Electronic Supplementary Material (ESI) for Polymer Chemistry.

Controllable and Facile Synthesis of Poly(2-oxazoline)s Using Trimethylsilyl Trifluoromethanesulfonate as the Initiator

Min Zhou,[†, a] Zhemin Ji,[†, b] Ximian Xiao,[b] Longqiang Liu,[b] Ruxin Cui,[b] Zhengjie Luo,[b] Zihao Cong[b] and Runhui Liu[*,a,b,c]

[a]State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China

[b]Key Laboratory for Ultrafine Materials of Ministry of Education, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai 200237, China

[c]East China University of Science and Technology Shenzhen Research Institute, Shenzhen, China

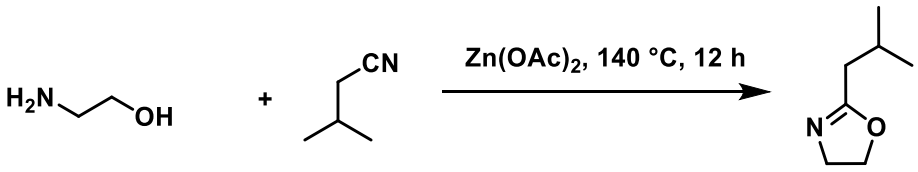
[†]These authors contribute equally to this work.

Correspondence should be addressed to R.L. (rliu@ecust.edu.cn)

EXPERIMENTAL SECTION

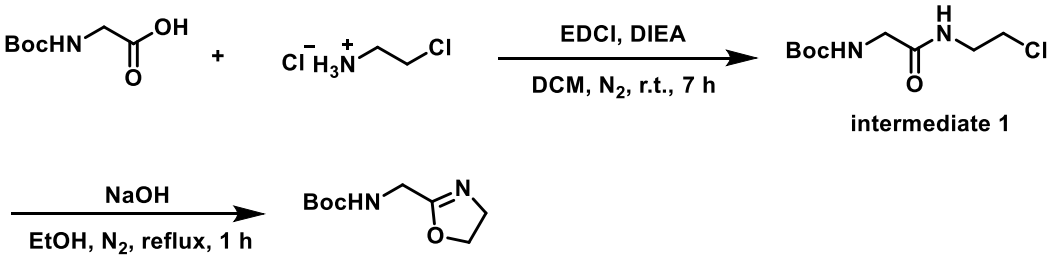
Materials. Anhydrous solvents of acetonitrile (MeCN), benzonitrile (PhCN), dimethyl sulfoxide (DMSO), *N*, *N*-Dimethylformamide (DMF), *N*, *N*-dimethylacetamide (DMAc), sulfolane, dichloromethane (DCM), tetrahydrofuran (THF), chloroform ($CHCl_3$) were purchased from Sigma-Aldrich and used without further purification. 2-Methyl-2-oxazoline (MeOx), 2-ethyl-2-oxazoline (EtOx), 2-phenyl-2-oxazoline (PhOx), trimethylsilyl trifluoromethanesulphonate (TMSOTf), methyl trifluoromethanesulfonate (MeOTf) , isovaleronitrile, ethanolamine, zinc acetate, *N*-Boc-aminoacetic acid, *N*-Boc-aminobutyric acid, *N*-Boc-aminovaleric acid, *N*-Boc-aminocaproic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), *N, N*-diisopropylethylamine (DIEA), 2-chloroethylamine hydrochloride, sodium cyanoborohydride ($NaBH_3CN$), citric acid, magnesium sulphate ($MgSO_4$), lithium bromide (LiBr), triphenylmethane (TPM), sodium hydroxide (NaOH) and other solvents 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. 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 electron ionization time of flight mass spectrometry (HREI-MS) was collected on a Waters GCT Premier 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 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 determined 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 using 2,5-dihydroxybenzoic acid (DHB) as matrix.

Synthesis of 2-iso-butyl-2-oxazoline (*i*BuOx):



Isovaleronitrile (10.0 g, 120.3mmol), ethanolamine (8.1 g, 132.3 mmol) and zinc acetate (2.2 g, 12.0 mmol) were added to a dried flask and the reaction mixture was stirred at 140 °C for 12 h under nitrogen atmosphere. The reaction solution was cooled down to room temperature (r.t.) and subsequently diluted with dichloromethane (DCM, 150 mL). The organic phase was washed with H_2O (50 mL × 3) and brine (50 mL × 1), followed by drying over anhydrous $MgSO_4$. The organic phase was then filtered and the filtration was concentrated under reduced pressure. The residue was distilled under diminished pressure to afford the product as colorless liquid (7.0 g, 45.8% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.24 (t, *J* = 9.2 Hz, 2H), 3.83 (t, *J* = 9.2 Hz, 2H), 2.16 (d, *J* = 7.6 Hz, 2H), 2.04 (m, *J* = 6.8 Hz, 1H), 0.98 (d, *J* = 6.4 Hz, 2H). The ^{1}H NMR spectrum was in agreement with the data in previous reports.[1] HRESI-MS: m/z calculated for $C_7H_{14}NO$ $[M+H]^+$: 128.1075; Found 128.1073.

Synthesis of 2-(*N*-Boc-1-aminomethyl)-2-oxazoline ($MeNH_{Boc}Ox$):

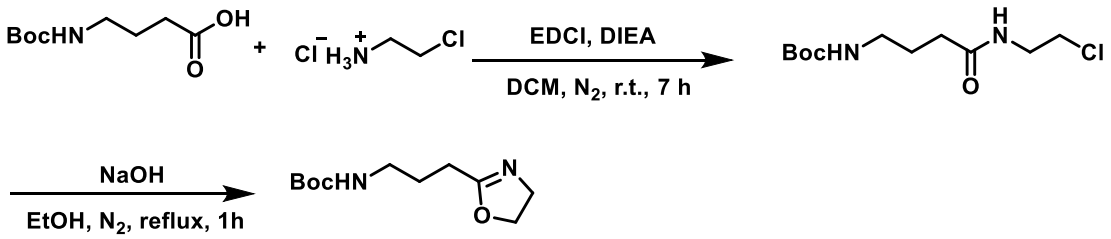


First, *N*-Boc-aminoacetic acid (10.0 g, 57.1 mmol) was dissolved in anhydrous DCM (200 mL) in a round bottom flask. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI, 16.4 g, 85.6 mmol) and *N*, *N*-diisopropylethylamine (DIEA, 28.9 mL, 171.3 mmol) was added to the solution subsequently at 0 °C under nitrogen atmosphere. The mixture was stirred for 20 minutes, 2-chloroethylamine hydrochloride (8.6 g, 74.2

mmol) was added and the reaction mixture was warmed up to r.t. and stirred for 8 h. The reaction mixture was washed with citric acid (2%, 100 mL × 3) and brine (100 mL × 1), followed by drying over anhydrous $MgSO_4$. The organic phase was then filtered and the filtration was concentrated under reduced pressure. The crude product was recrystallized in the mixture of DCM/hexane (v/v=1/3) to give the intermediate 1 as a colorless needle-like crystal (6.2 g, 45.9% yield).

