Electronic Supporting Information

Non-Invasive, Real-Time Reporting Drug Release *In Vitro* and *In Vivo*

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**Experimental section**

**Materials.** Camptothecin (CPT) and 7-ethyl-10-hydroxycamptothecin (EHCPT) were purchased from Wilshire Technologies, Inc. (Princeton, NJ, USA) and used as received. 2-hydroxyethyl disulfide (HEDS), 1,6-hexanediol, phosgene (15 wt% in toluene), triphosgene, sodium hydride (NaH), diisopropyl azodicarboxylate (DIAD), IR-780 iodide, and other reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. Tetrahydrofuran (THF) and dichloromethane (DCM) were dried with a column packed with alumina.

**Instrumentation.** NMR spectra were recorded on a Varian UI400 (400 MHz), UI500NB (500 MHz), or VXR-500 (500 MHz) spectrometer. Tandem gel permeation chromatography (GPC) was performed on a system equipped with an isocratic pump (Model 1100, Agilent Technologies, Santa Clara, CA, USA), a DAWN HELEOS 18-angle laser light scattering detector (also known as multi-angle laser light scattering (MALLS) detector; Wyatt Technology, Santa Barbara, CA, USA), and an Optilab rEX refractive index detector (Wyatt Technology). The detection wavelength of the HELEOS was set at 658 nm. Separations were performed on serially connected size exclusion columns (100 Å, 500 Å, 10^3 Å and 10^4 Å Phenogel columns, 5 µm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60°C with DMF containing 0.1 M LiBr as the mobile phase. The HELEOS detector was calibrated with pure toluene without the need for external polymer standards and was used for the determination of the absolute molecular weights. The molecular weight of each polymer was determined from the dn/dc value calculated offline by means of the internal calibration system processed by the ASTRA V software (Version 5.1.7.3, Wyatt Technology). Measurement of fluorescence spectra of compounds was carried out on a LS55 fluorescence spectrometer (Perkin Elmer, Santa Clara, CA, USA). The NIR fluorescence imaging in vivo were conducted on a whole-animal fluorescence imaging system (Maestro, CRi, Inc) coupled with a 615-665 nm excitation filter and a 680-950 nm emission filter. The images were then analyzed using the Maestro in vivo fluorescence imaging software. HPLC was performed on a System Gold system (Beckman Coulter, Fullerton, CA, USA) equipped with a 126P solvent module, a System Gold 128 UV detector, and an analytical C18 column (Luna C18, 250 mm × 4.6 mm, 5 µm, Phenomenex). The UV wavelengths for the
analysis of CPT and CyN were set at 370 nm and 590 nm, respectively. The liquid chromatography-multiple-reaction monitoring-mass spectrometry (LC-MRM/MS) was employed for the quantification of CPT using the internal standard EHCPT at a concentration of 200 ng/mL. A calibration curve was established between 1 and 100 ng/mL of CPT. The LC-MRM/MS system consists of an analytical high performance liquid chromatography separation module Waters Alliance 2795 (Waters, Milford, MA) coupled with an electrospray ionization mass spectrometer (Waters QuattroUltima, Waters, Milford, MA). Samples were analyzed using a reversed-phase C18 column (Luna C18, 250 mm × 4.6 mm, 5 µm, Phenomenex). The solvent system was composed of two solutions: solution A (95% H₂O, 5% acetonitrile (ACN) and 0.1% formic acid (FA)) and solution B (5% H₂O and 95% ACN, and 0.1% FA). The 30 min gradient LC separation included 5 steps: 75% solvent A in 0–5 min (isocratic); 75–10% solvent A for 5–15 min (linear); 10% solvent A for 15-25 min (isocratic); 10-75% solvent A for 25-28 min (linear); and 75% solvent A for 28-30 min (isocratic). The MRM data acquisition consisted of monitoring the following analytes in positive mode using these transitions (parent ion → fragment ion, cone voltage, collision voltage), CPT (349.0 → 219.0, 30 eV, 50 eV and 349.0 → 305.1, 30 eV, 20 eV), and EHCPT (393.0 → 197.0, 30 eV, 50 eV and 393.0 → 249.1, 30 eV, 50 eV) all with 100 ms dwell time. Quantification of CPT was determined using the peak areas normalized to the internal standard (EHCPT 200 ng/ml) and a previously calculated standard curve. Analytical data was processed using Waters Mass Lynx software (version 4.1).

