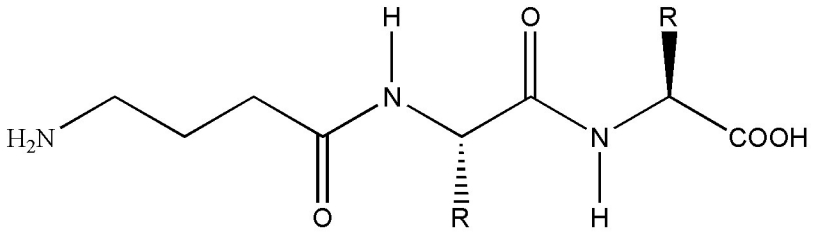
Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information for:

De novo designed aliphatic and aromatic peptides assemble into amyloid-like cytotoxic supramolecular nanofibrils

Contents

Chemical structure of peptides:



For Peptide 1, R = $CH(CH_3)_2$ Peptide 4, R = CH_2Ph
Peptide 2, R = $CH_2CH(CH_3)_2$ Peptide 5, R = CH_2Ph(*p*-OH)
Peptide 3, R = $CH(CH_3)(C_2H_5)$ Peptide 6, R = CH_2(1H-indole-3-yl)

Figure S1: The general chemical structure of the peptides.

Synthesis of peptides

All peptides have been synthesized by conventional solution phase methods using racemization free fragment condensation strategy.[1] The Boc group has been used for N-terminal protection and the C-terminus -COOH group is protected as methylester. Couplings are mediated by dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (DCC-HOBt). Deportation of OMe-group has performed using NaOH catalyzed saponification method and Boc-group by trifluoro aceticacid (TFA). All intermediates have been characterized by ^{1}H NMR spectroscopy and the final compounds are fully characterized by NMR, mass and IR spectroscopy.

Synthesis of peptide 1:

Synthesis of Boc-γ-Abu-OH

A mixture of 1, 4-dioxane (40 mL), water (20 mL) and 1(N) NaOH (20 mL) are stirred in the round bottom flask (R.B). Then 2.06 gm (20 mmol) γ-Abu is added to this mixture. When γ-Abu dissolves completely, the R.B is placed in ice cooled water bath and di-tertbutyldicarbonate (4.37 gm, 20 mmol) is added. The solution is allowed to come at room temperature under stirring and it is continued for 12 hour. Then the solution is concentrated in *vacuum* to about 10 to15 mL, cool in ice water bath, acidify with dilute solution of $KHSO_4$ to pH 2-3 (congo red) and cover

with the layer of ethyl acetate (about 10 mL). The aqueous phase is extracted with ethyl acetate and this operation is done thrice. The ethyl acetate extracts are pooled, wash with water and dry over anhydrous Na_2SO_4 and evaporate in *vacuum*. A white waxy material is obtained.

Chemical formula and molecular mass: $C_9H_{17}NO_4$, 203.1158
Yield: 3.35 gm, (~16.5 mmol, 82.5%).

Boc-γ-Abu-Val-OMe

3.24 gm (~16 mmol) of Boc-γ-Abu-OH is dissolved in 5 mL of dry DMF in R.B and 2.16 gm (16 mmol) of N-hydroxybenzotriazole (HOBt) is added to it. When HOBt is dissolved completely, R.B is placed in ice cooled water bath. 3-4 ml of ethyl acetate containing L-Val-OMe, isolated from 4.58 gm (35 mmol) of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 3.30 gm (16 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1 ×10 mL), 1(M) sodium carbonate (3×10 mL) and again with brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporate in *vacuum.* A white material is obtained.

Chemical formula and molecular mass: $C_{15}H_{28}N_2O_5$, 316.1998

Yield: 3.95 gm (~12.5 mmol, 78.12 %).

NMR analysis: ^{1}H NMR (400 MHz, $CDCl_3$, δ) **:** 6.70 (Val N<u>H</u>, 1H, *d, J*=7.6 Hz), 4.85 (γ-Abu N<u>H</u>, 1H, *b*), 4.47-4.43 (Val C$^{\alpha}$<u>H</u>, 1H, *m*), 3.66 (-OC<u>H</u>$_3$, 3H, *s*), 3.19-3.06 (γ-Abu C$^{\gamma}$<u>H</u>, 2H, *m*), 2.24-2.16 (γ-Abu C$^{\alpha}$<u>H</u>, 2H, *m)*, 2.15-2.08 (Val C$^{\beta}$<u>H</u>, 1H, *m*), 1.77-1.71 ((γ-Abu C$^{\beta}$<u>H</u>, 1H, *m*), 1.36 (Boc C<u>H</u>$_3$, 9H, *s)*, 0.89-0.85 (Val C$^{\gamma}$<u>H</u>, 6H, *m*).

Synthesis of Boc-γ-Abu-Val-OH

3.79 gm (12 mmol) Boc-γ-Abu-Val-OMe is dissolved in 5 mL MeOH at room temperature. At stirring condition, 3 mL 2 (N) NaOH is added gradually to it and the progress of saponification is monitored by thin layer chromatography (TLC). After 6h, methanol is removed under *vacuum*,

the residue is taken in 10 mL of water, wash with diethyl ether (2×15 mL), and pH of the aqueous layer is adjusted to 2-3 using $KHSO_4$ and it is extracted with ethyl acetate. The extracts are pooled, dry over anhydrous sodium sulphate and evaporate in *vacuum*. A white solid material is obtained.

Chemical formula and molecular mass: $C_{14}H_{26}N_2O_5$, 302.1842
Yield: 3.18 gm (10.5 mmol, 87.5 %).

NMR analysis: ^{1}H NMR (400 MHz, $CDCl_3$, δ)**:** 7.30 (Val NH, 1H, *d*, J=8 Hz), 6.60 (γ-Abu NH,1H, *b*), 4.67-4.65 (Val C$^{\alpha}$H, 1H, *m*), 3.17-3.13 (γ-Abu C$^{\gamma}$H, 2H, *m*), 3.08-3.05 (γ-Abu C$^{\alpha}$H, 2H, *m*), 2.32-2.28 (Val C$^{\beta}$H, 1H, *m*), 2.24-2.21 (γ-Abu C$^{\beta}$H, 2H, *m*), 1.36 (Boc CH_3, 9H, *s*), 0.89-0.82 (Val C$^{\gamma}$H, 6H, *m*).

Synthesis of Boc-γ-Abu-Val-Val-OMe

3.00 gm (9.9 mmol) of Boc-γ-Abu-Val-OH is dissolved in 5 mL of dry DMF in R.B and 1.34 gm (9.9 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. 3-4 ml of ethyl acetate containing L-Val-OMe, isolated from 2.62 gm of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 2.03 gm (9.9 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporate in *vacuum.* A white material is obtained.

Purification of Boc-γ-Abu-Val-Val-OMe

Purification of Boc-γ-Abu-Val-Val-OMe is done by column chromatography using chloroform: methanol (98:2) as a mobile phase. Silica gel 100-200 mesh is used as a stationary phase.

Chemical formula and molecular mass: $C_{20}H_{37}N_3O_6$, 415.2682
Yield: 3.05 gm (7.34 mmol, 74.14%).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$, δ**):** 6.82 (Val NH, 1H, *d, J*=10 Hz), 6.77 (Val NH, 1H, *d, J*=10 Hz), 4.91 (γ-Abu NH, 1H, *b*), 4.54-4.51 (Val $C^{\alpha}H$, 1H*, m*), 4.39-4.36 (Val $C^{\alpha}H$, 1H*, m*), 3.73 (OCH_3, 3H, *s*), 3.26-3.14 (γ-Abu $C^{\gamma}H$, 2H, *m*), 2.30-2.27 (Val $C^{\beta}H$, 1H, *m*), 2.18-2.13 (Val $C^{\beta}H$, 1H, *m*), 1.94 (γ-Abu $C^{\alpha}H$, 2H*, b*), 1.85-1.80 (γ-Abu $C^{\beta}H$, 2H *, m*), 1.43 (Boc CH_3, 9H, *s*), 0.98-0.90 (Val $C^{\gamma}H$, 12H*, m*).

H_2N-γ-Abu-Val-Val-OH (Peptide 1)

2.75 gm (6.63 mmol) of Boc-γ-Abu-Val-Val-OMe is saponified and then trifluoroacetic acid (TFA) is added, removal of Boc group is monitored by TLC. After 8 h, TFA is removed under *vacuum*. The residue is taken in water (20 ml) and washes with DCM (2×30 ml). The aqueous portion is evaporated in *vacuum* to yield peptide **1** as white solid.

Chemical formula and molecular mass: $C_{14}H_{27}N_3O_4$, 301.2002
Yield = 1.72 g (5.71 mmol, 86.12%).

NMR analysis: ^{1}H NMR (500 MHz, DMSO-d_6, *δ*)**:** 7.98 (Val NH, 1H, *d, J*=10 Hz), 7.88 (Val NH, 1H, *d, J*=10 Hz), 4.14-4.03 (Val $C^{\alpha}H$, 1H*, m*), 3.92-3.88 (Val $C^{\alpha}H$, 1H*, m*), 2.77-2.70 (γ-Abu $C^{\gamma}H$, 2H, *m*), 2.29-2.18 (Val $C^{\beta}H$, 2H, *m*), 2.02-1.94 (γ-Abu $C^{\alpha}H$, 2H*, m*), 1.78-1.70 (γ-Abu $C^{\beta}H$, 2H *, m*), 0.84-0.80 (Val $C^{\gamma}H$, 12H*, m*).

NMR analysis: ^{13}C NMR (125 MHz, DMSO-d_6, δ): 159.42, 159.13, 158.97, 74.39, 73.05, 37.60, 32.64, 31.30, 26.54, 25.20, 24.53, 23.86, 21.58, 19.10.

ESI-MS data: m/z $[M+H]^+_{calculated}$ = 302.2002, Observed = 302.2279; $[M+Na]^+_{calculated}$ =324.2002, Observed = 324.2120;

Synthesis of peptide 2:

Boc-γ-Abu-Leu-OMe

2.84 gm (14 mmol) of Boc-γ-Abu-OH is dissolved in 5 mL of dry DMF in R.B and 1.89 gm (14 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Leu-OMe, isolated from 4.35 gm (30

mmol) of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 2.88 gm (14 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporate in *vacuum.* A white material is obtained.

Chemical formula and molecular mass: $C_{16}H_{30}N_2O_5$, 330.2155
Yield: 3.65 gm (11.06 mmol, 79%).

NMR analysis: ^{1}H NMR (400 MHz, $CDCl_3$, δ) : 6.67 (Leu N$\underline{H}$, 1H, *d, J*=6.8 Hz), 4.84 (γ-Abu N$\underline{H}$, 1H, *b*), 4.58-4.57 (Leu C$^{\alpha}\underline{H}$, 1H, *m*), 3.72 (OC$\underline{H}_3$, 3H, *s*), 3.27-3.12 (γ-Abu C$^{\gamma}\underline{H}$, 2H, *m*), 2.28-2.24 (γ-Abu C$^{\alpha}\underline{H}$, 2H, *m)*, 1.84-1.77 (Leu C$^{\beta}\underline{H}$, 2H, *m*), 1.70-1.57 (γ-Abu C$^{\beta}\underline{H}$, 2H, *m*), 1.44 (Boc C$\underline{H}_3$, 9H, *s)*, 0.95-0.93 (Leu C$^{\gamma}\underline{H}$ and C$^{\delta}\underline{H}$, 7H, *m*).

