Electronic Supplementary Material (ESI) for Materials Advances.

Supporting Information

Hydrophobic nanofibers: peptide-based functional anti-fouling material

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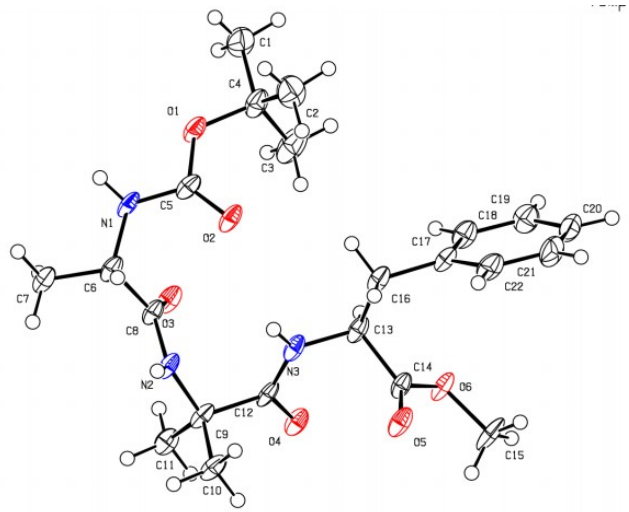

Fig. S1: The ORTEP diagram of peptide **1** including the atom numbering scheme. Thermal ellipsoids are shown at the level of 50% probability.

Table S1: Selected torsion angles (°) for peptide **1**

C6-N1-C5-O1	168.61 (3)	ω_0	N2-C9-C12-N3	26.74 (4)	ψ_2
C5-N1-C6-C8	-49.98 (4)	ϕ_1	C9-C12-N3-C13	174.64 (3)	ω_2
N1-C6-C8-N2	145.05 (4)	ψ_1	C12-N3-C13-C14	-56.38 (4)	ϕ_3
C6-C8-N2-C9	174.65 (3)	ω_1	N3-C13-C17-O6	137.63 (3)	ψ_3
C8-N2-C9-C12	62.52 (4)	ϕ_2			

Table S2: Intermolecular hydrogen bonding parameters of peptide **1**

D-H….A	H….A(Å)	D…A(Å)	D-H…A (°)
N1-H1…O5[a]	2.26(3)	3.107 (3)	169.0 (3)
N2-H2…O4[b]	2.02(3)	2.879 (3)	174.0 (3)
N3-H3…O2	2.57(3)	3.212(3)	132.0 (3)

Symmetry equivalent a = 1-x, -1/2+y, ½-z; b = 1+x, y, z

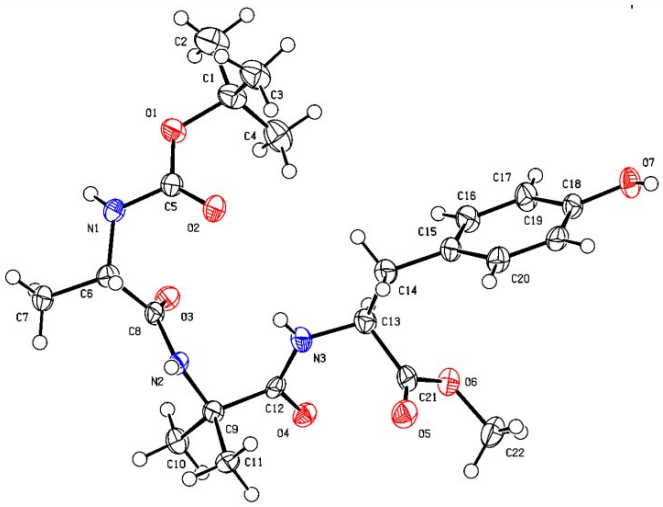

Fig. S2: The ORTEP diagram of peptide **2** including the atom numbering scheme. Thermal ellipsoids are shown at the level of 50% probability.

Table S3: Intermolecular hydrogen bonding parameters of peptide **2**

D-H….A	H….A(Å)	D…A(Å)	D-H…A (°)
N2-H2…O4[a]	2.0300	2.875 (5)	167.9 (4)
O7-H7…O3[b]	2.1100	2.880(4)	157.00

Symmetry equivalent a = 1+x, y, z; b = -3/2-x, -y, -1/2+z

Table S4: Selected torsion angles (°) for peptide **2**

C6-N1-C5-O1	162.3 (4)	ω_0	N2-C9-C12-N3	27.5 (5)	ψ_2
C5-N1-C6-C8	-52.4 (5)	ϕ_1	C9-C12-N3-C13	170.0 (4)	ω_2
N1-C6-C8-N2	145.2 (4)	ψ_1	C12-N3-C13-C21	-54.4 (5)	ϕ_3
C6-C8-N2-C9	168.0 (4)	ω_1	N3-C13-C21-O7	136.8 (4)	ψ_3
C8-N2-C9-C12	67.3 (5)	ϕ_2			

ESI Table S5: Crystal data and structure refinement for Peptide **1**.

