Electronic Supplementary Material (ESI) for Polymer Chemistry.

Controlled Copolymerization on α-NCAs and α-NNTAs for Preparing Peptide/Peptoid Hybrid Polymers with Adjustable Proteolysis

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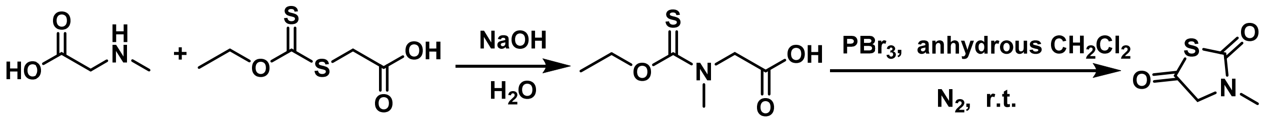
EXPERIMENTAL SECTION

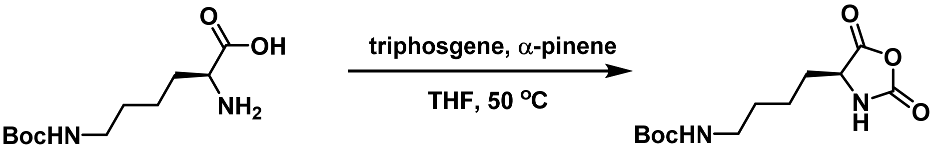
Materials. Anhydrous dichloromethane (DCM) and tetrahydrofuran (THF) were purchased from Sigma-Aldrich and used without further purification. N^{ε}-Boc-L-lysine, sarcosine and other reagents were purchased from Shanghai Adamas Reagent. The synthesized intermediates were purified using a SepaBean machine equipped with Sepaflash columns produced by Santai Technologies Inc. in China. High performance liquid chromatography (HPLC) analysis was performed with SHIMADZU LC 20AR HPLC System equipped with a Luna Omega 5 μm polar C18 column. Nuclear magnetic resonance (NMR) spectra were collected on a Bruker spectrometer at 400 MHz and 600 MHz and the corresponding chemical shifts are referenced to residual protons in the deuterated NMR solvents. High resolution electrospray ionization time-of-flight mass spectrometry (HRESI-MS) was collected on a Waters XEVO G2 TOF mass spectrometer. Gel permeation chromatography (GPC) was performed on a system equipped with a Waters 1515 isocratic HPLC pump, a Brookhaven BI-MwA multi-angle light scattering detector and a Waters 2414 refractive index detector. The Tosoh TSKgel Alpha-2500 column (particle size 7 μm, 300 × 7.8 mm), Tosoh TSKgel Alpha-3000 column (particle size 7 μm, 300 × 7.8 mm) and Tosoh TSKgel Alpha-4000 column (particle size 10 μm, 300 × 7.8 mm) connected in series were utilized for separation of polymers using N, N-Dimethylformamide (DMF), supplemented with 0.01 M LiBr, as the mobile phase, at a flow rate of 1 mL/min at 50 °C. Prior to analysis, the samples were filtered through a 0.22 μm polytetrafluoroethylene (PTFE) filter. The number-average molecular weight (M_n), degree of polymerization (DP) and dispersity index ($Đ$) were calculated from a calibration curve using polymethylmethacrylate (PMMA) as standards. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) experiments were conducted on a Bruker autoflex speed MALDI TOF/TOF.

Synthesis of sarcosine *N*-Thiocarboxy Anhydride (Sar NTA):



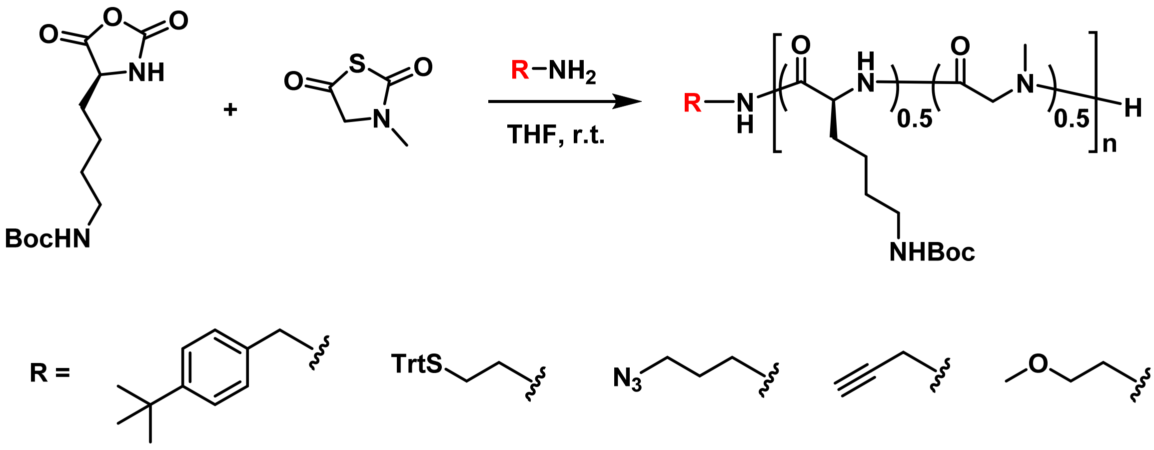
Sar NTA was synthesized according to the method in previous literature with slight modification.[1] *S*-ethoxythiocarbonyl mercapto-acetic acid (XAA, 5.00 g, 27.7 mmol) and sarcosine (2.3 g, 25.2 mmol) were added sequentially to an aqueous solution of NaOH (1.5 M, 50 mL) and the mixture was stirred at room temperature for 48 h. Then the solution was acidified with hydrochloric acid (1.0 M) to pH = 3 and extracted with ethyl acetate (EtOAc, 3 × 50 mL). The organic phase was collected and washed with brine (1 × 50 mL), followed by drying over anhydrous $MgSO_4$. The solution was then filtered and the filtration was concentrated under a reduced pressure to give the intermediate as a white solid (4.1 g, 91.0% yield). The intermediate (1.0 g, 5.6 mmol) was dissolved in anhydrous dichloromethane (60 mL) under a nitrogen atmosphere, followed by dropwise addition of PBr_3 (1.5 g, 5.6 mmol) to this solution through a syringe at 0 °C, and then the solution was stirred at room temperature for 1 h. Then the reaction mixture was washed with water (3 × 50 mL). The obtained organic layer was dried over anhydrous $MgSO_4$ and concentrated under a reduced pressure to give a crude product. The crude product was purified through a silica gel column chromatography to give an oily monomer (0.56 g, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$, Figure S1): δ 4.19 (s, 2H), 3.10 (s, 3H). The obtained ^{1}H NMR spectrum was consistent to the report in precedent literature.[1] EI-MS: m/z calculated for $C_4H_5NO_2S$ $[M]^+$: 131.0; Found 131.0.

Synthesis of N^{ε}-*tert*-butyloxycarbonyl-L-lysine *N*-Carboxy Anhydride (Boc-L-Lys NCA):



Boc-L-Lys NCA (BLL NCA) was synthesized according to the method in previous literature with slight modification.[2] N^{ε}-Boc-L-Lysine (5.0 g, 20.3 mmol) and α-pinene (8.4 mL, 54.0 mmol) were added into 160 mL anhydrous THF followed by addition of triphosgene (2.6 g, 8.9 mmol) dissolved in 40 mL anhydrous THF. The reaction was heated to 50 °C and stirred for 2 h under nitrogen atmosphere. The solvent was removed under a reduced pressure and then the residue was dissolved in 100 mL EtOAc and washed with water (3 × 100 mL) and cold brine (1 × 100 mL) sequentially. The organic phase was dried over anhydrous $MgSO_4$ and concentrated under a reduced pressure to 10 mL to give a crude product. The resulting crude product was recrystallized in anhydrous EtOAc/ n-hexane for three times to give the pure Boc-L-Lys NCA as a colorless acicular crystal (2.7 g, 49.6% yield). ^1H NMR (400 MHz, $CDCl_3$, Figure S2): δ 6.87 (s, 1H), 4.68 (br, 1H), 4.33 (dd, *J* = 6.8, 5.2 Hz, 1H), 3.13 (s, 2H), 1.95-2.08 (m, 1H), 1.90-1.79 (m, 1H), 1.61-1.45 (m, 4H), 1.45 (s, 9H). The obtained ^1H NMR spectrum was consistent to the report in precedent literature.[2] ESI-MS: m/z calculated for $C_{12}H_{19}N_2O_5[M\text{-}H]^-$: 271.1; Found 271.1.

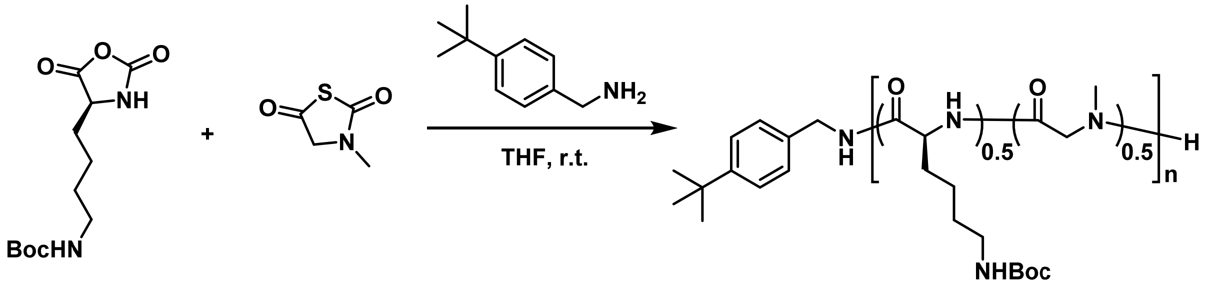
Synthesis of peptide/peptoid hybrid polymer using different initiators:



The peptide/peptoid hybrid polymers were synthesized using different initiators bearing

