Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information for:

Photo-triggerable Hydrogel-Nanoparticle Hybrid Scaffolds for Remotely Controlled Drug Delivery

Shreyas Shah[†], Pijus K. Sasmal[†], Ki-Bum Lee[*]

Department of Chemistry & Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, U.S.A.

***Correspondence:**

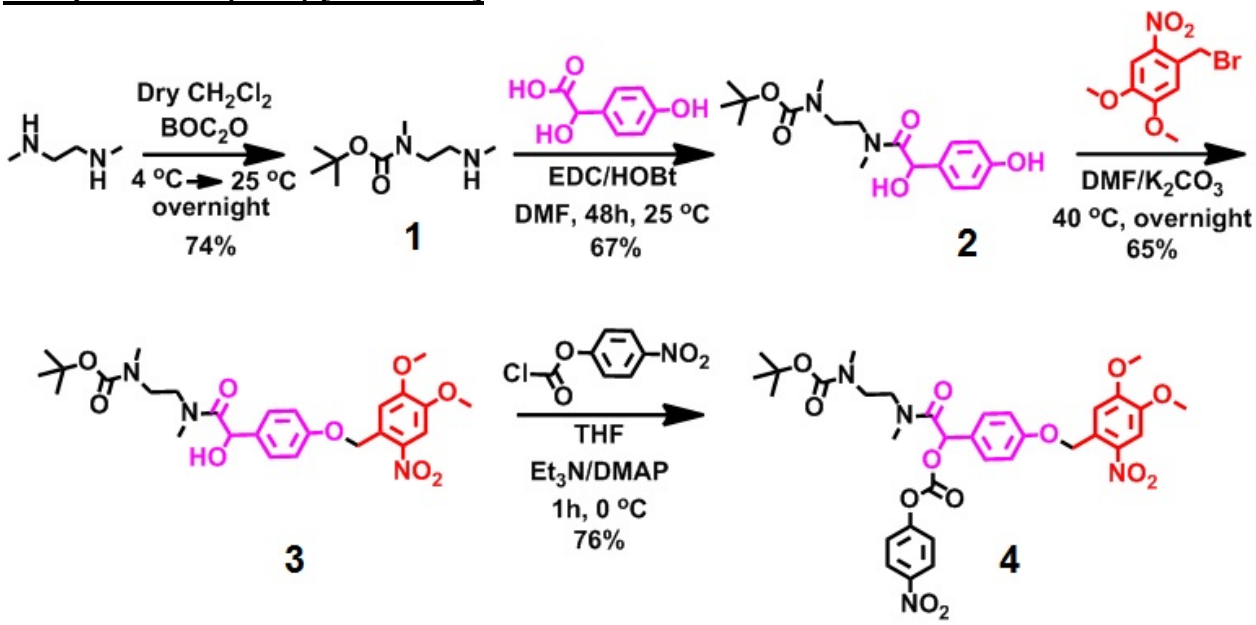
Prof. Ki-Bum Lee
Department of Chemistry and Chemical Biology
Rutgers, The State University of New Jersey
Piscataway, NJ 08854 (USA)
Phone: 848-445-2081
Fax: 732-445-5312
Email: kblee@rutgers.edu

[†] These authors contributed equally to this work.

GENERAL

The ^{1}H- and ^{13}C-NMR spectra were recorded on either a Varian-300 instrument (300 MHz), Varian-400 instrument (400 MHz) or a Varian-500 instrument (500 MHz) spectrometer at ambient temperature. NMR standards used were as follows: (^{1}H-NMR) $CDCl_3$ = 7.26 ppm; d_6-DMSO = 2.50. (^{13}C-NMR) $CDCl_3$ = 77.16 ppm; d_6-DMSO = 39.52. Data are reported as app = apparent, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, ddd = doublet of doublet of doublets, m = multiplet, comp = complex, br = broad; and coupling constant(s) are reported in Hz. Mass spectra were recorded on a Finnigan LCQ-DUO mass spectrometer.

Section 1: Synthesis of Chemical Adapator System (CAS) Conjugated with Camptothecin (CPT) [CAS-CPT]



Scheme 1. Synthetic scheme for chemical adaptor system (CAS).

Compound 1: Compound **1** was synthesized according to literature.[1] Briefly, *N,N'*-dimethylethylenediamine (3.674 g, 34 mmol) was dissolved in 45 mL dry CH_2Cl_2 and cooled to 0 °C. A solution of di-*tert*-butyl dicarbonate (Boc_2O, 2.597 g, 11.9 mmol) in 25 mL dry CH_2Cl_2 was added dropwise to the above cooled solution. After addition the mixture was allowed to warm at room temperature and stirred overnight. Then the solvent was removed under vacuum and the residur was extracted with EtOAc/water. The water layer was extracted two times with EtOAc and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford pale yellow oil (1.65 g, yield 74%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.33 (t, br, 2H), 2.87 (s, 3H), 2.73 (t, J = 6.5 Hz, 2H), 2.45 (s, 3H), 1.45 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 155.7, 79.5, 49.7, 48.3, 36.3, 34.7, 28.4. MS (m/z): calculated, 188.15 for $C_9H_{20}N_2O_2$; found, 211.15 for $[M + Na]^+$.

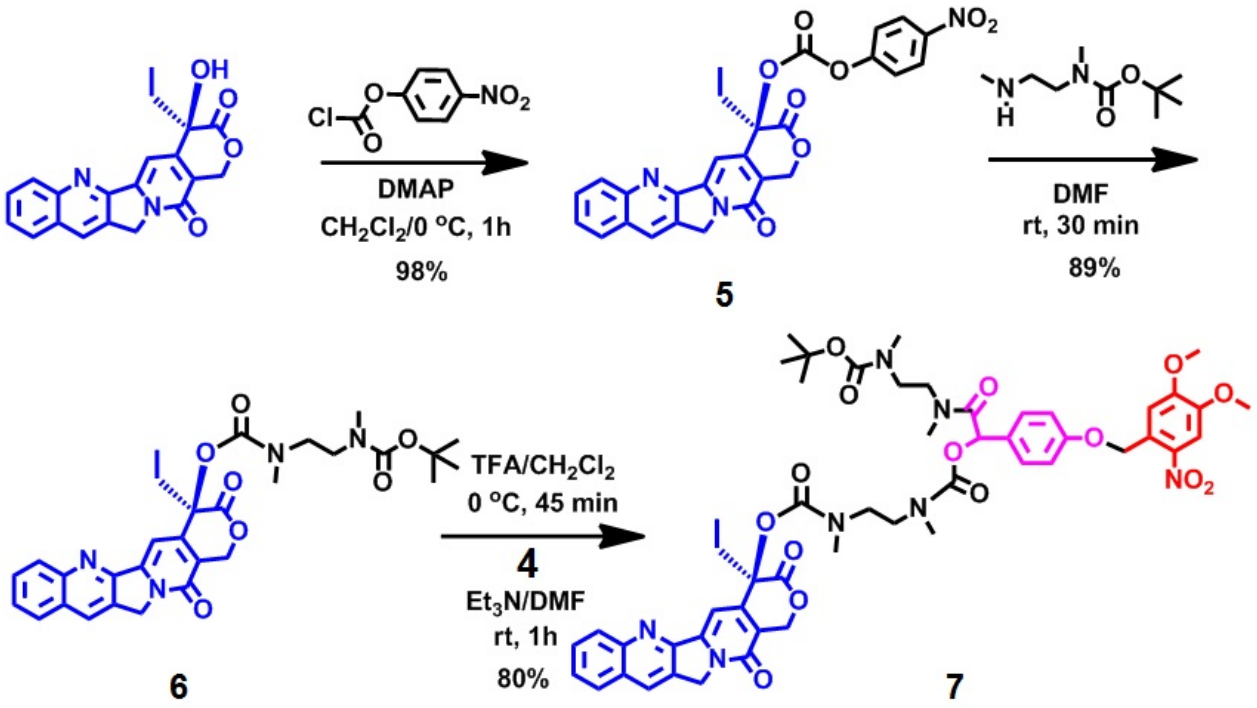
Compound 2: Compound **2** was prepared according to literature with slightly modification.[2] 4-Hydroxymandelic acid monohydrate (302 mg, 1.622 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 466 mg, 2.433 mmol) and 1-hydroxybenzotriazole (HOBt, 124 mg, 0.811 mmol) were dissolved in 3.0 mL dry DMF.

The reaction mixture was stirred at ambient temperature under nitrogen atmosphere. After 1h compound **1** was added to the above mixture and stirred at ambient temperature under N_2 atm. for 60h. Then, the reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic phases was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude oily material was subjected to silica gel flash column chromatography with ethyl acetate/hexane (3:1) as the eluting solvent. The resulting product was isolated as a white foam solid (368 mg, 67%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 6.76 (m, 2H), 6.36 (m, 2H), 5.11 (d, br, 1H), 4.70 (br, 1H), 3.73-3.19 (m, 4H), 3.03-2.77 (6H, the signal of the N-Me group appears as three singlets at 3.03, 2.86, and 2.77 ppm with their integrations corresponded to 1H, 2H, and 3H, respectively), 1.46 (9H, the signal of the Boc group appears as two singlets, the sum of their integrations corresponded to 9 hydrogens). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 173.1, 157.0, 156.2, 128.9, 116.2, 80.2, 71.0, 53.5, 46.6, 35.0, 28.4. MS (m/z): calculated, 338.18 for $C_{17}H_{26}N_2O_5$; found, 361.18 for $[M + Na]^+$.

Compound 3: To a solution of compound **2** (125 mg, 0.37 mmol) in DMF (2 mL) was added potassium carbonate (153 mg, 1.11 mmol) and 4,5-dimethoxy-2-nitrobenzyl bromide (102 mg, 0.37 mmol) and the reaction mixture was stirred in the dark at 40 °C overnight. The reaction mixture was cooled to room temperature, diluted with ~15 mL water and extracted from EtOAc (3x15 mL). The combined organic phases was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude oily material was subjected to silica gel flash column chromatography with ethyl acetate/hexane (1:1 to 2:1) as the eluting solvent. The resulting product was isolated as a pale yellow solid (128 mg, 65%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.77 (s, 1H), 7.32 (s, 1H), 7.25 (m, 2H), 6.98 (m, 2H), 5.48 (s, 2H), 5.14 (d, br, 1H), 4.66 (s, br, 1H), 3.97 (6H, the signal of the OMe group appears as two singlets, the sum of their integrations corresponded to 6 hydrogens), 3.86-3.15 (m, 4H), 3.04-2.74 (6H, the signal of the N-Me group appears as four singlets at 3.04, 2.85, 2.80, and 2.74 ppm with their integrations corresponded to 1H, 2H, 2H and 1H, respectively), 1.43 (9H, the signal of the Boc group appears as two singlets, the sum of their integrations corresponded to 9 hydrogens). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 173.1, 158.3, 157.1, 154.0, 148.0, 139.2, 129.0, 128.8, 116.0, 115.6,

109.5, 108.1, 80.0, 71.1, 67.2, 56.5, 53.5, 46.4, 45.6, 35.0, 28.4. MS (m/z): calculated, 533.57 for $C_{26}H_{35}N_3O_9$; found, 556.57 for $[M + Na]^+$.

Compound 4: Compound **3** (60 mg, 0.112 mmol) was dissolved in dried THF and cooled to 0 ºC. Triethylamine (31 µL, 0.225 mmol) and 4-dimethylaminopyridine (DMAP, 14 mg, 0.112 mmol) were added to above solution at 0 ºC, and followed by slow addition of 4-nitrophenyl chloroformate (45 mg, 0.225 mmol). Immediately, a light yellow solution was formed which was stirred in the dark at room temperature for 1h. The crude material was subjected to silica gel flash column chromatography with hexane/ethyl acetate (2:1 to 1:1) as the eluting solvent. The resulting product was isolated as a light yellow solid (59.4 mg, 76%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.27 (d, J = 9.2 Hz, 2H), 7.78 (s, 1H), 7.58-7.41 (m, 4H), 7.32 (s, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.10 (s, 1H), 5.51 (s, 2H), 3.98 (s, 6H), 3.79-3.24 (m, 4H), 3.06-2.82 (6H, the signal of the N-Me group appears as three singlets, the sum of their integrations corresponded to 6 hydrogens), 1.47 (9H, the signal of the Boc group appears as two singlets, the sum of their integrations corresponded to 9 hydrogens). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 171.4, 167.6, 162.8, 159.5, 155.6, 154.1, 148.2, 145.5, 139.3, 130.6, 126.2, 125.4, 121.8, 115.7, 109.6, 108.3, 80.3, 68.3, 67.3, 60.6, 56.6, 35.2, 28.4. MS (m/z): calculated, 698.24 for $C_{33}H_{38}N_4O_{13}$; found, 721.24 for $[M + Na]^+$.



Scheme 2. Synthetic scheme for conjugation of camptothecin (CPT) with chemical adaptor system (CAS) [CAS-CPT].

