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The synthesis of sphingomyelin-derived Paclitaxel protocol

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We use this protocol and it's working

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Abstract

The Sphingomyelin (SM)-derived Paclitaxel (PTX) conjugates (SM-PTX) were constructed by linking the 2'-hydroxyl group on PTX with SM's hydroxyl group. The conjugation was realized by using a sophisticated condensation reaction. The chemical structures and purity of SM-PTX conjugates were confirmed by ^1H , ^{13}C , and HSQC NMRs, high-performance liquid chromatography (HPLC) and high-resolution mass spectrometry (HRMS).

Materials

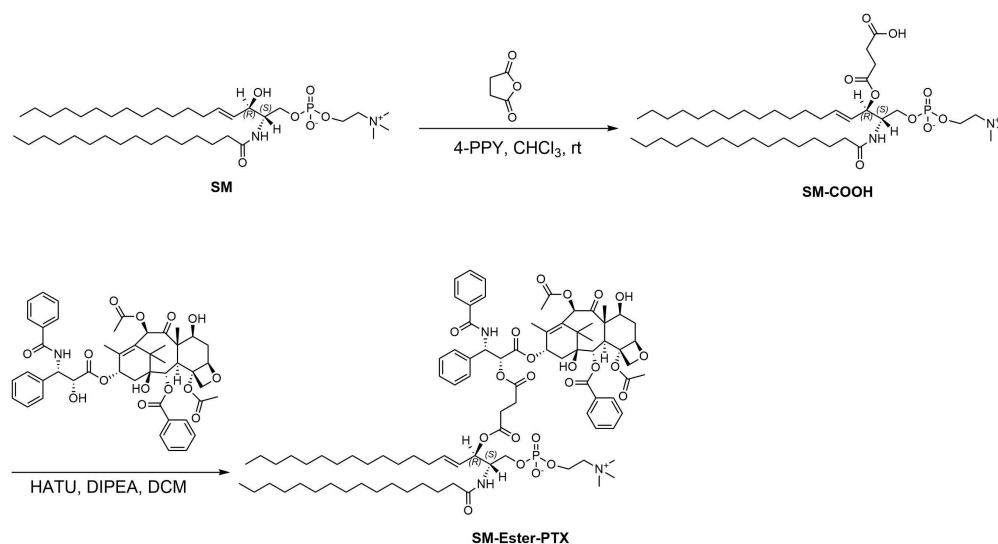
Paclitaxel (PTX, 98%), Gemcitabine hydrochloride (GEM, 98%), 4-Dimethylaminopyridine (DMAP, 98%), O-(7-Azabenzotriazol-1-yl)-N,N,N,N-tetramethyl uronium hexafluorophosphate (HATU, 98%), (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid (98%), 2-(azepan-1-yl)ethan-1-ol (98%), azocane (98%), 2-bromoethan-1-ol (98%) and (tert-butoxycarbonyl)glycine (98%) were purchased from BLDpharm (Shanghai, China). Sphingomyelin (SM, egg, 99%) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] sodium salt (DSPE-PEG_{2K}, 99%) were purchased from Lipoid LLC (Ludwigshafen, Germany). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-maleimide (DSPE-Maleimide, 98%), hydrogenated soya phosphatidylcholine (HSPC, 99%), 1,2-distearoyl-sn-glycero-3-phosphorylcholine (DSPC, 99%), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, 99%), soy phosphatidylcholine (SPC, 99%), lecithin (99%) and cholesterol (Chol, 99%) were purchased from Avanti Polar Lipids, LLC (Alabama, USA). Succinic anhydride (98%), N, N-diisopropylethylamine (98%), 4-pyrrolidinopyridine (4-PPY, 98%), 2-mercaptoacetic acid (99%), 4-nitrophenyl carbonochloridate (98%), triethylamine (TEA, 99%) and 2, 2'-dithiodiethanol (98%) were purchased from Sigma-Aldrich (MO, USA). Peptide cgggCERVIGTGWVRC (98%) was purchased from Yuan peptide Inc. (Nanjing, China). Matrigel was purchased from Corning, Discovery labware Inc. (USA). Trypsin-EDTA solution, Triton X-100, and Dulbecco's Modified Eagle's Medium (DMEM), RPMI-1640 medium, fetal bovine serum (FBS), and penicillin-streptomycin solution were all purchased from Gibco (MD, USA). All solvents used for chemical reactions were anhydrous, and the eluting solvents for compound purification were HPLC grade.

Chemical synthesis

- 1 The NMR spectra were acquired by Bruker topspin software (v. 2.1) using TMS (0 ppm) as the internal standard on a AVIII 400 or 500 MHz spectrometer and analysed by MestReNova (v. 6.0.2). ¹H NMR data were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, m = multiplet), coupling constant in Hertz (Hz) and hydrogen numbers based on integration intensities. ¹³C NMR chemical shifts are reported in ppm relative to the central peak of TMS (0 ppm) as internal standards. The high-resolution mass spectra (HRMS) were generated via a LTQ Orbitrap Velos mass spectrometer with an ESI source (Thermo Scientific). The low-resolution mass spectra were generated using a LCMS-2020 + DUIS-2020 (Shimadzu). The reactions were followed by thin-layer chromatography (TLC, Silica gel 60 F254, Merck KGaA) on glass-packed precoated silica gel plates and visualized in an iodine chamber or with a UV lamp. Flash column chromatography was performed using silica gel (SiliaFlash® P60, 230–400 mesh) purchased from Silicycle Inc.

The synthesis of SM-Ester-PTX

- 2 Synthetic route for SM-Ester-PTX with ester bond (SM-Ester-PTX) was indicated as below



Supplementary Scheme 1, Synthetic route for SM-Ester-PTX with ester bond (SM-Ester-PTX).

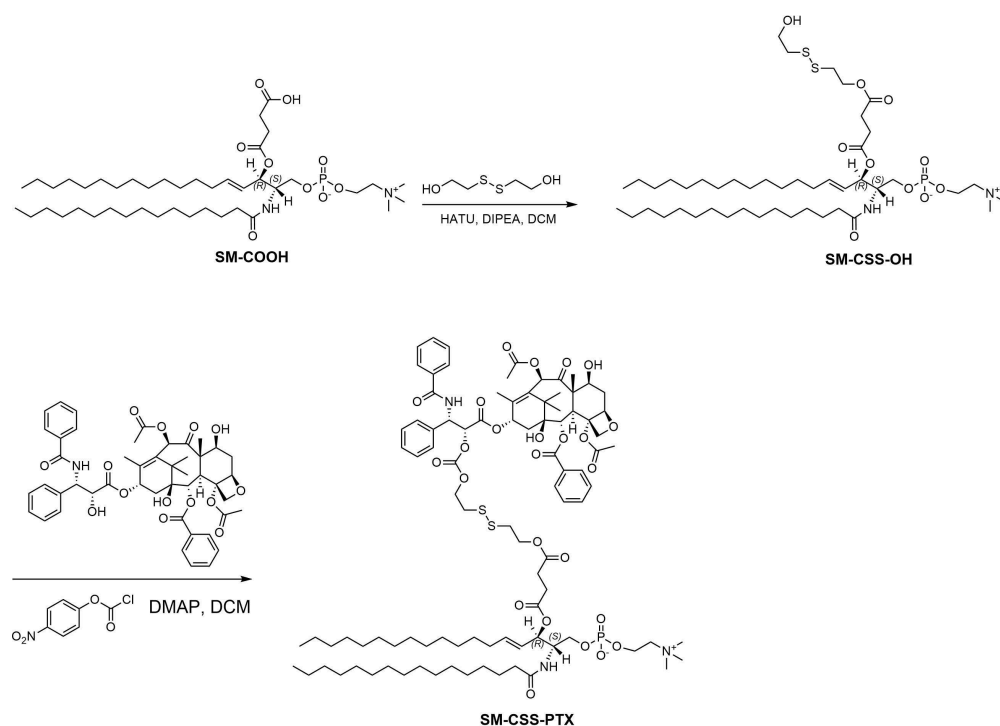
- 3 (2S,3R,E)-3-((3-carboxypropanoyl)oxy)-2-palmitamidooctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate (SM-COOH)

- 3.1 The synthesis of SM-COOH was according to our previous publication¹. 4-pyrrolidinopyridine (4-PPY, 44.4 mg, 0.3 mmol) was added to a solution of sphingomyelin (2.1 g, 3.0 mmol) and succinic anhydride (3 g, 30 mmol) in anhydrous CHCl_3 (100 mL). The solution was stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, CH_3OH (30 mL) was added into the mixture solution, the reaction was further stirred at room temperature for 12 h. The solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with $\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O}$ (v/v/v, 300/200/36) as the elution solvent. White solid with 93% yield was garnered. $R_f = 0.23$ ($\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O} = 300/200/36$). ^1H NMR (400 MHz, CDCl_3) δ 6.98 (d, $J = 6.7$ Hz, 1H), 5.75 – 5.68 (m, 1H), 5.39 (dd, $J = 15.0, 8.3$ Hz, 1H), 5.28 (t, $J = 8.8$ Hz, 1H), 4.29 (d, $J = 4.4$ Hz, 3H), 3.94 (s, 2H), 3.77 (s, 2H), 3.28 (s, 9H), 2.64 (dd, $J = 13.1, 6.3$ Hz, 2H), 2.39 (dd, $J = 14.3, 6.6$ Hz, 2H), 2.12 (q, $J = 13.9$ Hz, 2H), 1.97 (d, $J = 6.6$ Hz, 2H), 1.55 (s, 2H), 1.28 (d, $J = 19.0$ Hz, 46H), 0.88 (t, $J = 6.7$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.74, 173.10, 172.06, 137.75, 125.20, 73.28, 65.76, 65.70, 64.26, 64.23, 59.20, 59.15, 54.32, 50.66, 50.61, 36.66, 32.25, 31.88, 29.74, 29.71, 29.70, 29.69, 29.63, 29.60, 29.56, 29.50, 29.46, 29.33, 28.92, 25.79, 22.64, 14.06. HRMS (ESI) m/z $[\text{M} + \text{H}]^+$ for $\text{C}_{43}\text{H}_{84}\text{N}_2\text{O}_9\text{P}$ calculated 803.5909, found 803.5928.
- 4 (2S,3R,E)-3-(((4-(((1S,2R)-1-benzamido-3-(((2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-6,12b-diacetoxy-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-9-yl)oxy)-3-oxo-1-phenylpropan-2-yl)oxy)-4-oxobutanoyl)oxy)-2-palmitamidooctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate (SM-Ester-PTX)
- 4.1 DIPEA (2 mL) was added to a solution of SM-COOH (803.0 mg, 1.0 mmol) and HATU (456 mg, 1.5 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 30 min. A solution of PTX (386 mg, 1.0 mmol) in 10 mL anhydrous DCM was added into the reaction and further stirred for 12 h. The reaction was monitored by TLC, after completion of the reaction, the reaction mixture was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na_2SO_4 , the solvent was removed using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with $\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O}$ (v/v/v, 300/200/36) as the eluting solvent. White solid with 86% yield was achieved. $R_f = 0.32$ ($\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O} = 300/200/36$). ^1H NMR (400 MHz, CDCl_3) δ 9.76 (s, 1H), 8.05 (t, $J = 7.9$ Hz, 4H), 7.69 (t, $J = 7.4$ Hz, 2H), 7.57 (t, $J = 6.8$ Hz, 4H), 7.41 (d, $J = 6.7$ Hz, 3H), 7.33 (t, $J = 7.6$ Hz, 2H), 7.05 – 7.00 (m, 1H), 6.68 (d, $J = 8.7$ Hz, 1H), 6.29 (s, 1H), 5.94 (t, $J = 8.7$ Hz, 1H), 5.67 (tt, $J = 16.2, 8.1$ Hz, 4H), 5.57 (d, $J = 6.9$ Hz, 1H), 5.32 (dd, $J = 15.2, 8.1$ Hz, 1H), 5.03 (d, $J = 8.8$ Hz, 1H), 4.88 (d, $J = 9.4$ Hz, 1H), 4.29 (s, 3H), 4.23 (d, $J = 8.4$ Hz, 1H), 4.09 (d, $J = 8.3$ Hz, 1H), 4.02 (d, $J = 8.6$ Hz, 1H), 3.73 – 3.64 (m, 2H), 3.63 – 3.55 (m, 3H), 3.30 (d, $J = 8.1$ Hz, 1H), 3.22 (s, 9H), 2.97 – 2.83 (m, 2H), 2.68 (d, $J = 5.0$ Hz, 1H), 2.64 (s, 1H), 2.55 (s, 2H), 2.44 (d, $J = 9.1$ Hz, 5H), 2.18 (s, 3H), 2.12 – 2.07 (m, 2H),

1.97 – 1.90 (m, 3H), 1.80 (s, 3H), 1.67 (s, 1H), 1.63 (s, 3H), 1.54 (s, 2H), 1.26 (s, 46H), 1.16 (s, 3H), 1.09 (s, 3H), 0.88 (t, $J = 6.7$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 203.50, 173.01, 171.92, 171.31, 170.41, 169.56, 167.57, 166.79, 141.44, 137.86, 137.72, 134.45, 133.89, 132.69, 131.50, 130.12, 129.22, 128.67, 128.61, 128.50, 128.36, 128.25, 127.79, 124.91, 84.08, 80.89, 78.62, 77.27, 76.20, 75.76, 75.69, 74.65, 73.86, 71.32, 70.69, 66.82, 66.76, 63.75, 58.67, 58.63, 57.89, 55.05, 54.37, 50.81, 50.74, 46.54, 43.03, 36.71, 34.46, 32.29, 31.93, 30.11, 29.74, 29.68, 29.64, 29.61, 29.53, 29.48, 29.37, 29.20, 28.92, 26.52, 25.94, 23.30, 22.69, 21.57, 21.03, 14.77, 14.13, 10.02. HRMS (ESI) m/z $[\text{M} + \text{H}]^+$ for $\text{C}_{90}\text{H}_{133}\text{N}_3\text{O}_{22}\text{P}$ calculated 1638.91138, found 1638.91732.

The synthesis of SM-CSS-PTX

- 5 Synthetic route for SM-derived PTX with disulfide bond and longer linker (SM-CSS-PTX) was indicated as below



Supplementary Scheme 2, Synthetic route for SM-derived PTX with disulfide bond and longer linker (SM-CSS-PTX).

- 6 (2S,3R,E)-3-((4-(2-((2-hydroxyethyl)disulfaneyl)ethoxy)-4-oxobutanoyl)oxy)-2-palmitamidooctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate (SM-CSS-OH)
- 6.1 DIPEA (2 mL) was added to a solution of SM-COOH (1.6 g, 2.0 mmol) and HATU (912 mg, 3.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room

temperature for 30 min, then a solution of 2, 2'-dithiodiethanol (0.925 g, 6 mmol) in anhydrous THF (25 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid with 88% yield was acquired. *R*_f = 0.28 (CHCl₃/EtOH/H₂O (v/v/v, 300/200/36)). ¹H NMR (400 MHz, CDCl₃) δ 7.34 (d, *J* = 8.8 Hz, 1H), 5.74 (dd, *J* = 14.7, 6.9 Hz, 1H), 5.41 (dd, *J* = 15.0, 8.0 Hz, 1H), 5.33 (t, *J* = 7.8 Hz, 1H), 4.41 – 4.26 (m, 5H), 3.92 (s, 2H), 3.80 (t, *J* = 5.6 Hz, 4H), 3.34 (s, 9H), 2.91 (dd, *J* = 11.0, 5.1 Hz, 4H), 2.70 – 2.56 (m, 4H), 2.15 (t, *J* = 7.4 Hz, 2H), 2.02 – 1.94 (m, 2H), 1.56 (s, 2H), 1.27 (d, *J* = 19.5 Hz, 46H), 0.88 (t, *J* = 6.7 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 173.31 (s), 172.35 (s), 171.08 (s), 137.56 (s), 124.59 (s), 74.05 (s), 66.26 (d, *J* = 5.9 Hz), 63.91 (s), 62.64 (s), 59.98 (s), 59.26 (d, *J* = 4.8 Hz), 54.41 (s), 51.15 (d, *J* = 5.2 Hz), 42.35 (s), 36.84 (d, *J* = 18.8 Hz), 32.31 (s), 31.87 (s), 29.94 – 29.22 (m), 28.95 (s), 25.87 (s), 22.63 (s), 14.06 (s).

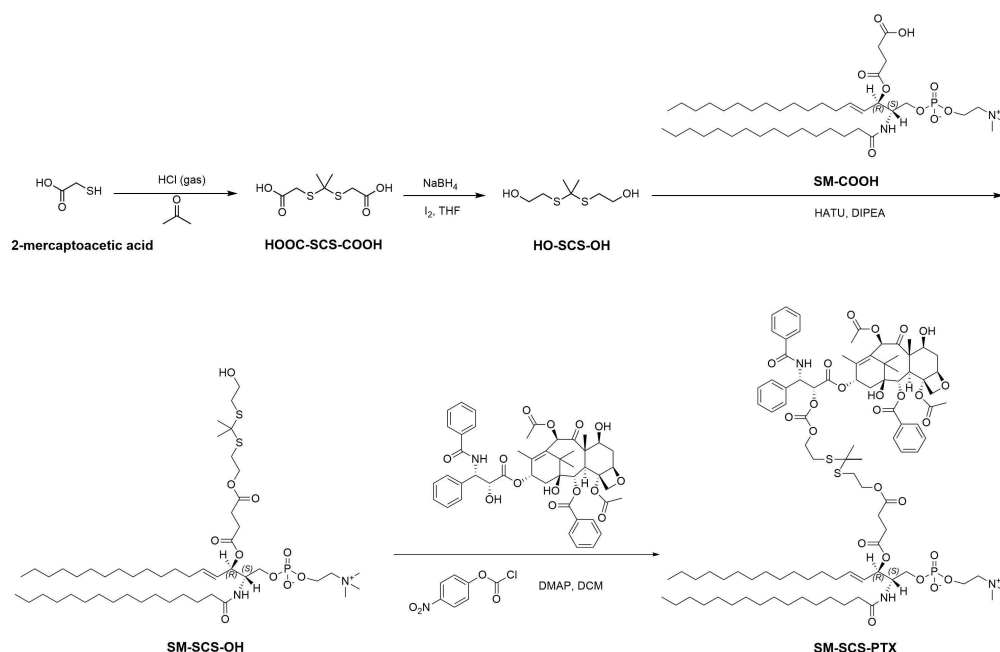
7 (3S,4R,20R,21S)-4-((((2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-6,12b-diacetoxy-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-9-yl)oxy)carbonyl)-1,6,15,18-tetraoxo-21-palmitamido-20-((E)-pentadec-1-en-1-yl)-1,3-diphenyl-5,7,14,19-tetraoxa-10,11-dithia-2-azadocosan-22-yl (2-(trimethylammonio)ethyl) phosphate (SM-CSS-PTX)

7.1 DMAP (244.4 mg, 2.0 mmol) was added to a solution of SM-CSS-OH (939.3 mg, 1.0 mmol) and 4-nitrophenyl carbonochloridate (403 mg, 1.5 mmol) in anhydrous DCM (50 mL). The solution mixture was stirred at room temperature for 30 min. A solution of PTX (854 mg, 1.0 mmol) in 10 mL anhydrous DCM was then added into the reaction mixture and further stirred for 12 h. After completion of the reaction, the reaction mixture was washed with 50 mM HCl aqueous solution and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with CHCl₃/EtOH/H₂O (v/v/v, 300/200/36) as the eluting solvent. White solid with 91% yield was attained. *R*_f = 0.34 (CHCl₃/EtOH/H₂O = 300/200/36). ¹H NMR (400 MHz, CDCl₃) δ 8.10 (d, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 7.4 Hz, 2H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.54 – 7.43 (m, 5H), 7.37 (dd, *J* = 13.1, 7.1 Hz, 4H), 7.26 – 7.21 (m, 1H), 6.33 (s, 1H), 6.14 (t, *J* = 8.7 Hz, 1H), 5.85 (dd, *J* = 8.3, 5.3 Hz, 1H), 5.75 – 5.67 (m, 1H), 5.63 (d, *J* = 6.6 Hz, 1H), 5.53 (d, *J* = 5.1 Hz, 1H), 5.39 (dd, *J* = 15.0, 7.9 Hz, 1H), 5.30 (t, *J* = 7.8 Hz, 1H), 4.93 (d, *J* = 9.5 Hz, 1H), 4.43 – 4.33 (m, 3H), 4.27 (s, 6H), 4.14 (d, *J* = 8.1 Hz, 1H), 3.91 (s, 3H), 3.75 (d, *J* = 6.5 Hz, 1H), 3.65 (s, 2H), 3.38 (d, *J* = 43.4 Hz, 1H), 3.23 (s, 9H), 2.93 (t, *J* = 6.3 Hz, 2H), 2.86 (t, *J* = 6.2 Hz, 2H), 2.74 – 2.47 (m, 6H), 2.43 (s, 3H), 2.18 (s, 4H), 2.11 (dd, *J* = 14.4, 6.8 Hz, 3H), 2.01 – 1.93 (m, 3H), 1.90 (s, 4H), 1.65 (s, 3H), 1.52 (s, 2H), 1.24 (d, *J* = 8.5 Hz, 46H), 1.20 (s, 3H), 1.13 (s, 3H), 0.87 (t, *J* = 6.7 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 203.76, 173.36, 172.26, 171.18, 170.59, 170.19, 168.55, 167.20, 166.86, 153.93,

141.76, 137.46, 136.96, 133.74, 132.94, 131.81, 130.17, 129.30, 128.99, 128.69, 128.54, 127.51, 127.32, 124.64, 84.41, 81.01, 78.79, 76.35, 75.62, 74.95, 74.10, 71.86, 71.76, 66.66, 66.54, 66.48, 63.92, 62.35, 59.17, 59.14, 58.34, 54.46, 53.71, 51.23, 46.06, 43.19, 37.16, 36.77, 36.69, 36.01, 35.17, 32.36, 31.91, 29.73, 29.72, 29.68, 29.66, 29.63, 29.57, 29.55, 29.42, 29.39, 29.36, 29.00, 26.68, 25.89, 25.15, 22.92, 22.67, 21.84, 20.93, 14.76, 14.11, 9.82. HRMS (ESI) m/z $[M + H]^+$ for $C_{95}H_{140}N_3O_{25}PS_2$ calculated 1819.90606, found 1819.90470.

