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Supporting Information

Bortezomib-Catechol Conjugated Prodrug micelles: Combining Bone Targeting and Aryl Boronate-based pH-Responsive Drug Release for Cancer Bone-metastasis Therapy

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S1 Characterization.

^{1}H NMR spectra were performed on a Bruker Avance 500 spectrometer operating at 500 MHz by using D_2O or DMSO-d_6 as the solvent depending on polymer solubility. The chemical shifts were calibrated against solvent signals. The morphology of nanoparticles was observed using transmission electronic microscopy (TEM, Hitachi, Japan). The particle size and zeta potential were investigated using dynamic light scattering (DLS, Zs90, Malvern, U.K.).

S2 Synthesis of PEG-*b*-P(LL-*g*-Cat-BTZ) conjugate.

Poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-*b*-PLL) was synthesized as our previous work[1]. The synthetic route of PEG-*b*-P(LL-*g*-Cat-BTZ) conjugate are divided in two steps and shown in Fig. S1A. Briefly, Cat (25.2 mg, 0.15 mM), EDC (86.4 mg, 0.45 mM) and NHS (52.0 mg, 0.45 mM) were dissolved in 15 mL anhydrous N,N-dimethylformamide(DMF) and stirred for 2 h to active carboxyl groups. PEG-*b*-PLL (700 mg, 0.1 mM) and 20 μL TEA were dissolved in 50 mL anhydrous DMF, and slowly added dropwise into the above reaction mixture. The reaction mixture was stirred at room temperature for 24 h. Subsequently, the reaction mixture was purified by dialyzed (MWCO: 3500 Da) against water for 48 h. The product was obtained by Lyophilization (yield 85.8%).

Then, PEG-*b*-P(LL-g-Cat) (500 mg, 0.06 mM) and BTZ (38.4 mg, 0.1 mM) were dissolved in 60 mL DMF, followed with adjusting pH value to 8-9. Next, the mixture was stirred for 12 h at room temperature. Finally, the reaction mixture was purified

by dialyzed against water (pH 8-9) for 24 h. The product was obtained by Lyophilization.

The amount of conjugated BTZ was quantified by HPLC (Shimadzu, Germany) at 268 nm after completely acidified and calculated by using the following formula:

$$BTZ\ content\ \% = \frac{weight\ of\ conjugated\ BTZ}{weight\ of\ coplymer} \times 100\%$$

S3 Synthesis of ALN-PEG-*b*-PLLZ.

The synthetic route of ALN-PEG-*b*-PLLZ is shown in Fig. S1B. Firstly, ALN was reacted with HOOC-PEG-NH_2 to obtain the ALN-PEG-NH_2. In brief, HOOC-PEG-NH_2 (500 mg, 0.1 mM), EDC (57.3 mg, 0.3 mM) and NHS (34.5 mg, 0.3 mM) were dissolved in 30 mL distilled water, and stirred for 4 h at room temperature for active carboxyl groups. Then, ALN (55.7 mg, 0.2 mM) and TEA (30 µL) were dissolved in 10 mL distilled water, and slowly added into the above reaction mixture under vigorous stirring. At the same time, the pH of the reaction solution was adjusted to 7-8 using 1 M NaOH[2].

ALN-PEG-*b*-PLLZ was synthesized by the ring-opening polymerization of Lys(Z)-NCA using ALN-PEG-NH_2 as a macrocinitiator[1]. ALN-PEG-NH_2 (1.51 g, 0.30 mM) was dissolved in 40 mL of dry DMF. Then, Lys(Z)-NCA (1.84 g, 6 mM) was dissolved in 50 mL of dry DMF and added to the ALN-PEG-NH_2 solution under a dry argon atmosphere. The reaction mixture was stirred for 48 h at 40 °C under a dry argon atmosphere and then the solvent was evaporated under reduced pressure. The product was precipitated into excess ice-cold diethyl ether to obtain ALN-PEG-*b*-PLLZ (yield 89.2%).

