Supporting Information

Synthesis and structural investigation of 2-aminomethyl-3-(4-methoxy-phenyl)-propionic acid containing peptide analogue of amyloidogenic AS(6-7) sequence: Inhibition of fibril formation

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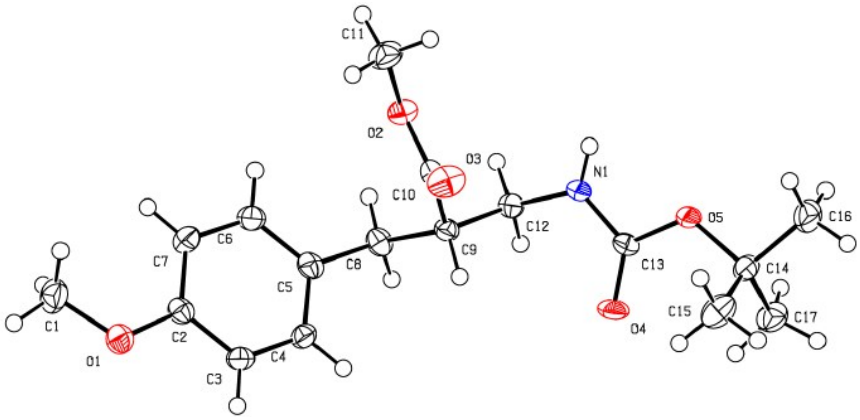
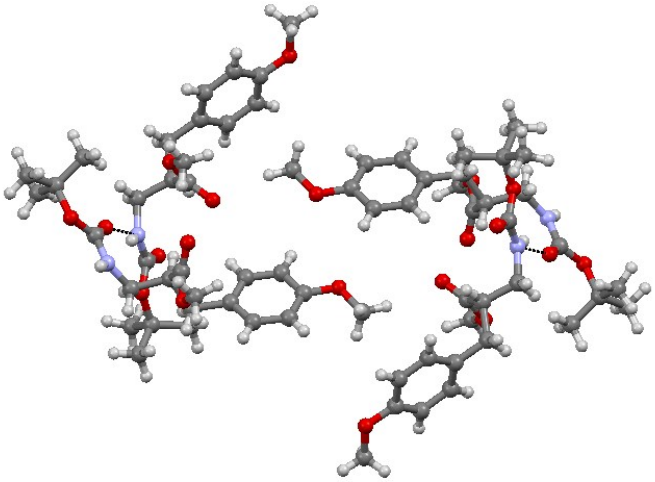
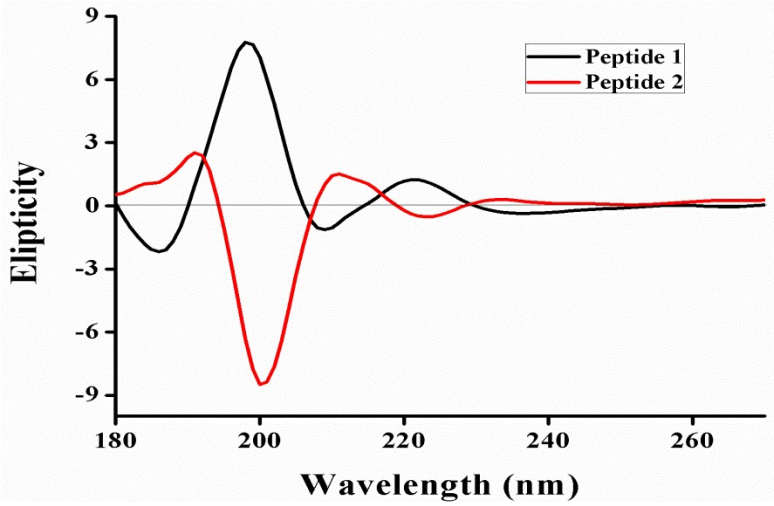


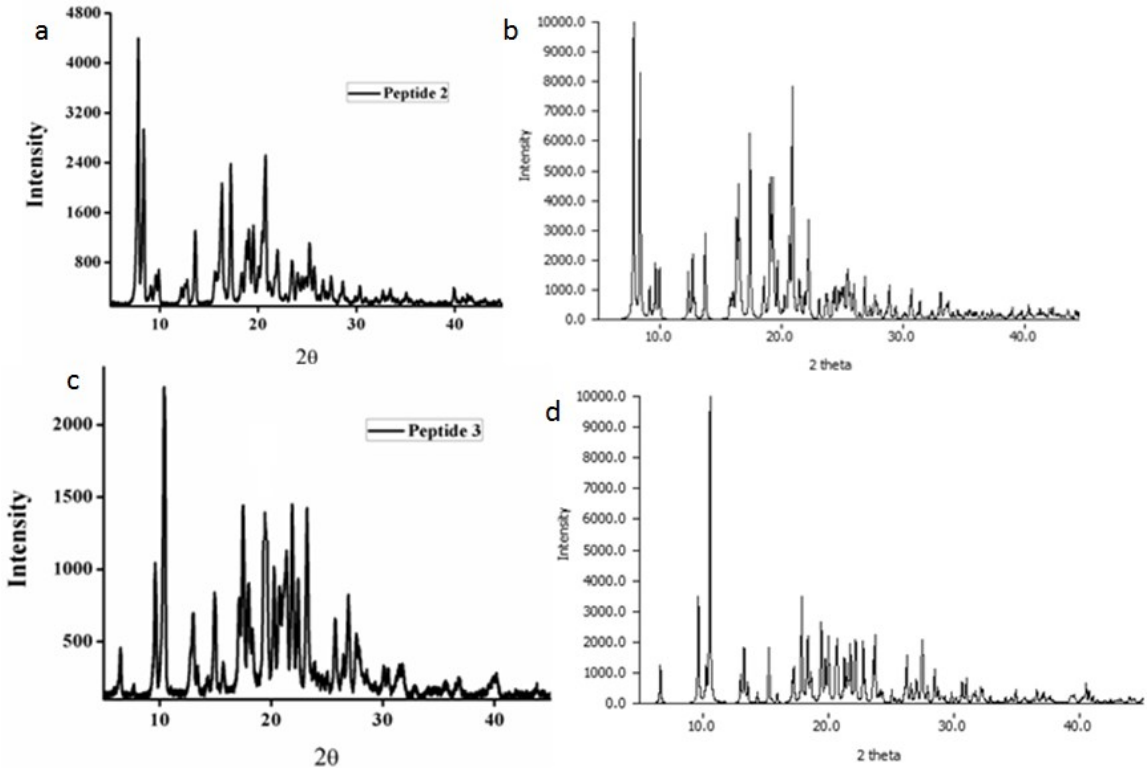
ESI Figure S1: The ORTEP diagram of compound **5**. Ellipsoids are drawn at the 50% probability level.



