

## Supporting Information

# Manipulating the Membrane Penetration Mechanism of Helical Polypeptides via Aromatic Modification for Efficient Gene Delivery

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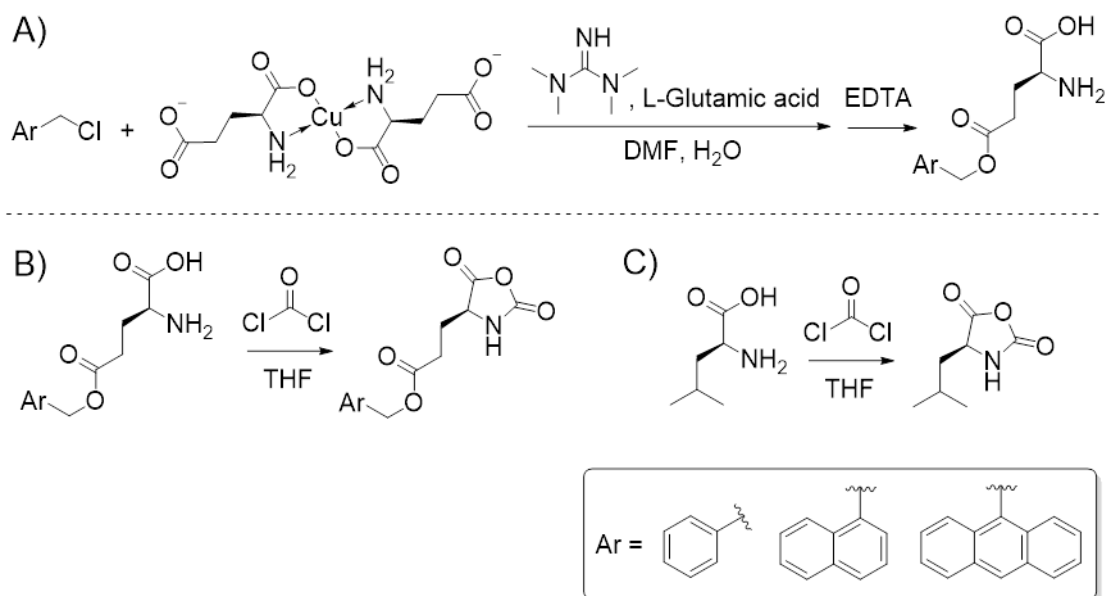
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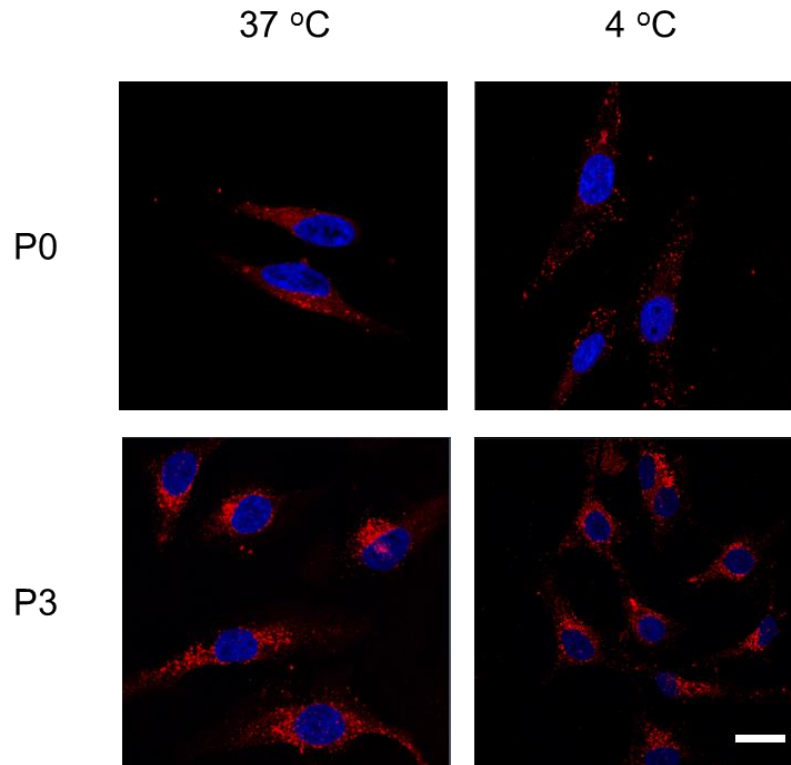


**Scheme S1.** Synthetic routes of aromatic glutamate (A), aromatic glutamate based NCA monomers (B), and the L-Leu-NCA monomer (C).

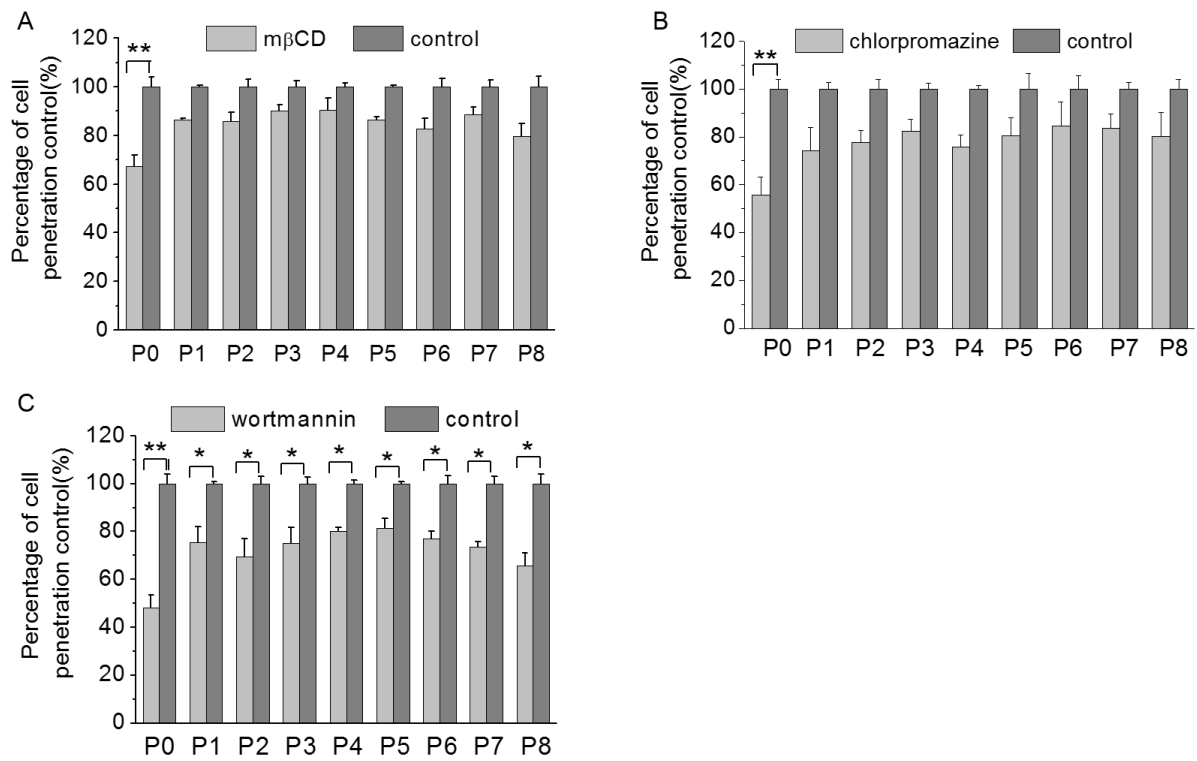
**Table S1.** Secondary conformational analysis of polypeptides

