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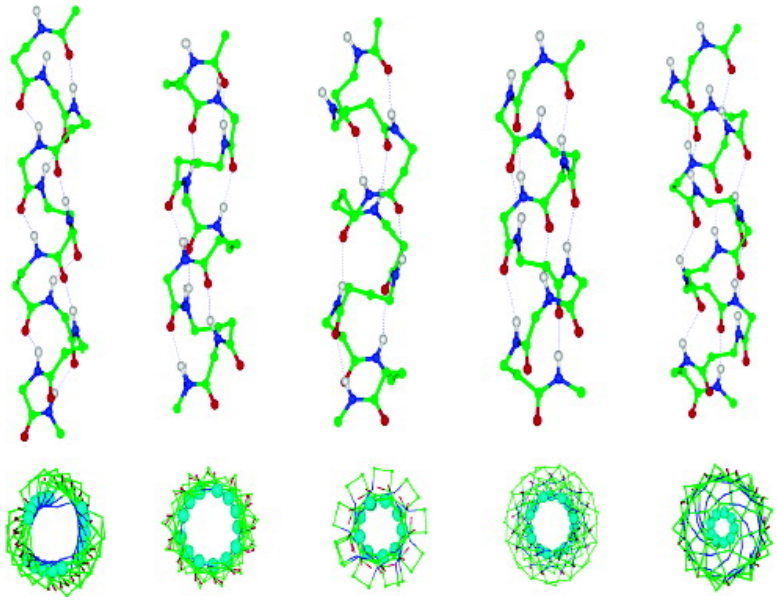

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Polypeptide Helices in Hybrid Peptide Sequences

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Abstract: A new class of polypeptide helices in hybrid sequences containing α-, *β*-, and *γ*-residues is described. The molecular conformations in crystals determined for the synthetic peptides Boc-Leu-Phe-Val-Aib-*β*Phe-Leu-Phe-Val-OMe **1** (*β*Phe: (*S*)-*β*[3]-homophenylalanine) and Boc-Aib-Gpn-Aib-Gpn-OMe **2** (Gpn: 1-(aminomethyl)cyclohexaneacetic acid) reveal expanded helical turns in the hybrid sequences $(\alpha\alpha\beta)_n$ and $(\alpha\gamma)_n$. In **1**, a repetitive helical structure composed of C_{14} hydrogen-bonded units is observed, whereas **2** provides an example of a repetitive C_{12} hydrogen-bonded structure. Using experimentally determined backbone torsion angles for the hydrogen-bonded units formed by hybrid sequences, we have generated energetically favorable hybrid helices. Conformational parameters are provided for C_{11}, C_{12}, C_{13}, C_{14}, and C_{15} helices in hybrid sequences.

Introduction

Structural diversity in protein structures is generated by using two different secondary structural elements, helices and extended strands. Variations in the mode of arrangement of the constituent structural units lead to a remarkable range of three-dimensional arrangements or folds. Essentially, two types of helical structures are found in proteins, the classical nonintegral Pauling α-(3.6_{13})-helix[1] and the integral 3_{10}-helix.[2] The original identification of stable helical structures for polypeptides was based on explicit considerations of chemistry, hydrogen bonding between backbone amide donors and acceptors and planarity of the peptide unit. Subsequent enumeration of the possible hydrogen-bonded structures of polypeptide chains was greatly facilitated by the use of two backbone torsional variables (ϕ and ψ) for the two degrees of freedom at each α-amino acid residue.[3] More recently, helical structures have emerged as optimally packed arrangements of compact strings, in a theoretical analysis which does not consider explicit chemical details.[4] The search for novel helical folds in polypeptides has been stimulated by the observation of two unique classes of helices in poly *β*-peptides, the 12-helix, and the 14-helix.[5−8] In principle, expansion of the polypeptide backbone by insertion of additional atoms can enhance the repertoire of intramolecularly hydrogen-bonded structures. We describe in this report new families of polypeptide helices in hybrid sequences containing α-, *β*-, and *γ*-residues.[9]

Conventional helices formed in polypeptides composed of α-amino acids are characterized by a pattern of repetitive hydrogen-bonded rings containing 10 atoms (C_{10}, 3_{10}-helix) and 13 atoms (C_{13}, α-helix) (Figure 1). A characteristic of these helical structures is the repetition of backbone torsion angles (ϕ and ψ) along the polypeptide chain (for 3_{10}-helix, $\phi = -49.3°$ and $\psi = -25.7°$ and for α-helix, $\phi = -57.8°$ and $\psi = -47°$ for right-handed structures formed by L-amino acids;[3] the values based on the analysis of the crystal structures of peptides and proteins[10] are 3_{10}-helix $\phi = -57°$ and $\psi = -30°$ and α-helix $\phi = -63°$ and $\psi = -42°$). When the hydrogen-bonded units are considered as the repeating feature, the two types of helices may be represented by the notations $(\alpha\alpha)_n$ and $(\alpha\alpha\alpha)_n$, which denote the number of residues determining the hydrogen-bonded turn. The more recently discovered helices in oligo *β*-peptides are formed by repeating *ββ* segments, with the 12- and 14-helices corresponding to different hydrogen-bonded directionalities.[5−8] The 12-helix hydrogen-bonded unit may be considered as a formal expansion of the parent αα unit, namely, 3_{10}-helix, by insertion of two additional backbone atoms (Figure 1). In hybrid sequences, the use of higher *ω*-amino acids permits insertion of additional atoms into the hydrogen-bonded units, which constitute defined helical folds. For example, an $(\alpha\beta)_n$ sequence can in principle generate a pattern of successive C_{11}