Compound 5: Compound **5** was prepared according to literature with slightly modification.[2] Briefly, camptothecin (CPT, 40 mg, 0.115 mmol), 4-nitrophenyl chloroformate (69.4 mg, 0.344 mmol), and DMAP (84 mg, 0.69 mmol) were suspended in 2.0 mL CH_2Cl_2 at 0 ºC. Then the clear solution was stirred at room temperature for 1 h. The reaction mixture was diluted with 30 mL CH_2Cl_2 and extracted from 0.1 N aqueous HCl solutions (20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude oily material was subjected to silica gel flash column chromatography from 100% CH_2Cl_2 to 100% EtOAc as the eluting solvent. The resulting product was isolated as an yellowish powder (57.8 mg, 98%).^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.43 (s, 1H), 8.23 (m, 3H), 7.96 (d, J = 7.5 Hz, 1H), 7.86 (t, J = 7.5 Hz, 1H), 7.70 (t, J = 6.0 Hz, 1H), 7.40 (m, 3H), 5.72 (d, J = 17.5 Hz, 1H), 5.42 (d, J = 17.5 Hz, 1H), 5.31 (d, J = 5.5 Hz, 2H), 2.40-2.34 (m, 1H), 2.28-2.22 (m, 1H), 1.07 (t, J = 7.5 Hz, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 166.9, 157.3, 155.2, 152.3, 151.4, 149.1, 147.0, 145.7, 144.9, 131.5, 131.0, 129.7, 128.6, 128.4, 125.4, 121.8, 120.5, 95.6,

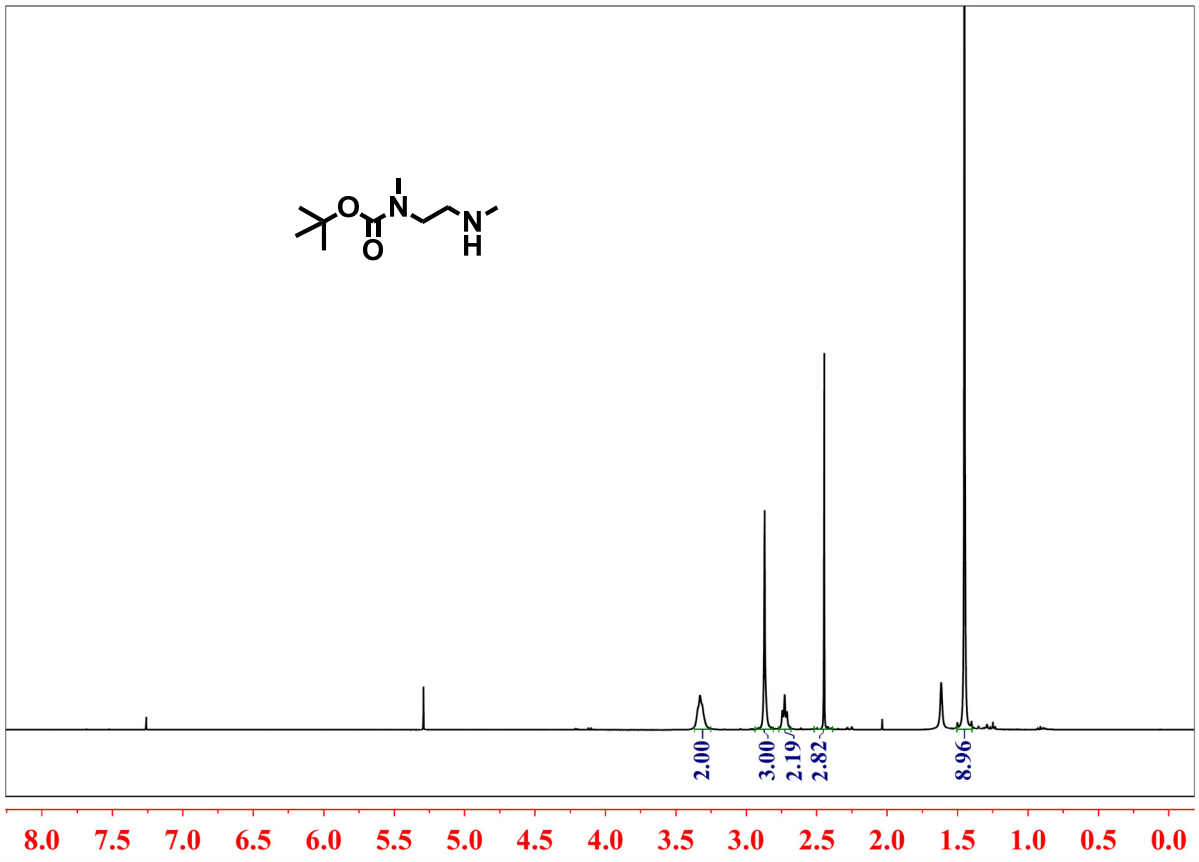
79.5, 67.4, 50.2, 32.1, 7.8. MS (m/z): calculated, 513.12 for $C_{27}H_{19}N_3O_8$; found, 536.12 for $[M + Na]^+$.

Compound 6: Compound **1** (21.44 mg, 0.114 mmol) and **5** (39 mg, 0.076 mmol) were suspended in 1.0 mL DMF at rt and the color of the reaction mixture turned from an off-white to light yellow. Finally a clear solution was formed which was stirred at rt for 30 min. The reaction mixture was diluted with brine solution and extracted from EtOAc (3 x 20 mL). The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The crude material was subjected to silica gel flash column chromatography from 100% EtOAC to EtOAc/MeOH (10:1) as the eluting solvent. The resulting product was isolated as an off-white powder (38.1 mg, 89%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.38 (s, 1H), 8.20 (d, J = 8.5 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.82 (t, J = 7.7 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.24 (s, 1H), 5.67 (d, J = 17.0 Hz, 1H), 5.41-5.36 (dd, J = 7.5, 7.0 Hz, 1H), 5.30-5.21 (m, 2H), 3.72-3.30 (m, 4H), 3.17-2.82 (6H, the signal of the N-Me group appears as four singlets, the sum of their integrations corresponded to 6 hydrogens), 2.32-2.22 (m, 1H), 2.16-2.08 (m, 1H), 1.46 (9H, the signal of the Boc group appears as two singlets, the sum of their integrations corresponded to 9 hydrogens), 0.99 (m, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 168.1, 157.5, 154.5, 152.7, 148.9, 146.2, 131.3, 130.8, 129.7, 128.6, 128.1, 120.1, 96.4, 79.8, 76.0, 67.3, 49.0, 47.7, 35.8, 32.3, 29.8, 28.5, 7.9. MS (m/z): calculated, 562.24 for $C_{30}H_{34}N_4O_7$; found, 585.24 for $[M + Na]^+$.

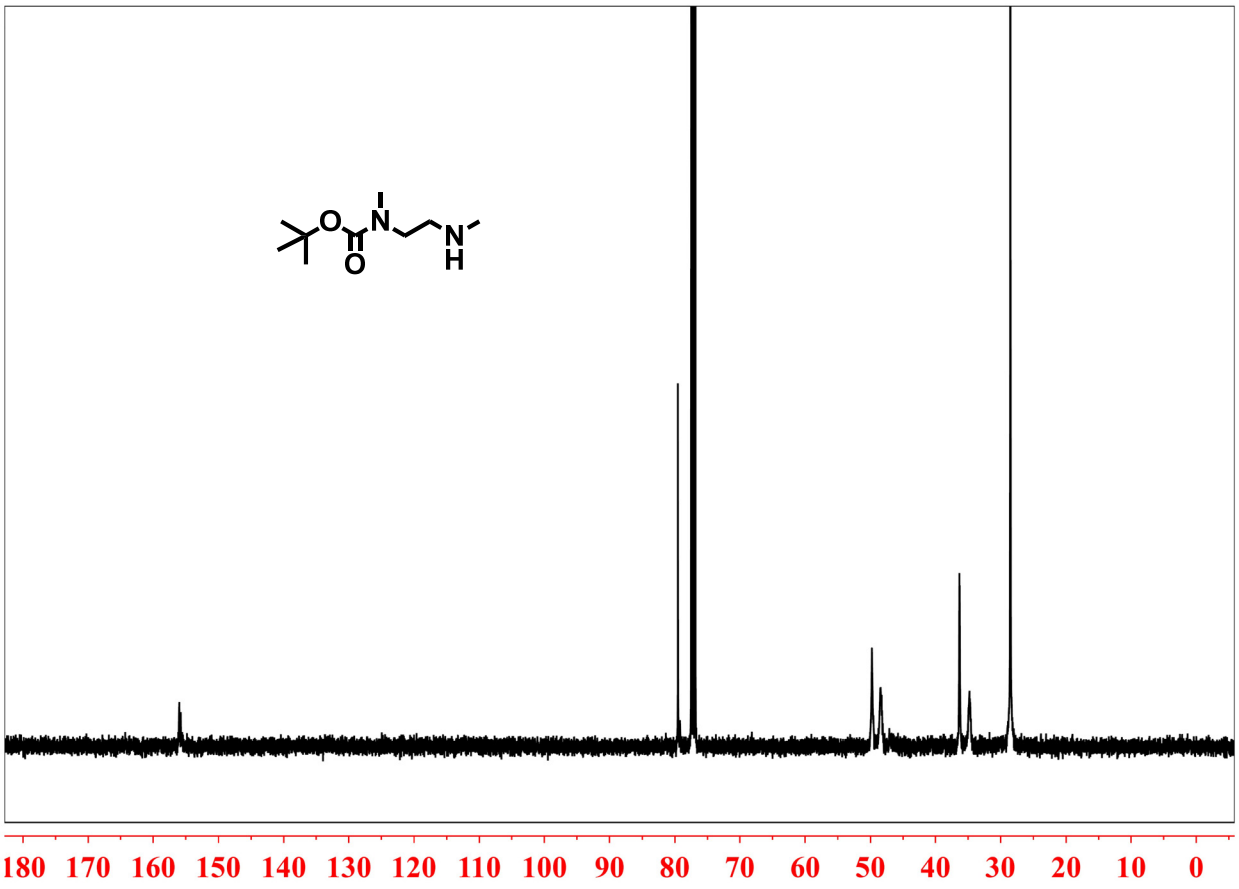
Compound 7 (CAS-CPT): Compound **6** (20 mg, 0.036 mmol) was dissolved in 800 µL CH_2Cl_2 and cooled to 0 ºC. To this was added trifluoroacetic acid (TFA, 200 µL) dropwise and the reaction was continued in the dark at 0 ºC for 45 min. The TFA with CH_2Cl_2 were removed under reduced pressure and the residue was dissolved in 750 µL DMF. Compound **4** (28 mg, 0.040 mmol) and Et_3N (300 µL) was added to the above solution and stirred in the dark at rt for 6h. The reaction mixture was diluted with brine solution (15 mL) and extracted from EtOAc (3 x 15 mL). The organic phases were dried over Na_2SO_4, and concentrated *in vacuo*. The crude material was subjected to silica gel flash column chromatography with 100% EtOAC to EtOAc/MeOH (10:1) as the eluting solvent. The resulting product was isolated as a light yellow solid (29.4 mg, 80%). ^{1}H-NMR (500 MHz, d_6-DMSO): δ (ppm) 8.69 (s, 1H), 8.12 (s, br, 1H), 7.84 (s, 1H), 7.73-

6.98 (m, 8H), 6.20 (m, 1H), 5.42 (m, 3H), 5.29 (m, 3H), 3.87 (s, 6H), 3.20-2.57 (m, 20H), 2.16 (m, 2H), 1.35 (9H, the signal of the Boc group appears as two singlets, the sum of their integrations corresponded to 9 hydrogens), 0.93 (m, 3H). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.38 (s, 1H), 8.20 (s, br, 1H), 7.95-7.25 (m, 7H), 7.04 (m, 3H), 6.13 (m, 1H), 5.69 (m, 1H), 5.50-5.25 (m, 5H), 3.97 (s, 6H), 3.90-2.72 (m, 20H), 2.18 (m, 2H), 1.43 (9H, the signal of the Boc group appears as two singlets, the sum of their integrations corresponded to 9 hydrogens), 0.99 (m, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 171.2, 169.1, 168.2, 162.6, 157.5, 154.1, 152.7, 148.9, 148.1, 146.1, 139.3, 131.3, 130.7, 130.3, 129.7, 128.3, 128.1, 119.8, 115.6, 109.6, 108.2, 96.3, 79.6, 76.0, 73.5, 67.3, 60.5, 56.6, 50.0, 36.6, 35.7, 32.0, 29.8, 29.4, 28.5, 7.9. MS (m/z): calculated, 1021.41 for $C_{52}H_{59}N_7O_{15}$; found, 1044.41 for $[M + Na]^+$.

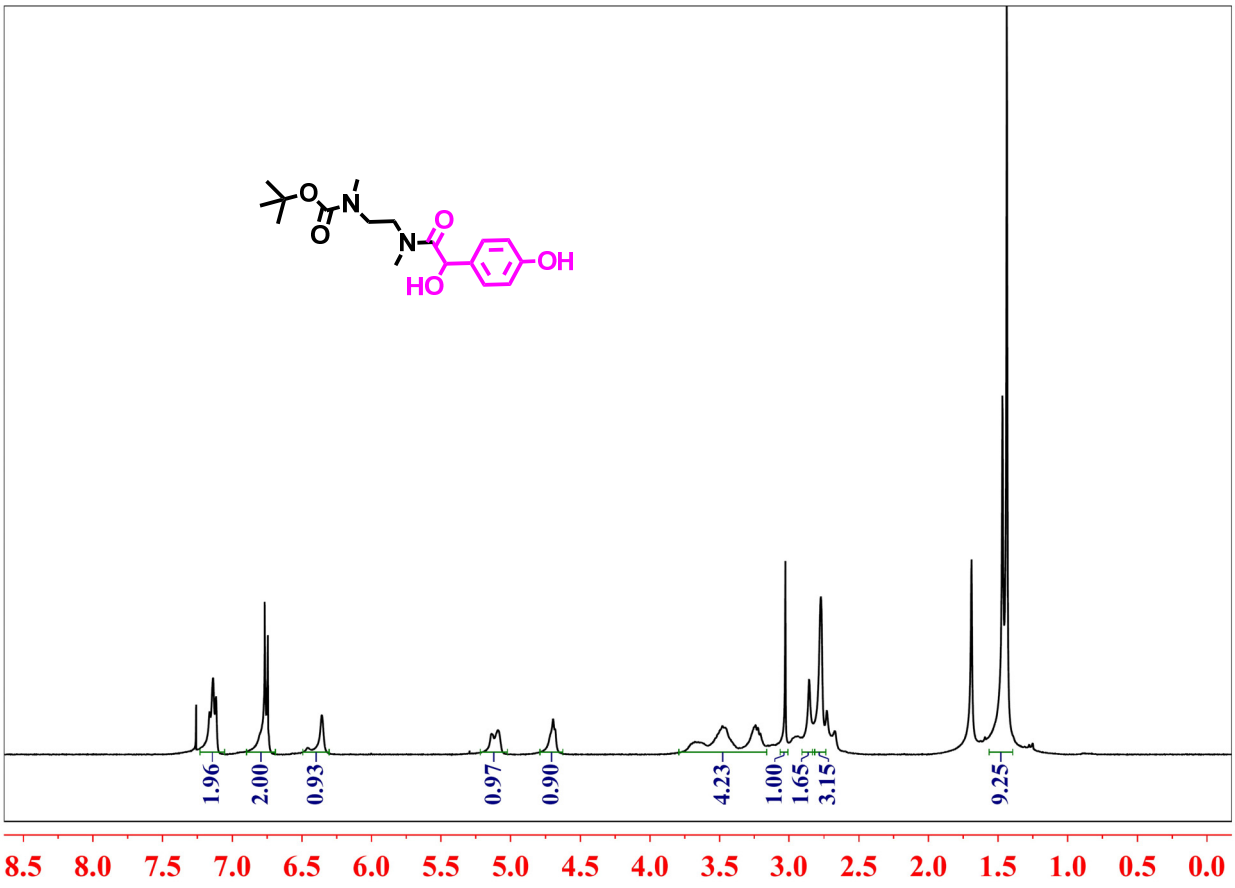
Drug Release from CAS-CPT Monitored using HPLC: Compound **7** (CAS-CPT) was dissolved in 1xPBS containing 2% DMF to make a 20 μM solution. Samples were irradiated with UV light (365 nm, 2.5 mW/cm^2) for 10 min. HPLC was carried out using C-18 column, wavelength: 368 nm; eluent: acetonitrile:water; 50:50; flow rate: 1 ml/min. The samples were monitored over 24 hrs (**Figure S1**).