ESI Figure S2: The packing diagram of compound **5**. Dotted lines are showing hydrogen bonds.



ESI Figure S3: CD spectra of peptides **1** (black) and **2** (red) in methanol.



ESI Figure S4: (a) PXRD pattern of peptide **2** in fibrils and (b) powder pattern from X-ray crystallography of a peptide 2 single crystal. (c) PXRD pattern of peptide **3** in spheres and (b) powder pattern from X-ray crystallography of a peptide 3 single crystal.

Experimental

General

All L-amino acids and D-Phe were purchased from Sigma chemicals. HOBt (1-hydroxybenzotriazole) and DCC (dicyclohexylcarbodiimide) were purchased from SRL.

Peptide Synthesis

The peptides were synthesized by conventional solution-phase methods using racemization free fragment condensation strategy. The Boc group was used for N-terminal protection, and the C-terminus was protected as a methyl ester. Coupling was mediated by dicyclohexylcarbodiimide/1-hydroxyl benzotriazole (DCC/HOBt). The products were purified by column chromatography using silica (100−200 mesh size) gel as a stationary phase and an n-hexane-ethyl acetate mixture as an eluent. The intermediates and final compounds were fully characterized by 500 MHz and 400 MHz

^{1}H NMR spectroscopy, 125 MHz ^{13}C NMR spectroscopy, FT-IR spectroscopy and mass spectrometry. The peptides **1**, **2** and **3** were characterized by X-ray crystallography.

(a) Boc-(L)-Phe(1)-OH (6). A solution of L-Phe (3.3 g, 20 mmol) in a mixture of dioxane (40 mL), water (10 mL) and 1 M NaOH (10 mL) was stirred and cooled in an ice-water bath. Ditert- butylpyrocarbonate (4.8 g, 22 mmol) was added and stirring was continued at room temperature for 6h. Then the solution was concentrated in vacuum to about 20–30 mL, cooled in an ice water bath, covered with a layer of ethyl acetate (about 50 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (Congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water and dried over anhydrous Na_2SO_4 and evaporated in a vacuum. The pure material was obtained as a waxy solid. Yield: 4.58 g (17.28 mmol, 86.4%)

^{1}H NMR (DMSO-d_6, 500 MHz, δ in ppm): 12.61 (b, 1H, COOH), 7.29–7.09 (m, 5H, aromatic ring protons), 6.76–6.74 (d, 1H, *J* ¼ 7 Hz, Phe NH), 4.11–4.02 (m, 1H, Phe C$^{\alpha}$H), 3.03–2.99 (d, 1H, *J* ¼ 20 Hz, Phe C$^{\beta}$H), 2.83–2.79 (d, 1H, *J* ¼ 20 Hz, Phe C$^{\beta}$H), 1.313 (s, 9H, BOC Hs). ^{13}C NMR (DMSO-d_6, 125 MHz, δ in ppm): 173.57, 155.41, 138.00, 129.05, 128.09, 126.27, 77.99, 55.10, 36.39, 20.73.

(b) Boc-(L)-Phe(1)-Leu(2)-OMe (1). 4.30 g (16.22 mmol) of Boc- Phe-OH was dissolved in 25 mL dry DCM in an ice-water bath. H-Leu-OMe 4.2 g (32 mmol) was isolated from the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate and solvent evaporation. It was then added to the reaction mixture, followed immediately by 3.34 g (16.22 mmol) dicyclohexylcarbodiimide (DCC) and 2.48 g (16.22 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3X50 mL), brine (2X50 mL), 1 M sodium bicarbonate (3X50 mL) and brine (2X50 mL) and dried over anhydrous sodium sulfate; and evaporated in a vacuum to yield Boc-Phe-Leu-OMe, **1**, as a white solid. The product was purified by silica gel (100–200 mesh) using n hexane–ethyl acetate (3 : 1) as eluent. Yield: 4.94 g (12.62 mmol, 77.8%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.3344-7.2295 [m, aromatic 5H], 6.3936-6.3726 [d, 1H, Leu(2) NH, *J*=8.4], 5.0863 [b, 1H, Boc NH], 4.6283-4.5730 [m, 1H, Phe $C^{\alpha}H$], 3.1054-3.0883 [m, 2H, Phe $C^{\beta}H$], 3.7197 [s, 3H. OMe], 1.6493–1.4795 [m, 3H, Leu $C^{\beta}H$ and $C^{\gamma}H$], 1.38 [s, 9H, Boc], 0.9451–0.9108 [t, 6H, Leu 2xMe]. ^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm): 174.28, 171.41, 155.60, 137.03, 129.82, 129.03, 127.32, 80.38, 56.05, 52.67, 51.15, 41.98, 38.50, 28.67, 25.07, 23.18.

(c) Boc-(D)-Phe(1)-Leu(2)-OMe (2). Similar procedure as Boc-(L)-Phe(1)-Leu(2)-OMe (1).

(d) Compound (4). Target designer amino ester 4 was synthesized by microwave assisted condensation reaction followed by borohydride reduction and insitu BOC protection. 2.359ml P-Anisaldehyde (19.39mmol), 2.132 ml Ethylcyanoacetate (18.06mmol) and 1.26g Ammonium formate taken in a 250ml conical flask and heated to microwave for 90sec. The mixture was cooled and poured into 100g crushed ice and stir by a glass rod till pale yellow precipitate appears. The product was filtered, washed several times by water and dried. Yield: 3.55g (15.4mmol,79.4%)

^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.1894 [s, 1H, Double bond proton], 8.0119-7.9909 [d, 2H, Aro Hs, *J*=8.4MHz], 7.01-7.0024 [d, 2H, Aro Hs, *J*=3.04 MHz], 4.3917-4.3363 [q, 2H, -OCH_2], 3.8898 [s, 1H, OMe proton], 1.4051-1.3688[t, 3H, CH_3 of OEt].

