Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supramolecular Hydrogels Mimicking Collagen for Tissue Engineering

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Supporting Information

Materials and general methods:

Chemicals: L-tyrosine, Fmoc-OSu and all Fmoc-amino acids 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. The solvents were dried according to regular protocols. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 300) using DMSO-d6 as the solvent and ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. Emission spectra were recorded on a Perkin-Elmer LS-55 luminance spectrometer at excitation wavelength of 272 nm; HPLC was conducted at LUMTECH HPLC (Germany) system using a C18 RP column with MeOH (0.1% of TFA) and water (0.1% 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 EM-400ST system, 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.

Peptide synthesis. All the peptide derivatives were prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding 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. At the final step, the N-terminus of the peptides was coupled with 2-naphthalene acetic acid to attach the aromatic group on the pentapeptides. 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 dichloromethane (DCM) for 2 min (5 ml per gram of resin). The peptide derivatives were cleaved from the resin by ice-cold reagent B and the mixture was stirred at room temperature, filtered, and poured into ice-cold diethylether. The resulting precipitate was centrifuged for 10 min at 2 ^{0}C at 10,000 rpm. Afterward the supernatant was decanted and dissolved in double-distilled (dd) water and lyophilized. The crude products were purified using preparation reverse-phase HPLC.

Determination of T_{gs} and pH_{gs}. The values of T_{gs} and pH_{gs} of all the hydrogels are determined by an inverted-tube method. After obtaining the hydrogels, the gels in vials are incubated in a water bath with a temperature increasing speed of 2 ^{o}C/minute, and the temperatures at which the gels started to flow upon inverting the vial was recorded as T_{gs}. The values of pHgs of the gels are determined by using a 0.1 N NaOH solutions.

Table S-1. Gel-sol phase transition temperature (T_{gs}) of the hydrogels

Gels	Control gel I	K-gel II	E-gel III	S-gel IV	A-gel V	P-gel VI
0.1 wt%	25 ^{0}C	53 ^{0}C	25 ^{0}C	33 ^{0}C	44 ^{0}C	33 ^{0}C
0.2 wt%	70 ^{0}C	67 ^{0}C	32 ^{0}C	58 ^{0}C	55 ^{0}C	53 ^{0}C
0.3 wt%	75 ^{0}C	80 ^{0}C	42 ^{0}C	66 ^{0}C	70 ^{0}C	62 ^{0}C
0.4 wt%	82 ^{0}C	84 ^{0}C	44 ^{0}C	74 ^{0}C	84 ^{0}C	68 ^{0}C
0.5 wt%	88 ^{0}C	88 ^{0}C	46 ^{0}C	86 ^{0}C	93 ^{0}C	72 ^{0}C

Table S-2. Gel-sol phase transition pH values (pH_{gs}) of the hydrogels

Gels	Control gel I	K-gel II	E-gel III	S-gel IV	A-gel V	P-gel VI
0.1 wt%	7.8	8.2	7.3	7.5	8.0	7.8
0.2 wt%	8.3	9.3	7.6	8.0	8.3	8.0
0.3 wt%	8.8	9.8	7.9	8.5	8.8	8.3
0.4 wt%	9.5	10.5	8.3	8.8	9.5	9.0
0.5 wt%	10.1	10.9	8.5	9.2	10.0	9.5

CD measurement. The CD was measured on an AVIV Model 410 spectrophotometer under a nitrogen atmosphere. The width of the quartz cuvette is 0.10 mm.

Table S-3. Summary of the shift of peaks (maxima) in the fluorescence spectra from the solution to the gel phase of all hydrogelators

Gelators	Maxima in solution phase (nm)	Maxima in gel phase (nm)	Change in wavelength (nm)
1	348	352	4
2	341	344	3
3	335	344	9
4	339	343	4

