Electronic Supplementary Material (ESI) for Soft Matter

Supporting information

β-Amino acid containing proteolitically stable dipeptide based Hydrogels: encapsulation and sustained release of some important biomolecules at physiological pH and temperature.

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Synthesis:

The dipeptides were synthesized by conventional solution phase methods using racemization free fragment condensation strategy. The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide-1-hydroxybenzotriazole (DCC/HOBt). C-terminal methyl group was removed using aqueous sodium hydroxide. Boc group was removed using formic acid. The details synthesis of β-alanine containing dipeptides was reported previously by our group.[S1] The Fmoc group protection had been performed in solution phase. Initially, 1 mmol dipeptide was taken in a round bottom flask and dissolved in the sodium bicarbonate solution. The dissolved solution was cooled to ice-salt mixture. Then, Fmoc-Cl (1.1 equivalent) was dissolved in the 1,4-dioxane solvent and added to the cold mixture. The reaction mixture was left for 8 hours. Then mixture solution was concentrated in rotary evaporator and diluted with water. The solution was washed with ether solution and aqueous solution was neutralized by dilute hydrochloric acid and it was extracted using ethyl acetate. The solid materials were subjected to column chromatography to get purified solid material.

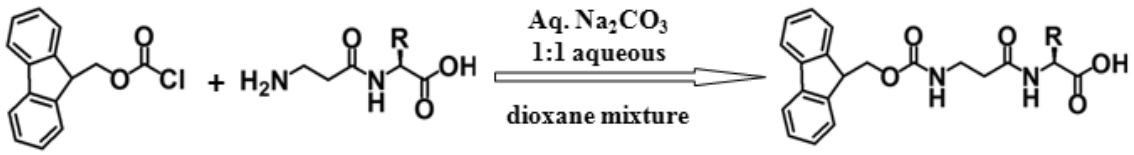

Figure. Schematic presentation of synthesis of Fmoc-dipeptides.

Peptide 1 (Fmoc-βAla-Val-OH): ^{1}H NMR ($(CD_3)_2SO$, 300MHz) δ 0.90-0.84 (6H, m), 2.37-2.26 (3H, m), 3.25-3.22 (2H, m), 4.22-4.17 (1H, m), 4.55-4.51 (3H, m), 8.12-7.92 (5H, m), 8.27-8.18 (4H, m), 8.39-8.35 (1H, d, *J*= 7.54), 12.55 (1H, br). ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 173.69, 171.13, 156.57, 144.47, 141.28, 128.17, 127.63, 125. 73, 120.68, 65.94, 57.66, 47.27, 39.26, 37.64, 35.85, 30.30, 19.69, 18.62.

Elemental analysis calculated (%) for $C_{23}H_{26}N_2O_5$ (410.1842): C, 67.30; H, 6.38; N, 6.82; found C, 67.35; H, 6.35; N, 6.86. HRMS (m/z): 433.1156 $(M + Na)^+$, 434.1236 $(M + Na+ H)^+$.

Peptide 2 (Fmoc-βAla-Phe-OH): ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 2.25–2.18 (2H, m), 2.85–2.82 (1H, m), 3.11–2.99 (1H, m), 3.37-3.33 (2H, m), 4.27–4.20 (2H, m), 4.42–4.38 (2H, m), 7.40–7.16 (11H, m), 7.68–7.65 (1H, d, *J*= 7.29), 7.88–7.86 (1H, d, *J* = 7.44), 8.21–8.18 (1H, m), 12.66 (1H, br). ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 173.69, 170.89, 156.57, 144.46, 143.13, 141.27, 139.98, 137.99, 135.09, 129.47, 128.15, 127.84, 127.62, 125.75, 121.98, 120.612, 65.94, 57.66, 47.27, 30.53, 19.84, 18.64. Elemental analysis calculated (%) for $C_{27}H_{26}N_2O_5$ (458.1842): C, 70.73; H, 5.72; N, 6.11; found C, 70.77; H, 5.78; N, 6.14. HRMS (m/z): 459.4669 $(M+ H)^+$, 481.4493 $(M+ Na)^+$.

Peptide 3 (Fmoc-Ala-Val-OH): ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 0.88-0.83 (6H, m), 1.28-1.21 (3H, m), 2.07-2.02 (1H, m), 4.29-4.14 (4H, m), 7.37-7.28 (2H, m), 7.43-7.39(2H, m), 7.52-7.51 (1H, d, *J*=7.5), 7.65-7.64 (1H, d, *J*=6.0), 7.73-7.67(2H, m), 7.91-7.84 (2H, m). ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 172.86, 172.74, 155.61, 143.90, 143.78, 140.69, 127.60, 127.11, 127.05,125.28, 125.24, 125.19, 120.06, 66.34, 65.59, 56.98, 49.80, 46.64, 29.95, 19.03, 18.15, 17.88.

Elemental analysis calculated (%) for $C_{23}H_{26}N_2O_5$ (410.1842): C, 67.30; H, 6.38; N, 6.82; found C, 67.33; 609H, 6.35; N, 6.84. HRMS (m/z): 411.1161$(M + H)^+$, 433.0862 $(M + Na)^+$, 449.0 $(M + K)^+$.

