Electronic Supplementary Material (ESI) for ChemComm.

Supporting Informtion

Thermo-responsive Recoverable Polymeric Inhibitor for the Resolution of Racemic Amino Acid

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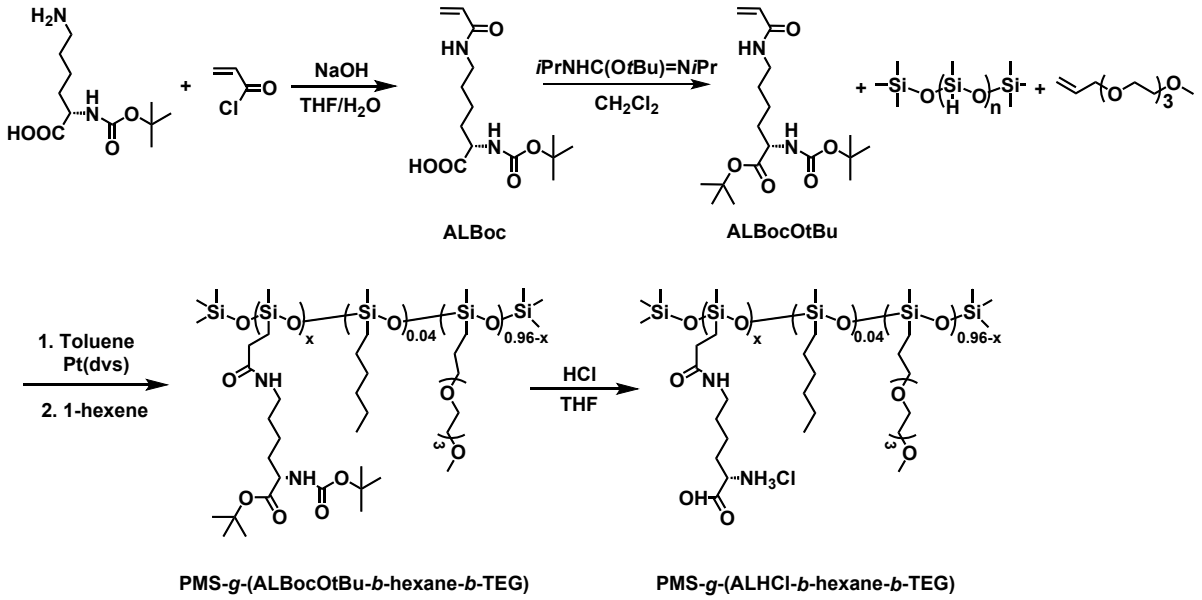
1. Experimental Section

1.1. Synthesis Procedures



Scheme S1. Synthetic route of TRPI-x

1.2. Materials.

Acryloyl chloride (97%, Energy Chemical), N^2-(*tert*-butoxycarbonyl)-*L*-lysine (98%, Energy Chemical), Pt(dvs) (dvs = 1,3-divinyl-1,1,3,3-tetramethyldisiloxane) (Karstedt catalyst, in xylene, Pt, ~2%, Macklin), allyloxy (triethylene glycol) monomethyl ether (allyl-TEG, 98%, Gecko Scientific Inc.), 1-hexene (99%, Aladdin Inc), Polymethylhydrogensiloxane (PMS, 15~40 cps, Bluestar Scientific Inc.), Cuprous chloride (CuCl, AR, Beijing Chemical Co.), *N,N'*-diisopropylcarbodiimide (98%, J & K Scientific Inc), Sodium hydroxide (NaOH, AR, Sinopharm

Chemical Reagent Co., Ltd), hydrochloric acid (36%~38%, Sinopharm Chemical Reagent Co., Ltd), *D*-asparagine monohydrate (*D*-Asn•H_2O), and *L*-asparagine monohydrate (*L*-Asn•H_2O) (98%, Alfa Aesar) were used as purchased. Tert-butanol (*t*-BuOH, AR, Sinopharm Chemical Reagent Co., Ltd) was refluxed with magnesium and distilled, Toluene and tetrahydrofuran (AR, Beijing Chemical Co.) were refluxed with sodium and distilled.

