Supporting Information

Materials and methods:

Chemicals: L-Tyrosine and Fmoc-OSu were obtained from GL Biochem (Shanghai). All the other Starting materials were obtained from *Alfa*. Commercially available reagents were used without further purification, unless noted otherwise. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

Proteins: Alkali phosphatase were from Sigma-Aldrich (Cat. No. P7640). Proteinase K tritirachium album was from Calbiochem (Cat.No:539480).

General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 300) using DMSO-d_6 as the solvent and ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. HPLC was conducted at LUMTECH HPLC (Germany) system using a C18 RP column with MeOH (0.05% of TFA) and water (0.05% of TFA) as the eluents, TEM samples were prepared as following: a copper coated with a thin layer of carbon layer was dipped into the hydrogel, and then it was kept in a desicator overnight. The dried sample was performed at the Tecnai G2 F20 system, operating at 200 kV. LC-MS was conducted at the LCMS-20AD (Shimadzu) system, and rheology was performed on an AR 2000ex (TA instrument) system using a parallel plates (40 mm) at the gap of 500 μm.

Syntheses and characterizations:

Compound 4 was synthesized by solid phase peptide synthesis:

Peptide Synthesis: The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding Fmoc-succinated cystamine and N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 0.6 mmol/g. 20% piperidine in

anhydrous N,N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 ml per gram of resin), followed by five steps of washing using DCM for 2 min (5 ml per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid in DCM for 30 minutes. All the solutions were combined and concentrated, and then 20 mL of ice-cold diethylether was added. The resulting precipitate was centrifuged for 10 min at 4 ^{0}C at 10,000 rpm. Afterward the supernatant was decanted and the resulting solid was dissolved in DMSO for HPLC separation.

Synthesis and Characterization of compound 4: The compound **4** was obtained by general solid phase peptide synthesis (spps). The resulting solid was dissolved in DMSO and purified by HPLC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14-8.26 (m, 5H), 8.00-8.02 (m,3H), 7.78-7.86 (m,3H), 7.72 (s, 1H), 7.38-7.47 (m, 3H), 7.13-7.19 (m, 11H), 7.02 (d, J=8.404, 2H), 6.62 (d, J=8.452, 2H), 4.44-4.51 (m, 3H), 4.25-4.36 (m, 2H), 4.13-4.18 (t, 1H), 3.54-3.74 (m, 5H), 2.87-3.01 (m, 3H), 2.61-2.80 (m, 3H), 2.53 (s, 1H), 2.24-2.29 (m, 3H), 1.88-1.93 (m, 1H), 1.74-1.80 (m, 1H), 1.19-1.28 (m, 1H). HR-MS: calc. M^{+} = 1028.43, obsvd. $(M+H)^{+}$ = 1029.22

