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Supporting Information

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Structure-Based Rational Design of Prodrugs to Enable Their Combination with Polymeric Nanoparticle Delivery Platforms for Enhanced Antitumor Efficacy**

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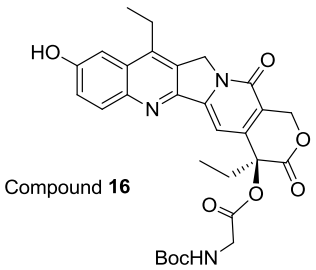


Compound **16**

Figure S1 Compound **16** in which the Boc-Gly-OH was modified on 20-hydroxyl.

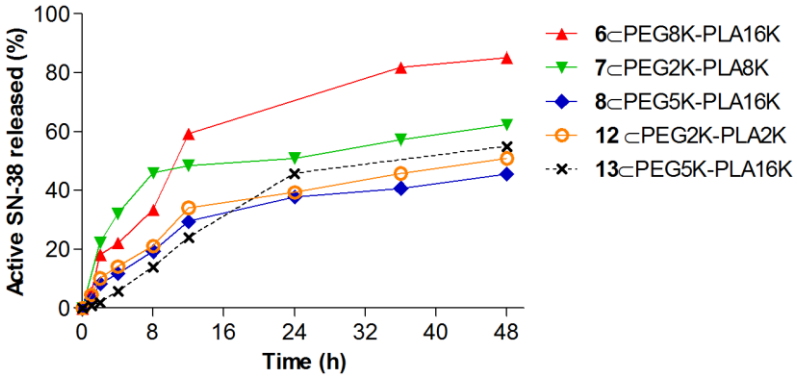


Figure S2 Free SN-38 release from PEG-PLA NPs in phosphate buffer solutions containing 0.2% Tween 80 at pH 7.4. The amounts of released active SN-38 were determined by HPLC analysis. The percentage of SN-38 released from the NPs and prodrugs was plotted against time and the cumulative amount of SN-38 was calculated.

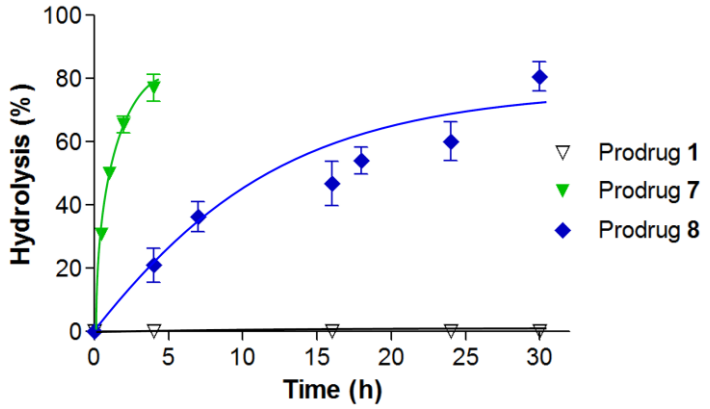


Figure S3 Prodrug hydrolysis in phosphate buffer solutions containing 0.2% Tween 80 at pH 7.4. The percentage of hydrolysis was determined by TLC analysis and plotted against time.

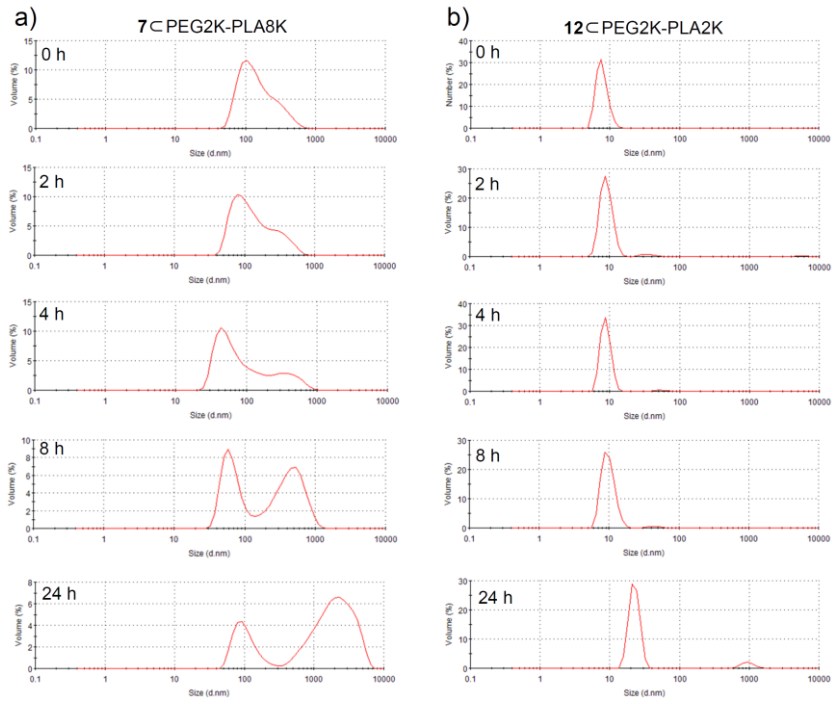


Figure S4 Stability of prodrug **7** (a) and **12** (b)-encapsulated nanoparticles in mouse serum. NPs with 0.2 mg/ml prodrugs were incubated in 50% mouse serum for 0 h, 2 h, 4 h, 8 h and 24 h, respectively and then the hydrodynamic diameters were measured by DLS.

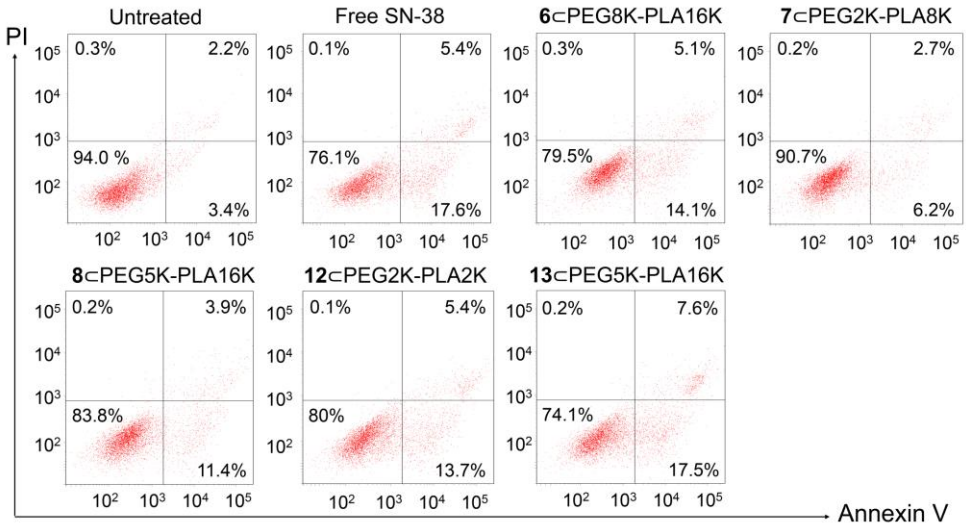


Figure S5 Apoptotic analysis of HCT-116 cells determined by FACS using Alexa Fluor® 488 Annexin V/PI staining kit after 12-h drug treatments. Four distinct phenotypes: viable cells (lower left quadrant); early apoptotic cells (lower right quadrant); late apoptotic cells (upper right quadrant); necrotic or dead cells (upper left quadrant).