Name	$-[\theta]_{222} \times 10^{-3}$ (cm <sup>2</sup> deg dmol <sup>-1</sup> ) <sup>a)</sup>	Helical content (%) <sup>b)</sup>
P0	30.0	84.7
P1	27.6	78.3
P2	24.6	70.8
P3	27.2	77.4
P4	22.6	65.6
P5	31.4	88.3
P6	32.9	92.0
P7	27.5	78.3
P8	19.9	58.7

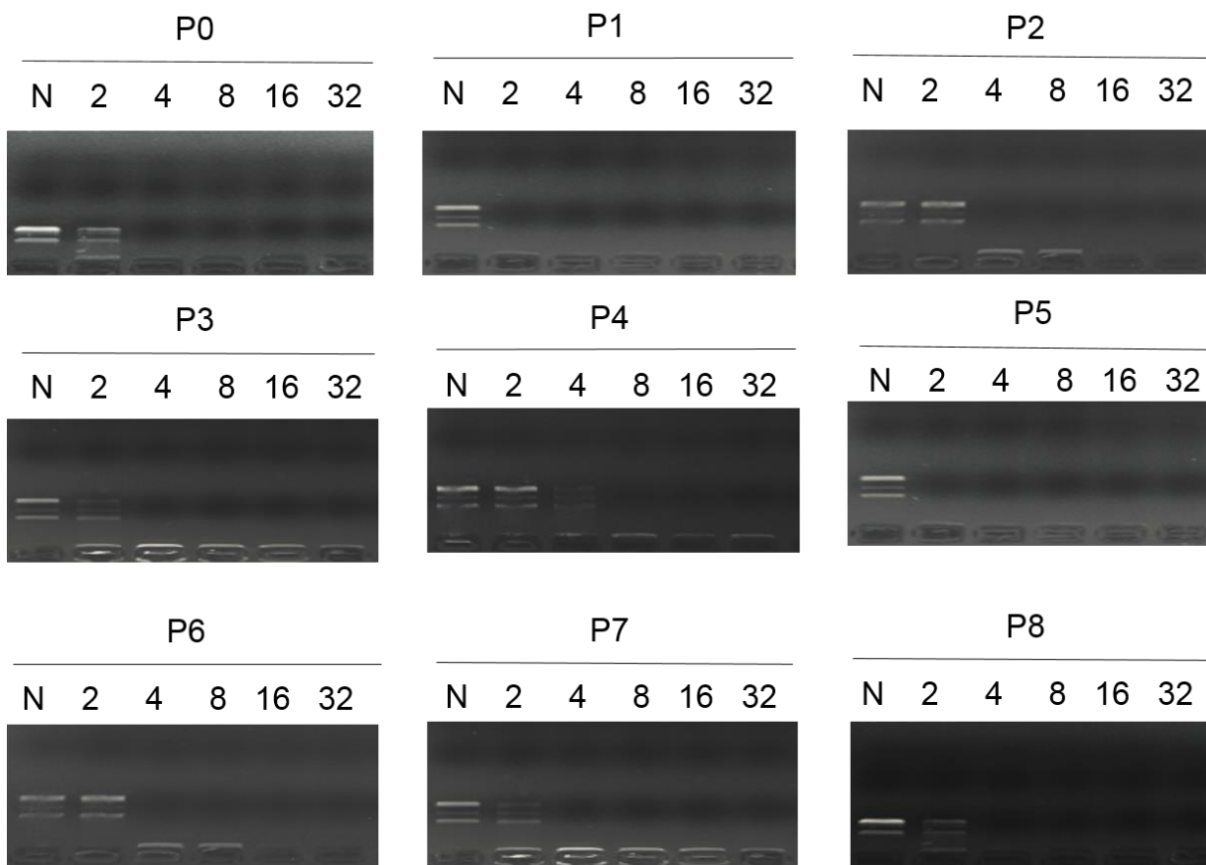
<sup>a)</sup> The mean residue ellipticity  $[\theta]$  was determined by following formula: Ellipticity ( $[\theta]$  in cm<sup>2</sup> deg dmol<sup>-1</sup>) = (millidegrees  $\times$  mean residue weight) / (path length in mm  $\times$  concentration of polypeptide in mg ml<sup>-1</sup>). <sup>b)</sup> The helical contents of the polypeptides were calculated by the following equation: helical content =  $(-[\theta_{222}] + 3000) / 39000$ .



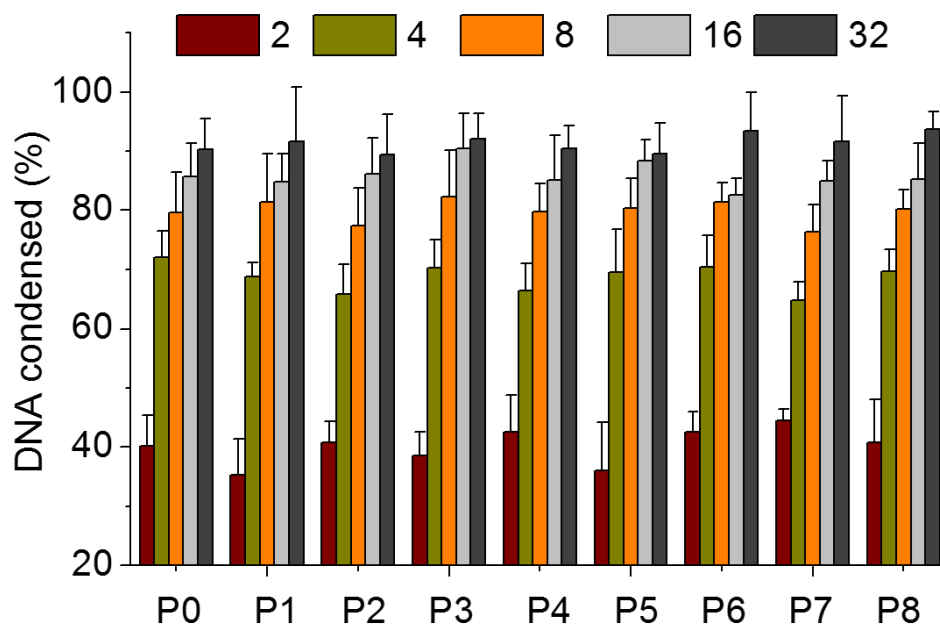
**Fig. S1.** CLSM images of HeLa cells following incubation with RhB-**P0** and RhB-**P3** at 37 °C or 4 °C for 2 h. Cell nuclei were stained with DAPI. Bar represents 20  $\mu$ m.



