Electronic Supplementary Information (ESI)

Boronate-Crosslinked Polysaccharide Conjugates for pH-Responsive and Targeted Drug Delivery

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Experimental Section

Materials. All chemicals were reagent grade unless noted otherwise. Sodium hyaluronate (M_w = 550 kDa), bortezomib, and fluorescein isothiocyanate isomer I (FITC) were purchased from commercial sources. Hyaluronic acid was prepared by treating sodium hyaluronate with cation exchange resin. *N*-(2-aminoethyl)-gluconamide[1] was synthesized according to the reported methods. NIH3T3 mouse embryonic fibroblast cell line, PC-3 human prostatic cancer cell line and MCF-7 human breast cancer cell line were obtained from China Infrastructure of Cell Line Resource.

Instruments. NMR spectrum were recorded on Bruker AV400 spectrometer at 25 °C. UV-Vis spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) on a Thermo Scientific EVOLUTION 300 spectrophotometer equipped with a HAAKE SC 100 temperature controller to keep the temperature at 25 °C. High-resolution transmission electron microscope (HR-TEM) images were obtained on a Tecnai G^2 F20 microscope (FEI) with an accelerating voltage of 200 keV, and the sample was prepared by dropping 4 μL sample solution on a carbon-coated copper grid and air-dried. SEM experiments were performed on a Shimadzu SS-550 scanning electron microscope at an accelerating voltage of 30 keV. DLS experiments were recorded on a laser light scattering spectrometer (Brookhaven company) at λ = 648 nm with a scattering angle of 90 °C. The zeta potential were examined on NanoBrook 173Plus at 25 °C. The fluorescent confocal images were recorded on a Leica TCS SP8 fluorescence microscope (λ_{ex} = 405 nm).

Synthesis of *N*-(2-aminoethyl)-gluconamide grafted hyaluronic acid（HAGlu）.

HA (500 mg, 1.32 mmol) was added to 50 mL DMSO, and the solution was heated at 60 °C for 4 h, then the solution was cooled to room temperature after HA was dissolved. Triethylamine (0.92 mL, 6.6 mmol) was added, the mixture was stirred for 10 min at room temperature. Then ethyl chloroformate (0.377 mL, 3.96 mmol) was added, the mixture was further stirred for another 1 h. Subsequently, *N*-(2-aminoethyl)-gluconamide (157 mg, 0.66 mmol) was added, and the mixture was continued to stir for 24 h at room temperature. The resulting solution was dialyzed against excess amount of deionized water for 5 days. After being freeze-dried, HAGlu was obtained as white powder. ^{1}H NMR (400 MHz, D_2O, ppm): δ 2.0 (s, 3H, H of methyl group of HA), 3.16-3.21 (q, 0.67H, H of methylene on ethanediamine of *N*-(2-aminoethyl)-gluconamide), 3.35-4.13 (m, 12.96H, H of HA and gluconamide), 4.45-4.54 (m, 2H, H of HA). According to the single-point method from integrated peak area of ethanediamine group and HA backbone in NMR spectrum, the degree of substitution (DS) was determine as 16.8%, indicating that *N*-(2-aminoethyl)-gluconamide was grafted every 6 repeating sugar units on average.

Synthesis of *N*-(2-aminoethyl)-gluconamide grafted hyaluronic acid/FITC（FITC@HAGlu）. The HAGlu (40 mg, 0.094 mmol) and triethylamine (21.84 μL, 0.156 mmol) were added into 10 mL DMSO, the mixture was stirred for 10 min at room temperature. Then ethyl chloroformate (8.94 μL, 0.094 mmol) was added, the mixture was further stirred for 1 h. Finally, ethylenediamino-modified FITC (7.04 mg, 0.156 mmol) was added, and the mixture was further stirred for 24 h at room temperature. The resulting solution was dialyzed against excess amount of deionized

water for 5 days. After being freeze-dried, FITC@HAGlu was obtained as pale yellow powder. The content of FITC in the HAGlu chain was calculated as 0.2% using a Uv-Vis calibration curve of ethylenediamino-modified FITC at 485 nm.

