Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Acid-Sensitive PEG-Removable Nanoscale Liposomes for Delivery of Doxorubicin in A549/ADR Therapy

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1. Synthesis of mPEG-N=CH-DOB and mPEG-NH-CO-DOB

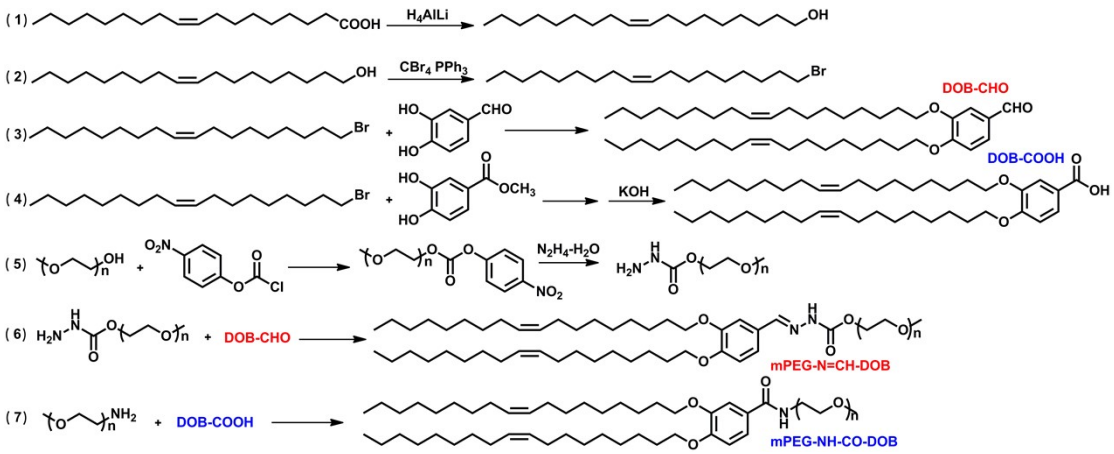


Fig. S1. Synthetic route of mPEG-N=CH-DOB and mPEG-NH-CO-DOB.

(1) Add 10 g of oleic acid and 120 mL of anhydrous ethyl ether to a 250 mL flask and stir well. Add 45 mL of anhydrous tetrahydrofuran solution of 3.36 g lithium aluminum hydride to the oleic acid in batches under ice bath conditions. Raise the temperature to 60°C, reflux the reaction for 4 h. Cool the reaction and quench the reaction by adding wet ether. The reaction solution was hydrolyzed by adding 2 mol/L

hydrochloric acid, centrifuged, partitioned, the organic phase was collected, dried by adding anhydrous Na_2SO_4, filtered, spun off and dried under vacuum to obtain 7.73 g (yield 81%) of the product **oleyl alcohol** as a colorless viscous liquid and was confirmed by GC-MS, and the purity was 98.72 %.

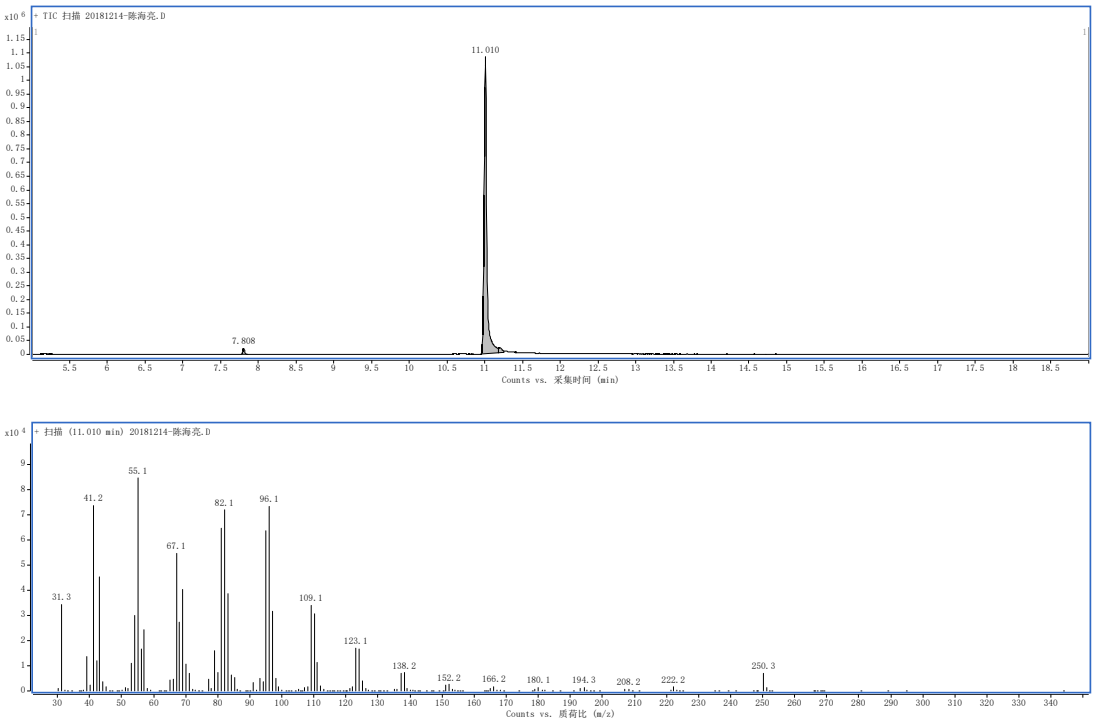


Fig. S2. GC-MS of Oleyl alcohol.

