Supporting information

Combating Drug-Resistant Bacteria with Sulfonium Cationic Poly(methionine)

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Materials

Chemical reagents including D,L-methionine, Phosphorus tribromide, methyl iodide and the solvents including dichloromethane (CH_2Cl_2), dioxane and methanol were purchased from the Sinopharm Chemical Reagent Co., Ltd. All chemicals were purchased from commercial suppliers and used without further purification. The bacteria of Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* ATCC12228, methicillin-resistant *Escherichia coli* (*E. coli*) ATCC25922 and *Pseudomonas aeruginosa* O1 (PA O1) were provided by Wuhan Yeasen Biotechnology Co., Ltd, China. All biological experimentations were tested three times independently.

Characterizations

^{1}H NMR spectra were recorded on a Bruker AVANCE NEO 400 MHz NMR spectrometer. Deuterated chloroform ($CDCl_3$) or deuteroxide (D_2O) was used as the solvents. Gel permeation chromatography (GPC) was conducted with 0.02 M LiBr in DMF as the eluent at a flow rate of 1.0 mL/min at 50 °C using detector Waters1525 detector. Calibration was done using polystyrene standards. The optical density (OD) reading was recorded on a microplate reader (TECAN, Switzerland).

Methods

Synthesis of D, L-Met NTA: D,L-methionine (14.9 g, 100 mmol) and XAA (18.0 g, 100 mmol) was dissolved in 500 mL deionized water, then the sodium hydroxide solution (4 M, 100 mL) were added to the mixture, the reaction mixture was stirred vigorously for two days at room temperature followed by acidification with 1 M HCl to pH ~ 3 and extraction with ethyl acetate (2 × 250 mL). The organic phase was combined then dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to afford a yellow oil.

The oil was dissolved in dichloromethane (500 mL), then the phosphorus tribromide (9.3 mL, 100 mmol) was dropwise into the reaction solution under nitrogen at 0 ^oC. The reaction mixture was stirred at room temperature for 12 h and then sequentially washed with deionized water (2 × 200 mL) and brine solution (200 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to afford the crude product, which was purified by silica gel column separation to afford the D, L-Met NTA as a white solid (9.67 g, 42.5 mmol, 50.6% yield). MS-HREI

(m/z): $[M+H]^+$ calculated for $C_6H_9NO_2S_2$ 191.0075, found 191.0077. ^1H NMR (400 MHz, $CDCl_3$, **Figure S1**) δ (ppm): 7.58 (s, 1H, NH), 4.50 (m, 1H, CH), 2.67 (m, 2H, CH_2), 2.28 & 2.04 (m, 2H, CH_2), 2.10 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, **Figure S2**) δ (ppm): 198.38, 167.65, 66.07, 31.58, 29.89, 15.21. The characterization results are consistent with the data in the literature reported by reported by Zhang *et. al*[1]

Polymerization of D, L-Met NTA: Firstly, P-tert butyl benzylamine as initiator and D, L-Met NTA as monomer were respectively dissolved in dioxane to 2 M final concentration. Then the different volumes of initiator solution (200 μL, 100 μL, 50 μL and 25 μL) were respectively added to 1 mL of D, L-Met NTA solution under an air environment according to the designed degree of polymerizations (DPs), the reaction mixtures were stirred for 3 days at 80 ^oC. The polymerization process was monitored by TLC. The reaction was concentrated under vacuum to afford the crude product polymers, which was purified by precipitation in ice petroleum ether. After drying, the final poly(D,L-methionine)s as the white solid were obtained and then characterized by GPC or MALDI-TOF-MS (**Figure S3, Table S1**).

General procedure for alkylation: The final poly(D,L-methionine)s were respectively dissolved in methanol at the concentration of 30 mg/mL, followed adding the methyl iodide (10 eq per of methylthio-group) with stirred vigorously for 60 h at 37°C, the solution was precipitated in ice ether. After drying, the final sulfonium ion-based poly(D,L-methionine)s were characterized by ^1H NMR.