Synthesis of N-(3,6,9-trioxadecyl)phthalimide (TEG-PA). Phthalimide (3.53 g, 24 mmol), triethylenglycol monomethylether (TEG-OH, 3.2 mL, 20 mmol), and PPh₃ (6.3 g, 24 mmol) were dissolved in THF (100 mL). After 15 min, DIAD (4.73 mL, 24 mmol) was added dropwise at room temperature. After 12 h, the reaction was quenched by EtOH (40 mL). The solvent was removed and the residue was re-dissolved in hexane/EtOAc (1:1, v/v, 20 mL) and stirred at 40 °C for 1 h. The white solid was filtered and washed with the same solvent mixture (10 mL). The filtrate was concentrated under vacuum and the residues was purified by silica gel column chromatography (hexane/EtOAc, 4:1, v/v) to afford TEG-PA as colorless oil (5.55 g, yield 92%).

¹H NMR (CDCl₃, 500 MHz): δ 7.81–7.77 (m, 2 H), 7.69–7.65 (m, 2 H), 3.83 (t, J = 5.5 Hz, 2 H), 3.71–3.67 (t, J = 5.5 Hz, 2 H), 3.62–3.51 (m, 6 H), 3.40 (m, 2 H), 3.28 (s, 1 H). ¹³C NMR (CDCl₃, 500 MHz): δ 168.1, 133.8, 132.0, 123.1, 71.7, 70.4, 69.9, 67.7, 58.8, 37.1. ESI-MS (low
resolution, positive mode): calculated for C_{15}H_{20}NO_{5}, m/z, 294.1 [M + H]^+; found 294.1 [M + H]^+.

**Scheme S1.** General synthetic route of activatable prodrug (CPT-SS-CyN) and non-activatable prodrug (CPT-CC-CyN).

**Scheme S2.** Synthetic route of TEG-NH₂.
Synthesis of 3,6,9-trioxadecylamine (TEG-NH₂).\textsuperscript{1} The TEG-PA (5.55 g, 18.9 mmol) and hydrazine monohydrate (1.16 mL, 22.4 mmol) were dissolved in EtOH (55 mL). The resulting mixture was refluxed for 5 h, whereupon a white precipitate formed. The slurry was allowed to cool and then treated with HCl (36 wt%, 4.8 mL), followed by refluxing again for 1 h. The slurry was allowed to cool to room temperature and the white solid was filtered off. The filtrate was concentrated under vacuum and the residue was dissolved in H₂O (30 mL). The solution was brought to pH 11 with 1 N NaOH (≈ 20 mL). The aqueous phase was saturated with NaCl and extracted with DCM (4 × 30 mL). The combined organic phases were dried over anhydrous Na₂SO₄. The slightly brownish liquid was obtained after removal of the solvent. The residue was purified by reduce pressure distillation, which afforded TEG-NH₂ as a colorless liquid (2.2 g, 68%). \textsuperscript{1}H NMR (CDCl₃, 500 MHz): δ 3.62 (s, 4H), 3.53 (s, 2H), 3.47 (s, 2H), 3.34 (s, 3H), 2.82 (s, 2H), 1.40 (s, 2H). \textsuperscript{13}C NMR (CDCl₃, 500 MHz): δ 73.2, 71.8, 70.5, 70.4, 70.1, 58.9, 41.6. ESI-MS (low resolution, positive mode): calculated for C₇H₁₈NO₃, m/z, 164.1 [M + H]⁺; found 164.1 [M + H]⁺.

**Scheme S3.** Synthetic route of CPT-SS-OH.

![Synthetic route of CPT-SS-OH](image)

