Supplementary Information

Facile preparation of polyester-polyglutamate diblock copolymers through regio-selective polymerization of *N*-carboxyanhydride

Wanying Wang[a,†], Pengfei Li[a,†], Yue Xu[a], Shiqi Wei[b], Xiangdie Hou[c], Ning Li[a], Zhichao Zhou[c], Xiaohong Li[c] and Ziyuan Song[a,*]

[a]Institute of Functional Nano & Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Joint International Research Laboratory of Carbon-Based Functional Materials and Devices, Soochow University, Suzhou 215123, China.

[b]Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States.

[c]The Key Lab of Health Chemistry and Molecular Diagnosis of Suzhou, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China.

*Corresponding email: zysong@suda.edu.cn

Table of Contents

Materials

All chemicals were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China) and used as received unless otherwise specified. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, USA). Organic solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Anhydrous tetrahydrofuran (THF) and hexane were dried in a column using alumina. Anhydrous *N*,*N*-dimethylformamide (DMF) was pre-treated with polymer-bound isocyanate (MilliporeSigma, St. Louis, USA) to remove any amino residues. The monomer γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA), L-phenylalanine NCA (Phe-NCA), and N^{ε}-benzyloxycarbonyl-L-lysine NCA (ZLL-NCA) were synthesized following literature procedures.[1, 2] The monomers L-lactide, δ-valerolactone, and ε-caprolactone were purified by recrystallization in anhydrous ethyl acetate before polymerization.[3]

Instruments

^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE NEO-400 MHz spectrometer or an Agilent Direct-Drive II 600 MHz spectrometer. The chemical shifts (δ) were reported in ppm and referenced to the residual protons in the deuterated solvents. MestReNova software (version 6.1.0, Mestrelab Research, Escondido, USA) was used for all NMR analysis. Gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (1260 Infinity II, Agilent, Santa Clara, USA), a multi-angle static light scattering (MALS) detector (DAWN, Wyatt Technology, Santa Barbara, USA), and a differential refractive index (dRI) detector (Optilab, Wyatt Technology, Santa Barbara, USA). The detection wavelength of the MALS detector was set at 658 nm. Separations were performed using serially connected size exclusion columns (KD-803, KD-804, and KD-806, 8 × 300 mm, Shodex, Yokohama, Japan) using DMF containing LiBr (0.1 mol/L) as the mobile phase at a flow rate of 1 mL/min at 60 °C. The MALS detector was calibrated using pure toluene and was used for the determination of the absolute molecular weights (MWs). The MWs of polymers were determined

based on the d*n*/d*c* value of each polymer sample calculated offline by using the internal calibration system processed by the ASTRA 8 software (version 8.1.0, Wyatt Technology, Santa Barbara, USA). Fourier transform infrared (FTIR) spectra were recorded using a Thermo Fisher Nicolet iS20 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, USA). The quantitative FTIR analysis was performed using a Pearl liquid transmission accessory (Specac Ltd, Orpington, UK) with a ZnSe window. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were collected on a Bruker ultrafleXtreme, with *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) as the matrix and sodium trifluoroacetate as the salt.

NCA stability study

The NCA stability was monitored by FTIR or NMR (the latter was typically used for samples with high NCA/alcohol concentrations). For the FTIR quantification, BLG-NCA (15.8 mg, 0.060 mmol) was dissolved in dichloromethane (DCM, 450 μL), into which the DCM solution of methanol (0.4 mol/L, 150 μL. $[NCA]_0 = [OH]_0 = 0.1$ mol/L) was added. At different time intervals, an aliquot of the solution was taken out and transferred into the Pearl liquid cell for FTIR analysis. The concentration of NCA was quantified according to the standard curve based on the absorbance of anhydride peaks at 1863 cm^{-1}. For the study with solubilized water, an equal volume of water was added into DCM, which was vortexed to promote solubilization. The mixture was then phase-separated, and the bottom DCM layer saturated with solubilized water was carefully taken out for the stability studies.

For the NMR quantification study, the sample preparation was similar, but in CD_2Cl_2 in an NMR tube. At different time intervals, the NMR tube was scanned to obtain the spectrum. The concentration of NCA was quantified according to the integration of α-H (δ = 4.41 ppm).

