Supplementary Material (ESI) for Chemical Communications

Self-Assembly of Cyclic Homo and Hetero β-Peptides with *Cis*-Furanoid Sugar Amino Acid and β-hGly as Building Blocks

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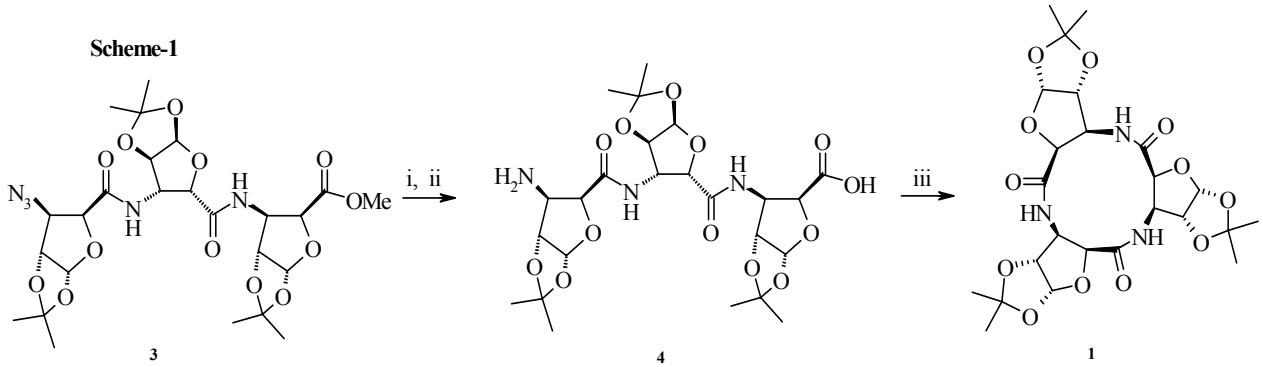
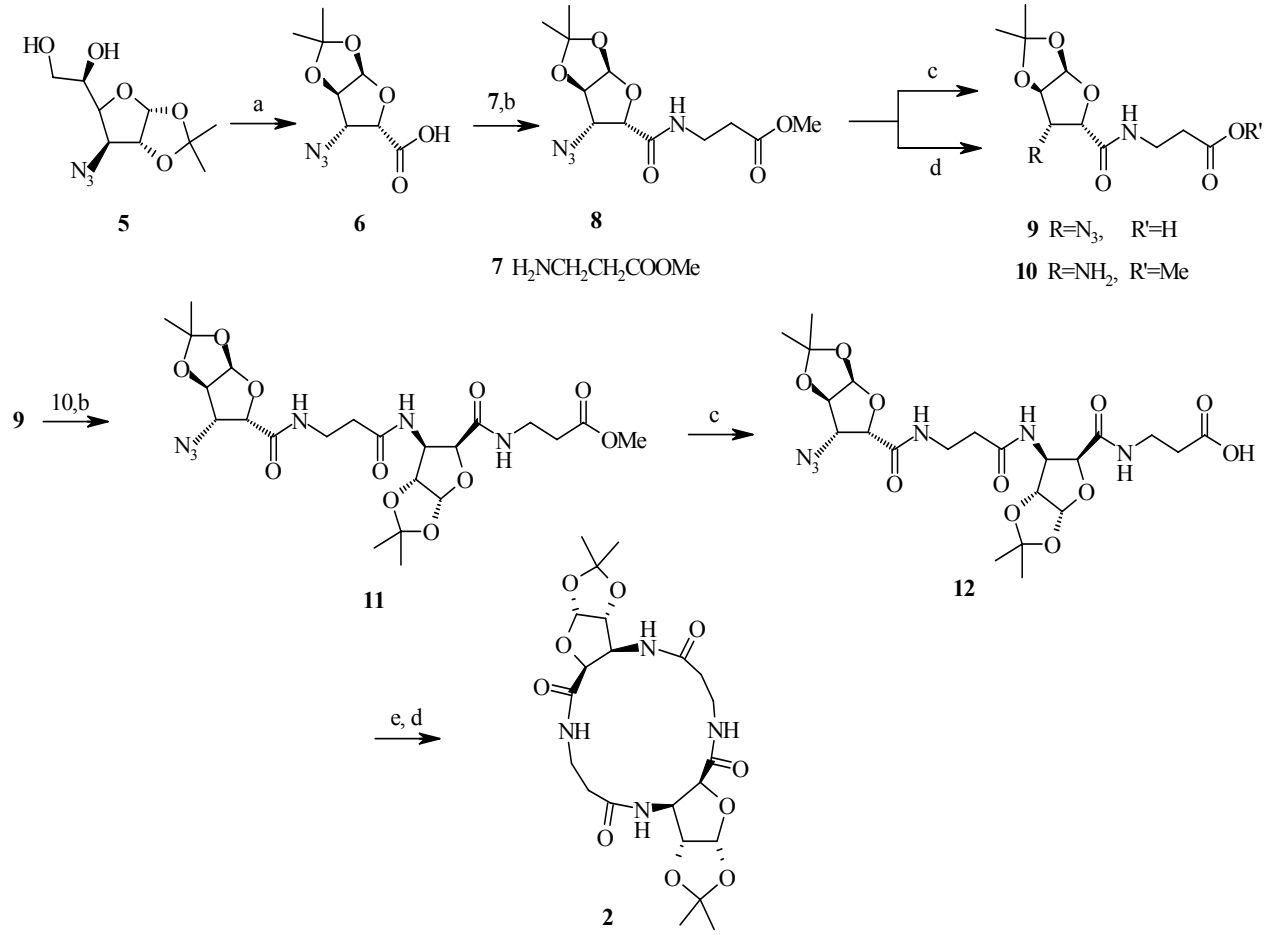
Synthesis of 1 and 2 :

Scheme-1



i) LiOH, THF-H_2O, 0°C ii) Pd-C/10%, EtOAc iii) EDCI-HOBt, CH_2Cl_2, 48h

Scheme-2



a) $NaIO_4$, THF-H_2O,0°C, $NaClO_2$, NaH_2PO_4, H_2O_2, CH_3CN b) EDCI, HOBt, CH_2Cl_2 c) LiOH, THF-H_2O (3:1)
d) Pd/C-10%, EtOAc e) C_6F_5OH, EDCI, CH_2Cl_2.

The cyclo-β-trimer **1** was readily synthesized from

the azido ester trimer **3**[1] in three steps. The methyl ester **3** was hydrolyzed using LiOH followed by the azido group was reduced with Pd/C (10%) under 1 atm hydrogen pressure resulted in the free amino acid trimer **4** in good yield, which is read for cyclization. The intramolecular peptidation of the crude trimer amino acid **4** was achieved with EDCI-HOBt[2a] conditions under high dilution in CH_2Cl_2 for 48 h to yield **1** in 60% yield. The synthesis of the cyclo-β-tetramer **2** began from the 3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose **5**[2b] which on oxidative cleavage furnished azido acid **6**. The coupling of beta alanine methyl ester **7** under EDCI-HOBt protocol on azido acid **6** furnished dimer azide **8**. The ester hydrolysis with LiOH gave **9** whereas reduction of azide with Pd-C/H_2 generated the free amino ester **10**. These two building blocks **9** and **10** were subjected to peptidation to realize **11** in good yield. The mild hydrolysis of ester group in **11** was realized using LiOH to furnish azido acid **12**, which was further activated with pentafluorophenol[2c] followed by hydrogenation generated the desired cyclic tetramer **2**. The cyclic peptides **1** (95%) and **2** (99%) were purified by HPLC.

Experimental Techniques

General Procedures: Melting points (mp) were obtained on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco Dip 360 digital polarimeter and are reported in degrees, concentrations are reported in gm/100mL. IR spectra were obtained on Perkin–Elmer 683 spectrometer or Nicholet-670. Absorption maxima are reported in wave numbers (cm^{-1}). ^1H NMR spectra and ^{13}C were recorded in deuterated solvents on Bruker Avance-300 MHz and Varian Inova-500 MHz spectrometers. ^1H NMR splitting pattern are designated as singlet (s), doublet (d), triplet (t), quartet (q), all first order splitting pattern are assigned on the basis of appearance of the multiplet by splitting pattern that could not be interpreted or easily visualized are designated as multiplet (m) or broad (br). Column chromatographic separations were carried out on silica gel (60–120 mesh) and the cyclic peptide **2** was purified by preparative HPLC. Inertsil ODS-3-V, 250X4.6 mm, 5 um (C18/AR/25) with methnol : water (30:70) as mobile phase. 1-Hydroxybenzotriazole (HOBt), 1-[3-(dimethyl

amino)propyl]-3-ethyl-carbodiimide hydrochloride (EDCI), were purchased from Lancaster. All other reagents and solvents were purchased from Aldrich or Merk. All solvents were purified by standard techniques.

Mass Spectrometry

Mass spectra were obtained on Finnegan MAT 1020B or micro-mass VG 70-70H spectrometer operating at 70 eV using direct inlet system.

Synthesis of C_3-symmetric *cyclo* [cis-β-fSAA]$_3$ 1:

To a stirred solution of compound **3** (0.50 g, 0.82 mmol) in THF–H_2O (3:1, 12 mL) was added LiOH.H_2O (0.10 g, 2.45 mmol) at 0 °C and stirred for 1 h at 0 °C. The reaction mixture was acidified (pH ~ 2.5) with 5% aqueous sodium bisulphate and extracted with ethyl acetate (3 x 25 mL). The organic layer was washed with water (20 mL), brine (20 mL) and dried over anhydrous sodium sulphate and evaporated to furnish the corresponding acid (0.46 g, 95%) of compound **3**, which was directly subjected to hydrogenation (1 atm) using 10% Pd/C (0.10 g) for 3 h at room temperature. The catalyst was filtered off and the filtrate was concentrated to give compound **4** (0.44 g, 100%). The compound **4** (0.44 g, 0.76 mmol) was dissolved in CH_2Cl_2 (100 mL, 0.008 M) to which were added HOBt (0.83 g, 6.1 mmol), EDCI (1.17 g, 6.1 mmol), and the reaction mixture was stirred at < 20 °C for 48 h. The reaction mixture was washed with water (2 x 50 mL), brine (1 x 50 mL) and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent purified (98%) by column chromatography (9:1 ethyl acetate: hexane) afforded cyclic compound **1** (0.25 g, 60%) as white solid; mp > 270 ^oC $[\alpha]^{26}_D$ = 2.5 (c 1.0, $CHCl_3$); ^{13}CNMR (75 MHz, $CDCl_3$) δ 167.7, 112.6, 105.4, 84.5, 80.3, 56.8, 26.7, 26.2. ESI-MS (*m/z*) $[M+H]^+$ 556; ESI-HRMS calcd for $C_{24}H_{34}N_3O_{12}$ $[M+H]^+$ 556.2142, found 556.2163.