Table S1 The maximum concentration of prodrugs **6**, **7**, **8**, **12**, and **13** encapsulated in PEG-PLA polymeric nanoparticles.

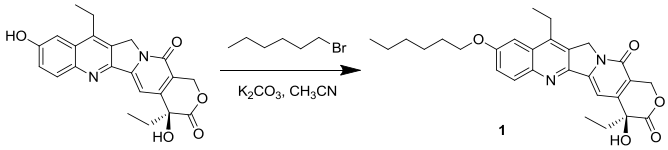
	Solubility (SN-38 equivalent)	Mean d_h (nm) ± SD	Encapsulation efficiency (%)
SN-38 in PEG-PLA	<0.01 mg/mL	–	–
6⊂PEG8K-PLA16K	1.6 mg/mL	41 ± 5	88
7⊂PEG2K-PLA8K	>3.0 mg/mL	82 ± 10	95
8⊂PEG5K-PLA16K	2.1 mg/mL	45 ± 6	96
12⊂PEG2K-PLA2K	1.6 mg/mL	20 ± 3	94
13⊂PEG5K-PLA16K	>3.0 mg/mL	43 ± 4	97

General materials and methods for organic synthesis

7-Ethyl-10-hydroxycamptothecin (SN-38) was purchased from Knowshine Pharmachemicals Inc. (Shanghai, China). PEG-PLA copolymers were obtained from Advanced Polymer Materials Inc. (Montreal, Canada). 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyl tetrazolium bromide (MTT) was purchased from Aladdin (Shanghai, China). All other compounds and solvents were purchased from J&K Chemical (Shanghai, China). Alexa Fluor® 488 Annexin V/PI apoptosis assay kit and cell culture reagents were purchased from Life Technologies (Shanghai, China).

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-d6 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. High-resolution mass spectrometry (HRMS)-ESI was recorded on Agilent 6460 LC/QQQ MS instruments. Reverse phase HPLC (RP-HPLC) was carried out on a Hitachi Chromaster 5000 system, and a YMC-Pack ODS-A column (5 μm, 250 × 4.6 mm) at a flow rate of 1.0 mL/min. UV detection for SN-38 was at 378 nm. All runs used linear gradients of acetonitrile (solvent A) and water (solvent B) containing 0.1% TFA.

Synthesis of SN-38 prodrugs 1-16

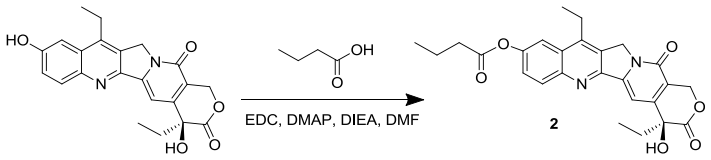


Supplementary Scheme 1 Synthetic scheme of **1**

To a solution of 1-bromohexane (215 μL, 1.5 mmol) and SN-38 (500 mg, 1.3 mmol) in 30 mL of CH_3CN were added anhydrous K_2CO_3 (352 mg, 2.5 mmol). The reaction mixture was heated to reflux for overnight. The reaction mixture was filtered to remove K_2CO_3, and then the solvent was evaporated under vacuum. The residual solid was purified with flash chromatography (DCM:MeOH = 75:1) to give compound **1** (336 mg, 55%).

^{1}H NMR (400 MHz, $CDCl_3$): 0.91-0.95 (t, 3H), 1.00-1.04 (t, 3H), 1.25 (t, 3H), 1.35-1.42 (m, 6H), 1.86-1.92 (m, 4H), 3.12-3.18 (q, 2H), 4.11-4.14 (t, 2H), 5.23 (s, 2H), 5.27-5.31 (d, 1H, *J* = 16.4), 5.71-5.75 (d, 1H, *J* = 16.4), 7.29 (s, 1H), 7.44-7.46 (d, 1H, *J* = 9.2), 7.45-7.47 (d, 1H, *J* =9.2), 7.69 (s, 1H).

HR-ESI Qq-LTMS: calcd for $C_{28}H_{32}N_2O_5$ $[M+H]^+$ = 475.2232; obsd 475.2275.

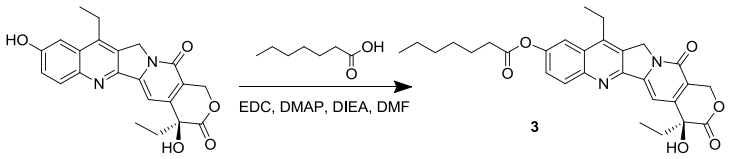


Supplementary Scheme 2 Synthetic scheme of **2**

To a solution of n-butanoic acid (116 μL, 1.3 mmol) and SN-38 (500 mg, 1.3 mmol) in 28 mL of anhydrous DMF were added EDC·HCl (267 mg, 1.4 mmol) and DMAP (172 mg, 1.4 mmol) and DIEA (232 μL, 1.4 mmol). The reaction mixture was stirred at 25 ℃ overnight. After removing the solvent, DCM was added and washed with 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 = 75:1) to give compound **2** (350 mg, 59%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02-1.05 (t, 3H), 1.08-1.12 (t, 3H), 1.42 (t, 3H), 1.83-1.90 (m, 4H), 2.64-2.67 (t, 2H), 3.18-3.20 (t, 2H), 5.30 (s, 2H), 5.28-5.32 (d, 1H, *J* = 16), 5.71-5.75 (d, 1H, *J* = 16.4), 7.57-7.59 (d, 1H, *J* = 7.6), 7.84 (s, 1H), 7.86 (s, 1H), 8.35-8.37 (d, 1H, *J* = 8.4).

