JOC Note

β-Sugar Aminoxy Peptides As Rigid Secondary Structural Scaffolds

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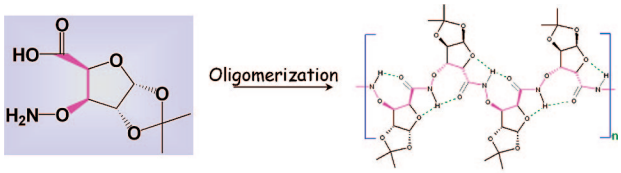


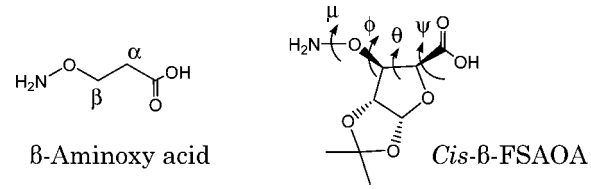
cis-β-FSAOA — Ribbon-like structures

Short homo-oligomers of a new building block, *cis*-$\beta^{2,3}$-furanoid sugar aminoxy acid, are designed, characterized, and found to exhibit rigid ribbon-like secondary structures composed of 5/7 bifurcated intramolecular hydrogen bonds.

β-Peptidic oligomers have emerged as highly versatile structural scaffolds in recent years, as they exhibit well-defined secondary structures, "foldamers",[1] such as helices, strands, and turns,[2] which bring about the appropriate spatial arrangements of the functional groups for biological applications.[3] Design and synthesis of conformationally restricted β-peptide building blocks[4] for deriving specific foldamers is topical in peptidomimetics.[5] By substituting oxygen atom in place of the Cγ atom of γ-amino acid, Yang and co-workers have designed novel rigid turn-inducing building blocks, β-aminoxy acids,[6] which are considered as extended β-amino acids or γ-amino acid analogues. Their studies have shown that di- and tripeptides of both linear β-aminoxy acids[7a] (Scheme 1) and *trans*-$\beta^{2,3}$-cycloalkane constrained aminoxy acids[7b] (aminoxy analogues of Gellman's classic *trans*-$\beta^{2,3}$-ACPC and *trans*-$\beta^{2,3}$-ACHC[1,2d]) preferentially adopt rigid N−O turns or helical folds stabilized by nine-membered inter-residue NH_i-CO_{i-2} hydrogen bonding (9-hb). These findings are consistent with Hoffman's theoretical predictions[8] that 9-helical and 14-helical folds are most favorable in the homo-oligomers of γ-amino acids. On the other hand, it is evident from earlier reports that the choice of *cis* over *trans* geometry around the $C_\alpha-C_\beta$ bond of $\beta^{2,3}$-amino acids results in a conformational switch in the backbone folding, by forming strand structures in oligomers of *cis*-$\beta^{2,3}$-aminocyclopentane carboxylic acid (*cis*-$\beta^{2,3}$-ACPC)[2e] and a right-handed 14-helix in oligomers of *cis*-$\beta^{2,3}$-cyclic furanoid sugar amino acid (*cis*-β-FSAA),[4c,d] in contrast to the left-handed 12-helix[9a] and 12/10-helix[9b] exhibited by *trans*-$\beta^{2,3}$-ACPC and β-FSAA oligomers, respectively. In light of the above theoretical and experimental findings, it is interesting to investigate the folding propensities of *cis*-$\beta^{2,3}$-cyclic aminoxy peptides in general. Furthermore, although the β-aminoxy acids reported so far are either aliphatic type or cycloalkane constrained, their analogues with carbohydrate rings on the backbone have not been explored.

As sugar amino acids[10] have been recognized as versatile structure building blocks, herein we report short oligopeptides based on a new class of building block, *cis*-$\beta^{2,3}$-furanoid sugar aminoxy acid (*cis*-FSAOA) (Scheme 1), which exhibit ribbon-like secondary structures that are unprecedented in β-aminoxy peptides. The present work focuses on the residue-based conformational control in deriving diverse secondary structural scaffolds.