Preparation of HAGlu-BTZ conjugates. HAGlu (25.3 mg) was dissolved in 9.6 mL deionized water, then the solution of BTZ (3.84 mg) in 400 μL DMSO was slowly added, and the mixture was stirred 24 h at room temperature. The resulting solution was dialyzed against excess amount of deionized water for 2 h to remove unbounded BTZ and freeze-dried. The drug loading efficiency and encapsulation efficiency of BTZ on HAGlu were estimated by the follow equations:

$$\text{Drug loading efficiency (\%)} = 100\ m_{\text{BTZ in HAGlu-BTZ}}/m_{\text{HAGlu-BTZ}}$$

$$\text{Drug encapsulation efficiency (\%)} = 100\ m_{\text{BTZ in HAGlu-BTZ}}/m_{\text{total BTZ}}$$

pH-sensitivity of HAGlu-BTZ conjugates. The pH responsive of HAGlu-BTZ was analyzed by ^{1}H NMR spectrum. *N*-(2-aminoethyl)-gluconamide was employed as a reference molecular to evaluate the pH responsiveness of Glu with BTZ. BTZ (3.07 mg, 8 mmol) in DMSO-d_6 (200 μL) was added to a solution Glu (1.91 mg, 8 mmol) in a solution of 0.01 M monosodium phosphate in D_2O (3.8 mL), and the pH of the solution was adjusted to 5.7, 6.5, 7.2 and 8.5. Then the solutions were measured with Bruker AV400 spectrometer at 25 °C.

pH-triggered BTZ release *in vitro*. The release of BTZ from HAGlu-BTZ *in vitro* was investigated using dialysis method in PBS buffer. The solution of HAGlu-BTZ (2.5 mL, [BTZ] = 0.1 mM) was placed into a dialysis membrane with a molecular weight

cut off of 8-14 kDa and dialyzed against 40 mL PBS buffer (0.01 M, 37 °C) with pH at 7.2, 6.5 and 5.7. 2.5 mL dialysate was collected at certain time intervals and an equal volume of fresh PBS buffer was added. The released drug was analyzed by the absorbance of BTZ at 270 nm.

Cytotoxicity experiments. NIH3T3 mouse embryonic fibroblast cell line was cultured in Dulbecco's modified Eagle's medium (DMEM), PC-3 human prostatic cancer cell line was cultured in RPMI-1640 medium, both of the medium were supplemented with 10% fetal bovine serum (FBS). NIH3T3 cells and PC-3 cells were seeded in 96-well plates (5×10^4 cells/mL, 100 μL per well) for 24 h, then the cells were incubated with HAGlu, BTZ, HAGlu-BTZ and HAGlu-BTZ + excess HA ([BTZ] = 1.0 μM, [HAGlu] = 27.8 μM) for 24 h, respectively. The relative cellular viability was determined by MTT assay. All data were presented as the mean ± standard deviation.

Fluorescent confocal imaging. NIH3T3 cells, PC-3 cells and MCF-7 cells were cultured in 6-well plates (5×10^4 cells mL^{-1}, 2 mL per well) for 24 h at 37 °C in 5% CO_2. The cells incubated with FITC@HAGlu-BTZ for 6 h, and then washed with PBS buffer for three times. After that the cells were fixed with 4% paraformaldehyde for 15 min. Then the cell nuclei were stained with DAPI (1 μg/mL) for 5 min and observed by a confocal laser scanning microscope (λ_{ex} = 405 nm for DAPI and 488 nm for FITC).

