SUPPLEMENTARY DATA

for

Revised Manuscript #B518420G

Oligomers of *cis*-β-norbornene amino acid: Formation of β-strand mimetics

Srivari Chandrasekhar*[†], Bathini Nagendra Babu[†], Anabathula Prabhakar[‡], Ambadi Sudhakar[†], Marepally Srinivasa Reddy[†], Marelli Udaya Kiran[‡], Bharatam Jagadeesh*[‡].

[†] *Division of Organic Chemical Sciences, Indian Institute of Chemical Technology(IICT), Hyderabad,   India.*

[‡] *Center for Nuclear Magnetic Resonance.   IICT*

x = MeOH, HCl (g)
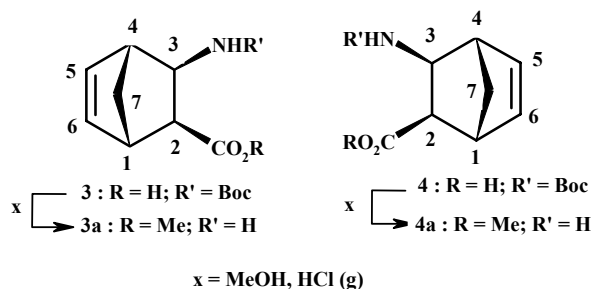
Synthesis of (1S,2S,3R,4R)-3-exo-N-tbutyloxycarbonylaminobicyclo[2,2,1]hept-5-ene-2-exo-carboxylic acid(3): N-Boc protected amino acid **2** (10 g, 39.5 mmol) and $R(+)$ phenylethylamine (4.78 g, 39.5 mmol) was dissolved in ethyl acetate (150 mL) and the solution was kept overnight at room temp. The resulting salt was filtered off and recrystalized from ethyl acetate. The fractional recrystalization procedure was repeated until the value of optical rotation was constant $[\alpha]^{20}_D$ +16° (C = 1, MeOH). The resolved salt was treated with 2N HCl (60 mL) and extracted with ethyl acetate (3x60mL). The ethyl acetate layer was dried over Na_2SO_4 and taken to dryness under reduced pressure. The desired free acid exo-(1S, 2S, 3R, 4R)-**3** was obtained (4.0 g, 40%), $[\alpha]^{20}_D$: +90.6 with 99%ee [determined by HPLC using Chiral pak AD, column(250x4.6 mm), 10um, hexane : 0.1% TFA in EtOH (90:10), t_R = 7.349]. Similar method (apart from S(-)-phenylethylamine was used in place of $R(+)$-phenylethylamine) used for another isomer exo (1R, 2R, 3S, 4S)-**4** with $[\alpha]^{20}_D$: -90.6 [determined by HPLC using Chiral pak AD, column(250x4.6 mm), 10um, hexane : 0.1% TFA in EtOH / 90:10, t_R = 8.213].

Synthesis of 3a and 4a: Acids **3/4** was dissolved in dry MeOH and HCl gas was passed until solvent was refluxed. After that reacton mixture was stirred for 30 min. Solvent was removed and dried under vaccum. The yield of the product was not calculated due to the presence of HCl, and the crude amine-HCl **3a /4a** was used for the next step.

Synthesis of compounds 5-10: The oligomerization of the resolved two enantiomers of this amino acid (**3** [2S,3R] and **4**[2R,3S]) was achieved following the conventional peptidation as described previously. Deprotection of Boc group was achieved with trifluoro acetic acid in dichloromethane (1:1) at 0 °C while esters were hydrolyzed with LiOH in THF, H_2O (3:1). The synthesis of oligomers **5-10** involved peptidation of monomers (**3/ 4**) and (**3a/ 4a**) in the requisite sequence using standard coupling reagents EDCI, HOBt and DIPEA in dry dichloromethane. All the compounds reported were purified by column chromatography over silica gel (60-120mesh; Ethyl acetate and hexane).

Circular Dichroism Spectroscopy:

CD spectra were recorded on JASCOJ-715 spectrometer at 25°C in methanol, using 1mm path length CD cell. All spectra represent the average of 5 scans. They are all background corrected. The concentration of peptides used is 0.5 mM, Scan Range: 195-250nm: band width: 2nm. CD spectra of **5-10** as shown in Figure **4**.

FT-IR-Spectroscopy.

IR spectra of **6** and **7** are recorded in solution ($CHCl_3$) and also as solid pellet (KBr) by using Thermo Nicolet-670 FT-IR spectrometer. 64 scans are made for each experiment with $2cm^{-1}$ resolution.
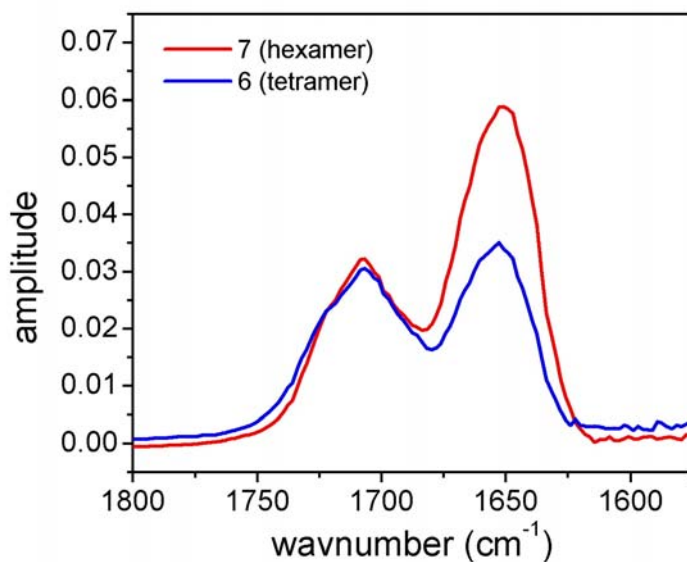

FT-IR bands of Amide-I : Signatures of H-bonding (~ 1650 cm^{-1})
in tetramer and hexamer

NMR Spectroscopy:

NMR spectra were recorded on Varian Unity Inova - 500 MHz at 303 K with 7-10 mM solutions in $CDCl_3$ solvents using tetramethylsilane as internal standard or the solvent signals as secondary standards, and the chemical shifts are shown in scales. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), br (broad), m (multiplet, for unresolved lines), etc. Two dimensional (2D) total correlation spectroscopy (TOCSY), and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments were carried out. All the experiments were carried out in the phase-sensitive mode.

The 2D spectra were acquired with 2 x 256 or 2 x 192 free induction decays (FID) containing 8-16 transients with relaxation delays of 2.0 s. The ROESY experiments were performed with mixing time of 0.2 to 0.3 s. For ROESY experiments a spin locking field of about 2 kHz and pulsed field locking with 30° pulses were used. The TOCSY experiments were performed with the spin locking field of about 10 kHz and a mixing time of 0.08 s. The two dimensional data were processed with Gaussian apodization in both the dimensions. In these oligomers the N-terminal amide proton was easily assigned as it shows nOe with intra residue CαH proton only, whereas the other amide protons show both intra residue and inter residue NH / CαH nOe. This assignment has further supported by the nOe between the amide and Boc group. Similarly the CαH of the C- terminal residue has been identified from the presence of nOe with the intra residue NH only. The spectra (One Dimensional, TOCSY and ROESY) and solvent titration studies plot are illustrated in the supporting Figures **1-17** and the chemical shifts, coupling constants are given in Supporting Tables **01-06**.

