Supporting Information

Multilayer vesicles, tubes, various porous structures and organo gel through solvent-assisted self-assembly of two modified tripeptides and their different applications

Pradyot Koley and Animesh Pramanik*

Department of Chemistry, University of Calcutta, 92, A. P. C. Road, Kolkata-700 009, India;
Fax: +91 33 2351 9755.
E-mail: animesh_in2001@yahoo.co.in

Table of contents

Figures	Page numbers	Figures	Page numbers
Fig. S1	2	Fig. S10	8
Fig. S2	2	Fig. S11	9
Fig. S3	3	Fig. S12	10
Fig. S4	3	Synthesis and characterization	
Fig. S5	4	of the peptides	10-12
Fig. S6	4	Fig. S13-S18	13-16
Fig. S7	5		
Fig. S8	6		
Fig. S9	7		

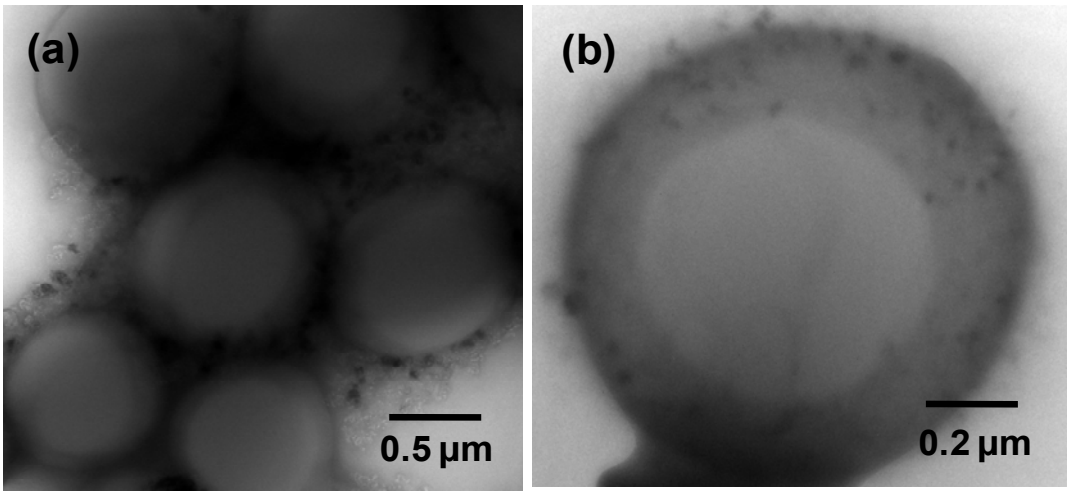

Fig. S1 TEM images showing the (a) hollow vesicular morphology and (b) layer like assemblies of peptide **I** from 5 mM methanolic solution.

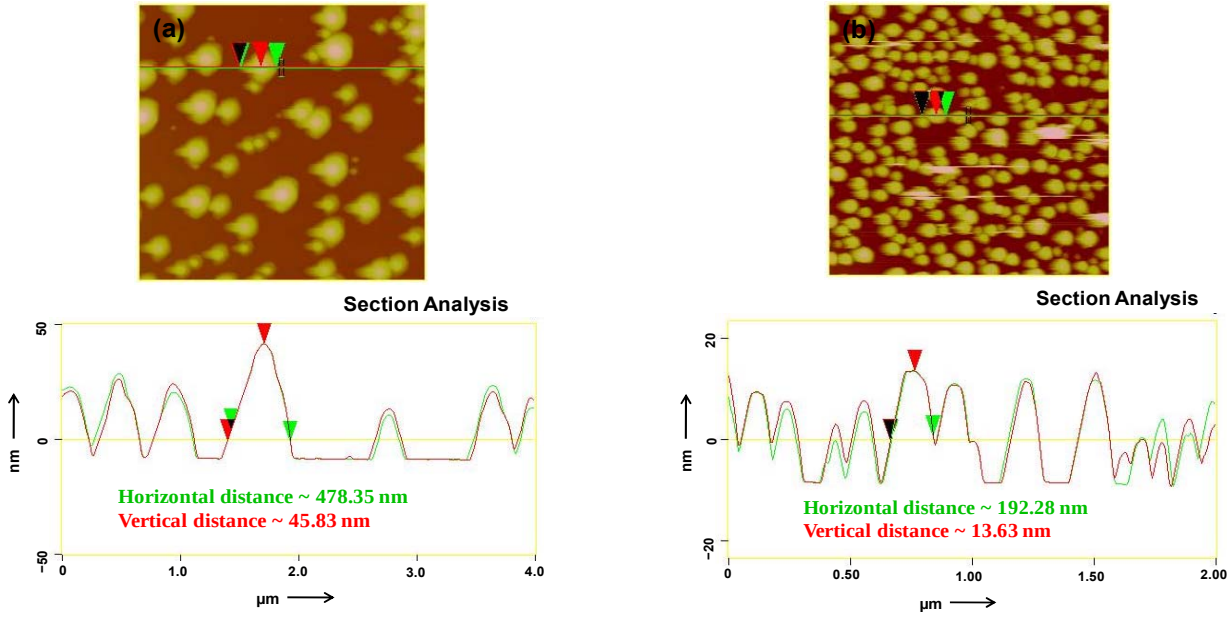

Fig. S2 AFM images of vesicular structures of (a) peptide **I** and (b) peptide **II** from 2 mM methanolic solution; (bottom pannel) respective section analysis plots.

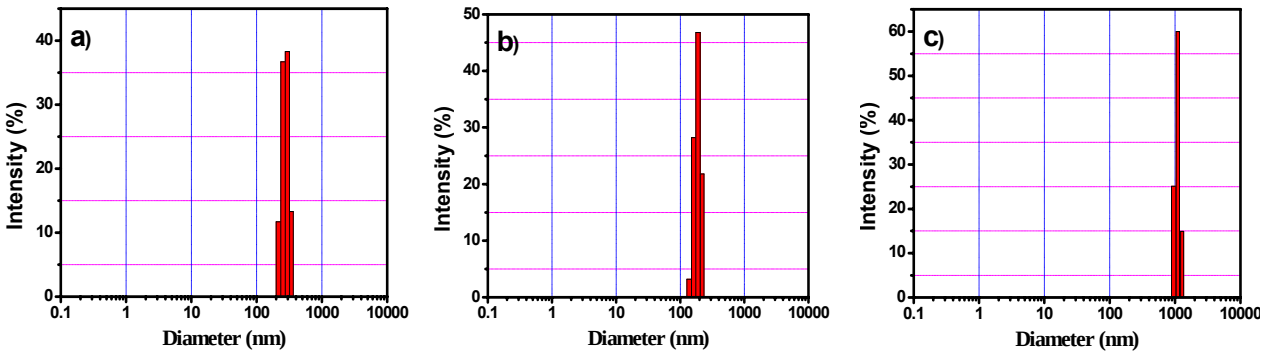

Fig. S3 DLS results show discrete peak intensities of (a) peptide **I** and (b) peptide **II** from 2 mM methanolic solutions; (c) indicates intensity distribution of peptide **I** at higher methanolic concentration (5 mM).

