Supporting Information

Ugi Reaction of Natural Amino Acids: A General Route toward Facile Synthesis of Polypeptoids for Bioapplications

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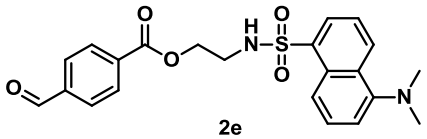
Experimental Section

1. Materials & Characterization

Amino acids, aldehydes and *t*-butyl isocyanide were purchased from Energy Chemical and used as received unless otherwise stated.

^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer. Number-average molecular weights (M_n) and polydispersity indexes (PDI) were determined by SEC on a Waters 1515 HPLC pump equipped with Waters Styragel HT3, HT4 columns and a Waters 2414 Refractive index Detector (eluent: DMF containing 0.01M LiBr; flowrate: 1 mL/min; temperature: 50 ^{o}C; standard: PS). UV-Vis absorption spectra were conducted on Shimadzu UV-2450 spectrophotometer. Fluorescence emission spectra were recorded on a PerkinElmer LS-55 phosphorescence spectrophotometer. TEM images were conducted by transmission electron microscopy (TEM, model JEOL JEM-1011). Size and size distribution of the nanoparticles were determined by dynamic light scattering (DLS) (Malvern Zeta-sizer Nano). Circular dichroism spectra were measured in a 1.0-mm quartz cell on a JASCO J-820 spectrometer.

2. Synthesis of 2-(5-(dimethylamino)naphthalene-1-sulfonamido)ethyl-4-formyl benzoate (2e)



2e

To a solution of dansyl chloride (1.0 g, 3.71 mmol) in CH_2Cl_2 (20 mL), Et_3N (1.08 mL, 7.79 mmol) and 2-aminoethanol (0.22 mL, 3.70 mmol) were added, the resulting mixture was stirred at room temperature for 2 h. The mixture was partitioned between CH_2Cl_2 and 6% aqueous HCl, the organic layer was separated and washed with aqueous Na_2CO_3 until neutral, dried with Na_2SO_4 and concentrated under reduced pressure. The residue purified by Column Chromatography to afford the product (1.0 g, 92% yield).

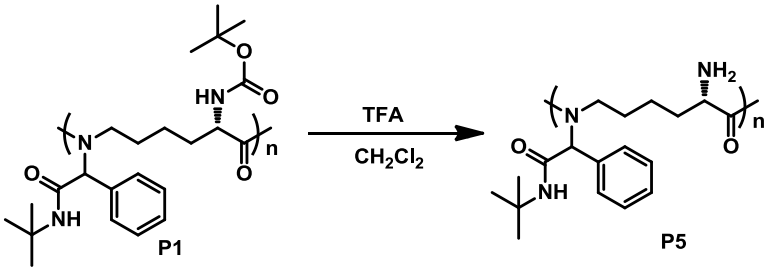
Above product (1.0 g, 3.4 mmol), 4-formylbenzoic acid (0.51 g, 4.08 mmol) and DMAP (83 mg, 0.68 mmol) were dissolved in 30 mL dry CH_2Cl_2 and 5 mL anhydrous THF. EDCI (0.72 g, 3.74 mmol) was added to the mixture under nitrogen atmosphere. The mixture was stirred at room temperature for 24 h. The insoluble white solid was removed by filtration and the filtrate was

purified by column chromatograph to get a pale yellow solid (1.26g, 87% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.08 (s, 1H), 8.49 (dd, dd, *J* = 6.0 Hz, 1H 1H), 8.26(m, 2H), 7.86(dd, *J* = 7.8 Hz, *J* = 23.4 Hz, 4H), 7.47(m, 2H), 7.10(dd, *J* = 6.0 Hz, 1H), 4.27(t, dd, *J* = 6.0 Hz, 2H), 3.34(m, 2H), 2.85(s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.09, 165.12, 151.92, 138.98, 134.62, 134.15, 130.72, 130.29, 129.86, 129.48, 129.40, 129.28, 128.42, 127.57, 124.05, 122.22, 116.31, 114.37, 62.42, 45.50, 42.16. ESI-MS: $[M + H]^+$ =427.1

3. Typical procedure for the Ugi reaction

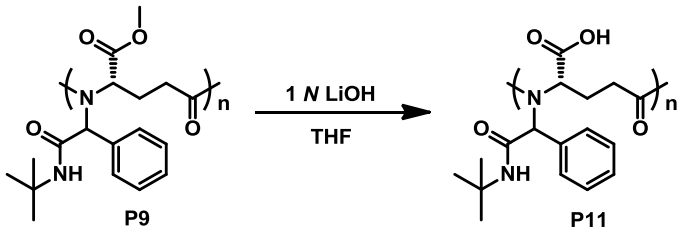
To a suspension of Amino acid (or derivatives) (1.0 mmol), aldehyde (2.2 mmol) in 2 mL MeOH was added *tert*-butyl isocyanide (2.2 mmol) under air atmosphere, the resulting mixture was stirred at 25 ^{o}C for 96 h. The volatile was removed in vacuum, and the residue was dissolved in 2 mL CH_2Cl_2. After precipitating in petroleum ether for three times and dried in vacuum at room temperature to afford polypeptoids **P1**, **P2, P3, P4**, **P6**, **P7**, **P9**, **P10, P12, P13, P15** as white or light yellow solid, the yield ranged from 35% to 88%.

4. Typical procedure for the Deprotection of P1



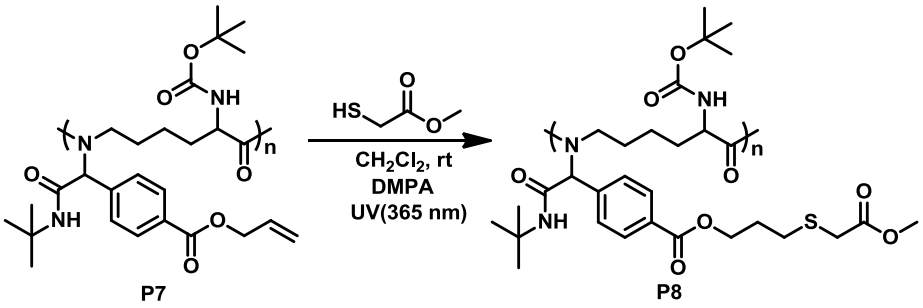
To a solution of **P1** (1.0 g) in dry CH_2Cl_2 (6 mL) was added TFA (1.2 mL) slowly at 0 ^{o}C. The mixture was allowed to stirred at 25 ^{o}C for 2 h. The polymer solution was concentration and precipitated into petroleum ether to yield **P5** (0.7 g, 92% yield).

