ESI

Self-assembled peptide microspheres for sustainable release of sulfamethoxazole†

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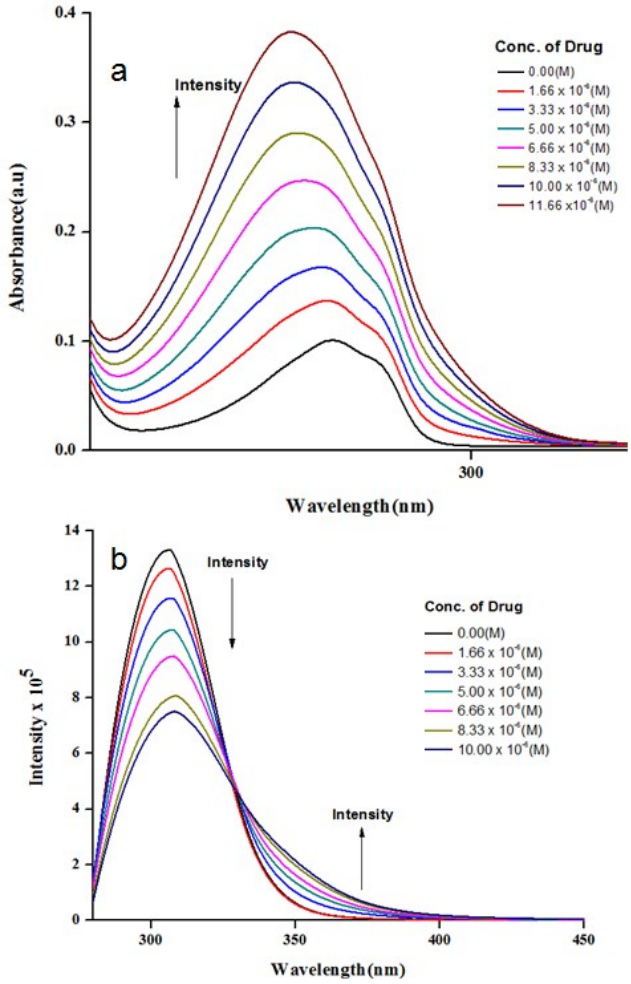
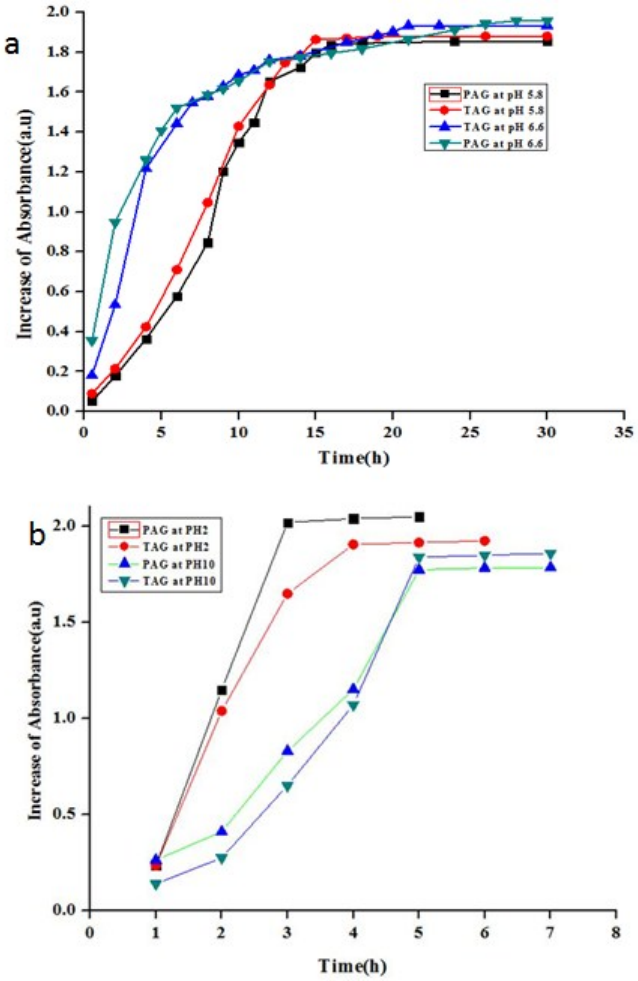
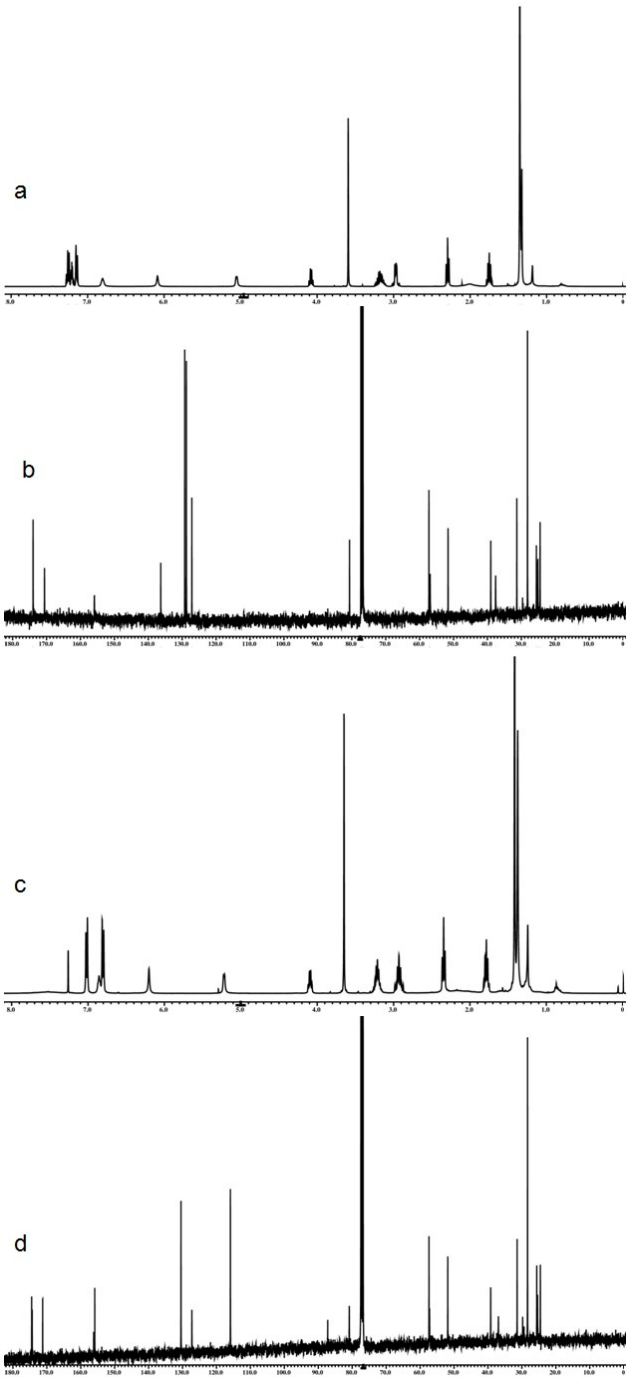


ESI Fig. S1: (a) UV-vis and (b) fluorescence spectra of peptide **2** (1.00 x 10^{-5}M) with increasing concentration of sulfamethoxazole ($\lambda_{excitation}$ = 275 nm).

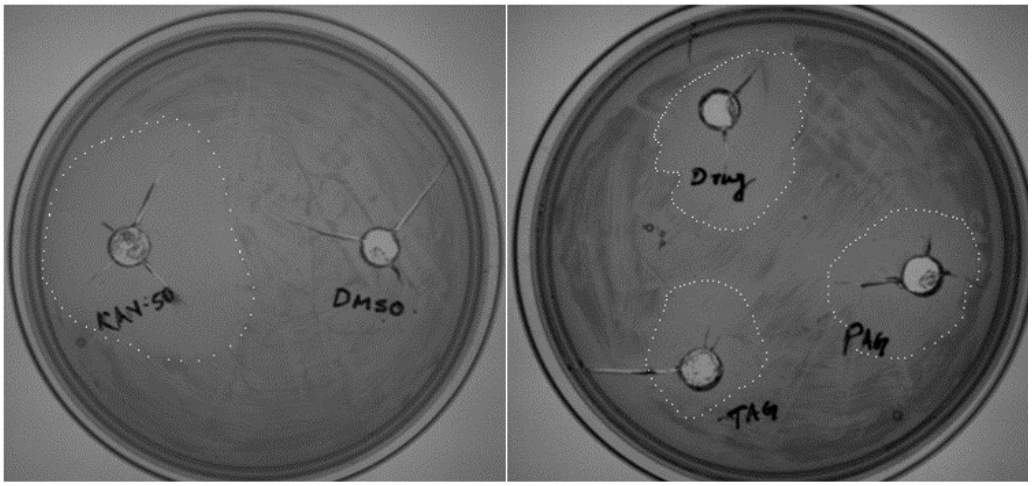


ESI Fig. S2: (a) Drug release profile of sulfamethoxazole loaded peptide **1** (PAG) and **2** (TAG) microspheres obtained from UV-Vis spectroscopy at pH 2 and pH 5.8 and pH 6.6. (b) Drug release profile of sulfamethoxazole loaded peptide **1** (PAG) and **2** (TAG) microspheres obtained from UV-Vis spectroscopy at pH 2 and pH 10.



