Supporting Information

Remarkable Thermoresponsive Nanofibers From γ-Peptides

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**Figure S1.** The packing of dipeptide P1 in unit cell as viewed down the crystallographic a-axis. Only polar H-atoms are shown for clarity. Ellipsoids are drawn to 50% probability. (CCDC No. 943867)

**Figure S2.** Arrangement of molecules of peptide P1 is shown in unit cell depicting the intermolecular hydrogen bonding (shown in dotted black lines). Arrows indicates the direction of H-bond dipole in the packing as viewed down crystallographic b-axis.
Figure S3: The ORTEP diagram of Boc-Aic-Aic-Aic-OEt (P2). H-atoms are not labeled for clarity. (CCDC No. 943868).
**Figure S4.** Arrangement of P2 depicting antiparallel double helical structure as viewed down crystallographic a-axis. Right panel gives insight into orientation of γ-peptide (P2) backbone in double helical form.

**Figure S5.** Arrangement of molecules of peptide P2 is shown in unit cell depicting the intermolecular hydrogen bonding (shown in dotted black lines). Arrows indicates the direction of H-bond dipole in adjacent layers as viewed down crystallographic c-axis.
Torsion angles for peptide P1 and P2

Table 1. Torsion angles [°] for P1

Boc-Aic-Aic-OEt

(There are four molecules in asymmetric unit of P1)

i) Molecule a

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Table S2. Torsion angles [°] for P2

Boc-Aic-Aic-Aic-OEt

(There are two molecules in asymmetric unit of P2)

i) Molecule a

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Figure S6. A) Thermoreversible gel formation is shown for Peptide P3 in Toluene. Also, all other solvents are able to form thermoreversible gels. P4 also forms thermoreversible gel in all above solvents. B) Inverted sample vial test to confirm the gel formation for the peptide P3 in a) Benzene, b) Toluene, c) Isopropanol, d) DMF/Water (95:5), e) DMSO/Water (95:5) and f) Diglyme/Water (95:5). C) Inverted sample vial test to confirm the gel formation for the peptide P4 in g) Benzene, h) Toluene, i) Isopropanol, j) DMF/Water (95:5), k) DMSO/Water (95:5) and l) Diglyme/Water (95:5).

Figure S7. AFM images of A) peptide P2 in methanol, B) xerogel of peptide P3 in toluene and xerogel of peptide P4 in toluene.
Figure S8. SEM image of A) Viscous solution of Peptide P2 in MeOH, B) Xerogel of P3 in DMSO/H\textsubscript{2}O (95:5) showing fibrous network, C) and D) Xerogel of Peptide P4 in MeOH showing self assembly which assembles into nanofibers.

Figure S9A. Schematic mechanism for gel formation by P3 and P4 in various solvents.
Details of gelation in various solvents.
(For the entire gelation attempt shown below, 2mg of respective peptide was dissolved in 1mL of solvent.)

**Table S3.** Gelation attempts for peptide P1, P2, P3 and P4 in various solvents

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TG = Transparent Gel, I = Insoluble, S= Soluble, OG = Opaque Gel.

* forms transparent gel on standing for 2-3 days in partially open sample vial.
Figure S9B. Experiment showing the utility of γ-peptide gel of P3 as water resistant proof.

i) Half part of the kitchen tissue paper (part B) was coated with xerogel of peptide P3 and another half (part A) was not coated.

ii) When drop of water casted on part B, it was not absorbed by tissue paper.

iii) As soon as water drop casted on part A, it spreads and absorbs immediately. Still water drop on part B was seen standing as it was.

iv) After 10 minutes, the water drop was completely absorbed on part A, while part B still resist water drop to get absorbed.

* Water drop contain 5% CuSO₄ to get visible blue color.
Figure S10. Upfield chemical shift for all amide protons is observed in $^1$H NMR for peptide P3 when temperature was varied from -40 to +40 °C. Spectra were recorded on 500 MHz spectrometer in CDCl$_3$ as solvent.
Figure S11: Partial TOCSY NMR spectrum of P3 (3 mM) in CDCl₃. The sequential assignment of -CH₂ protons were performed using ROESY.
Figure S12. Partial ROESY spectrum of P3 (3 mM) in CDCl$_3$ showing peaks for amide and methylene proton interactions depicting extended sheet structure.
Figure S13. Partial ROESY spectrum of P3 (3 mM) in CDCl₃ showing no interaction between amide protons through space.
Figure S14: Partial TOCSY NMR spectrum of P4 (2.0 mM) in CDCl₃. The sequential assignment of -CH₂ protons were performed using ROESY.
Figure S15. Partial ROESY spectrum of P4 (2.0 mM) in CDCl$_3$ showing peaks for amide and methylene proton interactions depicting extended sheet structure.
Figure S16. Partial ROESY spectrum of P4 (2.0 mM) in CDCl₃ showing no interaction between amide protons through space.
Materials and methods

