Synthesis and Conformational Studies of a Hybrid Cyclic Peptide Based on *cis*-β-Furanoid Sugar Amino Acid (FSAA) and Ornithine

by **Srivari Chandrasekhar***[a]), **Birudaraju Saritha**[a]), **Police Naresh**[b]), **Marelli Udayakiran**[b]), **Chada Raji Reddy**[a]), and **Bharatam Jagadeesh***[b])

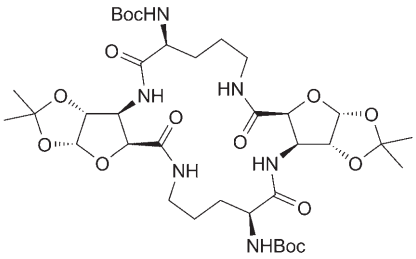
[a]) Organic Division-I, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, India
[b]) Centre for Nuclear Magnetic Resonance, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, India (fax: (+)91-40-27160512; e-mail: srivaric@iict.res.in and bj@iict.res.in)

The conformational control of a *14*-helix nucleating template, *cis*-β-furanoid sugar amino acid (FSAA), over a flexible δ-amino acid, ornithine is studied in a FSAA-ornithine cyclic tetrapeptide. Extensive NMR and MD studies reveal that the cyclic peptide adopts a three-dimentional bowl-shape cavity, which promotes six- and seven-membered intra- and inter-residue H-bonding, in polar and non-polar solvents, respectively.

Introduction. – Amongst the unnatural oligopeptides, β-peptides that exhibit well-defined secondary folding ('foldamers') [1] have gained immense scientific interest in recent years. The interests are mainly due to their ability to form stable secondary structural scaffolds with a relatively small number of β-amino acid residues compared to the natural α-peptides, and due to their potential applications in drug delivery. In this regard, sugar amino acids (SAA) have a special appeal as they serve as bridge between carbohydrates and proteins [2]. Recently, we have reported that homooligomers of a novel peptide building block, *cis*-β-furanoid sugar amino acid (FSAA) [3], adopt robust right handed *14*-helices. Subsequently, we have shown that the conformational space (represented by dihedral angles) of the FSAA can be expanded to a more flexible residue β-Ala in linear [4] and cyclic [5] heterooligomers consisting of FSAA and β-Ala residues at alternate positions. These linear and cyclic heteropeptides also exhibited robust *14*-helical folding [4] and C_2-symmetric β-sheet-like peptide rings, which self-assembled to form tubular structures [5], respectively, suggesting that FSAA exerts conformational control of the peptide backbone [6] and diverse secondary structures can be derived.

As a part of our ongoing research activity on SAA based molecular design, we are interested in developing cyclic hybrid peptides that can accommodate various combinations of functional side chains. In fact, the cyclization of linear peptides is a widely accepted strategy to constrain the conformational freedom of the backbone and to gain improved accessibility for molecular recognition, a process essential for biological activity [7]. The present work aims in this direction and reports the synthesis and conformation of a cyclic hybrid tetrapeptide consisting of an alternating FSAA and ornithine (equivalent of δ-amino acid), **1**. This example is also expected to provide insight into the conformational control exerted by the rigid FSAA over a more flexible and longer spacer ornithine, compared to β-Ala in a heterocyclic peptide [5]. To the

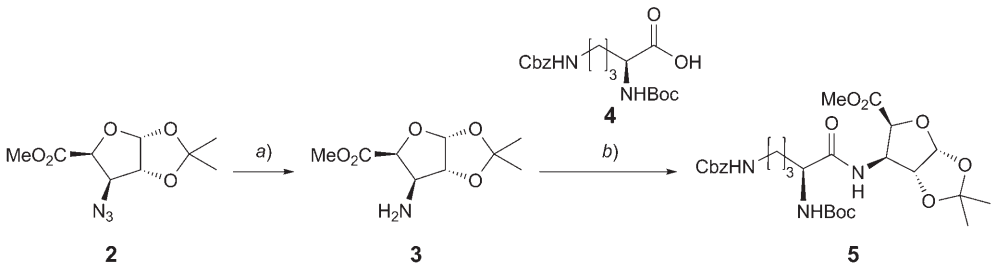
best of our knowledge, this is the first synthesis of a C_2-symmetric cyclic peptide with alternating SAA and δ-amino acid residues. Detailed NMR, ESI-MS, FT-IR, and restrained MD simulations techniques were employed to characterize the peptide **1**.



1

Results and Discussion. – The monomer of FSAA, **3**, was synthesized from the known azido sugar derivative **2** [8], which was used to prepare the dimer **5** by coupling with *Nα*-Boc-*Nδ*-Z-L-ornithine using the standard solution-phase coupling method using EDCI and HOBt as coupling agents in dry CH_2Cl_2. Initially, the free amine of FSAA **3** was coupled with the carboxylic acid group of the protected ornithine **4**, which afforded the protected dimer **5** in 85% yield (*Scheme 1*). The ester group of compound **2** was hydrolyzed with LiOH in THF/H_2O to afford the acid **6**. The benzyloxycarbonyl group of **5** was deprotected under hydrogenation reaction conditions to obtain the amine **7**, which was coupled with the dimer acid **6** under EDCI/HOBt coupling conditions to give tetramer **8** in 81% yield. After hydrolysis of the Me ester group in compound **8**, the obtained acid was esterified with pentafluoro phenol to afford **9** in 76% yield. Compound **9** was cyclized under standard hydrogenation conditions to get the desired 20-membered cyclic peptide **1** in 71% yield (*Scheme 2*).