As a control, the PEG-*b*-PLLZ was synthesized by the same method except that HOOC-PEG-NH_2 was set as macroinitiator instead of ALN-PEG-NH_2.

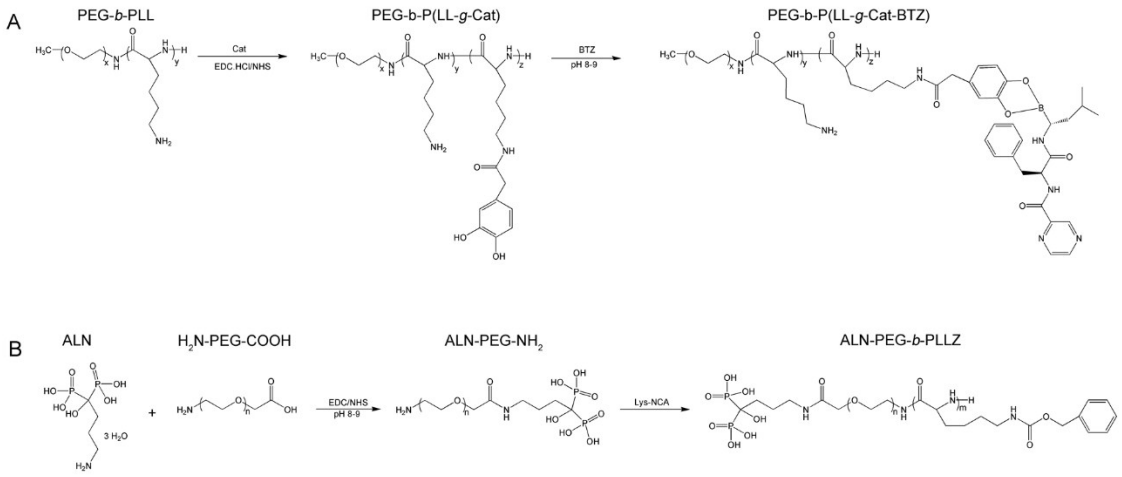

Fig. S1 Synthesis route of PEG-*b*-P(LL-*g*-Cat-BTZ) (A) and ALN-PEG-*b*-PLLZ (B).

S4 Characterizations of PEG-*b*-P(LL-*g*-Cat-BTZ) and ALN-PEG-*b*-PLL.

The PEG-*b*-PLL was synthesized by ring-opening polymerization and the protocols are as same as which are described in our previous work[1]. The chemical structures of PEG-*b*-P(LL-*g*-Cat-BTZ) were confirmed by ^{1}H NMR. As is shown in Fig S2, the peaks at 3.65 ppm (**2**) indicated the presence of PEG, the peaks at 6.8-7.2 ppm (**4**) were assigned to the protons in benzene of Cat; the characteristic chemical shifts at 7.3 ppm (**6**) assigned to the protons on benzene of BTZ, and at 8.6-9.1 ppm (**5**) attributed to the protons of pyrazine on BTZ. These results indicated the successful conjugation of BTZ with PEG-*b*-PLL linked by Cat. Besides, the chemical structures of ALN-PEG-*b*-PLLZ were also measured by ^{1}H NMR, and the results were shown in Fig. S3. The peak at 1.8-2.0 ppm (**1, 2**) attributed to the protons of ALN [2], the peak at 3.64 ppm (**5**) in presented the methylene of PEG; the peak at 1.2-1.6 ppm (**6**) showed the presence of lysine, and the peaks at 5.0 ppm and 7.2-7.4 ppm (**7**) indicated benzyloxycarbonyl [3].