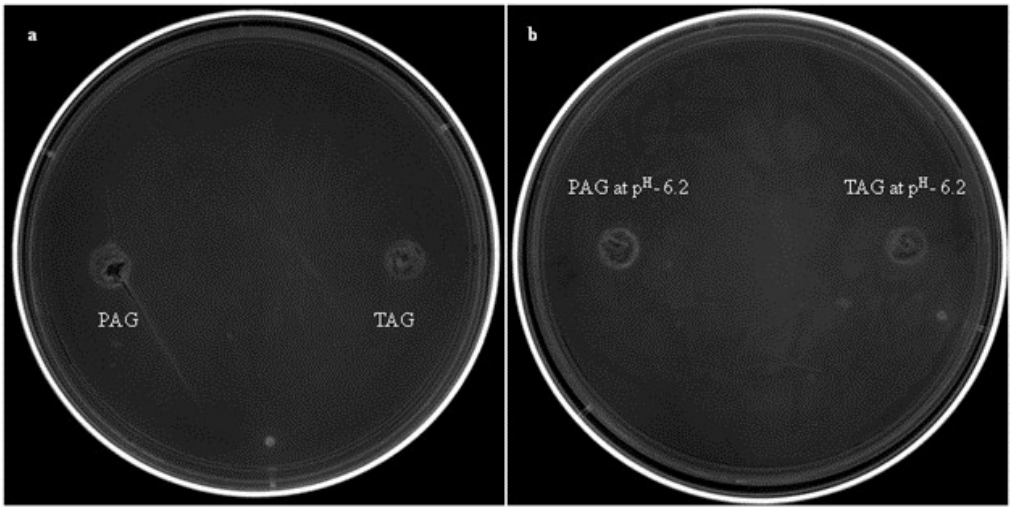
ESI Fig. S3: (a) ^{1}H NMR(400 MHZ, $CDCl_3$) spectra of peptide **1** at pH 6.2, (b) ^{13}C NMR(125 MHZ, $CDCl_3$) spectra of peptide **1** at pH 6.2, (c) ^{1}H NMR(400 MHZ, $CDCl_3$) spectra of peptide

2 at pH 6.2 and (d) ^{13}C NMR(125 MHZ, $CDCl_3$) spectra of peptide **2** at pH 6.2 showing no hydrolysis of Boc or methyl ester groups at pH 6.2.

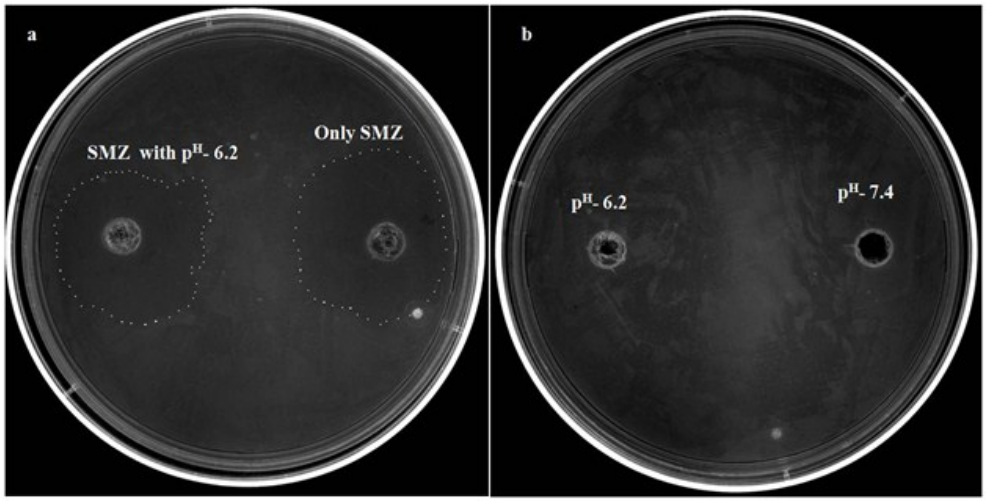


KAN-50(cm)	SMZ(cm)	PAG+SMZ(cm)	TAG+SMZ(cm)
r= 3.6	r= 3.1	r=2.7	r=2.5

ESI Fig. S4: The growth inhibition zone of E. Coli bacteria against the sulfamethoxazole (drug) and encapsulated peptide **1**-sulfamethoxazole formulation (PAG) and peptide **2**-sulfamethoxazole formulation (TAG) in Water-DMSO (10 %). Water-DMSO (10 %) exhibits no effect.



ESI Fig. S5: Antibacterial property of (a) only peptide **1** (PAG) and only peptide **2** (TAG) in water-DMSO (10%), (b) peptide **1** (PAG) and only peptide **2** (TAG) in buffer at pH 6.2 showing no bacteriostatic activity.



ESI Fig. S6: Antibacterial property of (a) only sulfamethoxazole and protonated sulfamethoxazole at pH 6.2 showing no change of bacteriostatic activity on protonation, (b) the buffer at pH 6.2 and 7.4 showing no bacteriostatic activity.

Experimental

General

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

Synthesis of peptide 1:

(a) Boc–Phe(1)–OH : A solution of L-phenylalanine (3.30 g, 20 mmol) in a mixture of dioxane (40 mL), water (20 mL) and 1(M) NaOH (20 mL) was stirred and cooled in an ice-water bath. Di-tert-butylpyrocarbonate (4.8 g, 22 mmol) was added and stirring was continued at room temperature for 6 h. 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 under vacuum. The pure material was obtained as a waxy solid. Yield 4.87 g, (18.35 mmol, 91.78%).

^{1}H NMR (DMSO-d_6, 500 MHz, δ in ppm); 12.75 (br, 1H, COOH); 7.28–7.09 (m, 5H, aromatic ring protons); 7.11–7.09 (s, 1H, Phe NH); 4.09–4.01 (m, 1H, C$^{\alpha}$H Phe); 3.02–2.87 (m, 2H, C$^{\beta}$H Phe), 1.36 (s, 9H, Boc). ^{13}C NMR (DMSO-d_6, 125 MHz, δ in ppm): 173.57, 155.41, 138.00, 129.05, 128.09, 126.27, 80.24, 55.10, 36.39, 20.73.

(b) Boc-Phe(1)-Aib(2)-OMe (3). 4.2 g (16 mmol) of Boc-Phe-OH was dissolved in 25 mL DCM in an ice-water bath. H-Aib-OMe was isolated from 4.91 g (32 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed immediately by 3.3 g (16 mmol) dicyclohexylcarbodiimide (DCC) and 2.2 g (16 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 2M HCl (3x50 mL), brine (2x50 mL), 1M sodium carbonate (3x50 mL) and brine (2x50 mL) and dried over

anhydrous sodium sulfate. It was evaporated in a vacuum to yield Boc-Phe-Aib-OMe as a white solid. Yield: 4.56 g (12 mmol, 75%).

^{1}H NMR (500MHz, $CDCl_3$,δ in ppm): 7.26–7.27 (m, 2 H, phenyl ring protons), 7.21–7.22 (m, 2 H, phenyl ring protons),7.22-7.20(m, 1H,phenyl ring proton) 6.48 (s, 1 H, Aib NH), 5.22-5.19(s, 1 H, Phe NH), 4.22 (m, 1 H, Phe C^{α} H), 3.70 (s, 3 H,–OCH_3), 2.95–2.90 (m, 2 H, Phe C^{β} H), 1.44 (s, 6 H, Aib C^{α} H),1.41 (s, 9 H, BOC CH_3). ^{13}C NMR(125MHz, $CDCl_3$, δ in ppm):174.17,170.44,156.31,136.86,129.54,128.26,126.95,80.2,56.43,56.4,52.65,38.54,28.32,24.76

(c) Boc-Phe(1)-Aib(2)-OH (4). To 4.37 g (12 mmol) of Boc-Phe-Aib-OMe, 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. After10 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 extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtained compound as a waxy solid. Yield: 3.8 g (10.38 mmol, 89.6%).

^{1}H NMR (500 MHz,DMSO-d_6, δ in ppm): 12.75 (br, 1 H, –COOH), 8.02 (s, 1 H, Aib NH), 7.01–7.03 (m, 2 H, phenyl ring protons), 6.87 (m, 1 H, Phenyl ring proton) 6.66-6.63 (s, 1 H, Phe NH), 6.61–6.63(m, 2 H, phenyl ring protons), 4.01-3.98 (m, 1 H, Phe C^{α} H), 2.78–2.81 (m, 2H, Phe C^{β} H), 1.34 (s, 3 H, Aib C^{β} H), 1.32 (s,3 H, Aib C^{β} H), 1.30 (s, 9H, BOC CH_3). ^{13}C NMR (125MHz, $CDCl_3$, δ in ppm):174.20,170.42,156.29,136.86,129.54,128.26,126.95,80.2,56.43, 52.67,38.54,28.32,24.72.