Polymerization kinetics and polymer characterization

The polymerization kinetics was monitored in situ by ^1H NMR in CD_2Cl_2. Typically, BLG-NCA

(52.6 mg, 0.20 mmol) was dissolved in CD_2Cl_2 (460 μL), into which the CD_2Cl_2 solution of ethanolamine (0.1 mol/L, 40 μL. $[M]_0$ = 0.4 mol/L, $[M]_0/[I]_0$ = 50) was added to start the polymerization. The mixture was vortexed and transferred into an NMR tube, and the NMR spectra were collected at different time intervals. The conversion of NCA was calculated based on the integral of α-H signal of BLG-NCA (δ = 4.41 ppm), which was normalized compared to the α-H signal at t = 0. The α-H signal at t = 0 was calculated based on the integral ratios of benzyl peaks between BLG-NCA (δ = 5.14 ppm) and poly(γ-benzyl-$_L$-glutamate) (PBLG) (δ = 5.07 ppm).

The obtained polypeptides were purified by precipitation in hexane/ether (1:1, v/v) and dried under vacuum. For the GPC characterization, the polypeptides were re-dissolved in DMF containing LiBr (0.1 mol/L), filtered through nylon membrane (0.22 μm), and injected into GPC for analysis.

Synthesis of polyester-polypeptide diblock copolymer

For the preparation of diblock copolymer, aminoalkyl alcohols with various alkyl lengths were used to initiate NCA monomers and lactide/lactone in a stepwise manner. Typically, BLG-NCA (52.6 mg, 0.20 mmol) was dissolved in DCM (460 μL), into which the DCM solution of 6-amino-1-hexanol (0.1 mol/L, 40 μL. $[M]_0$ = 0.4 mol/L, $[M]_0/[I]_0$ = 50) was added to start the polymerization. The polymer was purified by precipitation in hexane/ether (1:1, v/v) and dried under vacuum (yield: 86%).

After the polymerization of the polypeptide block, the terminal amino group was deactivated. Briefly, the polypeptides were incubated at 40 °C for 48 h (higher temperature at 50 °C also works) to promote the amidation reaction with side-chain glutamate esters, yielding deactivated pyroglutamate moieties that remained inert during the following polyester synthesis. The hydroxyl-capped, polypeptide macroinitiators with deactivated N terminus were precipitated in hexane/ether (1:1, v/v) and used for the preparation of polyester-polypeptide diblock copolymers. The deactivation of N terminus was confirmed by both MALDI-TOF MS (through end-group analysis) and NMR (check the loss of last benzyl ester groups using oligomeric PBLG).

In a glovebox, polypeptide macroinitiators (22 mg, 0.0010 mmol), $_L$-lactide (7.2 mg, 0.10 mmol),

and the toluene solution of stannous isooctanoate (0.1 mol/L, 30 μL.) were mixed into a Schlenk tube charged with a stir bar. Anhydrous toluene (1 mL) was then added into the Schlenk tube, and the tube was sealed and transferred out of the glove box ($[M]_0$ = 0.1 M, $[M]_0/[I]_0$ = 100). The polymerization solution was refluxed for 24 to 48 h depending on the $[M]_0/[I]_0$ value. The diblock copolymer was purified by precipitation in hexane/ether (1:1, v/v) and dried under vacuum (yield: 91-98%). The copolymer was then characterized by GPC.

