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Hybrid Peptides: Direct Transformation of α/α, β-Unsaturated γ-Hybrid Peptides to α/γ-Hybrid Peptide 12-Helices

Anupam Bandyopadhyay and Hosahudya N. Gopi*

Department of Chemistry, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pashan, Pune-411008, India

hn.gopi@iiserpune.ac.in

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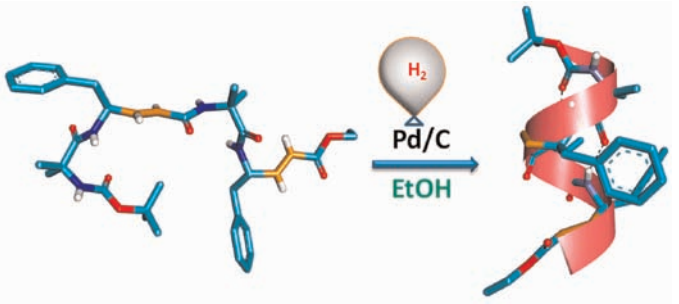
ABSTRACT



A smooth transformation of unusual planar structures of α/vinylogous hybrid peptides to ordered α/γ^4-hybrid peptide 12-helices and the stereochemical preferences of vinylogous amino acid residues in single crystals are studied.

The design of peptides containing non-natural amino acids that can mimic natural protein secondary structures is a challenging task for chemists and biochemists. A good deal of success has been achieved in this regard using the oligomers of β- and γ-amino acids or mixed sequences containing α/β, α/γ, and β/γ-hybrid peptides.[1] Various ordered helical conformations of γ-and mixed α/γ-hybrid peptides containing 3,3-dialkyl-γ-amino acids (Gpn, gabapentin),[1e,2] cyclic γ-amino acids,[3] β-aminooxy acids,[4] carbo-γ-amino acids,[5] and 2,3,4-substituted γ-amino acids[6] have been reported. However, the progress of γ^4-peptides (oligomers of double homologated α-amino acids) lags behind that of β- and other γ-peptides, due in part to the difficulty of obtaining stereochemically pure γ^4-amino acids.[7] Nonetheless, Seebach and colleagues[8] and Hanessian et al.[9] in their pioneering work recognized the 14-helical conformations of the oligomers of γ^4-amino acids. In addition, recently Hofmann and colleagues predicted the wide range of helical organizations from the

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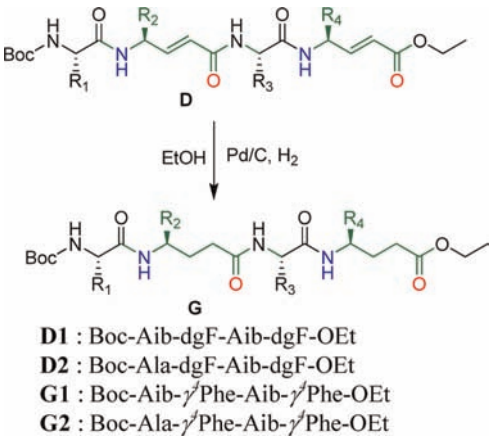
Scheme 1. Direct Transformation of α/Vinylogous Hybrid Peptides (D) to α/γ^4-Hybrid Peptides (G)[a]



[a] The sequences of α/vinylogous hybrid peptides (**D1** and **D2**) and α/γ^4-hybrid peptides (**G1** and **G2**) are shown.

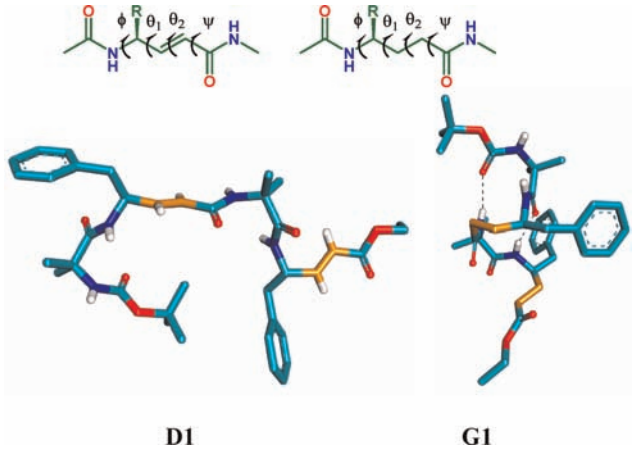


Figure 1. X-ray structures of hybrid α-vinylogous peptide (**D1**) and α/γ^4-hybrid peptide (**G1**). Local torsional variables of γ-residues are shown at the top.