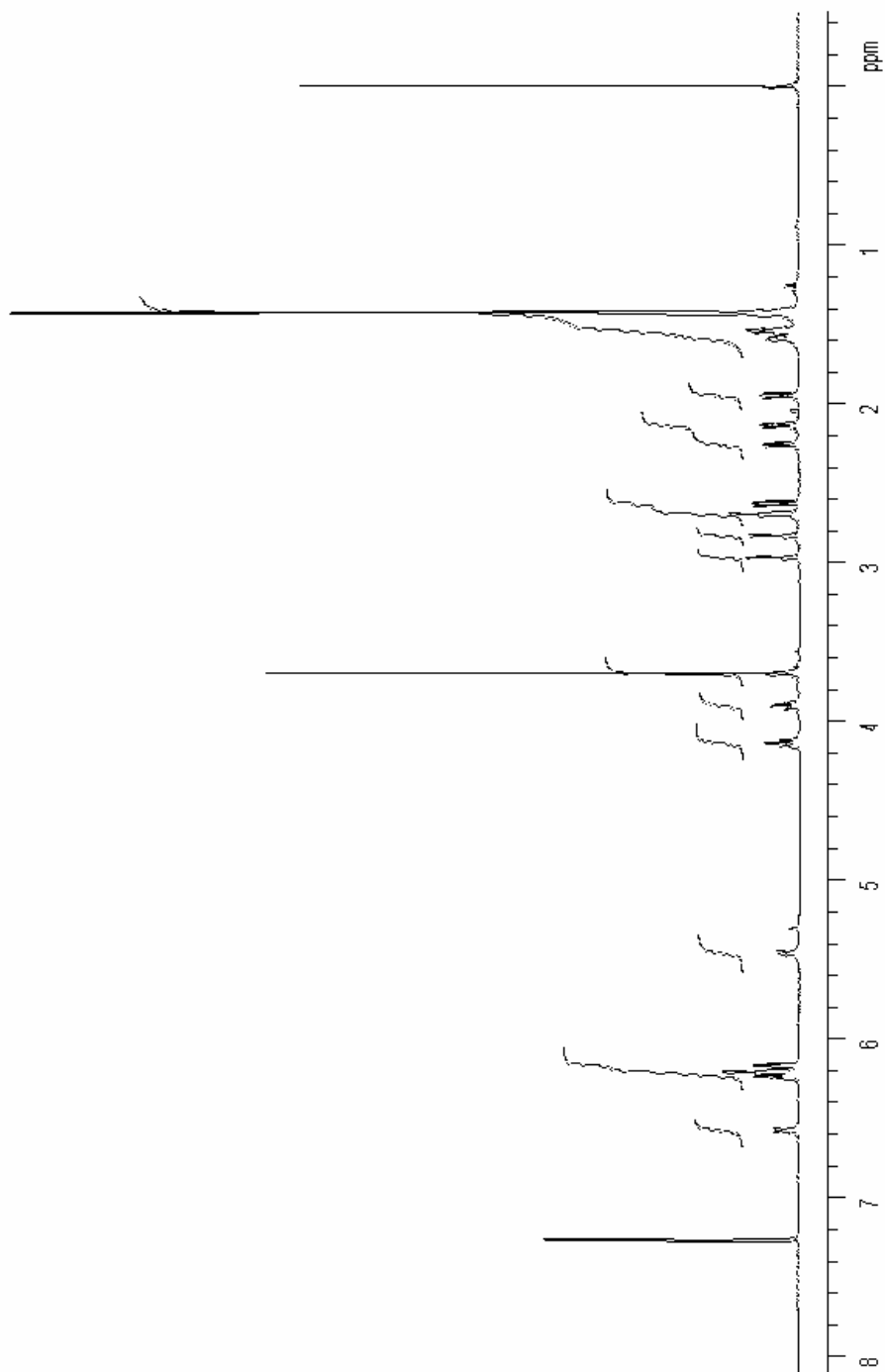**Newman projections for two enantiomers of norborene about Cα-Cβ axis**
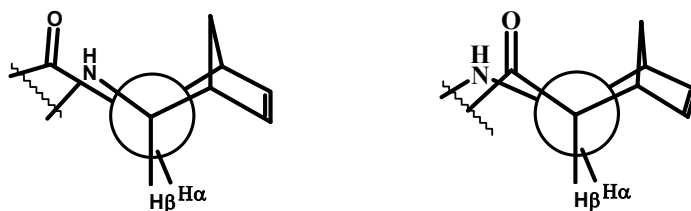
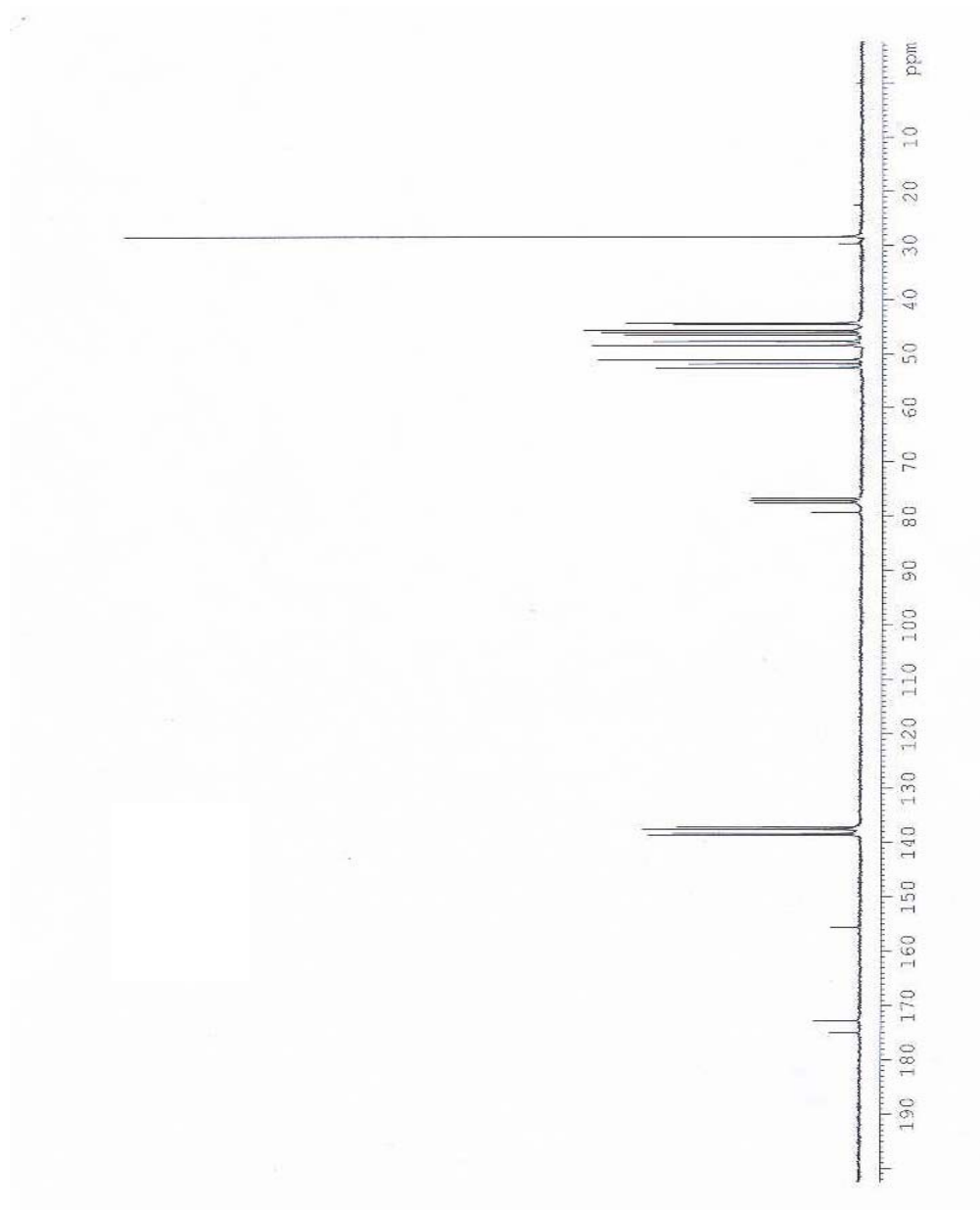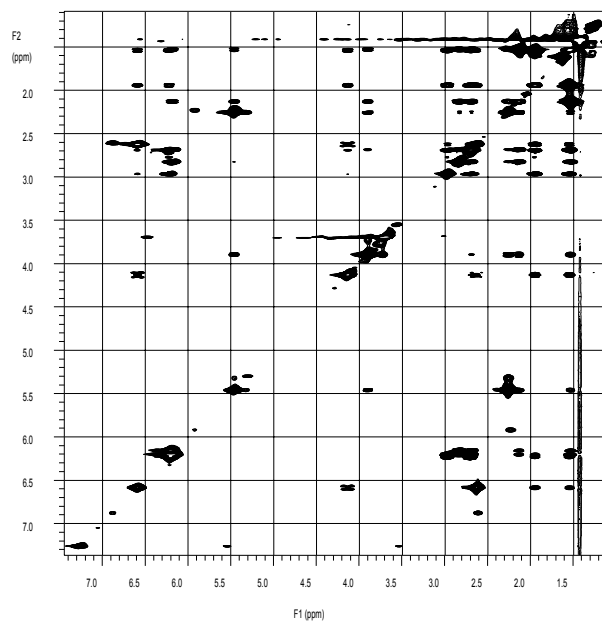Dihedral angles for norbornene
residue (Balaram's convention)
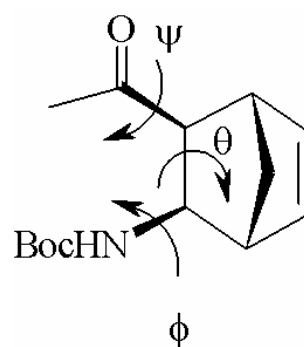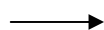
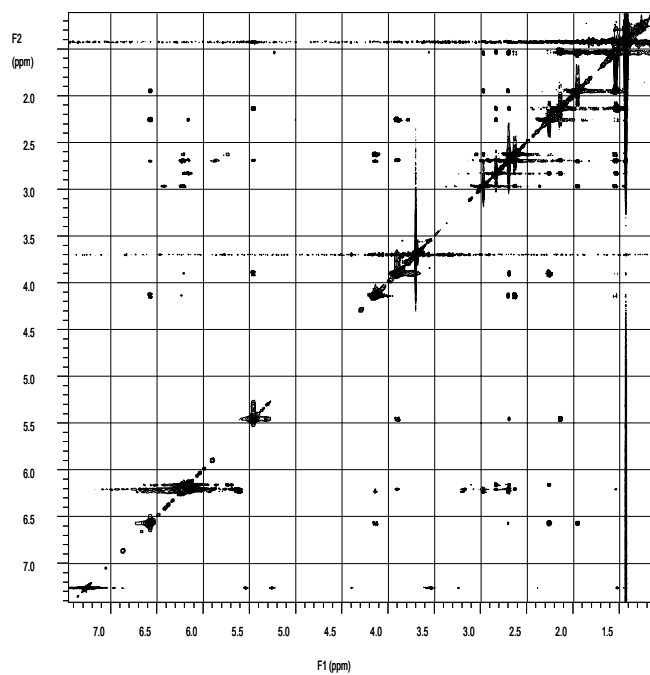**Supporting Figure-01: ^{1}H NMR spectrum of dimer-5[500MHz, 303K,CDCl$_3$]**

