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Supporting Information

An oral redox-sensitive self-immolating prodrug strategy

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1. Materials

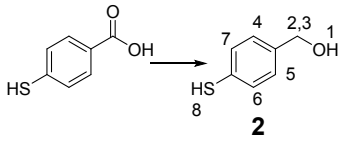
Sodium sulfate (Na_2SO_4, anhydrous), sodium chloride (NaCl), potassium carbonate (K_2CO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl, 37 wt. %, aq.), 4-(dimethylamino)-pyridine (DMAP), triethylamine (Et_3N, stored over solid NaOH after the redistillation), acetic acid (AcOH), formaldehyde solution (37 wt. % in water), *p*-nitrophenol (*p*NP), 4-nitrophenyl chloroformate, phosgene solution (~20 wt. % in toluene), pancreatin (from *porcine pancreas*, for the preparation of simulated intestinal fluid (SIF)), lipase (from *Candida rugosa*), pepsin (from *porcine gastric mucosa*, for the preparation of simulated gastric fluid (SGF)), silica gel for flash column chromatography (SilicaFlash F60, 230~400 mesh), (*S*)-(+)-camptothecin (~95%) as the high-performance liquid chromatography (HPLC) internal standard for 7-ethyl-10-hydroxycamptothecin (SN-38) analysis, (-)-riboflavin (≥98%) as the HPLC internal standard for mitomycin C (MMC) analysis, and solvents (including methanol (MeOH), ethanol (EtOH), dichloromethane (DCM), ethyl acetate, hexane, diethyl ether, acetonitrile (≥99.9% for HPLC) and *N*,*N*-dimethylacetamide (DMF, 99.8%, extra dry over molecular sieves)) were purchased from Sigma-Aldrich (Buchs, Switzerland). Carbamazepine (CBZ, 99%) as the HPLC internal standard for phenytoin analysis, lithium aluminum hydride ($LiAlH_4$) and tetrahydrofuran (THF, 99.8%, extra dry over molecular sieves) were obtained from Acros Organics (Geel, Belgium). Trypsin (from *bovine pancreas*) and alpha-chymotrypsin (from *porcine pancreas*, Mw. ~25000 g/mol) were from Applichem (Darmstadt, Germany). *N*-acetylcysteine (NAC) and phenytoin were obtained from ABCR Chemicals (Karlsruhe, Germany). Alpha-amylase (from *porcine pancreas*, 50~100 units/mg) was from Fisher Chemicals (Reinach, Switzerland). 4-Mercaptobenzoic acid and 2,2-dithiobispyridine were purchased from TCI Europe (Eschborn, Germany). TLC silica gel 60 F254 aluminum sheets and *ortho*-phosphoric acid (85% *w/v* aq.) were from Merck KGaA (Darmstadt, Germany), disodium hydrogen orthophosphate (>98%) and sodium dihydrogen phosphate (>98%) from Fluka Chemical (Buchs, Switzerland), argon (Ar, 99.999% purity) from Pangas (Dagmersellen, Switzerland), methoxy poly(ethylene glycol) thiol, Mw. 5000 ($mPEG_{5000}$-SH, Mw. value from supplier also confirmed by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)) from Jenkem Technology (Beijing, China), 7-ethyl-10-hydroxy-camptothecin (SN-38) from Knowshine Pharmachemicals Inc. (Shanghai, China), while mitomycin C (MMC) from HONCH

Pharmaceutical Co., Ltd (Hubei, China). Agents including D_2O, CD_3OD, $CDCl_3$, sodium deuteroxide (40% wt. solution in D_2O) and phosphoric acid-d3 (D, 99%) for nuclear magnetic resonance (NMR) spectroscopy were from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). All chemicals were used as received unless indicated otherwise.

2. Equipment

Proton-1 NMR (^{1}H NMR), carbon-13 NMR (^{13}C NMR) and 2D NMR correlation spectroscopy (NMR COSY) spectra were recorded on a Bruker Av400 spectrometer (Bruker BioSpin, Fällanden, Switzerland) operating at 400 MHz/25 °C. Fourier transform infrared spectroscopy (FTIR) spectra were obtained using ATR geometry on a Spectrum 65 infrared spectrophotometer (Perkin-Elmer, Schwerzenbach, Switzerland) at 25 °C. High resolution mass spectrometry (HRMS)-ESI data were obtained from Bruker maXis (Bruker Daltonics GmbH, Fällanden, Switzerland), HRMS-EI data from Micromass (Waters) AutoSpec Ultima (Milford, Germany), and MALDI-TOF-MS data from Bruker UltraFlex II (Bruker BioSpin, Fällanden, Switzerland). UV absorbance values were recorded on a plate reader (Infinite M200®, Tecan, Männedorf, Switzerland) at 37 °C. The reconversion of three prodrugs was monitored by analytical HPLC, with a Dionex system equipped with gradient flow control pump (Merck HITACHI pump L-7100, Dartford, UK), autosampler (Merck HITACHI L-7200, Dartford, UK), Merck HITACHI Interface L-7000 (Dartford, UK), solvent degasser (Merck L-7612, Dartford, UK), autosampler, diode array detector (DAD, Merck HITACHI L-7455, Dartford, UK), fluorescence detector (Merck HITACHI FL L-7485, Dartford, UK) and column oven (Merck L-7360, Dartford, UK). All the parameters of HPLC were controlled by LC solutions software Ezchrom Elite (SIM GmbH, Oberhausen, Germany).

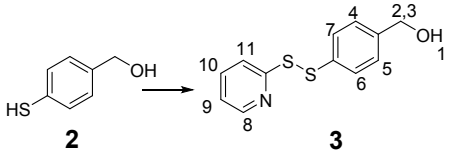
3. Synthesis



2

Compound **2**: (4-Mercaptophenyl)methanol (**2**) was prepared as described previously.[1] A solution of 4-mercaptobenzoic acid (5 g, 32.5 mol, 1 eq) in dry THF (65 mL) was added dropwise over 2 h to a suspension of $LiAlH_4$ (3.7 g, 97.4 mmol, 3 eq) in dry THF (65 mL) under an atmosphere of N_2 at 0 °C. After 16 h, the reaction was carefully quenched by slow addition of H_2O (2 mL). The mixture was then acidified to pH = 2 with HCl aq. (2 N) and extracted with ethyl acetate (3 × 50 mL). The organic layers were combined and washed with H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated to dryness *in vacuo*. The resulting residue was subjected to a flash column chromatography (SiO_2, ethyl acetate/hexane, from 1/4 to 1/1 *v/v*) to afford **2** (3.7 g, 82%) as a white powder.

Retention factor value (R_f) = 0.4 (ethyl acetate/hexane, 1/4 *v/v*); ^{1}H NMR (400 MHz, $\underline{D_2O}$, δ, ppm): 7.19 (d, J = 8.0 Hz, 2H, H-6, 7), 6.95 (d, J = 8.0 Hz, 2H, H-4, 5), 4.36 (s, 2H, H-2, 3); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 137.7, 129.3, 128.9 (2C), 127.1 (2C), 64.2; HRMS-EI Calc. for C_7H_8OS $[M]^{\cdot+}$ 140.0296, Found, 140.0291; IR (νcm^{-1}): 2554 (s, $\nu_{S\text{-}H}$), 3316 (vs, br, $\nu_{O\text{-}H}$). The characterization data are in agreement with those previously reported.[1]

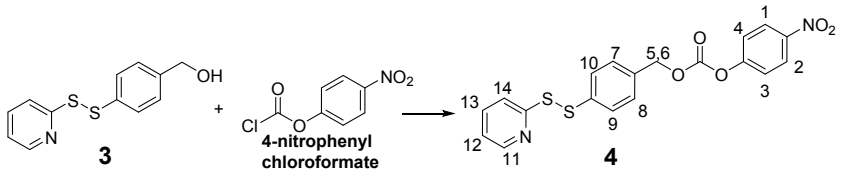


2 **3**

Compound **3**: To a stirred solution of 2,2'-dithiobispyridine (716 mg, 3.25 mmol, 2.3 eq) in AcOH/EtOH (1/20 *v/v*, 3 mL, degassed by N_2 for 3 min), **2** (230 mg, 1.6 mmol, 90% purity, 1 eq) in AcOH/EtOH (1/20 *v/v*, 5 mL, degassed with N_2 for 3 min) was added dropwise over 20 min under an atmosphere of N_2. The mixture was allowed to react at r.t. for 12 h. The solvents were then evaporated to dryness *in vacuo*. The resulting residue was subjected to a flash column chromatography (SiO_2, ethyl acetate/hexane, 1/3 *v/v*) to afford **3** (301 mg, 82%) as yellow oil.

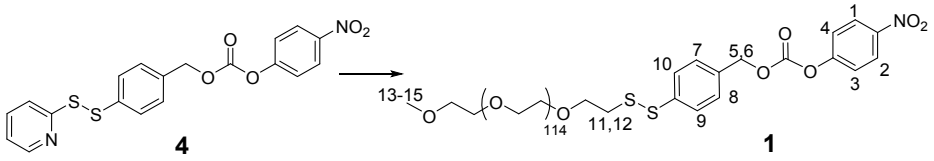
R_f = 0.3 (ethyl acetate/hexane, 1/1 *v/v*); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.35 (d, J = 4.8 Hz, 1H, H-8), 7.62-7.54 (m, 2H, H-10, 11), 7.42 (d, J = 8.4 Hz, 2H, H-6, 7), 7.23 (d, J = 8.4 Hz, 2H, H-4, 5), 7.05-7.02 (m, 1H, H-9), 4.58 (s, 2H, H-2, 3), 3.54 (br, s, 1H, H-1); ^{13}C NMR (400 MHz,

$CDCl_3$, δ, ppm): 159.1, 149.7, 137.5, 137.4, 128.1, 127.9, 127.8, 121.2, 121.1, 119.8, 119.8, 64.9; HRMS-EI Calc. for $C_{12}H_{11}NOS_2$ $[M]^{\cdot+}$ 249.0282, Found, 249.0276; IR (νcm^{-1}): 519 (w, $\nu_{S\text{-}S}$), 1118 (m, $\nu_{C\text{-}N}$), 3297 (vs, br, $\nu_{O\text{-}H}$).



Compound **4**: To a solution of 4-nitrophenyl chloroformate (255 mg, 1.27 mmol, 1.4 eq) in dry THF (5 mL) at 0 °C under an atmosphere of Ar, a solution of **3** (220 mg, 0.88 mmol, 1 eq), DMAP (45 mg, 0.37 mmol, 0.4 eq), Et_3N (0.7 mL, 5 mmol, 5.7 eq) in dry THF (5 mL) was added dropwise over 10 min. The mixture was warmed slowly to r.t. in 1 h and stirred for 15 h. THF was then evaporated *in vacuo*, and the resulting residue was diluted with DCM (20 mL), washed with HCl aq. (2 N, 15 mL) and brine (15 mL), dried over Na_2SO_4, and evaporated to dryness *in vacuo*. A flash column chromatography (SiO_2, ethyl acetate/DCM, 1/2 *v/v*) was used for the purification to afford **4** (356 mg, 97%) as a pale yellow powder.

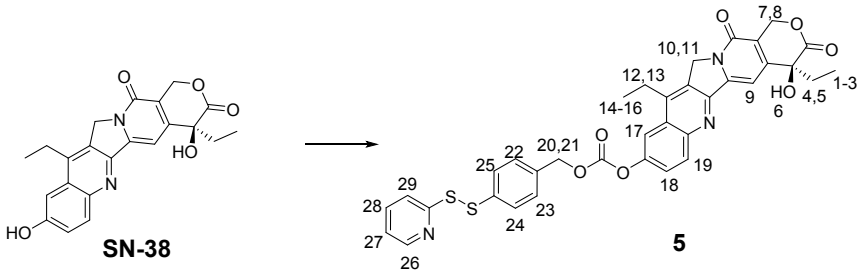
R_f = 0.5 (ethyl acetate/DCM, 1/5 *v/v*); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.44 (dt, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H, H-11), 8.20 (dt, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H, H-1, 2), 7.59 (m, 2H, H-13, 14), 7.53 (d, J = 9.2 Hz, 2H, H-9, 10), 7.36-7.31 (m, 4H, H-3, 4, 7, 8), 7.10-7.05 (m, 1H, H-12), 5.22 (s, 2H, H-5, 6); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 155.6, 152.5, 149.9, 137.7, 137.5, 133.4, 133.2, 129.6 (2C), 127.6 (2C), 125.5 (2C), 121.9 (2C), 121.2, 119.8, 70.4; HRMS-ESI Calc. for $C_{19}H_{15}N_2O_5S_2$ $[M+H]^+$ 415.0422, Found, 415.0420; IR (νcm^{-1}): 526 (w, $\nu_{S\text{-}S}$), 1257 (m, $\nu_{C\text{-}N}$), 1518 (s, ν_{NO_2}), 1577 (m, $\sigma_{N\text{-}H}$), 1758 (vs, br, $\nu_{C=O}$).



Compound **1**: $mPEG_{5000}$-SH (100 mg, 0.02 mmol, 1 eq) in AcOH/EtOH (1/20 *v/v*, 4 mL) was added dropwise into a stirred solution of **4** (36 mg, 0.087 mmol, 4.4 eq) in AcOH/EtOH (1/20 *v/v*, 10 mL) over 5 min under an atmosphere of N_2. The mixture was allowed to react at r.t. for 24 h. Most solvent was evaporated *in vacuo*, and the residue was precipitated in diethyl ether (20 mL). The suspension was centrifuged (4000 × *g*, 4 °C, 5 min) and the obtained solid was dissolved

into DCM (0.2 mL) and precipitated in diethyl ether (20 mL) again. The precipitation was performed a total of three times. The crude was further purified by a flash column chromatography (SiO_2, MeOH/DCM, from 1/20 to 1/5 *v/v*). **1** (59 mg, 54%) was obtained as a pale yellow powder.

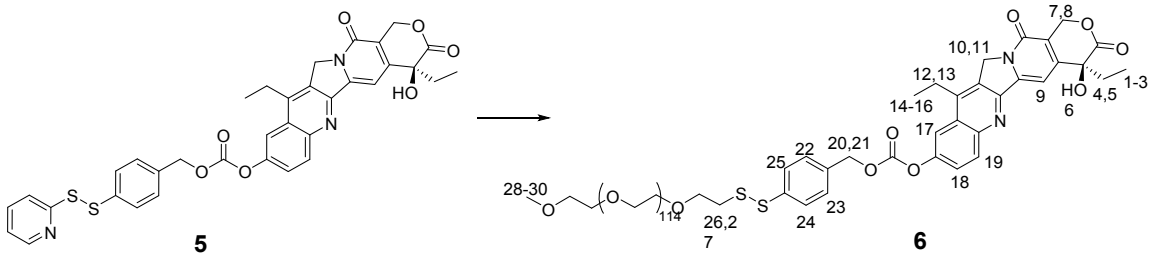
^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.25 (d, J = 9.2 Hz, 2H, H-1, 2), 7.56 (d, J = 8.4 Hz, 2H, H-9, 10), 7.39-7.35 (m, 4H, H-3, 4, 7, 8), 5.25 (s, 2H, H-5, 6), 3.80-3.43 (m, 460H, H-CH_2 of $mPEG_{5000}$), 3.35 (s, 3H, H-13~15), 2.91 (t, J = 6.4 Hz, 2H, H-11, 12); IR ($v$cm^{-1}): 1097 (vs, $v_{CH_2\text{-}O\text{-}CH_2}$), 1341 (m, σ_{NO_2}), 1767 (m, $v_{C=O}$).



