Supporting Information for

Self-Assembling Peptide-Etoposide Nanofibers for Overcoming Multidrug Resistance

Li-Song Zhang [†], Li-Xin Yan [†], Shan Gao [†], Hui Long [†], Zhen Xi [§], Lu-Yuan Li [†], and Zhi-Song Zhang *, [†]

[†] State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300350, China

[§] State Key Laboratory of Elemento-Organic Chemistry and College of Chemistry, Nankai University, Tianjin 300071, China

Corresponding Author:

*E-mail: zzs@nankai.edu.cn

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S1. Experiment Materials and Instruments

Roswell Park Memorial Institute-1640, Dulbecco's Modified Essential Medium (DMEM), Penicillin-Streptomycin Solution (100x), Trypsin-EDTA (0.25%), phosphate buffer saline and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Waltham, MA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), RedDot™2 Far-Red Nuclear Stain and 2-Cl-trityl chloride resin (1.0-1.2 mmol/g) were obtained from Gil Biochemical (Shanghai, China). N, N-diisopropylethylamine (DIPEA), Trifluoroacetic acid (TFA), Etoposide, DMSO-d6, Methanol-d4, 1-Hydroxybenzotriazole (HOBt), N-(3-(dimethylamino)propyl)propionimidamide hydrochloride (EDCI), 7-(diethylamino)coumarin-3-carboxylic acid (7ACC), 2-(1H-benzotriazole-1-yl)-1, 1, 3, 3-tetramethylur onium hexafluoro phosphate (HBTU), Fmoc-OSu and other Fmoc-amino acids were obtained from Bide Pharmaceutical (Shanghai, China); Alkaline phosphatase was purchased from Biomatik (Cat. No. A1130, [ALP] >1300U/mg, in 50% glycerol). Morphological analyses were conducted on a Thermo Scientific™ Talos™ F200C transmission electron microscopy. Cellular uptake and drug tracking images were taken by a confocal laser scanning microscopy (Leica TSC SP8, Germany). ^{1}H, ^{31}P and ^{13}C NMR spectra were recorded at 25 °C on a Bruker AV400 NMR spectrometer, operating at 400 and 100 MHz, respectively.

S2. Synthesis and Characterizations

Nap (7ACC)-GFFpYK was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin[1]. Fmoc-Lys(Boc)-OH was loaded onto the resin at the C-terminus, and then the Fmoc protecting group was removed by treatment with 20% piperidine. N,N-diisopropylethylamine/O-benzotriazole-N,N,N',N'-tetramethyluranium-hexafluorophosphate (DIPEA / HBTU) was used as coupling agent. Under a nitrogen atmosphere, the resin-bound peptide was cleaved using a mixture of TFA/triisopropylsilane/water (95:2.5:2.5) for 2 hours[2], and then the cleavage solution was collected. After adding cold ether to the concentrated filtrate, the crude product was obtained and purified by reverse-phase high performance liquid chromatography (HPLC). The obtained peptide solution was lyophilized to obtain a purified compound in a yield of about 40% to 50%. The chemical synthesis of the active ester of etoposide is shown in Figure S2. Both isomers of etoposide are purified and separated by high performance liquid chromatography. The hydrogen spectrum, carbon spectrum and phosphorus spectrum of the compound are as follows.

Nap-GFFpYK: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (t, J = 5.7 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.04 (t, J = 9.1 Hz, 3H), 7.81 – 7.74 (m, 3H), 7.68 (s, 2H), 7.41 (td, J = 7.4, 6.2, 4.3 Hz, 2H), 7.38 – 7.33 (m, 1H), 7.24 – 7.02 (m, 12H), 6.98 (d, J = 8.1 Hz, 2H), 4.52 – 4.39 (m, 3H), 4.12 – 4.04 (m, 1H), 3.66 (dd, J = 16.7, 5.8 Hz, 1H), 3.56 (s, 2H), 3.50 (d, J = 5.5 Hz, 1H), 2.88 (m, 4H), 2.66 (m, 4H), 1.52 (m, 4H), 1.25 – 1.15 (m, 2H). ^{31}P NMR (162 MHz, DMSO) δ -5.74.

7ACC-GFFpYK: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (t, J = 5.2 Hz, 1H), 8.65 (s, 1H), 8.27 (d, J = 8.2 Hz, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.11 (d, J = 7.8 Hz, 1H), 7.98 (s, 1H), 7.74 (s, 2H), 7.68 (d, J = 9.1 Hz, 1H), 7.28 – 7.11 (m, 12H), 7.02 (d, J = 8.1 Hz, 2H), 6.81 (dd, J = 9.1, 2.4 Hz, 1H), 6.62 (d, J = 2.3 Hz, 1H), 4.53 (m, 3H), 4.10 (d, J = 6.7 Hz, 1H), 3.91 (d, J = 5.1 Hz, 1H), 3.85 (d, J = 5.0 Hz, 1H), 3.05 – 2.87 (m, 6H), 2.75 (m, 6H), 1.49 (s, 4H), 1.25 (d, J = 9.5 Hz, 2H), 1.14 (t, J = 7.0 Hz, 6H). ^{31}P NMR (162 MHz, DMSO) δ -5.85.

Etoposide: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.01 (s, 1H), 6.53 (s, 1H), 6.18 (s, 2H), 6.10 – 5.96 (m, 2H), 5.26 (dd, J = 5.3, 1.5 Hz, 2H), 4.93 (d, J = 3.4 Hz, 1H), 4.72 (q, J = 5.0 Hz, 1H), 4.58 (d, J = 7.7 Hz, 1H), 4.49 (d, J = 5.4 Hz, 1H), 4.31 – 4.18 (m, 2H), 4.08 (dd, J = 10.1, 4.8 Hz, 1H), 3.61 (s, 6H), 3.51 (t, J = 10.1 Hz,

1H), 3.26 (td, J = 14.1, 7.5 Hz, 2H), 3.16 (t, J = 9.2 Hz, 1H), 3.06 (td, J = 8.2, 5.2 Hz, 1H), 2.89 (dtd, J = 13.6, 9.8, 9.2, 3.3 Hz, 1H), 1.24 (d, J = 5.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.19, 148.16, 147.54, 146.59, 135.04, 133.21, 130.63, 129.25, 110.29, 108.71, 101.97, 101.74, 99.03, 80.55, 74.82, 73.16, 72.28, 68.13, 67.79, 66.21, 56.38, 43.37, 40.94, 37.61, 20.78.

1E: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 8.30 (s, 1H), 7.04 (s, 1H), 6.51 (s, 1H), 6.15 (s, 2H), 6.02 (d, J = 40.6 Hz, 2H), 4.99 (d, J = 8.0 Hz, 1H), 4.84 (d, J = 3.2 Hz, 1H), 4.75 (p, J = 5.0 Hz, 1H), 4.60 – 4.46 (m, 2H), 4.27 (t, J = 7.9 Hz, 1H), 4.16 (dd, J = 10.2, 8.4 Hz, 1H), 4.07 (dd, J = 10.1, 4.8 Hz, 1H), 3.64 (s, 1H), 3.60 (s, 6H), 3.54 (d, J = 10.5 Hz, 1H), 3.40 (td, J = 9.7, 4.9 Hz, 2H), 3.29 (t, J = 9.3 Hz, 1H), 2.97 (dd, J = 14.2, 5.1 Hz, 1H), 2.89 (ddt, J = 10.8, 7.1, 3.2 Hz, 1H), 2.39 (dt, J = 14.4, 5.1 Hz, 1H), 2.20 (q, J = 6.8 Hz, 2H), 2.09 – 2.00 (m, 1H), 1.91 (s, 4H), 1.25 (d, J = 4.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.79, 173.4 , 172.53, 171.35, 148.21, 147.53, 146.61, 135.06, 132.79, 130.38, 129.51, 110.30, 109.63, 108.66, 101.84, 100.21, 99.13, 80.27, 75.01, 74.07 , 70.69, 67.93, 67.58, 66.25, 56.37, 43.21, 37.36, 29.00, 28.94, 21.55, 20.72.

2E: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H), 8.30 (s, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.52 (s, 1H), 6.18 (s, 2H), 6.03 (d, J = 13.9 Hz, 2H), 4.96 (dd, J = 15.2, 6.1 Hz, 2H), 4.78 (d, J = 7.7 Hz, 1H), 4.72 (d, J = 5.1 Hz, 1H), 4.49 (d, J = 5.2 Hz, 1H), 4.26 (d, J = 9.0 Hz, 2H), 4.11 (dd, J = 10.3, 3.8 Hz, 1H), 3.60 (d, J = 2.4 Hz, 6H), 3.53 (t, J = 9.4 Hz, 1H), 3.42 (d, J = 4.7 Hz, 2H), 3.29 – 3.21 (m, 2H), 2.94 – 2.86 (m, 1H), 2.45 (d, J = 6.7 Hz, 2H), 1.90 (d, J = 2.3 Hz, 2H), 1.23 (d, J = 3.0 Hz, 2H), 1.19 (d, J = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.12, 173.70, 171.97, 148.20, 147.54, 146.64, 135.04, 133.13, 130.54, 129.23, 110.39, 110.19, 108.69, 101.98, 101.74, 99.04, 77.82, 74.24, 72.99, 72.45, 68.12, 67.64, 66.00, 56.37, 43.35, 40.93, 40.61, 40.56, 40.41, 40.35, 40.20, 40.15, 39.99, 39.94, 39.73, 39.52, 39.31, 37.57, 29.36, 21.58, 20.65.

E1: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 6.99 (s, 1H), 6.52 (s, 1H), 6.15 (d, J = 4.8 Hz, 2H), 6.01 (d, J = 23.8 Hz, 2H), 5.53 (d, J = 5.6 Hz, 1H), 4.91 – 4.87 (m, 1H), 4.76 (d, J = 5.1 Hz, 1H), 4.62 – 4.54 (m, 1H), 4.48 (dd, J = 14.7, 5.1 Hz, 1H), 4.26 (q, J = 7.7, 6.6 Hz, 1H), 4.19 – 4.04 (m, 2H), 3.67 (td, J = 9.2, 5.6 Hz, 1H), 3.60 (s, 6H), 3.59 – 3.53 (m, 1H), 3.34 – 3.27 (m, 1H), 3.05 (dd, J = 14.3, 5.4 Hz, 1H), 2.89 (ddd, J = 11.1, 7.4, 3.5 Hz, 1H), 2.81 (s, 3H), 2.75 (d, J = 6.6 Hz, 1H), 2.39 – 2.02 (m, 2H), 1.25 (d, J = 5.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 174.89, 170.55, 170.27, 168.55, 148.25, 147.53, 146.54, 135.04, 133.24, 130.54, 128.78, 110.44, 109.82, 108.65, 101.86, 99.20, 99.14, 80.22, 75.37, 73.21, 70.64, 67.81, 67.57, 66.26, 56.37, 55.44, 51.61, 43.25, 37.43, 28.40, 25.90, 20.72.

E2: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 7.05 (s, 1H), 6.52 (s, 1H), 6.18 (s, 2H), 6.03 (d, J = 14.6 Hz, 2H), 5.55 (d, J = 5.7 Hz, 1H), 5.03 – 4.91 (m, 2H), 4.78 (d, J = 7.7 Hz, 1H), 4.70 (d, J = 5.1 Hz, 1H), 4.49 (d, J = 5.4 Hz, 1H), 4.27 (d, J = 9.2 Hz, 2H), 4.11 (dd, J = 10.0, 4.4 Hz, 1H), 3.61 (s, 6H), 3.58 (d, J = 1.1 Hz, 1H), 3.52 (q, J = 8.9, 7.9 Hz, 1H), 3.48 – 3.39 (m, 2H), 3.33 – 3.20 (m, 2H), 2.96 – 2.88 (m, 2H), 2.80 (s, 3H), 2.69 (t, J = 6.7 Hz, 1H), 1.28 – 1.20 (m, 2H), 1.19 (t, J = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.12, 170.83, 170.57, 168.60, 148.20, 147.54, 146.64, 135.04, 133.13, 130.53, 129.24, 110.39, 110.19, 108.69, 101.99, 101.74, 99.05, 77.71, 74.70, 73.06, 72.38, 68.12, 67.66, 65.98, 56.37, 55.39, 51.90, 43.35, 40.93, 37.57, 28.61, 26.10, 25.89, 20.62.

7ACC-GFFpYK-Etoposide1(AFE1): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (t, J = 5.0 Hz, 1H), 8.65 (s, 1H), 8.26 (d, J = 7.5 Hz, 1H), 8.21 (d, J = 8.4 Hz, 1H), 8.17 (d, J = 8.1 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 5.5 Hz, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.27 – 7.10 (m, 12H), 7.05 (d, J = 8.1 Hz, 2H), 7.01 (s, 1H), 6.80 (dd, J = 9.0, 2.3 Hz, 1H), 6.62 (d, J = 2.3 Hz, 1H), 6.49 (s, 1H), 6.16 (s, 2H), 6.00 (d, J = 39.1 Hz, 2H), 4.97 (d, J = 8.0 Hz, 1H), 4.84 (d, J = 3.2 Hz, 1H), 4.75 (d, J = 5.3 Hz, 1H), 4.56 (d, J = 8.6 Hz, 1H), 4.54 – 4.49 (m, 3H), 4.26 (t, J = 7.9 Hz,

1H), 4.16 (t, J = 9.5 Hz, 2H), 4.10 – 4.05 (m, 1H), 3.92 (dd, J = 17.2, 5.0 Hz, 1H), 3.82 (dd, J = 17.2, 5.2 Hz, 1H), 3.60 (s, 6H), 3.48 (d, J = 7.1 Hz, 4H), 3.44 – 3.34 (m, 2H), 3.28 (t, J = 9.2 Hz, 2H), 3.03 – 2.96 (m, 4H), 2.93 (d, J = 9.4 Hz, 2H), 2.80 (d, J = 10.0 Hz, 2H), 2.69 (dd, J = 13.9, 9.6 Hz, 2H), 2.18 – 2.12 (m, 1H), 2.03 (d, J = 11.3 Hz, 2H), 1.72 (s, 1H), 1.60 (s, 1H), 1.44 – 1.26 (m, 4H), 1.26 – 1.23 (m, 4H), 1.14 (t, J = 7.0 Hz, 6H). ^{31}P NMR (162 MHz, DMSO) δ -6.20.

7ACC-GFFpYK-Etoposide2(AFE2): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (t, J = 5.1 Hz, 1H), 8.65 (s, 1H), 8.25 (d, J = 7.6 Hz, 1H), 8.21 (d, J = 8.2 Hz, 1H), 8.17 (d, J = 8.2 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.88 (t, J = 5.5 Hz, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.29 – 7.10 (m, 12H), 7.06 (d, J = 8.3 Hz, 3H), 6.80 (dd, J = 9.1, 2.4 Hz, 1H), 6.62 (d, J = 2.3 Hz, 1H), 6.51 (s, 1H), 6.18 (s, 2H), 6.02 (d, J = 13.7 Hz, 2H), 5.00 – 4.93 (m, 2H), 4.78 (d, J = 7.6 Hz, 1H), 4.71 (d, J = 5.2 Hz, 1H), 4.58 (d, J = 4.9 Hz, 1H), 4.49 (d, J = 5.6 Hz, 3H), 4.26 (d, J = 9.4 Hz, 2H), 4.18 (q, J = 7.9 Hz, 1H), 4.10 (d, J = 7.8 Hz, 1H), 3.92 (dd, J = 17.3, 5.0 Hz, 1H), 3.82 (dd, J = 17.2, 5.2 Hz, 1H), 3.61 (s, 6H), 3.48 (q, J = 7.2 Hz, 4H), 3.42 (d, J = 6.8 Hz, 2H), 3.29 – 3.20 (m, 2H), 3.00 (t, J = 11.4 Hz, 4H), 2.94 (s, 2H), 2.85 – 2.76 (m, 2H), 2.76 – 2.63 (m, 2H), 2.33 (t, J = 7.4 Hz, 2H), 2.04 – 1.93 (m, 1H), 1.71 (s, 1H), 1.64 – 1.55 (m, 1H), 1.44 – 1.27 (m, 4H), 1.23 (s, 2H), 1.19 (d, J = 4.9 Hz, 2H), 1.14 (t, J = 7.0 Hz, 6H). ^{31}P NMR (162 MHz, DMSO) δ -6.20.

Nap-GFFpYK-Etoposide1(NFE1): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 – 8.21 (m, 2H), 8.12 (dd, J = 18.5, 7.6 Hz, 2H), 8.03 (d, J = 8.3 Hz, 1H), 7.88 – 7.79 (m, 4H), 7.75 (s, 1H), 7.48 (q, J = 6.2, 5.3 Hz, 2H), 7.42 (d, J = 8.5 Hz, 1H), 7.27 – 7.11 (m, 12H), 7.09 – 6.99 (m, 3H), 6.50 (s, 1H), 6.17 (s, 2H), 6.00 (d, J = 38.7 Hz, 2H), 4.97 (d, J = 8.0 Hz, 1H), 4.57 (d, J = 8.2 Hz, 1H), 4.50 (d, J = 5.0 Hz, 3H), 4.27 (q, J = 8.3, 7.5 Hz, 1H), 4.17 (d, J = 9.6 Hz, 2H), 4.07 (d, J = 6.8 Hz, 1H), 3.71 (dd, J = 16.7, 5.4 Hz, 1H), 3.61 (d, J = 8.7 Hz, 8H), 3.52 (s, 1H), 3.42 – 3.39 (m, 1H), 3.31 – 3.26 (m, 2H) 2.99 (d, J = 13.5 Hz, 5H), 2.94 – 2.75 (m, 4H), 2.74 – 2.61 (m, 2H), 2.40 (d, J = 7.6 Hz, 2H), 2.16 (d, J = 7.9 Hz, 1H), 2.01 (s, 2H), 1.72 (s, 1H), 1.59 (s, 1H), 1.28 – 1.19 (m, 6H). ^{31}P NMR (162 MHz, DMSO) δ -6.21.

Nap-GFFpYK-Etoposide2(NFE2): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 – 8.22 (m, 2H), 8.12 (dd, J = 16.7, 8.0 Hz, 2H), 8.03 (d, J = 8.3 Hz, 1H), 7.87 – 7.81(m, 4H), 7.75 (s, 1H), 7.49 – 7.45 (m, 2H), 7.43 – 7.40 (m, 1H), 7.25 – 7.14 (m, 12H), 7.06 (d, J = 9.2 Hz, 3H), 6.52 (s, 1H), 6.18 (s, 2H), 6.02 (d, J = 14.0 Hz, 2H), 4.94 (d, J = 3.5 Hz, 1H), 4.57 (d, J = 4.1 Hz, 1H), 4.49 (d, J = 5.7 Hz, 3H), 4.26 (d, J = 9.1 Hz, 2H), 4.17 (d, J = 6.5 Hz, 2H), 3.69 (d, J = 5.6 Hz, 1H), 3.61 (d, J = 4.2 Hz, 8H), 3.56 – 3.52 (m, 2H), 3.42 (d, J = 7.2 Hz, 2H), 3.28 – 3.22 (m, 2H), 3.00 (d, J = 9.4 Hz, 5H), 2.91 (d, J = 3.7 Hz, 2H), 2.86 – 2.72 (m, 4H), 2.70 – 2.61 (m, 2H), 2.33 (d, J = 7.4 Hz, 2H), 1.71 (s, 2H), 1.59 (d, J = 9.5 Hz, 2H), 1.39 – 1.29 (m, 4H), 1.19 (d, J = 5.0 Hz, 3H). ^{31}P NMR (162 MHz, DMSO) δ -6.20.

7ACC-NH_2-Boc: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (t, J = 5.8 Hz, 1H), 8.65 (s, 1H), 7.67 (d, J = 9.0 Hz, 1H), 6.95 (t, J = 5.5 Hz, 1H), 6.79 (dd, J = 9.1, 2.4 Hz, 1H), 6.60 (s, 1H), 3.47 (q, J = 7.0 Hz, 4H), 3.34 (t, J = 6.0 Hz, 2H), 3.09 (q, J = 6.0 Hz, 2H), 1.36 (s, 9H), 1.13 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.90, 162.02, 157.67, 156.19, 152.86, 148.13, 132.03, 110.54, 109.80, 108.05, 96.27, 78.12, 44.79, 40.21, 39.40, 28.67, 12.76.

7ACC-NH_2: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (t, J = 6.0 Hz, 1H), 8.68 (s, 1H), 7.70 (d, J = 9.0 Hz, 1H), 6.82 (dd, J = 9.0, 2.4 Hz, 1H), 6.63 (s, 1H), 3.56 (d, J = 6.1 Hz, 2H), 3.49 (d, J = 7.1 Hz, 4H), 2.97 (t, J = 6.2 Hz, 2H), 1.14 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.64, 161.90, 157.74, 152.98, 148.26, 132.13, 110.63, 109.62, 108.01, 96.28, 44.81, 39.05, 37.48, 12.77.

7ACC-Etoposide1(AE1): ^{1}H NMR (400 MHz,) δ 8.71 (t, J = 5.9 Hz, 1H), 8.66 (s, 1H), 8.24 (s, 1H), 7.96 (t, J

= 5.6 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.00 (s, 1H), 6.80 (dd, J = 9.1, 2.4 Hz, 1H), 6.60 (d, J = 2.3 Hz, 1H), 6.50 (s, 1H), 6.16 (s, 2H), 6.00 (d, J = 33.2 Hz, 2H), 5.47 (d, J = 5.6 Hz, 1H), 4.96 (d, J = 8.0 Hz, 1H), 4.84 (d, J = 3.3 Hz, 1H), 4.75 (q, J = 5.0 Hz, 1H), 4.61 – 4.49 (m, 2H), 4.26 (t, J = 7.9 Hz, 1H), 4.16 (dd, J = 10.4, 8.4 Hz, 1H), 4.08 (dd, J = 10.1, 4.8 Hz, 1H), 3.65 (dt, J = 9.3, 4.6 Hz, 1H), 3.60 (s, 6H), 3.48 (q, J = 7.0 Hz, 4H), 3.39 (dd, J = 9.9, 5.3 Hz, 2H), 3.27 (d, J = 9.3 Hz, 1H), 3.19 (dd, J = 12.2, 5.6 Hz, 2H), 3.01 (dd, J = 14.2, 5.3 Hz, 1H), 2.92 – 2.84 (m, 1H), 2.45 – 2.35 (m, 1H), 2.21 – 2.13 (m, 1H), 2.11 – 1.99 (m, 2H), 1.25 (d, J = 5.0 Hz, 3H), 1.14 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 174.81, 171.68, 171.14, 162.96, 162.05, 157.70, 152.91, 148.26, 148.13, 147.61, 146.65, 135.21, 132.89, 132.02, 130.49, 129.35, 110.59, 110.35, 109.93, 109.65, 108.87, 108.10, 101.86, 100.03, 99.99, 99.15, 96.33, 80.30, 74.99, 73.82, 70.80, 67.90, 67.63, 66.28, 56.45, 44.79, 43.24, 40.80, 39.13, 38.97, 37.44, 30.36, 29.45, 20.72, 12.77.

7ACC-Etoposide2(AE2): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (t, J = 5.9 Hz, 1H), 8.66 (s, 1H), 8.24 (s, 1H), 8.03 (t, J = 5.5 Hz, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.04 (s, 1H), 6.80 (dd, J = 9.1, 2.4 Hz, 1H), 6.61 (d, J = 2.3 Hz, 1H), 6.51 (s, 1H), 6.18 (s, 2H), 6.02 (d, J = 13.0 Hz, 2H), 5.47 (d, J = 5.5 Hz, 1H), 5.00 – 4.91 (m, 2H), 4.77 (d, J = 7.6 Hz, 1H), 4.71 (q, J = 5.0 Hz, 1H), 4.49 (d, J = 5.3 Hz, 1H), 4.31 – 4.21 (m, 2H), 4.10 (dd, J = 10.0, 4.0 Hz, 1H), 3.48 (q, J = 7.0 Hz, 4H), 3.41 (dt, J = 6.4, 2.6 Hz, 2H), 3.36 (d, J = 6.0 Hz, 2H), 3.22 (ddd, J = 14.9, 9.7, 6.6 Hz, 4H), 2.89 (dtd, J = 14.7, 7.6, 6.6, 4.0 Hz, 1H), 2.54 (t, J = 6.9 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 1.19 (d, J = 5.0 Hz, 3H), 1.14 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 175.11, 172.14, 171.37, 162.95, 162.06, 157.69, 152.89, 148.20, 147.56, 146.65, 135.08, 133.12, 132.07, 130.55, 129.29, 110.58, 110.36, 110.17, 109.81, 108.74, 108.08, 102.03, 101.74, 99.05, 96.29, 77.79, 74.22, 73.05, 72.53, 68.14, 67.64, 66.01, 56.39, 44.79, 43.36, 40.93, 39.07, 38.94, 37.60, 30.71, 29.82, 20.66, 12.77.

