Supporting Information

Water-Soluble Polypeptides with Elongated, Charged Side Chains Adopt Ultra-Stable Helical Conformations

Yanfeng Zhang,[†] Hua Lu,[†] Yao Lin,[‡] Jianjun Cheng[†]*

[†]Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801

[‡] Polymer Program, Institute of Materials Science and Department of Chemistry, University of Connecticut, Storrs, CT 06269

*Corresponding Author: jianjunc@illinois.edu

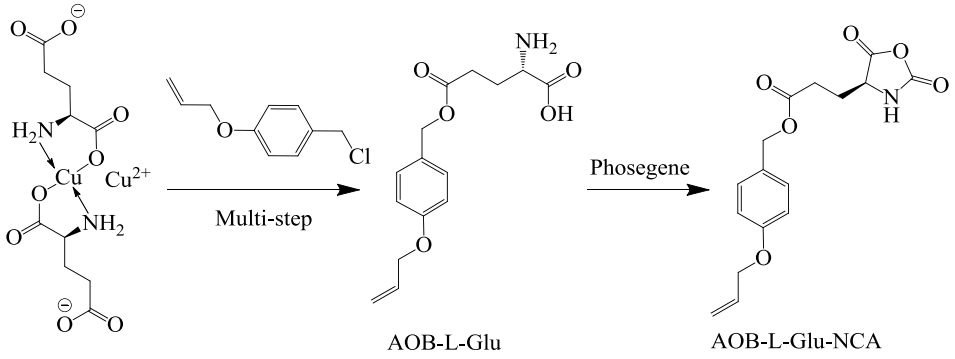
Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. Anhydrous dimethylformamide (DMF) was dried by a column packed with 4Å molecular sieves and stored in a glove box. Tetrahydrofuran (THF) and hexane were dried by a column packed with alumina and stored in a glove box. 4-Allyloxylbenzyl chloride,[1] L-glutamic acid copper(II) complex copper(II) salt tetrahydrate,[2] PLL_{60},[3] and $(PVBLG\text{-}1)_{10}$[4] were prepared by following the previously reported procedures.

Instrumentation

NMR spectra were recorded on a Varian UI400 MHz, a UI500NB MHz or a VXR-500 MHz spectrometer. Tandem gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA), a DAWN HELEOS 18-angle laser light scattering detector (also known as multi-angle laser light scattering (MALLS) detector, Wyatt Technology, Santa Barbara, CA) and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100Å, 500Å, 10^3Å and 10^4Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA) at 60°C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene with no need for external polymer standards and was used for the determination of the absolute molecular weights. The molecular weights (MWs) of all polymers were determined based on the dn/dc value of each sample calculated offline by using the internal calibration processed by the ASTRA V software (version 5.1.7.3, Wyatt Technology, Santa Barbara, CA). Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer equipped with universal attenuated total reflectance (ATR), which enabled the analysis of polymer sample in powder form. Circular dichroism (CD) measurements were carried out on a JASCO J-700 or a JASCO 720 CD Spectrometer. Lyophilization was performed on a FreeZone lyophilizer (Labconco, Kansas City, MO). UV light was generated from an OmiCure S1000 UV lamp (EXFO, Mississauga, Canada). Matrix Assisted Laser

Desorption/Ionization-Time Of Flight mass spectrometry (MALDI-TOF MS) spectra were collected on a Applied Biosystems Voyager-DE^{TM} STR system using 2,5-dihydroxybenzoic acid as matrix.



Synthesis of γ-(4-allyloxylbenzyl)-L-glutamate (AOB-L-Glu). In a 500-mL round-bottom flask, *N*, *N*, *N*', *N*'-tetramethylguanidine (3.4 mL, 2.7 mmol) was added slowly to a stirred mixture of L-glutamic acid copper(II) complex copper(II) salt tetrahydrate (3.29 g, 6.7 mmol) and L-glutamic acid (1.99 g, 13.4mmol) in a mixed solvent of dimethylformamide (DMF)/water (12 mL/1.9mL). The mixture gradually turned dark blue. After the dissolution of all solids, DMF (9.6 mL) was added. 4-Allyloxylbenzyl chloride (5.15 g, 28.3 mmol) was added to the deep blue solution in one portion. The mixture was stirred at rt for 24 h. Acetone (300 mL) was added to the slurry and the mixture was stirred until a fine precipitate was obtained. The precipitate was collected by filtration and washed with acetone (100 mL × 3) to yield crude AOB-L-Glu in violet powder form. The crude AOB-L-Glu was added a freshly-prepared EDTA disodium salt solution (EDTA (5.84 g) and sodium bicarbonate (3.36 g) in 40-45 mL water). The violet solid gradually turned white and the solution turned blue. The mixture is vigorously stirred for 3 h. The solid was collected by filtration, washed by DI water until the solid became white, recrystallized with water/isopropyl alcohol (1:2 v/v), and dried by lyophilization to give AOB-L-Glu (3.6 g, 46% yield). ^{1}H-NMR (DMSO-d_6 : DCl (20%) = 9:1 v/v, 500 MHz): δ 7.24 (d, 2H, Ar<u>H</u>), 6.89 (d, 2H, Ar<u>H</u>), 5.95 (m, 1H, $PhOCH_2C\underline{H}=CH_2$), 5.32 (dd, 1H, $PhOCH_2CH=C\underline{H}_2$), 5.19 (dd, 1H,