5	339	344	5
6	339	347	8

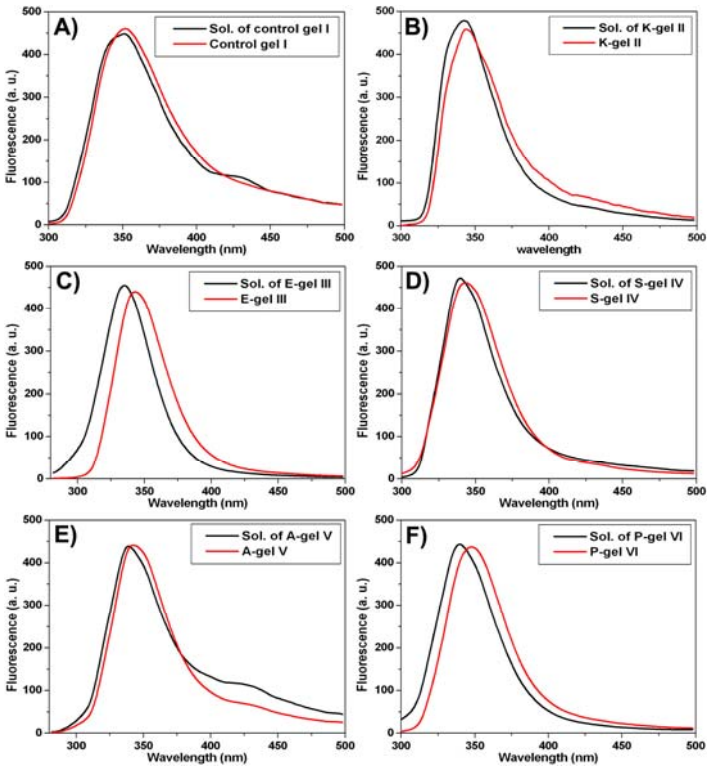
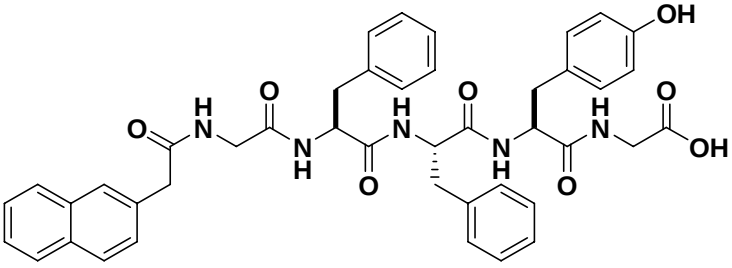


Figure S-1. The emission spectra of **1-6** in their corresponding solution and gel phase

Characterization of all compounds used for hydrogelation.



Scheme S-1. Chemical structure of compound **1**

*Characterization of compound **1***. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81-7.88 (m,3H), 7.75 (s, 1H), 7.46-7.48 (m, 2H), 7.40-7.43 (m, 1H), 7.13-7.22 (m, 10H), 7.04-7.07 (d, 2H), 6.65 (d, 2H), 4.47-4.52(m, 3H), 3.77(s, 2H), 3.62 (s, 2H), 3.42 (m, 2H), 2.90-3.01 (m, 3H), 2.62-2.78 (m, 3H). MS: calc. M^+ = 758.13, obsvd. $(M+Na)^+$ = 780.35

