Supporting Information

Supramolecular Polymersomes Constucted from Water-soluble Pillar[5]arene and Cationic Poly(glutamamide)s and Their Applications for Targeted Anticancer Drug Delivery

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Table of Contents

1. General information S2

2. The synthesis of WP5, Polymer 1, Polymer 3, and G_M S2

3. Job Plot for WP5⊃G_M S15

4. Determination of the association constant for WP5⊃G_M S15

5. Self-assembly of polymer 3 and polymer 1 in the presence of WP5 ..... S16

6. Drug loading and release behavior of polymersomes fabricated by WP5 and polymer 3 ..... S20

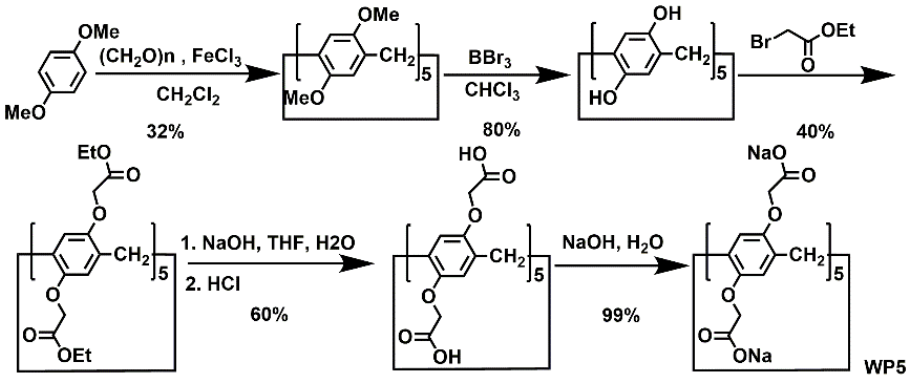
7. References S22

1. *General information*

All reactions were performed in air atmosphere unless otherwise stated. The commercially available reagents and solvents were either employed as purchased or dried according to procedures described in the literature. Column chromatography was performed with silica gel (200 – 300 mesh) produced by Qingdao Marine Chemical Factory, Qingdao (China). All yields were given as isolated yields. NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer (or Bruker DPX 400 MHz spectrometer) with internal standard tetramethylsilane (TMS) and solvent signals as internal references at room temperature, and the chemical shifts (δ) were expressed in ppm and J values were given in Hz. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on an Agilent 6540Q-TOF LCMS equipped with an electrospray ionization (ESI) probe operating in the positive-ion mode with direct infusion. Transmission electron microscope (TEM) investigations were carried out on a JEM-2100 instrument. Dynamic light scattering (DLS) measurements were carried out on a Brookhaven BI-9000AT system (Brookhaven Instruments Corporation, USA), using a 200-mW polarized laser source (λ = 514 nm). The UV-Vis absorption spectra were measured on a Perkin Elmer Lambda 35 UV-Vis Spectrometer. Gel permeation chromatograph (GPC) analysis was performed on a PL-GPC 50 integrated GPC equipped with refractive index detector at 25 °C. The column used in the GPC analysis was PL aquagel−OH 30 (300 mm × 7.5 mm, 8 μm) with DMF as the eluent.

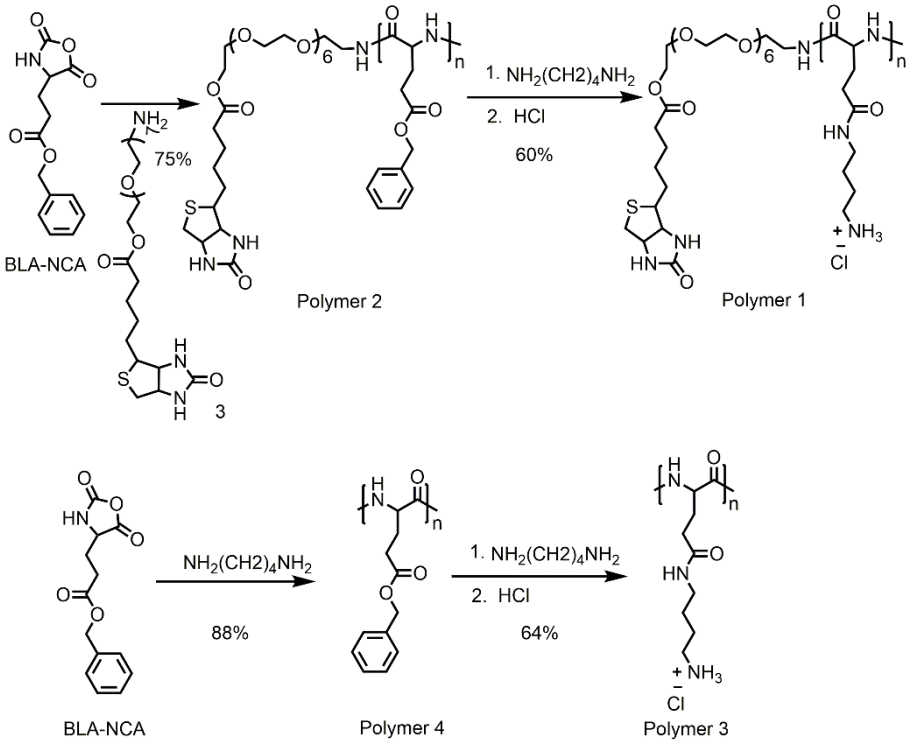
2. *The synthesis of WP5, Polymer 1, Polymer 3, and G_M*

1) **WP5** was synthesized and purified according to previously reported procedures (Scheme S1).[S1]



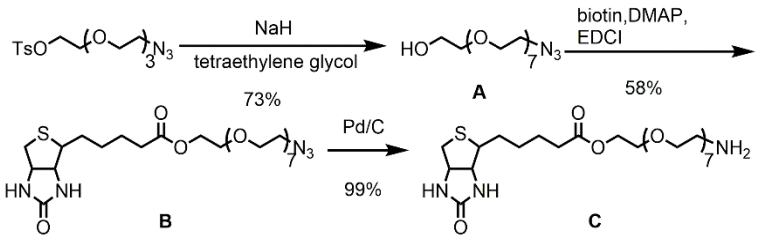
Scheme S1. The synthesis route of **WP5**.

2) Synthesis of **Polymer 1** and **Polymer 3**



Scheme S2. The synthesis route of **polymer 1** and **polymer 3**.

Synthesis of the initiating agent (compound **C**):



Scheme S3. The synthesis route of compound **C**.

Compound A:[S2] Tetraethylene glycol **1** (6.98 g, 3.6 mmol) dissolved in anhydrous THF (20 mL) was added dropwise to a suspension of 60% sodium hydride (1.6 g, 40 mmol) in anhydrous THF (90 mL) at 0 ℃ under nitrogen. The reaction was stirred at 0 ℃ for 1 h and continued at 25 ℃ for another 2 h. 2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (6.7 g, 1.8 mmol) dissolved in THF (20 mL) was added dropwise to the refluxing solution of sodium alcoholate. Then, the mixture was refluxed for another 10 h. After cooling to room temperature, the mixture was quenched with H_2O carefully. After removing the solvent in *vacuo*, the residue was extracted with EtOAc, washed with H_2O and saturated sodium chloride solution successively. The combined organic phase was concentrated in *vacuo* and then subjected to column chromatography using CH_2Cl_2/MeOH (40/1, *v*/*v*) as eluent to afford compound **A** (5.2 g, 1.3 mmol, 73%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm) = 3.72−3.59 (m, 30H), 3.37 (t, J = 4.2 Hz, 2H).

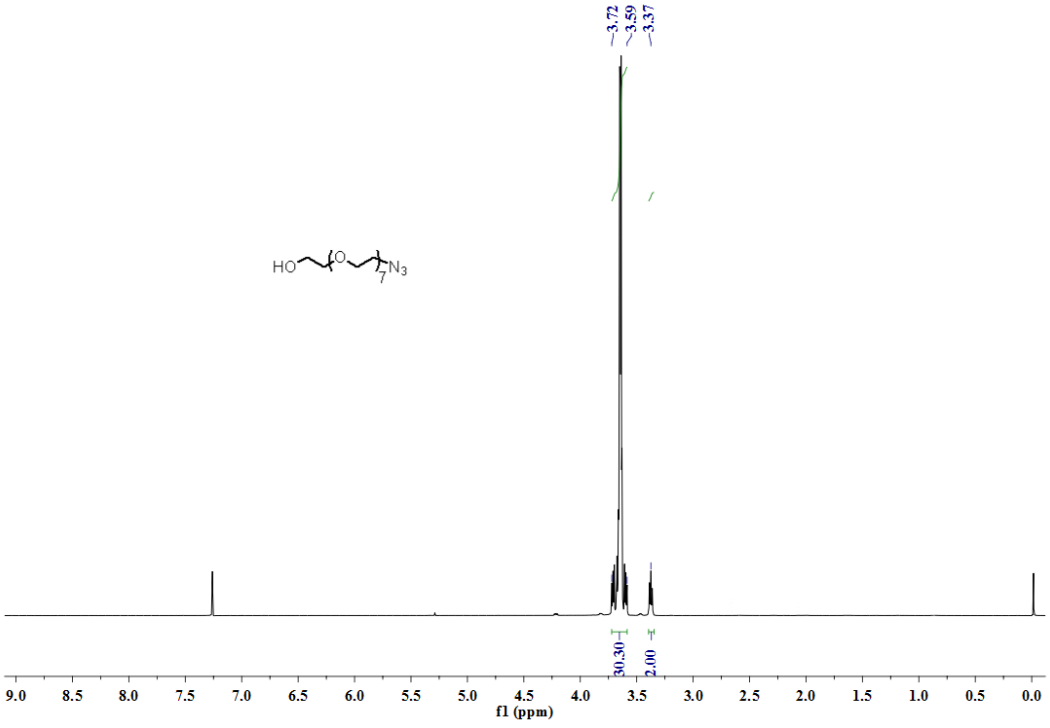


Figure S1. ^{1}H NMR spectrum of compound **A** (400 MHz, $CDCl_3$, 298 K).