Supplementary figures

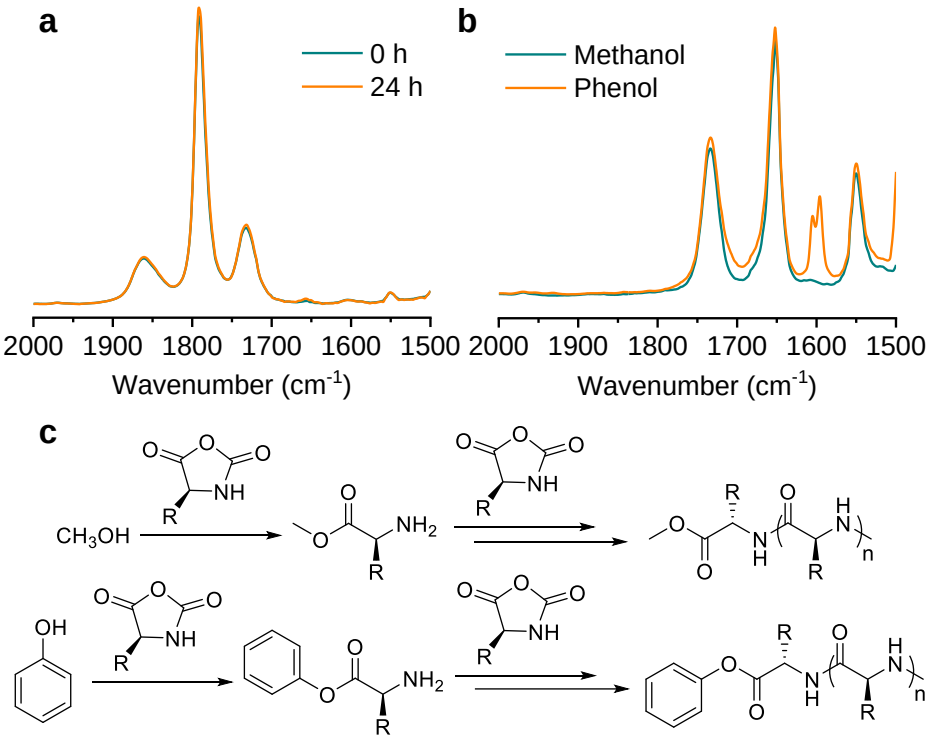


Figure S1. NCA stability in the presence of alcohols and phenol (PhOH). (a) Overlaid FTIR spectra of BLG-NCA in DCM in the presence of ethanol before and after 24-h treatment. (b) Overlaid FTIR spectra of BLG-NCA in DCM in the presence of methanol (MeOH) or PhOH after 24-h treatment. (c) Scheme illustrating the uncontrolled polymerization process of NCA in the presence of MeOH or PhOH. $[NCA]_0 = [OH]_0 = 0.1$ M.

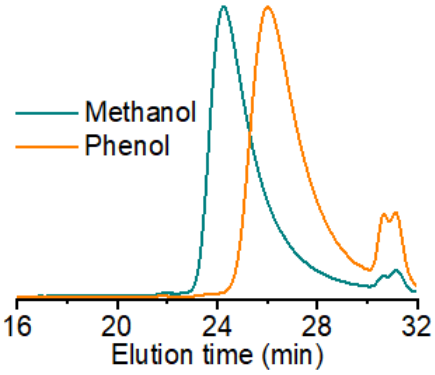


Figure S2. Normalized GPC-LS traces of degradation products of BLG-NCA in the presence of MeOH or PhOH. $[NCA]_0 = [OH]_0 = 0.1$ M

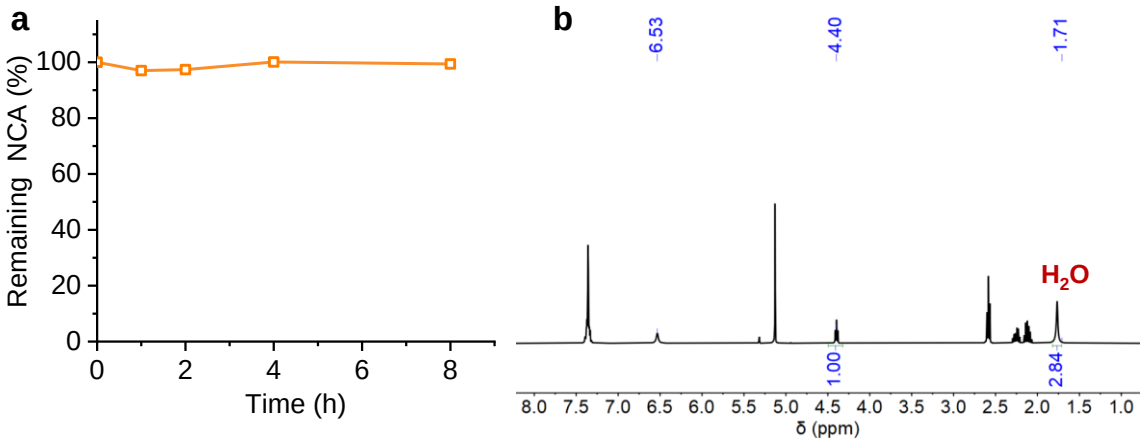


Figure S3. NCA stability against solubilized water in DCM. (a) Degradation profile of BLG-NCA in DCM in the presence of solubilized water ($[NCA]_0$ = 0.1 M, $[H_2O]_0$ = 0.14 M). (b) ^1H NMR spectrum (400 MHz) of BLG-NCA and solubilized water in CD_2Cl_2 for the calculation of saturated concentration of water in DCM.