PhCH=C$\underline{H_2}$), 4.95 (s, 2H, PhC$\underline{H_2}$), 4.50 (d, 2H, PhOC$\underline{H_2}$CH=CH$_2$), 3.86 (t, 1H, alpha-H), 2.50 (m, 2H, C$\underline{H_2}$CH$_2$COOCH$_2$ and DMSO), 2.03 (t, 2H, CH$_2$C$\underline{H_2}$COOCH$_2$). ^{13}C-NMR (DMSO-d$_6$: DCl (20%) = 9:1 v/v, 500 MHz): δ 172.3, 170.8, 158.6, 134.0, 130.5, 128.5, 118.3, 115.2, 68.7, 66.3, 51.5, 29.9 and 25.7. ESI-MS (m/z): calcd. $C_{15}H_{19}NO_5$ 293.1 (M); found 294.1 $(M+H)^+$.

Synthesis of γ-(4-allyloxylbenzyl)-L-glutamate *N*-carboxyanhydride (AOB-L-Glu-NCA)

AOB-L-Glu (2.93 g, 10 mmol) was dried under vacuum for 1 h. Anhydrous THF (30 mL) was added under nitrogen followed by the addition of phosgene (20% in toluene, 7 mL) over the course of 5 min. The suspension was stirred at 50^{o}C for about 2 h until a clear solution was obtained. The solvent was removed under vacuum. The crude AOB-L-Glu-NCA was recrystallized three times with anhydrous THF/hexane (10 mL/100 mL) in a glove box to yield AOB-L-Glu-NCA in needle crystalline form (2.6 g, 81%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.24 (d, 2H, Ar$\underline{H}$), 6.88 (d, 2H, Ar$\underline{H}$), 6.01 (m, 1H, PhOCH$_2$C$\underline{H}$=CH$_2$), 5.39 (dd, 1H, PhOCH$_2$CH=C$\underline{H_2}$), 5.26 (dd, 1H, PhCH=C$\underline{H_2}$), 5.03 (s, 2H, PhC$\underline{H_2}$), 4.51 (d, 2H, PhOC$\underline{H_2}$CH=CH$_2$), 4.37 (t, 1H, alpha-H), 2.52 (t, 2H, CH$_2$C$\underline{H_2}$COOCH$_2$), 2.14 (m, 2H, C$\underline{H_2}$CH$_2$COOCH$_2$). ^{13}C-NMR ($CDCl_3$, 500 MHz): δ 172.7, 169.6, 159.0, 152.4, 133.2, 130.5, 127.8, 118.0, 115.2, 69.1, 67.1, 56.9, 30.0, 26.8. ESI-MS (m/z): Calcd $C_{16}H_{17}NO_6$ 319.1 (M); found: 342.3 $(M+Na)^+$. Elemental analysis: calcd. $C_{16}H_{17}NO_6$ 60.18%C, 5.37%H, 4.39%N; found: 60.06%C, 5.28%H, 4.40%N.

General procedure for the polymerization of AOB-L-Glu-NCA

In a glove box, AOB-L-Glu-NCA (32 mg, 0.1 mmol) was dissolved in DMF (1 mL) followed by the addition of HMDS (20 μL, 2 μmol). The polymerization solution was stirred for 16 h at room temperature. An aliquot of the polymerization solution was diluted to 10 mg polymer (PAOBLG)/mL using DMF containing 0.1 M LiBr and then analyzed by GPC (M_n = 1.38 × 10^3 g/mol; M_w/M_n = 1.05). The majority of the DMF of the polymerization solution was removed under vacuum. The polymer was precipitated with ether (15 mL). The obtained PAOBLG was sonicated for 5 min in ether and centrifuged to remove the solvent. After the

sonication-centrifugation procedure was repeated two more times, PAOBLG was collected and dried under vacuum (22 mg, 80 % isolated yield). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.14 (d, 2H, Ar<u>H</u>),6.77 (d, 2H Ar<u>H</u>), 5.97 (m, 1H, $PhOCH_2C\underline{H}=CH_2$), 5.33 (dd, 1H, $PhOCH_2CH=C\underline{H_2}$), 5.22 (dd, 1H, $PhCH=C\underline{H_2}$), 5.03 (d, 2H, $PhOC\underline{H_2}CH=CH_2$), 4.51 (s, 2H, $PhC\underline{H_2}$), 3.96 (m, 1H, alpha-H), 2.63 (m, 2H, $CH_2C\underline{H_2}COOCH_2$), 2.16 (m, 2H, $C\underline{H_2}CH_2COOCH_2$).