Synthesis of Boc-γ-Abu-Leu-OH

3.50 gm Boc-γ-Abu-Leu-OMe (10.60 mmol) is dissolved in 5 mL MeOH at room temperature. At stirring condition, 3 mL 2 (N) NaOH is added gradually to it and the progress of saponification is monitored by thin layer chromatography (TLC). After 6h, methanol is removed under *vacuum*, the residue was taken in 10 mL of water, washed with diethyl ether (2 × 15 mL), and pH of the aqueous layer is adjusted to 2-3 using $KHSO_4$ and it is extracted with ethyl acetate. The extracts are pooled, dry over anhydrous sodium sulphate and evaporate in *vacuum*. A white solid material is obtained.
Chemical formula and molecular mass: $C_{15}H_{28}N_2O_5$, 316.1998
Yield: 2.83 gm (8.95 mmol, 84.43 %)

NMR analysis: ^{1}H NMR (400 MHz, $CDCl_3$, δ) **:** 7.23 (Leu N$\underline{H}$, 1H, *d, J*=8 Hz), 6.47 (γ-Abu N$\underline{H}$,1H, *b*), 4.54 (Leu C$^{\alpha}\underline{H}$, 1H, *m*), 3.26-3.18 (γ-Abu C$^{\gamma}\underline{H}$, 2H, *m*), 2.30-2.28 (γ-Abu C$^{\alpha}\underline{H}$, 2H, *m)*, 1.81-1.76 (Leu C$^{\beta}\underline{H}$, 2H, *m*), 1.71-1.66 (γ-Abu C$^{\beta}\underline{H}$, 2H, *m*), 1.43 (Boc C$\underline{H}_3$, 9H, *s)*, 0.95-0.94 (Leu C$^{\gamma}$H and C$^{\delta}\underline{H}$, 7H, *m*).

Synthesis of Boc-γ-Abu-Leu-Leu-OMe

2.70 gm (8.5 mmol) of Boc-γ-Abu-Leu-OH is dissolved in 5 mL of dry DMF in R.B and 1.15 gm (8.5 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Leu-OMe, isolated from 2.90 gm of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 1.75 gm (8.5 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporated in *vacuum.* A white material is obtained.

Purification of Boc-γ-Abu-Leu-Leu-OMe

Purification of Boc-γ-Abu-Leu-Leu-OMe has done by column chromatography using chloroform**:** methanol (98:2) as the mobile phase. Silica gel 100-200 mesh is used as a stationary phase.

Chemical formula and molecular mass: $C_{22}H_{41}N_3O_6$, 443.2995
Yield: 2.86 gm (6.45 mmol, 75.88 %).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$, *δ*)**:** 6.8 (Leu NH, 1H, *d*, *J*=10 Hz), 6.65 (Leu NH, 1H, *d, J*=5 Hz), 4.83 (γ-Abu NH, 1H, *b*), 4.60-4.56 (Leu $C^{\alpha}H$, 1H*, m*), 4.49-4.44 (Leu $C^{\alpha}H$, 1H*, m*), 3.72 (OCH_3, 3H, *s*), 3.25-3.11 (γ-Abu $C^{\gamma}H$, 2H, *m*), 2.26-2.23 (γ-Abu $C^{\alpha}H$, 2H, *m)*, 1.82-1.78 (γ-Abu $C^{\beta}H$, 2H, *m*),1.69-1.63 (Leu $C^{\beta}H$, 2H, *m*), 1.57-1.54 (Leu $C^{\beta}H$, 2H, *m*), 1.44 (Boc CH_3, 9H, *s)*, 0.95-0.90 (Leu $C^{\gamma}H$ and $C^{\delta}H$, 14H,*m*).

H_2N-γ-Abu-Leu-Leu-OH (Peptide 2)

2.6 gm (5.9 mmol) of Boc-γ-Abu-Leu-Leu-OMe is saponified and then trifluoroacetic acid (TFA) is added, removal of Boc group is monitored by TLC. After 8 h, TFA is removed under *vacuum*. The residue is taken in water (20 ml) and is washed with DCM (2 × 30 ml). The aqueous portion is evaporated in *vacuum* to yield peptide **2** as white solid.

Chemical formula and molecular mass: $C_{16}H_{31}N_3O_4$, 329.2315
Yield = 1.78 gm (5.41 mmol, 91.69%)

NMR analysis: ^{1}H NMR (500 MHz, DMSO-d_6, δ)**:** 8.08 (Leu NH, 1H, *d*, *J*=10 Hz), 7.77 (Leu NH, 1H, *d, J*=10 Hz), 4.24-4.19 (Leu $C^{\alpha}H$, 1H*, m*), 4.05-4.01 (Leu $C^{\alpha}H$, 1H*, m*), 2.75-2.71 (γ-Abu $C^{\gamma}H$, 2H, *m*), 2.21-2.13 (γ-Abu $C^{\alpha}H$, 2H, *m)*, 1.78-1.71 ((γ-Abu $C^{\beta}H$, 2H, *m*),1.61-1.53 (Leu $C^{\beta}H$, 2H, *m*), 1.45-1.38 (Leu $C^{\beta}H$, 2H, *m*), 0.85-0.79 (Leu $C^{\gamma}H$ and $C^{\delta}H$, 14H,*m*).
NMR analysis: ^{13}C NMR (125 MHz, DMSO-d_6, δ): 158.90, 158.59, 158.33, 51.24, 50.78, 42.39, 34.00, 33.43, 31.49, 29.76, 26.42, 25.35, 15.46, 15.21, 13.77, 12.46.

ESI-MS data: m/z $[M+H]^+_{calculated}$ = 330.2315, Observed = 330.2608; $[M+Na]^+_{calculated}$ =352.2315, Observed = 352.2444;

Synthesis of peptide 3:

Boc-γ-Abu-Ile-OMe

2.64 gm (13 mmol) of Boc-γ-Abu-OH is dissolved in 5 mL of dry DMF in R.B and 1.76 gm (13 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Ile-OMe, isolated from 4.35 gm (30 mmol) of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 2.68 gm (13 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporate in *vacuum.* A white material is obtained.

Chemical formula and molecular mass: $C_{16}H_{30}N_2O_5$, 330.2155
Yield: 3.45 gm (10.50 mmol, 80.77%).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$, δ) **:** 6.63 (Ile N<u>H</u>, 1H, *d, J*=10 Hz), 4.78 (γ-Abu N<u>H</u>, 1H, *b*), 4.58-4.55 (Ile C$^{\alpha}$<u>H</u>, 1H, *m*), 3.73 (OC<u>H</u>$_3$, 3H, *s*), 3.28-3.14 (γ-Abu C$^{\gamma}$<u>H</u>, 2H, *m*), 2.29-2.26 (γ-Abu C$^{\alpha}$<u>H</u>, 2H, *m)*, 1.92-1.89 (Ile C$^{\beta}$<u>H</u>, 1H, *m*), 1.84-1.79 (γ-Abu C$^{\beta}$<u>H</u>, 2H, *m*), 1.44 (Boc C<u>H</u>$_3$, 9H, *s)*, 0.94-0.91 (Ile C$^{\gamma}$<u>H</u> and C$^{\delta}$<u>H</u>, 8H, *m*).

Synthesis of Boc-γ-Abu-Ile-OH

3.30 gm Boc-γ-Abu-Ile-OMe (10 mmol) is dissolved in 5 mL MeOH at room temperature. At stirring condition, 3 mL 2 (N) NaOH is added gradually to it and the progress of saponification is monitored by thin layer chromatography (TLC). After 6h, methanol is removed under *vacuum*, the residue was taken in 10 mL of water, washed with diethyl ether (2 × 15 mL), and pH of the aqueous layer is adjusted to 2-3 using $KHSO_4$ and it is extracted with ethyl acetate. The extracts are pooled, dry over anhydrous sodium sulphate and evaporate in *vacuum*. A white solid material is obtained.

Chemical formula and molecular mass: $C_{15}H_{28}N_2O_5$, 316.1998

Yield: 2.79 gm (8.83 mmol, 88.3 %).

Synthesis of Boc-γ-Abu-Ile-Ile-OMe

2.62 gm (8.3 mmol) of Boc-γ-Abu-Ile-OH is dissolved in 5 mL of dry DMF in R.B and 1.12 gm (8.3 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Ile-OMe, isolated from 2.90 gm of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 1.71 gm (8.3 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporated in *vacuum.* A white material is obtained.

Purification of Boc-γ-Abu-Ile-Ile-OMe

Purification of Boc-γ-Abu-Ile-Ile-OMe has done by column chromatography using chloroform**:** methanol (98:1) as the mobile phase. Silica gel 100-200 mesh is used as a stationary phase.

Chemical formula and molecular mass: $C_{22}H_{41}N_3O_6$, 443.2995

Yield: 2.75 gm (6.20 mmol, 74.7%).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$,δ) **:** 6.75 (Ile N<u>H</u>, 1H, *d*, *J*=10 Hz), 6.66 (Ile N<u>H</u>, 1H, *d, J*=10 Hz), 4.89 (γ-Abu N<u>H</u>, 1H, *b*), 4.58-4.55 (Ile C$^{\alpha}$<u>H</u>, 1H*, m*), 4.38-4.35 (Ile C$^{\alpha}$<u>H</u>, 1H*, m*), 3.73 (OC<u>H</u>$_3$, 3H, *s*), 3.26-3.13 (γ-Abu C$^{\gamma}$<u>H</u>, 2H, *m*), 2.28-2.25 (γ-Abu C$^{\alpha}$<u>H</u>, 2H, *m)*, 1.91-1.87 (Ile C$^{\beta}$<u>H</u>, 2H, *m*), 1.83-1.80 ((γ-Abu C$^{\beta}$<u>H</u>, 2H, *m*), 1.44 (Boc C<u>H</u>$_3$, 9H, *s)*, 0.94-0.89 (Ile C$^{\gamma}$<u>H</u> and C$^{\delta}$<u>H</u>, 16H, *m*).

H_2N-γ-Abu-Ile-Ile-OH (Peptide 3)

2.55 gm (5.75 mmol) of Boc-γ-Abu-Ile-Ile-OMe is saponified and then trifluoroacetic acid (TFA) is added, removal of Boc group is monitored by TLC. After 8 h, TFA is removed under *vacuum*. The residue is taken in water (20 ml) and is washed with DCM (2 × 30 ml). The aqueous portion is evaporated in *vacuum* to yield peptide **3** as white solid.

Chemical formula and molecular mass: $C_{16}H_{31}N_3O_4$, 329.2315

Yield = 1.68 gm (5.10 mmol, 88.7%)

NMR analysis: ^{1}H NMR (500 MHz, DMSO-d_6,δ) **:** 8.00 (Ile N<u>H</u>, 1H, *d*, *J*=10 Hz), 7.76 (Ile N<u>H</u>, 1H, *d, J*=10 Hz), 4.23-4.19 (Ile C$^{\alpha}$<u>H</u>, 1H*, m*), 4.06-4.03 (Ile C$^{\alpha}$<u>H</u>, 1H*, m*), 2.77 (γ-Abu C$^{\gamma}$<u>H</u>, 2H, *b*), 2.27-2.24 (γ-Abu C$^{\alpha}$<u>H</u>, 2H, *m)*, 1.80-1.74 (Ile C$^{\beta}$<u>H</u>, 2H, *m*), 1.44-1.40 ((γ-Abu C$^{\beta}$<u>H</u>, 2H, *m*), 0.85-0.79 (Ile C$^{\gamma}$<u>H</u> and C$^{\delta}$<u>H</u>, 16H, *m*).

NMR analysis: ^{13}C NMR (125 MHz, DMSO-d_6, δ):173.22, 171.87, 171.68, 56.97, 56.78, 39.00, 37.17, 36.57, 32.37, 25.15, 24.75, 23.82, 15.91, 15.71, 11.71, 11.36.

ESI-MS data: m/z $[M+H]^+_{calculated}$ = 330.2315, Observed = 330.2612; $[M+Na]^+_{calculated}$ =352.2315, Observed = 352.2439;

Synthesis of peptide 4

Boc-γ-Abu-Phe-OMe

2.84 gm (14 mmol) of Boc-γ-Abu-OH is dissolved in 5 mL of dry DMF in R.B and 1.89 gm (14 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled

water bath. Then 3-4 ml of ethyl acetate containing L-Phe-OMe, isolated from 5.37 gm (30 mmol) of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 2.88 gm (14 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporate in *vacuum.* A white material is obtained.