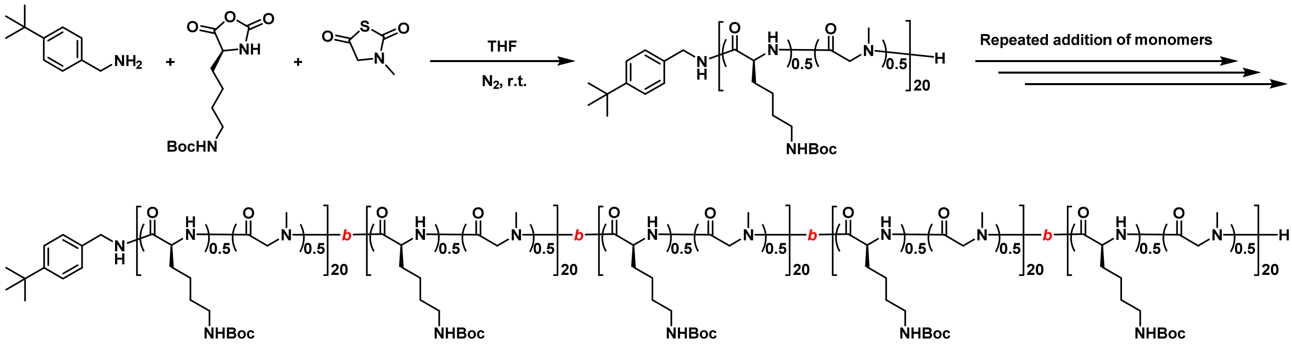
4-*tert*-butylbenzyl, trityl protected sulfhydryl group, azide group, alkynyl group and methoxy group, respectively. BLL NCA, Sar NTA and primary amines were individually prepared as stock solutions at a concentration of 0.5 M in anhydrous THF under a nitrogen environment and then mixed as needed for different copolymerization. The mixture of BLL NCA, Sar NTA and the initiators were stirred at room temperature for 3 days under a nitrogen atmosphere. Once the polymerization reaction was completed monitored by TLC, the reaction mixture was poured into 45 mL cold n-hexane to precipitate out the crude polypeptide as a light-yellow solid, which was collected after centrifugation and dried under air flow. The crude product was purified via the dissolution-precipitation process thrice (2.0 mL THF/45 mL n-hexane) and then dried under vacuum (66.4-84.6% yield).

Synthesis of peptide/peptoid hybrid polymer with variable chain lengths:



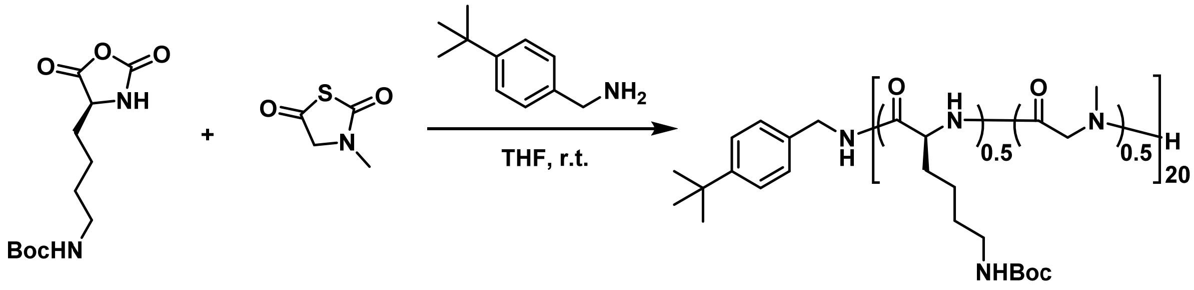
To prepare peptide/peptoid hybrid polymer with variable chain lengths, BLL NCA, Sar NTA and *t*BuBnNH$_2$ were individually prepared as stock solutions at a concentration of 1.0 M in anhydrous THF under nitrogen and then mixed as needed for different copolymerizations. The mixture of BLL NCA and Sar NTA and the initiator were stirred at room temperature for 2-4 days under nitrogen atmosphere. Once the polymerization reaction was completed monitored by TLC, the reaction mixture was poured into 45 mL cold n-hexane to precipitate out the crude polypeptide as a light-yellow solid, which was collected after centrifugation and dried under air flow. The crude product was purified via the dissolution-precipitation process thrice (2.0 mL THF/45 mL n-hexane) and then dried under vacuum (74.1−83.1% yield).

Synthesis of peptide/peptoid hybrid pentablock polymer:



For the synthesis of peptide/peptoid hybrid pentablock polymer, the BLL NCA, Sar NTA and *t*BuBnNH$_2$ were individually prepared as stock solutions to a final concentration of 0.7 M in anhydrous THF under nitrogen. 100 μL BLL NCA, 100 μL Sar NTA and 10 μL *t*BuBnNH$_2$ solution were mixed and reacted for 24 h to give the first block of peptide/peptoid hybrid polymer poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ (M_n = 2.7 kg/mol, $Đ$ = 1.22, characterized by GPC). Then an extra solution of 100 μL BLL NCA and 100 μL Sar NTA was added to the reaction solution and reacted for another 24 h to give the peptide/peptoid hybrid diblock copolymer poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ (M_n = 6.3 kg/mol, $Đ$ = 1.18, characterized by GPC). And the above steps were repeated to give the triblock poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ (M_n = 9.5 kg/mol, $Đ$ = 1.18, characterized by GPC), tetrablock poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ (M_n = 12.5 kg/mol, $Đ$ = 1.07, characterized by GPC) and the final pentablock copolymer poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$-*b*-poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ (M_n = 15.7 kg/mol, $Đ$ = 1.21, characterized by GPC). DMF was used as the mobile phase at a flow rate of 1 mL/min.

Kinetics study on copolymerization on BLL NCA and Sar NTA in THF:



Copolymerization kinetics of BLL NCA and Sar NTA in THF was measured as below. BLL NCA, Sar NTA and *t*BuBnNH$_2$ were individually prepared as stock solutions at a concentration of 0.2 M in anhydrous THF under nitrogen. Triphenylmethane (TPM, 12.0 mg) was added to a mixture of 1.0 mL BLL NCA, 1.0 mL Sar NTA and 100 µL *t*BuBnNH$_2$ solution, as internal standard. The reaction mixture was stirred at room temperature in a nitrogen charged glovebox. The remaining percentage of BLL NCA and Sar NTA was calculated by measuring the ratio of the absorbance peak area of the BLL NCA/TPM and Sar NTA/TPM at 215 nm using HPLC in real time. The mobile phase was a gradient of MeCN/H$_2$O, with MeCN increasing from 50% to 90% within 9 min, holding at 90% for 8 min, and then reducing from 90% to 50% within 3 min, at a flow rate of 1.0 mL/min using a Luna Omega polar C18 column at ambient temperature. The remaining percentage of NCA and NNTA over time was calculated according to the following equation:

$$\text{Remaining percentage (\%)} = \frac{[\mathrm{M}]_\mathrm{t}}{[\mathrm{M}]_0} \times 100\%$$

Whereas, $[M]_t$ is the concentration of the monomer in real time, $[M]_0$ is the initial concentration of the monomer.

The reactivity ratio of BLL NCA and Sar NTA in THF was calculated using the Lynd method by following a precedent procedure as shown in Figure 3a.[3] Lynd equation for the evaluation of reactivity ratio is given below:

$$\mathrm{X} = 1 - f_{1,o}\left[1 - \left(1 - \frac{[\mathrm{M}]_1}{[\mathrm{M}]_{1,0}}\right)\right] - (1 - f_{1,o})\left[1 - \left(1 - \frac{[\mathrm{M}]_1}{[\mathrm{M}]_{1,0}}\right)\right]^{r_2}$$

$$\mathrm{X} = 1 - f_{1,o}\left[1 - \left(1 - \frac{[\mathrm{M}]_\mathrm{x}}{[\mathrm{M}]_{\mathrm{x},0}}\right)\right]^{r_1} - (1 - f_{1,o})\left[1 - \left(1 - \frac{[\mathrm{M}]_\mathrm{x}}{[\mathrm{M}]_{\mathrm{x},0}}\right)\right]$$

Whereas, r_1 and r_2 means the reactivity ratio of BLL NCA and Sar NTA respectively.

$[M]_1$ and $[M]_x$ are the concentrations of NCA and NNTA in real time, respectively.

$[M]_{1,0}$ and $[M]_{x,0}$ is the initial concentration of NCA and NNTA, respectively.

$f_{1,0}$ is the initial mole fraction of NCA.

X is the total conversion.

The copolymer microstructure can be calculated by the reactivity ratio through the equations given below.[3] The instantaneous copolymer composition F_{NCA} is plotted against the total conversion X as shown in Figure 3c.

$$X = 1 - \frac{[M]_1 + [M]_x}{[M]_{1,0} + [M]_{x,0}}$$

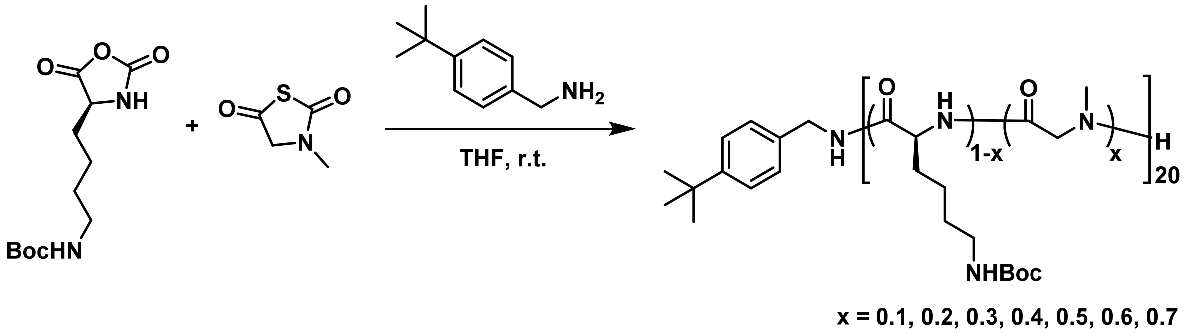
$$t = \frac{[M]_1}{[M]_x}$$

$$\varepsilon = \frac{1 + r_1 t}{1 + r_2 t^{-1}}$$

$$F_{NCA} = \frac{\varepsilon}{1 + \varepsilon}$$

Where, F_{NCA} is the instantaneous copolymer composition.