Compound B： D-Biotin (0.29 g, 1.2 mmol) was mixed with DMF (4 mL) and stirred at 70 ℃ for 15 min. Then, a mixture of compound **A** (0.40 g, 1.0 mmol), EDCI

(0.23 g, 1.2 mmol), and DMAP (0.24 g, 0.2 mmol) in chloroform (10 mL) was added dropwise. The obtained solution was stirred at 70 ℃ for 24 h. After cooling to room temperature, the solvent was evaporated under vacuum and the crude product was purified by silica gel chromatography using methanol/CH_2Cl_2 (1/10, *v*/*v*) as eluent to afford compound **B** (0.36 g, 0.58 mmol, 58%) as a light yellow oil. ^{1}H NMR (400 MHz, CD_3OD, 298 K) δ (ppm) = 4.48 (dd, J_1 = 12.0 Hz, J_2 = 8.0 Hz, 1H), 4.33 (dd, J_1 = 12.0 Hz, J_2 = 8.0 Hz, 1H), 4.21−4.23 (m, 2H), 3.71−3.69 (m, 2H), 3.68−2.64 (m, 26H), 3.38 (t, J = 4.0 Hz, 2H), 3.24−3.19 (m, 1H), 2.93 (dd, J_1 = 14.0 Hz, J_2 = 8.0 Hz, 1H), 2.71 (d, J = 14.0 Hz, 1H), 2.38 (t, J = 8.0 Hz, 2H), 1.79−1.55 (m, 4H), 1.50−1.44 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz, 298 K) δ (ppm) = 173.7, 164.0, 70.6, 70.5, 70.4, 69.9, 69.1, 63.4, 61.9, 60.1, 55.6, 50.6, 40.5, 33.7, 28.3, 28.1, 24.7. HR-ESI-MS: m/z Calcd for $C_{26}H_{47}O_{10}N_5SNa$ $[M + Na]^+$ 644.2941, found 644.2945.

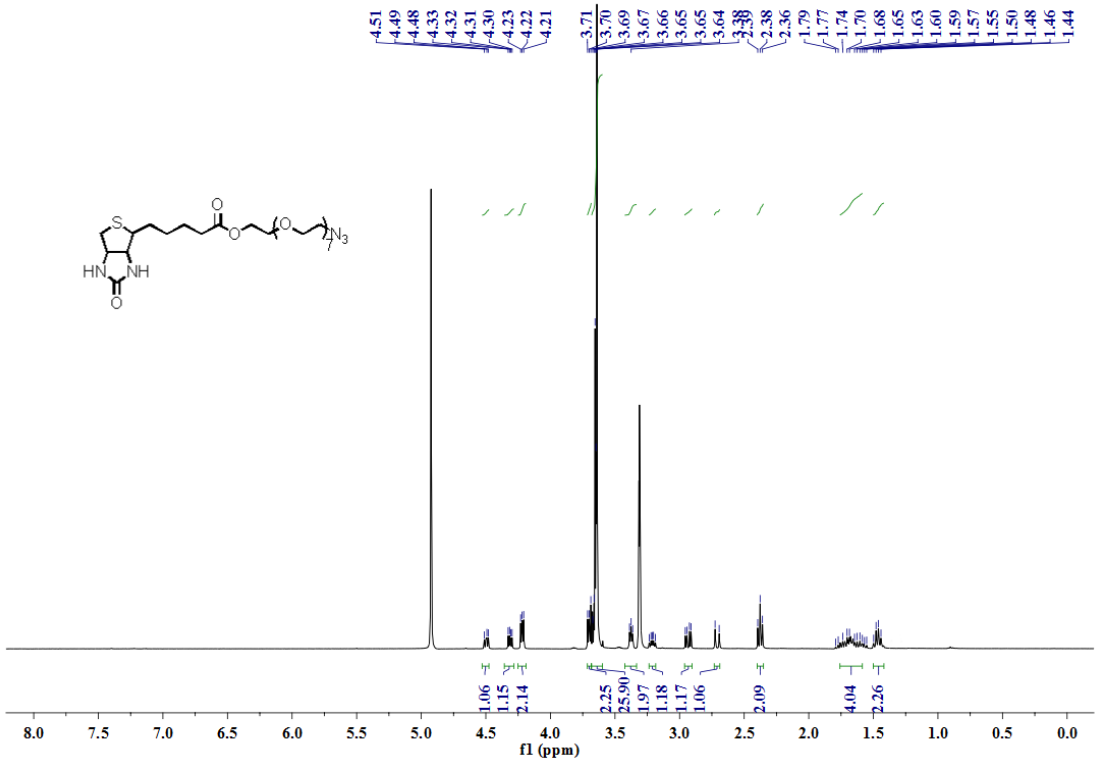


Figure S2. ^{1}H NMR spectrum of compound **B** (400 MHz, CD_3OD, 298 K).

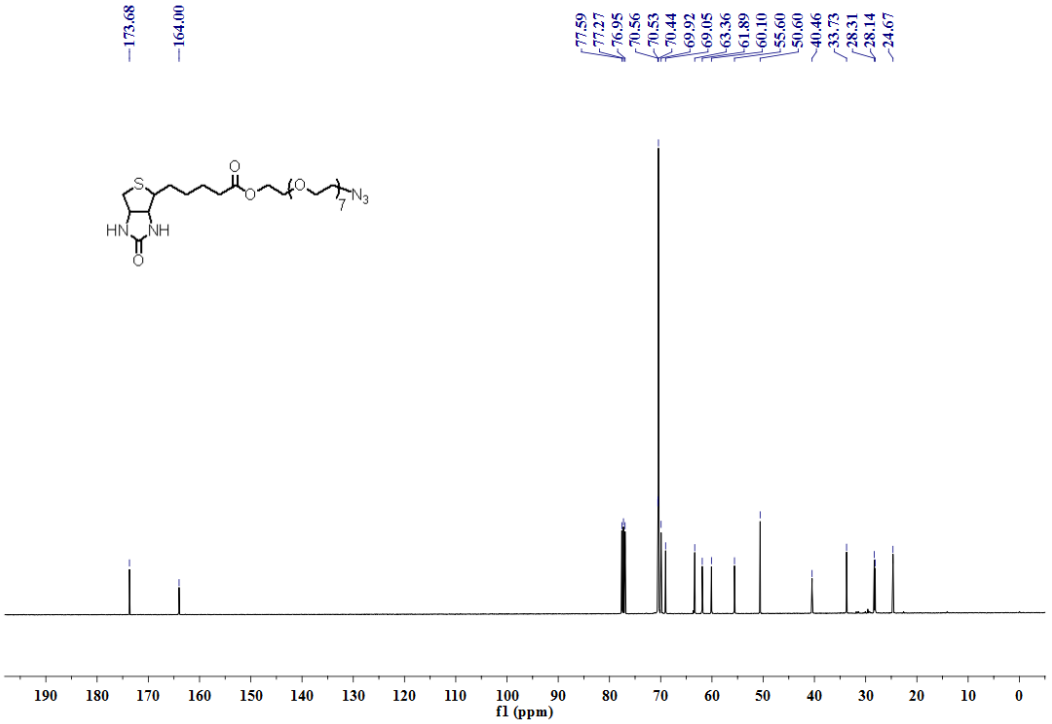


Figure S3. ^{13}C NMR spectrum of compound **B** (100 MHz, $CDCl_3$, 298 K).

Compound C： Compound **B** (0.31 g, 0.5 mmol) was dissolved in methanol (10 mL) and charged with 10% Pd/C (50 mg) and H_2 gas. After stirring at 25 ℃ for 2 h, the metal catalyst was removed by filtration through Celite pad, and rinsed with MeOH. The filtrate was condensed under reduced pressure and dried *in vacuo*. The resulting product (0.30 g, quantitative yield) was used for further reactions without purification. ^{1}H NMR (400 MHz, CD_3OD, 298 K) δ (ppm) = 4.51−4.48 (m, 1H), 4.33−4.30 (m, 1H), 4.23−4.21 (m, 2H), 3.72−3.56 (br, 28H), 3.24−3.18 (m, 1H), 2.92−2.85 (m, 3H), 2.70 (d, *J* = 14.0 Hz, 1H), 2.40−2.33 (m, 2H), 1.77−1.57 (m, 4H), 1.50−1.43 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz, 298 K) δ (ppm) = 173.6, 164.0, 70.41, 70.38, 70.3, 70.1, 69.0, 63.3, 61.9, 60.1, 55.55, 55.51, 51.46, 40.5, 33.7, 33.6, 28.33, 28.29, 28.15, 24.7, 24.6. HR-ESI-MS: m/z Calcd for $C_{26}H_{49}O_{10}N_3SNa$ $[M + Na]^+$ 618.3036, found 618.3040.