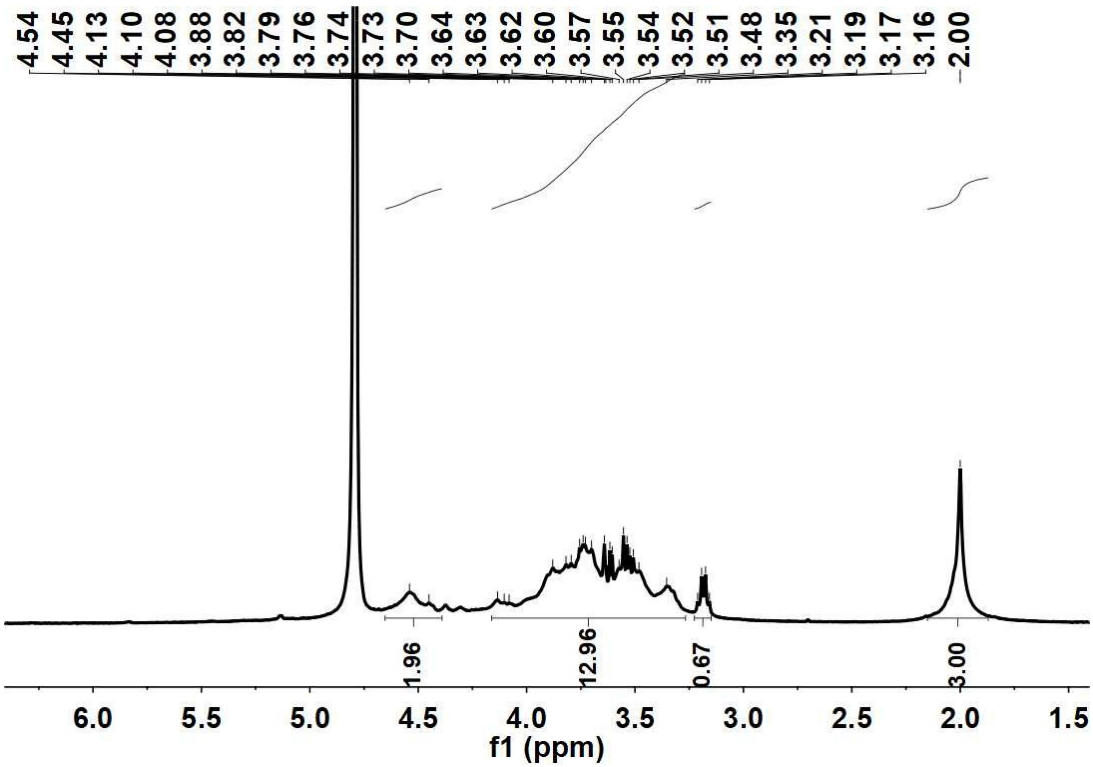

Fig. S1 ^{1}H NMR spectrum of HAGlu in D_2O at 25 °C.

