

SUPPORTING INFORMATION

Tetrapeptide based Hydrogels: for Encapsulation and Slow Release for an
Anticancer Drug at Physiological pH

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Preparation of peptide 1.

Boc-Ala-OH: reference *Tetrahedron*, **2007**, *63*, 7432- 7442.

Boc-Ala-Ile-OMe: 3.87g (20 mM) of Boc-Ala-OH was dissolved in 10 ml of DMF in an ice water bath. H-Ile-OMe was isolated from 7.22g (40 mM) 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 4.12 g (20 mM) of dicyclohexylcarbodiimide (DCC) and 2.7 g (20 mMol) of HOBr. The reaction mixture was allowed to come to room temperature and stirred for 3 days. The residue was taken up in ethyl acetate (40 ml) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 N HCl (3×30 ml), brine (1×30 ml), 1 M sodium carbonate (3×30 ml) and brine (2×30 ml) and dried over anhydrous sodium sulfate and evaporated in *vacuum*. A white material was obtained.

Yield: 4.74 g (15 mM, 75%)

^1H NMR (300 MHz, CDCl_3 , δ): 6.80 (Ile NH, 1H, d, $J= 6.00$), 5.16 (Ala NH, 1H, d, $J= 6.09$), 4.58-4.54 (Ile C^αH , 1H, m), 4.20-4.11 (Ala C^αH , 1H, m), 3.73 (COOCH_3 , 3H, s), 1.92-1.87 (Ile C^βH , 1H, m), 1.45 ($(\text{CH}_3)_3\text{C}$, 9H, s), 1.38-1.34 (Ala C^βH , 3H, d, $J= 6.00$), 1.28-1.10 (Ile $\text{C}^\gamma\text{H}_3$, 3H, m), 0.92-0.88 (Ile $\text{C}^\gamma\text{H}_2$ and $\text{C}^\delta\text{H}_3$, 5H, m)

ESI-MS: found m/z ($\text{M}+\text{Na}$) $^+ = 339.30$; Mcald = 316.39

Anal. Calcd for $\text{C}_{15}\text{H}_{28}\text{N}_2\text{O}_5$ (316.39): C, 56.94, H, 8.92, N, 8.85

Found: C, 56.77, H, 8.80, N, 8.82

Boc-Ala-Ile-OH: to 4.74g (15 mM) Boc-Ala-Ile-OMe, 35 ml MeOH and 25 ml 2 N NaOH were added 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*, the residue was taken in 50 ml of water, washed with diethyl ether (2×50 ml) then the pH of the aqueous layer was adjusted to 2 using drop wise addition of 1 N HCl and it was extracted with ethyl acetate (3×50 ml). The extracts were pooled, dried over anhydrous sodium sulphate and evaporated in *vacuum*. A white solid material was obtained.

Yield: 3.6g (12 mM, 80%)

^1H NMR (300 MHz, DMSO-d₆, δ): 12.64 (COOH, 1H, b), 7.72 (Ile NH, 1H, d, $J= 9$), 6.98 (Ala NH, 1H, d, $J= 7.5$), 4.21-4.16 (Ile C ^{α} H, 1H, m), 4.05-4.00 (Ala C ^{α} H, 1H, m), 1.79-1.76 (Ile C ^{β} H, 1H, m), 1.37 ((CH₃)₃C, 9H, s), 1.21-1.20 (Ile C ^{γ} H₂, 2H, m), 1.15 (Ala C ^{β} H, 3H, d, $J= 6$) 0.86-0.82 (Ile C ^{γ} H₃ and C ^{δ} H₃, 6H, m)

ESI-MS: found m/z (M+Na)⁺= 325.27; Mcald = 302.39

Anal. Calcd for C₁₄H₂₆N₂O₅ (302): C, 55.61, H, 8.67, N, 9.26

Found: C, 55.41, H, 8.52, N, 9.16

Boc-Ala-Ile-Leu-OMe: 3.6g (12 mM) of Boc-Ala-Ile-OH were dissolved in 10 ml of DMF in an ice water bath. H-Leu-OMe was isolated from 5.41g (30 mM) 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 2.47g (12 mM) of dicyclohexylcarbodiimide (DCC) and 1.62 g (12 mM) of HOBr. The reaction mixture was allowed to come to room temperature and stirred for 3 days. The residue was taken up in ethyl acetate (40 ml) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 N HCl (3×30 ml), brine (1×30 ml), 1 M sodium carbonate (3

\times 30 ml) and brine ($2 \times$ 30 ml) and dried over anhydrous sodium sulfate and evaporated in *vacuum*. A white material was obtained.

Yield: 3.8 g (9 mM, 75%)

^1H NMR (300 MHz, CDCl_3 , δ): 6.80 (Leu NH, 1H, d, $J= 6$), 6.58 (Ile NH, 1H, d, $J= 9$), 5.11 (Ala NH, 1H, d, $J= 6$), 4.62-4.55 (Leu C^αH , 1H, m), 4.33-4.28 (Ile C^αH , 1H, m), 4.19-4.17 (Ala C^αH , 1H, m), 3.72 (COOCH_3 , 3H, s), 1.92-1.88 (Leu C^βH , 2H, m), 1.77-1.74 (Ile C^βH , 1H, m), 1.44 ($(\text{CH}_3)_3\text{C}$, 9H, s), 1.34 (Ala C^βH , 3H, d, $J= 6$), 1.26-1.11 (Ile C^γH , 3H, m), 0.94-0.87 (Leu C^γH , Ile C^γH and C^δH , 12H, m)

ESI-MS: found m/z ($\text{M}+\text{Na}$) $^+ = 452.04$; ($\text{M}+\text{K}$) $^+ 468.12$; Mcald = 429.55

Anal. Calcd for $\text{C}_{21}\text{H}_{39}\text{N}_3\text{O}_6$ (429): C, 58.72, H, 9.15, N, 9.78

Found: C, 58.68, H, 9.05, N, 9.72

Boc-Gly-Ala-Ile-Leu-OMe: 0.9g (5 mM) of Boc-Gly-OH was dissolved in 10 ml of DMF in an ice water bath. H-Ala-Ile-Leu-OMe was isolated from 3.8g (9 mM) of the corresponding Boc-Ala-Ile-Leu-OMe by treatment with TFA, neutralization with NaHCO_3 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 1.01g (5 mM) of dicyclohexylcarbodiimide (DCC) and 0.68 g (5 mM) of HOBr. The reaction mixture was allowed to come to room temperature and stirred for 3 days. The residue was taken up in ethyl acetate (40 ml) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 N HCl ($3 \times$ 30 ml), brine ($1 \times$ 30 ml), 1 M sodium carbonate ($3 \times$ 30 ml) and brine ($2 \times$ 30 ml) and dried over anhydrous sodium sulfate and evaporated in *vacuum*. A white material was obtained. It was purified by column chromatography using chloroform-methanol (95:5) eluent.

