Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2024

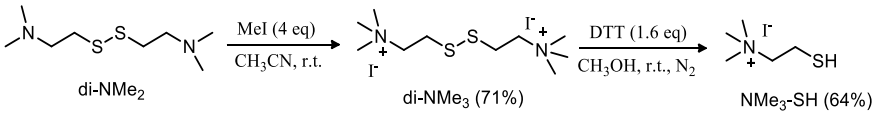
Electronic Supplementary Information

Synthesis and antibacterial properties of fluorinated biodegradable cationic polyesters

Zhao-Yue Li, Xiaoying Zhang, Yi-Lin Qian, Fu-Sheng Du,* Zi-Chen Li*

Syntheses

Synthesis of thiocholine iodide (NMe_3-SH)



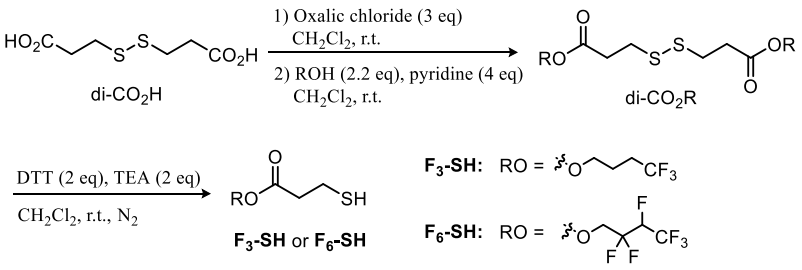
Scheme S1 Synthesis of thiocholine iodide (NMe_3-SH)

Thiocholine iodide was synthesized following the route shown in Scheme S1. Bis(2-dimethylaminoethyl) disulfide dihydrochloride (2.0 g, 7.2 mmol) was dissolved in ice-cooled aqueous NaOH (1 equiv.) solution (5.0 mL) and stirred for ~4 h. The react mixture was then extracted with DCM (50 mL × 3), the organic phase was dried with anhydrous Na_2SO_4 and concentrated in vacuo to afford bis(2-dimethylaminoethyl) disulfide (di-NMe_2) as colorless odor oil. This acid-free oil was dissolved in 15 mL acetonitrile, to which ethyl iodide (4.0 g, 28.2 mmol) was added slowly at -30 °C. The obtained mixture was stirred at ambient temperature overnight and poured into excess diethyl ether, generating white precipitate. After washing with diethyl ether and drying, white solid (di-NMe_3) was obtained with a yield of 71%. ^{1}H NMR (400 Hz, DMSO-d_6, ppm): δ 3.67-3.63 (m, 4H), 3.28-3.24 (m, 4H), 3.14 (s, 18H).

di-NMe_3 (2.5g, 5.1 mmol) was dissolved in 10 mL methanol, to which DTT (1.2 g, 7.8 mmol) was added. After being stirred at 25 °C for 12 h, the crude product was obtained by precipitation in excess diethyl ether. The crude product was washed by acetone (10 mL × 3) and dried in vacuo at room temperature for 2 days to generate NMe_3-SH with a yield of 64%. ^{1}H NMR (400 Hz, DMSO-d_6, ppm): δ 3.48-3.45 (m, 2H), 3.07 (s, 9H), 2.89-2.85 (m, 2H). ^{13}C NMR (100 Hz, DMSO-d_6, ppm): δ 67.38, 52.78, 17.22.

Synthesis of F_3-SH and F_6-SH

4,4,4-Trifluorobutyl 3-mercaptopropanoate (F_3-SH) and 2,2,3,4,4,4-hexafluorobutyl 3-mercapto-propanoate (F_6-SH) were synthesized following the route of Scheme S2



Scheme S2 Synthesis of thiol compounds F_3-SH and F_6-SH.

Take the synthesis of F_3-SH as an example. Oxalyl chloride (11 mL) and 4 drops of DMF were added to 3,3'-dithiodipropionic acid (di-CO_2H, 5.0 g, 23.8 mmol) dissolved in 100 mL of DCM. The mixture was stirred for 4 h at 25 °C and then concentrated under reduced pressure to give 3,3'-dithiodipropionyl chloride (di-COCl) as a colorless liquid. The obtained crude product was dissolved in 100 mL Et_2O/DCM (v/v =1/1), followed by adding 4,4,4-trifluoro-1-butanol (6.6 g, 52.0 mmol) and pyridine (7.53 g, 95.2 mmol) at 0 °C. The mixture was stirred at 25 °C for 24 h and filtered to remove the generated precipitates. The filtrate was concentrated under reduced pressure, the residual liquid purified by silica gel column chromatography using the mixed eluent of petroleum/ethyl acetate (v/v = 8/1) to give a colorless liquid (di-F_3) with a yield of 98%. ^{1}H NMR (400 Hz, $CDCl_3$, ppm): δ 4.17 (t, J = 6.3 Hz, 4H), 2.93 (t, J = 7.1 Hz, 4H), 2.76 (t, J = 7.1 Hz, 4H), 2.26-2.12 (m, 4H), 1.96-1.89 (m, 4H). ^{13}C NMR (100 Hz, $CDCl_3$, ppm): δ 171.46, 128.41, 125.59, 62.94, 33.78, 33.00, 30.65, 21.63.

Compound di-F_6 was prepared following the same procedure (yield = 83%). ^{1}H NMR (400 Hz, $CDCl_3$, ppm): δ 5.10-4.84 (m, 2H), 4.59-4.42 (m, 4H), 3.00-2.91 (m, 4H), 2.89-2.80 (m, 4H).

To prepare F_3-SH, dithiothreitol (5.4 g, 35 mmol) and triethylamine (3.5 g, 35 mmol) were added to compound di-F_3 (7.5 g, 17.4 mmol) dissolved in 35 mL DCM. The mixture was stirred for 2 h at 25 °C. After concentration under reduced pressure, the residual crude product was purified by silica gel column chromatography using a mixed eluent of petroleum/ethyl acetate (v/v = 6/1) to give the purified F_3-SH (5.7 g, 26.3 mol) as a colorless odor liquid, yield = 76%. ^{1}H NMR (400 Hz, $CDCl_3$, ppm): δ 4.18 (t, J = 6.3 Hz, 2H), 2.80 (dd, J = 7.7 Hz, 6.5 Hz, 2H), 2.76 (t, J = 6.5 Hz, 2H), 2.31-2.10 (m, 2H), 1.98-1.86 (m, 2H), 1.64 (t, J = 8.3 Hz, 1H). ^{13}C NMR (100 Hz, $CDCl_3$, ppm): δ 171.35, 128.35 (q, J = 275.9 Hz), 62.78, 30.54 (q, J = 29.3 Hz), 21.46, 19.57.

Compound F_6-SH was synthesized following the same procedure (yield = 56%). ^{1}H NMR (400 Hz, $CDCl_3$, ppm): δ 5.10-4.84 (m, 1H), 4.59-4.43 (m, 2H), 2.90-2.72 (m, 4H), 1.66 (t, J = 8.2 Hz, 1H). ^{13}C NMR (100 Hz, $CDCl_3$, ppm): δ 169.97, 118.57 (m), 85.04 (m), 83.10 (m), 60.76 (m), 37.85, 19.29.