SCHEME 1. Schematic Representation of β-Aminoxy Compounds and Corresponding Dihedral Angles



β-Aminoxy acid — *Cis*-β-FSAOA

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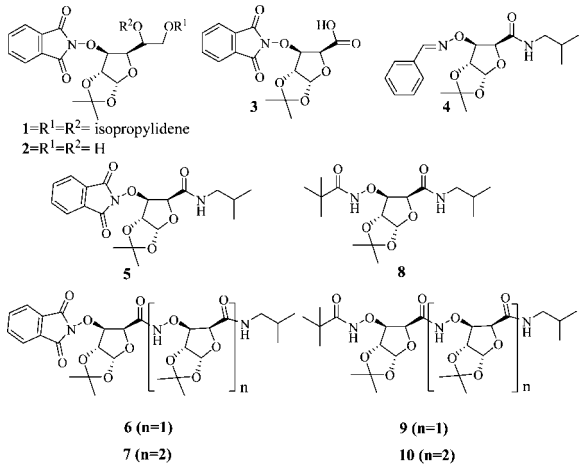
SCHEME 2. Schematic Representation of Compounds 1−10[a]



[a] Compounds **1**, **2**, **3**, **5**, **6**, and **7** are protected at the N-terminus by phthalimide (phth), whereas compounds **8**, **9**, and **10** are protected by pivoloyl (piv).

Monomer acid **3** was synthesized from the known *O*-phthalimido sugar derivative **1**.[11] Compounds **4**−**10**, which were derived from **3**, were synthesized by using EDCI and HOBt reagents (Scheme 2). Whereas **4** was synthesized to look for specific hydrogen bonding, **5**−**10** were explored for the folding behavior of the backbone. Detailed structural studies have been carried out by using NMR, ab initio DFT calculations, FT-IR, and restrained MD simulation techniques (Supporting Information).

To explore the folding propensities and hydrogen bond modes in *cis*-$\beta^{2,3}$-FSAOA homo-oligomers, we have carried out DFT calculations for the compounds **6**−**8** (Supporting Information). All geometries have been fully optimized using Gaussian 03[12] software, and the minima were confirmed by harmonic frequency calculations. Initially, the potential energy surface was scanned for minima with various starting structures and for different possible hydrogen bonding modes at the B3LYP/6-31G(d,p) level. The lowest two minima obtained for each molecule were further refined at the higher B3LYP/6-311++G(d,p) level. The relative energies were calculated, and unscaled ZPVE correction at the 6-31G (d,p) level has been applied. The resultant minimum energy structures of monomer (Piv)-**8**, dimer (Phth)-**6**, and trimer (Phth)-**7** have predominantly exhibited *gauche* conformation around HCα−CβH (~40°) and a secondary folding with aminoxy-NH groups involved in a bifurcated three-center hydrogen bonding (Figure 1): seven-membered intra-residue (NH_i−C=O_i) hydrogen bonding (7-hb) and five-membered inter-residue (NH_i−furan−O_{i-1}) hydrogen bonding (5-hb). This preferential three-center 5/7-hb backbone folding over the nine-membered (9-hb) helical turns[7b] may be attributed

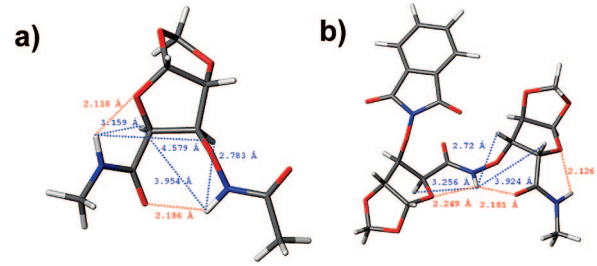


FIGURE 1. DFT calculated minimum conformers for **8** (a) and **6** (b).

to constrained geometry of the *gauche*-configured *cis*-FSAOA with rigid ∠NOC$_\beta$C$_\alpha$ (~108°), resulting in the positioning of furan-O_i, NH_{i+1}, and CO_{i+1} groups in the same plane. In trimer (Phth)-**7**, one of the two minimum energy conformers has exhibited an additional 12-membered hydrogen bonding (12-hb), which was not realized in NMR studies.