The synthesis of peptide/peptoid hybrid polymer library in THF:



The synthesis and characterization of poly-($BLL_{1\text{-}x}/Sar_x$)$_{20}$ libraries were performed as below. BLL NCA, Sar NTA and *t*BuBnNH$_2$ were individually prepared as stock solutions at a concentration of 0.2 M in anhydrous THF under nitrogen. 50 µL *t*BuBnNH$_2$ solution was added into 1.0 mL mixture of BLL NCA stock solution and Sar NTA stock solution in a volume ratio of 1-x/x (x = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7), and the reaction mixture was stirred at room temperature for 60 h under nitrogen atmosphere. Once the reaction was completed, the crude product was purified via a dissolution-precipitation process thrice (2.0 mL THF/45 mL n-hexane) and then drying under vacuum to give poly-($BLL_{1\text{-}x}/Sar_x$)$_{20}$. The purified poly-($BLL_{1\text{-}x}/Sar_x$)$_{20}$ was treated with 2 mL TFA at room temperature for 2 h under gentle shaking to remove the side-chain N-Boc protecting group, followed by removing TFA under air flow to give the crude product. The obtained crude product poly-(L-$Lys_{1\text{-}x}Sar_x$)$_{20}$ were purified by the dissolution-precipitation process (0.5 mL MeOH/45.0 mL methyl *tert*-butyl ether) for three times. The purified poly-(L-$Lys_{1\text{-}x}Sar_x$)$_{20}$ were dissolved in milli-Q and lyophilized to obtain fluffy white powder in the form of TFA salts (71.0-81.0% yield). The polymers were characterized by ^{1}H NMR and used for further proteolytic degradation test.

Proteolytic degradation test on poly-(L-$Lys_{1\text{-}x}/Sar_x$)$_{20}$

Proteolytic kinetics were measured through ^{1}H NMR analysis by following previously reported literature with modifications.[4] Specifically, poly-(L-$Lys_{1\text{-}x}/Sar_x$)$_{20}$ (x=0, 50%, 70%) and trypsin were individually dissolved in PBS buffer (pH = 7.4) to a final concentration 10 mg/mL using D_2O as solvent. The mixture of 0.5 mL of polymer solution and 12.5 µL of trypsin solution was detected by ^{1}H NMR at various time intervals. The integration of the CH proton on lysine backbone close to newly generated amino terminal group in ^1H NMR (labeled as Ha' in red) for each data collecting time is plotted against the time for data collecting. The proteolytic kinetic curves of poly-(L-$Lys_{1-x}Sar_x)_{20}$ (x=0, 50%, 70%) were obtained from fitting of the data to an exponential decay function. Half-life obtained from proteolytic kinetics was used to evaluate proteolytic stability.

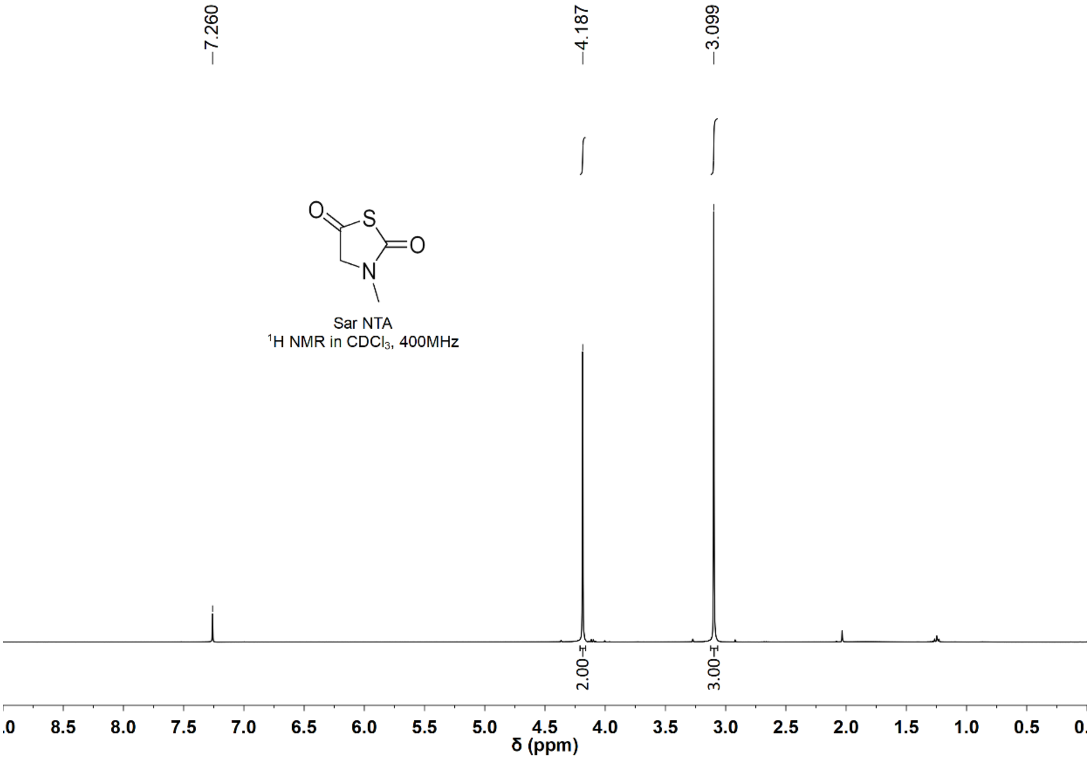


Figure S1. ^{1}H NMR spectrum of Sar NTA in CDCl$_3$ at 400 MHz.

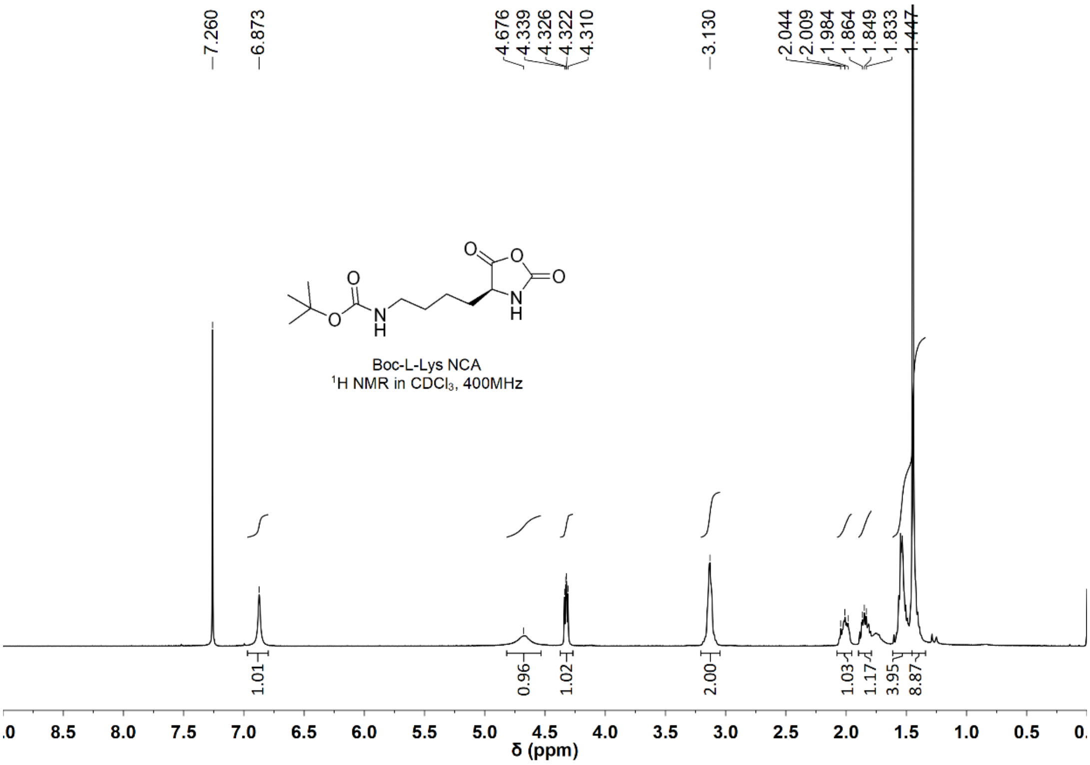


Figure S2. ^{1}H NMR spectrum of Boc-L-Lys NCA in CDCl$_3$ at 400 MHz.

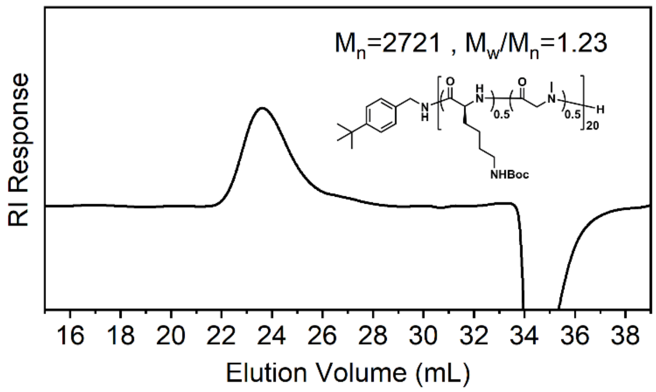


Figure S3. GPC trace of poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ obtained using *t*BuBnNH$_2$ as the initiator for polymerization.

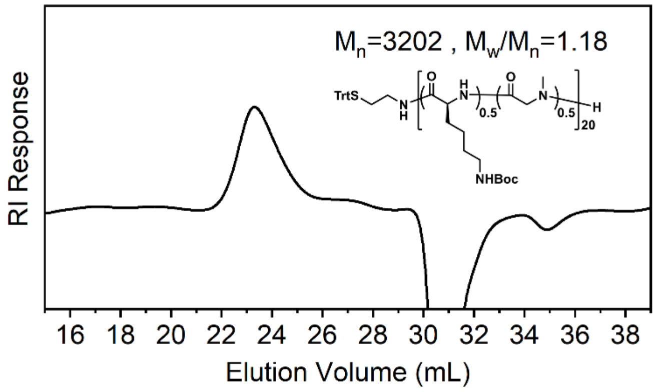


Figure S4. GPC trace of poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ obtained using trityl-protected cysteamine as the initiator for polymerization.

