

Supporting Information

for

Phenylboronic acid-functionalized unimolecular micelles based on a star polyphosphoester random copolymer for tumor-targeted drug delivery

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Synthesis of functional cyclic monomers, BEP and MP

The cyclic functional phosphoester monomers, 2-butenyl phospholane (BEP) and 2-methoxy phospholane (MP) were synthesized via nucleophilic substitution reaction between 2-chloro-1,3,2-dioxaphospholane-2-oxide (COP) and monohydric alcohols. A solution of COP (14.25 g, 100 mmol) in 50 mL of anhydrous THF was separately dropwise added into a stirred solution of 3-buten-1-ol (7.40 g, 106 mmol), methanol (3.85 g, 120 mmol) and triethylamine (12.14 g, 120 mmol) in 200 mL of anhydrous THF at 0 °C. After stirred for 12 h and the complete conversion of COP was confirmed by TLC, the reaction mixture was filtered and concentrated. The concentrated filtrate was distilled under reduced pressure to obtain a viscous liquid, with faint yellow for BEP, and transparent for MP. For the chemical structure characterization of BEP, ¹H NMR (DMSO-d₆, ppm): δ=4.22 (t, 4H, POCH₂CH₂OP), 4.20 (t, 2H, POCH₂CH₂C), 2.57 (t, 2H, POCH₂CH₂C), 2.48 (s, H, POCH₂CH₂C≡CH). ³¹P NMR (DMSO-d₆, ppm): δ=15.74. IR: 3300, 2200~2100, 1690, 1300~1140, 845~725 cm⁻¹. For the chemical structure characterization of MP, ¹H NMR (DMSO-d₆, ppm): δ=4.22 (t, 4H, POCH₂CH₂OP), 3.71 (s, 3H, POCH₃). ³¹P NMR (DMSO-d₆, ppm): δ=15.83. IR: 3300, 2200~2100, 1690, 1200~1100, 1300~1140, 845~725 cm⁻¹.

Synthesis of PAMAM-P(BEP-co-MP)

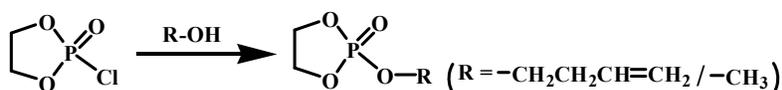
A solution of BEP, MP and a given amount of G4.0 PAMAM-OH in anhydrous DMF was transferred into a flame-dried 25 mL shell vial equipped with a rubber septum and a stir bar. At 25 °C, a solution of a given amount of DBU in anhydrous dimethylformamide was injected into the vial via syringe, while being maintained under a nitrogen gas atmosphere. The weight ratio of the ingredients was shown in Table S1. After being stirred for a certain period of time (1.25 h), a solution of acetic acid (excess) in dimethylformamide was added into the reaction mixture to quench the reaction. The resulting mixture was purified by precipitation from dimethylformamide into diethyl ether (3×) and was then dried under vacuum to the copolymer, PAMAM-P(BEP-co-MP), as a light yellow highly viscous liquid. NMR and FT-IR spectroscopy were used to testify the structure of prepared copolymer. GPC was used to determine the molar mass and its dispersity (Đ). ¹H NMR (DMSO-d₆, ppm): δ=4.22 (t, 4H, POCH₂CH₂OP), 3.71 (s, 3H, POCH₃), 2.46 (t, 4H, NCH₂CH₂N), 4.20 (t, 2H, POCH₂CH₂C), 2.57 (t, 2H, POCH₂CH₂C), 5.80 (s, H, POCH₂CH₂CH=CH₂), 5.08 (s, H, POCH₂CH₂CH=CH₂), 2.62 (m, 2H, NCH₂CH₂CO), 2.81 (m, 2H, NCH₂CH₂CO), 3.39 (m, 2H, HNCH₂CH₂OH), 3.79 (m, 2H, HNCH₂CH₂OH). FT-IR: 3600-3200, 3100~3000, 2953, 1648, 1564, 1455, 1207, 1020, 954, 798, 741 cm⁻¹.