2-Aminoisobutyric acid (Aib), N-hydroxsuccinimide, NaBH₄, oxone, DCC, HOBt, Ethyl bromoacetate, PPh₃, Pd/C (10%), were purchased from Sigma-Aldrich. Solvents THF, EtOAc, were obtained from Merck. 2-Iodobenzoic acid was purchased from Spectrochem. THF was distilled over sodium prior to use. Column chromatography was performed on Merck silica gel (120-200 mesh). Reactions were monitored by analytical thin layer chromatography using aluminium-backed plates coated with Merck Kieselgel 60 F254; Visualization was accomplished with UV light and KMnO₄ or ninhydrin stain. Yields refer to chromatographically pure compounds unless otherwise stated. Proton nuclear magnetic resonances (¹H NMR) were recorded in deuterated solvent on JEOL 400 MHz (for ¹³C, 100 MHz) and Bruker 500 MHz (for 13C, 125 MHz) spectrometer. Variable temperature experiment and all 2D NMR experiments are carried on 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00) or residual protio solvent (CHCl₃, δ 7.27 for ¹H and δ 77.0 for ¹³C) unless otherwise mentioned. ¹H NMR splitting patterns are designated as singlet (s), doublet (d), broad doublet (bd), triplet (t), or doublet of doublets (dd), quintet (qn). Coupling constants (J) are reported in Hertz (Hz). The Matrix Assisted LASER Desorption Ionization mass spectrometer (MALDI-TOF/TOF, Applied Biosciences) was used to obtain accurate mass. MCR-301 (Anton-Paar) rheometer was used for Rheological studies. IR spectra were recorded on ATR Bruker ALPHA FT-IR spectrometer. Data for X-ray structure determination were obtained from Bruker APEX II DUO diffractometer using Mo-Kα (λ= 0.71073 Å) graphite monochromated radiation.
General procedures

a) Synthesis of ethyl esters of N-Boc-protected 4-Aic (4-Amino isocaproic acid)

Scheme S1: Synthesis of 4-Aic

The suspension of activated Pd/C (20 % by weight) and (E)-ethyl 4-((tert butoxycarbonyl)amino)-4-methylpent-2-enoate(I) (1.028 g, 4 mmol) which was synthesized using reported method [1], in MeOH (20 mL) was stirred at room temperature in the presence of hydrogen. After completion of the reaction (TLC, ~5 Hrs), Pd/C was filtered through the bed of celite and the filtrate was evaporated to dryness under vacuum to get gummy N-Boc protected 4-Aic. The pure product was obtained after silica gel column chromatography 5% ethyl acetate in hexane in good yield (0.907 g, 90%).

b) Synthesis of peptides

Dipeptide, tripeptide, tetrapeptide and pentapeptides were synthesized by conventional solution-phase methods using a fragment-condensation strategy. The tert-butyloxycarbonyl group was used for N-terminus protection, and the C-terminus was protected as an ethyl ester. Deprotectons were performed with trifluoroacetic acid and saponification for the N- and C-termini, respectively. Couplings were mediated by dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBr). The dipeptide (P1) was prepared by coupling reaction between N-terminal Boc-Aic-OH and H-Aic-OEt. The tripeptide Boc-Aic-Aic-Aic-OEt (P2) was prepared by [2 + 1] condensation involving N-terminal dipeptide acid Boc-Aic-Aic-OH and H-Aic-OEt. The Tetrapeptide Boc-Aic-Aic-Aic-Aic-OEt (P3) was prepared by [2+2] condensation involving N-terminal dipeptide acid Boc-Aic-Aic-OH and H-Aic-Aic-OEt. The pentapeptide Boc-Aic-Aic-Aic-Aic-Aic-OEt (P4) was prepared by [3 + 2] condensation involving N-terminal tripeptide acid Boc-Aic-Aic-Aic-OH and H-Aic-Aic-OEt.

c) Preparation of gel from various solvents for P3 and P4

2 mg of peptide was dissolved in 1mL of respective solvent mentioned in Table S3. Solution was warmed to 50 °C. Gel in respective solvents was obtained on standing for 10-15 min. Sonication of warm solution accelerated the gelation process within 1-2 min. Gels
were obtained from Methanol and Isopropanol after standing the warm solution for 2-3 days.