Scheme 1. *Synthesis of* **5**

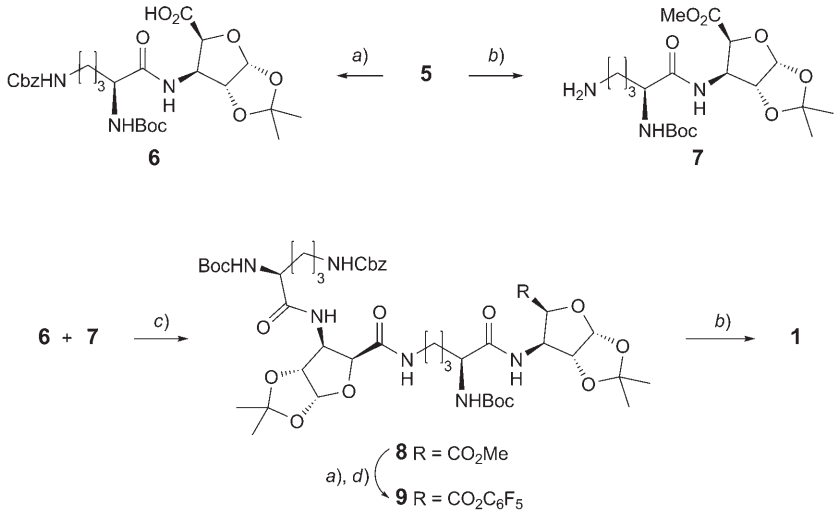


2 **3** **5**

a) Pd/C, H_2, AcOEt, r.t., 1 h, 95%. *b*) EDCI, HOBt, CH_2Cl_2; 0° to r.t., 24 h, 85%.

Preliminary molecular mechanics calculations carried out on the 20-membered backbone of **1** by using the conformational space search method [9] showed that FSAA maintains *gauche*-conformation [3] around $C_\alpha - C_\beta$, similar to that observed for *14*-helical folds. Information on the preferred conformation of **1** (4 mM) was obtained in both non-polar and polar solvents ($CDCl_3$, (D_6)DMSO) by carrying out 1D- and 2D-

Scheme 2. *Synthesis of* **1**



a) LiOH, THF/H_2O 3 : 1, 0° to r.t., 2 h. *b*) Pd/C, H_2, AcOEt, r.t. *c*) EDCI, HOBt, CH_2Cl_2, 0° to r.t., 24 h, 81%. *d*) C_6F_5OH, EDCI, CH_2Cl_2, 0° to r.t., 18 h, 76%.

(gDQCOSY, TOCSY, and ROESY) NMR studies. The 1D spectra in both solvents revealed a single set of resonances, at low (253–278 K, $CDCl_3$) and high (300–323 K) temperatures, and were consistent with the C_2 symmetry of the molecule. The complete resonance assignments were accomplished by using a combination of 1D-NMR (^1H), COSY, TOCSY, and ROESY data. The ROESY spectra were recorded at different mixing times ($\tau_{mix} = 180$ to 400 ms). The data was found to be consistent over the range, and the analysis of the data for $\tau_{mix} = 300$ ms is discussed here. The chemical shifts of amide H-atoms were found to be constant as a function of the sample dilution from 10 mM to *ca.* 0.5 mM, thereby confirming the absence of aggregation. ESI-MS data ($[\mathbf{1}+H]^+$, $[\mathbf{1}+Na]^+$), also support this possibility with the values 799 and 821 Da, respectively, corresponding to the monomeric cyclic tetramer **1**, and signals corresponding to aggregats were absent.

Conformational Analysis of Compound **1** *in CDCl₃*. The value of $^3J(HC_\alpha,HC_\beta)$ of FSAA is observed to be *ca.* 5.0 Hz, which is a clear sign for a *gauche* conformation around the $C_\alpha-C_\beta$ bond [3]. The other ^3J couplings along the backbone $J(HC_\beta,NH) = 6.0$ and $J(HC_\beta,C_\alpha H) = 5.0$ Hz were obtained by selective homonuclear decoupling (*Table 1*).

The observed coupling $J(NH,C_\beta H) = 6$ Hz, and the strong NOEs NH/C_αH and NH/C_βH of FSAA suggest a predominantly *gauche* conformation around NH–C_βH of FSAA as well (*Figs. 1* and *2*). Absence of an NH_{Orn}/NH_{FSAA} NOE and the presence of medium range NOEs, $NH_{Orn}/C_\alpha H_{FSAA}$ and $NH_{Orn}/C_\delta H_{FSAA}$ further indicate that the two NH groups (Orn and FSAA) are *anti* (phasing in opposite direction). These findings collectively infer a cyclic backbone conformation of **1** in $CDCl_3$, with the CO groups of Orn pointing towards the inner cavity, while the CO groups of FSAA being in opposite

Table 1. *^{1}H-NMR Chemical Shifts* (δ, in ppm) *and Coupling Constants* (J, in Hz) *of* **1** *in $CDCl_3$*

Residue	Sugar	Ornithine
NH	8.03 (d, $J = 6.0$)	7.09 (t, $J = 6.1$)
$C_\alpha H$	4.81 (d, $J = 5.0$)	4.67 (m)
$C_\beta H$	4.06 (dd, $J = 5.0, 6.0$)[a])	1.72 (m)
$C_{\beta 1} H$	–	1.26 (m)
$C_\gamma H$	5.03 (d, $J = 3.1$)	1.61 (m)
$C_{\gamma 1} H$	–	1.36 (m)
$C_\delta H$	6.31 (d, $J = 3.1$)	3.88 (m)
$C_{\delta 1} H$	–	2.99 (m)
BocNH	–	5.22 (d, $J = 8.1$)

[a]) The coupling constants were obtained by selective homonuclear decoupling.