Chemical formula and molecular mass: $C_{19}H_{28}N_2O_5$, 364.1998

Yield: 3.62 gm (9.94 mmol, 71 %).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$,δ) **:** 7.30-7.13 (Phe aromatic H, 5H, *m*), 6.59 (Phe NH, 1H, *d*, *J*=10 Hz), 5.09 (γ-Abu NH, 1H, *b*), 4.87-4.77 (Phe C^{α}H, 1H, *m*), 3.73 (OCH_3, 3H, *s*), 3.21-3.04 (γ-Abu C^{γ}H and Phe C^{β}H, 4H, *m*), 2.22-2.17 (γ-Abu C^{α}H, 2H, *m)*, 1.78-1.72 (γ-Abu C^{β}H, 2H*, m*), 1.43 (Boc CH_3, 9H, *s*).

Synthesis of Boc-γ-Abu-Phe-OH

3.45 gm Boc-γ-Abu-Phe-OMe (9.47 mmol) is dissolved in 5 mL MeOH at room temperature. At stirring condition, 3 mL 2 (N) NaOH is added gradually to it and the progress of saponification is monitored by thin layer chromatography (TLC). After 6h, methanol is removed under *vacuum*, the residue was taken in 10 mL of water, washed with diethyl ether (2 × 15 mL), and pH of the aqueous layer is adjusted to 2-3 using $KHSO_4$ and it is extracted with ethyl acetate. The extracts are pooled, dry over anhydrous sodium sulphate and evaporate in *vacuum*. A white solid material is obtained.

Chemical formula and molecular mass: $C_{18}H_{26}N_2O_5$, 350.1842

Yield: 3.05 gm (8.71 mmol, 91.97 %).

Synthesis of Boc-γ-Abu-Phe-Phe-OMe

2.87 gm (8.2 mmol) of Boc-γ-Abu-Phe-OH is dissolved in 5 mL of dry DMF in R.B and 1.10 gm (8.2 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Phe-OMe, isolated from 3.58 gm of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 1.69 gm (8.2 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporated in *vacuum.* A white material is obtained.

Purification of Boc-γ-Abu-Phe-Phe-OMe

Purification of Boc-γ-Abu-Phe-Phe-OMe has done by column chromatography using chloroform**:** methanol (98:2) as the mobile phase. Silica gel 100-200 mesh is used as a stationary phase.

Chemical formula and molecular mass: $C_{28}H_{37}N_3O_6$, 511.2682

Yield: 2.69 gm (5.26 mmol, 64.14 %).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$,δ) **:** 7.27-7.04 (Phe aromatic H, 10H, *m*), 6.79 (Phe NH, 1H, *d*, *J*=10 Hz), 6.71 (Phe NH, 1H, *d*, *J*=10 Hz), 4.81-4.67 (Phe $C^{\alpha}H$, 2H, *m*), 3.66 (OCH_3, 3H, *s*), 3.11-2.96 (γ-Abu $C^{\gamma}H$ and Phe $C^{\beta}H$, 6H, *m*), 2.14-2.12 (γ-Abu $C^{\alpha}H$, 2H, *m)*, 1.72-1.66 (γ-Abu $C^{\beta}H$, 2H*, m*), 1.44 (Boc CH_3, 9H, *s*).

H_2N-γ-Abu-Phe-Phe-OH (Peptide 4)

2.52 gm (4.93 mmol) of Boc-γ-Abu-Phe-Phe-OMe is saponified and then trifluoroacetic acid (TFA) is added, removal of Boc group is monitored by TLC. After 8 h, TFA is removed under *vacuum*. The residue is taken in water (20 ml) and is washed with DCM (2 × 30 ml). The aqueous portion is evaporated in *vacuum* to yield peptide **4** as white solid.

Chemical formula and molecular mass: $C_{22}H_{27}N_3O_4$, 397.2002

Yield = 1.54 gm (3.87 mmol, 78.49 %)

NMR analysis: ^{1}H NMR (500 MHz, DMSO-d_6, δ) **:** 8.24 (Phe N<u>H</u>, 1H, *d*, *J*=10 Hz), 7.83 (Phe N<u>H</u>, 1H, *d*, *J*=10 Hz), 7.38-7.15 (Phe aromatic <u>H</u>, 10H, *m*), 4.43 (Phe C$^{\alpha}$<u>H</u>, 1H, *b*), 4.23 (Phe C$^{\alpha}$<u>H</u>, 1H, *b*), 3.11-2.60 (γ-Abu C$^{\gamma}$<u>H</u> and Phe C$^{\beta}$<u>H</u>, 6H, *m*), 2.18-2.03 (γ-Abu C$^{\alpha}$<u>H</u>, 2H, *m)*, 1.70-1.63 (γ-Abu C$^{\beta}$<u>H</u>, 2H*, m*)

NMR analysis: ^{13}C NMR (125 MHz, DMSO-d_6, δ): 159.05, 158.82, 158.55, 138.58, 137.29, 131.15, 129.82, 129.49, 128.50, 128.36, 126.51, 121.13, 118.73, 116.42, 113.98, 57.72, 54.52, 38.62, 37.48, 32.29, 30.41, 23.77

ESI-MS data: m/z $[M+H]^{+}_{calculated}$ = 398.2002, Observed = 398.2391; $[M+Na]^{+}_{calculated}$ =420.2002, Observed = 420.2219;

Synthesis of peptide 5

Boc-γ-Abu-Tyr-OMe

2.64 gm (13 mmol) of Boc-γ-Abu-OH is dissolved in 5 mL of dry DMF in R.B and 1.76 gm (13 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Tyr-OMe, isolated from 5.85 gm (30 mmol) of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 2.68 gm (13 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporate in *vacuum.* A white material is obtained.

Chemical formula and molecular mass: $C_{19}H_{28}N_2O_6$, 380.1947

Yield: 3.48 gm (9.15 mmol, 70.38%).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$,δ) **:** 6.97 (Tyr aromatic H, 2H, *d, J*=10 Hz), 6.75 (Tyr aromatic H, 2H*, d, J*=10 Hz), 6.63 (Tyr NH, 1H, *d*, *J*=10 Hz), 4.84 (γ-Abu NH, 1H, *b*), 4.83 (Tyr C^{α}H, 1H, *m*), 3.73 (OCH_3, 3H, *s*), 3.12-3.08 (γ-Abu C^{γ}H, 2H, *m*), 2.96-2.92 (Tyr C^{β}H, 2H, *m*), 2.23-2.13 (γ-Abu C^{α}H, 2H*, m*), 1.75-1.70 (γ-Abu C^{β}H, 2H *, m*), 1.44 (Boc CH_3, 9H, *s*).

Synthesis of Boc-γ-Abu-Tyr-OH

3.35 gm Boc-γ-Abu-Tyr-OMe (8.81 mmol) is dissolved in 5 mL MeOH at room temperature. At stirring condition, 3 mL 2 (N) NaOH is added gradually to it and the progress of saponification is monitored by thin layer chromatography (TLC). After 6h, methanol is removed under *vacuum*, the residue was taken in 10 mL of water, washed with diethyl ether (2 × 15 mL), and pH of the aqueous layer is adjusted to 2-3 using $KHSO_4$ and it is extracted with ethyl acetate. The extracts are pooled, dry over anhydrous sodium sulphate and evaporate in *vacuum*. A white solid material is obtained.

Chemical formula and molecular mass: $C_{18}H_{26}N_2O_6$, 366.1791

Yield: 2.90 gm (7.92 mmol, 89.89 %).

Synthesis of Boc-γ-Abu-Tyr-Tyr-OMe

2.80 gm (7.6 mmol) of Boc-γ-Abu-Tyr-OH is dissolved in 5 mL of dry DMF in R.B and 1.03 gm (7.6 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Tyr-OMe, isolated from 3.90 gm of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 1.57 gm (7.6 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporated in *vacuum.* A white material is obtained.

Purification of Boc-γ-Abu-Tyr-Tyr-OMe

Purification of Boc-γ-Abu-Tyr-Tyr-OMe has done by column chromatography using chloroform**:** methanol (98:2) as the mobile phase. Silica gel 100-200 mesh is used as a stationary phase.

Chemical formula and molecular mass: $C_{28}H_{37}N_3O_8$, 543.2581

Yield: 2.25 gm (4.14 mmol, 54.47%).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$,*δ*) **:** 7.00 (Tyr aromatic H, 2H, *d, J*=5 Hz), 6.83 (Tyr aromatic H, 2H*, d, J*=5 Hz), 6.71 (Tyr aromatic H, 2H, *d, J*=5 Hz), 6.40 (Tyr NH, 1H, *d*, *J*=5 Hz), 6.23 (Tyr NH, 1H, *d*, *J*=5 Hz), 4.82 (γ-Abu NH, 1H, *b*), 4.78-4.73 (Tyr C^{α}H, 1H, *m*), 4.61-4.56 (Tyr C^{α}H, 1H, *m*), 3.73 (OCH_3, 3H, *s*), 3.05-3.01 (γ-Abu C^{γ}H, 2H, *m*), 2.93-2.83 (Tyr C^{β}H, 4H, *m*), 2.10-2.01 (γ-Abu C^{α}H, 2H*, m*), 1.70-1.64 (γ-Abu C^{β}H, 2H *, m*), 1.45 (Boc CH_3, 9H,

H_2N-γ-Abu-Tyr-Tyr-OH (Peptide 5)

2.17 gm (3.99 mmol) of Boc-γ-Abu-Tyr-Tyr-OMe is saponified and then trifluoroacetic acid (TFA) is added, removal of Boc group is monitored by TLC. After 8 h, TFA is removed under *vacuum*. The residue is taken in water (20 ml) and is washed with DCM (2 × 30 ml). The aqueous portion is evaporated in *vacuum* to yield peptide **5** as white solid.

Chemical formula and molecular mass: $C_{22}H_{27}N_3O_6$, 429.19

Yield = 1.25 gm (2.91 mmol, 72.93%)

NMR analysis: ^{1}H NMR (500 MHz, DMSO-d_6, *δ*) **:** 8.14 (Tyr NH, 1H, *d*, *J*=5 Hz), 7.89 (Tyr NH, 1H, *d*, *J*=5 Hz), 7.48-7.29 (Tyr aromatic H, 4H, *m*), 6.98 (Tyr aromatic H, 2H*, s*), 6.63 (Tyr aromatic H, 2H, *s*), 4.63 (Tyr C^{α}H, 1H, *b*), 4.23 (Tyr C^{α}H, 1H, *b*), 2.97-2.85 (γ-Abu C^{γ}H, 2H, *m*), 2.83-2.67 (Tyr C^{β}H, 4H, *m*), 2.11 (γ-Abu C^{α}H, 2H*, b*), 1.68 (γ-Abu C^{β}H, 2H *, b*)

NMR analysis: ^{13}C NMR (125 MHz, DMSO-d_6, δ): 159.46, 159.24, 159.00, 142.16, 141.26, 136.94, 130.56, 130.49, 128.28, 121.10, 118.65, 118.24, 116.20, 115.37, 114.10, 52.88, 51.98, 38.70, 36.52, 32.82, 32.41, 23.57

ESI-MS data: m/z $[M+H]^+_{calculated}$ = 430.19, Observed = 430.9402;

Synthesis of peptide 6

Boc-γ-Abu-Trp-OMe

2.84 gm (14 mmol) of Boc-γ-Abu-OH is dissolved in 5 mL of dry DMF in R.B and 1.89 gm (14 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Trp-OMe, isolated from 6.1 gm (28 mmol) of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 2.88 gm (14 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporate in *vacuum.* A white material is obtained.

Chemical formula and molecular mass: $C_{21}H_{29}N_3O_5$, 403.2107

Yield: 3.72 gm (9.23 mmol, 65.92%).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$,δ) **:** 7.53 (Indole aromatic NH, 1H, *d*, *J*=10 Hz), 7.36 (Trp aromatic H, 1H, *d, J*=10 Hz), 7.26 (Trp aromatic H, 1H, *s*), 7.21-7.18 (Trp aromatic H, 1H, *m,*), 7.13-7.10 (Trp aromatic H, 1H, *m,*) 7.01 (Indole aromatic H, 1H, *s*), 6.33 (Trp NH, 1H, *d*, *J*=10 Hz), 4.93 (γ-Abu NH, 1H, *b*), 4.71 (Trp C^{α}H, 1H, *b*), 3.71 (OCH_3, 3H, *s*), 3.37-3.08 (γ-Abu C^{γ}H, 2H, *m*), 3.14-3.04 (Trp C^{β}H, 2H, *m*), 2.19-2.15 (γ-Abu C^{α}H, 2H*, m*), 1.77-1.72 (γ-Abu C^{β}H, 2H *, m*), 1.43 (Boc CH_3, 9H, *s*).