Compound **5**: To a solution of **3** (150 mg, 0.6 mmol, 4.6 eq) in dry THF (10 mL) at 0 °C under Ar, a 20 wt. % phosgene solution in toluene (1.6 mL, 3.0 mmol, 23 eq) was quickly added. The mixture was stirred at 0 °C for 30 min and then r.t. for 1 h. A flow of Ar was bubbled through the solution to remove the unreacted phosgene for 1.5 h. A solution of DMAP (30 mg, 0.25 mmol, 2 eq), Et_3N (0.45 mL, 3.2 mmol, 25 eq) and SN-38 (50 mg, 0.13 mmol, 1 eq) in dry THF (15 mL) was slowly added to the purged suspension cooled to 0 °C over 15 min. The mixture was warmed slowly to r.t. in 1 h and stirred for 15 h. Most solvent was evaporated *in vacuo* and the resulting residue was diluted with DCM (15 mL), washed with HCl aq. (2 N, 15 mL) and brine (15 mL), dried over Na_2SO_4, and evaporated to dryness *in vacuo*. A flash column chromatography (SiO_2, ethyl acetate/DCM, 1/5 *v/v*) was used for the purification to afford **5** (28 mg, 33%) as a pale yellow powder.

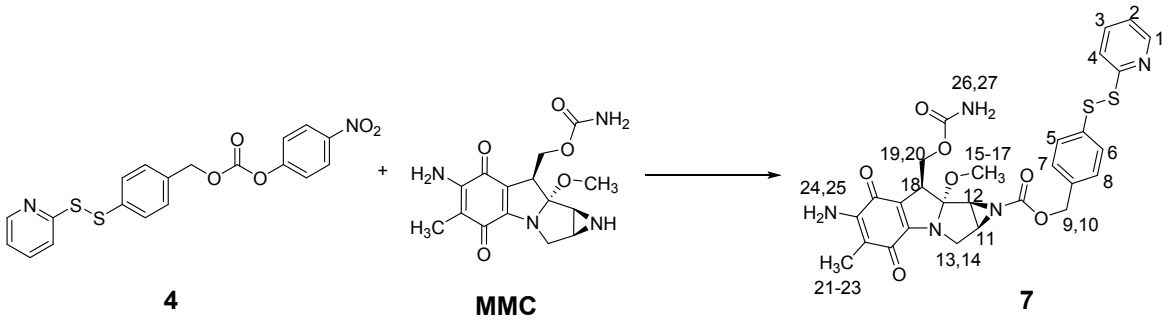
R_f = 0.4 (ethyl acetate/DCM, 1/2 *v/v*); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.48 (d, J = 4.4 Hz, 1H, H-26), 8.22 (d, J = 9.2 Hz, 1H, H-17), 7.86 (d, J = 2.4 Hz, 1H, H-18), 7.66-7.60 (m, 4H, H-9, 19, 28, 29), 7.57 (d, J = 8.4 Hz, 2H, H-24, 25), 7.41 (d, J = 8.4 Hz, 2H, H-22, 23), 7.11 (q, J = 4.4 Hz, 1H, H-27), 5.72 (d, J = 16.4 Hz, 1H, H-20), 5.27 (d, J = 16.4 Hz, 1H, H-21), 5.27 (s, 2H, H-7, 8), 5.23 (s, 2H, H-10, 11), 4.16 (s, 1H, H-6), 3.12 (q, J = 7.6 Hz, 2H, H-12, 13), 1.97-1.82 (m, 2H, H-4, 5), 1.38 (t, J = 7.6 Hz, 3H, H-14~16), 1.01 (t, J = 7.6 Hz, 3H, H-1~3); ^{13}C NMR

(400 MHz, $CDCl_3$, δ, ppm): 174.1, 157.9, 153.6, 152.4, 150.5, 150.1, 150.0 (2C), 147.7, 147.0, 145.7, 137.6 (2C), 133.9, 132.5, 129.7 (2C), 127.7 (3C), 127.6, 124.9, 121.4 (2C), 120.0, 119.1, 114.3, 98.4, 73.1, 70.4, 49.7, 32.0, 23.5, 14.3, 8.1; HRMS-ESI Calc. for $C_{35}H_{30}N_3O_7S_2$ $[M+H]^+$ 668.1525, Found, 668.1520; IR (νcm^{-1}): 1152 (w, $\nu_{C\text{-}OH}$), 1605 (m, $\nu_{C\text{-}N}$), 1747 (s, br, $\nu_{C=O}$).



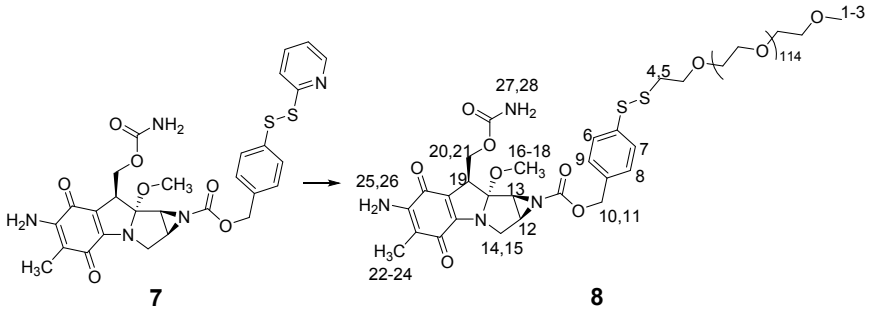
Compound **6**: $mPEG_{5000}$-SH (42 mg, 0.008 mmol, 1 eq) in AcOH/EtOH (1/20 *v/v*, 4 mL, degassed with N_2 for 3 min) was injected into a stirred solution of **5** (22.5 mg, 0.034 mmol, 4.2 eq) in AcOH/EtOH (1/20 *v/v*, 5 mL, degassed with N_2 for 3 min) over 5 min under an atmosphere of N_2. The whole mixture was allowed to react at r.t. for 24 h. Most solvent was evaporated *in vacuo*, and the residue was precipitated in diethyl ether (20 mL) under vigorous stirring. The suspension was centrifuged (4000 × *g*, 4 °C, 5 min) and the obtained solid was dissolved into DCM (0.2 mL) and precipitated in diethyl ether (40 mL) again. The precipitation was performed a total of three times. The crude was further purified by a flash column chromatography (SiO_2, MeOH/DCM, from 1/20 to 1/5 *v/v*). **6** (28 mg, 59%) was obtained as a pale yellow powder.

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.24 (d, J = 9.2 Hz, 1H, H-17), 7.92 (d, J = 2.4 Hz, 1H, H-18), 7.65-7.63 (m, 4H, H-9, 19, 28, 29), 7.58 (d, J = 8.0 Hz, 2H, H-24, 25), 7.42 (d, J = 8.0 Hz, 2H, H-22, 23), 5.74 (d, J = 16.0 Hz, 1H, H-20), 5.30 (d, J = 16.0 Hz, 1H, H-21), 5.30 (s, 2H, H-7, 8), 5.25 (s, 2H, H-10, 11), 3.81-3.44 (m, 502H, H-CH_2 of $mPEG_{5000}$), 3.36 (s, 3H, H-28~30), 3.15 (t, J = 8.0 Hz, 2H, H-26, 27), 2.93 (q, J = 6.8 Hz, 2H, H-12, 13), 1.96-1.81 (m, 2H, H-4, 5), 1.39 (t, J = 8.0 Hz, 3H, H-14~16), 1.03 (t, J = 8.0 Hz, 3H, H-1~3); IR (νcm^{-1}): 1095 (vs, $\nu_{CH_2\text{-}O\text{-}CH_2}$), 1143 (w, $\nu_{C\text{-}OH}$), 1653 (m, $\nu_{C\text{-}N}$), 1752 (m, br, $\nu_{C=O}$).



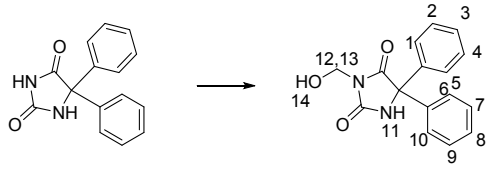
Compound **7: 4** (95 mg, 0.23 mmol, 1.05 eq), MMC (75 mg, 0.22 mmol, 1 eq) and DMAP (40 mg, 0.33 mmol, 1.5 eq) were dissolved in dry DMF (3 mL) under an atmosphere of N_2 at 0 °C. . The mixture was warmed slowly to r.t. in 1 h, stirred for 4 h and then slowly dripped into water (30 mL) under vigorous stirring. The precipitation was collected by filtration, washed by water (30 mL) and dried to dryness *in vacuo*. A flash column chromatography (SiO_2, MeOH/DCM, 1/10 *v/v*) was employed for the purification to afford **7** (130 mg, 95%) as a purple powder.

R_f = 0.45 (MeOH/DCM, 1/10 *v/v*); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.45 (d, J = 4.8 Hz, 1H, H-1), 7.61-7.60 (m, 2H, H-3, 4), 7.46 (d, J = 8.0 Hz, 2H, H-5, 6), 7.28 (d, J = 8.0 Hz, 2H, H-7, 8), 7.11-7.07 (m, 1H, H-2), 5.33 (s, br, 2H, H-24, 25), 5.07-4.96 (m, 2H, H-9, 10), 4.85 (dd, J_1 = 10.8, J_2 = 4.4 Hz, 1H, H-19), 4.81 (s, br, 2H, H-26, 27), 4.40 (d, J = 13.4 Hz, 1H, H-13), 4.25 (t, J = 10.8 Hz, 1H, H-20), 3.64 (dd, J_1 = 10.8, J_2 = 4.4 Hz, 1H, H-18), 3.46 (dd, J = 13.4, 1.6 Hz, 1H, H-14), 3.41 (d, J = 4.6 Hz, 1H, H-12), 3.28 (dd, J_1 = 4.6, J_2 = 1.6 Hz, 1H, H-11), 3.16 (s, 3H, H-15~17), 1.74 (s, 3H, H-21~23); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 178.4, 176.0, 160.8, 159.4, 156.5, 154.4, 149.8, 147.3, 137.5, 136.8, 134.6, 129.6 (2C), 127.5 (2C), 121.2, 119.9, 110.5, 105.5, 105.3, 68.1, 62.1, 49.9, 48.8, 43.7, 42.2, 40.2, 8.0; HRMS-ESI Calc. for $C_{28}H_{27}N_5NaO_7S_2$ $[M+Na]^+$ 632.1250, Found, 632.1239; IR (νcm^{-1}): 1319 (m, $\nu_{C\text{-}N}$), 1556 (s, $\nu_{C\text{-}N\ of\ pyridine}$), 1714 (s, $\nu_{C=O}$).



Compound **8:** $mPEG_{5000}$-SH (50 mg, 0.01 mmol, 1 eq) in AcOH/EtOH (1/20 *v/v*, 5 mL, degassed with N_2 for 3 min) was added dropwise into a stirred solution of **7** (26 mg, 0.043 mmol, 4.3 eq) in AcOH/EtOH (1/20 *v/v*, 5 mL, degassed by N_2 for 3 min) over 5 min under an atmosphere of N_2. The whole mixture was stirred at r.t. for 24 h. Most solvent was evaporated *in vacuo*, and the residue was precipitated in diethyl ether (20 mL) under vigorous stirring. The suspension was centrifuged (4000 × *g*, 4 °C, 5 min) and the obtained solid was dissolved into DCM (0.2 mL) and precipitated in diethyl ether (20 mL) again. The precipitation was performed a total of three times. The crude was then purified by a flash column chromatography (SiO_2, MeOH/DCM, from 1/20 to 1/5 *v/v*). **8** (34 mg, 61%) was obtained as a purple powder.

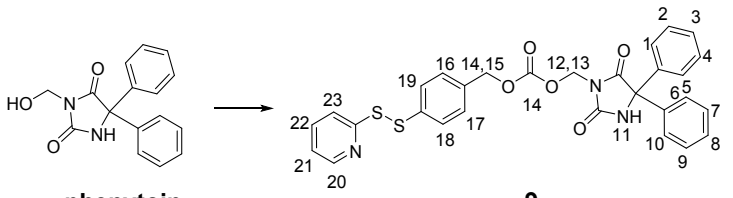
^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.44 (d, J = 8.4 Hz, 2H, H-6, 7), 7.27 (d, J = 8.4 Hz, 2H, H-8, 9), 5.48 (s, br, 2H, H-25, 26), 5.03-4.94 (m, 2H, H-10, 11), 5.00 (s, br, 2H, H-27, 28), 4.79 (dd, J_1 = 10.8, J_2 = 4.4 Hz, 1H, H-20), 4.36 (d, J = 13.2 Hz, 1H, H-14), 4.20 (t, J = 10.8 Hz, 1H, H-21), 3.76~3.35 (m, 534H, H-19, 15, 13, CH_2 of $mPEG_{5000}$), 3.31 (s, 3H, H-1~3), 3.28 (dd, J_1 = 4.8, J_2 = 1.6 Hz, 1H, H-12), 2.51 (s, 3H, H-16~18), 1.68 (s, 3H, H-22~24); IR (νcm^{-1}): 1105 (vs, $\nu_{CH2\text{-}O\text{-}CH2}$), 1347 (m, $\nu_{C\text{-}N}$), 1725 (m, $\nu_{C=O}$).



phenytoin **_pro_-phenytoin**

Compound ***pro*-phenytoin**: ***pro*-phenytoin** was synthesized as described in the literature.[2] A suspension of phenytoin (10 g, 0.04 mol, 1 eq), formalin (40 mL, 37 wt. % aq., 0.54 mol, 13.5 eq) and K_2CO_3 (0.5 g, 3.5 mmol, 0.09 eq) in water (360 mL) was stirred at r.t. for 24 h. The mixture was filtered and the resulting white solid was washed with 3% aqueous formaldehyde, air dried for 24 h to give ***pro*-phenytoin** (11 g, 98%) as a white powder.

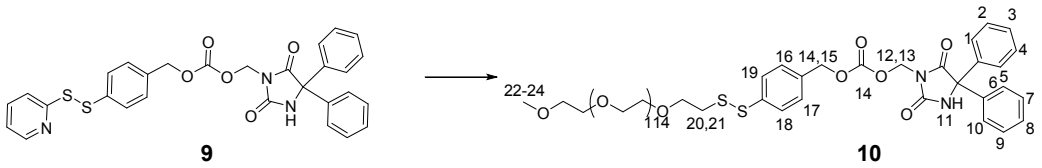
R_f = 0.4 (ethyl acetate/hexane, 1/1 *v/v*); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.44-7.34 (m, 10H, H-1~10), 6.21 (s, br, 1H, H-11), 5.14 (d, J = 8.0 Hz, 2H, H-12, 13), 3.21 (t, J = 8.0 Hz, 1H, H-14); ^{13}C NMR (400 MHz, DMSO, δ, ppm): 154.7, 139.6, 128.5 (4C), 128.1 (2C), 126.7 (4C), 126.6 (2C), 68.9, 61.1; HRMS-EI Calc. for $C_{16}H_{14}N_2O_3$ $[M]^{\cdot+}$ 282.1004, Found, 282.1010; IR (νcm^{-1}): 1700 (vs, $\nu_{C=O}$), 3336 (s, br, $\nu_{O\text{-}H}$). The characterization data are in agreement with those previously reported.[2]



***pro*-phenytoin** **9**

Compound **9**: To a solution of **3** (320 mg, 1.28 mmol, 1 eq) in dry THF (15 mL) at 0 °C under Ar, a 20 wt. % phosgene solution in toluene (3.2 mL, 6.4 mmol, 5 eq) was quickly added. The mixture was stirred at 0 °C for 30 min and then r.t. for 1 h. Ar was bubbled through the solution to remove unreacted phosgene for 1.5 h. A solution of DMAP (64 mg, 0.52 mmol, 0.4 eq), Et_3N (1.0 mL, 7.17 mmol, 5.6 eq) and ***pro*-phenytoin** (700 mg, 2.48 mmol, 2 eq, dried over P_2O_5 overnight) in dry THF (15 mL) was slowly added to the suspension cooled to 0 °C under an atmosphere of N_2. The mixture was warmed slowly to r.t. in 1 h and stirred for 15 h. Most solvent was evaporated *in vacuo* and the resulting residue was diluted with DCM (20 mL), washed with HCl aq. (2 N, 15 mL) and brine (15 mL), dried over Na_2SO_4, evaporated to dryness *in vacuo*. A flash column chromatography (SiO_2, ethyl acetate/DCM, from 1/10 to 1/6 *v/v*) was used for the purification to afford **9** (215 mg, 30%) as a pale yellow powder.