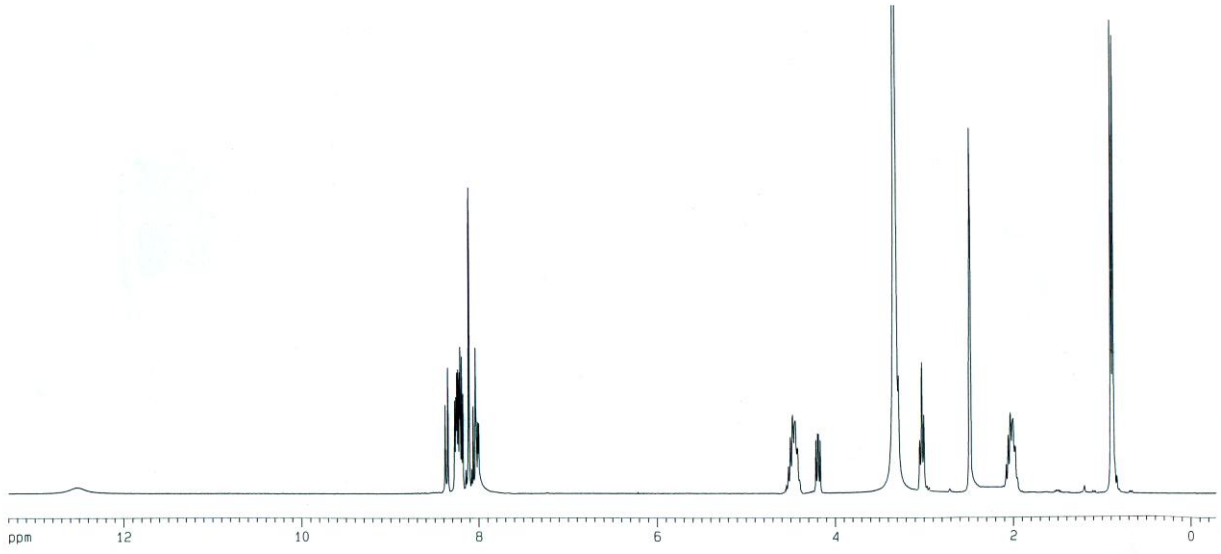

Figure S1: ^{1}H NMR spectra of peptide 1.

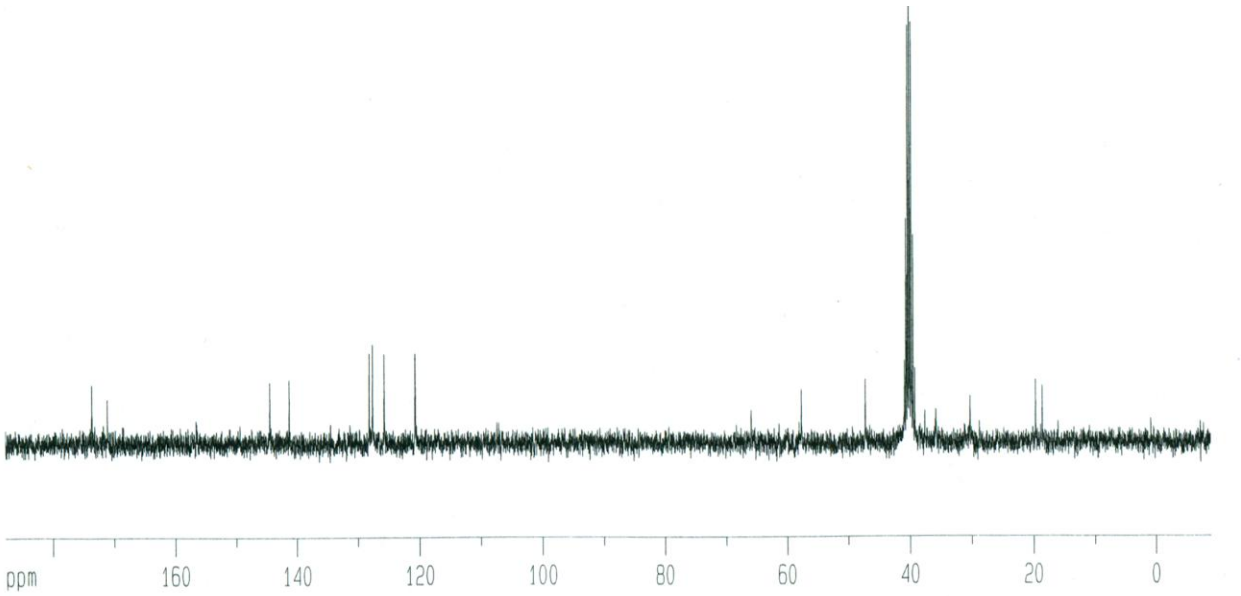

Figure S2: ^{13}C NMR spectra of peptide 1.

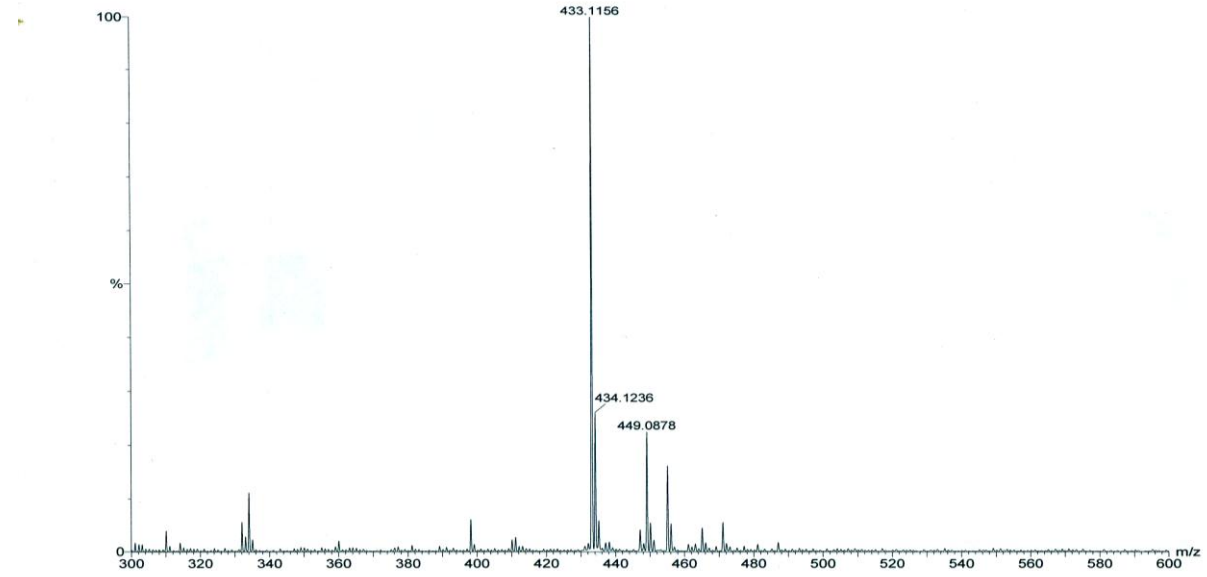

Figure S3: HRMS spectra of peptide 1.

