Supporting Information

Polymeric Engineering of Aptamer−Drug Conjugates for Targeted Cancer Therapy

Zhengyu Deng,[†#] Qiuxia Yang,[†#] Yongbo Peng,[†] Jiaxuan He,[†] Shujuan Xu,[†%] Dan Wang,[†] Tianhuan Peng,[†] Ruowen Wang,[§] Xue-Qiang Wang,[*†] and Weihong Tan[*†‡%]

[†] Molecular Science and Biomedicine Laboratory (MBL), State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, Aptamer Engineering Center of Hunan Province, Hunan University, Changsha, Hunan 410082, China

[‡] Institute of Cancer and Basic Medicine (IBMC), Chinese Academy of Sciences; The Cancer Hospital of the University of Chinese Academy of Sciences, Hangzhou, Zhejiang 310022, China

[§] Institute of Molecular Medicine, Renji Hospital, School of Medicine, College of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

% Foundation for Applied Molecular Evolution, 13709 Progress Boulevard, Alachua, FL 32615, USA.

***** To whom correspondence should be addressed. E-mail: tan@hnu.edu.cn; wangxq@hnu.edu.cn.

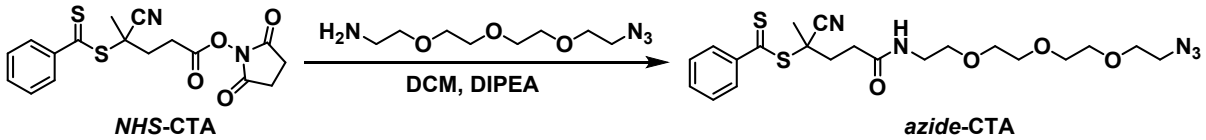
[#] These authors contribute equally.

Experimental Section

Materials.

All chemicals were used as received from Sinopharm Chemical Reagent Co. Ltd. unless otherwise specified. Oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA, M_n = 500 g/mol, mean degree of polymerization, DP, is 9) purchased from Aldrich was passed through a neutral alumina column to remove the inhibitor and then stored at -20 °C prior to use. All the oligonucleotides were obtained from Sangon Biotechnology (Shanghai) Co., Ltd. 7-Ethyl-10-hydroxycamptothecin (SN-38), paclitaxel (PTX), irinotecan, 11-azido-3,6,9-trioxaundecan-1-amine, glutathione reduced (GSH), methacryloylchloride, triphosgene, *N,N*-diisopropylethylamine (DIPEA), and 4-dimethylaminopyridine (DMAP) were purchased from Aladdin and used as received. 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid *N*-succinimidyl ester (*NHS*-CTA) was purchased from Sigma-Aldrich and used as received. Benzoyl peroxide (BPO) was recrystallized from chloroform. *N*,*N*-dimethylaniline (DMA) was distilled under reduced pressure and the pure DMA was collected below 135 °C. Dulbecco's Phosphate Buffered Saline solution (DPBS, calcium and magnesium-free), agarose and 10× PBS were purchased from Thermo Fisher Scientific. Fetal bovine serum (FBS), penicillin, streptomycin, RPMI-1640 medium, and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO and used as received. Water was deionized (DI) with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.4 MΩ cm. 2-((2-Hydroxyethyl)disulfaneyl)ethyl methacrylate (SSM-OH) was synthesized according to previously reported literature procedures.[1]

Synthesis of Azide-Modified Chain Transfer Agent (azide-CTA).



***NHS*-CTA** ***azide*-CTA**

Typical procedures employed for the preparation of *azide*-CTA are as follows. *NHS*-CTA (376 mg, 1 mmol) and 11-azido-3,6,9-trioxaundecan-1-amine (300 mg, 1.37 mmol) were dissolved in DCM (20 mL) containing DIPEA (129 mg, 1 mmol) and the reaction was stirred at room temperature for 2 h. The product was purified by flash chromatography (yield: 351 mg, 74%). ^{1}H NMR ($CDCl_3$, δ, ppm, Figure S1).

Synthesis of SN-38 Monomer, SN-38M (Scheme S1).

Typical procedures employed for the preparation of SN-38M monomer are as follows. SSM-OH (2.65 g, 11.93 mmol) was first activated using triphosgene (1.18 g, 3.98 mmol) in DCM (100 mL) containing DIPEA (3.85 g, 29.83 mmol). Then, to a stirred suspension of SN-38 (3.12 g, 7.95 mmol) in DMF (30 mL) at 4 ^{o}C, was added newly activated 2-((2-((chlorocarbonyl)oxy)ethyl)disulfaneyl)ethyl methacrylate; the cooling bath was removed and the clear solution was stirred at room temperature for 2 h. The product was purified by flash chromatography (yield: 4.43 g, 87%). ^{1}H and ^{13}C NMR ($CDCl_3$, δ, ppm, Figure S2 and S3). ESI-MS (m/z): $[M+H]^+$ calcd. for $C_{31}H_{32}N_2O_9S_2$ ([M]), 641.16; found: 641.16.

Synthesis of PTX Monomer, PTXM (Scheme S1).

Typical procedures employed for the preparation of PTXM monomer are as follows. SSM-OH (440 mg, 1.98 mmol) was first activated using triphosgene (197 mg, 0.66 mmol) in DCM (20 mL) containing DIPEA (642 mg, 4.97 mmol). Then, to a stirred suspension of PTX (1.13 g, 1.325 mmol) in DCM (30 mL) at 4 ^{o}C, was added newly activated 2-((2-((chlorocarbonyl)oxy)ethyl)disulfaneyl)ethyl methacrylate; the cooling bath was removed and the clear solution was stirred at room temperature for 8 h. The product was purified by flash chromatography (yield: 1.05 g, 72%). ^{1}H and ^{13}C NMR ($CDCl_3$, δ, ppm, Figure S4 and S5). ESI-MS (m/z): $[M+H]^+$ calcd. for $C_{56}H_{63}NO_{18}S_2$ ([M]), 1102.35; found: 1102.35.

Synthesis of Polyprodrug Copolymers (Scheme 1b and S2).

Polyprodrug copolymer was synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization. Typically, *azide*-CTA (8 mg, 0.017 mmol, 1 equiv.), OEGMA (1 g, 2.0 mmol, 120 equiv.), SN-38M (426 mg, 0.66 mmol, 40 equiv.), BPO (8.2 mg, 0.033 mmol, 2 equiv.), DMA (4.1 mg, 0.033 mmol, 2 equiv.), and DMSO (2 mL) were charged into a reaction tube equipped with a magnetic stirring bar. The tube was carefully degassed by three freeze-pump-thaw cycles and sealed under vacuum. After thermostating at 30 ^{o}C in an oil bath and stirring for 24 h, the reaction tube was quenched into liquid nitrogen and opened; the mixture was diluted with 1,4-dioxane then precipitated into an excess of ether and the precipitate was dissolved with $CHCl_3$ again. The above dissolution-precipitation cycle was repeated three times. The final product was dried in a vacuum oven overnight at room temperature, yielding a pink solid (975 mg, yield: 68%), *azide*-P(OEGMA$_{0.75}$-*co*-SN-

$38M_{0.25})_{160}$. The molecular weight and molecular weight distribution were determined by GPC using DMF as the eluent. Following similar procedures, *azide*-P($OEGMA_{0.75}$-*co*-$PTXM_{0.25})_{160}$ was also synthesized.

Polyprodrug copolymer containing two drugs was also synthesized via RAFT copolymerization. Typically, *azide*-CTA (4 mg, 0.0083 mmol, 1 equiv.), OEGMA (1 g, 2.0 mmol, 240 equiv.), SN-38M (213 mg, 0.33 mmol, 40 equiv.), PTXM (363 mg, 0.33 mmol, 40 equiv.), BPO (4.1 mg, 0.017 mmol, 2 equiv.), DMA (2.1 mg, 0.017 mmol, 2 equiv.), and DMSO (2 mL) were charged into a reaction tube equipped with a magnetic stirring bar. The tube was carefully degassed by three freeze-pump-thaw cycles and sealed under vacuum. After thermostating at 30 °C in an oil bath and stirring for 24 h, the reaction tube was quenched into liquid nitrogen and opened; the mixture was diluted with 1,4-dioxane then precipitated into an excess of ether and the precipitate was dissolved with $CHCl_3$ again. The above dissolution-precipitation cycle was repeated three times. The final product was dried in a vacuum oven overnight at room temperature, yielding a pink solid (1.02 g, yield: 65%), *azide*-P($OEGMA_{0.75}$-*co*-SN-$38M_{0.125}$-*co*-$PTXM_{0.125})_{320}$. The molecular weight and molecular weight distribution were determined by GPC using DMF as the eluent. The structural parameters of all as-synthesized copolymers are summarized in Table S1.

Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) for Aptamer-Polyprodrug Conjugates.

DBCO-modified DNA (0.2 mM) and *azide*-terminated polyprodrug (1 mM of azide group) were dissolved in DI water and the reaction mixture was shaken at 37 °C for 24 h. The mixture was electrophoresed with 2% agarose gel in 1× TBE buffer (89 mM Tris borate, 2 mM Na_2EDTA, pH 8.3). When the unreacted DNA and conjugated DNA showed clear separation, the electrophoresis was stopped, and the gel was analyzed with a molecular imager (BIO-RAD).

Gel Electrophoresis for Purification.

Agarose electrophoresis was used to purify the aptamer-polyprodrug conjugates. After electrophoresis with 2% agarose gel in 1× TBE buffer at different voltage for 60 min, the product band was cut and further extracted using Freeze 'N Squeeze DNA Gel Extraction Spin Column (BIO-RAD), followed by desalting with NAP column (GE Healthcare).

DNA Serum Stability.

DBCO-Sgc8c-*FAM* and *FAM*-Sgc8c-PSN_{40} (final concentration of DNA was 1 μM) were suspended in RPMI-1640 medium supplemented with 10 vol% fetal bovine serum (FBS), respectively, at 37 °C for 0, 4, and 8 h. After incubation, each sample was loaded in the 2% agarose gel and the gel was run in 1X TBE buffer. After predetermined time, the bands were captured using the FITC channel on ChampChemi imaging system. All experiments were performed independently for at least three times.

Cell Culture and Buffers.

HCT116, CCRF-CEM, and K562 cells were purchased from ATCC. The CCRF-CEM, HCT116, and K562 cell lines were cultured in RPMI-1640 medium. All types of media were supplemented with 10% FBS and 1% penicillin-streptomycin solution (PS) at 37 °C in 5% CO_2 atmosphere. DPBS was used to wash cells. The number of cells was determined by a hemocytometer prior to each experiment. Washing buffer was prepared with DPBS supplemented with 4.5 g/L glucose and 5 mM $MgCl_2$. Binding buffer was prepared with washing buffer supplemented with 0.1 mg/mL yeast tRNA (Sigma-Aldrich) and 1 mg/mL BSA (Fisher Scientific).

In Vitro Cytotoxicity Assay.

HCT116 and CCRF-CEM cells were seeded in a 96-well plate at a density of 1×10^5 (suspended cells) or 1×10^4 (adhesive cells) cells per well, respectively. After 24 h of culture, cells were treated with Sgc8c, Libs, Sgc8c-PSN_{40}, free SN-38, and irinotecan, respectively. After 72 h of incubation, the medium was removed, and the cells were treated with 100 μL of medium and 20 μL of Cell Counting Kit-8 (CCK-8) for 2-4 h. Finally, the absorbance of the solution at 450 nm was recorded with a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT). The results were repeated in quadruple, and the error bars represent the standard derivations.

Flow Cytometry Analysis.

A total of 4×10^5 cells (HCT116, CCRF-CEM, or K562 cells) were washed with washing buffer via centrifugation at 1000 rpm and then incubated with Cy5-labeled samples at 4 °C (using binding buffer for binding assay) or 37 °C (using RPMI-1640 medium for cellular uptake assay), respectively, for predetermined time. After washing twice with washing buffer, the samples were collected and then resuspended in DPBS for flow cytometry.

Confocal Laser Scanning Microscopy (CLSM) Measurement.