Supporting Figure-02: ^{13}C NMR spectrum of dimer-5[75MHz, 303K,CDCl$_3$]

Supporting Figure-03: TOCSY NMR spectrum of dimer-5[500MHz,303K,CDCl₃]

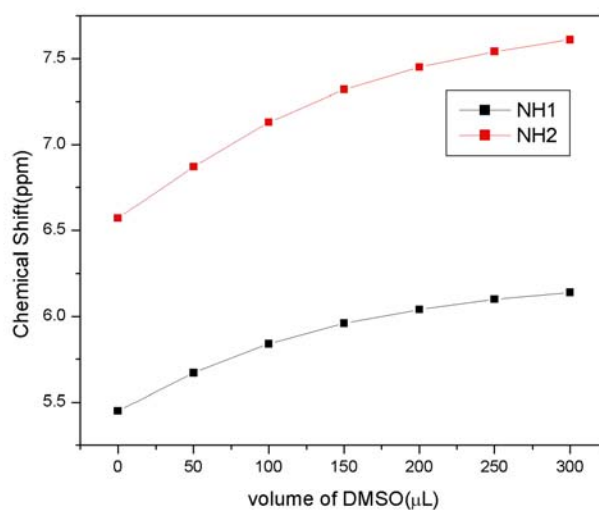**Supporting Figure-04: ROESY NMR spectrum of dimer-5 [500MHz, 303K,CDCl₃]**

Supporting Table-01: Chemical Shifts and Coupling Constants for dimer-5 in CDCL3 (500MHz)

Residue	NH	C1H	C2H (α)	C3H (β)	C4H	C7H	C7'H
1	5.45 $J_{NH,3}$=9.4	2.83 br.m	2.26(dd) $J_{2,3}$=8.2 $J_{2,1}$=1.3	3.90(ddd) $J_{NH,3}$=9.4 $J_{3,4}$=1.6 $J_{2,3}$=8.2	2.69 m	2.14 m	1.51 m
2	6.59 $J_{NH,3}$=8.7	2.97 br.m	2.63(dd) $J_{2,3}$=8.4, $J_{2,1}$=1.3	4.13(ddd) $J_{NH,3}$=8.7 $J_{3,4}$=1.7 $J_{2,3}$=8.4	2.70 m	1.94 m	1.52 m

Mass spectrometry data : (ESI/HRMS): cal.c. m/z 403.2232, found. 403.2243

Supporting Figure-05: Solvent Titration Studies of dimer-5 :