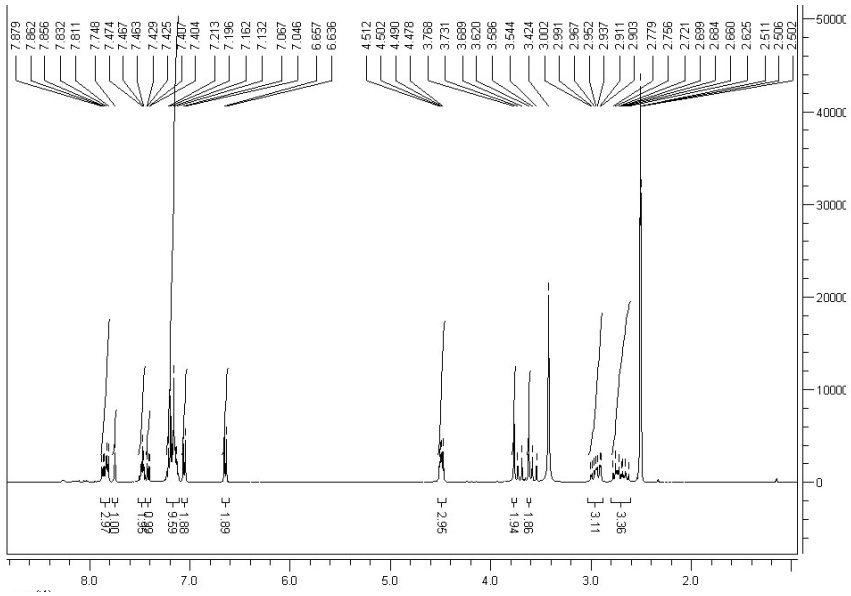


Figure S-2. ^{1}H NMR of compound **1**

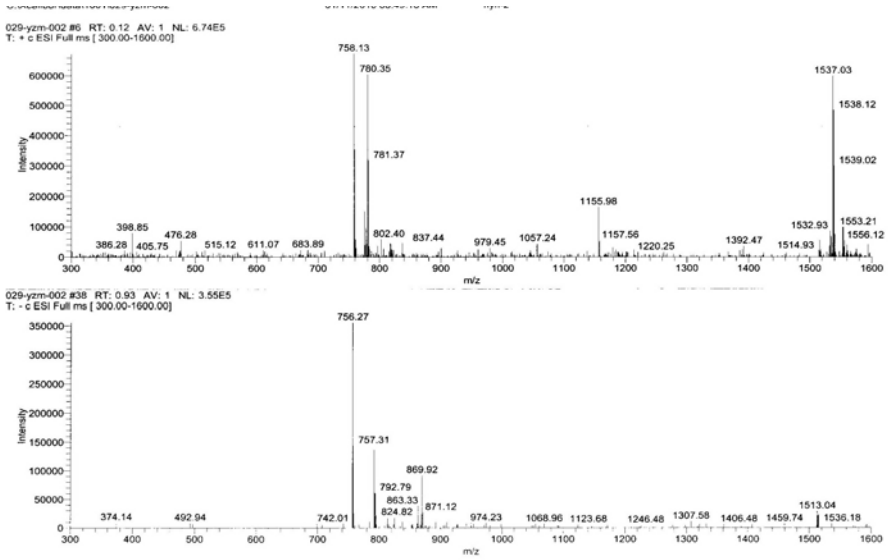
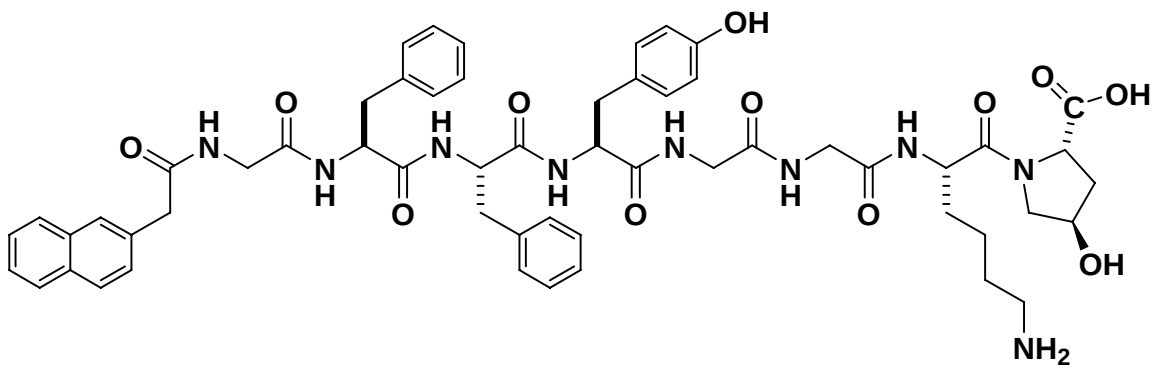


Figure S-3. MS of compound **1**



Scheme S-2. Chemical structure of compound **2**

Characterization of compound ***2***. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14-8.19 (m,2H), 7.81-7.87 (m, 4H), 7.75 (s, 1H), 7.40-7.48 (m, 4H), 7.16-7.19 (m, 12H), 7.03-7.06 (m, 2H), 6.64-6.66 (m, 3H), 4.47-4.50(m, 5H), 4.26-4.34(m, 2H), 3.62-3.73 (m, 10H), 2.92-2.94 (m, 3H), 2.72 (m, 6H), 2.08-2.11 (m, 2H),1.89-1.91 (m, 1H), 1.60-1.65 (m, 1H), 1.50-1.51 (m, 3H), 1.31-1.34 (m, 3H). MS: calc. M^+ = 1056.51, obsvd. $(M+Na)^+$ = 1078.62