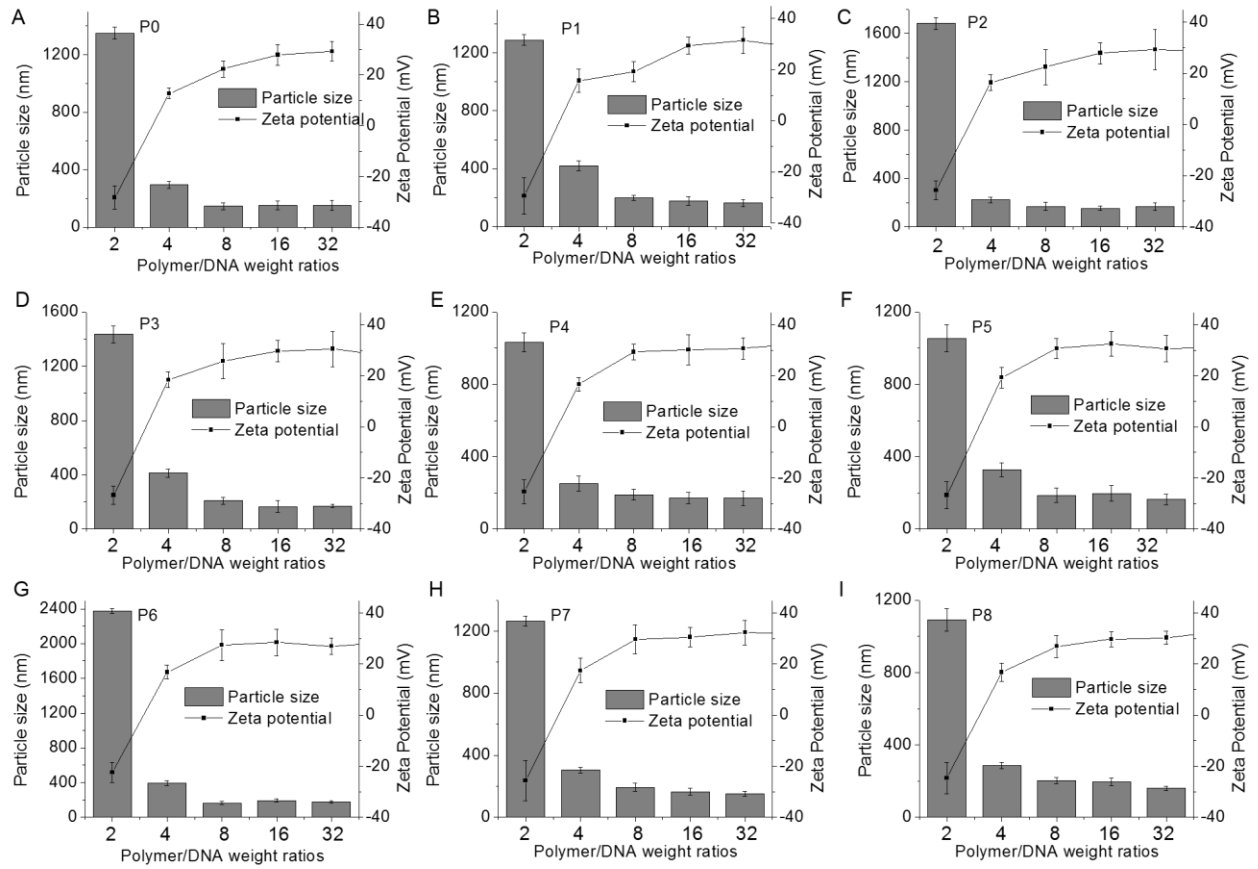
**Fig. S2.** Cell penetration levels of RhB-labeled polypeptides in HeLa cells in the presence of various endocytosis inhibitors including mβCD (A), chlorpromazine (B), and wortmannin (C) (n = 3).



**Fig. S3.** DNA condensation by polypeptides at various polypeptide/DNA weight ratios as evaluated by the gel retardation assay. N represents naked DNA.

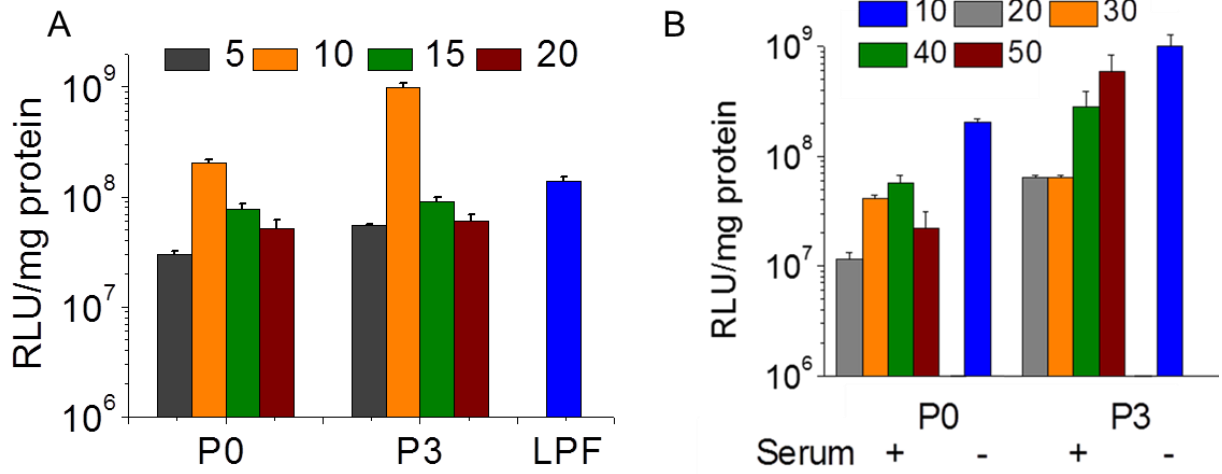


**Fig. S4.** DNA condensation by polypeptides at different polypeptide/DNA weight ratios as determined by the EB exclusion assay (n = 3).