Yield: 1.4g (3 mM, 60%)

¹H NMR (300 MHz, CDCl₃, δ): 8.16 (Leu NH, 1H, d, J=9), 7.97 (Ile NH, 1H, d, J=7.5), 7.88 (Ala NH, 1H, d, J=6), 5.96 (Gly NH, 1H, b), 4.99-4.95 (Leu C^αH, 1H, m), 4.62-4.59 (Ile C^αH, 1H, m), 4.62-4.53 (Ala C^αH, 1H, m), 4.03 (Gly C^αH, 2H, m), 3.71 (COOCH₃, 3H, s), 1.80- 1.78 (Leu C^βH, 2H, m), 1.68-1.65 (Ile C^βH, 1H, m), 1.43 (C(CH₃)₃, 9H, s), 1.33 (Ala C^βH, 3H, d, J=6), 0.96-0.94 (Ile C^γH, 2H, m), 0.90-0.82 (Ile C^γH, C^δH and Leu C^γH, C^δH, 13H, m)

ESI-MS: found m/z (M+Na)⁺= 509.12; Mcald = 486.60

Anal. Calcd for C₂₃H₄₂N₄O₇ (429): C, 56.77, H, 8.70, N, 11.51

Found: C, 56.68, H, 8.05, N, 11.72

Boc-Gly-Ala-Ile-Leu-OH: To 1.4g (3 mM) Boc-Gly-Ala-Ile-Leu-OMe, 10ml MeOH and 25ml 2(N) NaOH were added 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*, the residue was taken in 50 ml of water, washed with diethyl ether (2 × 50 ml) then the pH of the aqueous layer was adjusted to 2 using 1 N HCl and it was extracted with ethyl acetate (3 × 50 ml). The extracts were pooled, dried over anhydrous sodium sulphate and evaporated in *vacuum*. A white solid material was obtained.

Yield: 1.2g (2.6 mM, 86%)

¹H NMR (300 MHz, DMSO-d₆, δ): 12.44 (COOH, 1H, b), 8.19 (Leu NH, 1H, d, J=9), 7.89 (Ile NH, 1H, d, J=9), 7.83 (Ala NH, 1H, d, J=6), 6.98 (Gly NH, 1H, t, J=8.5), 4.38-4.34 (Leu C^αH, 1H, m), 4.33-4.20 (Ile C^αH, 1H, m), 4.17-4.15 (Ala C^αH, 1H, m), 3.52 (Gly C^αH, 2H, m), 1.72-1.67 (Leu C^βH, 2H, m), 1.54-1.57 (Ile C^βH, 1H, m), 1.38

(C(CH₃)₃, 9H, s), 1.15 (Ala C^βH, 3H, d, *J*=9), 1.07-0.98 (Ile C^γH, 2H, m), 0.89-0.80 (Ile C^γH, C^δH and Leu C^γH, C^δH, 13H, m)

ESI-MS: found m/z (M)⁺= 472.21; (M+Na)⁺ 495.98; Mcald = 472.57

Anal. Calcd for C₂₂H₄₀N₄O₇ (472): C, 55.91, H, 8.53, N, 11.86

Found: C, 55.41, H, 8.05, N, 11.6

H₃N⁺-Gly-Ala-Ile-Leu-COO⁻ (peptide **1**). To 1.2g (2.6 mM) of Boc-Gly-Ala-Ile-Leu-OH 4ml of trifluoroacetic acid (TFA) was added, removal of Boc group was monitored by TLC. After 8 h, TFA was removed under *vacuum*. The residue was taken in water (20 ml) and washed with diethyl ether (2 × 30 ml). The aqueous portion was evaporated in *vacuum* to yield peptide **1** as white solid.

Yield: 0.7g (2 mM, 76%)

FT-IR: 1434 (sym str COO⁻), 1500 (N-H bending and COO⁻ asym str), 1641, 1672 (C=O str), 3087, 3388 (N-H str) cm⁻¹

¹H NMR (300 MHz, DMSO-d₆, δ): 8.50 (Ala NH, 1H, d, *J*=6), 8.13 (Ile NH, 1H, d, *J*=6), 8.04 (Leu NH, 1H, d, *J*=6), 4.47-4.45 (Leu C^αH, 1H, m), 4.23-4.21 (Ile C^αH, 1H, m), 4.18-4.15 (Ala C^αH, 1H, m), 3.43-3.42 (Gly C^αH, 2H, m), 1.71-1.59 (Leu C^βH, 2H, m), 1.56-1.44 (Ile C^βH, 1H, m), 1.18-1.16 (Ala C^βH₃, 3H, d, *J*=12), 1.07-1.04 (Ile C^γH, 2H, m), 0.90-0.79 (Ile C^γH and C^δH, Leu C^γH and C^δH, 13H, m)

¹³C NMR (75 MHz, DMSO-d₆, δ): 170.06 (COOH), 171.75 (Ile C=O), 171.13 (Ala C=O), 165.44 (Gly C=O), 56.96 (Ile C^α), 50.16 (Leu C^α), 48.24 (Ala C^α), 40.33 (Gly C^α), 40.17 (Leu C^β), 36.63 (Ile C^β), 24.41 and 24.35 (Ile C^γ), 22.99 and 21.23 (Leu C^γ), 18.68 (Leu C^δ), 15.35 (Ala C^β), 11.07 (Ile C^δ)

ESI-MS: found m/z (M+Na)⁺ = 395.25; (M+H)⁺ = 373.27; (M+K)⁺ = 411.23; Mcald = 372.45

Anal. Calcd for C₁₇H₃₂N₄O₅ (372): C, 54.82, H, 8.66, N, 15.04

Found: C, 54.41, H, 8.05, N, 14.9

[α]_D²⁰ -9.2 (c = 0.5 M, H₂O).

Peptide 2.

Boc-Phe-OH: reference Org. Lett. 2004, 6, 4463-4465.

Boc-Phe-Ile-OMe: reference Soft Matter, 2008, 4, 1430-1437.

Boc-Phe-Ile-OH: reference Soft Matter, 2008, 4, 1430-1437.

Boc-Phe-Ile-Leu-OMe. 4.53g (12 mM) of Boc-Phe-Ile-OH were dissolved in 10ml of DMF in an ice water bath. H-Leu-OMe was isolated from 5.41g (30 mM) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and ethyl acetate extract was concentrated to 10ml. It was then added to the reaction mixture, followed immediately by 2.47g (12 mM) of dicyclohexylcarbodiimide (DCC) and 1.62g (12 mM) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 3 days. The residue was taken up in ethyl acetate (40ml) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1(N) HCl (3 × 30ml), brine (1 × 30ml), 1 m sodium carbonate (3 × 30ml) and brine (2 × 30ml) and dried over anhydrous sodium sulfate and evaporated in *vacuum*. A white material was obtained.

Yield: 4.5g (9 mM, 75%)

¹H NMR (300 MHz, CDCl₃, δ): 7.31-7.18 (Ar H, 5H, m), 6.55 (Leu NH, 1H, d, *J*=9), 6.33 (Ile NH, 1H, d, *J*=6), 4.96 (Phe NH, 1H, d, *J*=6), 4.60-4.52 (Leu C^αH, 1H, m), 4.37-

4.34 (Ile C ^{α} H, 1H, m), 4.29-4.24 (Phe C ^{α} H, 1H, m), 3.73 (COOCH₃, 3H, s), 3.10-3.06 (Phe C ^{β} H, 2H, m), 1.90-1.87 (Ile C ^{β} H, 1H, m), 1.63-1.58 (Leu C ^{β} H, 2H, m), 1.40 ((CH₃)₃C, 9H, s), 1.09-1.05 (Ile C ^{γ} H₂, 2H, m), 0.94-0.86 (Leu C ^{γ} H, Ile C ^{γ} H and C ^{δ} H, 12H, m)

ESI-MS: found m/z (M+Na)⁺= 528.06; Mcald = 505.64

Anal. Calcd for C₂₇H₄₃N₃O₆ (505): C, 64.13, H, 8.57, N, 8.31

Found: C, 53.68, H, 8.47, N, 8.22

Boc-Gly-Phe-Ile-Leu-OMe. 1.4g (8 mM) of Boc-Gly-OH were dissolved in 10ml of DMF in an ice water bath. H-Phe-Ile-Leu-OMe was isolated from 4.5g (9 mM) of the corresponding Boc-Phe-Ile-Leu-OMe by treatment with TFA, neutralization with NaHCO₃ and subsequent extraction with ethyl acetate and ethyl acetate extract was concentrated to 10ml. It was then added to the reaction mixture, followed immediately by 1.61g (8 mM) of dicyclohexylcarbodiimide (DCC) and 1.08g (8 mM) of HOBr. The reaction mixture was allowed to come to room temperature and stirred for 3 days. The residue was taken up in ethyl acetate (40ml) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1(N) HCl (3 × 30ml), brine (1 × 30ml), 1(M) sodium carbonate (3 × 30ml) and brine (2 × 30ml) and dried over anhydrous sodium sulfate and evaporated in *vacuum*. A white material was obtained. It was purified by column chromatography using chloroform-methanol (98:2) eluent.