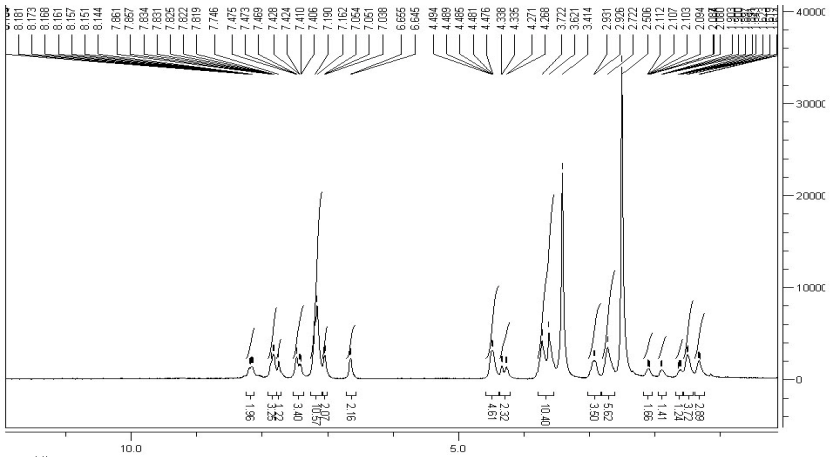


Figure S-4. ^{1}H NMR of compound **2**

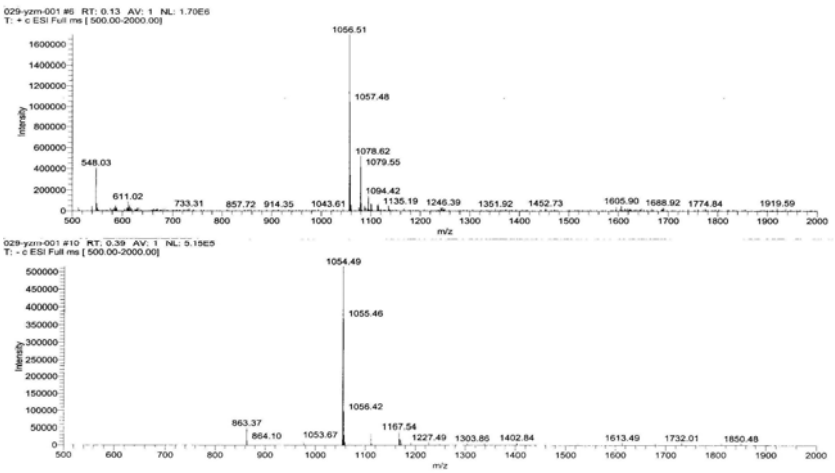
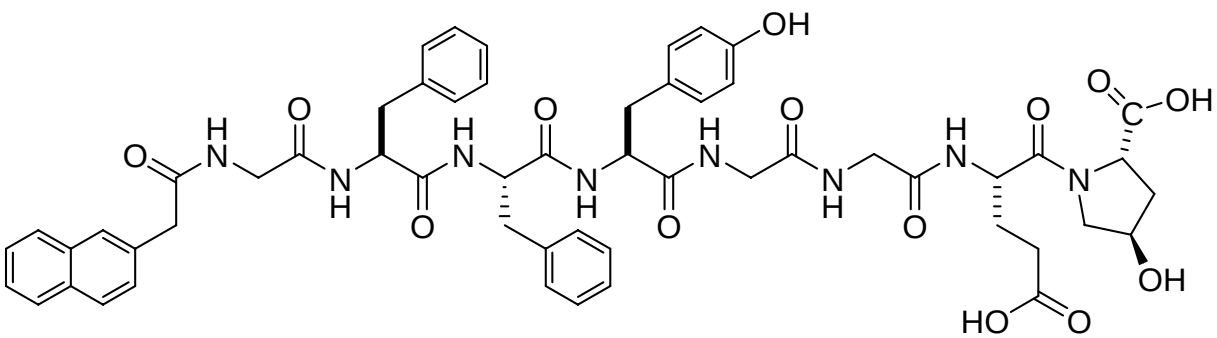


Figure S-5. MS of compound **2**



Scheme S-3. Chemical structure of compound **3**

*Characterization of compound **3***. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.11-8.198 (m,2H), 7.82-7.84 (m, 4H), 7.47 (s, 1H), 7.40-7.47(m, 4H), 7.16 (m, 12H), 7.05 (m, 3H), 6.64-6.65(m, 2H), 4.58-4.61(m, 1H), 4.46-4.48(m, 3H), 4.25-4.34 (m, 2H), 3.66-3.78 (m, 4H), 3.61(m, 5H), 2.91-2.94 (m, 3H),2.65-2.75 (m, 4H), 2.32 (s, 3H), 2.06-2.12 (m, 1H), 1.88-1.91 (m, 3H), 1.65-1.70 (m, 1H). MS: calc. M^+ = 1056.50, obsvd. $(M+Na)^+$ = 1075.80