HR-ESI Qq-LTMS: calcd for $[C_{26}H_{27}N_2O_6]^+$ $[M+H]^+$ = 463.1864; obsd 463.1893.

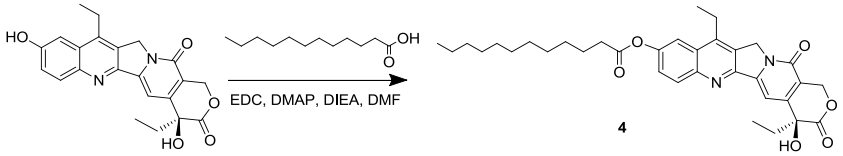


Supplementary Scheme 3 Synthetic scheme of **3**

To a solution of heptanoic acid (108 μL, 0.76 mmol) and SN-38 (300 mg, 0.76 mmol) in 20 mL of anhydrous DMF were added EDC·HCl (160 mg, 0.84 mmol) and DMAP (103 mg, 0.84 mmol) and DIEA (149 μL, 0.84 mmol). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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 = 75:1) to give compound **3** (157 mg, 41%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91-0.94 (t, 3H), 1.00-1.04 (t, 3H), 1.35-1.41 (m, 6H), 1.43-1.49 (t, 3H), 1.80-1.91 (m, 4H), 2.64-2.68 (t, 2H), 3.17-3.18 (q, 2H), 5.27 (s, 2H), 5.27-5.31 (d, 1H, *J* = 16.4), 5.70-5.74 (d, 1H, *J* = 16.4), 7.54-7.56 (d, 1H, *J* = 7.6), 7.79 (s, 1H), 7.83 (s, 1H) 8.29-8.31 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{29}H_{33}N_2O_6]^+$ $[M+H]^+$ = 505.2333; obsd 505.2289.

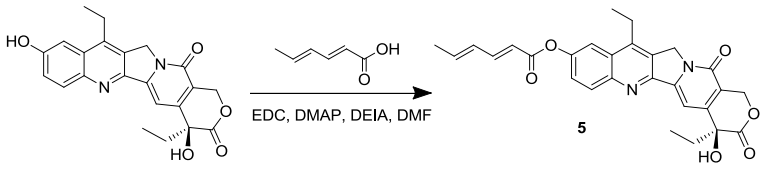


Supplementary Scheme 4 Synthetic scheme of **4**

To a solution of dodecanoic acid (153 mg, 0.76 mmol) and SN-38 (300 mg, 0.76 mmol) in 20 mL of anhydrous DMF were added EDC·HCl (160 mg, 0.84 mmol) and DMAP (103 mg, 0.84 mmol) and DIEA (149 μL, 0.84 mmol). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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** (220 mg, 50%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86-0.90 (t, 3H), 1.01-1.05 (t, 3H), 1.25-1.35 (m, 16H), 1.40-1.43 (t, 3H), 1.78-1.91 (m, 4H), 2.64-2.68 (t, 2H), 3.15-3.22 (q, 2H), 5.30 (s, 2H), 5.27-5.31 (d, 1H, *J* = 16.4), 5.70-5.74 (d, 1H, *J* = 16.4), 7.56-7.58 (d, 1H, *J* = 7.6), 7.85 (s, 1H), 7.86 (s, 1H), 8.34-8.37 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{34}H_{43}N_2O_6]^+$ $[M+H]^+$ = 575.3116; obsd 575.3091.

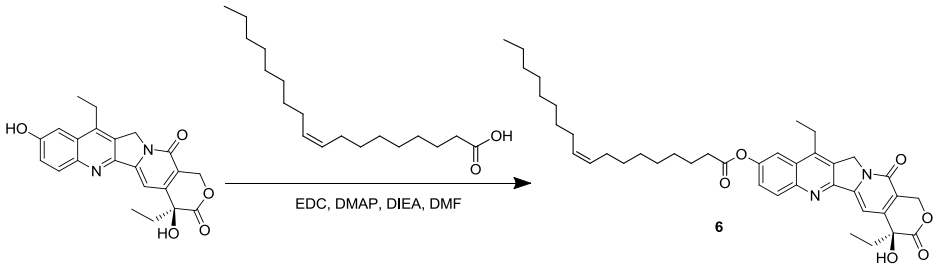


Supplementary Scheme 5 Synthetic scheme of **5**

To a solution of sorbic acid (85.7 mg, 0.76 mmol) and SN-38 (300 mg, 0.76 mmol) in 10 mL of anhydrous DMF were added EDC·HCl (160 mg, 0.84 mmol) and DMAP (103 mg, 0.84 mmol) and DIEA (149 μL, 0.84 mmol). The reaction mixture was stirred at 30 ℃ overnight. After removing the solvent, DCM was added and washed with 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 **5** (160 mg, 43%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02-1.06 (t, 3H), 1.39-1.42 (t, 3H), 1.87-1.94 (m, 5H), 3.14-3.18 (q, 2H), 5.27 (s, 2H), 5.30-5.34 (d, 1H, *J* = 16.0), 5.72-5.76 (d, 1H, *J* = 16.0), 6.02-6.06 (d, 1H, *J* = 15.2), 6.31-6.34 (m, 2H), 7.50-7.56 (m, 1H), 7.59-7.61 (d, 1H, *J* = 9.2), 7.68 (s, 1H), 7.87 (s, 1H), 8.23-8.25 (d, 1H, *J* = 8.8).

HR-ESI Qq-LTMS: calcd for $[C_{28}H_{27}N_2O_6]^+$ $[M+H]^+$ = 487.1864; obsd 487.1859.



Supplementary Scheme 6 Synthetic scheme of **6**

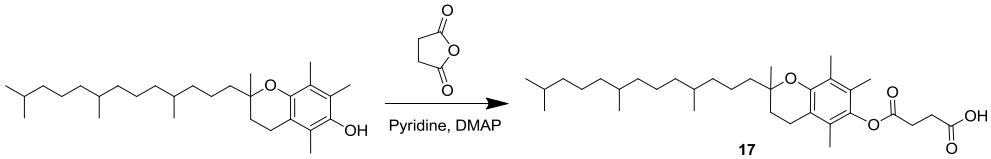
To a solution of oleic acid (21 mg, 0.076 mmol) and SN-38 (30 mg, 0.076 mmol) in 2 mL of anhydrous DMF were added EDC·HCl (16 mg, 0.084 mmol) and DMAP (10 mg, 0.084 mmol) and DIEA (15 μL, 0.084 mmol). The reaction mixture was stirred at 25 ℃ overnight. After removing the solvent, DCM was added and washed with 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 **6** (26 mg, 52%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79-0.82 (t, 3H), 0.95-0.99 (t, 3H), 1.20-1.32 (m, 23H), 1.73-1.95 (m, 4H), 1.99-2.04 (m, 4H), 2.64-2.68 (t, 2H), 3.15-3.21 (q, 2H), 5.28 (s, 2H),

5.30-5.37 (m, 2H), 5.32-5.36 (d, 1H, *J* = 14.8), 5.72-5.76 (d, 1H, *J* = 16.4), 7.55-7.57 (d, 1H, *J* = 7.6), 7.58 (s, 1H), 7.85 (s, 1H), 8.32-8.34 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{40}H_{53}N_2O_6]^+$ $[M+H]^+$ = 657.3898; obsd 657.3831.