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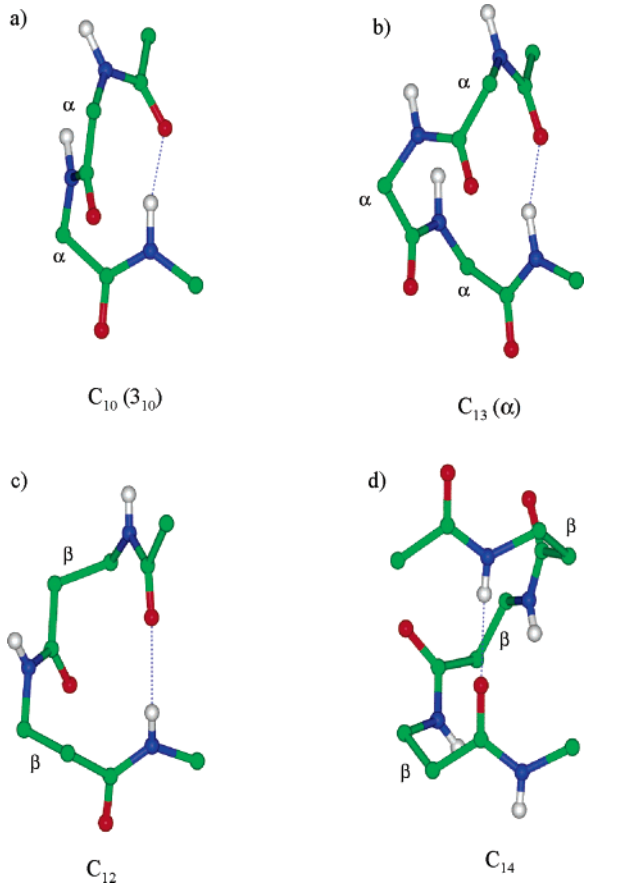

Figure 1. Schematic view of hydrogen-bonded rings formed by repeating two and three residues. (a) C_{10}, 3_{10}-helical turn in an αα segment. (b) C_{13}, α-helical turn in an ααα segment. (c) C_{12} helical turn in a 12-helix generated by a $\beta\beta$ segment. (d) C_{14} helical turn in a 14-helix generated by a $\beta\beta\beta$ segment. (a) and (b) are generated by using standard torsion angles, whereas (c) and (d) are generated by using torsion angles given in ref 7.

hydrogen bonds, constituting an expanded 3_{10}-helix. Similarly, an $(\alpha\alpha\beta)_n$ sequence may permit generation of a repetitive C_{14} hydrogen-bonding pattern, which results in an expanded analogue of the α-helix. The search for such structures is facilitated by the structural characterization of hybrid oligopeptides, as illustrated in the study described below.

The generation of synthetic peptide helices is promoted by the incorporation of stereochemically constrained residues, which are limited to local folded conformations. For example, the α,α-dialkylated residue α-aminoisobutyric acid, Aib (Figure 2), is largely restricted to backbone conformations, which correspond to 3_{10}- or α-helical structures.[10−13] Substitution at the backbone carbon atoms restricts the range of accessible conformations in the case of amino acid residues. Gabapentin (Gpn) is an achiral β,β-disubstituted γ-amino acid residue, which possesses four degrees of torsional freedom, with the energetically favorable conformations about the $C^{\gamma}-C^{\beta}$ and $C^{\beta}-C^{\alpha}$ bonds being restricted to the *gauche* form because of substituent effects.[14,15] In generating synthetic sequences, the monosubstituted β-residue, (S)-β^3-homophenylalanine (βPhe), and the unsubstituted γ-residue, γ-aminobutyric acid (γAbu), have also been used. The molecular conformations of the oligopeptides Boc-Leu-Phe-Val-Aib-βPhe-Leu-Phe-Val-OMe (**1**) and Boc-Aib-Gpn-Aib-Gpn-OMe (**2**) provide examples of repetitive C_{14} hydrogen-bonded structures formed by an $(\alpha\alpha\beta)_n$ segment and a repetitive C_{12} hydrogen-bond pattern generated in an $(\alpha\gamma)_n$ sequence, respectively. These structures, together with the conformational parameters determined for hybrid segments containing β- and γ-residues, permit the construction of models for hybrid helices.

Table 1. Crystal and Diffraction Parameters for Peptide **1** and Peptide **2**

	peptide **1**	peptide **2**
empirical formula	$C_{60}H_{88}N_8O_{11}$	$C_{32}H_{56}N_4O_7$
cryst habitat	clear plate	thin plate
cryst size (mm)	0.50 × 0.17 × 0.09	0.2 × 0.06 × 0.002
crystallizing solvent	methanol/water	methanol/water
space group	$P2_12_12_1$	$P2_1/c$
cell params		
a (Å)	12.36(13)	13.73(18)
b (Å)	18.94(2)	15.37(2)
c (Å)	27.12(3)	17.49(2)
α (deg)	90	90
β (deg)	90	101.3(3)
γ (deg)	90	90
V (Å^3)	6352.0(12)	3619.3(8)
Z	4	4
molecules/asymmetric unit	1	1
cocrystallized solvent	none	none
mol wt	1097.4	608.8
D (g/cm^3) (calcd)	1.148	1.117
F(000)	2368	1328
radiation (λ = 0.71073 Å)	Mo Kα	Mo Kα
T (°C)	20	20
2θ max (deg)	54.1	46.5
scan type	ω scan	ω scan
measured reflns	49368	22166
independent reflns	12666	5191
unique reflns	7167	5191
obsd reflns [$Fo \geq 4\sigma(\|Fo\|)$]	3706	3425
R_{int}	0.1474	0.0635
final R1 (%)	6.25	7.58
final wR2 (%)	12.74	16.07
GOF (S)	0.965	1.087
$\Delta\rho_{max}$ (e Å^{-3})	0.42	0.23
$\Delta\rho_{min}$ (e Å^{-3})	−0.21	−0.25
no. of restraints/params	6/712	0/388
data−param ratio	5.2:1	8.8: 1