Molecular weights and their distributions of PAMAM-P(BEP-co-MP)

The average molar mass and the dispersity (Đ) of the copolymers were measured by gel permeation chromatography (GPC) (Waters1515, America), calibrated against linear polystyrene (PS) standards with DMF as the mobile phase, the flow rate was 1.0 mL min⁻¹. The elution traces and obtained results were shown in Figure S3 and Table S2 respectively. The obtained copolymers PAMAM-P(BEP-co-MP) exhibited narrow molecular weight distribution ($M_w/M_n < 1.05$) and controlled molecular weight. According to the GPC results, the number-average molecular weight of PAMAM-P(BEP-co-MP) ranged in $5.86 \times 10^3 \sim 6.78 \times 10^3$ g mol⁻¹. However, the molecular weight of the copolymer core, G4.0 PAMAM, has already been known as 14280 Da, which is higher than the above-measured results of copolymers. The GPC result of G4.0 PAMAM (Figure S4) also showed a larger molecular weight than PAMAM-P(BEP-co-MP). In this case, there is no positive correlation in the GPC results between the G4.0 PAMAM core and copolymers for an equivalent calculation to obtain the molecular weight of the copolymers. The inaccurate anomaly may be due to the special and complex molecular structures of the synthesized copolymers. Their multiple, long arms and numerous terminal hydroxyl groups lead to a complex entanglement between intramolecular chain segments of copolymers, thereby the GPC results based on linear polymer standards were inaccurate. Although there is not an equivalent relationship in the GPC results between the G4.0 PAMAM core and copolymers, the consistency of the five copolymer samples in the GPC results with two mobile phases also can indicate the successful ROP.

Degradation of PAMAM-P(BEP-co-MP)

A certain amount of PAMAM-P(BEP-co-MP) was dissolved into ultrapure water and transferred into a dialysis cellulose membrane (MWCO of 10000 Da), then immersed in 100 mL of pH 5.0 PBS containing PDE I. Every 12 h polymer solution was taken out and purified for ^1H NMR characterization to track the degradation process of PAMAM-P(BEP-co-MP). As shown in Figure S5, at the first 12 h, the peaks at 4.2 ppm and 3.71 ppm that separately attributed to the hydrogen protons in the main chain of polyphosphoester and methoxy protons of MP segments decreased obviously. This may be caused by the rapid degradation of the outer hydrophilic segment of the copolymer exposed to the acid PBS solution containing enzymes. After 24 h of degradation, the peaks at 5.80, 5.08 and 2.38 ppm that assigned to the protons of the propenyl group in the PBEP segments were more obvious to be observed, which attributed to the increased proportion of components of PBEP. Whereafter, the peaks of PBEP gradually decreased over time. Therefore, the copolymer PAMAM-P(BEP-co-MP) could be degraded gradually and rapidly in an esterase environment.



Scheme S1. Functional modification of cyclic phosphoester monomers via nucleophilic substitution reaction.