NMR studies of **4**−**8** were carried out in $CDCl_3$ solvent at 303 and 279 K. The complete resonance assignments were accomplished by using a combination of 1D and 2D gDQF-COSY, TOCSY, and ROESY data (Supporting Information). The ^{1}H NMR spectra of all the compounds have shown a clear dispersion of amide-NH (δ_{NH} ~6.7 ppm) and aminoxy-NHs (δ_{NH} = 9.5−9.7 ppm) resonances. Solvent titration studies (Supporting Information) for these compounds (with sequential addition of aliquots of DMSO up to 10% v/v) showed chemical-shift changes ($\Delta\delta_{NH}$) of ~0.15 and 1.1−1.45 ppm for amide-NH and aminoxy-NHs, respectively. These findings suggest that the amide-NH is not solvent-accessible and participates in a strong intra molecular hydrogen bonding, whereas the aminoxy-NHs are involved only in a weak hydrogen bonding. The measured $^3J_{C\alpha H-C\beta H}$ coupling constant for all of the FSAOA residues is <4 Hz (~ −40°), which suggests a highly restricted *gauche* conformation around the Cα−Cβ bond and that the backbone folding is uniform along its length.[4d]

Explicit ROESY analysis of **6**−**8** could yield the preferred local backbone conformation and the possible hydrogen bonding modes it can favor. For the monomer (Piv)-**8** and dimer (Phth)-**6**, the presence of characteristic intra-residue NOEs, NH_i−C$_\beta H_i$ (medium), NH_i−C$_\alpha H_i$ (weak), and NH_i−C$_\gamma H_i$ (weak), and inter-residue NOEs, NH_i−C$_\delta H_{i-1}$ (medium), NH_i−C$_\alpha H_{i-1}$ (medium), and NH_i−C$_\beta H_{i-1}$ (weak) (Figure 2), rules out helical turns or random coil[2e] and can be assigned to a predominantly strand or ribbon-like secondary structure.[4f,13]

This spatial arrangement implicates the aminoxy-NHs to participate in a three-center bifurcated hydrogen bonding composed of intra-residue (NH_i−C=O_i) 7-hb and inter-residue (NH_i−furan−O_{i-1}) 5-hb, while the terminal NH_a can only be involved in two-center 5-hb, which is consistent with the DFT calculations. The NMR (ROESY) studies of β-peptidic strands stabilized by intramolecular bifurcated 7-hb γ-peptidic ribbons[13] and of 5/6-hb rings[14] also support our analysis. The inter-residue

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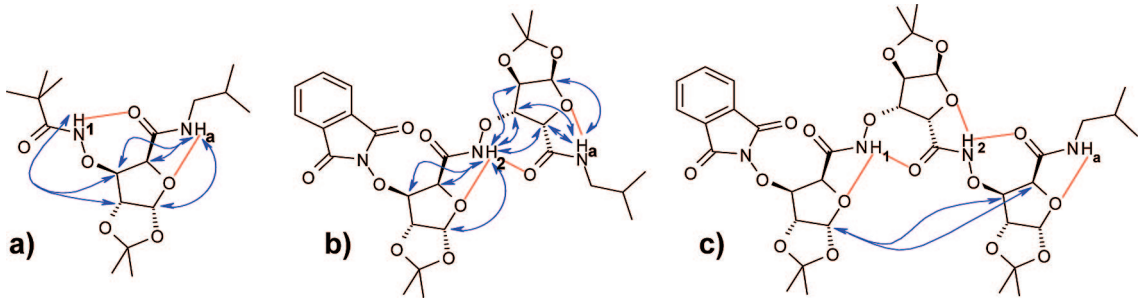

FIGURE 2. Schematic representation of ROEs (blue) and hydrogen bonding (dashed lines) in monomer (Piv)-**8** (a), dimer (Phth)-**6** (b), and trimer (Phth)-**7** (c) that characterize 5/7 hydrogen-bonded ribbons. For the sake of clarity, only the long-range $C_\delta H_{i-2}-C_\beta H_i$ and $C_\delta H_{i-2}-C_\alpha H_i$ are shown for **7**.

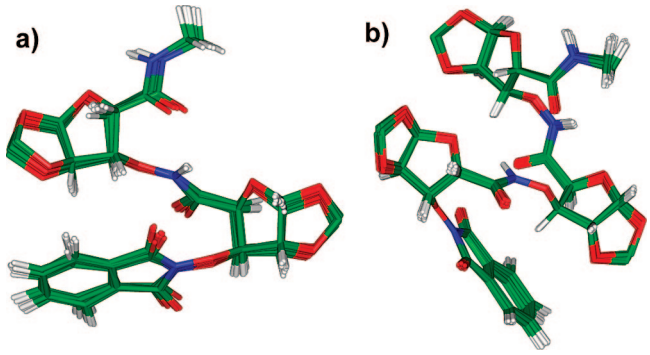

FIGURE 3. Superposition of 15 lower energy structures obtained in MD calculations for dimer **6** (a) and trimer **7** (b).

