The First CD73-Instructed Supramolecular Hydrogel

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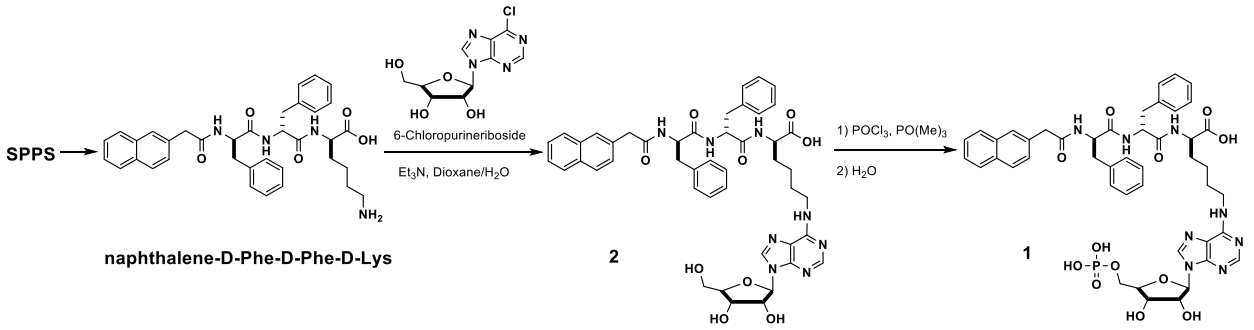
Supplementary material

Experimental Section

All of the chemical reagents and solvents were used as received from commercial sources without further purification, unless otherwise noted. Hydrogen and phosphate nuclear magnetic resonance spectra were recorded on a Varian Unity Inova 400 with DMSO as solvent. Data are reported as follows: chemical shift δ, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad), coupling constant in Hz, integration, and assignment. LC-MS was performed on a Waters Acouity ultra Performance LC with Waters MICRO-MASS detector. Transmission electron micrographs were obtained on Morgagni 268 transmission electron microscope.

Synthesis and Characterization



Scheme S1. The synthetic route for making precursor **1** and hydrogelator **2**.

Solid phase peptide synthesis (SPPS) of naphthalene-D-Phe-D-Phe-D-Lys. After 2-chlorotrityl chloride resin (100~200 mesh and about 1 mmol/g) was put in the SPPS reactor and bubbled in dry dichloromethane (DCM) using nitrogen gas (N_2) for 30 minutes, the resin swelled and was washed 3 times with dry *N*,*N*-dimethylformamide (DMF). Then a solution of Fmoc-D-Lys (Boc)-OH (D-Lys, 2 equiv.) and *N*, *N*-diisopropylethylamine (DIPEA 5 equiv.) in DMF was added to the reactor and bubbled with resin (using N_2) for 1 hour. After washing with DMF (5 times), the resin was bubbled with the blocking solution (DCM/MeOH/DIPEA = 8/1.5/0.5, 10 minutes × 2 times) to inactivate the unreacted sites. Following washing with DMF (3 times), the resin was treated with 20% piperidine (in DMF) for 0.5 hour to remove the Fmoc-protecting group and free the N-terminus. The resin was washed with DMF (5 times) again, a solution of Fmoc-D-Phe-OH (D-Phe, 2 equiv.), *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU, 2 equiv.), and DIPEA (5 equiv.) in DMF was added to the reactor and reacted with the resin for 1

hour using N_2 bubbling. After washing with DMF (5 times), 20% piperidine (in DMF) was added to remove the Fmoc-protecting group for 0.5 hour. The resin was washed with DMF for 5 times, and a solution of Fmoc-D-Phe-OH (2 equiv.), HBTU (2 equiv.) and DIPEA (5 equiv.) in DMF was added to react with the resin for 1 hour. After washing the resin with DMF (5 times), 20% piperidine (in DMF) was added again to remove Fmoc group. The resin was washed with DMF (5 times), and a solution of 2-(naphthalen-2-yl) acetic acid (2 equiv.), HBTU (2 equiv.), and DIPEA (5 equiv.) in DMF was added to the reactor and bubbled using N_2 for 1 hour. For the last step, the resin was washed with DMF (5 times), DCM (5 times), MeOH (5 times) and hexane (5 times) successively, and then the conjugate was cleaved using TFA/H_2O (95/5) for 3 hours and the resulting crude product was purified by reverse phase HPLC to afford the pure peptide naphthalene-D-Phe-D-Phe-D-Lys.

