Supplementary Material

Polylactide-tethered prodrugs in polymeric nanoparticles as reliable nanomedicines for the efficient eradication of patient-derived hepatocellular carcinoma

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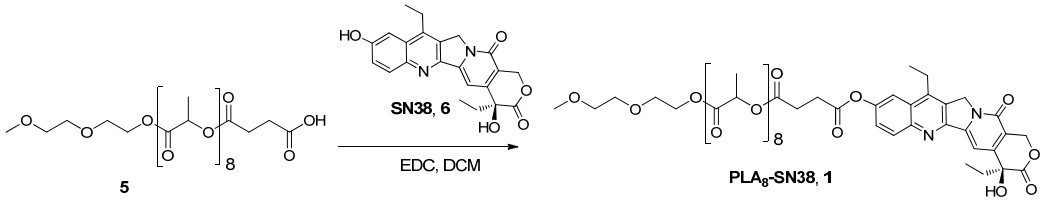
General methods for organic synthesis

All reactions were performed in a dry atmosphere. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} pre-coated aluminium sheets (Merck) and visualized by fluorescence quenching. Chromatographic purification was accomplished using flash column chromatography on silica gel (neutral, Qingdao Haiyang Chemical Co., Ltd). ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker 400 (400 MHz) spectrometer and calibrated to the residual solvent peak or tetramethylsilane (= 0 ppm). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, br = broad.

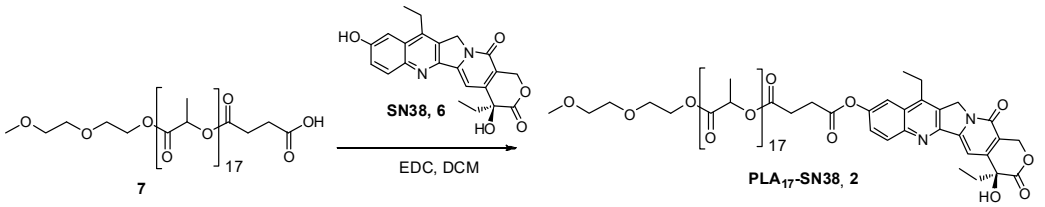
Synthesis of oligolactide (n = 8)-SN38 prodrug conjugate 1



To a solution of **oLA** (n = 8)-succinic acid (213 mg, 0.26 mmol) and SN38 (100 mg, 0.26 mmol) in 8 mL of anhydrous dichloromethane (DCM) were added EDC (59 mg, 0.38 mmol). The reaction mixture was stirred at 50ºC for 5 h and then washed with 5% citric acid, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 120:1) to give compound **1** (150 mg, 48%).

^{1}H NMR (400 MHz, $CDCl_3$): δ1.00-1.01 (t, 3H), 1.38-1.42 (t, 3H), 1.58 (s, 24H), 1.88-1.92 (q, 2H), 2.89-2.92 (t, 2H), 2.98-3.03 (m, 2H), 3.13-3.19 (q, 2H), 3.38 (s, 3H), 3.53-3.56 (t, 2H), 3.61-3.64 (t, 2H), 3.68-3.70 (t, 2H), 4.25-4.33 (m, 2H), 5.15-5.22 (m, 8H), 5.27 (s, 2H), 5.36 (s, 1H), 5.74-5.78 (d, 1H, J = 16.4), 7.57-7.59 (t, 1H), 7.65 (s, 1H), 7.85-7.85 (d, 1H, J = 2.4), 8.22-8.25 (d, 1H, J = 9.2).

Synthesis of polylactide (n = 17)-SN38 prodrug conjugate 2

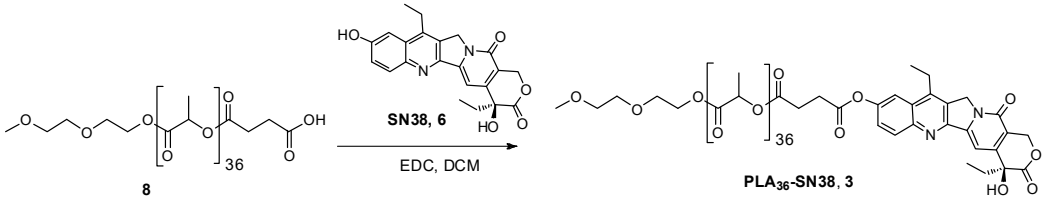


To a solution of **PLA** (n=17)-succinic acid (369 mg, 0.26 mmol) and SN38 (100 mg, 0.26 mmol) in 13 mL of anhydrous DCM were added EDC (59 mg, 0.38 mmol). The reaction mixture was stirred at 50ºC for 5 h and then washed with 5% citric acid, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by

flash column chromatography on silica gel (DCM:MeOH = 120:1) to give compound **2** (266 mg, 57%).