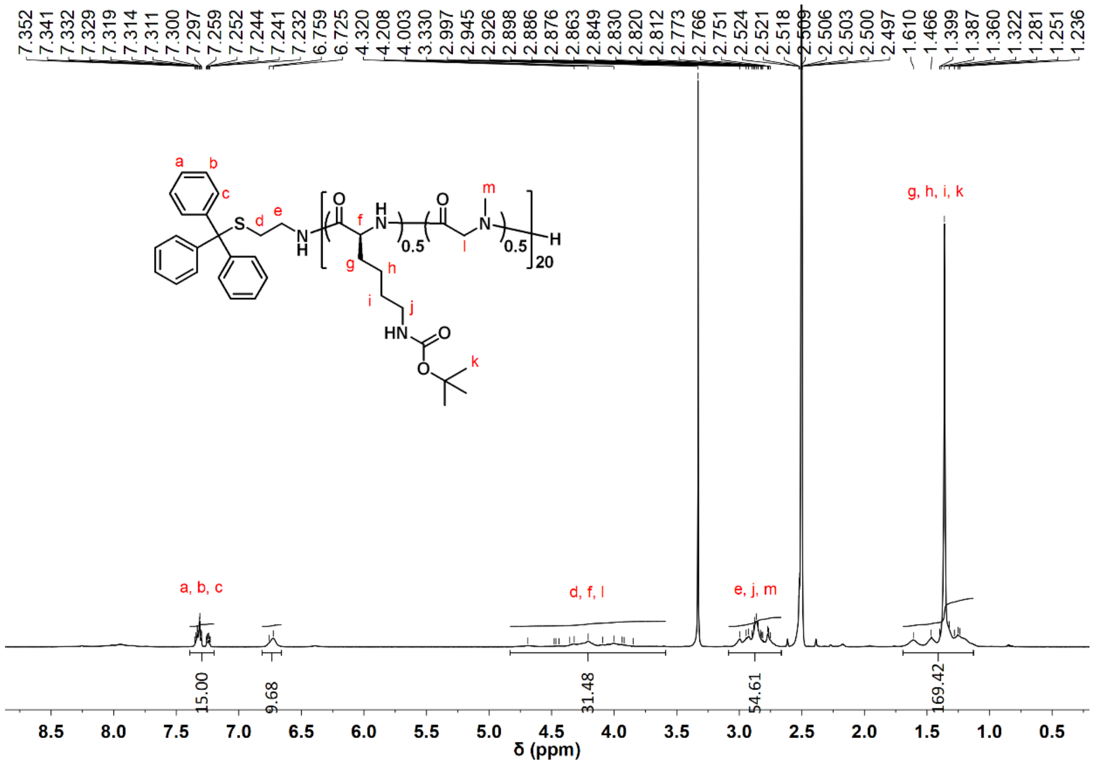


Figure S5. ^{1}H NMR spectrum of poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ obtained using trityl-protected cysteamine as the initiator for polymerization. Spectrum was collected in D_2O at 600 MHz.

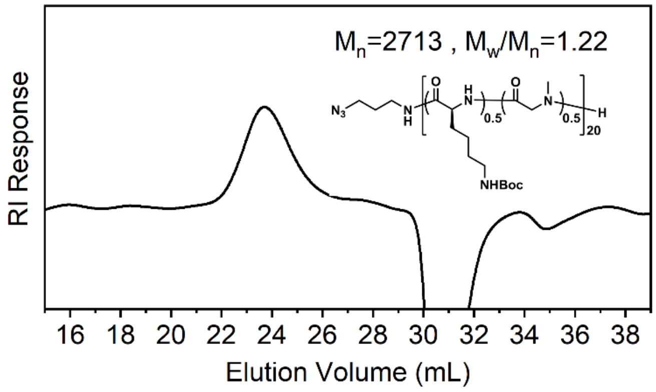


Figure S6. GPC trace of poly-(L-$Lys_{0.5}Sar_{0.5}$)$_{20}$ was obtained using 3-azidopropylamine as the initiator for polymerization.

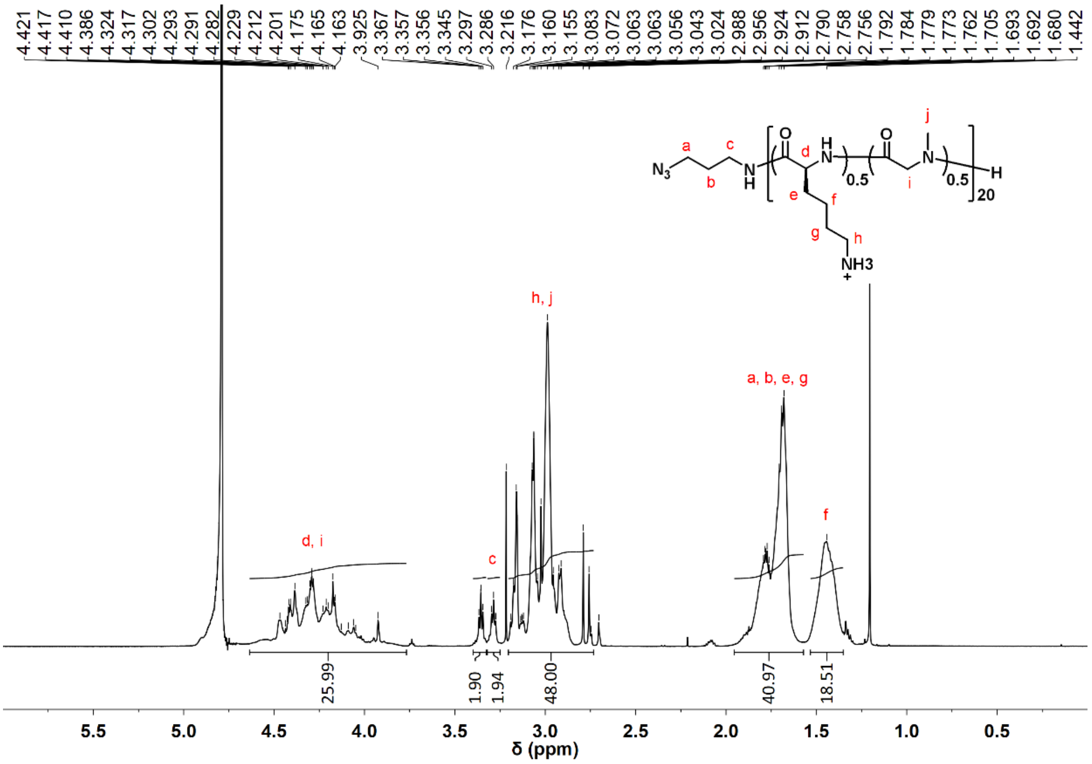


Figure S7. ^{1}H NMR spectrum of poly-(L-$Lys_{0.5}Sar_{0.5}$)$_{20}$ was obtained using 3-azidopropylamine as the initiator for polymerization. Spectrum was collected in D_2O at 600 MHz.

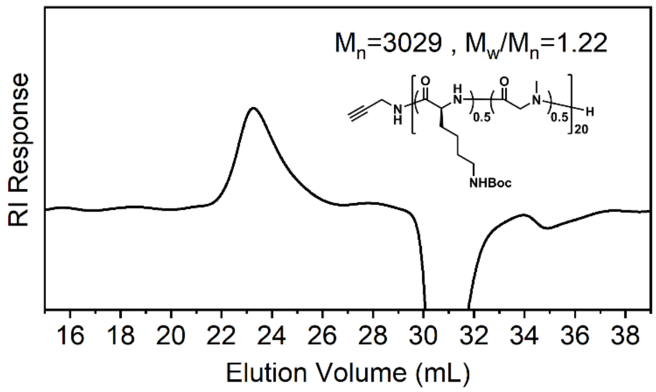


Figure S8. GPC trace of poly-(L-Lys$_{0.5}$Sar$_{0.5}$)$_{20}$ using propargylamine as the initiator for polymerization.

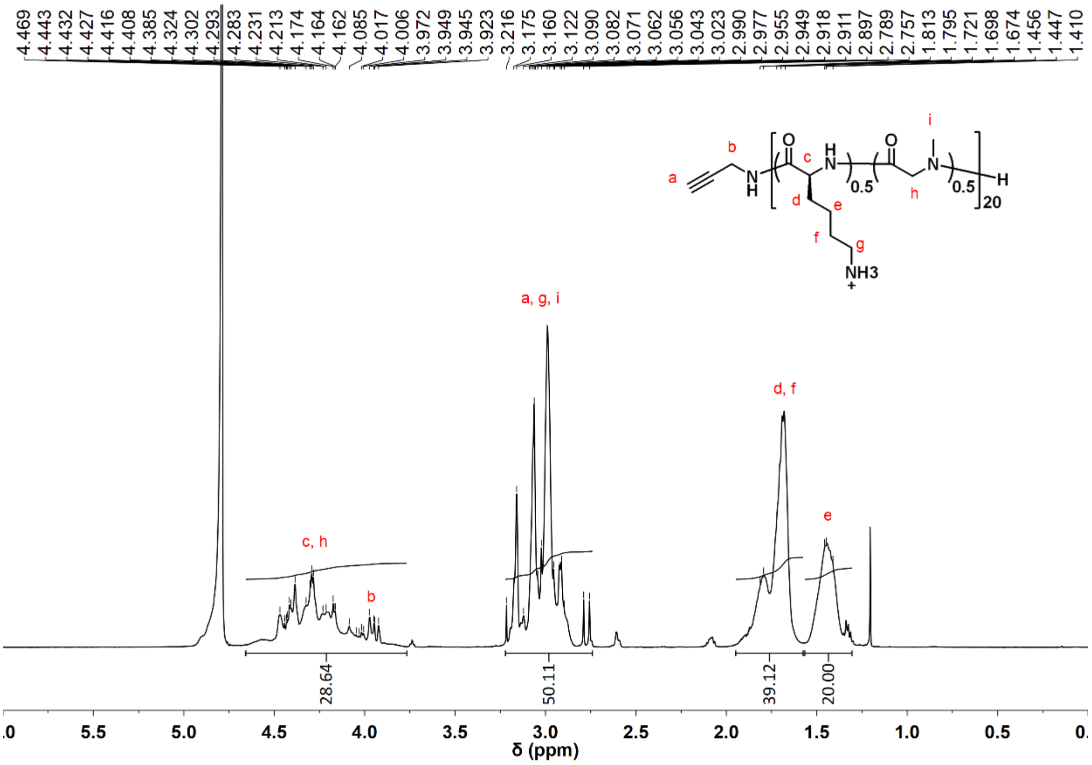


Figure S9. ^{1}H NMR spectrum of poly-(L-Lys$_{0.5}$Sar$_{0.5}$)$_{20}$ using propargylamine as the initiator for polymerization. Spectrum was collected in D$_2$O at 600 MHz.