ALP inhibitor (DQB = 2,5-dimethoxy-N-(quinolin-3-yl)benzenesulfonamide): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.71 (d, J = 2.6 Hz, 1H), 7.96 (d, J = 2.6 Hz, 1H), 7.94 – 7.86 (m, 2H), 7.63 (ddd, J = 8.3, 6.8, 1.4 Hz, 1H), 7.54 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.35 (d, J = 2.8 Hz, 1H), 7.17 – 7.06 (m, 2H), 3.81 (s, 3H), 3.71 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.67, 150.79, 145.24, 144.86, 132.08, 128.98, 128.73, 128.06, 128.01, 127.74, 126.86, 123.11, 120.98, 115.52, 114.80, 56.93, 56.25.

S3. ALP siRNA knocks down ALP protein on cell membrane surface

Depletion of ALP was performed using GenePharma siRNA. MDA-MB-231 cells and MCF-7 cells were seeded into a 6-well plate at a density of 1×10^5 cells/well with serum free culture medium. After incubation for 24 h, transfections at a final concentration of 20 nM of GenePharma siRNA were performed according to the manufacturer's instructions: Take 2 μL/well of Lipofectamine2000 and dilute with 100 μL Opti-MEM I Reduced Serum Medium. After gently mixing, incubate at room temperature for 5 min; Take 2 uL Control siRNA or 2 uL ALP siRNA , dilute with 100 μL Opti-MEM I Reduced Serum Medium, and mix gently; After incubating the diluted Lipofectamine 2000 for 5 minutes, mix gently with the diluted siRNA, and let stand at room temperature for 20 minutes to form a siRNA-transfection reagent mixture. Add the siRNA-transfection reagent mixtureto the well containing the cells and culture medium, and gently shake the well plate to mix; Cultivate in a CO_2 incubator at 37 ℃. After 4-6 hours, the medium can be changed to a complete medium containing serum; The transfection efficiency can be detected by fluorescence microscope 6 hours after transfection. Knockdown of ALP can be observed by western blot at 48 h after transfection.

ALP siRNA sequence: sense 5′-GCCCUCUGCCUCAUGGCAATT-3′

Control siRNA sequence: sense 5′-UUCUCCGAACGUGUCACGUTT-3′

S4. Hydrogel Preparation and TEM Sample Preparation.

Enzyme gelation: To evaluate Nap (7ACC)-GFFpYK-etoposide (NFE1/2 and AFE1/2) self-assembly ability in ALP catalysis. 4 kinds of etoposide gel molecules are added to PBS solution (1mL) at a concentration of 2 mmol/L. By adding an appropriate 1 mol/L sodium carbonate solution, the pH of the solution is adjusted to 7.4. Take 300 microliters of the solution and add recombinant alkaline phosphatase (2.0U/mL) and place it in a 37 ° C incubator for 4 hours.

Nap (7ACC)-GFFpYK-etoposide (NFE1/2 and AFE1/2) gelled sample solution (5μL) were added to the glow-discharged thin carbon-coated copper grid (400 mesh, Pacific Grid-Tech). After 30 seconds, the excess PBS solution was carefully removed from the edges of the grid with filter paper, and the grid was slowly rinsed with deionized water two or three times. After the thin carbon-coated copper grid was dried in air for 48 hours, a TEM image was obtained using a transmission electron microscope.

S5. Establishment of 4T1-MDR and LLC-MDR Cell Line

The gene coding sequence of mouse-derived MDR1 was obtained from the cDNA of LLC cells by PCR-based method, and connected with PEASY-Blunt Simple vector (Transgene, China). The coding sequences of restriction endonuclease sites NotI, SpeI and Flag tags were introduced into the specially designed primers by PCR, and then topologically cloned into PEASY vector. Then, the specific coding sequence of Flag-MDR1 was cloned into the PLVX eukaryotic expression vector between NotI/SpeI sites to obtain PLVX-Flag-MDR1. All inserted sequences of the construct were confirmed by Sanger DNA sequencing. In this experiment, the Flag-MDR1-over-expressed lentivirus with the expression of the Puromycin resistance gene was used to infect 4T1 cells and LLC cells. The EF-1α promoter drives the expression of the Flag-MDR1 gene. 48 hours after the infection of 4T1 cells and LLC cells, cells that were not effectively infected were killed by adding and maintaining 2 μg/mL of Puromycin. Under the maintenance of the Puromycin drug, 4T1-MDR cells and LLC-MDR cells with the stable expression of Flag-MDR1 were finally obtained.

S6. Cell Culture and Cell Viability Assay

MCF-7, MB-MDA-231, LLC, and 4T1 cells were purchased from the American Type Culture Collection (ATCC, USA) and cultured in a basic essential medium (MEM) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. The incubation conditions for the cells are at 37 °C in a humidified atmosphere of 5% CO_2. For cell viability assay, MCF-7, MB-MDA-231, LLC, LLC-MDR, 4T1, and 4T1-MDR in the logarithmic growth phase were seeded into 96-well plates at a density of 2000 cells/well and cultured for 6 hours. Then the cells were incubated with etoposide, Nap (7ACC)-GFFpYK-etoposide (NFE-1/2 and AFE-1/2) at gradient concentrations (0 μmol/L as a control) ranging from 0 to 160 μmol/L. After 48 h treatment, the medium was replaced with 200 μL 0.5 mg/mL MTT. After 3 hours the MTT solution was replaced with 200 μL DMSO solution. Measure the absorbance of each well at 570 nm with a microplate reader.

S7. Flow Cytometry

Apoptosis was evaluated by Annexin V-FITC apoptosis detection kit (Beyotime, China) following the manufacturer's instructions. MCF-7, MB-MDA-231, LLC, LLC-MDR, 4T1, and 4T1-MDR cells were seeded into a 6-well plate at a density of 1×10^5 cells/well. After incubation for 24 h, treat the cells with 10 μmol/L etoposide and etoposide hydrogel (NFE1/NFE2), respectively, using untreated cells as the negative control. After 12 hours, the cells were trypsinized and centrifuged at 1000g for 5 min. Remove the supernatant, wash the cells with ice-cold

PBS and re-suspend the cells in 195 μL of binding buffer. Thereafter, 5 μL of Annexin V-FITC and 10 μL of PI were added and the cells were incubated for 20 min in the dark at room temperature. The fluorescence of 10,000 events per sample was analyzed using flow cytometry (BD, USA).

S8. Sample Preparation for Confocal Microscopy

LLC cells and 4T1 cells in log phase growth were seeded at the density of 1×10^5 cells/well in a dedicated confocal dish. 7ACC-etoposide (AE1/2) and 7ACC-GFFpYK-etoposide (AFE1/2) were added to Petri dishes, maintaining the concentration at 20μmol/L, and incubated at 37 °C for 3 hours. Wash the cells three times with PBS buffer and stain the cells with 1xRedDot2 at 37 °C for 2 minutes. Confocal fluorescence imaging was performed on Leica TSC SP8 confocal laser scanning microscope (CLSM).

S9. *In Vivo* Therapy Assays of the Etoposide Nanofibers.

Female 7~week~old C57BL/6 mice (weight 18~20 g) and BALB/c mice (weight 17~19 g) were purchased from Chinese Academy of Medical Sciences (Beijing, China). To establish the tumor model, 4T1 cells and 4T1-MDR cells (1.0×10^5) were injected into the mammary fat pads of the BALB/c mice. LLC cells and LLC-MDR cells (2.0×10^5) were subcutaneously injected into the back skin of the C57BL/6 mice. After the tumors had been allowed to develop to approximately 50 mm^3, in vivo tumor suppression studies were carried out to examine the toxicity and tumor inhibition efficiency of Nap-GFFpYK-etoposide (NFE1 or NFE2). Using the same molar concentration method, 36 mice are divided into the following six experimental groups (N=6): PBS (control group), Nap-GFFpYK (7.72 mg/kg), etoposide (5 mg/kg), Nap-GFFpYK (7.72 mg/kg) + etoposide (5 mg/kg), Nap-GFFpYK-etoposide1/2 (NFE1 or NFE2) (13.41 mg/kg). Each group was treated with continuous paratumoral injection for 16 days. The weights and tumor sizes were recorded daily at the same time. Tumor sizes were measured by a vernier caliper. Tumor volume was calculated by the formula: $V = (L\times W^2)/2$ (L is for the longest and W is the shortest in tumor diameters (mm)).

All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85-23, revised in 2011). All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee at Nankai University, Tianjin, China. We made every effort to minimize animal suffering and to reduce the number of animals used.

S10. Supplementary Figures S1 to S16.

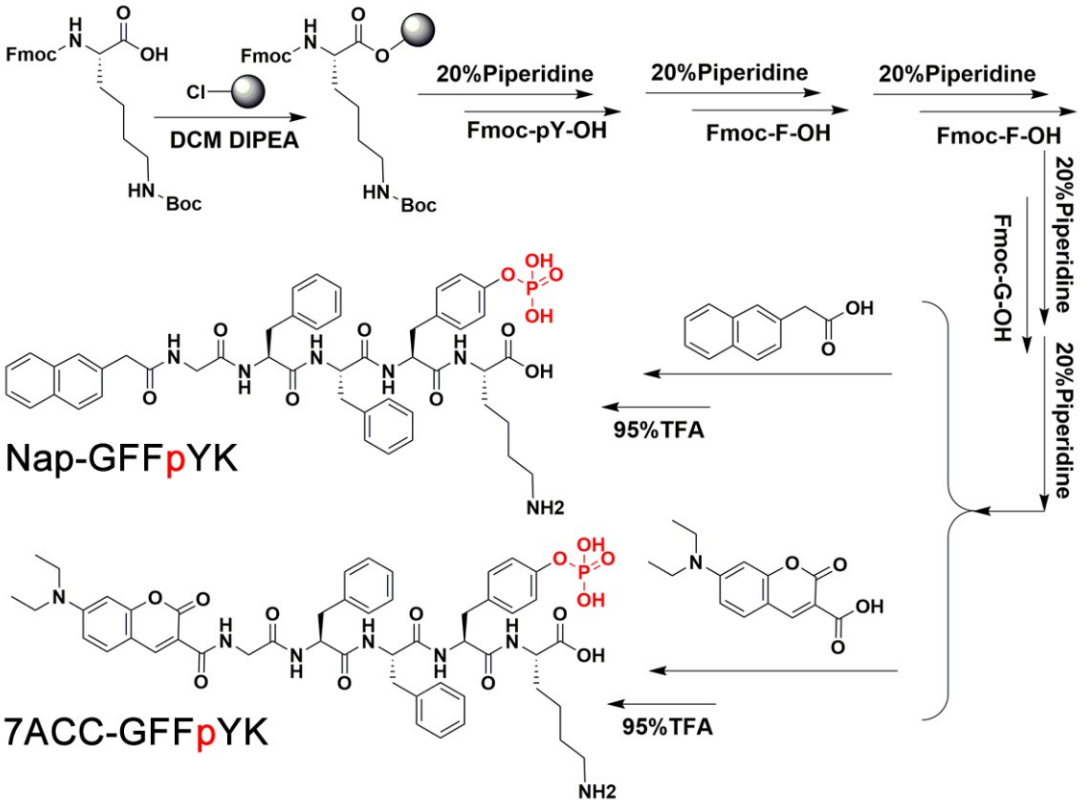

Figure S1. Standard solid-phase synthesis of Nap-GFFpYK and 7ACC-GFFpYK.

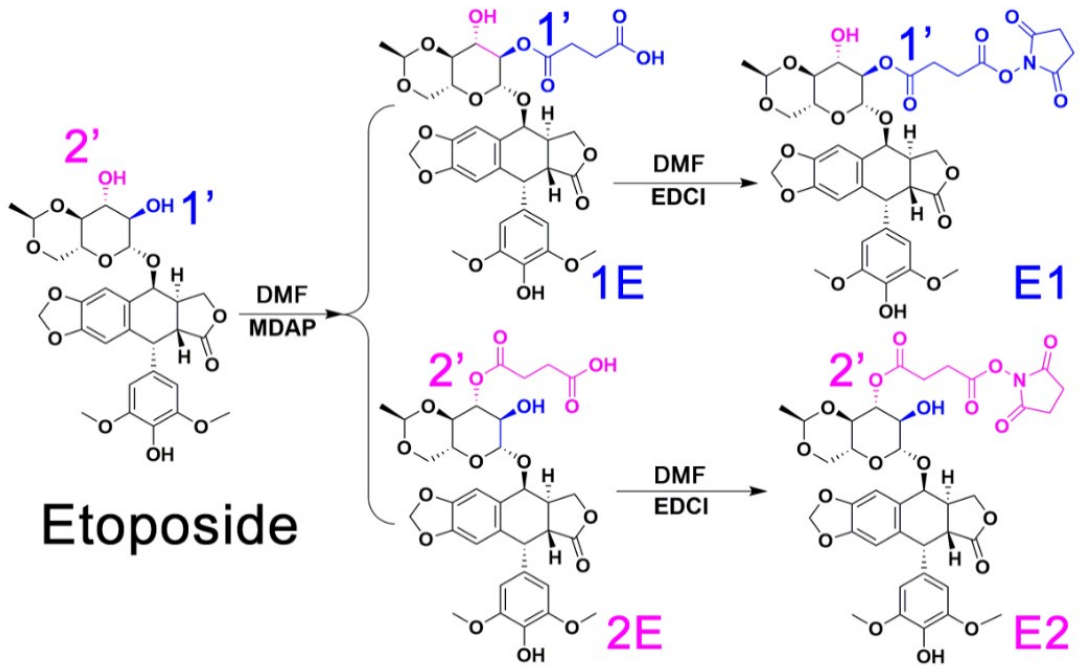

Figure S2. Synthesis routes of Etoposide1(E1) and Etoposide(E2).

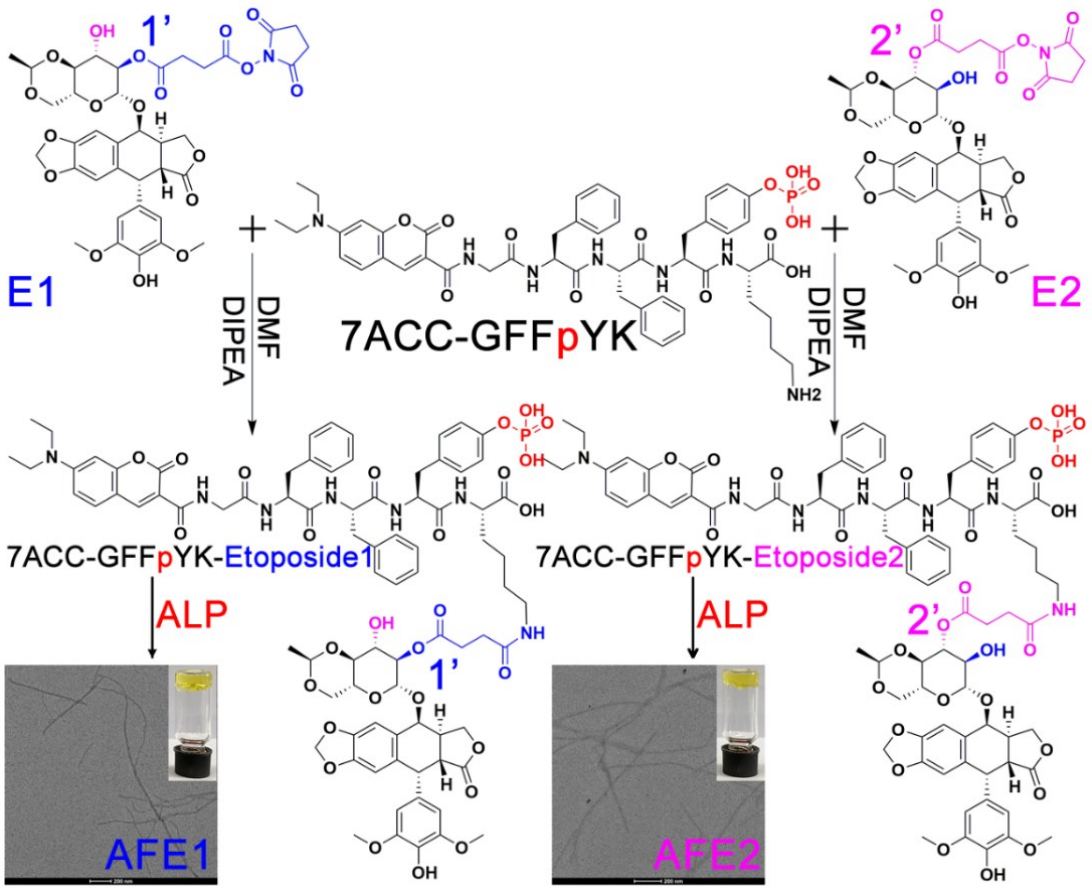

Figure S3. Synthesis routes of 7ACC-GFFpYK-Etoposide1 and 7ACC-GFFpYK-Etoposide2. TEM images of AFE1 and AFE2 after treatment with an alkaline phosphatase (ALP, 12 h) at 2U/mL Main Text Paragraph.

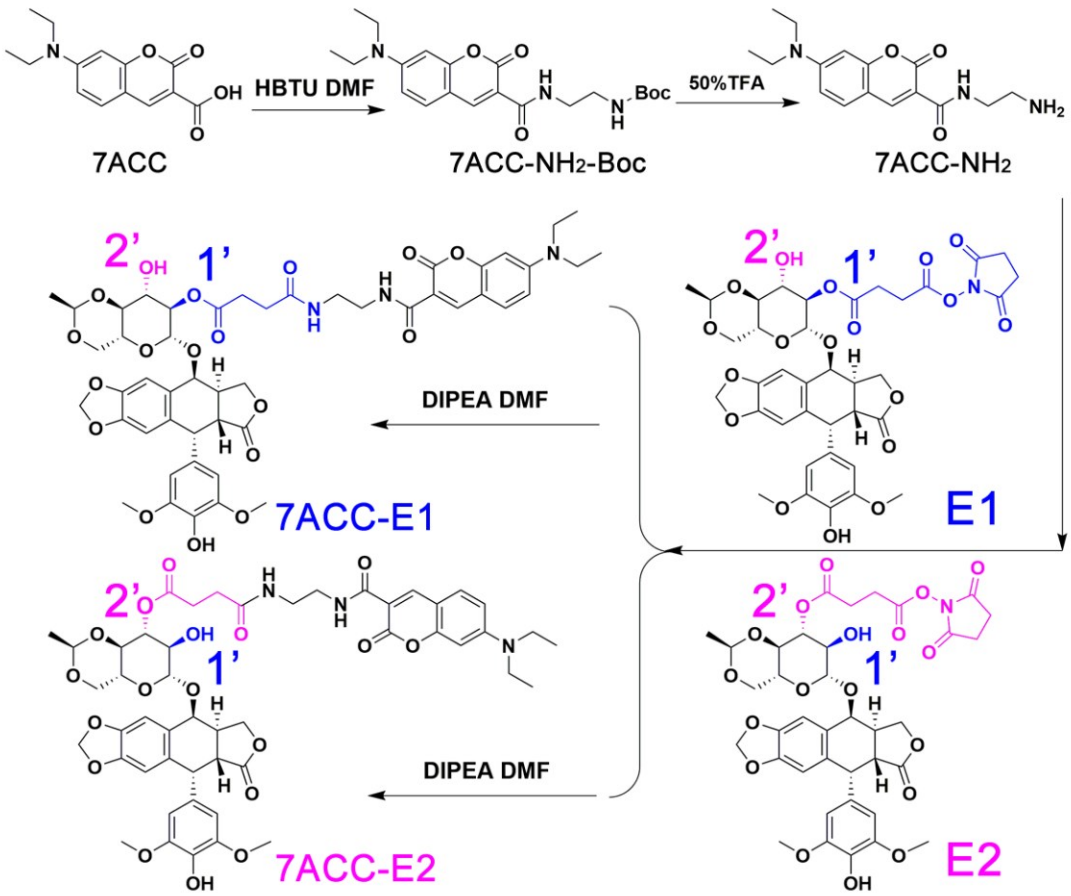

Figure S4. Synthesis routes of 7ACC-Etoposide1 (AE1) and 7ACC-Etoposide2 (AE2).

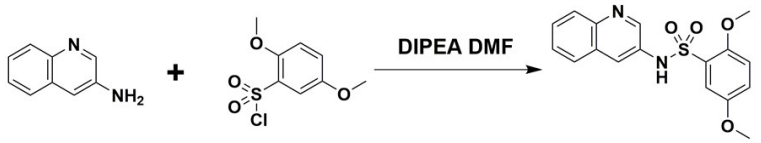

Figure S5. Synthesis routes of ALP inhibitor (DQB = 2,5-dimethoxy-N-(quinolin-3-yl)benzenesulfonamide).

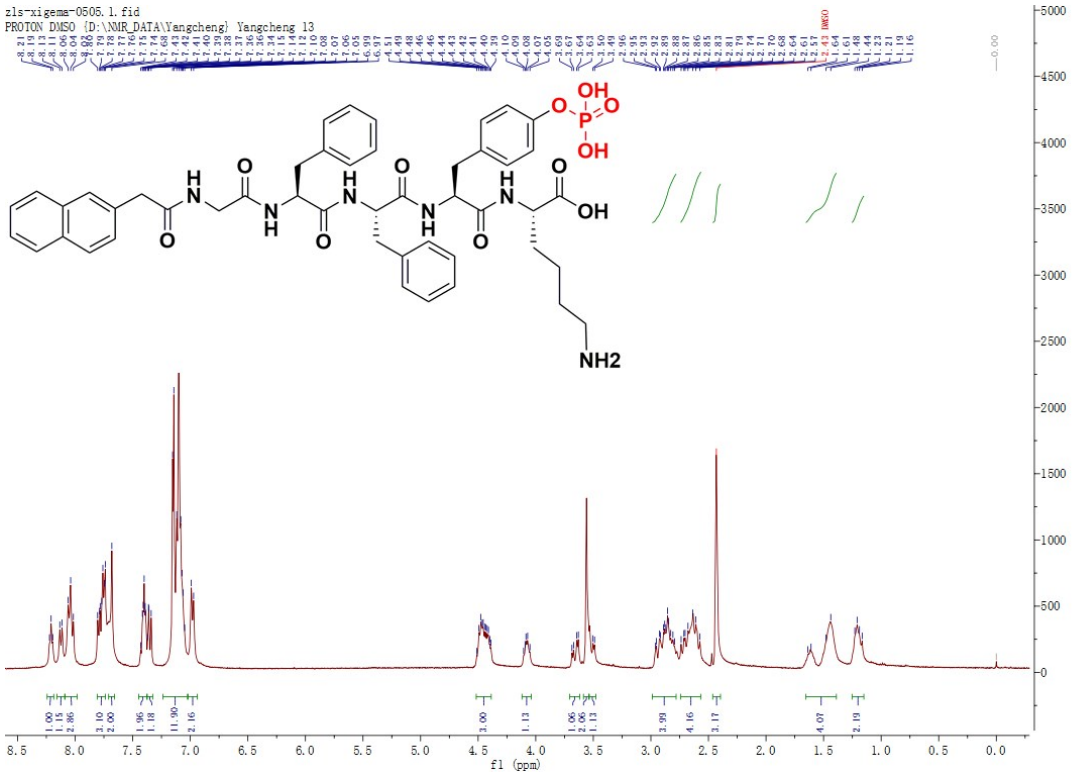

Figure S6A-1. ^{1}H NMR spectrum (400 MHz) of Nap-GFFpYK in DMSO-d6 at 25 ºC.