Preparation of PMDXO

In a N_2-filled glovebox, 0.64 g MDXO (5.0 mmol, 40 equiv.), 157 mg DPP (0.63 mmol, 5 equiv.) and 125 μL BnOH (1.0 mol/L in toluene, 1 equiv.) were dissolved in toluene (2.0 mL) in a 10 mL vial containing a micro stirring bar, the mixture was stirred at 60 °C for 24 h. After concentration, the crude product was purified by precipitation in excess diethyl ether thrice. Next, the purified polymer product, 137 mg TEA (in 14 mL DCM) and 98 mg acetyl chloride (in 10 mL DCM) were mixed together, the mixture was stirred at 0 °C for 2 h. Then, the mixture was washed by deionized water thrice, the organic phase was dried over anhydrous Na_2SO_4 and concentrated, the residual crude product was purified by precipitation from diethyl ether thrice.

Critical aggregation concentration (CAC) measurements

The CAC of the amphiphilic cationic polyesters in deionized (DI) water was determined using pyrene as a probe. Briefly, pyrene in acetone solution (0.06 mM, 10 μL) were added to a volumetric flask and the solvent was removed by volatilization. A desired volume of polymer solution (10 mg/mL) was added into the flask and diluted with DI water to a total volume of 10 mL. After equilibration at ambient temperature for 24 h, the fluorescence spectrum of the polymer solution was determined. The intensity ratio of I_{338}/I_{333} from the excitation spectra was plotted against polymer concentration, and CAC was determined as the inflection point of the obtained plots.

Cytotoxicity measurements.

CCK-8 assay was performed to evaluate the cytocompatibility of the cationic polyesters following the procedure in literature.[1] Poly(ethylene glycol) (PEG) and branched polyethylenimine (PEI, 25 kDa) were used as the negative and positive controls, respectively. The cells (293T, 1 x 10^4 cells/well) seeded in 96-well plates were co-incubated with polymer sample at 37 °C under a 5% CO_2 humidified atmosphere for 12 h prior to CCK-8 assay.

Table S1 Characterization results of cationic polyesters (PNs) with different substitution degree [a]

Sample	$[ene]_0/[NMe_3\text{-}SH]_0$ in feed [b]	Time (min)	NMe_3% in polymer [c]	Yield (%)
PN2	1/0.4	15	37	99
PN3	1/0.5	15	50	98
PN4	1/0.6	15	61	98
PN5	1/0.8	30	77	99
PN6	1/1	120	87	99
PN7	1/1.2	120	92	98
PN8	1/1.8	120	~100	98

[a] Reaction condition: $[ene]_0/[TEA]$ = 1/0.3, 25 °C in DMSO. [b] Molar feed ratio. [c] Percent molar content of quaternary ammonium group (NMe_3^+) in the purified cationic polyesters. The values were determined by comparing the integration intensities of peaks c' and k in the ^1H NMR spectra (Fig. S9).

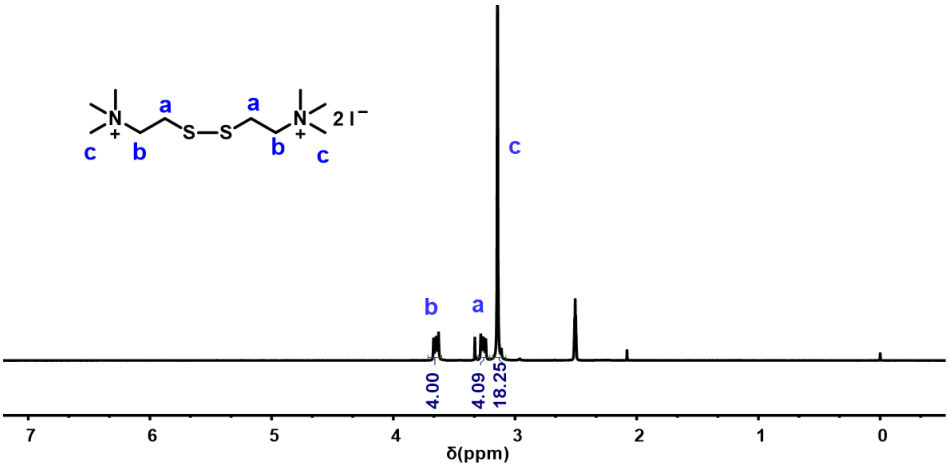


Fig. S1 ^{1}H NMR spectrum of di-NMe$_3$ in DMSO-d_6.

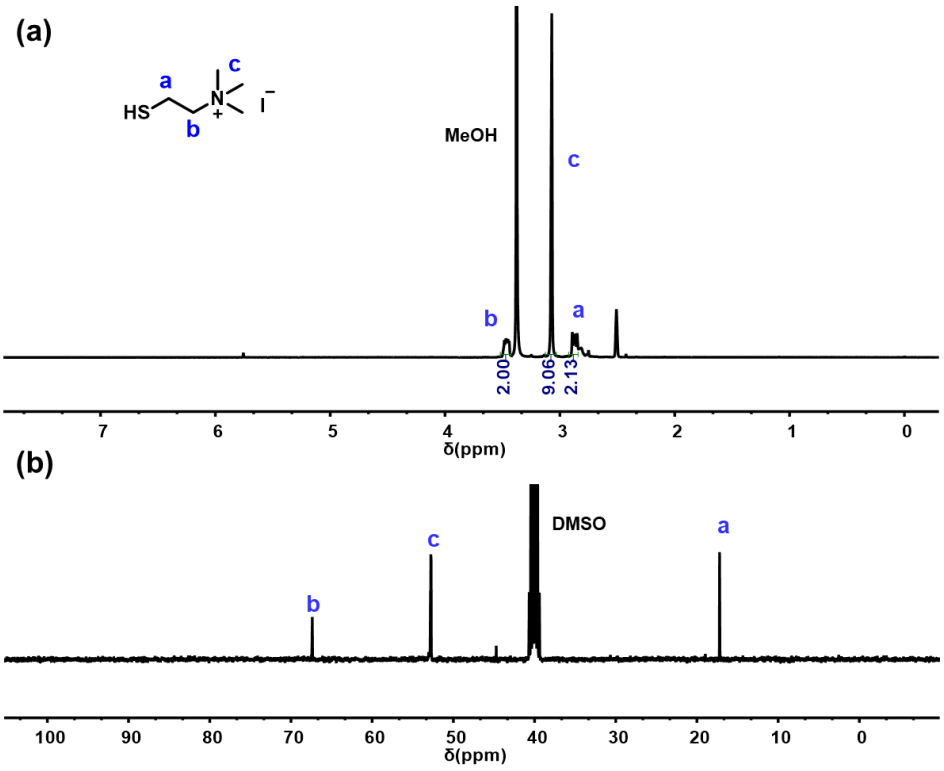


Fig. S2 ^{1}H and ^{13}C NMR spectra of thiocholine iodide (NMe$_3$-SH) in DMSO-d_6.