The concentration of saturated, solubilized water was calculated to be ~ 140 mM, based on the integral ratio of water protons (i.e., 1.71 ppm) to α-H of NCA (i.e., 4.40 ppm), assuming the concentration of NCA (i.e., 100 mM) was accurate.

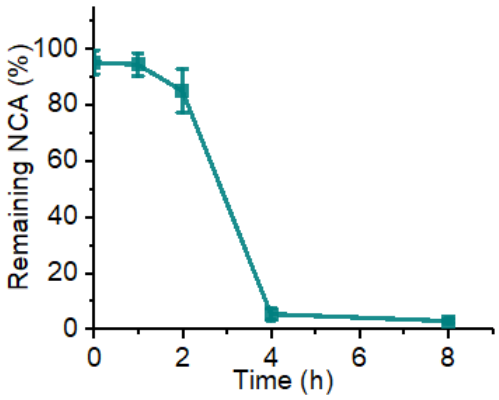


Figure S4. Degradation profiles of BLG-NCA in CD_2Cl_2 in the presence of HexOH at higher concentrations. $[NCA]_0 = [OH]_0 = 0.4$ M.

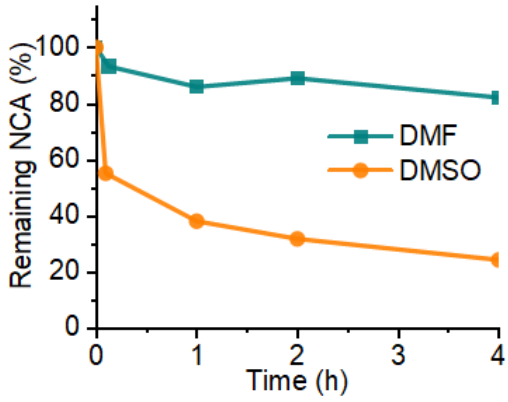


Figure S5. Degradation profiles of BLG-NCA in DMF-*d*7 or DMSO-*d*6 in the presence of HexOH. $[NCA]_0 = [OH]_0 = 0.4$ M.