For adhesive HCT116 cells, 1×10^5 cells were seeded in confocal dishes and cultured for 24 h, followed by incubation with Cy5-labeled samples (*Cy5*-Sgc8c, *Cy5*-Sgc8c-PSN_{40}, and *Cy5*-Libs-PSN_{40}), respectively, for 2 h at 37 °C. For suspended cells (CCRF-CEM or K562 cells), 2×10^5 cells were incubated with Cy5-labeled samples, respectively, for 2 h at 37 °C. Then, cells were washed with DPBS and imaged with confocal laser scanning microscope.

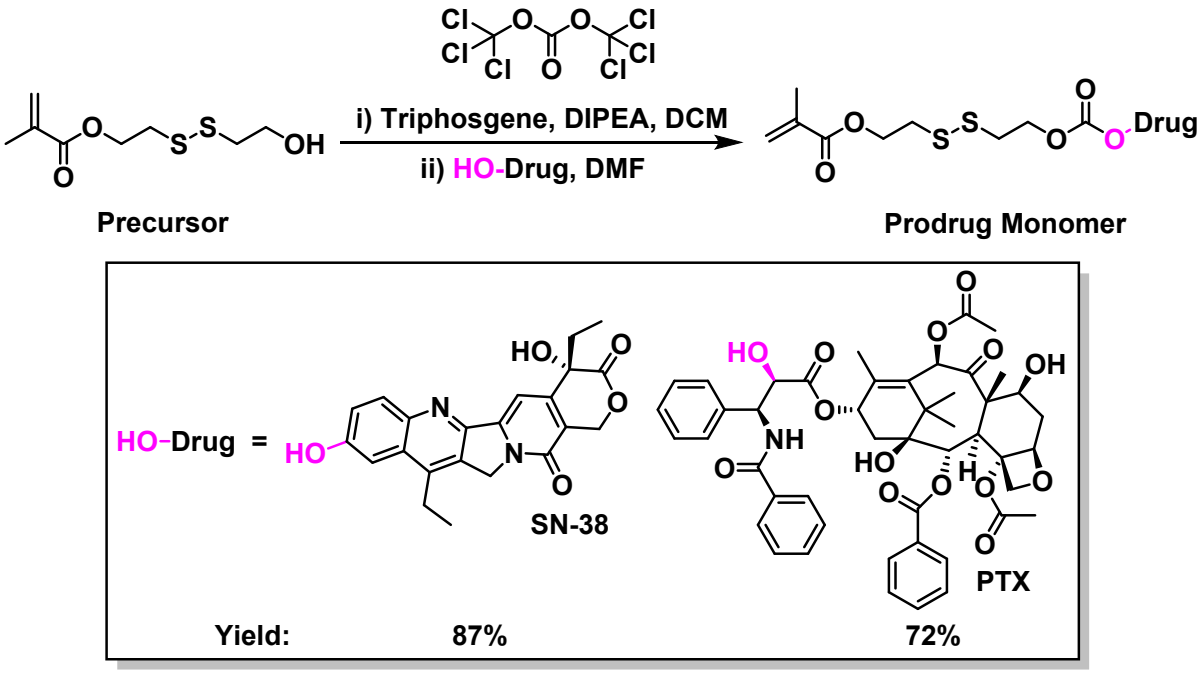
In Vivo Circulation Time Measurement

Sprague-Dawley (SD) rats (~120 g) were obtained from Hunan SJA Laboratory Animal Co., Ltd. and managed under protocols approved by the Hunan University Laboratory Animal Center. SD rats were divided into 3 groups, which were then treated with Cy5-labeled Sgc8c or Sgc8c-PSN_{40}, respectively, by single-bolus intravenous injection via the tail vein. At predetermined time points, blood samples were collected from retro-orbital plexus of the eye with heparin-coated tubes. The samples were then centrifuged to remove blood cells, followed by the fluorescence measurement of the supernatant solution to analyze the concentration of aptamer or ApPDC semi-quantitatively. The 100% of the fluorescence intensity was defined as the sample taken right after the injection, and the results were expressed as mean ± SD. Pharmacokinetic parameters (distribution phase half-life, $t_{1/2}(\alpha)$ and elimination phase half-life, $t_{1/2}(\beta)$) were analyzed using two-compartment compartmental analysis.

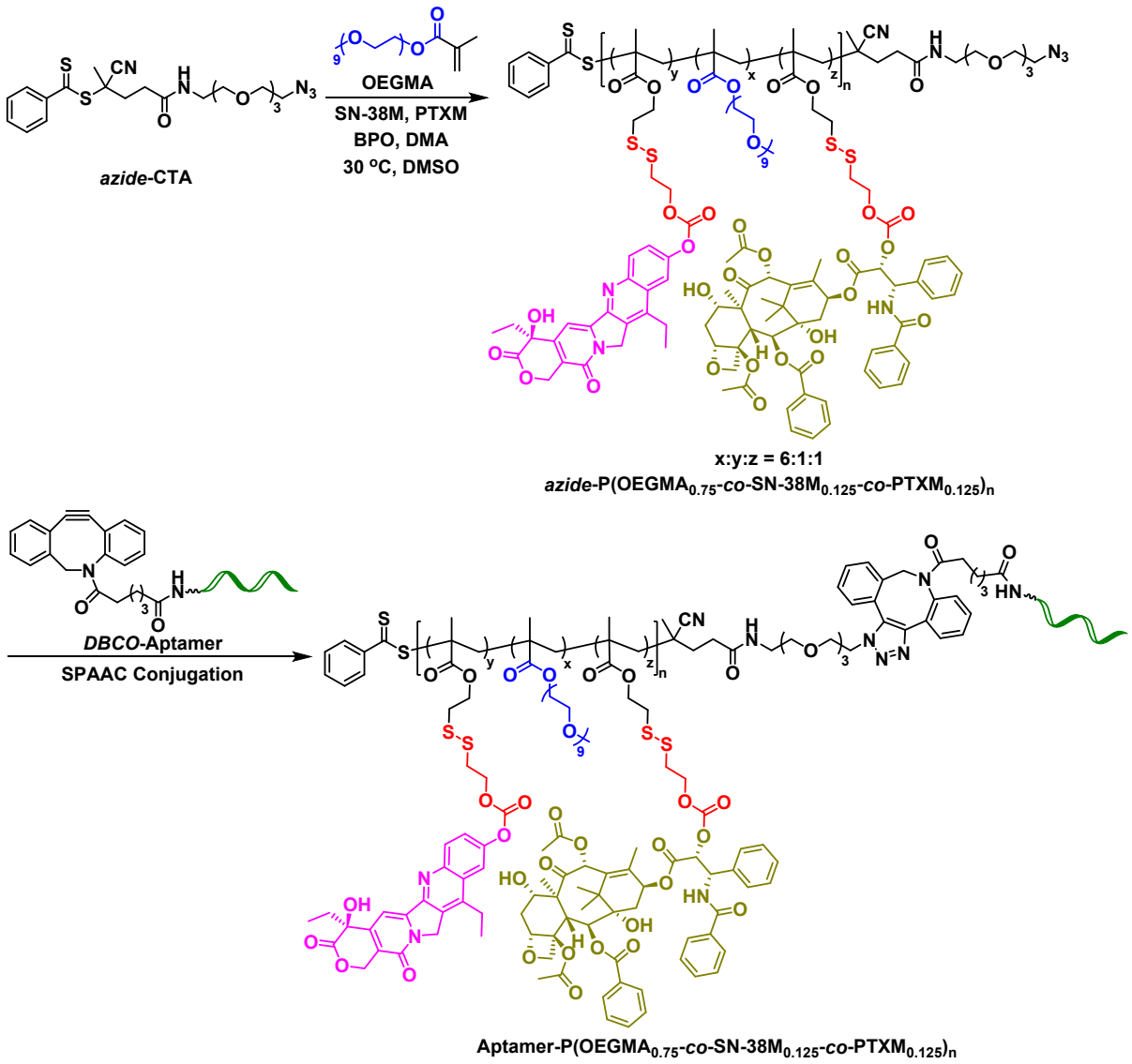
Characterization

All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz NMR spectrometer operated in the Fourier transform mode. $CDCl_3$ and DMSO-d_6 were used as the solvent. Chemical shifts (δ) were reported in ppm. Electrospray ionization mass spectrometry (ESI-MS) experiment was performed on Thermo Scientific LTQ Orbitrap Mass Spectrometer equipped with an electrospray interface. GPC was carried out on a Waters Breeze 2 GPC system equipped with two PLgel 5 μm MIXED-C, 300×7.5 mm columns and a RI detector. HPLC-grade DMF with 0.1% LiBr was used as the eluent, and samples were run at a flow rate of 1 mL/min and 60 °C. A series of low polydispersity polystyrene standards were employed for calibration. Fluorescence spectra were recorded on Fluoromax-4 spectrofluorometer (Horiba, JobinYvon, Edison, NJ). The slit widths were both set at 5 nm for excitation and

emission. All UV/Vis spectra were acquired on a UV-2450 double-beam UV/Vis spectrophotometer (Shimadzu, Japan). Dynamic light scattering (DLS) measurements were conducted on a commercial spectrometer Zetasizer Nano ZS90 (Malvern). Scattered light was collected at a fixed angle of 90^{o} for duration of ~3 min. All data were averaged over three consecutive measurements. Reversed-phase HPLC (RP-HPLC) analysis was performed on a Shimadzu HPLC system, equipped with a LC-20AP binary pump, a SPD-20A UV-Vis detector, and a Symmetry C18 column. Flow cytometry was performed with a BD FACSVerse™ or Cytek DxP Athena system. Confocal laser scanning microscopy (CLSM) images were acquired using a FV1000-X81 confocal microscope (Olympus).



Scheme S1. Synthetic routes for the preparation of polymerizable prodrug monomers, SN-38M and PTXM.



Scheme S2. Synthetic route for the construction of aptamer-polyprodrug conjugate containing two kinds of drug via combination of RAFT copolymerization and SPAAC.

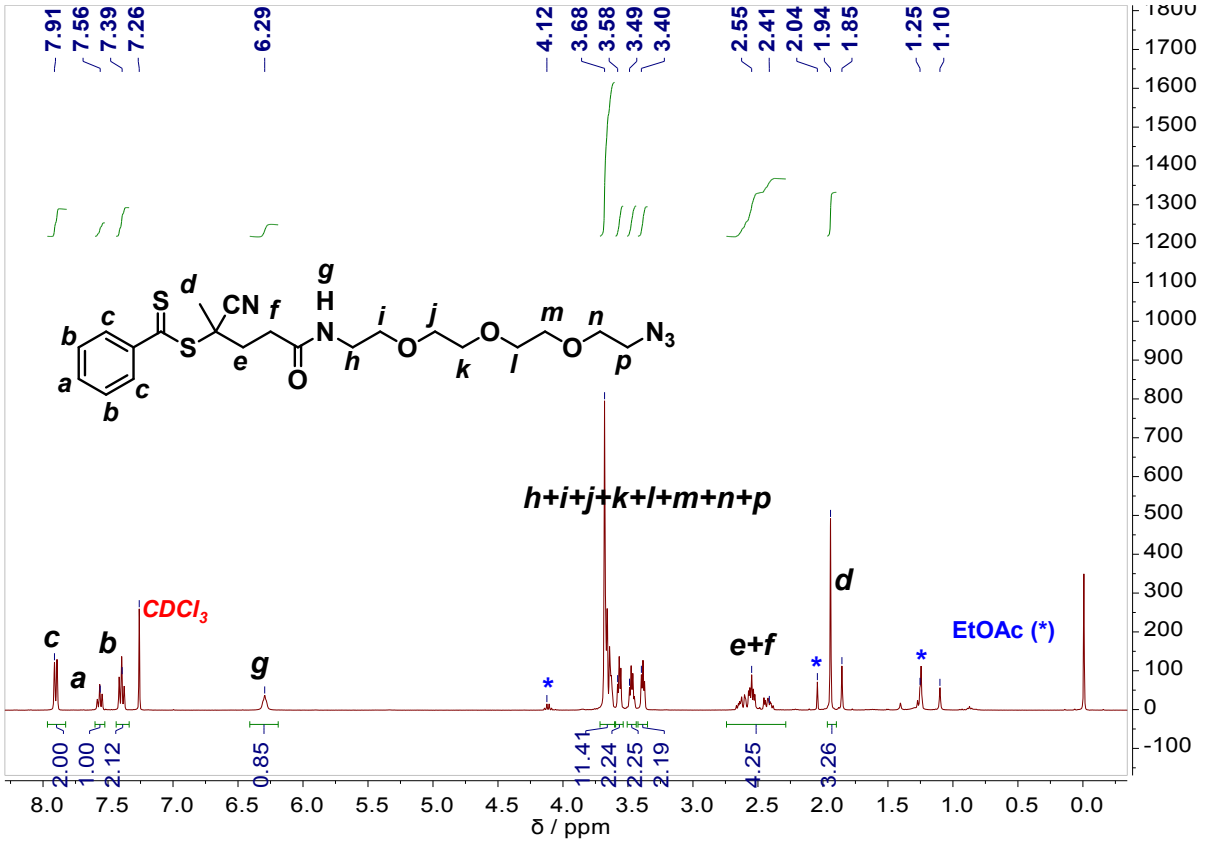


Figure S1. ^{1}H NMR spectrum of azide-modified chain transfer agent, *azide*-CTA in $CDCl_3$.