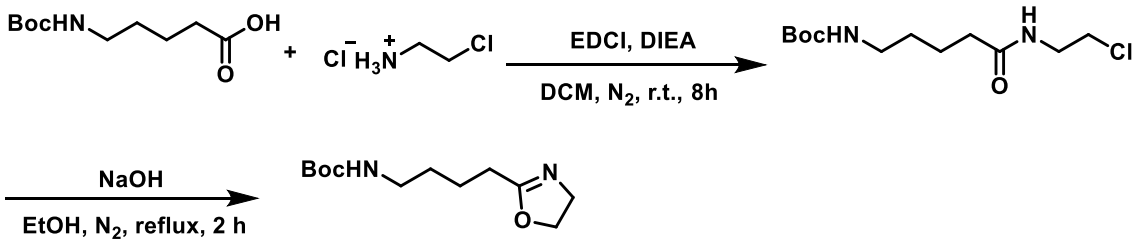
Secondly, intermediate 1 (6.0 g, 25.4 mmol) and sodium hydroxide (NaOH, 1.2 g, 30.0 mmol) was dissolved in ethanol (EtOH, 250 mL). The reaction mixture was stirred at reflux under nitrogen atmosphere for 1.5 h. Subsequently, the solvent was removed under reduced pressure. The remaining solid was dissolved in ethyl acetate (EA, 100 mL) and washed with H_2O (100 mL × 3), followed by drying over anhydrous $MgSO_4$. The organic phase was then filtered and the filtration was concentrated under reduced pressure. The crude product was recrystallized in the mixture of EA/hexane (v/v=1/7) to give $MeNH_{Boc}Ox$ as a colorless needle-like crystal (2.6 g, 51.2% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (br, 1H), 4.32 (t, J = 9.6 Hz, 2H), 3.95 (d, J = 5.2 Hz, 2H), 3.86 (t, J = 9.2 Hz, 2H), 1.45 (s, 9H). The ^{1}H NMR spectrum was in agreement with the data in previous reports.[2] EI-MS: m/z calculated for $C_9H_{16}N_2O_3$ $[M]^+$: 200.1; Found 200.1.

Synthesis of 2-(*N*-Boc-3-aminopropyl)-2-oxazoline ($ProNH_{Boc}Ox$):



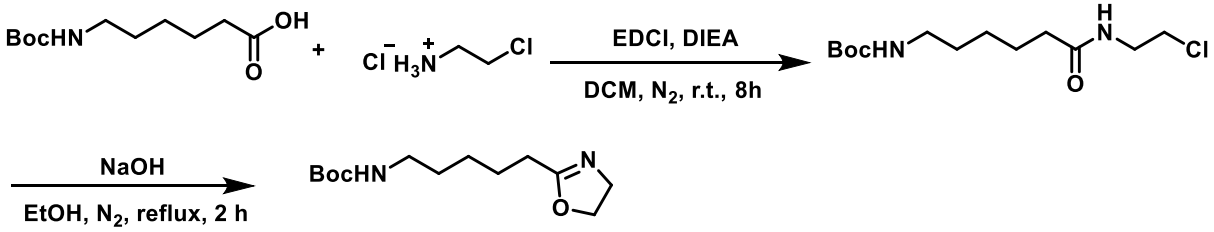
The $ProNH_{Boc}Ox$ was synthesized by following the aforementioned procedure for synthesis of $MeNH_{Boc}Ox$ using *N*-Boc-aminobutyric acid (11.6 g, 57.1 mmol) as the starting material to afford the final product as a white crystal (3.0 g, 23.0% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.26(br, 1H), 4.23 (t, J = 9.2 Hz, 2H), 3.83 (t, J = 9.2 Hz, 2H), 3.18 (dd, J = 6.0 Hz, 2H), 2.32 (t, J = 7.6 Hz, 2H), 1.83 (m, J = 7.2 Hz, 2H), 1.44 (s, 9H). The ^{1}H NMR spectrum was in agreement with the data in previous reports.[3] EI-MS: m/z calculated for $C_{11}H_{20}N_2O_3$ $[M]^+$: 228.1; Found 228.1.

Synthesis of 2-(*N*-Boc-4-aminobutyl)-2-oxazoline (BuNH$_{Boc}$Ox):



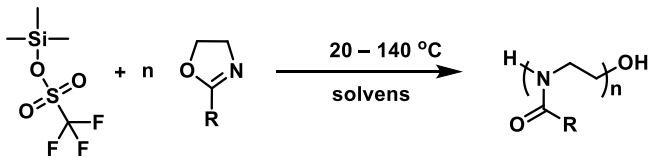
The BuNH$_{Boc}$Ox was synthesized by following the aforementioned procedure for synthesis of MeNH$_{Boc}$Ox using *N*-Boc-aminovaleric acid (20.0 g, 92.1 mmol) as the starting material, the final product was purified through distillation to give a colorless liquid (4.3 g, 19.3% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.68 (br, 1H), 4.24 (t, *J* = 9.6 Hz, 2H), 4.13 (t, *J* = 9.6 Hz, 1H),3.82 (m, *J* = 9.6 Hz, 2H), 3.14(dd, *J* = 6.8 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 1.67 (t, *J* = 8.4 Hz, 2H), 1.52 (m, *J* = 6.8 Hz, 2H), 1.41(s, 9H). The ^{1}H NMR spectrum was in agreement with the data in previous reports.[4] EI-MS: m/z calculated for $C_{12}H_{23}N_2O_3$ $[M+H]^+$: 243.1709; Found 243.1708.

Synthesis of 2-(*N*-Boc-5-aminopentyl)-2-oxazoline (AmNH$_{Boc}$Ox):



The AmNH$_{Boc}$Ox was synthesized by following the aforementioned procedure for synthesis of MeNH$_{Boc}$Ox using *N*-Boc-aminocaproic acid (10.0 g, 43.2 mmol) as the starting material, the final product was purified through basic alumina column chromatography to give a white solid (2.5 g, 22.6% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.78 (br, 1H), 4.12 (t, *J* = 9.2 Hz, 2H), 3.71 (t, *J* = 9.6 Hz, 2H), 3.00 (t, *J* = 6.0 Hz, 2H), 2.17 (t, *J* =7.6 Hz, 2H), 1.55 (m, *J* = 7.6 Hz, 2H), 1.29 (m, 13H). The ^{1}H NMR spectrum was in agreement with the data in previous reports.[5] HRESI-MS: m/z calculated for $C_{13}H_{25}N_2O_3$ $[M+H]^+$: 257.1865; Found 257.1866.

Polymer synthesis and characterization:



General synthetic procedure of poly(2-oxazoline)s is described as below. All the feeding processes were carried out in a nitrogen-regulated glove box. The polymer length was controlled via the monomer:initiator ratio. Take EtOx as an example, a certain amount of EtOx was weighed and dissolved in anhydrous *N*, *N*-dimethylacetamide (DMAc) to final concentration at 4 M in a pre-dried reaction vial equipped with a magnetic stirrer. Then a solution of trimethylslly trifluoromethanesulphonate (TMSOTf) in DMAc was added to the monomer solution. The reaction mixture was stirred at 100 °C for 1 h to 18 h until the monomer was completely consumed. The reaction mixture was diluted with tetrahydrofuran (THF, 0.3 mL) then precipitated with petroleum ether (PE, 47 mL). After centrifugation, the precipitate was collected and dried under air flow. The dissolution-precipitation (0.3 mL THF/47 mL PE) process was repeated thrice. The resulting polymer was dried under vacuum and dissolved with DMF (containing 0.01 M LiBr) to 5 mg/mL, and then was analyzed by GPC to measure the relative molecular weight of PEtOx using PMMA as standards.

Polymerization of EtOx in different solvents including acetonitrile (MeCN), benzonitrile (PhCN), dimethyl sulfoxide (DMSO), *N*, *N*-Dimethylformamide (DMF), sulfolane and DMAc were carried out at 30 °C for 7d at an initial monomer concentration of 4 M.

For the gram-scale polymerization demonstration, the mixture of EtOx (4.76 g, 48.0 mmol) and TMSOTf (533. 4 mg, 2.4 mmol) was stirred in DMAc at an initial monomer concentration of 4 M at 30 °C for 7 d. The reaction mixture was parted into ten centrifuge tubes. Then THF (0.3 mL) were added to each tube to dilute the mixture and iced PE (47 mL) was poured into the mixture to precipitate out the polymer. After centrifugation, the precipitate was collected and dried under air flow. The dissolution-precipitation (0.3 mL THF/47 mL PE) process was repeated thrice. The resulting polymer was characterized by GPC and MALDI-TOF-MS.

