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Furan-Based Locked *Z*-Vinylogous γ-Amino Acid Stabilizing Protein α-Turn in Water-Soluble Cyclic $\alpha_3\gamma$ Tetrapeptides

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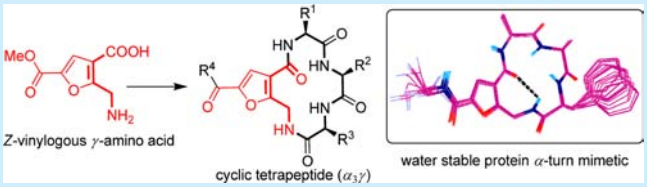
Supporting Information

ABSTRACT: Described here is the design, synthesis, and conformational analysis of cyclic tetrapeptides (CTPs) with $\alpha_3\gamma$ architecture containing a furan-based locked *Z*-vinylogous amino acid (Vaa). This unnatural amino acid locks into a γ-turn that induces type Iα_{RS}-turn in the CTPs. Stabilized by a 13-membered intramolecular H-bond, these CTPs show robust conformation in water and aprotic solvent irrespective of the sequence of tripeptide consisting of α-amino acids used.



Turns play an important structural role in the stability of globular proteins and, being exposed on the protein surfaces, often involve in a variety of molecular recognition processes.[1,2] Approximately, more than one hundred G-protein coupled receptors (GPCRs) have been identified that recognize ligand with turn structure.[2] Consequently, development of turn mimetics as the modulators of protein−protein interactions has become an attractive approach in drug discovery.[2,3] Depending on the number of residues involved, turns are classified[4] as δ-, γ-, β-, α-, and π- turns (Figure 1A), and among these, γ- and β-turns are very well explored.[2,5] α-Turn, one of the important turn motifs, occurs at many key sites (enzyme active site, metal binding domains, kinks of helices, etc.) of proteins like human lysozyme, glyceraldehydes-3 phosphate dehydrogenase, ferredoxin I, T-cell surface of glycoprotein, azurine, HIV gp120, *p*-hydroxybenzoate hydroxylate, glutathione peroxidase, etc.[4c,6] Structurally, α-turn involves five amino acid residues where the inner three-residue segment is stabilized by a H-bond between (*i*)CO and (*i*+4)NH, forming a 13-membered pseudocycle. Interestingly, while consecutive linking of α-turn with identical backbone dihedral angles (ϕ −58°, ψ −47°) among all the residues leads to the formation of α-helix,[7] α-turns in proteins (tight α-turn[4c]) do not follow this rule and frequently change the backbone dihedral angles, helical pitch, and orientation of the side chains (Figure 1B).[4a] Nine different types of tight α-turn have been identified on the basis of varying backbone dihedral angles, and type I-α_{RS} is the most widely occurring (238 occurrences out of 356 α-turns in 190 proteins).[4b,c] Surprisingly, while numerous reports have been described to mimic α-helices,[8−12] reports to stabilize protein α-turn using small molecules are rare. There are few approaches to stabilize single α-turn, although only for α-turn of an α-helix.[13] Very recently, Fairlie et al. reported[14] cyclic peptides that mimic tight α-turns. However, the side chain has been engaged in those peptides to stabilize the motifs.

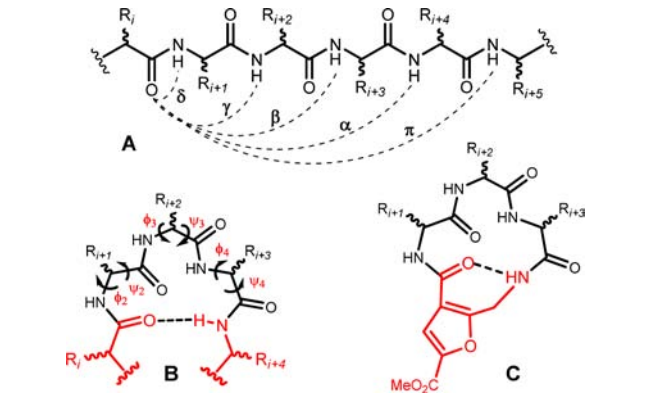


Figure 1. (A) Various tight turn motifs. (B) Schematic diagram of α-turn with variable dihedral angles of inner three residues.[4a,14] (C) Vaa as the surrogate of *i* and *i*+4 residues.

