

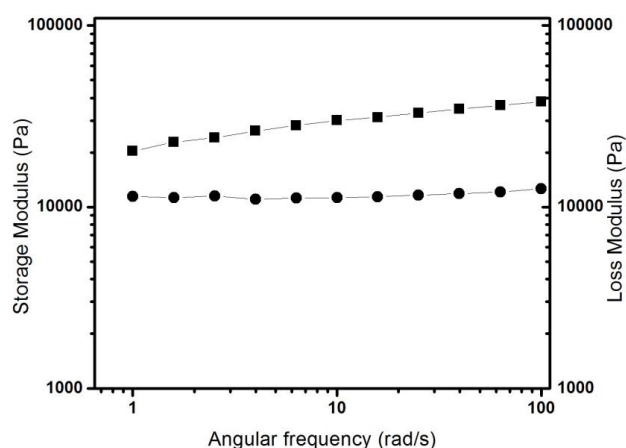
## Supplementary Information

# Redox-Triggered Changes in the Self-Assembly of a Ferrocene-Peptide Conjugate

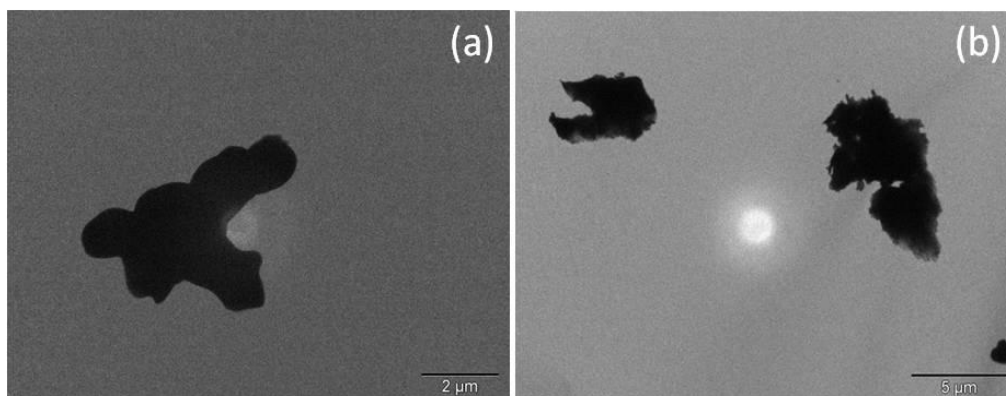
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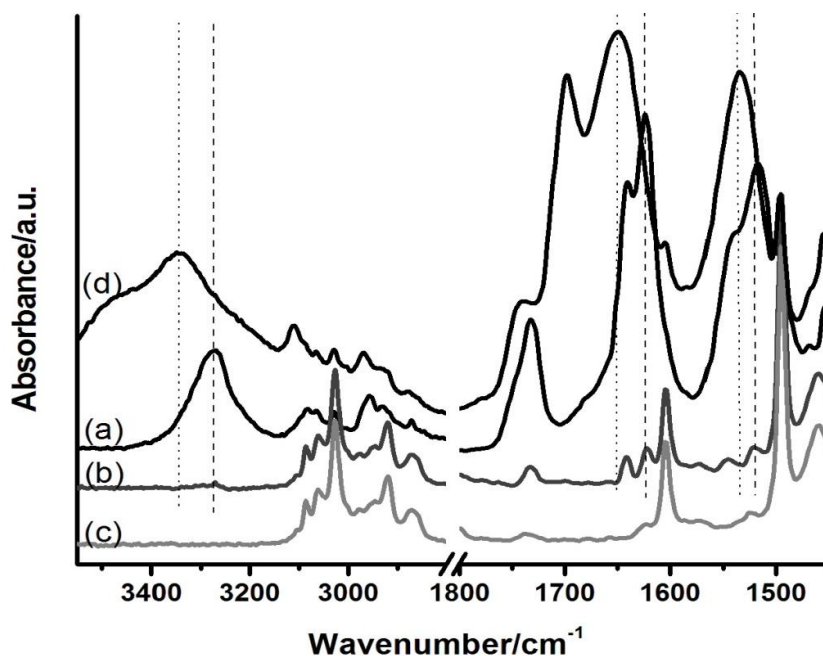
E-mail: [bernie.kraatz@utoronto.ca](mailto:bernie.kraatz@utoronto.ca)



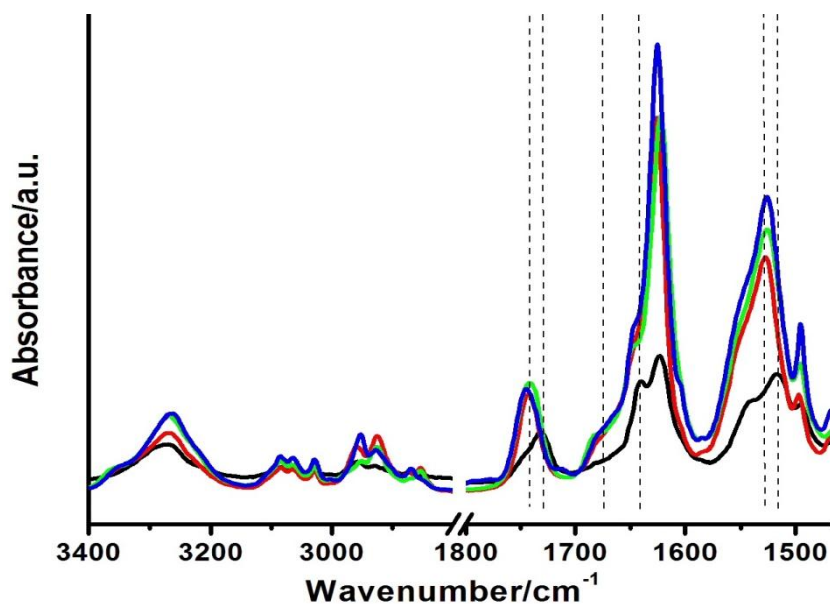
**Fig. S1** Frequency dependence of the dynamic storage moduli ( $G'$ ) and the loss moduli ( $G''$ ) of gel (concentration 2%, w/v) at a constant strain 0.1%. Square and circle represent the  $G'$  and  $G''$  respectively.



**Fig. S2** TEM images of non-gelator, non-amyloidogenic peptides: (a) Fc-peptides **3** and (b) Fc-peptides **4**. Images suggest that these non-amyloidogenic peptides are unable to form any ordered nanofibrillar network structures which is essential for gel formation.

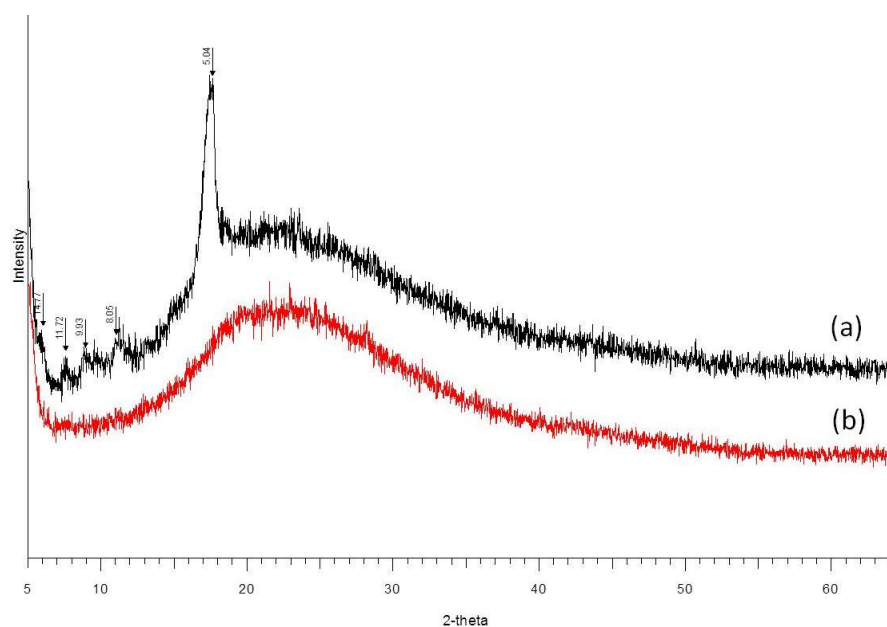


**Fig.S3** FTIR spectra of Fc-peptide **1**: (a) dried gel material; (b) wet gel phase material in toluene; (c) only toluene; (d) oxidized gel. Peaks marked by dashed lines are due to the hydrogen-bonded  $\beta$ -sheet structure of Fc peptide **1** in gel state before oxidation. In contrast, dotted lines represent non-hydrogen-bonded amides due to the disruption of  $\beta$ -sheet structure upon oxidation, evident by the absence of band at 1622 cm<sup>-1</sup>.