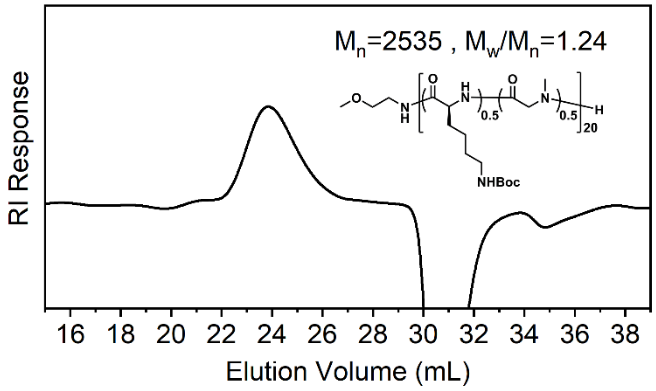


Figure S10. GPC trace of poly-(L-Lys$_{0.5}$Sar$_{0.5}$)$_{20}$ using 2-methoxyethylamine as the initiator for polymerization.

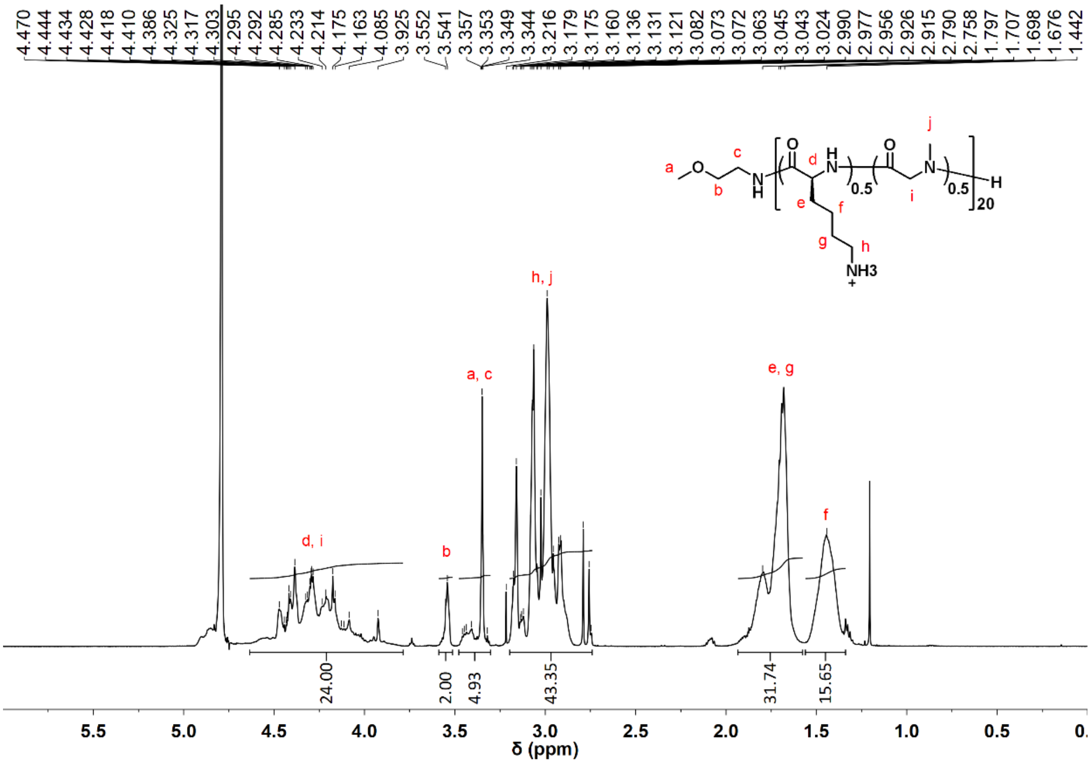
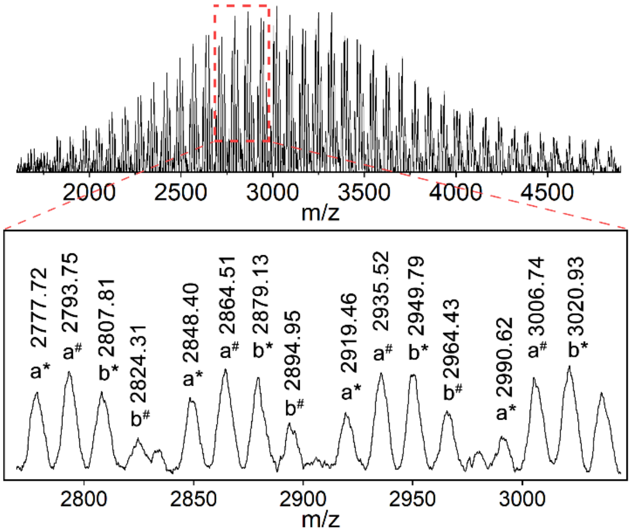


Figure S11. ^{1}H NMR spectrum of poly-(L-Lys$_{0.5}$Sar$_{0.5}$)$_{20}$ using 2-methoxyethylamine as the initiator for polymerization. Spectrum was collected in D_2O at 600 MHz.



a*: R-BLL$_7$Sar$_n$+Na$^+$ 162.13+7*228.15+n*71.04+1.01+22.99 (n=14,15,16,17)

a#: R-BLL$_7$Sar$_n$ +K$^+$ 162.13+7*228.15+n*71.04+1.01+38.96 (n=14,15,16,17)

b*: R-BLL$_9$Sar$_n$+Na$^+$ 162.13+9*228.15+n*71.04+1.01+22.99 (n=8,9,10,11)

b#: R-BLL$_9$Sar$_n$+K$^+$ 162.13+7*228.15+n*71.04+1.01+38.96 (n=8,9,10,11)

Figure S12. MALDI-TOF-MS spectrum of poly-(BLL$_{0.5}$Sar$_{0.5}$)$_{20}$ from tBuBnNH$_2$-initiated copolymerization on Boc-L-Lys NCA and Sar NTA using THF as the solvent at 25°C, [M]$_0$=0.2 M and [M$_{NCA}$]$_0$:[M$_{NNTA}$]$_0$:[I]$_0$=10:10:1. With the structural assignment for the observed mass ions.

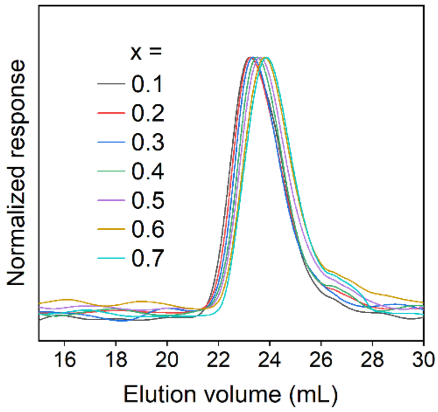


Figure S13. GPC traces of poly-(BLL$_{1-x}$Sar$_x$)$_{20}$ (x=0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7). DMF was used as the mobile phase at a flow rate of 1 mL/min.

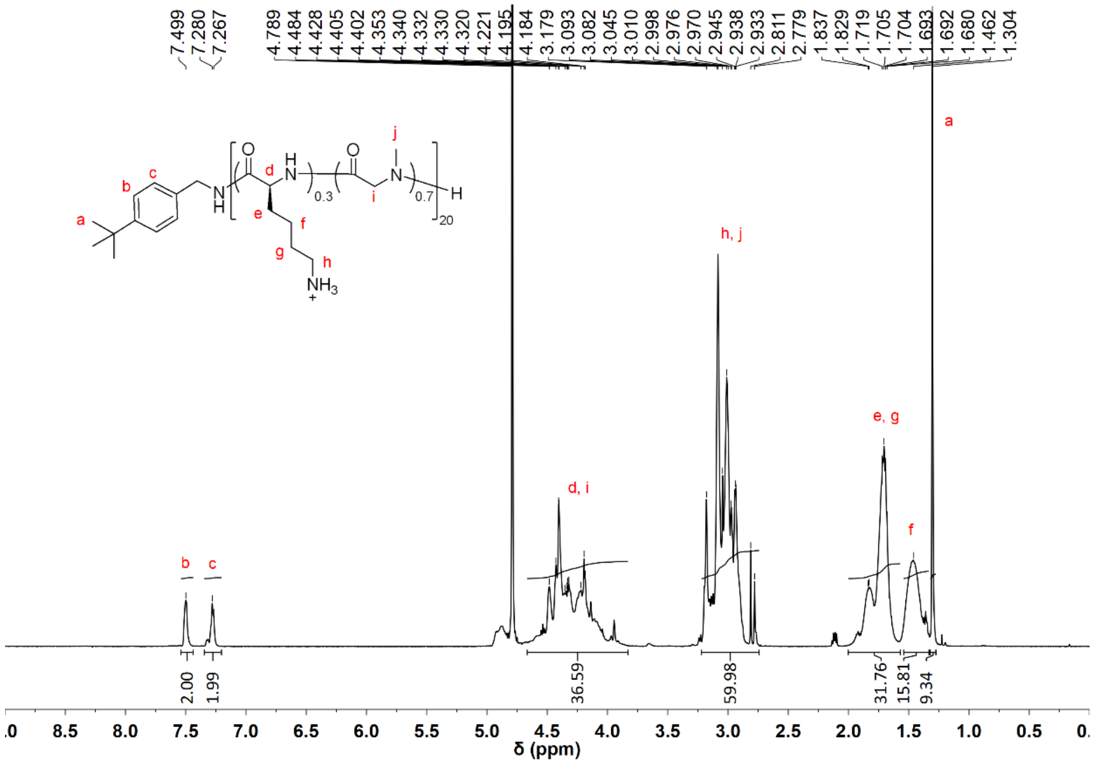


Figure S14. ^{1}H NMR spectrum of poly-(L-Lys$_{0.3}$Sar$_{0.7}$)$_{20}$ in D_2O at 600 MHz.