Kinetic study of the polymerization of AOB-L-Glu-NCA

In a glove box, AOB-L-Glu-NCA (192 mg, 0.6 mmol) was dissolved in DMF (3.0 mL). HMDS (0.1 M in DMF, 0.06 mL) (or HMDS (0.1 M in DMF, 0.06 mL) and TBD (0.01 M in DMF, 0.06 mL)) was added to the stirred NCA solution in one portion. The real-time concentration of NCA was quantified by measuring the intensity of the anhydride band at 1784 cm^{-1} by FT-IR. The conversion of NCA was determined by comparing the NCA concentration in the polymerization solution versus the initial NCA concentration.

General procedure for the synthesis of PAOBLG-AET via thiol-ene reaction

In a quartz bottle, $PAOBLG_n$ (16 mg, 0.06 mmol), 2-aminoethanethiol hydrochloride (34 mg, 0.3 mmol) and Irgacure®2959 photo-initiator (1 mg, 0.004 mmol) were dissolved in DMF/DI water (1.0 mL/0.1 mL). The quartz bottle was sealed with a rubber septum and the mixture was purged with nitrogen for 10 min. Irradiation with a 365 nm UV lamp (16 mW/cm^2) was carried out for 10 min. The crude product was dialyzed against water for 2-3 days and lyophilized (yield 70-90%). The modification efficiency was determined to be about 100 % based on ^{1}H-NMR analysis (Fig. S5 and S6).

Synthesis of PAOBLG-MPA via thiol-ene reaction

PAOBLG-MPA was synthesized by following similar protocol as described above for the synthesis of PAOBLG-AET by using 3-mercaptopropionic acid instead of 2-aminoethanethiol hydrochloride as starting materials. The isolated yield was about 90%. The modification efficiency was determined to be about 100 % based on ^{1}H-NMR analysis (Fig. S7).

General procedure for the analysis of polypeptide conformations by circular dichroism (CD)

The CD study was performed on a JASCO J-700 and J-720 CD spectrometer. The polymer samples were prepared at concentrations of 0.01-0.1 mg/mL in general unless otherwise specified. The solution was placed in a quartz cell with a path length of 0.5 cm. The mean residue molar ellipticity of each polymer was calculated based on the measured apparent ellipticity by following the literature reported formulas: Ellipticity ([θ] in deg·cm^2·dmol^{-1}) = (millidegrees × mean residue weight)/(path length in millimeters × concentration of polypeptide in mg·ml^{-1}).[5] For the helix-temperature dependency study, the temperature of the sample chamber where the quartz cell was placed was controlled by a water bath (from 4^{o}C to 70^{o}C). The sample was equilibrated at corresponding temperature for at least 10 min before the CD measurements. By following similar preparation method, the polymers were dissolved in DI water or in a solution containing NaCl or urea to analyse the conformation of polymers under denaturing conditions. For the helix-pH dependency study, the pH of the polymer solution was tuned by the addition of a concentrated HCl or NaOH solution. The α-helix contents of the polypeptides were calculated using the following equation: % α-helix = (-[θ_{222}] + 3000)/39,000.[6]

Table S1. HMDS-Initiated Polymerization of AOB-L-Glu-NCA.

entry	M/HMDS/TBD[a]	time (h)	conv. (%)	M_n (M_n^*) (× 10^{-4})[b]	MWD
1	10/1/0	8	>98	0.28 (0.30)	1.22
2	20/1/0	12	>98	0.55 (0.56)	1.12
3	50/1/0.1	16	>98	1.42 (1.38)	1.05
4	100/1/0.1	36	>98	2.68 (2.76)	1.06

[a]Molar ratio of M/HMDS/TBD (M = AOB-l-Glu-NCA, HMDS = hexamethyldisilazane, TBD =1,5,7-triazabicyclo [4.4.0]dec-5-ene); [b]The MW obtained (the MW expected*).