For the polymerization on 2-oxazolines with variable side chain functional groups demonstration, TMSOTf-initiated polymerization on 2-oxazolines monomers (MeOx, EtOx, PhOx, *i*BuOx, $MeNH_{Boc}Ox$, $ProNH_{Boc}Ox$, $BuNH_{Boc}Ox$ and $AmNH_{Boc}Ox$) were carried out at an initiator ratio of 20:1 (2-oxazoline : TMSOTf) in DMAc

at 100 °C. For the 2-oxazolines of MeOx, EtOx, *i*BuOx and PhOx, the initial monomer concentration was 4 M. For the 2-oxazolines of $MeNH_{Boc}Ox$, $ProNH_{Boc}Ox$, $BuNH_{Boc}Ox$ and $AmNH_{Boc}Ox$, the initial monomer concentration was 2 M because of the solubility.

Mechanism study of polymerization on MeOx using TMSOTf as the initiator in MeCN:

The in situ ^{1}H NMR characterization was carried out as follow. A 1.4 M solution of MeOx in deuterated acetonitrile (MeCN-d_3, 250 μL) and a 0.14 M solution of TMSOTf in MeCN-d_3 (250 μL) were mixed under nitrogen atmosphere. The reaction solution was stirred at 30 °C for 7 d, then transferred into nuclear magnetic tube for ^{1}H NMR characterization.

The 2-oxazoline polymerization using TMSOTf and methyl trifluoromethanesulfonate (MeOTf) as the initiator respectively were carried out as below. MeOx polymerization was carried out in DMAc at the $[M]_0/[I]_0$ ratio of 10/1 under 78 °C for 1 h. The reaction mixture was cooled down to r.t. then diluted in methanol (MeOH, 0.3 mL), subsequently precipitated in cold methyl tert-butyl ether (MTBE, 47 mL) thrice to give the purified polymer. The resulting polymer was dissolved in MeCN-d_3 and characterized by ^{1}H NMR (400 MHz) and ^{13}C NMR (600 MHz).

The reduction reaction of TMSOTf-initiated poly(2-oxazoline) was carried out as below. $PMeOx_{10}$ (60.0 mg, 0.07 mmol) that was prepared from TMSOTf-initiated polymerization and $NaBH_3CN$ (18.0 mg, 0.28 mmol) were dissolved in EtOH (5.0 mL). The reaction mixture was stirred at reflux under nitrogen atmosphere for 5 h. The solvent was removed under vacuum and the product was dissolved in MeOH (0.2 mL) and precipitated in MTBE (47 mL) thrice to give the purified polymer. The resulting poly(2-oxazoline)s were characterized by MALDI-TOF-MS.

Kinetic study of polymerization of 2-oxazolines:

For the polymerization under different temperatures, the kinetic study of MeOx in MeCN with $[M]_0/[I]_0$ ratio of 10/1 was operated as below. MeOx was dissolved in anhydrous MeCN which supplemented with triphenylmethane (TPM, 0.02 M) as the internal standard, then the initiator was added to the monomer solution

to achieve the initial monomer concentration of 0.7 M. The polymerizations were performed at 20 °C, 30 °C, 78 °C under nitrogen atmosphere.

For the polymerization at different initial monomer concentrations, MeOx was dissolved in anhydrous MeCN which supplemented with TPM (0.02 M) as the internal standard, then the initiator was added to the monomer solution to achieve the final monomer concentration at 0.7 M, 2 M and 4 M, respectively. The polymerizations were performed at 78 °C under nitrogen atmosphere.

The remaining MeOx was monitored by HPLC and the values of $\ln([M]_0/[M])$ and conversion was calculated according to the following formula with relative ratio of peak area between the MeOx and the TPM.

$$\ln\frac{[M]_0}{[M]} = \ln\frac{(A_{Ox}/A_{TPM})_{t=0}}{(A_{Ox}/A_{TPM})_t}$$

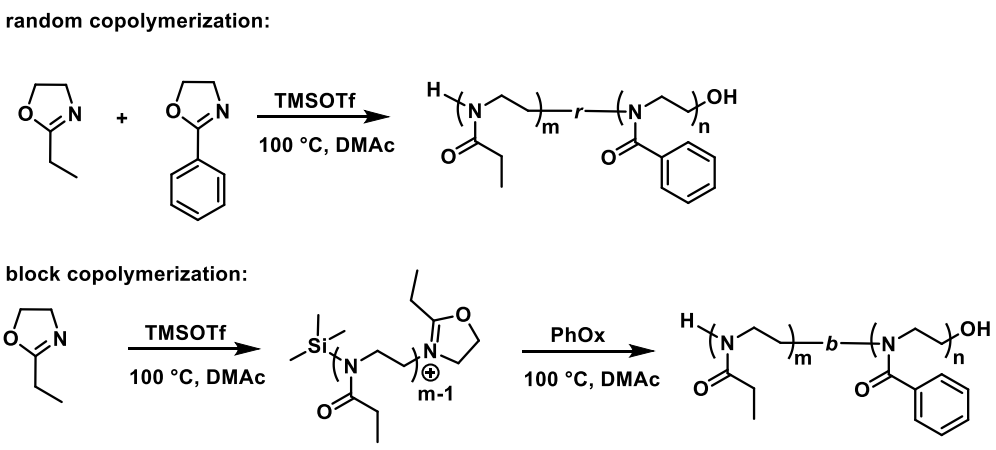
$$conversion(\%) = \frac{[M]_0 - [M]_t}{[M]_0} \times 100\% = \left(1 - \frac{(A_{Ox}/A_{TPM})_t}{(A_{Ox}/A_{TPM})_{t=0}}\right) \times 100\%$$

Then the polymerization rate was calculated by plotting the $\ln([M]_0/[M])$ vs. time according to the following formula.

$$-\frac{d[M]}{dt} = k_p[I][M]$$

$$\ln\frac{[M]_0}{[M]} = k_p\,[I]t$$

Copolymerization of EtOx and PhOx:



The random copolymerization of EtOx and PhOx was performed as below. EtOx (119 mg, 1.2 mmol) and PhOx (176.6 mg, 1.2 mmol) were dissolved in anhydrous DMAc to final concentration at 2 M in a pre-dried reaction vial equipped with a magnetic stirrer. Then a solution of TMSOTf in DMAc (1 M, 60 μL) was added to the monomer solution quickly. The reaction mixture was stirred at 100 °C for 18 h to give the random copolymer $PEtOx_{20}$-*r*-$PhOx_{20}$.

The block copolymerization of EtOx and PhOx was performed as below. The mixture of EtOx (119 mg, 1.2 mmol) and TMSOTf (1 M, 30 μL) in DMAc was stirred at 100 °C for 3 h to give the first block $PEtOx_{40}$. 10 μL of reaction mixture was diluted by DMF to 2 mg/mL then characterized by GPC. Then, an extra solution of PhOx (176.6 mg, 1.2 mmol) was added into the polymerization solution for another 24 h to give the block copolymer $PEtOx_{40}$-*b*-$PhOx_{40}$.