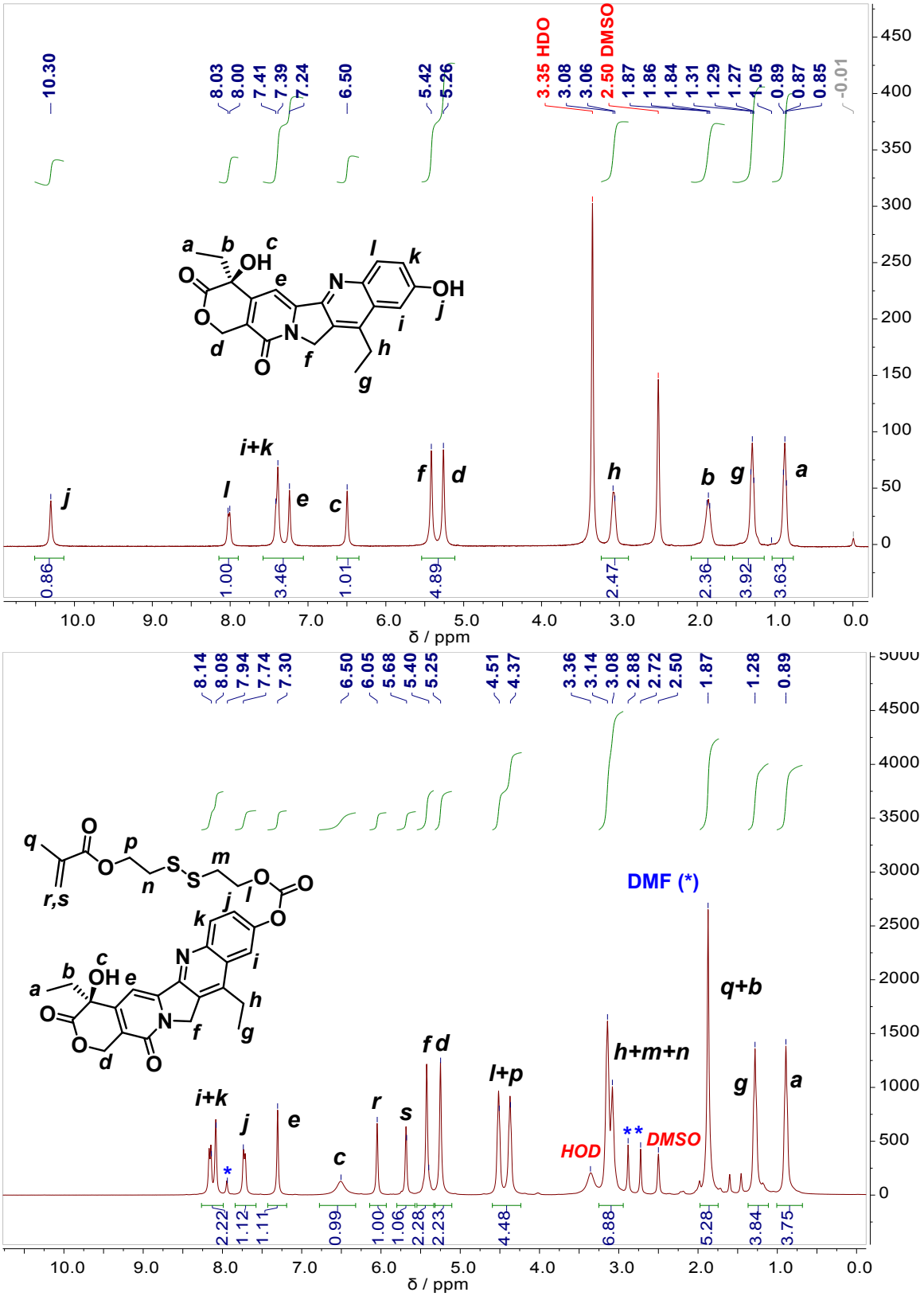


Figure S2. ^{1}H NMR spectra of SN-38 (upper) and SN-38M (lower) in DMSO-d_6.

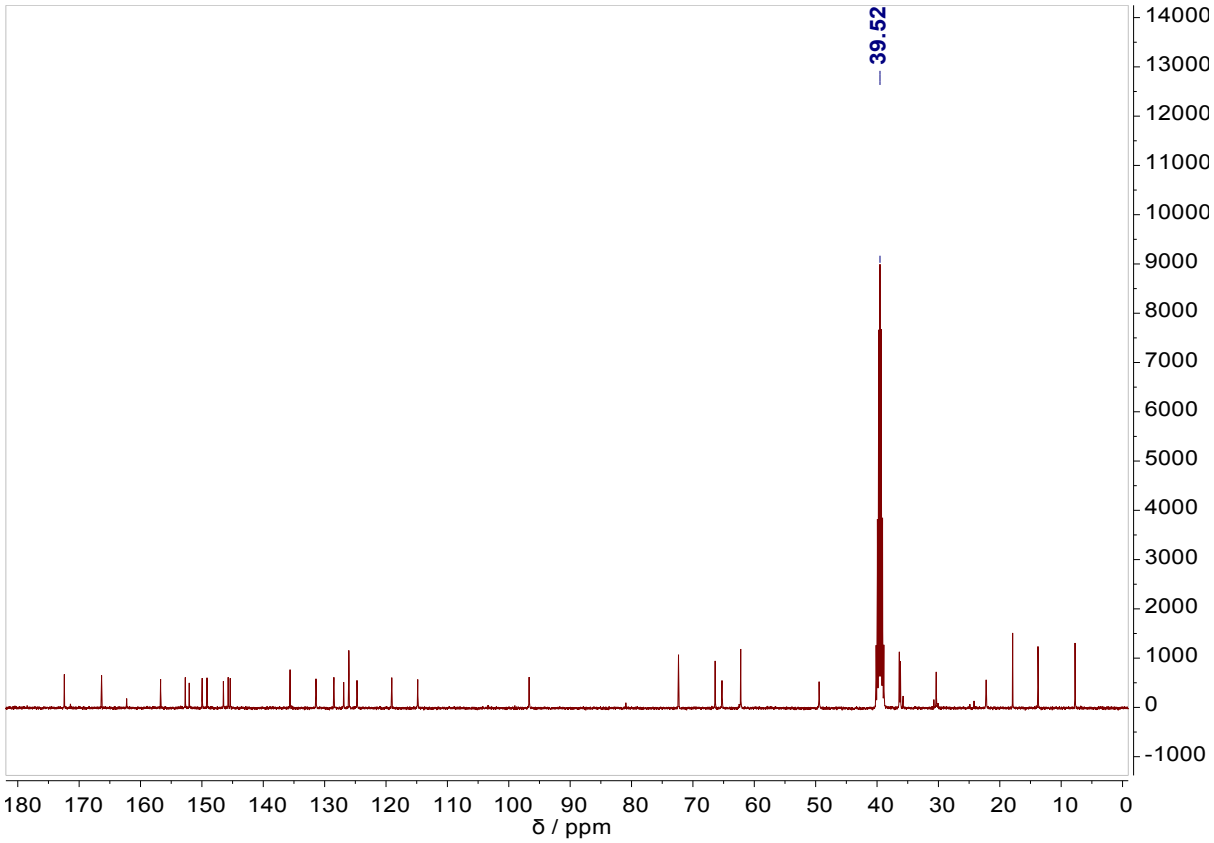


Figure S3. ^{13}C NMR spectrum of SN-38M in DMSO-d_6.

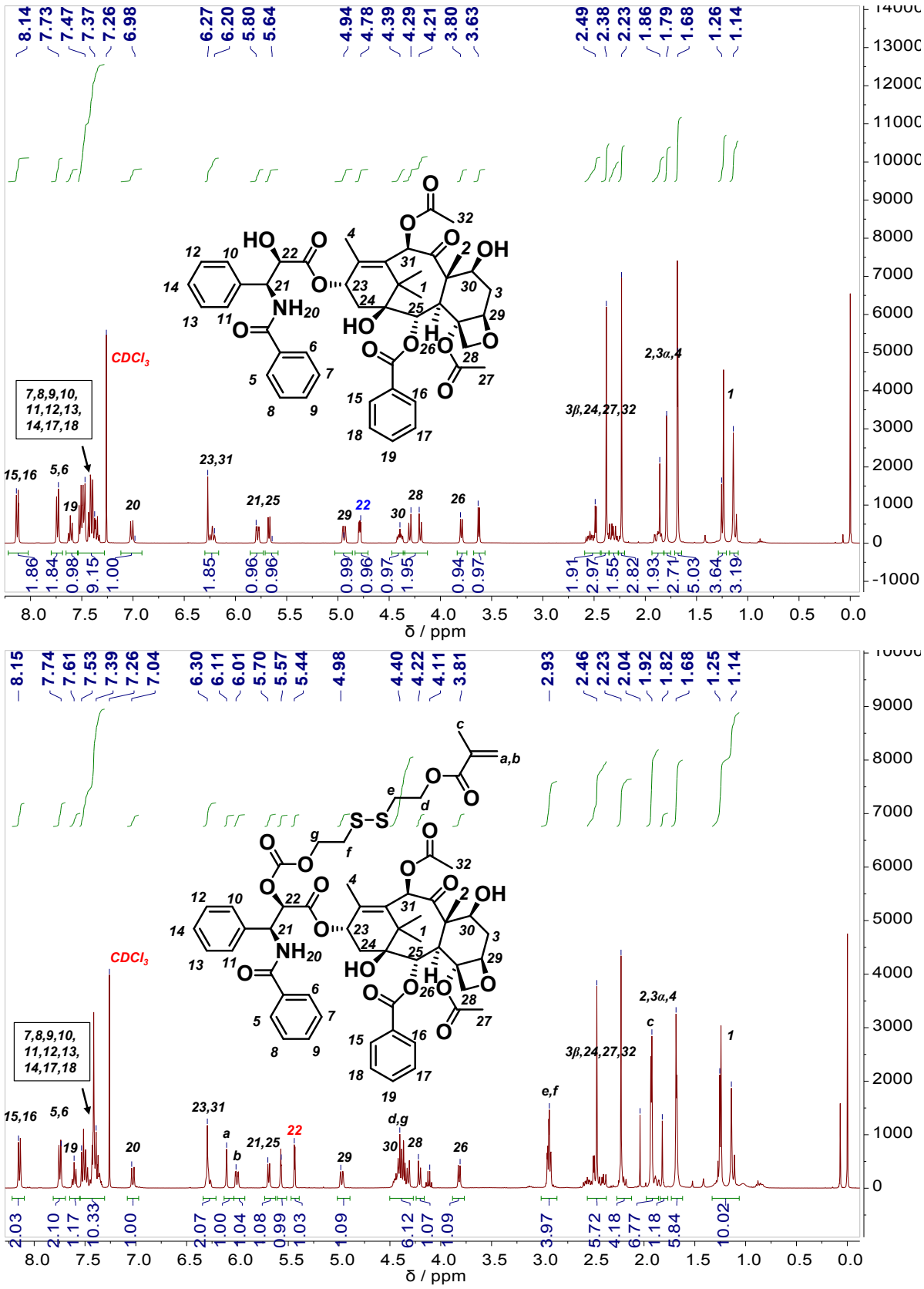


Figure S4. ^{1}H NMR spectra of PTX (upper) and PTXM (lower) in $CDCl_3$.

