Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

An efficient one pot ipso-nitration: Structural transformation of a dipeptide by N-terminus modification†

Rajib Sarkar, Krishnendu Maji and Debasish Haldar*

Department of Chemical Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur, West Bengal 741252, India,

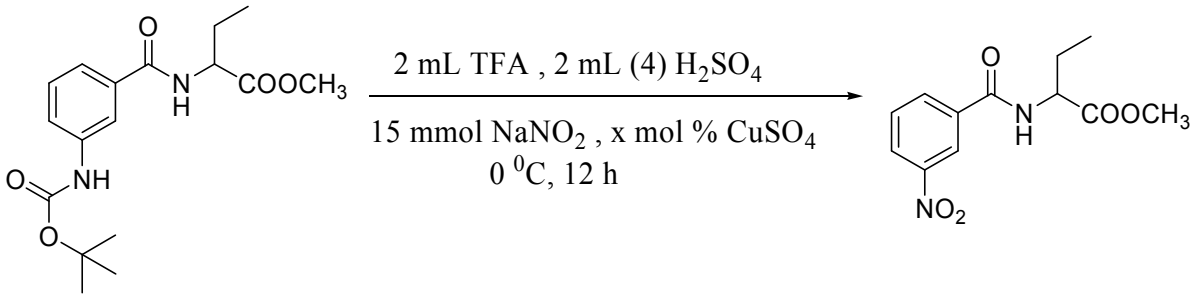
Fax: (+)913325873020; Tel: +913325873119;

E-mail: deba_h76@yahoo.com; deba_h76@iiserkol.ac.in

Table of contents

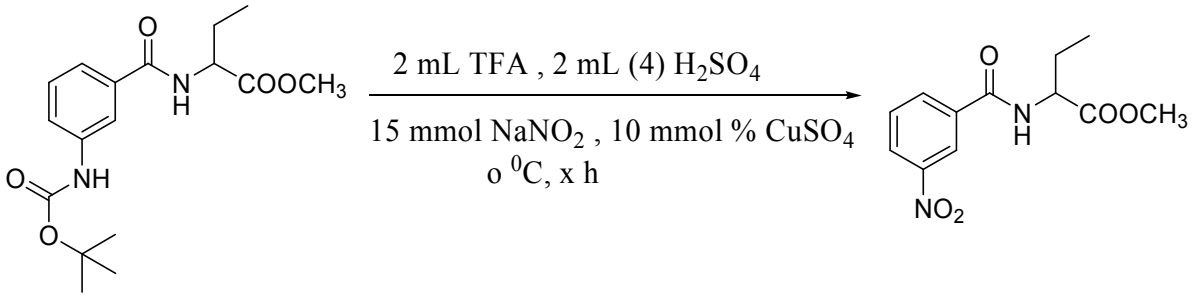
Optimization Details:

(A) Optimization of catalytic amount of $CuSO_4$:



Entry	Amount of $CuSO_4$ (mol %)	Yield (%)[a]
1	2	23
2	5	30
3	8	54
4	**10**	**60**
5	15	60

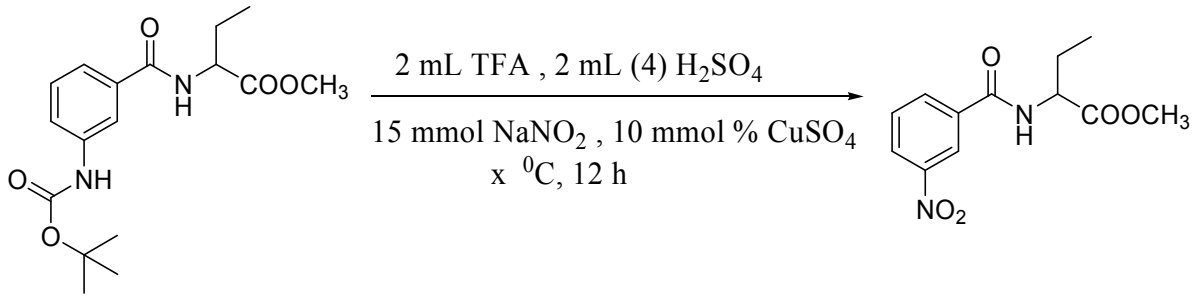
(B) Optimization of reaction time:



Entry	Reaction time (in hours)[b]	Yield (%)[a]
1	0.5	21
2	1	28
3	1.5	33
4	2	40
5	3	45

6	6	58
7	**12**	**60**
8	15	55

(C) Optimization of reaction Temperature:



Entry	Reaction temperature (in ^{0}C)	Yield (%)[a]
1	-5	60
2	**0**	**60**
3	5	53
4	10	20
5	20	0
6	27	0

[a] Isolated yield, [b] reaction time was counted after 2 hours from the addition of TFA.

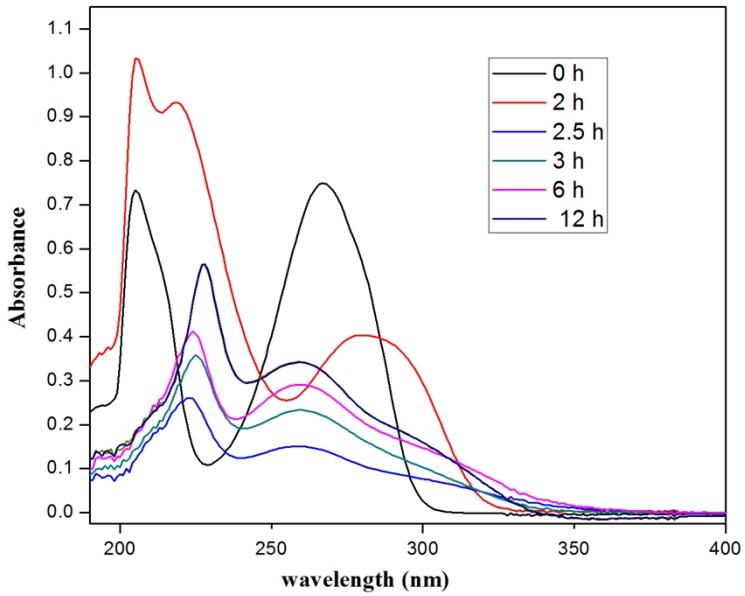


Figure S1: Reaction kinetics of the ipso-nitration of peptide 5 over time.

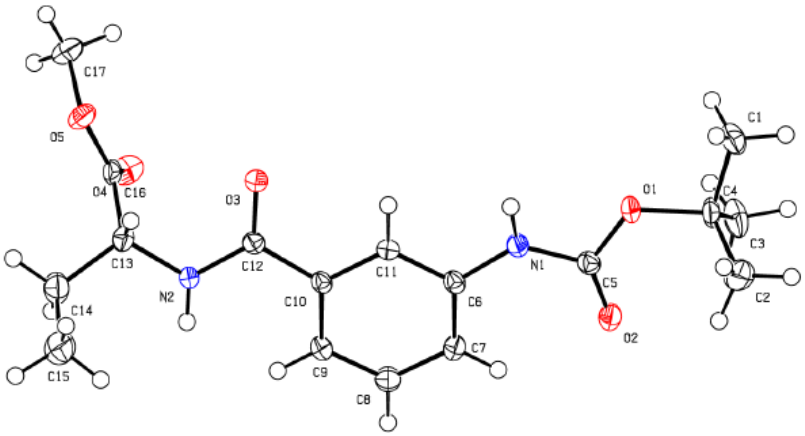


Figure S2: ORTEP diagram with atom numbering scheme of peptide **1**. 50% probability level.

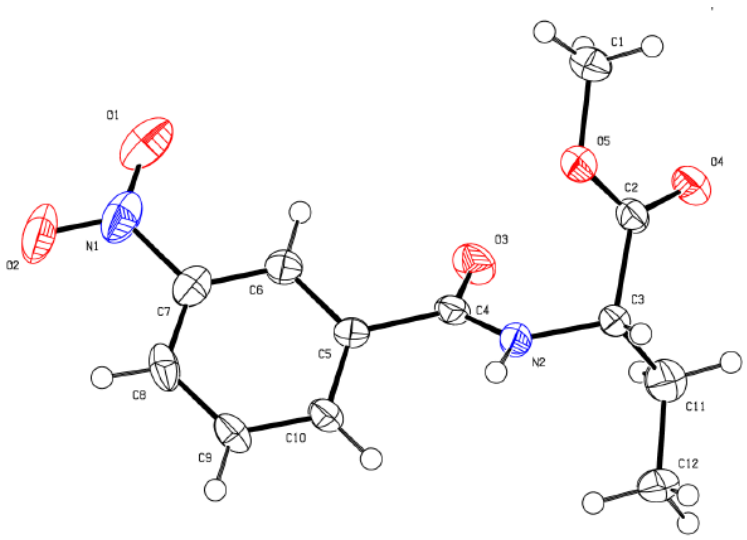
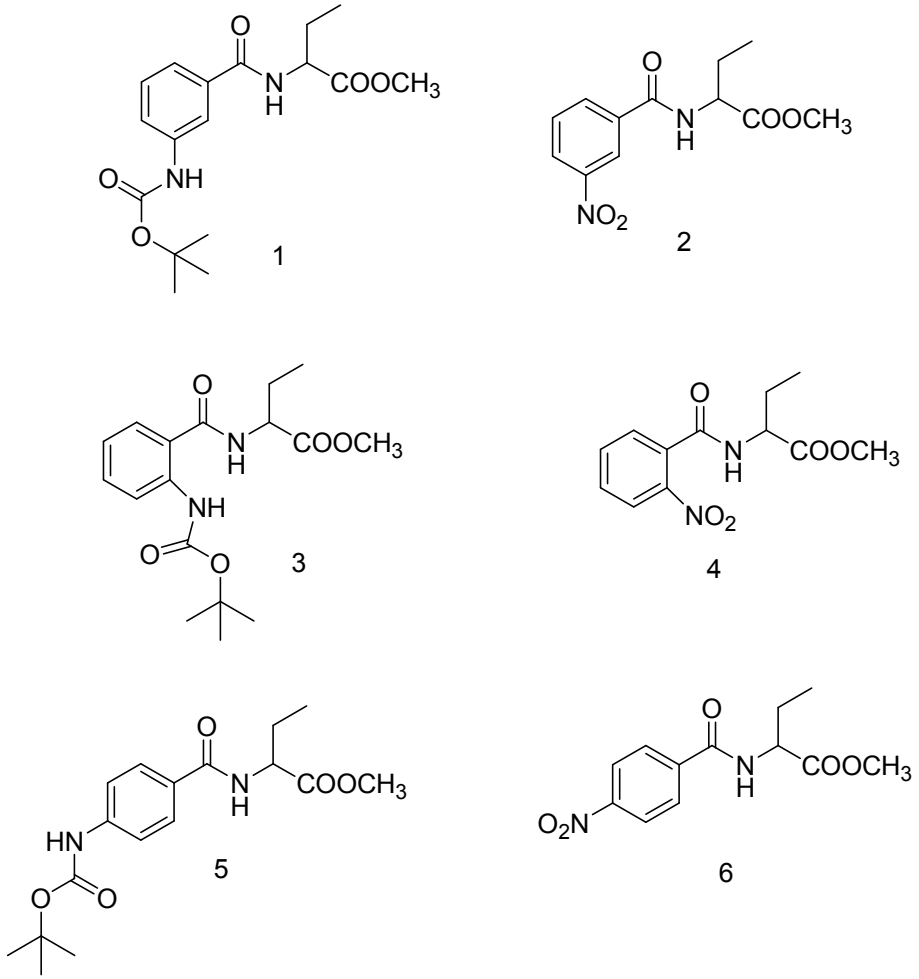


Figure S3: ORTEP diagram with atom numbering scheme of peptide **2**. 50% probability level.