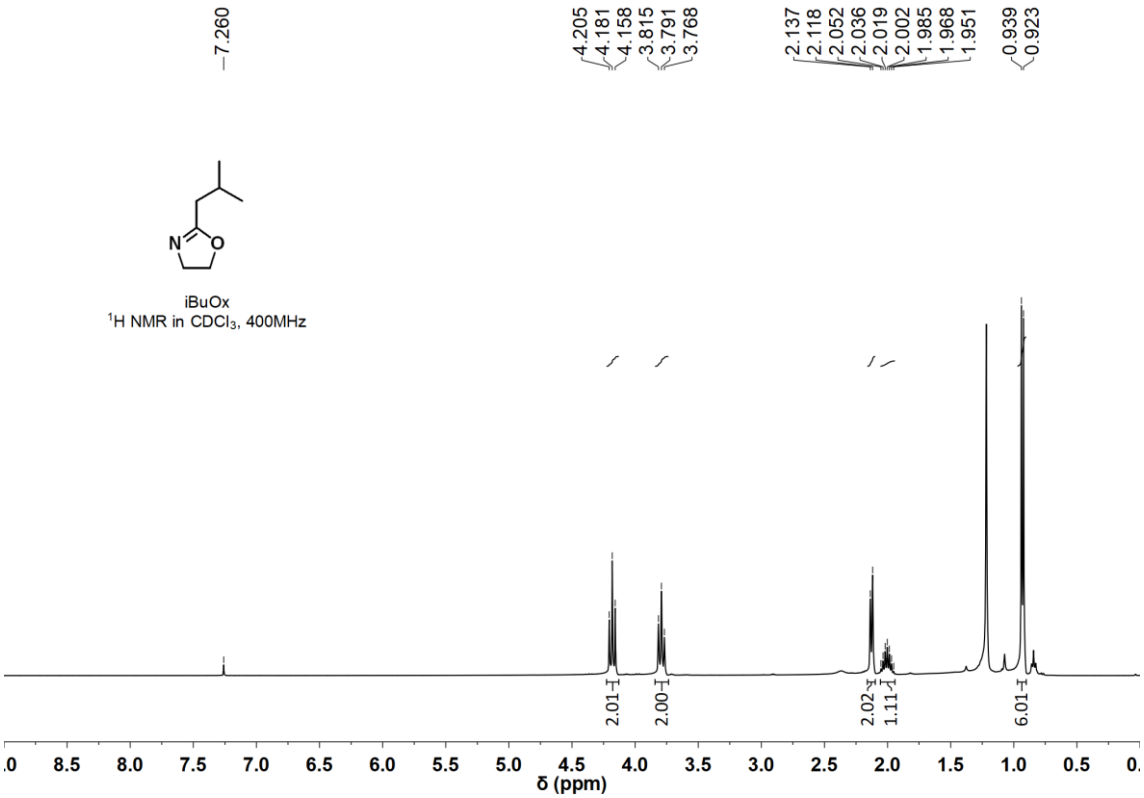


Figure S1. ^{1}H NMR spectrum of *i*BuOx in $CDCl_3$ at 400 MHz.

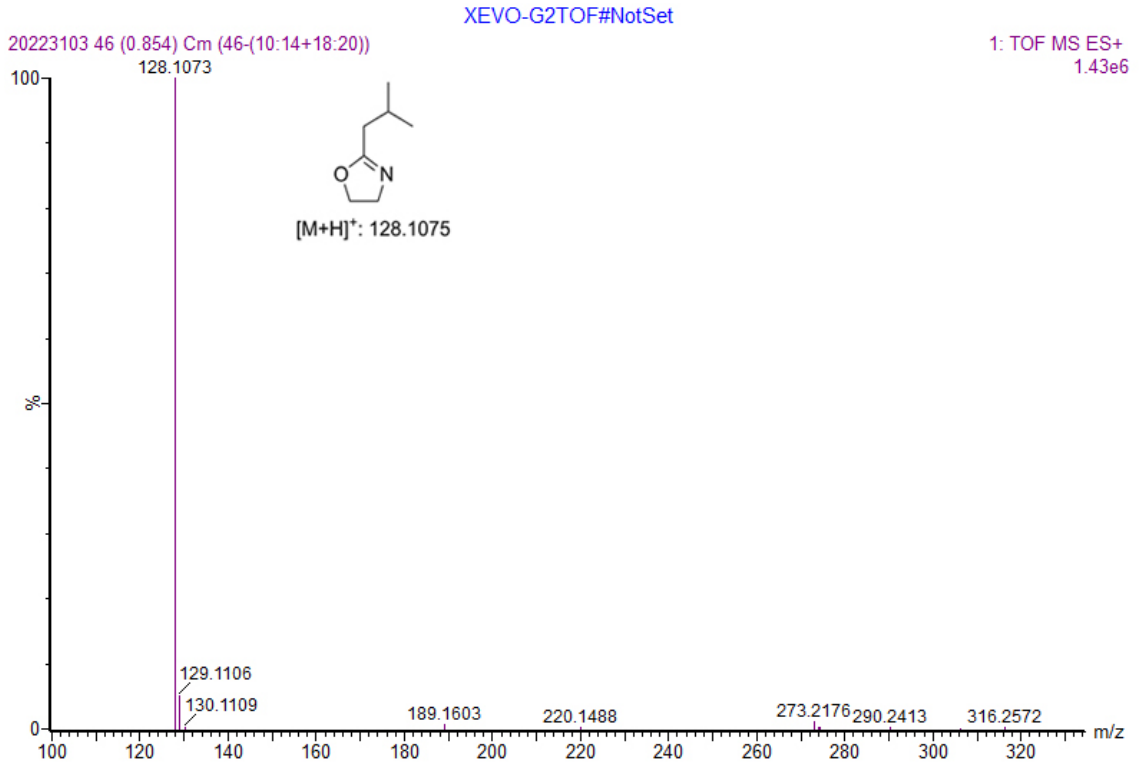


Figure S2. HRESI-MS spectrum of *i*BuOx.

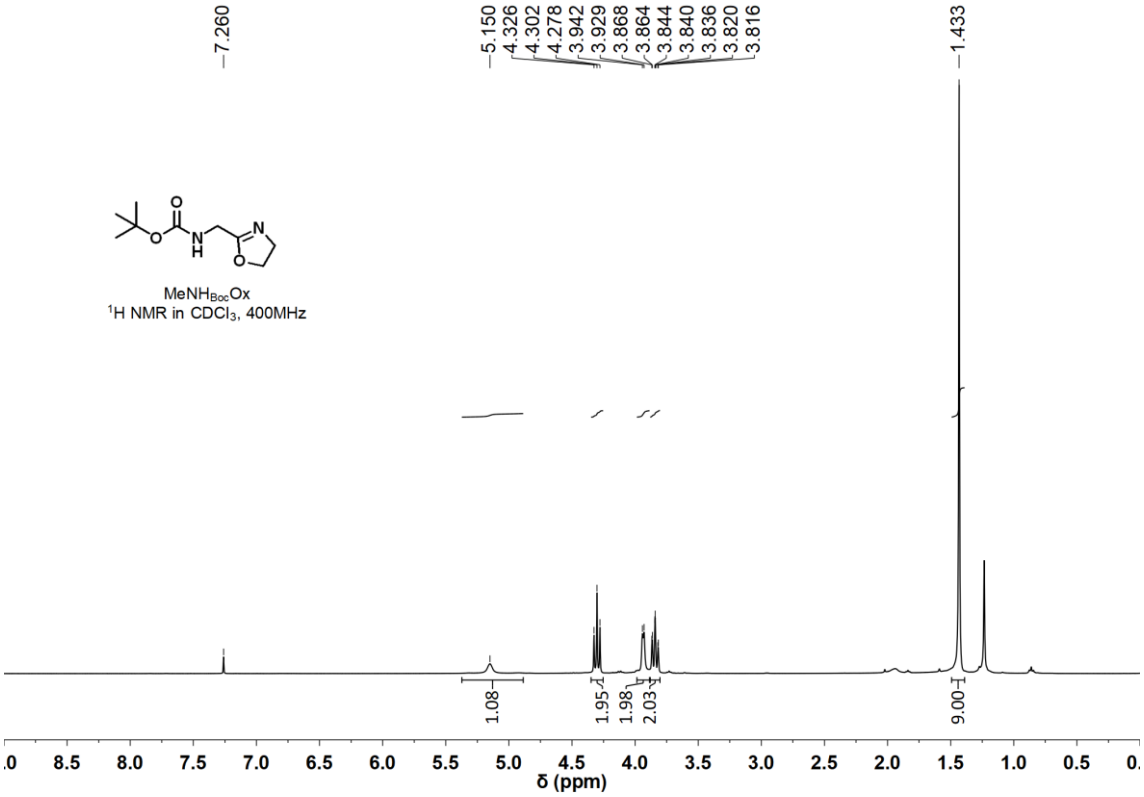


Figure S3. ^{1}H NMR spectrum of $MeNH_{Boc}Ox$ in $CDCl_3$ at 400 MHz.