5. Typical procedure for the Deprotection of P9



To a solution of **P9** (1.0 g) in THF (6 mL) was added LiOH (9 mL, 1 *N*) at 0 ^{o}C. The mixture was allowed to stir at 25 ^{o}C for 12 h. The polymer solution was dialyzed in H_2O for 7 days to afford carboxyl functionalized polymer **P11** (0.81 g, 85% yield).

6. Thiol-ene reaction of P7 with methyl 2-mercaptoacetate to generate the P8



P7 (95 mg, 0.2 mmol), methyl 2-mercaptoacetate (0.2 mL, 2 mmol), DMPA (5.2 mg, 0.02 mmol) and CH_2Cl_2 (1 mL) were introduced into a 10 ml quartz reactor, a purified nitrogen stream was introduced for about 30 min to degas the reaction mixture, The reaction flask was then sealed and subjected to UV irradiation (365 nm) for 2 h, After the reaction, the reaction solution was precipitated into petroleum ether to yield **P8** (100 mg, 86% yield).

7. Cytotoxicity test

HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells/well and incubated in DMEM for 24 h. Polypeptoids were diluted with fresh medium (Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) fetal bovine serum) to the desired concentrations and added to the corresponding well, respectively. After incubation for 48 h at 37 °C, cell viability was assessed using the standard MTT assay.

8. Preparation of Cur-loaded polypeptoid NPs

The self-assemble technique[1] was used to prepare Cur-loaded polypeptoid NPs. Briefly, certain amounts of Cur and the polypeptoid **P5** (3 mg) was dissolved in THF (5 mL). After stirred about 5 min, the solution was added dropwise to deionized water (10 mL) and stirred to evaporate the organic solvent followed by dialysis for 24 h.

9. Confocal Laser Scanning Microscope Studies

Cellular uptakes by HeLa cells were examined using a confocal laser scanning microscope (CLSM). HeLa cells were seeded in 6-well culture plates (a sterile cover slip was put in each well) at a density of 5×10^4 cells per well and allowed to adhere for 24 h. After that, the cells were treated with Cur-loaded polypeptoid NPs for 0.5 h at 37 ^{o}C. After that, the supernatant was carefully removed and the cells were washed three times with PBS. Subsequently, the cells were fixed with 800 μL of 4% formaldehyde in each well for 20 min at room temperature and washed twice with PBS again. The slides were mounted and observed with a confocal laser scanning microscope imaging system (Zeiss LSM 780).

10. Antimicrobial activity tests

S. aureus, a commercially available TCH-sensitive bacteria strain, was used in this test, and beef extract-peptone medium pre-pared according to the standard formulation was applied as the growth medium. Antimicrobial activity test was assessed according to the literature.[2] The bacterial inhibition percentage was calculated according to the following equation:

Bacterial inhibition (%) = $I_c - I_s/I_c \times 100$

where I_c and I_s are the absorbance of the control bacterial suspension and the bacterial suspension treated with different samples at the wavelength of 625 nm. Mean and standard deviation of triplicate samples for each sample were reported and all data were expressed as mean ± S.D.

References

(1) Lin, W. H.; Sun, T. T.; Xie, Z. G.; Gu, J. K.; Jing, X. B. *Chemical Science* **2016**, *7*, 1846-1852.
(2) Qi, R. L.; Guo, R.; Zheng, F. Y.; Liu, H.; Yu, J. Y.; Shi, X. Y. *Colloids and Surfaces B: Biointerfaces* **2013**, *110*, 148–155.

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Supplementary Figures

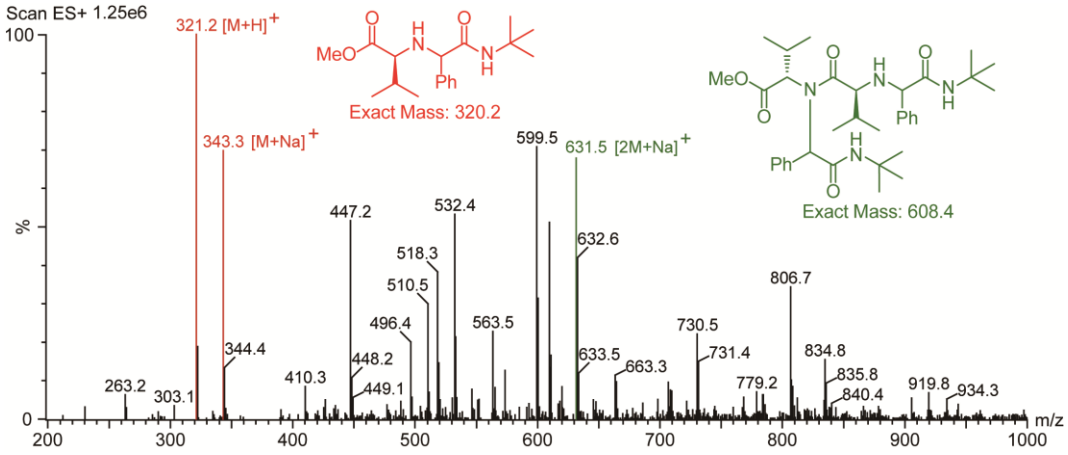

Figure S1. ESI-MS spectrum of the product from the Ugi reaction of Valine (**1c**) with benzaldehyde (**2a**) and *tert*-butyl isocyanide (**3**) in MeOH at 25 ^{o}C for 96 h. The monomer ester (red) and dimer ester (green) were detected as the main products.

