Supporting information

Amino-functionalized Poly(*N*-vinylcaprolactam) Derived from Lysine: Sustainable Polymer with Thermo and pH Dual Stimuli Response

Fan Jia,[a,b] Shixue Wang,[a,b] Xiaojie Zhang,[a] Chunsheng Xiao,[a] Youhua Tao*[a] and Xianhong Wang*[a]

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

[b]*University of Chinese Academy of Sciences, Beijing 100039, People's Republic of China.*

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

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Materials and Methods

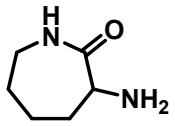
All solvents and reagents obtained from commercial sources were used as received without further purification unless stated otherwise. DMF were freshly distilled from CaH_2. *N*-Vinylcaprolactam (VCL, Alfa, 99%) was recrystallized from hexane before use. 2,2'-Azobis(2-methylpropionitrile) (AIBN, Aladdin, 98%) was recrystallized from ethanol before use. Methyl 2-bromopropionate (98%), potassium ethyl xanthogenate (98%), $_L$-Lysine monohydrochloride (99%) and *P*-toluenesulfonic acid monohydrate (98.5%) were obtained from Aladdin.

^1H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer and a Bruker AV-500 spectrometer, respectively. Deuterated chloroform ($CDCl_3$) and deuterium oxide (D_2O) were used as solvent. The spectra were measured at room temperature. Size Exclusion Chromatography (SEC) 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 eluent was DMF (containing 0.01M LiBr) at a flow rate of 1 mL/min at 50 ^oC and calibrated with PMMA standards. ESI-MS was conducted on a system composed of a Waters Quattro Premier XE. The system was operated with Full Scan function type, 3.0kV Capillary, 20V Cone, 110 ^oC Source Temperature, 80L/Hr Cone Gas Flow, 380 ^oC desolvation temperature and 600 L/Hr Desolvation Gas Flow. 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 trans-2-[3-(4-*tert* -butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB). Turbidimetric measurements were carried out on a UV-Vis spectrometer (Shimadzu UV-2401PC) equipped with a temperature controller (Shimadzu S-1700) at a heating rate of 1 ^{o}C min^{-1}. All samples were prepared at a concentration of 5 mg/mL. The cloud point was defined as the temperature corresponding to that of solutions reaching 50% of the initial transmittance. Ethyl 2-(ethoxycarbonothioylthio) propanoate (CTA) was synthesized according to literature.[1]

Synthesis of Monomers

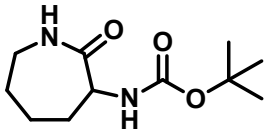
Synthesis of α-amino-ε-caprolactam (1)



The α-amino-ε-caprolactam (**1**) was synthesized according to our previous report.[2] Briefly, to a stirred solution of $_{L}$-lysine monohydrochloride (18.2 g, 0.1 mol) in 500 mL methanol at 0 °C was added thionyl chloride (35.7 g, 0.3 mol) dropwise. After that the mixture were refluxed for 4 h, the solution was cold down to room temperature. The crude product of lysine methyl ester dihydrochloride was precipitated out as white crystal. After filtrating and concentrating, the crude product was dissolved in 1000 mL methanol. NaOH (12 g, 0.3 mol) in 100 mL methanol was then added to the mixture slowly. The mixture was stirred over night at room

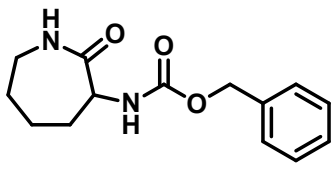
temperature. The crude product was purified by recrystallization from ethylacetate to get *α*-amino-*ε*-caprolactam (**1**) as a white solid (15.1 g, 59 %). ^{1}H NMR (400 MHz, DMSO) δ 3.44 (d, 1H); 3.06 (m, 2H); 1.83 (m, 1H); 1.64 (m, 2H,); 1.56(m, 1H); 1.36-1.13 (m, 2H).