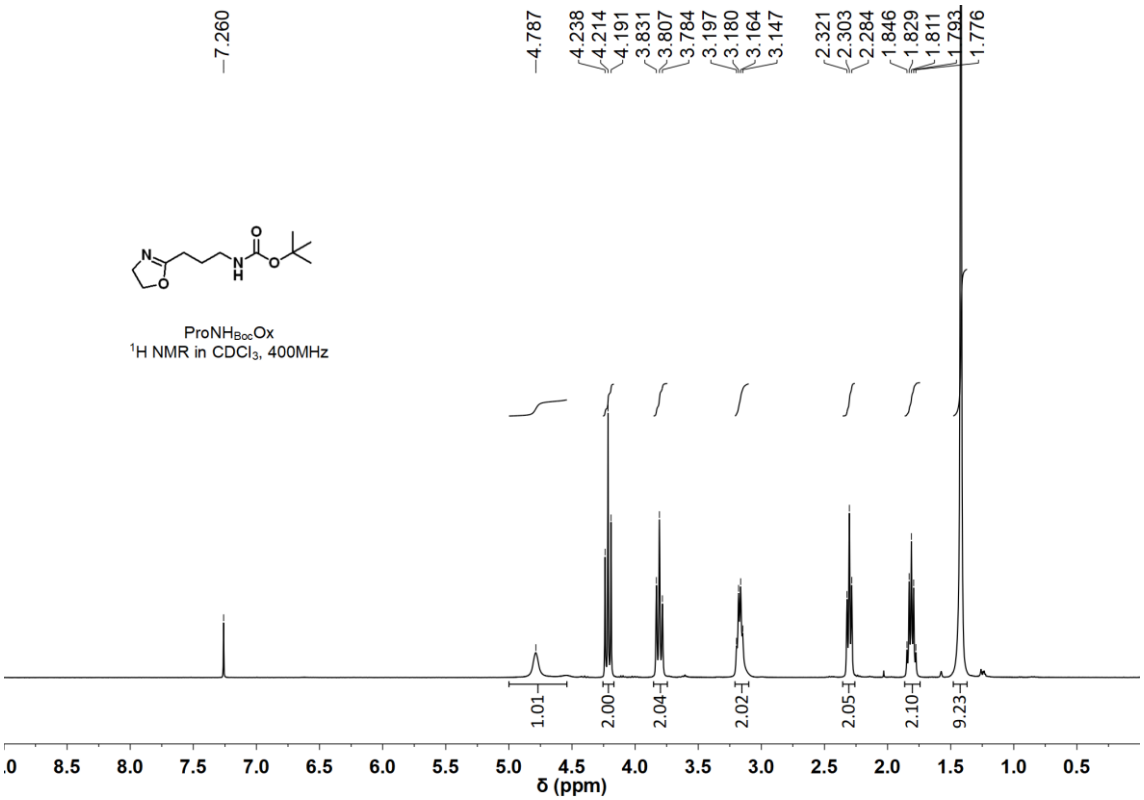


Figure S4. ^{1}H NMR spectrum of $ProNH_{Boc}Ox$ in $CDCl_3$ at 400 MHz.

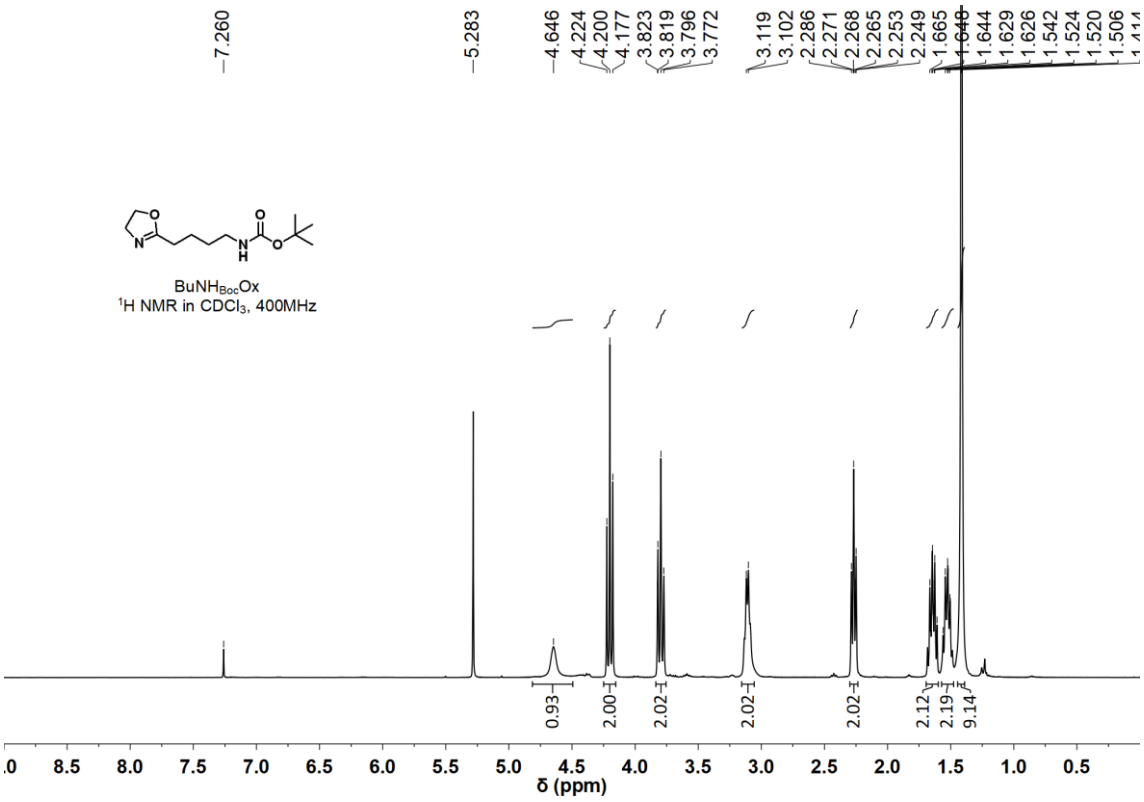


Figure S5. ^{1}H NMR spectrum of $BuNH_{Boc}Ox$ in $CDCl_3$ at 400 MHz.

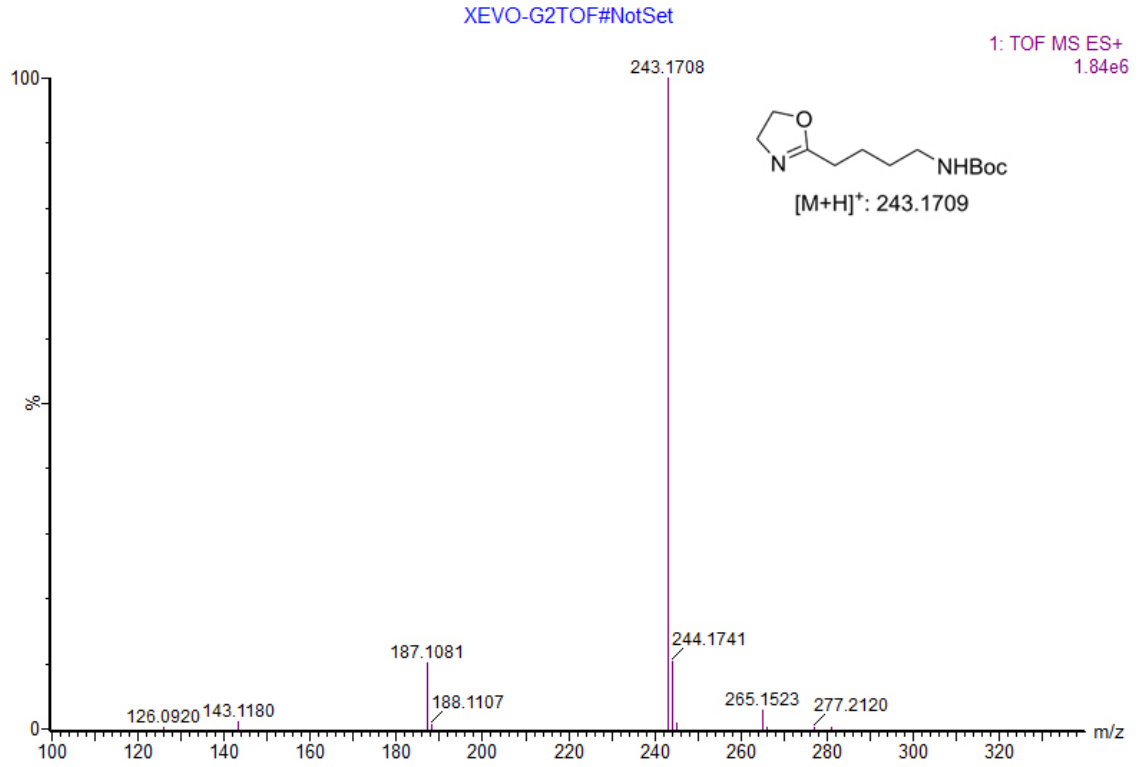


Figure S6. HRESI-MS spectrum of $BuNH_{Boc}Ox$.