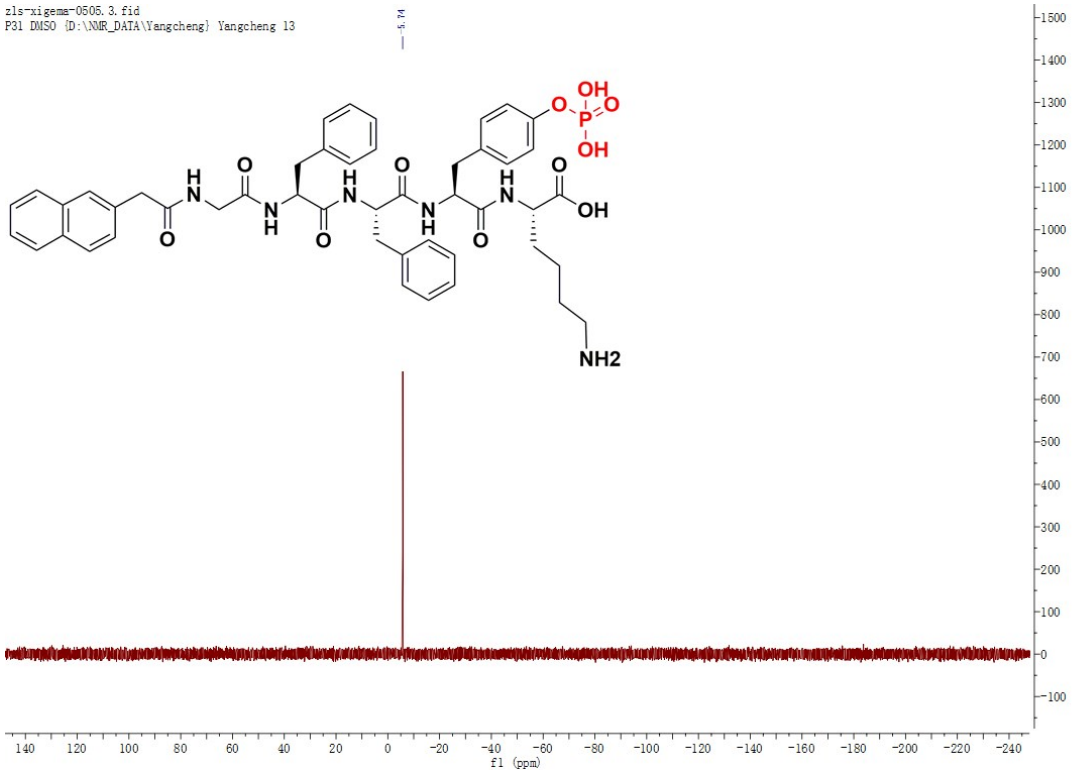

Figure S6A-2. ^{31}P NMR spectrum of Nap-GFFpYGK in DMSO-d6 at 25 ºC.

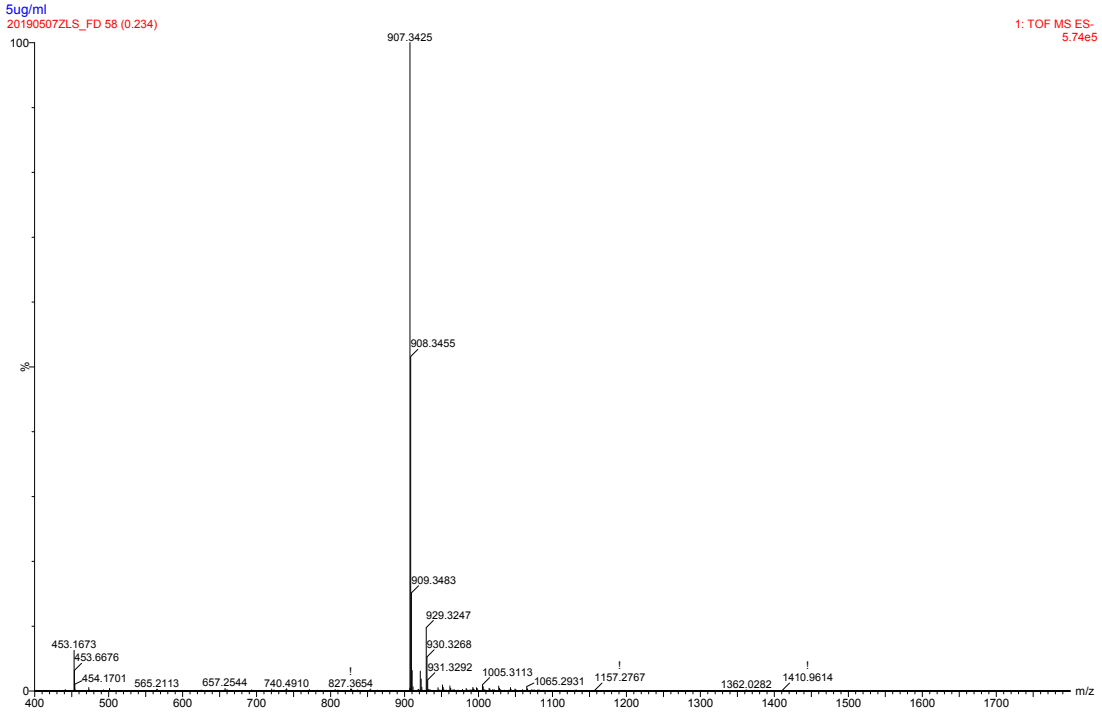

Figure S6A-3. HR-MS spectrum of Nap-GFFpYGK. M-1=907.3425.

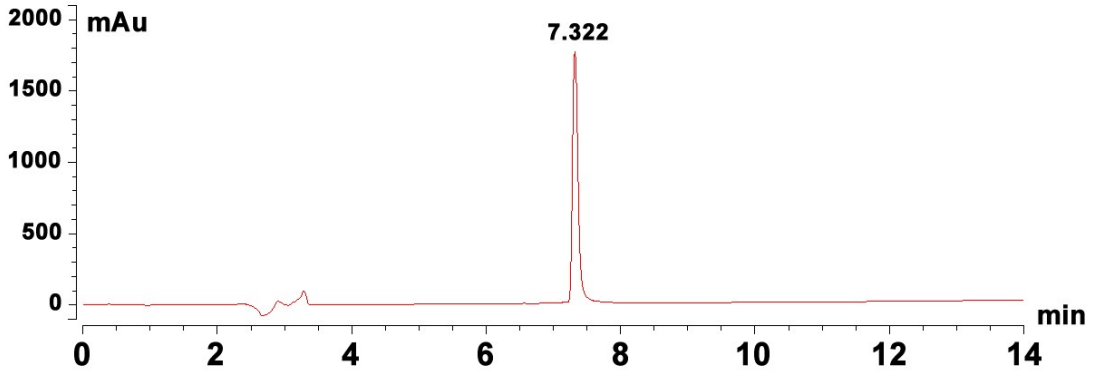

Figure S6A-4. HPLC graph of Nap-GFFpYGK.

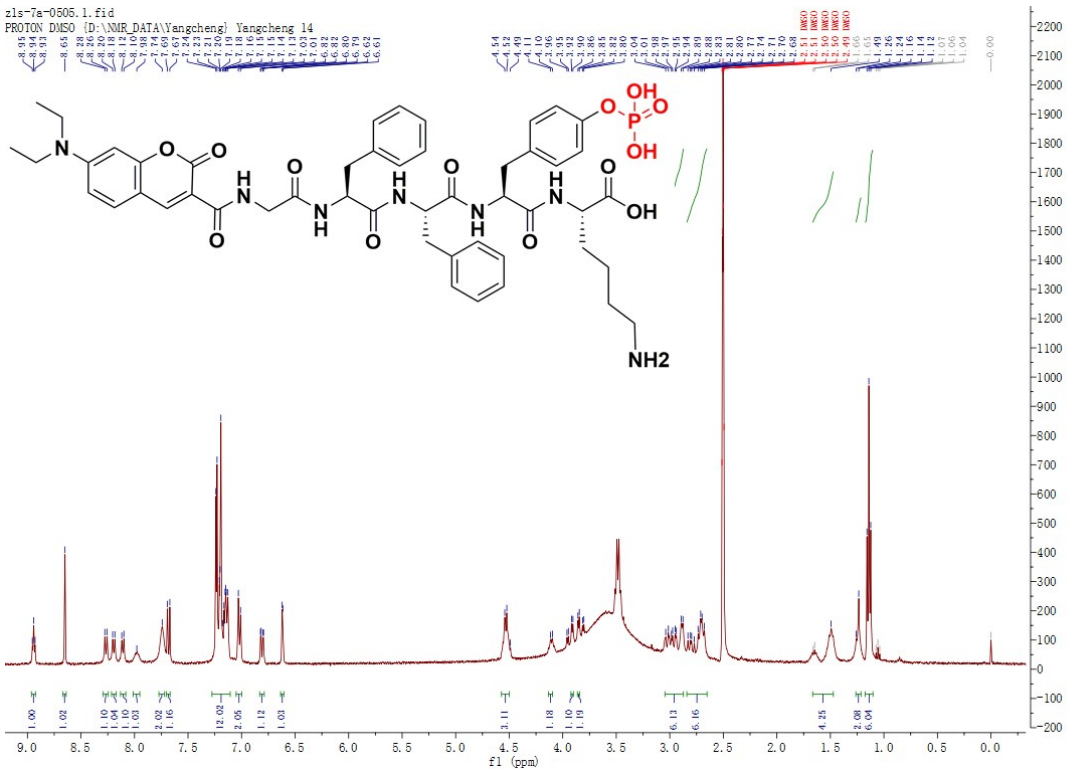

Figure S6B-1. ^{1}H NMR spectrum (400 MHz) of 7ACC-GFFpYK in DMSO-d6 at 25 ºC.

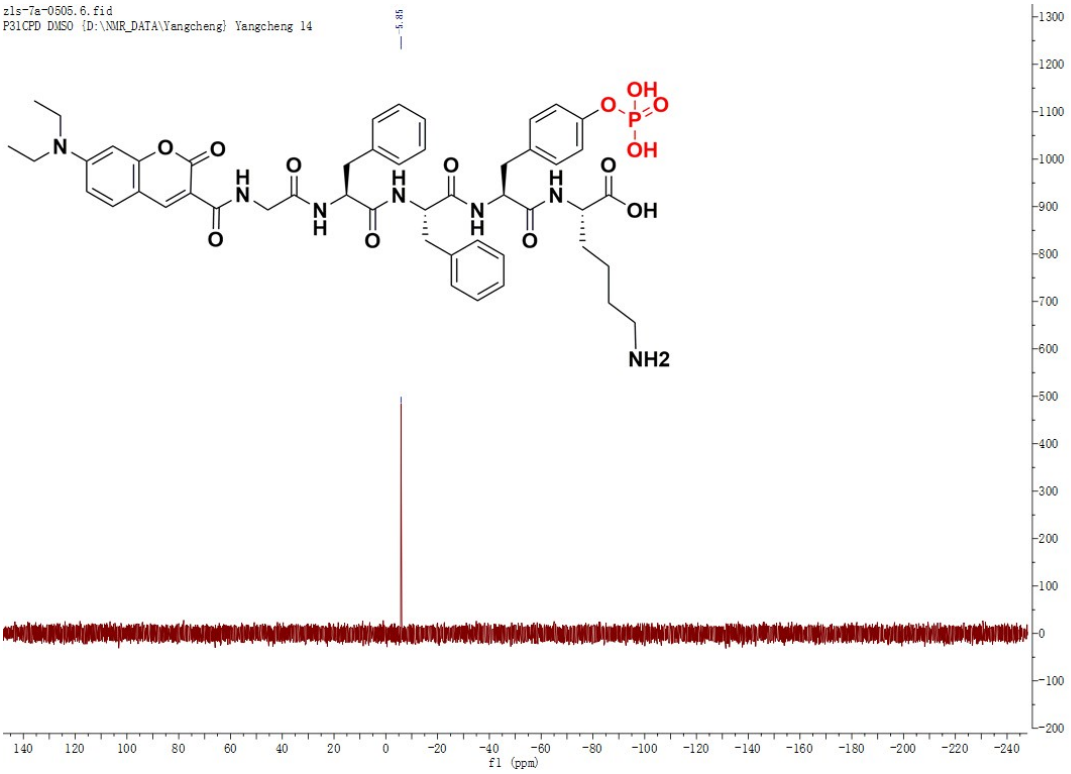

Figure S6B-2. ^{31}P NMR spectrum of 7ACC-GFFpYGK in DMSO-d6 at 25 ºC.

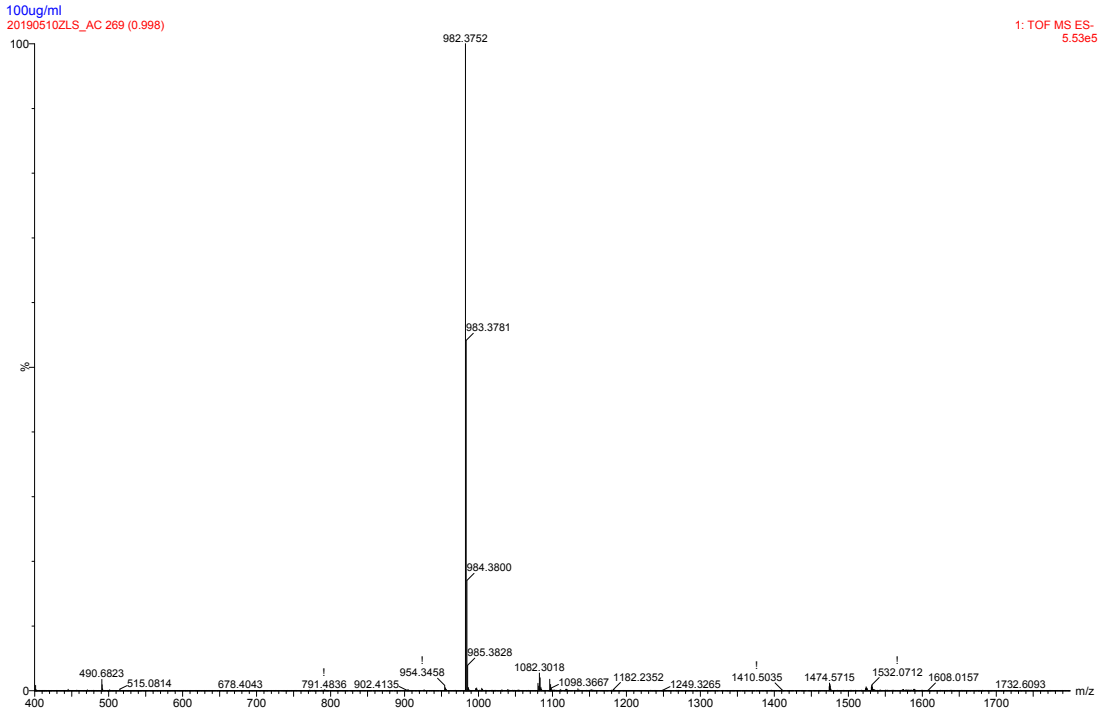

Figure S6B-3. HR-MS spectrum of 7ACC-GFFpYGK. M-1=982.3752.

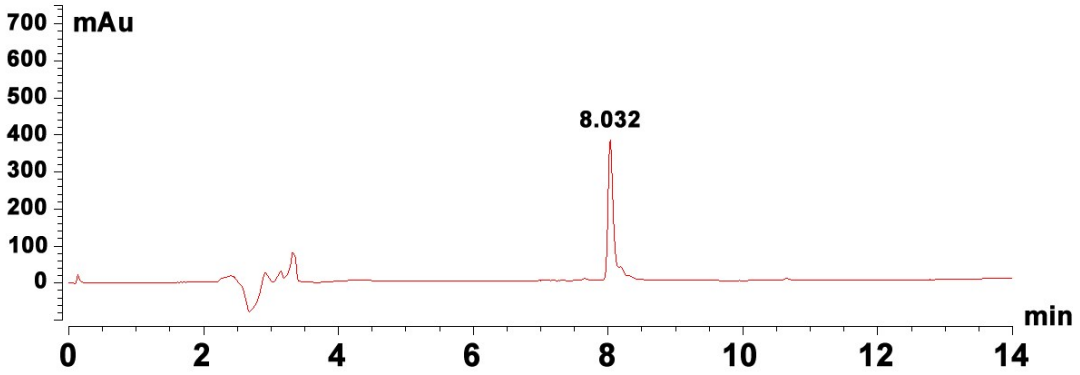

Figure S6B-4. HPLC graph of 7ACC-GFFpYGK.

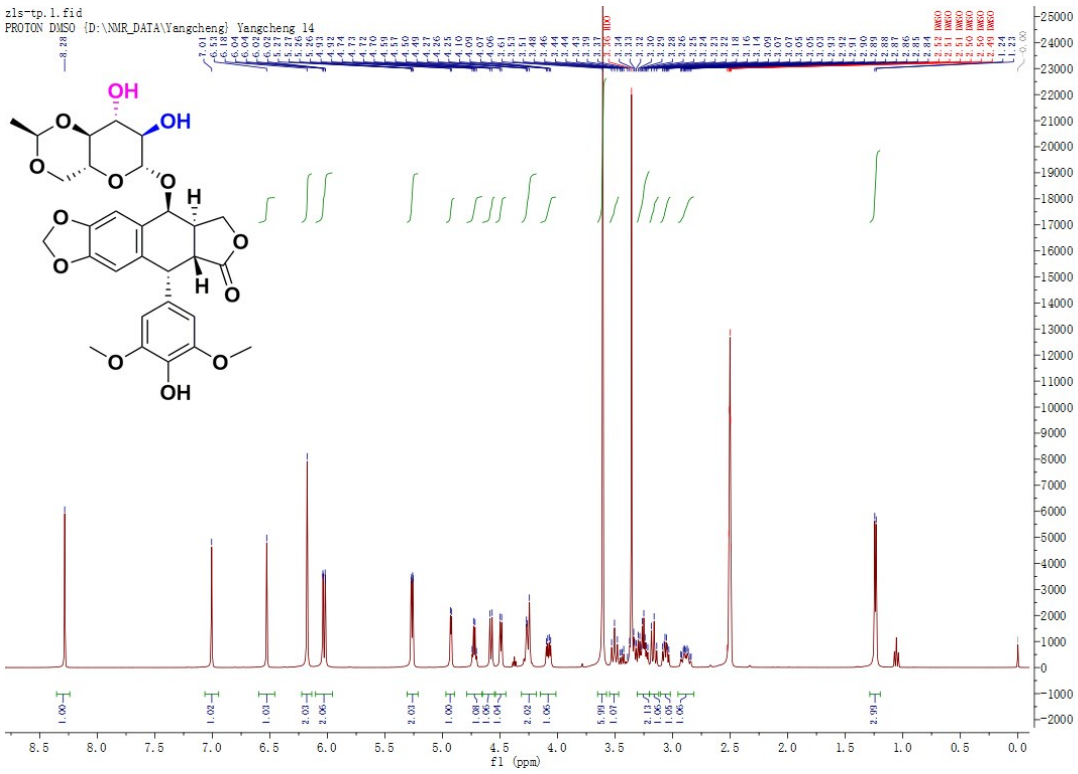

Figure S6C-1. [1]H NMR spectrum (400 MHz) of etoposide in DMSO-d6 at 25 ºC.

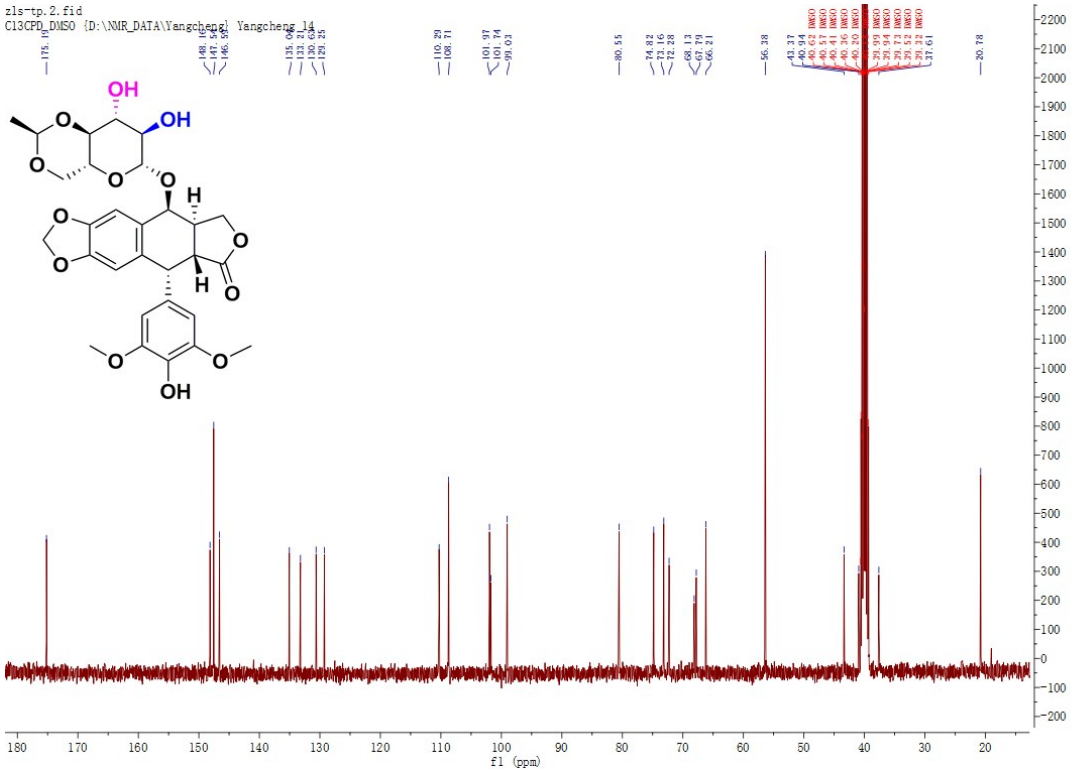

Figure S6C-2. [13]C NMR spectrum (100 MHz) of etoposide in DMSO-d6 at 25 ºC.

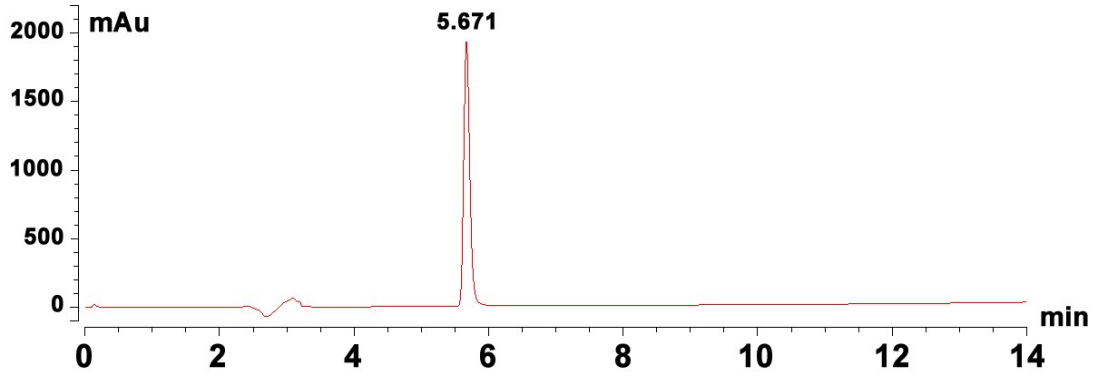

Figure S6C-3. HPLC graph of etoposide.

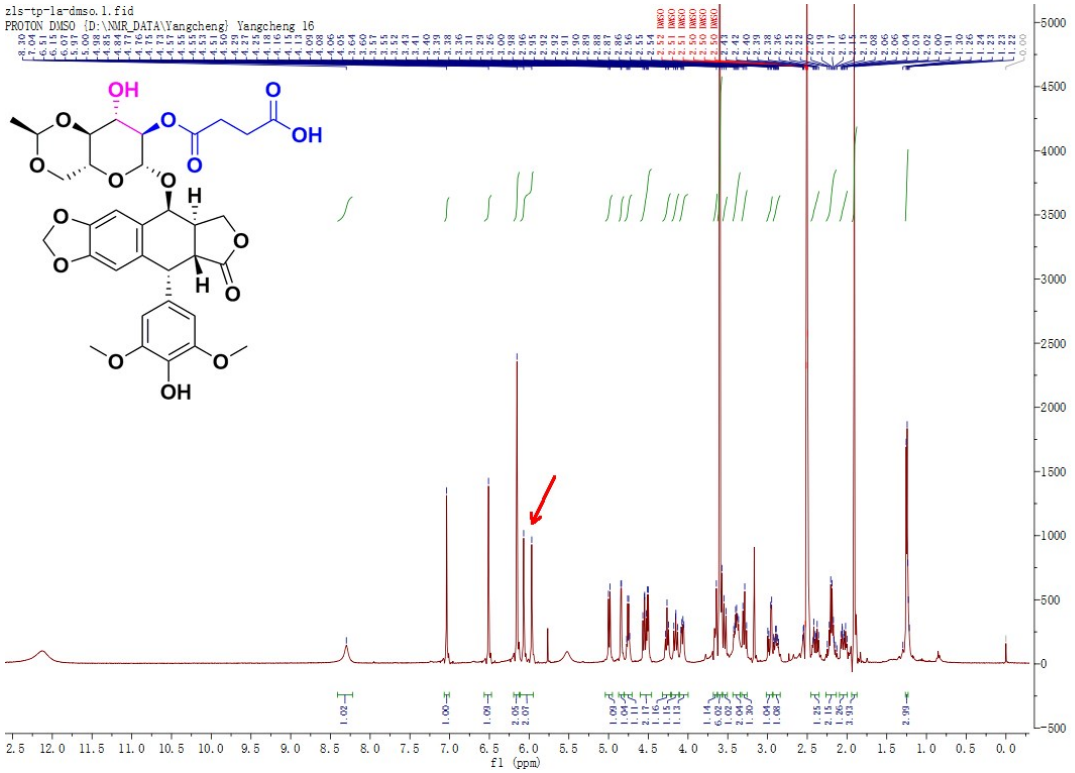

Figure S6D-1. ^{1}H NMR spectrum (400 MHz) of 1E in DMSO-d6 at 25 ºC.