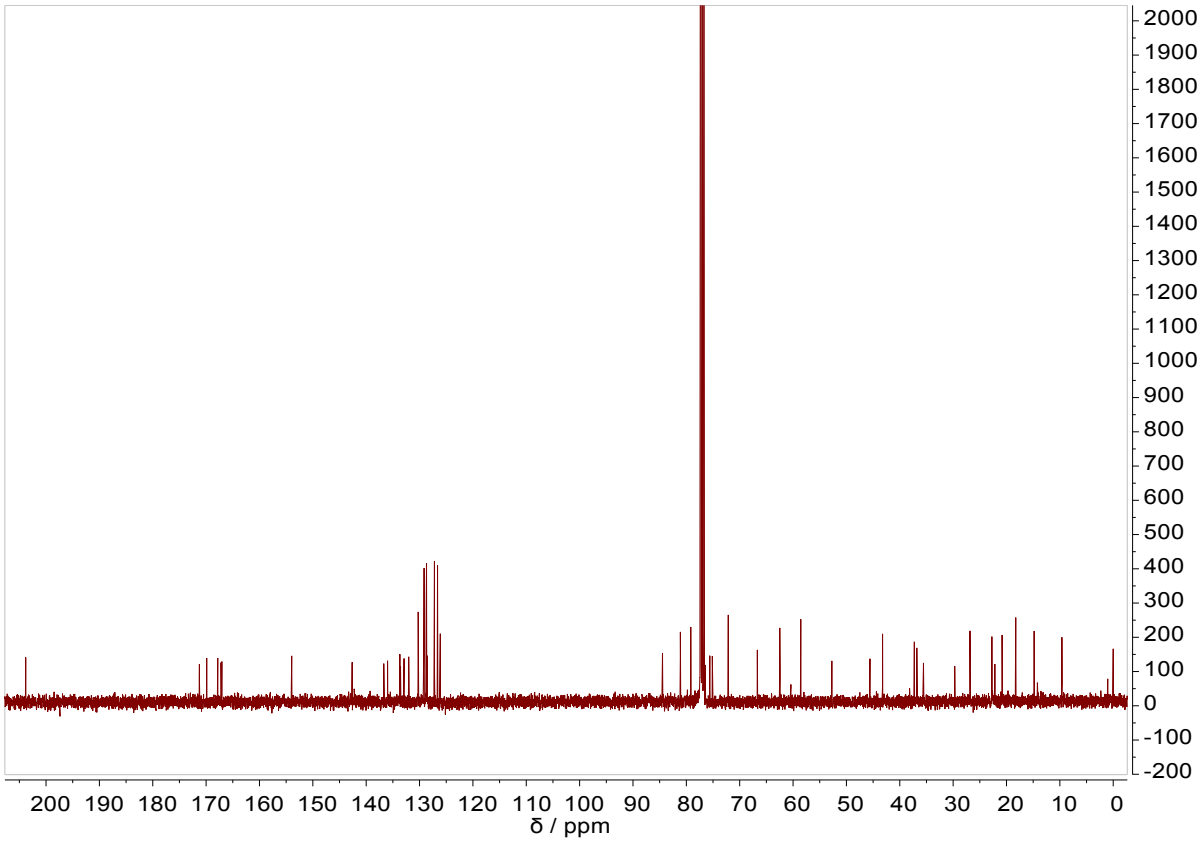


Figure S5. ^{13}C NMR spectrum of PTXM in $CDCl_3$.

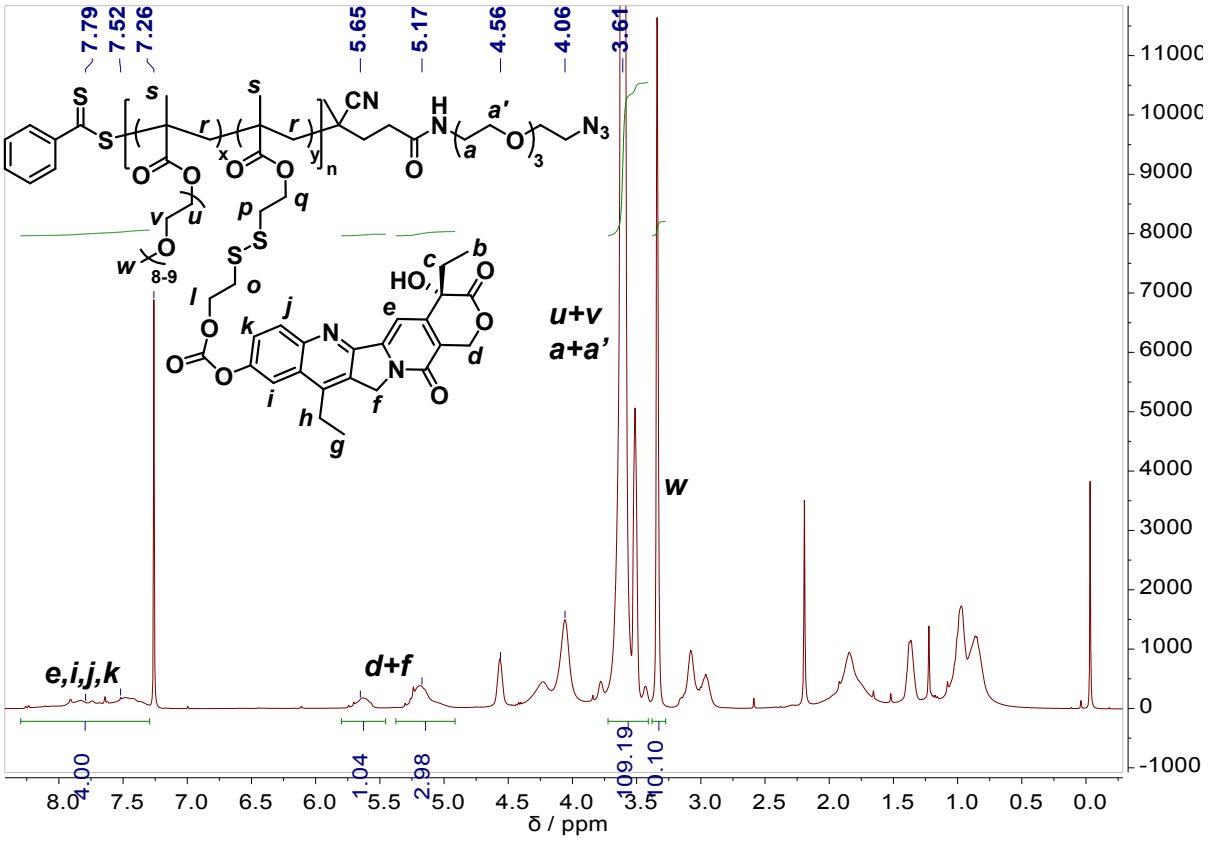


Figure S6. Typical ^{1}H NMR spectrum of polyprodrug, *azide*-P(OEGMA$_{0.75}$-*co*-SN-38M$_{0.25}$)$_{160}$ (*azide*-PSN$_{40}$) in $CDCl_3$.

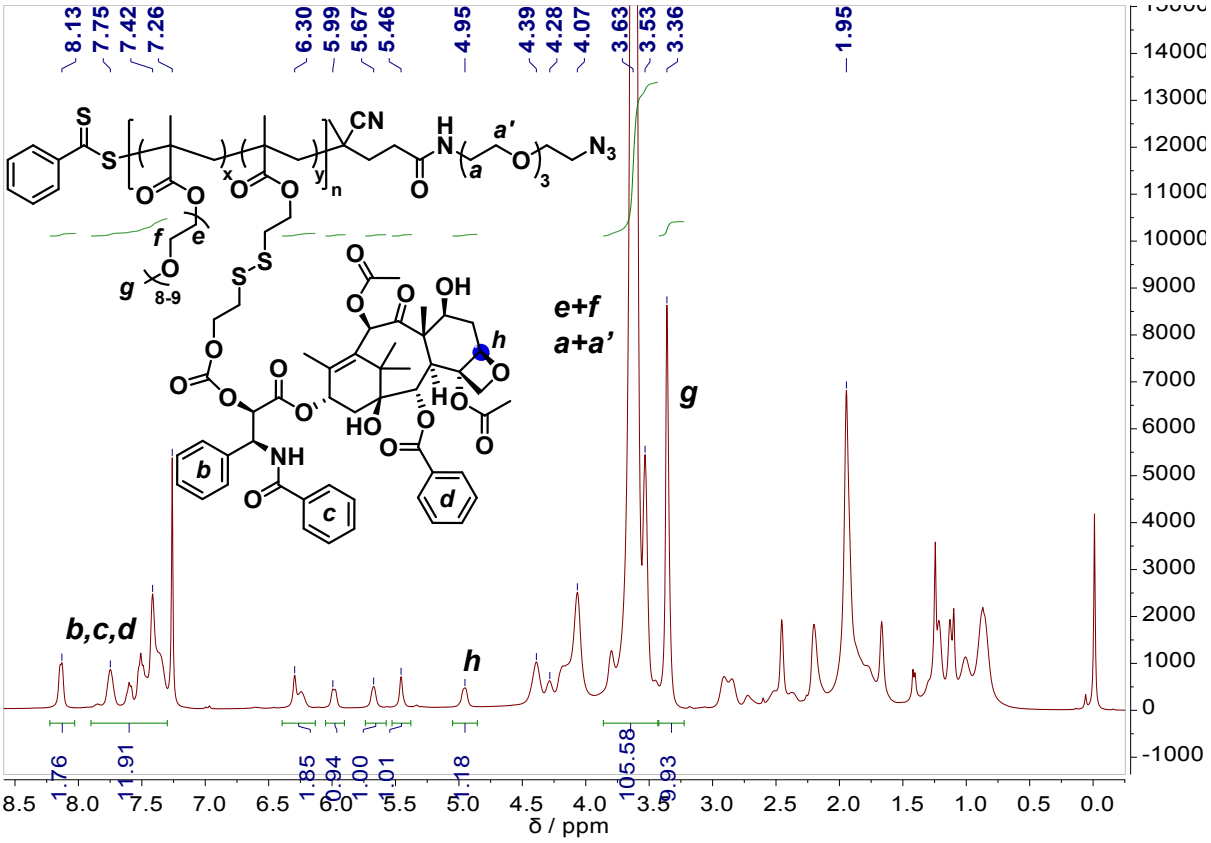


Figure S7. ^{1}H NMR spectrum of polyprodrug, *azide*-P(OEGMA$_{0.75}$-*co*-PTXM$_{0.25}$)$_{160}$ (*azide*-PTX$_{40}$) in $CDCl_3$.

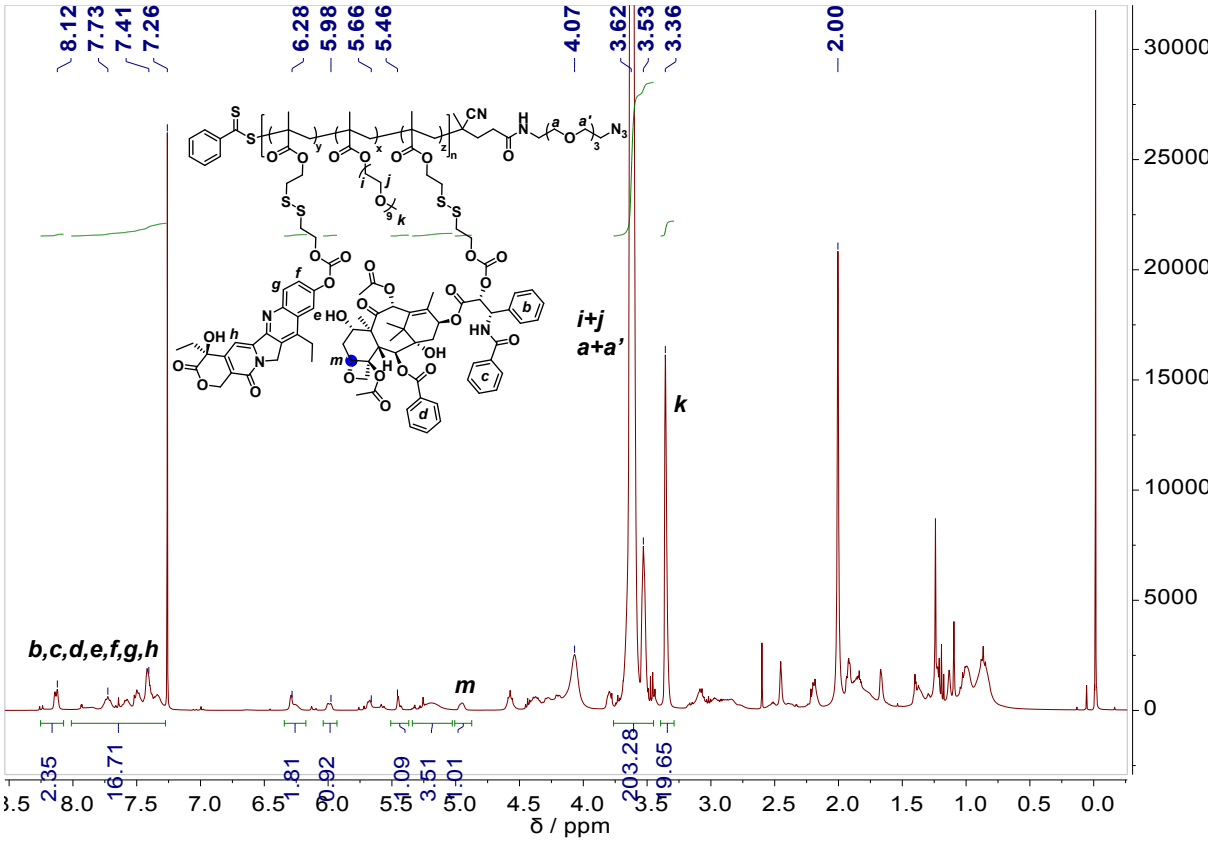


Figure S8. ^{1}H NMR spectrum of polyprodrug containing two kind of drugs, *azide*-P(OEGMA$_{0.75}$-*co*-SN-38M$_{0.125}$-*co*-PTXM$_{0.125}$)$_{320}$ (*azide*-P(SN$_{40}$-TX$_{40}$)) in $CDCl_3$.