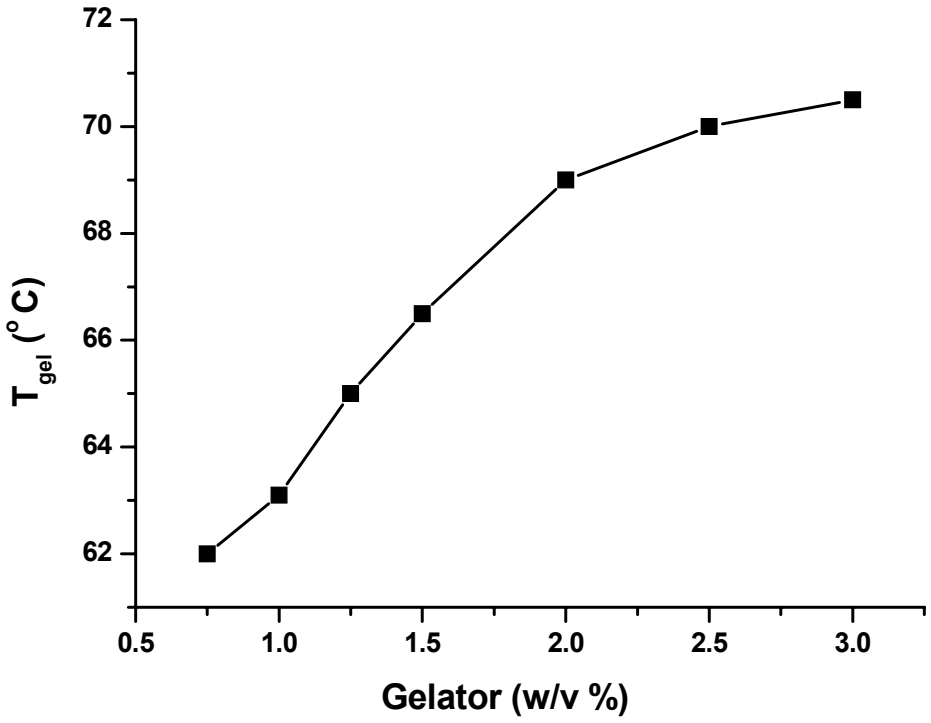

Fig. S4 Plot of gel-to-sol transition temperature (T_{gel}) against gelator concentration of peptide **I** from chloroform.

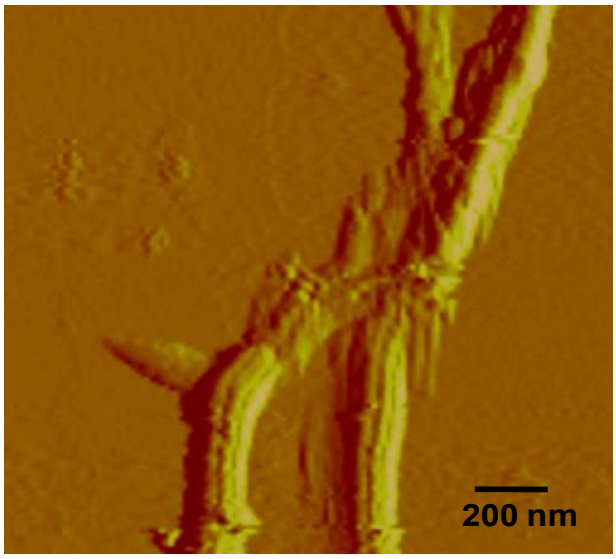

Fig. S5 AFM topographic image of peptide **I** tubular structure from 2 mM chloroform: petroleum ether (3:7) mixture.

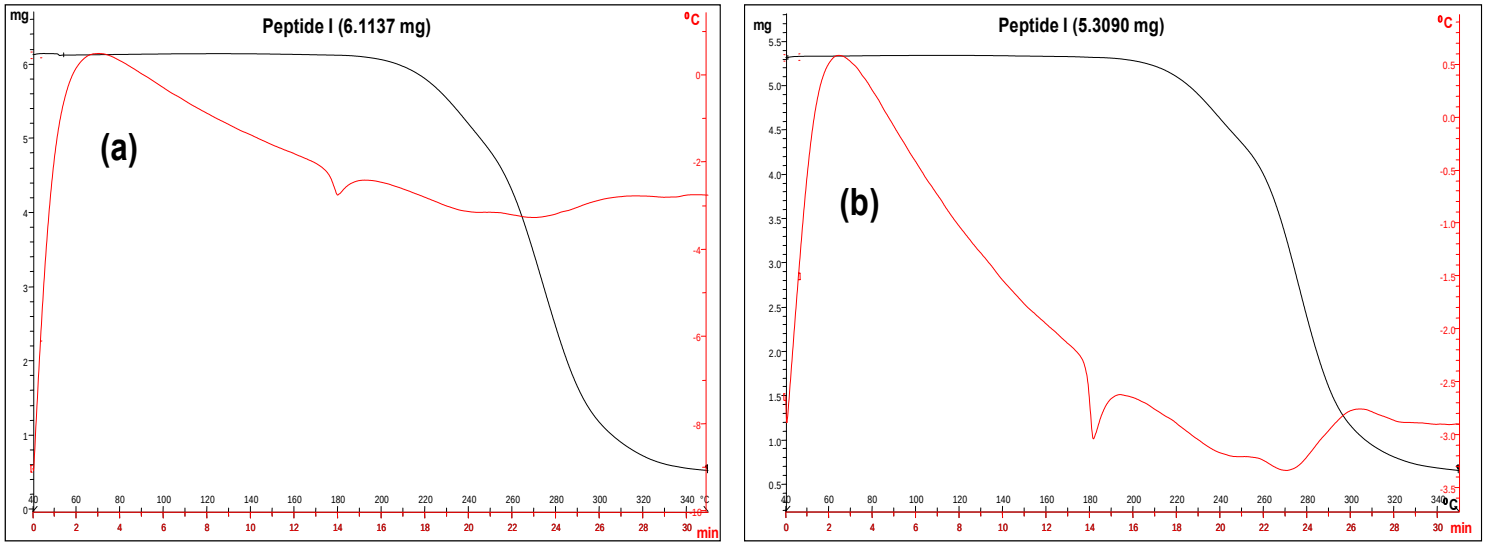

Fig. S6 TGA/SDTA thermograph of the (a) as synthesized compound and (b) vesicular structures of peptide **I**, showing their stability up to 180 °C.

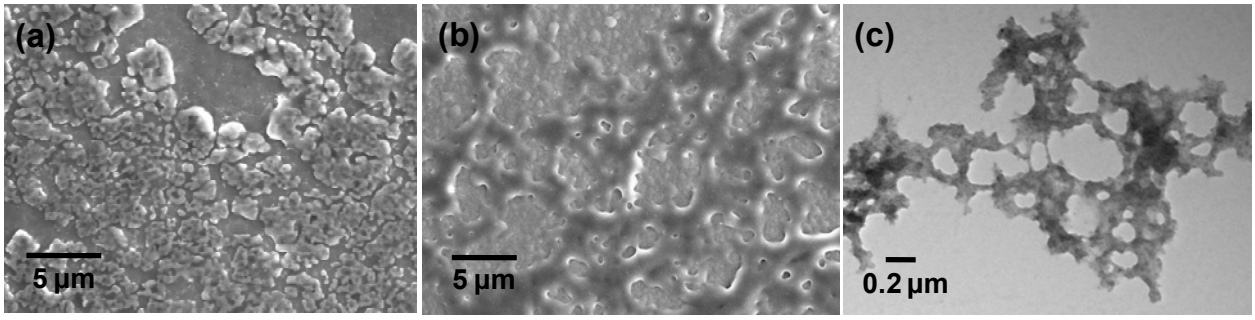

Fig. S7 SEM images show the rupture of peptide **I** vesicular structure in presence of (a) KCl and (b) $CaCl_2$ salts after 12 hours incubation. (c) TEM images of ruptured vesicles of peptide **I** after addition of KCl salts.