(e) Compound (5). Reaction procedure followed from *Tetrahedron*, 2003, **59**, 5417-5423. Yield:3.2g (9.90mmol, 62.5%)

^{1}H NMR (DMSO-, 400 MHz, δ in ppm) 7.0687-7.0477 [d, 2H, Aro Hs, *J*=8.4MHz], 7.0019-7.0477 [t, 1H, NH proton], 6.8339-6.8130 [d, 2H, Aro Hs, *J*=8.36MHz], 3.7061 [s, 3H, Aro OMe], 3.5038 [s, 3H, CO_2Me], 3.1221-3.081 [m, 2H, C^{β} Proton], 2.7538-2.6527 [m, 3H, C^{β}+C^{α} Proton], 1.3645 [s, 9H, Boc protons]. ^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm): 179.31, 158.30, 129.94, 114.06, 78.44, 60.38, 55.30, 47.45, 40.84, 34.77, 28.40. Mass spectra: m/z 346.28, $[M + Na]^+$; M calcd 323.12

(f) Saponifiaction of 5. To 2.98 (9.22 mmol) of compound **5**, 25 mL MeOH and 2 M 15 mL NaOH were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred and after 10 h, methanol was removed under vacuum, the residue was dissolved in 50 mL of water, and washed with diethyl ether (2X50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M

HCl and it was extracted with ethyl acetate (3X50 mL). The extract were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtained compound **7** as a white solid. Yield 2.45 g (7.57 mmol, 82.10%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm). 8.4394 [bs,1H, OH] 7.1131-7.0921 [d, 2H, Aro Hs, *J*=8.4MHz], , 6.8306-6.8077 [d, 2H, Aro Hs, *J*=9.16 MHz], 6.4394 [bs, 1H, NH proton] 3.7715 [s, 3H, Aro OMe], 3.5038 [s, 3H, CO_2Me], 3.365-2.6398 [m, 5H, C^{β}+C^{α} Proton], 1.4165 [s, 9H, Boc protons]. ^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm): 179.31, 158.30, 129.94, 114.06, 78.44, 60.38, 55.30, 47.45, 40.84, 34.77. Mass spectra: m/z 332.15, $[M + Na]^+$; M calcd 309.25.

(g) Boc-Xaa-Leu(2)-OMe (3). 2.30 g (7.44 mmol) of compound 7 was dissolved in 25 mL dry DCM in an ice-water bath. H-Leu-OMe 1.16 g (15 mmol) was isolated from the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate and solvent evaporation. It was then added to the reaction mixture, followed immediately by 3.07 g (14.88 mmol) dicyclohexylcarbodiimide (DCC) and 2.10 g (14.88 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3X50 mL), brine (2X50 mL), 1 M sodium bicarbonate (3X50 mL) and brine (2X50 mL) and dried over anhydrous sodium sulfate; and evaporated in a vacuum to yield peptide **3**, as a white solid. The product was purified by silica gel (100–200 mesh) using n hexane–ethyl acetate (3: 1) as eluent. Yield: 2.01 g (4.6 mmol, 61.82%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.0829-7.0615 [m, aromatic 2H], 6.7959-6.7653 [3, aromatic 2H], 5.6753-5.6554 [d, 1H, Boc NH], 5.8417-5.8218 [m, 1H, Leu NH], 4.5425-4.4325 [m, 1H, Leu C^{α}H], 3.6893 [s, 3H, Aromatic OMe], 3.6386 [s, 3H, CO_2Me], 3.4234-3.3211 and 3.26-3.1745 [m, 4H 2xC^{β} proton of amino acid], 2.8203-3.1745 [m, 1H C^{α} proton of amino acid], 1.87-1.6951 [m, 2H, Leu C^{β}H], 1.5806-1.5119 [m, 1H, Leu C^{γ}H] 1.4035 [s, 9H, Boc], 0.8967-0.8768 [m, 6H, Leu 2xMe]. ^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm): 174.00, 158.67, 156.20, 130.97, 130.27, 114.37, 79.75, 55.62, 52.62, 51.00, 43.27, 41.39, 41.84, 35.81, 28.80, 25.14, 24.80, 23.18. Mass spectra: m/z 459, $[M + Na]^+$; M calcd 436.17

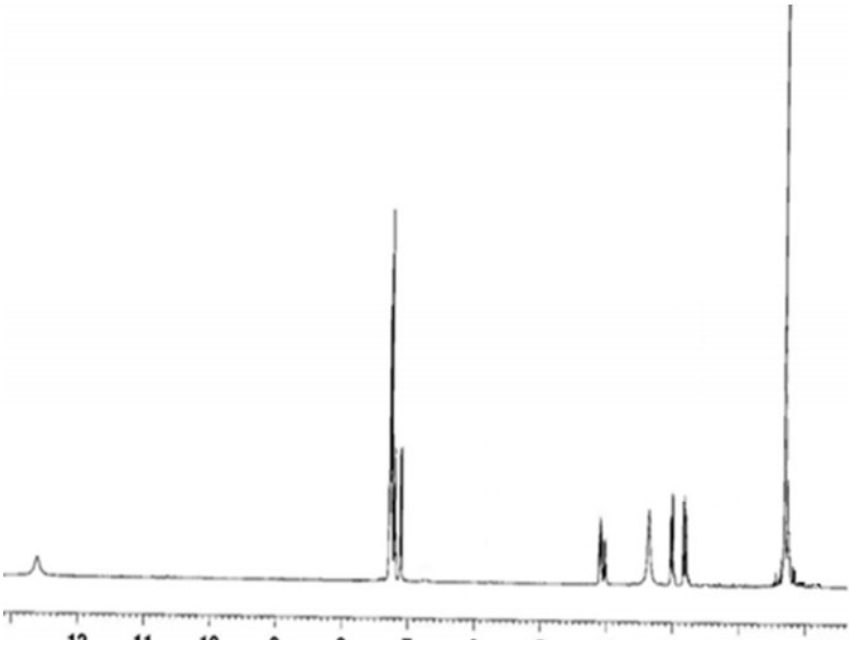


Figure S3: ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm) spectra of Boc-Phe-OH 6.