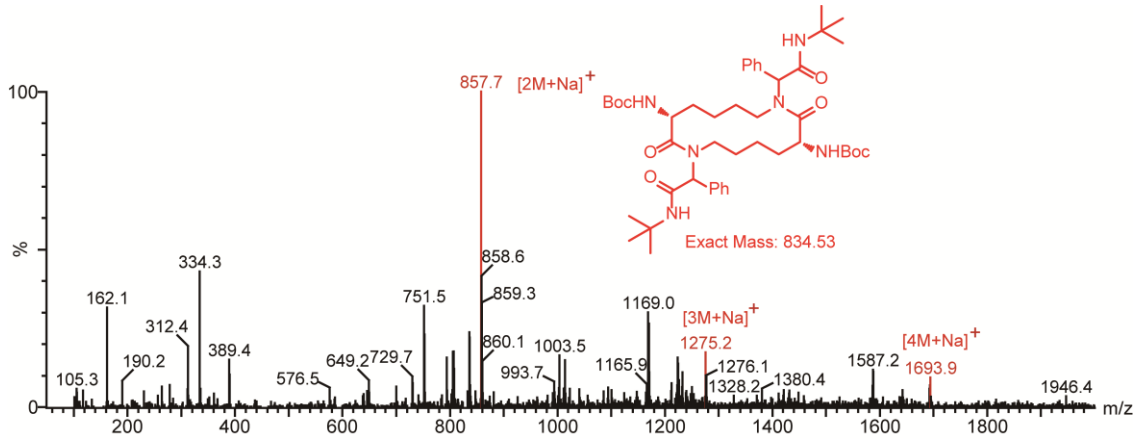

Figure S2. ESI-MS spectrum of the product from the Ugi reaction of N_{α}-Boc-L-lysine (**1f**) with benzaldehyde (**2a**) and *tert*-butyl isocyanide (**3**) in MeOH at 25 ^{o}C for 96 h. The red peaks revealed the existence of oligomeric cyclic species.

Figure S3. The image shows the total amount of **P1** obtained from the Ugi reaction of N_{α}-Boc-L-lysine with benzaldehyde and *tert*-butyl isocyanide in MeOH at room temperature.

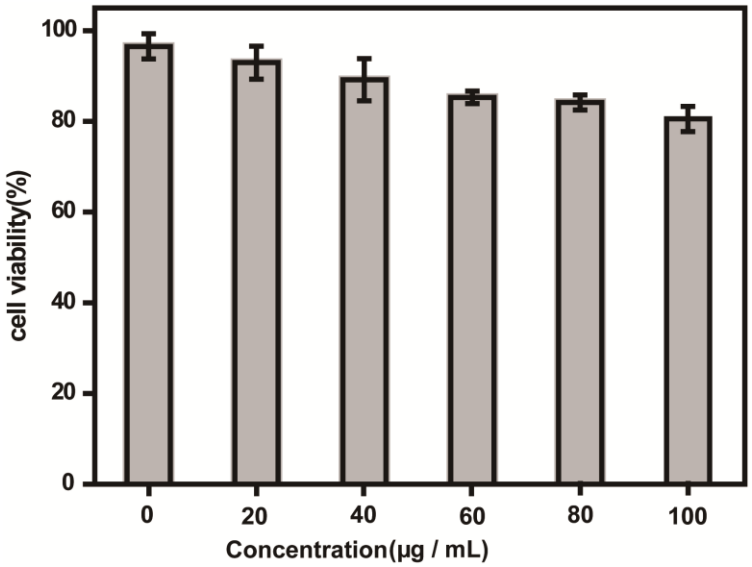

Figure S4. Viability of HeLa cell, and MTT assay was used to detect the toxicity of the **P5** in different concentrations.

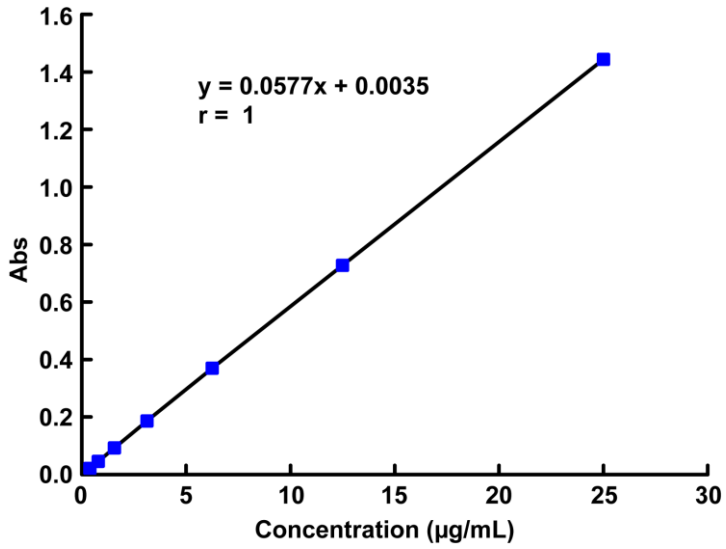

Figure S5. The UV−vis absorption of Cur at 425 nm, the liner range was 0.39~25μg/mL (r = 1) and the regression equation is y = 0.0577x + 0.0035. The UV−vis absorption intensity was 0.413 when the loading concentration of Cur is 100 μg/mL, the drug loading was 71% by calculation.