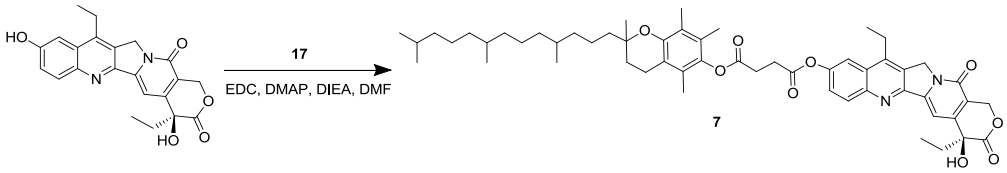
The synthesis of compound **7** has been reported previously.[S1]



Supplementary Scheme 7 Synthetic scheme of **17**

To a solution of vitamin E (1.2 g, 3 mmol) and succinic anhydride (0.902 g, 9 mmol) in 10 mL of anhydrous pyridine were added DMAP (367 mg, 3 mmol). The reaction mixture was stirred at 25 ℃ overnight. After removing the solvent, DCM was added and washed with 0.1 N HCl 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 **17** (1.5 g, 94%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85-0.87 (m, 12H), 1.06-1.16 (m, 6H), 1.23-1.39 (m, 16H), 1.52-1.54 (m, 2H), 1.96 (s, 3H), 2.01 (s, 3H), 2.08 (s, 3H), 2.56-2.59 (t, 2H), 2.82-2.84 (t, 2H), 2.91-2.94 (t, 2H), 3.00 (t, 2H).



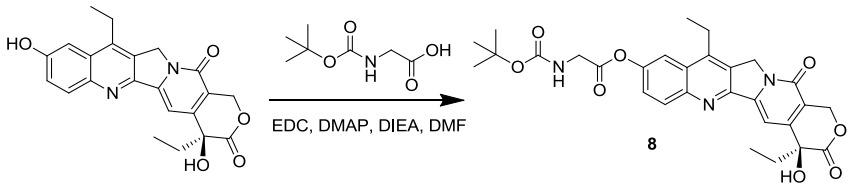
Supplementary Scheme 8 Synthetic scheme of **7**

To a solution of **17** (240 mg, 0.452 mmol) and SN-38 (177 mg, 0.452 mmol) in 10 mL of anhydrous DMF were added EDC·HCl (100 mg, 0.52 mmol) and DMAP (61 mg, 0.5 mmol) and pyridine (100 μL, 0.5 mmol). The reaction mixture was stirred at 25 ℃ overnight. After removing the solvent, DCM was added and washed with 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 **7** (285 mg, 70%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85-0.87 (m, 12H), 1.03-1.06 (t, 3H),1.03-1.14 (m, 6H),

1.23-1.38 (m, 16H), 1.35-1.39 (t, 3H), 1.52-1.59 (m, 2H), 1.77-1.92 (m, 2H), 1.99 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.57-2.60 (t, 2H), 3.11-3.16 (t, 6H), 3.48-3.50 (q, 2H), 5.25 (s, 2H), 5.29-5.33 (d, 1H, *J* = 16), 5.74-55.78 (d, 1H, *J* = 16.4), 7.55-7.58 (d, 1H, *J* = 9.2), 7.65 (s, 1H), 7.81 (s, 1H), 8.22-8.24 (d, 1H, *J* = 8.8).

HR-ESI Qq-LTMS: calcd for $[C_{56}H_{76}N_2O_9]^+$ $[M+H]^+$ =905.5311; obsd 905.5276.

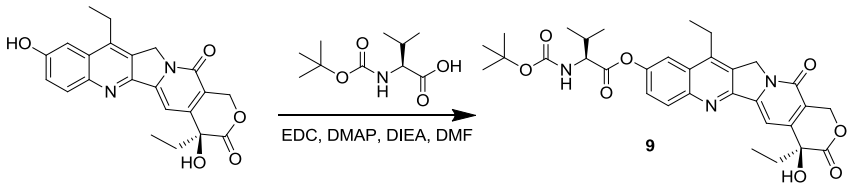


Supplementary Scheme 9 Synthetic scheme of **8**

To a solution of Boc-Gly-OH (144.8 mg, 0.76 mmol) and SN-38 (300 mg, 0.76 mmol) in 20 mL of anhydrous DMF were added EDC·HCl (160 mg, 0.84 mmol) and DMAP (103 mg, 0.84 mmol) and DIEA (139 μL, 0.84 mmol). The reaction mixture was stirred at 25 ℃ overnight and then the solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate and 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 = 75:1) to give compound **8** (242 mg, 58%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97-1.06 (t, 3H), 1.37-1.40 (t, 3H), 1.50 (s, 9H), 1.86-1.94 (m, 2H), 3.14-3.17 (q, 2H), 4.27-4.28 (d, 2H, *J* = 5.6), 5.16 (br, 1H), 5.26 (s, 2H), 5.39-5.43 (d, 1H, *J* = 17.2), 5.74-5.78 (d, 1H, *J* = 16.4), 7.55-7.58 (d, 1H, *J* = 7.6), 7.64 (s, 1H), 7.85 (s, 1H), 8.22-8.24 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{29}H_{32}N_3O_8]^+$ $[M+H]^+$ =550.2184; obsd 550.2222.



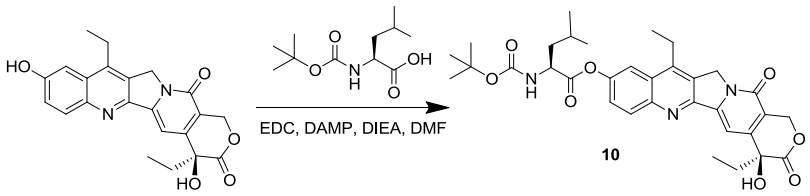
Supplementary Scheme 10 Synthetic scheme of **9**

To a solution of Boc-Val-OH (166 mg, 0.76 mmol) and SN-38 (300 mg, 0.76 mmol) in 20 mL of anhydrous DMF were added EDC·HCl (160 mg, 0.84 mmol) and DMAP (103 mg, 0.84

mmol) and DIEA (139 μL, 0.84 mmol). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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 = 50:1) to give compound **9** (215 mg, 46.4%).

