

Mussel-inspired fabrication of pH-sensitive biomimetic hydrogel based on greenhouse gas carbon dioxide

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Supporting Information for New Journal of Chemistry

1. Materials

Lysine, N-hydroxysuccinimide(NHS), dopamine hydrochloride (DA-HCl), and 1-ethyl-3-(3(dimethylamino)propyl)carbodiimide hydrochloride (EDC-HCl) were purchased from Aladdin Reagent (Shanghai) Co., Ltd. and used without further purification. Ethyl acetate (EA), tetrahydrofuran (THF), dichloromethane, methanol, triethylamine (TEA), anhydrous diethyl ether, HCl and NaHCO₃ were purchased from Sinopharm Reagent Co., Ltd. All solvents were dehydrated and dried before use.

2. Synthesis of Lysine-Dopamine (LDA)

2.1 Synthesis of (Boc)₂-Lysine (BLB)

Lysine (3.65 g 25 mmol) was dissolved in 150 mL of deionized water in a single-necked flask and tetrahydrofuran (50 ml) was added. NaHCO₃ (10.6 g) and di-t-butyl dicarbonate (Boc anhydride) (16.37 g 75 mmol) was added and stirred. The reaction was going on for 24h at room temperature under a N₂ atmosphere. After that, tetrahydrofuran was removed with a rotary evaporator. Then 1 mol/L HCl was added dropwise to the flask until the viscous material no longer appeared, and the dropwise addition was stopped. The mixture was extracted with ethyl acetate, and the organic phase was combined. The solvent was removed by a rotary evaporator and the product was dried at 60°C for 24 h with 90% yield. ¹H NMR(DMSO-d₆, 400MHz) (δ ppm): 12.35(br.s, 1H, COOH), 6.98(d, 1H, -OCONH-CH<), 6.75(t, 1H, -OCONH-CH₂-), 3.81(m, 1H, -OCONH-CH<), 2.87(m, 2H, -OCONH-CH₂-), 1.66-1.20(m, 24H, -CH₂- and -CH₃).

2.2 Synthesis of (Boc)₂-Lysine-NHS (BLN)

BLB (7.2 g, 20.8 mmol) and dichloromethane (150mL) were added to a one-necked flask. EDC-HCl (9.07 g, 50 mmol): NHS (2.83 g, 24.96 mmol): BLB were added at a molar ratio of 2.4:1.2:1. The mixture was stirred at 0°C for 0.5h. Then the temperature was raised to room temperature and the reaction was going on for 5.5 h under a N₂ atmosphere. Finally, deionized water was used to wash the solution 3

times, and the organic phase was combined and dried with anhydrous MgSO_4 . The solvent was removed with a rotary evaporator to yield light yellow solid. The product was dried at 60°C to give BLN (80%). ^1H NMR(DMSO- d_6 , 400MHz) (δ ppm): 7.55(d, 1H, $-\text{OCONH}-\underline{\text{CH}}<$), 6.78(t, 1H, $-\text{OCONH}-\underline{\text{CH}}_2-$), 4.27(m, 1H, $-\text{OCONH}<$), 2.96-2.65(m, 6H, $-\text{N}(\text{CO}-\text{CH}_2)_2$ and $-\text{OCONH}-\underline{\text{CH}}_2-$), 1.85-1.05(m, 24H, $-\text{CH}_2-$ and $-\text{CH}_3$).