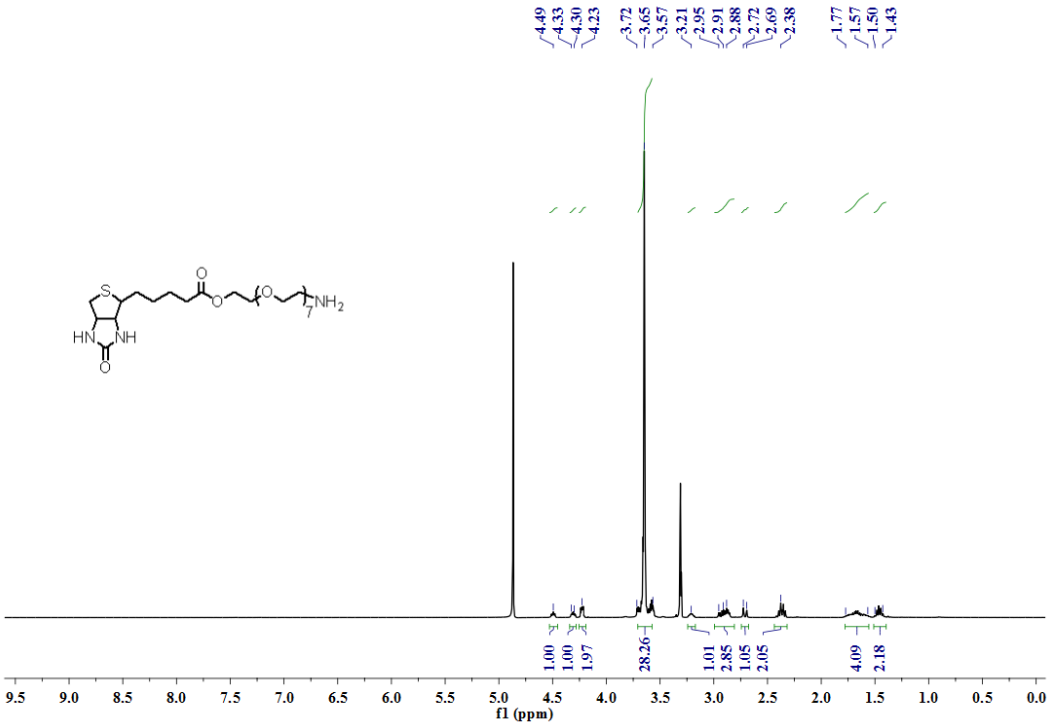


Figure S4. ^{1}H NMR spectrum of compound **C** (400 MHz, CD_3OD, 298 K).

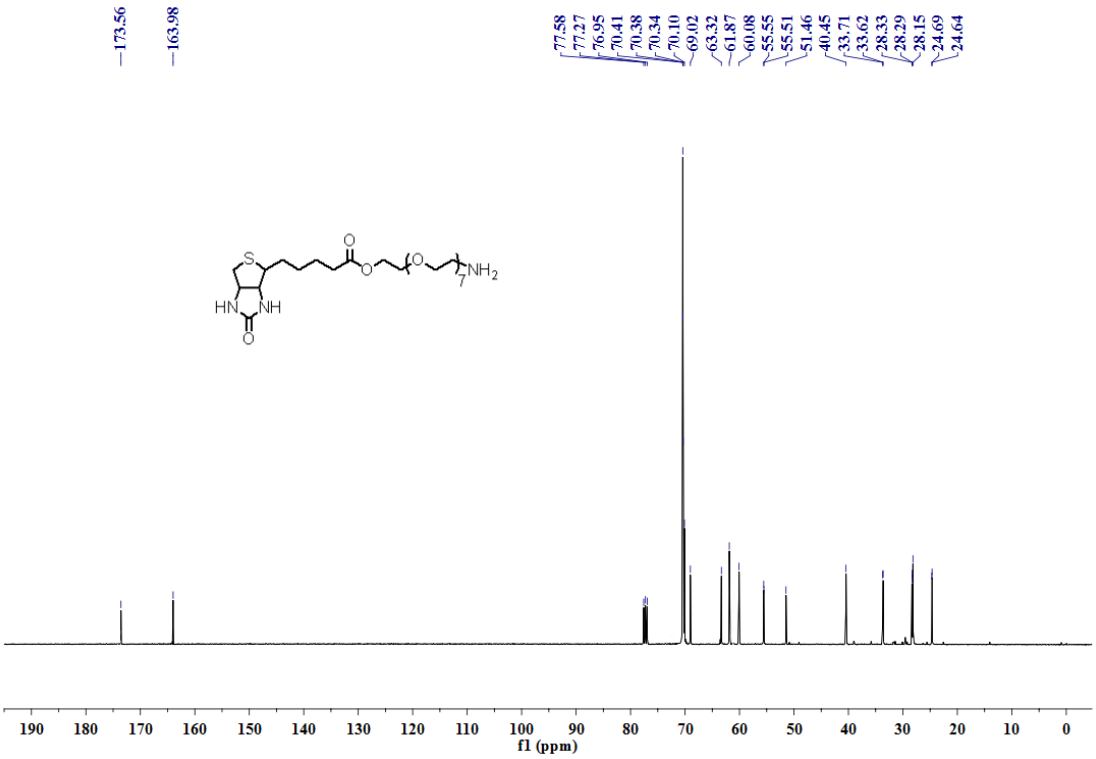


Figure S5. ^{13}C NMR spectrum of compound **C** (100 MHz, $CDCl_3$, 298 K).

Synthesis of **Polymer 1** and **Polymer 3**

Polymer 2: BLA-NCA (1.0 g, 3.8 mmol) was dissolved in 10 mL of CH_2Cl_2/DMF solution (10/1, *v*/*v*) under a nitrogen atmosphere. Then, compound **C** (0.038 g, 63 μmol) in 1 mL of CH_2Cl_2/DMF solution (10/1, *v*/*v*) was added dropwise. The obtained solution was stirred at 40 ℃ for 3 days. After that, the reaction solution was pulled into n-hexane. A white precipitate was obtained by centrifugation. The polymer was further purified by the two dissolution-precipitation cycles. The final precipitate was dried under vacuum and polymer **2** was obtained as white solid (0.75 g, 75%). The M_n, n and M_w/M_n were determined to be 37408, 170 and 1.25, respectively by GPC measurement. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm) = 8.85−8.01 (br, 1H, H_f), 7.51−7.09 (5H, H_a), 5.22−4.92 (2H, H_b), 4.10−3.80 (1H, H_e), 3.59−3.47 (0.17H, H_g), 2.47 (2H, H_c), 2.37−1.96 (2H, H_d).

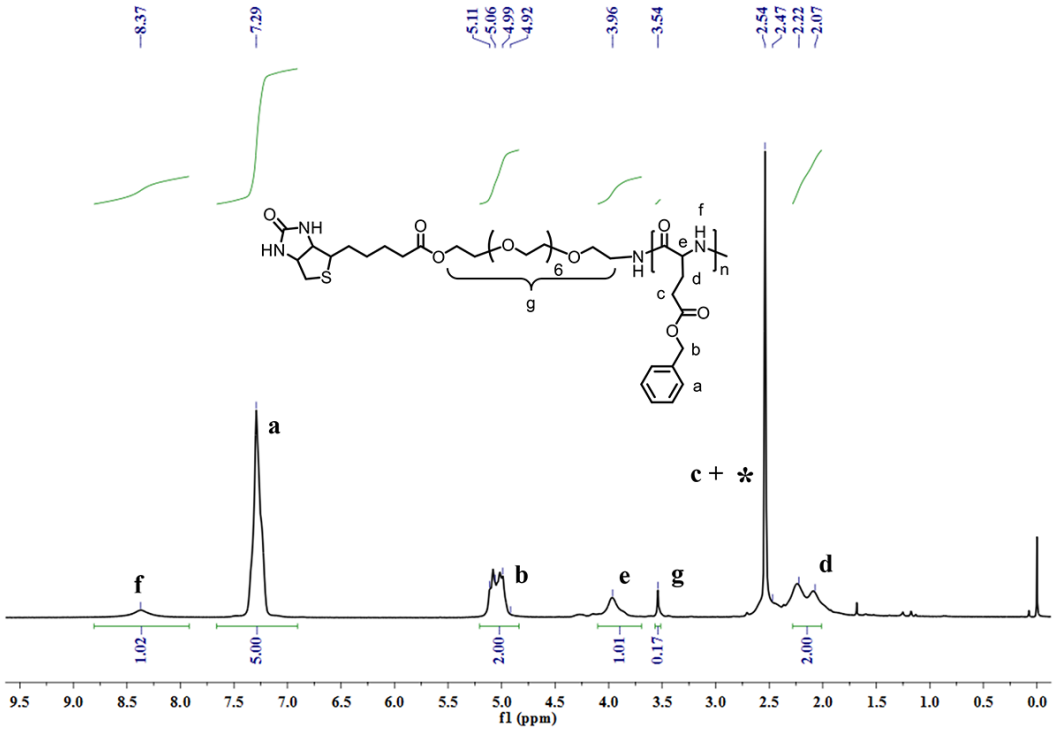


Figure S6. ^{1}H NMR spectrum of **Polymer 2** (400 MHz, DMSO-d_6, 298 K). Solvent peak is marked with asterisk.