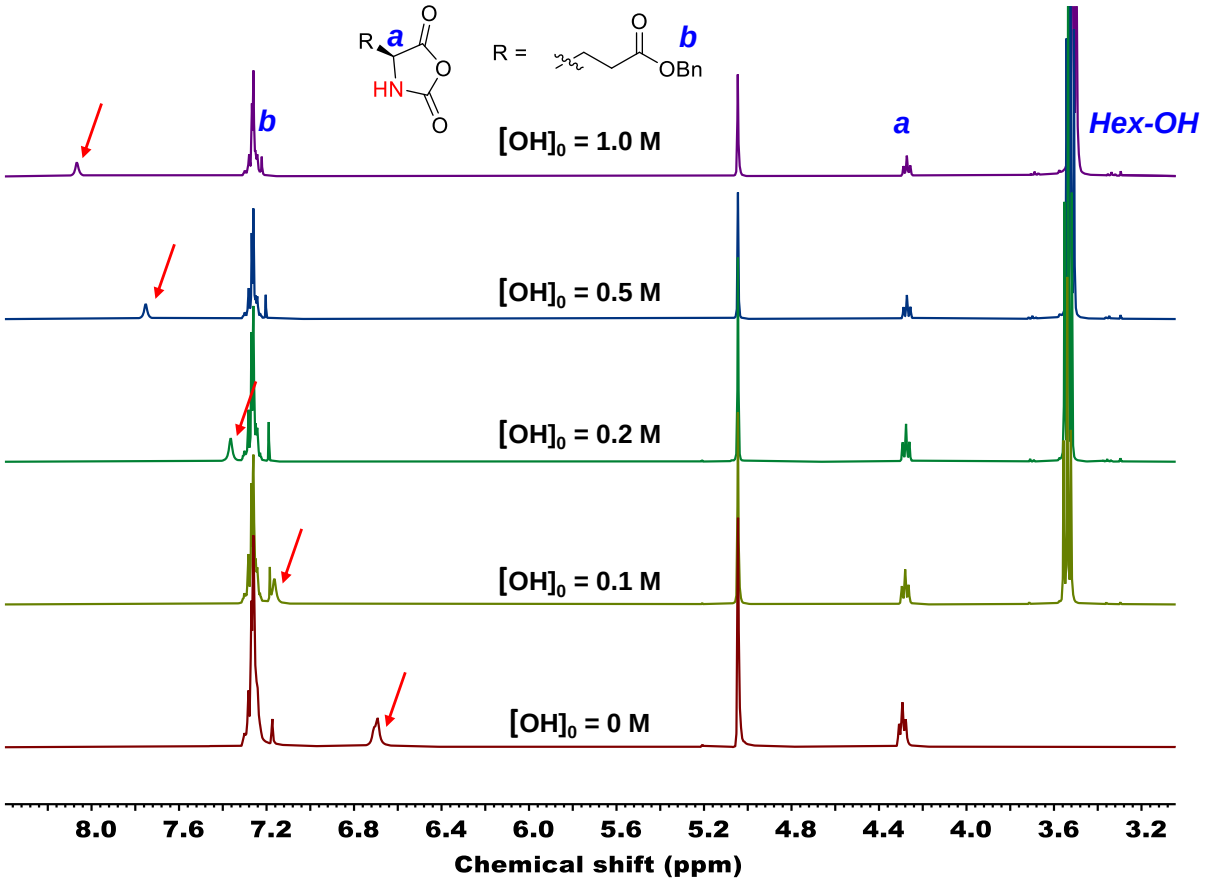


Figure S6. Overlaid ^{1}H NMR spectra (400 MHz) of BLG-NCA with various concentrations of HexOH in CD_2Cl_2. The signal of ring N-H proton of BLG-NCA was highlighted in red arrows $[NCA]_0 = 0.1$ M.

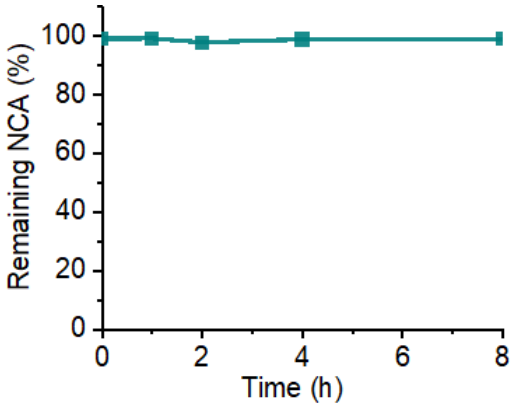


Figure S7. Degradation profile of BLG-NCA in DCM in the presence of HexOH at low concentration ($[NCA]_0$ = 0.4 M, $[HexOH]_0$ = 0.04 M).