Supporting Figure-06: ^{1}H NMR spectrum of tetramer-6[500MHz, 303K,CDCl$_3$]
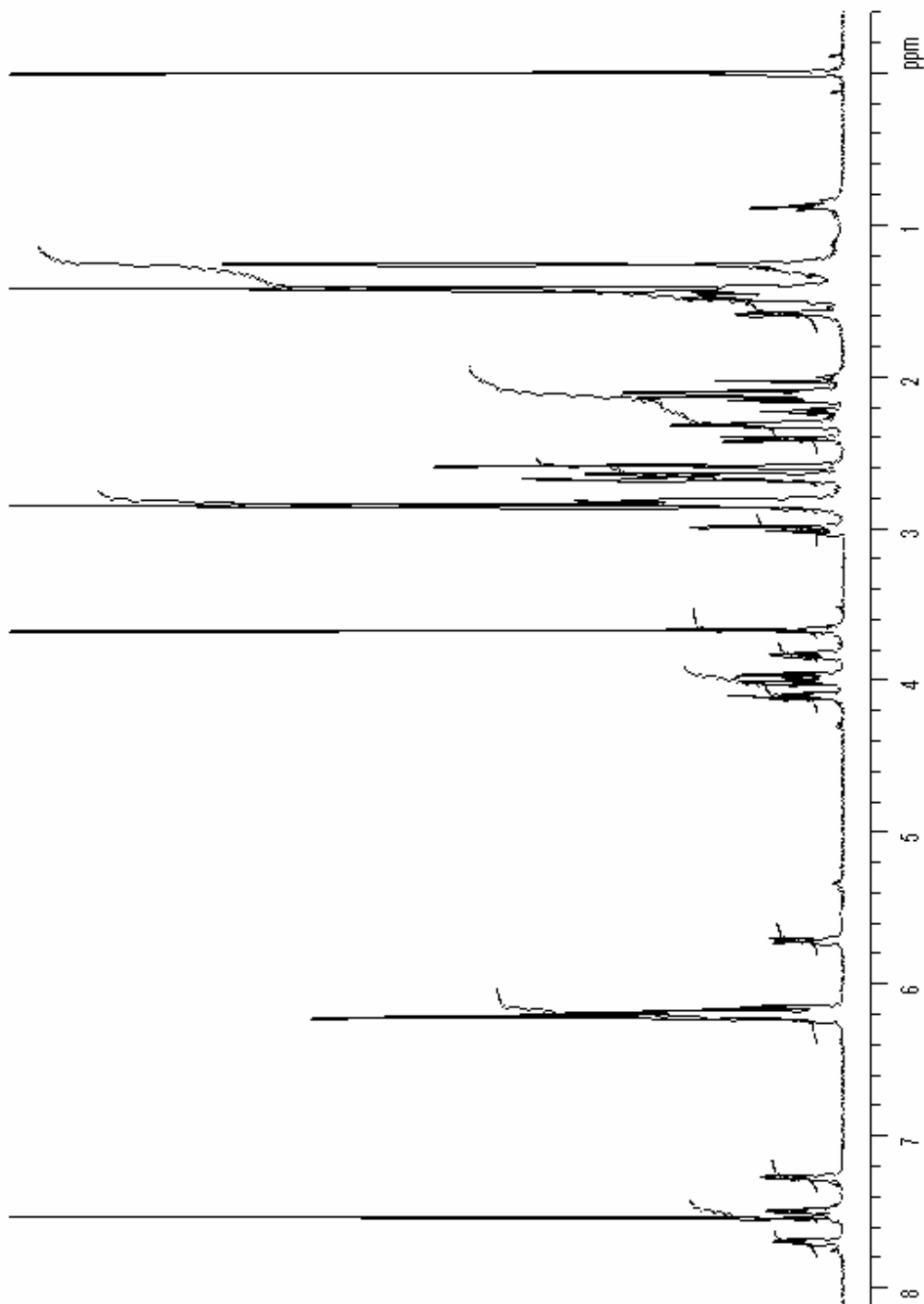
Supporting Figure-07: ^{13}C NMR spectrum of hexamer-6[75MHz, 303K,CDCl$_3$]
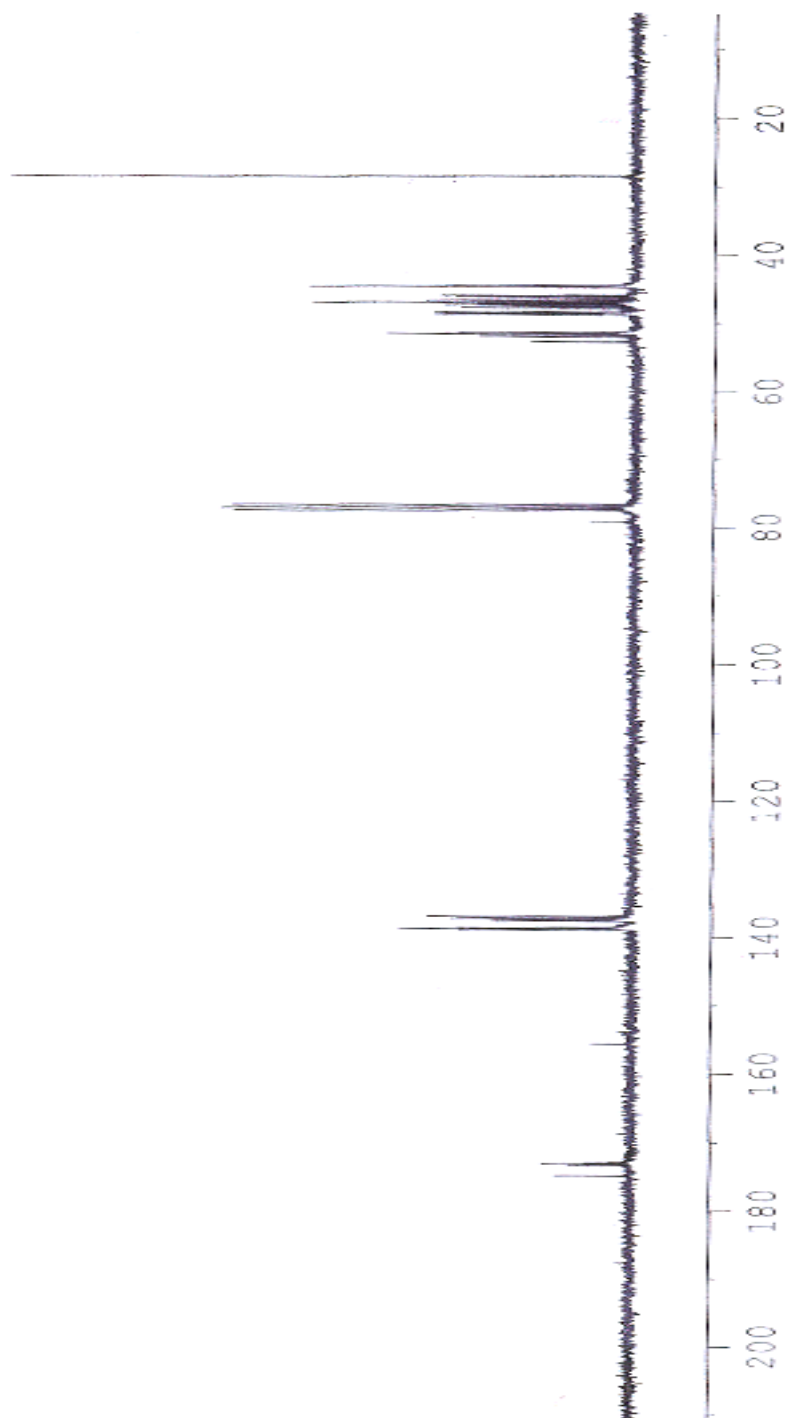
Supporting Figure-08: TOCSY NMR spectrum of tetramer-6[500MHz,303K,CDCl₃
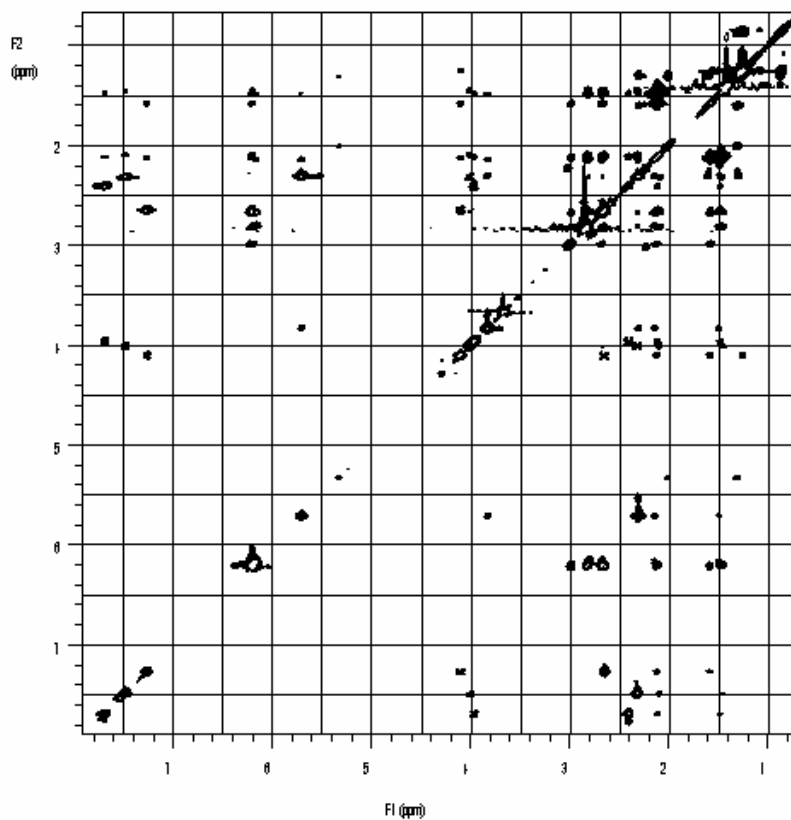
Supporting Figure-09: ROESY NMR spectrum of tetramer-6 [500MHz, 303K,CDCl₃]
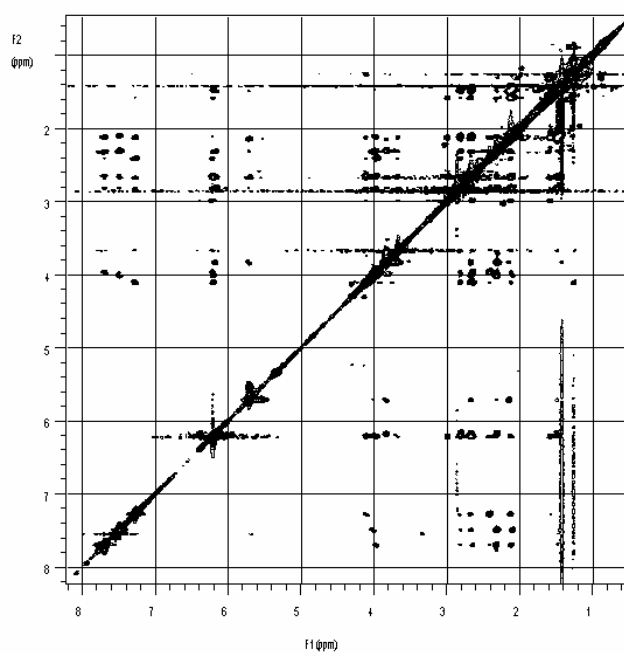
Supporting Table-02: Chemical Shifts and Coupling Constants for tetramer-6 in CDCl$_3$ (500MHz))