Polymer 1: **Polymer 2** (0.2 g) was dissolved in DMF (8 mL). Butane-1, 4-diamine (2.7 mL, 30 equiv. relative to the benzyl groups of PBLA) was then added and stirred at 40 ℃ for 24 h under a dry argon atmosphere. After that, 16 mL of 10% acetic acid

was added and stirred for 5 h at 0 ℃, and the resulting solution was dialyzed against 0.01 N HCl and then water using a Spectrapor dialysis membrane (MWCO 8000). **Polymer 1** (0.14 g, 60%) was obtained as the hydrochloride salt after lyophilization. From the ^{1}H-NMR measurement, the degree of polymerization (n) was calculated to be about 180 through the peak intensity ratio between the methylene protons **h** of PEG and the methyne protons **g**. Moreover, the grafting amount of butyl-ammonium (BA) units in **polymer 1**, comparing the peak intensity ratio between the protons **g** and the methylene protons in the side chain of **polymer 1** (**b** and **c**), was calculated to be 180, indicating the aminolysis was proceeded quantitatively. ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm) = 4.40−4.23 (1H, H_g), 3.73−3.70 (0.17H, H_h), 3.29−3.14 (2H, H_d), 3.07−2.96 (2H, H_a), 2.42−2.29 (2H, H_e), 2.14−1.99 (2H, H_f), 1.73−1.52 (4H, $H_{b,c}$).

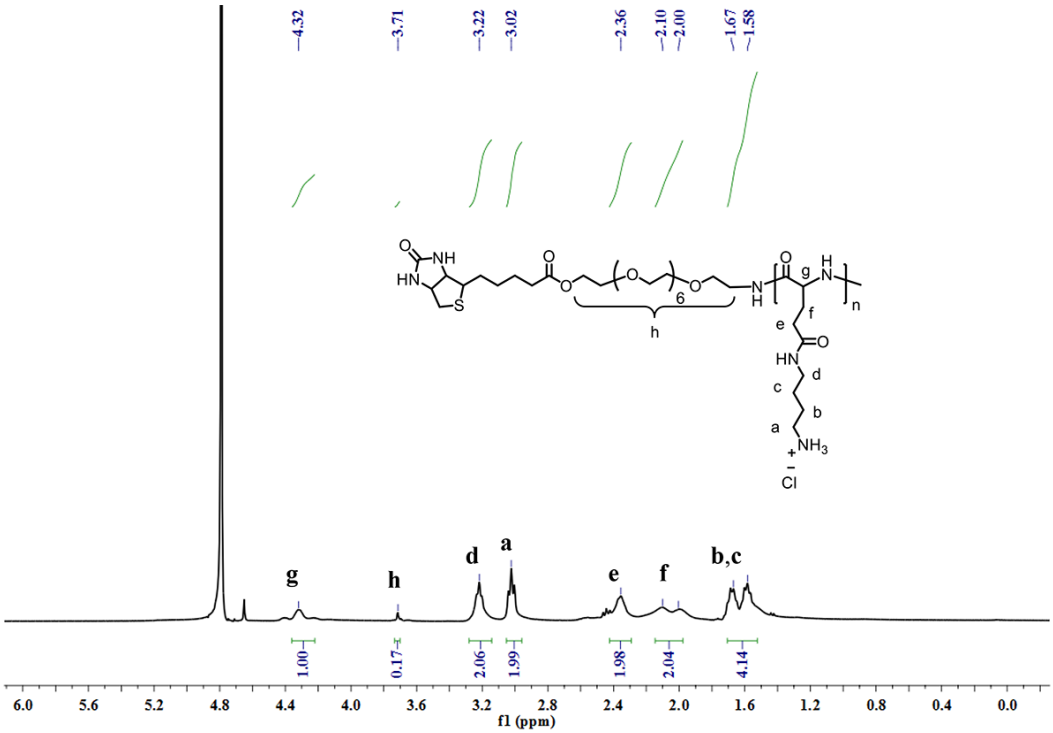


Figure S7. ^{1}H NMR spectrum of **Polymer 1** (400 MHz, D_2O, 298 K).

Polymer 4: BLG-NCA (1.0 g, 3.8 mmol) was dissolved in 10 mL of CH_2Cl_2/DMF solution (10/1, *v*/*v*) under nitrogen atmosphere. Then, butane-1, 4-diamine (4.2 mg, 48 μmol) in 1 mL of CH_2Cl_2/DMF (10/1, *v*/*v*) was added to the above solution dropwise.

The obtained solution was stirred at 40 ℃ for 3 days. Finally, the reaction solution was pulled into n-hexane. A white precipitate was obtained by centrifugation. The polymer was further purified by the two dissolution-precipitation cycles. The final precipitate was dried under vacuum and **polymer 4** was obtained as white solid (0.88 g, 88%). The M_n, n and M_w/M_n were determined to be 29298, 134 and 1.43, respectively by GPC measurement. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm) = 8.57−8.05 (br, 1H, H_f), 7.45−7.08 (5H, H_a), 5.18−4.86 (2H, H_b), 4.04−3.80 (1H, H_e), 2.47 (2H, H_c), 2.31−1.97 (2H, H_d).

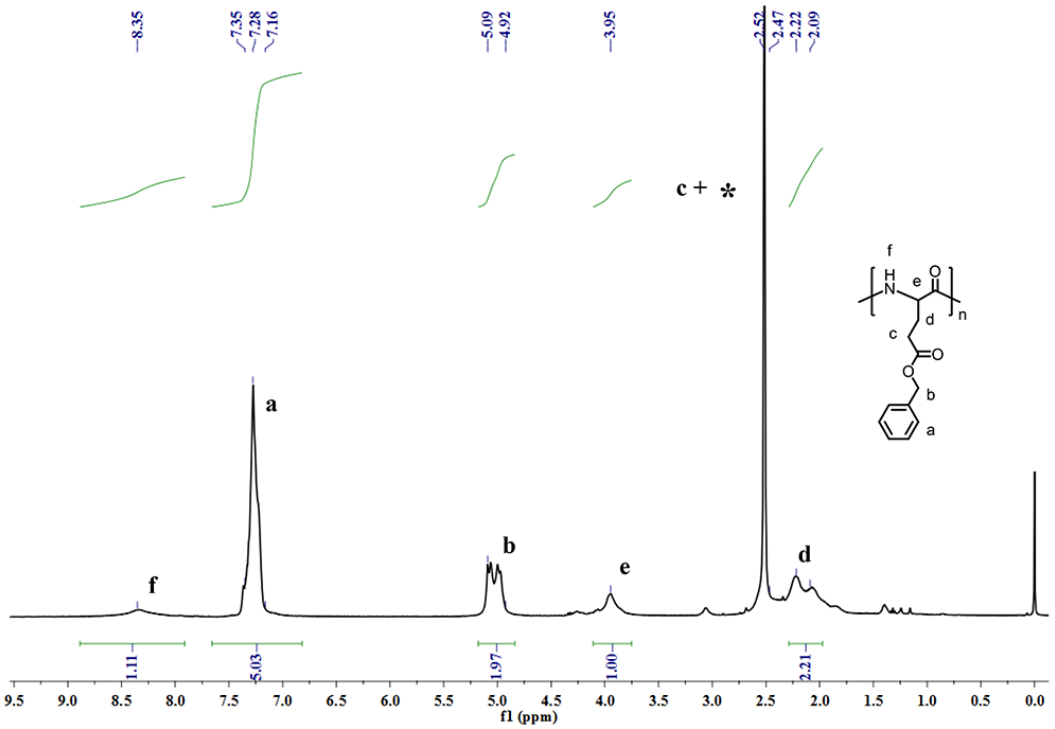


Figure S8. ^{1}H NMR spectrum of **Polymer 4** (400 MHz, DMSO-d_6, 298 K). Solvent peak is marked with asterisk.

Polymer 3: **Polymer 4** (0.2 g) was dissolved in DMF (8 mL). Butane-1, 4-diamine (2.7 mL, 30 equiv. relative to the benzyl groups of PBLG) was then added and stirred at 40 ℃ for 24 h under dry argon atmosphere. After that, 16 mL of 10% acetic acid was added and stirred at 0 ℃ for 5 h, and the resulting solution was dialyzed against 0.01 N HCl and then water using a Spectrapor dialysis membrane (MWCO 8000). **Polymer 3** (0.15 g, 64%) was obtained as the hydrochloride salt after the lyophilization. Similar to **polymer 1**, the peak intensity ratio between the methyne

protons **g** and the methylene protons in the side chain of **polymer 3** (**b** and **c**) was calculated to be 4, indicating the aminolysis was proceeded quantitatively. ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm) = 4.41−4.19 (1H, H_g), 3.27−3.14 (2H, H_d), 3.10−2.99 (2H, H_a), 2.44−2.25 (2H, H_e), 2.20−1.91 (2H, H_f), 1.74−1.48 (4H, $H_{b,c}$).