Synthesis of Lysine-NCA: $N^ε$-Boc-L-lysine (24.6 g, 100 mmol) and α-pinene (46.6 mL, 300 mmol) was dissolved in 600 mL anhydrous THF, then triphosgene (14.8 g, 50 mmol) was added to the mixture, the reaction mixture was stirred for 6 hours at 50 ^oC under N_2 environment. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (500 mL), followed by washing with ice deionized water (2 × 250 mL) then dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to afford a colorless oil. The crude product was purified by recrystallization three times using a mixture of dry ethyl acetate and hexane to give a white powder (12.6 g, 46.3% yield). MS-ESI (m/z): $[M+H]^+$ calculated for $C_{12}H_{21}N_2O_5$ 273.1, found 273.1. ^1H NMR (400 MHz, $CDCl_3$, **Figure S5**) δ (ppm): ^1H NMR (400 MHz, $CDCl_3$): δ 6.95 (s, 1H), 4.68 (br, 1H), 4.32 (dd, J = 6.8, 4.8 Hz, 1H), 3.14 (m, 2H), 1.96-2.04 (m, 1H), 1.80-1.90 (m, 1H), 1.48-1.60 (m, 4H), 1.45 (s, 9H).

Synthesis of Poly(L-Lysine): L-lysine NCA (2.72 g, 10 mmol) was dissolved in anhydrous dioxane to 2 M final concentration, followed by addition of 2 M solution (250 μL) of P-tert butyl benzylamine in dioxane. The reaction mixture was stirred for three days at room temperature under N_2 environment until the complete consuming of L-lysine NCA. The mixture was concentrated under vacuum to afford a yellow oil that was purified by precipitation in ice petroleum ether.

After drying, the *N*-Boc protected poly(L-Lysine)$_{20}$ and 5 mL trifluoroacetic acid was mixed, the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted by the addition of 50 mL DCM, followed by concentration under vacuum to afford a yellow oil that was purified by precipitation in ice ether. The *N*-Boc deprotected poly(L-Lysine)$_{20}$ was dried via vacuum to obtain the white solid (2.2 g, 88.0% yield) and then characterized by ^{1}H NMR (**Figure S6**).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay: Antibacterial testing was conducted by following the reported methods by Chen *et. al*[2]. Briefly, bacteria in logarithmic phase were diluted to 3×10^5 colony-forming units (CFU) per milliliter in MH medium as the working suspension. The sulfonium ion-based poly(D,L-methionine)s aqueous solution was diluted to the desired concentration (0.49-250 μg/mL) in MH medium via two-fold serial dilution in a 96-well plate. 100 μL of bacterial suspension and 100 μL of polymer solution were mixed in each well, then the 96-well plate was incubated at 37 °C for 18 h, the OD values at 600 nm were collected on a microplate reader. MH medium was considered as the blank, whereas, the bacteria suspension was considered as the positive control. The percentage of bacterial survival was calculated by the equation:

$$(\%\ \text{cell growth} = \frac{OD_{600}^{polymer} - OD_{600}^{blank}}{OD_{600}^{control} - OD_{600}^{blank}} \times 100)$$

After MIC assay, 5 μL of bacterial suspension in a 96-well plate was transferred onto LB agar plates, which was incubated at 37 °C for 18 h again, the final values were determined by visually observing the viability of the bacteria.

Hemolysis assay: The human red blood cells (hRBCs) was washed with phosphate buffer saline (PBS) for three times then diluted to 5% (v/v) in PBS. The sulfonium ion-based poly(D,L-methionine)s aqueous solution (20 mg/mL) were diluted to the desired concentration using PBS via two-fold serial dilution in a 96-well plate. 100 μL of hRBCs suspension and 100 μL of polymer solution in PBS were mixed in

each well, PBS buffer was considered as the blank, whereas, the hRBCs suspension containing 0.5% (v/v) Triton X-100 was as the positive control. Then the 96-well plate was incubated at 37 °C. After 1 h, 100 μL of supernatant in each well was transferred to a new 96-well plate after centrifugation. The OD values at 540 nm were collected on a microplate reader. The percentage of hemolysis was calculated by the equation:

$$(\%\ \text{Hemolysis} = \frac{OD_{540}^{Sample} - OD_{540}^{blank}}{OD_{540}^{control} - OD_{540}^{blank}} \times 100)$$