Synthesis of hydrogelator 2. 6-Chloropurineriboside (1.5 mmol), naphthalene-D-Phe-D-Phe-D-Lys (0.5 mmol) and triethylamine (1 mmol) were added to a mixed solvent of dioxane (20 mL) and water (20 mL) to form a suspension. The suspension was stirred overnight at room temperature to give a clear solution, which was evaporated by blowing dry nitrogen. Finally, the residue was purified by column chromatography (silica gel: methanol/chloroform) to give **2**. **2**, white powder; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.43 (s, 1H), 8.27 (s, 1H), 8.22 (d, J = 8.0 Hz, 2H), 8.09 (d, J = 8.4 Hz, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.54 (s, 1H), 7.44-7.38 (m, 3H), 7.22-7.08 (m, 10H), 5.87 (d, J = 5.6 Hz, 1H), 4.56-4.46 (m, 3H), 4.18-4.10 (m, 2H), 3.94 (d, J = 3.2 Hz, 1H), 3.64 (dd, J = 12.4 and 3.6 Hz, 1H), 3.55-3.43 (m, 5H), 3.04-2.90 (m, 2H), 2.80-2.64 (m, 2H), 1.77-1.59 (m, 4H), 1.37-1.33 (m, 2H) ppm; MS: calc. M^+ = 858.37, obsvd. $(M-1)^-$ = 857.50.

Synthesis of precursor 1. 2 (0.2mmol) was suspended in 1 mL of trimethylphosphate at 0 ^{o}C. Phosphorus oxychloride (0.6 mmol) was added and the suspension was stirred at 0 ^{o}C for 8 h. When the suspension became a clear solution, water was added to hydrolyze the 5′- phosphoryl chloride to 5′-monphosphate. The solution was then neutralized by saturated ammonium carbonate at 0 ^{o}C. The crude products were purified by semi-preparative HPLC. **1**, white powder; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.42 (s, 1H), 8.29-8.25 (m, 3H), 8.13 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.53 (s, 1H), 7.44-7.38 (m, 3H), 7.20-7.10 (m, 10H), 5.92 (d, J = 4.4 Hz, 1H), 4.63-4.48 (m, 3H), 4.21-3.92 (m, 4H), 3.66-3.42 (m, 5H), 3.08-2.90 (m, 2H), 2.80-2.63 (m, 2H), 1.75-1.59 (m, 4H), 1.37-1.34 (m, 2H) ppm; MS: calc. M^+ = 938.34, obsvd. $(M-1)^-$ = 937.45.

