Electronic Supplementary Information

For

Cancer-targeted Design of Bioresponsive Prodrug with Enhanced Cellular Uptake to Achieve Precise Cancer Therapy

Methods

Synthesis of OH-ss-CPT. To a mixture of **CPT** (70 mg, 0.2 mmol) and triphosgene (24 mg, 0.08 mmol) in 30 mL of dry chloroform was added DMAP (60 mg, 0.48 mmol) in CH_2Cl_2 dropwise. The solution was allowed to react for 4 h, then flushed with argon for 5 min, following by adding of 2,2'-dithiodiethanol (1 mmol, 154 mg) and DIPEA (25 μL, 0.1 mmol) in 6 ml anhydrous THF. The reaction mixture was allowed to stir for 5 h. Then the solvent was evaporated, the resulted solid was washed with water for three times. The crude product was purified over silica gel using MeOH / CH_2Cl_2 (v/v, 1:40) as the eluent to yield **OH-ss-CPT** as yellowish solid (86 mg, 82%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ8.46 (s, 1H), 8.27 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.2 Hz, 1H), 7.91-7.87 (m, 1H), 7.74-7.70 (m, 1H), 7.47 (s, 1H), 5.72 (d, J = 7.6 Hz, 1H), 5.42 (d, J = 7.6 Hz, 1H), 5.34 (s, 2H), 4.46-4.35 (m, 2H), 3.94 (s, 2H), 3.34 (s, 1H), 3.07-2.86 (m, 4H), 2.37-2.15 (m, 2H), 1.05 (t, 3H，J = 7.4 Hz). Mass spectrometry (HR-MS, m/z): [M + H^+] calcd for $C_{25}H_{25}N_2O_7S_2$, 529.1103; found 529.1079.

Synthesis of Biotin-ss-CPT. **OH-ss-CPT** (53 mg, 0.1 mmol), DAMP (15 mg, 0.12 mmol), EDC (21 mg, 0.11 mmol) and biotin (25 mg, 0.1 mmol) was suspended in 30 ml CH_2Cl_2, the mixture was allowed to stirred for 13 h at room temperature. The solvent was evaporated and washed with 10% hydrochloric acid and water twice respectively. The resulting crude product was subject to HPLC to yield **Biotin-ss-CPT** as white solid (45 mg, 60%). Purity: 99.7% (Figure S19). HPLC: Agilent 1260 infinity pre-system, YMC-Pack ODS-A Column (250*20 mml. D. S-5μm, 12nm). Mobile phase A: H_2O, B: MeOH. 0-26 min: 30%-70% B, 26-34 min: 70%-100% B. Absorption wavelength, 365

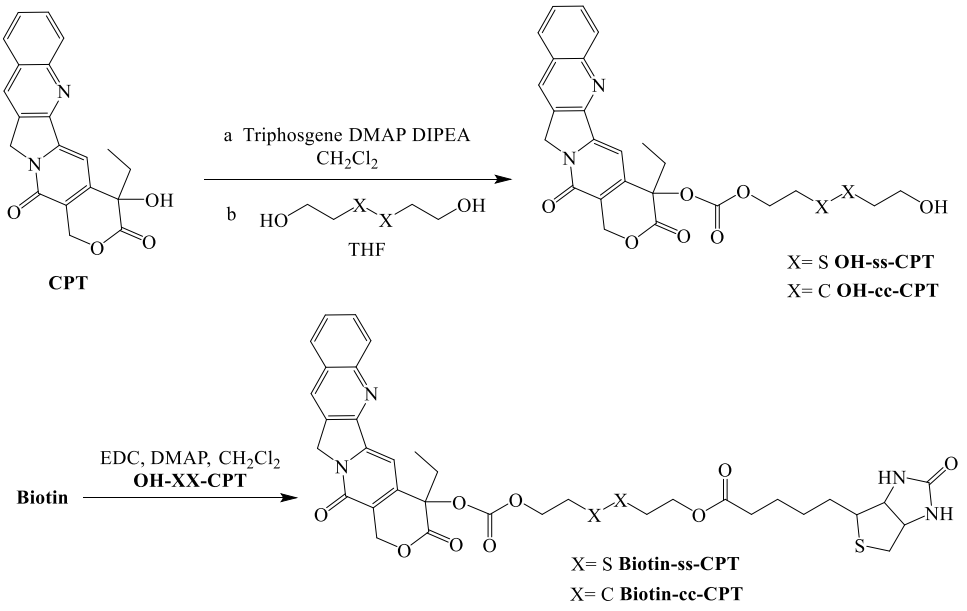
nm (based on the UV spectrum in Figure S20). ^{1}H NMR ($CDCl_3$, 500 MHz): δ8.46 (s, 1H), 8.29 (d, *J* = 8.6 Hz, 1H), 7.98 (d, *J* = 8.4 Hz, 1H) 7.88-7.85 (m, 1H), 7.71-7.69 (m, 1H), 7.44 (s, 1H), 5.68(d, *J* = 7.6 Hz, 1H), 5.41 (d, *J* = 7.6 Hz, 1H), 5.34 (s, 2H), 4.53-4.51 (m, 1H), 4.42-4.32 (m, 3H), 4.27-4.23 (m, 2H), 3.17-3.16 (m, 1H), 2.98-2.88 (m, 5H), 2.72 (d, *J* = 6.3 Hz, 1H), 2.33-2.26 (m, 3H), 2.20-2.13 (m, 1H), 1.71-1.60 (m, 4H), 1.47-1.39 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) : δ173.2, 167.3, 162.9, 157.3, 153.5, 151.9, 148.3, 146.1, 145.7, 131.8, 131.0, 129.2, 128.7, 128.3, 128.3, 120.4, 96.64, 78.1, 67.1, 66.6, 61.97, 61.96, 60.2, 55.2, 53.4, 52.7, 50.2, 40.5, 37.5, 36.6, 33.7, 31.9, 28.2, 24.7, 7.7. Mass spectrometry (HR-MS, m/z): [M + H^+] calcd for $C_{35}H_{39}N_4O_9S_3$, 755.1879; found 755.1862.

Synthesis of OH-cc-CPT. To a mixture of **CPT** (70 mg, 0.2 mmol) and triphosgene (24 mg, 0.08 mmol) in 30 mL of dry chloroform was added DMAP (60 mg, 0.48 mmol) in CH_2Cl_2 dropwise. The solution was allowed to react for 4 h, then flushed with argon for 5 min, following by adding of 1,6-hexanediol (1 mmol, 118 mg) and DIPEA (25 μL, 0.1 mmol) in 6 ml anhydrous THF. The reaction mixture was stirred for 5 h. Then the solvent was evaporated. Then the solvent was evaporated, the resulted solid was washed with water for three times. The crude product was purified over silica gel using MeOH / CH_2Cl_2 (v/v, 1:55) as the eluent to yield **OH-cc-CPT** as yellowish solid (76 mg, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ8.51 (s, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.00 (d, *J* = 8.2 Hz, 1H), 7.94-7.88 (m, 1H), 7.77-7.72 (m, 1H), 7.62 (s, 1H), 5.70 (d, *J* = 7.6 Hz, 1H), 5.45 (s, *J* = 7.6 Hz, 1H), 5.35 (s, 2H), 4.16 (m, 2H), 3.63 (t, *J* = 6.8 Hz, 2H), 3.27 (s, 1H), 2.38-2.15 (m, 2H), 1.72-1.67 (m, 2H), 1.61-1.52 (m, 2H), 1.45-1.36 (m, 4H), 1.03 (t, *J* = 7.4 Hz, 3H). Mass spectrometry (HR-MS, m/z): [M + H^+] calcd for $C_{27}H_{29}N_2O_7$, 493.1975; found 493.1926.