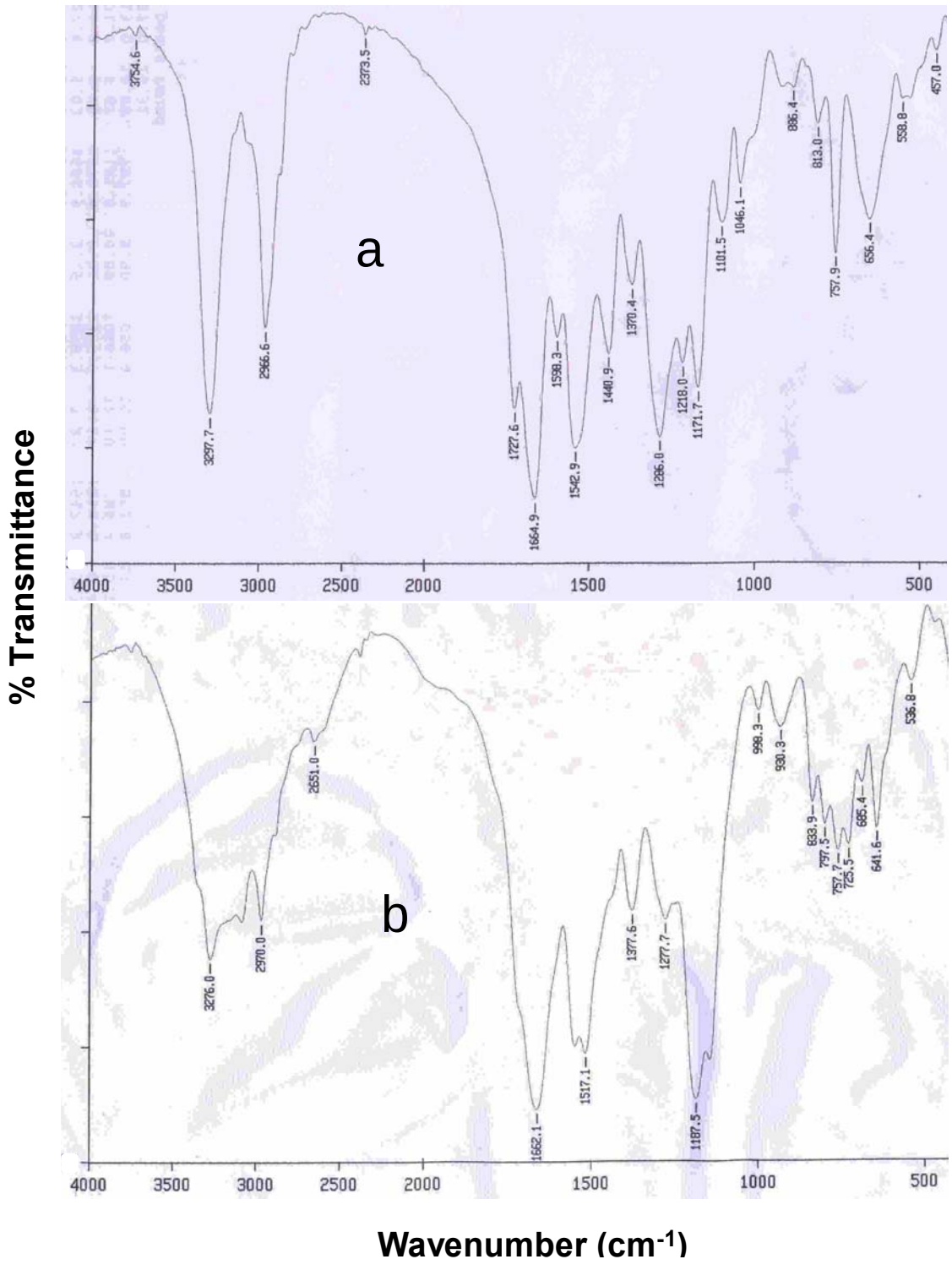

Fig. S8 FT-IR spectra of (a) as synthesized peptide **I** and (b) peptide **I** vesicles from methanol.

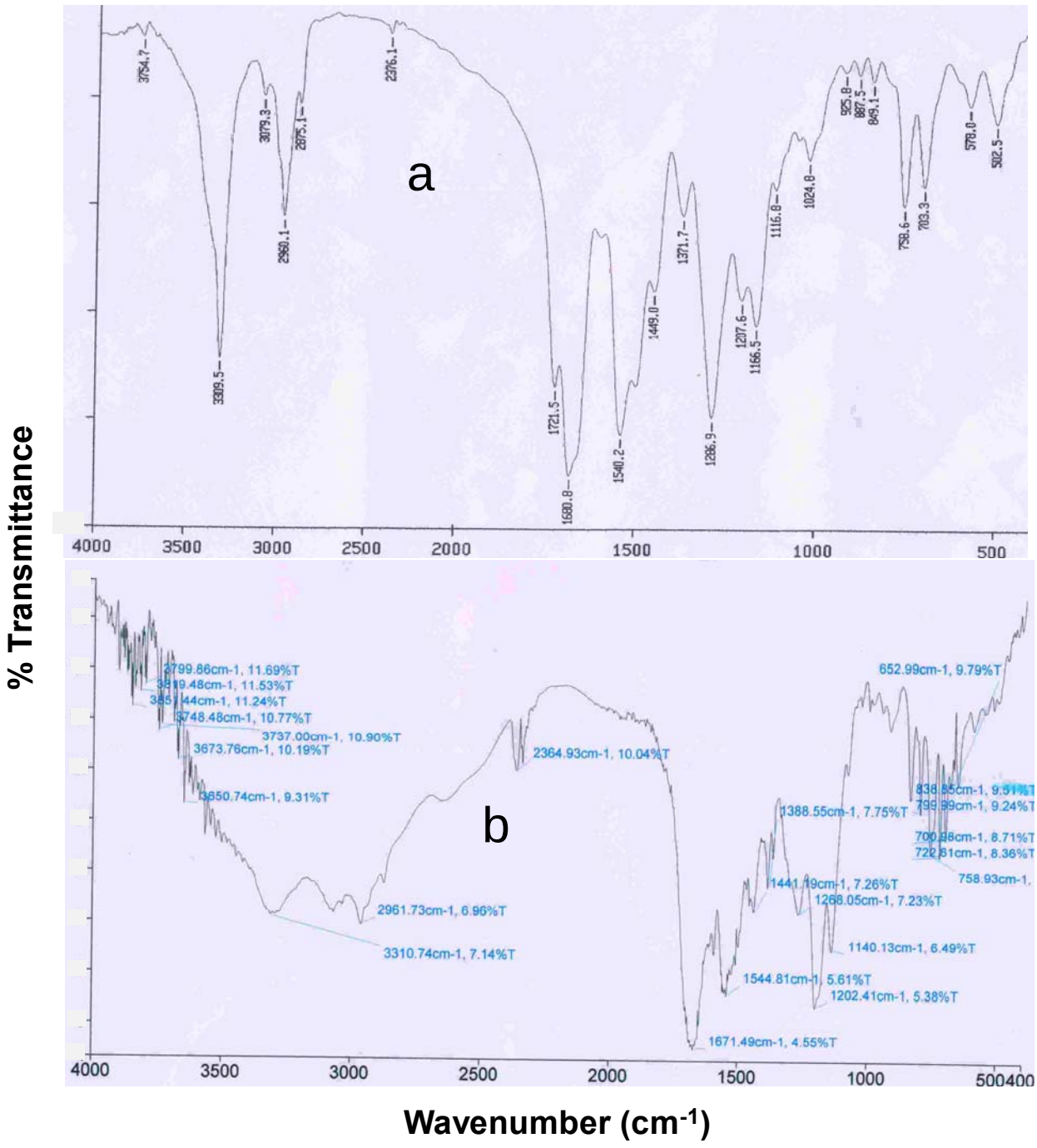

Fig. S9 FT-IR spectra of (a) as synthesized peptide **II** and (b) peptide **II** vesicles from methanol.