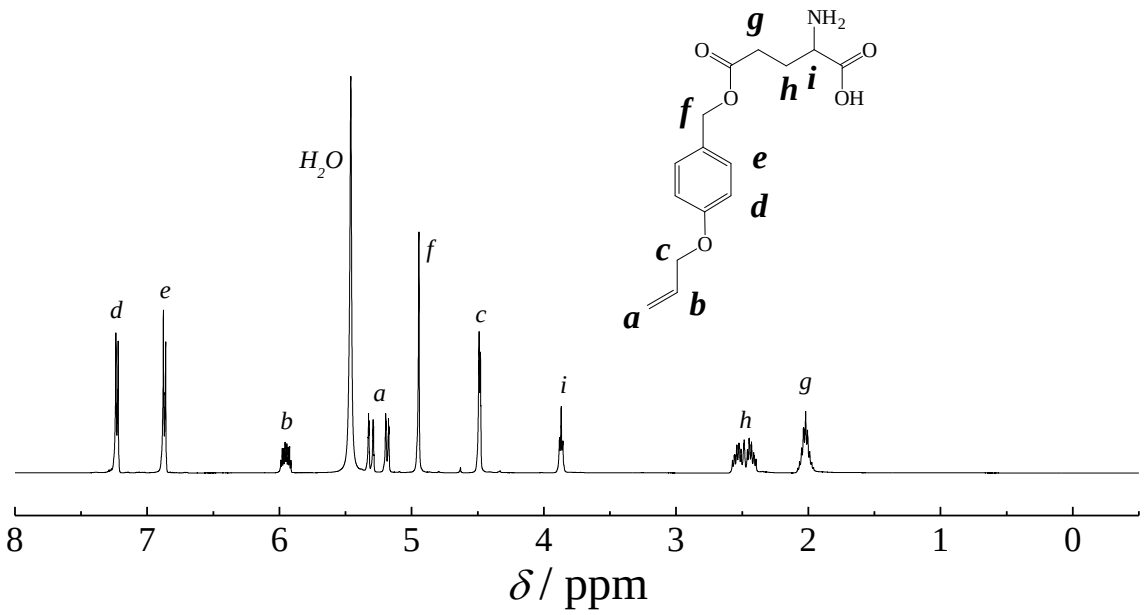


Figure S1. ^{1}H-NMR spectrum of AOB-L-Glu in DMSO-d_6 : DCl (20%) = 9 : 1, v/v.

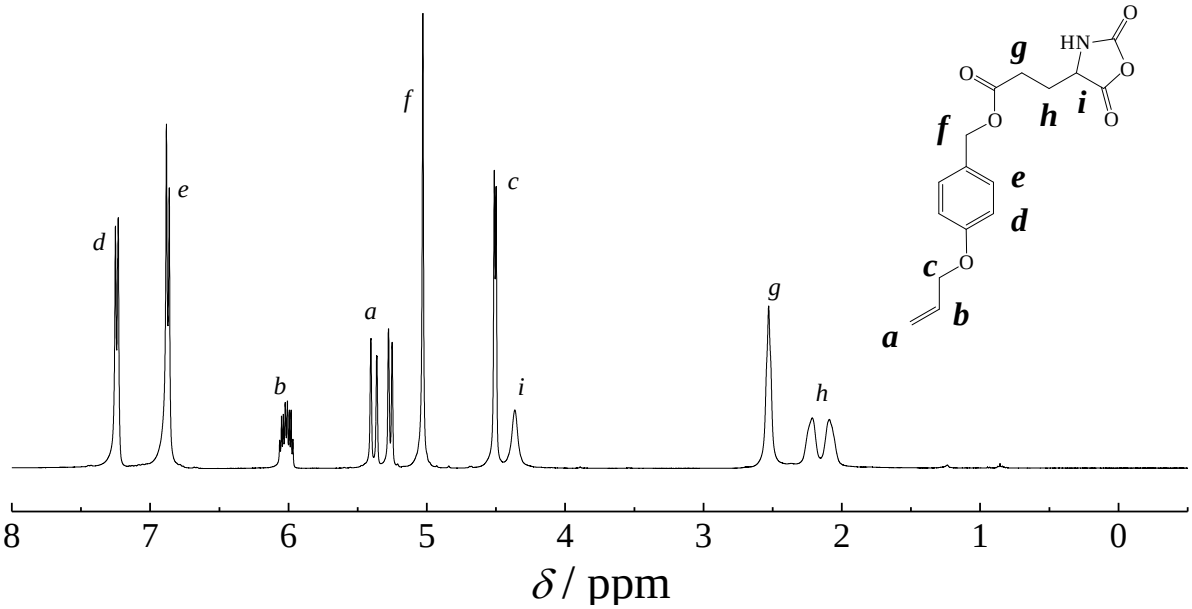


Figure S2. ^{1}H-NMR spectrum of AOB-L-Glu-NCA in $CDCl_3$.