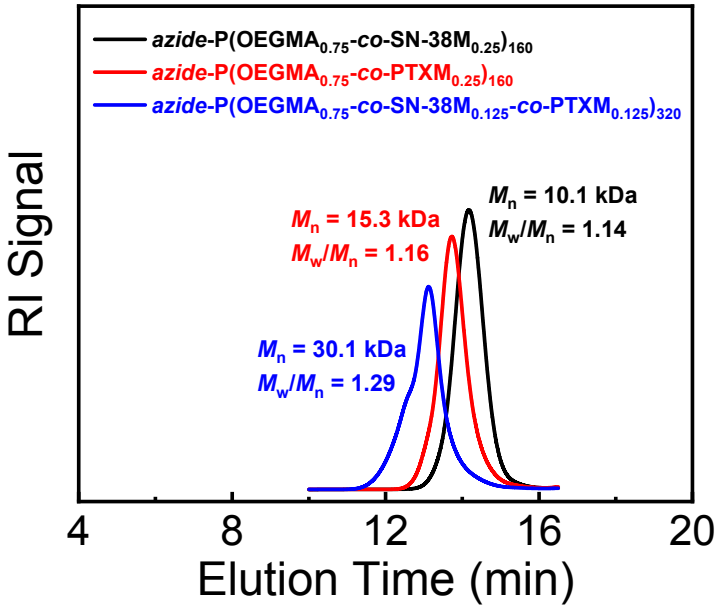


Figure S9. DMF GPC (1 mL/min) traces of as-synthesized polyprodrugs.

Table S1. Summary of Polyprodrugs Synthesized in This Study.

Code	Samples	$M_{n,\,GPC}$ (kDa)	M_w/M_n
azide-PSN$_{40}$	*azide*-P(OEGMA$_{0.75}$-*co*-SN-38M$_{0.25}$)$_{160}$	10.1	1.14
azide-PTX$_{40}$	*azide*-P(OEGMA$_{0.75}$-*co*-PTXM$_{0.25}$)$_{160}$	15.3	1.16
azide-P(SN$_{40}$-TX$_{40}$)	*azide*-P(OEGMA$_{0.75}$-*co*-SN-38M$_{0.125}$-*co*-PTXM$_{0.125}$)$_{320}$	30.1	1.29

Table S2. DNA Sequences Used in This Study.

Code	Sequence [a] (5'→3')
DBCO-Sgc8c	*DBCO*-TTT TTT **ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA**
DBCO-Sgc8c-*FAM*	*DBCO*-TTT TTT **ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-*FAM***
DBCO-Sgc8c-*Cy5*	*DBCO*-TTT TTT **ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-*Cy5***
DBCO-Libs	*DBCO*-TTT TTT NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NN
DBCO-Libs-*FAM*	*DBCO*-TTT TTT NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NN-***FAM***
DBCO-Libs-*Cy5*	*DBCO*-TTT TTT NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NN-***Cy5***
DBCO-XQ-2d-*FAM*	*DBCO*-TTT TTT **ACT CAT AGG GTT AGG GGC TGC TGG CCA GAT ACT CAG ATG GTA GGG TTA CTA TGA G-*FAM***
DBCO-Libx-*FAM*	*DBCO*-TTT TTT NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN N-***FAM***

[a] Bold letters indicate the aptamer sequence. N denote the random base for library sequence.

Table S3. Aptamer-PolyproDrug Conjugates (ApPDCs) and Control Samples Constructed in This Study.

Samples	DS [a]
Sgc8c-PSN_{40}	40
FAM-Sgc8c-PSN_{40}	40
Cy5-Sgc8c-PSN_{40}	40
Libs-PSN_{40}	40
FAM-Libs-PSN_{40}	40
Cy5-Libs-PSN_{40}	40
FAM-XQ2d-PSN_{40}	40
FAM-Libx-PSN_{40}	40
FAM-Sgc8c-PTX_{40}	40
FAM-Sgc8c-P(SN_{40}-TX_{40})	80

[a] Average cytotoxic drug stoichiometry (DS) within conjugates. For ApPDCs, DS also represents drug to aptamer ratio (DApR).

Figure S10. Agarose gel analysis (2%; 110 V for 60 min) for the conjugation reaction of DBCO-modified aptamer, *DBCO*-Sgc8c-*FAM* and *azide*-PSN_{40}, in DI water at 37 ^{o}C for 24 h. Lanes: 1, *DBCO*-Sgc8c-*FAM;* 2, *azide*-PSN_{40}; 3, crude mixture. The formation of a new band with green fluorescence emission in lane 3 was attributed to the aptamer-polyprodrug conjugate, *FAM*-Sgc8c-PSN_{40}.

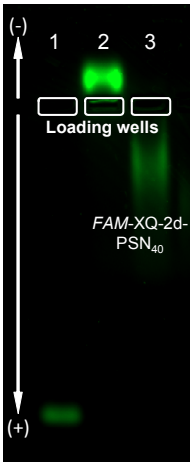


Figure S11. Agarose gel analysis (2%; 120 V for 60 min) of the purified aptamer-polyprodrug conjugate, *FAM*-XQ-2d-PSN_{40}. Lanes: 1, *DBCO*-XQ-2d-*FAM*; 2, *azide*-PSN_{40}; 3, purified *FAM*-XQ-2d-PSN_{40}. The disappearance of aptamer and polyprodrug bands verified the purity of *FAM*-XQ-2d-PSN_{40}. Note that XQ-2d DNA aptamer was selected against PL45 cells (pancreatic ductal adenocarcinoma cell line) with high affinity and specificity.[2]

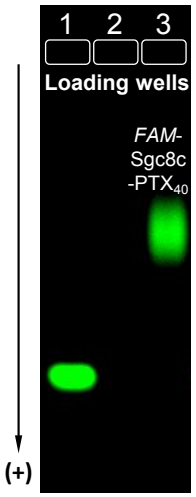


Figure S12. Agarose gel analysis (2%; 110 V for 60 min) for the conjugation reaction of *DBCO*-Sgc8c-*FAM* and *azide*-PTX_{40}, in water at 37 °C for 24 h. Lanes: 1, *DBCO*-Sgc8c-*FAM*; 2, *azide*-PTX_{40}; 3, crude mixture. The formation of a new band with green emission in lane 3 was attributed to the aptamer-polyprodrug conjugate, *FAM*-Sgc8c-PTX_{40}.

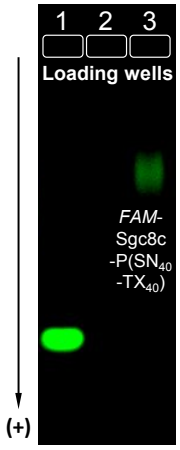


Figure S13. Agarose gel analysis (2%; 110 V for 60 min) for the conjugation reaction of *DBCO*-Sgc8c-*FAM* and *azide*-P(SN_{40}-TX_{40}), in water at 37 °C for 24 h. FAM-Sgc8c-PTX40. Lanes: 1, *DBCO*-Sgc8c-*FAM*; 2, *azide*-P(SN_{40}-TX_{40}); 3, crude mixture. The formation of a new band with green emission in lane 3 was attributed to the aptamer-polyprodrug conjugate, *FAM*-Sgc8c-P(SN_{40}-TX_{40}).

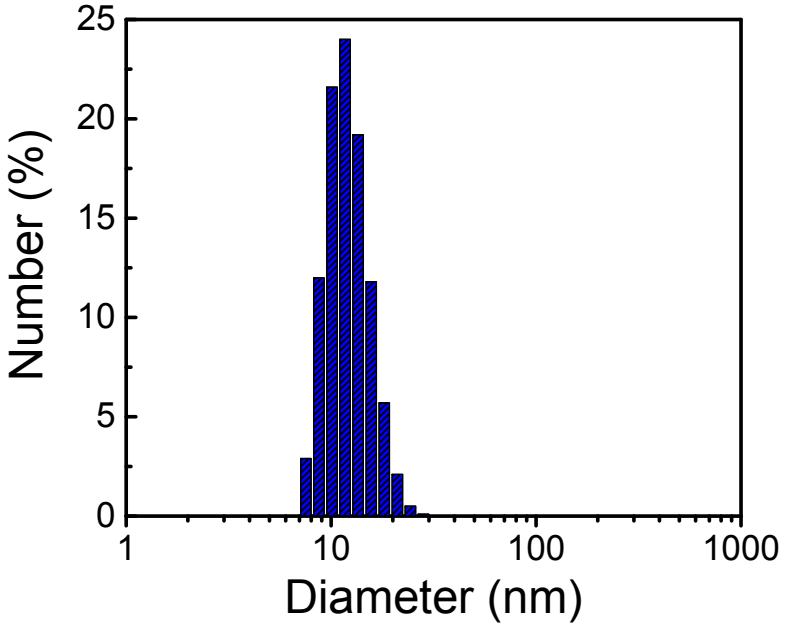
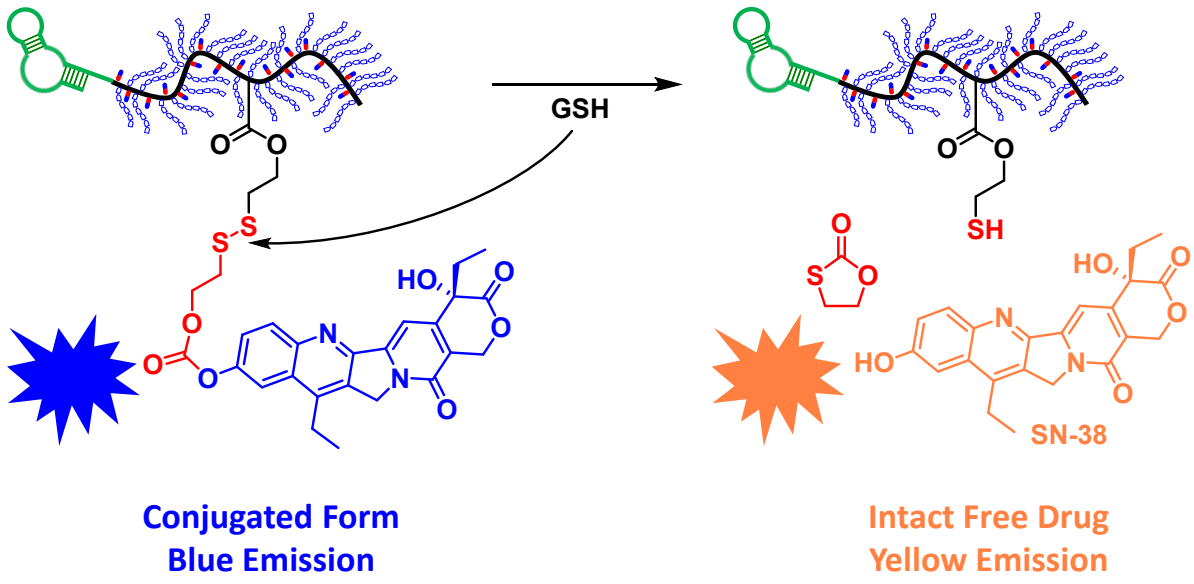


Figure S14. DLS data of *FAM*-Sgc8c-PSN_{40} (DNA concentration was 1 μM) in PBS buffer (1 mM).



