Electronic Supplementary Material (ESI) for RSC Advances.

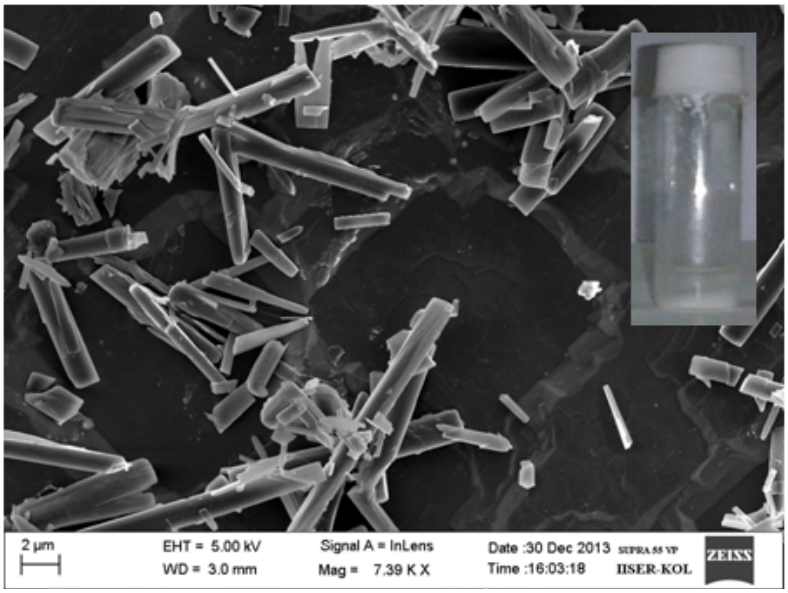
Sonication-induced instant fibrillation and fluorescent labeling of tripeptide fibers†

Apurba Pramanik, Arpita Paikar, and Debasish Haldar*

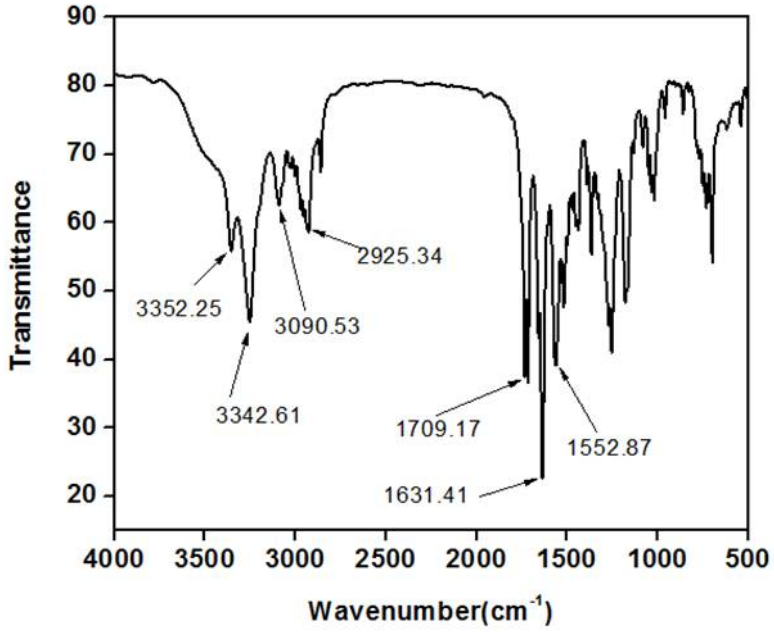
Department of Chemical Sciences, Indian Institute of Science Education and Research – Kolkata, Mohanpur, Nadia, West Bengal – 741252, India.

