Supporting Information

Aβ$_{1\text{-}42}$ C-Terminus Fragment Derived Peptides Prevent the Self Assembly of Parent Peptide

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1. Chemistry

Peptide synthesis: All chemicals were purchased from Sigma-Aldrich, Missouri, U.S.A. and Chem-Impex International, Illinois, U.S.A., and used without further purification, unless otherwise specified. The solvents used for synthesis and purification were of analytical grade and used without further purification. Peptides were synthesized on Aapptec Focus XC automated peptide synthesizer following the solid phase peptide synthesis protocol (SPPS) using Fmoc chemistry on 0.1 mM scale. Wang resin preloaded with the C-terminal amino acid was used as solid support. Resin was first swelled in DMF for 15 minutes in a reaction vessel equipped with a sintered glass bottom. Fmoc group on the amino acid coupled with the linker was then removed by using 20% piperidine in DMF for 15 minutes (2 x 10 mL). Subsequently, Fmoc protected amino acid was first activated *in situ* by the treatment with TBTU (3.0 equiv., 0.5 M in DMF) and DIEA (3.0 equiv., 1.0 M in DMF) and then coupled to the resin-bound amino acid by mechanical shaking at ambient temperature for 2.5 h. In case of α-aminoisobutyric acid (Aib), HATU was used as coupling reagent instead of TBTU. Successful completion of each coupling was routinely monitored by Kaiser's test. The cycle of Fmoc removal and coupling was repeated with all subsequent Fmoc protected amino acids to obtain resin-bound desired pentapeptides. Each coupling was followed by removal of Fmoc group and washing the resin by DMF. Cleavage of peptides from resin and concomitant deprotection of side chain protecting groups was carried out by using TFA: triisopropylsilane:water (95:3:2) as cleavage cocktail (15 mL/g); stirred magnetically at ambient temperature for 2.5 h. Filtration under vacuum afforded the peptides in filtrate. The volume of filtrate was reduced up to 0.5 mL. The crude peptides were precipitated by adding cold diethyl ether.

Purification: Crude peptides were purified and analyzed for purity by the reverse-phase high performance liquid chromatography, and characterized for identity by high-esolution mass spectroscopy. Crude peptides were dissolved in a mixture of H_2O-CH_3OH (1:1, v/v) and loaded on a preparative RP-HPLC (Shimadzu Prominence LC-8A model using a Phenomenex™ column, LC-18, 250 × 21.2 mm, 10 μm), operating at 215 nm with a flow rate of 21 mL/min. The mobile phases used were: (A) 0.1% trifluoroacetic acid (TFA) in acetonitrile and (B) 0.1% TFA in water. A linear gradient of 10-70% of mobile phase A was run for 50 minutes. Desired fractions containing pure peptide were pooled and concentrated. The peptides were dissolved in 80% acetic acid (aq.) and lyophilized to obtain peptides as fluffy white powder. The purity of the peptides was checked by analytical RP-HPLC (Shimadzu Prominence RP-HPLC system using a Phenomenex™ column (LC-18, 250 × 4.6 mm, 5 μm). The peptides were analyzed in a solvent system composing (A) CH_3CN (0.1% TFA) and (B) H_2O (0.1% TFA) using a 30 minutes gradient of 10-70% of mobile phase A, at 215 nm with a flow rate of 1.0 mL/min. The final purity of the peptides varied in the range of 95-100%. The identity of the peptides was further ascertained by the high-resolution mass spectroscopy (HRMS) taken on a Maxis Bruker spectrometer.

2. MTT cell viability assay

Aβ Peptides were purchased from AnaSpec Inc., USA. PC-12 cells were purchased from National Centre for Cell Science (NCCS), Pune, India. Horse serum was purchased from HiMedia Laboratories, India. 96-Well, flat-bottomed microtiter plates (Costar), Corning®

tissue-culture treated dishes, MTT, sodium bicarbonate, fetal bovine serum (heat inactivated), penicillin/streptomycin and F-12K growth media were purchased from Sigma-Aldrich Chemicals, Saint Louis, MO, USA. HPLC grade dimethyl sulphoxide (DMSO) was purchased from Alfa Aesar. Phosphate buffered saline (PBS, pH 7.4) was purchased from Gibco (Life Technologies). All the solutions were pre-sterilized using 0.2 μm syringe filters. The optical density (OD_{570}) measurements were taken using a microtiter plate reader (VERSA max tunable; Molecular Devices, Sunnyvale, CA).

Cell culture: PC-12 cells were cultured in F-12K growth media supplemented with 10% horse serum, 5% FBS and 1% penicillin/streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. Cells were sub-cultured and grown in tissue-culture treated dishes and used for the experiments when 70% confluent. On the day of study, the media was replaced with F-12K containing 10% FBS and 1% penicillin/streptomycin.

Aβ pretreatment: Since conformational homogeneity of Aβ samples is a pre-requisite for reproducibly monitoring Aβ self-assembly, the Aβ peptides were brought in a completely monomeric state following Zagorski's protocol.[1] Briefly, the peptides were first dissolved in TFA at an approximate 1:1 ratio (mg:mL) and sonicated for 10 min. The solution was kept at ambient temperature for 1-2 h before drying under dry N_2 gas. The peptides formed a thin coat on walls of the glass vial. The cycle was repeated three times. To remove the traces of TFA, hexafluoroisopropanol (HFIP) was added and the cycle of sonication and removal of the HFIP with dry N_2 gas was repeated three times. Traces of HFIP were removed under vacuum (0.5 mm Hg, 2 h). Finally, 1 mL of HFIP was added and peptides were aliquoted in 100 μg stocks, and dried under vacuum. To remove any metal impurities that could promote aggregation of the Aβ peptides, TFA and HFIP were pre-distilled under an inert atmosphere of nitrogen and stored in opaque bottles at 5 °C.

Assay: An aliquot of Aβ peptide was taken and immediately before the experiment, 20 mM NaOH was added to make up a concentration of 200 μM. The Aβ peptides were diluted in 10 mM sodium phosphate buffer (SPB, pH 7.4) to 20 μM. Cells in exponential growth phase were seeded in 96-well plates, at a rate of 17000 cells per well per 80 μL, and incubated overnight. Test peptides were dissolved in dimethylsulfoxide (DMSO) as 5 mM stock solutions. Just before the experiments, test peptides were diluted in phosphate buffered saline (PBS) to the concentrations of 200 μM, 100 μM and 20 μM. Next morning, Aβ (10 μL) was added in each well with and without the test peptides (10 μL). The final concentration of Aβ was kept 2 μM and that of test peptides as 2, 10, and 20 μM so that their ratios were as 1:1, 1:5, and 1:10 (Aβ: test peptides). The plates were incubated for 6 h. Untreated cell samples without Aβ or test peptides with other components in the same concentration as in the test wells were taken as control. After 6 h, 20 μL of MTT (5 mg/mL in PBS) was added in each well and incubated for 4 h. The plate was centrifuged at 4 °C for 10 min. Supernatant was carefully removed from the wells and DMSO (200 μL, per well) was added. The resulting suspension was mixed well, and OD_{570} were measured. Statistical analysis was performed by one way anova test followed by dunnett's multiple comparison test ($^{\$}p < 0.05$, $^{\#}p < 0.01$, $^{*}p < 0.001$, Vs Aβ) using software Graph pad Prism, ISI, San Diego, CA. *P* Values less than 0.05 were considered as significant.

3. Cytotoxicity study

PC-12 cells in their exponential growth phase were seeded in 96-well plates, at a rate of 17000 cells per well per 90 µL and incubated overnight. Next morning, the inhibitor pentapeptides (**6**, **9** and **15**, 10 µL, 200 µM in 10% DMSO in PBS), were added to make the final nominal concentration of 20 µM. The well-plate was incubated for 6 h. After 6 h, 20 µL of MTT (5 mg/mL in PBS) was added and incubated for 4 h. The plate was centrifuged at 4 °C for 10 minutes. Supernatant was carefully removed from the wells and DMSO (200 µL, per well) was added. The resulting suspension was mixed well and OD_{570} was measured using a microtiter plate reader.
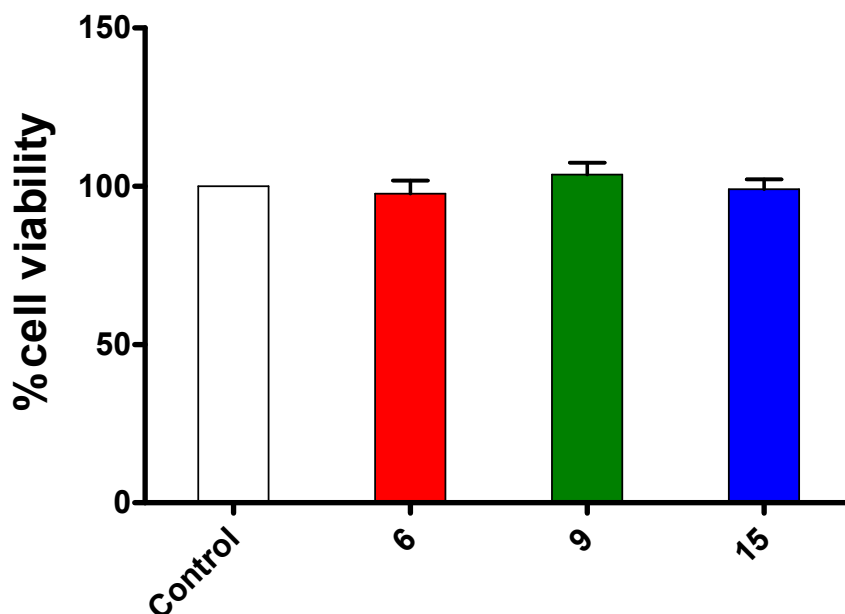
Figure S1. Effect of peptides **6**, **9** and **15** on the viability of PC-12 cells. PC-12 cells were treated with 20 µM of test peptides for 6 h, after which their ability to reduce MTT was measured. Bar graphs represent the cell viability of untreated cells (colorless, control) and cells treated with peptides **6**, **9** and **15** (red, green and blue respectively). Each experiment was performed in triplicates (n= 3). ODs (absorbance) of samples with untreated cells were set to 1. The percentage of MTT cellular reduction in the presence of test peptides was determined by comparing the OD_{570} of each test sample to the OD_{570} of the cells alone in control. Blank ODs were subtracted from each sample OD and the triplicate sample ODs were averaged.

