Supporting information

A New Strategy to Bottlebrushes with Helical Polyglutamate Backbone *via N*-Carboxyanhydride Polymerization and RAFT

Haiwang Lai, Xiaoyu Chen, Qi Lu, Zheng Bian, Youhua Tao*, Xianhong Wang*

Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Renmin Street 5625, Changchun 130022, People's Republic of China

* Corresponding author. E-mail: youhua.tao@ciac.ac.cn, xhwang@ciac.ac.cn

Experimental Section

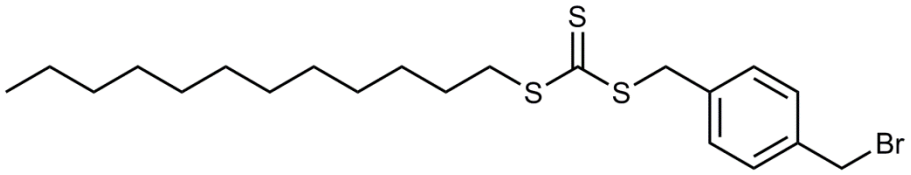
Materials & Methods

Tetrahydrofuran (THF) was refluxed with sodium and distilled under nitrogen before use. Ethyl acetate (EtOAc), dichloromethane (DCM) and petroleum ether were freshly distilled from CaH_2. N, N-dimetylformamide (DMF) was stirred over calcium hydride (CaH_2) and purified by vacuum distillation with CaH_2. AIBN (2, 2'-Azobis(2-methylpropionitrile)) was recrystallized from ethanol three times and stored under N_2 atmosphere in the dark at 4 °C. All the other reagents and solvents were used as received without further purification unless otherwise stated. ^1H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker AV-300 spectrometer and a Bruker AV-400 spectrometer, respectively. Matrix-assisted laser desorption/ionization time-of-flight

mass spectroscopy (MALDI-TOF/MS) was performed on a Bruker atuoflex III mass spectrometer in linear, positive ion mode equipped with 355nm smartbeam laser. The matrix was dithranol (DIT), and solvent was chloroform. Gel permeation chromatography (GPC) was conducted on a system composed of a Waters 2414 Refractive Index Detector equipped with a series of linear Shodex columns (KD-802.5, KD-804 and KD-G). The system was operated with DMF (containing 0.02M LiBr) as the eluent at a flow rate of 1 mL/min at 50 ^{o}C and calibrated with polymethyl methacrylate standards in the molecular weight range from 1860 to 3.3×10^{5} Da. Fourier transform infrared spectra were performed on a Bruker TENSOR-27 spectrophotometer. Circular dichroism spectra were recorded on a JASCO J-820 spectrometer using a quartz cell with a pathlength of 1mm in deionic water and THF at a concentration of 2 mg/mL at 25 ^{o}C. Ellipticity ([θ] in deg.cm^{2}.dmol^{-1}) = (millidegrees×mean residue weight) / (pathlength in millimeters×concentration of polypeptide in mg·mL^{-1}). The α-helix contents of the polypeptides were calculated by following equation: % α-helix = (-[θ_{222}] + 3000)/39,000.[1] The tapping mode AFM images were obtained with a MultiMode Scanning Probe Microscope coupled to a Nanoscope IIIa controller (Digital Instruments/Veeco, Santa Barbara, CA, USA) under ambient conditions by casting dilute solution of PMESLG$_{36}$-*g*-POEGA$_{7}$ bottlebrush on a mica surface. 2-(2-methoxyethoxy)ethanethiol and $_{L}$-glutamic acid copper(II) complex copper(II) salt tetrahydrate were synthesized according to literature.[2-3]

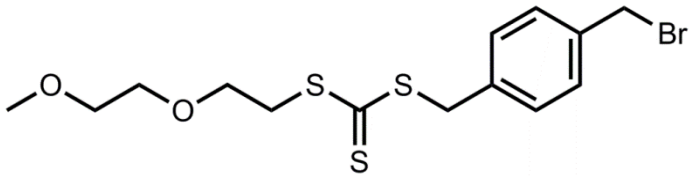
Synthetic protocols

Synthesis of 4-(bromomethyl)benzyl dodecyl carbonotrithioate (1)