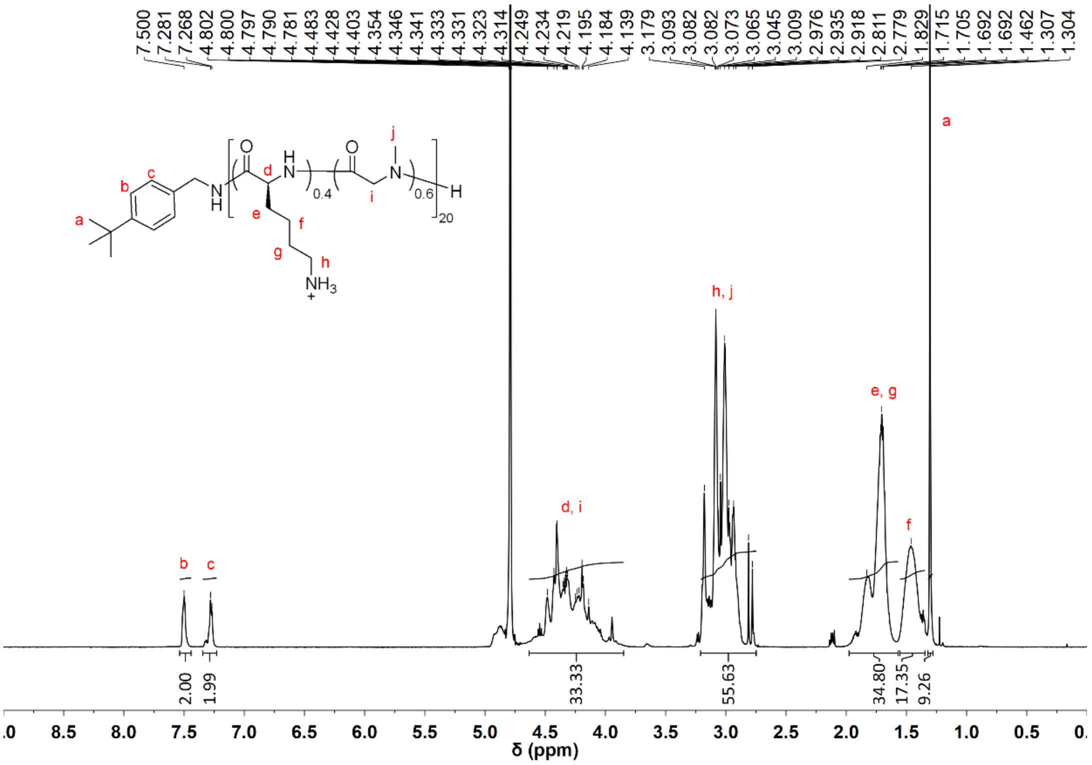


Figure S15. ^{1}H NMR spectrum of poly-(L-Lys$_{0.4}$Sar$_{0.6}$)$_{20}$ in D_2O at 600 MHz.

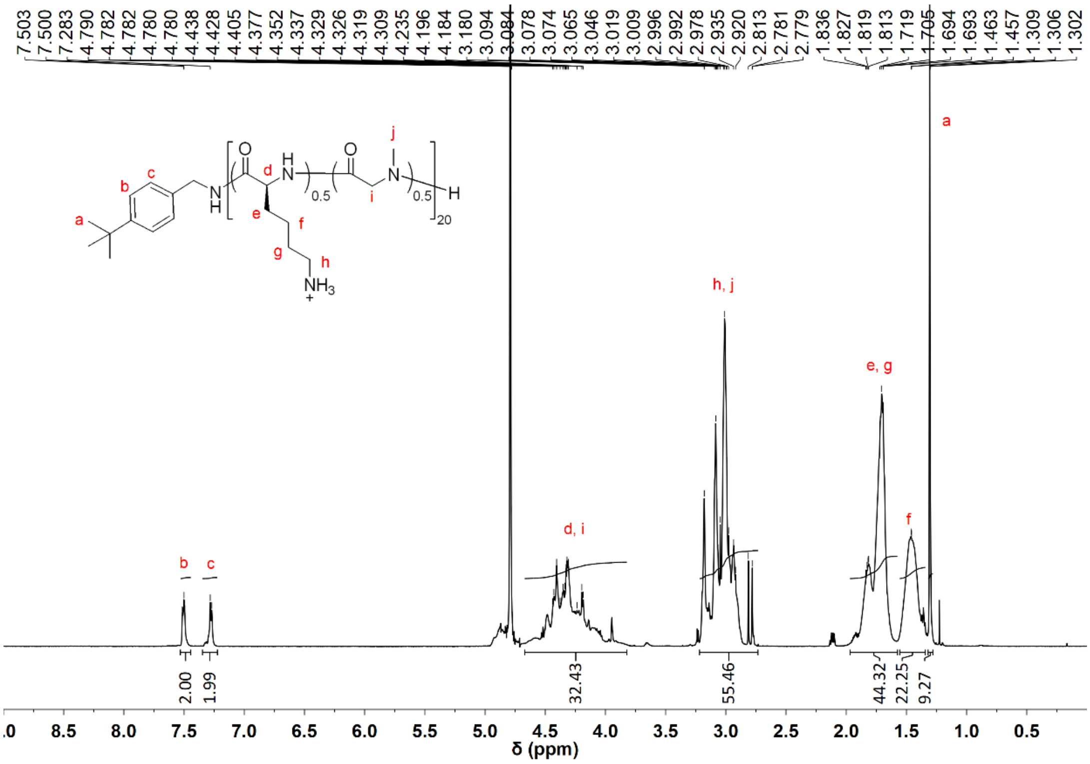


Figure S16. ^{1}H NMR spectrum of poly-(L-$Lys_{0.5}Sar_{0.5})_{20}$ in D_2O at 600 MHz.

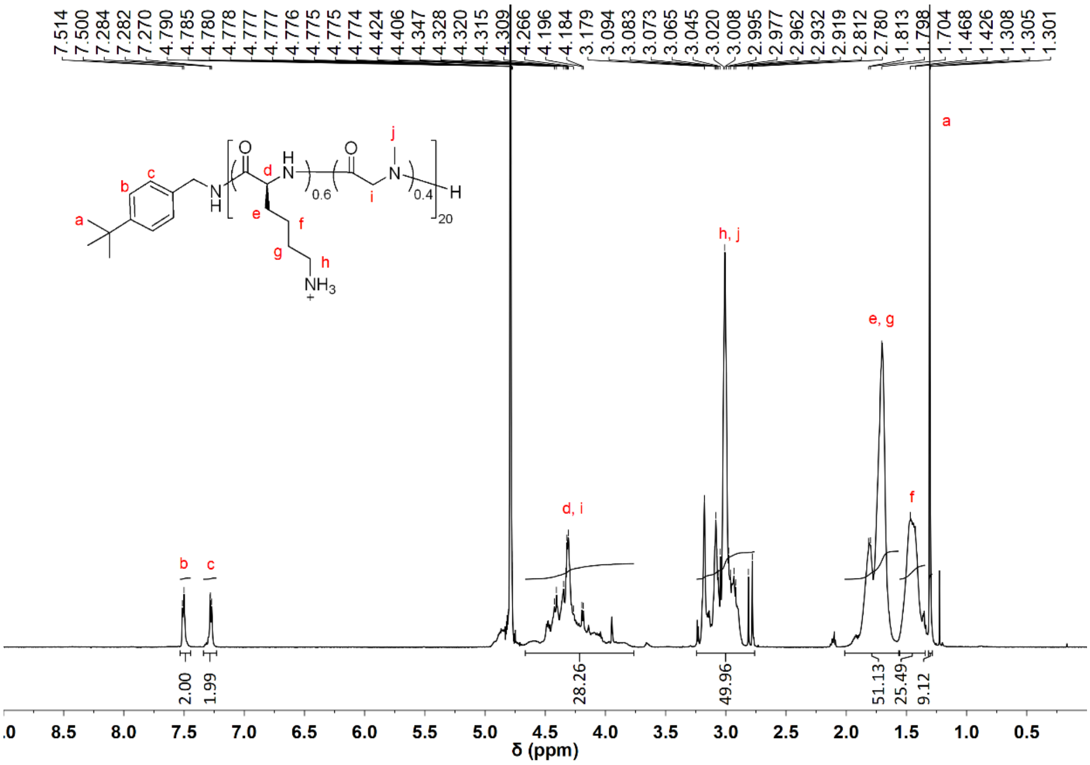


Figure S17. ^{1}H NMR spectrum of poly-(L-$Lys_{0.6}Sar_{0.4})_{20}$ in D_2O at 600 MHz.

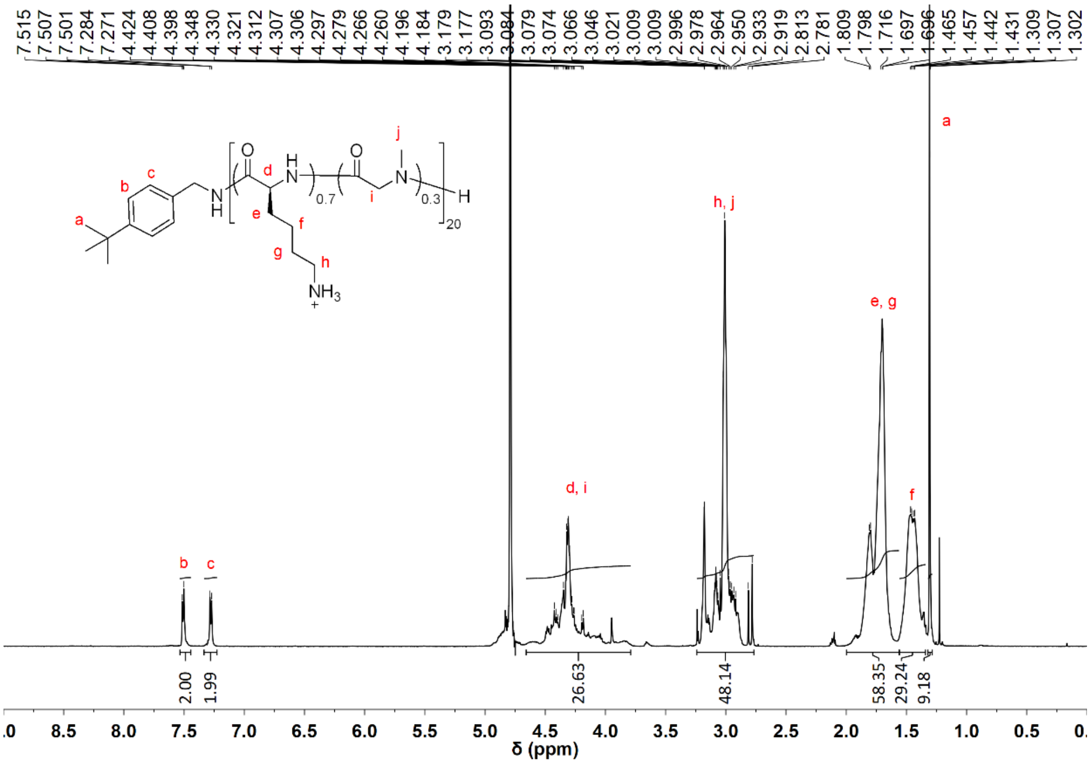


Figure S18. ^{1}H NMR spectrum of poly-(L-Lys$_{0.7}$Sar$_{0.3}$)$_{20}$ in D_2O at 600 MHz.

