

A naphthalene-containing amino acid enables hydrogelation of a conjugate of nucleobase-saccharide-amino acids

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Supporting Information

Experimental Section

All of the chemical reagents and solvents were used as received from the commercial sources without further purification unless otherwise noted. Hydrogen 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 Acuity ultra Performance LC with Waters MICRO-MASS detector. Transmission electron micrographs were obtained on Morgagni 268 transmission electron microscope.

Synthesis and Characterizations

The *o*-diol-protected adenosine acid derivative **5** was prepared according to the reported method.¹

Solid-phase peptide synthesis (SPPS) of conjugate 1. After 2-chlorotriptyl chloride resin (100~200 mesh and about 1 mmol/g) was put in the SPPS reactor and bubbled in dry dichloromethane (DCM) by nitrogen gas (N₂) for 30 minutes, the resin swelled and was washed with dry *N,N*-dimethylformamide (DMF) (3 times). Then the solution of *N*-Fmoc-L-phenylalanine (L-Phe, 2 equiv.) and *N,N*-diisopropylethylamine (DIPEA 5equiv.) in DMF was added to the reactor and bubbled with resin by N₂ for 1 hour. After washed with DMF (5 times), the resin was bubbled with the blocking solution (DCM/MeOH/DIPEA = 8/1.5/0.5, 10 minutes \times 2) to inactivate the unreacted sites. Followed by 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 make the amine group free. The resin was washed with DMF (5 times) again, the solution of L-Phe (2 equiv.), *O*-benzotriazole-*N,N,N',N'*-tetramethyluronium-hexafluoro-phosphate (HBTU, 2 equiv.), and DIPEA (5 equiv.) in DMF was added to the reactor and reacted with the resin for 1 hour by N₂ bubbling. After washed with DMF (5 times), the 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. Then the solution of *o*-diol-protected adenosine acid derivative **5** (2 equiv.), HBTU (2 equiv.) and DIPEA (5 equiv.) in DMF was added to react with the resin for 4 hours. At the final 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 with TFA/H₂O (95/5) for 3 hours and the resulted crude product was

purified by reverse phase HPLC to afford the pure conjugate **1**.

1, white powder; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 12.74 (br, 1H), 9.02 (d, $J = 9.2$ Hz, 1H), 8.42 (d, $J = 9.2$ Hz, 1H), 8.26 (s, 1H), 8.17 (s, 1H), 7.23-7.07 (m, 10H), 5.83 (d, $J = 7.7$ Hz, 1H), 4.84-4.80 (m, 1H), 4.41-4.33 (m, 2H), 4.15 (s, 1H), 3.66 (d, $J = 4.6$ Hz, 1H), 3.13-2.98 (m, 2H), 2.89-2.75 (m, 2H) ppm; MS: calc. $M^+ = 575.21$, obsvd. $(M+1)^+ = 576.31$.

SPPS of conjugate 2. After 2-chlorotriethyl chloride resin was put in the SPPS reactor and bubbled in dry DCM by N_2 for 30 minutes, the resin swelled and was washed with dry DMF (3 times). Then the solution of Fmoc-3-(2-naphthyl)-L-alanine (L-Nal, 2 equiv.), and DIPEA (5 equiv.) in DMF was added to the reactor and bubbled by N_2 for 1 hour. After washed with DMF (5 times), the resin was bubbled with the blocking solution (10 min \times 2). Followed by washing with DMF (3 times), the resin was treated with 20% piperidine (in DMF) for 0.5 hour. The resin was washed with DMF (5 times) again, the solution of L-Phe (2 equiv.), HBTU (2 equiv.) and DIPEA (5 equiv.) in DMF was added to the reactor and reacted with the resin for 1 hour. After washed with DMF (5 times), the 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 once more, and the solution of L-Phe (2 equiv.), HBTU (2 equiv.) and DIPEA (5 equiv.) in DMF was added to react with the resin for 1 hour again. After washed the resin with DMF (5 times), the solution of **5** (2 equiv.), HBTU (2 equiv.), and DIPEA (5 equiv.) in DMF was added to reactor and bubbled by N_2 for 4 hours. 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 with TFA/ H_2O (95/5) for 3 hours and the resulted crude product was purified by reverse phase HPLC to afford the pure conjugate **2**.