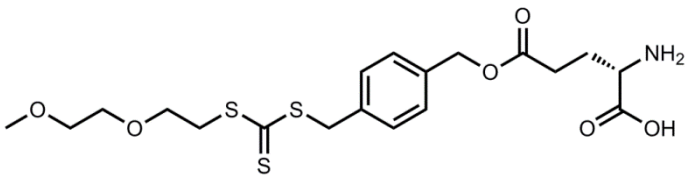
4-(bromomethyl)benzyl dodecyl carbonotrithioate was ynthesized according to that previously reported.[4] Briefly, dodecanethiol (5.1 g, 24.5 mmol) was dissolved in acetone/H_2O (32 mL/12 mL) under N_2 purge followed with addition of NaOH (2.0g, 50%, 25.0 mmol). The mixture was stirred at 0 ° C for 0.5 h before CS_2 (1.5 mL, 24.8 mmol) was added to the mixture. The resultant yellow mixture was added dropwise to a vigorously strirred solution of *p*-xylylene dibromide (10g, 37.9 mmol) in THF (50mL). The reaction was stirred at room temperature overnight, then 1N HCl (20mL) was added to the mixture and the mixture was extracted with DCM (50 mL×3). The organic layer was washed with water three times and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and the residue was purified by column chromatography using petroleum ether (R_f = 0.24) as eluent to afford the product as yellow solid (4.5 g, 40.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 4H); 4.60 (s, 2H); 4.47 (s, 2H); 3.37 (t, 2H, *J*=7.5Hz); 1.70 (quin, 2H, *J*=7.5Hz); 1.26 (m, 18H); 0.88 (t, 3H, *J*=7.2Hz).

Synthesis of 4-(bromomethyl)benzyl (2-(2-methoxyethoxy)ethyl) carbonotrithioate (2)



2-(2-methoxyethoxy)ethanethiol (10.4 g, 76.3 mmol) was dissolved in dry THF (100 mL) under N_2 purge followed with addition of sodium hydride (4.1 g, 60%, 102.5mmol). The mixture was stirred at 0 ° C for 0.5 h before CS_2 (4.6 mL, 76.1 mmol) was added to the mixture. The resultant yellow mixture was added dropwise to a vigorously strirred solution of p-xylylene dibromide (20.1 g, 76.0 mmol) in THF (50 mL). The reaction was stirred at room temperature overnight, then 1N HCl (200 mL) was added to the mixture and the mixture was extracted with DCM (100 mL×3). The organic layer was dried over anhydrous Na_2SO_4.The solvent was evaporated under vacuum and the residue was purified by column chromatography using petroleum ether/DCM (v:v = 1:1, R_f = 0.47) ~ DCM (R_f = 0.83) as eluent to afford the product as an orange oil (8.44g, 27.9%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 4H); 4.61 (s, 2H); 4.48 (s, 2H); 3.76 (t, 2H); 3.65 (m, 4H); 3.56 (m, 2H); 3.40 (s, 3H).

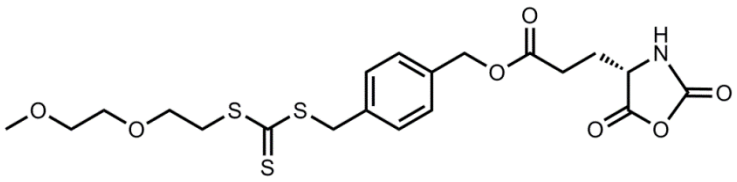
Synthesis of 2-amino-5-oxo-5-((4-(3-thioxo-7,10-dioxa-2,4-dithiaundecyl)benzyl) oxy)pentanoic acid (3):



In a 500-mL round-bottom flask, *N*, *N*, *N*', *N*'-tetramethylguanidine (2.0 mL, 1.6 mmol) was added slowly to a stirred mixture of $_L$-glutamic acid copper(II) complex copper(II) salt tetrahydrate(2.61 g, 5.3 mmol) and $_L$-glutamic acid (1.57g, 10.6 mmol) in a mixed solvent of dimethylformamide(DMF)/water(10 mL/1.5 mL). The mixture gradually turned dark blue and the solid dissolved. After 2h stirring, RAFT agent **2** (8.44 g, 21.3 mmol) was added to the deep blue solution in one portion. DMF/water (10 mL/1.5 mL) was added to improve mixing. The mixture was stirred at rt for 48h. The crude product was added a freshly-prepared EDTA disodium salt solution (EDTA (5.84 g) and sodium bicarbonate (3.36 g) in 40-45 mL water). Water (60 mL) was added to the

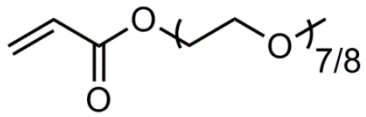
mixture and the product was extracted with $CHCl_3$ (50 mL×3). The organic layer was dried over anhydrous Na_2SO_4. After evaporation of solvent under vacuum, the crude product was purified by column chromatography using chloroform/methanol (v:v=2:1, R_f = 0.43) as eluentto give the product as a yellow solid (3.2 g, 32.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 4H); 5.00 (s, 2H); 4.70 (m, 1H); 4.62 (s, 2H); 3.75 (m, 2H); 3.65 (m, 4H); 3.55 (m, 2H); 3.40 (s, 3H); 2.61 (m, 2H); 2.20 (m, 2H).