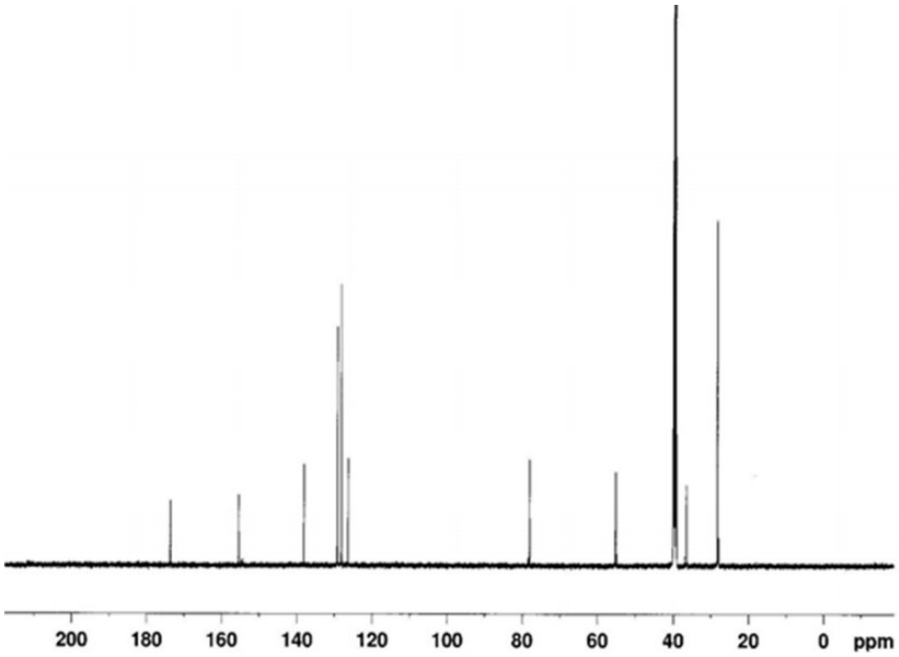


Figure S4: ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm) spectra of Boc-Phe-OH 6.

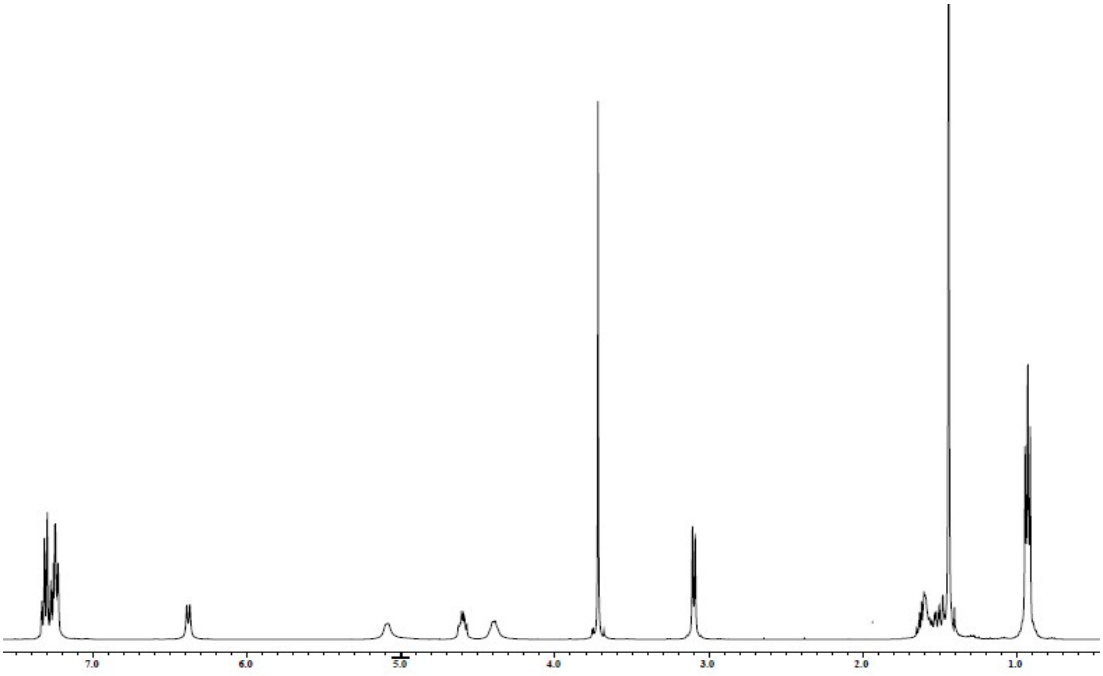


Figure S5: $^{1}HNMR$ ($CDCl_3$, 400 MHz, δ in ppm) spectra of Boc-Phe-Leu-OMe **1**