Synthesis of CPT-SS-OH. CPT (700 mg, 2 mmol) and triphosgene (220 mg, 0.67 mmol) were dispersed in DCM (100 mL), to which DMAP (780 mg, 6.4 mmol) was added. The mixture was stirred at room temperature for 15 min. 2-Hydroxyethyl disulfide (HEDS) (1.54 g, 10 mmol) in THF (20 mL) was added. The solution was stirred at room temperature overnight, diluted with DCM (400 mL) and washed with HCl (pH 1, 3 × 40 mL) and then with water (3 × 40 mL). The organic phase was dried with Na₂SO₄. After the solvent was removed under vacuum, CPT-SS-OH was purified by silica gel column chromatography (EtOAc/MeOH = 10:1 v/v) to give a slight yellow solid (560 mg, yield 57%). \textsuperscript{1}H NMR (CDCl₃, 500 MHz): δ 8.45 (s, 1H), 8.26 (d, J = 8.5 Hz, 1H), 7.98 (dd, J = 8.2, 1.3 Hz, 1H), 7.88 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 7.71 (ddd, J = 8.0, 6.8, 1.1 Hz, 1H), 7.46 (s, 1H), 5.74–5.45 (d, J = 17.2 Hz, 2H), 5.27 (s, 2H), 4.47–4.33 (m,
2H), 3.93 (tq, J = 11.5, 5.9 Hz, 2H), 3.07–2.84 (m, 4H), 2.32 (dq, J = 14.9, 7.4 Hz, 2H), 2.19 (dq, J = 14.9, 7.5 Hz, 2H), 1.05 (q, J = 7.6 Hz, 3H). 13C NMR (CDCl3, 500 MHz): δ 168.2, 160.3, 156.2, 154.1, 149.0, 147.8, 145.9, 145.6, 134.8, 134.6, 131.9, 130.7, 130.0, 129.0, 128.4, 127.2, 126.5, 125.1, 124.3, 119.8, 98.3, 81.2, 68.2, 65.0, 62.5, 52.6, 32.6, 9.5. ESI-MS (low resolution, positive mode): calculated for C25H25N2O7S2, m/z, 529.1 [M + H]+; found 529.1 [M + H]+.

**Scheme S4.** Synthetic route of CyN.

**Synthesis of CyN.** IR-780 iodide (130 mg, 0.2 mmol), TEG-NH2 (98 mg, 0.6 mmol), and N,N-diisopropylethylamine (DIPEA, 52 µL, 0.4 mmol) were dissolved in acetonitrile (ACN) (3 mL). The mixture was stirred at 80 °C for 1 h. The solution was cooled to room temperature. The residues were purified by silica gel column chromatography (EtOAc/MeOH, 100:1 to 20:1, v/v) to afford the CyN as blue solid (110 mg, yield 69%). 1H NMR (CDCl3, 500 MHz): δ 7.76 (d, J = 13.1 Hz, 2H), 7.35–7.26 (m, 4H), 7.14–7.06 (m, 2H), 6.90 (d, J = 7.8 Hz, 2H), 5.70 (d, J = 12.9 Hz, 2H), 4.05–3.95 (m, 2H), 3.87–3.80 (m, 4H), 3.77–3.63 (m, 2H), 3.61–3.47 (m, 2H), 3.42 (s, 3H), 2.51 (t, J = 6.4 Hz, 4H), 1.92 (m, 4H), 1.89–1.66 (m, 12H), 1.59 (m, 4H), 1.37–1.25 (m, 2H), 1.06 (t, J = 7.4 Hz, 6H). 13C NMR (CDCl3, 500 MHz): δ 173.2, 146.5, 144.4, 142.3, 141.2, 140.5, 128.3, 126.6, 116.8, 108.6, 104.8, 71.6, 71.2, 70.1, 59.0, 54.4, 51.2, 49.5, 45.1, 30.3, 27.7, 26.8, 25.8, 24.7, 21.4, 11.9, 10.6. ESI-MS (low resolution, positive mode): calculated for C43H60N3O3, m/z, 666.4 [M]+; found 666.3 [M]+.

**Scheme S5.** Synthetic route of CPT-SS-CyN.
Synthesis of CPT-SS-CyN. CPT-SS-Cl (Scheme S4) was first prepared. Phosgene solution in toluene (2 mL, 15% w/w, 2.88 mmol) was added to the solution of CPT-SS-OH (100 mg, 0.19 mmol) in dry THF (5 mL) with stirring. The mixture was stirred for 16 h at room temperature. The excess phosgene and solvents were removed under reduced pressure, and the phosgene in the vacuum traps was deactivated by aqueous NaOH. The resulting yellowish solid residue (CPT-SS-Cl) was used in the subsequent reaction without further purification.