Synthesis of (S)-4-(3-thioxo-7,10-dioxa-2,4-dithiaundecyl)benzyl 3-(2,5-dioxoox azolidin-4-yl)propanoate (4):



The obtained amino acid with a chain transfer side group (**3**) (1.60g, 3.5 mmol) was mixed with triphosgene (0.68g，2.3mmol) and THF (15mL) in a 100mL flask. The solution was stirred at 50℃ for about 3 h. The solvent was concentraed under vacuum. The residue was washed with anhydrous petroleum ether three times and then purified using column chromatography with EtOAc/PE (v:v=1:1, R_f = 0.25) as eluent to give MES-$_L$-Glu-NCA monomer (**4**) as yellow oil (1.13g, 67.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 4H); 6.56 (s, 1H); 5.10 (s, 2H); 4.60 (s, 2H); 4.38 (t, 1H, *J*=6.3Hz); 3.74 (m, 2H); 3.62 (m, 4H); 3.56 (m, 2H); 3.38 (s, 3H); 2.58 (t, 2H, *J*=6.9Hz); 2.20 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 222.6, 171.8, 168.9, 151.2, 135.2, 134.2, 129.1, 128.3, 71.4, 69.9, 68.1, 66.2, 58.6, 56.4, 40.5, 35.9, 29.3, 26.5. HRMS (MALDI-TOF): calculated for C20H25NO7S3, 487.0793; found for M+Na (C20H25NO7S3Na), 510.0678.

Synthesis of oligoethylene glycol acrylate (OEGA)



To a solution of oligoethyleneglycol monomethyl ether (Mw = 350) (9.6 g, 27.4 mmol) in DCM (100 ml) was added triethylamine (4.19 g, 41.4 mmol). This solution was cooled to 0°C and acryloyl chloride (2.5 g, 27.6 mmol) was added dropwise over 1h. After addition, the reaction mixture was stirred overnight at ambient temperature. The reaction mixture was washed with water, dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by column chromatography on alkaline aluminum oxide using DCM (R_f = 0.49~0.55) as eluent to give the product as a pale yellow liquid (8.32g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.40 (dd, 1H, J_1=1.5 Hz, J_2=17.1 Hz); 6.13 (dd, 1H, J_1=10.5 Hz, J_2=17.1 Hz); 5.82 (dd, 1H, J_1=1.5 Hz, J_2=10.5 Hz); 4.29 (t, 2H, J=4.8 Hz); 3.72 (t, 2H, J=4.8 Hz); 3.63 (m, 4H); 3.52 (m, 2H); 3.35 (s, 3H).

Synthesis of PMESLG

The ROP of MES-$_L$-Glu-NCA (**4**) was performed in THF using Et_3N as initiator in a glove box. Briefly, NCA was dissolved in THF (2 mL) to give a yellow solution. Et_3N/THF (20 mg/mL) solution was injected into NCA/THF solution (50−100 mg/mL) via a syringe, and the solution was then stirred at room temperature for 72 h. According to literatures work,[5-7] for Et_3N-initiated ROP of NCA, 3 days of polymerization is necessary for the higher conversion of NCA monomer. The product was precipitated using excess petroleum ether and collected via centrifiguation. The collected product was diluted with H_2O and transferred to dialysis bag with a cutoff molecular weight of 1000 g/mol. The sample was dialyzed against deionized water for 4 days with water changed per 8h. Dialyzed polymers were lyophilized to yield PMESLG homopolypeptides as yellow solids (63 % yield for $PMESLG_{18}$ and 69 % yield for $PMESLG_{36}$).

Synthesis of PMESLG-*g*-POEGA

The PMESLG-*g*-POEGA bottlebrushes were prepared using PMESLG as macro-CTA for RAFT polymerization of OEGA. Typically, in a glovebox under argon atomosphere, 3.1mg AIBN, 91.1mg of PMESLG, 1.50 g of OEGA, and 0.62 mL of DMF were mixed in a 10mL flask. The flask was then immersed in a preheated oil bath at 70 ° C for 7 h. After that, the solution was cooled to RT and quenched by exposure to air. The polymer was precipitated using excess diethyl ether, collected via centrifiguation, and the collected product was dissolved in H_2O and transferred to dialysis bag with a cutoff molecular weight of 5000 g/mol. The sample was dialyzed against deionized water for 5 days with water changed per 8 h. The samples were then dried under reduced pressure to give a white solid with yield about 52%.