Synthesis of Biotin-cc-CPT. OH-cc-CPT (49 mg, 0.1 mmol), DAMP (15 mg, 0.12 mmol), EDC (21 mg, 0.11 mmol) and biotin (25 mg, 0.1 mmol) was suspended in 30 ml CH_2Cl_2, the mixture was allowed to stirred for 13 h at room temperature. The solvent was evaporated and washed with 10% hydrochloric acid and water twice respectively. The resulting crude product was subject to HPLC to yield **Biotin-cc-CPT** as white solid (42 mg, 58%). Purity: 98.9%. HPLC: Agilent 1260 infinity pre

system, YMC-Pack ODS-A Column (250*20 mml. D. S-5μm, 12nm). Mobile phase A: H_2O, B: MeOH. 0-27 min: 30%-70% B, 27-35 min: 70%-100% B. Absorption wavelength, 365 nm (based on the UV spectrum in Figure S19). ^1H NMR ($CDCl_3$, 500 MHz): δ8.45 (s, 1H), 8.28 (d, *J* = 8.6 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.89-7.86 (m, 1H), 7.73-7.69 (m, 1H), 7.40 (s, 1H), 5.72 (d, *J* = 7.6 Hz, 1H), 5.44 (d, *J* = 7.6 Hz, 1H), 5.34 (s, 2H), 4.56-4.53 (m, 1H), 4.36-4.33 (m, 1H), 4.20-4.09 (m, 2H), 4.02 (t, *J* = 6.6 Hz, 2H), 3.20-3.16 (m, 1H), 2.95 (dd, *J* = 2.6, 6.8 Hz, 1H), 2.76 (d, *J* = 6.9 Hz, 1H), 2.33-2.27 (m, 3H), 2.22-2.15 (m, 1H), 1.74-1.59 (m, 8H), 1.48-1.35 (m, 6H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ173.6, 167.5, 157.3, 153.8, 152.2, 148.7, 146.3, 145.9, 131.5, 130.9, 129.5, 128.6, 128.2, 128.2, 120.4, 96.3, 77.7, 77.2, 76.8, 69.0, 67.1, 64.2, 62.0, 60.2, 55.3, 50.1, 40.5, 33.9, 31.9, 28.4, 28.4, 28.3, 28.3, 25.5, 25.2, 24.8, 7.7. Mass spectrometry (HR-MS, m/z): ($M + H^+$) calcd for $C_{37}H_{43}N_4O_9S$ 719.2751; found 719.2717.

Results



Scheme S1. Synthetic route to **Biotin-cc-CPT** and **Biotin-ss-CPT.**

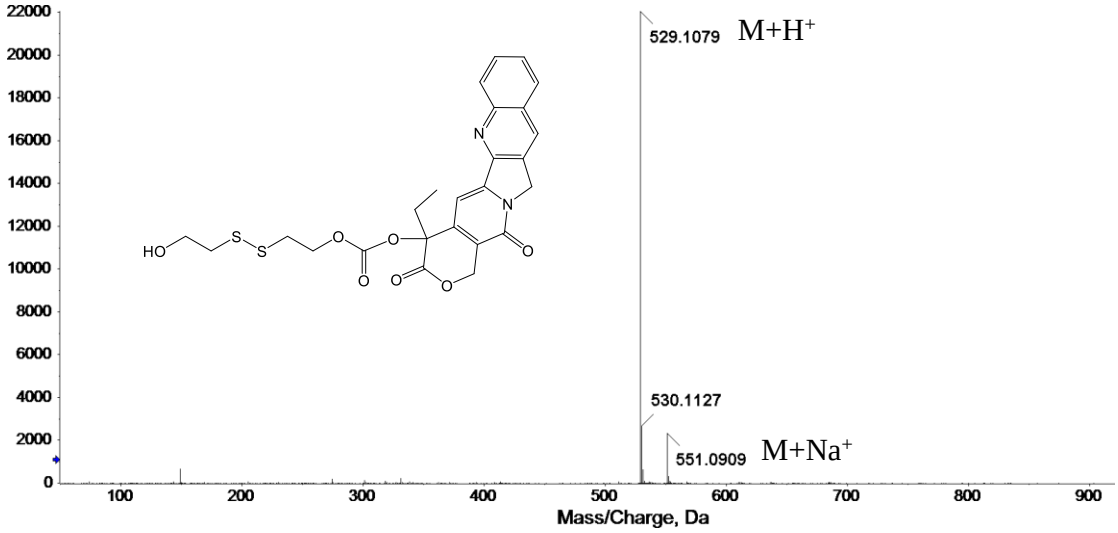


Figure S1. HRMS for **OH-ss-CPT**

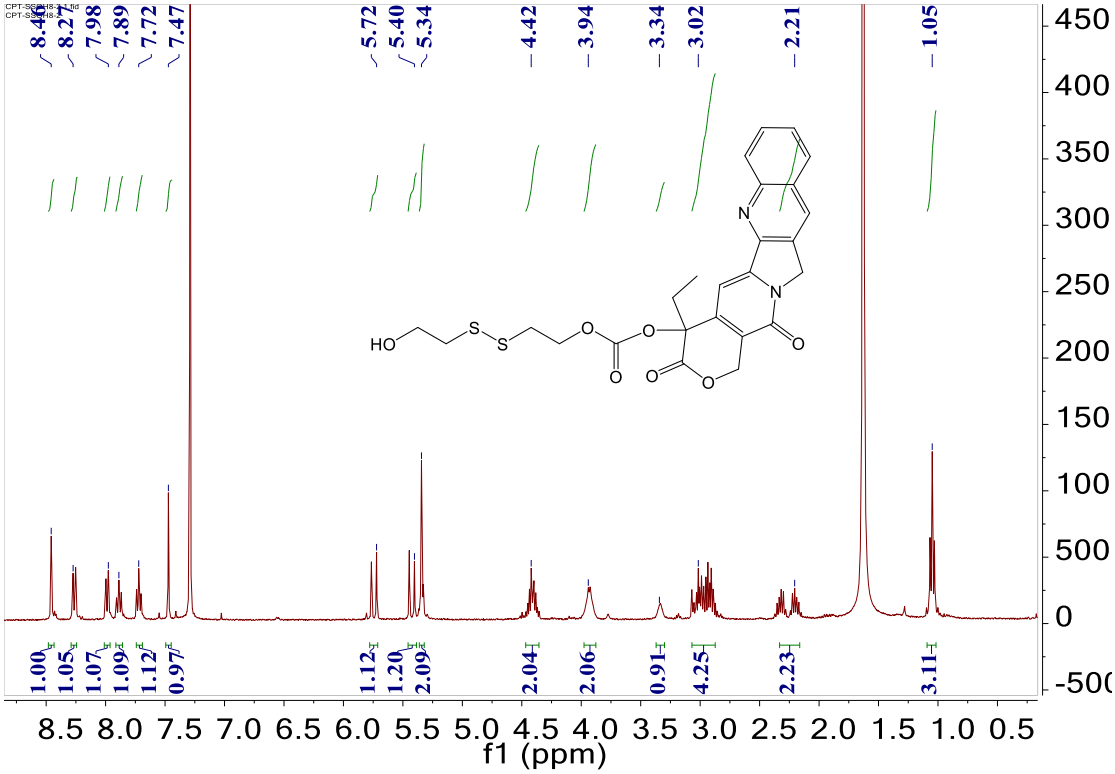


Figure S2. ^{1}H NMR spectrum of **OH-ss-CPT** in $CDCl_3$.