E-mail: deba_h76@yahoo.com

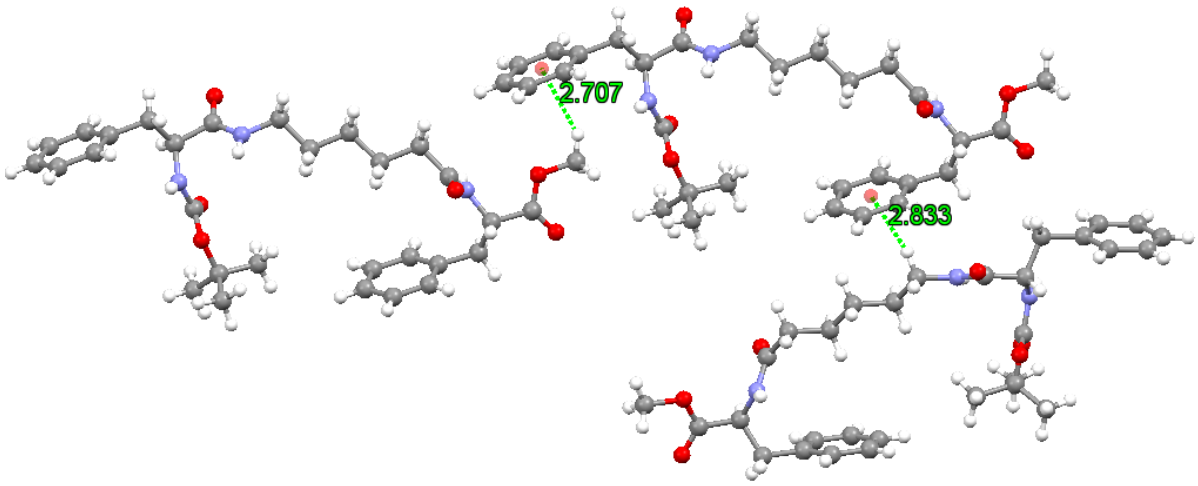
Table of contents



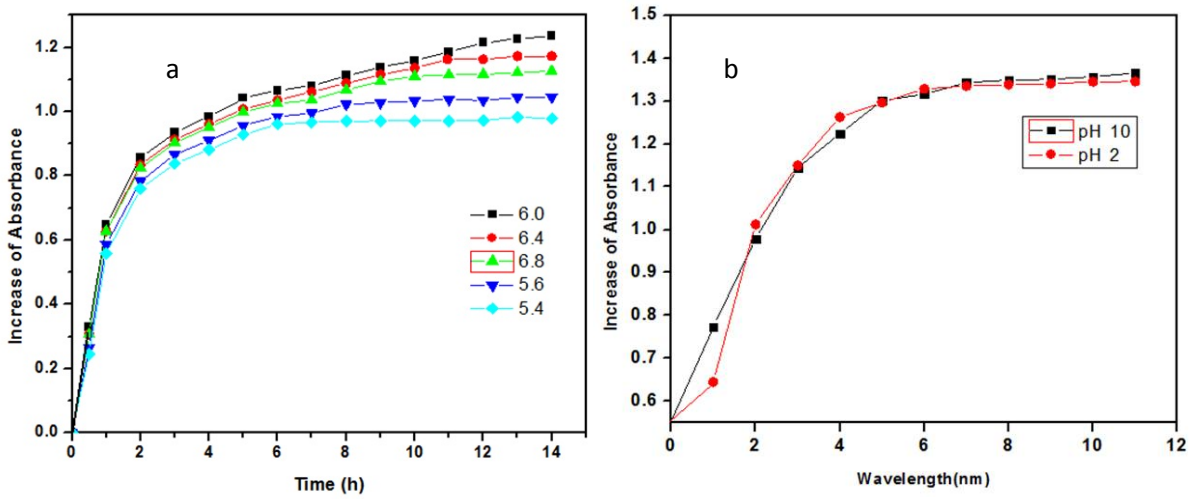
ESI Figure S1: FE-SEM image of the peptide **1** crystals by ultrasound exposure in cyclohexane.



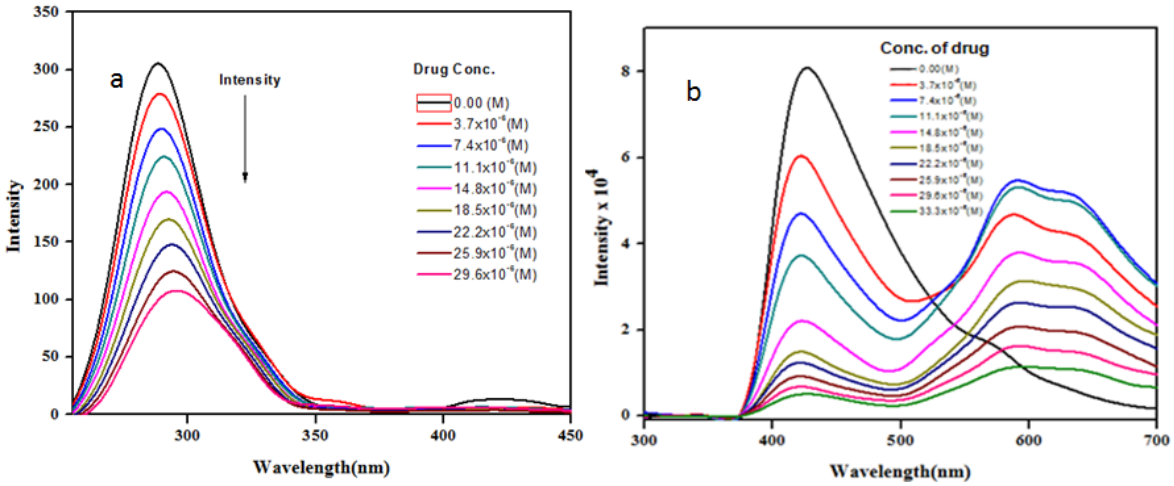
ESI Figure S2: FT-IR-Spectra of Boc-Phe-Acp-Phe-OMe.



ESI Figure S3: The C-H-π interactions between Phe (1) ring and ester methyl and Phe (3) ring and ε-methylene of caproic acid.



ESI Figure S4: We have checked the drug release from peptide**1** fibers at different pH like 2.0, 5.4, 5.6, 6.0, 6.4, 6.8, 10.0. From these we can conclude that the release of drug is more efficient at the range of pH 6.0-6.8. The above figure (a) shows that the peptide fibers is slowly releasing the encapsulated drug at pH 6.0-6.8 and the drug release undergo completion in the time period 14 hr. But when the pH is in the range of 5.4-5.6 the time required for the complete drug released is 11-12 hr. The above figure (b) shows that the in the higher acidic and basic pH (i.e. pH- 2, 10) the encapsulated drugs are complete release in the time period 5-6 hr.