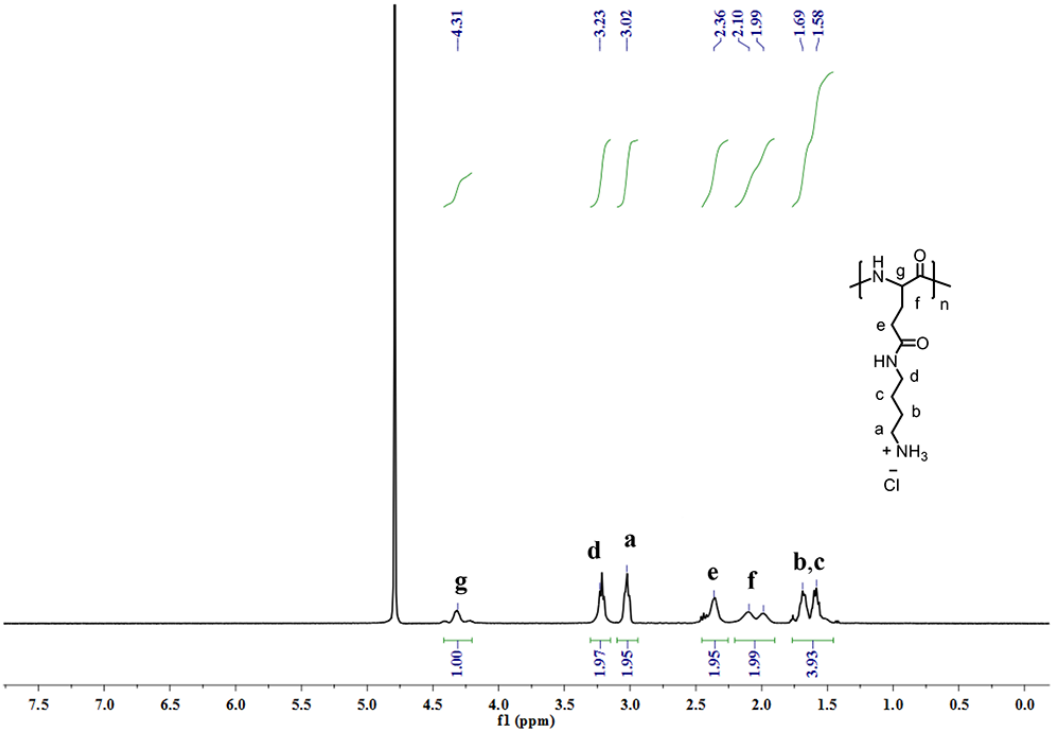
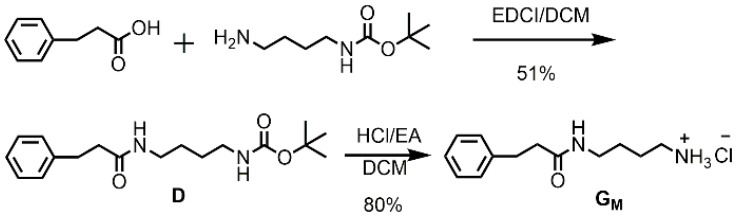


Figure S9. ^{1}H NMR spectrum of **Polymer 3** (400 MHz, D_2O, 298 K).

Synthesis of model guest $\mathbf{G_M}$



Scheme S4. The synthesis route of $\mathbf{G_M}$.

Compound **D**: 3-phenylpropanoic acid (0.3 g, 2.0 mmol), tert-butyl (4-aminobutyl)carbamate (0.45 g, 2.4 mmol), and EDCI (0.46 g, 2.4 mmol) were dissolved in dry CH_2Cl_2 (20 mL). The resulting solution was stirred at room temperature for 24 h. The solution was washed by 1 M HCl and saturated $NaHCO_3$ solution, and then the organic phase was dried by Na_2SO_4. Removal of the solvent and further purification by column chromatograph on silica gel (CH_2Cl_2/ Methanol from

100:1 to 40:1) gave **4** as a white solid (0.35 g, 1.02 mmol, 51%)。^{1}H NMR (CD_3OD, 400 MHz, 298 K) δ (ppm) = 7.92 (br s, 1H), 7.27-7.24 (m, 2H), 7.21−7.15 (m, 3H), 6.54 (br s, 1H), 3.12 (t, *J* = 4.0 Hz, 2H), 2.99 (q, *J* = 8.0 Hz, 2H), 2.90 (t, *J* = 8.0 Hz, 2H), 2.46 (t, *J* = 8.0 Hz, 2H) 1.43 (s, 9H), 1.41−1.32 (m, 4H). ^{13}C NMR ($CDCl_3$, 400 MHz, 298 K) δ (ppm) = 172.3, 156.2, 141.0, 128.5, 128.4, 126.2, 79.2, 40.1, 39.1, 38.4, 31.8, 29.7, 28.5, 27.5, 26.6. HR-ESI-MS: m/z Calcd for $C_{18}H_{28}O_3N_2Na$ [M + Na]$^{+}$ 343.1998, found 343.2001.

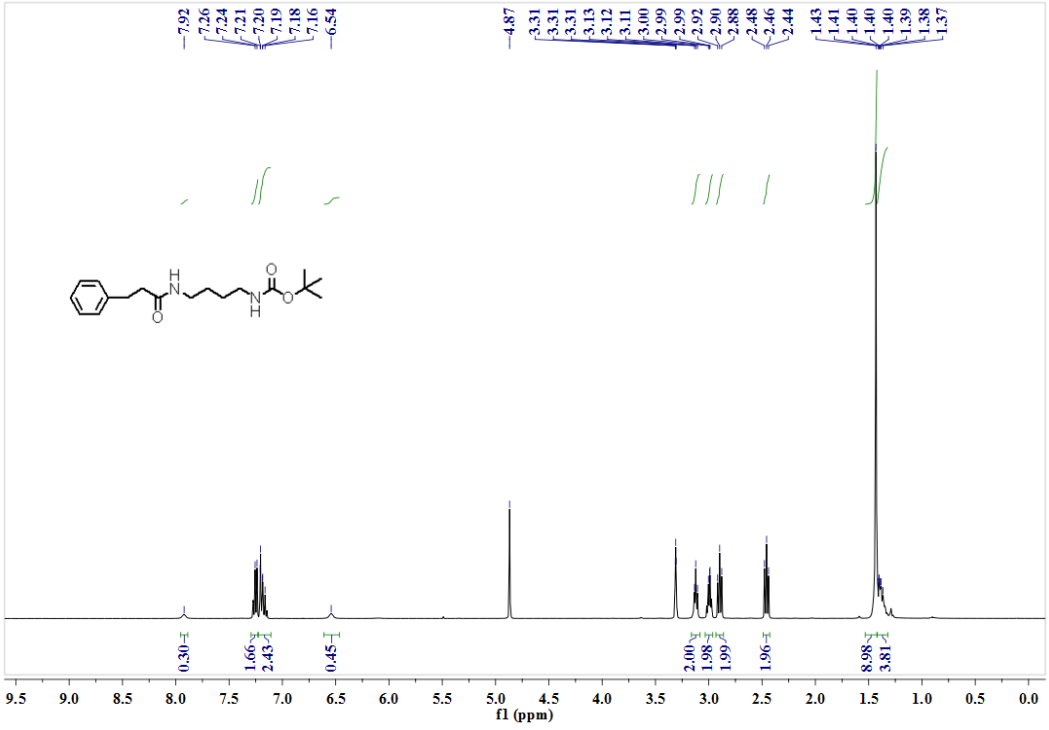


Figure S10. ^{1}H NMR spectrum of **4** (400 MHz, CD_3OD, 298 K).

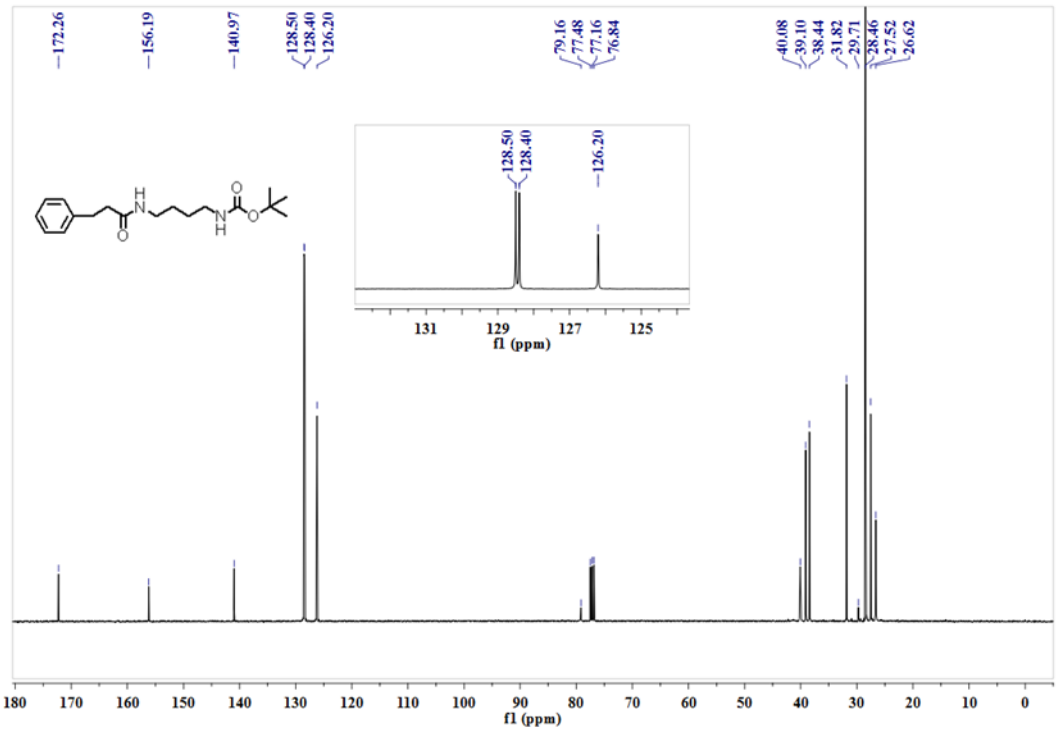


Figure S11. ^{13}C NMR spectrum of **4** (100 MHz, $CDCl_3$, 298 K)

G_M: Compound **D** (160 mg, 0.5 mmol) was dissolved in ethanol (2 mL), and 0.5 mL of 4M HCl/EA was added. The solution was stirred at room temperature for 4 h. Removal of the solvent under reduced pressure, and **G_M** was obtained as a yellow solid (103 mg, 0.4 mmol, 80%). ^{1}H NMR (D_2O, 400 MHz, 298 K) δ (ppm) = 7.35 (t, *J* = 8.0 Hz, 2H), 7.27−7.25 (m, 3H), 3.08 (t, *J* = 8.0 Hz, 2H), 2.92 (t, *J* = 8.0 Hz, 2H), 2.88 (t, *J* = 8.0 Hz, 2H), 2.55 (t, *J* = 8.0 Hz, 2H), 1.43−1.34 (m, 4H). ^{13}C NMR (CD_3OD, 400 MHz, 298 K) δ (ppm) = 175.3, 142.0, 129.39, 129.35, 127.1, 40.3, 39.4, 38.8, 32.9, 27.2, 25.7. HR-ESI-MS: m/z Calcd for $C_{13}H_{21}ON_2$ $[M - Cl]^+$ 221.1648, found 221.1652.