2, white powder; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.73 (d, $J = 8.8$ Hz, 1H), 8.39 (s, 1H), 8.34 (d, $J = 7.8$ Hz, 1H), 8.27-8.24 (m, 2H), 7.81-7.79 (m, 3H), 7.72 (s, 1H), 7.40-7.38 (m, 3H), 7.12-6.99 (m, 10H), 5.88 (d, $J = 7.8$ Hz, 1H), 4.74-4.68 (m, 1H), 4.61-4.47 (m, 2H), 4.34 (dd, $J = 7.6, 4.7$ Hz, 1H), 4.21 (s, 1H), 3.59 (d, $J = 4.2$ Hz, 1H), 3.26-3.21 (m, 1H), 3.12-3.07 (m, 1H), 3.00-2.90 (m, 2H), 2.74-2.65 (m, 2H) ppm; MS: calc. $M^+ = 772.30$, obsvd. $(M+1)^+ = 773.28$.

SPPS of conjugates 3 and 4. **3** and **4** were synthesized by following the same procedure of **1** and **2** respectively, but using D-amino acids.

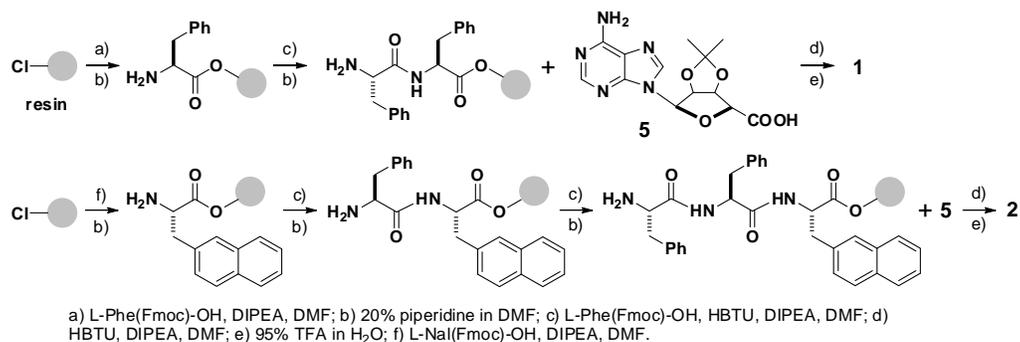
3, white powder; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.60 (d, $J = 8.5$ Hz, 1H), 8.53 (s, 1H), 8.41 (d, $J = 7.7$ Hz, 1H), 8.20 (s, 1H), 7.28-7.08 (m, 10H), 5.93 (d, $J = 7.5$ Hz, 1H), 4.67-4.61 (m, 1H), 4.45-4.39 (m, 1H), 4.37-4.34 (m, 1H), 4.29 (s, 1H), 3.87 (d, $J = 4.1$ Hz, 1H), 3.06-3.00 (m, 2H), 2.92-2.86 (m, 1H), 2.77-2.71 (m, 1H) ppm; MS: calc. $M^+ = 575.21$, obsvd. $(M+1)^+ = 576.12$.

4, white powder; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 12.74 (br, 1H), 8.56 (s, 1H), 8.54 (d, $J = 8.7$ Hz, 1H), 8.36 (d, $J = 7.8$ Hz, 1H), 8.23 (s, 1H), 8.21 (d, $J = 8.5$ Hz, 1H), 7.80-7.78 (m, 3H), 7.71 (s, 1H), 7.40-7.38 (m, 3H), 7.17-7.04 (m, 10H), 5.94 (d, $J = 7.4$ Hz, 1H), 4.60-4.53 (m, 3H), 4.34-4.30 (m, 2H), 3.83 (d, $J = 4.6$ Hz, 1H), 3.24-3.20 (m, 1H), 3.11-3.06 (m, 1H), 2.98-2.89 (m, 2H), 2.77-2.61 (m, 2H) ppm; MS: calc. $M^+ = 772.30$, obsvd. $(M+1)^+ = 773.22$.