^{1}H NMR (400 MHz, DMSO): δ 0.87-0.93 (t, 3H), 1.05-1.07 (d, 6H, *J* = 6.4), 1.27-1.31 (t, 3H), 1.45 (s, 9H), 1.86-1.90 (m, 2H), 2.24-2.29 (m, 1H),4.09-4.17 (m, 1H), 3.15-3.20 (m, 2H), 5.34 (s, 2H), 5.44 (s, 2H), 7.24 (s, 1H), 7.55-7.57 (d, 1H, *J* = 7.2), 7.90 (s, 1H), 8.23-8.25 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{32}H_{38}N_3O_8]^+$ $[M+H]^+$ = 592.2653; obsd 592.2637.

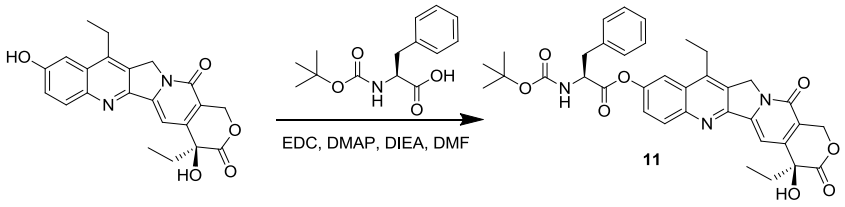


Supplementary Scheme 11 Synthetic scheme of **10**

To a solution of Boc-Leu-OH (318 mg, 1.3 mmol) and SN-38 (500 mg, 1.3 mmol) in 25 mL of anhydrous DMF were added EDC·HCl (269 mg, 1.4 mmol) and DMAP (171.4 mg, 1.4 mmol) and DIEA (232 μL, 1.4 mmol). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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 = 80:1) to give compound **10** (540 mg, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02-1.04 (t, 3H), 1.06-1.08 (m, 6H), 1.39-1.43 (t, 3H), 1.49 (s, 9H), 1.72-1.77 (m, 1H), 1.86-1.93 (m, 4H), 2.11 (s, 1H), 3.15-3.20 (q, 2H), 4.59 (br s, 1H), 5.31 (s, 2H), 5.30-5.34 (d, 1H, *J* = 16.4), 5.71-5.75 (d, 1H, *J* = 16.4), 7.56-7.58 (d, 2H, *J* = 9.2), 7.68 (s, 1H), 7.86 (s, 1H), 8.22-8.25 (d, 2H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{33}H_{40}N_3O_8]^+$ $[M+H]^+$ = 606.2810; obsd 606.2788.

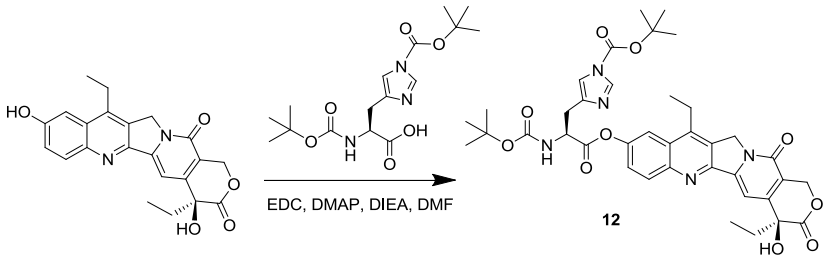


Supplementary Scheme 12 Synthetic scheme of **11**

To a solution of Boc-Phe-OH (340 mg, 1.3 mmol) and SN-38 (500 mg, 1.3 mmol) in 25 mL of anhydrous DMF were added EDC·HCl (269 mg, 1.4 mmol) and DMAP (171.4 mg, 1.4 mmol) and DIEA (232 μL, 1.4 mmol). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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 = 75:1) to give compound **11** (450 mg, 55%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00-1.04 (t, 3H), 1.24-1.26 (t, 3H), 1.46 (s, 9H), 1.90-1.94 (m, 2H), 3.11-3.16 (q, 2H), 3.25-3.29 (m, 3H), 4.84 (s, 1H), 5.32 (s, 2H), 5.32-5.36 (d, 1H, *J* = 16.8), 5.69-5.74 (d, 1H, *J* = 16.8), 7.33-7.38 (m, 5H), 7.40-7.42 (d, 1H, *J* = 7.6), 7.63 (s, 1H), 7.68 (s, 1H), 8.18-8.20 (d, 1H, *J* = 9.6).

HR-ESI Qq-LTMS: calcd for $[C_{36}H_{38}N_3O_8]^+$ $[M+H]^+$ = 640.2653; obsd 640.2634.



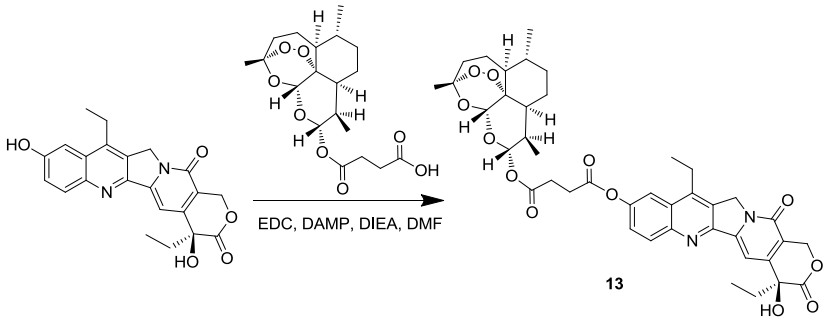
Supplementary Scheme 13 Synthetic scheme of **12**

To a solution of Boc-His(Boc)-OH (433 mg, 1.3 mmol) and SN-38 (500 mg, 1.3 mmol) in 25 mL of anhydrous DMF were added EDC·HCl (269 mg, 1.4 mmol) and DMAP (171.4 mg, 1.4 mmol) and DIEA (232 μL, 1.4 mmol). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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 = 75:1) to give compound **12** (575 mg, 62.8%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02-1.06 (t, 3H), 1.38-1.42 (t, 3H), 1.48 (s, 9H), 1.63 (s, 9H), 1.86-1.94 (m, 2H), 3.12-3.18 (q, 2H), 3.49-3.78 (m, 2H), 4.90 (m, 1H), 5.26 (s, 2H),

5.29-5.33 (d, 1H, *J* = 16.0), 5.74-5.78 (d, 1H, *J* = 16.4), 7.29 (s, 1H), 7.59-7.61 (d, 1H, *J* = 9.2), 7.64 (s, 1H), 7.90 (s, 1H), 8.07 (s, 1H), 8.21-8.23 (d, 1H, *J* = 8.8).

