Electronic Supplementary Information (ESI) for Polymer Chemistry
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for

Synthesis and Characterization of a New Multifunctional Polymeric Prodrug Paclitaxel-Polyphosphoester-Folic Acid for Targeted Drug Delivery

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Characterization

^{1}H NMR measurement

^{1}H NMR and ^{31}P NMR spectra were recorded on a 400 MHz NMR instrument (INOVA-400) at room temperature with DMSO-d_6 and $CDCl_3$ as the solvents and TMS as internal standard. From Figure 1(B), the chemical shifts (δ, ppm) of the protons on PTX can be ascribed as follows: δ ~8.14 (Ar-***H***, 2H), 7.75 (Ar-***H***, 2H), 7.62 (Ar-***H***, 1H), 7.48-7.39 (Ar-***H***, 10H), 7.01 (peak z,1H, -N***H***), 6.26 (peak s, 1H, -O-C***H***-), 6.24 (peak g, 1H, -O-C***H***-), 5.80 (peak x, 1H, -C***H***-), 5.68 (peak i, 1H, -C_2***H***-), 4.90 (peak n, 1H, -C***H***-O-), 4.80(peak f, -C2′***H***-OH), 4.39 (peak q, -C7***H***-OH), 4.30 and 4.18 (peak m, 2H, -C***H***$_2$-), 3.70 (peak j, 1H, -C***H***-) 2.54 (peak p, 1H, -C***H***H-), 2.38 (peak t, 3H, -(C=O)-C***H***$_3$), 2.30 (peak h, 2H, -C***H***$_2$-), 2.24 (peak k, 3H, -(C=O)-C***H***$_3$), 1.88 (peak p′ 1H, -CH***H***-), 1.79 (peak y, 3H, =C-C***H***$_3$), 1.68 (peak r, 3H, -C-C***H***$_3$), 1.24 (peak u, 3H, -C-C***H***$_3$), and 1.14 (peak v, 3H, -C-C***H***$_3$).

According to the ^{1}H NMR measurement, the chemical shifts (δ, ppm) of protons in FA can be ascribed as follows: 1.88 and 1.78 (peak 2, -COCCH$_2$C***H***$_2$-), 2.33 (peak 1, -COC***H***$_2$-), 4.35 (peak 3, -COC***H***$_2$NH-), 4.50 (peak 8, =C-C***H***$_2$NH), 5.58 (peak 10, -N***H***$_2$), 6.59 (peak 6, Ar-***H***), 7.71 (peak 5, Ar-***H***), 6.69 (peak 7, -CH$_2$N***H***-), 8.21 (peak 4, =C***H***-N), 11.50 (peak 12, -COO***H***), 8.65 (peak 9, =C***H***-N=), 9.25 (peak 11, -N***H***-).

^{31}P NMR measurement

^{31}P NMR measurement was carried out to confirm the structure of PTX-PEEP, and the comparison of ^{31}P NMR spectra of cyclic phosphoester monomer (EOP) and PTX-PEEP is depicted in Figure S1. From Figure S1(A), we can find that EOP has a strong resonance at δ 17.98

ppm (peak a), whereas PTX-PEEP in Figure S1(B) shows a strong peak at δ -0.22 ppm (peak b) and a weak resonance at δ -0.02 ppm (peak c) assigning to the phosphorus atoms in PEEP backbone.