1.3. Measurements.

Fourier-transform infrared (FTIR) spectra were recorded on a PE 100 spectrometer with a disc of KBr. ^{1}H NMR spectra were carried out on a Bruker ARX400 spectrometer at room temperature using TMS as an internal standard. Transmittance was recorded on a Varian Cary 1E UV-Vis spectrometer. The enantiomeric excess (ee%) was obtained by the method of specific optical rotation, ee% =($[\alpha]_{obs}/[\alpha]_{max}$)×100%, c = 1-2 in 5 M HCl, in a 50 mm cell at 25 °C on a JASCO Model P-1030 digital polarimeter. Dynamic light scatter (DLS) measurements were performed on a commercialized spectrometer from Brookhaven Instrument Corporation (BI-200SM Goniometer, Holtsville, NY). A vertically polarized, 100 mW solid-state laser (GXC-III, CNI, Changchun, China) operating at 633 nm was used as the light source, and a BITurboCo digital correlator (Brookhaven Instruments Corp.) was used to collect and process data. The samples were filtered through 450 nm filters. TEM images were obtained on a JEM-2100 (JEOL, Japan) transmission electron microscopy operated at 200 KV. The samples were prepared by dipping a drop of solution onto copper grids coated with amorphous carbon membranes and then drying in air.

1.4. Synthesis of N^6-acryloyl-N^2-(tert-butoxycarbonyl)-*L*-lysine (ALBoc)

N^2-(tert-butoxycarbonyl)-*L*-lysine (8.0 g, 32.5 mmol) was dissolved in 55 mL water. The solution of the acryloyl chloride (3.5 g, 39.0 mmol) in 37 mL dry THF was added dropwise into the N^2-(tert-butoxycarbonyl)-*L*-lysine solution at 0 ℃ with vigorous stirring, and keeping the *p*H close to 9 by gradual addition of 1 M NaOH aqueous solution. Kept stirring at room temperature for 1 day, followed by washing with 3 ×150 mL portions of diethyl ether. The aqueous solution was acidified with 0.5 M HCl until pH close to 3. Then the mixture was extracted with 3 × 150 mL portions of ethyl acetate. The organic layer was collected and dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, the crude product was purified further by column chromatography (silica gel, dichloromethane/methanol (20/1, v/v) as eluent) and recrystallized in CH_2Cl_2 to give 7.9 g of white solids. Yield: 78%.

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.44 (m, 11H; -C*(CH$_3$)$_3$* & -CH_2CH_2*CH_2*CH_2CH-), 1.55-1.62 (m, 2H; -CH_2*CH_2*CH_2CH_2CH-), 1.86-2.05 (m, 2H; -$CH_2CH_2CH_2$*CH_2*CH-), 3.33 (m, 2H; -*CH_2*$CH_2CH_2CH_2CH$-), 4.25-4.30 (m, 1H; -$CH_2CH_2CH_2CH_2$*CH*-), 5.35-5.40 (m, 1H; NH), 5.63-5.69 (d, 1H; vinyl), 6.10-6.25 (m, 1H; vinyl), 6.25-6.30 (d, 1H; vinyl), 6.46-6.50 (m, 1H; NH), 9.94 (broad, 1H; -COOH).

1.5. Synthesis of 2-Tert-Butyl-1,3-Diisopropylisourea

The isourea was prepared according to literature:[1] CuCl (63.4 mg, 0.6 mmol) was added into *N,N'*-diisopropylcarbodiimide (10.0 mL, 63.9 mmol). The solution was cooled to 0 ℃, then *t*-BuOH (7.0 mL, 73.5 mmol) was added to the mixture dropwise in 30 min. The reaction mixture

was stirred for another one hour at 0 ℃, then gradually heated to 25 ℃ and stirred for 20 h. After being distilled under reduced pressure (80 ℃, 25 mmHg) the title compound was obtained as a colorless oil (9.6 g, 75%).