Yield: 3.3g (6 mM, 75%)

¹H NMR (300 MHz, CDCl₃, δ): 7.67 (Leu NH, 1H, d, J =6), 7.45 (Ile NH, 1H, d, J =6), 7.26-7.10 (Ar H, 5H, m), 7.03 (Phe NH, 1H, d, J =6), 5.60 (Gly NH, 1H, b), 4.88-4.86 (Leu C ^{α} H, 1H, m), 4.59-4.57 (Ile C ^{α} H, 1H, m), 4.48-4.43 (Phe C ^{α} H, 1H, m), 4.02-3.97

(Gly C ^{α} H, 2H, m), 3.71 (COOCH₃, 3H, s), 2.97-2.95 (Phe C ^{β} H, 2H, m), 1.77-1.73 (Leu C ^{β} H, 2H, m), 1.68-1.66 (Ile C ^{β} H, 1H, m), 1.42 (C(CH₃)₃, 9H, s), 1.10-1.04 (Ile C ^{γ} H, 2H, m), 0.95-0.82 (Ile C ^{γ} H, C ^{δ} H and Leu C ^{γ} H, C ^{δ} H, 13H, m)

ESI-MS: found m/z (M+Na)⁺= 585.11; (M+H)⁺ 563.16; Mcald = 562.69

Anal. Calcd for C₂₉H₄₆N₄O₇ (562): C, 61.90, H, 8.24, N, 9.96

Found: C, 61.68, H, 8.05, N, 9.72

Boc-Gly-Phe-Ile-Leu-OH: To 3.3g (6 mM) Boc-Gly-Phe-Ile-Leu-OMe, 10ml MeOH and 25ml 2(N) NaOH were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10h, methanol was removed under *vacuum*, the residue was taken in 50ml of water, washed with diethyl ether (2 × 50ml) then the pH of the aqueous layer was adjusted to 2 using 1(N) HCl and it was extracted with ethyl acetate (3 × 50ml). The extracts were pooled, dried over anhydrous sodium sulphate and evaporated in *vacuum*. A white solid material was obtained.

Yield: 2.1g (4 mM, 66%)

¹H NMR (300 MHz, DMSO-d₆, δ): 12.49 (COOH, 1H, b), 8.18 (Leu NH, 1H, d, J=6), 8.05 (Ile NH, 1H, d, J= 9), 7.79 (Phe NH, 1H, d, J= 9), 7.18 (Ar H, 5H, m), 6.93 (Gly NH, 1H, b), 4.62-4.60 (Leu C ^{α} H, 1H, m), 4.28-4.24 (Ile C ^{α} H, 1H, m), 4.21-4.19 (Phe C ^{α} H, 1H, m), 3.35 (Gly C ^{α} H, 2H, m), 2.98-2.72 (Phe C ^{β} H, 2H, m), 1.73-1.68 (Leu C ^{β} H, 2H, m), 1.53-1.49 (Ile C ^{β} H, 1H, m), 1.36 (C(CH₃)₃, 9H, s), 1.08-1.01 (Ile C ^{γ} H, 2H, m), 0.86-0.79 (Ile C ^{γ} H, C ^{δ} H and Leu C ^{γ} H, C ^{δ} H, 13H, m)

ESI-MS: found m/z (M+Na)⁺= 571.17; (M+K)⁺ = 58.18; Mcald = 548.67

Anal. Calcd for C₂₈H₄₄N₄O₇ (548): C, 61.29, H, 8.08, N, 10.21

Found: C, 61.01, H, 8.0, N, 10.22

H₃N⁺-Gly-Phe-Ile-Leu-COO⁻ (peptide **2**). To 2.1g (4 mM) of Boc-Gly-Phe-Ile-Leu-OH 4ml of trifluoroacetic acid (TFA) was added, removal of Boc group was monitored by TLC. After 8h, TFA was removed under *vacuum*. The residue was taken in water (20ml) and washed with diethyl ether (2 × 30ml). The aqueous portion was evaporated in *vacuum* to yield peptide **2** as white solid.

Yield: 1.3g (2 mM, 50%)

FT-IR: 1400 (sym str COO⁻), 1550 (N-H bending and COO⁻ asym str), 1641, 1674 (C=O str), 3112, 3305 (N-H str) cm⁻¹

¹H NMR (300 MHz, DMSO-d₆, δ): 8.51 (Phe NH, 1H, d, *J*=6), 8.26 (Ile NH, 1H, d, *J*=9), 7.95 (Leu NH, 1H, d, *J*=9), 7.23-7.18 (Ar H, 5H, m), 4.65 (Leu C^αH, 1H, m), 4.18-4.15 (Ile C^αH, 1H, m), 4.13-4.10 (Phe C^αH, 1H, m), 3.48-3.25 (Gly C^αH, 2H, m), 3.04-2.72 (Phe C^βH, 2H, m), 1.65-1.63 (Leu C^βH, 2H, m), 1.52-1.48 (Ile C^βH, 1H, m), 1.12-1.00 (Ile C^γH, 2H, m), 0.90-0.79 (Ile C^γH and C^δH, Leu C^γH and C^δH, 13H, m)

¹³C NMR (75 MHz, DMSO-d₆, δ): 175.2 (COOH), 171.5 (Ile CO), 171.2 (Phe CO), 167.8 (Gly CO), 138.4, 130.0, 128.9, 127.2 (Ar C), 58.2 (Ile C^α), 55.0 (Phe C^α), 52.0 (Leu C^α), 41.8 (Gly C^α), 25.28-22.57 (Leu C^γ, C^δ and Ile C^γH₂), 16.20 (Ile C^δH₃).

ESI-MS: found m/z (M+Na)⁺ = 471.27; Mcald = 448.55

Anal. Calcd for C₂₃H₃₆N₄O₅ (448): C, 61.59, H, 8.08, N, 12.49

Found: C, 69.41, H, 8.05, N, 11.9

[α]_D²⁰ -10.2 (c = 0.5 M, H₂O).