3-Azido–3-deoxy-1,2-O-isopropylidene-α-D-xylo-furanoic acid 6:

To a stirred solution of compound **5** (2.00 g, 8.0 mmol) in 80% aqueous THF (20 mL), was added sodium meta periodate (6.90 g, 32.0 mmol) in three portions at 0 ^oC. After 6

h, the reaction mixture was filtered and washed with ethyl acetate. The filtrate was concentrated and taken in ether, washed with water, brine, dried over anhydrous Na_2SO_4 and evaporated to afford crude aldehyde (1.20 g, 70% yield) as yellow syrup which was used without any further purification. The aldehyde (1.20 g, 5.7 mmol) was dissolved in acetonitrile (6 mL) and to this were added solution of $NaClO_2$ (0.70 g, 8.5 mmol) in 9 mL of water, NaH_2PO_4 (1 g, 8.5 mmol) in 5 mL of water and 30% H_2O_2 (3 mL, 28.4 mmol) at 0^{o}C. The mixture was stirred overnight at room temperature, solvent was removed under reduced pressure and the remaining content was basified by adding 5% aqueous solution of $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (3 x 25 mL) followed by acidification of the remaining aqueous layer with sodium bisulfate. The acidic layer was further extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent at reduced pressure gave acid **6** (1.17 g, 90%) as colorless oil. $[\alpha]^{26}_D$ = -27.2 (c 1.0, $CHCl_3$); IR (film) 3477, 2375, 2119, 1707, 1634 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.03 (d, *J* = 3.3 Hz, 1H), 4.88 (d, *J* = 3.6 Hz, 1H), 4.71 (d, *J* = 3.3 Hz, 1H), 4.36 (d, *J* = 3.6 Hz, 1H), 3.96 (br s, 1H), 1.51 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 113.1, 105.2, 83.0, 78.2, 66.5, 26.6, 26.1. ESI-MS (*m/z*) $[M+Na]^+$ 252.

Dimer Azide 8: To a stirred solution of 3-Azido-3-deoxy–1,2-*O*-isopropylidene-α-D-xylo-furanoic acid **6** (1.17 g, 5.1 mmol) in dry CH_2Cl_2 (30 mL) were added HOBt (0.69 g, 5.1 mmol) and EDCI (0.97 g, 5.1 mmol) at 0 ^{o}C. After being stirred at 0 ^{o}C for 0.5 h, amine **7** (0.52 g, 5.1 mmol) was added and the reaction mixture was stirred under N_2 atm at room temperature for 12 h. The reaction mixture was washed with 1N HCl (15 mL), 5% aq.$NaHCO_3$ (15 mL) and water (2 x 10 mL) , brine (1 x 10 mL) and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure to give a residue, which was purified by column chromatography (2:3) ethyl acetate: hexane to afford dimer azide **9** (1.5 g, 94%) as colorless oil. $[\alpha]^{26}_D$ = -24.3 (c 1.1, $CHCl_3$); IR (film) 3427, 2116, 1734, 1662, 1538 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.08-6.95 (m, 1H), 5.95 (d, *J* = 3.3 Hz, 1H), 4.75 (d, *J* = 3.4 Hz, 1H), 4.61 (d, *J* = 3.3 Hz, 1H), 3.38 (d, *J* = 3.4 Hz, 1H), 3.71 (s, 3H), 3.61 (q, *J* = 6.1 Hz, 2H), 2.63-2.56 (m, 2H), 1.49 (s, 3H), 1.33 (s, 3H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 172.3, 166.9, 112.9, 105.1, 83.0, 79.5, 66.3, 51.6, 34.3,

33.5, 26.6, 26.1; ESI-MS (*m/z*) $[M+Na]^+$ 337 ESI-HRMS calcd for $C_{12}H_{19}N_4O_6$ $[M+H]^+$ 315.1304, found 315.1296.

Dimer Azido Acid 9: To a solution of dimer azide **8** (0.7 g, 2.2 mmol) in 28 mL of THF-H_2O (3:1), was added $LiOH.H_2O$ (0.28 g, 6.6 mmol) at 0 ^oC. After being stirred at 0 ^oC for 1 h, the reaction mixture was acidified with 5% sodium bisulphate (pH ~ 2) and extracted with ethyl acetate (6 x 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solvent was filtered and evaporated at reduced pressure to afford acid **9** (0.66 g, 100%) as a colorless oil. $[\alpha]^{26}_D$ = -18.7 (c 1.6, $CHCl_3$); IR (film) 3456, 2360, 2117, 1636, 1662, 1219 cm^{-1}; ^{1}HNMR (300MHz, $CDCl_3$) δ 7.04 (t, *J* = 6.0 Hz, 1H), 5.95 (d, *J* = 3.0 Hz, 1H), 4.75 (d, *J* = 3.7 Hz, 1H), 4.61 (d, *J* = 3.0 Hz, 1H), 4.39 (d, *J* = 3.7 Hz, 1H), 3.60 (q, *J* = 6.0 Hz, 2H), 2.69-2.62 (m, 2H), 1.50 (s, 3H), 1.33 (s, 3H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 176.1, 167.5, 113.2, 105.2, 83.1, 79.6, 66.3, 34.4, 33.6, 26.7, 26.3; ESI-MS (*m/z*) $[M+Na]^+$ 323; ESI-HRMS calcd for $C_{11}H_{17}N_4O_6$ $[M+H]^+$ 301.1148, found 301.1145.

Dimer Amine 10: A solution of compound **9** (0.7 g, 2.2mmol) and 10% Pd/C (140 mg) in ethyl acetate (30 mL) was stirred at room temperature overnight under 1atm hydrogen pressure. The reaction mixture was filtered through a small pad of celite and washed with CH_2Cl_2 (2 x 25 mL). The combined filtrates were concentrated under reduced pressure to afford amine **10** (0.64 g, 100%) as colorless oil, which was used in the coupling step without further purification. $[\alpha]^{26}_D$ = -70.9 (c 1.0, $CHCl_3$); IR (film) 3452, 1651, 1443 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.08 (t, *J* = 6.0, 1H), 6.00 (d, *J* = 3.3 Hz, 1H), 4.70 (d, *J* = 3.6 Hz, 1H), 4.44 (d, *J* = 3.4 Hz, 1H), 3.86 (d, *J* = 3.6 Hz, 1H), 3.70 (s, 3H), 3.68-3.48 (m, 2H), 2.58-2.54 (m, 2H), 1.50 (s, 3H), 1.32 (s, 3H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 172.3, 168.9, 112.2, 105.1, 85.8, 81.6, 58.0, 51.7, 34.4, 33.8, 26.8, 26.1; ESI-MS (*m/z*) $[M+H]^+$ 289 ESI-HRMS calcd for $C_{12}H_{21}N_2O_6$ $[M+H]^+$ 289.1399, found 289.1409.

Tetramer Azide 11: Following the similar procedure described above for the preparation of **9**, The amine **10** (0.58 g, 2.0mmol) was coupled with acid **9** (0.61g, 2.0 mmol) to yield **11** as a white solid (0.75 g, 65%) mp 204-208 ^oC $[\alpha]^{26}_D$ = –30.2 (c 1.0, $CHCl_3$); IR (film) 3412, 2991, 2120, 1740, 1664, 1542 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$)

δ 7.28-7.22 (m, 1H), 7.04 (t, *J* = 6.9 Hz, 1H), 6.74 (d *J* = 6.8 Hz, 1H) 5.94-5.90 (m, 2H), 4.74 - 4.68 (m, 2H), 4.63 (d, *J* = 3.4 Hz, 1H), 4.58-4.52 (m, 2H),4.33 (d, J = 3.0 Hz, 1H), 3.75-3.62 (m, 4H), 3.58-3.49 (m, 2H), 3.47-3.34 (m, 1H), 2.66-2.34 (m, 4H), 1.52-1.46 (m, 6H), 1.34 - 1.28 (m, 6H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 172.8, 171.5, 167.2, 113.0, 112.6, 105.1, 84.0, 83.0, 79.5, 78.8, 66.3, 57.1, 51.9, 35.3, 35.1, 34.6, 33.5, 26.6, 26.2, 26.1; ESI-HRMS calcd for $C_{23}H_{35}N_6O_{11}$ $[M+H]^+$ 571.2363, found 571.2382.

Tetramer azido acid 12: Following the similar procedure as described above for the preparation of **9** tetramer azido acid **12** was synthesized from tetramer azide **11** (0.50 g, 2.0mmol) as a white solid (0.48g). mp 140-145 ^oC $[\alpha]^{26}_D$ = -21.2 (c 1.0, $CHCl_3$); IR (film) 3414, 2990, 2119, 1660, 1545 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.24 (t, *J* = 6.0 Hz, 1H), 6.96 (dd, *J* = 7.5 Hz, *J* = 3.7 Hz ,1H), 6.60(d, *J* = 6.7 Hz, 1H), 5.96-5.91 (m, 2H), 4.80 (d, *J* = 3.7 Hz, 1H), 4.76 (d, *J* = 3.7 Hz, 1H), 4.65-4.59 (m, 3H), 4.36 (d, *J* = 3.7 Hz, 1H), 4.05-3.92 (m, 1H), 3.63-3.47 (m, 2H), 3.25 (m, 1H), 2.24-2.63 (m, 2H), 2.51-2.40 (m, 2H), 1.50 (br.s, 6H), 1.33,1.30 (acetonide methyls); ^{13}CNMR (75 MHz, $CDCl_3$) δ 174.5, 171.6, 168.2, 167.8, 113.2, 112.7, 105.2, 105.04, 84.1, 83.1, 79.5, 78.8, 66.4, 56.9, 35.8, 35.0, 33.5, 26.7, 26.3, 26.2; ESI-HRMS calcd for $C_{22}H_{33}N_6O_{11}$; $[M+H]^+$ 557.2207, found 557.2222.