Identification code	KHBAAPOME
Empirical formula	$C_{22}H_{33}N_3O_6$
Formula weight	435.51
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	5.8857(1)
b/Å	18.9299(5)
c/Å	21.3905(6)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2383.24(10)
Z	4
ρ_{calc}g/cm^3	1.214
μ/mm^{-1}	0.729
F(000)	936.0
Crystal size/mm^3	0.20× 0.23× 0.28
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	3.1 to 66.3
Index ranges	$-6 \leq h \leq 6$, $-22 \leq k \leq 22$, $-25 \leq l \leq 25$
Reflections collected	20483
Independent reflections	4152
Goodness-of-fit on F^2	1.053
Largest diff. peak/hole / e Å^{-3}	0.25/-0.34
Flack parameter	0.04(19)
R	0.0541
WR2	0.1399

ESI Table S6: Crystal data and structure refinement for Peptide **2**.

Identification code	BALAIBTYR
Empirical formula	$C_{22}H_{33}N_3O_7$
Formula weight	451.51
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	5.9599(4)
b/Å	19.6632(13)
c/Å	20.5253(10)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2405.4(3)
Z	4
ρ_{calc}g/cm^3	1.247
μ/mm^{-1}	0.773
F(000)	968
Crystal size/mm^3	0.24× 0.26× 0.27
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	3.1 to 66.1
Index ranges	$-7 \leq h \leq 6$, $-23 \leq k \leq 21$, $-12 \leq l \leq 24$
Reflections collected	6038
Independent reflections	3551
Goodness-of-fit on F^2	1.032
Largest diff. peak/hole / e Å^{-3}	0.26/-0.31
Flack parameter	-0.3(4)
R	0.0629
WR2	0.1756

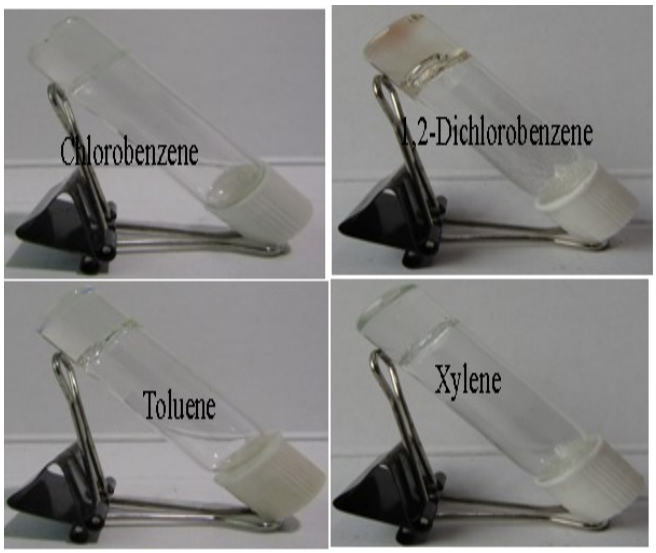

Fig. S3: Photograph of peptide **1** transperent gel in different solvents.

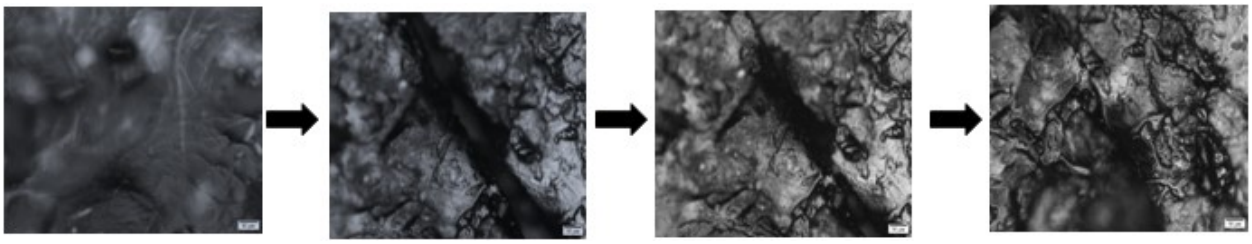

Fig. S4: Images showing self-healing nature of organo gel of peptide **1** investigated using POM.