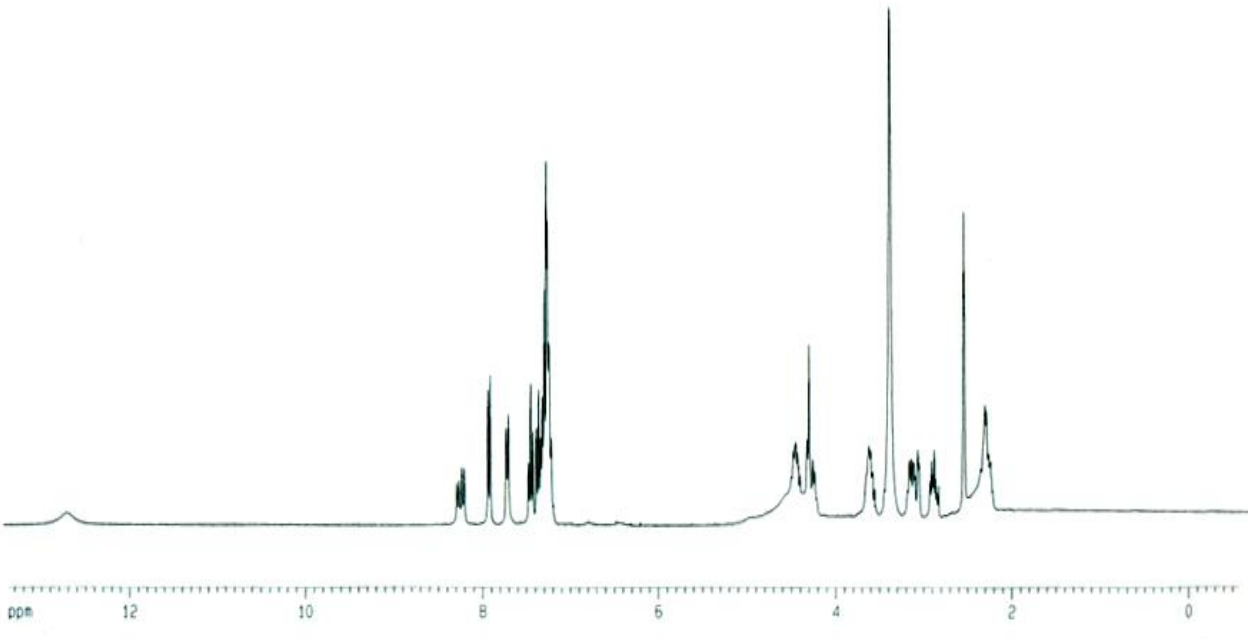

Figure S4: ^{1}H NMR spectra of peptide 2.

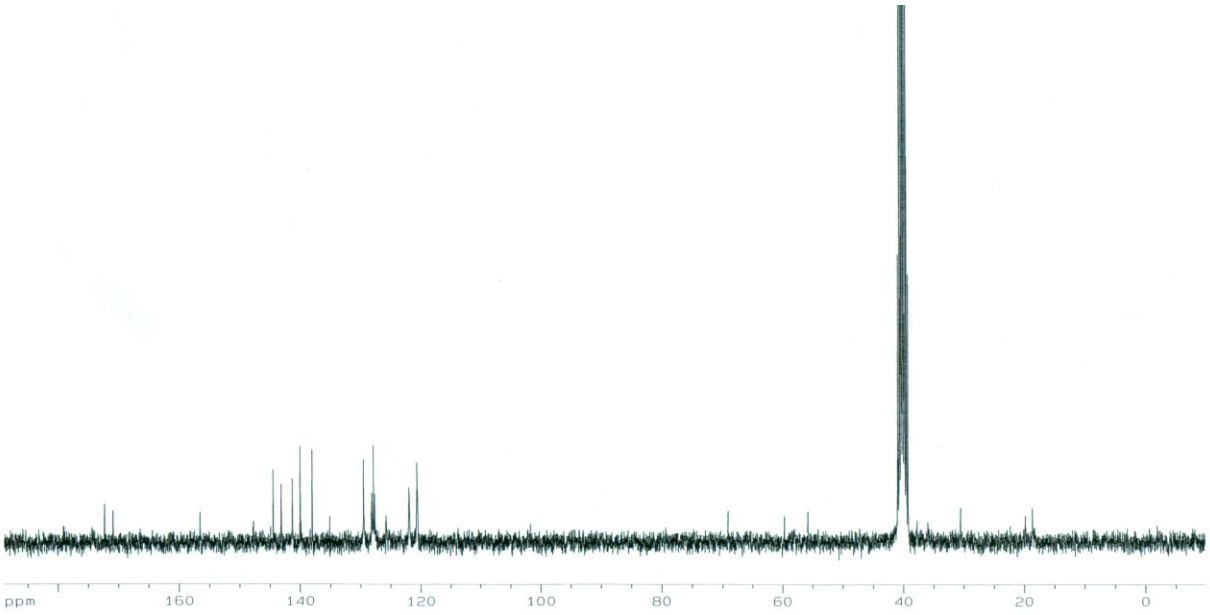

Figure S5: ^{13}C NMR spectra of peptide 2.