***Cyclo* [cis-β-Fsaa-β-hGly]$_2$ 2 :** To a solution of compound **12** (0.47 g, 0.85 mmol) in dry CH_2Cl_2 (15 mL) were added pentafluorophenol (0.17 g, 0.92 mmol) and EDCI (0.16 g, 0.85 mmol) at 0 ^oC and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was extracted with CH_2Cl_2 (2 x 25 mL) and the organic layer was washed with water (1 x 20 mL), brine and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure. The resulting pentafluorophenyl ester (0.59 g, 98%) was taken directly in ethyl acetate (250 mL) and 10% Pd/C (100 mg) was added, the reaction mixture was stirred at room temperature over night under 1 atm hydrogen pressure. The reaction mixture was filtered, washed with CH_2Cl_2. solvents were removed under reduced pressure and the crude cyclic product was purified (99%) by preparative HPLC using methanol : water (30:70) as mobile phase to afford cyclic product **2** (0.16 g, 40%Yield). $[\alpha]^{26}_D$ = -17.5 (c 1.0, $CHCl_3$); Mp > 270 ^oC; ^{13}CNMR (75 MHz, $CDCl_3$) δ 171.7, 168.7, 111.8, 106.8, 84.1, 80.6, 59.4, 34.7, 34.4, 26.9, 26.2; ESI-

MS (*m/z*) $[M+H]^+$ 513 ESI-HRMS calcd for $C_{22}H_{32}N_4O_{10}$; $[M+H]^+$ 513.2196, found 513.2211.

NMR studies:

NMR spectra were recorded on Varian Inova - 500 MHz at 303 K in 2:3 $CDCl_3/CCl_4$ and DMSO solvents (10 mM) using tetramethylsilane as internal standard or the solvent signals as secondary standards, and the chemical shifts are shown in ppm scales. 10mM concentrated samples are used in these studies. Two dimensional (2D) double quantum filtered COSY (DQCOSY), total correlation spectroscopy (TOCSY), and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments were also 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 sec. 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. The spectra (One Dimensional, DQCOSY and ROESY) are given in the supporting information.

FT-IR studies:

FT-IR investigations were carried out on Nicholet-670 spectrometer. Dry films of samples **1** and **2** were deposited on KBr pellet and dried in vacuum for one hour before they were studied. The solution state studies were carried out in CaF2 cell. The measurements were taken using a 2 cm^{-1} resolution and averaging of 2000 scans. Absorbance spectra were displayed by the OMNIC analysis program (Nicolet). By the OMNIC analysis program (Nicolet). The obtained absorption spectra were smooth by applying the Savitzky–Golay function to eliminate noise.

DIC studies:

The samples were also examined by polarized light microscopy in the differential interference contrast (DIC) mode employing a Zeiss polarized light microscope equipped with an oil immersion objective (63X, 1.4 NA), a rotatable sample holder mounted between the polarizer and analyzer, and a iezomotor.

SEM and TEM studies:

SEM and TEM images were recorded on FEI–ESEM (XL-30) and FEI-Tecnai F12 (Philips Electron Optics, Holland), respectively. A drop of an aqueous dispersion of self-assembled peptides 1 and 2 were placed on a Formvar-coated copper TEM grid (200 mesh) and after 1 min, excess fluid was removed was allowed to air-dry.

Molecular Modeling:

Conformational preferences of the cyclo β tripeptide **1** and the cyclo β- tetra peptide **2** were studied using a highly efficient sampling technique based on local non stochastic deformations and the MM2(91) force field. An exhaustive conformational search was carried out using the Conflex program [1] at the molecular mechanics level using the MM2 (91) force field. The search was done using the Conflex algorithm [2, 3]. The program was further modified incorporating subroutines to calculate the point group symmetries[4] and incorporating restraints. The method uses a three-step procedure: structure generation, minimization and duplicate detection for generating the low-energy conformers (LECs). Reservoir filling and a gradual increase in the energy window ensures that the conformational space search is directed towards LECs. Duplicates were detected and eliminated using root-mean-squared difference (RMSD) of the torsion angles.

The initial structure of **1** and **2** were constructed using arbitrarily chosen geometries. The default dielectric constant of 1.5, which is standard for gas phase calculations, was used and the convergence limit for energy gradient was set to be less than 10^{-4} kcal mol^{-1} $Å^{-1.}$ The selected conformers were further minimized using torsion angles from the coupling constant data.

Figure 1: Electro Spray Ionization mass spectrometry for compound 1 :

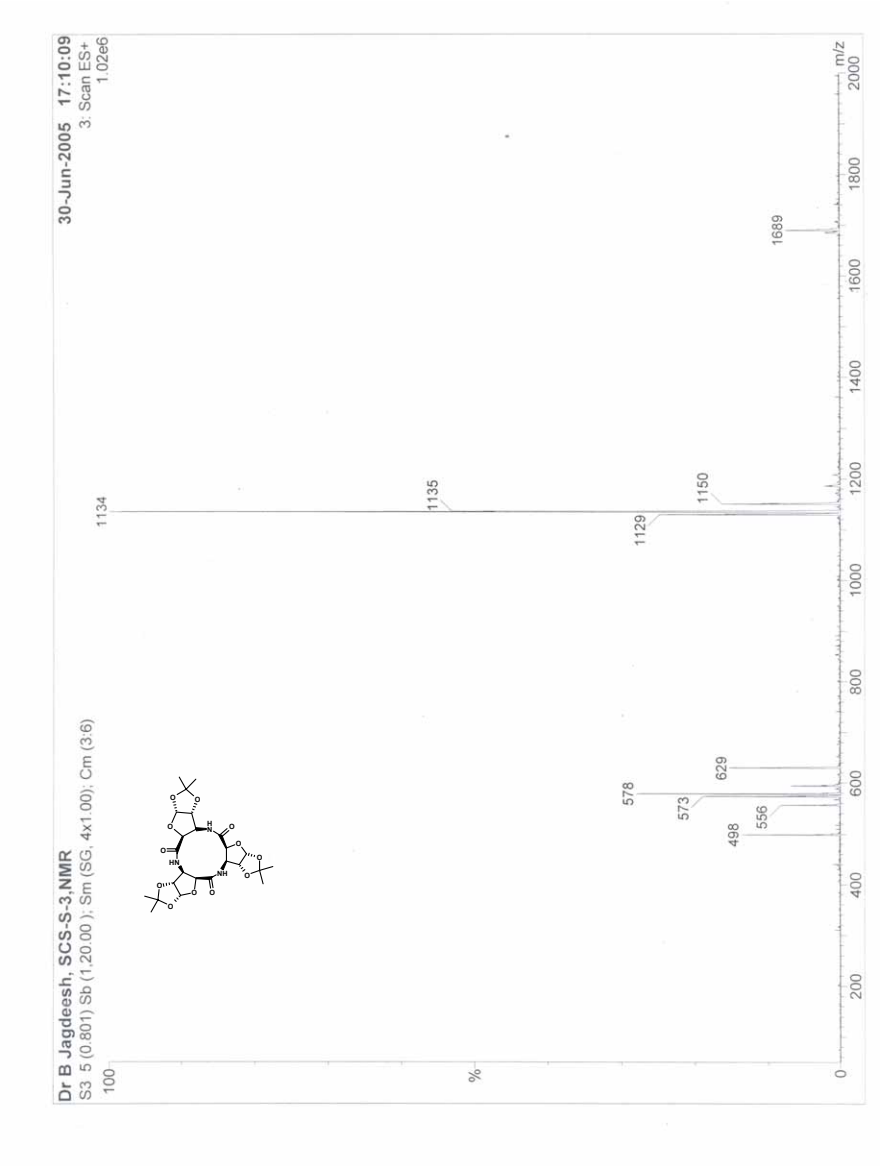
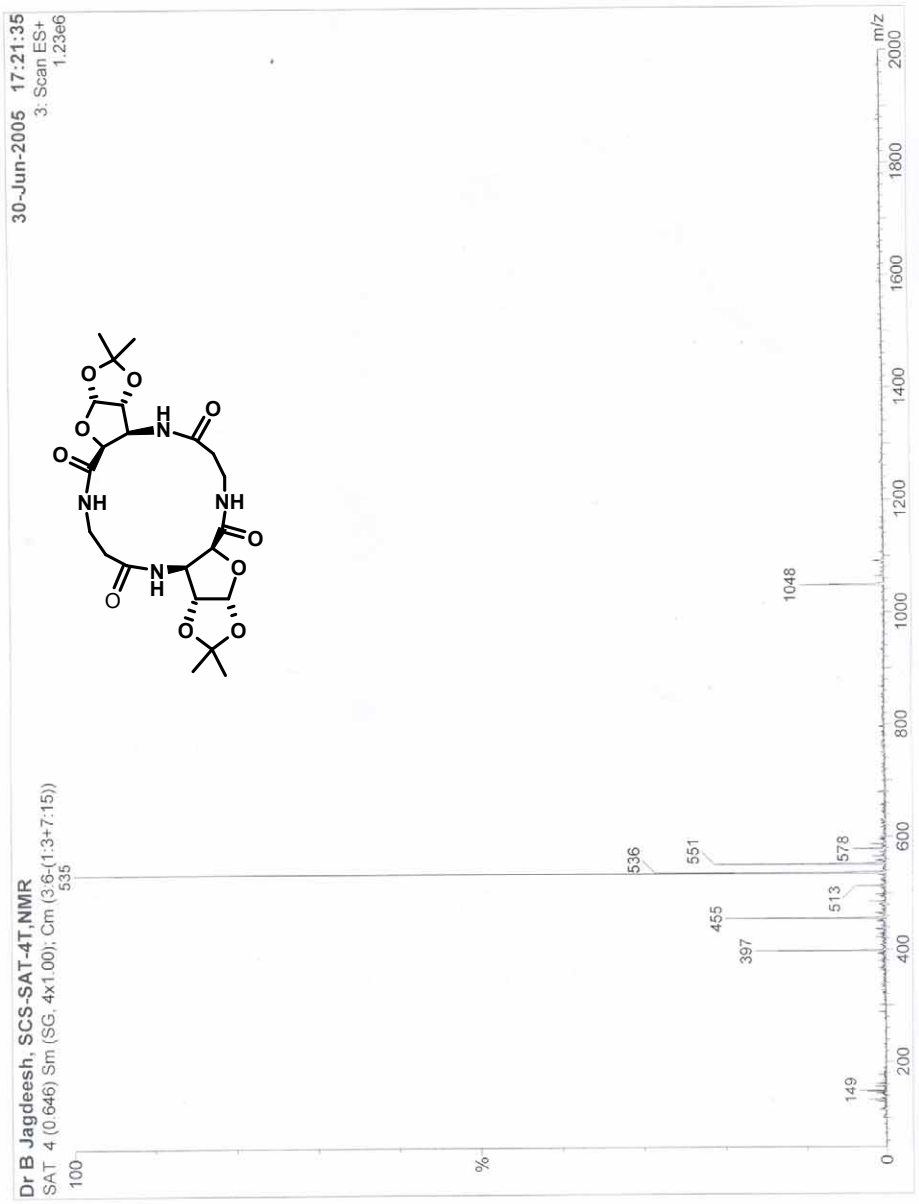