Cytotoxicity assay: Cytotoxicity of sulfonium ion-based poly(D,L-methionine)s against NIH/3T3 fibroblast cells was determined by methyl thiazolyl tetrazolium (MTT) assay. About 2×10^4 NIH/3T3 fibroblast cells in DMEM medium were respectively added into each well of 96-well plate, then cultured in a humidified 5% CO_2 in air incubator. After 24h, 100 μL of supernatant in each well was removed, and 100 μL of polymer solution in DMEM with desired concentration was added into in each well. The 96-well plate was incubated for 24h again. 10 μL of MTT solution in PBS (5 mg/mL) was added to each well of 96-well plate and the plate was incubated for 4 h again. After aspirating original solution, 150 μL of DMSO was added into each well of each well of 96-well plate. After shaking 96-well plate for 30 min, cells in Tween 20 solution were used as negative control, whereas, cells in DMEM were used as the positive control. The OD values at 490 nm were collected on a microplate reader. The percentage of cell viability was calculated by the equation:

$$(\%\ \text{Cell viability} = \frac{OD_{490}^{polymer} - OD_{490}^{blank}}{OD_{490}^{control} - OD_{490}^{blank}} \times 100)$$

Study on bacterial killing kinetics: Briefly, bacteria in logarithmic phase were washed three times with PBS, and diluted to 3×10^5 CFU/mL as the working suspension, and sulfonium ion-based poly(D,L-methionine)s aqueous solution (5 mg/mL) was diluted to the desired concentration in PBS. Then, 500 μL polymer solution and 500 μL bacterial suspension were mixed in the 96-well plate, which was incubated at 37 °C. At different time intervals, the bacterial suspension was sampled and diluted for plating on LB agar plates. After incubation for 18 h at 37 °C, the final values were calculated by CFU counting of the bacteria.

Reference

1. J. B. Cao, D. Siefker, B. A. Chan, T. Y. Yu, L. Lu, M. A. Saputra, F. R. Fronczek, W. W. Xie and D. H. Zhang, *ACS Macro. Lett.*, 2017, **6**, 836-840.
2. J. Sun, M. Li, M. Lin, B. Zhang and X. Chen, *Adv. Mater*, 2021, **33**, e2104402.

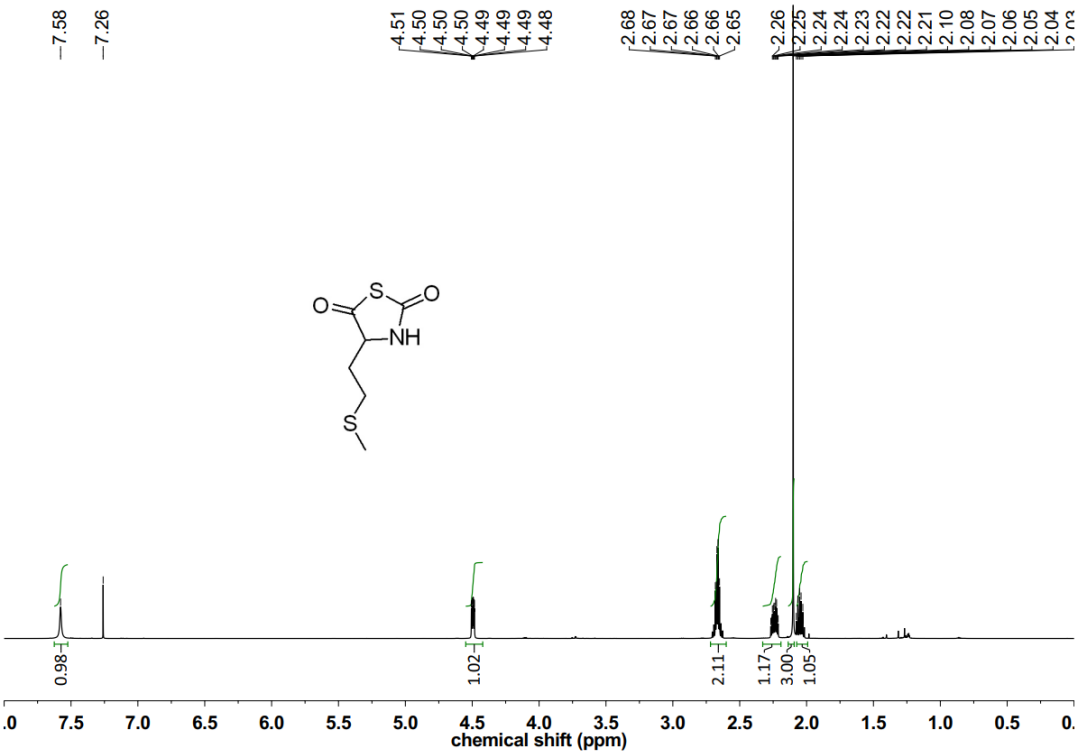

Figure S1. ^{1}H NMR spectra of D, L-Met NTA in $CDCl_3$ solvent.