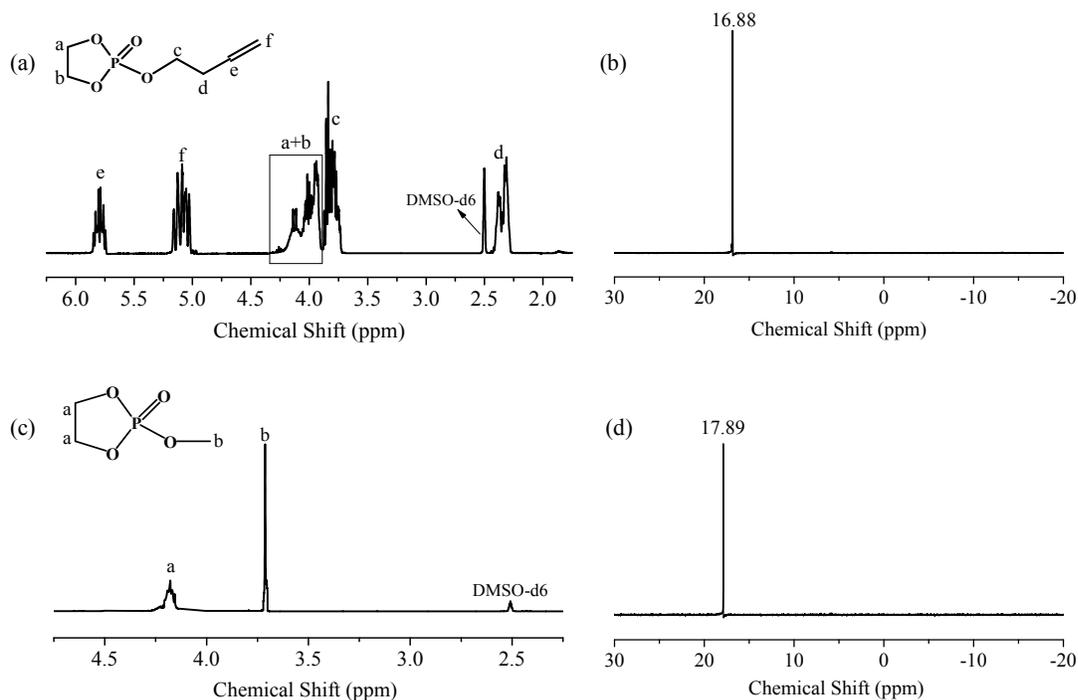
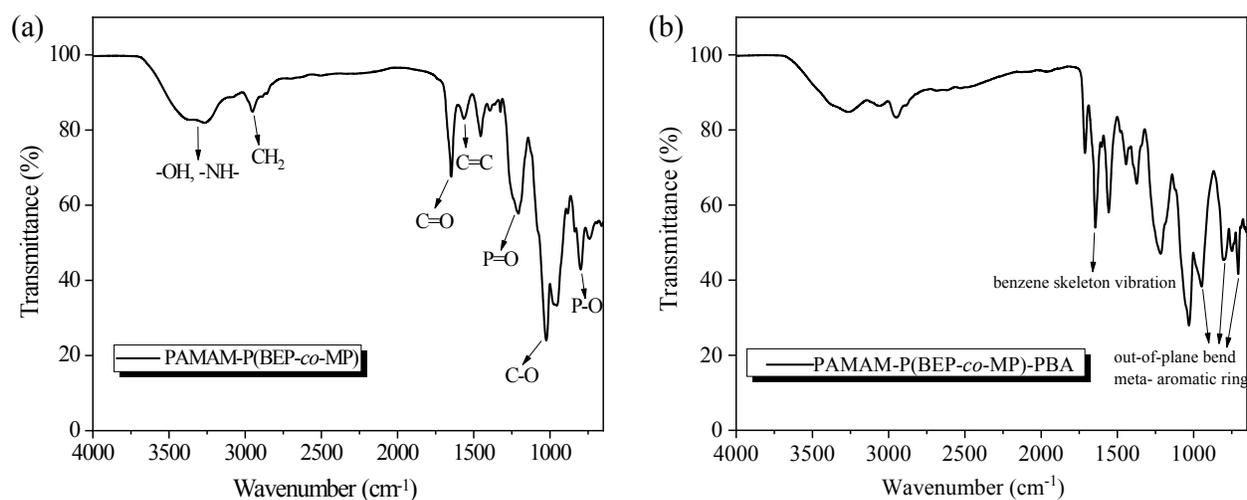


Figure S1. NMR spectra (400 MHz) of functional monomers in DMSO-d₆ at room temperature: ^1H -NMR (a, c) and ^{31}P -NMR (b, d) spectra of BEP (a, b) and MP (c, d).