oligomers of unsubstituted γ-amino acids, α,β-unsaturated γ-amino acids and 1:1 heterooligomers of α/γ-amino acids using ab initio theoretical calculations.[10] Here we report the unusual planar structures of the α/vinylogous hybrid peptides Boc-Aib-dgF-Aib-dgF-OEt (**D1**, dgF = α,β- dehydro γ^4-phenylalanine) and Boc-Ala-dgF-Aib-dgF- OEt (**D2**) and their direct transformation to ordered α/γ^4-hybrid peptide 12-helices, Boc-Aib-γ^4Phe-Aib-γ^4Phe-OEt (**G1**) and Boc-Ala-γ^4Phe-Aib-γ^4Phe-OEt (**G2**), respectively, using catalytic hydrogenation.

Vinylogous amino acids (insertion of −CH═CH− between C_αH and CO, α,β-unsaturated-γ-amino acids) have been frequently found in many peptide natural products.[11] The structures of α,β-unsaturated γ-amino acids and their oligomers have little explored. Nevertheless, Schreiber and colleagues[12] and others[13] reported the β-sheet, β-turn, and unusual helical type of structures for the peptides containing vinylogous amino acids. Recently, we reported the utility of *E*-vinylogous amino acids in the construction of a stable and functionalizable hybrid β-hairpin.[14] In continuation of our studies in the design of hybrid peptides containing *E*-vinylogous amino acids, we designed the peptides **D1** and **D2** to understand the behavior of vinylogous amino acids in the presence of conformationally constrained, helix favoring Aib residues[15] and to transform into their saturated γ-peptide analogues (Scheme 1). Further, this approach also provides an unprecedented opportunity to analyze and understand the conformational preferences of both α,β-unsaturated and saturated γ^4-amino acids in oligopeptides. The *E*-vinylogous amino acid dgF [(*S*,*E*)-4-amino-5-phenylpent-2-enoic acid] was synthesized using the Wittig reaction starting from *N*-Boc-(*S*)-phenylalanal.[16] Peptides **D1** and **D2** were synthesized in the solution-phase method using Boc chemistry.

We began our analysis with **D1**; the ^{1}H NMR shows the wide dispersion of NH and vinylic protons. Surprisingly, the 2D NMR (ROESY) analysis reveals that no characteristic intramolecular NOEs corresponding to either the helix, sheet or reverse turn conformations (see the Supporting Information), indicating no secondary structure in the hybrid peptide. Similarly, the solution structure analysis of **D2** suggests no regular secondary structures. Further, we were able to get single crystals of **D1** from the slow evaporation of methanol/toluene solution, and its X-ray structure is shown in Figure 1. Interestingly, **D1** adopted an unusual planar structure in crystalline state and as anticipated did not show any protein secondary structural properties. No intramolecular H-bonding is observed in the crystal structure. Examination of the torsional angles of helix favoring Aib residues reveal that both residues adopted opposite right and left handed helical conformations with the ϕ and ψ values −51, −48, and 64 and 52, respectively. The local conformations of the vinylogous residues were determined by introducing additional torsional variables θ_1 (N−C_γ−C_β═C_α) and θ_2 (C_γ−C_β═C_α−C) as shown in Figure 1. The vinylogous residue dgF2 adopted a fully extended conformation by having the torsional angles $\phi = -139$, $\theta_1 = 121$, $\theta_2 = 178$, and $\psi = -161°$. Interestingly, another vinylogous residue, dgF4 adopted N−C_γ−C_β═C_α

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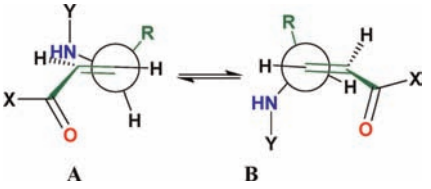
Table 1. Backbone Torsional Variables of α/*E*-Vinylogous and α/γ^4-Hybrid Peptides

peptide	residue	ϕ	θ_1	θ_2	ψ
D1	dgF2	−139	121	178	−161
	dgF4	−81	12	180	−168
G1	γPhe2	−127 ± 6	50 ± 3	62.5 ± 0.5	−117 ± 7
	γPhe4	−104 ± 3	61 ± 2	−175 ± 3	−155 ± 7
G2	γPhe2	−123	51	63	−123
	γPhe4	−113	57	−177	−130
G3	γPhe2	−117	49	68	−125
	γPhe4	−111	43	56	−137

Scheme 2. Stable Eclipsed Conformers of Vinylogous Residues Observed in the Crystal Structures



eclipsed conformation by having θ_1 close to 0°. Further, the torsional angle ϕ adopted a semiextended conformation with the value −81° and the other torsional variables θ_2 and ψ of dgF4 showed the extended conformations. The torsional angles of vinylogous residues are tabulated in Table 1. In addition, the local *s-cis* conformations (ψ = ∼180°) of the conjugated ester (dgF4) and the amide (dgF2), favoring the extended planar structure.