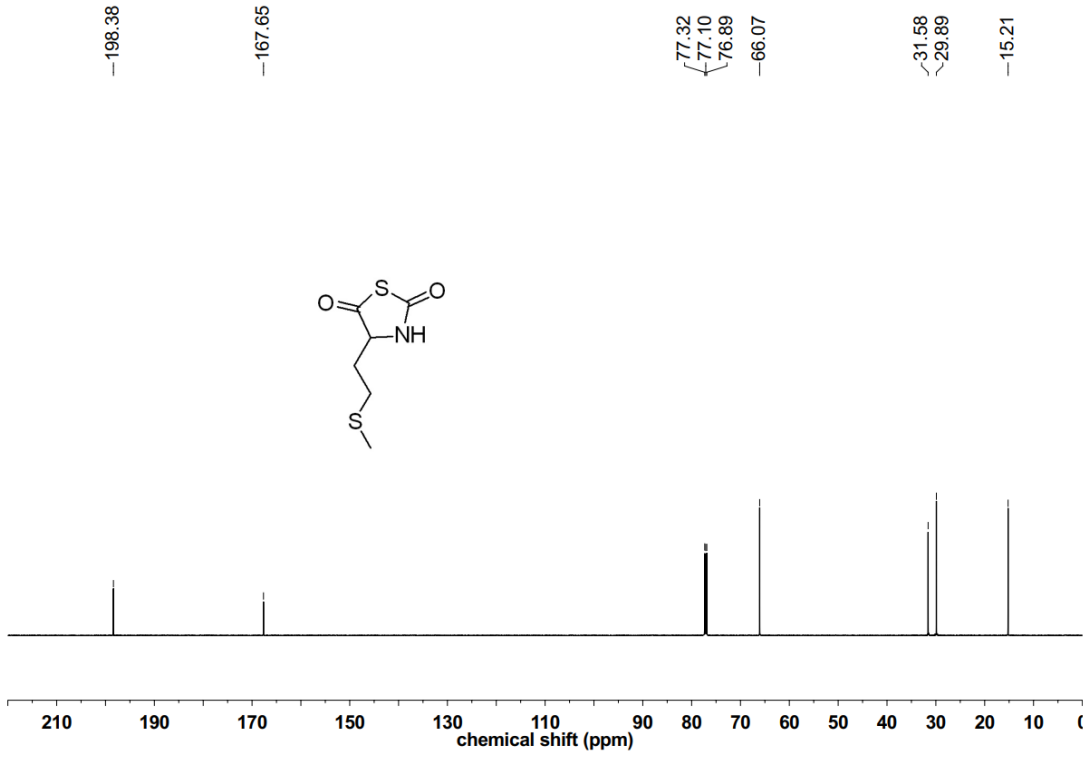

Figure S2. ^{13}C NMR spectra of D, L-Met NTA in $CDCl_3$ solvent.