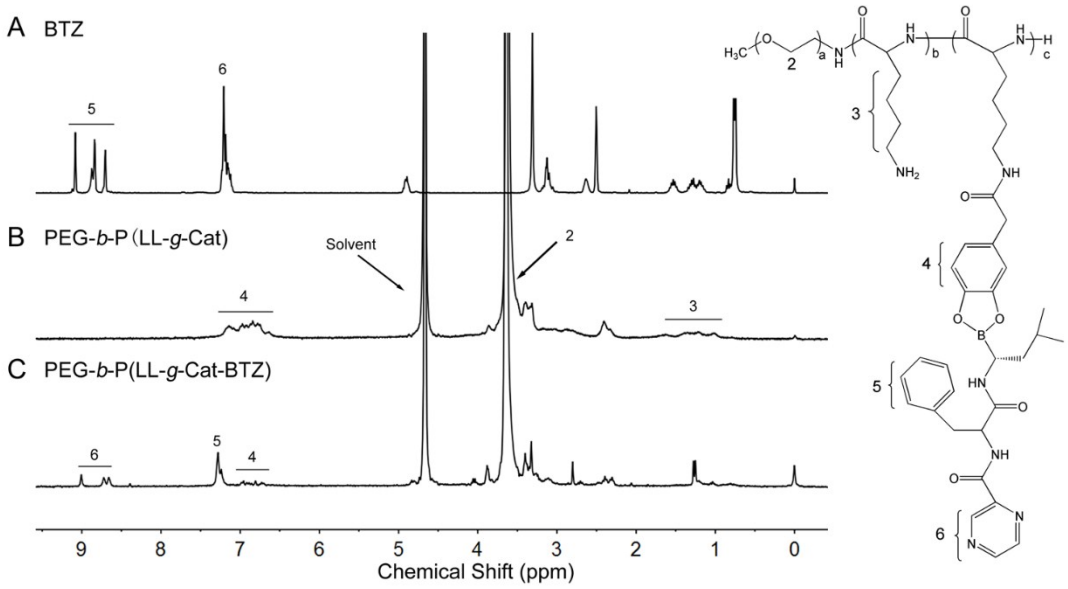

Fig. S2 The H^1 NMR of (a) BTZ, (b) PEG-*b*-P(LL-*g*-Cat) and (c) PEG-*b*-P(LL-*g*-Cat-BTZ) in DMSO-d_6.