^{1}H NMR (400 MHz, $CDCl_3$): δ1.02-1.06 (t, 3H), 1.38-1.42 (t, 3H), 1.57-1.61 (m, 51H), 1.88-1.92 (q, 2H), 2.89-2.94 (m, 2H), 2.98-3.07 (m, 2H), 3.13-3.18 (q, 2H), 3.38 (s, 3H), 3.54-3.56 (q, 2H), 3.63-3.65 (q, 2H), 3.68-3.70 (t, 2H), 4.25-4.33 (m, 2H), 5.15-5.22 (m, 17H), 5.27 (s, 2H), 5.33 (s, 1H), 5.73-5.77 (d, 1H, *J* = 16.0), 7.55-7.58 (q, 1H), 7.66 (s, 1H), 7.84-7.84 (d, 1H, *J* = 2.4), 8.22-8.24 (d, 1H, *J* = 9.2).

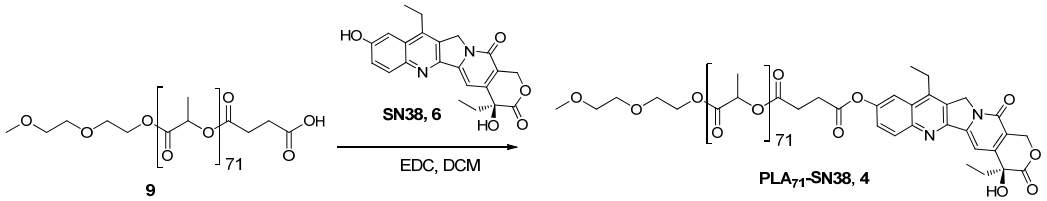
Synthesis of polylactide (n = 36)-SN38 prodrug conjugate 3



To a solution of **PLA** (n = 36)-succinic acid (564 mg, 0.20 mmol) and SN38 (79 mg, 0.20 mmol) in 15 mL of anhydrous DCM were added EDC (47 mg, 0.30 mmol). The reaction mixture was stirred at 50ºC for 5 h and then washed with 5% citric acid, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 120:1) to give compound **3** (404 mg, 48%).

^{1}H NMR (400 MHz, $CDCl_3$): δ1.02-1.06 (t, 3H), 1.38-1.42 (t, 3H), 1.53-1.61 (m, 108H), 1.86-1.92 (m, 2H), 2.89-2.92 (m, 2H), 2.98-3.03 (m, 2H), 3.13-3.19 (q, 2H), 3.38 (s, 3H), 3.53-3.56 (q, 2H), 3.63-3.65 (t, 2H), 3.68-3.70 (t, 2H), 4.23-4.34 (m, 2H), 5.13-5.19 (m, 36H), 5.27 (s, 2H), 5.33 (s, 1H), 5.74-5.78 (d, 1H, *J* = 16.0), 7.55-7.58 (q, 1H), 7.65 (s, 1H), 7.84-7.85 (d, 1H, *J* = 2.4), 8.22-8.24 (d, 1H, *J* = 8.8).

Synthesis of polylactide (n = 71)-SN38 prodrug conjugate 4



To a solution of **PLA** (n = 71)-succinic acid (1.38 g, 0.26 mmol) and SN38 (100 mg, 0.26 mmol) in 15 mL of anhydrous DCM were added EDC (59 mg, 0.38 mmol). The reaction mixture was stirred at 50ºC for 5 h and then washed with 5% citric acid, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by

flash column chromatography on silica gel (DCM:MeOH = 100:1) to give compound **4** (640 mg, 49 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ1.02-1.06 (t, 3H), 1.40-1.42 (t, 3H), 1.53-1.61 (m, 213H), 1.88-1.92 (m, 2H), 2.89-2.92 (m, 2H), 2.98-3.07 (m, 2H), 3.13-3.19 (q, 2H), 3.38 (s, 3H), 3.54-3.56 (q, 2H), 3.63-3.65 (t, 2H), 3.68-3.70 (t, 2H), 4.27-4.31 (m, 2H), 5.13-5.13 (m, 71H), 5.27 (s, 2H), 5.33 (s, 1H), 5.73-5.77 (d, 1H, J = 16.4), 7.55-7.58 (q, 1H), 7.65 (s, 1H), 7.84-7.85 (d, 1H, J = 2.4), 8.22-8.24 (d, 1H, J = 9.2).