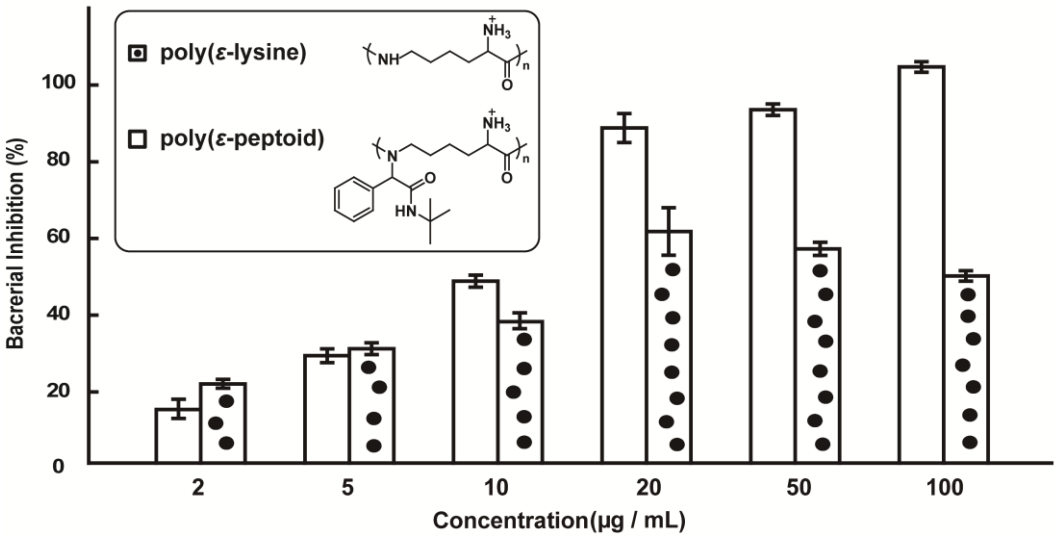

Figure S6. Comparison of bacterial inhibition of poly(ε-peptoid) **P5** and Commercial antibacterial agent poly(ε-lysine) using *Staphylococcus aureus* as a model in different concentrations.

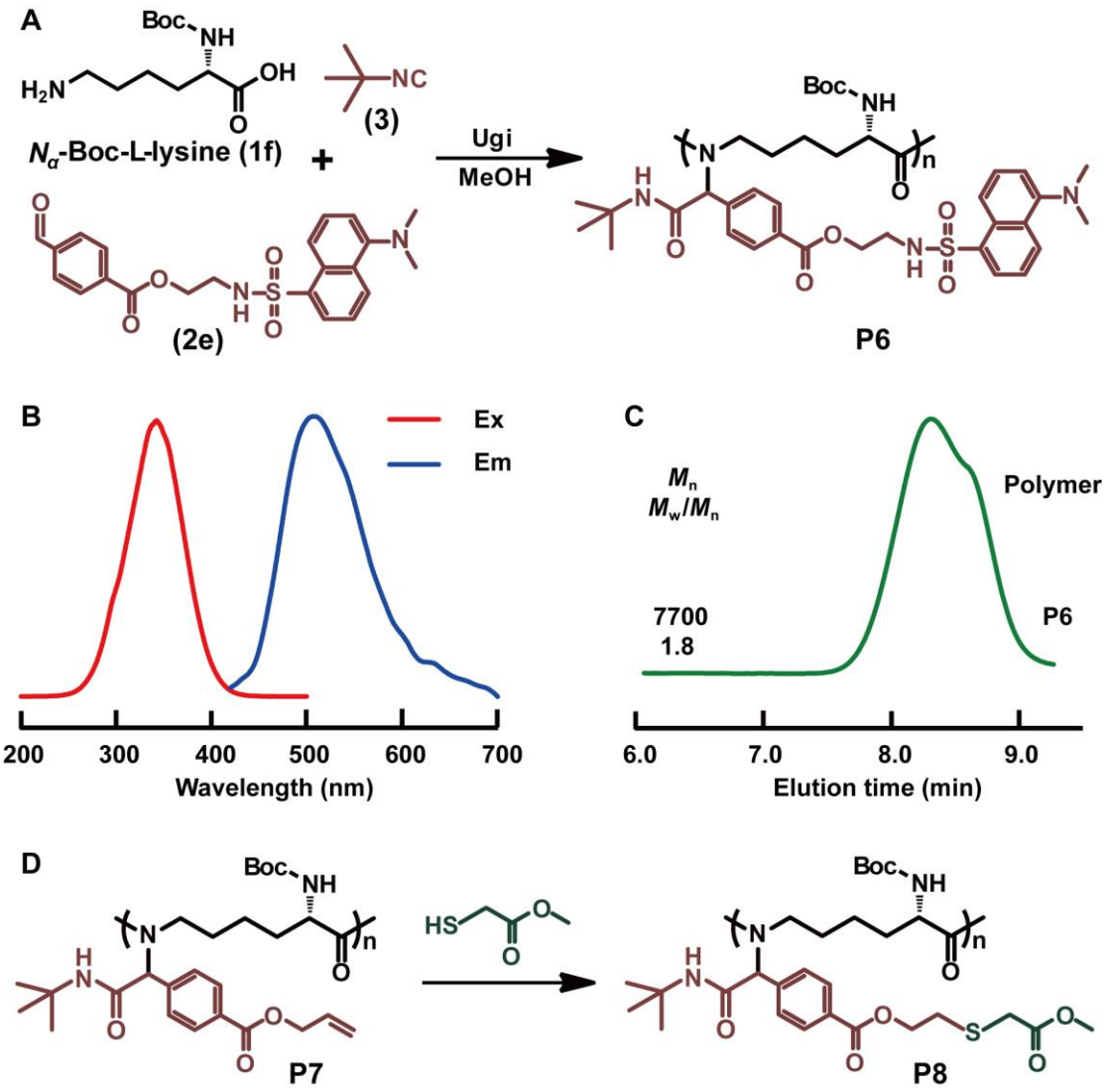

Figure S7. (A) Synthesis of fluorescent polypeptoid **P6** *via* Ugi reaction. (B) Fluorescence spectrum of **P6**. (C) SEC trace (DMF at 50 ^{o}C) of **P6** (M_n =7700, PDI=1.8). (D) Post polymerization functionalization of polypeptoid **P7** with thiols.

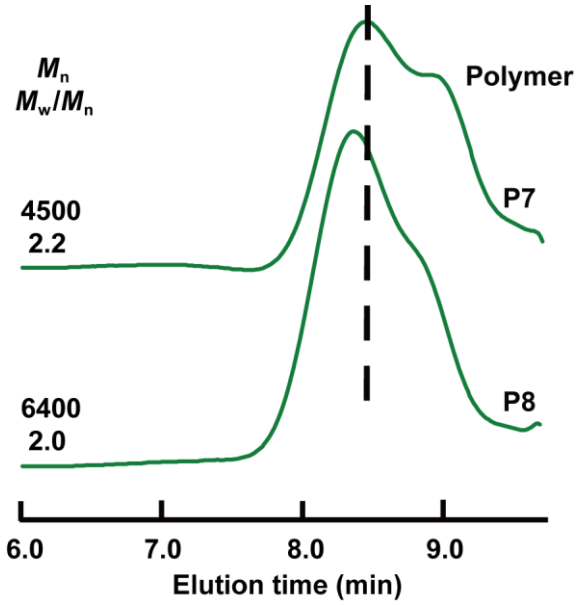

Figure S8. SEC traces of the **P7** and **P8**, **P8** was produced *via* the click reaction of **P7** with methyl 2-mercaptoacetate at the presence of DMPA and irradiated by 365 nm UV light for 2 h.