Figure 2: Electro Spray Ionization mass spectrometry for compound 2



NMR STUDIES:

^{1}H NMR spectra (DMSO) of all entries in Table-1 were assigned from the corresponding double-quantum-filtered 2D DQCOSY spectrum acquired at the concentration and temperature indicated. Spectra were acquired using Varian's standard pulse sequence on INOVA-500MHz spectrometer as indicated for **1** and **2** and were referenced to residual $CHCl_3$ solvent peak (7.24ppm).

Residue	NH	CαH	Cα^{1}H	CβH	Cβ^{1}H	CγH	CδH
S	7.48 *JNH,β*=10.2	4.43(d) *Jα,β*=4.1	-	4.42(dd) *Jα,β* = 4.1 *JNH,β*=10.2		4.58(d) *Jγ*,δ=3.5	6.0(d) *Jγ*,δ=3.5

Table-1

^{1}H Chemical Shifts (ppm) (DMS0-d6, 500MHz, 300K) and coupling constants(Hz) of 1.

Residue	NH	CαH	Cα^{1}H	CβH	Cβ^{1}H	CγH	CδH
S	7.60(d) *JNH,β*=8.5	4.58(d) *Jα,β*=4.8	-	4.28(dd) *JNH,β* = 8.5 *Jα,β* = 4.8	-	4.40(d) *Jγ*,δ=3.7	5.85(d) *Jγ*,δ=3.7
A	8.00(t) *JNH,β* = 5.7 *JNH,β'*=5.7	2.28(ddd) *Jαα'*=17.0 *Jα,β'* =9.9 *Jα,β* =2.8	2.13(ddd) *Jαα'*= 17.0 *Jα',β* = 5.6 *Jα',β'*=2.3	3.20(dddd) *Jβ,β* =14.6 *JNH,β* = 5.7 *Jα,β* =2.8 *Jα',β* = 5.6	3.12(dddd) *Jβ,β* =14.6 *JNH,β'*=5.7 *Jα,β'* =9.9 *Jα',β'*=2.3		

Others: methyls; 1.37, 1.22, S=sugar,

Table-2

^{1}H Chemical Shifts (ppm) (DMS0-d6, 500MHz, 300K) and coupling constants(Hz) of 2

Others: methyls,1.36, 1.19 . S=*cis* fSAA, A= β-hGly

Figure 3: ^{1}H NMR of 1 (DMS0-d6, 500MHz, 300K)

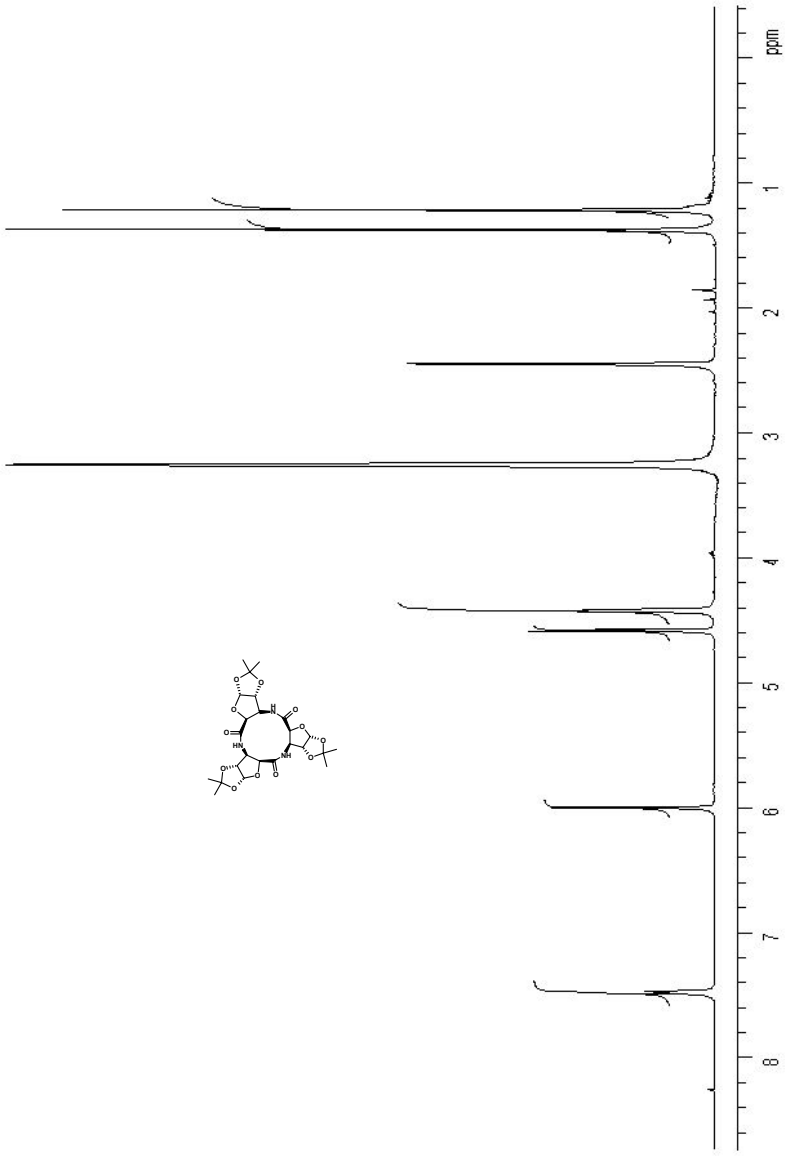


Figure 4: ^{13}C NMR of 1 ($CDCl_3$, 75MHz, 300K)

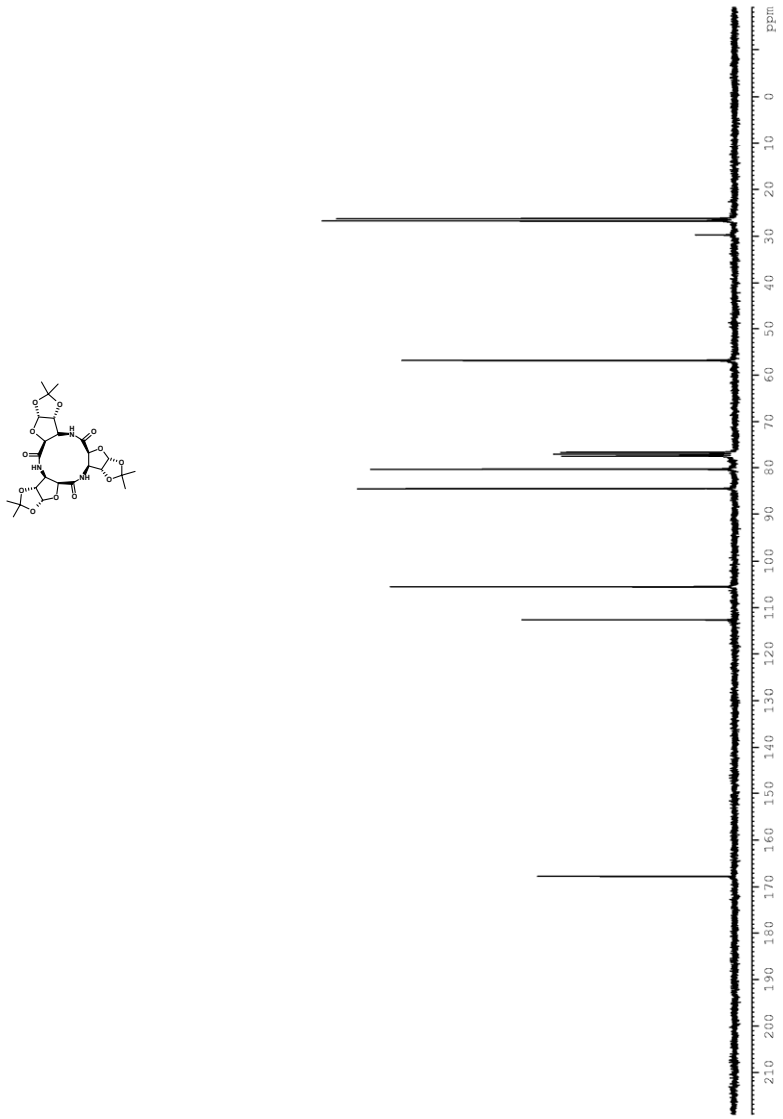


Figure 5: ^{1}H NMR of 2(2:3 CDCl3/CCl4, 500MHz, 300K)