Synthesis of *tert*-butoxycarbonyl (Boc) protected *α*-amino-*ε*-caprolactam monomer (2a)



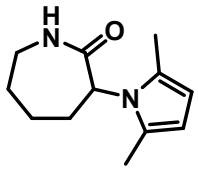
The *tert*-butoxycarbonyl (Boc) protected α-amino-ε-caprolactam monomer (**2a**) was synthesized as described earlier.[3] Briefly, to a stirred solution of α-amino-ε-caprolactam (**1**) (3.2 g, 25 mmol) and K_2CO_3 (3.45 g, 25 mmol) in 30 mL tetrahyrofuran (THF) cooled in an ice bath was added di-*tert*-butyl dicarbonate (4.9 mL, 37.5 mmol) dropwise. After 15 min, the ice bath was removed and the reaction was allowed to proceed overnight at room temperature. The precipitate was removed by filtration, THF was concentrated. The crude product was then purified by recrystallized from petroleum ether and ethyl acetate to afford the product as white solid (4 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.45 (s, 1H); 5.93 (s, 1H); 4.27 (m, 1H); 3.25 (s, 2H,); 2.05(m, 2H); 1.81(m, 2H); 1.51(m, 2H); 1.45(s, 9H).

Synthesis of carbobenzyloxy (Cbz) protected *α*-amino-*ε*-caprolactam monomer (2b)



The carbobenzyloxy (Cbz) protected α-amino-ε-caprolactam monomer (**2b**) was synthesized as described earlier.[4] Briefly, to a stirred solution of α-amino-ε-caprolactam (**1**) (5 g, 39 mmol) in 10 mL tetrahyrofuran (THF) and 4 mL water at 0 ^{o}C was added $NaHCO_3$ (4.75 g, 57 mmol) and benzyl chloroformate (5 mL, 39 mmol), and the mixture was stirred for 18h at room temperature. After concentrated of solvent under vacuum, the crude product was purified by recrystallization from diethyl ether and chloroform to afford the product as white solid (8.7 g, 84 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (m, 5H); 6.17 (m, 2H); 5.12 (s, 2H); 4.38 (m, 1H,); 3.25(m, 1H); 2.11 (dd, 2H); 1.86(m, 2H); 1.57-1.39(m, 2H).

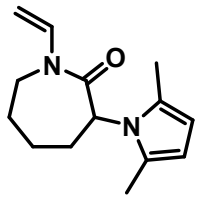
Synthesis of 2,5-dimethylpyrrole protected *α*-amino-*ε*-caprolactam monomer (3)



The 2,5-dimethylpyrrole protected α-amino-ε-caprolactam monomer (**3**) was synthesized by a modified procedure as described earlier.[2] Briefly, to a stirred solution of *α*-amino-*ε*-caprolactam (**1**) (12.8 g, 0.1 mol) in 100 mL toluene was added *P*-toluenesulfonic acid monohydrate (0.19 g, 0.001 mol) and 2,5-hexanedione (13.68 g, 0.12 mol) respectively. The mixture was refluxed for 4 hours and subsequently concentrated *in vacuo*. The product was then dissolved in dichloromethane (100 mL) and washed with saturated $NaHCO_3$ and NaCl aqueous solutions for several times. The crude product was subjected to column chromatography using ethyl acetate as eluent and silica as solid phase to afford the product as a white solid (14.5 g, 70%). ^{1}H

NMR (400 MHz, $CDCl_3$) δ 5.85 (s, 2H); 4.91 (d, 1H); 3.29 (s, 2H); 2.32(s, 6H); 2.19-1.92 (m, 4H); 1.73-1.54(dd, 2H).

Synthesis of MPVCL monomer (4)



2,5-dimethylpyrrole protected α-amino-ε-caprolactam monomer (**3**) (1.03 g, 0.05 mol) and potassium *tert*-butanolate (56 mg, 0.5 mmol) were added to a stainless-steel autoclave with a magnetic stirrer in a glove box. After 2 hours of pre-reaction at 150 °C, 30 μL 1,4-diethoxybutane was added to the mixture. Then, the autoclave was pressurized to 1 MPa with acetylene and the mixture was stirred at 170 °C for 8 hours. The autoclave was cooled to room temperature, and the acetylene pressure was released by opening the outlet valve. The crude product was subjected to column chromatography using petroleum ether and ethyl acetate (3:1) as eluent and silica as solid phase to afford the product as a white powder (0.72 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, 1H); 5.80 (s, 2H); 5.09 (dd, 1H); 4.52 (dd, 2H); 3.90 (dd, 1H); 3.50 (dd, 1H); 2.32(s, 6H); 2.19-1.92 (m, 4H); 1.73-1.54(dd, 2H). ^{13}C NMR (125 MHz,$CDCl_3$) δ 170.68, 132.34, 128.68, 106.56, 93.81, 58.43 43.77, 32.29, 29.15, 26.69, 14.29. Elemental analysis calcd for $C_{14}H_{20}N_2O$ (%): C,72.38; H,8.68; N,12.06; found: C,72.30; H,8.72; N,12.04.

