Electronic Supplementary Material (ESI) for CrystEngComm


**Supporting information**

# Fabrication of microspheres from self-assembled γ-peptides

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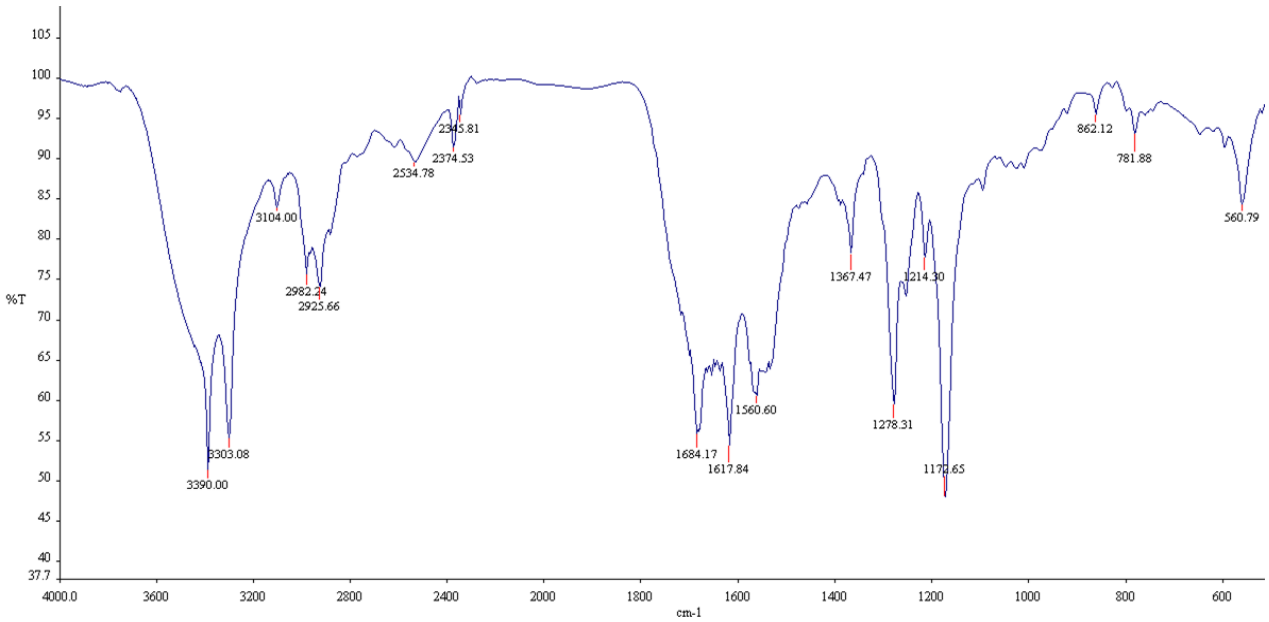


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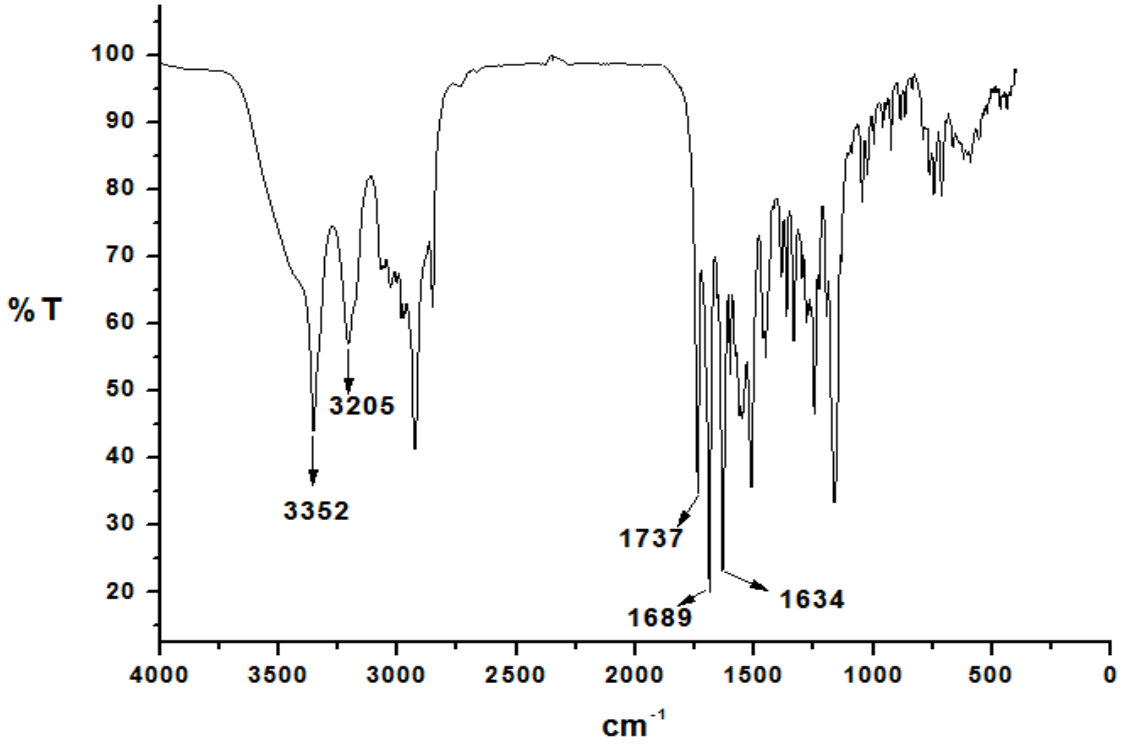


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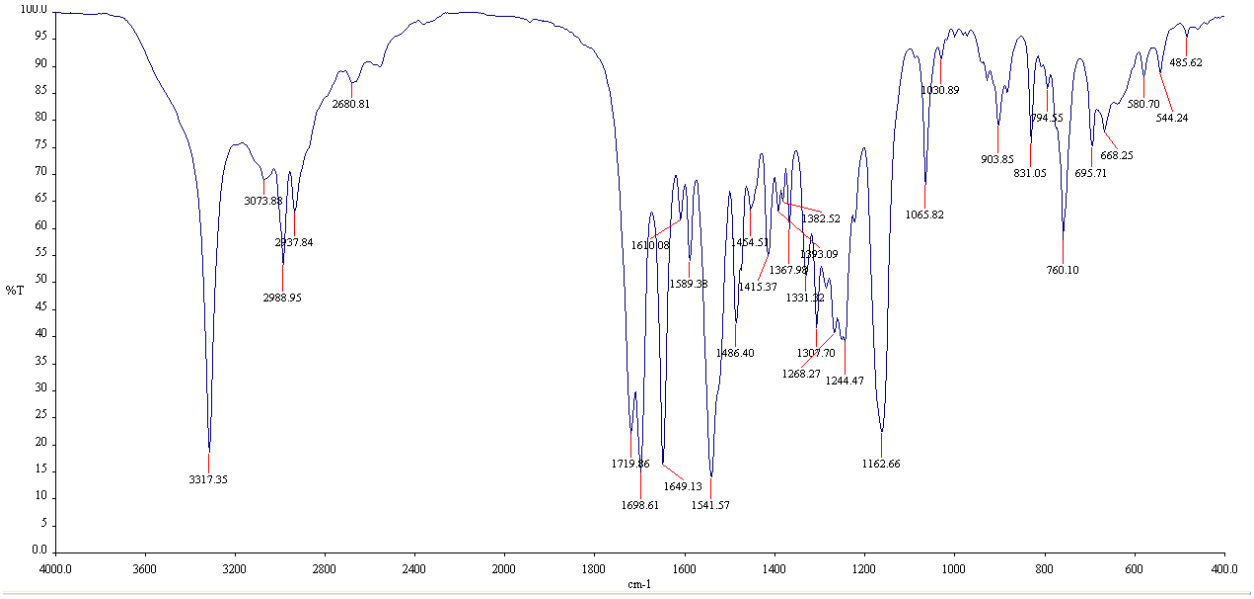
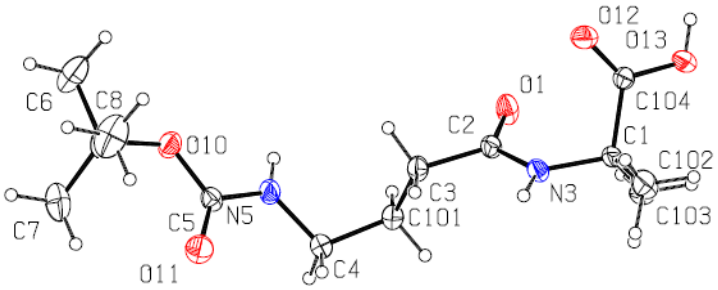
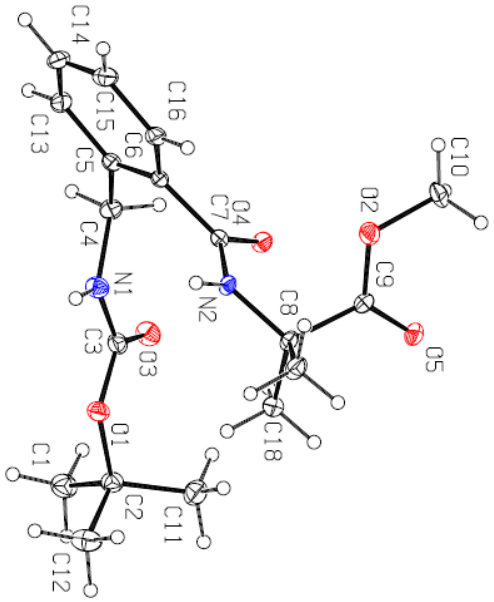