Synthesis of Boc-γ-Abu-Trp-OH

3.60 gm Boc-γ-Abu-Trp-OMe (8.93 mmol) is dissolved in 5 mL MeOH at room temperature. At stirring condition, 3 mL 2 (N) NaOH is added gradually to it and the progress of saponification is monitored by thin layer chromatography (TLC). After 6h, methanol is removed under *vacuum*, the residue was taken in 10 mL of water, washed with diethyl ether (2 × 15 mL), and pH of the aqueous layer is adjusted to 2-3 using $KHSO_4$ and it is extracted with ethyl acetate. The extracts

are pooled, dry over anhydrous sodium sulphate and evaporate in *vacuum*. A white solid material is obtained.

Chemical formula and molecular mass: $C_{20}H_{27}N_3O_5$, 389.1951

Yield: 3.05 gm (7.84 mmol, 87.79 %).

Synthesis of Boc-γ-Abu-Trp-Trp-OMe

2.92 gm (7.5 mmol) of Boc-γ-Abu-Trp-OH is dissolved in 5 mL of dry DMF in R.B and 1.03 gm (7.6 mmol) of HOBt is added to it. When HOBt dissolved completely, R.B is placed in ice cooled water bath. Then 3-4 ml of ethyl acetate containing L-Trp-OMe, isolated from 3.90 gm of the corresponding methyl ester hydrochloride by neutralization with Na_2CO_3 and subsequent extraction with ethyl acetate, is added to it and followed immediately by 1.57 gm (7.6 mmol) of dicyclohexylcarbodimide (DCC). The reaction mixture is allowed to come to room temperature and stirring is continued for 48 h. The residue is taken up in ethyl acetate (25 mL) and dicyclohexylurea (DCU) is filtered off. The organic layer is washed with 1(N) HCl (3×10 mL), brine (1×10 mL), 1(M) sodium carbonate (3×10 mL) and again brine (2×10 mL) by the use of separating funnel. Then the ethyl acetate extracts are pooled and dry over anhydrous sodium sulphate and evaporated in *vacuum.* A white material is obtained.

Purification of Boc-γ-Abu-Trp-Trp-OMe

Purification of Boc-γ-Abu-Trp-Trp-OMe has done by column chromatography using chloroform**:** methanol (98:5) as the mobile phase. Silica gel 100-200 mesh is used as a stationary phase.

Chemical formula and molecular mass: $C_{32}H_{39}N_5O_6$, 589.29

Yield: 2.05 gm (3.48 mmol, 46.4 %).

NMR analysis: ^{1}H NMR (500 MHz, $CDCl_3$,δ) **:** 8.53 (Indole aromatic N<u>H</u>, 1H, *b*), 8.10 (Indole aromatic N<u>H</u>, 1H, *b*), 7.64 (Trp aromatic <u>H</u>, 1H, *d, J*=10 Hz), 7.35-7.31 (Trp aromatic <u>H</u>, 2H, *m*), 7.21-7.09 (Trp aromatic <u>H</u>, 4H, *m*), 6.98 (Indole aromatic <u>H</u>, 1H, *s*), 6.84-6.84 (Trp aromatic <u>H</u>, 1H, *m*), 6.75 (Indole aromatic <u>H</u>, 1H, *s*), 6.40 (Trp N<u>H</u>, 1H, *d*, *J*=10 Hz), 6.32(Trp N<u>H</u>, 1H, *b*),4.83-4.81 (Trp C$^{\alpha}$<u>H</u>, 1H, *m*), 4.77-4.73 (Trp C$^{\alpha}$<u>H</u>, 1H, *m*), 3.65 (OC<u>H</u>$_3$, 3H, *s*), 3.33-3.28 (γ-

Abu C$^{\gamma}$H, 2H, *m*), 3.17-3.09 (Trp C$^{\beta}$H, 2H, *m*), 2.94-2.92 (Trp C$^{\beta}$H, 2H, *m*), 2.02-1.80 (γ-Abu C$^{\alpha}$H, 2H, *m*), 1.56-1.53 (γ-Abu C$^{\beta}$H, 2H , *m*), 1.44 (Boc CH$_3$, 9H, *s*).

H_2N-γ-Abu-Trp-Trp-OH (Peptide 6)

1.90 gm (3.22 mmol) of Boc-γ-Abu-Trp-Trp-OMe is saponified and then trifluoroacetic acid (TFA) is added, removal of Boc group is monitored by TLC. After 8 h, TFA is removed under *vacuum*. The residue is taken in water (20 ml) and is washed with DCM (2 × 30 ml). The aqueous portion is evaporated in *vacuum* to yield peptide **6** as white solid.

Chemical formula and molecular mass: $C_{26}H_{29}N_5O_4$, 475.222

Yield = 1.12 gm (2.35 mmol, 72.98 %)

NMR analysis: ^{1}H NMR (500 MHz, DMSO-d_6, δ) **:** 8.60 (Indole aromatic NH, 1H, *d, J*=10 Hz), 8.06 (Indole aromatic NH, 1H, *b*), 7.14-6.93 (Trp aromatic H, 8H, *m*), 6.60-657 (Indole aromatic H, 1H, *m*), 6.50 (Trp NH, 1H, *d*, *J*=10 Hz), 6.45 (Trp NH, 1H, *d*, *J*=10 Hz), 4.60 (γ-Abu NH, 1H, *b*), 4.39 (Trp C$^{\alpha}$H, 2H, *b*), 3.53-3.27 (γ-Abu C$^{\gamma}$H, 2H, *m*), 3.24-3.19 (Trp C$^{\beta}$H, 2H, *m*), 2.78-2.75 (Trp C$^{\beta}$H, 2H, *m*), 2.22-2.17 (γ-Abu C$^{\alpha}$H, 2H, *m*), 1.75-1.72 (γ-Abu C$^{\beta}$H, 2H , *m*),

NMR analysis: ^{13}C NMR (125 MHz, DMSO-d_6, δ): 172.50, 171.83, 170.85, 136.40, 127.53, 127.36, 123.55, 123.26, 122.23, 122.06, 119.77, 119.32, 118.80, 118.29, 111.44, 111.22, 110.35, 109.22, 108.01, 53.65, 52.64, 37.78, 33.06, 27.53, 27.27, 25.75.

ESI-MS data: m/z $[M+H]^+_{calculated}$ = 476.222, Observed = 476.2565; $[M+Na]^+_{calculated}$ =498.222, Observed = 498.2385.

NMR experiments

NMR spectra of final compounds are recorded on 500 MHz spectrometer in DMSO-d_6. Other intermediates NMR studies are carried out on a Brüker DPX 400 MHz spectrometer in $CDCl_3$.

Atomic Force Microscopy

Atomic Force Microscopy (AFM) was performed to investigate the morphology of the peptide aggregates. Samples were prepared using a small amount of the solution of the corresponding

compounds on a mica foil and then allow evaporating in vacuum. AFM images were recorded on a AUTOPROBE CP BASE UNIT. di CP-II instrument.

Congo Red (CR) Binding assay

For the CR binding studies, CR is dissolved in phosphate buffer (pH 7.41). It is filtered through a 0.22 μm syringe filter and immediately used for the CR binding study. A fixed amount of CR solution is added with the peptide solution such that final volume reached 1000 μL and the final peptide concentration reached 3000 μM. CR absorbance spectra are recorded in the wavelength range of 200-700 nm using a Hitachi spectrophotometer (UH 5300). For CR birefringence assay, 5 mg of peptide is dissolved in 200 μL of ethanol and a saturated solution of Nacl is added to this solution. A freshly prepared CR solution is mixed with peptide solution and incubated at room temperature in the dark. Finally, the solution is spread evenly onto a glass slide, air-dried at room temperature, and imaged using a microscope (Olympus U-CMAD3) equipped with polarizer and a CCD camera (SC180).

Thioflavin T binding assay

A stock solution of ThT in deionised water is prepared. 20 μL of this stock solution is mixed with the peptide solution such that final volume reached 1000 μL and the final peptide concentration reached 3000 μM. Then the fluorescence is measured. The fluorescence experiments were conducted using a Hitachi (F7000) spectrofluorometer with excitation at 450 nm and an emission range of 470-650 nm.

FT-IR spectra of Fibril

Aged peptide solutions are suspended on a CaF_2 plate and dried in vacuum. The peptide deposits are re-suspended with D_2O and subsequently dried to form thin films and spectra are collected at 25 ℃.

Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) experiments were carried out on a TA nano ITC instrument. Before starting the experiment, all the solutions were degassed for 10 minutes in the degassing chamber.

Dynamic light scattering

Dynamic light scattering experiments were carried out in a MALVERN ZS Instrument UK to determine the self-assembled particle presence in solutions. For DLS studies peptides solution were prepared in milli-Q water to a concentration of 0.3 mM and taken in cuvette and scanned.

MTT Cell Viability Assay

The cells were seeded in 12-well plates and grown before treatment to obtain >70% confluency and exposed to either various concentrations of peptides. After 48 h of incubation at 37 °C in a humidified atmosphere of 5% CO_2, the medium was replaced with the fresh medium containing 10% FBS. Then, 0.5 mg/mL methyl thiazolyl tetrazolium (MTT) reagent was added. The formazan crystals were dissolved in DMSO, and the absorbance values were recorded using the ELISA plate reader. The percentage of cell viability was calculated from the absorbance values. The results are presented as the mean ± SD of three independent experiments.

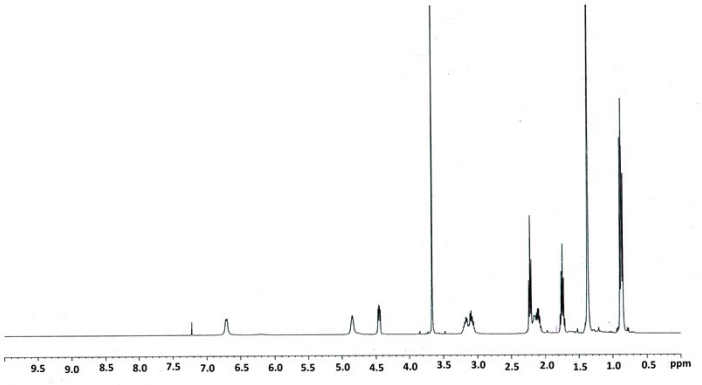


Figure S2: ^{1}H NMR spectrum of peptide Boc-γ-Abu-Val-OMe (400 MHz, $CDCl_3$, 300 K).

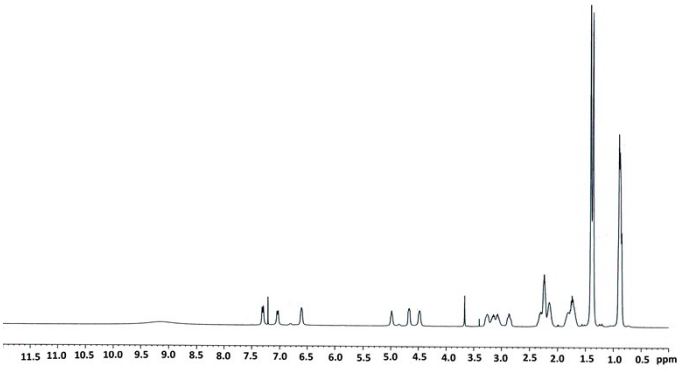


Figure S3: ^{1}H NMR spectrum of peptide Boc -γ-Abu-Val-OH **(**400 MHz, $CDCl_3$, 300 K).