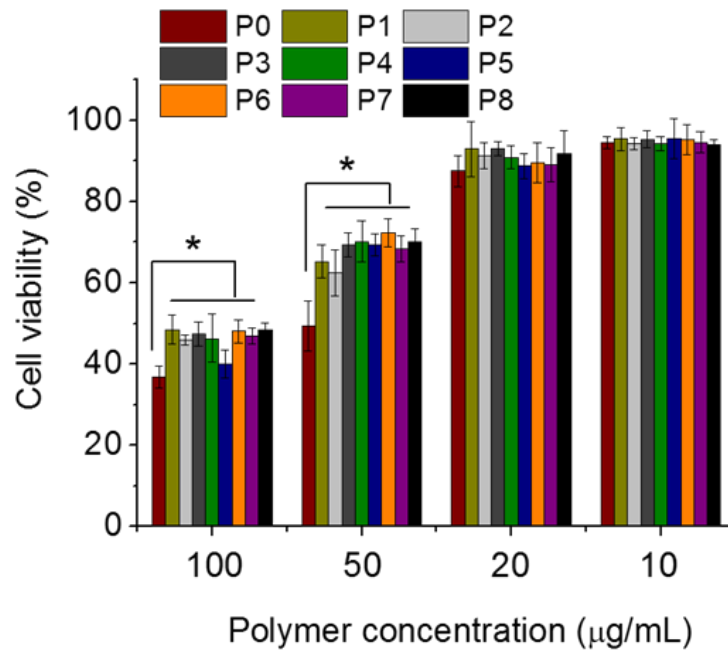


**Fig. S5.** Particle size and zeta potential of polypeptide/DNA complexes at different polypeptide/DNA weight ratios.

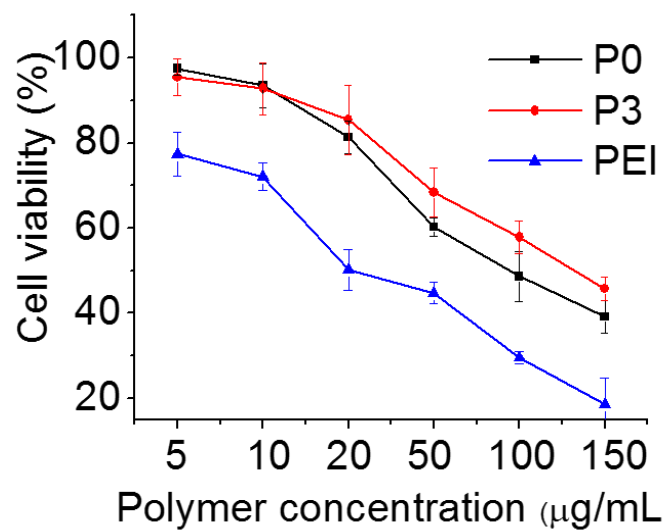




**Fig. S6.** Transfection efficiencies of **P0** and **P3** at various polypeptide/DNA weight ratios in B16F10 cells in the absence (A) or presence (B) of 10% serum (n = 3).

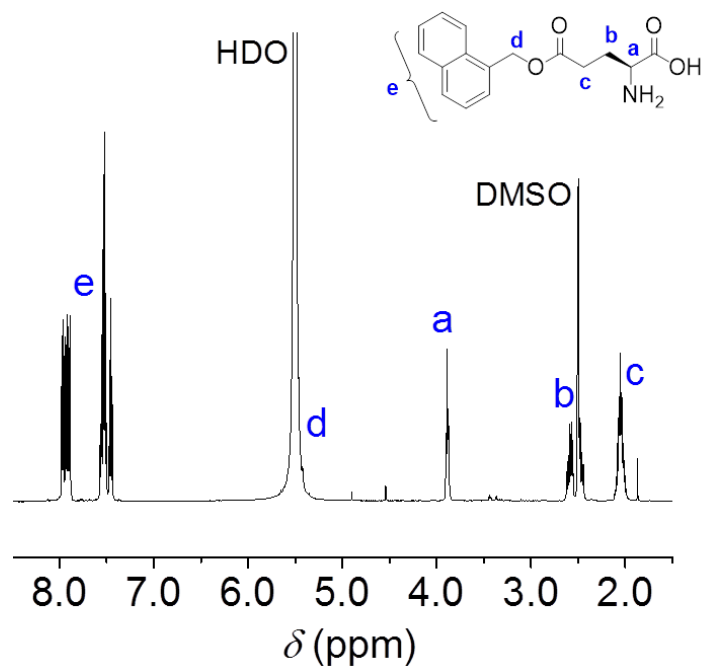


**Fig. S7.** Cytotoxicity of polypeptide/DNA polyplexes following 24-h incubation in HeLa cells as determined by the MTT assay (n = 3).

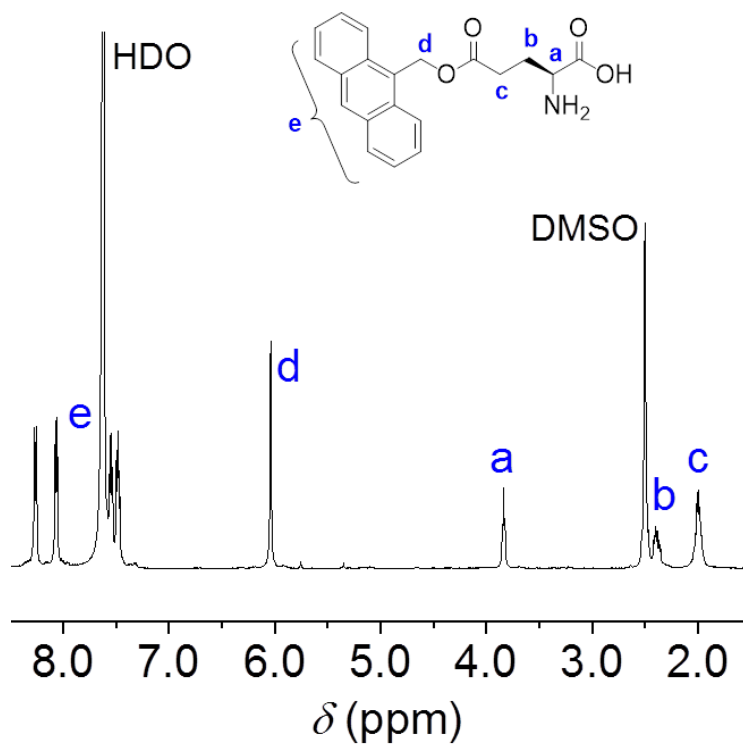


**Fig. S8.** Cytotoxicity of **P0**, **P3**, and PEI (25 kDa) at various concentrations in HeLa cells following 24-h incubation as determined by the MTT assay (n = 3).

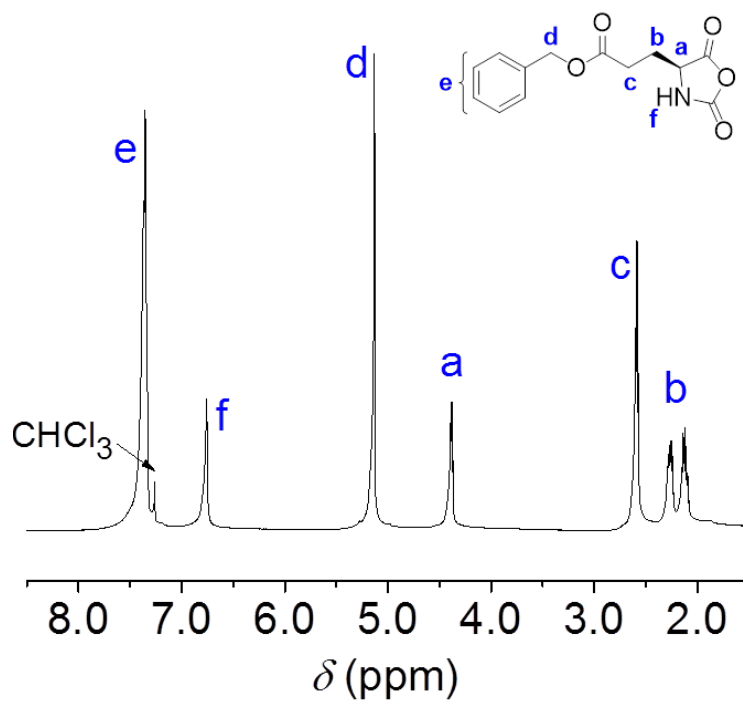
$^1\text{H}$  NMR spectra of new compounds.