ESI Figure S5: The fluorescence measurements of peptide 1 with carbamazepine. (a) With increasing carbamazepine drug concentration, the intensity of the phenyl group (287 nm) pick is decreased (excitation at 255nm). (b) Fluorescence spectra of peptide **1** with increasing concentration of Carbamazepine. The excitation wavelength is 287 nm.

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 2: 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 (d, 1H, *J* ¼ 10 Hz, 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)-ε-Acp(2)-OMe 3. 2 g (7.53 mmol) of Boc-Phe-OH was dissolved in 25 mL DCM in an ice-water bath. H-ε-Acp-OMe was isolated from 2.18 g (15.06 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 1.6 g (7.53 mmol) dicyclohexylcarbodiimide (DCC) and 1 g (7.53 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), 1(M) sodium carbonate (3x50 mL) and brine (2x50 mL) and dried over anhydrous sodium sulphate. It was evaporated in a vacuum to yield Boc-Phe-ε-Acp-OMe as a white solid.

Yield: 2.66 g (6.77 mmol, 88.97%).

^{1}H NMR (500 MHz, $CDCl_3$,δ in ppm): 7.26–7.15 (m, 5H, phenyl ring protons), 6.13-6.11 (t, 1H, ε-Acp(2) NH), 5.25 (br, 1 H, Phe NH), 4.27 (br, 1 H, Phe C^{α} H), 3.61 (s, 3 H,–OCH_3), 3.14-3.12 (m, 2 H, Phe C^{β} H), 3.00-2.97 (br, 2 H, ε-Acp C^{δ} H), 2.24-2.21 (m, 2H, ε-Acp $C^{\varepsilon}H$), 1.55-1.53 (m, 2H, ε-Acp $C^{\alpha}H$), 1.50-1.48 (m, 2H, ε-Acp $C^{\beta}H$), 1.35 (s, 9H, Boc -CH_3), 1.28-1.24 (m, 2H, ε-Acp $C^{\gamma}H$). $^{13}CNMR$ (125 MHz, $CDCl_3$, δ in ppm): 174.35, 171.56, 155.82, 137.30, 129.69, 128.90, 127.17, 80.32, 56.36, 51.85, 39.48, 39.35, 34.33, 30.03, 29.31, 26.56, 25.33.

(c) Boc-Phe(1)-ε-Acp(2)-OH 4. To 2.5 g (6.36 mmol) of Boc-Phe-ε-Acp-OMe, 25 mL MeOH and 2(M) 10 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: 2.2 g (5.8 mmol, 91.19%).

^{1}H NMR (500 MHz, DMSO-d_6, δ in ppm): 12.01 (br, 1 H, –COOH), 7.85-7.83 (m, 1 H, ε-Acp(2) -NH), 7.26–7.17 (m, 5 H, phenyl ring protons) 6.83-6.82 (d, *J*=5, 1H, Phe -NH), 4.09 (m, 1H, Phe C^{α} H), 3.06-3.04 (m, 2H, Phe C^{β} H), 3.02-2.99 (m, 2H, ε-Acp-C^{δ}H), 2.75-2.50 (m, 1H, ε-Acp C^{ε}H), 2.18-2.15 (m, 2H, ε-Acp C^{α}H),1.48-1.45 (m,2H, ε-Acp C^{β}H)1.34 (s, 9H,Boc -CH_3),1.31-1.30(m, 3H, ε-Acp C^{γ}H and ε-Acp C^{ε}H). ^{13}C NMR (125 MHz, $CDCl_3$, δ in ppm): 174.42, 171.28, 155.13, 138.14, 129.17, 127.96, 126.12, 77.92, 55.76, 39.16, 38.99, 37.77, 33.60, 28.72, 25.81, 24.20.

(d) Boc-Phe(1)-ε-Acp(2)-Phe(3)-OMe 1. 2.2 g (5.8 mmol) Boc-Phe-ε-Acp–OH was dissolved in 10 mL of DMF in an ice–water bath. H-Phe-OMe 2.1 g (11.6 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 1.2 g (5.8 mmol) of dicyclohexylcarbodiimide (DCC) and 784 mg (5.8 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 : 1.45 g (2.68 mmol, 46.32%).

^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm): 7.26-7.08 (m, 10 H, 2 phenyl ring protons), 6.22 (s, 2H, Phe -NH), 5.30 (s, 1 H, ε-Acp -NH), 4.85 (m, 1H, Phe C^{α} H),4.31 (br, 1H, Phe C^{α} H), 3.70 (s, 3 H,-OCH_3),3.14-3.09 (m, 4 H, Phe C^{β}H) , 3.06-3.02 (m, 2H, ε-Acp C^{ε} H), 2.11-2.09 (t, 2H, ε-Acp C^{α}H), 1.52-1.50(m, 2H, ε-Acp C^{δ}H), 1.51 (s, 9 H, Boc -CH_3), 1.38-1.36 (m, 2H, ε-Acp C^{β}H), 1.24-1.18 (m, 2H, ε-Acp C^{γ}H), ^{13}CNMR (125 MHz, $CDCl_3$, δ in ppm): 172.97, 172.92, 171.65, 155.81, 137.38, 136.40, 129.71, 129.58, 128.93, 127.47, 127.19, 80.36, 56.39, 53.44, 52.72, 39.51, 39.32, 38.20, 36.35, 30.12, 29.22, 28.68, 26.48, 25.28.