(d) Boc-Phe(1)-Aib(2)-Gaba(3)-OMe (5). 3.53 g (10.1 mmol) Boc-Phe-Aib–OH was dissolved in 10 mL of DMF in an ice–water bath. H-Gaba-OMe 3.07 g (20 mmol) was isolated from the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 7 mL. Then it was added to the reaction mixture, followed immediately by 2.08 g (10.11 mmol) of dicyclohexylcarbodiimide (DCC) and 1.37 g (10.11 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and then stirred for 72 h. The residue was taken in 30 mL ethyl acetate and dicyclohexylurea (DCU) was filtered off.The organic layer was washed with 2(M) HCL (3x50 mL), brine (2x50

mL), then 1 M sodium carbonate (3x30 mL) and brine (2x30 mL) and dried over anhydrous sodium sulfate and evaporated under vacuum to yield the tripeptide **1** as a white solid. Purification was done by silica gel column (100–200 mesh size) with an ethyl acetate and hexane mixture 1 : 2 as the eluent. Yield: 4.4g (9.17 mmol, 90%).

^{1}H NMR(500 MHz, $CDCl_3$, δ in ppm): 7.18–7.27 (m, 2 H, phenyl ring protons), 7.17-7.27 (m, 2 H, phenyl ring protons), 6.87-7.17 (m, 1 H, phenyl ring protons), 6.87 (s,1 H, Aib NH), 6.27-6.25 (s, 1 H, Gabu NH), 5.26 (s, 1 H,Phe NH), 4.14-4.11 (m, 1 H, Phe C^{α} H), 3.62 (s, 3 H,-OCH_3),3.22-3.19 (m, 2 H, Phe C^{β}H) 3.00-3.02 (m, 2 H, Gabu C^{γ} H), 2.32-2.33 (m, 2 H, Gabu C^{β} H), 1.79-2.20 (m, 1 H, Gabu C^{β} H), 1.38 (s, 9 H, BOC CH_3), 1.35 (s, 6 H, Aib C^{β} H), HRMS m/z 449.54 $[M + Na]^+$, Mcalcd 472.08. ^{13}CNMR (125 MHz, $CDCl_3$, d in ppm): 174.38, 171.01,156.24, 136.71, 129.56, 129.02, 127.39, 77.61, 57.53, 57.16, 51.85, 39.31 , 37.91,31.61,28.49,25.88,25.34,24.79.

Synthesis of peptide 2:

(e) Boc-Tyr-OH. A solution of L-tyrosine (3.62 g, 20 mmol) in a mixture of dioxane (40 mL), water (20 mL) and 1M NaOH(20 mL) was stirred and cooled in an ice–water bath. Di-tertbutylpyrocarbonate (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 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 (congored). 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 under vacuum. The pure material was obtained as a waxy solid. Yield 4.87 g (17 mmol, 85%).

^{1}H NMR (500MHz, $CDCl_3$, δ in ppm): 12.75 (br, 1 H, –COOH), 9.21 (s,1 H, Tyr –OH), 7.02–7.00 (m, 2 H, Tyr phenyl ring protons), 6.65–6.63 (m, 2 H, Tyr phenyl ring protons),4.04-4.01 (m, 1H, Tyr C^{α}H), 3.91-3.89 (s, 1H, Tyr NH), 2.88-2.87 (m,2 H, Tyr C^{β} H), 1.42 (s, 9 H, BOC CH3). ^{13}C NMR (125 MHz, $CDCl_3$, δin ppm): 173.74, 155.78, 129.96, 127.98, 114.87, 55.50, 35.61, 28.13.

(f) Boc-Tyr(1)-Aib(2)-OMe. 4.9 g (16 mmol) of Boc-Tyr-OH was dissolved in 25 mL DCM in an ice-water bath. H-Aib-OMe was isolated from 4.91 g (32 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture,

followed immediately by 3.3 g (16 mmol) dicyclohexylcarbodiimide(DCC) and 2.2 g (16 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), 1M sodium carbonate (3x50 mL) and brine (2x50 mL) and dried over anhydrous sodium sulfate. It was evaporated in a vacuum to yield Boc-Tyr-Aib-OMe as a white solid. Yield 4.56 g (12 mmol, 75%).

^{1}H NMR (500MHz, $CDCl_3$, δ in ppm): 9.21 (s, 1 H, Tyr –OH), 7.03–7.02(m, 2 H, Tyr phenyl ring protons), 6.75–6.74 (m, 2 H, Tyr phenyl ring protons), 6.48 (s, 1 H, Aib NH), 5.22(s, 1 H, Tyr NH), 4.22 (m, 1 H, Tyr C^{α} H), 3.70 (s, 3 H,–OCH3), 2.95–2.90 (m, 2 H, Tyr C^{β} H), 1.44 (s, 6 H, Aib C^{β} H),1.41 (s, 9 H, BOC CH3). HRMS m/z 403.19 $[M + Na]^{+}$, Mcalcd 380.19, ^{13}C NMR (125 MHz, $CDCl_3$, δ in ppm): 174.17, 170.74, 155.66, 130.58, 127.97, 115.56, 56.45, 53.45, 37.58, 29.70, 24.74.

(g) Boc-Tyr(1)-Aib(2)-OH. To 4.4 g (12 mmol) of Boc-Tyr-Aib-OMe, 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. After10 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 using1M HCl and it was extracted with ethyl acetate (3x50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to obtained compound as a waxy solid. Yield 3.8 g (10.38 mmol, 89.6%).

^{1}H NMR (500 MHz, DMSO-d_6, δ in ppm): 12.75 (br, 1 H, –COOH), 9.14 (s, 1 H, Tyr–OH), 8.02 (s, 1 H, Aib NH), 7.01–7.03 (m, 2 H, Tyr phenyl ring protons), 6.66-6.62 (s, 1 H, Tyr NH), 6.61–6.63(m, 2 H, Tyr phenyl ring protons), 4.01-3.97 (m, 1 H, Tyr C^{α}H), 2.78–2.81 (m, 2H, Tyr C^{β} H), 1.34 (s, 3 H, Aib C^{β} H), 1.32 (s,3 H, Aib C^{β} H), 1.30 (s, 9 H, BOC CH3). ^{13}C NMR (125 MHz, DMSO-d_6, δ in ppm):175.43, 170.95, 155.64, 130.14, 128.02, 114.69, 177.91, 155.61, 28.10, 24.70.

(h) Boc-Tyr(1)-Aib(2)-Gaba(3)-OMe. 3.7 g (10.1 mmol) Boc-Tyr-Aib–OH was dissolved in 10 mL of DMF in an ice–water bath. H-Gaba-OMe 3.07 g (20 mmol) was isolated from the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl

acetate and the ethyl acetate extract was concentrated to 7 mL. Then it was added to the reaction mixture, followed immediately by 2.08 g (10.11 mmol)of dicyclohexylcarbodiimide (DCC) and 1.37 g (10.11 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and then stirred for 72 h. The residue was taken in 30 mL ethyl acetate and dicyclohexylurea (DCU) was filtered off.The organic layer was washed with 2(M) HCL (3x50 mL), brine (2x50 mL), then 1 M sodium carbonate (3x30 mL) and brine (2x30 mL) and dried over anhydrous sodium sulfate and evaporated under vacuum to yield the tripeptide **1** as a white solid. Purification was done by silica gel column (100–200 mesh size) with an ethyl acetate and hexane mixture 1: 2 as the eluent. Yield 4.4g (9.17 mmol, 90%).

^{1}H NMR(500MHz, $CDCl_3$, δ in ppm): 9.20 (s, 1 H, Tyr-OH),7.18–7.27 (m, 2 H, phenyl ring protons), 7.17-7.27 (m, 2 H, phenyl ring protons), 6.87-7.17 (m, 1 H, phenyl ring protons), 6.87 (s,1 H, Aib NH), 6.27 (s, 1 H, Gabu NH), 5.26 (s, 1 H, Tyr NH), 4.14-4.11 (m, 1 H, Tyr C^{α} H), 3.62 (s, 3 H,-OCH3),3.22 (m, 2 H, Tyr C^{β}H) 3.00-3.02 (m, 2 H, Gabu C^{γ} H), 2.32-2.33 (m, 2 H, Gaba C^{β} H), 1.79-2.2 (m, 1 H, Gaba C^{β} H), 1.38 (s, 9 H, BOC CH3), 1.35 (s, 6 H, Aib C^{β} H), HRMS m/z 465.54 $[M + Na]^{+}$, Mcalcd 488.11, ^{13}C NMR (125 MHz, $CDCl_3$,δ in ppm):174.38, 171.01, 156.24, 136.71, 129.56, 129.02, 127.39, 77.61, 57.53, 57.16, 51.85, 39.31, 37.91, 31.61, 28.49, 25.88, 25.34, 24.79.