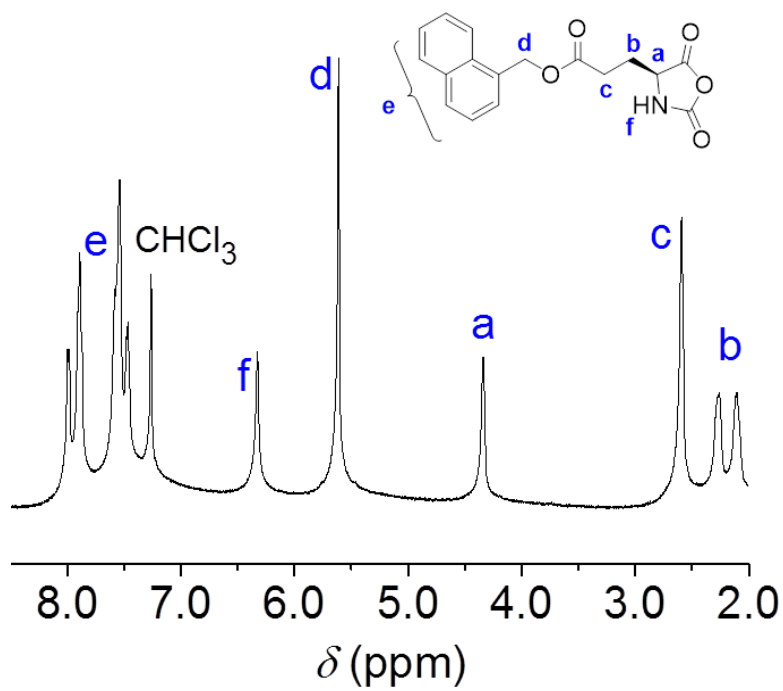
**Fig. S9.**  $^1\text{H}$  NMR spectrum of Naph-L-Glu in DMSO- $d_6$ :DCI- $\text{D}_2\text{O}$  (9:1, v/v).



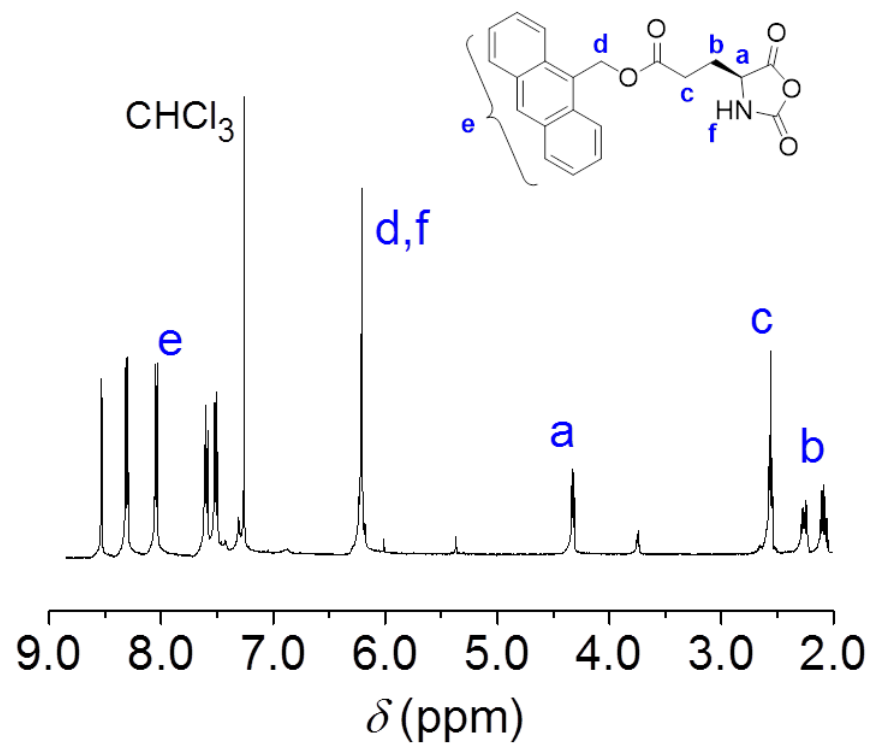
**Fig. S10.**  $^1\text{H}$  NMR spectrum of Anth-L-Glu in DMSO- $d_6$ :DCI- $\text{D}_2\text{O}$  (9:1, v/v).