As part of our ongoing program on peptidomimetics,[15] we desired to use a furan-based *Z*-vinylogous γ-amino acid (Vaa) as the general template (surrogate of the terminal residues of a tight α-turn) in which a tripeptide will be grafted without disturbing its side chains and the template would preorganize the resultant cyclic tetrapeptide (CTP) into a stable conformation irrespective of the sequence used (Figure 1C). The rationalities for choosing Vaa are as follows: (1) the furan scaffold of Vaa would bring conformational rigidity in the CTP by locking the *cis* geometry in **2**; (2) an intraresidual 7-membered H-bond in the Vaa (as predicted theoretically by Hoffmann et al.[16]) would eventually replicate the (*i*,*i*+4) 13-membered H-bonding feature of α-turn; (3) the flat Vaa scaffold would not allow the peptide to maintain the pitch corresponding to the α-turn of an α-helix; (4) the resultant 14-membered cyclic ring would be released from the strain of a 12-

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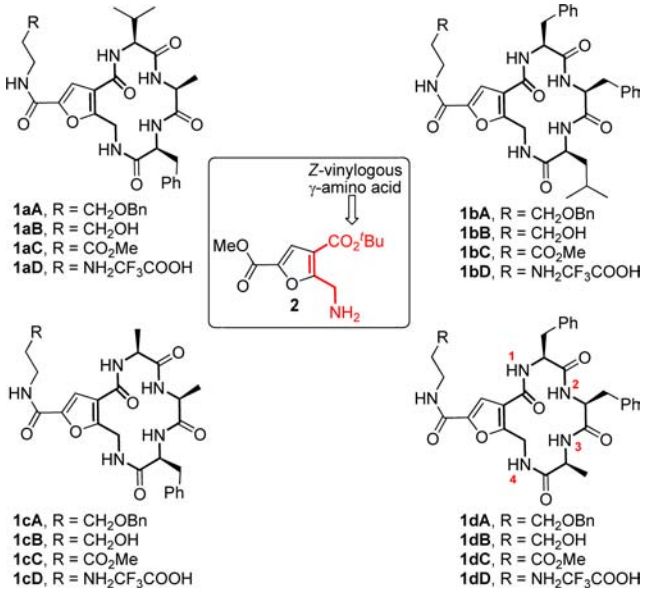
membered classical α_4 CTP, and it would also circumvent the associated limitations of classical α_4 CTP, i.e., conformational heterogeneity, metabolic instability;[17] (5) the integrated additional carboxylic acid could be used for any postcyclization modification. Herein, we describe synthesis and conformational analysis of water-stable highly robust CTPs with $\alpha_3\gamma$ architecture (Scheme 1).