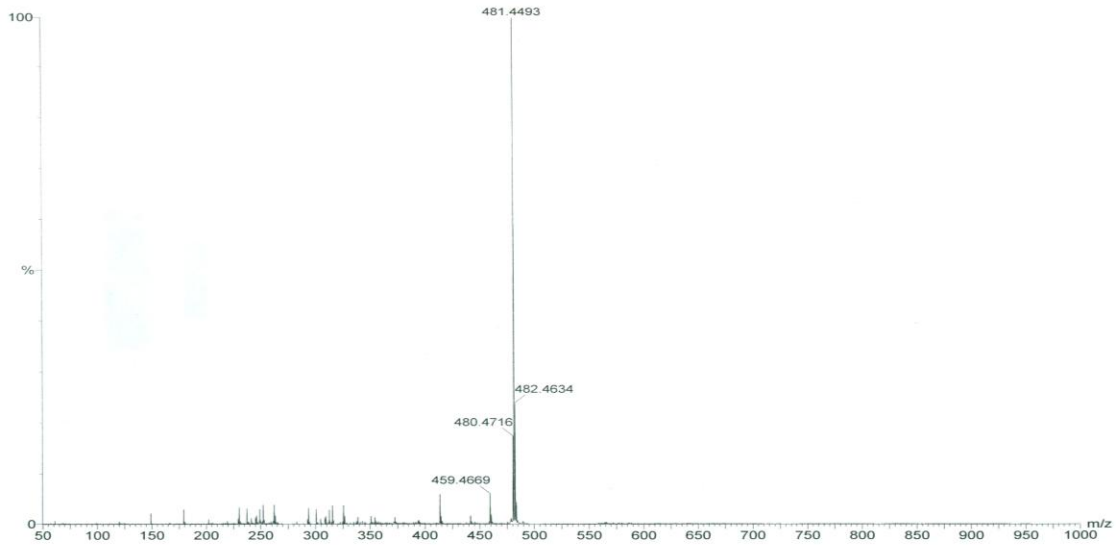

Figure S6: HRMS spectra of peptide 2.

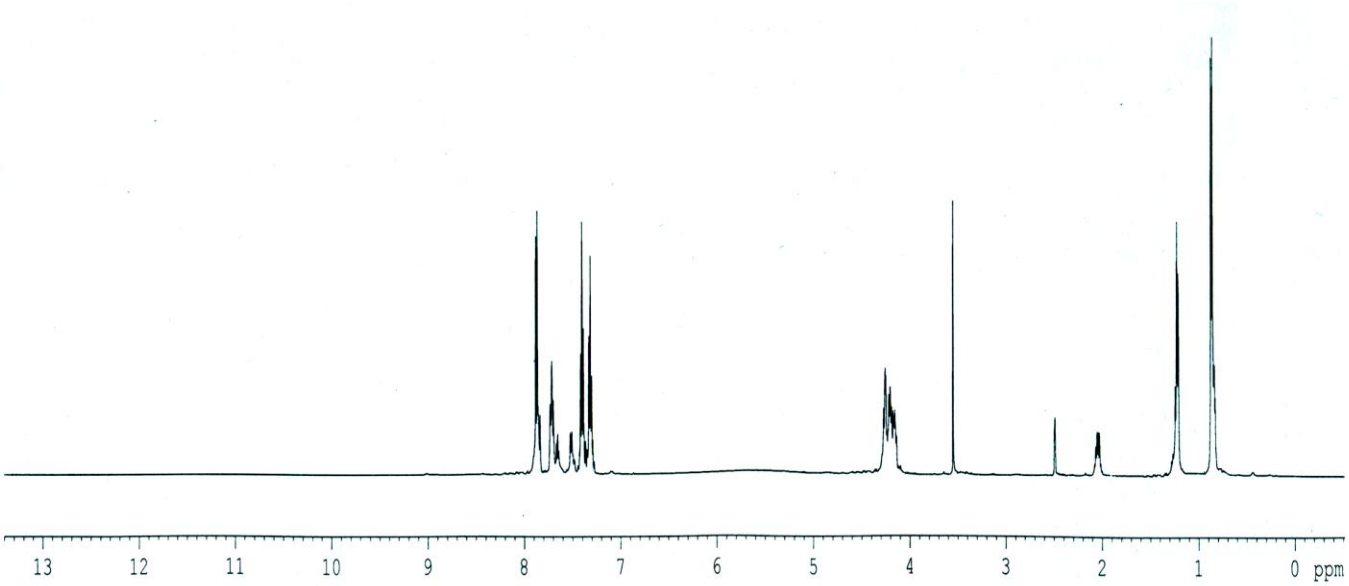

Figure S7: ^{1}H NMR spectra of peptide 3.