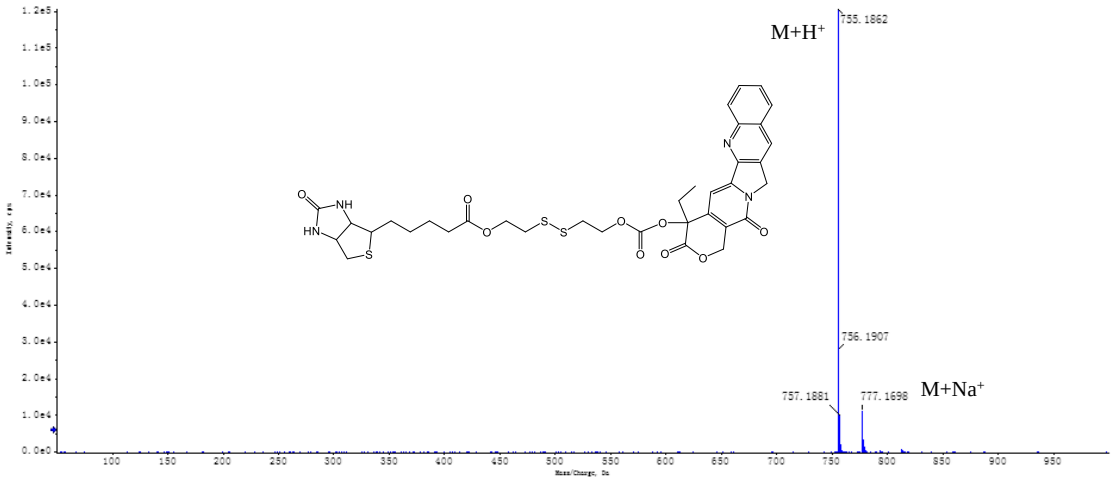


Figure S3. HRMS for **Biotin-ss-CPT**

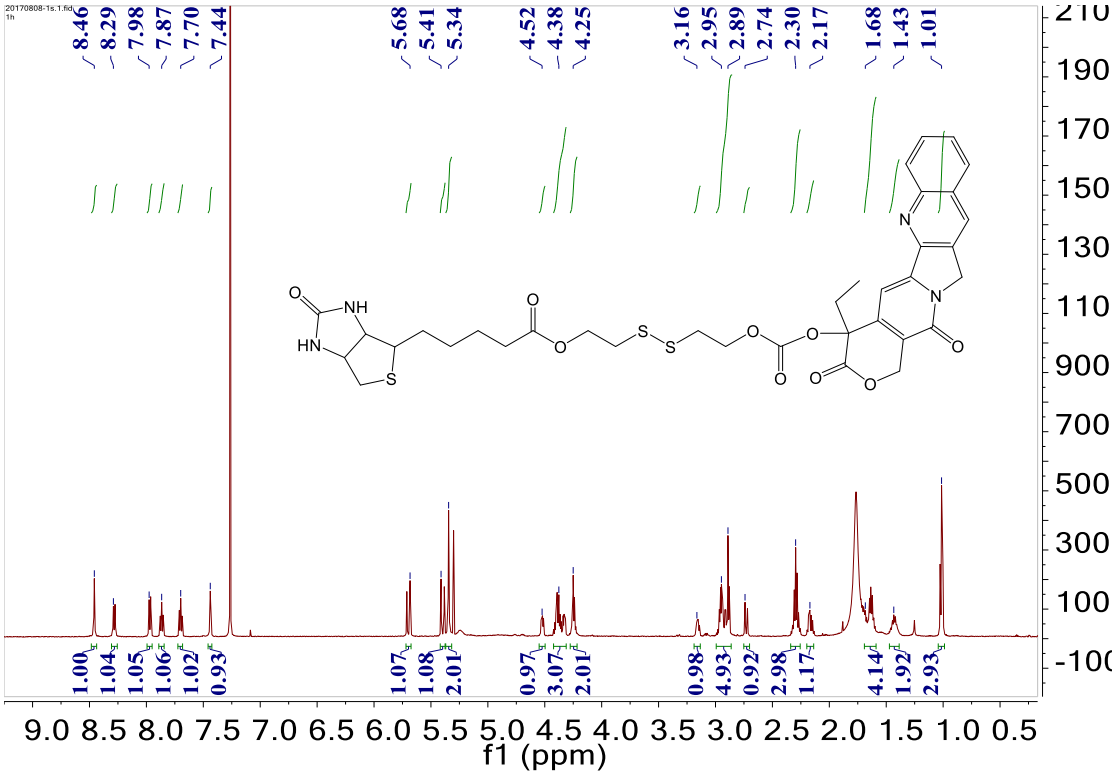


Figure S4. ^{1}H NMR spectrum of **Biotin-ss-CPT** in $CDCl_3$.

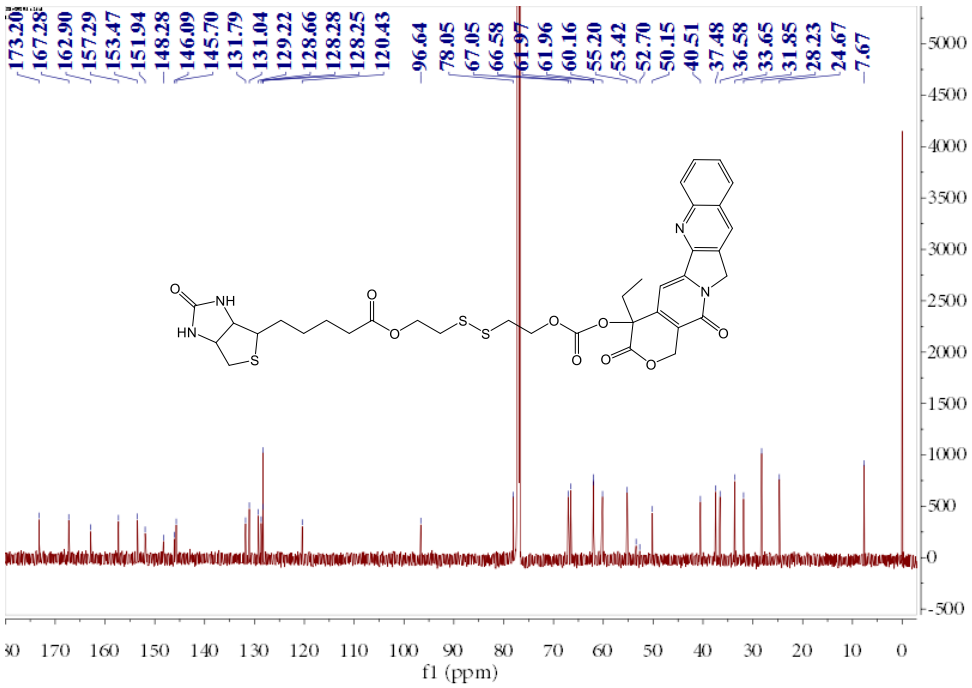


Figure S5. ^{13}C NMR spectrum of **Biotin-ss-CPT** in $CDCl_3$.