Scheme 1. Vinylogous Amino Acid-Based CTPs

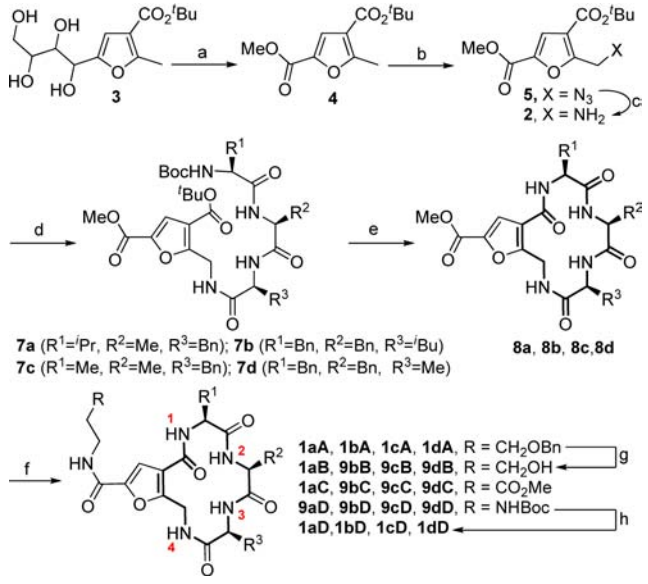


Syntheses of **2** and CTPs are described in Scheme 2. The vicinal 1,2 diol of **3**[18] was oxidatively cleaved to furnish aldehyde, which was oxidized to carboxylic acid and esterified as methyl ester **4** using diazomethane in 75% yield over three steps. Allylic bromination of **4** using *N*-bromo succinimide followed by azidation by NaN_3 under heating condition delivered azido diester **5** in 68% overall yield. The furan amino ester derivative **2** was obtained in a quantitative yield from **5** upon catalytic hydrogenation and coupled with the carboxylic acids derivatives **6a**–**d** using EDCI and HOBt as coupling reagent in dichloromethane to furnish the linear tetrapeptides **7a**–**d** in 75–85% overall yield. TFA-mediated one-pot deprotection of *tert*-butyl ester and Boc in DCM from **7a**–**d** followed by intramolecular cyclization in acetonitrile using pentafluorophenyl diphenylphosphinate (FDPP)/DIPEA under dilute condition (0.5×10^{-2} M) furnished the CTPs **8a**–**d**, having $\alpha 3\gamma$ architecture in 52–60% overall yield. To determine the conformational preferences of the CTPs in nonaqueous and aqueous medium various hydrophobic and hydrophilic side chains were attached to synthesize **1aA**–**dD**.

In CD spectroscopy, the electronic transition of the backbone amide of a peptide is expected to occur in the far-UV region, and delightfully, the CD spectra of **1aB**–**1dB** showed absorption maxima at 222 and 208 nm and a large minimum at 195 nm, characteristic of a helical conformation.[19] In addition, the absorption minimum observed at 258 nm (near-UV region) is possibly due to the electronic transition of the aromatic groups (furan and phenyl).[20]

Encouraged by the characteristic CD spectrum of a helical peptide, we analyzed the conformational preferences of the CTPs using NMR spectroscopic techniques. Sharp and well resolved amide NH signals of the CTPs suggested the presence

Scheme 2. Synthesis of Cyclic Peptidomimetics[a]



[a]Reagents and conditions: (a) (i) $NaIO_4$, MeOH:H_2O (1:1), 0 °C to rt, 3 h, (ii) $NaClO_2$, NaH_2PO_4, H_2O_2, CH_3CN, 0 °C to rt, 3 h, (iii) CH_2N_2, diethyl ether, 75% over three steps; (b) (i) NBS, AIBN, CCl_4, reflux, 2 h, (ii) NaN_3, DMF, 65 °C, 30 min, 68% over two steps; (c) H_2, Pd/C, ethyl acetate, quantitative; (d) **6a**/**6b**/**6c**/**6d**, 1-hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI), diisopropylethylamine (DIPEA), DCM, then **2**, 0 °C to rt, 8 h, 75–85%; (e) (i) TFA:DCM (1:1), 0 °C to rt, 3 h, (ii) FDPP, DIPEA, CH_3CN, −5 °C to rt, 60–72 h, 52–60%; (f) (i) LiOH, THF:MeOH:H_2O, (ii) HOBt, EDCI, DCM, $RCH_2CH_2NH_2$, DIPEA, 71–79%; (g) H_2, Pd/C, MeOH, quantitative; (h) (i) TFA:DCM (1:1), 0 °C to rt, 1 h, quantitative; **6a** = Boc-Val-Ala-Phe-OH; **6b** = Boc-Phe-Phe-Leu-OH; **6a** = Boc-Ala-Ala-Phe-OH; **6b** = Boc-Phe-Phe-Ala-OH.

of predominant well-defined folded structures in aqueous and aprotic (DMSO-d_6) solvents.[20] Variable-temperature (VT) ^{1}H NMR spectra of water-soluble CTPs (**1aD**, **1bD**, **1cD**, **1dD**) showed small changes in the amide NH chemical shift of Vaa ($\Delta\delta_{NH}/T$ −4.3 ppb/°C in **1dD**) and large changes that of other amide protons ($\Delta\delta_{NH}/T$ −7.1, −6.7, and −6.4 ppb/°C for ^{1}Phe, ^{2}Phe, ^{3}Ala, respectively, in **1dD**), indicating the involvement of ^{4}VaaNH in the H-bonding (Figure 2A).[20,21] Further the invariance of the amide NH chemical shifts observed in the concentration-dependent-aggregation studies clearly suggested the participation of ^{4}VaaNH in the intramolecular H-bonding (Figure 2B).[16] Additional support for the intramolecular H-bonding of VaaNH appeared from hydrogen/deuterium exchange experiment. ^{1}H NMR spectra of **1dD** in

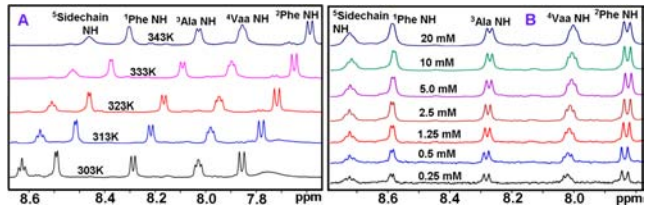


Figure 2. VT and concentration-dependent-aggregation studies. (A) VT ^{1}H NMR spectra of amide protons of **1dD** in H_2O:D_2O (9:1). (B) Concentration independence of amide proton chemical shifts of **1dD** in H_2O:D_2O (9:1).