Residue	NH	C1H	C2H (α)	C3H (β)	C4H	C7H	C7'H
1	5.71 $J_{NH,3}$=9.0	2.80 br.m	2.30(dd) $J_{2,3}$=9.0 $J_{2,1}$=1.3	3.83(dt) $J_{NH,3}$=9.0 $J_{3,4}$=1.9 $J_{2,3}$=9.0	2.66 m	2.14 m	1.48 m
2	7.48 $J_{NH,3}$=8.4	2.80 br.m	2.31(dd) $J_{2,3}$=8.4, $J_{2,1}$=1.3	4.01(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.9 $J_{2,3}$=8.4	2.66 m	2.09 m	1.44 m
3	7.69 $J_{NH,3}$=8.4	2.82 br.m	2.40(dd) $J_{2,3}$=8.4, $J_{2,1}$=1.3	3.97(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.9 $J_{2,3}$=8.4	2.64 m	2.11 m	1.47 m
4	7.26 $J_{NH,3}$=8.4	2.81 br.m	2.64(dd) $J_{2,3}$=8.4 $J_{2,1}$=1.3	4.10(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.9 $J_{2,3}$=8.4	2.67 m	2.12 m	1.58 m

Others: 6.14-6.28(m, H5&H6)

Supporting Table-03: Chemical Shifts and Coupling Constants for tetramer-6 in DMSO (500MHz)

Residue	NH	C1H	C2H(α)	C3H(β)	C4H	C7H	C7'H
1	6.32 $J_{NH,3}$=8.3	2.69 br.m	2.32(dd) $J_{2,3}$=8.3 $J_{2,1}$=1.5	3.61(dt) $J_{NH,3}$=8.3 $J_{3,4}$=1.9 $J_{2,3}$=8.3	2.51 m	2.06 m	1.30 m
2	7.76 $J_{NH,3}$=8.4	2.71 br.m	2.35(dd) $J_{2,3}$=8.4, $J_{2,1}$=1.4	3.84(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.8 $J_{2,3}$=8.4	2.51 m	2.12 m	1.41 m
3	7.59 $J_{NH,3}$=8.4	2.65 br.m	2.40(dd) $J_{2,3}$=8.4, $J_{2,1}$=1.4	3.81(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.3 $J_{2,3}$=8.4	2.50 m	2.15 m	1.31 m
4	7.61 $J_{NH,3}$=8.4	2.87 br.m	2.48(dd) $J_{2,3}$=8.4 $J_{2,1}$=2.0	3.99(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.7 $J_{2,3}$=8.4	2.52 m	2.10 m	1.42 m