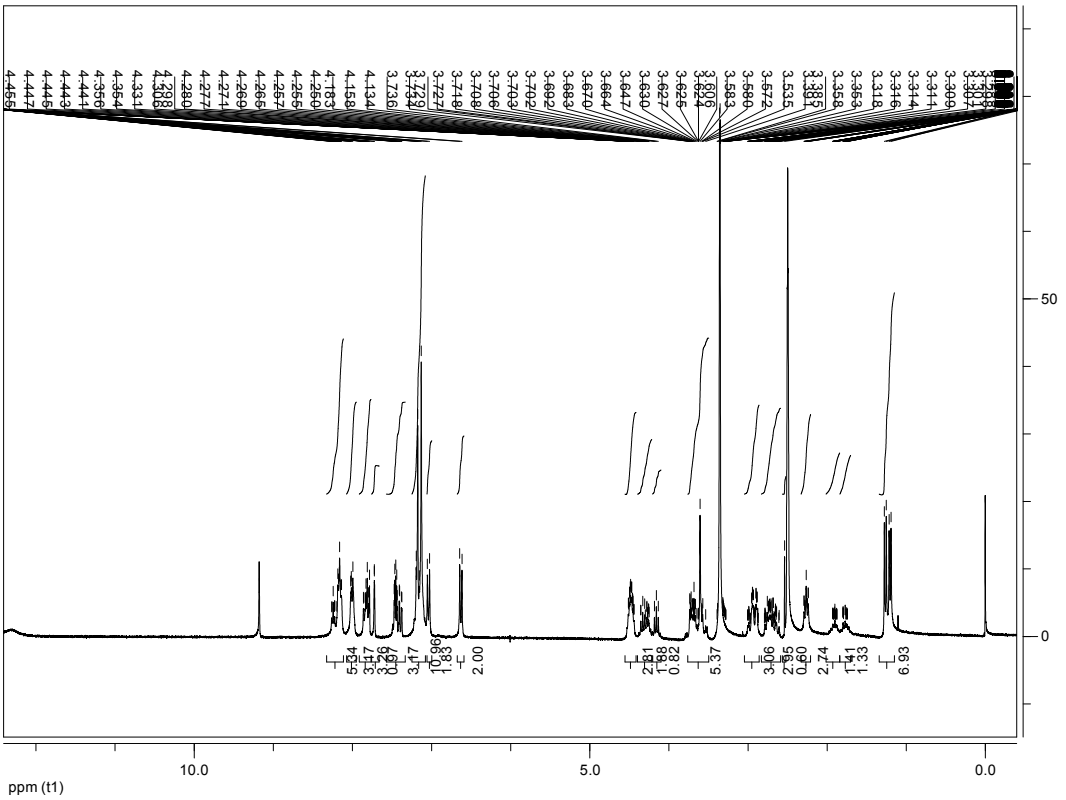

Figure S-1. ^{1}H NMR of 4

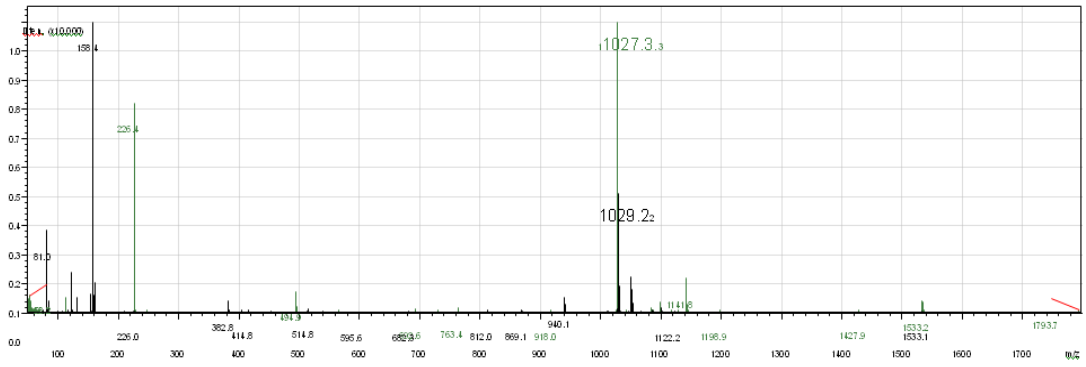

Figure S-2. HR-MS of 4

Purification of 4

The crude peptide was dissolved at 50 mg mL^{-1} in DMSO, the filtrate was purified by reverse-phase high-performance liquid chromatography (RP-HPLC) on a preparative Venusil XBP C18 peptide/protein column (5µm, 100Å, 21.5 X 250 mm, Cat: VX952520-0, Agela technologies), employing a flow rate of 8 mL min^{-1}. HPLC

solvents: standard A is 0.05% TFA in Water, and Standard B is 0.05% TFA in MeOH.

ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System.,the column was Ultro C18Q (5μm, 50 X 4.6mm, Peekescientific), LC-MS solvents: standard A is 0.035% TFA in Water, and Standard B is 0.05% TFA in acetonitrile

***Table S-1*. Eluting gradient for HPLC analysis of compound 4**

Time (minute)	Flow (mL/min.)	H_2O %	MeOH %
0	8	70	30
10	8	90	10
15	8	0	100
24	8	0	100
25	8	70	30
30	8	70	30

***Table S-2*. Eluting gradient for LC-MS analysis of compound 4**

Time (minute)	Flow (mL/min.)	H_2O %	acetonitrile %
0.01	0.8	90	10
2.50	0.8	55	45
5.00	0.8	0	100
8.00	0.8	0	100
9.00	0.8	90	10
10.00	0.8	90	10

.

Dynamic strain/time sweeps

Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiment at the gap of 500 μm.