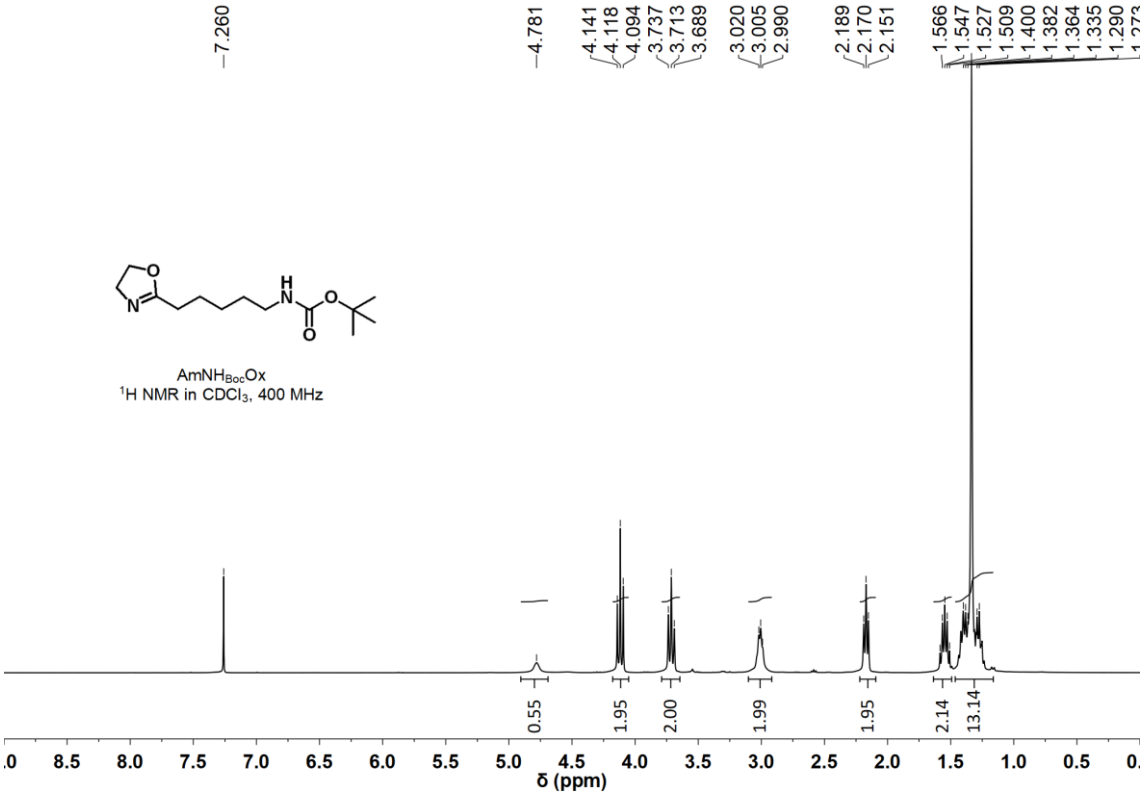


Figure S7. ^{1}H NMR spectrum of $AmNH_{Boc}Ox$ in $CDCl_3$ at 400 MHz.

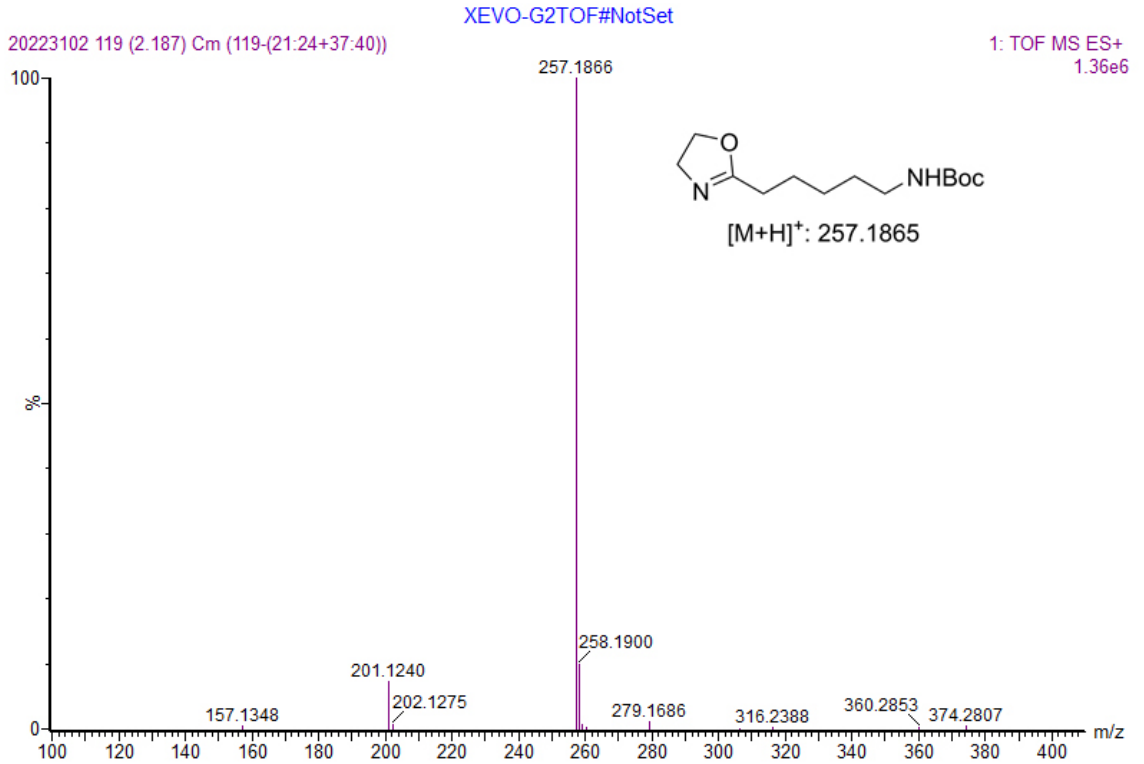


Figure S8. HRESI-MS spectrum of $AmNH_{Boc}Ox$.