Preparation of hydrogel of 2 + T₁₀ (Add T₁₀ to the hydrogel of **2**). T₁₀ was dissolved in water at the concentration of 10 mM. The hydrogel of **2** was prepared by the addition of 5 mg

2 in 436 μL H_2O , then, 64 μL T_{10} was added to the hydrogel to make the final concentration of **2** as 1 wt % and the molar ratio of **2**: T_{10} as 10:1. After being sonicated and slightly heated, the mixed gel was equilibrated overnight at room temperature before characterization.

Supplementary figures and schemes



Scheme S1. The solid phase synthetic route for making compound **1** and hydrogelator **2** (**3** and **4** follow the same procedure except using D-amino acids).

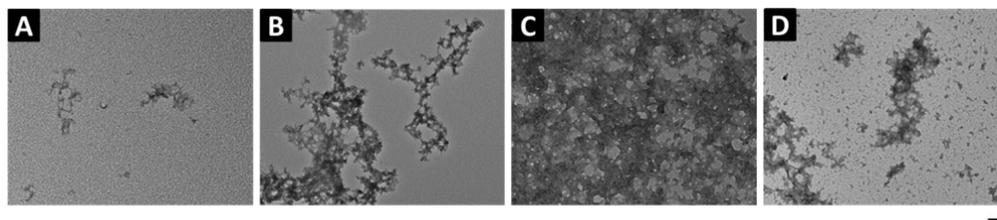
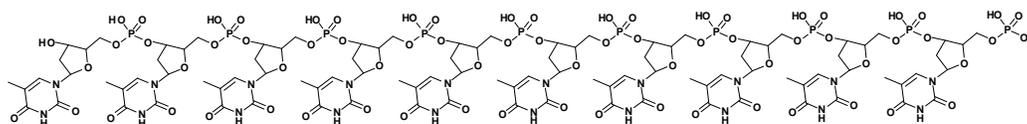


Figure S1. Transmission electron micrographs (TEM) of the solutions of (A) **1** (pH 7.0, 1.0 wt %), (B) **3** (pH 7.0, 1.0 wt %), (C) **4** (pH 7.0, 1.0 wt %), and (D) T_{10} . Scale bar is 100 nm.



Scheme S2. The structure of T_{10} .

Preparation and characterization of hydrogel of T_{10} + **2** (Add **2** to T_{10} solution). 64 μL 10 mM T_{10} was diluted to 500 μL , then 5 mg solid **2** was added to the solution. After sonication and heat to make **2** dissolved, the solution was self-assembled for overnight at room temperature to afford the hydrogel.

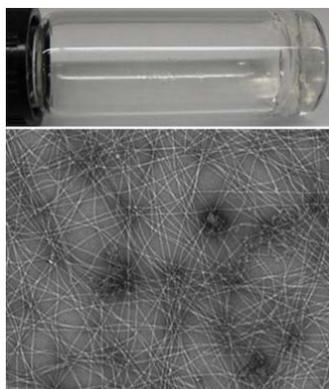
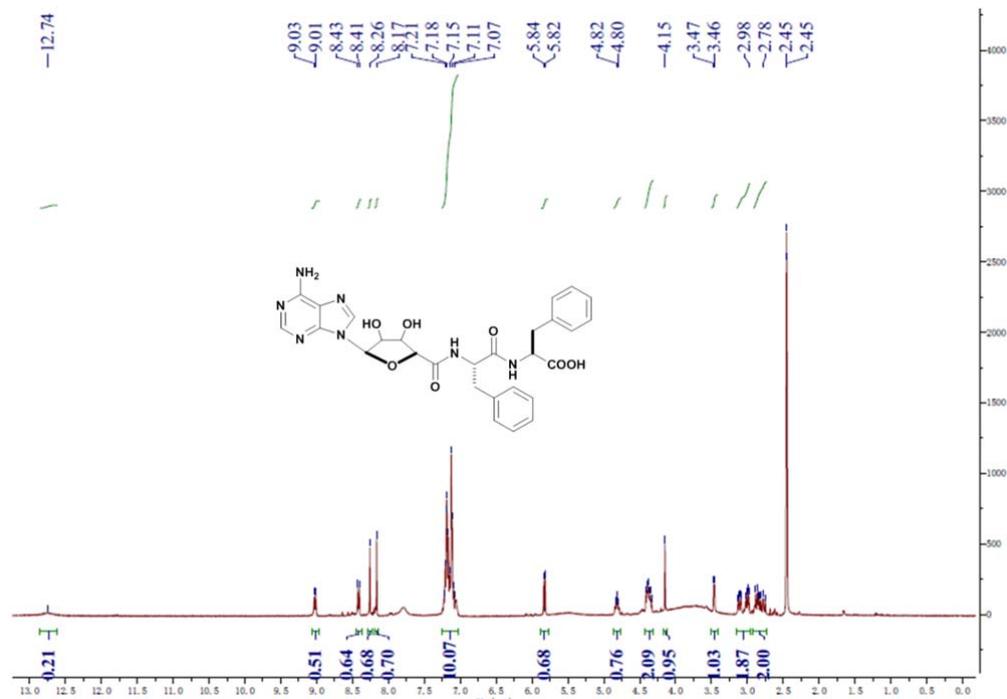