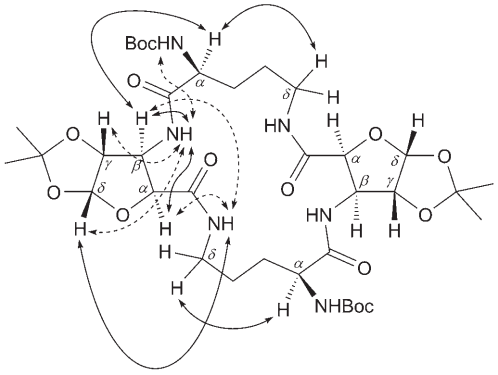


Fig. 1. *Schematic representation of observed NOEs of* **1** *in $CDCl_3$*. The solid and dotted arrows represent strong and weak NOEs, resp.

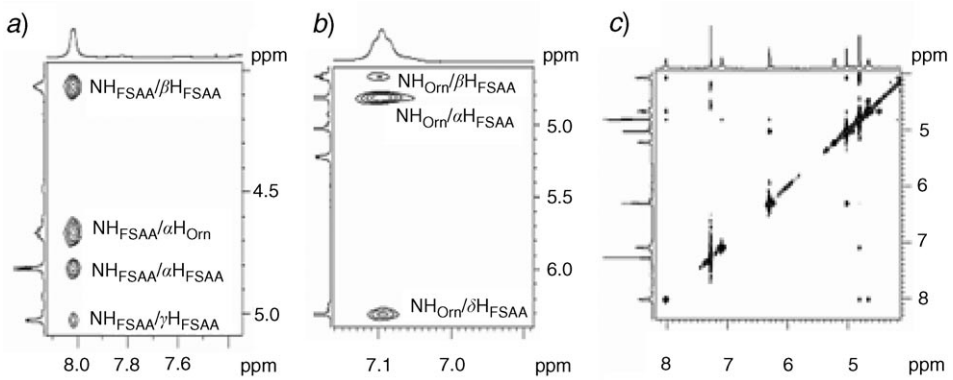


Fig. 2. *Selected NOESY regions for* **1** *in $CDCl_3$*. *a*) Intra-residue NOEs in FSAA involving the NH_{FSAA} H-atom. *b*) Inter-residue NOEs involving the $C_\beta H$ and $C_\alpha H$ of FSAA, and NH_{Orn} H-atoms. *c*) In contrast to the data in DMSO, inter-residue NH/NH NOEs are not observed.

direction. This conformation brings the NH_{FSAA} to a proximity to CO_{Boc}, an arrangement that can favor seven-membered inter-residue H-bonding. The downfield

shift of amide H-atoms of FSAA (8.03 ppm) supports the possibility of its participation in H-bonding [3].

In order to assess the involvement of amide H-atoms in H-bonding, DMSO titration studies were carried out by sequentially adding DMSO (up to 33% *v/v*) to the $CDCl_3$ solution. The variations of NH chemical shifts over the range of titration are observed to be -0.4, 1.0, and 1.6 ppm for NH_{FSAA}, NH_{Orn}, and NH_{Boc}, respectively, which imply that NH_{FSAA} is involved in H-bonding (possibly inter-residue H-bond), while the NH_{Orn} and NH_{Boc} seem to be solvent-exposed.

Conformational Analysis of Compound **1** *in DMSO*. The $C_\beta H$ of FSAA shows a resolved *doublet* of *doublet* with $^3J(C_\beta H,NH) = 7.5$, $J(C_\beta H,C_\alpha H) = 4.5$ Hz (*Table 2*), which suggests that the *gauche* conformation is retained around the $C_\alpha - C_\beta$ bond of FSAA, while the $NH - C_\beta H$ bond strongly deviates from *gauche* conformation, contrary to the observation in $CDCl_3$.