**(c)**

**ESI Fig. 1:** IR spectra of (a) peptide **1** (b) peptide **2** (c) peptide **3.**




**(a)**

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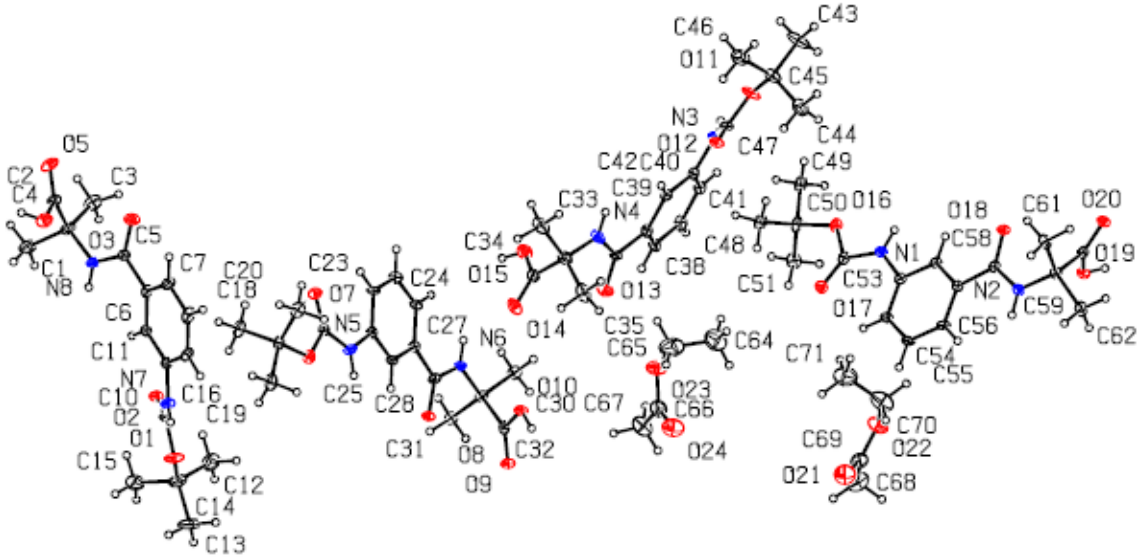
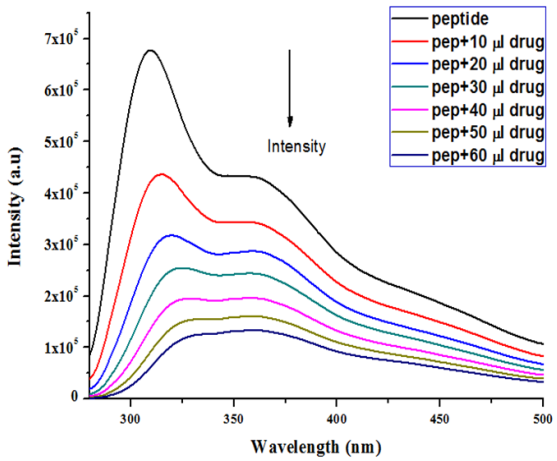
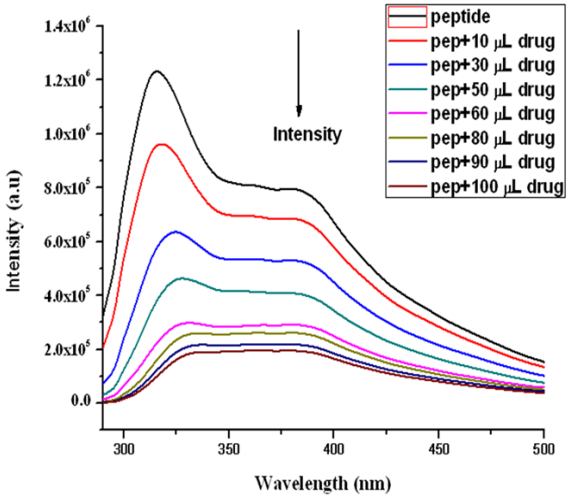



**(c)**

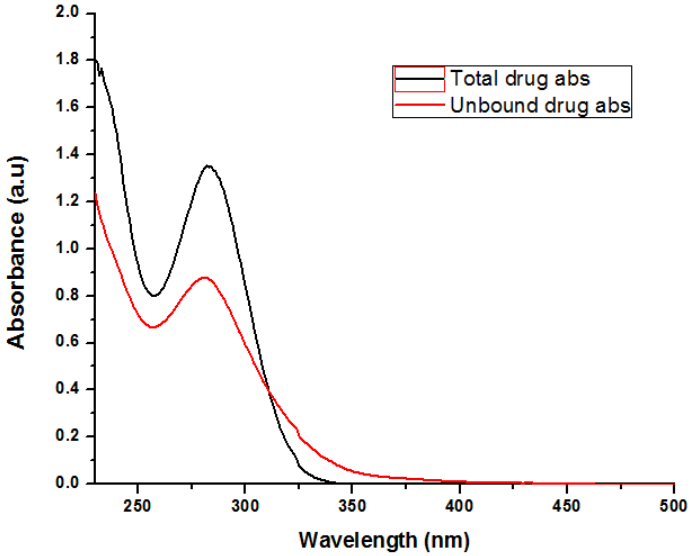
**ESI Fig. 2:** ORTEP diagram of (a) peptide **1** (b) peptide **2** (c) peptide **3** showing the atomic numbering scheme with 30% probability level.



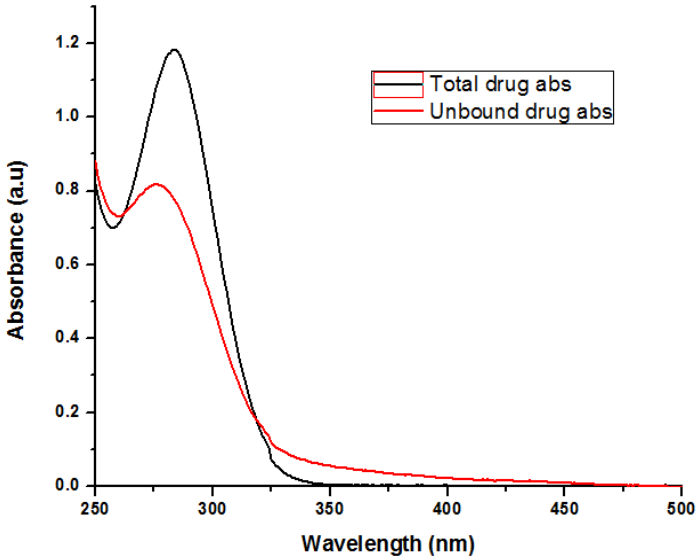

**ESI Fig. 3:** Emission spectra of peptide **1** with increasing concentration of carbamazepine (peptide concentration $1.0x10^{-3}$ M and drug concentration $1.1x10^{-2}$ M). Excitation wavelength is 255 nm.




**ESI Fig. 4:** Emission spectra of peptide **2** with increasing concentration of carbamazepine (peptide concentration $1.0x10^{-3}$ M and drug concentration $1.1x10^{-2}$ M). Excitation wavelength is 267 nm.

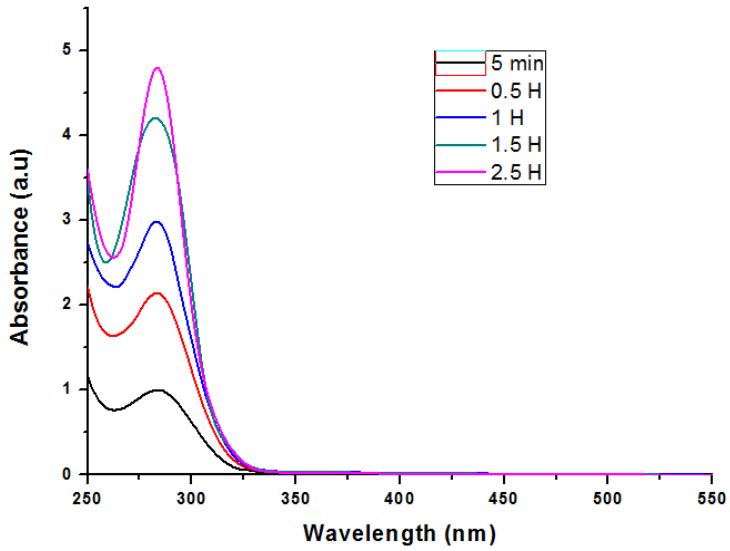



**(a)**




**(b)**

**ESI Fig. 5:** Absorption spectra of total drug (black) and unbound drug (red) of (a) peptide **1** and (b) peptide **2**.




**ESI Fig. 6:** Time dependent absorption spectra of water dispersion of drug loaded spheres from peptide **2.**

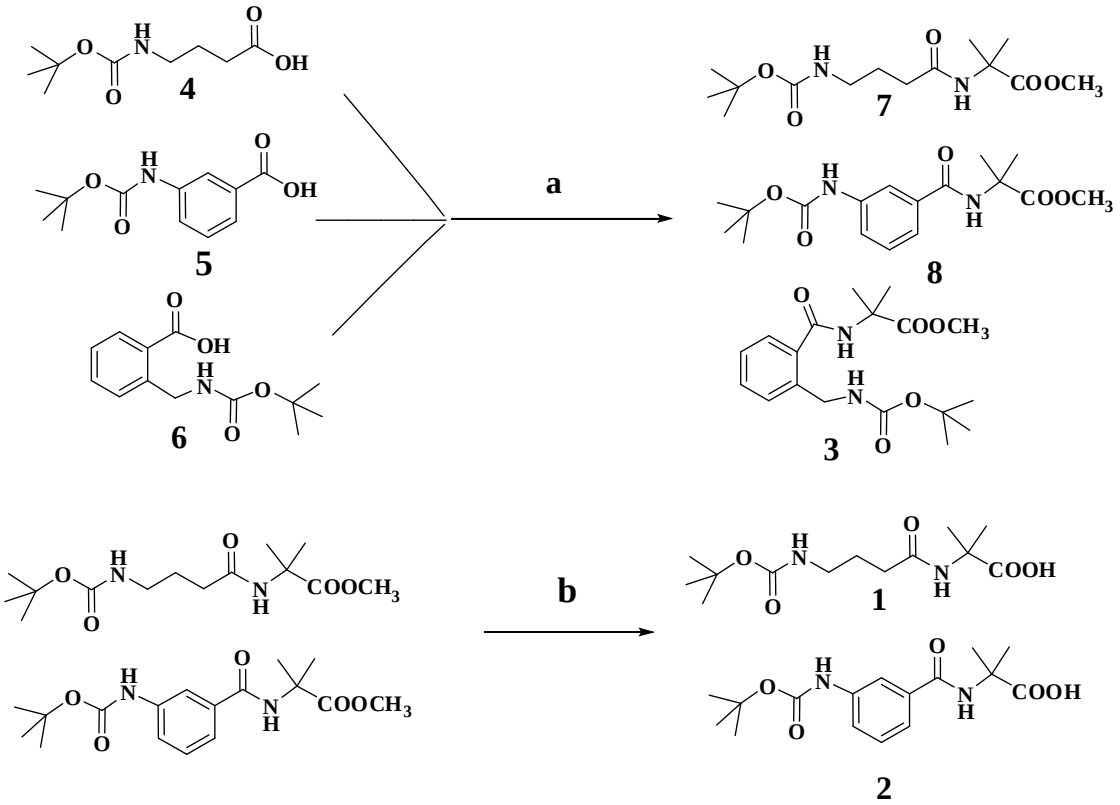



**Fig S1:** Reactions and conditions: (a) dry DCM, H-Aib-COOCH3, DCC, HOBt, 0 ºC, (b) NaOH, MeOH, HCl.

**Peptide synthesis:**

***(a) Boc-γ-Abu-OH 4.*** A solution of γ-aminobutyric acid (4.12 g, 40 mmol) in a mixture of dioxan (80 mL), water (40 mL), and 1 M NaOH (40 mL) was stirred and cooled in an ice-water bath. Di-tert-butyl pyrocarbonate (9.6 g, 44 mmol) was added, and stirring was continued at room temperature for 6 h. Then, the solution was concentrated under vacuum to about 40–60 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 repeatedly extracted with ethyl acetate. 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: 7.5 g (37 mmol, 92%).

$^1$H NMR (400 MHz, $CDCl_3$, δppm): 8.37 [1H, b, s, COOH], 4.82 [1H, s, γ-Abu NH], 3.13–3.16 [2H, m, Cγ Hs of γ-Abu], 2.33–2.37 [2H, m, Cα Hs of γ-Abu], 1.75–1.82 [2H, m, Cβ Hs of γ-Abu], 1.40 [9Hs, s, Boc $CH_3$]. $^{13}$C NMR (100 MHz, $CDCl_3$, δppm): 178.07, 156.18, 78.53, 39.69, 31.21, 28.28, 25.03.

***(b) Boc-γ-Abu(1)-Aib(2)-OMe 5***. A 5.0 g (24.6 mmol) sample of Boc-γ-Abu-OH was dissolved in a mixture of 30 mL of dichloromethane (DCM) in an ice-water bath. H-Aib-OMe was isolated from 7.6 g (50 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 5.07 g (24.6 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 M HCl (3 x 50 mL), brine, 1 M sodium carbonate (3 x 50 mL), and brine (2 x 50 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield dipeptide 5 as a white solid. Purification was done on a silica gel column (100–200 mesh) using ethyl acetate–hexane (3:1) as the eluent. Yield: 5.2 g (17.21 mM, 70%).