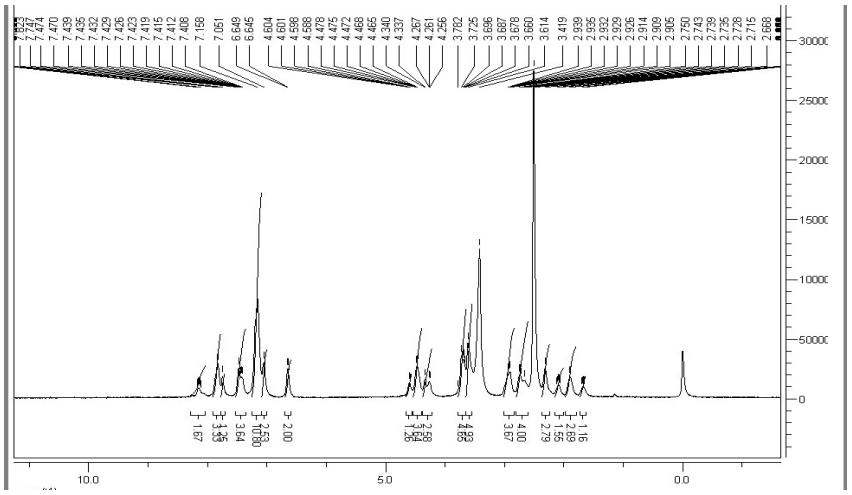


Figure S-6. ^{1}H NMR of compound **3**

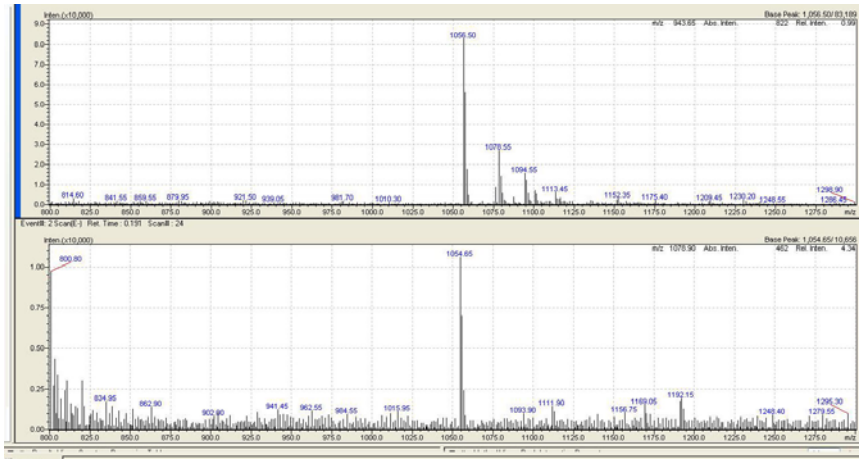
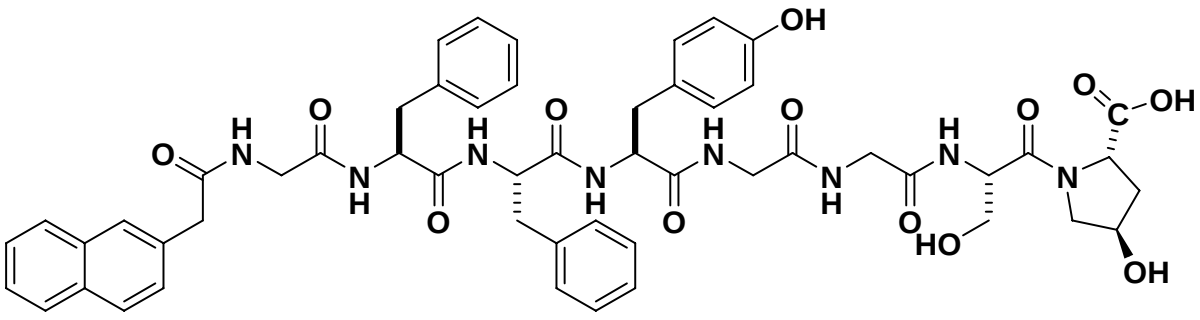


Figure S-7. MS of compound **3**



Scheme S-4. Chemical structure of compound **4**

*Characterization of compound **4***. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.162 (m,1H), 7.82-7.86 (m,