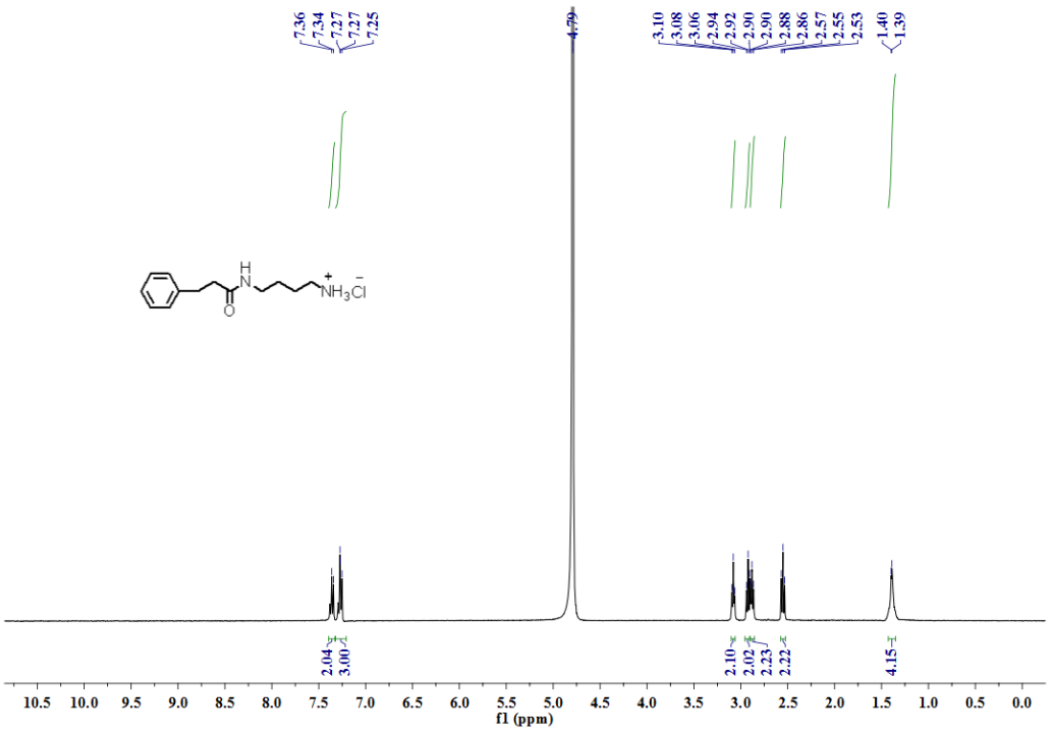


Figure S12. ^{1}H NMR spectrum of $\mathbf{G_M}$ (400 MHz, D_2O, 298 K).

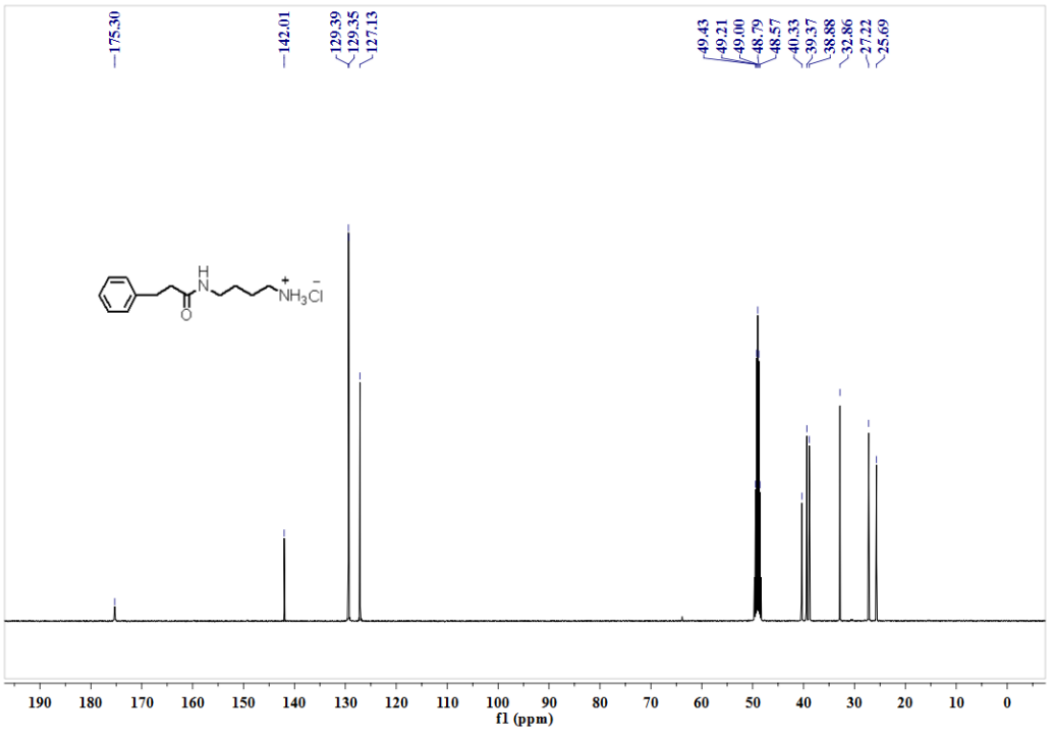


Figure S13. ^{13}C NMR spectrum of $\mathbf{G_M}$ (100 MHz, CD_3OD, 298 K).

3. *Job Plot for WP5⊃G$_M$*

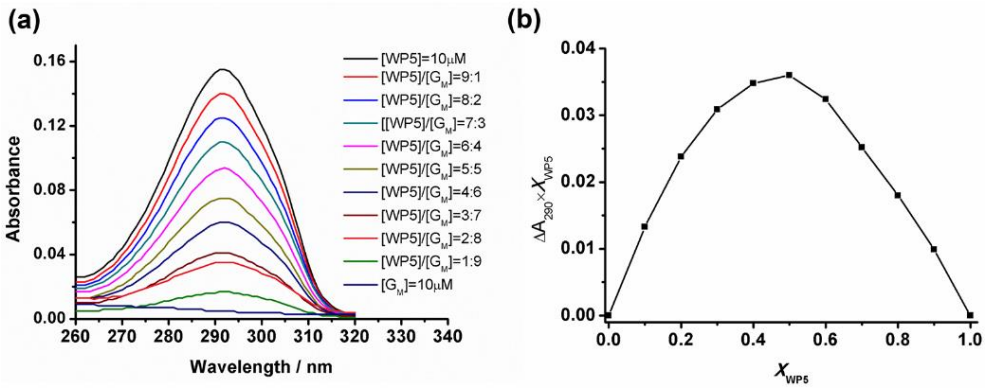


Figure S14. (a) UV-Vis absorption spectra of **WP5⊃G$_M$** complex with different molar ratios in water while [**WP5**] + [**G$_M$**] = 10 μM. (b) Job plots of the complex **WP5⊃G$_M$** showing a 1:1 stoichiometry between **WP5** and **G$_M$** by plotting the absorbance differences at 290 nm (a characteristic absorption peak of **WP5**) against the molar fraction of **G$_M$**.

4. *Determination of the association constant for WP5⊃G$_M$*

To determine the association constant for the complexation between **WP5** and **G$_M$**, ^{1}H NMR titration experiments were carried out in D_2O, which had a constant concentration of **G$_M$** (3.0 mM) and varying concentrations of **WP5**. By a non-linear curve-fitting method, the association constant (K_a) of **WP5⊃G$_M$** was estimated to be (9.28 ± 4.42) × 10^3 M^{-1}, which was approximated as the K_a value between **WP5** and **G$_M$**. The non-linear curve-fittings were based on the equation:

$\Delta\delta = (\Delta\delta_\infty/[G]_0)\ (0.5[H]_0 + 0.5([G]_0 + 1/K_a) - (0.5([H]_0^2 + (2[H]_0(1/K_a - [G]_0)) + (1/K_a + [G]_0)^2)^{0.5}))$ (eq. S1)

Where $\Delta\delta$ is the chemical shift change of $H_{b,c}$ on **G$_M$** at $[H]_0$, $\Delta\delta_\infty$ is the chemical shift change of $H_{b,c}$ when the guest is completely complexed, $[G]_0$ is the fixed initial concentration of the guest, and $[H]_0$ is the varying concentration of **WP5**.