(2) Dissolve 7.73 g of oleyl alcohol in 144 mL of dichloromethane in an ice water bath, add 12.42 g of CBr_4 and PPh_3 in batches and stir for 40 min. Dilute with hexane, filter, spin and repeat until no precipitation, dry under vacuum to obtain 8.78 g of product **(Z)-1-bromooctadec-9-ene** (yield 92%) as a colorless viscous liquid and was confirmed by GC-MS, and the purity was 99.13%.

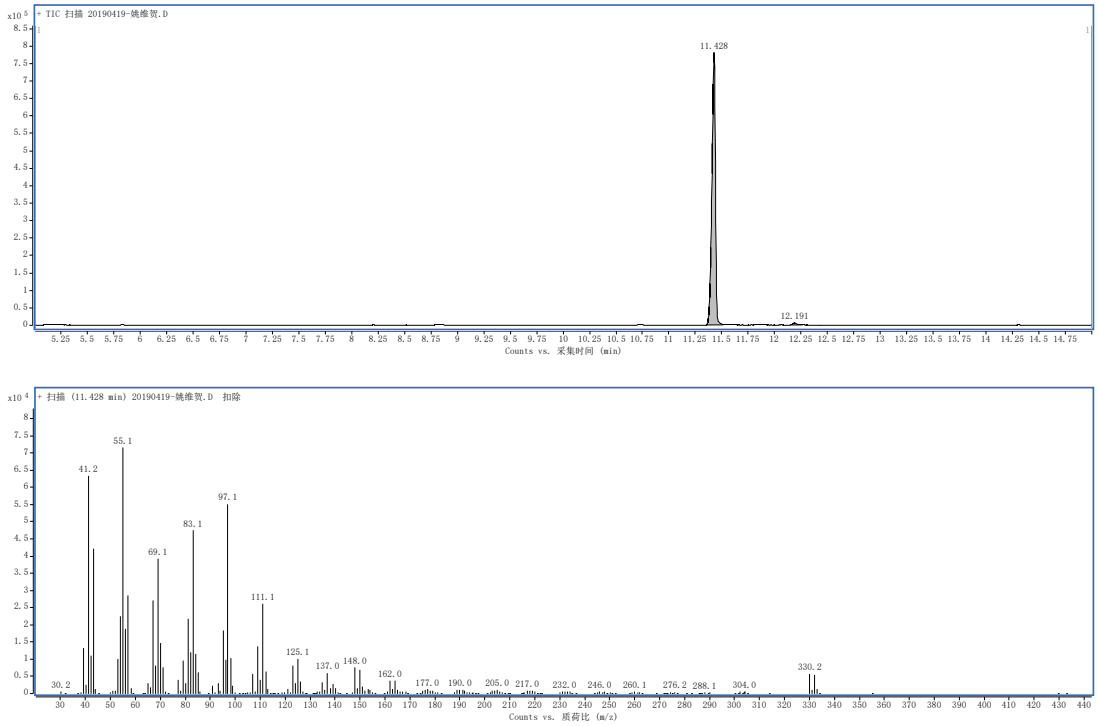


Fig. S3. GC-MS of (Z)-1-bromooctadec-9-ene.

(3) 4.17 g (Z)-1-bromo-octadec-9-ene was dissolved in 25 mL cyclohexanone, added 0.80 g 3,4-dihydroxybenzaldehyde, 2.39 g K_2CO_3, 0.12 g KI, protected from light and N_2, and reacted at 100°C for 18 h. The residue was dissolved in dichloromethane, washed twice with water, collected the organic phase, dried with anhydrous Na_2SO_4, filtered, and purified by column chromatography with petroleum ether/ethyl acetate, dried under vacuum, and obtained 1.70 g **DOB-CHO** (yield 46.2%) as a yellow solid and confirmed by ^{1}H NMR. ^{1}H NMR (500MHz, $CDCl_3$): δ = 9.83 (s, 1H, -*C(O)H*), 7.41 (m, 2H, H_{ar} o-C(O)), 6.95 (d, 1H, H_{ar} m-C(O)), 5.35 (s, 4H, =*CH*-), 4.06 (m, 4H, O-*CH_2*), 2.02 (s, 8H, -*CH_2*-CH=), 1.85 (d, 4H, -*CH_2*-CH_2 -O-ph), 1.60-1.10 (m, 44H, alkyl-*CH_2*), 0.88 (t, 6H, -*CH_3*).