Peptide Synthesis:



Scheme 1: The schematic presentation of reported peptides **1-6**.

General

All amino acids were purchased from Sigma chemicals. HOBt (N-hydroxybenzotriazole) and DCC (dicyclohexylcarbodiimide) were purchased from SRL.

Entry	peptide	Yield (%)
1	Boc-Oaba-Aba-OMe (3)	63
2	Boc-Maba-Aba-OMe (1)	71
3	Boc-Paba-Aba-OMe (5)	78
4	Boc-Maba-Aib-OMe (7)	75

***(a) Boc-Maba-Aba-OMe 1*:** See the experimental section of the manuscript.

(b)Boc-Oaba-OH: A solution of o-amino benzoic acid (2.7 g, 20 mmol) in a mixture of dioxane (40 mL), water (20 mL), and 1(N) NaOH (20 mL) was stirred and cooled in an ice-water bath. Di tertiarybutylpyrocarbonate (4.8 g, 22 mmol) was added, and stirring was continued at room temperature for 6 h. Then, the solution was concentrated under vacuum to about 30-40 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, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The pure material was obtained. Yield: 3.79 g (16 mmol, 80%).
^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): [COOH, 1H, s], 10.52 [Oaba NH, 1H, s], 8.29-8.22 [Oaba CH, 1H, d], 7.96-7.94 [Oaba CH, 1H, d], 7.55-7.53 [Oaba CH, 1H, m], 7.08-7.04 [Oaba CH, 1H, m], 1.48 [Boc CH_3, 9H, s]. ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 169.69, 152.02, 141.56, 143.29, 131.27, 121.47, 118.23, 115.10, 81.16, 27.93.

(c) Boc-Oaba-Aba-OMe 3: A 2.37 g (10 mmol) sample of Boc-Oaba-OH was dissolved in 30 mL dry dichloromethane (DCM) in an ice-water bath. H-Aba-OMe was isolated from 2.34 g (20 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration (10 mL), and this was added to the reaction mixture, followed immediately by 2.47 g (12 mmol) of dicyclohexylcarbodiimide (DCC). The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated, and the residue was taken in ethyl acetate (60 mL). dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 (N) HCl (3 × 50 mL), brine, 1 (M) sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield dipeptide 3 as a white solid. Purification was done on a silica gel column (60-120 mesh) using ethyl acetate: hexane (1:4) as the eluent. Yield: 2.1 g (63 mM, 63%).

^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm): 10.05 [1H, s, Oaba NH], 8.38-8.36 [1H, d, J=8 Hz, Oaba CH], 7.52-7.50 [1H, d, Oaba CH], 7.46-7.44 [1H, t, Oaba CH], 7.02-6.99 [1H, t, Oaba CH], 6.72 [1H, b, Aba NH], 4.77-4.73 [1H, m, Aba Cα H] 3.79 [3H, s, OCH3], 2.04-2.00[1H, m, Aba Cβ H], 1.89-1.84[1H, m, Aba Cβ H] 1.51 [9H, s, Boc CH_3], 0.99-0.96 [3H, t, Aba Cγ H]. ^{13}C NMR (125 MHz, CDCl3, δ in ppm): 173.21, 169.01, 153.53, 140.97, 133.30, 127.23, 121.91, 120.37, 119.81, 80.78, 54.01, 53.01, 28.83, 26.16, 9.97. Mass spectra: $[M+Na]^+$: 359.075, Expected mass: 359.16.

(d) Boc-Paba-OH: A solution of p-amino benzoic acid (2.7 g, 20 mmol) in a mixture of dioxane (40 mL), water (20 mL), and 1(N) NaOH (20 mL) was stirred and cooled in an ice-water bath. Di tertiarybutylpyrocarbonate (4.8 g, 22 mmol) was added, and stirring was continued at room temperature for 6 h. Then, the solution was concentrated under vacuum to about 30-40 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, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The pure material was obtained. Yield: 4.26 g (18 mmol, 90%).

^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 12.87 [COOH, 1H, broad], 9.71 [Paba NH, 1H, s], 7.84-7.82 [Paba CH, 2H, m], 7.52-7.55 [Paba CH, 2H, m], 1.485 [Boc CH_3, 9H, s]. ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 167.07, 152.56, 146.24, 143.81, 130.37, 124.0, 122.23, 117.24, 85.54, 79.68, 28.04.

***(e) Boc-Paba-Aba-OMe 5*:** A 2.37 g (10 mmol) sample of Boc-Paba-OH was dissolved in 30 mL dry dichloromethane (DCM) in an ice-water bath. H-Aba-OMe was isolated from 2.34 g (20 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration (10 mL), and this was added to the reaction mixture, followed immediately by 2.47 g (12 mmol) of dicyclohexylcarbodiimide (DCC). The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated, and the residue was taken in ethyl acetate (60 mL); dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 (N) HCl (3 × 50 mL), brine, 1 (M) sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield dipeptide 1 as a white solid. Purification was done on a silica gel column (60-120 mesh) using ethyl acetate: hexane (1:2) as the eluent. Yield: 2.62 g (7.8 mM, 78%).

^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm): 7.76-7.74 [2H, d, J=8 Hz, Paba CH], 7.44-7.42 [2H, d, J=8 Hz, Paba CH], 6.71 [1H, s, Paba NH], 6.62 [1H, b, Aba NH], 4.79-4.75 [1H, m, Aba Cα H], 3.78 [3H, s, OCH_3], 2.04-1.98 [1H, m, Aba Cβ H], 1.86-1.81 [1H, m, Aba Cβ H] 1.52 [9H, s, Boc CH_3], 0.98-0.95 [3H, t, Aba Cγ H]. ^{13}C NMR (125 MHz, $CDCl_3$, δ in ppm): 173.98, 167.0, 153.16, 142.23. 128.79, 134.75, 118.20, 81.70, 54.17, 52.97, 49.73, 34.50, 28.91, 26.40, 26.18, 25.49, 10.09. Mass spectra: $[M+Na]^+$: 359.04, Expected mass: 359.16, $[2M+ Na]^+$: 695.08.

Ipso-Nitration:

Entry	Reactant	Product	Yield (%)
1	Boc-Oaba-Aba-OMe (3)	Onba-Aba-OMe (4)	23
2	Boc-Maba-Aba-OMe (1)	Mnba-Aba-OMe (2)	60
3	Boc-Paba-Aba-OMe (5)	Pnba-Aba-OMe (6)	65
4	Boc-Maba-Aib-OMe (7)	Mnba-Aib-OMe (8)	58

(a) Ipso-nitration for peptide 3: To 0.34 g (1 mmol) of Boc-Oaba-Aba-OMe 3, 2 mL of TFA was added and the resulting solution was stirred for 2 hours at room temperature. Then, 2 mL 4(N) H_2SO_4 was added into it and the resulting solution was cooled to 0 ^oC. After that, $NaNO_2$ (1.04 g, 15 mmol) and $CuSO_4$ (10 mol %) were added into the reaction mixture. During the addition of the reagents temperature of the reaction mixture was maintained to 0-5 0C. Then, the resulting solution was stirred for further 12 hours. After that, the reaction was quenched with Na_2CO_3 and pH of the solution was maintained to 7-8.The solution was extracted with ethyl acetate and the ethyl acetate solution was washed with brine (3x15 mL), dried over anhydrous Na_2SO_4 and evaporated under vacuum to get off white solid product **4**. The product was purified with 60-120 mesh silica gel column using ethyl acetate: hexane (1:4) as the eluent. Yield: 0.06 g (0.23 mM, 23%).

^1H NMR (500 MHz, $CDCl_3$, δ in ppm): 8.38-8.36 [1H, d, J=8 Hz, Onba CH], 7.52-7.5 [1H, d, J=8 Hz, Onba CH], 7.46-7.44 [1H, t, Onba CH], 7.03-7.00 [1H, t, Onba CH], 6.72 [1H, b, Aba NH], 4.77-4.74 [1H, m, Aba Cα H] 3.81 [3H, s, OCH_3], 2.06-2.00 [1H, m, Aba Cβ H], 1.89-1.82 [1H, m, Aba Cβ H] 0.99-0.96 [3H, t, Aba Cγ H]. ^{13}C NMR (125 MHz, $CDCl_3$, δ in ppm): 173.27, 165.17, 148.74, 136.1, 133.62, 130.25, 126.75, 122.55, 54.45, 53.07, 26.1, 10.1. Mass spectra: $[M+Na]^+$: 289.09 Expected mass: 289.08.

(b)Ipso-nitration for peptide 1: See the experimental section of the manuscript.

(c) Ipso-nitration for peptide 5: To 0.34 g (1 mmol) of Boc-Paba-Aba-OMe 5, 2 mL of TFA was added and the resulting solution was stirred for 2 hours at room temperature. Then, 2 mL 4(N) H_2SO_4 was added into it and the resulting solution was cooled to 0 ^oC. After that, $NaNO_2$ (1.04 g, 15 mmol) and $CuSO_4$ (10 mol %) were added into the reaction mixture. During the addition of the reagents temperature of the reaction mixture was maintained to 0-5 0C. Then, the resulting solution was stirred for further 12 hours. After that, the reaction was quenched with Na_2CO_3 and pH of the solution was maintained to 7-8.The

solution was extracted with ethyl acetate and the ethyl acetate solution was washed with brine (3x15 mL), dried over anhydrous Na_2SO_4 and evaporated under vacuum to get off white solid product **6**. The product was purified with 60-120 mesh silica gel column using ethyl acetate: hexane (1:3) as the eluent. Yield: 0.17 g (0.65 mM, 65%).

^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm): 8.30-8.28 [2H, d, J=8 Hz, Pnba CH], 7.98-7.96 [2H, d, J=8 Hz, Paba CH], 6.83 [1H, b, Aba NH], 4.82-4.77 [1H, m, Aba Cα H], 3.81 [3H, s, OCH_3], 2.07-2.01 [1H, m, Aba Cβ H], 1.9-1.83 [1H, m, Aba Cβ H], 0.99-0.95 [3H, t, Aba Cγ H]. ^{13}C NMR (125 MHz, $CDCl_3$, δ in ppm): 173.23, 165.49, 150.21, 139.99, 128.81, 124.34, 54.36, 53.14, 26.16, 10.05. Mass spectra: [M+Na]+ : 289.31 Expected mass: 289.08.