Table 2. *^{1}H-NMR Chemical Shifts* (δ, in ppm) *and Coupling Constants* (*J*, in Hz) *of Compound* **1** *in (D$_6$)DMSO*

Residue	Sugar	Ornithine
NH	7.32 (*d*, *J* = 7.5)	8.11 (*t*, *J* = 5.4)
$C_\alpha H$	4.61 (*d*, *J* = 4.5)	3.78 (*m*)
$C_\beta H$	4.25 (*dd*, *J* = 4.5, 7.5)	1.44 (*m*)
$C_{\beta 1} H$	–	1.44 (*m*)
$C_\gamma H$	4.43 (*d*, *J* = 3.9)	1.44 (*m*)
$C_{\gamma 1} H$	–	1.44 (*m*)
$C_\delta H$	5.88 (*d*, *J* = 3.9)	3.04 (*m*)
$C_{\delta 1} H$	–	3.04 (*m*)
BocNH	–	7.08 (*d*, *J* = 6.3)

Strong NOEs, $(NH/C_\gamma H)_{FSAA}$, $(NH/C_\delta H)_{FSAA}$, and a weak NOE, $(NH/C_\beta H)_{FSAA}$ imply that NH_{FSAA} and $C_\beta H_{FSAA}$ are in *trans* conformation. On the other hand, a strong NOE between NH_{Orn} and $C_\alpha H_{FSAA}$ indicates that these two H-atoms are *syn* to each other. This flip in the orientation of NH_{Orn} and NH_{FSAA} is reflected in their corresponding dihedral angles (Φ). The temperature dependence of the NH_{FSAA} chemical shifts showed a small coefficient ($\Delta\delta/\Delta T$) of -4.0 ppb/K compared to that of NH_{Orn} and NH_{Boc} (-6.5 ppb/K, and -10.5 ppb/K, resp.). This temperature coefficient for NH_{FSAA} and the corresponding NOEs (*Figs. 3* and *4*) suggest a possibility of six-membered $(NH-CO)_{FSAA}$ H-bonding (intra-residue).

The involvement of NH groups in H-bonding is also further corroborated by FT-IR studies, which show a characteristic broad NH-stretching band around 3297 cm^{-1} and C=O stretching peaks around 1670 cm^{-1} [10].

Restrained Molecular Dynamics. The distance restraints used in molecular dynamics were derived from the ROESY cross-peak intensities, which are volume-integrated and normalized with respect to the CH_2 H-atoms (1.8 Å) or FSAA-γ- and -δ-H-atoms (2.43 Å) (*Table 3*).

The restrained MD calculations were carried out following a simulated annealing protocol [11]. However, ambiguity in ornithine residue assignments has limited us to use only eight NOEs for restrained molecular dynamics. Dielectric constants of 4.7 and

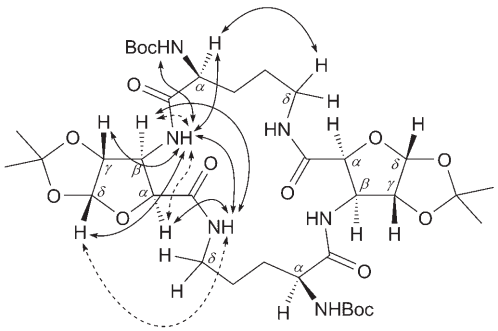


Fig. 3. *Schematic representation of observed NOEs of* **1** *in DMSO.* The solid and dotted arrows represent strong and weak NOEs, resp.

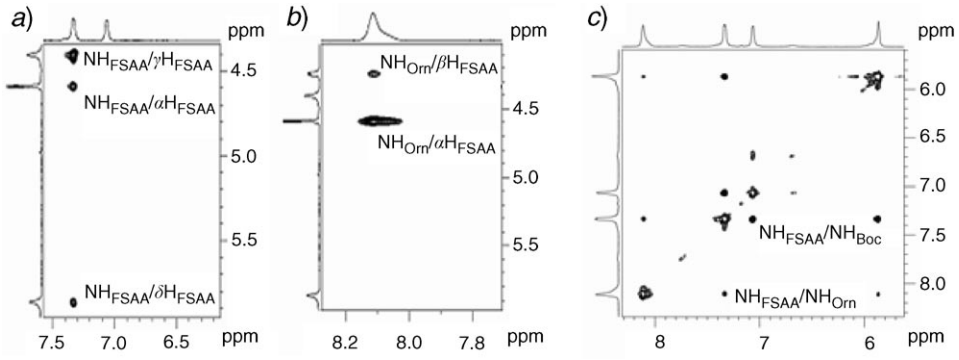


Fig. 4. *Selected NOESY regions for* **1** *in DMSO. a*) Intra-residue NOE in FSAA involving the NH_{FSAA} H-atom. *b*) Inter-residue NOEs involving the $C_{\beta}H$ and $C_{\alpha}H$ of FSAA, and NH_{Orn} H-atoms. *c*) Inter-residue NH/NH NOEs.

47.0 were used for $CDCl_3$ and DMSO, respectively. Initially, the molecule was heated up to 500 K and gradually cooled down to 300 K in two steps, for a 1 ns time period. At 300 K, a prolonged dynamics (5 ns) was run. By collecting a snapshot for every 50000 history files, 100 structures were obtained, which were energy minimized by using the conjugate method. Among these structures, the lowest energy structures were subjected to restrained (distances and torsion angles) MD studies.

During the MD trajectory over a period of 500000 fs, 100 structures were collected by taking a snapshot for every 5000 fs. Superposition of these structures showed a good convergence (*Fig. 5*), where at least the backbone is well-defined, suggesting a predominant single conformation for **1** in both solvents during the timescale of observation.