^{1}H NMR(400 MHz, $CDCl_3$, δ, ppm): 3.80-3.58 (m, 1H, CH), 3.31-2.97 (m, 1H, CH), 1.44 (s, 9H, tBu), 1.19-1.01 (12H, m, Me2×2)

1.6. Synthesis of tert-butyl *N*^{6}-acryloyl-*N*^{2}-(tert-butoxycarbonyl)-*L*-lysinate (ALBocO*t*Bu)

Isourea (2.4 g, 12.0 mmol) was added to a 0 ℃ solution of ALBoc (2 g, 8.1 mmol) in CH_2Cl_2 (10 mL). After stirring for 24 h, an additional amount of 2-*tert-butyl*-1,3-diisopropylisourea (2.4 g, 12.0 mmol) was added at room temperature. After stirring for 24 h, the mixture was filtered through a short column of silica gel eluting with ethyl acetate (EtOAc). The product was purified by flash column (silica gel, dichloromethane/ ethyl acetate (10/1, v/v) as eluent) as a colorless viscous liquid (1.8 g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.45 (s, 9H; -NHOO-C*(CH$_3$)$_3$*), 1.46 (s, 9H; -COO-C*(CH$_3$)$_3$*), 1.50-2.05 (m, 11H; -CH_2*CH$_2$CH$_2$CH$_2$*CH-), 3.34 (m, 2H; -*CH$_2$*$CH_2CH_2CH_2$CH-), 4.15 (m, 1H; -$CH_2CH_2CH_2CH_2$*CH*-), 5.09 (broad, 1H; NH), 5.62-5.66 (d, 1H; vinyl), 5.85 (broad, 1H; NH),6.10-6.25 (m, 1H; vinyl), 6.25-6.30 (d, 1H; vinyl).

1.7. Synthesis of PMS graft [tert-butyl *N*^{6}-acryloyl-*N*^{2}-(tert-butoxycarbonyl)-*L*-lysinate-*b*-hexane-*b*-tri(ethylene glycol)] [TRPIBocOtBu]

Taking PMS-g-(ALBocO*t*Bu$_{0.05}$-*b*-hexane-*b*-TEG) as an example, the typical procedure was presented as follows: PMS (6.1 g, 100.0 mmol) was added into a pre-dried three-neck flask

equipped with a condenser under N_2. ALBocO*t*Bu (1.8 g, 5.2 mmol) and allyl-TEG (15.5 g, 75.9 mol) were charged into the flask. 200 mL anhydrous toluene was added and the solution was stirred for 15 min, then cooled to 0 ℃. 1.2 mL Pt(dvs) xylene solution (0.2_{wt}%) was added dropwise in to the mixture. The reaction mixture was gradually heated to 25 ℃. After stirring for 8 h, an additional amount of allyl-TEG (10 g, 49.0 mmol) was added to the mixture. After stirring for 24 h, 1-hexene (14 mL, 240 mmol) was added to the mixture. Keep stirring until the Si-H signal was disappeared confirmed by ^{1}H NMR.

The mixture was condensed by a rotovap and the residue was dissolved in 20 mL CH_2Cl_2 and precipitated in 250 mL hexane for 3 times. Afterwards, the product was dried in vacuum at 50 ℃ for 24 hours. 10.0 g viscous liquid was obtained, Yield: 40%.

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0-0.35 (-*CH*$_3$, in PMS), 0.38-0.61 (-*CH*$_2$-CH_2-CH_2-$(OCH_2CH_2)_3$-OCH_3), 0.80-0.91 (-*CH*$_2$-CH_2-CH_2-CH_2-CH_2-CH_3), 0.95-1.06 (-*CH*$_2$-CH_2-CONH-), 1.14-1.27 (-CH_2-CH_2-CH_2-CH_2-CH_2-*CH*$_3$), 1.35-1.48 (-$C(CH_3)_3$), 1.47-1.74 (-CH_2-*CH*$_2$-CH_2-$(OCH_2CH_2)_3$-OCH_3, -CH_2-*CH*$_2$-*CH*$_2$-*CH*$_2$-*CH*$_2$-CH_3, and -*CH*$_2$- in lys), 3.25-3.37 (--*CH*$_3$ in TEG) and 3.43-3.64 (-*CH*$_2$- in TEG). FTIR (KBr plate, wavenumber, cm^{-1}): 3471, 2918, 2868, 2100, 1957, 1732, 1643, 1452, 1415, 1351, 1198, 1109, 1020, 804, 538.