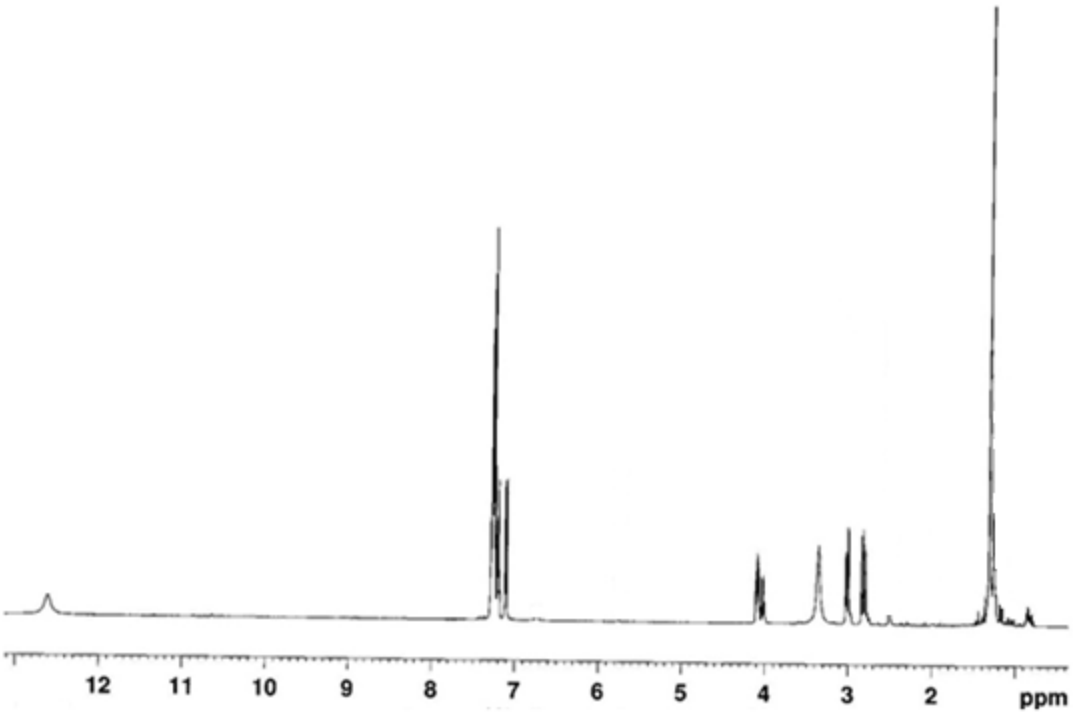


Fig S4: ^{1}H NMR (500MHz, DMSO-d_6) spectra of Boc-Phe-OH

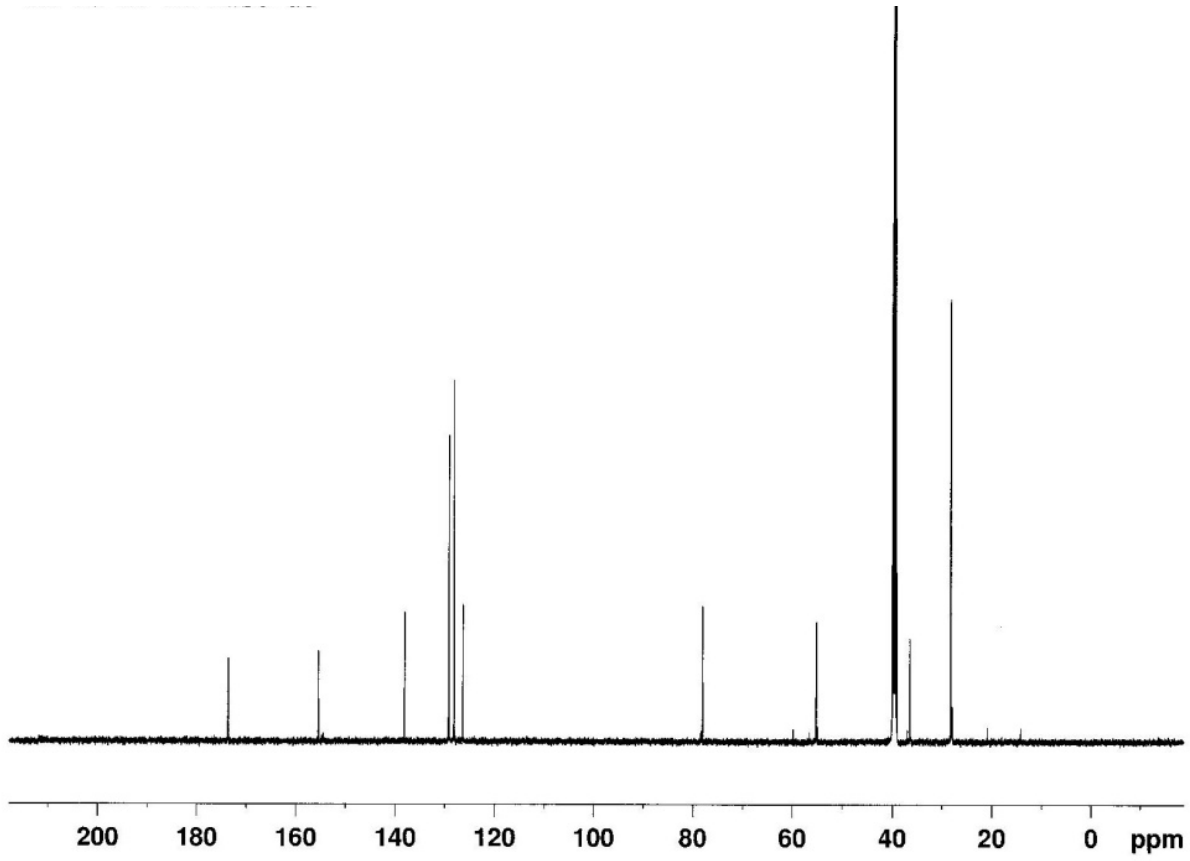


Fig S5: ^{13}C NMR (125 MHz, DMSO-d_6) spectra of Boc-Phe-OH