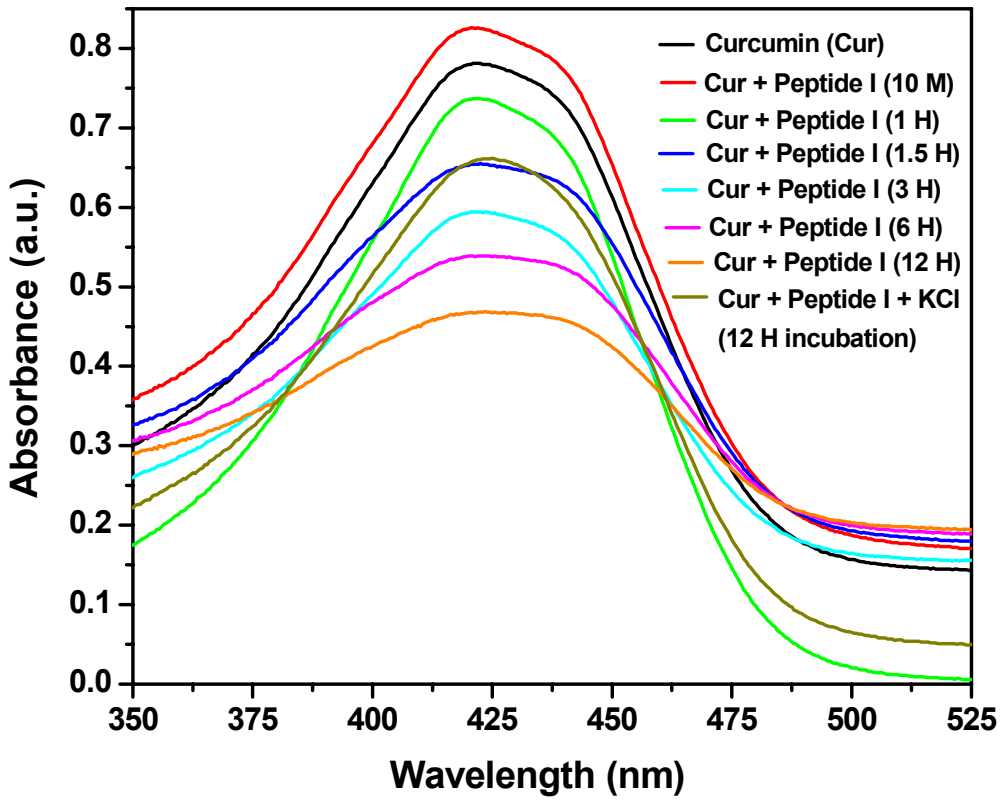

Fig. S10 UV absorption spectra showing the drug (curcumin) encapsulation and release with time of peptide **I** from 70% methanol.

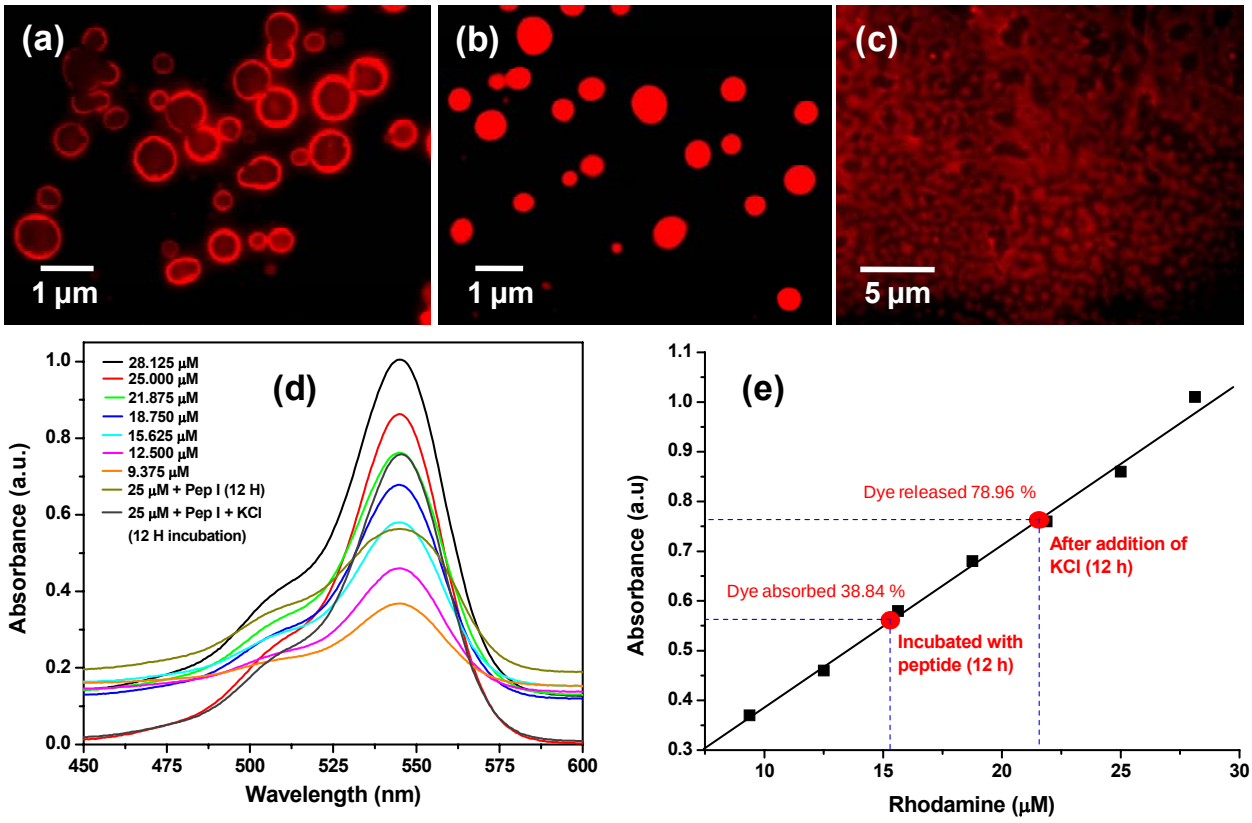

Fig. S11 Fluorescence microscopy and UV-absorption studies show the encapsulation of the dye rhodamine B by peptide **I** vesicles at different incubation times. Fluorescence microscopic images showing the (a) intensified bright red fluorescence of rhodamine B at the periphery of the circular structures compared to the rest of the surface after 15 mins incubation; (b) uniformly distributed bright red fluorescence of the rhodamine B loaded vesicles after 12 hr incubation; and (c) release of rhodamine B after overnight incubation with KCl salt. (d) Concentration dependent UV-spectra of rhodamine B (9.375-28.125 μM) and (e) corresponding calibration curve plotted quantifying the encapsulation efficiency and release in presence of KCl.