**Fig. S11.** <sup>1</sup>H NMR spectrum of B-L-Glu-NCA in CDCl<sub>3</sub>.

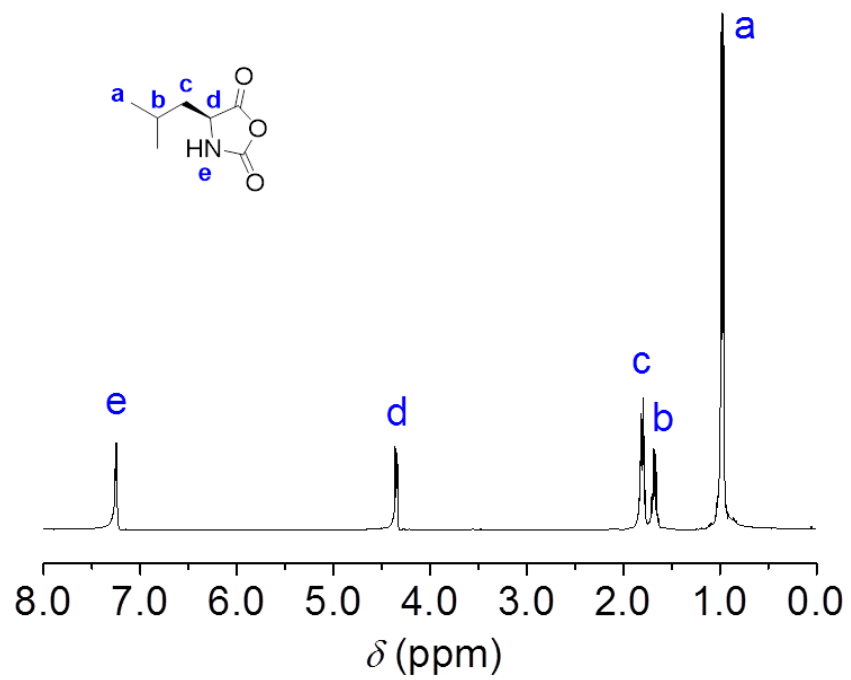


**Fig. S12.** <sup>1</sup>H NMR spectrum of Naph-L-Glu-NCA in CDCl<sub>3</sub>.

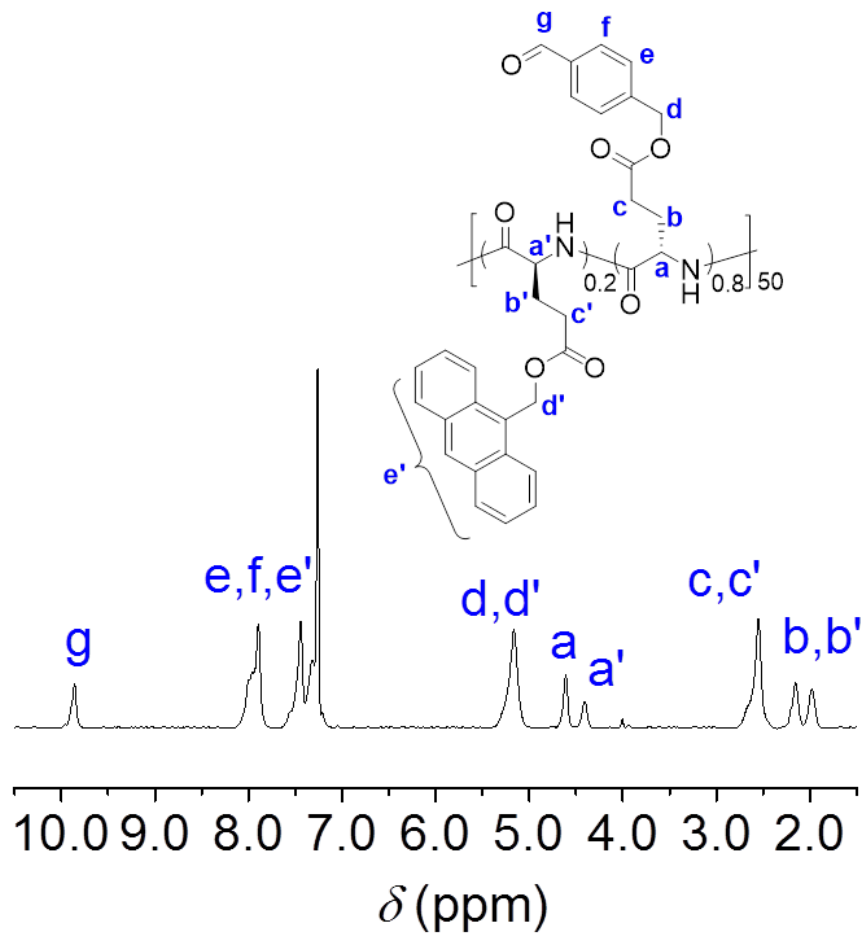


**Fig. S13.** <sup>1</sup>H NMR spectrum of Anth-L-Glu-NCA in CDCl<sub>3</sub>.

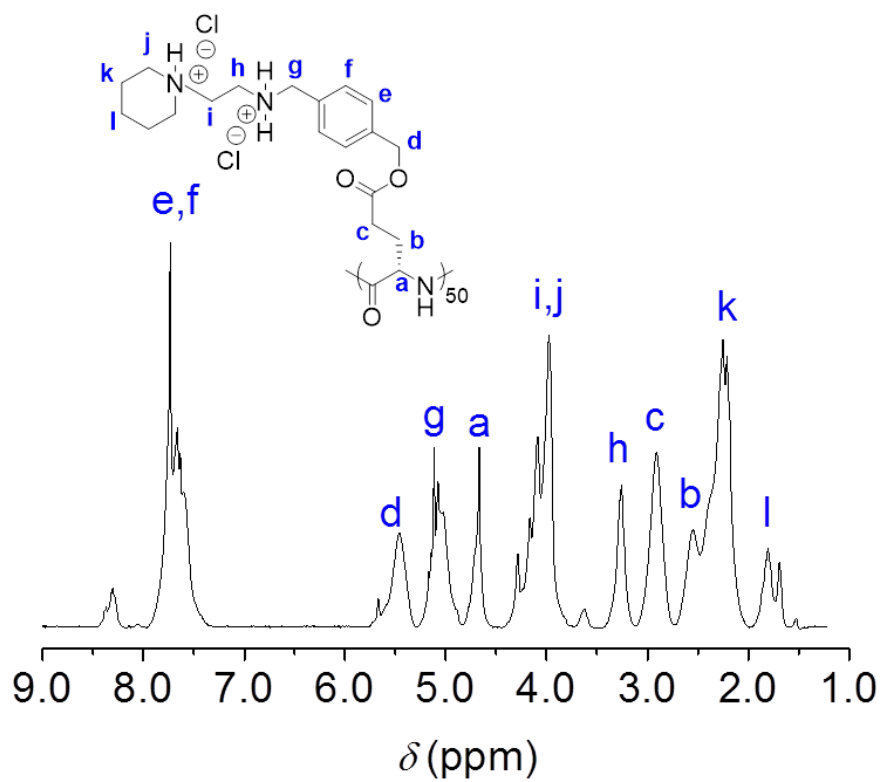




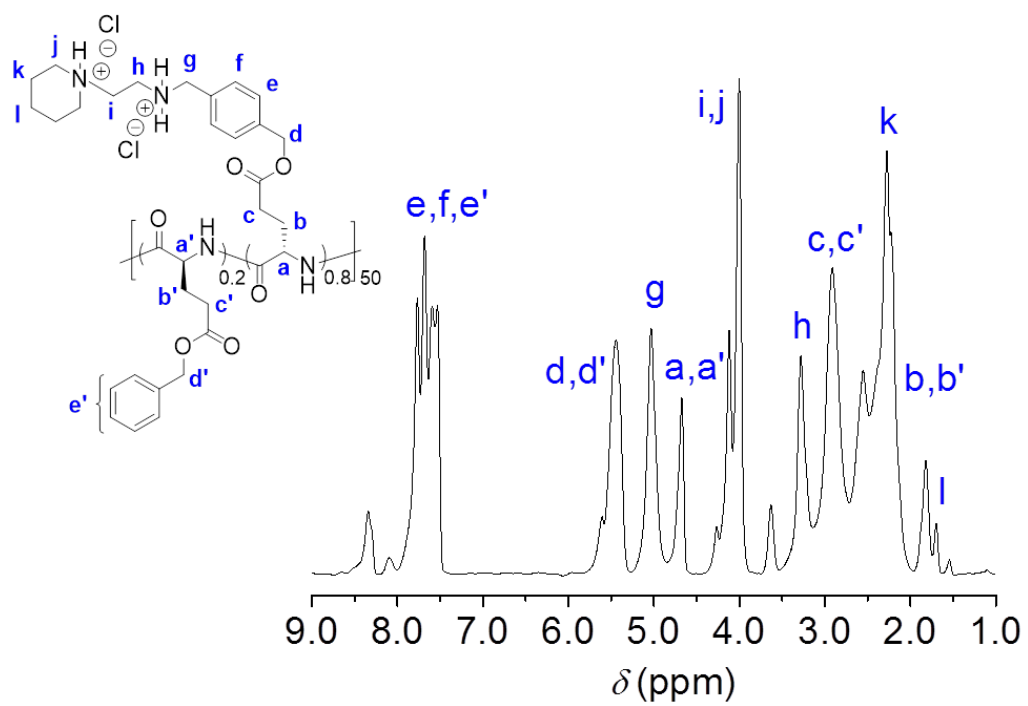
**Fig. S14.**  $^1\text{H}$  NMR spectrum of L-Leu-NCA in  $\text{CDCl}_3$ .