The individual structures were further subjected to energy minimization using the conjugate method (*Fig. 6*). No violations larger than 0.15 Å occurred for any distance restraints except for the $NH_{FSAA}/C_{\alpha}H_{Orn}$ (−0.25 Å) in DMSO. Analysis of the snapshots of MD of **1** showed that the backbone dihedral angles, Φ, θ, and Ψ, of FSAA were −70.25, −26.55, and 138.43 in $CDCl_3$, and 167.64, −37.16, and −91.87 in DMSO,

Table 3. *Distance Restraints Used in Molecular Dynamics for* **1**

Residue	Atom	Residue	Atom	Distance range [Å]
$CDCl_3$ as the solvent medium				
FSAA	NH	Ornithine	H(α)	3.56 – 2.91
FSAA	NH	Ornithine	H(β)	3.63 – 2.97
FSAA	NH	FSAA	H(α)	4.07 – 3.33
Ornithine	NH	FSAA	H(δ)	4.36 – 3.56
Ornithine	NH	FSAA	H(α)	3.32 – 2.72
FSAA	H(γ)	FSAA	H(α)	4.26 – 3.48
FSAA	H(δ)	FSAA	H(α)	3.48 – 2.84
Ornithine	H(α)	Ornithine	H(δ)	2.64 – 2.16
DMSO as the solvent medium				
FSAA	NH	FSAA	H(δ)	3.89 – 3.18
FSAA	NH	FSAA	H(β)	3.25 – 2.66
FSAA	NH	Ornithine	H(α)	3.20 – 2.6
Ornithine	NH	FSAA	NH	4.19 – 3.80
Ornithine	NH	FSAA	H(α)	2.77 – 2.27
Ornithine	NH	FSAA	H(β)	4.08 – 3.3
FSAA	H(β)	Ornithine	H(α)	4.48 – 3.67
FSAA	H(δ)	FSAA	H(α)	4.08 – 3.34
Ornithine	H(α)	Ornithine	H(δ)	3.20 – 2.6

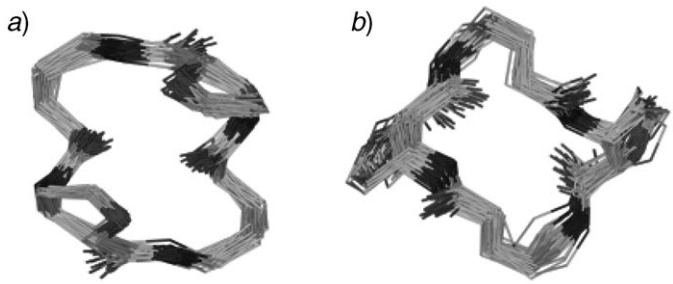


Fig. 5. *Superposition of 40 snapshots* (MD) *for* **1** *in* $CDCl_3$ (*a*) *and DMSO* (*b*). H-Atoms and Boc groups are omitted for the sake of clarity.

respectively. While the rigid FSAA motif continued to maintain a *gauche* conformation around $C_\alpha - C_\beta$ (θ), the change in the solvent polarity caused a flip in the orientation of the C=O and NH groups of FSAA and Orn, which is clearly reflected in their Φ and Ψ values. The resultant conformations favor a seven-membered inter-residue ($NH_{FSAA} - CO_{Boc}$) H-bond (2.16 Å) in $CDCl_3$, and a relatively weak 6-membered intra-residue (FSAA) H-bond (2.63 Å) in DMSO. Nevertheless, FSAA seems to have significant control over the backbone folding, which is stabilized by either six- or seven-membered rings by H-bonding, and the cyclization enhanced the rigidity of the overall peptide macrocycle in both solvents (*Fig. 6*). These findings were consistent with the analysis of the NMR data.

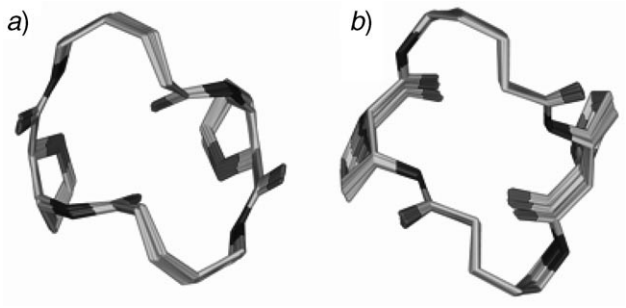


Fig. 6. *Superposition of the 40 minimum energy structures* (MD simulated) *of* **1** *in* $CDCl_3$ (*a*) *and DMSO* (*b*). H-Atoms and Boc groups are omitted for the sake of clarity.

To explore the extent of the influence of rigid FSAA scaffold over a more flexible ornithine, we have also studied the linear analogue of the cyclic peptide **1**, **8**. The data exhibited *gauche* conformation for θ (-40°). The observed $^3J(\mathrm{NH},\mathrm{C}_\beta\mathrm{H}) = ca.$ 9 Hz for the FSAA residues corresponds to an antiperiplanar arrangement between these H-atoms, similar to the earlier observations in FSAA-based *14*-helix oligomers [3]. However, no clearly determined secondary structure could be derived from the CD spectrum for the linear analog of **1**. Furthermore, except the third residue NH-5 (Boc-NH of ornithine) all the NH H-atoms of the oligomer seem to be participating only in weak H-bonds, as suggested by their temperature coefficients ($\Delta\delta/\Delta T$) of *ca.* 5.5 ppb/K. One can infer that the FSAA can still modulate the conformational space of the adjacent flexible spacer to some extent. Nevertheless, a detailed structural study on this linear oligomer along with the $C_\gamma - C_\delta$ constrained ornithine residues would be necessary for gaining insight into the conformational propensities.