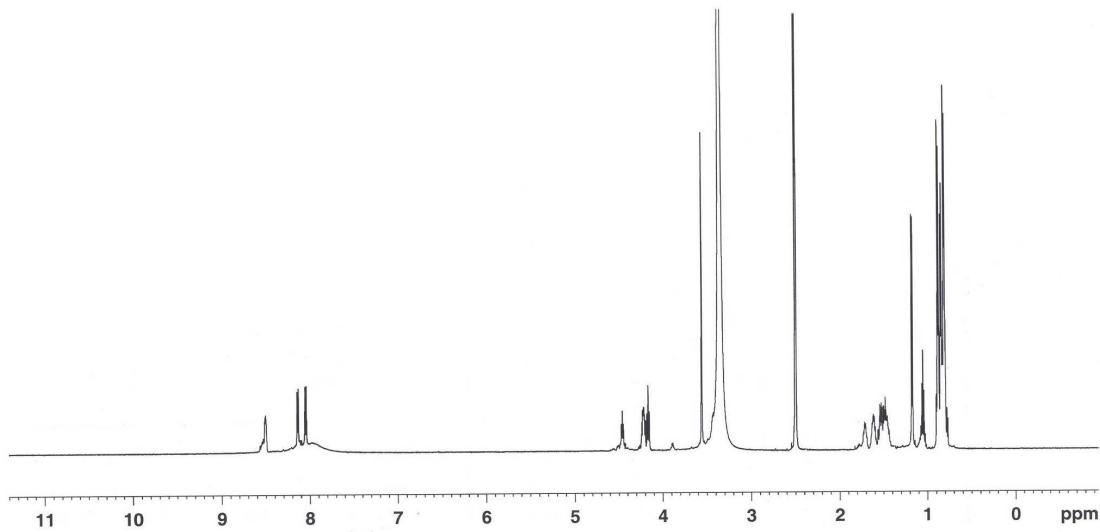


Figure S1. ¹H NMR spectra of peptide **1**

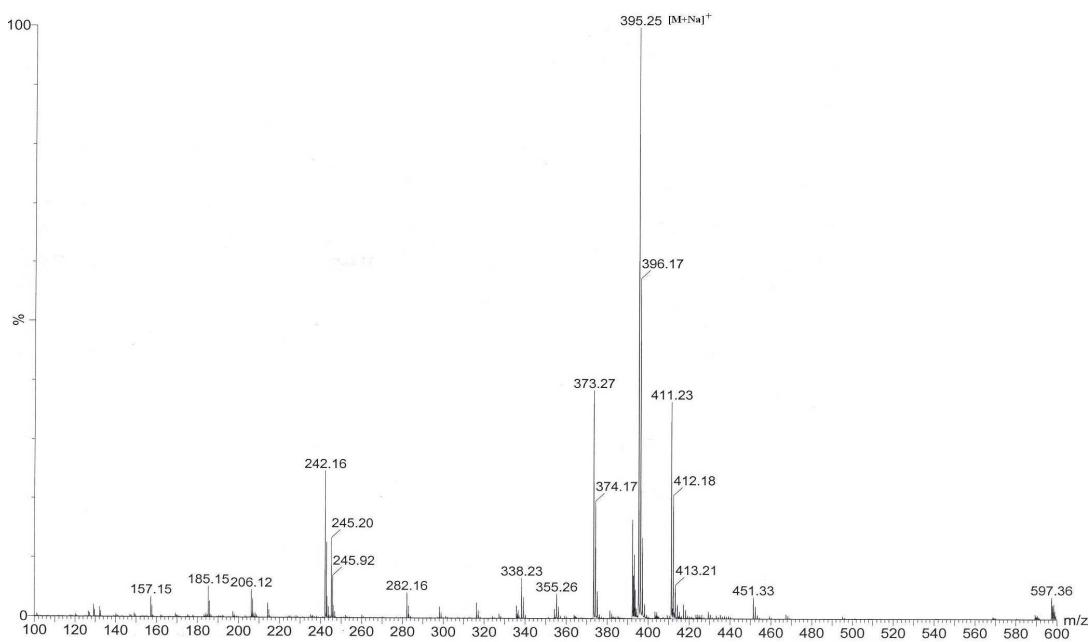


Figure S2. Mass spectra of peptide 1

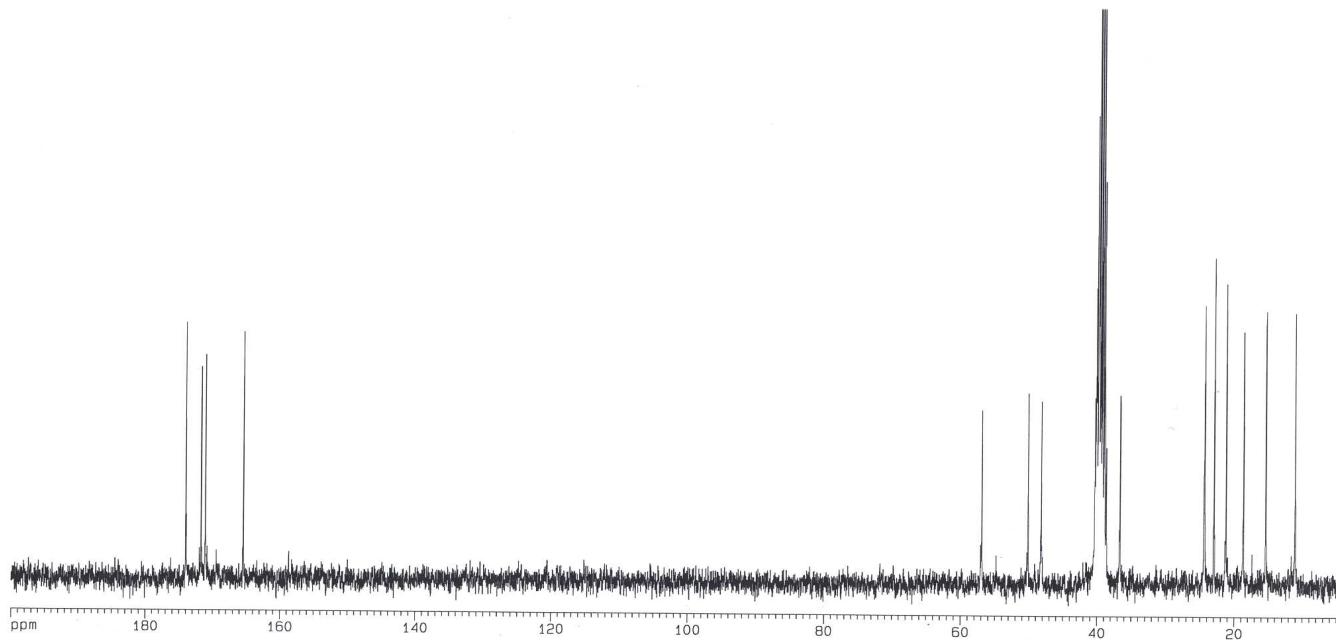


Figure S3. ^{13}C spectra of peptide 1

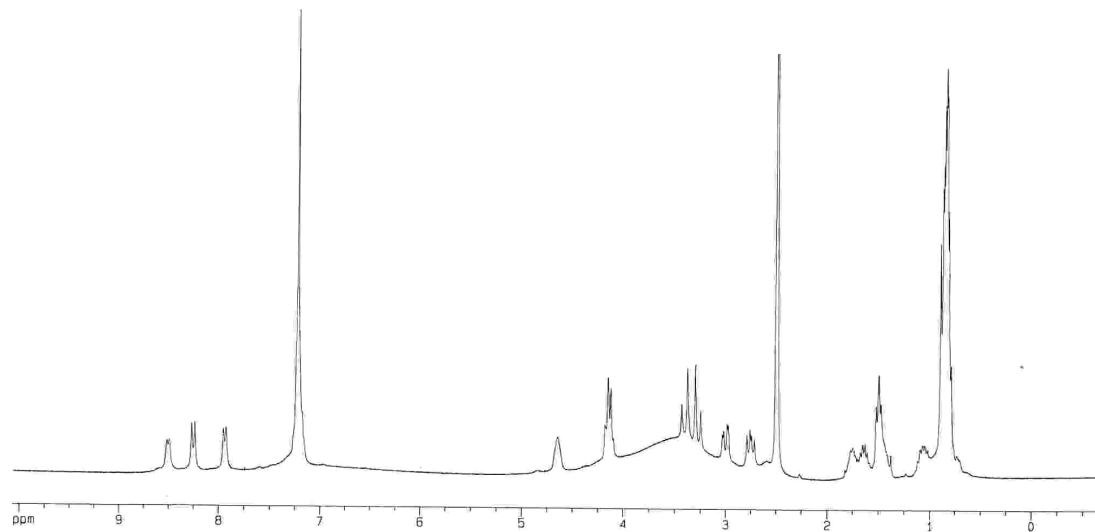