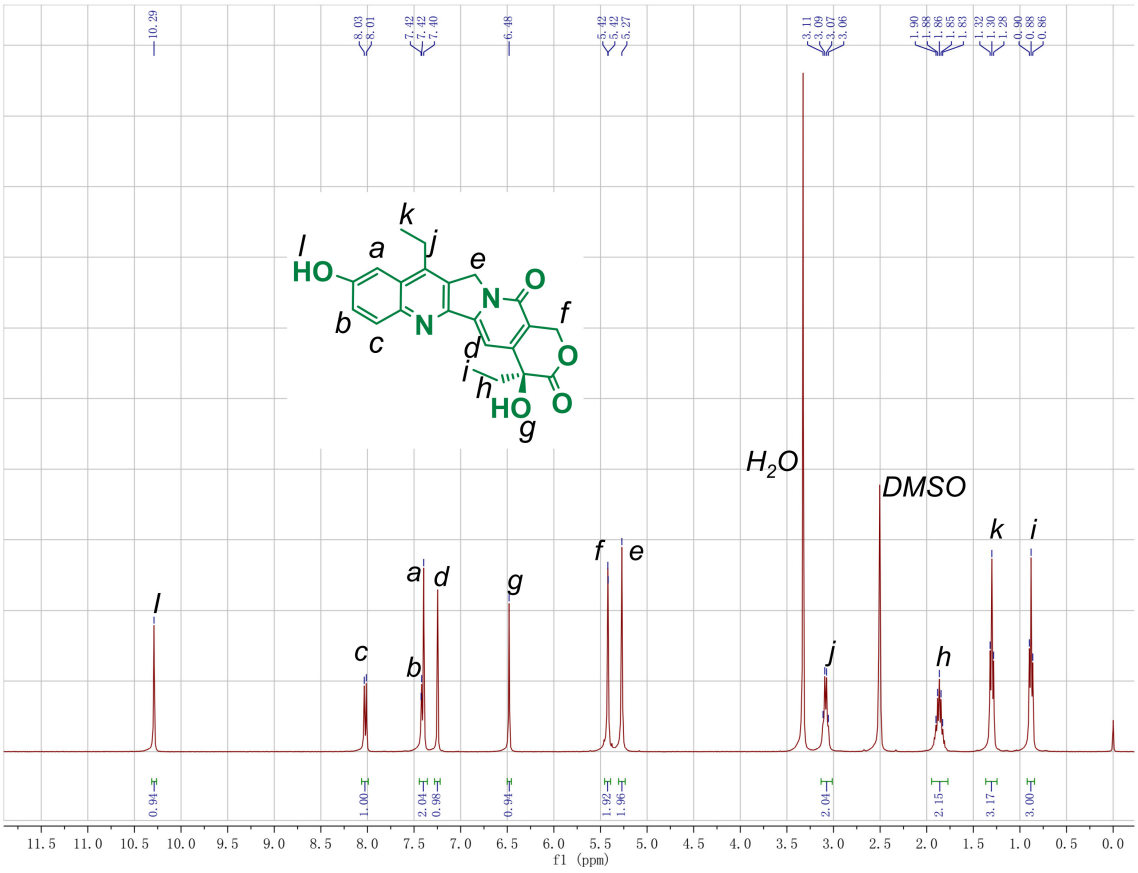


Figure S1. The ^{1}H NMR spectrum of SN38 agent in DMSO-d_6.