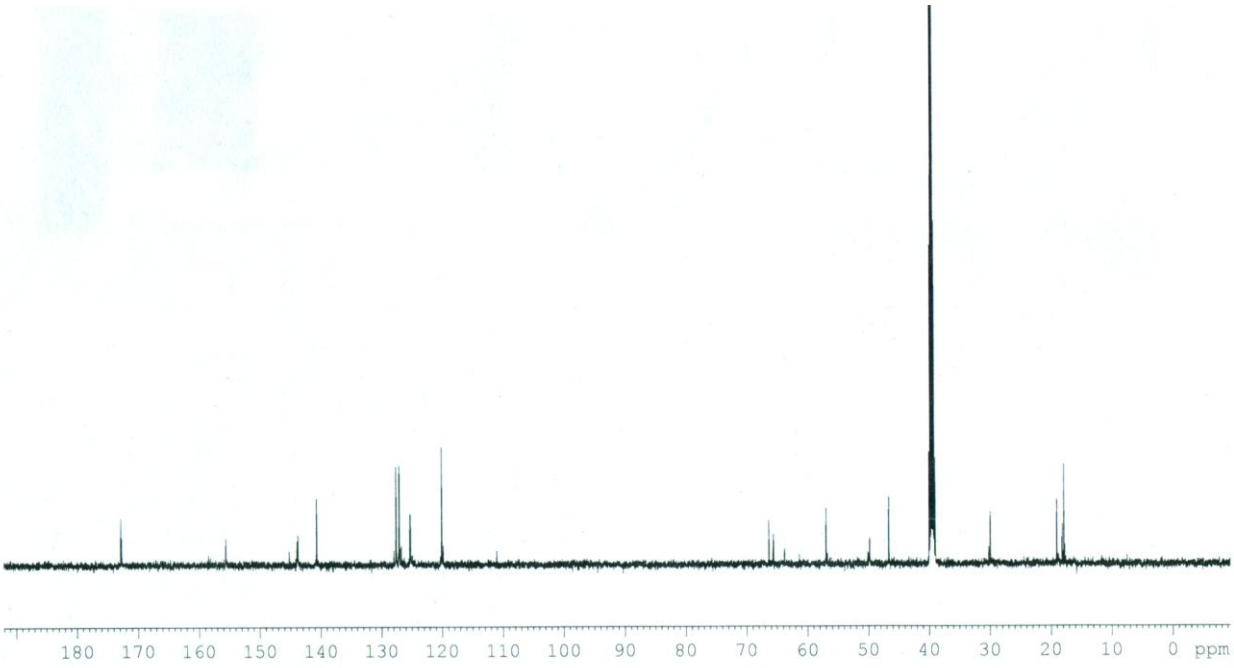

Figure S8: ^{13}C NMR spectra of peptide 3.

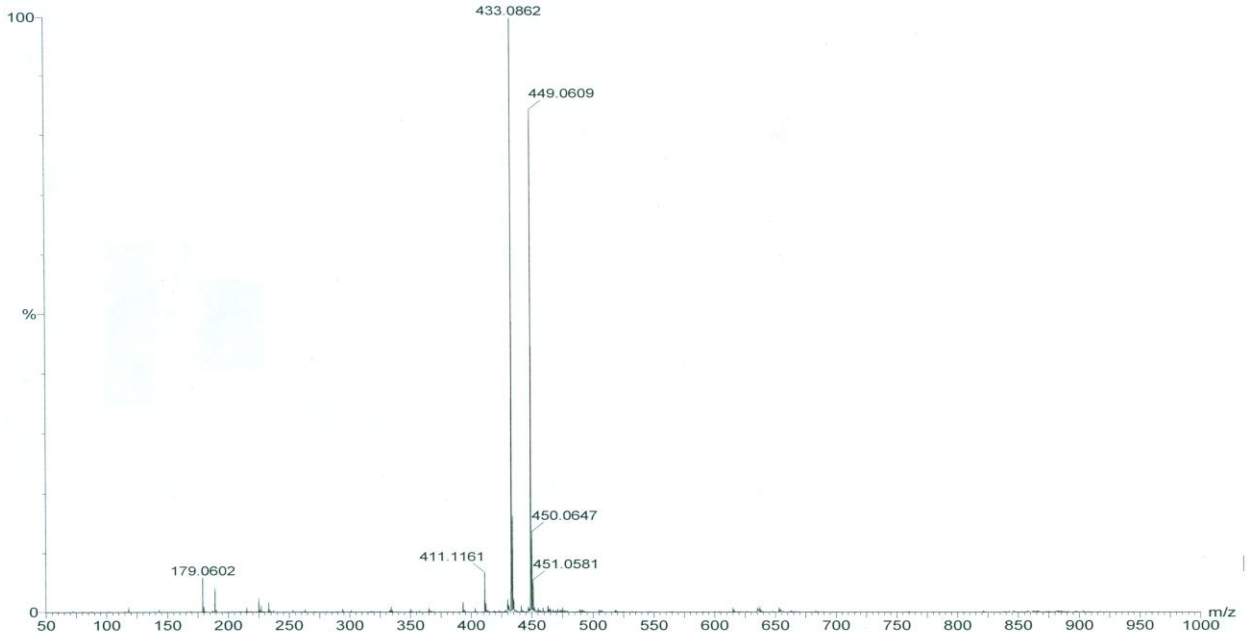

Figure S9: HRMS spectra of peptide 3.

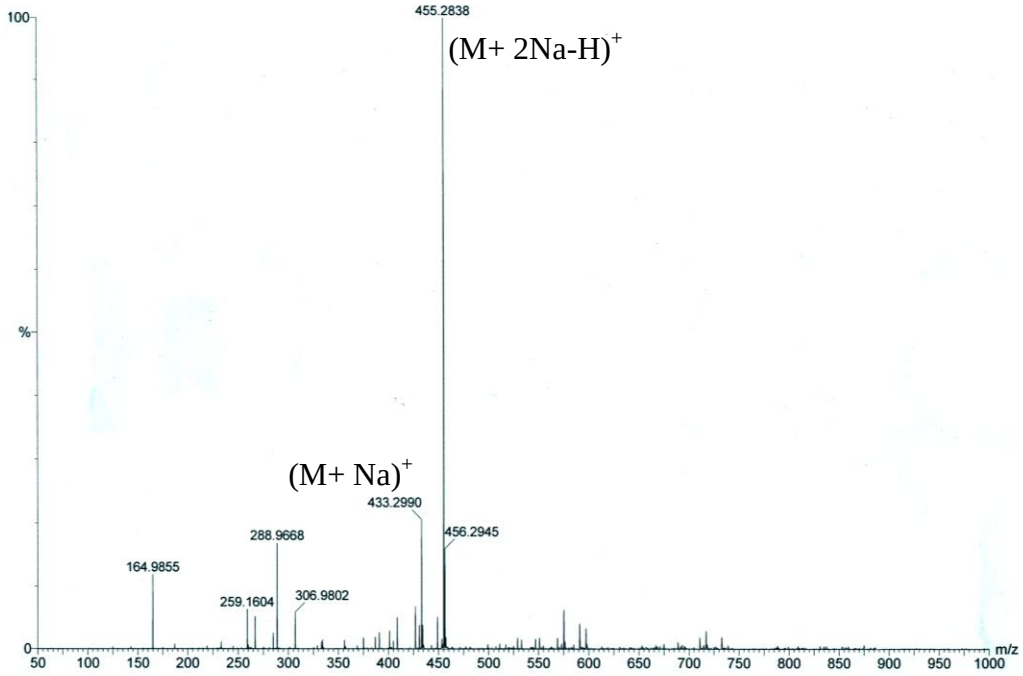

Figure S10: HRMS spectra of peptide 3 under treatment of protenase K enzyme (time 0 hour)