4H), 7.75 (m, 1H), 7.75 (m, 1H), 7.40-7.48 (m, 5H), 7.16-7.20 (m, 12H), 7.04-7.06 (m, 3H), 6.64-6.66 (m, 2H), 4.58-4.61 (m, 1H), 4.47-4.48(m, 3H), 4.33-4.34 (m, 1H), 4.25-4.26 (m, 1H), 3.69-3.74 (m, 6H), 3.58-3.62 (m, 6H), 2.89-2.97 (m, 4H), 2.50-2.69 (m, 3H), 2.05-2.10 (m, 2H), 1.89-1.92 (m, 2H) MS: calc. M^+ = 1015.24, obsvd. $(M+Na)^+$ = 1037.64

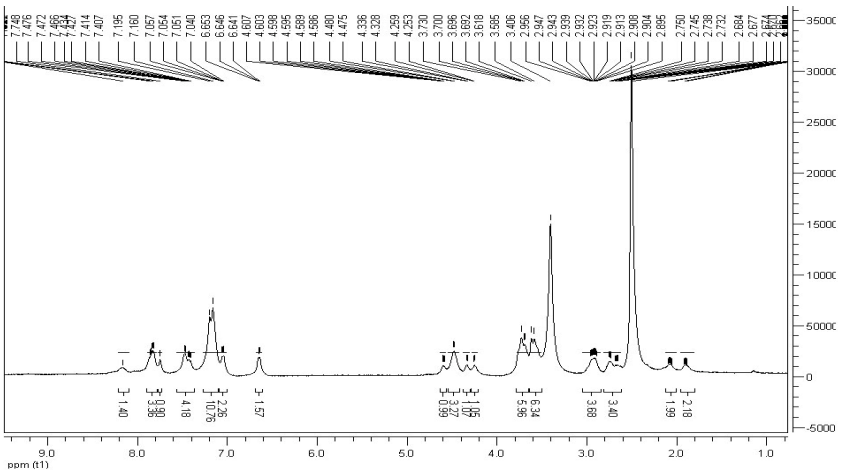


Figure S-8. ^{1}H NMR of compound **4**

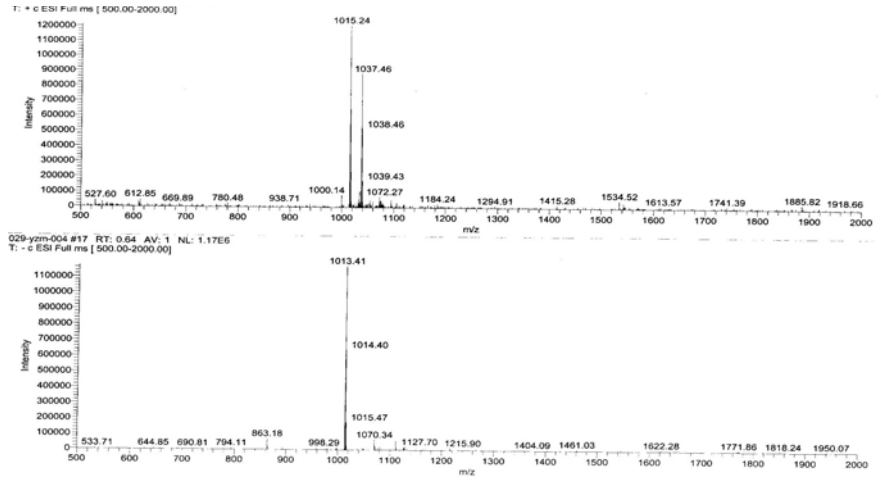
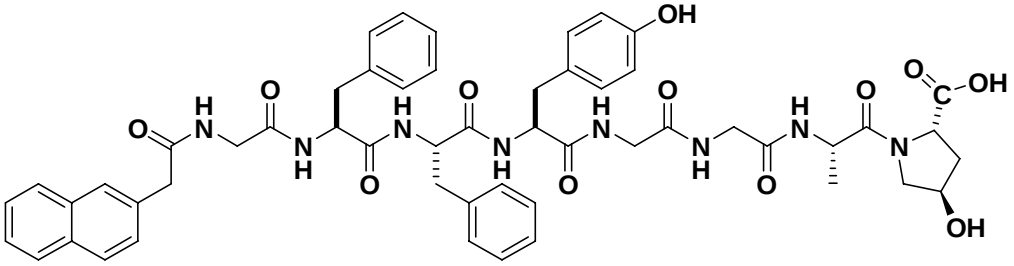