Figure S4. ^1H NMR spectra of peptide 2

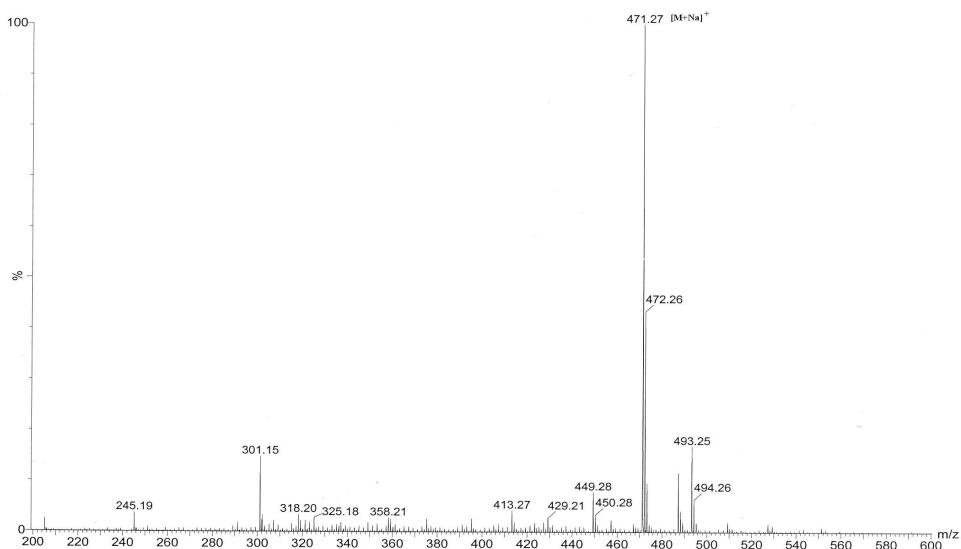


Figure S5. Mass spectra of peptide 2

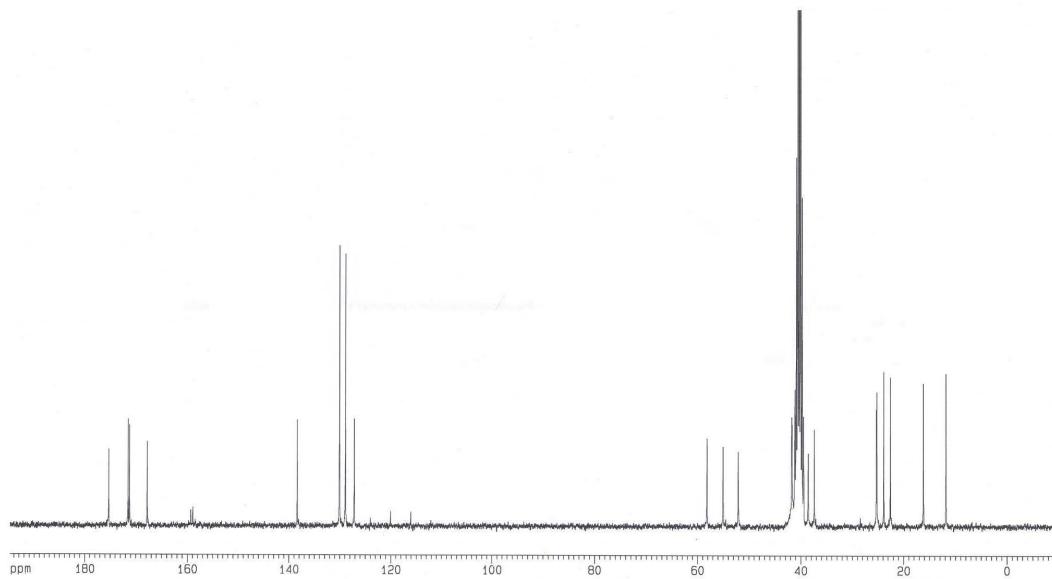


Figure S6. ¹³C spectra of peptide 2

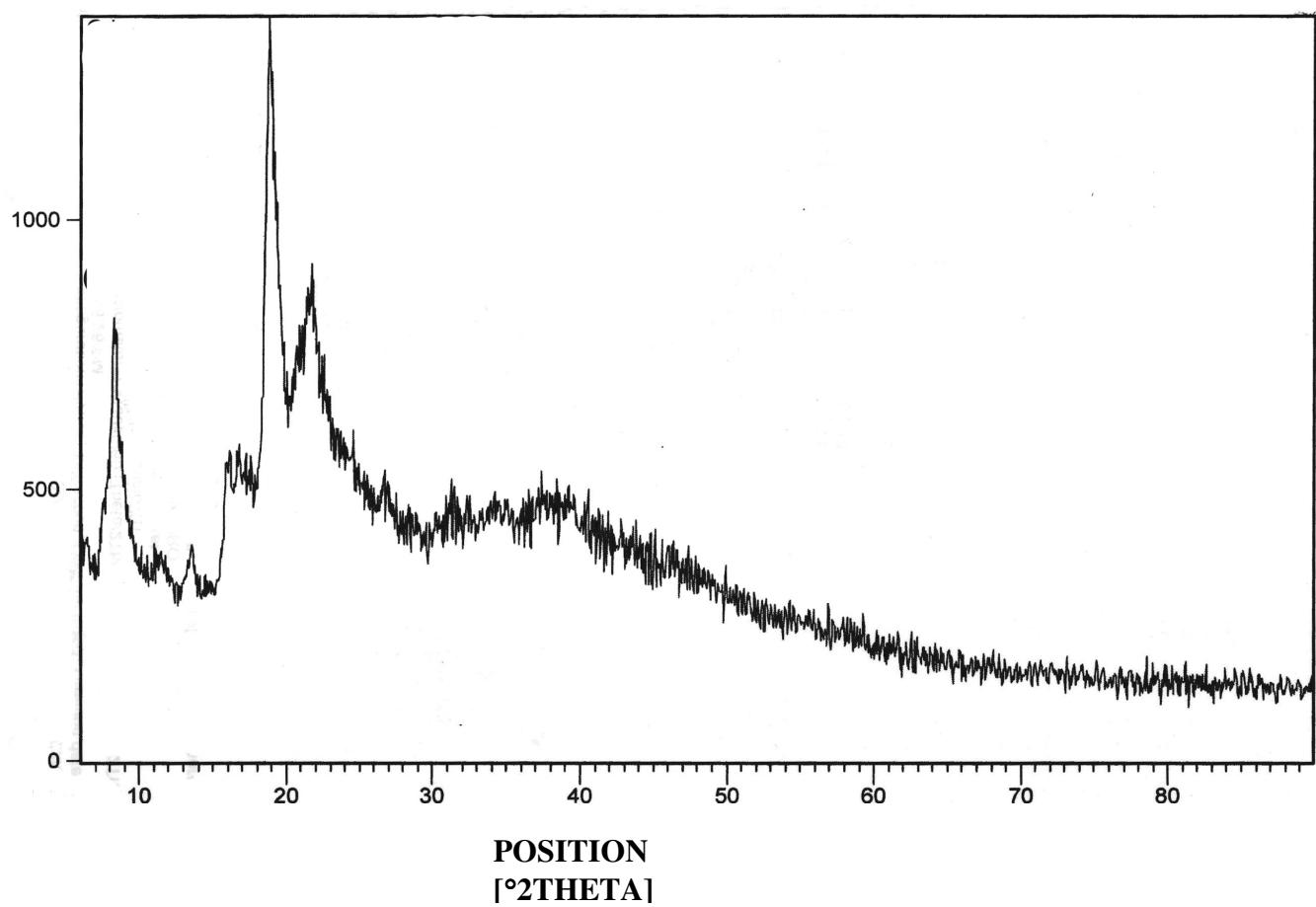


Figure S7. Wide angle X-ray powder diffraction pattern of xerogel 1.

