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Nonstandard Amino Acids in Conformational Design of Peptides. Helical Structures in Crystals of 5−10 Residue Peptides Containing Dipropylglycine and Dibutylglycine

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Abstract: Nonstandard amino acids dipropylglycine (Dpg) and dibutylglycine (Dbg) have been incorporated into penta- to decapeptides in order to impose local restrictions on the polypeptide chain stereochemistry. In each case, the Dpg and Dbg residues showed similar helix-forming propensity as the Aib (α-aminoisobutyric) residue. Further, the 3_{10}- and α-helices containing the Dbg and Dpg residues had crystal packing motifs quite similar to those found for Aib-containing peptides. Crystal structure analyses are presented for Boc-Aib-Ala-Leu-Ala-Leu-Dpg-Leu-Ala-Leu-Aib-OMe (**I**), space group $P2_1$ with a = 11.313(3) Å, b = 28.756(5) Å, c = 11.884 Å, β = 103.74(1)°; Boc-Leu-Dpg-Leu-Ala-Leu-Aib-OMe, polymorph a (**IIa**), space group $P1$ with a = 10.205(5) Å, b = 10.996(5) Å, c = 21.393(9) Å, α = 81.92(3)°, β = 88.20(3)°, γ = 89.74(3)° and polymorph b (**IIb**), space group $P2_12_12_1$ with a = 9.291(1) Å, b =23.003(5) Å, c = 23.085(6) Å; and Boc-Leu-Dbg-Val-Ala-Leu-OMe (**III**), space group $P2_1$ with a = 9.907(2) Å, b = 16.078(3) Å, c = 13.543(3) Å, β = 104.48(2)°. The observation of helical conformations at all Dpg/Dbg residues is not entirely expected on the basis of conformational energy calculations and crystal structure observations on small homooligopeptides.

Introduction

The incorporation of nonstandard amino acids with well-defined stereochemical and functional properties can greatly enhance the scope of peptide design and protein engineering[1] by imposing local restrictions on polypeptide chain stereochemistry, thus conferring stability on specific regions of secondary structure. Further benefits may include enhanced binding to a molecular target, improved oral bioavailability, and longer

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persistence in circulation in the case of analogs of biologically active peptides. The strong helix-inducing properties of α-aminoisobutyric acid (Aib),[2] the first member of the series of α,α-di-*n*-alkylglycines, have been well documented by numerous crystal structure analyses of short peptides[3] that form 3_{10}-helices and longer peptides[4] that form mixed 3_{10}-/α-helices and α-helices. The systems studied include both model and synthetic sequences and naturally occurring peptides like alamethicin[5] and zervamicin,[6] some containing as few as one Aib residue per 10 standard residues.[4a]

Extensions of these studies to peptides containing cycloalkyl and linear alkyl side chains have been reported. For peptides containing 1-aminocycloalkane-1-carboxylic acids (Ac_nc, where *n* is the ring size), helical conformations have been observed at the Ac_nc residue, with the crystallographically characterized structures being restricted to relatively small peptides.[7] In the case of linear alkyl side chains, theoretical calculations[8] for α,α-diethylglycine (Deg) and α,α-di-*n*-propylglycine (Dpg) suggest that *fully extended* (C_5) conformations ($\phi \sim \psi \sim 180°$,) are energetically favored over helical conformations ($\phi \sim \pm 60°$, $\psi \pm 30°$).[9] Indeed, early crystal structure analyses performed on the homopeptides Tfa-$(Deg)_n$-OtBu and Tfa-$(Dpg)_n$-OtBu, with $n = 5$ or less, showed fully extended backbones ($\phi \sim \psi \sim 180°$).[10] Incorporation of a Dpg residue into a biologically active chemotactic tripeptide sequence, *N*-formyl-Met-Dpg-Phe-OMe, also yielded a fully extended backbone at the Dpg residue in crystals.[11] More recently, the structure determination of Tfa-$(Dpg)_3$-dibenzylhydrazide provided an example of all three Dpg residues adopting helical backbone conformations, resulting in a type III β-turn structure centered at residues 1 and 2.[12] Crystals of the tripeptide Boc-Leu-Dpg-Val-OMe contained two molecules in the asymmetric unit, one occurring in an extended conformation at Dpg ($\phi = 176°$, $\psi = 190°$) while the other adopted a conformation in the helical region ($\phi = 62°$, $\psi = 40°$) at this residue.[13]

In order to clarify the stereochemical preferences of α,α-dialkyl residues with linear alkyl side chains and to explore the context dependence of local residue conformations, we have undertaken a systematic analysis of the crystal structures of oligopeptides incorporating Dpg and Dbg residues. We describe in this report the conformations in crystals of three peptides ranging in length from 5 to 10 residues:

Boc-Aib-Ala-Leu-Ala-Leu-*Dpg*-Leu-Ala-Leu-Aib-OMe **(I)**

Boc -Leu-*Dpg*-Leu-Ala-Leu-Aib-OMe **(II)**

Boc -Leu-*Dbg*-Val-Ala-Leu -OMe **(III)**

The Dpg and Dbg peptides are heteropeptides (in contrast to Deg and Dpg homopeptides, which have been studied earlier[8,10]) and include Ala, Val, and Leu, nonpolar residues with side chains of varying length, and Aib. These types of residues are common in membrane-active, naturally occurring peptides. The decapeptide **I** was designed as an analog of Aib containing helices previously studied.[17b] The central Aib was replaced by Dpg to evaluate the effect of this residue on the peptide helix. Peptide **II** is a synthetic fragment of **I**. Peptide **III** is a related Dbg sequence. As established earlier, the precise positioning of Val, Ala, and Leu residues exerts only minimal structural influences in Aib peptides. Peptides **I**–**III** are therefore good models for investigating the stereochemistry of Dpg/Dbg-containing sequences. Crystal packing and sequence effects are likely to exert minimal influences in *long* peptides containing α,α-dialkylated residues.

(2) Abbreviations used: Aib, α-aminoisobutyric acid; Ac_nc, 1-aminocycloalkanecarboxylic acid with *n* atoms in cycloalkane ring; Deg, α,α-diethylglycine; Dpg, α,α-di-*n*-propylglycine; Dbg, α,α-di-*n*-butylglycine; Boc, *tert*-butyloxycarbonyl; Tfa, trifluoroacetyl. All chiral amino acids are of the L-configuration.

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Table 1. Crystal and Diffraction Parameters[a]

	I	IIa	IIb	III
empirical formula	$C_{55}H_{100}N_{10}O_{13}$	$C_{39}H_{72}N_6O_9$	$C_{39}H_{72}N_6O_9$	$C_{36}H_{72}N_5O_8$
cocrystallized solvent	$2C_3H_7OH$		H_2O	
crystal habit	irregular plate[b]	colorless plate	elongated prism[c]	prism
crystal size (mm)	0.65 × 0.08 × 1.00	0.75 × 1.0 × 0.10		0.30 × 0.32 × 0.50
crystallizing solvent	$^iPrOH/H_2O$	$MeOH/H_2O$	CH_3CN/H_2O	$MeOH/H_2O$
space group	$P2_1$	$P1$	$P2_12_12_1$	$P2_1$
a (Å)	11.313(2)	10.205(3)	9.291(2)	9.907(2)
b (Å)	28.756(5)	10.996(5)	23.003(5)	16.078(3)
c (Å)	11.884(2)	21.393(9)	23.085(6)	13.543(3)
α (deg)	90	81.92(3)	90	90
β (deg)	103.74(1)	88.20(3)	90	104.48(2)
γ (deg)	90	89.74(3)	90	90
volume (Å^3)	3755.4	2375.5	4933.75	2088.7
Z	2	2	4	2
molecules/asymmetric unit	1	2	1	1
mol wt	1109.48 + 120.19	769.05	769.05 + 18.02	703.01
density (g/cm^3) (calcd)	1.087	1.075	1.059	1.118
F(000)	1344	840	1720	774
temperature (°C)	−50	−50	20	−50
independent reflcns	5035	6347	3523	2759
obsd reflcns ($\|F\| > 3\sigma(F)$)	4170	4248	1745	2638
final *R* (obsd data)	7.7	12.3	10.8	5.8
data/parameter	5:36:1.0	4.90:10	4.07:1.0	6.00:1.0

[a] For all the crystals, Cu Kα radiation (λ = 1.541 78 Å) was used with a $\theta/2\theta$ scan, a scan speed of 14 deg/min (except for **IIa**), a scan width of 2.0° ± $2\theta(\alpha_1 - \alpha_2)$, and $2\theta_{max}$ = 112° (resolution 0.93 Å, except for crystal **IIb**, where half of the data were measured at less than 3σ, mostly at the higher 2θ values). [b] Inclusions were present in the crystal. [c] The crystal was dried in air for ~3 months. It had lost ~400 Å in volume, and the *c* axis decreased by ~2 Å from the original measurements on a fresh crystal which was unstable either at room or low temperature.

Three determinations have been made of the conformation(s) of peptide **II**, since two different crystal forms (space grops P_1 and $P2_12_12_1$) were obtained from different solvents and the triclinic form had two independent molecules in the cell. Despite the different crystalline environments, peptide **II** has nearly the same conformation in each. Peptides **I**−**III** differ not only in length and choice of Dpg or Dbg but also in the Aib content, ranging from two in **I** to none in **III**. The presence or absence of Aib allowed an evaluation of the propensity of the strong helix-forming property of Aib as opposed to the suggested backbone extension property of Dpg or Dbg. Actually all three peptides folded into a 3_{10}- or α-helix.

Experimental Procedures

The peptides were synthesized by conventional solution-phase procedures as described elsewhere for Aib peptides[14] and purified by HPLC on a reverse phase C_{18} (10 μm) column using methanol−water gradient. Crystals were grown by slow evaporation from organic solvent/H_2O mixtures as listed in Table 1. X-ray diffraction data were obtained readily from crystals of **I** and **III** on an automated four-circle diffractometer. Crystals of **II**, for which two polymorphs were obtained, presented difficulties for obtaining data. Each polymorph was unstable in the X-ray beam both at room temperature and at −50 °C. For the triclinic polymorph **IIa**, a "best" set of data was obtained with a scan of 29 deg/min at −50 °C. There was no solvent in the cell. For the orthorhombic polymorph **IIb**, a "best" set of data was obtained at room temperature after the crystal had dried in air for 3 months. During that time the cell parameters changed by Δa = +0.1 Å, Δb = −0.1 Å, Δc = −1.97 Å, and ΔV = −391 Å^3. The large change in volume can correspond to a loss of three H_2O molecules per asymmetric unit or one molecule each of H_2O and CH_3CN. The refined structure still retained one H_2O molecule per asymmetric unit.