SUPPLEMENTARY FIGURES

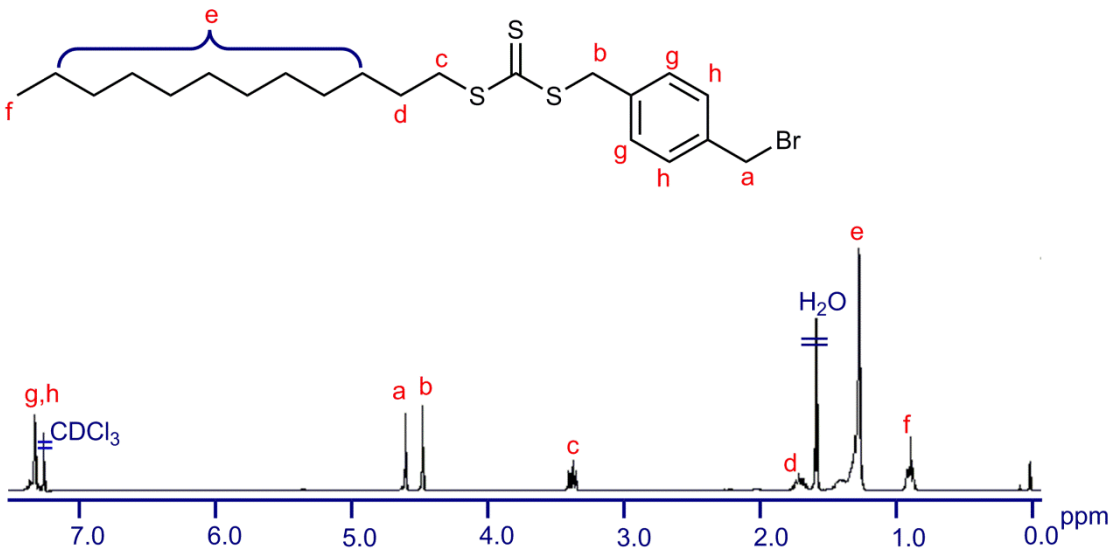


Figure S1. ^{1}H NMR spectrum of (**1**) (300 MHz, $CDCl_3$).

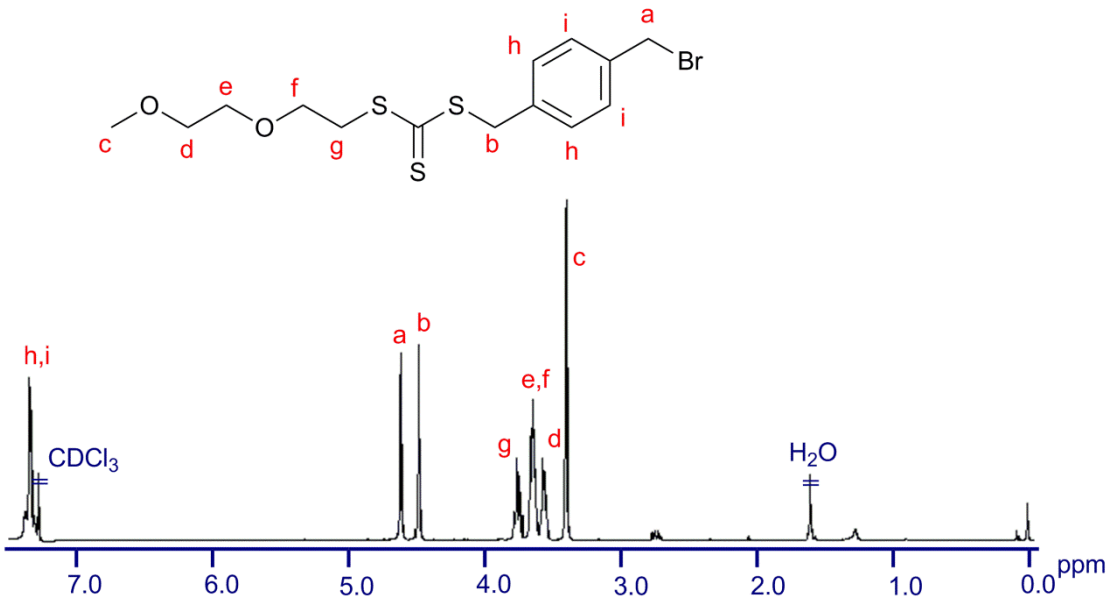


Figure S2. ^{1}H NMR spectrum of (**2**) (300 MHz, $CDCl_3$).