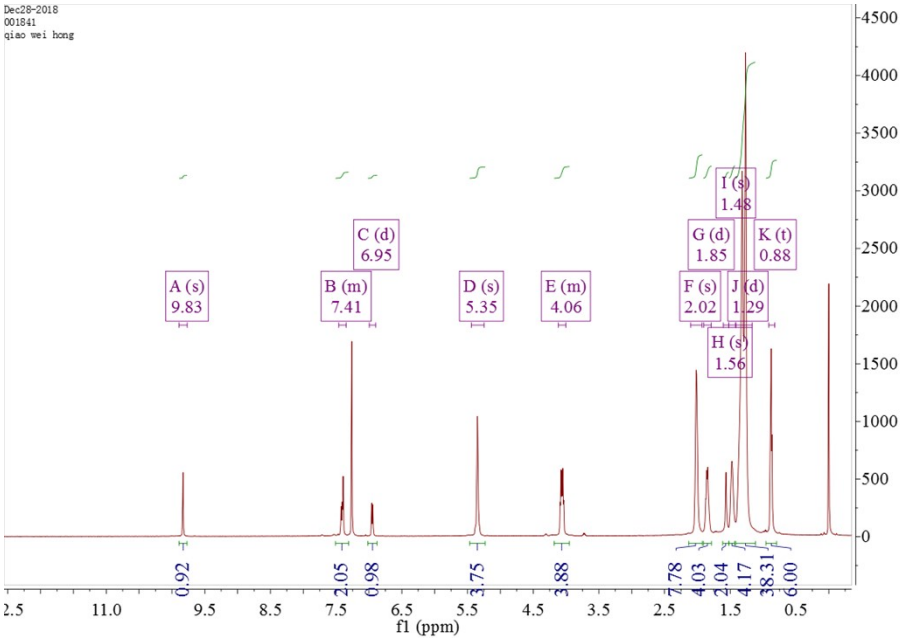


Fig. S4. ^{1}H-NMR spectra of DOB-CHO.

(4) Dissolve 3.51 g (Z)-1-bromo-octadec-9-ene in 25 mL cyclohexanone, add 0.82 g methyl 3,4-dihydroxybenzoate, 2 g K_2CO_3, 0.98 g KI, protect from light, N_2, and react at 100°C for 18 h. Filter while hot, spin evaporate, and dissolve the residue in 2.20 g KOH in 82.50 mL ethanol solution, reflux at 80°C for 4 h. Add the reaction solution to 640 mL water, precipitate a white solid, filter, wash twice with water, recrystallized with ethanol, filter and vacuum dry to obtain 1.67 g DOB-COOH (yield 52%) as a white solid and confirmed by MS and ^{1}H NMR. MS: M=654.56; m/z=653.68 is $[M-H]^-$. ^{1}H NMR (500MHz, $CDCl_3$): δ = 7.72 (d, 1H, H_{ar} o-C(O), m-OR, p-OR), 7.58 (s, 1H, H_{ar} o-C(O), o-OR, m-OR), 6.88 (t, 1H, H_{ar} m-C(O)), 5.35 (m, 4H, =*CH*-), 4.05 (dd, 4H, O-CH_2), 2.02 (d, 8H, -CH_2-CH=), 1.84 (m, 4H, -CH_2-CH_2 -O-ph),1.53-1.15 (m, 44H, alkyl-CH_2), 0.88 (t, 6H, -CH_3).