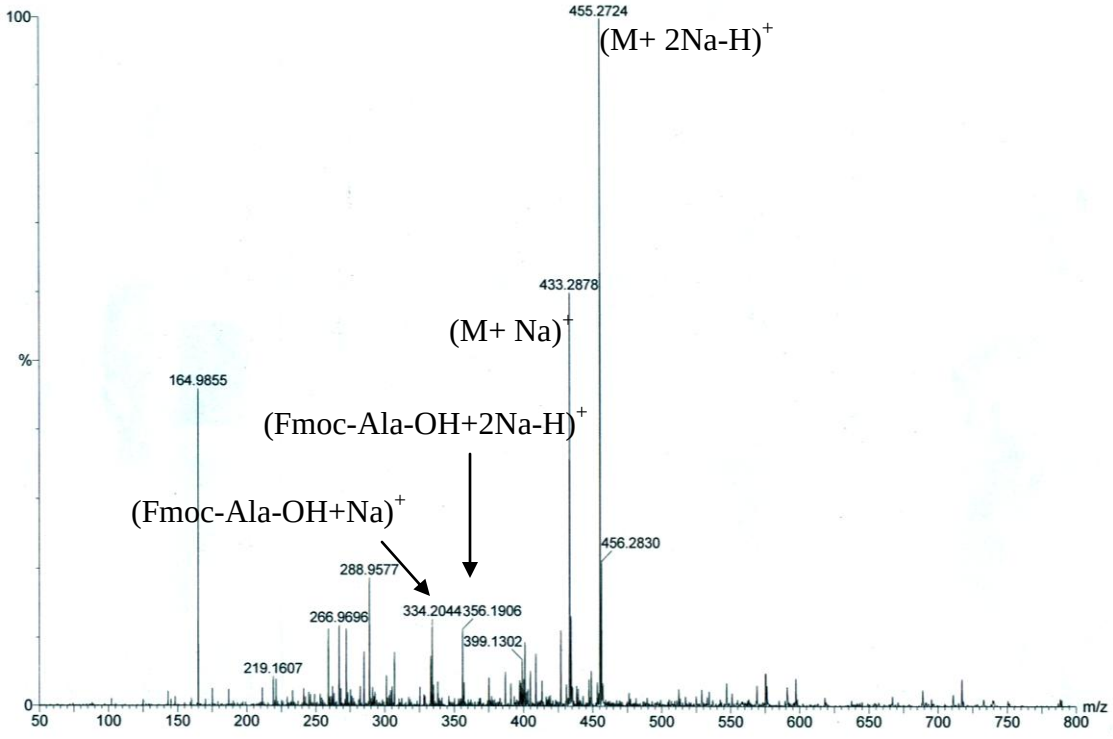

Figure S11: HRMS spectra of peptide 3 under treatment protenase K enzyme (time 4 hour)

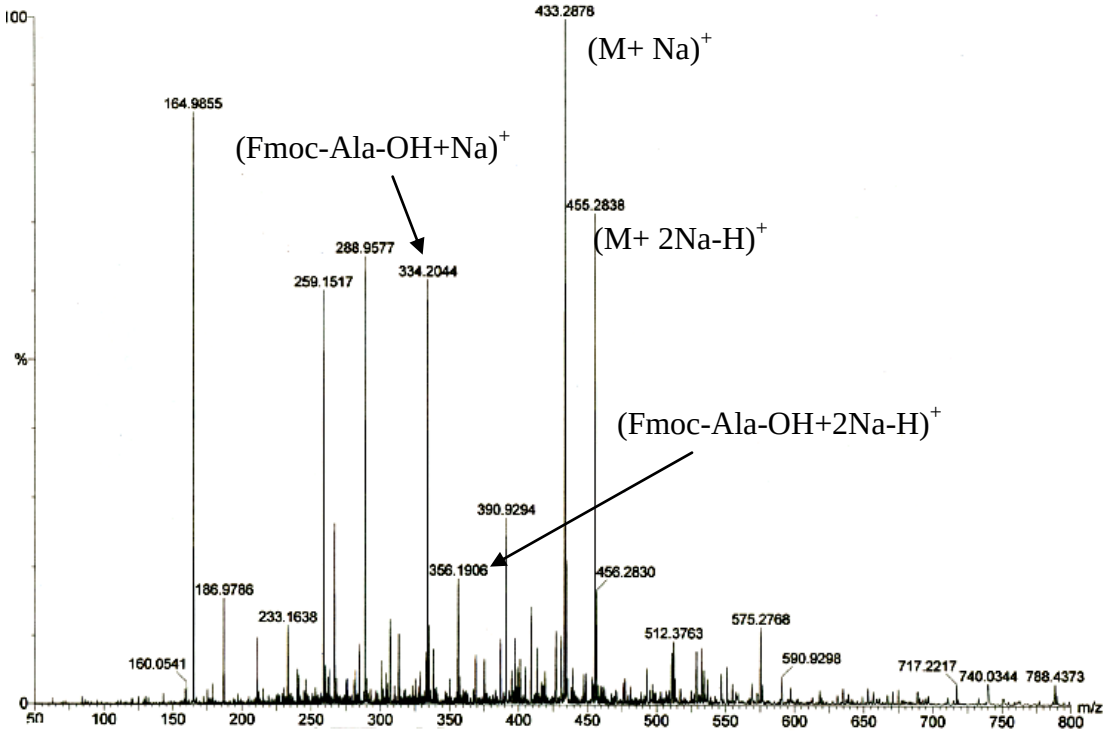

Figure S12: HRMS spectra of peptide 3 under treatment of protenase K enzyme (time 8 hours).