The synthesis of SM-SCS-PTX

- 8 Synthetic route for SM-derived PTX with thioketal bond and longer linker (SM-SCS-PTX) was indicated as below



Supplementary Scheme 3, Synthetic route for SM-derived PTX with thioketal bond and longer linker (SM-SCS-PTX).

- 9 2,2'-(propane-2,2-diylbis(sulfanediy))diacetic acid ($HOOC-SCS-COOH$)²

- 9.1 2-mercaptoacetic acid (92 g, 1 mol) was dissolved in 500 mL anhydrous acetone at room temperature, HCl gas was bubbled into the mixture solution for 24 h. After the completion of the reaction, the precipitation was filtered under reduced pressure, the solid was collected. White solid with 77% yield was attained. 1H NMR (400 MHz, DMSO) δ 12.61 (s, 2H), 3.38 (s, 4H), 1.55 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 171.83, 56.68, 33.26, 30.58.

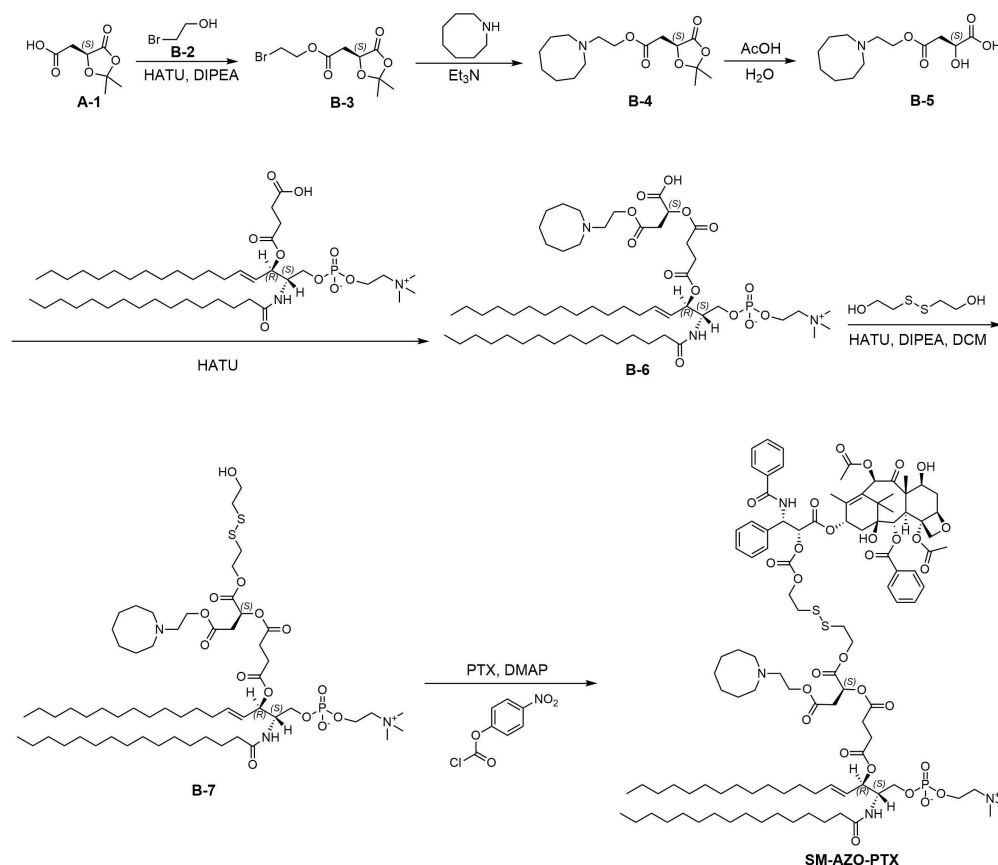
- 10 2,2'-(propane-2,2-diylbis(sulfanediy))bis(ethan-1-ol) (HO-SCS-OH)
- 10.1 Sodium borohydride (5.0 g, 0.132 mol), dry THF (100 mL), and HOOC-SCS-COOH (5.0 g, 0.022 mol) were charged into a 500 mL flame-dry three-necked flask fitted with magnetic stir bar and a reflux condenser. The flask was cooled in an ice bath. Subsequently, iodine (20.0 g, 0.057 mol) in dry THF (100 mL) was added via an addition funnel slowly over 1 h. Then, the flask was heated to reflux for 24 h and cooled to room temperature. Methanol (50 mL) was then added cautiously until the mixture become clear. After stirring for 45 min, the solvent was removed under vacuum. The mixture was further dissolved in NaOH solution (25%, 200 mL). The resultant solution was stirred for 5 h and extracted with ethyl acetate (5 × 100 mL) and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. Colorless oil with 52% yield was acquired. *R_f* = 0.34 (Hexane/EtOAc = 2/1). ¹H NMR (400 MHz, CDCl₃) δ 3.82 (t, *J* = 6.1 Hz, 4H), 2.90 (t, *J* = 6.1 Hz, 4H), 2.46 (s, 2H), 1.66 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 61.29, 55.89, 33.69, 31.23.
- 11 (14*R*,15*S*)-1-hydroxy-4,4-dimethyl-9,12-dioxo-15-palmitamido-14-((*E*)-pentadec-1-en-1-yl)-8,13-dioxo-3,5-dithiahexadecan-16-yl (2-(trimethylammonio)ethyl) phosphate (SM-SCS-OH)
- 11.1 DIPEA (2 mL) was added to a solution of SM-COOH (1.6 g, 2.0 mmol) and HATU (912 mg, 3.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 30 min, then a solution of HO-SCS-OH (1.17 g, 6 mmol) in anhydrous THF (25 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid with 81% yield was acquired. *R_f* = 0.29 (CHCl₃/EtOH/H₂O = 300/200/36). ¹H NMR (400 MHz, CDCl₃) δ 7.41 (d, *J* = 7.9 Hz, 1H), 5.76 – 5.62 (m, 1H), 5.37 (dd, *J* = 15.1, 7.9 Hz, 1H), 5.28 (dd, *J* = 15.1, 7.4 Hz, 1H), 4.34 – 4.12 (m, 5H), 3.86 (s, 2H), 3.74 (s, 2H), 3.71 – 3.50 (m, 2H), 3.30 (s, 9H), 2.83 (t, *J* = 6.9 Hz, 2H), 2.73 (dd, *J* = 21.5, 14.8 Hz, 2H), 2.66 – 2.49 (m, 4H), 2.20 – 2.05 (m, 2H), 2.00 – 1.89 (m, 2H), 1.71 – 1.50 (m, 6H), 1.51 (d, *J* = 6.0 Hz, 2H), 1.28 – 1.16 (m, 46H), 0.84 (t, *J* = 6.8 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 173.43 (s), 172.31 (s), 171.16 (s), 137.43 (s), 124.59 (s), 74.04 (s), 66.21 (d, *J* = 5.6 Hz), 63.98 (d, *J* = 3.7 Hz), 63.68 (s), 61.06 (s), 59.24 (d, *J* = 4.1 Hz), 56.10 (s), 54.34 (s), 51.18 (d, *J* = 5.1 Hz), 36.69 (s), 33.24 (s), 32.33 (s), 31.87 (s), 31.00 (s), 29.98 – 29.21 (m), 28.94 (d, *J* = 4.4 Hz), 25.93 (s), 22.63 (s), 14.06 (s).
- 12 (3*S*,4*R*,21*R*,22*S*)-4-((((2*aR*,4*S*,4*aS*,6*R*,9*S*,11*S*,12*S*,12*aR*,12*bS*)-6,12*b*-diacetoxy-12-(benzyloxy)-4,11-dihydroxy-4*a*,8,13,13-tetramethyl-5-oxo-2*a*,3,4,4*a*,5,6,9,10,11,12,12*a*,12*b*-dodecahydro-1*H*-7,11-methanocyclodeca[3,4]benzo[1,2-

b]oxet-9-yl)oxy)carbonyl)-11,11-dimethyl-1,6,16,19-tetraoxo-22-palmitamido-21-((E)-pentadec-1-en-1-yl)-1,3-diphenyl-5,7,15,20-tetraoxa-10,12-dithia-2-azatricosan-23-yl (2-(trimethylammonio)ethyl) phosphate (SM-SCS-PTX)

- 12.1 DMAP (244.4 mg, 2.0 mmol) was added to a solution of SM-CSS-OH (981.4 mg, 1.0 mmol) and 4-nitrophenyl carbonochloridate (403 mg, 1.5 mmol) in anhydrous DCM (50 mL). The solution mixture was stirred at room temperature for 30 min. A solution of PTX (854 mg, 1.0 mmol) in 10 mL anhydrous DCM was then added into the reaction mixture and further stirred for 12 h. After completion of the reaction, the reaction mixture was washed with 50 mM HCl aqueous solution and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with CHCl₃/EtOH/H₂O (v/v/v, 300/200/36) as the eluting solvent. White solid with 82% yield was attained. *R*_f = 0.35 (CHCl₃/EtOH/H₂O = 300/200/36). ¹H NMR (500 MHz, DMSO) δ 9.43 (d, *J* = 8.3 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 7.97 (dd, *J* = 12.0, 4.3 Hz, 3H), 7.91 – 7.79 (m, 2H), 7.73 (t, *J* = 7.4 Hz, 1H), 7.65 (dd, *J* = 10.4, 4.7 Hz, 2H), 7.57 – 7.40 (m, 8H), 7.20 – 7.14 (m, 1H), 6.30 (s, 1H), 5.85 – 5.75 (m, 1H), 5.67 – 5.60 (m, 1H), 5.59 – 5.45 (m, 2H), 5.39 (ddd, *J* = 18.6, 12.7, 7.6 Hz, 3H), 5.20 (t, *J* = 7.2 Hz, 1H), 5.15 – 5.04 (m, 1H), 5.01 (d, *J* = 6.9 Hz, 1H), 4.91 (d, *J* = 9.4 Hz, 1H), 4.66 (s, 1H), 4.30 – 4.27 (m, 1H), 4.20 – 4.08 (m, 3H), 4.01 (dd, *J* = 15.1, 8.4 Hz, 6H), 3.66 (d, *J* = 6.5 Hz, 2H), 3.62 – 3.43 (m, 4H), 3.12 (s, 9H), 2.85 (dd, *J* = 14.4, 7.1 Hz, 2H), 2.79 (t, *J* = 6.8 Hz, 2H), 2.53 (d, *J* = 3.8 Hz, 3H), 2.25 (s, 3H), 2.10 (s, 3H), 2.03 (td, *J* = 14.1, 7.0 Hz, 2H), 1.94 (dd, *J* = 14.1, 7.1 Hz, 2H), 1.82 – 1.74 (m, 4H), 1.68 – 1.59 (m, 1H), 1.51 (d, *J* = 13.3 Hz, 9H), 1.47 – 1.40 (m, 2H), 1.22 (s, 45H), 1.00 (t, *J* = 10.4 Hz, 6H), 0.84 (t, *J* = 6.9 Hz, 6H). ¹³C NMR (126 MHz, DMSO) δ 202.83, 172.54, 172.17, 171.16, 170.19, 169.47, 169.18, 166.70, 165.67, 154.14, 139.66, 137.52, 135.61, 134.51, 133.95, 131.99, 130.43, 130.05, 129.20, 129.15, 128.78, 128.68, 128.57, 128.16, 128.13, 127.96, 125.54, 84.10, 80.74, 77.65, 77.13, 75.15, 74.95, 74.03, 71.62, 70.90, 67.99, 65.94, 63.61, 58.79, 57.88, 56.54, 53.57, 46.53, 43.42, 36.08, 32.23, 31.77, 31.06, 29.58, 29.56, 29.52, 29.49, 29.45, 29.42, 29.32, 29.19, 29.10, 28.98, 28.95, 28.87, 26.81, 25.86, 23.01, 22.56, 21.82, 21.12, 14.40, 10.23. HRMS (ESI) *m/z* [M + H]⁺ for C₉₈H₁₄₆N₃O₂₅PS₂ calculated 1861.95301, found 1861.95522.

The synthesis of SM-AZO-PTX

- 13 Synthetic route for SM-AZO-PTX was indicated as below



Supplementary Scheme 4, Synthetic route for SM-AZO-PTX.

14 2-bromoethyl (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetate (B-3)

- 14.1** DIPEA (20 mL) was added to a solution of (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid (6.96 g, 40 mmol) and HATU (22.8 g, 60 mmol) in anhydrous DCM (200 mL). The reaction mixture was stirred at room temperature for 30 min, then a solution of 2-bromoethanol (6.0 g, 48 mmol) in anhydrous THF (30 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with Hexane/EtOAc = 3/1 as the eluting solvent. Colorless oil with 86% yield was acquired. *R*_f = 0.37 (Hexane/EtOAc = 3/1). ¹H NMR (500 MHz, CDCl₃) δ 4.72 (dd, *J* = 6.7, 3.9 Hz, 1H), 4.47 – 4.40 (m, 2H), 3.50 (t, *J* = 6.2 Hz, 2H), 2.97 (dd, *J* = 17.0, 3.9 Hz, 1H), 2.83 (dd, *J* = 17.0, 6.7 Hz, 1H), 1.61 (s, 3H), 1.55 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 171.86, 168.78, 111.28, 70.53, 64.41, 36.09, 28.14, 26.79, 25.84.

15 2-(azocan-1-yl)ethyl (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetate (B-4)

15.1 TEA (6 mL) was added to a solution of 2-bromoethyl (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetate (**B-3**, 1.40 g, 5 mmol) and azocane (0.56 g, 15 mmol) in anhydrous DCM (50 mL). The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with Hexane/EtOAc = 2/1 as the eluting solvent. Colorless oil with 92% yield was acquired. R_f = 0.28 (Hexane/EtOAc = 2/1). ^1H NMR (500 MHz, CDCl_3) δ 4.74 (dd, J = 6.9, 3.7 Hz, 1H), 4.24 – 4.15 (m, 2H), 2.94 (dd, J = 16.9, 3.7 Hz, 1H), 2.79 (dd, J = 17.0, 7.0 Hz, 1H), 2.75 (t, J = 6.2 Hz, 2H), 2.60 (d, J = 4.8 Hz, 4H), 1.62 (s, 5H), 1.57 (s, 5H), 1.55 – 1.53 (m, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 172.11, 169.27, 111.16, 70.73, 63.77, 56.87, 54.21, 36.43, 28.10, 27.40, 26.83, 26.17, 25.87.

16 (S)-4-(2-(azocan-1-yl)ethoxy)-2-hydroxy-4-oxobutanoic acid (B-5)

16.1 AcOH (20 mL) was added to a solution of 2-(azocan-1-yl)ethyl (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetate (3.13 g, 10 mmol), 3 mL H_2O was added into the solution. The mixture was stirred at 60 °C for 6 h and monitored by TLC. After completion of the reaction, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with Hexane/EtOAc = 1/1 as the eluting solvent. Colorless oil with 92% yield was acquired. R_f = 0.13 (Hexane/EtOAc = 2/1). ^1H NMR (500 MHz, CDCl_3) δ 4.51 (ddd, J = 12.7, 6.5, 4.2 Hz, 1H), 4.41 – 4.34 (m, 1H), 4.32 (t, J = 6.0 Hz, 1H), 3.32 (ddd, J = 13.1, 9.7, 5.4 Hz, 6H), 2.92 (dd, J = 14.6, 5.4 Hz, 1H), 2.75 (dd, J = 14.6, 6.5 Hz, 1H), 1.96 (s, 4H), 1.69 (s, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 178.36, 170.45, 68.65, 57.50, 52.63, 50.02, 40.41, 26.46, 24.27, 22.05.

17 (2S,3R,E)-3-((4-((S)-3-(2-(azocan-1-yl)ethoxy)-1-carboxy-3-oxopropoxy)-4-oxobutanoyl)oxy)-2-palmitamidooctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate (B-6)

17.1 DIPEA (2 mL) was added to a solution of SM-COOH (4.8 g, 6.0 mmol) and HATU (2.8 g, 9.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 12 h, then a solution of (S)-4-(2-(azocan-1-yl)ethoxy)-2-hydroxy-4-oxobutanoic acid (1.77 g, 6.5 mmol) in anhydrous DCM (25 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na_2SO_4 , the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid

with 63% yield was acquired. $R_f = 0.21$ ($\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O} = 300/200/36$). ^1H NMR (500 MHz, CDCl_3) δ 5.89 – 5.54 (m, 1H), 5.39 (dd, $J = 15.0, 8.0$ Hz, 1H), 5.31 (dd, $J = 16.2, 8.2$ Hz, 1H), 5.22 (dd, $J = 8.4, 3.2$ Hz, 1H), 4.29 (d, $J = 5.6$ Hz, 5H), 3.94 (s, 2H), 3.74 (s, 2H), 3.29 (s, 9H), 3.16 – 2.73 (m, 8H), 2.73 – 2.58 (m, 3H), 2.50 (dd, $J = 11.5, 5.1$ Hz, 1H), 2.14 (t, $J = 7.1$ Hz, 2H), 2.02 – 1.92 (m, 2H), 1.89 – 1.36 (m, 12H), 1.30 – 1.20 (m, 46H), 0.88 (t, $J = 6.9$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.33, 172.27, 172.06, 171.12, 170.31, 137.49, 125.12, 74.09, 70.84, 66.27, 64.12, 61.61, 59.44, 55.00, 54.36, 52.44, 51.97, 51.15, 45.45, 36.70, 36.52, 32.35, 31.95, 29.81, 29.76, 29.71, 29.70, 29.67, 29.57, 29.50, 29.42, 29.39, 29.00, 28.71, 26.32, 25.90, 22.70, 14.13, 8.67.