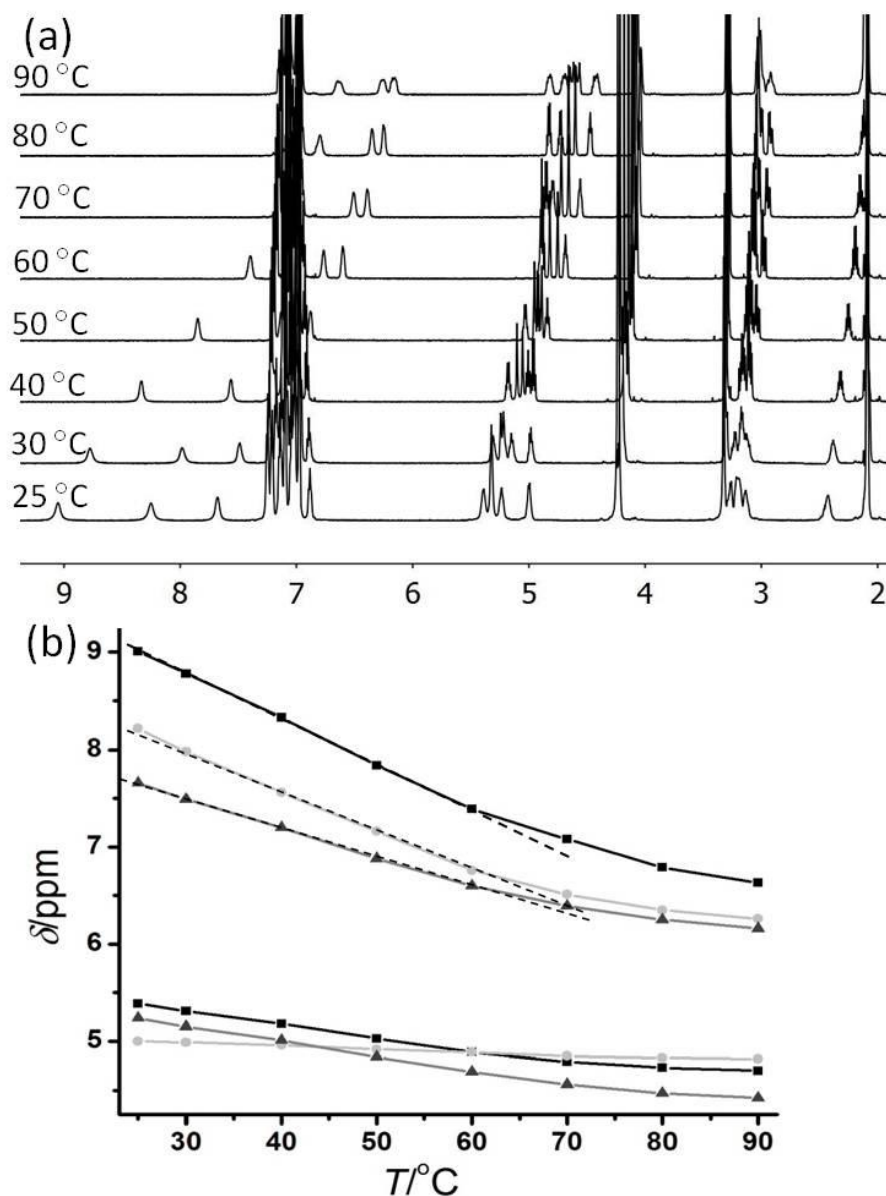


**Fig.S4** FTIR spectra of dried material obtained from toluene for comparison: Fc-peptide **1** (black), Fc-peptide **2** (red), (c) Fc-peptide **3** (green) and Fc-peptide **4** (blue). Figure suggests the significant difference

in peak position and intensities between amyloidogenic gelator peptide and non-amyloidogenic peptides. Some of these peaks are marked by dashed lines. For example, the amide II band at  $1514\text{ cm}^{-1}$  for gelator peptide **1** shifted to  $1529\text{ cm}^{-1}$  for non-gelator peptide **2/3/4**. A small shoulder at  $1682\text{ cm}^{-1}$  was only observed for non-gelator peptide **2/3/4** suggesting their contribution in antiparallel  $\beta$ -sheet structure. In contrast, the absence of this peak in -gelator peptide **1** indicates parallel  $\beta$ -sheet conformation. This might explain their differences in assembling properties for gel formation.



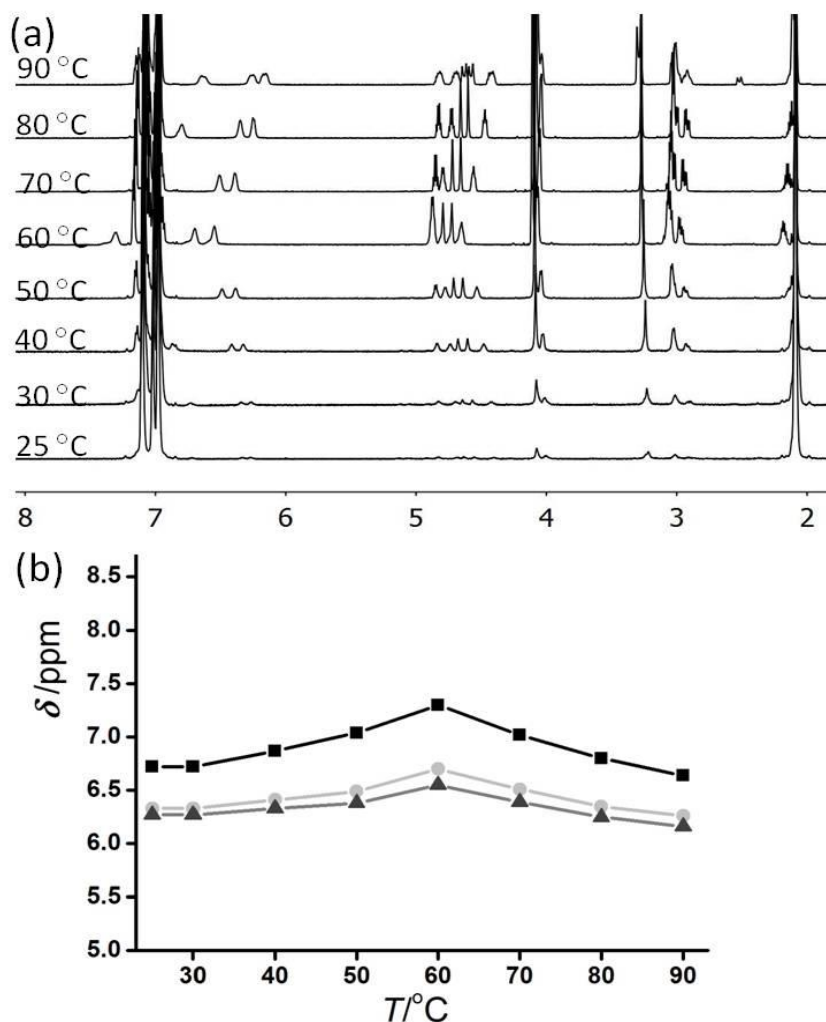
**Fig.S5** XRD studies of Fc-peptide **1**: (a) dried gel phase and (b) oxidized material.



**Fig. S6** VT-NMR of Fc-peptide **1** in Toluene D8 at a concentration of 8 mg/0.5 mL: a solution without treatment of ultrasound, on cooling starting from 90 °C (a) stacked spectra and (b) Plots of chemical shift for three amides (upper) and alpha protons (lower) obtained from spectra; square, circle and triangle represent for Phe(2), Phe(3) and Val(1) respectively. The temperature coefficient values of amide protons were calculated using respective straight lines (30-60 °C)

**Explanation:** It can be noted that, the temperature coefficient is higher for two phenyl alanine than valine, presumably due to presence of additional  $\pi$ - $\pi$  stacking effect. The considerable change in  $\delta$  value (0.68–1.18) of alpha protons were also observed as a consequence of secondary effect of hydrogen bonds.

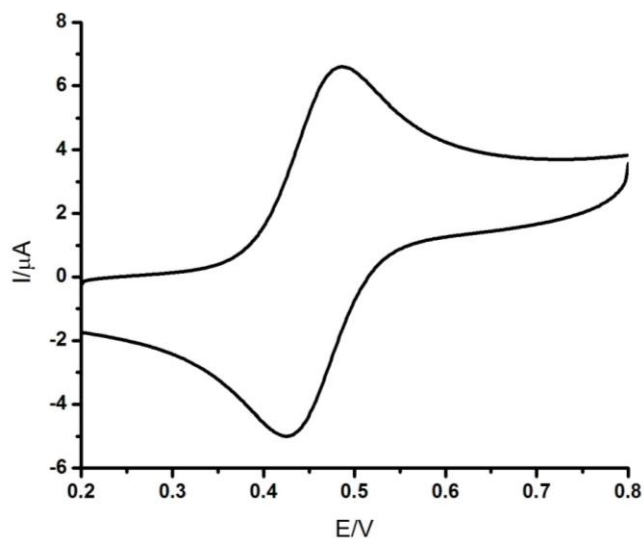
Unlike amides, plots of alpha protons were crossed to each other presumably suggesting a change in conformation of peptides occurred with temperature. It can be note that the NMR peaks are enough sharp even at lower temperature where most of the molecules remain in hydrogen bonded and they are dynamic in nature as they do not form gel in absence of ultrasound.



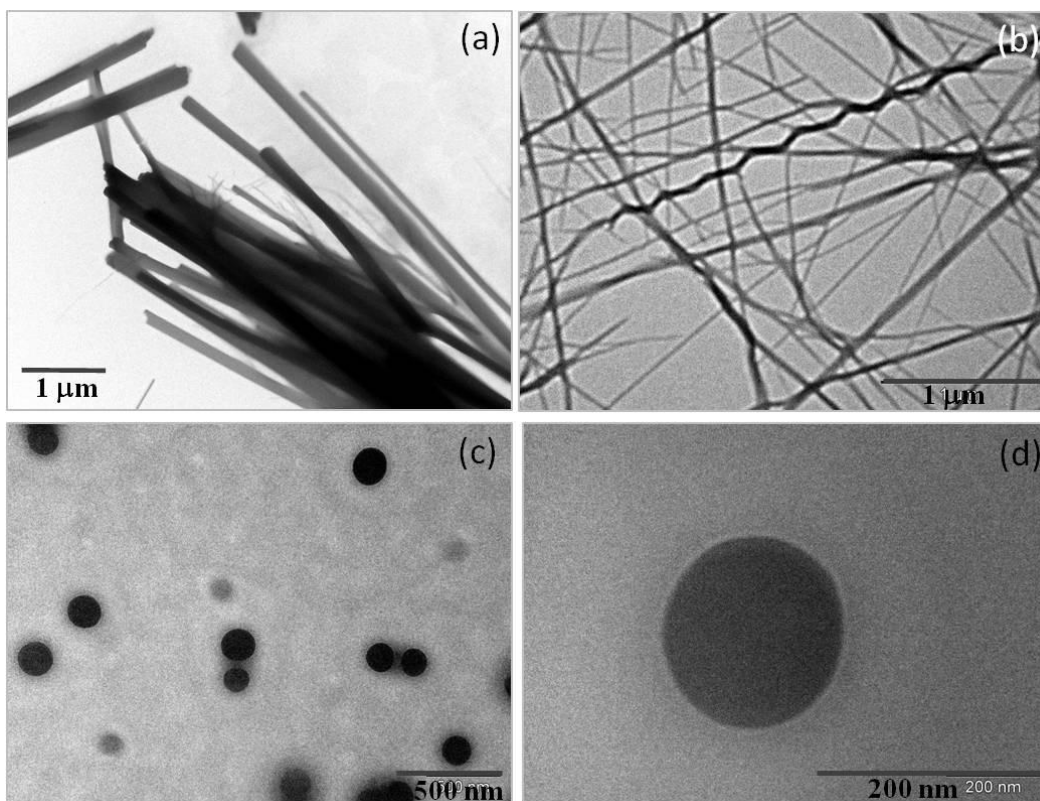
**Fig. S7** VT-NMR of Fc-peptide **1** in Toluene D8 at a concentration of 8 mg/0.5 mL: ultrasound induced gel phase material, on heating starting from 25 °C (a) stacked spectra and (b) Plots of chemical shift for three amide protons obtained from spectra; square, circle and triangle represent for Phe(2), Phe(3) and Val(1) respectively.