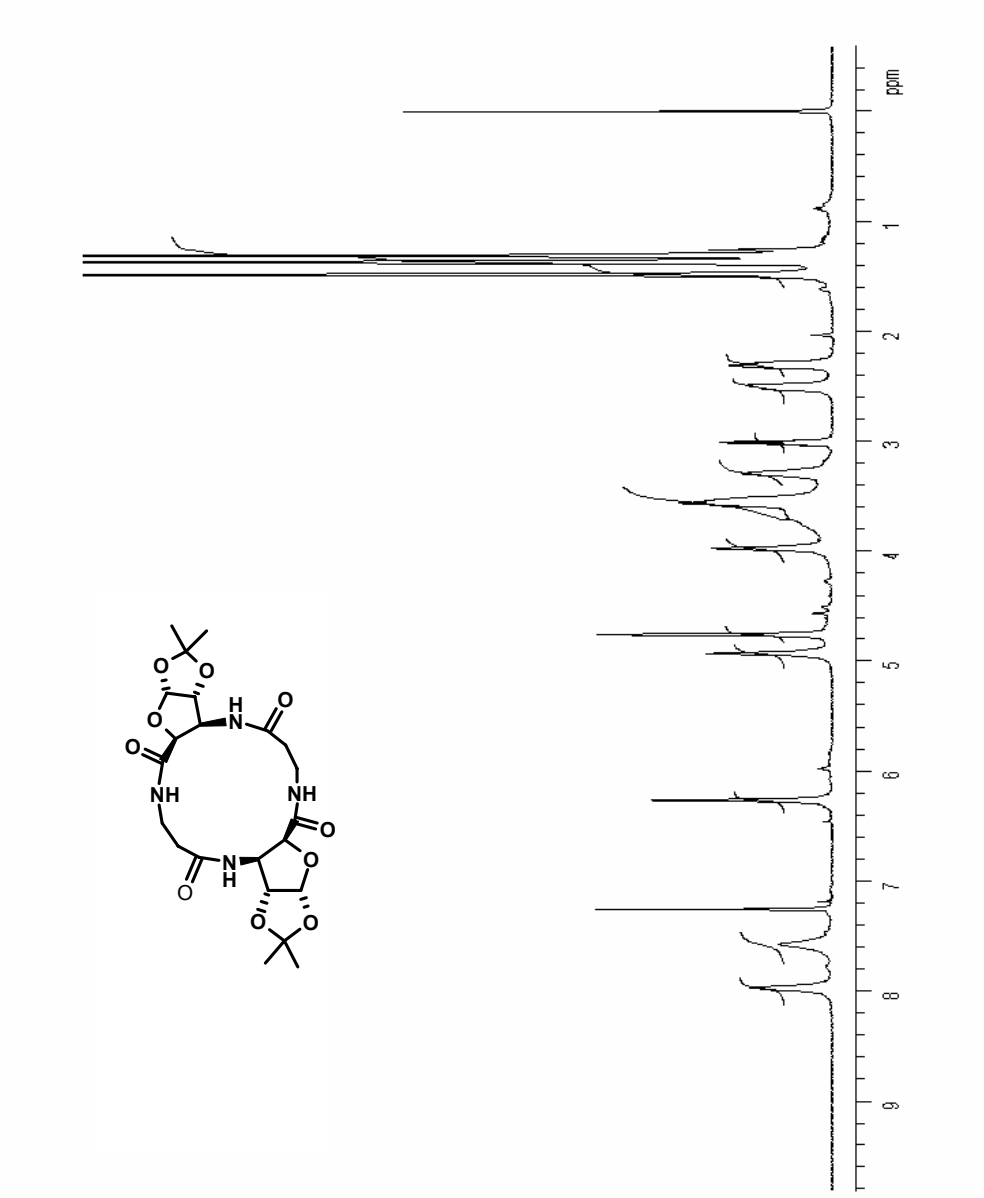


Figure 6: ^{13}C NMR of 2 ($CDCl_3$, 75MHz, 300K)

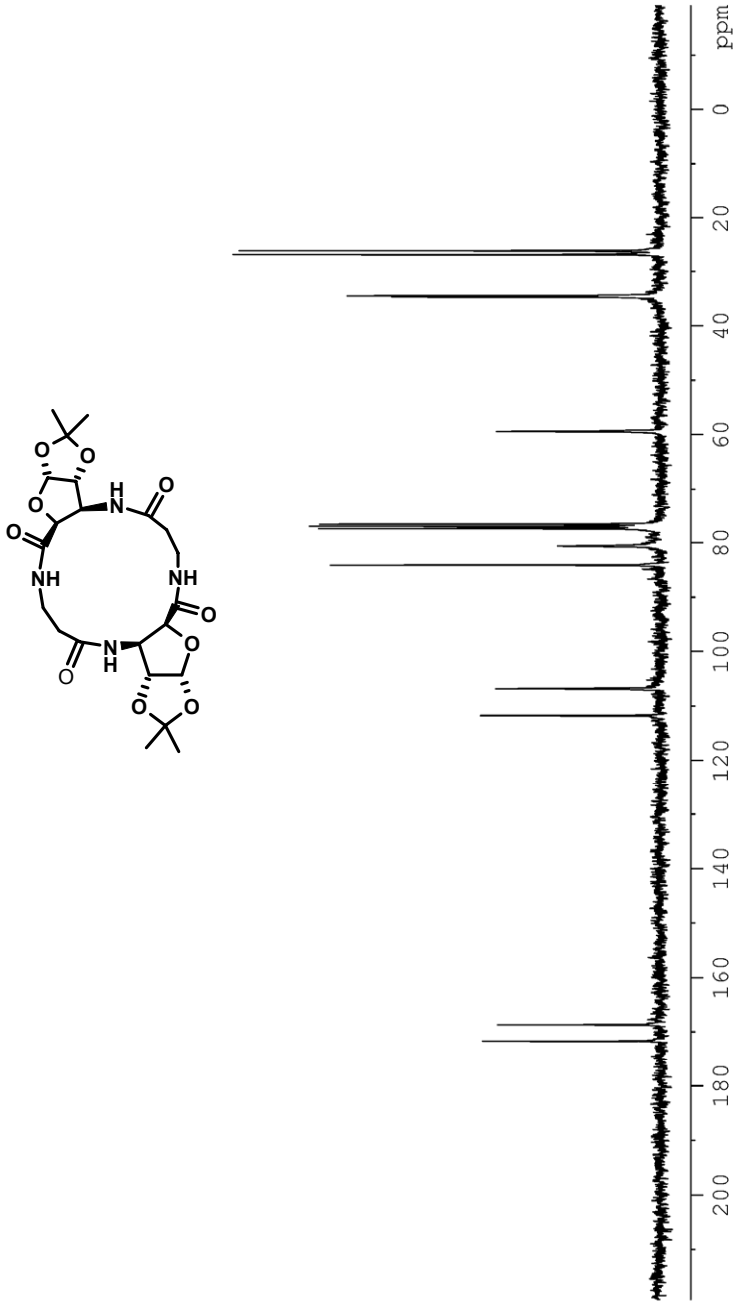


Figure 7: 2D-DQCOSY spectrum of 2 in DMSO(500MHz)