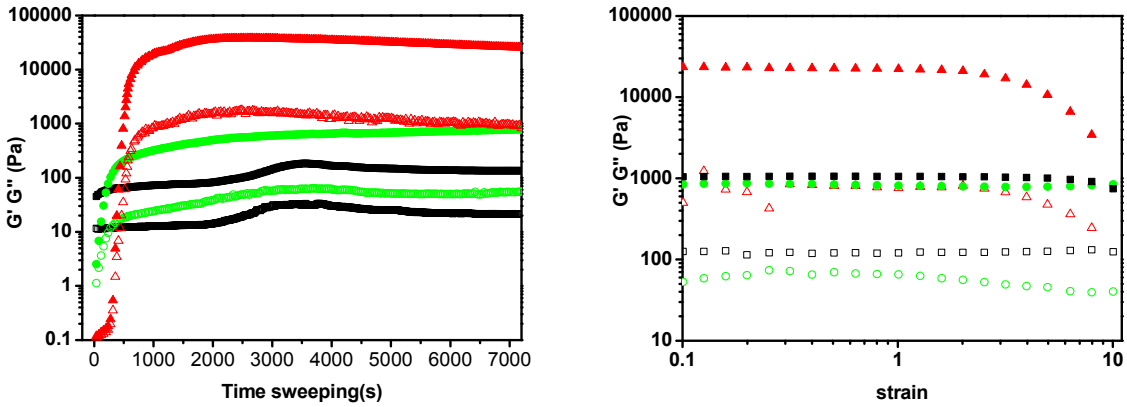

Fig. S-3. Dynamic time (left) and strain (right) sweeps of gels (triangles: SH-gel, circles: OMe-gel, and squares: OH-gel, filled symbols: G' and open symbols: G")

Formation of hydrogels

Formation of SH-gel: 3.5 mg of **1** (2.65μmol) and 1.5mg of 4 (1.46 μmol) was dissolved in 0.45 mL of PBS buffer solution containing 1.4 mg (5 equiv. to **1**) of Na_2CO_3 (3 equiv. of Na_2CO_3 were used to neutralize the carboxylic acids on **1** and the additional 2 equiv. of Na_2CO_3 were used to neutralize DTT to make the final pH value of the resulting gel to about 7.4). And then 0.05 mL of PBS buffer solution containing 0.82 mg of DTT (5.3 μmol, 2.0 equiv. to **1**) was added. Gels would form after being kept at room temperature (22-25^{0}C) for about 10-20 minutes.

Formation of OMe-gel: To a mixed PBS solution (pH = 7.4) of compound 4 and Nap-GFFpY-OMe (Compound 4 was 30 wt% to total amounts of Nap-GFFY-OMe and 4), 30 U/mL of alkali phosphatase was added in 20-25 ^{0}C. The OMe-gel could be obtained after 10 minutes.

Formation of OH-gel: The PBS suspension containing 0.3 wt% of compound 4 and 0.7 wt% of Nap-GFFY-COOH was heated to become a clear solution. The OH-gel was formed after cooling back to room temperature.

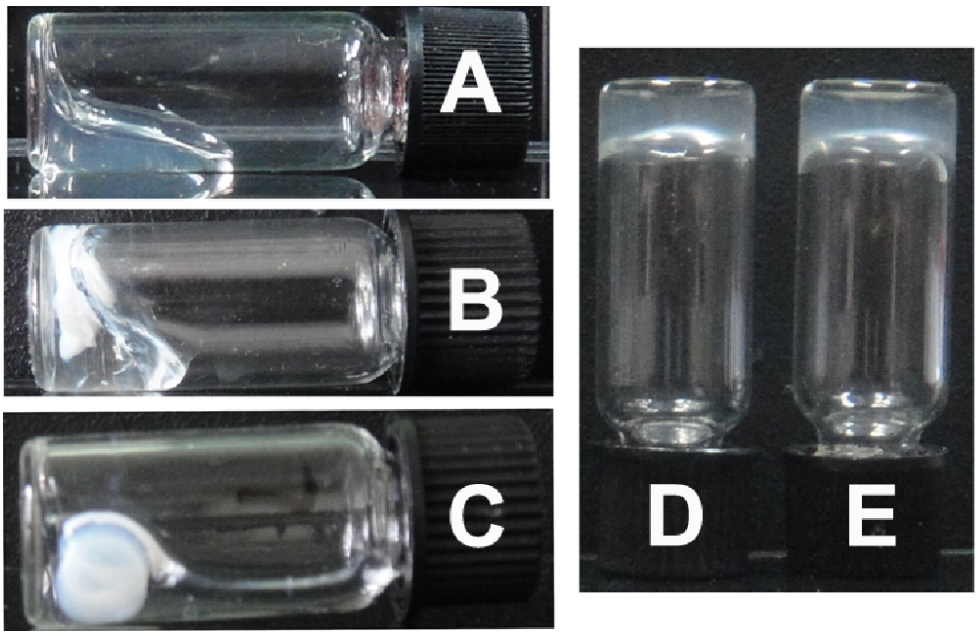

Fig. S-4. Optical images of A) solution of 1.0 wt% of Nap-GFFY-ss-EE, B) precipitate formed at 1 hour after the addition of DTT to solution in A), C) the partial gel containing 20 wt% of 4 to total amount of compounds 1 and 4, D) a stable gel containing 25 wt% of 4 to total amount of compounds 1 and 4, and E) a stable gel containing 40 wt% of 4 to total amount of compounds 1 and 4