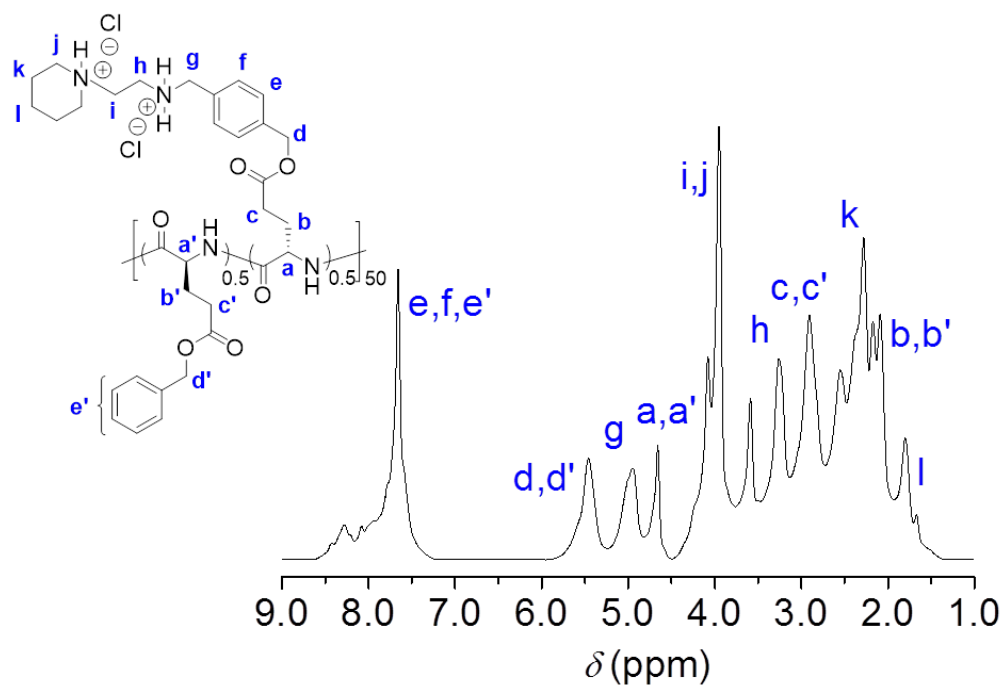
**Fig. S15.** Representative <sup>1</sup>H NMR spectrum of copolymer precursor for composition calculation (PALG-*r*-PABLG as an example, **P5** precursor) in CDCl<sub>3</sub>:TFA-*d* (85:15, v/v). The block composition was calculated by the integration ratio of the  $\alpha$ -protons in PALG residues (proton *a*) to the  $\alpha$ -protons in PABLG residues (proton *a'*).



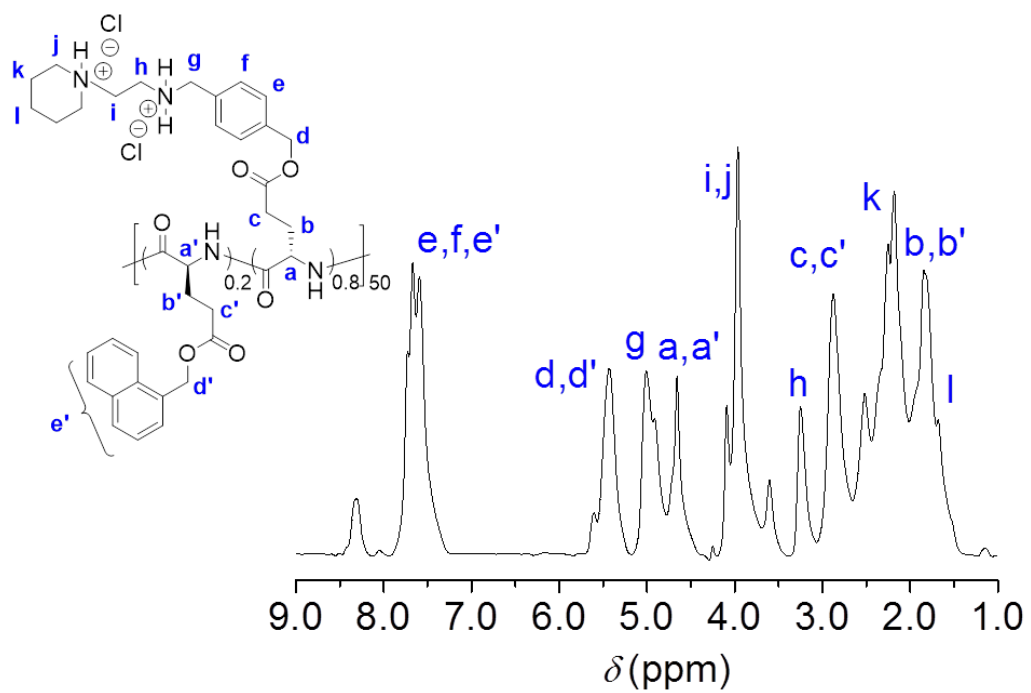
**Fig. S16.**  $^1\text{H}$  NMR spectrum of **P0** in  $\text{TFA-}d$ .



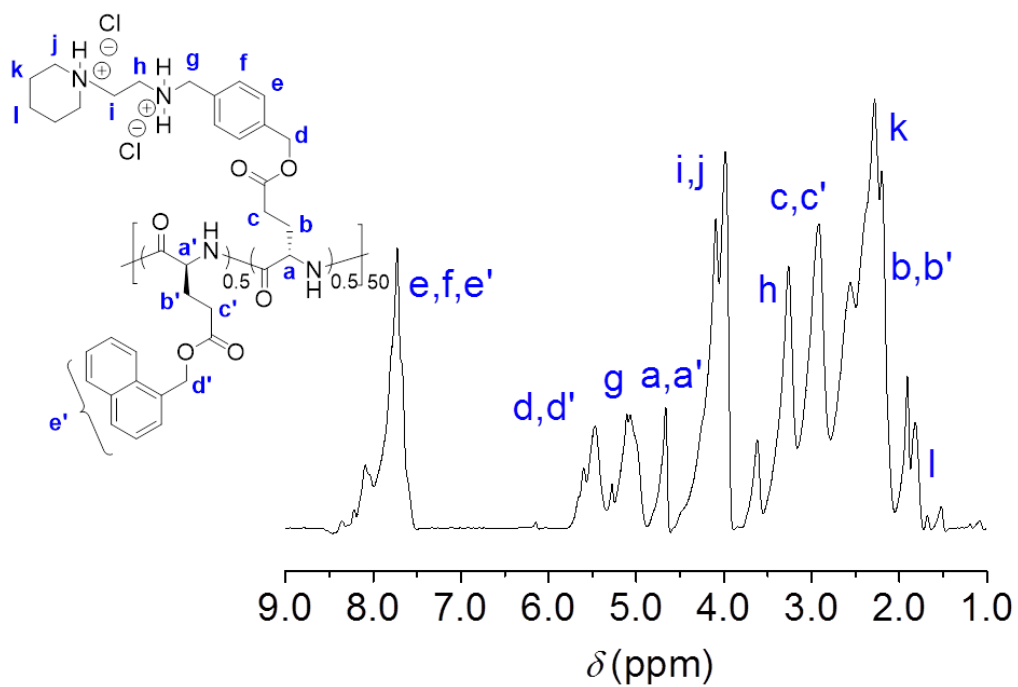
**Fig. S17.**  $^1\text{H}$  NMR spectrum of **P1** in  $\text{TFA-d}$ .