CyN (126 mg, 0.19 mmol) was dissolved in THF (10 mL), to which NaH (8 mg, 0.33 mmol) was added. The resulting mixture was stirred at room temperature for 20 min, with the color of the solution changing from dark blue to orange. The THF solution of CPT-SS-Cl (10 mL) was added dropwise over the course of 5 min, followed by the addition of DIPEA (52 µL, 0.4 mmoL). The mixture was stirred at room temperature overnight. After that, the solution was diluted with DCM (400 mL), washed with HCl (pH 1, 3 × 40 mL) and then with water (3 × 40 mL). The organic phase was dried with Na2SO4. After the solvent was removed under vacuum, CPT-SS-CyN was purified by silica gel column chromatography (EtOAc/MeOH = 50:1 to 3:1, v/v) to give a green solid (36 mg, yield 15.9%). 1H NMR (CDCl3, 500 MHz): δ 8.56 (s, 1H), 8.25 (d, 1H), 8.03 (t, 1H), 7.86 (d, 1H), 7.73–7.55 (m, 3H), 7.44–7.12 (m, 5H), 7.01–6.91 (m, 2H), 6.31–6.17 (m, 2H), 5.74–5.54 (m, 4H), 4.50–4.06 (m, 4H), 3.94–3.51 (m, 10H), 3.34 (s, 3H), 2.94–2.72 (m, 4H), 2.60 (m, 4H), 2.31–2.05 (m, 2H), 2.00–1.83 (m, 4H), 1.83–1.65 (s, 12H), 1.33–0.88 (m, 9H). 13C NMR (CDCl3, 500 MHz): δ 173.4, 168.5, 160.3, 156.7, 154.9, 151.0, 147.8, 145.9, 144.7, 142.6, 141.8, 140.9, 134.2, 131.3, 130.7, 129.0, 128.4, 127.2, 126.0, 125.1, 124.5, 119.8, 116.0, 108.4, 104.5, 98.5, 81.7, 71.0, 70.1, 68.6, 65.0, 62.3, 59.3, 54.8, 52.1, 51.2, 49.5, 45.2, 36.4, 30.7, 27.0, 26.8, 25.6, 24.8, 21.4, 11.9, 10.3, 9.0. ESI-MS (low resolution, positive mode): calculated for C69H82N5O11S2, m/z, 1220.5 [M]+; found 1220.5 [M]+. The purity of CPT-SS-CyN was over 95% by Prep-HPLC.

Synthesis of CPT-CC-CyN. The non-activatable prodrug CPT-CC-CyN (Scheme S1) was synthesized by following the same method as CPT-SS-CyN preparation using 1,6-hexanediol instead of 2-hydroxyethyl disulfide as the starting materials. After purification, a green solid was obtained (30 mg, overall yield 8.2%). 1H NMR (CDCl3, 500 MHz): δ 8.60 (s, 1H), 8.30 (d, 1H), 8.08 (t, 1H), 7.92 (d, 1H), 7.75–7.53 (m, 3H), 7.44–7.10 (m, 5H), 7.03–6.95 (m, 2H), 6.32–6.11
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(m, 2H), 5.75–5.57 (m, 4H), 4.52–4.01 (m, 4H), 3.90–3.53 (m, 10H), 3.34 (s, 3H), 2.92–2.70 (m, 4H), 2.34–2.10 (m, 2H), 2.04–1.88 (m, 4H), 1.85–1.55 (m, 16H), 1.42–0.98 (m, 13H). ESI-MS (low resolution, positive mode): calculated for C_{71}H_{86}N_{5}O_{11}, \text{m}/\text{z}, 1184.6 [M]^+; found 1184.7 [M]^+.

The purity of CPT-SS-CyN was over 95% by Prep-HPLC.

**General procedure for the degradation of CPT-SS-CyN and analysis of drug release by HPLC**

To the solution of CPT-SS-CyN (20 uM, 1 mL) in DMF/PBS (1:49, v/v), DTT in PBS (2 mM, 1 mL) was added. And the solution was incubated at 37 °C for a specified period of time. The solution was used for HPLC analysis.

**MTT assay of CPT, CPT-SS-CyN, and CyN.**

The HeLa cells (ATCC, Manassas, VA, USA) used for MTT assays were cultured in MEM medium containing 10% Fetal Bovine Serum (FBS), 100 units/mL aqueous Penicillin G and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA). HeLa cells were seeded in a 96-well plate for 24 h (3, 000 cells per well). They were then washed once with PBS (100 μL). Freshly prepared solutions of CPT, CPT-SS-CyN, and CyN of different concentrations in cell medium (100 μL/well) were added to the cells. After incubation at 37 °C for 72 h, the medium was removed. Standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay protocols were followed.\(^2\) The absorbance wavelength on a microplate reader (Perkin Elmer, Victor\(^{3\,\text{TM}}\) V, Waltham, MA, USA) was set at 590 nm for the MTT assay. The half maximal inhibitory concentration (IC\(_{50}\)) was determined by half-cell viability concentration from the MTT assay test.