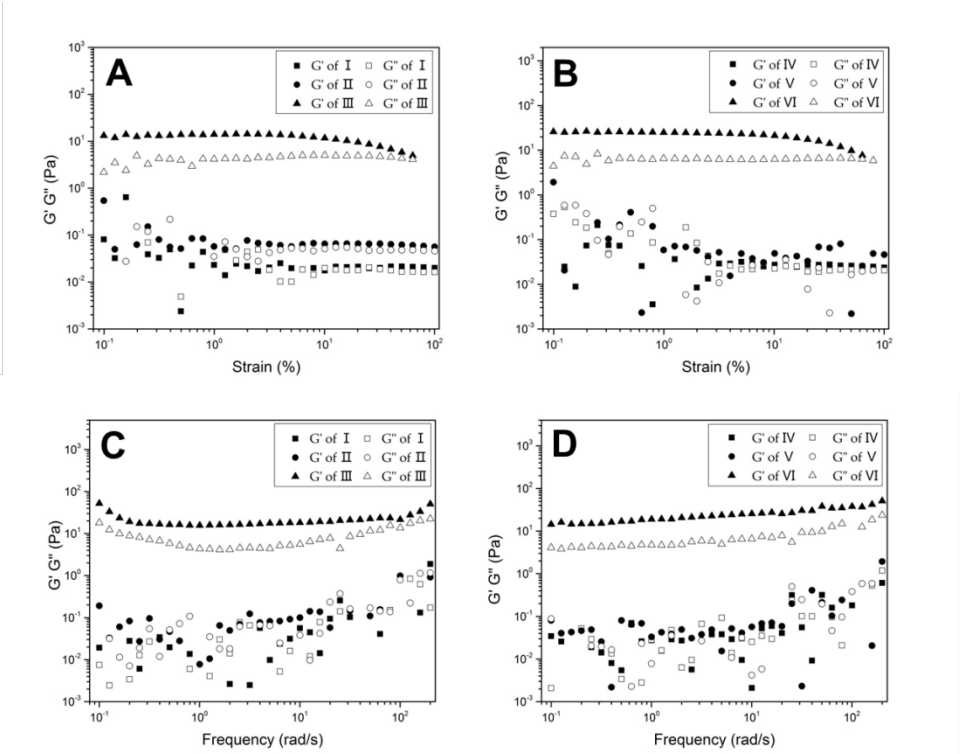
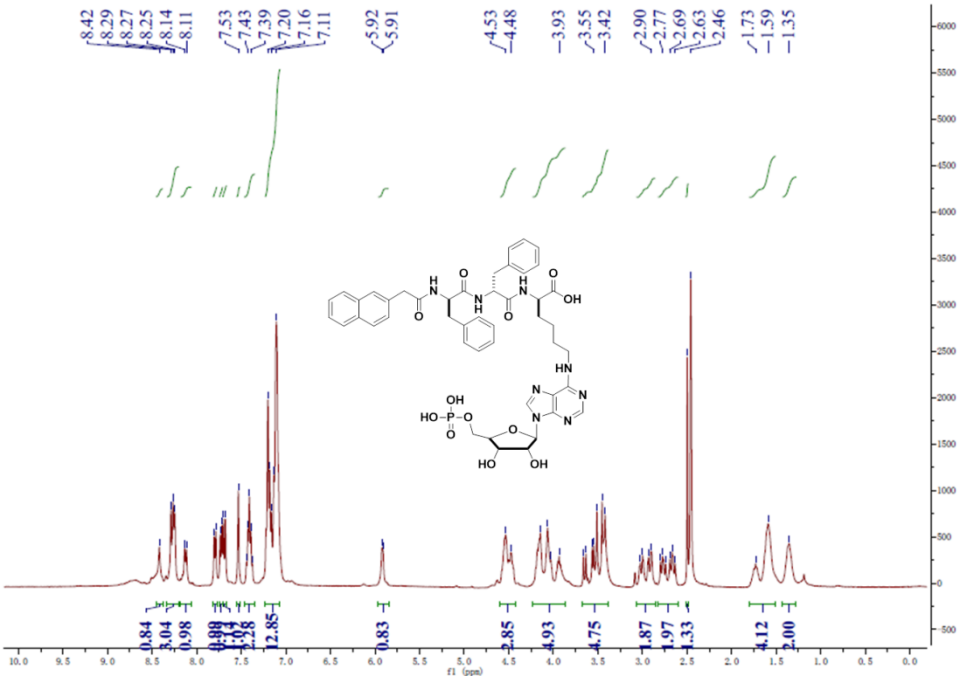
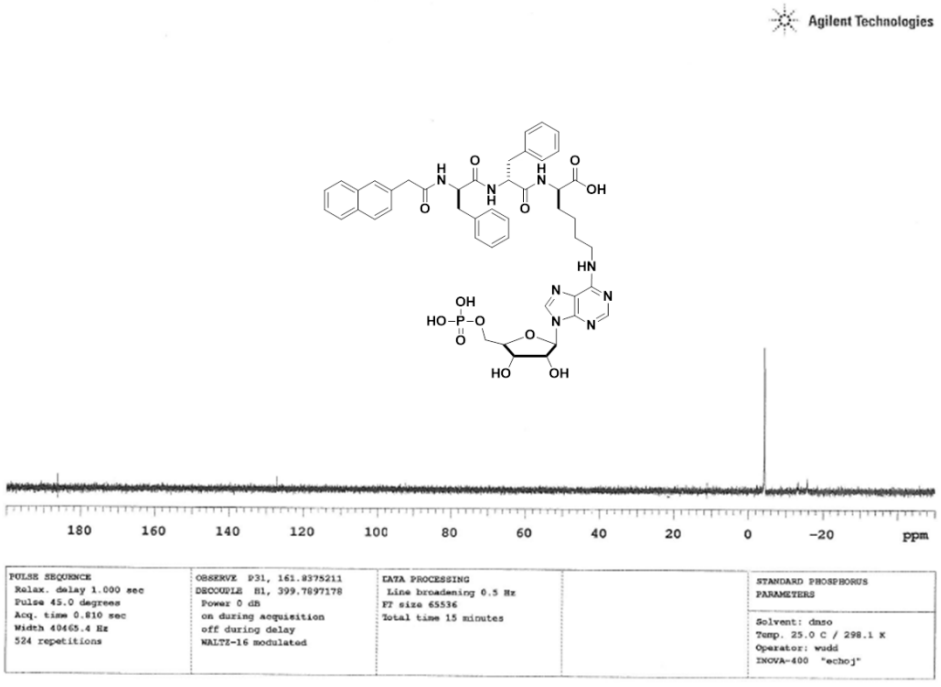