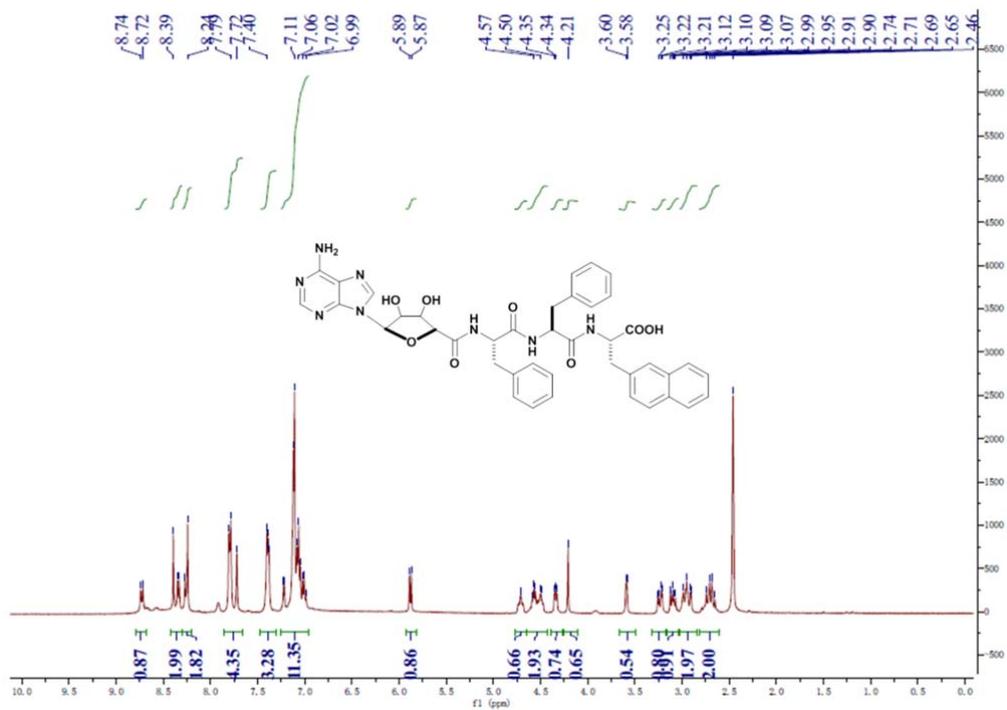
Figure S2. The optical image and TEM of the hydrogel of $T_{10} + 2$ (pH 7.0, 1.0 wt %, and the molar ratio of T_{10} to 2 is 1:10). Scale bar is 100 nm.