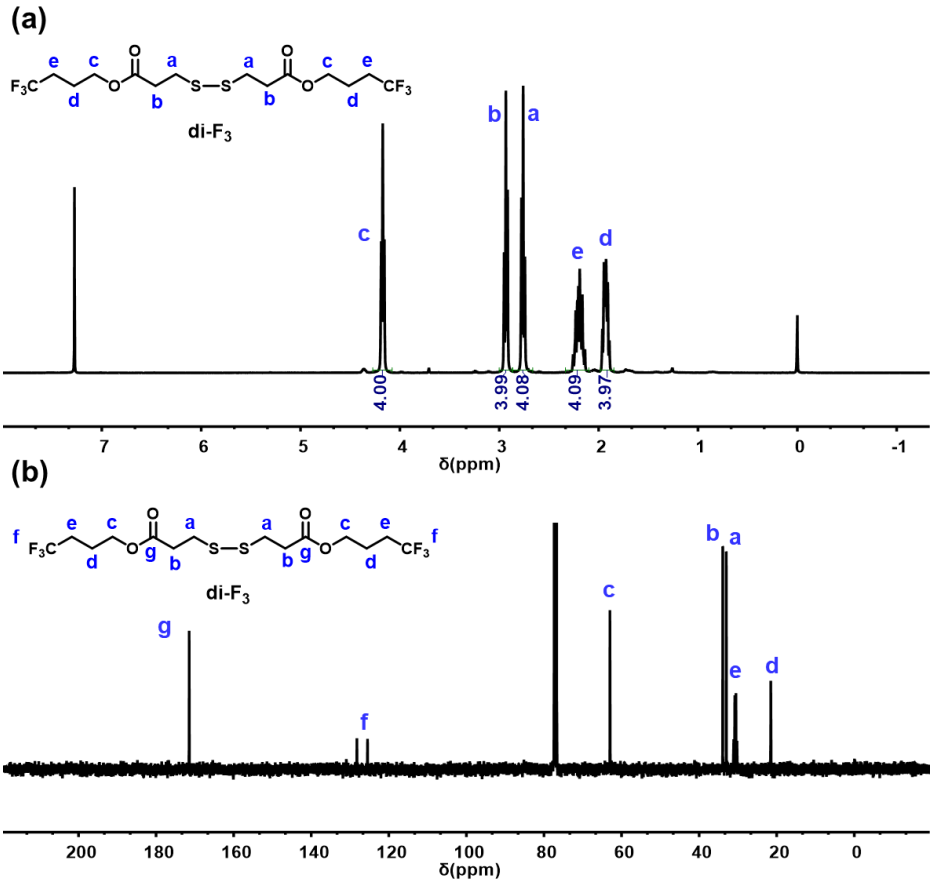


Fig. S3 ^{1}H and ^{13}C NMR spectra of di-F_3 in $CDCl_3$.

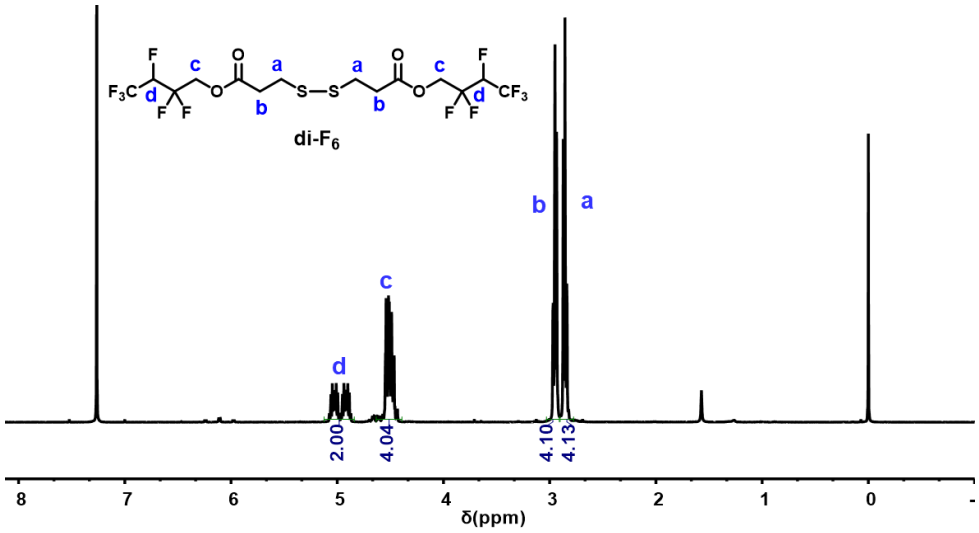


Fig. S4 ^{1}H NMR spectrum of di-F_6 in $CDCl_3$.

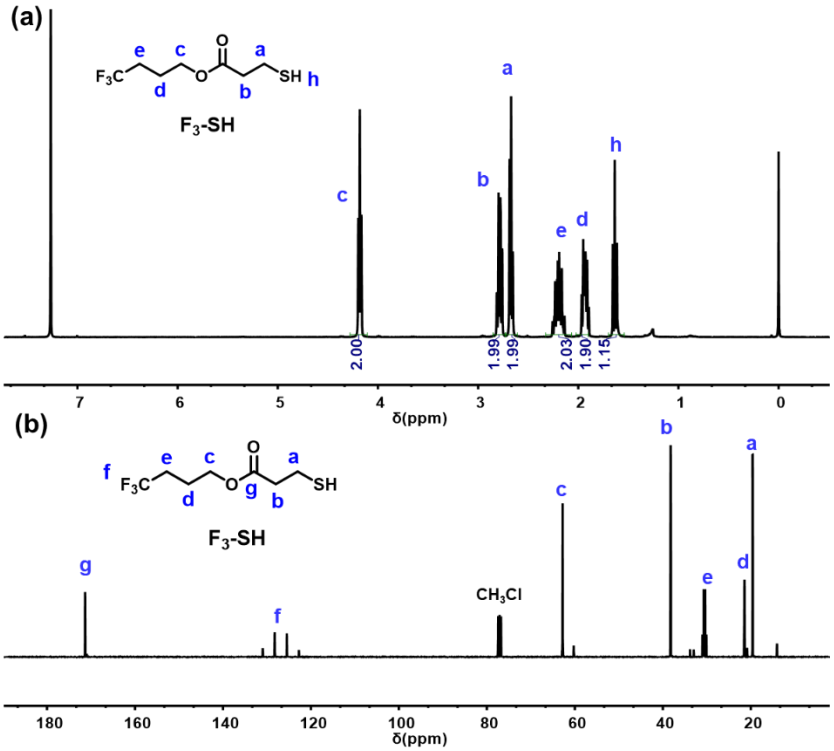


Fig. S5 ^{1}H and ^{13}C NMR spectra of F_3-SH in $CDCl_3$.

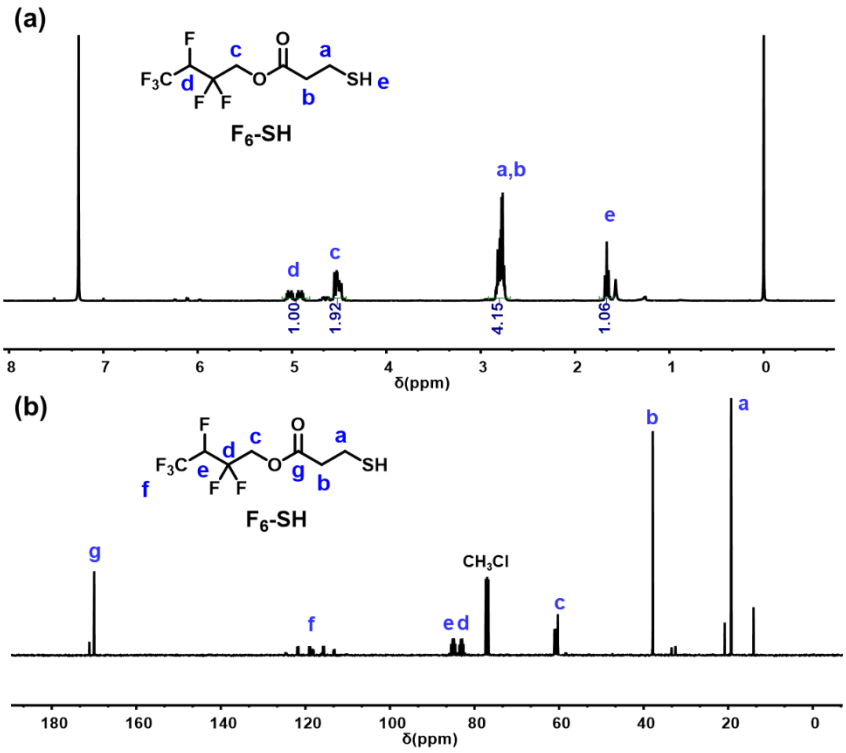


Fig. S6 ^{1}H and ^{13}C NMR spectra of F_6-SH in $CDCl_3$.