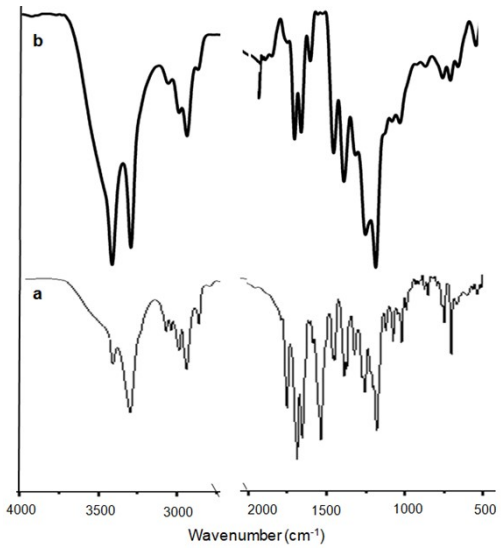

Fig. S5: FT-IR spectra of (a) peptide **1** and (b) peptide 1 xerogel.

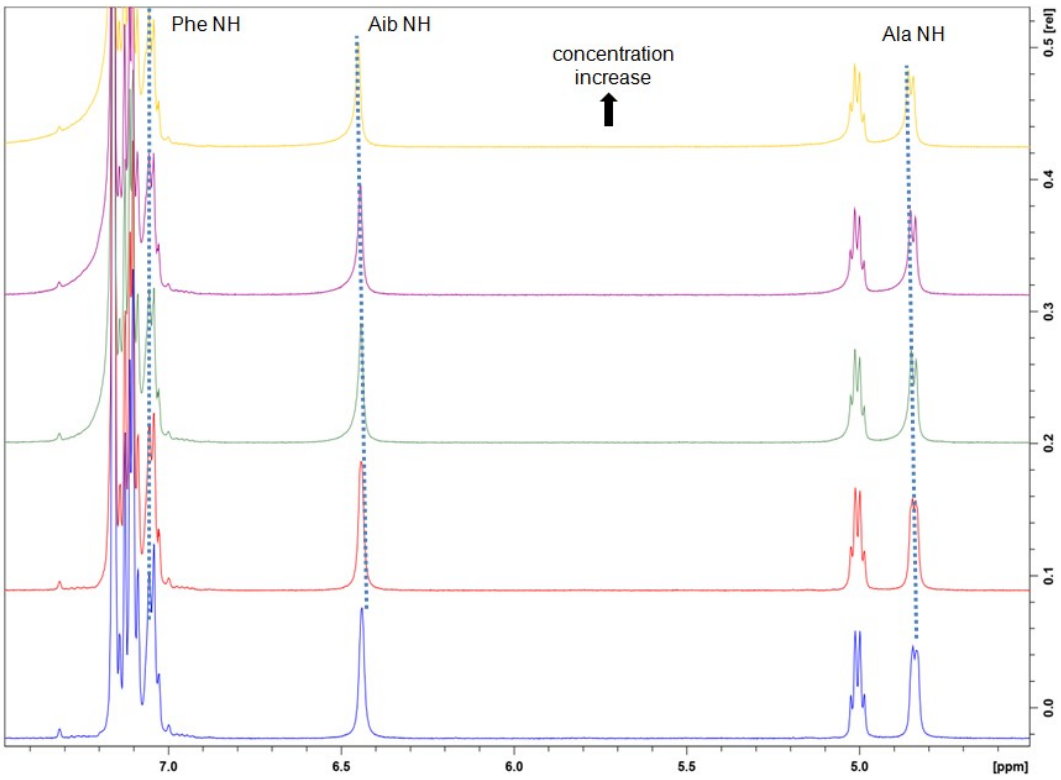

Fig. S6: Part of the ^{1}H NMR spectra of peptide 1 in C_6D_6 exhibits downfield shift of the Ala NH and Aib NH protons with increasing concentration, which suggest that the NH protons are intermolecular hydrogen bonded.