Table S1. Synthesis conditions of PAMAM-P(BEP-*co*-MP)

Samples	I[-OH]: BEP: BMP: DBU	PAMAM _{4,0}		BEP		MP		DBU		reaction time (h)
		m (g)	n (mmol)	m (g)	n (mmol)	m (g)	n (mmol)	m (g)	n (mmol)	
1	1: 10: 30: 1.5	0.150	0.671	1.248	6.707	2.778	20.122	0.153	1.006	1.25
2	1: 15: 30: 1.5	0.150	0.671	1.872	10.061	2.778	20.122	0.153	1.006	1.25
3	1: 20: 30: 1.5	0.150	0.671	2.496	13.414	2.778	20.122	0.153	1.006	1.25
4	1: 25: 30: 1.5	0.150	0.671	3.120	16.768	2.778	20.122	0.153	1.006	1.25
5	1: 30: 30: 1.5	0.150	0.671	3.744	20.122	2.778	20.122	0.153	1.006	1.25

**Figure S2.** FT-IR spectra of PAMAM-P(BEP-*co*-PMP) and PAMAM-P(BEP-*co*-PMP)-PBA**Table S2.** Molecular weights and their distributions of PAMAM-P(BEP-*co*-MP)

Samples	I[-OH]: BMP: DBU	BEP:	M _n [×10 ⁻³]	M _w [×10 ⁻³]	M _w /M _n
PAMAM-P(BEP- <i>co</i> -MP)-1	1: 10: 30: 1.5		6.78	7.00	1.03
PAMAM-P(BEP- <i>co</i> -MP)-2	1: 15: 30: 1.5		6.64	6.85	1.03
PAMAM-P(BEP- <i>co</i> -MP)-3	1: 20: 30: 1.5		7.16	7.41	1.04
PAMAM-P(BEP- <i>co</i> -MP)-4	1: 25: 30: 1.5		5.86	6.08	1.04
PAMAM-P(BEP- <i>co</i> -MP)-5	1: 30: 30: 1.5		6.17	6.33	1.03

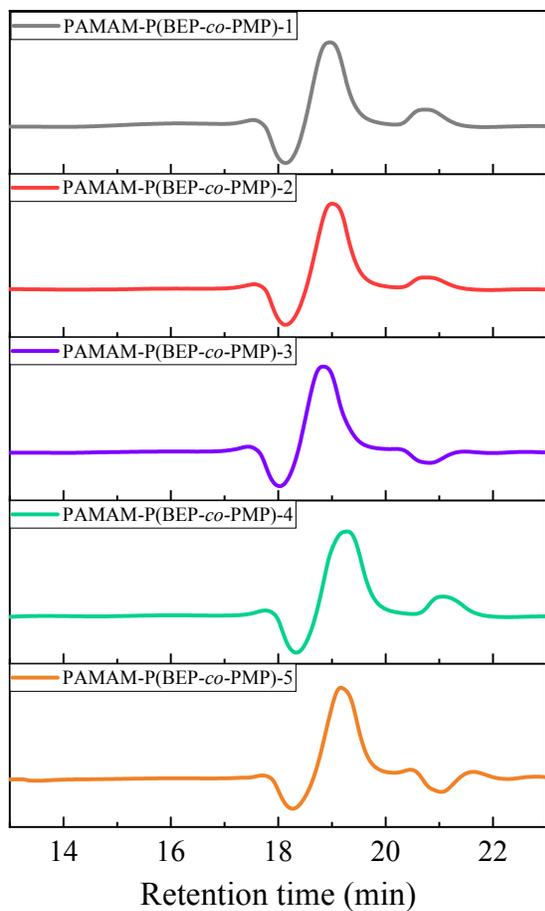


Figure S3. GPC traces calibrated against linear polystyrene standards with DMF as the mobile phase.

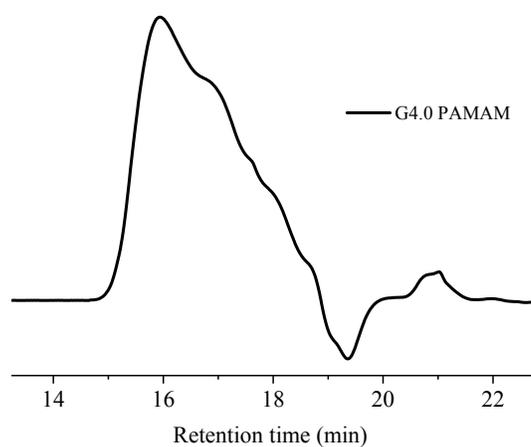


Figure S4. GPC traces of G4.0 PAMAM and PAMAM-P(BEP-co-MP)