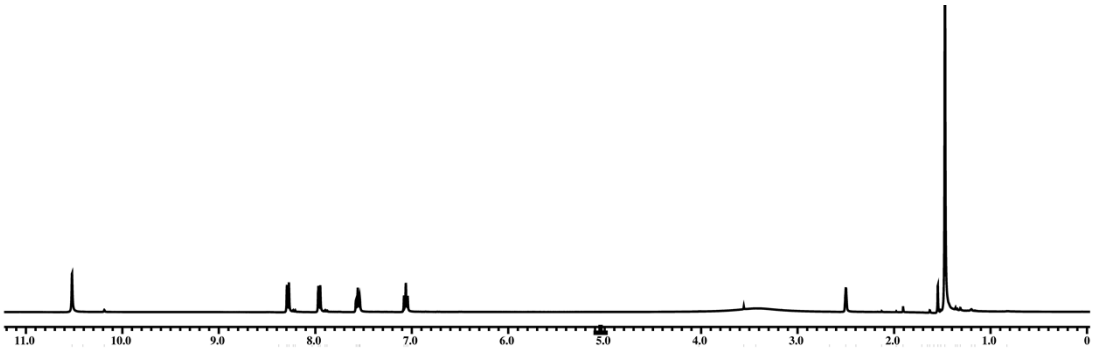


Figure S4: ^{1}H NMR Spectra (DMSO-d_6, 400 MHz, δ in ppm, 298 K) of Boc-Oaba-OH.

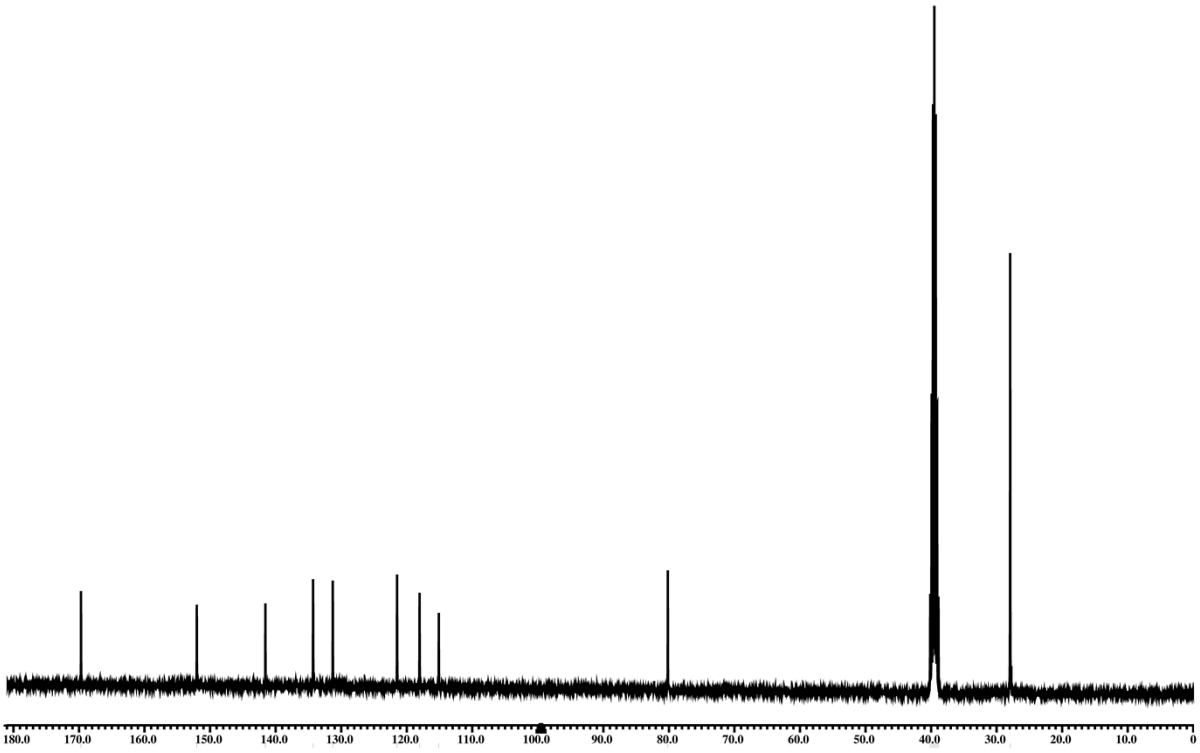


Figure S5: ^{13}**C** NMR Spectra (DMSO-d_6, 100 MHz,δ in ppm, 298 K) of Boc-Oaba-OH.

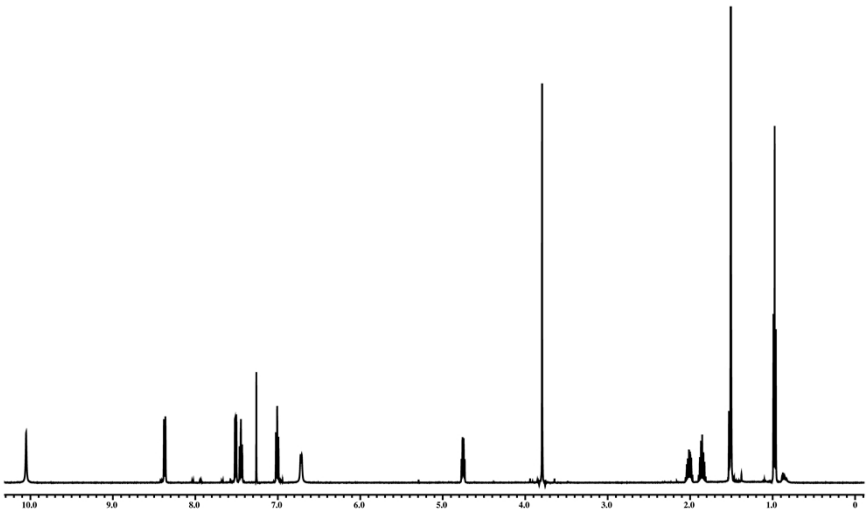


Figure S6: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz,δ in ppm, 298 K) of peptide **3.**

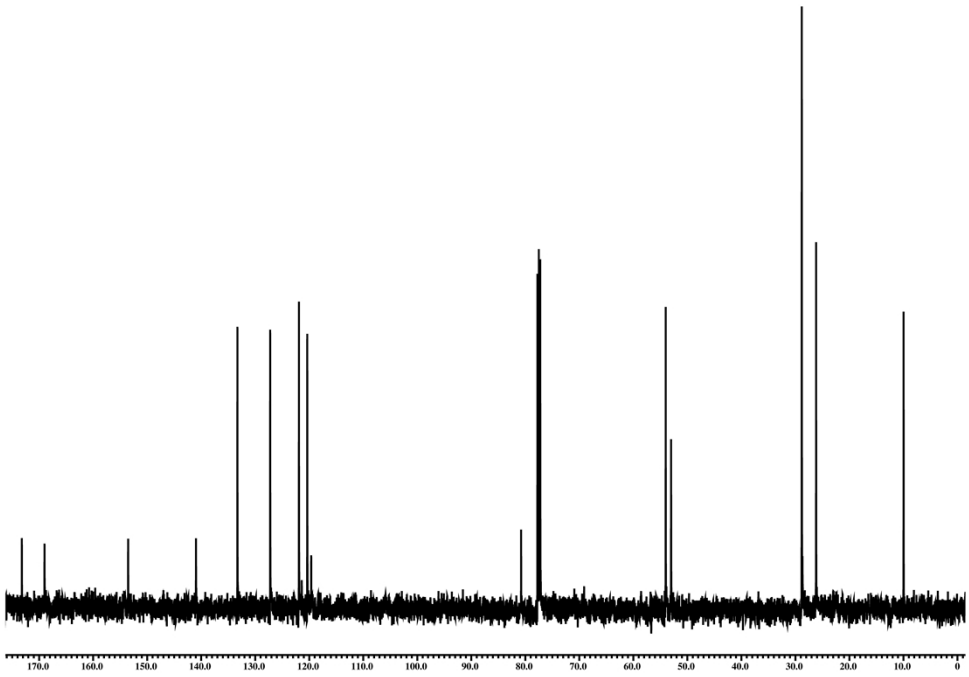


Figure S7: ^{13}**C** NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **3.**