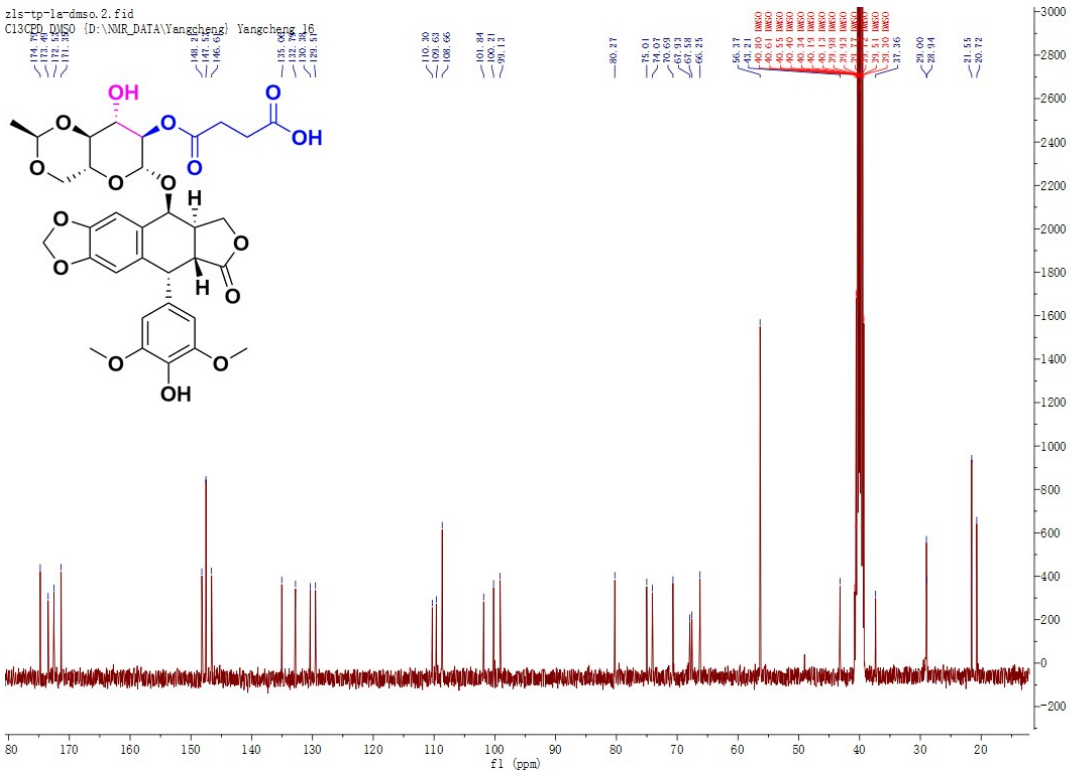

Figure S6D-2. ^{3}C NMR spectrum (100 MHz) of 1E in DMSO-d6 at 25 ºC.

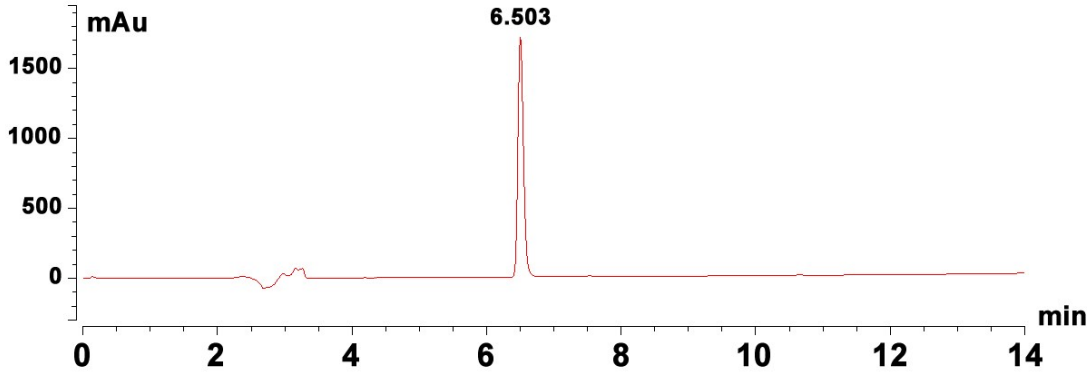

Figure S6D-3. HPLC graph of 1E.

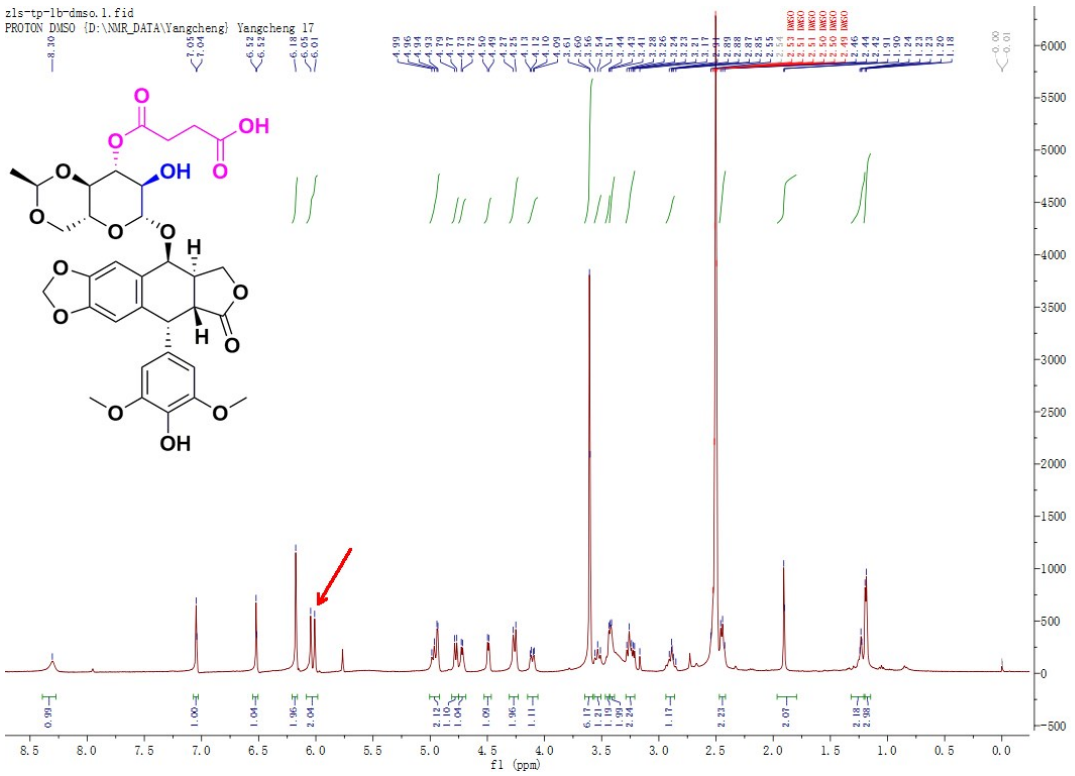

Figure S6E-1. [1]H NMR spectrum (400 MHz) of 2E in DMSO-d6 at 25 ºC.

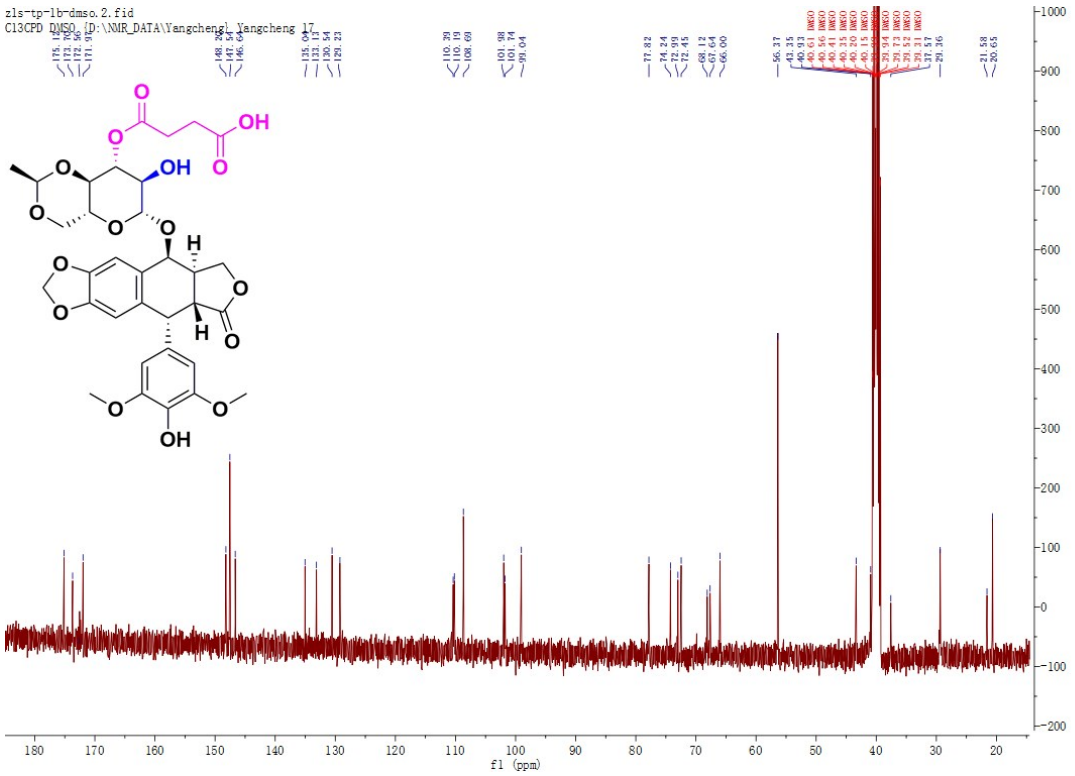

Figure S6E-2. [13]C NMR spectrum (100 MHz) of 2E in DMSO-d6 at 25 ºC.

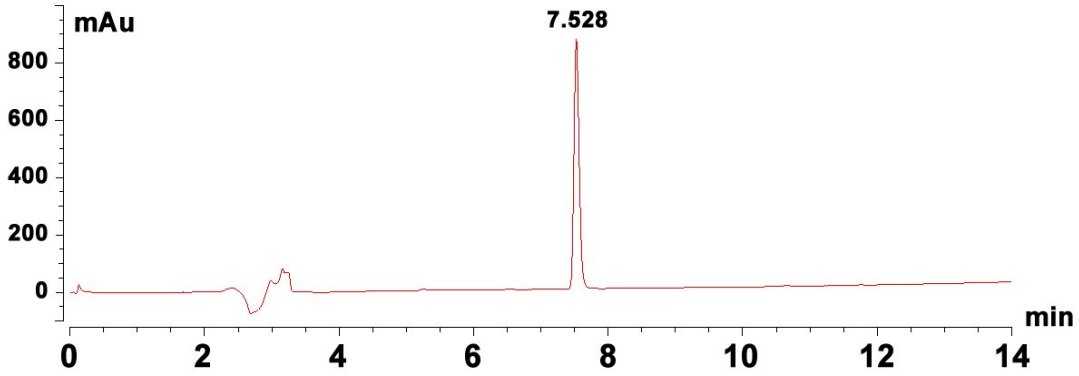

Figure S6E-3. HPLC graph of 2E.

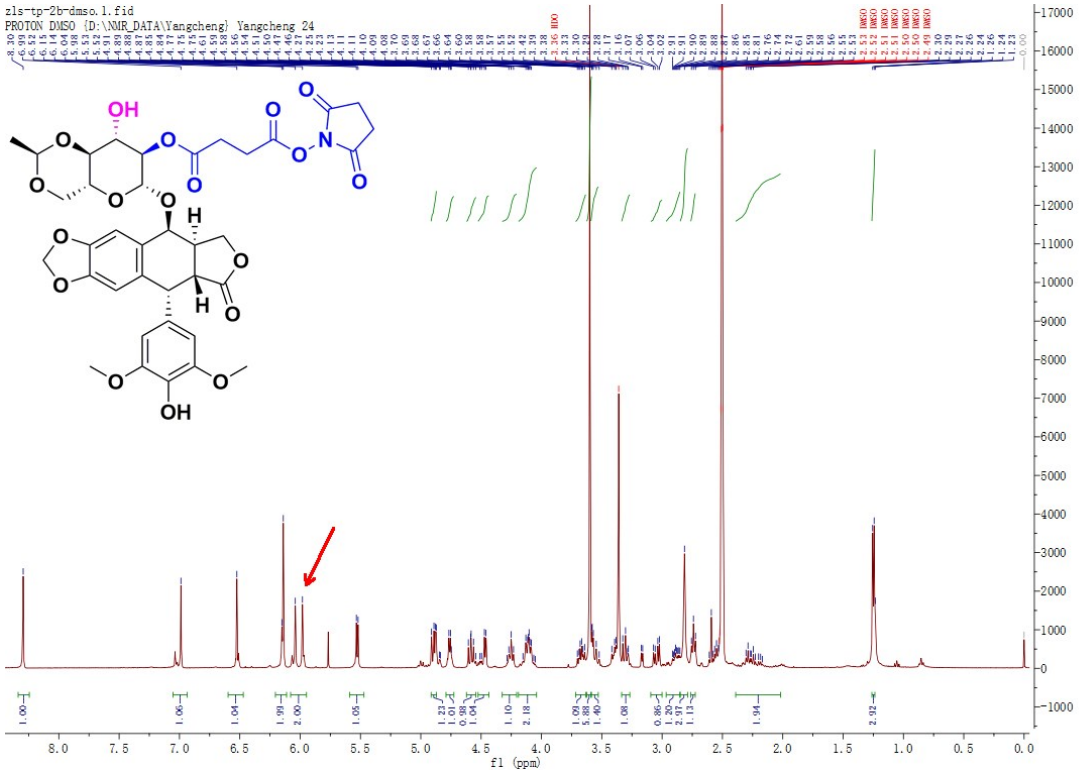

Figure S6F-1. [1]H NMR spectrum (400 MHz) of E1 in DMSO-d6 at 25 ºC.

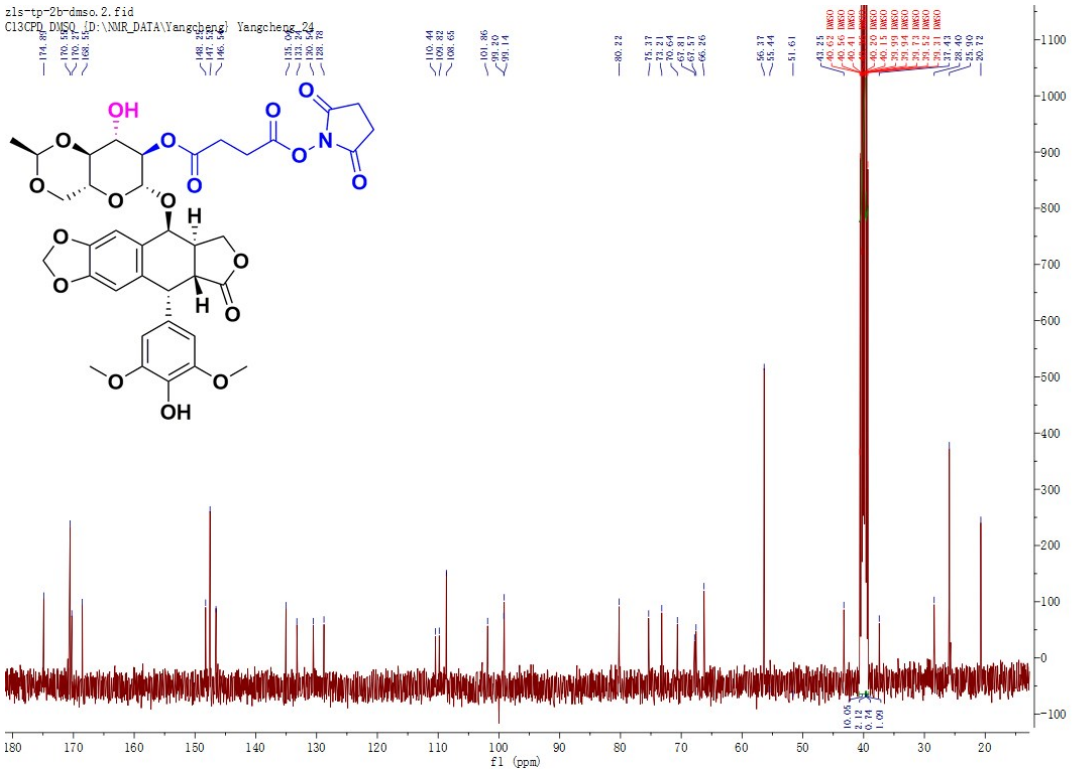

Figure S6F-2. ^{13}C NMR spectrum (100 MHz) of E1 in DMSO-d6 at 25 ºC.

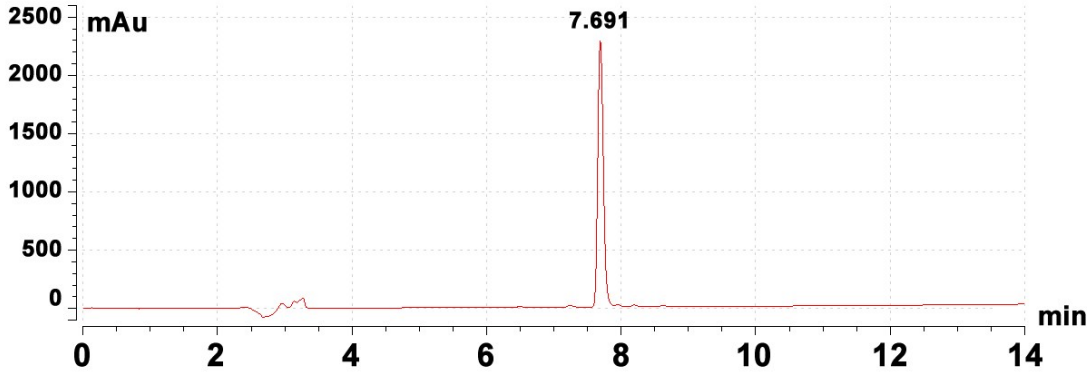

Figure S6F-3. HPLC graph of E1.

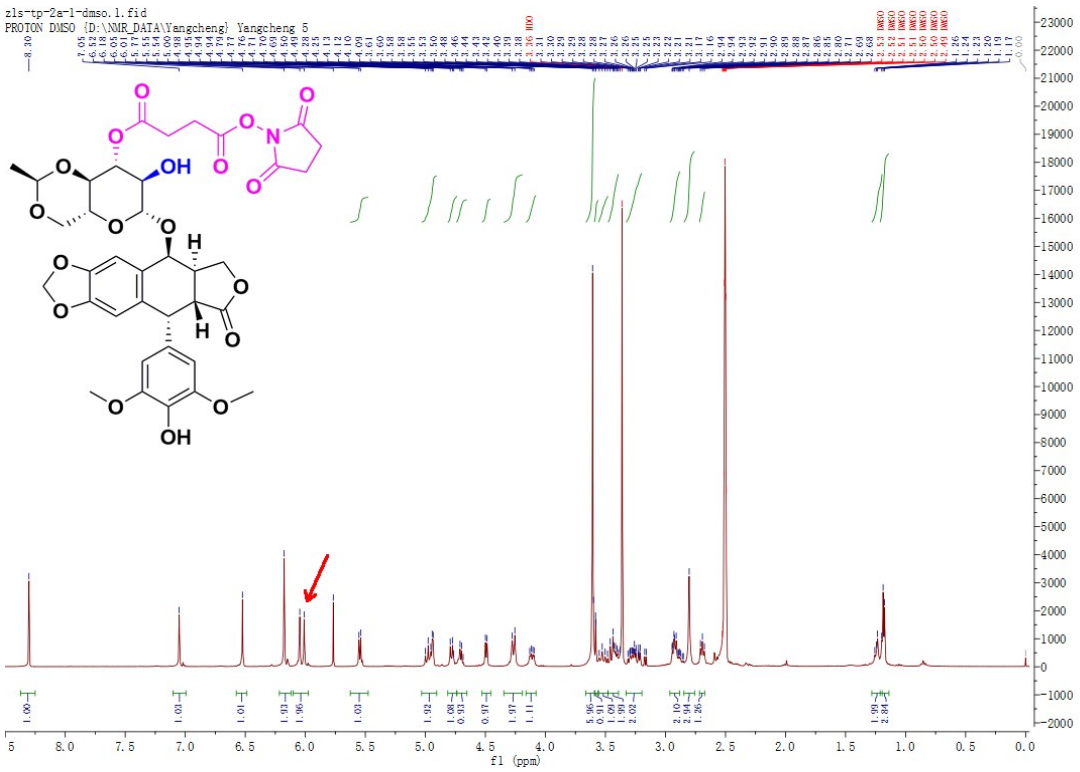

Figure S6G-1. [1]H NMR spectrum (400 MHz) of E2 in DMSO-d6 at 25 ºC.

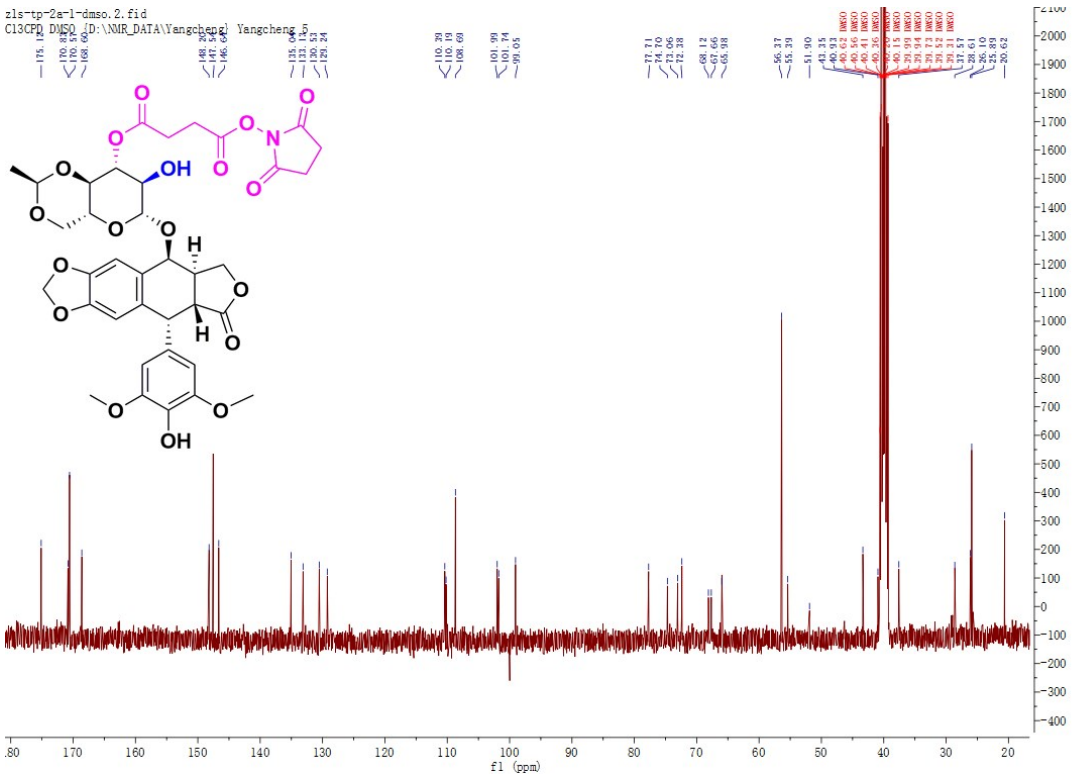

Figure S6G-2. [13]C NMR spectrum (100 MHz) of E2 in DMSO-d6 at 25 ºC.