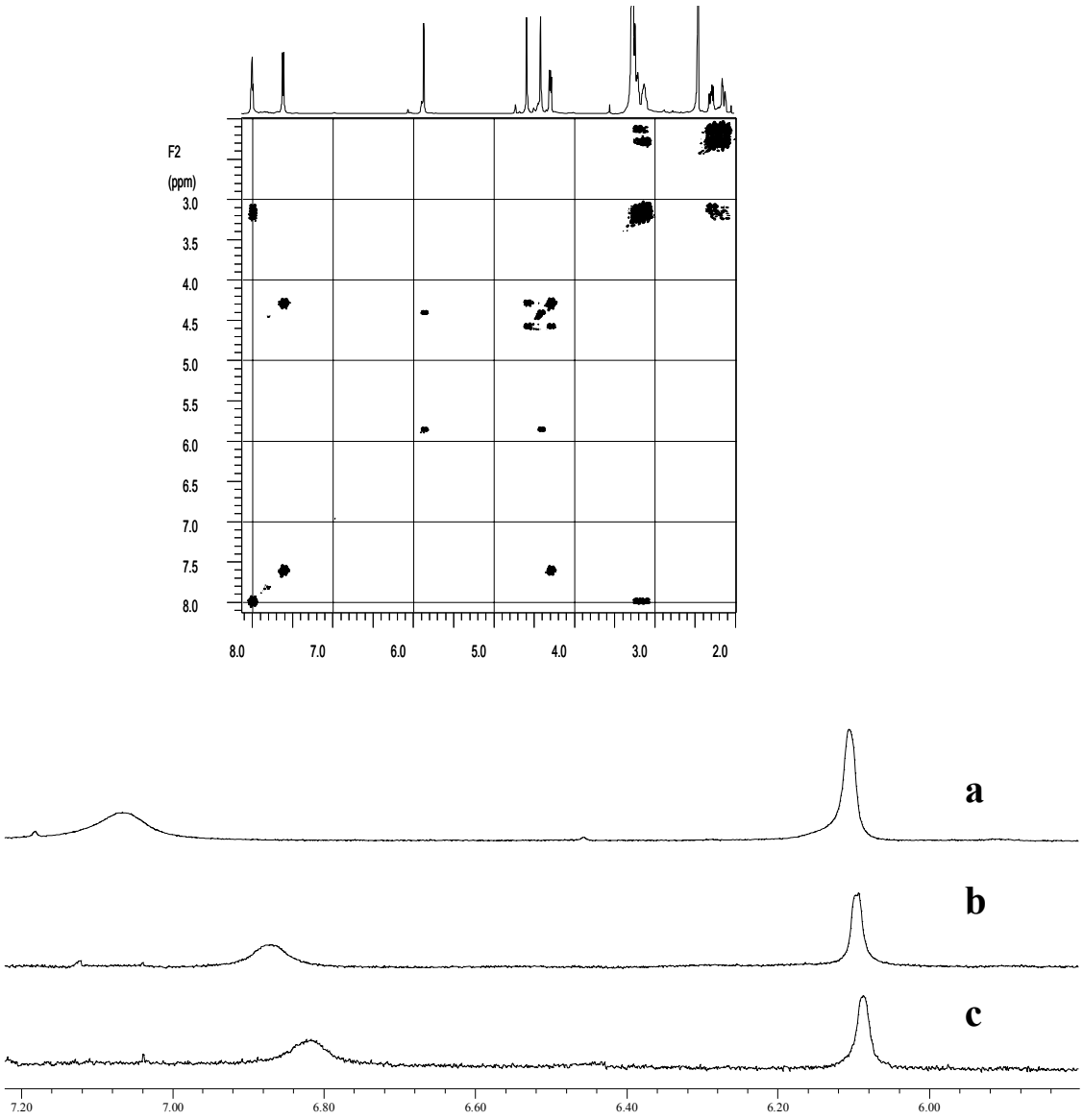


Figure 8. Selected region of ^{1}H NMR spectrum showing NH chemical shifts in **1**, as a function of concentration**:** (a) 20 mM (b) 10 mM (c) 1mM in $CDCl_3/CCl_4$.

Figure 9: 2D-ROESY spectrum of 2 in DMSO(500MHz) :

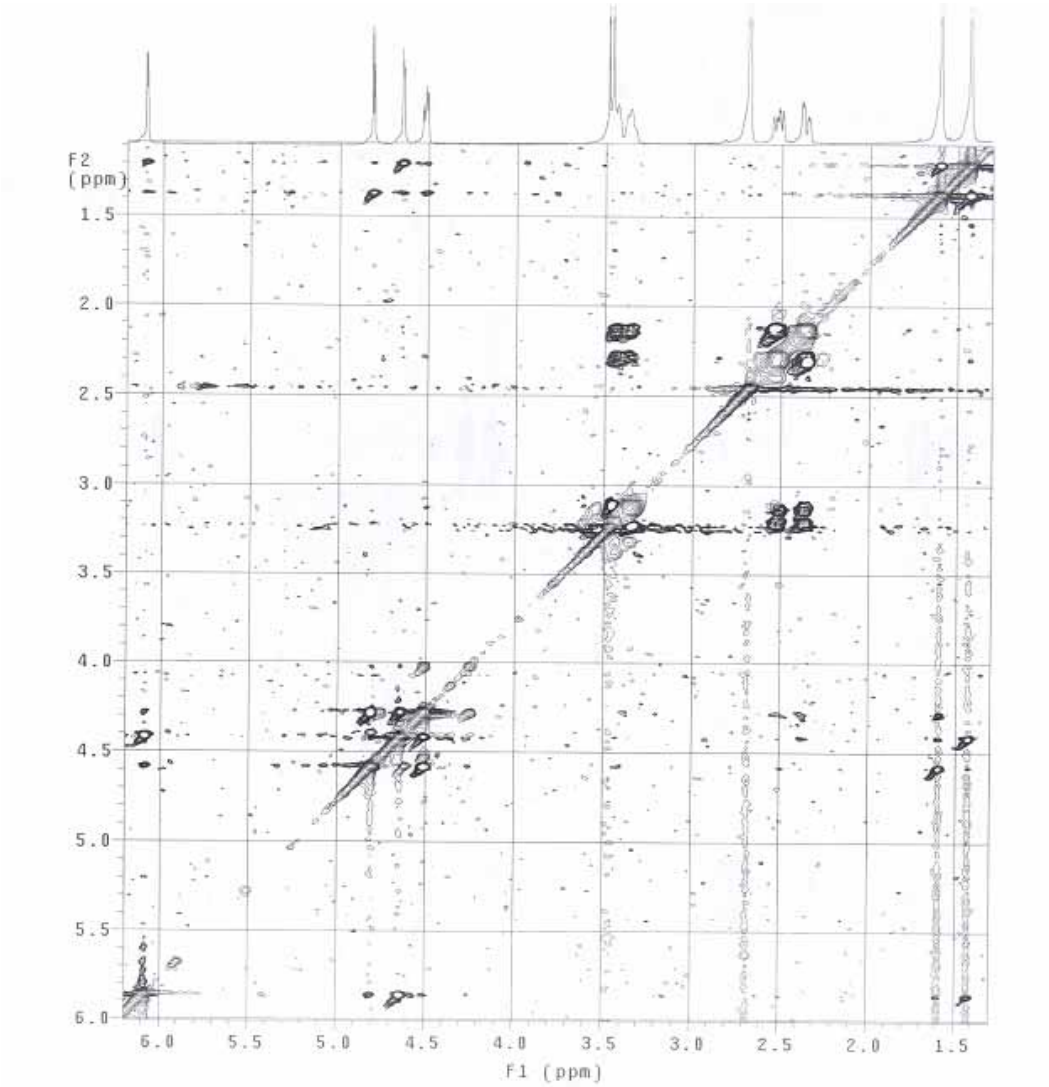
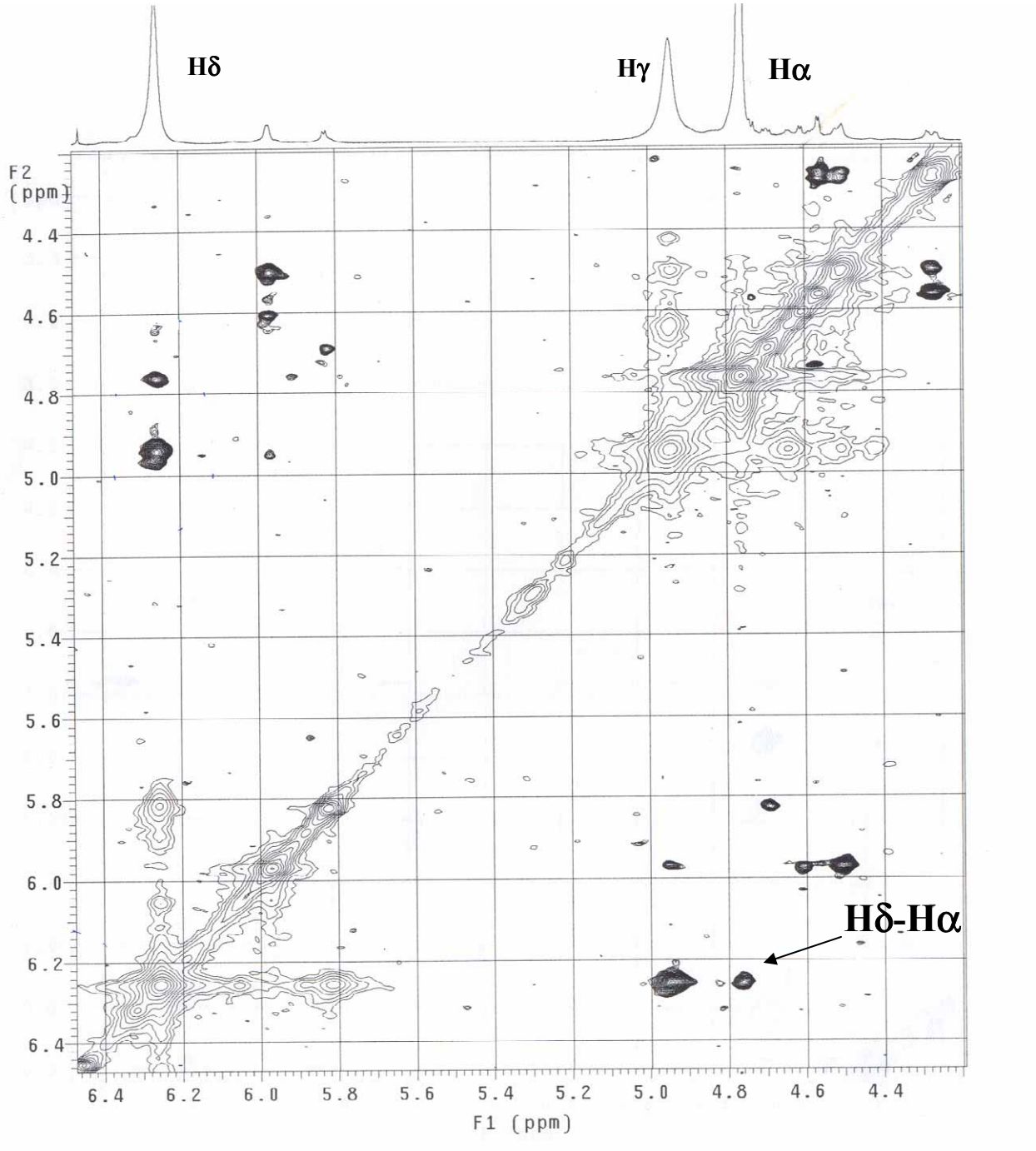


Figure 10: Expaned ROESY spectrum of 2 in $CDCl_3/CCl_4$



ROESY and Relative orientation of peptide rings.

The relative orientation of the peptide rings in the peptide tube has been studied for **2** by using NMR spectroscopy. The hybrid cyclic peptide **2** has two faces, NH and C=O groups of the sugar amino acid (FSAA face) and NH and C=O groups of β-hGly amino acid (β-hGly face). The peptide rings can in principle, vertically stack by hydrogen bonding either with the faces of FSAAs aligned (inter-subunit homostacking) or with the faces of FSAA and β-hGly aligned (inter-subunit heterostacking). In order to elucidate the probable alignment, NOE studies are carried out for monomer unit of **2** in DMSO, and for the corresponding aggregated units in $CDCl_3/CCl_4$ mixture. These studies infer that the self-assembly favours FSAA-FSAA and the βhGly-βhGly inter-subunit homostacking. The details are given below.