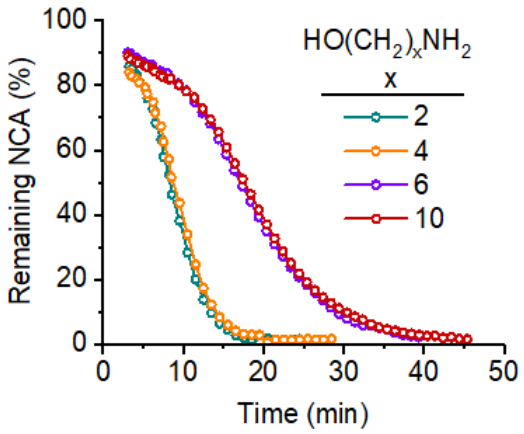
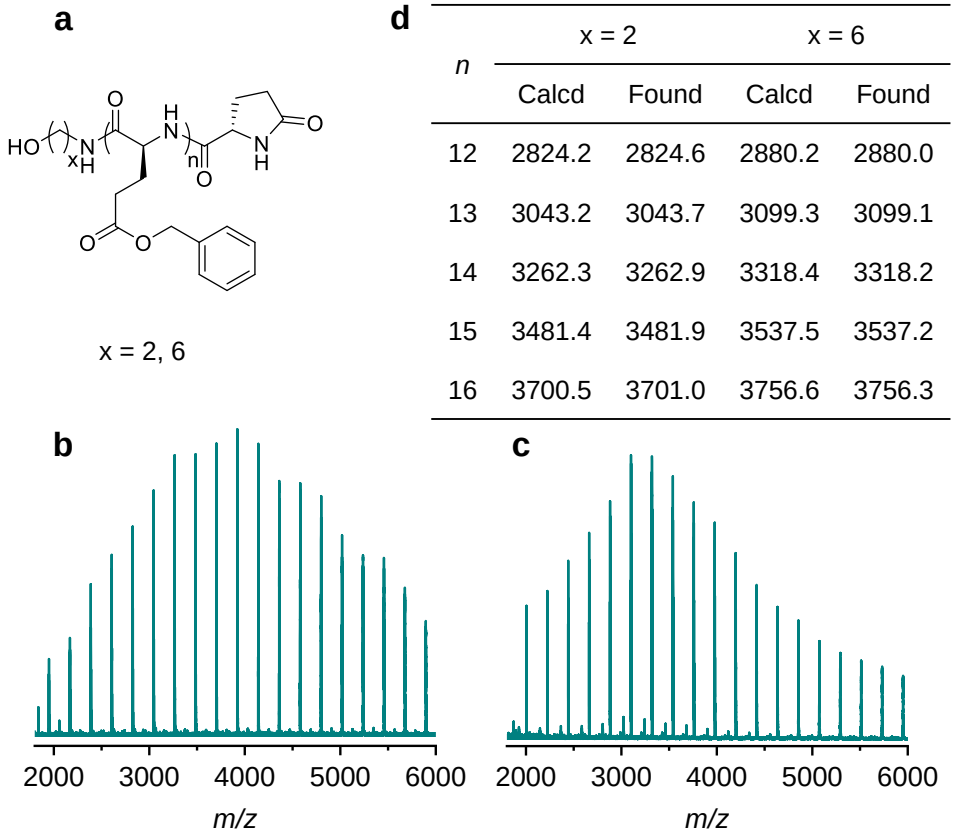


Figure S8. Polymerization kinetics of BLG-NCA initiated by various bifunctional aminoalkyl alcohols as initiators. $[M]_0$ = 0.4 M, $[M]_0/[I]_0$ = 100.



d

n	x = 2		x = 6	
	Calcd	Found	Calcd	Found
12	2824.2	2824.6	2880.2	2880.0
13	3043.2	3043.7	3099.3	3099.1
14	3262.3	3262.9	3318.4	3318.2
15	3481.4	3481.9	3537.5	3537.2
16	3700.5	3701.0	3756.6	3756.3

Figure S9. End-group analysis of polypeptides initiated by bifunctional aminoalkyl alcohols. (a) Chemical structure of the polypeptides detected by MALDI-TOF MS. (b, c) MALDI-TOF mass spectra of resulting polypeptides with ethanolamine (b) or 6-amino-1-hexanol (c) as initiators. (d). Comparison between the obtained and calculated *m*/*z* signals in the MALDI-TOF MS spectra.

The obtained *m/z* signals agreed well with the calculated values, which were (195.07 + 219.09n) ($[M+Na]^+$) for ethanolamine-initiated polypeptides and (251.14 + 219.09n) ($[M+Na]^+$) for 6-amino-1-hexanol-initiated polypeptides.