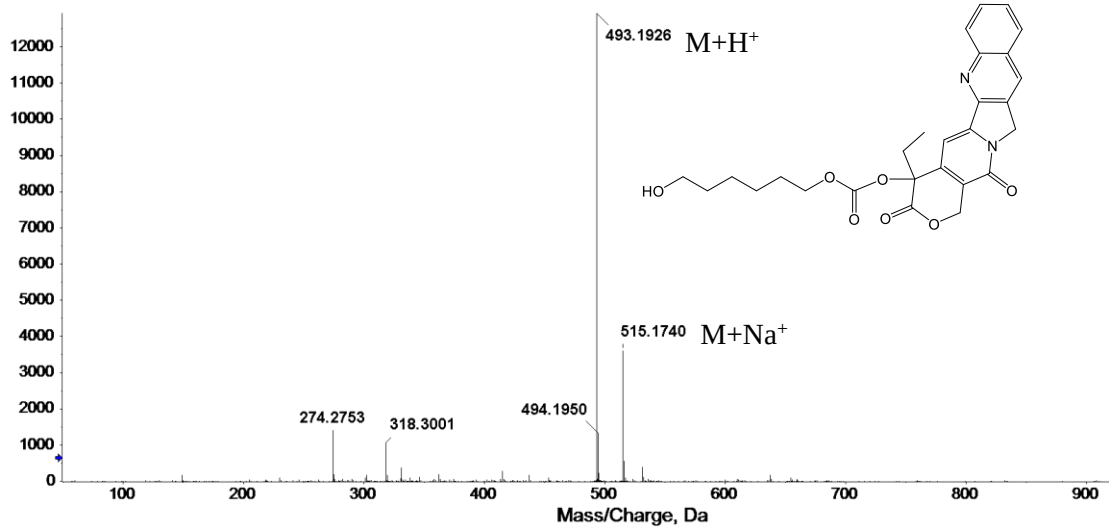


Figure S6. HR-MS for **OH-cc-CPT**

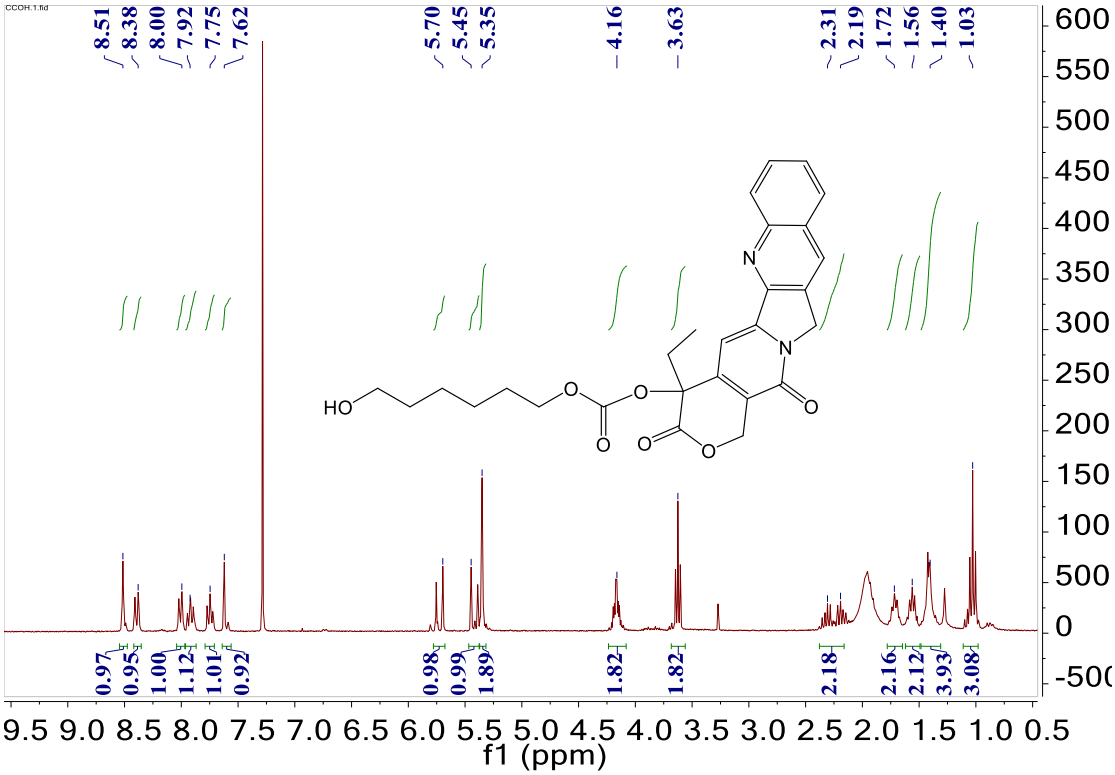


Figure S7. ^{1}H NMR spectrum of **OH-cc-CPT** in $CDCl_3$.