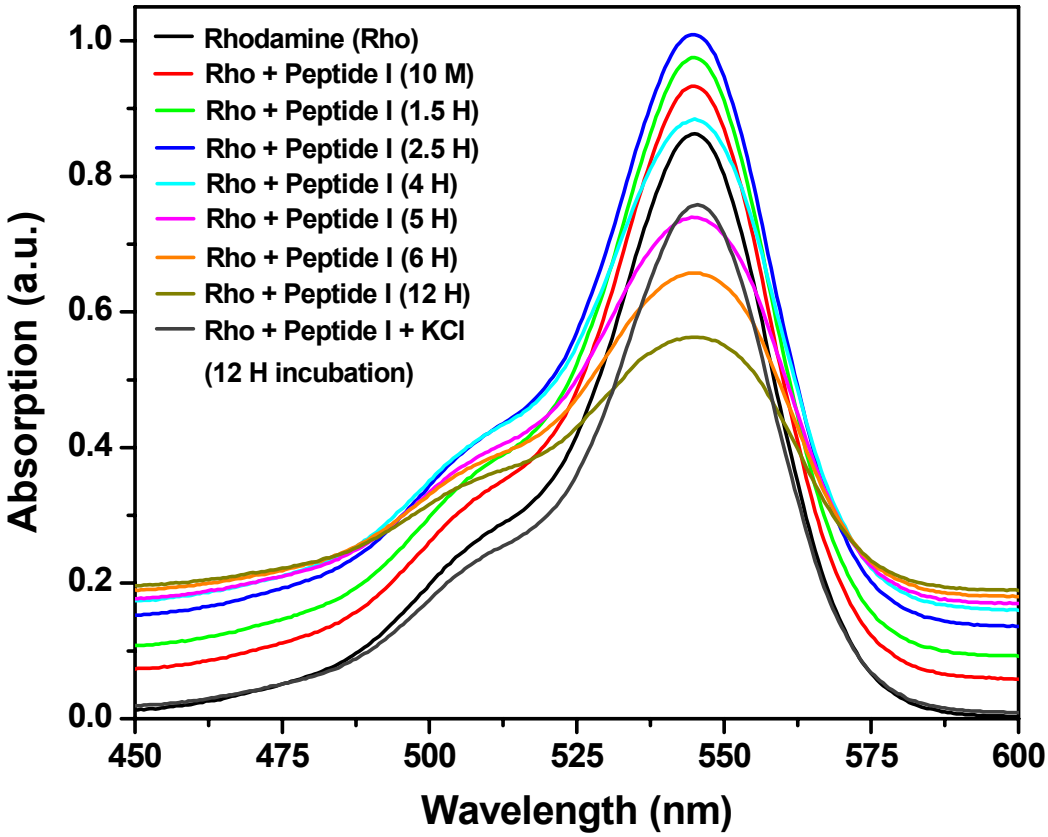

Fig. S12 UV absorption spectra showing the dye rhodamine B encapsulation and release with time of peptide **I** from 70% methanol.

Synthesis and Characterization of the peptides

Peptides **I** (Boc-Leu-Aib-Val-*m*-ABA-OMe) & **II** (Boc-Phe-Aib-Leu-*m*-ABA-OMe) were synthesised by conventional solution phase procedures using a racemization free, fragment condensation strategy.[1] The *t*-butyloxycarbonyl and methyl ester group were used for amino and carboxyl protections respectively whereas dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) used as coupling agents. Deprotection of the methyl ester was performed using the saponification method. Methyl ester hydrochlorides of Aib, Val, Leu and *meta*-aminobenzoic acid (*m*-ABA-OH) were prepared by the thionyl chloride-methanol procedure. All the intermediates obtained were checked for purity by thin layer chromatography

(TLC) on silica gel and used without further purification. All the final peptides were purified by column chromatography using silica gel (100-200 mesh) as the stationary phase and ethyl acetate and petroleum ether mixture as the eluent. The reported peptides **I** & **II** were fully characterised by ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, FT-IR spectroscopy, HR-MS spectroscopy and microanalysis.

Synthesis of Peptide I (Boc-Leu-Aib-Val-*m*-ABA-OMe): Peptide Boc-Leu-Aib-Val-COOH [2] (2.0 g, 4.8 mmol) was dissolved in DMF (6 ml). H-*m*-ABA-OMe obtained from its hydrochloride (1.8 g, 9.6 mmol) was added, followed by DCC (1.48 g, 7.2 mmol) and HOBT (0.65 g, 4.8 mmol). The reaction mixture was stirred at room temp for 2 days. The precipitated N,N'-dicyclohexylurea (DCU) was filtered off. The organic layer was diluted with 30 ml ethyl acetate and washed with 1N HCl (3x30 ml), brine, 1M Na2CO3 solution (3x30 ml) and then again with brine. The solvent was then dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, giving a light yellow gum. Purification was carried out using silica gel as the stationary phase and ethyl acetate petroleum ether mixture as the eluent. Yield: 2.4 g (4.37 mmol, 90.9 %). Mp = 176-178 °C.

^{1}H NMR (300 MHz, $CDCl_3$, δ_{ppm}): 9.19 (s, 1H, *m*-ABA (4) NH); 8.54 (s, 1H, *m*-ABA (4) Ha); 8.11 (d, 1H, *J* = 8.1 Hz, *m*-ABA (4) Hd); 7.75 (d, 1H, *J* = 7.8 Hz, *m*-ABA (4) Hb); 7.36 (t, 1H, *J* = 7.95 Hz, *m*-ABA (4) Hc); 6.99 (br s, 2H, Aib (2) NH and Val (3) NH); 5.35 (d, 1H, *J* = 4.2 Hz, Leu (1) NH); 4.51-4.55 (m, 1H, Val (3) C^{α} Hs); 3.93-3.99 (m, 1H, Leu (1) C^{α} Hs); 3.89 (s, 3H, -OCH_3); 2.60-2.66 (m, 1H, Val (3) C^{β} Hs); 1.68–1.72 (m, 2H, Leu (1) C^{β} Hs); 1.55 (s, 3H, Aib (2) $C1^{\beta}$ Hs); 1.50 (s, 3H, Aib (2) $C2^{\beta}$ Hs); 1.42 (s, 9H, Boc CH_3s); 0.85-1.02 (m, 13H, Leu (1) and Val (3) C^{γ} Hs and C^{δ} Hs). ^{13}C NMR (75 MHz, $CDCl_3$, δ_{ppm}): 174.16, 173.37, 170.19, 167.05, 156.33, 138.86, 130.46, 128.58, 124.89, 124.61, 121.17, 80.98, 59.39, 57.03, 54.76, 52.00, 40.24, 29.22, 28.09 (3C), 27.36, 24.83, 23.86, 22.69, 21.75, 19.43, 17.03. FT-IR (KBr): 3298, 2967, 1728, 1665, 1543, 1441, 1286, 1178.

HR-MS (M^{+} Na^{+}) = 571.3386, *M*calcd = 548.3210.