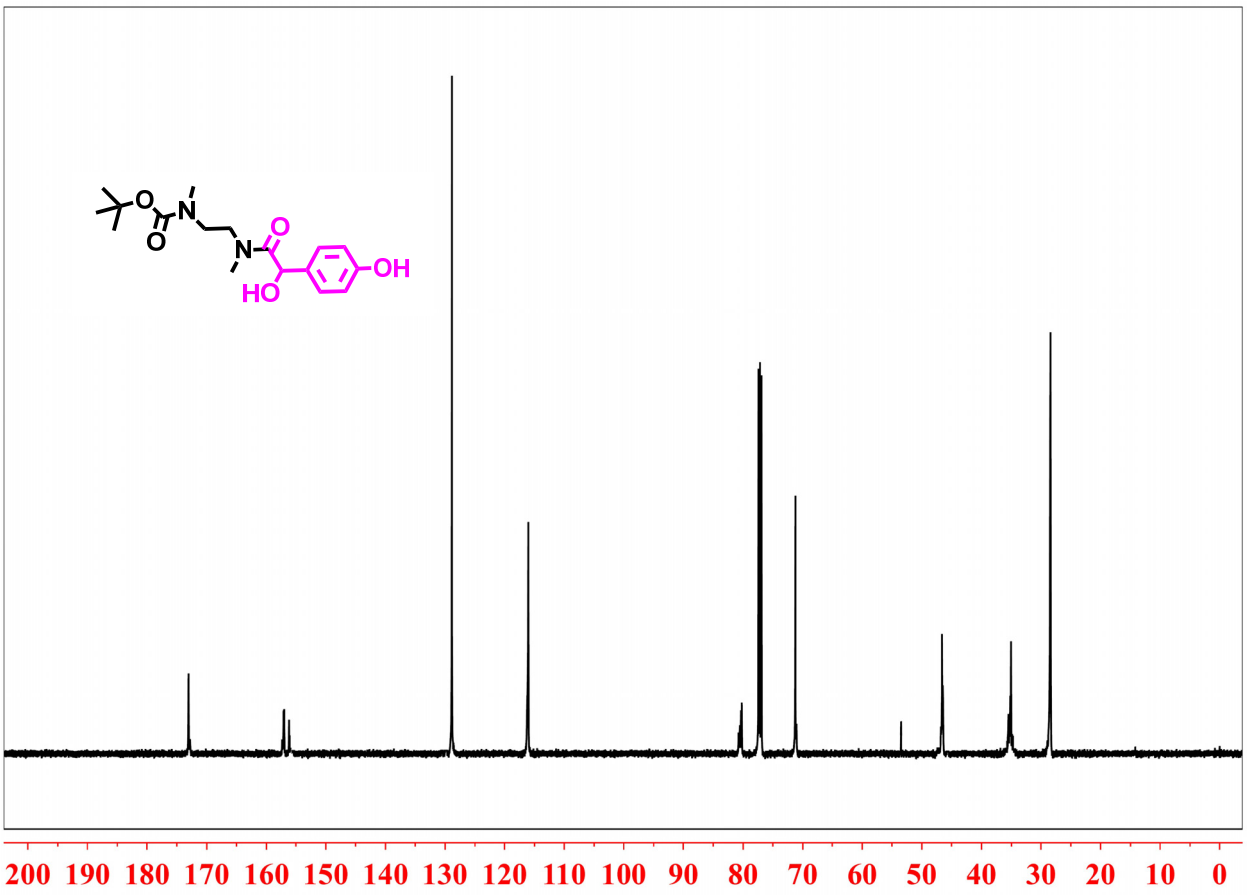
^{1}H NMR of compound **1** in $CDCl_3$.



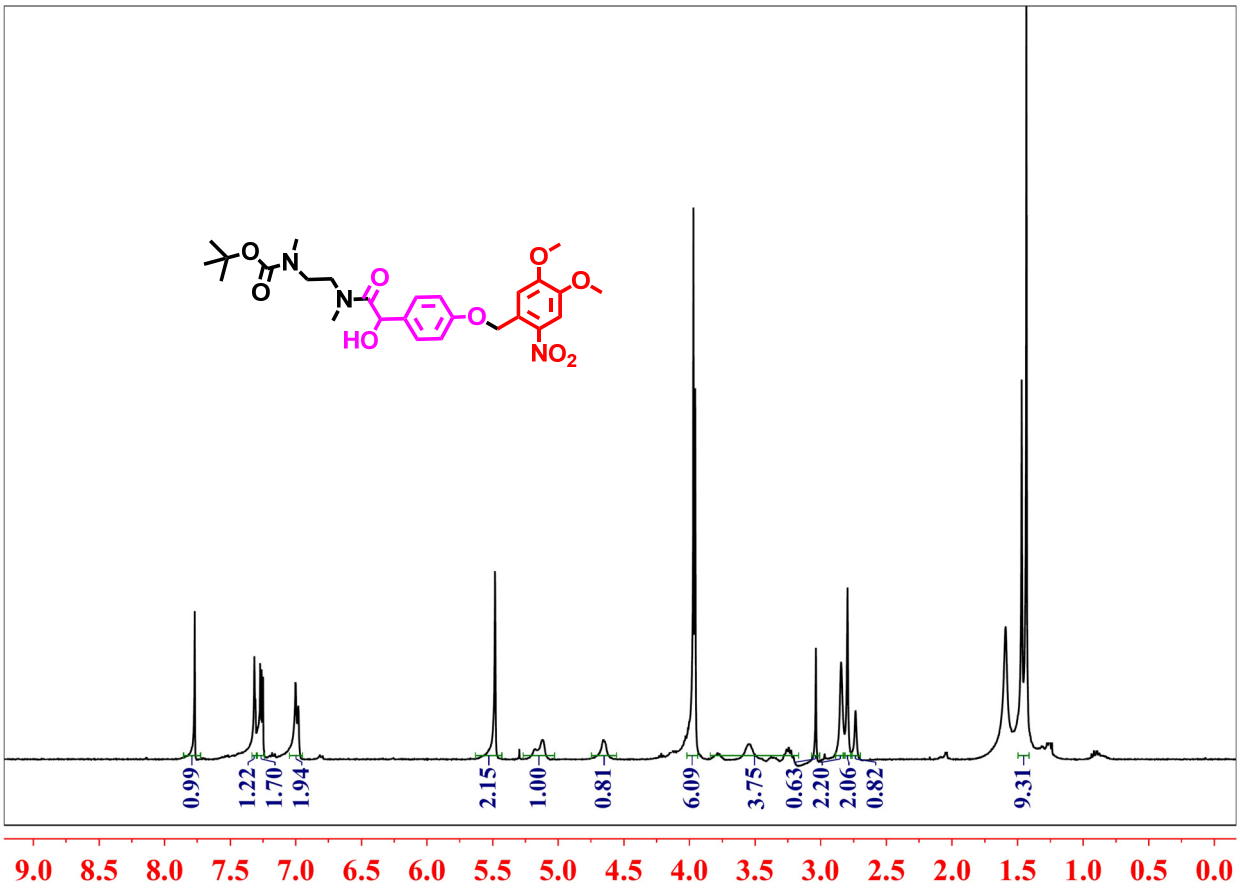
^{13}C NMR of compound **1** in $CDCl_3$.



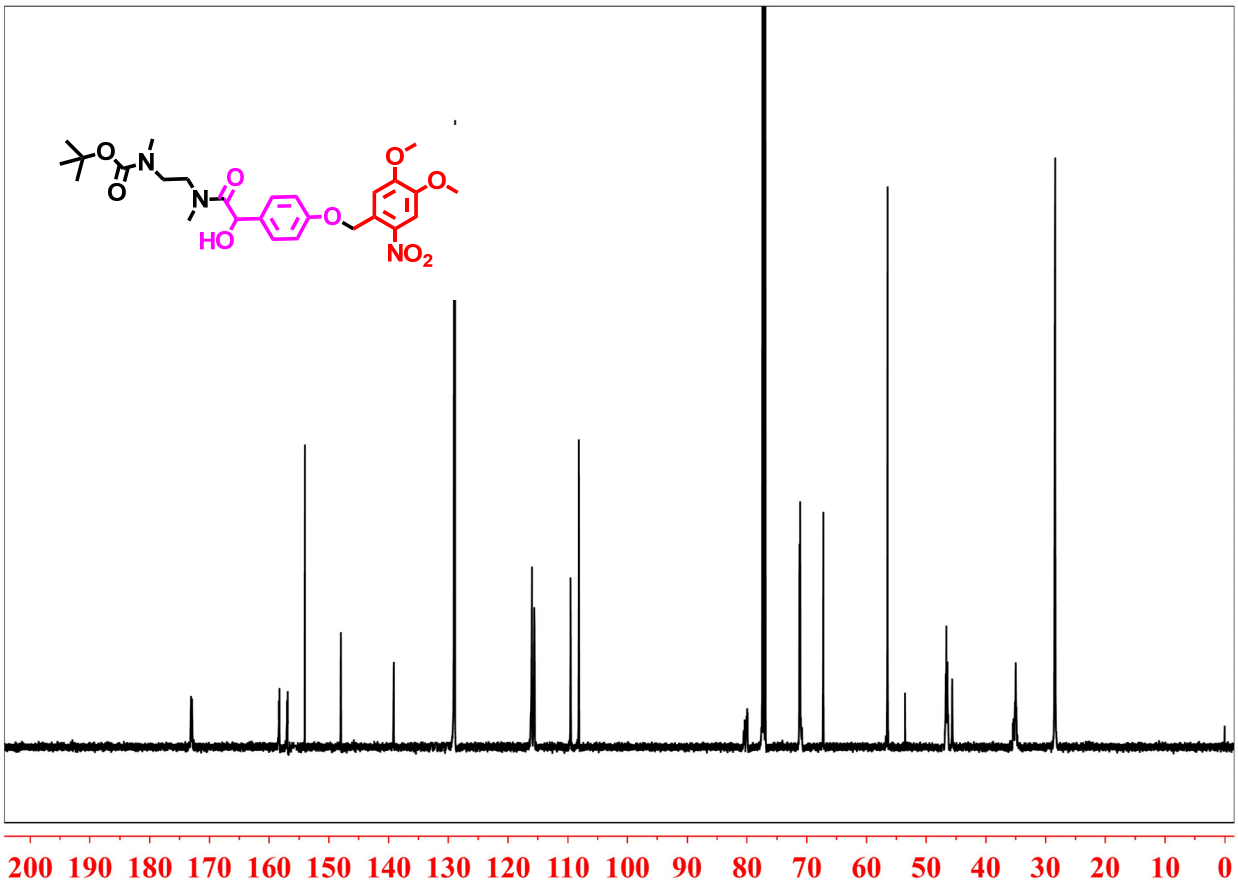
^{1}H NMR of compound **2** in $CDCl_3$.



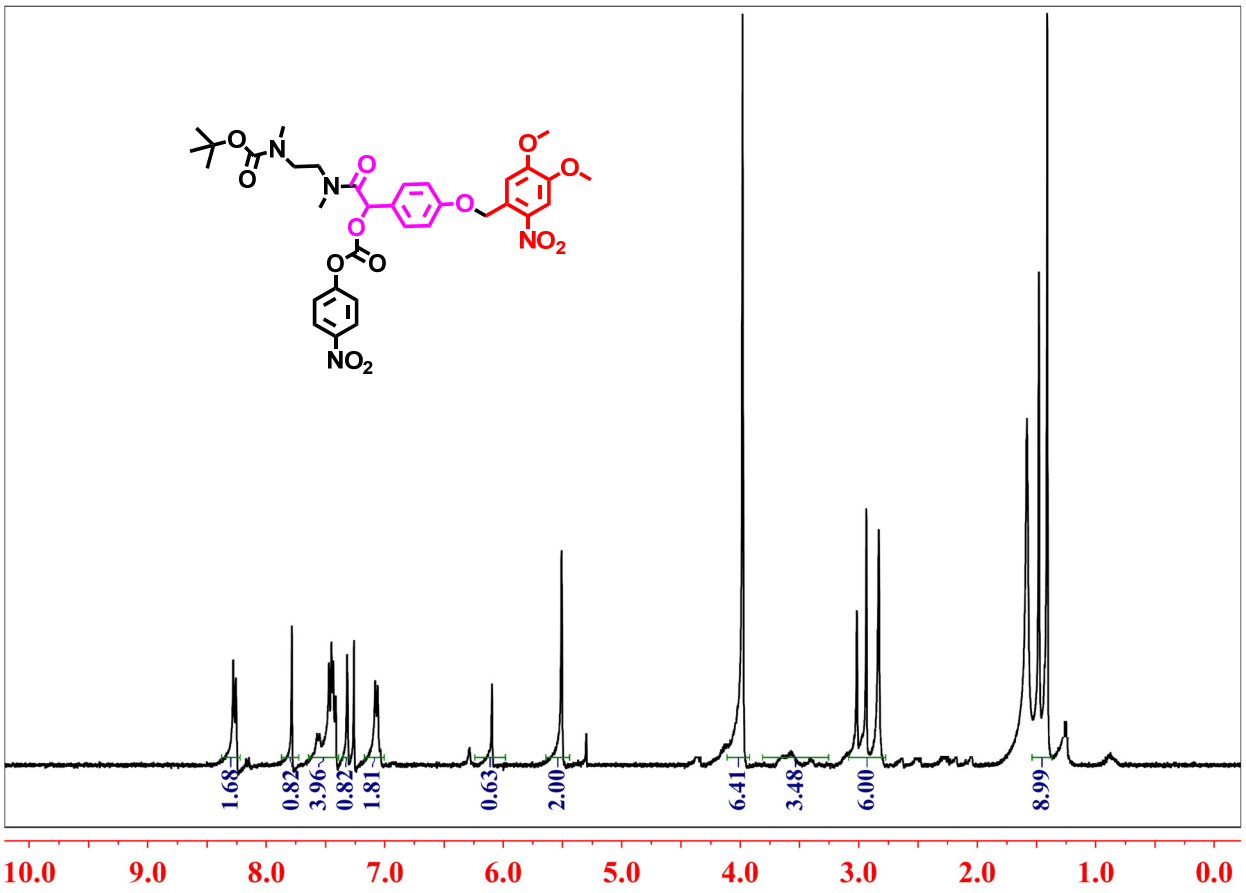
^{13}C NMR of compound **2** in $CDCl_3$.