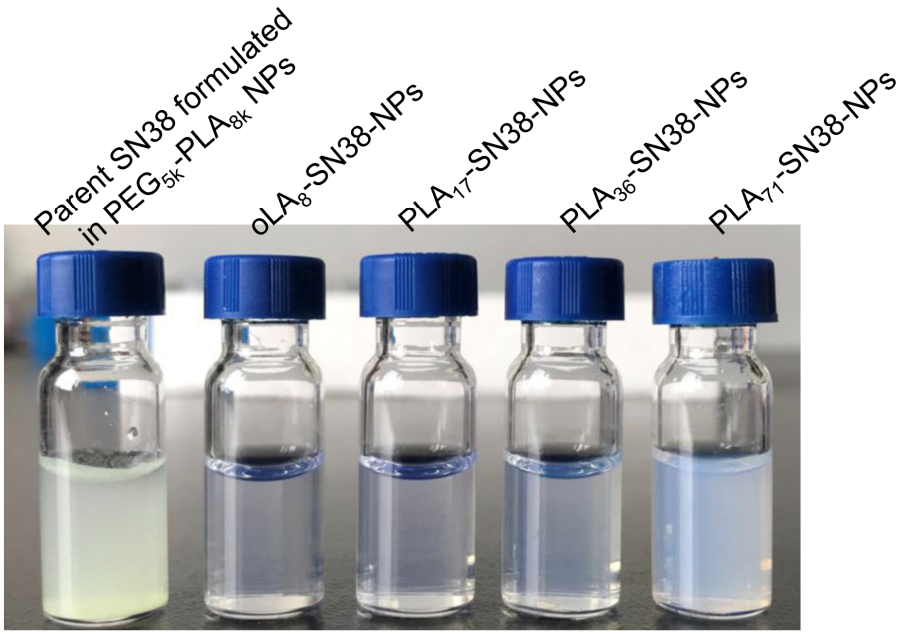


Figure S2. The photographs of parent SN38 and prodrugs **1**-**4** formulated in PEG_{5k}-PLA_{8k} polymeric nanoparticles in deionized water (the drug concentration is 1 mg/mL, at an SN38 equivalence). Obviously, the parent SN38 molecule cannot be encapsulated in PEG_{5k}-PLA_{8k} nanoparticles, leading to the formation of large precipitates in water.