Elemental analysis calculated for $C_{28}H_{44}N_4O_7$ (548.32): C, 61.29; H, 8.08; N, 10.21 %; Found: C, 61.24; H, 8.03; N, 10.17 %

Synthesis of Peptide II (Boc-Phe-Aib-Leu-*m*-ABA-OMe): (a) Boc-Phe-Aib-Leu-OH: Peptide Boc-Phe-Aib-Leu-OMe [3] (2.0 g, 4.19 mmol) was dissolved in methanol (20 ml) and 2N NaOH (10 ml) was added. The reaction mixture was stirred for 1 day at room temperature. The progress of the reaction was monitored by TLC. After completion of reaction the methanol was evaporated. The residue was diluted with water and washed with diethyl ether. The aqueous layer was cooled in an ice-bath and then neutralized by using 2N HCl and extracted with ethyl acetate. The solvent was evaporated *in vacuo* to give a waxy colorless solid. Yield: 1.7 g (3.67 mmol, 87.63 %).

(b) Boc-Phe-Aib-Leu-*m*-ABA-Ome: The as synthesized peptide Boc-Phe-Aib-Leu-OH (1.7 g, 3.67 mmol) was dissolved in DMF (6 ml). H-*m*-ABA-OMe obtained from its hydrochloride (1.37 g, 7.34 mmol) was added, followed by DCC (1.13 g, 5.51 mmol) and HOBT (0.50 g, 3.67 mmol). The reaction mixture was stirred at room temp for 2 days. The precipitated N,N'-dicyclohexylurea (DCU) was filtered off. The organic layer was diluted with 30 ml ethyl acetate and washed with 1N HCl (3x30 ml), brine, 1M Na2CO3 solution (3x30 ml) and then again with brine. The solvent was then dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, giving a light yellow gum. Purification was carried out using silica gel as the stationary phase and ethyl acetate petroleum ether mixture as the eluent. Yield: 2.4 g (4.37 mmol, 90.9 %). Mp = 158-160 °C.

^{1}H NMR (300 MHz, $CDCl_3$, δ_{ppm}): 9.08 (s, 1H, *m*-ABA (4) NH); 8.59 (s, 1H, *m*-ABA (4) Ha); 8.16 (d, 1H, *J* = 6.6 Hz, *m*-ABA (4) Hd); 7.76 (d, 1H, *J* = 6.6 Hz, *m*-ABA (4) Hb); 7.21-7.42 (m, 6H, *m*-ABA (4) Hc and Phe (1) phenyl ring protons); 7.03 (d, 1H, *J* = 8.1 Hz, Leu (3) NH); 6.43 (s, 1H, Aib (2) NH); 5.05 (d, 1H, *J* = 3.6 Hz, Phe (1) NH); 4.53-4.61 (m, 1H, Leu (3) C^{α} Hs); 4.19-4.25 (m, 1H, Phe (1) C^{α} Hs); 3.89 (s, 3H, -OCH_3); 3.06-3.18 (m, 2H, Phe (1) C^{β}Hs); 1.64-1.68 (m, 2H, Leu (3) C^{β} Hs); 1.41 (s, 9H, Boc CH_3s); 1.38 (s, 6H, Aib (2) C^{β} Hs); 0.85-0.95 (m, 7H, Leu (3) C^{γ} Hs and C^{δ} Hs). ^{13}C NMR (75 MHz, $CDCl_3$, δ_{ppm}): 173.51, 172.04, 171.07, 167.11, 156.50, 139.04, 135.43, 130.51, 129.09 (2C), 128.69 (2C), 127.61, 124.83, 124.55, 121.25, 118.14, 81.60, 57.36, 57.15, 52.69, 52.02, 39.95, 37.02, 28.07 (3C), 27.36, 25.13, 23.62, 23.30, 20.76. FT-IR (KBr): 3310, 2960, 1722, 1681, 1540, 1287, 1167 cm^{-1}.

HR-MS (M^+ Na^+) = 619.3425, *M*calcd = 596.3210.

Elemental analysis calculated for $C_{32}H_{44}N_4O_7$ (596.32): C, 64.41; H, 7.43; N, 9.39 %; Found: C, 64.37; H, 7.38; N, 9.34 %.