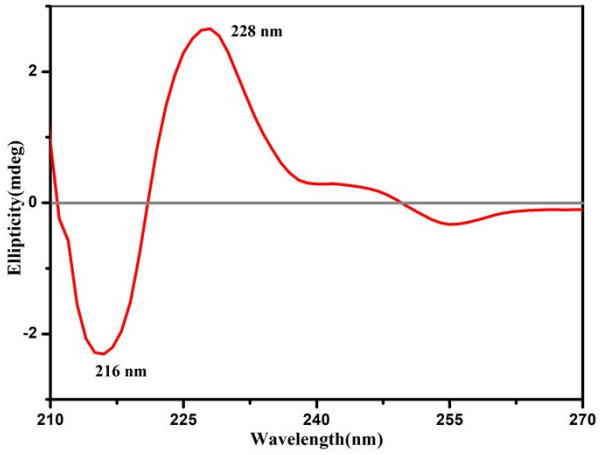

Fig. S7: CD spectra of peptide 1 exhibits positive band at 228 nm and negative bands at 216 and 255 nm.

Experimental

Peptide Synthesis: The reported peptide was synthesized by traditional solution-phase reaction using racemisation free fragment condensation strategy by N,N'-dicyclohexylcarbodiimide /1-hydroxybenzotriazole (DCC/ HOBt). The C-terminus was protected as a methyl ester and tertiary-butoxycarbonyl group was used for N-terminal protection. The products were purified by column chromatography using silica (100-200-mesh size) gel as stationary phase and n-hexane-ethylacetate 9:1 as eluent. The compounds were characterized by 400 MHz ^{1}H NMR spectroscopy, 100 MHz ^{13}C NMR spectroscopy, solid-state FT-IR Spectroscopy and mass spectrometry.

Synthesis of Boc-Ala-Aib-OMe:

0.47g (2.5 mmol) of Boc-Ala was dissolved in 20 mL dry DCM and minimum DMF to make it soluble and kept in an icewater bath. Aib-OMe was isolated from 0.47 g (4 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate, and ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed immediately by 0.79 g (3.8 mmol) N,N′-Dicyclohexylcarbodiimide (**DCC)** and 0.55 g (4.1 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). The organic layer was washed with 2 M HCl (3 × 50 mL), brine (2 × 50 mL), 1M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL) dried over anhydrous sodium sulfate; and evaporated in a vacuum to yield compound Boc-Ala-Aib-OMe as a gelly like compound. The product was purified by silica gel (60-120mesh) using hexane-ethyl acetate as eluent.

Yield: 0.67g (2.3 mmol, 92%)

^{1}H NMR (500 MHz, DMSO-d_6, δ ppm)

7.39[s, Aib-NH], 5.6[s, Ala-NH], 3.44[s, 3H-OMe], 4.1[m, C^{α}-H], 1.18[s, 9H], 1.25[s, 6H], 1.07[s, 3H]

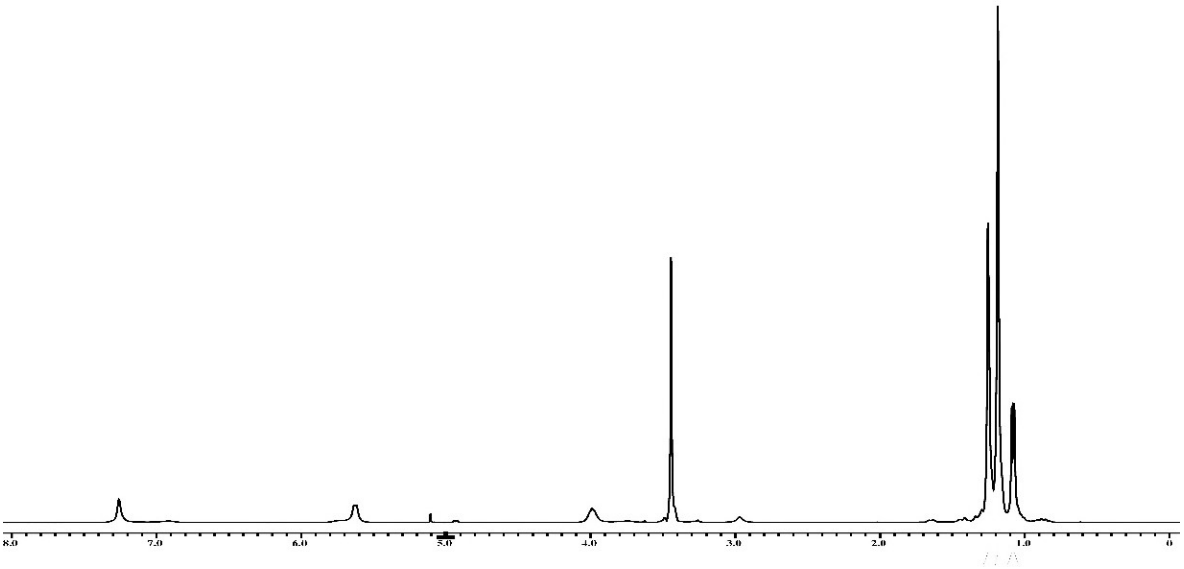

Fig. S8: ^{1}H NMR spectra of Boc-Ala-Aib-OMe

^{13}C NMR (125 MHz, DMSO-d_6, δ ppm)