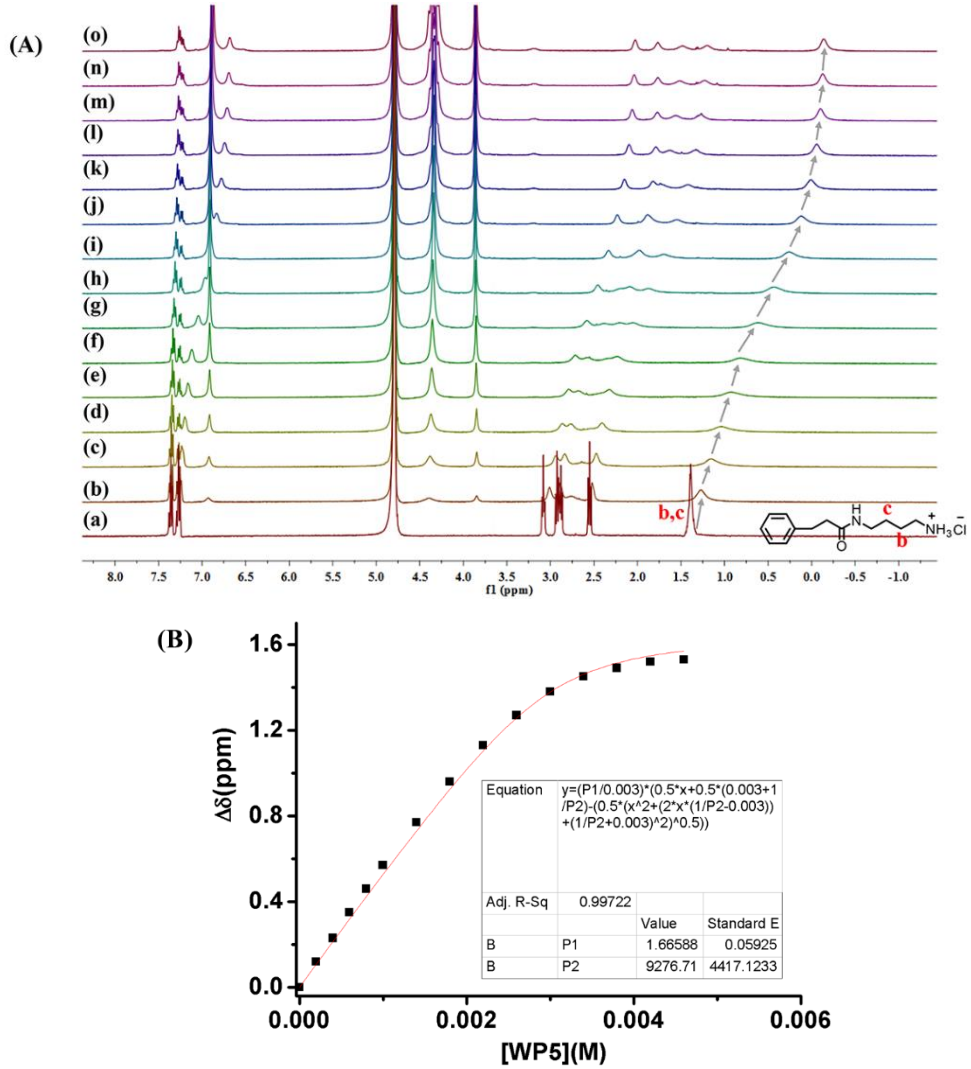


Figure S15. (A) ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of $\mathbf{G_M}$ at a constant concentration of 3.0 mM with different concentrations of **WP5** (mM): (a) 0.0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.0, (g) 1.4, (h) 1.8, (i) 2.2, (j) 2.6, (k) 3.0, (l) 3.4, (m) 3.8, (n) 4.2, and (o) 4.6. (B) The chemical shift changes of $H_{b,c}$ on $\mathbf{G_M}$ upon the addition of **WP5**. The red solid line was obtained from the non-linear curve-fitting using eq. S1. The association constant of **WP5** and $\mathbf{G_M}$ was estimated to be $(9.28 \pm 4.42) \times 10^3$ M^{-1}.

5. *Self-assembly of polymer 3 and polymer 1 in the presence of WP5*

Obvious Tyndall effect was also observed upon adding **WP5** to the solution of **polymer 3**, implying the existence of plenty of nanoparticles. The morphology and size of the aggregates were investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM) experiments. When **polymer 3** (50.4 μg/mL)

mixed with **WP5** solution (15.4 μM), DLS result showed that the generated **WP5-polymer 3** complex formed well-defined aggregates with a narrow size distribution, giving an average diameter of 234 nm (Figure S16b). Moreover, TEM images clearly showed the formation of hollow spherical structures with diameters range from 180 nm to 300 nm (Figure S16c). Based on the DLS and TEM data, we know that the self-assembly behavior of **WP5-polymer** complex is similar with the inclusion complex formed by **WP5** and **polymer 1** (Scheme S5), indicating that the short polyethylene glycol side chain in **polymer 1** has negligible influence on the assembly process.

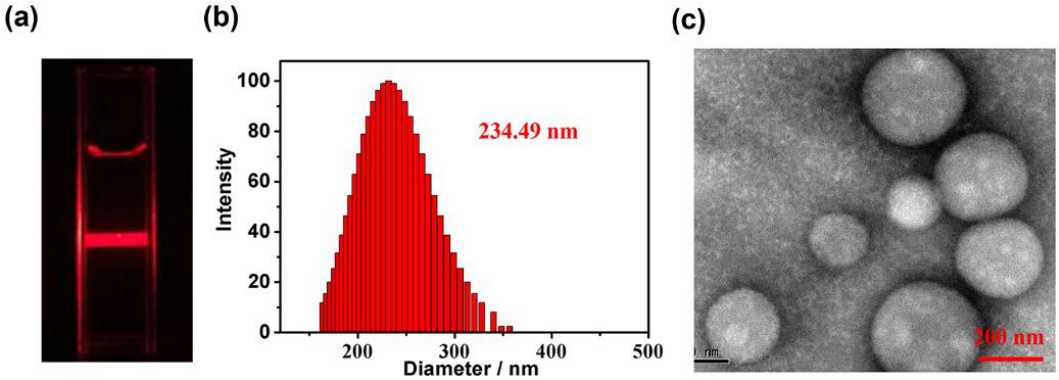
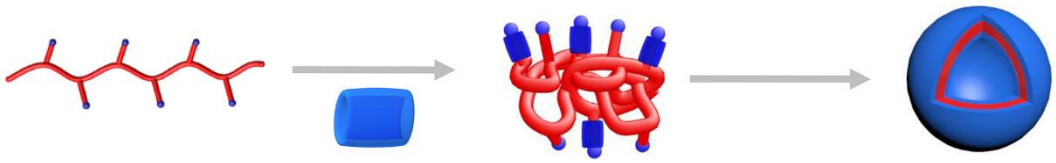


Figure S16. (a) Tyndall effect of **WP5-polymer 3** aggregates; (b) DLS data of the **WP5**–**polymer 3** aggregates; (c) TEM images of **WP5-polymer 3** aggregates.



Scheme S5. Illustration of **WP5**-induced assembly process of the polymer.

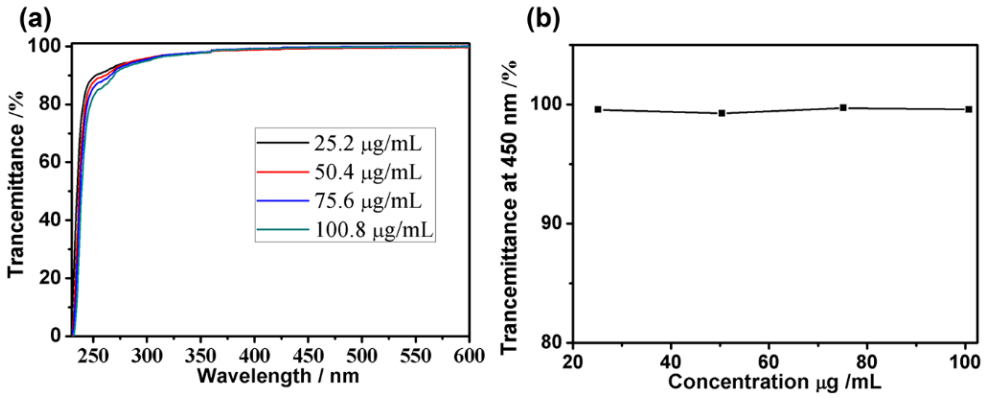


Figure S17. (a) Optical transmittance of the aqueous solutions of **polymer 1** with different concentrations at 25 ℃. (b) Dependence of the optical transmittance at 450 nm on the concentration of **polymer 1**.

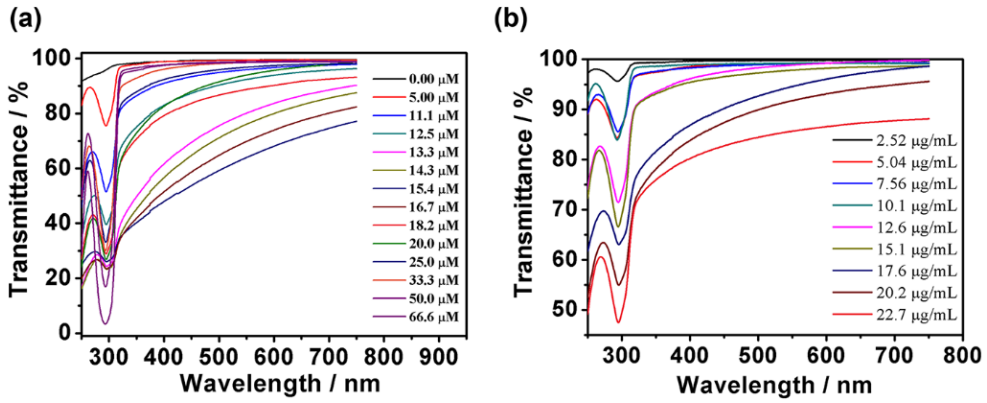


Figure S18. (a) Optical transmittance of **polymer 1** (50.4 μg/mL) upon increasing the concentration of **WP5** (0 – 66 μM) at 25 ℃ in water; (b) Optical transmittance of the **WP5**–**polymer 1** aggregates at the best mixing ratio.