Mass spectrometry data : (ESI/HRMS): calc. m/z 673.3601, found. 673.3600
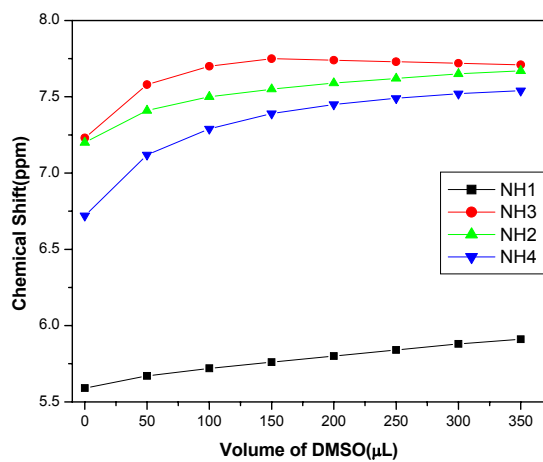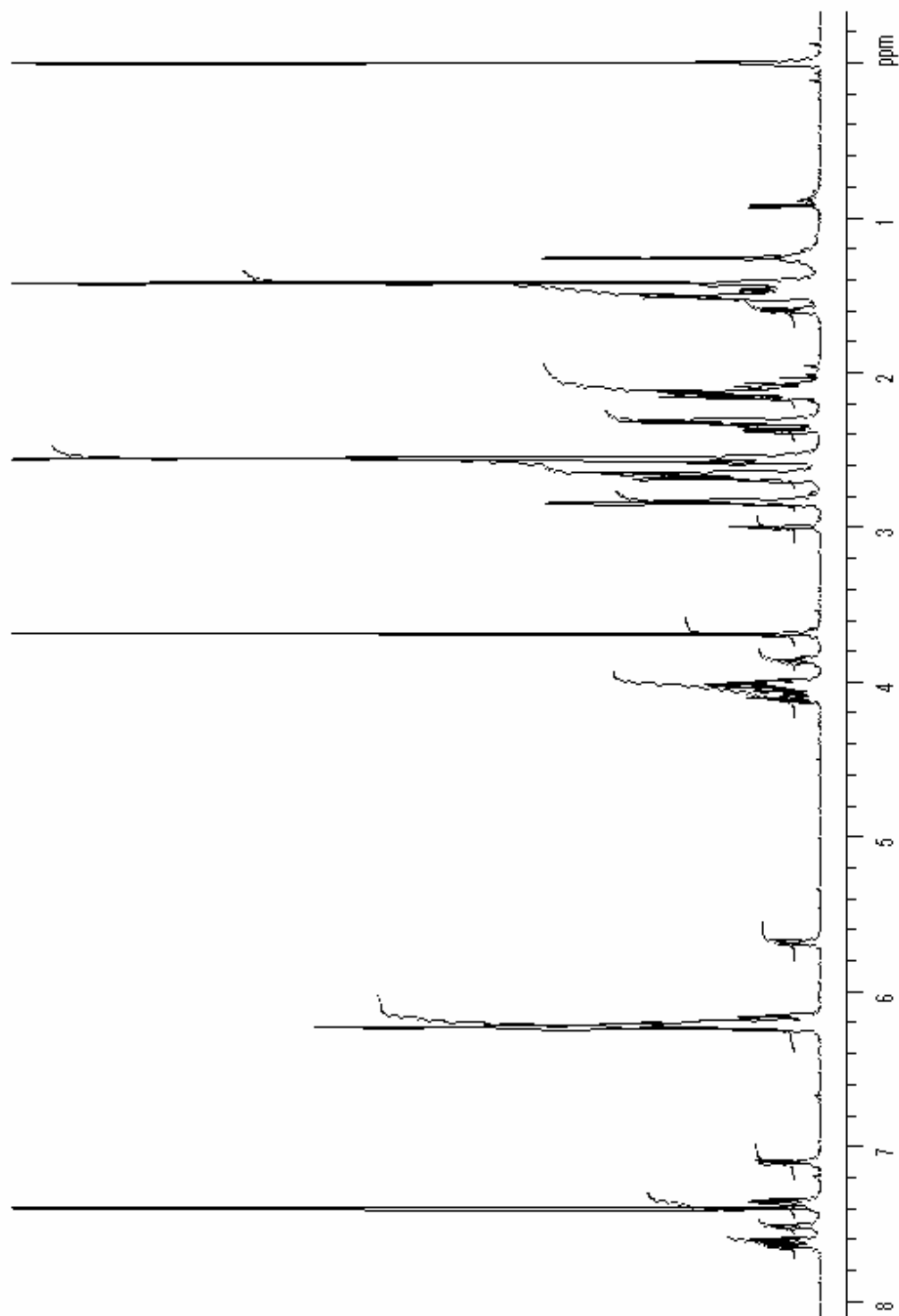
Supporting Figure-10: Solvent Titration Studies of tetramer-6:

Supporting Figure-11: ^{1}H NMR spectrum of hexamer-7[500MHz, 303K,CDCl$_3$]

Supporting Figure-12: ^{13}C NMR spectrum of hexamer-7 [75MHz, 303K,CDCl$_3$]
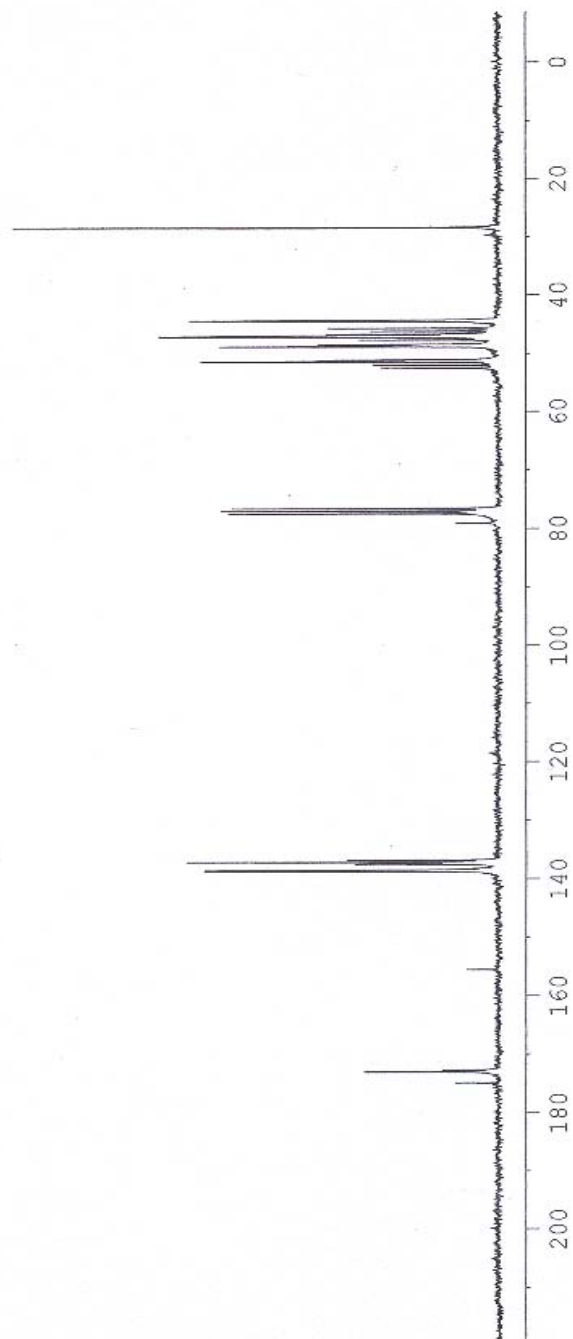
Supporting Figure-13: TOCSY NMR spectrum of hexamer-7 [500MHz,303K,CDCl₃]