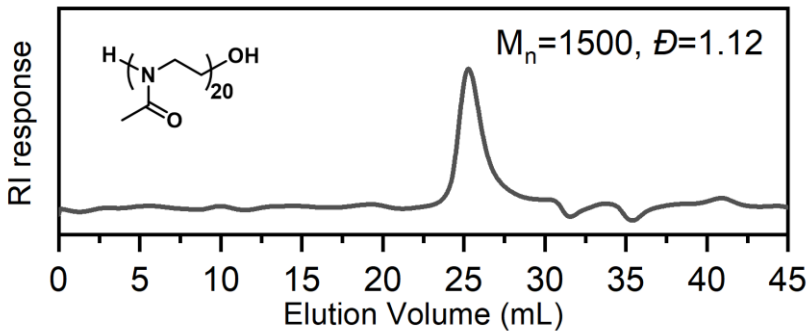


Figure S9. GPC traces of $PMeOx_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

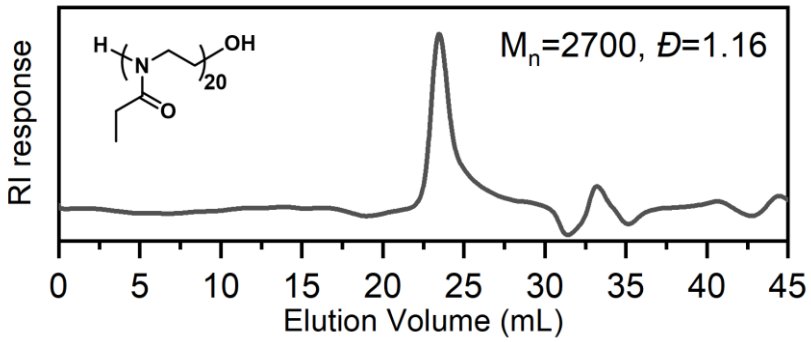


Figure S10. GPC traces of $PEtOx_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

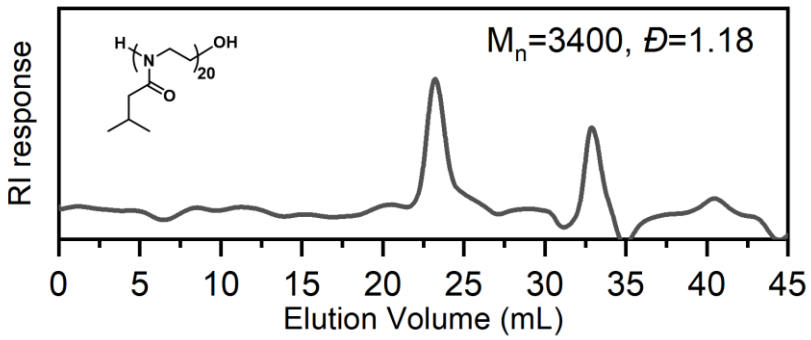


Figure S11. GPC traces of P*i*$BuOx_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

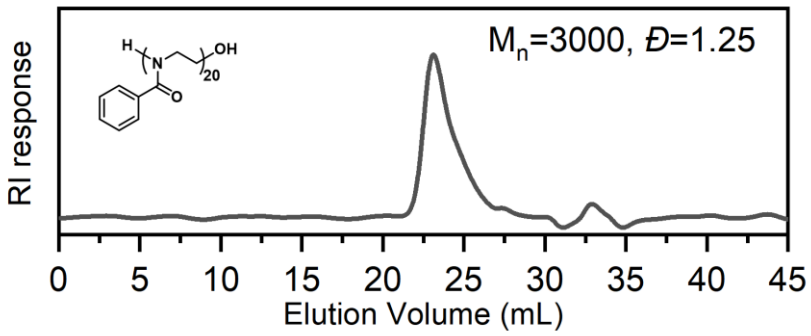


Figure S12. GPC traces of $PPhOx_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

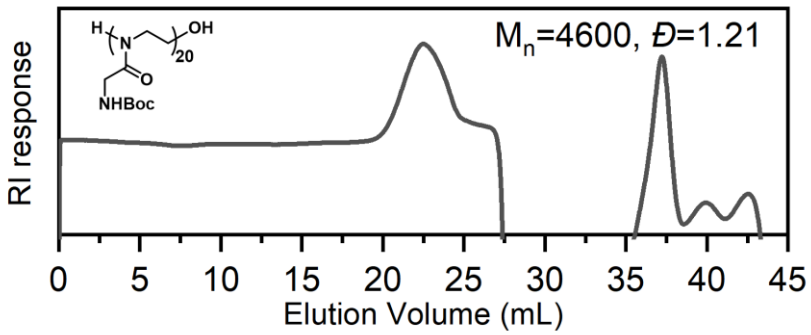


Figure S13. GPC traces of $PMeNH_{Boc}Ox_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

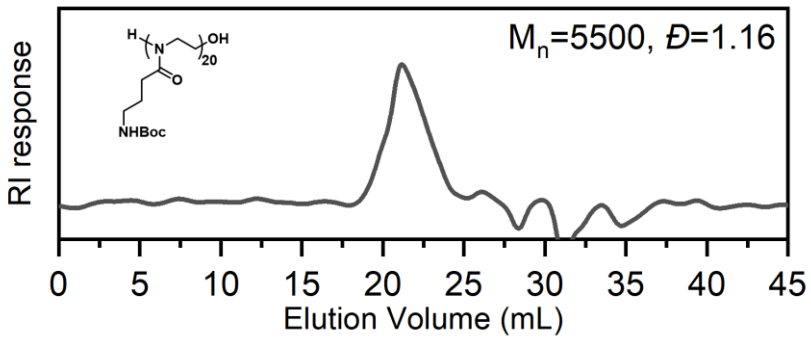


Figure S14. GPC traces of $PProNH_{Boc}Ox_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

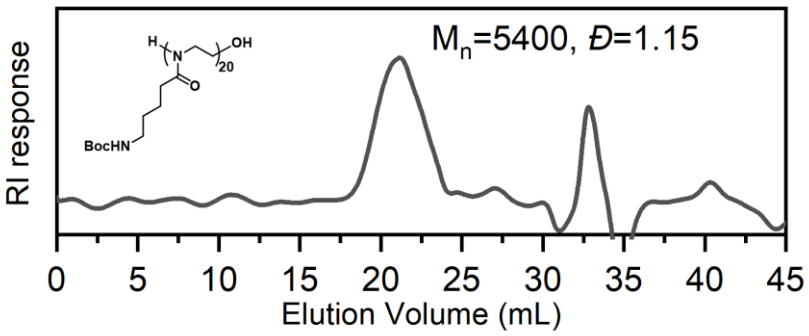


Figure S15. GPC traces of $PBuNH_{Boc}Ox_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

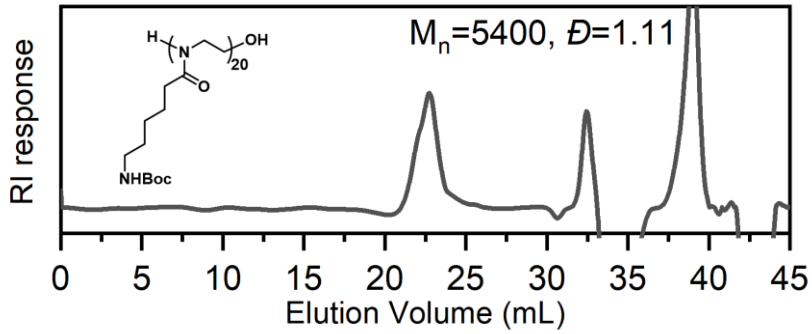


Figure S16. GPC traces of $PAmNH_{Boc}Ox_{20}$ prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$. DMF was used as the mobile phase at a flow rate of 1 mL/min.