2.3 Synthesis of (Boc)₂-Lysine-Dopamine (BLDA)

BLN (2.4 g, 5.31 mmol) was dissolved in anhydrous methanol (125 mL) and then added with DA-HCl (1.53 g 7.96 mmol) and triethylamine (Et_3N) (1.15 mL). The molar ratio of Et_3N : DA-HCl: BLN is 1.3: 1.5: 1. The mixture was stirred at room temperature for 12h under a N_2 atmosphere. The solvent and Et_3N were removed with a rotary evaporator. Ethyl acetate was added to dissolve and washed with deionized water. Combined organic phase and solvent was removed with a rotary evaporation, then product was dried by vacuum at 60°C for 24 h to give the BLDA(70%). ^1H NMR(DMSO- d_6 , 400MHz) (δ ppm): 8.90-8.60(d, 2H, $-\text{Ar}(\text{OH})_2$), 7.75(t, 1H, $-\text{CONH}-\underline{\text{CH}}_2-$), 6.90-6.65(m, 3H, $-\text{OCONH}-\text{CH}<$ and $-\text{OCONH}-\underline{\text{CH}}_2-$), 6.64-6.30(m, 3H, $-\text{Ar}$), 3.80(m, 1H, $-\text{OCONH}-\underline{\text{CH}}<$), 3.28-3.04(m, 4H, $-\text{CONH}-\underline{\text{CH}}_2-$ and $-\text{OCONH}-\underline{\text{CH}}_2-$), 2.85(m, 2H, $-\text{CONH}-\text{CH}_2-\underline{\text{CH}}_2-$), 1.80-1.20(m, 24H, $-\text{CH}_2-$ and $-\text{CH}_3$).

2.4 Synthesis of lysine-dopamine (LDA)

BLDA (2.5g) was dissolved in ethyl acetate (100mL), HCl/ethyl acetate saturated solution (50 mL) was added, and the mixture was stirred at room temperature for 3h under a N_2 atmosphere. The solvent was removed by rotary evaporation. The product was dried under vacuum at 60°C for 24 h, and a white LDA solid was obtained with 96% yield (2.4g). ^1H NMR(DMSO- d_6 , 400MHz) (δ ppm): 8.80-8.60(d, 2H, $-\text{Ar}(\text{OH})_2$), 8.46(t, 1H, $-\text{CONH}-\underline{\text{CH}}_2$), 8.24(s, 2H, $>\text{CH}-\underline{\text{NH}}_2$), 8.01(s, 2H, $-\text{CH}_2-\underline{\text{NH}}_2$), 6.67-6.40(m, 3H, $-\text{Ar}$), 3.68(m, 1H, $>\underline{\text{CH}}-\text{NH}_2$), 3.32-3.10(m, 2H, $-\text{CONH}-\underline{\text{CH}}_2-$), 2.73(m, 2H, $-\underline{\text{CH}}_2-\text{NH}_2$), 2.56(m, 2H, $-\text{CONH}-\text{CH}_2-\underline{\text{CH}}_2-$), 1.85-1.05(m, 6H, $-\text{CH}_2-$).