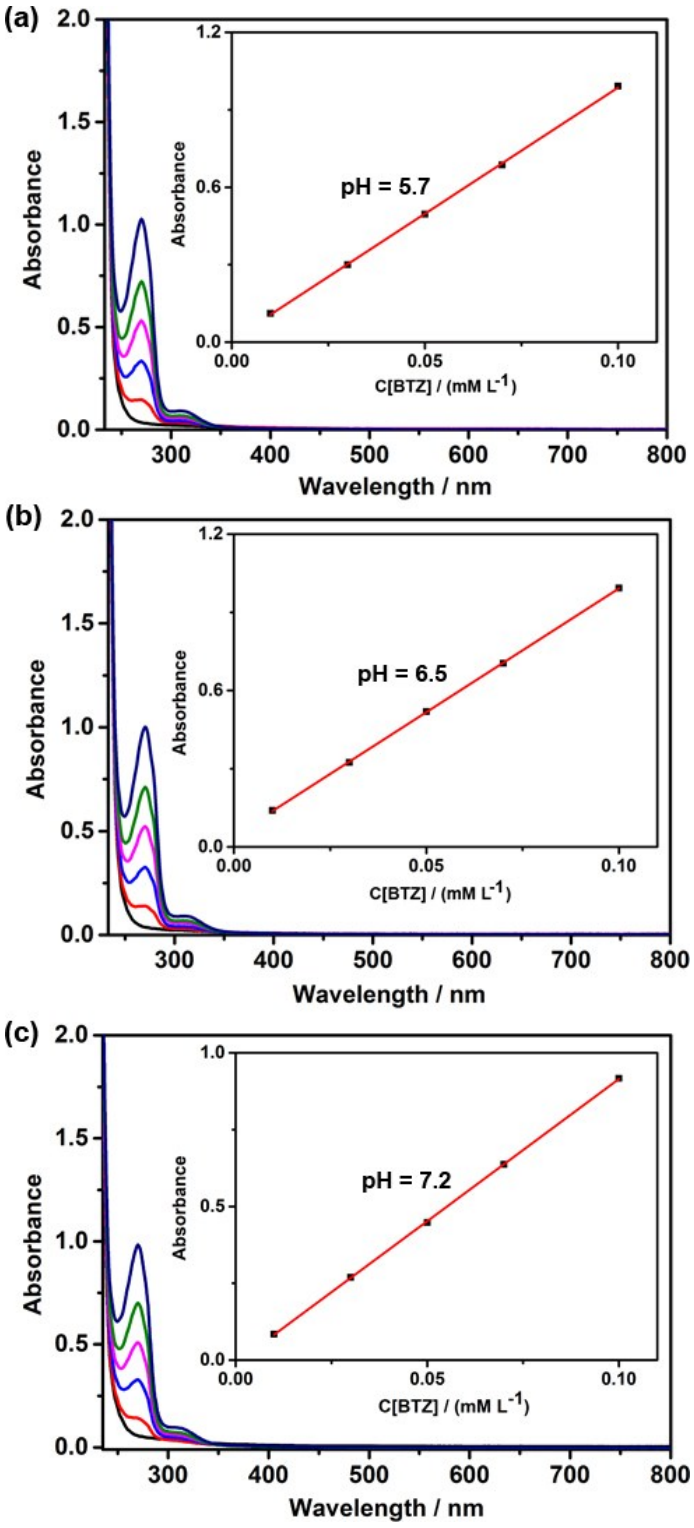

Fig. S2 Uv-Vis spectra of HAGlu-BTZ in PBS (I = 0.01 M) at pH (a) 5.7, (b) 6.5, (c) 7.2. Inset: Standard curve of HAGlu-BTZ at different pH with absorption at λ = 270 nm vs concentration.

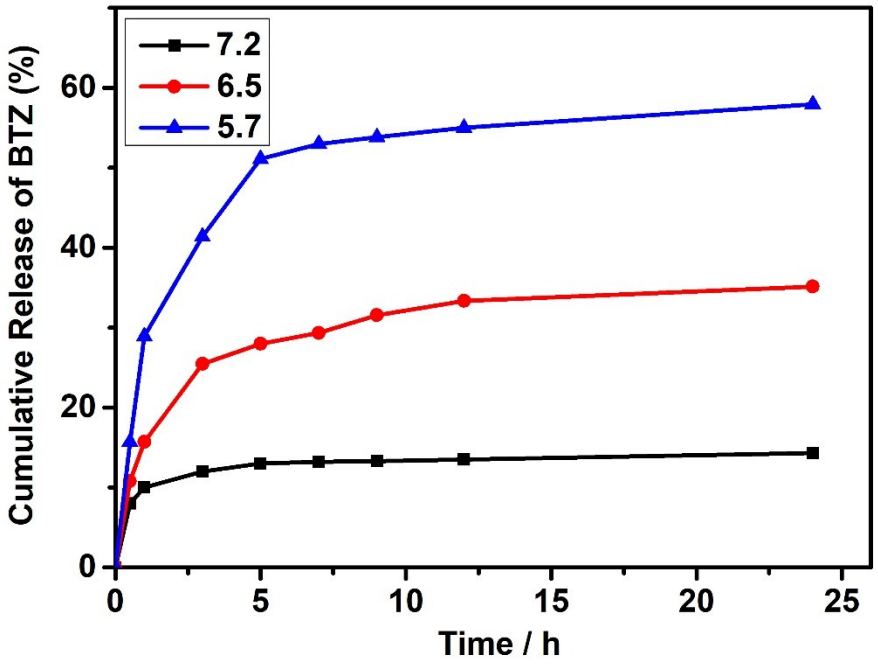

Fig. S3 The cumulative release profile of BTZ from polysaccharide conjugates at pH 5.7, 6.5, and 7.2 in PBS (I = 0.01 M) *in vitro* at 37 °C.