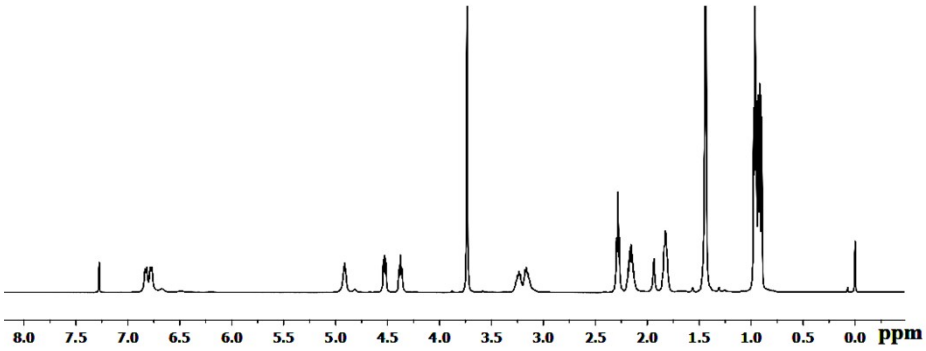


Figure S4: ^{1}H NMR spectrum of peptide Boc -γ-Abu-Val-Val-OMe **(**500 MHz, $CDCl_3$, 300 K).

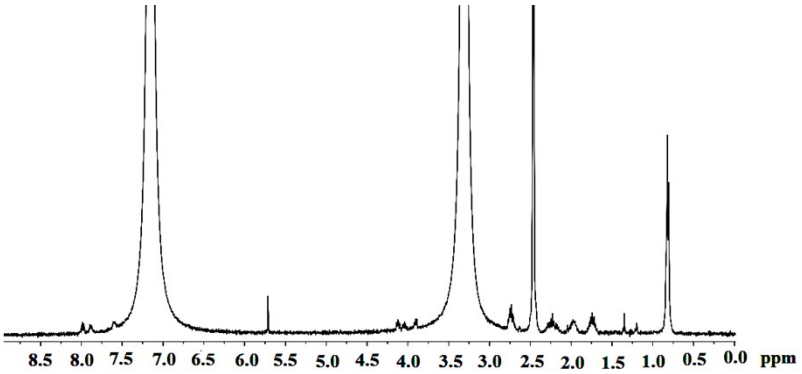


Figure S5: ^{1}H NMR spectrum of peptide **1** (500 MHz, DMSO-d_6, 300 K)

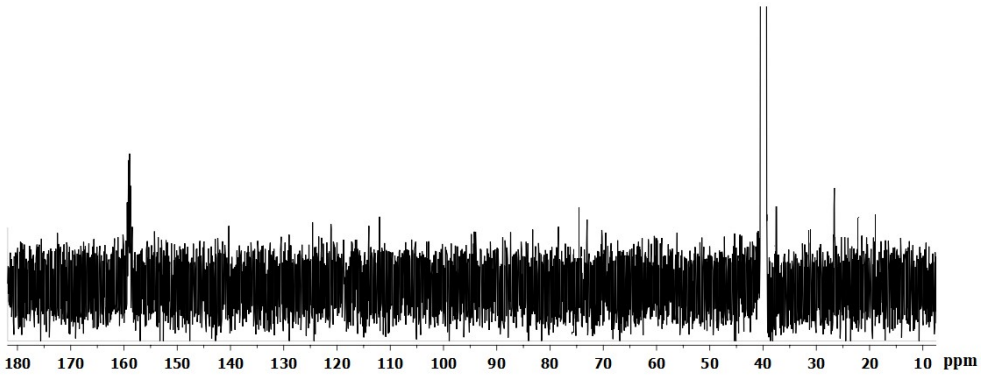


Figure S6: ^{13}C NMR spectrum of peptide **1**(125 MHz, DMSO-d_6, 300 K)

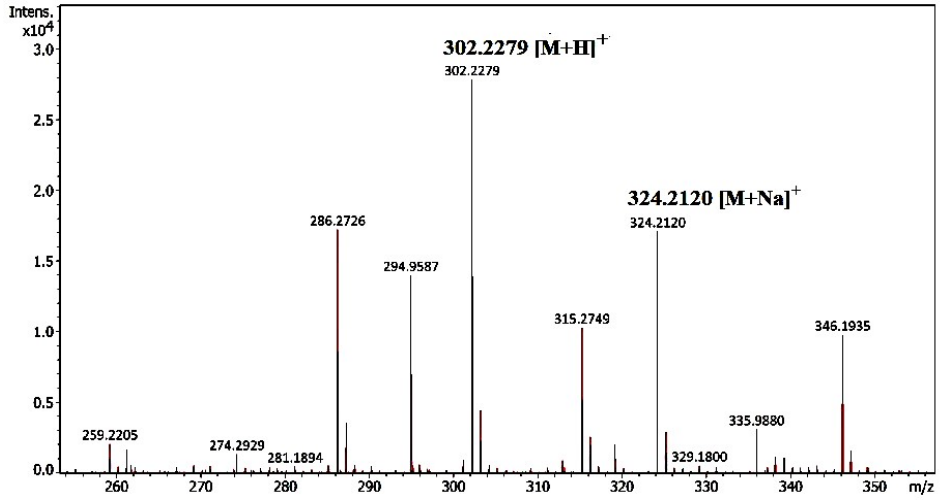


Figure S7: HR-MS mass spectrum of peptide **1.**

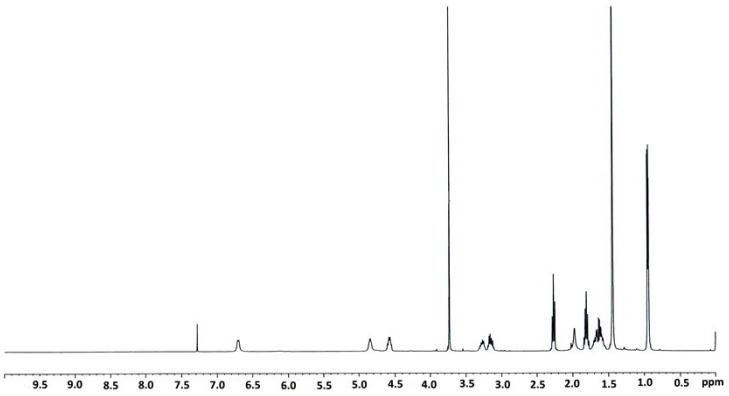


Figure S8: ^{1}H NMR spectrum of peptide Boc-γ-Abu-Leu-OMe (400 MHz, $CDCl_3$, 300 K).

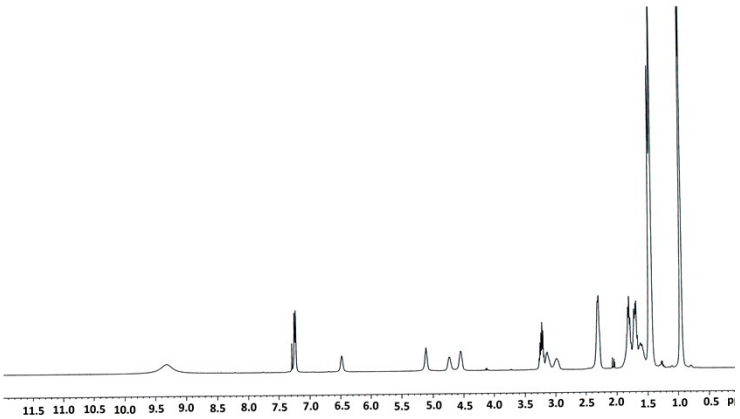


Figure S9: ^{1}H NMR spectrum of peptide Boc-γ-Abu-Leu-OH **(**400 MHz, CDCl3, 300 K).

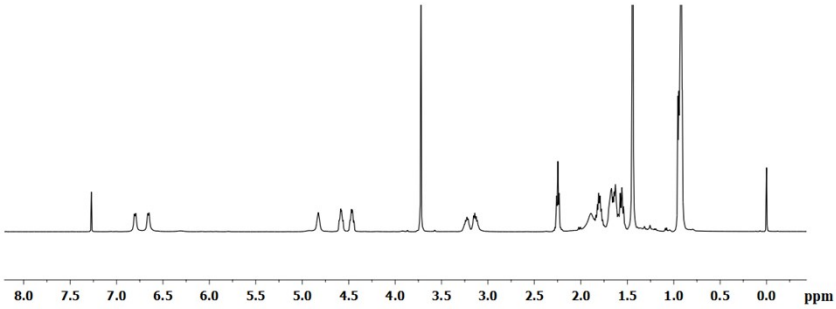


Figure S10: ^{1}H NMR spectrum of peptide Boc-γ-Abu-Leu-Leu-OMe **(**500 MHz, $CDCl_3$, 300 K).

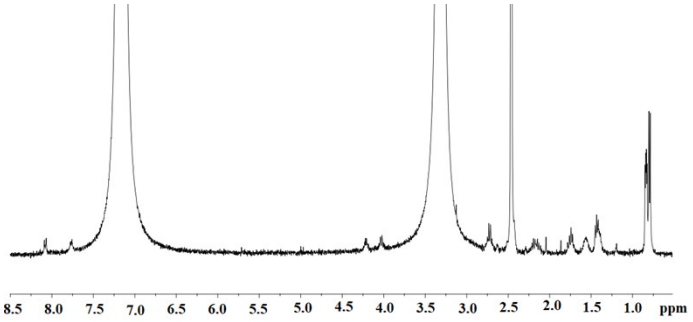


Figure S11: ^{1}H NMR spectrum of peptide **2** (500 MHz, DMSO-d_6, 300 K)

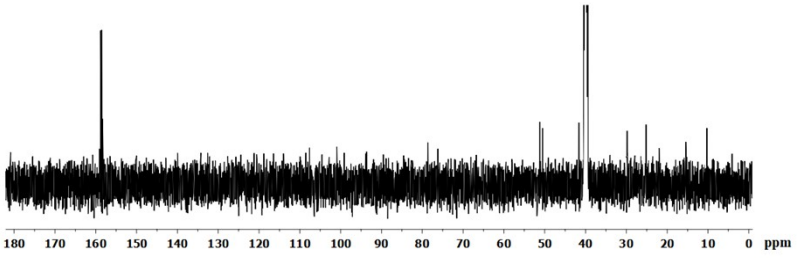


Figure S12: ^{13}C NMR spectrum of peptide **2** (125 MHz, DMSO-d_6, 300 K)

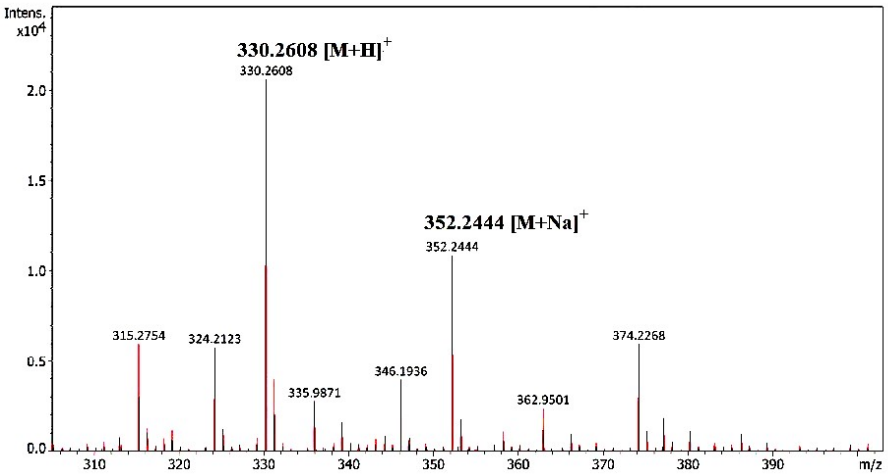


Figure S13: HR-MS mass spectrum of peptide **2.**

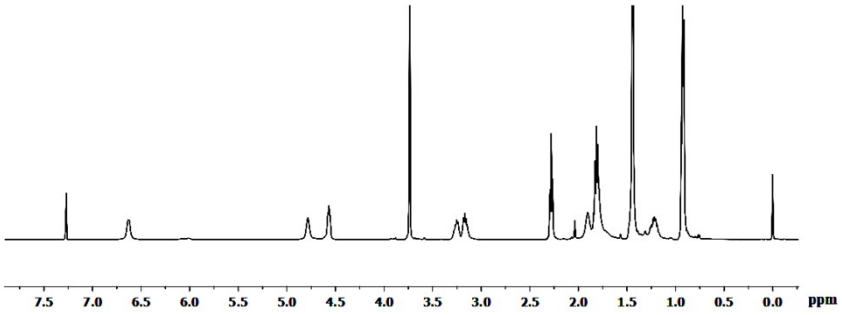


Figure S14: [1]H NMR spectrum of peptide Boc-γ-Abu-Ile-OMe **(**500 MHz, CDCl3, 300 K).