18 (9*S*,16*R*,17*S*)-9-(2-(2-(azocan-1-yl)ethoxy)-2-oxoethyl)-1-hydroxy-8,11,14-trioxo-17-palmitamido-16-((*E*)-pentadec-1-en-1-yl)-7,10,15-trioxa-3,4-dithiaoctadecan-18-yl (2-(trimethylammonio)ethyl) phosphate (B-7)

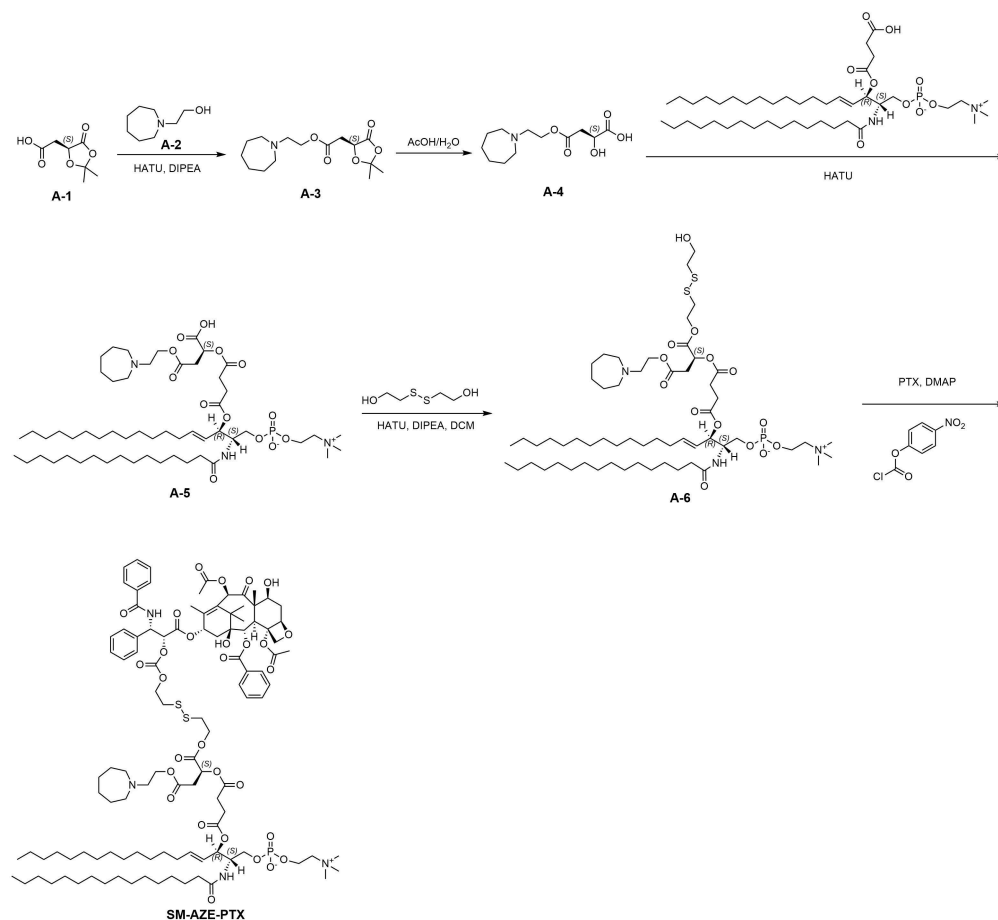
18.1 DIPEA (2 mL) was added to a solution of B-6 (2.1 g, 2.0 mmol) and HATU (912 mg, 3.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 30 min, then a solution of 2, 2'-dithiodiethanol (0.925 g, 6 mmol) in anhydrous THF (25 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na_2SO_4 , the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid with 81% yield was acquired. $R_f = 0.23$ ($\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O}$ (v/v/v, 300/200/36)). ^1H NMR (500 MHz, CDCl_3) δ 5.72 (dd, $J = 9.2, 5.6$ Hz, 1H), 5.52 (d, $J = 2.9$ Hz, 1H), 5.45 – 5.38 (m, 1H), 5.31 (dd, $J = 17.8, 9.1$ Hz, 1H), 4.72 – 4.15 (m, 7H), 3.92 (s, 2H), 3.83 (dd, $J = 14.2, 6.4$ Hz, 4H), 3.41 (s, 9H), 3.37 – 2.71 (m, 12H), 2.73 – 2.45 (m, 4H), 2.15 (t, $J = 7.1$ Hz, 2H), 1.98 (d, $J = 6.6$ Hz, 2H), 1.94 – 1.33 (m, 12H), 1.31 – 1.21 (m, 46H), 0.88 (t, $J = 6.8$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.32 (s), 171.52 (d, $J = 12.0$ Hz), 170.90 (d, $J = 29.7$ Hz), 168.97 (d, $J = 7.9$ Hz), 168.54 (d, $J = 8.0$ Hz), 137.71 (d, $J = 21.7$ Hz), 124.84 (d, $J = 9.4$ Hz), 74.01 (s), 68.45 (d, $J = 8.6$ Hz), 66.32 (s), 64.26 (s), 63.54 (s), 60.24 (s), 59.98 (s), 59.49 (d, $J = 4.4$ Hz), 57.95 (s), 54.44 (s), 51.12 (s), 41.94 (d, $J = 6.4$ Hz), 36.80 (s), 35.91 (s), 32.14 (d, $J = 52.8$ Hz), 31.93 (s), 29.85 – 29.62 (m), 29.58 (d, $J = 4.3$ Hz), 29.46 (s), 29.38 (s), 29.22 (s), 29.01 (s), 28.72 (s), 26.28 (d, $J = 3.0$ Hz), 25.91 (s), 22.69 (s), 18.51 (s), 14.13 (s).

19 (3*S*,4*R*,16*S*,23*R*,24*S*)-16-(2-(2-(azocan-1-yl)ethoxy)-2-oxoethyl)-4-(((2*aR*,4*S*,4*aS*,6*R*,9*S*,11*S*,12*S*,12*aR*,12*bS*)-6,12*b*-diacetoxo-12-(benzoyloxy)-4,11-dihydroxy-4*a*,8,13,13-tetramethyl-5-oxo-2*a*,3,4,4*a*,5,6,9,10,11,12,12*a*,12*b*-dodecahydro-1*H*-7,11-methanocyclodeca[3,4]benzo[1,2-*b*]oxet-9-yl)oxy)carbonyl)-1,6,15,18,21-pentaoxo-24-palmitamido-23-((*E*)-pentadec-1-en-1-yl)-1,3-diphenyl-5,7,14,17,22-pentaoxa-10,11-dithia-2-azapentacosan-25-yl (2-(trimethylammonio)ethyl) phosphate (SM-AZO-PTX)

- 19.1 DMAP (244.4 mg, 2.0 mmol) was added to a solution of B-7 (1.2 g, 1.0 mmol) and 4-nitrophenyl carbonochloridate (403 mg, 1.5 mmol) in anhydrous DCM (50 mL). The solution mixture was stirred at room temperature for 30 min. A solution of PTX (854 mg, 1.0 mmol) in 10 mL anhydrous DCM was then added into the reaction mixture and further stirred for 12 h. After completion of the reaction, the reaction mixture was washed with 50 mM HCl aqueous solution and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with CHCl₃/EtOH/H₂O (v/v/v, 300/200/36) as the eluting solvent. White solid with 73% yield was attained. R_f = 0.31 (CHCl₃/EtOH/H₂O = 300/200/36). ¹H NMR (500 MHz, CDCl₃) δ 8.11 (d, J = 7.5 Hz, 2H), 7.97 – 7.90 (m, 2H), 7.64 (t, J = 7.5 Hz, 1H), 7.58 – 7.47 (m, 5H), 7.41 – 7.36 (m, 4H), 7.20 (dd, J = 10.8, 7.3 Hz, 1H), 6.31 – 6.28 (m, 1H), 6.15 – 6.07 (m, 1H), 5.87 – 5.78 (m, 1H), 5.73 – 5.68 (m, 1H), 5.63 (t, J = 7.9 Hz, 1H), 5.55 (dd, J = 13.9, 6.2 Hz, 1H), 5.41 (dd, J = 15.0, 8.0 Hz, 2H), 5.33 (dd, J = 14.6, 8.0 Hz, 1H), 4.94 (d, J = 9.5 Hz, 1H), 4.44 – 4.25 (m, 10H), 4.15 (dd, J = 8.3, 3.9 Hz, 2H), 4.08 – 3.77 (m, 3H), 3.72 (dd, J = 12.7, 7.3 Hz, 1H), 3.63 (s, 2H), 3.25 (d, J = 15.9 Hz, 9H), 2.80 (dddd, J = 26.2, 16.9, 12.5, 7.6 Hz, 12H), 2.64 – 2.51 (m, 6H), 2.46 (d, J = 6.7 Hz, 3H), 2.24 – 2.17 (m, 4H), 2.17 – 2.08 (m, 3H), 1.97 (s, 3H), 1.90 – 1.82 (m, 10H), 1.66 (s, 3H), 1.62 (d, J = 4.9 Hz, 2H), 1.54 (s, 6H), 1.24 (d, J = 7.3 Hz, 46H), 1.20 (d, J = 2.6 Hz, 3H), 1.12 (d, J = 6.5 Hz, 3H), 0.88 (t, J = 6.9 Hz, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 203.77, 173.42, 173.36, 173.30, 172.28, 171.73, 171.54, 171.19, 171.12, 171.00, 170.67, 170.59, 170.23, 170.13, 169.29, 169.13, 168.68, 168.60, 168.49, 168.44, 167.28, 166.82, 153.96, 141.86, 141.69, 137.58, 137.43, 136.94, 136.89, 133.70, 132.96, 132.91, 131.84, 130.17, 129.31, 128.99, 128.68, 128.56, 128.33, 127.87, 127.45, 127.28, 127.22, 124.72, 124.66, 124.61, 84.39, 81.00, 78.74, 76.34, 75.60, 74.96, 74.11, 74.04, 71.92, 71.81, 68.51, 66.65, 66.49, 63.88, 63.09, 62.36, 59.14, 58.34, 56.64, 54.42, 54.24, 54.01, 53.55, 51.16, 51.02, 46.07, 45.96, 43.18, 37.14, 36.72, 35.90, 35.21, 32.35, 31.90, 29.72, 29.66, 29.57, 29.54, 29.40, 29.35, 28.98, 28.57, 28.46, 27.83, 27.22, 26.67, 26.16, 25.88, 22.85, 22.67, 21.88, 20.90, 14.75, 14.11, 9.78. HRMS (ESI) m/z [M + 2H]²⁺ for C₁₀₈H₁₆₁N₄O₂₉PS₂ calculated 1038.03019, found 1038.02984.

The synthesis of SM-AZE-PTX

- 20 Synthetic route for SM-AZE-PTX was indicated as below



Supplementary Scheme 5, Synthetic route for SM-AZE-PTX

21 2-(azepan-1-yl)ethyl (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetate (A-3)

- 21.1 DIPEA (20 mL) was added to a solution of (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid (6.96 g, 40 mmol) and HATU (22.8 g, 60 mmol) in anhydrous DCM (200 mL). The reaction mixture was stirred at room temperature for 30 min, then a solution of 2-(azepan-1-yl)ethan-1-ol (6.9 g, 48 mmol) in anhydrous THF (30 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with Hexane/EtOAc = 3/1 as the eluting solvent. Colorless oil with 91% yield was acquired. *R_f* = 0.31 (Hexane/EtOAc = 3/1). ¹H NMR (500 MHz, CDCl₃) δ 4.73 (dd, *J* = 6.7, 3.8 Hz, 1H), 4.29 – 4.18 (m, 2H), 2.95 (dd, *J* = 16.9, 3.7 Hz, 1H), 2.79 (dd, *J* = 11.8, 5.8 Hz, 3H), 2.75 –

2.64 (m, 4H), 1.63 (s, 8H), 1.57 (d, $J = 6.0$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 172.08, 169.22, 111.16, 70.72, 63.28, 55.71, 55.55, 36.38, 28.00, 27.03, 26.82, 25.87.

22 (S)-4-(2-(azepan-1-yl)ethoxy)-2-hydroxy-4-oxobutanoic acid (A-4)

22.1 AcOH (20 mL) was added to a solution of 2-(azepan-1-yl)ethyl (S)-2-(2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl)acetate (3.0 g, 10 mmol), 3 mL H_2O was added into the solution. The mixture was stirred at 60 °C for 6 h and monitored by TLC. After completion of the reaction, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with Hexane/EtOAc = 1/1 as the eluting solvent. Colorless oil with 92% yield was acquired. $R_f = 0.25$ (Hexane/EtOAc = 1/1). ^1H NMR (500 MHz, CDCl_3) δ 4.55 – 4.46 (m, 1H), 4.42 – 4.34 (m, 1H), 4.29 (t, $J = 6.0$ Hz, 1H), 3.29 (d, $J = 3.5$ Hz, 6H), 2.88 – 2.75 (m, 2H), 1.92 (s, 4H), 1.71 (s, 4H). ^{13}C NMR (126 MHz, CDCl_3) δ 178.15, 170.91, 68.53, 58.15, 54.57, 54.44, 40.29, 26.98, 23.13.

23 (2S,3R,E)-3-((4-((S)-3-(2-(azepan-1-yl)ethoxy)-1-carboxy-3-oxopropoxy)-4-oxobutanoyl)oxy)-2-palmitamidooctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate (A-5)

23.1 DIPEA (2 mL) was added to a solution of SM-COOH (4.8 g, 6.0 mmol) and HATU (2.8 g, 9.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 12 h, then a solution of (S)-4-(2-(azepan-1-yl)ethoxy)-2-hydroxy-4-oxobutanoic acid (1.68 g, 6.5 mmol) in anhydrous DCM (25 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na_2SO_4 , the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid with 68% yield was acquired. $R_f = 0.18$ ($\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O} = 300/200/36$). ^1H NMR (500 MHz, MeOD) δ 5.86 – 5.76 (m, 1H), 5.41 (dd, $J = 15.2, 8.0$ Hz, 1H), 5.33 (dt, $J = 15.6, 7.7$ Hz, 1H), 5.21 (t, $J = 6.4$ Hz, 1H), 4.66 – 4.46 (m, 1H), 4.41 (dt, $J = 10.1, 4.7$ Hz, 1H), 4.29 (dd, $J = 12.3, 4.7$ Hz, 3H), 4.27 – 4.07 (m, 2H), 3.98 – 3.88 (m, 2H), 3.64 (s, 2H), 3.56 – 3.40 (m, 2H), 3.37 (s, 2H), 3.23 (s, 9H), 3.02 – 2.88 (m, 1H), 2.87 – 2.74 (m, 1H), 2.71 – 2.56 (m, 4H), 2.23 – 2.15 (m, 2H), 2.03 (d, $J = 6.7$ Hz, 2H), 1.95 (s, 2H), 1.75 (s, 2H), 1.59 (d, $J = 6.4$ Hz, 2H), 1.37 (s, 2H), 1.33 – 1.25 (m, 49H), 0.90 (t, $J = 6.8$ Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 174.72 (s), 172.03 (s), 171.61 (s), 137.36 (s), 124.50 (s), 73.59 (s), 71.32 (s), 66.10 (d, $J = 3.4$ Hz), 63.89 (d, $J = 5.3$ Hz), 59.19 (d, $J = 5.2$ Hz), 57.79 (s), 54.97 (s), 54.51 (s), 53.33 (d, $J = 3.5$ Hz), 51.27 (d, $J = 7.5$ Hz), 37.23 (s), 35.87 (s), 32.05 (s), 31.70

(s), 29.72 – 28.28 (m), 28.16 (s), 28.16 (s), 28.16 (s), 26.33 (s), 25.77 (s), 22.86 (s), 22.35 (s), 13.06 (s).

24 (9*S*,16*R*,17*S*)-9-(2-(2-(azepan-1-yl)ethoxy)-2-oxoethyl)-1-hydroxy-8,11,14-trioxo-17-palmitamido-16-((*E*)-pentadec-1-en-1-yl)-7,10,15-trioxa-3,4-dithiaoctadecan-18-yl (2-(trimethylammonio)ethyl) phosphate (A-6)

24.1 DIPEA (2 mL) was added to a solution of A-5 (2.0 g, 2.0 mmol) and HATU (912 mg, 3.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 30 min, then a solution of 2, 2'-dithiodiethanol (0.925 g, 6 mmol) in anhydrous THF (25 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid with 81% yield was acquired. *R*_f = 0.23 (CHCl₃/EtOH/H₂O (v/v/v, 300/200/36)). ¹H NMR (500 MHz, CDCl₃) δ 5.77 – 5.66 (m, 1H), 5.56 – 5.43 (m, 1H), 5.39 (dd, *J* = 18.4, 9.5 Hz, 1H), 5.33 (t, *J* = 7.7 Hz, 1H), 4.76 – 4.07 (m, 7H), 3.94 (s, 2H), 3.89 – 3.74 (m, 4H), 3.38 (s, 9H), 3.24 – 2.79 (m, 12H), 2.75 – 2.49 (m, 4H), 2.22 – 2.06 (m, 2H), 1.95 (t, *J* = 13.2 Hz, 2H), 1.94 – 1.57 (m, 8H), 1.54 (s, 2H), 1.25 (d, *J* = 5.1 Hz, 46H), 0.88 (t, *J* = 6.7 Hz, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 173.33, 172.27, 172.06, 171.12, 170.31, 137.49, 125.12, 74.09, 70.84, 66.27, 64.12, 61.61, 59.44, 55.00, 54.36, 52.44, 51.97, 51.15, 45.45, 36.70, 36.52, 32.35, 31.95, 29.81, 29.76, 29.71, 29.70, 29.67, 29.57, 29.50, 29.42, 29.39, 29.00, 28.71, 26.32, 25.90, 22.70, 14.13, 8.67.