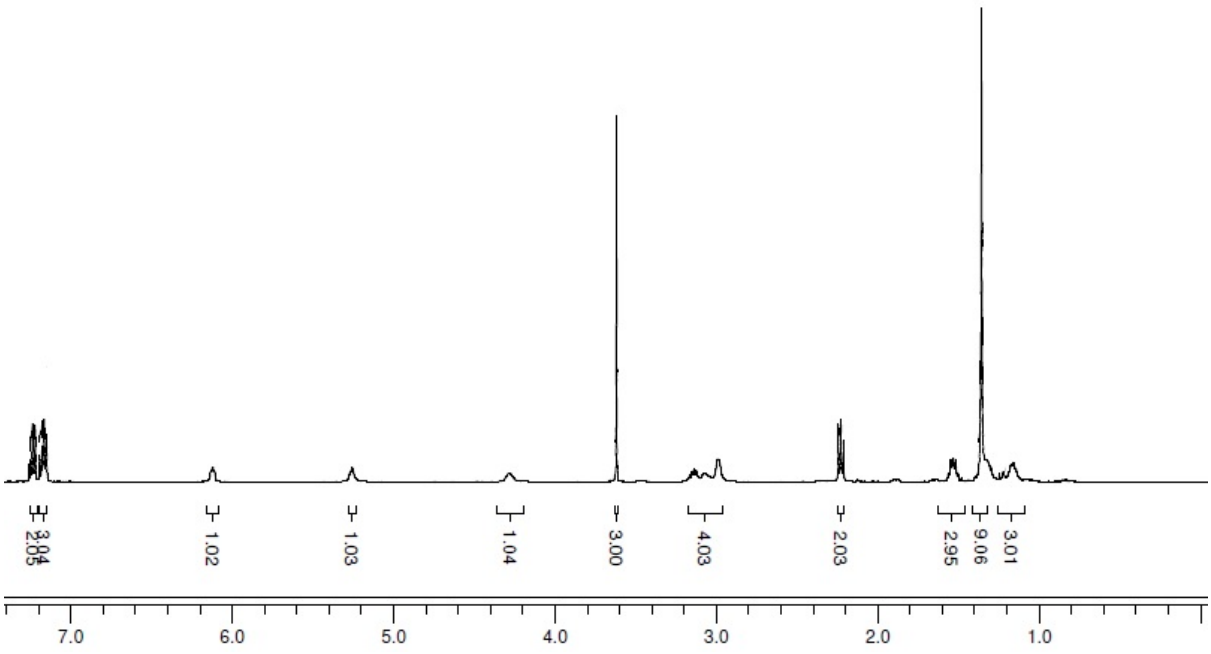


Fig S6: ^{1}H NMR(500 MHz, $CDCl_3$) of Boc-Phe-Acp-OMe

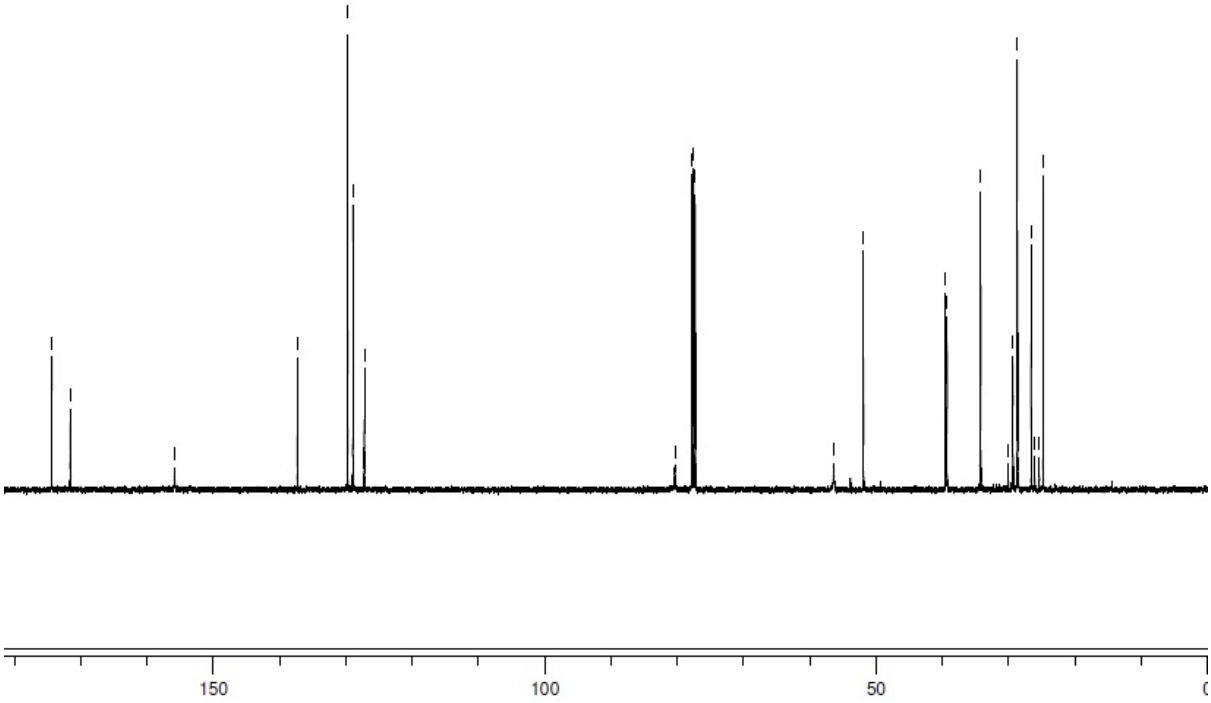