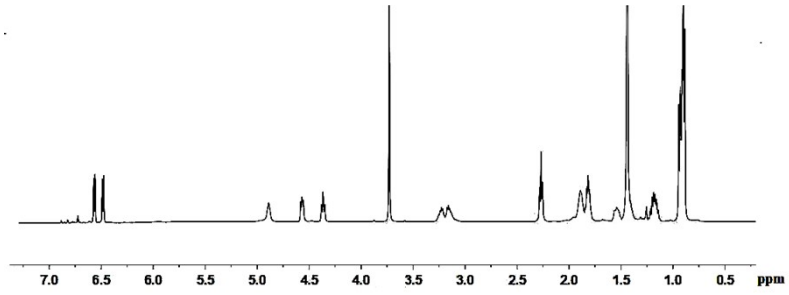


Figure S15: [1]H NMR spectrum of peptide Boc-γ-Abu-Ile-Ile-OMe **(**500 MHz, CDCl3, 300 K)

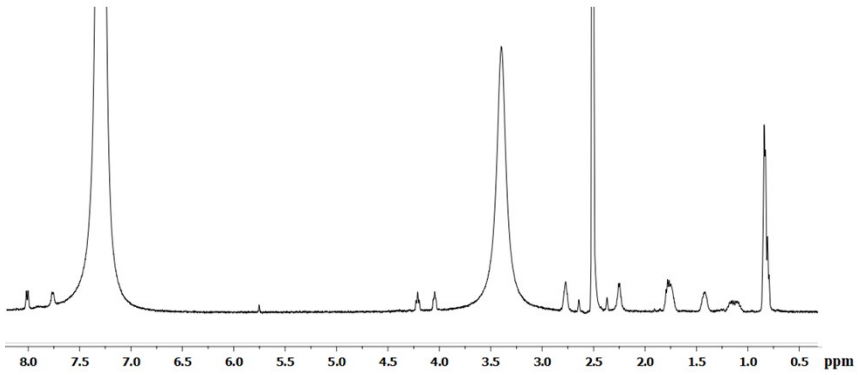


Figure S16: ^{1}H NMR spectrum of peptide **3** (500 MHz, DMSO-d_6, 300 K)

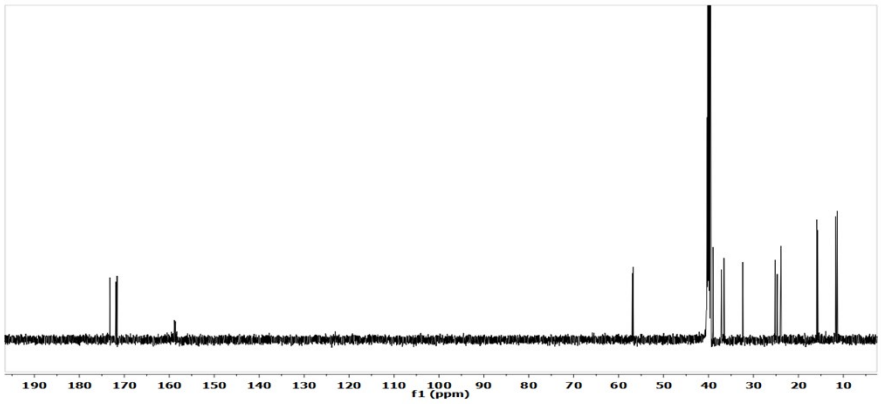


Figure S17: ^{13}C NMR spectrum of peptide **3** (125 MHz, DMSO-d_6, 300 K)

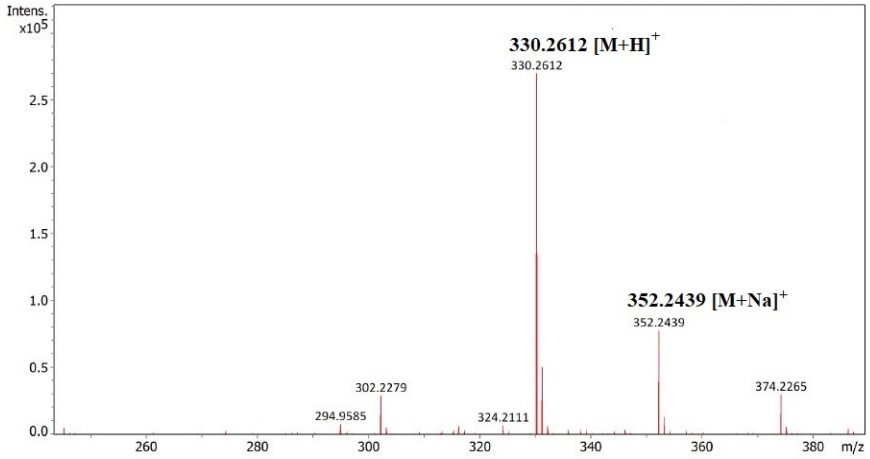


Figure S18: HR-MS mass spectrum of peptide **3.**

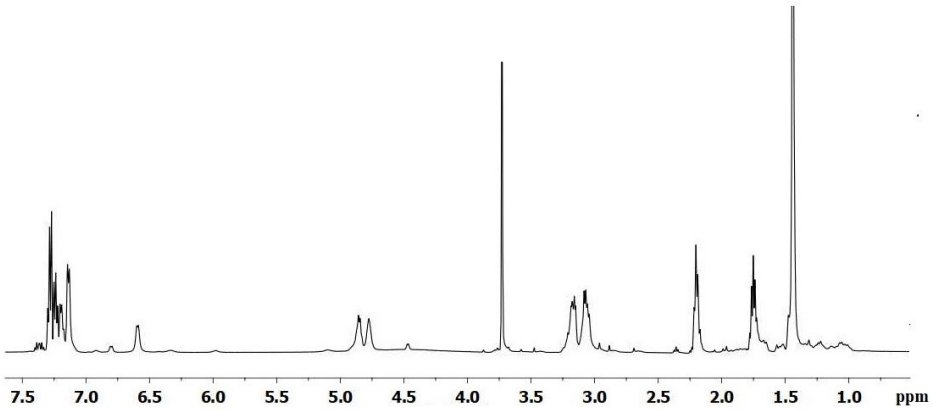


Figure S19: [1]H NMR spectrum of peptide Boc-γ-Abu-Phe-OMe **(**500 MHz, CDCl3, 300 K).

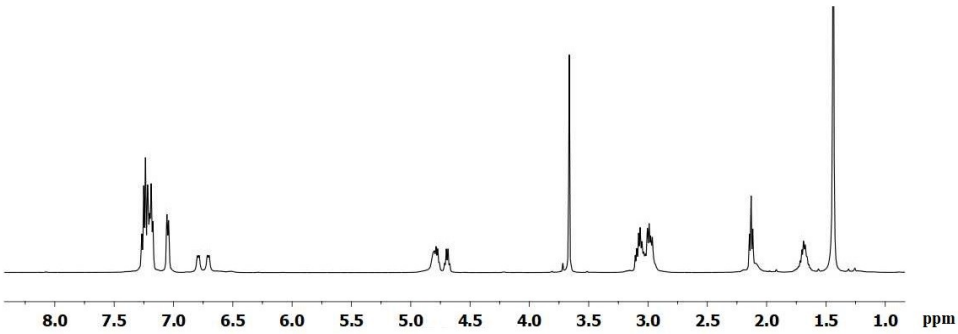


Figure S20: [1]H NMR spectrum of peptide Boc-γ-Abu-Phe-Phe-OMe **(**500 MHz, CDCl3, 300 K).

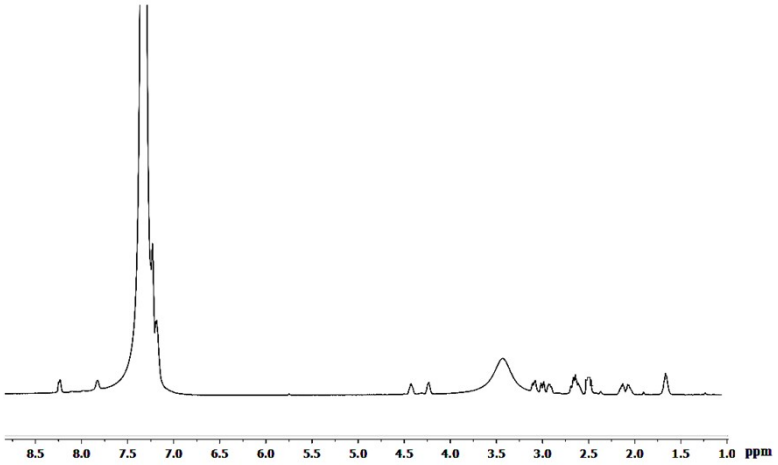


Figure S21: ^{1}H NMR spectrum of peptide 4 (500 MHz, DMSO-d_6, 300 K)

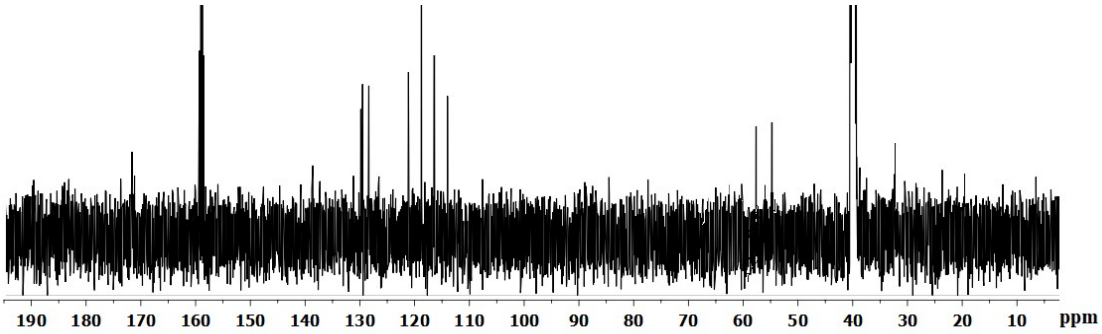


Figure S22: ^{13}C NMR spectrum of peptide **4** (125 MHz, DMSO-d_6, 300 K)

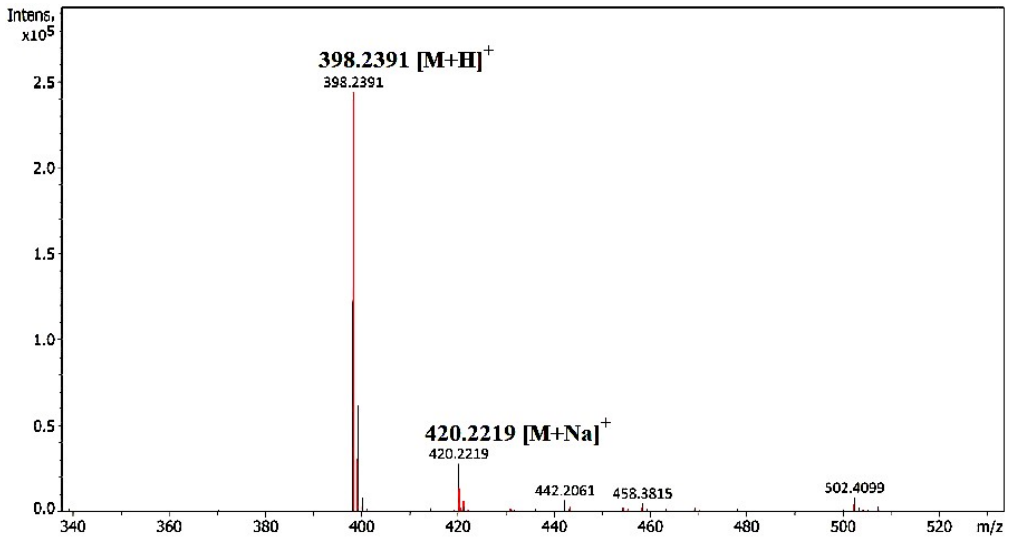


Figure S23: HR-MS mass spectrum of peptide **4.**

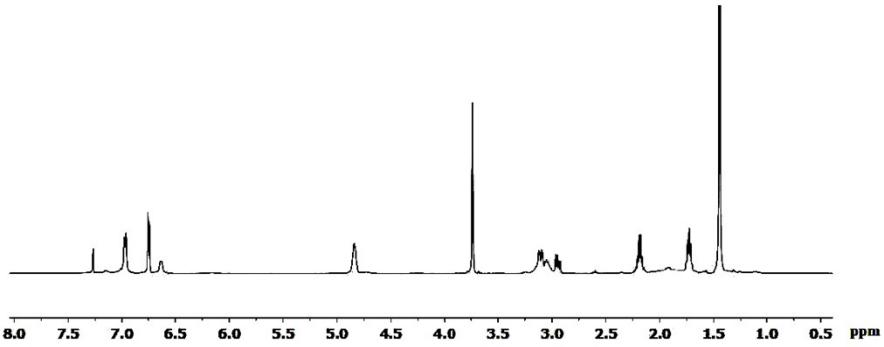


Figure S24: [1]H NMR spectrum of peptide Boc-γ-Abu-Tyr-OMe **(**500 MHz, CDCl3, 300 K).