References

1 M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*; Springer: New York, 1984, pp. 1

2 I.L. Karle, A. Banerjee, S. Bhattacharya and P. Balaram, *Biopolymers*, 1996, **38**, 515.

3 A. Dutt, R. Frohlich and A. Pramanik, *Org. Biomol. Chem.*, 2005, **3**, 661.

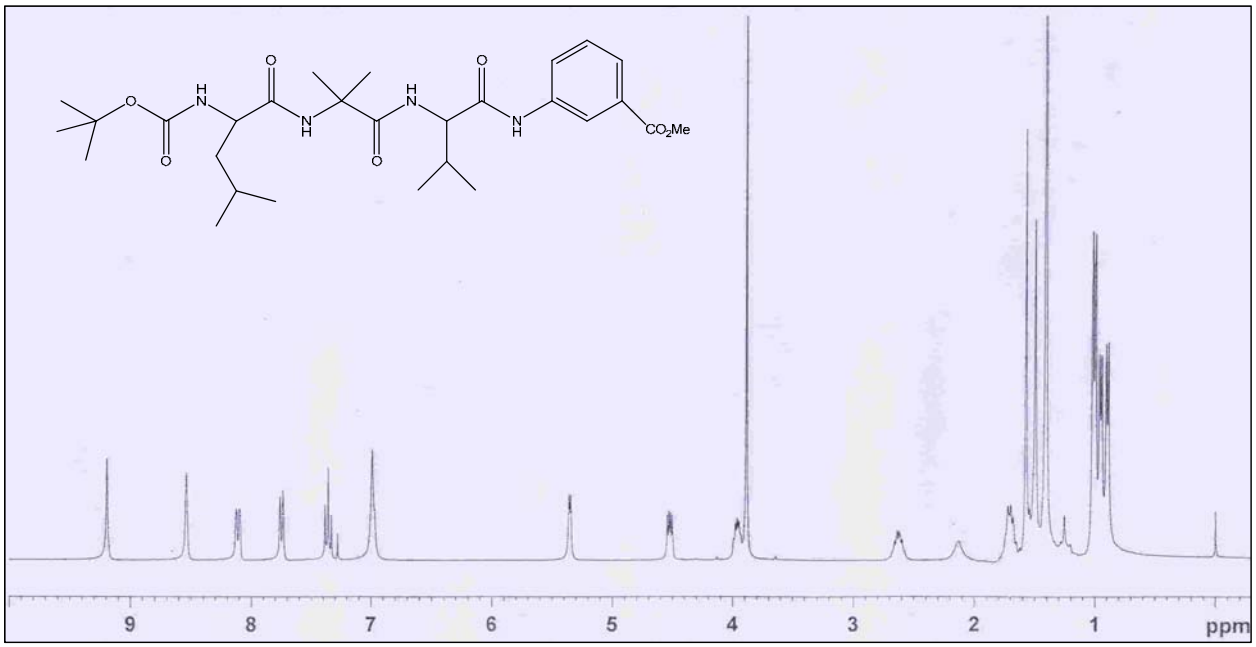

Fig. S13 ^{1}H NMR (300 MHz, $CDCl_3$,) spectra of peptide **I**

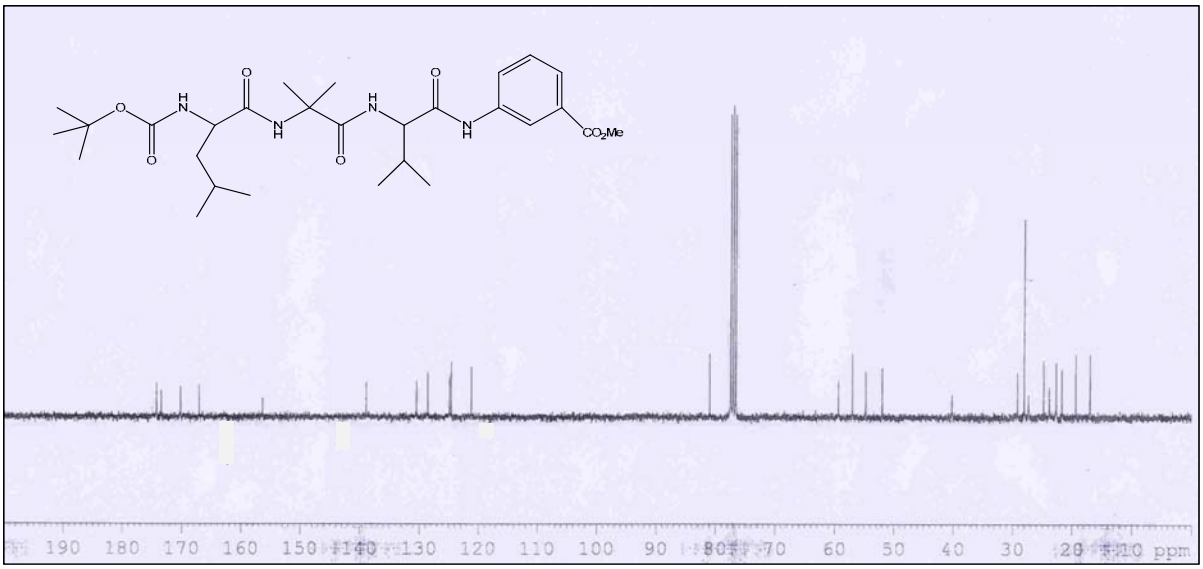