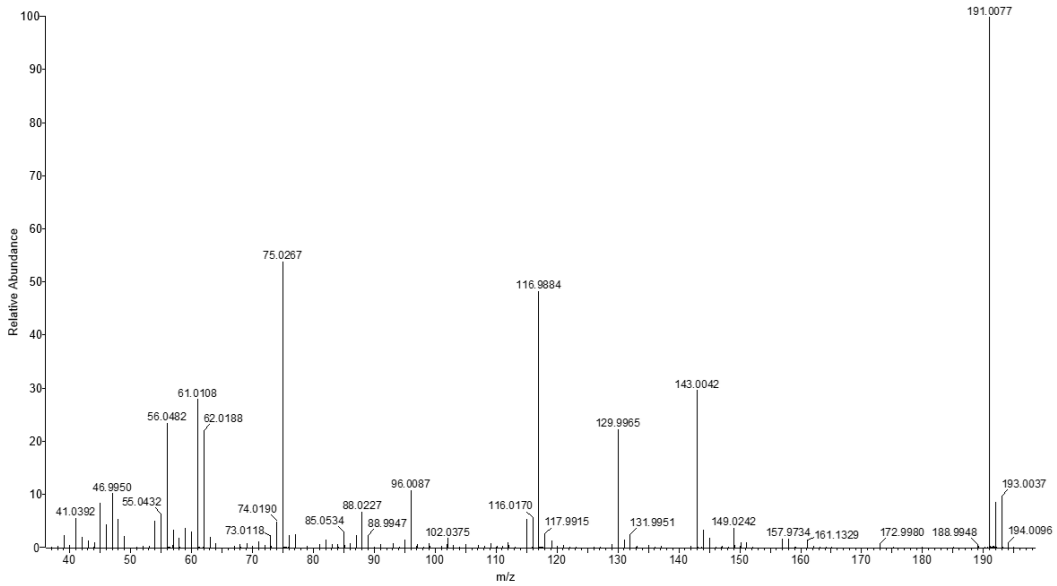

Figure S3. HREI-MS spectra of D, L-Met NTA.

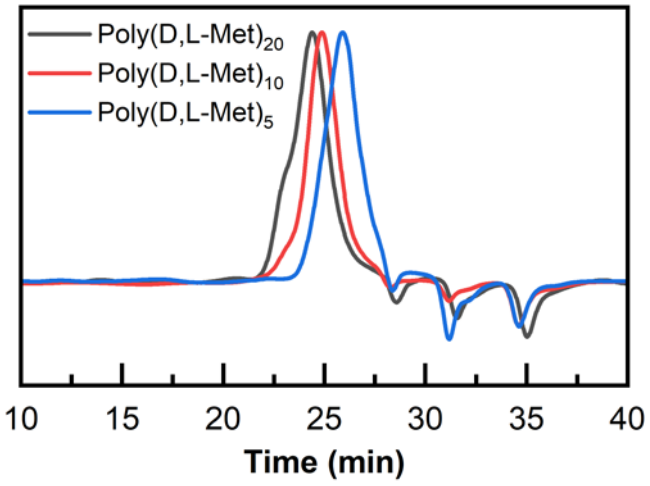

Figure S4. GPC traces of poly(D,L-methionine)s. In the case of poly(D,L-methionine)s with the initial feeding ratio of monomer/initiator at 5/1-20/1, the polymers was soluble in DMF solvent. The polymerization product showed the narrow molecular weight distribution with dispersities (Đ) < 1.20 shown by GPC, indicating the well-controlled polymerization.