In DMSO, the intra and inter-residual NOEs are clearly seen for an isolated monomer unit of **2**, which served as reference data for analyzing the self-assembled units that were studied in $CDCl_3/CCl_4$.[23] It may be noted that due to the symmetry of the cyclic tetra peptide **2**, in a self-assembled array one cannot expect additional resonance signals and the observed NMR spectra are consistent with this. However, depending upon the type of self-assembly, either FSAA-FSAA (inter-subunit homostacking) or FSAA-β-hGly (inter-subunit heterostacking) pairs of two vertically stacked rings come closer. These two types of molecular stackings should result in a relatively intense NOE cross-peaks for proximal inter-ring protons, compared to the similar NOEs within the monomer unit.

This implies that in a homostacking, the Hδ proton of FSAA of the bottom ring comes closer to Hα proton of FSAA of the upper ring (Figure 4). Similarly, the Hγ and Hβ protons of two vertically stacked FSAA residues will come closer as well. The ROESY data did not show NOE cross-peaks between Hδ-Hα and/or -Hβ protons of FSAA-β-hGly residues, which suggest that the heterostacking can be ruled out. On the other hand, relatively intense NOE cross-peaks between Hα-Hδ protons of FSAA

residues are observed compared to that of in the isolated monomer units, which correspond to a spatially averaged distance of ~ 2.3 Å. This distance is considerably shorter than the distance (~3.4 Å) between similar proton pairs within the sugar ring.[16,17] These findings support the homostacking in the present tubular assemblies (Figure 4).

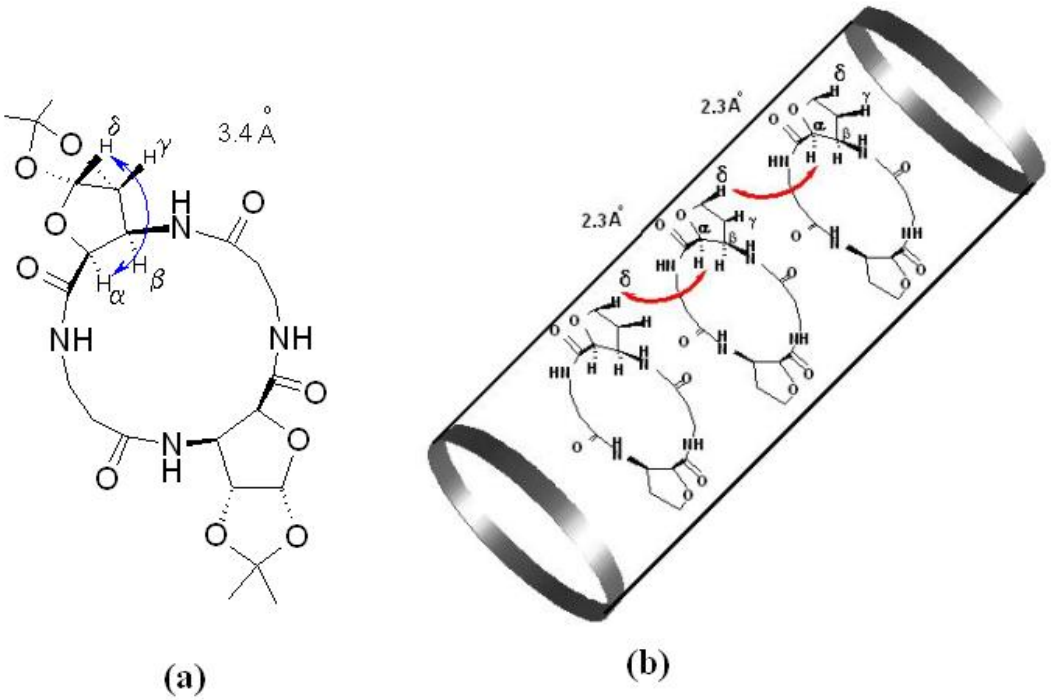
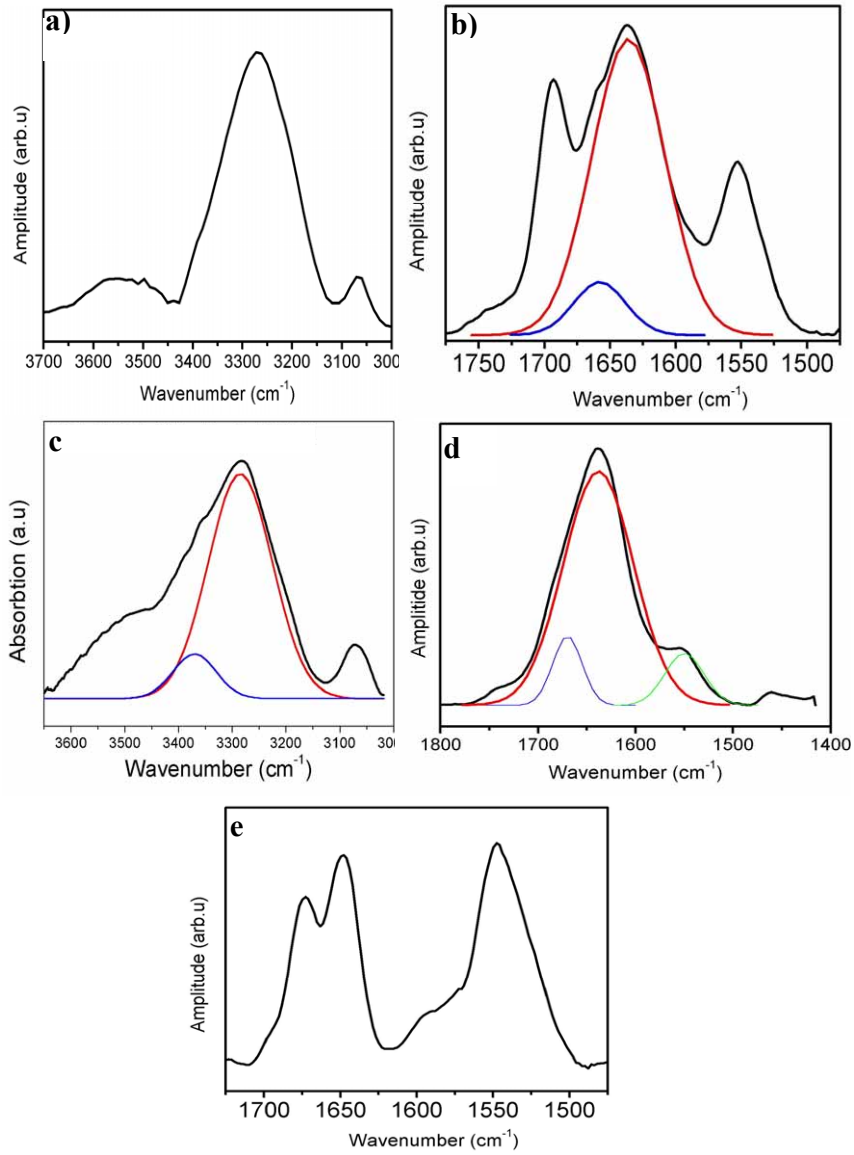


Figure 11. (a) Schematic diagram of the observed NOE connectivities between Hα-Hδ protons in an isolated monomer unit of **2;** (b) Cartoon representation showing a closer proximity (~~ 2.3 Å) of inter Hα- Hδ protons of the vertically stacked sugar rings in the tubular assembly, compared to that in the isolated unit (~3.4 Å).

Figure 12: FT-IR results



FT-IR spectra of a) and c) amide-A region b) and d) the amide-I and amide-II region of **1** and **2**. e) amide I and amide II region of **2** in $CDCl_3$. The spectra are deconvoluted to show the two possible bands of amide I.

Molecular Modeling :

Conformational preferences of the cyclo β tripeptide **1** and the cyclo β- tetra peptide **2** were studied using a highly efficient sampling technique based on local non stochastic deformations and the MM2(91) force field. An exhaustive conformational search was carried out using the Conflex program [3] at the molecular mechanics level using the MM2 (91) force field. The search was done using the Conflex algorithm [4, 5]. The program was further modified incorporating subroutines to calculate the point group symmetries [6] and incorporating restraints. The method uses a three-step procedure: structure generation, minimization and duplicate detection for generating the low-energy conformers (LECs). Reservoir filling and a gradual increase in the energy window ensures that the conformational space search is directed towards LECs. Duplicates were detected and eliminated using root-mean-squared difference (RMSD) of the torsion angles.

The initial structure of **1** and **2** were constructed using arbitrarily chosen geometries. The default dielectric constant of 1.5, which is standard for gas phase calculations, was used and the convergence limit for energy gradient was set to be less than 10^{-4} kcal mol^{-1} $Å^{-1.}$ The selected conformers were further minimized using torsion angles from the coupling constant data.