$^1$H NMR (500 MHz, $CDCl_3$, δppm): 6.73 [1H, s, Aib(2) NH], 4.79 [1H, s, γ-Abu(1) NH], 3.72 [3H, s, OCH3], 3.18–3.21 [2H, m, Cγ Hs of γ-Abu(1)], 2.19–2.22 [2H, m, Cα Hs of γ-Abu(1)], 1.77–1.82 [2H, m, Cβ Hs of γ-Abu(1)], 1.53 [6H, s, $CH_3$ Hs of Aib(2)], 1.44 [9Hs, s, Boc $CH_3$]. $^{13}$C NMR (100 MHz, CDCl3, δppm): 175.05, 172.08, 156.54, 78.53, 56.18, 52.46, 39.40, 33.48, 28.34, 26.33, 24.85.

***(c) Boc-γ-Abu(1)-Aib(2)-OH 6.*** To 5.00 g (16.5 mmol) of 5 were added 40 mL MeOH and 17 mL of 2 M NaOH, and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 x 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1 M HCl, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuum to yield 4.5 g of 5. Yield: 4.5 g, (27.77 mmol, 95%).

$^1$H NMR (500 MHz, DMSO-$d_6$, δppm): 12.06 [1H, s, COOH], 7.97 [1H, s, Aib(2) NH], 6.79 [1H, t, γ-Abu(1) NH], 2.87–2.91 [2H, m, Cγ Hs of γ-Abu(1)], 1.99–2.04 [2H, m, Cα Hs of γ-Abu(1)], 1.53–1.56 [2H, m, Cβ Hs of γ-

Abu(1)], 1.37 [9Hs, s, Boc $CH_3$], 1.30 [6H, s, $CH_3$ Hs of Aib(2)]. $^{13}$C NMR (100 MHz, DMSO-$d_6$, δppm): 175.64, 171.29, 155.58, 77.45, 54.61, 32.24, 28.29, 25.85, 24.96.

***(d) Boc-2-(aminomethyl)benzoic acid (1)-Aib(2)-OMe 2.*** A 113 mg (0.5 mmol) sample of Boc-2-(aminomethyl) benzoic acid was dissolved in a mixture of 30 mL of dichloromethane (DCM) in an ice-water bath. H-Aib-OMe was isolated from 153.60 mg (1 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 123.8 mg (0.6 mmol) of dicyclohexylcarbodiimide (DCC) and 81 mg (0.6 mmol) of HOBt.. 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 (100-200 mesh) using ethyl acetate: hexane (3:1) as the eluent. Yield: 150 mg (0.43 mmol, 86%).

$^1$H NMR (500 MHz, $CDCl_3$, δppm): 7.44-7.46 [2H, d, J=8 Hz, 2-(aminomethyl) benzoic acid (1) aromatic CH], 7.39-7.42 [1H, m, 2-(aminomethyl) benzoic acid (1) aromatic CH], 7.28-7.32 [1H, m, 2-(aminomethyl) benzoic acid aromatic (1) CH], 6.92 [1H, s, Aib(2) NH], 5.69 [1H, s, 2-(aminomethyl) benzoic acid (1) NH], 4.32-4.33 [2H, d, benzylic $CH_2$ of 2-(aminomethyl) benzoic acid (1)], 3.78 [3H, s, $OCH_3$], 1.64 [6H, s, Aib(2) CβH], 1.40 [9H, s, Boc $CH_3$]. $^{13}$C NMR (125 MHz, $CDCl_3$, δppm): 174.88, 169.26, 156.03, 137.80, 135.59, 130.71, 130.57, 127.49, 127.13, 79.26, 56.82, 52.70, 33.91, 28.40, 24.93.

(***e) Boc-Maba-OH 7***: A solution of *m*-amino benzoicacid (2.7 g, 20 mmol) in a mixture of dioxan (40 mL), water (20 mL), and 1N 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%).

1H NMR (400 MHz, DMSO-$d_6$, δppm): 12.87 [1H, s, COOH], 9.54 [1H, s, Maba NH], 8.14 [1H, s, Maba CH], 7.60-7.62 [1H, d, Maba CH], 7.52-7.54 [1H, d, Maba CH], 7.34-7.39 [1H, m, Maba CH], 1.48 [9Hs, s, Boc $CH_3$]. 13C NMR (100 MHz, DMSO-$d_6$, δppm): 167.25, 152.73, 139.76, 131.22, 128.77, 122.87, 122.23, 118.74, 79.28, 28.05.

***(f) Boc-Maba(1)-Aib(2)-OMe 8.*** A 2.37 g (10 mmol) sample of Boc-Maba-OH was dissolved in a mixture of 30 mL of dichloromethane (DCM) in an ice-water bath. H-Aib-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 8 as a white solid. Purification was done on a silica gel column (100-200 mesh) using ethyl acetate: hexane (3:1) as the eluent. Yield: 2.6 g (7.1 mM, 71%). Mp: 175-176ºC.

1H NMR (500 MHz, CDCl3, δppm): 7.71 [1H, S, Maba(1) CH], 7.57-7.59 [1H, d, J=8 Hz, Maba(1) CH], 7.40-7.42 [1H, d, J=8 Hz, Maba(1) CH], 7.30-7.33 [1H, m, Maba(1) CH], 6.83 [1H, s, Aib(2) NH,], 6.76 [1H, s, Maba(1) NH], 3.76 [3H, s, OCH3], 1.65 [6H, s, Aib CβH], 1.50 [9H, s, Boc CH3]. 13C NMR (125 MHz, CDCl3, δppm): 175.17, 166.32, 152.67, 138.79, 135.27, 129.27, 121.47, 121.36, 116.97, 80.78, 56.81, 52.69, 28.28, 24.77.

***(g) Boc-Maba(1)-Aib(2)-OH 3.*** To 3.36 g (10 mmol) of **8,** 25 mL MeOH and 15 mL of 2 N NaOH were added and stirred. The progress of saponification was monitored by thin-layer chromatography (TLC). After 10 h, methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1 N HCl, and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuum to yield 2.57 g of **2**. Yield: 2.57 g, (8 mmol, 80%).

$^{1}$H NMR (500 MHz, DMSO-$d_6$, δppm): 12.14 [1H, s, COOH], 9.45 [1H, s, Aib(2) NH], 8.35 [1H, s, Maba(1) NH] 7.91 [1H, S, Maba(1) CH], 7.53-7.55 [1H, d, J=8 Hz, Maba(1) CH], 7.41-7.43 [1H, d, J=8 Hz, Maba(1) CH], 7.30-7.33 [1H, m, Maba(1) CH], 1.44 [6H, s, Aib(2) CβH], 1.48 [9H, s, Boc $CH_3$].$^{13}$C NMR (125 MHz, DMSO-$d_6$, δppm): 175.58, 166.13, 152.81, 139.51, 135.22, 128.31, 120.97, 120.87, 117.60, 79.21, 55.40, 28.12, 24.97.