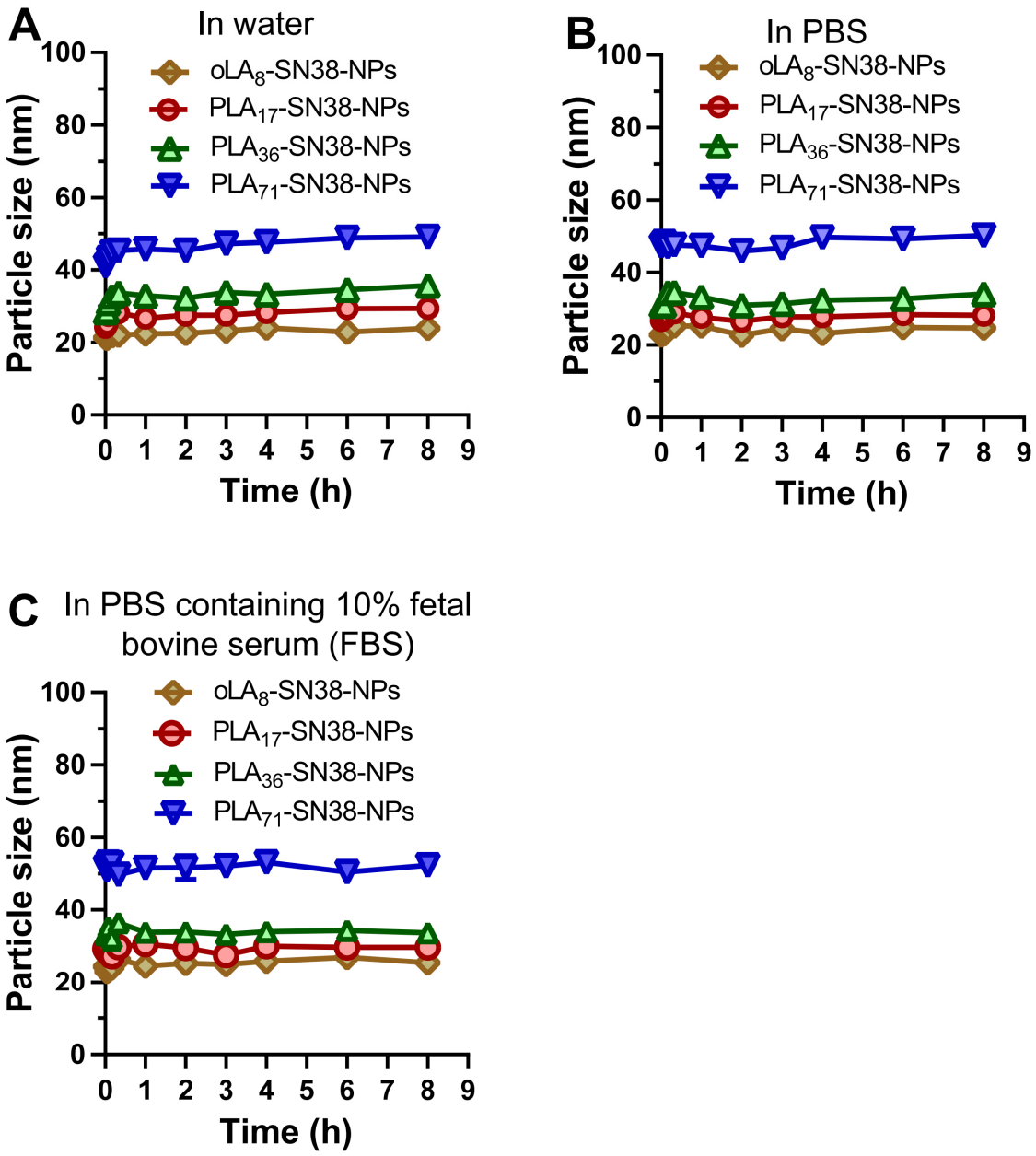


Figure S3. The stability of the prepared nanoparticles in different media including DI water, phosphate buffered saline (PBS, pH 7.4) and PBS containing 10% FBS for 8 days.

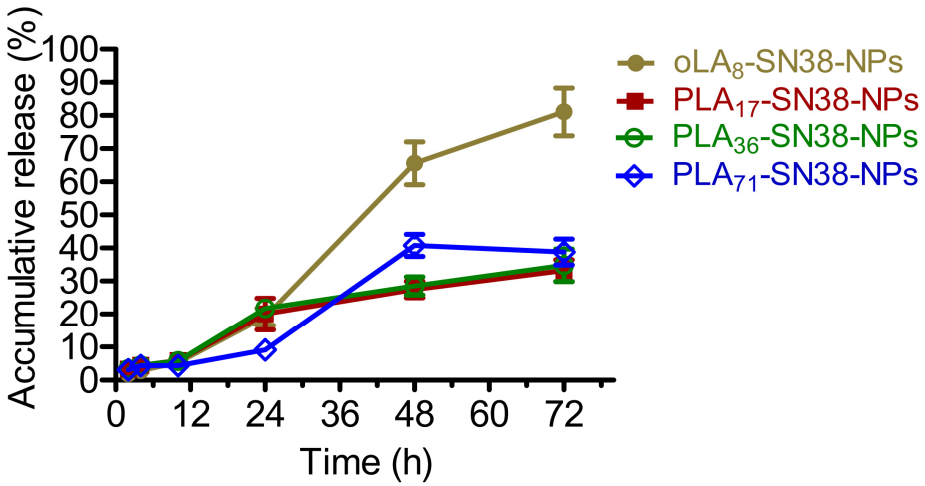


Figure S4. *In vitro* drug release profiles of total SN38 amounts including free SN38 or SN38 prodrugs from NPs composed of PEG_{5k}-PLA_{8k} over a 72-h period at PBS (pH 7.4) containing 20% mouse serum.