HR-ESI Qq-LTMS: calcd for $[C_{38}H_{44}N_5O_{10}]^+$ $[M+H]^+$ =730.3083; obsd 730.3137.

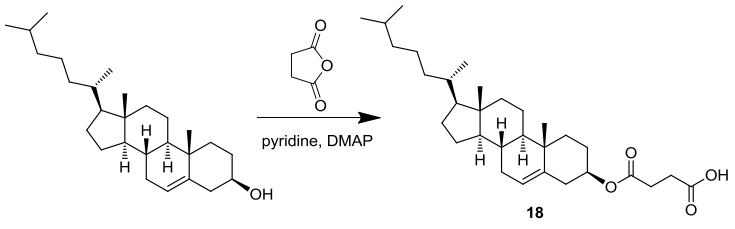


Supplementary Scheme 14 Synthetic scheme of **13**

To a solution of artesunate (19 mg, 0.051 mmol) and SN-38 (20 mg, 0.051 mmol) in 2 mL of anhydrous DMF were added EDC·HCl (12 mg, 0.056 mmol) and DMAP (8 mg, 0.056 mmol). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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=50:1) to give compound **13** (23 mg, 59.5%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86-0.87 (m, 3H), 0.94-0.97 (m, 6H), 1.25-1.28 (m, 3H), 1.29-1.31 (m, 2H), 1.37-1.38 (m, 4H), 1.40 (s, 2H), 1.41-1.43 (m, 3H), 1.62-1.65 (m, 2H), 1.75 (m, 1H), 1.85-1.89 (m, 2H), 1.90-1.95 (m, 1H), 2.02 (s, 1H), 2.94-2.97 (t, 2H), 2.98-3.07 (m, 2H), 3.15-3.20 (t, 2H), 5.27 (s, 2H), 5.28-5.32 (d, 1H, *J* = 16), 5.71-5.75 (d, 1H, *J* = 16.4), 7.58-7.61 (d, 1H, *J* = 7.6), 7.77 (s, 1H), 7.84 (s, 1H), 8.29-8.31 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{41}H_{46}N_2O_{12}]^+$ $[M+H]^+$ = 759.3124; obsd 759.3125.

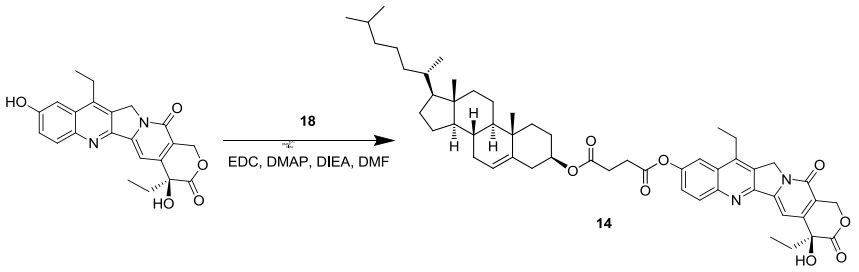


Supplementary Scheme 15 Synthetic scheme of **18**

To a solution of cholesterol (1.16 g, 3 mmol) and succinic anhydride (0.9 g, 9 mmol) in

10 mL of anhydrous pyridine were added DMAP (367 mg, 3 mmol). The reaction mixture was stirred at 25 ℃ overnight. After removing the solvent, ethyl acetate was added and washed with 0.1 N HCl 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 **18** (950 mg, 65%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67-1.57 (m, 37H), 1.83-2.02 (m, 6H), 2.30-2.32 (m, 1H), 2.60-2.62 (d, 2H, *J* = 6.8), 2.66-2.68 (d, 2H, *J* = 6.4), 5.36-5.37 (m, 1H).

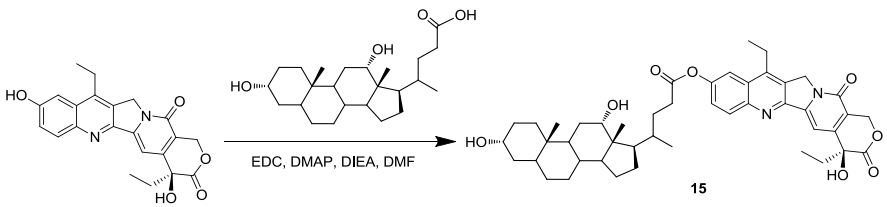


Supplementary Scheme 16 Synthetic scheme of **14**

To a solution of **18** (300 mg, 0.62 mmol) and SN-38 (241 mg, 0.62 mmol) in 18 mL of anhydrous DMF were added EDC·HCl (140 mg, 0.73 mmol) and DMAP (80 mg, 0.73 mmol) and pyridine (200 μL, 1 mmol). The reaction mixture was stirred at 25 ℃ overnight. After removing the solvent, DCM was added and washed with 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 **14** (381 mg, 71.8%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64-1.59 (m, 37H), 0.95-0.98 (t, 3H), 1.32-1.43 (t, 3H), 1.84-1.89 (m, 2H), 1.87-2.02 (m, 6H), 2.35-2.36 (m, 1H), 2.78-2.80 (d, 2H, *J* = 7.2), 2.96-2.98 (d, 2H, J = 6.8), 3.11-3.16 (q, 2H), 5.05-5.15 (m, 1H), 5.23 (s, 2H), 5.26-5.30 (d, 1H, *J* = 16), 5.71-5.75 (d, 1H, *J* = 16.4), 7.53-7.56 (d, 1H, *J* = 9.2), 7.64 (s, 1H), 7.80 (s, 1H), 8.16-8.18 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $[C_{53}H_{69}N_2O_8]^+$ $[M+H]^+$ = 861.5048; obsd 861.5019.