1.8. Synthesis of PMS graft [*N*^{6}-acryloyl-*L*-lysine•HCl)-*b*-hexane-*b*-tri(ethylene glycol)] [TRPI-x]

A typical process for the preparation was presented as follows: The TRPIBocO*t*Bu obtained in the previous reaction was dissolved into 250 mL THF and stirred for 1 h. 9 mL concentrated

hydrochloric acid (36~38%) was then added into the solution. After stirring for 24 h at room temperature, the most solvent was removed by a rotovap. The residual was diluted by 30 mL deionized water and neutralized to pH≈5.5 with 0.2 M aqueous ammonia. The solution was then transferred into a dialysis bag with a molecular weight cutoff of 3500 and dialyzed against distilled water for 72 h, during which the water was refreshed every 12 h to remove unreacted small molecules. The solution was then filtered over celite and freeze-dried to get the viscous oil. Yield: 9.2 g, 95%.

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0-0.28 (-*CH_3*, in PMS), 0.38-0.65 (-*CH_2*-CH_2-CH_2-$(OCH_2CH_2)_3$-OCH_3), 0.84-0.92 (-*CH_2*-CH_2-CH_2-CH_2-CH_2-CH_3), 0.95-1.04 (-*CH_2*-CH_2-CONH-), 1.22-1.36 (-CH_2-CH_2-CH_2-CH_2-CH_2-*CH_3*), 1.51-1.75 (-CH_2-*CH_2*-CH_2-$(OCH_2CH_2)_3$-OCH_3, -CH_2-*CH_2*-*CH_2*-*CH_2*-CH_3, and -*CH_2*- in lys), 3.31-3.50 (-*CH_3* in TEG) and 3.51-3.78 (-*CH_2*- in TEG). FTIR (KBr plate, wavenumber, cm^{-1}): 3438, 2939, 2866, 1748, 1638, 1528, 1449, 1391, 1349, 1250, 1202, 1103, 1045, 798, 609, 542.

1.9. Fractional crystallization of racemic asparagine monohydrate (*rac*-Asn•H_2O) and the recycling of TRPI-x

Crystallization experiments were carried out from supersaturated solutions of *rac*-Asn•H_2O containing the PMS-*g*-TEG or PMS-*g*-(ALHCl-*b*-hexane-*b*-TEG). The supersaturated solution (1.0 g of *rac*-Asn•H_2O in 15 mL H_2O) was heated at 50 ℃ until complete dissolution occurred, filtered, cooled to 25 ℃, and then 7 g supersaturated solution was transferred to a hot penicillin bottle, 1.33 g TRPI-5 was added in. The whole solution was cooled down to 25 ℃ and

then kept shaking until be homogeneous. After being left stand at 25 ℃ for 30 min, seeds of *D*-Asn•H_2O were added in. After a period of time, the formed crystals were separated by filtration, washed with ethanol/water (v/v, 50/50) ^{x} 3, and then dried under vacuum.

The filtrate was left stand at 50 ℃ for 30 min for obvious phase separation. After decanting the supernatant, the oil phase was cooled down to 25 ℃. The oil phase was washed with 2 mL deionized water × 3, and then freeze-dried under vacuum.