Explanation: Initially, at 20/30 °C gel phase material exhibited broad peaks for amide protons as expected because most of the gelator molecules remain in aggregated (polymeric) state and only a few non-aggregated molecules present in gel phase which contribute to NMR signal. With increasing temperature up to gel melting point, aggregated (polymeric) gelators become more soluble/dynamic presumably by transferring into lower aggregated or low molecular weight polymeric state and they

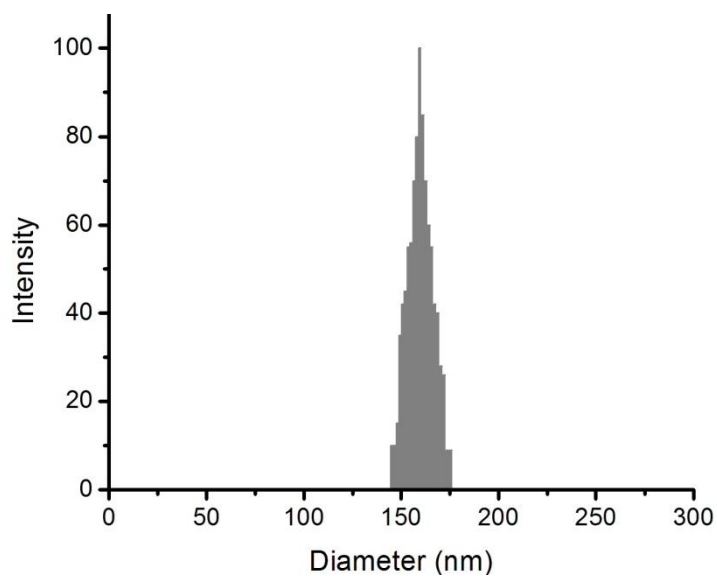
contribute to NMR showing gradual downfield shift as those soluble low molecular weight polymers still remain intermolecularly hydrogen. Then further increase in temperature, the expected gradual upfield shift is due to the gradual transition of aggregated to non-aggregated state by breaking of intermolecular hydrogen bonds.



**Fig. S8** Cyclic voltammogram of Fc-peptide **1** at a scan rate of 0.1 V (0.5 mM solute in MeCN) using LiClO<sub>4</sub> (0.5M) as supporting electrolyte. Glassy carbon was used as working electrode, Ag/AgCl as reference and Pt as counter electrode.



**Fig. S9** TEM images of self-assembled Fc-peptide **1**: (a) without (b) with treatment of ultrasound; (c, d) fully oxidized Fc-peptide **1** with increasing magnification.



**Fig. S10** Dynamic light scattering (DLS) plot of fully oxidized Fc-peptide **1**.



**General Considerations.** Ferrocene and toluene were purchased from Aldrich. Amino acids were purchased from Advanced ChemTech. We used the chemicals as received without further purification. Ferrocene-monodicarboxylic acid was synthesized according to a modified literature procedure (by Perry C. Reeves. C. Reeves in Organic Syntheses, Coll. Vol. 6, p.625 (1988); Vol. 56, p.28 (1977).).

**Fc-Peptide synthesis.** Fc-Peptides were synthesized by conventional solution-phase methodology by using a racemisation-free fragment condensation strategy. The N terminus was protected by a Boc group and the C terminus was protected as a methyl ester. Coupling was mediated by using EDC/HOBt. Deprotection of the methyl ester was performed by using the saponification method. Deprotection of the Boc group was conducted by treatment with TFA. Finally, the ferrocene (Fc) group was introduced into the deprotected dipeptide by coupling with ferrocene monocarboxylic acid. All the compounds were fully characterised by mass spectrometry,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy.

*General Procedure of Peptide Coupling*– In a round bottom flask, 5 mmol of Boc-AA1-OH was dissolved in 50 mL of dichloromethane by stirring and the mixture was cooled around  $0^\circ\text{C}$ . To this mixture, 10 mmol of EDC and 10 mmol of HOBt were added. Then 10 mmol triethylamine (TEA) was added into the reaction mixture and the stirring was continued for 15 min. H-AA2-OMe was isolated from 10 mmol of the corresponding methyl ester hydrochloride by neutralization with TEA and added into the reaction mixture and stirred at room temperature for one day. Then the mixture was worked up with saturated sodium bicarbonate, 10% citric acid solution and finally with brine. This washed organic solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The resulting peptide Boc-AA1-AA2-OMe was purified by flash chromatography.

*General Procedure for the Hydrolysis of Methyl Ester*–To 4 mmol of Boc-AA1-AA2-OMe were added 20 mL MeOH and 5 mL of 1(M) NaOH and the reaction mixture was stirred. After 10 h, methanol was removed under vacuum and the residue was taken in 30 mL of water and washed with diethyl ether. The pH of the aqueous layer was then adjusted to 2-3 using 1(N) HCl, the aqueous layer was extracted with ethyl acetate. The extract were pooled, dried over anhydrous sodium sulfate and evaporated in vacuum.

*General Procedure for the Deprotection of Boc*–2mmol of Boc-protected peptide was dissolved in minimum amount of TFA and stirred at room temperature for 2 hours. The mixture was then evaporated to dryness. It was dissolved in dichloromethane (20 mL) and the remaining TFA was neutralized with TEA. The resulting neutralized solution was concentrated by rotary evaporation in vacuum and used for coupling with ferrocene monocarboxylic acid.

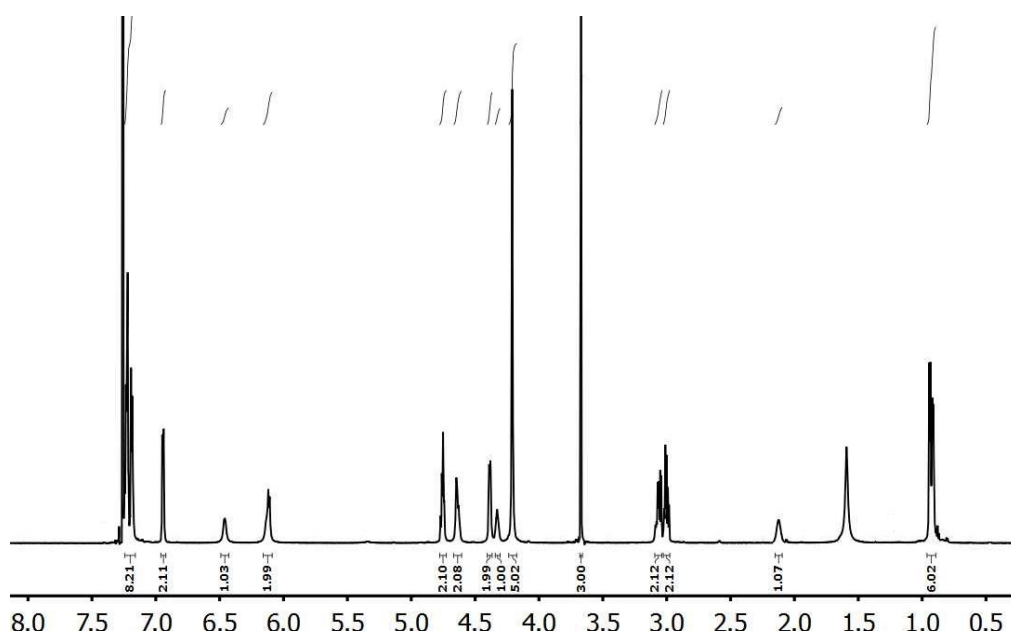
$^1\text{H}$  NMR assignments were made on the basis of chemical shift, relative integration, signal multiplicity, and 2D-NMR experiments such as  $^1\text{H}$ - $^1\text{H}$  gCOSY. The  $^1\text{H}$  and  $^{13}\text{C}\{^1\text{H}\}$  signals produced by the Fc moiety follow the typical signal pattern of a monosubstituted Fc group with the unsubstituted Cp ring producing a singlet near 4.2 ppm, with the meta proton signals further downfield and the ortho protons furthest downfield. The Cp meta protons are relatively far from the stereocenter of the first amino acid residue, and so these diastereotopic protons appear together as a broadened singlet like in all Fc-peptides synthesized. The Cp ortho protons, however, are close enough to the chiral center that it is possible to distinguish these protons spectroscopically. It was important to clearly assign the NH proton resonances of gelator Fc-peptide 1, because the interpretation of NMR titration data depends on these assignments. Hence, we carried out gHMBCAD experiment.