Scheme S3. Schematic illustration of GSH-triggered intact SN-38 release from Sgc8c-PSN$_{40}$, leading to changes of fluorescent emission.

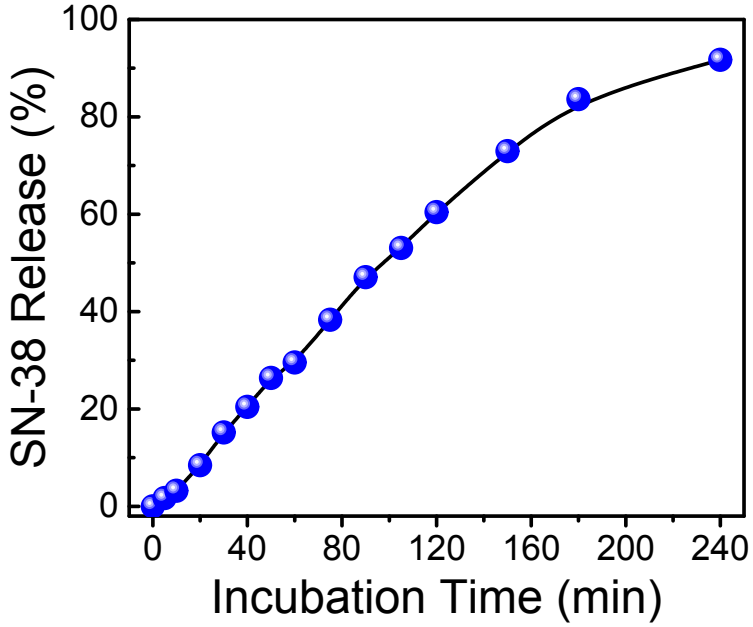


Figure S15. Cumulative release profile of SN-38 drug from Sgc8c-PSN_{40} (DNA concentration was 1 μM) upon incubation with GSH (10 mM) in PBS buffer (20 mM, pH = 7.4; 37 ^{o}C).

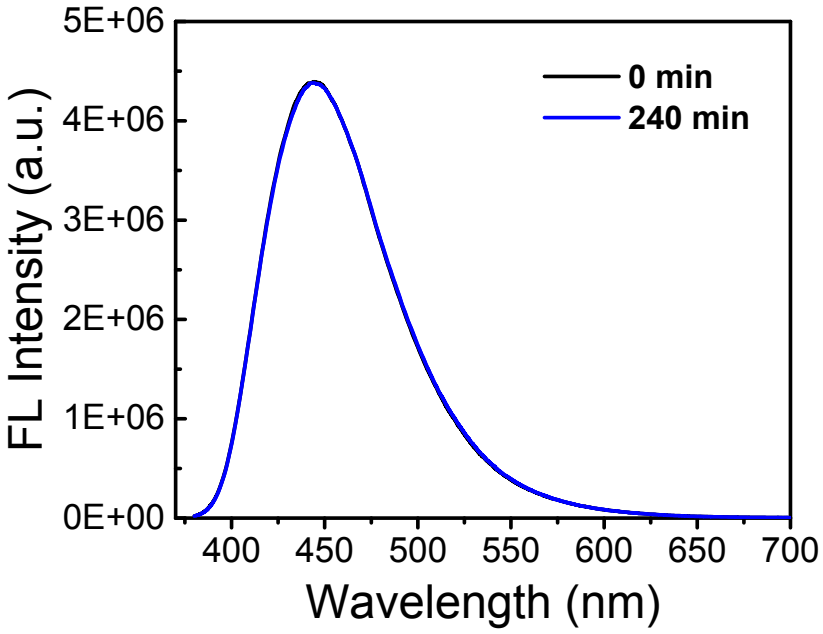


Figure S16. Variations of fluorescence spectra (λ_{ex} = 365 nm, slit width = 5 nm/5 nm, 37 °C) of Sgc8c-PSN_{40} (DNA concentration was 1 μM) without GSH in PBS buffer (20 mM, pH = 7.4).

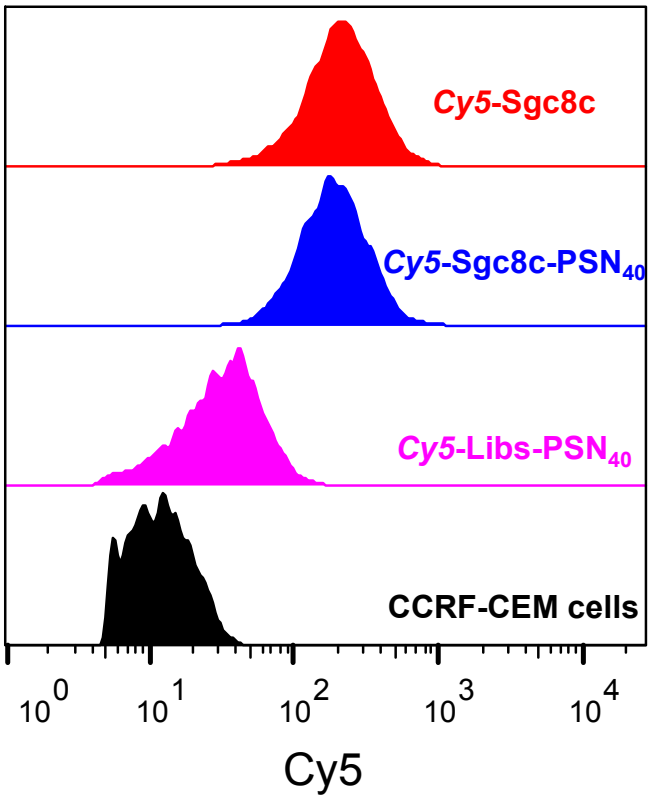


Figure S17. Flow cytometry measurements (total cell counts: 10,000) of CCRF-CEM cells upon incubation with *Cy5*-Sgc8c, *Cy5*-Sgc8c-PSN_{40}, and *Cy5*-Libs-PSN_{40}, respectively, for 1 h at 4 ^{o}C. The DNA concentration of all the samples was fixed at 500 nM.

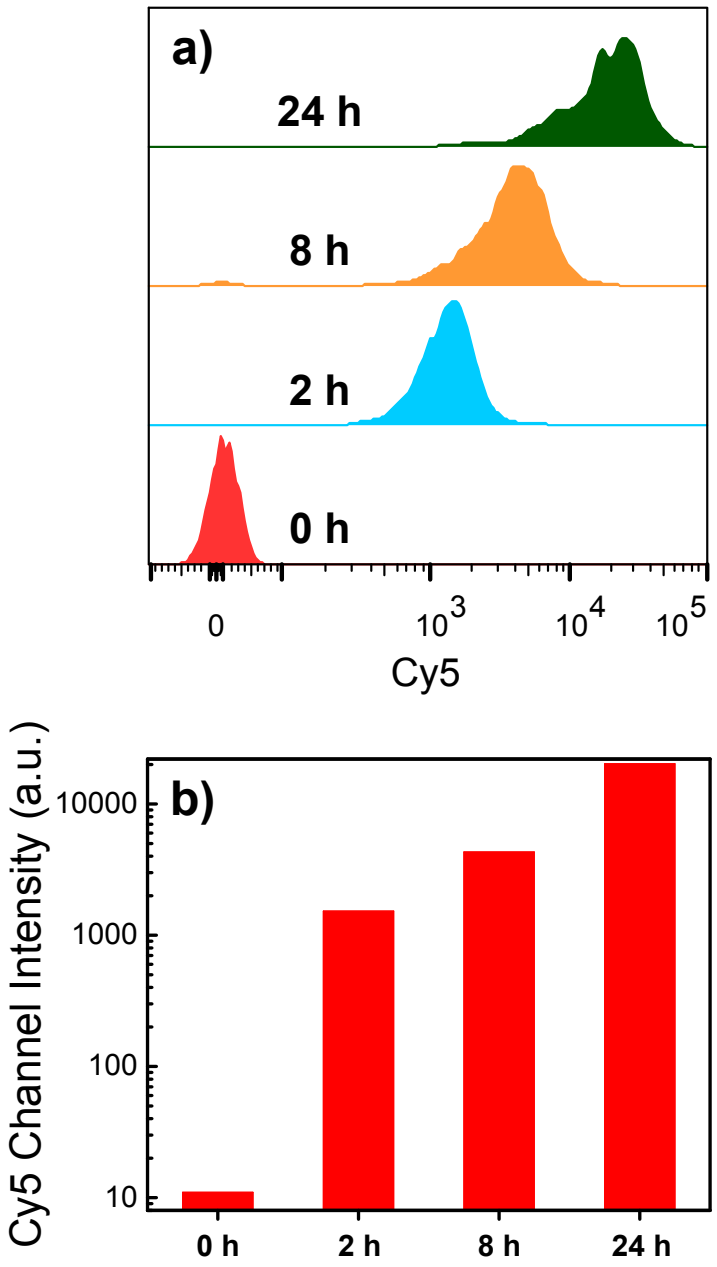


Figure S18. Flow cytometry measurements (total cell counts: 10,000; Cy5 channel) of HCT116 cells upon incubation with *Cy5*-Sgc8c-PSN_{40} for different time at 37 °C. The DNA concentration was fixed at 500 nM.

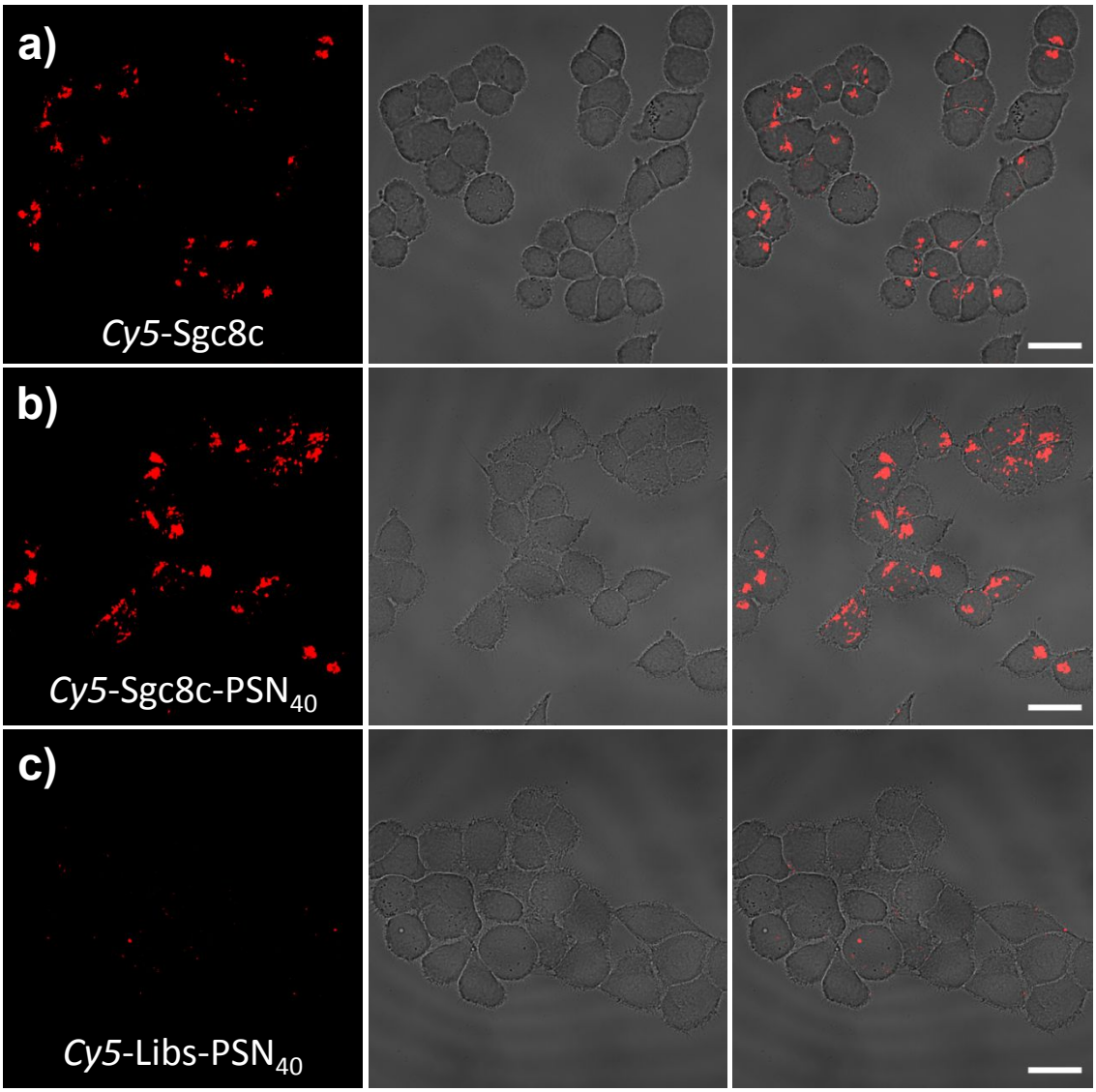


Figure S19. Representative CLSM images (scale bar = 20 μm) of HCT116 cells upon incubation with (a) *Cy5*-Sgc8c, (b) *Cy5*-Sgc8c-PSN_{40}, and (c) *Cy5*-Libs-PSN_{40} for 2 h at 37 °C. The DNA concentration of all the samples was fixed at 500 nM.