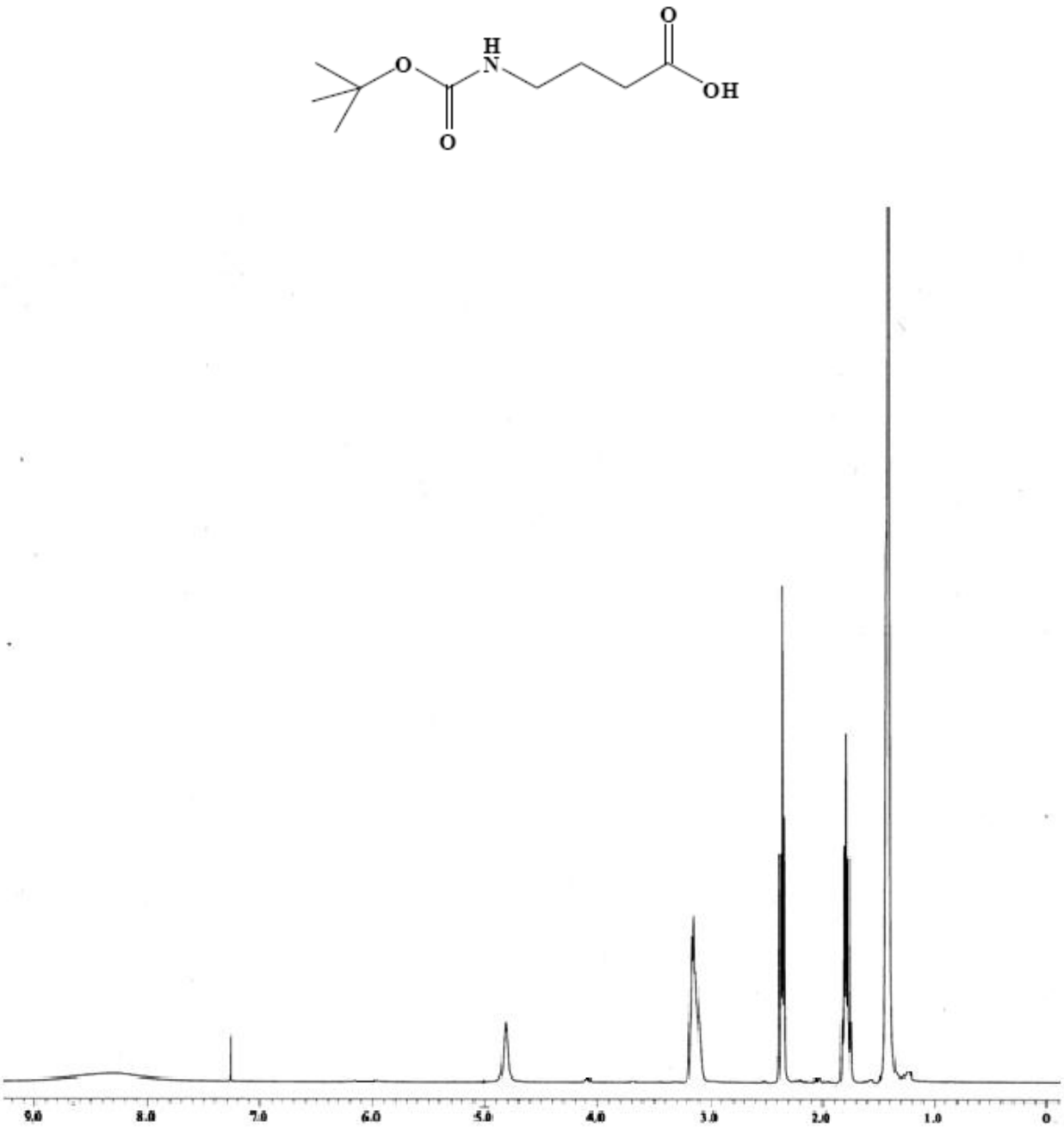



**Figure S2:** $^{1}H$ NMR ($CDCl_3$, 400 MHz, $\delta_{ppm}$) spectra of Boc- γ-Abu-OH.

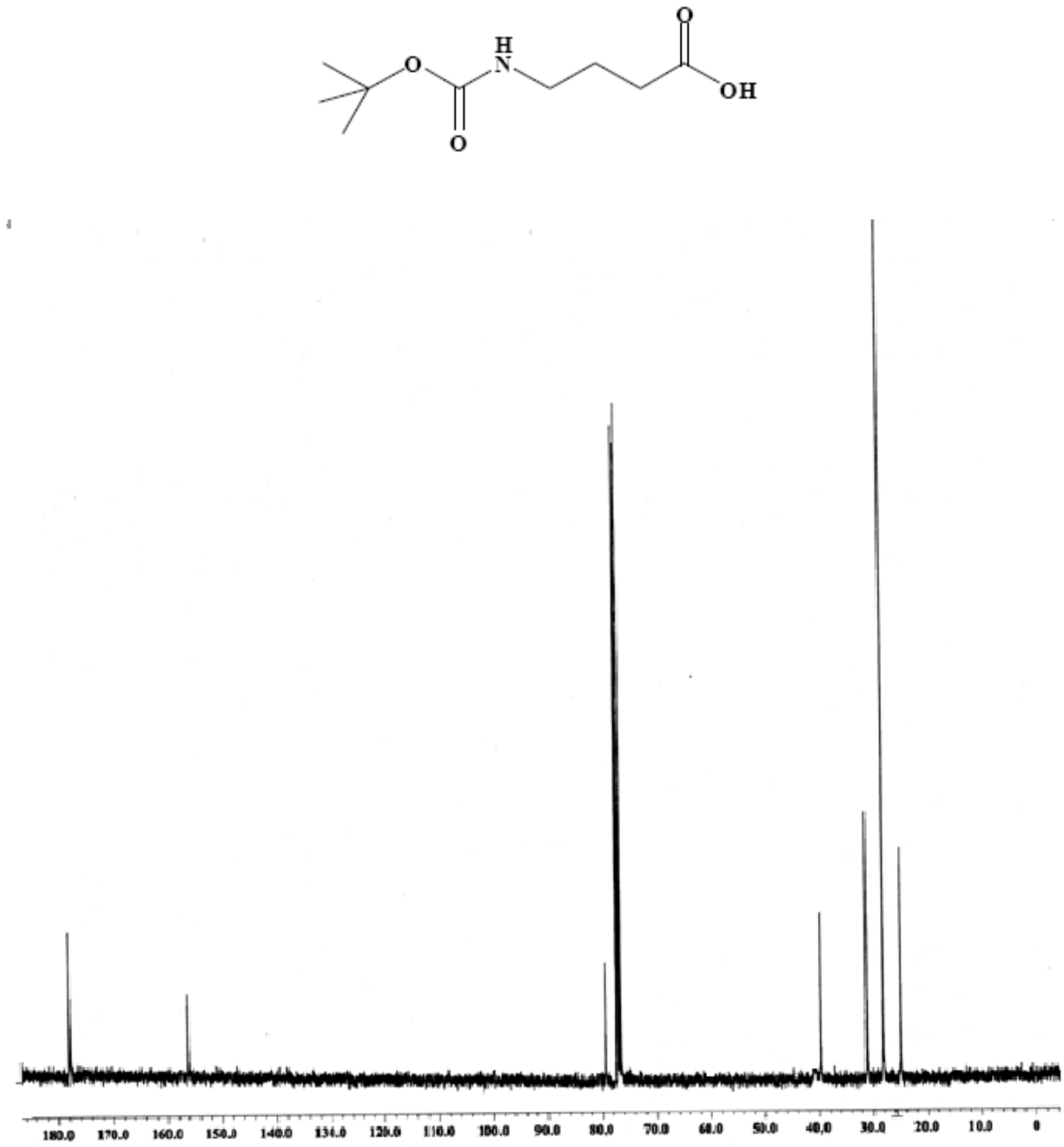
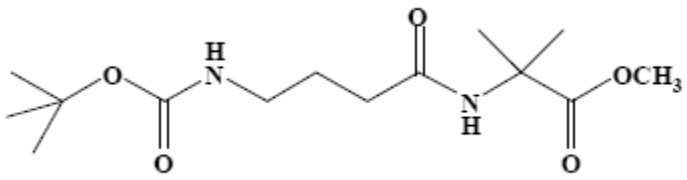