R_f = 0.4 (ethyl acetate/DCM, 1/5 *v/v*); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.46 (dt, J_1 = 4.4 Hz, J_2 = 1.6 Hz, 1H, H-20), 7.61-7.59 (m, 2H, H-22, 23), 7.49 (d, J = 8.4 Hz, 2H, H-18, 19), 7.35 (m, 10H, H-1~10), 7.29 (d, J = 8.4 Hz, 2H, H-16, 17), 7.09 (q, J = 4.4 Hz, 1H, H-21), 7.03 (br, s, 1H, H-11), 5.61 (s, 2H, H-12, 13), 5.11 (s, 2H, H-14, 15); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 172.1, 154.3, 153.2, 149.7, 138.5, 137.4 (2C), 136.9, 133.8, 129.3 (2C), 129.0 (4C), 128.9 (2C), 127.3 (2C), 126.9 (4C), 121.1, 119.7, 70.5, 69.4, 64.6; HRMS-ESI Calc. for $C_{29}H_{24}N_3O_5S_2$ $[M+H]^+$ 558.1157, Found, 558.1151; IR (νcm^{-1}): 519 (w, ν_{S-S}), 1231 (m, ν_{C-N}), 1577 (m, σ_{N-H}), 1724 (vs, br, $\nu_{C=O}$).



9 **10**

Compound **10**: $mPEG_{5000}$-SH (23.9 mg, 0.005 mmol, 1 eq) in AcOH/EtOH (1/20 *v/v*, 4 mL, degassed with N_2 for 3 min) was injected into a stirred solution of **9** (10.6 mg, 0.02 mmol, 4 eq) in AcOH/EtOH (1/20 *v/v*, 1 mL) over 5 min under an atmosphere of N_2. The whole mixture was

stirred at r.t. for 24 h. Most solvent was evaporated *in vacuo*, and the residue was allowed to precipitate in diethyl ether (20 mL) under vigorous stirring. The suspension was centrifuged (4000 × *g*, 4 °C, 5 min) and the obtained solid was dissolved into DCM and precipitated in diethyl ether (20 mL) again. The precipitation was performed a total of three times. The crude was then purified by a flash column chromatography (SiO_2, MeOH/DCM, from 1/20 to 1/5 *v/v*). **10** (18 mg, 67%) was obtained as a white powder.

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.52 (d, J = 8.4 Hz, 2H, H-18, 19), 7.35-7.30 (m, 12 H, H-1~10, 16, 17), 6.49 (br, s, 1H, H-11), 5.63 (s, 2H, H-12, 13), 5.14 (s, 2H, H-14, 15), 3.82-3.44 (m, 464H, H-CH_2 of $mPEG_{5000}$), 3.37 (s, 3H, H-22~24), 2.91 (t, J = 6.4 Hz, 2H, H-20, 21); IR ($v$cm^{-1}): 531 (w, $\nu_{S\text{-}S}$), 1095 (vs, $\nu_{CH_2\text{-}O\text{-}CH_2}$), 1568 (w, $\sigma_{N\text{-}H}$), 1733 (m, br, $\nu_{C=O}$).

4. Methods:

4.1. *p*NP standard curves at pH 4.5 and 6.8

*p*NP was dissolved at different concentrations (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 mM) in 10 mM phosphate buffer (pH 4.5 and 6.8) and the UV absorbance spectra of 100 µL were recorded on 96-well plate (Costar® 3370, flat bottom, sterile polystyrene, Corning Lifesciences, New York, US) with an Infinite® M200 plate reader at 37°C (Tecan, Männedorf, Switzerland). At pH 6.8, the balance of *p*NP between the phenol and ionic state results two peaks at 320 nm and 400 nm (Figure S1a). The maximum absorbance values at 320 nm and 400 nm were recorded to obtain the standard curves for pH 4.5 and 6.8, respectively (Figure S1b and Figure S1d). All the measurements were performed in independent triplicates.

4.2. Kinetic study of prodrug 1 by UV

NAC solutions at different concentrations (0, 0.1, 0.2, 0.5, 1, 10, 100 mM) in 10 mM phosphate buffer (pH 4.5 and 6.8) were prepared and degassed by N_2 for 5 min, and 2 mL of each was transferred into a polypropylene tube (2.5 mL) containing prodrug **1** (1.07 mg) under N_2 atmosphere. Samples were incubated in N_2 atmosphere at 37 °C under continuous mixing over the period of the experiment. At the desired incubation times, 100 µL of the sample was transferred to a 96 well UV plate (Costar® 3370, flat bottom, sterile polystyrene) and measured with the plate reader at 37 °C. The measured intensity was blank corrected and normalized to the t = 0 min time point. Absorbance was measured at 320 or 400 nm for samples at pH 4.5 and 6.8, respectively. Finally, to obtain the full conversion product amount, 0.1 mg of solid NAC was added to the sample and incubated at 37 °C for 2 h. 100 µL of the sample was transferred to the plate and measured. The experiment was performed in triplicate.

The UV spectra of 0.2 mM prodrug **1** /1 mM NAC in 10 mM phosphate buffer (pH 4.5) is shown as an example here (Figure S2). The same protocol can also be applied to the enzyme-mediated drug reconversion study in presence of single enzymes (without NAC, Figure S5).

The NAC-triggered reconversion study at pH 1.2 was also performed on the plate reader as described above. The pH 1.2 solution (pH of the stomach fluid *ante cibum*) was prepared by

adding NaCl (20 mg) and HCl aq. (37 wt. %, 70 µL) to H_2O (10 mL), followed by a pH-adjustment to 1.2 by HCl aq. (0.2 N).

4.3. Stability and reconversion study of prodrug 1 by ^{1}H NMR

D_2O buffers (100 mM, pH 4.5/6.8) were prepared by mixing NaOD (40%, D_2O) and D_3PO_4 (99%) in D_2O. NAC solution (2 mL, degassed 5 min by N_2, in D_2O buffers) with a certain concentration (0, 0.2 or 10 mM) was transferred into an NMR tube containing prodrug **1** (2.1 mg) under a N_2 flow. The tube was sealed and incubated at 37 °C with continuous mixing over the period of the experiment. ^{1}H NMR spectra were recorded at different time points with a Bruker Av400. The integral of peak of the methyl group of the PEG extremity was used as the internal standard and the integration area of the released *p*NP peaks was calculated. At the end of the kinetics, NAC (1 mg) was added to the NMR tube, the solution was incubated at 37 °C for 2 h and then submitted to ^{1}H NMR analysis to obtain the value of a complete reconversion.

4.4. Preparation of SGF/SIF

SGF and SIF were prepared as described previously.[3] **For SGF**: NaCl (20 mg) and pepsin (32 mg) were dissolved into acetate buffer (pH 4.5, 10 mL, 100 mM) and the pH was adjusted back to 4.5 using HCl aq. (0.2 N) or NaOH aq. (0.2 N). **For SIF**: pancreatin (68 mg) was dissolved in 10 mL of phosphate buffer (pH 6.8, 10 mM) and the pH was adjusted to 6.8 using HCl aq. (0.2 N) or NaOH aq. (0.2 N).

For buffers containing single enzymes a protocol similar to the one for SIF was used, except for the replacement of 68 mg pancreatin by single enzymes (amylase: 31.5 mg, lipase: 5.8 mg, chymotrypsin: 4.4 mg, trypsin: 26.3 mg).[4] Before the test, all suspensions were filtered through a 0.45 µm membrane.

4.5. Prodrug reconversion study by HPLC

A LiChroCART® 250-4 column (100 RP-18 (5 µm), Merck KGaA, Darmstadt, Germany) was employed for the HPLC analysis of all three prodrugs.

For the analysis of SN-38 prodrug reconversion, a fluorescence detector with $\lambda_{exc}/\lambda_{em}$ = 377/550 nm was used. A mobile phase of constant 40-60% acetonitrile in phosphate buffer (10 mM, pH

3.0) gradient in 18 min at 1 mL/min flow rate was used for the separation. The retention times (R_t) of the analyte (SN-38) and the internal standard (camptothecin) were found to be 7.0 and 8.4 min, respectively. NAC solution (0.5 mL, degassed by N_2 for 2 min, 0-100 mM) was transferred to a polypropylene tube containing 0.25 mg prodrug and incubated at 37 °C with continuous mixing over the period of the experiment. At the indicated incubation times, 50 μL of the solution was transferred into an HPLC vial containing 900 μL mixed acetonitrile/phosphate buffer (1/1 *v/v*, 10 mM phosphate buffer, pH 3.0) and 50 μL camptothecin solution (in acetonitrile, 93.5 μg/mL). After a 20-s vortex, 20 μL of the solution was submitted to HPLC analysis at the indicated incubation times. The integral of the peaks of SN-38 and camptothecin was recorded. To determine the 100% reconversion, 1 mg NAC solid was added to the polypropylene tube and incubated at 37 °C overnight, then 360 μL acetonitrile was added to the tube followed by a 20-s vortex. 100 μL of the solution was transferred into an HPLC vial containing 850 μL mixed acetonitrile/phosphate buffer (1/1 *v/v*, 10 mM phosphate buffer, pH 3.0) and 50 μL camptothecin solution (in acetonitrile, 93.5 μg/mL), 20 μL of which was withdrawn for HPLC analysis.

For MMC prodrug analysis, a UV detector with wavelength from 200 to 600 nm was used and the absorbance was recorded at 365 nm. An isocratic mobile phase of 15% acetonitrile in 10 mM phosphate buffer (pH 6.5) in 18 min at a 1 mL/min flow rate was used. The R_t of the analyte (MMC) and the internal standard (riboflavin) are 7.8 and 6.7 min, respectively. A MMC prodrug solution (0.2 mM, 0.5 mL, in buffers or SGF/SIF, freshly prepared/degassed by N_2 for 2 min) and NAC solution in the same medium (0.5 mL, degassed by N_2 for 2 min) were transferred into a closed tube containing riboflavin (25 μg). The tube was incubated at 37 °C with continuous mixing over the period of the experiment. At the indicated incubation times, 20 μL solution was withdrawn for HPLC analysis. The integral of the peaks of MMC and riboflavin was recorded. To determine the 100% reconversion, 1 mg NAC solid was added to the vial and incubated for 2 h at 37 °C, then 860 μL acetonitrile was added to the solution followed by a 20-s vortex. 40 μL of the solution was submitted for HPLC analysis.

For phenytoin prodrug analysis, the same UV detector as for MMC analysis was used and the absorbance was recorded at 210 nm. An isocratic mobile phase of 40% acetonitrile in 10 mM phosphate buffer (pH 3.0) in 18 min at a 0.8 mL/min flow rate was used. The R_t of the analyte (phenytoin) and the internal standard (CBZ) were found to be 6.7 and 7.9 min, respectively. It

should be noted that the released ***pro*-phenytoin** will go through a rapid reconversion to phenytoin with a half-life of 1.6 s at 37 °C in aqueous solution.[2] As predicted, no peak for ***pro*-phenytoin** was found from the HPLC experiments. A phenytoin prodrug solution (0.2 mM, 0.5 mL, in buffers or SGF/SIF, freshly prepared/degassed by N_2 for 2 min) and NAC solution in the same medium (0.5 mL, degassed by N_2 for 2 min) were transferred into a closed tube containing CBZ (8.64 μg). The tube was incubated at 37 °C with continuous mixing over the period of the experiment. At the indicated incubation times, 20 μL was withdrawn for HPLC analysis. The integral of the peaks of phenytoin and CBZ was recorded. To determine the 100% reconversion, 1 mg NAC solid was added to the vial and incubated for 2 h at 37 °C, then 860 μL acetonitrile was added to the solution followed by a 20-s vortex. 40 μL of the above solution was submitted for HPLC analysis.

All above HPLC experiments were performed in triplicate.

4.6. Drug oversaturation upon controlled cleavage by NAC

With CBZ as the internal standard (4.32 μg/mL), the standard curve of phenytoin from 0 to 150 μg/mL was obtained by HPLC (Figure S6). Considering the limited solubility of phenytoin and CBZ, a mixed solvent of acetonitrile/H_2O (1/1 *v/v*) was used to prepare the samples with different concentrations. The experiment was performed in triplicate. NAC aqueous solution (2 mL, 0.5 mM, pH 6.8, degassed by N_2 for 2 min) was added to a vial containing 5.5 mg phenytoin prodrug **10** solid (the phenytoin equivalent concentration is 89 μg/mL) and 8.64 μg CBZ (dried *in vacuo* from CBZ solution in acetonitrile (100 μL, 86.4 μg/mL)). After a 20-s vortex, the suspension was incubated at 37 °C and, at the appropriate times, 20 μL of the above suspension (filtered through a 0.22 μm filter membrane) was directly withdrawn for HPLC analysis. The experiment was replicated 5 times independently.

With riboflavin as the internal standard, the standard curve of MMC from 0 to 0.5 mg/mL was obtained by HPLC (the absorbance was recorded at 282 nm, Figure S8). Considering the limited solubility of MMC and riboflavin, acetonitrile was used to prepare the samples with different concentrations. The experiment was performed in triplicate. NAC aqueous solution (1 mL, 6 mM, pH 6.8, degassed by N_2 for 2 min) was added to a vial containing 35 mg MMC prodrug **8** solid (the MMC equivalent concentration is 2.13 mg/mL). After a 20-s vortex, the suspension was

incubated at 37 °C and, at the appropriate times, 15 μL of the above suspension (filtered through a 0.22 μm filter membrane) was diluted into 135 μL riboflavin solution in acetonitrile (0.4 mg/mL). 5 μL of the above solution was withdrawn for HPLC analysis. The experiment was carried out in triplicates.

With camptothecin as the internal standard (4.66 μg/mL), the standard curve of SN-38 from 0 to 40 μg/mL was obtained by HPLC (Figure S10). Considering the limited solubility of SN-38 and camptothecin, a mixed solvent of acetonitrile/H_2O (1/1 *v/v*) was used to prepare the samples with different concentrations. The experiment was performed in triplicate. NAC aqueous solution (1 mL, 0.1 mM, pH 6.8, degassed by N_2 for 2 min) was added to a vial containing 1.1 mg SN-38 prodrug **6** solid (the SN-38 equivalent concentration is 78.4 μg/mL) and 4.66 μg camptothecin (dried *in vacuo* from camptothecin solution in acetonitrile (37 μL, 126 μg/mL)). After a 20-s vortex, the suspension was incubated at 37 °C and, at the appropriate times, 10 μL of the above solution was directly withdrawn for HPLC analysis. The experiment was carried out in triplicates.