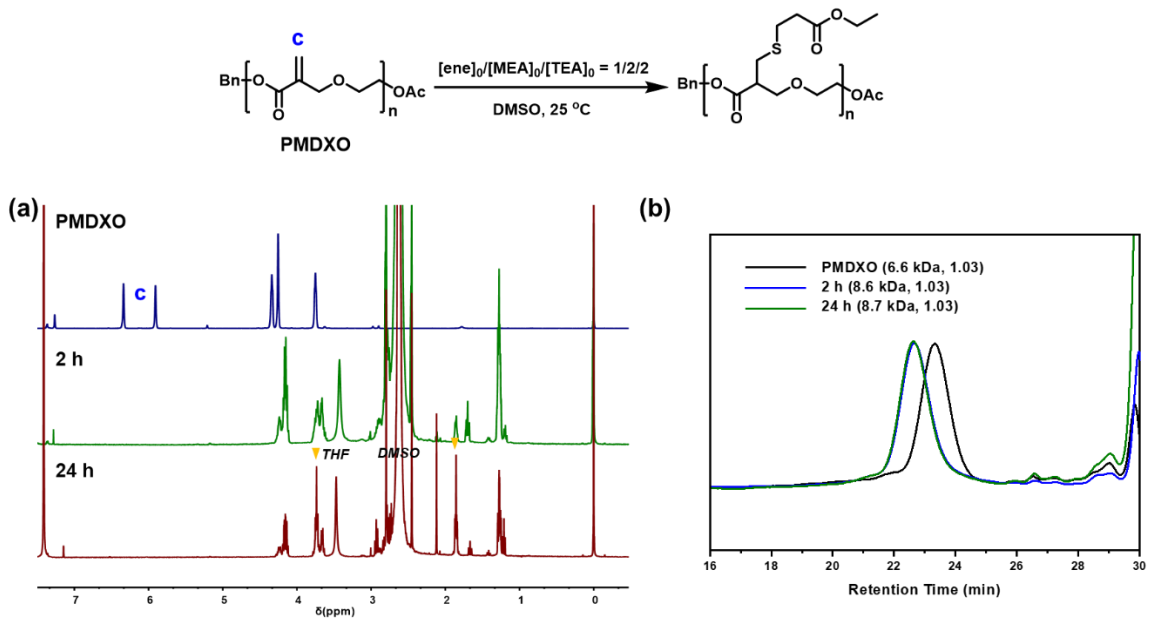


Fig. S7 ^{1}H NMR spectra and SEC traces of PMDXO modified by MEA.

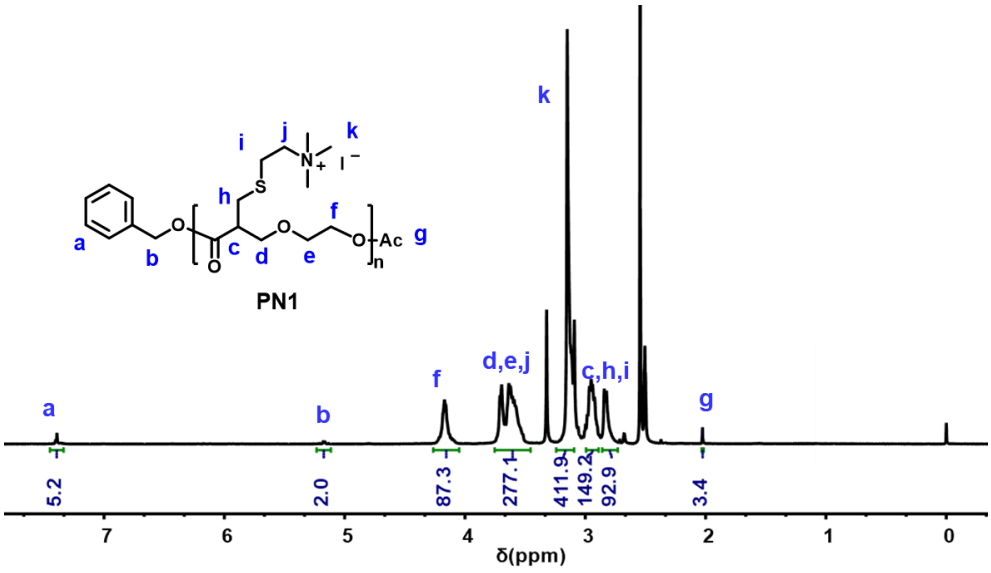


Fig. S8 ^{1}H NMR spectrum of cationic polyester PN1. Reaction condition: $[ene]_0/[NMe_3\text{-}SH]_0/[TEA]$ = 1/2/2, 25 °C, 2 h, in DMSO.