Figure S-9. MS of compound **4**



Scheme S-5. Chemical structure of compound **5**

Characterization of compound 5. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15-8.20 (m,1H), 7.82-7.83 (m, 4H), 7.75 (s, 1H), 7.41-7.48 (m, 4H), 7.16-7.20 (m, 12H), 7.03-7.06 (m, 3H), 6.64-6.66 (m, 3H),

4.47-4.49(s, 4H), 4.33 (s, 1H), 4.25 (s, 10H), 3.721 (m, 5H), 3.41-3.62 (m, 6H), 2.90-2.92 (m, 2H),2.65-2.75 (m, 3H), 2.07-2.09 (m, 2H). MS: calc. M^+ = 999.33, obsvd. $(M+Na)^+$ =1021.58

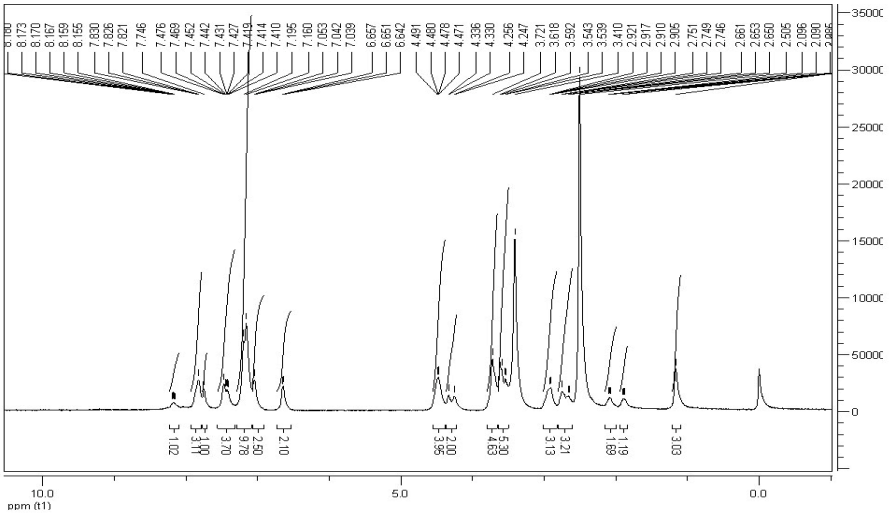


Figure S-10. ^{1}H NMR of compound **5**

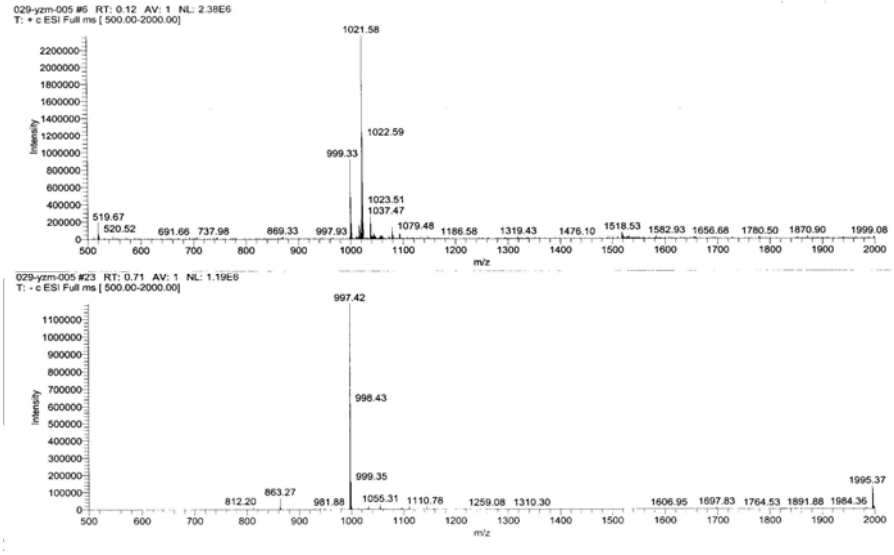
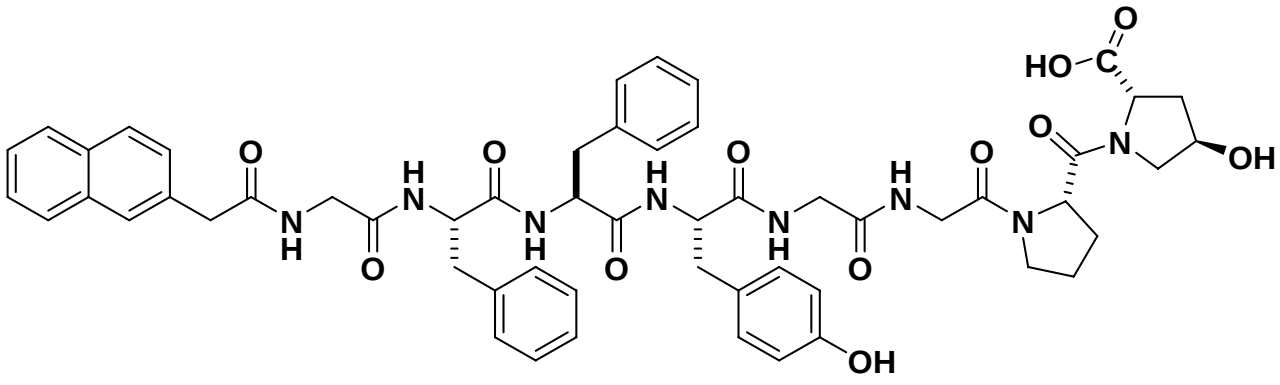