5. Supporting results

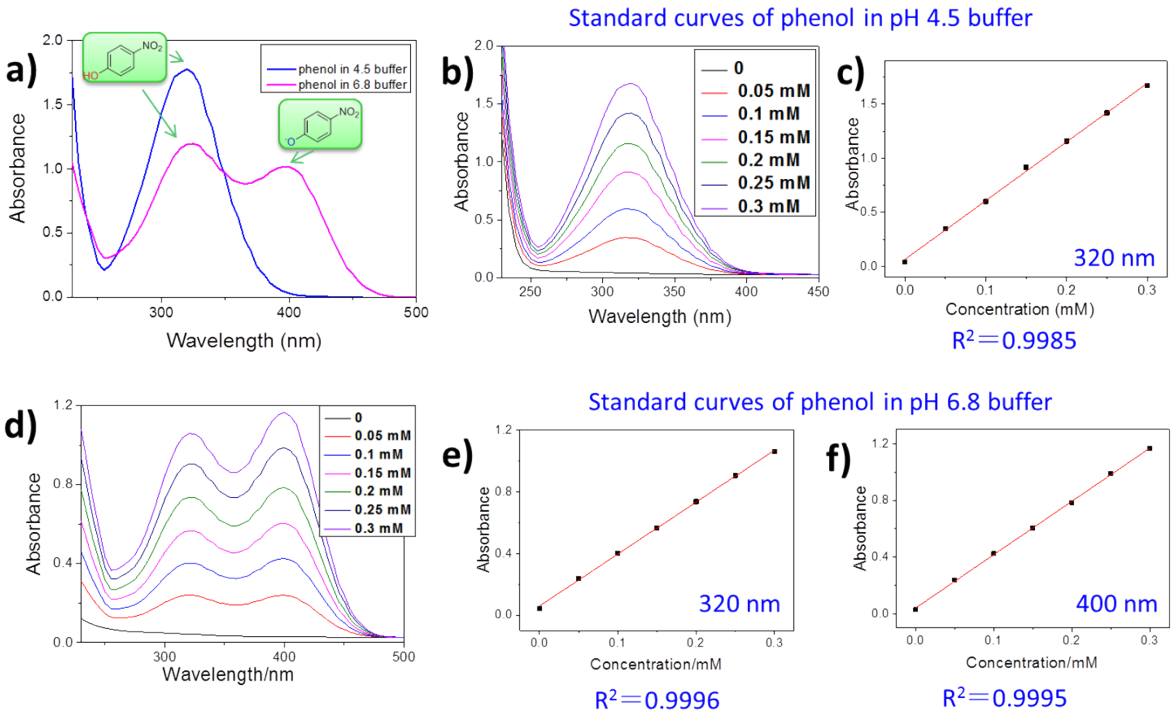


Figure S1. a) UV absorbance of *p*NP at pH 4.5 and 6.8 at 37 °C; b) UV absorbance of increasing concentrations of *p*NP at pH 4.5 at 37 °C; c) standard curve of *p*NP at pH 4.5 at 37 °C at 320 nm; d) UV absorbance of *p*NP with an increasing concentration at pH 6.8 at 37 °C; e) standard curve of *p*NP at pH 6.8 at 37 °C at 320 nm; f) standard curve of *p*NP at pH 6.8 at 37 °C at 400 nm. Values are all represented as a mean ± SD (n = 3).

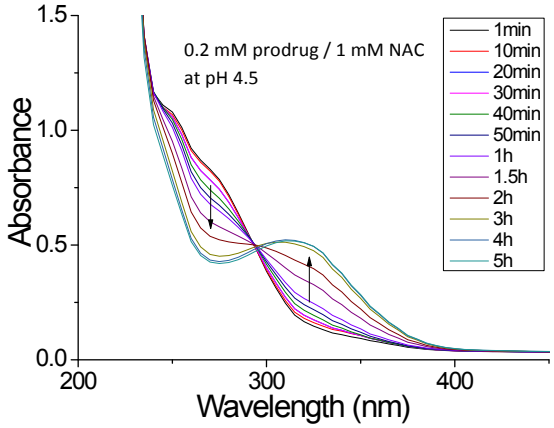


Figure S2. The UV spectra over time of 0.2 mM prodrug **1** in presence of 1 mM NAC at pH 4.5 at 37 °C evaluated by UV spectroscopy.

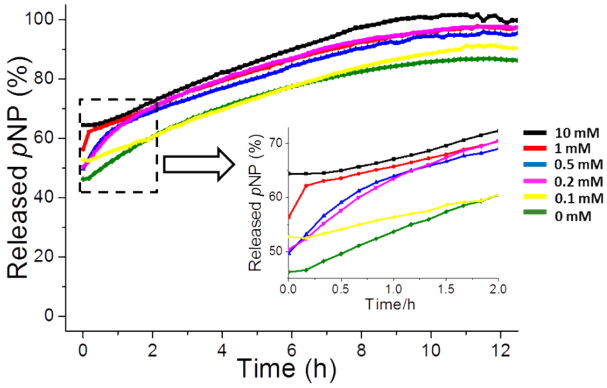


Figure S3. Stability of the prodrug **1** at pH 1.2 in the presence of NAC at 37 °C determined by UV spectroscopy.

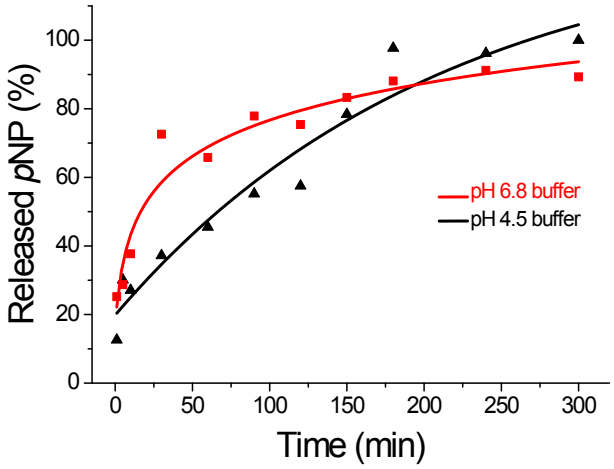


Figure S4. Reconversion of the prodrug **1** in buffers (0.1 mM prodrug **1**, 10 mM NAC for pH 4.5 buffer, and 0.1 mM prodrug **1**, 0.2 mM NAC for pH 6.8 buffer) at 37 °C determined by ^{1}H NMR spectroscopy.

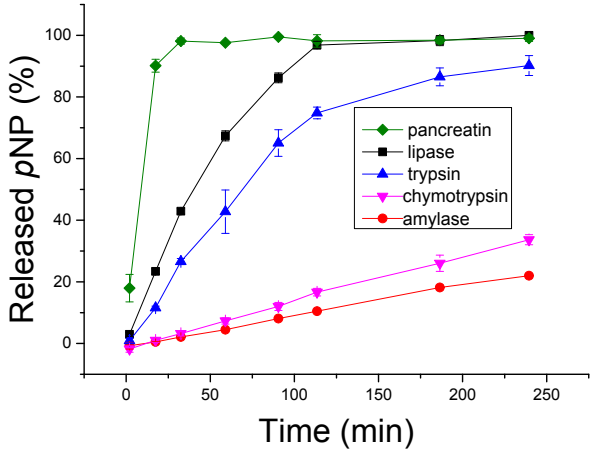


Figure S5. Reconversion of the prodrug **1** in the presence of pancreatin, lipase, trypsin, chymotrypsin and amylase (0.1 mM prodrug **1** in pH 6.8 buffer) at 37 °C determined by UV spectroscopy. Values are represented as means ± SD (n = 3).

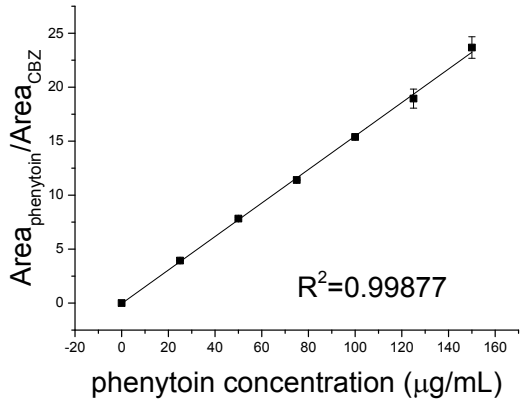


Figure S6. The standard curve of phenytoin concentration with CBZ as the internal standard determined by HPLC. Values are represented as a mean ± SD (n = 3).

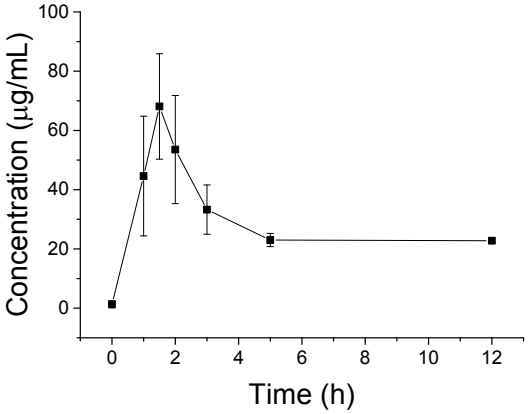


Figure S7. Phenytoin oversaturation upon controlled cleavage by NAC at pH 6.8/37 °C measured by HPLC (with a peak value 70 μg/mL, 3.5 times its solubility). Values are represented as means ± SD (n = 5). The experimentally finding solubility is in accordance with those reported previously.[5]

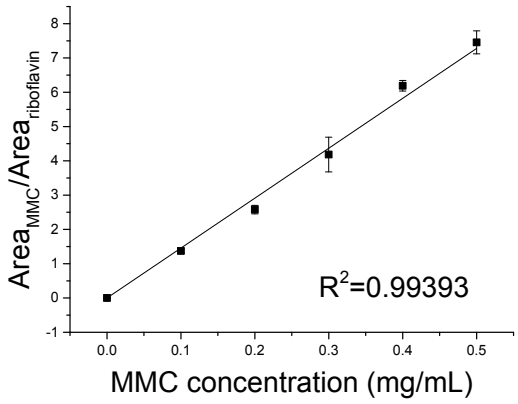


Figure S8. The standard curve of MMC concentration with riboflavin as the internal standard determined by HPLC. Values are represented as a mean ± SD (n = 3).

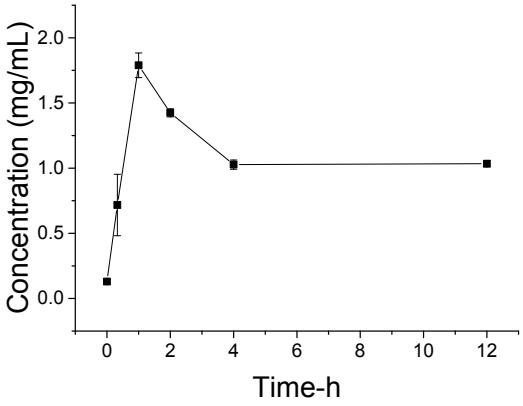


Figure S9. MMC oversaturation upon controlled cleavage by NAC at pH 6.8/37 °C measured by HPLC (with a peak value 1.79 mg/mL, 1.94 times its solubility). Values are represented as means ± SD (n = 3). The experimentally finding solubility is in accordance with those reported previously.[6]

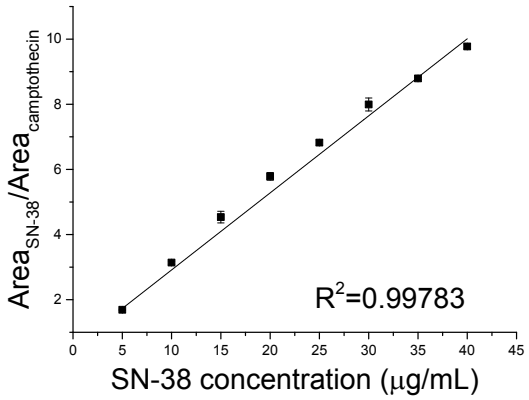


Figure S10. The standard curve of SN-38 concentration with camptothecin as the internal standard determined by HPLC. Values are represented as a mean ± SD (n = 3).

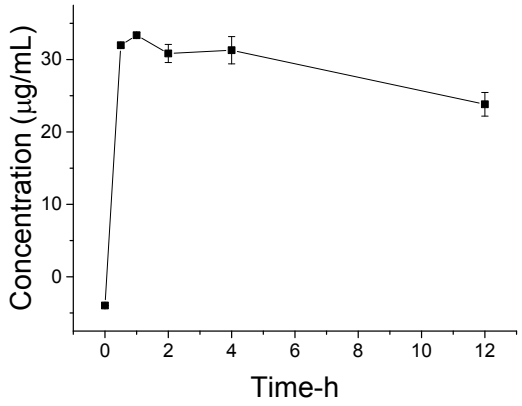


Figure S11. SN-38 oversaturation upon controlled cleavage by NAC at pH 6.8/37 °C measured by HPLC (with a peak value 33.3 μg/mL, 1.4 times its solubility). Values are represented as means ± SD (n = 3). The experimentally finding solubility is in accordance with those reported previously.[7]

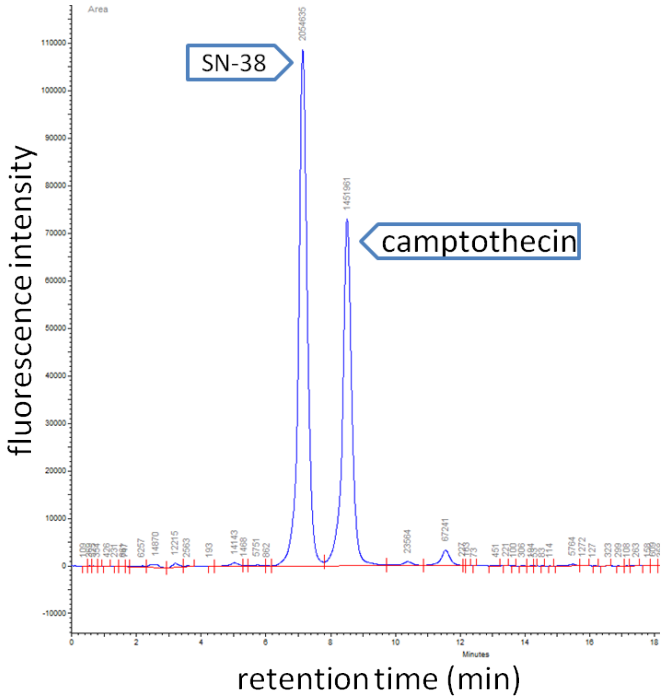


Figure S12. Representative HPLC chromatogram of SN-38 and camptochecin (internal standard)