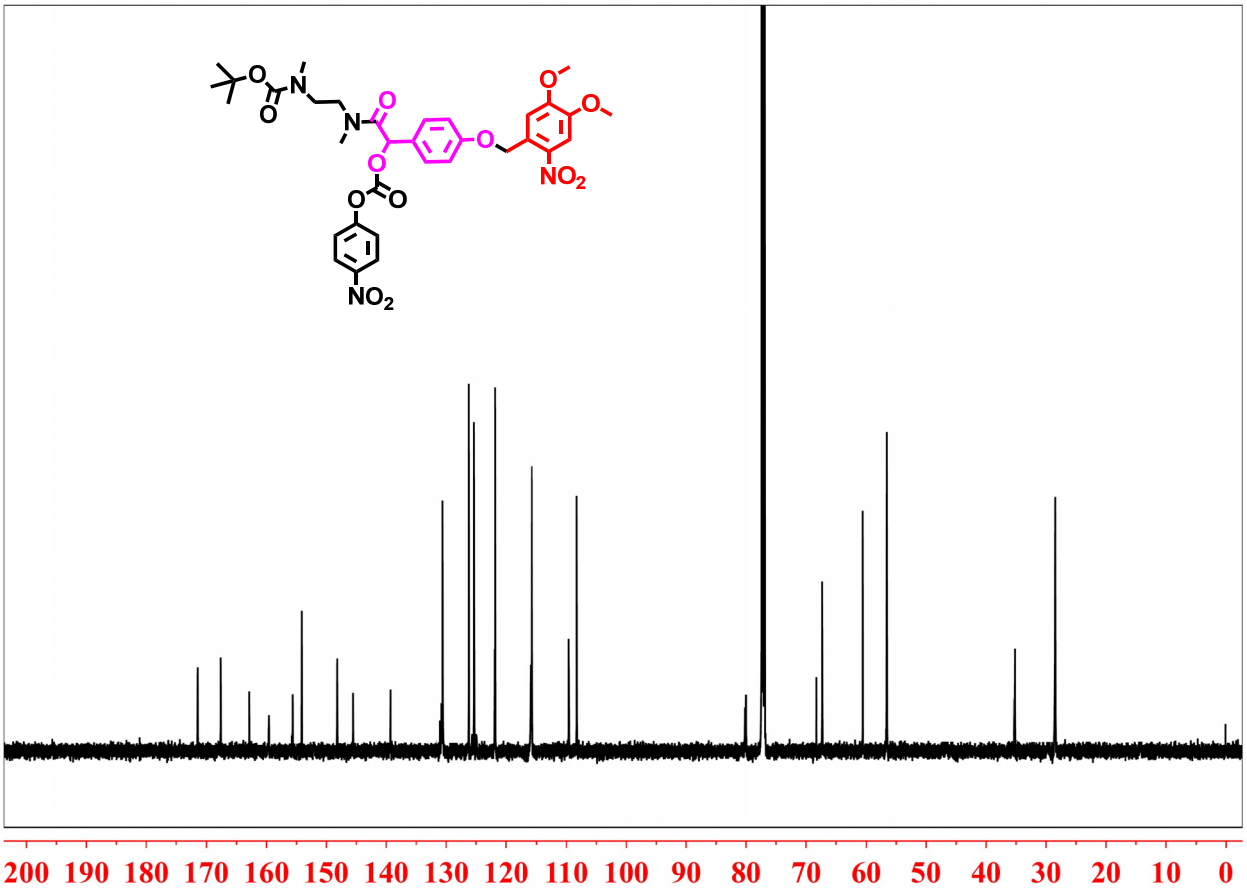
^{1}H NMR of compound **3** in $CDCl_3$.



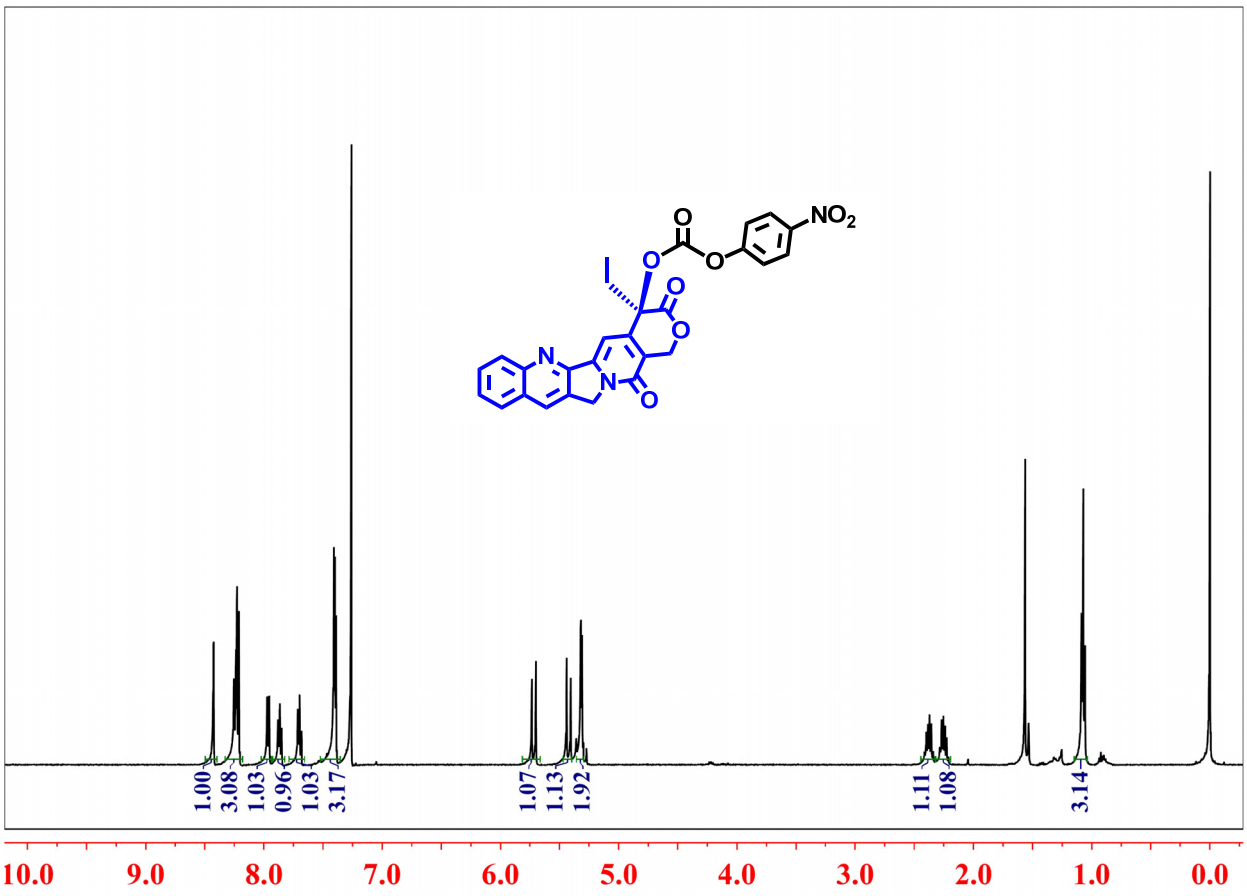
^{13}C NMR of compound **3** in $CDCl_3$.



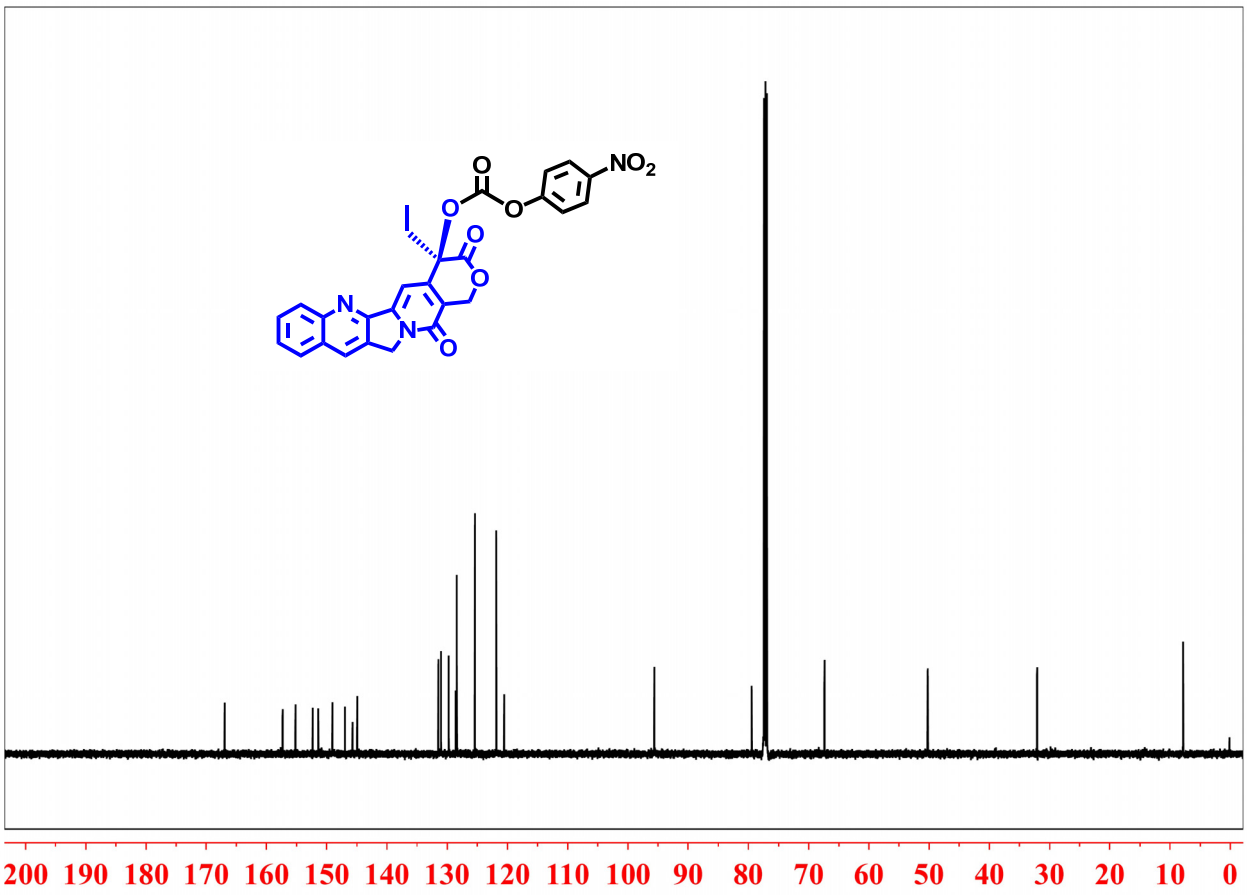
^{1}H NMR of compound **4** in $CDCl_3$.



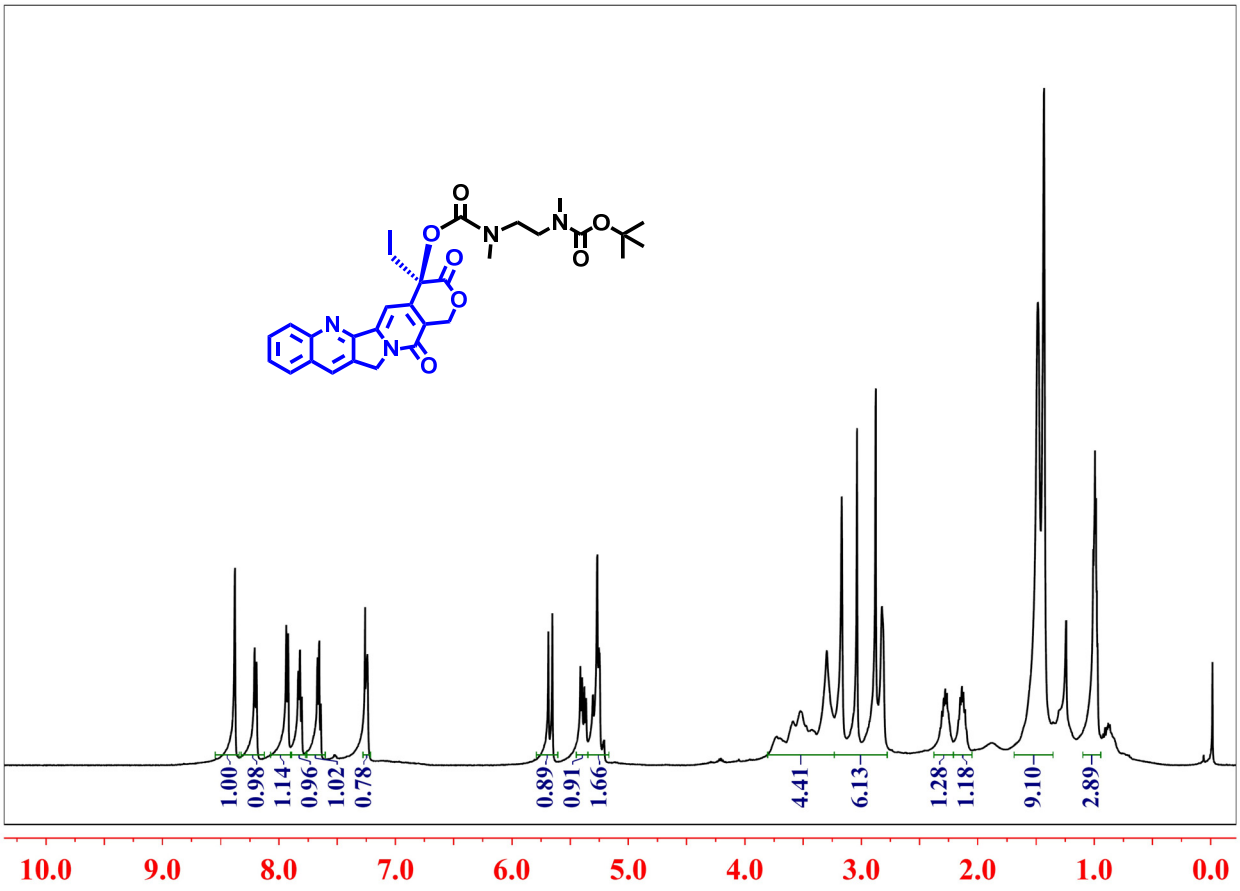
^{13}C NMR of compound **4** in $CDCl_3$.