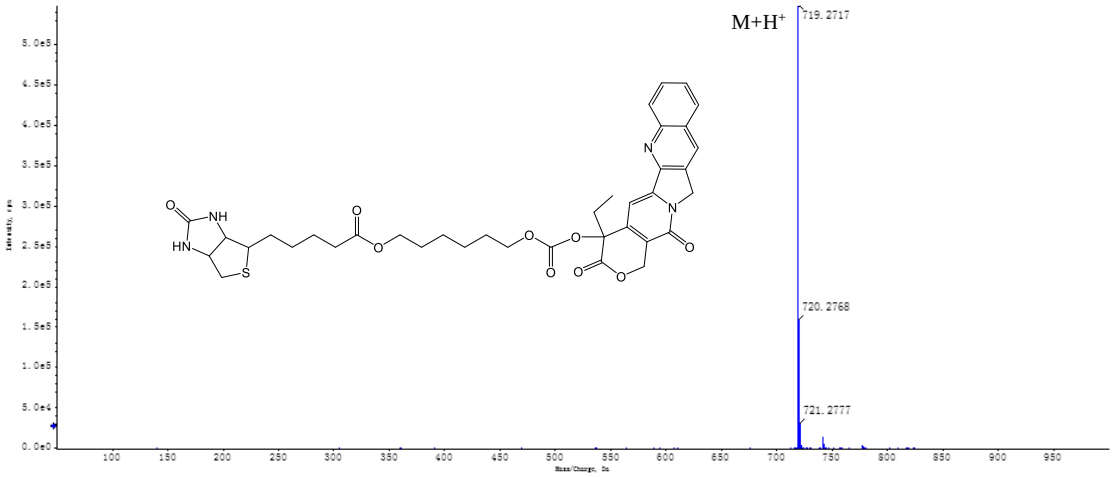


Figure S8. HRMS for **Biotin-cc-CPT**

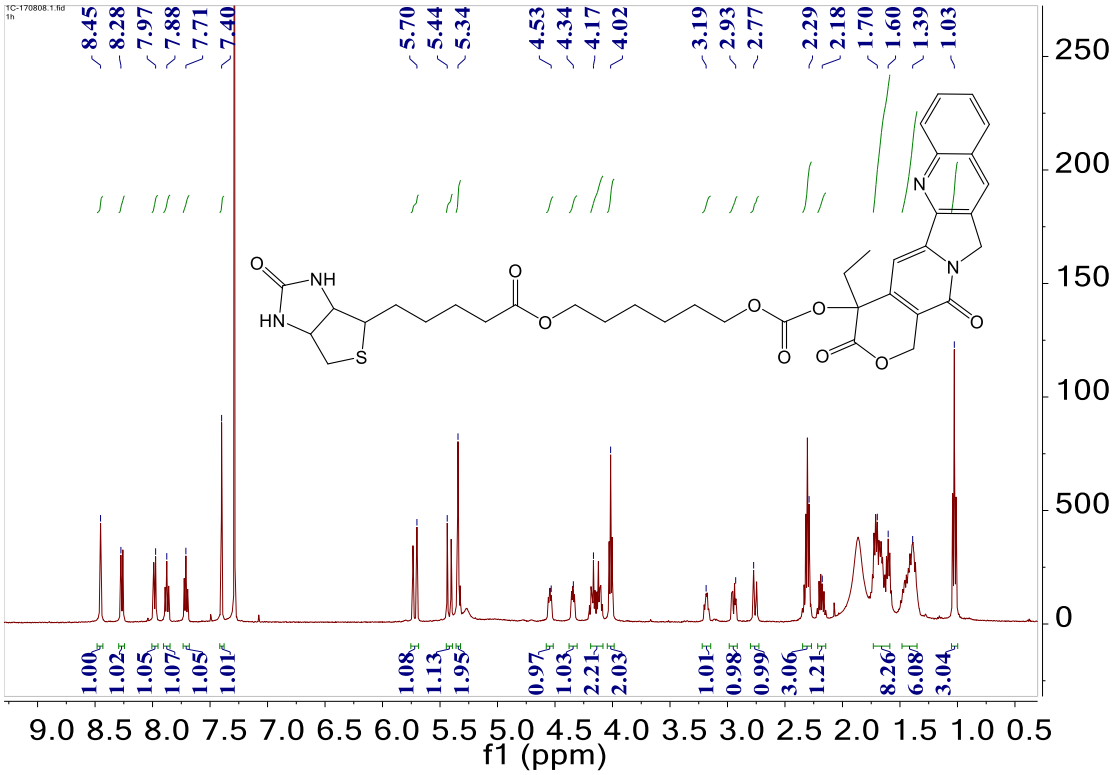


Figure S9. ^{1}H NMR spectrum of **Biotin-cc-CPT** in $CDCl_3$.

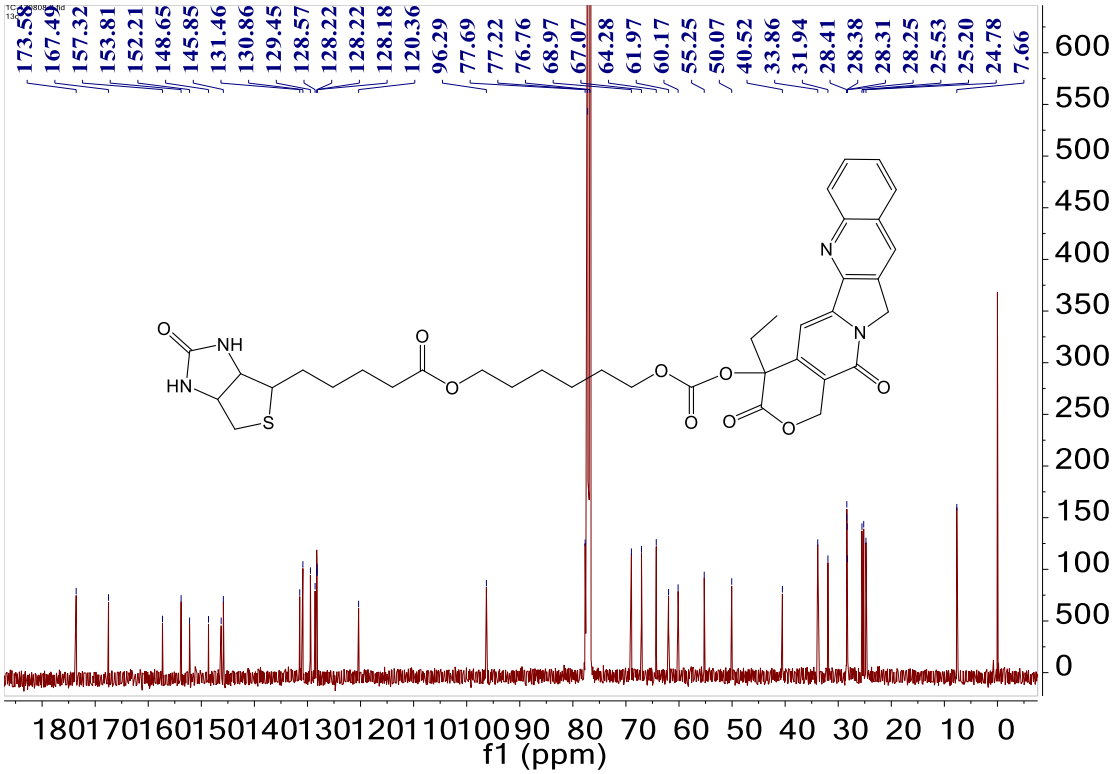


Figure S10. ^{13}C NMR spectrum of **Biotin-cc-CPT** in $CDCl_3$.

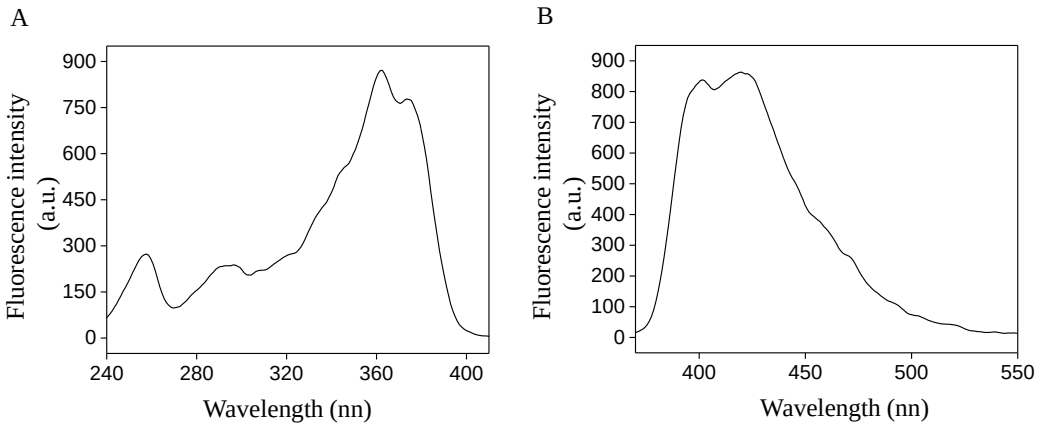


Figure S11. Excitation (A) and Emission spectrum (B) of **Biotin-cc-CPT** in $CHCl_3$.