Experimental Methods

Peptide Synthesis. Peptides **1** and **2** were synthesized by conventional solution phase procedures using the Boc group and the methoxy group for protecting N- and C-termini, respectively (Boc: *tert*-butyloxycarbonyl). Couplings were mediated by *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu).[16] Boc deprotection was carried out using formic acid, and saponification was used to remove the C-terminal methyl ester. The final step in the synthesis of **1** was a 3 + 5 coupling, whereas in the synthesis of **2**, a 2 + 2 coupling was used. The final peptides were purified by medium-pressure liquid chromatography on a reverse-phase C_{18} column; their homogeneity was established by HPLC and their identity was established by 500 MHz ^{1}H NMR spectroscopy and electrospray ionization mass spectrometry (M_{cal} = 1097.4 and M_{Na+} = 1120.1 for peptide **1**, and M_{cal} = 608.8 and M_{Na+} = 631.7 for peptide **2**). Gabapentin was obtained as a

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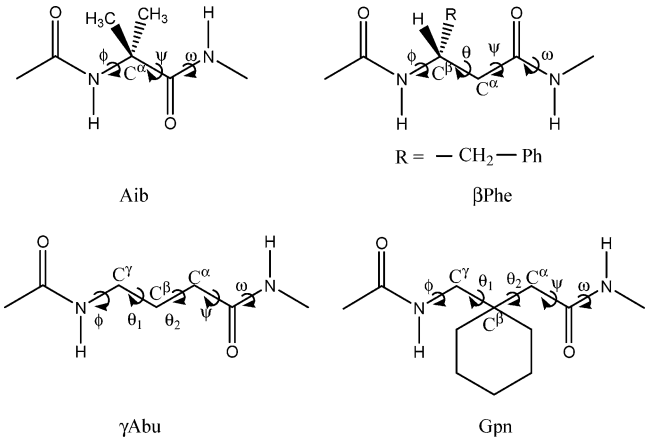

Figure 2. Chemical structures of nonstandard amino acids used in synthetic hybrid peptides.

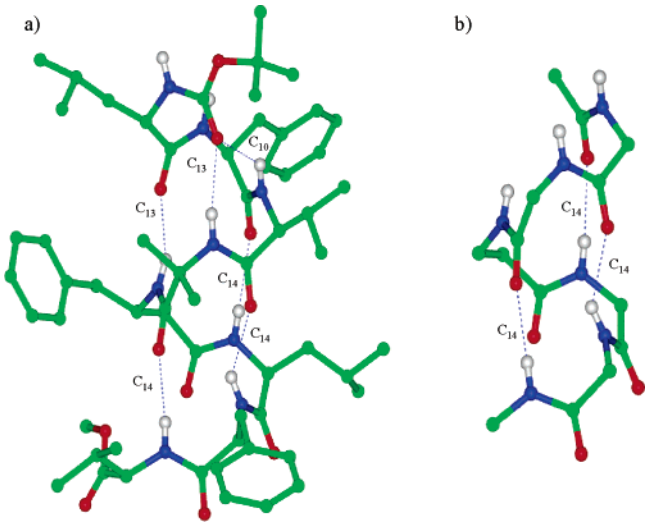

Figure 3. Three-dimensional structure of peptide **1** revealing an incipient C_{14} helix. (a) Molecular conformation of the octapeptide **1**, Boc-Leu-Phe-Val-Aib-*β*Phe-Leu-Phe-Val-OMe, determined in the solid state. (b) Expanded view of the αα*β*αα segment, which is stabilized by three successive C_{14} hydrogen bonds constituting a segment of a C_{14} helix.

Table 2. Backbone Torsion Angles (deg) for Peptide **1** and Peptide **2**[a]

	ϕ	θ_1	θ_2	ψ	ω
		Peptide **1**			
Leu (1)	−60.1			−28.4	179.3
Phe (2)	−60.6			−39.2	177.1
Val (3)	−76.6			−45.2	179.9
Aib (4)	−51.3			−49.4	−168.2
*β*Phe (5)	−122.4	81.0		−98.2	−176.5
Leu (6)	−62.4			−37.8	−171.0
Phe (7)	−86.7			−44.0	−172.1
Val (8)	−123.8			−41.9	175.9
		Peptide **2**			
Aib (1)	−59.8			−37.8	−175.7
Gpn (2)	−126.8	52.1	63.8	−107.9	−178.1
Aib (3)	−51.5			−48.8	−179.1
Gpn (4)	109.9	60.8	62.7	146.3	177.7

[a] See Figure 2 for torsion angle definitions. For Peptide **2**, the choice of signs is arbitrary and represents one enantiomeric form present in the centrosymmetric crystals. Estimated standard deviations are ≈0.5°.

gift from the Hikal R&D Centre (Bangalore, India), and *β*-phenylalanine was synthesized by standard procedures.[8]

Structure Determination. Single crystals of **1** and **2**, suitable for X-ray diffraction, were grown from a methanol/water mixture. **1** crystallized in the orthorhombic space group $P2_12_12_1$, and the achiral peptide **2** crystallized in the monoclinic, centrosymmetric space group $P2_1/c$. X-ray intensity data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer, using Mo Kα radiation (λ = 0.71073 Å). A ω-scan type was used. Structures of **1** and **2** were solved by direct methods of phase determination using the programs SHELXD[17] and SHELXS-97,[18] respectively. Both the structures were refined against F^2 by the full matrix least-squares method using SHELXL-97.[19] All the hydrogen atoms were fixed geometrically in the idealized positions and refined in the final cycle of refinement as riding over the atoms to which they are bonded. After the final refinement, the R factor (R1) was 6.25% for **1** and 7.58% for **2**. The crystal and diffraction parameters of **1** and **2** are summarized in Table 1.