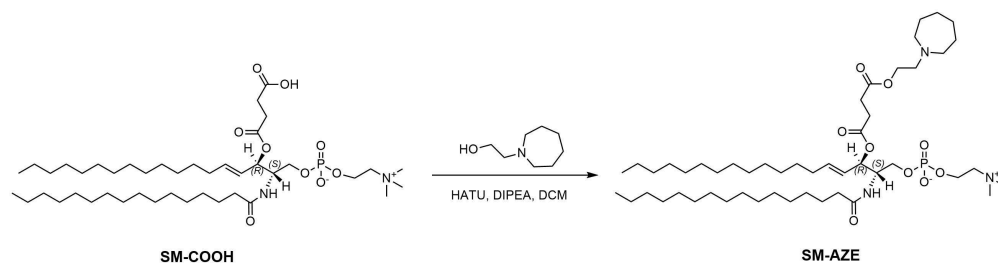
25 (3*S*,4*R*,16*S*,23*R*,24*S*)-16-(2-(2-(azepan-1-yl)ethoxy)-2-oxoethyl)-4-(((2*aR*,4*S*,4*aS*,6*R*,9*S*,11*S*,12*S*,12*aR*,12*bS*)-6,12*b*-diacetoxy-12-(benzoyloxy)-4,11-dihydroxy-4*a*,8,13,13-tetramethyl-5-oxo-2*a*,3,4,4*a*,5,6,9,10,11,12,12*a*,12*b*-dodecahydro-1*H*-7,11-methanocyclodeca[3,4]benzo[1,2-*b*]oxet-9-yl)oxy)carbonyl)-1,6,15,18,21-pentaoxo-24-palmitamido-23-((*E*)-pentadec-1-en-1-yl)-1,3-diphenyl-5,7,14,17,22-pentaoxa-10,11-dithia-2-azapentacosan-25-yl (2-(trimethylammonio)ethyl) phosphate (SM-AZE-PTX)

25.1 DMAP (244.4 mg, 2.0 mmol) was added to a solution of A-6 (1.2 g, 1.0 mmol) and 4-nitrophenyl carbonochloridate (403 mg, 1.5 mmol) in anhydrous DCM (50 mL). The solution mixture was stirred at room temperature for 30 min. A solution of PTX (854 mg, 1.0 mmol) in 10 mL anhydrous DCM was then added into the reaction mixture and further stirred for 12 h. After completion of the reaction, the reaction mixture was washed with 50 mM HCl aqueous solution and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography with CHCl₃/EtOH/H₂O (v/v/v, 300/200/36) as the eluting solvent. White solid with 68% yield was attained. *R*_f = 0.27 (CHCl₃/EtOH/H₂O = 300/200/36). ¹H NMR (500 MHz, CDCl₃) δ 8.18 (d, *J* = 6.8 Hz, 2H), 8.14 – 8.04 (m, 2H), 7.99 – 7.93 (m, 1H), 7.71 – 7.44 (m, 5H), 7.44 –

7.29 (m, 4H), 7.24 – 7.18 (m, 1H), 6.63 (d, $J = 7.1$ Hz, 2H), 6.47 – 6.23 (m, 1H), 6.21 – 5.99 (m, 1H), 5.89 – 5.75 (m, 1H), 5.74 – 5.69 (m, 1H), 5.63 (dd, $J = 8.3, 4.6$ Hz, 1H), 5.47 – 5.38 (m, 2H), 5.34 (dd, $J = 15.2, 7.7$ Hz, 1H), 5.00 – 4.87 (m, 1H), 4.48 – 4.22 (m, 9H), 4.23 – 4.11 (m, 3H), 3.99 – 3.92 (m, 2H), 3.81 (d, $J = 4.8$ Hz, 1H), 3.79 – 3.65 (m, 3H), 3.37 – 3.31 (m, 10H), 3.11 (s, 8H), 2.89 (ddd, $J = 20.5, 10.3, 4.6$ Hz, 4H), 2.73 – 2.50 (m, 6H), 2.50 – 2.34 (m, 3H), 2.25 – 2.04 (m, 6H), 2.01 – 1.92 (m, 3H), 1.84 (dt, $J = 11.2, 8.5$ Hz, 4H), 1.64 (d, $J = 15.1$ Hz, 3H), 1.54 (s, 2H), 1.29 (d, $J = 5.8$ Hz, 4H), 1.25 (d, $J = 9.4$ Hz, 48H), 1.21 (d, $J = 5.5$ Hz, 3H), 1.11 (d, $J = 11.9$ Hz, 3H), 0.87 (d, $J = 7.2$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 203.80, 173.22, 172.27, 171.58, 171.14, 169.34, 168.87, 168.69, 167.12, 166.89, 155.88, 153.94, 144.19, 137.66, 133.76, 132.84, 131.70, 130.19, 129.32, 128.92, 128.67, 128.46, 127.70, 127.61, 124.81, 106.71, 84.43, 80.95, 78.83, 75.65, 74.94, 74.10, 71.75, 68.55, 66.65, 64.06, 62.34, 61.99, 61.31, 59.40, 58.29, 58.24, 55.31, 54.55, 51.06, 43.17, 39.59, 36.83, 36.04, 32.33, 31.93, 29.74, 29.68, 29.62, 29.55, 29.44, 29.37, 29.00, 28.59, 26.94, 26.70, 25.84, 23.06, 22.69, 21.87, 20.94, 18.46, 14.81, 14.13, 14.07, 9.80. HRMS (ESI) m/z $[\text{M} + 2\text{H}]^{2+}$ for $\text{C}_{107}\text{H}_{159}\text{N}_4\text{O}_{29}\text{PS}_2$ calculated 1031.02237, found 1031.02332.

The synthesis of SM-AZE

26 Synthetic route for SM-AZE was indicated as below



Supplementary Scheme 6, Synthetic route for SM-AZE

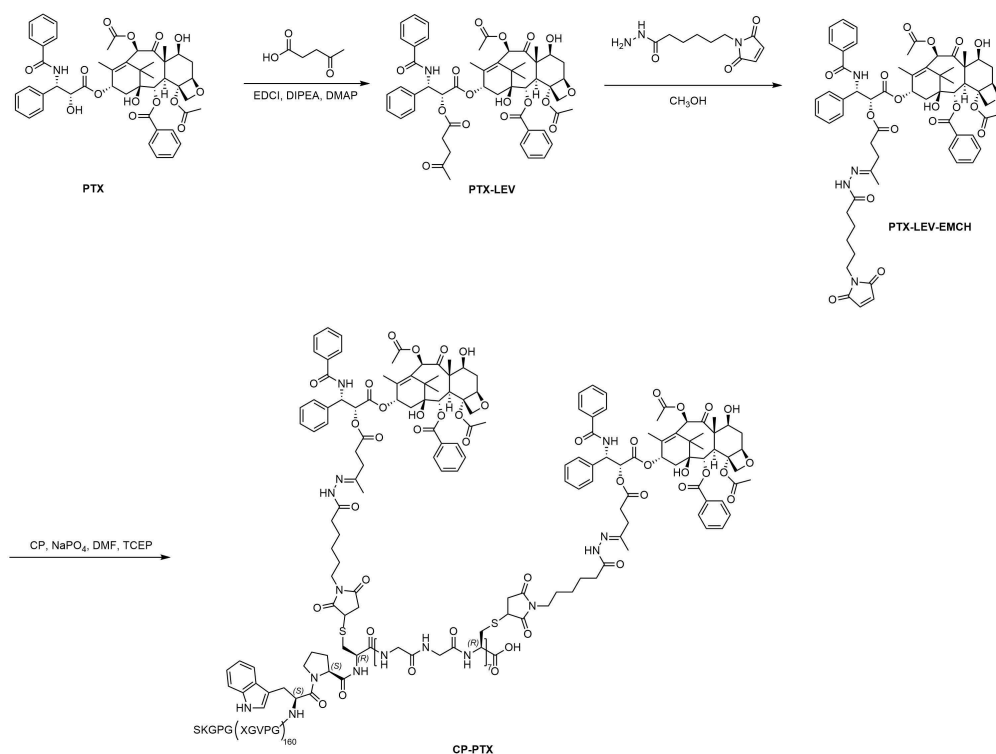
27 (2*S*,3*R*,*E*)-3-(((4-(2-(azepan-1-yl)ethoxy)-4-oxobutanoyl)oxy)-2-palmitamidooctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate (SM-AZE)

27.1 DIPEA (2 mL) was added to a solution of SM-COOH (4.8 g, 6.0 mmol) and HATU (2.8 g, 9.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 12 h, then a solution of 2-(azepan-1-yl)ethan-1-ol (0.86 g, 6 mmol) in anhydrous THF (30 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na_2SO_4 , the solvent was evaporated using

rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid with 73% yield was acquired. $R_f = 0.21$ ($\text{CHCl}_3/\text{EtOH}/\text{H}_2\text{O} = 300/200/36$). ^1H NMR (400 MHz, CDCl_3) δ 7.40 (d, $J = 6.8$ Hz, 1H), 5.77 – 5.65 (m, 1H), 5.43 – 5.31 (m, 1H), 5.24 (t, $J = 7.8$ Hz, 1H), 4.56 (s, 2H), 4.41 – 4.23 (m, 3H), 3.95 (s, 4H), 3.42 (s, 9H), 3.29 (s, 6H), 2.71 – 2.52 (m, 4H), 2.13 (t, $J = 5.9$ Hz, 2H), 1.94 (s, 6H), 1.70 (s, 4H), 1.53 (s, 2H), 1.23 (s, 46H), 0.86 (dd, $J = 6.3, 4.6$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.53, 172.25, 171.26, 137.77, 124.86, 74.05, 66.16, 66.11, 64.41, 64.38, 59.94, 59.55, 59.53, 55.23, 54.33, 51.21, 51.16, 36.69, 32.39, 31.95, 29.84, 29.80, 29.77, 29.74, 29.71, 29.59, 29.48, 29.40, 28.99, 26.79, 26.00, 23.32, 22.71, 14.14. HRMS (ESI) m/z $[\text{M} + \text{H}]^+$ for $\text{C}_{51}\text{H}_{98}\text{N}_3\text{O}_9\text{P}$ calculated 928.71134, found 928.71085.

The synthesis of CP-PTX

28 Synthetic route for CP-PTX was indicated as below



Supplementary Scheme 7, Synthetic route for CP-PTX

29 (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((2R,3S)-3-benzamido-2-((4-oxopentanoyl)oxy)-3-phenylpropanoyloxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-3,4,4a,5,6,9,10,11,12,12a-decahydro-1H-7,11-

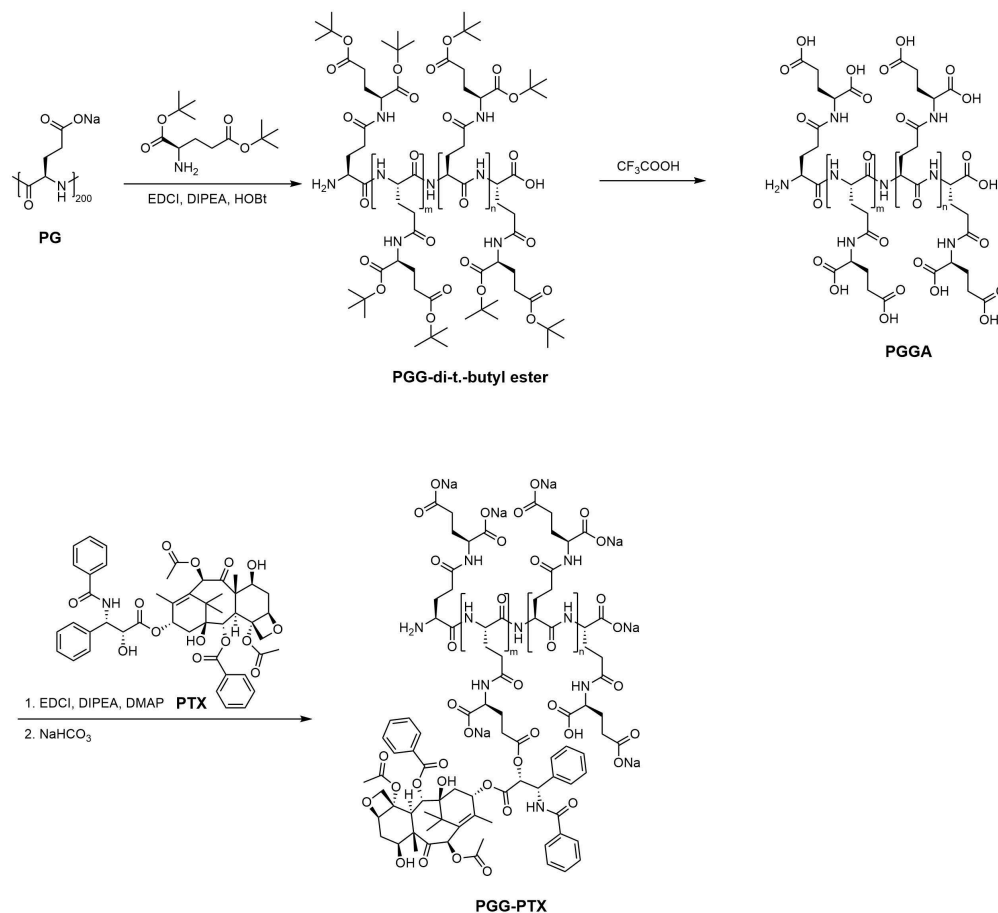
methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b(2aH)-diyl diacetate (PTX-LEV)

- 29.1 The synthesis of CP-PTX was according to the reported method³. We obtained chimeric polypeptide (CP-PTX) from GenScript Biotech Co., Ltd. (USA) based on the reported method³. To synthesize PTX-LEV, Levulinic acid (LEV, 240 mg, 2.2 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDCI, 342 mg, 2.2 mmol), 4-dimethylaminopyridine (268 mg, 2.2 mmol) and N,N-Diisopropylethylamine (1 mL) were dissolved in 20 mL anhydrous DMF, and then the mixture of solution was stirred for 1h at room temperature, Paclitaxel (1.7 g, 2 mmol) was added into the mixture reaction, and the solution was then stirred for 24 h at room temperature and monitored by TLC. After the completing of the reaction, the solvent was evaporated under reduced pressure, and the residue was washed with 50 mM HCl aqueous solution. The product was extracted by ethyl acetate (50 mL × 4 times), and the organic solvent was washed with saturated brine, and dried over anhydrous Na₂SO₄. The solvent was then evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid with 79% yield was acquired. R_f = 0.33 (Hexane/EtOAc = 1/1). ¹H-NMR (400 MHz, CDCl₃) δ 8.11 (d, J = 7.5 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H), 7.59 (t, J = 7.3 Hz, 1H), 7.49 (dd, J = 13.4, 7.2 Hz, 3H), 7.39 (t, J = 7.5 Hz, 6H), 7.32 (t, J = 6.7 Hz, 1H), 6.99 – 6.93 (m, 1H), 6.27 (s, 1H), 6.21 (t, J = 8.8 Hz, 1H), 5.92 (dd, J = 8.8, 2.9 Hz, 1H), 5.66 (d, J = 7.0 Hz, 1H), 5.45 (d, J = 3.1 Hz, 1H), 4.95 (d, J = 8.8 Hz, 1H), 4.48 – 4.36 (m, 1H), 4.29 (d, J = 8.4 Hz, 1H), 4.17 (d, J = 8.4 Hz, 1H), 3.78 (d, J = 6.9 Hz, 1H), 2.76 – 2.59 (m, 4H), 2.58 – 2.46 (m, 2H), 2.41 (s, 3H), 2.32 (dd, J = 15.4, 9.4 Hz, 1H), 2.20 (s, 3H), 2.11 (s, 3H), 1.90 (s, 3H), 1.86 (s, 1H), 1.83 (s, 1H), 1.21 (s, 3H), 1.11 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 206.38, 203.85, 171.79, 171.25, 169.80, 168.05, 167.10, 167.00, 142.81, 136.98, 133.68, 133.59, 132.73, 131.99, 130.22, 129.18, 129.07, 128.73, 128.67, 128.49, 127.19, 126.63, 84.43, 81.01, 79.10, 77.25, 76.42, 75.60, 75.08, 74.15, 72.12, 71.82, 60.40, 58.49, 52.86, 45.56, 43.15, 37.89, 35.51, 29.64, 27.70, 26.78, 22.66, 22.11, 21.05, 20.83, 14.81, 14.19, 9.59. HRMS (ESI) m/z [M + H]⁺ for C₅₂H₅₇NO₁₆ calculated 952.37501, found 952.37459.
- 30 (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((2R,3S)-3-benzamido-2-(((E)-4-(2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)hydrazineylidene)pentanoyl)oxy)-3-phenylpropanoyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-3,4,4a,5,6,9,10,11,12,12a-decahydro-1H-7,11-ethanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b(2aH)-diyl diacetate (PTX-LEV-EMCH)
- 30.1 To synthesize PTX-LEV-EMCH, PTX-LEV (952 mg, 1.0 mmol) and EMCH (315.3 mg, 1.4 mmol) were dissolved in 20 mL anhydrous CH₃OH. The mixture solution was stirred at 45 °C under dark conditions for 24 hours. The reaction was monitored by TLC. Upon completing of the reaction, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. White solid

with 68% yield was acquired. $R_f = 0.28$ ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH} = 30/1$). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.73 (d, $J = 7.8$ Hz, 1H), 8.33 (s, 1H), 8.12 (d, $J = 6.6$ Hz, 1H), 8.06 (d, $J = 7.7$ Hz, 1H), 7.82 (d, $J = 7.7$ Hz, 1H), 7.79 – 7.61 (m, 2H), 7.59 – 7.48 (m, 4H), 7.42 – 7.29 (m, 7H), 7.09 – 6.99 (m, 1H), 6.65 (s, 1H), 6.58 (s, 1H), 6.34 – 6.22 (m, 1H), 6.25 – 6.18 (m, 1H), 5.98 – 5.82 (m, 1H), 5.65 (dd, $J = 15.8, 7.7$ Hz, 1H), 5.52 (dd, $J = 21.1, 5.1$ Hz, 1H), 5.23 (d, $J = 9.8$ Hz, 1H), 4.93 (dd, $J = 23.2, 9.2$ Hz, 1H), 4.38 (t, $J = 21.8$ Hz, 1H), 4.30 (d, $J = 8.4$ Hz, 1H), 4.21 (dd, $J = 13.5, 8.5$ Hz, 1H), 4.14 – 4.06 (m, 1H), 3.80 (d, $J = 6.9$ Hz, 1H), 3.65 (d, $J = 8.2$ Hz, 1H), 3.53 – 3.41 (m, 3H), 2.81 – 2.64 (m, 2H), 2.62 – 2.45 (m, 6H), 2.42 (d, $J = 10.7$ Hz, 2H), 2.34 (dd, $J = 15.7, 9.5$ Hz, 1H), 2.22 (dd, $J = 18.5, 8.2$ Hz, 6H), 2.12 (dd, $J = 15.1, 9.0$ Hz, 1H), 1.89 (dd, $J = 11.3, 8.7$ Hz, 3H), 1.86 – 1.78 (m, 4H), 1.74 – 1.63 (m, 8H), 1.58 (q, $J = 8.5$ Hz, 5H), 1.40 – 1.18 (m, 5H), 1.13 (d, $J = 8.5$ Hz, 3H), 1.05 (s, 2H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 204.06, 203.91, 175.38, 172.40, 171.99, 171.31, 171.00, 170.94, 170.24, 169.97, 169.89, 169.20, 168.19, 167.41, 167.26, 167.08, 166.97, 154.43, 148.46, 143.84, 142.75, 137.95, 137.06, 134.89, 134.20, 134.16, 134.13, 134.06, 133.91, 133.81, 133.78, 133.74, 132.90, 132.27, 132.12, 132.08, 131.26, 130.31, 129.39, 129.31, 129.28, 129.14, 128.85, 128.81, 128.77, 128.64, 128.56, 128.14, 128.04, 127.66, 127.28, 127.23, 126.72, 126.59, 84.53, 81.15, 80.79, 79.19, 79.02, 76.52, 76.36, 75.76, 75.68, 75.45, 75.16, 74.20, 72.16, 72.13, 71.84, 71.04, 58.56, 58.44, 55.37, 52.85, 50.91, 45.69, 45.58, 43.27, 42.98, 37.98, 37.79, 37.42, 35.63, 35.48, 35.13, 34.63, 33.64, 32.83, 32.36, 30.08, 29.55, 28.34, 28.16, 27.79, 26.86, 26.76, 26.46, 26.28, 24.97, 24.06, 22.81, 22.73, 22.22, 21.97, 20.92, 15.93, 15.55, 15.06, 14.87, 9.71, 9.59. HRMS (ESI) m/z $[\text{M} + \text{H}]^+$ for $\text{C}_{62}\text{H}_{70}\text{N}_4\text{O}_{18}$ calculated 1159.47579, found 1159.47671. The conjugation of PTX-LEV-EMCH with CP were performed following the reported method. Purified CP was first reduced by 1 mL of TCEP under pH = 7.0 (100 mM) at $\sim 5 \times$ excess to thiol in the reaction buffer (0.1 M Na_3PO_4 , 1 mM EDTA, pH 7.0). The extra TCEP was removed by initiating the phase transition with sodium chloride (2.5 M) and centrifuged (4,000 rpm) for 10 min at 25 °C. The CP solid pellet was resuspended in 2 mL reaction buffer and PTX-LEV-EMCH in DMF (2 mL) was added into the solution with stirring. After that, 1 mL of pH-neutral TCEP (100 mM) was added into the mixture solution, and the reaction was stirred for 16h under dark conditions at 20 °C. After the reaction was completed, the excess PTX-LEV-EMCH precipitate was separated by centrifugation (13,000 rpm) for 10 min under. at 10 °C. The supernatant was collected and diluted with 20% acetonitrile in PBS. The solution was further centrifuged in an Amicon Ultra-15 Centrifugal Filter Units (MWCO: 10 kDa, Millipore) under 2,500 r.p.m. at 10 °C. The CP-PTX was washed with NH_4HCO_3 solution twice and then lyophilized. The conjugation ratio of PTX to CP was determined by MALDI-TOF-MS of the CP-PTX conjugates and free CP, the purity was analyzed by HPLC.