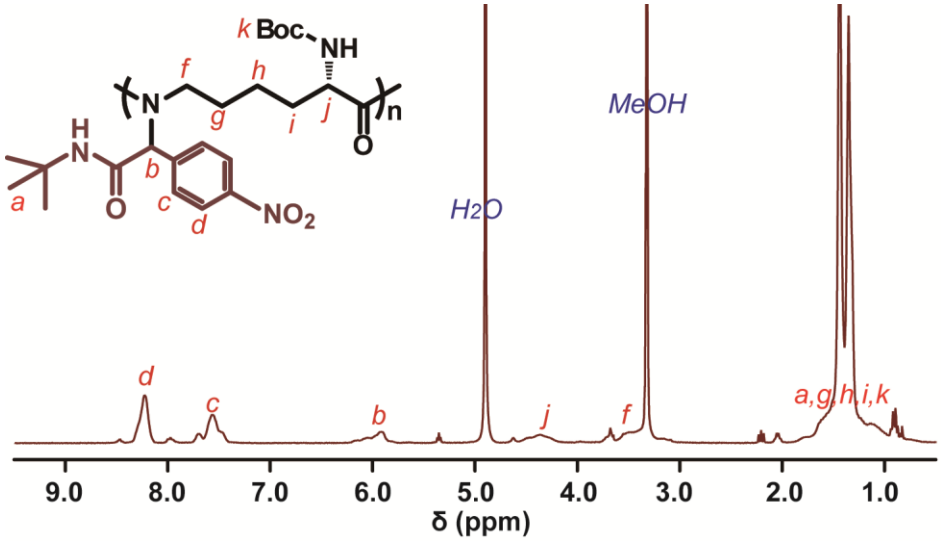

Figure S9. ^{1}H NMR spectra (in CD_3OD at 25 °C) of **P2**

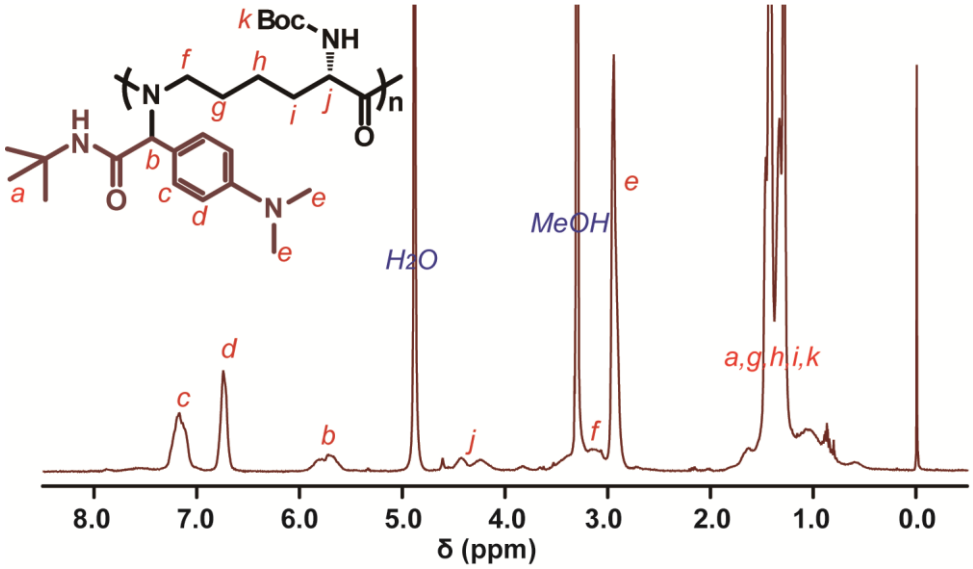

Figure S10. ^{1}H NMR spectra (in CD_3OD at 25 °C) of **P3**

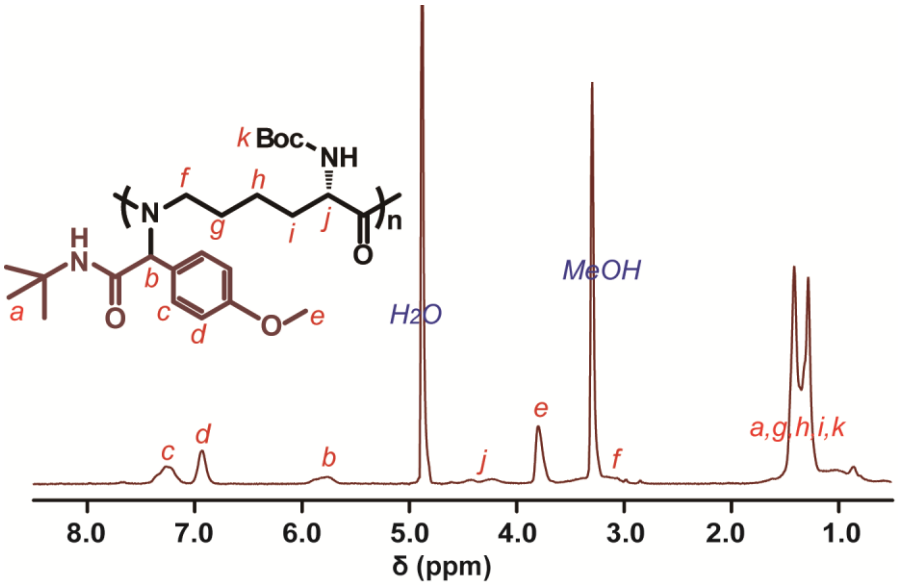

Figure S11. ^{1}H NMR spectra (in CD_3OD at 25 °C) of **P4**

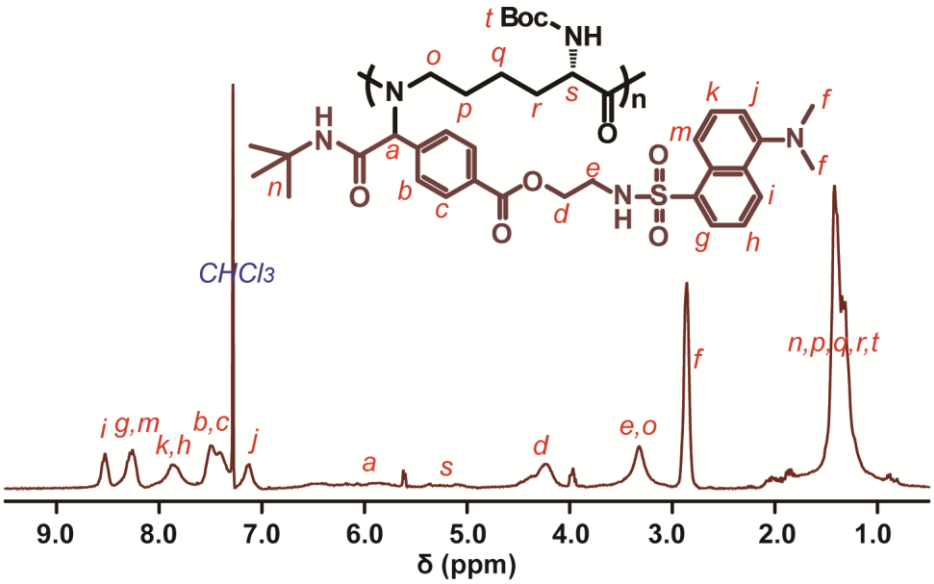

Figure S12. ^{1}H NMR spectra (in $CDCl_3$ at 25 °C) of **P6**

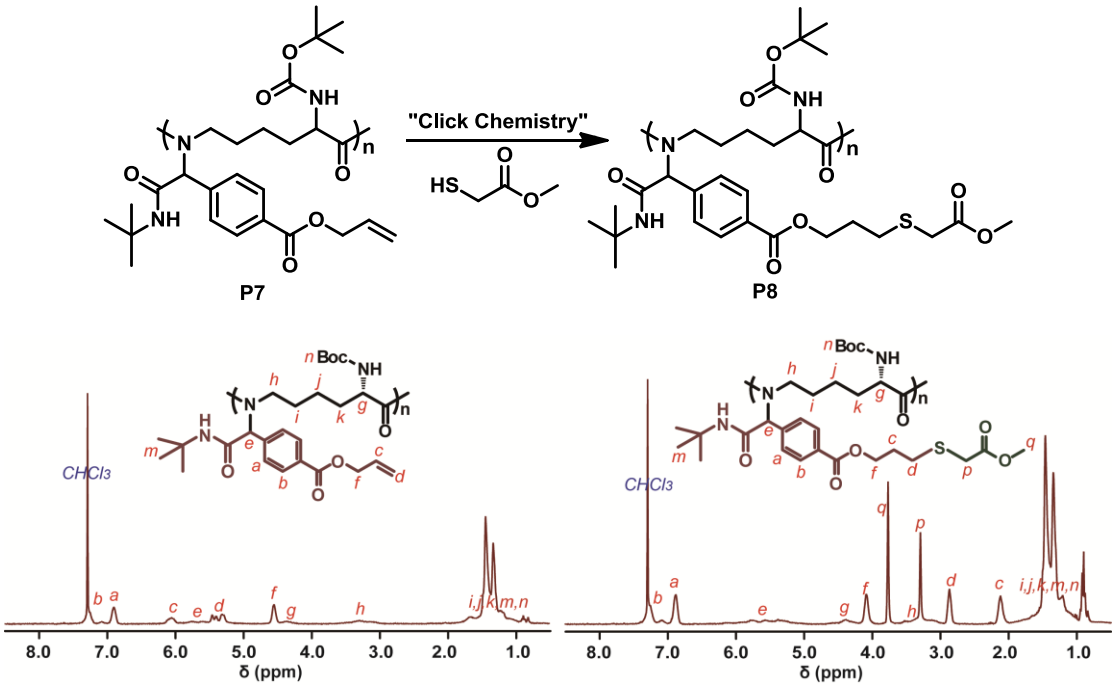