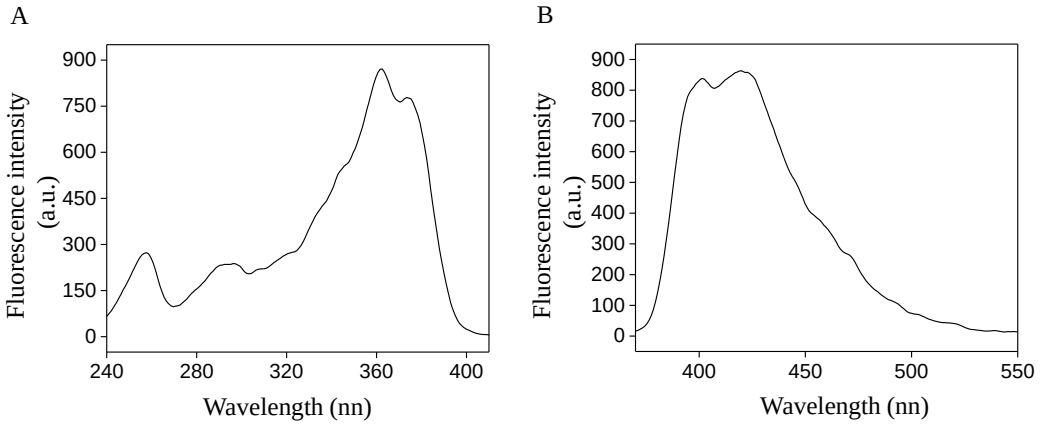


Figure S12. Excitation (A) and Emission spectrum (B) of **Biotin-ss-CPT** in $CHCl_2$.

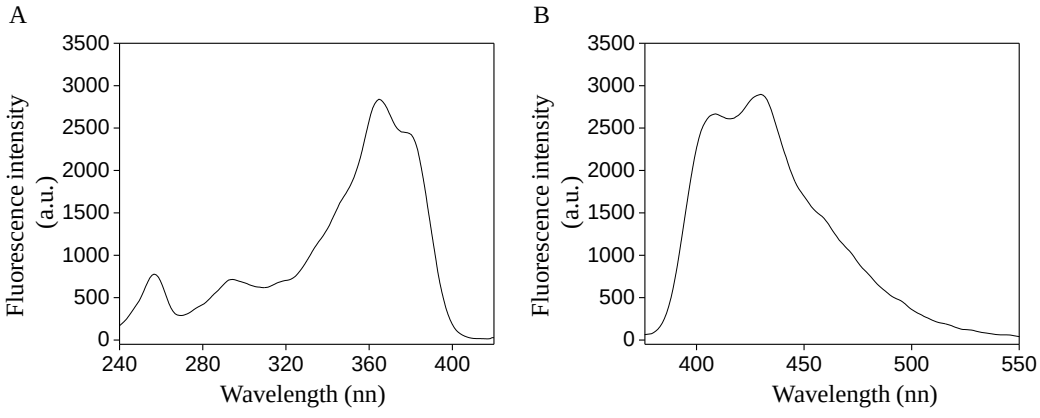


Figure S13. Excitation (A) and Emission spectrum (B) of **CPT** in $CHCl_2$.

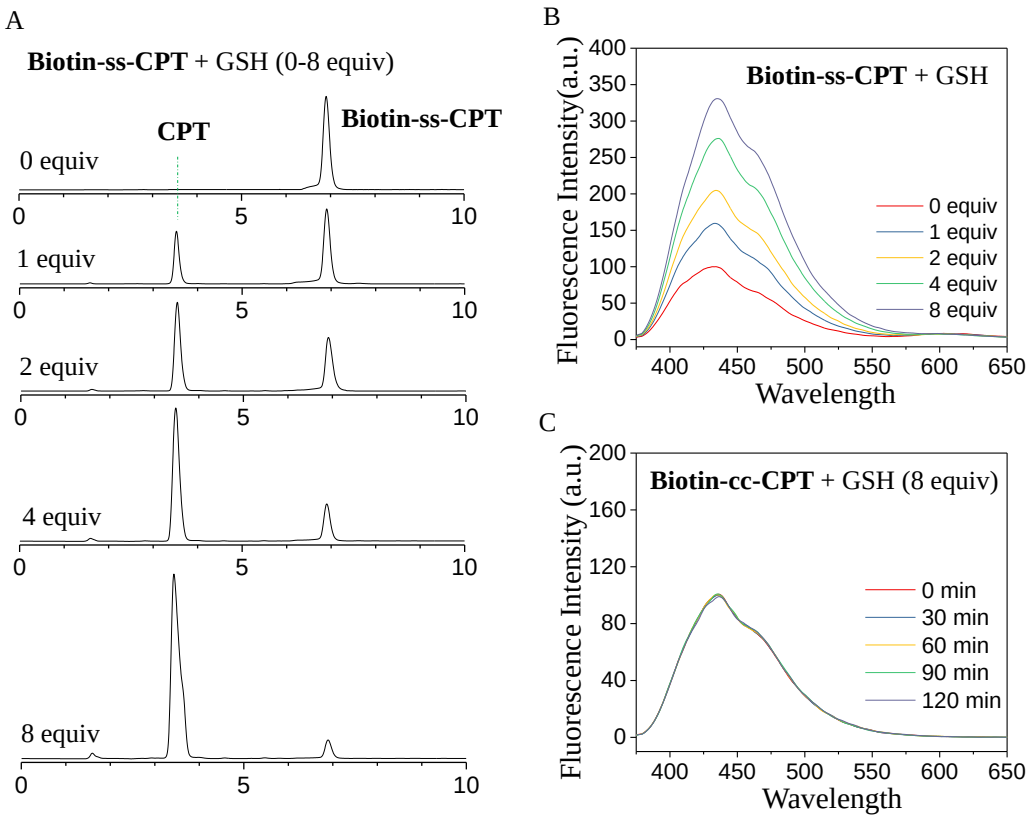


Figure S14. HPLC analysis (A) and fluorescence spectra of **CPT** release from the **Biotin-ss-CPT** (B) and **Biotin-cc-CPT** (C) at different equivalent of GSH. Peak area ratio rise of **CPT** release form **Biotin-ss-CPT** in HPLC and fluorescence enhancement of **Biotin-ss-CPT** witnessed on treatment with increasing concentrations of GSH (0−8 equiv) in fluorescence spectra respectively. **Biotin-ss-CPT** (20 μM) was treated with GSH in mixed solution of PBS buffer and DMSO (v/v: 4/1) at pH=7.4. Peaks in the chromatograms were detected by monitoring the UV/Vis absorption at 365 nm. HPLC: Agilent 1260 infinity II system, Agilent ZORBAX SB C18 (250*4.6mm, 5μm) Mobile phase A: H_2O, B: CH_3CN. 0-20 min: 50%-100% B. Flow rate: 1 ml/min.