Ironically, the crystal structure of **IIa**, with the largest number of atoms (two molecules per asymmetric unit) and the poorest data, was solved most readily, probably because direct phase determination for noncentrosymmetric space groups works best for space group $P1$. Structures for **I** and **IIb** were obtained by the location of a fragment of known geometry (the backbone and C^β atoms of **IIa**) by integrated Patterson, packing, and direct phasing methods in the PATSEE program. Peptide **III** was solved by direct phase determination. Full-matrix anisotropic least-squares refinement was performed on the C, N, and O atoms. In the final stages of refinement, H atoms were added in idealized positions and allowed to ride with the C or N atom to which each was bonded. The *R* factors for crystals **IIa** and **IIb**, Table 1, are high owing to the instability of the hexapeptide crystals. The instability has been reflected mostly in the thermal parameters of individual atoms rather than in the bond lengths and folding of the molecule, as can be ascertained by comparing the hexapeptide molecules with peptides **I** and **III**. Fractional coordinates for **I**, **IIa**, **IIb**, and **III** are available in the supplementary material. Conformational angles are listed in Table 2 and hydrogen bond parameters in Table 3.

Results and Discussion

Peptide Molecules. The backbones in **I**, **IIa**(1 and 2), **IIb**, and **III** all fold into a helix as shown in Figures 1 and 2. The decapeptide **I** is predominantly an α-helix with two 3_{10}-type hydrogen bonds at the N-terminus. The three independent molecules of the hexapeptide **II** form 3_{10}-helices, and the pentapeptide **III** forms a helix that is near a 3_{10}-type. The change from a 3_{10}-helix for the short peptides to an α-helix for the decapeptide is consistent with earlier observations.[4a] A graphical comparison of the torsional angles ϕ (about N−C^α) and ψ (abut C^α−C′) is shown in Figure 3, where the sequences in **II** and **III** have been shifted to the right so that the residues correspond to those in **I**. The top of each bar corresponds to the ψ value, and the bottom corresponds to the ϕ value. In each case, distortions from ideal ϕ and ψ values for helices increase toward the C-terminus, although the backbones remain helical and acceptable hydrogen bonds are formed. The Dpg and Dbg residues have ϕ and ψ values comparable to those for Aib residues and have the least variation from idealized values. An argument could be proposed for the Aib residues being

Table 2. Torsional Angles[a,b] in Peptides I, II, and III

angle		I	IIa(1)	IIa(2)	IIb	III
Aib	ϕ	−58[c]				
	ψ	−39				
	ω	−176				
Ala	ϕ	−60				
	ψ	−26				
	ω	175				
Leu	ϕ	−65				
	ψ	−41				
	ω	175				
Ala	ϕ	−61				
	ψ	−42				
	ω	177				
Leu	ϕ	−60	−64[c]	−56[c]	−45[c]	−59[c]
	ψ	−49	−33	−42	−46	−31
	ω	−177	−177	−169	−170	−173
Dpg (Dbg)[d]	ϕ	−56	−55	−53	−43	−59
	ψ	−47	−43	−43	−37	−25
	ω	−178	−173	−168	−178	−179
Leu (Val)[d]	ϕ	−64	−70	−68	−51	−92
	ψ	−38	−20	−26	−31	−2
	ω	179	174	175	−177	180
Ala	ϕ	−64	−70	−72	−56	−102
	ψ	−28	−20	−10	−30	−21
	ω	−175	−176	174	−176	−179
Leu	ϕ	−110	−104	−110	−77	−120
	ψ	1	−44	−35	−17	−48[e]
	ω	−173	−165	−172	−177	−178[f]
Aib	ϕ	−48	+45	+51	+56	
	ψ	−43[e]	+47[e]	+35[e]	−153[e]	
	ω	179[f]	178[f]	−174[f]	−178[f]	
		Side Chains				
Leu	χ^1	180				
	χ^2	−176				
		61				
	κ_L[g]	168				
Leu	χ^1	−176	−178	−177	179	−176
	χ^2	−169	−169	−169	−168	−175
		69	63	77	62	62
	k_L	177	−179	177	−176	176
Dpg (Dbg)[d]	χ^{1L}	178	−178	176	69	66
	χ^{2L}	−170	−172	−173	160	174
	χ^{3L}					−169
	κ_L	−171	−166	−164	−171	−177
	χ^{1D}	60	67	66	55	54
	χ^{2D}	−163	−178	−172	−173	−175
	χ^{3D}					−66
	κ_D	65	68	63	75	62
Leu (Val)[d]	χ^1	−69	−73	−83	77	−58
						−69
	χ^2	−68	−178	178	−158	
		168	51	71	79	
	k_L	173	165	166	173	143
Leu	χ^1	−58	−66	−60	−67	−61
	χ^2	−52	−51	−30	−71	−59
		−174	−179	−176	167	178
	k_L	126	135	130	161	118