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H_2O:D_2O (5:1) showed a slower rate of exchange of ^{4}VaaNH and ^{2}PheNH than the ^{1}PheNH, ^{3}AlaNH, and side chain amide protons.[20]

Being short peptide, the characteristic NOE correlations of an α-helical peptide [e.g., (i)CαH ↔ (i+3)NH, (i)CαH ↔ (i+4)NH] were absent. However, characteristic NOEs observed in the ROESY spectra of these CTPs between VaaNH ↔ (i+3)VaaNH, VaaNH ↔ (i+2)VaaNH, and (i+1)VaaNH ↔ (i+2)VaaNH suggested the close proximity of the amide protons in the preferred single conformation (Figure 3A). Absence of

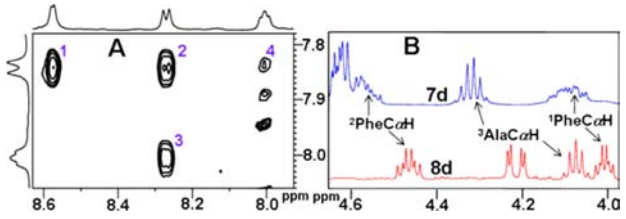

Figure 3. (A) ROESY spectrum of **1dD** in H_2O:D_2O (9:1) where NOEs between ^{1}PheNH ↔ ^{2}PheNH, ^{2}PheNH ↔ ^{3}AlaNH, ^{3}AlaNH ↔ ^{4}VaaNH, and ^{4}VaaNH ↔ ^{2}PheNH are marked as **1**, **2**, **3**, and **4**, respectively. (B) Comparison of δ(CαH) region of cyclic peptide (**8d**) and linear peptide (**7d**).

NOE between any two consecutive CαH protons suggested the nonexistence of *cis*-amide bond. Importantly, the δ(CαH) of all the residues in CTPs displayed an upfield chemical shift (0.08, 0.11, and 0.24 ppm for ^{1}Phe, ^{2}Phe, and ^{3}Ala, respectively, in **8D**) relative to the corresponding residues in linear peptides, thus supporting the fact that these CTPs folded into helical conformation (Figure 3B).[22]

The $^3J_{NH\text{-}C\alpha H}$ values of (i+1)Vaa, (i+2)Vaa, and (i+3)Vaa residues (~4.5, ~9.0, and ~8.0 Hz, respectively) of the water-soluble CTPs obtained from the ^{1}H NMR spectra suggested that the CTPs have nonidentical backbone dihedral angles (ϕ) (Table 1), which implied that the CTPs do not adopt the α-

Table 1. Dihedral Angles of (i+1)Vaa, (i+2)Vaa, (i+3)Vaa Calculated from $^3J_{NH\text{-}C\alpha H}$ Values

CTP	ϕ(i+1)	ϕ(i+2)	ϕ(i+3)	d(Å) C(i)–C(i+3)	d(Å) O(i)–N(i+4)
1aD	−60 ± 5	−115 ± 5	−110 ± 5	4.88	2.88
1bD	−65 ± 5	−110 ± 10	−110 ± 5	4.94	2.86
1cD	−65 ± 5	−110 ± 10	−105 ± 10	4.84	2.89
1dD	−55 ± 5	−100 ± 5	−100 ± 10	4.94	2.90

turn conformation of an α-helix. On the other hand, the ϕ(i+1) values of all the CTPs lie within the α-helical region of the Ramachandran plot.[14,23] Solution structural calculations of these CTPs were carried out using the ROESY spectral data acquired in H_2O:D_2O (9:1) at 300 K. Both distance and torsional angle values were applied as constraints in the restrained molecular dynamics (MD) studies and carried out in Accelrys Discover studio (3.0) using CHARMm force field.[24] The 20 lowest energy structures of **1(a–d)D** are superimposed, and the ensemble is presented in Figure 4. These 20 structures converged very well, suggesting the rigidity in their conformational preferences. Therefore, considering the CD and VT ^{1}H NMR spectroscopic properties, distance between O(i)–N(i+4) (<3.5 Å) and (ϕ) values, it is suggested that the CTPs are stabilized by a 13-membered H-bond between VaaCO (i) and