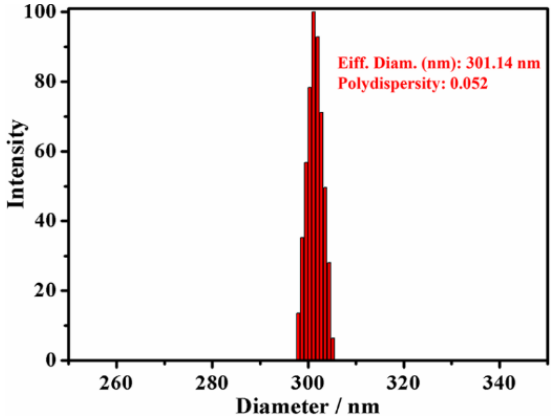


Figure S19. DLS data of MTZ-loaded polymersomes constructed by **WP5** and **polymer 1**.

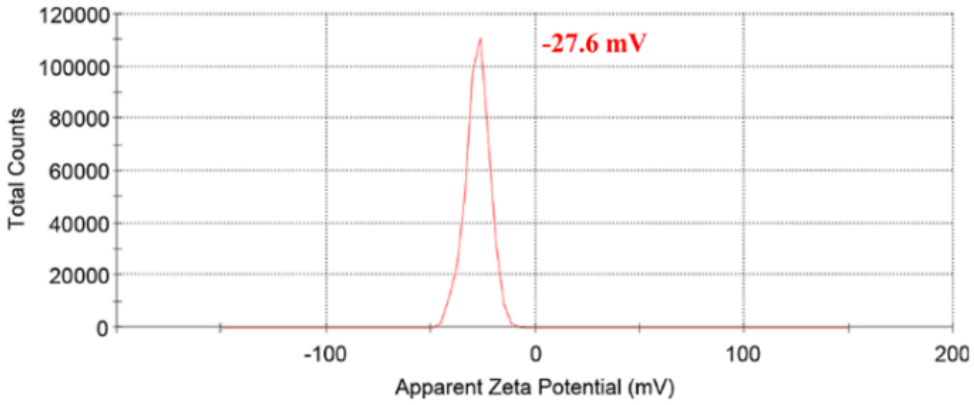


Figure S20. zeta-Potential of the MTZ-loaded polymersomes constructed by **WP5** and **polymer 1**.

6. *Drug loading and release behavior of polymersomes fabricated by WP5 and polymer 3*

Under the same conditions as preparing MTZ-loaded polymersomes by **WP5-polymer 1** complex, MTZ-loaded polymersomes formed by **WP5-polymer 3** was prepared. As shown in Figure S21e, the absorbance of MTZ-loaded solution becomes much stronger from to 610 to 660 nm (the characteristic absorption peak of MTZ) compare with the unloaded polymersome solution (Figure S21e). Meanwhile, compared with the transparent light blue solution of free MTZ, MTZ-loaded polymersome solution turned to royal purple after removing unloaded MTZ by dialysis (Figure S21a). Moreover, the MTZ-loaded polymersome showed larger size (290 nm) and darker interior from TEM images (Figure S21b, c), confirming that MTZ molecules were loaded into the polymersome cavities. In addition, zeta-potential measurement indicated that the MTZ-loaded polymersomes also have good stability (ζ-potential = − 30.3 mV, Figure S23). According to UV-Vis absorption spectra, the drug encapsulation efficiency was calculated to be 52%. Then, the drug release behavior from the MTZ-loaded polymersome formed by **polymer 3-WP5** was investigated (Figure S22). Under physiological condition (pH 7.4), the cumulative leakage of MTZ was only 13% within 12 h. However, a gradual release of MTZ from the MTZ-loaded polymersome was observed under acidic conditions, with the

cumulative drug release amount of 42% at pH 6.0 and 83% at pH 5.2 within 12 h, indicating an efficient pH-triggered drug release.

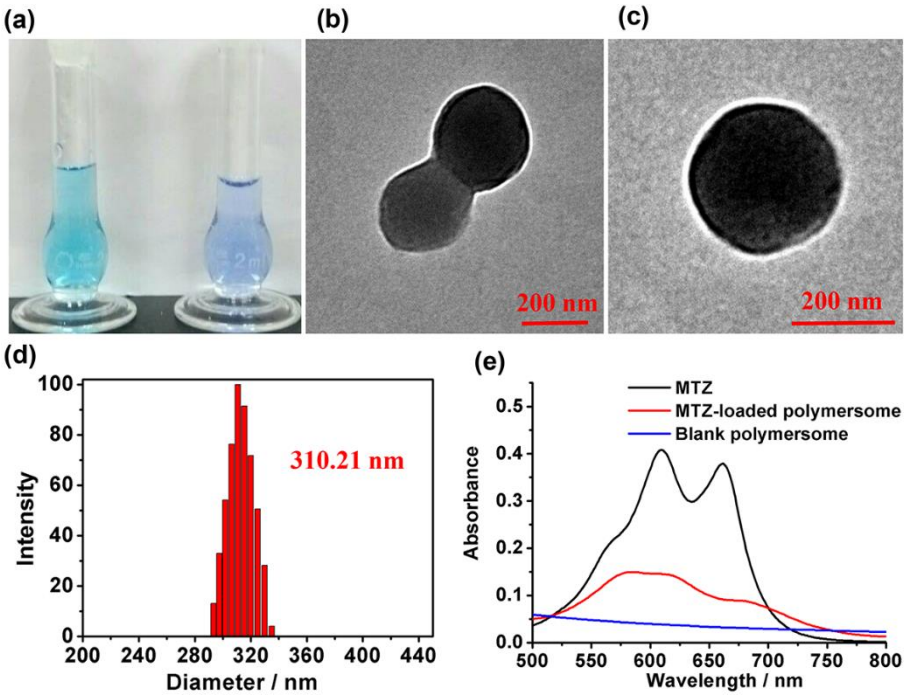


Figure S21. (a) Images of MTZ-loaded vesicular solution (right) and free MTZ solution (left), (b) and (c) TEM images of MTZ-loaded polymersomes formed by **WP5** and **polymer 3**, (d) DLS data of MTZ-loaded polymersomes constructed by **WP5** and **polymer 3**, and (e) UV-Vis absorption spectra of blank polymersomes, MTZ-loaded polymersomess, and free MTZ.

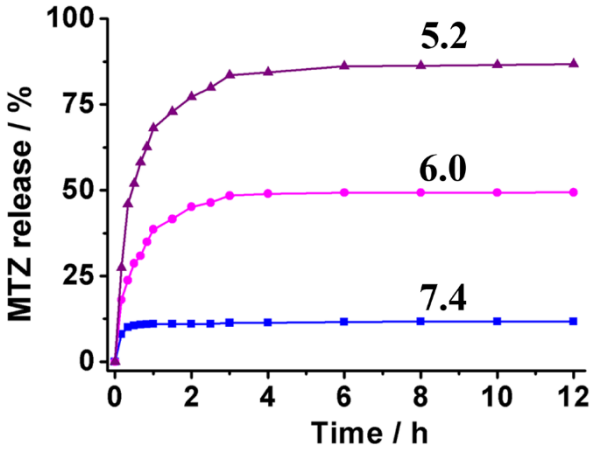


Figure S22. MTZ-release profiles from the MTZ-loaded polymersomes in release media with different pH values.

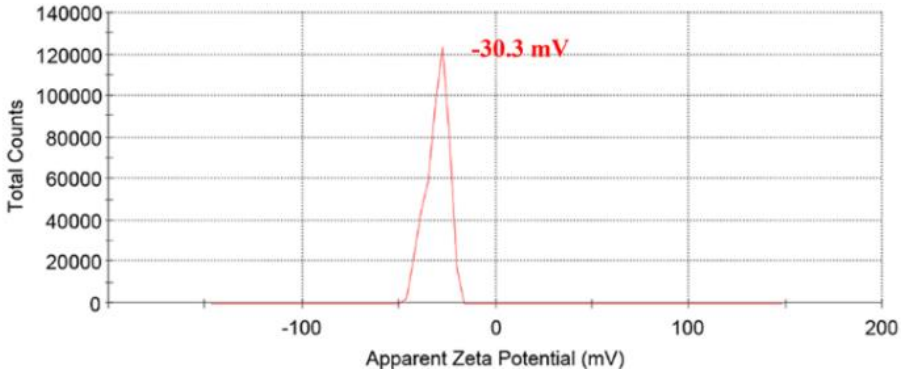


Figure S23. zeta-Potential of the MTZ-loaded polymersomes constructed by **WP5** and **polymer 3**.

7. *References*

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S2. X. Yue, Z. Wang, L. Zhu, Y. Wang, C. Qian, Y. Ma, D. O. Kiesewetter, G. Niu, X. Chen, *Mol. Pharmaceutics* **2014**, *11*, 4208-4217.