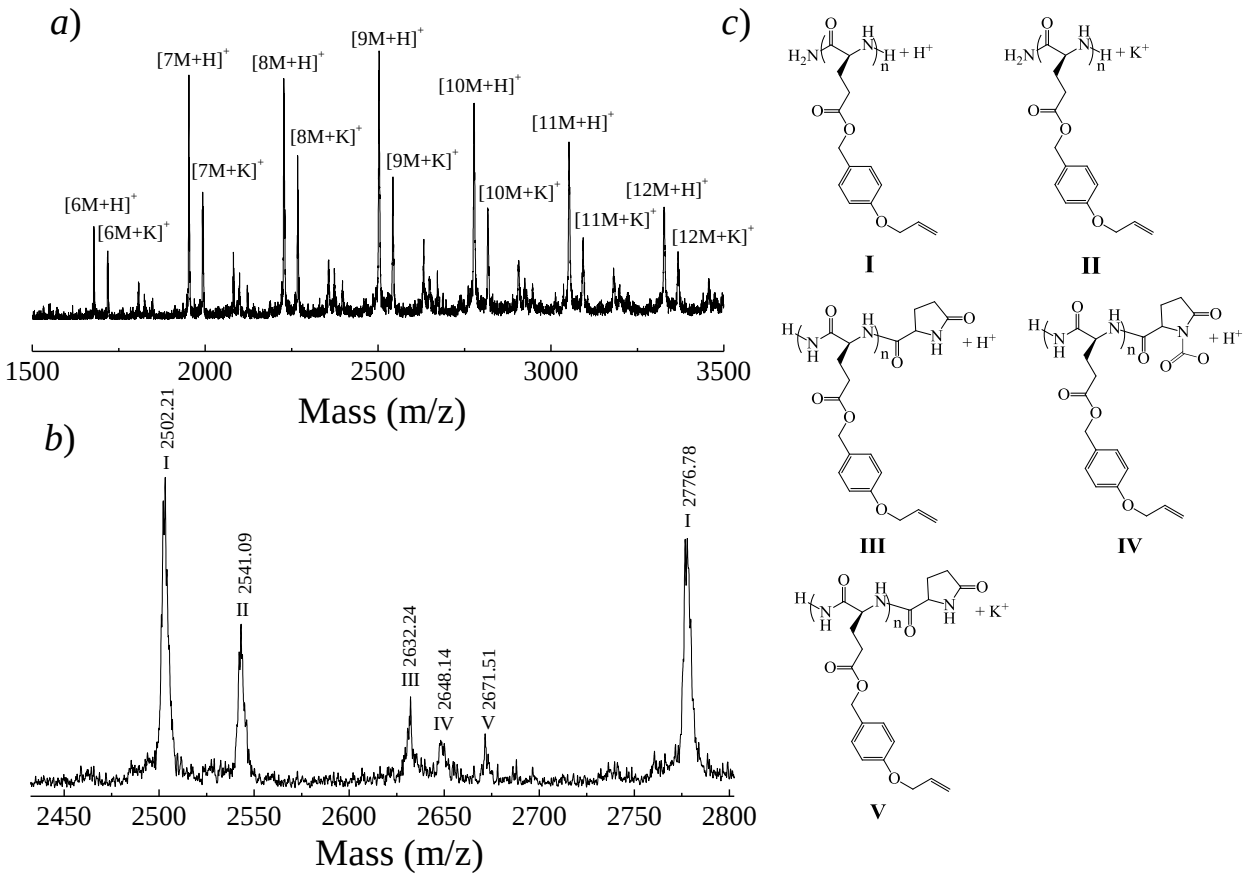


Figure S3. Representative full (a) and expanded (b) MALDI-TOF MS spectrum of PAOBLG$_{10}$ (2,5-dihydroxybenzoic acid as matrix) and the molecular structures of PAOBLG with assigned end groups (c).[7]

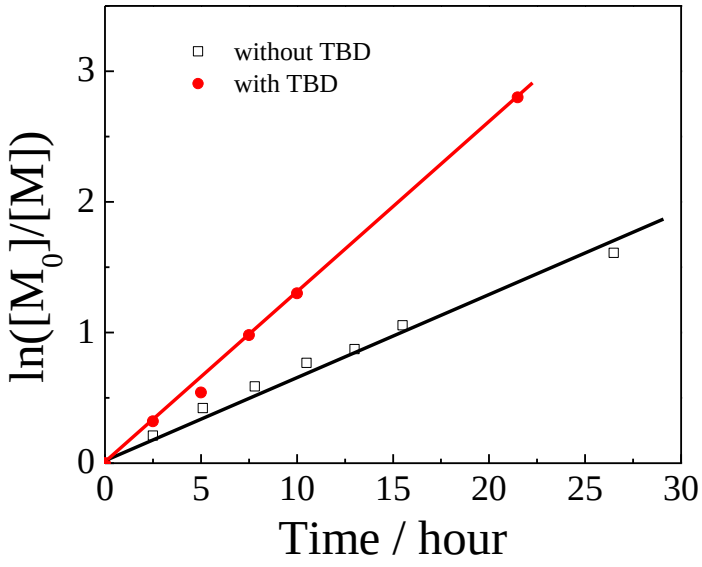


Figure S4. Kinetic study of ROP of AOB-L-Glu-NCA initiated by HMDS with or without TBD (1/10 of HMDS) as a co-catalyst in DMF at room temperature. The initial NCA concentration were 0.2 mmol/mL and the ratio of $[M]_0/[I]_0$ were 100/1. The conversion of the NCA was measured by FTIR.