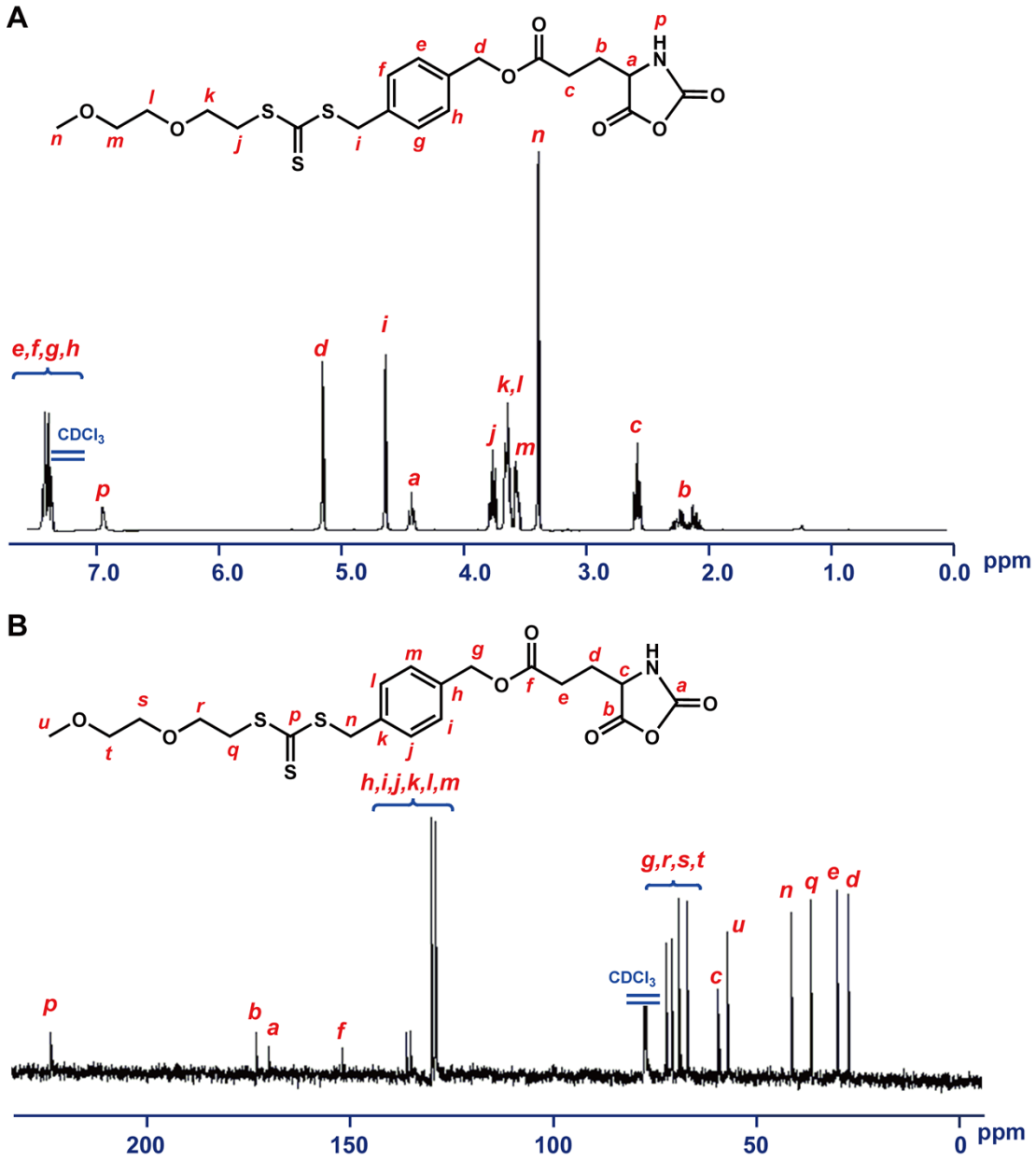


Figure S3. (A) ^{1}H NMR spectrum of MES-$_{L}$-Glu-NCA monomer (300 MHz, $CDCl_3$). (B) ^{13}C NMR spectrum of MES-$_{L}$-Glu-NCA monomer (100 MHz, $CDCl_3$).

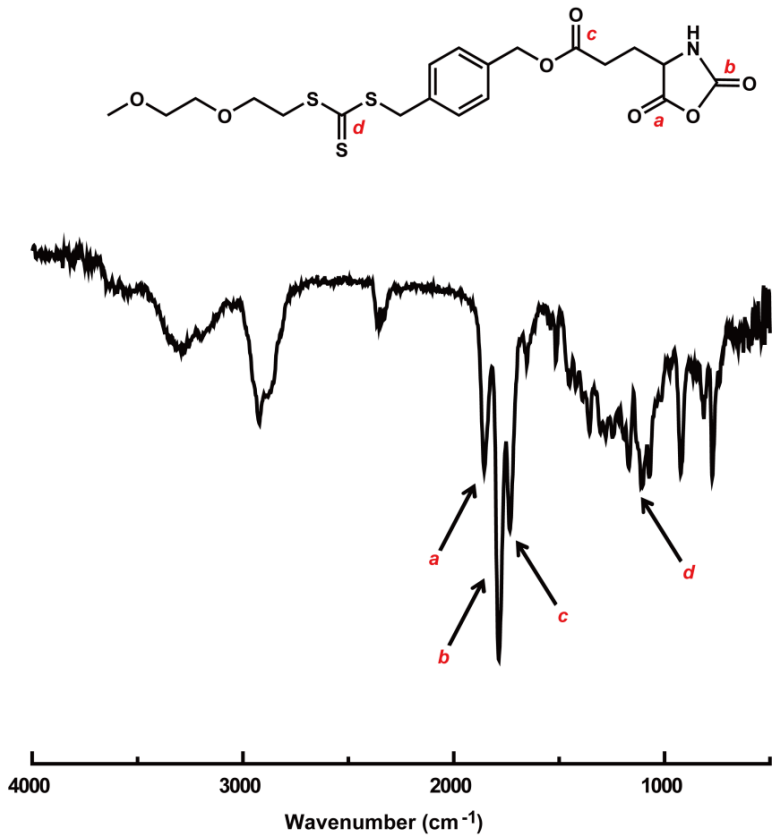


Figure S4. The FTIR spectrums of MES-$_L$-Glu-NCA monomer.