Figure S1. Strain dependence (A and B) and frequency dependence (C and D) of the dynamic storage moduli (G′) and loss moduli (G″) of (Ⅰ) the solution of 0.5 wt% of **2**, (Ⅱ) the solution of 1 wt% **2**, (Ⅲ) the hydrogel of 2 wt% of **2**, (Ⅳ) the CD73-treated solution of 0.5 wt% of **1**, (Ⅴ) the CD73-treated solution of 1 wt% of **1**, and (Ⅵ) the CD73-instructed enzymatic hydrogel of 2 wt% of **1**. All samples are at pH 7.0.

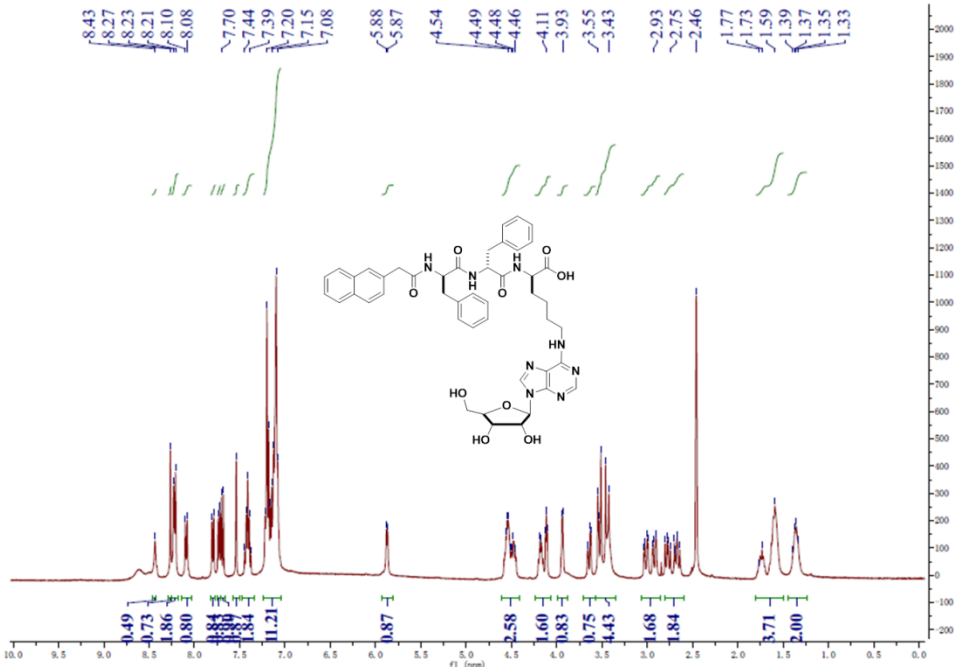
^{1}H NMR of 1



^{31}P NMR of 1



^{1}H NMR of 2


8.43
8.27
8.23
8.21
8.10
8.08
7.70
7.44
7.39
7.20
7.15
7.08
5.88
5.87
4.54
4.49
4.48
4.46
4.11
3.93
3.55
3.43
2.93
2.75
2.46
1.77
1.73
1.59
1.39
1.37
1.35
1.33
0.49
0.73
1.86
0.80
11.21
0.87
2.58
1.60
0.83
0.75
4.43
1.68
1.84
3.71
2.00
f1 (ppm)