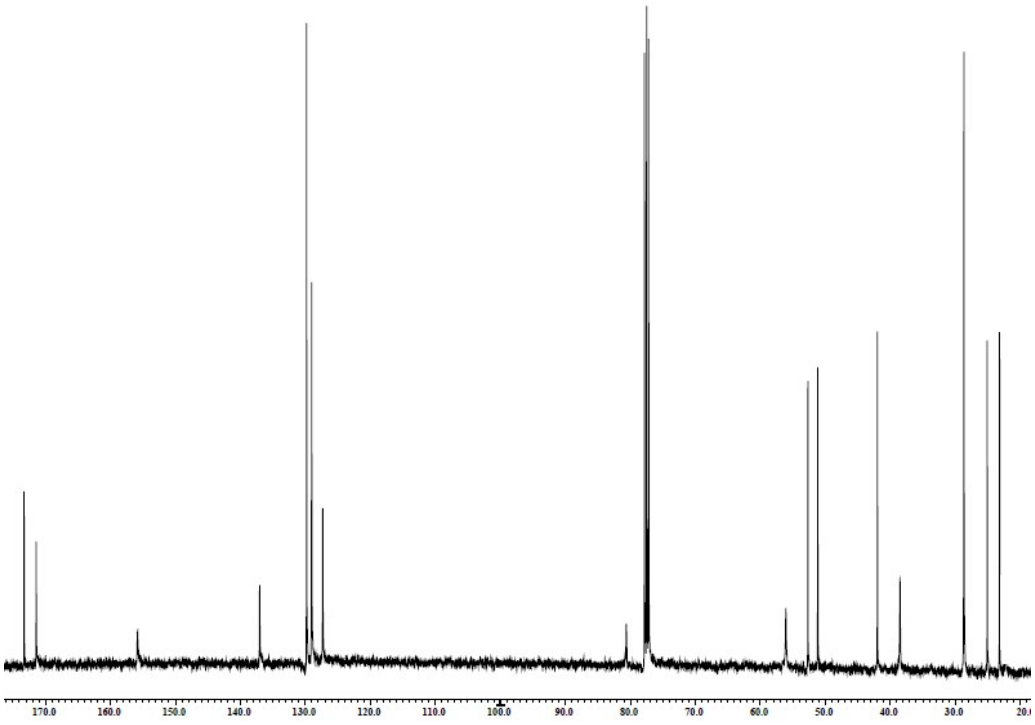


Figure S6: ^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm) spectra of Boc-Phe-Leu- OMe **1**

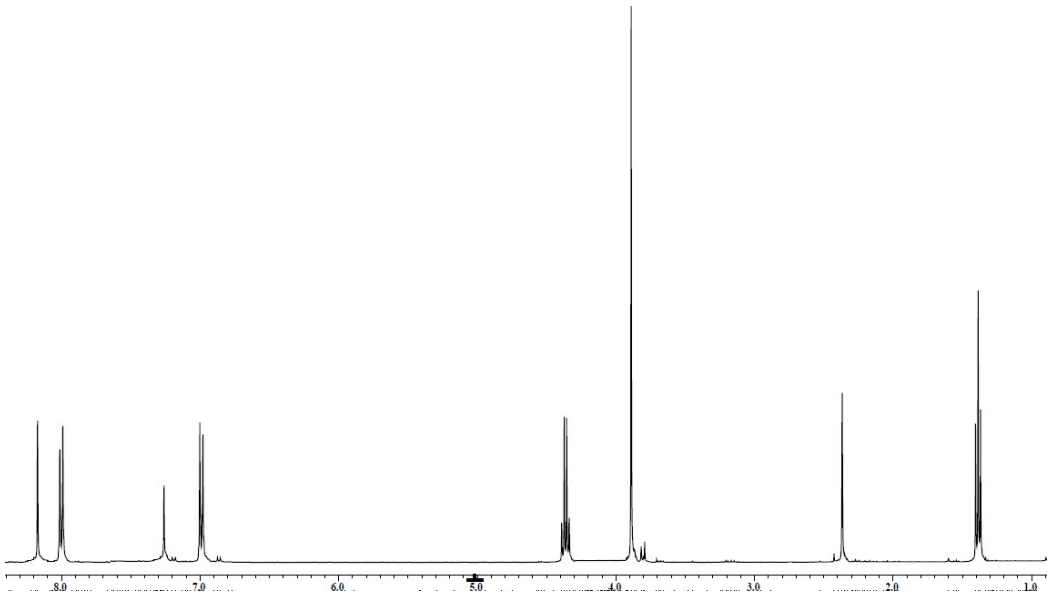


Figure S7: ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm) spectra of compound 4.

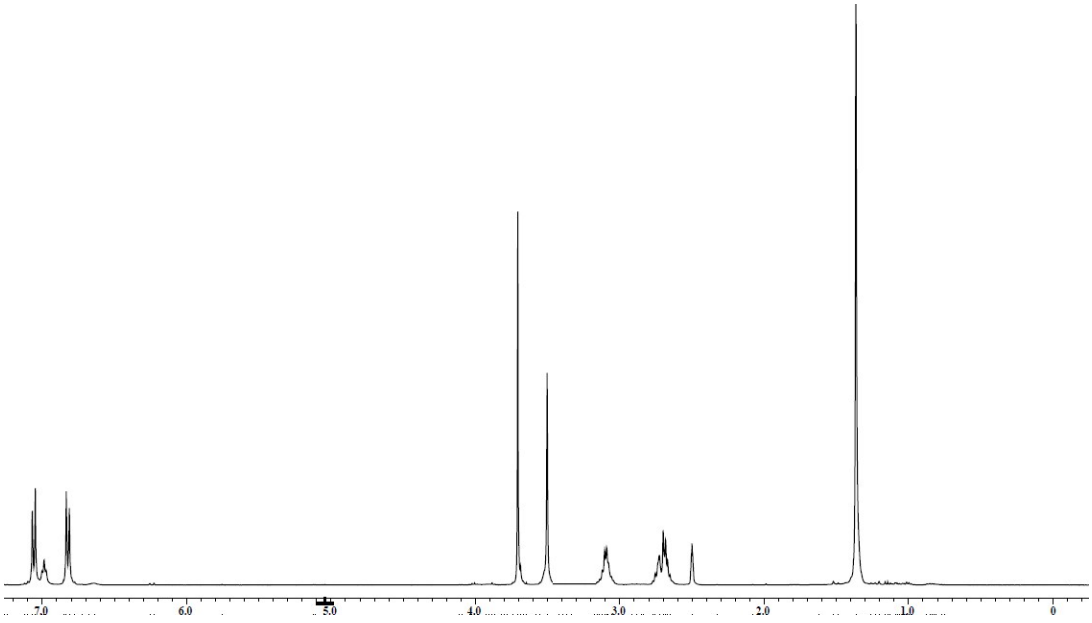


Figure S8: ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm) spectra of **5**.