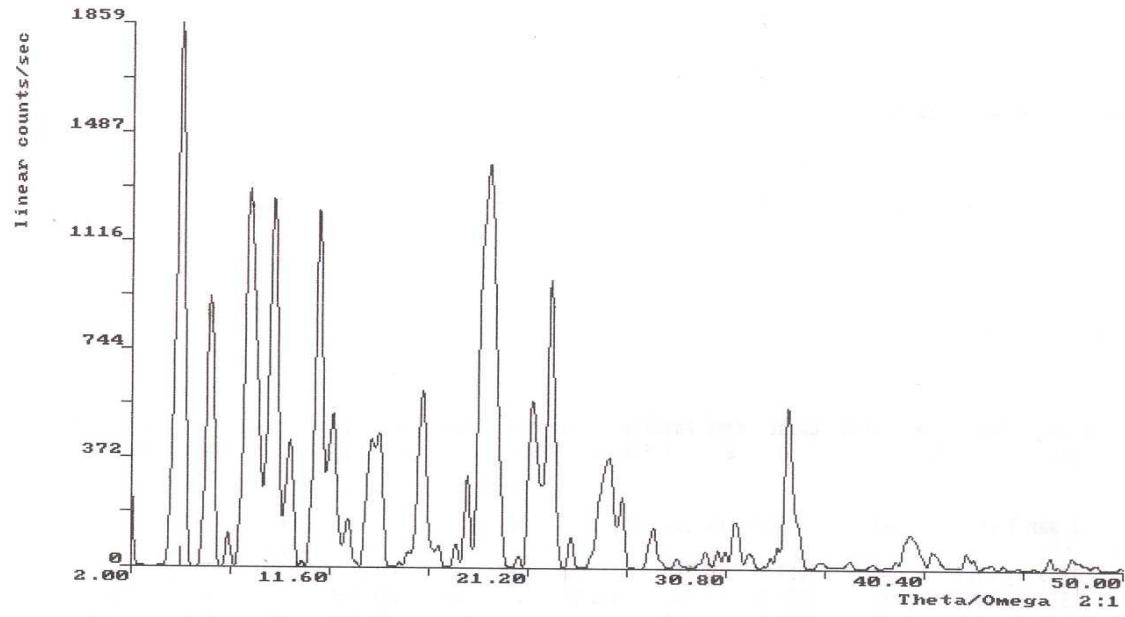


Figure S8. Wide angle X-ray powder diffraction pattern of xerogel 2.

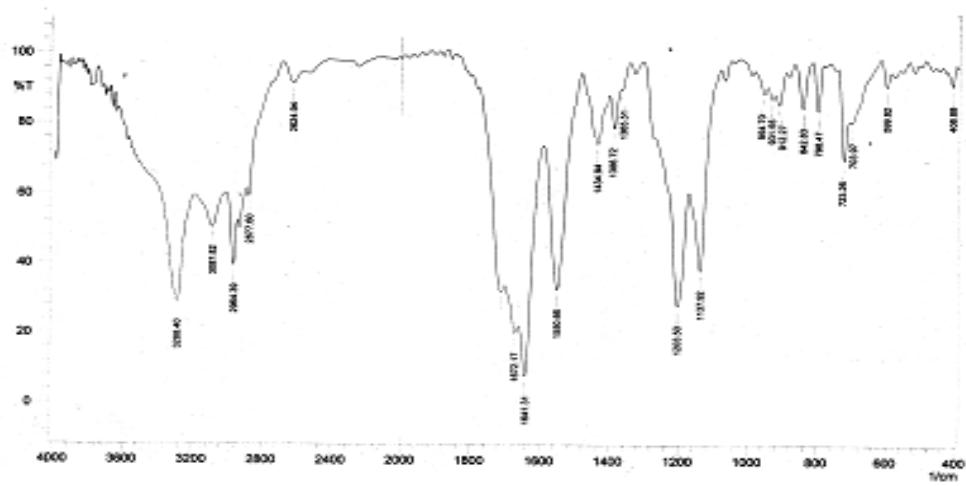


Figure S9. FTIR spectra of peptide **1** in solid amorphous state

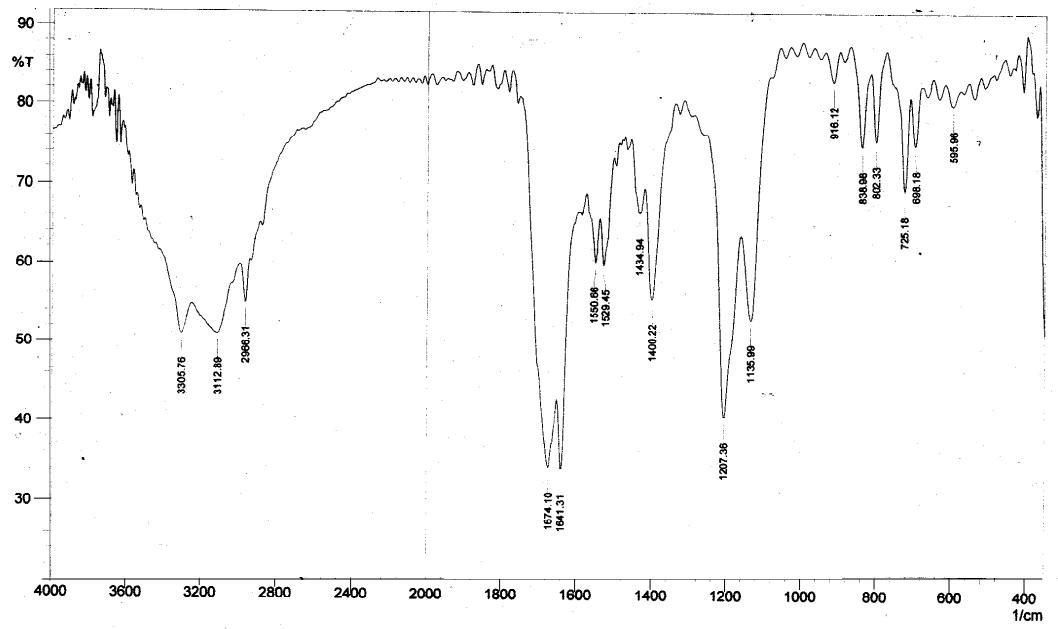


Figure S10. FT-IR spectra of peptide **2** in solid amorphous state.