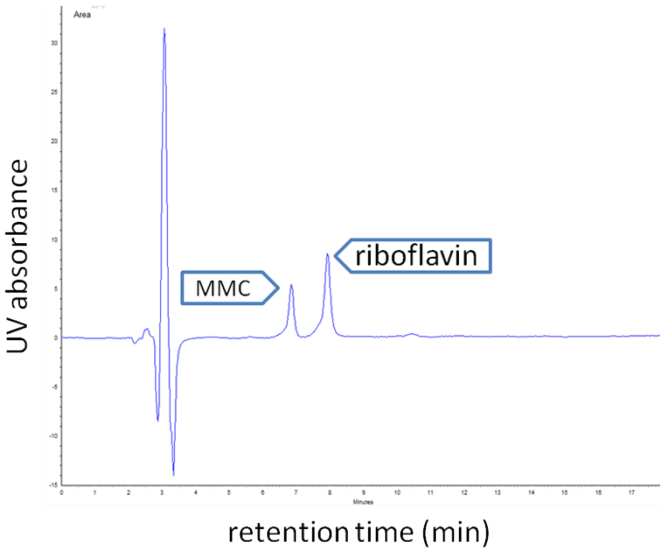


Figure S13. Representative HPLC chromatogram of MMC and riboflavin (internal standard)

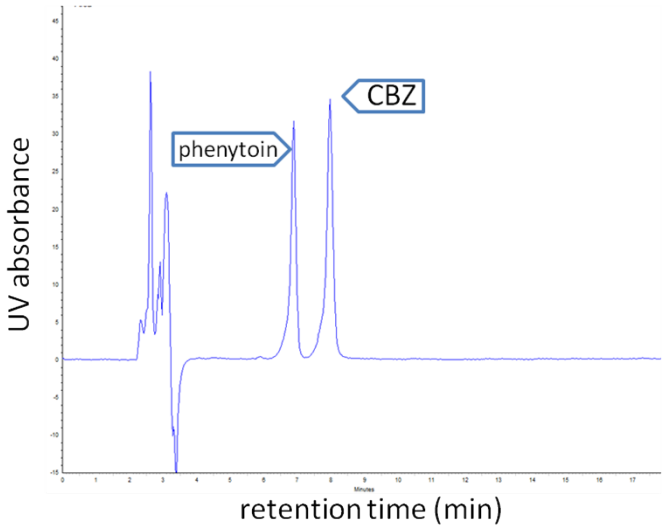
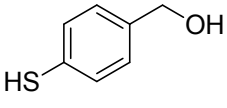


Figure S14. Representative HPLC chromatogram of phenytoin and CBZ (internal standard)

6. Characterizations of all compounds



2

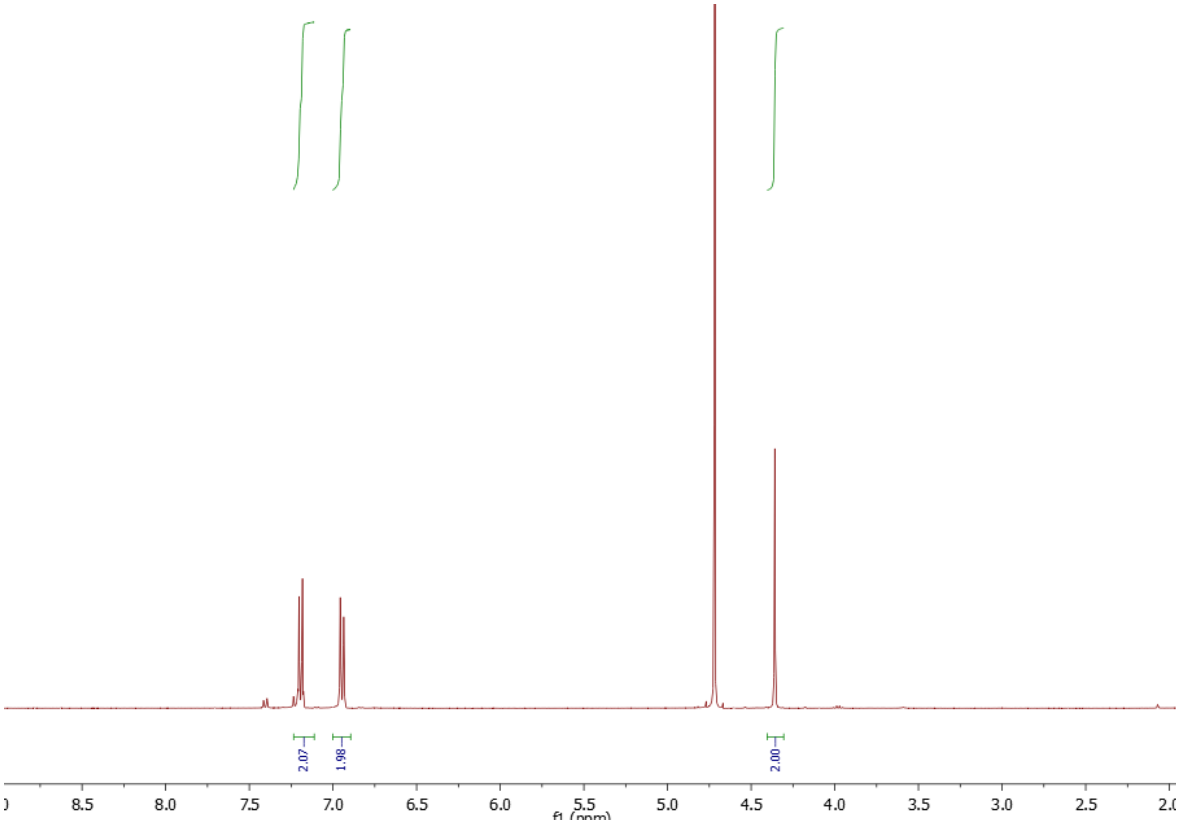


Figure S15. ^{1}H NMR spectrum of **2** in D_2O at ambient temperature.

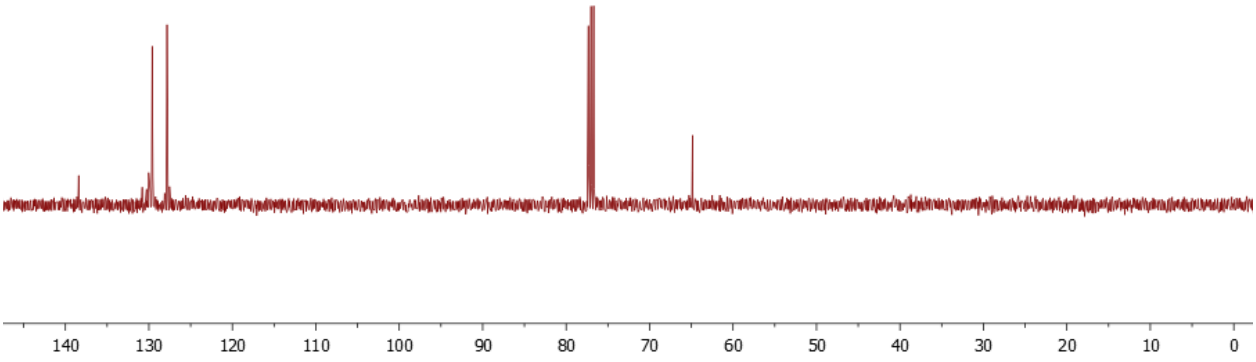
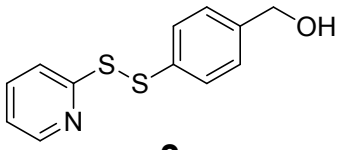


Figure S16. ^{13}C NMR spectrum of **2** in $CDCl_3$ at ambient temperature.



3

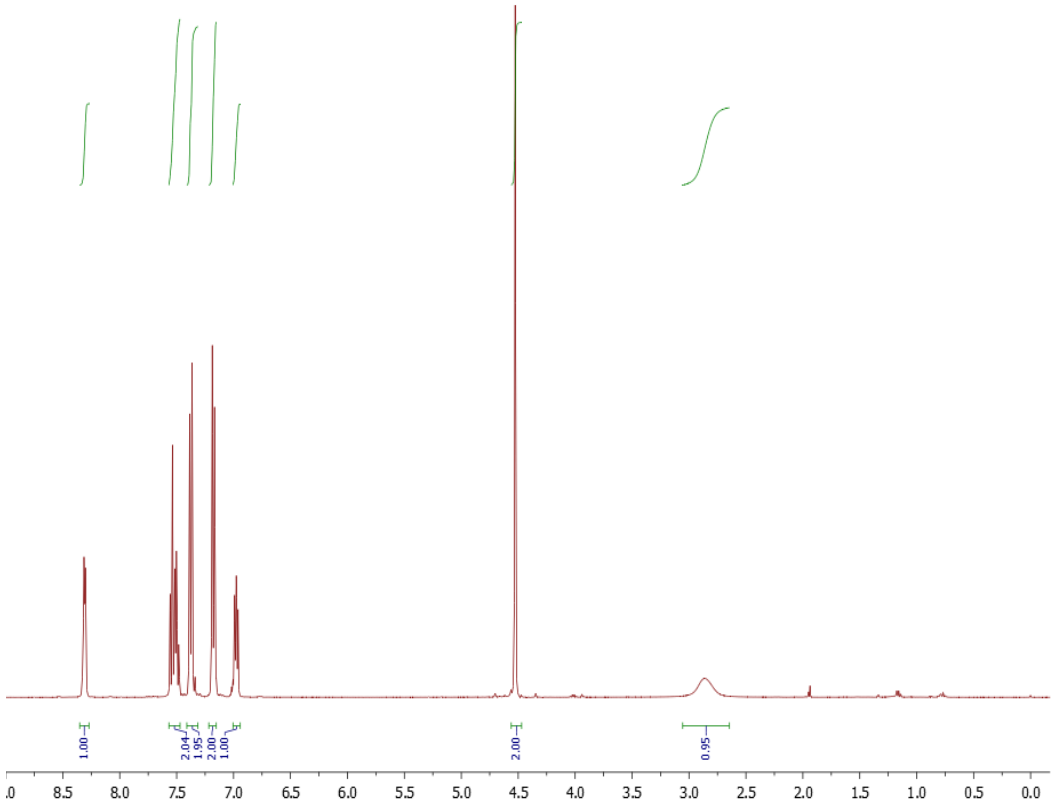


Figure S17. ^{1}H NMR spectrum of **3** in $CDCl_3$ at ambient temperature.

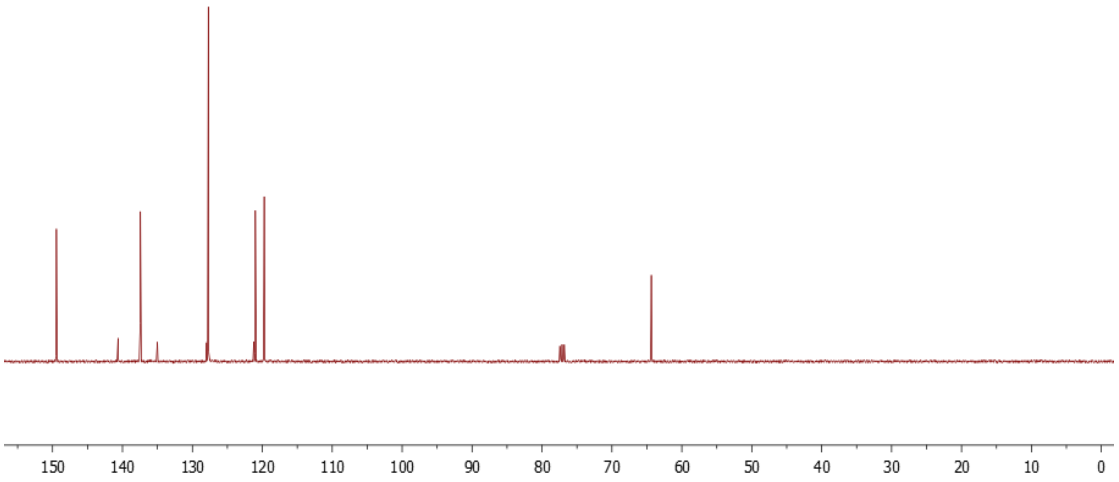


Figure S18. ^{13}C NMR spectrum of **3** in $CDCl_3$ at ambient temperature.

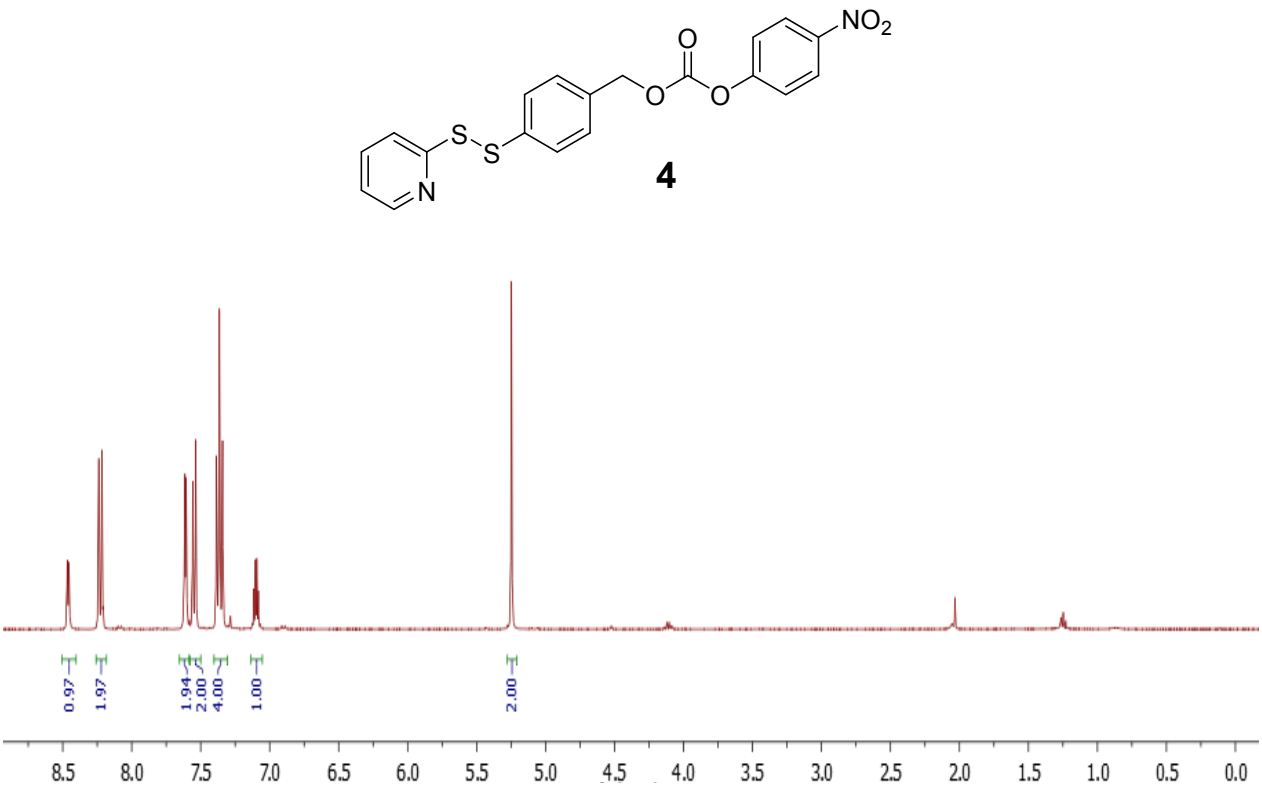


Figure S19. ^{1}H NMR spectrum of **4** in $CDCl_3$ at ambient temperature.