4. Thioflavin T fluorescence assay

Black, clear bottomed, 96-well plates and thioflavin T were purchased from Sigma-Aldrich Chemicals, Saint Louis, MO, USA. All the solutions were pre-sterilized using 0.2 µm syringe filters. Measurements were performed on Tecan M200 plate reader. $A\beta_{1-42}$ and $A\beta_{1-40}$ were taken as 2 µM and 10 µM, respectively, and the pentapeptides **6**, **9**, **14**, **15** and **21** were studied in concentrations ratios of 1:1, 1:5 and 1:10 ($A\beta$: test peptides). The samples were incubated for 24 and 72 h for $A\beta_{1-42}$ and $A\beta_{1-40}$, respectively. Further, the pentapeptides (**6**, **9**, **14**, **15** and **21)** were also studied against the aggregation of the eleven-residue fragment peptide $A\beta_{25-35}$. The concentration of $A\beta_{25-35}$ was fixed at 25 µM and inhibitor pentapeptides were taken in concentrations as 25, 125 and 250 µM to keep the ratios as 1:1, 1:5, 1:10. All test samples were incubated for 5 days prior to the fluorescence measurements.

Assay: Monomeric $A\beta_{1-42}$ was dissolved in 20 mM NaOH to obtain a concentration of 400 µM, and then diluted in sodium phosphate buffer (pH 7.4) to 20 µM. 120 µL of ThT (2.5 µM), dissolved in glycine-NaOH buffer (pH 8.5) was added in each well of a black, 96-well plate with clear bottom. Peptides **6**, **9**, **14**, **15** and **21** were dissolved in DMSO at 5 mM stock and diluted in PBS to obtain concentrations of 200 µM, 100 µM and 20 µM. 15 µL of inhibitor peptides was added to each well, followed by the addition of 15 µL of $A\beta_{1-42}$ to bring the final nominal concentrations of $A\beta_{1-42}$: test peptides in ratios of 1:1, 1:5 and 1:10. The plate was incubated for 24 h at 37 °C and shaken at 200 rpm. Plate was read at excitation and emission wavelengths of 445 nm and 485 nm, respectively. Except for the concentrations and the incubation periods, similar protocol was followed for the ThT study on $A\beta_{1-40}$ and $A\beta_{25-35}$ peptides. Statistical analysis was performed by one way anova test followed by dunnett's multiple comparison test ($^\$p < 0.05$, $^\#p < 0.01$, $^*p < 0.001$, Vs Control) using software Graph pad Prism, ISI, San Diego, CA. *P* Values less than 0.05 were considered as significant. The enhancement in fluorescence (RFU) caused by $A\beta$ binding was taken as 100% and percentage inhibition of amyloid beta aggregation was calculated from the corresponding reduction in ThT fluorescence by using the formula: $100* [1-\{RFU_{485} (test)-RFU_{485} (control)/RFU_{485} (A\beta)-RFU_{485}(control)\}]$, and the data obtained in triplicates were averaged (<5% variation). The percentage inhibition of aggregation in the presence of the inhibitor pentapeptides against the aggregation of $A\beta_{1-42}$ and $A\beta_{25-35}$ has been described in Table T1 and Table T2 respectively.

Table T1. RFU values and calculated % inhibition by inhibitor peptides against $A\beta_{1-42}$ peptide aggregation

Peptides		RFU Values			(%) Inhibition of $A\beta_{1-42}$		
No.	Sequence	20 µM	10 µM	2 µM	20 µM	10 µM	2 µM
6	Pro-Val-Val-Ile-Ala	78.1	28.5	48.9	30.0	98.0	70.0
9	Gly-Gly-Val-Ile-Ala	27.0	84.0	86.1	100	22.0	19.0
14	Gly-Aib-Val-Ile-Ala	34.3	35.8	56.2	90.0	88.0	60.0
15	Gly-Phe-Val-Ile-Ala	27.0	74.5	78.1	100	35.0	30.0
21	Gly-Val-Aib-Ile-Ala	79.6	49.7	56.2	28.0	69.0	60.0
Control		27.0					
$A\beta_{1-42}$		100					

Table T2. RFU values and calculated % inhibition by inhibitor peptides against $A\beta_{25-35}$ peptide aggregation

Peptides		RFU Values			(%) Inhibition of $A\beta_{25-35}$		
No.	Sequence	250 µM	125 µM	25 µM	250 µM	125 µM	25 µM
6	Pro-Val-Val-Ile-Ala	89.3	57.7	71.2	25.0	98.0	67.0
9	Gly-Gly-Val-Ile-Ala	100	100	100	0	0	0
14	Gly-Aib-Val-Ile-Ala	100	99.1	99.6	0	2.0	1.0
15	Gly-Phe-Val-Ile-Ala	89.0	100	95.7	25.0	0	10
21	Gly-Val-Aib-Ile-Ala	100	100	100	0	0	0
Control		57.0					
$A\beta_{25-35}$		100					

In the time-dependent study, peptide **15** was incubated with the $A\beta_{1-42}$ peptide and fluorescence was measured at regular intervals for duration of 7 days. Inhibition activity was

calculated by the relative percent enhancement in the RFU of the amyloid-bound ThT fluorescence of the target-inhibitor mixture compared to that in the absence of the inhibitor. The percentage inhibition of the $A\beta_{1-42}$ aggregation is given by $[1-\{(Fb-Fc)/(Fa-Fc)\}]\times100$, where Fa is the ThT fluorescence of the target peptide alone, Fb is the ThT fluorescence of the target-inhibitor complex, and Fc is the background ThT fluorescence with no target or inhibitor present.

5. Circular dichroism spectroscopy study

The instrument (Jasco, J-815 CD spectrometer) was calibrated using the freshly prepared (0.6% w/v) ammonium salt of (+)-camphor-10-sulfonic acid as a reference. CD studies were performed using a 1 mm quartz cell in the far-UV wavelength range of 190-260 nm at 37 °C. Data points were collected in 0.2 nm intervals at a speed of 50 nm per minute with a response time of 1 second and a band width of 2 nm. An average of 3 scans was taken to generate the data.

Assay: Samples for circular dichroism study were prepared much like the thioflavin T assay with the only difference being that trifluoroethanol (TFE) was used to dissolve peptide **15** in place of DMSO. $A\beta_{1-42}$ was dissolved in NaOH to a concentration of 200 µM and then diluted in SPB to a concentration of 20 µM. The peptide **15** was dissolved in TFE and diluted in SPB to a concentration of 100 µM. Equal volumes of $A\beta_{1-42}$ and peptide **15** were mixed to result in a concentration ratio of 1:5 ($A\beta_{1-42}$: **15**) and incubated at 37 °C for 12 h ($t_{12\ h}$) in an eppendorf. The eppendorf tube was sealed tightly to avoid evaporation of TFE. The final CD spectra for a test samples were determined by subtracting the buffer blank spectrum from that acquired for the samples. The far-UV CD spectra were smoothed by noise reduction. The direct CD measurements (θ, in mdeg) were converted to molar ellipticity, using $[\theta] = \theta/(10\cdot C\cdot l)$, where C is the molar concentration (mol/L) and l, the path length. The molar ellipticity $[\theta]$ is in units, deg cm^2 $dmol^{-1}$. At least three scans were recorded for each run sample and data were averaged. Deconvolution of CD spectra was performed using software spectra manager II and the relative percentages of various forms of secondary structures were calculated. Aβ and the inhibitor pentapeptide **15** were mixed in a ratio of 1:5 and incubated for 12 h ($t_{12\ h}$) before the secondary structure analysis was performed. The effect of peptide **15** on the conformation of the $A\beta_{1-42}$ peptide was analyzed by subtracting the inhibitor spectrum from that of inhibitor-treated $A\beta_{1-42}$. For conformational study protocol similar to the one described for the activity study of inhibitory peptide **15** was used. Peptides **6** and **29** were taken in 10-fold excess relative to $A\beta_{1-42}$ and incubated at 37 °C for 12 h ($t_{12\ h}$). A control run of $A\beta_{1-42}$ peptide alone was subtracted from the test sample so that the resultant shows the CD spectrograph of the peptides **6** and **29** alone.