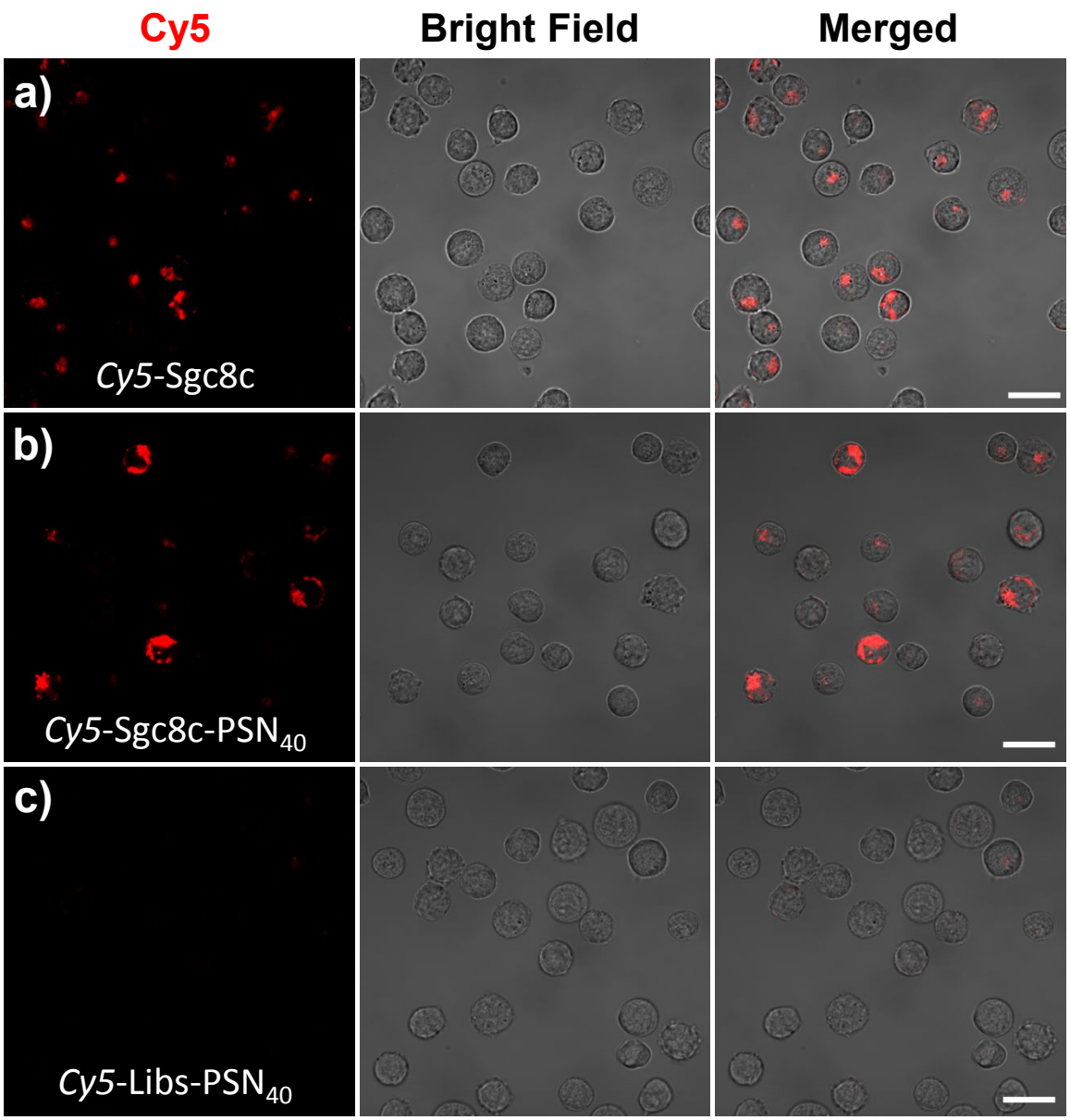


Figure S20. Representative CLSM images (scale bar = 20 μm) of CCRF-CEM cells upon incubation with (a) *Cy5*-Sgc8c, (b) *Cy5*-Sgc8c-PSN_{40}, and (c) *Cy5*-Libs-PSN_{40} for 2 h at 37 °C. The DNA concentration of all the samples was fixed at 500 nM.

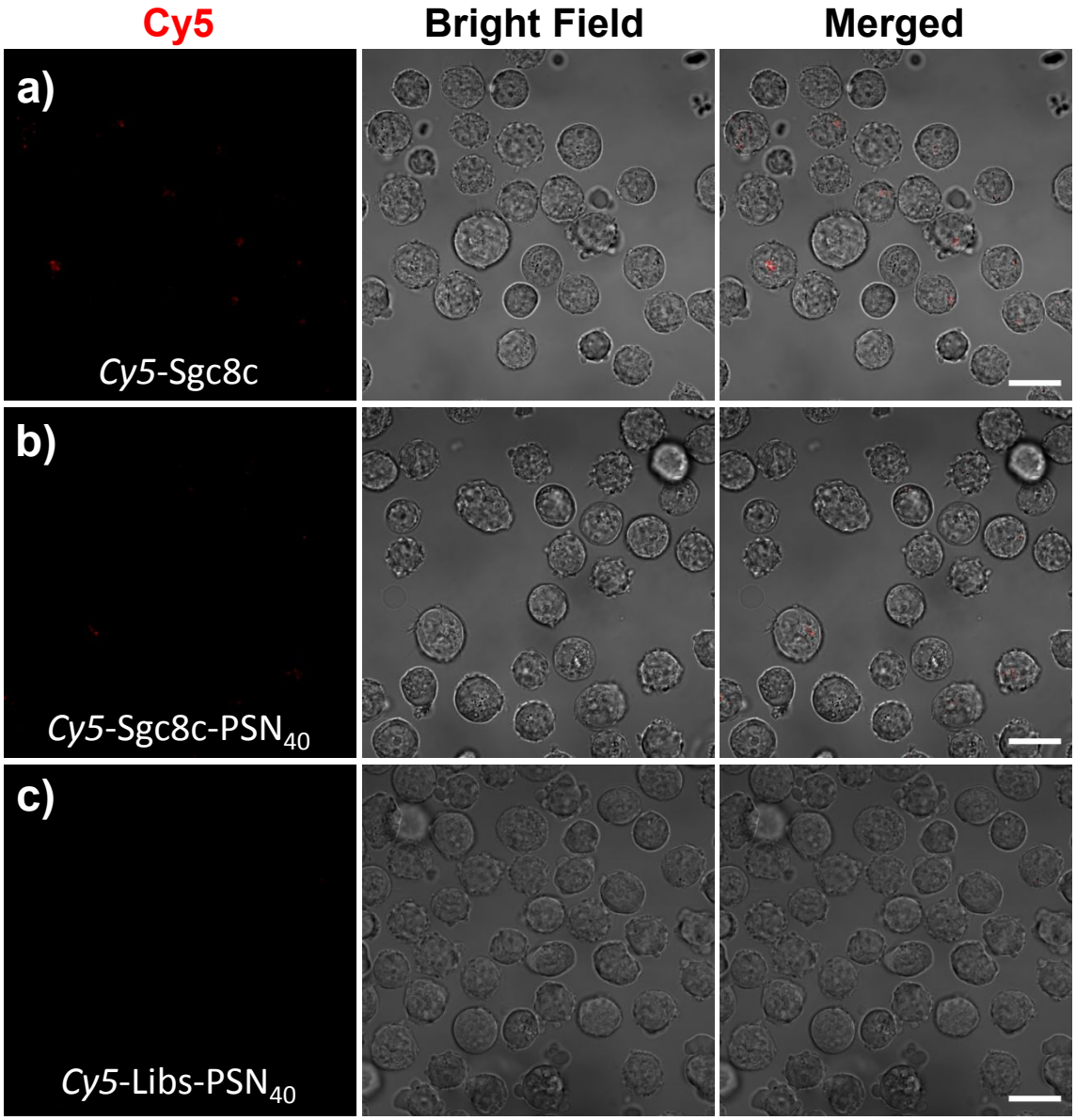


Figure S21. Representative CLSM images (scale bar = 20 μm) of K562 cells upon incubation with (a) *Cy5*-Sgc8c, (b) *Cy5*-Sgc8c-PSN_{40}, and (c) *Cy5*-Libs-PSN_{40} for 2 h at 37 °C. The DNA concentration of all the samples was fixed at 500 nM.

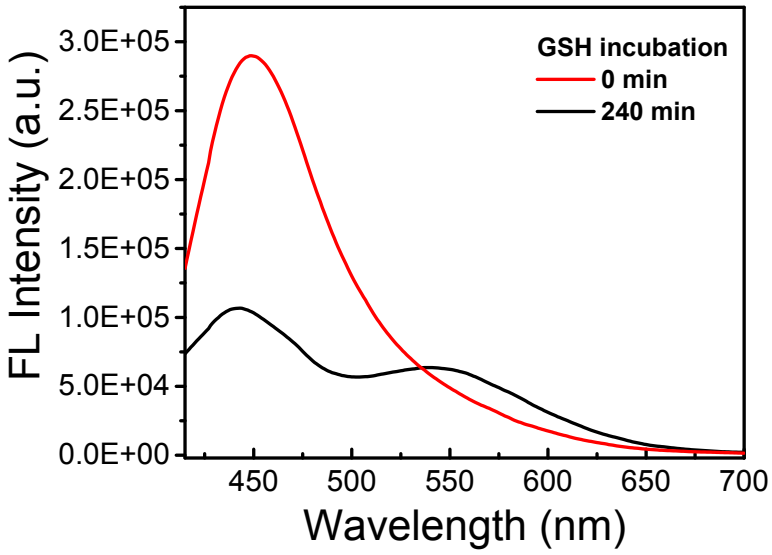


Figure S22. Fluorescence spectra (λ_{ex} = 407 nm, slit width = 5/5, 37 °C) of Sgc8c-PSN$_{40}$ (1 μM of DNA) upon incubation with GSH (10 mM) in DPBS buffer (20 mM, pH = 7.4) for 0 and 240 min.