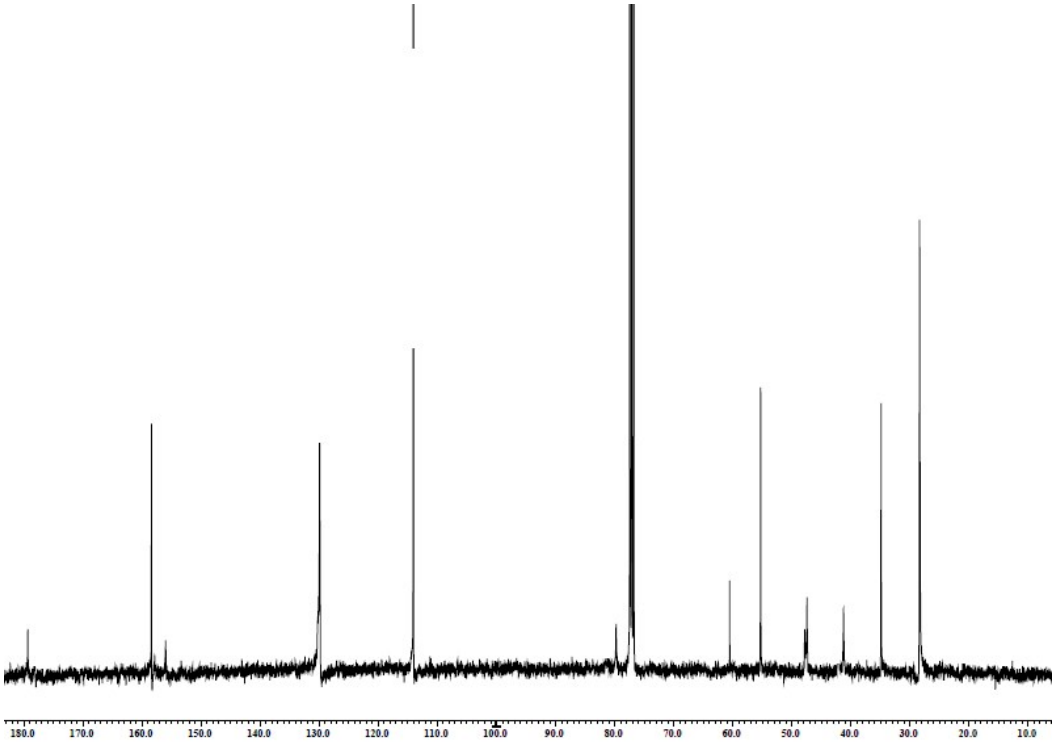


Figure S9: ^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm) spectra of compound 5.

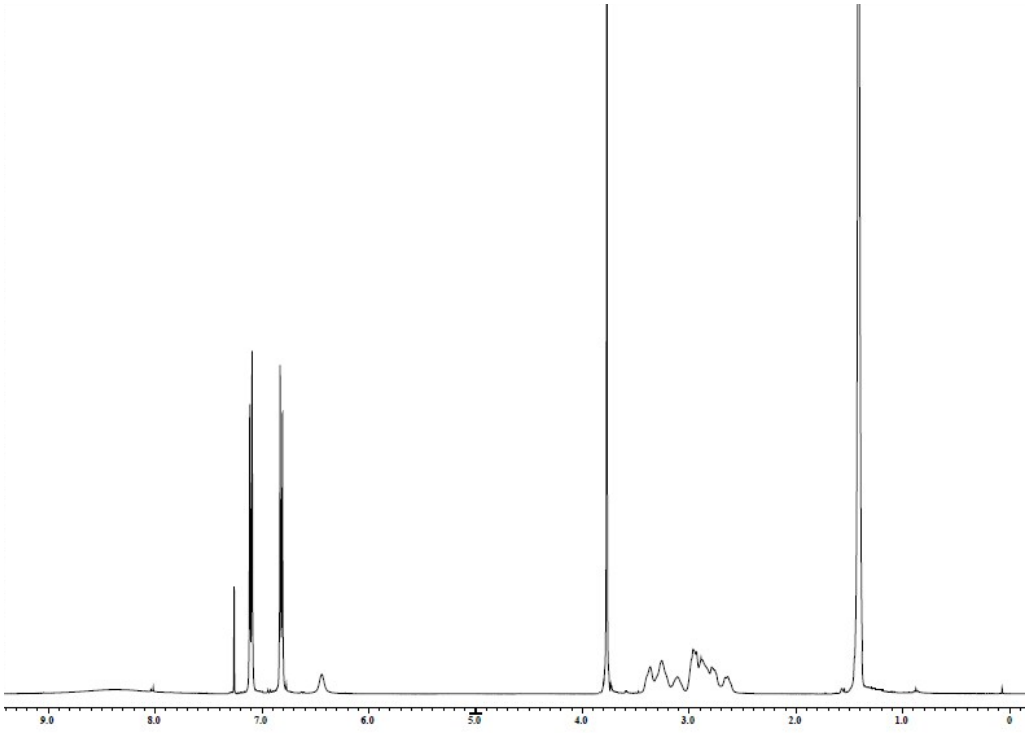


Figure S10: ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm) spectra of compound 7.