Table 3. Hydrogen-Bond Parameters in Peptide **1** and Peptide **2**[a]

type	donor (D)	acceptor (A)	D···A (Å)	H···A (Å)	C=O···H (deg)	C=O···N (deg)	NH···O (deg)
			Peptide **1**				
			Intramolecular				
4→1	N3	O0	3.04	2.42	117.5	127.1	129.8
5→1	N4	O0	3.05	2.19	151.8	154.4	170.9
5→1	N5	O1	2.93	2.13	144.3	149.5	156.5
5→1	N6	O2	3.09	2.29	138.1	144.4	154.4
5→1	N7	O3	2.96	2.25	153.4	163.1	140.0
5→1	N8	O4	2.99	2.25	143.8	152.8	144.8
			Intermolecular				
	N1	O6[b]	2.89	2.06	144.0	147.7	163.9
	N2	O7[b]	2.91	2.07	144.3	146.6	166.0
			Peptide **2**				
			Intramolecular				
	N3	O0	2.93	2.11	140.9	144.6	159.6
	N4	O1	3.05	2.19	138.0	139.8	172.7
			Intermolecular				
	N1	O2[c]	2.88	2.04	156.0	159.6	167.7
	N2	O3[c]	3.03	2.27	148.8	149.4	148.6

[a] Estimated standard deviations in the hydrogen-bond lengths and angles are approximately 0.03 Å and 0.5°, respectively. [b] Symmetrically related by the relation ($-x + 3/2$, $-y$, $z - 1/2$). [c] Symmetrically related by the relation (x, $-y + 1/2$, $z + 1/2$).

Energy Minimization. All the polypeptide models were built in the model-builder module of INSIGHT II, using the crystallographically determined coordinates for the backbone atoms. Unsubstituted α-, *β*-, and *γ*-residues were used for model building, with the exception of the (Aib-Gpn)$_n$ copolymer, where a crystallographically determined fragment was repeated. The coordinates of the highlighted segment of Boc-Val-Ala-Phe-Aib-***β*Val-*β*Phe-Aib**-Val-Ala-Phe-Aib-OMe[20] were used to generate the C_{11} (βα) helix, Ac−(*β*Gly-Gly)$_4$−NHMe, and the C_{15} (α*ββ*) helix, Ac−(Gly-*β*Gly-*β*Gly)$_3$−NHMe. The C_{12} (*γ*α) helix, Ac−(*γ*Abu-Gly)$_4$−NHMe, and the C_{13} (*βγ*) helix, Ac−(*β*Gly-*γ*Abu)$_4$−NHMe, were generated using the highlighted segment of the peptide Boc-Leu-Aib-Val-***β*Gly-*γ*Abu-Leu**-Aib-Val-Ala-Leu-Aib-OMe.[21] The crystal structure of Boc-Leu-Phe-**Val-Aib-*β*Phe**-Leu-Phe-Val-OMe was used to generate the C_{14} helix, Ac−(Gly-Gly-*β*Gly)$_3$−NHMe. A model C_{12} helix, Ac−(Aib-Gpn)$_{10}$−NHMe, was also generated using the coordinates of the first two residues of the tetrapeptide, Boc-Aib-Gpn-Aib-Gpn-OMe. An unconstrained energy minimization was then carried out on the models using a dielectric constant of 1.0 and the AMBER

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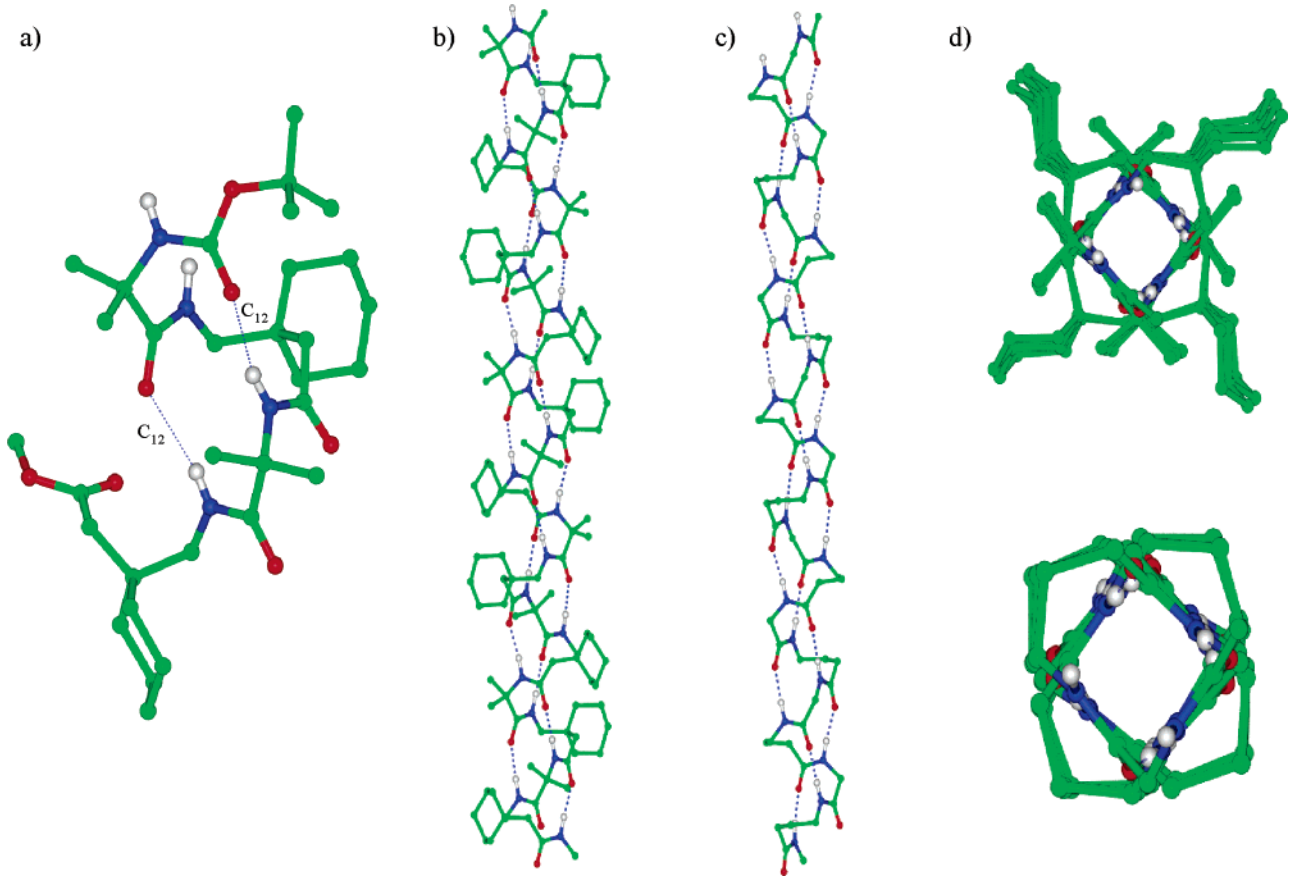