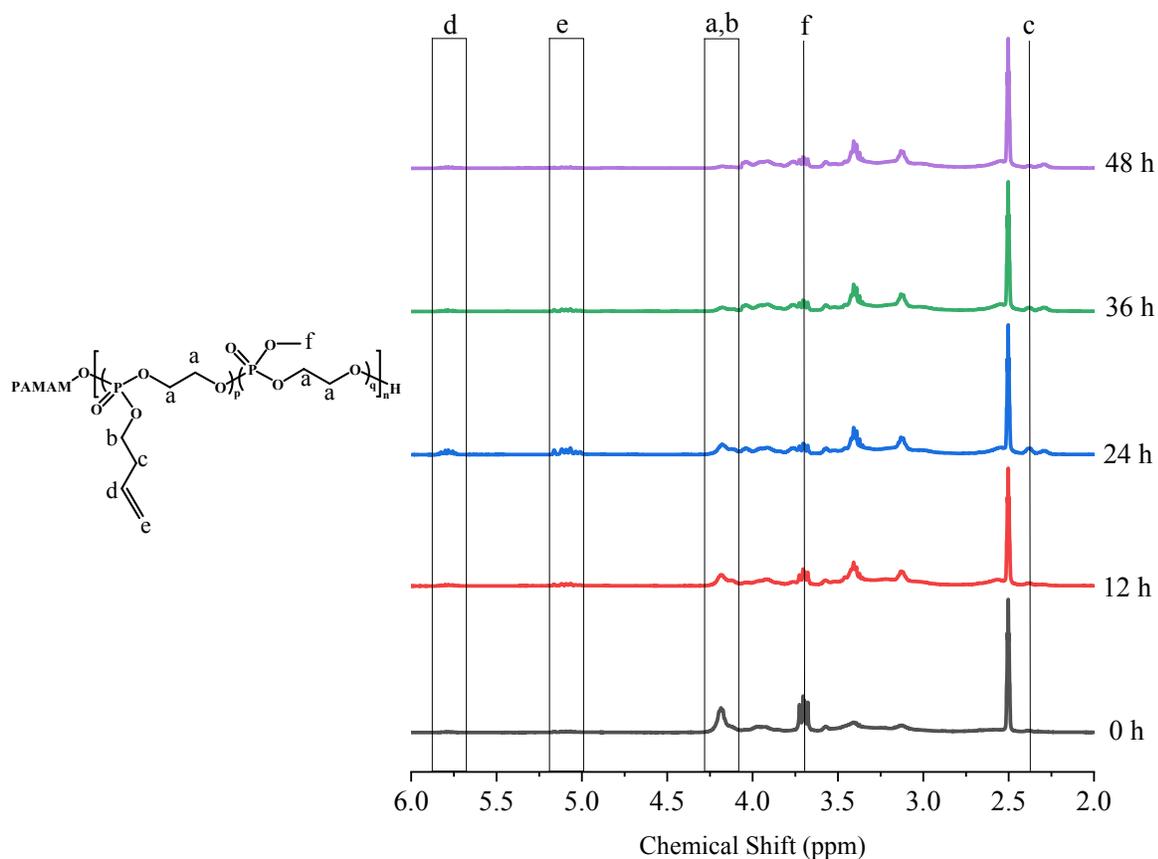


Figure S5. ^1H NMR spectra (400 MHz) of PAMAM-P(BEP-co-PMP) in DMSO- d_6 at room temperature before and after 12, 24, 36 and 48 h of degradation in an enzyme solution.