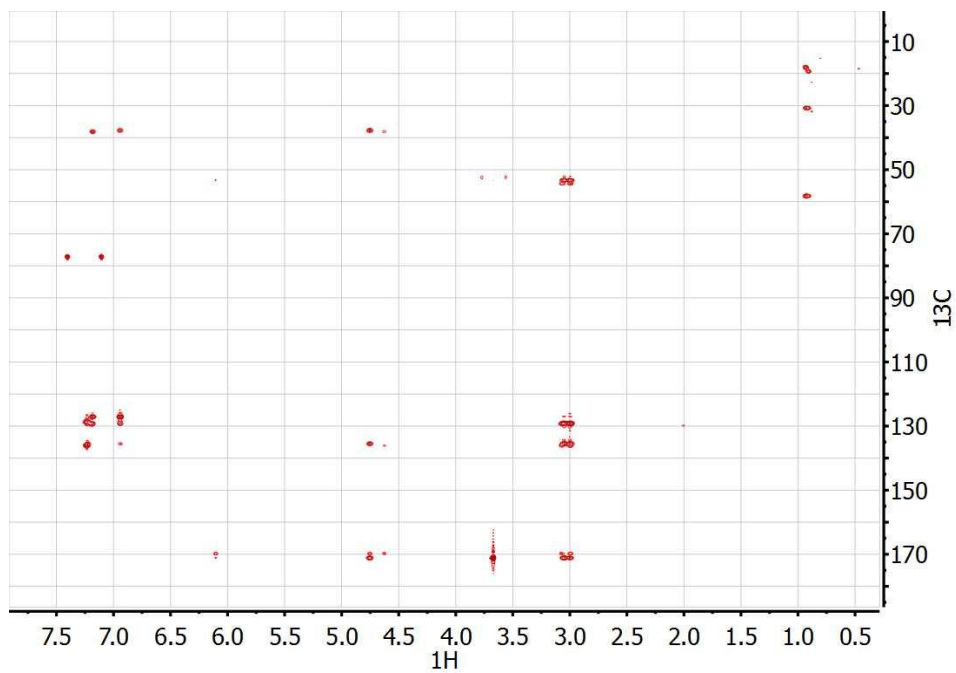
Characterization of Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe:

$^1\text{H}$  NMR (700 MHz,  $\text{CDCl}_3$ , 22° C, Concentration of 2.2 mg/0.6 mL):  $\delta$  7.25–7.18 (m, 8H; aromatic H of two phenyl rings),  $\delta$  6.95–6.94 (m, 2H; aromatic ortho-H of two phenyl rings),  $\delta$  6.47 (d,  $^3J(\text{H}, \text{H}) = 7$  Hz, 1H; NH of Phe(2)),  $\delta$  6.12 (d,  $^3J(\text{H}, \text{H}) = 7$  Hz, 2H, NH of Phe(3) and NH of Val(1)), 4.77–4.74 (2H; m, ortho-H of Cp ring which is directly attached to peptide segment and  $\alpha$  CH of Phe(3)), 4.65–4.63 (2H; m, ortho-H of Cp ring which is directly attached to peptide segment and  $\alpha$  CH of Phe(2)), 4.39–4.38 (2H; m, meta-H of Cp ring which is directly attached to peptide segment), 4.33 (1H, m;  $\alpha$  CH of Val(1)),  $\delta$  4.21 (br, 5H; H of another Cp ring which is not directly attached to peptide segment),  $\delta$  3.67 (s, 3H,  $\text{OCH}_3$ ), 3.07 (m, 1H;  $\beta$   $\text{CH}_2$  of Phe(2)),  $\delta$  3.05 (m, 1H;  $\beta$   $\text{CH}_2$  of Phe(3)),  $\delta$  3.00 (m, 1H;  $\beta$   $\text{CH}_2$  of Phe(3)),  $\delta$  2.99 (m, 1H;  $\beta$   $\text{CH}_2$  of Phe(2)),  $\delta$  2.13 – 2.12 (m, 2H;  $\beta$   $\text{CH}_2$  of Val(1)),  $\delta$  0.94–0.91 (m, 6H,  $\gamma$   $\text{CH}_3$ ); Anal. Calcd. for  $\text{C}_{35}\text{H}_{39}\text{FeN}_3\text{O}_5$  (637.55): MS (ESI)  $m/z$  637.22  $[\text{M}]^+$ , 660.21  $[\text{M}+\text{Na}]^+$ .

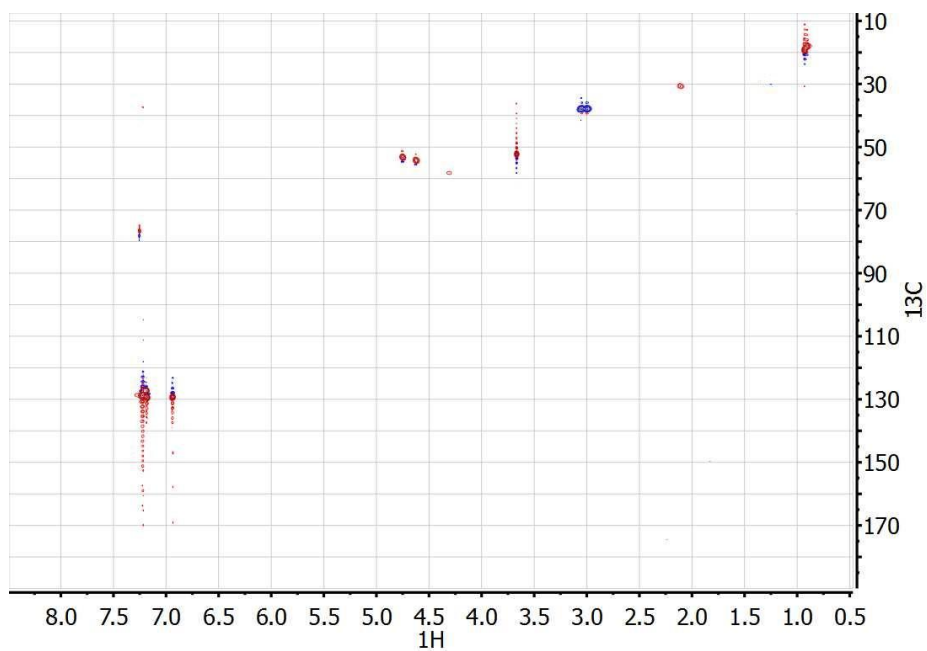
$^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ , 22° C, Concentration of 6 mg/0.6 mL):  $\delta$  171.06 (C of  $\text{COOMe}$ ; Phe(3)),  $\delta$  169.83 (C of  $\text{CONH}$ ; Phe(2)),  $\delta$  136.11 (ipso carbon of phenyl ring; Phe(2)),  $\delta$  135.48 (ipso carbon of phenyl ring; Phe(3)),  $\delta$  129.34 (2C;  $o$ -C of phenyl ring),  $\delta$  129.16 (2C;  $o$ -C of phenyl ring),  $\delta$  128.73 (2C;  $m$ -C of phenyl ring),  $\delta$  128.59 (2C;  $m$ -C of phenyl ring),  $\delta$  127.12 (2C;  $p$ -C of phenyl ring),  $\delta$  77.57–76.72 (C of  $\text{CDCl}_3$ ),  $\delta$  70.13 (carbon of aromatic ferrocene),  $\delta$  59.22 ( $\alpha$  C of Val(1)),  $\delta$  54.26 ( $\alpha$  C of Phe(2)),  $\delta$  53.25 ( $\alpha$  C of Phe(3)),  $\delta$  52.26 (C of  $\text{OCH}_3$ ),  $\delta$  30.69 ( $\beta$   $\text{CH}_2$  of Val(1)),  $\delta$  37.9 ( $\beta$   $\text{CH}_2$  of Phe(3)),  $\delta$  38.12 ( $\beta$   $\text{CH}_2$  of Phe(2)),  $\delta$  19.33 ( $\gamma$   $\text{CH}_3$  of Val(1)),  $\delta$  18.02 ( $\text{CH}_3$  of Val(1)).



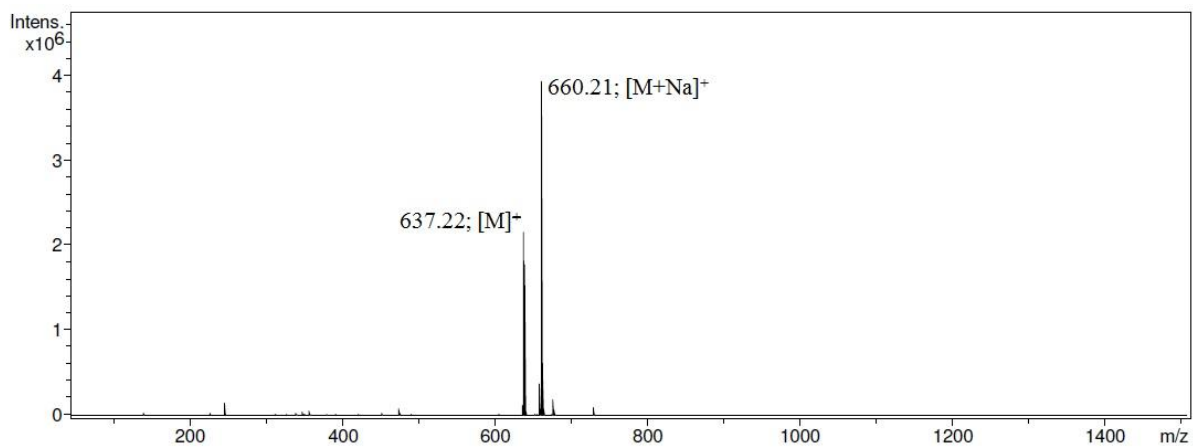
**Fig.S11** <sup>1</sup>H NMR spectrum of Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe in CDCl<sub>3</sub>.