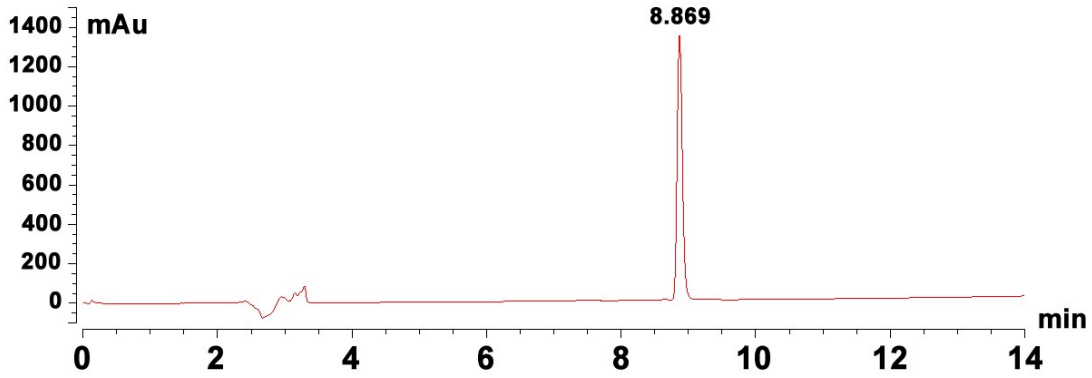

Figure S6G-3. HPLC graph of E2.

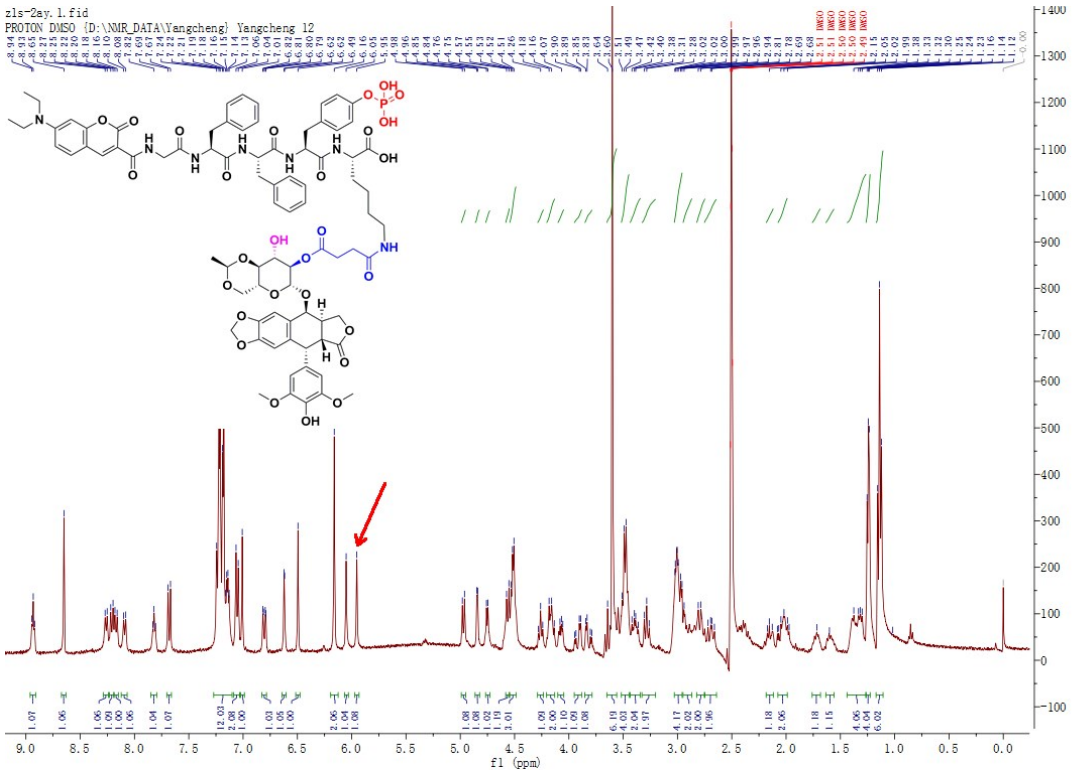

Figure S6H-1. [1]H NMR spectrum (400 MHz) of 7ACC-GFFpYK-Etoposide1 (AFE1) in DMSO-d6 at 25 ºC.

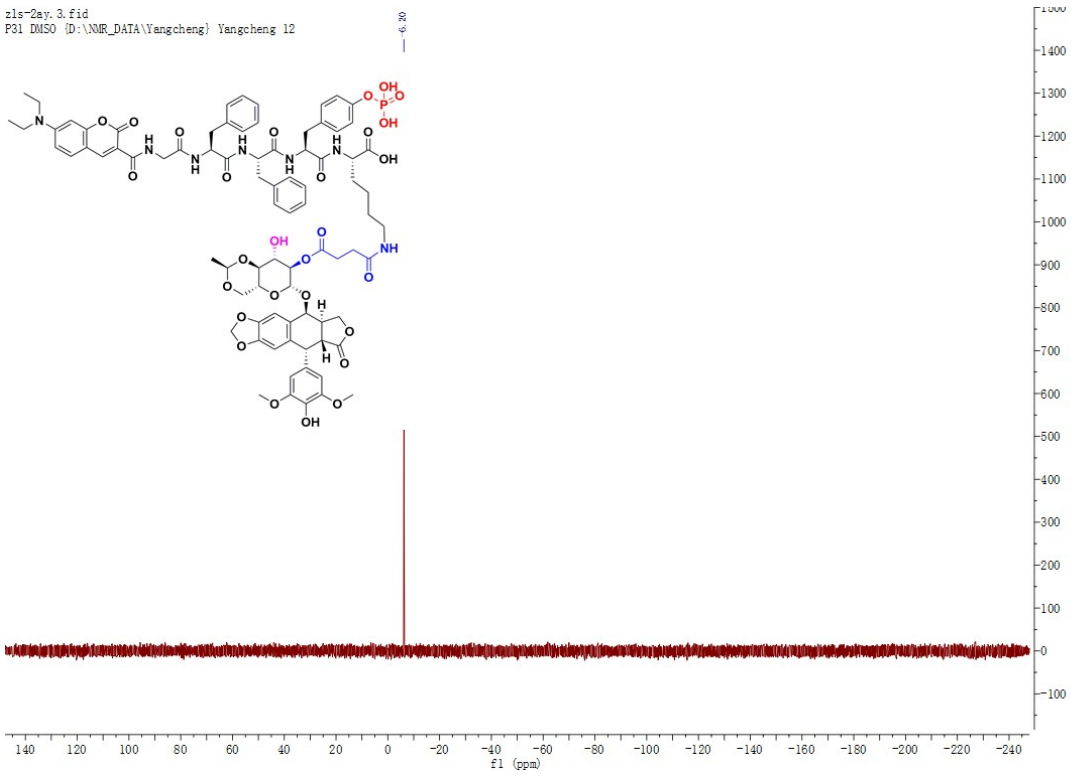

Figure S6H-2. ^{31}P NMR spectrum of 7ACC-GFFpYGK-Etoposide1 (AFE1) in DMSO-d6 at 25 ºC.

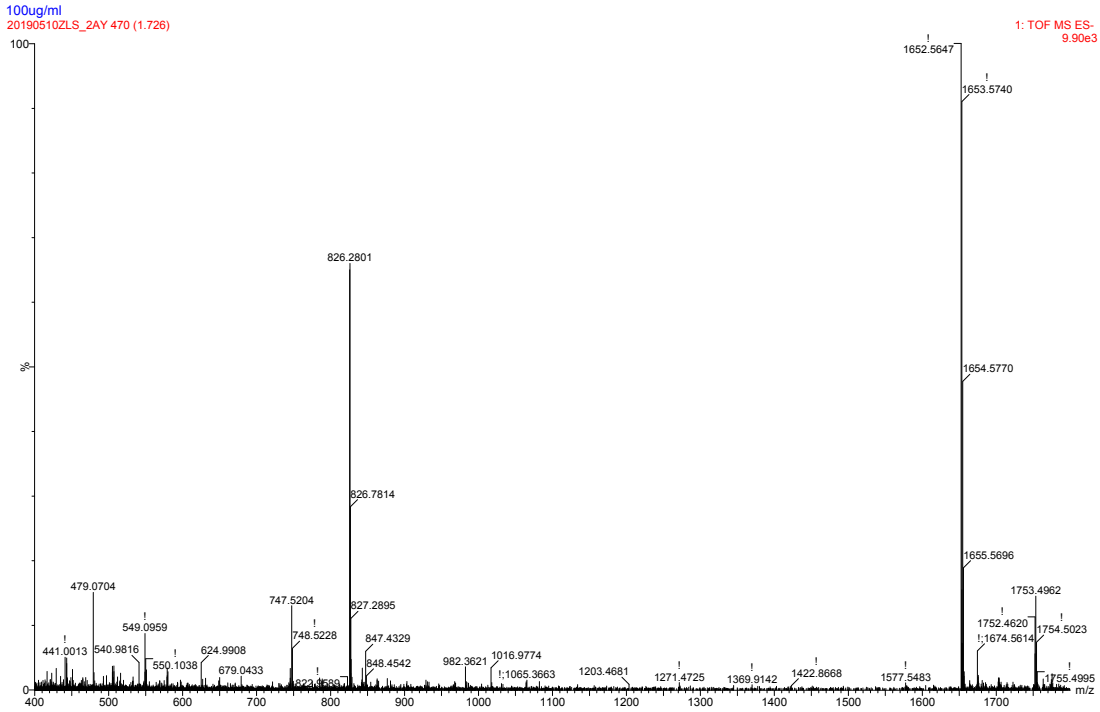

Figure S6H-3. HR-MS spectrum of 7ACC-GFFpYGK-Etoposide1 (AFE1). M-1=1652.5647.

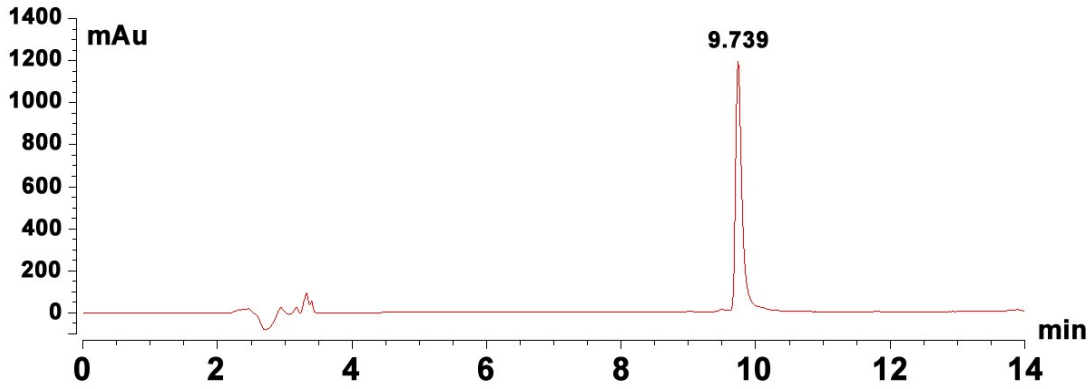

Figure S6H-4. HPLC graph of 7ACC-GFFpYGK-Etoposide1 (AFE1).

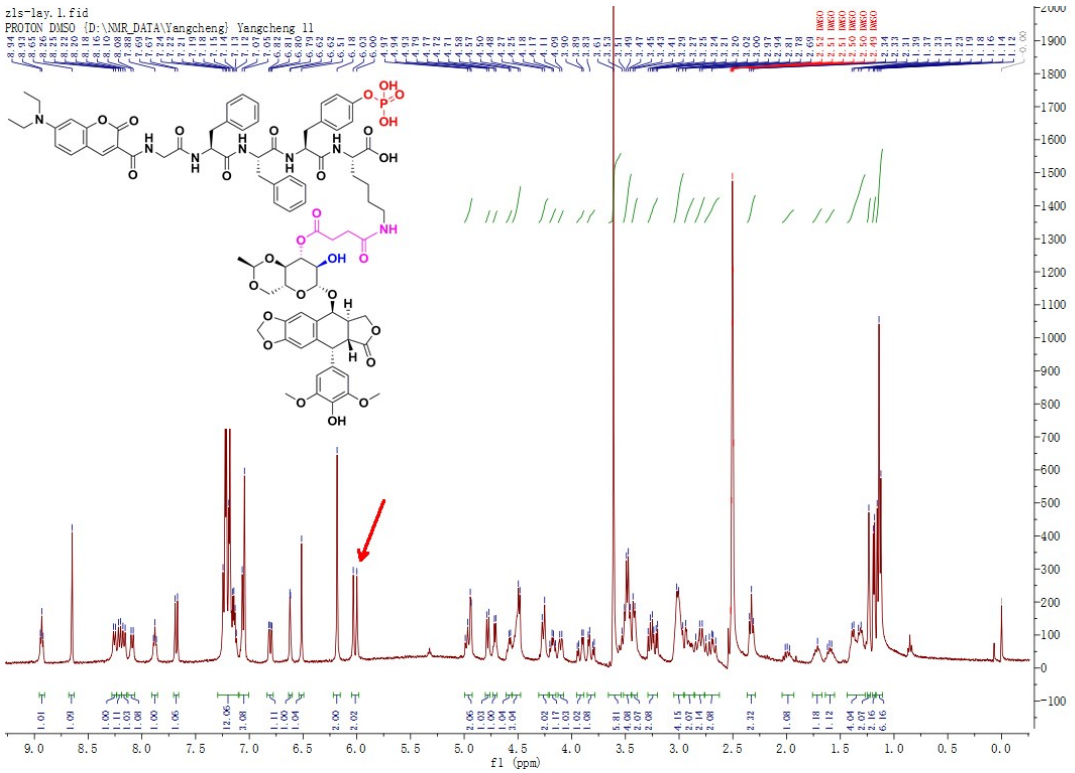

Figure S6I-1. ^{1}H NMR spectrum (400 MHz) of 7ACC-GFFpYK-Etoposide2 (AFE2) in DMSO-d6 at 25 ºC.

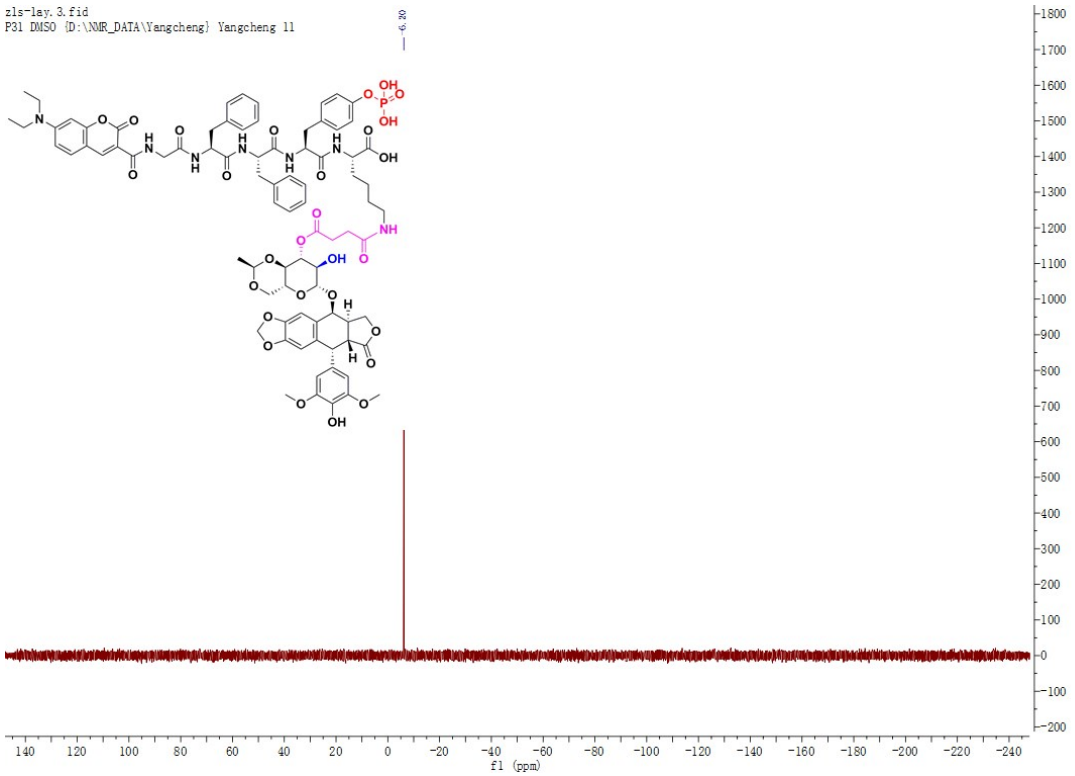

Figure S6I-2. ^{31}P NMR spectrum of 7ACC-GFFpYGK-Etoposide2 (AFE2) in DMSO-d6 at 25 ºC.

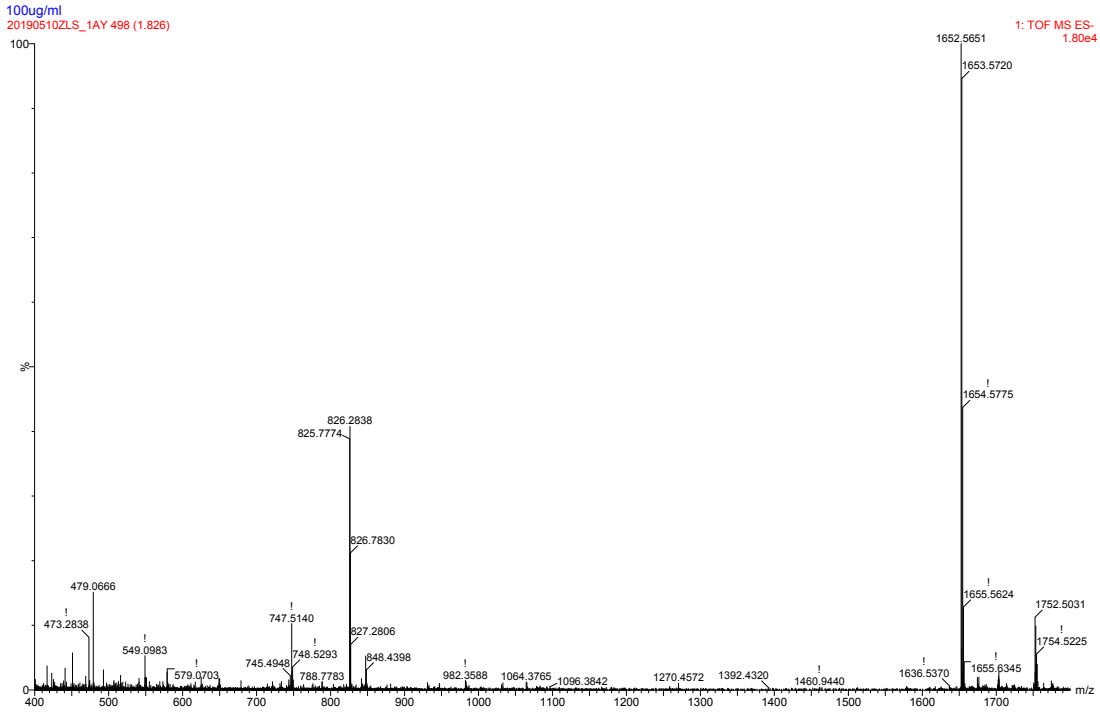

Figure S6I-3. HR-MS spectrum of 7ACC-GFFpYGK-Etoposide2 (AFE2). M-1=1652.5651.

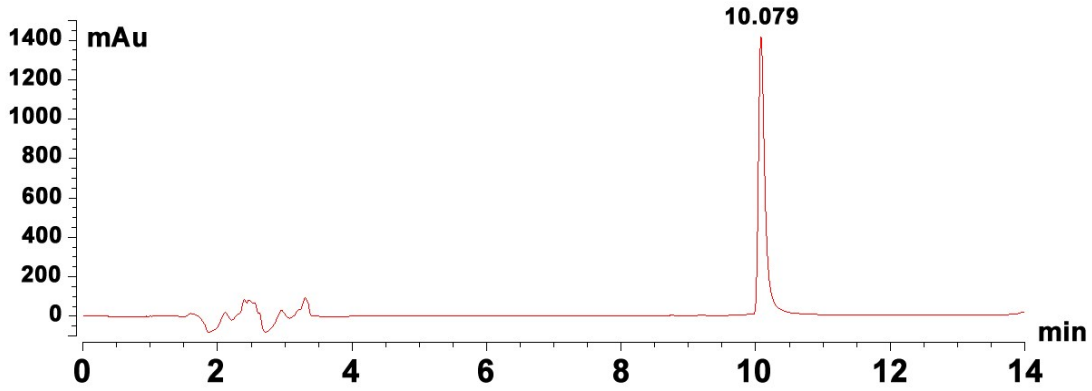

Figure S6I-4. HPLC graph of 7ACC-GFFpYGK-Etoposide2 (AFE2).

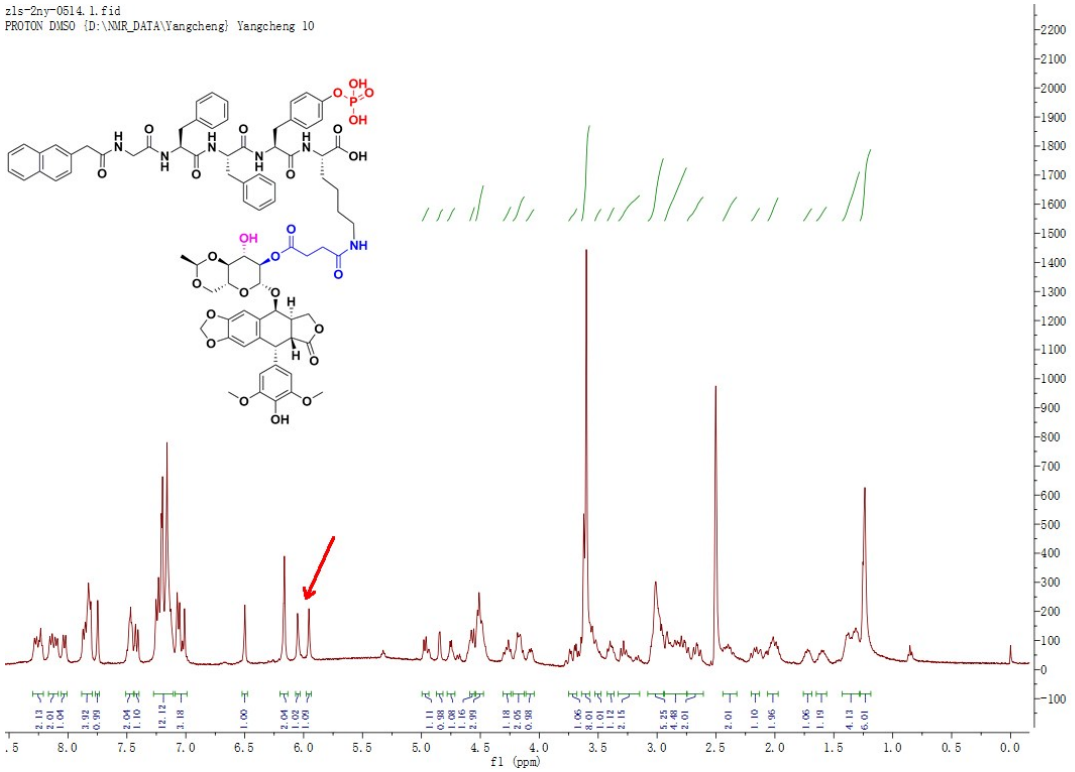

Figure S6J-1. ^{1}H NMR spectrum (400 MHz) of Nap-GFFpYK-Etoposide1 (NFE1) in DMSO-d6 at 25 ºC.