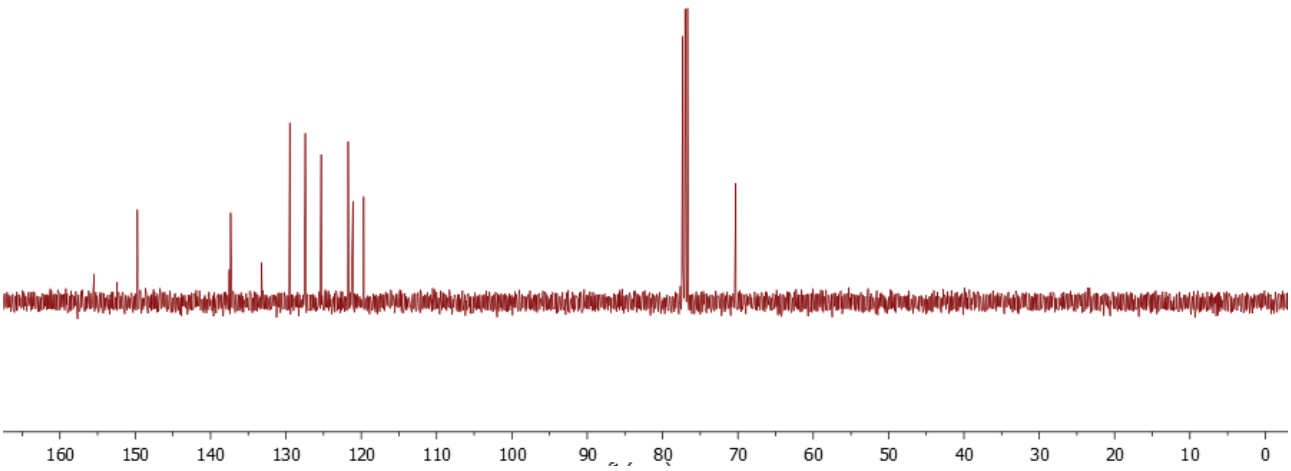


Figure S20. ^{13}C NMR spectrum of **4** in $CDCl_3$ at ambient temperature.

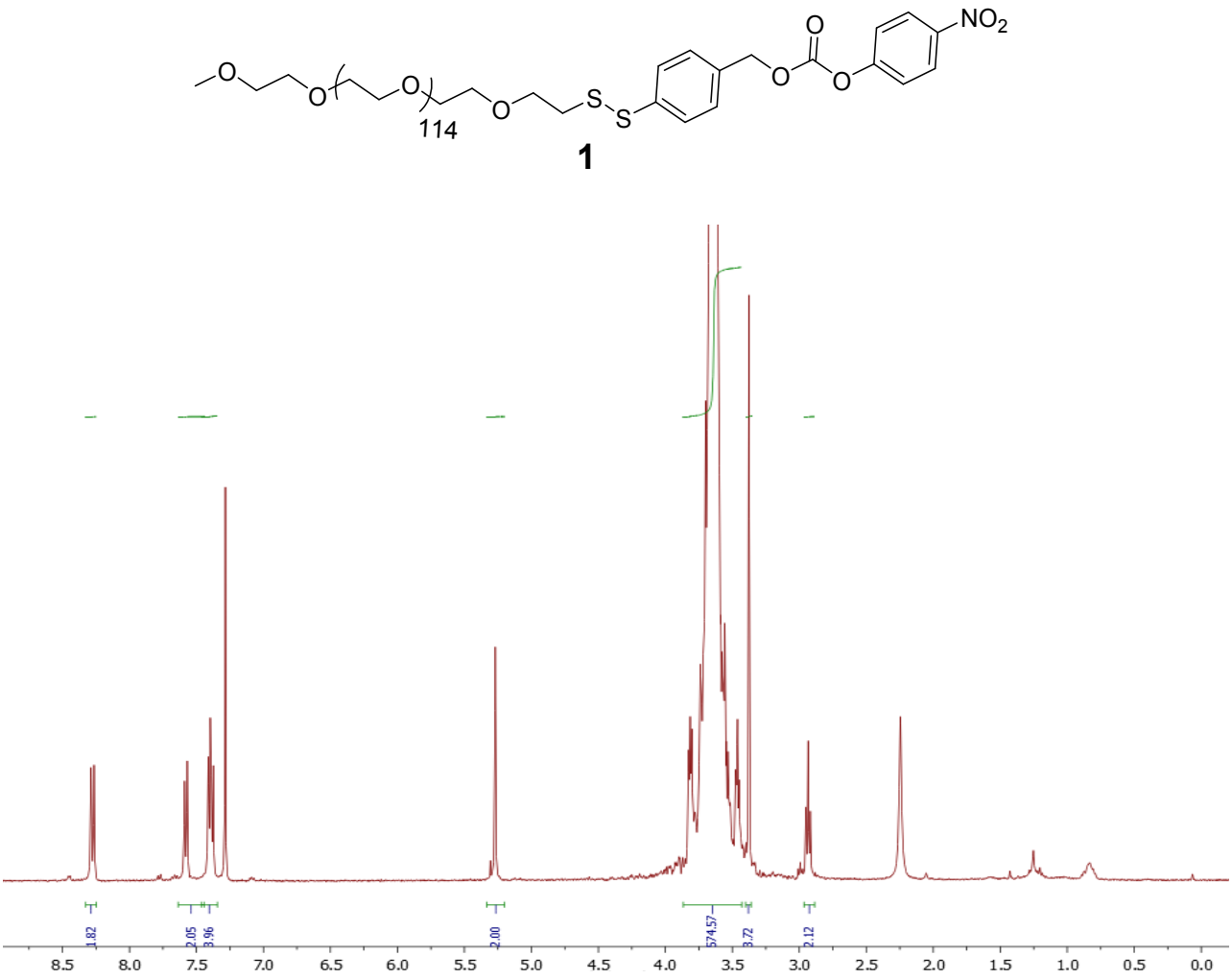


Figure S21. ^{1}H NMR spectrum of **1** in $CDCl_3$ at ambient temperature.

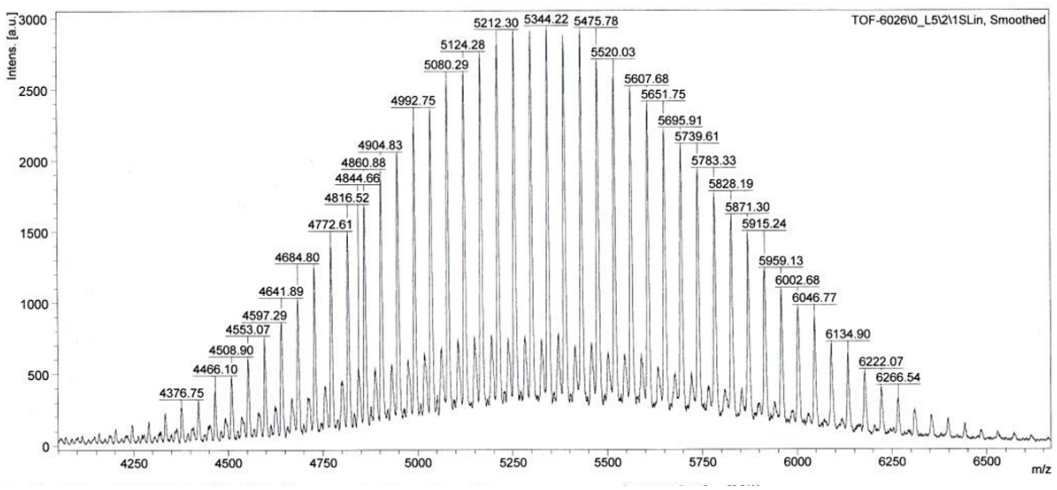


Figure S22. MALDI-TOF-MS spectrum of **1**.

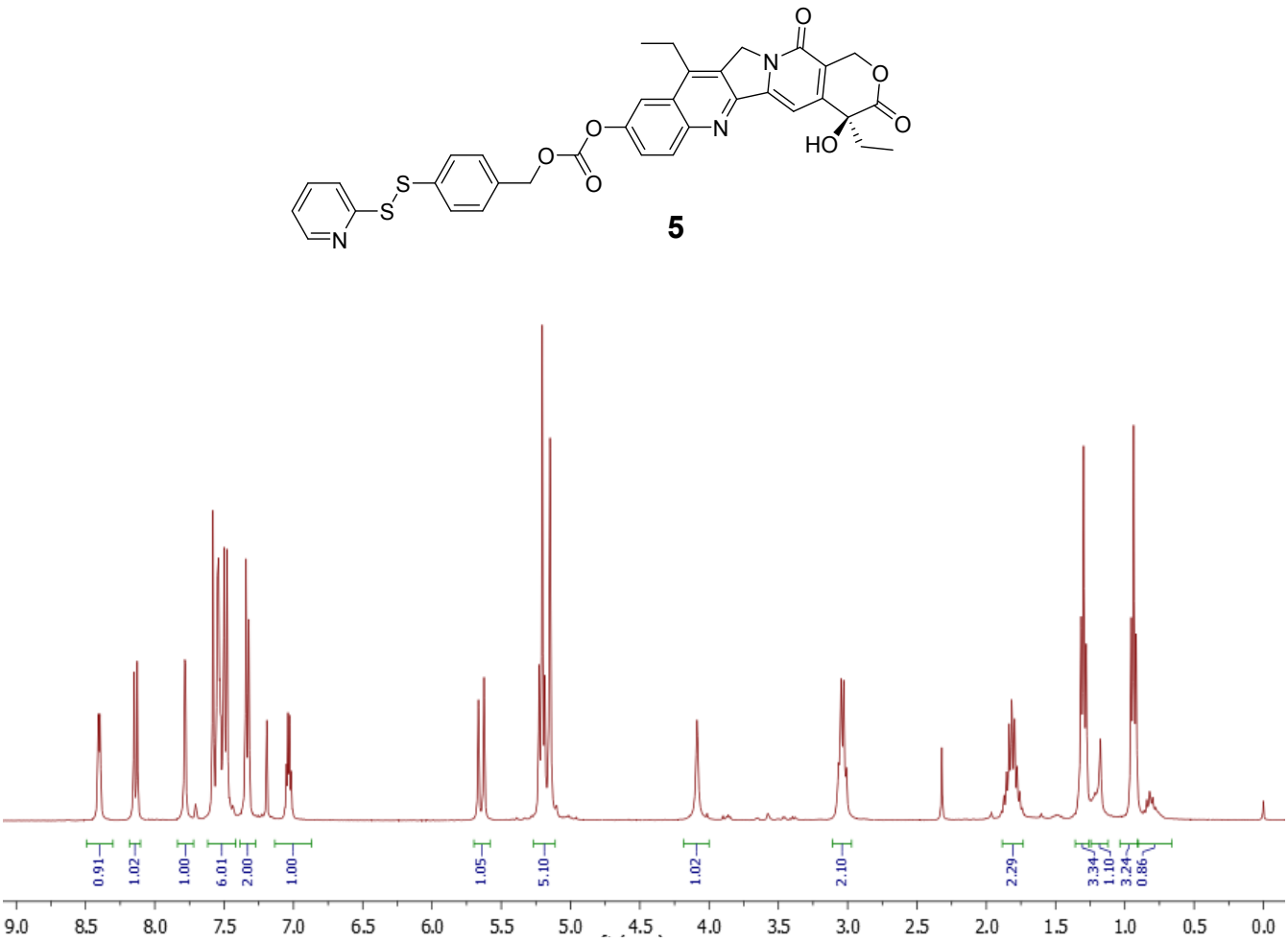


Figure S23. ^{1}H NMR spectrum of **5** in $CDCl_3$ at ambient temperature.

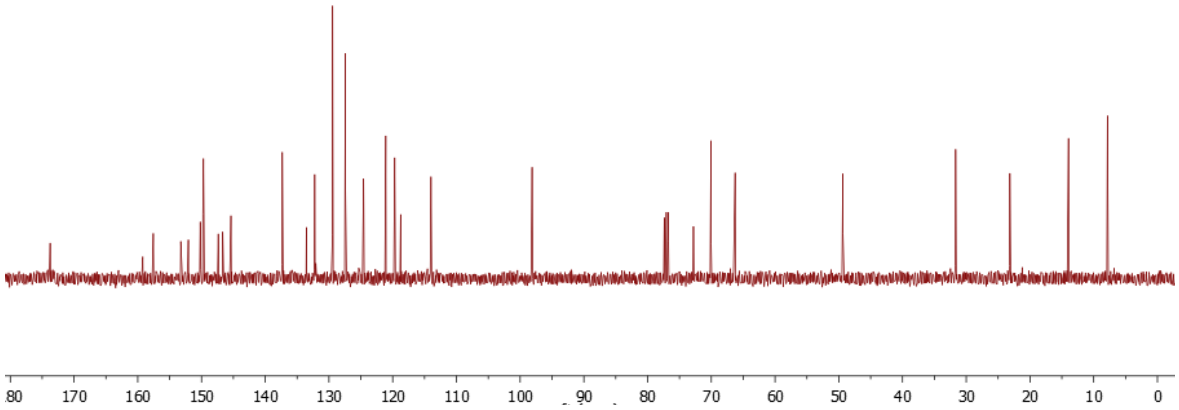


Figure S24. ^{13}C NMR spectrum of **5** in $CDCl_3$ at ambient temperature.

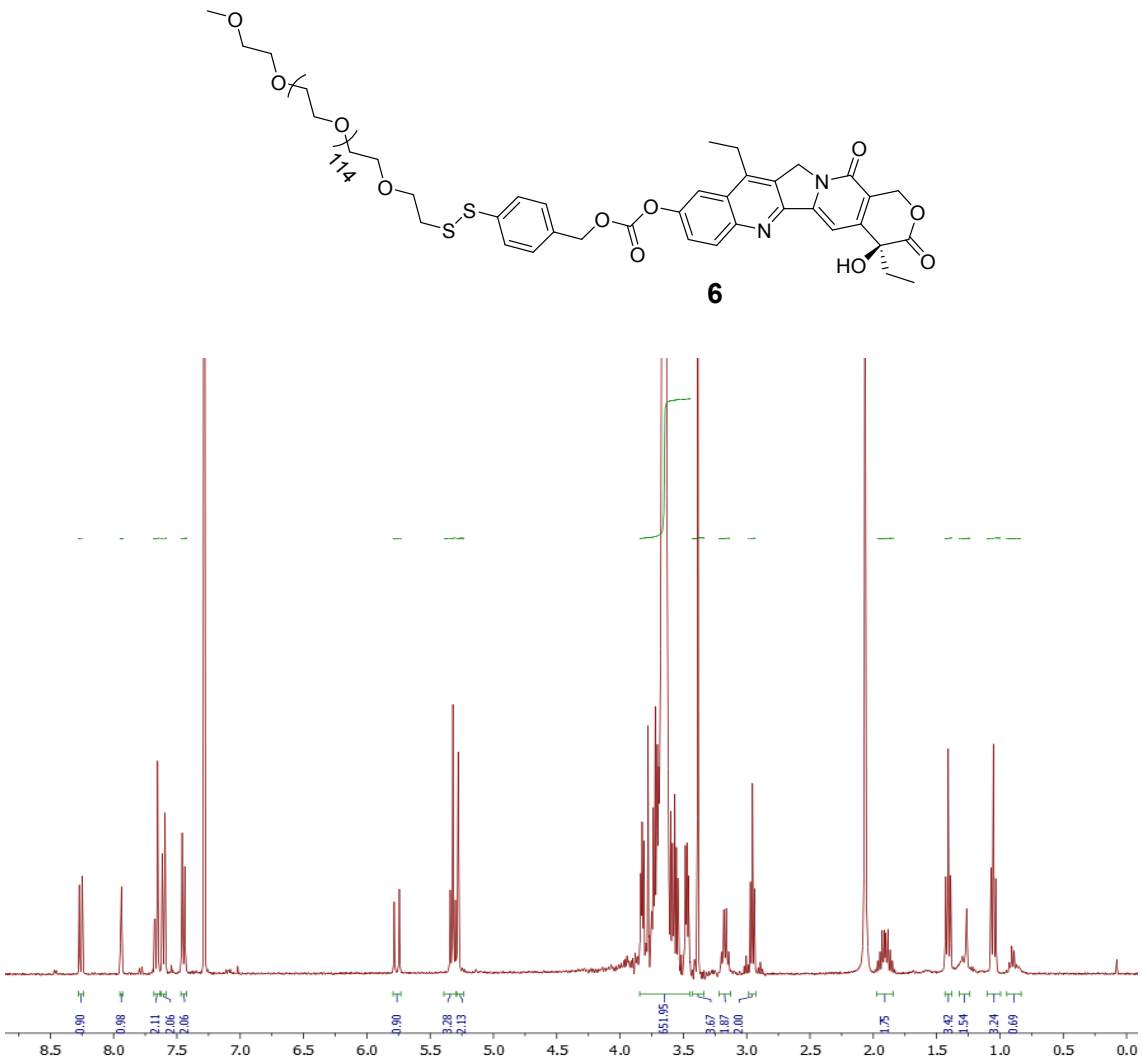


Figure S25. ^{1}H NMR spectrum of **6** in $CDCl_3$ at ambient temperature.