CD spectra: Zoomed section (210-230 nm)
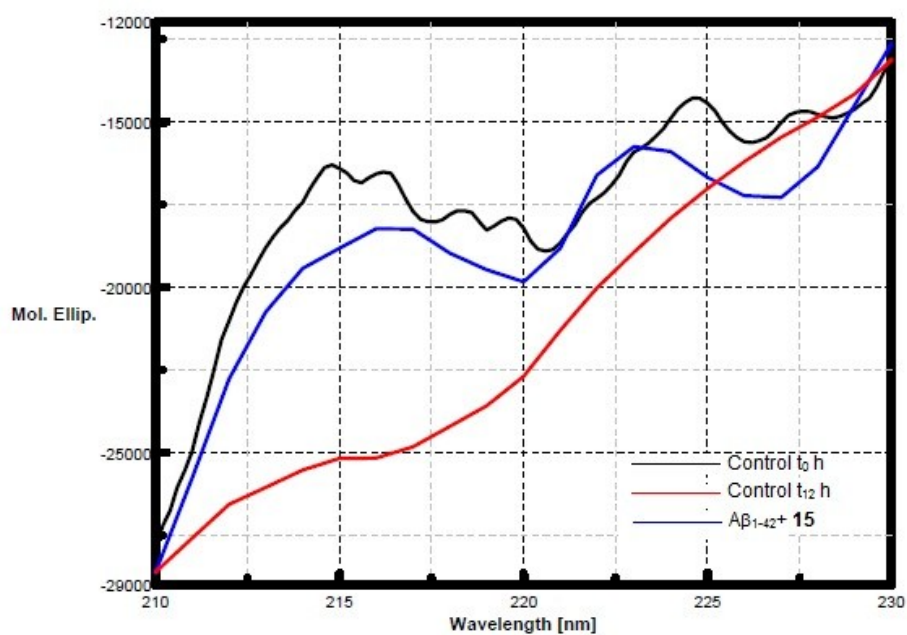
Figure S2. A zoomed section (210-230 nm) of full CD spectra (190-260 nm, full text) showing the inhibitory effects of peptide (**15**) on $A\beta_{1-42}$ conformation. Differently colored curves show the $A\beta_{1-42}$ sample at t_{0h} (black), $A\beta_{1-42}$ sample at t_{12h} (red) and $A\beta_{1-42}$ peptide, co-incubated with peptide (**15**, blue) at t_{12h}.
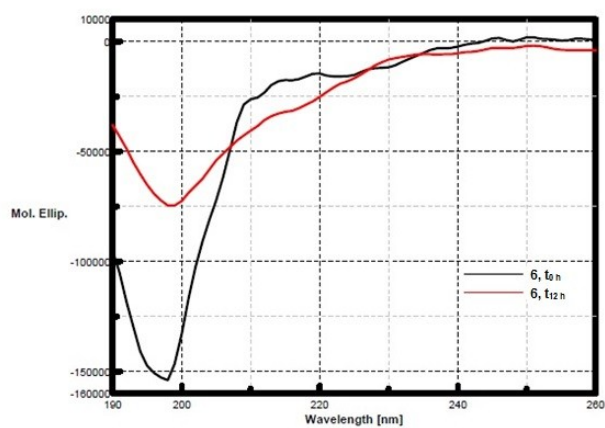

Figure S3. CD spectral analysis to study the conformational behaviour of pentapeptide **6**. Initial spectrum at t_{0h} is shown by black while red line shows CD spectrum of peptide **6** after 12 h of incubation. Relatively deeper minima at 217 nm after 12 hrs of incubation of peptide **6** compared to the curve at start time shows the formation of β-sheet.
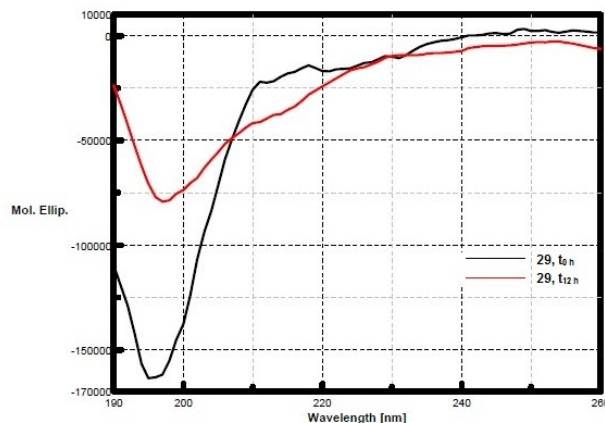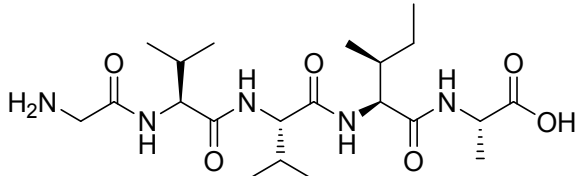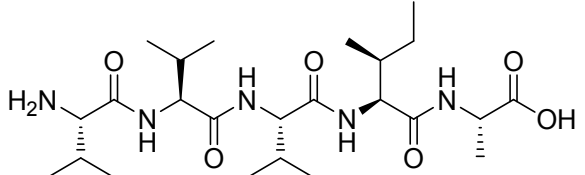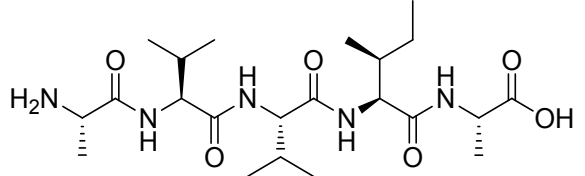
Figure S4. CD spectral analysis to study the conformational behaviour of pentapeptide **29**. Initial spectrum at t_{0h} is shown by black while red line shows CD spectrum of peptide **29** after 12 h of incubation. Relatively deeper minima at 217 nm after 12 hrs of incubation of peptide **29** compared to the curve at start time shows the formation of β-sheet.
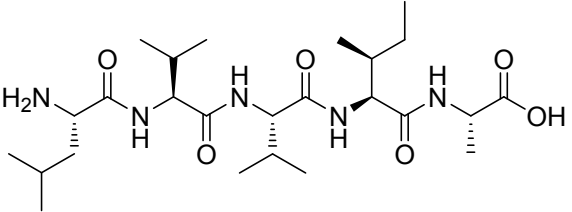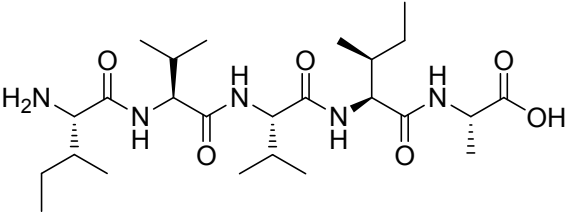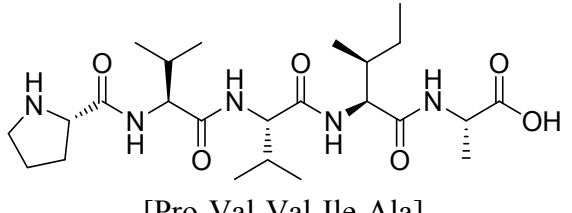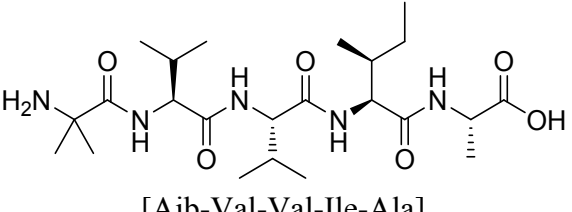
6. Transmission electron microscopy study
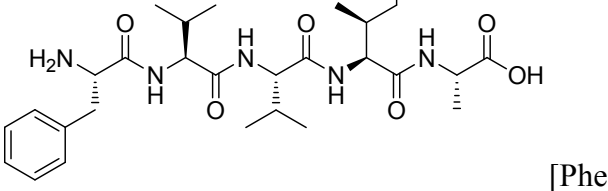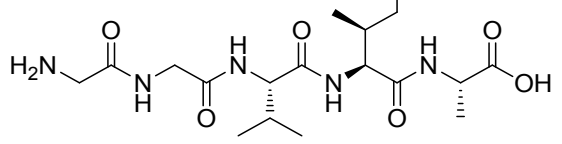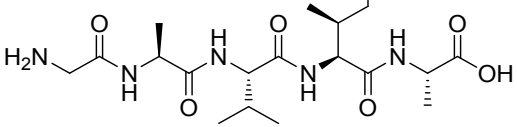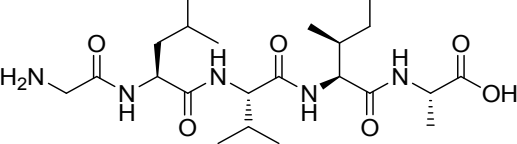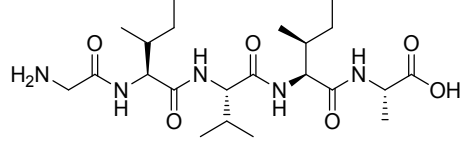
For high resolution transmission electron microscopy (HR-TEM) images and scanning transmission electron microscopy (STEM) analysis, FEI Tecnai (G2 F20) operating at 120 keV was used. Uranyl acetate and glutaraldehyde (EM grade) were purchased from Sigma-aldrich chemicals, Saint Louis, MO, USA. Electron microscopy grids of copper (carbon coated, 200 mesh) were purchased from Electron Microscopy Sciences.
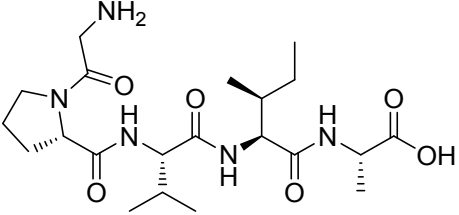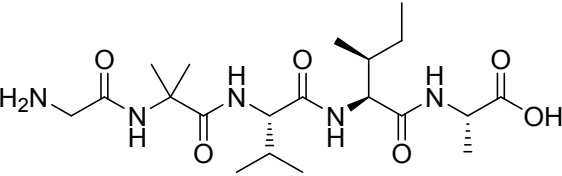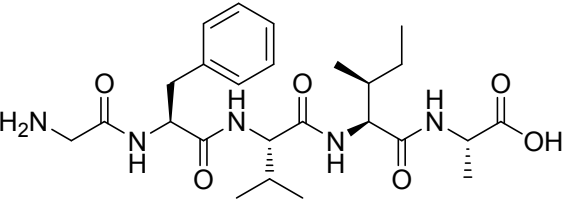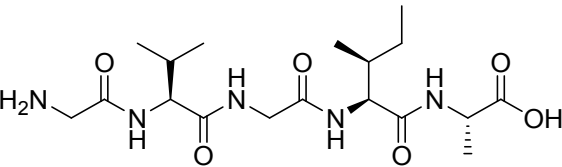
Assay: An aliquot of Aβ$_{1-42}$ peptide was dissolved in 20 mM NaOH to make it 500 μM and then diluted in 10 mM of SPB (pH 7.4) to reach a concentration of 50 μM. The pentapeptides **15** and **17** (negative control), pre-dissolved in DMSO as 5 mM stock solutions were diluted in SPB to a final concentration of 250 μM. 25 μL of Aβ$_{1-42}$ was mixed with 25 μL of peptides **15** and **17**, to result in a concentration ratio of 1:5 (Aβ$_{1-42}$:test peptides). The solutions were mixed well and incubated at 37 °C. After 72 h, 3-5 μL of sample was placed on a glow discharged grid. The sample was fixed on the grid by applying equal volume of 0.5% of glutaraldehyde solution by droplet procedure 3-5 times. The grid was washed with ultrapure water (3-5 μL) three times by single droplet method. Grids were negatively stained by 2% uranyl acetate. The sample was air dried. Excess liquid was wicked away at every step using filter paper carefully through the grid edges, without letting the grid dry. At last, the grid was examined under the electron microscope. Aβ$_{1-42}$ alone plus the buffers in similar ratios and concentrations was used as a control. Each EM grid was examined at several positions (>10) for the unbiased classification of fibril density and morphology.
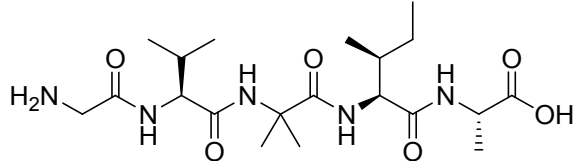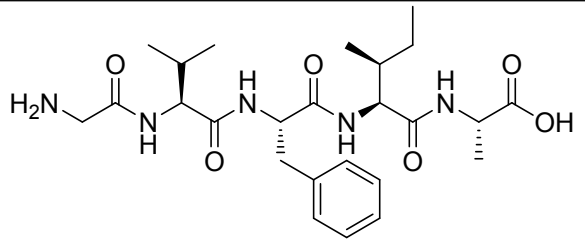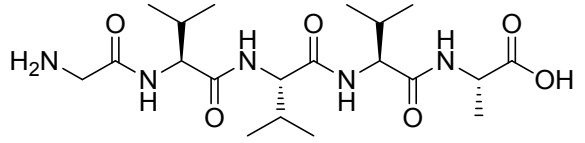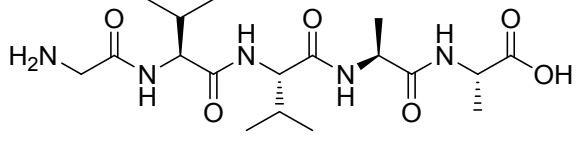
7. Table T3.