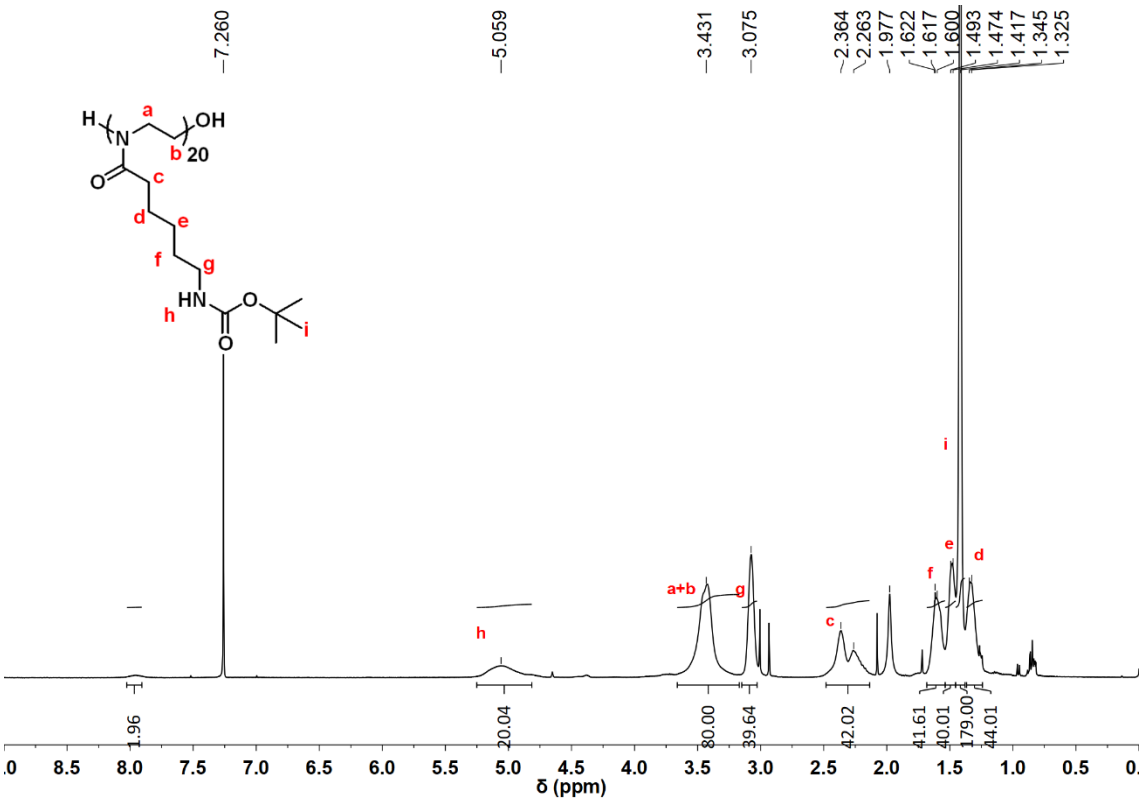


Figure S17. ^{1}H NMR spectrum of $PAmNH_{Boc}Ox_{20}$ in $CDCl_3$ at 400 MHz that was prepared by TMSOTf-initiated polymerization with 20:1 molar ratio of $[M]_0/[I]_0$.

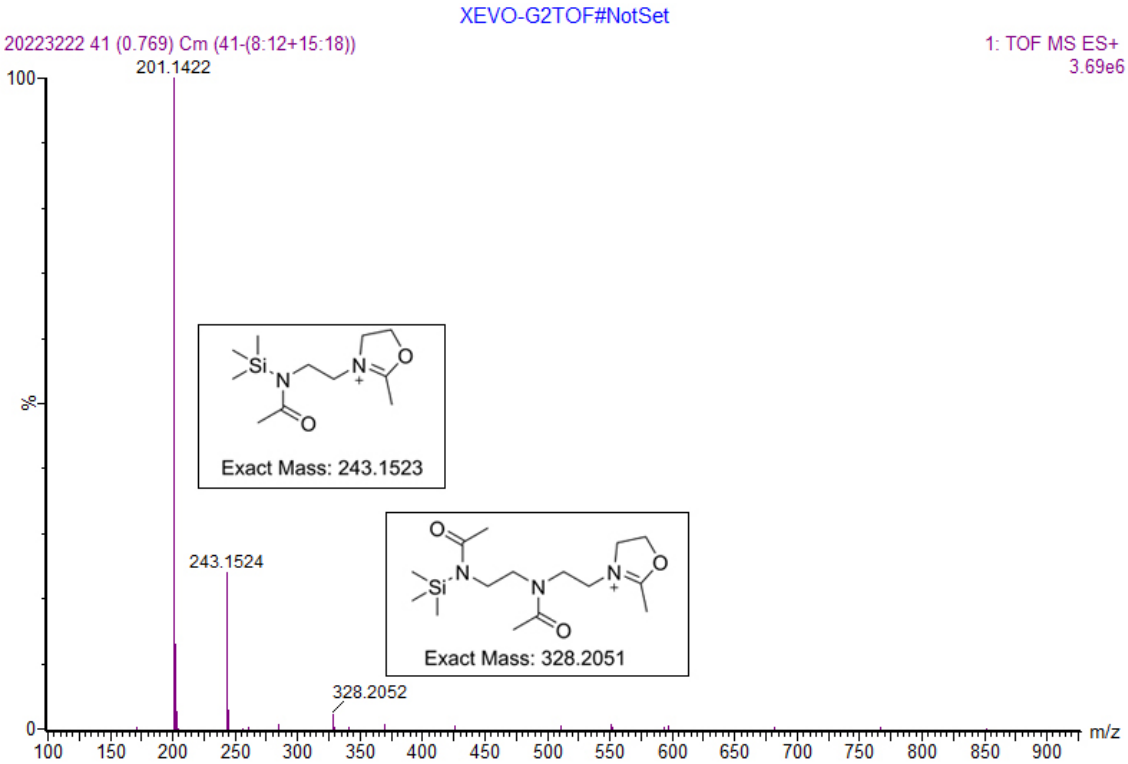


Figure S18. HRESI-MS characterization of TMSOTf-initiated polymerization reaction $(M]_0:[I]_0$ = 4:1, $[M]_0$ = 4 M, T=78 °C).

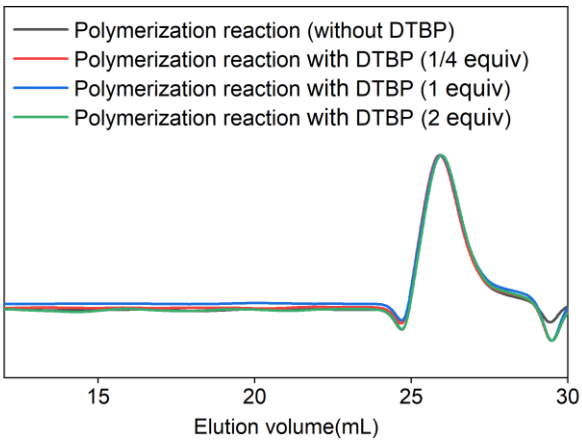


Figure S19. GPC trace of TMSOTf-initiated polymerization reaction mixtures $(M]_0:[I]_0$ = 10:1, $[M]_0$ = 4 M, T=78 °C) with initial ratios of DTBP/initiator at 0/1, 1/4, 1/1 and 2/1.

REFFERENCES

1. Lubtow, M. M.; Haider, M. S.; Kirsch, M.; Klisch, S.; Luxenhofer, R., Like Dissolves Like? A Comprehensive Evaluation of Partial Solubility Parameters to Predict Polymer-Drug Compatibility in Ultrahigh Drug-Loaded Polymer Micelles. *Biomacromolecules* **2019,** *20* (8), 3041-3056.
2. Zhou, M.; Qian, Y.; Xie, J.; Zhang, W.; Jiang, W.; Xiao, X.; Chen, S.; Dai, C.; Cong, Z.; Ji, Z.; Shao, N.; Liu, L.; Wu, Y.; Liu, R., Poly(2-Oxazoline)-Based Functional Peptide Mimics: Eradicating MRSA Infections and Persisters while Alleviating Antimicrobial Resistance. *Angew. Chem., Int. Ed.* **2020,** *59* (16), 6412-6419.
3. Dai, C.; Zhou, M.; Jiang, W.; Xiao, X.; Zou, J.; Qian, Y.; Cong, Z.; Ji, Z.; Liu, L.; Xie, J.; Qiao, Z.; Liu, R., Breaking or following the membrane-targeting mechanism: Exploring the antibacterial mechanism of host defense peptide mimicking poly(2-oxazoline)s. *J. Mater. Sci. Technol.* **2020,** *59*, 220-226.
4. Hartlieb, M.; Pretzel, D.; Kempe, K.; Fritzsche, C.; Paulus, R. M.; Gottschaldt, M.; Schubert, U. S., Cationic poly(2-oxazoline) hydrogels for reversible DNA binding. *Soft Matter* **2013,** *9* (18), 4693–4704
5. Cesana, S.; Auernheimer, J.; Jordan, R.; Kessler, H.; Nuyken, O., First Poly(2-oxazoline)s with Pendant Amino Groups. *Macromol. Chem. Phys* **2006,** *207* (2), 183-192.