The synthesis of PGG-PTX

31 Synthetic route for PGG-PTX was indicated as below



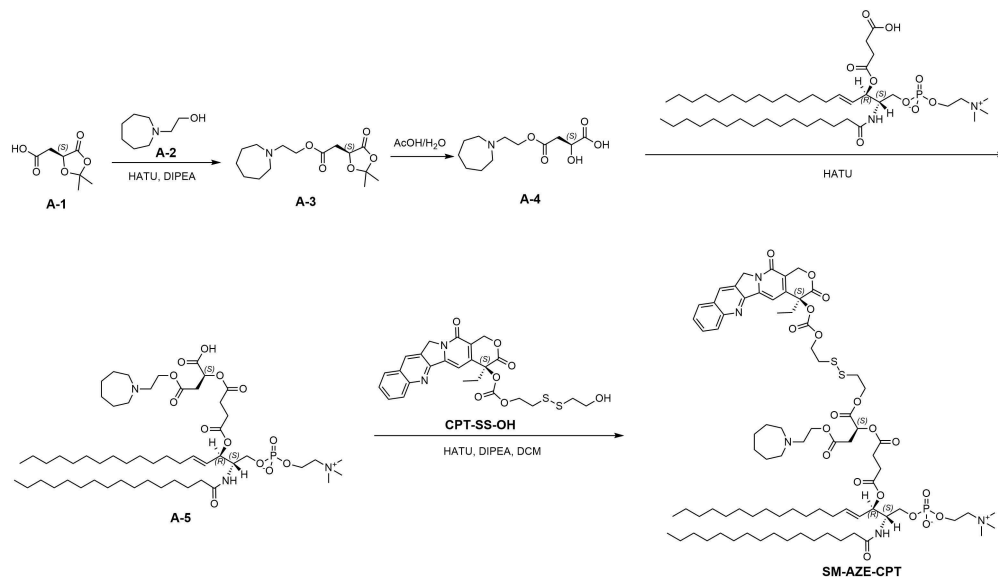
Supplementary Scheme 8, Synthetic route for PGG-PTX

- 32 The synthesis of PGG-PTX was performed according to the reported method^{4,5}. 1.0 g of Commercial available poly(L-glutamic acid) sodium salt (purchase from Alamanda Polymers) with an average molecular weight of 25000 Da was dissolved in 50 mL anhydrous N,N-dimethylformamide (DMF). 3.7g of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDCI), and 1.06g of 1-hydroxybenzotriazole (HOBt) were added into the mixture solution, which was stirred at room temperature for 1 hour. After that, 3.83 g of L-glutamic acid di-tert-butyl ester hydrochloride was added into in the reaction, which was further stirred for 24 hours at room temperature. The mixture solution was poured slowly into the distilled water (300 mL) with stirring. After 30 minutes, the precipitation was filtered, and the solid was washed with distilled water. The solid was dried under vacuum and the product was characterized by ¹H-NMR. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 4.19 (s, 1H), 4.05 (s, 1H), 2.46 (s, 4H), 2.21 – 2.16 (m, 3H), 1.88 – 1.79 (m, 1H), 1.33 (s, 24H).

- 33 The above dry solid was transferred to a 100 mL round-bottom glass flask, 20 mL of trifluoroacetic acid was added into the flask, the reaction was stirred under nitrogen atmosphere for 5 hours at room temperature. The solvent trifluoroacetic acid was evaporated under reduced pressure by rotary evaporation. Then, 80 mL of distilled water was added into the flask, and the mixture solution was stirred at room temperature for 30 minutes until the solid was completely dissolved. The solution was dialyzed (10,000 MWCO cassette) against distilled water for 24 hours (8 L water * 4 times) and then filtered through a 0.45 μ m filter. The solution was lyophilized to obtain the PGG and characterized by $^1\text{H-NMR}$. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 4.29 (s, 1H), 4.14 (s, 1H), 2.24 (d, J = 43.8 Hz, 4H), 1.91 (s, 3H), 1.71 (s, 1H).
- 34 To synthesize PGG-PTX, 1.0 g of PGG was dissolved in 50 mL anhydrous N,N-dimethylformamide (DMF). The solution was then stirred at room temperature to allow complete dispersion. 0.94 g of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide and 0.26 g of 4-dimethylaminopyridine were added to the solution, which was stirred at room temperature for 15 min to allow complete dispersion. Afterwards, 0.54 g of PTX was added into the mixture reaction, which was further stirred at room temperature for 24 hours until the PTX was unable to be detected by thin layer chromatography ([TLC], 100% ethyl acetate). The solution was then poured into 0.2 M aqueous HCl solution (150 mL) with vigorously stirring. Then the precipitation was isolated by centrifuging the solution for 10 minutes at 5,000 rpm. The solid precipitation was dissolved in 150 mL of 0.5 M sodium bicarbonate solution and dialyzed against distilled water for 24 hours (8 L water * 4 times). The solution was then filtered by 0.45 μ m filter and lyophilized. PGG-PTX (0.8 g) was obtained and characterized by $^1\text{H-NMR}$. The PTX content was determined to be 36.1% by the reported ultraviolet-visible method⁶. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 8.20 – 7.87 (m, 3H), 7.87 – 7.74 (m, 2H), 7.68 (dd, J = 9.5, 4.8 Hz, 1H), 7.60 (dd, J = 14.5, 7.0 Hz, 2H), 7.54 – 7.48 (m, 1H), 7.44 (dd, J = 16.2, 9.0 Hz, 3H), 7.36 (t, J = 3.4 Hz, 3H), 7.23 – 7.06 (m, 1H), 6.25 (s, 1H), 5.82 (dd, J = 30.3, 21.3 Hz, 1H), 5.51 – 5.27 (m, 2H), 4.89 (t, J = 14.0 Hz, 1H), 4.73 – 4.46 (m, 2H), 4.15 (d, J = 54.8 Hz, 4H), 3.97 (dd, J = 14.6, 7.9 Hz, 3H), 3.63 (dd, J = 46.6, 7.5 Hz, 4H), 3.26 – 3.21 (m, 6H), 2.99 – 2.82 (m, 4H), 2.68 (d, J = 41.7 Hz, 4H), 2.30 (d, J = 5.7 Hz, 3H), 2.21 – 2.03 (m, 16H), 1.84 – 1.70 (m, 7H), 1.44 (d, J = 15.5 Hz, 4H), 1.19 (s, 1H), 1.07 – 0.90 (m, 9H).

The synthesis of SM-AZE-CPT

- 35 Synthetic route for SM-AZE-CPT was indicated as below



Supplementary Scheme 9, Synthetic route for SM-AZE-CPT

36 (11*S*,18*R*,19*S*)-11-(2-(2-(azepan-1-yl)ethoxy)-2-oxoethyl)-1-(((*S*)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-4-yl)oxy)-1,10,13,16-tetraoxo-19-palmitamido-18-((*E*)-pentadec-1-en-1-yl)-2,9,12,17-tetraoxa-5,6-dithiaicosan-20-yl (2-(trimethylammonio)ethyl) phosphate (SM-AZE-CPT)

36.1 DIPEA (2 mL) was added to a solution of A-5 (2.0 g, 2.0 mmol) and HATU (912 mg, 3.0 mmol) in anhydrous DCM (50 mL). The reaction mixture was stirred at room temperature for 30 min, then a solution of CPT-SS-OH (1.58 g, 3.0 mmol) in anhydrous DCM (25 mL) was added into the mixture solution. The reaction was further stirred at room temperature for 12 h and monitored by TLC. After completion of the reaction, the mixture solution was washed with 50 mM HCl aqueous solution, and then with saturated brine. The organic layer was dried with anhydrous Na₂SO₄, the solvent was evaporated using rotary evaporator under vacuum, and the residue was purified by silica gel flash chromatography. Pale yellow solid with 76% yield was acquired. R_f = 0.25

(CHCl₃/EtOH/H₂O (v/v/v, 300/200/36)). ¹H-NMR (400 MHz, CDCl₃) δ 8.44 (s, 1H), 8.22 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.85 (t, J = 7.7 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.38 (d, J = 9.0 Hz, 1H), 7.33 (s, 1H), 5.69 (dd, J = 19.5, 7.3 Hz, 2H), 5.57 – 5.44 (m, 1H), 5.41 (t, J = 13.9 Hz, 2H), 5.33 (d, J = 9.0 Hz, 3H), 4.61 – 4.41 (m, 2H), 4.40 – 4.32 (m, 5H), 4.29 (dd, J = 11.7, 6.0 Hz, 2H), 4.03 – 3.87 (m, 4H), 3.44 (s, 9H), 3.39 (s, 6H), 3.07 (dd, J = 16.4, 4.1 Hz, 1H), 2.94 (d, J = 6.5 Hz, 2H), 2.88 (dd, J = 9.2, 6.7 Hz, 2H), 2.77 – 2.67 (m, 2H), 2.64 – 2.49 (m, 3H), 2.16 (dd, J = 14.1, 7.6 Hz, 3H), 2.09 – 1.78 (m, 7H), 1.71 (s, 4H), 1.55 (s, 2H), 1.24 (d, J = 3.7 Hz, 46H), 1.01 (t, J = 7.4 Hz, 3H), 0.88 (t, J = 6.7 Hz, 6H).

¹³C-NMR (101 MHz, CDCl₃) δ 173.23, 171.40, 171.12, 168.60, 68.19, 167.43, 157.27, 153.49, 152.26, 148.88, 146.64, 145.53, 137.71, 131.34, 130.80, 129.62, 128.54, 128.29, 128.24,

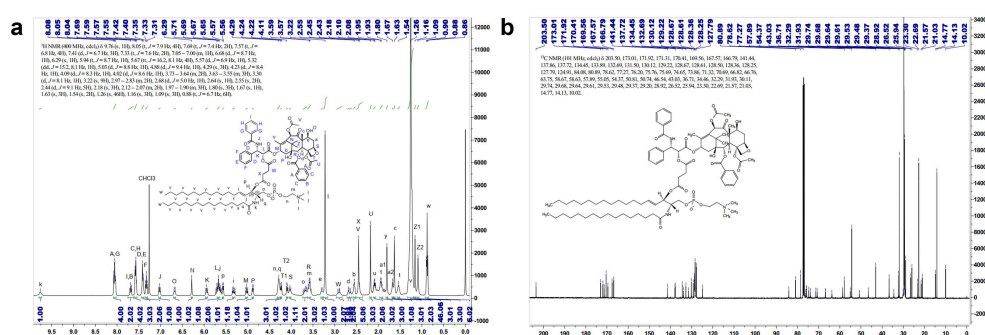
128.17, 124.93, 120.06, 95.86, 78.12, 77.23, 73.91, 68.22, 67.10, 66.40, 63.21, 54.96, 54.65, 50.11, 36.84, 32.32, 31.94, 31.81, 29.74, 29.69, 29.62, 29.55, 29.38, 29.01, 26.87, 25.85, 23.35, 22.70, 14.14. HRMS (ESI) m/z $[M + H]^+$ for $C_{80}H_{124}N_5O_{19}PS_2$ calculated 1554.81423, found 1554.81627.

The synthesis of DSPE-CD47p

- 37 DSPE-CD47p was synthesized by conjugation of self-peptide (cgggCERVIGTGWVRC) with DSPE-Maleimide. The self-peptide CD47p and DSPE-Maleimide (1:1, mol/mol) were dissolved in DMF/ H_2O = 5/1 (v/v), and then the mixture solution was stirred at 37 °C for 48 h. The reaction was monitored by TLC, after the completion of the reaction, the reaction mixture was purified by the reverse phase HPLC and then lyophilized by benchtop freeze dryer (Labconco, FreeZone 4.5 Liter, #710402000) under minus 80 °C.

Supplementary Note Fig. 1

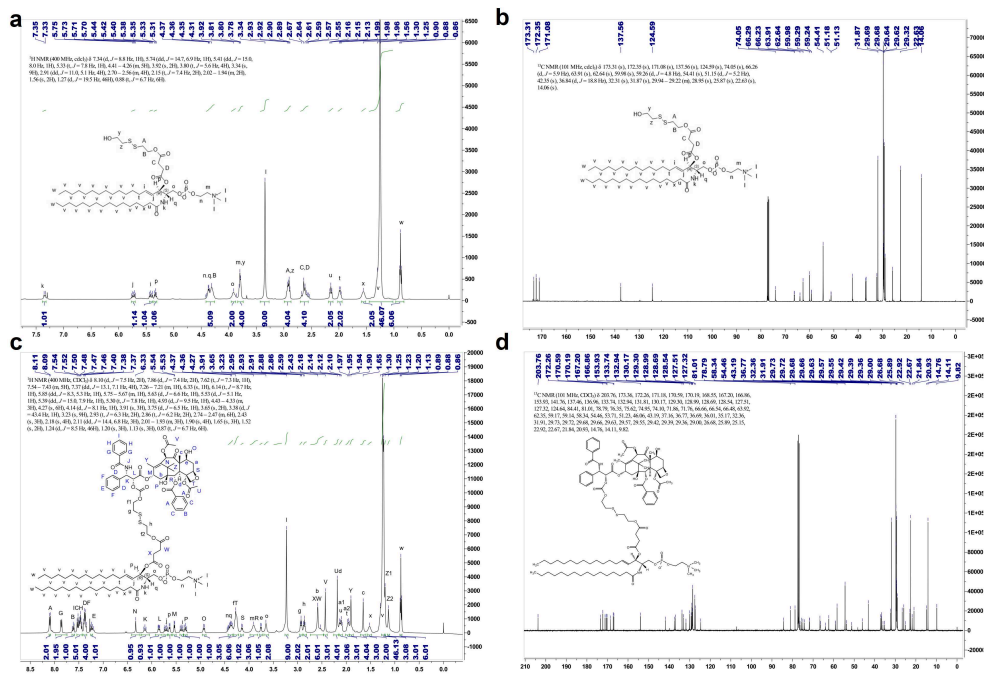
38



Supplementary Note Fig. 1. 1H NMR (a) and ^{13}C NMR (b) spectra for SM-Ester-PTX.

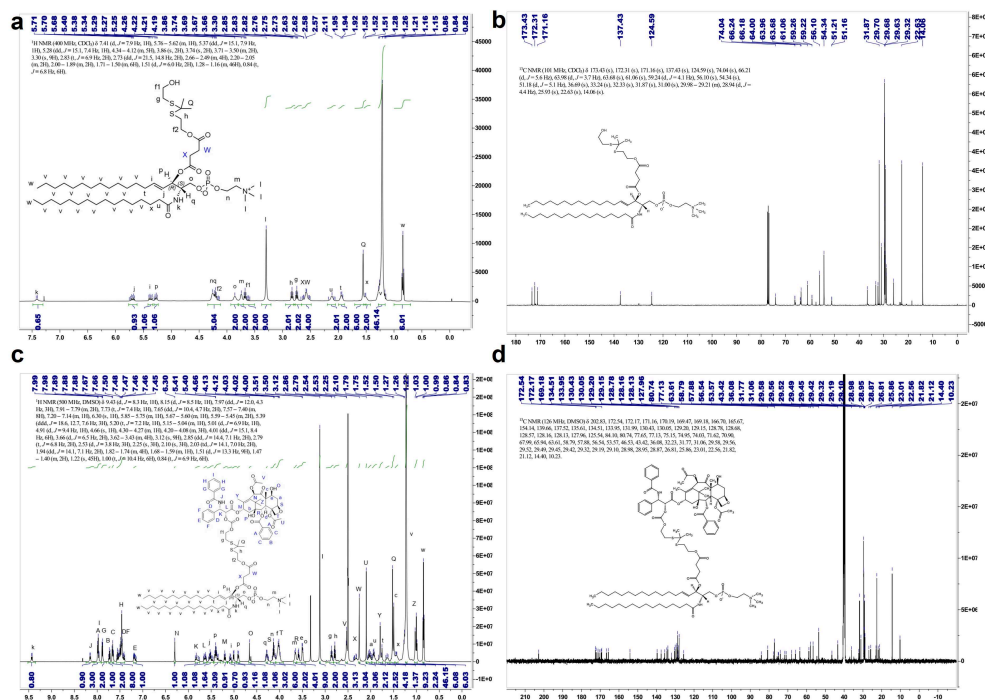
Supplementary Note Fig. 2.

39



Supplementary Note Fig. 2. ^1H NMR and ^{13}C NMR spectra for SM-CSS-OH (a,b), SM-CSS-PTX (c,d).

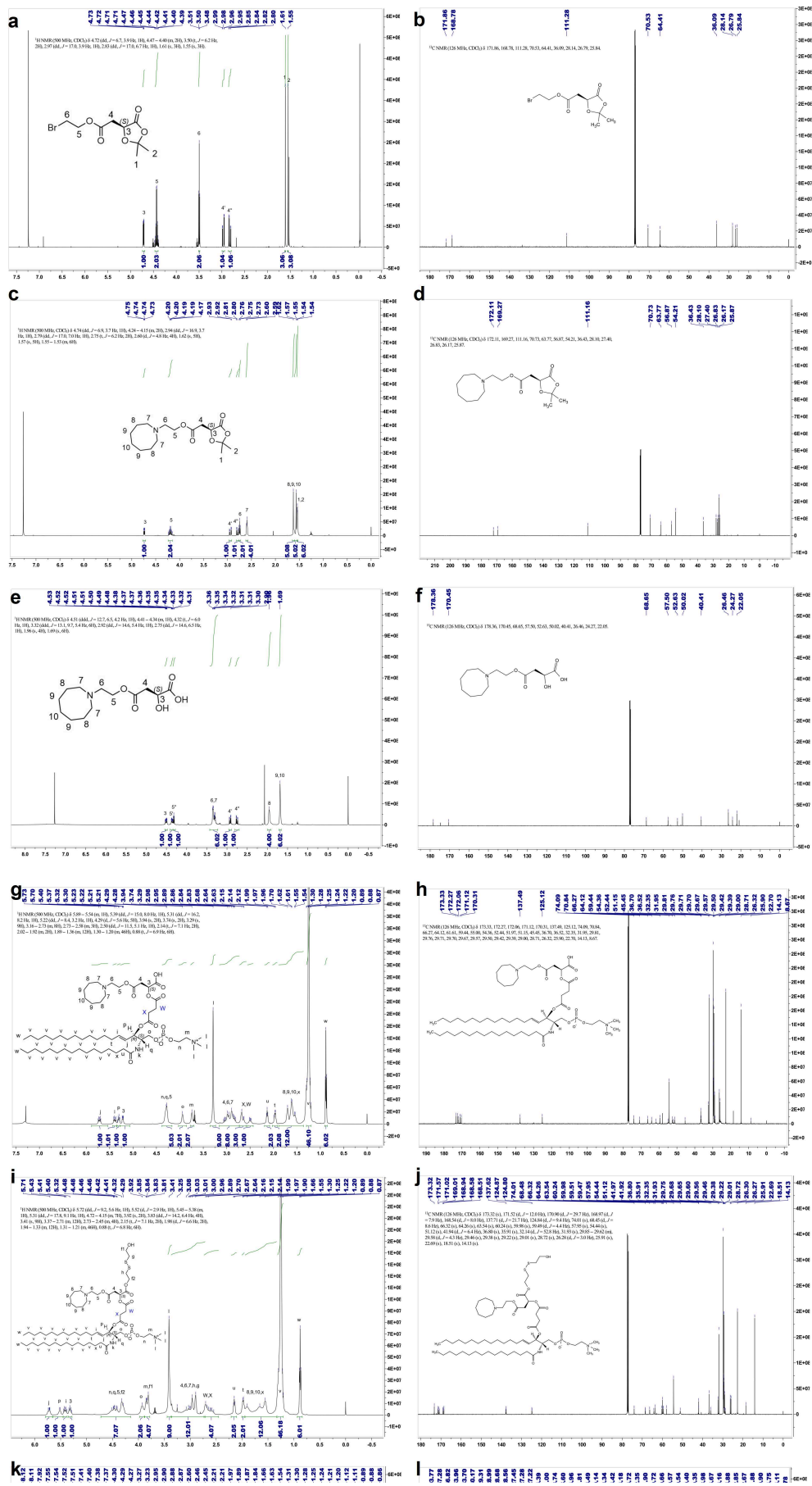
Supplementary Note Fig. 3

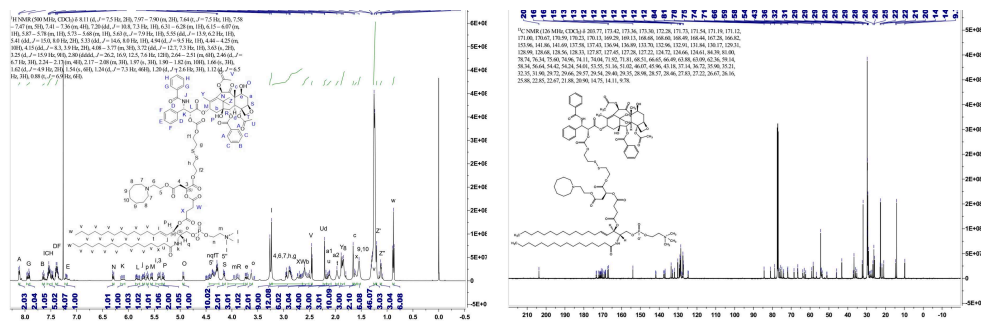


Supplementary Note Fig. 3. ^1H NMR and ^{13}C NMR spectra for SM-SCS-OH (a,b), SM-SCS-PTX (c,d).