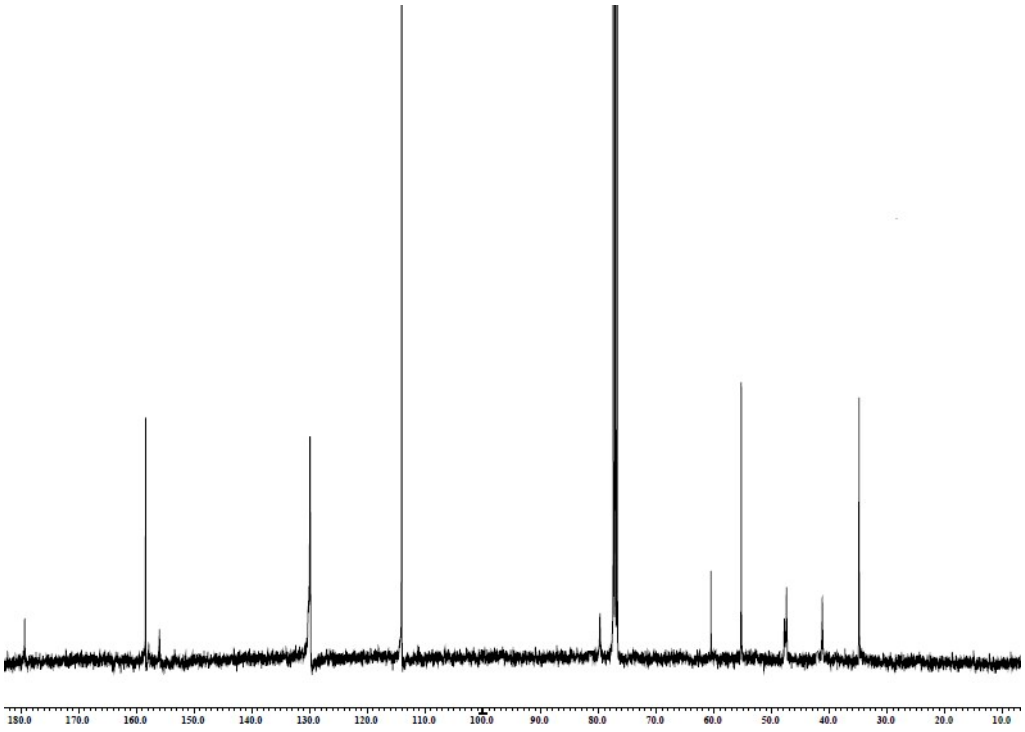


Figure S11: ^{13}C NMR ($CDCl_3$,100 MHz, δ in ppm) spectra of compound **7**

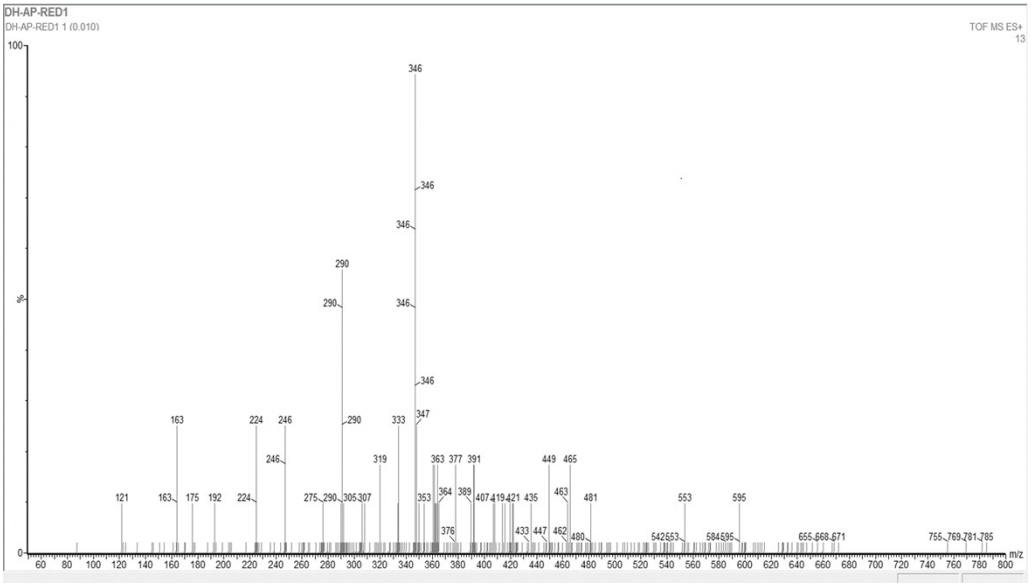


Figure S12: Mass Spectra of compound 5

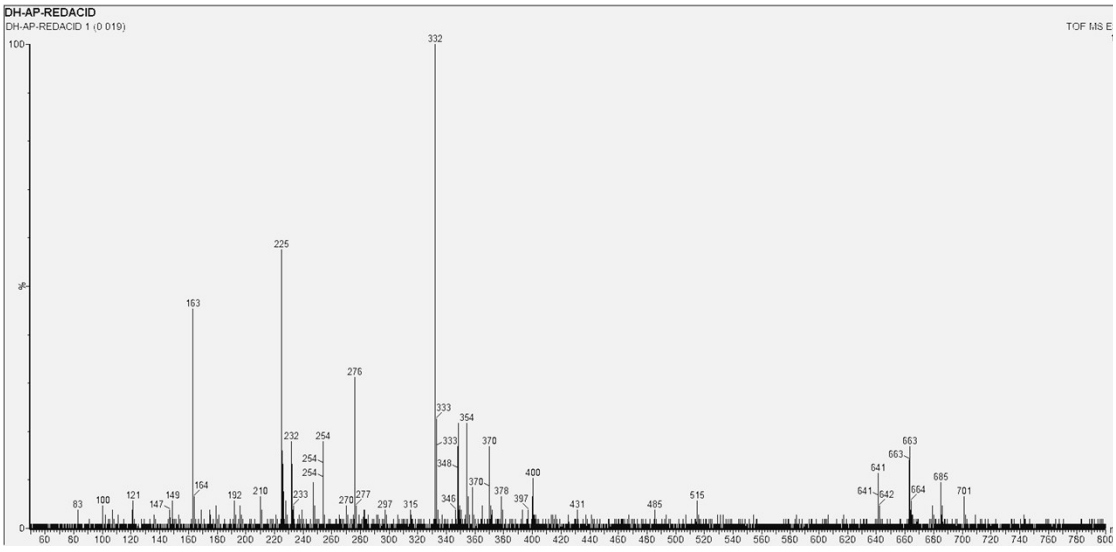


Figure S13: Mass Spectra of compound 7.