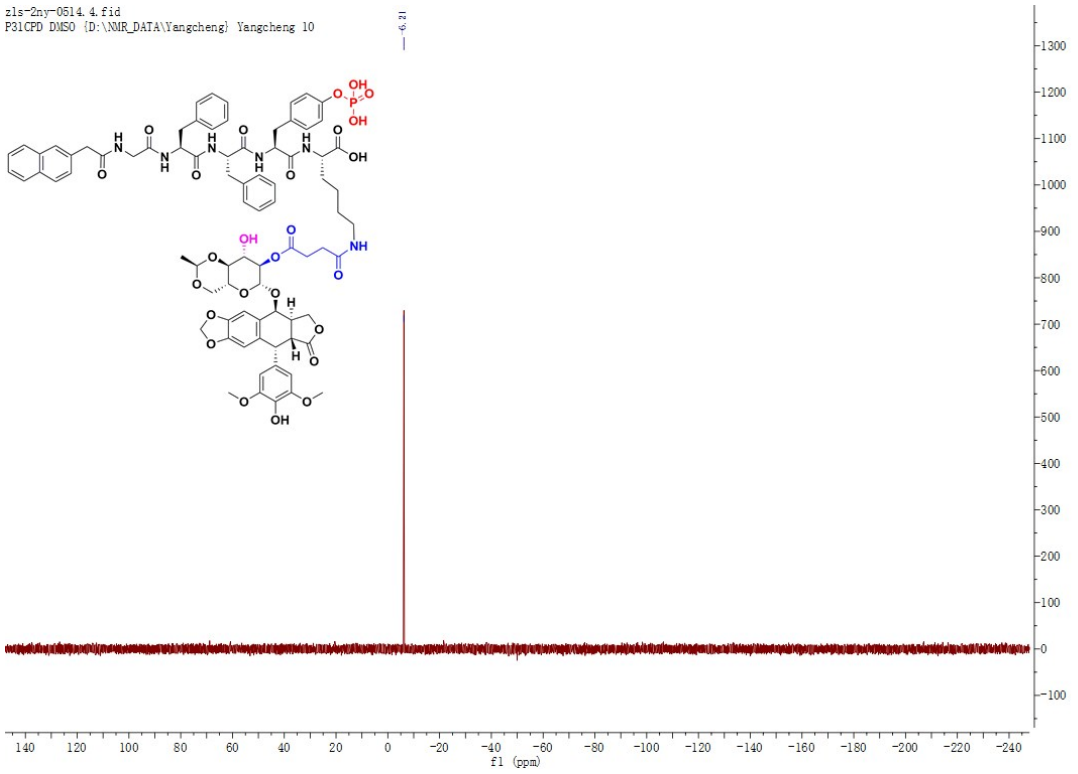

Figure S6J-2. ^{31}P NMR spectrum of Nap-GFFpYGK-Etoposide1 (NFE1) in DMSO-d6 at 25 ºC.

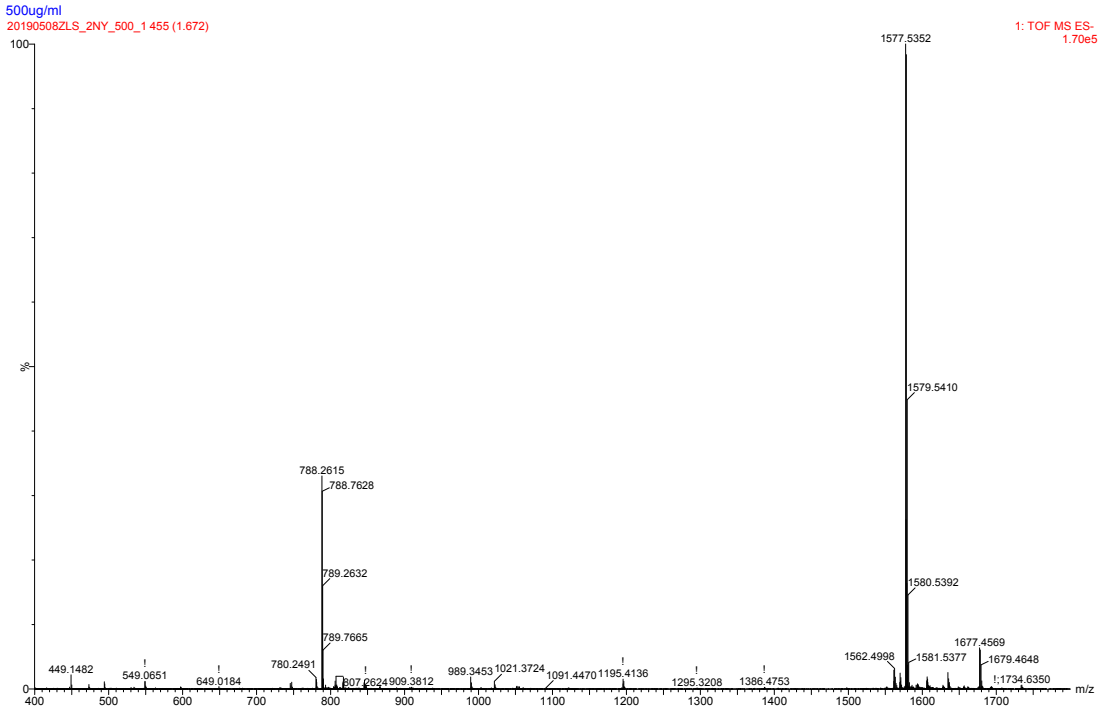

Figure S6J-3. HR-MS spectrum of Nap-GFFpYGK-Etoposide1 (NFE1). M-1=1577.5352.

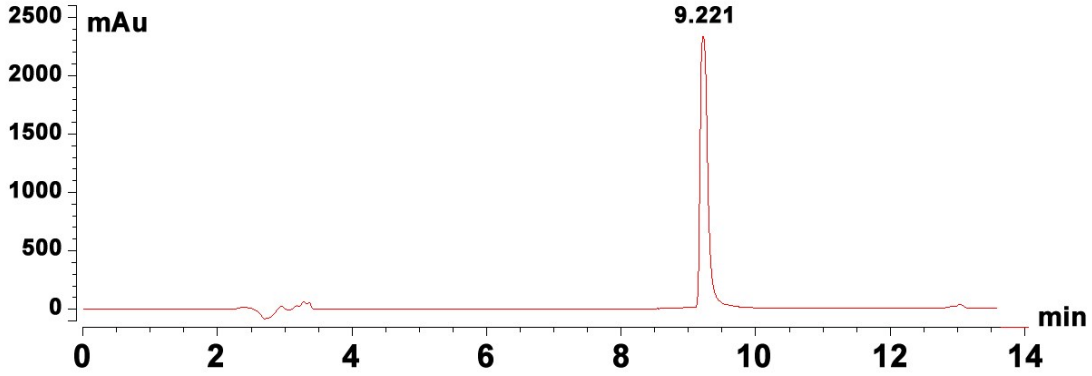

Figure S6J-4. HPLC graph of Nap-GFFpYGK-Etoposide1 (NFE1).

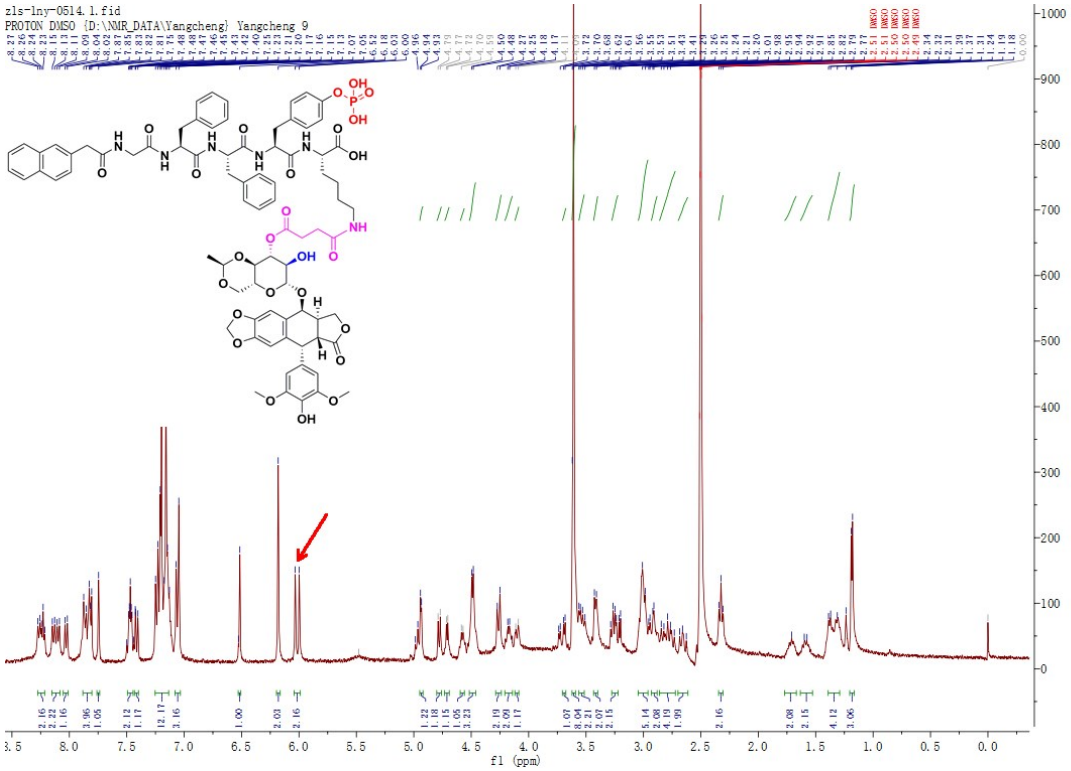

Figure S6K-1. ^{1}H NMR spectrum (400 MHz) of Nap-GFFpYK-Etoposide2 (NFE2) in DMSO-d6 at 25 ºC.

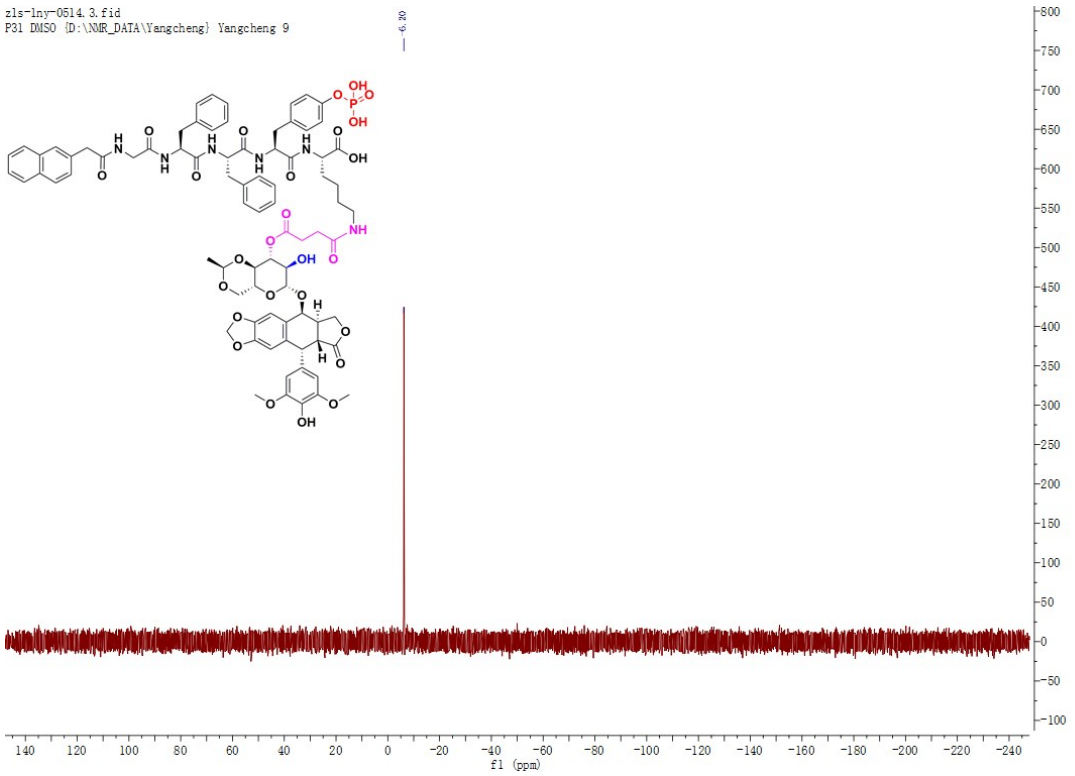

Figure S6K-2. ^{31}P NMR spectrum of Nap-GFFpYGK-Etoposide2 (NFE2) in DMSO-d6 at 25 ºC.

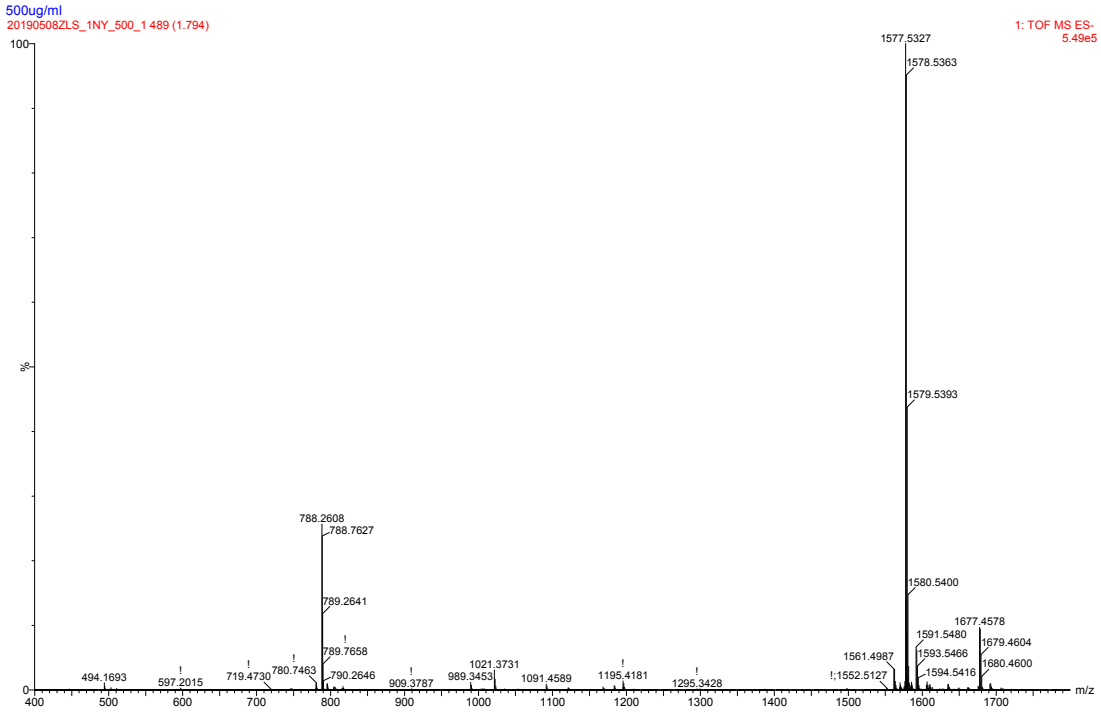

Figure S6K-3. HR-MS spectrum of Nap-GFFpYGK-Etoposide2 (NFE2). M-1=1577.5327.

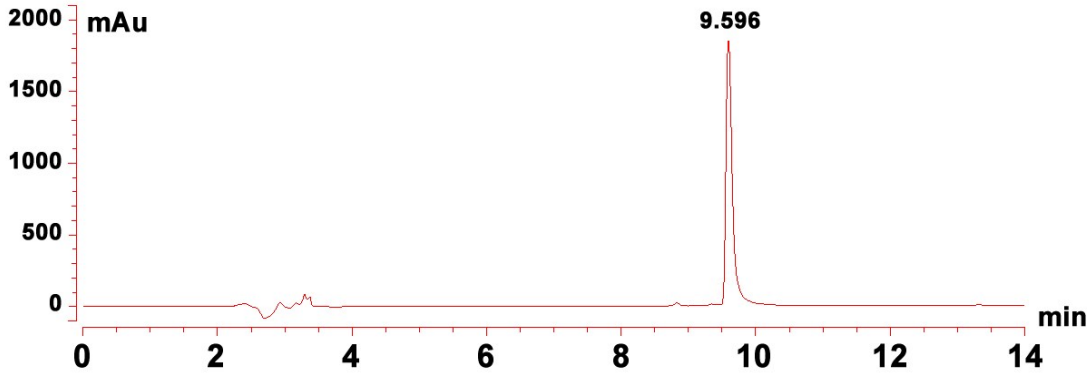

Figure S6K-4. HPLC graph of Nap-GFFpYGK-Etoposide2 (NFE2).

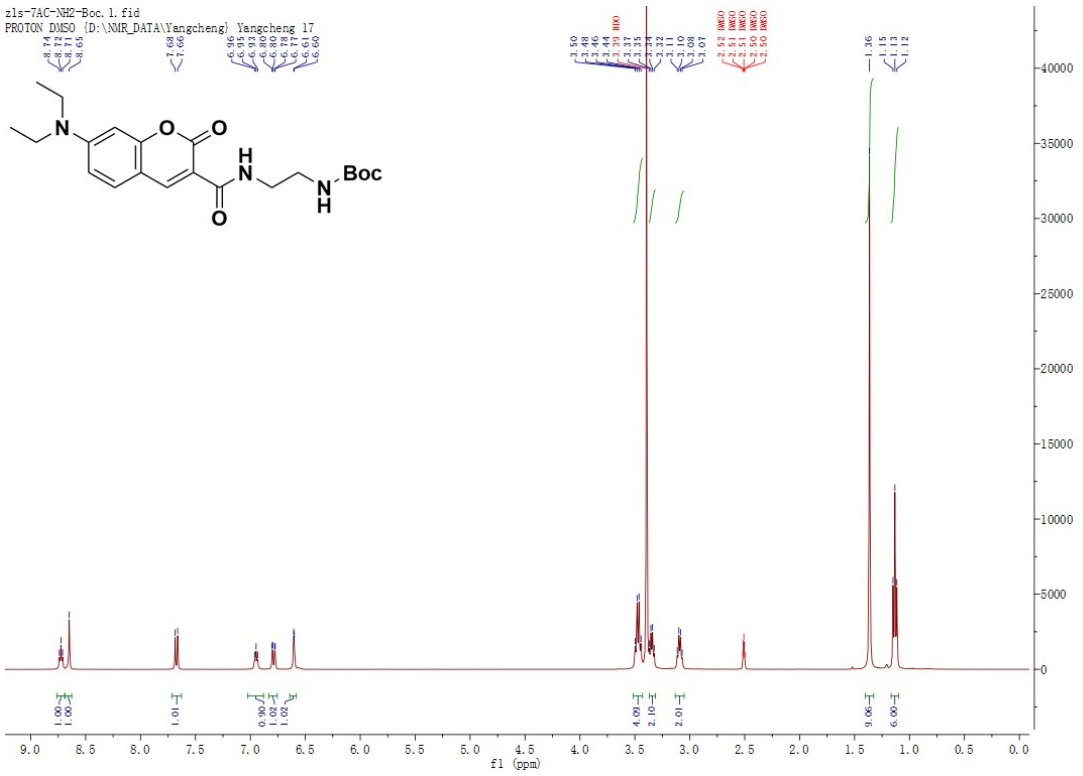

Figure S6L-1. ^{1}H NMR spectrum (400 MHz) of 7ACC-NH_2-Boc in DMSO-d6 at 25 ºC.

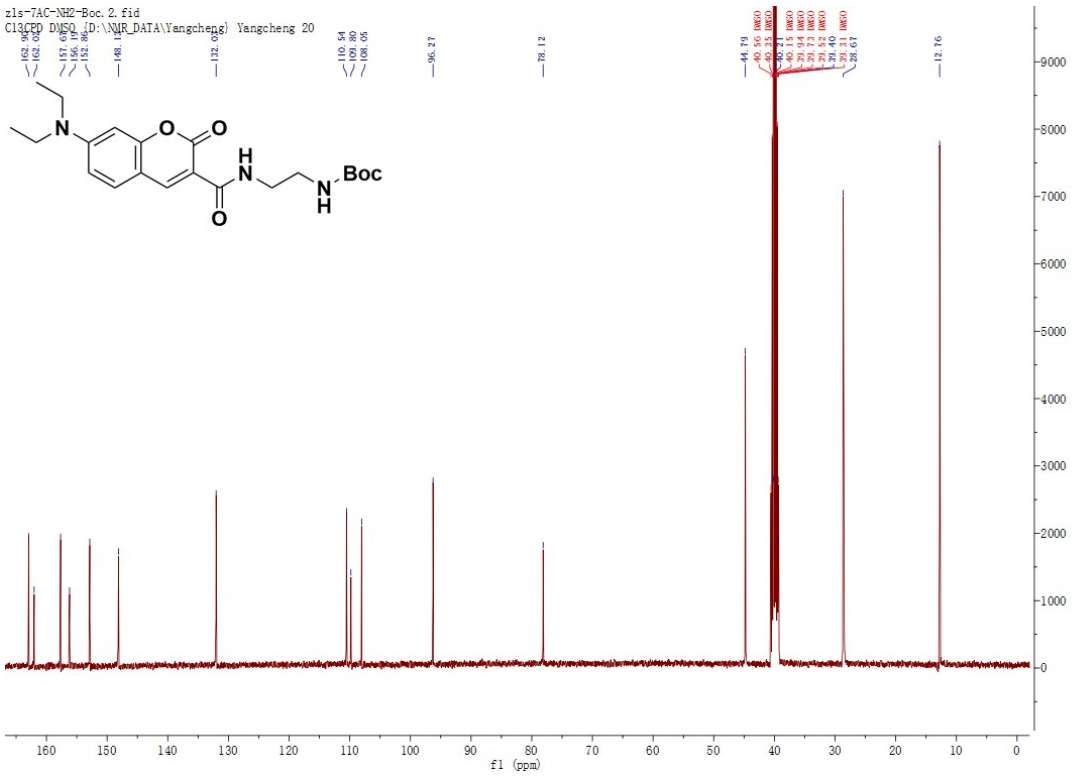

Figure S6L-2. ^{13}C NMR spectrum (100 MHz) of 7ACC-NH_2-Boc in DMSO-d6 at 25 ºC.

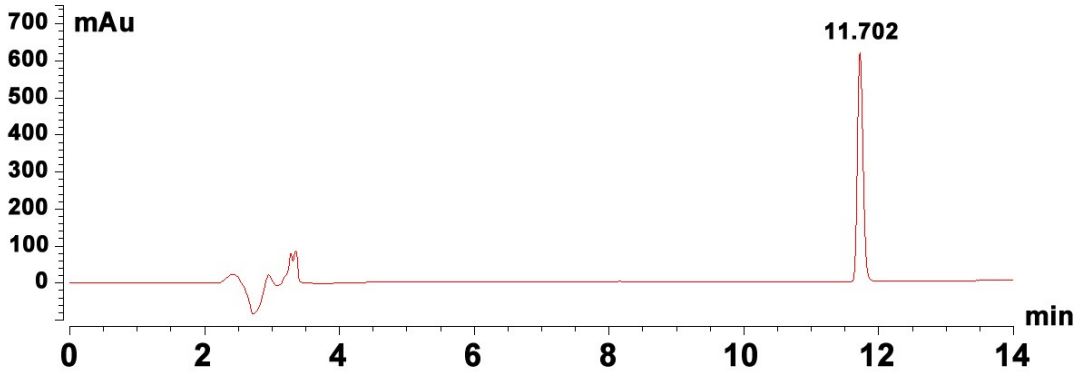

Figure S6L-3. HPLC graph of 7ACC-NH_2-Boc.