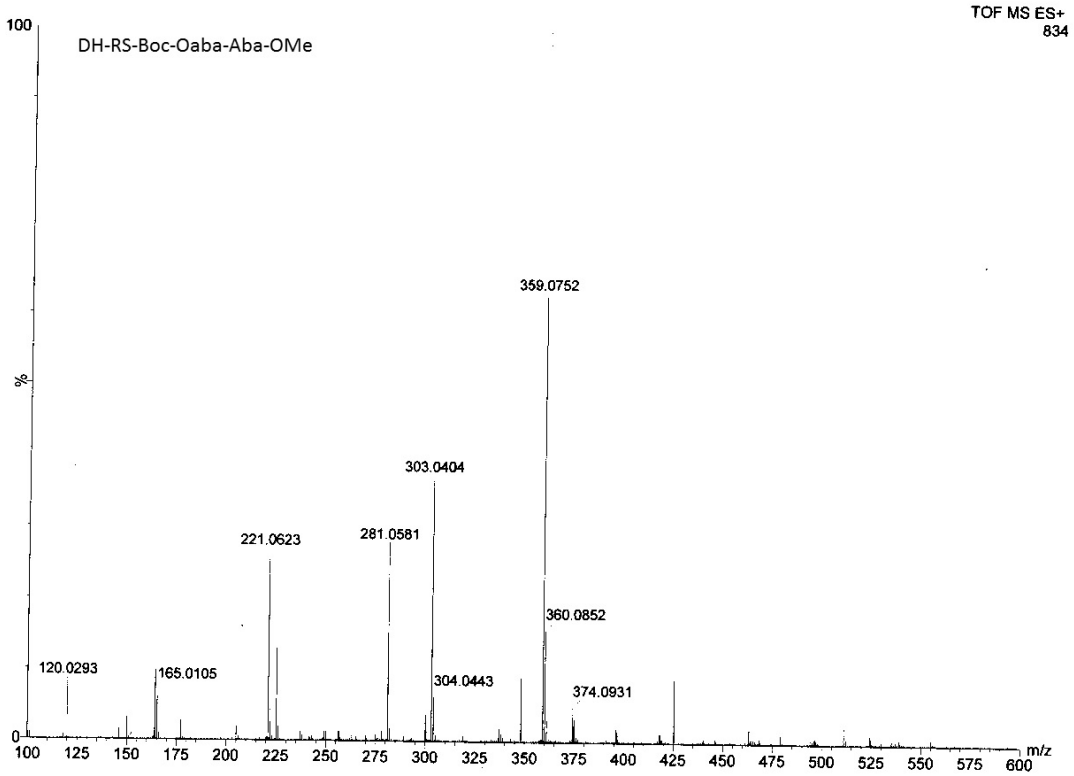


Figure S8: Mass spectra of dipeptide **3.**

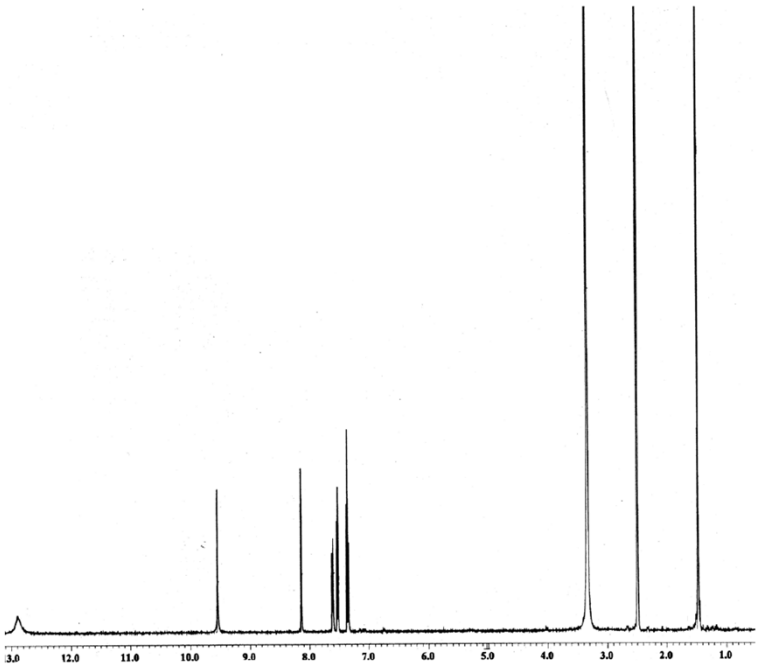


Figure S9: ^{1}H NMR Spectra ($CDCl_3$, 400 MHz, δ in ppm, 298 K) of Boc-Maba-OH :

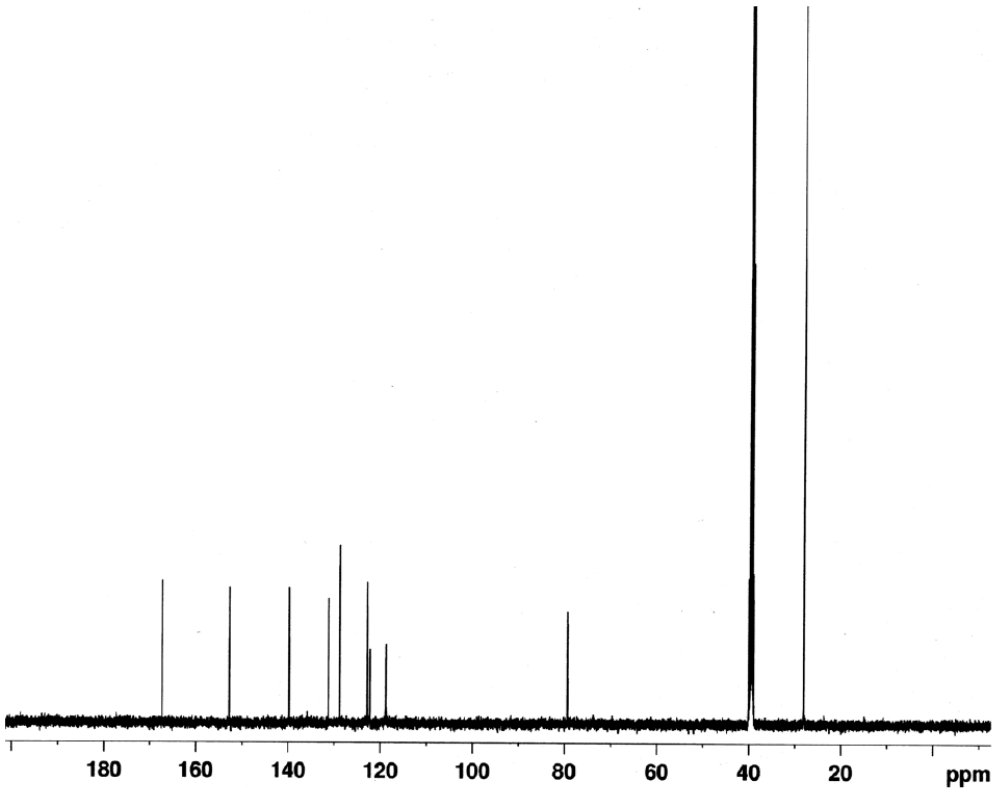


Figure S10: ^{13}**C** NMR Spectra ($CDCl_3$, 100 MHz,δ in ppm, 298 K) of Boc-Maba-OH.

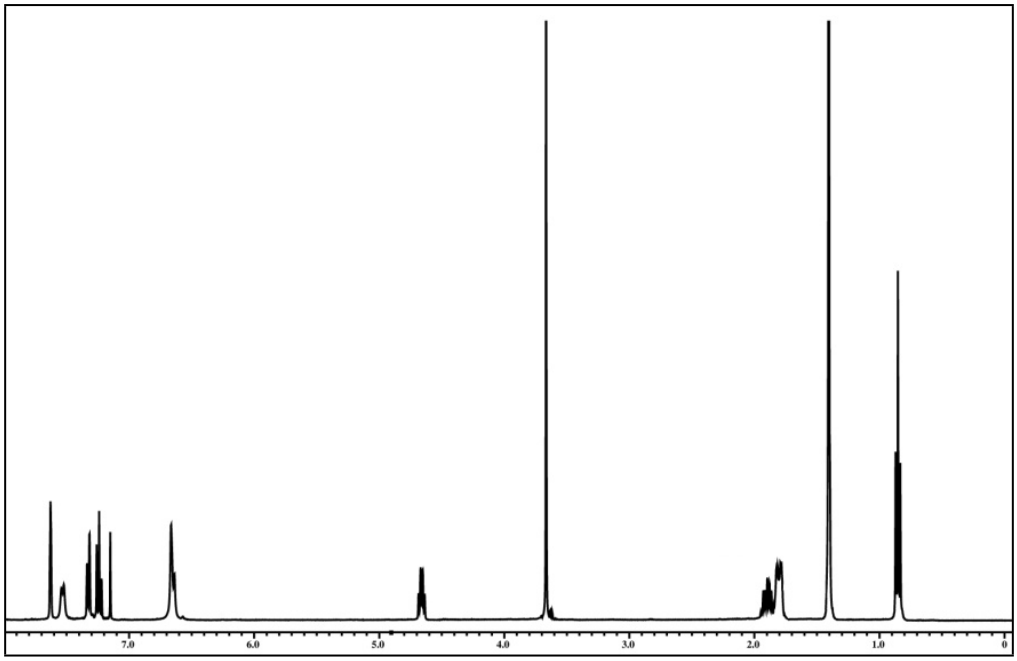


Figure S11: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **1.**

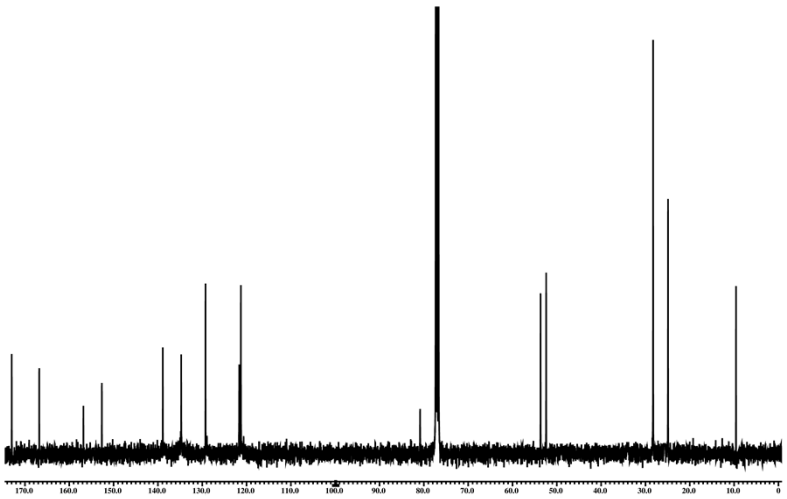


Figure S12: ^{13}C NMR Spectra ($CDCl_3$, 125 MHz, δ in ppm, 298 K) of peptide **1.**