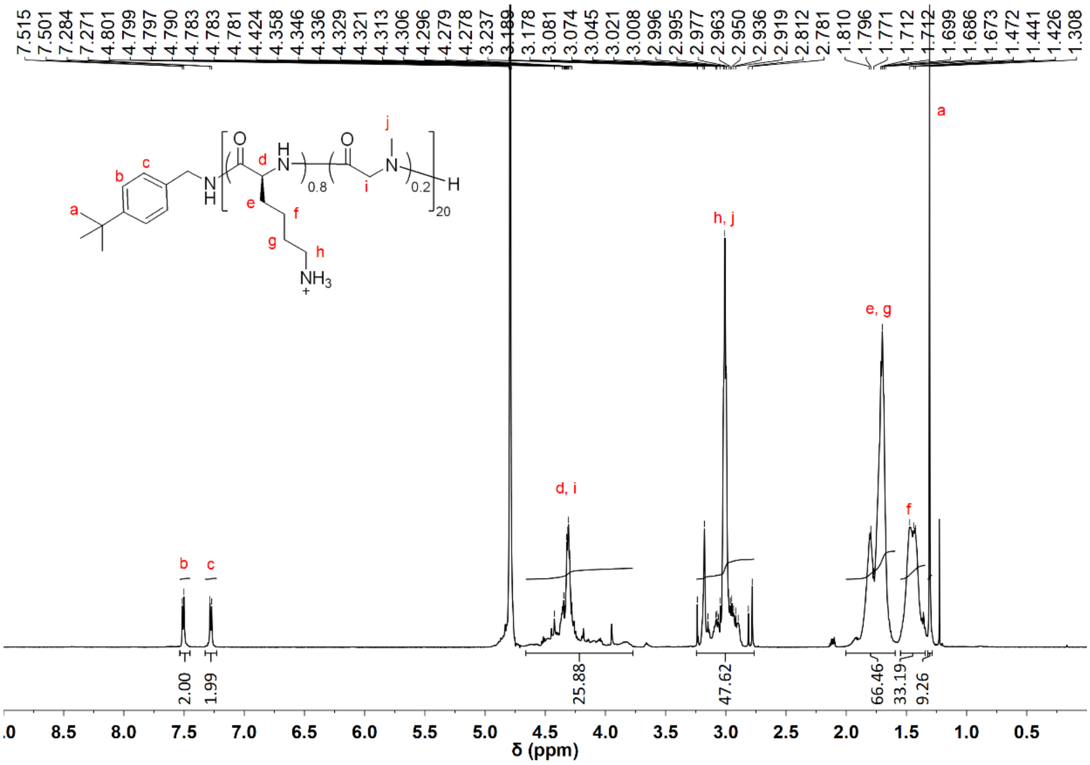


Figure S19. ^{1}H NMR spectrum of poly-(L-Lys$_{0.8}$Sar$_{0.2}$)$_{20}$ in D_2O at 600 MHz.

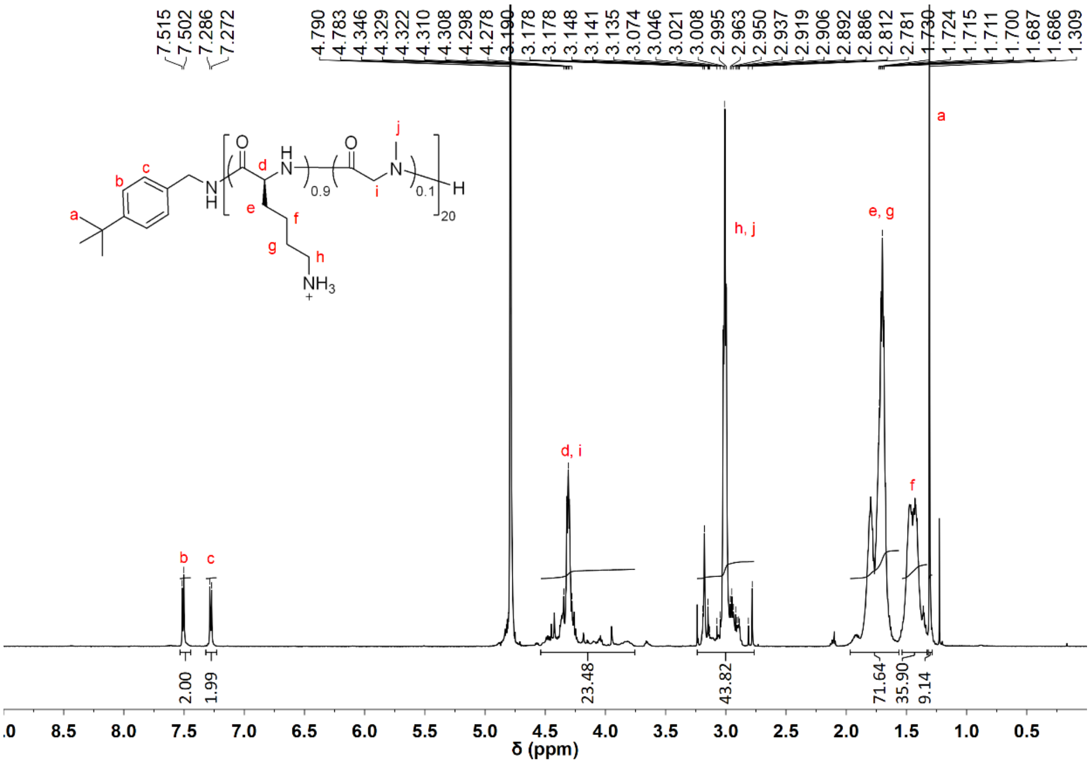


Figure S20. ^{1}H NMR spectrum of poly-(L-Lys$_{0.9}$Sar$_{0.1}$)$_{20}$ in D$_2$O at 600 MHz.

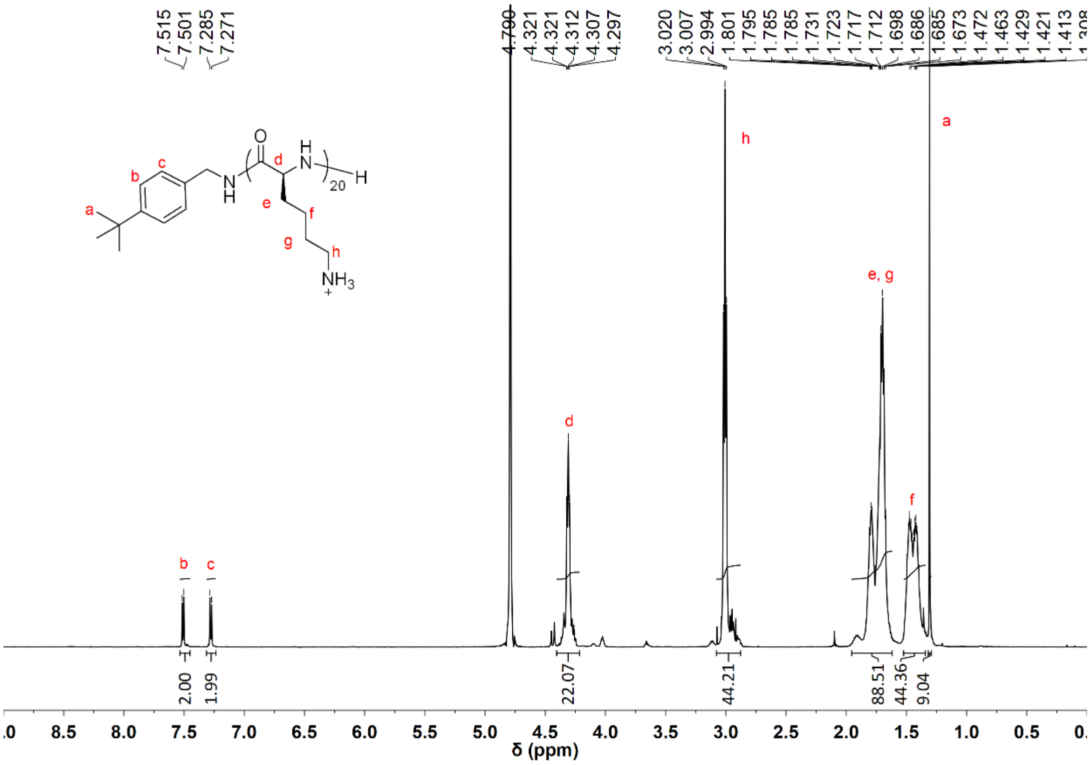


Figure S21. ^{1}H NMR spectrum of poly-(L-Lysine)$_{20}$ in D$_2$O at 600 MHz.