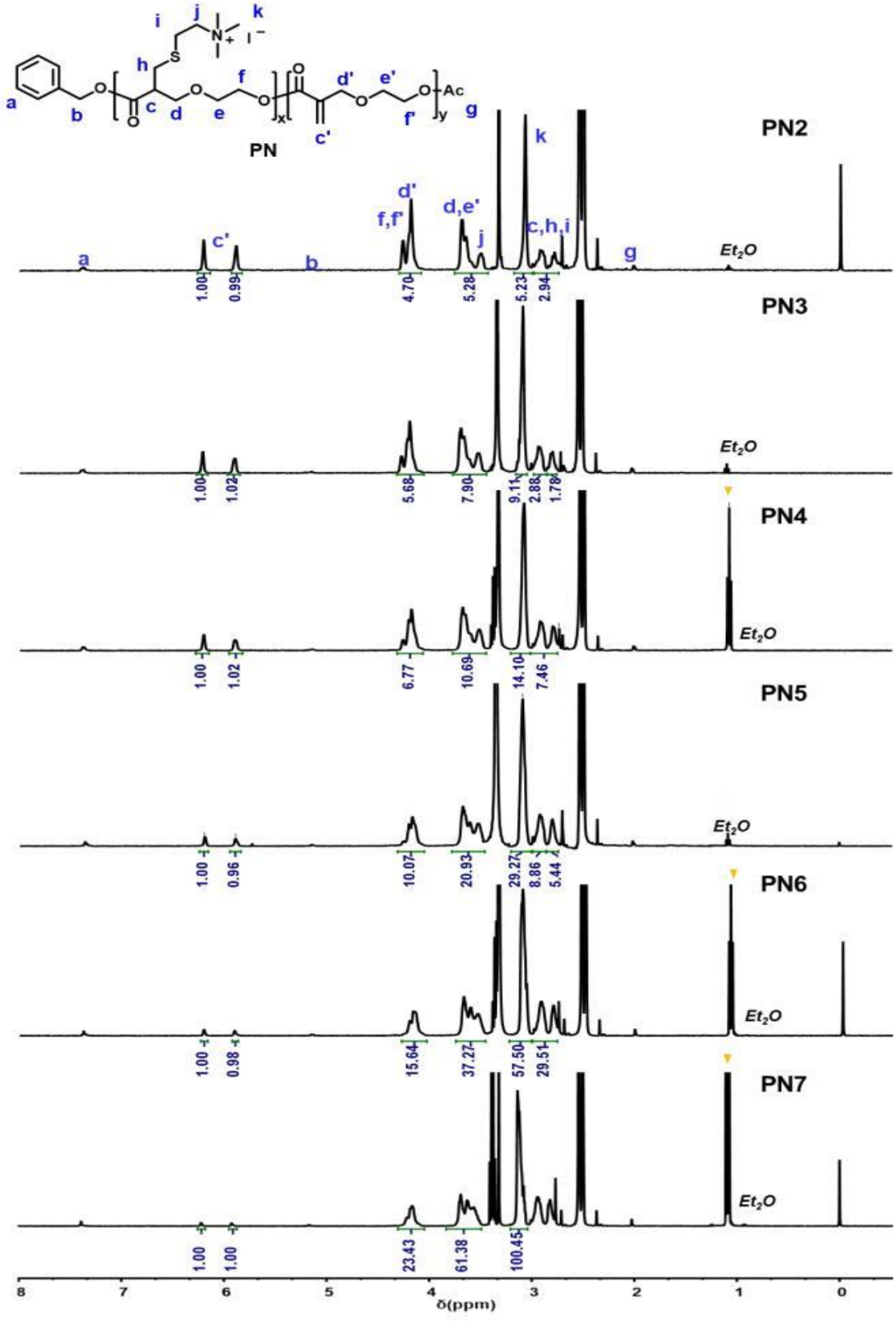


Fig. S9 ^{1}H NMR spectra of cationic polyesters (PNs) with different substitution degree. Reaction condition: $[ene]_0/[TEA]$ = 1/0.3, 25 °C in DMSO.

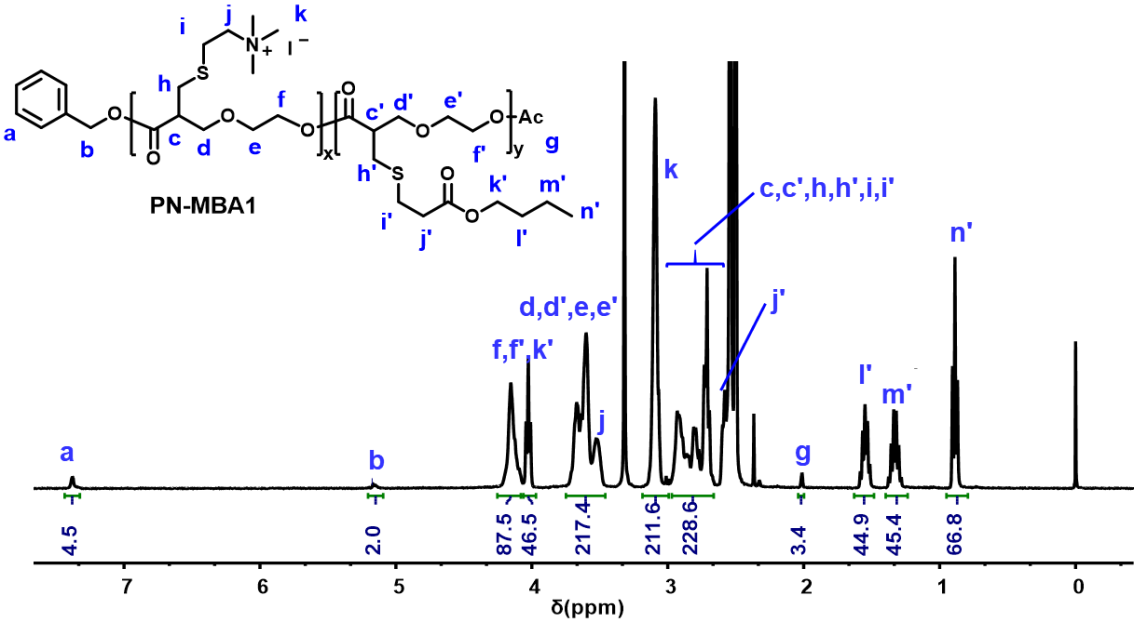


Fig. S10 ^{1}H NMR spectrum of PN-MBA1 in DMSO-d_6. The molar ratio of NMe_3 to n-butyl ester was determined by comparing the peak intensities of k (~3.1ppm) and l' (~1.6 ppm).

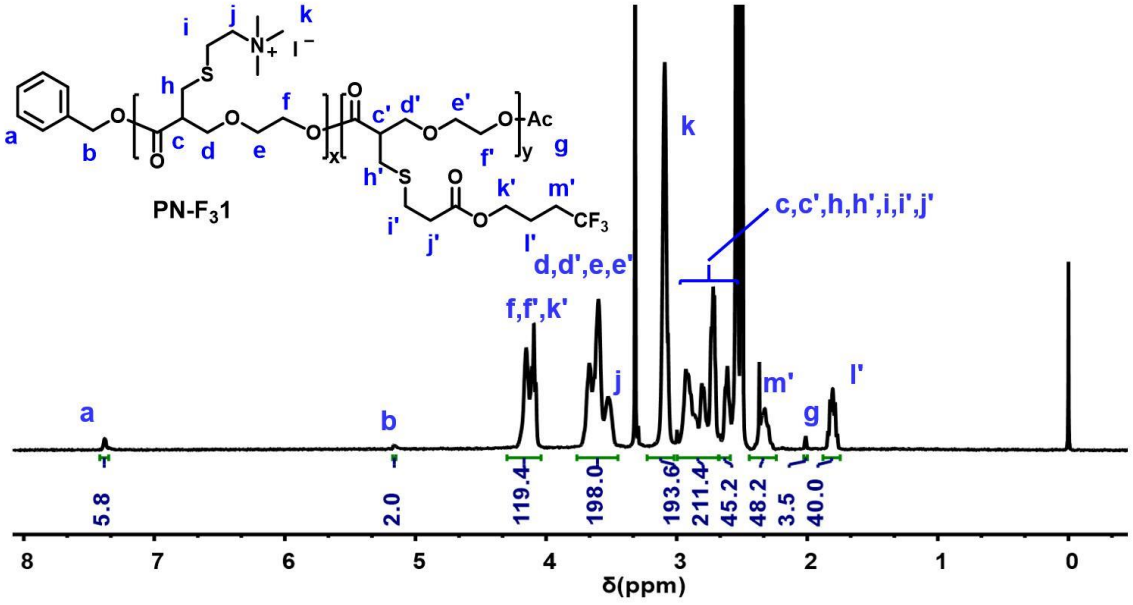


Fig. S11 ^{1}H NMR spectrum of PN-F$_3$1 in DMSO-d_6. The molar ratio of NMe_3 to 4,4,4-trifluoro-1-butyl ester was determined by comparing the peak intensities of k (~3.1ppm) and l' (~1.8 ppm).