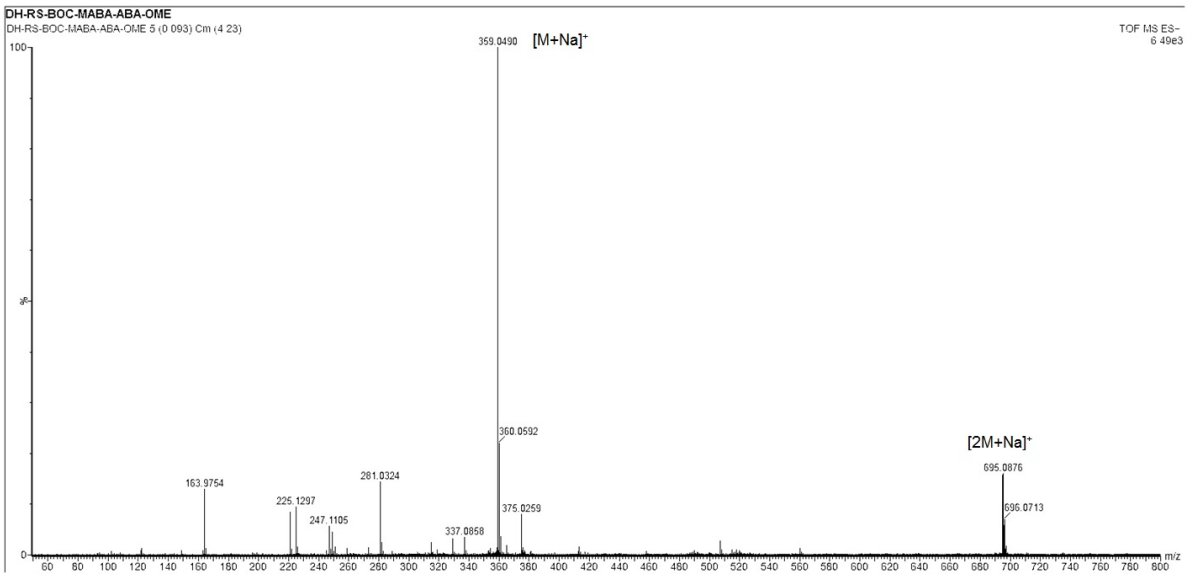


Figure S13: Mass spectra of dipeptide **1.**

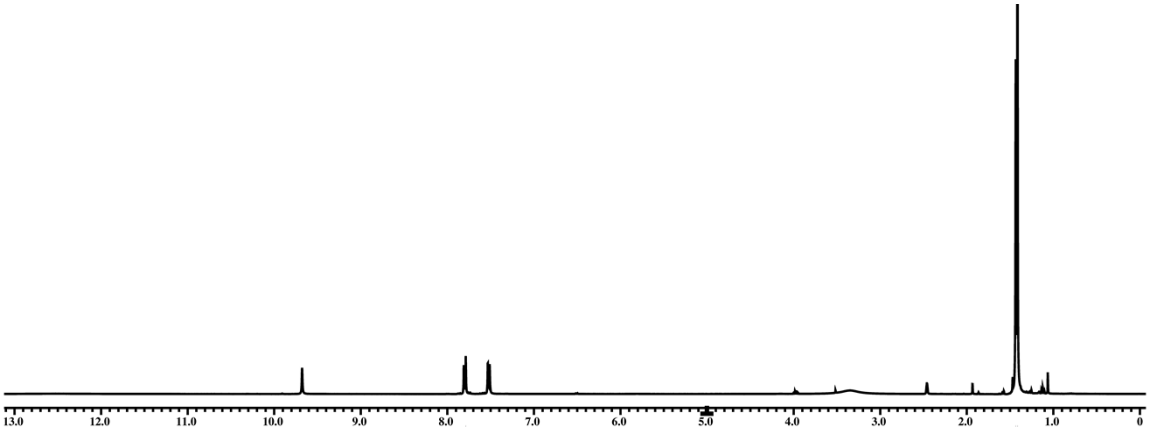


Figure S14: ^{1}H NMR Spectra (DMSO-d_6, 400 MHz, δ in ppm, 298 K) of Boc-Paba-OH:

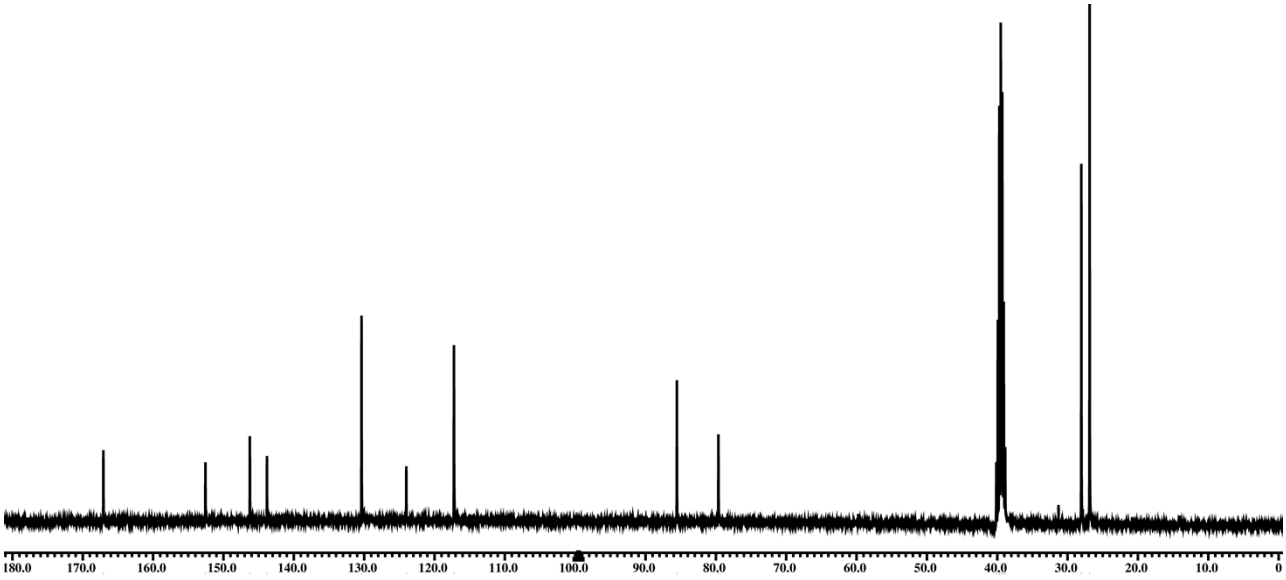


Figure S15: ^{13}C NMR Spectra (DMSO-d_6, 100 MHz, δ in ppm, 298 K) of Boc-Paba-OH.

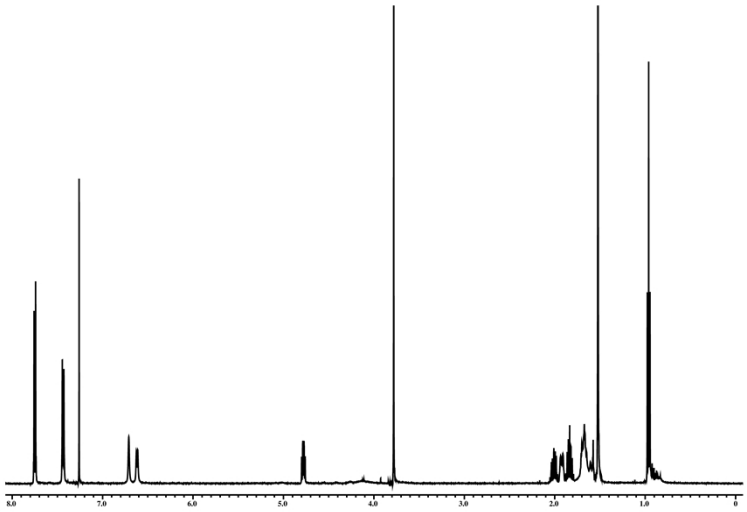


Figure S16: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **5.**

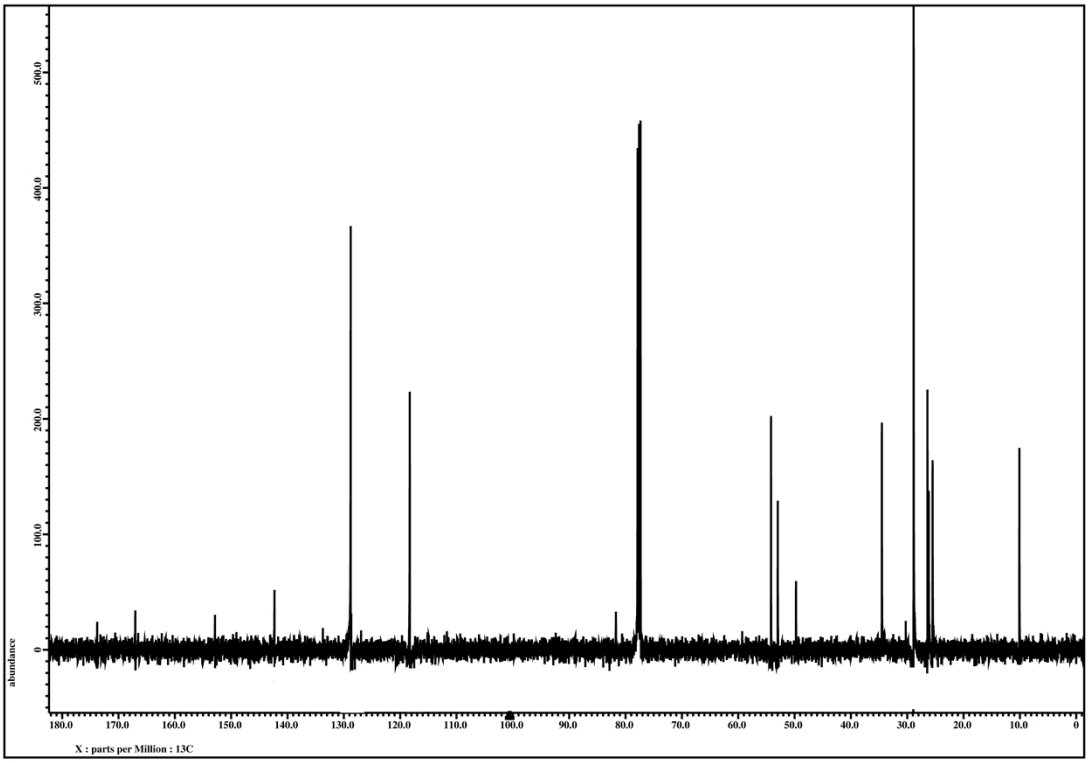


Figure S17: ^{13}**C** NMR Spectra ($CDCl_3$, 125 MHz, δ in ppm, 298 K) of peptide **5.**