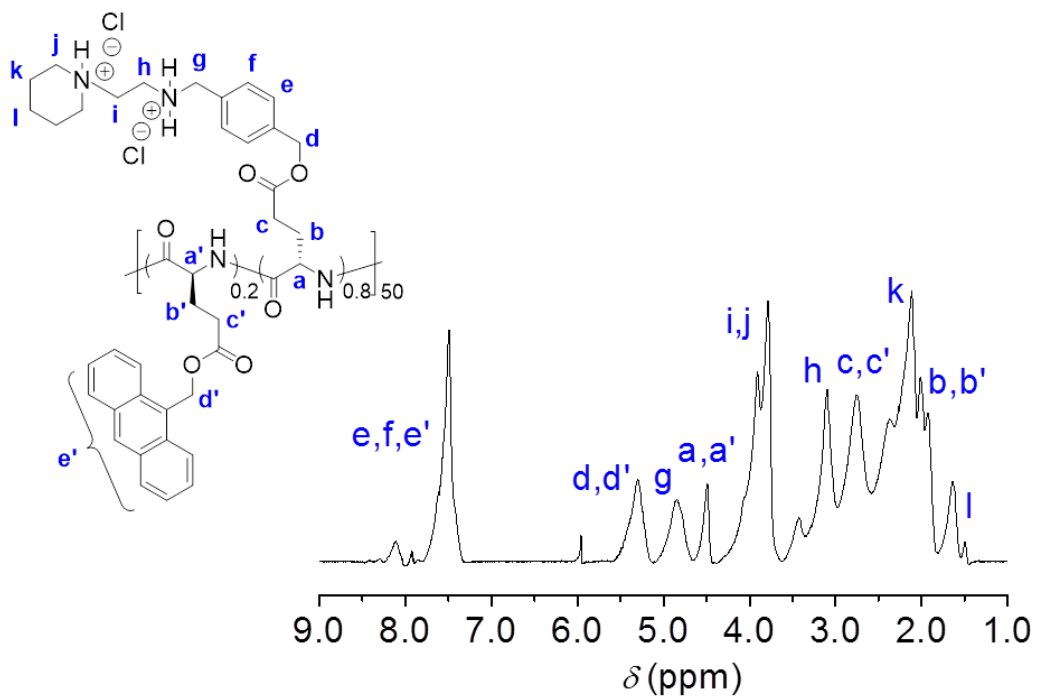
**Fig. S18.**  $^1\text{H}$  NMR spectrum of **P2** in  $\text{TFA-d}$ .



**Fig. S19.**  $^1\text{H}$  NMR spectrum of **P3** in  $\text{TFA-}d$ .



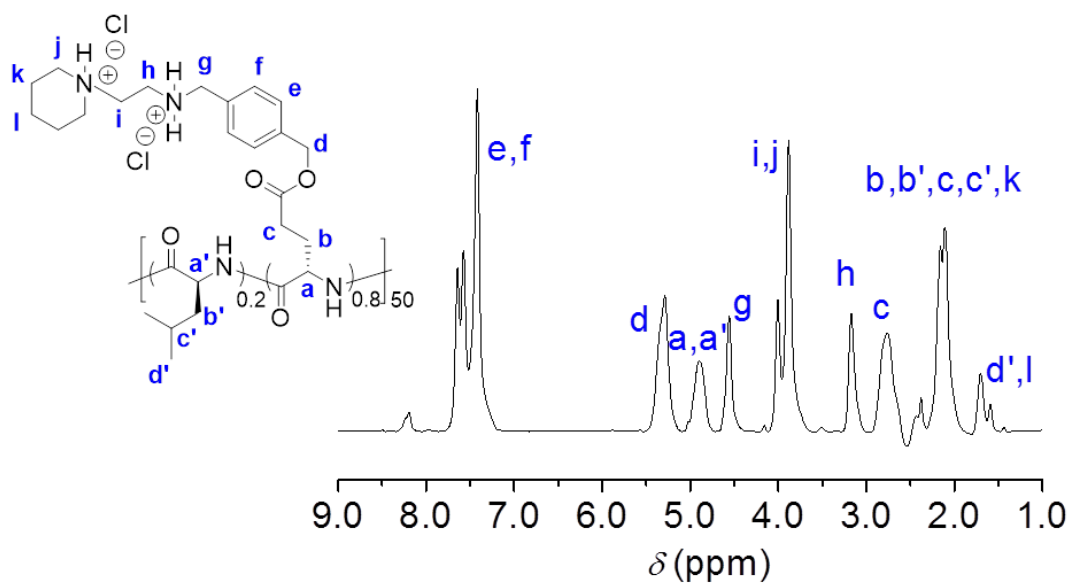
**Fig. S20.**  $^1\text{H}$  NMR spectrum of **P4** in TFA-*d*.



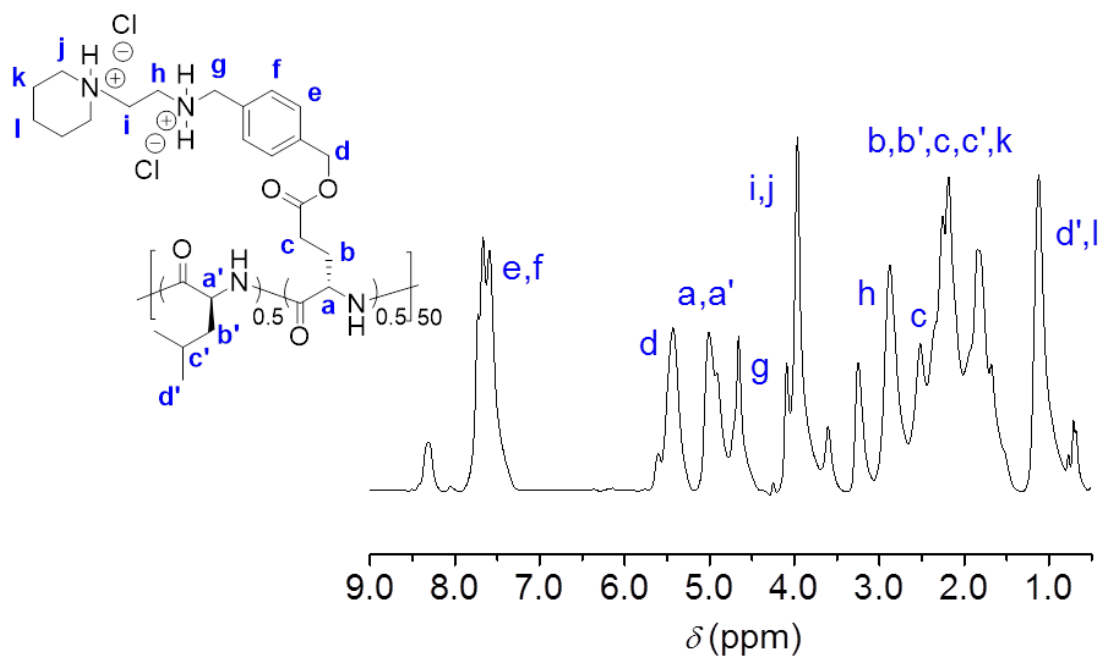
**Fig. S21.** <sup>1</sup>H NMR spectrum of **P5** in TFA-*d*.







**Fig. S23.**  $^1\text{H}$  NMR spectrum of **P7** in  $\text{TFA-d}$ .



**Fig. S24.**  $^1\text{H}$  NMR spectrum of **P8** in  $\text{TFA-d}$ .