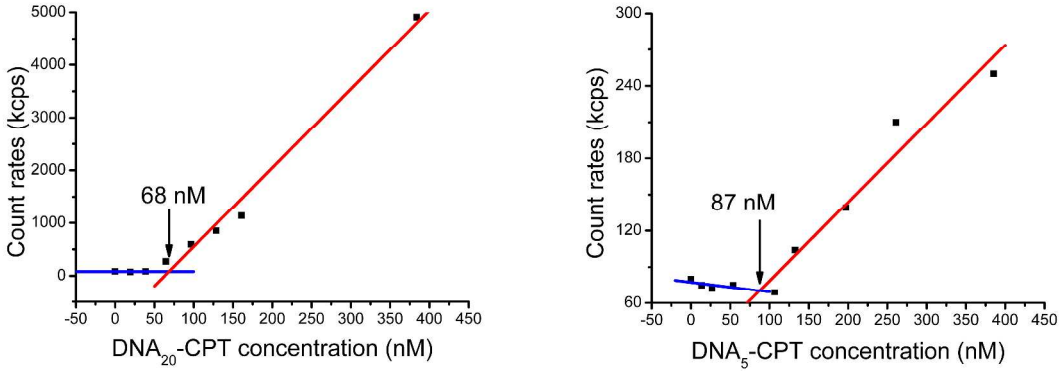


Figure S7. Determination of critical micelle concentrations for DNA_{20}-CPT in PBS containing 5.0 $MgCl_2$ (left) and DNA_5-CPT in PBS (right).

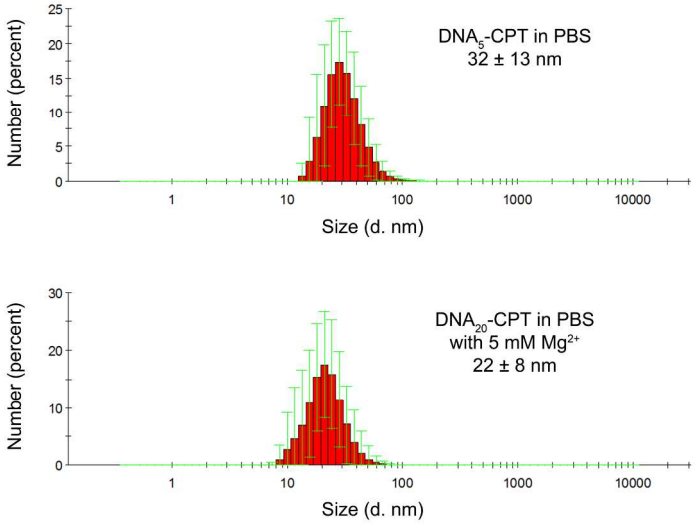


Figure S8. Number-average hydrodynamic diameters of nanostructures consisting of DNA_5-CPT in PBS (top) and DNA_{20}-CPT in PBS containing 5 mM $MgCl_2$ (bottom), as measured by DLS. Samples were measured at 25 ^{o}C, and the histograms are averages of 20 measurements.

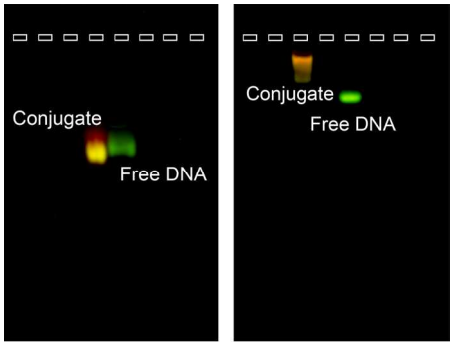


Figure S9. Multiplex gel imaging showing that free DNA_{20}-CPT undergoes self-assembly to form nanostructures with high MW in the absence (left) or presence (right) of Mg^{2+} (2 mM). CPT fluorescence is assigned a red color and Cy3-DNA a green color. The two colors co-localize in a single band to produce a yellow color. The top of the gel lane is marked with dashed line.

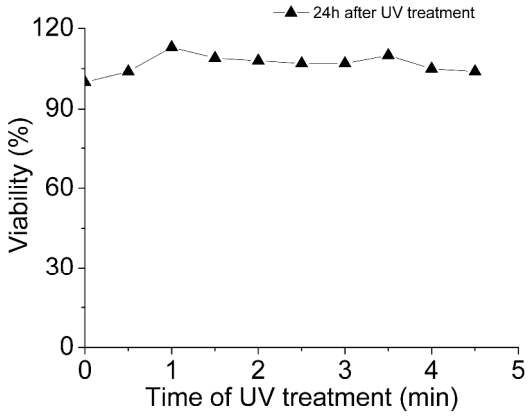


Figure S10. Cell viability as a function of UV dosage. The UV light used has a wavelength of 365 nm and intensity of 10 mJ/s•cm^2.

Reference:

(1) Haba, K.; Popkov, M.; Shamis, M.; Lerner, R. A.; Barbas, C. F., 3rd; Shabat, D. *Angew. Chem. Int. Ed.* **2005**, *44*, 716.