Fig. S14 ^{13}C NMR (75 MHz, $CDCl_3$,) spectra of peptide **I**

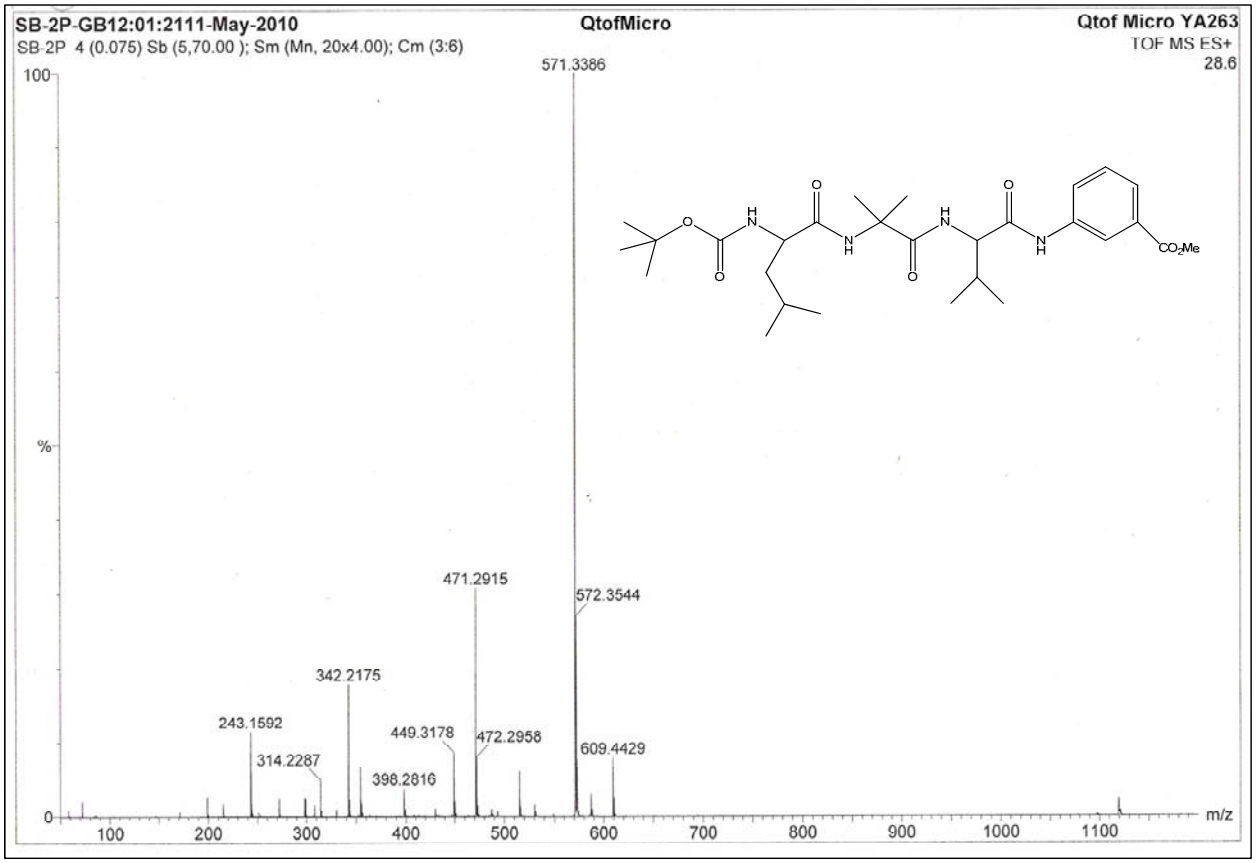

Fig. S15 Mass spectra of peptide **I**

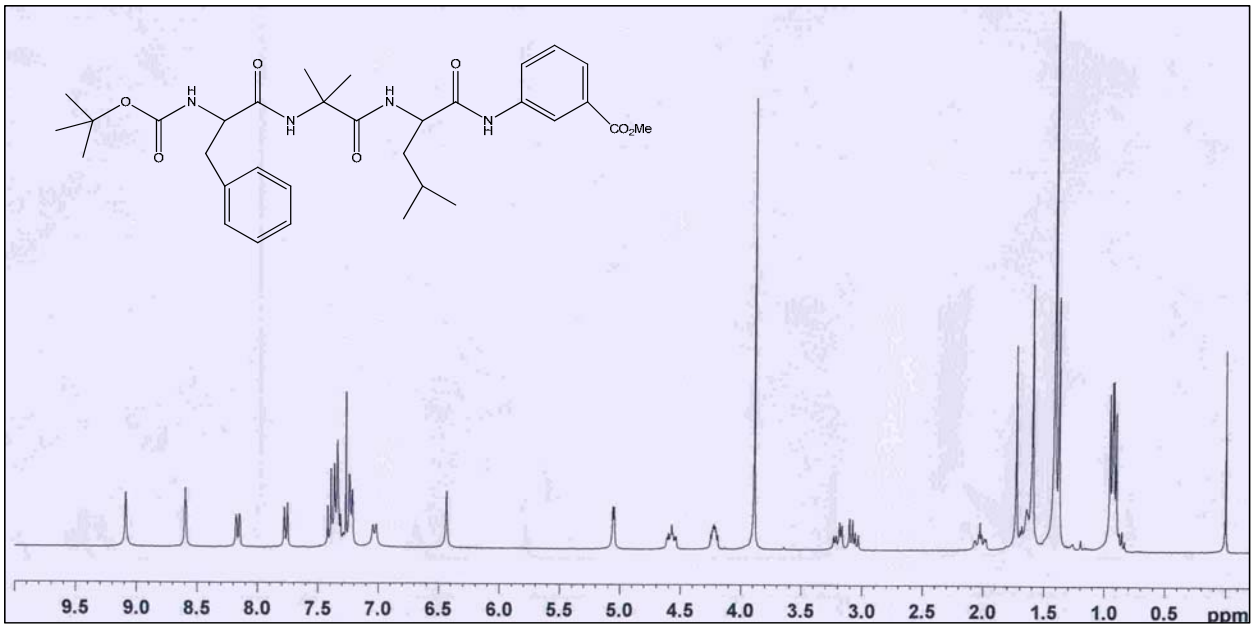

Fig. S16 ^{1}H NMR (300 MHz, $CDCl_3$,) spectra of peptide **II**

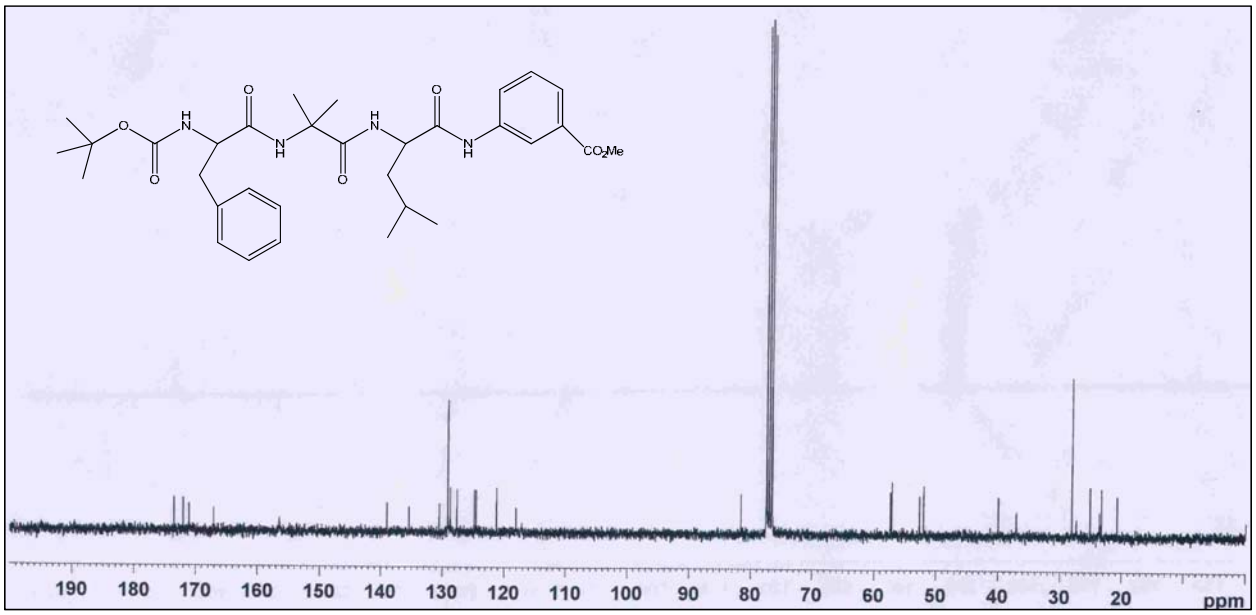

Fig. S17 ^{13}C NMR (75 MHz, $CDCl_3$,) spectra of peptide **II**

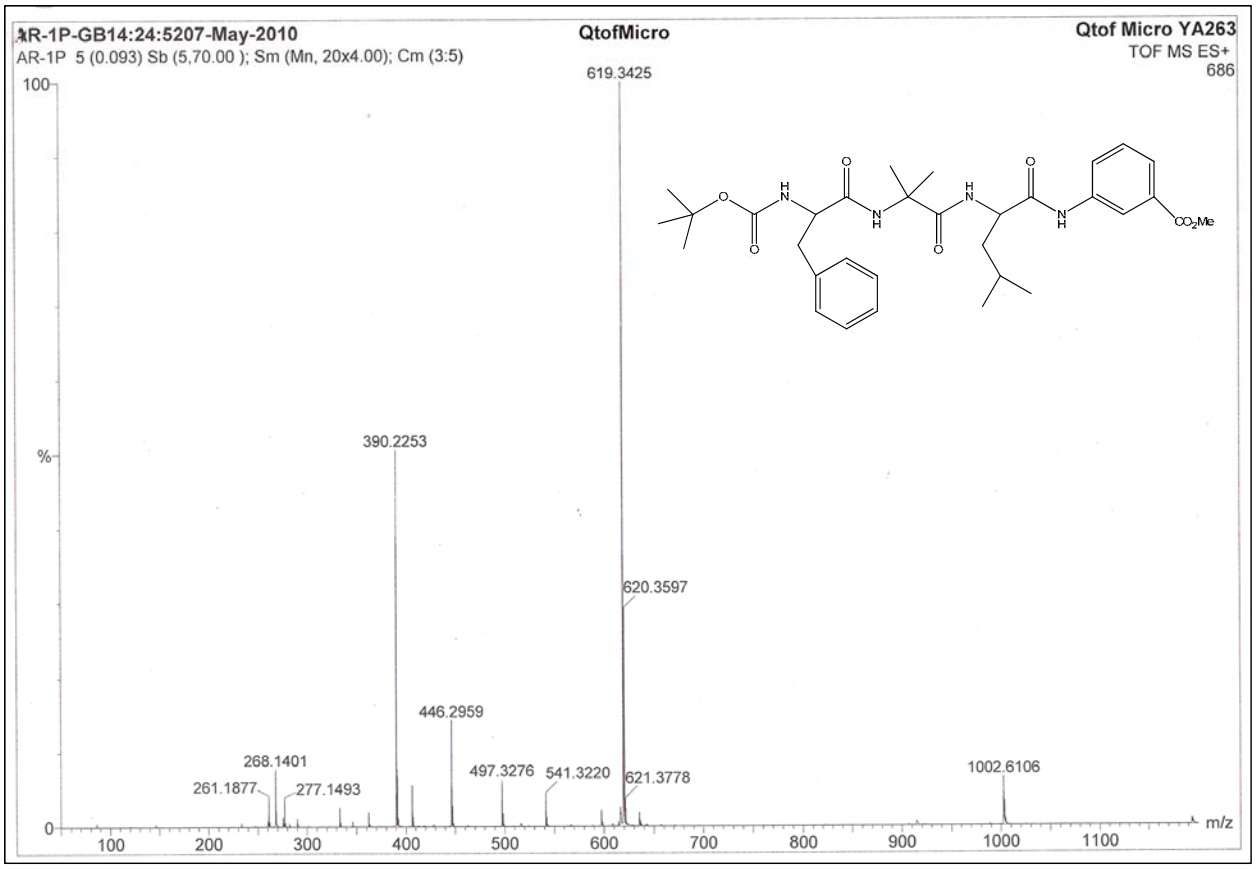

Fig. S18 Mass spectra of peptide **II**