Figure 4. C_{12} helix in a repetitive $\alpha\gamma$ hybrid peptide. (a) Molecular conformation determined in the solid state for the peptide Boc-Aib-Gpn-Aib-Gpn-OMe (**2**). Two successive C_{12} hydrogen bonds stabilizing the helical fold. (b) Computer generated model for the polymer $(Aib\text{-}Gpn)_n$ illustrating a continuous C_{12} $\alpha\gamma$ helix. (c) View of the polypeptide backbone in the C_{12} helix. (d) View of the C_{12} helix viewed down the helix axis, illustrating the approximate 4-fold nature of the helix. Projection of the (Aib-Gpn) polymer (top). Projection of the C_{12} helix without any side chains (bottom).

force field, in the DISCOVER module of INSIGHT II. The steepest descent or conjugate gradient algorithms were used for minimization. The torsion angles for the central segment in the minimized models were considered as the ideal values of torsion angles for these hybrid helices. The minimization of the model peptide Ac−$(Aib\text{-}Gpn)_{10}$−NHMe generated using the idealized residues from the INSIGHT II library also yielded the same result.

Results and Discussion

Conformation of 1 and 2 in Crystals. Figure 3 shows the molecular conformation determined in crystals for the synthetic octapeptide, Boc-Leu-Phe-Val-Aib-βPhe-Leu-Phe-Val-OMe (**1**), which contains a single, centrally positioned βPhe residue. The molecule adopts a helical fold stabilized by six intramolecular hydrogen bonds: one is a C_{10} hydrogen bond, two are C_{13} hydrogen bonds, and three are C_{14} hydrogen bonds, where the subscripts refer to the number of atoms in the hydrogen-bonded rings. The carbonyl oxygen O0 of the Boc group is a double acceptor, taking part in C_{10} and C_{13} hydrogen bonds. Backbone torsion angles are listed in Table 2, and hydrogen-bond parameters are listed in Table 3. The expanded view of the three successive C_{14} hydrogen bonds formed by the peptide segment Val(3)-Aib(4)-βPhe(5)-Leu(6)-Phe(7) illustrates a C_{14} helical structure formed by the hybrid sequence $\alpha\alpha\beta\alpha\alpha$, where the Greek letters refer to the nature of the amino acid residue. For α-residues, each unit contributes three atoms to the peptide backbone (N, C^{α}, and C′), whereas β- and γ-residues contribute four and five atoms, respectively. A single C_{13} hydrogen bond, which is characteristic of the α-helix, is formed by the triplet $\alpha\alpha\alpha$. The repetitive C_{14} hydrogen-bonded structure characterized in the octapeptide constitutes a formal expansion of the α-helix, with the repetitive units now being represented as $(\alpha\alpha\beta)_n$.

Figure 4 shows the molecular conformation in crystals for the tetrapeptide Boc-Aib-Gpn-Aib-Gpn-OMe (**2**). Relevant torsion angles and hydrogen-bond parameters are summarized in Tables 2 and 3, respectively. The cyclohexane rings of both the Gpn residues adopt perfect chair conformation. However, in Gpn (2), the aminomethyl group orients equatorially to the cyclohexane ring, and it orients axially in Gpn (4). A notable feature of the structure is the observation of two successive C_{12} hydrogen-bonded turns, which results in the formation of an incipient C_{12} helix. Inspection of the backbone torsion angles in Table 2 reveals that both the Aib residues adopt helical ϕ and ψ values, with Gpn (2) occupying the $i + 2$ position of the first C_{12} turn and the $i + 1$ position of the second C_{12} turn. This structure represents an expansion of the consecutive β-turn or incipient 3_{10}-helical structure, previously characterized in all α-peptides.[22−24]

Crystal Packing. Figure 5 shows the views of crystal packing in peptides **1** and **2**. The parameters for intermolecular hydrogen

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Table 4. Parameters for Polypeptide Helices[a]

residue repeat	helix type	hydrogen-bond ring/residue	torsion angles (deg) α ϕ	α ψ	β/γ ϕ	β/γ θ_1	β/γ θ_2	β/γ ψ	n	*h* (Å)
α	3_{10}	10/αα	−49.3	−25.7					3.00	2.00
α	3.6_{13}	13/ααα	−57.8	−47.0					3.60	1.50
α	4.4_{16}	16/αααα	−57.1	−69.7					4.40	1.15
αβ	C_{11}	11/αβ[b]	−62.0	−44.0	−105.0	80.0		−73.0	3.15	3.80
αγ	C_{12}	12/αγ	−57.0	−53.0	−126.0	59.0	57.0	−99.0	4.30	4.00
ββ	C_{12}	12/ββ[c]			−95.0	94.3		−103.0	2.50	2.10
βγ	C_{13}	13/βγ			−106.0	75.0		−115.0	4.50	4.20
					−117.0	66.0	62.0	−120.0		
ααβ	C_{14}	14/ααβ	−61.0	−50.0	−114.0	74.0		−91.0	7.50	4.63
			−66.0	−48.0						
αββ	C_{15}	15/αββ	−72.0	−70.0	−101.0	71.0		−128.0	8.70	4.47
					−83.0	84.0		−100.0		