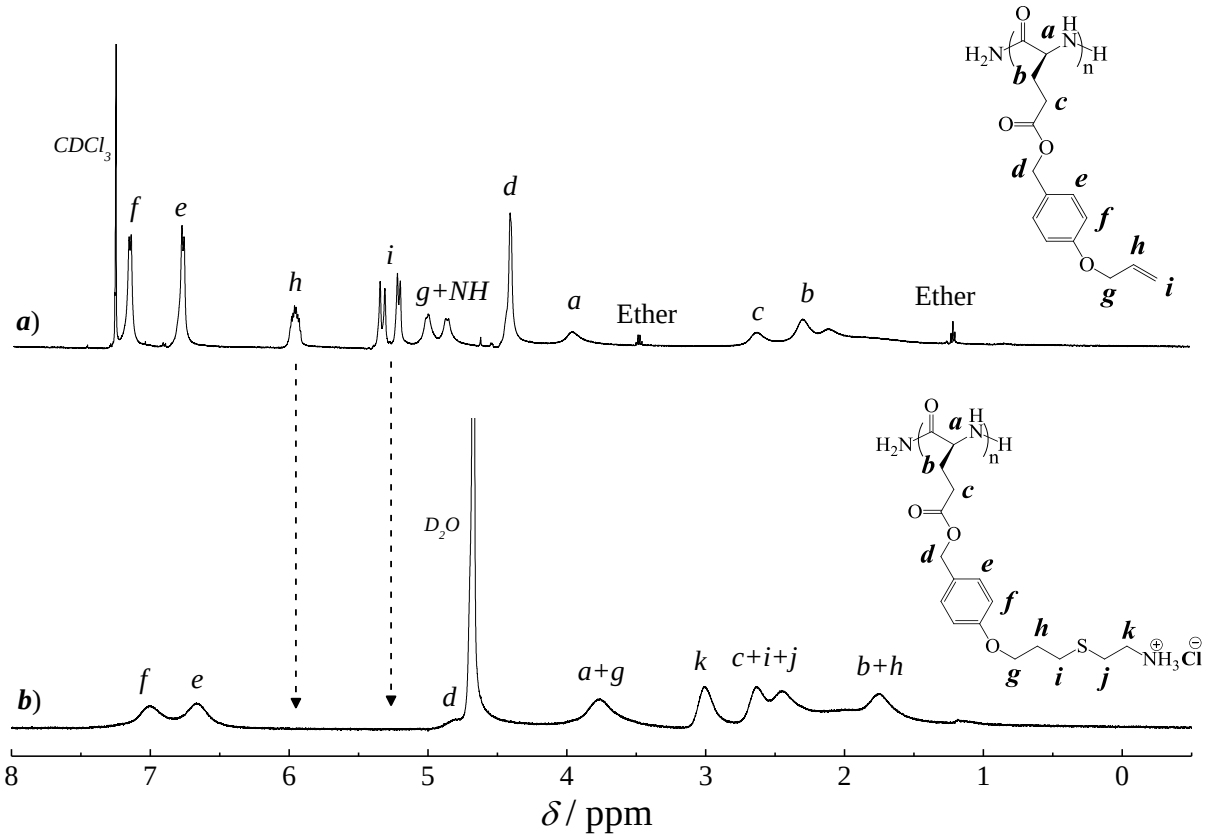


Figure S5. ^{1}H-NMR spectra of (a) $PAOBLG_{50}$ in $CDCl_3$ and (b) $(PAOBLG\text{-}AET)_{50}$ in D_2O/DCl (pH 4).

Brief discussion:

The ^{1}H-NMR studies showed that the thiol-ene reaction completed with nearly 100% efficiency, evidenced by the complete disappearance of the allyl protons of PAOBLG (δ 5.3 and 5.9 ppm (*i and h*) after the reaction (Figure S5b)) and the ratio of the normalized integration value of peak-*k* versus peak-*f* and *e* (0.63) (Figure S5b). $(PAOBLG\text{-}AET)_{50}$ has excellent water solubility (> 20 mg/mL) while $(PAOBLG)_{50}$ is insoluble in water.