**Characterization of peptides**

4-((tert-butoxycarbonyl)amino)-4-methylpentanoic acid: $^1$H NMR (400 MHz, Chloroform d) δ 4.5 (bs, 1H), 2.36 (m, 2H), 2.01 (m, 2H), 1.44 (s, 9H), 1.27 (s, 6H); $^{13}$C NMR (100 MHz, Chloroform d) δ 179.12, 154.23, 78.82, 51.94, 34.66, 30.92, 29.41, 28.38, 27.19; MALDI TOF/TOF - m/z calcd. for C$_{11}$H$_{21}$N$_{4}$O$_{4}$ [M+Na]$^+$ 254.1368, obsrd. 254.1085.

**ethyl 4-(4-((tert-butoxycarbonyl)amino)-4-methylpentanamido)-4-methylpentanoate (P1):** $^1$H NMR (400 MHz, Chloroform d) δ 5.72 (bs, 1H), 4.62 (bs, 1H), 4.12 (q, $J = 8$ Hz, 2H), 2.32 (t, $J = 8$ Hz, 2H), 2.12 (t, $J = 8$ Hz, 2H), 2.03 (t, $J = 8$ Hz, 2H), 1.96 (t, $J = 8$ Hz, 2H), 1.43 (s, 9H), 1.32 (s, 6H), 1.25 (m, 9H); $^{13}$C NMR (100 MHz, Chloroform d) δ 174.02, 172.79, 154.71, 78.84, 60.53, 53.03, 52.23, 36.30, 34.89, 32.71, 29.55, 28.42, 27.30, 26.70, 14.18; MALDI TOF/TOF - m/z calcd. for C$_{19}$H$_{36}$N$_{2}$O$_{5}$ [M+Na]$^+$ 395.2522, obsrd. 395.2507.

**ethyl 2,2,6,6,11,11,16,16-octamethyl-4,9,14-trioxo-3-oxa-5,10,15-triazanonaodecan-19-oate (P2):** $^1$H NMR (400 MHz, Chloroform d) δ 6.32, (bs, 1H), 5.89 (bs, 1H), 4.70 (bs, 1H), 4.13 (q, $J = 8$ Hz, 2H), 2.31 (t, $J = 8$ Hz, 2H), 2.14 (m, 4H), 2.02 (t, $J = 8$ Hz, 2H),
1.93 (m, 4H), 1.42 (s, 9H), 1.31 (m, 13H), 1.25 (m, 8H); $^{13}$C NMR (100 MHz, Chloroform d) $\delta$ 174.06, 173.05, 172.96, 155.41, 78.70, 60.56, 53.13, 52.19, 36.33, 34.88, 32.31, 28.42, 27.13, 26.65, 14.16; MALDI TOF/TOF- m/z calcd. for C$_{25}$H$_{47}$N$_3$O$_6$ [M+Na]$^+$ 508.3363, obsrvd. 508.3342.

ethyl 2,2,6,6,11,11,16,16,21,21-decamethyl-4,9,14,19-tetraoxo-3-oxa-5,10,15,20-tetraazatetracosan-24-oate (P3) : $^1$H NMR (400 MHz, Chloroform d) $\delta$ 6.72 (bs, 1H), 6.55, (bs, 1H), 6.07 (bs, 1H), 4.75 (bs, 1H), 4.13 (q, $J = 8$ Hz, 2H), 2.03 (m, 16H), 1.42 (s, 9H), 1.30 (m, 27H); $^{13}$C NMR (100 MHz, Chloroform d) $\delta$ 174.06, 173.27, 173.11, 172.96, 154.65, 78.73, 53.11, 52.16, 36.56, 36.42, 34.87, 32.50, 32.15, 32.04, 29.49, 28.40, 27.04, 26.61, 26.53, 26.40, 20.95, 20.59, 14.14; MALDI TOF/TOF- m/z calcd. for C$_{31}$H$_{58}$N$_4$O$_7$ [M+Na]$^+$ 621.4203, obsrvd. 621.4250.

ethyl 2,2,6,6,11,11,16,16,21,21,26,26-dodecamethyl-4,9,14,19,24-pentaoxo-3-oxa-5,10,15,20,25-pentaazanonacosan-29-oate (P4): $^1$H NMR (400 MHz, Chloroform d) $\delta$ 6.85 (bs, 2H), 6.55, (bs, 1H), 6.05 (bs, 1H), 4.75 (bs, 1H), 4.12 (q, $J = 8$ Hz, 2H), 2.33 (m, 3H), 2.10 (m, 8H), 1.80 (m, 10H), 1.43 (s, 9H), 1.33 (m, 24H), 1.26 (m, 9H); $^{13}$C NMR (100 MHz, Chloroform d) $\delta$ 173.45, 173.38, 173.27, 173.20, 172.93, 158.11, 79.44, 60.64, 53.14, 52.24, 36.64, 36.44, 34.97, 32.08, 30.94, 29.53, 28.46, 26.62, 26.40, 14.19; MALDI TOF/TOF- m/z calcd. for C$_{37}$H$_{69}$N$_5$O$_8$ [M+Na]$^+$ 734.5044, obsrvd. 734.5131.
Crystal structure information