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, ω) and about the bonds in the amino acid side chains (χ) are described in IUPAC−IUB Commission on Biochemical Nomenclature.[9] The values are in degrees. [b] The estimated standard deviations are ~1.5° for **I** and **III**, ~2° for **IIa**, and ~3° for **IIb**. [c] Initial C′(0)N(1)C$^\alpha$(1)C′(1), where 0 refers to the Boc group. [d] The residues in parentheses refer to peptide **III**. [e,f] The terminal torsional angles N(i)C$^\alpha$(i)C′(i)O(OMe) (*e*) and C$^\alpha$(i)C′(i)O(OMe)C(OMe) (*f*). [g] The κ_L and κ_D torsional angles, C′(i − 1)N(i)C$^\alpha$(i)C$^\beta$(i), distinguish the chirality between the L-hand ($\kappa \sim 180°$) and the D-hand ($\kappa \sim 60°$) in the helix. Near the C-terminal, where the helices begin to fray, the κ_L values (bottom line of Table IV) are near 130°.

responsible for the helix promotion; however, the number of Aib residues is two, one, and none in peptides **I**, **II**, and **III**, and peptide **III** still forms a helix despite its short length. Hence the Dbg residue must be responsible for promoting the helix.

Table 3. Hydrogen Bonds in Helices

type	donor	acceptor	N···O or O···O, Å	H···O, Å[a]	angle (deg) C=O···N
		Peptide **I**			
head-to-solvent	N(1)	O(2s)[b,c]	2.871	2.15	
head-to-tail	N(2)	O(8)[c]	2.910	2.22	
4 → 1	N(3)	O(0)	2.872	2.05	136
4 → 1, transition	N(4)	O(1)	2.934	2.37	122
5 → 1	N(5)	O(1)	3.184	2.25	160
	N(6)	O(2)	2.924	2.01	149
	N(7)	O(3)	2.967	2.03	152
	N(8)	O(4)	3.075	2.17	151
	N(9)	O(5)	3.152	2.41	159
	N(10)	O(6)	2.874	2.46	164
peptide-to-solvent	O(1s)[b]	O(7)[d]	2.761		
	O(2s)[b]	O(9)	2.713		
		Peptides **IIa(1)** and **IIa(2)**			
head-to-tail	N(1)	O(4)[e]	2.918	2.05	128
	N(1s)	O(4s)[f]	2.903	2.00	132
	N(2)	O(5)[e]	3.086	2.27	145
	N(2s)	O(5s)[f]	3.038	2.23	145
4 → 1	N(3)	O(0)	3.020	2.26	137
	N(3s)	O(0s)	3.016	2.22	134
	N(4)	O(1)	2.952	2.15	127
	N(4s)	O(1s)	2.969	2.22	121
	N(5)	O(2)	3.112	2.24	116
	N(5s)	O(2s)	3.072	2.17	116
5 → 1	N(6)	O(2)	2.972	2.11	162
	N(6s)	O(2s)	2.977	2.11	159
		Peptide **IIb**			
head-to-tail	N(1)	O(4)[g]	2.823	1.90	146
head-to-solvent	N(2)	W(1)[h]	2.945	2.01	
4 → 1	N(3)	O(0)	3.194	2.29	133
	N(4)	O(1)	2.944	2.02	137
	N(5)	O(2)	3.113	2.19	128
	N(6)	O(3)	3.041	2.13	121
	W(1)	O(5)[i]	2.855		
	W(1)	O(4)[j]	2.944		
		Peptide **III**			
head-to-tail	N(1)	O(4)[k]	2.982	2.58	134
	N(2)	O(4)[k]	3.001	2.19	142
4 → 1	N(3)	O(0)	*3.372*	2.45	120
	N(4)	O(1)	2.867	1.96	126
5 → 1	N(5)	O(1)	*3.333*	*2.56*	169

[a] The H atoms have been placed in idealized position with an N−H bond length of 0.96 Å. Carbonyl oxygens O(10) in peptide **I**, O(3) and O(6) in peptide **IIa**, O(6) in peptide **IIb**, and O(2), O(3), and O(5) in peptide **III** do not participate in hydrogen bonding. [b] Hydroxyl oxygen from 2-propanol. [c] Symmetry equivalent 1 − x, 1/2 + y, −z to coordinates listed in supplementary material. [d] Symmetry equivalent x, y, 1 + z. [e] Symmetry equivalent x, 1 + y, z. [f] Symmetry equivalent x, −1 + y, z. [g] Symmetry equivalent 1/2 − x, −y, −1/2 + z. [h] Symmetry equivalent −1/2 + x, 1/2 − y, 1 − z. [i] Symmetry equivalent 2 − x, 1/2 + y, −3/2 − z. [j] Symmetry equivalent 1 + x, y, z.