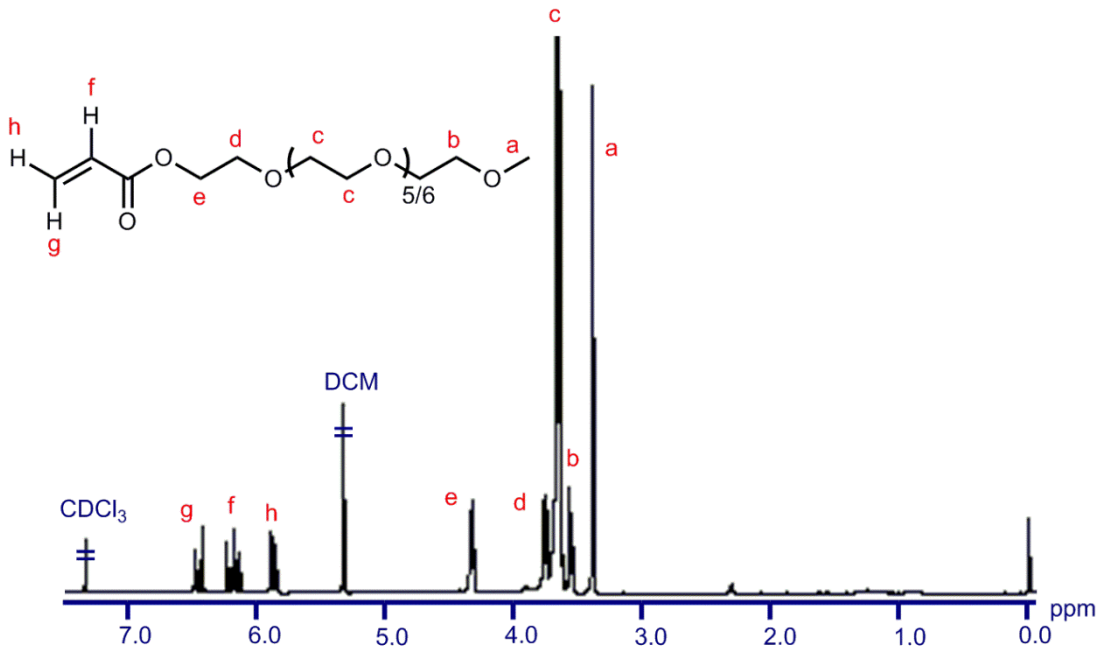


Figure S5. ^{1}H NMR spectrum of oligoethylene glycol acrylate (300 MHz, $CDCl_3$).

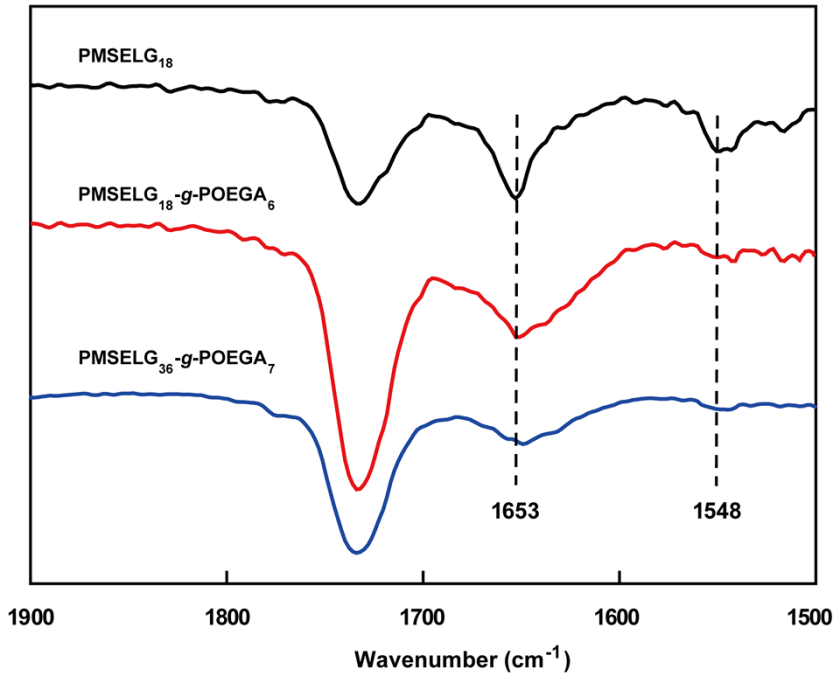


Figure S6. FTIR spectra of PMESLG macro-transfer agent and PMESLG-*g*-POEGA polypeptide bottlebrushes.