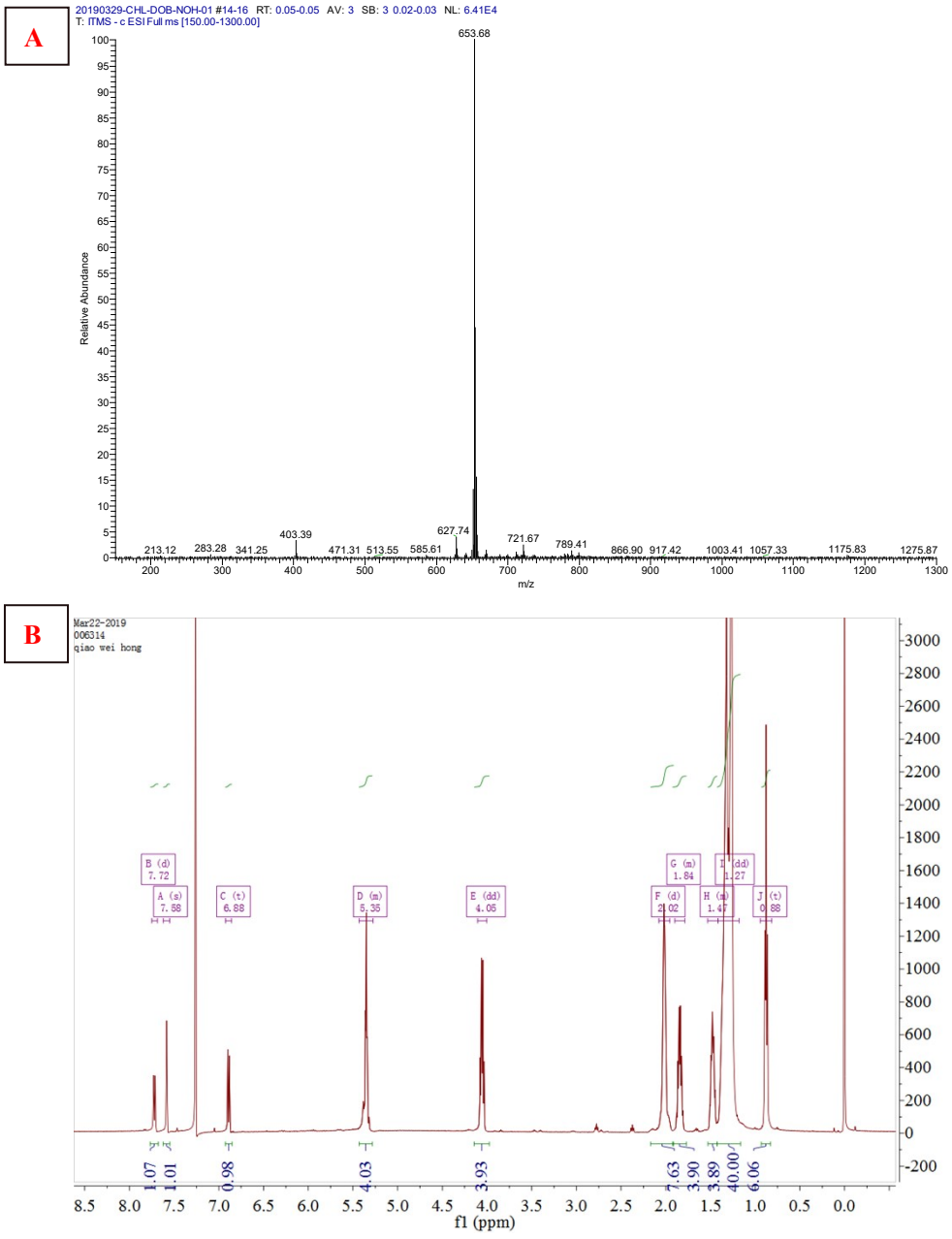


Fig. S5. MS (A) and ^{1}H-NMR spectra (B) of DOB-COOH.

(5) 4 g (2 mmol) PEG_{2000} monomethyl ether was dissolved in 40 mL dichloromethane. The dichloromethane solution of 48.9 mg (0.4 mmol) DMAP, 0.89 g (4.4 mmol) 4-nitrophenyl chloroformate (NPCF) was added to the reaction flask in turn, and the reaction was carried out at 35°C for 24 h under the protection of N_2. The residue was dissolved in ethyl acetate, added with an equal amount of ether, stood overnight at

4°C, filtered, and dried in vacuum to give a white solid **mPEG-NPCF** of 3.90 g (yield 90%) and confirmed by ^{1}H NMR. ^{1}H NMR (500MHz, $CDCl_3$):δ=8.28 (m, 2H, H_{ar} o-NO_2), 7.39 (m, 2H, H_{ar} m-NO_2), 4.43 (m, 2H, -CH_2-O-C(O)-), 3.60-3.66 (m, main peak: 3.65, 188H, -O-CH_2), 3.37 (s, 3H, -CH_3).

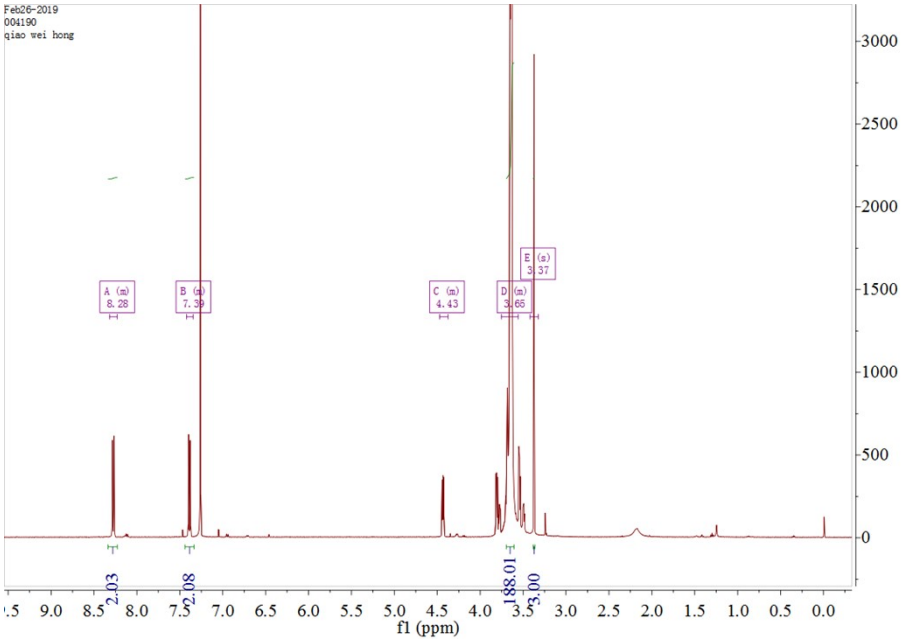


Fig. S6. ^{1}H-NMR spectra of mPEG-NPCF.

3.90 g (1.95 mmol) mPEG-NPCF and 1 g (20 mmol) hydrazine hydrate were dissolved in 10 mL anhydrous dichloromethane at room temperature under N_2 protection for 24 h. 1M hydrochloric acid, saturated NaCl, water washed 2 times, anhydrous Na_2SO_4 dry, rotary evaporation. Dissolve the residue with ethyl acetate, add the same amount of ether, 4°C overnight, filter, vacuum drying, get the product **mPEG-NH-NH$_2$** 3.50 g (yield 85%), white solid and confirmed by ^{1}H NMR. ^{1}H NMR (500MHz, $CDCl_3$): δ=3.63-3.70(d, main peak: 3.64,180H,-O-CH_2), 3.38 (s,3H,-CH_3).