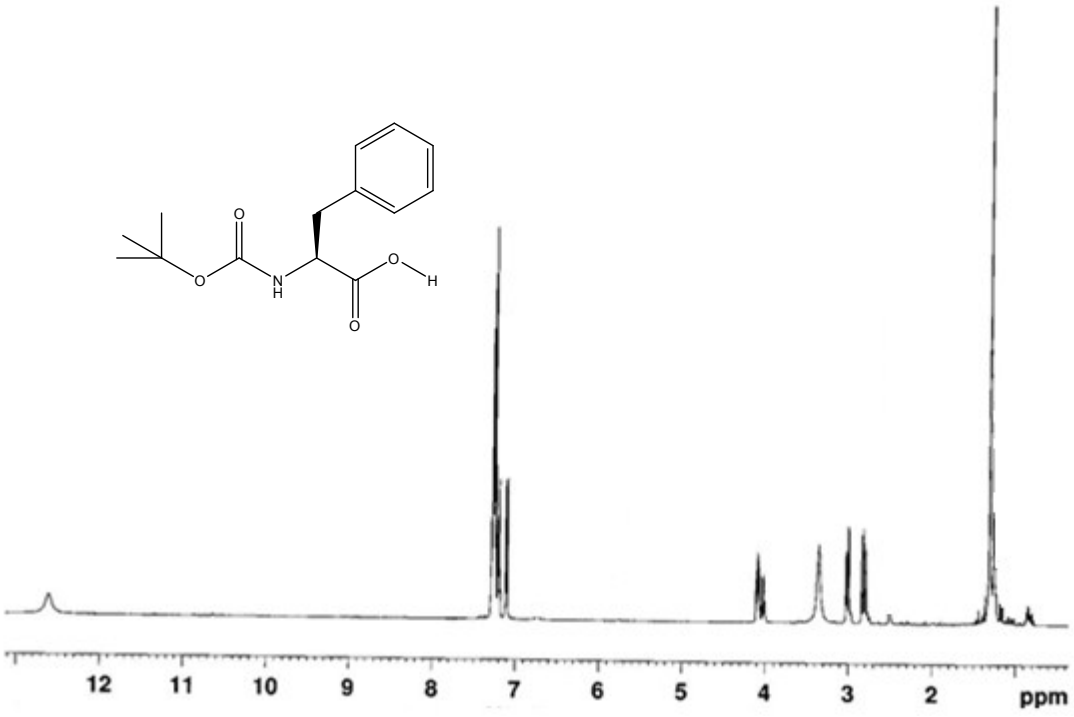


Figure S1: ^{1}H NMR (500MHz, DMSO-d_6) spectra of Boc-Phe-OH

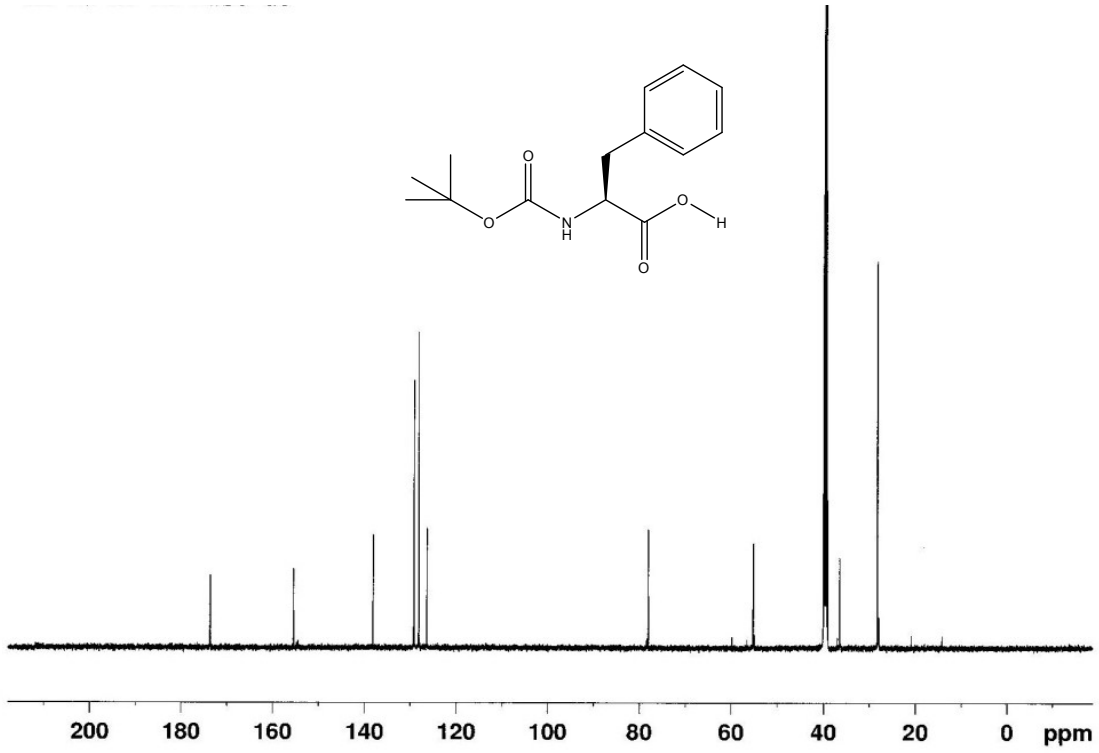


Figure S2: ^{13}C NMR (125 MHz, DMSO-d_6) spectra of Boc-Phe-OH

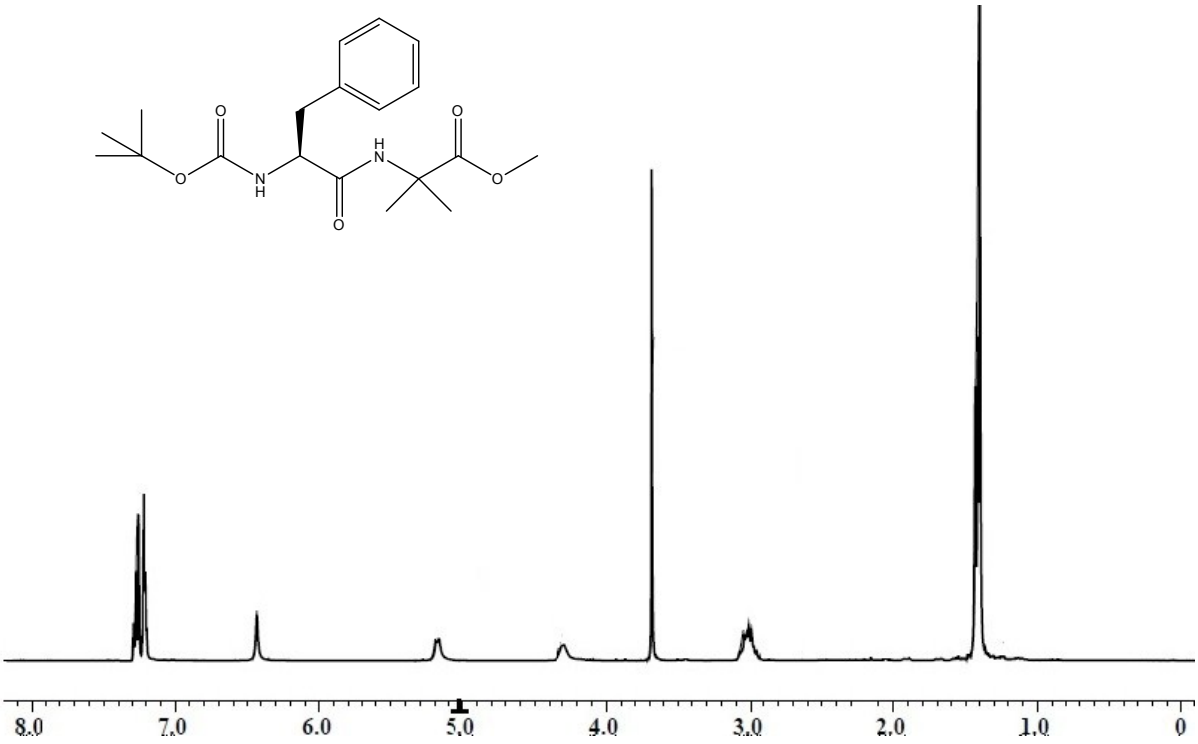


Figure S3: ^{1}H NMR (500 MHz, $CDCl_3$) spetra of Boc-Phe-Aib-OMe