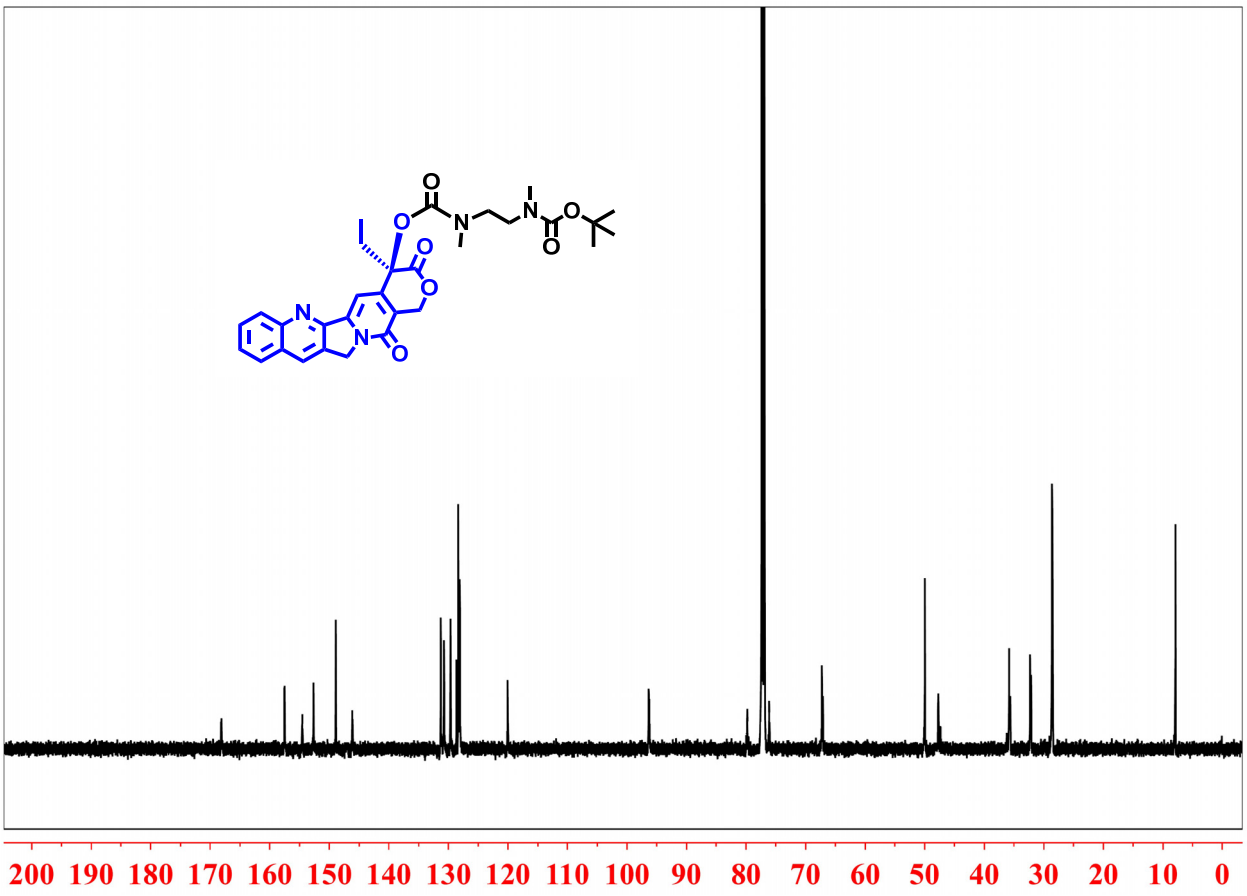
^{1}H NMR of compound **5** in $CDCl_3$.



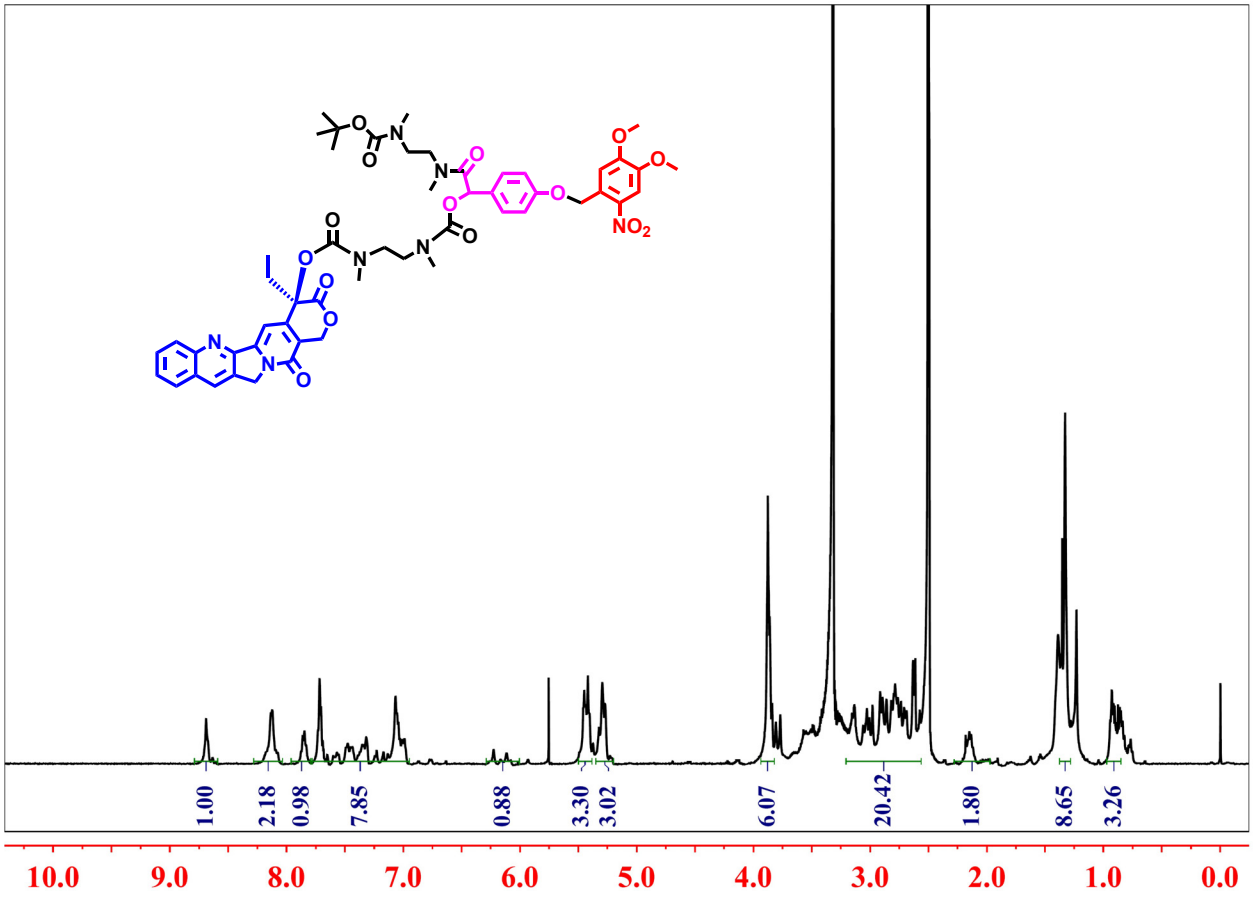
^{13}C NMR of compound **5** in $CDCl_3$.



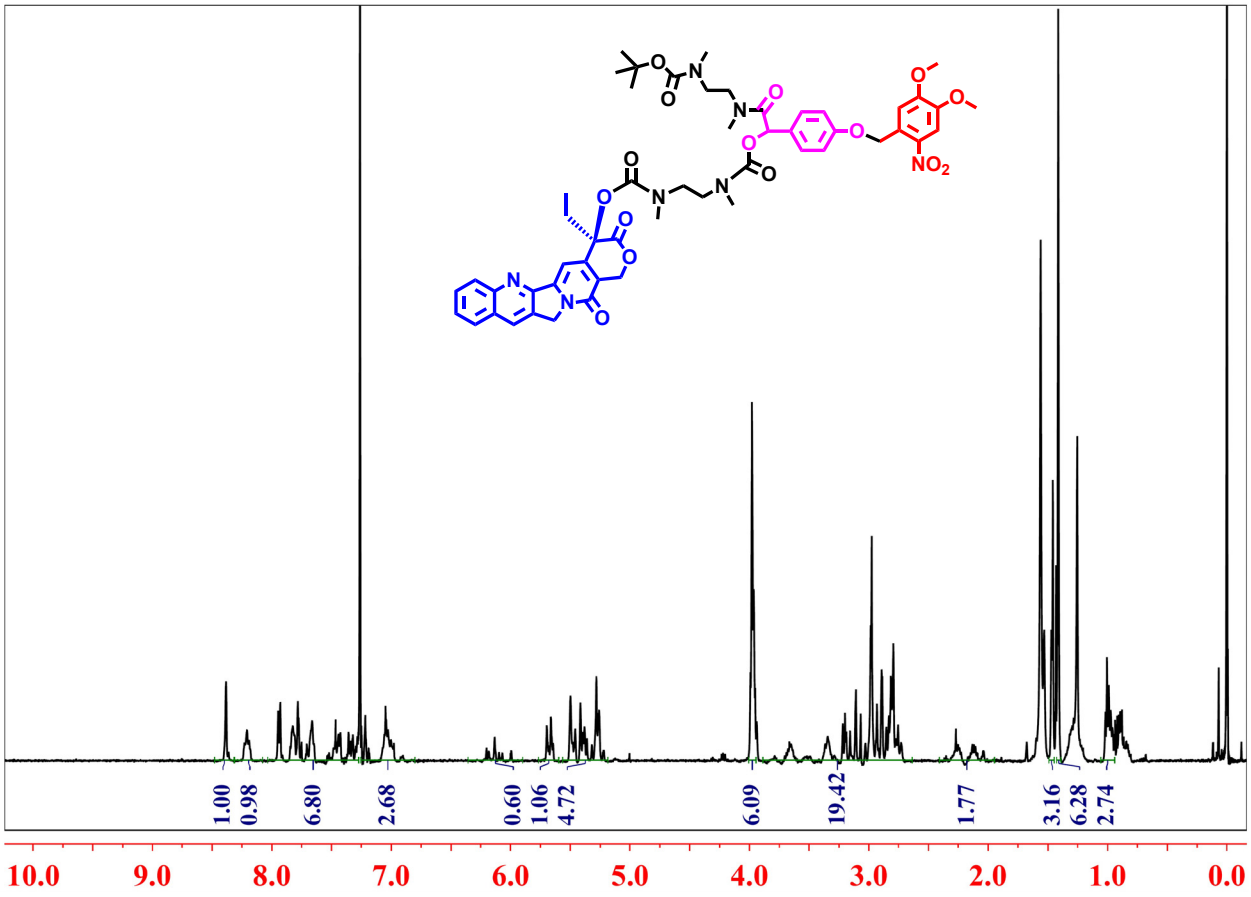
^{1}H NMR of compound **6** in $CDCl_3$.



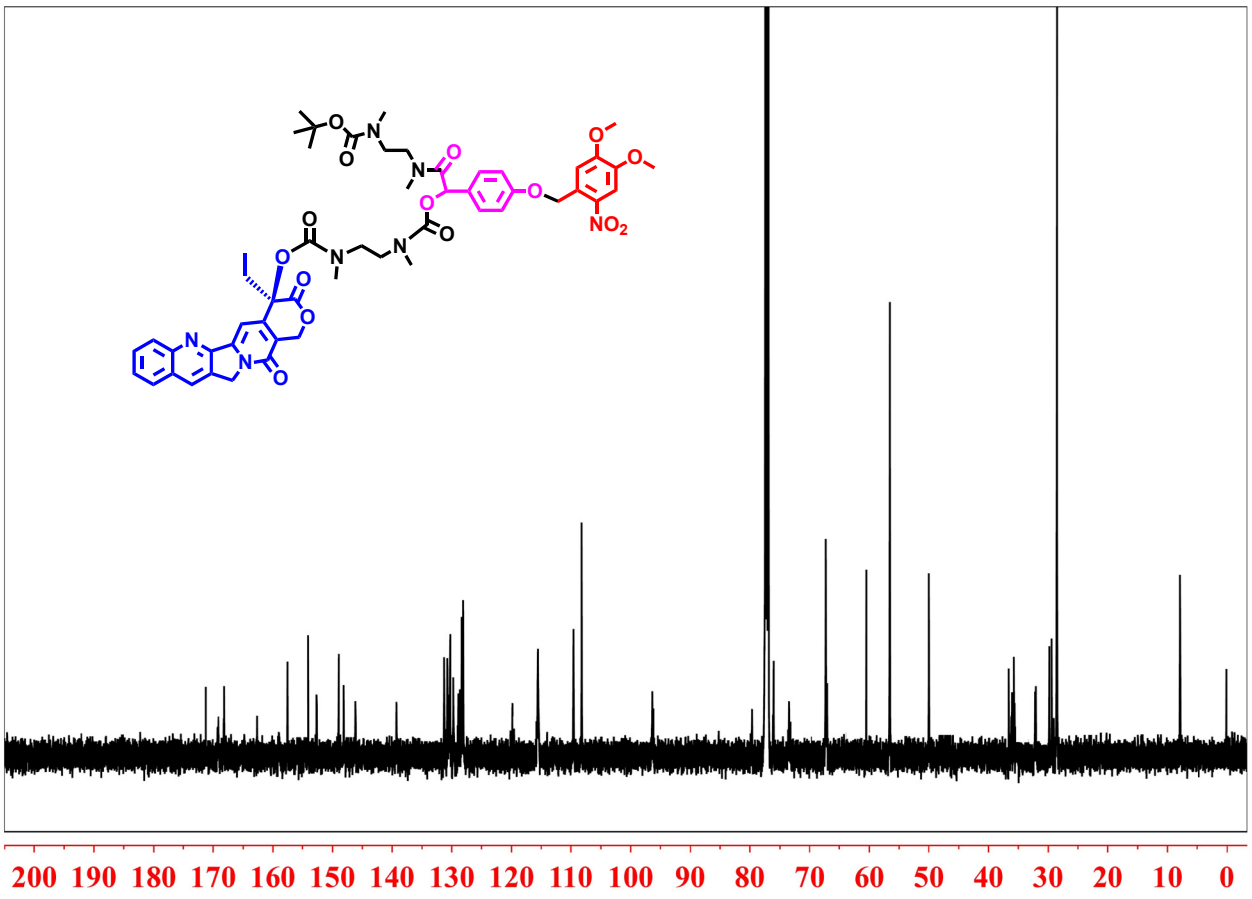
^{13}C NMR of compound **6** in $CDCl_3$.