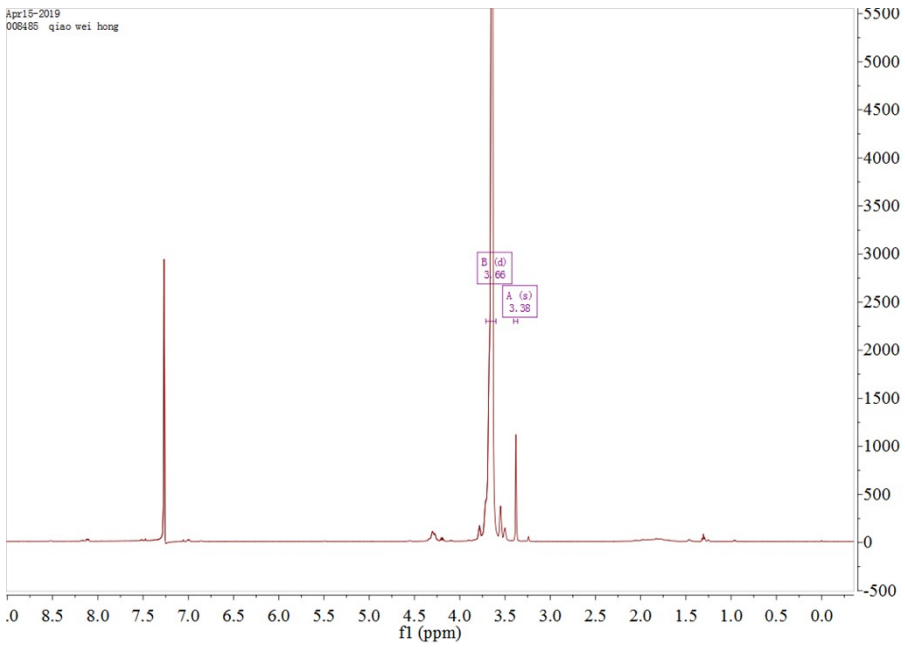


Fig. S7. ^{1}H-NMR spectra of mPEG-NH-NH_2.

(6) 3.50 g (1.75 mmol) mPEG-NH-NH_2 and 1.30 g (2 mmol) DOB-CHO were dissolved in 70 mL anhydrous dichloromethane, 35 μL trifluoroacetic acid was added, N_2 protection, reflux reaction for 24 h. After the reaction, 35 μL triethylamine was added and evaporated. The product **mPEG-N=CH-DOB** was separated and purified by gradient dichloromethane/methanol (30:1-15:1) column chromatography and vacuum drying to obtain 2.37 g (yield 52%), which was a white solid and confirmed by ^{1}H NMR and MALDI-TOF-MS. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.50 (s, 1H, -*CH*=N-), 7.80 (s, 1H, H_{ar} o-CH=N-, o-OR, m-OR), 7.03 (d, 1H, H_{ar} o-CH=N-, m-OR, p-OR), 6.82 (d, 1H, H_{ar} m-C(O)), 5.34 (d, 4H, =*CH*-), 4.27 (m, 2H, -C(O)-O-*CH_2*-O-), 4.01 (m, 4H, -*CH_2*-O-ph), 3.50-3.78 (s, main peak: 3.64, 180H, O-*CH_2*), 3.38 (s, 3H, -O-*CH_3*), 2.02 (m, 8H, -*CH_2*-CH=CH-), 1.72 (s, 4H, -*CH_2*-CH_2-O-ph), 1.45-1.29(m, 44H, alkyl-*CH_2*), 0.87 (t, 6H, alkyl-*CH_3*).