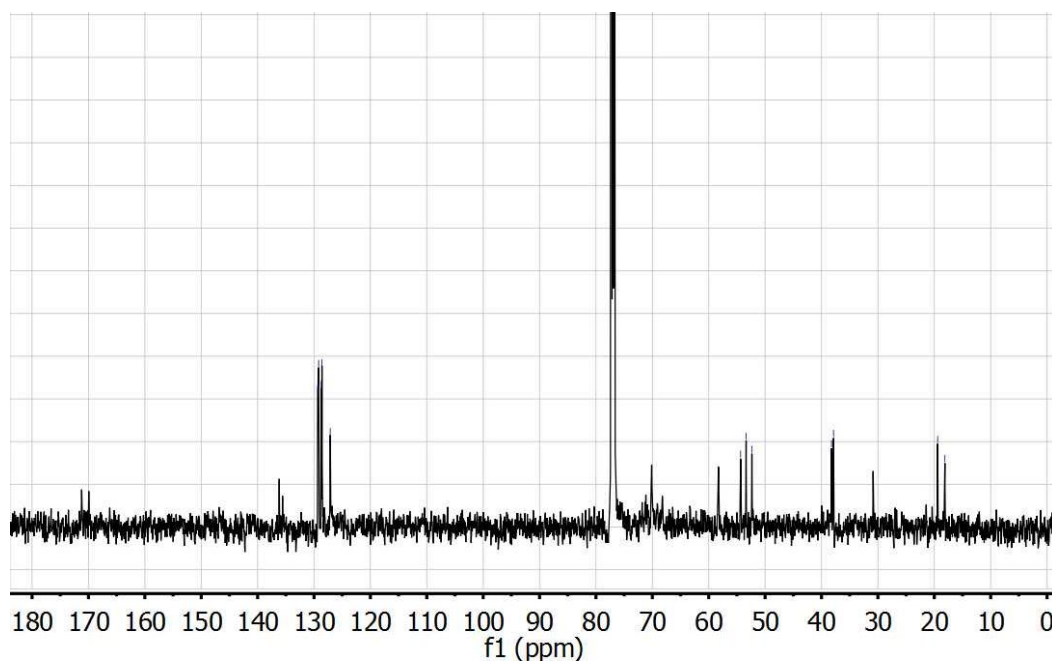
**Fig.S12** gHMBCAD spectrum of Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe in CDCl<sub>3</sub>.



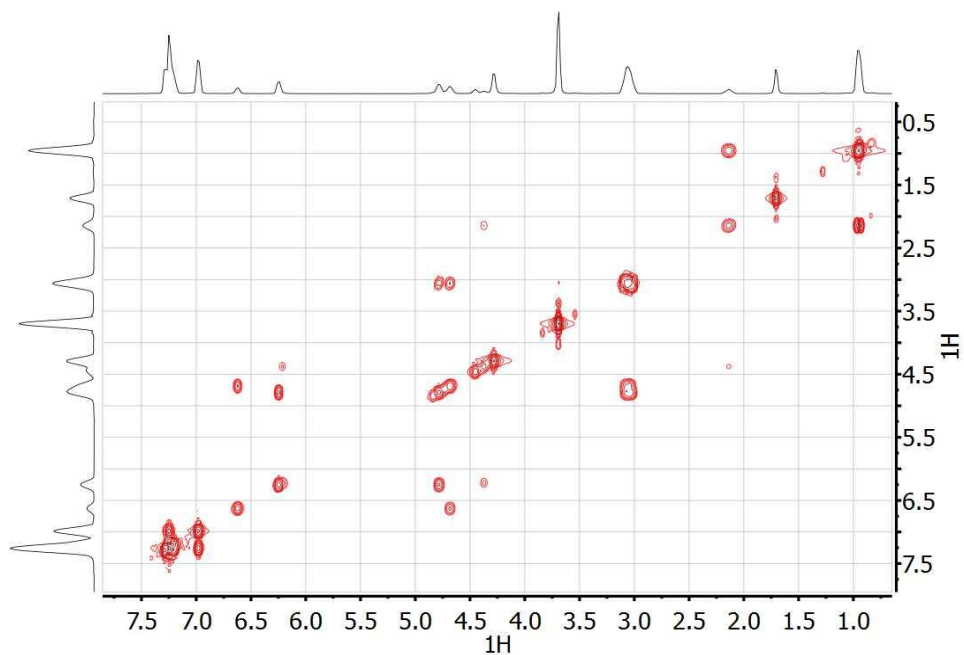
**Fig.S13** Gc2hsqcse spectrum of Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe in  $\text{CDCl}_3$ .



**Fig.S14** MS spectrum of Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe.



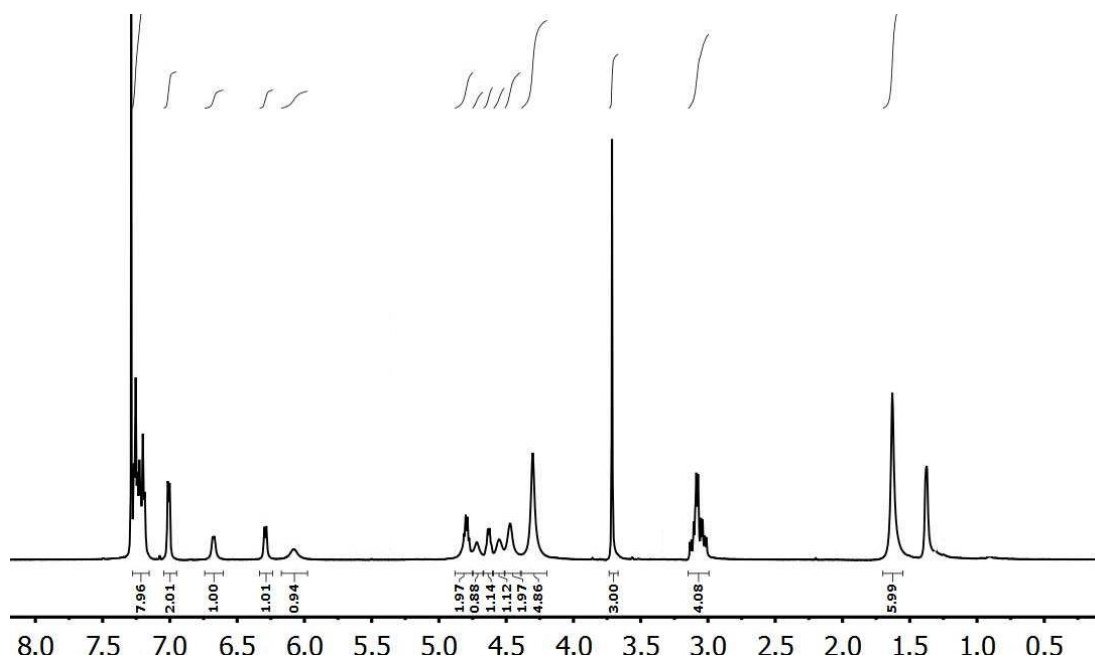
**Fig.S15**  $^{13}\text{C}$  spectrum of Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe in  $\text{CDCl}_3$ .



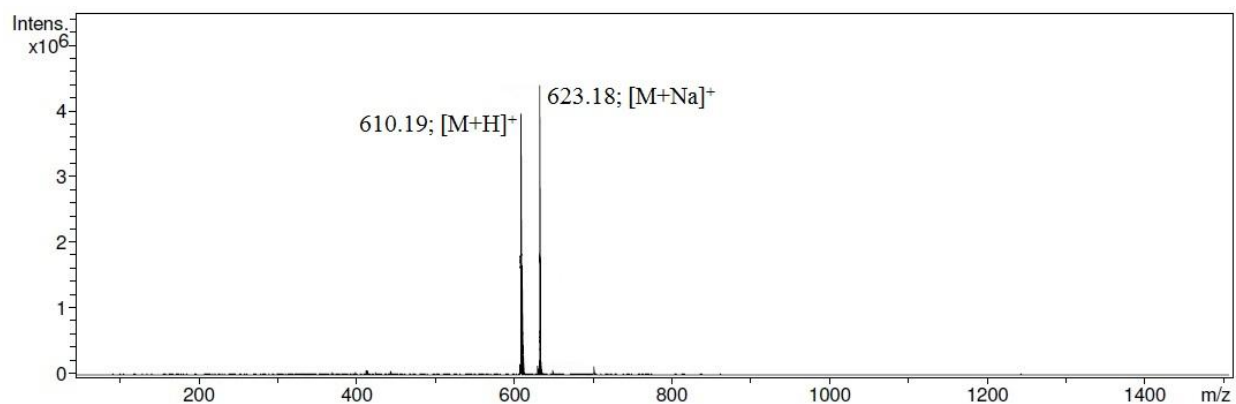
**Fig.S16**  $^1\text{H}$ - $^1\text{H}$  gCOSY spectrum of Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe in  $\text{CDCl}_3$ .

Characterization of Fc-CO-Ala(1)-Phe(2)-Phe(3)-OMe:

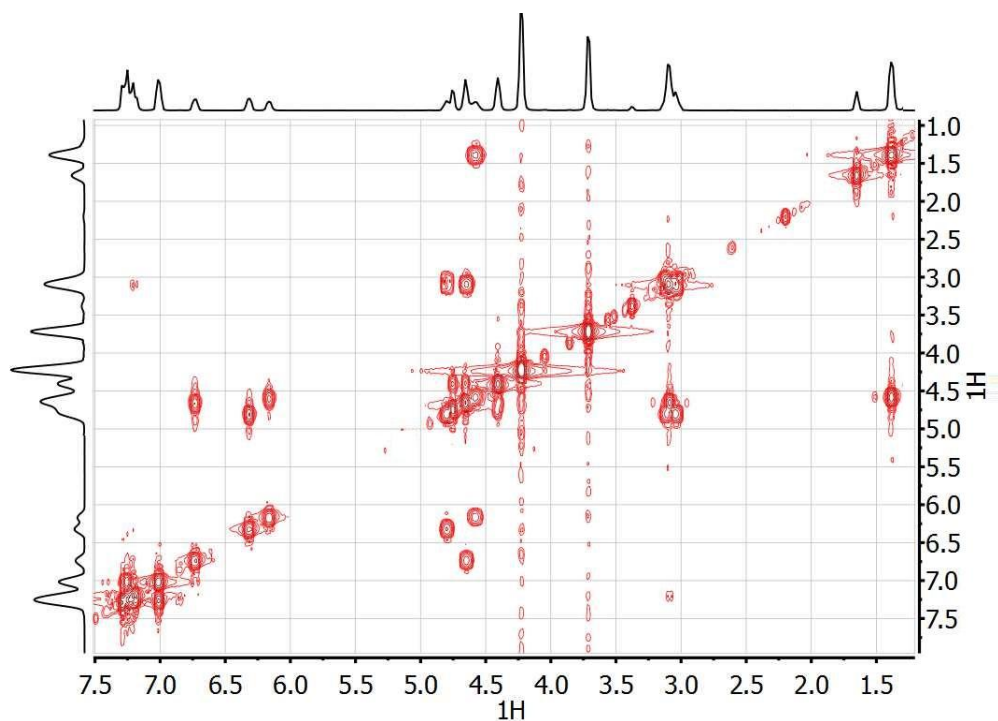
$^1\text{H}$  NMR (700 MHz,  $\text{CDCl}_3$ ,  $22^\circ\text{C}$ ):  $\delta$  7.27–7.19 (m, 8H; aromatic H of two phenyl rings),  $\delta$  7.02–7.00 (m, 2H; aromatic ortho-H of two phenyl rings),  $\delta$  6.68 (d,  $^3J(\text{H}, \text{H}) = 7\text{ Hz}$ , 1H; NH of Phe(2)),  $\delta$  6.30 (d,  $^3J(\text{H}, \text{H}) = 9\text{ Hz}$ , 1H, NH of Phe(3)),  $\delta$  6.08 (br, 1H, NH of Ala(1)), 4.82–4.77 (2H; m, ortho-H of Cp ring which is directly attached to peptide segment and  $\alpha$  CH of Phe(3)), 4.72 (1H; br, ortho-H of Cp ring which is directly attached to peptide segment), 4.65–4.61 (1H; m,  $\alpha$  CH of Phe(2)), 4.55 (1H, m;  $\alpha$  CH of Ala(1)), 4.47 (2H; br, meta-H of Cp ring which is directly attached to peptide segment),  $\delta$  4.30 (br, 5H; H of another Cp ring which is not directly attached to peptide segment),  $\delta$  3.71 (s, 3H,  $\text{OCH}_3$ ), 3.13–3.01 (m, 4H;  $\beta$   $\text{CH}_2$  of Phe),  $\delta$  1.38–1.37 (d, 2H;  $\beta$   $\text{CH}_3$  of Ala(1)); Anal. Calcd. for  $\text{C}_{33}\text{H}_{35}\text{FeN}_3\text{O}_5$  (609.49): MS (ESI)  $m/z$  610.19 $[\text{M}]^+$ , 632.18 $[\text{M}+\text{Na}]^+$ .