Supplementary Note Fig. 4

41

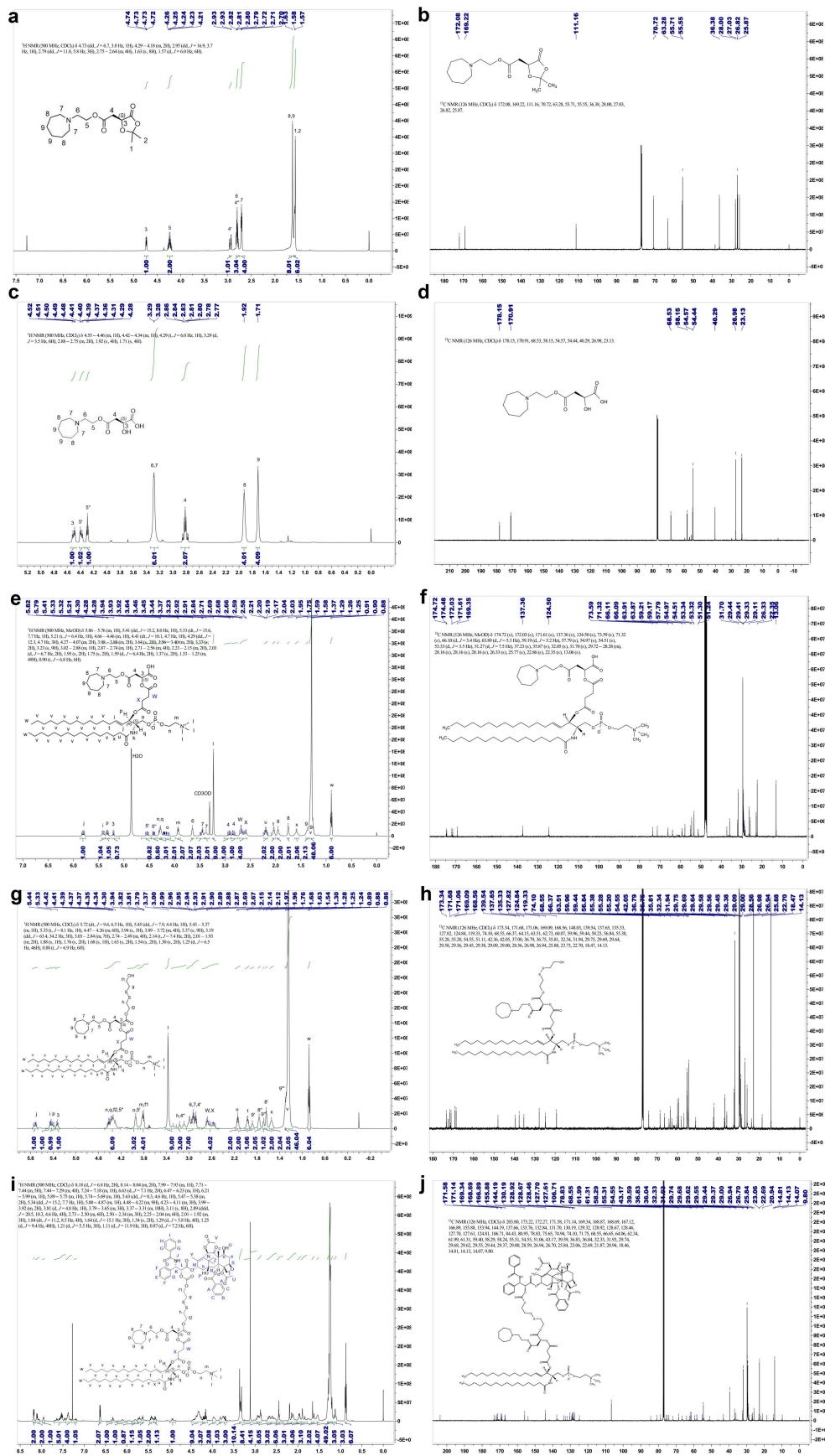




Supplementary Note Fig. 4. ^1H NMR and ^{13}C NMR spectra for B-3 (a,b), B-4 (c,d), B-5 (e,f), B-6 (g,h), B-7 (i,j), SM-AZO-PTX (k,l).

Supplementary Note Fig. 5

42

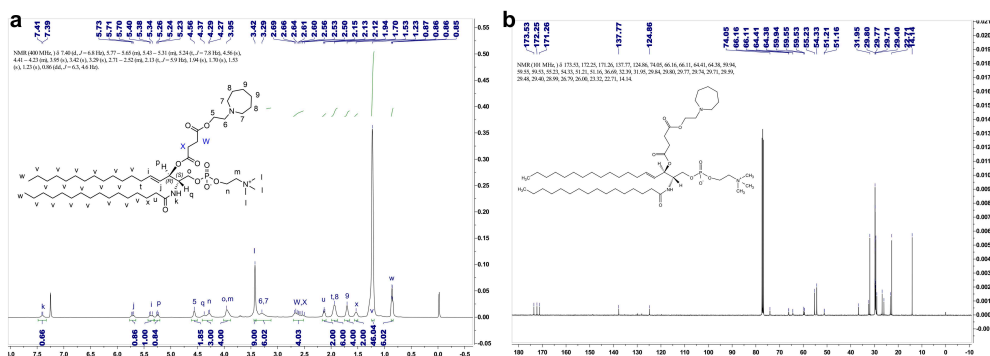


Supplementary Note Fig. 5 ^1H NMR and ^{13}C NMR spectra for A-2 (a, b), A-4 (c, d), A-5

Supplementary Note Fig. 5. ^1H NMR and ^{13}C NMR spectra for A-3 (a,b), A-4 (c,d), A-5 (e,f), A-6 (g,h), and SM-AZE-PTX (i,j).

Supplementary Note Fig. 6

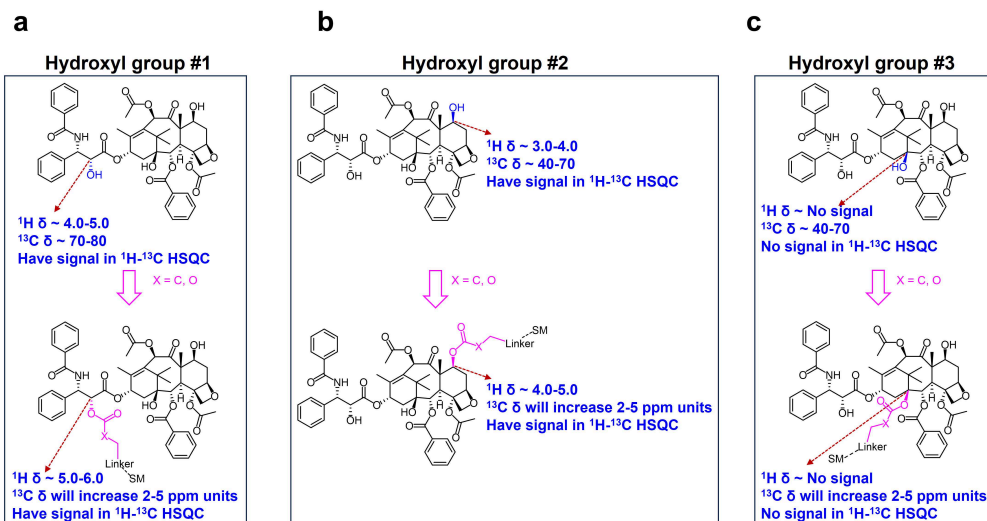
43



Supplementary Note Fig. 6. ^1H NMR and ^{13}C NMR spectra for SM-AZE (a,b).

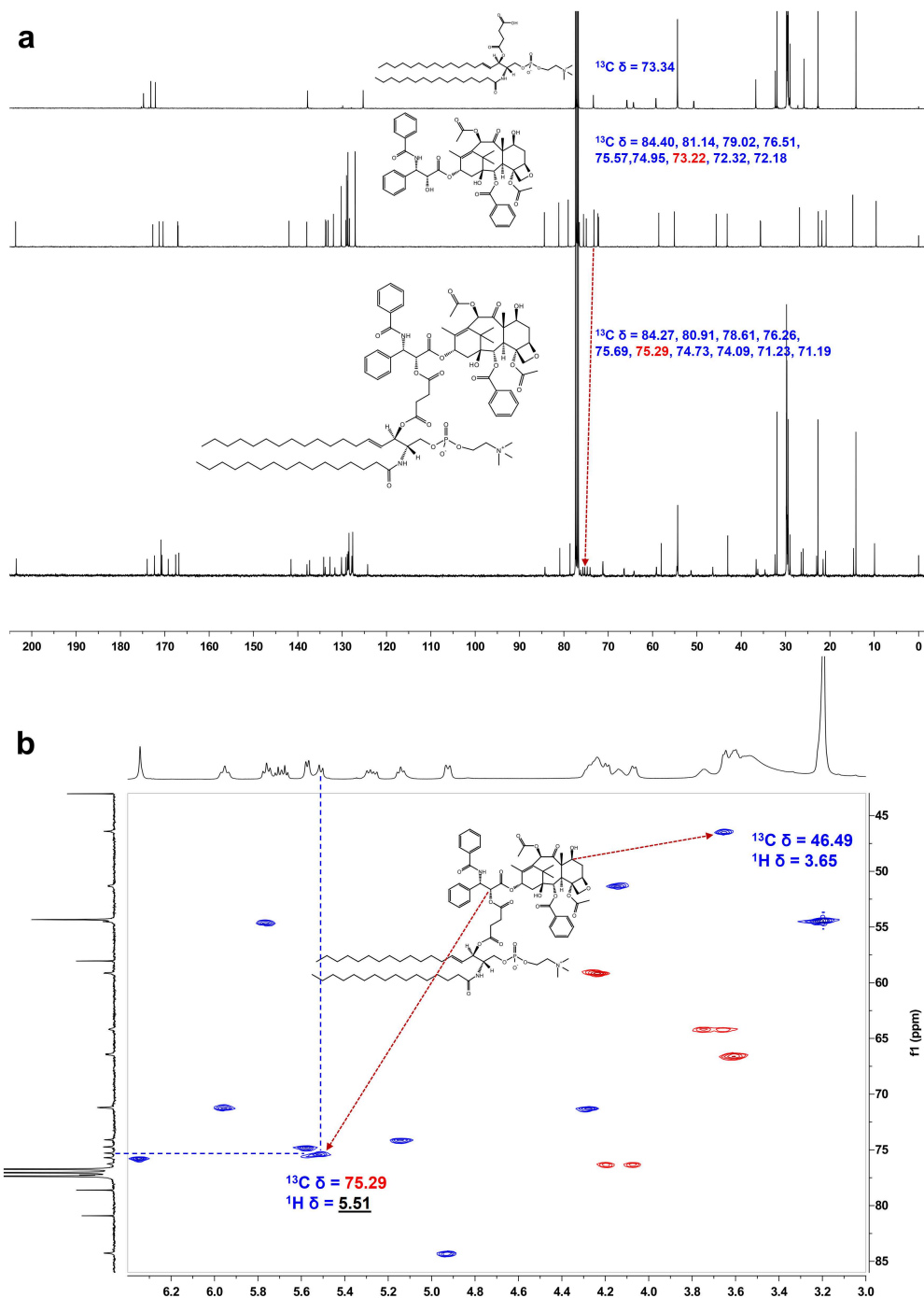
Supplementary Note Fig. 7

44



Supplementary Note Fig. 7. Chemical shift analysis of methine group (#1 and #2) and quaternary group (#3) attached to hydroxyl group in PTX and its corresponding SM-PTX conjugations by ^1H & ^{13}C -NMR and $^1\text{H}-^{13}\text{C}$ HSQC NMR. **a**, Hydroxyl group #1 is an alpha substituted hydroxyl group on ester bond, the chemical shift of ^1H -NMR and ^{13}C -NMR for methine group is around 4.0-5.0 and 70-80, respectively. Upon conjugating with SM to form an ester or carbonate ester group, the chemical shift of ^1H NMR for methine group will increase to 5.0-6.0 and the ^{13}C -NMR will increase by 2-5 ppm units. **b**, Hydroxyl group #2 is a routine hydroxyl group, the ^1H -NMR and ^{13}C -NMR chemical shift of methine group is around 3.0-4.0 and 40-70, respectively. Upon conjugating with SM to form an ester or carbonate ester group, the chemical shift of ^1H NMR for methine group will increase to 4.0-5.0 and the ^{13}C -NMR will increase by 2-5 ppm units. **c**, Hydroxyl group #3 is a hydroxyl group on quaternary carbon, the quaternary group only has signal in ^{13}C NMR but no signal in ^1H NMR and $^1\text{H}-^{13}\text{C}$ HSQC NMR, the ^{13}C -NMR chemical shift of quaternary group is around 40-70, upon conjugating with SM to form an ester or carbonate ester group, the chemical shift of ^{13}C -NMR will increase by 2-5 ppm units.

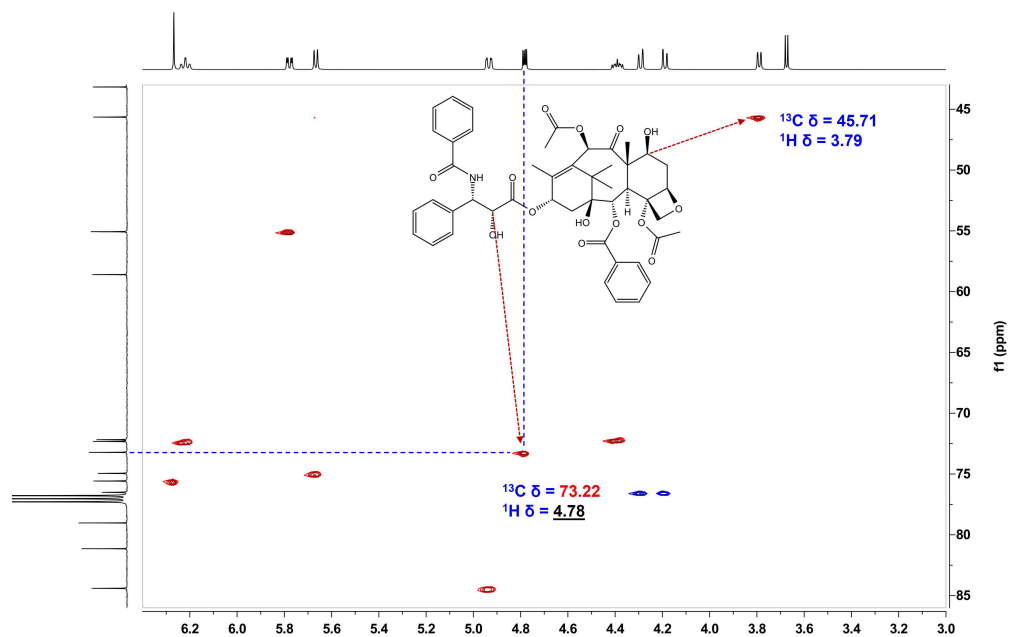
Supplementary Note Fig. 8



Supplementary Note Fig. 8. a, ^{13}C NMR of SM-COOH, PTX and SM-Ester-PTX in CDCl_3 . After conjugating SM-COOH with PTX to form SM-Ester-PTX, the ^{13}C NMR chemical shift δ 73.22 in PTX was increased to 75.29. **b,** ^1H - ^{13}C HSQC NMR of SM-Ester-PTX indicated that ^{13}C NMR δ 75.29 was associated with ^1H NMR δ 5.51, which demonstrated that the conjugated site was at hydroxyl group #1 of PTX.