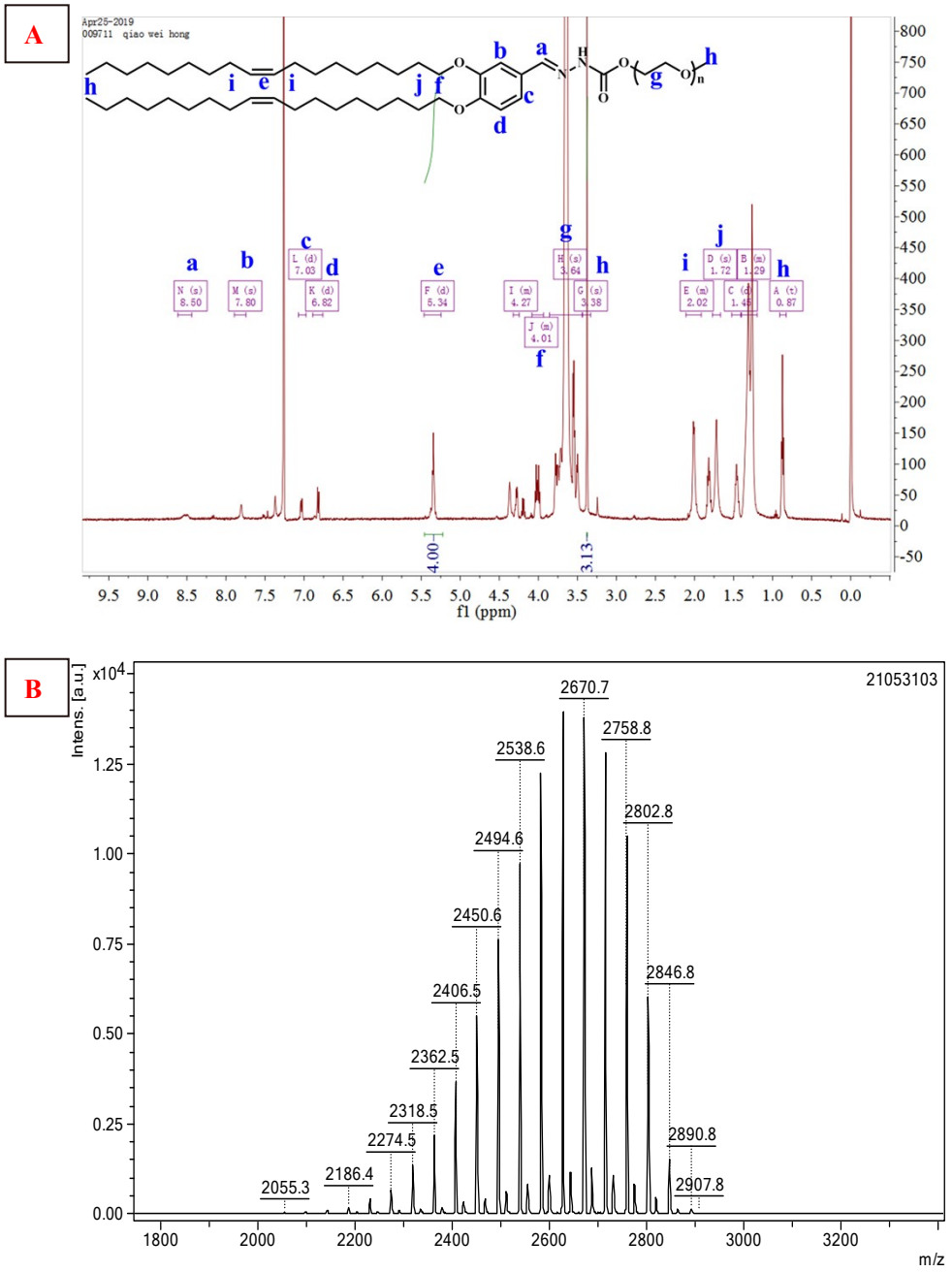
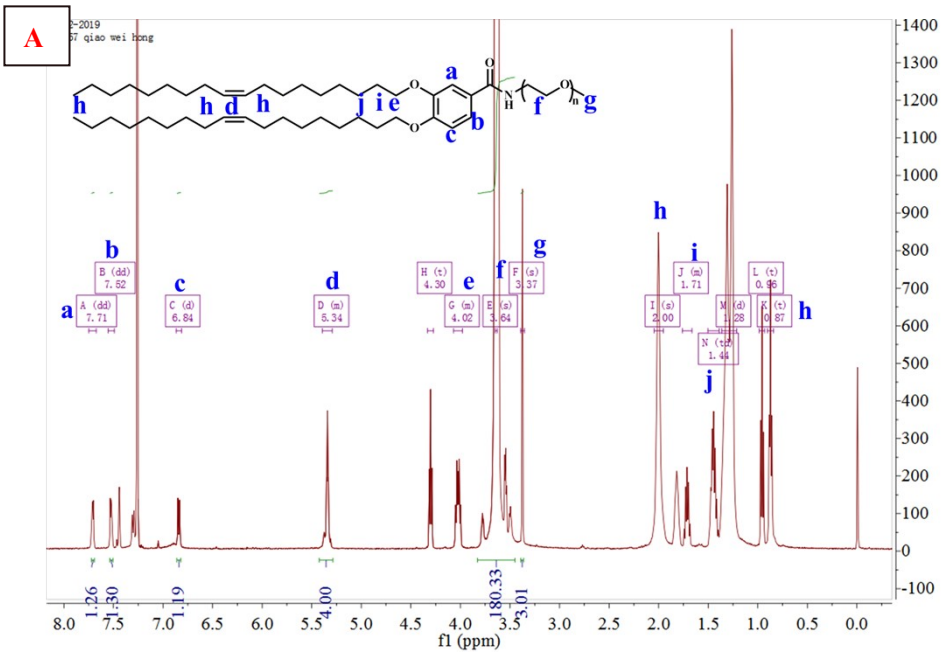


Fig. S8. ^{1}H-NMR spectra(A) and MALDI-TOF-MS (B)of mPEG-N=CH-DOB.