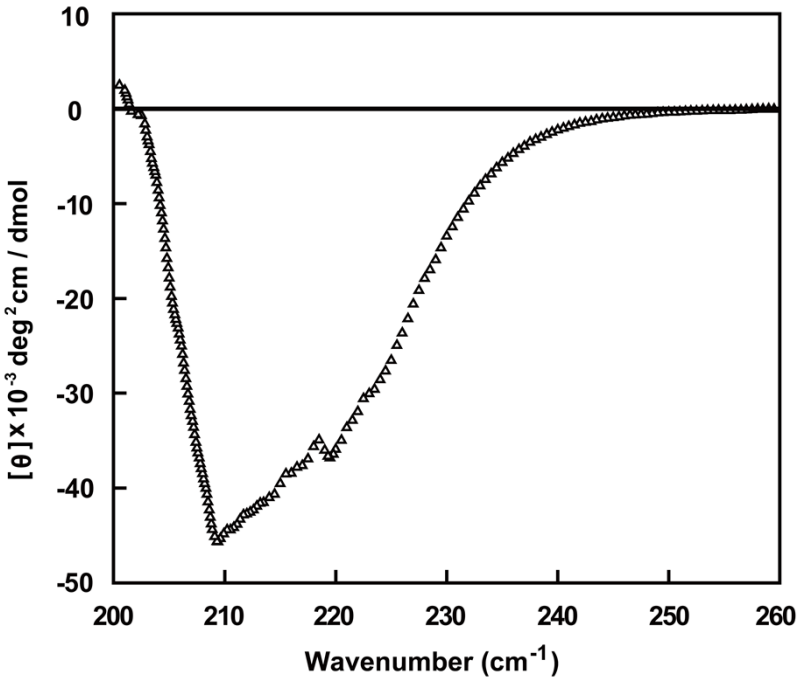


Figure S7. CD spectra of $PMESLG_{36}$ in THF.

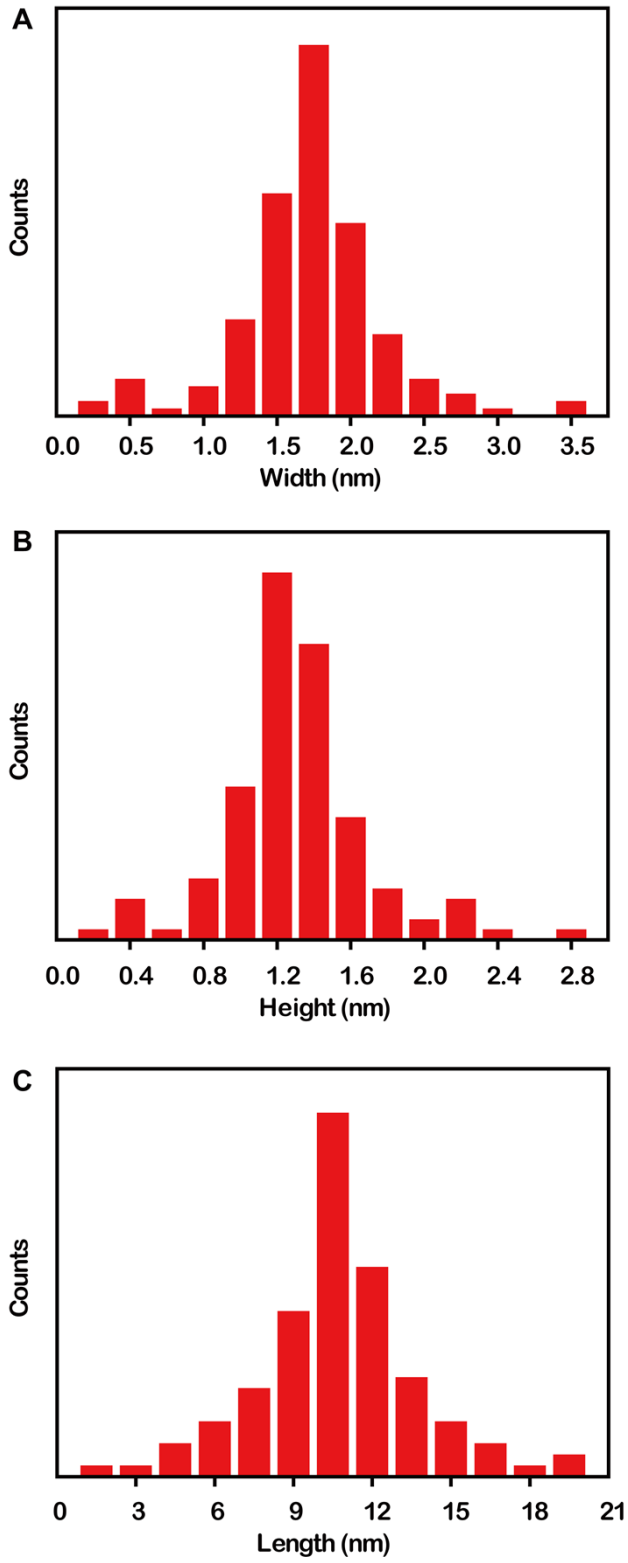


Figure S8. Statistical analysis of AFM data. (A) width distribution; (B) height distribution; (C) length distribution.