General procedure for crystallization of peptides

All crystallization attempts were conducted at room temperature. All oligomers of 4-Aic were purified carefully before keeping for crystallization. Glass sample vials (2 mL) were washed with acetone and dried under a nitrogen gas stream before use. PARAFILM “M” was used to close the vials. HPLC-grade solvents were used for crystallization.

Slow evaporation of a methanol/water mixture (P1)

Peptide Boc-Aic-Aic-OEt (P1) (8-10 mg) was dissolved in methanol (1 mL). The solution was transferred through a syringe filter into a glass vial. A few drops of water were added. The vial was closed with a PARAFILM and then pricked gently with clean and sharp needle to introduce 4 to 5 pores on PARAFILM so as to let the solvent mixture evaporate slowly. Reasonably good quality crystals were obtained after few days.

Slow evaporation of a methanol (P2)

Peptide Boc-Aic-Aic-Aic-OEt (P2) (2-5 mg) was dissolved in methanol (1 mL). The solution was transferred through a syringe filter into a glass vial. A few drops of water were added. The vial was closed with a PARAFILM and then pricked gently with clean and sharp needle to introduce 4 to 5 pores on PARAFILM so as to let the solvent mixture evaporate slowly. Peptide is prone to form gel during the process of crystallization. After several attempts, we could identify reasonably good quality crystal suitable for X-ray diffraction, after few days.

Crystal Structure Report of Peptide P1 and P2

i) Boc-Aic-Aic-OEt (P1)

Data Collection

A colorless crystal with approximate dimensions 0.3 x 0.1 x 0.05 mm³ was selected under oil under ambient conditions and attached on nylon CryoLoops with Paraton-N (Hampton Research). The crystal was mounted in a stream of cold nitrogen at 100(2) K and centered in the X-ray beam by using a video camera.

The crystal evaluation and data collection were performed on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) with Mo Kα (λ = 0.71073 Å) radiation and the diffractometer to crystal distance of 4.0 cm. The initial cell constants were obtained from three series of ω scans at different starting angles. Each series consisted of 12 frames.
collected $\omega$ with the exposure time of 20 seconds per frame. Obtained reflections were successfully indexed by an automated indexing routine built in the SMART program. The final cell constants were calculated from a set of 55478 strong reflections from the actual data collection.

The data were collected to a resolution of 0.75 Å, with an exposure time 20 sec per frame. The data integration and reduction were processed with SAINT [2] software. A multi-scan absorption correction was applied to the collected reflections.

**Structure Solution and Refinement**

The systematic absences in the diffraction data were uniquely consistent for the space group P -1 that yielded chemically reasonable and computationally stable results of refinement.[3]

A successful solution by the direct methods provided most non-hydrogen atoms from the $E$-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighbouring atoms with relative isotropic displacement coefficients. No molecule participates in any intramolecular hydrogen bond.

The final least-squares refinement of 970 parameters against 21030 data resulted in residuals $R$ (based on $F^2$ for $I \geq 2 \sigma$) and $wR$ (based on $F^2$ for all data) of 0.0914 and 0.2195, respectively.

Table S4. Crystal data and structure refinement for Boc-Aic-Aic-OEt (P1)

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<tr>
<td>Volume</td>
<td>$4383(3)\AA^3$</td>
</tr>
</tbody>
</table>

$a = 89.932(8)^\circ$. $\beta = 89.996(10)^\circ$. $\gamma = 82.953(10)^\circ$. 
Z 8
Density (calculated) 1.129 Mg/m³
Absorption coefficient (µ) 0.081 mm⁻¹
F (000) 1632
Crystal size 0.30 x 0.10 x 0.5 mm³
Theta range for data collection 1.00 to 28.44°
Index ranges -12<=h<=7, -14<=k<=15, -53<=l<=54
Reflections collected 55478
Independent reflections 21030 [R(int) = 0.2147]
Completeness to theta = 28.44° 95.2 %
Absorption correction Empirical with SADABS
Max. and min. Transmission 0.996 and 0.990
Refinement method Full-matrix least-squares on F²
Data / restraints / parameters 21030 / 0 / 970
Goodness-of-fit on F² 0.798
Final R indices [I>2sigma(I)] R1 = 0.0914, wR2 = 0.2195
R indices (all data) R1 = 0.2952, wR2 = 0.3591
Largest diff. peak and hole 0.361 and -0.368 e.Å⁻³

ii) Boc-Aic-Aic-Aic-OEt (P2)

Data Collection

A colorless crystal with approximate dimensions 0.45 x 0.4 x 0.2 mm³ was selected under oil under ambient conditions and attached on nylon CryoLoops with Paratone-N (Hampton Research). The crystal was mounted in a stream of cold nitrogen at 100(2) K and centered in the X-ray beam by using a video camera.