[a] Helices with the same hydrogen-bond directionality (C_i = O···H−N_{i+n}) such as the canonical α-peptide helices only are included in the table. [b] For a recent crystallographic characterization of an 11-helix in a short αβ peptide sequence, see ref 32. The torsional parameters calculated from the deposited coordinates are in good agreement with the idealized values for the 11-helix listed above. [c] Regular helical structures with reverse hydrogen-bond directionality have also been characterized in the β-homopolypeptides (cf. 14-helix).[33,34]

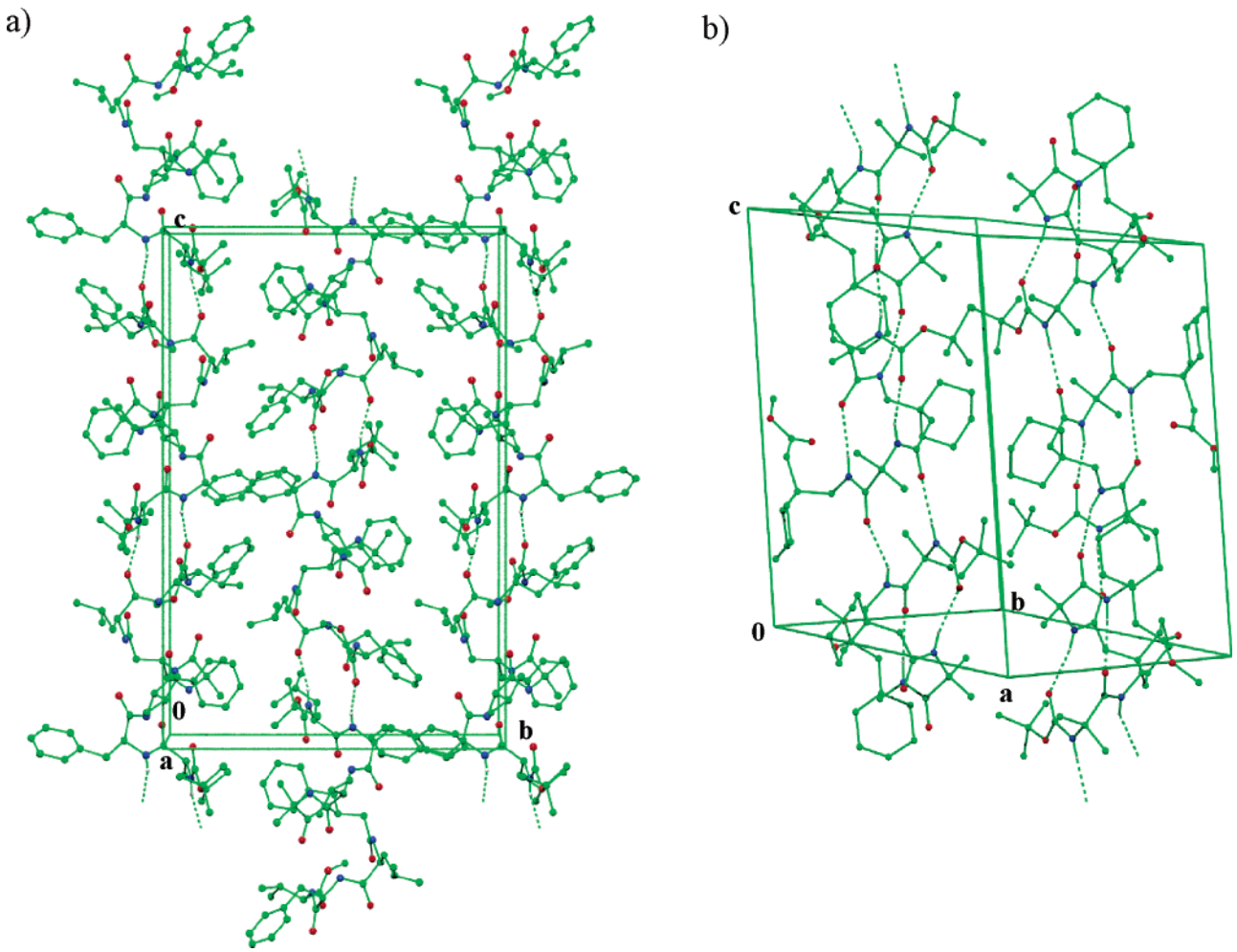

Figure 5. Molecular packing of peptides **1** and **2** in crystals. (a) **1**, Boc-Leu-Phe-Val-Aib-βPhe-Leu-Phe-Val-OMe, viewed down the crystallographic *a*-axis. Only intermolecular hydrogen bonds are shown. (b) **2**, Boc-Aib-Gpn-Aib-Gpn-OMe.

bonds are given in Table 3. Figure 5a shows the molecular packing in the crystals of peptide **1**, as viewed perpendicular to the helix axis. The helix aggregation into columns is achieved through two head−tail hydrogen bonds, N(1)···O(6) [$-x + 3/2$, $-y$, $z - 1/2$] and N(2)···O(7) [$-x + 3/2$, $-y$, $z - 1/2$]. The helical columns are arranged in pseudohexagonal grid fashion. The positioning of three aromatic residues along the sequence leads to favorable aromatic−aromatic interactions, which further stabilize the crystal packing. The centrally positioned βPhe residue is not involved in aromatic interactions.