2. Supplementary Figures and Tables

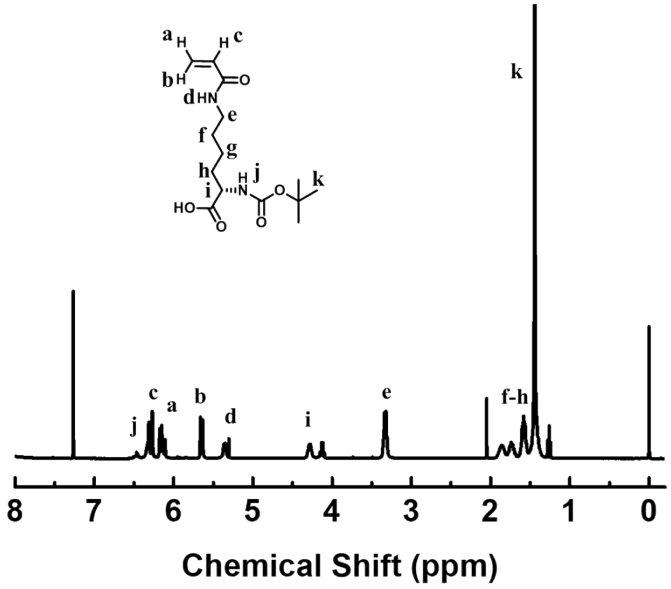


Figure S1 ^{1}H NMR spectrum of ALBoc in $CDCl_3$.

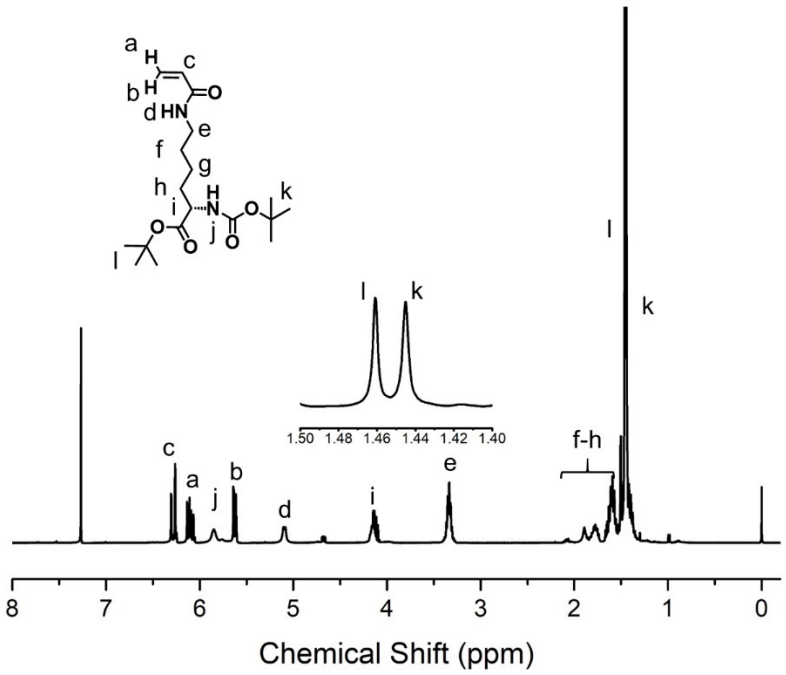


Figure S2 ^{1}H NMR spectrum of ALBocO*t*Bu in $CDCl_3$.

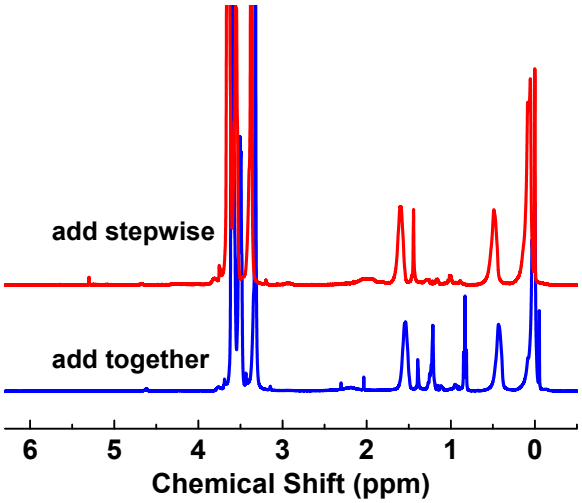


Figure S3 ^{1}H NMR spectrum of TRPI synthesized through gradual feeding method and one-time feeding method in CD_3OD.