The crystal evaluation and data collection were performed on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) with Mo Kα (λ = 0.71073 Å) radiation and the diffractometer to crystal distance of 4.0 cm. The initial cell constants were obtained from three series of ω scans at different starting angles. Each series consisted of 12 frames collected ω with the exposure time of 25 seconds per frame. Obtained reflections were successfully
indexed by an automated indexing routine built in the SMART program. The final cell constants were calculated from a set of 55478 strong reflections from the actual data collection.

The data were collected to a resolution of 0.75 Å, with an exposure time 25 sec per frame. The data integration and reduction were processed with SAINT [2] software. A multi-scan absorption correction was applied to the collected reflections.

**Structure Solution and Refinement**

The systematic absences in the diffraction data were uniquely consistent for the space group P2 1 2 1 2 1 that yielded chemically reasonable and computationally stable results of refinement. [3]

A successful solution by the direct methods provided most non-hydrogen atoms from the E-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighbouring atoms with relative isotropic displacement coefficients. No molecule participates in any intramolecular hydrogen bond.

The final least-squares refinement of 604 parameters against 10226 data resulted in residuals $R$ (based on $F^2$ for $I \geq 2\sigma$) and $wR$ (based on $F^2$ for all data) of 0.1057 and 0.2218, respectively.

**Table S5. Crystal data and structure refinement for Boc-Aic-Aic-Aic-OEt (P2)**

<table>
<thead>
<tr>
<th>Compound Identity</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C$<em>{25}$H$</em>{47}$N$_3$O$_6$</td>
</tr>
<tr>
<td>Formula weight</td>
<td>485.66</td>
</tr>
<tr>
<td>Temperature</td>
<td>100(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>P 2 1 2 1 2 1</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>$a = 9.633(5)$Å, $\alpha = 90^\circ$.</td>
</tr>
<tr>
<td></td>
<td>$b = 18.305(8)$Å, $\beta = 90^\circ$.</td>
</tr>
<tr>
<td></td>
<td>$c = 33.028(15)$Å, $\gamma = 90^\circ$.</td>
</tr>
<tr>
<td>Volume</td>
<td>5824(5)Å$^3$</td>
</tr>
<tr>
<td>Z</td>
<td>8</td>
</tr>
</tbody>
</table>
Density (calculated)                                             1.108Mg/m³
Absorption coefficient (µ)                                      0.078mm⁻¹
F (000)                                                        2128
Crystal size                                                   0.45 x 0.4 x 0.2mm³
Theta range for data collection                                1.27 to 25.0°
Index ranges                                                  -10<=h<=11, -21<=k<=21, -39<=l<=39
Reflections collected                                         80005
Independent reflections                                       10226 [R(int) = 0.2720]
Completeness to theta = 25.0°                                   99.4 %
Absorption correction                                          Empirical with SADABS
Max. and min. Transmission                                     0.985 and 0.966
Refinement method                                              Full-matrix least-squares on F²
Data / restraints / parameters                                  10226/ 0 / 604
Goodness-of-fit on F²                                           1.033
Final R indices [I>2sigma(I)]                                  R1 = 0.1057, wR2 = 0.2218
R indices (all data)                                           R1 = 0.196, wR2 = 0.2667
Absolute structure parameter                                   1(2)
Largest diff. peak and hole                                     0.470 and -0.357 e.Å⁻³

References
$^1$H, $^{13}$C and mass spectra of compounds
Calcd. [M+Na]+ = 254.1368

Mass (m/z)

270.0829(R4465,5811)

254.1085(R4356,5293)

2322.6
Calcd. \[\text{[M+Ne]}^+ \] = 395.2522
Calcd. [M+Na]^+ = 508.3363

524.3092(R7023,S4312)

508.3542(R6730,S1617)

408.2603(R3966,S1800)

424.2500(R555,1657)
Calcd. [M+Na]^+ = 621.4203

621.4250(R7296.5442)

637.3985(R7781.6317)

521.3696(R5584.3274)
IR spectra for P3 and P4