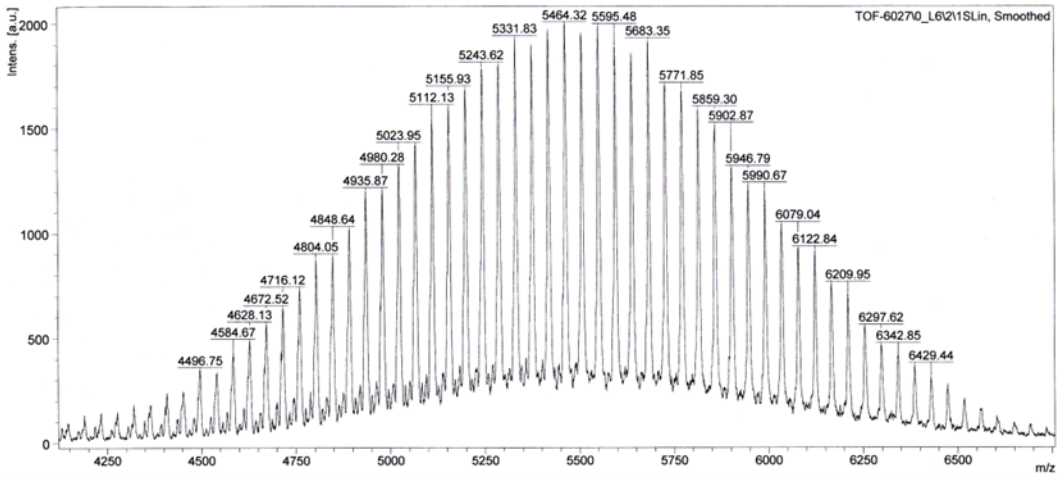


Figure S26. MALDI-TOF-MS spectrum of **6**.

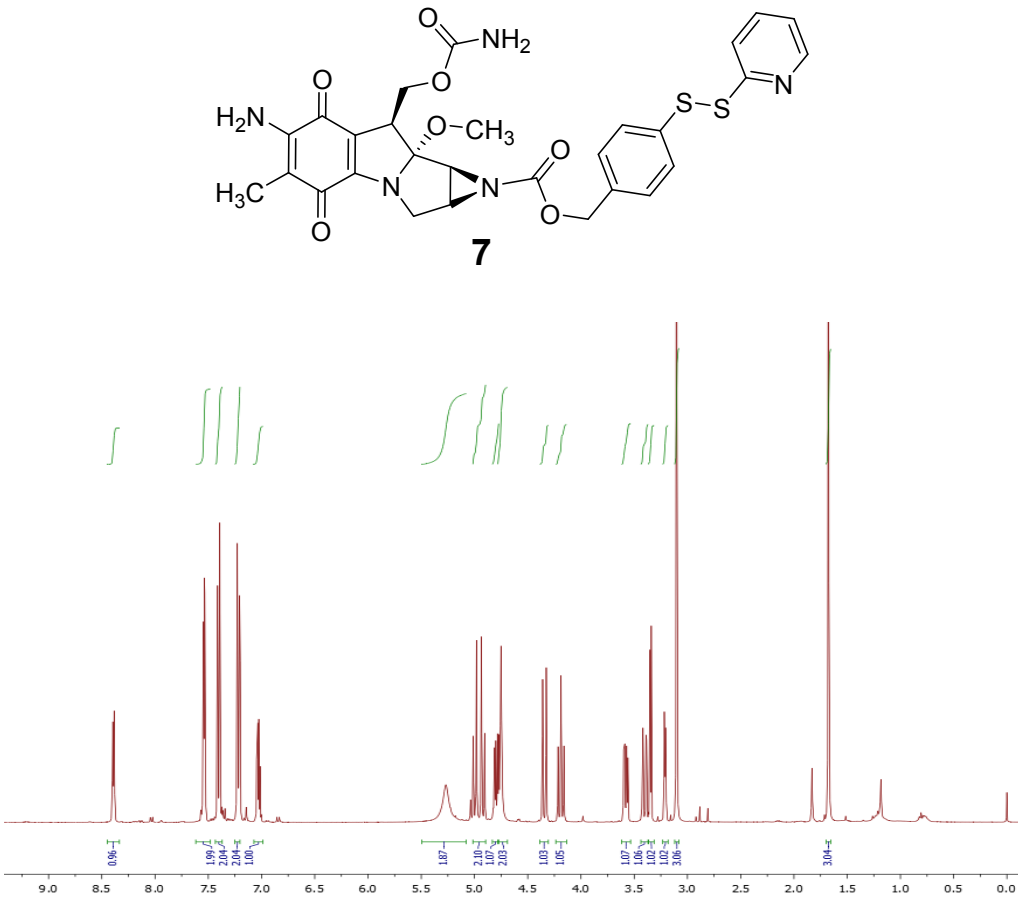


Figure S27. ^{1}H NMR spectrum of **7** in $CDCl_3$ at ambient temperature.

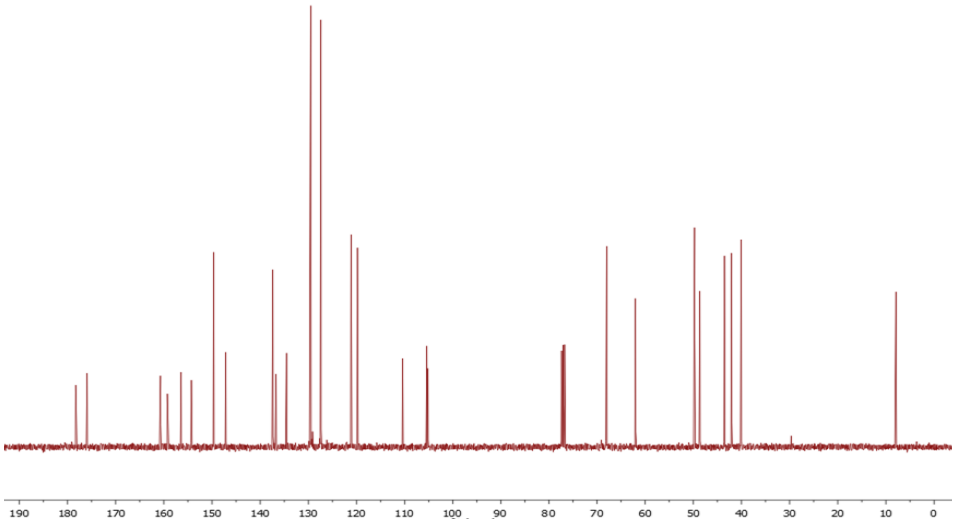
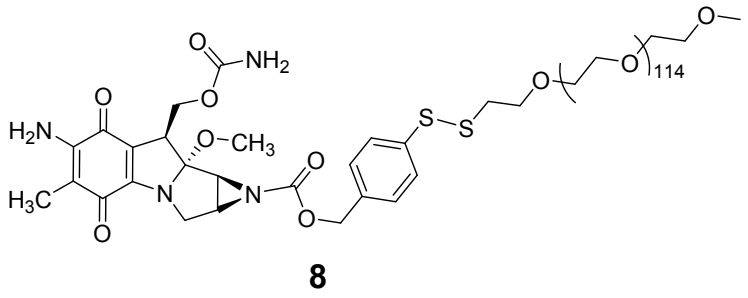


Figure S28. ^{13}C NMR spectrum of **7** in $CDCl_3$ at ambient temperature.



8

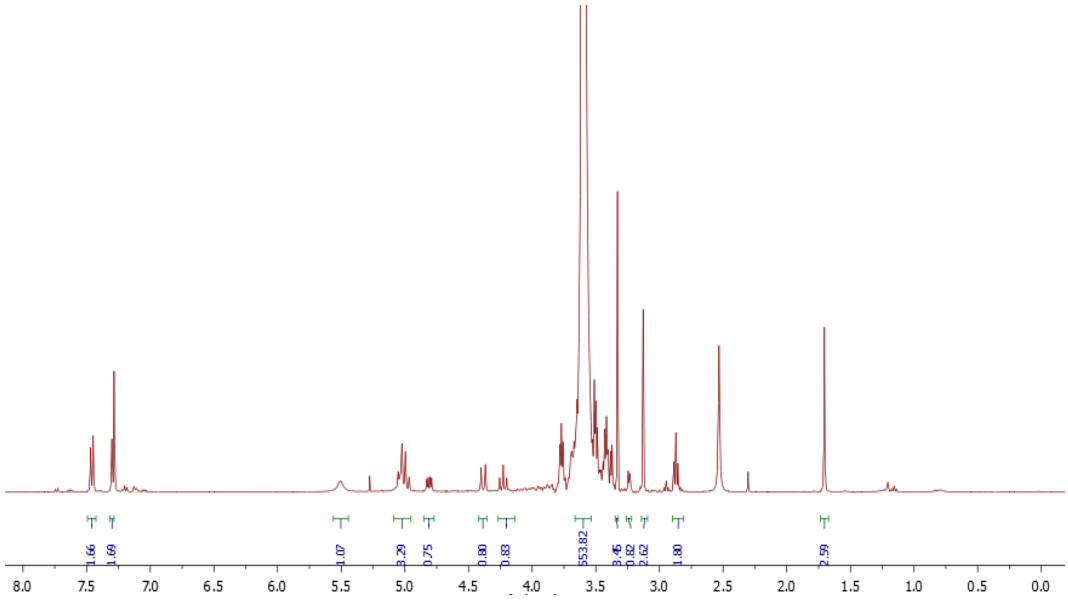


Figure S29. ^{1}H NMR spectrum of **8** in $CDCl_3$ at ambient temperature.

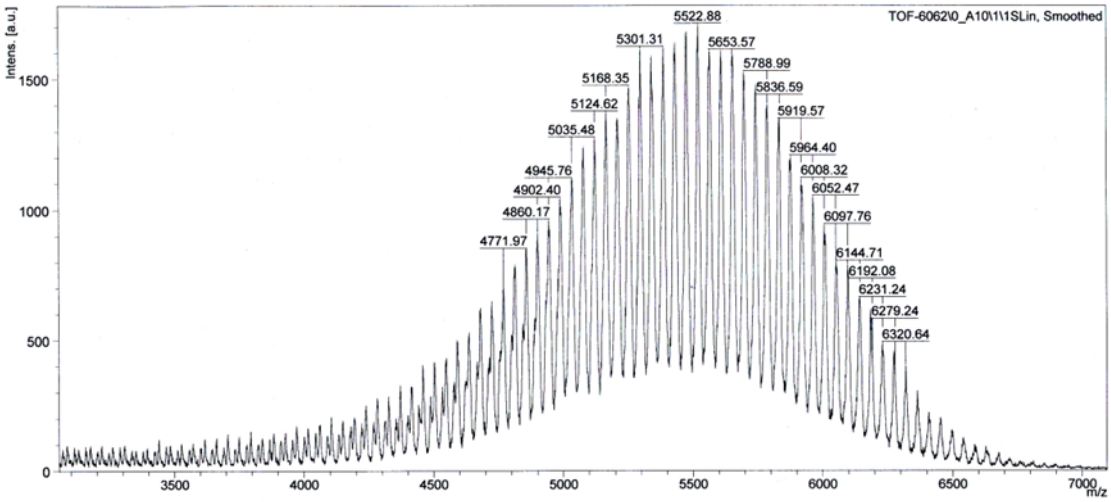
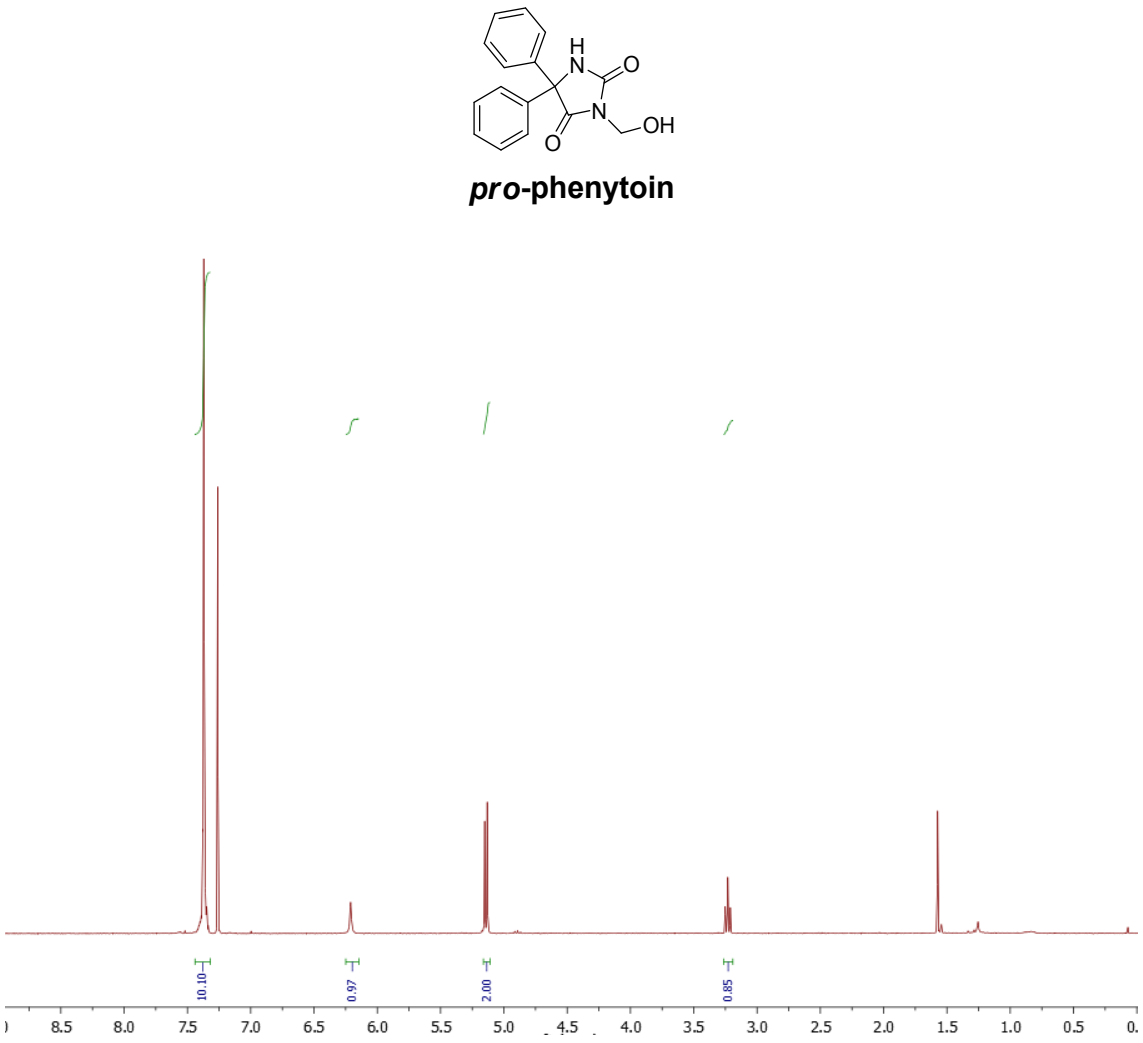


Figure S30. MALDI-TOF-MS spectrum of **8**.