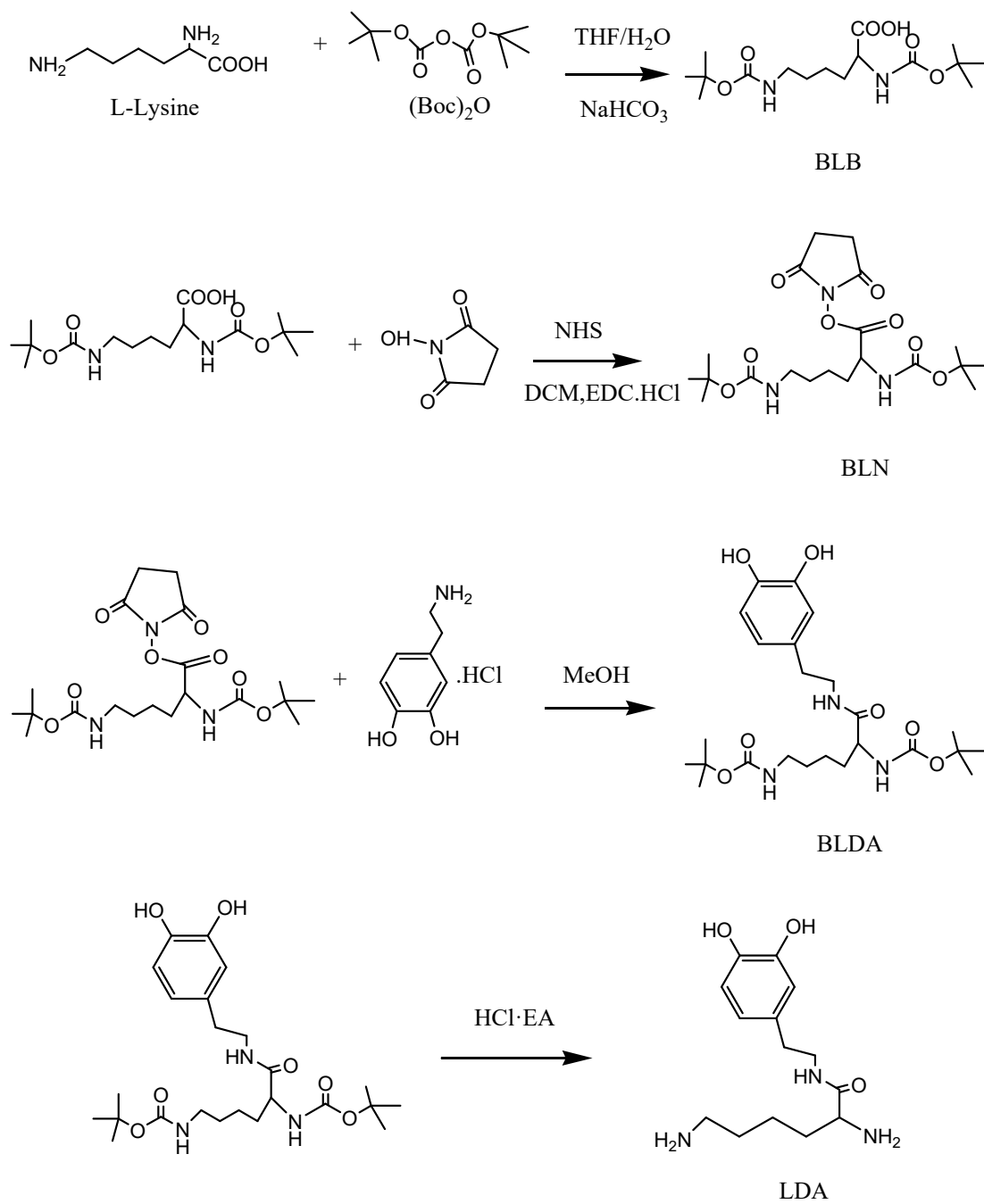
3. Characterization of PPC-diol

In order to obtain an oligomeric poly(propylene carbonate) (PPC) diol, the effect

of the EG/Cat molar ratio on the copolymerization reaction was investigated. Through a series of experiments with different EG/Cat ratios, the results were shown in Table S1. When the mole ratio of EG/Cat was from 10:1 to 800:1, these M_n were measured to be from 16321 to 979 g/mol by GPC measurement. In Figure S5, the ^1H NMR spectra also confirmed the copolymerization results of the three PPC-diols. It can be seen that the single peak at 4.90-5.10 ppm was the proton of -CH- in the PPC segment, and the triplet peaks at 4.10-4.30 ppm were the proton of the -CH₂- group. The peak at 3.30-3.70 ppm was the proton of the end -OH group. The doublet peaks at 1.3 ppm were protons of -CH₃ in the PPC segment. Comparing the ^1H NMR of the three PPC-diols, the proportion of the diol block can also be judged from the -OH peak strength. When M_n was 3260 g/mol, the proportion of diol block was the lowest. When M_n was 979 g/mol, the ratio of the diol block was the highest. The result was coincident with that of GPC.