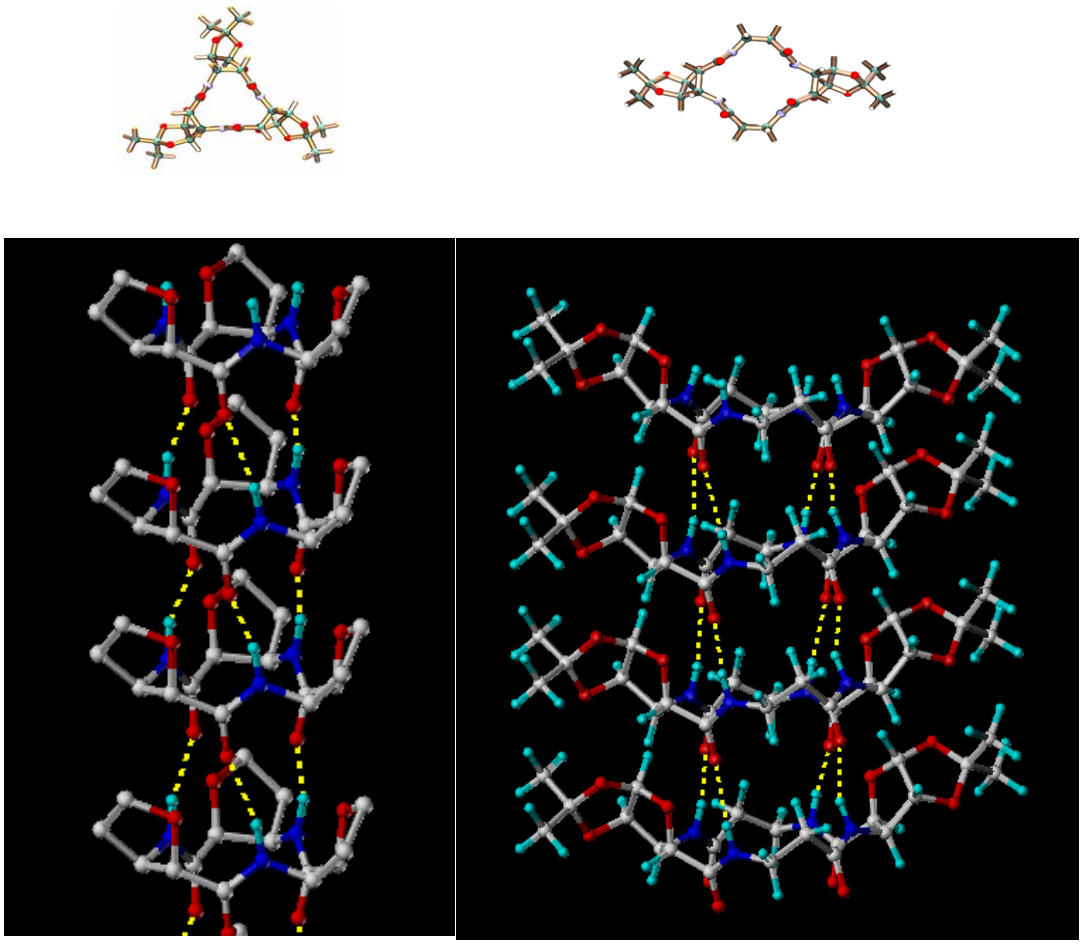


Figure 13: Low energy conformations of **1** (a) and **2** (b) and their corresponding schematic side views of self-assembled tubular aggregations are shown in (c) and (d), respectively.

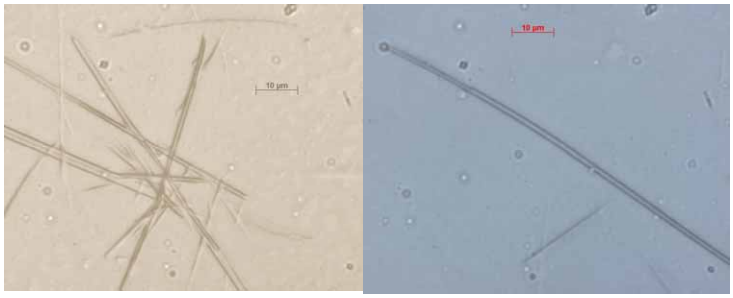


Figure 14: Transmitted light images of 2 taken with differential interference contrast (DIC) optics. Images show uniform molecular assemblies over several micron length.

Coordinates of compound 1 *cyclo* [*cis*-β-fSAA]$_3$

N	-1.71100	-0.39600	-1.19500
O	-0.67500	-2.83500	0.03500
C	-0.46200	-2.51900	-1.32400
C	-1.70400	-1.74200	-1.74000
C	-2.76800	-2.55600	-1.01900
C	-2.05800	-3.03900	0.21200
C	0.85300	-1.79800	-1.55000
O	-2.15000	-4.43700	0.19000
O	-3.03800	-3.74000	-1.72500
C	-3.12300	-4.77800	-0.77300
C	-4.50400	-4.82800	-0.14000
C	-2.73400	-6.08600	-1.43300
O	1.25500	-1.58300	-2.68200
N	1.52900	-1.41800	-0.39800
H	1.09400	-1.54700	0.44900
O	2.51400	0.81500	1.21100
C	2.79100	0.69000	-0.16700
C	2.84800	-0.81100	-0.41600
C	3.59400	-1.24900	0.83600
C	3.14000	-0.26100	1.87100
C	1.79500	1.44700	-1.02400
O	4.30400	0.34300	2.36700
O	4.96600	-0.97900	0.70700
C	5.39700	-0.43700	1.93600
C	5.69700	-1.53400	2.94400
C	6.58700	0.47000	1.68600
O	1.97200	1.56400	-2.22600
N	0.70500	1.97200	-0.34200
H	0.60200	1.75300	0.58700
O	-2.18900	1.91200	0.03200
C	-1.59000	2.05700	-1.23800
C	-0.29600	2.81400	-0.97100
C	-0.79300	3.81400	0.06200
C	-1.86400	3.05400	0.79000
C	-1.42200	0.73200	-1.95400
O	-3.03100	3.82100	0.67300
O	-1.50000	4.85400	-0.56400
C	-2.64800	5.10000	0.21800
C	-2.32700	6.00900	1.39200
C	-3.74100	5.65900	-0.67300
O	-1.05300	0.69700	-3.11600
H	-1.90700	-0.27900	-0.26200
H	-0.39600	-3.49100	-1.86600
H	-1.85800	-1.73600	-2.84600
H	-3.72900	-2.02100	-0.84300
H	-2.38500	-2.63700	1.19500
H	-4.77000	-3.88200	0.38400
H	-4.56100	-5.64700	0.61300

H	-5.28400	-5.01800	-0.91100
H	-1.71800	-6.01800	-1.88800
H	-3.45100	-6.35500	-2.24200
H	-2.71900	-6.91900	-0.69400
H	3.78700	1.16400	-0.33200
H	3.40500	-1.07200	-1.34800
H	3.46700	-2.32200	1.10700
H	2.50100	-0.63000	2.70200
H	4.81000	-2.17100	3.16100
H	6.03400	-1.09800	3.91200
H	6.50700	-2.20100	2.56900
H	6.33600	1.26300	0.94400
H	7.45200	-0.10800	1.28700
H	6.91200	0.97300	2.62500
H	-2.29500	2.66900	-1.84900
H	0.10600	3.31700	-1.88300
H	0.00100	4.26100	0.70300
H	-1.67800	2.77600	1.84900
H	-1.55800	5.57600	2.07100
H	-3.23800	6.19500	2.00500
H	-1.94800	6.99400	1.03600
H	-3.95400	4.97100	-1.52400
H	-3.44500	6.64500	-1.09800
H	-4.68900	5.79900	-0.10500

Coordinates of compound 2 *cyclo* [*cis*-β-fSAA-β-hGly]$_2$

H	-1.49700	2.90400	-0.54000
C	-1.49200	3.00700	0.57000
C	-2.57400	2.13600	1.20900
H	-3.56200	2.55700	0.89900
N	-2.59400	0.76300	0.72900
C	-0.10300	2.80300	1.14900
O	0.09900	2.91900	2.33400
O	3.28500	1.51000	-1.02900
C	3.18700	1.62900	0.37900
C	2.31000	2.85700	0.61600
C	2.90300	3.78500	-0.45000
C	3.32100	2.82300	-1.55700
N	0.90800	2.62500	0.27200
C	2.74500	0.35400	1.07000
O	4.64100	3.16300	-1.90800
O	4.11900	4.34400	-0.00800
C	4.99600	4.28200	-1.11600
C	4.86100	5.55700	-1.95800
C	6.42400	4.08600	-0.59900
O	2.61700	0.30200	2.27000
H	0.68300	2.45800	-0.64500
H	1.32600	-2.91100	-0.71200
C	1.51800	-2.99400	0.38200
C	2.68000	-2.09900	0.81000
H	3.60400	-2.52700	0.34700
N	2.61600	-0.74100	0.29400
C	0.25200	-2.78800	1.19700
O	0.24400	-2.97900	2.39000
H	2.65800	-0.62400	-0.65600
O	-3.43100	-1.50800	-0.46400
C	-3.16100	-1.60500	0.92300
C	-2.21600	-2.79900	1.05600
C	-2.91100	-3.76200	0.08700
C	-3.48800	-2.82900	-0.97200
N	-0.88300	-2.53200	0.51300
C	-2.68200	-0.30900	1.54500
O	-4.82800	-3.21500	-1.16000
O	-4.04800	-4.34500	0.68300
C	-5.05300	-4.32700	-0.31300
C	-4.98400	-5.61400	-1.14500
C	-6.41300	-4.15900	0.37000
O	-2.46100	-0.22800	2.73000

H	-0.80600	-2.31400	-0.41800
H	-2.72700	0.62100	-0.21000
H	-1.70300	4.07500	0.82700
H	-2.58800	2.24000	2.31600
H	4.22700	1.81600	0.74300
H	2.42800	3.27500	1.64100
H	2.23500	4.61600	-0.76700
H	2.68400	2.85300	-2.46800
H	3.83300	5.67800	-2.36800
H	5.56300	5.53700	-2.82200
H	5.09300	6.46000	-1.34900
H	6.50200	3.15600	0.00900
H	6.73700	4.94000	0.04300
H	7.14800	4.00400	-1.44200
H	1.78300	-4.05600	0.61500
H	2.89300	-2.17800	1.89900
H	-4.14200	-1.82400	1.41200
H	-2.17900	-3.19900	2.09400
H	-2.26500	-4.58100	-0.30000
H	-2.96300	-2.85100	-1.95200
H	-4.01000	-5.71500	-1.67400
H	-5.78600	-5.62900	-1.91700
H	-5.11500	-6.51100	-0.49800
H	-6.44400	-3.22000	0.96800
H	-6.62300	-5.00900	1.05800
H	-7.23500	-4.11200	-0.38000

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