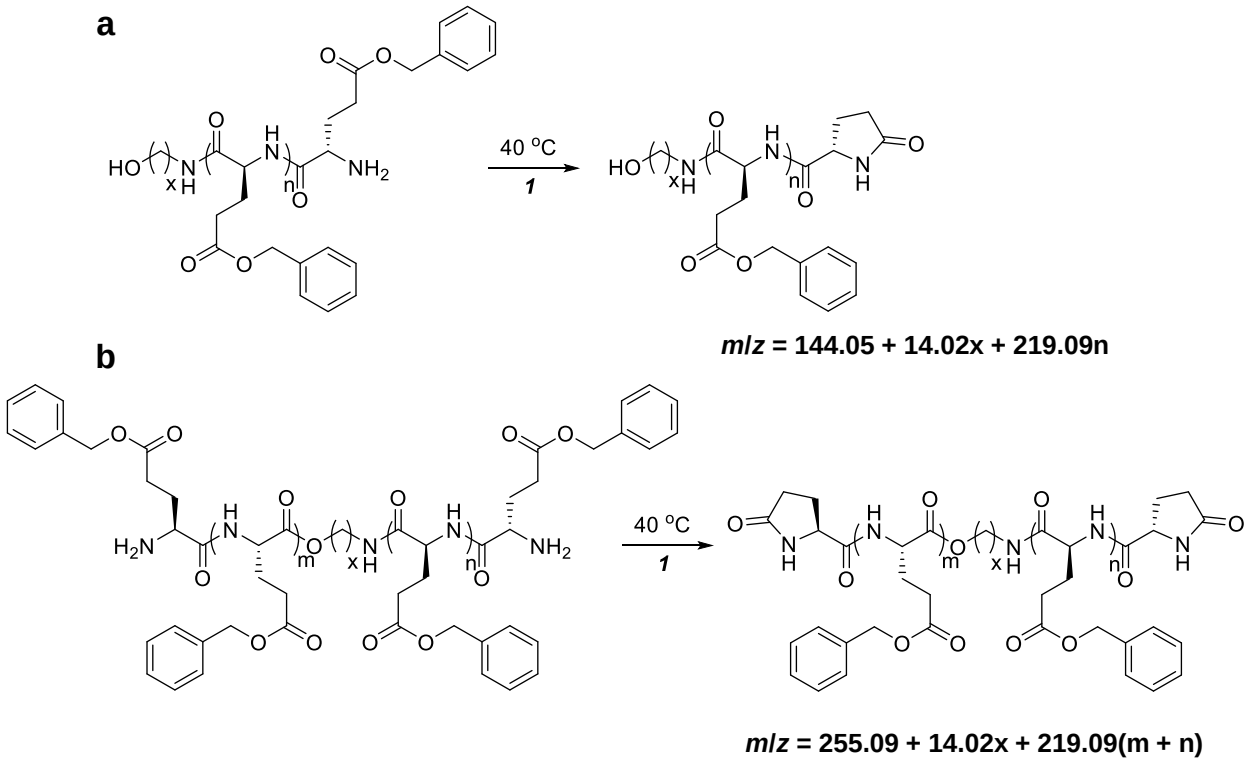


Figure S10. Schemes illustrating the pyroglutamate product after 40 °C deactivation of PBLG if the hydroxyl chain-ends remained inert (a) or participated in the NCA polymerization as initiating sites (b). The calculation of *m*/*z* did not consider the mass of cations.

With the two-side initiations/polymerizations, the obtained end-groups of polypeptides from MALDI-TOF analysis will be different compared to those with regio-selective initiations.