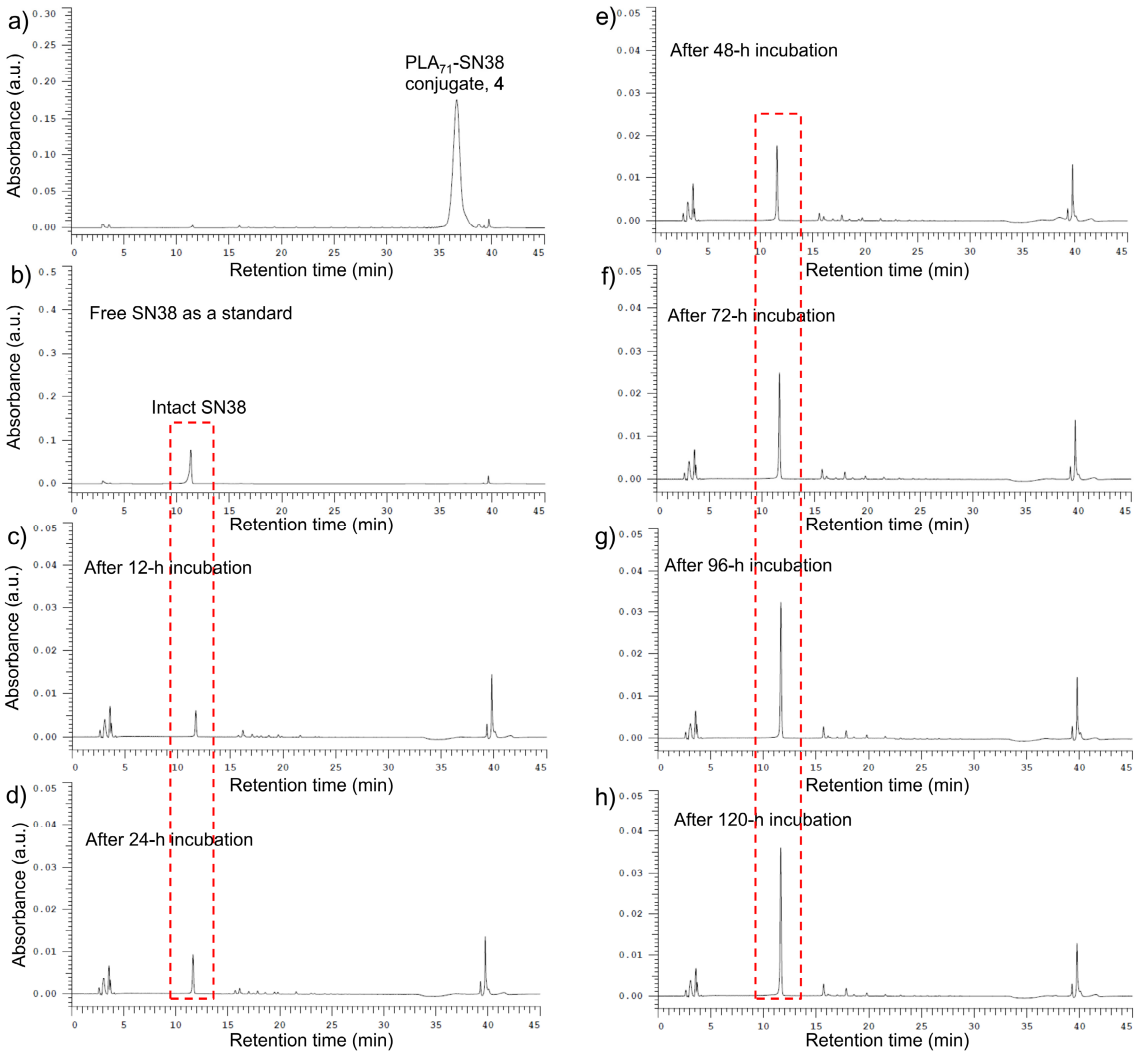


Figure S5. Intact SN38 molecules were released from **PLA_{71}-SN38-NPs**, which was confirmed by HPLC analysis. PLA_{71}-SN38 conjugate **4** was used as a reference (a). Free SN38 dissolved in DMSO was injected as a standard (b). RP-HPLC was performed using a Hitachi Chromaster 5000 system with a YMC-Pack ODS-A column (5 µm, 250 × 4.6 mm) at a flow rate of 1.0 mL/min. UV detection for SN38 was performed at 378 nm. All of the runs used linear gradients of acetonitrile (solvent A) and water (solvent B) containing 0.1% TFA as follows: A:B (20:80) at time 0 to A:B (80:20) at 30 min.

Table S1. The encapsulation efficiency (EE) and percentages of drug loading (DL%) in PEG_{5k}-PLA_{8k} NPs; the results were repeated three times, and all data were expressed as the mean ± SD.[a]

	oLA_8-SN38 (**1**)	PLA_{17}-SN38 (**2**)	PLA_{36}-SN38 (**3**)	PLA_{71}-SN38 (**4**)
EE (%)	92.3±5.6	96.7±3.2	97.8±4.5	98.2±5.5
DL (%)	4.4±0.3	4.6±0.2	4.7±0.2	4.7±0.3

[a] To ensure the DL% to be approximately 5%, we fed matrices/SN38 at a weight ratio of 19:1 (i.e., the matrices refer to total materials except for SN38 agent).