two-atom 5-hb as a part of a three-center bifurcated hydrogen bond is not uncommon in peptidomimetics,[15] biopolymers,[16] and natural products[17] and also has been found to be crucial in stabilizing the side chain of taxol.[18] The formation of 5-hb in the present compounds has been further confirmed by analyzing the monomeric reference **4**, which showed NOE pattern and DMSO titration ($\Delta\delta_{NHa}$ ~0.15 ppm) very similar to that observed for NH_a of **5**−**8** (Supporting Information). The ROESY data of trimer (Phth)-**7** has also exhibited unambiguous and similar periodic ROEs as in **6** and **8**, which supported the propagation of 5/7-hb ribbon-like backbone in this higher oligomer. Furthermore, the observed long-range ROEs (weak) $C_\delta H_{i-2}-C_\beta H_i$ and $C_\delta H_{i-2}-C_\alpha H_i$ suggest that the backbone is not fully stretched[4f] (Figure 2c). The NMR spectra of dimer (Piv)-**9** and trimer (Piv)-**10** also showed signatures of secondary folding (Supporting Information). However, a detailed structural elucidation has been hampered due to the presence of rotamers, which is not the case with **4**−**8**.

The minimum energy structures derived from ROE-restrained MD calculations for **5**−**8** by following simulated annealing protocol (Insight-II) (Supporting Information) are found to be in good agreement with those discussed above. Superposition of these structures (Figure 3) showed good convergence, suggesting a predominantly single backbone conformation composed of bifurcated 5/7-hb rings. The measured values of 5/7-hb distances (~2.3 Å), $\angle NOC_\beta C_\alpha$ (112 ± 10°), θ (40 ± 5°), three-center ∠NHO (112 ± 5° and 124 ± 5° for 5- hb and 7-hb, respectively), and two-center 5-hb ∠NHO (104 ± 3°) for terminal NH_a are in agreement with our DFT studies. These nonlinear ∠NHO values are characteristic of bifurcated hydrogen bonding between adjacent residues[15] and match well with those observed in 5/6-hb strands.[14]

However, normally, three-center hydrogen bonds and nonlinear ∠NHO are not optimal for strong hydrogen bonding. Nevertheless, it is noteworthy that the conformational stability is not necessarily related to the stability of hydrogen bonding. It is reasonable to speculate that the resultant 5-hb and the consecutive 5/7-hbs in these peptides are due to the manifestation of the rigid conformation[19] of the *cis*-FSAOA motif and that the strong 5-hb of terminal NH_a over the weak 5/7-hb of aminoxy-NHs is due to the energetic superiority of an isolated 5-hb interaction, relative to 5-hb as part of a three-center hydrogen bonding.[15] Our preliminary molecular mechanics calculations on a simulated tetramer also showed an increased population of ribbon conformation (Supporting Information). The striking feature revealed from these findings is that despite the presence of 14-helix nucleating motif, furanoid sugar ring as a backbone constraint, the *cis*-$\beta^{2,3}$-FSAOA oligomers do not support the formation of either 9- or 14-helical folds, which otherwise are favorable for γ-amino acid oligomers,[8] but rather preferentially adopt ribbon-like structures. These findings may shed more light on understanding the mechanism scope for generating diverse secondary scaffolds.

The FT-IR spectra showed resolved N−H stretching bands for amide-NH and aminoxy-NHs and suggested their participation in hydrogen bonding (Supporting Information). The dimer (Phth)-**6** and trimer (Phth)-**7** exhibited predominantly two distinct peaks (3318 and 3224 cm^{-1}) and (3321 and 3235 cm^{-1}), respectively, whose relative intensities are in accordance with the population of possible hydrogen bonding groups in the compounds. The bands at 3318 and 3321 cm^{-1} correspond to the hydrogen-bonded amide-NH, whereas the bands at 3224 and 3235 cm^{-1} can be attributed to the hydrogen-bonded aminoxy-NHs[7b,20] for **6** and **7**, respectively. The assignments have been supported by the observed single band at ~3325 for the control samples, monomer imine-**4** and monomer (Phth)-**5** (which do not have aminoxy groups), that predominantly correspond to the hydrogen-bonded amide-NH. Weak signatures of non-hydrogen-bonded amide-NH and aminoxy-NHs are also noticed around ~3450 and ~3390 cm^{-1}, respectively. The increase in

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the population of the hydrogen-bonded aminoxy-NHs in dimer (Phth)-**6** is reflected in the intensity of its band at 3235 cm^{-1}. However, the relatively higher values (by ~10 cm^{-1}) of aminoxy-NH bands compared to those in other β-aminoxy peptides may be due to the weaker bifurcated hydrogen bonding, which is consistent with the NMR data.