Figure 5b shows the arrangement of molecules in the crystals of peptide **2**. The aggregation of helices into columns, which is a common packing motif in α-peptide helices, is observed in the crystals of peptide **2**, an α,γ-hybrid peptide helix. The helical

portions of the molecules are connected by head−tail hydrogen bonds, N(1)···O(2) [x, $-y + 1/2$, $z + 1/2$] and N(2)···O(3) [x, $-y + 1/2$, $z + 1/2$], such that the helices appear to be continuous. The helical columns extend along the crystallographic c-axis, with adjacent columns orienting in antiparallel fashion. Notably, the C-terminal residue Gpn (4) adopts a backbone conformation different from that of the Gpn (2) residue (Table 2), facilitating the formation of columns of helices. The Gpn (4) residue along with the C-terminal methyl ester group projects outward from the column, promoting favorable hydrophobic interactions.

Hybrid Helices. Reexamination of previously published crystal structures of hybrid peptides provides additional examples for the formation of novel helices containing α- and ω-amino acids. Figure 6a illustrates the expansion of a conventional α-peptide helix by insertion of a central $\beta\beta$ segment.[20] C_{11} and C_{15} hydrogen-bonded rings are formed by $\beta\alpha$ and $\alpha\beta\beta$ segments, respectively. The insertion of a $\beta\gamma$ segment into an 11-residue peptide (Figure 6c) reveals C_{14}, C_{13}, and C_{12} hydrogen-bonded rings formed by $\alpha\alpha\beta$, $\beta\gamma$, and $\gamma\alpha$ segments.[21] (The number of atoms in the hydrogen-bonded ring is obtained by adding 4 to the number of backbone atoms in the turn segment; e.g., $\alpha\gamma = 3 + 5 + 4$.)

The structures of the synthetic hybrid peptides provide information on geometrical parameters for β- and γ-residues and the range of torsion angles obtained in the analogues of conventional α-polypeptides. Models of hybrid helices can now be constructed by repeating the crystallographic units or by model building using idealized residue geometries. The (Aib-Gpn-Aib) unit may be repeated to generate an $\alpha\gamma$ C_{12} helix, which has approximately four residues per turn resulting in the 4-fold helix illustrated in Figure 4. Using the torsion angle parameters determined in the hybrid helical peptide, optimized structures are shown in Figure 7 for C_{11}, C_{12}, C_{13}, C_{14}, and C_{15} helices formed in hybrid sequences. The relevant torsional and helical parameters are listed in Table 4. All the helices depicted in Figure 7 have well-packed interiors, suggesting that these would be stable structures for polypeptide backbones. In the case of all α-peptides, helices with large hydrogen-bonded rings such as the 4.4_{16}-(π)- helix[25] are unstable and unobserved in polypeptides because of the development of a cavity in the helix interior, which diminishes the energetic contributions of favorable nonbonded interactions. However, isolated C_{16} turns occur in the Schellman motif found at the C-termini of helices in proteins and peptides.[26−28]

Conclusions

Helical polypeptide folding patterns are compatible with variations in the number of backbone atoms. Repetitive structures may be generated using dipeptide and tripeptide units as "residues" for helix generation. β- and γ-residues can also be incorporated into antiparallel strands in designed β-hairpin peptides.[29−31] Thus, the overall fold of the major secondary structural elements observed in proteins can be maintained even in hybrid sequences, suggesting that globular polypeptide structures may indeed be accessible for mixed sequences containing α-, β-, and γ-residues. The attributes of folding and

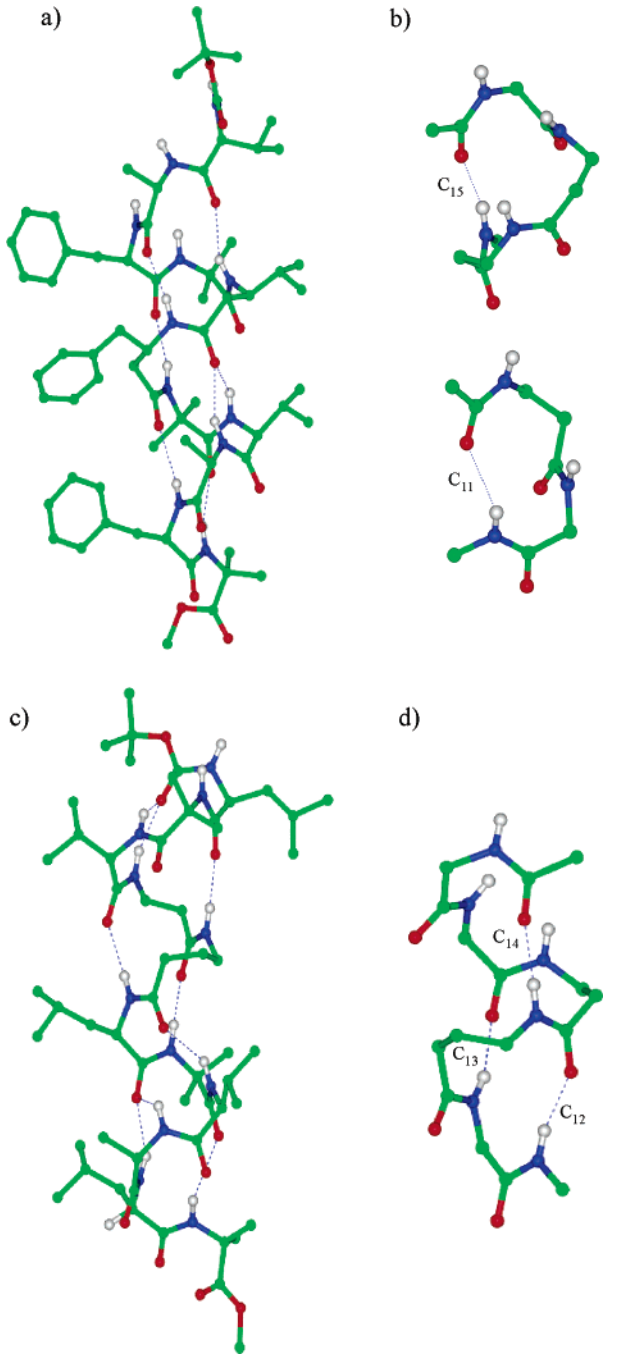

Figure 6. Expanded hydrogen-bonded helical turns observed in hybrid peptides. (a) Solid-state conformation of the peptide, Boc-Val-Ala-Phe-Aib-βVal-βPhe-Aib-Val-Ala-Phe-Aib-OMe.[20] (b) View of the C_{15} turn formed by an $\alpha\beta\beta$ segment (top) and of a C_{11} hydrogen bond formed by a $\beta\alpha$ segment (bottom). (c) Solid-state conformation of the 11-residue hybrid peptide, Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-Ala-Leu-Aib-OMe.[21] (d) Expanded view of residues 2−6 showing a successive pattern of C_{14}, C_{13}, and C_{12} hydrogen-bonded helical turns.