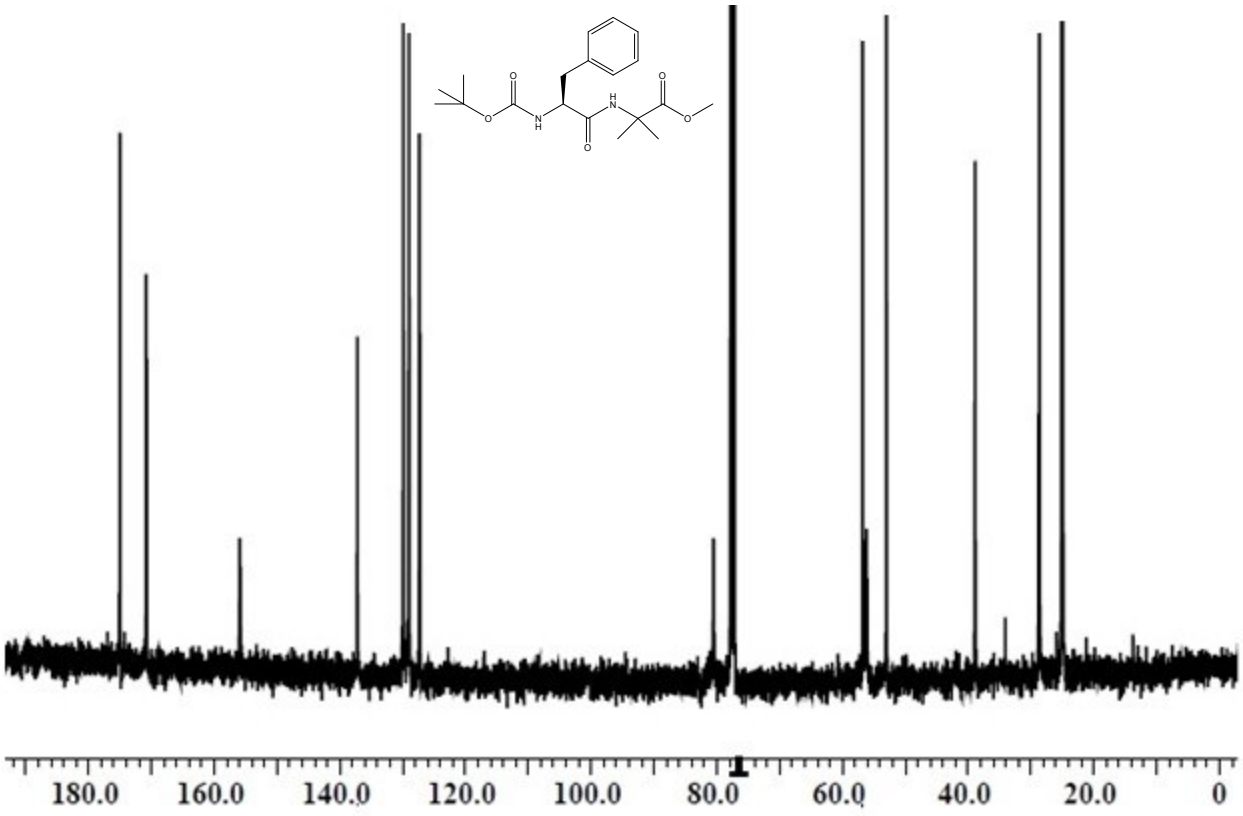


Figure S4: ^{13}C NMR (125 MHz, $CDCl_3$) spectra of Boc-Phe-Aib-OMe

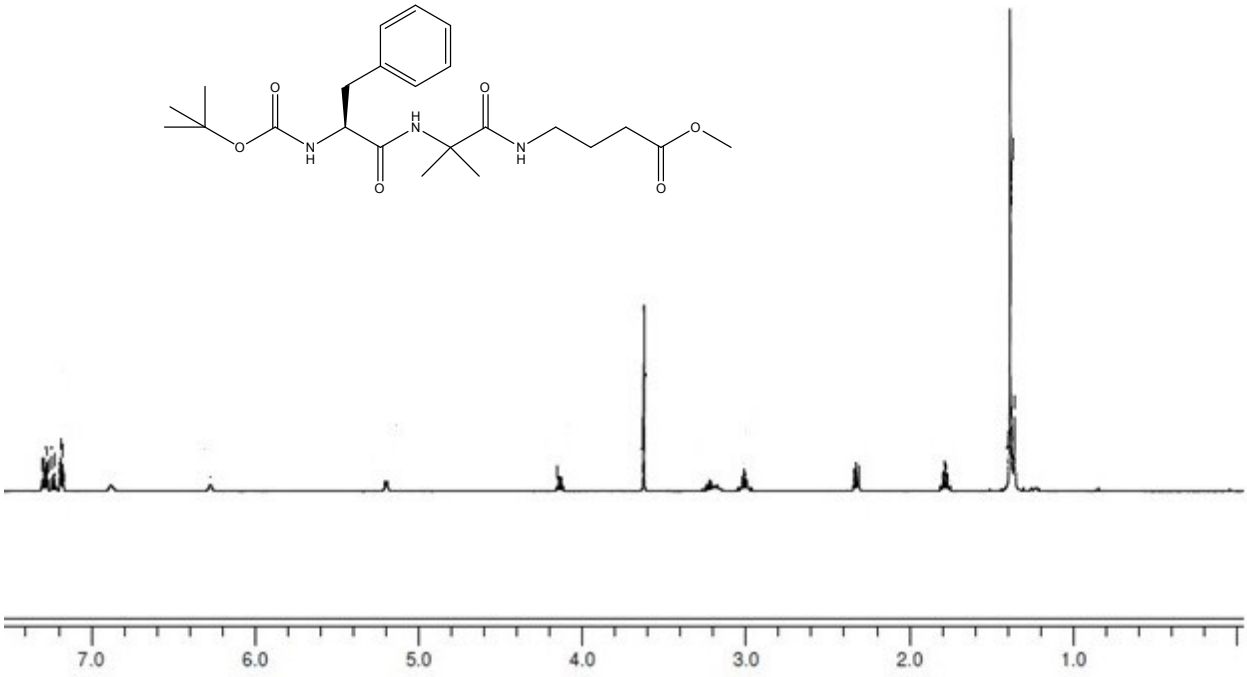


Figure S5: ^{1}H NMR (500MHz, $CDCl_3$) spectra of Boc-Phe-Aib-Gaba-OMe

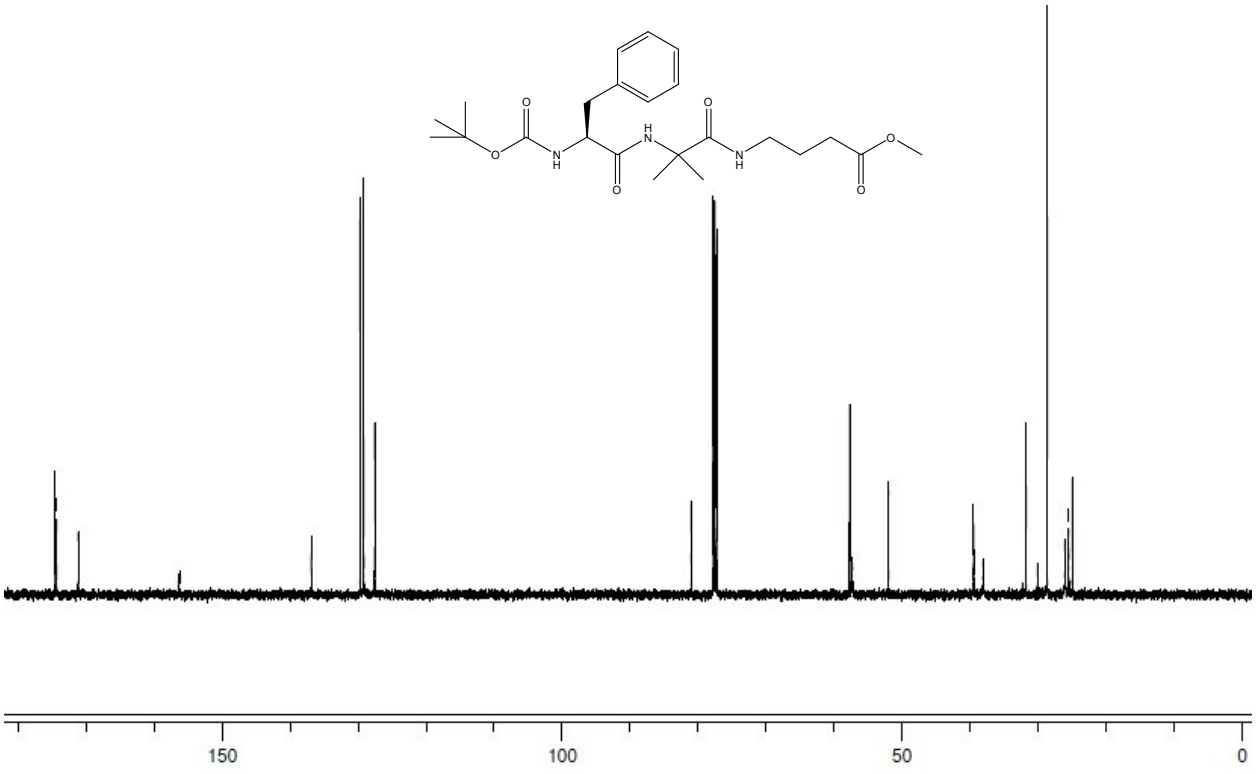


Figure S6: ^{1}H NMR (500MHz,$CDCl_3$) spectra of Boc-Phe-Aib-Gaba-OMe