Polymerization

MADIX/RAFT homopolymerization of MPVCL momomer

All polymerizations were carried out with AIBN as initiator and *O*-ethyl-*S*-(1-methoxycarbonyl) ethyl dithiocarbonate as CTA at 65 °C in DMF solution under dry nitrogen with standard Schlenk techniques. A typical procedure is described below. MPVCL (233 mg, 1 mmol), CTA (4.2 mg, 0.02 mmol) and AIBN (1.1 mg, 0.006 mmol) were dissolved in 0.1 mL DMF and placed in a Schlenk flask with a magnetic stirring bar. The mixed solution in the flask was degassed three freeze−pump−thaw cycles followed by dry nitrogen. The reaction was carried out at 65 °C on oil bath for 20 h and was quenched on liquid nitrogen. The polymer was purified by precipitation in hexane and washed by hexane 3 times to yield light yellow solid (80 mg, 34%).

MADIX/RAFT copolymerization of MPVCL with PVCL

All polymerizations were carried out with AIBN as initiator and *O*-ethyl-*S*-(1-methoxycarbonyl) ethyl dithiocarbonate as CTA at 65 °C in DMF solution under dry nitrogen protect with standard Schlenk techniques. A typical procedure is described below. VCL (285.6 mg, 2.4 mmol), MPVCL (139.2 mg, 0.6 mmol), CTA (4.2 mg, 0.02 mmol) and AIBN (1.1 mg, 0.006 mmol) were dissolved in 1 mL DMF and placed in a Schlenk flask with a magnetic stirring bar. The mixed solution in the flask was degassed three freeze−pump−thaw cycles followed by dry nitrogen. The reaction

was carried out at 65 °C on oil bath for 20 h and quenched on liquid nitrogen. The polymer was purified by precipitation in hexane and washed by hexane 3 times to yield light yellow solid (340 mg, 80 %).

Deprotection

A sample of 100 mg of polymer was dissolved in 2 mL of THF. Hydroxylamine hydrochloride (1g) dissolve in water (1mL) were added to the above mixture under nitrogen. The solution was heated at 80 °C and stirred for 96 h. The reaction mixture was then allowed to cool to room temperature and quenched by pouring it into a solution of concentrated NaOH (50%). The mixture was concentrated and dialyzed (MWCO 1000, Spectrum Laboratories, Inc.) against water for a total of 3 days to yield light yellow powder (32 mg, 48%).

Cell viability assays

The cytotoxicity of amino-PVCL was evaluated in L929 cells (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) using the MTT assay. L929 cells were seeded in a 96-well plate at 1×10^4 cells per well and incubated for 24 h at 37 °C. Amino-PVCLs were diluted with fresh medium (Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) fetal bovine serum) to the desired concentrations and added to the corresponding well, respectively. After incubation for 48 h at 37 °C, cell viability was assessed using the standard MTT assay.

Computational Details

The geometry structures of MPVCL was optimized using density functional theory B3LYP with 6-31G+(d, p) basis set implanted in Gaussian 09 suite of programs. Frequency calculations have been done at the same theory level to characterize the nature of the stationary points, that is, the minima with all real frequencies.