4. MTT assay

The cytotoxicity of PPC-PU-LDA was studied by MTT assay. (PPC-PU-LDA uses PPC-PU-LDA1) HeLa cells (105 cell/mL, 10 mL) were dispersed with 96-well microtiter plates to a total volume of 100 μL /well. Plates were maintained at 37 °C in 5% CO₂/95% air incubator for 24 h. The cells were incubated for 24 h with different concentrations of PPC-PU-LDA (5, 10, 15, 20, 25, 30 μm) in the medium at 37 °C. Then, 10 μL MTT (5 mg/mL) solution was added into each well and incubated for additional 4 h. After removal of supernatant and addition of 100 μL of DMSO into each well, the cells was shaken for 10 min and the absorbance in each well was measured at 492 nm using a microplate reader (Biotek, USA). The cell viability (%) was calculated according to the equation: cell viability % = $A/B \times 100\%$, where A represents the absorbance of each well treated with PPC-PU-LDA, and B represents that of the control wells. At the same time, another group of MTT assay were performed using the same experimental method using the complex of PPC-PU-LDA with Fe³⁺ at six concentrations (5, 10, 15, 20, 25, 30 μm) .



Scheme S1 Synthesis of lysine - dopamine (LDA)

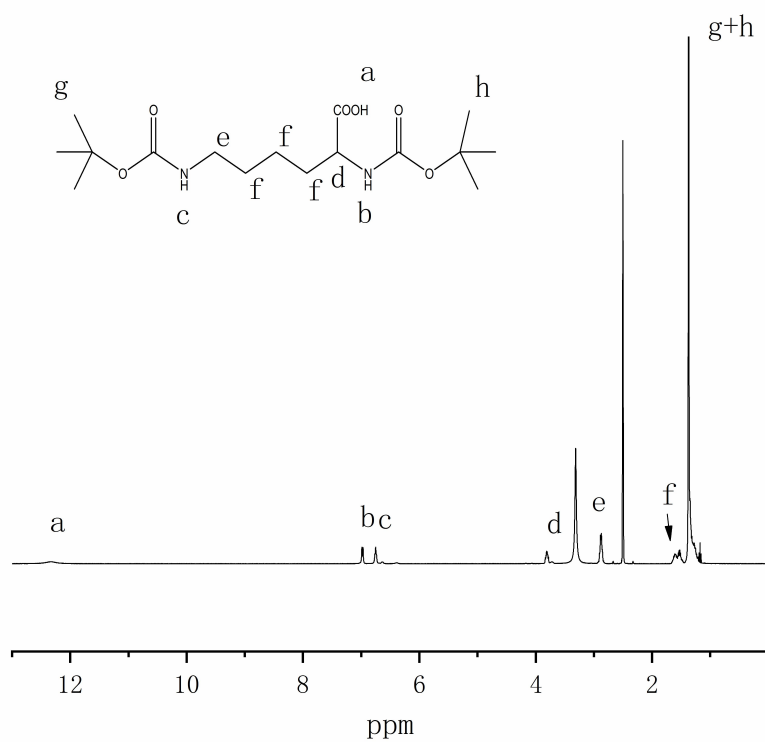


Fig. S1 ^1H NMR spectrum of the BLB in DMSO-d_6 .