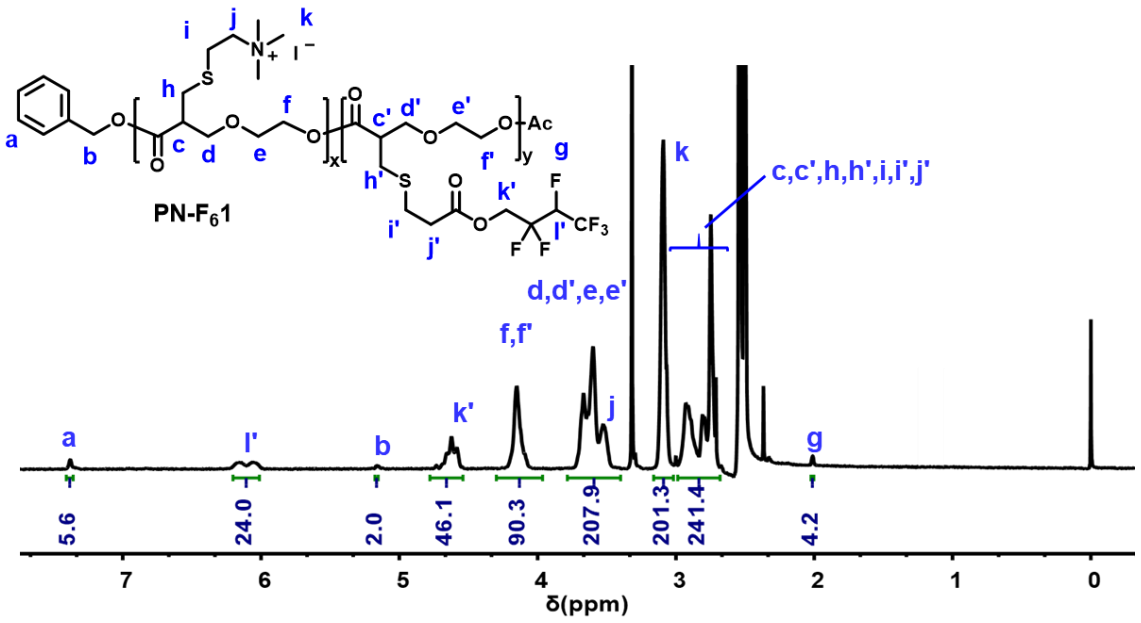


Fig. S12 ^{1}H NMR spectrum of PN-F$_6$1 in DMSO-d_6. The molar ratio of NMe$_3$ to 2,2,3,4,4,4-hexafluoro-1-butyl ester was determined by comparing the peak intensities of k (~3.1ppm) and k' (~4.6 ppm).

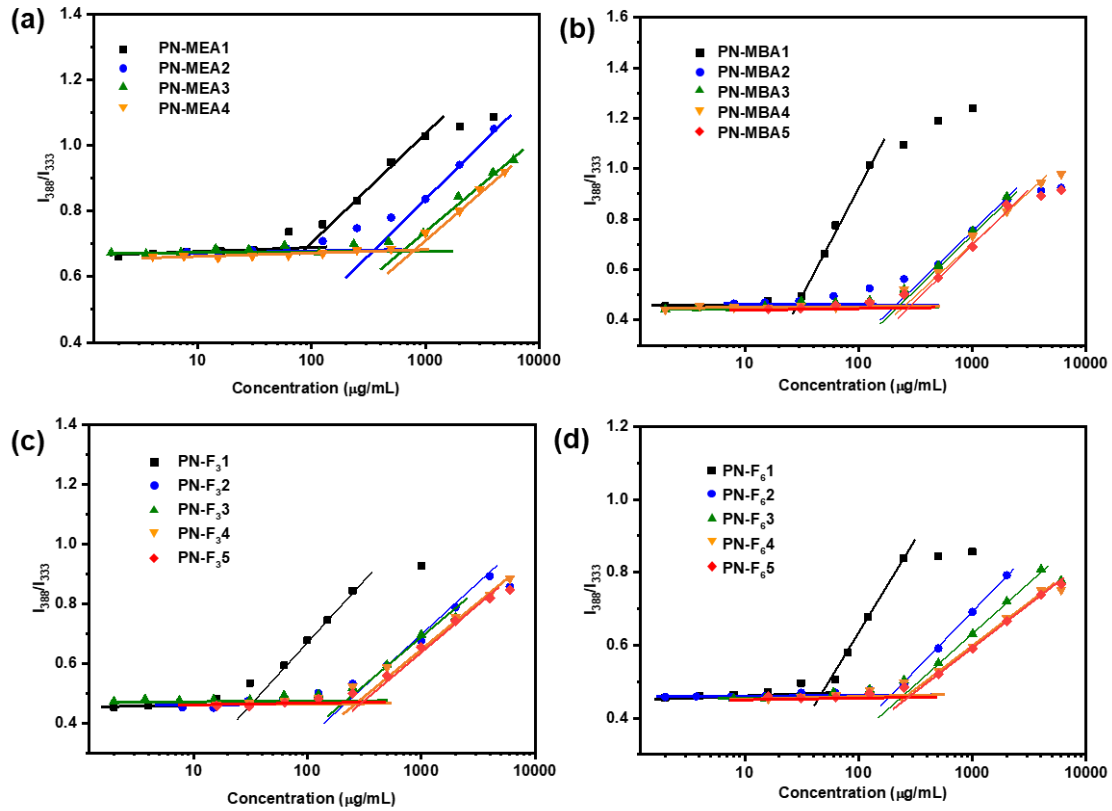
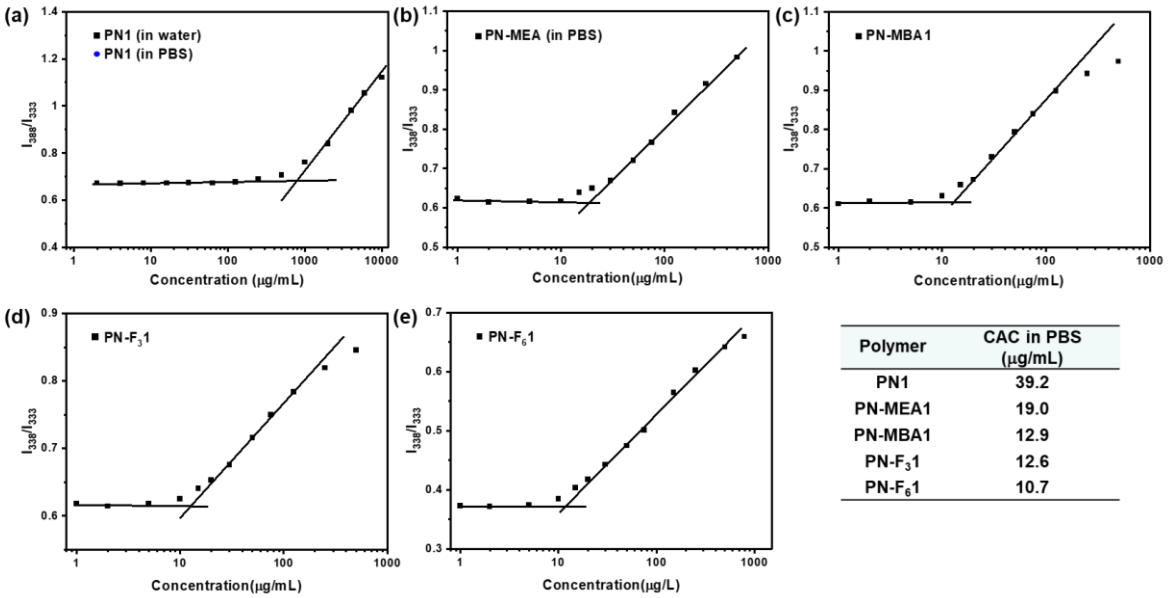


Fig. S13 The plots of I_{338}/I_{333} vs polymer concentration obtained from the pyrene excitation spectra in deionized water. Condition: 25 °C， concentration of pyrene = 6.0 × 10^{-7} mol/L.



Polymer	CAC in PBS (μg/mL)
PN1	39.2
PN-MEA1	19.0
PN-MBA1	12.9
PN-$F_3$1	12.6
PN-$F_6$1	10.7

Fig. S14 The plots of I_{338}/I_{333} vs polymer concentration obtained from the pyrene excitation spectra in PBS (pH = 7.4， 10 mM). Condition: 25 °C， concentration of pyrene = 6.0×10^{-7} mol/L.