Coordinates:

MPVCL:

O	0.68389600	-0.78633600	-1.76330700
N	2.11570400	0.57314500	-0.57812200
C	1.96515700	-1.11663500	1.70489700
C	2.88843400	-1.51216200	0.52691300
C	0.45011100	-1.09799200	1.39466200
C	3.25587600	-0.33665900	-0.40228600
C	-0.05442800	0.01353400	0.42076100
C	0.86095700	-0.05540800	-0.81045500
C	2.23292000	1.94157700	-0.36868900
C	1.38362900	2.87816700	-0.82751700
H	2.10678800	-1.84740000	2.50952500
H	2.27963500	-0.14757300	2.11585800
H	3.82244500	-1.93564300	0.91403100
H	2.41119500	-2.31021700	-0.05428700
H	-0.10671000	-0.96471300	2.32889300
H	0.16864300	-2.07529200	0.99488500
H	3.62323500	-0.70187800	-1.36876300
H	4.05544500	0.26021600	0.04840500
H	0.13302500	0.97189700	0.90298500
H	3.10666600	2.22781800	0.21467600
H	1.52966600	3.91716100	-0.55818900
H	0.54863000	2.63657700	-1.47757700
N	-1.49017400	-0.05171000	0.17078500
C	-2.33606700	1.03545100	0.42307400
C	-2.25063000	-1.10412600	-0.35427800
C	-3.61042100	0.67122900	0.05056700
C	-1.88330100	2.32431000	1.03820000
C	-3.55482000	-0.66337100	-0.43610600
C	-1.74694400	-2.47062300	-0.71271400
H	-4.48554200	1.30226500	0.13026800

H	-1.13118000	2.85383800	0.44166800
H	-2.75058200	2.98305400	1.13379800
H	-1.46658500	2.19241500	2.04626600
H	-4.38076000	-1.25652100	-0.80560300
H	-0.84605700	-2.43739500	-1.32825700
H	-1.54274000	-3.09070100	0.17059400
H	-2.52793700	-2.98101400	-1.28353500

Supplementary Figures

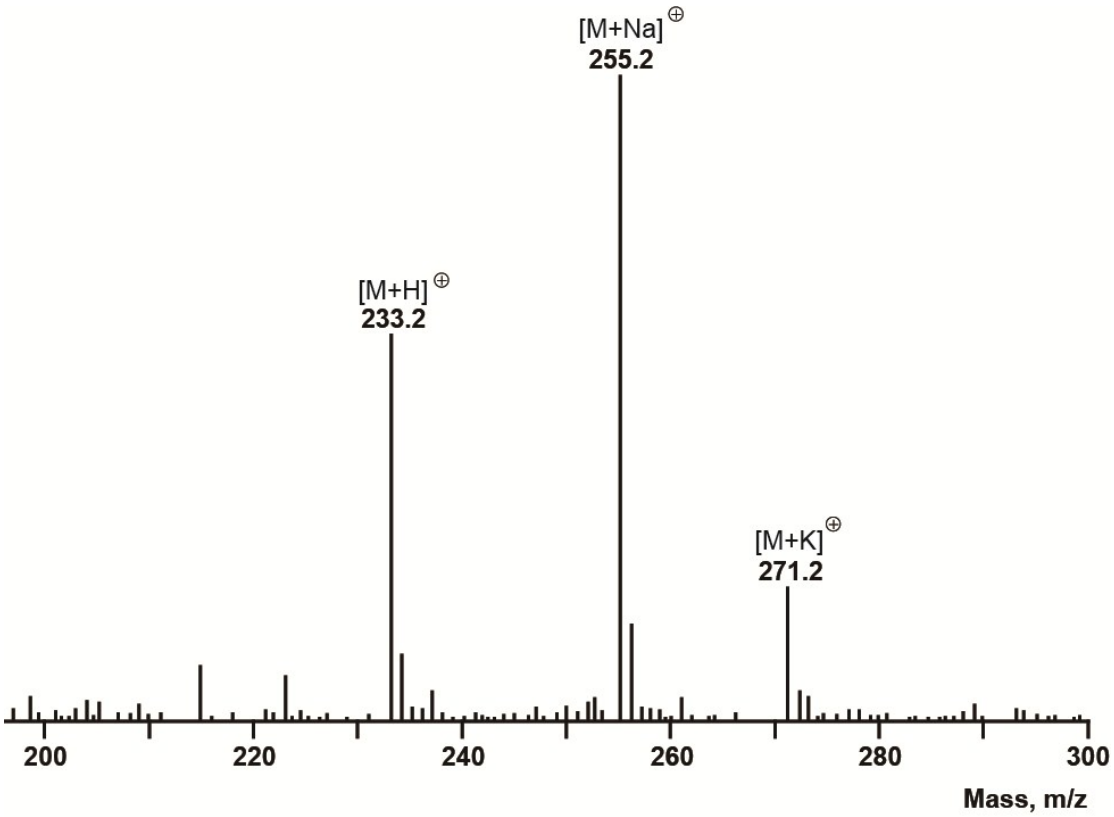


Fig. S1 ESI-MS spectra of the MPVCL monomer.