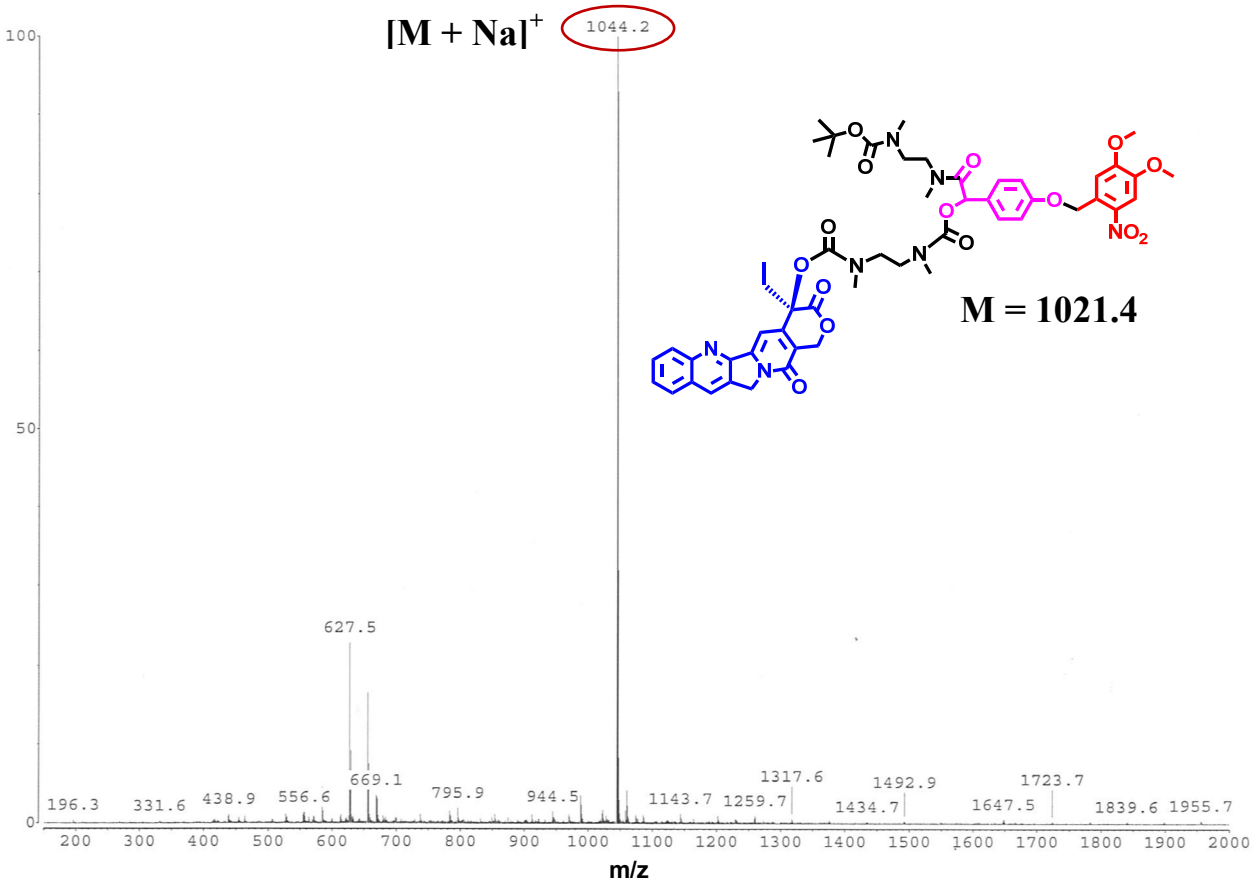
^{1}H NMR of compound **7** in d_6-DMSO.



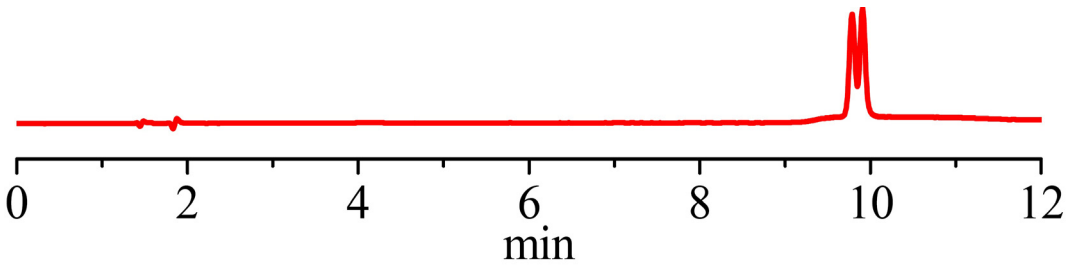
^{1}H NMR of compound **7** in $CDCl_3$.



^{13}C NMR of compound **7** in $CDCl_3$.

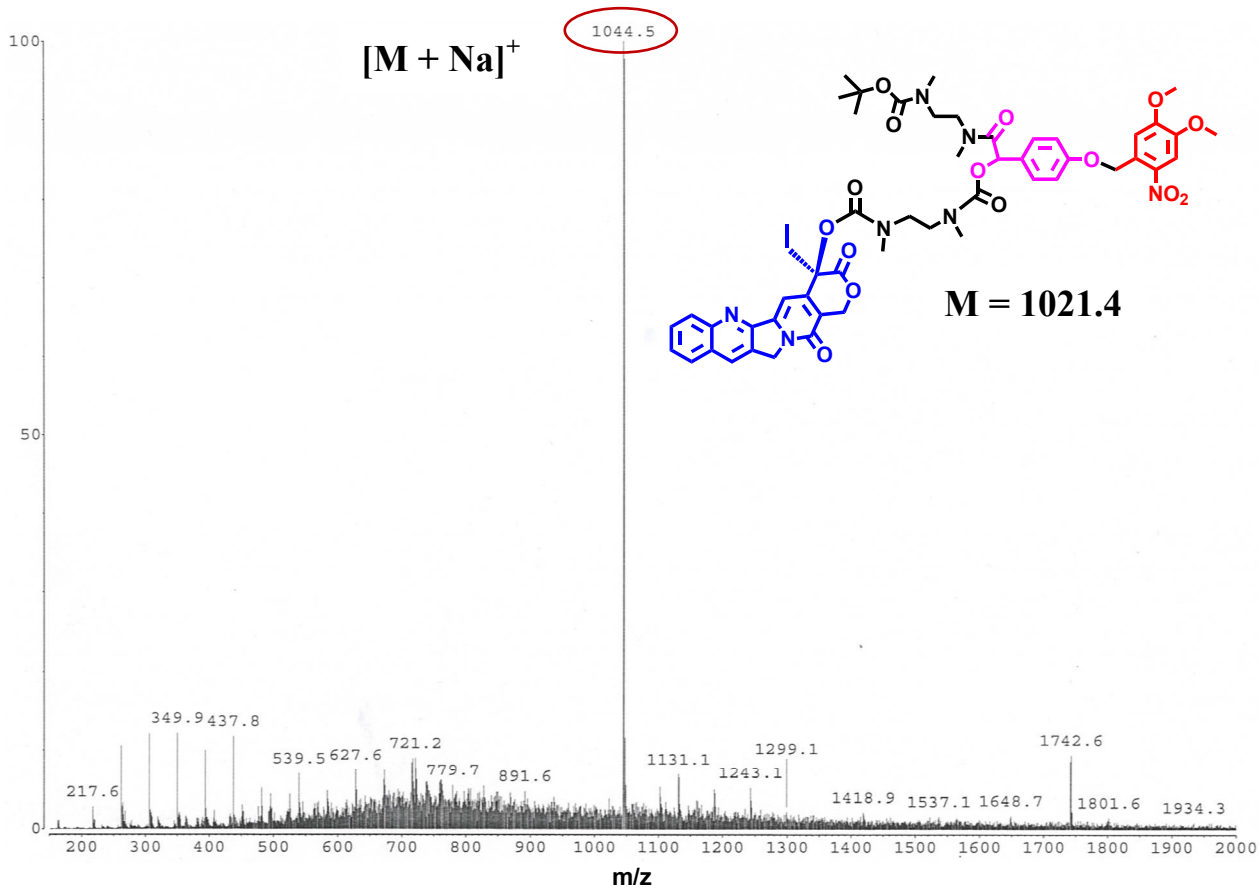


Mass spectrum of compound **7 (CAS-CPT)**.

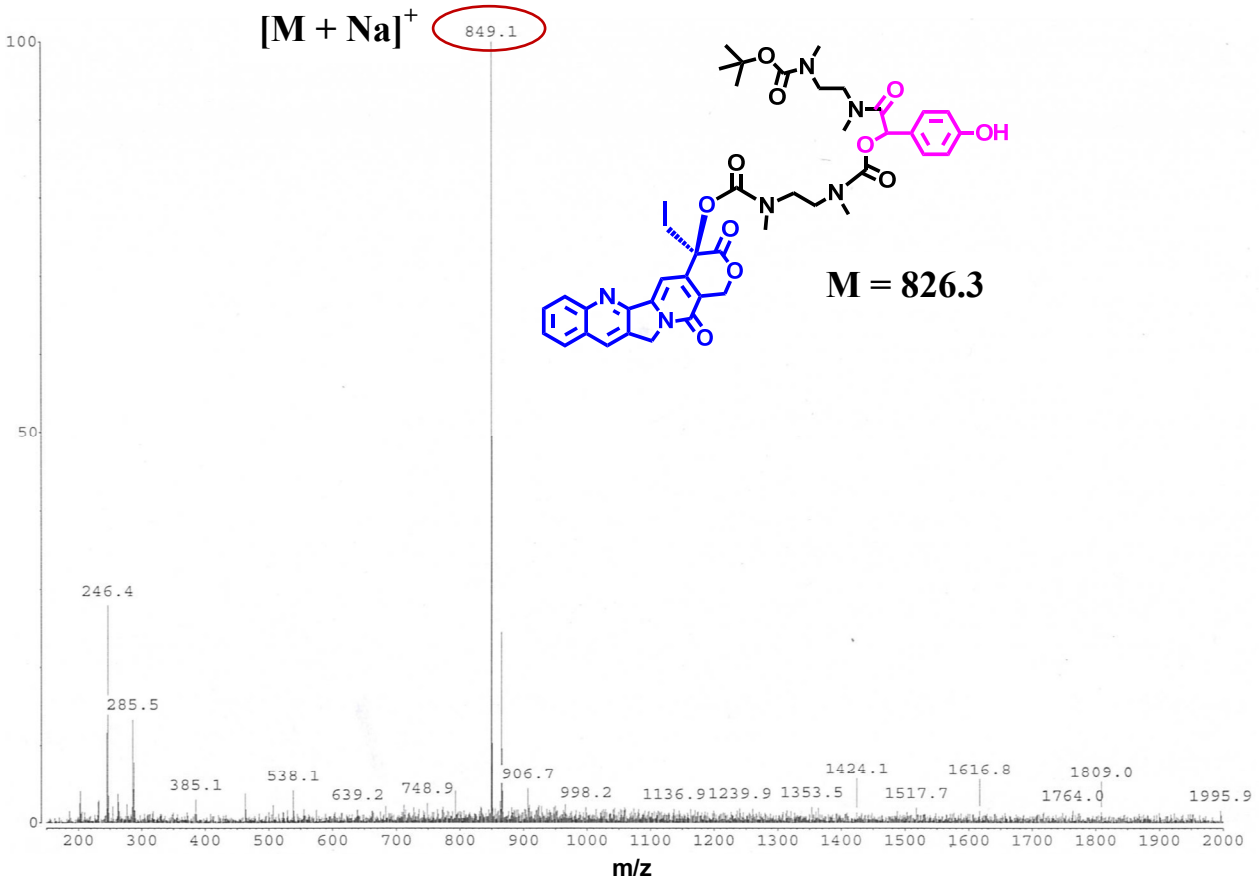


HPLC Chromatogram of compound **7** (**CAS-CPT**) in MeCN/H_2O; 7/1; v/v. Wavelength; 368 nm, eluent; acetonitrile:water; 50:50, flow rate; 1 ml/min.