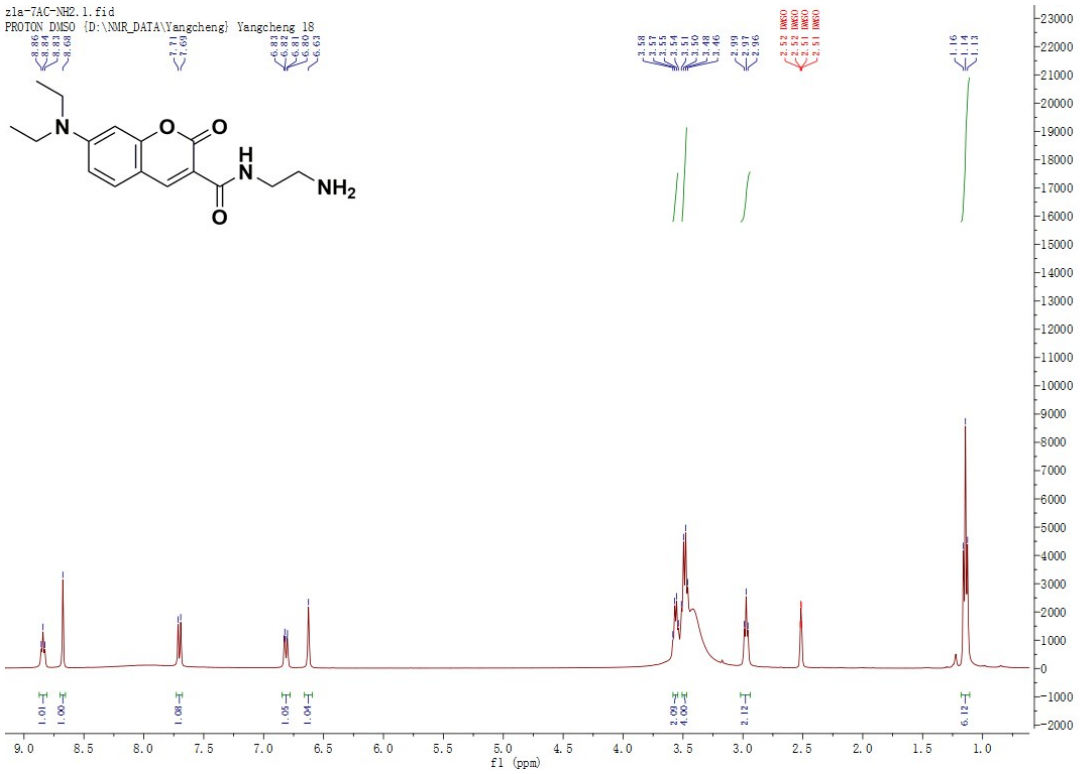

Figure S6M-1. ^{1}H NMR spectrum (400 MHz) of 7ACC-NH_2 in DMSO-d6 at 25 ºC.

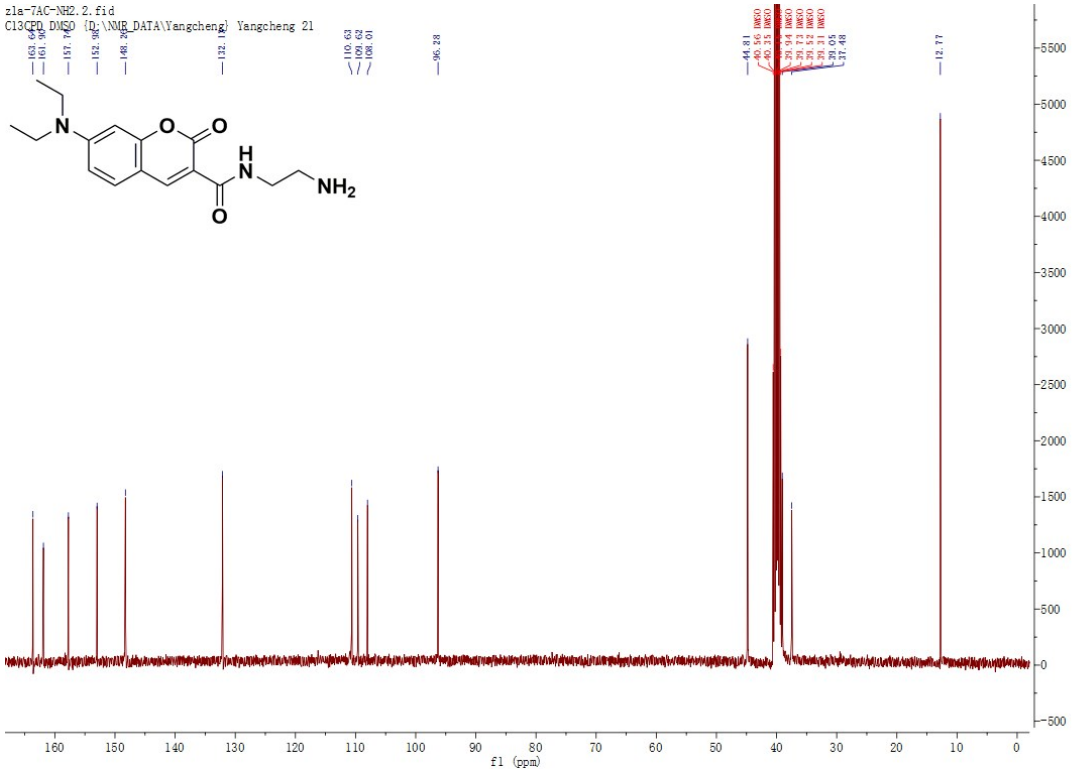

Figure S6M-2. ^{13}C NMR spectrum (100 MHz) of 7ACC-NH_2 in DMSO-d6 at 25 ºC.

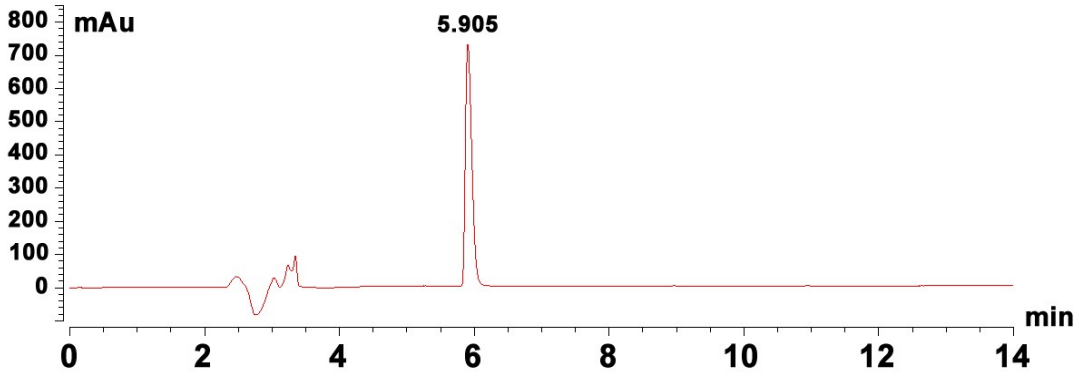

Figure S6M-3. HPLC graph of 7ACC-NH_2.

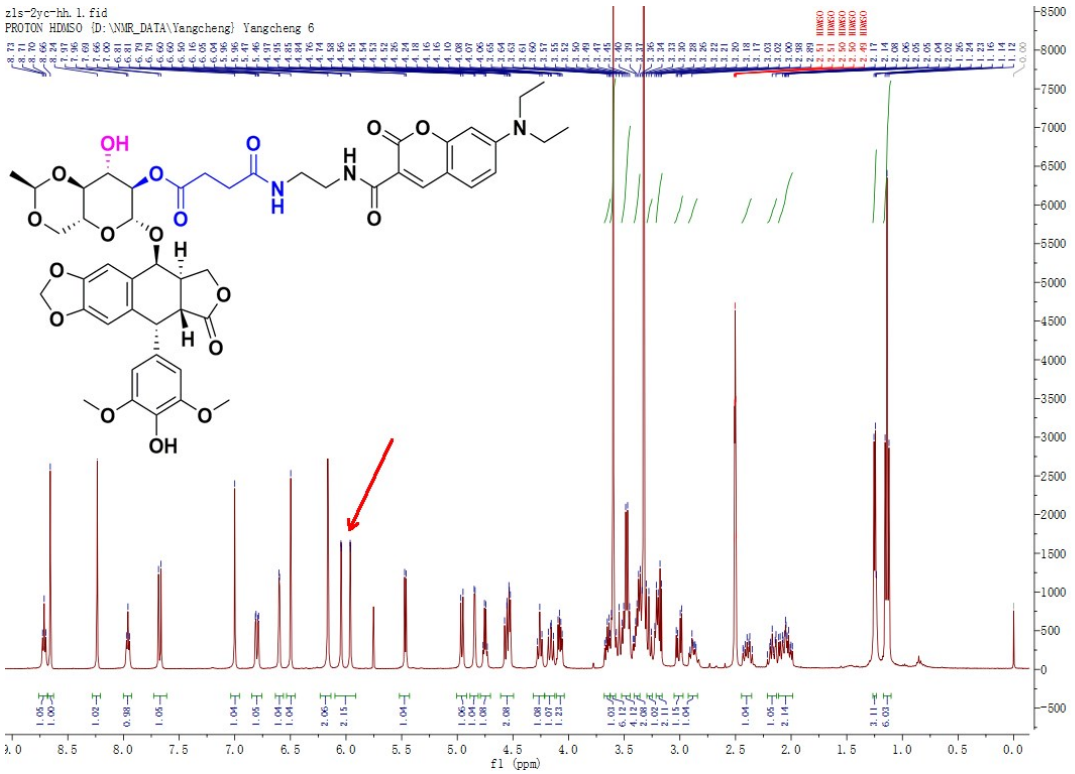

Figure S6N-1. ^{1}H NMR spectrum (400 MHz) of AE1 in DMSO-d6 at 25 ºC.

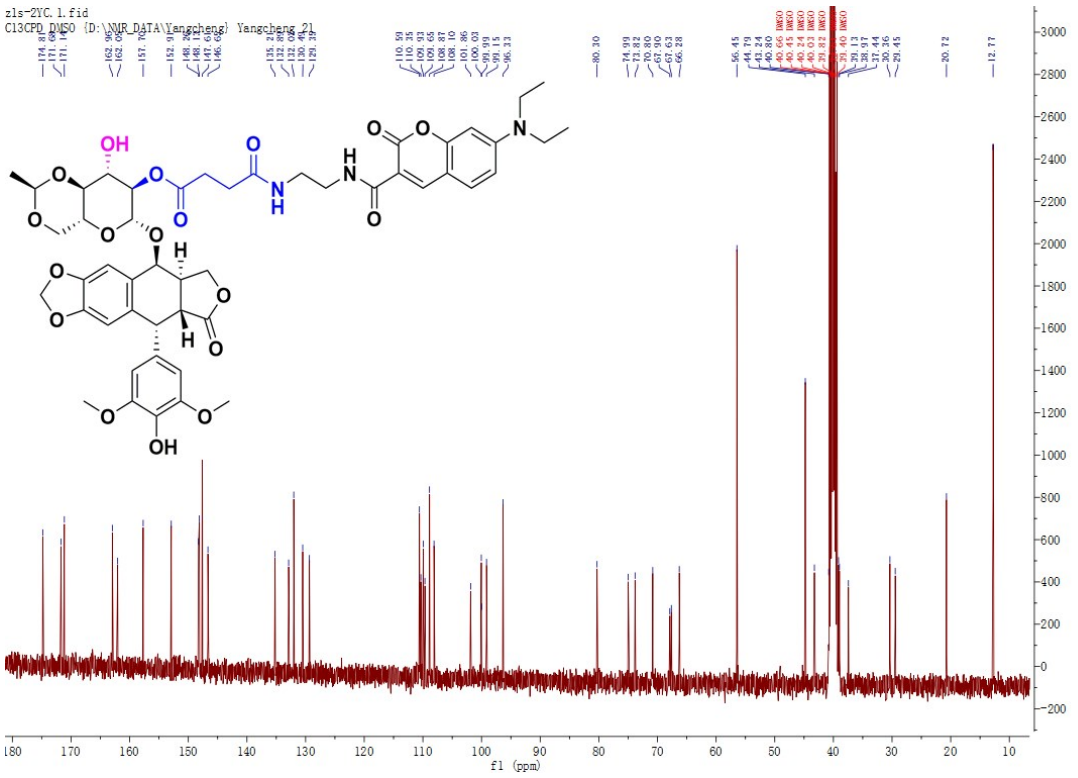

Figure S6N-2. ^{13}C NMR spectrum (100 MHz) of AE1 in DMSO-d6 at 25 ºC.

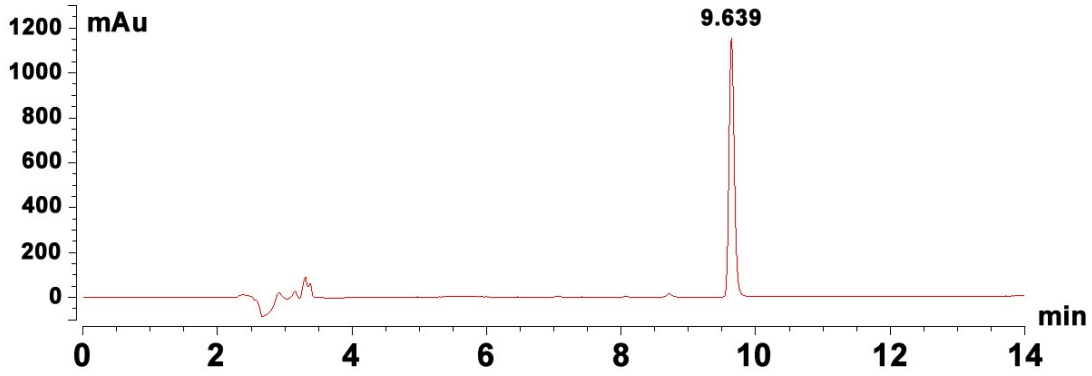

Figure S6N-3. HPLC graph of AE1.

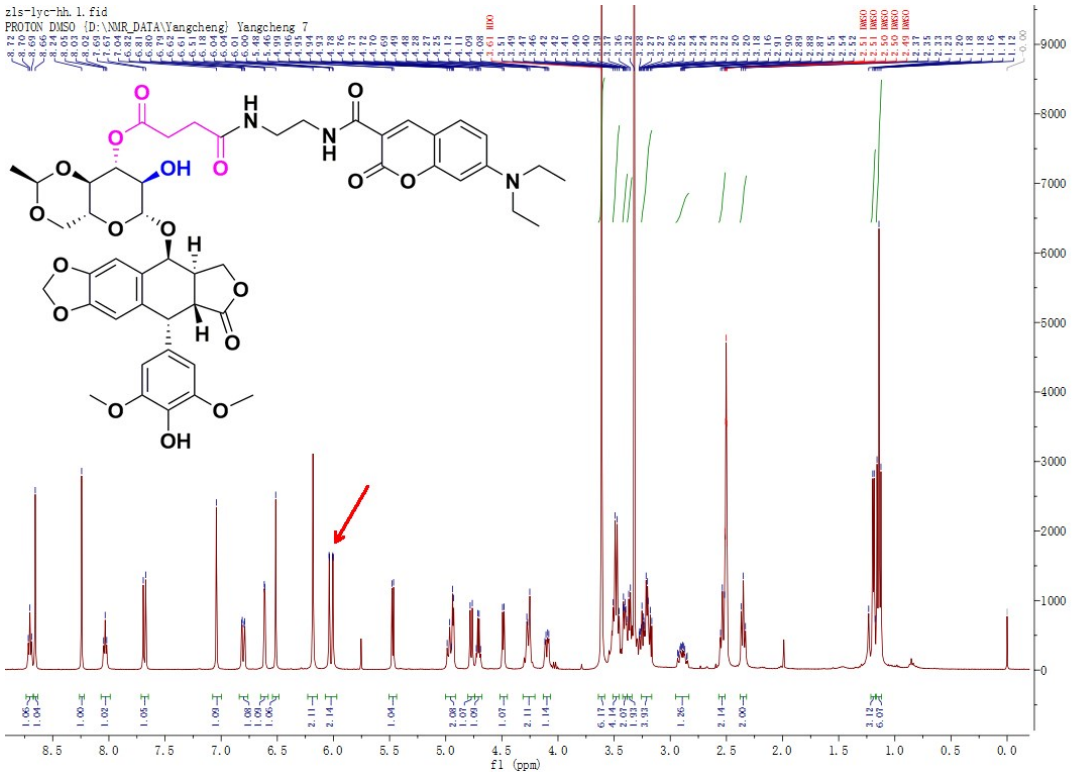

Figure S6O-1. [1]H NMR spectrum (400 MHz) of AE2 in DMSO-d6 at 25 ºC.

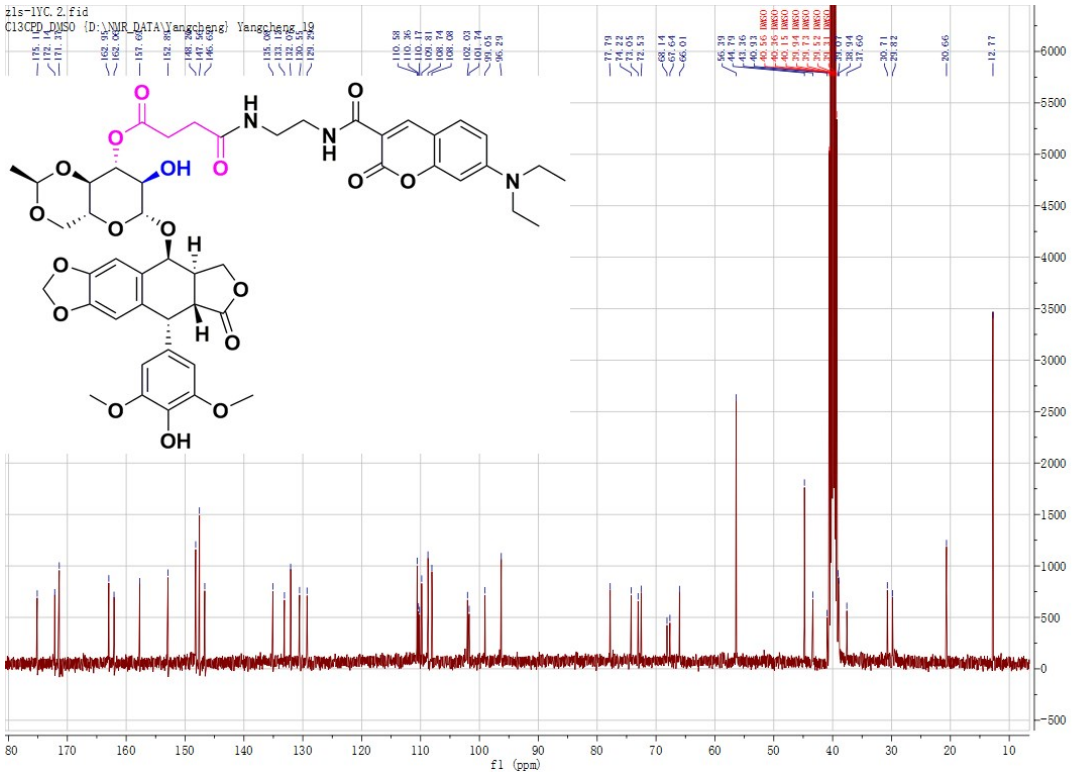

Figure S6O-2. [13]C NMR spectrum (100 MHz) of AE2 in DMSO-d6 at 25 ºC.

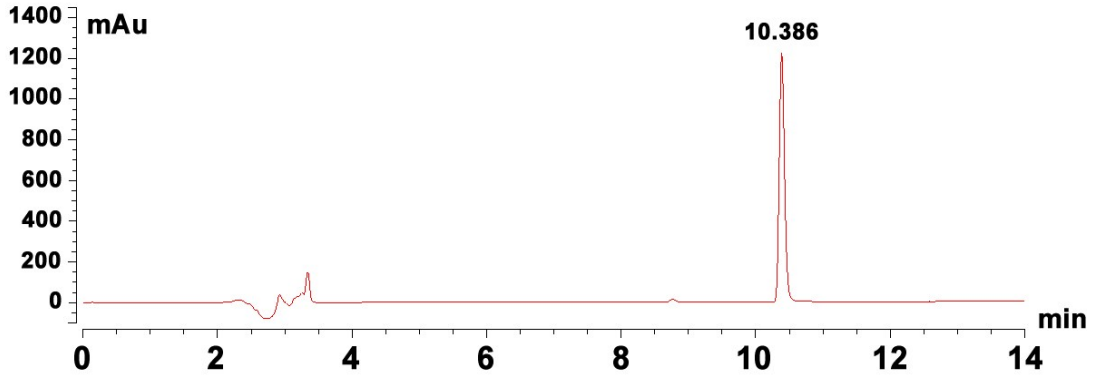

Figure S6O-3. HPLC graph of AE2.

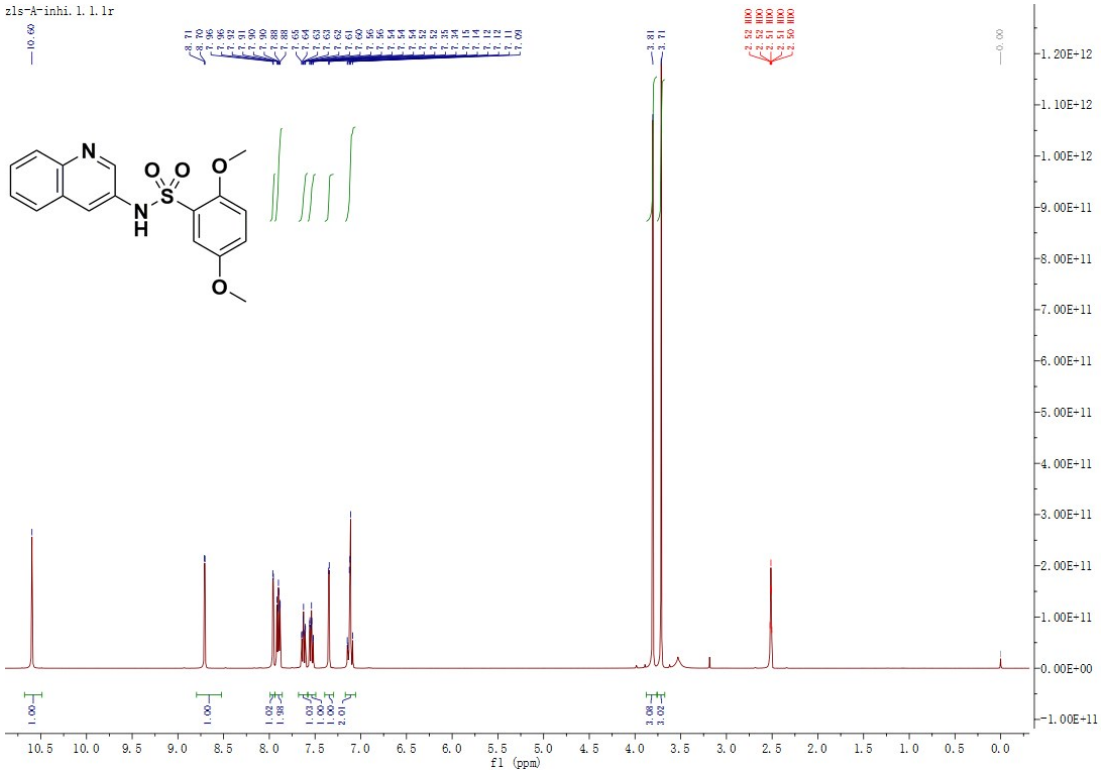

Figure S6P-1. ^{1}H NMR spectrum (400 MHz) of ALP inhibitor (DQB) in DMSO-d6 at 25 ºC.

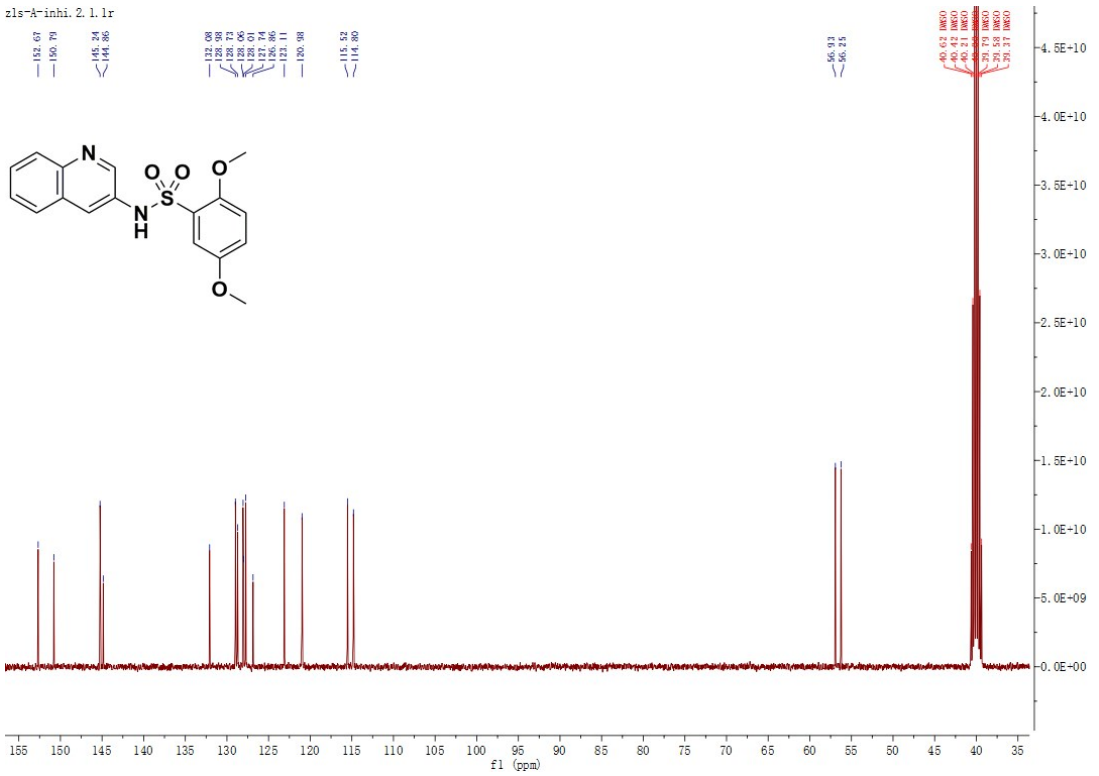

Figure S6P-2. ^{13}C NMR spectrum (100 MHz) of ALP inhibitor (DQB) in DMSO-d6 at 25 ºC.