174.43, 172.35, 155.30, 55.6, 79.48, 55.6, 51.95,49.46, 27.93, 24.47, 17.8

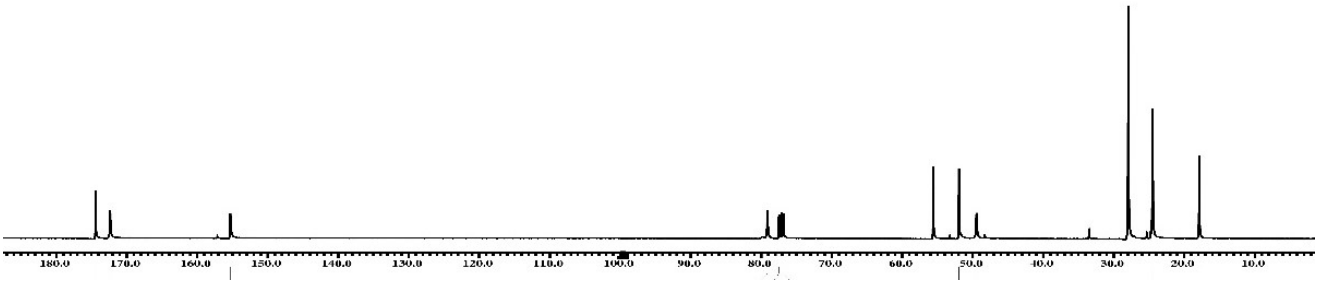

Fig. S9: ^{13}C NMR spectra of Boc-Ala-Aib-OMe

Synthesis of Boc-Ala-Aib-OH: **(Saponification)**

To 0.61 g (2.12 mmol) of compound Boc-Ala-Aib-OMe, 10 mL MeOH and 2M 10mL NaOH were added in an ice bath and the progress of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After10 h, the methanol was removed under vacuum; the residue was dissolved 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 1M HCl and it was extracted

with ethyl acetate (3 x 50 mL). The extracts were pooled, dried over anhydrous sodium sulphate, and evaporated under vacuum to obtain the compound as a white solid.

Yield: 0.56g (2.0 mmol, 98 %).

Synthesis of Boc-Ala-Aib-Phe-OMe 1

0.56g (2.07 mmol) of Boc-Ala-Aib-OH was dissolved in 20 mL dry DCM and minimum DMF to make it soluble and kept in an icewater bath. Phe-OMe was isolated from 0.81 g (4.5 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate, and ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed immediately by 0.64 g (3.1 mmol) N,N′-Dicyclohexylcarbodiimide (**DCC)** and 0.55 g (4.1 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). The organic layer was washed with 2 M HCl (3 × 50 mL), brine (2 × 50 mL), 1M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL) dried over anhydrous sodium sulfate; and evaporated in a vacuum to yield compound Boc-Ala-Aib-Phe-OMe as a solid. The product was purified by silica gel (60-120mesh) using hexane-ethyl acetate as eluent.

Yield: 0.71g (1.6 mmol, 78%)

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm)

7.25-7.04[m,6H, 5H aromatic, 1 NH], 6.94-6.87[b,1H,NH], 6.78-6.71[b,1H,NH], 5.07-5.01[m,1H, C^{α}-1H-Ala], 4.81-4.75[m,1H, C^{α}-1H-Phe], 3.66[s,3H,OMe], 3.15-3.01[m, 2H,beta-Phe], 1.44[s, 6H], 1.40[s, 9H], 1.27[m, 3H].