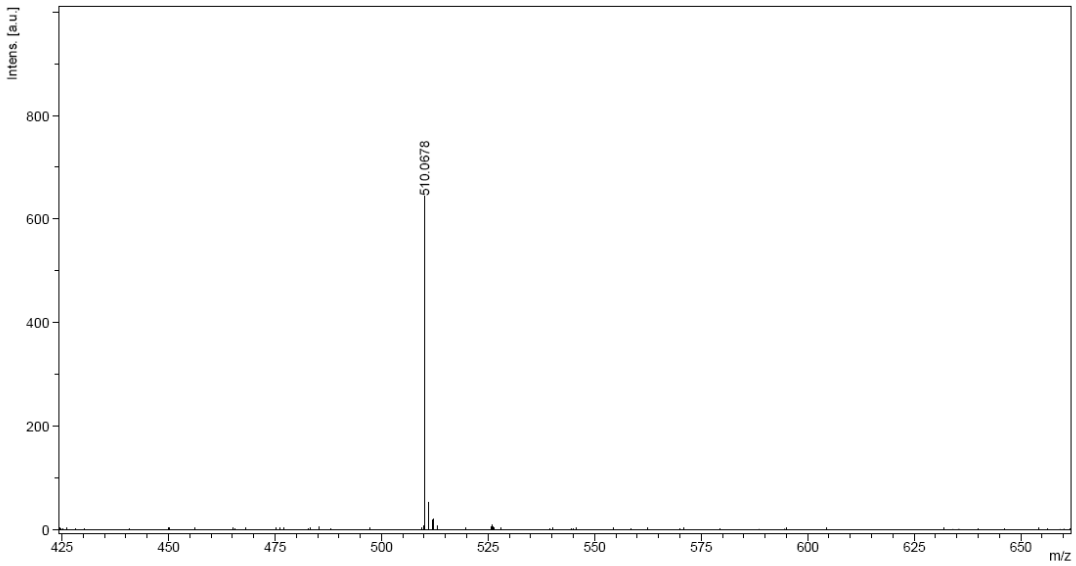


Figure S9. MS spectra of the NCA monomer.

SUPPLEMENTARY TABLES

Table S1. Solubility of PMESLG and PMESLG-*g*-POEGA [a]

sample	THF	DCM	$CHCl_3$	DMSO	DMF	acetone	MeOH	EtOAc	H_2O
PMESLG	S	S	S	S	S	S	I	S	I
PMESLG-g-POEGA	S	S	S	S	S	S	S	S	S

[a] S= soluble; I= insoluble

Table S2. Characterization of homopolypeptide of PMESLG [a]

Entry	Polymers	M_n (g/mol)[b]	M_w/M_n [b]	DP [c]
1	$PMESLG_{18}$	8210	1.12	18
2	$PMESLG_{36}$	16000	1.11	36

[a] polymerization in THF at room temperature with Et_3N as the initiator. [b] Determined by GPC analysis. [c] Degree of Polymerization, determined by GPC.

Table S3. Molar Ellipticity ($[\theta]_{222}$) and α-helix % of PMESLG and PMESLG-*g*-POEGA [a]

sample	Solvent	$[\theta]_{222}$	α-helix %
$PMESLG_{36}$	THF	-32952	92
$PMESLG_{18}$-*g*-$POEGA_6$	H_2O	-11316	37
$PMESLG_{36}$-*g*-$POEGA_7$	H_2O	-5945	23

[a] CD spectra were collected with a polymer concentration of 2 mg/mL at 25 °C.

(1) Lu, H.; Wang, J.; Bai, Y.; Lang, J. W.; Liu, S.; Lin, Y.; Cheng, J. *Nat Commun* **2011**, *2*, 206.
(2) Snow, A. W.; Foos, E. E. *Synthesis-Stuttgart* **2003**, 509.
(3) Vanheeswijk, W. A. R.; Eenink, M. J. D.; Feijen, J. *Synthesis-Stuttgart* **1982**, 744.
(4) Samakande, A.; Sanderson R. D.; Hartmann P. C. *Synthetic Communications* **2007,** 3861.
(5) Lutz, J.-F.; Schütt, D.; Kubowicz, S. *Macromolecular Rapid Communications* **2005**, 26, 23.
(6) Zhang, X.; Li, J.; Li, W.; Zhang, A. *Biomacromolecules* **2007**, 8, 3557.
(7) Ding, J.; Xiao, C.; Tang, Z.; Zhuang, X.; Chen, X. *Macromolecular Bioscience* **2011**, 11, 192.