The τ angles about the C$^\alpha$ atom in peptide residues deviate from tetrahedral values. Toniolo et al.[16] observed that, for an Aib residue in a right-handed 3_{10}-helix, the bond angles N−C$^\alpha$−C$^\beta$ (D) and C′−C$^\alpha$−C$^\beta$ (D) become larger than the tetrahedral value of 109.5°, and the angles N−C$^\alpha$−C$^\beta$ (L) and C′−C$^\alpha$−C$^\beta$ (L) become smaller than the tetrahedral value (where L and D distinguish by handedness the two methyl groups in Aib). For the peptides reported in this paper, the six τ angles for each C$^\alpha$ atom in a dialkyl residue are shown in Table 4. The above observation holds for all the Aib values except the pair marked by the footnote *e* and all the Dpg or Dbg values except those in molecule **IIb**. Also, the τ angle N−C$^\alpha$−C′ has been implicated in the choice between extended or helical conformations: "helical structures are favored by standard values (111°) of the N−C$^\alpha$−C′ angle, while fully extended conformations are favored by smaller values of the same angle, as experimentally observed, for instance, in the case of α,α-di-*n*-propylglycyl".[8b]

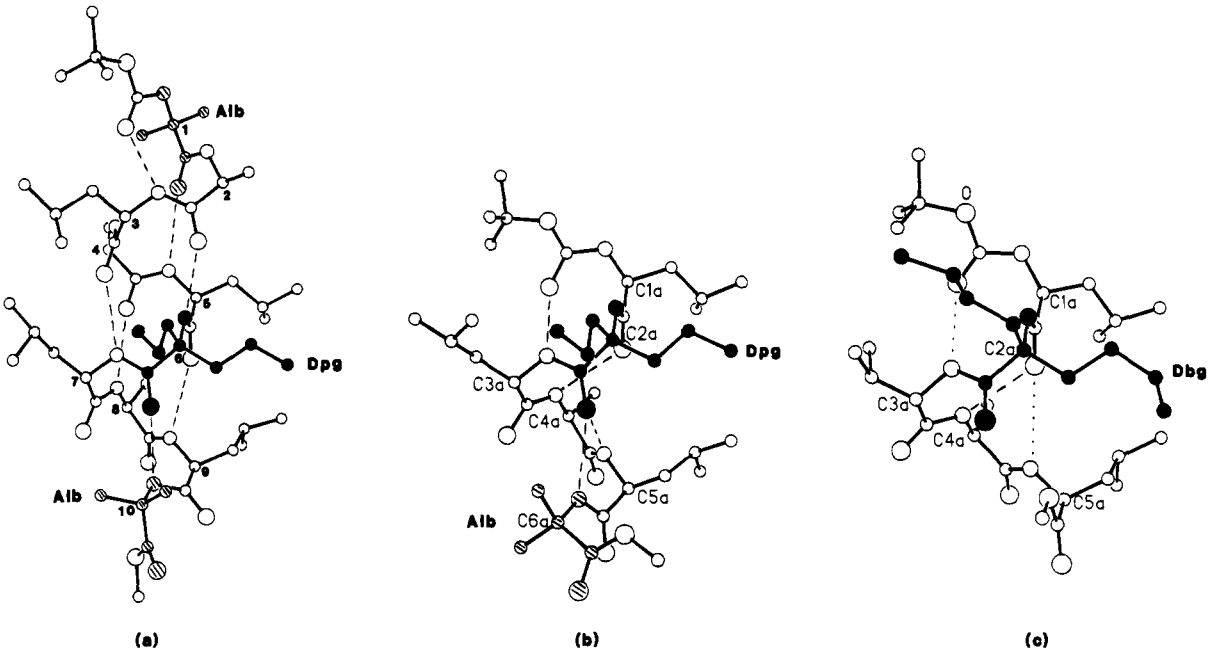

Figure 1. Views perpendicular to helix. Dpg and Dbg residues are darkened; Aib residues are striped. (a) Boc-Aib-Ala-Leu-Ala-Leu-Dpg-Leu-Ala-Leu-Aib-Ome (I). (b) Boc-Leu-Dpg-Leu-Ala-Leu-Aib-OMe (II); **IIa(1)**, **IIa(2)**, and **IIb** have nearly the same conformation (only one is shown). (c) Boc-Leu-Dbg-Val-Ala-Leu-OMe (**III**).

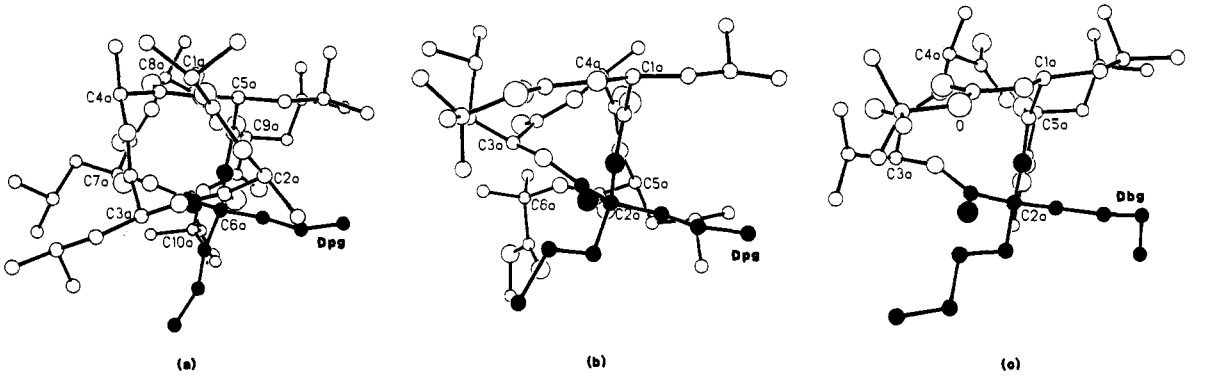

Figure 2. Views into the helices of **I**, **II**, and **III** shown in Figure 1.