Peptide No.	Structure and Sequence	Mol. formula (Mol. weight)	HRMS data		HPLC data	
			Calcd.	Obsd.	$t_{R \text{ (min)}}$	Purity (%)
1	[Gly-Val-Val-Ile-Ala]	$C_{21}H_{39}N_5O_6$ (457.290)	458.2978	458.2978	18.81	97.4
2	[Val-Val-Val-Ile-Ala]	$C_{24}H_{45}N_5O_6$ (499.337)	500.3448	500.3454	12.49	98.9
3	[Ala-Val-Val-Ile-Ala]	$C_{22}H_{41}N_5O_6$ (471.3057)	472.3135	472.3140	10.47	99.4
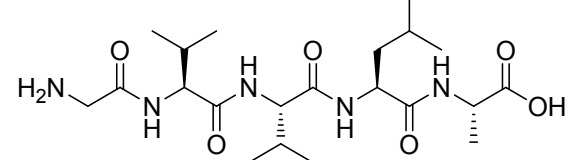
HRMS and HPLC data of synthesized peptides
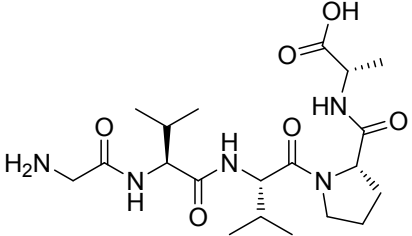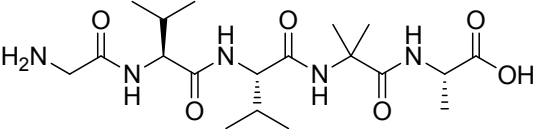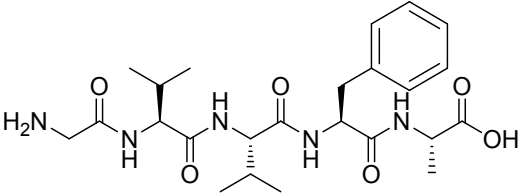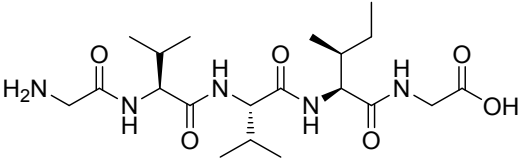
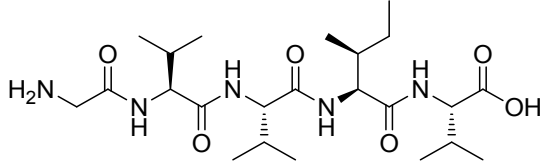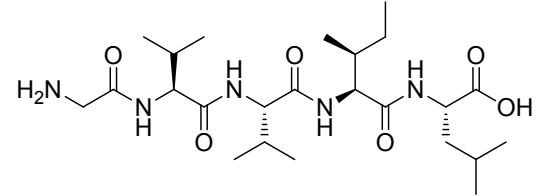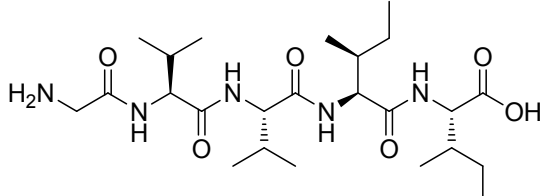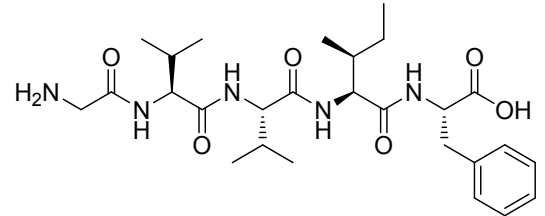| 4	[Leu-Val-Val-Ile-Ala]	$C_{25}H_{47}N_5O_6$ (513.3526)	514.3604	514.3608	15.55	99.4
5	[Ile-Val-Val-Ile-Ala]	$C_{25}H_{47}N_5O_6$ (513.3526)	514.3604	514.3599	14.74	100
6	[Pro-Val-Val-Ile-Ala]	$C_{24}H_{43}N_5O_6$ (497.3213)	498.3291	498.3308	12.15	100
7	[Aib-Val-Val-Ile-Ala]	$C_{23}H_{43}N_5O_6$ (485.3213)	486.3291	486.3331	12.23	98.1

8	[Phe -Val-Val-Ile-Ala]	$C_{28}H_{45}N_5O_6$ (547.337)	548.3448	548.3453	17.70	97.5
9	[Gly-Gly-Val-Ile-Ala]	$C_{18}H_{33}N_5O_6$ (415.2431)	416.2509	416.2510	7.37	100
10	[Gly-Ala-Val-Ile-Ala]	$C_{19}H_{35}N_5O_6$ (429.2587)	430.2665	430.2659	7.26	95.1
11	[Gly-Leu-Val-Ile-Ala]	$C_{22}H_{41}N_5O_6$ (471.3057)	472.3135	472.3150	14.58	95.7
12		$C_{22}H_{41}N_5O_6$ (471.3057)	472.3135	472.3143	13.67	98.8

	[Gly-Ile-Val-Ile-Ala]				
13	 [Gly-Pro-Val-Ile-Ala]	$C_{21}H_{37}N_5O_6$ (455.2744)	456.2822	456.2832	8.86	97.1
14	 [Gly-Aib-Val-Ile-Ala]	$C_{20}H_{37}N_5O_6$ (443.2744)	444.2832	444.2825	9.55	100
15	 [Gly-Phe-Val-Ile-Ala]	$C_{25}H_{39}N_5O_6$ (505.2900)	506.2978	506.2983	16.97	96.9
16	 [Gly-Val-Gly-Ile-Ala]	$C_{18}H_{33}N_5O_6$ (415.2431)	416.2509	416.2523	7.93	97.9