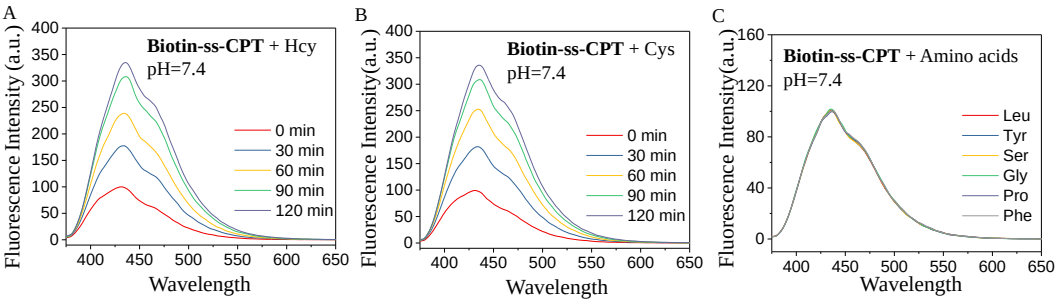


Figure S15. Fluorescence spectra of Biotin-ss-CPT (20.0 μM) toward 10 equiv of Homocysteine (Hcy) (A), Cysteine (Cys) (B) and some amino acids (C). The data were recorded 2 h after incubation with Hcy, Cys (at interval of 30 min) or amino acids in mixed solution of PBS buffer and DMSO (v/v:4/1) at pH=7.4. Excitation was set at 365 nm.

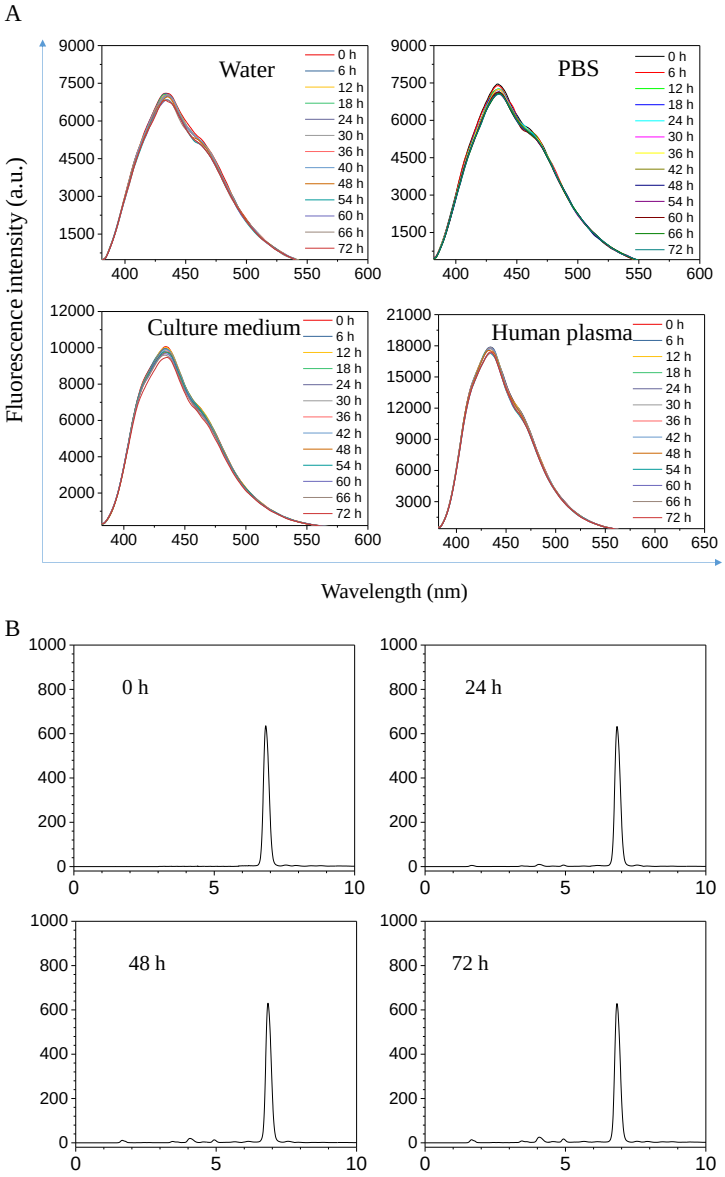


Figure S16. (A) Stability of **Biotin-ss-CPT** in Milli-Q water, PBS, culture medium and human plasma measured by fluorescence spectra. (B) Reverse-phase HPLC chromatograms analysis of **Biotin-ss-CPT** (30 μM) in plasma after incubation for 0, 24, 48 and 72 h respectively. Peaks in the chromatograms were detected by monitoring the UV/Vis absorption at 365 nm. HPLC: Agilent 1260 infinity II system, Agilent ZORBAX SB C18 (250*4.6 mm, 5 μm) Mobile phase A: H_2O, B: CH_3CN. 0-20 min: 50%-100% B. Flow rate: 1 ml/min.