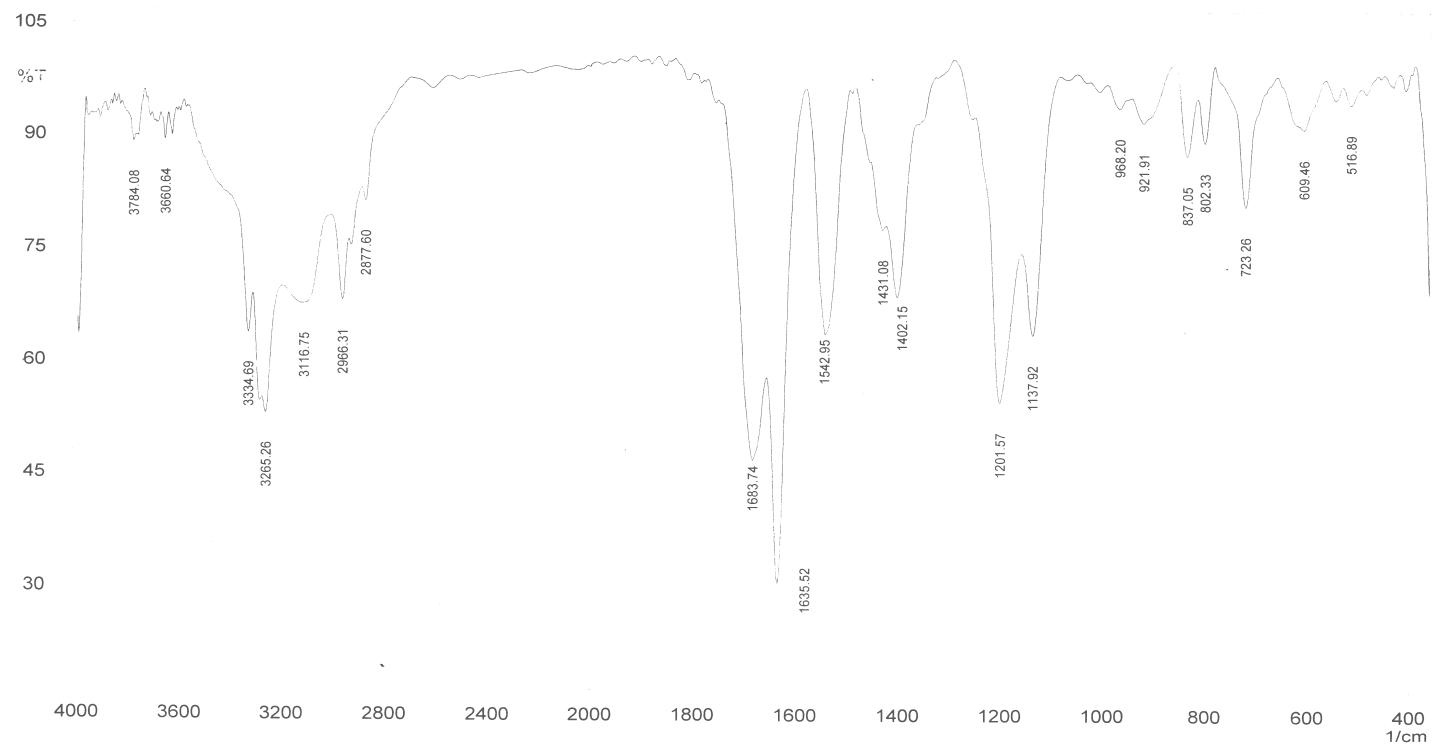


Figure S11: FTIR spectra of xerogel obtained from peptide **1**

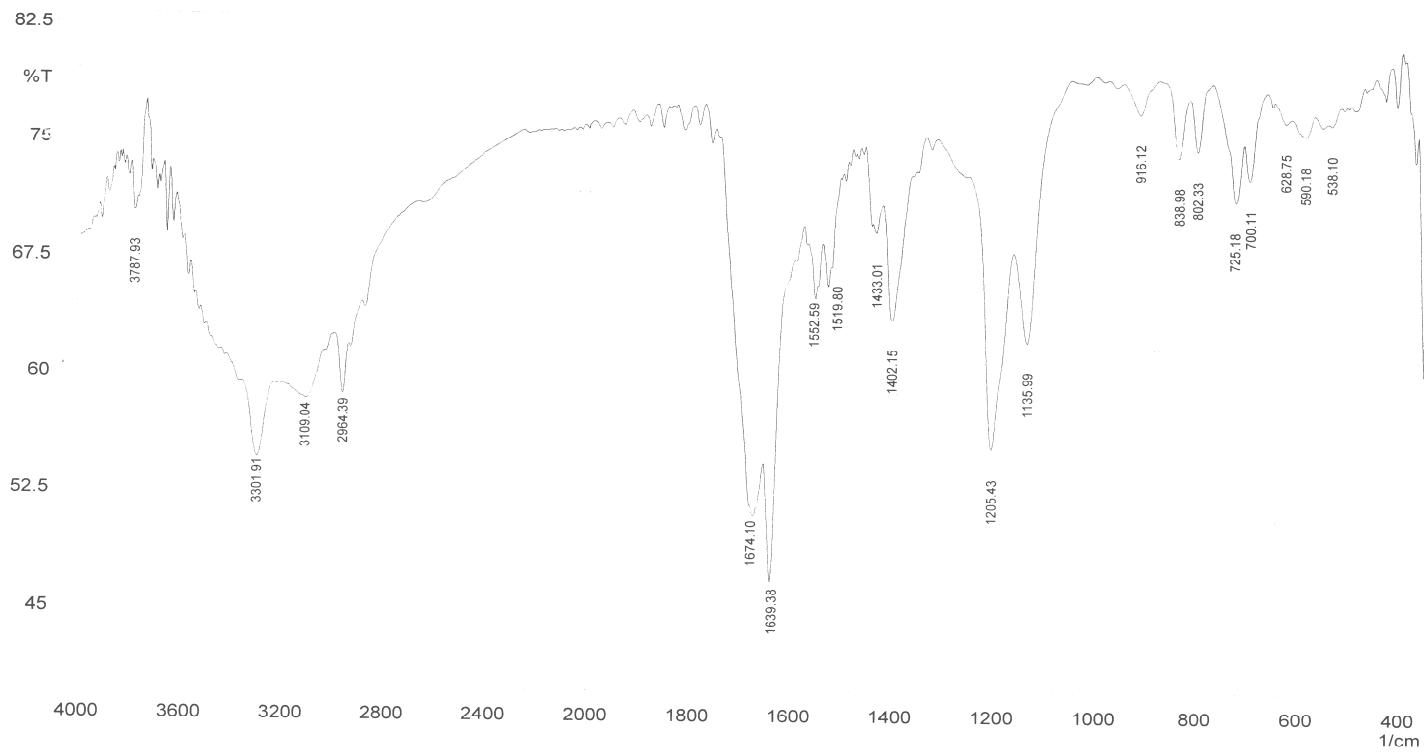


Figure S12: FTIR spectra of xerogel obtained from peptide **2**

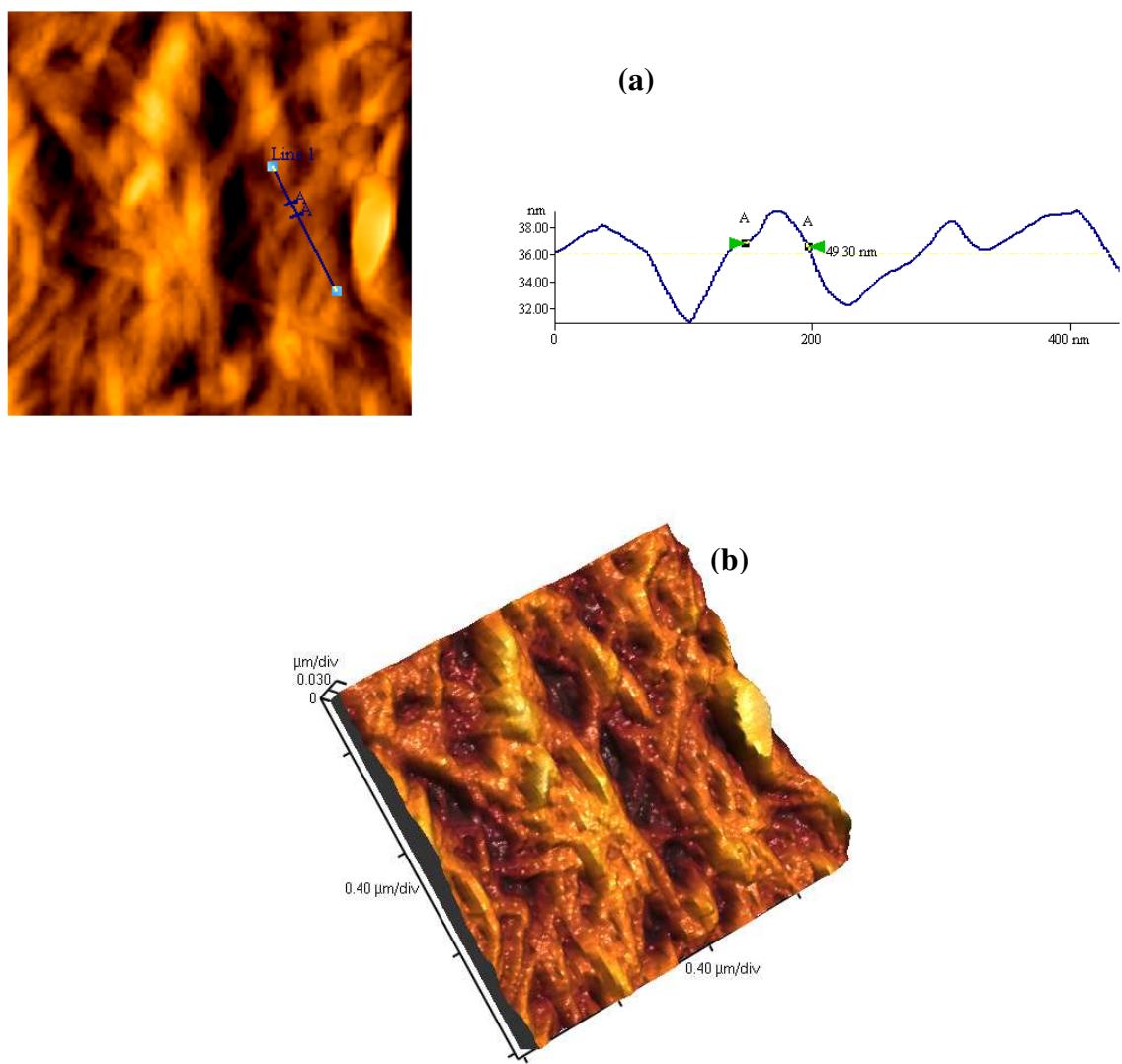


Figure S13. (a) Height profile plot and (b) 3D topographic view (along Z axis) of the gel network obtained form the hydrogel **1**.