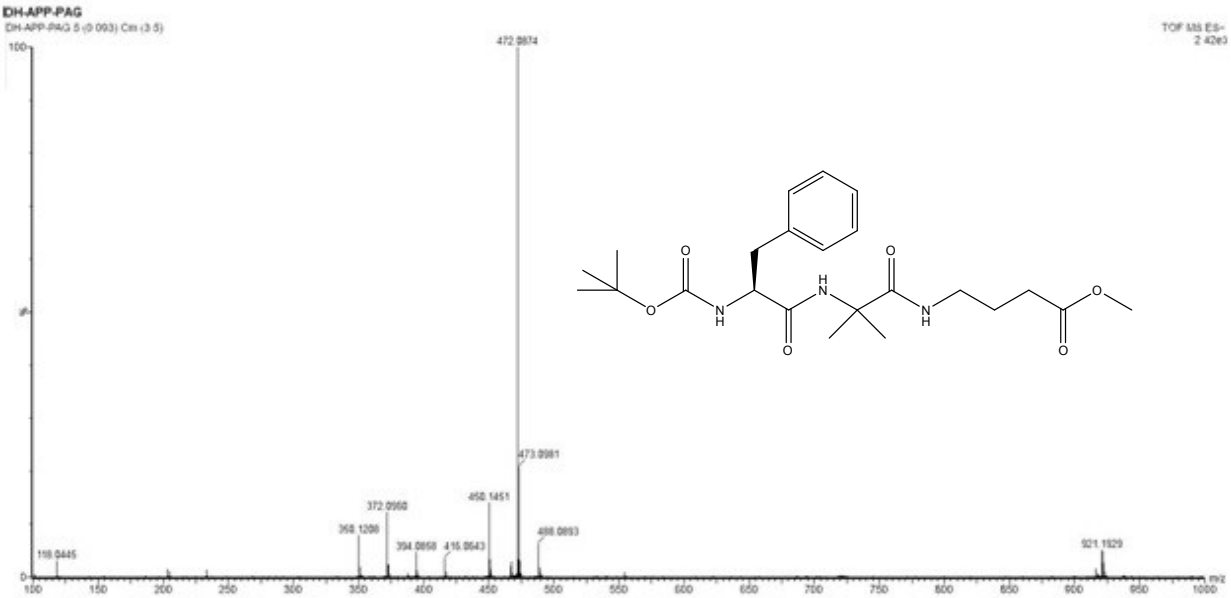


Figure S7: Mass spectra of peptide Boc-Phe-Aib-Gaba-OMe

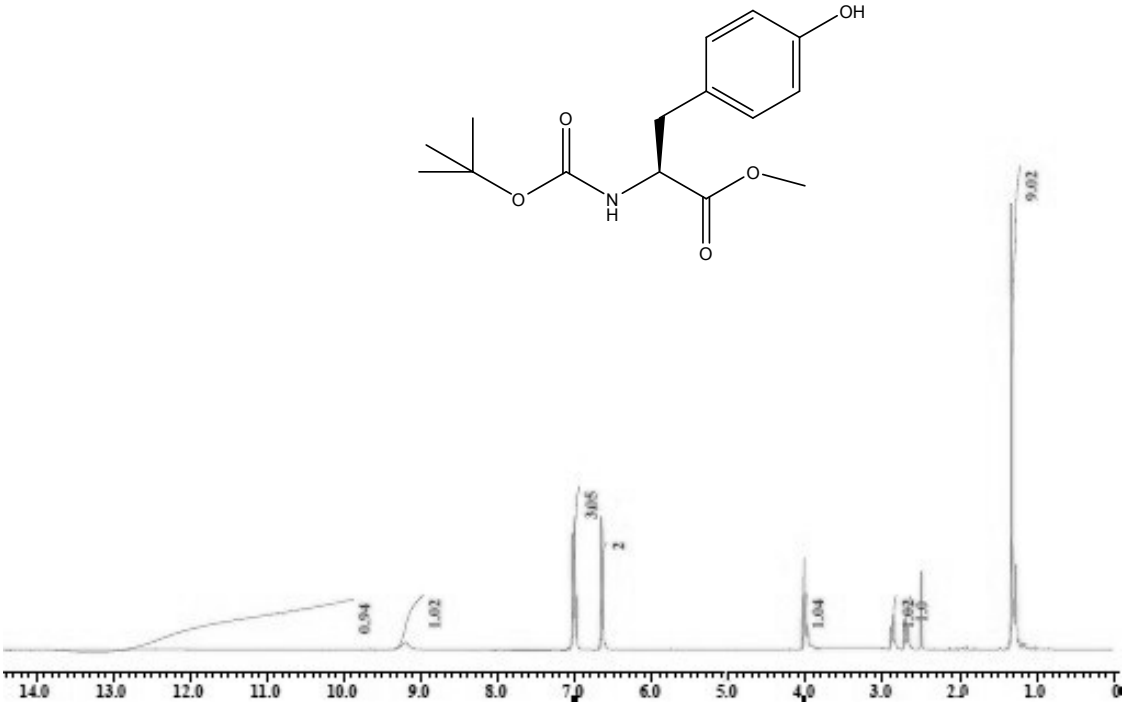


Figure S8: ^{1}H NMR (400MHz, DMSO-d_6) spectra of Boc-Tyr-OH

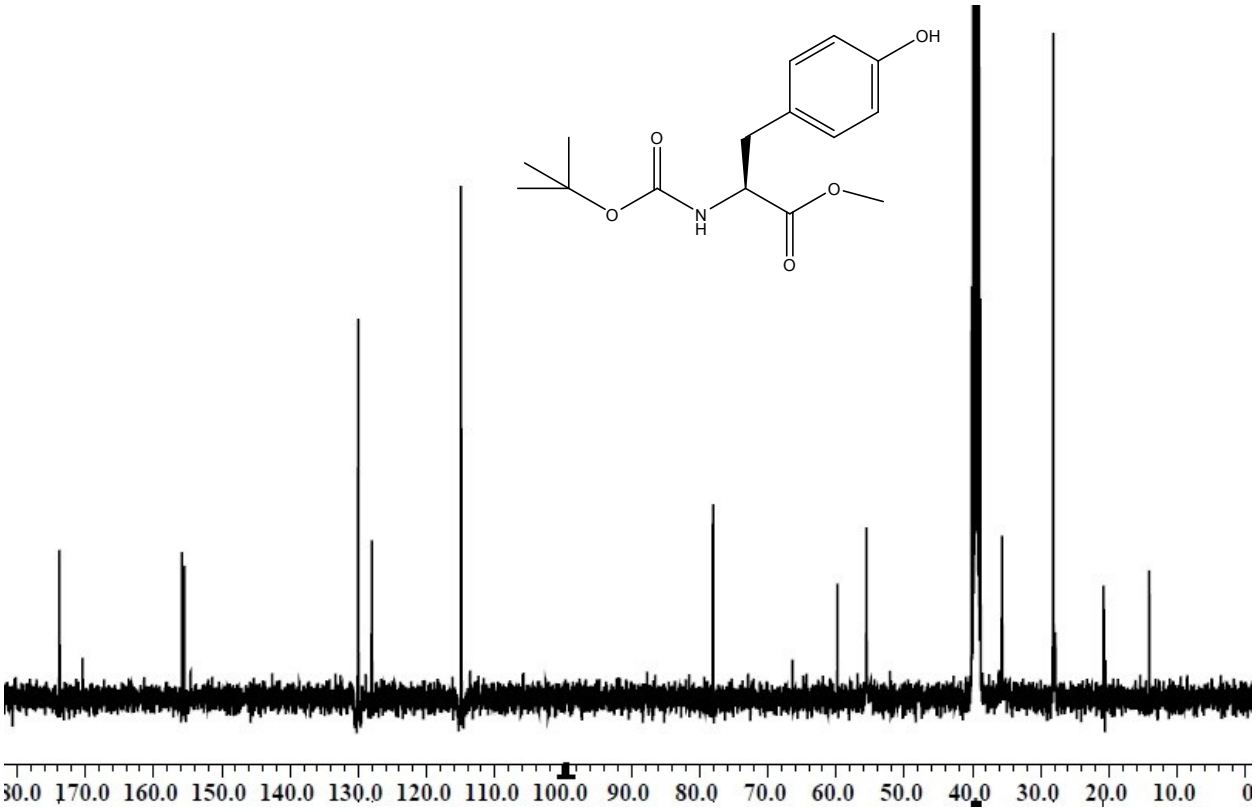


Figure S9: ^{13}C NMR (125MHz, DMSO-d_6) of Boc-Tyr-OH