**Determination of the relationship between the fluorescence intensity per cell in the images analyzed by GE In-Cell Analyzer, the amount of CyN released from CPT-SS-CyN analyzed by fluorescence spectrometer and the amount of CPT released from CPT-SS-CyN analyzed by LC-MRM/MS.**

HeLa cells were plated in a 6-well plate at a confluency of 150, 000 cells per well (6 wells per group) for 48 h. Cells were then washed once with PBS (1 ×, 1.0 mL) and the medium was...
replaced with Opti-MEM (1.0 mL). Freshly prepared solutions of CPT-SS-CyN with different concentrations in cell medium (1.0 mL/well) were added to the cells directly and mixed by gentle shaking. After incubation at 37 °C for 3.5 h, the Hoechst (1 µg/mL) was added to the cells, and incubated for another 30 minutes. And then the medium was removed, cells were then washed 3 times with PBS (1 ×, 1.0 mL) and the media was replaced with Opti-MEM (2.0 mL) and placed directly into the GE In Cell Analyzer 2000 at 20 × objective magnification for imaging. The imager was set to take 6 random fields in each well in bright field, DAPI (excitation at 320-370 nm, emission at 430-580 nm) and Cy5 channel (excitation at 640-660 nm, emission at 680-750 nm) every 10 min. The images were then stored and analyzed using the GE In Cell Investigator Workstation software. In brief, a protocol was established to identify cells by the nuclear stain and the fluorescence intensity of the CY5 channel was obtained in the cell and quantified. For each group, 3 wells were treated by Trypsin containing MEM medium (0.25%, 1.0 mL) for 5 min, and diluted to 2 mL with MEM medium. The cell numbers were determined by flow cytometry. The other 3 wells were treated by RIPA buffer (0.5 mL) for 20 min, and diluted to 2 mL with PBS buffer (1 ×). The solutions were analyzed by fluorescence spectrometer (excitation at 640 nm). The concentrations of CyN in solution was determined from concentration dependent fluorescence intensity standard curve of CyN in RIPA/PBS (1/3, v/v) buffer (excitation at 640 nm). Then, the solutions were dried via lyophilization for 48h, and the mixtures were redispersed in methanol (2.0 mL). The suspension solution was centrifuged at 15,000 rpm for 15 min and the upper layer of the solutions was used for LC-MRM/MS analysis.

**Real time monitoring the fluorescence enhancement from CPT-SS-CyN and CPT-CC-CyN in HeLa cells using GE In Cell Analyzer.**

HeLa cells were plated in 24 well plates at a confluency of 50,000 cells per well. They were then stained with Hoechst (1 µg/mL) for 30 minutes and then washed with PBS (3 × 1.0 mL). The media was replaced with Opti-MEM (1.0 mL). The CPT-SS-CyN or CPT-CC-CyN (0.5 µM) were then added to the cells, mixed by gentle shaking, and placed directly into the GE In Cell Analyzer 2000 at 20 × objective magnification for imaging. The imager was set to take 6 random fields in each well in bright field, DAPI, and CY5 channels every 10 min. The images were then stored and analyzed using the GE In Cell Investigator Workstation software. In brief, a protocol
was established to identify cells by the nuclear stain and the fluorescence intensity of the CY5 channel was obtained in the cell and quantified.

**Real-time monitoring of the fluorescence enhancement from CPT-SS-CyN in A375 melanoma tumor implanted athymic nude mice using Maestro in vivo whole-animal fluorescence imaging system.**