**Figure S3:** $^{13}C$ NMR ($CDCl_3$, 100 MHz, $\delta_{ppm}$) spectra of Boc- γ-Abu -OH.



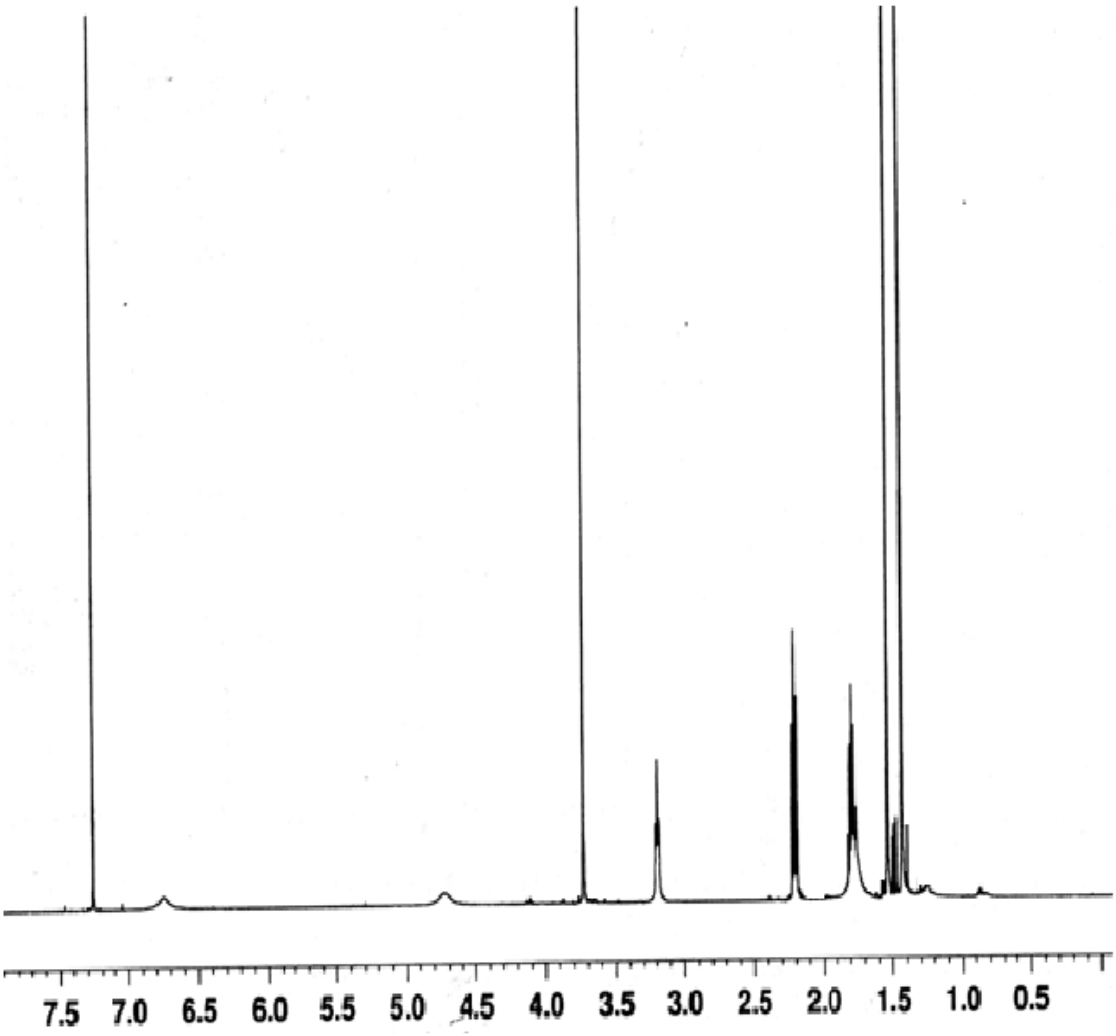




**Figure S4:** $^{1}$H NMR ($CDCl_3$, 500 MHz, $\delta_{ppm}$) spectra of Boc- γ-Abu -Aib-OMe.

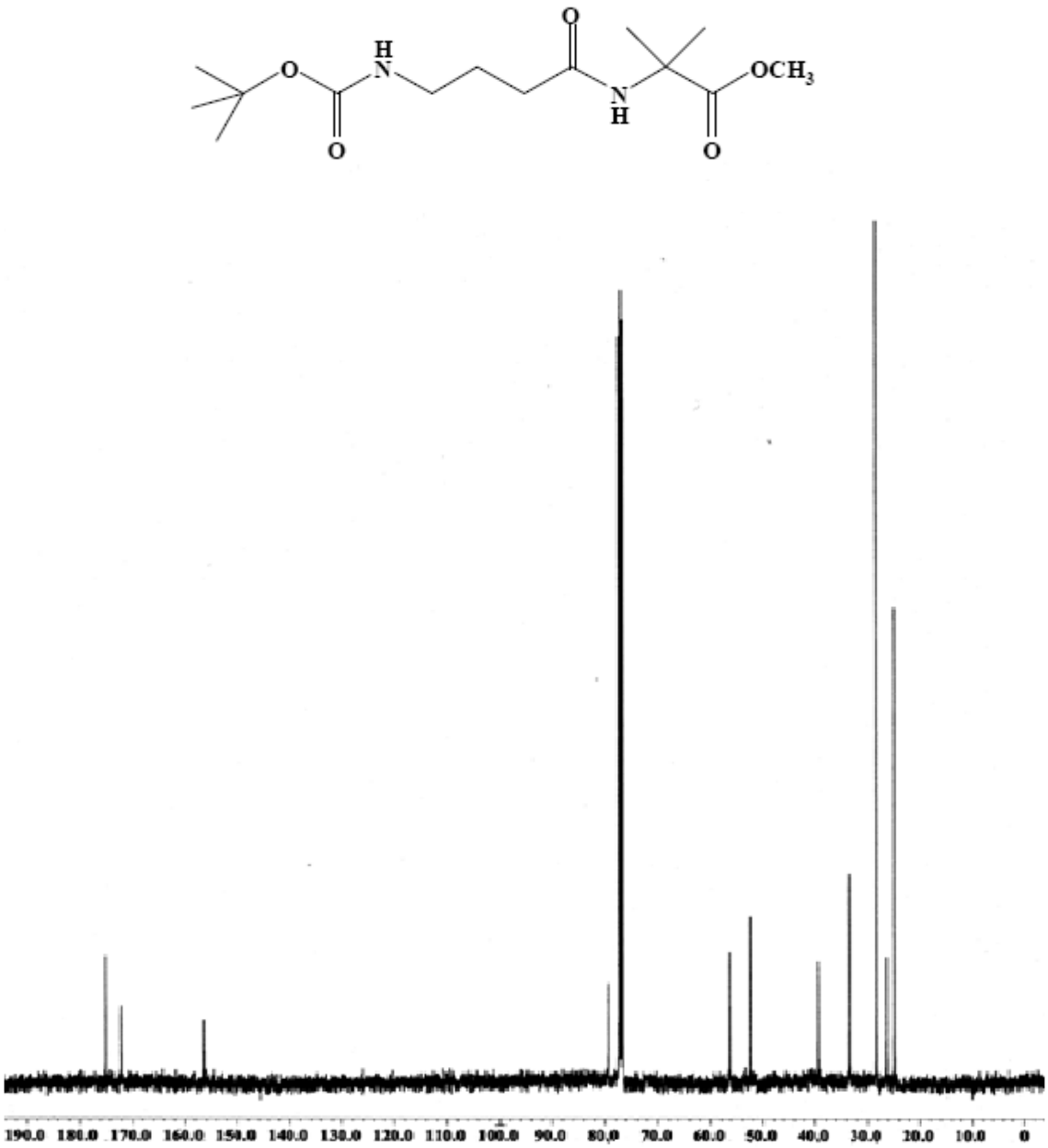



**Figure S5:** $^{13}C$ NMR ($CDCl_3$, 100 MHz, $\delta_{ppm}$) spectra of Boc- γ-Abu -Aib-OMe.

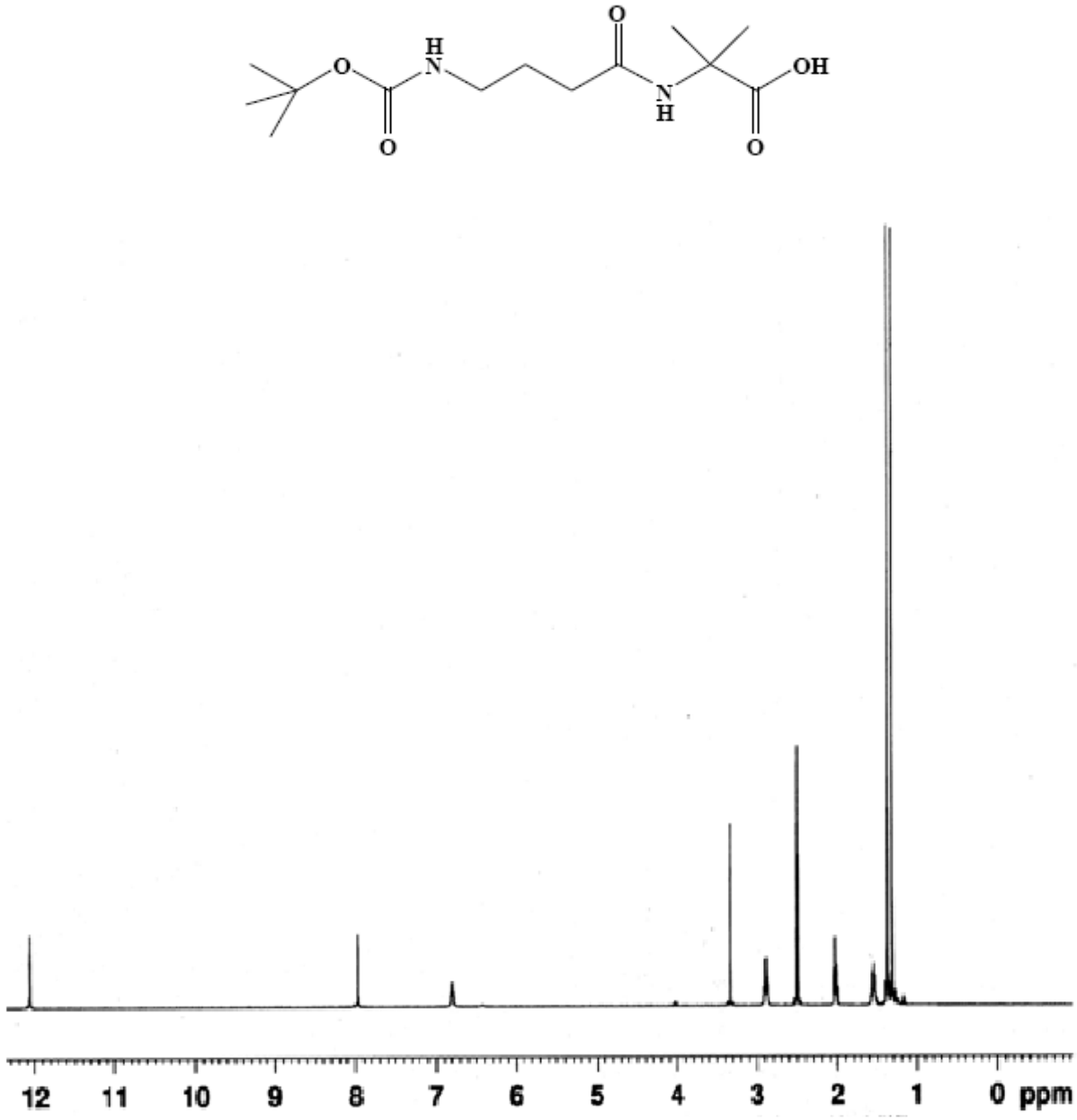



**Figure S6:** $^{1}$H NMR (DMSO-*d6*, 500 MHz, $\delta_{ppm}$) spectra of Boc- γ-Abu -Aib-OH.

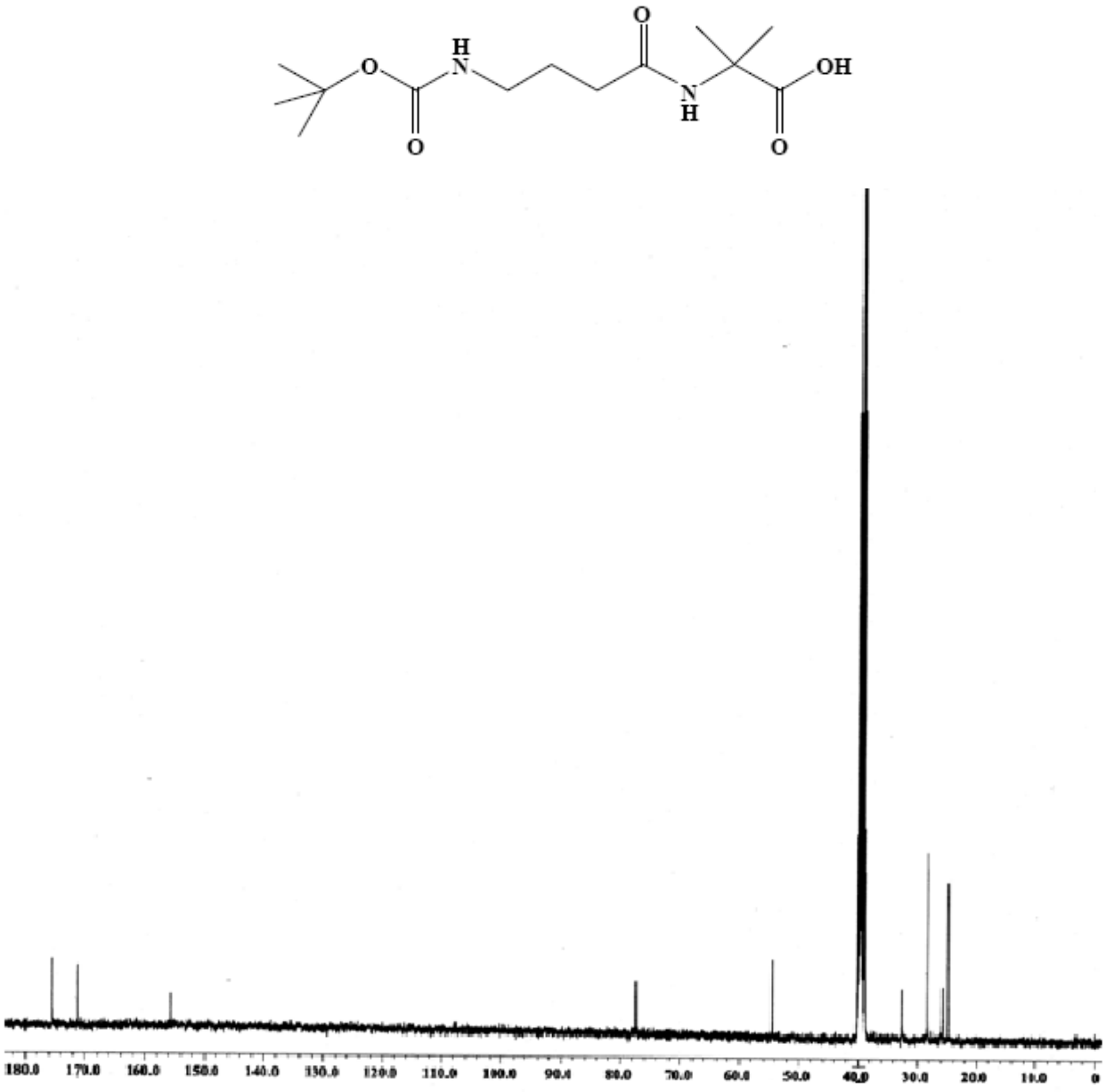