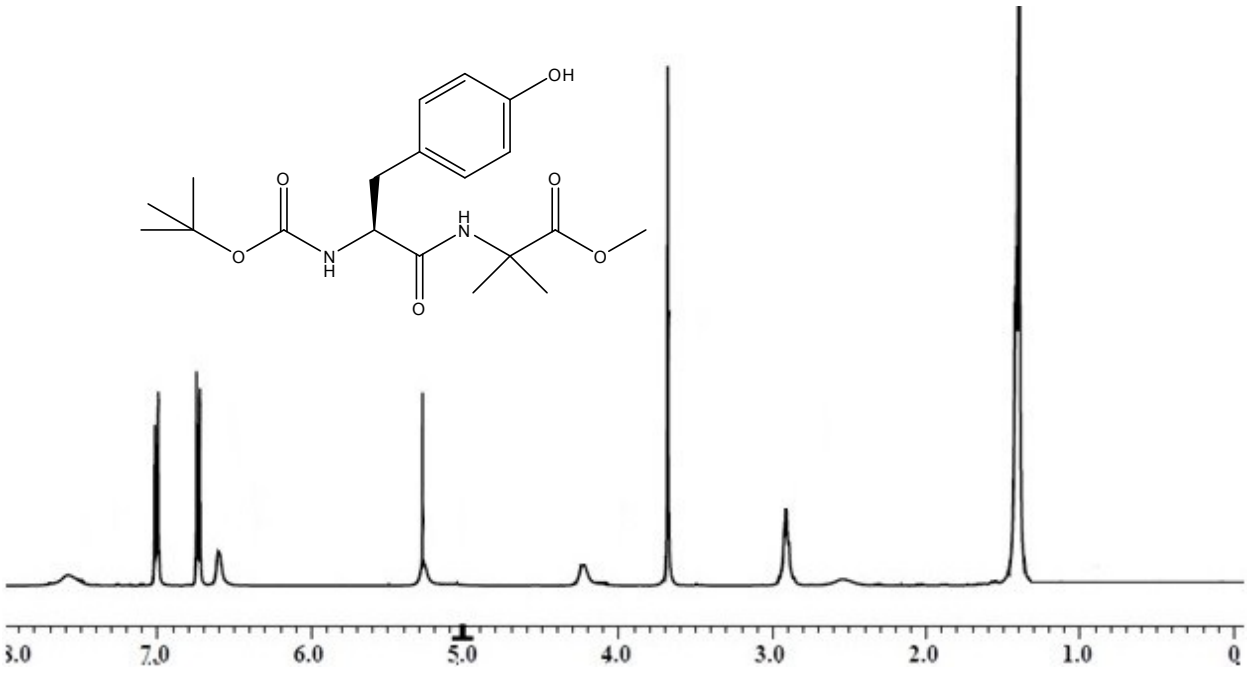


Figure S10: ^{1}H NMR (400MHz, $CDCl_3$) spectra of Boc-Tyr-Aib-OMe

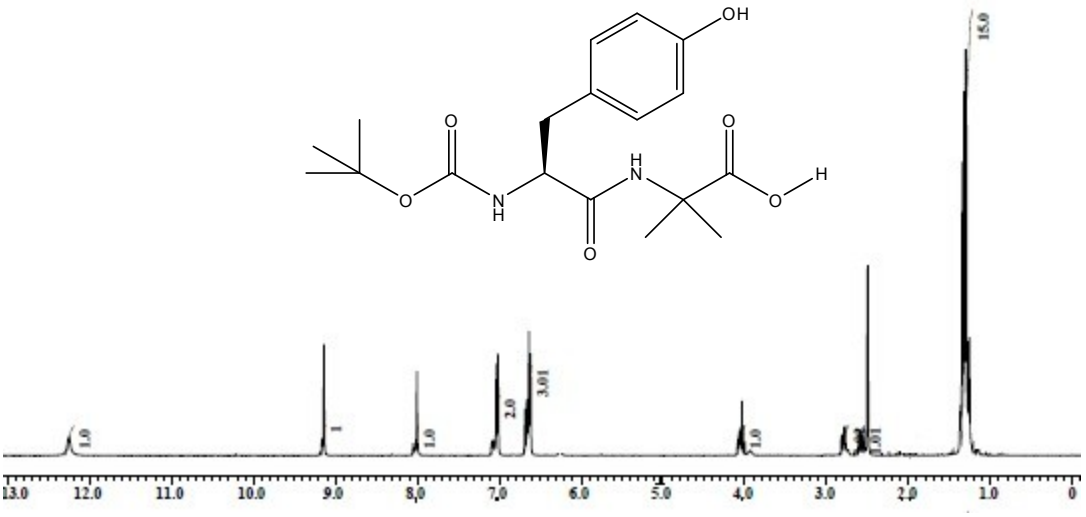


Figure S11: ^{1}H NMR (400MHz, DMSO-d_6) spectra of Boc-Tyr-Aib-OH

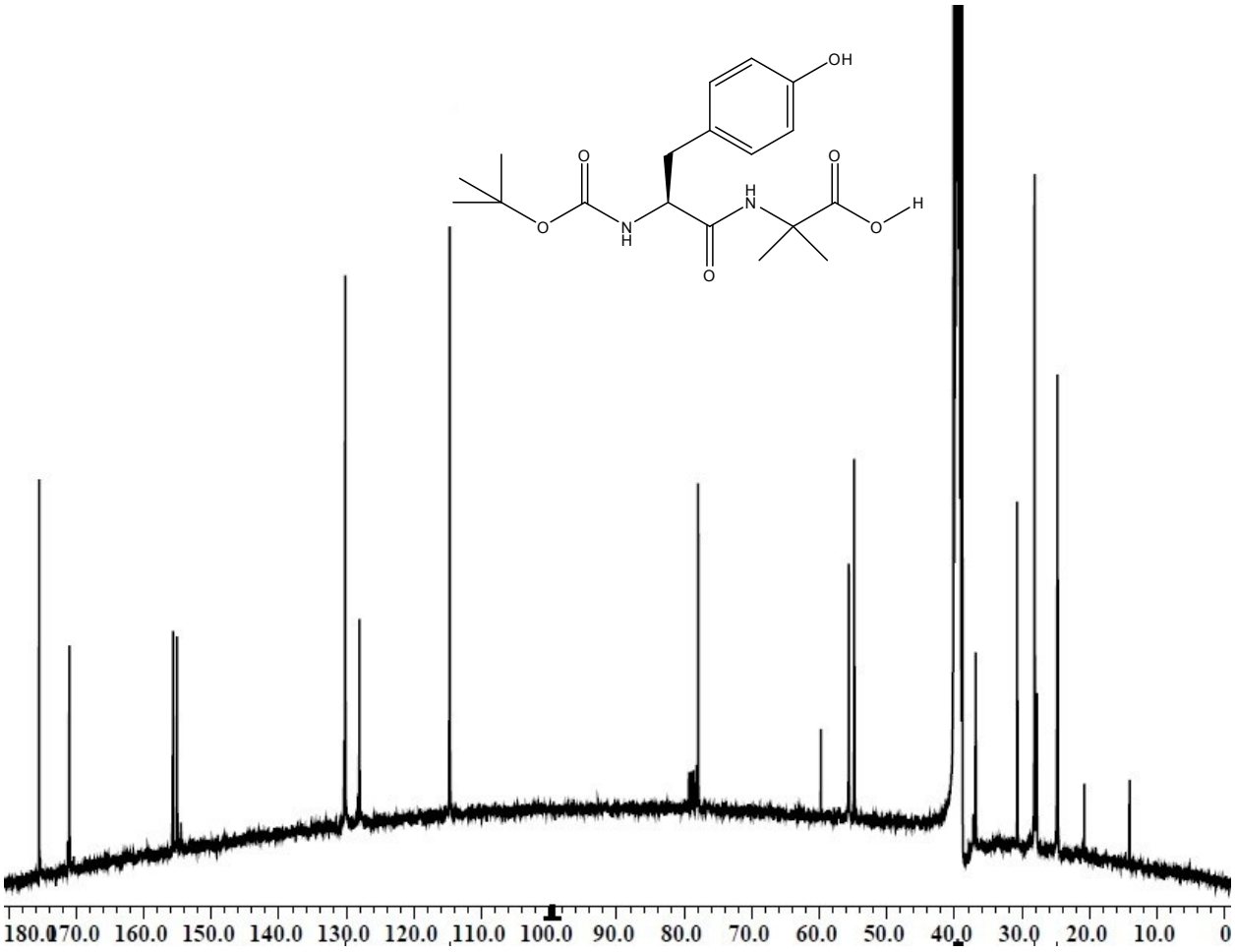


Figure S12: ^{13}C NMR (125MHz,DMSO-d_6) spectra of Boc-Tyr-Aib-OH