17	 [Gly-Val-Ala-Ile-Ala]	$C_{19}H_{35}N_5O_6$ (429.2587)	430.2665	430.2667	13.16	95.7
18	 [Gly-Val-Leu-Ile-Ala]	$C_{22}H_{41}N_5O_6$ (471.3057)	472.3135	472.3156	14.13	95.7
19	 [Gly-Val-Ile-Ile-Ala]	$C_{22}H_{41}N_5O_6$ (471.3057)	472.3135	472.3143	13.04	99.5
20	 [Gly-Val-Pro-Ile-Ala]	$C_{21}H_{37}N_5O_6$ (455.2744)	456.2822	456.2833	9.05	97.3

21	[Gly-Val-Aib-Ile-Ala]	$C_{20}H_{37}N_5O_6$ (443.2744)	466.2635	466.2627	11.83	96.5
22	[Gly-Val-Phe-Ile-Ala]	$C_{25}H_{39}N_5O_6$ (505.2900)	506.2978	506.2990	16.39	98.9
23	[Gly-Val-Val-Val-Ala]	$C_{20}H_{37}N_5O_6$ (443.2744)	444.2822	444.2829	7.44	99.2
24	[Gly-Val-Val-Ala-Ala]	$C_{18}H_{33}N_5O_6$ (415.2431)	416.2509	416.2508	5.40	96.1
25		$C_{21}H_{39}N_5O_6$ (457.2900)	458.2978	458.2995	11.13	95.6

	[Gly-Val-Val-Leu-Ala]					
26	 [Gly-Val-Val-Pro-Ala]	$C_{20}H_{35}N_5O_6$ (441.2587)	442.2665	442.2661	6.66	99.3
27	 [Gly-Val-Val-Aib-Ala]	$C_{19}H_{35}N_5O_6$ (429.2587)	430.2665	430.2669	9.99	98.2
28	 [Gly-Val-Val-Phe-Ala]	$C_{24}H_{37}N_5O_6$ (491.2744)	492.2822	492.2833	19.54	96.5
29	 [Gly-Val-Val-Ile-Gly]	$C_{20}H_{37}N_5O_6$ (443.2744)	444.2822	444.2821	13.45	96.2

30	[Gly-Val-Val-Ile-Val]	$C_{23}H_{43}N_5O_6$ (485.3213)	486.3291	486.3291	14.88	96.7
31	[Gly-Val-Val-Ile-Leu]	$C_{24}H_{45}N_5O_6$ (499.3370)	500.3448	500.3457	18.76	98.3
32	[Gly-Val-Val-Ile-Ile]	$C_{24}H_{45}N_5O_6$ (499.3370)	500.3448	500.3465	18.40	98.2
33	[Gly-Val-Val-Ile-Phe]	$C_{27}H_{43}N_5O_6$ (533.3213)	534.3291	534.3544	21.15	96.9

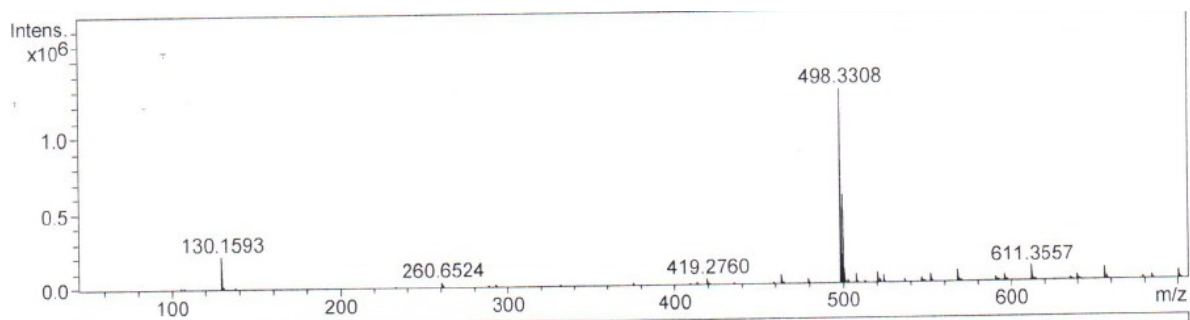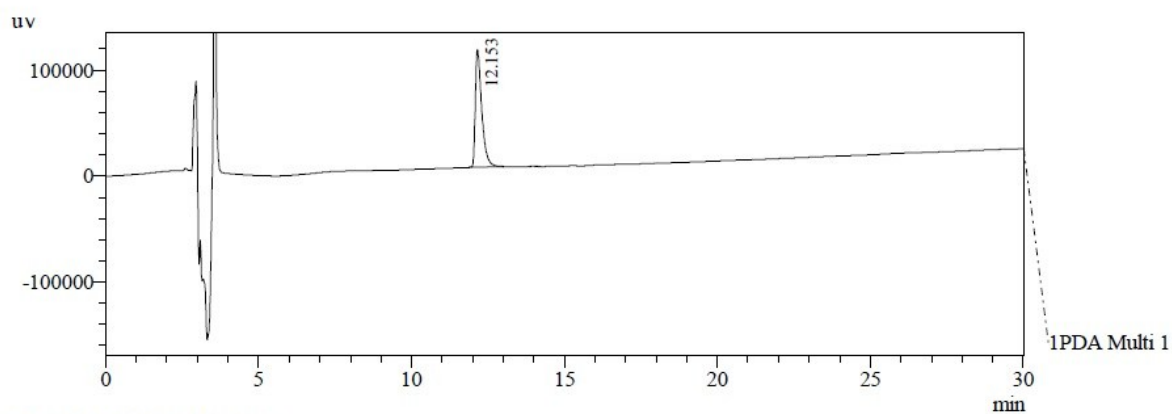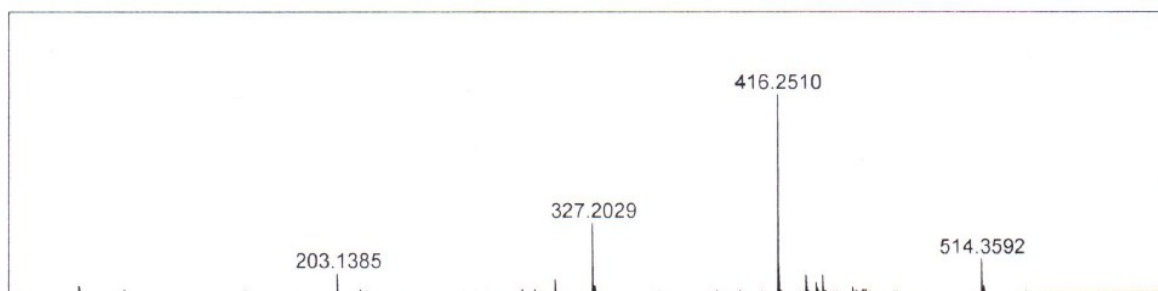## 8. HRMS and HPLC chromatograms of representative peptides
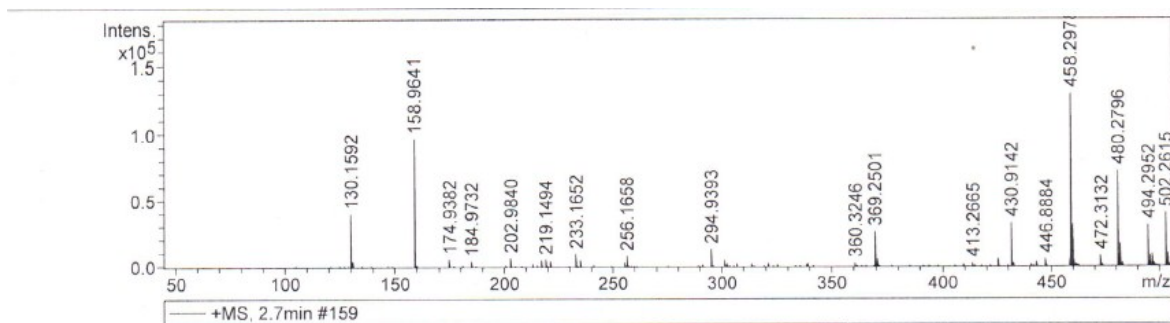
Figure S5. HRMS spectra of peptide (**1**)
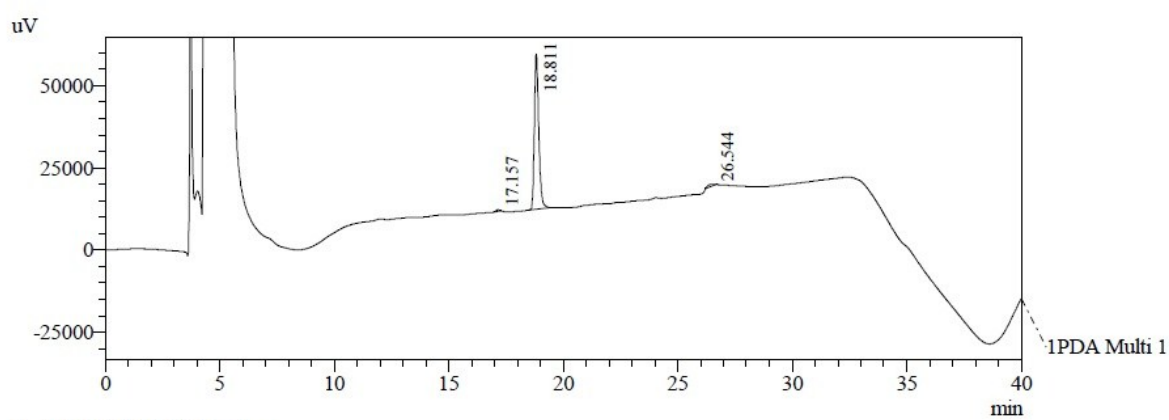
1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	17.157	4055	396	0.628
2	18.811	629161	47131	97.483
3	26.544	12192	382	1.889
Total		645407	47908	100.000