Supporting Figure-14: ROESY NMR spectrum of hexamer-7 [500MHz, 303K,CDCl₃]

Supporting Figure-14 : ROESY expansion for hexamer-7 showing the possible inter-residue H_1-H_4 NOE's which are overlapped (shown in circles)

Supporting Table-04: Chemical Shifts and Coupling Constants for HEXAMER-7 in CDCL$_3$ (500MHz)

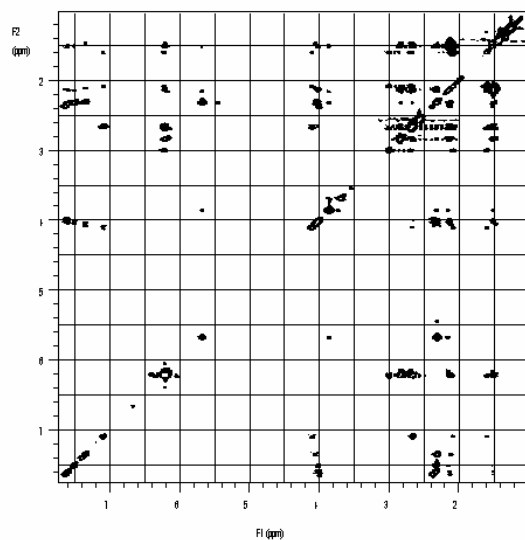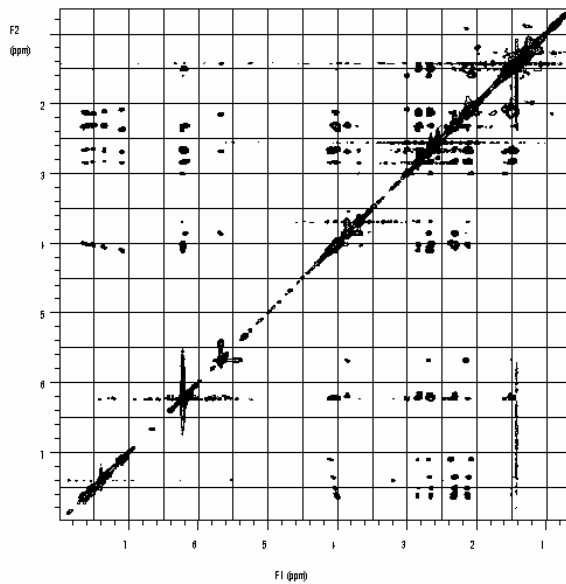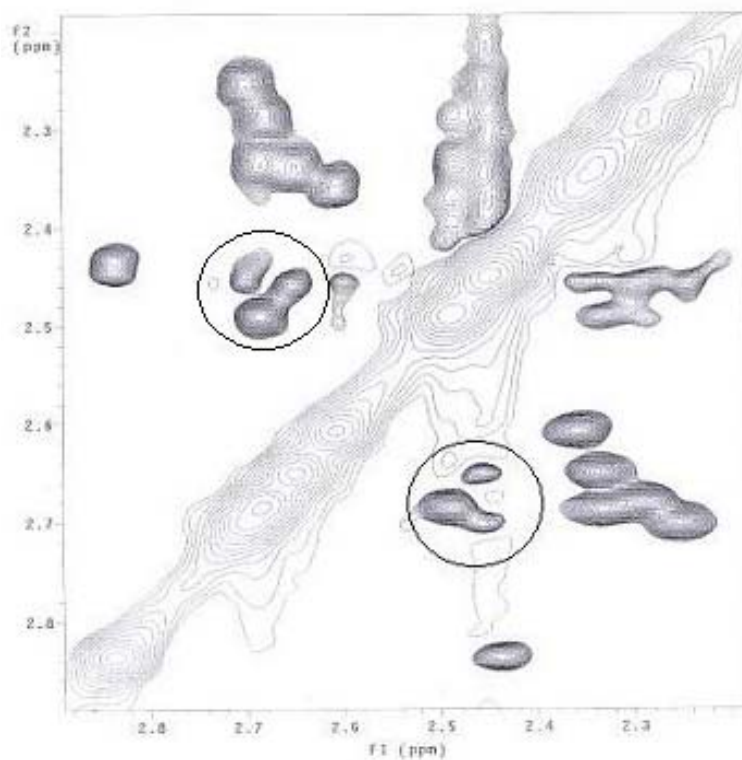| Residue	NH	C1H	C2H(α)	C3H(β)	C4H	C7H	C7'H
1	5.68 $J_{NH,3}$=9.0	2.82 br.m	2.305(dd) *overlap*	3.86(dt) $J_{NH,3}$=9.0 $J_{3,4}$=1.2 $J_{2,3}$=9.0	2.69 m	2.15 m	1.50 m
2	7.36 $J_{NH,3}$=8.4	2.82 br.m	2.30(dd) *overlap*	4.05(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.6 $J_{2,3}$=8.4	2.66 m	2.10 m	1.46 m
3	7.52 $J_{NH,3}$=8.4	2.84 br.m	2.31(dd) *overlap*	4.02(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.6 $J_{2,3}$=8.4	2.63 m	2.14 m	1.50 m
4	7.61 $J_{NH,3}$=8.4	2.83 br.m	2.32dd) *overlap*	4.01(dt) $J_{NH,3}$=8.4 $J_{3,4}$=2.0 $J_{2,3}$=8.4	2.65 m	2.13 m	1.50 m
5	7.64 $J_{NH,3}$=8.4	2.85 br.m	2.37(dd) *overlap*	4.00(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.9 $J_{2,3}$=8.4	2.65 m	2.12 m	1.51 m
6	7.10 $J_{NH,3}$=8.4	2.99 br.m	2.66(dd) *overlap*	4.10(dt) $J_{NH,3}$=8.4 $J_{3,4}$=2.0 $J_{2,3}$=8.4	2.79 m	2.08 m	1.59 m

Others: 6.14-6.28(m, H5&H6)

Supporting Table-05: Chemical Shifts and Coupling Constants for hexamer 7 in DMSO (500MHz)