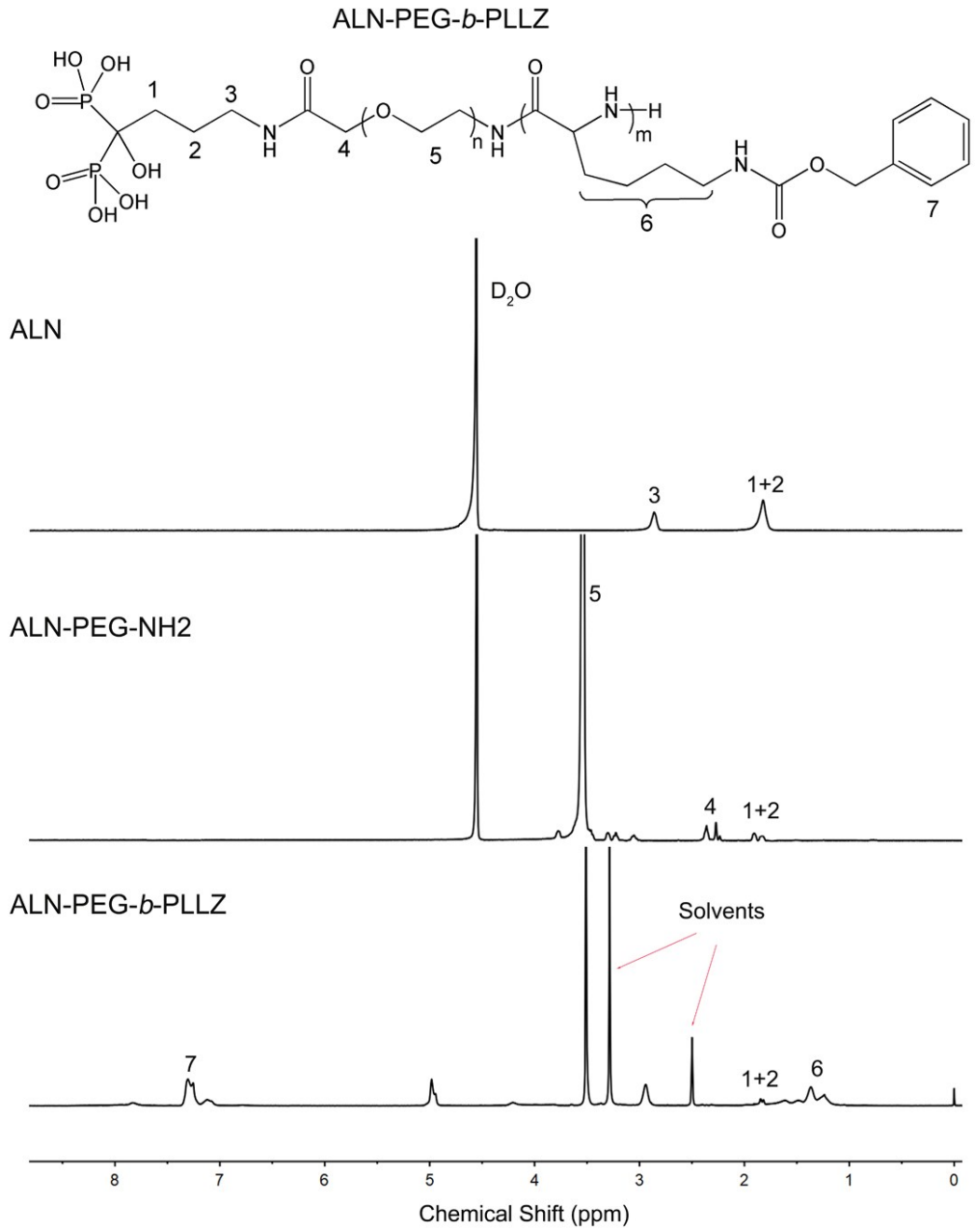

Fig. S3 The H^1 NMR of ALN and ALN-PEG-NH_2 in D_2O, and ALN-PEG-*b*-PLLZ in DMSO-d_6.

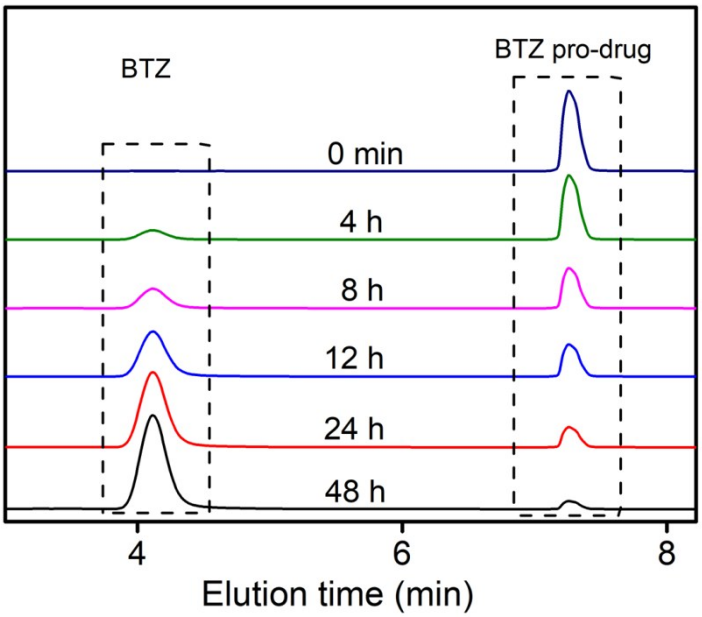

Fig. S4 The degradation of PEG-b-P(LL-g-Cat-BTZ) triggered by acid, and monitored by HPLC.

References

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