Figure S13. ^{1}H NMR spectra (in $CDCl_3$ at 25 °C) of **P7** and **P8**

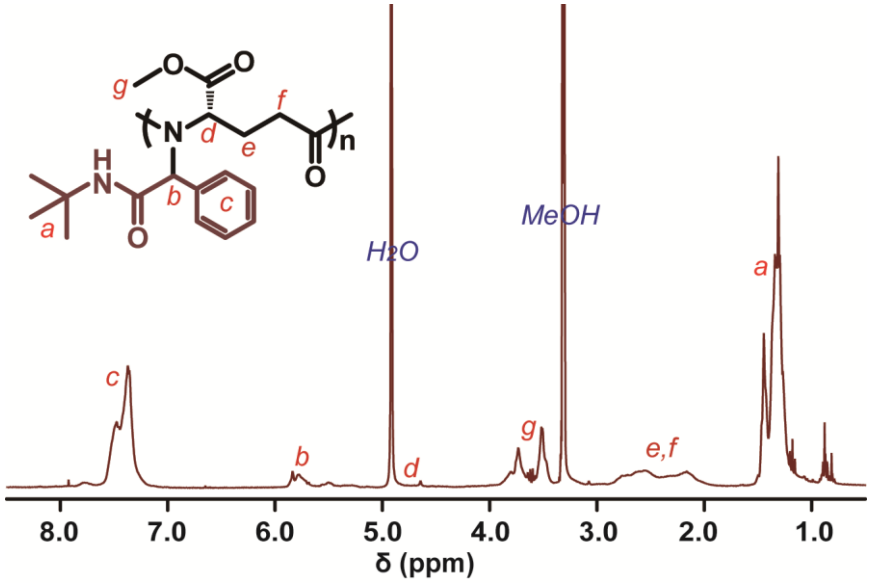

Figure S14. ^{1}H NMR spectra (in CD_3OD at 25 °C) of **P9**

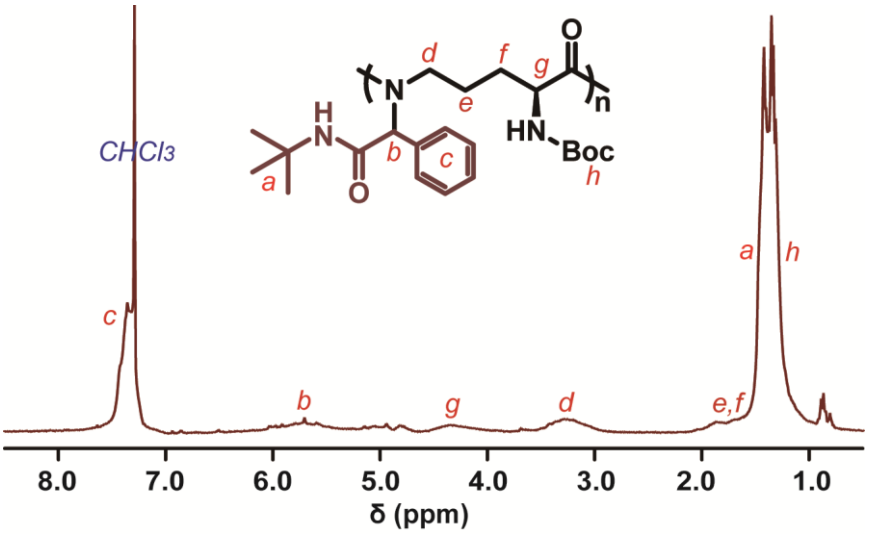

Figure S15. ^{1}H NMR spectra (in $CDCl_3$ at 25 °C) of **P10**

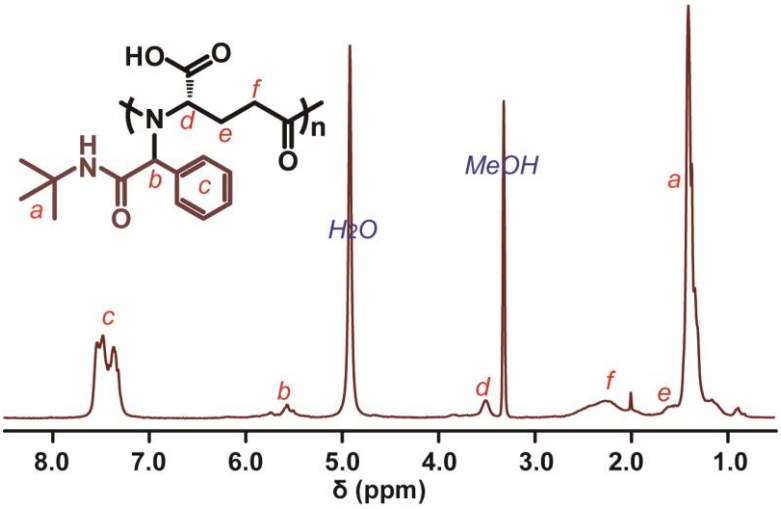

Figure S16. ^{1}H NMR spectra (in CD_3OD at 25 °C) of **P11** which was produced *via* deprotection of the sample **P9.**