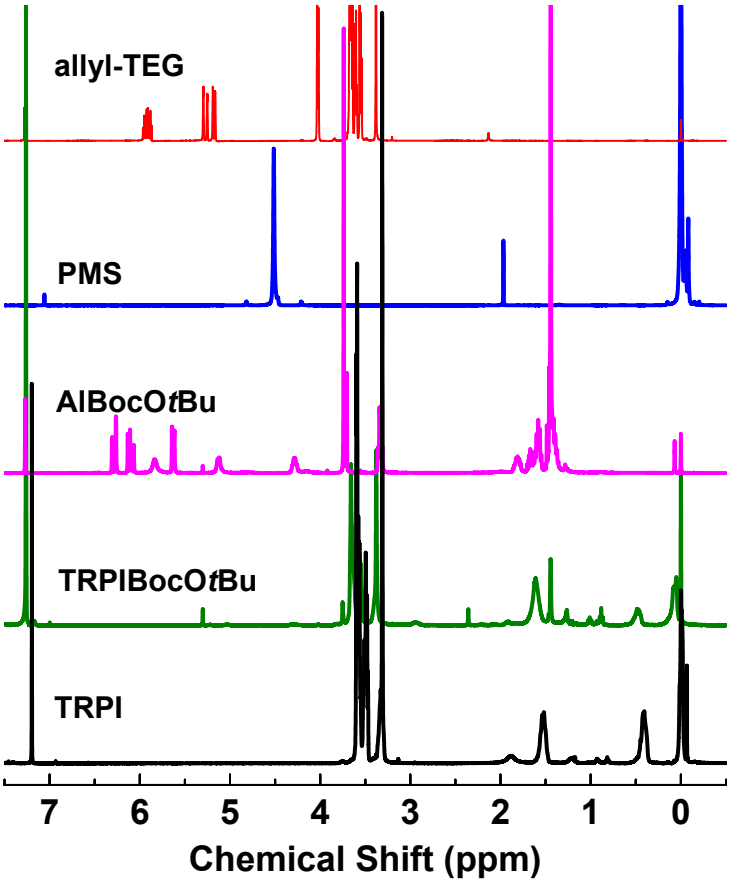


Figure S4 ^{1}H NMR of PMS-*g*-(ALBocO*t*Bu-*b*-hexane-*b*-TEG), ALBocO*t*Bu, PMS and allyl-TEG.

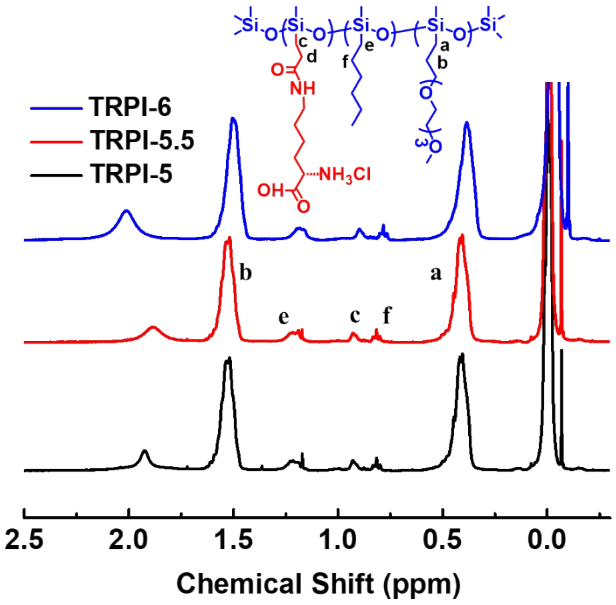


Figure S5 ^{1}H NMR of TRPI-5, TRPI-5.5, and TRPI-6 in CD_3OD.