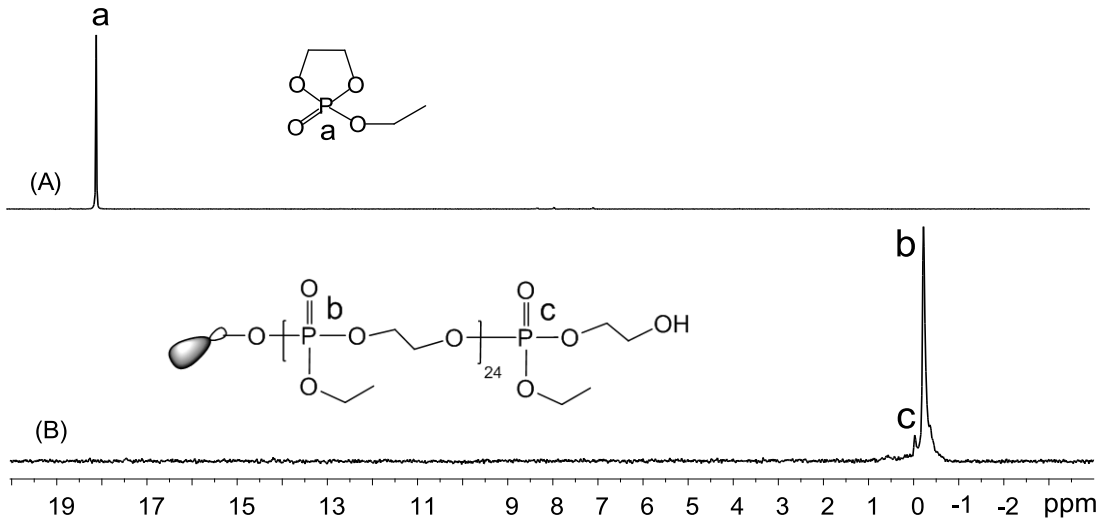

Figure S1. ^{31}P NMR spectra of (A) EOP monomer and (B) PTX-PEEP$_{25}$ in $CDCl_3$.

FT-IR measurement

FT-IR spectra were recorded on a Nicolet 6700 FT-IR Spectrometer (Thermo Scientific) using the KBr disk method. The FT-IR spectra of PTX, PTX-PEEP and PTX-PEEP-FA were exhibited in Figure S2. The absorption peaks at ~1270 and ~1030 cm^{-1} in Figure S2(B) and Figure S2(C) are the asymmetrical and symmetrical P=O stretching, respectively. The absorption peak at 980 cm^{-1} can be ascribed to P-O-C stretching.[1] Meanwhile, the skeleton vibration of benzene ring at 1600, 1500, and 1450 cm^{-1}, the bending vibration and stretching vibration of conjugate benzene ring at ~750 cm^{-1} and ~2900 cm^{-1} were accordingly found in the FT-IR spectra. Furthermore, the N-H stretching vibration at 3300 and 2800 cm^{-1} as well as the bending vibration at 1550 cm^{-1} confirmed the presence of folic acid as shown in Figure S2(C).

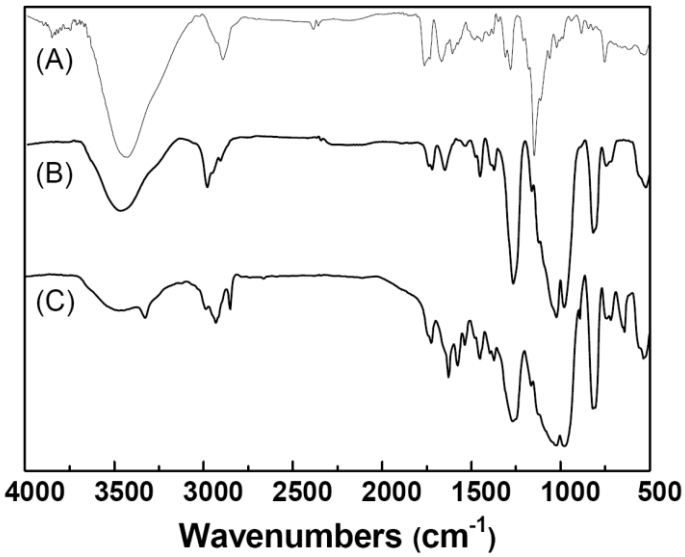

Figure S2. FT-IR spectra of (a) PTX, (b) PTX-PEEP$_{34}$, and (c) PTX-PEEP$_{34}$-FA.