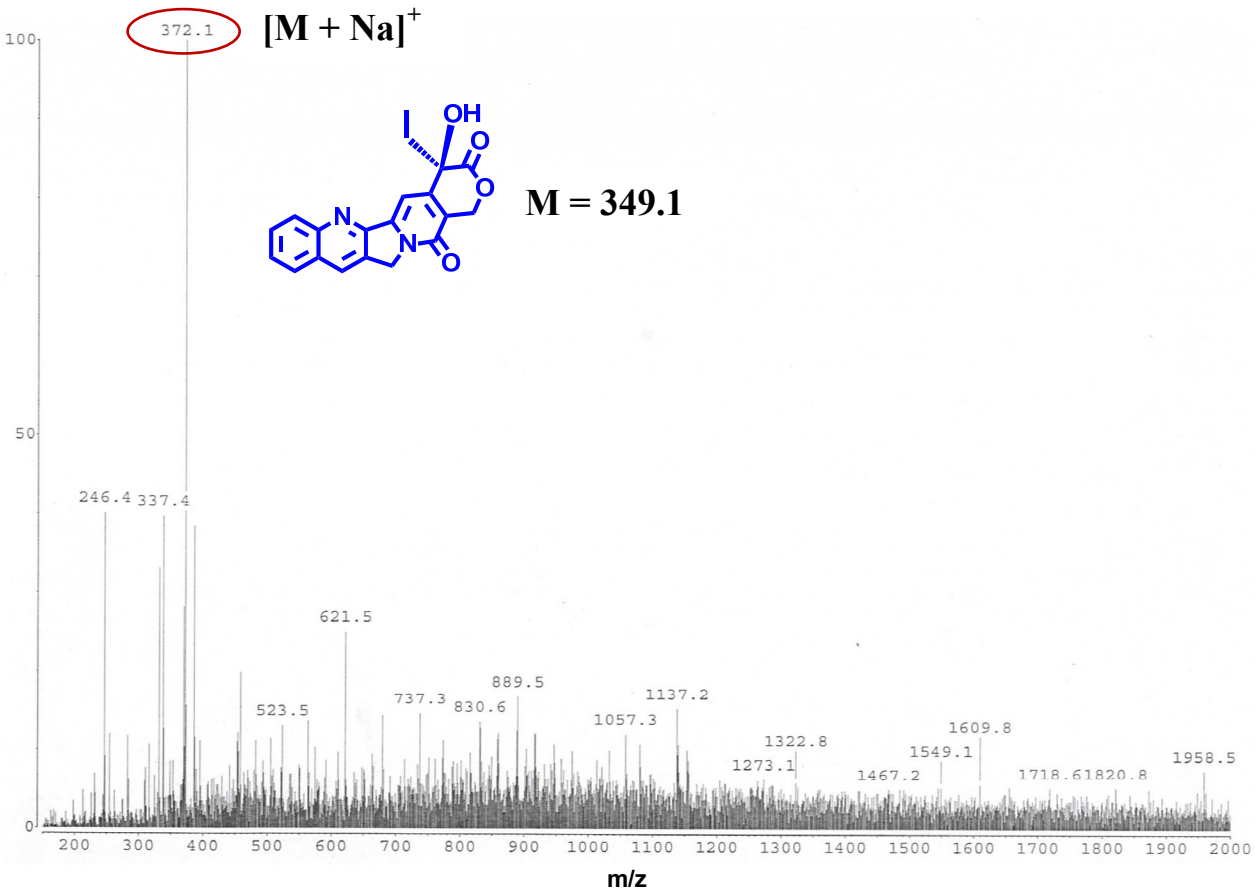
Section 2: Characterization of CPT Release from CAS-CPT



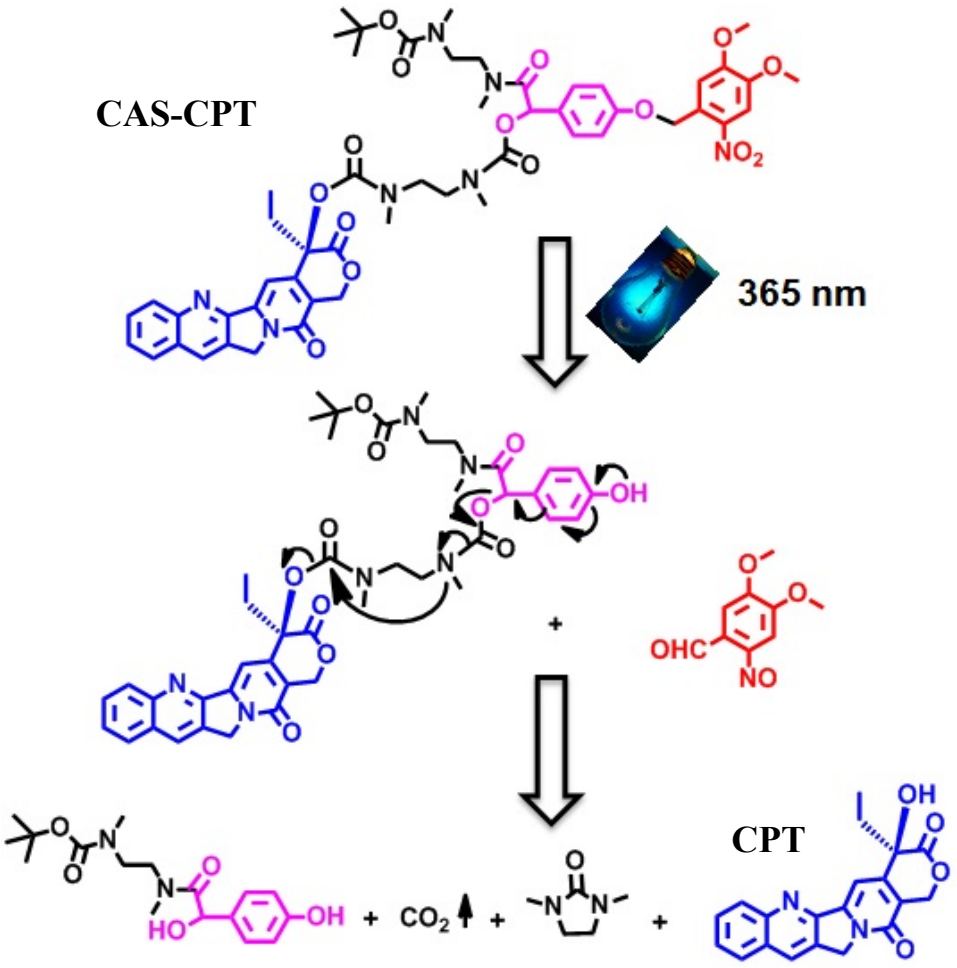
Mass spectrum of CAS-CPT before photo-irradiation.



Mass spectrum of CAS-CPT immediately after photoirradiation (365 nm, 2.5 mW/cm^2, 10 mins).

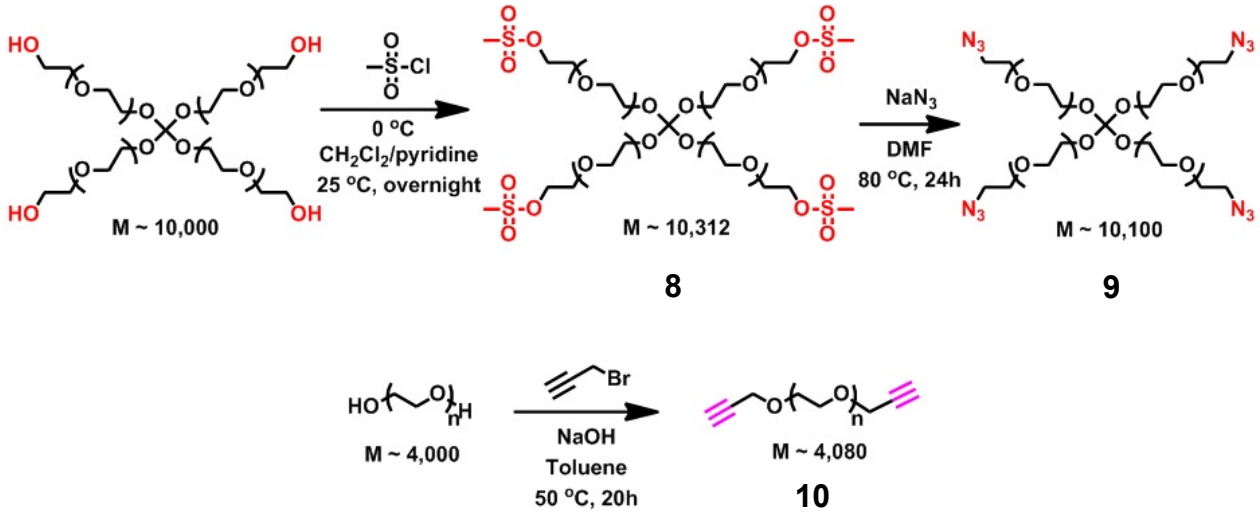


Mass spectrum of CAS-CPT 24h after photoirradiation.



Scheme 3. Proposed Mechanism of Drug Release from CAS-CPT.

Section 3: Synthesis of Hydrogel Monomers



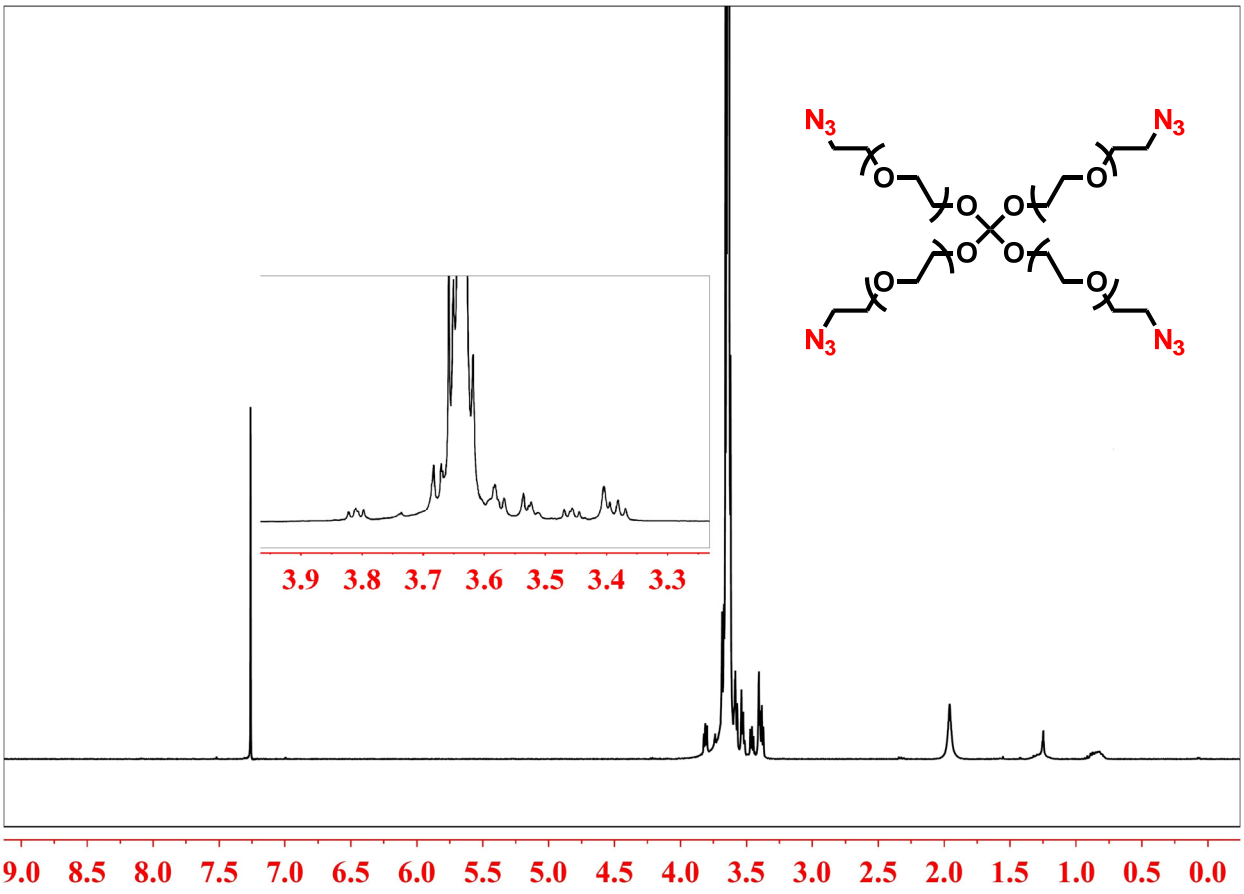
Scheme 4. Synthetic Scheme for 4-arm-PEG-tetraazide and 2-arm-PEG-dialkyne.

Synthesis PEG-tetramesylate (8): A 4-arm-poly(ethylene glycol) (MW ~ 10 kD, 2.0 g, 0.8 mmol OH groups) was dissolved in a mixture of 30 mL dry dichloromethane / pyridine (4/1; v/v) under argon atm. The mixture was then cooled to 0°C. To the above cooled solution, mesyl chloride (308 µL, 4 mmol) in 5 mL CH_2Cl_2 was added dropwise during 10 min. The reaction was allowed to warm to room temperature overnight with constant stirring under argon atmosphere. The solvent was evaporated and the residue was dissolved in $CHCl_3$. The organic layer was extracted from saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and filtered through suction. The filtrate was concentrated *in vacuo* to obtain oily colorless liquid. To the above oily liquid was added diethyl ether to precipitate the intermediate compound (Yield: 2.04 g). ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 4.30 (m, 8H, 4 x $MsOCH_2$), 3.68 (m, 8H, 4 x $MsOCH_2CH_2$), 3.47-3.57 (m, $[CH_2CH_2O]_n$), 3.19 (s, 12H, 4 x CH_3SOO-).

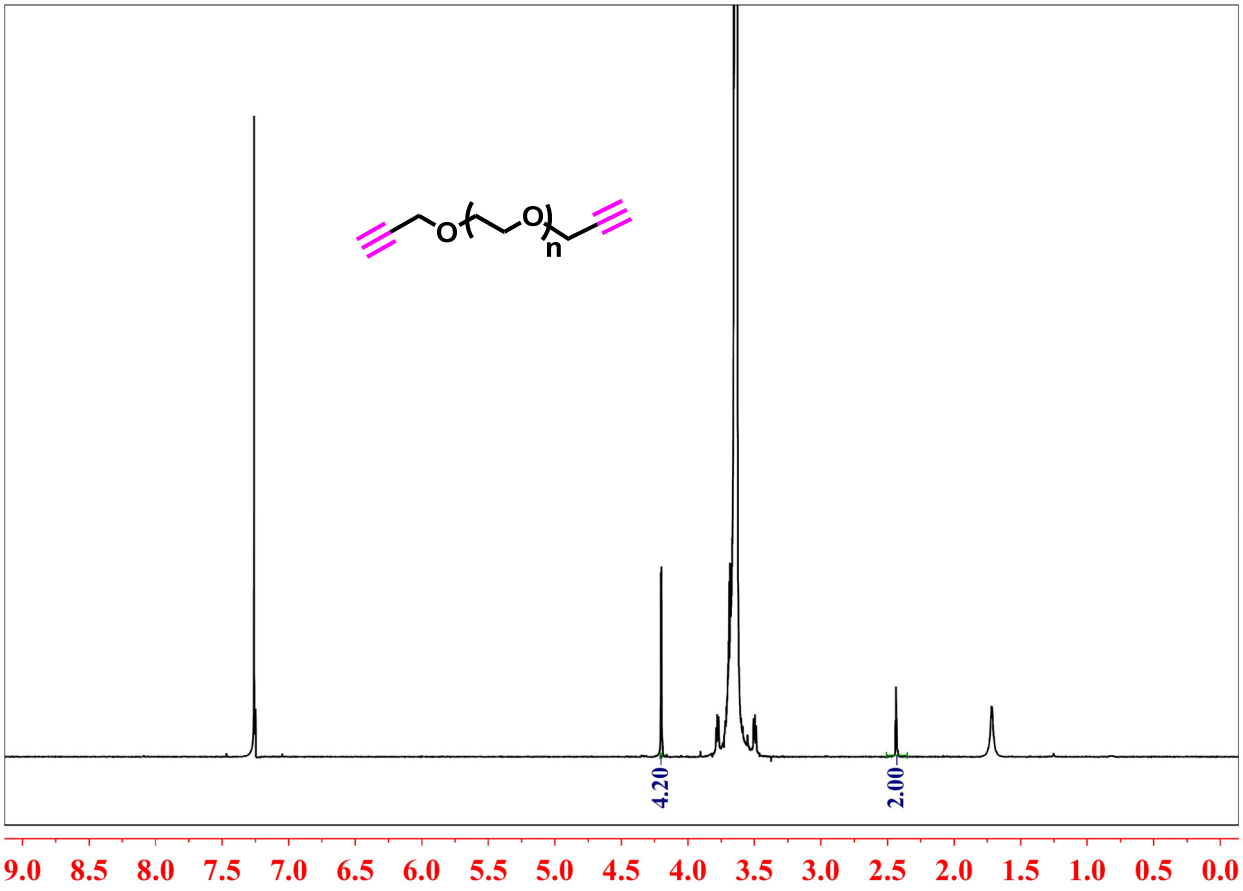
Synthesis of 4-arm-PEG-tetraazide (9): A mixture of compound **8** (2.04 g, 0.79 mmol CH_3SO_2O-) and sodium azide (257 mg, 3.91 mmol) in 15 mL dry DMF was stirred under argon atm. at 85°C for 24 h. The solid salts were removed *via* filtration through Celite, and the filtrate was concentrated *via* evaporation. The polymer was recovered *via*

precipitation with diethyl ether and filtration. The precipitate was then dissolved in distilled water and dialyzed against water for 3 days. The dialyzed solution was then freeze-dried to yield the desired product as a white powder. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.50-3.83 (m, $CH_2[CH_2CH_2O]_nCH_2$), 3.36-3.41 (m, 8H, 4 x CH_2N_3).