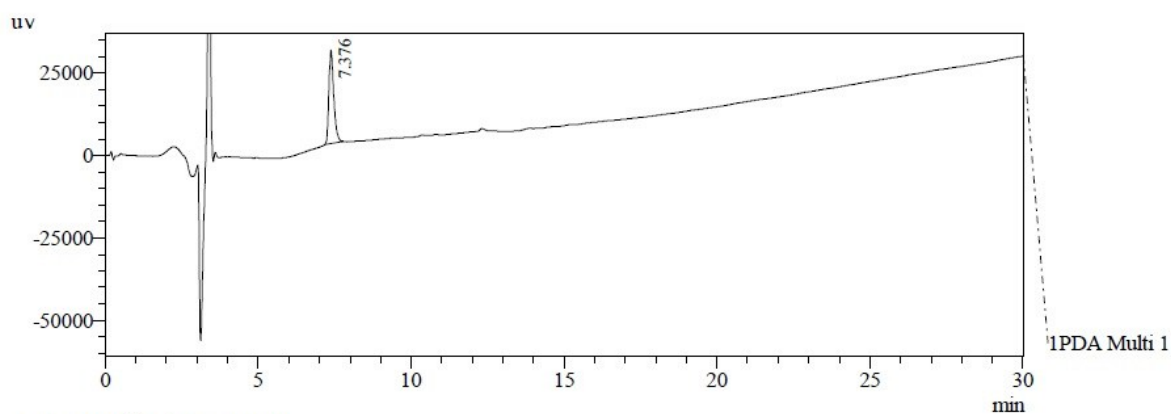**Figure S6.** HPLC chromatogram of peptide (**1**)
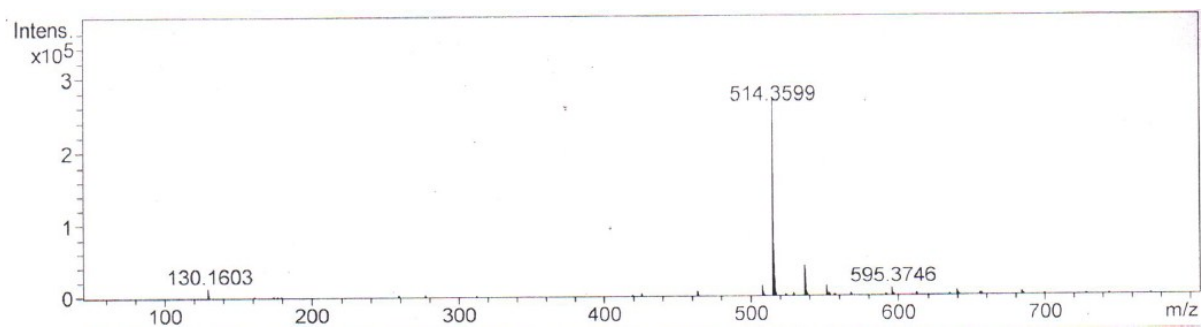
Figure S7. HRMS spectra of peptide (**5**)
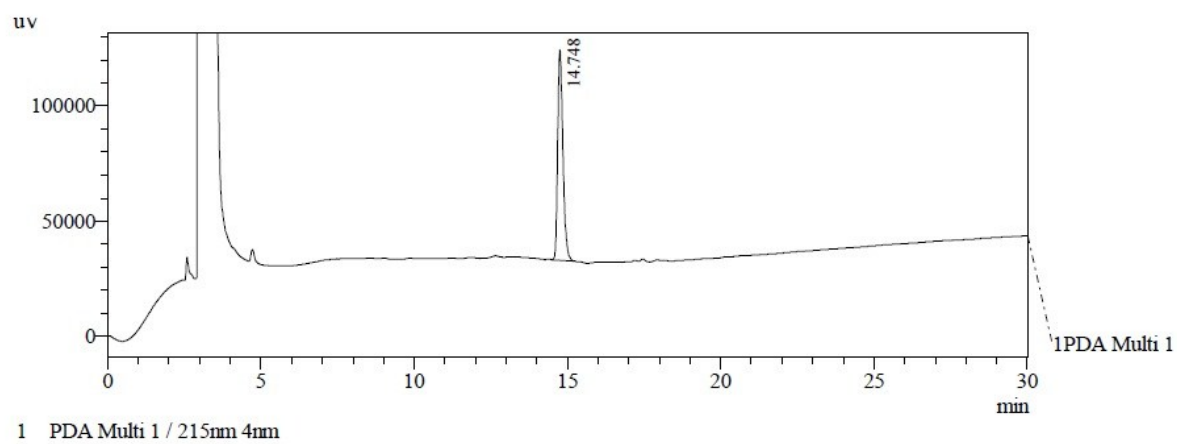
1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	14.748	1079651	91402	100.000
Total		1079651	91402	100.000

Figure S8. HPLC chromatogram of peptide (**5**) (Gly$_{38}$ scan)

Figure S9. HRMS spectra of peptide (**6**)

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	12.153	1612419	111135	100.000
Total		1612419	111135	100.000

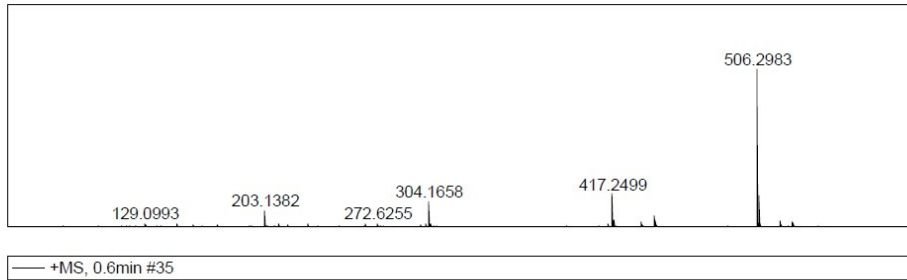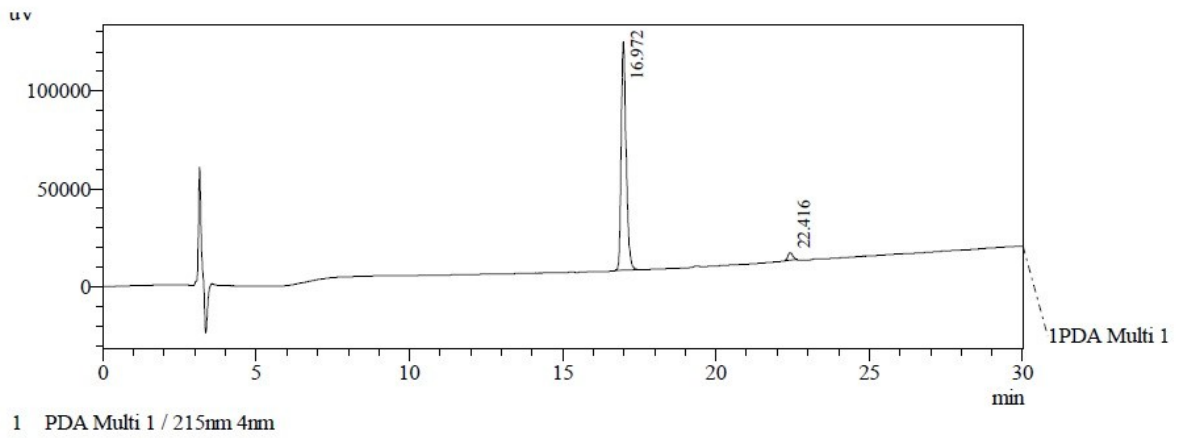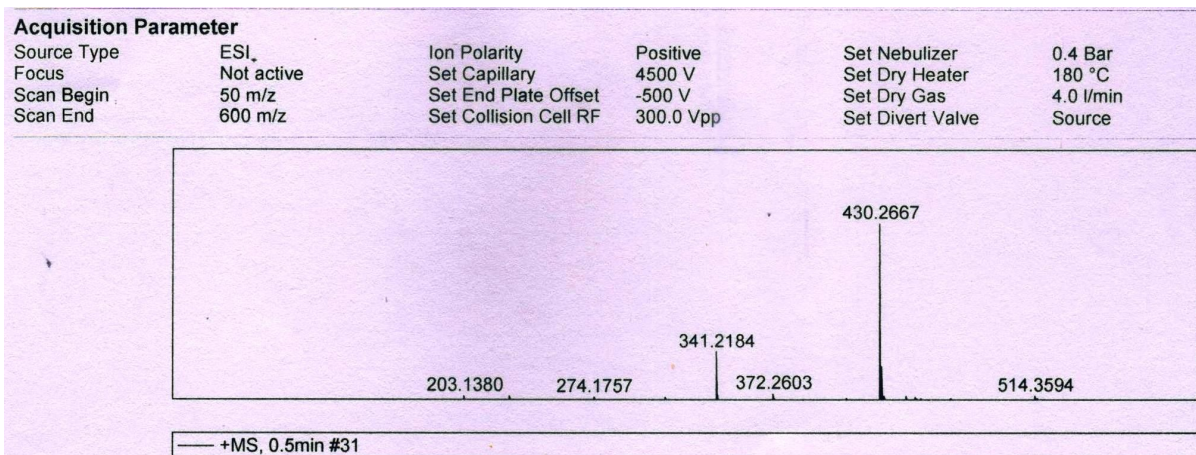**Figure S10.** HPLC chromatogram of peptide (**6**) (Gly$_{38}$ scan)

Figure S11. HRMS spectra of peptide (**9**)

1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	7.376	314561	28271	100.000
Total		314561	28271	100.000

Figure S12. HPLC chromatogram of peptide (**9**) (Val$_{39}$ scan)