(7) 1.11 g (1.70 mmol) DOB-COOH, 0.65 g (1.71 mmol) HBTU and 0.44 g (3.40 mmol) DIEPA were added to a round bottom flask and dissolved in 50 mL dichloromethane. After stirring at room temperature for 2 h, 2.70 g (1.35 mmol) mPEG-NH_2 was added, protected by N_2, and stirred at room temperature for 24 h. The samples were washed twice with 0.1M hydrochloric acid, saturated $NaHCO_3$ and

saturated salt water, respectively. The organic phase was collected, dried with anhydrous Na_2SO_4, filtered, and rotary evaporated. The samples were separated and purified by silica gel column chromatography with a gradient of dichloromethane/methanol (30:1 to 6:1). The product 3.25 g (yield 90%) was white solid and confirmed by ^1H NMR and MALDI-TOF-MS. R_f =0.88 (dichloromethane/methanol 6:1 v/v). ^1H NMR (500MHz, $CDCl_3$): δ = 7.71 (dd, 1H, H_{ar} o-C(O), o-OR, m-OR), 7.52 (dd, 1H, H_{ar} o-C(O), m-OR, p-OR), 6.84 (d, 1H, H_{ar} m-C(O)), 5.34 (m, 4H, =*CH*-), 4.30 (t, 4H, O-*CH_2*-*CH_2*-NH-); 4.02 (m, 4H, -*CH_2*-O-ph), 3.50-3.78 (s, main peak: 3.64, 180H, O-*CH_2*), 3.38 (s, 3H, -O-*CH_3*), 2.00 (s, 8H, -*CH_2*-CH=CH-), 1.71 (m, 4H, -*CH_2*-CH_2-O-ph), 1.44 (td, 4H, -*CH_2*-CH_2-CH_2-O-ph);1.28 (d, 44H, alkyl-*CH_2*), 0.87 (t, 6H, alkyl-*CH_3*).



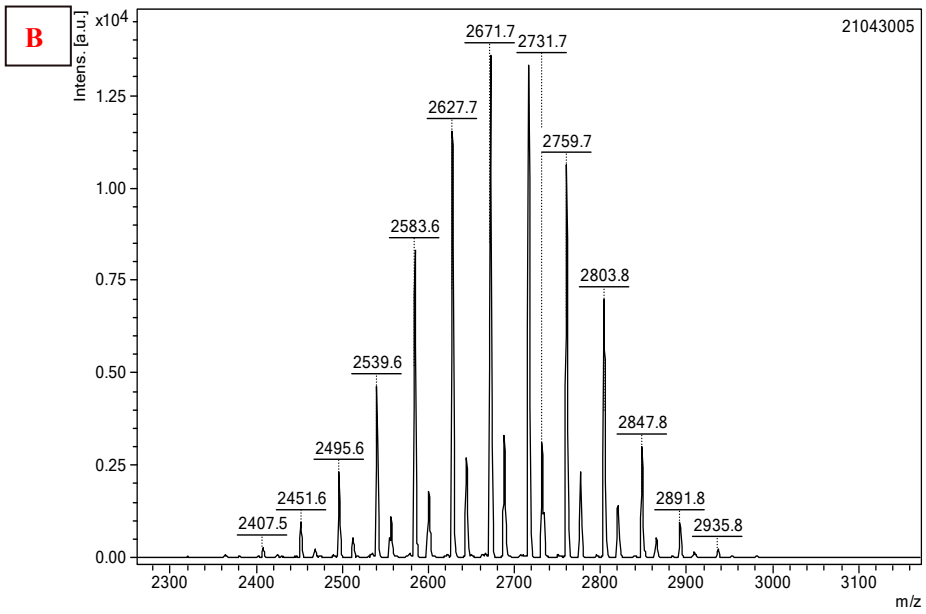


Fig. S9. [1]H-NMR spectra (A) and MALDI-TOF-MS (B)of mPEG-NH-CO-DOB.

2. Experimental supplementary materials

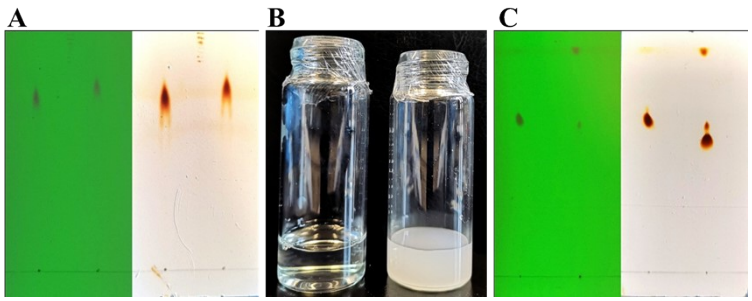


Fig. S10. pH fracture performance study of mPEG-N=CH-DOB.

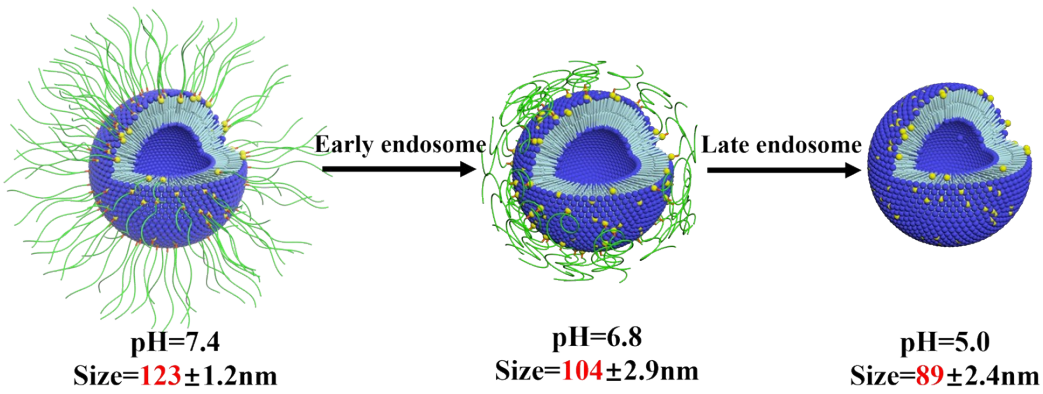


Fig. S11. Morphological changes of PHDG with decreasing pH.