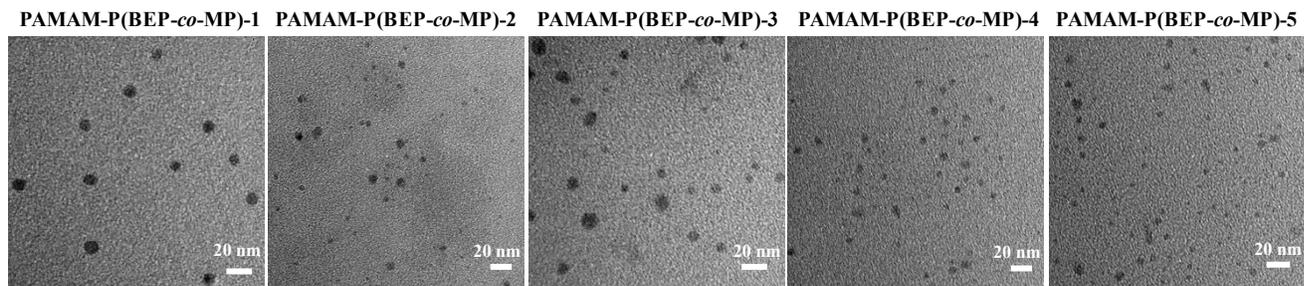


Figure S6. Morphologies of unimolecular micelles formed by PAMAM-P(BEP-co-MP) with different molar ratios of monomers observed by TEM.

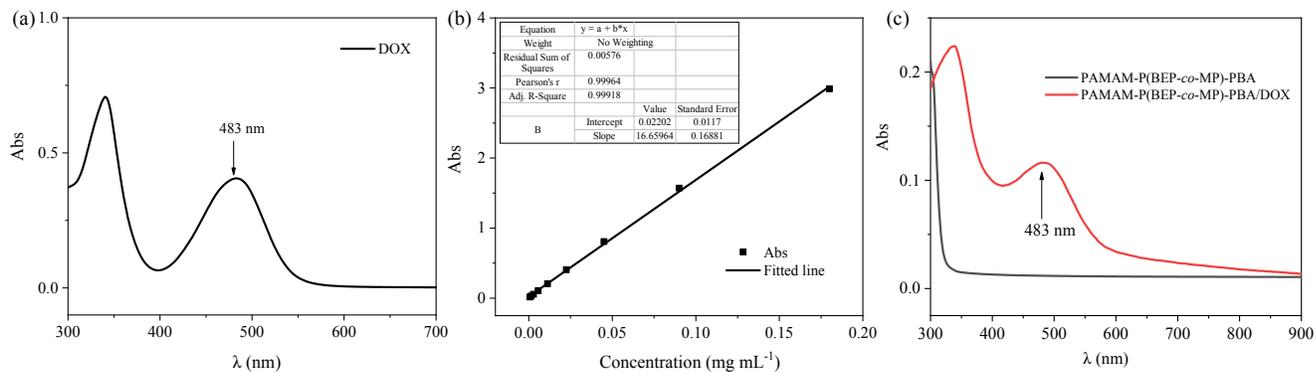


Figure S7. a) UV absorption curve of DOX measured by UV-vis spectrometer. b) The standard curve of DOX with a correlation function between absorbance and concentration. c) UV absorption curves of PAMAM-P(BEP-co-MP)-PBA and PAMAM-P(BEP-co-MP)-PBA/DOX in DMSO.

Table S3. Drug loading content (DLC) and drug loading efficiency (DLE) of unimolecular micelles