***pro*-phenytoin**

Figure S31. ^{1}H NMR spectrum of ***pro*-phenytoin** in $CDCl_3$ at ambient temperature.

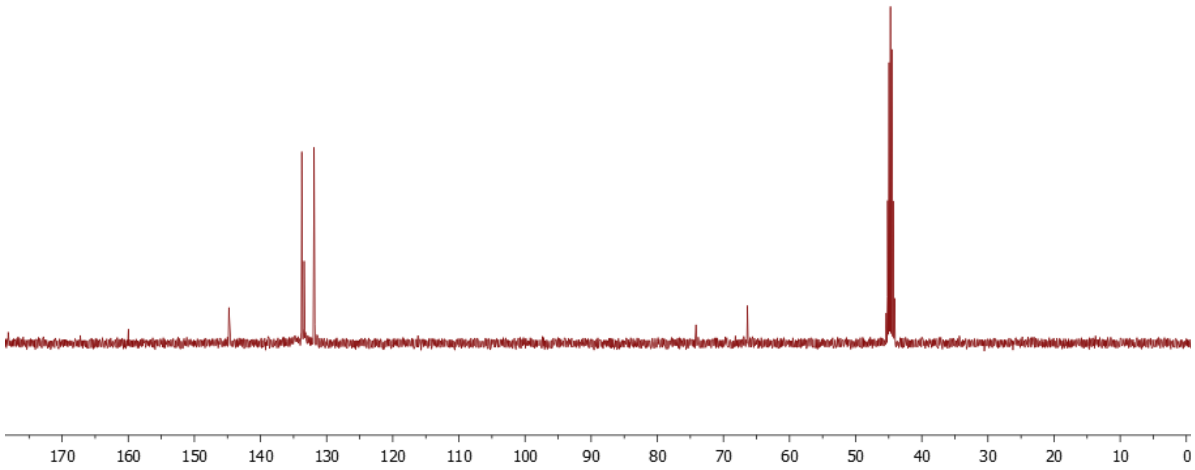
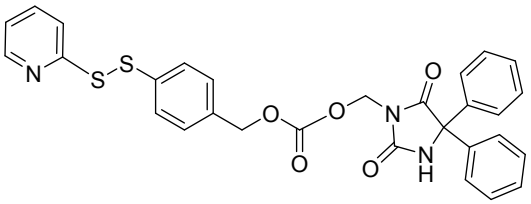


Figure S32. ^{13}C NMR spectrum of ***pro*-phenytoin** in DMSO at ambient temperature.



9

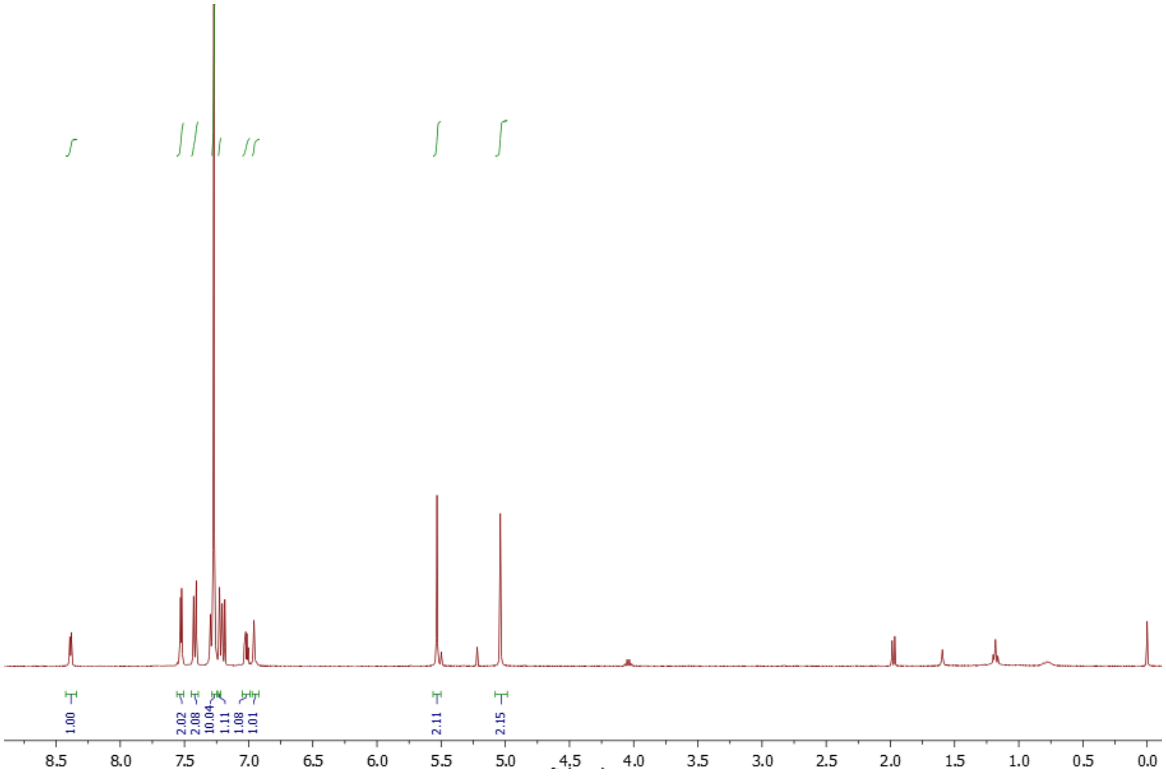


Figure S33. ^{1}H NMR spectrum of **9** in $CDCl_3$ at ambient temperature.

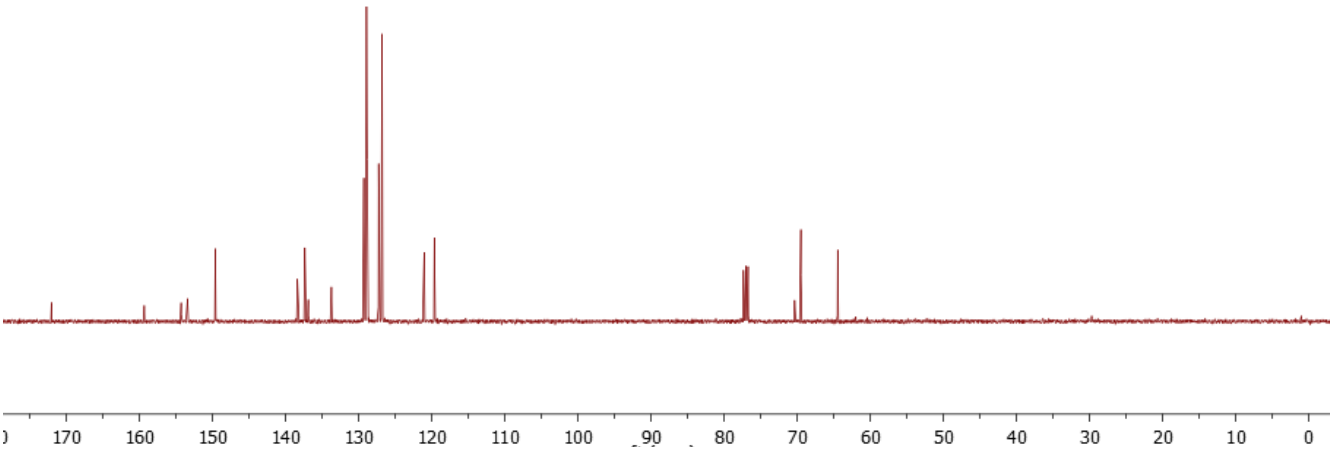
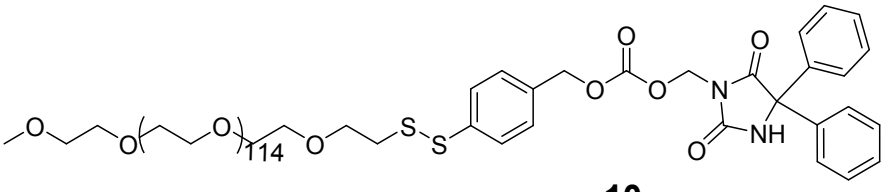


Figure S34. ^{13}C NMR spectrum of **9** in $CDCl_3$ at ambient temperature.



10

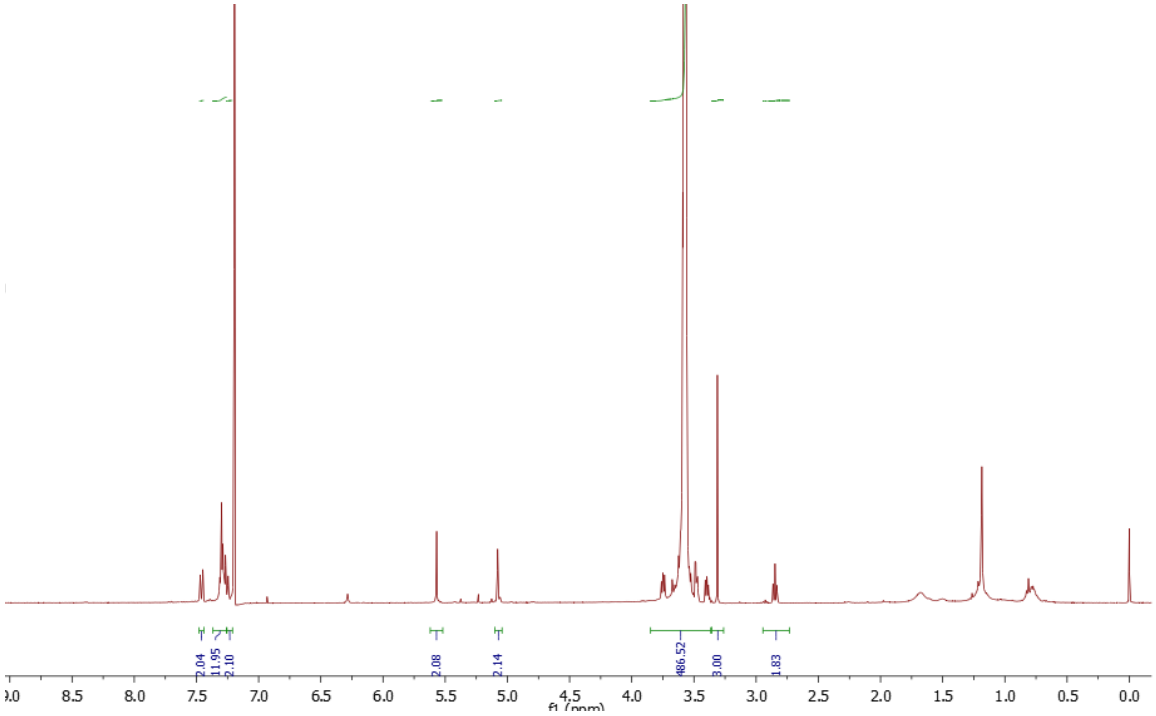


Figure S35. ^{1}H NMR spectrum of **10** in $CDCl_3$ at ambient temperature.

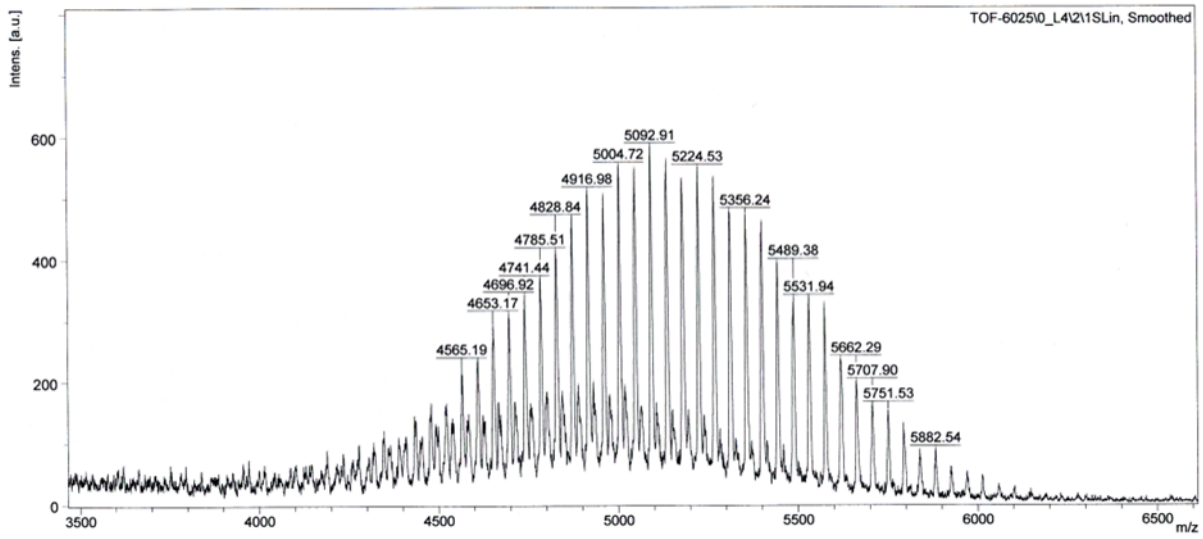


Figure S36. MALDI-TOF-MS spectrum of **10**.

7. References of supporting information

[1] T. V. DeCollo, W. J. Lees, *J. Org. Chem.* **2001,** *66,* 4244-4249.

[2] S. A. Varia, S. Schuller, K. B. Sloan, V. J. Stella, *J. Pharm. Sci.* **1984**, *73*, 1068-1073.

[3] T.-J. Fu, U. R. Abbott, C. Hatzos, *J. Agric. Food Chem.* **2002**, *50*, 7154-7160.

[4] P. R. Regmi, N. S. Ferguson, R. T. Zijlstra, *J. Anim. Sci.* **2009**, *87*, 3620-3629.

[5] S. A. Varia, S. Schuller, K. B. Sloan, V. J. Stella, *J. Pharm. Sci.* **1984**, *73*, 1068-1073.

[6] T. Kakutani, R. Atsumi, E. Sumimoto, M. Hashida, *Chem. Pharm. Bull. (Tokyo)* **1987**, *35*, 4907-4914.

[7] J. A. Zhang, T. Xuan, M. Parmar, L. Ma, S. Ugwu, S. Ali, I. Ahmad, *Int. J. Pharm.* **2004**, *270*, 93-107.