Athymic nude mice (female, 6-8 weeks old) bearing subcutaneous A375 melanoma tumors (average tumor size ≈500 mm³) were grouped to minimize the difference of tumor size and body weight in all the groups (n=3). The mice received intratumoral injection of CPT-SS-CyN (5 nmol in 25 μL DMSO/PBS (1:10, v/v)). Mice were anesthetized 5 min post-injection with isoflurane/oxygen for 3 min, and then imaged under anesthesia using a whole-body fluorescence imaging system (Maestro, CRi, Inc.) coupled with a 615–655 excitation filter and a 680–950 nm emission filter. General anesthesia was maintained by isoflurane/oxygen during the experiment and the mice were allowed to recover after imaging. The same mice were then re-imaged in the imaging system every 30 min for 5 h. After the imaging study was complete, the mice were euthanized. The fluorescence images of the mice were processed using the Maestro in vivo fluorescence imaging software. Based on the fluorescence spectra of the dyes obtained from the tumor areas, the fluorescence intensity at 740 nm at different time points were used to semi-quantitatively analyze the CPT release profiles in each tumor tissue.
Figure S1. $^1$H NMR spectrum of TEG-NH$_2$ in CDCl$_3$. 
Figure S2. $^1$H NMR spectrum of CPT-SS-OH in CDCl$_3$. 
Figure S3. $^1$H NMR spectrum of CyN in CDCl$_3$. 
Figure S4. $^1$H NMR spectrum of CPT-SS-CyN in CDCl$_3$. 
Figure S5. ESI-MS spectrum of CPT-SS-CyN.
Figure S6. HPLC curves of CPT (a), CyN (b) CPT-SS-CyN before (c) and after (d) DTT treatment (10 μM of CPT-SS-CyN and 1 mM of DTT in DMF/H₂O (1:99, v/v) at 37 °C for 2 h).
Figure S7. (a) The absorption spectra and (b) excitation spectra (dashed line) and emission spectra (solid line) of CyN, CPT-SS-CyN (10 µM) with and without DTT treatment (1 mM).
Figure S8. (a) Fluorescence intensity change of CPT-SS-CyN (10 µM) at 760 nm treated with DTT at various DTT/CPT-SS-CyN ratios in PBS (pH 7.4). (b) Fluorescence intensity change of CPT-SS-CyN at 760 nm after treatment of different amino acids (amino acid/CPT-SS-CyN molar ratio of 100:1) and GSH in PBS (pH 7.4).
Figure S9. (a) Chemical scheme for the non-activatable prodrug CPT-CC-CyN treated with DTT. (b) Fluorescence spectra of the non-activatable prodrug CPT-CC-CyN with and without DTT treatment. (c) Fluorescence images of HeLa cells treated with CPT-SS-CyN on a GE InCell Analyzer with QUAD 1 Polychroic. The cells were incubated with serum free DMEM medium containing CPT-SS-CyN (0.5 μM); the images were collected after 5 h incubation at 37 °C. The nucleus was stained with Hoechst. The cells images were obtained using DAPI channel (excitation at 320-370 nm, emission at 430-580 nm) and Cy5 channel (excitation at 640-660 nm, emission at 680-750 nm).
Figure S10. (a) Schematic illustration for the analysis of amounts of CyN per cell and fluorescence intensity per cell using GE In Cell Analyzer. (b) Fluorescence curves of lysed cells in the mixture of RIPA lysis buffer and PBS (1/3, v/v) after 4 h incubation with different dosages of CPT-SS-CyN in HeLa cells (~ $1 \times 10^7$/well). (c) The amounts of CyN per cell at different amount of CPT-SS-CyN applied to HeLa cells. The cell numbers per well were determined by flow cytometry. The total amounts of CyN in the RIPA/PBS (1/3, v/v) buffer solution were determined by standard curve of the CyN dye (excitation wavelength 640 nm, emission wavelength 740 nm). The amount of CyN per cell was calculated by dividing the total amount of CyN divided by the number of cells per well.
**Figure S11.** Fluorescence images of HeLa cells treated with CPT-SS-CyN by GE In-Cell Analyzer with QUAD 1 Polychroic at DAPI and Cy5 channels with analysis areas.
Figure S12. Correlation of the average fluorescence intensity per cell analyzed by GE In-Cell Analyzer with QUAD 1 Polychroic with the number of CyN per cell in HeLa cells analyzed by fluorescence spectroscopy.
Figure S13. Correlation of the average amount of CyN per cell analyzed by fluorescence spectroscopy with the average amount of CyN per cell analyzed by LC-MRM/MS
**Figure S14.** Standard curve of the integration ratios of CPT/7-ethyl-10-hydroxycamptothecin (EHCPT) at different CPT concentrations in methanol using EHCPT (200 ng/mL) as the internal standard of LC-MS analysis
Figure S15. Viability of HeLa cells treated with CPT, CyN, CPT-SS-CyN, and CPT-CC-CyN at different concentrations analyzed by microculture tetrazolium (MTT) assay.
Reference