Samples	DLC%	DLE%
PAMAM-P(BEP-co-MP)-PBA/DOX	17.6%	59.0%

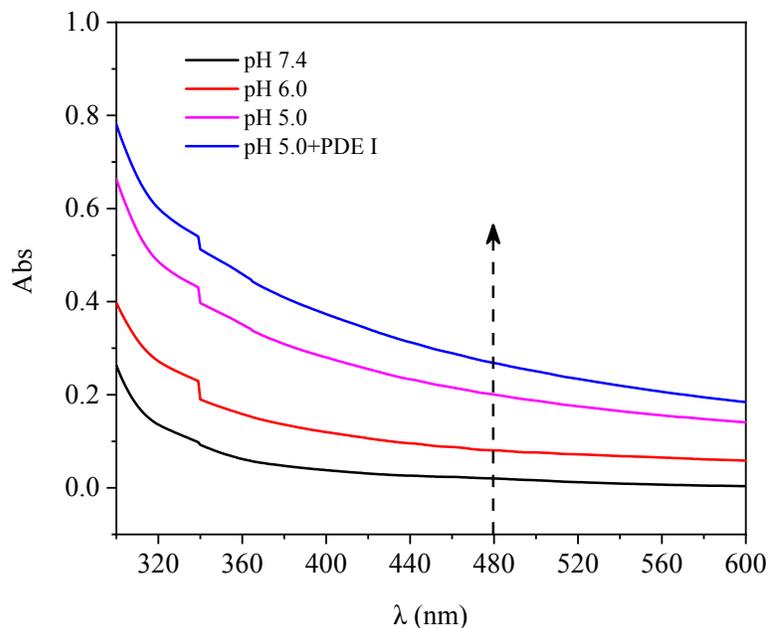


Figure S8. Ultraviolet absorption curves of PAMAM-(PBEP-co-PMP)-PBA/DOX release media after 312 h measured by UV-vis spectrometer.

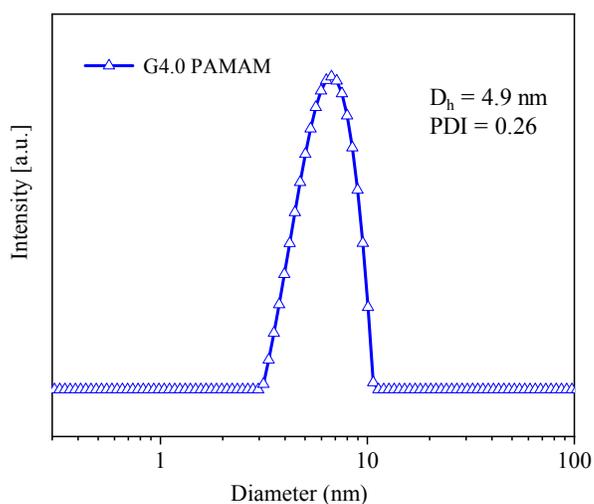


Figure S9. Hydrodynamic diameter of hydroxyl-terminated G4.0 PAMAM measured by DLS.

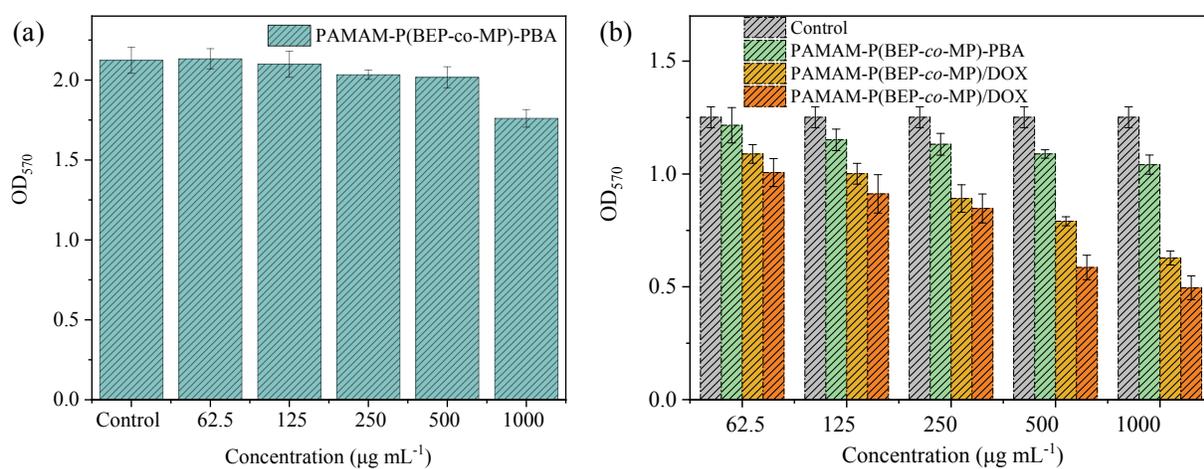


Figure S10. Optical density of live cells at 570 nm (OD_{570}) for the calculation of the in-vitro cell viability of HepG2 cells in different samples treated culture media. Data were shown as mean \pm SD (n=5).