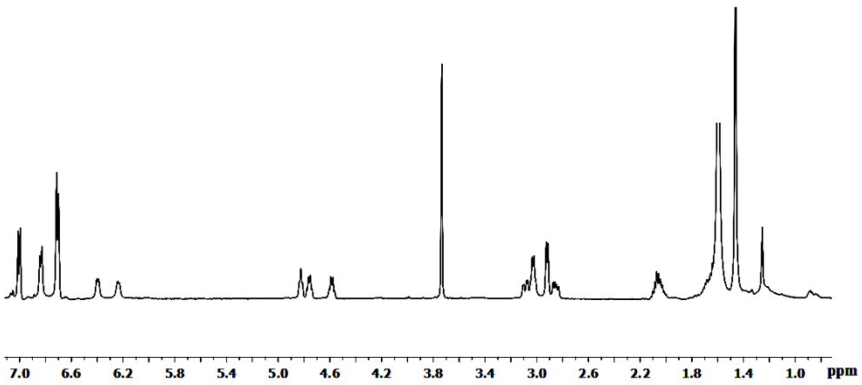


Figure S25: [1]H NMR spectrum of peptide Boc-γ-Abu-Tyr-Tyr-OMe **(**500 MHz, CDCl3, 300 K).

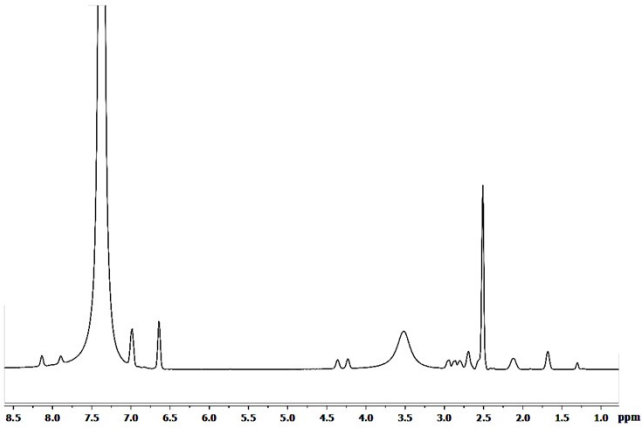


Figure S26: [1]H NMR spectrum of peptide 5 (500 MHz, DMSO-d_6, 300 K)

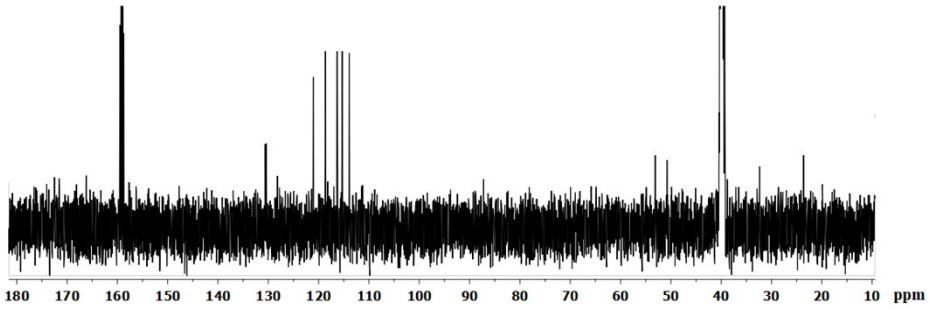


Figure S27: ^{13}C NMR spectrum of peptide **5** (125 MHz, DMSO-d_6, 300 K)

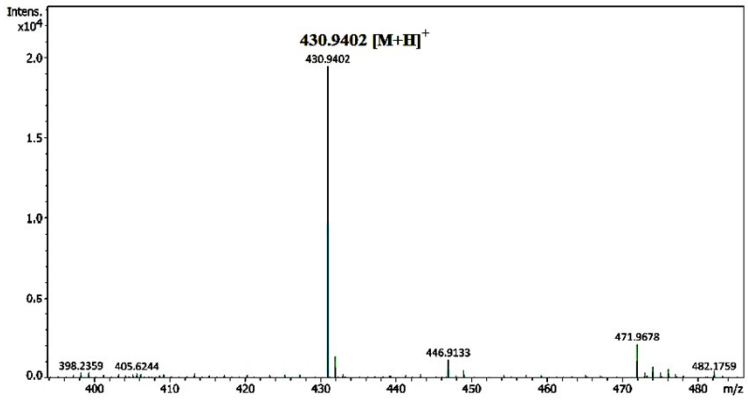


Figure S28: HR-MS mass spectrum of peptide **5.**

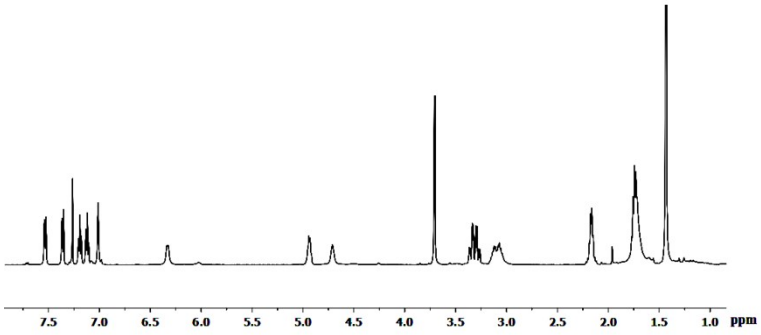


Figure S29: ^{1}H NMR spectrum of peptide Boc-γ-Abu-Trp-OMe **(**500 MHz, CDCl3, 300 K).

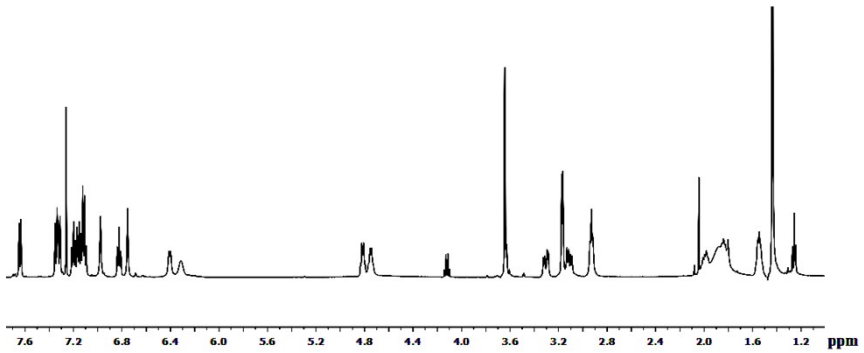


Figure S30: ^{1}H NMR spectrum of peptide Boc-γ-Abu-Trp-Trp-OMe **(**500 MHz, CDCl3, 300 K).

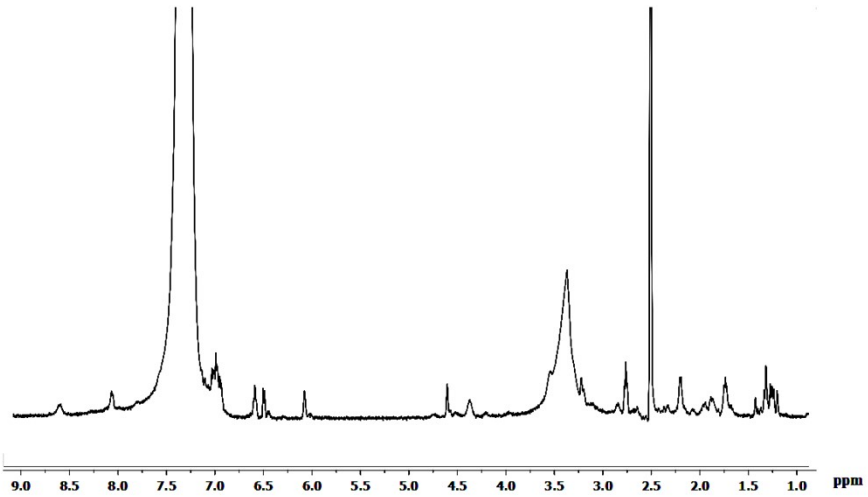


Figure S31: ^{1}H NMR spectrum of peptide 6 (500 MHz, DMSO-d_6, 300 K)

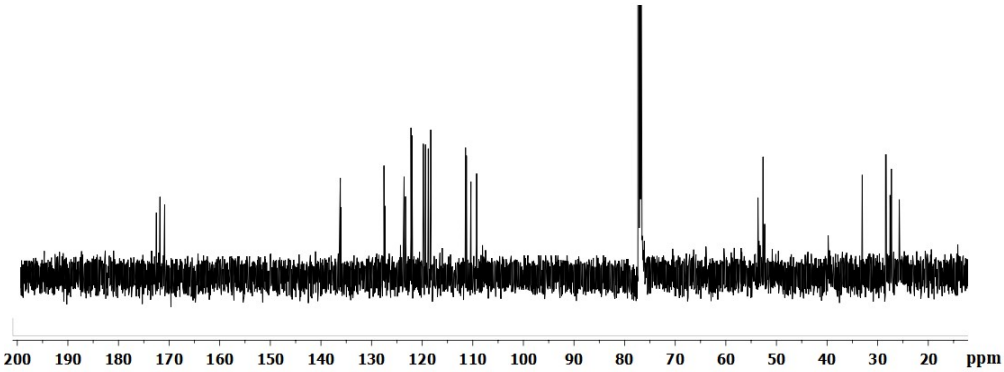


Figure S32: ^{13}C NMR spectrum of peptide **6** (125 MHz, DMSO-d_6, 300 K)