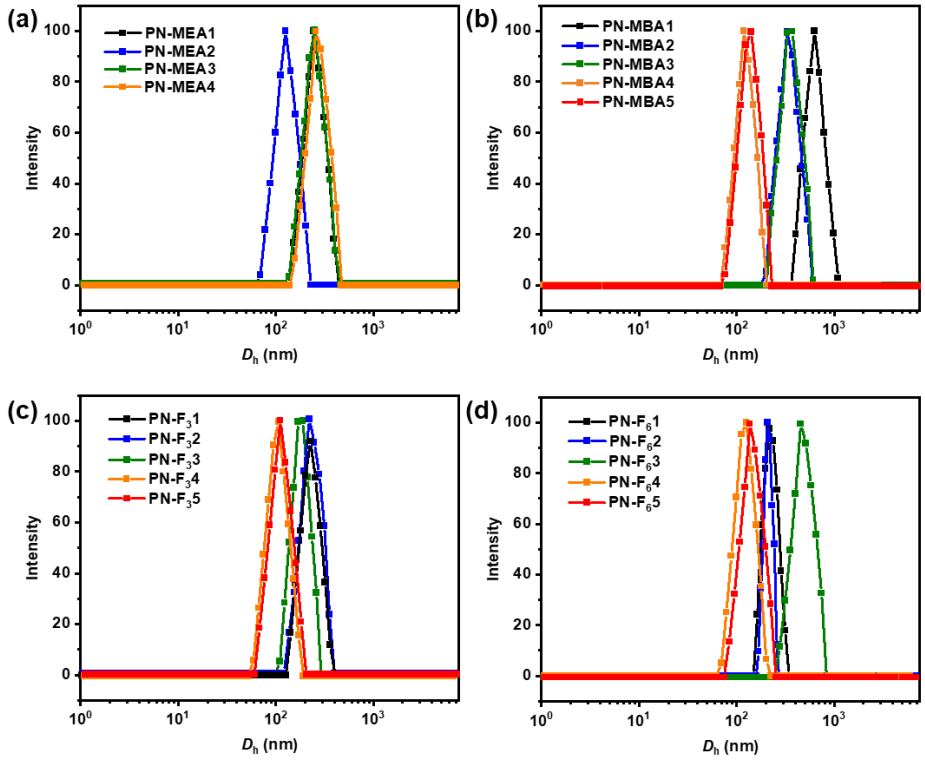


Fig. S15 Size distribution of the amphiphilic cationic polyester aggregates in water measured by DLS. Condition: 0.5 mg/mL, 25 °C.

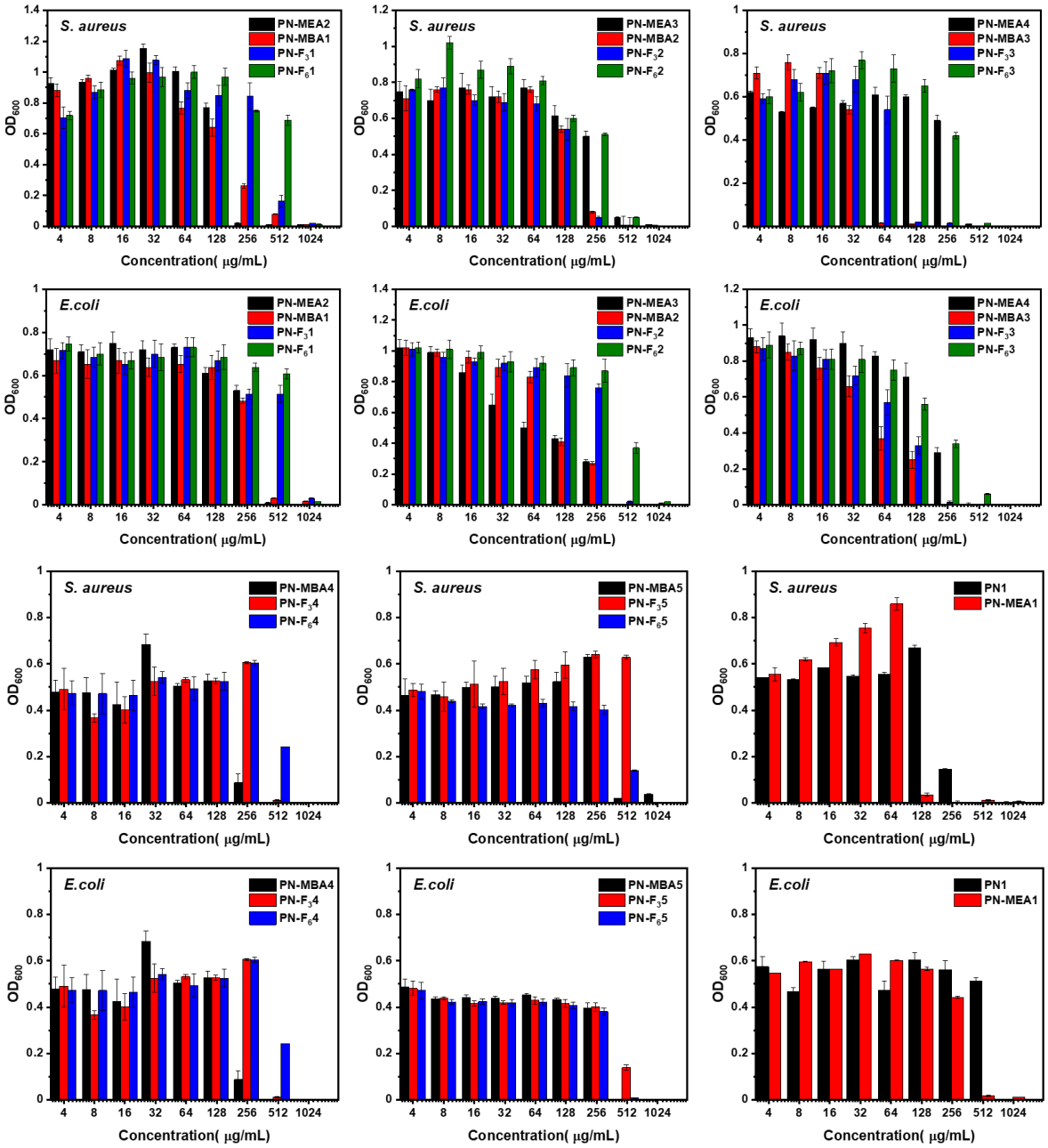


Fig. S16 MIC measurements of the dually modified amphiphilic cationic polyesters against *S. aureus* and *E. coli*.

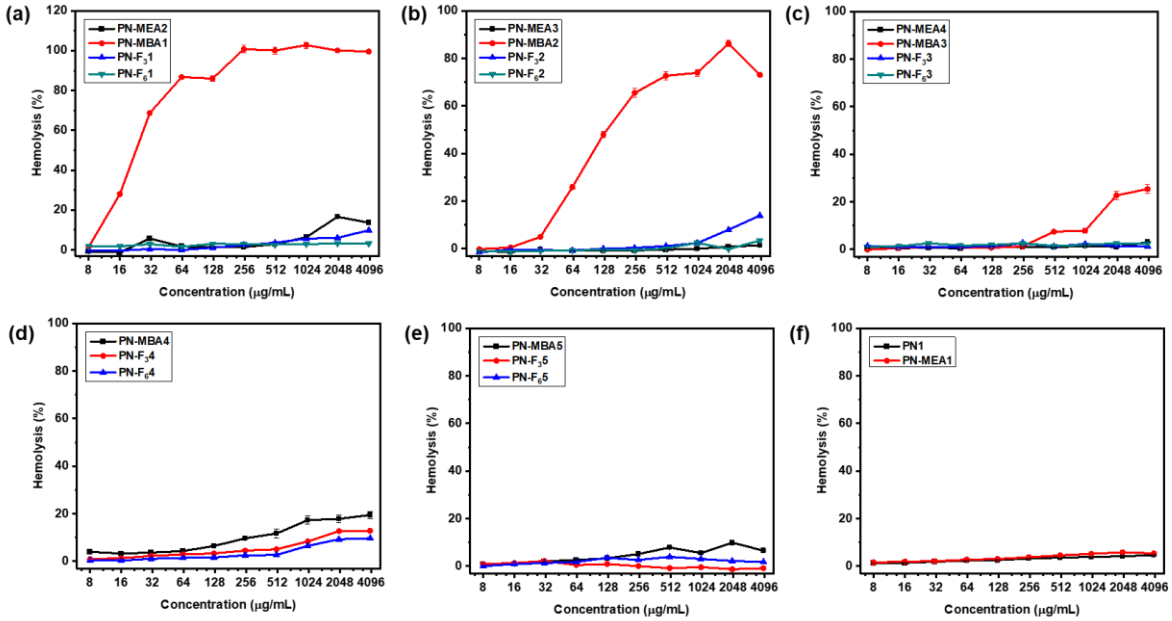


Fig. S17 Hemolysis assays of the dually modified amphiphilic cationic polyesters.