Conclusions. – In summary, the present work describes the synthesis and conformational analysis of a C_2-symmetric cyclic tetrapeptide **1**, with alternating β- and δ-amino acids, which adopts a 3D-bowl shape structure in both polar and non-polar solvents. The study could also reveal that in a linear analogue of **1**, FSAA can nominally modulate the conformational space of the flexible ornithine. Further investigations on the linear and cyclic peptide with constrained ornithine (by introducing side chains at selected positions) may yield more information on various folding propensities.

Experimental Part

General. Commercial reagents were used without further purification. All solvents were purified by standard techniques. Column chromatography (CC) was carried out on silica gel (SiO_2, 60–120 mesh). Optical rotations: *Jasco Dip 360* digital polarimeter. CD Spectra: *Jasco J-75* spectrometer at 25° in MeOH, using a 1 mm path-length CD cell. IR Spectra: *Perkin-Elmer 683* spectrometer. NMR Spectra: in $CDCl_3$ or DMSO on a *Varian Unity Inova-500* MHz or an *Avance-II 600* MHz spectrometer. Chemical shifts (δ) in ppm, referenced to the tetramethylsilane (TMS) as internal standard; coupling constants (J) in Hz. 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 phase-sensitive mode. MS: *Finnigan MAT 1020B* or *Micromass VG 70–70 H* spectrometer at 70 eV with a direct inlet system. Model building and molecular dynamics simulations were carried out

using Insight II (97.0)/Discover1 program on a SiliconGraphics Octane and Fuel workstations using IRIX64 (6.5) operating system.

N*α-Boc*-N*δ-Cbz-Orn-fSAA-OMe* (= *Methyl 3-{[*N^2-*(*tert-*Butoxycarbonyl)*-N^5-*(phenoxycarbonyl)*-L-*ornithyl]amino}-3-deoxy-1,2*-O-*(1-methylethylidene)*-α-D-*xylofuranoate*; **5**). To a soln. of *methyl 3-azido-3-deoxy-1,2*-O-*(1-methylethylidene)*-α-D-*xylofuranoate* (**2**; 1 g, 4.6 mmol) in AcOEt (20 ml) was added 10% Pd/C (0.05 g). After hydrogenation of the mixture for 1 h at atm. pressure, the catalyst was filtered off, and the filtrate was concentrated *in vacuo* to give *methyl 3-amino-3-deoxy-1,2*-O-*(1-methylethylidene)*-α-D-*xylofuranoate* (**3**; 0.92 g). The compound N^2-*(*tert-*butoxycarbonyl)*-N^5-*(phenoxycarbonyl)*-D-*ornithine* (**4**; 1.39 g, 3.8 mmol) was dissolved in CH_2Cl_2 and treated with 1-hydroxybenzotriazole (HOBt; 0.52 g, 4.2 mmol) and *N*-[3-(dimethylamino)propyl]-*N′*-ethylcarbodiimide (EDCI; 0.97 g, 5 mmol) at 0°. The mixture was stirred at r.t. for 30 min, and the free amine **3** (0.92 g, 4.2 mmol) was added. The mixture was stirred at r.t. for further 24 h and diluted with aq. HCl (1M, 50 ml), and extracted with CH_2Cl_2 (3 × 50 ml). The combined org. layers were extracted with aq. $NaHCO_3$ (50 ml) and brine (50 ml), dried, concentrated in *vacuo*, and purified by CC (SiO_2, AcOEt/hexanes 2 :3) to yield **5** (2.03 g, 85%) as a white solid. M.p. 153–155°. $[\alpha]_D^{25} = -7.5$ (*c* = 1, $CHCl_3$). IR (neat): 3348, 2980, 1701, 1660, 1521, 1246, 1029, 754. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.45–7.30 (*m*, 5 H); 7.17 (*d*, *J* = 8.7, 1 H); 5.99–5.90 (*m*, 1 H); 5.25–5.20 (*m*, 1 H); 5.10–5.00 (*m*, 3 H); 4.95–4.90 (*m*, 1 H); 4.82–4.80 (*m*, 1 H); 4.52–4.50 (*m*, 1 H); 4.20–4.18 (*m*, 1 H); 3.73 (*s*, 3 H); 3.45–3.30 (*m*, 1 H); 3.21–3.18 (*m*, 1 H); 2.14–1.92 (*m*, 3 H); 1.81–1.79 (*m*, 1 H); 1.50 (*s*, 3 H); 1.40 (*s*, 9 H); 1.26 (*s*, 3 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.0; 168.4; 156.6; 155.5; 136.4; 128.4; 128.07; 128.0; 112.7; 104.8; 83.8; 76.3; 66.6; 56.4; 52.4; 40.1; 29.9; 28.2; 26.6; 26.1. HR-MS: 588.2532 ($[M + Na]^+$, $C_{27}H_{39}N_3NaO_{10}^+$; calc. 588.2533).