UV-Vis measurement

UV-Vis measurements were conducted using a U-3900 spectrophotometer (Hitachi). As shown in Figure S3, in the wavelength range from 400 to 200 nm, the maximum absorption peak of free PTX and FA are at 229 nm and 286 nm, while pure PEEP rarely has any UV absorption in this range. The maximum absorption peak near 226 nm for PTX-PEEP$_{34}$ and the two maximum absorption peaks at about 226 nm and 278 nm for PTX-PEEP$_{34}$-FA revealed the successful combination of PTX-PEEP and FA. In addition, the blue shift occurred in the spectrum of PTX-PEEP$_{34}$-FA may be attributed to the covalent linkage of FA to PTX-PEEP$_{34}$ by one of its carboxyl groups.

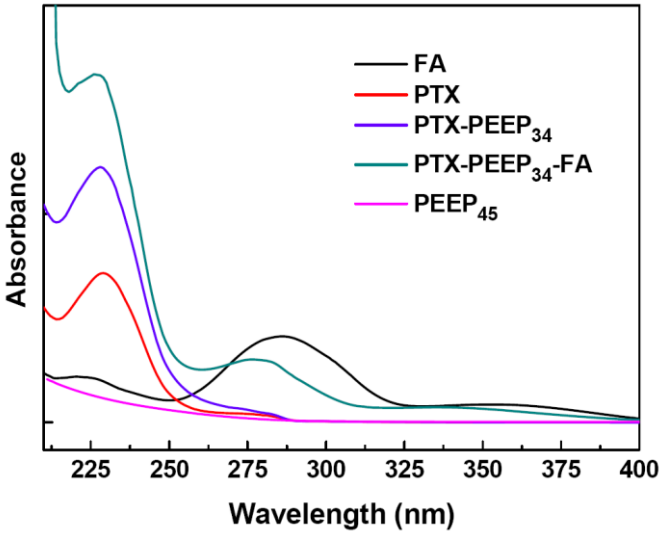

Figure S3. The UV absorption spectra of FA, PTX, PTX-$PEEP_{34}$, PTX-$PEEP_{34}$-FA, and $PEEP_{45}$.

DLS measurement

In order to achieve lower level of reticuloendothelial system (RES) uptake, minimal renal excretion, and realize effective EPR effect for passive tumor-targeting, keeping a small size (< 200 nm) of micelles is significant for nanotherapeutic delivery system.[2-4] The size and size distribution of different PTX-PEEP-FA micelles were measured by DLS and the results are shown in Table S1. All the micelles assembled from polymeric drugs with different PEEP chain length possess the average diameters of less than 150 nm and size PDI less than 0.26 in aqueous solution. In addition, the average diameters of micelles from TEM images are less than those obtained from DLS analysis. This is because TEM images were recorded under dry condition where those hydrophilic segments were easy to shrink, while they were fully stretched in aqueous solution for DLS measurement.

Table S1 Particle sizes ($\overline{D}_z$) and size polydisperisity indices (size PDIs) of micelles self-assemble from various PTX-PEEP-FA prodrugs.

Samples	$\overline{D}_z$ (nm)	size PDI
PTX-PEEP$_{18}$-FA	132	0.26
PTX-PEEP$_{24}$-FA	130	0.23
PTX-PEEP$_{25}$-FA	132	0.21
PTX-PEEP$_{34}$-FA	143	0.26

Drug encapsulation experiment

Table S2 shows the DLC and DLE with different feed ratio of PTX. When the feed ratio of PTX was increased from 5.15% to 19.96%, the DLC was increased from 1.05% to 3.48%, while the DLE didn't show apparent increase. That's because there is only one PTX molecule as the hydrophobic moiety, which is weak to load much more drugs.

Table S2. PTX loading experiment of PTX-PEEP$_{40}$ with different feed ratios.

Entry	PTX/polymer (%)	DLC (%)	DLE (%)
1	5.15	1.05	20.16
2	15.05	3.27	21.00
3	19.96	3.48	16.84

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