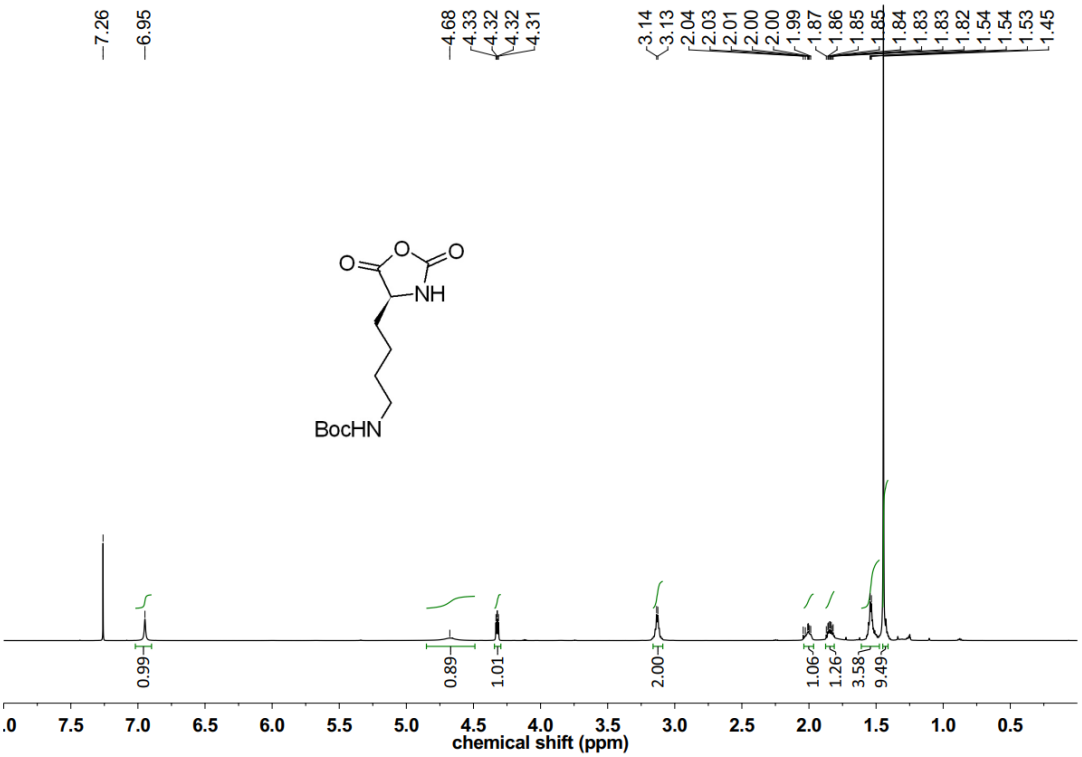

Figure S5. ^{1}H NMR spectra of L-Lysine NCA in $CDCl_3$ solvent.

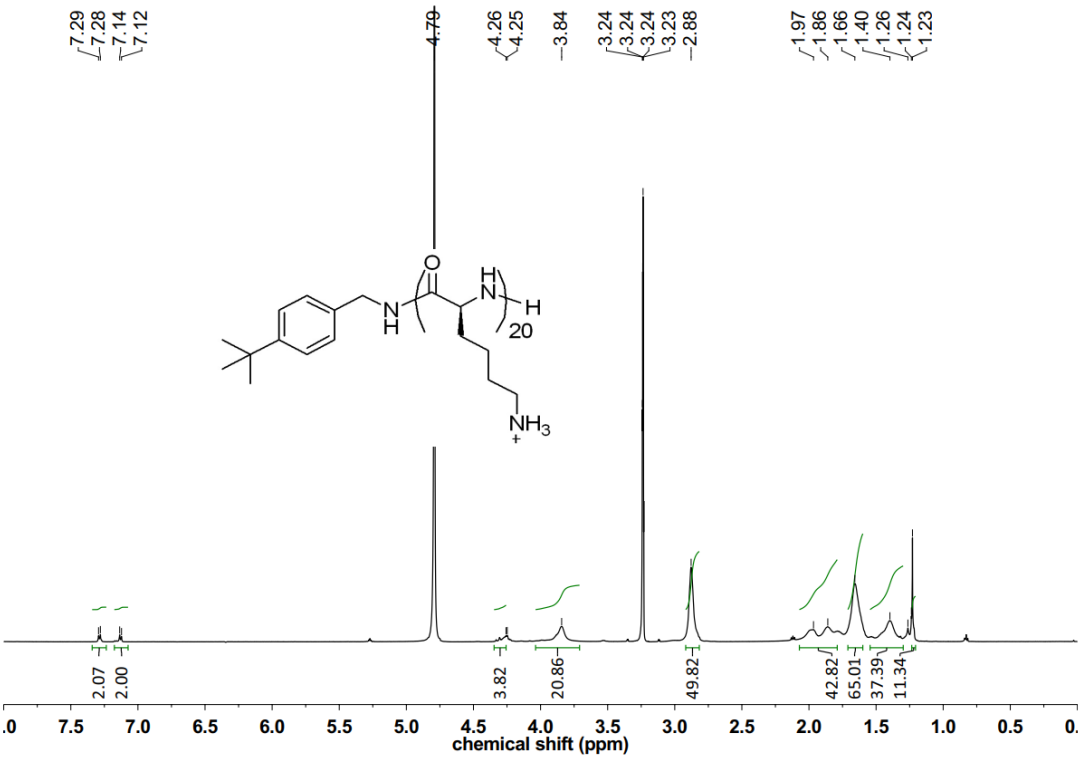

Figure S6. ^{1}H NMR spectra of Poly(L-Lysine)$_{20}$ in D_2O solvent.

Table S1. Characterization of sulfonium ion-based poly(D,L-methionine)s [a].

Entry	monomers/initiator	M_n [a] (g/mol)	M_n [b] (g/mol)	DP	Đ
1	5:1	818	1100	7	1.16
2	10:1	1473	1600	11	1.16
3	20:1	2783	2900	21	1.19
4	40:1	5403	5000 [c]	37 [c]	1.15 [c]

[a]M_n means theoretical molecular weight, [a]M_n means molecular weight calculated from GPC characterization using DMF as solvent. [c]M_n means molecular weight calculated from MALDI-TOF-MS characterization, [c]DP mean degree of polymerization that calculated from MALDI-TOF-MS characterization.