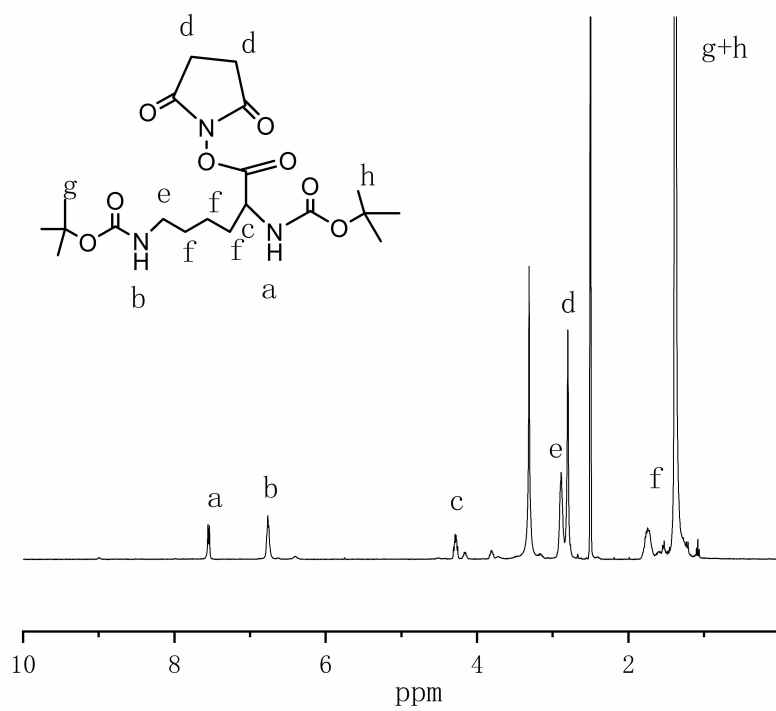


Fig. S2 ^1H NMR spectrum of the BLN in DMSO-d_6 .

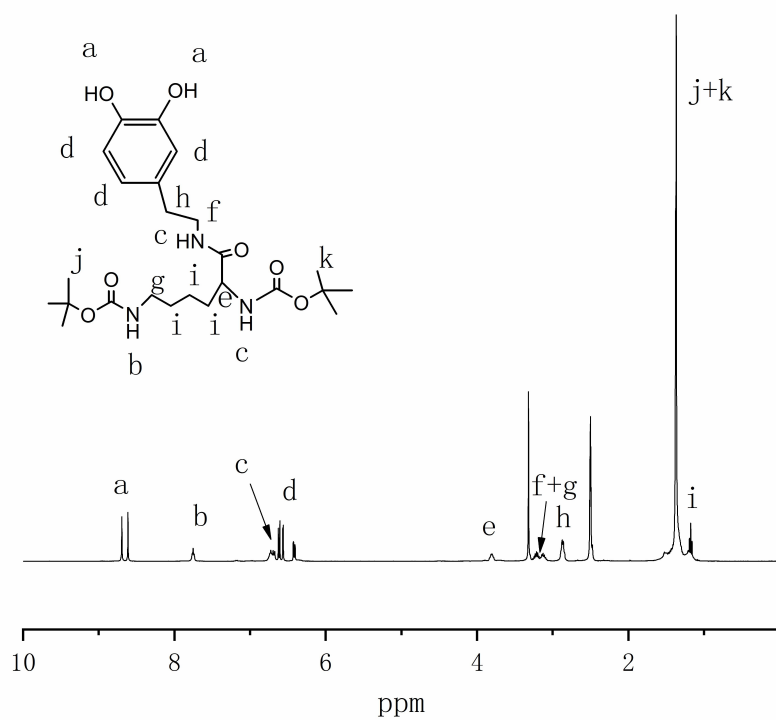


Fig. S3 ¹H NMR spectrum of the BLDA in DMSO-d₆.