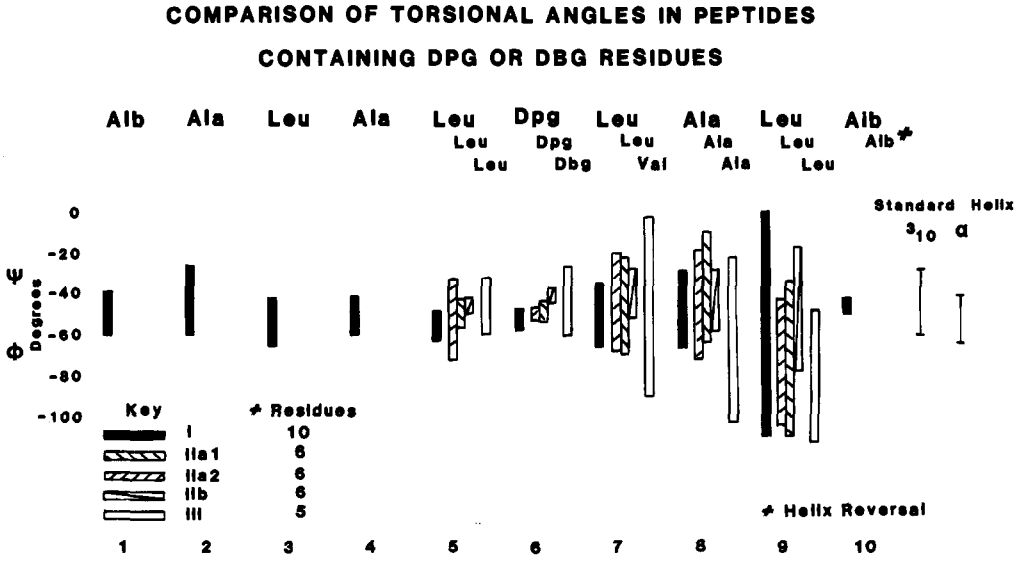

Figure 3. Torsional ϕ and ψ values for peptides **I**, **IIa(1)**, **IIa(2)**, **IIb**, and **III** as well as the "standard" 3_{10}- and α-helices. The bottom of each bar represents the ϕ value (torsion about N–C^{α}), and the top of each bar represents the ψ value (torsion about C^{α}–C′).

For the helical peptides reported in Table 4, the averaged value for the N–C^{α}–C′ angle of the Aib residues is near 111°, consistent with previous observations in helical structures. However, the N–C^{α}–C′ angle in the Dpg residue has a large range of values. The reason for the inconsistencies of the τ angles of **IIb** when compared with those of the other four

Table 4. τ Values (Bond Angles) for C^{α} with Two Side Chains

angle	residue	I	IIa(1)	IIa(2)	IIb	III	av
$N-C^{\alpha}-C^{\beta}(L)$	Aib[a]	107					107
$N-C^{\alpha}-C^{\beta}(D)$		110					110
$C'-C^{\alpha}-C^{\beta}(L)$		108					108
$C'-C^{\alpha}-C^{\beta}(D)$		110					110
$N-C^{\alpha}-C^{\beta}(L)$	Dpg or Dbg[b]	109	106	112	114[d]	108	109
$N-C^{\alpha}-C^{\beta}(D)$		110	112	117	110[d]	111	112
$C'-C^{\alpha}-C^{\beta}(L)$		106	105	102	114[d]	108	105
$C'-C^{\alpha}-C^{\beta}(D)$		110	112	105	106[d]	109	109
$N-C^{\alpha}-C^{\beta}(L)$	Aib[c]	106	112[e]	109	107		108
$N-C^{\alpha}-C^{\beta}(D)$		111	105[e]	112	111		110
$C'-C^{\alpha}-C^{\beta}(L)$		110	110	105	103		107
$C'-C^{\alpha}-C^{\beta}(D)$		109	114	112	112		112
$N-C^{\alpha}-C'$	Aib[a]	112					112
$C^{\beta}(L)-C^{\alpha}-C^{\beta}(D)$		110					110
$N-C^{\alpha}-C'$	Dpg or Dbg[b]	109	107	105	113[d]	111	108
$C^{\beta}(L)-C^{\alpha}-C^{\beta}(D)$		113	115	113	102[d]	110	113
$N-C^{\alpha}-C'$	Aib[c]	111	108	113	105[d]		111
$C^{\beta}(L)-C^{\alpha}-C^{\beta}(D)$		111	107	104	117[d]		107

[a] Aib(1) in **I**. [b] Dpg(6) in **I**, Dpg(2) in **II**, Dbg(2) in **III**. [c] Terminal Aib. [d] Not included in averages. Values in molecule **IIb** are not consistent with the other four molecules. [e] Values not consistent with other molecules.

molecules is not immediately apparent. Similar inconsistencies in τ values have also been found for the series of decapeptides Boc-Gly-Dpg-X-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (where X = Leu, Pro, Ala) (to be published).