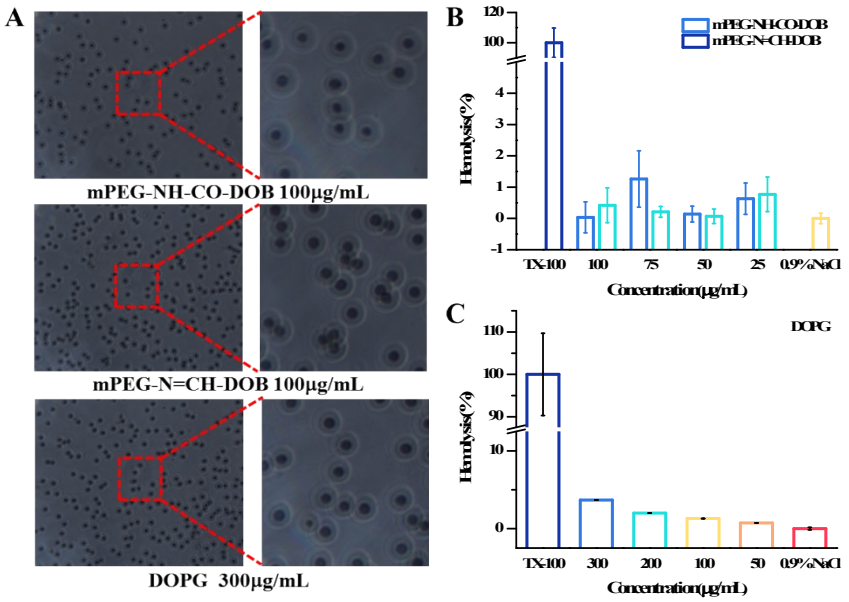


Fig. S12. Hemolysis test results.

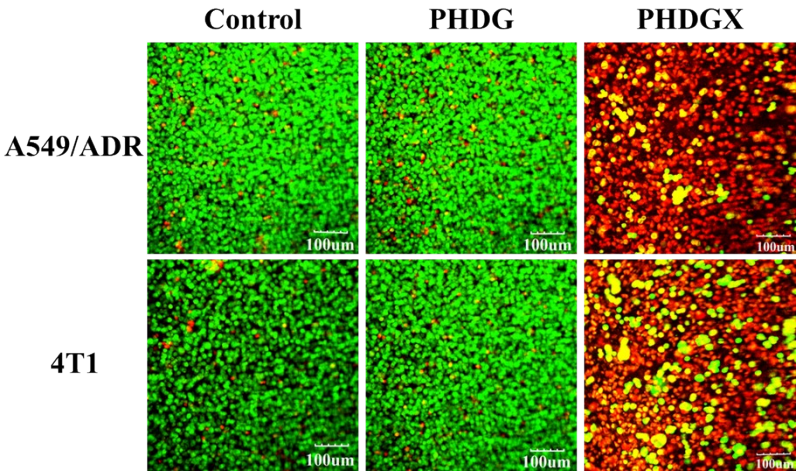


Fig. S13. Live-dead cell staining assay of A549/ADR with 4T1 cells.

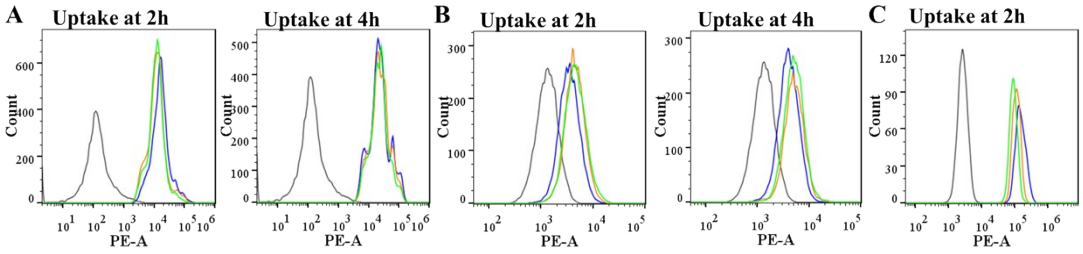


Fig. S14. (A) Flow cytometric analysis results of 4T1 cells on free DOX, PDGX and

PHDGX. The concentration of DOX was 3 μg/mL. (B) Flow cytometric analysis results of A549/ADR cells on free DOX, PDGX and PHDGX. The concentration of DOX was 3 μg/mL. (C) Flow cytometric analysis results of A549/ADR cells on free DOX, PDGX and PHDGX. The concentration of DOX was 20 μg/mL. The gray line is the negative control group. The blue line is DOX, the green line is PDGX, and the orange line is PHDGX.