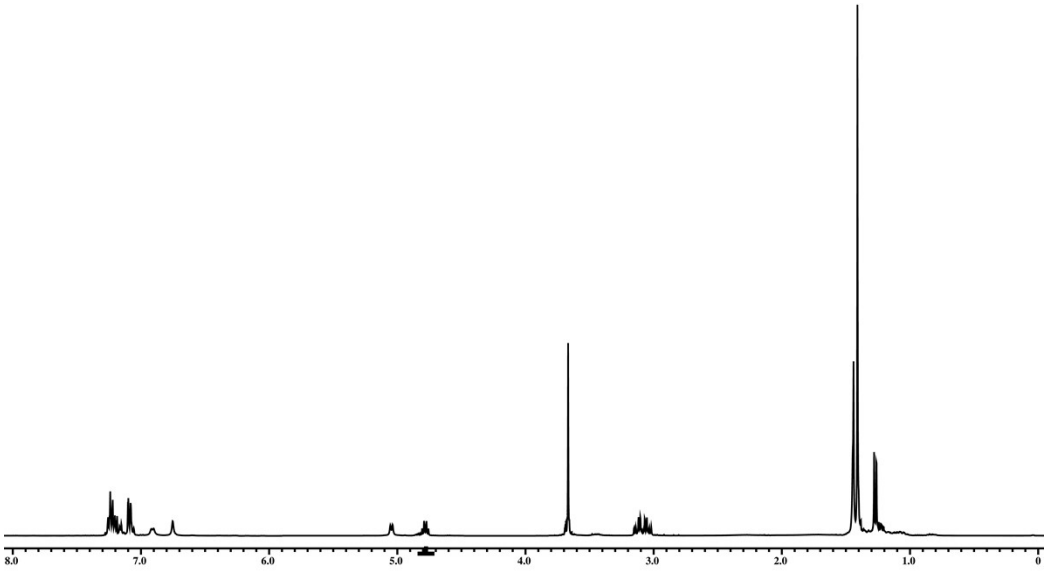

Fig. S10: ^{1}H NMR Spectra of Boc-Ala-Aib-Phe-OMe **1**.

^{13}C NMR (125 MHz, $CDCl_3$, δ ppm):

173.99,172.05,171.94,155.79,136.24,129.37,128.50,127.04,80.30,57.13,52.26,38.82,28.35,25.19,17.88.

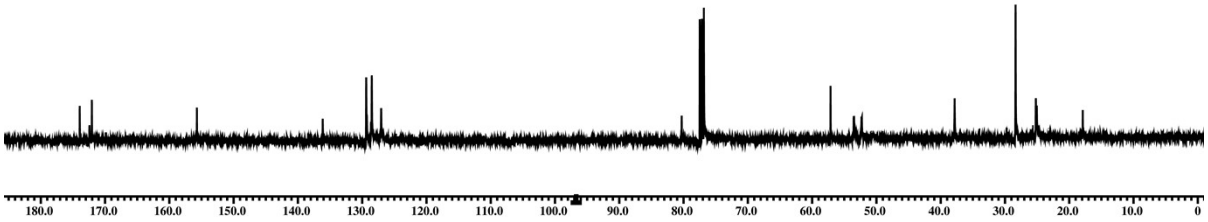

Fig. S11: ^{13}C NMR Spectra of Boc-Ala-Aib-Phe-OMe **1**.

Mass Spectrometry

Mass spectrometry was carried out on a Waters Corporation Q-Tof Micro YA263 high-resolution mass spectrometer by electrospray ionization (positive-mode). Molar mass **435.2427** g/mol