506.2983

129.0993   203.1382   272.6255   304.1658   417.2499

+MS, 0.6min #35

Figure S13. HRMS spectra of peptide (**15**)

1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	16.972	1318174	116611	96.941
2	22.416	41599	3751	3.059
Total		1359774	120362	100.000

Figure S14. HPLC chromatogram of peptide (**15**) (Val$_{39}$ scan)

430.2667

341.2184

203.1380   274.1757   372.2603   514.3594

+MS, 0.5min #31

Figure S15. HRMS spectra of peptide (**17**)

Chromatogram
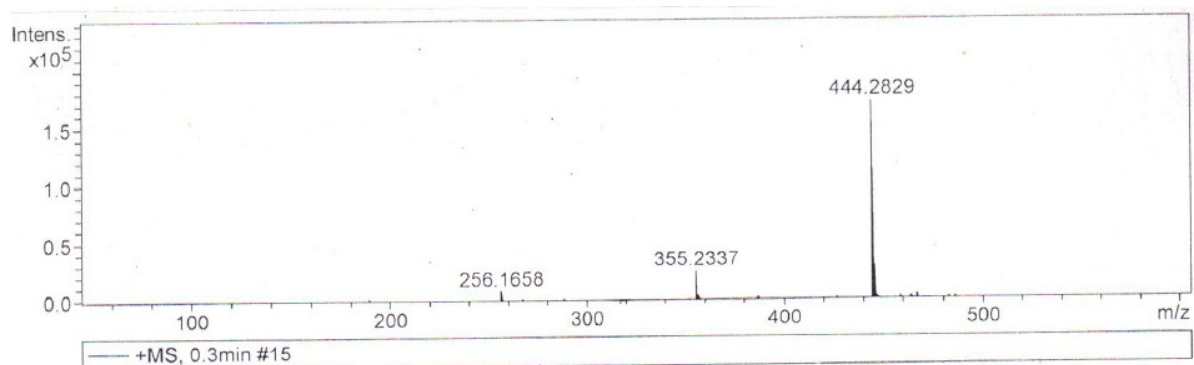SB-120-F1R E:\HPLC\DATA\sunil\SBP-Series\SB-120-F1R.lcd

uV

14.134

19.614

1PDA Multi 1

1   PDA Multi 1 / 215nm 4nm
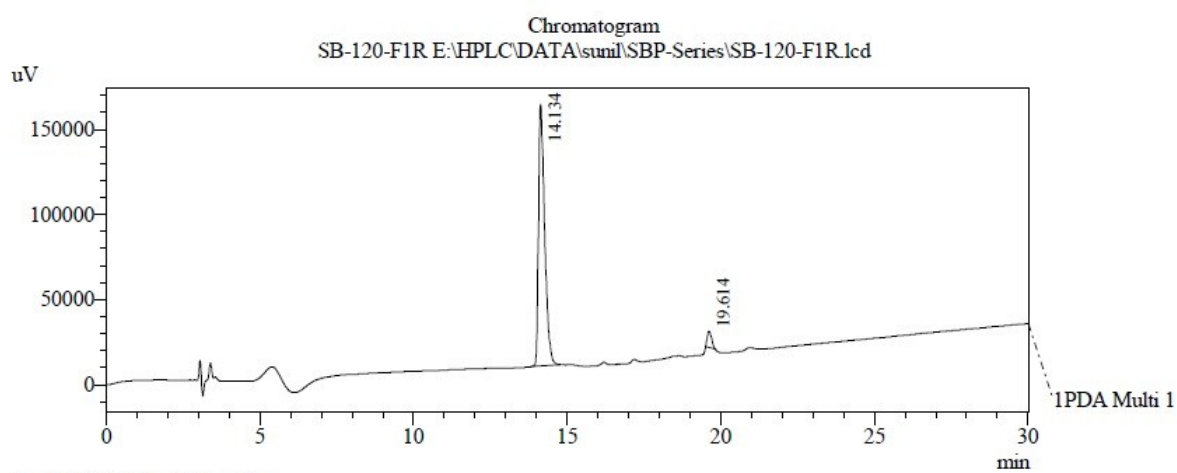
PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	14.134	2149405	153823	95.749
2	19.614	95432	9558	4.251
Total		2244837	163381	100.000

Figure S16. HPLC chromatogram of peptide (**17**) (Val_{40} scan)
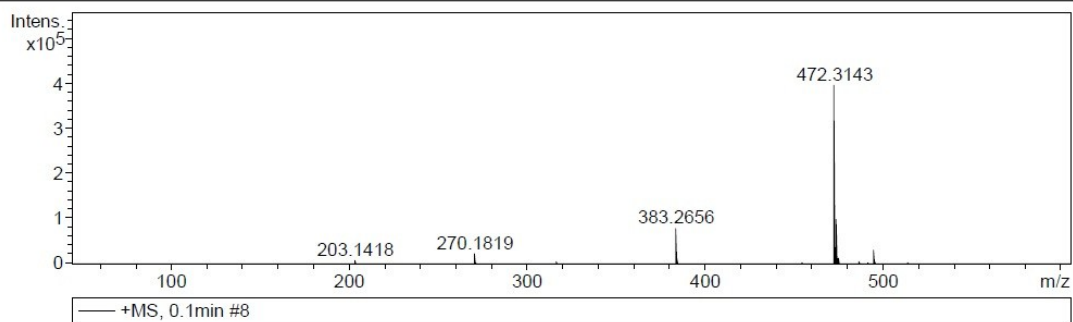
Figure S17. HRMS spectra of peptide (**19**)
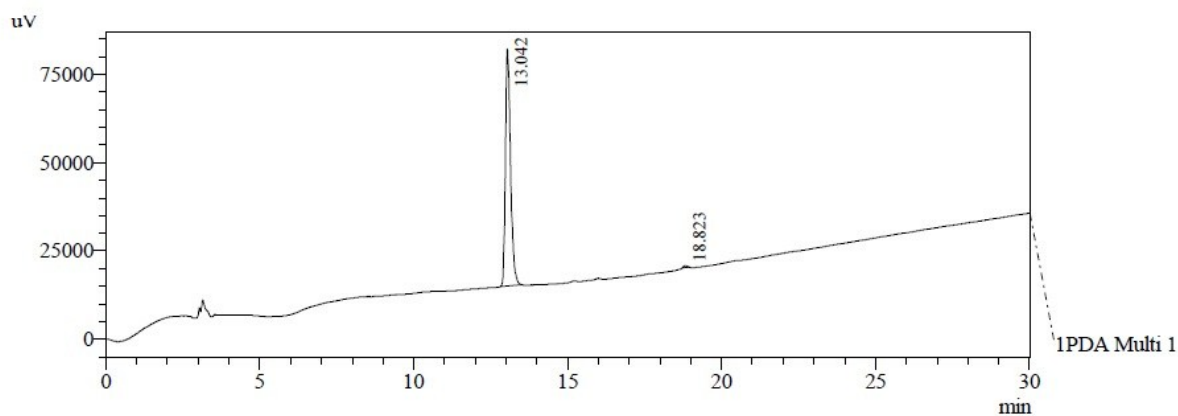
PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	13.042	780093	66985	99.479
2	18.823	4085	515	0.521
Total		784178	67500	100.000

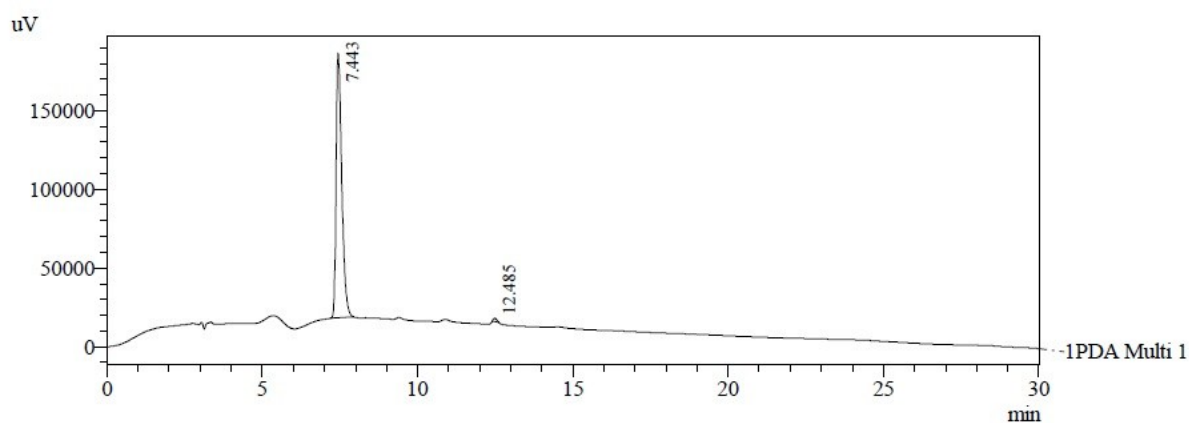**Figure S18.** HPLC chromatogram of peptide (**19**) (Val_{40} scan)

Figure S19. HRMS spectra of peptide (**23**)

1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	7.443	2148885	168151	99.208
2	12.485	17159	2286	0.792
Total		2166043	170438	100.000