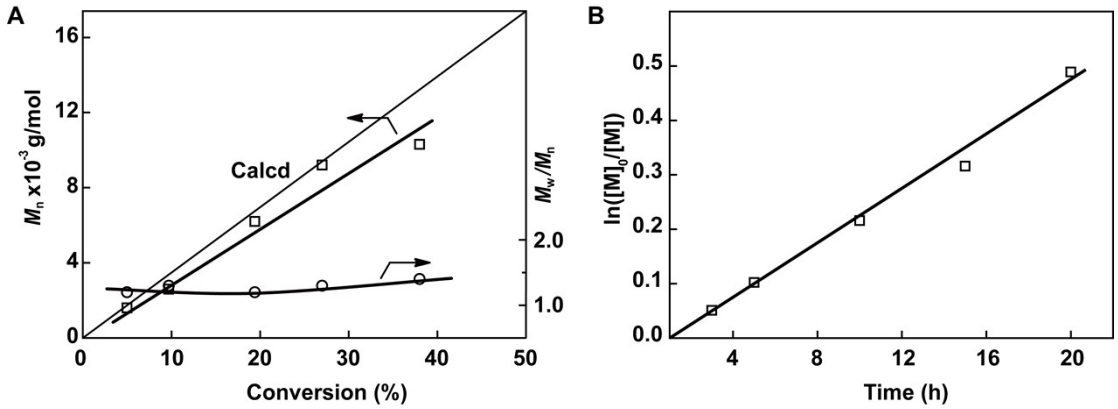


Fig. S2 (A) M_n and M_w/M_n of the PMPVCL *via* MADIX/RAFT polymerization at 65 ^oC in DMF with *O*-ethyl-S-(1-methoxycarbonyl) ethyl dithiocarbonate as CTA initiated by AIBN; [M]/[CTA]/[AIBN]=3 M/20 mM/6 mM. (B) 1st-order kinetic plots for MADIX/RAFT polymerization at 65 ^oC in DMF with *O*-ethyl-S-(1-methoxycarbonyl) ethyl dithiocarbonate as CTA initiated by AIBN; [M]/[CTA]/[AIBN]=3M/20mM/6mM.