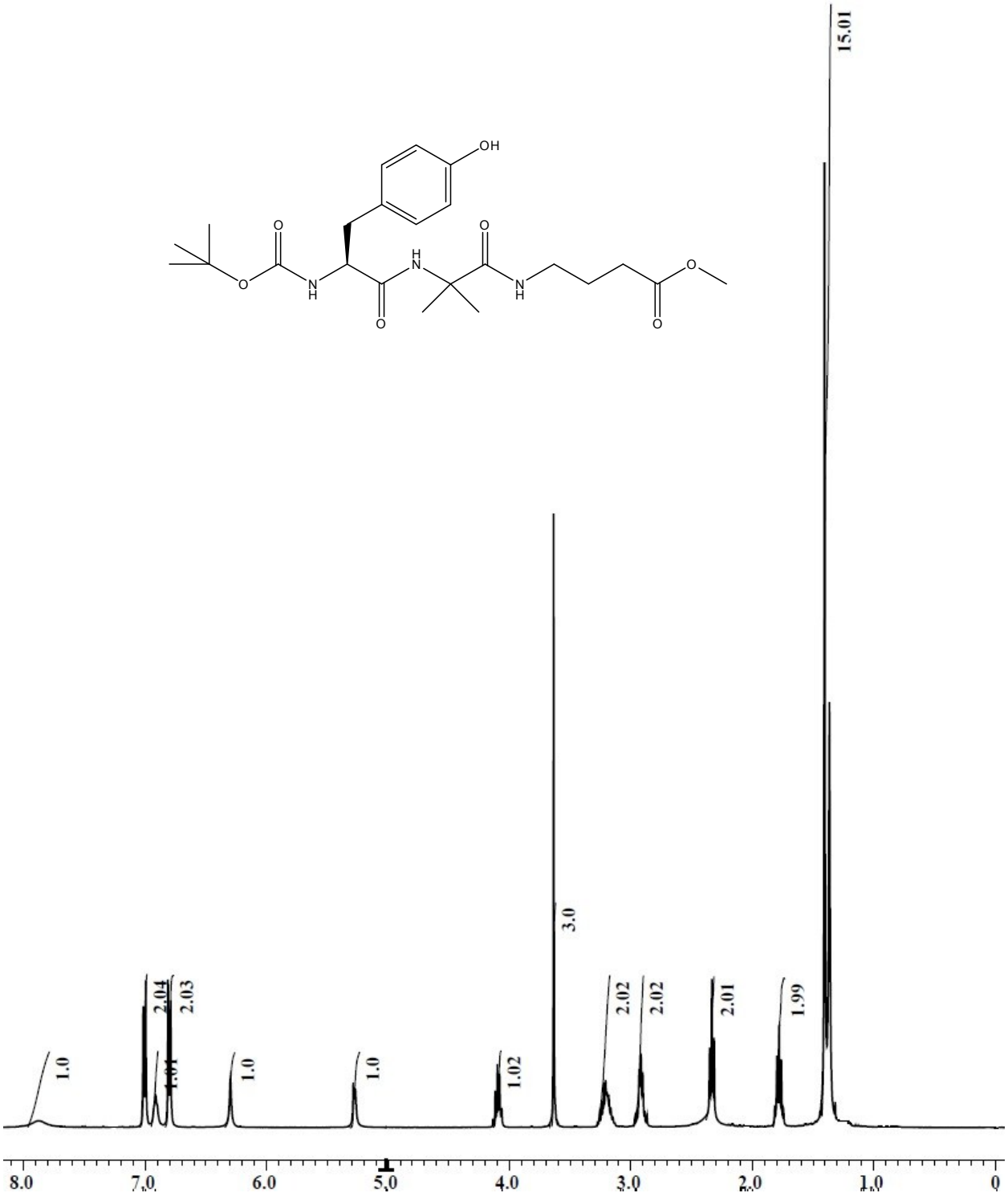


Figure S13: ^{1}H NMR (400MHz, $CDCl_3$) spectra of Boc-Tyr-Aib-Gaba-OMe

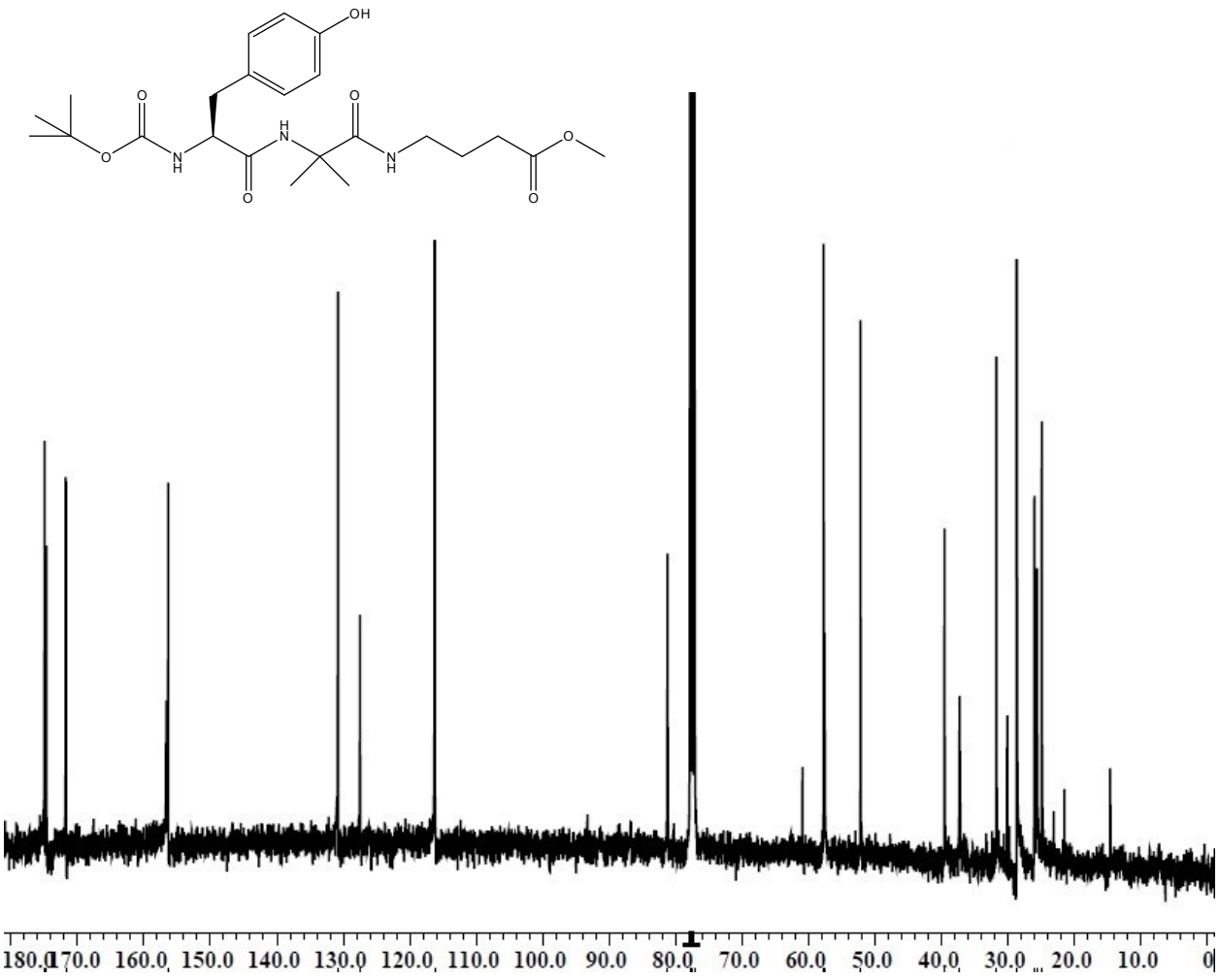


Figure S14: ^{13}C NMR (125MHZ, $CDCl_3$) spectra of Boc-Tyr-Aib-Gaba-OMe

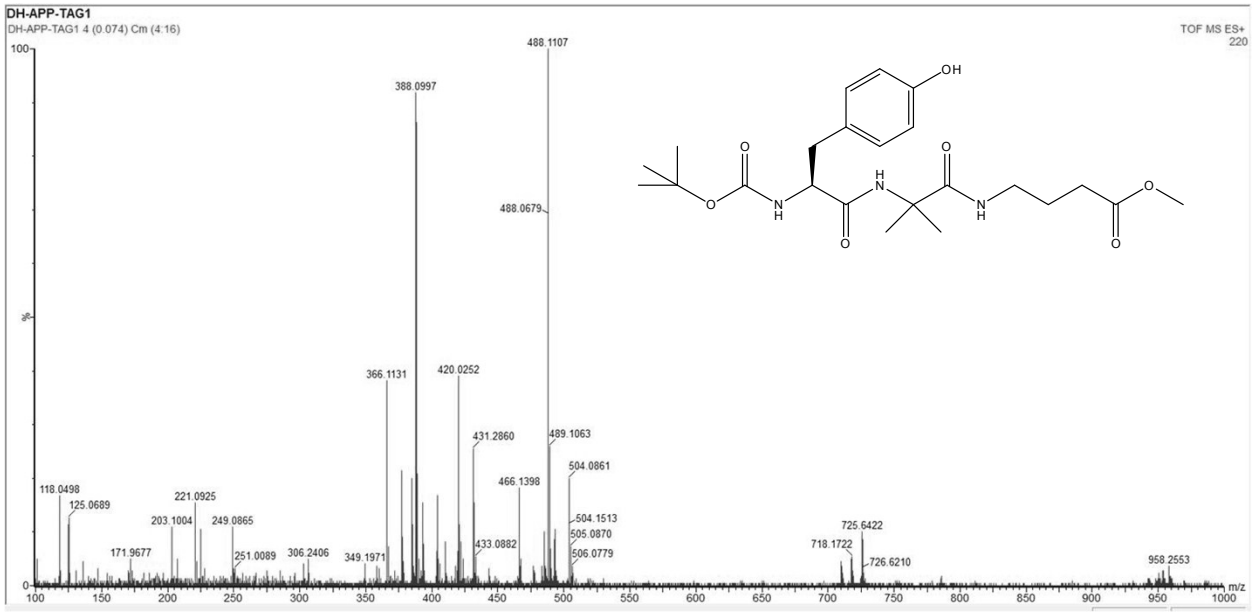


Figure S15: Mass spectra of peptide Boc-Tyr-Aib-Gaba-OMe 2.