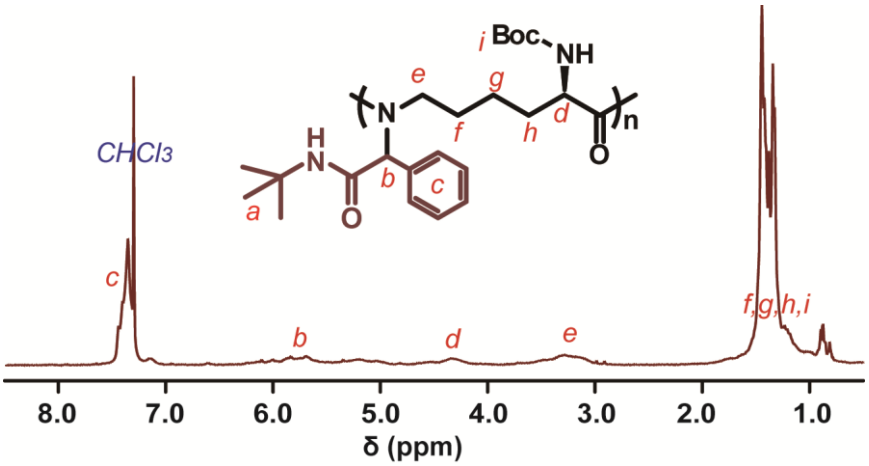

Figure S17. ^{1}H NMR spectra (in $CDCl_3$ at 25 °C) of **P12**

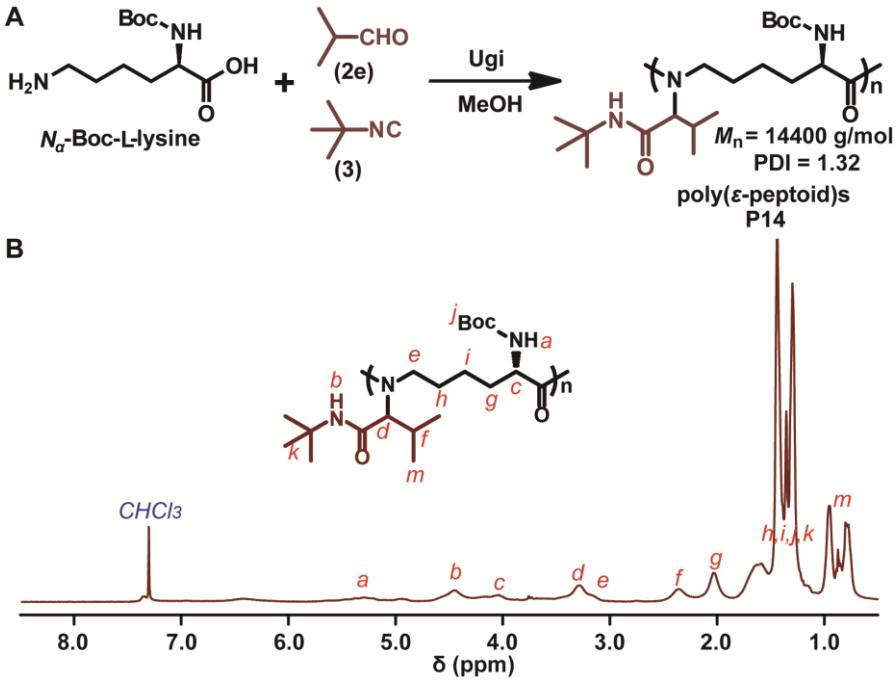

Figure S18. (A) Polymerization of isobutyraldehyde and *tert*-butyl isocyanide with N_α-Boc-L-lysine. Reactions were carried out at room temperature for 96 h in MeOH, [N_α-Boc-L-lysine] = 0.5 M, [**2e**] = 1.1 M, [**3**] = 1.1 M; (B) ^{1}H NMR spectra (in $CDCl_3$ at 25 °C) of **P14.**

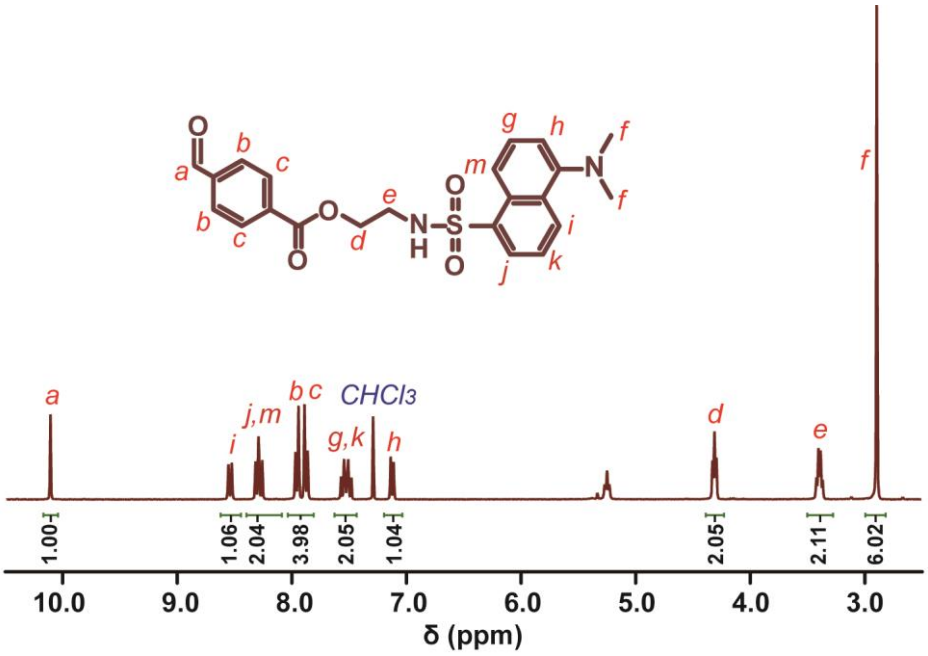

Figure S19. ^{1}H NMR spectra (in $CDCl_3$ at 25 °C) of fluorescent aldehyde **2e**

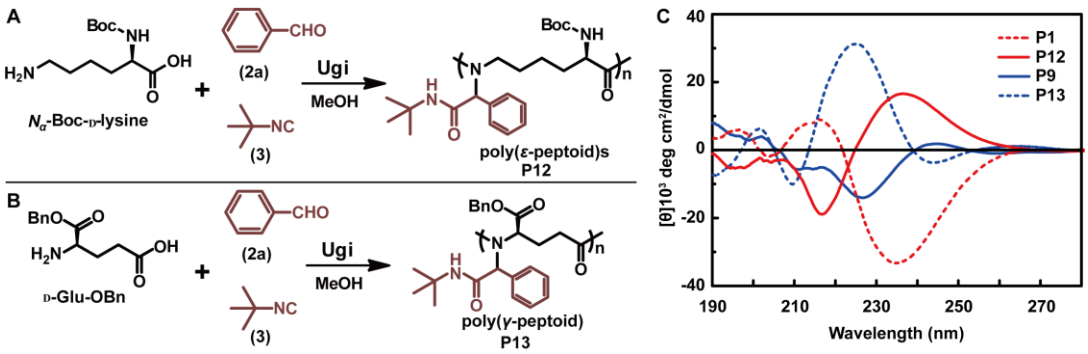

Figure S20. Polymerization of benzaldehyde and *tert*-butyl isocyanide with N_α-Boc-D-lysine (A) and D-glutamic acid benzylester (B), respectively, through Ugi reaction. (C) CD spectra (measured in THF at 25 ^{o}C, c = 1 mg mL^{-1}) of **P1**, **P12**, **P9**, **P13** showed that enantiomer of L-amino acid was similarly polymerized to afford polypeptoids with approximate opposite cotton effect.