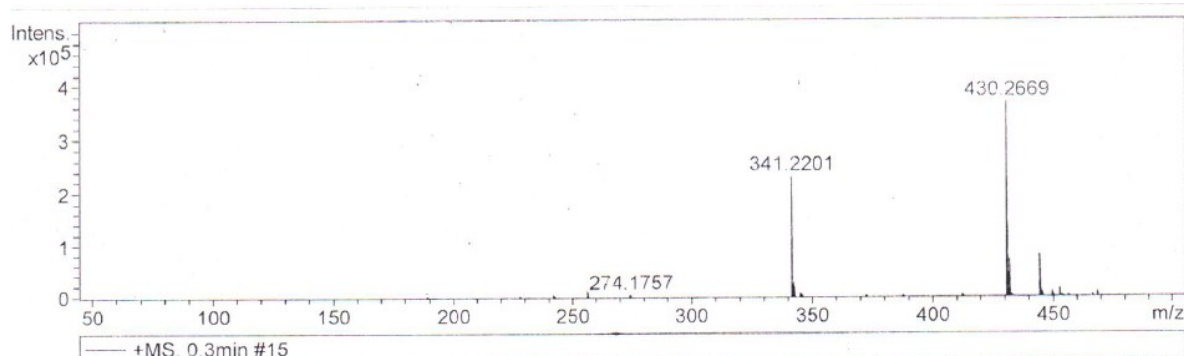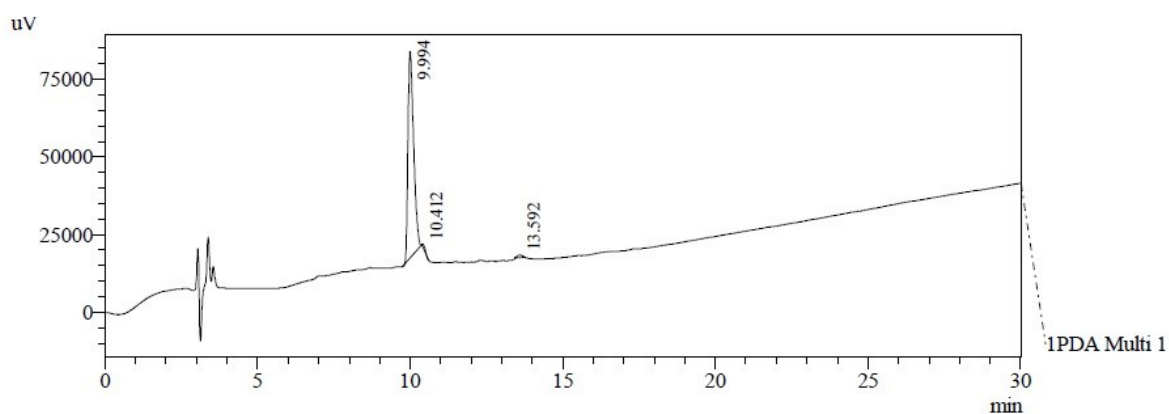**Figure S20.** HPLC chromatogram of peptide (**23**) (Ile_{41} scan)

Figure S21. HRMS spectra of peptide (**27**)

1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	9.994	853199	66446	98.212
2	10.412	5339	726	0.615
3	13.592	10190	893	1.173
Total		868728	68065	100.000

Figure S22. HPLC chromatogram of peptide (**27**) (Ile$_{41}$ scan)

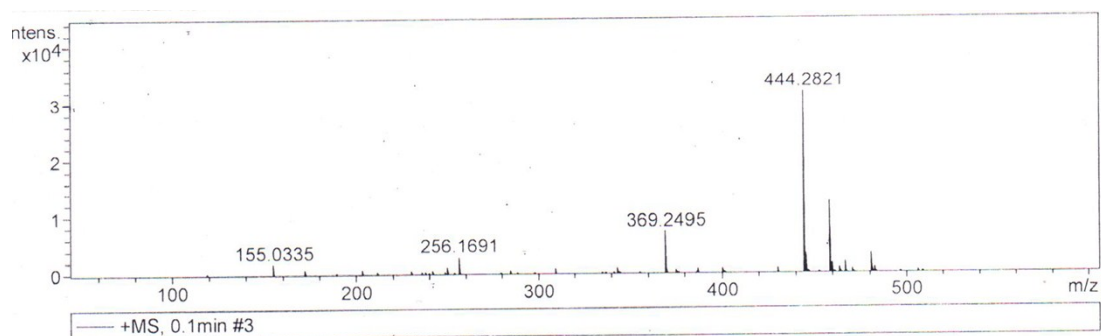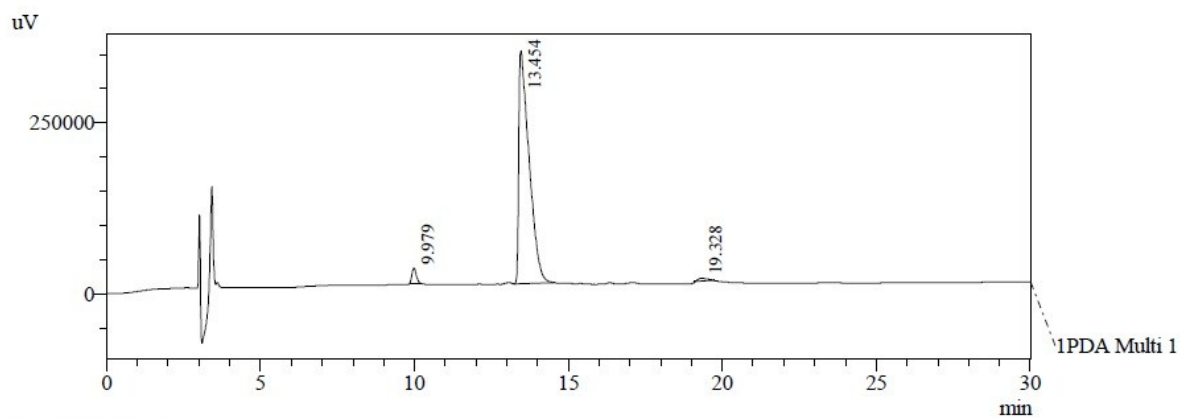**Figure S23.** HRMS spectra of peptide (**29**)

1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	9.979	217924	22530	2.643
2	13.454	7932408	340253	96.211
3	19.328	94446	4097	1.146
Total		8244778	366879	100.000

Figure S24. HPLC chromatogram of peptide (**29**) (Ala$_{42}$ scan)
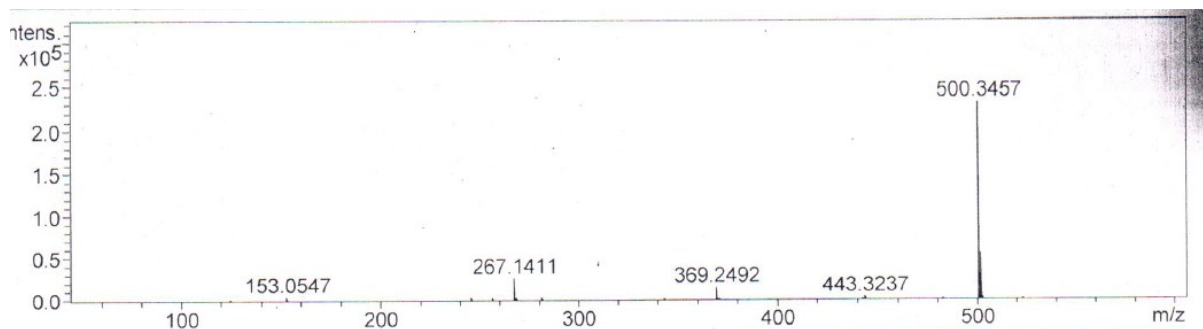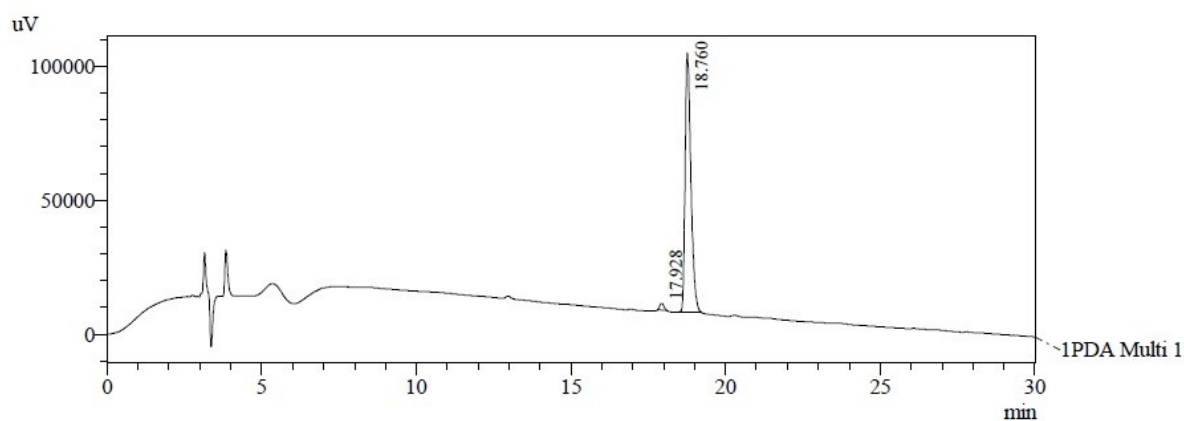
Figure S25. HRMS spectra of peptide (**31**)

1   PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	17.928	21652	2388	1.707
2	18.760	1246421	96534	98.293
Total		1268074	98922	100.000

Figure S26. HPLC chromatogram of peptide (**31**) (Ala$_{42}$ scan)

References

1. Zagorski, M. G., Yang, J., Shao, H., Ma, K., Zeng, H., and Hong, A. (1999) Methodological and chemical factors affecting amyloid-β peptide amyloidogenicity. *Methods Enzymol. 309*, 189–204.