All the side chains of the Leu residues and the Dpg and Dbg residues are extended away from the helical backbone, Figures 1 and 2. Of the 15 Leu side chains in the molecular studies, six have the t(tg^-)conformation (all occurring near or toward the N-terminus), six have the g(gt) conformation (all occurring near or toward the C-terminus), and three (occurring in the middles of **IIa**(1), **IIa**(2), and **IIb**) have a g^-(tg^-) or g(tg^-) conformation. The Dpg and Dbg residues do not seem to influence the conformation of neighboring Leu residues.

In the Dpg and Dbg residues, the side chains on the L- and D-positions of the C^{α} atom are distinguished by κ_L (near 180°) and κ_D (near 60°), where κ is the torsional angle defined by $C'(i-1)-N(i)-C^{\alpha}(i)-C^{\beta}(i)$. The alkyl chains assume values for the χ^1 and χ^2 torsions near *trans–trans* (tt) for three L-chains and near *gauche–trans* (g^-t) for two L-chains and all the D-chains.

Intramolecular Hydrogen Bonds. The decapeptide **I** has two 4 → 1 hydrogen bonds involving N(3) and N(4) and six 5 → 1 hydrogen bonds involving N(5) to N(10), Table 3. Carbonyl oxygen O(1) is the acceptor of a hydrogen bond from N(4) and N(5) and is the point of transition from a 3_{10}- to an α-helix. The N···O distances range from 2.87 to 2.97 Å, except for N(5)···O(1) = 3.18 Å, a helix transition region, and N(9)···O(5) = 3.15 Å, a helix distortion area at Leu.[9] The two indepedent hexapeptide molecules in crystal **IIa** are very similar, in that each has three 4 → 1 hydrogen bonds involving N(3) to N(5) and a transition to a 5 → 1 type bond at N(6). The N···O distances range from 2.95 to 3.11 Å. The same hexapeptide in crystal **IIb** has four 4 → 1 hydrogen bonds with N···O distances ranging from 2.94 to 3.19 Å. The helix type for the pentapeptide **III** is not as well defined. There is one strong 4 → 1 bond (N(4)···O(1)) and a very weak 4 → 1 type bond between N(3) and O(0) from the Boc end group. Further, there may be a weak 5 → 1 type bond between N(5) and O(1).

Head-to-Tail Hydrogen Bonds. All the helical peptides described in this paper align themselves into columns by head-to-tail hydrogen bonding, as has been found in almost all other

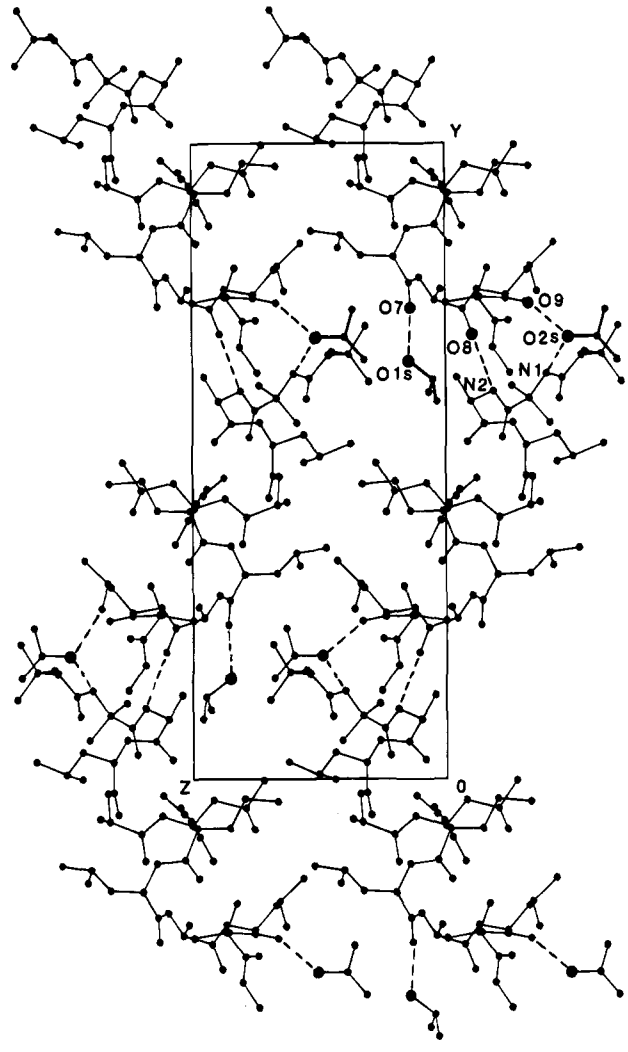

Figure 4. Packing diagram for Boc-Aib-Ala-Leu-Ala-Leu-Dpg-Leu-Ala-Leu-Aib-OMe·2^{i}PrOH. Head-to-tail hydrogen bonds join helices (related by a 2-fold screw) into an infinite column. All the helices have the N-terminus pointing up to form an all-parallel packing motif. There is one direct head-to-tail hydrogen bond, N(2)H···O(8).