In summary, we have reported the synthesis and characterization of a new class of β-aminoxy acid motif, *cis*-$\beta^{2,3}$-FSAOA and its short oligomers. The DFT, NMR, and MD studies highlight that the combination of geometry around $\beta^{2,3}$ positions on the sugar ring constrained backbone and rigid N−O conformation exerts significant control of the folding preferences in the compounds studied. The results showed that the peptidic backbone accesses unusual ribbon-like secondary structure favoring 5/7 bifurcated intramolecular hydrogen-bonded rings, in contrast to the 9-helical turns in *trans*-cyclo alkane constrained β-aminoxy acid oligomers. These findings offer scope for generating diverse rigid scaffolds with residue based conformational control of the folding propensities.

Experimental Section

(3a*R*,5*S*,6*R*,6a*R*)-6-(1,3-dioxoisoindolin-2-yloxy)-*N*-isobutyl-2,2-dimethyl-tetrahydrofuro[2,3-*d*][1,3]dioxole-5-carboxamide (5). To a stirred solution of compound **2** (3.2 g, 8.76 mmol) in 80% aqueous THF (35 mL) was added $NaIO_4$ (3.75 g, 17.5 mmol) in three portions at 0 °C. After 6 h, the reaction mixture was filtered and washed with EtOAc (50 mL). The filtrate was concentrated, taken in ether, washed with water (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. This protocol afforded the intermediate aldehyde, which was dissolved in CH_3CN (18 mL), and to this were added a solution of $NaClO_2$ (1.3 g, 14.4 mmol) in 10 mL of water, NaH_2PO_4 (1.72 g, 14.4 mmol) in 7 mL of water, and 30% H_2O_2 (4.35 mL, 38.4 mmol) at 0 °C. The mixture was stirred for overnight at room temperature, solvent was removed under reduced pressure, and the mixture was washed with brine (10 mL) and extracted with EtOAc (4 × 60 mL). The combined organic solution was dried (Na_2SO_4) and concentrated in vacuo. This treatment afforded the intermediate acid **3**, which was dissolved in dry CH_2Cl_2/DMF (1:1, 40 mL), to which were added sequentially HOBt (1.88 g, 13.7 mmol) and EDCI (2.63 g, 13.7 mmol) at 0 °C under N_2 atmosphere. After 10 min, isobutyl amine (1.1 mL, 11 mmol) was added to the reaction mixture. The reaction mixture was allowed to warm room temperature and stirred further for 12 h under N_2 atmosphere. It was diluted with $CHCl_3$ and washed with 5% aqueous $NaHCO_3$ solution (15 mL), water (15 mL), and brine (15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Silica gel column chromatography (EtOAc/hexanes, 30:70) of the residue gave **5** (2.46 g, 69% yield) as a white solid, mp 230.5−234.8 °C; $[\alpha]_D = -37.0$ (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.88−7.74 (m, 4H), 6.62 (t, J = 5.8 Hz, 1H), 6.21 (d, J = 3.6 Hz, 1H), 5.10 (d, J = 3.6 Hz, 1H), 4.90 (d, J = 3.6 Hz, 1H), 4.85 (d, J = 3.6 Hz, 1H), 3.24 (qn, J = 6.5 Hz, 1H), 3.10 (qn, J = 6.5 Hz, 1H), 1.87−1.76 (m, 1H), 1.52 (s, 3H), 1.34 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.2, 163.2, 134.7, 128.7, 123.7, 113.1, 105.3, 88.7, 81.5, 79.5, 46.4, 28.3, 26.8, 26.3, 20.0. ESI (MS): *m*/*z* 427 $[M + Na]^+$, ESI-HRMS calcd for $C_{20}H_{24}N_2O_7Na$ $[M + Na]^+$ 427.1481, found 427.1465.

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Supporting Information Available: Experimental procedures, copies of ^1H and ^{13}C NMR spectra, NMR details, solvent titration plots, and MD studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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