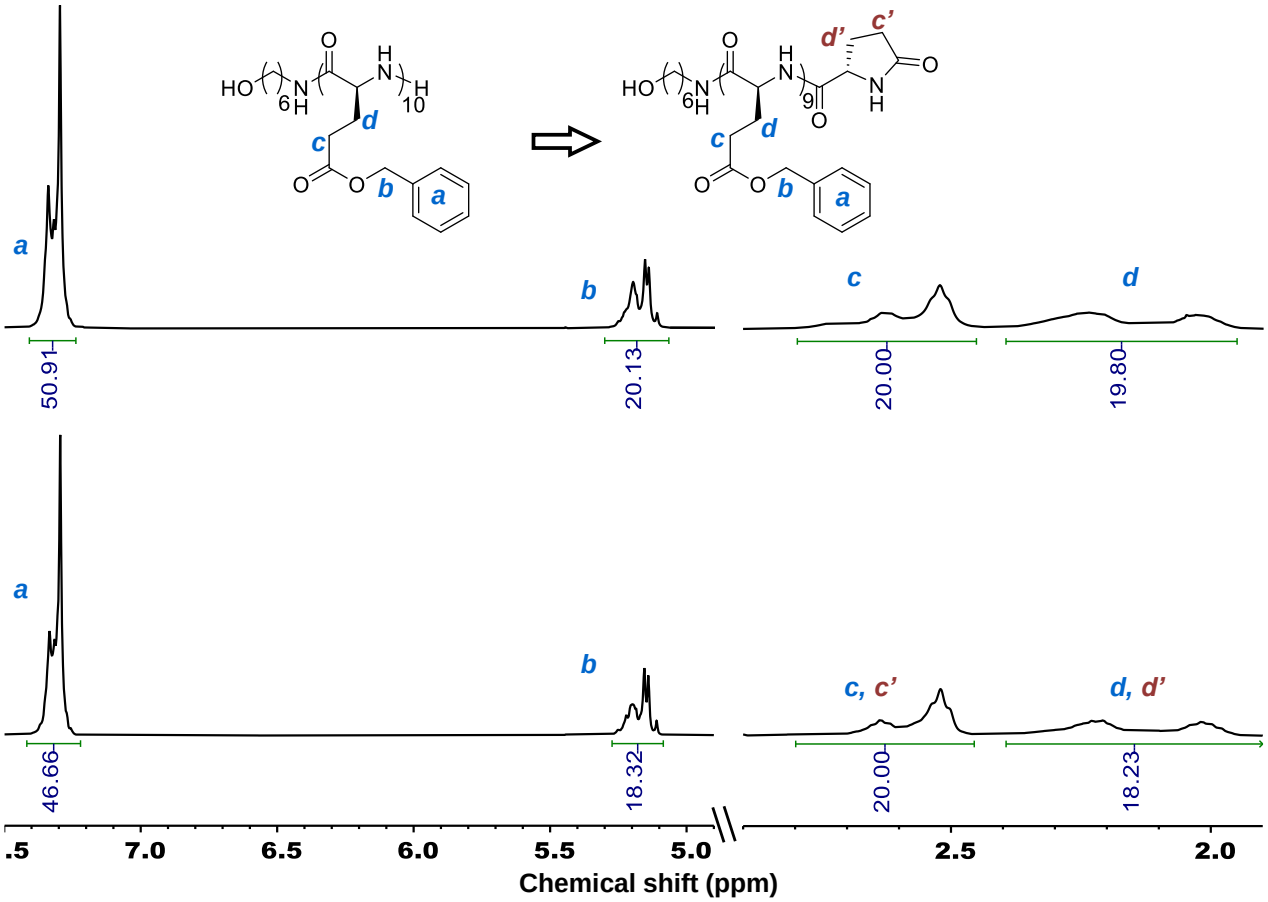


Figure S11. Overlaid ^{1}H NMR spectra (400 MHz) of PBLG 10-mer before and after the pyroglutamate formation.

Significant decrease in the proton signals in aromatic regions (i.e, 7.29 ppm) and benzyl regions (5.15 ppm) was observed, which corresponded to the loss of one benzyl ester side chains due to the formation of pyroglutamate.

Supplementary tables

Table S1. Preparation of polypeptide in the presence of equimolar alcohols and amines.[a]

Entry	Amine/Alcohol	$M_{n,\,GPC}$ (kDa)[b]	$Đ$[b]
1	Hex-NH_2/Pyr-OH	22.3	1.05
2	Pyr-NH_2/HexOH	18.9	1.06

[a]Both polymerizations were conducted in DCM at room temperature with BLG-NCA as the monomer. The theoretical MW was 22.0 kDa, calculated from $[M]_0/[NH_2]_0$ rather than $[M]_0/([NH_2]_0 + [OH]_0)$. [b]Determined by GPC; dn/dc = 0.095.

Supplementary references

1. R. Baumgartner, H. Fu, Z. Song, Y. Lin and J. Cheng, *Nat. Chem.*, 2017, **9**, 614–622.

Z. Song, H. Fu, J. Wang, J. Hui, T. Xue, L. A. Pacheco, H. Yan, R. Baumgartner, Z. Wang, Y. Xia,

2. X. Wang, L. Yin, C. Chen, J. Rodríguez-López, A. L. Ferguson, Y. Lin and J. Cheng, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 10658–10663.

3. O. Thillaye du Boullay, E. Marchal, B. Martin-Vaca, F. P. Cossío and D. Bourissou, *J. Am. Chem. Soc.*, 2006, **128**, 16442–16443.