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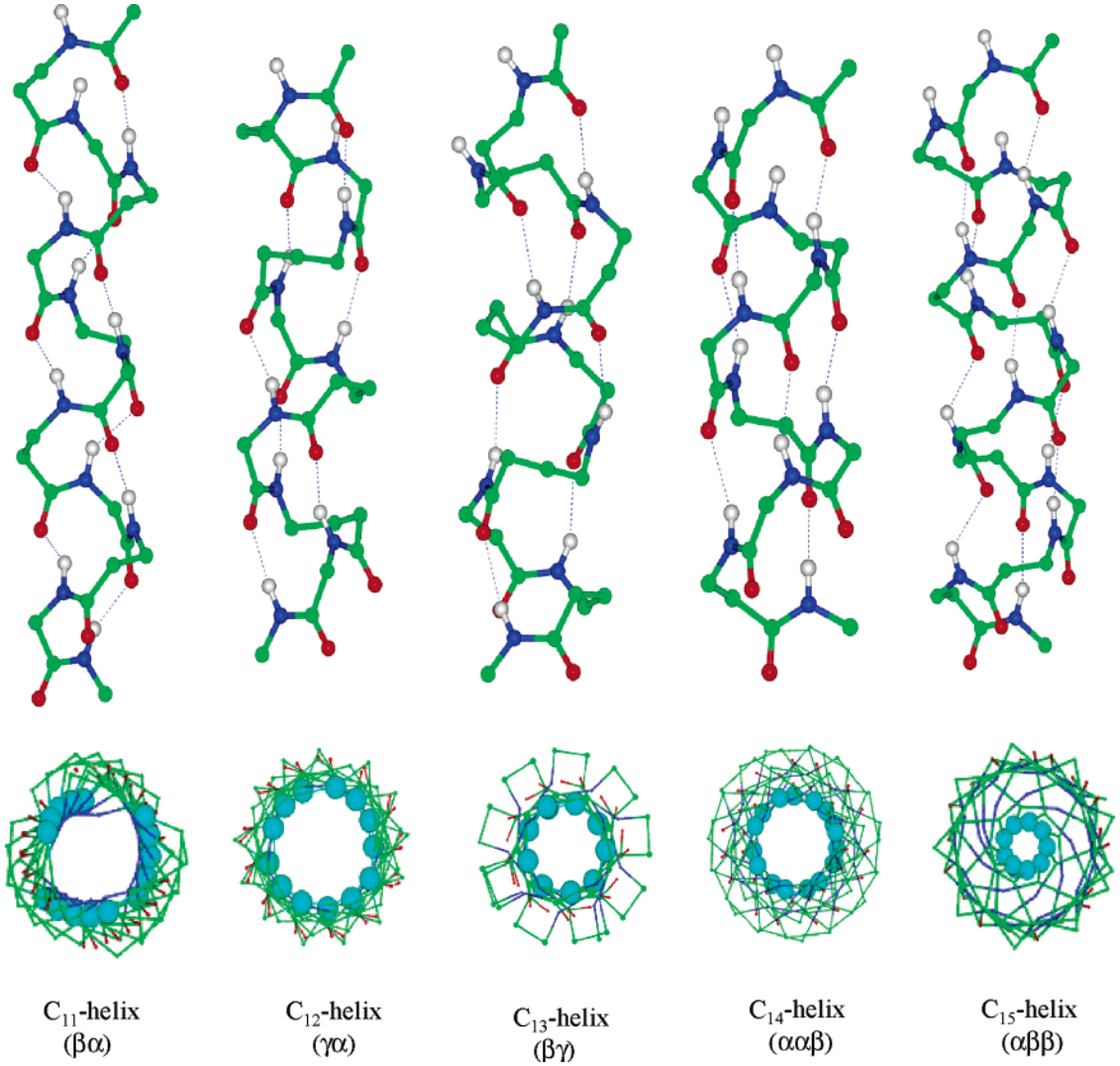

Figure 7. Computer-generated models of idealized hybrid helices with hydrogen-bonded ring sizes ranging from 11−15. Projection viewed perpendicular to the helix axis (top). Projection viewed down the helix axis (bottom). The shaded circles represent the atoms that form the inner core of the helix.

globularity are clearly not restricted to heteropolymers of α-amino acids. Hybrid polypeptides constitute a new class of heteropolymers in which the monomer units contribute different numbers of backbone atoms. The structure space of hybrid peptides appears to be considerably greater than that of their α-amino acid counterparts. Hybrid structures must necessarily be produced by chemical synthesis of designed sequences. The emerging design principles suggest that rational construction will be a practical possibility. Designed hybrid peptides also provide a direct route to proteolytically stable analogues of biologically active peptides, which largely retain the overall fold of the parent sequence.

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Supporting Information Available: X-ray crystallographic file for peptides **1** and **2** (CIF format). This material is available free of charge via the Internet at http://pubs.acs.org. The X-ray crystallographic files (CIF) have also been deposited with the Cambridge Structural Database with accession numbers 271599 (**1**) and 271598 (**2**).

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