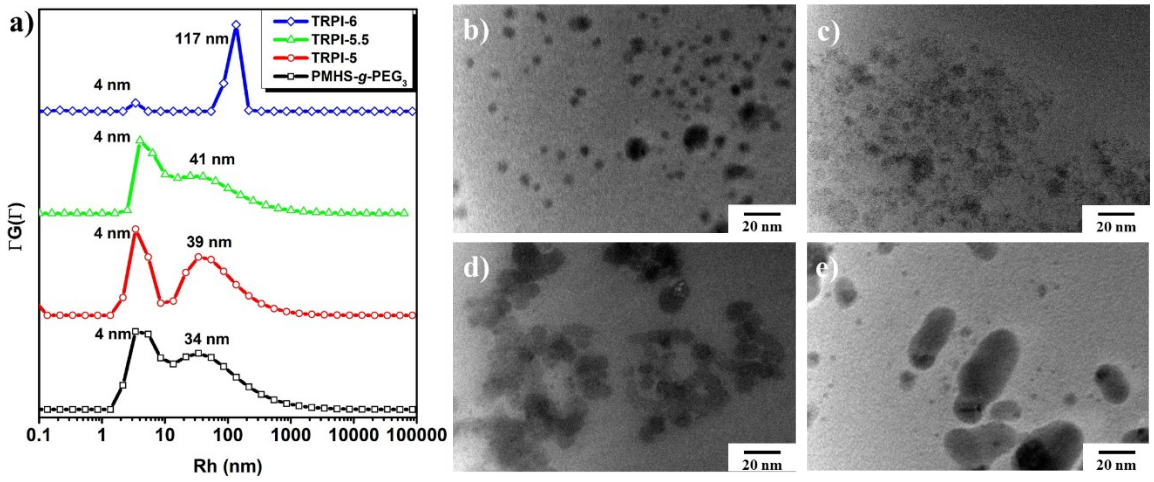


Figure S6 (a) Dynamic light scattering data of TRPI-x and PMS-*g*-TEG, polymer concentration: 0.1 mg•mL^{-1}, Representative TEM images of (b) PMS-*g*-TEG, (b) TRPI-5, (c) TRPI-5.5 and (d) TRPI-6 assemblies.

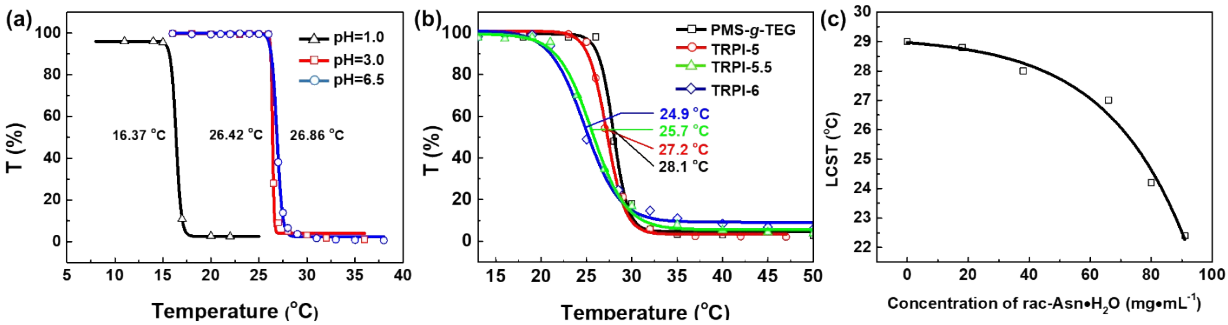


Figure S7 (a) Transmittance versus temperature plots of TRPI-5.5 at different *p*H. Polymer concentration: 1.0 mg•mL^{-1}. (b) Transmittance versus temperature plots of TRPI in 66 mg•mL^{-1} *rac*-Asn aqueous solution. Polymer concentration: 1.0 mg•mL^{-1} (c) The LCST of TRPI-5 versus concentration of *rac*-Asn. Polymer concentration: 1.0 mg•mL^{-1}

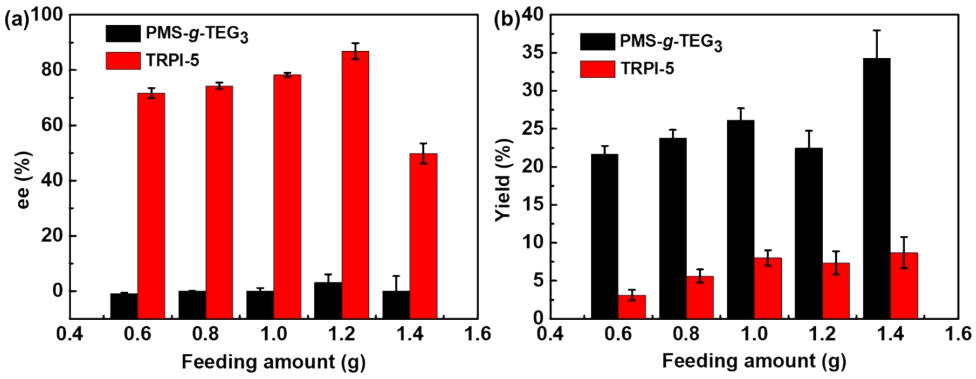


Figure S8 Blank control experiments using TRPI-0 as an additive.