**Fig.S17**  $^1\text{H}$  NMR spectrum of Fc-CO-Ala(1)-Phe(2)-Phe(3)-OMe in  $\text{CDCl}_3$ .



**Fig.S18** MS spectrum of Fc-CO-Ala(1)-Phe(2)-Phe(3)-OMe.

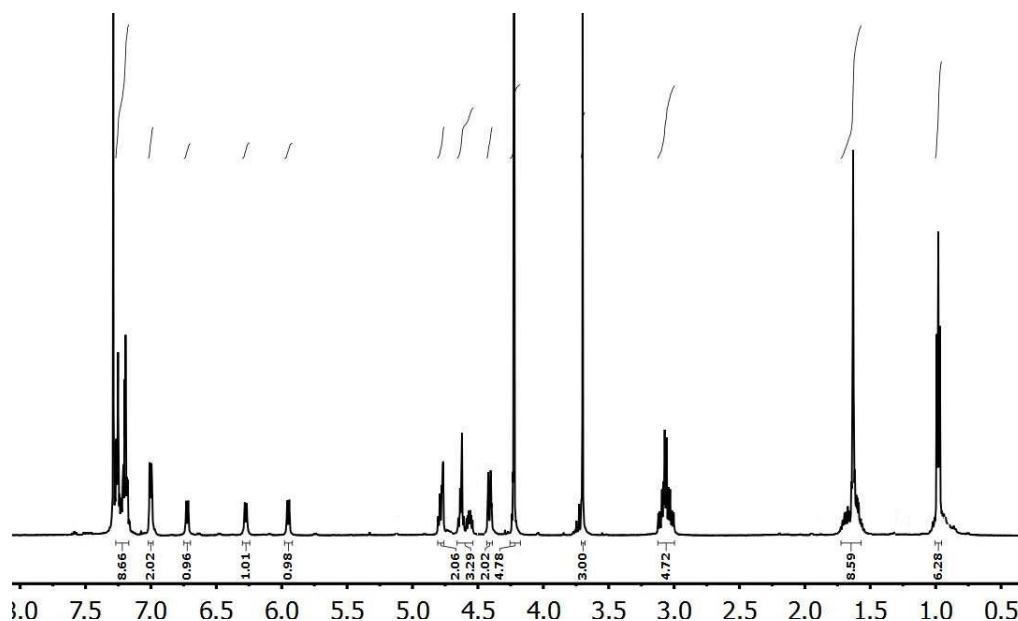


**Fig.S19** <sup>1</sup>H-<sup>1</sup>H gCOSY spectrum of Fc-CO-Ala(1)-Phe(2)-Phe(3)-OMe in CDCl<sub>3</sub>.

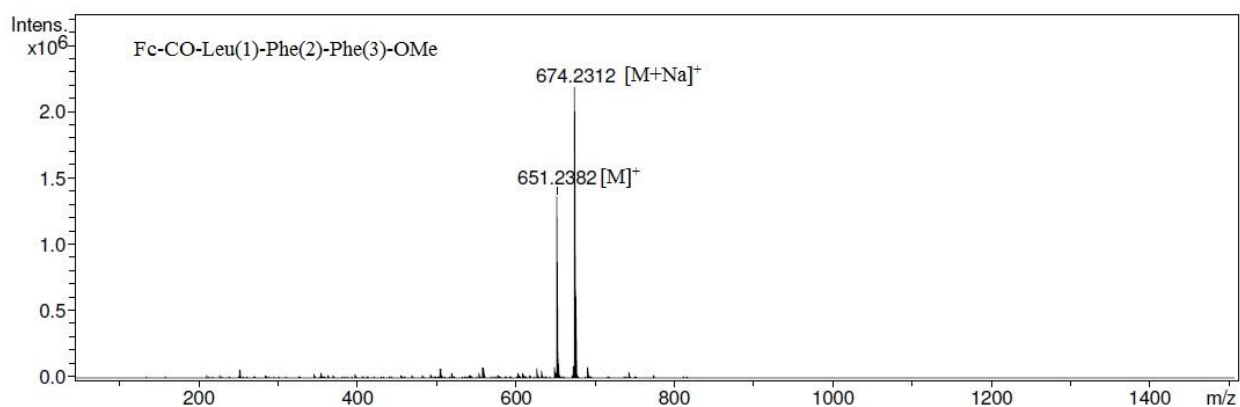


Characterization of Fc-CO-Leu(1)-Phe(2)-Phe(3)-OMe:

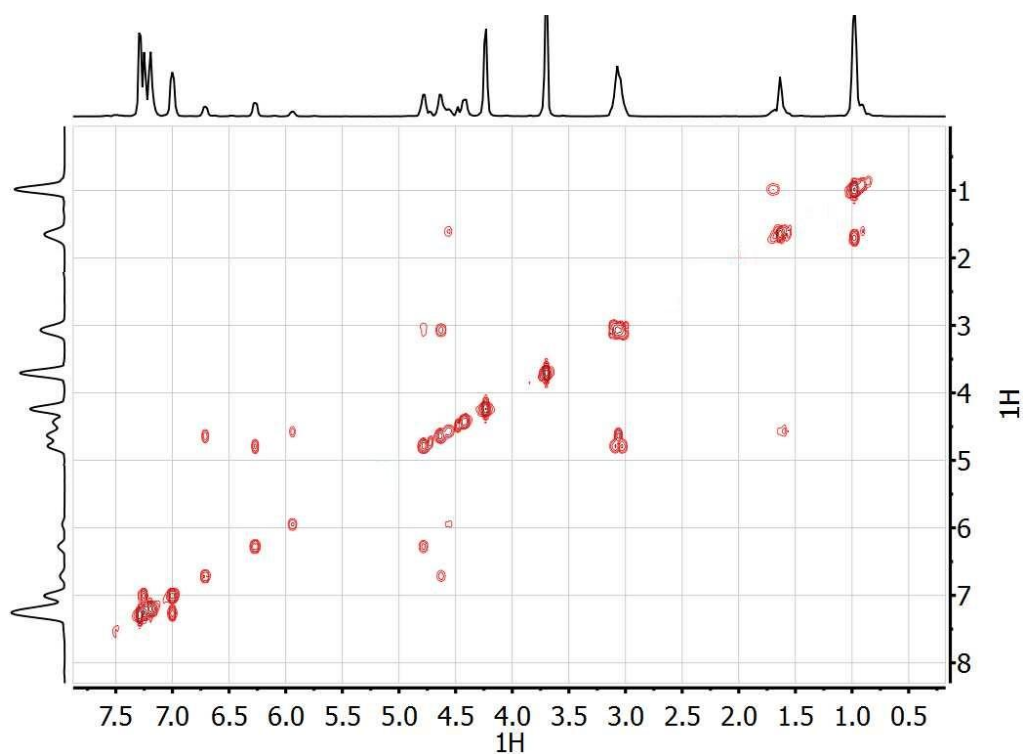
$^1\text{H}$  NMR (700 MHz,  $\text{CDCl}_3$ ,  $22^\circ\text{C}$ ):  $\delta$  7.26–7.16 (m, 8H; aromatic H of two phenyl rings),  $\delta$  7.00–6.99 (m, 2H; aromatic ortho-H of two phenyl rings),  $\delta$  6.72 (d,  $^3J(\text{H}, \text{H}) = 7.5\text{ Hz}$ , 1H; NH of Phe(2)),  $\delta$  6.28 (d,  $^3J(\text{H}, \text{H}) = 7.5\text{ Hz}$ , 1H, NH of Phe(3)),  $\delta$  5.95 (d,  $^3J(\text{H}, \text{H}) = 8\text{ Hz}$ , 1H; NH of Leu(1)), 4.78–4.76 (2H; m, ortho-H of Cp ring which is directly attached to peptide segment and  $\alpha$  CH of Phe(3)), 4.63–4.53 (3H; m, ortho-H of Cp ring which is directly attached to peptide segment,  $\alpha$  CH of Phe(2) and  $\alpha$  CH of Leu(1)), 4.42–4.39 (2H, m; meta-H of Cp ring which is directly attached to peptide segment),  $\delta$  4.21 (br, 5H; H of another Cp ring which is not directly attached to peptide segment),  $\delta$  3.70 (s, 3H,  $\text{OCH}_3$ ), 3.12–3.00 (m, 4H;  $\beta$   $\text{CH}_2$  of Phe),  $\delta$  1.70–1.57 (m, 3H;  $\beta$  and  $\gamma$  H of Leu(1),  $\delta$  0.99–0.96 (m, 6H;  $\delta$  H of Leu(1)); Anal. Calcd. for  $\text{C}_{36}\text{H}_{41}\text{FeN}_3\text{O}_5$  (651.57): MS (ESI)  $m/z$  674.23 $[\text{M}+\text{Na}]^+$ .