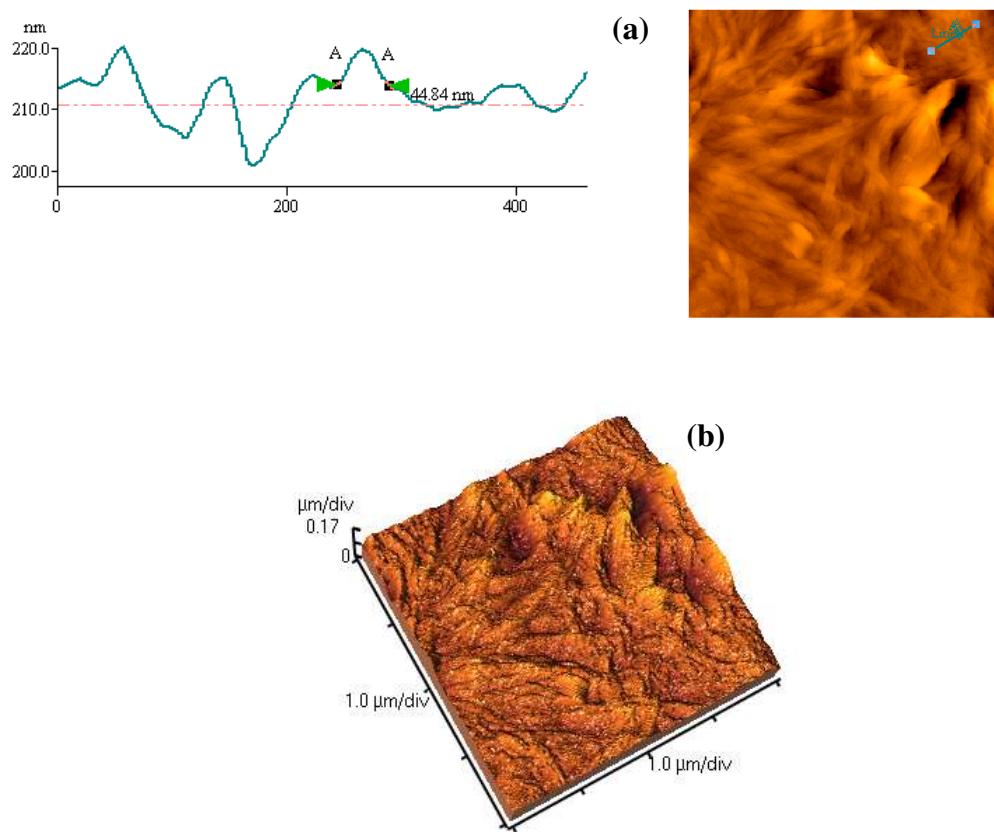


Figure S14. (a) Height profile plot and (b) 3D topographic view (along Z-axis) of the gel network obtained form the hydrogel **2**.

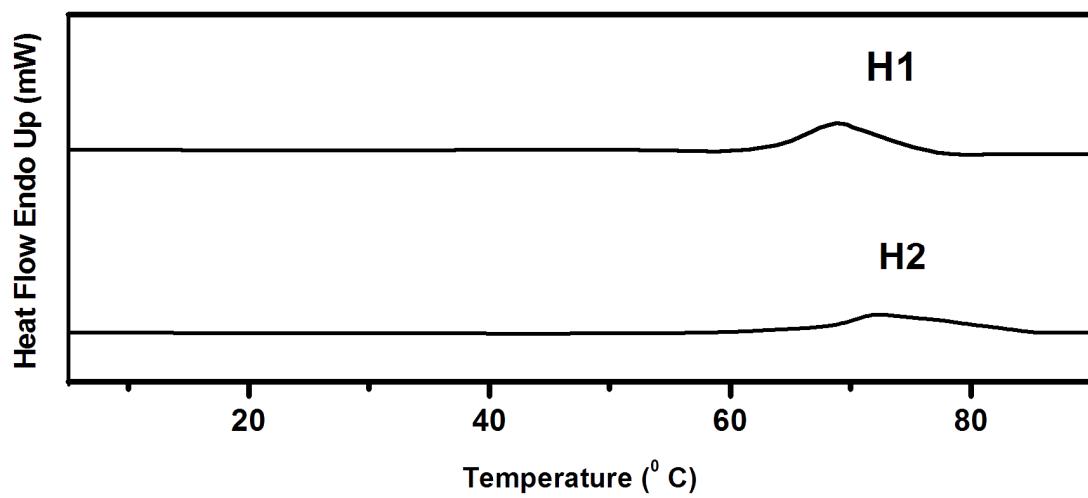


Figure S15: DSC thermogram: for the hydrogel 1 at 17% w/v (H1), for the hydrogel 2 at 2.65% w/v (H2).