Synthesis of 2-arm-PEG-dialkyne (10): A 2-arm-PEG-dialkyne was synthesized *via* reaction between propargyl bromide (1.782 mL, 20 mmol) and PEG (M ~ 4000, 2.0 g, 0.5 mmol) in the presence of NaOH powder (0.8 g, 20 mmol) in 25 mL toluene for 17 h at 60 ºC. The solvent was evaporated under vacuum and the residue was dissolved in water. The aqueous layer was extracted with CH_2Cl_2 (3 x 40 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The product was precipitated out after treatment with diethyl ether. The precipitate was re-dissolved in CH_2Cl_2 and re-precipitated with diethyl ether. This procedure was repeated three times to purify the products. The 2-arm-PEG-dialkyne was isolated as a white powder (1.65 g, 81%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 4.20 (d, J = 2.5 Hz, 4H, $CH_2C{\equiv}CH$), 3.55-3.73 (m, $[CH_2CH_2O]_n$), 2.44 (t, J = 2.5 Hz, 2H, $C{\equiv}CH$).



^{1}H NMR of 4-arm-PEG-tetraazide (**9**) in $CDCl_3$. The inset is showing enlarged view of the spectrum.



^{1}H NMR of 2-arm-PEG-alkyne (**10**) in $CDCl_3$.

REFERENCES:

1. Meyer, Y., Richard, J.-A., Delest, B., Noack, P., Renard, P.-Y., and Romieu, A. “A comparative study of the self-immolation of para-aminobenzylalcohol and hemithioaminal-based linkers in the context of protease-sensitive fluorogenic probes”. *Org. Biomol. Chem.*, **2010**, 8, 1777–1780.

2. Gopin, A., Pessah, N., Shamis, M., Rader, C., and Shabat, D. "A chemical adaptor system designed to link a tumor-targeting device with a prodrug and an enzymatic trigger". *Angew. Chem. Int. Ed. Engl.*, **2003**, 42, 327-332.

SUPPLEMENTARY FIGURES

Figure S1	HPLC Chromatogram showing CPT release over time from CAS-CPT after photoirradiation.
Figure S2	HPLC Chromatogram showing stability of CAS-CPT.
Figure S3	Elastic moduli of hydrogels with varying loadings of NP-CAS-CPT.
Figure S4	Fluorescence emission spectra of CPT release from hydrogel-NP hybrid scaffolds.
Figure S5	CPT Release from hydrogels loaded with varying amount of NP-CAS-CPT.

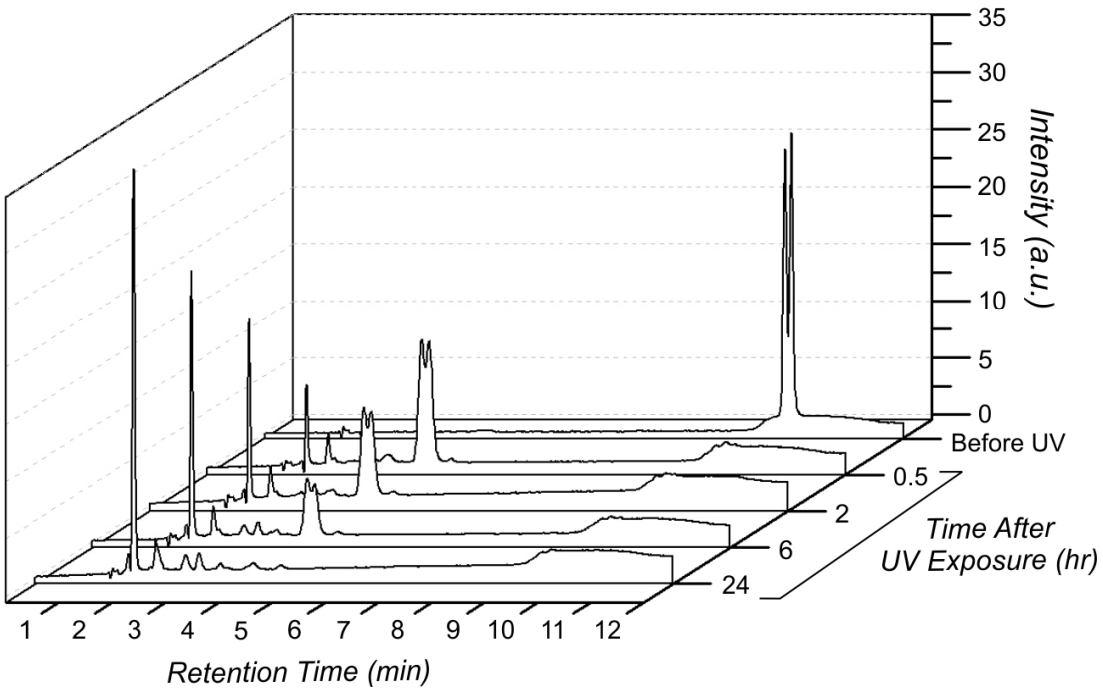


Figure S1. HPLC Chromatograms showing CPT release over time from CAS-CPT after photoirradiation. CAS-CPT (20 μM) was dissolved in aqueous medium (1xPBS containing 2% DMF). The solution was exposed to UV light (365 nm, 2.5 mW/cm^2) for 10 mins and then monitored over 24 hrs.

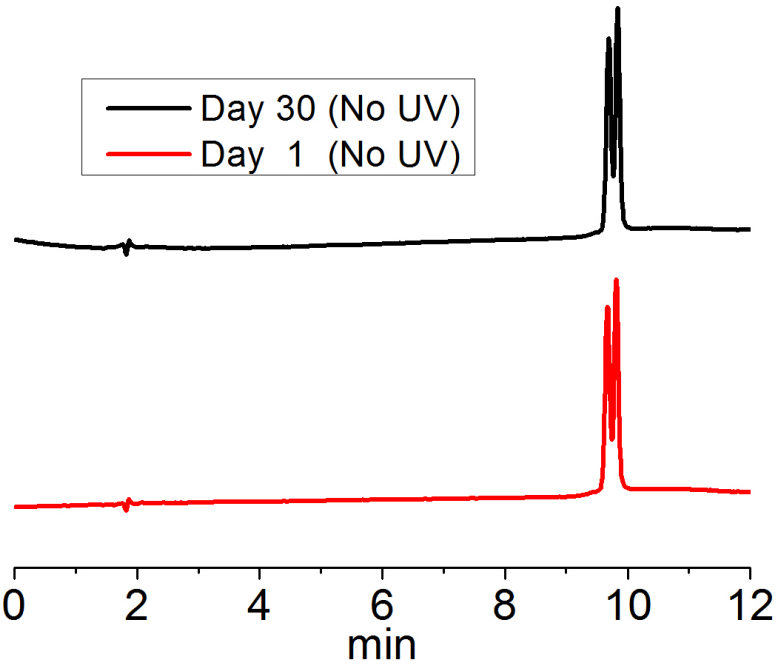


Figure S2. HPLC Chromatograms showing stability of CAS-CPT in 1xPBS in the absence of photo-irradiation, monitored over 30 days.

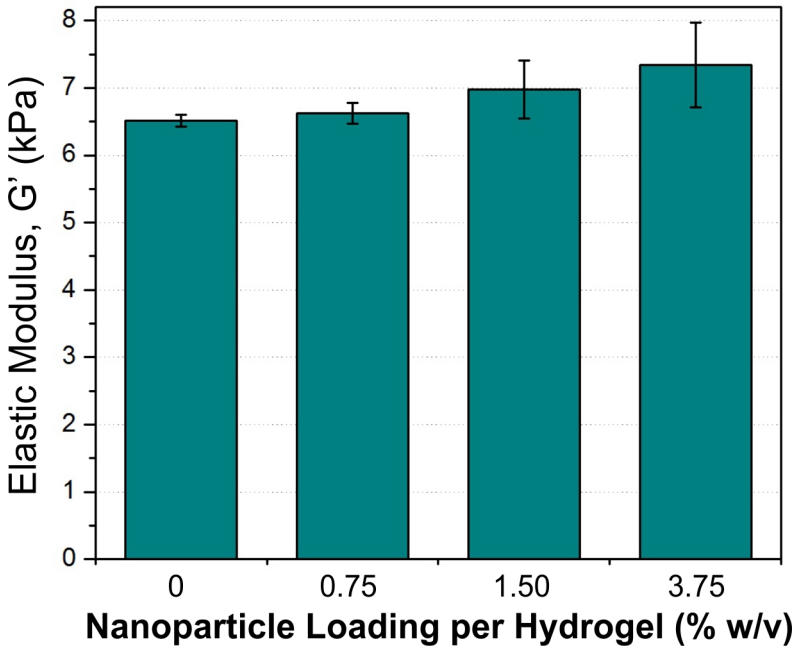


Figure S3. Elastic moduli of hydrogels with varying loadings of NP-CAS-CPT. Rheometry measurements were acquired at a 0.1% shear rate and the moduli reported at a 1 Hz frequency.

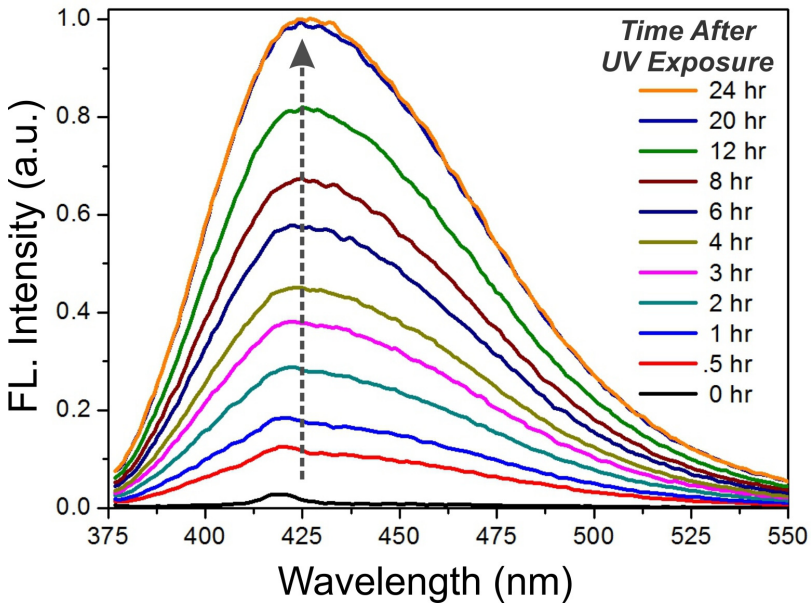


Figure S4. Fluorescence emission spectra of CPT release from hydrogel-NP hybrid scaffolds. The emission spectra (λ_{ex} = 368 nm) was monitored over 24 hrs after 10 min irradiation with UV light (365 nm, 2.5 mW/cm^2).

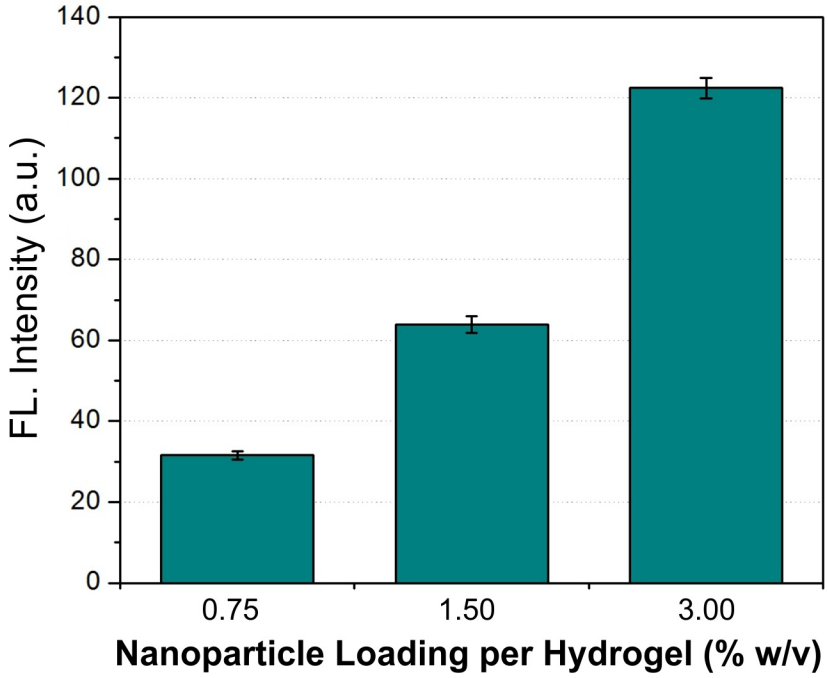


Figure S5. CPT Release from hydrogels loaded with varying amounts of NP-CAS-CPT. The samples were irradiated with UV light (365 nm, 2.5 mW/cm^2) for 10 mins. After 24 hrs, the release was measured using fluorescence spectroscopy (λ_{ex} = 368 nm, λ_{em} = 426 nm).