Fig S7: ^{13}C NMR (125 MHz, $CDCl_3$) of Boc-Phe-Acp-OMe

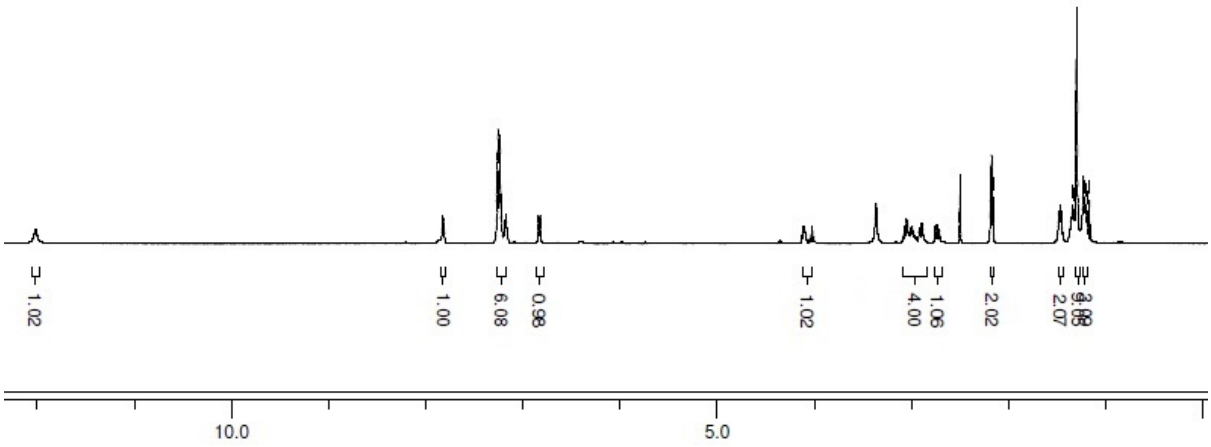


Fig S8: ^{1}H NMR(500 MHz, DMSO-d_6) of Boc-Phe-Acp-OH

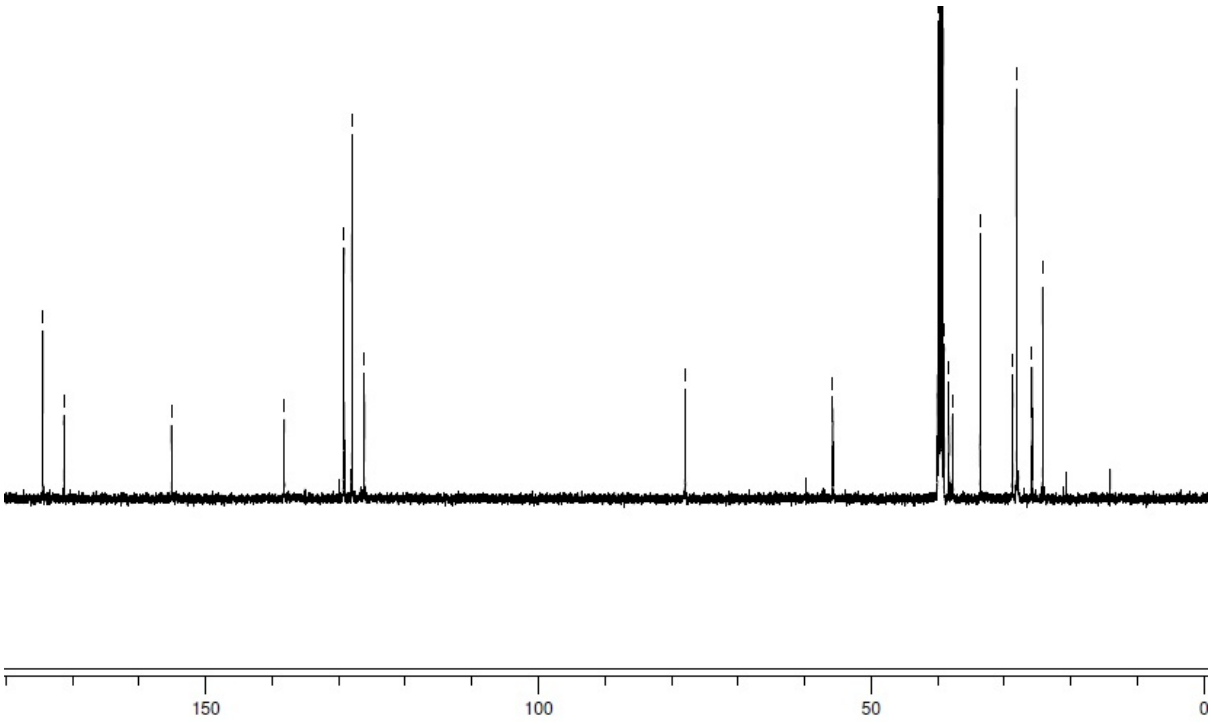