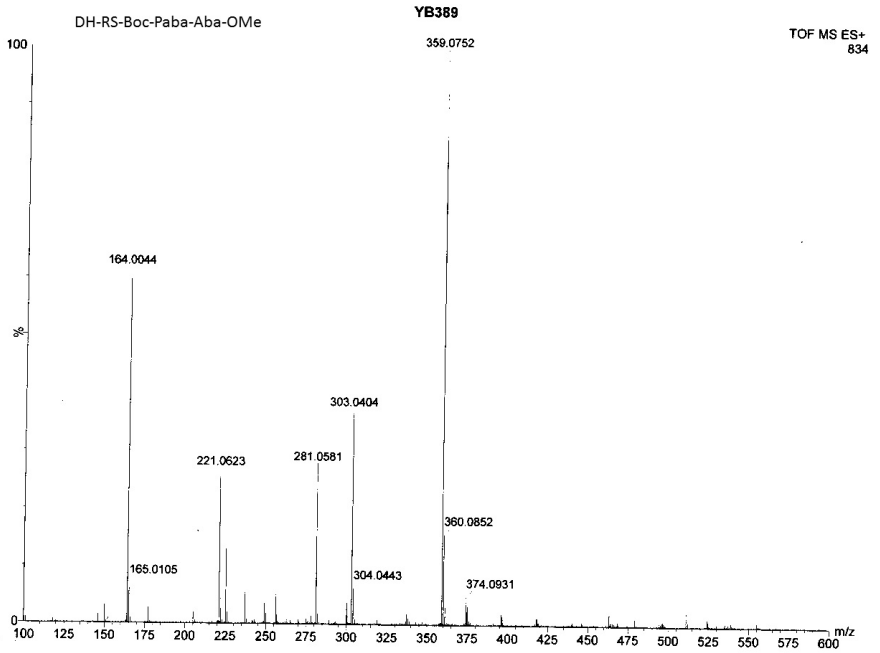


Figure S18: Mass spectra of dipeptide **5.**

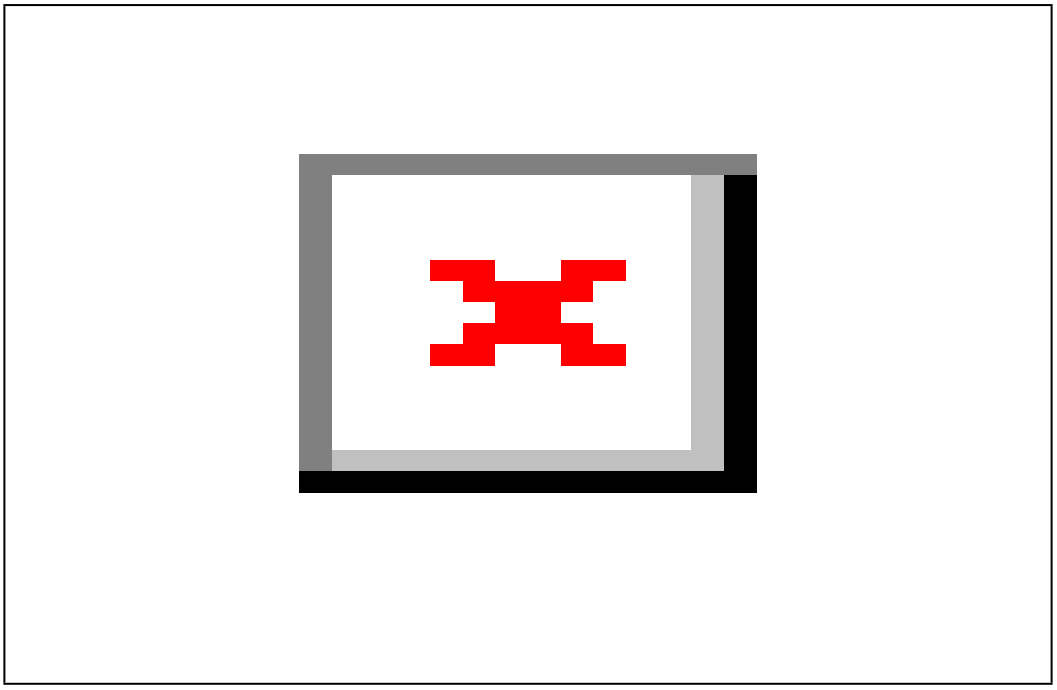


Figure S19: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **7.**

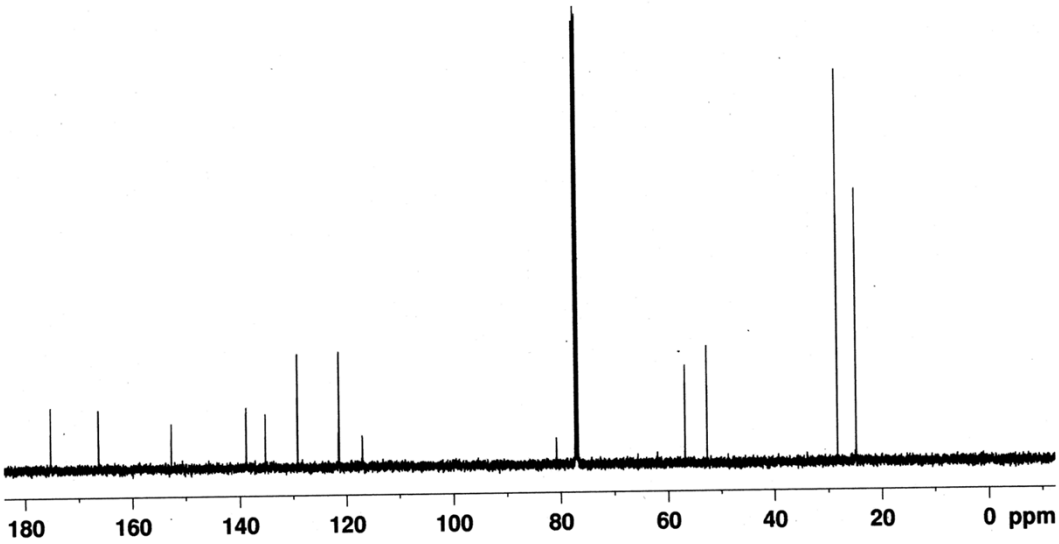


Figure S20: ^{13}**C** NMR Spectra ($CDCl_3$, 125 MHz, δ in ppm, 298 K) of peptide **7.**

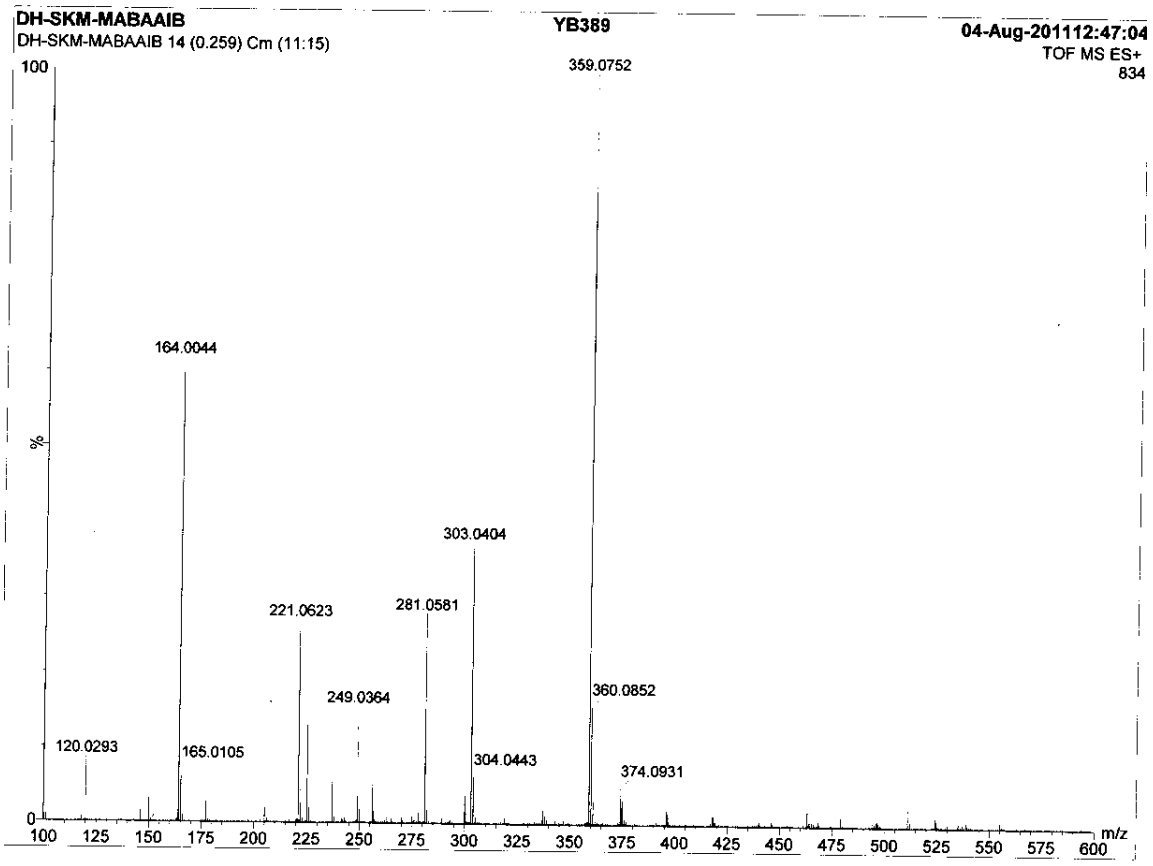


Figure S21: Mass spectra of dipeptide **7.**

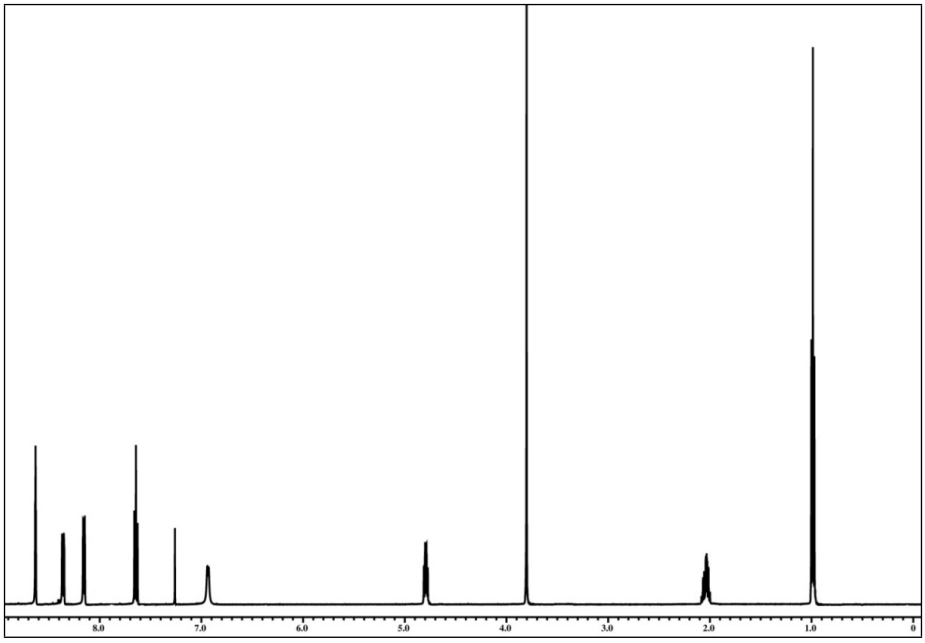


Figure S22: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **2.**

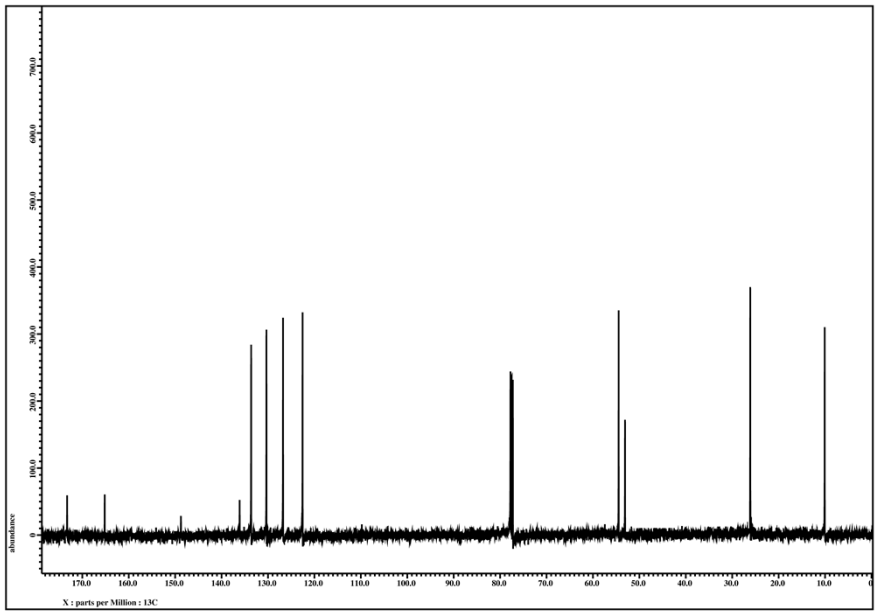


Figure S23: ^{13}C NMR Spectra ($CDCl_3$, 125 MHz, δ in ppm, 298 K) of peptide **2.**