N*δ-Cbz(*N*α-Boc-Orn-fSAA)$_2$-OMe* (= *Methyl (3a*R,*5*S,*6*R,*6a*R*)-6-{[(2*S*)-2-[(*tert-*Butoxycarbonyl)amino]-5-({[(3a*R,*5*S,*6*R,*6a*R*)-6-({(2*S*)-2-[(*tert-*butoxycarbonyl)amino]-5-[(phenoxycarbonyl)amino]-pentanoyl}amino)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl]carbonyl}amino)pentanoyl]amino}-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole-5-carboxylate*; **8**). Compound **5** (1 g, 1.7 mmol) was dissolved in 12 ml of THF/H_2O (3 :1), followed by the addition of LiOH (0.22 g, 5.3 mmol). After stirring at r.t. for 2 h, the mixture was acidified to pH 2 with $NaHSO_4$ and extracted with AcOEt (2 × 50 ml). Then, the combined org. layers were dried over anh. Na_2SO_4 and concentrated under vaccum to give *3-{[*N^2-*(*tert-*butoxycarbonyl)*-N^5-*(phenoxycarbonyl)*-L-*ornithyl]amino}-3-deoxy-1,2*-O-*(1-methylethylidene)*-α-D-*xylofuranoic acid* (**6**; 0.87 g), which was used without further purification for the next reaction.

To a soln. of **5** (1.2 g, 2.1 mmol) in AcOEt (10 ml) 10% Pd/C (0.05 g) was added. After hydrogenation of the mixture for 12 h at atm. pressure, the catalyst was filtered off, and the filtrate was concentrated *in vacuo* to give *methyl 3-{[*N^2-*(*tert-*butoxycarbonyl)*-L-*ornithyl]amino}-3-deoxy-1,2*-O-*(1-methylethylidene)*-α-D-*xylofuranoate* (**7**; 0.85 g). Compound **6** (0.83 g, 1.5 mmol) was dissolved in CH_2Cl_2 and treated with HOBt (0.24 g, 1.7 mmol) and EDCI (0.4 g, 2 mmol) at 0°. This mixture was stirred at r.t. for 30 min, and the free amine **7** (0.75 g, 1.7 mmol) was added. The mixture was stirred at r.t. for 24 h and diluted with aq. HCl (1M, 50 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The combined org. layers were extracted with aq. $NaHCO_3$ (50 ml) and brine (50 ml), dried, concentrated *in vacuo*, and purified by CC (SiO_2, AcOEt/hexane 4 :1) to yield compound **8** (1.36 g, 81%) as a white solid. M.p. 183–185°. $[\alpha]_D^{25} = -14.0$ (*c* = 0.5, $CHCl_3$). IR (neat): 3326, 2981, 1660, 1530, 1255, 1165, 1029, 753. ^{1}H-NMR (500 µl $CDCl_3$ + 20 µl DMSO, 600 MHz): 8.2 (*d*, *J* = 9.64, 1 H); 7.83 (*d*, *J* = 8.77, 1 H); 7.82 (*t*, *J* = 6.07, 1 H); 7.35 (*m*, 4 H); 7.30 (*m*, 1 H); 7.10 (*t*, *J* = 5.5, 1 H); 6.60 (*d*, *J* = 7.97, 1 H); 6.53 (*d*, *J* = 7.84, 1 H); 6.0 (*d*, *J* = 3.15, 1 H); 5.94 (*d*, *J* = 3.20, 1 H); 5.03 (*s*, 2 H); 4.78 (*d*, *J* = 4, 1 H); 4.72 (*dd*, *J* = 4, 9.6, 1 H); 4.64 (*d*, *J* = 3.9, 1 H); 4.53 (*dd*, *J* = 3.95, 8.77, 1 H); 4.46 (*d*, *J* = 3.32, 1 H); 4.45 (*d*, *J* = 3.27, 1 H); 3.93 (*m*, 2 H); 3.66 (*s*, 3 H); 3.24 (*m*, 1 H); 3.04 (*m*, 3 H); 1.58 (*m*, 2 H); 1.50 (*m*, 4 H); 1.47 (*m*, 8 H); 1.41 (*s*, 9 H); 1.39 (*s*, 9 H); 1.29 (*s*, 6 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.6; 172.1; 168.4; 167.0; 156.9; 155.5; 136.5; 128.4; 127.8; 112.3; 104.9; 84.0; 79.4; 66.4; 56.6; 52.3; 30.5; 28.5; 26.6; 26.2. HR-MS: 987.4509 ($[M + Na]^+$; $C_{45}H_{68}N_6NaO_{17}^+$; calc. 987.4538).

*Di(*tert-*butyl) [(3a*R,*4a*R,*10*S,*12a*R,*12b*R,*15a*R,*16a*S,*22*S,*24a*R,*24b*R*)-Tetracosahydro-2,2,14,14-tetramethyl-5,11,17,23-tetraoxobis[1,3]dioxolo[4,5]furo[3,2-*b*:3′,2′-*l*][1,5,11,15]tetraazacycloicosine-10,22-diyl]biscarbamate* (**1**; [12]). Compound **8** (0.317 g, 0.3 mmol) was dissolved in 12 ml THF/H_2O (3 :1), followed by the addition of LiOH (0.041 g, 0.9 mmol). After stirring at r.t. for 2 h, the mixture was acidified to pH 2.0 with $NaHSO_4$ and extracted with AcOEt (2 × 50 ml). Then, the combined org. layers