Fig S9: ^{13}C NMR (125 MHz, DMSO-d_6) of Boc-Phe-Acp-OH

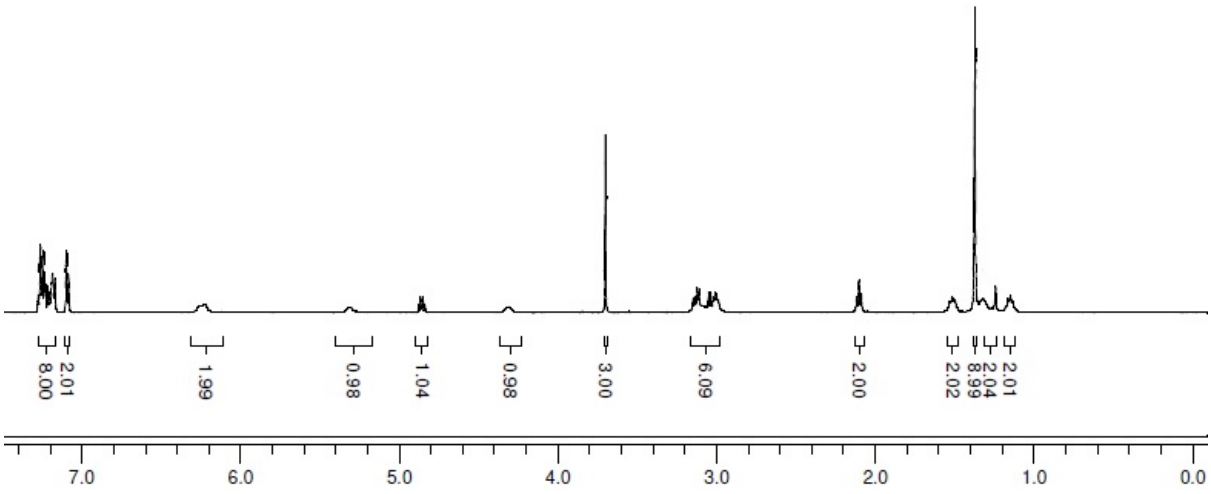


Fig S10: ^{1}H NMR(500 MHz, $CDCl_3$) of Boc-Phe-Acp-Phe-OMe