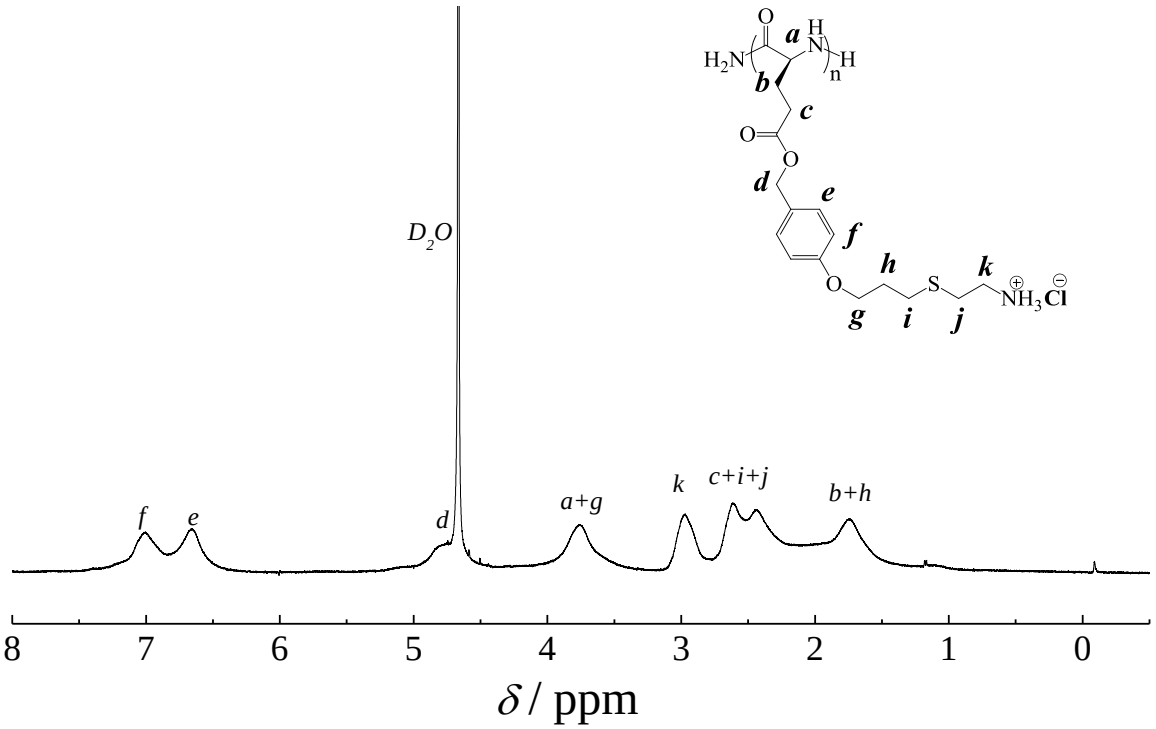


Figure S6. ^{1}H-NMR spectrum of $(PAOBLG\text{-}AET)_{10}$ in D_2O/DCl (pH 4).