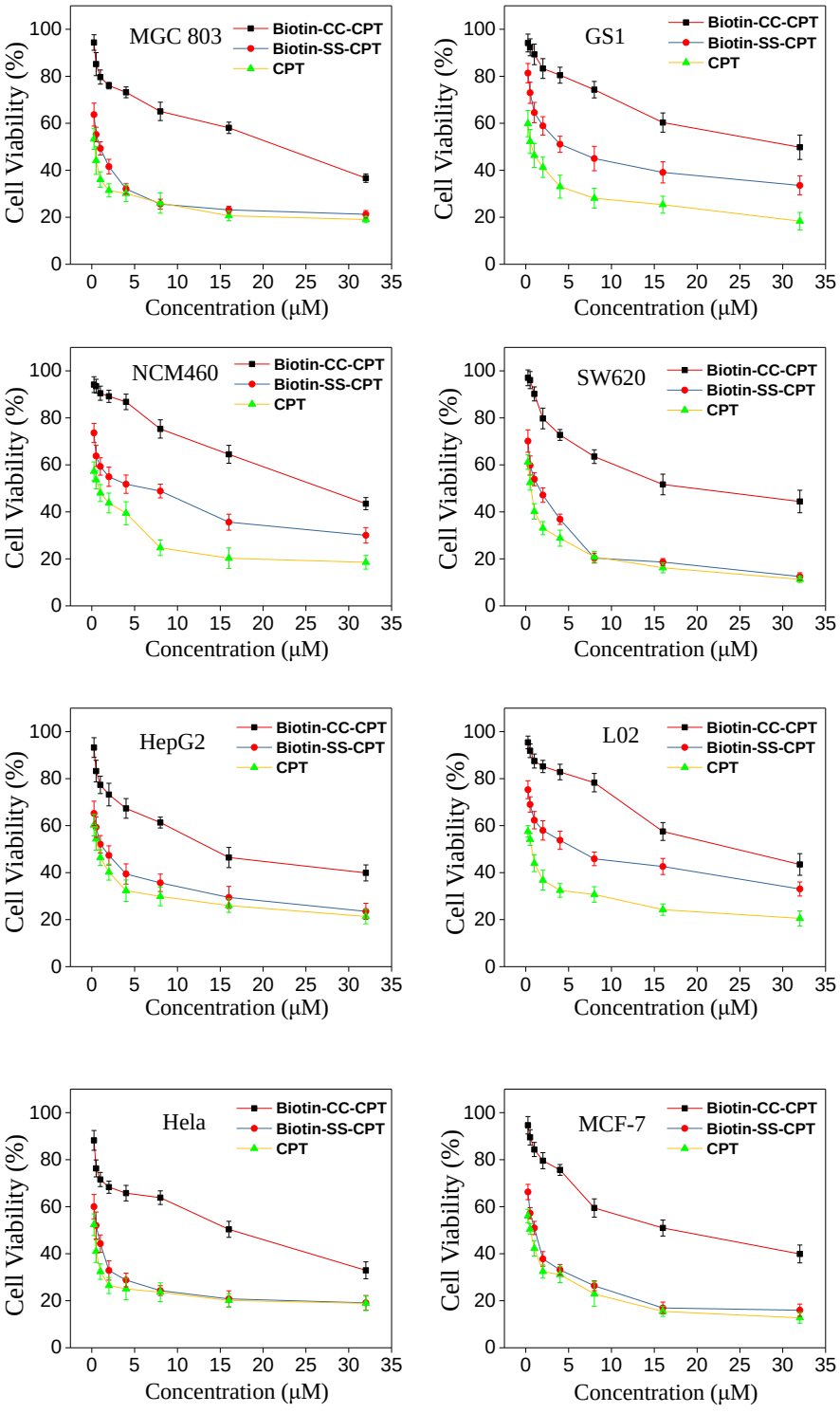


Figure S17. The inhibitory effects **of Biotin-cc-CPT**, **Biotin-ss-CPT** and **CPT** on the proliferation of tumor cells MGC803, NCM460, HepG2, Hela and MCF-7 and normal cells GS1, SW620 and L02. Cell viability was determined by MTT assay after treatment for 72 h.

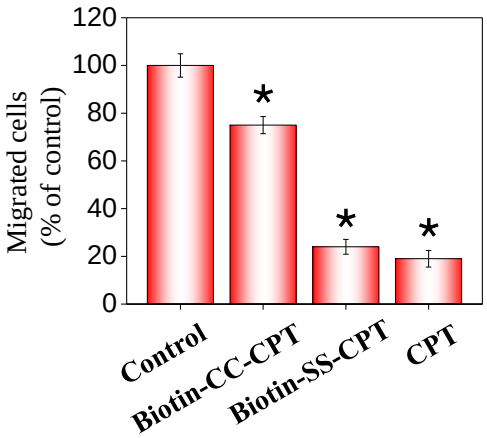


Figure S18. Quantitative analysis of the migrated cells subjected to **Biotin-ss-CPT**, **Biotin-cc-CPT** and **CPT** at 24 h by manual counting. Values expressed are the mean ±SD of 3 independent experiments.

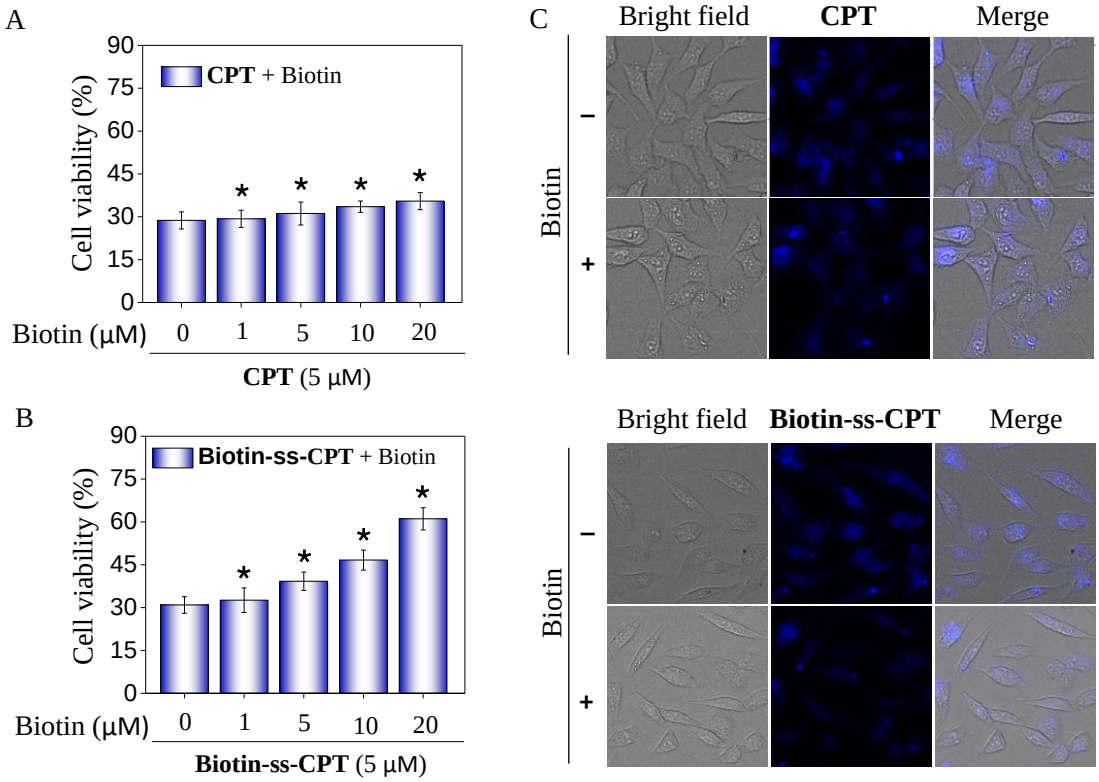


Figure S19. Effects of biotin on the anticancer activity of **CPT** (A) or **Biotin-ss-CPT** (B). The cells were pretreated with different concentration of biotin (0-20 μM) in 96-well plates for 2 h then exposed to **CPT** (5 μM) or **Biotin-ss-CPT** (5 μM) for 72 h. Cell viability was measured by MTT assay. (C) Effects of biotin on the fluorescence intensity of **CPT** or **Biotin-ss-CPT**. The cells were pretreated with biotin (20 μM) in 2-cm dish for 2 h then exposed to **CPT** (10 μM) or **Biotin-ss-CPT** (10 μM) for 5 h then the fluorescence was measured under fluorescence microscope.

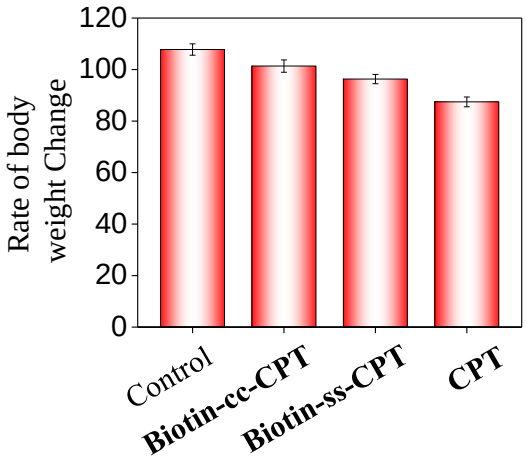


Figure S20. Rate of body weight change. Values represented were means ± SD of triplicates.

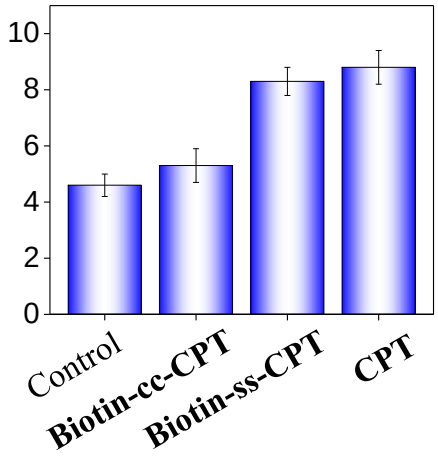


Figure S21. The quantitative analysis of ADCs.