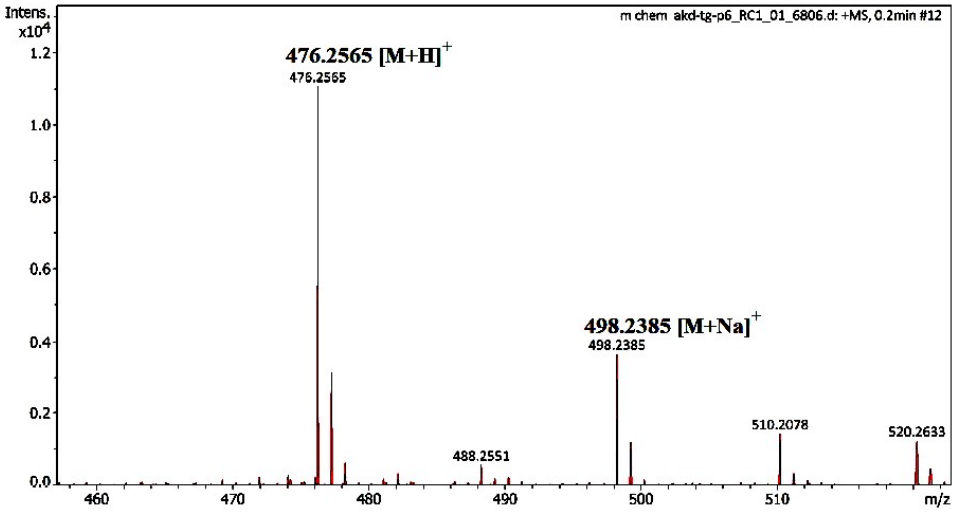


Figure S33: HR-MS mass spectrum of peptide **6.**

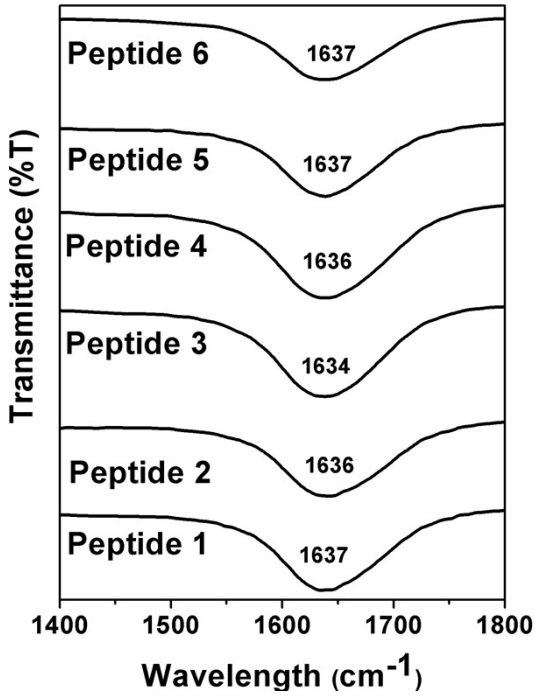


Figure S34: FT-IR spectra of the aggregated peptide in solution.

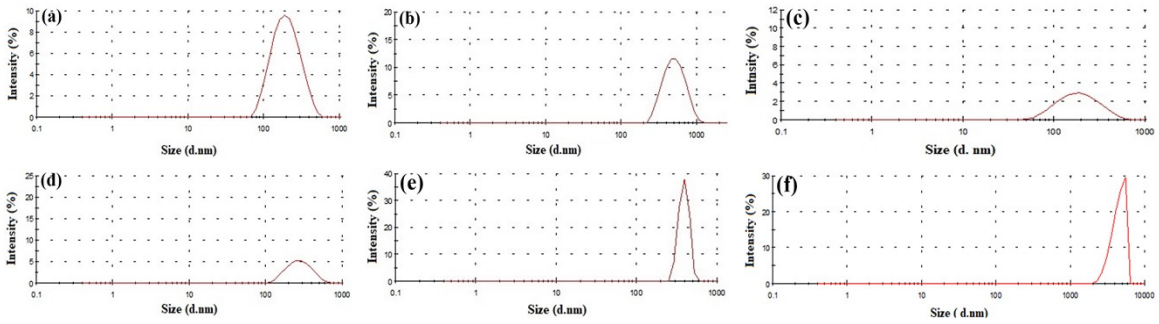


Figure S35: DLS study. (a), (b), (c), (d), (e) and (f) are for peptide 1, 2, 3, 4, 5, and 6 respectively.

Table S1: The hydrodynamic diameter of the peptides at concentration 300 µM.

Peptide	Concentration	Diameter
Gaba-Val-Val (Peptide 1)	300 µM	280.9 nm
Gaba-Leu-Leu (Peptide 2)	300 µM	732.4 nm
Gaba-Ile-Ile (Peptide 3)	300 µM	278.2 nm
Gaba-Phe-Phe (Peptide 4)	300 µM	386.3 nm
Gaba-Tyr-Tyr (Peptide 5)	300 µM	690.1 nm
Gaba-Trp-Trp (Peptide 6)	300 µM	3433.8 nm

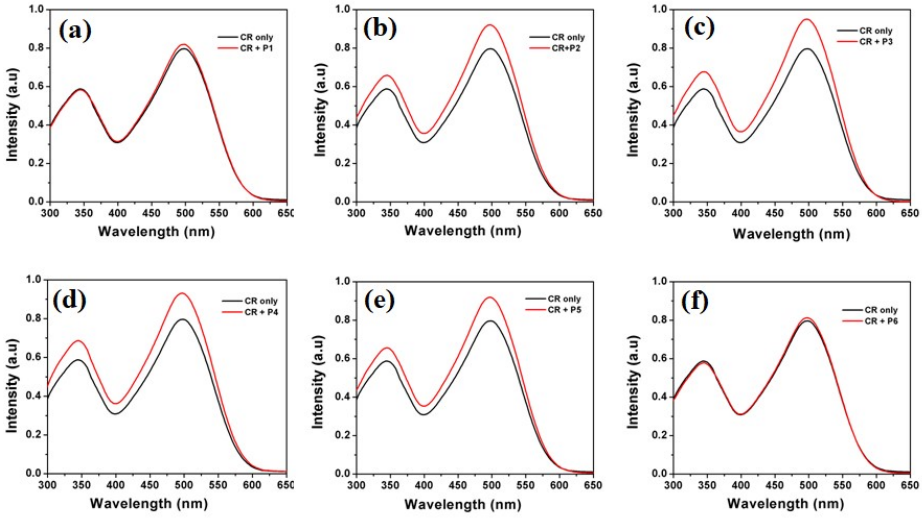


Figure S36: CR absorption study. (a), (b), (c), (d), (e) and (f) are the representative of peptide 1, 2, 3, 4, 5 and 6.

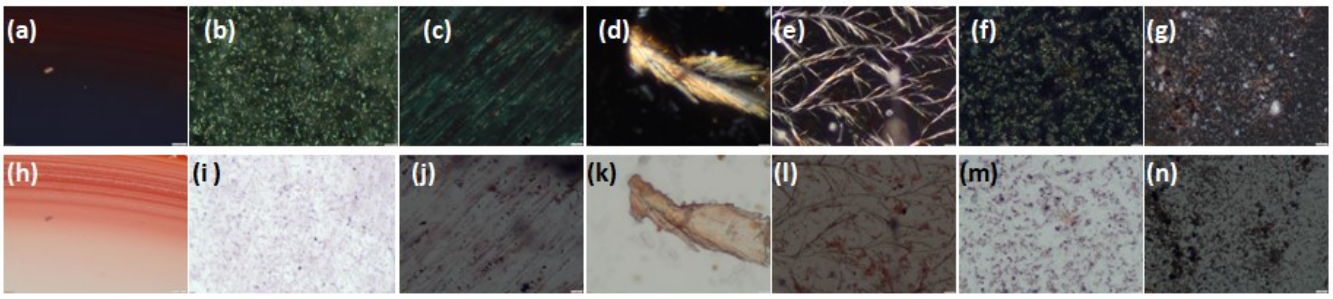


Figure S37: CR birefringence assay. Upper panel represents CR-peptide aggregate under polarized light whereas the lower panel is the corresponding bright field images. (a) and (h) for control , (b) and (i) for peptide 1, (c) and (j) for peptide 2, (d) and (k) for peptide 3, (e) and (l) for peptide 4, (f) and (m) for peptide 5, (g) and (n) for peptide 6 respectively.

Table S2: The thermodynamic parameters of CR-peptide interaction.

Peptide	Binding constant (Ka x 10^4) M^{-1}	ΔH (KJ/mol)	TΔS (KJ/mol)	ΔG (KJ/mol)
1	2.46	13.01	39.76	-26.75
2	9.28	8.26	36.62	-28.06
3	4.05	9.22	35.52	-26.30
4	5.02	-79.4	-52.00	-26.83
5	4.64	10.9	30.54	-26.6
6	8.9	-100	-66.04	-33.96

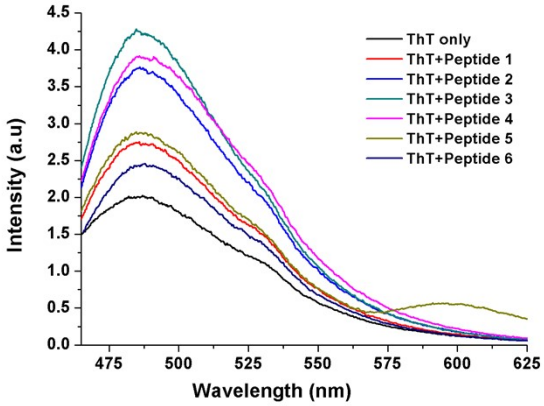


Figure S38: ThT binding study. The characteristic emission of ThT-peptide complex upon excitation at 450 nm after 4 days of incubation.

Table S3: The cytotoxic effect of the peptides on both HeLa and HEK 293 cell lines. The percentage of viable cells after 48 h exposure to the 7.5 mM concentration of peptides.

Peptide (7.5 mM)	HeLa cell	HEK 293
1	79%	78%
2	64%	68%
3	77%	90%
4	50%**	67%
5	54% **	61%

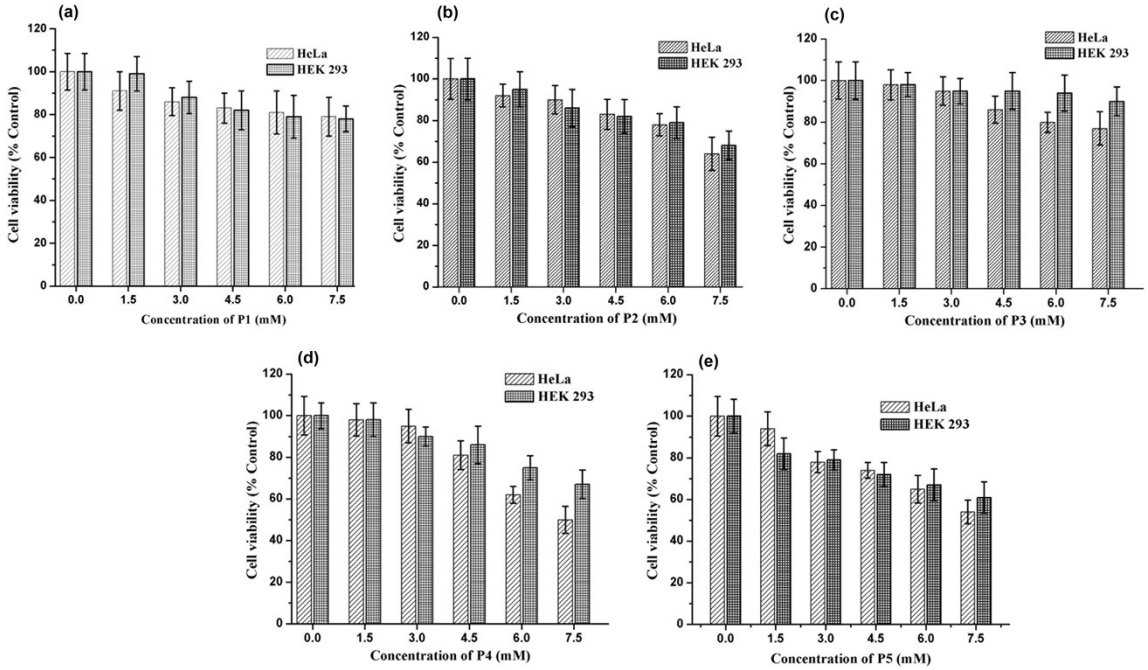


Figure S39: Cytotoxic effects of aggregated peptide variants as evaluated by MTT cell viability assay. Panels (a), (b), (c), (d) and (e) represent the cytotoxicity displayed by peptides 1, 2, 3, 4 and 5, respectively, on cancer (HeLa) and non-cancer (HEK 293) cell lines after 48 h exposure. The results show the mean ± SD of three independent experiments.

References:

[1]. Bodanszky M, Bodanszky A, The practice of peptide synthesis. *Springer*: *New York* (1984), 1–282.