**Fig.S20**  $^1\text{H}$  NMR spectrum of Fc-CO-Leu(1)-Phe(2)-Phe(3)-OMe in  $\text{CDCl}_3$ .



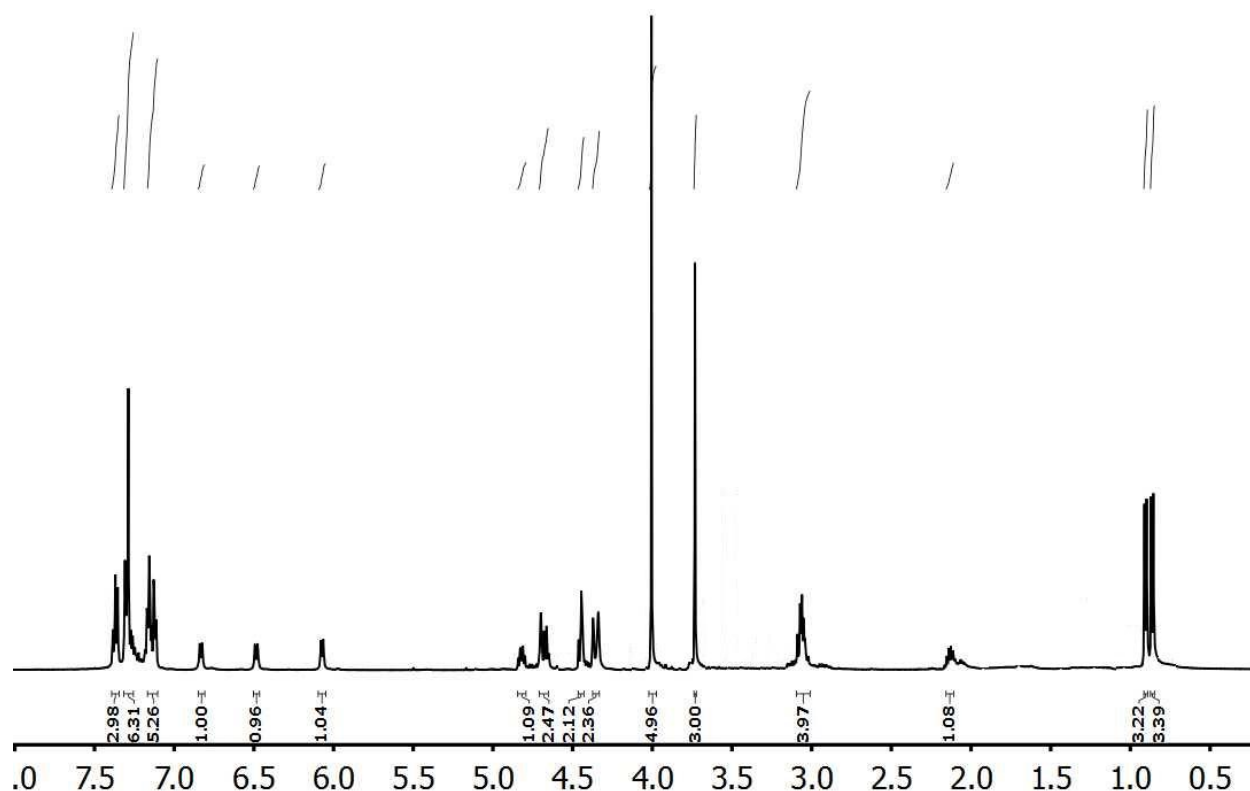
**Fig.S21** MS spectrum of Fc-CO-Leu(1)-Phe(2)-Phe(3)-OMe.



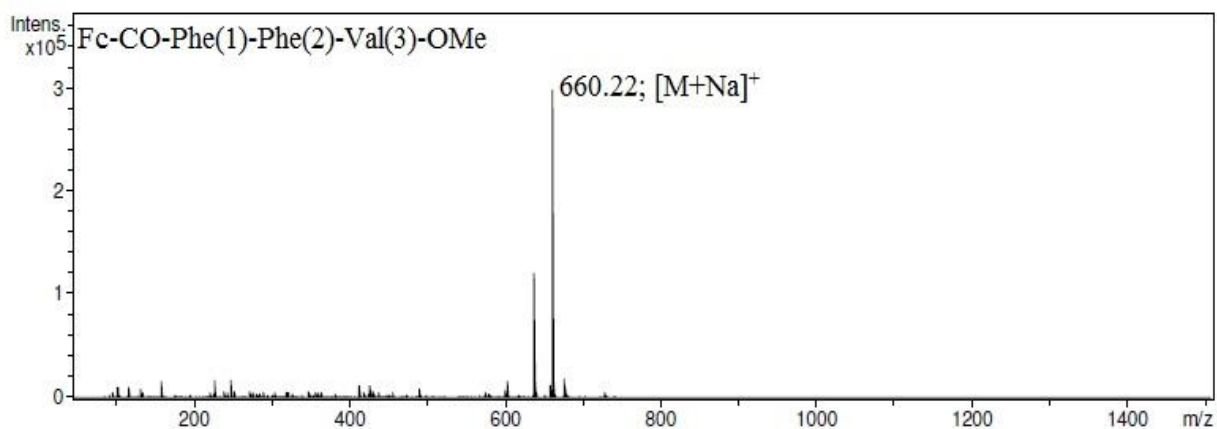
**Fig.S22**  $^1\text{H}$ - $^1\text{H}$  gCOSY spectrum of Fc-CO-Leu(1)-Phe(2)-Phe(3)-OMe in  $\text{CDCl}_3$ .

Characterization of Fc-CO- Phe(1)-Phe(2)-Val(3)-OMe:

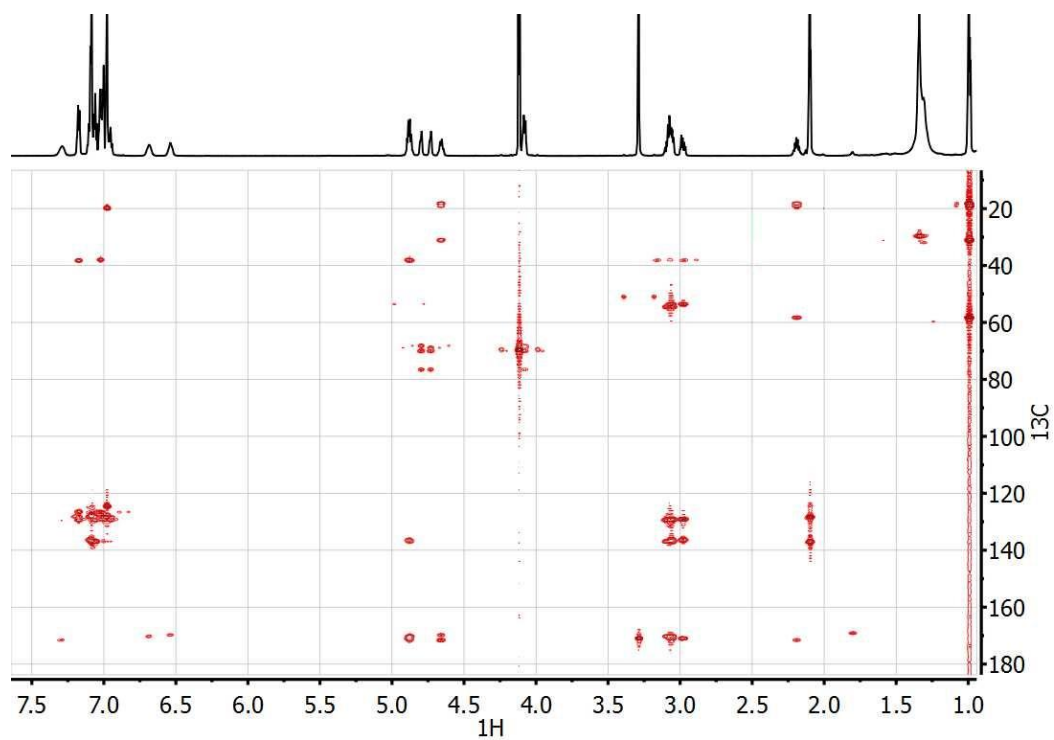
$^1\text{H}$  NMR (700 MHz,  $\text{CDCl}_3$ ,  $22^\circ\text{C}$ ):  $\delta$  7.38–7.11 (m, 10H; aromatic H of two phenyl rings),  $\delta$  6.83 (d,  $^3J(\text{H}, \text{H}) = 7.5$  Hz, 1H; NH of Phe(2)),  $\delta$  6.49 (d,  $^3J(\text{H}, \text{H}) = 7.5$  Hz, 1H, NH of Phe(1)),  $\delta$  6.07 (d,  $^3J(\text{H}, \text{H}) = 7.5$  Hz, 1H; NH of Val(3)), 4.84–4.80 (1H; m,  $\alpha$  CH of Phe(1)), 4.70–4.66 (2H; m, ortho-H of Cp ring which is directly attached to peptide segment,  $\alpha$  CH of Phe(2)), 4.46–4.44 (2H; m, ortho-H of Cp ring which is directly attached to peptide segment,  $\alpha$  CH of Val(3)), 4.37–4.34 (2H, m; meta-H of Cp ring which is directly attached to peptide segment),  $\delta$  4.01 (br, 5H; H of another Cp ring which is not directly attached to peptide segment),  $\delta$  3.73 (s, 3H,  $\text{OCH}_3$ ), 3.09–3.04 (m, 4H;  $\beta$   $\text{CH}_2$  of Phe),  $\delta$  2.15–2.11 (m, 1H;  $\beta$  H of Val(3)),  $\delta$  0.91–0.95 (m, 6H;  $\gamma$  H Val(3)); Anal. Calcd. for  $\text{C}_{35}\text{H}_{39}\text{FeN}_3\text{O}_5$  (637.22): MS (ESI)  $m/z$  660.21 $[\text{M}+\text{Na}]^+$ .