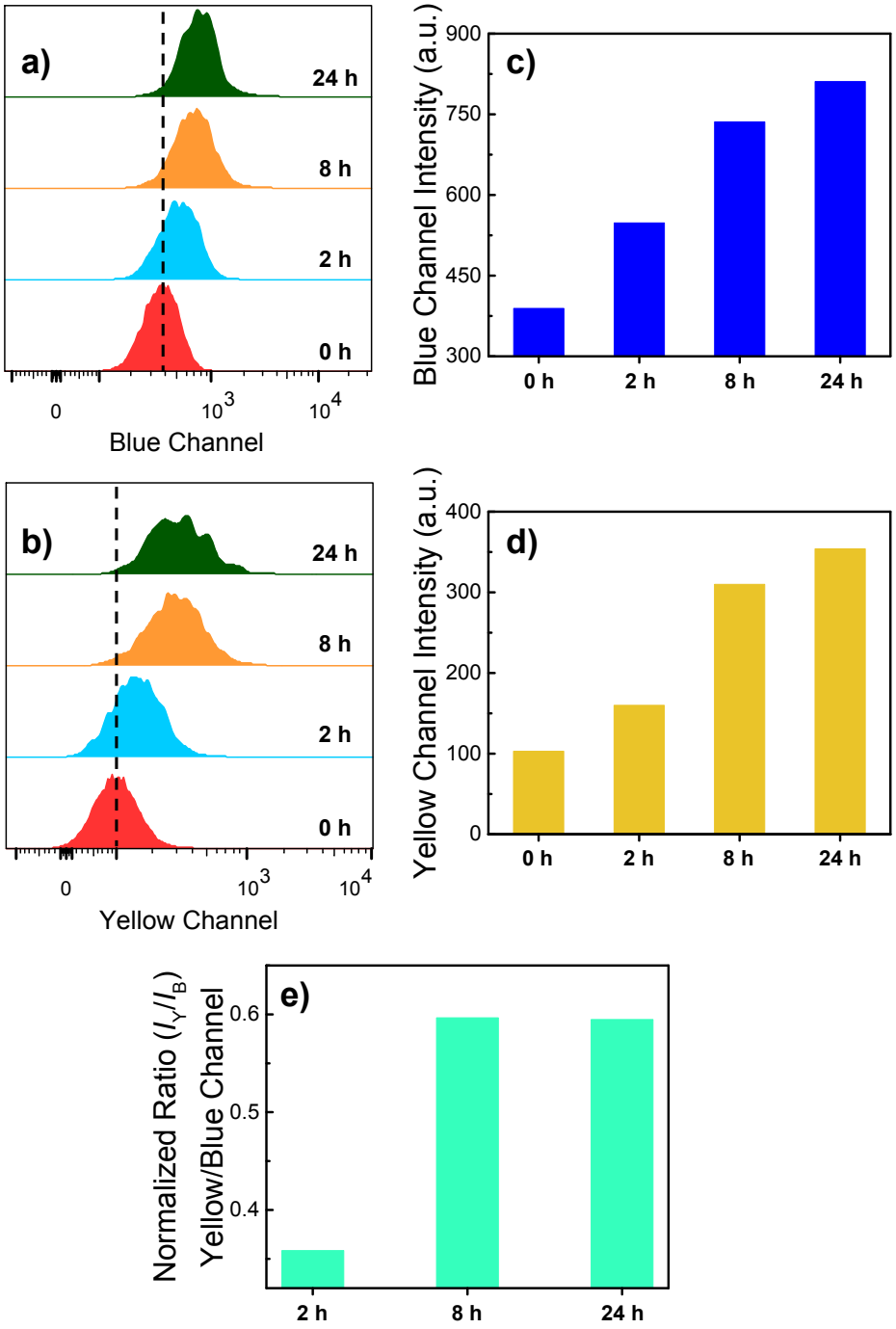


Figure S23. Flow cytometry measurements (total cell counts: 10,000; blue and yellow channels) of HCT116 cells upon incubation with *Cy5*-Sgc8c-PSN$_{40}$ for different time at 37 °C. (a-d) The excitation wavelength was 407 nm and fluorescence filters were 450 ± 25 nm (a,b; blue channel) and 525 ± 25 nm (c,d; yellow channel). The DNA concentration was fixed at 500 nM. (e) Normalized ratio of mean fluorescence intensity of yellow and blue channel.

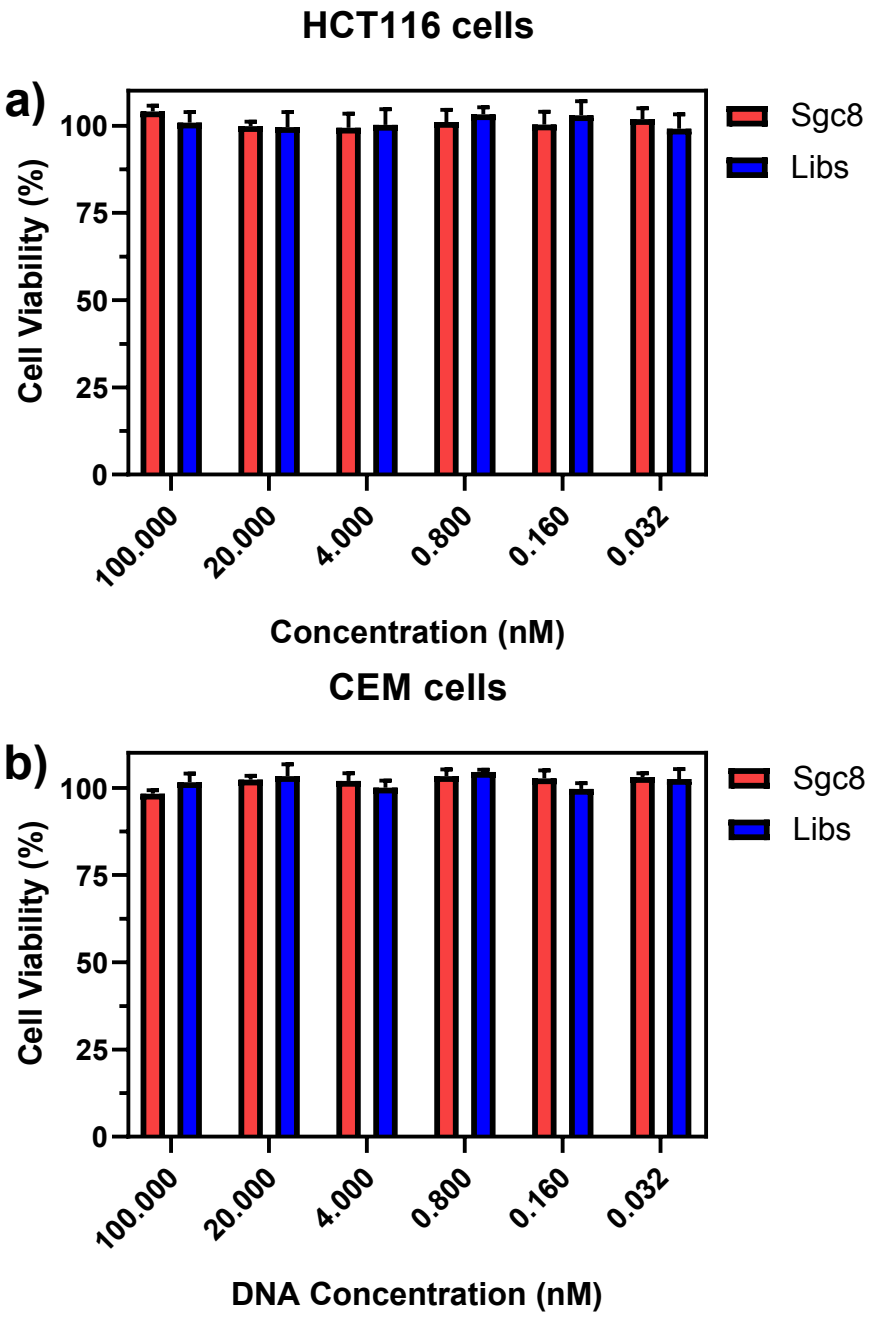


Figure S24. Relative cell viabilities of *DBCO*-Sgc8c and *DBCO*-Libs against (a) HCT116 and (b) CCRF-CEM cells for 72 h.

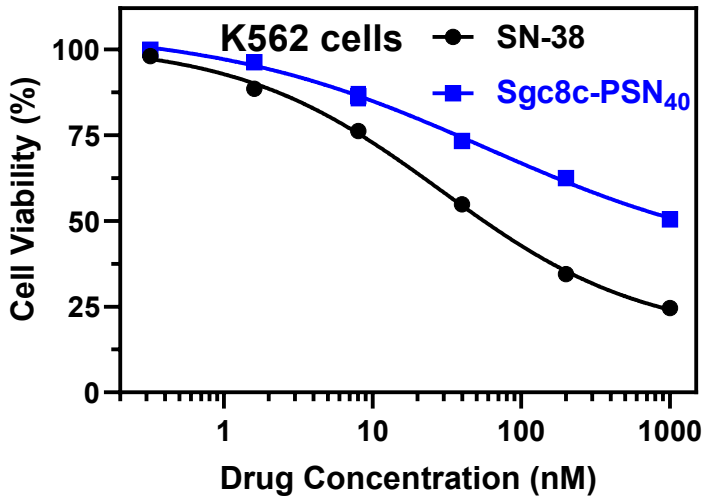


Figure S25. Relative cell viabilities of SN-38 and Sgc8c-PSN_{40} against K562 cells for 72 h.

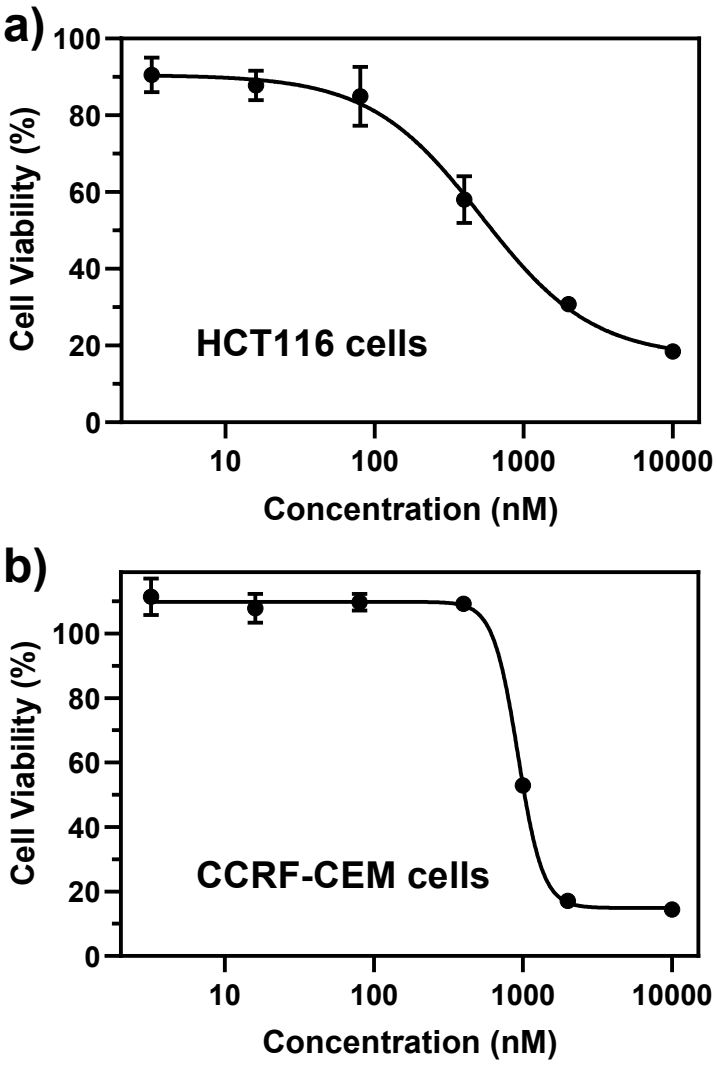


Figure S26. Relative cell viabilities of SN-38 prodrug, irinotecan, against (a) HCT116 and (b) CCRF-CEM cells for 72 h.

(1) Hu, X., Hu, J., Tian, J., Ge, Z., Zhang, G., Luo, K., and Liu, S. (2013) Polyprodrug amphiphiles: hierarchical assemblies for shape-regulated cellular internalization, trafficking, and drug delivery. *J. Am. Chem. Soc. 135*, 17617-17629.
(2) Wu, X., Zhao, Z., Bai, H., Fu, T., Yang, C., Hu, X., Liu, Q., Champanhac, C., Teng, I. T., Ye, M., et al. (2015) DNA Aptamer Selected against Pancreatic Ductal Adenocarcinoma for in vivo Imaging and Clinical Tissue Recognition. *Theranostics 5*, 985-994.