Supplementary Note Fig. 9

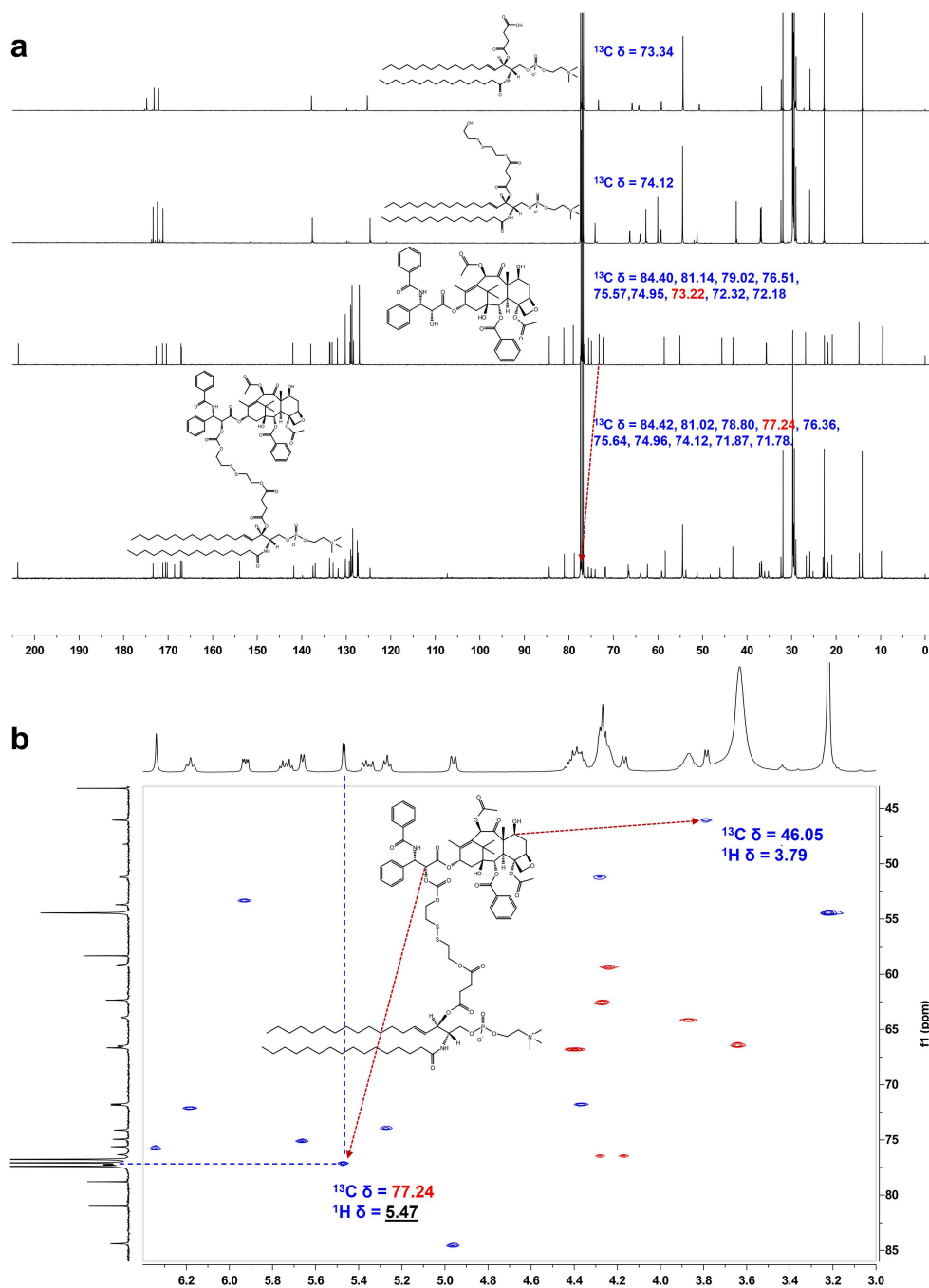
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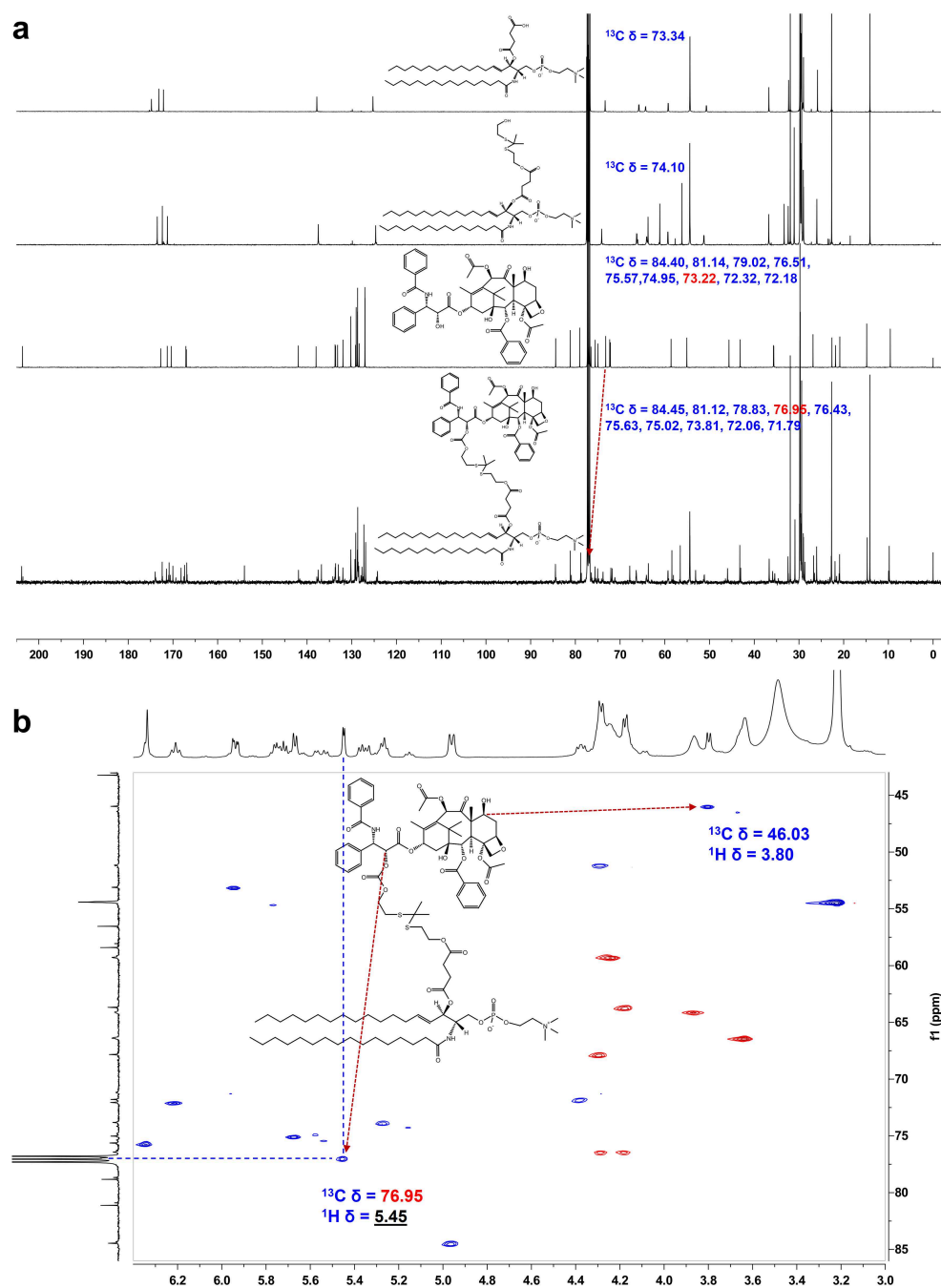
Supplementary Note Fig. 9. ^1H - ^{13}C HSQC NMR of PTX in CDCl_3 indicated that ^{13}C NMR δ 73.22 was associated with ^1H NMR δ 4.78, which further demonstrated that the conjugated site was at hydroxyl group #1 of PTX.

Supplementary Note Fig. 10

47



Supplementary Note Fig. 11

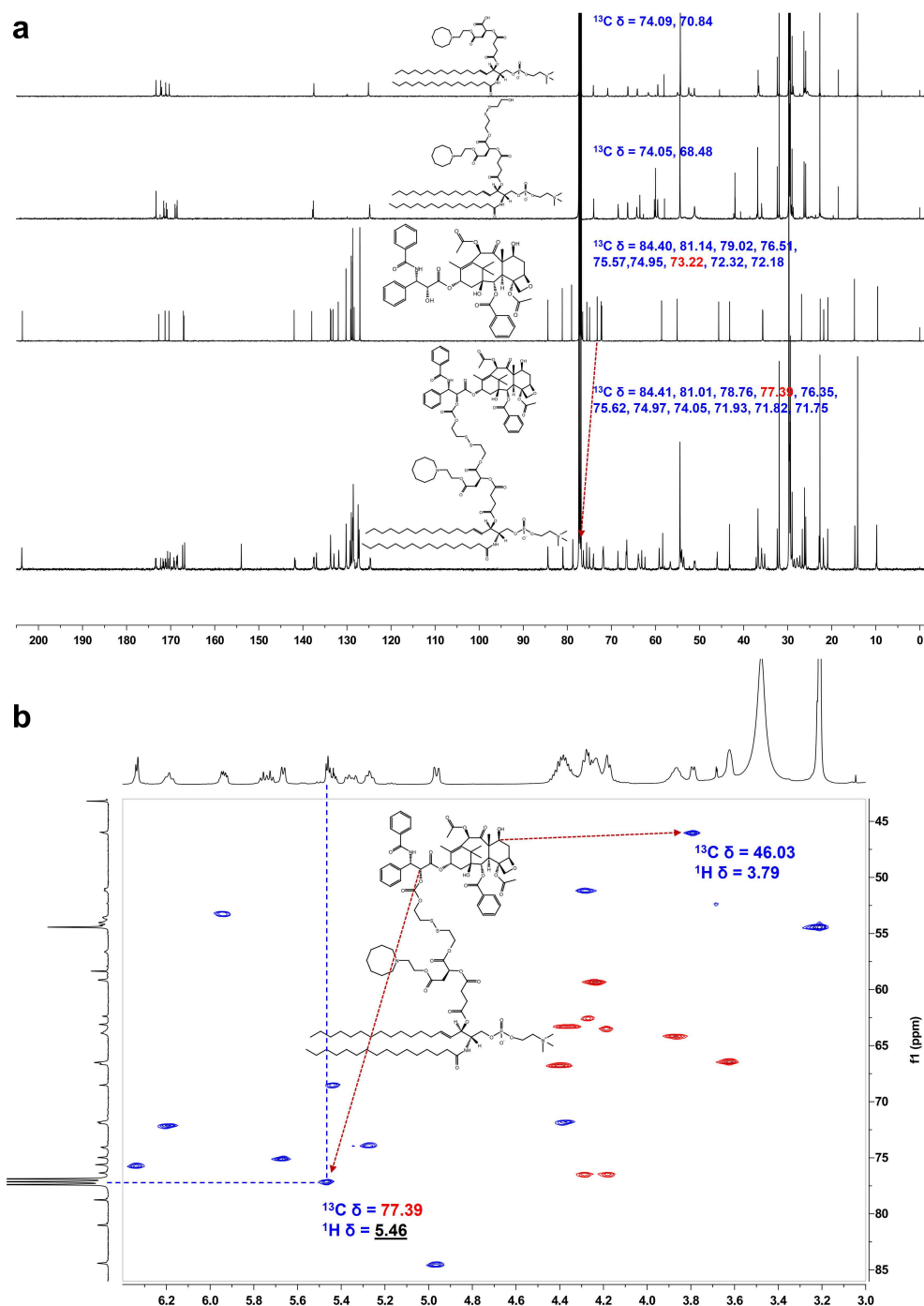


Supplementary Note Fig. 11. a, ^{13}C NMR of SM-COOH, SM-SCS-OH, PTX and SM-SCS-PTX in CDCl_3 . After conjugating SM-COOH with PTX to form SM-SCS-PTX, the ^{13}C NMR chemical shift δ 73.22 in PTX was increased to 76.95. **b,** ^1H - ^{13}C HSQC NMR of SM-SCS-PTX indicated that ^{13}C NMR δ 76.95 was associated with ^1H NMR δ 5.45, which demonstrated that the conjugated site was at hydroxyl group #1 of PTX.



Supplementary Note Fig. 12

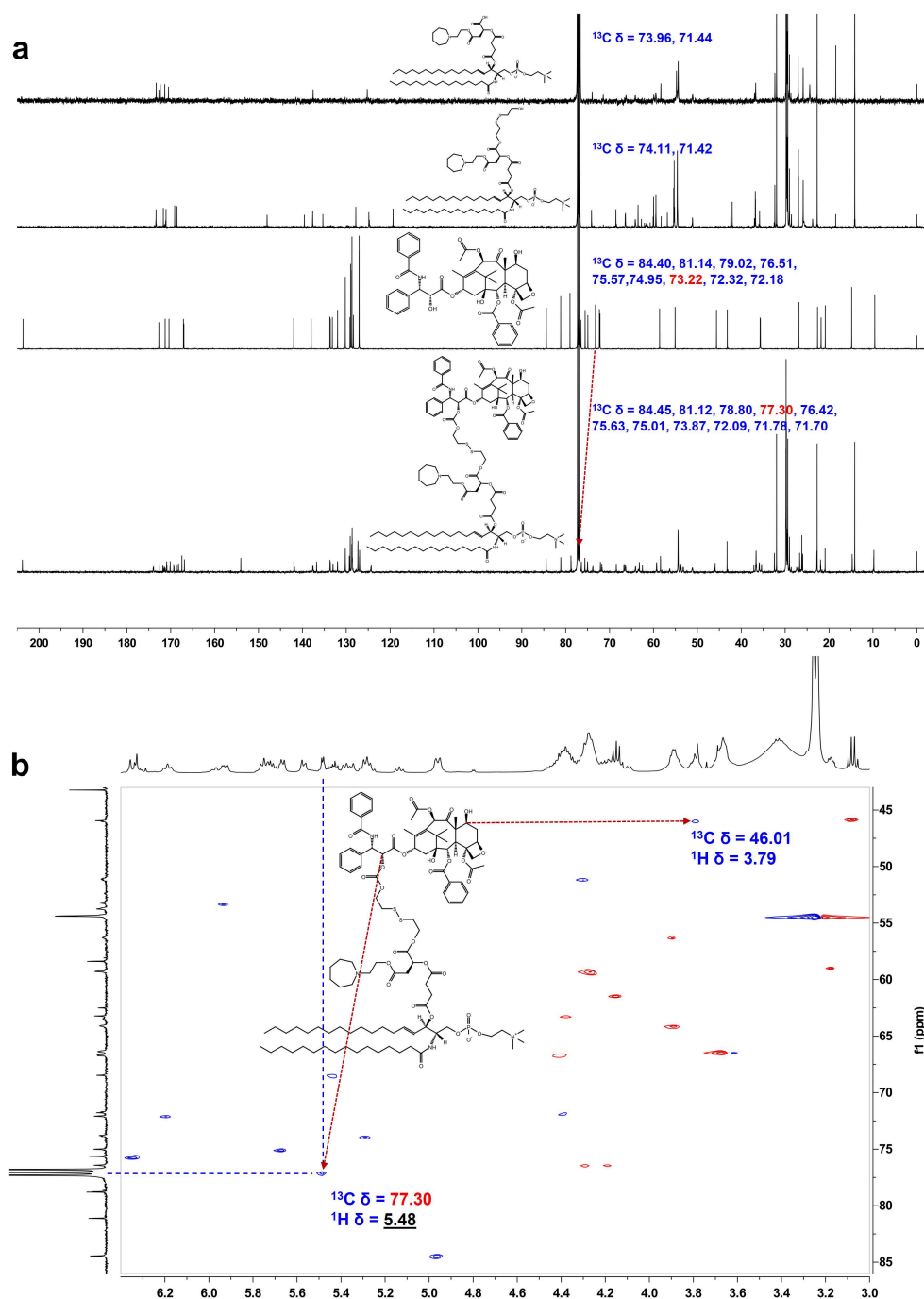
49



Supplementary Note Fig. 12. **a**, ^{13}C NMR of B-6, B-7, PTX and SM-AZO-PTX in CDCl_3 . After conjugating B-7 with PTX to form SM-AZO-PTX, the ^{13}C NMR chemical shift δ 73.22 in PTX was increased to 77.39. **b**, ^1H - ^{13}C HSQC NMR of SM-AZO-PTX indicated that ^{13}C NMR δ 77.39 was associated with ^1H NMR δ 5.46, which demonstrated that the conjugated site was at hydroxyl group #1 of PTX.

Supplementary Note Fig. 13

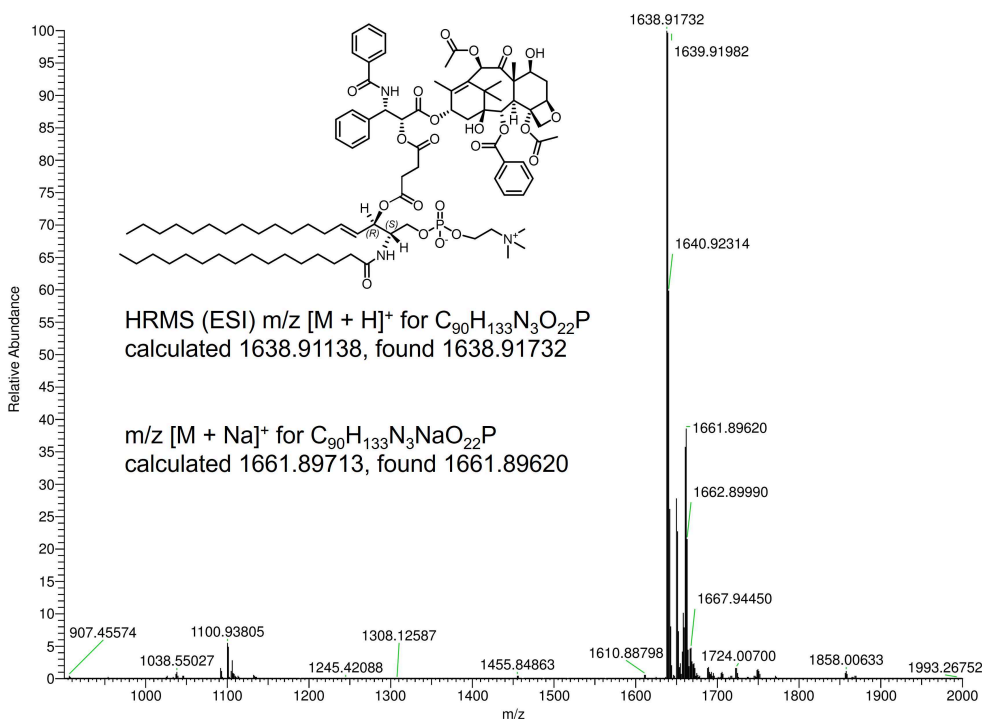
50



Supplementary Note Fig. 13. **a**, ^{13}C NMR of A-5, A-6, PTX and SM-AZE-PTX in CDCl_3 . After conjugating A-6 with PTX to form SM-AZE-PTX, the ^{13}C NMR chemical shift δ 73.22 in PTX was increased to 77.30. **b**, ^1H - ^{13}C HSQC NMR of SM-AZE-PTX indicated that ^{13}C NMR δ 77.30 was associated with ^1H NMR δ 5.48, which demonstrated that the conjugated site was at hydroxyl group #1 of PTX.

Supplementary Note Fig. 14

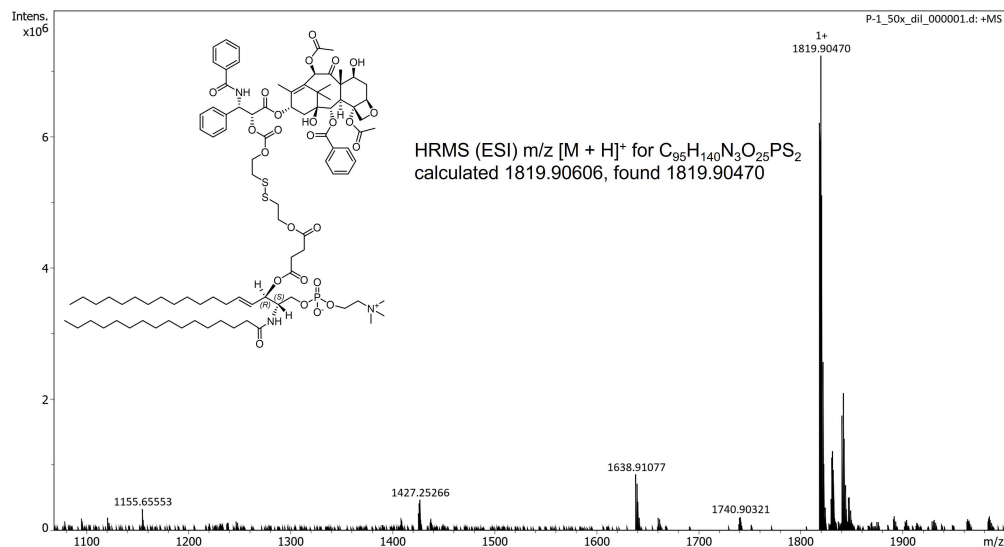
51



Supplementary Note Fig. 14. HRMS (ESI) spectrum of SM-Ester-PTX.