**Figure S7:** $^{13}$C NMR (DMSO-*d*$_6$, 100 MHz, $\delta_{ppm}$) spectra of Boc- γ-Abu -Aib-OH.

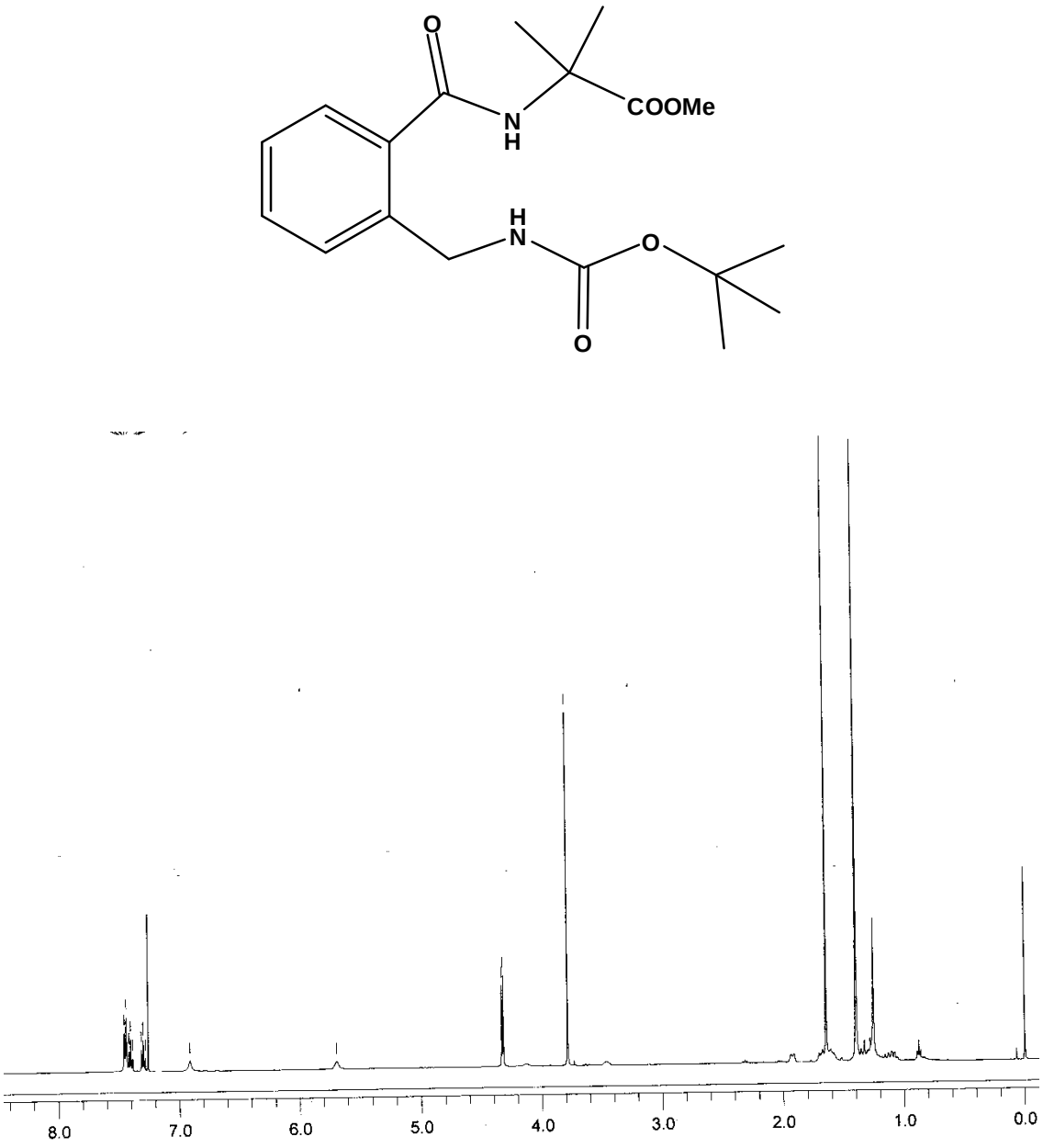



**Figure S8:** $^{1}H$ NMR ($CDCl_3$, 500 MHz, $\delta_{ppm}$) spectra of Boc-2-(aminomethyl)benzoic acid-Aib-OMe.

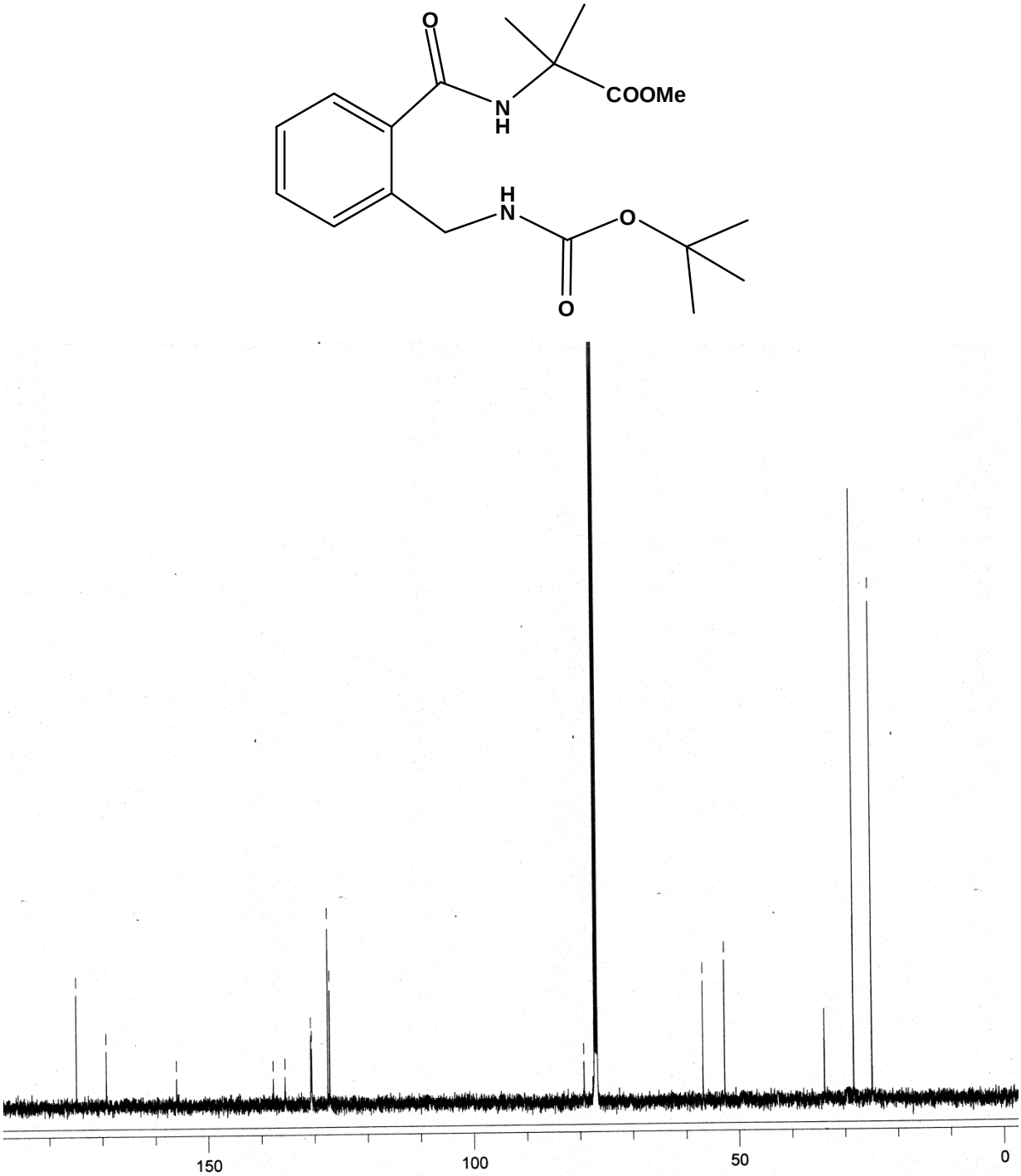



**Figure S9:** $^{13}C$ NMR ($CDCl_3$, 125 MHz, $\delta_{ppm}$) spectra of Boc-2-(aminomethyl)benzoic acid-Aib-OMe.

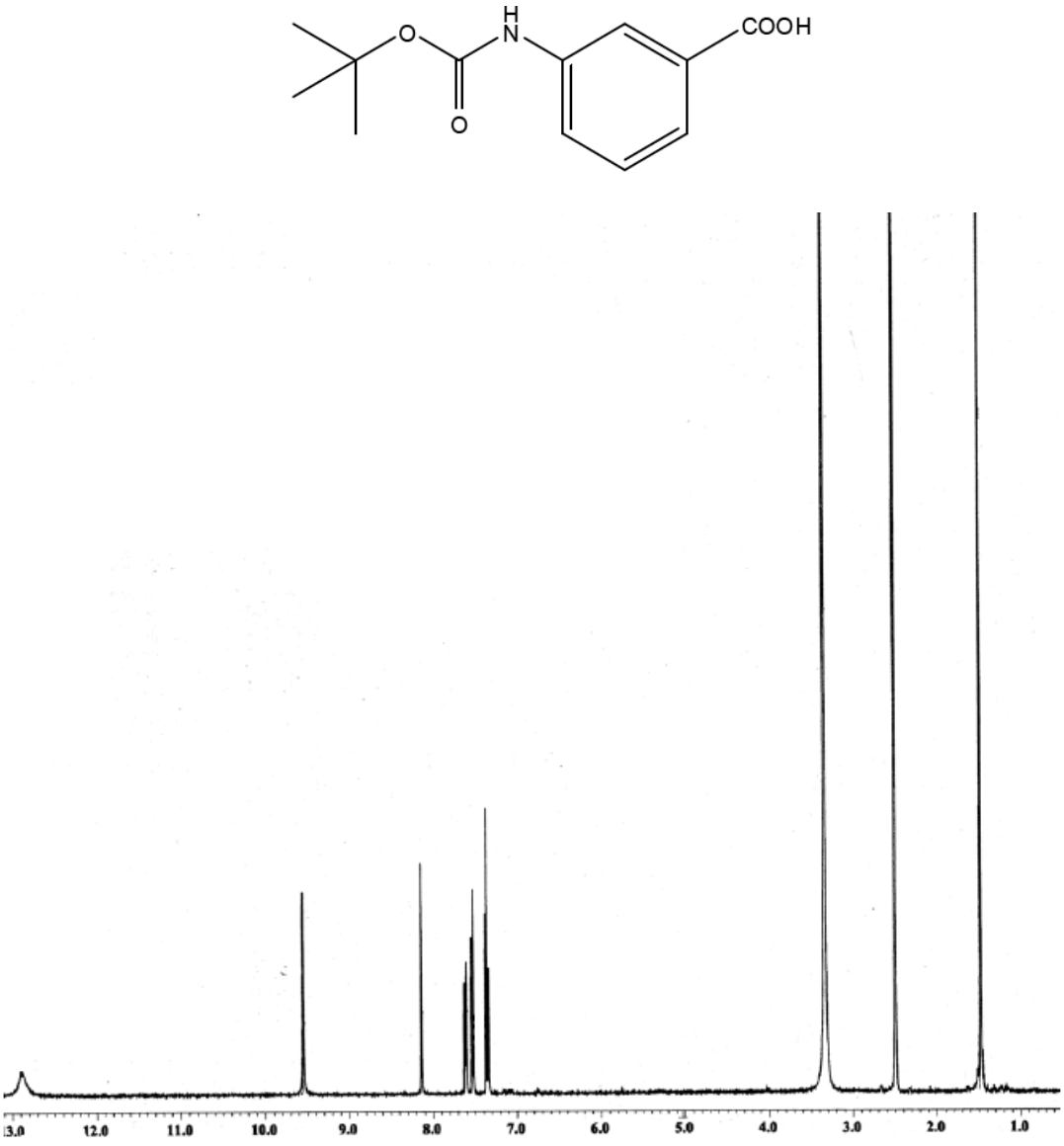



**Figure S10:** $^{1}$H NMR (DMSO-$d_6$, 400 MHz, δppm ) spectra of Boc-Maba-OH.

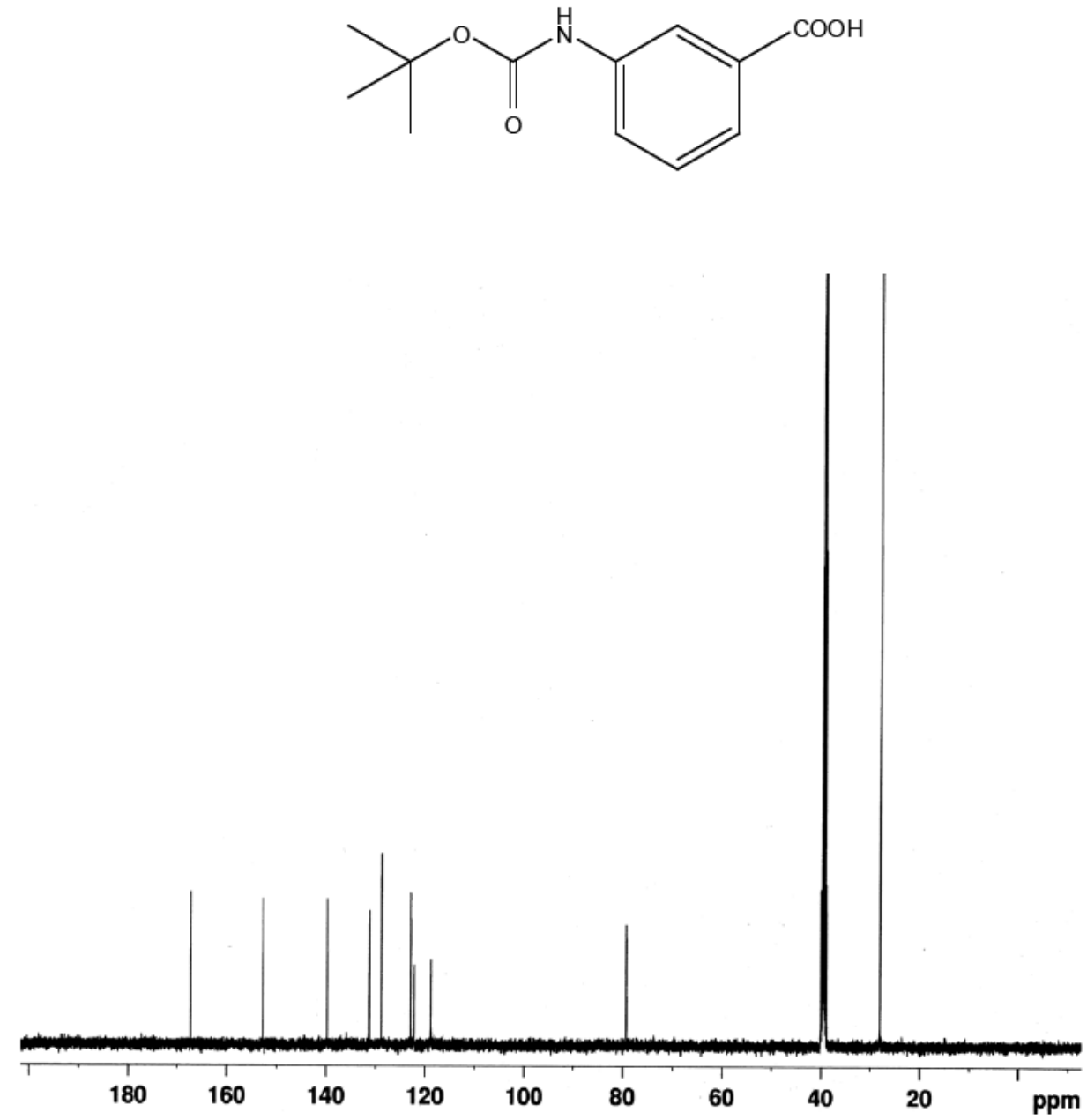