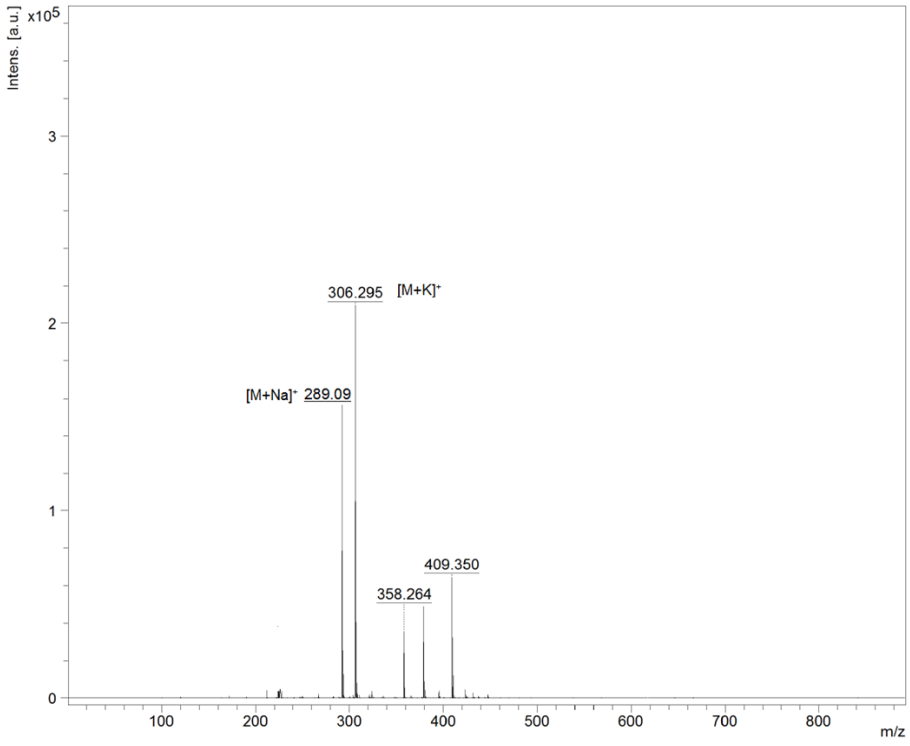


Figure S24: Mass spectra of peptide **2.**

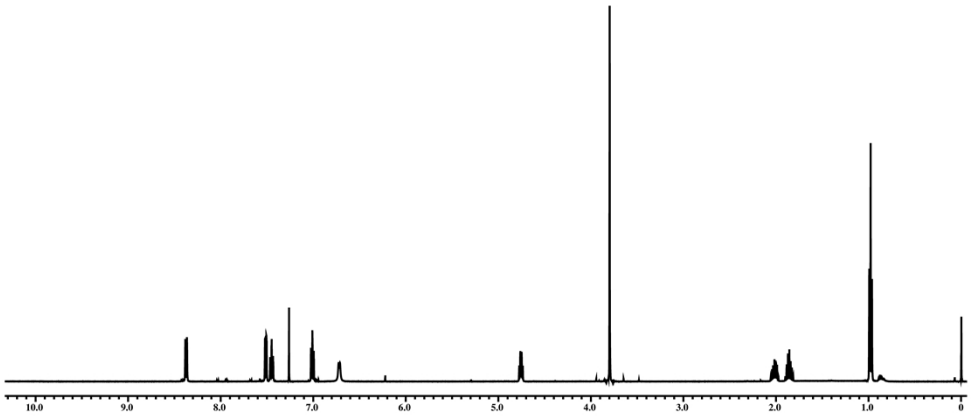


Figure S25: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **4.**

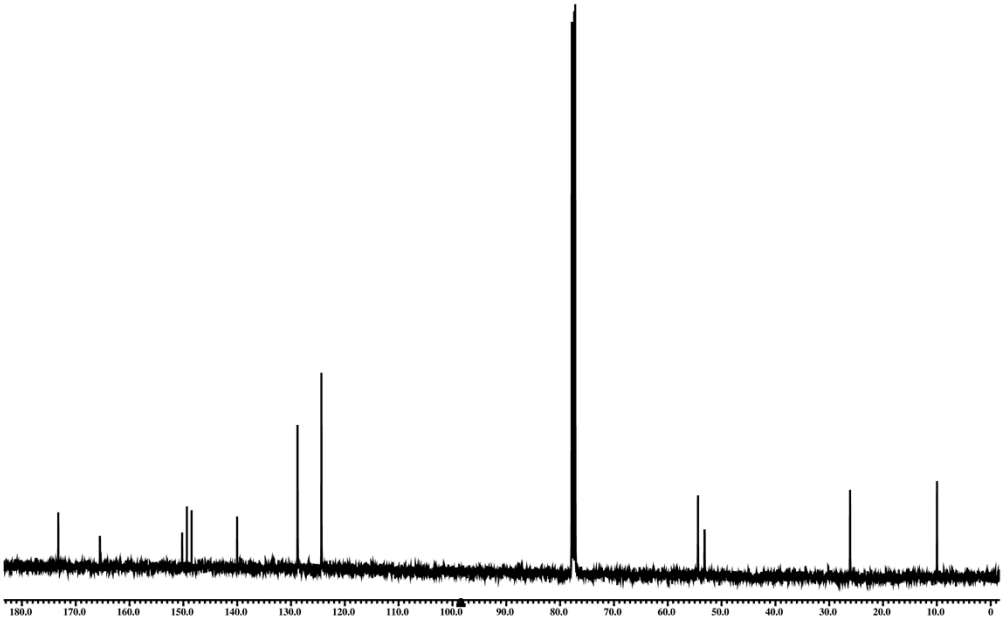


Figure S26: ^{13}**C** NMR Spectra ($CDCl_3$, 125 MHz, δ in ppm, 298 K) of peptide **4.**