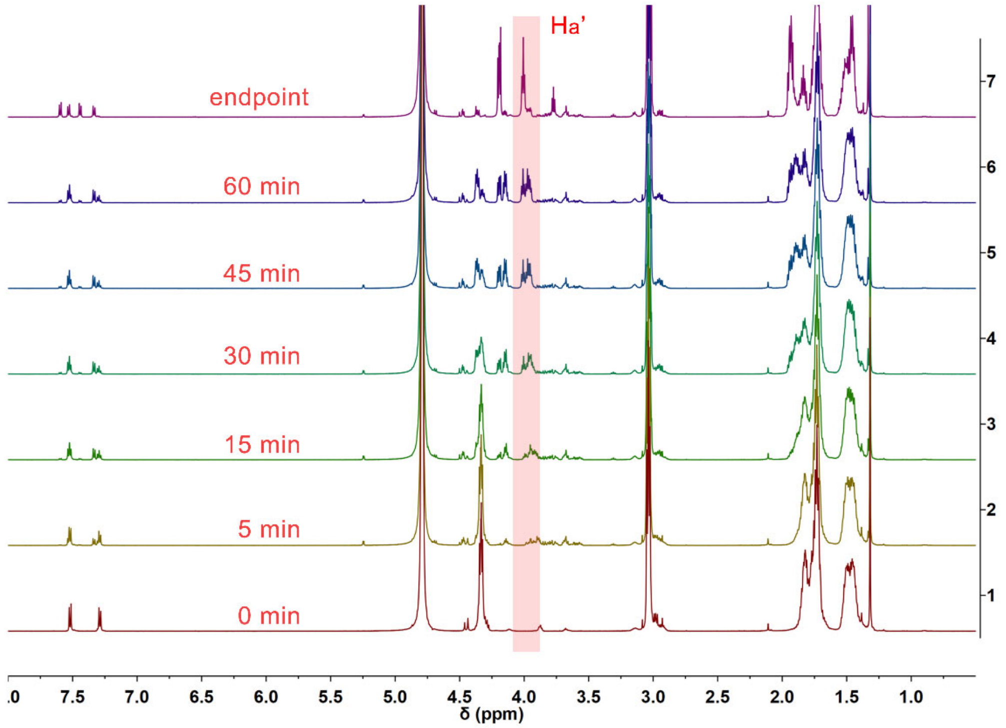


Figure S22. ^{1}H NMR spectra of poly-(L-Lysine)$_{20}$ in the presence of trypsin to analyze polypeptide degradation at 600 MHz.

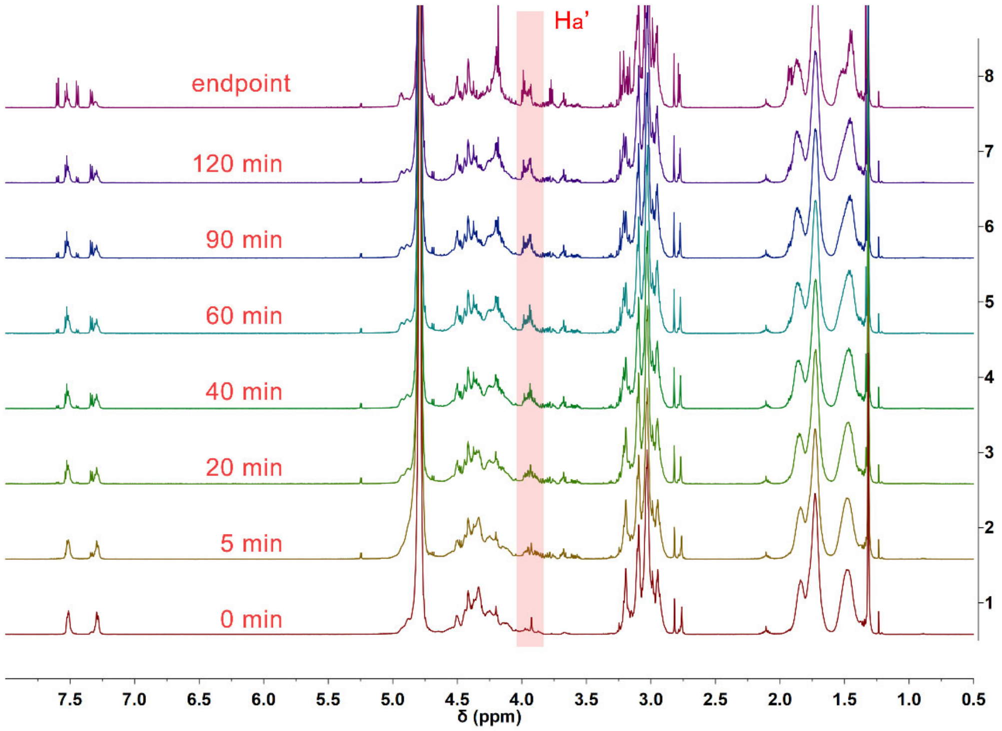


Figure S23. ^{1}H NMR spectra of poly-(L-Lys$_{0.5}$Sar$_{0.5}$)$_{20}$ in the presence of trypsin to analyze polypeptide degradation at 600 MHz.

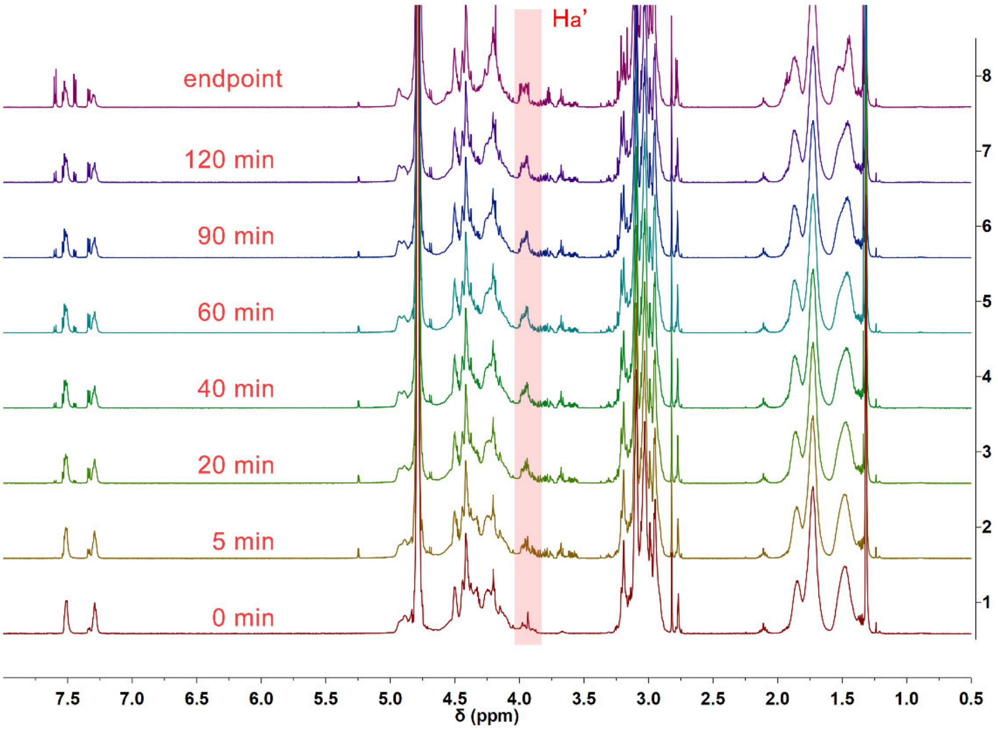


Figure S24. ^{1}H NMR spectra of poly-(L-Lys$_{0.3}$Sar$_{0.7}$)$_{20}$ in the presence of trypsin to analyze polypeptide degradation at 600 MHz.

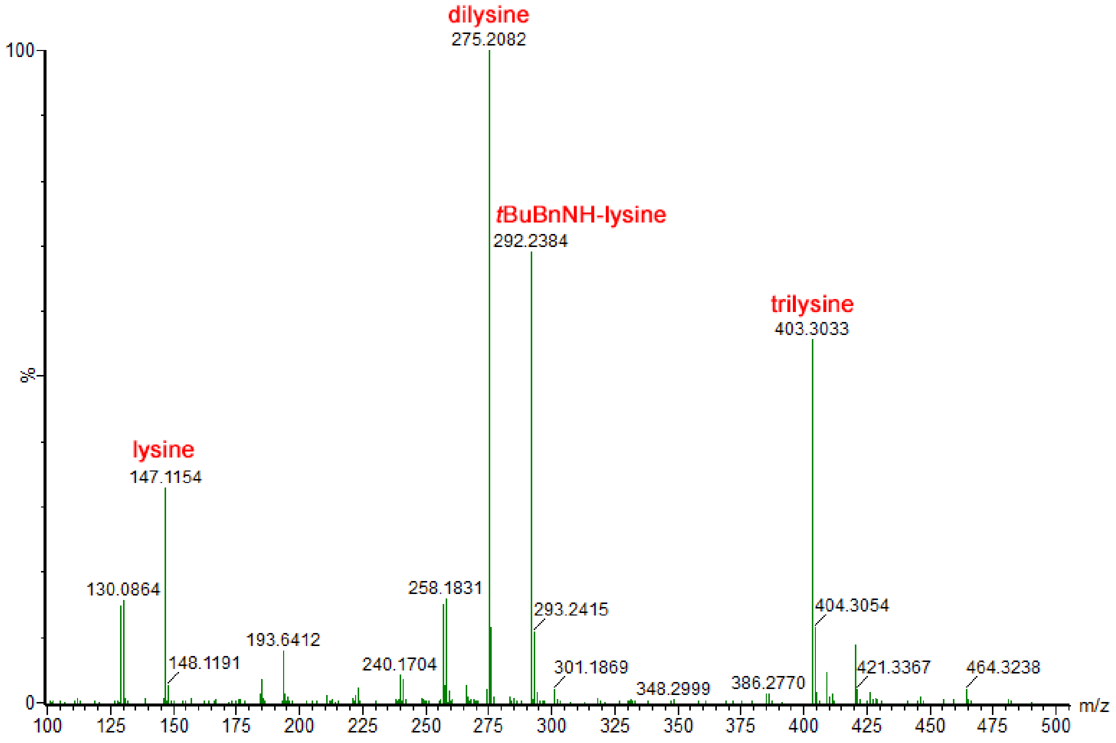


Figure S25. ESI-MS characterization result of poly-(L-Lysine)$_{20}$ incubated with trypsin at the endpoint (polymer/trypsin=40/1, w/w).

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