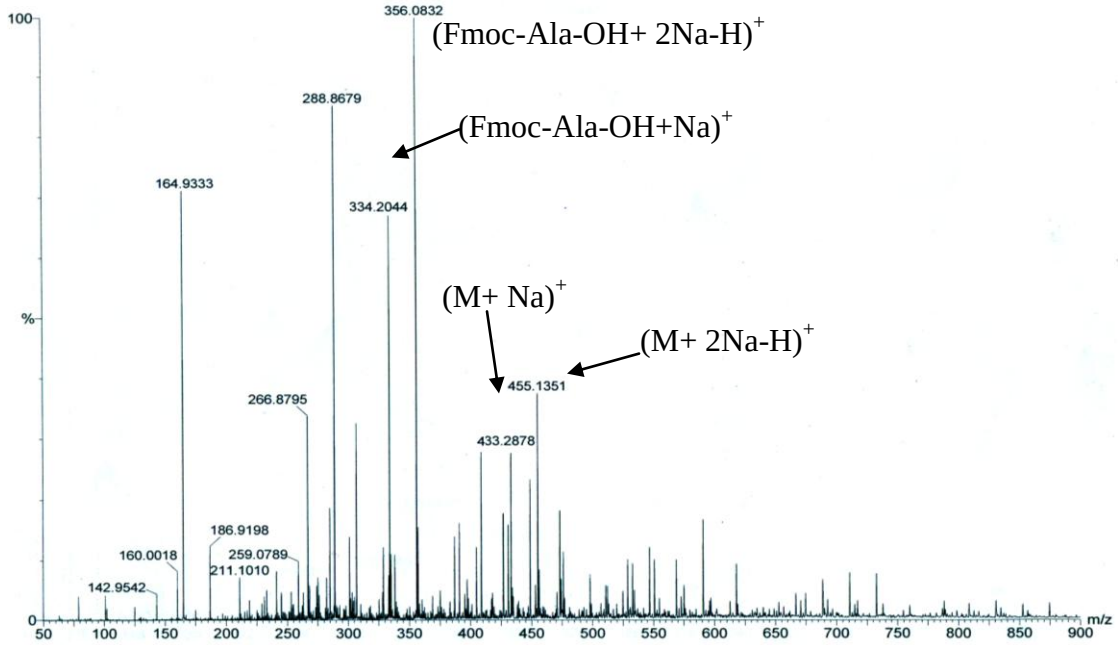

Figure S13: HRMS spectra of peptide 3 under treatment of protenase K enzyme (time 24 hours).

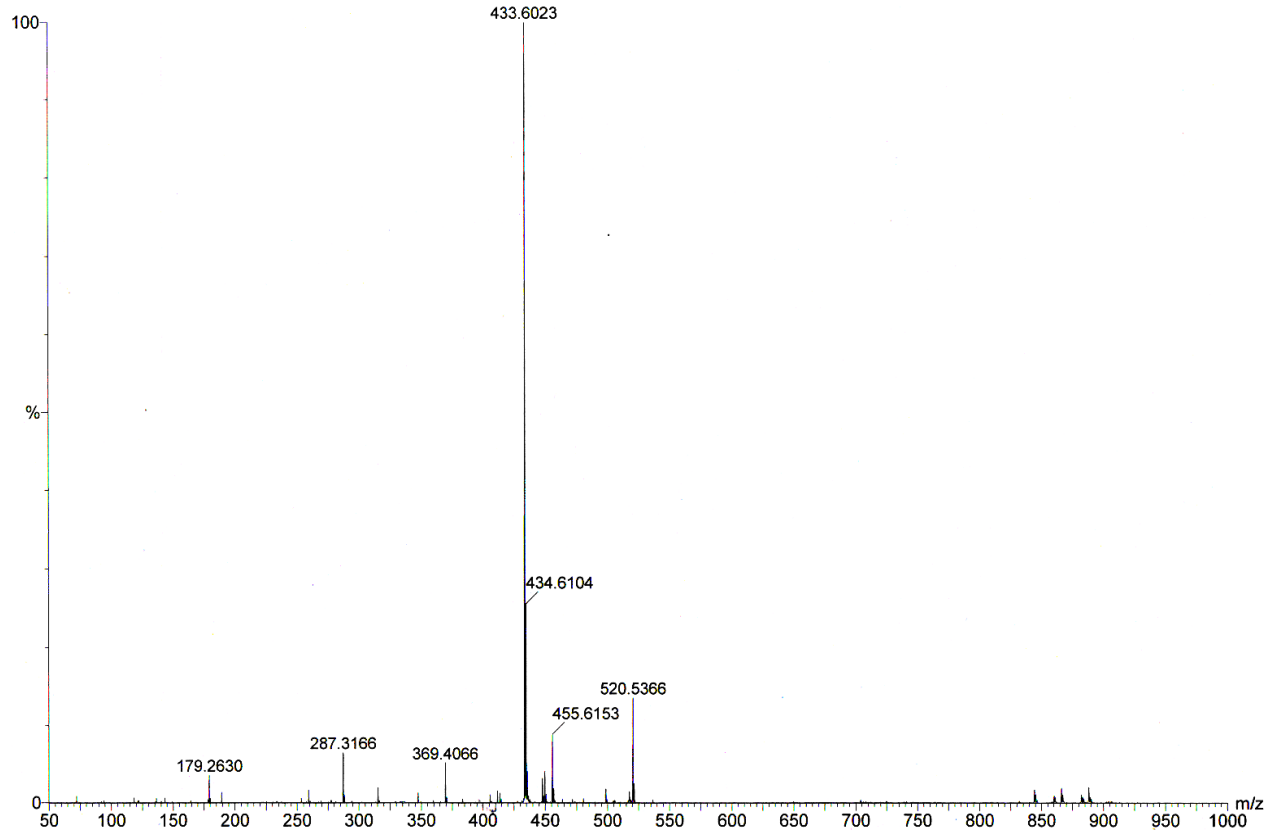

Figure S14: HRMS spectra of peptide 1 under treatment of protease p-5380 enzyme (time 24 hours).