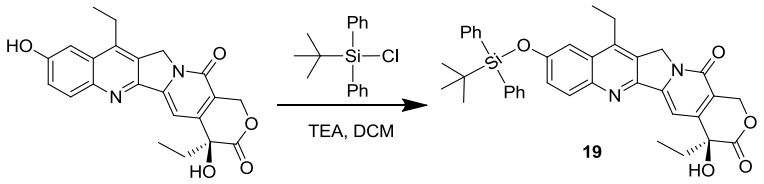
Supplementary Scheme 17 Synthetic scheme of **15**

To a solution of deoxycholic acid (300 mg, 0.76 mmol) and SN-38 (300 mg, 0.76 mmol) in 13 mL of anhydrous DMF were added EDC·HCl (160 mg, 0.84 mmol) and DMAP (103 mg, 0.84 mmol) and DIEA (149 μL, 0.84 mmol).The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 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 = 30:1) to give compound **15** (267 mg, 45%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (s, 3H), 0.91-0.94 (t, 6H), 1.03-1.07 (t, 3H), 1.09-1.10 (m, 4H), 1.26 (s, 6H), 1.39-1.43 (t, 7H), 1.53-1.59 (m, 10H), 1.77-1.81 (m, 2H), 3.16-3.18 (q, 2H), 3.64-3.68 (m, 2H), 3.98-4.07 (m, 2H), 5.27 (s, 2H), 5.30-5.34 (d, 1H, *J* = 16.4), 5.74-5.78 (d, 1H, *J* = 16.4), 7.54-7.57 (dd, 1H, *J* = 9.2, 2.8), 7.66 (s, 1H), 7.825-7.832 (d, 1H, *J* = 2.8), 8.23-8.25 (d, 1H, *J* = 9.2).

HR-ESI Qq-LTMS: calcd for $C_{46}H_{59}N_2O_8$ $[M+H]^+$ = 767.4266; obsd 767.4259.

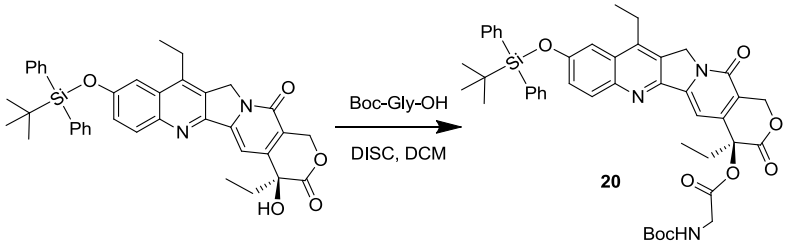
The synthesis of compound **16** has been reported previously.[S2]



Supplementary Scheme 18 Synthetic scheme of **19**

To a solution of tert-butyldiphenylchlorosilane (TBDPSCl, 2.6 mL, 10.2 mmol) and SN-38 (1 g, 2.55 mmol) in 30 mL of anhydrous DCM were added TEA (1.6 mL, 11.5 mmol). The reaction mixture was reflux at 45ºC overnight and then washed with 0.1 N HCl, 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 = 200:1) to give compound **19** (1.511g, 94%).

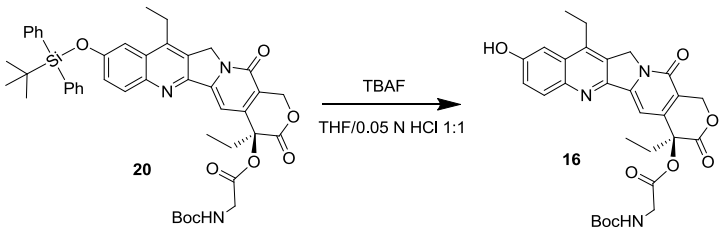
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82-0.86 (t, 3H), 0.91-0.94 (t, 3H), 1.11 (s, 9H), 2.02-2.16 (m, 2H), 2.59-2.64 (q, 2H), 5.08 (s, 2H), 5.26-5.30 (d, 1H, *J* = 17.6), 5.57-5.61 (d, 1H, *J* = 17.2), 7.04 (s, 1H), 7.32-7.35 (m, 4H), 7.38-7.40 (m, 2H), 7.50-7.52 (d, 1H), 7.67-7.69 (d, 4H, *J* = 6.4), 7.95 (s, 1H), 8.32-8.33 (d, 1H, *J* = 6.8).



Supplementary Scheme 19 Synthetic scheme of **20**

To a solution of the compound **19** (200 mg, 0.32 mmol) and Boc-Gly-OH (66.7 mg, 0.38 mmol) in 5 mL of anhydrous DCM were added DISC (52 mg, 0.38 mmol) and DMAP (39 mg, 0.32 mmol). The reaction mixture was stirred at 25ºC overnight and 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 = 200:1) to give compound **16** (220 mg, 87%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87-0.91 (t, 3H), 1.00-1.03 (t, 3H), 1.07 (s, 9H), 1.17 (s, 9H), 1.59 (s, 2H), 1.84-1.90 (m, 2H), 2.27 (s, 1H), 2.61-2.67 (q, 2H), 3.70 (s, 1H), 5.10 (s, 2H), 5.25-5.29 (d, 1H, *J* = 16.0), 5.70-5.74 (d, 1H, *J* = 16.4), 7.08 (s, 1H), 7.37-7.40 (m, 6H), 7.44-7.46 (d, 1H, *J* = 7.2), 7.57 (s, 1H), 7.76-7.78 (m, 4H), 8.04-8.06 (d, 1H, *J* = 9.2).



Supplementary Scheme 20 Synthetic scheme of **16**

Compound **20** (122 mg, 0.15 mmol) was dissolved in a solution of TBAF (630 mg, 2.38 mmol) in a 1:1 mixture of THF and 0.05 N HCl (12.5 mL). The reaction mixture was stirred at room temperature for 4 h. DCM was added and the target compound was extracted. After washing with 5% citric acid 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 **16** (66 mg, 78%).

H NMR (400 MHz, $CDCl_3$): δ 0.97-0.99 (t, 3H), 1.31-1.33 (t, 3H), 1.36 (s, 9H), 2.06-2.14 (m, 2H), 3.09-3.12 (q, 2H), 3.25 (bs, 1H), 5.12-5.23 (m, 2H), 5.26 (s, 2H), 5.27-5.32 (d, 1H, *J* = 17.2), 5.65-5.69 (d, 1H, *J* = 17.2), 7.46 (s, 1H), 7.56-7.58 (d, 1H, J = 8.0), 8.08-8.10 (d, 1H, J = 8.8), 8.32 (s, 1H).

HR-ESI Qq-LTMS: calcd for $[C_{29}H_{30}N_3O_8]^-$ $[M-H]^-$ = 548.2038; obsd 548.2096.