crystals of helices. The individual helices are repeated either by a simple translation along the helix axis or by a 2-fold screw translation. The helix axis may not correspond to the axis of the column but may have a considerable tilt, as in Figure 4. In all the crystals, there is direct hydrogen-bonding between the peptides in a column, i.e., N(2)···O(8) in **I**, N(1)···O(4) and N(2)···O(5) in **IIa**, N(1)···O(4) in **IIb**, and N(1)···O(4) and N(2)···O(4) in **III**. Further, in two of the crystals, cocrystallized solvent molecules act as hydrogen-bonding bridges between the head and tail of neighboring helical peptides. Specifically, in **I**, the OH group in 2-propanol acts like a bridge by forming N(1)H···O(2s) and O(2s)H···O(9) hydrogen bonds. Similarly, in crystal **IIb**, a water molecule acts as a hydrogen-bonding bridge between N(2) of one molecule and O(4) and O(5) of two other molecules in the head-to-tail region. Crystals **IIa** and **III** do not contain any solvent.

Aggregation of Peptides. The crystal packing of helices in **I**, **IIa**, **IIb**, and **III** does not display any features that have not been observed for helical Aib-containing peptides.[17] The lateral surfaces of the helices are covered by hydrophobic side chains and have no possibility to engage in lateral hydrogen bonds to neighboring molecules. Except for $CH_3···CH_3$ van der Waals attractions (with C···C > 3.9 Å), there are no specific attractions between the columns of helical molecules. The crystal packing

appears to be an optimum filling of the space available. As a result, the columns of helices pack either in an all-parallel motif, as in crystal **I**, Figure 4, or in an antiparallel motif, as in crystals **IIa**, **IIb**, and **III**.

The presence of two long alkyl chains on the C^{α} atom in Dpg and Dbg residues does not appear to cause much difficulty or unusual assemblies. However, the crystal density for the Dpg- or Dbg-containing peptides is smaller, by up to 7% for **IIa** and **IIb**, as compared to densities of 1.13−1.15 gm/cm^3 for similar Aib-containing peptides. The atomic displacement values U for the terminal atoms in Leu or Dpg residues in **IIa** and **IIb** are larger than those in similar peptide crystals. The U values are a measure of positional disorder and/or libration, movements that can become more probable with "loose" packing associated with low density. Voids in the lattice often occur with molecules that do not fit together well. Crystal **I**, Figure 4, shows such an example in the head-to-tail region, where the fit of the peptides leaves a large enough void for two 2-propanol molecules.

Conclusions

The present investigation was motivated by the possibility of specifically stabilizing fully extended conformations at residues centrally positioned in a polypeptide sequence by employing α,α-dialkyl residues with long linear hydrocarbon chains. Despite the propensity of such residues to adopt extended structures in short homooligopeptides[10] and in a couple of heteromeric sequences,[11,13] the results presented above demonstrate the ready incorporation of Dpg and Dbg residues into helical sequences. Indeed, in pentapeptide **III**, a single Dbg residue appears to be sufficient to stabilize helical folding. The extra length of the alkyl chain in Dbg/Dbg does not appear to disturb the formation of good 3_{10}- or α-helices. It is noteworthy that in all cases the Dpg/Dbg residues adopt chiral geometries with the two alkyl chains occurring in different conformations in all cases. The observation of several *gauche* conformations in the linear alkyl chains may be directed by local packing interactions.

Theoretical calculations on Dpg residues have clearly shown the occurrence of two energy minima corresponding to the fully extended and helical regions of ϕ,ψ space, with the former being marginally more favorable, energetically.[8] The accumulating body of crystal structures of peptides containing Dpg/Dbg residues suggests that both conformations are indeed experimentally observable, with helices being more probable in long heteromeric sequences, containing residues with high helix propensities. In contrast, in Aib-containing peptides, helical conformations are almost always observed in both short and long sequences in both homopeptides and heteromers.[1,3−6] The behavior of the linear alkyl side chain-containing residues may also be contrasted with the 1-aminocycloalkane-1-carboxylic acid (Ac_nc)-containing peptides, where exclusively helical conformations have been observed at those residues in small peptides.[7] The use of Dpg/Dbg and related α,α-dialkylamino acids would be attractive in introducing conformational constraints into synthetic modules for *de novo* design or in developing analogs of biologically active peptides. The greater side chain extension as compared to Aib may make these residues useful in probing hydrophobic interaction sites on receptors, as exemplified in studies of analogs of the chemotactic tripeptide, For-Met-Leu-Phe-OH, where Leu has been substituted.[11] Studies presently underway in these laboratories address the issue of stabilizing extended conformers at Dpg and Dbg using flanking sequences containing poor helix-forming residues.

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Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic temperature factors, and H atom coordinates for **I**, **IIa**, **IIb**, and **III** (29 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.