Residue	NH	C1H	C2H(α)	C3H(β)	C4H	C7H	C7'H
1	6.30 $J_{NH,3}$=9.3	2.66 br.m	2.34(dd) *overlap*	3.56(dt) $J_{NH,3}$=9.3 $J_{3,4}$=1.9 $J_{2,3}$=9.3	2.48 m	2.03 m	1.28 m
2	7.55 $J_{NH,3}$=8.7	2.65 br.m	2.33(dd) $J_{2,3}$=8.1, $J_{2,1}$=1.3	3.85(ddd) $J_{NH,3}$=8.7 $J_{3,4}$=1.9 $J_{2,3}$=8.1	2.45 m	2.07 m	1.28 m
3	7.46 $J_{NH,3}$=8.3	2.70 br.m	2.25(dd) $J_{2,3}$=8.3, $J_{2,1}$=1.3	3.86(dt) $J_{NH,3}$=8.3 $J_{3,4}$=1.8 $J_{2,3}$=8.3	2.46 m	2.07 m	1.28 m
4	7.56 $J_{NH,3}$=8.4	2.68 br.m	2.29(dd) $J_{2,3}$=8.1 $J_{2,1}$=1.3	3.78(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.9 $J_{2,3}$=8.4	2.47 m	2.07 m	1.28 m
5	7.53 $J_{NH,3}$=8.4	2.61 br.m	2.35(dd) $J_{2,3}$=8.2 $J_{2,1}$=1.3	3.77(dt) $J_{NH,3}$=8.4 $J_{3,4}$=1.9 $J_{2,3}$=8.4	2.46 m	2.07 m	1.28 m
6	7.60 $J_{NH,3}$=8.7	2.83 br.m	2.44(dd) *overlap*	3.95(dt) $J_{NH,3}$=8.7 $J_{3,4}$=1.9 $J_{2,3}$=8.7	2.49 m	2.07 m	1.38 m

Others: 6.07-6.20(m, 2H, H5&H6)
Mass spectrometry data : (ESI/HRMS): calc. m/z 943.4969, found. 943.5000

Supporting Figure-15: Solvent Titration Studies of hexamer −7:
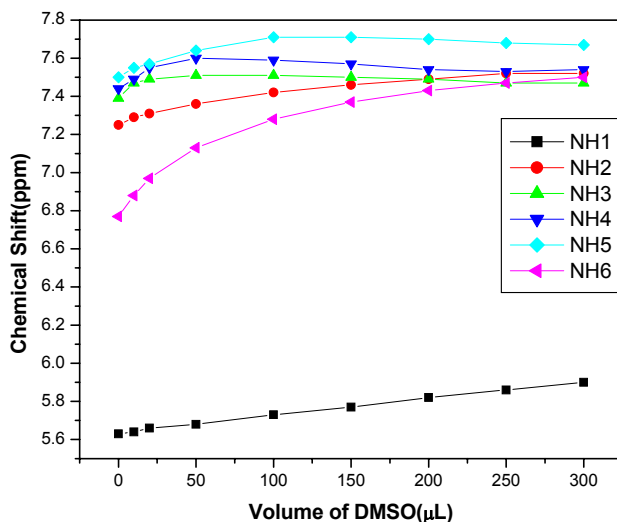
Molecular dynamics calculations:

The cvff force field with default parameters was used throughout the simulations. Minimization's were done first with steepest decent, followed by conjugate gradient methods for a maximum of 1000 iterations each or RMS deviation of 0.001 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD simulations. A number of inter atomic distance and torsional angle constraints obtained from NMR data were used as restraints in the minimization as well as MD runs. For MD runs, a temperature of 300 K was used. The molecules were initially equilibrated for 20 ps and subsequently subjected to a 500 ps dynamics with a step size of 1 fs, sampling the trajectory at equal intervals of 5 ps. In trajectory 100 samples were generated and the best structures, which forming the terminal hydrogen bonding, were again energy minimized with above protocol and superimposed these strucures.

Supporting Table-06: Distance constraints used in MD calculations for tetramer-6 derived from ROESY experiment (dmso)

Residue No.	Hydrogen Atom	Residue No.	Hydrogen Atom	Lower Bound	Upper Bound
1	NH	1	H_7	2.78	3.39
1	NH	1	H_4	2.88	3.52
1	NH	1	H_3 β)	3.13	3.83
1	NH	2	H_3 (β)	4.34	5.00
1	H1	3	H_4	2.33	2.85
2	NH	2	H_7	2.90	3.55
2	NH	2	H_4	2.88	3.52
2	NH	2	H_3 (β)	3.11	3.80
2	NH	1	H_3 (β)	4.24	5.00
2	NH	3	H_3 (β)	3.9	4.8
2	NH	1	H_2 (α)	2.46	3.00
2	NH	1	H_1	3.80	4.64
2	H1	4	H_4	2.38	2.90
2	H2	4	H_3	3.79	4.63
3	NH	3	H_7	2.81	3.44
3	NH	3	H_4	2.68	3.27
3	NH	3	H_3 (β)	3.08	3.76
3	NH	2	H_2 (α)	2.43	2.97
3	NH	2	H_1	3.61	4.41
3	NH	4	H_3 (β)	3.90	4.80
3	NH	2	H_3 (β)	4.2	5.0

4	NH	4	H_7	2.82	3.45
4	NH	4	H_4	2.72	3.32
4	NH	4	H_3 (β)	3.13	3.83
4	NH	3	H_2 (α)	2.42	2.95
4	NH	3	H_3 (β)	4.3	5.2
4	NH	3	H_1	3.93	4.81
4	OME	3	H_1	2.34	2.86

Dynamics pictures of 6, 7, 9, 10 :

a)

b)

Figure 16 : Side view of a) 9 b) 7

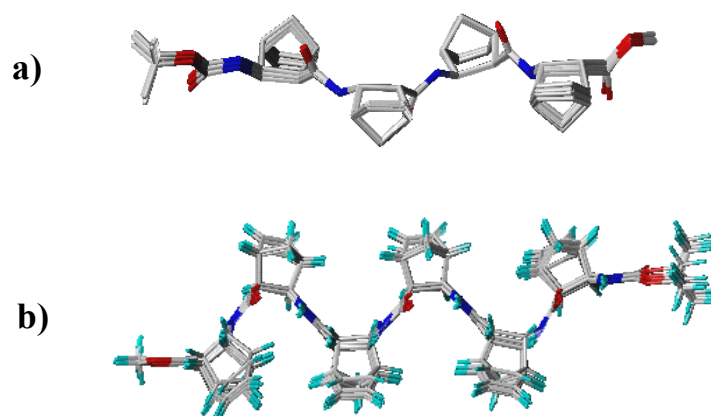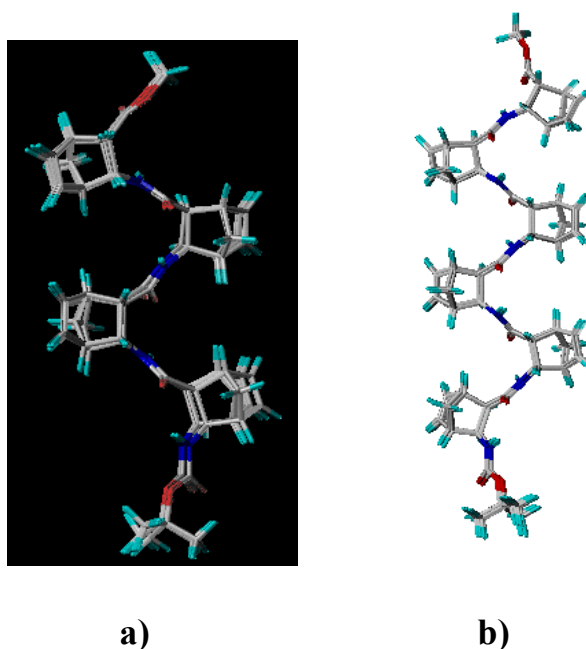a)   b)

Figure 17 : top view of a) 6 b) 10