^1H NMR of conjugates 1, 2, 3 and 4

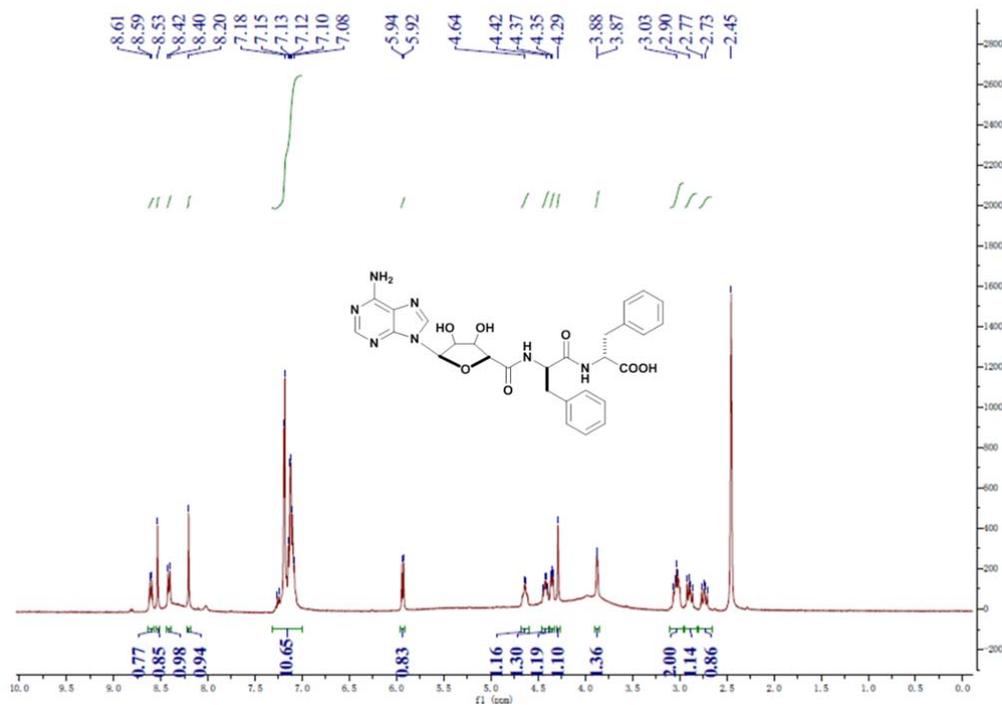
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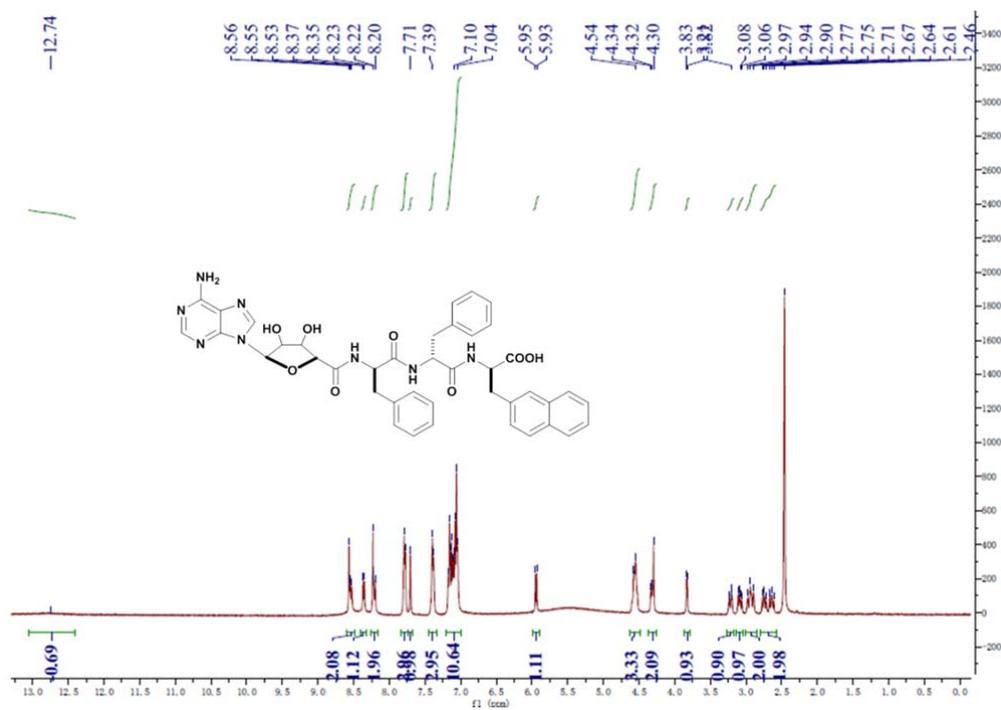
2:



3:



4:



Reference

1. J. Debnath, S. Dasgupta and T. Pathak, *Bioorganic & Medicinal Chemistry*, 2010, 18, 8257.