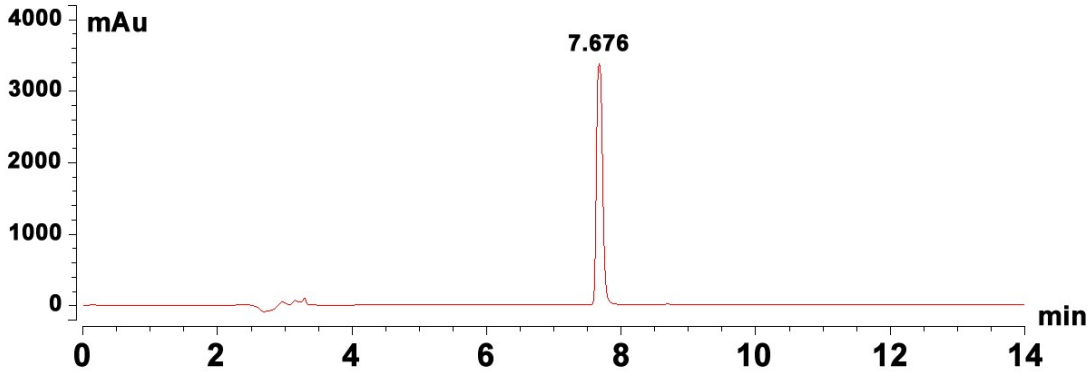

Figure S6P-3. HPLC graph of ALP inhibitor (DQB).

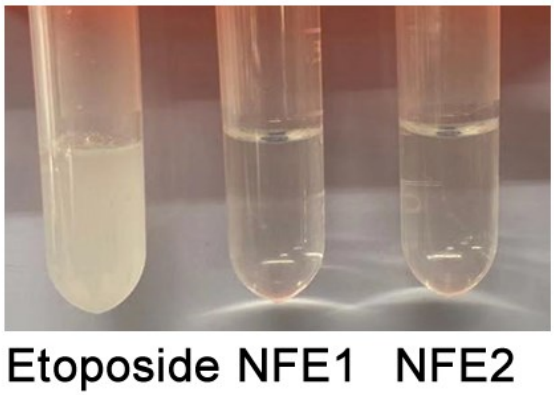

Figure S7. Solubility of etoposide (10mmol/L) and NFE1/2 (10mmol/L) in PBS. Etoposide forms cloudy emulsion in PBS. The solution of the NFE1and NFE2 are clear and transparent.

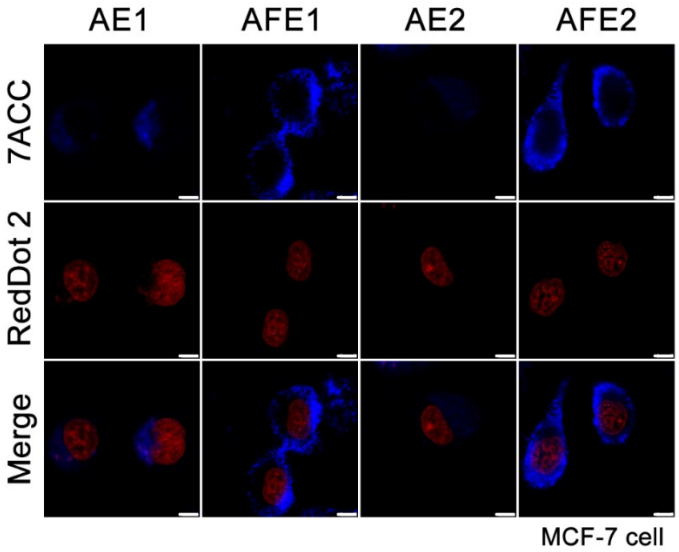

Figure S8. Cell uptake efficiency of 7ACC-GFFpYK-Etoposide1/2 (AFE1/2) and 7ACC-Etoposide1/2 (AE1/2) (20 μmol/L, 4h) in MCF-7 cells. 7ACC: λex = 408nm, λex = 468nm; RedDot 2: λex =561nm, λem = 700nm. Scale bar = 10μm.

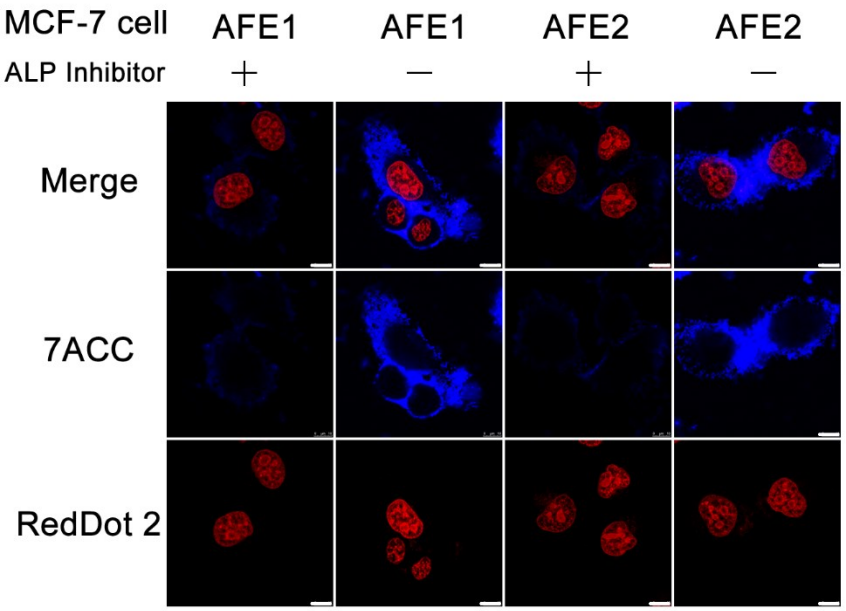

Figure S9. Cell uptake efficiency of 7ACC-GFFpYK-Etoposide1/2 (AFE1/2) (20 μmol/L, 4h) in MCF-7 cells with ALP inhibitor (2,5-dimethoxy-N-(quinolin-3-yl)benzenesulfonamide) (50 μmol/L, 6h). 7ACC: λex = 408 nm, λex = 468 nm; RedDot 2: λex =561 nm, λem = 700 nm. Scale bar = 5 μm.

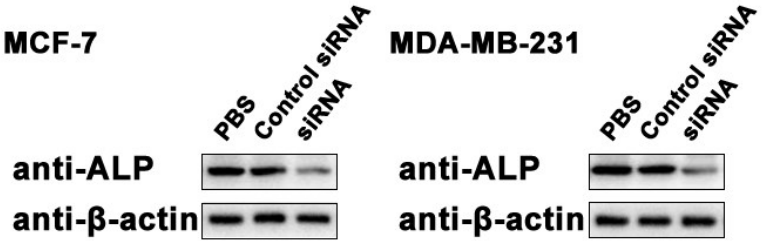

Figure S10. Western Blotting analyses of the expression of the ALP protein in MCF-7 and MDA-MB-231 cells. Lane: PBS, Control siRNA, ALP siRNA.

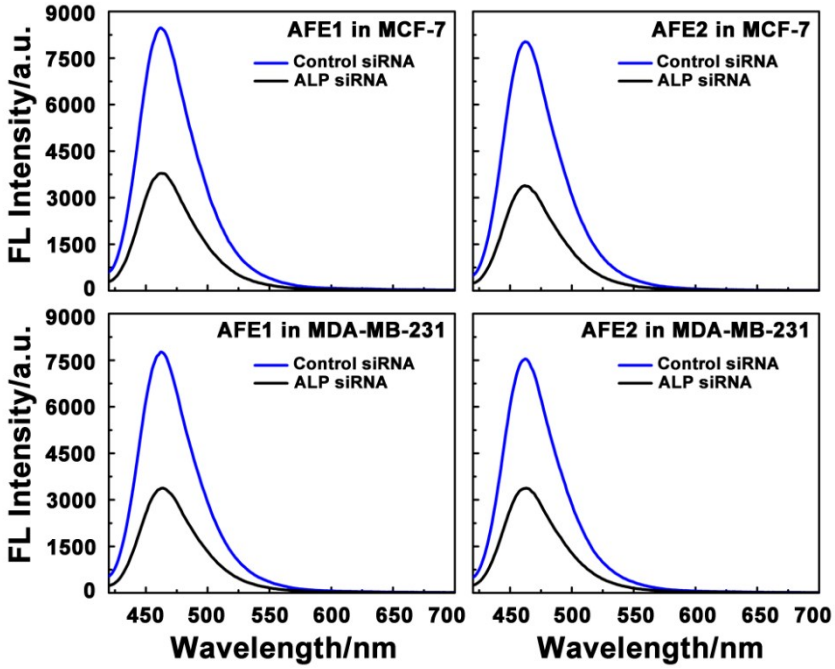

Figure S11. The fluorescence intensity of AFE1 and AFE2 in MCF-7 and MDA-MB-231 cells transfected with ALP siRNA or Control siRNA.

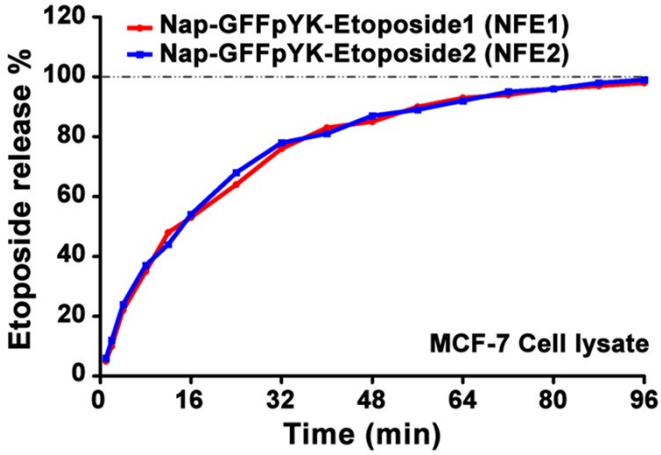

Figure S12. The ratio of etoposide released from self-assembly precursor molecules NFE1 (1 mmol/L) and NFE2 (1 mmol/L) in MCF-7 cell lysate. Sampling time points were 1, 2, 4, 8, 12, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, and 96 min.

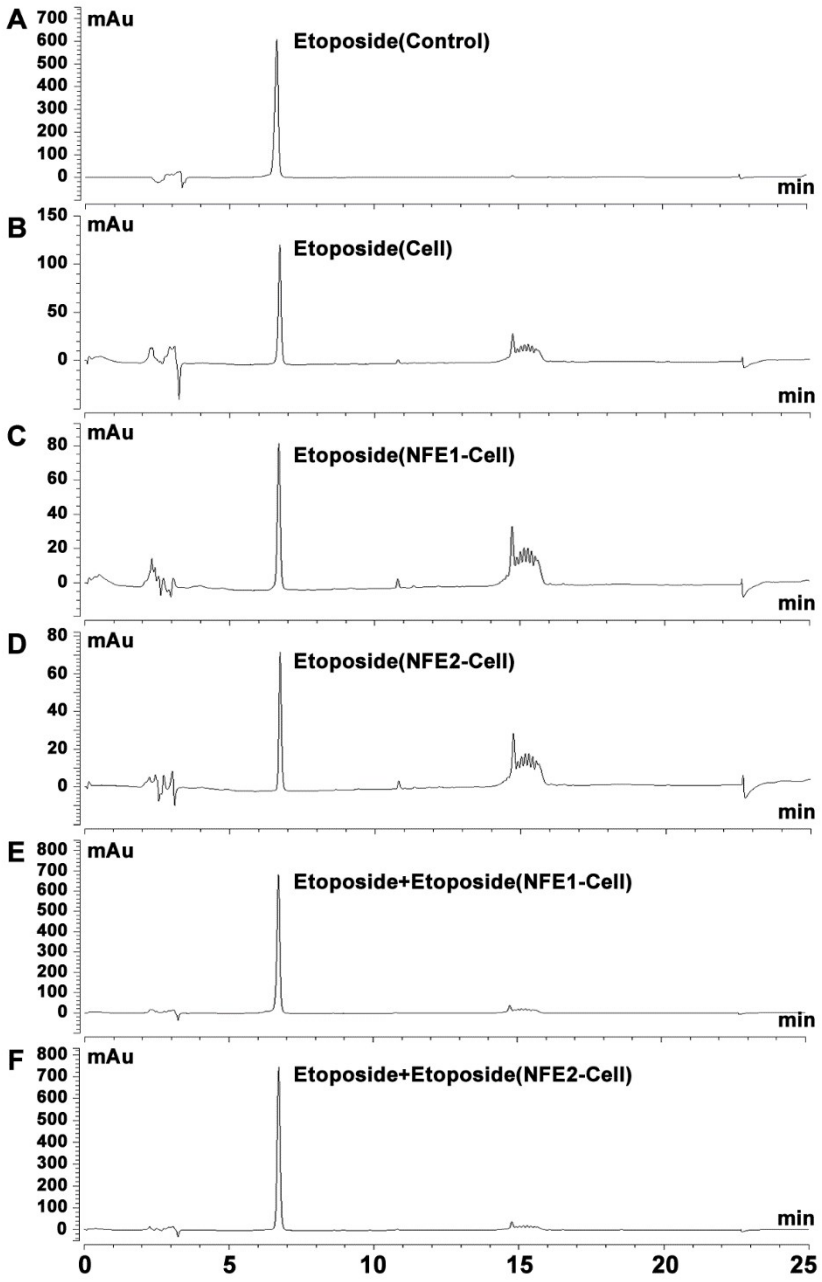

Figure S13. Release of free etoposide from etoposide self-assembly precursor molecules NFE1 and NFE2 in MCF-7 cells. A) Retention time of etoposide standard by HPLC test. B) Retention time of etoposide isolated from MCF-7 cells. C) Retention time of etoposide released by NFE1 in MCF-7 cells. D) Retention time of etoposide released by NFE2 in MCF-7 cells. E) Retention time of etoposide released by NFE1 in MCF-7 cells and etoposide standard. F) Retention time of etoposide released by NFE2 in MCF-7 cells and etoposide standard.

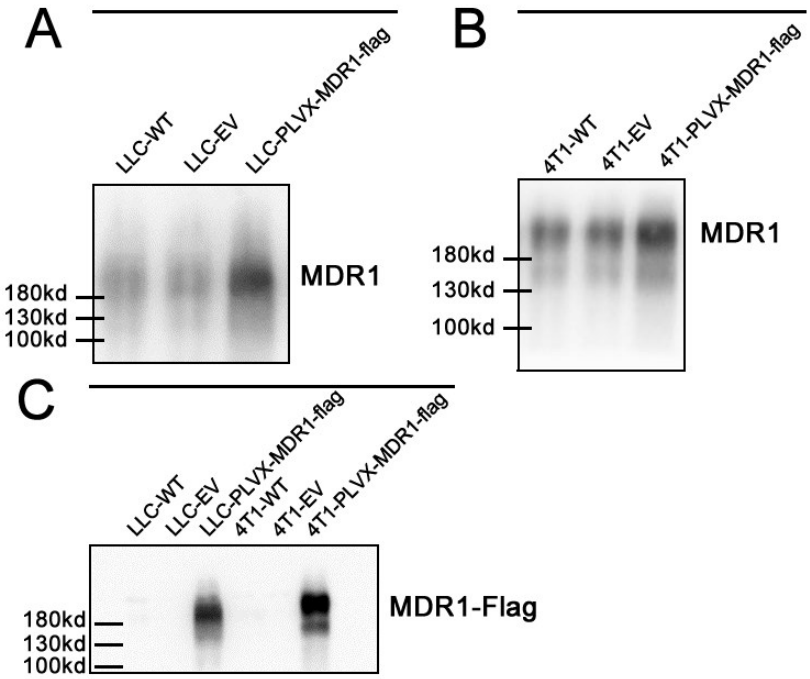

Figure S14. Establishment of MDR1-Flag overexpressing LLC and 4T1 cell lines. MDR1 antibody detects the expression of MDR1 protein in LLC-MDR (A) and 4T1-MDR (B) cell lines. (C) Flag antibody detects the expression of MDR1-Flag protein in LLC-MDR and 4T1-MDR cell lines.

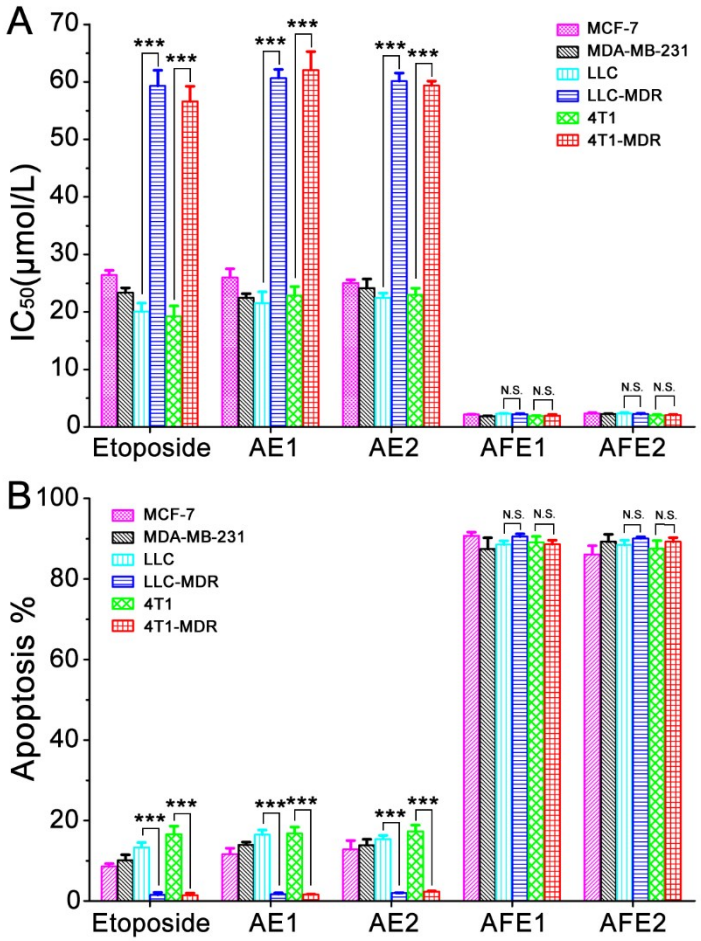

Figure S15. (A) 48-hour cytotoxicity of etoposide-nanofibers (Etoposide, AE1, AE2, AFE1, AFE2) on cancer cell lines (MCF-7, MDA-MB-231, LLC, LLC-MDR1, 4T1, and 4T1-MDR1) at different concentration. T test ***P<0.001. (A) Apoptosis induced by five drugs (Etoposide, AE1, AE2, AFE1, AFE2) with a concentration of 10μmol/L in six different cell lines (MCF-7, MDA-MB-231, LLC, LLC-MDR1, 4T1, and 4T1-MDR1) within 12 hours. T test ***P<0.001. N.S., no-significance.

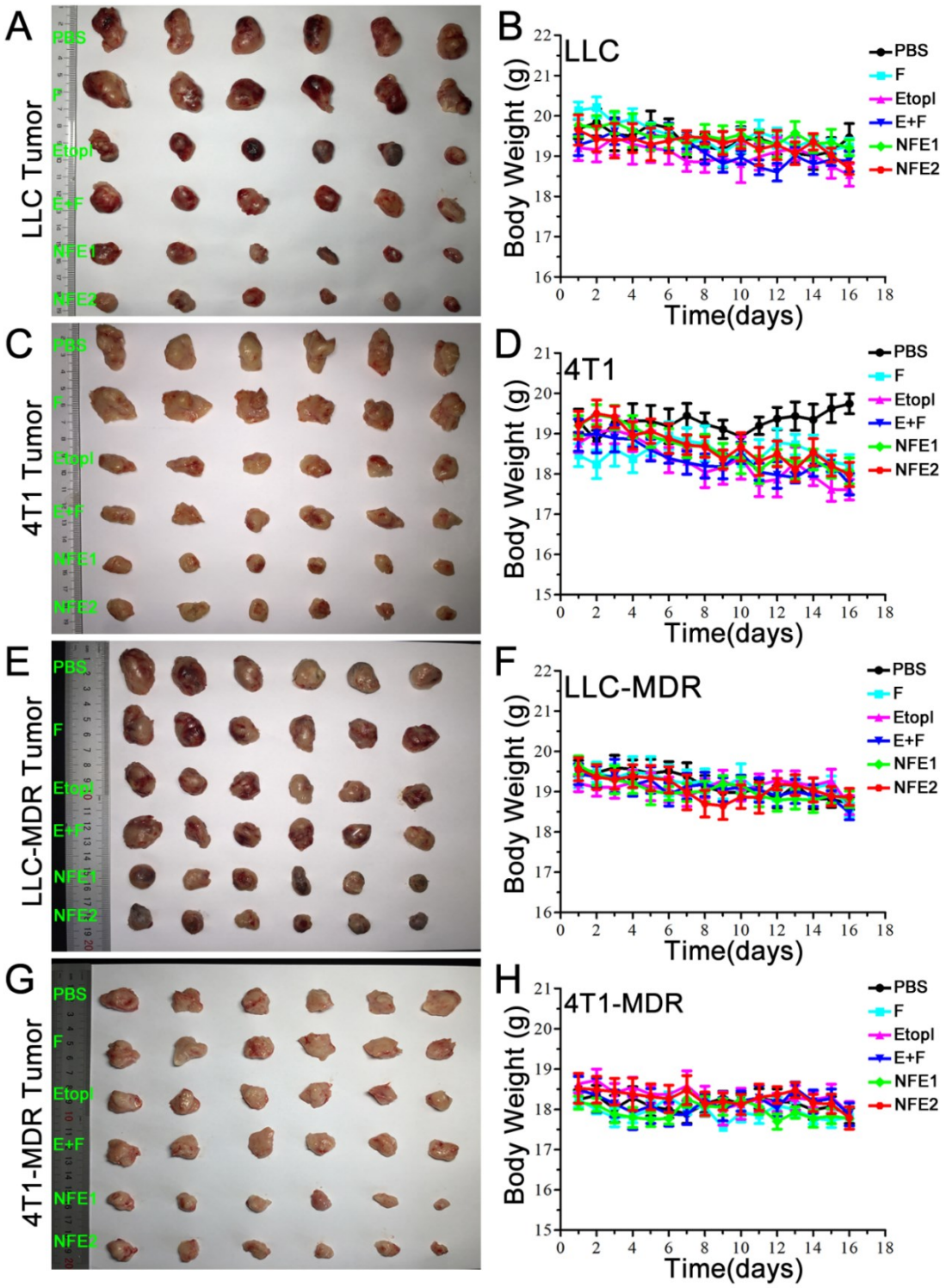

Figure S16. Anti-tumor activity of Nap-GFFpYK-Etoposide1/2 (NFE1/2) in LLC/4T1 Cells and LLC/4T1 multi-drug resistant Cells tumor model. (A,C,E,G) Photograph of tumor after treatment. (B,D,F,H) The curve of mouse body weight with time during treatment. The doses of etoposide, F and NFE1/2 at paratumoral injection are 5, 7.72 and 13.41mg/kg (equimolar concentration), respectively.

S11. References

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