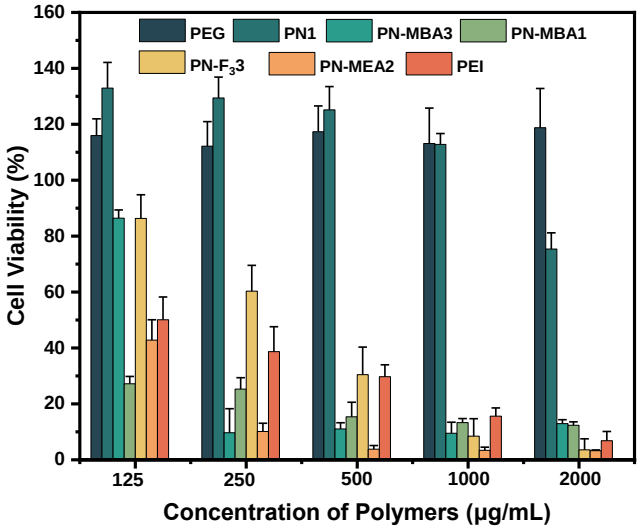


Fig. S18 Cell viability of 293T measured by CCK-8 assay at 37 °C. The cells were incubated with different concentrations of polymers for 12 h. Results are presented as the mean ± standard deviation in triplicate.

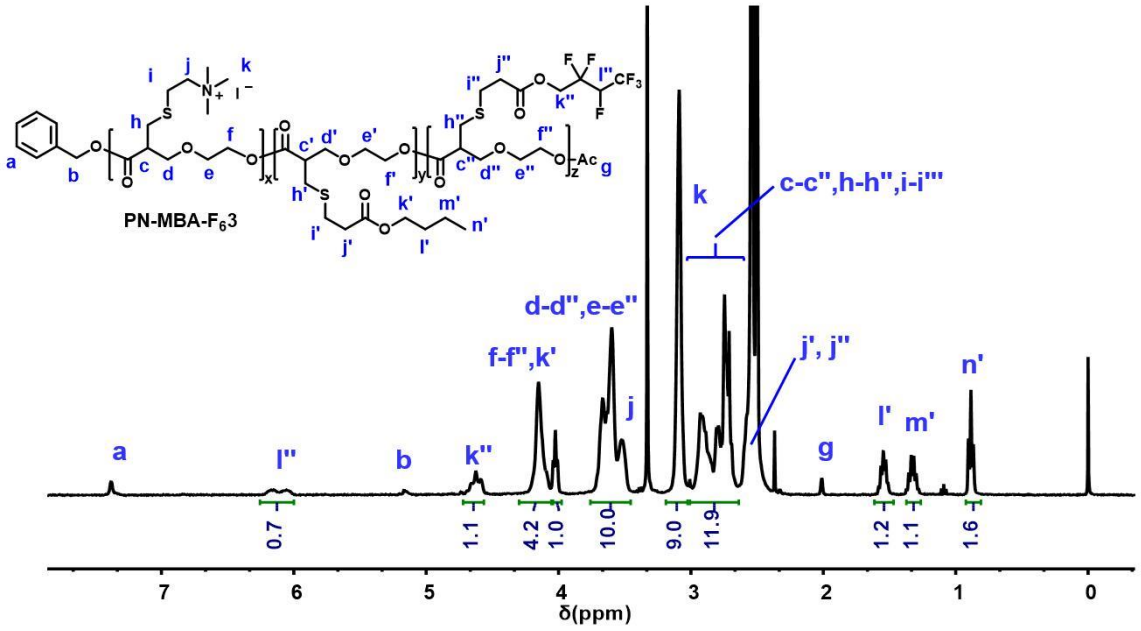


Fig. S19 Representative ^{1}H NMR spectrum of the triply modified amphiphilic cationic polyester PN-MBA-F$_6$3 in DMSO-d_6. The copolymer composition was determined by comparing the peak intensities of k" (~4.6ppm), k (~3.1ppm) and l' (~1.8 ppm).

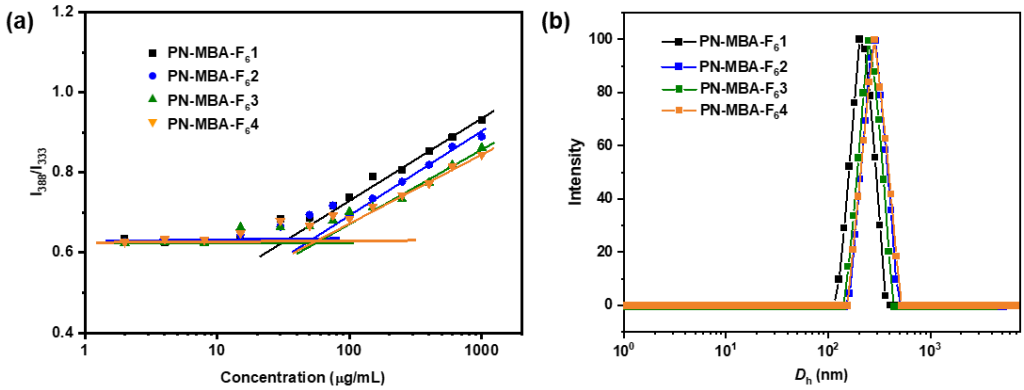


Fig. S20 (a) The plots of I_{338}/I_{333} vs polymer concentration for the triply modified polyesters in deionized water. Condition: 25 °C，concentration of pyrene = 6.0 × 10^{-7} mol/L. (b) Size distribution of the triply modified polyester aggregates in water measured by DLS. Condition: 0.5 mg/mL, 25 °C

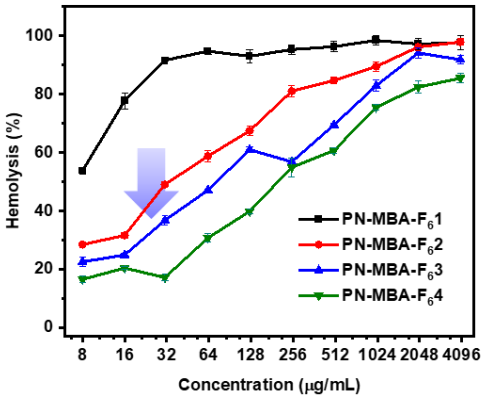


Fig. S21 Hemolysis assays of the triply modified polyesters

Reference.

1. L. Yu, H. L. Ke, F. S. Du and Z. C. Li, *Biomacromolecules,* **2019**, 20, 2809-2820.