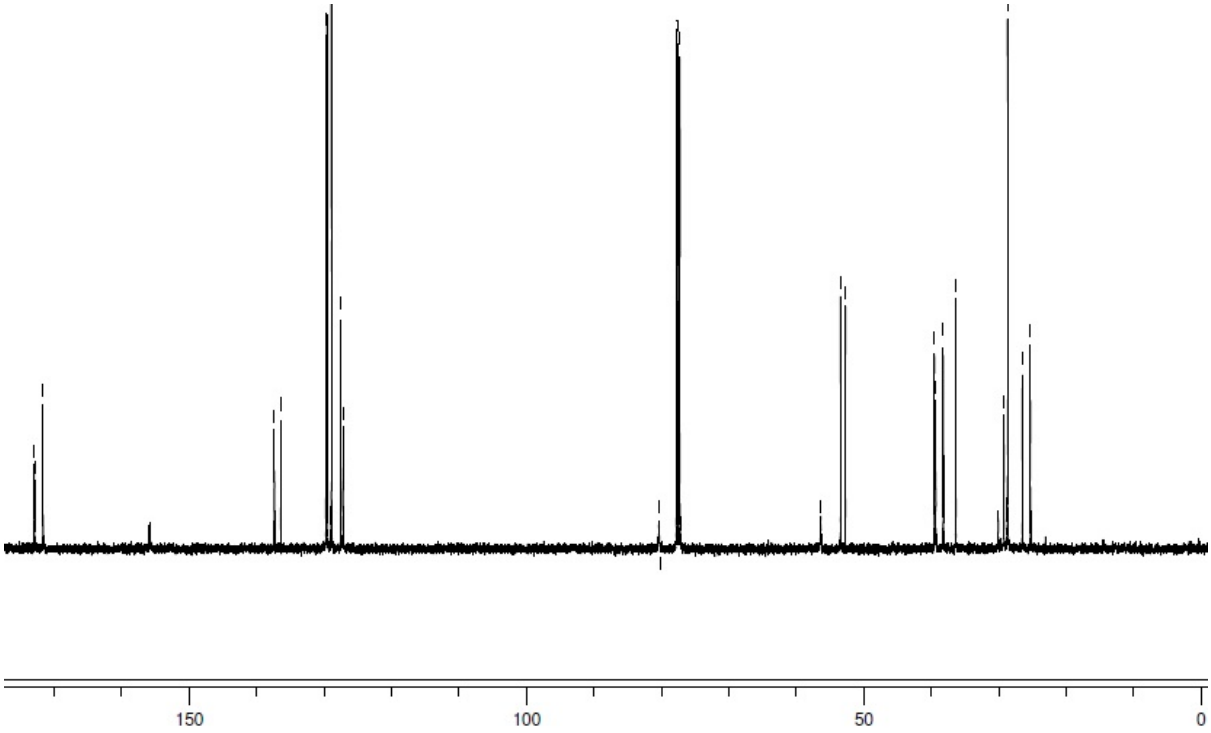


Fig S11: ^{13}C NMR (500 MHz, $CDCl_3$) of Boc-Phe-Acp-Phe-OMe

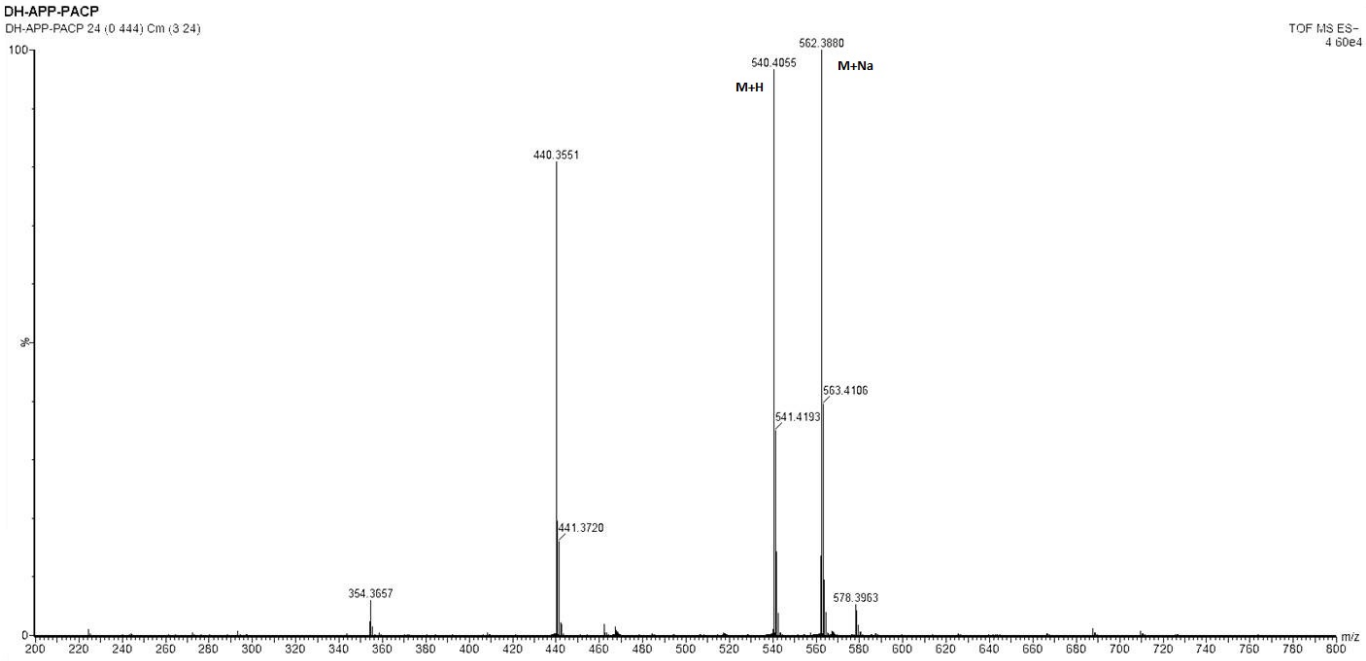


Fig S12: Mass spectra of Boc-Phe-Acp-Phe-OMe