Figure S-11. MS of compound **5**



Scheme S-6. Chemical structure of compound **6**

Characterization of compound ***6***. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13-8.21 (m,2H), 7.82-7.86 (m, 4H), 7.75 (s, 1H), 7.41-7.48 (m, 4H), 7.16-7.20 (m, 13H), 7.04-7.06 (m, 2H), 6.64-6.66 (m, 2H), 4.48 (s, 4H), 4.35 (s, 2H), 4.22-4.24 (m, 1H), 3.96-4.01 (m, 1H), 3.69-3.74 (m, 4H), 3.54-3.62 (m, 4H), 2.90-2.94 (m, 3H), 2.65-2.76 (m, 3H), 2.05-2.08 (m, 3H), 1.73-1.80 (m,6H).

MS: calc. M^+ = 1025.32, obsvd. $(M+Na)^+$ =1047.49

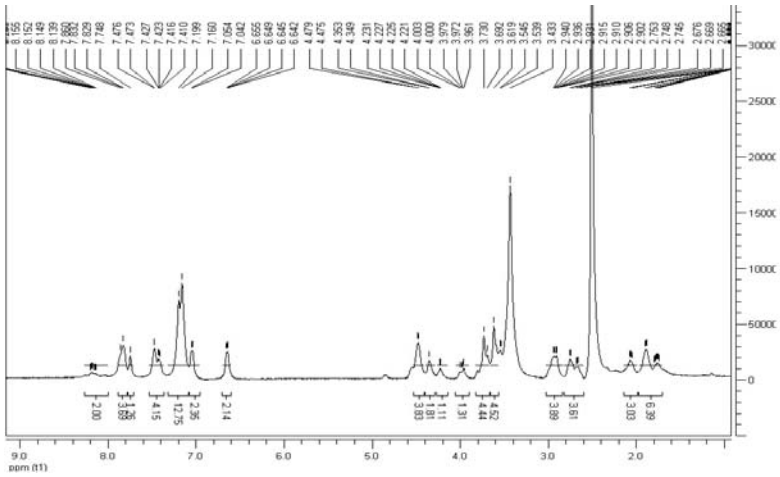


Figure S-12. ^{1}H NMR of compound **6**

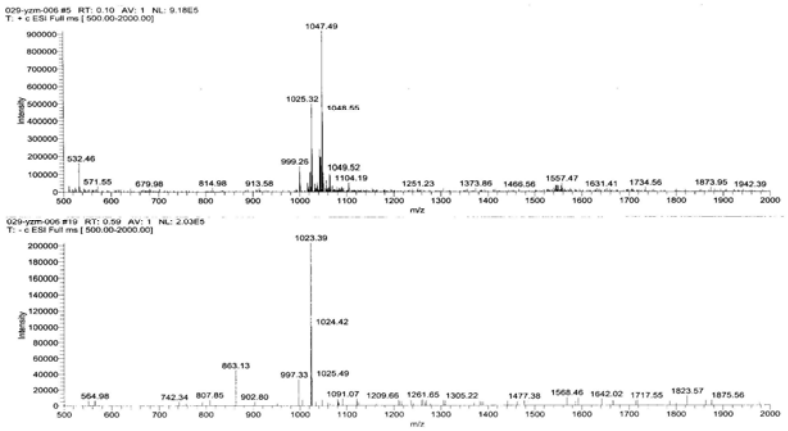


Figure S-13. MS of compound **6**