**Figure S11:** $^{13}$CN MR (DMSO-$d_6$, 400 MHz, δppm ) spectra of Boc-Maba-OH.

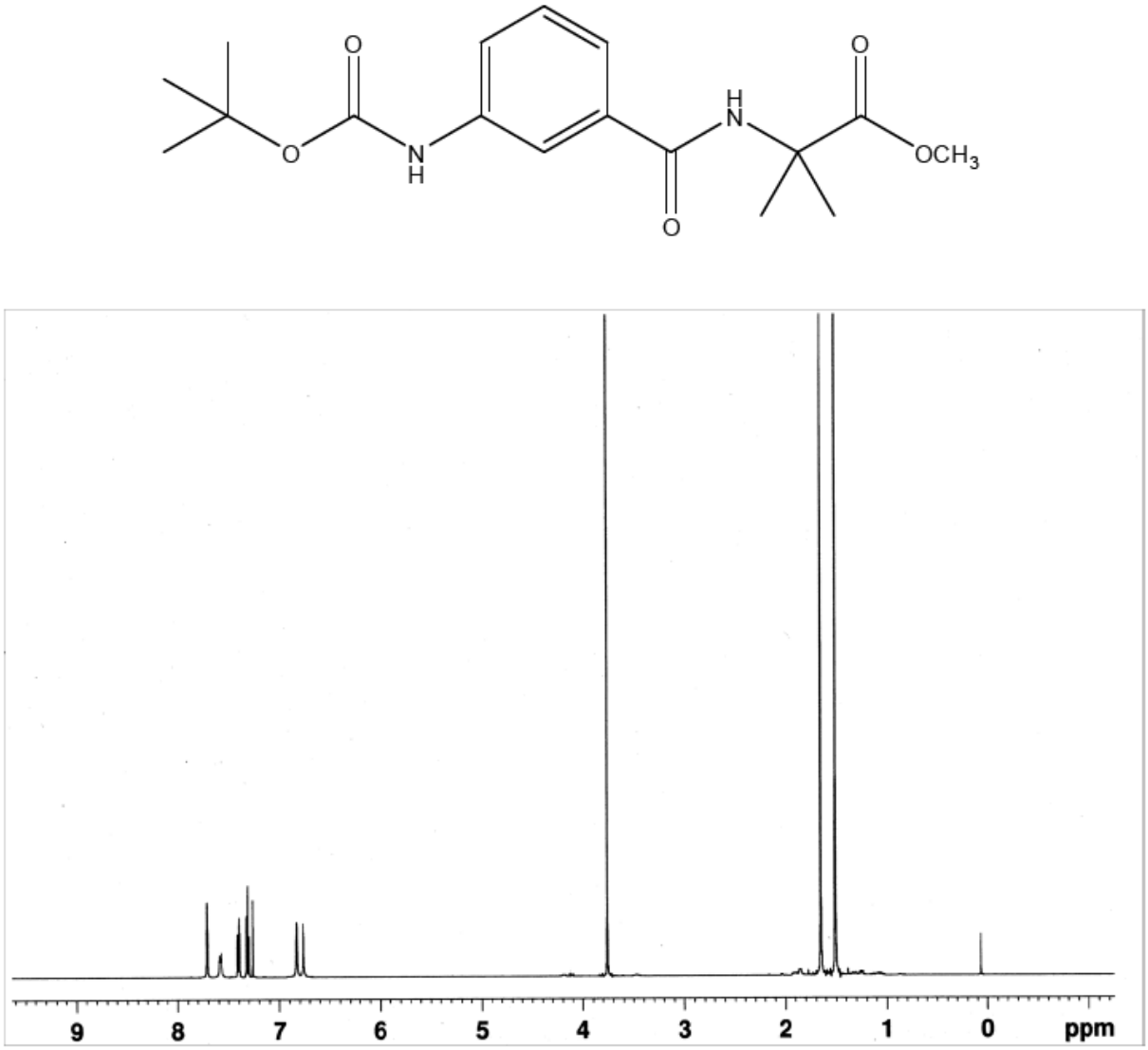



**Figure S12:** $^{1}$H NMR ($CDCl_3$, 500 MHz, δppm ) spectra of Boc-Maba-Aib-OMe .

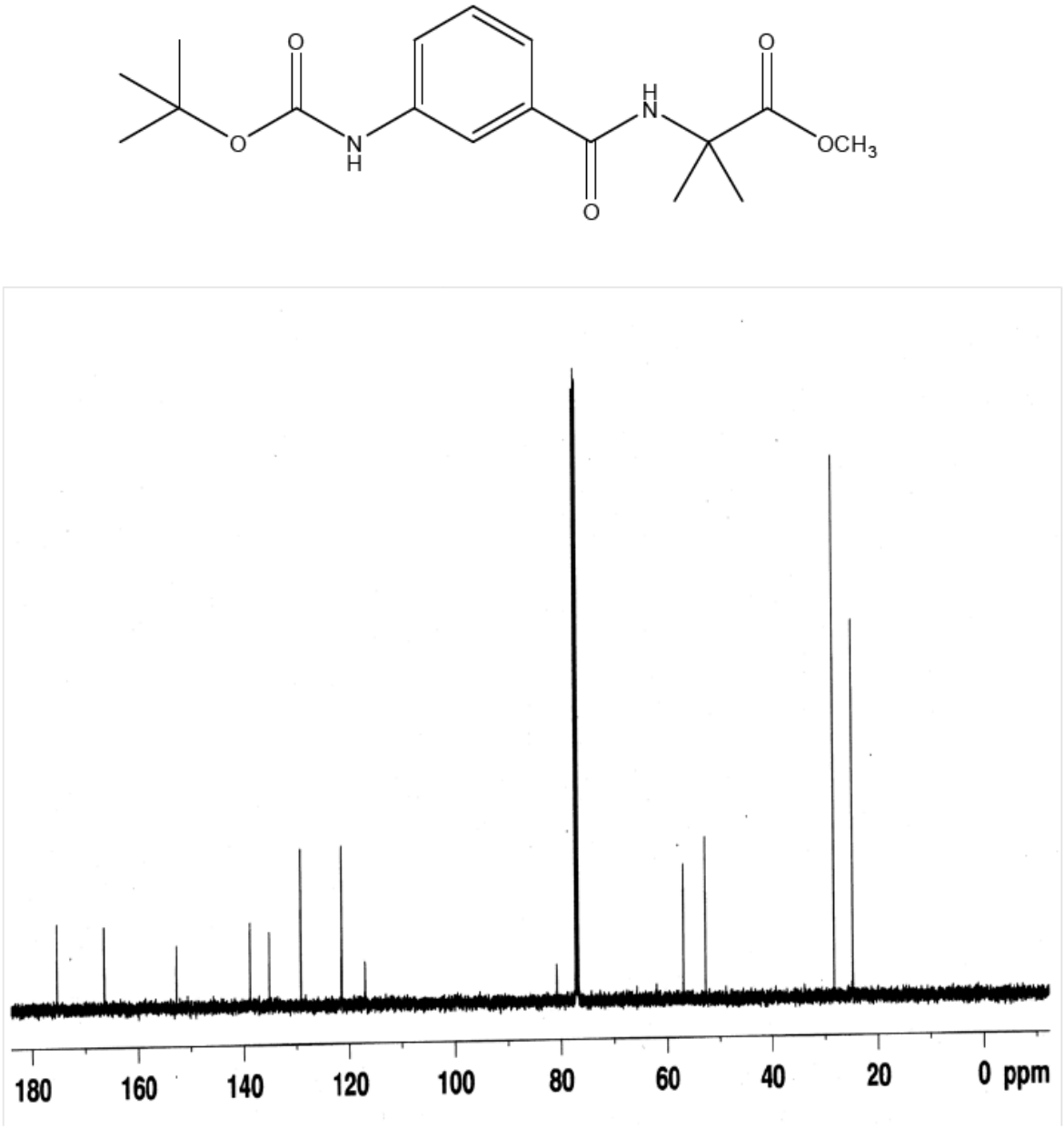



**Figure S13:** $^{13}C$ NMR ($CDCl_3$ 125 MHz, δppm ) spectra of Boc-Maba-Aib-OMe .

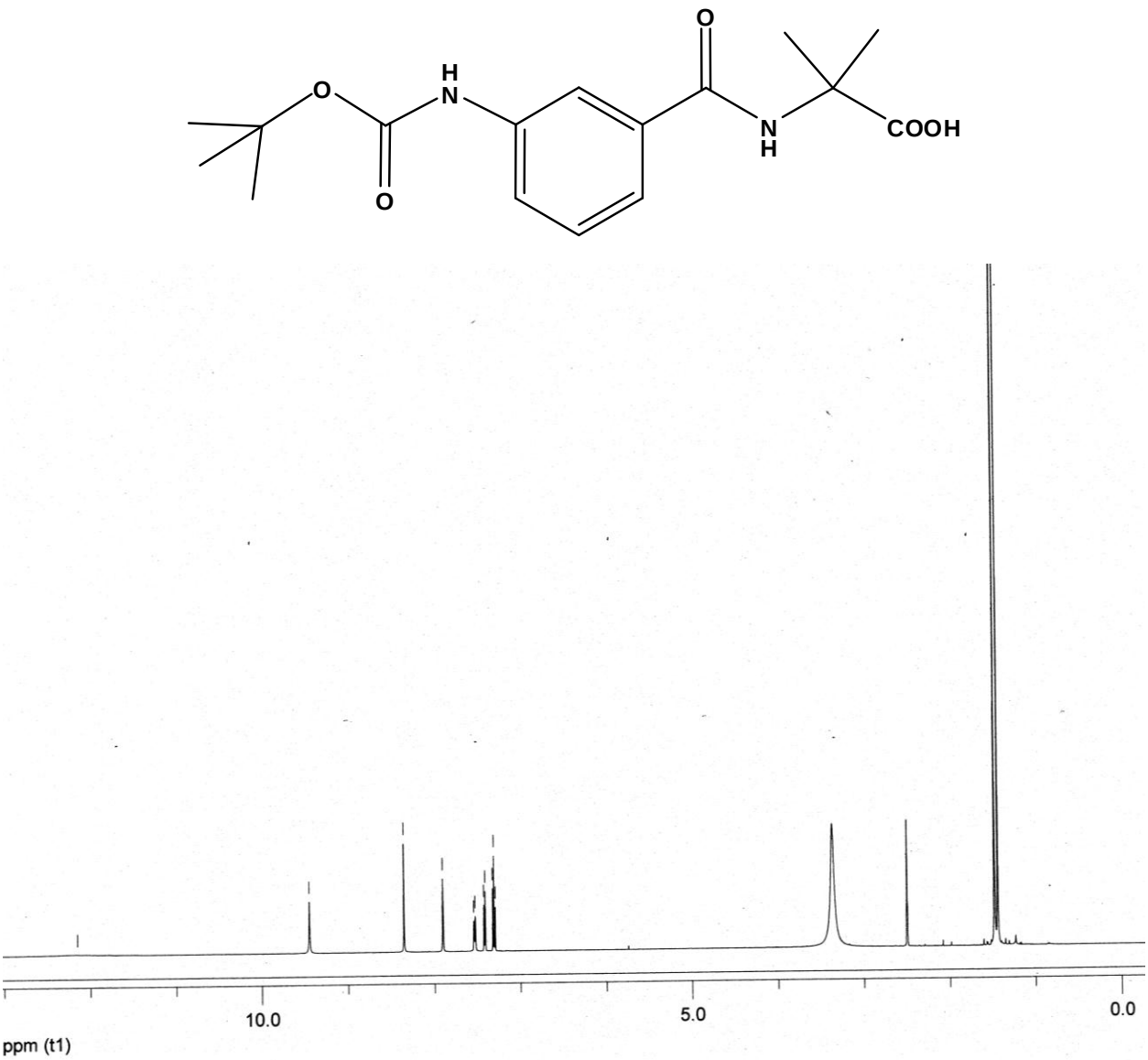



**Figure S14:** $^{1}$H NMR ($CDCl_3$, 500 MHz, δppm) spectra of Boc-Maba-Aib-OH.

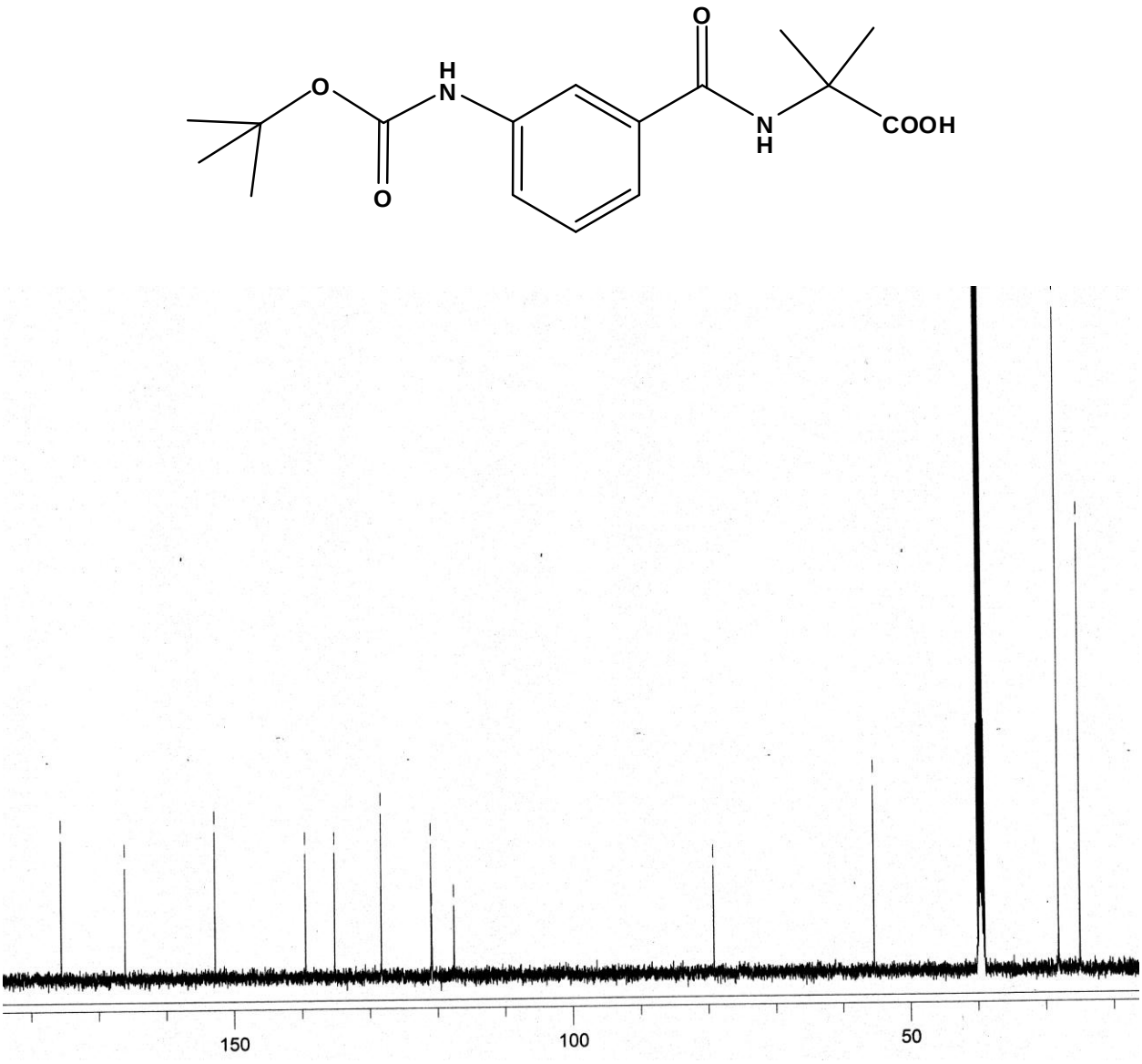