Table S1. T_g and T_d data of **P1**, **P9**, and **P10**.

Entry	Monomer	Polymer	T_g [a] (°C)	T_d [b] (°C)
1	N_α-Boc-L-Lysine+**2a**+**3**	**P1**	128	219
2	L-Glu-OMe+**2a**+**3**	**P9**	129	283
3	N_α-Boc-L-ornithine+**2a**+**3**	**P10**	129	219

[a]Determined by DSC, 10 °C/min scan rate. Values are recorded from the second scan data. [b]Determined by TGA, 10 °C/min scan rate. T_d is defined as the temperature when 5% weight loss occurs.

Table S2. Ugi reaction using $_{D}$-amino acid [a]

Entry	Monomer	Polymer	Yield (%) [b]	M_n [c]	PDI [c]
1	N_α-Boc-D-Lysine+**2a**+**3**	**P12**	75	5800	1.47
2	D-Glu-OBn+**2a**+**3**	**P13**	66	2800	1.44

[a] Reactions were carried out at room temperature for 96 h in MeOH, [amino acid] = 0.5 M, [**2**] = 1.1 M, [**3**] = 1.1 M. [b] Calculated based on polymers recovered after precipitation in petroleum ether. [c] Measured by SEC with polystyrene as standard and DMF as eluent.