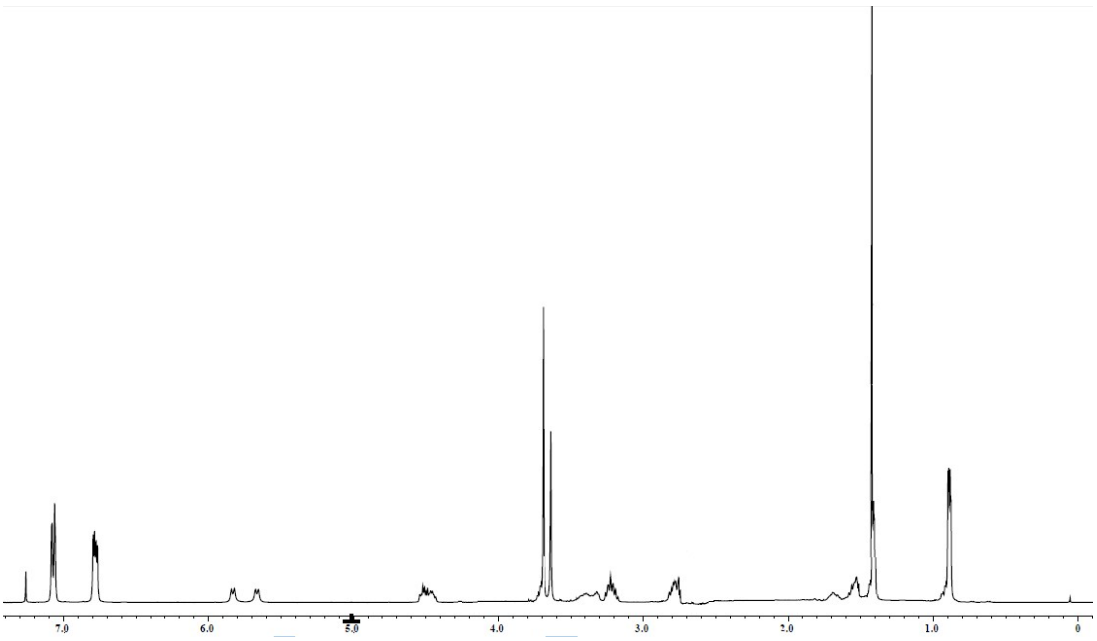


Figure S14: ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm) spectra of peptide 3.

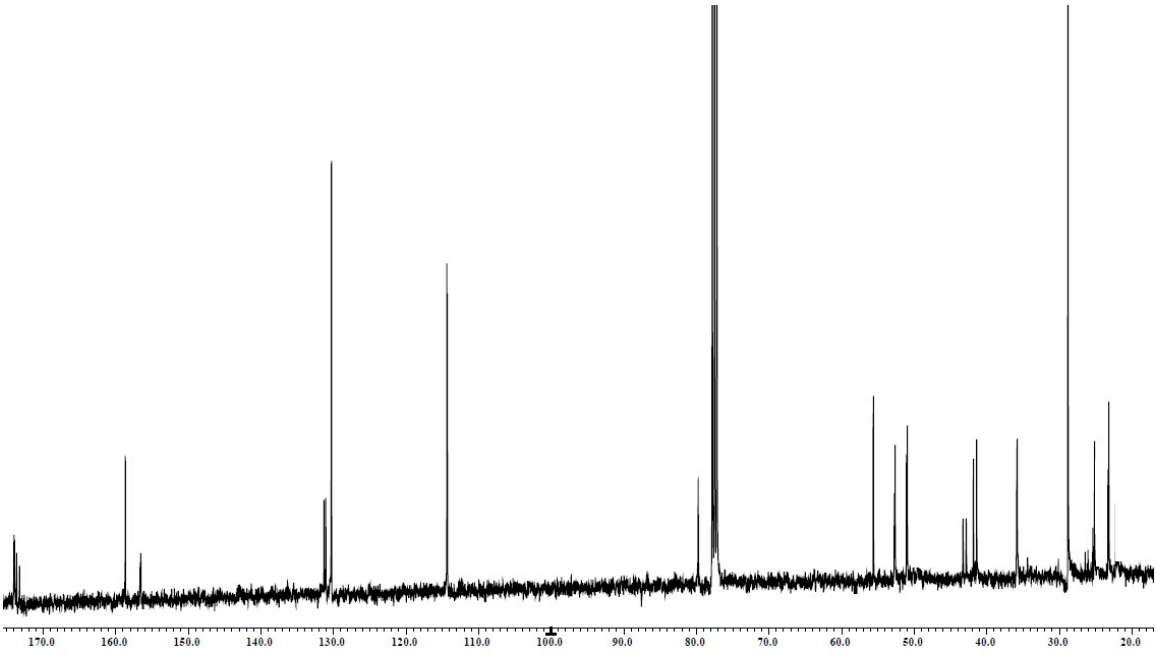


Figure S15: ^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm) spectra of peptide 3.

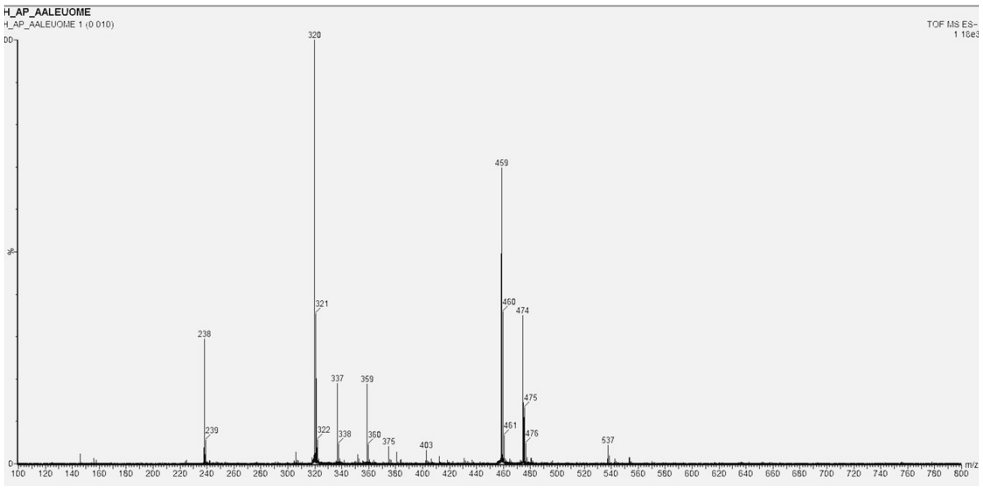


Figure S16: Mass spectra of peptide 3.