were dried over Na_2SO_4 and concentrated under vaccum to give the acid, which was dissolved in CH_2Cl_2 and treated with EDCI (0.05 g, 0.3 mmol) and pentafluorophenol (0.05 g, 0.3 mmol) at 0°. The mixture was stirred at r.t. for 18 h and diluted with H_2O (20 ml), and extracted with CH_2Cl_2 (2×50 ml). The combined org. layers were extracted with brine (50 ml), dried, and concentrated *in vacuo* to give *pentafluorophenyl (3a*R,*5*S,*6*R,*6a*R*)-6-{[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-5-({[(3a*R,*5*S,*6*R,*6a*R*)-6-({[(*tert*-butoxycarbonyl)amino]propyl}amino]acetyl}amino)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl]carbonyl}amino)pentanoyl]amino}-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole-5-carboxylate* (**9**). To the soln. of **9** (0.317 g, 0.2 mmol) in AcOEt was added 10% Pd/C (0.050 g). After hydrogenation of the mixture for 24 h at atm. pressure, the catalyst was filtered off, and the filtrate was concentrated *in vacuo* and purified by CC (SiO_2, $CHCl_3$/MeOH 25 :1) to yield **1** (0.16 g, 71%) as a white solid. M.p. 230–232. $[\alpha]_D^{25} = -43.3$ ($c = 0.015$, $CHCl_3$). IR (neat): 3297, 2919, 2850, 1690, 1677, 1532, 1451, 1368, 1217, 1165, 102, 861. ^{1}H-NMR($CDCl_3$, 500 MHz): 8.0 (*d*, *J* = 5.0, 1 H); 7.09 (*t*, *J* = 6.08, 1 H); 6.31 (*d*, *J* = 3.1, 1 H); 5.22 (*d*, *J* = 8.0, 1 H); 5.03 (*d*, *J* = 3.14, 1 H); 4.81 (*d*, *J* = 5.05, 1 H); 4.67 (*m*, 1 H); 4.06 (*t*, *J* = 5.0, 1 H); 3.88 (*m*, 1 H); 2.99 (*m*, 1 H); 1.72 (*m*, 1 H); 1.62 (*m*, 1 H); 1.51 (*s*, 3 H); 1.43 (*s*, 9 H); 1.36 (*m*, 1 H); 1.33 (*s*, 3 H); 1.26 (m, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.5; 170.0; 156.0; 111.5; 106.3; 84.5; 81.5; 79.7; 59.1; 50.6; 37.3; 33.0; 28.2; 26.7; 26.0; 25.7. HR-MS: 821.3931 ($[M + Na]^+$, $C_{36}H_{58}N_6NaO_{14}^+$; calc. 821.3908).

B. S., *P. N.*, and *M. U.* are thankful to CSIR, New Delhi, for fellowship.

REFERENCES

[1] C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252.
[2] S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173; D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015.
[3] S. Chandrasekhar, M. S. Reddy, B. Jagadeesh, A. Prabhakar, M. H. V. Ramana Rao, B. Jagannadh, *J. Am. Chem. Soc.* **2004**, *126*, 13586.
[4] S. Chandrasekhar, M. S. Reddy, B. N. Babu, B. Jagadeesh, A. Prabhakar, B. Jagannadh, *J. Am. Chem. Soc.* **2005**, *127*, 9664.
[5] B. Jagannadh, M. S. Reddy, C. L. Rao, A. Prabhakar, B. Jagadeesh, S. Chandrasekhar, *Chem. Commun.* **2006**, 4847.
[6] T. A. Martinek, I. M. Mándity, L. Fülöp, G. K. Tóth, E. Vass, M. Hollósi, E. Forró, F. Fülöp, *J. Am. Chem. Soc.* **2006**, *128*, 13539.
[7] J. Auernheimer, R. Haubner, M. Schottelius, H.-J. Wester, H. Kessler, *Helv. Chim. Acta* **2006**, *89*, 833.
[8] S. Gruner, G. Kéri, R. Schwab, A. Venetianer, H. Kessler, *Org. Lett.* **2001**, *3*, 3723; E. Dalcanale, F. Montanari, *J. Org. Chem.* **1986**, *51*, 567; S. Saito, H. Nakajima, M. Inaba, T. Moriwake, *Tetrahedron Lett.* **1989**, *30*, 837; G. W. J. Fleet, J. M. Peach, P. W. Smith, G. N. Austin, P. D. Baird, D. J. Watkin, *Tetrahedron* **1987**, *43*, 3095.
[9] B. Jagannadh, A. C. Kunwar, R. P. Thangavelu, E. Osawa, *J. Phys.Chem.* **1996**, *100*, 14339; B. Jagannadh, J. A. R. P. Sarma, *J. Phys. Chem. A* **1999**, *103*, 10993.
[10] S. Krimm, J. Bandekar, in 'Advances in Protein Chemistry', Eds. C. B. Anfinsed, J. T. Edsall, and F. M. Richards, Academic Press, Orlando, FL, 1986, pp. 181–364.
[11] R. M. van Well, L. Marinelli, C. Altona, K. Erkelens, G. Siegal, M. van Raaij, A. L. Llamas-Saiz, H. Kessler, E. Novellino, A. Lavecchia, J. H. van Boom, M. Overhand, *J. Am. Chem. Soc.* **2003**, *125*, 10822.

Received February 6, 2008