Hydrophobicity and pK_a value calculation:

The replacement of Valine residue in peptide 1 (Fmoc-βAla-Val-OH) by Phenylalanine (peptide 2; Fmoc-βAla-Phe-OH) increases the hydrophobicity of the gelator. The hydrophobicity of these two gelators in presence of the aromatic group (Fmoc) has been estimated from a predicted value of log *P* value, the partition coefficient. The log *P* has a direct correlation with the hydrophobicity of the gelators as described by others earlier [C. Tang *et. al. Langmuir, 2011, 27, 14438–14449* and Adams *et. al.* Soft Matter, 2010, 6, 1971-1980]. The prediction of the hydrophobicity was done using software (http://www.molinspiration.com). The predicted hydrophobicity of peptide 1 and peptide 2 are respectively 1.76 and 2.256. C. Tang *et. al.* in their recent report [C. Tang *et. al. Langmuir, 2011, 27, 14438–14449*] proposed that the apparent increase of pK_a values (experimentally calculated) from the theoretically predicted pK_a indicates the higher hydrophobicity of the gelator than expected and this difference in hydrophobicity have a profound impact on the self-assembly and the gelation process of the gelators. The theoretical pKa values of these two gelators have been calculated using SPARC web calculator to be found at http://ibmlc2.chem.uga.edu/sparc. The predicted pK_a values for the –COOH are 3.85 (for Fmoc-βAla-Val-OH) and 3.70 (for Fmoc-βAla-Phe-OH). Then, the pKa values of the gelator were calculated by titration method[S2].

For titration, 40 mg of Fmoc-dipeptides were taken into 20 ml distilled water (Milli Q) and it was dissolved completely by the slow addition of diluted NaOH solution. After complete dissolution, the final pH of this basic aqueous gelator solution was set at pH 10.26 using excess NaOH solution. At this pH, almost all the Fmoc conjugated dipeptides

are expected to be ionized. The titration experiment was performed by stepwise addition of small volumes of dilute HCl. After each addition of HCl, mixture was shaken well and pH values were recorded using a pH meter. Then, the pH of the solution was plotted against the volume of the dilute HCl added. From the plot, the pKa values were calculated.

The calculated pKa of the peptide 1 (Fmoc-βAla-Val-OH) is 5.9 and hence shift of pKa value from the theoretically calculated pKa value (3.85) is 2.05 unit.

For peptide 2 (Fmoc-βAla-Phe-OH), the pKa value is 6.1 and hence the shift of apparent pka value from the theoretically calculated pKa is 2.4. Our result is in accordance with the previous report [*Langmuir, 2011, 27, 14438–14449*] showing the higher difference in pKa values indicates the higher hydrophobicity. That is why the hydrophobicity of the gelator peptide 2 is more that that of the peptide 1.

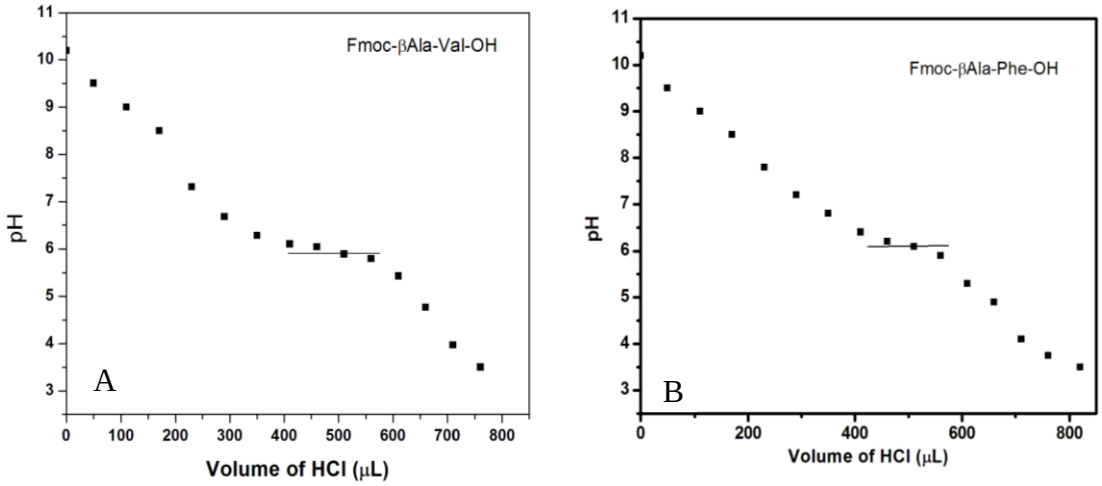

Figure S15: Titration curves for pK_a determination for (a) Fmoc-βAla-Val-OH and (b) Fmoc-βAla-Phe-OH."

Reference S1. S. Guha and A. Banerjee, *Adv. Funct. Mater.* **2009**, *19*, 1949–1961.

S2. B. Adhikari, J. Nanda and A. Banerjee, *Soft Matter*, **2011**, *7*, 8913–8922.