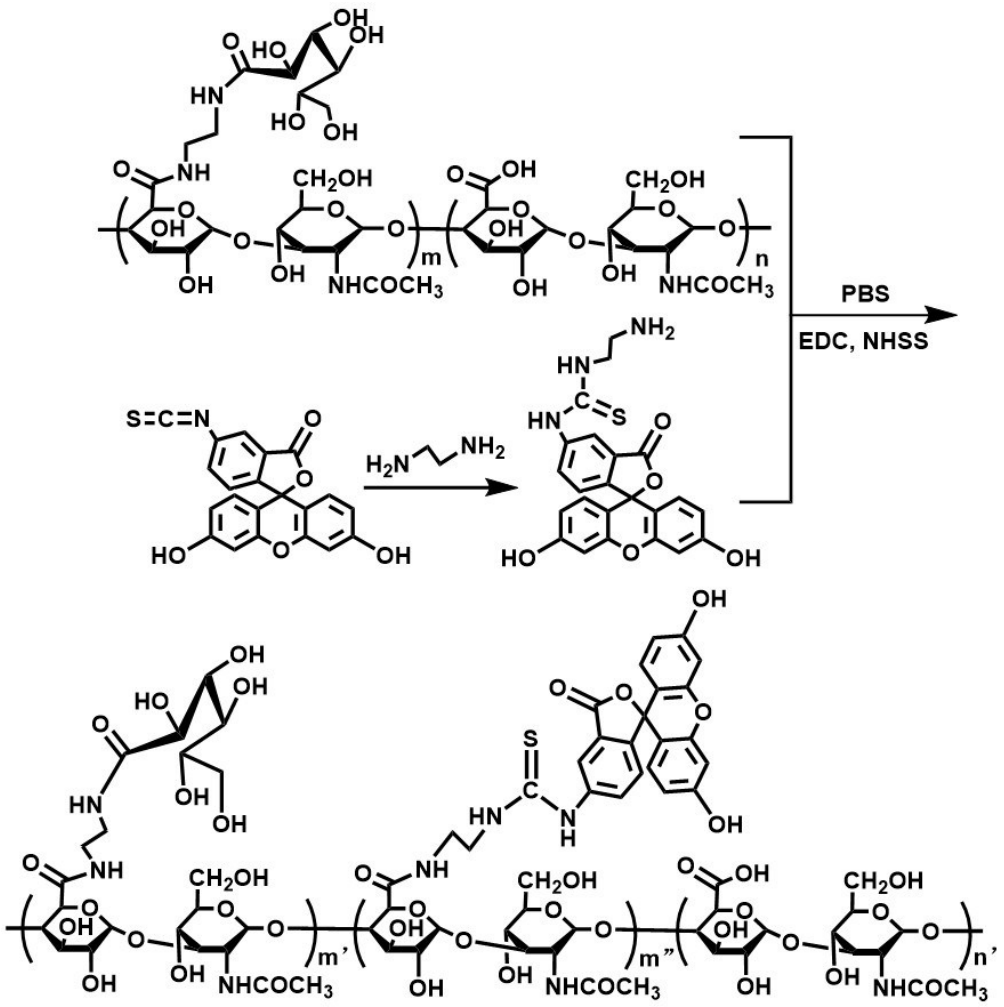

Fig. S4 Synthetic route of FITC@HAGlu.

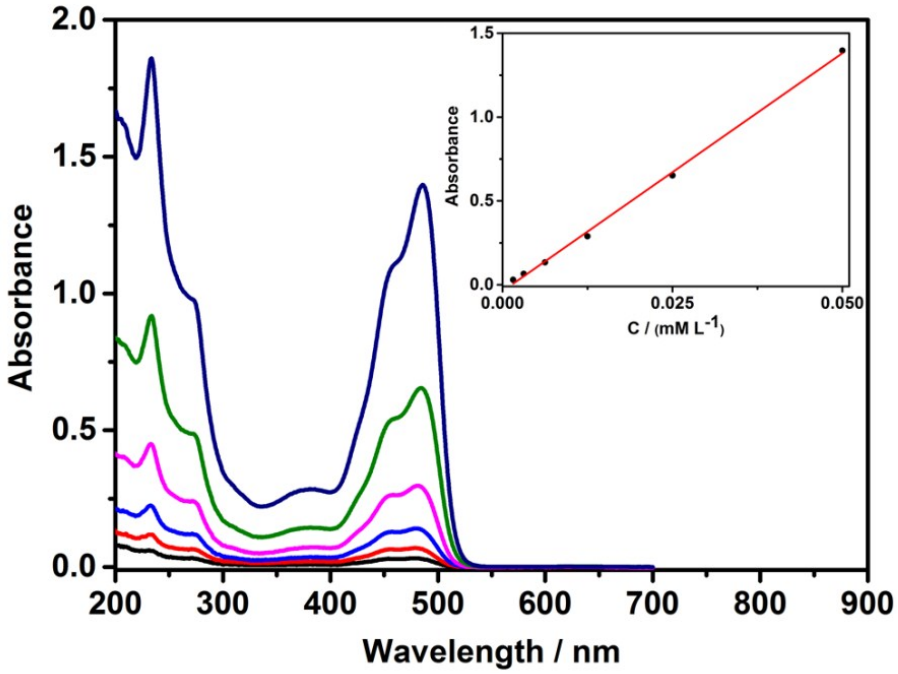

Fig. S5 Uv-Vis spectra of ethylenediamino-modified FITC in water. Inset: Standard curve of ethylenediamino-modified FITC with absorption at λ = 485 nm vs concentration.

References

1 R. G. Nuzzo, S. L.H aynie, M. E. Wilson, G. M. Whitesides, *J. Org. Chem.* 1981, **46**, 2861.