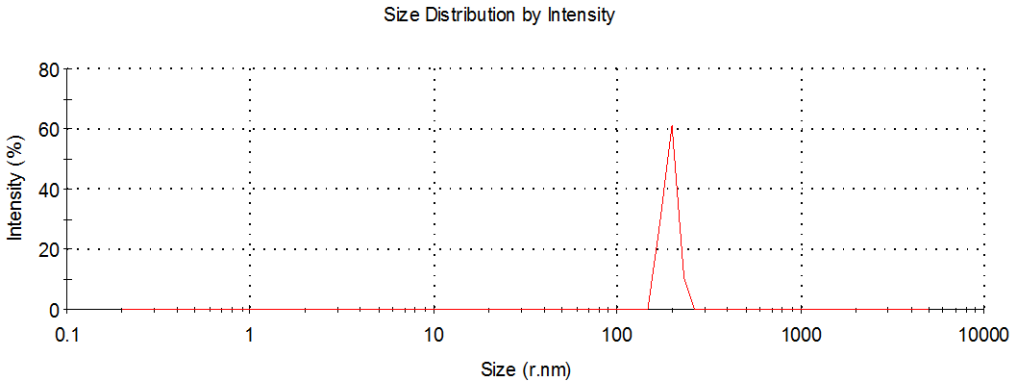
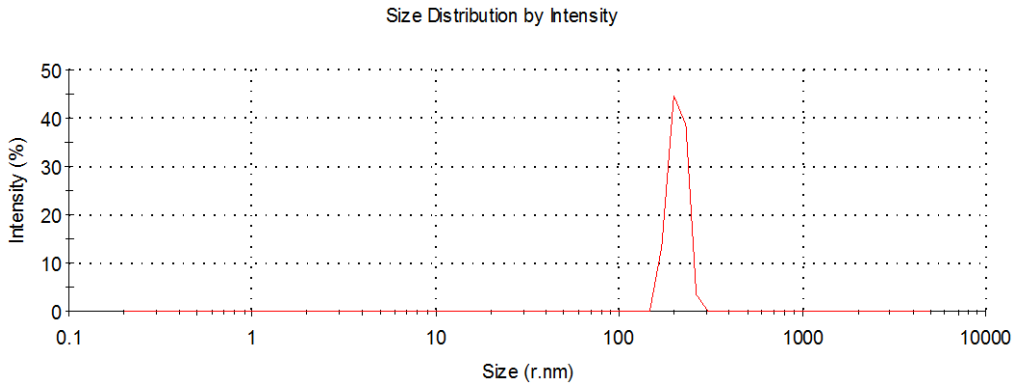



**Figure S15:** $^{13}C$ NMR ($CDCl_3$, 125 MHz, δppm ) spectra of Boc-Maba-Aib-OH.

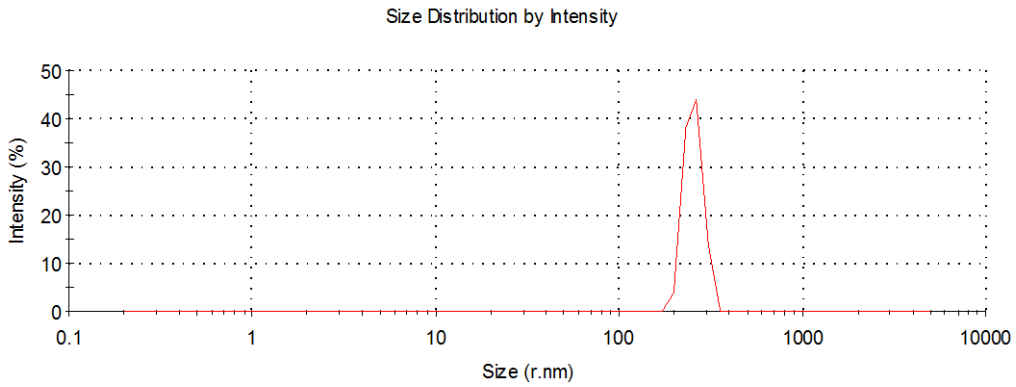
Figure S16. The dynamic light scattering (DLS) experiments of peptide **1**, **2** and **3** were performed in methanol water solution.



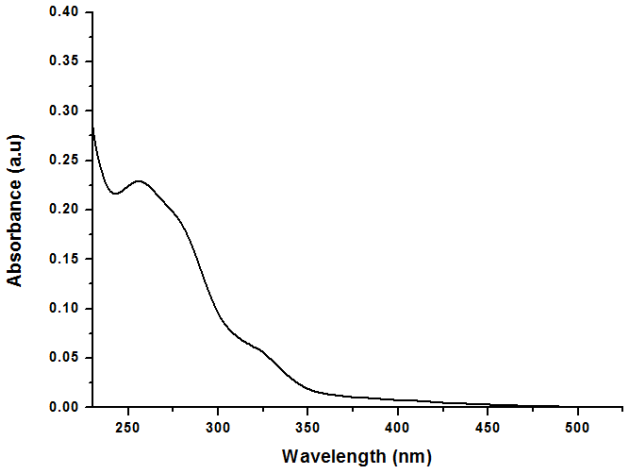

Peptide **1**



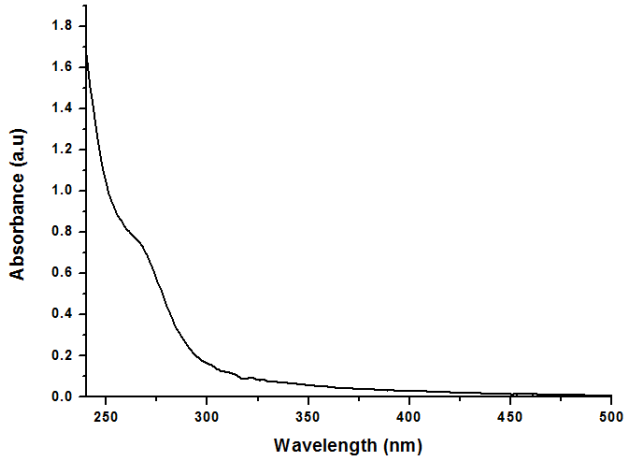

Peptide **2**




Peptide **3**




(a)




(b)

Figure S17: Absorption spectra of peptide **1** (a) and peptide **2** (b)