Surprised by the unusual planar crystal structure of **D1**, we sought to analyze the conformations adopted by the vinylogous residues in crystal structures. A careful analysis of the crystal structures of *E*-vinylogous residues reported earlier[12,14,16] and from the present work revealing very intriguing results. From the analysis of total twenty one units of vinylogous residues in the single crystals of both monomers and peptides (see ESI), two lowest energy eclipsed conformations are observed (Scheme 2). The N−C_γ−C_β=C_α eclipsed conformation **A** (θ_1 = 0°) is normally observed in the vinylogous esters, while H−C_γ−C_β=C_α eclipsed conformation **B** (θ_1 = ± 120°) is observed in the vinylogous amides. In all the cases, θ_1 takes the value either close to 0 or ±120°. The conjugated carbonyl functional group mostly adapted local *s-cis* conformation (ψ = ∼±180°) compared to the *s-trans* conformation (ψ = ∼±0°). Results from the analysis reveal that vinylogous residues (amides) prefer the extended conformations and may serve as ideal candidates for the construction of β-sheet structures.[14]

We further investigated the possibilities of transforming a hybrid unsaturated peptide to its saturated analogue using catalytic hydrogenation. The pure peptide **D1** was subjected to catalytic hydrogenation using 20% Pd/C in ethanol. The reaction was monitored by MALDI-TOF and HPLC. The complete conversion of peptide **D1** to **G1**

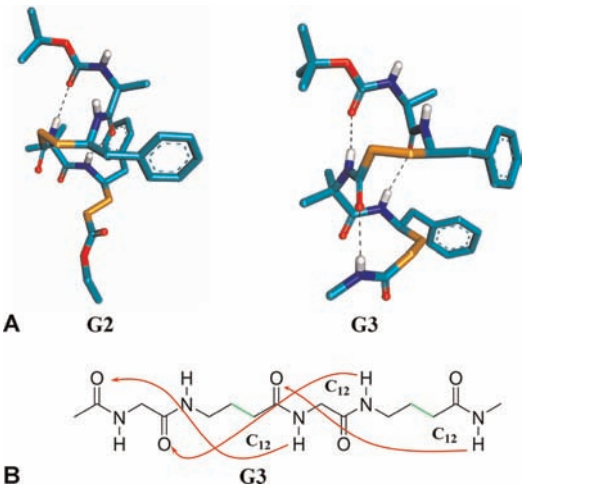


Figure 2. (A) X-ray structures of **G2** and **G3** (Boc-Ala-γ^4Phe-Aib-γ^4Phe-CONHMe). (B) Backward 12-membered H-bonds observed in the crystal structures is depicted in the extended model **G3**.

was achieved within six hours and the pure α/γ^4-hybrid peptide was isolated in 95% yield. Fully assigned ^{1}H NMR (amide and vinylic region) before and after the catalytic hydrogenation of peptide **D1** is shown in the Supporting Information. Single crystals of α/γ^4-hybrid peptide **G1** obtained from the slow evaporation of ethanol solution yield the structure shown in Figure 1. Four molecules of peptide **G1** were observed in the asymmetric unit with slight variation in the torsional values. Instructively, the peptide adopted a right-handed helical conformation in the crystalline state. Moreover, Hofmann and co-workers have predicted the formation of 12-helix [12-atom ring H-bonds of C=O(i)···H−N(*i*+3)] by 1:1 alternative α and unsubstituted γ-residues using ab initio MO theory.[10c] In complementary work, Balaram and colleagues[2] and Gellman et al.[3] showed the formation of 12-helix in the α/γ-hybrid peptides containing 3, 3-dialkyl γ-amino acids and cyclic γ-amino acids, respectively. Indeed peptide **G1** adopted a 12-helix conformation in single crystals. The analysis of the crystal structure reveals that the two cooperative twelve membered H-bonds C=O(Boc)···NH(3) [C=O···H−N dist. 2.064 Å, O···N dist. 2.920 Å and ∠O···H−N 172°] and C=O(1)···NH(4) [C=O···H−N dist. 1.984 Å, O···N dist. 2.819 Å and ∠O···H−N 163°] stabilizing the helical conformation.