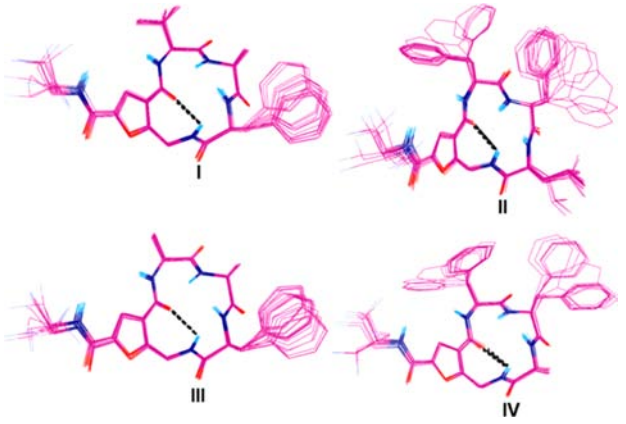

Figure 4. Structure ensemble of CTPs in H_2O:D_2O (9:1) superimposed 20 lowest energy structures of **1aD** (**I**), **1bD** (**II**), **1cD** (**III**), and **1dD** (**IV**). The H-bond is shown as dotted lines, and for clarity all protons are removed except for amides.

VaaNH (i+4), characteristic of a tight α-turn. Further, the ϕ values calculated from the CTPs fit very well with the region defined by Ramachandran plot of the type I-α_{RS} found in proteins and previously described by Chou et al.[4c]

In aprotic solvent, these CTPs displayed almost identical coupling constant values, NOE correlations. However, the VT studies[15] revealed that both VaaNH and (i+2)VaaNH were H-bonded. The 20 lowest energy structures of **1aB–1dB** are superimposed and presented in Figure 5. This suggested that all

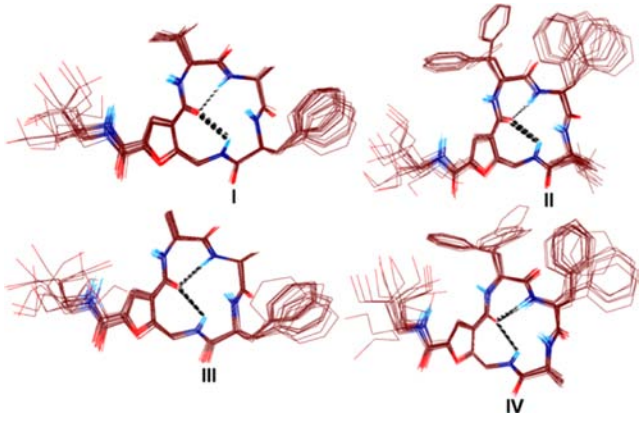

Figure 5. Structure ensemble of CTPs in DMSO-d_6. Superimposed 20 lowest energy structures of **1aB** (**I**), **1bB** (**II**), **1cB** (**III**) and **1dB** (**IV**). The H-bond is shown as dotted lines, and for clarity all protons are removed except for amides.

the CTPs possess similar type of secondary structure as observed in aqueous solution. However, an additional H bonding between (i)CO and (i+2)NH was also observed, leading to a reverse γ-turn structure.

In conclusion, reconstruction of protein α-turn was accomplished using small molecule-based peptidomimetic. The template Vaa with its rigid frame and intraresidual H-bonding propensity dictated the CTP to mimic the most widely occurring tight α-turn of type I-α_{RS}. Importantly, the structure is stable in both aqueous and aprotic solvents and does not depend on the sequence of inner three residues, which implied that the stability of the secondary structure is governed by the template of Vaa. Consequently, the liberty to alter the side-chains of the amino acids and scope to introduce a variety of

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functionalities on the Vaa side chain highlight the usefulness of this scaffold and could be utilized as α-turn mimetic for the inhibition of protein−protein interaction.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and characterization data of all new compounds, 2D-ROESY spectra for the cyclic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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