Preparation and Characterization of the SN-38 Prodrug-Loaded Nanoparticles

SN-38 prodrug-loaded PEG-PLA nanoparticles were prepared by using the nanoprecipitation method. Briefly, PEG-PLA (10 mg/ml) and prodrugs (0.5 mg/ml) were dissolved in acetonitrile and together added dropwise into water, providing a final polymer concentration of 3 mg/ml. After stirring for 30 min, the remaining organic solvent was removed in a rotary evaporator at reduced pressure. The NPs were concentrated by using Amicon Ultra-4 centrifugal filters (Millipore, Mw=10,000) and washed with deionized water. Prodrug contents encapsulated in the NPs were determined by reverse phase HPLC by using a UV detector at 378 nm. The quantification of encapsulation efficiency (EE) was performed by HPLC method.

Dynamic Light Scattering (DLS)

The hydrodynamic diameters of the prodrug-loaded nanoparticles were measured on a Malvern NanoS90 instrument (Malvern Instruments, Malvern, UK) in 25ºC.

Transmission Electron Microscopy (TEM)

TECNAL 10 (Philips) was used to obtain transmission electron microscopy (TEM) images, operating at an acceleration voltage of 80 kV. The sample solution of SN-38 prodrug-loaded NPs at a concentration with 0.5 mg/ml (SN-38 equivalent) was placed onto a 300-mesh copper grid coated with carbon. Approximately 2 min after deposition, the surface water was removed with filter paper and then air-dried. Positive staining was performed using a 2 wt % aqueous uranyl acetate solution.

In Vitro Active SN-38 Release Assay

To evaluate the in vitro release profile of prodrugs **6**, **7**, **8**, **12**, and **13** from the PEG–PLA nanoparticles, NPs with 0.1 mg/mL prodrug loaded were dialyzed against phosphate buffer solutions (PBS, pH 7.4, 0.2% tween 80). The dialysis tubes (Spectrum, molecular weight cutoff 6-8 kDa) were continuously stirred in an orbital sharking water bath at 37ºC. At pre-determined time intervals, the release media were collected and the fresh media were supplemented. The amounts of released active SN-38 were determined by HPLC analysis. The percentage of SN-38 released from the NPs was plotted against time and the

cumulative amount of SN-38 was calculated.

Cell Culture

Human colon carcinoma cell line HCT-116, SW480, A549 cells were maintained in RPMI medium 1640. MCF-7 cells were grown in Minimum essential medium (MEM). All media were supplemented with 10% (v/v) heat-inactivated fetal calf serum (FBS) (56°C, 30min), penicillin (100 μg/mL) and streptomycin (100 μg/mL). Cells were maintained in a humid atmosphere at 37 °C with 5% CO_2.

Cytotoxicity assay (MTT)

The cells were plated in flat-bottomed 96-well plates (5000 cells/well) and incubated at 37°C for 24 h. The cells were added by serial dilution of CPT-11, free SN-38 and prodrug-loaded NPs and further incubated for 48h. At the end of the exposure, each well was added by 30 μL MTT solution (5 mg/ml in PBS). After incubating for 4 h in a humidified atmosphere at 37°C with 5% CO_2, the MTT solution was removed from the wells, and the purple MTT-formazan crystals were dissolved by addition of 100 μL DMSO. The absorbance in each individual well was determined at 490 nm on a SynergyHT platereader (BioTek, Winooski, VT, USA).

Apoptosis induced by SN-38 and Prodrug-loaded NPs Using Flow Cytometric Analysis

HCT-116 cells were seeded at a density of 1 x 10^6 cells/mL on each well of a six well plate and allowed to grow overnight. Medium was changed and the cells were incubated with SN-38 (3 μM) and prodrug **6**, **7**, **8**, **12**, **13**-loaded NPs (3 μM SN-38 equivalent) for 12 or 24 h at 37°C, respectively. The cells without drug treatment were used as a control. Cells were harvested from adherent cultures by trypsinization. Following centrifugation at 1000 rpm for 5 min, cells were washed with cold PBS repeatedly. For apoptosis analysis, an Alexa Fluor® 488 annexin V and propidium iodide (PI) apoptosis detection kit was used according to the manufacture's protocol. Briefly, treated and untreated cells (1x10^5) were suspended in 1 x annexin V binding buffer (100 μL) (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4), then 5 μL Alexa Fluor® 488 annexin V and 1 μL PI (100 μg/mL) were added to each sample and incubated at room temperature for 15 min. After then, more binding buffer (400 μL) was added while gently mixing. The samples were kept on ice prior to analysis with the BD

FACSCanto™ II flow cytometer.

Animal Studies

BALB/c nude mice (5 weeks old) were used in animal studies and were purchased from Shanghai Experimental Animal Centre, Chinese Academy of Science. All studies on mice were conducted in accordance with the National Institute Guide for the Care and Use of Laboratory Animal. They were housed under aseptic conditions and given autoclaved rodent diet and sterile water.

Human colon carcinoma HCT-116 cells were grown to 80% confluence in 90 mm tissue culture dishes. After harvesting, cells were suspended in PBS at 4°C to a final concentration of 2.5×10^7 cells/mL. Mice were subcutaneous injection with 200 μL cell suspension containing 5×10^6 cells in a 1 mL disposable syringe. After the tumor reached ~100 mm^3 in volume, 14 days after transplantation, the animals were randomized into six groups.

The mice were treated by administering saline and Irinotecan (CPT-11) as controls, and prodrug-loaded NPs every three days intravenously in 200 μL at a dose of 10 mg/kg. Tumor growth and body weight were monitored and recorded every three days. The length (*L*) and width (*W*) of tumors were measured with calipers and tumor volume was calculated using the following formula: $V = (L \times W^2)/2$, with *W* being smaller than *L*. Mice were sacrificed by CO_2 inhalation when their tumors reached the 1500 mm^3 endpoint value or after 3 weeks.

Statistical Analysis

All quantitative data are presented as means ± SD of three independent experiments. The statistical significance of compared measurements was evaluated using the two-tailed unpaired Student's t test. A *P*-value less than 0.05 was considered as statistically significant, while a *P*-value less than 0.01 was considered as highly significant.

References

S1. Y. Zhang, L. C. Gold, Tocopherol modified therapeutic drug compounds, U.S. Pat. Appl. Publ. (2008), 67pp.

S2. H. Zhao, B. Rubio, P. Sapra, D. Wu, P. Reddy, P. Sai, A. Martinez, Y. Gao, Y. Lozanguiez, C. Longley, L. M. Greenberger, I. D. Horak, *Bioconjugate Chem.* **2008**, *19*, 849–859.