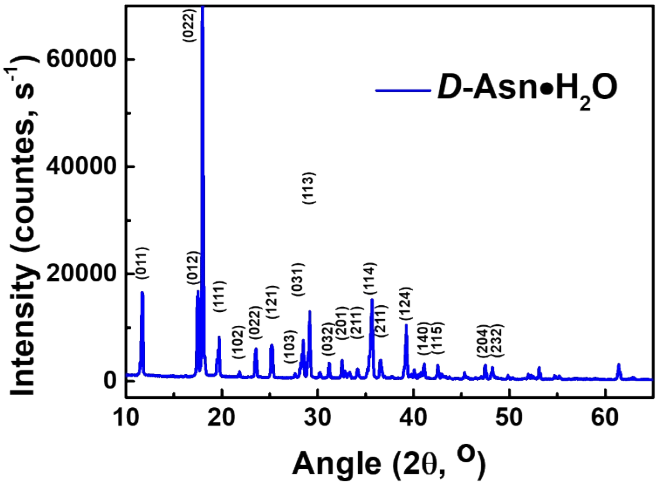


Figure S9 Powder X-ray diffraction of the abstained *D*-Asn•H_2O

Table S1 Effect of TRPI-5 adding amount on the fractional crystallization of *rac*-Asn•H_2O[a]

Weight %[b] of lysine unit	Yield (%)[c]	$[\alpha]_D^{25}$[d]	ee (%)
0	1	-13.3	46.0
6	3	-20.7	71.4
10	7	-22.9	79.1
12	7	-25.7	88.6
14	8	-14.6	50.3
15	8	-20.3	70.1

[a] Concentration of Asn•H_2O, 66 mg•mL^{-1}; Crystallization temperature, 25 °C; Crystallization time, 18 h;

[b] Expressed in weight % of racemic mixture.

[c] The yield is defined as the weight ratio of precipitated crystals / total racemic mixture.

[d] Measured by polarimeter, *c* = 1-2 in 5 *M* HCl, *ee*% = $([\alpha]_{obs}/[\alpha]_{max}) \times 100$; $[\alpha]_{max}$ = –29.0 (*c* = 2, 5 *M* HCl).

Table S2. Effect of polymer adding amount on the fractional crystallization of *rac*-Asn•H_2O[a]

Type of additives	Weight%[b] of lysine unit	Yield (%)[c]	$[\alpha]_D^{25}$[d]	ee (%)
TRPI-5.5	3	26	-10.82	37.30
TRPI-5.5	6	29	-7.77	26.79
TRPI-5.5	9	26	-10.62	36.61
TRPI-6	3	20	-13.92	48.00
TRPI-6	6	39	-1.57	5.42
TRPI-6	9	34	-1.90	6.54

[a] Concentration of Asn•H_2O, 66 mg•mL^{-1}; Crystallization temperature, 25 ℃; Crystallization time, 5 h; Content of ethanol: 30_{wt}%.
[b] Expressed in weight% of racemic mixture.
[c] The yield is defined as the weight ratio of precipitated crystals / total racemic mixture.
[d] Measured by polarimeter, *c* = 1-2 in 5 *M* HCl, *ee*% = ($[\alpha]_{obs}/[\alpha]_{max}$) × 100; $[\alpha]_{max}$ = –29.0 (*c* = 2, 5 *M* HCl).

Reference:

1 T. Sugimura, A. Komatsu, Y. Koseki, T. Usuki, *Tetrahedron Lett.*, 2014, **55**, 6343