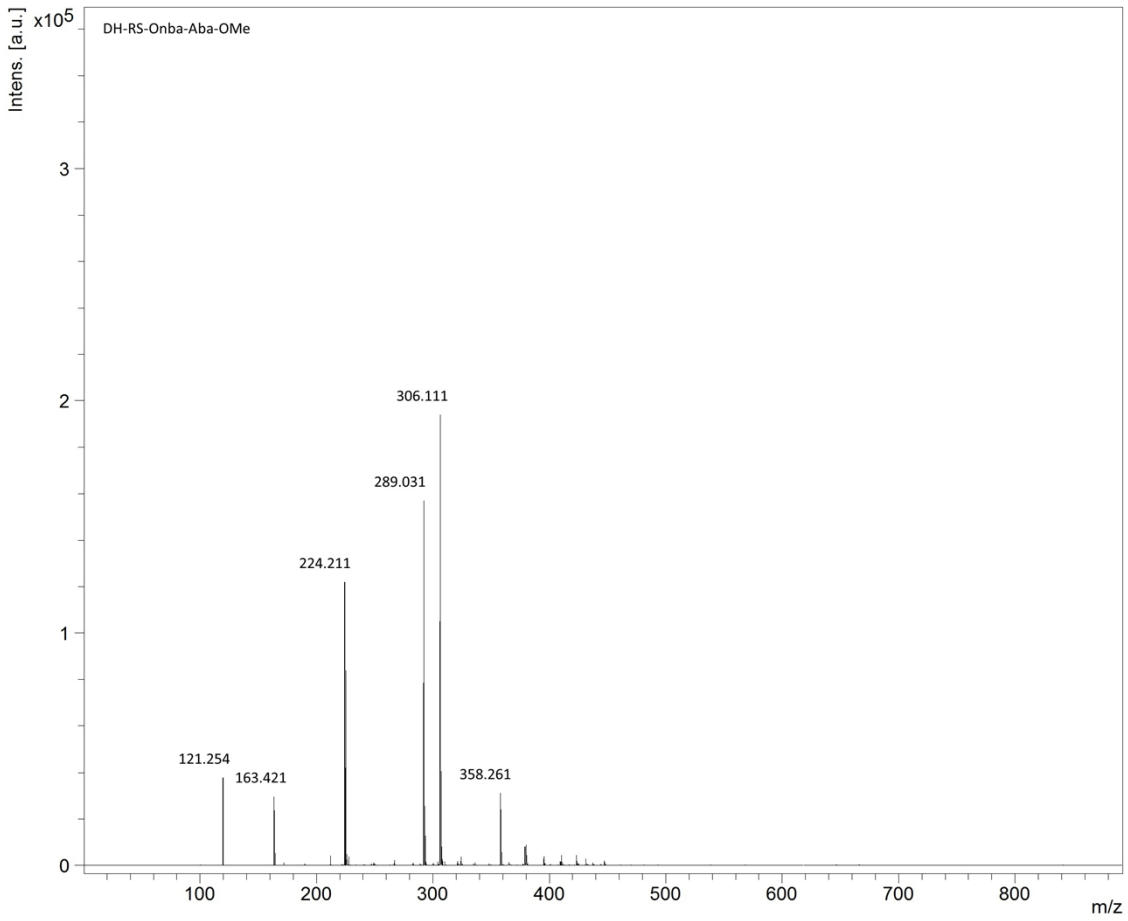


Figure S27: Mass spectra of dipeptide **4.**

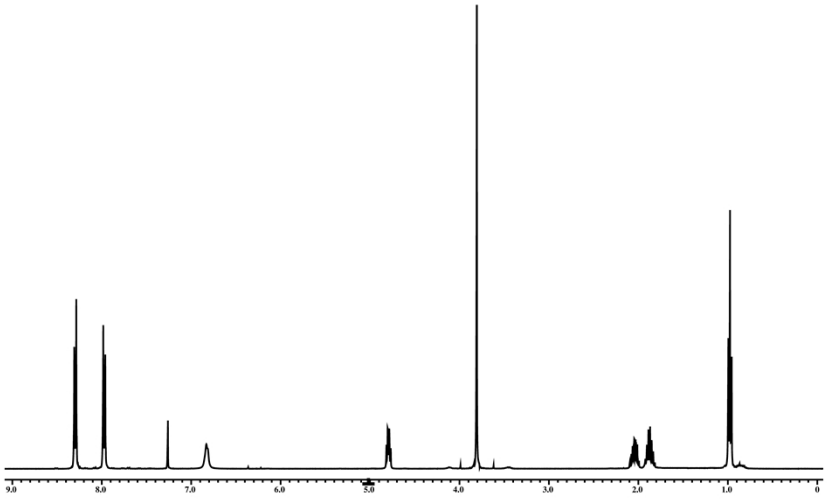


Figure S28: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **6.**

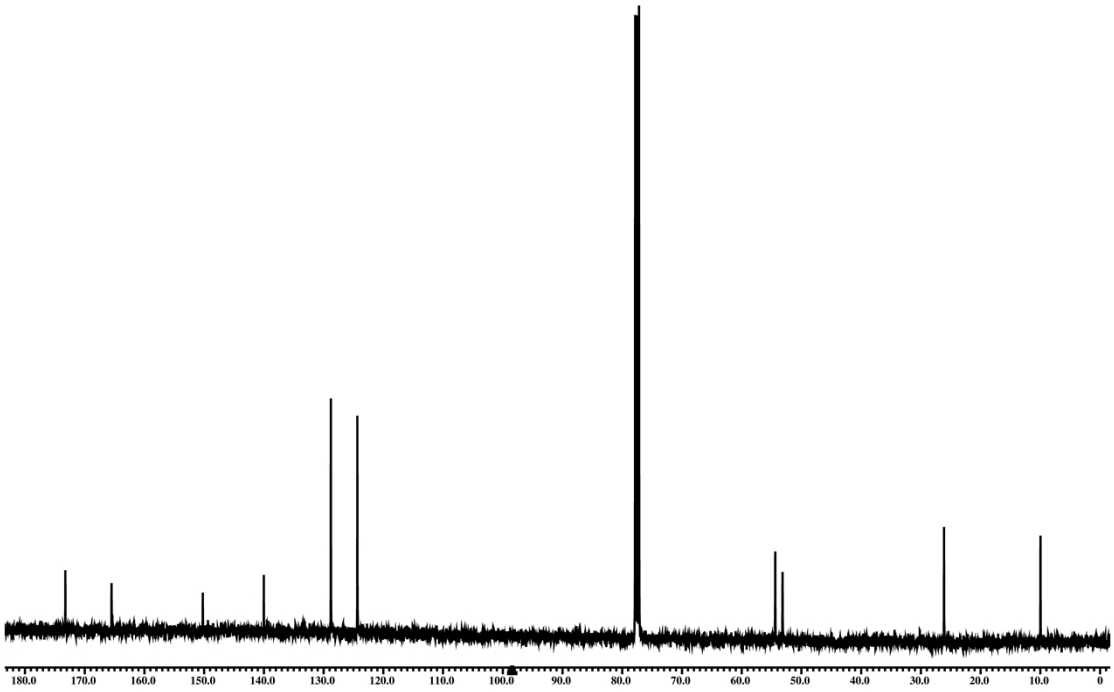


Figure S29: ^{13}**C** NMR Spectra ($CDCl_3$, 125 MHz, δ in ppm, 298 K) of peptide **6.**

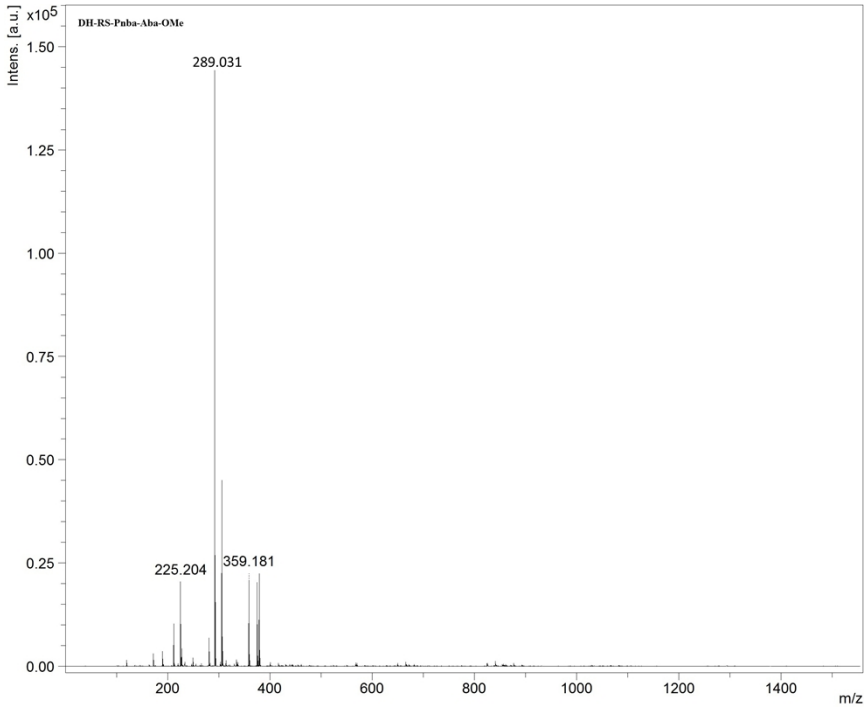


Figure S30: Mass spectra of dipeptide **6.**

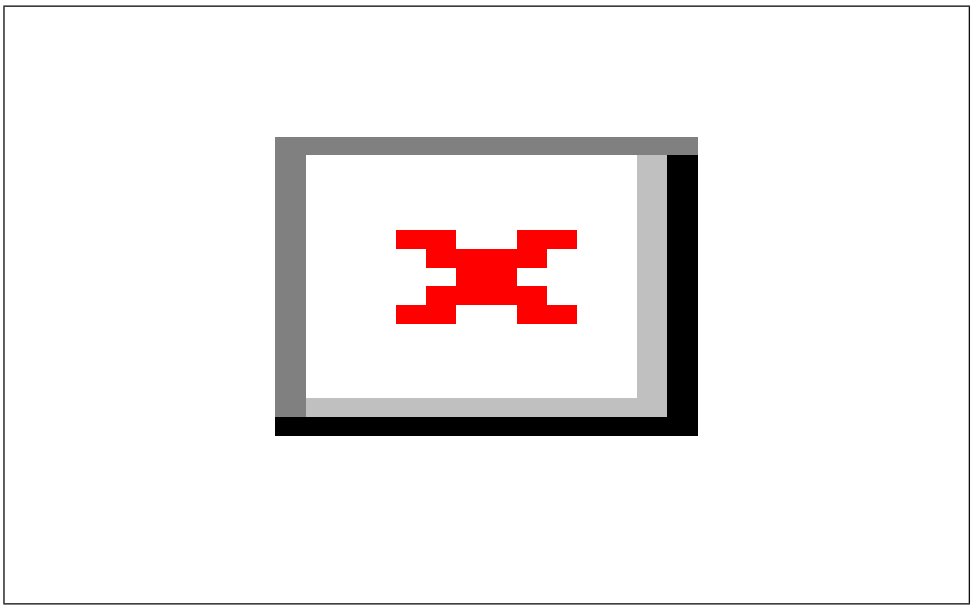


Figure S31: ^{1}H NMR Spectra ($CDCl_3$, 500 MHz, δ in ppm, 298 K) of peptide **8.**

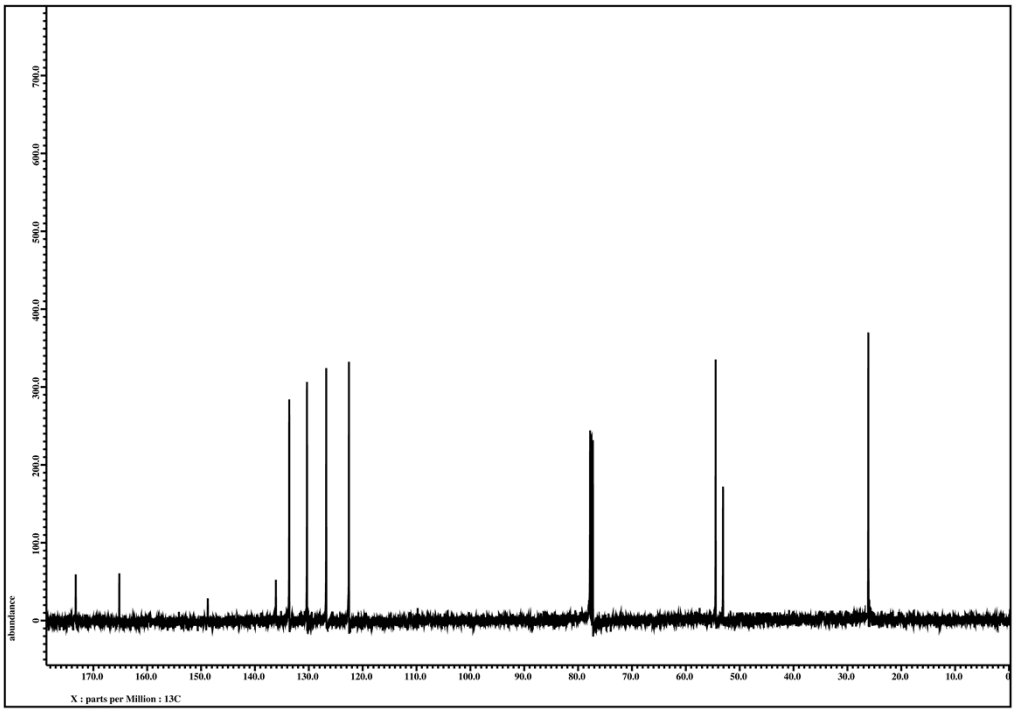


Figure S32: ^{13}**C** NMR Spectra ($CDCl_3$, 125 MHz, δ in ppm, 298 K) of peptide **8.**

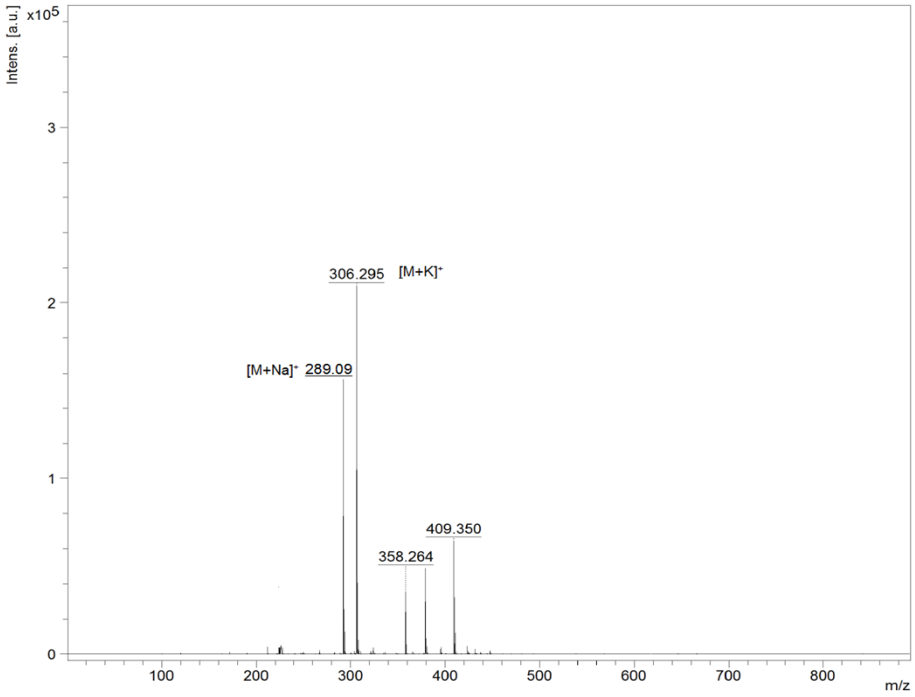


Figure S33: Mass spectra of dipeptide **8.**