The transformation of **D1** to **G1** represents an interesting example of nonfolded to H-bond guided folded state of peptides. Inspired by the 12-helical conformation of **G1**, we further subjected **D2** to the catalytic hydrogenation to give saturated peptide **G2**. The crystal conformations of **G2** is shown in Figure 2A. The crystal structure analysis suggests that similar to **G1,** peptide **G2** also adopted 12-helical conformation in single crystals. The stereochemical analysis of γ^4-residues in both **G1** and **G2** reveal that γ^4Phe2 adopted *gauche*$^+$, *gauche*$^+$ (g^+, g^+, $\theta_1 \approx \theta_2 \approx$ 60°) local conformation about the C_β−C_γ and C_α−C_β

bonds, while the C-terminal γ^4Phe4 residues displayed *gauche*$^+$ ($C_\beta-C_\gamma$), *anti* ($C_\alpha-C_\beta$) conformation due to the lack of terminal H-bond donor (NH). However, a weak 10 membered C−H···O interaction between the acidic α-hydrogens of γ^4Phe4 and the C=O of γ^4Phe2 (1←3) is observed. We anticipate that helix favoring g^+, g^+ conformation can be induced in the terminal γ^4-amino acids by introducing H-bond donor (NH) at the C-terminus. Peptide **G3** (Boc-Ala-γ^4Phe-Aib-γ^4Phe-CONHMe) was synthesized from **G2** to evaluate the hypothesis. The treatment of aqueous methyl amine to the *N*-hydroxy succinimidyl ester of **G2** leads to the formation of **G3** and its X-ray structure is shown in Figure 2A. As anticipated, the terminal γ^4-residue adopted g^+, g^+conformation and accommodated into the helix. The helical structure is stabilized by three consecutive intramolecular backward C_{12} H-bonds (Figure 2B). The H-bonding between the 1←4 residues in all α/γ^4 hybrid peptides indicating the backbone expanded version of a 3_{10}-helix. The H-bond parameters of all the peptides (**G1-G3**) are tabulated in the Supporting Information. In contrast to peptide **D1**, α-residues in **G1-G3** showed the right handed helical conformations by having average ϕ and ψ values -58 ± 3 and $-39 \pm 5°$, respectively. The dihedral angles of γ^4-residues were measured by introducing two additional variables θ_1 ($N-C_\gamma-C_\beta-C_\alpha$) and θ_2 ($C_\gamma-C_\beta-C_\alpha-C$) (Figure 1) and are given in the Table 1. The circular dichroism spectra of all C_{12}-helical peptides (**G1**−**G3**) along with their unsaturated analogues **D1** and **D2** are shown in Figure 3. Similar to the β-peptide 12-helices, α/γ^4-hybrid peptide 12-helices also showed CD maxima at ~205 nm and a weak minima at 218 nm.[17] The facile transformation and the structural analysis of α/γ-hybrid peptides containing backbone homologated γ^4-amino acids presented here may be useful in the design of functional foldamers similar to the α/β-hybrid peptides.[1c]

In conclusion, we presented the stereochemical analysis of hybrid α/α,β-unsaturated γ-peptides and their smooth transformation to α/γ^4-hybrid peptides. The crystal structure analyses of vinylogous residues reveal the two lowest energy eclipsed conformations, which restrict the rotations around $C_\gamma-C_\beta$ and $C_\alpha-C=O$ bonds. The presence of geometrical constrains of the double bonds in the hybrid vinylogous peptides forced the molecules to adopt unusual planar conformations. After the geometrical constrains of the double bonds were released, the H-bond strength overrode the conformational flexibility of the saturated γ^4-amino acids to accommodate into the helical conformations. The conformational analysis of the hybrid peptides presented here may provide basic information for the structure-based designs with specific functions.

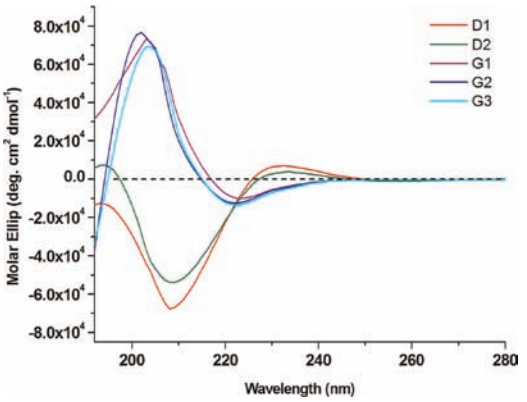


Figure 3. CD spectra of α/vinylogous hybrid peptides and α/γ^4 hybrid peptide 12-helices in MeOH ($c = 200\ \mu$M).

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Supporting Information Available. Experimental procedures, compound characterization, and crystallographic information. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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