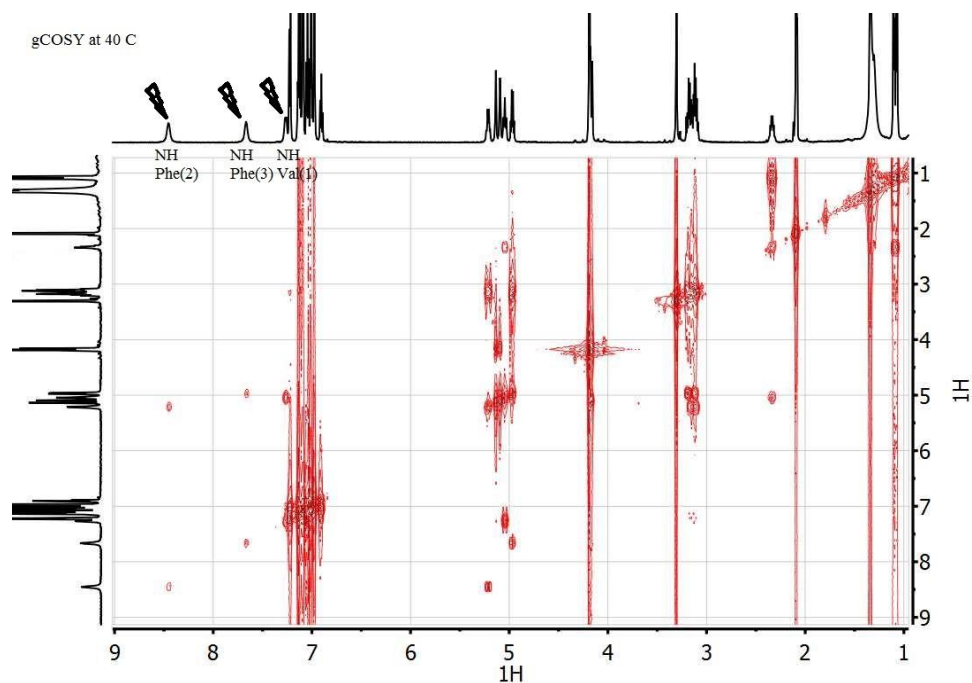
**Fig.S23**  $^1\text{H}$  NMR spectrum of Fc-CO- Phe(1)-Phe(2)-Val(3)-OMe in  $\text{CDCl}_3$ .



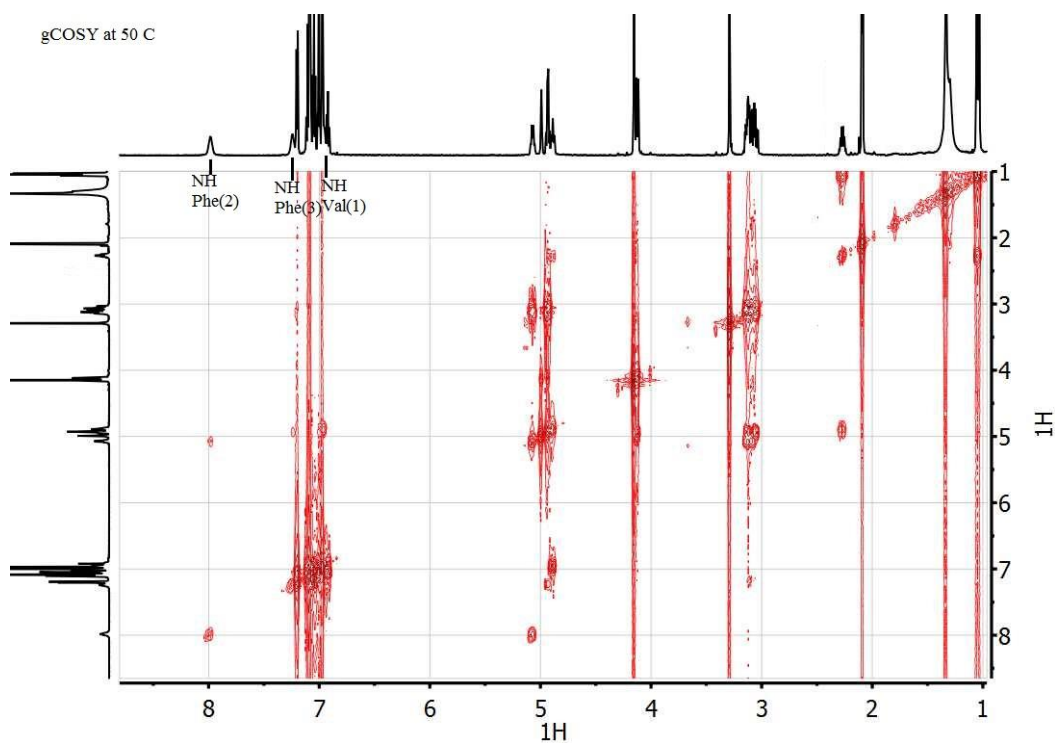
**Fig.S24** MS spectrum of Fc-CO- Phe(1)-Phe(2)-Val(3)-OMe.



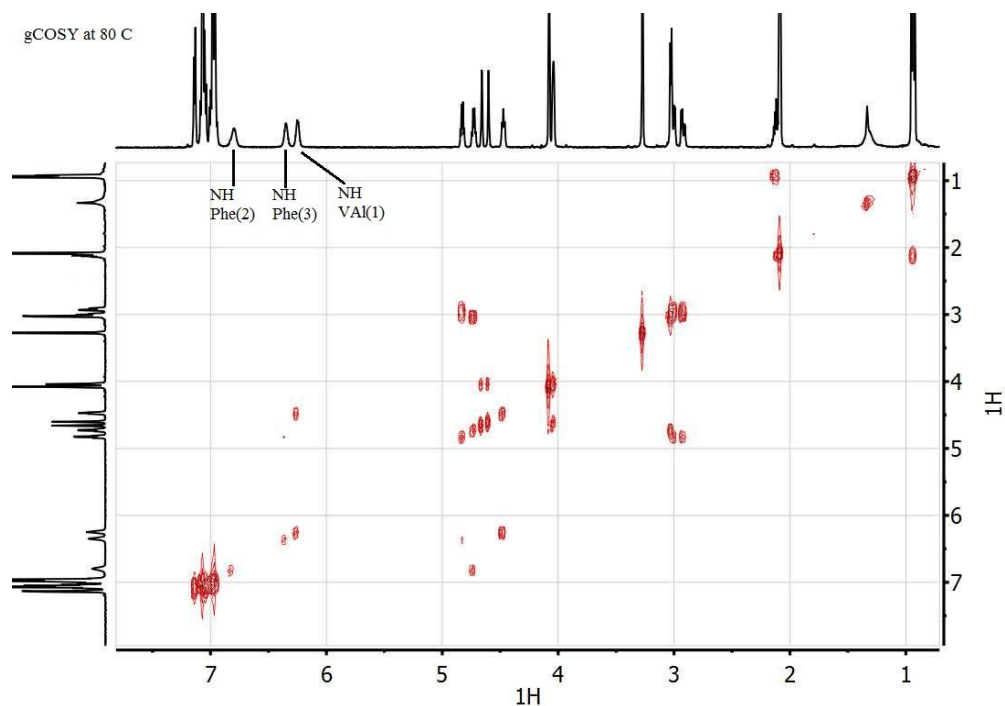
**Fig. S25** gHMBCAD of Fc peptide 1 (Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe) in toluene  $D_8$  at 60 °C.



**Fig. S26** gCOSY of Fc peptide 1 (Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe) in toluene D8 at 40 °C.



**Fig. S27** gCOSY of Fc peptide 1 (Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe) in toluene D8 at 50 °C.



**Fig. S28** gCOSY of Fc peptide **1** (Fc-CO-Val(1)-Phe(2)-Phe(3)-OMe) in toluene D8 at 80 °C.

#### Instrumentation.

**UV/Vis spectroscopic study.** UV-Visible experiments were performed to probe the oxidation–reduction reaction of ferrocene by dissolving Fc-peptide **1** in acetone. UV/Vis absorption spectra of the Fc-peptide **1** were recorded in the wavelength range 300 to 800 nm using a UV/Vis spectrophotometer (Agilent 8453). Sample was prepared by dissolving Fc-peptide in acetone.

**NMR study.** All synthetic Fc-peptides were characterized using NMR spectroscopy (Bruker spectrometer 500 MHz).

**FTIR study.** FTIR spectra of wet gel material, dried gel, oxidized gel and toluene were recorded in the range 3400-1400  $\text{cm}^{-1}$  using a Bruker ALPHA FTIR spectrometer equipped with a diamond ATR. Wet gel sample was prepared by dissolving Fc-peptide **1** in toluene at mgc and dried material was obtained by drying the wet sample.

**Transmission Electron Microscopic (TEM) study.** The morphologies of the assemblies of Fc-peptides have been studied using TEM at room temperature (20 °C). The samples have been prepared by



depositing a small amount of assembling viscous solution of Fc-peptide 1 in toluene on a TEM grid (300 mesh size Cu grid) coated with Formvar and a carbon film. The grid was allowed to dry by slow evaporation in air, and then allowed to dry separately in a vacuum for few hours. Images were taken using a Hitachi 7500 transmission EM.

***Circular dichroism (CD) study.*** CD spectra were recorded between 300 and 600 nm using a JASCO J-810 spectrometer.

**X-ray diffraction (XRD).** The experiment was carried out by using an X ray diffractometer (Bruker AXS D8 Discovery) equipped with a conventional CuK $\alpha$  X-ray radiation source and Bragg diffraction setup (Seifert 3000P).

**Dynamic Light Scattering (DLS) Study.** DLS study was performed in MELLERS GRIOT HeNe-LASER TERBO-CORR CORILATOR Instrument using dilute solution of fully oxidized Fc-peptide 1.