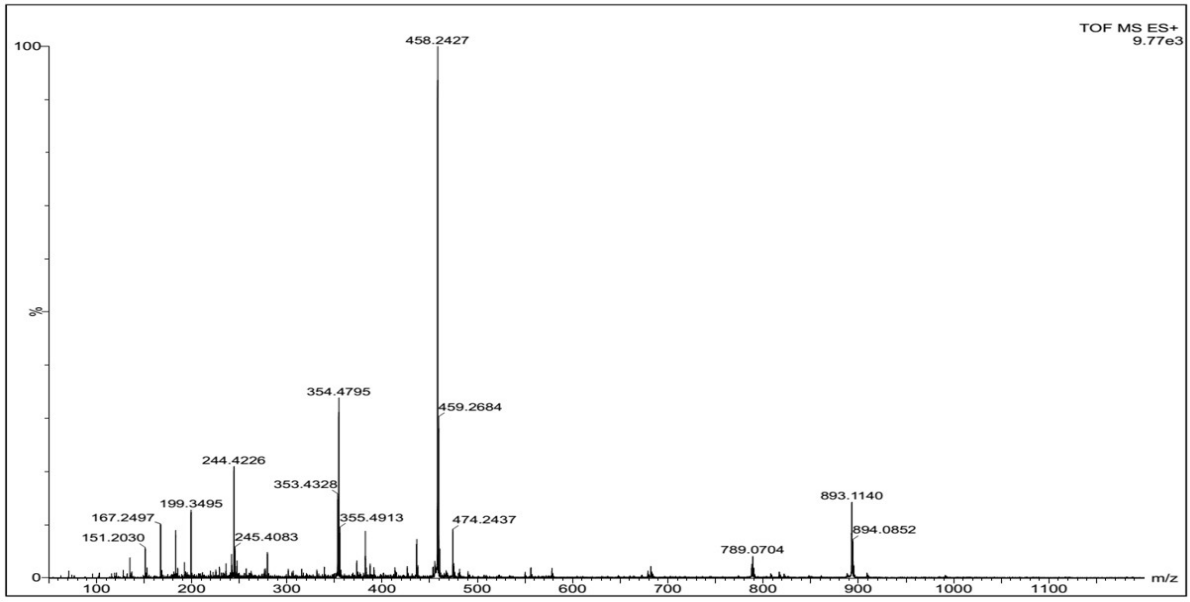

Fig. S12: Mass spectrum of Boc-Ala-Aib-Phe-OMe **1**.

Synthesis of Boc-Ala-Aib-Tyr-Ome 2

0.7g (2.54 mmol) of Boc-Ala-Aib-OH was dissolved in 20 mL dry DCM and minimum DMF to make it soluble and kept in an icewater bath. Tyr-OMe was isolated from 0.99g (5.1 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate, and ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed immediately by 0.8g (3.8 mmol) N,N′-Dicyclohexylcarbodiimide (**DCC)** and 0.68 g (5 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). The organic layer was washed with 2 M HCl (3 × 50 mL), brine (2 × 50 mL), 1M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL) dried over anhydrous sodium sulfate; and evaporated in a vacuum to yield compound Boc-Ala-Aib-Tyr-OMe as a solid. The product was purified by silica gel (60-120mesh) using hexane-ethyl acetate as eluent.

Yield: 0.68g (1.5 mmol, 59%)

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm)

7.07-6.94[m,3H, 2H aromatic, 1 NH], 6.88-6.82[b,1H,NH], 6.75-6.70[m,2H,aromatic], 6.68-6.62[b,1H,NH], 5.05-4.99[m,1H, C^{α}-1H-Ala], 4.81-4.75[m,1H, C^{α}-1H-Phe], 4.03-

4.10[b,1H,OH], 3.72[s,3H,OMe], 3.12-3.00[m, 2H,beta,Tyr], 1.48[s, 6H], 1.44[s, 9H], 1.31[m, 3H].