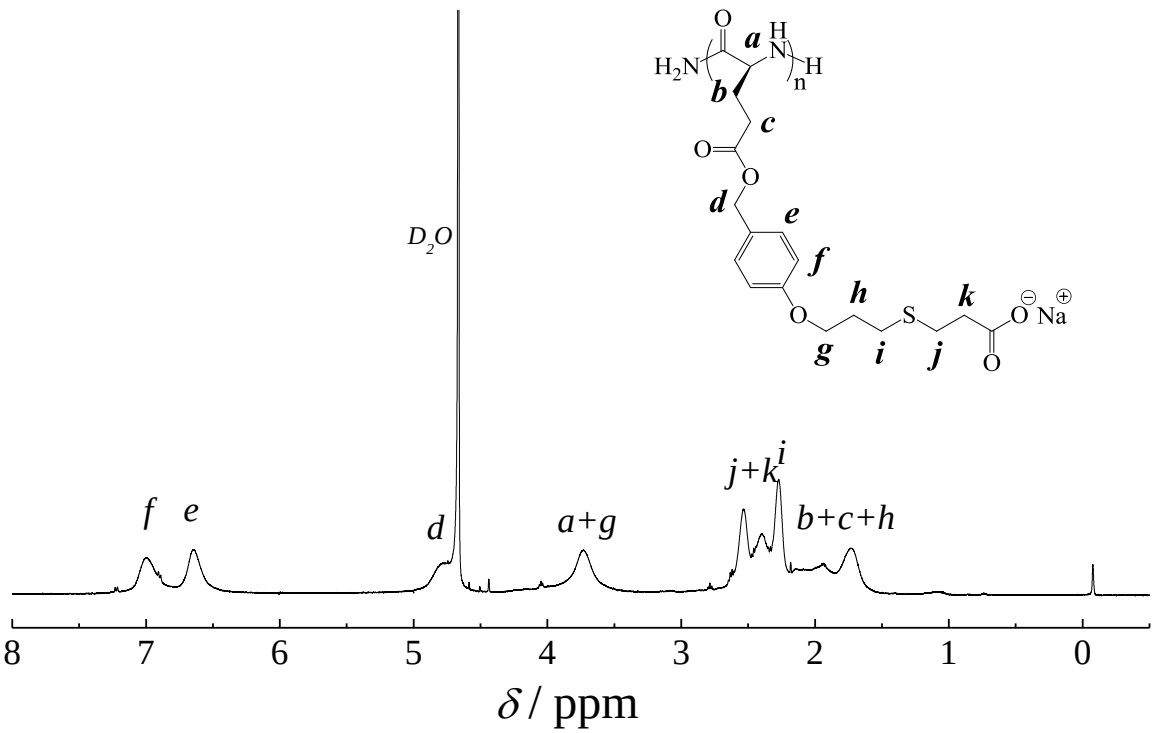


Figure S7. ^{1}H NMR spectrum of $(PAOBLG\text{-}MPA)_{10}$ in D_2O/NaOD (pH 9).

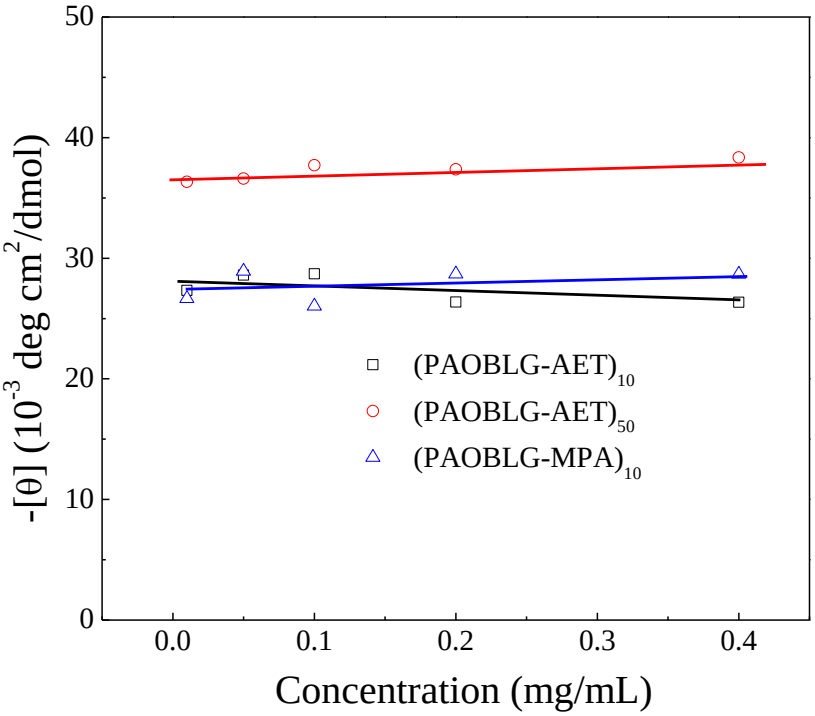


Figure S8. Concentration dependence of residue molar ellipticity at 222 nm for $(PAOBLG\text{-}AET)_{10}$ and $(PAOBLG\text{-}AET)_{50}$ at pH 2 and $(PAOBLG\text{-}MPA)_{10}$ at pH 10.

References:

(1) Podichetty, A. K.; Wagner, S.; Schroer, S.; Faust, A.; Schafers, M.; Schober, O.; Kopka, K.; Haufe, G. *J. Med. Chem.* **2009,** 52, 3484-3495.

(2) Vanheeswijk, W. A. R.; Eenink, M. J. D.; Feijen, J. *Synthesis-Stuttgart* **1982,** 744-747.

(3) Lu, H.; Cheng, J. J. *J. Am. Chem. Soc.* **2007,** 129, 14114-14115.

(4) Lu, H.; Wang, J.; Bai, Y.; Lang, J. W.; Liu, S.; Lin, Y.; Cheng, J. *Nature Communications* **2011,** 2, 206.

(5) Greenfield, N. J. *Nat. Prot.* **2006,** 1, 2876-2890.

(6) Morrow, J. A.; Segall, M. L.; Lund-Katz, S.; Phillips, M. C.; Knapp, M.; Rupp, B.; Weisgraber, K. H. *Biochemistry* **2000,** 39, 11657-11666.

(7) Tang, H. Y.; Zhang, D. H. *Biomacromolecules* **2010,** 11, 1585-1592.