Supplementary Note Fig. 15

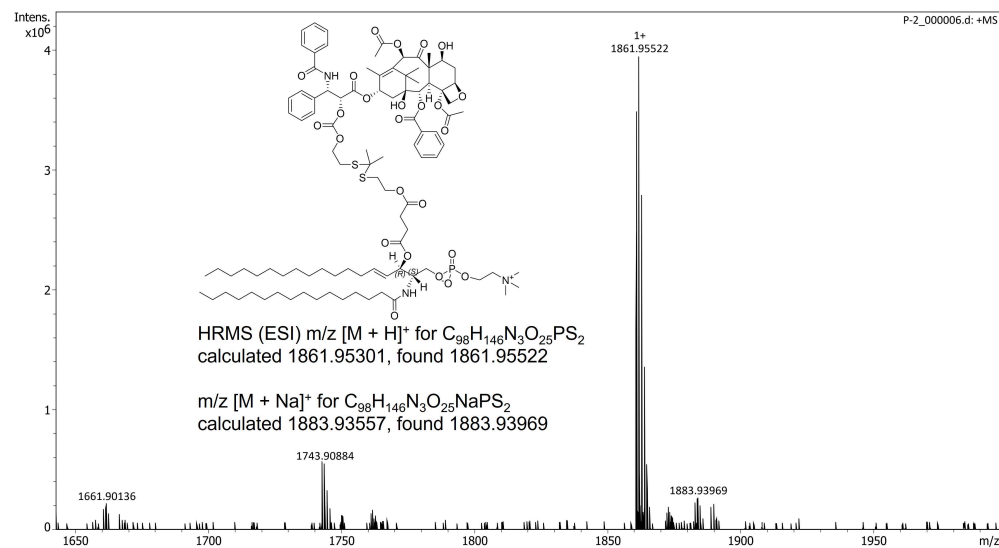
52



Supplementary Note Fig. 15. HRMS (ESI) spectrum of SM-CSS-PTX.

Supplementary Note Fig. 16

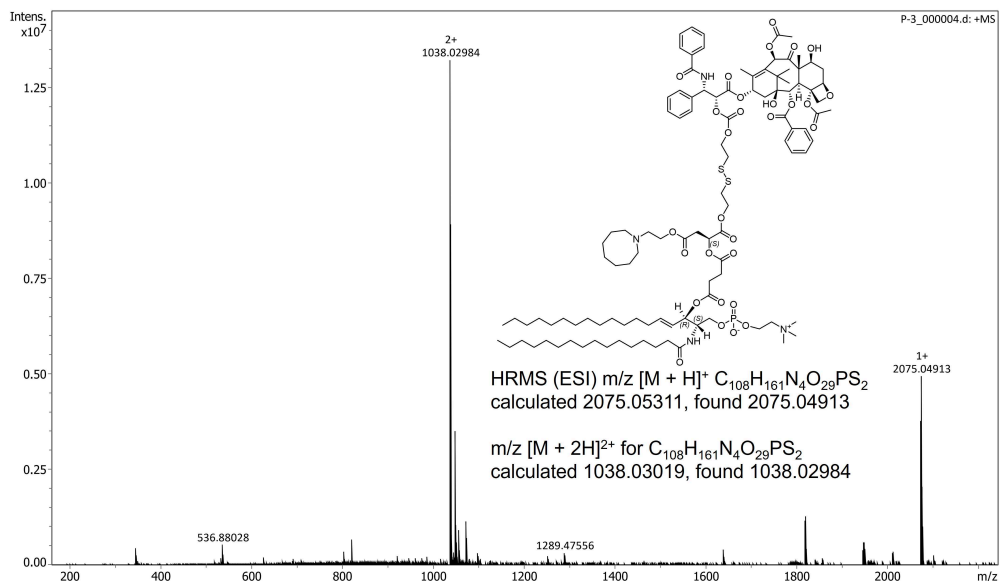
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Supplementary Note Fig. 16. HRMS (ESI) spectrum of SM-SCS-PTX.

Supplementary Note Fig. 17

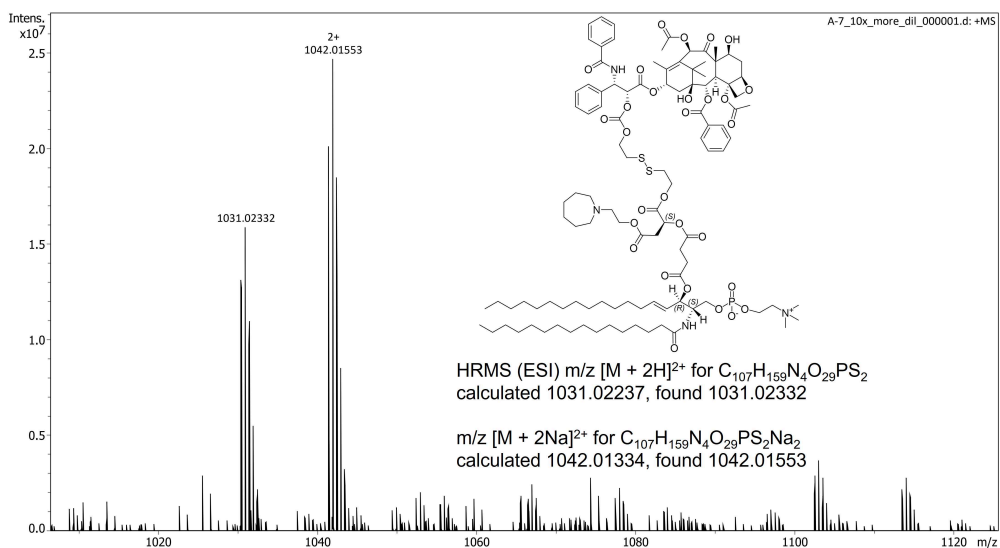
54



Supplementary Note Fig. 17. HRMS (ESI) spectrum of SM-AZO-PTX.

Supplementary Note Fig. 18

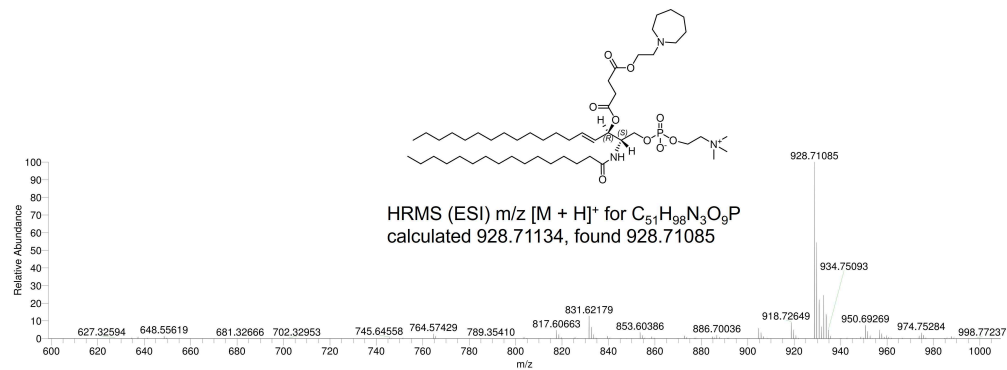
55



Supplementary Note Fig. 18. HRMS (ESI) spectrum of SM-AZE-PTX.

Supplementary Note Fig. 19

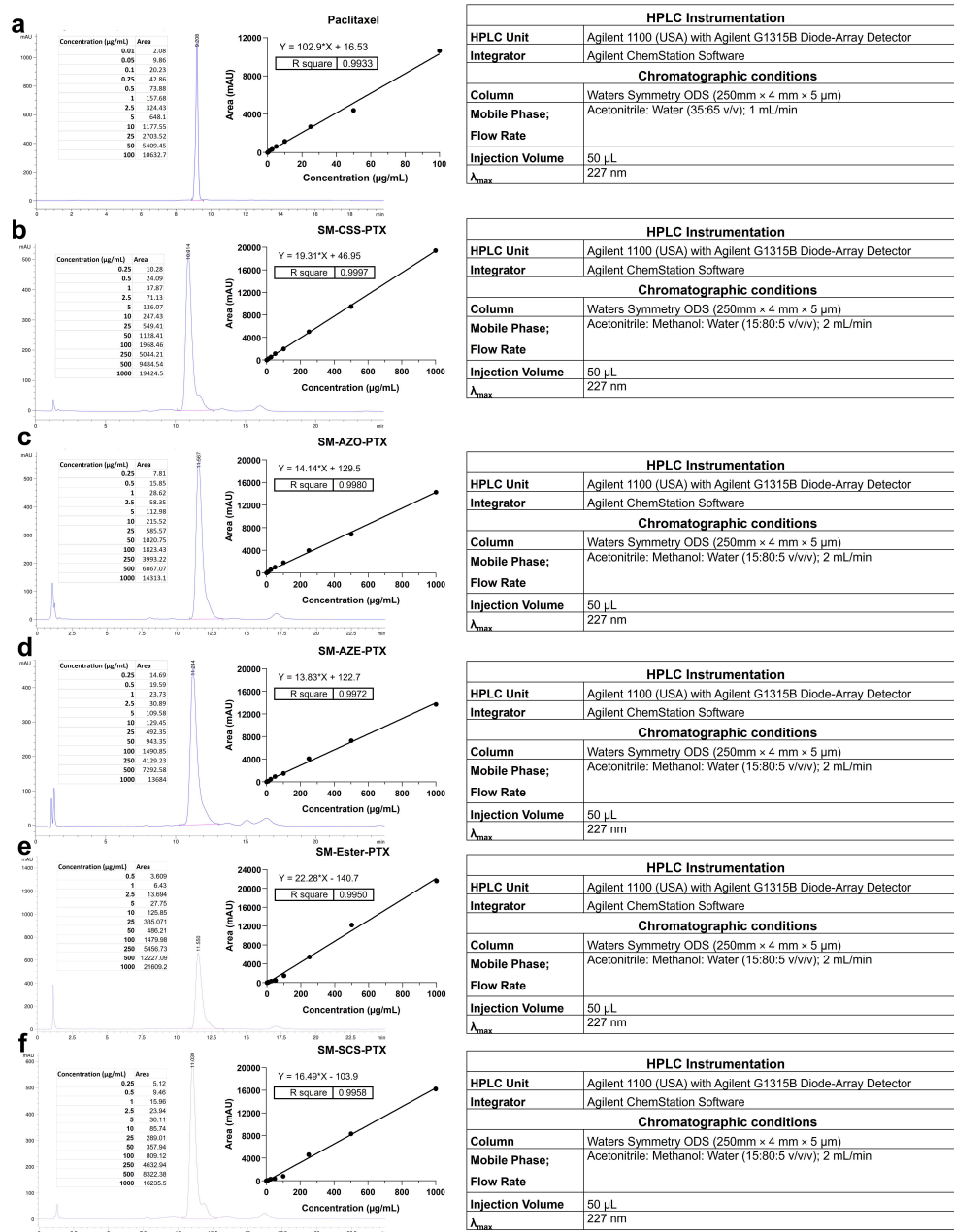
56



Supplementary Note Fig. 19. HRMS (ESI) spectrum of SM-AZE.

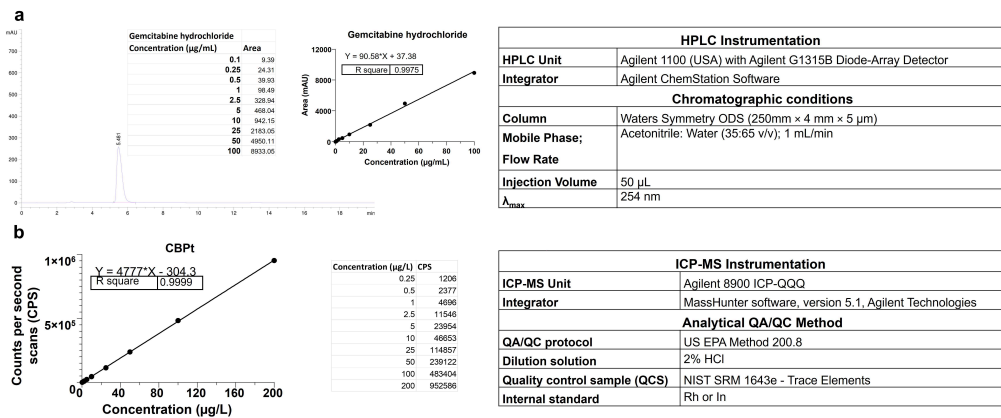
Supplementary Note Fig. 20

57



Supplementary Note Fig. 20. a-f, The Analytic Reverse-phase High Performance Liquid Chromatography (HPLC) method development for free PTX (a), SM-CSS-PTX (b), SM-AZO-PTX (c), SM-AZE-PTX (d), SM-Ester-PTX (e) and SM-SCS-PTX (f) concentration measurement in pharmacokinetics and biodistribution studies. Representative HPLC chromatogram, standard curve, and HPLC instrumentation and chromatographic conditions.

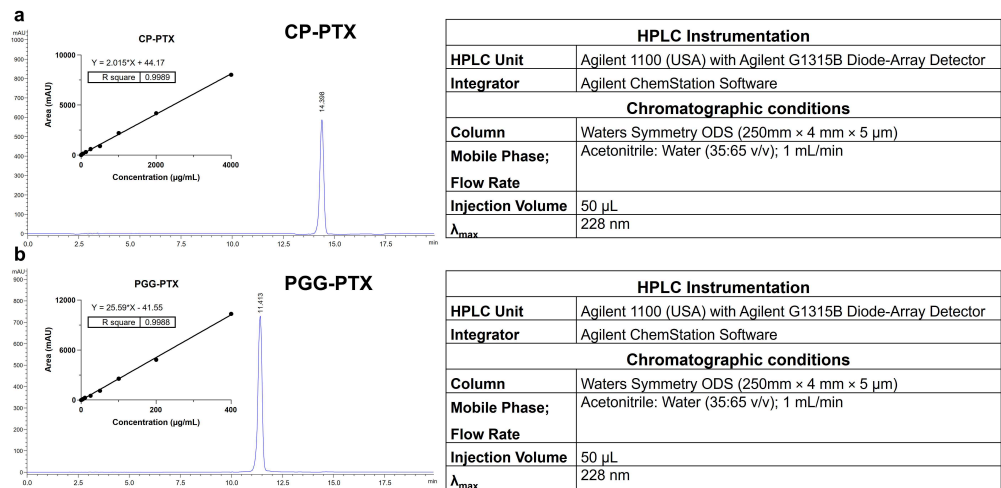
Supplementary Note Fig. 21



Supplementary Note Fig. 21. a, The Analytic Reverse-phase High Performance Liquid Chromatography (HPLC) method development for Gemcitabine hydrochloride concentration measurement in pharmacokinetics and biodistribution studies. Representative HPLC chromatogram, standard curve, and HPLC instrumentation and chromatographic conditions. **b**, The ICP-MS method development for CBP concentration measurement in pharmacokinetics and biodistribution studies. Standard curve, and ICP-MS instrumentation and conditions.

Supplementary Note Fig. 22

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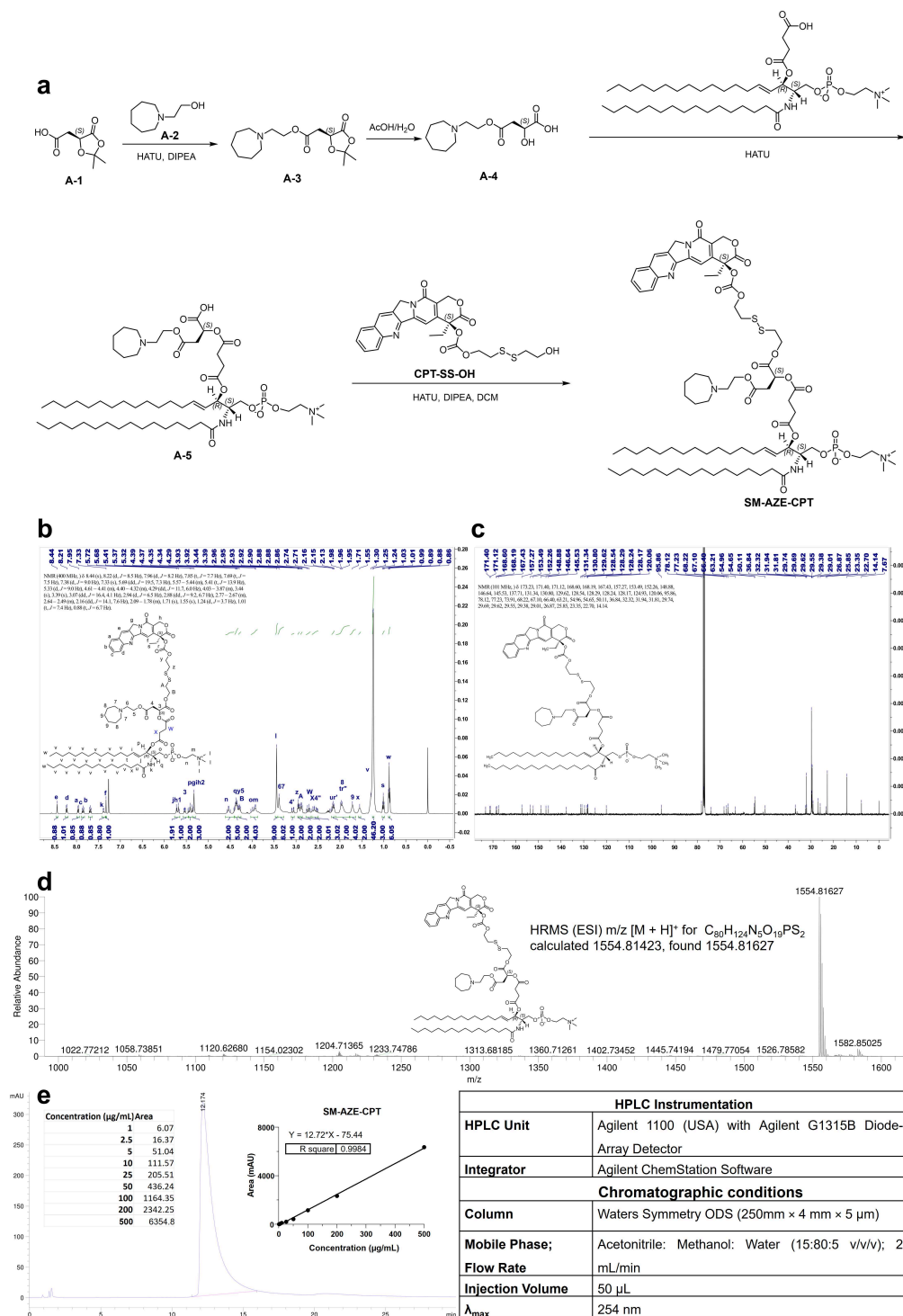


Supplementary Note Fig. 22. a, b, The Analytic Reverse-phase High Performance Liquid Chromatography (HPLC) method development for CP-PTX (**a**) and PGG-PTX (**b**) concentration measurement in pharmacokinetics and biodistribution studies. Representative HPLC chromatogram, standard curve, and HPLC instrumentation and chromatographic conditions.



Supplementary Note Fig. 23

60



Supplementary Note Fig. 23, a, Synthetic route for SM-AZE-CPT. **b,c**, ^1H NMR (**b**) and ^{13}C NMR (**c**) spectra for SM-AZE-CPT. **d**, HRMS (ESI) spectrum of SM-AZE-CPT. **e**, The Analytic Reverse-phase High Performance Liquid Chromatography (HPLC) method development for SM-AZE-CPT concentration measurement in pharmacokinetics and biodistribution studies. Representative HPLC chromatogram, standard curve, and HPLC instrumentation and chromatographic conditions.

Protocol references

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- 2 Yin, W., Ke, W., Chen, W., Xi, L., Zhou, Q., Mukerabigwi, J. F. & Ge, Z. Integrated block copolymer prodrug nanoparticles for combination of tumor oxidative stress amplification and ROS-responsive drug release. *Biomaterials* **195**, 63-74 (2019).
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