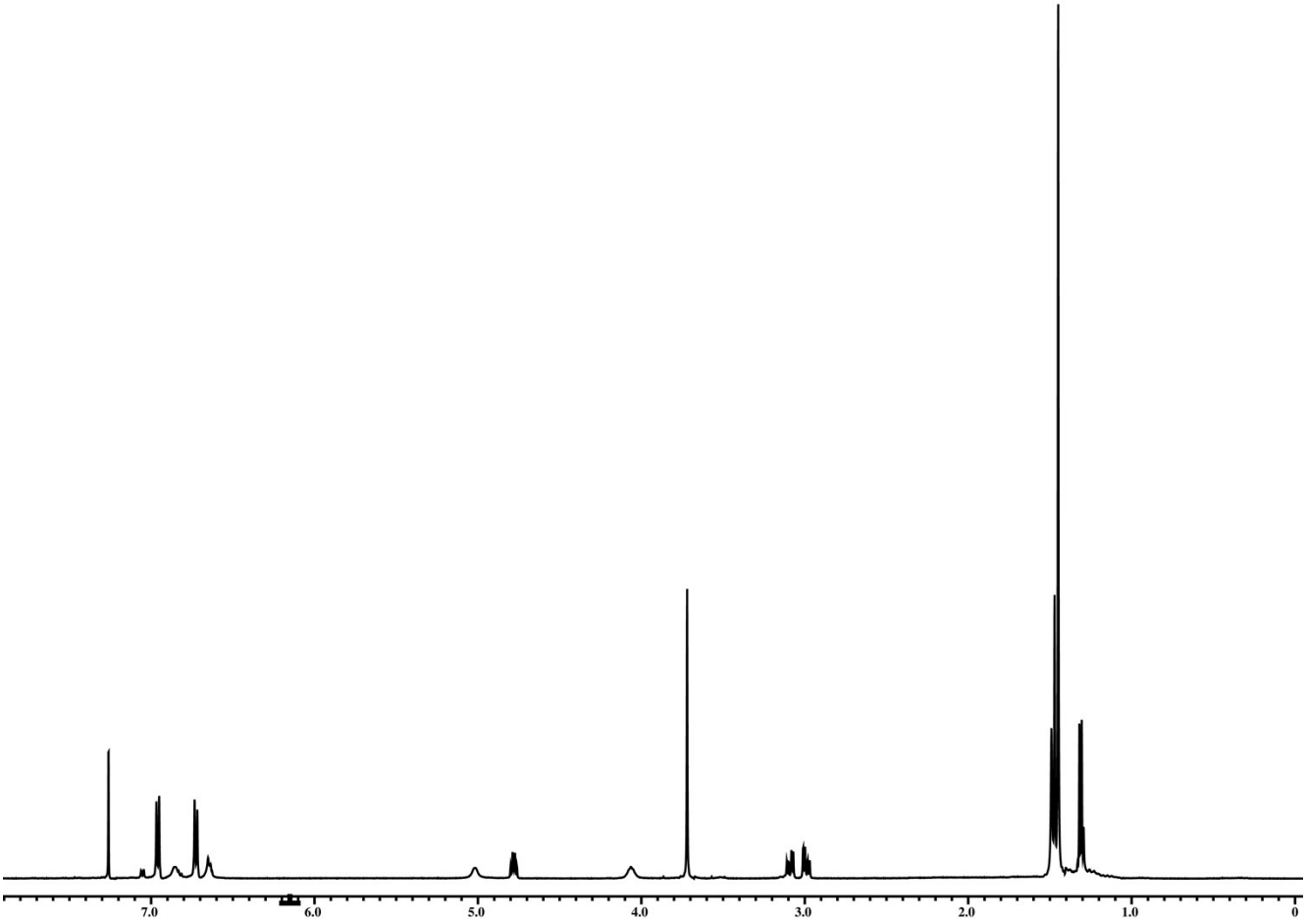

Fig. S13: ^{1}H NMR Spectra of Boc-Ala-Aib-Tyr-OMe **2**.

^{13}C NMR(125 MHz, $CDCl_3$, δ ppm)

174.04, 172.59, 172.33, 155.61, 130.59, 127.50, 115.76, 80.15,57.34, 53.76, 52.55, 51.47,37.22, 28.50,25.51, 25.39, 17.92.

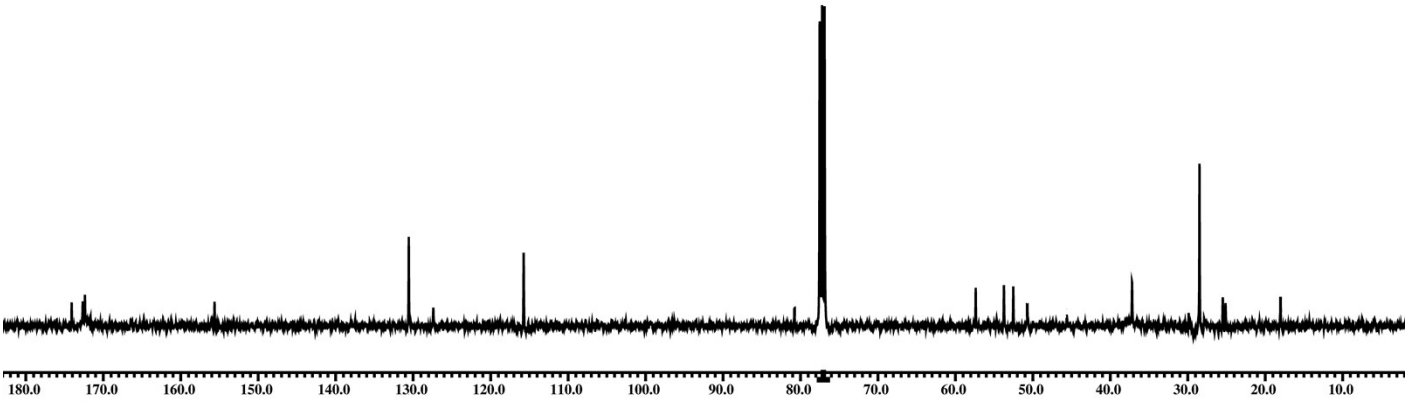

Fig. S14: ^{13}C NMR Spectra of Boc-Ala-Aib-Tyr-OMe **2**.