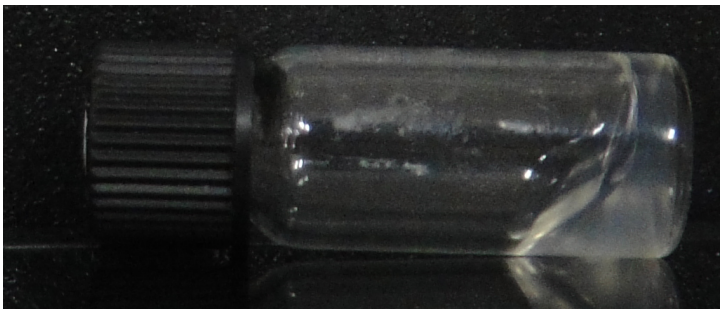

Fig. S-5. An optical image of a weak gel containing 0.5 wt% of 4 itself in PBS solution (pH = 7.4)

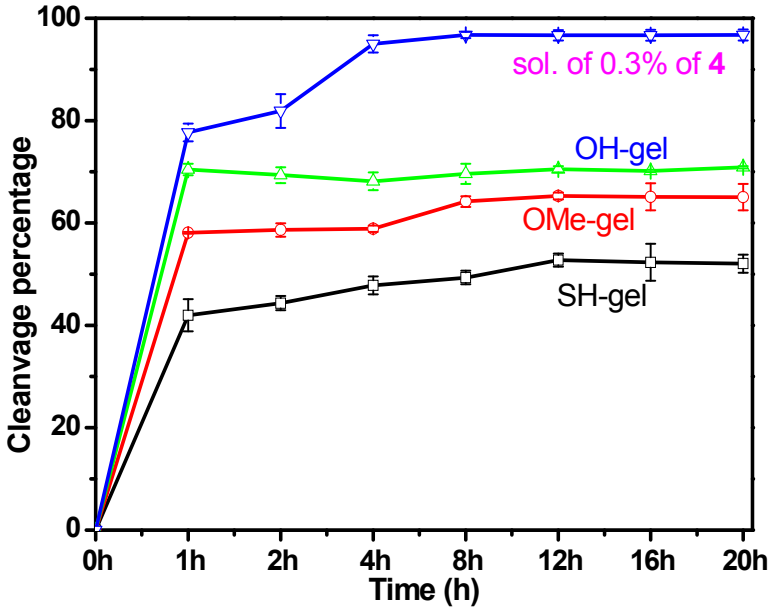

Fig. S-6. Cleavage percentage of 4 in different gels and the PBS solution (the concentration of 4 is 0.3 wt% in all samples)

Analysis of Proteolytic Stabilities

Three gels containing proteinase K (10U/mL) were incubated at 37 °C with gentle shaking (40rad/min). At selected time intervals, gels were dissolved in DMSO for LC-MS determination. The experiment was repeated for three times and SD was determined.

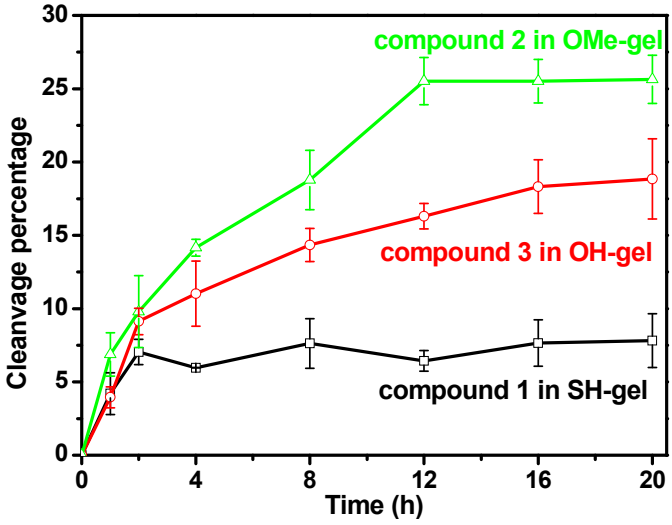

Fig. S-7. Cleavage percentage of compounds 1-3 in their corresponding gels