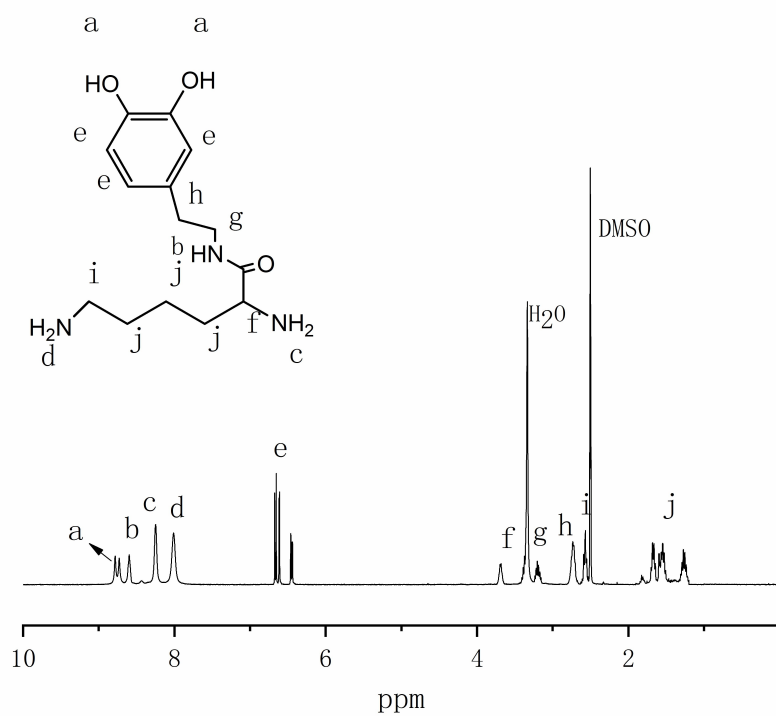


Fig. S4 ¹H NMR spectrum of the LDA in DMSO-d₆.

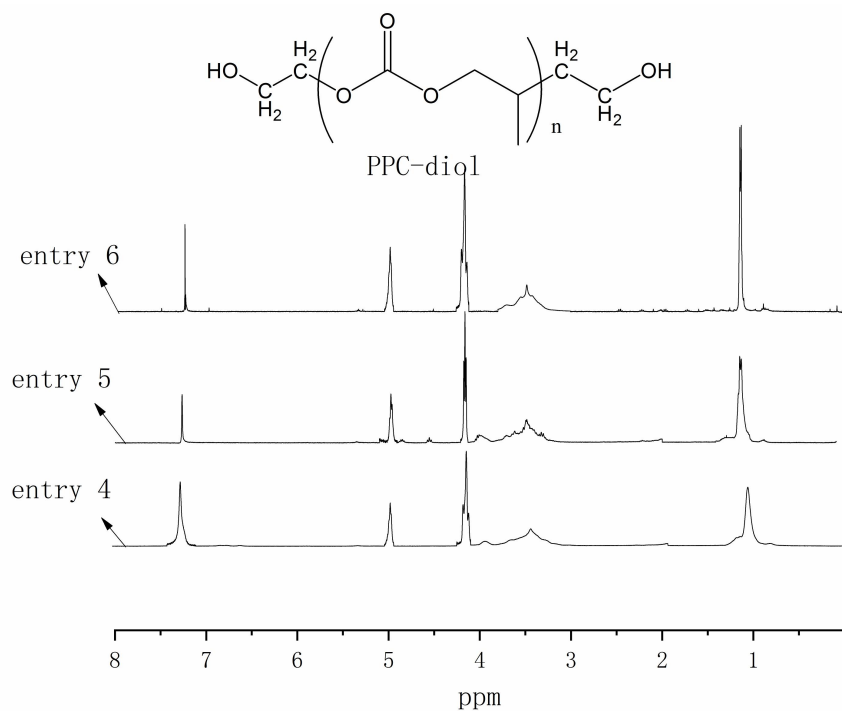


Fig. S5 ^1H NMR spectrum of the different molecular weight PPC-diols in CDCl_3 .

Table S1 Copolymerization results of PPC-diols formed by different EG contents.^a

Entry	EG/Cat (molar ratio)	PO Conv. ^b (%)	yield(g)	M_n^c (g/mol)	M_w/M_n
1	10:1	14.8	2.6	16321	1.4
2	20:1	13.7	2.4	13139	1.2
3	100:1	14.3	2.5	8699	1.3
4	400:1	12.0	2.1	3260	1.3
5	600:1	11.5	1.9	1718	1.9
6	800:1	12.8	2.2	979	1.2

a: Copolymerization conditions: PO(10.0 g, 172 mmol), Cat(9.4 mg, 6.8 μmol), [PO]/Cat=25000, CO_2 (2 MPa), bath temperature of 80 $^\circ\text{C}$.

b: 100*(Mole of the reacted PO)/(mole of the charged PO).

c: Determined by GPC using a polystyrene standard.