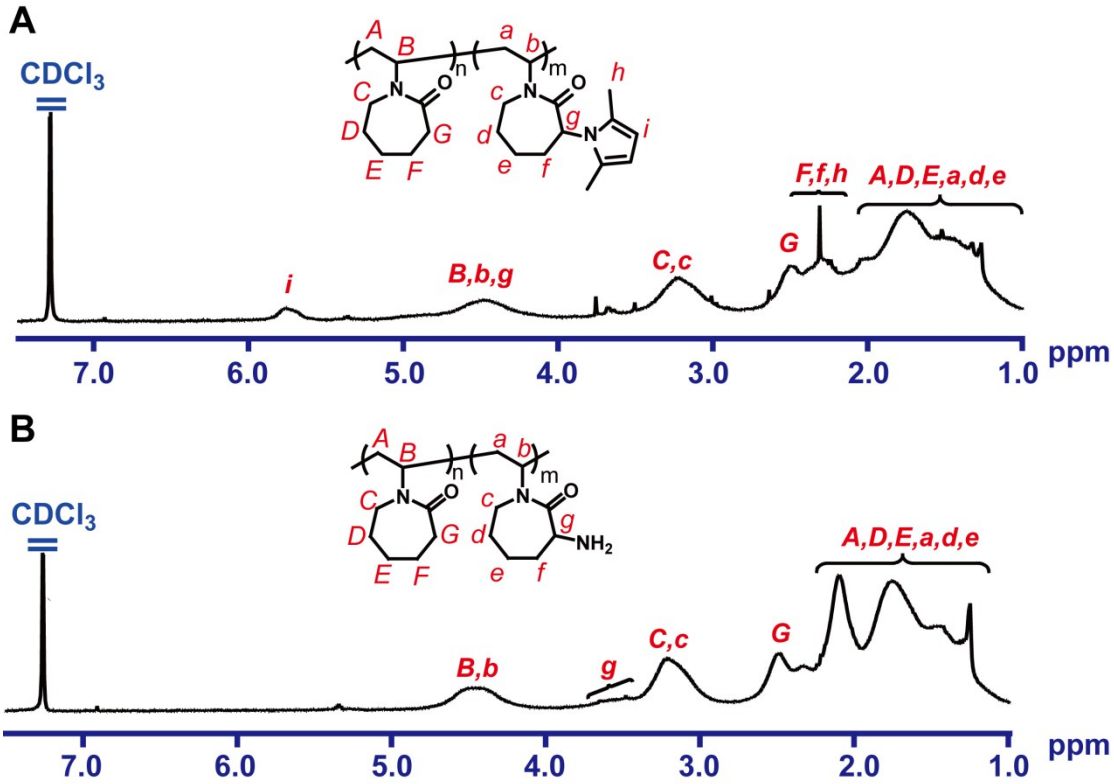


Fig. S3 (A) ^{1}H NMR spectrum of P(VCL-*co*-MPVCL) in $CDCl_3$. (B) ^{1}H NMR spectrum of Amino-PVCL14 in $CDCl_3$.

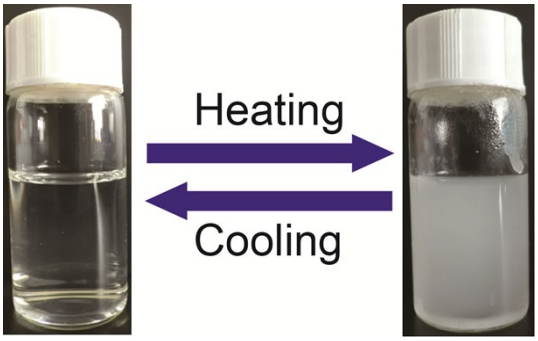


Fig. S4 Visual turbidity change of Amino-PVCL7 upon heating or cooling the aqueous solution.

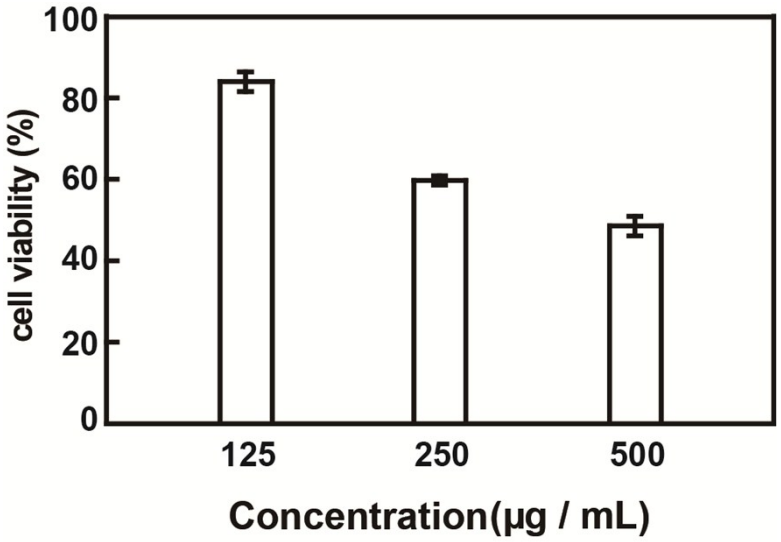


Fig. S5 Viability of L929 cells through the MTT assay for 48 hours incubation with Amino-PVCL100.

Supplementary Tables

Table S1. Solubility of PMPVCL and Amino-PVCL[a]

sample	THF	$CHCl_3$	DMF	EtOAc	MeOH	H_2O
PMPVCL	S	S	S	I	I	I
Deprotected PMPVCL	I	I	S	I	S	S
P(VCL-*co*-MPVCL)	S	S	S	I	S	I
Amino-PVCL20	I	S	S	I	S	S

[a] S= soluble; I= insoluble.

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